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Inhibition of monoamine oxidase by indole-5,6-dicarbonitrile derivatives

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ABSTRACT

Recent studies have found that phthalonitrile derivatives are remarkably potent inhibitors of human monoamine oxidase (MAO) A and B. In an attempt to further determine the structure-activity relationships (SARs) for MAO inhibition by this class of compounds and to discover novel potent MAO inhibitors, the present study investigated the MAO inhibition properties of a series consisting of indole-5,6-dicarbonitrile derivatives. The results document that 3-chloro-1*H*-indole-5,6-dicarbonitrile derivatives exhibited potent inhibition of the MAOs. For example, 3-chloro-2-(4-methylphenyl)-1*H*-indole-5,6-dicarbonitrile inhibited MAO-A and MAO-B with IC_{50} values of 0.014 μM and 0.017 μM, respectively. It was further shown that this compound acts as a reversible and competitive inhibitor of both MAO isoforms. An analysis of the SARs for MAO inhibition by 3-chloro-1*H*-indole-5,6-dicarbonitriles showed that methylation of the indole nitrogen eliminates MAO-B inhibition activity, and replacement of the 2-phenyl ring with the thienyl results in a 9-fold reduction of MAO-B inhibition activity. A series of 3-bromo-1-hydroxy-1*H*-indole-5,6-dicarbonitriles are, in turn, comparatively weaker MAO inhibitors. It may be concluded that indole-5,6-dicarbonitrile derivatives are suitable leads for the design MAO inhibitors for the treatment of disorders such as Parkinson's disease and depression.

The monoamine oxidase (MAO) enzymes play vital roles in several biochemical processes and have thus been drug targets for several decades.[1,2] The biological role of the MAOs is to metabolise neurotransmitter amines in the peripheral and central tissues, and thereby terminating their physiological actions.[3] The MAOs oxidise amine substrates by a 2-electron process at the α-carbon to yield an imine product. For most substrates, the imine products are subsequently hydrolysed non-enzymatically to yield the corresponding aldehyde.[4] The MAOs consist of two distinctive isoforms, MAO-A and MAO-B, which are products of two different but related genes. As a result, the two isoforms are approximately 70% identical with respect to their amino acid sequences, and overlapping substrate specificities are frequently observed.[5] For example, dopamine, tyramine and norepinephrine are equally well metabolised by the two MAO isoforms while serotonin is considered to be MAO-A selective substrate. The dietary amines, benzylamine and phenylethylamine are, in turn, selectively metabolised by the MAO-B isoform.[6]

The pharmacological importance of the MAOs is based on their roles in the breakdown of neurotransmitter amines in the central nervous system.[3] Since the central deficiency of serotonin, and to a lesser extent norepinephrine, has been implicated in depressive illness, inhibitors of MAO-A are used as antidepressant agents.[7,8] Inhibitors of MAO-B are employed in the therapy of Parkinson's disease where the central deficiency of dopamine is responsible for the characteristic motor deficits of this disease.[9] MAO-B inhibitors are thought to conserve central dopamine reserves, and possibly promote the elevation of dopamine levels after treatment with the metabolic precursor of dopamine, L-dopa.[10,11] Although MAO-A also metabolises central dopamine, inhibitors have not been used in Parkinson's disease therapy, foremost because MAO-A inhibition is associated with tyramine-induced hypertension, a potentially fatal adverse effect when foods rich in tyramine (such as wine, cheese and fermented products) are combined with MAO-A inhibitors.[12] This adverse effect is, however, observed with irreversible MAO-A inhibitors and reversible inhibitors, such as

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moclobemide and toloxatone, do not provoke the tyramine-induced hypertensive response.[13,14] Reversible MAO-A inhibitors, however, remain effective antidepressant agents.[8] Based on this analysis, the development of MAO-A inhibitors for depression should focus on agents that interact reversibly with MAO-A. In addition, since MAO-A also metabolises dopamine, non-selective MAO-A/B inhibitors may be more effective for the therapy of Parkinson's disease. Since Parkinson's disease patients often exhibit signs of depression, the antidepressant action of MAO-A inhibitors may be of additional value to these patients.[15]

