The use of MAO-B inhibitors in the treatment of Parkinson’s disease is well established since MAO-B is known to be one of the main enzymes responsible for the oxidation of dopamine. Increased activity of MAO-B results in the overproduction of aldehydes and hydrogen peroxide which may lead to neuronal damage and contribute to the neurodegeneration in PD (Youdim & Bahkle, 2006). The role of MAO-B in the oxidation of MPTP to the mitochondrial toxin MPP⁺ (Chiba et al., 1984; Heikkila et al., 1984b), suggests that MAO-B inhibitors may also protect against environmental toxins. MAO-B inhibitors may be viewed as a promising option in PD treatment as they may offer both symptomatic and neuroprotective effects.

In this study, structurally related classes of compounds, the isatins, phthalimides and phthalonitriles, were synthesized and evaluated as inhibitors of recombinant human MAO-A and MAO-B. Stryrylisatins have previously been shown to be inhibitors of MAO-B (Van der Walt et al., 2009), but to the best of our knowledge, this study is the first to report on the inhibition activities of phthalimide and phthalonitrile derivatives. One goal of this research was to study the MAO-B inhibition of C5- and C6-substituted analogues of isatin. In the present work, C5- and C6-substituted isatin analogues were shown to act as moderate to potent inhibitors of recombinant human MAO-A and MAO-B. The most potent MAO-B inhibitor, 5-(4-phenylbutyl)isatin, exhibited an IC₅₀ value of 0.66 nM, approximately 13-fold more potent than (E)-5-styrylisatin and 18,500-fold more potent than isatin. The most potent MAO-A inhibitor was found to be 5-phenylisatin with an IC₅₀ value of 562 nM. The results document that substitution at C5 with a variety of substituents is a general strategy for enhancing the MAO-B inhibition potency of isatin.

Another goal of this study was to investigate the inhibition potencies of substituted phthalimide derivatives. A series of N-aryl substituted and C5-substituted phthalimides were synthesized and evaluated as inhibitors of recombinant human MAO-A and –B. While phthalimide and N-aryl-substituted phthalimides were found to be weak MAO inhibitors, this study showed that phthalimide homologues containing C5-substituents were potent reversible inhibitors of recombinant human MAO-B, with IC₅₀ values ranging from 0.007 to 2.5 µM and moderately potent reversible inhibitors of recombinant human MAO-A, with IC₅₀ values ranging from 0.22 to
9.0 µM. The structure-activity relationships (SAR) studies reveal that increasing the length or size of the C5 substituent enhances the MAO-B inhibition potencies of the phthalimide analogues. Halogen substitution on the ring system of the C5 side chain also enhances MAO-B inhibition potency, particularly of the weaker phthalimide inhibitors.

An important outcome of the modeling studies was the finding that the phthalimide rings of the inhibitors are stabilized via hydrogen bonding in the MAO-B active site. This finding is in accordance with the hypothesis that hydrogen bond acceptors of highly potent inhibitors may stabilize such enzyme-inhibitor complexes (Novaroli et al., 2006). The difference in activities of the N-aryl substituted analogues and the C5-substituted analogues may be dependent upon the ability of the phthalimide heterocyclic system of C5-substituted phthalimide derivatives to act as a hydrogen bond acceptor. The phthalimide rings of N-substituted homologues are not able to interact with the MAO-B active site via hydrogen bonding.

This research also examined the MAO-B inhibition properties of a series of C4-substituted phthalonitrile derivatives. In general, the C4-substituted phthalonitrile analogues were shown to be highly potent reversible MAO-B inhibitors with most analogues exhibiting IC_{50} values in the low nM range. It was observed that increasing size or length of the C4 substituent led to enhanced MAO-B inhibition. The most potent inhibitor among the derivatives considered was 4-(4-bromobenzyloxy)phthalonitrile, with an IC_{50} value of 0.0048 µM for MAO-B, while 4-(phenylpropenylxyloxy)phthalonitrile was the most potent inhibitor of MAO-A (IC_{50} value of 0.399 µM). This study demonstrates that potent MAO inhibition can be readily achieved by making use of nitrile substitution. Substituted phthalonitrile analogues represent a new class of lead compounds for the development of antiparkinsonian drugs.

Based on the finding that C4-substituted phthalonitriles display exceptionally potent inhibition towards recombinant human MAO-A and MAO-B, an evaluation of the MAO inhibitory activities of C3- and C4-substituted benzonitrile derivatives was carried out. Generally, the benzonitrile analogues were good MAO-A and MAO-B inhibitors, with selectivities mostly towards the B isoform. It was observed that C3-substituted benzonitriles are better reversible MAO-B inhibitors than the C4-substituted analogues. For example, the C3-substituted benzonitrile, 3-(benzyloxy)benzonitrile, with an IC_{50} value of 0.249 µM, is 3 fold more potent than its corresponding C4-substituted isomer, 4-(benzyloxy)benzonitrile, which has an IC_{50} value of 0.785 µM.
However, comparing the inhibitory activities of the benzonitrile analogues with the phthalonitriles, they were found to be weaker MAO-B inhibitors than the phthalonitrile analogues. For example, the IC$_{50}$ value of 4-(benzyloxy)benzonitrile is 0.785 µM, while that of 4-(benzyloxy)phthalonitrile is 0.0079 µM. A similar trend was observed among the homologous series of benzonitrile analogues considered. The findings from this study has established that the phthalonitrile moiety is more optimal for MAO-B inhibition than the corresponding benzonitrile moiety, and that C3-substituted benzonitriles are better MAO-B inhibitors than C4-substituted benzonitriles.

Also in this study, a series of benzyl phenyl ether analogues, which are devoid of the nitrile functional group, was also investigated as potential MAO inhibitors. In general, these compounds were weaker inhibitors than their nitrile containing homologues (phthalonitriles and benzonitriles) towards both MAO-A and MAO-B. Comparing the inhibition potencies of the benzyl phenyl ether analogues to that of the phthalonitriles and benzonitriles, they were found to be weak inhibitors of MAO-B with IC$_{50}$ values ranging from 1.3–11.8 µM. Of note, benzyl phenyl ethers were found to be devoid of MAO-A inhibition properties. The finding that the removal of nitrile functional group yielded compounds with only moderate MAO-B inhibition potencies has established that the nitrile functional group is a requirement for high affinity binding of the phthalonitrile and benzonitrile analogues to the MAO-B active site. This study highlights the significance of the nitrile functional groups of phthalonitriles and benzonitriles for enhanced binding affinity to the MAO-B active site.

In conclusion, the high inhibition potencies of the isatin, phthalimide and phthalonitrile derivatives indicate that these compounds may be lead compounds for the development of new drugs that may be used in the treatment of PD.