Selective and Potent Monoamine Oxidase Type B Inhibitors: 2-Substituted 5-Aryltetrazole Derivatives

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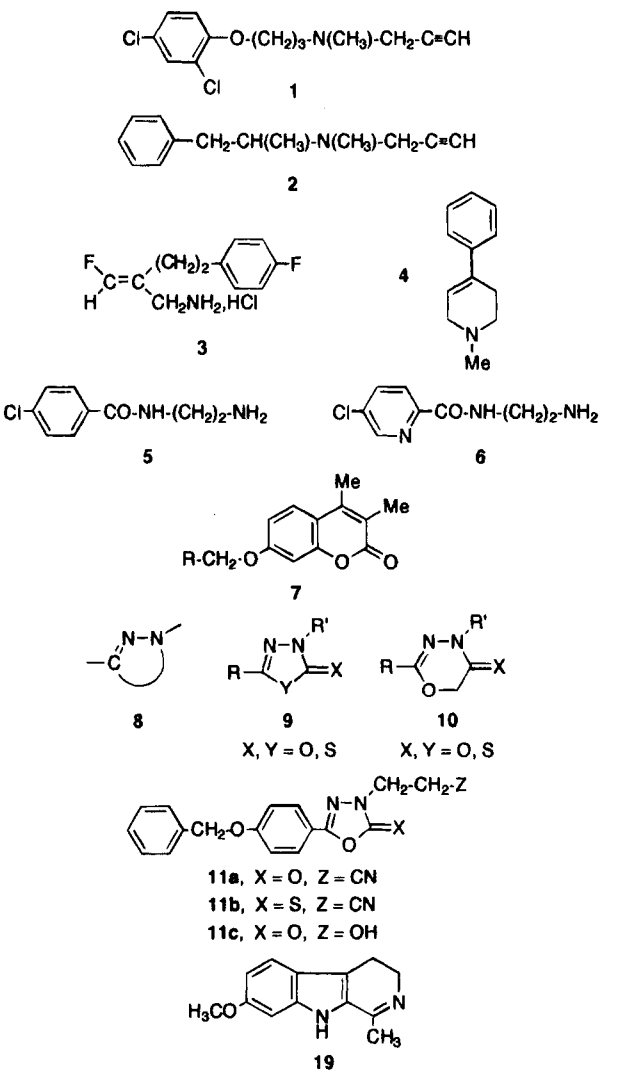
Twenty new 2-(cyanoalkyl)tetrazoles (**15** and **16**) and twenty new 2-(hydroxyalkyl)tetrazoles (**17** and **18**) were synthesized and investigated in vitro for their abilities to inhibit selectively rat brain monoamine oxidase (MAO) B over MAO A. Most of them were MAO B inhibitors and those bearing a substituted 4-(arylmethoxy)phenyl group in the position 5 of the tetrazole ring had IC_{50} values between 8 μM for **18d** and 2 nM for **16a** (30 nM for lazabemide) with a selectivity toward MAO B of 37 000 for **16a**. The reversibility of their inhibitory activity was demonstrated by in vitro dialysis tests. The 5-[4-(phenylmethoxy)phenyl]-2-(2-cyanoethyl)-tetrazole (**16a**) its derivative **16h** and the 5-[4-(phenylmethoxy)phenyl]-2-(2-hydroxyethyl)-tetrazole (**18a**) and its derivative **18h** were found to be potent, in vitro selective, and competitive MAO B inhibitors. Tetrazole **16a** can be considered one of the most active and selective competitive MAO B inhibitors known up to now. This compound was selected for ex vivo experiments and was shown to be a strong and reversible MAO B inhibitor with a short duration of action after oral administration at 5 mg/kg. The structure–activity approach gives rise to the great importance of lipophilicity over electronic effects of the compounds in these series.

Introduction

The mitochondrial enzyme monoamine oxidase (MAO, EC 1.4.3.4) is a FAD-containing enzyme[1] implicated in the oxidative deamination of a variety of biogenic and exogenic monoamines. It exists as two isoforms, MAO A and MAO B,[2,3] and it has been well established that they are encoded by separate genes[4] located on the human X chromosome.[5] MAO A selectively deaminates biogenic amines and is irreversibly inhibited by clorgyline (**1**). MAO B preferentially deaminates β-phenylethylamine and is irreversibly inhibited by L-deprenyl (**2**). The MAO's and their inhibitors have been recently reviewed.[6–8] MAO B inhibitors such as L-deprenyl (**2**), alone or with an antioxidant, are used with L-dopa to retard the progression of the degenerative process of Parkinson's disease.[9–11] They could also be very active drugs for the treatment of age-mediated neurodegenerative disorders such as depressed mood, anxiety, and severely inhibited sexual performance.[7,12]

L-Deprenyl (**2**) belongs to the well-known class of *N*-methylpropargylamine suicide inhibitors of MAO B.[13] The fluoroallylamines such as MDL 72,974 A[14] (**3**) are also selective irreversible MAO B inhibitors. It has been reported that some analogues of the nigrostriatal toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine or MPTP[15] (**4**) and some molecules extracted from *Himatanthus sucuuba*, a brazilian plant,[16] had MAO B inhibitory activities.

