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Novel sulfanylphthalimide analogues as highly potent inhibitors of monoamine oxidase B

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ABSTRACT

Monoamine oxidase (MAO) plays an essential role in the catabolism of neurotransmitter amines. The two isoforms of this enzyme, MAO-A and -B, are considered to be drug targets for the therapy of depression and neurodegenerative diseases, respectively. Based on a recent report that the phthalimide moiety may be a useful scaffold for the design of potent MAO-B inhibitors, the present study examines a series of 5-sulfanylphthalimide analogues as potential inhibitors of both human MAO isoforms. The results document that 5-sulfanylphthalimides are highly potent and selective MAO-B inhibitors with all of the examined compounds possessing IC_{50} values in the nanomolar range. The most potent inhibitor, 5-(benzylsulfanyl)phthalimide, exhibits an IC_{50} value of 0.0045 μM for the inhibition of MAO-B with a 427-fold selectivity for MAO-B compared to MAO-A. We conclude that 5-sulfanylphthalimides represent an interesting class of MAO-B inhibitors and may serve as lead compounds for the design of antiparkinsonian therapy.

Monoamine oxidase A and B (MAO-A and -B) are flavine adenine dinucleotide (FAD) containing enzymes, bound to the outer membrane of mitochondria.[1,2] These enzymes catalyze the oxidative deamination of neurotransmitter and dietary amines thereby terminating their physiological actions.[3] Although MAO-A and -B share 70% sequence identity, they exhibit different substrate and inhibitor specificities.[4] MAO-A metabolizes serotonin, adrenaline and noradrenaline and inhibitors of this enzyme are in use for the treatment of clinical depression and anxiety.[5] MAO-B preferentially metabolizes the dietary amine, 2-phenylethylamine, and may therefore act as a metabolic brain barrier, limiting the entry of this false neurotransmitter into the central nervous system.[6,7] Since MAO-B also catabolyzes dopamine in the brain, inhibitors of this enzyme are used in the treatment of Parkinson's disease.[5,7,8] In Parkinson's disease, MAO-B inhibitors conserve the depleted supply of central dopamine and enhance dopamine levels following administration of levodopa, the metabolic precursor of dopamine.[9] For these reasons, MAO-B inhibitors are frequently combined with levodopa in Parkinson's disease therapy. It should be noted that MAO-A also metabolizes dopamine in the primate brain, and MAO-A inhibitors may consequently also elevate dopamine levels in the central nervous system.[9] In addition, MAO-A inhibitors may be employed to treat non-motor symptoms of Parkinson's disease such as depression and anxiety.[3,10] MAO-A inhibitors in conjunction with levodopa should, however, be used with caution since this may lead to a severe hypertensive response.[11]

A wide variety of heterocyclic moieties have been employed in the design of MAO inhibitors. Among these are isatin (**1**), an endogenous small molecule inhibitor of MAO-A and -B, and caffeine (**3**) (Fig. 1).[12–14] Phthalimide (**2**), an isomer of isatin, has also recently been reported to be a potentially useful scaffold for the design of MAO-B selective inhibitors.[15] Although phthalimide is a weak MAO inhibitor, substitution on C5 yields structures endowed with highly potent and selective MAO-B inhibitory activities. In contrast, N-substitution yields structures that are essentially devoid of MAO inhibitory properties.[15] The MAO-B inhibitory properties of isatin

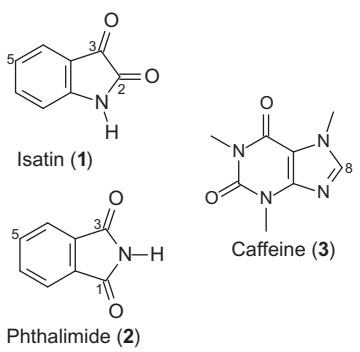

Figure 1. The structures of isatin (**1**), phthalimide (**2**) and caffeine (**3**).

