Chapter 2

Literature overview

2.1. Monoamine oxidase

2.1.1. General background

Monoamine oxidase (MAO) was first discovered in 1928 when Mary Hare-Bernheim described it as tyramine-oxidase. She found that it catalyzed the degradation of tyramine through the process of oxidative deamination (Youdim & Bakhle, 2006). It was then found to catalyze the metabolism of other amines like serotonin, noradrenaline and dopamine, and was named monoamine oxidase. MAO is found on the outer membrane of mitochondria of various cell-types and contains flavin adenine dinucleotide (FAD) as co-factor. As mentioned above, MAO plays a critical role in the degradation of important amine neurotransmitters.

As mentioned in Chapter 1, MAO is not a single enzyme, but exists as two isoforms. These two isoforms are named MAO-A and MAO-B. MAO-A and MAO-B have different substrate and inhibitor specificities and their localization in the human body also differ. MAO-A selective substrates include serotonin, adrenaline and noradrenaline (Chen et al., 1994), and it is inhibited by the irreversible inhibitor, clorgyline. MAO-B employs benzylamine as substrate and is inhibited by (R)-deprenyl (Fowler & Tipton, 1984). Dopamine is a substrate for both MAO forms.

MAO-B activity increases with age, a process which is dependent on the compartmentalization of MAO-B in glial cells. The amount of glial cells increases with age (Westlund et al., 1988). Since $H_2O_2$ is generated in the MAO catalytic cycle, the increase of MAO-B activity with age may lead to an increase in oxidative stress. An increase in oxidative stress may accelerate the development of neurodegenerative disorders like Parkinson’s disease (Knoll, 1988). The discovery that MAO increases with age, aids in the understanding of the mechanism of expression of MAO (Nicotra et al., 2004). As more information is collected about the behaviour and mechanism of this enzyme, more effective inhibitors can be developed (Tipton et al., 2004).
2.1.2. Monoamine oxidase B

2.1.2.1. Role of MAO-B in Parkinson’s disease

Parkinson’s disease is a neurodegenerative disorder and is the result of the death of dopaminergic neurons in the substantia nigra of the brain (Dauer & Przedborski, 2003). The loss of dopaminergic neurons is due, in part, to the metabolism of dopamine by MAO which generates toxic byproducts and reactive oxygen species (ROS). These ROS may cause oxidative stress and may lead to the misfolding and aggregation of proteins such as α-synuclein. Misfolded α-synuclein may, in turn, lead to the damage of dopaminergic neurons because misfolded and aggregated proteins are toxic (Chesselet, 2003). The symptoms associated with Parkinson’s disease are tremors, rigidity, bradykinesia, monotonous speech and problems with coordination. The early onset of these symptoms is rarely noticed by the patient (Lees et al., 2009).

![Figure 2.1](image)

*Figure 2.1. Neuropathology of Parkinson’s disease. (A) Schematic representation of the normal nigrostriatal pathway. (B) Schematic representation of the diseased nigrostriatal pathway. There is a marked loss of dopaminergic neurons that project to the putamen (dashed line) and a much more modest loss of those that project to the caudate. (Dauer & Przedborski, 2003).*
The current treatment for Parkinson’s disease only relieves the symptoms and does not cure the disease. There have been many hypotheses about the mechanism of development of Parkinson’s disease and the role of MAO in the pathogenesis of Parkinson’s disease. These mechanisms include misfolding and aggregation of proteins, oxidative stress and the observation that MAO-B increases with age (Fowler et al., 1997). Age is therefore a risk factor for the development of Parkinson’s disease.

MAO-B catalyzes the oxidative deamination of dopamine and inhibitors of MAO-B are used in the treatment of Parkinson’s disease. MAO-B inhibitors are thought to prevent the metabolism of dopamine thereby elevating dopamine levels. This treatment aims at relieving the symptoms of Parkinson’s disease by compensating for dopamine loss (Foley et al., 2000). Another role that MAO-B plays in Parkinson’s disease is the formation of toxic substances and free radicals that damage dopaminergic neurons. This may also be prevented by MAO-B inhibitors, thus yielding a neuroprotective effect (Novaroli et al., 2005). Although dopamine is a substrate for both MAO isoforms, MAO-B inhibitors are used in Parkinson’s disease because the basal ganglia, which contain 80% of brain dopamine, predominantly contain MAO-B (Bertler & Rosengren, 1959). The use of MAO-A inhibitors in the treatment of Parkinson’s disease may also be an option, since as mentioned above, dopamine is also a substrate for MAO-A. MAO-A inhibitors are, however, associated with adverse side effects such as the serotonin syndrome (serotonin toxicity) and the cheese reaction. Therefore little attention has been paid in applying MAO-A inhibitors for the treatment of Parkinson’s disease and more emphasis is put on the development of selective MAO-B inhibitors.

![Figure 2.2](image.png)

**Figure 2.2.** The oxidation of dopamine by MAO-A and MAO-B.

As mentioned above, MAO-B inhibitors may also exert a neuroprotective effect. The MAO-catalyzed metabolism of dopamine leads to the formation of H$_2$O$_2$ and other toxic
free radicals, which may cause neurodegeneration. MAO-B inhibitors reduce the formation of these toxic by-products. The first study of the neuroprotective properties of MAO-B was the DATATOP study, a double blind, randomized, placebo controlled study of (R)-deprenyl and tocopherol in 800 patients not previously treated and which were in the first stages of Parkinson’s disease. The study showed that (R)-deprenyl postponed the necessity for L-dopa therapy for up to nine months (Foley et al., 2000). Another study has been carried out where patients received (R)-deprenyl or placebo treatment for 14 months (12 months treatment and 2 months washout period). It was found that (R)-deprenyl improved the symptoms of Parkinson’s disease and simultaneous administration of L-dopa and MAO-B inhibitors, for example (R)-deprenyl, elevated the beneficial effects of L-dopa. MAO-B inhibition also resulted in a 30-40% reduction in the required L-dopa dosage (Foley et al., 2000).

![Figure 2.3. The decarboxylation of L-dopa to yield dopamine.](image)

Environmental factors have been investigated as potential causes for the development of Parkinson’s disease. To study Parkinson’s disease, various animal models are available. Toxin based models that have been studied are the 6-hydroxydopamine, paraquat, rotenone and MPTP animal models. These models have given evidence that environmental factors may have a role to play in the development of Parkinson’s disease and that MAO inhibitors may possess neuroprotective properties (Dauer & Przedborski, 2003).

