Chapter 1
Research rationale and aims

1.1 Introduction

Parkinson’s disease (PD) is a progressive neurodegenerative disorder (Prediger et al., 2012), characterized pathologically by a marked loss of dopaminergic nigrostriatal neurons (Nagatsu & Sawada, 2006) and clinically by disabling movement disorders (Henderson et al., 2003; Yazdani et al., 2006). In this thesis the focus is directed towards two strategies to improve the treatment of PD. It is known that PD may be treated by inhibiting monoamine oxidase (MAO), specifically MAO type B (MAO-B), since this is a major enzyme involved in the catabolism of dopamine in the substantia nigra of the brain (Nagatsu & Sawada, 2006; Youdim et al., 2006). Accordingly, the first aim of this study was to develop inhibitors of MAO-B that may enhance the levels of dopamine in the brain and may therefore provide symptomatic relief for PD patients. While dopamine has long been the neurotransmitter most closely associated with PD, several other neurotransmitters, which are active in the basal ganglia, are also affected (Trevitt et al., 2009). According to literature, adenosine A_2A receptors have an important role in the modulation of dopamine-mediated responses and thus the control of motor behaviour (Pinna et al., 2005). In the brain, adenosine A_2A receptors are almost exclusively expressed in the striatum of the basal ganglia (Tanganelli et al., 2004; Pinna et al., 2005). It is speculated that adenosine A_2A receptor antagonists may have value in the treatment of PD for their abilities to reverse motor impairment (symptomatic treatment). In addition, A_2A antagonists may also possess neuroprotective properties (disease modifying). The second aim of this thesis is thus the discovery of new antagonists of the adenosine A_2A receptor.

In this chapter a brief overview of the research rationale and aims are provided. The subsequent chapters may be summarized as follows: Chapter 2 provides a literature background on PD, while Chapters 3 to 5 discuss the unique roles of the neurotransmitter dopamine, monoamine oxidase and the neurotransmitter adenosine in the therapy of PD. The research is presented as three papers, which are given in Chapters 6, 7 and 8. In the first paper, presented in Chapter 6, the objective was to discover novel phthalimide derived inhibitors of MAO. To achieve this, a series of 5-sulfanylphtalimide analogues was synthesized and evaluated as inhibitors of both human MAO-A and MAO-B. A subsequent project, described in Chapter 7, investigated a series of phthalo- and benzonitriles as potential inhibitors of human MAO-A and MAO-B. For this paper, several sulfanylphthalonitriles and sulfanylbenzonitriles were synthesized in an attempt to identify potent and selective MAO-B
inhibitors. These projects are aimed at discovering novel MAO-B inhibitors for the treatment of PD.

The third project focused on adenosine A2A receptor antagonists as a novel therapeutic strategy in PD. A wide variety of 8- styrylxanthines, with the styryl double bond in the (E)-configuration, have been employed in the development of adenosine A2A receptor antagonists (Jacobsen et al., 1993; Suzuki et al., 1996; Massip et al., 2006). Taking this into consideration, series of (E)-8- styrylxanthines, 8-(phenoxy methyl)xanthines and 8-(3- phenylpropyl)xanthines were synthesized and evaluated as antagonists of the adenosine A2A receptor. An important goal of this study was to explore the importance of the styryl-side chain at the C8 position of the xanthine core for A2A antagonism and to contribute to the structure- activity relationships (SARs) of A2A antagonism by the xanthine class of compounds. In conclusion, the research described in this thesis is aimed at identifying new agents for the treatment of PD.

1.2 Rationale for the three research projects

1.2.1 First article: Novel sulfanylphthalimide analogues as highly potent inhibitors of monoamine oxidase B

In the past, a wide range of structures have been employed as inhibitors of the MAO enzyme. Among these isatin (1) and caffeine (2) (Figure 1.1) have been used as scaffolds in the search for MAO-B inhibitors (Van der Walt et al., 2009; Strydom et al., 2010; Manley-King et al., 2011a). Recently, it has been reported that phthalimide (3) (Figure 1.1), an isomer of isatin, may also be a potentially useful scaffold for designing MAO-B selective inhibitors (Manley-King et al., 2011b).

