Bioorganic & Medicinal Chemistry Letters 26 (2016) 1200–1204

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Inhibition of monoamine oxidase by benzoxathiolone analogues

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ARTICLE INFO

Article history:
Received 17 November 2015
Revised 12 January 2016
Accepted 14 January 2016
Available online 16 January 2016

Keywords:
Monoamine oxidase
MAO
Inhibition
Reversible
Benzoxathiolone

ABSTRACT

Inhibitors of the monoamine oxidase (MAO) enzymes are considered useful therapeutic agents, and are used in the clinic for the treatment of depressive illness and Parkinson's disease. In addition, MAO inhibitors are also under investigation for the treatment of certain cardiovascular pathologies and as possible aids to smoking cessation. In an attempt to discover novel classes of compounds that inhibit the MAOs, the current study examines the human MAO inhibitory properties of a small series of 2*H*-1,3-benzoxathiol-2-one analogues. The results show that the benzoxathiolones are potent MAO-B inhibitors with IC_{50} values ranging from 0.003 to 0.051 μM. Although the benzoxathiolones are selective for the MAO-B isoform, two compounds display good MAO-A inhibition with IC_{50} values of 0.189 and 0.424 μM. Dialysis studies show that a selected compound inhibits the MAOs reversibly. It may thus be concluded that the benzoxathiolone class is suitable for the design and development of MAO-B inhibitors, and that in some instances good MAO-A inhibition may also be achieved.

The monoamine oxidase (MAO) enzymes are responsible for the oxidation of a variety of amine substrates.[1,2] MAO consists of two isoforms, MAO-A and MAO-B, which share overall similar structures and possess covalently attached flavin adenine dinucleotide (FAD) cofactors.[3] Substrate oxidation occurs by two half reactions, the reductive half reaction where the FAD cofactor is reduced when accepting two electrons from the substrate amine, and an oxidative half reaction where the reduced FAD is reoxidised by molecular oxygen to yield hydrogen peroxide as by-product.[4–6] In most instances, the product of amine oxidation, the corresponding imine, is hydrolysed to yield an aldehyde. Since the MAOs metabolise neurotransmitter amines, they have become targets for the treatment of neuropsychiatric and neurodegenerative disorders.[3] Drugs that inhibit MAO-A are established antidepressants, and are thought to act by elevating central serotonin levels.[7,8] Examples of clinically used agents are phenelzine (**1**), isocarboxazid (**2**), tranylcypromine (**3**) and iproniazid (**4**) (Fig. 1). Phenelzine and iproniazid (now discontinued) are non-selective MAO inhibitors. Drugs that inhibit the MAO-B isoform, in turn, are used in the treatment of Parkinson's disease, often in combination with L-Dopa.[9] Since MAO-B metabolise dopamine in the brain, inhibitors are thought to enhance dopamine levels, particularly following therapy with L-Dopa.[10,11] Examples of clinically used agents are (*R*)-deprenyl (**5**) and rasagiline (**6**). MAO-B inhibitors are also thought to protect against neurodegeneration in Parkinson's disease. In this respect, central inhibition of MAO-B reduces the formation of hydrogen peroxide and aldehydes, species which may cause neuronal injury if not adequately cleared from the brain.[1] This risk may be particularly important in the aged brain where MAO-B activity is significantly increased.[12]

The MAOs have also attracted attention as targets for the development of therapy for Alzheimer's disease. Laboratory evidence suggests that MAO inhibitors improve cognitive deficits and reverse amyloid β peptide (Aβ) pathology.[13] In cardiovascular pathophysiology, MAO inhibitors may reduce the formation of hydrogen peroxide and thus improve cardiac function in patients with congestive heart failure.[14] MAO-A appears to be the relevant isoform here since cardiac MAO-A activity and hydrogen peroxide generated by MAO-A show an age-dependent increase in the hearts of rats. MAO-A could be a major source of hydrogen peroxide in the ageing heart, and thus a contributor to cardiac cellular degeneration.[15] Another potential application of MAO inhibitors, particularly MAO-B inhibitors, is the possible treatment of smoking cessation. This is based on reports that MAO-B activity is reduced in smokers, and by increasing synaptic monoamines, MAO-B inhibitors may mimic the effects of smoking, at least in part.[16] Interestingly, MAO-A levels are reported to be elevated in certain types of cancer tissue such as prostate cancer, and MAO-A inhibition may, in synergism with survivin suppressants, inhibit cancer cell growth, migration and invasion.[17,18]

