Chapter 1

Introduction

1.1 Overview and background

Parkinson's disease (PD) is a neurodegenerative disorder which is progressive in nature and is characterized by motor disturbances such as bradykinesia and muscular rigidity. It is normally associated with the elderly and leads to a lowered life quality. These disturbances are due to the degeneration of the dopaminergic neurons originating in the substantia nigra pars compacta (SNpc), with the subsequent loss of their terminals in the striatum that differs from the normal loss due to ageing (Petzer *et al.*, 2003; Sauer & Oertel, 1994; Lang & Lozano, 1998). This degenerative process leads to the depletion of the dopamine (DA) normally found in SNpc neurons. Although uncertainties still exist about how the disorder precipitates, mutations in a number of genes have been proposed to be the cause of PD. They include α-synuclein and leucine rich repeat kinase 2 (LRRK-2) (Tugwell, 2008). Although the environmental agents, paraquat and rotenone, have been shown to induce dopaminergic cell degeneration in animal models, it is still unclear whether they can lead to the development of PD (Betarbet *et al.,* 2000*;* Brooks *et al.,* 1999; Chun *et al*., 2001).

The monoamine oxidases (MAO) A and B are mitochondrial outer membrane bound, flavin adenine dinucleotide (FAD) containing enzymes that are responsible for the oxidation of a variety of different substrates (Youdim & Bakhle, 2006; Binda *et al*., 2002b). Both isoforms metabolize DA, which plays an integral role in PD (Youdim *et al*., 2005). The two isoforms share a 70% sequence identity (Kearney *et al*., 1971) suggesting that they share a common ancestral gene (Grimsby *et al*., 1991). As mentioned, the MAO enzymes are responsible for metabolizing different substrates and inhibitors of the two MAO isoforms may be used to treat different types of disorders. MAO-A catalyzes the oxidation of serotonin (5-HT), which plays a role in neuropsychiatric disorders such as depression. Thus, by inhibiting 5-HT metabolism, MAO-A inhibitors may elevate central 5-HT levels and are therefore used in the treatment of depressive disorders (Johnston, 1968; Youdim & Bakhle, 2006). MAO-B is the principal enzyme that catalyzes the oxidation of DA in the brain. This process produces toxic by-products namely hydrogen peroxide, ammonia and an aldehyde, which may lead to DNA damage and neuronal death (Youdim *et al*., 2006; Riederer *et al*., 2004a; Standaert & Young, 2006). A number of drugs are used to treat the symptoms associated with PD such as levodopa (L-Dopa) and DA

agonists, but slowing the disease progression remains the ultimate goal for effective therapy (Lees, 2005).

MAO-B inhibitors are thought to possess the ability to act as neuroprotective agents. One of the proposed mechanisms of neuroprotection involves the inhibition of the MAO-B catalyzed oxidation of DA and thus prevention of the formation of hydrogen peroxide and aldehyde species, which are generated in the catalytic cycle of MAO-B. This will in turn reduce oxidative stress and apoptotic processes (Binda *et al.,* 2002a). By blocking the MAO-B catalysed metabolism of DA, MAO-B inhibitors may also conserve DA in the brain and provide symptomatic relief of PD. MAO-B inhibitors also elevate DA levels derived from the DA precursor, L-Dopa, and are therefore frequently used in combination with L-Dopa in PD therapy. The MAO-B inhibitors that are currently being used in the treatment of PD are selegiline and rasagiline. Selegiline, once metabolized, forms amphetamine derivatives which are potentially neurotoxic (Montastruc *et al*., 2000; Churchyard *et al*., 1999; Gill *et al*., 1967; Churchyard *et al*., 1997). Rasagiline, on the other hand, is not metabolized to harmful amphetamine derivatives (Chen & Swope, 2005). Both these drugs are selective, irreversible inhibitors of MAO-B. Following treatment with these drugs, enzyme activity is recovered only after several weeks following withdrawal of the irreversible MAO-B inhibitor (Riederer *et al*., 2004b). Reversible MAO-B inhibitors may be considered to be safer than irreversible inhibitors since enzyme activity is regained as soon as drug treatment is terminated and the drug is cleared from the tissues.

The goal of this research project is to design new reversible inhibitors of MAO-B, which may potentially be used for the symptomatic treatment of PD as well as possibly possessing neuroprotective properties.

1.2 Rationale of this study

The lead compound for the design of the new MAO inhibitors in this study is caffeine (**1**) (figure 1.1).

