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Original paper

Acetylenic and allenic derivatives of 2-(5-methoxyindolyl) methylamine: synthesis and evaluation as selective inhibitors of the monoamine oxidases A and B

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Summary — A series of acetylenic and allenic derivatives of 2-(5-methoxyindolyl)methylamine has been synthesized. The new compounds were studied as inhibitors of the A and B forms of the mitochondrial monoamine oxidase (MAO) from bovine brain, using ^{14}C-tyramine as the substrate and clorgyline and *l*-deprenyl as references. All the studied compounds were MAO inhibitors. However, these compounds either did not show selectivity (compounds **3a–3d, 4c, 4e, 4m** and **4o**) or they were selective for MAO-A (compounds **4a, 4b, 4d, 4f–4l, 4n** and **4p**). Some of the compounds showed a similar inhibitory potency for MAO-A and lower for MAO-B than clorgyline and the higher selectivity for MAO-A was about 2.5 times that of clorgyline. Selectivity was shown only by acetylenic and allenic potent inhibitors, but no simple relationship between inhibitory potency and selectivity was found.

Résumé — Dérivés acétyléniques et alléniques de la 2-(5-méthoxy-indolyl) méthylamine: synthèse et évaluation comme inhibiteurs sélectifs des monoamines oxydases A et B. *Une série de dérivés acétyléniques et alléniques de la 2-(5-méthoxy-indolyl)-méthylamine a été préparée. Les composés ont été étudiés comme inhibiteurs des formes A et B de la monoamine oxydase mitochondriale de cerveau de bœuf, en utilisant la ^{14}C-tyramine comme substrat et la clorgyline et la l-deprenyl comme références. Tous les composés étudiés se sont montrés être des inhibiteurs de MAO. Certains composés se sont montrés dénués de sélectivité (composés **3a–3d, 4c, 4e, 4m** et **4c**), d'autres étant sélectifs vis-à-vis de la MAO-A (composés **4a, 4b, 4d, 4f–4l, 4n** et **4p**). Relativement à la clorgyline, quelques composés ont montré une activité inhibitrice similaire pour la MAO-A et inférieure pour la MAO-B, la plus grande sélectivité vis-à-vis de la MAO-A étant 2,5 fois celle de la clorgyline. Seuls les inhibiteurs acétyléniques et alléniques puissants se sont montrés sélectifs, sans que nous puissions trouver une relation simple entre activité inhibitrice et sélectivité.*

acetylenic and allenic indolylmethylamine derivatives / monoamine oxidase inhibitors

Introduction

The mitochondrial monoamine oxidase [MAO; monoamine; O_2 oxidoreductase (deaminating), EC. 1.4.3.4] is a FAD-containing enzyme which catalyzes the oxidative deamination of important neuronal monoamines, such as catecholamines and serotonin. Inhibitors of MAO have been shown to be molecules of therapeutic interest [1]. At present, the enzyme is known in two forms, namely MAO-A and MAO-B. These two forms have recently been shown to be similar, and it has been suggested they might derive from a common progenitor gene [2]. The two forms of MAO are characterized by their different sensitivities to inhibitors and their different specificities for substrates. MAO-A is sensitive to inhibition by lower concentrations of clorgyline, and MAO-B is sensitive to inhibition by lower concentrations of *l*-deprenyl. Serotonin, adrenalin and noradrenalin are preferably metabolized by MAO-A, whereas benzylamine and ß-phenylethylamine are predominantly metabolized by MAO-B, and tyramine is a common substrate for both forms of the enzyme [3].

A number of MAO inhibitors are useful in clinical psychiatry for the treatment of depression. However, the usefulness of these compounds has been questioned due to the 'cheese effect', a serious hypertensive response to the tyramine and other pressor amines present in some common foodstuffs [1]. A potential and attractive strategy to obtain MAO inhibitors free of that effect takes in consideration the selective inhibition of the different forms of MAO. The 'cheese effect' seems to result from MAO-A inhibition,

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whereas selective inhibition of MAO-B do not produce this effect. Antidepressant activity seems to occur when MAO-A is inhibited, while the role of MAO-B in depression remains controversial. However, *l*-deprenyl, a selective MAO-B inhibitor, has found application in the L-Dopa treatment of Parkinson's syndrome [4, 5].

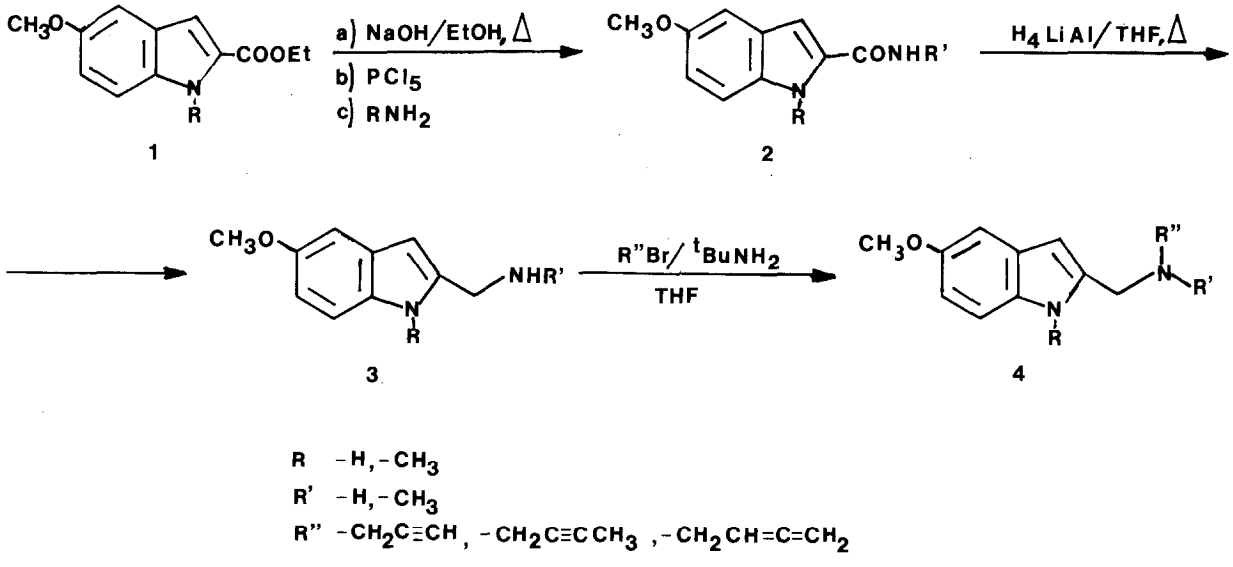
Though numerous substrates and reversible and irreversible inhibitors of both forms of MAO are known, 'no simple patterns have yet emerged which will allow the rational design of potent monoamine oxidase inhibitors with predictable selectivity' [3].

