CHAPTER 1.
INTRODUCTION AND RESEARCH PROPOSAL

1.1. INTRODUCTION

Idiopathic Parkinson’s disease (PD) is a neurodegenerative disorder characterized pathologically by a marked loss of dopaminergic nigrostriatal neurons and clinically by disabling movement disorders (Dauer & Przedborski, 2003; Youdim et al., 2006). At present the mainstay for the treatment of PD relies on dopamine replacement therapy with the dopamine precursor levodopa. Levodopa, however, only provides symptomatic relief (Youdim et al., 2006). The therapeutic role of MAO-B inhibitors in PD is particularly of interest. The MAO-B isoform plays an important role in the pathophysiology of PD (Youdim, 1988) since it is the major dopamine metabolising enzyme in the basal ganglia (Collins et al., 1970; Youdim et al., 2006). Inhibition of this enzyme in the brain may thus conserve the depleted supply of dopamine and lead to enhanced dopaminergic neurotransmission (Youdim & Weinstock 2004; Alexi et al., 2000).

MAO-B inhibitors are currently used in the symptomatic treatment of PD (Youdim et al., 2006; Finberg et al., 1998). Furthermore, MAO-B inhibitors also may exert a neuroprotective effect by reducing the concentrations of potentially hazardous by-products of MAO-B catalyzed dopamine oxidation (Youdim & Weinstock 2004; Youdim & Bahkle, 2006). Such products include aldehyde and hydrogen peroxide, which are neurotoxic if not rapidly metabolized to inactive compounds (Youdim & Bahkle, 2006; Jenner, 2003). Although the concentration of MAO-B is much higher than MAO-A in the human basal ganglia (Collins et al., 1970), MAO-A inhibitors may also enhance dopamine levels in this region. A selective irreversible inhibitor of MAO-A, clorgyline, enhances dopamine levels in the striatum of primates, treated with levodopa, to a similar degree than the elevation obtained with (R)-depenyl, a selective irreversible inhibitor of MAO-B (Di Monte et al., 1996). MAO-A inhibitors are currently used in the treatment of depression.

Standard dopamine replacement therapy for PD frequently involves combination therapy. For example (R)-depenyl, may be used with levodopa (Rabey et al., 2000). However, some safety considerations arise with the use of (R)-depenyl (Gnerre et al., 2000) since it is a propargyl amphetamine derivative and undergoes extensive metabolism to amphetamine metabolites such as (R)-methamphetamine (Mahmood, 1997; Riederer et al., 2004). These amphetamine...
metabolites are potentially neurotoxic and may possess adverse cardiovascular and psychiatric effects (Churchyard et al., 1997). Another limitation of (R)-deprenyl includes the loss of selectivity for MAO-B at higher doses, which may result in the occurrence of the “cheese reaction” (Youdim & Bahkle, 2006). Also, upon treatment with (R)-deprenyl, which is an enzyme inactivator, enzyme activity is only regained via de novo synthesis of the MAO-B protein, which may require several weeks (Riederer et al., 2004). For these reasons, reversible inhibitors may be therapeutically more desirable than inactivators, since MAO-B activity can be regained more quickly following withdrawal of a reversible inhibitor. Reversible inhibitors may therefore be safer than irreversible inhibitors of MAO-B (Novaroli et al., 2006).

Drugs that target the mechanism of neuronal cell death and therefore delay or even halt the progression of this disease may offer an improved therapeutic approach for the treatment of PD (Nicotra et al., 2004; Fowler et al., 1997). Inhibitors of MAO may represent a strategy for neuroprotection in PD. As mentioned, MAO-B inhibitors may reduce toxic by-products that may form during the metabolism of dopamine. Since MAO-B inhibitors reduce the formation of these toxic metabolites, they may act as neuroprotective agents. This study focuses on the design of new reversible inhibitors of MAO-B that may be used in the symptomatic treatment of PD, by blocking dopamine metabolism, as well as providing neuroprotection by reducing the levels of potentially toxic metabolites derived from the MAO catalytic cycle.

