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Original paper

Inhibition of monoamine oxidase types A and B by 2-aryl-4*H*-1,3,4-oxadiazin-5(6*H*)-one derivatives

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Summary — Monoamine oxidase (MAO) assay specificity based on substrate specificity was investigated by substrate competition experiments. 10 μM serotonin (5-HT) and 5 μM β-phenylethylamine (PEA) were found to ensure total substrate specificity for, respectively, MAO types A and B.

Twenty-five 2-aryl-4*H*-1,3,4-oxadiazin-5(6*H*)-one derivatives were synthesized and tested *in vitro* for their inhibitory effects on MAO A and B. Most of them inhibited preferentially MAO B. The 2-(4-biphenylyl)-4-(2-cyanoethyl)-4*H*-1,3,4-oxadiazin-5(6*H*)-one **32** was the most efficient MAO B inhibitor and acted as a competitive inhibitor on the two enzymes. Its K_i values for MAO A and B were 11 and 0.15 μM, respectively.

Structure—activity relationships suggest that these oxadiazinones should interact with a hydrophobic site and a nucleophilic site on MAO B for binding, while the functional group of the N-4 substituent should compete with the substrate for the active site of the enzyme.

Résumé — **Inhibition des monoamine oxydases A et B par des dérivés d'aryl-2 4*H*-oxadiazine-1,3,4 (6*H*) ones-5.** *La spécificité du dosage de la monoamine oxydase (MAO) a été étudiée par des expériences de compétition. Les concentrations de 10 μM pour la 5-HT et de 5 μM pour la PEA assurent une spécificité totale du dosage respectivement pour les MAO A et B.*

Vingt cinq dérivés d'aryl-2 4H-oxadiazine-1,3,4(6H)ones-5 ont été synthétisés et testés in vitro *pour leur activité inhibitrice sur les MAO A et B. La plupart de ces composés inhibent préférentiellement la MAO B. La (biphénylyl-4)-2 (cyano-2 éthyl)-4 4H-oxadiazine-1,3,4(6H)one-5* **32** *est l'inhibiteur le plus efficace de la série sur la MAO B et agit de façon compétitive sur les deux types d'enzyme. Les valeurs des K_i pour ce composé* **32** *vis-à-vis des MAO A et B sont égales respectivement à 11 et 0,15 μM.*

Les relations structure—activité suggèrent que des interactions avec un site hydrophobe et un site nucléophile de la MAO B interviendraient dans la fixation de l'inhibiteur alors que le groupe fonctionnel du substituant en position 4 entrerait en compétition avec le substrat pour le site actif de l'enzyme.

reversible monoamine oxidase inhibitors / 4*H*-1,3,4-oxadiazin-5(6*H*)-ones

Introduction

Monoamine oxidase (MAO, EC 1.4.3.4) is the enzyme responsible, in the central nervous system, for the metabolic inactivation of amine neurotransmitters, serotonin (5-HT), noradrenaline (NA) and dopamine (DA), which are implicated in many neurological and psychiatric disorders [1]. Since Johnston's discovery in 1968 of two types of MAO, called MAO A and MAO B [2], a resurgence in interest has occurred in all aspects of MAO and, especially, in MAO inhibitors as potential therapeutic agents in depressive illnesses or Parkinson's disease. More particularly, a new approach has been devised in the last past decade and consists of developing a new generation of MAO inhibitors mostly selective as well as reversible or short-acting [3].

Following these criteria, many MAO A inhibitors have been obtained: toloxatone [4, 5], MD 780515 (cimoxatone) [6—9], FLA 336+ and its derivatives [10, 11], Ro 11-1163 (moclobemide) [12, 13], CGP 11305 A (bromofaremine) [14, 15], 5-FMT [16], SR 95191 [17, 18], RS-2232 [19, 20], bifemelane [21]. However, only a few reversible or short-acting MAO B inhibitors have been described: the racemic MD 780236 and its two enantiomers [22, 23], p-CMP

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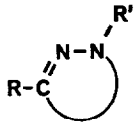
Abbreviations : **MAO:** monoamine oxidase; **5-HT:** serotonin; **DA:** dopamine; **NA:** noradrenaline; **PEA:** β-phenylethylamine.

[16], Ro 16-6491 [24], the later being the most selective.

Such inhibitors are believed to be safer in therapy than the first known ones, acyclic hydrazine derivatives, which were discarded because of their hepatic toxicity and mainly because of their blood pressure potentiating effect of indirectly activating sympathomimetic amines, *e.g.*, tyramine on the sympathetic nervous system leading to severe hypertensive crisis, namely the 'cheese effect' [25]. This last side effect seemed directly related to the non-selectivity of these inhibitors and, to a lesser degree, to their irreversibility towards the two enzymes [26].

Furthermore, from preliminary clinical results, it now seems well established that selective MAO A inhibitors are effective drugs for the treatment of 5-HT and NA deficits observed in depressive illnesses [1, 27], whereas selective MAO B inhibitors, when combined with the DA precursor L-dopa, are useful for the management of DA depletion in Parkinson's disease [1, 28—31].

Considering that the N—N group was implicated in the activity of the hydrazinic MAO inhibitors, it seemed interesting to examine whether heterocycles containing the N—N group and related to the following general structure could afford new classes of MAO inhibitors.



We report, in this first paper, the inhibitory activity *in vitro* of new 4*H*-1,3,4-oxadiazin-5(6*H*)-one derivatives on the two types of MAO and the structural requirements in this series for maximal inhibition and selectivity towards MAO B.

Chemistry

Van Alphen's method [32, 33] was used to synthesize 4*H*-1,3,4-oxadiazin-5(6*H*)-ones. According to this method, 1-acyl-2-(α-chloroacyl)hydrazines were cyclized in alkaline solution (Scheme 1).

These hydrazines were prepared by reaction of acylhydrazines with an α-chloroacyl chloride. This reaction was applied to aroylhydrazines and afforded hydrazines **1—5** (Table I). It was also applied to 1-aroyl-2-(2-cyanoethyl)hydrazines **6—9** (Table I) which were first prepared by Michael reaction of acrylonitrile with aroylhydrazines.

A few examples of this type of Michael reaction have been reported [37] but the simultaneous formation of secondary products was not mentioned. These products, detected by analytical chromatography, were isolated and identified as 1-aroyl-2,2-bis(2-cyanoethyl)hydrazines **10—13** (Table I). They resulted, in fact, from the addition of a second molecule of acrylonitrile to hydrazines **6—9**. In particular, when acrylonitrile was in excess, only compounds **10—13** were isolated.

2-Aryl-4*H*-1,3,4-oxadiazin-5(6*H*)-ones **14—18** (Table I) were synthesized by cyclization of hydrazines **1—5** on treatment with sodium bicarbonate in dry dimethylformamide (DMF). In this case, tarry products were formed from secondary reactions and low yields were obtained. However, when 2-substituted 1-acylhydrazines were cyclized to the corresponding 2,4-disubstituted oxadiazinones, better yields were observed.

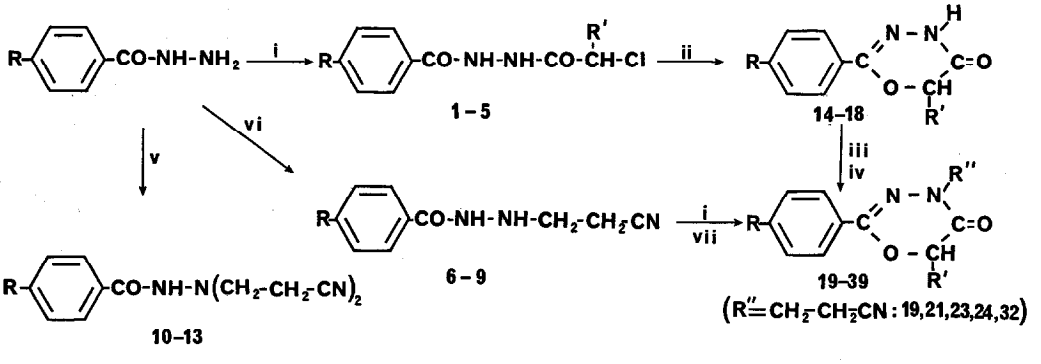
4-Substituted 2-aryl-4*H*-1,3,4-oxadiazin-5(6*H*)-ones **19—39** (Tables II) were generated either by classical *N*-alkylation of oxadiazinones **14—18** or by Van Alphen's reaction applied to substituted hydrazines **6—9** when the N-4 substituent was a 2-cyanoethyl group. *N*-Alkylation was achieved by the successive reaction of **14—18** with sodium hydride and a halogen derivative. When the latter was 3-chloropropionitrile, an acrylonitrile elimination occured and yields were very low.