The MPTP model has given insights into the events that take place during neurodegeneration of dopaminergic pathways and have shown that MAO-B inhibitors may possess neuroprotective properties (Dauer & Przedborski, 2003). This model also provides an understanding of the importance of MAO-B in the treatment of Parkinson’s
disease and provides a rationale for the development of new MAO-inhibitors to treat Parkinson’s disease, especially as neuroprotective therapy. MPTP was first discovered when young drug users developed a parkinsonian syndrome after accidental self-administration of MPTP. It was subsequently found that MPTP is a pro-neurotoxin and required bioactivation. The first step of the bioactivation of MPTP is catalyzed by MAO-B (Chiba et al., 1984). The ultimate product, MPP⁺, is a mitochondrial toxin which causes selective degeneration of nigrostriatal dopaminergic neurons in humans and experimental animals (Langston et al., 1983). Inhibitors of MAO-B protect against the neurotoxic effects of MPTP, an effect that is almost certainly linked to the blockade of the metabolic bioactivation of MPTP. MPP⁺ is thought to exert its neurotoxin action by inhibiting complex 1 of the electron transport chain. It is thought that the mechanisms of the development of Parkinson’s disease may include mitochondrial dysfunction and oxidative stress. Complex 1 of the mitochondrial electron transport chain has been linked to the development of Parkinson’s disease (Abou-Sleiman et al., 2006). For example complex 1 deficiency in Parkinson’s disease may lead to a decrease in ATP production and an increase in oxidative stress through free radical production (Khan, 2006). Oxidative stress is caused when electron flow through complex 1 is inhibited. The decrease in ATP together with increased oxidative stress may lead to neuronal toxicity. Since MPP⁺ is also a complex 1 inhibitor, it is thought that the mechanism by which MPP⁺ induces neurotoxicity is similar to the mechanism responsible for Parkinson’s disease.
2.1.2.2. Inhibitors of MAO-B

MAO inhibitors have been investigated for many years because of their potential in the treatment of neurodegenerative disorders. Inhibitors of this enzyme have been used in the treatment of depressive illnesses. Tranylcypromine and iproniazid (which is structurally related to isoniazide) are examples of such drugs. The adverse effects of these inhibitors lessened interest in MAO inhibitors, specifically MAO-A, as a treatment for depression (Youdim & Bakhle, 2006).

![Tranylcypromine and Isoniazide structures](image)

Tranylcypromine | Isoniazide
--- | ---

**Figure 2.4.** The structures of tranylcypromine and isoniazide.

MAO-B inhibitors were initially investigated as a treatment strategy for depression. This led to the discovery of (R)-deprenyl, a selective inhibitor of MAO-B, which does not cause the cheese reaction. The application of this inhibitor in the treatment of depression, however, was not successful and little attention has subsequently been paid to the potential of these inhibitors in the treatment of Parkinson’s disease (Youdim & Bakhle, 2006).

Peter Riederer was the first to study the potential of (R)-deprenyl as adjunct to L-dopa therapy in the treatment of Parkinson’s disease. Hereafter (R)-deprenyl was used successfully for the treatment of Parkinson’s disease (Youdim & Bakhle, 2006). (R)-Deprenyl is a selective irreversible inhibitor of MAO-B which increases the concentration of dopamine. (R)-Deprenyl also inhibits the re-uptake of dopamine into the nerve endings of catecholaminergic neurons and the uptake of tyramine into the noradrenergic nerve terminals. Since (R)-deprenyl does not inhibit the metabolism of tyramine in intestinal endothelial cells it does not cause the cheese reaction (Knoll, 1988).
Irreversible inhibitors of MAO-B:

The following are examples of well known irreversible MAO-B inhibitors

(a) (R)-Deprenyl

The R-enantiomer of deprenyl, also known as selegiline, is a potent selective, irreversible inhibitor of MAO-B that does not potentiate a sympathomimetic reaction associated with the inhibition of MAO-A, also called the cheese reaction. (R)-Deprenyl inhibits MAO-B thereby decreasing $H_2O_2$ and oxidative stress, while potentiating the effects of dopamine. By inhibiting the oxidative deamination of dopamine, (R)-deprenyl reduces the damage to dopaminergic neurons caused by the formation of ROS which is associated with the MAO-catalyzed oxidation of dopamine (Magyar et al., 2010). The expression of antioxidant enzymes, such as superoxide dismutase, is stimulated by the chronic treatment with (R)-deprenyl which, in turn, leads to an increase in the metabolic destruction of ROS (Magyar et al., 2010). As mentioned above, (R)-deprenyl also inhibits the re-uptake of dopamine into the nerve endings of catecholaminergic neurons and inhibits the metabolism of dopamine, thus elevating dopamine levels.

The hypothesis that apoptosis plays a role in the development of Parkinson’s disease can be demonstrated by the MPTP model of Parkinson’s disease where neuronal death is caused by the triggering of apoptosis (Blandini, 2005). (R)-Deprenyl, in concentrations lower than is needed to inhibit MAO-B activity, reduces apoptosis and may act as a neuroprotective agent in diseases such as Parkinson’s disease (Magyar et al., 2010).

As mentioned, (R)-deprenyl is also combined with L-dopa in the treatment of Parkinson’s disease. L-dopa, the metabolic precursor of dopamine, relieves the symptoms associated with Parkinson’s disease and improves morbidity and mortality in Parkinson’s disease patients. The long term use of L-dopa in Parkinson’s disease patients leads to the development of the “wearing off” effect and L-dopa induced dyskinesias. As mentioned above, (R)-deprenyl has neuroprotective properties and may delay the onset of disability and the necessity of L-dopa treatment (Olanow et al., 1995).
Furthermore, in early PD, treatment with (R)-deprenyl allows for a reduction in levodopa and dopamine agonist doses required for a therapeutic effect.

(b) Rasagiline

Rasagiline, similar to (R)-deprenyl, is a propargylamine, but is tenfold more potent than (R)-deprenyl as a MAO-B inhibitor. Rasagiline does not have sympathomimetic activity because, unlike (R)-deprenyl, it is not metabolized to amphetamine-like metabolites (Blandini, 2005). Rasagiline is an irreversible and selective inhibitor of MAO-B and also demonstrates neuroprotective properties. The neuroprotective properties of rasagiline may be unrelated to the inhibitor activity on MAO-B. This effect may be linked to the propargyl moiety’s anti-apoptotic properties (Blandini, 2005). Rasagiline enhances the release of dopamine, slows down the catabolism of dopamine and additionally blocks the action of an endogenous neurotoxin, N-methyl-(R)-salsolinol, which is an inducer of apoptosis (LeWitt & Taylor, 2008). Rasagiline was approved in 2006 as the first once daily oral treatment for Parkinson’s disease, as initial monotherapy in early Parkinson’s disease and as adjunct to L-dopa in moderate to advanced stages of Parkinson’s disease. In this regard, rasagiline decreases the need to add L-dopa in the early treatment regimens (Geldenhuys et al., 2011).