Few examples of reversible and selective MAO B inhibitors are available. Some analogues of benzylamine, aliphatic amines, primary alcohols, or benzyl



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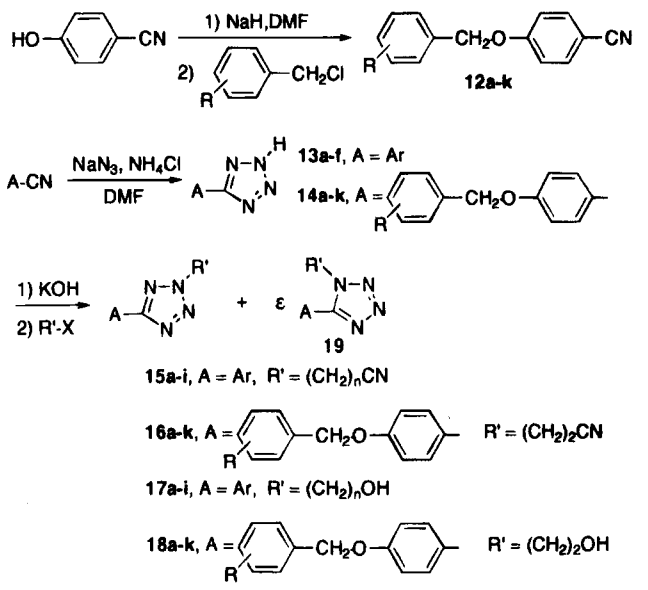
Scheme 1



cyanide[17] and also numerous psychotropic drugs including the tricyclic antidepressants (amitriptyline, clomipramine, desipramine, and imipramine)[18] are compounds with low in vitro activity and selectivity. Compounds Ro 16−6491 (**5**) and Ro 19−6327 or lazabemide (**6**) are highly selective and reversible MAO B inhibitors[19,20] and are under clinical investigation.[21,22] Recently, it was reported that some ether derivatives of coumarin (**7**) had the same properties.[23]

Another new promising class of reversible MAO B inhibitors structurally related to a cyclic hydrazone moiety **8** was studied in our laboratory as described in recent papers on the activities of 1,3,4-oxadiazol-2(3*H*)-one derivatives **9**[24,25] and of 4*H*-1,3,4-oxadiazin-3(6*H*)-one derivatives **10**.[26] The 5-[4-(phenylmethoxy)phenyl]-3-(2-cyanoethyl)-1,3,4-oxadiazol-2(3*H*)-one (**11a**), its analogue the oxadiazolethione **11b**, and the 5-[4-(phenylmethoxy)phenyl]-3-(2-hydroxyethyl)-1,3,4-oxadiazol-2(3*H*)-one (**11c**) were shown to act as reversible, highly potent, and selective MAO B inhibitors. Their IC_{50} (MAO B) values were in the low nanomolar range of 1.4−4.6 nM and their selectivities, estimated from the IC_{50}(MAO A)/IC_{50}(MAO B) ratio, were from 3200 to >71 000.

In the course of this study, new 2,5-disubstituted tetrazoles structurally close to compounds **9** and suspected to be more stable to hydrolysis than oxadiazolone derivatives were prepared and tested in vitro for their MAO A and B inhibitory activities. The ex vivo properties of some of the most potent and selective in vitro MAO B inhibitors were evaluated.

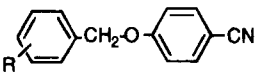
Chemistry

The synthesis of tetrazole derivatives was performed as illustrated in Scheme 1. Commercially unavailable 4-(arylmethoxy)benzonitriles **12a−k** were prepared in good yield by treatment of the 4-cyanophenol sodium salt with the corresponding substituted benzyl chloride in anhydrous dimethylformamide. According to a classical method,[27] benzonitrile derivatives were converted into 5-substituted tetrazoles **13a−f** and **14a−k** by treatment with azothydric acid generated in situ from sodium azide and ammonium chloride in dimethylformamide. Alkylation of **13** and **14** by reaction of the appropriate alkyl halogenide derivative with their potassium salts, prepared by action of KOH in 1-propanol, gave a mixture of isomeric *N*-1 and *N*-2 substituted 5-aryltetrazoles. In all cases, the latter were found to be the major products (the molar ratio was greater than 10:1).

Pure *N*-2 isomers **15−18** were obtained by selective recrystallization, and some *N*-1 isomers **19** were isolated by chromatographic methods. To confirm our assignment, we have prepared some *N*-1-substituted isomers by an unambiguous method of synthesis which will be presented with their MAO inhibitory activities in an upcoming paper.

The physicochemical characteristics of the new compounds are given in Tables 1−3. Satisfactory ^1H NMR spectra and analytical data were obtained for all compounds. IR data of compounds **15−18** have shown

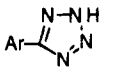
Table 1. Physicochemical Data of 4-(Arylmethoxy)benzonitriles **12a−k**



no.	R	mp,[a] °C	rec solv[b]	% yield[c]	formula[d]
12a	H	96	A	87	$C_{14}H_{11}NO$
12b	2-Me	81	A	90	$C_{15}H_{13}NO$
12c	3-Me	105	B	55	$C_{15}H_{13}NO$
12d	4-Me	111	B	60	$C_{15}H_{13}NO$
12e	3-MeO	98	B	61	$C_{15}H_{13}NO_2$
12f	4-MeO	130	B	83	$C_{15}H_{13}NO_2$
12g	2-Cl	88	C/D	69	$C_{14}H_{10}ClNO$
12h	3-Cl	91	C/D	76	$C_{14}H_{10}ClNO$
12i	4-Cl	101	C/D	82	$C_{14}H_{10}ClNO$
12j	4-F	120	E	78	$C_{14}H_{10}FNO$
12k	2-I	90	B	77	$C_{14}H_{10}INO$