1.2 BACKGROUND

In the present study, three classes of compounds will be examined as potential MAO inhibitors.

- Isatins
- Phthalimides
- Phthalonitriles

1.2.1. Isatins

Figure 1: Structure of isatin

Indoles have unique structural features which are believed to be responsible for their various biological activities (Smith et al., 1988). Serotonin, for example, is a neurotransmitter which plays an important role in a variety of physiological processes (Kikuchi et al., 1999) and contains
the basic skeleton of an indole. Indoles are valuable therapeutic agents and are found in drugs such as indomethacin, a non-steroidal anti-inflammatory agent (Reinicke, 1977).

Isatin (1H-indole-2,3-dione) is an indole derivative of special interest (Da Silva et al., 2001) and possesses unique biological and pharmacological properties. In nature, isatin is found in plants such as in the genus *Isatis* (Guo & Chen, 1986). Synthetic isatin was discovered early in the nineteenth century as an oxidation product of indigo (Erdmann, 1841). It is a bright orange-coloured compound which was, in 1988, identified in human urine and rat brain tissue as an endogenous MAO inhibitor (Glover et al., 1988; Medvedev et al., 1992). It has a distinctive distribution in brain tissue and the highest levels are found in the hippocampus (Glover et al., 1988). In recent years, Schiff and Mannich bases of isatin were reported to exhibit a broad-spectrum of pharmacological properties such as antiviral (Sriram & Yogeeswari, 2003), anti-TB (Karah, 1998), antifungal and antibacterial activities (Pandeya et al., 1999; Pandeya et al., 2000). Pharmacological studies show that isatin can be both anxiogenic and sedative and may cause an increase in brain monoamine levels since it is a competitive inhibitor of both MAO-A and -B (Medvedev et al., 1995; Hamaue et al., 1992). The discovery that isatin can inhibit both MAO-A and MAO-B is of interest to researchers. Isatin inhibits human MAO-B with an enzyme-inhibitor dissociation constant (K_i value) of 3 µM and human MAO-A with a K_i value of 15 µM (Hubàlek et al., 2005). Structural analogues of isatin have also been found to be inhibitors of both MAO-A and MAO-B. Of note is the discovery that (E)-5-styrylisatin and (E)-6-styrylisatin are potent inhibitors of MAO-B (Van der Walt et al., 2009). This finding has led us to believe that structural derivatives of this unique compound may represent potential therapeutic agents for the treatment of PD.

The crystal structure of human MAO-B in complex with isatin has been reported (Binda et al., 2003). Inspection of this complex shows that, in the substrate cavity, the dioxoindolyl ring of isatin is orientated with the 2-oxo group directed towards the flavin cofactor, while the C-2 carbonyl oxygen forms hydrogen bonds with ordered water molecules in the active site. This binding mode leaves the entrance cavity of MAO-B unoccupied (Binda et al., 2003). Also, molecular docking of (E)-5-styrylisatin and (E)-6-styrylisatin in the active site of MAO-B, indicates that the styryl side chain extends into the entrance cavity, while the dioxoindolyl ring is located in the substrate cavity (Van der Walt et al., 2009). The binding orientation of the dioxoindolyl ring of (E)-5-styrylisatin and (E)-6-styrylisatin is similar to that of isatin, with the 2-oxo and NH functional groups hydrogen bonded to water molecules present in the substrate
Based on these considerations, it was concluded that potent MAO-B inhibitors may result from structures that bind to both the entrance and substrate cavities (Binda et al., 2002).

