

CHAPTER 6

Summary:

In Chapter 1, it was stated that the goal of this study is the identification of drugs that can be repurposed as pharmacological agents in the treatment of PD. PD has a high prevalence under the aging population, and due to an increased life expectancy, PD is likely to become a larger problem in the future. The current treatment strategies are only symptomatic and there is a great need for the development of new drugs.

One of the drug targets in PD is MAO. The MAO enzymes are responsible for the metabolism of amine neurotransmitters, including dopamine, the primary neurotransmitter involved in the pathology of PD. In particular, selective MAO-B inhibitors are in clinical use for the treatment of PD as adjuvants to levodopa therapy. Non-selective and selective MAO-A inhibitors are in clinical use for the treatment of depression and other neurological disorders (Aminoff, 2009).

Identifying MAO inhibitors is also important from a toxicological perspective. Inhibition of MAO-A, especially irreversible inhibition, has been linked with potentially fatal drug and food interactions such as the serotonin syndrome and the so-called 'cheese reaction' (Yamada & Yasuhara, 2004).

Developing new drugs is a long and costly process. Repurposing existing drugs that are already approved for other indications greatly increases the speed of the drug discovery process. Drug repurposing is based on the theory of polypharmacology, which states that drugs may act as master keys that unlock various targets, rather than the traditional view of a drug as a key that only opens a single lock (Medina-Franco *et al.*, 2013).

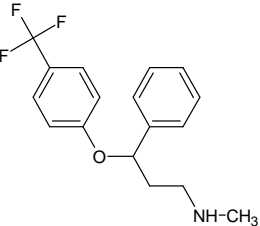
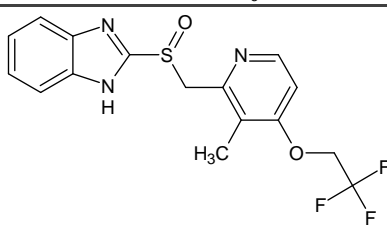
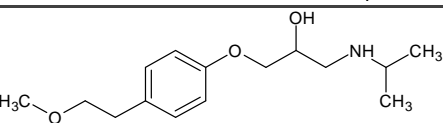
The virtual screening of libraries of drugs is a convenient method for sifting through large amounts of drugs in order to identify promising new leads so that development can be focussed on them. Traditionally, new drugs have been discovered by luck, rather than by rational design. Researchers typically screen thousands of molecules *in vitro* before finding compounds with significant activity. A pharmacophore is an abstract concept that describes the common steric and electrostatic complementarities of bio-active molecules with the target of interest and a pharmacophore model is a 3D representation of a selection of pharmacophore groups. The pharmacophore model may be built from the crystal structure of the protein co-crystallized with a ligand and certain hydrogen bond donor, hydrogen bond acceptor and hydrophobic interactions between the ligand and the protein receptor are

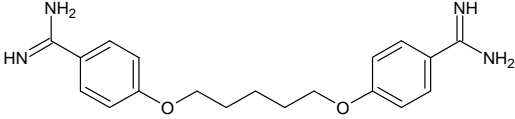
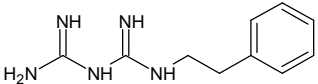
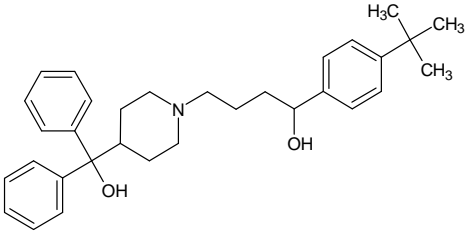
selected. Screening libraries for compounds that have interactions with the receptor at the predetermined points of importance greatly reduces the amount of potential compounds to a manageable amount so that they can be tested further with *in vitro* screening (Seidel *et al.*, 2010).

In this study pharmacophore models for MAO-A and MAO-B were built with Discovery Studio 3.1[®] using a *structure-based* approach. For these models the X-ray crystallographic structures of MAO-A co-crystallized with harmine and MAO-B co-crystallized with safinamide were obtained from the Protein Data Bank. After construction, the models were validated and subsequently used to screen virtual libraries of the FDA's approved drugs and the EPA's maximum daily dose databases for potential MAO-A and MAO-B inhibitors. Among the compounds which mapped to the pharmacophore models of MAO-A and MAO-B, 26 compounds with good Fit-Values, which were readily available and affordable, were selected for *in vitro* evaluation.

The *in vitro* screening protocol was based on the principle that the MAO enzymes oxidize kynuramine, a non-fluorescent substrate, to 4-hydroxyquinoline, a fluorescent product. The amount of fluorescence can be measured to quantify the amount of 4-hydroxyquinoline that was formed, and thus the level of activity of the MAO-enzymes (Legoabe *et al.*, 2011). The IC₅₀ values for the inhibition of MAO-A and MAO-B of the 26 selected hits were determined *in vitro*. 6 of the compounds were found to possess *in vitro* MAO inhibitory activities, and among these pentamidine and phenformin were selected for further evaluation.