Physicochemical data were in agreement with the proposed structures (Tables I, II and III).

Results and Discussion

MAO assay specificity

The MAO assay specificity, based on 5-HT specificity for MAO A and PEA specificity for MAO B, was investigat-



Scheme 1. Reagents: i: Cl—CO—CH(R′)—Cl; ii: NaH CO_3; iii: NaH, DMF; iv: R″X; v: $2CH_2 = CH—CN$; vi: $CH_2 = CH—CN$; vii: K_2CO_3.

Table I.

1-Aroyl-2-(α-chloroacyl)hydrazines 1-5 R-C6H4-CO-NH-NH-CO-CH(R')-Cl

N°	R	R'	mp (°C)	Rec. S.[a]	Yield (%)[b]	Formula	PMR δ (ppm)
1	H	H	165[c]	A	85	$C_9H_9ClN_2O_2$	4.2 (s, 2H), 7.4-7.9 (m, 5H), 10.3 and 10.4 (2s, 2H)
2	4-MeO	H	188[d]	B	90	$C_{10}H_{11}ClN_2O_3$	3.8 (s, 3H), 4.3 (s, 2H), 7.9-8.6 (m, 4H), 10.4-11 (m, 2H)
3	4-NO_2	H	204	C	82	$C_9H_8ClN_3O_4$	4.3 (s, 2H), 8.1 and 8.4 (2d, 4H), 10.6 and 11 (2s, 2H)
4	4-NO_2	Me	212	B	84	$C_{10}H_{10}ClN_3O_4$	1.6 (d, 3H), 4.75 (q, 1H), 8.1 and 8.4 (2d, 4H), 10.3-10.8 (m, 2H)
5	4-Ph	H	244	C/D	76	$C_{15}H_{13}ClN_2O_2$	4.2 (s, 2H), 7.3-8.1 (m, 9H), 10.2-10.6 (m, 2H)

1-Aroyl-2-(2-cyanoethyl)hydrazines 6-9 R-C6H4-CO-NH-NH-CH2-CH2-CN

N°	R	mp (°C)	Rec. S.[a]	Yield (%)[b]	Formula	PMR δ (ppm)
6	H	121[e]	A	65	$C_{10}H_{11}N_3O$	2.7 (t, 2H), 3 (q, 2H), 5.5 (q, 1H), 7.4-8.1 (m, 5H), 10.2 (d, 1H)
7	4-MeO	137	C	27	$C_{11}H_{13}N_3O_2$	2.7 (t, 2H), 3.1 (q, 2H), 3.9 (s, 3H), 5.5 (bs, 1H), 7.1 and 7.9 (2d, 4H), 10 (bs, 1H)
8	4-NO_2	162[f]	E	72	$C_{10}H_{10}N_4O_3$	2.7 (t, 2H), 3.2 (q, 2H), 5.7 (q, 1H), 7.7-8.6 (m, 4H), 10.6 (d, 1H)
9	4-Ph	174	C/F	51	$C_{16}H_{15}N_3O$	2.7 (t, 2H), 3 (q, 2H), 5.5 (q, 1H), 7.3-8.1 (m, 9H), 10.2 (d, 1H)

1-Aroyl-2,2-bis(2-cyanoethyl)hydrazines 10-13 R-C6H4-CO-NH-N(CH2-CH2-CN)2

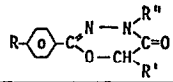
N°	R	mp (°C)	Rec. S.[a]	Yield (%)[b]	Formula	PMR δ (ppm)
10	H	145.5	A	88	$C_{13}H_{14}N_4O$	2.7 (t, 4H), 3.3 (t, 4H), 7.6-8.1 (m, 5H), 9.8 (s, 1H)
11	4-MeO	141	A	53	$C_{14}H_{16}N_4O_2$	2.7 (t, 4H), 3.3 (t, 4H), 3.9 (s, 3H), 7 and 7.8 (2d, 4H), 9.5 (s, 1H)
12	4-NO_2	146-7	C	72	$C_{13}H_{13}N_5O_3$	2.7 (t, 4H), 3.3 (t, 4H), 8.1 and 8.4 (2d, 4H), 10 (s, 1H)
13	4-Ph	192.5	A/G	58	$C_{19}H_{18}N_4O$	2.7 (t, 4H), 3.3 (t, 4H), 7.3-8 (m, 9H), 9.7 (s, 1H)

2-Aryl-1,3,4-oxadiazin-5(4H)-ones 14-18 R-C6H4-C(=N-N(H)-C(=O)-O-CH(R'))

N°	R	R'	mp (°C)	Rec. S.[a]	Yield (%)[b]	Formula	PMR δ (ppm)
14	H	H	160[g]	B	35	$C_9H_8N_2O_2$	4.8 (s, 2H), 7.2-8 (m, 5H), 11.2 (s, 1H)
15	4-MeO	H	158[h]	C/F	31	$C_{10}H_{10}N_2O_3$	3.8 (s, 3H), 4.7 (s, 2H), 6.9 and 7.6 (2d, 4H), 10.7 (s, 1H)
16	4-NO_2	H	230[i]	H	65	$C_9H_7N_3O_4$	4.9 (s, 2H), 8 and 8.3 (2d, 4H), 11.2 (s, 1H)
17	4-NO_2	Me	170	C	63	$C_{10}H_9N_3O_4$	1.6 (d, 3H), 5 (q, 1H), 8 and 8.3 (2d, 4H), 11.1 (s, 1H)
18	4-Ph	H	216	B	53	$C_{15}H_{12}N_2O_2$	4.8 (s, 2H), 7.4-8.1 (m, 9H), 11.2 (s, 1H)

[a]Recrystallization solvent: A = water; B = ethanol; C = ethyl acetate; D = petroleum ether 40—60; E ‖ methanol; F = diethyl ether; G = 1-propanol; H = 1-butanol.
[b]Yields were not optimized.
[c]Lit. [33], mp: 165°C.
[d]Lit. [34], mp:185—188°C.
[e]Lit. [35], mp: 122—124°C.
[f]Lit. [36], mp: 164.5—166°C.
[g]Lit. [33], mp: 161°C.
[h]Lit. [34], mp: 161—163°C.
[i]Lit. [38], mp: 220—222°C.

