Substituted Xanthones as Selective and Reversible Monoamine Oxidase A (MAO-A) Inhibitors

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1,3-Dihydroxy-2-methylxanthone (X1), its 4-chloro and 4-bromo derivatives (X1-Cl and X1-Br), and 1,3-dihydroxy-4-methylxanthone were investigated for their inhibition activities toward MAO. A hyperbolic function was derived to fit the data and to calculate IC_{50} values. The compounds proved to be reversible and selective inhibitors of MAO-A, with X1 displaying the highest activity (IC_{50} = 3.7 μM).

KEY WORDS: dihydroxymethylxanthones; monoamine oxidase (MAO); MAO-A; selective reversible inhibitors.

INTRODUCTION

Monoamine oxidase (MAO) (amine:oxygen oxidoreductase, deaminating, flavin-containing; EC 1.4.3.4) is an enzyme of the outer mitochondrial membrane, which plays a key role in the regulation of some physiological amines and is the target of inhibitors used as antidepressive drugs (1). MAO exists as two isoenzymes, MAO-A and MAO-B, classically differentiated by the inhibition of the irreversible inhibitors clorgyline for MAO-A and (−)-deprenyl for MAO-B (2). The nucleotide sequence of the two forms is determined and is characterized by an overall homology of 70% and a difference in molecular weight (3).

Significant components of the activity of inhibitors are selectivity and mechanism of inhibition (i.e., competitive, reversible, and time-independent versus irreversible or mechanism-based and time-dependent). While the first generation of MAO inhibitors is mechanism-based inactivators, causing irreversible inhibition and displaying a range of unwanted side effects (the well-known "cheese effect"), short-acting reversible MAO inhibitors are now receiving much attention and are of considerable interest in the management of a variety of MAO-related disorders (2).

Most MAO inhibitors are synthetic compounds prepared as potential drugs, but a number of phytochemicals display the same activity. This is particulary true of xanthone derivatives, occurring mainly in the two families Gentianaceae and Guttiferae. A number of them are reversible MAO inhibitors showing modest or high activity and selectivity (4–6). In the present paper, we describe the activity and selectivity of four synthetic xanthone derivatives. Their IC_{50} values were calculated by means of a hyperbolic function derived here, relating degree of inhibition and inhibitor concentration.

MATERIALS AND METHODS

Chemicals

The synthesis of 1,3-dihydroxy-2-methylxanthone and 1,3-dihydroxy-4-methylxanthone (X1 and X2 in Fig. 1, respectively) has been described (7). In contrast, the 4-chloro and 4-bromo derivatives of X1 (X1-Cl and X1-Br in Fig. 1, respectively) are new compounds.

The following products were obtained from commercial sources and were used without further purification: Na_2HPO_4, KCl, KH_2PO_4, DMSO, and sucrose (Fluka Chemie AG, Buchs, CH); kynuramine and clorgyline (Sigma Chemical Co., St. Louis, MO); and (−)-deprenyl (RBI, Natick, MA). The following reagents and solvents were obtained from E. Merck (Darmstadt, D): acetone, chloroform, diethyl ether, ethanol, ethyl acetate, hydrochloric acid 37%, methanol, phosphorous oxide trichloride, salicylic acid, and silica gel 60 for column chromatography (35 to 70-mesh ASTM); silica gel 60 HF_{254} for thin-layer chromatography, NaOH pellets, $NaSO_4$ anhydrous, THF, $ZnCl_2$ dry, and potassium bromide for spectroscopy (Uvasol); and DMSO-d6 (Uvasol). The additional reagents were purchased from other commercial sources: C-methylphloroglucinol (Pfaltz & Bauer, USA), FeNa(EDTA) (Grace, Hampshire, USA), hydrobromic acid puriss. p.a. (Fluka Chemie AG, Buchs, CH), and acetone-d6 (Stohler Isotope Chemicals).

