Chapter 1

Introduction and Rationale

1.1. Monoamine oxidase – general background

Monoamine oxidase (MAO) is an enzyme located on the outer mitochondrial membrane. MAO may be classified into two isoforms, MAO-A and MAO-B (Youdim *et al*., 2006). MAO acts as a catalyst for the metabolism of neurotransmitter amines such as serotonin, noradrenaline and dopamine. These neurotransmitter amine substrates are oxidatively deaminated by MAO, a process which terminates their biological actions. The oxidative deamination of these amines is associated with the formation of hydrogen peroxide (H_2O_2), an aldehyde and free amine or ammonia (Figure 1.1) (Youdim & Bakhle, 2006). The aldehyde and the H_2O_2 that forms may be cytotoxic and toxic, respectively. The aldehyde is metabolized by aldehyde dehydrogenase, which is an important mechanism to detoxify the aldehyde since it may be highly reactive and neurotoxic. H_2O_2, in turn, is deactivated in tissues by glutathione peroxidase (Grünblatt *et al*., 2004).

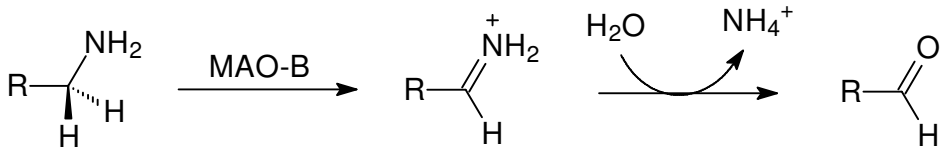


Figure 1.1. The oxidative metabolism of a primary amine by MAO.

MAO-A and MAO-B share a 70% sequence identity at the amino acid level (Bach *et al*., 1988). In spite of this, the MAO isoforms have different substrate and inhibitor specificities. Type A MAO is inhibited by low concentrations of clorgyline and catalyzes the oxidative deamination of serotonin and noradrenaline (Youdim *et al*., 2006; Johnston, 1968). Type B MAO prefers benzylamine as substrate and is inhibited by (R)-deprenyl (Chen *et al*., 1994). Dopamine is a substrate for both forms of MAO.

The localization of the two MAO isoforms also differs. MAO-B is mostly found in serotonergic and histaminergic neurons, while MAO-A is most abundant in

catecholaminergic neurons (Jahng *et al*., 1997). It has also been found that, in the fetus, MAO-A appears before MAO-B (Youdim *et al*., 2006) and that MAO-B activity increases with age (Novaroli *et al*., 2005).

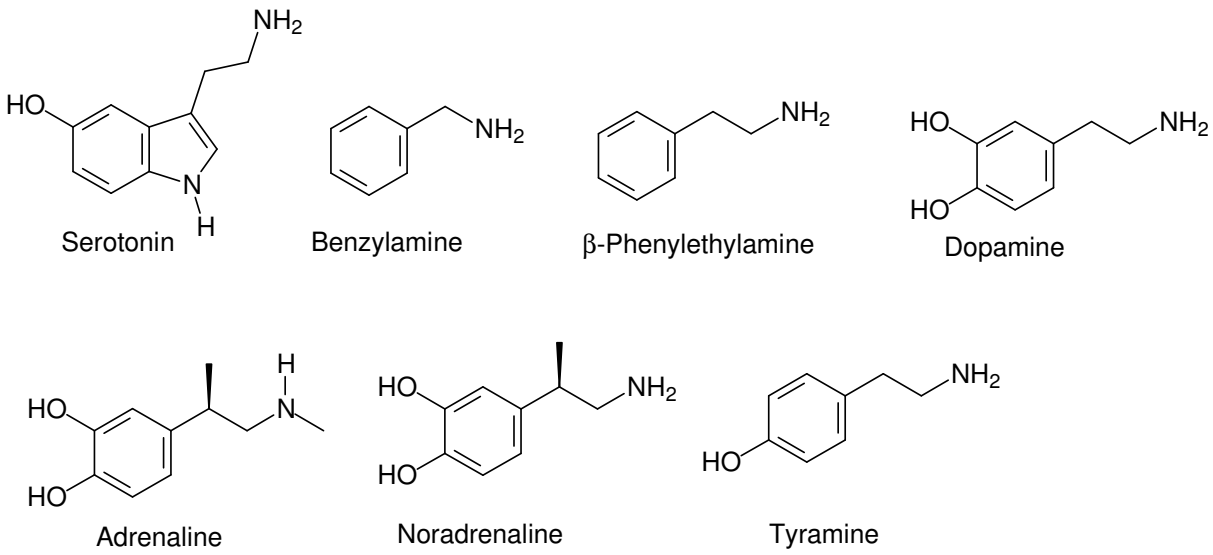
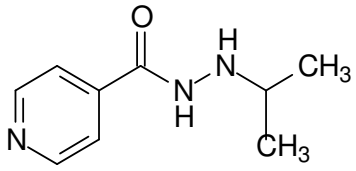


