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Inhibition of human monoamine oxidase A and B by 5-phenoxy 8-aminoquinoline analogs

Narayan D. Chaurasiya [a], Shobana Ganesan [c], N.P. Dhammika Nanayakkara [a], Luiza R.S. Dias [d], Larry A. Walker [a,b], Babu L. Tekwani [a,b,*]

[a] National Center for Natural Products Research, School of Pharmacy, University of Mississippi, MS 38677, USA
[b] Department of Pharmacology, School of Pharmacy, University of Mississippi, MS 38677, USA
[c] OSU/OHSU College of Pharmacy, 3303 SW Bond Ave, Portland, OR 97239, USA
[d] Laboratório de Química Medicinal, Faculdade de Farmácia, Universidade Federal Fluminense, Niterói, RJ, Brazil

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ABSTRACT

8-Aminoquinolines (8-AQs) are important class of anti-infective therapeutics. 5-Phenoxy 8-aminoquinoline analogs have shown improved metabolic stability compared to primaquine. In view or predictive role of monoamine oxidases (MAO) in metabolism of 8-aminoquinolines the 5-phenoxy analogs were evaluated in vitro for the inhibition of recombinant human MAO-A and MAO-B. The analogs were several folds more potent inhibitors of MAO-A and MAO-B compared to primaquine, the parent drug, with selectivity for MAO-B. 5-(4-Trifluoromethylphenoxy)-4-methylprimaquine (**6**) Inhibited MAO-B with IC_{50} value of 150 nM (626-fold more potent than primaquine). These results will have important implications in optimizing metabolic stability of 8-AQs to improve therapeutic value and also indicate scope for development of 8-AQs as selective MAO inhibitors.

Monoamine oxidases (MAOs) are flavin adenine dinucleotide (FAD) containing enzymes located at the outer membrane of mitochondria in various cells found in nerve terminals, the liver, intestinal mucosa and other tissues. They regulate the levels of biogenic and xenobiotic amines in the brain and the peripheral tissues by catalyzing their oxidative deamination.[1] On the basis of their substrate specificities and inhibitor sensitivities two forms of MAOs have been identified and designated as MAO-A and MAO-B. MAO-A preferentially catalyzes the oxidation of serotonin (5-hydroxytryptamine, 5-HT) and is inhibited by low concentration of clorgyline[2] and RO41-1049,[3] whereas MAO-B preferentially oxidizes β-phenylethylamine (PEA) and is inhibited by low concentrations of deprenyl[4] and RO16-6491.[5] Both isoforms utilize dopamine, epinephrine and tyramine and tranylcypromine is a non-selective inhibitor of MAO-A and MAO-B.[6] MAO-A and MAO-B play important roles in the central nervous system and peripheral organs. Imbalances in the levels of biogenic amines have been associated with several neuronal and metabolic disorders.[7] Abnormalities in MAO-B activities are implicated in neurological disorders such as Parkinson's and Alzheimer's disorders, whereas MAO-A plays important roles in psychiatric conditions such as depression.[6] Elevated levels of MAO-B have been demonstrated to induce apoptosis in neurons[8] and kidney cells.[9] Due to their roles in maintenance and regulation of levels of biogenic amines in neuronal and peripheral tissues. Both MAO-A and B have been of considerable pharmacological interest and the MAO inhibitors (MAOIs) have found wide clinical use for treatments of several neurological and psychiatric disorders. MAOIs although in wide use for the therapy of neuropsychiatric diseases, have various side effects. The first generation MAOIs (non-selective and irreversible) tranylcypromine, phenelzine, isocarboxazid and nilamide have serious side effects including hepatotoxicity, orthostatic hypotension, and most importantly hypertensive crises 'the cheese effect', that occurs following the ingestion of food containing tyramine.[10] Selagiline, with or without levodopa, used for the control of Parkinson's disease, can cause anorexia/nausea, dry mouth, dyskinesia, orthostatic hypotension, musculoskeletal injuries and cardiac arrhythmias.[11] The adverse effects reported with moclobemide, a reversible inhibitor of MAO-A used for the treatment of depression, are sleep disturbance, anxiety, restlessness and headache.[12]

The role of MAO-A and MAO-B in the oxidative deamination of endogenous biogenic amines and regulation of levels of neurotransmitter amines in the neuronal tissues has been studied in detail. However, contribution of MAOs to the metabolism of xenobiotics and drugs has been largely neglected. The involvement of MAOs in the metabolism of xenobiotics has frequently been demonstrated in laboratory animals or in in vitro systems, such as animal or human hepatocytes[13] or rat liver fractions,[14] rather

* Corresponding author.
E-mail address: btekwani@olemiss.edu (B.L. Tekwani).

