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Different stereoselective inhibition of monoamine oxidase-B by the *R*- and *S*-enantiomers of MD 780236

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MD 780236, a selective inhibitor of the B form of MAO behaves as an irreversible inhibitor in in-vitro conditions and mainly as a short-acting inhibitor in ex-vivo experiments. The enantiomer with the *R* absolute configuration (MD 240928) is fully reversible in ex-vivo conditions, whereas the *S*-enantiomer (MD 240931) has kept the irreversible component of the inhibition seen with MD 780236. The corresponding racemic alcohol derivative (MD 760548) is also a short-acting inhibitor of the B form of MAO; its *S*-enantiomer, which has an absolute configuration corresponding to that of MD 240928, has a selectivity towards the B form superior to that of the other enantiomer. The mechanism of the MAO inhibition by MD 780236 is discussed.

MD 780236, 3-[4-((3-chlorophenyl)methoxy)-phenyl]-5[(methylamino)methyl]-2-oxazolidinone, is a selective inhibitor of the B form of the enzyme monoamine oxidase (MAO; monoamine O_2: oxidoreductase; EC 1.4.3.4) in rat tissues (Strolin Benedetti et al 1982a,b; Tipton et al 1982b). This compound is an irreversible inhibitor in in-vitro experiments, whereas it behaves ex-vivo mainly as a short-acting inhibitor, although some residual inhibition is still present 72 h after administration, indicating the presence of an irreversible component. Preliminary results on the metabolism of this molecule in the rat have shown that the acidic derivative formed from oxidative deamination is one of the major urinary metabolites (Strolin Benedetti & Dow 1982).

These results have led us to hypothesize that MD 780236, which is a secondary amine, might act not only as a 'suicide inhibitor' of monoamine oxidase (analogous to the inhibition of MAO by some *N*-cyclopropyl-*N*-arylalkylamine derivatives [Silverman & Hoffman 1980]), but also as a substrate for the enzyme.

The interaction between both forms of monoamine oxidase and their substrates and inhibitors is very sensitive to the absolute configuration of the molecules; for discussion see Fowler (1982) and Williams (1982). For example, (+)-amphetamine is a much more potent and selective inhibitor of MAO-A than the (−)-enantiomer (Mantle et al 1976). For the acetylenic 'suicide' MAO-B selective inhibitor selegiline (deprenyl), it is the (−)-enantiomer that is the more potent (Knoll & Magyar 1972).

Since MD 780236 is a racemic compound the two enantiomers have been synthesized in order to investigate whether they both have the same irreversible and reversible inhibitory properties in-vivo or whether one of them alone is responsible for the irreversible characteristics of MD 780236. In addition, the aldehyde, alcohol and acid derivatives of MD 780236 have been synthesized and tested for their MAO inhibitory properties.

MATERIALS AND METHODS

The compounds studied have been synthesized in the Department of Organic Chemistry of the Delalande Research Centre (†). Their structural formulae as well as their respective code numbers are presented in Table 1. MD 780236 and its enantiomers were prepared as methane sulfonate salts.The enantiomers of MD 780236 and MD 760548 were synthesized unambiguously from compounds of known absolute configuration and their *R* or *S* configuration is indicated. The aldehyde was synthesized and used in the hydrated form because of the stability of the adduct.

Male Sprague Dawley rats (Charles River, CD, France), 160≃190 g, fasted for approximately 16 h for the ex-vivo experiments, were used. The compounds were administered orally at the dose of 5 mg kg^{-1} (expressed in terms of the free base for the amine derivatives). The salts were administered in aqueous solution and the other compounds as a suspension in 0·5% methylcellulose.

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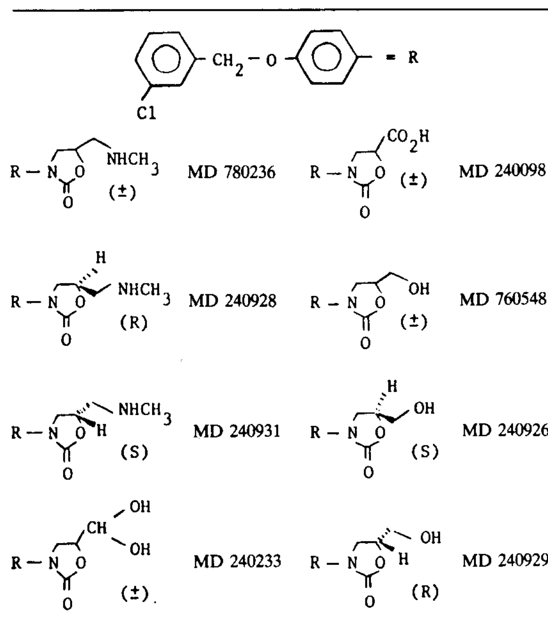
* Delalande French patents, n° 2381.037 (26.8.1977), n° 2428.032 (9.6.1978), n° 2458.547 (4.6.1980).

Table 1. Structural formulae of the studied compounds.



