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Original article

5-Aryl-1,3,4-oxadiazol-2(3*H*)-one derivatives and sulfur analogues as new selective and competitive monoamine oxidase type B inhibitors

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Summary — Eighteen new 5-aryl-1,3,4-oxadiazol-2(3*H*)-one derivatives and sulfur analogues were prepared and evaluated *in vitro* for their inhibitory properties on monoamine oxidase (MAO) types A and B. The most active compounds in these series acted preferentially against MAO B with IC_{50} values in the range of 1.8–0.056 μM. The 5-(4-biphenylyl)-3-(2-cyanoethyl)-1,3,4-oxadiazol-2(3*H*)-one **23** and its oxadiazolethione analogue **33** were found to act as potent, selective and competitive MAO B inhibitors with a slight slow-binding character. Both compounds inhibited MAO A in a classical competitive manner. According to their K_i (MAO B) values of 2.6 and 4 x 10^{-8} M, respectively, and their K_i (MAO A)/K_i (MAO B) ratios of 270 and500, respectively, **23** and **33** can be placed among the most active and selective competitive MAO B inhibitors known up to now. The structure–activity relationships are discussed.

Résumé — Dérivés d'aryl-5 3*H*-oxadiazone-1,3,4 ones-5 et d'analogues soufrés comme nouveaux inhibiteurs sélectifs et compétitifs de la monoamine oxydase B. *Dix huit dérivés d'aryl-5 3*H*-oxadiazole-1,3,4 ones-2 et d'analogues soufrés ont été synthétisés et testés* in vitro *pour leur activité inhibitrice sur les monoamines oxydases (MAO) A et B. Les produits les plus actifs inhibent préférentiellement la MAO B avec des CI_{50} dans l'intervalle 1,8–0,056 μM. La (cyano-2 éthyl)-3 (biphénylyl-4)-5 3*H*-oxadiazole-1,3,4 one-2 **23** et son analogue, l'oxadiazolethione **33** se sont révélées très actives, sélectives et compétitives vis-à-vis de la MAO B, avec un léger caractère de "slow-binding". Ces deux produits inhibent la MAO A de façon compétitive classique. Compte tenu de leurs K_i (MAO B), respectivement de 2,6 et 4,10^{-8} M et de leurs rapports K_i (MAO A)/K_i (MAO B), respectivement de 270 et de 500, les produits **23** et **33** peuvent être classés parmi les inhibiteurs les plus actifs et sélectifs que l'on connaisse actuellement dans la catégorie des inhibiteurs compétitifs de la MAO B. Les relations structure–activité sont discutées.*

competitive monoamine oxidase B inhibitors / 1,3,4-oxadiazol-2(3*H*)-one derivatives / 1,3,4-oxadiazole-2(3*H*)-thione derivatives

Introduction

Monoamine oxidase (MAO, EC1.4.3.4) is a flavoprotein enzyme tightly bound to the outer mitochondrial membrane [1] and implicated in the oxidative deamination of a variety of biogenic and exogenic monoamines [2]. In the central nervous system, the MAO is a crucial enzyme involved in the metabolism of the major amine neurotransmitters, serotonin (5-HT), noradrenaline (NA) and dopamine (DA) which are implicated in many neuropsychiatric disorders [3].

Originally, Johnston in 1968 developed the concept of MAO A and MAO B subtypes after the discovery of the first selective MAO A inhibitor, clorgyline [4]. Subsequent pharmacological studies with L-deprenyl, the first selective MAO B inhibitor [5] as well as biochemical, peptide mapping and immunological investigations have provided substantial data supporting this concept [6]. More recently, determination of the nucleotide and the corresponding amino acid sequences for the cloned MAO A and B cDNAs [7, 8] have provided the molecular basis for the existence of 2 physically and genetically independent enzymes.

It is widely assumed that symptoms of Parkinson's disease are related to a deficit in the brain of DA, a mixed substrate for both types of MAO [4]. Although

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Abbreviations: MAO: monoamine oxidase; 5-HT: serotonin; DA: dopamine; NA: noradrenaline; PEA: ß-phenylethylamine
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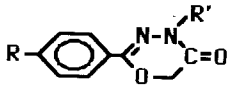
DA in the brain is metabolized by MAO A within the dopaminergic neurons [9], it is in fact substantially inactivated in other cells, mainly the glial cells, by MAO B [10], the major extraneuronal subtype of MAO in human brain [11].

In the last few years, accumulated data indicating the effectiveness of L-deprenyl in neurodegenerative diseases, such as Parkinson's [12, 13] and Alzheimer's [14] diseases, have brought great credit to MAO B inhibitors [15] which are thought to be valuable in senescence [16, 17].

For reasons of safety [18–20], new approaches, in particular in the field of MAO B inhibitors as potential therapeutic drugs, have led to the development of a new generation of selective [21] as well as reversible or short-acting [22] inhibitors.

Nowadays, the most potent and selective MAO B inhibitors are the long-lasting allylamine MDL 72145 [23], the propargylamines L-deprenyl and AGN 1135 [24], or the short-acting amine derivatives MD 240928 [25], Ro 16-6491 [26] and Ro 19-6327 [27]. Other reversible and competitive MAO B inhibitors, such as pCMPEA [28] and the endogenous isatin [29], have only a moderate degree of activity. In general, their K_i (MAO B) value is not lower than 10^{-7} M.

We have reported previously that 2-aryl-4*H*-1,3-4-oxadiazin-5(6*H*)-one derivatives with the following general structure:



could lead to selective and competitive MAO B inhibitors, particularly when the R substituent was a lipophilic phenyl group and the R' substituent a 2-cyanoethyl group [30]. Further to these preliminary results which confirmed in particular the prominent role of hydrophobicity for the affinity and selectivity of the inhibitors against MAO B, an examination was performed to determine whether other heterocyclic rings with an aromatic character and which were hence more lipophilic, could provide potent inhibitors.

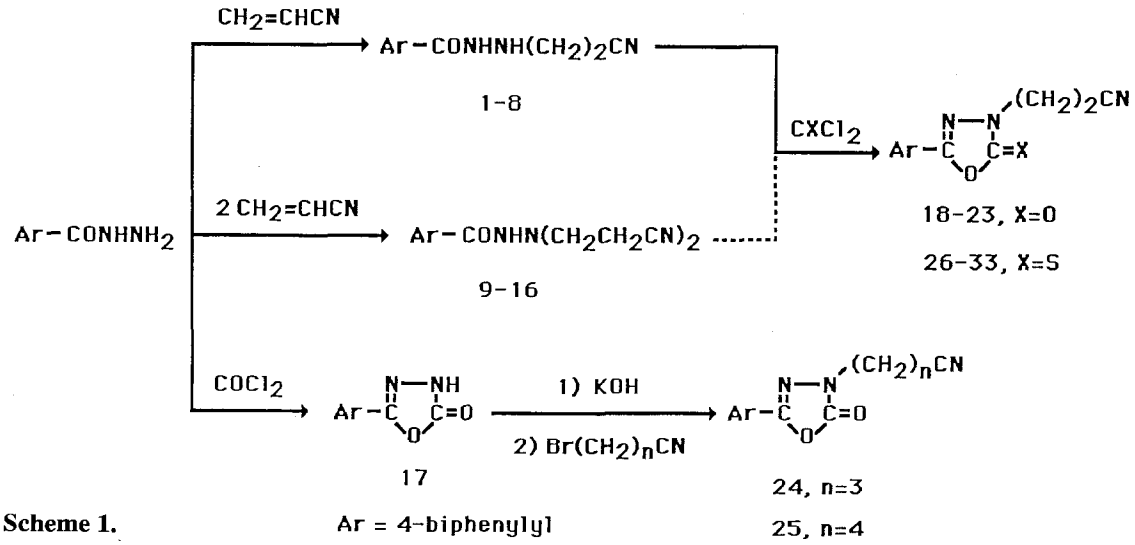
In the present study, new 1,3,4-oxadiazol-2(3*H*)-one derivatives and sulfur analogues were synthesized and investigated *in vitro* on rat brain MAO types A and B.