(c) Ladostigil

Ladostigil is a dual acetylcholinesterase inhibitor and non-selective MAO inhibitor. It had been developed following the finding that the propargylamine moiety is a key feature of certain MAO inhibitors’ neuroprotective properties (Geldenhuys et al., 2011). Ladostigil, similar to other selective inhibitors of MAO-B, prevents the neurodegeneration and depletion of dopamine as a result of the MPP⁺ toxin in the brain and, because it inhibits both forms of MAO, markedly increases dopamine levels (Youdim & Bakhle, 2006). Low dosage of ladostigil also prevents oxidative stress, astrocytosis and microglial activation in rats (Weinstock et al., 2011).
Figure 2.5. The structure of ladostigil.

Reversible inhibitors of MAO-B:

The following are examples of well-known reversible MAO-B inhibitors

(a) Isatin

Isatin is an endogenous reversible MAO-B inhibitor present in human urine. Endogenous MAO inhibitory activity was first discovered in normal human urine and was given the name tribulin. Isatin is a major constituent of tribulin (Glover et al., 1988). The concentrations of isatin in human urine are sufficient to account for the inhibitory effect on MAO. Exogenously administered isatin elevate dopamine levels in the rat striatum. In rats with a parkinsonian syndrome induced by the Japanese encephalitis virus, exogenously administered isatin relieves bradykinesia (Hamaue et al., 2004). Isatin may also be a marker for Parkinson’s disease since there is an increased urinary isatin excretion in Parkinson’s disease patients (Hamaue et al., 2004). Higher urinary isatin excretion may thus indicate a compensatory response to a lower cerebral dopamine level. Isatin is also found in higher levels in patients with neuropathological conditions (Binda et al., 2003).

Figure 2.6. The structure of isatin.
(b) Trans-trans-farnesol

Trans-trans-farnesol is a reversible inhibitor of MAO-B and a component of tobacco smoke. This compound is a moderately potent competitive inhibitor of human MAO-B with a $K_i$ value of 2.3 µM. It has been established that trans-trans-farnesol also modulates neuronal voltage-gated calcium channels, and that trans-trans-farnesol has antibacterial and anti-inflammatory activity in addition to the inhibitory activity on MAO-B (Khallil et al., 2006).

![Figure 2.7. The structure of trans-trans-farnesol.]

(c) Safinamide

Safinamide is a highly selective and reversible inhibitor of MAO-B. Safinamide is also a dopamine modulator, an inhibitor of dopamine metabolism by MAO-B, an inhibitor of dopamine reuptake and has an effect on the glutamate pathway by inhibiting glutamate release (Binda et al., 2007). The inhibition of glutamate release is achieved by blocking the N-type Ca$^{2+}$ mobilization. Glutamate release is one of the excitotoxic inputs leading to neuronal death (Caccia et al., 2006). Studies have shown the positive effects of safinamide include the increase in dopamine levels when co-administered with L-dopa and the potentiation of L-dopa response and reduction in the “wearing off” effect. It also prevents dopaminergic neuron death caused by the neurotoxin MPTP, suggesting a neuroprotective activity for safinamide (Caccia et al., 2006).
Figure 2.8. The structure of safinamide.

2.1.3. Monoamine oxidase A

2.1.3.1. The cheese reaction

The cheese reaction is a fatal hypertensive reaction that is caused when MAO-A is inhibited and tyramine, which is found in fermented foods like cheese and wine, cannot be metabolized in the epithelial cells of the intestine. Tyramine is then released into the systemic circulation and taken up by the peripheral adrenergic neurons. Tyramine subsequently induces the release of noradrenaline. The release of noradrenaline into the synaptic cleft leads to the development of a hypertensive reaction called the cheese reaction. Tyramine is inactivated by MAO-A, and to a lesser extent MAO-B, in the gut wall and liver. The tyramine that reaches the systemic circulation is further metabolized by MAO in the vascular endothelial cells and lungs (Youdim & Bakhle, 2006). The cheese reaction is mostly potentiated by irreversible inhibitors of MAO-A.
Figure 2.9. The cheese reaction. Tyramine can enter the circulation if not adequately metabolized by MAO. The presence of tyramine in the systemic circulation causes the release of noradrenaline. Noradrenaline release into the synaptic cleft is initiated by tyramine uptake into the adrenergic neurons. Noradrenaline can be degraded, in the absence of MAO-A, by catechol-O-methyltransferase (COMT) (Youdim et al., 2006).

As mentioned in the previous chapter, the use of reversible MAO-A inhibitors may overcome the occurrence of the cheese reaction. Reversible inhibitors of MAO-A may be displaced by tyramine, which is then metabolized normally. Although reversible inhibitors do not cause major hypertensive reactions, they still potentiate the effects of ingested tyramine (Anderson et al., 1993). An example of this behaviour is obtained with competitive, reversible inhibitors. The inhibitory effect of a competitive inhibitor is
relieved when the substrate concentration is increased as a result of the inhibition of substrate metabolism. Thus, when tyramine concentration increases it leads to the decrease in the inhibitory effect of the competitive inhibitor. This may restrict the degree of the hypertensive response caused by tyramine (Anderson et al., 1993). It is thus important to consider that dietary restrictions may be needed, even when reversible MAO-A inhibitors are used. It is also important to consider the type of inhibitor in addition to the reversibility of an inhibitor (Anderson et al., 1993). Another approach that may prevent the development of the cheese reaction is the co-administration of tricyclic inhibitors with MAO inhibitors and the use of inhibitors specific for MAO-B. The tricyclic inhibitors prevent the neuronal uptake of tyramine.

Adverse effects may also occur when irreversible MAO-A inhibitors are co-administered with other drugs. Examples of drug interactions with irreversible MAO-A inhibitors are:

1. MAO-A inhibitors combined with directly acting sympathomimetic amines such as noradrenaline potentiates pressor effects.

2. MAO-A inhibitors combined with L-dopa and amphetamine may lead to a hypertensive crises.

3. MAO-A inhibitors combined with fluoxetine, pethidine, reserpine and tricyclic antidepressants may lead to the fatal serotonin syndrome (Callingham, 1993).