Table II. 4-Substituted 2-aryl-1,3,4-oxadiazin-5(4*H*)-ones **19—39.**



N°	R	R'	R"	mp (°C)	Rec. S.[a]	Yield (%)[b]	Formula	PMR δ (ppm)
19	H	H	$(CH_2)_2CN$	112-4	A	57	$C_{12}H_{11}N_3O_2$	2.9 (t, 2H), 3.9 (t, 2H), 4.8 (s, 2H), 7.4-7.9 (m, 5H)
20	H	H	$(CH_2)_4CN$	66-7	B/C	57	$C_{14}H_{15}N_3O_2$	1.6-2 (m, 4H), 2.7 (t, 2H), 3.8 (t, 2H), 4.9 (s, 2H), 7.4-8.1 (m, 5H)
21	4-MeO	H	$(CH_2)_2CN$	167-8	C/D	77	$C_{13}H_{13}N_3O_3$	3 (t, 2H), 3.9 (s, 3H), 4 (t, 2H), 4.9 (s, 2H), 7.1 and 7.8 (2d, 4H)
22	4-MeO	H	$(CH_2)_4CN$	86	E	83	$C_{15}H_{17}N_3O_3$	1.5-1.9 (m, 4H), 2.6 (t, 2H), 3.7 (t, 2H), 3.8 (s, 3H), 4.8 (s, 2H), 7.1 and 7.8 (2d, 4H)
23	4-NO_2	H	$(CH_2)_2CN$	161.5	C/D	73	$C_{12}H_{10}N_4O_4$	3 (t, 2H), 4 (t, 2H), 5 (s, 2H), 8.1 and 8.4 (2d, 4H)
24	4-NO_2	Me	$(CH_2)_2CN$	123	F/G	76	$C_{13}H_{12}N_4O_4$	1.5 (d, 3H), 3 (t, 2H), 4 (t, 2H), 5 (q, 1H), 7.8-8.4 (m, 4H)
25	4-NO_2	H	$(CH_2)_4CN$	74	A	86	$C_{14}H_{14}N_4O_4$	1.5-1.9 (m, 4H), 2.6 (t, 2H), 3.8 (t, 2H), 4.9 (s, 2H), 8 and 8.3 (2d, 4H)
26	4-NO_2	Me	$(CH_2)_4CN$	64	F/G	74	$C_{15}H_{16}N_4O_4$	1.5 (d, 3H), 1.6-2 (m, 4H), 2.6 (t, 2H), 3.9 (t, 2H), 5 (q, 1H), 7.8-8.4 (m, 4H)
27	4-NO_2	H	$(CH_2)_4Br$	88	E	91	$C_{13}H_{14}BrN_3O_4$	1.6-2.1 (m, 4H), 3.4-4.1 (m, 4H), 5 (s, 2H), 8.1 and 8.4 (2d, 4H)
28	4-NO_2	H	$(CH_2)_4COOMe$	104	E	74	$C_{15}H_{17}N_3O_6$	1.4-1.8 (m, 4H), 2.4 (t, 2H), 3.6 (s, 3H), 3.7 (t, 2H), 4.9 (s, 2H), 8 and 8.3 (2d, 4H)
29	4-Ph	H	Et	125	H	71	$C_{17}H_{16}N_2O_2$	1.15 (t, 3H), 3.7 (q, 2H), 4.9 (s, 2H), 7.4-8 (m, 9H)
30	4-Ph	H	n-Pr	93-4	H	51	$C_{18}H_{18}N_2O_2$	0.9 (t, 3H), 1.3-1.8 (m, 2H), 3.7 (t, 2H), 4.9 (s, 2H), 7.4-8 (m, 9H)
31	4-Ph	H	CH_2CN	124-5	I	60	$C_{17}H_{13}N_3O_2$	4.9 (s, 2H), 5 (s, 2H), 7.4-8 (m, 9H)
32	4-Ph	H	$(CH_2)_2CN$	146-7	H/J	66	$C_{18}H_{15}N_3O_2$	3 (t, 2H), 4 (t, 2H), 4.9 (s, 2H), 7.3-8.1 (m, 9H)
33	4-Ph	H	$(CH_2)_3CN$	115-6	I	88	$C_{19}H_{17}N_3O_2$	1.9-2.2 (m, 2H), 2.6 (t, 2H), 3.9 (t, 2H), 4.9 (s, 2H), 7.4-7.9 (m, 9H)
34	4-Ph	H	$(CH_2)_4CN$	91-2	I/K	93	$C_{20}H_{19}N_3O_2$	1.5-2 (m, 4H), 2.6 (t, 2H), 3.8 (t, 2H), 4.9 (s, 2H), 7.3-8 (m, 9H)
35	4-Ph	H	$(CH_2)_2Br$	127	H	63	$C_{17}H_{15}BrN_2O_2$	3.8 (t, 2H), 4.15 (t, 2H), 4.9 (s, 2H), 7.5-8.1 (m, 9H)
36	4-Ph	H	$(CH_2)_3Br$	114	G/K	22	$C_{18}H_{17}BrN_2O_2$	2-2.3 (m, 2H), 3.6 (t, 2H), 3.8 (t, 2H), 4.8 (s, 2H), 7.3-7.9 (m, 9H)
37	4-Ph	H	$(CH_2)_4Br$	98	I/K	84	$C_{19}H_{19}BrN_2O_2$	1.7-2 (m, 4H), 3.4-3.9 (m, 4H), 4.9 (s, 2H), 7.3-8.1 (m, 9H)
38	4-Ph	H	$(CH_2)_2COOMe$	123	H	53	$C_{19}H_{18}N_2O_4$	2.7 (t, 2H), 3.6 (s, 3H), 4 (t, 2H), 4.9 (s, 2H), 7.4-8 (m, 9H)
39	4-Ph	H	$(CH_2)_2OH$	154	G/K	46	$C_{17}H_{16}N_2O_3$	3.3 (t, 2H), 3.7 (t, 2H), 4.5-5 (bs, 1H), 4.8 (s, 2H), 7.3-7.9 (m, 9H)

[a]Recrystallization solvent: A = methanol; B = 1-propanol; C = water; D = acetonitrile; E = 1-butanol; F = diethyl ether; G = petroleum ether 40—60; H = cyclohexane; I = ethanol; J = xylene; K = ethyl acetate.
[b]Yields were not optimized.

Table III. IR frequencies (cm^{-1}).

No.	N—H	C—N	C=O	C=N cyclic
1—5	3250—3200	—	1690—1680 1650—1615	—
6—9	3380—3270 3280—3240	2260—2220	1640—1620	—
10—13	3280—3200	2240	1650—1620	—
14—18	3280—3200	—	1690—1675	1660—1620
19—39	—	—	1680—1640	1660—1620

ed since substrate specificity for one type of MAO depends upon the substrate concentration when the two enzymes are present [39]. Substrate competition experiments were performed in order to estimate the non-specific interactions (Table IV).

In MAO A assays with 10 μM 5-HT*, 100% activity was observed in the presence of 5 μM unlabeled PEA (line 2 compared to line 1) indicating the absence of MAO A—5 μM PEA and MAO B—10 μM 5-HT* interactions. In other words, neither 5 μM PEA competes with 10 μM 5-HT* for MAO A nor 10 μM 5-HT* with 5 μM PEA

Table IV. Substrate competition experiments.

MAO A (labeled 5-HT* deamination)			MAO B (labeled PEA* deamination)		
5-HT* (μM)	PEA (μM)	MAO activity (%)	PEA* (μM)	5-HT (μM)	MAO activity (%)
10	0	100	5	0	100
10	5	100	5	10	100
10	50	66	5	100	95
10	500	13	5	1000	70
100	0	100			
100	5	95			

MAO activity was measured under standard conditions (Experimental protocols) in the absence or presence of different concentrations of the unspecific unlabeled-substrate. Unspecific unlabeled-substrate and specific labeled-substrate were added simultaneously. Specific activity of 100 μM 5-HT* was 0.48 μCi/μmol. MAO activity was expressed as % of the control.

for MAO B. This result was confirmed in MAO B assays (line 2 compared to line 1).

In the absence of MAO A—5 μM PEA* interactions in MAO B assays, the decrease of MAO B activity, with the increase of unlabeled 5-HT will reflect only the MAO B—5-HT interactions, suggesting more likely an MAO B contribution in the 5-HT deamination rather than a MAO B inhibition by 5-HT, at least for 5-HT concentrations lower than K_m^{5-HT} (MAO B) = 1170 μM [40]. Thus, with 100 μM 5-HT ($\approx K_m^{5-HT}$ (MAO A), line 3) the contribution of MAO B was 5%. This result was confirmed in MAO A assays with 100 μM 5-HT* (line 2 compared to line 1).

Moreover, deprenyl (selective MAO B inactivator [41]) inhibited 100 μM 5-HT deamination in a biphasic manner, suggesting the participation of the 2 enzymes, whereas a single sigmoid was obtained with 10 μM 5-HT, indicating that only MAO A was implicated (Fig. 1B). Analysis of these MAO inhibition curves suggested that MAO B participation in the 100 μM 5-HT deamination was 3—4%, which is almost in accordance with the above value of 5%. However, for unknown reasons, less concluding results were obtained with clorgyline (selective MAO A inactivator [2]) inhibition curves (Fig. 1A). In this case, the MAO B contribution was found to be only 2%.

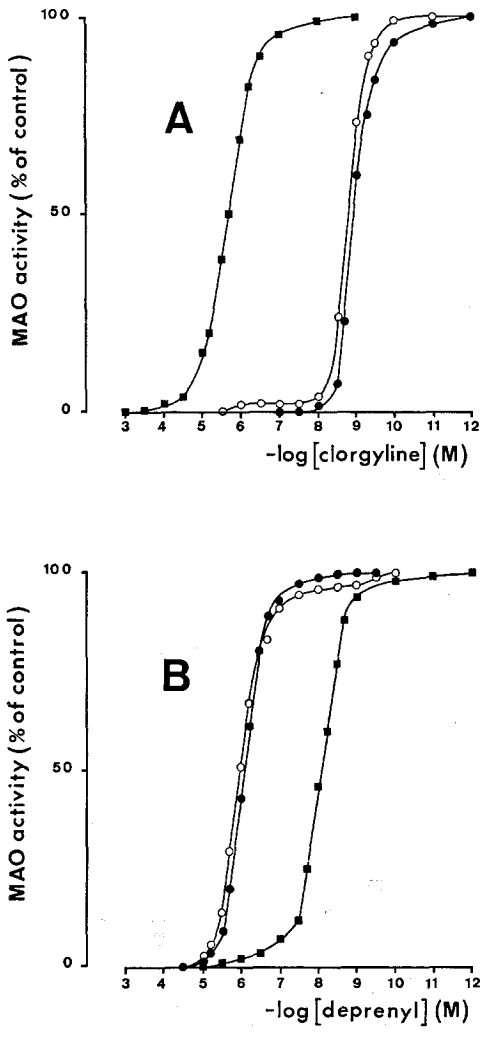


Fig. 1. Inhibition of rat brain MAO by clorgyline and deprenyl. MAO activity was measured under standard conditions (Experimental protocols) with using 10 (●) or 100 μM 5-HT (○) or 5 μM PEA (■) as substrates in the absence or presence of different concentrations of clorgyline (**A**) or deprenyl (**B**). Specific activity of 100 μM 5-HT was 0.48 μCi/μmol. MAO activity was expressed as % of the control.