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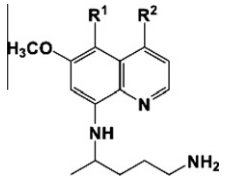


Figure 1. 8-Aminoquinoline analogs tested for inhibition (IC_{50}) of human MAO-A and MAO-B activities in vitro.

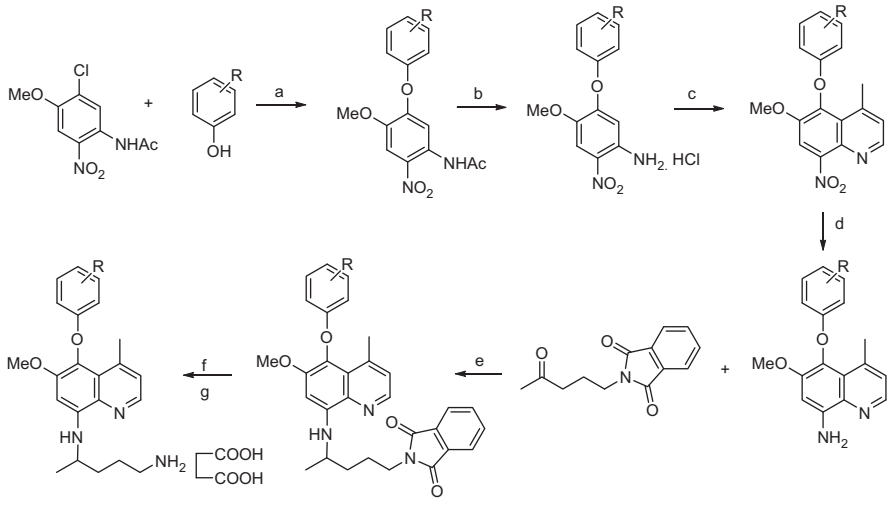
than in vivo in humans. Recently, involvement of MAOs in in vivo metabolism of citalopram,[15] sertraline[16] and the tryptan[17] in humans has been demonstrated. Role of MAO in metabolism of primaquine and generation of carboxyprimaquine, a major plasma metabolite has also been suggested.[14] Short plasma half-life and hemolytic toxicities in individuals with glucose 6-phosphate dehydrogenase deficiencies are major drawbacks, which have limited clinical use primaquine. As a part of our new 8-aminoquinoline drugs discovery program, we have synthesized several new analogs. The 5-phenoxy analogs showed significant improvement in antimalarial efficacy compared to primaquine.[18] A 5-(3,4-dicloro-phenoxy)-8-aminoquinoline analog (**14**) (NPC1161C) was selected for further preclinical development due its improved efficacy, metabolic stability, reduced hemolytic toxicity and selective pharmacologic profile of the enantiomers of this analogs.[19] NPC1161C was observed to cause significantly more potent inhibition of human MAO-A and B in vitro than primaquine. This observation led us to test several 5-phenoxy 8-aminoquinoline analogs as a new class of MAO inhibitors.

In the present study we have determined the IC-50 values for in vitro inhibition of human MAO-A and B with primaquine (PQ) (**15**) and its 5-phenoxy analogs (Fig. 1) (**1**–**13**). Compounds **1**–**14** were synthesized by the method outlined in Scheme 1. Appropriate halogenated phenol was coupled to commercially available *N*-(5-chloro-4-methyoxy-2-nitrophenyl) acetamide to yield diphenylethers. Hydrolysis of these yielded aniline hydrochlorides, which were condensed with methyl vinyl ketone to give nitroquinoline intermediates. Catalytic hydrogenation of the nitroquinwolines and subsequent attachment of the sidechain, 4-oxo-1-phthalimidopentane, afforded primaryamine-protected 8-aminoquinoline analogs. Deprotection of the terminal amine and treatment of the resulting amines with succinic acid gave compounds **1**–**13** as monosuccinates. Synthesis of NPC116C (**14**) was reported previously.