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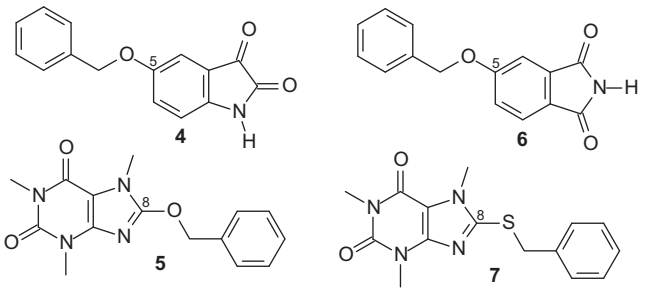
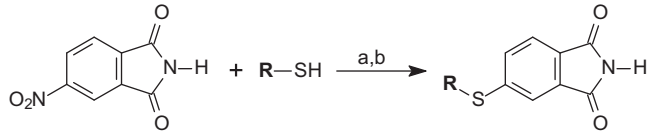


Figure 2. The structures of 5-benzyloxyisatin (**4**), 8-benzyloxycaffeine (**5**), 5-benzyloxyphthalimide (**6**) and 8-(benzylsulfanyl)caffeine (**7**).



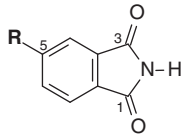
Scheme 1. Synthetic pathway to 5-sulfanylphthalimide analogues. Reagents and conditions: (a) K_2CO_3, acetone, reflux, 24 h; (b) HCl (6 N).

and caffeine may similarly be enhanced by substitution on the C5 and C6 positions of isatin and the C8 position of caffeine. In this regard, the benzyloxy substituent has been shown to be particularly favorable, and benzyloxy substitution of isatin, caffeine and phthalimide yields compounds **4**–**6** which are several orders of magnitude more potent MAO-B inhibitors than the parent compounds (Fig. 2).[13–15] In all instances, halogen substitution on the benzyloxy ring further improves inhibition potency. Modeling studies have shown that productive interactions of the benzyoxy side chain with the MAO-B entrance cavity may be responsible for this behavior. Interestingly, the benzylsulfanyl side chain appears to exhibit similar properties to that of the benzyloxy moiety, since a series of 8-(benzylsulfanyl)caffeine (**7**) analogues has recently been shown to exhibit similar MAO-B inhibition potencies to those of the 8-benzyloxycaffeine (**5**) analogues.[16] Based on these analyses, the present study examines the possibility that benzylsulfanyl substitution on C5 of phthalimide (to yield **8a**) would also lead to highly potent MAO-B inhibition. For this purpose, the effect that substitution (Cl, Br, F and OCH_3) on the benzylsulfanyl ring has on MAO inhibition will be explored. In addition, this study also determines the effect on MAO inhibition by the phenylsulfanyl, (2-phenylethyl)sulfanyl, cyclohexylsulfanyl and (3-methylbutyl)sulfanyl substituents. This study therefore aims to discover new highly potent MAO-B inhibitors and to contribute to the structure–activity relationships (SARs) of MAO inhibition by phthalimide derived compounds.

The 5-sulfanylphthalimides (**8a**–**k**) were conveniently synthesized according to a previously described protocol (Scheme 1).[17] The appropriate thiol reagents were reacted with 5-nitrophthalimide in the presence of K_2CO_3 to yield the target compounds in low to good yields (4–76%). The 5-sulfanylphthalimides were purified via crystallization from an appropriate solvent. In each instance, the structures and purities of the target compounds were verified by ^{1}H NMR, ^{13}C NMR, mass spectrometry and HPLC analysis as cited in the supplementary data. The presence of two ^{13}C NMR signals at 167.6–168.8 ppm, which corresponds to the carbonyl carbons at C1 and C3, and a ^{1}H NMR signal at 8.12–8.20 ppm ($CDCl_3$) or 11.28–11.36 ppm (DMSO-d_6), which corresponds to the phthalimide NH proton, confirmed that the presence of the phthalimide ring (Table 1).