Based on these considerations, several research efforts are aimed at the discovery and development of new MAO inhibitors, in particular compounds that bind reversibly and with high binding affinities to the MAOs.[16] Among the various classes of compounds that have been reported to inhibit the MAOs are nitrile containing compounds. In this respect, both phthalonitriles[17] and benzonitriles[17,18] act as potent and selective MAO-B inhibitors, with several compounds also possessing good MAO-A inhibitory activities. For example, 4-{[(2*E*)-3-phenylprop-2-en-1-yl]oxy}phthalonitrile (**1**) is a potent inhibitor of both MAO-A and MAO-B with IC_{50} values of 0.399 μM and 0.0066 μM, respectively (Fig. 1).[17] Similarly, 4-(4-bromobenzyloxy)phthalonitrile (**2**) also is a potent MAO inhibitor with IC_{50} values of 0.642 μM (MAO-A) and 0.0048 μM (MAO-B).[17] The nitrile functional group appears to be a requirement for potent MAO inhibition since the removal of the nitriles is associated with a large reduction in MAO inhibitory activity. Furthermore, phthalonitriles are reported to be more potent MAO inhibitors than the corresponding benzonitriles, which suggests that the enhancement of MAO inhibition potency by the nitrile group is additive.[17,19] The present study aims to expand the known structure-activity relationships (SARs) for MAO inhibition by the phthalonitrile class of compounds and to discover novel highly potent MAO inhibitors. To explore chemical space and potentially discover phthalonitrile-derived MAO inhibitors with divergent structures from those already reported, the present study investigated the MAO inhibition properties of a series consisting of indole-5,6-dicarbonitrile derivatives. As will be show below certain indole-5,6-dicarbonitrile derivatives (**3**–**6**) proved to be significant inhibitors, with good potencies recorded for the inhibition of both MAO isoforms. A limited SAR study for this class revealed interesting effects, particularly with respect to alterations of isoform selectivity as relatively small structural changes are made.

The structures of the indole-5,6-dicarbonitrile derivatives that were examined in this study are given in Table 1. The 3-chloro-1*H*-indole-5,6-dicarbonitriles derivatives (**3a**–**d**) were synthesized from the corresponding 1-hydroxy-1*H*-indole-5,6-dicarbonitrile derivatives (**7**), which in turn were synthesised by a known method (Scheme 1).[20] Chlorination of indoles **7** was performed using a twofold excess of PCl_5 in DMF. Reactions were carried out at 60–80 °C for 2 h, followed by neutralization of the reaction mixture with a 3% $NaHCO_3$ solution. These conditions led to a high product yield of up to 73% with the occurrence of only minor side reactions, such as hydrolysis of the cyano groups. The only exception was for the 2-thienyl substituted derivative (**3d**), for which the reaction temperature was lowered to 50 °C and the reaction time increased to 4 h, in order to reduce side reactions. The mechanism proposed for the formation of the 3-chloroindoles involves firstly the replacement of the *N*-hydroxy group by chlorine, after which rearrangement occurs, resulting in the migration of the chlorine to the third position of the heterocycle. Substitution of the hydroxy group after treatment with chlorinating agents has been observed previously for 10-hydroxy-indolo[3,2-b]quinoline derivatives,[21] and migration of chlorine is a well-known reaction of *N*-chloroindoles[22] and *N*-chlorocarbazole.[23] The structures of the products, **3a**–**d**, obtained were confirmed by IR-, NMR-spectroscopy and mass spectrometry, and for **3a** by X-ray diffraction (Fig. 2). In the mass spectra, the molecular ion is represented as two strong peaks (3:1), in accordance to the isotopic composition of a chlorine atom. In the ^{1}H NMR spectra, all of the expected characteristic signals of the hydrogen atoms of the chloroindoles were observed. The chemical shifts of the N–H signals of the products are markedly different from the signal of the hydroxy group of the hydroxyindole reagents, approximately 13.0 ppm and 12.2 ppm, respectively. With IR-spectroscopy, the characteristic absorption bands of the cyano groups were observed at 2220–2245 cm^{-1}.