Figure 1.2. The structures of selected MAO substrates.

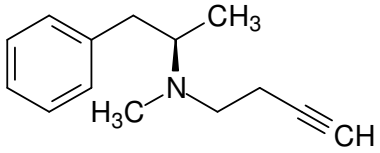
MAO inhibitors are useful in the treatment of Parkinson's disease and depression because they inhibit the MAO-catalyzed metabolism of dopamine and serotonin. The initial search for MAO inhibitors has been met with mixed success. MAO inhibitors such as iproniazid (Figure 1.3), which was first developed for the treatment of tuberculosis (Youdim *et al*., 2006) are hydrazine derivatives, which were associated with adverse side effects such as liver toxicity. Another complication of the older nonselective and irreversible MAO inhibitors is the "cheese reaction" which prompted scientists to seek new MAO inhibitors with an improved toxicity profile. The cheese reaction occurs when tyramine, which is found in fermented foods, enters the circulation and causes a release of noradrenaline. This may lead to a severe hypertensive reaction. Normally tyramine is metabolized in the gut by MAO-A, but when MAO-A is inhibited, the systemic concentrations of tyramine may increase (Youdim & Bakhle, 2006).

This problem may be overcome by the development of selective MAO-B inhibitors and reversible MAO-A inhibitors. Selective MAO-B inhibitors, of which (R)-deprenyl and rasagiline are examples, do not cause the cheese reaction because, in the intestines,

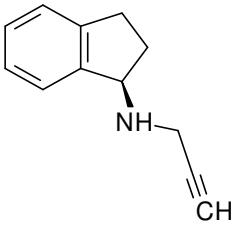
tyramine is metabolized by MAO-A. Tyramine also has the ability to displace reversible MAO-A inhibitors such as moclobemide, and can subsequently be metabolized normally, thus not causing the cheese reaction (Youdim & Bakhle, 2006).



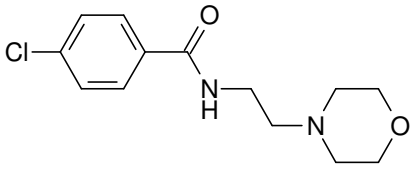
Iproniazid



(R)-Deprenyl



Rasagiline



Moclobemide

Figure 1.3. The structures of iproniazid, (R)-Deprenyl, rasagiline and moclobemide.

MAO-A inhibitors may also cause a syndrome called the serotonin syndrome when administered in combination with selective serotonin reuptake inhibitors (SSRIs). The symptoms of serotonin syndrome are restlessness, hallucinations, loss of coordination, rapid heartbeat, vomiting and nausea, changes in blood pressure, and body temperature spikes (Fernandez & Chen, 2007).