Table 6.1.1 The structures of compounds that inhibited MAO-A and/or MAO-B *in vitro*.

Name	Structure
Fluoxetine	
Lansoprazole	
Metoprolol	

Pentamidine	
Phenformin	
Terfenadine	

This study found that phenformin is a moderately potent MAO-A selective inhibitor with an IC_{50} value of 40.5 μM . It was also shown that phenformin interacts reversibly with MAO-A. To determine whether the MAO inhibition by phenformin is physiologically significant at normal therapeutic doses, the plasma levels were considered. Following a single oral dose of 50 mg phenformin hydrochloride to 8 human subjects, plasma levels of 0.33–0.74 μM were obtained (Oates *et al.*, 1983). Considering that the IC_{50} value for the inhibition of MAO-A is at least 54-fold higher than the plasma levels obtained by phenformin, a pharmacological relevant interaction between MAO-A and phenformin is unlikely. It also does not seem likely that phenformin would accumulate in tissues where MAO-A are found to levels above its IC_{50} value.

In contrast to phenformin, pentamidine was found to be a potent MAO-A and MAO-B inhibitor with K_i values of 0.41 μM and 0.224 μM , respectively. Similar to phenformin, pentamidine is a reversible and competitive inhibitor of both human MAO isoforms. This finding is in contrast to literature, which reports that pentamidine acts as an irreversible inhibitor of rabbit and rat liver MAO (Blaschko & Duthie, 1945; Davison, 1958). It is uncertain why the findings of this laboratory differ from those in literature. To determine whether the MAO inhibition of pentamidine is clinically significant, the plasma levels were considered. Following intramuscular injection of pentamidine isethionate at a dose of 4 mg/kg in humans, plasma levels of 0.51–2.36 μM were obtained (Wisplewey & Pearson, 1991). The maximal plasma concentration (2.36 μM) is well above the K_i values for the inhibition of MAO-A and MAO-B, and assuming similar concentrations are attained in tissues, inhibition of the MAOs in such tissues are possible. This is of significance since pentamidine may inhibit the metabolic breakdown of tyramine and other sympathomimetic dietary amines by MAO-A present in the gut wall and vascular endothelial cells (Da Prada *et al.*, 1988). Pentamidine may therefore

theoretically lead to a tyramine-associated hypertensive crisis when combined with tyramine-containing foods. It should however be noted that, in contrast to irreversible MAO-A inhibitors, reversible inhibitors are in general not associated with a hypertensive crisis (Harfenist *et al.*, 1996). The MAO-A inhibitory properties of pentamidine may also be of significance since MAO-A inhibitors may lead to serotonin toxicity when combined with serotonin-releasing agents or selective serotonin reuptake inhibitors (Ramsay *et al.*, 2007). Pentamidine is not expected to inhibit MAO-A in the brain because it does not appear to penetrate the central nervous system to a large extent and therefore it is not expected precipitate serotonin toxicity when used in conjunction with 5-hydroxytryptaminergic agents (Wisplewey & Pearson, 1991). The inhibition of MAO-B by pentamidine is unlikely to contribute to its toxicological profile due to the excellent safety profile of therapeutically used MAO-B inhibitors (Pae *et al.*, 2012).

Although both MAO-A and MAO-B inhibitors are clinically useful, the side effect profile of pentamidine and phenformin make it unlikely that they will be repurposed as therapeutic MAO inhibitors. However this research expanded the understanding of their pharmacological profiles and highlighted potential unknown adverse effects that patients taking these drugs should be aware of. This research also demonstrated the potential of the amidine and biguanide functional groups as potential lead moieties in the future design of MAO inhibitors, and potentially in the development of multifunctional inhibitors that inhibit both MAO and NOS (Xian *et al.*, 2001).

Other compounds that had reasonably good MAO inhibitory activities *in vitro*, but were not further explored in this study include metoprolol, fluoxetine, lansoprazole and terfenadine. Further evaluation could not be done on lansoprazole and terfenadine due to their low aqueous solubility. Fluoxetine was also not investigated further because Mukherjee & Yang (1999) have described its inhibitory activity in rats extensively. Metoprolol was not evaluated further because it may be viewed as a weak MAO inhibitor.

In conclusion, this study has achieved the objectives set out in chapter 1 by screening libraries of the FDA's approved drugs and EPA's maximum daily dose databases and identifying compounds that also inhibit the MAO enzymes. Two of these compounds, pentamidine and phenformin, were further evaluated to determine whether they interact with the MAO enzymes in a reversible, competitive manner. Even though pentamidine and phenformin will probably not be repurposed as agents in the treatment of Parkinson's disease, the discovery of their MAO inhibitory activity may be of value to scientists by contributing to a better understanding of the pharmacology and toxicology of these compounds.