Chapter 1
Introduction

1.1 Background

Parkinson's disease (PD) is a neurodegenerative disease characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) of the brain. Degeneration of dopaminergic neurons leads to decreased levels of dopamine (DA) in the striatum, which in turn results in the debilitating movement disorders observed in PD patients (Dauer & Przedborski, 2003; Przedborski, 2005).

Treatment of PD is mainly focussed on symptomatic management by replacing DA or stimulating DA release in the brain (Schapira & Olanow, 2008). Currently, the popular treatment of PD involves the administration of the DA precursor, levodopa (L-dopa) (Lees, 2005). L-dopa provides symptomatic relief for PD patients by increasing striatal DA. Unfortunately, long-term use of L-dopa is associated with motor fluctuations and dyskinesia mostly due to the 'wearing off' effect (Lees, 2005; Rezak, 2007). Monoamine oxidase (MAO) inhibitors are also used in the symptomatic treatment of PD since these drugs inhibit the metabolism of DA in the brain (Yamada & Yasuhara, 2004). There are two isoforms of MAO: MAO-A and MAO-B. Although DA is primarily metabolized by MAO-B in the brain, reversible inhibition of both isoforms may increase DA concentrations in the brain more effectively (Youdim & Bakhle, 2006). In addition to symptomatic relief, MAO inhibitors may also be neuroprotective by preventing the formation of neurotoxic metabolic by-products (Youdim *et al.*, 2006). There are two MAO-B inhibitors currently in use for the treatment of PD, namely selegiline and rasagiline. The use of selegiline is associated with cardiovascular and psychiatric side effects due to the amphetamine metabolites of selegiline (Yamada & Yasuhara, 2004; Tipton *et al.*, 2004). Rasagiline has a better safety profile, but is still an irreversible inhibitor of MAO-B. Irreversible inhibition of MAO is associated with a slow enzyme recovery rate after drug withdrawal since *de novo* synthesis of the enzyme is required. In contrast, the recovery of enzyme activity with reversible inhibitors is almost immediate after drug withdrawal (Riederer *et al.*,

2004; Tipton *et al.*, 2004). Development of reversible MAO inhibitors may thus provide a treatment strategy for PD which combines symptomatic treatment with neuroprotective properties.

1.2 Rationale

In a previous study, (*E*)-8-(3-chlorostyryl)caffeine (CSC) (Figure 1) was reported to be a potent, selective inhibitor of MAO-B with a K_i value of 70 nM (Chen *et al.*, 2002; Pretorius *et al.*, 2008). CSC consists of a caffeine moiety with an (*E*)-styryl substituent on C8 of caffeine. Unsubstituted caffeine (Figure 1) is a weak inhibitor of MAO. As demonstrated with CSC, substitution on C8 of caffeine may increase its inhibitory activity towards MAO (Vlok *et al.*, 2006; Petzer *et al.*, 2003).

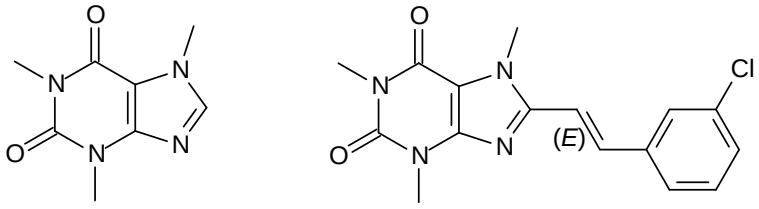
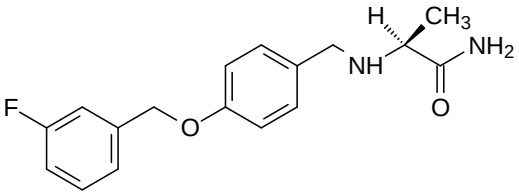