4-Chloro-1,3-dihydroxy-2-methylxanthone (X1-Cl)

A mixture of 1,3-dihydroxy-2-methylxanthone (X1) (20 mg) and 3.8 mg of FeNa(EDTA) was dissolved with 10.0 mL of a solution of THF/1 *N* HCl (1:1). This solution was diluted to 100.0 mL with EtOH and exposed to diffuse daylight (8). The development of the reaction was monitored by TLC (silicagel, chloroform/acetone, 95:5). Although the photoperiod was extended for 120 days, a complete transformation of X1 into X1-Cl was not observed. The solution was partially evaporated under reduced pressure. After dilution with AcOEt (50 mL), the mixture was washed with H_2O to neutrality and the organic layer was dried (Na_2SO_4) and then evaporated. The crude product was then purified by column chromatography (silicagel, chloroform). The fractions containing the halogenated derivative were combined and recrystallized from aq. ethanol to give X1-Cl as yellow needles (9 mg, 39.3%; mp 260–263°C, with decomposition).

The identity of the product was established by IR, ^{1}H-NMR, ^{13}C-NMR, and MS (spectra available from the authors upon request). The purity of the product was >97% (by TLC).

4-Bromo-1,3-dihydroxy-2-methylxanthone (X1-Br)

This compound was prepared from X1 (102.7 mg) and 3.7 mg FeNa(EDTA) dissolved in 10.0 mL THF/1 *N* HBr (1:1). The reaction conditions were as described for X1-Cl.

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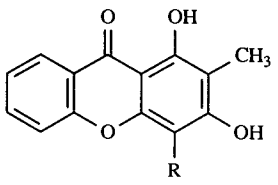
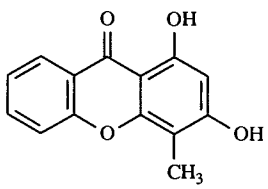


X1 : R=H
X1-Cl: R=Cl
X1-Br: R=Br



X2

Fig. 1. Structures of X1 (1,3-dihydroxy-2-methylxanthone), X1-Cl (4-chloro-1,3-dihydroxy-2-methylxanthone), X1-Br (4-bromo-1,3-dihydroxy-2-methylxanthone), and X2 (1,3-dihydroxy-4-methylxanthone).

After a photoperiod of 18 days, X1 was completely transformed into X1-Br. As this halogenated derivative was partly insoluble in the reactional medium, it was filtered under reduced pressure and washed with cold ethanol, giving X1-Br (40 mg). The mother liquors were collected and partially evaporated under reduced pressure. After dilution with AcOEt (80 mL), the mixture was washed with H_2O to neutrality and the organic layer was dried (Na_2SO_4), filtered, and then evaporated. The crude product was then recrystallized (EtOH) to give 80 mg of X1-Br as yellow needles (120 mg, 89.3%, mp 240–243%C, with decomposition). Identity by IR, ^{1}H-NMR, ^{13}C-NMR, and MS; purity >94% (by TLC).

Preparation of Brain Mitochondria

Fifteen Sprague–Dawley rats with a body weight of 200–250 g (Madörin Kleintierfarm, Füllinsdorf, CH) were anesthetized with CO_2 and decapitated. The brains were removed into ice-cold isolation medium (pH 7.4; Na_2HPO_4/KH_2PO_4 isotonized with sucrose). Blood vessels and pial membranes were removed. The method of Clark and Nicklas (9) modified by Walther *et al.* (10) was then applied to isolate brain mitochondria. The crude mitochondrial pellet was homogenized with 15 mL buffer solution in the tissue grinder and washed by further centrifugation. The washed mitochondrial pellet was resuspended to give a final volume of 25 mL.

Protein Determination

According to the procedure of Lowry *et al.* (11), the protein content of washed mitochondria fraction was determined with bovine serum albumin as a standard.