To examine the MAO inhibitory properties of the 5-sulfanylphthalimides, recombinant human MAO-A and -B were employed.[18]

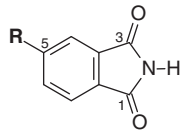
Table 1
The ^{1}H NMR and ^{13}C NMR chemical shifts for the NH proton and carbonyl C1 and C3 of phthalimide analogues **8a**–**k**



	R	NH	C1/C3	
8a	–S-(CH_2)-C_6H_5	11.28[a]	168.8[a]	168.8[a]
8b	–S-(CH_2)-(4-Cl-C_6H_4)	11.29[a]	168.8[a]	168.8[a]
8c	–S-(CH_2)-(4-Br-C_6H_4)	11.29[a]	168.7[a]	168.8[a]
8d	–S-(CH_2)-(4-F-C_6H_4)	11.29[a]	168.8[a]	168.8[a]
8e	–S-(CH_2)-(4-OCH_3-C_6H_4)	11.28[a]	168.8[a]	168.8[a]
8f	–S-C_6H_5	8.20[b]	167.7[b]	167.8[b]
8g	–S-(4-Cl-C_6H_4)	11.35 [a]	168.5[a]	168.6[a]
8h	–S-(4-Br-C_6H_4)	11.36 [a]	168.5[a]	168.6[a]
8i	–S-$(CH_2)_2$-C_6H_5	11.28[a]	168.8[a]	168.8[a]
8j	–S-C_6H_{11}	11.30 [a]	167.6[b]	167.8[b]
8k	–S-$(CH_2)_2$-$CH(CH_3)_2$	8.12[b]	167.9[b]	168.0[b]

[a] NMR experiments conducted in DMSO-d_6.
[b] NMR experiments conducted in $CDCl_3$.

Table 2
The IC_{50} values for the inhibition of recombinant human MAO-A and -B by 5-sulfanylphthalimides **8a**–**k**



	R	IC_{50} (μM)[a]		SI[b]
		MAO-A	MAO-B	
8a	–S-(CH_2)-C_6H_5	1.92 ± 0.172	0.0045 ± 0.0004	427
8b	–S-(CH_2)-(4-Cl-C_6H_4)	0.506 ± 0.032	0.0056 ± 0.0003	90
8c	–S-(CH_2)-(4-Br-C_6H_4)	0.273 ± 0.041	0.0074 ± 0.0029	37
8d	–S-(CH_2)-(4-F-C_6H_4)	0.958 ± 0.003	0.0068 ± 0.0009	141
8e	–S-(CH_2)-(4-OCH_3-C_6H_4)	1.63 ± 0.023	0.020 ± 0.0042	82
8f	–S-C_6H_5	8.03 ± 0.622	0.986 ± 0.026	8.1
8g	–S-(4-Cl-C_6H_4)	1.68 ± 0.343	0.457 ± 0.093	3.7
8h	–S-(4-Br-C_6H_4)	1.01 ± 0.053	0.364 ± 0.050	2.8
8i	–S-$(CH_2)_2$-C_6H_5	2.27 ± 0.136	0.030 ± 0.0094	76
8j	–S-C_6H_{11}	1.03 ± 0.062	0.179 ± 0.0082	5.8
8k	–S-$(CH_2)_2$-$CH(CH_3)_2$	0.380 ± 0.042	0.015 ± 0.0033	26

[a] All values are expressed as the mean ± SD of triplicate determinations.
[b] The selectivity index is the selectivity for the MAO-B isoform and is given as the ratio of [IC_{50}(MAO-A)]/[IC_{50}(MAO-B)].

