134. Synthesis and Monoamine Oxidase Inhibitory Activity of 3-Substituted 5*H*-Indeno[1,2-*c*]pyridazines

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The one-pot synthesis of nine 5*H*-indeno[1,2-*c*]pyridazines is described. These compounds are shown to be potent, reversible inhibitors of monoamine oxidase B (MAO-B) with little or no effect on monoamine oxidase A (MAO-A). Qualitative structure-activity relations indicate that the MAO-B inhibitory activity is strongly influenced by electronic and bulk properties of substituents.

1. Introduction. – Monoamine oxidase (MAO, *EC 1.4.3.4*) exists in two forms, MAO-A and MAO-B, which differ by their primary structure, substrate specificity, and sensitivity to inhibitors [1–3].

Clinical results have suggested that MAO-A inhibitors could be effective for treating serotonin (5-HT) and noradrenaline (NA) deficits observed in depressive illnesses, whereas MAO-B inhibitors, when combined with the dopamine (DA) precursor L-DOPA, could be useful in alleviating the debilitating symptoms of *Parkinson*'s disease [4–6].

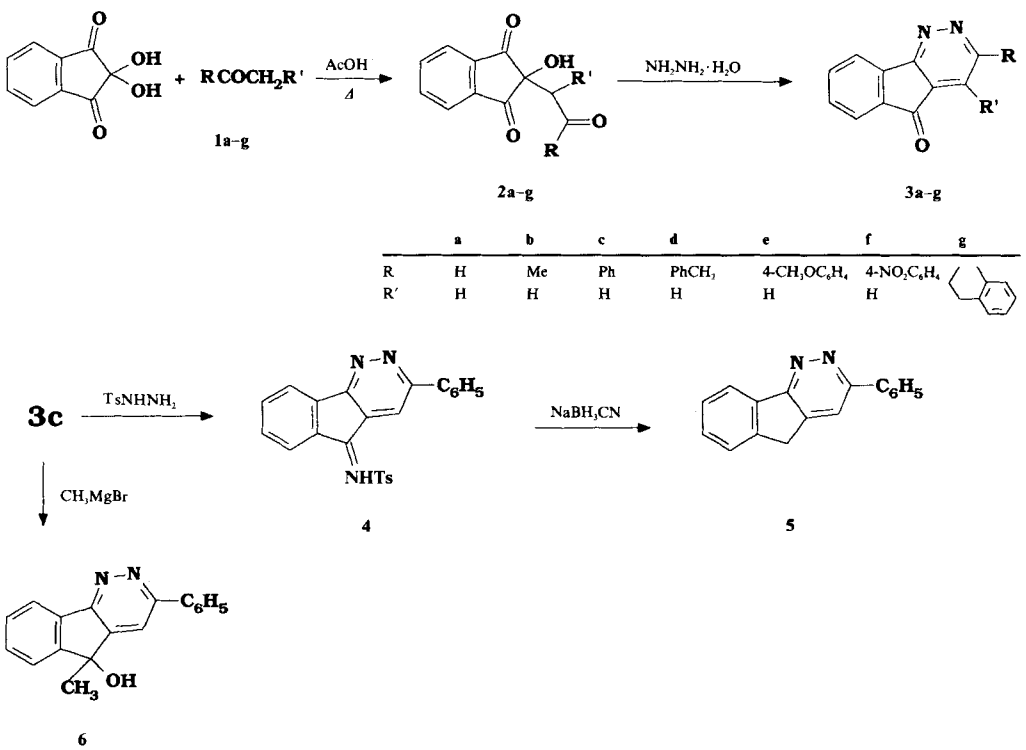
Aryl-oxadiazine and aryl-oxadiazole derivatives have recently been described as selective and competitive MAO inhibitors [7] [8], an activity probably linked in part to the endocyclic 'hydrazine' functionality (=N–N=) they feature. On our side, we have been interested in the convenient synthesis and properties of indeno[1,2-*c*]pyridazine derivatives [9] which also contain the endocyclic hydrazine functionality.

Here, we describe the one-pot synthesis of some 3-substituted 5*H*-indeno[1,2-*c*]-pyridazin-5-ones and analogs and report their MAO-A and MAO-B inhibitory activities.

