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Screening of unsubstituted cyclic compounds as inhibitors of monoamine oxidases

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Abstract—A number of unsubstituted aromatic hydrocarbons, azaheterocycles, oxaheterocycles and cyclic ketones were screened for their inhibitory potency towards monoamine oxidases (MAO; EC 1.4.3.4.) A and B. Fair activities (IC_{50} 10–100 μM) and selectivities were found for, e.g. naphthalene, anthracene, phenanthrene, isoquinoline and acridine. The most active inhibitors are oxygen-containing compounds (e.g. coumarin, flavone, dibenzofuran, xanthene, thioxanthone and acridone), with xanthone emerging as a potent (IC_{50} 0.8 μM) and reversible MAO-A inhibitor. All tested inhibitors seem to act in a reversible and time-independent manner.

Key words: monoamine oxidases (MAO); unsubstituted arenes; unsubstituted azaheterocycles; unsubstituted oxaheterocycles; xanthone

Monoamine oxidase (MAO; EC 1.4.3.4.) is a flavin adenine dinucleotide (FAD) containing enzyme of the outer mitochondrial membrane existing as two isoenzymes (MAO-A and MAO-B) which differ in specificity for substrates, sensitivity to inhibitors, and primary amino acid sequence. However, little is known of the topographical differences in the active sites of the two enzymes [1].

There is at present a considerable pharmacological and therapeutic interest in reversible inhibitors of MAO-A and MAO-B [2]. Recently, moclobemide, a selective, slow-binding MAO-A inhibitor [3] has been introduced as an antidepressant. In Parkinson's disease substitutive therapy together with a selective MAO-B inhibitor like selegiline is a useful approach to overcome the lack of dopamine [4]. Reversible MAO inhibitors belong to a very large variety of chemical classes, e.g. isoquinolines, tetrahydroisoquinolines [5], 4-(2-benzofuranyl)piperidines [6], oxadiazoles [7], phenoxathin-10,10-dioxides [8] and natural xanthones [9]. All these compounds feature various substituents and functionalized side-chains which, in a perspective of structure–activity relationships, make it impossible to assess the constitution of the molecular (hetero)cyclic system to the enzyme–inhibitor interaction. In this work, we investigated the reversible MAO-A and -B inhibitory activity of simple (hetero)cyclic systems found in well-known inhibitors. The unexpected results prompted us to examine other ring systems as potential new lead compounds.

Materials and Methods

Chemicals. The following products were purchased from commercial sources in the highest possible degree of purity (>97%) and were used without further purification: acridine, anthracene, anthraquinone, anthrone (purity ~95%), carbazole, DMSO, flavone, KCl, KH_2PO_4, Na_2HPO_4, naphthalene, phenanthrene, pyridine, sucrose, thioxanthone, xanthene, xanthene-9-carboxylic acid, xanthone (Fluka Chemie AG, Buchs, Switzerland); 9(10*H*)-acridone, 4-chromanone, coumarin, dibenzosuberone, 9-hydroxyxanthene, 4*H*-pyran-4-one, quinoline, tetrahydro-4*H*-pyran-4-one (Aldrich-Chemie, Steinheim, Germany); benzene, dibenzofuran (purity ~97%), isoquinoline, 1,2,3,4-tetrahydroisoquinoline hydrochloride (Merck, Darmstadt, Germany); kynuramine and clorgyline (Sigma Chemical Co., St Louis, MO, U.S.A.), selegiline (RBI, Natick, MA, U.S.A.).

Preparation of rat brain mitochondria.

The method of Clark and Nicklas [10] modified by Walther *et al.* [11] was used to isolate rat brain mitochondria. Sprague–Dawley rats with a a body weight of 200–250 g (Madörin Kleintierfarm, Füllinsdorf, Switzerland) were anaesthetised with CO_2 and decapitated. The brains were placed in an ice-cold isolation medium (pH 7.4; Na_2HPO_4/KH_2PO_4 isotonized with sucrose). Blood vessels and pial membranes were removed, and the brains homogenized with 3.75 mL buffer solution per gram tissue in a manual Dounce (Wheaton Scientific, Millville, NJ, U.S.A.) glass–tissue grinder kept on ice. Homogenates were centrifuged at 4° for 3 min at 1700 *g*. The supernatant was collected and centrifuged at 11,000 *g* for 20 min. The crude mitochondrial pellet was washed by further centrifugation. The protein content of the washed mitochondria fraction was determined according to Lowry *et al.* [12] with bovine serum albumin as standard.