This paper is a part of a more extensive research program on the synthesis and enzymatic and pharmacological studies of acetylenic and allenic derivatives of indoleamines as selective MAO inhibitors. We report here the synthesis and preliminary assays of inhibition and selectivity for a new series of acetylenic and allenic derivatives of 2-(5-methoxy-indolyl)methylamines. In previous papers [6, 7] we have reported on similar studies for indolylmethylamine [6] and (1-methylindolyl) methylamine [7] derivatives.

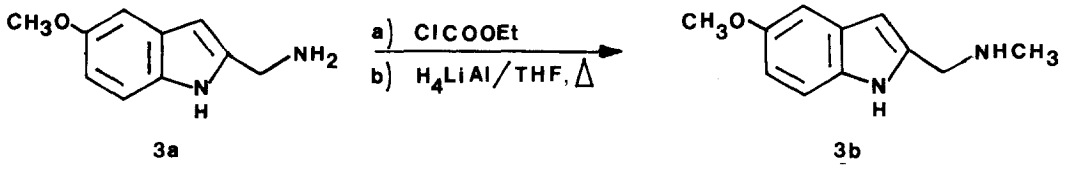
Chemistry

All compounds were obtained according to the synthetic route shown in scheme 1, from the respective ethyl 2-indolecarboxylates **1**, through saponification to the respective acids and reaction with phosphorous pentachloride to give the respective acyl chlorides and further aminolysis with ammonia or methylamine to obtain the corresponding amides **2**. These last compounds were reduced with lithium aluminium hydride in boiling tetrahydrofuran in a similar manner to that previously reported by us for a series of 2-indolecarboxamides [6], yielding the amines **3a, 3c** and **3d**. Amine **3b** could not be obtained in this way, as the respective amide **2b** was not reduced by the lithium aluminium hydride in the above conditions. Compound **3b** was obtained by the alternative route showed in scheme 2.

The amines **3a–3d** were *N*-alkylated in the presence of *tert*–butylamine by the respective alkyl (2-proponyl, 2,3-butadienyl and 2-butynyl) bromides to give the corresponding amines **4a–4p**, under similar conditions to those previously reported by us for



Scheme 1.



Scheme 2.

related compounds [6, 7]. The mixture of the bis-alkylation product (starting with **3a** or **3c**), the monoalkylation product and the residual starting amine (**3a–3d**) were easily separated by thin-layer or preparative column chromatography. Structure of all compounds were confirmed by spectral data (IR, ^{1}H NMR, UV) and elementary analysis (see tables I and II).

Table I. Analytical and spectral data of compounds **3** and **4**.

Comp No	*R*	*R'*	*R''*	*Salt* [a]	*mp °C (recrys)*	*Formula*[b]	*Yield*	*IR (KBr) ν (cm^{-1})*
3a	H	H	H	HCl	>295[c]	$C_{10}H_{13}N_2OCl$	96	3310(NH-indole); 3200–2500(NH^+_3)
3b	H	CH_3	H	HCl	212[c]	$C_{11}H_{15}N_2OCl$	91[f]	3280(NH-indole); 3100–2300(NH^+_2)
3c	CH_3	H	H	HCl	>250[c]	$C_{11}H_{15}N_2OCl$	96	3100–2310(NH^+_3)
3d	CH_3	CH_3	H	HCl	226[c]	$C_{12}H_{16}N_2OCl$	96	3100–2310(NH^+_2)
4a	H	H	$HC{\equiv}CCH_2$	HCl	183[c]	$C_{13}H_{15}N_2OCl$	40	3280(≡C-H); 3250(NH-indole); 3000–2300 (NH^+_2); 2220(C≡C)
4b	H	H	$CH_3{\equiv}CCH_2$	$H_2C_2O_4$	192[d]	$C_{16}H_{19}N_2O_5$	30	3700–2300(NH^+_2, NH-indole, COOH); 2220(C≡C); 1725, 1705(COOH); 1550 (COO^-)
4c	H	$CH_3C{\equiv}CCH_2$	$CH_3C{\equiv}CCH_2$	$H_2C_2O_4$	159[c]	$C_{20}H_{22}N_2O_5$	20	3700–2250(NH^+, NH-indole, COOH); 2220 (C≡C); 1720, 1700 (COOH); 1550 (COO^-)
4d	H	H	$CH_2{=}C{=}CHCH_2$	$H_2C_2O_4$	168[e]	$C_{16}H_{18}N_2O_5$	25	3700–2300(NH^+_2, COOH, NH-indole, C=C=CH); 1980, 1960(C=C=C); 1725, 1705(COOH); 1595(COO^-); 855 (=C=CH_2)
4e	H	$CH_2{=}C{=}CHCH_2$	$CH_2{=}C{=}CHCH_2$	$H_2C_2O_4$	130[c]	$C_{20}H_{22}N_2O_5$	7	3700–2200(NH^+, COOH, NH-indole, =C=CH); 1980, 1960(C=C=C); 1725, 1705(COOH); 1590 (COO^-); 855 (=C=CH_2)
4f	H	CH_3	$HC{\equiv}CCH_2$	$H_2C_2O_4$	119[d]	$C_{16}H_{18}N_2O_5$	74	3700–2280(NH^+, COOH, NH-indole, C≡CH); 2125 (C≡C); 1720, 1700-(COOH); 1585(COO^-)
4g	H	CH_3	$CH_3C{\equiv}CCH_2$	$H_2C_2O_4$	170[c]	$C_{17}H_{20}N_2O_5$	75	3700–2300(NH^+, COOH); 2220(C≡C); 1725, 1705(COOH); 1595(COO^-)
4h	H	CH_3	$CH_2{=}C{=}CHCH_2$	$H_2C_2O_4$	52[c]	$C_{17}H_{20}N_2O_5$	67	3700–2200(COOH, NH^+, C=C=CH); 1980, 1960(C=C=C); 1730, 1710-(COOH); 1590(COO^-); 850(=C=CH_2)
4i	CH_3	H	$HC{\equiv}CCH_2$	HCl	205[c]	$C_{14}H_{17}N_2OCl$	50	3220(C≡CH); 3100–2300(NH^+_2); 2120-(C≡C);
4j	CH_3	H	$CH_3C{\equiv}CCH_2$	HCl	210[d]	$C_{15}H_{19}N_2OCl$ (1/2 H_2O)	75	3000–2310(NH^+_2); 2240(C≡C)
4k	CH_3	$CH_3C{\equiv}CCH_2$	$CH_3C{\equiv}CCH_2$	HCl	117[c]	$C_{19}H_{23}N_2OCl$ (1 H_2O)	12	2700–2260(NH^+); 2240(C≡C)
4l	CH_3	H	$CH_2{=}C{=}CHCH_2$	HCl	204[d]	$C_{15}H_{19}N_2OCl$ (1/2 H_2O)	55	3000–2320(NH^+_2, =C=CH); 1980, 1960(C=C=C)
4m	CH_3	$CH_2{=}C{=}CHCH_2$	$CH_2{=}C{=}CHCH_2$	HCl	172[c]	$C_{19}H_{23}N_2OCl$ (1/3 H_2O)	21	3000–2200(NH^+, =C=CH); 1980, 1960-(C=C=C); 850(=C=CH_2)
4n	CH_3	CH_3	$HC{\equiv}CCH_2$	HCl	188[c]	$C_{15}H_{19}N_2OCl$	74	3300–2300(NH^+, C≡CH); 2120(C≡C)
4o	CH_3	CH_3	$CH_3C{\equiv}CCH_2$	HCl	199[d]	$C_{16}H_{21}N_2OCl$	72	2700–2260(NH^+); 2240(C≡C)
4p	CH_3	CH_3	$CH_2{=}C{=}CHCH_2$	HCl	185[d]	$C_{16}H_{21}N_2OCl$	70	3000–2300(NH^+, =C=CH); 1980, 1960-(C=C=C); 840(=C=CH_2)