2.1.3.2. The role of MAO-A in Parkinson’s disease and depression

MAO-A is found in sites where catecholaminergic neurons are located (Westlund et al., 1988). Dopamine is just as well metabolized by MAO-A, as by MAO-B (Youdim & Bakhle, 2006). In the past, MAO-A inhibitors were not considered for the treatment of Parkinson’s disease, because of adverse-effects such as the cheese reaction and serotonin syndrome. The metabolism of dopamine is dependent on the relative tissue concentration of the two enzymes and the dopamine concentration (Foley et al., 2000). As mentioned in previous chapters, MAO-B is predominantly expressed in the basal ganglia, the affected area in Parkinson’s disease (Bertler & Rosengren, 1959). Thus MAO-B inhibitors are mainly used in the treatment of Parkinson’s disease.
The development of reversible inhibitors of MAO-A (RIMAs), not causing the cheese reaction, made it a possibility that MAO-A inhibitors may be useful in the treatment of Parkinson’s disease. The cheese reaction does not occur when reversible inhibitors, such as moclobemide, are used because these inhibitors are displaced by ingested tyramine. The addition of a RIMA as adjunct to L-dopa, in the treatment of Parkinson’s disease, increases dopamine levels and it has mild symptomatic effects on motor functions.

A problem that may arise with moclobemide is the displacement of moclobemide from the enzyme by dopamine, which leads to the metabolism of dopamine and a lesser degree of elevation of dopamine levels. Also, when selective inhibitors of MAO-A are used, and one form of the enzyme is inhibited, it leads to the metabolism of the substrate by the other form (MAO-B) of the enzyme. Thus, the increase in dopamine levels may not be significant because dopamine is metabolized by both MAO isoforms (Youdim et al., 2006). The development of non-selective inhibitors of MAO, without the cheese reaction, may thus be an important consideration to overcome this problem.

As mentioned, MAO-A inhibits the oxidative deamination of amines such as serotonin, adrenaline and noradrenaline. MAO-A inhibitors have been used in the treatment of depression for decades. The aim of the therapy is to increase the concentration of serotonin in the brain by inhibiting MAO-A in the central nervous system, consequently relieving the symptoms of depressive illnesses. In Parkinson’s disease it is recognized that depression is the most frequent psychopathologic symptom of the disease (Jansen Steur & Ballering, 1999). It has been reported that, in brain samples of patients with Parkinson’s disease, there is a reduction of noradrenaline and serotonin (Youdim & Bakhle, 2006). These findings explain the prevalence of depression in patients with Parkinson’s disease. MAO-A inhibitors, used in Parkinson’s disease as antidepressants, show good tolerance, a shortened latency of motor response as well as a longer duration of action of L-dopa (Youdim & Weinstock, 2004).

It had, however, been suggested in the past that reversible MAO-A inhibitors are not as effective in the treatment of depression, compared to other antidepressants. Studies have shown that, when given in adequate doses, it may be as effective as other
antidepressants (Riederer et al., 2004). The main indications of MAO-inhibitors in the treatment of depressive illnesses are in patients with atypical and dysthymia depression, in patients who do not respond to re-uptake inhibitors and in patients with tricyclic resistant depression (Bergström et al., 1997).

MAO-A inhibitors are preferentially used in atypical depression and is less effective in the treatment of endogenous and melancholic depression (Thase et al., 1995). In the treatment of endogenous depression, it had been found that the tricyclic antidepressants are more effective than the MAO inhibitors. The use of MAO-A inhibitors in the treatment of endogenous depression, requires a larger dosage and is effective when the initial response to the tricyclic antidepressants are weak. The use of MAO-inhibitors in the treatment of psychotic depression and chronic depression shows fewer efficacies than treatment with the tricyclic antidepressants (Thase et al., 1995).

The use of irreversible MAO-A inhibitors have been met with adverse-effects, leading to poor patient compliance. As mentioned, irreversible MAO-A inhibitors are less safe to use and may lead to hypertension, thus dietary restrictions are needed when irreversible MAO-A inhibitors are used. These problems seem to be overcome by the reversible inhibitors, which are regarded as the first line MAO-A inhibitors (Riederer et al., 2004). Other complications that may occur are the development of behavioural toxicity and when MAO inhibitors are combined with serotonin uptake inhibitors, it may lead to a fatal syndrome, the serotonin syndrome. As mentioned, when MAO inhibitors, including selective MAO inhibitors, are co-administered with SSRIs, a complication which is called the serotonin syndrome may develop. The symptoms of serotonin syndrome are severe and may be fatal. Antidepressants may also cause behavioural toxicity. Behavioural toxicity occurs when a psychoactive substance impairs the cognitive and psychomotor abilities that are necessary to complete everyday tasks (Kerr et al., 1992). To avoid the development of behavioural toxicity MAO-inhibitors may be used in the treatment of depression. MAO-A inhibitors such as moclobemide do not cause behavioural toxicity. The application of moclobemide in the treatment of depression is thus important, especially in older patients whom are more susceptible to the development of behavioural toxicity.
2.1.3.3. Inhibitors of MAO-A

The first MAO-A inhibitors to be used in the treatment of major depressive disorders was tranylcypromine and phenelzine. These inhibitors are irreversible non-selective inhibitors of MAO-A, and cause the cheese reaction, which leads to poor patient compliance. The MAO-A inhibitors are thus not considered as first-line treatment for depression, and the SSRIs and tricyclic inhibitors are used instead. The development of reversible inhibitors of MAO-A sparked new interest in the potential that MAO-A inhibitors may have in the treatment of depression and even Parkinson's disease, because they do not, in general, cause the cheese reaction.

(a) Clorgyline

Clorgyline is an acetylenic, irreversible inhibitor of MAO-A, and was the first subtype selective inhibitor of MAO-A to be developed (Fowler et al., 2001). Although clorgyline is a potent antidepressant, the application of clorgyline in the treatment of depression is overshadowed by the cheese reaction. MAO-A is inactivated when clorgyline binds covalently to the flavin co-factor of the enzyme (Fowler et al., 2001). Clorgyline is thus known as a suicide substrate. The enzyme activity is only recovered via de novo enzyme synthesis, which may require several weeks. Irreversible inhibitors such as clorgyline need a “wash out” period of up to 7-14 weeks and the effects of clorgyline may last for several days after termination of treatment (Thase et al., 1995).

Figure 2.10. The structure of clorgyline.

