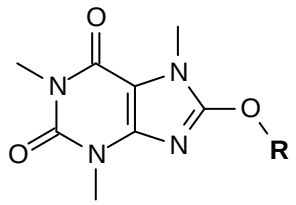
Chapter 6
Conclusion

In this study, three series of novel compounds were synthesized and evaluated as reversible inhibitors of MAO-A and MAO-B. The results were presented as three journal articles.

The first article describes a series of caffeine analogues as MAO inhibitors. In this series, caffeine was substituted on the C8 position with alkyloxy side chains to yield potent MAO inhibitors. These inhibitors were especially potent towards the B isoform. For example, 8-[2-(4-bromophenoxy)ethoxy]caffeine was found to be a highly potent MAO-B inhibitor with an IC_{50} value of 0.166 µM. Interestingly, this compound was also the most potent MAO-A inhibitor with an IC_{50} value of 1.65 µM. The effects that different alkyloxy side chains have on the inhibitory activity were investigated and it was found that the 8-(2-phenoxyethoxy) side chain resulted in particularly potent inhibitors of both MAO-A and –B, especially with halogen substitution on C4 of the phenyl ring. Compounds with shorter side chains on C8, such as the phenylethoxy side chain, as well as compounds with longer side chains, such as the benzyloxyethoxy side chain, proved to be weaker inhibitors of MAO-B. This suggests that the optimal side chain length for MAO-B inhibition is a linker containing 4 atoms between the phenyl ring and the caffeine moiety. An example of such a side chain is the phenoxyethoxy moiety. To establish the reversibility of inhibition of these compounds, a representative inhibitor, 8-[2-(4-bromophenoxy)ethoxy]caffeine, was selected and the time dependence of the inhibition of the oxidation of kynuramine by MAO-A and –B were determined. The results showed that this compound is not a time dependent MAO inhibitor, which is an indication of reversible inhibition. Sets of Lineweaver-Burk plots were also constructed and the interception of the plots on the y-axis was further confirmation that inhibition is competitive and therefore reversible for MAO-A and –B. Docking studies were carried out to clarify the binding modes of these compounds within the respective binding sites of MAO-A and MAO-B. The

docking studies illustrated that 8-(3-phenylpropoxy)caffeine binds in an extended conformation within MAO-B. The caffeine moiety is oriented towards the FAD co-factor while the phenylpropoxy side chain extends into the entrance cavity. By docking the same inhibitor into the smaller active site cavity of MAO-A, it was shown that the phenylpropoxy side chain binds in a bended conformation to avoid unfavourable interactions within the smaller active site. The ability of these compounds to bind to both the MAO-A and –B active sites can possibly be attributed to the flexibility of these molecules. The ether oxygen in the side chain of the 8-alkyloxycaffeine analogues may be responsible for the flexibility observed in these compounds.

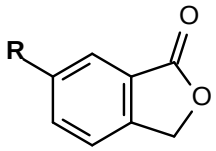
In the second article, the 8-(2-phenoxyethoxy)caffeine analogues were further investigated. A series of ten 8-(2-phenoxyethoxy)caffeine analogues (of which 7 were newly synthesized and 3 derived from series 1) with different substituents on C4 of the phenyl ring were investigated. QSAR studies were carried out to correlate the physicochemical properties of the C4 substituents with the inhibitory activity of the compounds towards MAO-A and –B. Compounds with substituents on C4, which are lipophilic, electron withdrawing and bulky (such as the iodo group), resulted in potent MAO-A inhibition. For example, 8-[2-(4-iodophenoxy)ethoxy]caffeine inhibited MAO-A with an IC_{50} value of 0.924 µM. For increased MAO-B inhibition, it was found that C4 substituents, which are more electron withdrawing (such as the CF_3 group), was optimal. For example, 8-{2-[4-(trifluoromethyl)phenoxy]ethoxy}caffeine exhibits an IC_{50} value of 0.061 µM for the inhibition of MAO-B. Similarly to the first series, docking studies showed that the 8-(2-phenoxyethoxy)caffeine analogues bind in a twisted conformation within the smaller active site cavity of MAO-A. The flexibility of these compounds may be partly responsible for the fact that they inhibit both MAO-A and –B.



R	IC_{50} MAO-A (µM)	IC_{50} MAO-B (µM)	SI
$C_6H_5CH_2$-	1.24	1.77	0.7
4-$BrC_6H_4OCH_2CH_2$-	1.65	0.166	9.94
4-$CF_3C_6H_4OCH_2CH_2$-	2.22	0.061	36
4-$IC_6H_4OCH_2CH_2$-	0.924	0.128	7.2
$C_6H_5OCH_2CH_2$-	20.35	0.383	53.133

The third article described the synthesis and evaluation of a novel series of 6-alkyloxyphthalide analogues. These compounds were potent inhibitors of both MAO-A and –B, but displayed greater selectivity towards MAO-B. A small series of 6-benzyloxyphthalide analogues, with different substituents on C4 of the phenyl ring, showed that lipophilic, electron withdrawing and bulky substituents enhance MAO-B inhibitory activity. For example, 6-[4-(trifluormethyl)benzyloxy]phthalide were the most potent MAO-B inhibitor in the series with an IC_{50} value of 0.0014 µM. The most potent MAO-A inhibitor was 6-(3-phenylpropoxy)phthalide with an IC_{50} value of 0.096 µM. To investigate the importance of an oxy moiety in the side chain, a compound with benzylamino substitution on the C6 position of phthalide was synthesized and evaluated as an inhibitor of MAO-A and –B. This compound proved to be the weakest inhibitor of MAO-A and –B with an IC_{50} value of 59.9 µM towards MAO-A and displaying no inhibition towards MAO-B. A benzylamino side chain is thus not suitable for the inhibition of MAO by phthalide analogues. To determine if inhibition was reversible for this class of compounds, 6-(3-phenylpropoxy)phthalide and 6[4-(trifluormethyl)benzyloxy]phthalide were selected as representative inhibitors of MAO-A and –B, respectively. The results showed that, for both the inhibition of MAO-A and MAO-B, the enzyme activities are recovered upon dilution of the enzyme-inhibitor complexes. Therefore, inhibition of MAO-A and –B by the 6-alkyloxyphthalide analogues is reversible. Lineweaver-Burk plots were also

constructed and since the plots intercepted at the y-axis, inhibition was competitive and thus reversible for MAO-A and –B.



R	IC_{50} MAO-A (µM)	IC_{50} MAO-B (µM)	SI
H-	44.9	28.6	1.6
4-$CF_3C_6H_4CH_2CH_2O$-	0.304	0.0014	214
$C_6H_5CH_2CH_2CH_2O$-	0.096	0.0062	16

In conclusion, this study showed that the alkyloxy substitution of caffeine on the C8 position and of phthalide on the C6 position are good strategies for the development of novel, reversible inhibitors of MAO for the treatment of PD. As postulated in Chapter 1, this study shows that highly potent MAO inhibitors may be designed using caffeine and phthalide as scaffolds, and that a variety of alkyloxy substituents on the C8 and C6 positions of caffeine and phthalide, respectively, yield compounds with potent MAO-A and –B inhibitory activities. It should be noted that these caffeine and phthalide derived compounds display, in almost all cases, selectivity for the inhibition of MAO-B over MAO-A. Also as postulated, these caffeine and phthalide derived inhibitors act reversibly with the MAO enzymes.