Chapter 5

Conclusion

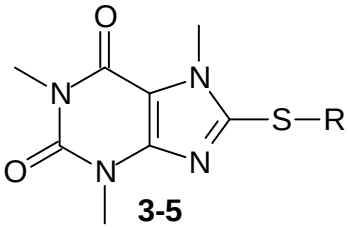
In the current study, a series consisting thirteen of 8-sulfanylcaffeine derivatives were successfully synthesized and evaluated as inhibitors of recombinant human MAO-A and –B. MAO-A is mainly responsible for the metabolism of the neurotransmitters 5-HT and NA, which plays a major role in depression. MAO-B is mainly responsible for the metabolism of the neurotransmitter DA in the brain, which plays a major role in neurodegenerative disorders such as PD. As mentioned, inhibitors of MAO-A and –B may be used in the treatment of depression and PD.

In this study, caffeine was used as a lead compound for the synthesis of novel inhibitors of MAO-A and -B. Caffeine is a weak inhibitor of MAO-B but substitution at the C8 moiety with a variety of groups, yield compounds with greatly enhanced MAO-B inhibition potencies. A particularly promising inhibitor is 8-[(phenylethyl)sulfanyl]caffeine (**2a**). This compound is a highly potent MAO-B inhibitor with an IC_{50} value of 0.223 μM (Booysen *et al*., 2011). This compound therefore represents a possible lead for the design of MAO-B inhibitors with exceptionally high binding affinities. We have therefore synthesized a series of five 8-[(phenylethyl)sulfanyl]caffeine analogues (**3a**–**e**) and evaluated the analogues as inhibitors of human MAO-A and –B. To further explore the MAO inhibitory properties of 8-sulfanylcaffeines, three selected 8-[(phenylpropyl)sulfanyl]caffeine (**4a**–**c**) and two 8-(benzylsulfanyl)caffeine analogues (**5a**–**b**) were synthesized and evaluated as MAO inhibitors. Furthermore, a series of two 8-sulfinylcaffeine analogues (**6a**–**b**) and one 8-sulfonylcaffeine analogue(**7**) were also synthesized and their MAO inhibitory potencies were measured. The structures of the compounds that were examined are presented in table 5.1.

Chemistry: Ten 8-sulfanylcaffeine analogues, **3a**–**e**, **4a**–**c** and **5a**–**b**, were successfully synthesized by reacting a mercaptan with 8-chlorocaffeine. Those mercaptans that were not commercially available were synthesized by reacting a commercially available alkylbromide with thiourea in the presence of sodium hydroxide and ethanol. Two 8-sulfinylcaffeine analogues, **6a**–**b**, and the 8-sulfonylcaffeine, **7**, were synthesized by treating the appropriate 8-sulfanylcaffeine analogues with hydrogen peroxide in the presence of acetic anhydride and glacial acetic acid. All of the newly synthesized compounds were verified by NMR and MS, and the purities were estimated with HPLC analysis. The ^{1}H NMR and ^{13}C NMR spectra were found

to be in agreement with the proposed structures, and the experimental exact masses corresponded to the theoretical masses for each compound. HPLC analysis indicated that the synthesized compounds are of good purity, and with the exception of analogue **7** (85%), the purities of the compounds ranged from 94–99%.

Table 5.1. Structures of the 8-[(phenylethyl)sulfanyl]caffeine analogues (**3a**–**e**), 8-[(phenylpropyl)sulfanyl]caffeine analogues (**4a**–**c**), 8-(benzylsulfanyl)caffeine analogues (**5a**–**b**), 8-sulfinylcaffeine analogues (**6a**–**b**) and 8-sulfonylcaffeine analogues (**7**) synthesized in this study.



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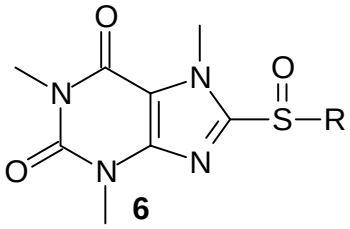

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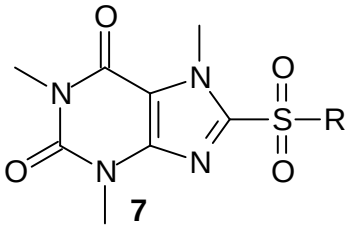

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Compound	R-Group	Compound	R-Group
3a	**–$(CH_2)_2$-(3-Cl-C_6H_4)**	**3b**	**–$(CH_2)_2$-(3-Br-C_6H_4)**
3c	**–$(CH_2)_2$-(3-CF_3-C_6H_4)**	**3d**	**–$(CH_2)_2$-(3-CH_3-C_6H_4)**
3e	**–$(CH_2)_2$-(3-OCH_3-C_6H_4)**	**4a**	**–$(CH_2)_3$-C_6H_5**
4b	**–$(CH_2)_3$-(3-Cl-C_6H_4)**	**4c**	**–$(CH_2)_3$-(4-Cl-C_6H_4)**
5a	**–CH_2-(3-Cl-C_6H_4)**	**5b**	**–CH_2-(3-Br-C_6H_4)**
6a	**–CH_2-C_6H_5**	**6b**	**–CH_2-(4-F-C_6H_4)**
7	**–$(CH_2)_2$-C_6H_5**		

MAO inhibition studies: The test inhibitors were evaluated as inhibitors of recombinant human MAO-A and –B. A fluorometric method was used to measure the inhibition potencies of the test inhibitors and the activities were expressed as IC_{50} values. Kynuramine was used as substrate for both MAO-A and –B. Kynuramine is non-fluorescent and undergoes MAO-catalyzed oxidation to yield 4-HQ as metabolite, which is fluorescent. The amount of the 4-HQ produced was measured using a fluorescence spectrophotometer at an excitation wavelength of 310 nm and an emission wavelength of 400 nm.