(b) Tranylcypromine

Tranylcypromine is a non-selective irreversible inhibitor of MAO-A, and was first used as an antidepressant in the late 1950s. Adverse effects such as liver toxicity and death due to hypertensive crises and intracranial hemorrhages were reported later
(Ramachandraih et al., 2011). The liver toxicity may be due to the metabolic pathway of tranylcypromine which may yield small amounts of amphetamines, but under usual circumstances tranylcypromine may preclude its metabolism to amphetamine (Thase et al., 1995). The dosages needed in the treatment of depression is higher than required for the treatment of Parkinson’s disease and may account for the adverse effects experienced when using tranylcypromine in depression (Baker et al., 1992). The inhibition of MAO-A is more effective with the (+)-enantiomer of tranylcypromine, whereas more effective inhibition of the uptake of catecholamines is achieved with the (-)-enantiomer of tranylcypromine (Baker et al., 1992).

(c) Moclobemide

Moclobemide is a benzamide derivative and a reversible inhibitor of MAO-A. It is short-acting which, together with the reversibility and selectivity of moclobemide, lead to fewer problems such as the cheese reaction and hepatotoxicity compared to other MAO-inhibitors (Kerr et al., 1992). As mentioned above, some antidepressants cause behavioural toxicity which may be dangerous. Moclobemide does not cause behavioural toxicity and is safe to use in the elderly. Moclobemide is considered to be a mild antidepressant and also provides a mild symptomatic benefit in Parkinson’s disease. In patients with Parkinson’s disease who also presents with depression, moclobemide influences cognitive function (Youdim & Weinstock, 2004). Other important advantages of moclobemide are that moclobemide does not have anticholinergic effects, does not cause sedation and shows similar pharmacokinetics in younger and older patients (Yamada & Yasuhara, 2004). This makes moclobemide an appropriate MAO inhibitor in the treatment of depression in the elderly. A study has shown that moclobemide, in combination with (R)-deprenyl, had substantial effects on the symptoms associated with depression. These findings suggest that the combination of MAO-A and MAO-B inhibition may be beneficial for the treatment of depression (Jansen Steur & Ballering, 1999).
2.1.4. Substrate specificities and localization of MAO

Two decades ago the discovery was made that MAO exists as two isoforms (Westlund et al., 1988). This discovery led to the examination of the substrate specificities of the MAO isoforms. Furthermore, the discovery was made that there are species dependent differences in the substrate specificities of MAO, as well as differences in the tissue distribution of MAO. In the rat brain, both isoforms of MAO occur, while in the rat heart only MAO-A is found. In human platelets and lymphocytes only the MAO-B isoform is found while MAO-A is expressed exclusively in placental tissue (Fowler & Tipton, 1984). Both isoforms are present in the human brain, although they are differently distributed (Westlund et al., 1985) with MAO-B present in higher concentrations (Fowler et al., 1980; Kalaria et al., 1988). In subhuman primates, MAO-B also has been shown to be the dominant isoform in the brain (Willoughby et al., 1988). MAO-B is the main form in human liver tissue (Inoue et al., 1999) while MAO-A is the main form in gut tissues (Saura et al., 1996).

Substrate specificity is defined by using kinetic parameters of MAO-A and MAO-B towards different substrates. When substrates have a high Michaelis constant ($K_m$) for MAO-B and a high maximal velocity ($V_{max}$) value for MAO-A, they may be considered to be good substrates for MAO-A, and the converse applies to substrates for MAO-B. As mentioned previously, adrenaline, serotonin and noradrenaline are substrates for MAO-A, and benzylamine is a substrate for MAO-B, while dopamine is a substrate for both isoforms of MAO. Many substrates have affinities, to some extent, for both forms of MAO, even though it had been previously reported to be substrates for only one form of MAO (Fowler & Tipton, 1984). Given in the table below are the $K_m$ and $V_{max}$ values of well known substrates of MAO-A and MAO-B.
Table 2.1. The $K_m$ and $V_{\text{max}}$ values for well known substrates of MAO-A and MAO-B

<table>
<thead>
<tr>
<th>Substrate</th>
<th>MAO-A</th>
<th>MAO-B</th>
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<tbody>
<tr>
<td></td>
<td>$K_m$ (µM)</td>
<td>$V_{\text{max}}$ (pmol min$^{-1}$mg protein$^{-1}$)</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>125</td>
<td>379</td>
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<tr>
<td>Dopamine</td>
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<td>680</td>
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<td>Serotonin</td>
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<td>Noradrenaline</td>
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<tr>
<td>Tyramine</td>
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</tr>
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2.1.5. The three-dimensional structure of MAO

The two isoforms of MAO have a 70% identical amino-acid sequence and the backbone structure of MAO-A is also nearly identical to that of MAO-B. It had also been discovered that the similarity between rat MAO-A and human MAO-A is 87% over the entire molecule and 90% over residues 108-118 and 210-216 (Son et al., 2008). The reports of the X-ray crystallographic structures of MAO-A and MAO-B have aided in our understanding of structures of these important enzymes. For example, an important structural feature is the carboxyl-terminal transmembrane helix. The C-terminal amino acid residues are important in the anchoring of MAO-A and MAO-B to the outer mitochondrial membrane (Rebrin et al., 2001). C-terminal anchoring is important for the biological function of MAO.

Another important structural feature is the “aromatic cage” found in the active sites of both MAO isoforms. The aromatic cage is formed by the structures of the covalent FAD coenzymes and two tyrosines (Tyr) and is nearly identical in the two isoforms of MAO (De Colibus et al., 2005). The similarity of the active sites, may contribute to the fact that
the two isoforms follow the same catalytic mechanism. A major difference between the two isoforms of MAO is the natures of their respective oligomeric states in the crystal structures. MAO-A is a monomer and human MAO-B and rat MAO-A are dimers. There are also differences in the shapes and sizes of the active site cavities between MAO-A and MAO-B. This is the result of the amino acid replacements and conformational alterations (De Colibus et al., 2005). Another important finding is that the entrance and substrate cavities become fused when certain inhibitors are bound to MAO-B. This is not seen with MAO-A since the MAO-A active site is a single cavity. Human MAO-B and MAO-A may be divided into three domains (Binda et al., 2002). These domains include the flavin binding domain, the substrate binding domain and the membrane binding domain (Edmondson et al., 2004).