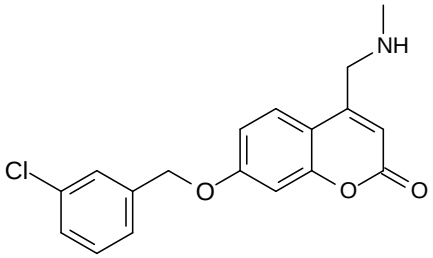
Figure 1 The chemical structures of caffeine and (*E*)-8-(3-chlorostyryl) caffeine (CSC)

Safinamide and 7-(3-chlorobenzyloxy)-4-(methylamino)methyl-coumarin (Figure 2) are very potent, reversible inhibitors of MAO-B with K_i values of 0.5 µM and 0.1 µM, respectively. Both of these compounds have a benzyloxy moiety. It was found that safinamide and the coumarin derivative bind similarly within the active site cavity of MAO-B. The active site of MAO-B consists of two cavities, an entrance cavity and a substrate cavity. The two inhibitors mentioned, bind in an extended conformation, traversing both cavities with the benzyloxy moiety in the entrance cavity (Binda *et al.*, 2007). The benzyloxy substituent is also present in other inhibitors of MAO. For example, 8-benzyloxycaffeine (Figure 2) was reported to be a potent inhibitor of MAO with a K_i value of 0.59 µM towards MAO-B and 0.43 µM towards MAO-A. 8-Benzyloxycaffeine consists of a caffeine moiety substituted on C8 with a benzyloxy side chain (Strydom *et al.*, 2010). The fact that 8-benzyloxycaffeine potently inhibits both MAO-A and –B may be attributed to the flexibility of the benzyloxy side chain.

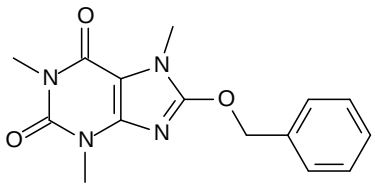
Inhibitors with a larger degree of conformational freedom are thought to have the ability to interact with both the active sites of MAO-A and –B (Strydom *et al.*, 2010; Van der Walt *et al.*, 2009). Freedom of rotation around the ether oxygen in the benzyloxy side chain may be responsible for the flexibility observed for this moiety (Strydom *et al.*, 2010).



Safinamide



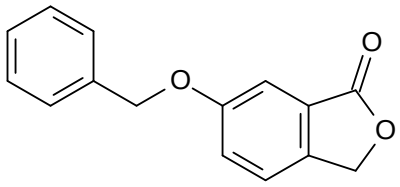
7-(3-Chlorobenzyloxy)-4-(methylamino)methyl-coumarin



8-Benzyloxycaffeine

Figure 2 The chemical structures of safinamide, 7-(3-chlorobenzyloxy)-4-(methylamino)methyl-coumarin and 8-benzyloxycaffeine. All of these MAO inhibitors contain the benzyloxy moiety.

In another study, the benzyloxy side chain was conjugated with different bicyclic structures containing a benzene ring fused with a heterocycle. These novel compounds were tested as MAO inhibitors. The most potent inhibitor among these was 6-benzyloxyphthalide (Figure 3), an inhibitor of rat MAO-A and –B with K_i values of 1.8 µM and 0.12 µM, respectively (Gnerre *et al.*, 2000).



6-Benzyloxyphthalide

Figure 3 The chemical structure of 6-benzyloxyphthalide.

When conjugated to an appropriate scaffold the benzyloxy moiety can therefore be considered as an efficient substituent to enhance both MAO-A and –B activity of a potential inhibitor. Caffeine and phthalide, in turn, can be employed as scaffolds for the design of MAO inhibitors with substitution on the C8 and C6 positions, respectively. This study is an exploratory study to discover novel highly potent caffeine and phthalide derived inhibitors.

1.3 Hypothesis

Based on literature it is postulated that highly potent MAO inhibitors may be designed using caffeine and phthalide as scaffolds. For this purpose, substitution with alkyloxy moieties on the C8 and C6 positions of caffeine and phthalide, respectively, will yield structures that are particularly potent inhibitors. In addition, it is postulated that these caffeine and phthalide derived inhibitors will act reversibly with the MAO enzymes.