[a]Salt: hydrochloride or acid oxalate; [b]Analyses were within ±0.4% for C, H, N and, where appropriate, for Cl. [c]Recrystallized from ethanol/ethyl ether; [d]Recrystallized from ethanol; [e]Recrystallized from ethanol; [f]Total yield for the synthesis of the respective urethane and its reduction.

Table II. UV and ^1H NMR data for compounds **3** and **4**.

Comp No	*UV (ethanol) λ, nm (log ε)*	*Solvent (MHz)*	*^1H NMR spectra[a] δ (ppm), J (Hz)*
3a	222(4.14), 269(3.91) 299(3.62), 308(3.56)	D_2O (90)	3.75(s, 3H, CH_3O), 4.43(s, 2H, CH_2N^+), 6.40(s, 1H-3), 6.96(dd, 1H-6, J = 9 Hz, 1.5 Hz), 7.27(d, 1H-4, J = 1.5 Hz), 7.50(d, 1H-7, J = 9 Hz)
3b	222(4.23), 269(4.02) 299(3.74), 307(3.69)	DMSO–d_6 (90)	2.43(s, 3H, CH_3N^+), 3.43(bs, NH_2^+)[b], 3.75(s, 3H, CH_3O), 4.15(t, 2H, CH_2N^+, J = 6 Hz), 6.35(s, 1H-3), 6.50(dd, 1H-6, J = 9 Hz, 1.5 Hz), 6.85(d, 1H-4, J = 1.5 Hz), 7.10(d, 1H-7, J = 9 Hz), 9.20–9.70(bs, 1H, NH-indole)[b]
3c	228(4.11), 275(4.02) 304(3.69), 312(3.65)	DMSO–d_6 (60)	3.8(s, 6H, CH_3O, CH_3N-indole), 4.3(s, 2H, CH_2N^+), 6.6(s, 1H-3), 6.9(dd, 1H-6, J = 9 Hz, < 3 Hz), 7.2(d, 1H-4, J < 3 Hz), 7.5(d, 1H-7, J = 9 Hz)
3d	228(4.12), 275(4.06) 305(3.72)	DMSO–d_6 (60)	2.6(s, 3H, CH_3N^+), 3.5(s, 3H, CH_3N-indole), 3.8(s, 3H, CH_3O), 4.4(s, 2H, CH_2N^+), 6.7–7.7(m, 4H aromat)
4a	223(3.27), 270(3.08) 300(2.78), 307(2.73)		3.17(t, 1H, C≡CH, J = 3 Hz), 3.30(bs, NH_2^+)[b], 3.75(s, 3H, CH_3O), 3.90(d, 2H, C≡CH_2), 4.30(s, 2H, CH_2N^+), 6.55(s, 1H-3), 6.80(dd, 1H-6, J = 9 Hz, 1.5 Hz), 7.05(d, 1H-4, J = 1.5 Hz), 7.30(d, 1H-7, J = 9 Hz), 9.90–10.4(bs, NH-indole)[b]
4b	227(4.28), 271(4.13) 295(3.86), 307(3.76)	DMSO–d_6 (90)	1.83(t, 3H, C≡CCH_3, J = 2.7 Hz), 3.80(m, 5H, CH_3O, C≡CCH_2), 4.25(s, 2H, CH_2N^+), 6.45(s, 1H-3), 6.67–7.13(m, 3H, 1H-4, 1H-6, NH-indole)[c], 7.23(d, 1H-7, J = 9 Hz), 11.3(s, 1H, COOH)[b]
4c	224(4.18), 272(3.97) 295(3.72), 307(3.57)	DMSO–d_6 (90)	1.83(t, 6H, C≡CCH_3, J = 2.7 Hz), 3.35(c, 4H, C≡CCH_2, J = 2.7 Hz), 3.75(s, 5H, CH_3O, CH_2N^+), 6.25(s, 1H-3), 6.70(dd, 1H-6, J = 9 Hz, 1.5 Hz), 6.95(d, 1H-4, J = 1.5 Hz), 7.23(d, 1H-7, J = 9 Hz), 7.90–8.60(bs, 1H, NH-indole)[b], 10.63(s, 1H, COOH)[b]
4d	225(4.11), 270(3.95) 298(3.65), 307(3.57)	DMSO–d_6 (90)	3.30–3.90(m, 2H, C=CHCH_2), 3.76(s, 3H, CH_3O), 4.25(s, 2H, CH_2N^+), 4.95–5.15(m, 2H, C=CH_2), 5.15–5.50(m, 1H, C=C=CH), 6.50(s, 1H-3), 6.77(dd, 1H-6, J = 9 Hz, 1.5 Hz), 7.05(d, 1H-4, J = 1.5 Hz), 7.31(d, 1H-7, J = 9 Hz), 7.67(bs, NH_2^+, NH-indole)[b], 11.5(s, 1H, COOH)[b]
4e	226(4.14), 272(3.97) 295(3.69), 307(3.58)	DMSO–d_6 (90)	3.34(dt, 4H, C=CHCH_2, J = 6 Hz, 3 Hz), 3.75(s, 3H, CH_3O), 3.97(s, 2H, CH_2N^+), 4.70–5.00(m, 4H, C=C=CH_2), 5.10–5.50(m, 2H, C=C=CH), 6.31(s, 1H-3), 6.41–6.83(m, 3H, H-6, NH^+, NH-indole)[d], 6.95(d, 1H-4, J = 1.5 Hz), 7.23(d, 1H-7, J = 9 Hz), 10.95(s, 1H, COOH)[b]
4f	226(4.13), 272(3.99) 296(3.72), 308(3.61)	DMSO–d_6 (90)	2.40(s, 3H, CH_3N^+), 3.40(t, 1H, C≡CH, J = 3 Hz), 3.55(d, 2H, C≡CCH_2, J = 3 Hz), 3.75(s, 3H, CH_3O), 3.90(s, 2H, CH_2N^+), 6.35(s, 1H-3), 6.80(dd, 1H-6, J = 9 Hz, 1.5 Hz), 6.96(d, 1H-4, J = 1.5 Hz), 7.23(d, 1H-7, J = 9 Hz), 8.55(bs, NH-indole)[b], 11.03(s, 1H, COOH)[b]
4g	228(4.16), 273(4.11) 297(3.80), 308(3.73	DMSO–d_6 (90)	1.80(t, 3H, C≡CCH_3, J = 3 Hz), 2.40(s, 3H, CH_3N^+), 3.55(c, 2H, C≡CCH_2, J = 3 Hz), 3.75(s, 3H, CH_3O), 3.85(s, 2H, CH_2N^+), 6.30(s, 1H-3), 6.80(dd, 1H-6, J = 9 Hz, 1.5 Hz), 6.96(d, 1H-4, J = 1.5 Hz), 7.23(d, 1H-7, J = 9 Hz), 7.50(bs, NH-indole)[b], 11.20(s, 1H, COOH)[b]