In conclusion, substrate competition experiments seem to be more appropriate than MAO inhibition curve studies for the evaluation of enzyme selectivity in the MAO assays, since experimental values were more reproducible. In addition, complete specificity of MAO assays was ensured in our system when using 10 μM 5-HT* for MAO A or 5 μM PEA* for MAO B. Other authors have reported the specificity of these substrate concentrations [42].

On the other hand, a higher decrease of activity occurred with unlabeled PEA than with 5-HT in these MAO assays (Table IV, lines 3 and 4 in MAO A assays compared to the corresponding lines in MAO B assays) revealing a higher interference of MAO A in PEA deamination than of MAO B in 5-HT deamination. This is in accordance with the low specificity of 5-HT towards MAO A (K_m^{5-HT} (MAO B)/K_m^{5-HT} (MAO A) $\approx$ 11) compared to the greater specificity of PEA towards MAO B (K_m^{PEA} (MAO A)/K_m^{PEA} (MAO B) $\approx$ 40) (Table V).

Table V. Substrate specificity towards MAO types A and B.

Substrate	K_m(MAO A) (μM)	K_m(MAO B) (μM)	Specificity
5-HT	105	1170[a]	11
PEA	158[b]	4	41

K_m(MAO A) and K_m(MAO B) were calculated graphically, respectively, from Figs. 3A and 4A. 5-HT specificity towards MAO A and PEA specificity towards MAO B were estimated, respectively, from the ratio $K_m^{5\text{-HT}}$ (MAO B)/$K_m^{5\text{-HT}}$ (MAO A) and the ratio K_m^{PEA} (MAO A)/K_m^{PEA} (MAO B).
[a]Literature value [40].
[b]Literature value [8].

Selectivity of the inhibitors

Twenty-five 2-aryl-4*H*-1,3,4-oxadiazin-5(6*H*)-one derivatives were tested for their abilities to inhibit MAO A and B, using the MAO standard assays (Experimental protocols).

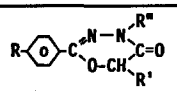
I_{50} (MAO A) and I_{50} (MAO B) values of these compounds (Table VI) were determined graphically from the corresponding MAO inhibition curves (Experimental protocols). I_{50} values of clorgyline and deprenyl were given solely for information and not for comparison purposes, as clorgyline and deprenyl are inactivators, whereas several oxadiazinones are thought to act as reversible inhibitors (see below). Inhibitor selectivity for MAO B was estimated from the ratio [I_{50} (MAO A)/I_{50} (MAO B)] (Table VI).

It should be observed that for competitive inhibitors, I_{50} was correlated with K_i, according to the rearranged Henri—Michaelis—Menten equation:

$$[I_{50} = (1 + [S]/K_m) \times K_i]$$

where S is the substrate. From this relationship and the

Table VI. Activity and selectivity of oxadiazinones **14—16, 18—39** towards MAO A and B.



N°	R	R'	R"	I_{50}(MAO A) (μM)	I_{50}(MAO B) (μM)	$\frac{I_{50}(\text{MAO A})}{I_{50}(\text{MAO B})}$
14	H	H	H	17	47	0.36
15	4-MeO	H	H	31	70	0.44
16	4-NO_2	H	H	140	130	1.1
18	4-Ph	H	H	94	6.7	14
19	H	H	$(CH_2)_2CN$	6.6	56	0.12
20	H	H	$(CH_2)_4CN$	36	67	0.54
21	4-MeO	H	$(CH_2)_2CN$	50	250	0.20
22	4-MeO	H	$(CH_2)_4CN$	0.92	17	0.05
23	4-NO_2	H	$(CH_2)_2CN$	230	4.5	51
24	4-NO_2	Me	$(CH_2)_2CN$	1800	2500	0.72
25	4-NO_2	H	$(CH_2)_4CN$	18	16	1.1
26	4-NO_2	Me	$(CH_2)_4CN$	380	100	3.8
27	4-NO_2	H	$(CH_2)_4Br$	50	38	1.3
28	4-NO_2	H	$(CH_2)_4COOMe$	90	23	3.9
29	4-Ph	H	Et	14	3.9	3.6
30	4-Ph	H	n-Pr	66	12	5.5
31	4-Ph	H	CH_2CN	1.1	0.21	5.2
32	4-Ph	H	$(CH_2)_2CN$	32	0.63	51
33	4-Ph	H	$(CH_2)_3CN$	14	5	2.8
34	4-Ph	H	$(CH_2)_4CN$	19	18	1.1
35	4-Ph	H	$(CH_2)_2Br$	38	24	1.6
36	4-Ph	H	$(CH_2)_3Br$	7.6	3.4	2.2
37	4-Ph	H	$(CH_2)_4Br$	15	3.1	4.8
38	4-Ph	H	$(CH_2)_2COOMe$	90	29	3.1
39	4-Ph	H	$(CH_2)_2OH$	10	0.83	12
deprenyl				0.79	0.0079	100
clorgyline				0.0012	1.9	0.0006

I_{50}, inhibitor concentration that produces 50% inhibition of enzyme activity.

experimental conditions, the following equation may be derived:
$K_i(\text{MAO A})/K_i(\text{MAO B}) \approx 2 \times [I_{50}(\text{MAO A})/I_{50}(\text{MAO B})]$. Thus, selectivity of competitive inhibitors towards MAO B was underestimated by a factor of about 2 when I_{50} ratios were taken into account instead of K_i ratios.

The results showed that most of the compounds acted preferentially as MAO B inhibitors (except **14**, **15**, **19**, **20**, **21**, **22** and **24**) and maximal efficiency was obtained with compound **32** (Fig. 2).

Inhibition mechanism

Because of the medium activity and selectivity of the inhibitors in this series, the nature of the inhibition was investigated only for **32**.

Different methods were used to check the inhibition mechanism. The mitochondrial suspension was preincubated

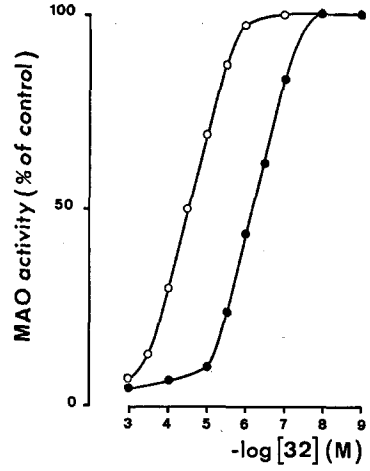


Fig. 2. Inhibition of rat brain MAO by compound **32**. MAO A (O) and MAO B (●) activities were measured under the standard conditions (Experimental protocols) in the absence or presence of different concentrations of **32**. MAO activity was expressed as % of the control.

in the absence or presence of **32** at a concentration which inhibited partially MAO A and B. 100% MAO A and B activities were recovered after either dialysis, washing or dilution of the mitochondria (Experimental protocols), revealing that **32** acted as a reversible inhibitor.

Also, the time dependency of MAO inhibition by **32** was evaluated. Prolonged preincubation of **32** with mitochondria up to 30 min (Experimental protocols) did not potentiate MAO inhibition. This confirmed the preceding results and indicated that reversible inhibition by **32** did not require prior metabolization of the inhibitor or a slow-binding process.

It was concluded from all these results that **32** acted as a classical reversible inhibitor.