2. Results. – *Chemistry.* Compounds **2–6** were prepared according to the reaction pathway shown in the *Scheme*. Intermediate aldol adducts **2c**, **e**, **g** [10], indeno-pyridazinones **3a** [11], **3b** [12], and **3c** [13] were previously synthesized by others through different procedures, as reported in the patent literature. Here, we used an efficient one-pot procedure to prepare the 5*H*-indeno[1,2-*c*]pyridazin-5-ones **3a**, **c**, **e**, **f** from ninhydrin and the corresponding ketones **1** (or acetaldehyde **1a**) as reported in a preliminary communication [9].

As expected, two isomeric compounds, namely **2d** and 2-hydroxy-2-(2-oxo-3-phenylpropyl)indane-1,3-dione were obtained from benzyl methyl ketone **1d** and separated by column chromatography on silica gel. Reductive transformation of CO at C(5) was performed on compound **3c** through *Grignard* addition of MeMgBr leading to **6** and

Scheme



through the *Caglioti* reaction [14] on **3c** leading to **5**. The yields and properties of the compounds are reported in *Table 1*.

MAO Inhibition. Our method, mainly based on that reported by *Weissbach et al.* [22] was validated by measuring the inhibitory activity of harman. The results obtained for MAO-A and MAO-B (IC_{50} 0.6 μM and 120 μM, respectively) compare very well with the literature data (IC_{50} 0.5 μM and 80 μM, respectively) [15].

Preliminary tests having shown the inhibitory activity of some of the investigated compounds, detailed studies were conducted. It was first established that the mechanism of inhibition is of a competitive type (*Fig. 1*) [16] [17], and, in contrast to other competitive inhibitors (*e.g.*, moclobemide, [2]), no time dependence was seen (data not shown).

Because the inhibition is of a competitive type, K_i values could be determined and are reported in *Table 2*. The MAO-B inhibitory activities range from nil to high (compound **3f**), whereas the MAO-A inhibitory activities are usually nil with only three compounds acting weakly.

3. Discussion. – Structural modifications of the indeno[1,2-*c*]pyridazine skeleton were carried out at C(3) and C(5) to obtain a preliminary evaluation of the physicochemical features mainly responsible for modulating MAO-B inhibitory activity. The property space at C(3) was thus explored for lipophilicity and bulk (compounds **3a–d**) and strong electronic effects (compounds **3e, f**), whereas the influence of conformational rigidity was

Table 1. *Yields, Crystallization Solvents, Physicochemical and Spectral Data of Compounds* **2**, **3**, **5**, *and* **6**