Modifications at this position are considered important in view of predictive role of 5-hydroxylated metabolites, generated through CYP mediated metabolic pathways[23] and are responsible for hemolytic toxicity of primaquine. All the analogs were testes were tested in vitro for inhibition of recombinant human MAO-A and B.[20,21] Primaquine (**15**) showed only moderate inhibition of both MAO-A and B. Similar to findings of Brossi et al.,[24] who reported similar and weak inhibition of both MAO-A and B in vitro by primaquine (K_i 103–225 μM), we observed a marginally higher inhibition of MAO-A than MAO-B by primaquine. The oxidative deamination of primaquine to carboxyprimaquine by MAO[14] indicates that it also act as a substrate for this enzyme. Substitutions at C-5 position of the quinoline ring with a phenoxy group containing halides at different position and at C-4 position with methyl group produced structures that were highly more potent inhibitors of MAO-A and B compared to PQ (Table 1). The potency for MAO-B inhibition was increased much more potently (24–626 folds) than for MAO-A, for which the inhibition potency increased to only 2–19-fold when compared with inhibition of MAO-A and B by PQ (Table 1).

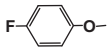
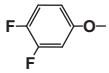
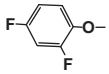
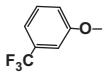
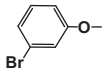
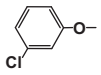
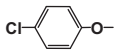
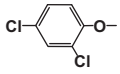
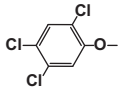
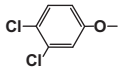
Among the series of 8-AQ analogs tested the most potent inhibitor for MAO-A was 5-(3-fluorophenoxy)-4-methylprimaquine (**2**) with IC_{50} of 3.2 μM and the least active was 5-(2-fluorophenoxy)-4-methylprimaquine (**1**) with IC_{50} of 38 μM. Similar to the finding of Van den Berg et al.,[25] who reported that styryl substituent's at C-8 position of caffeine with fluoro, chloro or bromo were weaker inhibitors of MAO than analogs bearing substituent trifluoromethyl group. The results presented here also indicate that 5-phenoxy substituted primaquine analogs with fluoro, chloro or bromo substitutions on the phenyl ring were less prominent inhibitors of MAO-B than the analog bearing trifluoromethyl group (**6**). It is also pertinent to mention about the findings of Ogunrombi et al.,[32] that among the fluoro, chloro, bromo or triflurometyl derivatives of 1-methyl-3-phenyl-3-pyrrolinyl the most potent inhibitor of MAO-B was 1-methyl-3-(4-trifluoromethylphenyl)-pyrrole.

From the crystal structure of human recombinant MAO-A and MAO-B complexed with several pharmacologically important inhibitors,[26–30] it is known that although the overall chain fold of the two isozymes is quite similar but some specific structural differences contribute to differential substrate utilization and inhibitor sensitivities of the two isoforms. There are significant similarities as well as differences in the active site of MAO-A and B.[29] The FAD coenzyme and two tyrosine residues constituting the 'aromatic cage' in the active site of both MAO-A and B are iden-



Scheme 1. Reagents and conditions: (a) KOH, DMF, 100 °C; (b) HCl, EtOH, reflux; (c) methyl vinyl ketone, H_3PO_4, 100 °C; (d) NH_2NH_2 H_2O, Pd/C, EtOH reflux; (e) $NaBH_4$, AcOH, 20 °C; (f) NH_2NH_2 H_2O, EtOH, reflux; (g) succinic acid, ethanol.

Table 1
8-Aminoquinoline analogs tested for inhibition (IC_{50}) of human MAO-A and MAO-B activities in vitro

Nos.	R^1	R^2	MAO-A[a] (μM)	MAO-B[a] (μM)	MAO-A/MAO-B index[b]
1		$-CH_3$	38.7 ± 3.2	3.83 ± 0.43	10.10
2		$-CH_3$	3.22 ± 0.24	10.8 ± 1.3	0.30
3		$-CH_3$	9.7 ± 1.3	33.4 ± 2.3	0.29
4		$-CH_3$	22.7 ± 2.1	3.65 ± 0.41	6.22
5		$-CH_3$	21.2 ± 1.8	11.7 ± 2.1	1.81
6		$-CH_3$	8.54 ± 0.91	0.15 ± 0.02	56.93
7		$-CH_3$	31.3 ± 4.1	1.58 ± 0.21	19.81
8		$-CH_3$	20.4 ± 2.2	1.54 ± 0.21	13.25
9		$-CH_3$	18.3 ± 2.1	2.25 ± 0.19	8.13
10		$-CH_3$	6.55 ± 0.62	2.53 ± 5.44	2.59
11		$-CH_3$	11.3 ± 2.1	4.3 ± 3.9	2.63
12		$-CH_3$	7.53 ± 2.2	1.54 ± 0.21	4.89
13		$-CH_3$	3.77 ± 0.22	3.53 ± 0.35	1.07
14 (NPC1161C)		$-CH_3$	6.26 ± 0.73	0.55 ± 0.06	11.36
15 (Primaquine)	–H	–H	75.7 ± 6.1	94.5 ± 5.1	0.80