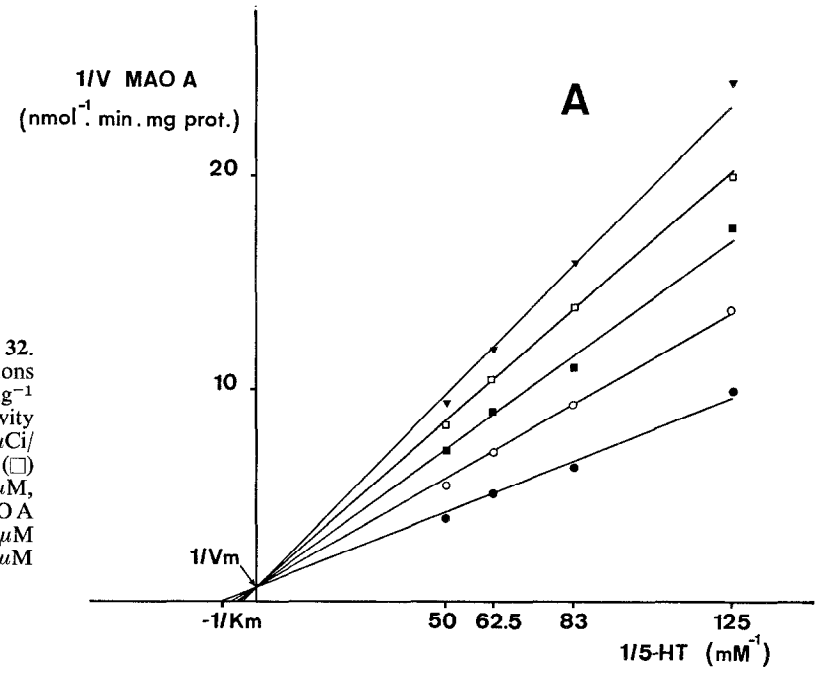
Inhibition type

Lineweaver—Burk and Dixon plots, used for analysis of kinetic inhibition of MAO A and B by compound **32**, showed that the latter was a competitive inhibitor. Its K_i values for MAO A and B were, respectively, 11 and 0.15 μM (Figs. 3 and 4).

Structure—activity approach

Analysis of the inhibition results was complex but some observations can be reported. The R substituent of the 2-aryl group seemed to direct the inhibitor selectivity. When it was a hydrogen atom (**14**, **19**, **20**) or an electron-releasing methoxy group (**15**, **21**, **22**), the inhibitors were more active on MAO A. On the contrary, when the R substituent was either an electron-withdrawing nitro group (**16**, **23—28** except **24**) or a phenyl group (**18**, **29—39**), selectivity was directed towards MAO B. Consequently, for the purpose of finding especially MAO B inhibitors, several nitrophenyl and biphenylyl derivatives were synthesized.

It is interesting to note that **32**, the most potent among



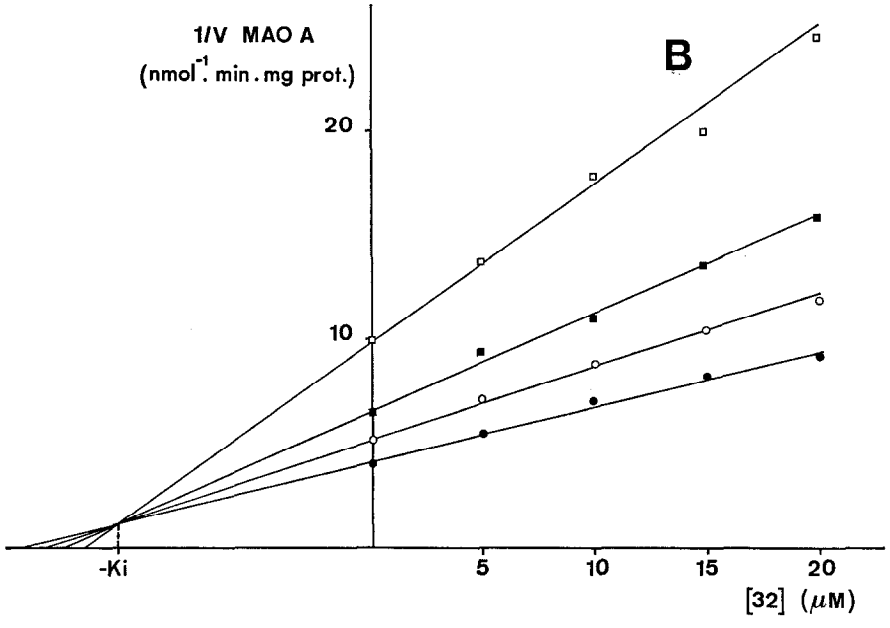


Fig. 3. Inhibition of rat brain MAO A by compound **32**. MAO A activity was measured under standard conditions (Experimental protocols) and expressed in nmol·min^{-1}·mg^{-1} of protein. **A.** Lineweaver–Burk plot: MAO A activity was measured with 8, 12, 16 and 20 μM [^{14}C] 5-HT (4.8 μCi/μmol) in the absence (●) or presence of 5 (○), 10 (■), 15 (□) or 20 μM (▼) of **32**. Control kinetic constants: K_m = 105 μM, V_m = 1.8 nmol·min^{-1}·mg^{-1} of protein. **B.** Dixon plot: MAO A activity was measured with 8 (□), 12 (■), 16 (○) and 20 (●) μM [^{14}C] 5-HT in the absence or presence of 5, 10, 15 or 20 μM of **32**. K_i = 11.5 μM.

the most selective B inhibitors (**18**, **23**, **32**, **39**) bears, in its structure, a 4-biphenylyl group which is very lipophilic (hydrophobic constant f = 3.842 [43]). Thus, it seemed that inhibitor potency on MAO B was more particularly provoked by a hydrophobic than an electron-withdrawing character of the R substituent (**32** compared to **23**).

The introduction of a 6-methyl substituent caused a significant decrease of the inhibitor potency on MAO B (**24** and **26** compared, respectively, to **23** and **25**).

Generally, the oxadiazinone preference for MAO A or B did not seem to be affected by N-4 substitution.

The length of the N-4 substituent appeared to influence MAO B inhibition which was maximal with an aliphatic chain of 1 carbon unit (**31**), while best selectivity and thus best efficiency was observed with 2 carbon units (**32** compared to **31**, **33** and **34**). The values obtained with the bromo derivatives (**35**, **36**, **37**) were less significant. The replacement of the cyano group in **32** by a hydroxyl group (**39**), a methyl