The enzyme catalytic activities in the absence and presence of the test inhibitors were determined by fluorometrically measuring the MAO-catalyzed formation of 4-hydroxyquinoline from the mixed MAO-A/B substrate, kynuramine.[14,18] This approach was suitable for evaluating the MAO inhibitory properties of all the 5-sulfanylphthalimides. The inhibition potencies of the test compounds were calculated from the sigmoidal dose–response curves and are expressed as the corresponding IC_{50} values. These IC_{50} values as well as the selectivity index (SI) values [SI = IC_{50}(MAO-A)/(IC_{50}(MAO-B)] are given in Table 2. The results document that all of the 5-sulfanylphthalimides are potent inhibitors of human MAO-B with IC_{50} values ranging from 0.0045 to 0.986 μM. In accordance with expectation (see introduction), benzylsulfanyl substitution of phthalimide to yield **8a**, resulted in highly potent MAO-B inhibition. An IC_{50} value of 0.0045 μM was recorded for **8a**. In fact **8a** proved to be the most potent MAO-B inhibitor among the compounds of the present series. Compared to its C5 benzyloxy

substituted phthalimide homologue, compound **6** (IC_{50} = 0.043 μM), compound **8a** is approximately eightfold more potent as a MAO-B inhibitor.[15] Compound **8a** also proved to be a more potent MAO-B inhibitor than 5-benzyloxyisatin (**4**) (IC_{50} = 0.103 μM),[13] 8-benzyloxycaffeine (**5**) (IC_{50} = 1.77 μM)[14] and 8-(benzylsulfanyl)caffeine (**7**) (IC_{50} = 1.86 μM).[16] Substitution on the benzylsulfanyl ring with Cl, Br, F and OCH_3, to yield **8b**–**e**, also resulted in highly potent inhibition, with these homologues exhibiting IC_{50} values of 0.0056–0.020 μM. It is interesting to note that particularly the halogen substituted homologues **8b**–**d** are weaker MAO-B inhibitors than compound **8a**. This is in contrast to the results obtained with 8-benzyloxycaffeine (**5**), 5-benzyloxyphthalimide (**6**) and 8-(benzylsulfanyl)caffeine (**7**), where particularly bromine substitution on the phenyl ring leads to an enhancement in MAO-B inhibitory potency.[14–16] From these results it may therefore be concluded that 5-(benzylsulfanyl)phthalimides are highly potent MAO-B inhibitors and superior to the lead structures, compounds **4**–**7**, of this study.

Although still considered as a potent MAO-B inhibitor, the phenylsulfanyl homologue **8f** (IC_{50} = 0.986 μM) was the weakest inhibitor of the present series. In this case, however, halogen substitution in the phenyl ring to yield compounds **8g** (IC_{50} = 0.457 μM) and **8h** (IC_{50} = 0.364 μM), resulted in enhanced MAO-B inhibition. The (2-phenylethyl)sulfanyl substituted homologue **8i** was also found to be a potent MAO-B inhibitor with an IC_{50} value of 0.030 μM, approximately sixfold weaker than **8a**. Since both **8f** and **8i** are weaker MAO-B inhibitor than **8a**, it may be concluded that the benzylsulfanyl side chain is particularly suitable for enhancing the MAO-B inhibitory potency of phthalimide, and that neither a reduction in side chain length (to yield **8f**), nor an increase in chain length (to yield **8i**) would further increase inhibitory activity. The general suitability of 5-sulfanylphthalimides for MAO-B inhibition was further demonstrated with the finding that the cyclohexylsulfanyl (**8j**) and (3-methylbutyl)sulfanyl (**8k**) substituted homologues are also potent MAO-B inhibitors with IC_{50} values of 0.179 μM and 0.015 μM, respectively.

The 5-sulfanylphthalimides were found to also act as inhibitors of MAO-A. Four homologues, **8b**–**d** and **8k**, exhibited IC_{50} values in the nanomolar range, with **8c** being the most potent MAO-A inhibitor with an IC_{50} value of 0.273 μM. As evident from the SI values, all of the 5-sulfanylphthalimides were, however, selective inhibitors of the MAO-B isoform. The most potent MAO-B inhibitor of the series, **8a**, is also the most selective inhibitor with an SI value of 427. This compound may therefore be considered the most suitable homologue of the series where MAO-B selectivity is desired. As already noted, MAO-A inhibition may lead to adverse effects when combined with certain antiparkinsonian therapies. In contrast, **8c** may be considered as a potent MAO inhibitor with comparatively low isoform selectivity.