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http://dx.doi.org/10.1016/j.bmcl.2016.01.034
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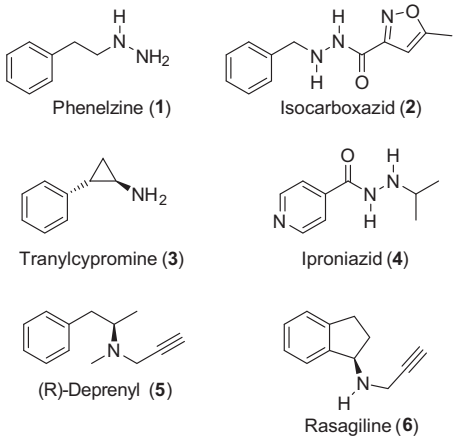


Figure 1. The structures of known MAO inhibitors.

Based on the current therapeutic value and future potential of MAO inhibitors, the discovery of new classes of compounds that inhibit the MAOs are justified. In the present study we have investigated a small series of 2*H*-1,3-benzoxathiol-2-one analogues (**7**) as potential human MAO inhibitors (Fig. 2). These compounds are structurally related to a number of heterocycles that have been found in previous studies to be MAO inhibitors. For example, both 5-benzyloxyisatin (**8**) and 5-benzyloxyphthalimide (**9**) are potent and selective (over the MAO-A isoform) inhibitors of human MAO-B.[19,20] A recent study has reported that 3-coumaranone derivatives, such as compound **10**, also are potent MAO-B inhibitors.[21] The benzyloxy side chains of these heterocycles appear to be required for MAO inhibition since the removal thereof results is significant reduction or abolishment of MAO inhibition. The first benzoxathiolone analogue of the present study, **7a**, has thus been substituted on the C6 position with the benzyloxy moiety (Table 1). Since substitution on the benzyloxy ring frequently enhances MAO inhibition we have included the chlorine and methyl substituted homologues, **7b–d**. The effect of side chain elongation was investigated with phenylpropoxy substitution (**7e**). Although the series is limited, the objective was to determine if the benzoxathiolone class of compounds may possess MAO inhibition activity. This study will also investigate the reversibility of MAO inhibition by selected benzoxathiolones. For MAO-A inhibition, reversibility is an important consideration since irreversible acting MAO-A inhibitors are associated with a potentially fatal

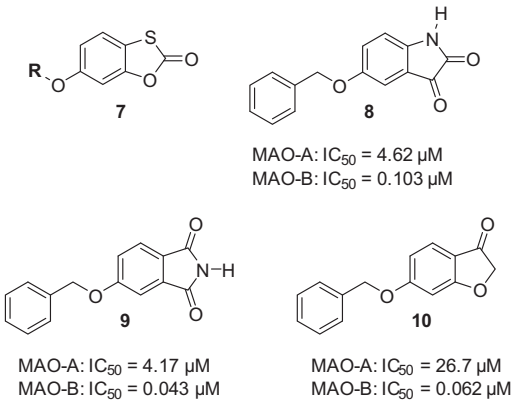
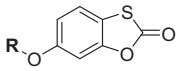


Figure 2. The structures of the 2*H*-1,3-benzoxathiol-2-one analogues (**7**) that will be investigated in this study as well as those of the lead compounds, 5-benzyloxyisatin (**8**), 5-benzyloxyphthalimide (**9**) and 3-coumaranone derivative **10**.[19–21]