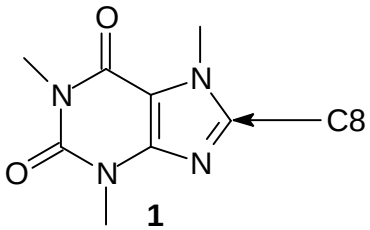


Figure 1.1. The chemical structure of caffeine showing the position of the C-8 carbon.

Caffeine is a weak inhibitor of MAO-B with an IC_{50} value of 5084 µM. Substitution, however, of the caffeine moiety at C-8 with a variety of groups, yields compounds with greatly enhanced MAO-B inhibition. Substitution on C-8 of caffeine with a 3-chlorostyryl substituent forms a derivative of caffeine namely (E)-8-(3-chlorostyryl)caffeine (CSC) (figure 1.2), which is a potent, reversible inhibitor of MAO-B with an IC_{50} value of 0.15 µM, approximately 32000 times more potent than caffeine (Vlok *et al*., 2006).

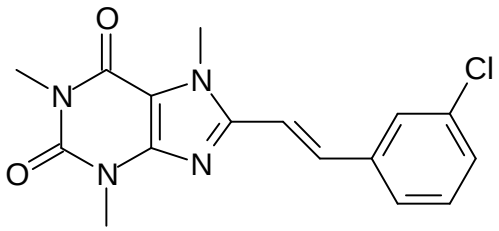
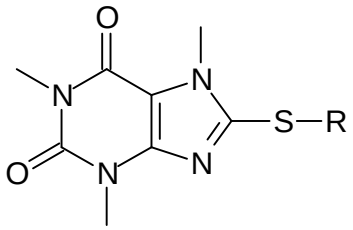
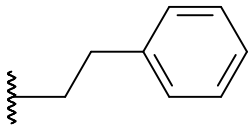
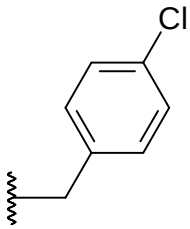
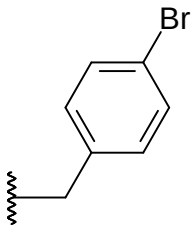
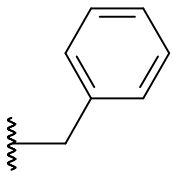
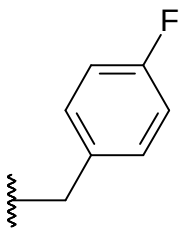


Figure 1.2. The chemical structure of CSC.

Recent studies have shown a series of 8-sulfanylcaffeine derivatives to be exceptionally potent MAO-B inhibitors and moderate MAO-A inhibitors. Results by Booysen *et al.* (2011) show that a series of 8-sulfanylcaffeine derivatives acts as highly potent inhibitors of MAO-B as well as moderately potent inhibitors of MAO-A. Examples of these compounds with their IC_{50} values for the inhibition of MAO-A and –B are shown in table 1.1.

Table 1.1. Illustration of selected 8-sulfanylcaffeine derivatives that were synthesized in a previous study.



Compound	R-Group	MAO-A (μM)	MAO-B (μM)
2a		20.5	0.223
2b		2.76	0.192
2c		2.61	0.167
2d		8.22	1.86
2e		4.79	0.348