Chemistry

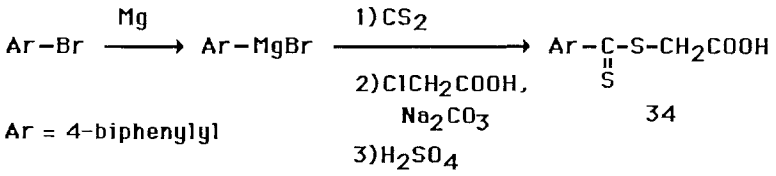
The synthesis of the molecules explored in this study is outlined in schemes 1 and 2. The monosubstituted aroylhydrazines, *eg*, 1-aroyl-2-(2-cyanoethyl)hydrazines **1–8** as well as the disubstituted ones, *eg*, 1-aroyl-2,2-bis(2-cyanoethyl-hydrazines **9–16**, obtained initially by Michael addition of acrylonitrile on the corresponding aroylhydrazines, were used as starting materials (table I). Some of these products have been previously described [30].

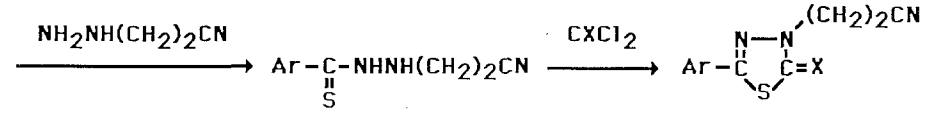
Synthesis of 5-aryl-1,3,4-oxadiazol-2(3*H*)-ones **17–23** (table II) was achieved by reaction of phosgene with the appropriate monosubstituted hydrazines [31].

5-Aryl-3-(2-cyanoethyl)-1,3,4-oxadiazol-2(3*H*)-ones **18–23** were prepared directly from the corresponding hydrazines **1–3**, **5**, **6**, **8** whereas the homologues 5-(4-biphenylyl)-3-(cyanoalkyl)-1,3,4-oxadiazol-2(3*H*)-ones **24** and **25** were synthesized indirectly by N-alkylation of the oxadiazolone **17**, *via* the potassium salt. This last high yield method could not be



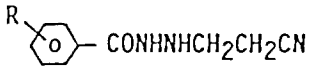
Scheme 1.





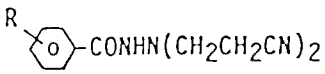
Scheme 2.

Table I. 1-Aroyl-2-(2-cyanoethyl)hydrazines **1–8**.



No	*R*	*mp (°C)*	*Rec S*[a]	*Yield (%)*[b]	*Formula*	*PMR δ (ppm)*
1	H	121[c]				
2	4-MeO	137[c]				
3	4-Me	125	A/B	50	$C_{11}H_{13}N_3O$	2.4 (s, 3H), 2.7 (t, 2H), 3.2 (q, 2H), 5.6 (q, 1H), 7.3 and 8 (2d, 4H), 10.2 (d, 1H)
4	3-Cl	114.5	A/B	40	$C_{10}H_{10}ClN_3O$	2.6 (t, 2H), 3 (q, 2H), 5.5 (q, 1H), 7.3-7.9 (m, 4H), 10.2 (d, 1H)
5	4-Cl	120	C	90	$C_{10}H_{10}ClN_3O$	2.6 (t, 2H), 3 (q, 2H), 5.5 (q, 1H), 7.5 and 7.9 (2d, 4H), 10.2 (d, 1H)
6	4-NO2	162[c]				
7	2-MeO, 5-Cl	78	A/B	26	$C_{11}H_{12}ClN_3O_2$	2.6 (t, 2H), 3 (q, 2H), 3.8 (s, 3H), 5.5 (q, 1H), 6.9-7.7 (m, 3H), 9.6 (d, 1H)
8	4-Ph	174[c]				

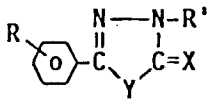
1-Aroyl-2,2-bis(2-cyanoethyl)hydrazines **9–16**.



No	*R*	*mp (°C)*	*Rec S*[a]	*Yield (%)*[b]	*Formula*	*PMR δ (ppm)*
9	H	145.5[c]				
10	4-MeO	141[c]				
11	4-Me	146	C	32	$C_{14}H_{16}N_4O$	2.4 (s, 3H), 2.6 (t, 4H), 3.3 (t, 4H), 7.2 and 7.8 (2d, 4H), 9.6 (s, 1H)
12	3-Cl	152	C/D	50	$C_{13}H_{13}ClN_4O$	2.65 (t, 4H), 3.2 (t, 4H), 7.4-8 (m, 4H), 9.75 (s, 1H)
13	4-Cl	165	D	75	$C_{13}H_{13}ClN_4O$	2.7 (t, 4H), 3.3 (t, 4H), 7.6 and 8 (2d, 4H), 9.85 (s, 1H)
14	4-NO2	146-7[c]				
15	2-MeO, 5-Cl	100	D/E	86	$C_{14}H_{15}ClN_4O_2$	2.75 (t, 4H), 3.25 (t, 4H), 3.9 (s, 3H), 7-7.6 (m, 3H), 9.3 (s, 1H)
16	4-Ph	192.5[c]				

[a]Recrystallization solvent: A = ethyl acetate; B = petroleum ether 40–60; C = ethanol; D = water; E = 1-propanol. [b]Yields were not optimized. [c]These compounds have been previously reported [30].

Table II. Oxadiazolones **17–25**, oxadiazolethiones **26–33**, thiadiazolone **36** and thiadiazolethione **37**.