Compound	Yield [%]	Cryst. solv. (M.p. [°C])	IR[a] (KBr [cm^{-1}])	^{1}H-NMR[b]
2b	95	AcOEt/Hexane (142–143)	3320, 1750, 1710	1.77 (*s*, CH_3(3′)); 3.08 (*s*, CH_2(1′)); 6.04 (br. *s*, HO–C(2)); 7.40–7.80 (*m*, 4H–C(arom.))
2d	15	$CHCl_3$/Hexane (139–140)	3380, 1750, 1730, 1727	3.26 (*s*, CH_2(1′)); 3.52 (br. *s*, HO–C(2)); 3.62 (*s*, CH_2(3′)); 7.00–7.30 (*m*, 5 arom. H); 7.70–8.00 (*m*, 4 arom. H)
2g	90	$CHCl_3$/Hexane (174–175)	3380, 1750, 1710, 1680	2.45–2.80 (*m*, CH_2(3′)); 3.00–3.30 (*m*, CH_2(4′)); 3.60 (*dd*, *J* = 13, 5, H–C(2′)); 6.83 (*s*, HO–C(2)); 7.13–7.70 (*m*, 4 arom. H); 7.90–8.20 (*m*, 4 arom. H)
3a	41	EtOH (164–165)[c]	1720, 1390	7.55 (*td*, *J* = 7.5, 1.1, H–C(8)); 7.58 (*d*, *J* = 4.8, H–C(4)); 7.72 (*td*, *J* = 7.5, 1.1, H–C(7)); 7.78 (*dt*, *J* = 7.5, 1.1, H–C(9)); 8.15 (*dt*, *J* = 7.5, 1.1, H–C(6)); 9.24 (*d*, *J* = 4.8, H–C(3))
3b	76	EtOH (190–191)[d]	1725, 1420	2.80 (*s*, CH_3–C(3)); 7.43 (*s*, H–C(4)); 7.54 (*td*, *J* = 7.2, 1.5, H–C(8)); 7.70 (*td*, *J* = 7.2, 1.5, H–C(7)); 7.81 (*dt*, *J* = 7.2, 1.5, H–C(9)); 8.14 (*dt*, *J* = 7.2, 1.5, H–C(6))
3c	49	EtOH (218–219)[e]	1720, 1410	7.49–7.59 (*m*, 4H–C(3′–5′,8)); 7.73 (*td*, *J* = 7.5, 1.1, H–C(7)); 7.84 (*dt*, *J* = 7.5, 1.1, H–C(9)); 7.98 (*s*, H–C(4)); 8.08–8.14 (*m*, 2H–C(2′,6′)); 8.17 (*dt*, *J* = 7.5, 1.1, H–C(6))
3d	90	EtOH (167–168 (dec.))	1725, 1415	4.20 (*s*, CH_2–C(3)); 7.30 (*s*, 5 arom. H); 7.36 (*s*, H–C(4)); 7.40–7.80 (*m*, 3H–C(7–9)); 8.14 (*dt*, *J* = 8, 1.5, H–C(6))
3e	66	Dioxane/EtOH (219–220)[d]	1725, 1420	3.87 (*s*, CH_3O–C(4′)); 7.04 (*dt*, *J* = 9.1, 2.2, 2H–C(3′,5′)); 7.53 (*td*, *J* = 7.5, 1.0, H–C(8)); 7.72 (*td*, *J* = 7.5, 1.0, H–C(7)); 7.83 (*dt*, *J* = 7.5, 1.0, H–C(9)); 7.93 (*s*, H–C(4)); 8.09 (*dt*, *J* = 9.1, 2.2, 2H–C(2′,6′)); 8.15 (*dt*, *J* = 7.5, 1.0, H–C(6))
3f	70	THF (318–320 (dec.))	1720, 1515, 1415, 1340	7.49–7.54 (*m*, 2H–C(2′,6′)); 7.59 (*td*, *J* = 7.5, 1.1, H–C(8)); 7.74–7.86 (*m*, 2H–C(7,9)); 8.17 (*dt*, *J* = 7.5, 1.1, H–C(6)); 8.23 (*s*, H–C(4)); 8.26–8.31 (*m*, 2H–C(3′,5′))
3g	70	EtOH (180 (dec.))	1715, 1390	2.96 (*t*, *J* = 7.2, CH_2(11)); 3.43 (*t*, *J* = 7.2, CH_2(12)); 7.23–7.28 (*m*, H–C(10)); 7.37–7.41 (*m*, 2H–C(8,9)); 7.50 (*td*, *J* = 7.5, 1.2, H–C(3)); 7.68 (*td*, *J* = 7.5, 1.2, H–C(2)); 7.78 (*dt*, *J* = 7.5, 1.2, H–C(4)); 8.11 (*dt*, *J* = 7.5, 1.2, H–C(1)); 8.48–8.53 (*m*, H–C(7))
5	20	EtOH (139–140)	1470, 1420	3.93 (*m*, CH_2(5)); 7.40–7.60 (*m*, 6H–C(3′–5′, 7–9)); 7.89 (*t*, *J* = 1.1, H–C(4)); 8.06–8.12 (*m*, 2H–C(2′,6′)); 8.28–8.33 (*m*, H–C(6))
6	55	AcOEt (190–191)	3250, 1415	1.75 (*s*, CH_3–C(5)); 5.85 (*s*, HO–C(5)); 7.46–7.51 (*m*, 5H–C(7,8,3′–5′)); 7.65–7.67 (*m*, H–C(9)); 8.03 (*s*, H–C(4)); 8.08–8.14 (*m*, 3H–C(6,2′,6′))

[a]) Only the most significant absorption bands are listed. [b]) Recorded in the following solvents, $CDCl_3$: **2d**, **3a**, **b**, **c**, **d**, **e**, **g**, and **5**; (D_6)DMSO: **2b**, **g**, and **6**; (D_8)THF: **3f**. [c]) [11]: 160–161°. [d]) [12]: 185–187°. [e]) [13]: 216°.