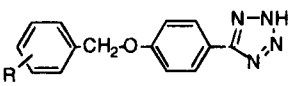
[a] Literature values: **12a**, 94 °C;[28] **12d**, 113 °C;[29] **12f**, 127−128 °C;[30] **12i**, 101−102 °C;[31] **12j**, 119 °C;[29]. [b] Recrystallization solvent: A = methanol, B = 1-propanol, C = ethanol, D = water, E = 1-butanol. [c] Yields were not optimized. [d] All compounds had C, H, N elemental analyses within ±0.4% of the theoretical values.

Table 2. Physicochemical Data of 5-Aryltetrazoles **13a−f** and 5-(4-(Arylmethoxy)phenyl)tetrazoles **14a−k**



13a-f

no.	Ar	mp,[a] °C	rec solv[b]	% yield[c]	formula[d]
13a	Ph	216	A	71	$C_7H_6N_4$
13b	4-MeO-Ph	235	A	76	$C_8H_8N_4O$
13c	4-Cl-Ph	263	A	88	$C_7H_5ClN_4$
13d	4-NO_2-Ph	220	A	80	$C_7H_5N_5O_2$
13e	4-biphenylyl	242	A	72	$C_{13}H_{10}N_4$
13f	4-pyridinyl	258 dec	B	55	$C_6H_5N_5$

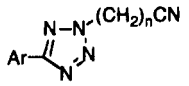


14a-k

no.	R	mp,[a] °C	rec solv[b]	% yield[c]	formula[d]
14a	H	228	A	55	$C_{14}H_{12}N_4O$
14b	2-Me	190	C	43	$C_{15}H_{14}N_4O$
14c	3-Me	184	A	37	$C_{15}H_{14}N_4O$
14d	4-Me	240	C	78	$C_{15}H_{14}N_4O$
14e	3-MeO	228	A/C	50	$C_{15}H_{14}N_4O_2$
14f	4-MeO	220	D	30	$C_{15}H_{14}N_4O_2$
14g	2-Cl	188	A	52	$C_{14}H_{11}ClN_4O$
14h	3-Cl	200	A	60	$C_{14}H_{11}ClN_4O$
14i	4-Cl	240	A	61	$C_{14}H_{11}ClN_4O$
14j	4-F	204	E	80	$C_{14}H_{11}FN_4O$
14k	2-I	207	C	46	$C_{14}H_{11}IN_4O$

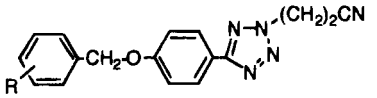
[a] Literature values: **13a**, 217−218 °C;[27] **13b**, 238−239 °C;[27] **13c**, 262−263 °C;[27] **13d**, 214−216 °C;[32] **13f**, 254 °C dec.[33] [b] Recrystallization solvent: A = methanol, B = water, C = 1-butanol, D = 1-propanol, E = xylene. [c,d] See corresponding footnotes in Table 1.

Table 3. Physicochemical Data and MAO Inhibitory Properties in Vitro for 5-Aryl-2-(cyanoalkyl)tetrazoles **15a–i**, 5-(4-Arylmethoxyphenyl)-2-(2-cyanoethyl)tetrazoles **16a–k**, 5-Aryl-2-(hydroxyalkyl)tetrazoles **17a–i**, and 5-(4-Arylmethoxyphenyl)-2-(2-hydroxyethyl)tetrazoles **18a–k**



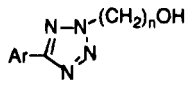
15a-i

no.	Ar	*n*	mp,[a] °C	rec solv[b]	% yield[c]	formula[d]	IC_{50},[e] μM MAO A	IC_{50},[e] μM MAO B	B selectivity[f]
15a	Ph	2	52	A/B	48	$C_{10}H_9N_5$	54	>100	<0.5
15b	4-MeO-Ph	2	84	C	40	$C_{11}H_{11}N_5O$	16.6	37	0.4
15c	4-Cl-Ph	2	100	C	55	$C_{10}H_8ClN_5$	6.5	3.6	1.8
15d	4-NO_2-Ph	2	162	C	52	$C_{10}H_8N_6O_2$	18	13.4	1.3
15e	4-biphenylyl	2	134	C	56	$C_{16}H_{13}N_5$	7.8	0.84	9.3
15f	4-biphenylyl	3	86	C	38	$C_{17}H_{15}N_5$	9.6	1.2	8
15g	4-biphenylyl	4	132	C	43	$C_{18}H_{17}N_5$	26	6	4.3
15h	4-biphenylyl	6	100	C	40	$C_{20}H_{21}N_5$	46	5.4	8.5
15i	4-pyridinyl	2	166	A	50	$C_9H_8N_6$	>100	30	>3.3