No	*X*	*Y*	*R*	*R'*	*mp (°C)*	*Rec Sa*	*Yield (%)b*	*Formula*	*PMR δ (ppm)*
17	O	O	4-Ph	H	270	A	86	$C_{14}H_{10}N_2O_2$	7.4-7.9 (m, 9H), 12.5 (bs, 1H)
18	O	O	H	$(CH_2)_2CN$	121	B	70	$C_{11}H_9N_3O_2$	3.1 (t, 2H), 4.1 (t, 2H), 7.4-8 (m, 5H)
19	O	O	4-MeO	$(CH_2)_2CN$	117	B	41	$C_{12}H_{11}N_3O_3$	3 (t, 2H), 3.9 (s, 3H), 4.1 (t, 2H), 7.1 and 7.8 (2d, 4H)
20	O	O	4-Me	$(CH_2)_2CN$	126	B	90	$C_{12}H_{11}N_3O_2$	2.4 (s, 3H), 3 (t, 2H), 4 (t, 2H), 7.4 and 7.7 (2d, 4H)
21	O	O	4-Cl	$(CH_2)_2CN$	137	B	95	$C_{11}H_8ClN_3O_2$	3 (t, 2H), 4.05 (t, 2H), 7.6 and 7.75 (2d, 4H)
22	O	O	4-NO_2	$(CH_2)_2CN$	162	B	45	$C_{11}H_8N_4O_4$	3 (t, 2H), 4.1 (t, 2H), 8 and 8.35 (2d, 4H)
23	O	O	4-Ph	$(CH_2)_2CN$	174	B/C	82	$C_{17}H_{13}N_3O_2$	3 (t, 2H), 4.05 (t, 2H), 7.3-7.9 (m, 9H)
24	O	O	4-Ph	$(CH_2)_3CN$	139	D/E	98	$C_{18}H_{15}N_3O_2$	2.1 (q, 2H), 2.7 (t, 2H), 3.9 (t, 2H), 7.4-8 (m, 9H)
25	O	O	4-Ph	$(CH_2)_4CN$	118	B/D	94	$C_{19}H_{17}N_3O_2$	1.5-2.1 (m, 4H), 2.8 (t, 2H), 3.9 (t, 2H), 7.4-8 (m, 9H)
26	S	O	H	$(CH_2)_2CN$	123c	B	65	$C_{11}H_9N_3OS$	3.15 (t, 2H), 4.4 (t, 2H), 7.4-8 (m, 5H)
27	S	O	4-MeO	$(CH_2)_2CN$	135	N	23	$C_{12}H_{11}N_3O_2S$	3.15 (t, 2H), 3.9 (s, 3H), 4.4 (t, 2H), 7.2 and 7.9 (2d, 4H)
28	S	O	4-Me	$(CH_2)_2CN$	153	B	70	$C_{12}H_{11}N_3OS$	2.4 (s, 3H), 3.15 (t, 2H), 4.4 (t, 2H), 7.4 and 7.8 (2d, 4H)
29	S	O	3-Cl	$(CH_2)_2CN$	92	B	45	$C_{11}H_8ClN_3OS$	3.1 (t, 2H), 4.4 (t, 2H), 7.65-8.05 (m, 4H)
30	S	O	4-Cl	$(CH_2)_2CN$	126.5	B	60	$C_{11}H_8ClN_3OS$	3.1 (t, 2H), 4.4 (t, 2H), 7.7 and 8 (2d, 4H)
31	S	O	4-NO_2	$(CH_2)_2CN$	138	B	43	$C_{11}H_8N_4O_3S$	3.15 (t, 2H), 4.45 (t, 2H), 8.15 and 8.45 (2d, 4H)
32	S	O	2-MeO, 5-Cl	$(CH_2)_2CN$	138	B	41	$C_{12}H_{10}ClN_3O_2S$	3.1 (t, 2H), 3.95 (s, 3H), 4.45 (t, 2H), 7.4-7.9 (m, 3H)
33	S	O	4-Ph	$(CH_2)_2CN$	159	B	47	$C_{17}H_{13}N_3OS$	3.2 (t, 2H), 4.45 (t, 2H), 7.4-8.1 (m, 9H)
36	O	S	4-Ph	$(CH_2)_2CN$	178	F	42	$C_{17}H_{13}N_3OS$	2.9 (t, 2H), 4.35 (t, 2H), 7.1-7.95 (m, 9H)
37	S	S	4-Ph	$(CH_2)_2CN$	152	G	26	$C_{17}H_{13}N_3S_2$	3.25 (t, 2H), 4.65 (t, 2H), 7.3-7.95 (m, 9H)

[a]Recrystallization solvent: A = 1-butanol; B = ethanol; C = ethyl acetate; D = 1-propanol; E = water; F = diethyl ether; G = benzene. [b]Yields were not optimized. [c]Lit [32], mp: 121°C.

used for the oxadiazolones **18–23**. Most presumably, in this case, the alkylating agent 3-bromopropionitrile underwent an elimination instead of a substitution reaction.

5-Aryl-3-(2-cyanoethyl)-1,3,4-oxadiazole-2(3*H*)-thiones **26–33** (table II) were generated directly by treatment of the corresponding aroylhydrazines **1–8** with thiophosgene [31].

Because of the difficulties in separating the monosubstituted hydrazines **1–8** from the corresponding disubstituted ones formed during the Michael reaction, an alternative route was investigated in order to obtaion easier access to oxadiazolones **18–23** and oxadiazolethiones **26–33**. The disubstituted hydrazines **9–16**, which were obtained in fair yields, were brought into reaction with phosgene or thiophosgene under conditions described by Meyer and Cummings [33]. However, this method was discarded since products were obtained in low yields.

Two additional oxadiazolone analogues were prepared following scheme 2. The starting material 4-biphenylyldithiocarbonyloxyacetic acid **34** was prepared *via* its sodium salt by reaction of the 4-biphenylylmagnesium bromide with carbon disulfide followed by addition of chloroacetic acid in the presence of sodium carbonate, then acidification [34]. Treatment of **34** with (2-cyanoethyl)hydrazine [35] gave 1-(4-biphenylylthiocarbonyl)-2-(2-cyanoethyl)hydrazine **35**, together with an unknown side product. The (2-cyanoethyl)hydrazine was obtained by reaction of hydrazine with acrylonitrile [35]. Subsequent reaction of **35** with phosgene or thiophosgene [31] afforded the corresponding substituted thiadiazolone **36** and thiadiazolethione **37**, respectively (table II).

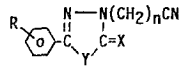
All physicochemical and analytical data were in agreement with the proposed structures (tables I–III).

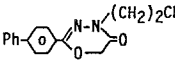
Results and Discussion

The 18 new oxadiazolones **18–25** and sulfur analogues **26–33**, **36** and **37** were tested *in vitro* for their inhibitory effects on rat brain MAO types A and B using a specific MAO assay.

Table IV. Activity and selectivity of oxadiazolones **18–23** and sulfur analogues **26–33**, **36** and **37** towards MAO A and B.

IC_{50}, inhibitor concentration that produces 50% inhibition of enzyme activity. The IC_{50} values were determined from experiments where the inhibitors were initially preincubated with the enzymes before adding the substrate. Under conditions where the reaction was initiated by adding enzyme to the mixture containing the inhibitor and the substrate, *eg*, without preincubation, oxadiazolones **18–22**, **24** and **25** showed the same IC_{50} values. For the oxadiazolone **23** and the oxadiazolethione **33**, the IC_{50} (MAO B) were 0.082 and 0.12 μM, respectively.



N°	X	Y	R	n	IC_{50}(MAO A) (μM)	IC_{50}(MAO B) (μM)	$\frac{IC_{50}(MAO\ A)}{IC_{50}(MAO\ B)}$
18	O	O	H	2	28.5	39	0.73
19	O	O	4-MeO	2	28	30	0.93
20	O	O	4-Me	2	21.5	128	0.17
21	O	O	4-Cl	2	12	27	0.44
22	O	O	4-NO_2	2	270	23	11.7
23	O	O	4-Ph	2	7.8	0.056	139
24	O	O	4-Ph	3	9.75	1.5	6.7
25	O	O	4-Ph	4	20	1.7	11.8
26	S	O	H	2	2.1	37	0.057
27	S	O	4-MeO	2	17.5	4.05	4.3
28	S	O	4-Me	2	16	13	1.23
29	S	O	3-Cl	2	5.5	22	0.25
30	S	O	4-Cl	2	13	12.5	1.04
31	S	O	4-NO_2	2	47	21.5	2.2
32	S	O	2-MeO, 5-Cl	2	7.5	<20	> 0.4
33	S	O	4-Ph	2	22.5	0.074	304
36	O	S	4-Ph	2	49	0.15	327
37	S	S	4-Ph	2	280	1.8	155
oxadiazinone					32	0.63	51
deprenyl					0.8	0.008	100
clorgyline					0.0012	1.9	0.0006

IC_{50} (MAO A) and IC_{50} (MAO B) values for these compounds (table IV) were calculated graphically

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

No	*N-H*	*C≡N*	*C=O*	*C=N cyclic*
1–8	3380-3270	2260-2220	1650-1620	–
	3280-3240			
9–16	3290-3200	2250-2230	1655-1620	–
18–25	–	2260-2240	1790-1770	1620-1610
26–33, 37	–	2250-2240	–	1620-1600
36	–	2240	1670	1600

from the respective MAO inhibition curves. Selectivity of the inhibitors towards MAO B (table IV) was estimated from the ratio IC_{50} (MAO A)/IC_{50} (MAO B). IC_{50} values for the inactivators clorgyline and L-deprenyl were given only for information since oxadiazolones and their derivatives, at least the most potent, have proved to act as competitive inhibitors (see below). The results indicated that oxadiazolones and sulfur analogues could lead to selective MAO B inhibitors (**22–25**, **33**, **36** and **37**). The most potent inhibitors **23** (fig 1) and **33**, in these series, inhibited selectively MAO B with IC_{50} values of 5.6 and 7.4 X 10^{-8} M, respectively. Their selectivity for MAO B is illustrated by the IC_{50} ratios between MAO A and MAO B of 139 and 304, respectively. These inhibitors were then selected to investigate the inhibition mechanism towards the 2 enzymes. Since **23** and **33** were found to act in a similar manner, only detailed studies with **23** are described herein.