MAO has also been implicated in the activation of environmental neurotoxins. The parkinsonian inducing toxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), has given insight into the theory that toxins may have a role to play in the development of lesions of the nigrostriatal pathway (Singer *et al*., 1988). MAO-B catalyzes the oxidation of MPTP to 1-methyl-4-phenylpyridinium (MPP^+), which damages dopaminergic neurons. ß-carbolines and tetrahydroisoquinolines are endogenous substances, which are also metabolized to neurotoxic products by MAO (Foley *et al*., 2000). MAO inhibitors

therefore, could protect neurons by inhibiting the activation of these harmful neurotoxins, and thus may potentially be neuroprotective.

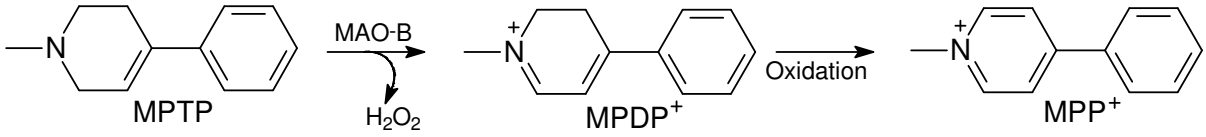


Figure 1.4. The metabolic activation of MPTP by MAO-B.

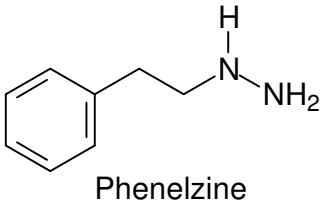
1.2. Inhibitors of MAO

As mentioned above, the inhibition of MAO is important in the treatment of several disorders, including Parkinson's disease, Alzheimer's disease and depressive illnesses. MAO-A inhibitors are used in depressive conditions because they inhibit the central metabolism of serotonin. MAO-B inhibitors are mainly used in Parkinson's disease because MAO-B is the predominant isoform in the basal ganglia, the affected area in Parkinson's disease (Bertler & Rosengren, 1959). In the brain, MAO-B inhibitors are thought to elevate dopamine levels and thus provide symptomatic relief in Parkinson's disease. The mechanism by which MAO inhibitors act is to directly inhibit MAO so that the metabolism of those neurotransmitters which are depleted in diseases, such as Parkinson's disease and depression, may be avoided. Studies have shown that the effects of MAO inhibitors may be much more diverse. Examples include the (+)-enantomer of tranylcypromine which is a poor antidepressant, despite the inhibitory effects it has on MAO. Also, MAO inhibitors may block the reuptake of certain neurotransmitters and chronic treatment with a number of MAO inhibitors leads to the reduction in the number of α- and ß-adrenergic and serotonin post-synaptic binding sites in the brain (Thase *et al*., 1995).

Inhibitors of MAO may be classified by several means: their chemical structures, their selectivities for the isoforms of MAO, by their modes of inhibition and by their reversibility of inhibition (Thase *et al*., 1995). Based on the reversibility of inhibition, inhibitors may be classified as irreversible, reversible or tight binding inhibitors. Irreversible inhibition occurs when an inhibitor binds covalently to the enzyme and

recovery of enzyme activity requires the synthesis of new enzyme. Repeated administration may lead to a loss of selectivity and substrate concentration changes do not effect inhibition. Reversible inhibitors do not bind covalently to the enzyme and recovery occurs after drug withdrawal and clearance of the drug from the tissues. There is less risk of the loss of selectivity and if the inhibitor is competitive, inhibition may be relieved if substrate concentration is increased (Tipton *et al*., 2004). Tight-binding occurs when the inhibition is reversible, but dissociates relatively slowly from the enzyme. This usually is the result of a conformational change which occurs after the inhibitor has bound to the enzyme.