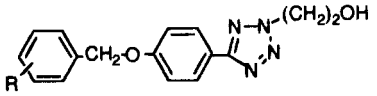
16a-k

no.	R	mp,[a] °C	rec solv[b]	% yield[c]	formula[d]	IC_{50},[e] μM MAO A	IC_{50},[e] μM MAO B	B selectivity[f]
16a	H	128	C	61	$C_{17}H_{15}N_5O$	86	0.002	37000
16b	2-Me	92	C	17	$C_{18}H_{17}N_5O$	>100	0.11	>910
16c	3-Me	106	C	46	$C_{18}H_{17}N_5O$	>100	0.2	>500
16d	4-Me	140	C	40	$C_{18}H_{17}N_5O$	>100	0.57	>180
16e	3-MeO	109	C	34	$C_{18}H_{17}N_5O_2$	>100	0.4	>250
16f	4-MeO	118	C	10	$C_{18}H_{17}N_5O_2$	>100	3.4	>30
16g	2-Cl	100	C	45	$C_{17}H_{14}ClN_5O$	>100	0.03	>3300
16h	3-Cl	93	C	41	$C_{17}H_{14}ClN_5O$	>100	0.10	>1000
16i	4-Cl	154	C	46	$C_{17}H_{14}ClN_5O$	>100	0.08	>1200
16j	4-F	142	C	45	$C_{17}H_{14}FN_5O$	>100	0.21	>480
16k	2-I	126	C	32	$C_{17}H_{14}IN_5O$	>100	0.38	>270



17a-i

no.	Ar	*n*	mp,[a] °C	rec solv[b]	% yield[c]	formula[d]	IC_{50},[e] μM MAO A	IC_{50},[e] μM MAO B	B selectivity[f]
17a	Ph	2	66	C/D	65	$C_9H_{10}N_4O$	>100	89	>1.2
17b	4-MeO-Ph	2	112	A	42	$C_{10}H_{12}N_4O_2$	46	>100	<0.5
17c	4-Cl-Ph	2	113	A	58	$C_9H_9ClN_4O$	66	38	1.7
17d	4-NO_2-Ph	2	124	A	60	$C_9H_9N_5O_3$	>100	>100	
17e	4-biphenylyl	2	148	A	52	$C_{15}H_{14}N_4O$	11	0.96	11.4
17f	4-biphenylyl	3	102	C	45	$C_{16}H_{16}N_4O$	4.6	1.36	3.4
17g	4-biphenylyl	4	105	C	48	$C_{17}H_{18}N_4O$	13	9.4	1.4
17h	4-biphenylyl	6	102	C	40	$C_{19}H_{22}N_4O$	80	16.4	4.9
17i	4-pyridinyl	2	123	C	63	$C_8H_9N_5O$	>100	>100	



18a-k

no.	R	mp,[a] °C	rec solv[b]	% yield[c]	formula[d]	IC_{50},[e] μM MAO A	IC_{50},[e] μM MAO B	B selectivity[f]
18a	H	113	C	63	$C_{16}H_{16}N_4O_2$	64	0.03	2100
18b	2-Me	95	E	22	$C_{17}H_{18}N_4O_2$	>100	0.29	>350
18c	3-Me	100	E	17	$C_{17}H_{18}N_4O_2$	>100	0.3	>340
18d	4-Me	125	C	60	$C_{17}H_{18}N_4O_2$	>100	8	>13
18e	3-MeO	105	C	21	$C_{17}H_{18}N_4O_3$	80	0.8	100
18f	4-MeO	131	C	52	$C_{17}H_{18}N_4O_3$	>100	4.7	>22
18g	2-Cl	118	A	41	$C_{16}H_{15}ClN_4O_2$	88	0.09	1000
18h	3-Cl	109	A	36	$C_{16}H_{15}ClN_4O_2$	58	0.2	290
18i	4-Cl	132	A	49	$C_{16}H_{15}ClN_4O_2$	60	0.32	200
18j	4-F	122	C	52	$C_{16}H_{15}FN_4O_2$	>100	0.27	>370
18k	2-I	133	C	23	$C_{16}H_{15}IN_4O_2$	>100	0.35	>290

Table 3 (Continued)

no.	R	mp,[a] °C	rec solv[b]	% yield[c]	formula[d]	IC_{50},[e] μM MAO A	IC_{50},[e] μM MAO B	B selectivity[f]
MAOI's references								
harmaline (**19**)						0.012	150	5×10^{-5}
lazabemide (**6**)						>10	0.03	>333

[a] Literature values: **15a**, 62 °C;[34] **15c**, 100 °C;[34] **17a**, 65 °C;[35] **17c**, 107–109 °C;[32] **17d**, 125–126 °C.[36] [b] Recrystallization solvent: A = ethyl acetate, B = petroleum ether (bp 40–60 °C), C = methanol, D = water, E = ethanol. [c,d] See corresponding footnotes in Table 1.[e] IC_{50} values were determined from experiments where the inhibitors were initially preincubated with the enzyme before adding the substrate and were graphically obtained from −log concentration/MAO inhibition plots based on at least four or five different inhibitor concentrations ranked in the pseudolinear part of the inhibition curve. IC_{50} value refers to the assay concentration of drug which produced 50% inhibition of enzyme activity. Data represent means of 3 separate experiments carried out in duplicates. Standard errors, not shown, were 10–25%. [f] Selectivity for the B form was estimated by the IC_{50}(MAO A)/IC_{50}(MAO B) ratio.

absorption bands at 2240–2260 cm^{-1} for C≡N and at 3200–3500 cm^{-1} for O–H. The ^1H NMR spectra of compounds **15a–e,i** and **16** showed two triplets (2 CH_2) at 3.3–3.35 ppm and 5–5.2 ppm. Compounds **17a–e,i** and **18** showed one triplet (OH) at 4.5–5.2 ppm, a triplet (N-CH_2) at 4.65–4.8 ppm, and a quadruplet (CH_2-O) at 3.3–4 ppm.