Figure 2.11. Localization of the substrate binding domain, the membrane binding domain and the FAD binding domain. FAD is coloured yellow. The substrate binding domain is coloured red, the FAD binding domain is coloured blue and the membrane binding domain is coloured green. The substrate and entrance cavities are coloured in cyan (Edmondson et al., 2009).
FAD is the co-factor of MAO. It plays an important role in the stabilization of MAO and is the site of covalent binding for irreversible inhibitors. Human MAO-A and MAO-B have identical FAD structures and the cysteine site for thioether linkage to the flavin ring is located at cysteine (Cys) 397 and Cys406 for MAO-B and MAO-A, respectively (Edmondson et al., 2004). The site of covalent FAD binding is near the C-terminal. Truncation of the C-terminal has effects on the solubility and the catalytic activity of MAO, but the inhibition of MAO is not influenced by the truncation of the C-terminal. As mentioned, the C-terminal anchors the enzymes to the mitochondrial membrane. Truncation studies suggest that MAO-B may contain additional membrane interaction sites, because deletion of C-terminal residues does not completely abolish the ability of the enzyme to bind to the membrane (Binda et al., 2002). In both MAO-A and MAO-B, the flavin rings exists in a “bent” rather than a planar configuration (Edmondson et al., 2009).

The active site of MAO-B consists of the substrate cavity and the entrance cavity. The substrate cavity is a 420 Å³-hydrophobic space and is interconnected with the 290 Å³ entrance cavity (Binda et al., 2002). The substrate cavity is located in front of the flavin and the entrance cavity underneath the protein surface and closed by the loop formed by residues 99-112 (Binda et al., 2007). There is also water molecules present in the active site of MAO-B. Furthermore the active site of MAO-B is hydrophobic. This allows apolar substrates and inhibitors to bind tightly to the active site. The only hydrophilic site is near the flavin and located between Tyr398 and Tyr435, which together forms the aromatic cage. This feature is important for amine recognition (Binda et al., 2003).

The three-dimensional structure of isatin bound to MAO-B shows that the dioxoindole ring is perpendicular to the flavin ring with the oxo groups on the pyrrole ring pointing towards the flavin. The 2-oxo group and the pyrrole NH are hydrogen bonded to the water present in the active site, whereas the 3-oxo group does not show any hydrogen bonding (Binda et al., 2003). When comparing the binding mode of isatin to MAO-B with the binding mode of 1,4-diphenyl-2-butene, it may be noted that when isatin is bound, the Ile199 side chain separates the two cavities. In contrast, when 1,4-diphenyl-2-butene is bound, the side chain of Ile199 is rotated so that the two cavities are no longer
separated and are fused to form a single cavity (Binda et al., 2003). 1,4-Diphenyl-2-butene, therefore, occupies both the substrate and the entrance cavities (Binda et al., 2003). These data show that Ile199, the “gating residue”, may adopt an open or closed conformation, which depends on the nature of the bound ligand (Binda et al., 2003).

Figure 2.12. Structure of MAO-B bound to 1,4-diphenyl-2-butene (Edmondson et al., 2004).
For the substrate to reach the active site it is important that loop 99-112 moves so that the entrance cavity may be reached. The entrance cavity is located between the active site and the protein surface and is protected by loop 99-112. When the entrance cavity is reached, residues Tyr326, Ile199, Leu171 and Phe168, which separate the substrate cavity and the entrance cavity, must undergo a slight movement. Hereafter the substrate may diffuse into the active site (Binda et al., 2002). The negatively charged membrane surface electrostatically facilitates the attraction of positively charged amine substrates to MAO-B (Edmondson et al., 2004). It is interesting to note that MAO-B catalyzes the oxidative deamination of smaller exogenous amines and MAO-A degrades larger endogenous amine neurotransmitters. In contrast, larger inhibitors are better accommodated in MAO-B than in MAO-A (Binda et al., 2002).

The structure of MAO-A is similar to that of MAO-B. A unique feature of human MAO-A is that the conformation of the cavity loop 210-216 differs from that of human MAO-B and rat MAO-A. Another important feature of MAO-A is that it is a monomer. The monomeric form may result because of a mutation unique to human MAO-A. The mutation occurs at glutamate (Glu) 151 → lysine (Lys). Lys151 is located proximal to a cluster of charged residues involved in monomer-monomer contacts to form a dimer in human MAO-B and rat MAO-A (De Colibus et al., 2005). This mutation may be the cause of the monomeric form of human MAO-A.
The active site of MAO-A is hydrophobic, which is similar to that of MAO-B. The cavity is 550 Å³ and is lined by 11 aliphatic and 5 aromatic residues, which demonstrates the hydrophobic character of MAO-A (De Colibus et al., 2005). Two Cys-residues (Cys321 and Cys323) are located near the catalytic site. Mutations of these Cys-residues do not influence the catalytic activity of MAO-A. The catalytic activity may, however, be influenced by the oxidation of the thiol groups to a disulfide, because MAO-A activity is sensitive to thiol oxidation and thiol reagents (De Colibus et al., 2005).

Harmine, a MAO-A inhibitor, interacts with a number of residues in MAO-A, including Ile335, a residue located in the center of the MAO-A active site. The structure of the harmine molecule is not accommodated by MAO-B because of the structural overlap with Tyr326 of MAO-B. The key residues that determine the inhibitor specificities of MAO-A and MAO-B are Ile335 and Tyr326, respectively (Ma et al., 2004). The inhibitor selectivity is thus greatly influenced by Ile335 of MAO-A and Tyr326 of MAO-B. Phe208 and Ile199 in MAO-A and MAO-B, respectively, also play a role in determining inhibitor
specificities. For example, 1,4-diphenyl-2-butene, does not bind to MAO-A since this inhibitor would overlap with Phe208 of MAO-A, which corresponds to Ile199 of MAO-B (Son et al., 2008). Inhibitors occupy a common, limited space between Gln215 and Ile180 in MAO-A and Gln206 and Leu171 in MAO-B.

Figure 2.14. Comparison of the active sites of MAO-A and MAO-B. Residue names and numbers are labeled according to MAO-A; the residues differing in MAO-B are labeled in parentheses. (Ma et al., 2004).

The structural data on MAO will greatly assist pharmacophore design and hit selection in this study. The knowledge of the interactions that an inhibitor undergoes with the MAO active sites and the structural features required for binding will facilitate the rational selection of drugs that will be evaluated as human MAO inhibitors.
2.1.6. *In vitro* measurement of MAO activity

Many of the methods developed for the determination of MAO activity was developed in the 1960s and was improved on in later years. Currently there are numerous methods available for the determination of MAO activity. These methods include radiometric, fluorometric, spectrophotometric, polarographic, chromatographic, and luminometric methods (Nicotra & Parvez, 1999).