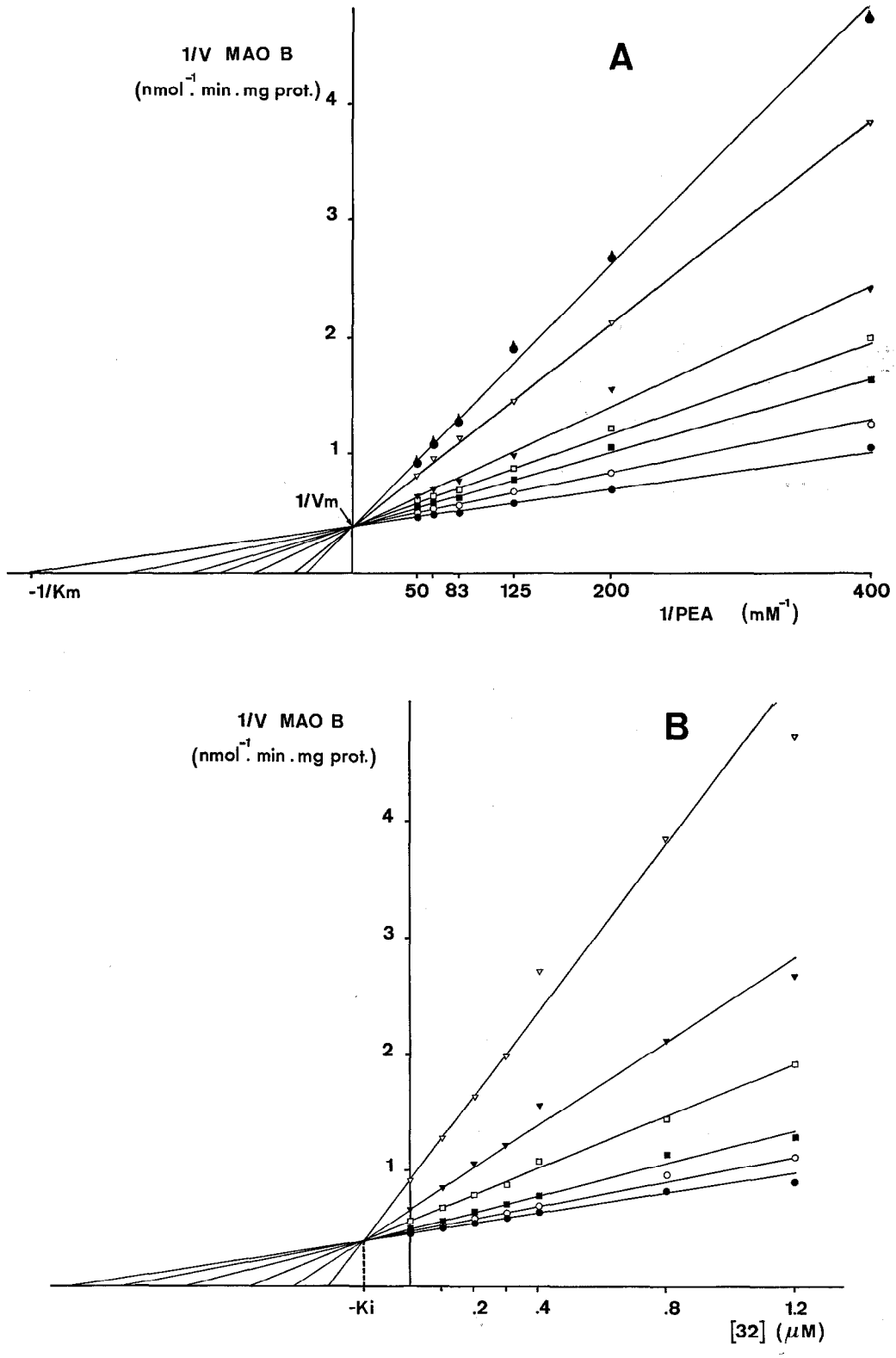


Fig. 4. Inhibition of rat brain MAO B by compound **32**. MAO B activity was measured under the standard conditions (Experimental protocols) and expressed in nmol·min^{-1}·mg^{-1} of protein. **A.** Lineweaver—Burk plot: MAO B activity was measured with 2.5, 5, 8, 12, 16 and 20 μM [^{14}C] PEA (26.6 μCi/μmol) in the absence (●) or presence of 0.1 (○), 0.2 (■), 0.3 (□), 0.4 (▼), 0.8 (▽) or 1.2 μM (◆) of **32**. Control kinetic constants: K_m = 4 μM, V_m = 2.7 nmol·min^{-1}·mg^{-1} of protein. **B.** Dixon plot: MAO B activity was measured with 2.5 (▽), 5 (▼), 8 (□), 12 (■), 16 (○) and 20 (●) μM [^{14}C] PEA in the absence or presence of 0.1, 0.2, 0.3, 0.4, 0.8 or 1.2 μM of **32**. K_i = 0.15 μM.

group (**30**), a hydrogen atom (**29**), a methoxycarbonyl group (**38**) or a bromine atom (**35**) caused a significant decrease of activity and selectivity on MAO B. This indicated that the N-4 substituent requires a 2-cyanoethyl group for maximal efficiency in this series.

The above results showed that oxadiazinone abilities to inhibit MAO B seemed to be most conclusive when the R substituent was a hydrophobic group, such as a phenyl, when the N-4 substituent was a 2-cyanoethyl and when there was no C-6 substituent.

The 1 or 2 carbon unit requirement for the aliphatic chain of the N-4 substituent agreed with the aliphatic chain length of benzylamine (1 carbon unit) and PEA (2 carbon units), both specific substrates for MAO B. Furthermore, other authors have observed reversible MAO inhibition by various cyanide [44, 45] or alcohol [46] derivatives. In particular, like the cyanide derivative **32**, the substrate analogue benzyl cyanide [44] and several cyanobenzenoids [44, 45] have been shown to be competitive MAO B inhibitors. Therefore, it was postulated that the cyano group should compete with the amino group of the substrate for the active site and 4-(cyanoalkyl) oxadiazinones (**19—26**, **31—34**) should act as competitive MAO B inhibitors.

In order to investigate the electronic contribution of the R substituent, the MAO B inhibitory potencies of 4-(2-cyanoethyl)-oxadiazinones (**19**, **21**, **23**, **32**) were related to the corresponding Hammett σ values [47] (Fig. 5). A positive correlation was observed, showing that the MAO B inhibitor potency increased with the electron-withdrawing character. The abnormal behavior of compound **32**, which showed more enhanced potency than expected by the corresponding σ value, could be explained by the lipophilic contribution of the phenyl group as mentioned above. Similar observations have been reported with other MAO B inhibitors [45]. These observations suggest that 2 types of interactions might be involved in the binding of the inhibitor to the enzyme: electronic interactions with a nucleophilic site and especially hydrophobic interactions with a hydrophobic site of the enzyme, while the cyano group interacted with the catalytic site of the enzyme. However, since little is known about the amino acid sequence and the topography of the MAO B active site, it is difficult to speculate on the essential groups of the enzyme which could be implicated in these interactions.

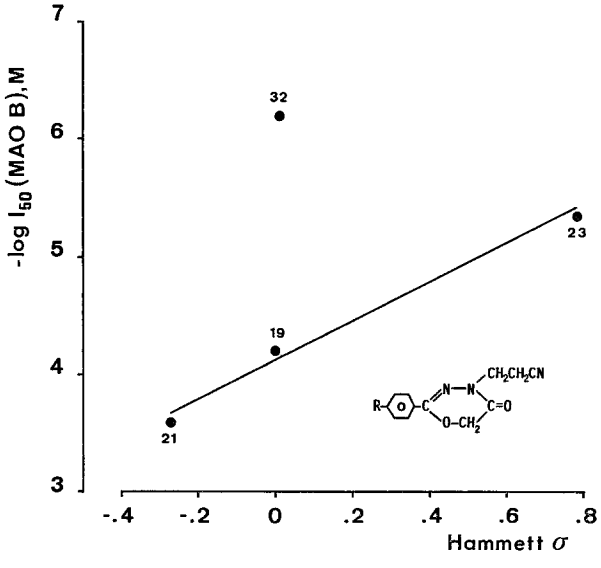


Fig. 5. Correlation between rat brain MAO B inhibition by 4-(2-cyanoethyl)oxadiazinones **19** (R = H), **21** (R = 4-MeO), **23** (R = 4-NO_2), **32** (R = 4-Ph) and the corresponding Hammett σ values of the R substituent of the 2-aryl group.

Experimental protocols

Chemistry

Melting points (uncorrected) were determined with using a Büchi oil-heated apparatus. IR spectra were recorded as KBr disks with a Perkin—Elmer 1310 spectrophotometer. PMR spectra were recorded for solutions in DMSO-d_6 on a Brucker WP 80 spectrometer. They are reported as δ values (ppm) relative to tetramethylsilane as an internal standard. Analytical results obtained for C, H, N of new compounds were within $\pm 0.4\%$ of the calculated values.

1-Aroyl-2-(α-chloroacyl)hydrazines ***1—5***
To a stirred refluxing solution of aroylhydrazine (10 mmol) in dry ethyl acetate (200 ml) was added dropwise a solution of α-chloroacyl chloride (12 mmol) in dry ethyl acetate (15 ml). After a few minutes, a strong evolution of gaseous hydrogen chloride started. The mixture was refluxed for 30 min then cooled. The solid was filtered and the solvent evaporated *in vacuo*. All solid residues were recrystallized from the appropriate solvent (Table I).

1-Aroyl-2-(2-cyanoethyl)hydrazines ***6—9***
A solution of aroylhydrazine (20 mmol) and of freshly distilled acrylonitrile (1.32 g, 25 mmol) in ethanol (*V* ml) was refluxed for *t* h. After removal of the solvent *in vacuo*, hydrazines were recrystallized from the appropriate solvent (Table I) except **7** and **9** which were first purified by column chromatography on Macherey—Nagel silica gel 60 (0.05—0.2 mm) using ethyl acetate—petroleum ether, respectively, 3:2 and 19:1 as the eluent. For each compound, *V* and *t* were: **6**: 40 ml, 15 h; **7**: 20 ml, 5 h; **8**: 40 ml, 72 h; **9**: 30 ml, 22 h.

1-Aroyl-2,2-bis(2-cyanoethyl)hydrazines ***10—13***
A solution of aroylhydrazine (20 mmol) and of freshly distilled acrylonitrile (2.65 g, 50 mmol) in water or in an aqueous organic solvent (*V* ml) was refluxed for *t* h. After removal of the solvent, the resulting hydrazine was recrystallized from the appropriate solvent (Table I). For each compound, reaction solvent, *V* and *t* were: **10**: 40 ml water, 23 h; **11**: 50 ml water—DMF (1:1), 12 h; **12**: 40 ml water, 2.5 h; **13**: 75 ml water, 12 h.