Table 1
The IC_{50} values for the inhibition of recombinant human MAO-A and MAO-B by 2*H*-1,3-benzoxathiol-2-one analogues, **7a–e**



	R	IC_{50} (μM)[a]		SI[b]
		MAO-A	MAO-B	
7a	$C_6H_5CH_2$–	5.14 ± 0.928	0.051 ± 0.011	101
7b	3-$ClC_6H_4CH_2$–	0.424 ± 0.092	0.004 ± 0.0003	106
7c	4-$ClC_6H_4CH_2$–	0.189 ± 0.012	0.003 ± 0.001	63
7d	4-$CH_3C_6H_4CH_2$–	2.55 ± 0.258	0.005 ± 0.001	510
7e	$C_6H_5(CH_2)_3$–	21.3 ± 0.826	0.033 ± 0.006	645
11	H–	9.77 ± 0.480	9.57 ± 1.07	1

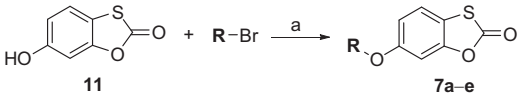
[a] All values are expressed as the mean ± standard deviation (SD) of triplicate determinations.
[b] The selectivity index is the selectivity for the MAO-B isoform and is given as the ratio of IC_{50}(MAO-A)/IC_{50}(MAO-B).

increase in blood pressure when taken with certain foods and can only be used in the clinic if dietary restrictions are followed.[22] Reversible MAO-A inhibitors, on the other hand, appear to be safe in this regard.[23,24]

The benzoxathiolone analogues were synthesized in poor to fair yields (4–53%) by reacting commercially available 6-hydroxy-1,3-benzoxathiol-2-one (**11**) with an appropriate substituted arylalkyl bromide in acetone (Scheme 1). Potassium carbonate served as base. The reactions were carried out at 60 °C for 5–24 h and monitored with thin-layer chromatography (TLC). After completion, ethyl acetate was added to the reaction and the resulting mixture was washed with water and brine. The crude obtained after evaporation of the organic phase was purified by recrystallization. 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 MAO inhibitory properties of the benzoxathiolone analogues **7a–e** were evaluated using the recombinant human MAO-A and MAO-B enzymes. Inhibition potencies are expressed as the IC_{50} values, which were determined from sigmoidal plots of residual enzyme activity versus the logarithm of inhibitor concentration. Residual enzyme activity, in turn, was measured by using kynuramine as substrate for both MAO isoforms. Kynuramine is oxidised by the MAOs to yield 4-hydroxyquinoline, which was quantified by fluorescence spectrophotometry.[25,26] Figure 3 provides examples of sigmoidal plots obtained for the inhibition of the MAOs by compound **7c**.

The inhibition potencies of the benzoxathiolone analogues are given in Table 1. From the results it is evident that these compounds are selective for MAO-B over the MAO-A isoform. In this respect selectivity index (SI) values range from 63 to 645. All compounds may be viewed as potent MAO-B inhibitors with IC_{50} values <0.051 μM. For comparison, the known MAO-B inhibitor, lazabemide (**12**; IC_{50} = 0.091 μM), is a weaker inhibitor than the benzoxathiolones examined here, while the reversible MAO-B inhibitor safinamide (**13**; IC_{50} = 0.048 μM) is approximately equipotent to **7a** (Fig. 4).[27] It should be noted that the IC_{50} values of the reference inhibitors have been measured and reported in previous



Scheme 1. Synthetic route to the 2*H*-1,3-benzoxathiol-2-one analogues, **7a–e**. Reagents and conditions: (a) acetone, K_2CO_3, 60 °C, 5–24 h.