**Radiometric:** The assay mostly used to determine MAO activity, is the discontinuous radiochemical assay. This method uses the $^{14}$C-labeled substrates and successive extraction of the labeled metabolites. This method is very sensitive and specific, and is mostly used because of the availability of the $^{14}$C-labeled physiological substrates. This discontinuous direct method allows MAO-A and MAO-B activity to be determined when only a very small quantity of tissue is available. The labeled products of the enzyme degradation are separated by HPLC and measured directly by a coupled radioisotope detector (Nicotra & Parvez, 1999). This method is a sensitive and reliable method for determining MAO activity in very small sample tissues.

**Luminometric:** A luminometric method has been developed which is discontinuous and measures the $\text{H}_2\text{O}_2$ produced by MAO activity. This method is based on the principle that the oxidation of luminal in the presence of peroxidase and $\text{H}_2\text{O}_2$ produces light (Nicotra & Parvez, 1999). The quantity of light is measured, which is an indication of the quantity of hydrogen peroxide produced by MAO activity. Although this method is more time consuming, a wider range of substrates are available to be used. Importantly, substrates that are more readily oxidized than luminol in the presence of horseradish peroxidase cannot be used. These substrates include adrenaline, noradrenaline and serotonin (Nicotra & Parvez, 1999).
Figure 2.15. The oxidation of luminal in the presence of \( \text{H}_2\text{O}_2 \) and HRP produces light.

Fluorometric: Various different fluorometric methods have been developed. Kynuramine displays similar \( K_m \) values towards the two enzymes of MAO with values of 16.1 µM and 22.7 µM for MAO-A and MAO-B, respectively. The MAO-catalyzed oxidation of kynuramine yields 4-hydroxyquinoline, a fluorescent compound which is readily measured in basic solutions at excitation and emission wavelengths of 310 nm and 400 nm, respectively (Petzer et al., 2012). This method will be used in this study.

Figure 2.16. The oxidative deamination of kynuramine to yield 4-hydroxyquinoline by MAO-A or MAO-B.

Another fluorometric method is a one-step indirect method allowing both end-point measurements and kinetic experiments and is based on the measurement of MAO generated \( \text{H}_2\text{O}_2 \) in a horseradish peroxidase coupled reaction. This method employs Amplex Red, as probe for the detection of \( \text{H}_2\text{O}_2 \). In the presence of horseradish peroxidase and \( \text{H}_2\text{O}_2 \), Amplex Red is oxidized to resorufin, a highly fluorescent product. This method is highly sensitive.
Figure 2.17. The oxidation of Amplex Red to resorufin in the presence of horse radish peroxidase (HRP).

Spectrophotometric: A continuous peroxidase-linked spectrophotometric method for measuring MAO activity uses a chromogenic solution containing 4-aminoantipyrine. This compound acts as the proton donor in the peroxidase-catalyzed reaction and then condenses with vanillic acid producing a red quinoneimine dye (Nicotra & Parvez, 1999). The amount of $\text{H}_2\text{O}_2$ produced is directly proportional to the absorbance. This method is inexpensive and does not use highly toxic reagents. This method is suitable for measuring MAO and diamine oxidase activities, as well as semicarbazide sensitive amine oxidase activities in tissue homogenates (Holt et al., 1997).
2.1.7. Mechanism of MAO catalysis

MAO oxidizes primary aliphatic and aromatic amines. This reaction results in the reduction of the enzyme-bound FAD and the formation of an imine product and ammonia. Hereafter the enzyme-bound FAD is reoxidated by $O_2$. This leads to the formation of $H_2O_2$. The C-H bond cleavage reaction may occur by three possible mechanisms. These mechanisms include heterolytic hydride transfer, heterolytic $H^+$ abstraction (including the ammonium cation radical as well as the polar nucleophilic mechanism), and homolytic $H^+$ abstraction (Edmondson et al., 2009). The stereochemistry of the hydrogen transfer from the $\alpha$-CH$_2$ is strictly pro-R for both MAO-A and MAO-B (Edmondson et al., 2004). The C-H bond cleavage is a rate limiting step in each catalytic reaction with the benzylamine class of substrates. There may also be
an H-tunneling contribution to the C-H cleavage. Four mechanisms have been proposed for MAO catalysis, the single electron transfer (SET) mechanism, the hydrogen atom transfer mechanism, the hydride transfer mechanism and the polar nucleophilic mechanism.

The SET mechanism

This mechanism is based on the premises that the pKa of the α-C-H protons of an amine requires a very strong base for abstraction. The first step of this mechanism is the one-electron oxidation of the lone pair on the amine nitrogen. This forms an aminium cation radical and a flavin radical. The α-C-H has a lower pKa as a result of the aminium radical formation. This is proposed to permit the abstraction of the H⁺ by an active site base in the catalytic site (Edmondson et al., 2004). In the second part of the reaction, radical recombination in the substrate takes place and a second electron is transferred from the amine lone pair to the FAD. This yields the reduced FAD and the iminium product.
The SET mechanism may be improbable. This is due to the fact that there is no structural data that shows amino acid residues in the catalytic site that may serve as active site bases for $\text{H}^+$ abstraction. Furthermore, the oxidation-reduction potential of the FAD cofactor is too low to be an effective oxidant of the deprotonated amine. The potential for the one electron reduction for the flavin in MAO-B is +0.04 V. The potential required for one electron oxidation of primary amines are 1.5 V (Edmondson et al., 2004). This leaves an energy barrier of 21-34 kcal/mol. This will result in an equilibrium constant of $10^{15}$-$10^{25}$ in favour of the reactants (Edmondson et al., 2004). Finally no flavin radical intermediates are detected spectrally in stop flow experiments and there is no observation of an influence of magnetic fields on the rate of enzyme reduction (Edmondson et al., 2009).
The polar nucleophilic mechanism

The initial step of catalysis in this mechanism is the nucleophilic attack of the C(4a) position of the FAD by the deprotonated amine to form an adduct. The adduct formation leads to the N(5) position of the flavin becoming a strong base. The N(5) would thus exhibit sufficient basicity to abstract the α-pro-R-H from the substrate (Edmondson et al., 2004). The abstraction of H⁺ from the pro-R α-CH is the preferred mode of C-H bond cleavage (Edmondson et al., 2009). This mechanism is consistent with current structural data available for MAO-B. The flavin is in a bent non-planar conformation which is expected to facilitate C(4a) adduct formation with the substrate (Edmondson et al., 2004). After proton abstraction, the iminium product is released from the reduced FAD.

Figure 2.20. Polar nucleophilic mechanism of MAO catalysis.
The hydrogen atom transfer mechanism

Hydrogen transfer from the α-C is strictly pro-R for MAO-A and MAO-B. Hydrogen transfer exhibits a large deuterium kinetic isotope effect. This demonstrates an H-tunneling effect contribution to the C-H bond cleavage and that the H acceptor is situated such that the distance traversed by the transferred H is comparable with the de Broglie wavelength for H (0.6 Å when E=20 kJ/mole) (Edmondson et al., 2004). The H-tunneling has not yet been determined for MAO-A.