1.2.2. Cyclic imides

Synthetic heterocyclic compounds and their derivatives have unique structural features which are responsible for their biological activities and pharmaceutical use. Compounds known as imides such as succinimides, maleimides, glutarimides and phthalimides are known to possess potent biological activities and include antibacterial, (Cechinel Filho et al., 1994; Cechinel Filho et al., 1995), antifungal (Dantas et al., 2000), analgesic, anti-viral (Hashimoto, 2002), anticonvulsant (Bailleux et al., 1994a; Bailleux et al., 1994b) and antitumor action (Wang et al., 2000). Extensive studies have demonstrated that isoindoline-1,3-dione appears to be the pharmacophoric structure responsible for these diverse biological activities. Recent studies of the anticonvulsant effect of N-substituted-isoindolinediones (Abdel-Hafez, 2004), showed 100% protection against convulsions in mice without neurotoxicity and mortality. Beside these interesting biological effects, some cyclic imides e.g., chlorophthalim (Adomat & Börger, 2000), \(N\) aryltetrahydrophthalimide (Birchfield & Casida, 1997) and \(N\)-(4-chloro-2-fluoro-5-propargyloxy)-phenyl-3,4,5,6-tetrahydrophthalimide (Watanabe et al., 1998) are peroxidizing herbicides, a class of herbicides that inhibit protoporphyrinogen IX oxidase, a key enzyme of heme and chlorophyll biosynthesis.

The cyclic imide class of compounds generally have an imide ring, with the general structure \(-\text{CO-N(R)-CO}-\). They are therefore both hydrophobic and neutral and can cross biological membranes \textit{in vivo} (Haergreaves et al., 1970). The biological properties of cyclic imides appear to be related to the size and electrophilic characteristics of substituent groups on the imide ring (Cechinel Filho et al., 1995; Lima et al., 1999; López et al., 2003). Increasing electronic density on the nitrogen atom of the imides, may result in the participation in the activation of redox cycles, that play a major role in a variety of biological activities (Andricopulo et al., 1999). The famous isoindoline-1,3-dione derivative, thalidomide, was synthesized in 1953, by the Swiss pharmaceutical company Ciba and marketed in 1954 by the German company Chemie Grünenthal as an anticonvulsant agent for the treatment of epilepsy (Hashimoto, 2002). Thalidomide was later withdrawn due to its serious teratogenic effects. However, thalidomide does have some therapeutic value: (1) as an immunosuppressive agent in the treatment of graft versus host disease (Arora et al., 2001), (2) in the treatment of leprosy (Teo et al., 2002) and (3)
for inflammatory dermatoses (Grosshans & Illy, 1984). Since the pioneering discovery of the anticonvulsant properties of thalidomide, the isoindoline-1,3-dione ring system became an important building block, that led to the discovery of a number of imide derivatives. In spite of the diversity of biological effects that have been assigned to cyclic imides, much of their biological and toxicological action mechanisms at molecular and cellular levels remain to be elucidated. However, studies have shown that N-methyl-2-phenylmaleimides can act as potent reversible inhibitors of MAO-B (Manley-King et al., 2009).

This study will examine the possibility that the derivatives of isoindoline-1,3-dione, phthalimide, may act as inhibitors of the MAO isozymes.

1.2.3. Phthalonitriles
Phthalonitrile consists of a phenyl ring containing two adjacent nitrile groups with a formula C_6H_4(CN)_2. At room temperature, it exists as an off-white crystal solid, partially soluble in water and soluble in acetone. In 1896, Johannes Pinnow reported the first formation of phthalonitrile which is believed to be a by-product of the reaction between orthamidobenzonitrile hydrochloride, sodium nitrite and hydrochloric acid in the synthesis of orthodicyanodiazoamidobenzene (Pinnow & Samann, 1896). To date, the biological properties of phthalonitriles remain unknown. This study will investigate the MAO inhibition properties of a synthetic series of phthalonitriles.

1.3. RATIONALE AND SELECTION OF COMPOUNDS
Due to their role in the metabolism of monoamine transmitters, MAO-A and -B are of considerable pharmacological interest. Inhibitors of MAO represent a useful tool for the treatment of neurological and psychiatric diseases (Youdim et al., 2006). The primary aim of this study is to synthesize compounds that may act as monoamine oxidase inhibitors and can be used in the treatment of PD. To achieve this goal, we will synthesize derivatives of the indole, isatin, and the imide, phthalimide, and examine their MAO inhibition properties. In addition, derivatives of phthalonitriles and benzonitriles will be synthesized and investigated as MAO inhibitors.