![Isatin (1), Caffeine (2), Phthalimide (3)](image)

**Figure 1.1:** The structures of isatin (1), caffeine (2) and phthalimide (3).

Even though phthalimide is a weak inhibitor of MAO, substitution on the C5 position of phthalimide yields structures endowed with highly potent and selective MAO-B inhibitory activities. Similarly, the MAO-B inhibitory properties of isatin and caffeine may be enhanced by substitution on the C5 and C6 positions of isatin and the C8 position of caffeine. The introduction of a benzyloxy substituent at these positions of the isatin, caffeine and phthalimide moieties can efficiently enhance the MAO affinities of these scaffolds. In fact, benzyloxy substitution of isatin, caffeine and phthalimide result in compounds 4–6 (Figure 1.2), which have IC50 values for MAO-B inhibition several orders of magnitude more potent than those reported.
for the parent compounds (Strydom et al., 2010; Manley-King et al., 2011a; Manley-King et al., 2011b). In addition, halogen substitution on the benzyloxy ring has been reported to further enhance the inhibition potencies of 4–6. Modelling studies have indicated that productive interactions of the benzyloxy side chain with the MAO-B entrance cavity may be responsible for the high potency MAO-B inhibition by these compounds. As part of our interest in identifying novel potent and selective MAO-B inhibitors, the observation that the benzylsulfanyl side chain possesses similar properties to that of the benzyloxy moiety is of significance. For example, a series of 8-(benzylsulfanyl)caffeine analogues (7) exhibits similar MAO-B inhibition properties to a series of 8-benzyloxycaffeine analogues (5) (Booysen et al., 2011).

![Figure 1.2](image1.png)

**Figure 1.2:** The structures of 5-benzyloxyisatin (4), 8-benzyloxycaffeine (5), 5-benzyloxyphthalimide (6) and 8-(benzylsulfanyl)caffeine (7).

The above considerations suggested that benzylsulfanyl substitution on C5 of the phthalimide moiety may yield structures which are highly potent and selective MAO-B inhibitors. With this in mind a series of 5-(benzylsulfanyl)phthalimide analogues will be synthesized and examined as MAO inhibitors in this study. Various substituents (Cl, Br, F and OCH$_3$) on the benzylsulfanyl ring will also be investigated in an attempt to discover highly potent MAO-B inhibitors. To explore the SARs of MAO-B inhibitors by the sulfanylphthalimide class of compounds, additional C5 substituents were considered. These include phenylsulfanyl, (2-phenylethyl)sulfanyl, cyclohexylsulfanyl and (3-methylbutyl)sulfanyl substituents.

![Figure 1.3](image2.png)

**Figure 1.3:** The chemical structure of 5-(benzylsulfanyl)phthalimide (right) which is derived from the known potent MAO-B inhibitor, 5-benzyloxyphthalimide (left).
In conclusion, the present study aims to discover new highly potent MAO-B inhibitors and to contribute to the SARs of MAO inhibition by phthalimide derived compounds.

1.2.2 Second article: Sulfanylphthalonitrile analogues as selective and potent inhibitors of monoamine oxidase B

As mentioned above, several potent MAO-B inhibitors have been documented to possess a benzylxy side chain. Some of these include safinamide, 5-benzylloxysatins, 8-benzylxoycaffeine and 5-benzylxyphthalimide (Binda et al., 2007; Strydom et al., 2010; Manley-King et al., 2011a; Manley-King et al., 2011b). Recent findings revealed that 4-benzylxyphthalonitrile (IC$_{50}$ = 0.0079 µM) and 4-benzylxybenzonitrile (IC$_{50}$ = 0.785 µM) analogues also display potent and selective inhibition of the MAO-B enzyme. These homologues were reported to possess 227- and 41-fold selectivities towards the MAO-B enzyme over MAO-A (Manley-King et al., 2012). 4-Benzyloxyphthalonitrile, in particular, may serve as a lead compound for the design of potent and selective MAO-B inhibitors. The nitrile groups are of importance for MAO-B inhibitory activity. The high binding affinities of nitrile containing compounds to MAO-B may be explained by the highly polar nature of the nitrile functional group. In the MAO-B active site, polar functional groups such as the nitrile groups may undergo hydrogen bonding and thus enhance the binding affinities of such compounds to MAO-B. Literature also documents that phthalonitriles are in general more potent MAO-B inhibitors than benzonitriles, which suggests that the productive interactions between the nitrile groups and the MAO-B enzyme are additive (Manley-King et al., 2012).

![Figure 1.4: The chemical structure of 4-(benzylsulfanyl)phthalonitrile (right) which is derived from 4-benzyloxyphthalonitrile (left).](image1)

![Figure 1.5: The chemical structure of 4-(benzylsulfanyl)benzonitrile (right) which is derived from 4-benzyloxybenzonitrile (left).](image2)

Based on the above considerations, the present study will attempt to enhance the selectivity and potency of the phthalonitrile and benzonitrile moieties with a benzylsulfanyl substitution to
yield 4-(benzylsulfanyl)phthalonitrile (Figure 1.4) and 4-(benzylsulfanyl)benzonitrile (Figure 1.5), respectively. Such compounds may act as highly potent and selective MAO-B inhibitors. Since halogen substitution on the benzylsulfanyl ring of 8-(benzylsulfanyl)caffeine was reported to enhance MAO-B inhibition (Booyse et al., 2011), various substituents (Cl, Br, F and OCH₃) on the benzylsulfanyl ring of the phthalonitrile and benzonitrile moieties will also be considered. The SARs of MAO-B inhibition by the sulfanylphthalonitrile and sulfanylbenzonitrile analogues will be further investigated by C4 substitution of the phthalonitrile and benzonitrile moieties with phenylsulfanyl, (2-phenylethyl)sulfanyl, cyclohexylsulfanyl, cyclopentylsulfanyl and (3-methylbutyl)sulfanyl substituents.