Measurements of inhibitor activities.

A continuous assay was chosen to monitor monoamine oxidase activity to ensure linearity during the reaction time. Directly monitoring the time-course of reaction avoids several problems which can be caused by a discontinuous assay [13]. Thus, the method of Weissbach *et al.* [14] was modified to measure inhibitory activities. Incubations were carried out at pH = 7.4 (NA_2HPO_4/KH_2PO_4 isotonized with KCl) at 37°. The mitochondrial suspension had a final protein concentration of 1.0 mg/mL. The mitochondria were preincubated at 37° for 5 min with either clorgyline (250 nM; MAO-A: IC_{50} 3.79 nM) or selegiline (250 nM; MAO-B: IC_{50} 61.9 nM). The inhibitor under study was solubilized in DMSO, added to give a final DMSO concentration of 5% (v/v), and incubated for 5 min. Preliminary experiments had verified that DMSO at this concentration does not affect MAO activity. The non-selective substrate kynuramine was then added to a concentration equal to its K_m (90 μM for MAO-A, 60 μM for MAO-B). Kynuramine is deaminated by MAO to an aldehyde that spontaneously cyclizes to 4-hydroxyquinoline, the formation of which was monitored continuously at 314 nm for 6 min using a Kontron UVIKON 941 spectrophotometer. Substrate oxidation did not exceed 10% in the absence of inhibitors and 4-hydroxyquinoline had shown no inhibitory effect at 150 μM.

We tested the compounds, when soluble in the medium mitochondrial suspension/DMSO (95:5), up to a concentration of 150 μM. If no inhibitory effect was seen at 150 μM, the compound was defined as inactive. To measure IC_{50} values, incubations were carried out in duplicate with at least five concentrations of inhibitor