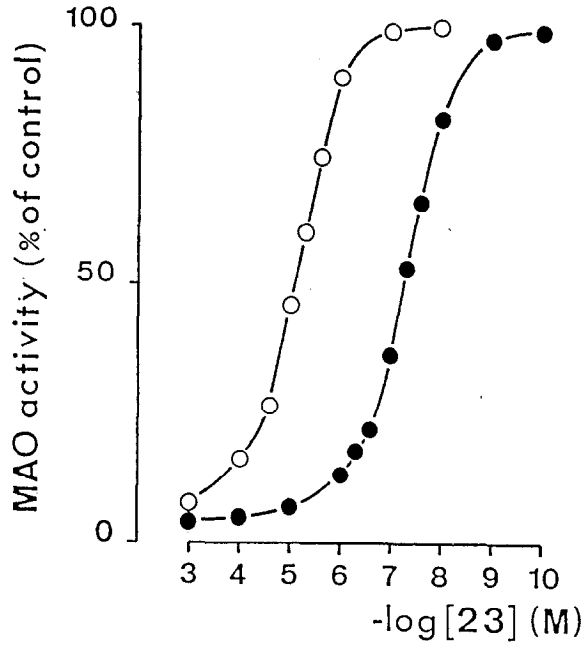


Fig 1. Inhibition of rat brain MAO by compound **23**. MAO A (○) and MAO B (●) activities were measured under standard conditions (see *Experimental protocols*) in the absence or presence of different concentrations of **23**. MAo activity was expressed as % of the control.

Inhibition mechanism

Partial inhibition of MAO B by **23** was fully reversed either after dialysis or washing of the mitochondria (table V). This indicated that **23** is a reversible inhibitor of MAO B and excluded any inactivation of the enzyme.

Table V. Reversibility tests for **23**, **33** and the MAOIs references, deprenyl and clorgyline.

	MAO activity, cpm (% of control)		
	Before washing or dialysis	After washing	After dialysis
MAO B			
control	16 220	11 354	5 064
23 (0.1 μM)	5 839 (36%)	11 126 (98%)	5 114 (101%)
33 (0.1 μM)	6 985 (43%)	11 400 (100%)	5 004 (99%)
deprenyl (20 nM)	**4 379 (27%)**	**3 185 (28%)**	**1 392 (27.5%)**
MAO A			
control	14 318	11 454	5 728
23 (10 μM)	6 486 (45%)	11 510 (100%)	5 745 (100%)
33 (200 μM)	7 330 (51%)	11 362 (99%)	5 710 (100%)
clorgyline (2 nM)	6 421 (45%)	5 268 (46%)	2 589 (45%)

However, time courses of MAO B inhibition by **23** showed a slight time dependency (fig 2). A maximal of 11% potentiation of the inhibition was observed with preincubation of the inhibitor at 10^{-7} M with the enzyme for 3 min. No further potentiation of the inhibition was detected with prolonged preincubation up to 30 min.

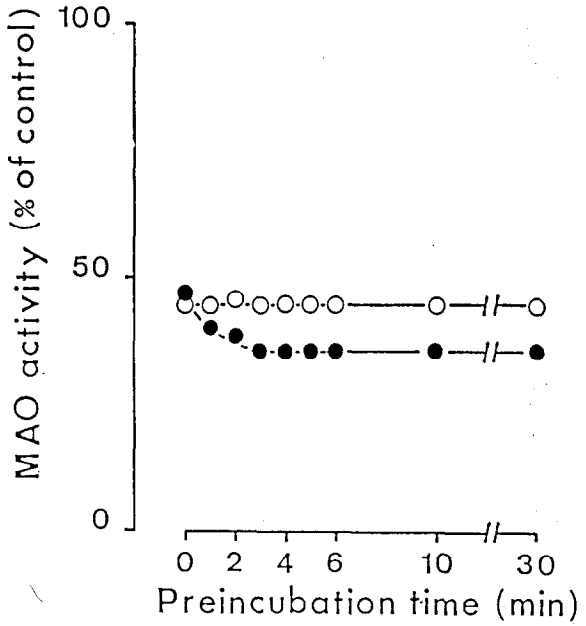


Fig 2. Time-courses of rat brain MAO inhibition by **23**. MAO activity was determined under standard conditions (see *Experimental protocols*) after various periods of preincubation at 37°C in the absence or presence of **23** at 100 nM for MAO B (●) and 10 μM for MAO A (○). Remaining MAO activities were expressed as % of the controls.

Moreover, MAO B inhibition by **23** without and with preincubation of the inhibitor with the enzyme, gave kinetic patterns typical for a reversible and competitive inhibitor. Both Lineweaver–Burk and Dixon plots, as well as the secondary replots, of initial and final steady-state velocities, respectively without (fig 3) and with preincubation of the inhibitor with the enzyme (fig 4), established that **23** is a linear competitive inhibitor of MAO B with an initial dissociation constant K_i = 3.6 X 10^{-8} M (figs 3a and 3B) and a steady-state constant K_i^* = 2.6 X 10^{-8} M (figs 4a and 4B). Linear competitivity is clearly demonstrated with Dixon secondary replots which give a straight line going through the origin [37] (figs 3b and 4b).

In the absence of inactivation of the enzyme, a time-dependent increase in inhibition may result from a slow isomerization or metabolization of the inhibitor to a more potent species or from a slow-binding process.

If **23** did undergo isomerization or metabolization during the preincubation period, we may expect a similar pathway for its analogues **18–25**, and especially for its closely related **24** and **25**. In particular, the IC_{50} (MAO B) values for these analogues would be lower with preincubation than without preincubation. In fact, such a mechanism seemed unlikely since the analogues showed the same IC_{50} (MAO B) values with and without preincubation (table IV; comments).