Measurements of Inhibitor Activities

A modified Weissbach *et al.* (12) method was applied to measure inhibitor activities: Incubations were carried out at pH 7.4 (Na_2HPO_4/KH_2PO_4 isotonized with KCl) at 37°C. The mitochondrial suspension was set to a final protein concentration of 1.0 mg/mL. The mitochondria were preincubated at 37°C for 5 min with either clorgyline (250 n*M*) or (−)-deprenyl (250 n*M*). The inhibitor under study was solubilized in DMSO, added to give a final DMSO concentration of 5% (v/v), and further incubated for 5 min. Preliminary experiments had verified that DMSO at this concentration does not affect MAO activity. The nonselective substrate kynuramine was then added to a concentration equal to its K_m (90 μ*M* for MAO-A, 60 μ*M* for MAO-B). Kynuramine is deaminated by MAO to an aldehyde that spontaneously cyclizes to 4-hydroxyquinoline. Formation of the latter was monitored continuously at 314 nm using a Kontron UVIKON 941 spectrophotometer.

In preliminary experiments, IC_{50} values were estimated. Incubations were then carried out with at least five concentrations of inhibitor ranging from 0.5 to 16 times the estimated IC_{50} value (13). The more precise IC_{50} was then calculated from the hyperbolic Eq. (5) developed below.

Reversibility can be shown by dialysis, gel filtration, or dilution techniques (14). We have evaluated the reversibility by measuring the inhibitor activity at IC_{50}, adding the substrate at concentrations of K_m and $6K_m$.

Determination of Inhibitor Activity

The common types of reversible inhibition obey the following equation:

$$v_I = \frac{V_{max}[S]}{K_m\left(1 + \frac{[I]}{K_{I1}}\right) + [S]\left(1 + \frac{[I]}{K_{I2}}\right)} \quad (1)$$

where v_I is the reaction rate in the presence of inhibitor (conditions to be specified), and K_{I1} and K_{I2} are the dissociation constants of enzyme–inhibitor (EI) and enzyme–substrate–inhibitor (ESI) complex, respectively.

Here, we define a degree of inhibition such that

$$i_I = 1 - \frac{v_I}{v_0} \quad (2)$$

where v_0 is the reaction rate in the absence of inhibitor.

A general equation to determine IC_{50} for reversible inhibitors in relation to Eq. (1) is (16)

$$IC_{50} = (K_m + [S]) \Big/ \left(\frac{K_m}{K_{I1}} + \frac{[S]}{K_{I2}}\right) \quad (3)$$

To establish the relationship between i_I and [I], K_{I2} is resolved in Eq. (3) and substituted into Eq. (1); setting $[S] = nK_m$ yields

$$v_I = V_{max} \frac{n}{(n+1)\left(1 + \frac{[I]}{IC_{50}}\right)} \quad (4)$$

From Eqs. (2) and (4) it follows that the degree of inhibition i_I shows a hyperbolic dependence only upon [I] at a given concentration of substrate:

$$i_I = \frac{[I]}{IC_{50} + [I]} \quad (5)$$

Once the hyperbolic relation is calculated, IC_{50} is given for $i_I = 0.5$.

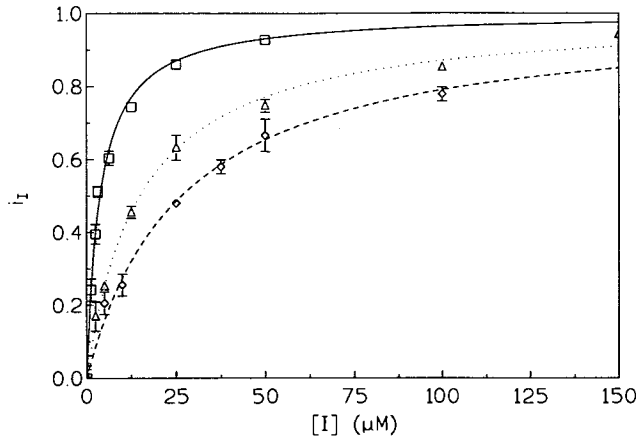


Fig. 2. MAO-A inhibition curves of 1,3-dihydroxy-2-methylxanthone derivatives: squares, X1; triangles, X1-Br; diamonds, X1-Cl.