Since it is reported that the benzyloxyphthalimide class of MAO inhibitors interacts reversibly with MAO, the current study examined if this property is also shared by sulfanylphthalimides.[15] For this purpose, the reversibility of MAO-B inhibition by the most potent sulfanylphthalimide MAO-B inhibitor, compound **8a**, was investigated by measuring the degree of enzyme recovery after dilution of the enzyme-inhibitor complex. MAO-B was preincubated with **8a** at concentrations of $10 \times IC_{50}$ and $100 \times IC_{50}$ for 30 min and then diluted to $0.1 \times IC_{50}$ and $1 \times IC_{50}$, respectively.[19] The results show that after dilution of the enzyme-inhibitor complexes to concentrations of **8a** equal to $0.1 \times IC_{50}$ and $1 \times IC_{50}$, the MAO-B catalytic activities are recovered to levels of approximately 72% and 28%, respectively, of the control value (Fig. 3). This behavior is consistent with a reversible interaction of **8a** with MAO-B. In contrast, incubation of MAO-B with the irreversible inhibitor (R)-deprenyl

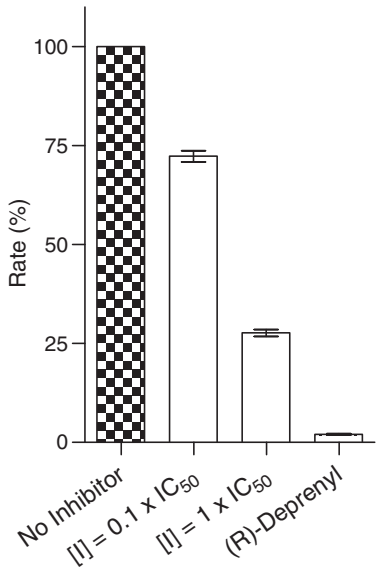


Figure 3. The reversibility of the inhibition of MAO-B by **8a**. The enzyme was preincubated with **8a** at $10 \times IC_{50}$ and $100 \times IC_{50}$ for 30 min and then diluted to $0.1 \times IC_{50}$ and $1 \times IC_{50}$, respectively. For comparison, (R)-deprenyl, at $10 \times IC_{50}$ was similarly incubated with MAO-B and diluted to $0.1 \times IC_{50}$. The residual enzyme activities were subsequently measured.

($10 \times IC_{50}$), the MAO-B activities were not recovered (2.1% of control). Interestingly, after dilution of the **8a**–MAO-B complex to $0.1 \times IC_{50}$ and $1 \times IC_{50}$, the enzyme activities are not recovered to 90% and 50%, respectively, as expected. This result suggests that, for the inhibition of MAO-B, **8a** may possess a quasi-reversible or tight-binding component.

In conclusion, the present study shows that 5-sulfanylphthalimides are potent and selective inhibitors of MAO-B. In this regard, the benzylsulfanyl side chain is particularly suitable for enhancing the MAO-B inhibitory potency of phthalimide. It is noteworthy that compound **8a** (IC_{50} = 0.0045 μM) is approximately 30,000-fold more potent than phthalimide (**2**) (IC_{50} = 134 μM).[15] This illustrates the importance of the C5 side chain for MAO-B inhibitory activity. Based on their MAO-B inhibition potencies and appropriate selectivity profiles, this study concludes that 5-sulfanylphthalimides are suitable lead compounds for the development of antiparkinsonian drugs. From a design point of view it is noteworthy that a wide variety of C5 substituents yield 5-sulfanylphthalimides with potent MAO-B inhibitory actions. This suggests that structural modifications made to the C5 side chain in order to improve the properties of the compound are less likely to reduce MAO-B inhibition potency.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.08.113.

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