Also, the observed slow establishment of the full inhibition (fig 2) cannot be caused by a depletion of the inhibitor by the enzyme or a slow bimolecular collision between the enzyme and the inhibitor. Under our experimental conditions, concentration of the enzyme E, estimated at ≈ 10^{-9} (results to be published), is 100-fold lower than that of the inhibitor I. On the other hand, the bimolecular rate constant k_1 for the formation of the collision complex EI is usually in the range of 10^6–10^8 M^{-1} s^{-1} [38]. Given a concentration

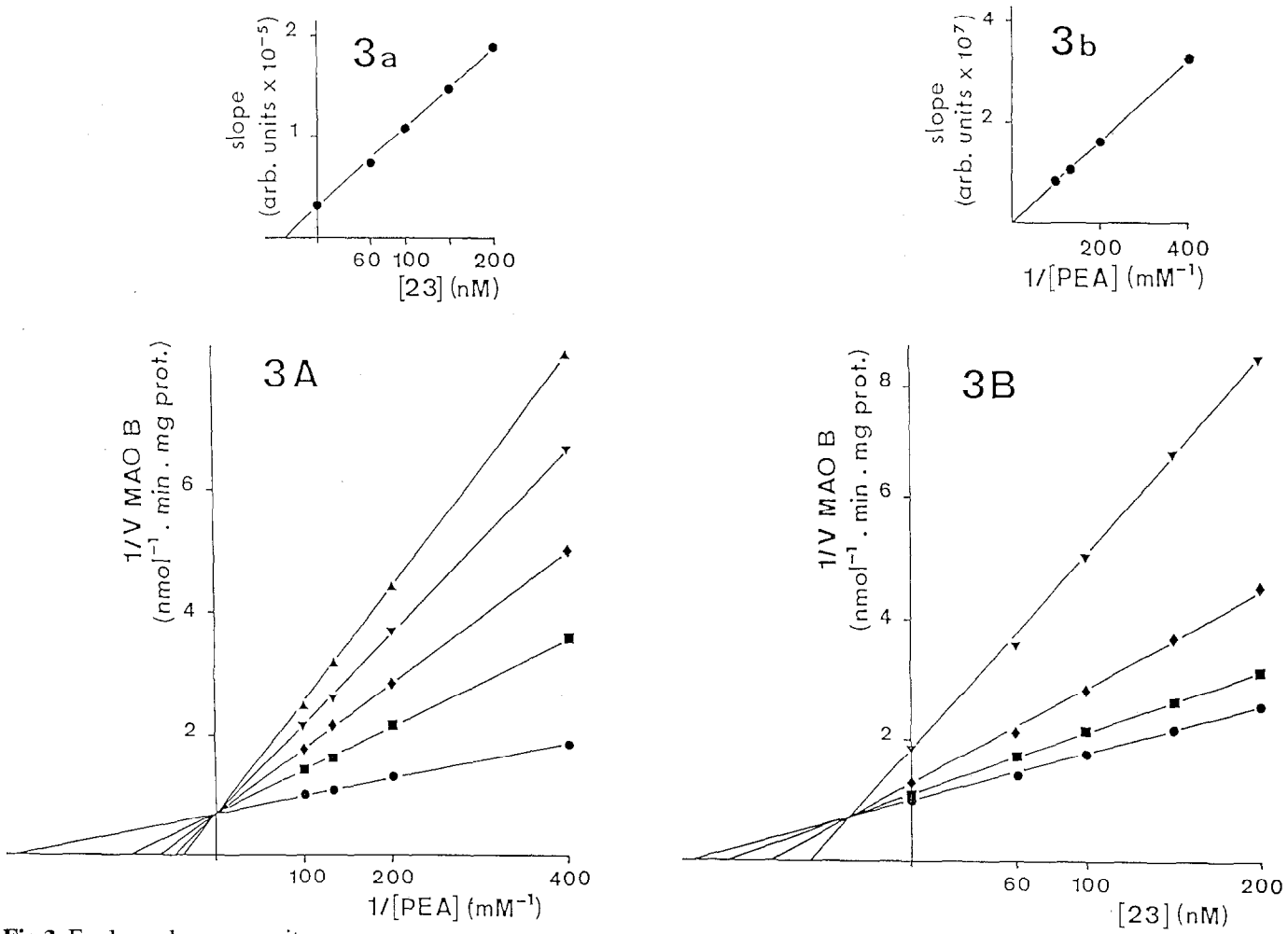
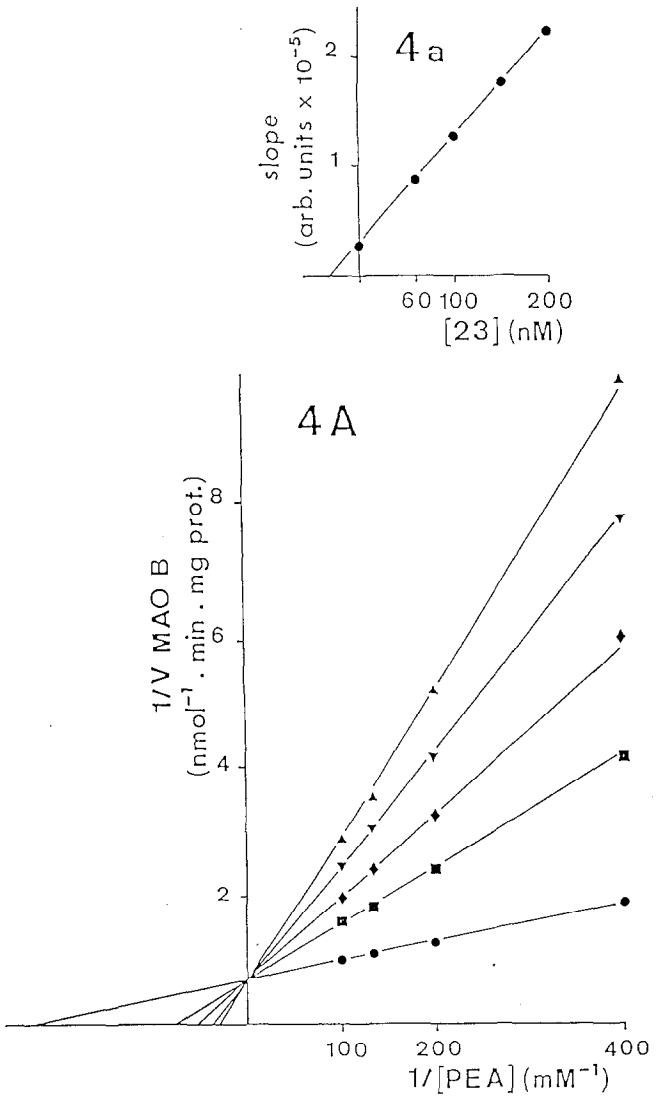
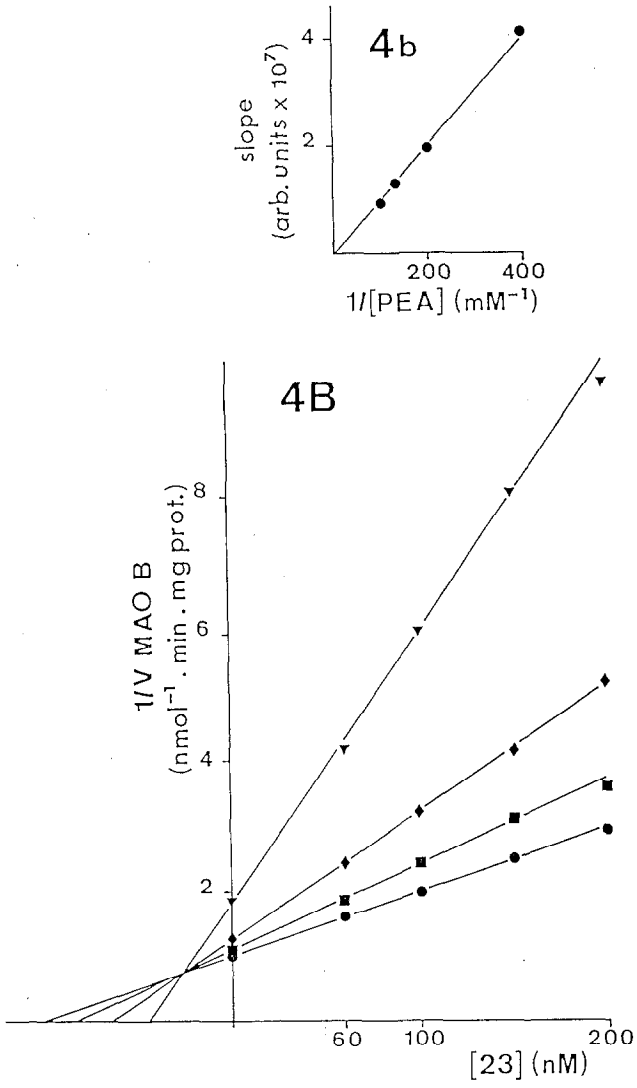


Fig 3. For legend, see opposite page.