Results and Discussion

In Vitro Evaluation. The 5-aryl-2-(cyano(or hydroxy)alkyl)tetrazoles **15–18** were tested in vitro for their inhibitory effects on rat brain MAO types A and B using previously described specific MAO assays.[24–26] IC_{50} (MAO A and MAO B) values for these compounds (Table 3) were graphically determined from the respective MAO inhibition curves. Selectivity of inhibitors toward MAO B (Table 3) was estimated by the IC_{50} (MAO A)/IC_{50}(MAO B) ratio. IC_{50} values for the inactivators harmaline[37] (**19**) and **6**, respectively reversible inhibitors of MAO A and B, were given as reference for comparison with the tetrazole derivatives.

The results indicated that most of the compounds acted preferentially as MAO B inhibitors except for **15a,b** and **17b,d,i** being inactive. The most potent compounds, **16a,g–i** and **18a,g** in these series, inhibited MAO B selectively with IC_{50} values between 10 μM for **16h** and 2 nM for **16a**.

The selectivity of some inhibitors was difficult to assess when their IC_{50} (MAO A) values were higher than 100 μM. Indeed, the percentage of DMSO used to solubilize the compounds above a concentration of 100 μM affected MAO activity. The selectivity of the most potent inhibitor **16a** was 37 000.

Among the most in vitro active inhibitors, compounds **16a,h** and **18a,h** were selected to investigate more deeply both the in vitro inhibition mechanism of MAO A and B and the ex vivo inhibition of the enzymes.

Inhibition Mechanism. Dialysis experiments show that in our experimental conditions, MAO B inhibition by compounds **18a** and **18h** was fully reversed whereas that induced by **16a** and **16h** was partially reversed, suggesting that in vitro, these compounds are more slowly reversible MAO B inhibitors (Table 4).

The time course of MAO B inhibition by **16a** showed a slight time dependency (Figure 1). A maximum of 35% potentiation of the inhibition was observed when preincubating the inhibitor at 10 nM with the enzyme for 10 min. No significant further potentiation of the inhibition was detected with prolonged preincubation for up to 30 min. In the absence of inactivation of the enzyme, a time-dependent increase of inhibition may result from a slow isomerization or metabolism of the inhibitor to a more potent species or from a slow-binding process.

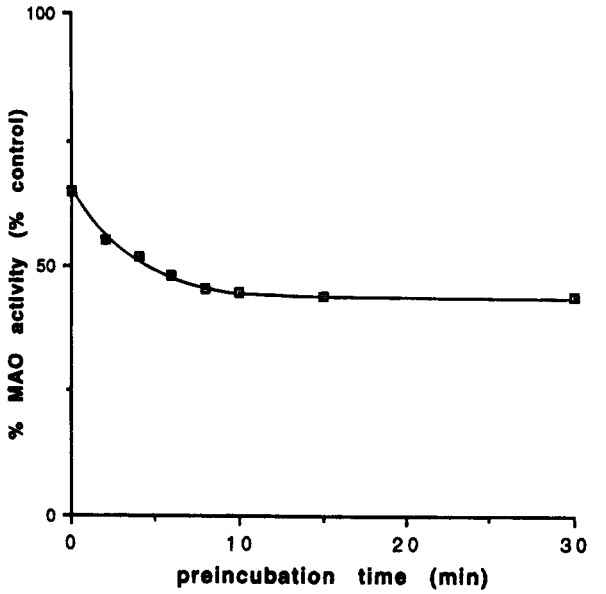


Figure 1. Time courses of rat brain MAO inhibition by **16a**. MAO activity was determined under standard conditions after various periods of preincubation at 37 °C in the absence or presence of **16a** at 10 nM for MAO B (▲), proceeding as described in the Experimental Section. Remaining MAO activities were expressed as percentages of the control.

The kinetics of MAO B inhibition by **16a** were investigated without preincubation of this compound with the enzyme. Lineweaver–Burk plots showed that it acts as a competitive inhibitor (Figure 2A). Its initial dissociation constant (K_i) value for MAO B determined from the secondary replot was 6.4 nM (Figure 2B). Moreover, as for oxadiazolone derivatives,[26] this inhibitor seems to be competitive.

Ex Vivo Inhibition. To confirm the previous in vitro results of selectivity and reversibility of four inhibitors (**16a,h** and **18a,h**) bearing a *N*-(2-cyanoethyl) or *N*-(2-hydroxyethyl) group, an ex vivo study at 5 mg/kg on rat brain MAO was undertaken (Table 5). As observed in vitro, these compounds were found to be selective and to produce 74, 81, 84, and 71% MAO B inhibition, respectively, 1 h after administration. Moreover, the inhibitory effect of **16h**, which lasted at least 4 h, had almost disappeared 24 h later, indicating a short-lasting action compared to irreversible inhibitors[38] like **2** for which the action depends on the enzyme turnover, lasting up to 8 days. No apparent toxic or behavioral effects were observed with **16a** at very high doses (a single dose of 1000 mg/kg po, or a daily dose of 100 mg/kg po for 9 days).