1.3.1 Isatins
This study is an extension of a previous investigation which found that the isatin derivatives (E)-5-styrylisatin and (E)-6-styrylisatin, are reversible inhibitors of human MAO-A and -B (Van der
Walt et al., 2009). In this study additional analogues of isatin will be prepared as part of an effort to define the structural requirements of this class of compounds to act as inhibitors of MAO. For this purpose, isatin derivatives with substitution at the C5 and C6 position of the isatin ring will be considered. One goal of this study, is to determine if C5-substituted isatin analogues are in general better MAO-B inhibitors than the corresponding C6 isomers as observed with the (E)-styrylisatin analogues (Van der Walt et al., 2009).

In addition, C3- and C4-substituted anilines, which are synthetic precursors in the synthesis of isatin derivatives, will be examined as potential MAO inhibitors. The purpose of examining the aniline derivatives is to determine the importance of the isatin ring for MAO inhibition. Among the C5 and C6 substituents chosen for this study is the benzyloxy side chain which has been shown to enhance the binding affinity of caffeine to the active site of both MAO-A and MAO-B (Strydom et al., 2010). Other substituents considered include the phenoxy, benzyloxy, 2-phenylethyl, 4-phenylpropenylxy and 4-bromophenoxy groups. This study may contribute to the discovery of a new class of potent reversible MAO inhibitors.

The structures of the isatin and aniline derivatives that will be investigated in this study are shown in figure 2.

Figure 2: General structures of the C5- and C6-substituted isatins (a) and C3- and C4-substituted anilines (b) that will be investigated in this study.
Table 1: The substituents considered for the design of the isatin and aniline derivatives in this study.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R$^1$</th>
<th>R$^2$</th>
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<tbody>
<tr>
<td>a</td>
<td>C$_6$H$_5$CH$_2$O</td>
<td>H</td>
</tr>
<tr>
<td>b</td>
<td>H</td>
<td>C$_6$H$_5$CH$_2$O</td>
</tr>
<tr>
<td>c</td>
<td>C$_6$H$_5$CH$_2$CH$_2$</td>
<td>H</td>
</tr>
<tr>
<td>d</td>
<td>H</td>
<td>C$_6$H$_5$CH$_2$CH$_2$</td>
</tr>
<tr>
<td>e</td>
<td>C$_6$H$_5$O</td>
<td>H</td>
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<tr>
<td>f</td>
<td>H</td>
<td>C$_6$H$_5$O</td>
</tr>
<tr>
<td>g</td>
<td>C$_6$H$_5$</td>
<td>H</td>
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<tr>
<td>h</td>
<td>H</td>
<td>C$_6$H$_5$</td>
</tr>
<tr>
<td>i</td>
<td>C$_6$H$_5$(CH$_2$)$_4$</td>
<td>H</td>
</tr>
<tr>
<td>j</td>
<td>4-ClC$_6$H$_5$O</td>
<td>H</td>
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</tbody>
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1.3.2. Phthalimides

The discovery that isatin analogues, containing C5 and C6 substituents, are potent reversible MAO-B inhibitors (Van der Walt et al., 2009), has provided a scaffold for further development of novel compounds that may inhibit MAO. The high inhibition potency of isatin may be attributed to its binding orientation within the active site, which allows for favourable interactions that stabilize the ligand. Based on these observations, the present study examines a series of phthalimide analogues as potential inhibitors of MAO-A and -B. As shown in figure 3, phthalimide is an isomer of isatin. A series of alkyl- and arylxy side chains shown in table 2 were selected for this study. The study aims to examine the possibility that phthalimides may inhibit MAO and to determine whether C5-substituted phthalimides are in general better reversible inhibitors of recombinant human MAO-B than the corresponding isatin analogues.

