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Inhibition of monoamine oxidase by benzoxathiolone analogues

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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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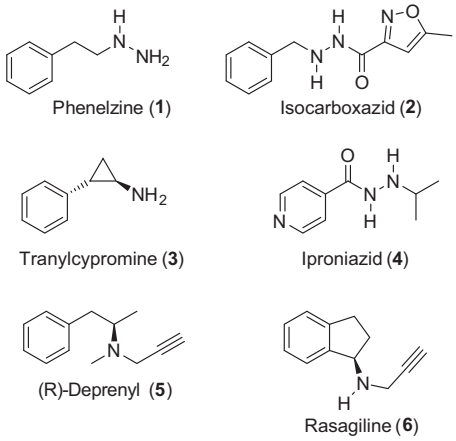


Figure 1. The structures of known MAO inhibitors.

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

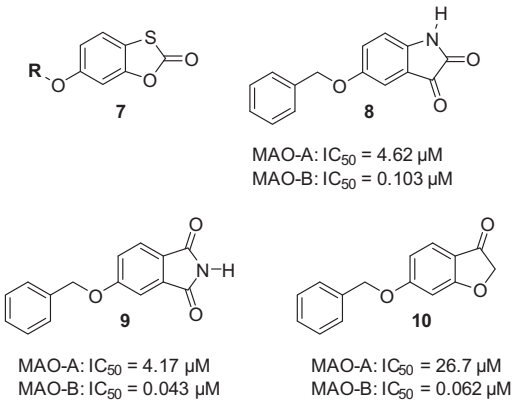
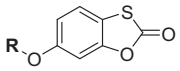


Figure 2. The structures of the 2*H*-1,3-benzoxathiol-2-one analogues (**7**) that will be investigated in this study as well as those of the lead compounds, 5-benzyloxyisatin (**8**), 5-benzyloxyphthalimide (**9**) and 3-coumaranone derivative **10**.[19–21]

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

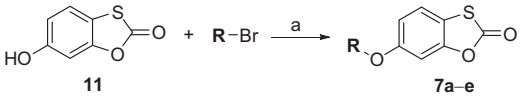
[a] All values are expressed as the mean ± standard deviation (SD) of triplicate determinations.
[b] The selectivity index is the selectivity for the MAO-B isoform and is given as the ratio of IC_{50}(MAO-A)/IC_{50}(MAO-B).

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.