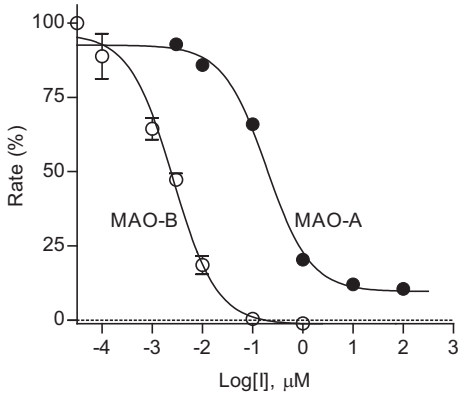


Figure 3. The sigmoidal plots for the inhibition of MAO-A (filled circles) and MAO-B (open circles) by **7c**.

studies employing identical experimental conditions to the current study. Although weaker as MAO-A inhibitors, two compounds display good MAO-A inhibition. These are **7b** and **7c** with IC_{50} values of 0.424 μM and 0.189 μM, respectively. For comparison, the clinically used reversible MAO-A inhibitor, toloxatone (**14**), inhibits MAO-A with an IC_{50} value of 3.92 μM.[27] These compounds as well as **7d** (IC_{50} = 2.55 μM) are thus more potent MAO-A inhibitors than toloxatone. This study also finds that 6-hydroxy-1,3-benzoxathiol-2-one (**11**) is a weak MAO-B inhibitor with an IC_{50} of 9.57 μM. This shows that the C6 substituent is indeed a requirement for potent MAO-B inhibition by the benzoxathiolone analogues, and is in agreement with similar findings with other heterocycles, such as isatins (e.g., **8**) and phthalimides (e.g., **9**), for which an appropriate side chain also is a requirement for MAO-B inhibition.[19,20] Based on the inhibition potencies it may thus be concluded that, similar to the isatins and phthalimides reported previously, benzoxathiolones represent a class of highly potent MAO-B inhibitors. When comparing **7a** with the corresponding isatin and phthalimide homologues, it is evident that **7a** is a more potent MAO-B inhibitor than 5-benzyloxyisatin (**8**; IC_{50} = 0.103 μM) and equipotent to 5-benzyloxyphthalimide (**9**; IC_{50} = 0.043 μM).[19,20] The results also show that with the appropriate substitution good MAO-A inhibition may be obtained with the benzoxathiolones. Good MAO-A inhibitors also exist among the reported isatins and phthalimides as exemplified by **15** (IC_{50} = 0.233 μM) and **16** (IC_{50} = 0.22 μM) (Fig. 5).[19,20]

As mentioned, reversibility of MAO inhibition is an important consideration in inhibitor design. This study thus investigates the reversibility of MAO-A and MAO-B inhibition by a selected benzoxathiolone analogue, compound **7c**. To examine the reversibility of inhibition, the MAO enzymes and test inhibitor (at a concentration of 4 × IC_{50}) were combined for 15 min and subsequently dialysed for 24 h. As negative control, similar dialysis of the MAOs in absence of inhibitor was carried out. As positive controls, the MAOs

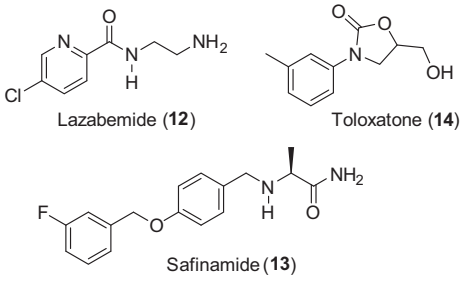


Figure 4. The structures of reference MAO inhibitors.

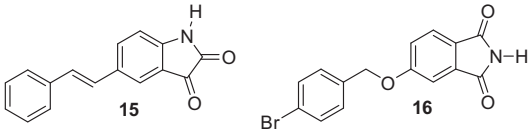


Figure 5. The structures of isatin (**15**) and phthalimide (**16**) analogues, both reported MAO inhibitors.[19,20]

were combined with the irreversible inhibitors, pargyline (MAO-A) and (*R*)-deprenyl (MAO-B), and dialysed. For reversible inhibitors, enzyme activity is expected to recover to 100% of the negative control value following dialysis. For irreversible inhibition, enzyme activity is not recovered by dialysis. The results are given in Figure 6, and show that, upon inhibition by **7c**, both MAO-A and MAO-B activities are recovered by dialysis to 93% and 78%, respectively. The MAO activities in undialysed mixtures of the MAOs and **7c**, in contrast are 73% and 39%, respectively. This result show that **7c** is a reversible inhibitor of both MAO isoforms. As expected, after dialysis of mixtures of the MAOs and the irreversible inhibitors, pargyline and (*R*)-deprenyl, enzyme activity is not recovered with MAO-A and MAO-B at 0.9% and 4.8%, respectively, of the negative control.