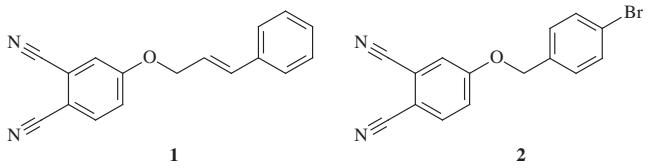

Figure 1. The structures of 4-{[(2*E*)-3-phenylprop-2-en-1-yl]oxy}phthalonitrile (**1**) and 4-(4-bromobenzyloxy)phthalonitrile (**2**).

The synthesis of the 3-chloro-1-methyl-1*H*-indole-5,6-dicarbonitriles derivatives (**4a**–**d**) was carried out by reacting **3a**–**d** with methyl iodide in the presence of potassium carbonate at room temperature. The structures of **4a**–**d** were determined by NMR-spectroscopy and mass spectrometry. A characteristic feature of the ^{1}H NMR spectra of the methylindoles is the relatively large chemical shift of N–CH_3 group (3.80–3.90 ppm), which is similar to the chemical shift expected for the signals of O–CH_3 groups. Interestingly, the signal of the N-CH_3 group on ^{13}C NMR spectra appeared at the expected chemical shift of 31–33 ppm. For compound **4a**, the structure was also solved by X-ray diffraction (Fig. 3).

For the synthesis of the of the 3-bromo-1-hydroxy-1*H*-indole-5,6-dicarbonitriles (**5a**–**c**), bromination of the corresponding hydroxyindoles **7** was carried out with *N*-bromosuccinimide in a solution of glacial acetic acid, with the addition *N*-bromosuccinimide and hydrogen peroxide at a temperature of 40–50 °C (Scheme 2). This led to the substitution of the hydrogen atom on the third position of the indole with bromine, while the 1-hydroxyl and cyano groups remained unchanged. Further methylation of **5b** under the conditions described above gave compound **6** in good yield. The structures of compounds **5a**–**c** and **6** were found to be consistent with the data obtained by IR-, NMR-spectroscopy and mass spectrometry. In the ^{1}H NMR spectra of indoles **5a**–**c**, the chemical shift of the O–H signal remained virtually unchanged at 12.25–12.32 ppm. Fragmentation of **5a**–**c** under electron impact ionization yielded low-intensity $[M]^+$ ions, with the base peak pair at $[M-16]^+$ in a ratio of 1:1, as expected for the isotopic composition of a bromine atom.[24]

To investigate the MAO inhibitory properties of the indole-5,6-dicarbonitriles, the recombinant human MAO-A and MAO-B enzymes were used.[25] The activities of both MAO-A and MAO-B were measured by monitoring the MAO-catalysed oxidation of kynuramine to ultimately yield 4-hydroxyquinoline, a fluorescent metabolite (λ_{ex} = 310 nm; λ_{em} = 400 nm).[26] The enzyme-catalysed formation of 4-hydroxyquinoline can thus be measured by spectrofluorometric analysis. With the inclusion of the appropriate control reactions, it was verified that neither kynuramine nor the indole-5,6-dicarbonitriles interferes with the fluorometric measurement by fluorescing under the assay conditions. By constructing sigmoidal dose-response curves from the residual MAO activities in the presence of various concentrations of the test inhibitors, IC_{50} values were calculated.

The IC_{50} values for the inhibition of the human MAO isoforms are provided in Table 1. From the results, it is evident that