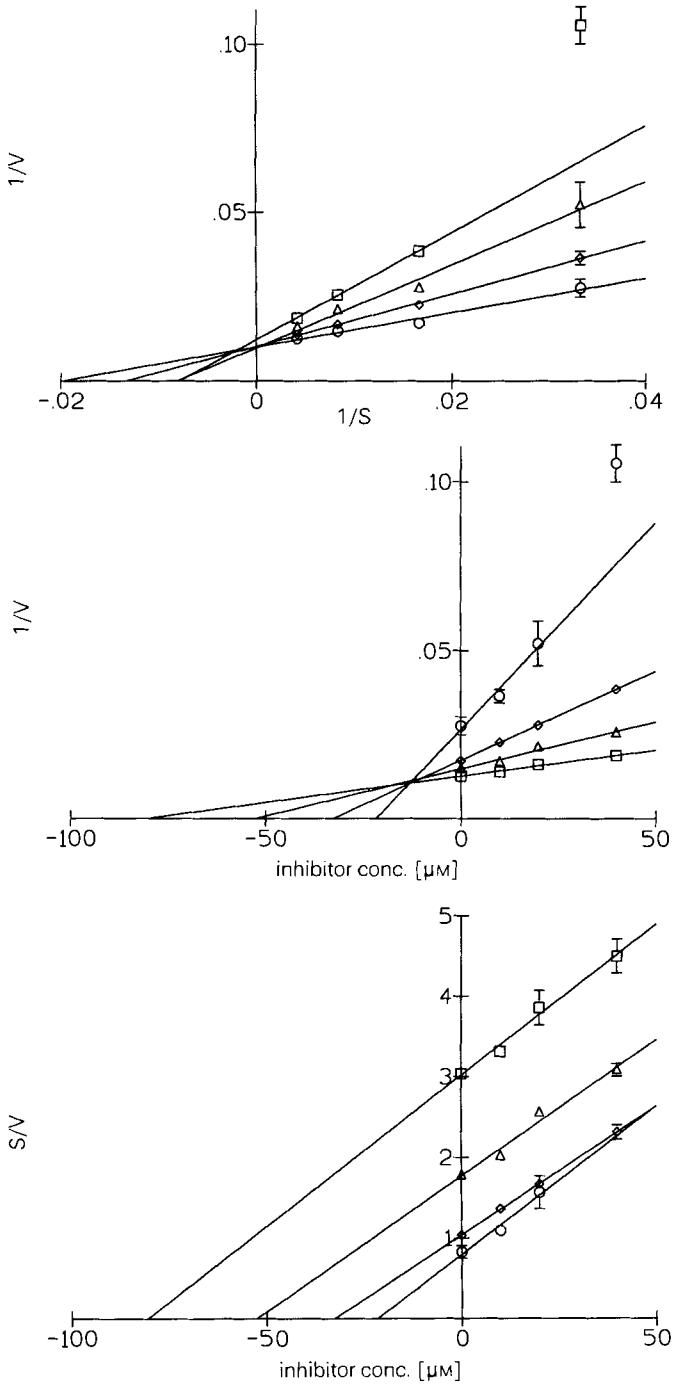
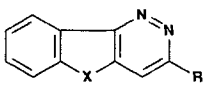
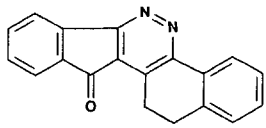


Fig. 1. Lineweaver-Burk (a), Dixon (b), *and* Cornish-Bowden (c) *plots for compound* **3a**

Table 2. *Inhibition of MAO-A and MAO-B by 5H-Indeno[1,2-c]pyridazines*



3a–f,5,6



3g

Compound	X	R	MAO-B		MAO-A
			IC_{50} [μM]	pK_I	Inhibition[a])
3a	C=O	H	27.5 ± 5.1	4.87	50%[b])
3b	C=O	Me	74.4 ± 2.5	4.33	20%[b])
3c	C=O	Ph	21.0 ± 0.6	5.15	none[d])
3d	C=O	$PhCH_2$	30.6 ± 0.3	4.82	none[b])
3e	C=O	4′-MeO–C_6H_4	3.22 ± 0.04	5.86	none[e])
3f	C=O	4′-NO_2–C_6H_4	0.50 ± 0.02	6.57	none[d])
3g			no inhibition[c])		none[c])
5	CH_2	Ph	23.4 ± 2.4	5.04	31%[c])
6	$C(CH_3)OH$	Ph	no inhibition[b])		none[b])