Structure–Activity Relationships. Only tetrazole derivatives substituted at *N*-2 by a cyanoalkyl or hydroxyalkyl chain were synthesized. The reason is that,

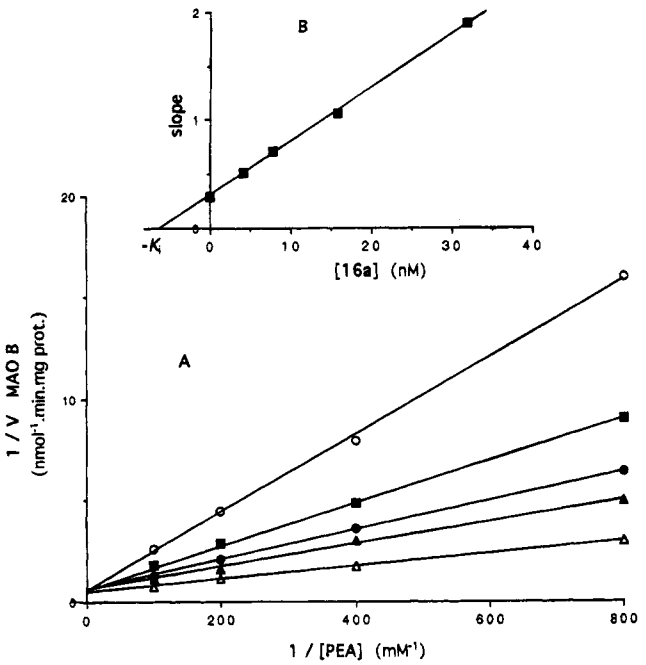


Figure 2. Kinetics of rat brain MAO B inhibition by compound **16a**. MAO B activity was determined under standard conditions and expressed in $nmol^{-1}$ min mg of protein, proceeding as described in the Experimental Section. (A) Lineweaver-Burk plot: MAO B activity was measured with 1.25, 2.5, 5.0, and 10 μM of [^{14}C]PEA in the absence (△) or presence of 4 (▲), 8 (●),16 (■), and 32 (○) nM of **16a** without preincubation with the enzyme. (B) Lineweaver-Burk secondary replot. K_i = 6.4 nM. Reactions without preincubation of the inhibitor with the enzyme were started by addition of the enzyme to the mixtures containing the substrate and the inhibitor. All values represent means of duplicate determinations in three homogenates.

Table 4. Reversibility Tests for **16a,h** and **18a,h** by Dialysis[a]

		MAO B activity in % of control	
no.	concn, μM	before dialysis	after dialysis
16a	0.1	12 ± 3	54 ± 2
16a	0.001	65 ± 2	85 ± 2
16h	0.1	15 ± 4	87 ± 1
16h	0.001	69 ± 2	95 ± 2
18a	0.1	19 ± 4	107 ± 1
18h	0.1	25 ± 4	109 ± 1

[a] The results are means ±SE of three separate experiments.

in other analogous chemical series described in previous papers,[24-26] it was shown that 2-cyanoethyl and 2-hydroxyethyl groups gave the best results among the various substituents tested.

Analysis of the inhibition results was complex but some observations can be reported. All new active compounds were selective MAO B inhibitors except for **15a,b** and **17b**. The less active inhibitors were compounds **15** and **17**, bearing a phenyl, a substituted phenyl, or a pyridinyl group. However, the most active ones were also the most hydrophobic compounds (**15c,e–h**, and **17e–g**) with a chlorophenyl or a biphenylyl group.

Electronic effects of the various substituents on the aryl part of the molecule were difficult to rationalize. For example, compounds **15d** and **17d**, which bear an electron-withdrawing nitro group, have an inhibitory activity close to that of compounds **15b** and **17b**, which have an electron-donating methoxy group.

A poor electronic contribution to the inhibitory activity was also observed in series **16** and **18**. Compounds

Table 5. Ex vivo Time Course of Rat Brain MAO Inhibition by Tetrazole Derivatives **16a,h** and **18a,h** at 5 mg/kg[a]

		% MAO A and B inhibition[b]			
no.	MAO	0.5 h	1 h	4 h	24 h
16a	A	6 ± 2 NS	12 ± 9 NS	0 ± 2 NS	2 ± 2 NS
16a	B	76 ± 6**	74 ± 6**	69 ± 4**	24 ± 4**
16h	A	13 ± 13 NS	18 ± 12 NS	8 ± 14 NS	1 ± 3 NS
16h	B	72 ± 5**	81 ± 4**	63 ± 6**	9 ± 4*
18a	A	5 ± 15 NS	4 ± 15 NS	4 ± 15 NS	
18a	B	84 ± 2**	84 ± 2**	63 ± 5**	
18h	A	15 ± 13 NS	15 ± 14 NS	14 ± 13 NS	
18h	B	69 ± 6**	71 ± 4**	55 ± 7**	
reference	MAO	1 h	8 h	24 h	
L-deprenyl (**2**)	A	3 ± 2 NS	5 ± 1 NS	2 ± 1 NS	
	B	83 ± 6**	89 ± 1**	81 ± 2**	

[a] Compounds were given by oral gavage. A single dose was administered. After treatment and at indicated times, groups of five animals were sacrificed and brain MAO activity was assayed, proceeding as described in the Experimental Section. [b] The results are expressed as percent inhibition vs the control. Values represent means ± SEM of determinations in five homogenates. Statistical significance is indicated by asterisks: $^{**}p < 0.001$; $^{*}p < 0.05$ (Bonferroni's test); NS, not significant (Wilcoxon's test).