To investigate the mode (e.g., competitive) of MAO-A and MAO-B inhibition by **7c**, Lineweaver–Burk plots were constructed. For each MAO isoform a set consisting of six Lineweaver–Burk plots was constructed by measuring the enzyme activities in the presence of the following inhibitor concentrations: 0 μM, ¼ × IC_{50}, ½ × IC_{50}, ¾ × IC_{50}, 1 × IC_{50} and 1¼ × IC_{50}. For each plot, eight different substrate (kynuramine) concentrations were used, ranging from 15 to 250 μM. The results are given in Figure 7 and show that, for both MAO-A and MAO-B inhibition, the lines are linear and intersect on the *y*-axis. This is in agreement with a competitive mode of inhibition of both MAO-A and MAO-B by **7c**. By plotting the slopes of the Lineweaver–Burk plots versus the inhibitor concentration, K_i values of 0.36 μM and 0.0035 μM for the inhibition of MAO-A and MAO-B, respectively, are estimated. The K_i values can also be determined by global (shared) fitting of the inhibition data directly to the Michaelis–Menten equation. This yielded similar results with K_i values of 0.56 ± 0.07 μM (R^2 = 0.99) and 0.0039 ± 0.0005 μM (R^2 = 0.98) μM for the inhibition of MAO-A and MAO-B, respectively.

Since benzoxathiolone analogues show good promise as MAO inhibitors, selected properties were measured for the series as part of a preliminary investigation of the suitability of these compounds as drugs. For this purpose, the lipophilicity (log*P*) and aqueous solubility of the compounds were experimentally determined and are listed in Table 2. Log *P* was determined with the shake-flask method and represents the partitioning of the test compounds between *n*-octanol and water. The log*P* value, in general, provides an estimation of how readily a compound may diffuse passively across the lipid bilayer, an important process for absorption from the gastrointestinal tract and permeation across the blood–brain barrier. It is generally accepted that, for a compound to display good oral bioavailability and blood–brain barrier permeation, the log*P* value should be in a range of 0–3.[28] The log*P* values of the benzoxathiolone analogues are 3.80–5.61, indicating that a high degree of lipophilicity may be a concern, particularly from a solubility point of view. Indeed, the aqueous solubilities are <0.081 μM and may be considered to be in the very weak solubility range. To improve the properties of the benzoxathiolone analogues, this study recommends that in future studies polar, and possibly ionisable groups should by incorporated in the C6 side chain to reduce lipophilicity and improve solubility. This would yield compounds