[a]) % Inhibition at maximum solubility. [b]) 100 μM. [c]) 50 μM. [d]) 25 μM. [e]) 5 μM.

studied through the synthesis of compound **3g**. Position 5 instead was only partly explored through suitable reductions of the ketone group leading to compounds **5** and **6**.

Table 2 clearly shows that the tested compounds are active inhibitors of MAO-B, with little or no activity towards MAO-A. In fact, the most active compound (**3f**) has an inhibitory activity towards MAO-B that is comparable to that of some highly active MAO-B inhibitors reported by *Da Prada et al.* [2].

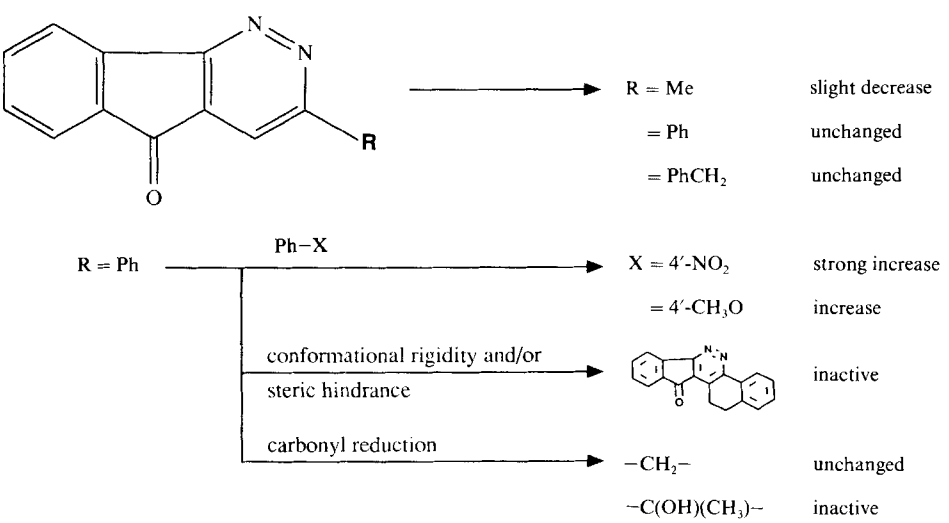


Fig. 2. *Qualitative structure-activity relationships of indeno[1,2-c]pyridazines for inhibition of MAO-B, relative to compound* **3a** (R = H)

Evaluation of the data in *Table 2* allows to derive some preliminary structure-activity relations, as summarized in *Fig. 2*. Among the 5*H*-indeno[1,2-*c*]pyridazine-5-one derivatives **3a–g**, the more potent MAO-B inhibitors are those with a Ph ring at C(3) (compounds **3c**, **e**, **f**). The electron-withdrawing NO_2 group (**3f**) as well as the electron-donating MeO group (**3e**) on the Ph ring give rise to a significant increase in inhibition potency. In particular, the NO_2 substituent in *para*-position increases activity by a factor of *ca.* 40.

While the influence of substituents on the Ph ring deserves further investigations, the example of compound **3g** could suggest that MAO-B inhibitory activity may also depend on the dihedral angle between the Ph ring and the tricyclic system.

Reduction of the C(5)=O group to a CH_2 yielded compound **5** which showed a MAO-B inhibition comparable to that of compound **3c**. This suggests that the presence of C=O in position 5 is not a critical requisite for MAO-B inhibition. Interestingly, the loss of C=O in position 5 produces a measurable IMAO-A activity (compared to **3c**).