Although they have been used to a considerable extent, irreversible and non-selective inhibitors of MAO have potential disadvantages. One of the first adverse-effects was hepatotoxicity caused by the use of MAO-A inhibitors in combination with barbiturates. The hepatotoxicity is caused by the hydrazine structure of certain MAO-A inhibitors such as iproniazid and phenelzine. Another profound disadvantage is the “cheese reaction”. As mentioned this is caused when MAO-A is irreversibly inhibited, usually when the older non-selective irreversible inhibitors of MAO are used. Tyramine is released into the systemic circulation and a hypertensive reaction occurs. Similarly, when MAO-A inhibitors are used in combination with SSRIs, patients may develop the serotonin syndrome, which can be fatal. Another problem with irreversible inhibitors of MAO is covalent binding. After drug withdrawal, the rate at which enzyme activity returns may require several weeks (Tipton *et al*., 2004). Also, repeated drug administration or high doses of irreversible inhibitors can cause loss of selectivity.



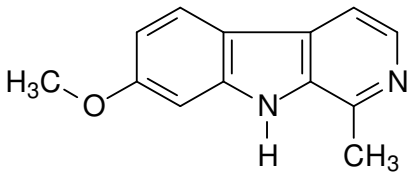
Phenelzine

Figure 1.5. The structure of phenelzine.

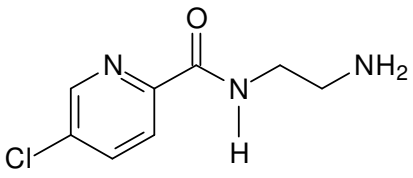
To avoid the “cheese reaction” and the other disadvantages (such as serotonin toxicity and slow rate of recovery) of irreversible inhibitors, reversible MAO-A inhibitors or

selective MAO-B inhibitors are used. Reversible inhibitors do not cause the cheese reaction, because tyramine displaces the inhibitor from the enzyme at the intestinal epithelial cells so that it could be metabolized normally. MAO-B selective inhibitors do not bind to MAO-A, thus allowing for tyramine to be normally metabolized in the intestines by MAO-A (Youdim & Bakhle, 2006). Similarly, serotonin toxicity usually does not occur when reversible MAO-A inhibitors are combined with SSRIs. To overcome covalent binding and facilitate the timely recovery of enzymatic activity, reversible inhibitors are used. After terminating the use of reversible inhibitors, enzyme activity recovers after the drug has been cleared and selectivity is not lost, even at higher concentrations (Legoabe *et al*., 2011). It is important to consider that the metabolism of a substrate by MAO-A or MAO-B is dependent on the tissue concentration of the two enzymes and the substrate. For example high concentrations of a selective inhibitor may inhibit the other MAO form and at high concentration of the substrate, metabolism by the less sensitive MAO form may occur (Foley *et al*., 2000).

Another type of MAO inhibitor is tight-binding inhibitors. The term also used to describe these types of inhibitors are slow-binding. Slow-binding occurs when inhibitors act as reversible inhibitors but recovery is time dependent because of their high affinities and tight binding characteristics (Tipton *et al*., 2004). These inhibitors bind rapidly and the resulting enzyme-inhibitor complex then slowly rearranges, causing it to bind tightly to the enzyme (Chimenti *et al*., 2010). Examples of tight binding inhibitors are harmine and lazabemide. These are MAO-A and MAO-B selective inhibitors, respectively.



Harmine



Lazabemide

Figure 1.6. The structures of harmine and lazabemide.

Reversible inhibitors can further be categorized as competitive, non-competitive, un-competitive and mixed-competitive inhibitors:

(a) Competitive inhibitors bind to the binding site of the enzyme and compete with the enzyme substrate. Competitive inhibitors are displaced when the substrate concentration is increased. Administration of high concentrations of competitive inhibitors is usually required because, when the enzyme is inhibited, the substrate concentration increases leading to the displacement of the competitive inhibitor (Tipton *et al*., 2004). If the inhibitor does not possess a high binding affinity for the enzyme, these high concentrations of a competitive inhibitor may lead to a higher risk of side-effects.

(b) Non-competitive inhibitors are not affected by changes in substrate concentrations. These inhibitors bind to a site that is distinct from the active site. Binding to a site that is distinct from the active site causes a change in the enzyme which reduces the affinity of the enzyme for the substrate (Tipton *et al*., 2004).