1.2.3 Third article: The adenosine A₂A antagonistic properties of selected C8-substituted xanthines

Adenosine is a purine nucleoside that is involved in numerous functions of the central nervous system (CNS) with four identified adenosine receptor subtypes (A₁, A₂ₐ, A₂ₐ, and A₃) (Ongini et al., 2001). A₂ₐ receptors have an important role in the modulation of dopamine-mediated responses and in the control of motor behaviour in PD (Pinna et al., 2005). Adenosine A₂ₐ receptor antagonists are attracting interest as a potent and new therapy of PD. To date some of the most effective adenosine receptor antagonists are substituted xanthines (Massip et al., 2006; Bansal et al., 2009).

Most adenosine A₂ₐ receptor antagonists belong to two different chemical classes, the xanthine derivatives and the amino-substituted heterocyclic compounds. Over the past decades, several xanthine derived A₂ₐ antagonists have been shown to possess a styryl moiety of the (E)-configuration at C8 of the xanthine ring. Examples of such structures are the frequently used reference A₂ₐ antagonists (E)-1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methylxanthine (KW-6002, istradefylline) and (E)-8-(3-chlorostyryl)caffeine (CSC).

![Figure 1.6: Chemical structure of xanthine.](image)

![Figure 1.7: Chemical structures of KW-6002 and CSC.](image)
Previous studies performed with various \((E)\)-8-styrylxanthines indicated that these compounds can tolerate a wide variety of substituents on the styryl phenyl ring, however, modification of the styryl double bond is usually associated with loss of \(A_{2A}\) antagonistic activity (Bansal et al., 2009). This is demonstrated by the finding that \((E)\)-8-styrylcaffeine \((K_i = 94\ nM)\) displays a greater binding affinity for the \(A_{2A}\) receptor than the corresponding phenyl substituted homologue \((K_i = 19\ \mu M)\) (Müller et al., 1997). In addition, SAR studies have revealed that a diverse range of substituents on the N1, N3 and N7 positions of the xanthine ring are appropriate for \(A_{2A}\) antagonism (Massip et al., 2006). These substituents include the methyl, ethyl, propyl and propargyl functional groups (Jacobson et al., 1993; Müller et al., 1997; Shimada et al., 1997; Massip et al., 2006).

The present study intends to identify novel high affinity xanthine derived \(A_{2A}\) antagonists and to further explore the SARs of \(A_{2A}\) antagonism by the xanthine class of compounds. A series of \((E)\)-8-styrylxanthines will be synthesized and compared to two chemical classes, which have not been investigated for \(A_{2A}\) antagonistic properties previously. These two chemical classes are the 8-(phenoxy)methyl)xanthine and 8-(3-phenylpropyl)xanthine derivatives. As a further step of our investigation of the \((E)\)-8-styrylxanthines, we examined the effect of different \(\text{CH}_3/\text{C}_2\text{H}_5\) substitution patterns on N1, N3 and N7 of the xanthine ring and the effect on \(A_{2A}\) antagonism by various halogen (Cl, Br) and halogen containing (CF\(_3\)) substituents on the styryl phenyl ring.

### 1.3 Aims

As mentioned above, the focus of this thesis is directed towards two strategies to improve the treatment of PD, namely to design inhibitors of the MAO-B enzyme and antagonists of the adenosine \(A_{2A}\) receptor. In addition, this study aims to publish the research presented in this thesis in academic journals.

The first aim is to synthesize and evaluate novel inhibitors of MAO by using phthalimide, phthalonitrile and benzonitrile as scaffolds. For the purpose of this study these moieties will be substituted with the benzylsulfanyl side chain and analogues thereof. This research may contribute to the SARs of MAO inhibition by phthalimide, phthalonitrile and benzonitrile derived compounds. The second aim is to synthesize a series of \((E)\)-8-styrylxanthine, 8-(phenoxy)methyl)xanthine and 8-(3-phenylpropyl)xanthine derivatives and examine their \(A_{2A}\) antagonistic properties. In addition, the SARs of the \((E)\)-8-styrylxanthine class will be further investigated by exploring the effect on \(A_{2A}\) antagonism of different \(\text{CH}_3/\text{C}_2\text{H}_5\) substitution patterns on N1, N3 and N7 of the xanthine ring and various halogen (Cl, Br) and halogen containing (CF\(_3\)) substituents on the styryl phenyl ring.
1.4 References