16a and **18a**, which bear no substituent on the arylmethoxy group, were the most potent and selective MAO B inhibitors in these series. Generally, compounds bearing a methyl, a methoxy, a fluoro, or an iodo substituent on the arylmethoxy fragment (**16b–f,j,k** and **18b–f,j,k**) were less active than those with a chloro substituent (**16g–i** and **18g–i**). The decrease in activity of compounds bearing a substituted arylmethoxy fragment could be due to steric hindrance if a hydrophobic interaction exists between the arylmethoxy group of these tetrazole derivatives and a putative hydrophobic pocket of the enzyme active site, as we have previously proposed for analogous compounds.[24-26]

Thus, the hydrophobic character of the phenylmethoxy group seems to be important for the increase of the activity and selectivity toward MAO B. The adjunction of a flexible CH_2O bridge between the two phenyls dramatically increased the activity and selectivity of the inhibitors **16a** and **18a** compared to **15e** and **17e**. These new results confirmed some structure–activity correlations previously observed with oxadiazinone[26] and oxadiazolone derivatives.[24,25]

On the other hand, the R' substituent was also important. The length of the R' substituent chain influenced the inhibition potency. For example, in the biphenylyl series, activity and selectivity were optimal with an alkyl chain of two carbon units (**15e** and **17e**) and a significant decrease was observed when lengthening this chain (**15f–h** and **17f–h**). Concerning activity and especially selectivity toward MAO B, these in vitro findings suggested that the cyano group is better than the hydroxyl group for the interaction with the catalytic site of the enzyme.

Ex vivo evaluation confirmed a better inhibition for compounds bearing the cyanoethyl group than for those with the hydroxyethyl group. The decrease of ex vivo inhibition is more rapid with this latter group.

Conclusion

According to its activity and selectivity towards MAO B, nitrile **16a** can be ranked among the most potent, and selective inhibitors of MAO B. Of particular interest, this compound was characterized as a reversible, potent, and fully selective in vitro and ex vivo MAO B

competitive inhibitor. Further studies concerning the kinetic parameters, time-dependence related to its mechanism of action, and its toxicity remain to be investigated. The biological results described in this paper and the higher chemical stability of the tetrazole ring compared to the oxadiazolone one[24] allow us to consider **16a** as valuable candidate for the symptomatic treatment of Parkinson's desease, and perhaps for the treatment of age-mediated neurodegenerative disorders. It can also be considered as a tomographic tool for diagnosis of this kind of disease.

Experimental Section

Chemistry. Anhydrous DMF was prepared by standard methods. All other solvents and reagents were reagent grade and used without purification. Melting points were determined in open capillary tubes on a Büchi 510 apparatus and are uncorrected. Column chromatography separation was carried out on Macherey-Nagel silica gel 60 (0.05-0.20 mm). Infrared spectra (KBr) were recorded on a Perkin-Elmer 1310 spectrophotometer. ^{1}H NMR spectra were recorded at 80 MHz (Bruker WP 80) in DMSO-d_6 with tetramethylsilane as the internal standard. Elemental analysis was performed in the Paris 6 Université Structural Chemistry Department. Analytical data were within 0.4% of the calculated values.

4-(Arylmethoxy)benzonitrile 12a–k. These compounds were prepared following a procedure slightly different from that described in the literature.[29] To a stirred suspension of sodium hydride (1.2 g, 50 mmol) in dry DMF (100 mL) was slowly added a solution of 4-hydroxybenzonitrile (5.96 g, 50 mmol) in dry DMF (25 mL) at room temperature. When hydrogen gas evolution ceased, a solution of a suitable benzyl chloride derivative (50 mmol) in dry DMF (20 mL) was added dropwise at 0 °C under stirring. Then, the reaction mixture was heated at 40–60 °C for 1 h and poured into cold water (300 mL). The resulting precipitate **12** was collected, dried, and recrystallized from the appropriate solvent (Table 1). The structures of compounds (**12**) were confirmed by analytical data, IR, and ^{1}H NMR spectroscopy.

5-Aryltetrazoles 13a–f and 5-[4-(Arylmethoxy)phenyl]-tetrazoles 14a–k. Compounds **13** and **14** were synthesized as described in the literature.[27] Their structures were confirmed by analytical data, IR, and ^{1}H NMR spectroscopy.

General Procedure for 5-Aryl-2-[cyano(or hydroxy)-alkyl]tetrazoles 15a–i or 17a–i and 5-[4-(Arylmethoxy)-phenyl]-2-[2-cyano(or hydroxy)ethyl]tetrazoles 16a–k or 18a–k. To a solution (or a suspension) of a 2-unsubstituted tetrazole **13** or **14** (10 mmol) in 1-propanol (30 mL) was added a solution of KOH (0.56 g, 10 mmol) in 1-propanol (30 mL). After heating at 80 °C for 1 h, a solution of 12 mmol of 3-bromopropionitrile (or its halogenous homologous nitriles) in 1-propanol (30 mL) for **15** or **16** or 10 mmol of pure 2-bromoethanol (or its halogenous homologous alcohols) for **17** or **18** was added. After refluxing the mixture for 1 day and removal of the solvent, diethyl ether (100 mL) was added and the mixture was stirred for 30 min. The ethereal solution was filtered and washed first with 0.5 M aqueous NaOH solution (20 mL) and then twice with water (20 mL) before being dried over sodium sulfate. After removal of the solvent, the resulting residue **15**, **16**, **17**, or **18** was recrystallized twice from the appropriate solvent (Table 3).