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Experimental Part

General. Ninhydrin, acetaldehyde **1a**, and ketones **1b–g** were commercial products from *Aldrich Chem. Co.* The following products were obtained from commercial sources and used without further purification: Na_2HPO_4, KCl, KH_2PO_4, DMSO, and sucrose (*Fluka*, CH–Buchs), kynuramine and clorgyline (*Sigma Chemical Co.*, St. Louis, USA), (–)-deprenyl (*RBI*, Natick, USA), and harman (*Aldrich Chemie*, D–Steinheim).

M.p.: in an open capillary with a *Mark II* electrothermal apparatus, uncorrected. Column chromatography: on silica gel *60* (70–230 mesh, *Merck*). IR Spectra: as KBr pellets on a *Perkin-Elmer 283* spectrophotometer. ^{1}H-NMR Spectra: at 200 MHz on a *Varian XL-200* instrument; chemical shifts in δ values, downfield from TMS used as internal standard; exchange with D_2O was used to identify OH protons. Elemental analyses were carried out by *G. Di Pinto* on a *Carlo Erba 1106* analyzer, and the results for C, H, N were within ±0.40% of the theoretical values.

*3-Phenyl-5*H*-indeno[1,2-c]pyridazine-5-one* (**3c**), *3-(4-Methoxyphenyl)-5*H*-indeno[1,2-c]pyridazine-5-one* (**3e**), *and 3-(4-Nitrophenyl)-5*H*-indeno[1,2-c]pyridazin-5-one* (**3f**). *One-Pot Procedure.* A soln. of ninhydrin (1.78 g, 10 mmol) and 10 mmol of the appropriate methyl ketone (**1c**, **e**, **f**) in 20 ml of glacial AcOH was heated to reflux over a period of 3–4 h. Upon cooling to r.t., the mixture was diluted with 60 ml of MeCN and added with hydrazine hydrate 98% (0.74 ml, 15 mmol) under magnetic stirring. After 1–2 h, the yellow solid so formed was collected by filtration and recrystallized.

*5*H*-Indeno[1,2-c]pyridazin-5-one* (**3a**). Compound **3a** was obtained as above with the following slight modifications: a soln. of ninhydrin (1.78 g, 10 mmol) and acetaldehyde (0.79 ml, 14 mmol) in 20 ml of glacial AcOH was heated in a suitably protected sealed tube at 125° for 5 h. The mixture, upon dilution with 200 ml of MeCN, was reacted with hydrazine hydrate 98% (0.74 ml, 15 mmol) for 1 h at r.t., under magnetic stirring. The mixture was evaporated to dryness and the residue chromatographed on a silica-gel column with hexane/AcOEt to give pure **3a**.

2-Hydroxy-2-(2-oxopropyl)indane-1,3-dione (**2b**), *2-Hydroxy-2-(2-oxo-3-phenylpropyl)indane-1,3-dione* (**2d**), *and 2-Hydroxy-2-(1,2,3,4-tetrahydro-1-oxonaphthalen-2-yl)indane-1,3-dione* (**2g**). A soln. of ninhydrin (1.78 g, 10 mmol) and ketone **1b**, **d**, **g** (50, 10, 10 mmol, resp.) in 20 ml of AcOH was heated to reflux over 1 h (5 h for **1g**). The mixture was evaporated to dryness to give a solid residue which was recrystallized to yield pure **2b** and **2g** or chromatographed on silica-gel column with hexane/AcOEt/i-PrOH 75:20:5 to yield **2d** (R_f 0.36) and the isomeric 2-hydroxy-2-(2-oxo-3-phenylpropyl)indane-1,3-dione (R_f 0.43; 65% yield; m.p. 140–141°, from acetone/hexane).

*3-Methyl-5*H*-indeno[1,2-c]pyridazin-5-one* (**3b**), *3-Benzyl-5*H*-indeno[1,2-c]pyridazin-5-one* (**3d**), *and Benzo[h]-13*H*-indeno[1,2-c]cinnolin-13-one* (**3g**). CF_3COOH (0.1 ml) was added to a soln. of hydrazine hydrate 98%

(0.74 ml, 10 mmol) and **2b**, **d**, **g** in anh. EtOH (100–200 ml) under magnetic stirring. The yellow solid which was formed within 1 h was collected by filtration and recrystallized.