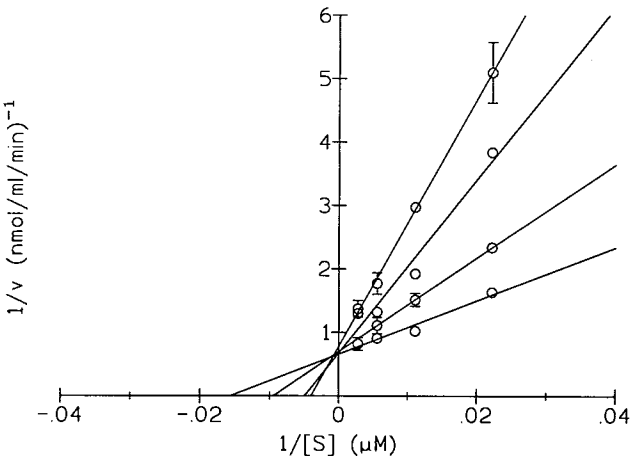


Fig. 3. Lineweaver–Burk plot of MAO-A inhibition by X2 showing a competitive mechanism.

RESULTS AND DISCUSSION

Inhibitory Potency and Selectivity

A familiar mathematical model to determine inhibitor activity is to measure the inhibition at different concentrations around the IC_{50} and to fit the data to a straight line. Using nonlinear regression is another approach to describe the degree of inhibition i_I depending upon the inhibitor concentration [I]. Since MAO appears to obey Michaelis–Menten kinetics (14), we used this theory to investigate a hyperbolic dependence between i_I and [I], as shown in Fig. 2 for the three 2-methylxanthones. The hyperbolic fits are indeed good and give experimental evidence for the validity of Eq. (5). The results presented in Table I indicate that the four xanthones are MAO inhibitors with a very marked preference for MAO-A.

With only four compounds, no meaningful structure–activity relationship can be derived, but some trends are apparent. Thus, the nonhalogenated xanthones are more active than the halogenated ones, but the latter are more selective. The 4-halogenation appears to decrease MAO inhibitory activity but to increase MAO-A selectivity. In contrast, the position of the methyl group (2- or 4-) is without influence on the activity and selectivity. Thus electronic rather than steric factors might perhaps be operative, but this remains to be established with a large series of compounds.

Table I. Inhibition of MAO-A and MAO-B by Four Synthetic Xanthone Derivatives

	MAO-A		MAO-B,
Substance	IC_{50} (μ*M*), [S] = 90 μ*M*	i_I[a] at [S] = 540 μ*M*	i_I[b] at [I] = 50 μ*M*
X1	3.7 ± 0.2	0.18	0.22
X2	4.3 ± 0.4	0.20	0.16
X1-Br	14.9 ± 0.6	0.20	—[c]
X1-Cl	26.5 ± 1.1	0.32	—[d]

[a] Inhibitor concentration = IC_{50}; calculated i_I = 0.22.
[b] Substrate concentration = 60 μ*M* (K_m).
[c] No inhibition at maximum solubility: 150 μ*M*.
[d] No inhibition at maximum solubility: 100 μ*M*.

Mechanism of Inhibition

Lineweaver–Burk plots of MAO-A inhibition by X2 prove the competitive mechanism of inhibition (Fig. 3). This is also documented by measuring inhibition at a sixfold higher substrate concentration (Table I). Markedly reduced inhibitions were seen for all four inhibitors, the values of which correspond to these predicted by Eqs. (2), (3), (4), and (5), assuming a competitive mechanism of inhibition (see Table I, footnote *a*).

If the inhibition is partly reversed, the type of inhibition is not an uncompetitive, but, in any case, a reversible one. Furthermore, the inhibition by some MAO inhibitors, for example, moclobemide (17), is affected by the duration of preincubation. Preincubating the xanthones for 15 rather than 5 min at IC_{50} and K_m had no influence on the degree of inhibition (results not shown). Thus all four xanthones appear to act on MAO-A by a competitive, reversible mechanism.

CONCLUSION

In the present paper, a hyperbolic dependence of i_I versus [I] has been established theoretically and demonstrated experimentally for the inhibition of MAO-A by xanthone derivatives. All four compounds tested behaved as selective, competitive, and reversible inhibitors of MAO-A.

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