[a]s = singlet; d = doublet; t = triplet; m = multiplet; bs = broad signal; dd = double doublet; dt = double triplet. [b]These signals disappear on addition of D_2O. [c]By the addition of D_2O to the solution in DMSO–d_6 the corresponding signal was simplified to 6.73(dd, 1H-6, J = 9 Hz, 1.5 Hz), 6.96(d, 1H-4, J = 1.5 Hz). [d]By the addition of D_2O to the solution in DMSO–d_6 the corresponding signal was simplified to 6.73(dd, 1H-6, J = 9 Hz, 1.5 Hz).

Table II. *(continued)*

Comp No	*UV (ethanol) λ, nm (log ε)*	*Solvent (MHz)*	*^1H NMR spectra[a] δ (ppm), J (Hz)*
4h	227(4.22), 272(4.14) 296(3.82), 307(3.76)	DMSO–d_6 (90)	2.40(s, 3H, CH_3N^+), 3.30–3.50(m, 2H, C=CHCH_2), 3.75(s, 3H, CH_3O), 4.10(s, 2H, CH_2N^+), 4.80–5.00(m, 2H, C=C=CH_2), 5.20–5.45(m, 1H, C=C=CH), 5.50–6.10(bs, NH^+, NH-indole)[b], 6.40(s, 1H-3), 6.75(dd, 1H-6, J = 9 Hz, 1.5 Hz), 6.96(d, 1H-4, J = 1.5 Hz), 7.23(d, 1H-7, J = 9 Hz), 11.15(s, 1H, COOH)[b]
4i	229(4.16), 277(4.11) 306(3.77)	DMSO–d_6 (200)	3.73–3.78(m, 1H, C≡CH), 3.74(s, 3H, CH_3N-indole), 3.76(s, 3H, CH_3O), 3.92(d, 2H, C≡CCH_2, J = 2.1 Hz), 4.39(s, 2H, CH_2N^+), 6.62(s, 1H-3), 6.83(dd, 1H-6, J = 9.0 Hz, 2.4 Hz), 7.06(d, 1H-4, J = 2.4 Hz), 7.38(d, 1H-7, J = 9.0 Hz), 9.96–10.00(bs, NH_2^+)[b]
4j	228(4.19), 276(4.10) 305(3.74)	DMSO–d_6 (90)	1.90(t, 3H, C≡CCH_3, J = 2.5 Hz), 3.76(s, 6H, CH_3O, CH_3N-indole), 3.83(t, 2H, C≡CCH_2, J = 2.5 Hz), 4.37(s, 2H, CH_2N^+), 6.67(s, 1H-3), 6.85(dd, 1H-6, J = 9 Hz, 1.5 Hz), 7.05(d, 1H-4, J = 1.5 Hz), 7.40(d, 1H-7, J = 9 Hz)
4k	230(4.31), 278(4.28) 301(3.91), 312(3.85)	DMSO–d_6 (60)	1.9(t, 6H, C≡CCH_3, J < 6 Hz), 3.7(s, 3H, CH_3N-indole), 3.8(s, 3H, CH_3O), 3.9–4.1(m, 4H, C≡CCH_2), 4.5(s, 2H, CH_2N^+), 6.8–7.7(m, 4H aromat)
4l	227(4.19), 276(4.17) 305(3.82)	D_2O (200)	3.58–3.69(m, 4H, C=CHCH_2), 3.63(s, 3H, CH_3N-indole), 3.75(s, 3H, CH_3O), 4.38(s, 2H, CH_2N^+), 4.90–4.97(m, 2H, C=C=CH_2), 5.16–5.30(m, 1H, C=C=CH), 6.56(s, 1H-3), 6.89(dd, 1H-6, J = 9.0 Hz, 2.5 Hz), 7.12(d, 1H-4, J = 2.5 Hz), 7.32(d, 1H-7, J = 9.0 Hz)
4m	226(4.30), 278(4.09) 307(3.69), 313(3.47)	DMSO–d_6 (60)	3.7–4.0(m, 2H, C=CHCH_2), 3.8(s, 3H, CH_3O), 3.9(s, 3H, CH_3N-indole), 4.5(s, 2H, CH_2N^+), 5.1–5.4(m, 4H, C=C=CH_2), 5.5–5.9(m, 2H, C=C=CH), 6.8(s, 1H-3), 7.0(dd, 1H-6, J = 9 Hz, < 3 Hz), 7.3(d, 1H-4, J < 3 Hz), 7.6(d, 1H-7, J = 9 Hz)
4n	224(4.27), 277(4.07) 307(3.68)	DMSO–d_6 (90)	2.76(s, 3H, CH_3N^+), 3.75(s, 3H, CH_3O), 3.80(s, 3H, CH_3N-indole), 3.83(t, 1H, C≡CH, J = 3 Hz), 4.06(d, 2H, C≡CCH_2, J = 3 Hz), 4.53(s, 2H, CH_2N^+), 6.73(s, 1H, H-3), 6.86(dd, 1H-6, J = 9 Hz, 2.5 Hz), 7.06(d, 1H-4, J = 2.5 Hz), 7.36(d, 1H-7, J = 9 Hz)
4o	228(4.19), 278(4.14) 307(3.76)	DMSO–d_6 (200)	1.92(t, 3H, C≡CCH_3, J = 2 Hz), 2.74(s, 3H, CH_2N^+), 3.38–3.48(bs, 1H, NH^+)[b], 3.75(s, 3H, CH_3O), 3.80(s, 3H, CH_3N-indole), 3.97(m, 2H, C≡CCH_2), 4.50(s, 2H, CH_2N^+), 6.71(s, 1H-3), 6.88(dd, 1H-6, J = 8.95 Hz, 2.50 Hz), 7.08(d, 1H-4, J = 2.50 Hz), 7.39 (d, 1H-7, J = 8.95 Hz)
4p	228(4.30), 278(4.24) 307(3.86)	DMSO–d_6 (200)	2.69(s, 3H, CH_3N^+), 3.70–3.80(m, 2H, C=CHCH_2), 3.76(s, 3H, CH_3O), 3.81(s, 3H, CH_3N-indole), 4.52(m, 2H, CH_2N^+), 5.11(m, 2H, C=C=CH_2), 5.56(m, C=C=CH), 6.72(s, 1H-3), 6.87(dd, 1H-6, J = 8.95 Hz, 2.4 Hz), 7.08(d, 1H-4, J = 2.4 Hz), 7.40(d, 1H-7, J = 8.95 Hz)