2-Aryl-4H-1,3,4-oxadiazin-5(6H)-ones ***14—18***
To a solution of hydrazine **1—5** (30 mmol) in dry DMF (60 ml) was added anhydrous sodium bicarbonate (2.94 g, 35 mmol). The resulting suspension of **14**, **15**, **16**, **17** or **18** was stirred at 60°C for, respectively, 5, 20, 4, 6 or 36 h. After filtration and removal of the solvent, the resulting residue was recrystallized from the appropriate solvent (Table I) except for compound **15** which was first chromatographed on alumina using ethyl acetate—petroleum ether (19:1) as the eluent.

4-Substituted 2-aryl-4H-1,3,4-oxadiazin-5(6H)-ones ***19—39***
Preparation of compounds ***19***, ***21***, ***23***, ***24*** *and* ***32***. Chloroacetyl chloride (or 2-chloropropionyl chloride for **24**) (2 mmol) was added dropwise at room temperature, to a solution of hydrazine **6—9** (2 mmol) in dry acetonitrile (15 ml). The mixture was refluxed for *t* h. After cooling, anhydrous potassium carbonate (0.5 g, 3.6 mmol) was added and the suspension was refluxed for further *t'* h. The hot mixture was filtered and the solvent evaporated *in vacuo* to give an oil, which, on cooling, solidified slowly and was recrystallized from the appropriate solvent (Table II). For each compound, *t* and *t'* were: **19**: 2 h, 1h; **21**: 0.75 h, 3 h; **23**: 1h, 2h; **24**: 1 h, 2 h; **32**: 0.5 h, 2 h.

N-Alkylation of 2-aryl-4H-1,3,4-oxadiazin-5(6H)-ones ***14—18***
Sodium hydride (50% in oil) (1.20 g, 25 mmol) was added in portions at 0°C to a stirred solution of **14—18** (10 mmol) in dry DMF (40 ml). When gaseous hydrogen evolution became very slow, the reaction mixture was heated at 40°C until the hydrogen evolution ceased. Then the halogen derivative (25 mmol) was added dropwise to the suspension of the oxadiazinone sodium derivative. The mixture was stirred for 3 h at 40°C. After filtration and removal of the solvent, the oily residue was chromatographed on silica gel using ethyl acetate—petroleum ether (19:1) as the eluent.

Biochemistry

Chemicals
Radioactive substrates, 5-hydroxytryptamine-[side chain-2-^{14}C]creatinine sulfate (56 mCi/mmol) and β-phenylethylamine-[ethyl-1-^{14}C]-hydrochloride (60 mCi/mmol) were purchased from Amersham Laboratories, Amersham, England. Clorgyline hydrochloride was purchased from May and Baker, Dagenham, England. L-Deprenyl hydrochloride was kindly provided by the 'Centre de Recherche Delalande'. Rueil-Malmaison, France. Oxadiazinones to be tested were dissolved in dimethyl sulfoxide (DMSO) to 100 mM. The weaker inhibitors were diluted in DMSO—water (3:1, v/v). The more potent inhibitors were diluted in DMSO—water (3:1, v/v). The more potent inhibitors were diluted in 4% aqueous DMSO, which caused no MAO inhibition. Clorgyline and L-deprenyl were dissolved in water.

Mitochondrial preparation
A crude mitochondrial fraction was prepared according to the procedure of Eichberg *et al.* [48] slightly modified. All operations were carried at 4°C. Male and female Sprague—Dawley rats (Iffa Credo, L'Arbresle, France), weighing 100—120 g were decapitated. The brains were removed rapidly and homogenized (15:1, v/w) in medium A (0.32 M sucrose, 10 mM Tris, pH 7.4) with an Ultra-Turrax homogenizer. The homogenates were centrifuged at 1000×*g* twice for 5 min at 4°C. The resulting supernatant was centrifuged at 20 000×*g* for 20 min. The mitochondrial pellet was suspended (6:1, v/w) in medium B (0.32 M sucrose, 10 mM Tris-HCl, 8% Ficoll, pH 7.4) and centrifuged at 20 000×*g* for 20 min. The mitochondrial fraction was finally washed with medium A (4:1, v/w) and centrifuged at 20 000×*g*. The washed mitochondria were resuspended (4:1, v/w) in 100 mM potassium phosphate buffer, pH 7.4, fractionated and stored at —80°C. Before use, mitochondria were diluted with phosphate buffer to 0.79 mg of protein/ml.

MAO standard assays
MAO activities were determined using a slight modification of Baker's method [49]. The total volume mixture was 200 μl. An aliquot of 50 μl of mitochondrial suspension (0.79 mg of protein/ml), 90 μl of 100 mM phosphate buffer, pH 7.4 and 20 μl of solubilizing solution (control) or inhibitor solution were preincubated for 20 min at 37°C. The reaction was started by adding 40 μl of either 50 μM [^{14}C] 5-HT (4.8 μCi/μmol) for MAO A assays or 25 μM [^{14}C] PEA (26.6 μCi/μmol) for MAO B assays. The final concentrations were 10 μM 5-HT (≈ 0.1 K_m (MAO A)) or 5 μM PEA (≈ K_m (MAO B)). After incubation at 37°C for 40 min (5-HT) or for 10 min (PEA), the reaction was stopped by adding 200 μl of cooled 2 N HCl. The metabolites were extracted by adding 1 ml of toluene—ethyl acetate (v/v), vortexing for 10 s and centrifugation at 5000×*g* for 2 min. Aliquots of 500 μl were withdrawn from the organic phases and counted in toluene containing 0.4% (w/v) 2,5-diphenyloxazole (PPO) in a SL20 Intertechnique scintillation counter. The assays were routinely duplicated. A blank (where the mitochondria were inactivated by adding 2 N HCl before the substrate) and a control were run for each test series. The MAO activity was corrected from the blank. MAO activities used in the standard assays were 2.09 pmol·min^{-1}·mg^{-1} of protein for MAO A and 1.52 pmol·min^{-1}·mg^{-1} of protein for MAO B.

Under these standard conditions, MAO A and B activities were linear with time and enzyme concentrations and there was no interference between the two activities (see Results and Discussion).

Protein determination
Protein concentration of the mitochondrial preparation was determined by the Biuret method as described by Layne [50] with bovine serum albumin as the standard.

I_{50} determination
MAO activity was measured under standard conditions in the absence or presence of different inhibitor concentrations and expressed as % of the control. I_{50} (MAO A) and I_{50} (MAO B) values for each inhibitor were determined graphically from the corresponding MAO inhibition curves. For each determination, at least 4—5 different inhibitor concentrations were ranged in the linear part of the inhibition curve.

Dialysis experiments
1 ml of mitochondrial suspension (0.79 mg of protein/ml) was incubated at 37°C for 20 min in the absence or presence of 10 μM **32** (concentration which inhibits 90% MAO B and 30% MAO A, Fig. 2). Mitochondria were dialyzed for 24 h at 4°C against 1 l of 100 mM phosphate buffer, pH 7.4. MAO A and B activities of the samples were then measured under standard conditions (without preincubation) and expressed as % of the respective control.

Washing experiments
5 ml of mitochondrial suspension (0.79 mg of protein/ml) were incubated at 37°C for 20 min in the absence or presence of 10 μM **32** (concentration which inhibits 90% MAO B and 30% MAO A). The mitochondria were then diluted in 50 ml of 100 mM phosphate buffer, pH 7.4, and centrifuged at 20 000×*g* for 20 min. Mitochondria were washed 3 times by the same procedure, resuspended in 5 ml of the same phosphate buffer and tested for their MAO A and B activities under standard conditions (without preincubation). MAO A and B activities were expressed as % of the respective control.

Dilution experiments
10 ml of mitochondrial suspension (0.79 mg of protein/ml) were concentrated by centrifugation at 20 000×*g* for 20 min (concentration factor = 100) and incubated at 37°C for 20 min in the absence or presence of **32** at 1 μM (concentration which inhibits 56% MAO B) or 300 μM (concentration which inhibits 50% MAO A). An aliquot of 10 μl was then withdrawn and added to a mixture containing 790 μl of 100 mM phosphate buffer, pH 7.4, and 200 μl of [^{14}C] 5-HT or [^{14}C] PEA at the standard concentration to give a total final volume of 1 ml. The reaction was stopped by adding 1 ml of 2 N HCl and metabolites were extracted with 5 ml of toluene—ethyl acetate (v/v). MAO A and B activities were then determined as under standard conditions and expressed as % of the respective control.