Biochemistry. [^{14}C]-5-HT creatine sulfate (1.8–2.2 GBq/mmol) was supplied by Amersham (Buckinghamshire, UK). [^{14}C]PEA hydrochloride (1.8–2.2 GBq/mmol) was supplied by New England Nuclear (Boston, MA). 5-HT creatine sulfate, PEA hydrochloride, and 2,5-diphenyloxazole (PPO) were supplied by Sigma (St. Louis, MO). Toluene, EDTA, and ethyl acetate were purchased from Labosi (OSI, Paris, France) and Biofluor from New England Nuclear. Inactivator **2**·HCl was donated by Synthélabo Recherche, Rueil-Malmaison, France. Inactivators **6** and **19** were supplied by Sigma and Chinoin, respectively. Drugs to be tested were dissolved in dimethyl sulfoxide (DMSO) to 1 or 10 mM, in DMSO/water (1:1, v/v) to 0.5 mM, and for the weaker concentrations in 10% DMSO/water (v/v), which caused no MAO inhibition (the final concentration of DMSO being 1% in the inhibition kinetic studies). Prior to use, radiolabeled compounds were diluted with corresponding unlabeled amines to give solutions of known specific radioactivity.

In Vitro MAO Inhibition. A crude mitochondrial fraction was prepared following the slightly modified Eichberg procedure.[39] All operations were carried out at 4 °C. Male and female adult Sprague-Dawley rats (Iffa Credo, L'Arbresle, France) weighing 100–120 g, were decapitated. All brains were rapidly removed and homogenized with an Ultra-Turrax homogenizer in cold 0.32 M sucrose and 10 mM Tris hydrochloride, 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 20000*g* for 20 min. The crude mitochondrial pellet obtained was suspended (4:1, v/w) in 10 mM Tris hydrochloride, pH 7.4 (8% Ficoll), and centrifuged again at 20000*g* for 20 min. Finally, the resulting pellet was suspended (4:1, v/w) in the same buffer solution and centrifuged a third time at 20000*g* for 20 min. The mitochondrial pellet was suspended (4:1, v/w) in 100 mM sodium 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 sodium phosphate buffer (pH 7.4) to give a working solution of 0.79 mg of protein/mL. For IC_{50} determinations, rat brains were homogenized in 20 volumes of 100 mM sodium phosphate buffer (pH 7.4) at 4 °C. MAO activities were determined as previously described by Fowler and Strolin-Benedetti[40] with minor modifications. Briefly, 100 μL homogenates were preincubated for 20 min at 37 °C with different concentrations of inhibitors in a total volume of 400 μL. After preincubation, the reaction was started by addition of [^{14}C]-5-HT (final concentration 125 μM) as the specific MAO A substrate or [^{14}C]PEA (final concentration 8 μM) as the specific MAO B substrate.[41–44] The incubation times were 5 min for MAO A and 1 min for MAO B. The reaction was stopped by addition of 200 μL of 4 N HCl. Deaminated metabolites were extracted by vigorous shaking for 10 min in 7 mL of toluene/ethyl acetate (v/v). Following extraction, the aqueous phase was frozen with liquid nitrogen and the organic layer was poured into a scintillation vial to which 10 mL of toluene containing 0.4% (w/v) 2,5-diphenyloxazole was subsequently added. After 5 min of agitation, radioactivity was measured in a scintillation spectrometer (LS-1801, Beckmann, USA). Blank values were obtained by adding HCl prior to the substrate.

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

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 four or five different inhibitor concentrations ranked in the pseudo-linear part of the inhibition curve.

Dialysis Experiments. One milliliter of mitochondrial working solution was incubated at 37 °C for 20 min in the absence or presence of 0.1 μM of **16a,h** or **18a,h** or 1 nM of **16a,h** (MAO B inhibition). Mitochondria were dialyzed for 15 h at 4 °C against 1 L of 100 mM sodium phosphate buffer (pH 7.4). MAO B activities of the samples were then measured under standard conditions (without preincubation) and expressed as a percentage of the respective control.

Time Course of MAO B Inhibition. MAO B activity of the mitochondrial suspension (0.79 mg of protein/mL) was measured under standard conditions in the absence or presence of **16a** (10 nM) for various periods of preincubation (0, 2, 4, 6, 8, 10, 15, and 30 min). MAO activity was expressed as a percentage of the controls.

Ex Vivo MAO Inhibition. Sprague-Dawley rats weighing 120–140 g were used. After 24 h of fasting, animals were weighed. Compounds were tested at 5 mg/kg. For each compound, 25 mg were solubilized in 50 μL of Tween 80 and then in 25 mL of 0.5% methyl cellulose. The compounds were orally administered in a volume of 5 mL/kg. At given times, rats were decapitated and their brains removed. A cerebral hemisphere was homogenized with a polytron (Kinematika CH 6010) in 100 mM sodium phosphate buffer (pH 7.4), (20:1, v/w).

The total volume of the standard assay was 500 μL. A mixture of 300 μL of 100 mM sodium phosphate buffer (pH 7.4) and 100 μL of brain suspension were preincubated and stirred in a water bath for 5 min at 37 °C. MAO A and B inhibitory activities were determined as previously described.

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