Enzymatic assays

Compounds **3** and **4** as well as clorgyline and *l*-deprenyl were studied as inhibitors of MAO-A and MAO-B from bovine brain mitochondria. Each compound, at several concentrations, was pre-incubated with the enzyme for 20 min at 37°C in 30 mM potassium phosphate buffer, pH 7.30, and the residual enzymatic activity was determined with ^{14}C-tyramine·HCl by suitable adaptation of previously reported methods [8, 9]. I_{50} values were determined from the plots of residual activity percentage, calculated in relation to a sample of the enzyme treated under the same conditions without inhibitor, *versus* –log I. Results are summarized in table III. When biphasic diagrams were obtained, which indicate selectivity in the inhibition for both forms of MAO, both I_{50} values were determined using clorgyline and *l*-deprenyl as references. Figures 1 and 2 are illustrative examples of those diagrams. Table III also shows the selectivity obtained for each compound, expressed as the ratio I_{50}–B / I_{50}–A = B / A.

Results and Discussion

All the studied compound proved to be MAO inhibitors. Comparison of the I_{50} values for this series (table III) with those of the corresponding series of 5-unsubstituted indole derivatives previously reported by us [6, 7] showed that the substitution at the 5 position of the indole by a methoxy group, occasionally enhanced the inhibitory activity or the selectivity for the inhibition of MAO-A. However, these effects were very variable, depending on the compound considered, and we are not able at present to give general rules to predict the inhibitory activity or the selectivity of a given compound. Up to the moment, of all the compounds we have prepared and studied, the most selective but not the most potent inhibitor for MAO-A was compound **4i** (table III), with a ratio I_{50}–B / I_{50}–A of 2100, ≈ 2.5-fold more selective for MAO-A than clorgyline, while its 5-demethoxy analogue [7] showed a ratio of 1600.

The non acetylenic or allenic amines (**3a–3d**) were weak reversible inhibitors of MAO, presumably being substrates. I_{50} values ranged from 18 μM–0.1 mM, and none of these compounds showed selectivity for MAO-A or MAO-B. Further studies will be necessary to investigate the behaviour of these compounds as substrates and reversible inhibitors.

Table III. I_{50} values for inhibition of MAO-A and MAO-B by compounds **3** and **4**.

Compound No	*I_{50} for MAO-A(M)* [a]	*I_{50} for MAO-B(M)* [a]	*I_{50}–B/ I_{50}–A*
3a	$5.0\ 10^{-5}$	$5.0\ 10^{-5}$	1
3b	$\geq 1.0\ 10^{-4}$	$>1.0\ 10^{-4}$	≥1
3c	$1.8\ 10^{-5}$	$1.8\ 10^{-5}$	1
3d	$1.3\ 10^{-4}$	$1.3\ 10^{-4}$	1
4a	$2.2\ 10^{-7}$	$1.1\ 10^{-4}$	500
4b[b]	$6.3\ 10^{-7}$	$>3.3\ 10^{-4}$	>530
4c[b]	$\geq 2.0\ 10^{-5}$	$>2.0\ 10^{-5}$	≥1
4d	$4.5\ 10^{-8}$	$2.5\ 10^{-6}$	56
4e	$4.5\ 10^{-7}$	$4.5\ 10^{-7}$	1
4f	$7.1\ 10^{-9}$	$5.4\ 10^{-7}$	63
4g	$8.9\ 10^{-9}$	$1.3\ 10^{-5}$	1400
4h	$1.8\ 10^{-8}$	$5.0\ 10^{-6}$	280
4i	$2.1\ 10^{-8}$	$4.5\ 10^{-5}$	2100
4j	$1.3\ 10^{-7}$	$7.9\ 10^{-5}$	610
4k[b]	$4.0\ 10^{-6}$	$>5.0\ 10^{-4}$	>130
4l	$2.2\ 10^{-8}$	$4.0\ 10^{-7}$	180
4m	$3.0\ 10^{-7}$	$3.0\ 10^{-7}$	1
4n	$8.9\ 10^{-9}$	$8.9\ 10^{-7}$	100
4o	$3.6\ 10^{-8}$	$3.6\ 10^{-8}$	1
4p	$5.4\ 10^{-9}$	$5.0\ 10^{-7}$	93
clorgyline	$5.3\ 10^{-9}$	$4.5\ 10^{-6}$	840
l-deprenyl	$1.3\ 10^{-6}$	$1.0\ 10^{-8}$	0.0077

[a]Mean values from at least 2 independent experiments. [b]These compounds were added in dimethylsulfoxide solution to the enzymatic reaction mixture.