1.4 Study Aim

The aim of this study is to synthesize and evaluate novel, reversible inhibitors of MAO, using caffeine and phthalide as scaffolds. These scaffolds will be substituted with different alkyloxy side chains and the effects of these substituents on the MAO inhibitory potencies of the resulting compounds will be explored. This study also aims to produce three high quality journal articles from the three projects discussed below.

Using caffeine as a scaffold, a series of 8-aryl- and alkyloxycaffeine analogues (Figure 4) will be synthesized and evaluated as inhibitors of MAO-A and –B. In this study the effect of a variety of side chains on inhibition potency will be investigated. In certain instances the effect of halogen substitution on the phenyl rings of selected inhibitors will also be examined. The mode of inhibition and reversibility of the interactions between the MAO enzymes and inhibitors will be determined. Molecular modelling studies will be carried out to gain insight into the possible modes of binding of these compounds to the active site cavities of MAO-A and –B.

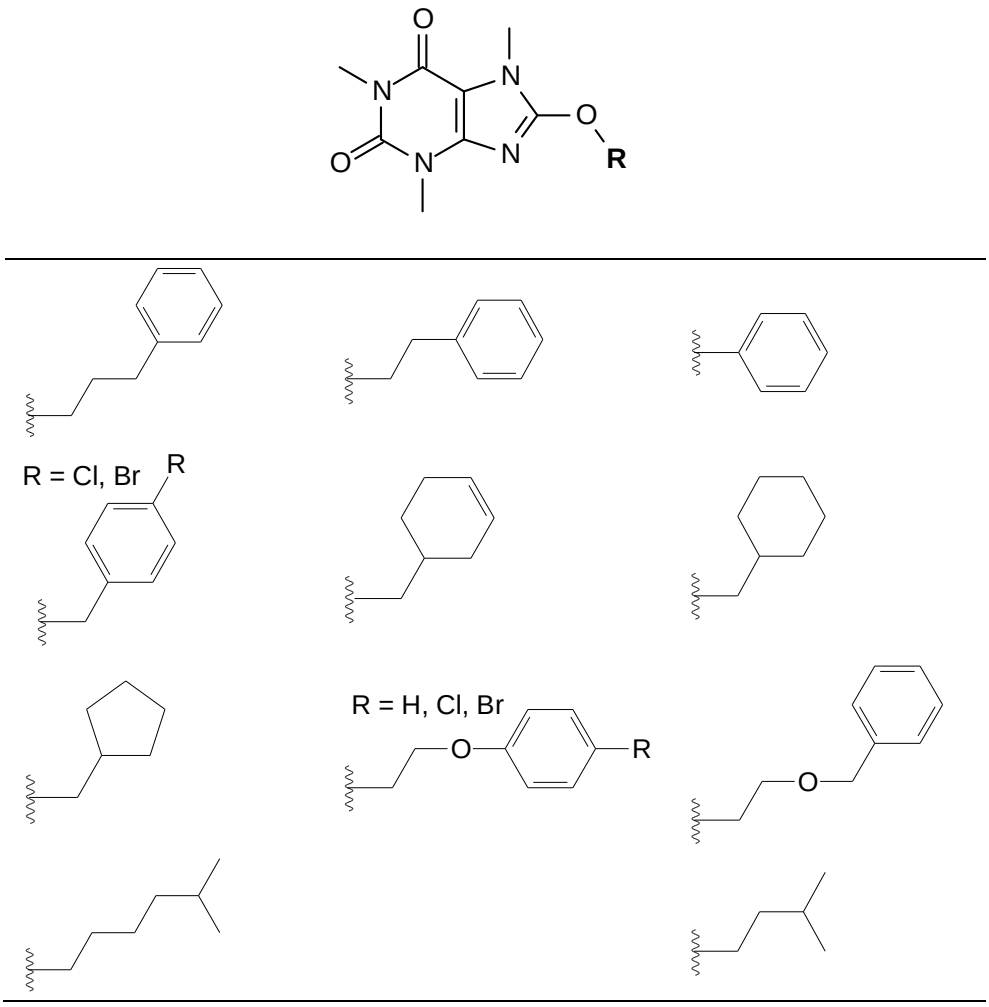
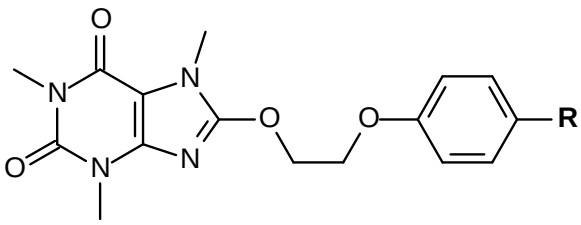