(c) Uncompetitive inhibitors bind to the enzyme-substrate complex which makes the development of an uncompetitive inhibitor difficult. The effects of uncompetitive inhibitors increase as the substrate concentration rises. These inhibitors have no effect when the substrate concentration is low. These inhibitors may thus be seen as substrate buffers (Tipton *et al*., 2004).

(d) Mixed competitive inhibition is a combination between competitive and uncompetitive inhibition. The response of mixed competitive inhibitors to changes in substrate concentration will depend on the magnitudes of the enzyme-inhibitor dissociation constant (K_i) for inhibitor binding to the free enzyme and the enzyme-substrate complex (Anderson *et al*., 1993).

1.3. Rationale of the study

Based on the above discussion, it may be concluded that MAO-A and MAO-B inhibitors are useful agents in the therapy of depression and Parkinson's disease. In the United States, (R)-deprenyl and rasagiline, are approved as adjunctive therapy to levodopa, or as monotherapy in Parkinson's disease. These inhibitors are both mechanism-based irreversible MAO-B inhibitors. Lazabemide, a reversible MAO-B inhibitor, has been shown to delay the need for levodopa in early untreated Parkinson's disease patients (The Parkinson study group, 1996). Phase III trials have shown that another reversible inhibitor, safinamide, results in significant improvements of motor scores when co-administered with dopamine agonist drugs (Fernandez & Chen, 2007; Stocchi, 2006). Moclobemide, a reversible MAO-A inhibitor is currently in use for the treatment of depression. It may thus be useful to identify novel MAO-A and MAO-B inhibitors which may find clinical application in depression and Parkinson's disease. One approach is to screen drugs which are already on the market for secondary inhibitory activities towards the MAO isoforms. The advantages of this approach are summarized as follows.

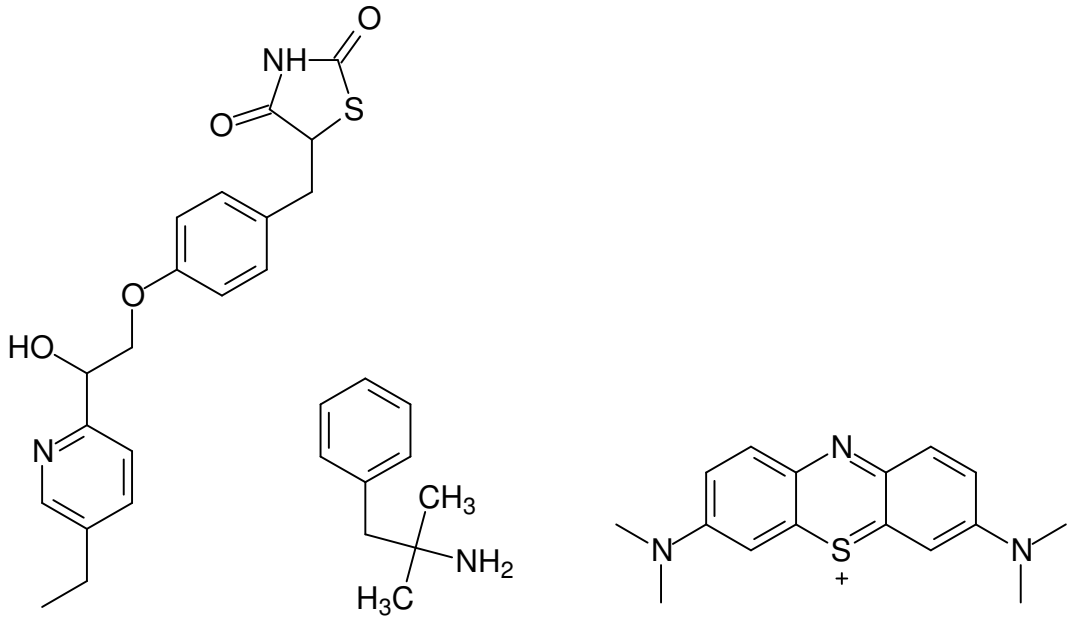
- Since such drugs are already approved for administration in human subjects it is a relatively simple process to re-register them for therapy in depression and Parkinson's disease. For these drugs only clinical efficacy has to be proven and preclinical and clinical development is not required.