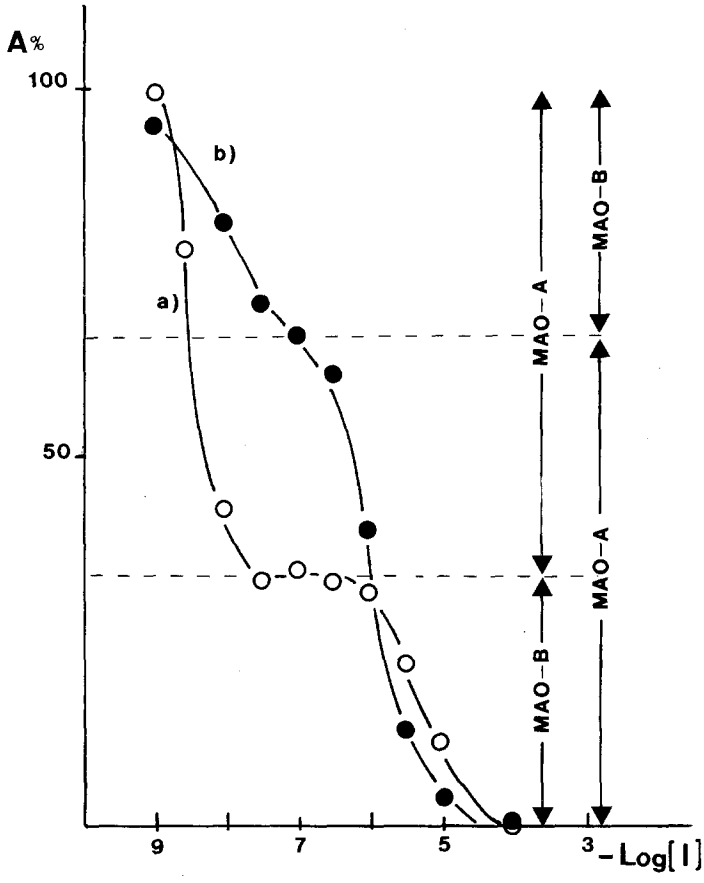


Fig 1. Inhibition of MAO-A and MAO-B by clorgyline and *l*-deprenyl. a) Clorgyline (MAO-A selective inhibitor). b) *l*-deprenyl (MAO-B selective inhibitor).

All the allenic and acetylenic compounds, with the exception of the bisacetylenic and bisallenic products (**4c, 4e, 4k** and **4m**) were potent inhibitors of MAO-A, with I_{50} values from 0.63 μM to 5.4 nM, and some of them (**4f, 4n** and **4p**) were as potent as clorgyline. In addition, all compounds, with the exception of **4o**, were selective for MAO-A. Compounds **4g** and **4i** were some 1400- and 2100-fold respectively, more active for MAO-A than MAO-B, and about twice more selective than clorgyline.

The lack of selectivity of the bisacetylenic and bisallenic products (**4c, 4e** and **4m**) and the weak inhibitory potency of these compounds and **4k** are probably due to steric effects in the active site of the enzyme. However, in the case of **4k** we found MAO-A selective behaviour, but the I_{50} value for MAO-B could not be determined due to the solubility limit of the product in the reaction mixture: about 0.5 mM.

Comparison of the I_{50} values of compounds **4a–4h** (R=H) with **4i–4p** (R=CH_3) showed that the inhibitory activity increased or remained about the same on the *N*-methylation of the indole moiety. However, the selectivity for the inhibition remained similar, increased or decreased depending on the compound considered. The introduction of a methyl group into the aminomethyl group (compounds **4a–4e** and **4i–4m** (R'=H) against **4f–4h** and **4n–4p** (R'=CH_3) increased the inhibitory activity, but the effect on selectivity was variable, depending on the compound considered. Also, we could not find any simple relationship between the characteristics of the reactive group (allene or acetylene) and potency or selectivity. The 2-propynyl and 2,3-butadienyl groups seemed to be better than the 2-butynyl group with regard to potency, but for selectivity 2-propynyl and 2-butynyl groups seemed to be better than the 2,3-butadienyl group. A practically similar type of behaviour has been observed in the previously studied series of 2-aminomethylindole [6] and 2-aminomethyl (1-methylindole) [7] derivatives.

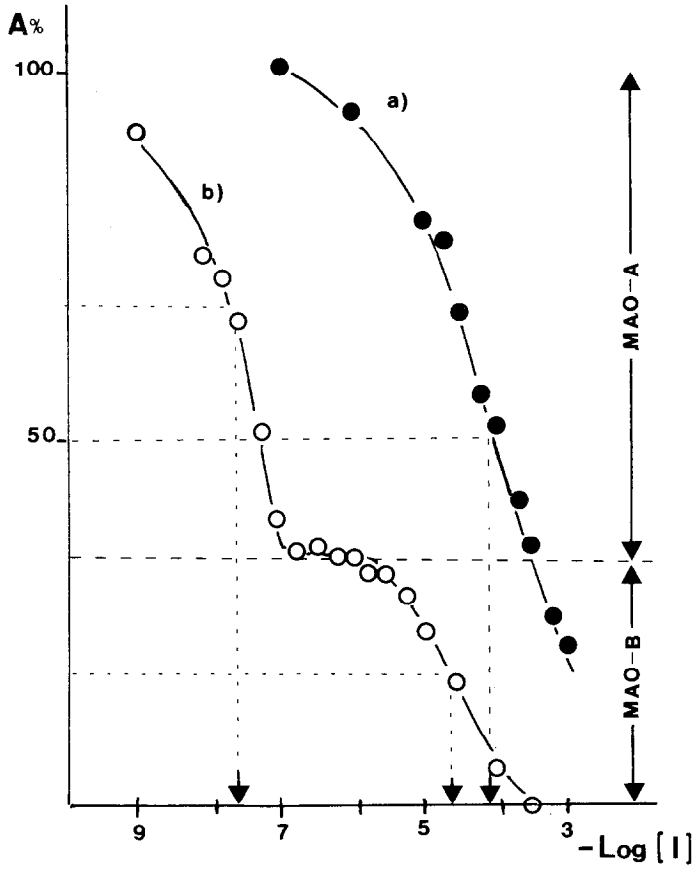


Fig 2. Representative examples of inhibition of MAO-A and MAO-B by 2-(5-methoxyindolyl)methylamine derivatives. a) Non selective compounds: **3d**. b) Selective compounds for MAO-A: **4i**.

As shown in table III, selectivity has been only observed with acetylenic and allenic derivatives, this being an unexpected result. On the other hand, it is well known [1, 3] that acetylenic and allenic amines react in an irreversible manner with the flavin component in the active site of the enzyme. All these facts suggest that the selectivity for MAO inhibition observed with acetylenic and allenic derivatives of indoleamines reported in this and previous papers [6, 7] is determined by the sequence of reactions between the reactive acetylenic or allenic group and the flavin component.

More detailed biochemical and pharmacological studies are in progress in our and other laboratories with the compounds reported in this paper and with related products [6, 7], to investigate the mechanism of inhibition, to establish quantitative structure–activity relationships and to assess the pharmacological and therapeutic potential of this new group of MAO inhibitors. The results of these studies will be published elsewhere. However, we can indicate in advance that at least two of the studied compounds have shown good potential antidepressant properties in animal models, without a significant 'cheese effect' at low active doses.