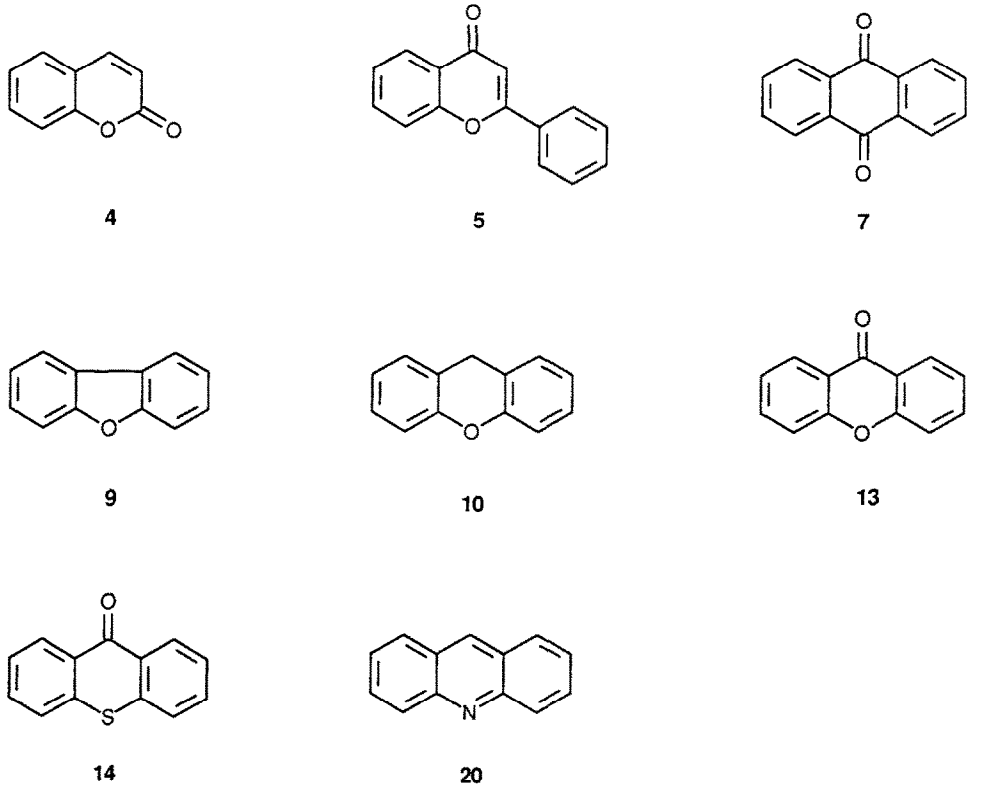

Fig. 1. Structure of the most active compounds ($IC_{50} \leq 20\ \mu M$).

ranging from 0.5 to 16 times the previously estimated IC_{50} value [15]. The IC_{50} was then calculated by a hyperbolic function [16]. The IC_{50} was verified in a separate experiment monitoring the formation of product and the disappearance of substrate at 360 nm. To validate the method harmane was used as a standard inhibitor (MAO-A: IC_{50} 0.6 μM; MAO-B: IC_{50} 119 μM). These results correspond quite well with the literature data: MAO-A: IC_{50} 0.5 μM; MAO-B: IC_{50} 80 μM [17].

Reversibility was evaluated either by displacement or by dilution [18]. In the former procedure inhibitory activity was measured at the IC_{50}, adding the substrate at a concentration of five times K_m. If the inhibition is partly reversed, the type of inhibition is reversible [19]. In the dilution procedure [18], the activity of the inhibitor is assayed twice (a) after preincubation for 30 min of MAO plus inhibitor at the 10 times IC_{50}, followed by a 10-fold dilution upon addition of substrate, and (b) after 30 min preincubation of mitochondria and buffer alone, followed by a 10-fold dilution and the addition of inhibitor at the IC_{50} plus substrate. Thus the final concentration of inhibitor is the same in the assay mixtures incubated in the absence and presence of inhibitor. Reversible inhibitors display the same activity in both assays, whereas irreversible inhibitors are considerably more potent in assay (a) than (b) [18].

Time-dependency was tested for all inhibitors by comparing preincubations of 5 and 15 min.

Results

The major group of investigated compounds comprises monocyclic, bicyclic and tricyclic oxaheterocycles and cyclic ketones (Fig. 1), several of which display marked inhibitory activities and even MAO-A versus MAO-B selectivity (Table 1). While monocyclic compounds (**1** and **2**) are inactive, bicyclic compounds (**3** and **4**) show moderate to good activities, particularly towards MAO-B. Flavone **5**, which can be regarded as both bicyclic and tricyclic, is MAO-A selective, as are the tricyclic compounds in Table 1, with activities ranging from weak (anthrone) to high.

The results we obtained with some oxaheterocycles and cyclic ketones show a marked influence of the number and nature of atoms in the central ring, but clear structure–activity relationships are not apparent. To obtain further insights, we also examined some azaheterocycles and aromatic hydrocarbons. Again, monocyclic compounds were inactive (**16** and **22**), while bicyclic compounds (**17, 18, 19** and **23**) displayed weak to good activities, with sometimes a selectivity towards MAO-B. Tricyclic compounds **20, 24** and **25**, but not carbazole **21**, showed good activities towards MAO-A. The MAO inhibitory activity of compounds **17, 18** and **19** has already been reported [5, 20] but comparison between these results and ours is difficult since in most cases IC_{50} could not be measured.

None of the active compounds in Table 1 inhibited MAO in a time-dependent manner. Preincubation of the inhibitors at their IC_{50} for 15 min rather than 5 min had no influence on the activity (results not shown). However, when the substrate kynuramine was introduced in higher concentrations, a marked decrease in inhibitory activity was seen for all inhibitors. Thus all inhibitors in this study appear to act in a reversible manner. Moreover, the three most active inhibitors of this study were assayed by the dilution technique [18]. Again, xanthone, flavone and coumarin showed a reversible mechanism of inhibition (Table 2).