Figs 3 and **4.** Kinetics of rat brain MAO B inhibition by compound **23**. MAO B activity was determined under standard conditions (see *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, 7.5 and 10 µM [^{14}C] PEA in the absence (●) or presence of 60 (■), 100 (◆), 150 (▼) and 200 (▲) nM of **23** without (fig 3A) and with (fig 4A) preincubation of the inhibitor at 37°C for 20 min with the enzyme. a, Lineweaver–Burk secondary replot. B, Dixon plot: MAO B activity was measured with 2.5 (▼), 5 (◆), 7.5 (■) and 10 (●) µM [^{14}C] PEA in the absence or presence of 60, 100, 150 and 200 nM of **23** without (fig 3B) and with (fig 4B) preincubation of the inhibitor at 37°C for 20 min with the enzyme. b, Dixon secondary replot. K_i = 36 nM. K_i* = 26 nM. Reactions without preincubation of the inhibitor were started by addition of the enzyme to the mixtures containing substrate and inhibitor.

of the inhibitor of 10^{-7} M and, even though, with a lower limit value of k_1, *eg* 10^6, k_{obs} for the diffusion-controlled bimolecular collision would be $10^6 \times 10^{-7} = 0.1$ s^{-1}. Under these conditions, the half-time for combination E-I, $T^{1/2} = 0.693/0.1 \approx 7$ s, is too short to account for the observed slow process.

Hence, assuming a rapid equilibrium between the enzyme and the inhibitor, the slow-binding process, as for the majority of slow-binders, could be attributed to a slow conformational change in the initial collision complex EI to a new complex EI* in which the inhibitor is more tightly bound to the enzyme [39]:

$$E + I \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EI \underset{k_4}{\overset{k_3}{\rightleftharpoons}} EI^*,$$

rapid slow

with $K_i = k_2/k_1$ and K_i* = overall equilibrium constant.

However, confirmation of the 2-step interaction between enzyme and inhibitor would require analysis of the presteady-state kinetics with rapid reaction techniques [39].

Identical studies indicated that the oxadiazolethione analogue **33** is a pure competitive inhibitor of MAO B with slow-binding characteristics similar to those of **23**. In particular, full inhibition of MAO B by 100 μM **33** was reached with preincubation for 3 min, and potentiation of the inhibition was estimated at 14%.

On the other hand, both **23** and **33** were found to inhibit MAO A in a simple competitive manner. The kinetic constants for **23** and **33** are listed in table VI.

Structure–activity correlations

From the data summarized in table IV some conclusions may be drawn, particularly on the structural requirements for effective inhibition of MAO B by oxadiazolone derivatives.

When the R substituent was a hydrogen atom (**18**) or an electron-donating group, such as Me (**20**) and MeO (**19**), the inhibitors were more active on MAO A. The behaviour of the Cl group (**21**) in parallel to that of electron-donating groups could be explained by the mesomeric effect. Conversely, when R was an electron-withdrawing NO_2 group (**22**) or a hydrophobic phenyl group (**23–25**), the inhibitors acted preferentially on MAO B. However, a hydrophobic character of the R substituent seems to be much more important in increasing activity and selectivity against MAO B(**23** *vs* **22**). To investigate this hydrophobic contribution, oxadiazolone activities against MAO B were related to the corresponding Rekker hydrophobic constants [40]. In fact, the data could be resolved into a biphasic curve in a semilog plot (fig 5). The upward linear curve indicates a net increase of activity with the lipophilic character of R. The downward linear curve shows a greater potency of the inhibitors than expected which seems to follow the electrophilic character of the R group: NO_2 (**22**) > H (**18**) > Me (**20**). The reasons for the abnormal behaviour of the MeO group (**19**) remain obscure.

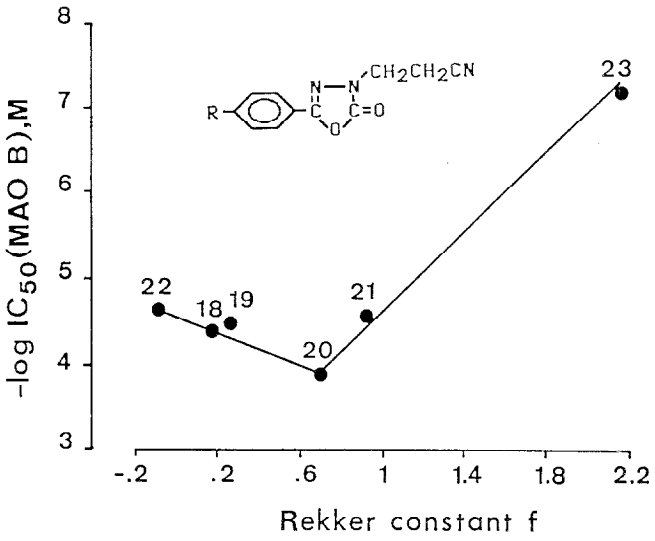


Fig 5. Correlation between rat brain MAO B inhibition by oxadiazolones **18** (R = H), **19** (R = 4-MeO), **20** (R = 4-Me), **21** (R = 4-Cl), **22** (R = 4-NO_2), **23** (R = 4-Ph) and the corresponding Rekker *f* values of the R substituent of the 5-aryl group.

As for oxadiazinones [30], above observations suggest that electrostatic interactions with a nucleophilic site of the enzyme and more particularly hydrophobic interactions with the active site might be involved in the binding of the inhibitors to MAO B.

In addition to the cyanide derivatives **23**, **33** and their oxadiazinone homologue [30], other cyanides unrelated to the above ones, including the substrate analogue benzyl cyanide [41] and various cyanobenzenoids [42], have all proved to inhibit MAO B in a reversible and competitive manner. These findings suggested that the cyano group, the only common active group with these inhibitors, interacts preferentially with the catalytic site of the enzyme and thereby is responsible for the competitivity of the inhibitors.

Table VI. Dissociation constants for **23** and **33** towards MAO A and B. Selectivity of the inhibitors was estimated from the ratio K_i (MAO A)/K_i* (MAO B). The K_i (MAO) values were determined graphically from the corresponding Lineweaver–Burk secondary replot and Dixon plot (see figs 3a and 3B, for instance). The MAO A inhibition patterns for **23** and **33** were obtained with 5, 10, 15 and 20 μM [^{14}C] 5-HT and 2.5, 5, 7.5 and 10 μM of the inhibitor. MAO B inhibition patterns for **33** were obtained with 2.5, 5, 7.5 and 10 μM [^{14}C] PEA and 100, 150, 200 and 300 nM of the inhibitor. For both **23** and **33**, K_i* (MAO A) = K_i (MAO A).