Figure 3: Structure of C5-substituted phthalimide.
Table 2: The substituents considered for the design of the phthalimide derivatives in this study.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
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<tbody>
<tr>
<td>a</td>
<td>C₆H₅O</td>
</tr>
<tr>
<td>b</td>
<td>C₆H₅CH₂O</td>
</tr>
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<td>c</td>
<td>C₆H₅CH₂CH₂O</td>
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<tr>
<td>d</td>
<td>C₆H₅(CH₂)₂O</td>
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<td>e</td>
<td>(E)-C₆H₅CH=CHCH₂O</td>
</tr>
<tr>
<td>f</td>
<td>2-Naphthalenyl-O</td>
</tr>
<tr>
<td>g</td>
<td>4-BrC₆H₅CH₂O</td>
</tr>
<tr>
<td>h</td>
<td>4-BrC₆H₅CH₂CH₂O</td>
</tr>
<tr>
<td>i</td>
<td>4-BrC₆H₅O</td>
</tr>
</tbody>
</table>

1.3.3. Phthalonitriles and benzonitriles

In the third part of this study, phthalonitriles and benzonitriles will be synthesized and evaluated as inhibitors of MAO. These include:

C₄ substituted phthalonitriles

The observation that the nitrile functional group is a bioisostere of water has been widely exploited in drug design to displace water molecules from the binding sites of proteins (Meanwell, 2011). This suggests that nitriles may undergo polar interactions with the active site of MAO and thus facilitate potent inhibition of MAO. In this study, a series of phthalonitriles will be examined as potential inhibitors of MAO-A and –B. Alkyl-and aryloxy side chains will be selected for substitution on the phthalonitrile ring since these have been shown to enhance the MAO-A and –B binding affinities of a variety of scaffolds, including caffeine, isatin and phthalimide (Manley-King et al., 2011a; Strydom et al., 2010; Manley-King et al., 2011b). Previous studies have suggested that alkyl-and aryloxy side chains, with a relatively larger degree of conformational freedom as a result of rotation about the carbon-oxygen ether bond, may be better suited for binding to MAO-A than relatively rigid structures (Strydom et al., 2010; Van der Walt et al., 2009). Therefore, oxy containing substituents considered for this study include the benzyloxy side chain, phenoxy, 2-phenylethoxy, 4-phenylpropenylxy and 4-bromophenoxy groups.
Figure 4: Structure of C4-substituted phthalonitriles.

Table 3: The substituents considered for the design of the phthalonitrile derivatives in this study.

<table>
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<tr>
<th>Compound</th>
<th>R</th>
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<tbody>
<tr>
<td>a</td>
<td>C₆H₅O</td>
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<tr>
<td>b</td>
<td>C₆H₅CH₂O</td>
</tr>
<tr>
<td>c</td>
<td>C₆H₅CH₂CH₂O</td>
</tr>
<tr>
<td>d</td>
<td>C₆H₅(CH₂)₃O</td>
</tr>
<tr>
<td>e</td>
<td>(E)-C₆H₅CH=CHCH₂O</td>
</tr>
<tr>
<td>f</td>
<td>2-Naphthalenyl-O</td>
</tr>
<tr>
<td>g</td>
<td>4-BrC₆H₄O</td>
</tr>
<tr>
<td>h</td>
<td>4-BrC₆H₄CH₂O</td>
</tr>
<tr>
<td>i</td>
<td>4-BrC₆H₄CH₂CH₂O</td>
</tr>
</tbody>
</table>

C3 and C4 substituted benzonitriles

In this study, C3- and C4-substituted benzonitriles will be synthesized and evaluated for MAO inhibitory activity. The inhibitory properties of C3- and C4-substituted benzonitrile derivatives will be compared with the C4-substituted phthalonitriles, in an attempt to examine the potential role that nitrile groups may play in the binding of inhibitors to MAO-A and –B.