Time-courses of MAO inhibition by ***32***
MAO A and B activities of the mitochondrial suspension (0.79 mg of protein/ml) were measured under standard conditions in the absence or presence of 10 μM **32** (concentration which inhibits 90% MAO B and 30% MAO A) for various periods of preincubation (0, 1, 2, 3, 4, 5, 6, 10, 15, 20, 25 and 30 min). MAO activities were expressed as % of the controls.

References

1 Youdim M. B. H. & Finberg J. P. M. (1985) *in: Psychopharmacology 2, Part I: Preclinical Psychopharmacology* (Grahame-Smith D. G. & Cowen P. J., eds.), Elsevier, Amsterdam, pp. 35—70
2 Johnston J. P. (1968) *Biochem. Pharmacol.* 17, 1285—1297
3 Fowler C. J. & Ross S. B. (1984) *Med. Res. Rev.* 4, 323—358
4 Kan J. P., Malnoe A. & Strolin Benedetti M. (1978) *J. Pharm. Pharmacol.* 30, 190—192
5 Keane P. E., Kan J. P., Sontag N. & Strolin Benedetti M. (1979) *J. Pharm. Pharmacol.* 31, 752—754
6 Strolin Benedetti M., Kan J. P. & Keane P. E. (1979) *in: Monoamine Oxidase: Structure, Function and Altered Functions* (Singer T. P., Von Korff R. W. & Murphy D. L., eds.), Academic Press, New York, pp. 335—340
7 Kan J. P. & Strolin Benedetti M. (1981) *J. Neurochem.* 36, 1561—1571
8 Fowler C. J. & Strolin Benedetti M. (1983) *J. Neurochem.* 40, 1534—1541
9 Fowler C. J. & Strolin Benedetti M. (1983) *J. Neurochem.* 40, 510—513
10 Oegren S.-O., Ask A.-L., Holm A.-C., Florvall L., Lindbom L.-O., Lundström J. & Ross S. B. (1981) *in: Monoamine Oxidase*

Inhibitors — The State of the Art (Youdim M. B. H. & Paykel E. S., eds.), John Wiley & Sons, Chichester, pp. 103—112
11 Ask A.-L., Hellström W., Norrman S., Oegren S.-O. & Ross S. B. (1982) *Neuropharmacology* 21, 299—308
12 Da Prada M., Keller H. H., Kettler R., Schaffner R., Pieri M., Burkard W. P., Korn A. & Haefely W. E. (1982) *in: Monoamine Oxidase: Basic and Clinical Frontiers* (Kamijo K., Usdin E. W. & Nagatsu T., eds.), Excerpta Medica, Amsterdam, pp. 183—196
13 Da Prada M., Kettler R., Keller H. H. & Haefely W. E. (1983) *Mod. Probl. Pharmacopsychiatr.* 19, 231—245
14 Waldmeier P. C., Baumann P. A., Delini-Stula A., Bernasconi R., Sigg K., Buech O. & Felner A. E. (1983) *Mod. Probl. Pharmacopsychiatr.* 19, 31—52
15 Waldmeier P. C. (1985) *Naunyn-Schmiedeberg's. Arch. Pharmacol.* 329, 305—310
15 Waldmeier P. C. (1985) *Naunyn-Schmiedeberg's Arch. Pharmacol.* 329, 305—310
17 Kan J. P., Steinberg R., Mouget-Goniot C., Worms P. & Bizière K. (1987) *J. Pharm. Exp. Ther.* 240, 251—258
18 Worms P., Kan J. P., Wermuth C. G., Roncucci R. & Bizière K. (1987) *J. Pharm. Exp. Ther.* 240, 241—250
19 Yokoyama T., Iwata N. & Kobayashi T. (1987) *Jpn. J. Pharmacol.* 44, 421—427
20 Yokoyama T., Kamioka T., Iwata N. & Kobayashi T. (1987) *Jpn. J. Pharmacol.* 44, 413—420
21 Naoi M., Nomura Y., Ishiki R., Suzuki H. & Nagatsu T. (1988) *J. Neurochem.* 50, 243—247
22 Strolin Benedetti M., Dostert P., Boucher T. & Guffroy C. (1982) *in: Monoamine Oxidase: Basic and Clinical Frontiers* (Kamijo K., Usdin E. W. & Nagatsu T., eds.), Excerpta Medica, Amsterdam, pp. 209—220
23 Dostert P., Strolin Benedetti M. & Guffroy C. (1983) *J. Pharm. Pharmacol.* 35, 161—165
24 Da Prada M., Kettler R., Keller H. H., Bonetti E. P. & Imhof R. (1986) *Adv. Neurol.* 45, 175—178
25 Dostert P. (1984) *in: Monoamine Oxidase and Disease* (Tipton K. F., Dostert P. & Strolin Benedetti M., eds.), Academic Press, London, pp. 9—24
26 Finberg J. P. M. & Youdim M. B. H. (1983) *Neuropharmacology* 22, 441—446
27 Murphy D. L., Sunderland T., Garrick N. A., Aulakh C. S. & Cohen R. M. (1987) *in: Psychopharmacology Series 3: Clinical Pharmacology in Psychiatry* (Dhal S. G., Gram L. F., Paul S. M. & Potter W. Z., eds.), Springer, Berlin, pp. 135—146
28 Youdim M. B. H. (1986) *J. Neural. Transm. Suppl.* 22, 91—105
29 Youdim M. B. H. & Finberg J. P. M. (1986) *Adv. Neurol.* 45, 127—136
30 Knoll J. (1986) *Adv. Neurol.* 45, 107—110
31 Youdim M. B. H., Finberg J. P. M., Riederer P. & Heikkila R. E. (1986) *Adv. Behav. Biol.* 29, 655—668
32 Van Alphen J. (1928) *Rec. Trav. Chim.* 47, 673—682
33 Van Alphen J. (1929) *Rec. Trav. Chim.* 48, 417—421
34 Brown D., Hargreaves R. B., McLoughlin B. J. & Mills S. D. (1983) Eur. Patent Appl. No. 80, 296; (1984) *Chem. Abstr.* 100, 22672f
35 Ebnöther A., Jucker E., Lindenmann A., Rissi E. & Steiner R. (1959) *Helv. Chim. Acta* 42, 533—563
36 Grudzinski S. (1965) *Lodz Towarz. Nauk., Wydzial III, Acta Chim.* 10, 85—93; (1966) *Chem. Abstr.* 65, 13599a
37 Heininger S. A. (1959) U.S. Patent No. 2,909,556; (1960) *Chem. Abstr.* 54, 2169c
38 Brown D., Dowell R. I., Hargreaves R. B. & Main B. G. (1982) Eur. Patent Appl. No. 52, 442; (1982) *Chem. Abstr.* 97, 144886u
39 Kinemuchi H., Fowler C. J. & Tipton K. F. (1984) *in: Monoamine Oxidase and Disease* (Tipton K. F., Dostert P. & Strolin Benedetti M., eds.), Academic Press, London, pp. 53—62
40 Fowler C. J. & Tipton K. F. (1982) *J. Neurochem.* 38, 733—736
41 Knoll J. & Magyar K. (1972) *Adv. Biochem. Psychopharmacol.* 5, 393—408
42 Zreika M., McDonald I. A., Bey P. & Palfreyman M. G. (1984) *J. Neurochem.* 43, 448—454
43 Rekker R. F. (1977) *in: The Hydrophobic Fragmental Constant.* Elsevier, Amsterdam, pp. 350, 354
44 Houslay M. D. & Tipton K. F. (1974) *Biochem. J.* 139, 645—652
45 Kimes A. S. & Carr D. O. (1982) *Biochem. Pharmacol.* 31, 2639—2642
46 Fowler C. J., Callingham B. A., Mantle T. J. & Tipton K. F. (1980) *Biochem. Pharmacol.* 29, 1177—1183
47 Perrin D. D. (1980) *in: Physical Chemical Properties of Drugs* (Yalkowsky S. H., Sinkula A. A. & Valvani S. C., eds.), Marcel Dekker, New York, pp. 10—11
48 Eichberg J. Jr., Whittaker V. P. & Dawson R. M. C. (1964) *Biochem. J.* 92, 91—100
49 Baker P. C. (1966) *Dev. Biol.* 14, 267—277
50 Layne E. (1957) *in: Methods in Enzymology: Preparation and Assay of Substrates* (Colowich S. & Kaplan N., eds.), Academic Press, New York, pp. 447—454