![Figure 2.21](image.png)

Figure 2.21. The first step of the hydrogen atom transfer mechanism of MAO catalysis.

The hydride transfer mechanism

This mechanism suggests that a hydride ion from the α-C of the amine migrates to the N(5) atom of the flavin. Hydride transfer is a high energy process and the oxidation of a hydrazine to a diazine by abstraction of a hydride ion from a nitrogen atom would be difficult to envision (Edmondson et al., 2009).
Figure 2.22. Hydride transfer mechanism of MAO catalysis.

Of all the mechanisms proposed, the most likely is the polar nucleophilic mechanism. The abstraction of a pro-R α-C group as the mode of C-H bond cleavage is exhibited by rat MAO-A and mutants of both human and rat MAO-A. The different mechanisms are each met with controversy. The proposed mechanisms need to be consistent with the mechanistic and structural experimental data (Edmondson et al., 2004). For the future design of MAO inhibitors it is important that further research be done to understand the structural and catalytic characteristics of MAO.

2.1.8. Pharmacophores and modeling studies

Computer-aided molecular design methods, as well as pharmacophore design and three-dimensional (3D) database searches, aims at reducing the overall cost associated with the discovery and development of a new drug by identifying the most promising candidates to focus the experimental efforts on (Langer & Wolber, 2004). The use of pharmacophores aids in the understanding of key interactions between a target and a ligand. A pharmacophore is an ensemble of steric and electrostatic features of different compounds which are necessary to ensure optimal supramolecular interactions with a specific biological target structure and to trigger or to block its biological response (Langer & Wolber, 2004). Pharmacophores are not real molecules, but a representation of interactions of a group of compounds towards a receptor, and may be considered as the largest common denominator by a set of active molecules. Pharmacophores may be
applied in several areas. These include the search of databases, scaffold hopping, virtual screening, ligand profiling, predict activity, fragment design and pose filtering.

There are two approaches that may be taken to develop a pharmacophore. These approaches are the structure-based approach and ligand-based approach. Furthermore the pharmacophore generation may be done manually or automatically. Manual construction includes the use of aligned analogs for ligand-based design and aligned receptor active sites for structure-based design. Automatic ligand-based construction may be divided into the qualitative approach and the quantitative approach. The qualitative approach finds features shared by a set of similarly active ligands, whereas quantitative approach finds features that relate to activity. Structure-based automatic construction is done from a receptor-ligand complex. In this study, structure-based pharmacophore generation will be done from the structures of human MAO-A and MAO-B.

The possibility of deriving a structure-based pharmacophore model depends on the availability of the 3D structure of the binding site of the target (Langer & Wolber, 2004). The structure-based model may be used only when the 3D structure of the binding site is available. Structure-based pharmacophore generation consists of three important steps. First the calculation of the interaction sites, second the clustering of the vectors for H-bonding donating and accepting and of the hydrophobic regions and, third the transformation of the obtained clusters into a feature-based pharmacophore hypothesis (Langer & Wolber, 2004). Ligand-based pharmacophore generation consists of two important steps. The first step is the analysis of the training set molecules to identify pharmacophore features and the second step is the alignment of the assumed bioactive conformations of the molecules to determine the best overlay of the corresponding features (Langer & Wolber, 2004).
Figure 2.23. An example of a pharmacophore model. The green spheres represent the H-bond acceptor feature, the purple spheres represent the H-bond donor feature and the cyan spheres are hydrophobic features.

Pharmacophore models or hypotheses contain features which may represent atoms, H-bond acceptors, H-bond donors, and ionizable groups, hydrophobic or aromatic rings. Together with these features geometrical constraints may be formed, which may include distances and angles (Langer & Wolber, 2004). Features will only map if they are solvent accessible. After features have been added it is important to add location constraints. Location constraints define the relative location of the features.

A shape constraint may also be used to constrain a pharmacophore or refine a hit list. This may be divided into two groups, shape-only and mixed queries. With shape-only constraints the shape is aligned to the ligand, whereas with mixed queries the shape is aligned to the ligand and the location constraints. When a shape constraint is added, the ligand will match all features of the pharmacophore if a matching atom is found in the right location of the features and if the overall shape of the molecule, after being
aligned, is similar to the shape. Additionally an exclusion constraint may be added, which specifies one or more spherical spaces in a pharmacophore that must not contain any atoms or bonds.

Figure 2.24. An illustration of shape constraints in a mixed query (left) and shape-only query (right).

After a pharmacophore model is generated, new molecules which map to its features may be identified by two ways, de novo design and 3D database search (Langer & Wolber, 2004). The de novo approach seeks to link the parts of the pharmacophore together with fragments to generate structures that are chemically reasonable and novel (Langer & Wolber, 2004). 3D database search is capable of identifying compounds that may exhibit properties outside of the set of compounds used for building the pharmacophore, which aids in the identification of novel chemical structures and molecular features. These are termed scaffold hopping and lead hopping, respectively (Langer & Wolber, 2004).

The quality of the match between a ligand and a pharmacophore may be given by a fit value. The fit value gives a measure of how well the ligands fit to the pharmacophore. Two parameters are important, the weights assigned to the pharmacophore features and how close the features correspond to the location constraints. When mapping a ligand to a pharmacophore, there may be multiple mappings to the pharmacophore for
one ligand. The best alignment may be achieved when less pharmacophore features are mapped. For example, best mapping is defined as the ligand that fits the most features or has the highest fit, which does not necessarily match all the features.

![Pharmacophore-based virtual screening workflow](image)

**Figure 2.25.** Pharmacophore-based virtual screening workflow. (Langer & Wolber, 2004).
2.1.9. Conclusion

This chapter shows that MAO-A and MAO-B are important drug targets since they participate in the metabolic inactivation of neurotransmitter amines. Inhibitors of these enzymes are useful in the treatment of depression and Parkinson's disease. As such the identification of inhibitors of these enzymes may be of value. One approach is to screen a virtual library of existing drugs for potential binding to MAO-A and MAO-B. This approach has the advantage that an existing drug which displays MAO inhibition may be re-appropriated for the treatment of depression and Parkinson’s disease. In this way expensive preclinical and initial clinical trials are avoided. Also noteworthy is the observation that MAO-A inhibition may lead to serious side effects when combined with certain drugs and food. It is therefore important to identify those clinically used drugs that also inhibits MAO-A.