*3-Phenyl-5*H*-indeno[1,2-c]pyridazin-5-one Tosylhydrazone* (**4**). A soln. of tosylhydrazine (3.72 g, 20 mmol) and **3c** (2.58 g, 10 mmol) in 100 ml of $CHCl_3$ was stirred at r.t. over 24 h. The resulting orange solid was collected and used without further purification for the subsequent reduction.

*3-Phenyl-5*H*-indeno[1,2-c]pyridazine* (**5**). A soln. of **4** (0.426 g, 1 mmol), $TsOH \cdot H_2O$ (40 mg, 0.21 mmol), and $NaCNBH_3$ (0.251 g, 4 mmol) in 40 ml of EtOH was heated at reflux for 1 h. The solid residue was filtered off, and the soln., evaporated to dryness and chromatographed on silica-gel column with $CHCl_3$/hexane 90:10, afforded the title compound.

*5-Hydroxy-5-methyl-3-phenyl-5*H*-indeno[1,2-c]pyridazine* (**6**). 3M soln. (2 ml) of CH_3MgCl in THF was added dropwise under N_2 to an ice-cooled, stirred soln. of **3c** (2 mmol) in THF (20 ml). After 10 min, the mixture was allowed to warm to r.t., stirred for 1 h, and added with 10 ml of a 0.35M aq. soln. of $KHSO_4$. The resulting mixture was extracted with $CHCl_3$, and the org. layers washed with brine and H_2O, dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed on silica gel with hexane/AcOEt 60:40 to yield pure **5**.

Preparation of Rat Brain Mitochondria. Rat brain mitochondria were isolated according to the method of *Clark* and *Nicklas* [18] modified by *Walther et al.* [19]. For further details, see *Thull et al.* [20].

The protein content of the washed mitochondrial fraction was determined according to the procedure of *Lowry et al.* [21] with bovine serum albumin as a standard.

MAO Inhibition. The method of *Weissbach et al.* [22] was modified in order to measure inhibitor activities [23]. Incubations were carried out at pH 7.4 (Na_2HPO_4/KH_2PO_4 isotonized with KCl) at 37°. The mitochondrial suspension was set to a final protein concentration of 1.0 mg/ml. The mitochondria were preincubated at 37° for 5 min with clorgyline (250 nM) or (−)-deprenyl (250 nM) to test MAO-B or MAO-A activity, respectively. 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 is deaminated by MAO to an aldehyde that spontaneously cyclizes to quinolin-4-ol. 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 four different substrate concentrations (0.5 K_M, 1 K_M, 2 K_M, 4 K_M; K_M = 90 μM for MAO-A, 60 μM for MAO-B) without inhibitor or with an inhibitor concentration of 0.5 IC_{50}, 1 IC_{50}, or 2 IC_{50}, respectively. K_I values were determined by *Lineweaver-Burk* and *Dixon* plots. IC_{50} values were calculated from a hyperbolic equation described in [20]. Time-dependence was tested by preincubation times of 5 and 15 min at 37° for one inhibitor concentration.

A second method used was a fluorimetric assay described by *Snyder* and *Hendley* [24] with a peroxidase substrate recommended by *Zaitsu* and *Ohkura* [25] and modified in our laboratory to monitor the reaction continuously. Incubations were carried out at pH 8.0 (Na_2HPO_4/KH_2PO_4 isotonized with KCl) at 37°. The mitochondrial suspension was set to a final protein concentration of 0.1 mg/ml. The mitochondria were preincubated at 37° for 5 min with horseradish peroxidase (HRP, 2 U/ml) and either clorgyline (250 nM) or (−)-deprenyl (250 nM). 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 nor HRP activities. Then the fluorogenic substrate hydroxyphenylpropionic acid (HPPA, 10^{-4} M) and finally the nonselective MAO substrate decylamine – at a concentration of K_M (14 μM for MAO-A, 6 μM for MAO-B) – were added and the formation of the fluorescent HPPA-dimer followed at the wavelengths λ_{ex} 320 nm, λ_{em} 404 nm.

Calibration was carried out under the same conditions, H_2O_2 being added instead of decylamine at concentrations ranging from 0.025 to 0.25 μM. For each inhibitor concentration a separate calibration was necessary.

The UV and fluorimetric methods led to comparable IC_{50} values; in *Table 2*, IC_{50} values coming from the former assay are reported.

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