Table 1. MAO inhibitory potency of oxaheterocycles and cyclic ketones (**1–15**), azaheterocycles (**16–21**), and aromatic hydrocarbons (**22–25**)

Number	Substance*	MAO-A IC_{50} in μM	MAO-B IC_{50} in μM	Ratio IC_{50} (A/B)
3	4-Chromanone	167 ± 36	21.7 ± 2.2	7.7
4	Coumarin	41.2 ± 5.8	12.0 ± 0.8	3.4
5	Flavone	2.66 ± 0.29	23.7 ± 5.5	0.11
6	Anthrone	25% (10 μM)†	Inact. (10 μM)‡	—
7	Anthraquinone	8.19 ± 0.35	27% (10 μM)†	—
8	Dibenzosuberone	51.6 ± 5.4	Inact. (100 μM)‡	—
9	Dibenzofurane	7.56 ± 0.38	55.5 ± 4.5	0.14
10	Xanthene	8.92 ± 0.56	22.5 ± 2.2	0.40
11	9-Hydroxyxanthene	49% (2.5 μM)†	Inact. (2.5 μM)‡	—
12	Xanthene-9-carboxylic acid	137 ± 15	Inact. (150 μM)‡	—
13	Xanthone	0.84 ± 0.08	122 ± 14	0.007
14	Thioxanthone	3.37 ± 0.43	15% (10 μM)‡	—
15	Acridone	20.4 ± 3.1	Inact. (75 μM)‡	—
17	Quinoline	23% (75 μM)†	14% (75 μM)†	—
18	Isoquinoline	38% (40 μM)†	44.9 ± 1.6§	—
19	Tetrahydroisoquinoline	90.4 ± 1.9	47.7 ± 6.8	1.9
20	Acridine	12.4 ± 1.2	112 ± 3	0.11
21	Carbazole	42% (50 μM)†	13% (50 μM)†	—
23	Naphthalene	20% (50 μM)†	28.5 ± 1.2	—
24	Anthracene	20.2 ± 0.8§	Inact. (15 μM)‡	—
25	Phenanthrene	20.8 ± 1.4	Inact. (37.5 μM)‡	—

* This table does not list the four MAO-A and MAO-B inactive compounds 4*H*-pyran-4-one (**1**), tetrahydro-4*H*-pyran-4-one (**2**), pyridine (**16**) and benzene (**22**).

† Per cent inhibition at maximal solubility.

‡ Inactive at maximal solubility or at 150 μM.

§ Estimated IC_{50}.

Table 2. Reversibility test (dilution technique [18]) for selected compounds

Number/ Concentration		% MAO-A inhibition 0 min*	% MAO-A inhibition 30 min†	% MAO-B inhibition 0 min*	% MAO-B inhibition 30 min†
3	(12 μM)	ND	ND	46 ± 2	52 ± 1
5	(3 μM)	41 ± 3	42 ± 2	ND	ND
13	(1 μM)	56 ± 4	55 ± 3	ND	ND
Clorgyline	(4 nM)	97 ± 2	56 ± 2	ND	ND
Selegeline	(60 nM)	ND	ND	100	44 ± 4

* Addition of inhibitor (10 times IC_{50}) before incubation and dilution.

† Addition of inhibitor (IC_{50}) after 30 min incubation and dilution.

ND, not determined.

Discussion

We report the rather unexpected finding that a number of bicyclic and tricyclic aromatic hydrocarbons, azaheterocycles, oxaheterocycles and cyclic ketones are reversible and selective MAO inhibitors. The most active inhibitors are some of the tricyclic compounds, and these show a clear selectivity towards MAO-A. In contrast, bicyclic compounds are moderately active, and slightly MAO-B selective. The nature of the ring system and the presence of a carbonyl group markedly influence activity. However, more elaborate structure–activity relationships are not apparent at this stage.

Some of the inhibitors examined here exist as molecular core in drugs or their metabolites, e.g. pyridines, acridines, coumarins and anthraquinones. More interesting is the fact that some of the compounds examined may be of interest as lead compounds for the development of new reversible and selective MAO inhibitors. A case in point is that of xanthone, the most active compound in this study, which could gainfully be compared with natural or synthetic xanthones bearing various substituents. Such work is in progress in our laboratory.

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