IC_{50} values: The results showed that the 8-[(phenylethyl)sulfanyl]caffeines (**3a**–**e**) are highly potent MAO-B inhibitors with all analogues exhibiting higher MAO-B inhibition potencies than the lead compound, 8-[(phenylethyl)sulfanyl]caffeine **2a**. The most potent inhibitor of this study, the 3-CF_3 substituted homologue (**3c**), had an IC_{50} value of 0.017 μM. Compounds **3a**–**e** are twofold to 13-fold more potent as MAO-B inhibitors than **2a**. The IC_{50} values recorded for **3a**–**e** ranged from 0.017 μM to 0.125 μM. Compounds **3a**–**e** also displayed higher inhibition potencies towards MAO-B than MAO-A. In fact, none of the analogues exhibited IC_{50} values in the submicromolar range for the inhibition of MAO-A.

The 8-(benzylsulfanyl)caffeines (**5a**–**b**) were also found to be potent inhibitors of MAO-B with IC_{50} values ranging from 0.199 μM to 0.227 μM. Compared to the 8-[(phenylethyl)sulfanyl]caffeine analogues (**3a**–**e**), the 8-(benzylsulfanyl)caffeines (**5a**–**b**), were, however, found to be significantly less potent as MAO-B inhibitors.

Interestingly, the 8-[(phenylpropyl)sulfanyl]caffeine analogues (**4a**–**c**) were also found to be highly potent MAO-B inhibitors with IC_{50} values ranging from 0.061 μM to 0.500 μM. These values are comparable to those of the 8-[(phenylethyl)sulfanyl]caffeines (**3a**–**e)**. The 8-[(phenylpropyl)sulfanyl]caffeine analogues (**4a**–**c)** were, however, less selective inhibitors of MAO-B. It was interesting to note that **4c**, the 4-Cl substituted 8-[(phenylpropyl)sulfanyl]caffeine analogue, was a potent MAO-A inhibitor with an IC_{50} value of 0.708 μM.

In contrast to the high MAO-B inhibition potencies of the 8-sulfanylcaffeines, the 8-sulfinylcaffeines (**6a**–**b**) and 8-sulfonylcaffeine (**7**) exhibited comparatively weak MAO-B inhibition. These compounds should thus not be further pursued for the design of potent MAO inhibitors.

Reversibility studies: The reversibility of inhibition of MAO-B by a selected representative 8-sulfanylcaffeine derivative, compound **3c**, was evaluated. The reversibility of MAO-B inhibition was investigated by measuring the degree of enzyme recovery after dilution of the enzyme-inhibitor complex.The results indicated that the MAO-B catalytic activities are partially recovered after dilution. After dilution of the enzyme-inhibitor complexes to 0.1 x IC_{50} and 1 x IC_{50}, respectively, the MAO-B catalytic activities were recovered to levels of approximately 35% and 22%, respectively. For reversible enzyme inhibition, the enzyme activities are expected to recover to levels of approximately 90% and 50%, respectively. These results show that while **3c** acts as a reversible inhibitor, the inhibition may be quasi-reversible with a possible tight-binding component.

Hansch-type structure activity relationship studies: A limited Hansch-type QSAR study was performed for the inhibition of MAO-B by the 8-[(phenylethyl)sulfanyl]caffeine analogues. The results showed that the MAO-B inhibition potencies of the 8-[(phenylethyl)sulfanyl]caffeine analogues correlated best with the Taft steric parameter (E_s). The R^2 (0.912) and statistical F (41.27) values suggested that the correlation is significant. The correlation suggests that MAO-B inhibition potency may be enhanced with placement of sterically bulky C3 substituents on the phenyl ring of 8-[(phenylethyl)sulfanyl]caffeine.

Perspective: Based on the potent MAO-B inhibitory properties of the 8-(phenylethyl)sulfanyl]caffeines (**3a**–**e**) and the 8-[(phenylpropyl)sulfanyl]caffeine analogues (**4a**–**c**), these compounds may be viewed as promising leads for the development of therapies for PD. These compounds are as potent, or even more potent than the reversible MAO-B selective inhibitor, lazabemide, which has an IC_{50} value of 0.091 µM for the inhibition of MAO-B. The 8-[(phenylpropyl)sulfanyl]caffeines (**4a**–**c)**, however, display lower degrees of selectivity for MAO-B than the corresponding 8-[(phenylethyl)sulfanyl]caffeines. For example, compounds **4a**–**c** exhibit SI values of 12–57, while the 8-[(phenylethyl)sulfanyl]caffeines display SI values of 132–8294. Although DA is metabolized by both MAO-A and –B in the human brain, the inhibition of MAO-A is associated with potentially dangerous side effects, and highly selective MAO-B inhibitors may therefore be more desirable for the treatment of PD. Based on this analysis, 8-[(phenylethyl)sulfanyl]caffeines may be better suited as antiparkinsonian drugs than 8-[(phenylpropyl)sulfanyl]caffeines, since several 8-[(phenylethyl)sulfanyl]caffeines are highly potent (IC_{50} < 0.05 µM) MAO-B inhibitors with SI values in excess of 100. The finding that a representative 8-[(phenylethyl)sulfanyl]caffeine (**3c**) interacts reversibly with MAO-B is also of significance. As discussed, reversibility is a desired characteristic of MAO inhibitors.