No	K_i*(MAO A)* (μM)	K_i*(MAO B)* (μM)	K_i** (MAO B)* (μM)	K_i*(MAO A)* / K_i** (MAO B)*
23	7	0.036	0.026	270
33	20	0.053	0.04	500

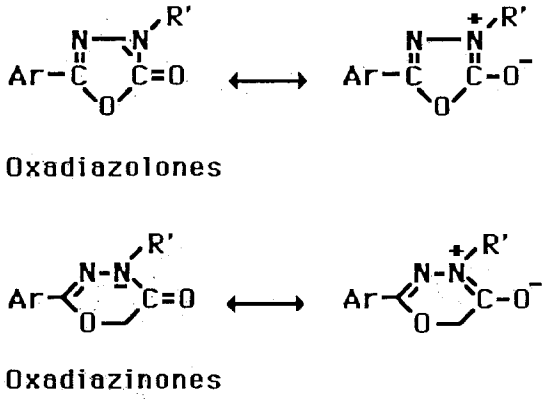
A significant decrease of activity and selectivity of the inhibitors towards MAO B was observed with the length of the cyano-alkyl chain in position 3. Optimum activity and selectivity were obtained with an alkyl chain of 2 carbon units similar to that of the specific MAO B substrate ß-phenylethylamine (**23** *vs* **24** and **25**).

Oxadiazolones were found to be more active and selective than oxadiazinones. In particular, the oxadiazolone **23** is approximately 10-fold more active and 2-fold more selective against MAO B than its oxadiazinone analogue.

In general, the replacement of the carbonyl group in oxadiazolones by thiocarbonyl (**26–28**, **30**, **31**, **33**) resulted in complex and contradictory effects.

Concerning the hetero-ring, it can be observed that the activity of the inhibitors seems to increase together with the electronegative character of the X – C = Y group: O – C = S (**33**) < O – C = O (**23**) and S – C = S (**37**) < S – C + O (**36**). These modulations in the heterocycle ring support the existence of a nucleophilic binding site on MAO B for the anchoring of the inhibitors.

A higher electronegative effect of the X – C = Y group should induce a higher decrease of the electronic density on the N-3 atom of the hetero-ring probably leading to stronger electronic interactions of the inhibitor woth the enzyme. Such a mechanism could explain, at least partly, the enhanced activity of oxadiazolones with regard to oxadiazinones (scheme 3).



Scheme 3.

In conclusion, as already noted for oxadiazinones, with a 2-cyanoethyl group as a side chain, an effective and selective inhibition of MAO B by oxadiazolones and oxadiazolethiones was only observed when the R substituent was a hydrophobic group. On the other hand, oxadiazolone **23** and, to a lesser extent, oxadiazolethione **33**, which both have a pseudoaromatic ring with a potentially delocalized positive charge, were found to be more potent and selective than the non aromatic oxadiazinone analogue. More particularly, **23** and **33** have proved to act as reversible and competitive MAO B inhibitors with a slight slow-binding character. Their steady-state K_i values of 2.6 and 4×10^{-8} M, respectively, and their K_i (MAO A/MAO B) ratios of 270 and 500, respectively, rank them among the most potent, selective and competitive MAO B inhibitors.

More significant in this study is perhaps the finding of a new class of potent and competitive inhibitors which are not amine derivatives. Indeed at present, potent and reversible MAO B inhibitors such as MD 240928, Ro 16-6491 and Ro 19-6327 belong to the amine classes. Nevertheless, like monoamines, these inhibitors should be deaminated by MAO B or/and A. For instance, MD 240928 has been shown to be a good substrate for MAO A [43, 44], and its hydroxylated metabolite is a strong inhibitor of MAO B [25]. Consequently, the use of such inhibitors as biochemical tools may complicate kinetic or binding studies on MAO B. Moreover, an eventual relatively rapid inactivation of the inhibitor *in vivo* may not be desirable for therapeutic uses.

Compounds **23** and **33**, which show promising features, are at present under molecular modifications in view to improve their affinity and selectivity towards MAO B. These studies will be reported when completed.

Experimental protocols

Chemistry

Melting points were taken on a Büchi 510 capillary melting point apparatus and are uncorrected. IR spectra recorded as KBr disks and reported in cm^{-1} were obtained on a Perkin–Elmer 1310 spectrophotometer. PMR spectra were recorded on a Brucker WP 80 spectrometer in DMSO–d_6 with tetramethyl silane as the internal reference. Column chromatography was carried out on Macherey–Nagel silica gel 60 (0.05–0.20 mm). Analyses indicated by the symbols of the elements or functions were within ± 0.4% of theoretical values.

*1-Aroyl-2-(2-cyanoethyl)hydrazines **3–5** and **7***
A suspension of aroylhydrazine (20 mmol) in 25 ml ethanol was treated with freshly distilled acrylonitrile (1.32 g, 25 mmol). The stirred mixture was refluxed for 25 h. After removal of the solvent *in vacuo*, the product was first purified by column chromatography using ethyl acetate-petroleum ether (9:1) as the eluent, then recrystallized from the appropriate solvent (table I).

*1-Aroyl-2,2bis(2-cyanoethyl)hydrazines **11–13** and **15***
Freshly distilled acrylonitrile (2.65 g, 50 mmol) was added to a suspension of aroylhydrazine (20 mmol) in 20 ml ethanol. The mixture was refluxed under stirring for 72 h, then cooled. The solid was filtered and recrystallozed from the appropriate solvent (table I).

5-(4-Biphenylyl)-1,3,4-oxadiazol-2(3H)-one ***17***
Phosgene was bubbled for 30 min into a cooled and stirred solution of 4-biphenylylcarbonylhydrazine (5.5 g, 25 mmol) in 200 ml of dry dioxane. The solvent was evaporated under a hood and the residue was recrystallized from 1-butanol (table II).

5-Aryl-3-(2-cyanoethyl)-1,3,4-oxadiazol-2(3H)-ones ***18–23***
25 ml of a fresh solution of phosgene in toluene (10%, w/v) was added to hydrazines **1–3**, **5**, **6** and **8** (5 mmol). The suspension was stirred for 3 h at room temperature. The solid obtained after evaporation to dryness under a hood was recrystallized from the appropriate solvent (table II).

5-(4-Biphenylyl)-3-(3-cyanopropyl)-1,3,4-oxadiazol-2(3H)-one ***24*** *and 5-(4-biphenylyl)-3-(4-cyanobutyl)-1,3,4-oxadiazol-2(3H)-one* ***25***
To a stirred solution of **17** (0.5 g, 3 mmol) in DMF (10 ml) was added, dropwise at room temperature, 2 ml of 1 M aqueous KOH solution. Stirring was continued for further 30 min to achieve the salt formation, then dry DMF (2 ml) containing the halogen derivative (3 mmol, 4-bromobutyronitrile for **24** or 5-bromovaleronitrile for **25**) was added dropwise. The reaction was stirred at room temperature for an additional 1 h, then heated at 50°C in water bath for 4 h. The resulting solution was cooled, poured into 50 ml of ice water and the crude solid was collected and recrystallized from the appropriate solvent (table II).

5-Aryl-3-(2-cyanoethyl)-1,3,4-oxadiazole-2(3H)-thiones ***26–33***
A solution of hydrazine **1–8** (5 mmol) in dry chloroform (50 ml) was treated with freshly distilled thiophosgene (0.58 g, 5 mmol). The stirred mixture was refluxed for 3 h under a hood. The residue obtained by evaporation to dryness was chromatographed on a silica gel column using dichloromethane as the eluent to remove byproducts. The products were recrystallized from ethanol (table II).

Alternative procedures

Oxadiazolones ***18–23***
Into a solution of hydrazine **9–11**, **13**, **14** and **16** (5 mmol) in dioxane (25 ml) was passed an excess of phosgene at gentle reflux. A white solid, which precipitated initially, dissolved within 2–3 h. The resulting solution was evaporated to dryness. The residue was chromatographed on a silica gel column using ethyl acetate–petroleum ether (19:1) as the eluent to remove the unreacted hydrazine. The oxadiazolone was recrystallized from the appropriate solvent (table II).

Oxadiazolethiones ***26–33***
To a solution of hydrazine **9–16** (5 mmol) in dry chloroform (25 ml) was added freshly distilled thiophosgene (0.58 g, 5 mmol). The stirred mixture was refluxed for 4 h then filtrated and evaporated. The crude product was recrystallized from ethanol (table II).

