Bioorganic & Medicinal Chemistry Letters 22 (2012) 6632–6635

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Novel sulfanylphthalimide analogues as highly potent inhibitors of monoamine oxidase B

Mietha M. Van der Walt[a], Gisella Terre'Blanche[a], Anél Petzer[b], Jacobus P. Petzer[a,*]

[a] Pharmaceutical Chemistry, School of Pharmacy, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa
[b] Unit for Drug Research and Development, School of Pharmacy, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

ARTICLE INFO

Article history:
Received 12 July 2012
Revised 16 August 2012
Accepted 28 August 2012
Available online 7 September 2012

Keywords:
Isatin
Phthalimide
Sulfanylphthalimide
Monoamine oxidase
MAO
Inhibition

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

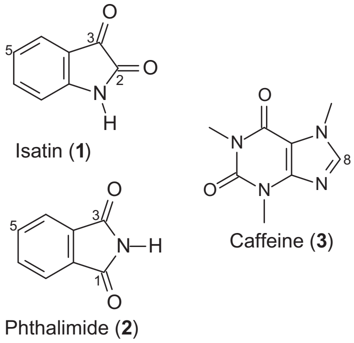


Figure 1. The structures of isatin (**1**), phthalimide (**2**) and caffeine (**3**).

---

[*] Corresponding author. Tel.: +27 18 2992206; fax: +27 18 2994243.
E-mail address: jacques.petzer@nwu.ac.za (J.P. Petzer).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.08.113

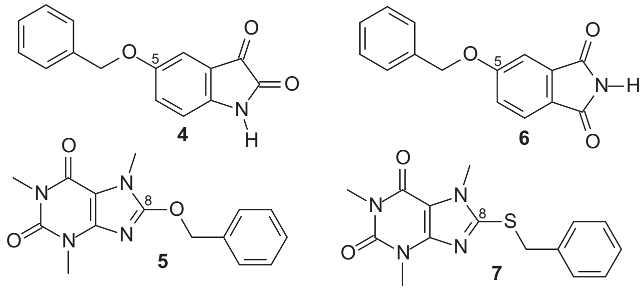
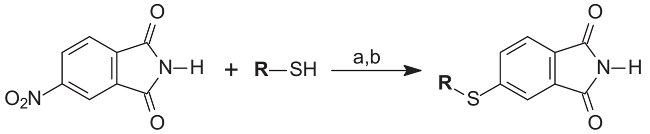


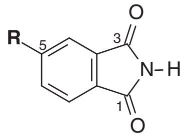
Figure 2. The structures of 5-benzyloxyisatin (**4**), 8-benzyloxycaffeine (**5**), 5-benzyloxyphthalimide (**6**) and 8-(benzylsulfanyl)caffeine (**7**).



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

Table 1

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



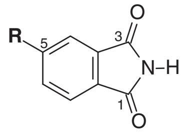
	R	NH	C1/C3	
8a	$-S-(CH_2)-C_6H_5$	11.28[a]	168.8[a]	168.8[a]
8b	$-S-(CH_2)-(4-Cl-C_6H_4)$	11.29[a]	168.8[a]	168.8[a]
8c	$-S-(CH_2)-(4-Br-C_6H_4)$	11.29[a]	168.7[a]	168.8[a]
8d	$-S-(CH_2)-(4-F-C_6H_4)$	11.29[a]	168.8[a]	168.8[a]
8e	$-S-(CH_2)-(4-OCH_3-C_6H_4)$	11.28[a]	168.8[a]	168.8[a]
8f	$-S-C_6H_5$	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 $[IC_{50}(MAO-A)]/[IC_{50}(MAO-B)]$.

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 ^1H 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 ^1H 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] 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