[a] The values are IC_{50}s computed from dose–response curve and are mean ± S.D. of at least triplicate observations.
[b] The MAO-A/MAO-B index shows the isoform selectivity of the analogs, values <1 indicate selectivity for MAO-A and >1 indicate selectivity for MAO-B.

tical and both enzymes follow the same catalytic mechanism for oxidative deamination. The major differences between the two enzymes exist in the area of the active sites opposite to the flavin moiety, which govern substrate recognition and confer differential substrate utilization and inhibitor sensitivities to MAO-A and B.[29] The human MAO-A has a single substrate cavity of ~550 A°, which is shorter in length and wider than the longer and narrow cavity in human MAO-B, which has a size of ~700 A°. Substrate entry into human MAO-B involves entrance and substrate cavities that become fused when certain inhibitors (including deprenyl) are bound.[31] Human MAO-A does not show such a dipartite nature.[29] These structural differences in MAO-A and B explain the molecular basis for the specificity of clorgyline for MAO-A and deprenyl for MAO-B. The small molecules of inhibitor such as isatin bind within the substrate cavity of enzyme, but the larger inhibitors such as 1,4-diphenyl-2-butane bind both the entrance and substrate cavities.[31] The tremendous increase, observed in present study, in the potency of inhibition of substituted 5-phenoxy 8-AQ analogs for MAO-B but not that much for MAO-A especially by 5-(4-trifluoromethylphenoxy)-4-methylprimaquine (**6**) may also be explained by a similar mode of binding that involves both the entrance and substrate cavity. In view of the role of MAO in metabolism of 8-aminoquinolines,[14] more potent inhibition of MAO-A and MAO-B by the 5-phenoxy PQ analogs compared to PQ, the parent drug, may affect their pharmacokinetic profile. The 5-(4-trifluoromethylphenoxy)-4-methylprimaquine analog (**6**) inhibited MAO-B with IC_{50} value of 150 nM (626-fold more potent than primaquine) and was more 56-fold selective for MAO-B than MAO-A. These observations indicate potential for further development of 8-AQ analogs as selective and highly potent inhibitors of MAO-B and potential therapeutic value for treatment of neurological disorders.

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20. *Materials*: Recombinant human MAO-A and B were obtained from BD Biosciences (Bedford, MA, USA). Kynuramine bromide, 4-hydroxyquinoline, clorgyline and *R* (−) deprenyl were purchased from Sigma (St. Louis, MO, USA).
21. *Methods*: An in vitro assay was designed to measure the effect of primaquine analogs on MAO-A and B activity. Primaquine analogs (10^{-9} to 10^{-2} M), clorgyline and deprenyl (10^{-12} to 10^{-5} M) were tested for inhibition of human MAO-A and B activity. MAO-activity was assessed by a modification of the fluorometric method of Krajl[22] and was adopted for 96 well plate's format. The 200 μl reaction mixtures containing recombinant human MAO-A or MAO-B (5 μg/ml) and the test compounds in potassium phosphate buffer (100 mM; pH 7.4) were pre-incubated at 37 °C for 15 min. The reactions with positive control wells with standard MAO inhibitors and controls without inhibitors were also set up simultaneously. The reaction was initiated by addition of kynuramine (250 μM) in potassium phosphate buffer (100 mM; pH 7.4) and incubated further at 37 °C for 20 min. After incubation the reaction was stopped by the addition of 75 μl of 2 N NaOH. The deaminated product of kynuramine, which spontaneously cyclizes to 4-hydroxyquinoline, was determined fluorometrically at 320 nm excitation and 460 nm emission wavelengths in a plate reader (SpectraMax M5, Molecular Devices, Sunnyvale, CA, USA). Wells receiving no test compounds were used as controls to calculate the inhibition percentage. The IC_{50} values were computed from the dose response inhibition curves prepared by GraphP.
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