Experimental protocols

Chemistry

Melting points were determined on a Gallenkamp apparatus (capillary) or a Kofler apparatus (heating block) and are uncorrected. Elemental analyses were obtained for vacuum-dried samples on a Perkin–Elmer automatic analyzer, accurate within ± 0.4% for each indicated element. IR spectra were recorded on a Perkin–Elmer 657 spectrometer, using potassium bromide pellets or Nujol dispersion and the frequencies (ν) are given in cm^{-1}. ^1H NMR spectra were recorded on a Perkin–Elmer R-12 (60 MHz), a Varian EM 390 (90 MHz) or a Brucker Am-200 (200 MHz) instrument, in the indicated solvents, and the chemical shifts are given in δ (ppm) units with tetramethylsilane as internal standard. UV spectra were recorded in ethanol as solvent on a Perkin–Elmer 402 apparatus. Thin layer chromatography (TLC) were carried out on silica gel (Merck, DC-Alufolien, Kieselgel 60 F_{254}) with the solvent indicated in each case, and the plates were scanned under ultraviolet light, λ = 254 nm.

The following compounds were prepared according to previously reported procedures: ethyl 2-(5-methoxyindole)-carboxylate, 75%, mp 154° [lit [10], mp 156°] from *p*-anisidine and ethyl 2-methylacetoacetate by the Japp–Klingemann reaction [11]; ethyl 2-(5-methoxy-1-methylindole)-carboxylate, 93%, mp 95°, from 2-(5-methoxyindole)-carboxylate by methylation with dimethyl sulfate / K_2CO_3 in dry boiling acetone, as previously reported for similar compounds [12]. From the above esters we obtained the respective acids by hydrolysis with sodium hydroxide in boiling ethanol: 2-(5-methoxyindole)-carboxylic acid, 97%, mp 200°, [lit [13], mp 199.5°] and 2-(5-methoxy-1-methyl-

indole)carboxylic acid, 85%, mp 215° [lit [14], mp 215°]. 2-Butynyl bromide, bp 58°/60 mm [lit [15], 60°/80 mm] was obtained from 2-butynyl-1-ol [15] and phosphorus tribromide; 2,3-butadienyl bromide, bp 64–65°/180 mm [lit [16], bp 64–66°/181 mm] was obtained in a similar way [15, 16] from 2,3-butadienyl-1-ol [17]; 2-propynyl bromide was commercially available (Fluka AG).

General procedures for the synthesis of 2-(5-methoxyindole)carboxamides and 2-(5-methoxy-1-methylindole)-carboxamides ***(2a–2b)***

A solution of the corresponding acid (10 mmol) and phosphorus pentachloride (15 mmol) in dry ethyl ether (100 ml) was stirred at room temperature for about 12 h. Solvent was removed in vacuum, the residue treated with a new portion of dry ethyl ether and solvent removed in vacuum again. This operation was repeated twice with ethyl ether and then twice with chloroform. The crude acyl chloride obtained in this way was dissolved in ethyl ether (75 ml) and the solution cooled in an ice-bath. To the solution was added dropwise with stirring, concentrated ammonium hydroxide or 40% aqueous methylamine (20 mmol) in dioxane (25 ml). The mixture was stirred at room temperature for 3 h. Solvents were removed in vacuum, the residue treated with water and the solid material collected and dried. In this way the following compounds were obtained.

2-(5-Methoxyindole)carboxamide ***2a.*** From ammonium hydroxide and 2-(5-methoxyindole)carboxylic acid. Yield 90%, mp 205° (ethanol / water). IR (KBr): 3480 (NH-indole), 3440–3000 (NH_2), 1680, 1650 (C=O). ^{1}H NMR (DMSO–d_6): 3,80 (s, 3H, CH_3O); 6.80–8.10 (m, 6H, $CONH_2$ + 4H-aromat). UV (ethanol), λ (log ε): 223 (4.24), 294 (4.27). Analysis: $C_{10}H_{10}N_2O_2$ (C, H, N).

N-*Methyl-2-(5-methoxyindole)carboxamide* ***2b.*** From 2-(5-methoxyindole)-carboxylic acid and 40% aqueous methylamine. Yield 95%, mp 226° (ethanol). IR (Nujol): 3400 (NH-indole), 3300–3000 (NH_2), 1700, 1630 (C=O). ^{1}H NMR (DMSO–d_6); 2.90 (d, 3H, CH_3NH, J = 6 Hz); 3.75 (s, 3H, CH_3O); 6.80–7.60 (m, 4H-aromat); 8.30–8.70 (bs, 1H, CONH). UV (ethanol), λ (log ε): 222 (4.29), 294 (4.29). Analysis: $C_{11}H_{12}N_2O_2$ (C, H, N).

2-(5-Methoxy-1-methylindole)carboxamide ***2c.*** From 2-(5-methoxy-1-methylindole)carboxylic acid and ammonium hydroxide. Yield 98%, mp 220° (ethanol). IR (KBr): 3420–3200 (NH_2), 1650 (C=O). ^{1}H NMR (DMSO–d_6): 3.75 (s, 3H, CH_3O), 4.05 (s, 3H, CH_3N), 6.90–8.00 (m, 6H, $CONH_2$ + 4H-aromat). UV (ethanol), λ (log ε): 220 (4.42), 294 (4.26). Analysis: $C_{11}H_{12}N_2O_2$ (C, H, N).

N-*Methyl-2-(5-methoxy-1-methylindole)carboxamide* ***2d.*** From 2-(5-methoxy-1-methylindole)carboxylic acid and 40% aqueous methylamine. Yield 96%, mp 183° (ethanol). IR (KBr): 3700–3300 (NH), 1645 (C=O). ^{1}H NMR (DMSO–d_6): 2.85 (d, 3H, CH_3NH, J = 6 Hz), 3.75 (s, 3H, CH_3O), 4.05 (s, 3H, CH_3N), 6.90–7.70 (m, 4H-aromat), 8.30–8.70 (bs, 1H, CONH). UV (ethanol), λ (log ε): 222 (4.37), 293 (4.26). Analysis: $C_{12}H_{14}N_2O_2$ (C, H, N).

General procedure for the preparation of 2-(5-methoxyindolyl) methylamines and 2-(5-methoxy-1-methylindolyl)methyl-amines ***(3a–3d)***

Compounds ***3a, 3c*** *and* ***3d.*** A suspension of lithium aluminium hydride (0.6 mol) in dry tetrahydrofuran (1000 ml) was cooled at 0° and a solution of the respective amide **2a, 2c** or **2d** (0.1 mol) in dry tetrahydrofuran was added dropwise with stirring (occasionally we used the Sohxlet technique for this addition). The temperature was allowed to rise and when the addition was finished the mixture was boiled to complete reduction of the amide (5–12 h, controlled by TLC with chloroform / methanol, 6:1 v / v as eluent). The mixture was cooled in an ice-bath and, with stirring, was hydrolyzed by careful dropwise addition of water. The aluminium hydroxide was removed by centrifugation and the precipitate extracted twice with tetrahydrofuran. Solvent was removed in vacuum from the collected extracts and the residue dissolved in ether. The amines were generally collected as their hydrochlorides by addition of dry 2 M HCl / ethanol solution to the dry ethereal solution. The analytical and spectral data of the obtained compounds are summarized in tables I and II.