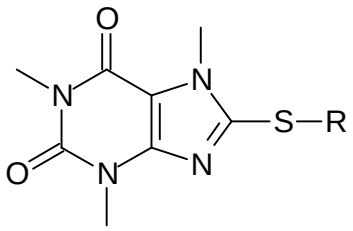
As can be seen from table 1.1, 8-sulfanylcaffeine analogues may be considered potent inhibitors of MAO-B, and moderately potent inhibitors of MAO-A. Noteworthy is the observation that, 8-[(phenylethyl)sulfanyl]caffeine (**2a**) is a highly potent MAO-B inhibitor with an IC_{50} value of 0.223 µM. This compound therefore represents a possible lead for the design of MAO-B inhibitors with exceptionally high binding affinities. Based on this analysis we aim to synthesize a series of five 8-[(phenylethyl)sulfanyl]caffeine analogues and evaluate the analogues as inhibitors of human MAO-A and –B. For the purpose of this study 8-[(phenylethyl)sulfanyl]caffeine homologues containing C-3 alkyl (CF_3, CH_3 and OCH_3) and halogen (Cl and Br) substituents on the phenyl ring were considered. These substituents were selected based on a report that similar substitution of a series of 8-benzyloxycaffeine analogues is beneficial for MAO-B inhibition (Strydom *et al*., 2010; Strydom *et al*., 2011). In addition, the human MAO-B inhibition potency of 8-(benzylsulfanyl)caffeine (**2d**; IC_{50} = 1.86 µM) may be improved with halogen (Cl, Br and F) substitution on the *para* position of the benzyl ring, yielding compounds **2b**, **2c** and **2e** with IC_{50} values ranging from 0.167–0.348 µM (Booysen *et al*., 2011). Furthermore, a series of two 8-sulfinylcaffeine analogues and one 8-sulfonylcaffeine analogue will also be synthesized and their MAO inhibitory potencies will be measured. The purpose with these compounds is to compare the MAO inhibitory properties of the 8-sulfinylcaffeine analogues and 8-sulfonylcaffeine with those of the 8-sulfanylcaffeine analogues. This study also investigates the MAO inhibition properties of three selected 8-[(phenylpropyl)sulfanyl]caffeine and two 8-(benzylsulfanyl)caffeine analogues. The 8-(benzylsulfanyl)caffeines that will be investigated in this study differ from those that were previously synthesized (table 1.1) in that they are substituted on the *meta* position of the phenyl ring, while **2b**, **2c** and **2e** are *para* substituted on the phenyl ring.

The structures of the compounds that will be synthesized in this study are illustrated in tables 1.2 and 1.3. These are the:

1. 8-[(phenylethyl)sulfanyl]caffeines (**3a**–**e**),
2. 8-[(phenylpropyl)sulfanyl]caffeines (**4a**–**c**),
3. 8-(benzylsulfanyl)caffeines (**5a**–**b**),
4. 8-sulfinylcaffeines (**6a**–**b**)
5. and 8-sulfonylcaffeine (**7**)

These compounds will subsequently be evaluated as inhibitors of human MAO-A and –B.

Table 1.2. Illustration of the 8-sulfanylcaffeine derivatives that will be synthesized in this study.



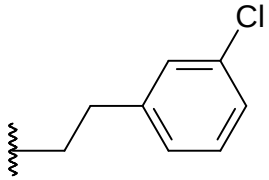
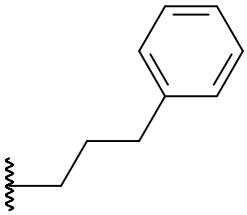
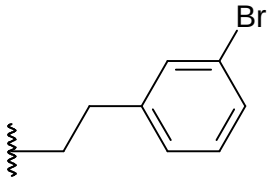
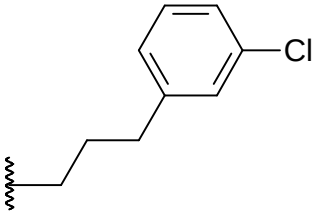
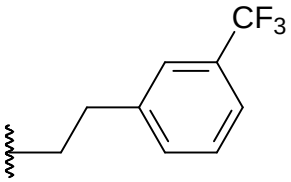
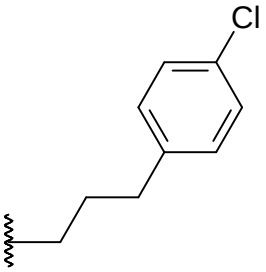
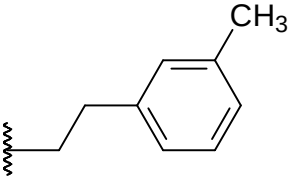
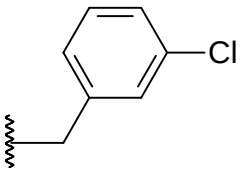
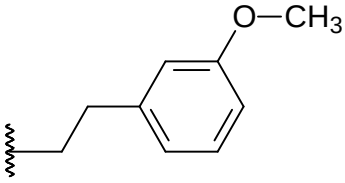
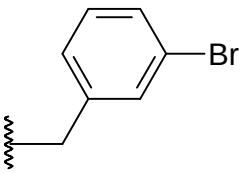
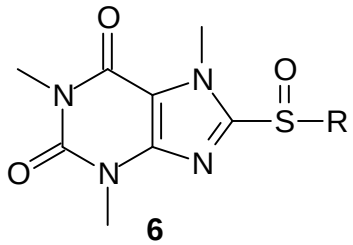
	R-Group		R-Group
3a		**4a**	
3b		**4b**	
3c		**4c**	
3d		**5a**	
3e		**5b**	

Table 1.3. Illustration of the 8-sulfinyl- and 8-sulfonylcaffeine derivatives that will be synthesized in this study.