- If the original pharmacological effect of the drug is useful in depression and Parkinson's disease, it provides the possibility for the discovery of drugs with a multi-target directed mode of action. In principle such drugs would have several advantages over multiple-medication therapy. For example, a single drug which modulates more than one target reduces the possibility of drug-drug interactions and simplifies the side effect profile and pharmacokinetic considerations of the therapy.

This approach is termed the re-appropriation or repurposing of existing drugs and is a relatively new and exciting development in drug design. Two examples where existing drugs were found to also display MAO inhibitory effects are with pioglitazone (Binda *et*

al., 2011) and phentermine (Nandigama *et al*., 2002). Pioglitazone is a potent MAO-B inhibitor with a K_i value of 0.5 µM, although it was originally designed for activity at nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-γ). The anorectic drug, phentermine, was found to inhibit MAO-A and MAO-B with K_i values of 498 µM and 375 µM, respectively.

From the toxicological viewpoint it may be of interest to screen virtual drug libraries for drugs that may act as inhibitors of MAO-A and MAO-B. As mentioned above, MAO-A inhibitors may lead to serious adverse effects when combined with certain drugs and food. It is therefore useful to determine if any existing drugs act as MAO-A inhibitors and thus possess the potential for serious adverse effects. An example of this behaviour is with methylene blue, a drug used for methemoglobinemia. A second pharmacological effect of methylene blue is to potently and reversible inhibit MAO-A with an IC_{50} value of 70 nM (Harvey *et al*., 2010). The high potency inhibition of MAO-A by methylene blue was only discovered after serotonin toxicity was observed in patients receiving an SSRIs and methylene blue (Ramsay *et al*., 2007).



Pioglitazone Phentermine Methylene blue

Figure 1.7. The structures of pioglitazone, phentermine and methylene blue.

1.4. Hypothesis of the study:

It is postulated that active MAO-A and MAO-B inhibitors may be identified among a virtual library of approved drugs using a molecular modeling approach. For this purpose, the Discovery studio 3.1 modeling software will be used to predict whether structures included in the virtual library of drugs may act as inhibitors of MAO-A and MAO-B.

1.5. Aim and Objectives

The aim of this study is to screen a virtual drug library of approved drugs for potential inhibitory activities towards MAO-A and MAO-B using molecular modeling. The structures will subsequently be evaluated *in vitro* as inhibitors of human MAO-A and MAO-B.

The objectives of the study may be summarized as follows:

- Structure-based pharmacophore models will be constructed for both MAO-A and MAO-B by using the known crystallographic structures of human MAO-A and MAO-B. Discovery studio 3.1 will be employed for these studies.
- The abilities of the models to distinguish between known MAO-A and MAO-B inhibitors and compounds known not to bind to these enzymes will be determined.
- These models will be used to screen a virtual drug library of Food and Drug Administration (FDA) approved drugs for drugs that may act as potential inhibitors.
- The drugs that are found to be potential MAO inhibitors will be evaluated *in vitro* as inhibitors of recombinant human MAO-A and MAO-B.
- Studies will be carried out to determine the reversibility of inhibition of MAO-A and MAO-B by active inhibitors. For this purpose, the recovery of enzyme activity after dilution and dialysis of the enzyme-inhibitor complexes will be evaluated to further evaluate reversibility.

- Lineweaver-Burk plots for the inhibition of MAO-A and MAO-B by active inhibitors will be constructed. This will be done in order to determine the mode of inhibition of the inhibitors.
- The structures of active MAO inhibitors will be docked into the active sites of MAO-A and MAO-B. This will be done in order to determine possible binding orientations and to confirm that the inhibitor fits within the active site cavities.
- Based on the results, letters will be written to report the findings of this study.