N-*2-(5-Methoxyindolyl)methylamine* ***3b***

N-*Ethoxycarbonyl-2-(5-methoxyindolyl)methylamine.* To a suspension of 2-(5-methoxyindolyl)methylamine (5.28 g, 30 mmol) in 2 N sodium hydroxide (30 ml), ethyl ether (30 ml) was added. The mixture was cooled at 0° and a solution of ethoxycarbonyl chloride (3.9 g, 36 mmol) in dry ether (15 ml) was added dropwise with stirring. The temperature was allowed to rise and stirring was continued for 4 h at room temperature. When the reaction was complete (TLC, ether as the eluent), the organic layer was separated and the aqueous solution was extracted twice with ether. The combined organic extracts were washed with water, dried (Na_2SO_4) and the solvent removed in vacuum. Yield 7.03 g (95%), mp 51° (from toluene / petroleum ether). IR (KBr): 3420 (NH), 1690 (C=O), 1030 (C–O). ^{1}H NMR (Cl_3CD): 1.23 (t, 3H, CH_3–C, J = 7 Hz), 3.83 (s, 3H, CH_3O), 4.15 (c, 2H, CH_2, J = 7 Hz), 4.36 (d, 2H, CH_2N, J = 3 Hz), 5.20 (bs, 1H, CONH), 6.23 (s, 1H, H–3), 6.80 (dd, 1H, H–6, J = 9 Hz and 1.5 Hz), 7.00 (d, 1H, H–4, J = 1.5 Hz), 7.17 (d, 1H, H–7, J = 9 Hz), 8.50–8.85 (bs, 1H, NH-indole). UV (ethanol), λ (log ε): 222 (4.20), 271 (3.87), 295 (3.72), 305 (3.59). Analysis: $C_{13}H_{16}N_2O_3$ (C, H, N).

N-*Methyl-2-(5-methoxyindolyl)methylamine* ***3b.*** N-Ethoxycarbonyl-2-(5-methoxyindolyl)methylamine (9.0 g, 36 mmol) was reduced with lithium aluminium hydride (180 mmol) in boiling tetrahydrofuran (300 ml) for 2 h as described above for the amides **2.** The compound was characterized as hydrochloride. Yield 7.8 g (96%). The properties of the compound are given in Tables I and II.

General procedure for the synthesis of allenic and acetylenic derivatives of 2-(5-methoxyindolyl)methylamines and N-*methyl-2(5-methoxy-1-methylindolyl)methylamines* ***4a–4p***

A solution of the respective amine 3 (1.0 mmol) in dry tetrahydrofuran (50 ml) and *tert*-butylamine (1.5 mmol) was cooled at 0–5°. To the stirred mixture, a solution of the appropriated allenic or acetylenic bromide (2,3-butadienyl-, 2-proponyl- and 2-butynyl bromides were employed) (1.0 mM) in tetrahydrofuran (15 ml) was added dropwise. The mixture was stirred at room temperature until no further evolution was observed by TLC. Then, the solvent was removed in vacuum and the residue treated with water and extracted with ethyl ether. The concentrated etheral solution was fractionated by column chromatography (silica gel 0.063–0.20 mm, Merck 7734 or 0.04–0.063 Merck 9385). The order of elution was always: bisalkylation product (only starting with **3a** or **3c**), monoalkylation product and finally the residual starting amine. The following eluents were employed: ethyl ether for **4a–4c**, **4f–4h** and **4n**; ethyl ether / benzene (10:1 v/v) for **4i–4m**; ethyl

ether / methanol (2:1 v/v) for **4d** and **4e**; ethyl ether / toluene (3:1 v/v) for **4o**; ethyl ether / toluene (1:1 v/v) for **4p**. Solvents were removed in vacuum from the collected solution of the respective amine and the residue dissolved in dry ethyl ether. The corresponding hydrochlorides or oxalates were prepared by addition to the respective dry ethereal solution of a 2 N HCl / dry ethanol solution or anhydrous oxalic acid in ethyl ether, respectively. Analytical and spectral data of compounds **4** are summarized in tables I and II.

Enzymatic assays

Mitochondria from bovine brain were prepared as described [18] and dispersed in ice-cold (≈ 4°C) 5 mM potassium phosphate buffer, pH 7.3, to give a preparation containing some 68 mg / ml of protein, determined by the microbiuret method [19], using bovine serum albumin as standard. This suspension was divided into aliquots and kept at –20° until needed. For standardization, MAO-B activity was estimated with benzylamine•HCl as the substrate by the spectrophotometric method or Tabor [20]. Immediately prior to use, the mitochondrial suspension was thawed and diluted with 5 mM potassium phosphate buffer, pH 7.3, to give a suspension containing 2.0 Tabor units / ml and 1.4 mg / ml of protein for each assay. This suspension was preincubated for 20 min at 37° with different inhibitor concentrations (from 0.01 nM–1.0 mM), the inhibitor being dissolved in water or dimethylsulfoxide according to its solubility. The remaining activity was determined at 37° for 30 min with 0.5 mM ^{14}C-tyramine•HCl (0.8 Ci / mol, Amersham) as the substrate in a final volume of 0.5 ml, in 30 mM potassium phosphate buffer pH 7.3. The reaction was stopped by the addition to the reaction mixture of 0.2 ml of 1 M perchloric acid or 2.5 M citric acid, and the products were extracted into toluene / ethyl acetate (1:1 v/v) [8, 9]. Aliquots of this extract were treated in the usual way and submitted for scintillation counting.

In order to determine the proportion of MAO-A and MAO-B within the enzyme preparations, similar assays were carried out with clorgyline and *l*-deprenyl as inhibitors. Figure 1 is illustrative of this control. The mitochondrial preparations of bovine brain always contained ≈30–35% of activity for MAO-B and 65–70% of activity for MAO-A.

In all the enzymatic assays initial velocities of product formation were measured, as the assays were carried out under conditions with good linear plots between product formation against reaction time and against enzyme concentration. Also, when the inhibitor was incorporated to the reaction mixture dissolved in dimethylsulfoxide, it was verified that this solvent does not affect the enzymatic activity at the concentrations employed (maximum 4% v/v).

Molar concentrations of the inhibitor producing 50% of inhibition (I_{50}) were determined graphically as illustrated in figures 1 and 2 and are presented in table III.

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