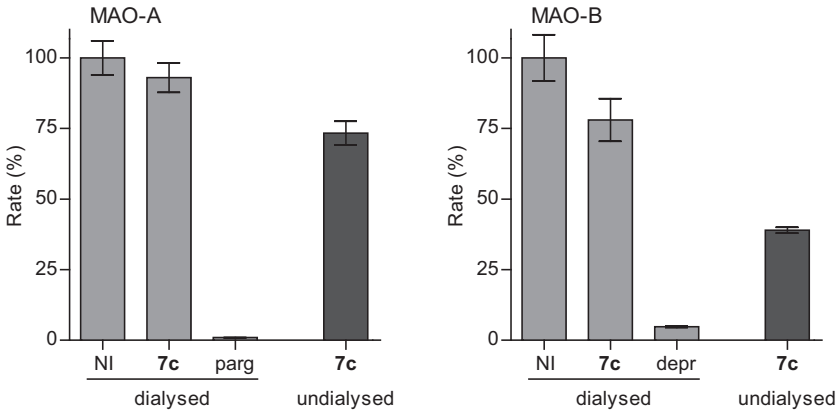


Figure 6. Dialysis reverses MAO-A and MAO-B inhibition by **7c**. The MAO enzymes and **7c** (at a concentration of 4 × IC_{50}) were combined for 15 min, dialysed for 24 h and the residual enzyme activity was measured (**7c** dialysed). Similar incubation and dialysis of the MAOs in the absence (NI dialysed) and presence of the irreversible inhibitors, pargyline (parg dialysed) and (*R*)-deprenyl (depr dialysed) were also carried out. The residual MAO activity of undialysed mixtures of the MAOs with **7c** was also recorded (**7c** undialysed).

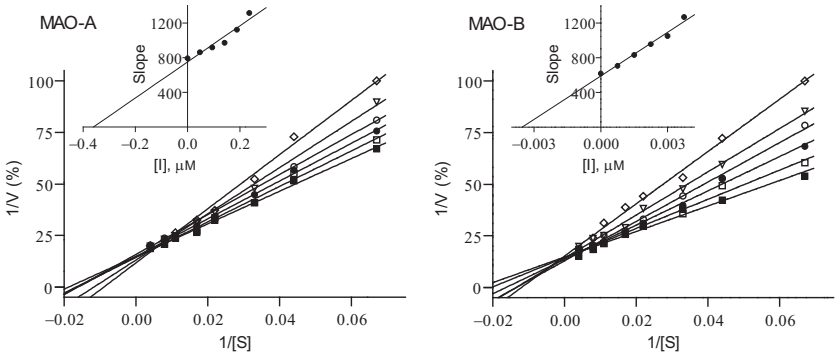


Figure 7. Lineweaver–Burk plots for the inhibition of human MAO-A and MAO-B by **7c**. The insets are plots of the slopes of the Lineweaver–Burk plots versus inhibitor concentration.

Table 2
The log*P* values and aqueous solubilities of 2*H*-1,3-benzoxathiol-2-one analogues, **7a**–**e**

	Log*P*	Solubility (μM)
7a	3.80 ± 0.03	0.064 ± 0.066
7b	4.72 ± 0.12	0.081 ± 0.015
7c	5.61 ± 0.24	0.072 ± 0.013
7d	4.54 ± 0.25	0.051 ± 0.005
7e	4.70 ± 0.17	0.013 ± 0.006

All values are expressed as the mean ± SD of triplicate determinations.

with improved properties. The effect of polar groups on MAO inhibition, however, remains to be determined.

In conclusion, the present study shows that a small series of 2*H*-1,3-benzoxathiol-2-one analogues are potent human MAO-B inhibitors, and may thus act as leads for the future design for therapies for disorders such as Parkinson's disease. The physicochemical properties of these compounds, however, are a concern and the high degree of lipophilicity and low aqueous solubilities require optimisation. Two of the 2*H*-1,3-benzoxathiol-2-one analogues also are relatively potent human MAO-A inhibitors, and may thus be suitable as leads for drugs aimed at the treatment of depression, certain cardiovascular pathologies and certain types of cancer. In this regard, the observation that a selected analogue is a reversible MAO-A inhibitor is of significance. Reversible MAO-A inhibitors are not likely to cause tyramine-induced changes in blood pressure and have better safety profiles than irreversible MAO-A inhibitors.[23,24] Compounds with dual MAO-A/B inhibition properties may be particularly relevant where depression is a comorbidity of Parkinson's disease.

Acknowledgements

The NMR and MS spectra were recorded by André Joubert and Johan Jordaan of the SASOL Centre for Chemistry, North-West University. This work is based on the research supported in part by the Medical Research Council and National Research Foundation of South Africa (Grant specific unique reference numbers (UID) 85642, 96180). The Grantholders acknowledge that opinions, findings and conclusions or recommendations expressed in any publication generated by the NRF supported research are that of the authors, and that the NRF accepts no liability whatsoever in this regard.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.01.034.

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