Figure 4 The structures of the 8-alkyl- and aryloxycaffeine analogues that will be synthesized in this study. This will constitute part of the first project.

The second part of this study is an extension of the first part. A series of 8-(2-phenoxyethoxy)caffeine analogues (Figure 5) with different substituents on C4 of the phenyl ring will be synthesized and evaluated as inhibitors of MAO. Structure-activity relationship (SAR) studies will be carried out to investigate the properties of the C4 substituents that result in potent MAO inhibition. Molecular modelling studies will also be carried out for these compounds to clarify the mode of binding within MAO-A and –B.



R = H, Cl, Br, F, CF_3, CH_3, OCH_3, I, CN, NO_2

Figure 5 Structures of the 8-(2-phenoxyethoxy)caffeine analogues that will be synthesized in this study. This will constitute part of the second project.

For the third part of this study, 6-benzyloxyphthalide will be used as lead compound. 6-Benzyloxyphthalide consists of a phthalde moiety with a benzyloxy side chain on the C6 position of phthalide. A series of 6-benzyloxyphthalide analogues (Figure 6) with different substituents on C4 of the benzyloxy ring will be synthesized. These compounds will be evaluated as reversible, competitive inhibitors of MAO-A and –B. As shown, to explore the SARs of this series, a variety of C6 side chains will be considered (Figure 6).

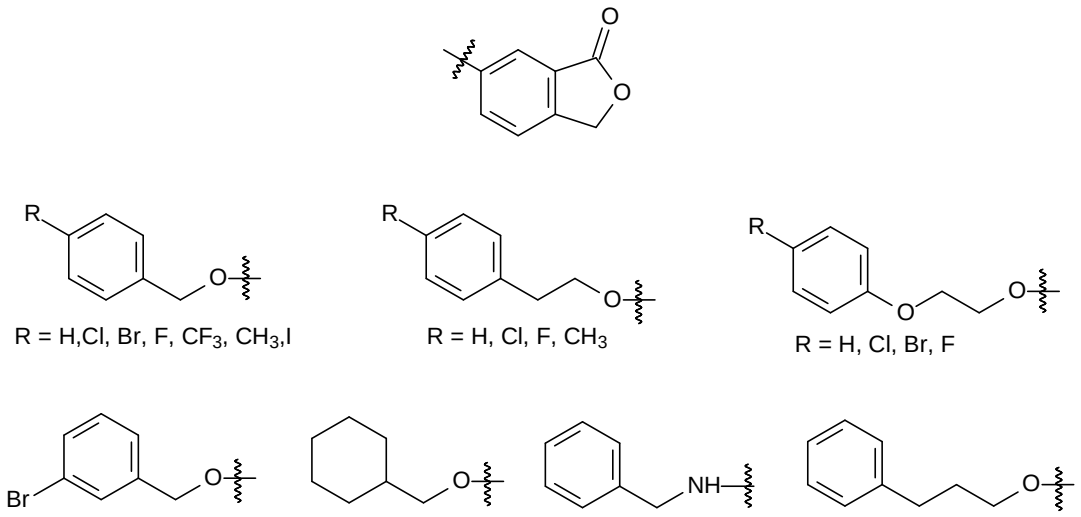


Figure 6 The structures of the phthalide analogues that will be synthesized in this study. This will constitute part of the third project.

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