Both procedures gave products which were identical, in every respect, to the samples prepared from the monosubstituted hydrazines and which are described in tables II and III. However, much lower yields were obtained, ranging from 10–20%.

4-Biphenylyldithiocarbonyloxyacetic acid ***34***
To a cold and vigorously stirred of 4-biphenylylmagnesium bromide solution prepared from 4-biphenylylbromide (126 g, 0.54 mol) and magnesium (14.5 g, 0.6 mol) in dry THF (300 ml), was added dropwise a solution of carbon disulfide (50 ml, 0.83 mol) in dry THF (100 ml). The mixture was stirred for 12 h at room temperature, then treated with a solution of chloroacetic acid (50 g, 0.53 mol) and Na_2CO_3 (76 g, 0.72 mol) in H_2O (500 ml). Stirring at room temperature was continued for one week. The precipitate was filtered and washed with water. The filtrate was extracted with diethyl ether. The aqueous layer containing the sodium salt of the product was acidified with sulfuric acid and extracted with ether. The red organic phases were dried over sodium sulfate and evaporated *in vacuo*. The obtained raspberry-red colored solids were combined and recrystallized from benzene to yield 125 g of **34**, mp: 170°C; yield 80%. IR (KBr) cm^{-1}: 2600 (HO), 1650 (CO). PMR δ ppm: 4.3 (s, 2H), 7.25–8.15 (m, 9H), H(HO) diffuse signal. Anal $C_{15}H_{12}O_2S_2$ (C, H).

1-(4-Biphenylylthiocarbonyloxy)-2-(2-cyanoethyl)hydrazine ***35***
To a stirred solution of 4-biphenylyldithiocarbonyloxyacetic acid **34** (5.76 g, 20 mmol) in N aqueous NaOH solution (60 ml), was added dropwise at 0°C a solution of freshly distilled (2-cyanoethyl)hydrazine (1.7 g, 20 mmol) prepared by the method of Hoffmann [36]. Stirring was continued for 2 h at 0°C. The precipitate was collected by filtration. The filtrate was acidified with acetic acid and the second precipitate was also collected. The resulting filtrate was extracted with diethyl ether and evaporated. Collected crude solids, which contained small amounts of an additional product, were recrystallized from diethyl ether to yield 2.87 g of **35**, mp: 132°C; yield 51%. IR (KBr) cm^{-1}: 3230 (NHNH), 2240 (CN). PMR δ ppm: 2.75 (t, 2H), 3.3 (t, 2H), 7.2–7.9 (m, 9H), H(NHNH) diffuse signal. Anal $C_{16}H_{15}N_3S$ (C, H, N).

5-(4-Biphenylyl)-3-(2-cyanoethyl)-1,3,4-thiadiazol-2(3H)-one ***36***
50 ml of fresh solution of phosgene in toluene (10%, w/v) was added to **35** (2.8 g, 10 mmol). The suspension was stirred for 3 h at room temperature. The solid obtained after evaporation to dryness under a hood was recrystallized from diethyl ether (table II).

5-(4-Biphenylyl)-3-(2-cyanoethyl)-1,3,4-thiadiazole-2(3H)-thione ***37***
A solution of **35** (1.4 g, 5 mmol) in dry chloroform (50 ml) was treated with freshly distilled thiophosgene (0.58 g, 5 mmol). The stirred mixture was refluxed for 3 h then evaporated to dryness under a hood. The residue obtained was recrystallized from benzene (table II).

Biochemistry

Chemicals
The radioisotopically labeled substrates, 5-hydroxytryptamine-[side chain-2-^{14}C]creatinine sulfate (54 mCi/mmol) and ß-phenylethylamine-[ethyl1-^{14}C]hydrochloride (55 mCi/mmol), were purchased from Amersham Laboratories, Amersham, England. Clorgyline hydrochloride was obtained from May and Baker, Dageham, England. L-Deprenyl hydrochloride was donated by the Centre de Recherche Delalande, Rueil-Malnaison, France. Drugs to be tested were dissolved in dimethyl sulfoxide (DMSO) to 100 mM. The weaker inhibitors were diluted in DMSO–water (1:1, v/v). The more potent inhibitors were diluted in 10% aqueous DMSO, which caused no MAO inhibition. Clorgyline and L-deprenyl were dissolved in

water. Prior to use, isotopes were diluted with corresponding unlabeled amines to give working solutions of known specific radioactivity.

Mitochondrial homogenate preparation
A crude mitochondrial fraction was prepared following the slightly modified procedure previously described [36]. All operations were carried out at 4°C. Male and female adult Sprague–Dawley rats (Iffa Credo, L'Arbresle, France), weighing 200–250 g, were decapited. All brains were removed rapidly and homogenized with an Ultra-Turrax homogenizer in cold 0.32 M sucrose and 10 mM potassium phosphate buffer, pH 7.4 (15:1, v/w). The homogenate was centrifuged twice at 1000 *g* for 5 min at 4°C. The resulting supernatant was centrifuged at 20 000 x *g* for 20 min. The crude mitochondrial pellet obtained was suspended (4:1, v/w) in 10 mM potassium phosphate buffer, pH 7.4, fractionated in plastic vials to 500 µl samples and stored at – 80°C. Before use, mitochondria were diluted with 100 mM phosphate buffer, pH 7.4, to give a working solution of 0.79 mg of protein/ml.

Protein determination
Protein content of the mitochondrial homogenate was determined according to the method of Lowry [45], using bovine serum albumin as the standard.

MAO activity determination
MAO types A or B activity was determined with [^{14}C] 5-HT (4.8 µCi/µmol) or [^{14}C] PEA (26.6 µCI/µmol), respectively as substrates, by the standard radioassay procedure previously described [30]. Both MAO activities were linear with time and enzyme concentrations, and substrate metabolisation did not exceed ≈ 10% of the available substrate in the absence of any inhibitor. All the assays were routinely duplicated.

IC_{50} determinations
For each inhibitor, IC_{50} (MAO A) and IC_{50} (MAO B) values were obtained graphically from log concentration/MAO inhibition plots based on at least 4–5 different inhibitor concentrations ranked in the pseudolinear part of the inhibition curve.

Reversibility tests
1 ml of mitochondrial working solution (0.79 mg of protein/ml) was incubated at 37°C for 20 min in the absence or presence of **23**, **33** and the irreversible MAOI references clorgyline and L-deprenyl, at concentrations which inhibited partially MAO A or B, as indicated in table V. For washing tests, mitochondria were diluted in 50 ml of cold 100 mM phosphate buffer, pH 7.4, and centrifuged at 20 000 *g* for 20 min at 4°C. The pellet was resuspended in 50 ml of the same phosphate buffer and the same procedure was repeated 3 times. Washed mitochondria were diluted in 1 ml of phosphate buffer. For dialysis tests, mitochondria were dialyzed for 24 h at 4°C against 1 liter of 100 mM phosphate buffer, pH 7.4.

For each test series, MAO activity of the samples was measured under standard conditions (without preincubation) and expressed as % of the respective control (table V).

Time-courses of MAO inhibition
MAO A and B activities of the mitochondrial working solution were determined under standard conditions after various periods of preincubation (0, 1, 2, 3, 4, 5, 6, 10 and 30 min) at 37°C in the absence or presence of 100 nM and 10 µM of **23** (concentrations which inhibit 64% MAO B and 55% MAO A, respectively). Remaining MAO activities were expressed as % of the controls. The same procedure was performed for **33** at 100 nM and 20 µM (concentrations which inhibit 57% MAO B and 49% MAO A, respectively).

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