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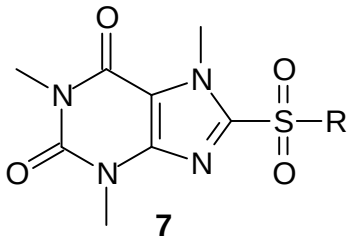
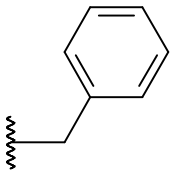
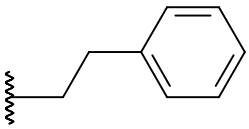
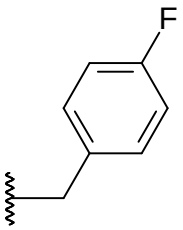


7

	R-Group		R-Group
6a		**7**	
6b			

1.3. Hypothesis of this study

Based on the finding that 8-[(phenylethyl)sulfanyl]caffeine (**2a**) is a potent MAO-B inhibitor, it is postulated that this compound is a promising lead for the design of potent MAO-B inhibitors, and that the addition of simple alkyl (CF_3, CH_3 and OCH_3) and halogen (Cl and Br) substituents on the phenyl ring of **2a** will significantly enhance its inhibition potency. It is further postulated that, based on the potent MAO-B inhibitory properties of both 8-[(phenylethyl)sulfanyl]caffeine (**2a**) and 8-(benzylsulfanyl)caffeine (**2d**), the 8-[(phenylpropyl)sulfanyl]caffeine homologue, and derivatives thereof may also act as potent MAO-B inhibitors.

As part of an exploratory study, the effects of MAO inhibition by substitution on the *meta* position of the phenyl ring of 8-(benzylsulfanyl)caffeine with chlorine (**5a**) and bromine (**5b**) will be compared to substitution on the *para* position on the phenyl ring with chlorine (**2b**) and bromine (**2c**). Furthermore, the MAO inhibitory properties of two 8-sulfinylcaffeine analogues and one 8-sulfonylcaffeine analogue will also be examined.

1.4 Objectives of this study

Based on the discussion above the objectives of this study are summarized below:

- A series consisting of thirteen 8-sulfanylcaffeine analogues and derivatives thereof will be synthesized. These compounds are:
    - 8-[(phenylethyl)sulfanyl]caffeines (**3a**–**e**),
    - 8-[(phenylpropyl)sulfanyl]caffeines (**4a**–**c**),
    - 8-(benzylsulfanyl)caffeines (**5a**–**b**),
    - 8-sulfinylcaffeines (**6a**–**b**)
    - and 8-sulfonylcaffeine (**7**)
- The target 8-sulfanylcaffeine analogues will be synthesized by reacting 8-chlorocaffeine with an appropriate mercaptan in the presence of NaOH. The 8-sulfinylcaffeine analogues and 8-sulfonylcaffeine will be synthesized by reacting the 8-sulfanylcaffeines with H_2O_2 in the presence of glacial acetic acid and acetic anhydride.
- The 8-sulfanylcaffeine analogues and derivatives thereof will be evaluated as inhibitors of MAO-A and MAO-B. For this purpose the recombinant human enzymes, which are commercially available, will be employed. The inhibition potencies will be expressed as the IC_{50} values (concentration of the inhibitor that produces 50% inhibition). A fluorometric assay will be used to measure the enzyme activities using kynuramine as substrate. The MAO activity measurements will be based on measuring the amount of 4-hydroxyquinoline (4-HQ) that is produced in the oxidation process. The quantity of 4-HQ in the reactions may be conveniently determined by measuring the fluorescence of this metabolite at an excitation wavelength of 310 nm and an emission wavelength of 400 nm.
- The reversibility of the inhibition of MAO-B for a selected inhibitor will be evaluated. This will be done in order to determine if the inhibitor interacts reversibly or irreversibly with MAO-B. As stated above, reversible inhibitors are more desirable than irreversible enzyme inhibitors. The reversibility of inhibition will be determined by evaluating the recovery of the enzymatic activity after dilution of the enzyme-inhibitor complex.
- A limited Hansch-type QSAR study will be performed for the inhibition of MAO by the 8-[(phenylethyl)sulfanyl]caffeines. The purpose of this study is to determine the effect

that different substituents on the phenyl ring will have on MAO-B activity of 8-[(phenylethyl)sulfanyl]caffeine.