Figure 5: Structures of C3-substituted (a) and C4-substituted benzonitriles (b).
Table 4: The substituents considered for the design of the benzonitrile derivatives in this study.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
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<tbody>
<tr>
<td>a</td>
<td>C₆H₅CH₂O</td>
</tr>
<tr>
<td>b</td>
<td>(E)-C₆H₅CH=CHCH₂O</td>
</tr>
<tr>
<td>c</td>
<td>4-BrC₆H₄CH₂O</td>
</tr>
<tr>
<td>d</td>
<td>4-BrC₆H₅CH₂CH₂O</td>
</tr>
<tr>
<td>e</td>
<td>4-C₆H₅CH₂O(CH₂)₂O</td>
</tr>
</tbody>
</table>

1.3.4. OBJECTIVES OF THIS STUDY

Since MAO-B activity is increased in both AD and PD, the use of MAO inhibitors may be of further therapeutic benefit (Youdim & Bahkle, 2006). For this reason it is of importance to develop and study compounds such as isatin, phthalimide and phthalonitrile derivatives which may represent new potent inhibitors of MAO-B. Based on the discussion above, the objectives of this study are summarized below:

- In the present study, C5- and C6-substituted isatin analogues will be synthesized and evaluated as inhibitors of recombinant human MAO-A and MAO-B. One of the goals of this study is to determine if C5-substituted isatin analogues are in general better MAO-B inhibitors than the corresponding C6 isomers as observed with the (E)-styrylisatin analogues. Furthermore, this study also aims to determine the effect of C5 and C6 substitution of isatin on MAO-A inhibition activity.

- A series of phthalimide analogues will also be synthesized and evaluated as inhibitors of recombinant human MAO-A and MAO-B. A goal of this study is to investigate the effect on MAO inhibition of the substituent position on the phthalimide ring. For this purpose, C5-substituted and N-substituted phthalimides will be synthesized.

- Thirdly, a series of phthalonitrile and benzonitrile analogues will be synthesized and evaluated as inhibitors of recombinant human MAO-A and –B. One of the goals of this study is to compare the MAO activities of the benzonitriles with the phthalonitriles to determine the requirement of the nitrile groups for MAO inhibition activity.
All the compounds will be evaluated as inhibitors of MAO-A and –B. For this purpose the recombinant human enzymes, which are commercially available, will be employed. The inhibition potencies will be expressed as the IC\textsubscript{50} values (concentration of the inhibitor that produces 50% inhibition). A fluorometric assay will be used to measure the enzyme activities. The MAO activity measurements are based on measuring the concentrations of the MAO-A or –B generated 4-hydroxyquinoline product formed from the oxidation process.

The time-dependency of inhibition of both MAO-A and –B, by selected analogues, will also be evaluated. This is done in order to determine if the inhibitors interact reversibly or irreversibly with the MAO isozymes.

If the inhibition is found to be reversible, sets of Lineweaver-Burke plots will be generated for those inhibitors selected above, in order to determine if the mode of inhibition is competitive.

Molecular docking studies will be carried out using the Windows based Discovery Studio 1.7 molecular modeling software (Accelrys Discovery Studio). The models generated from these studies may provide insight into the binding modes of these compounds and also assist future design of potent reversible inhibitors of MAO-A and -B.

The findings of this study will be compiled into three articles to be submitted to peer-reviewed journals. The first article will describe the MAO inhibitory activities of substituted isatin derivatives (figure 2), the second article, the MAO inhibitory activities of substituted phthalimide analogues (figure 3), and the third article will detail the MAO inhibitory activities of substituted phthalonitrile and benzonitrile derivatives (figure 4 and 5).

1.4. SUMMARY
The enzyme, MAO, has been implicated in the neurodegenerative processes associated with PD (Collins \textit{et al.}, 1970). MAO-B inhibitors may have a therapeutic role in PD since inhibition of this enzyme in the brain may conserve the depleted supply of dopamine. New compounds that could offer both symptomatic relief and neuroprotection in PD are currently the focus of many
research groups. Investigation of the MAO inhibitory activity of isatin, phthalimide and phthalonitrile analogues may therefore lead to the discovery of drugs for the treatment of PD.