Monoamine oxidase-A and -B inhibition was studied in-vitro in rat brain homogenates and ex-vivo in rat brain and liver homogenates as described previously (Strolin Benedetti et al 1982a,b), with 5-hydroxytryptamine (5-HT) and β-phenethylamine (PEA) as substrates for MAO-A and -B, respectively. Both 5-HT and PEA can be metabolized by both forms of MAO when high substrate concentrations are used (for discussion, see Suzuki et al 1982; Tipton et al 1982a). In the brain, however, at the substrate concentrations used in the present study (480 and 12 μM for 5-HT and PEA, respectively), 5-HT was essentially metabolized by MAO-A alone and PEA by MAO-B alone. In the liver, the concentration of PEA was higher (75 μM), but even at this concentration the deamination of this substrate is brought about almost entirely by the MAO-B of this tissue, since the V_{max} of rat liver MAO-A towards PEA is only 13% of the total V_{max} (Tipton et al 1982a). PEA has also been described as a time-dependent inhibitor of MAO-B at high substrate concentration, but no such inhibition is found when low substrate concentrations are used and initial velocities are measured (Kinemuchi et al 1982), as is the case for the present study. For the in-vitro experiments, compounds other than the amine derivatives were dissolved in dimethylsulphoxide. Although high concentrations of dimethylsulphoxide (≥2%) have been found to be inhibitory towards the two forms, and particularly the B form, of MAO (Strolin Benedetti, M., Bufkens, F. & Tipton, K. F., unpublished results), the solvent was not inhibitory at the concentrations used in the present experiments. All the compounds were preincubated with the homogenates for 20 min at 37 °C. The standard error of the percentage of inhibition (s.e.) was computed following the classical formula for the variance of a ratio (see Armitage 1973).

RESULTS AND DISCUSSION

All the compounds tested were inhibitory towards rat brain MAO-A and -B, and the IC50 values for the in-vitro experiments are given in Table 2. The ratio of the IC50 values for the two enzyme forms give a rough idea of the selectivity of the compounds towards MAO-B, although too detailed an interpretation of these figures is perhaps inadvisable, since they are dependent to some extent upon preincubation times and substrate concentrations; for discussion see Fowler et al (1982).

Table 2. In-vitro inhibitory activity of the tested compounds towards the A and B forms of MAO.

	Rat brain homogenates (1 g tissue/16 ml buffer) IC50 (M)	
Inhibitor	PEA (12 μM)	5-HT (480 μM)
MD 780236	$3·4\,10^{-8}$	$1·4\,10^{-5}$
MD 240928	$3·0\,10^{-8}$	$5·0\,10^{-5}$
MD 240931	$2·2\,10^{-8}$	$7·3\,10^{-6}$
MD 240233	$3·5\,10^{-7}$	10^{-5}
MD 240098	$4·5\,10^{-6}$	$>10^{-4}$
MD 760548	$3·3\,10^{-8}$	$6·0\,10^{-7}$
MD 240926	$8·5\,10^{-8}$	$9·0\,10^{-6}$
MD 240929	$1·8\,10^{-8}$	$3·5\,10^{-7}$

The two enantiomers of MD 780236 are roughly equipotent towards MAO-B, but their potencies towards MAO-A differ. The *R*-enantiomer of MD 780236 (MD 240928) appears to have the greater MAO-B selectivity. The alcohol with the *S*-configuration (MD 240926) is more MAO-B selective than the *R*-isomer. Interestingly enough, MD 240926 has the same absolute configuration as the *R*-enantiomer of the parent amine, in line with other studies which have shown the stereoselective nature of the active centre of monoamine oxidase (Mantle et al 1976; Fowler & Oreland 1981). The aldehyde (MD 240233) and the acid (MD 240098) compounds are both inhibitory towards MAO-B, but neither compound has the potency of the amine or alcohol compounds (Table 2).

Table 3. Time course of MAO inhibition ex-vivo after acute administration of MD 780236 and its enantiomers in the rat.

Inhibitor (5 mg kg^{-1} oral)	Substrate	Tissue	% Inhibition MAO ± s.e. Time after administration (h) 1	2	4	8	24	48	72
MD 780236	PEA	brain	84 ± 1	85 ± 0	78 ± 1	67 ± 3	31 ± 2	29 ± 3	21 ± 5
		liver	88 ± 1	86 ± 1	82 ± 1	70 ± 4	25 ± 3	18 ± 3	12 ± 2
	5-HT	brain	7 ± 2	8 ± 2	10 ± 2	8 ± 3	1 ± 2	1 ± 3	1 ± 2
		liver	17 ± 2	10 ± 3	15 ± 2	7 ± 2	−2 ± 3	0 ± 2	0 ± 3
MD 240928	PEA	brain	74 ± 3	77 ± 1	71 ± 1	61 ± 2	6 ± 3	5 ± 3	0 ± 3
		liver	84 ± 1	82 ± 1	78 ± 1	68 ± 2	9 ± 5	3 ± 4	−3 ± 5
	5-HT	brain	−1 ± 1	0 ± 2	0 ± 2	1 ± 2	−1 ± 2	−3 ± 2	3 ± 2
		liver	7 ± 4	3 ± 3	4 ± 4	11 ± 3	3 ± 3	5 ± 3	2 ± 5
MD 240931	PEA	brain	84 ± 2	87 ± 1	88 ± 1	86 ± 1	56 ± 2	49 ± 2	44 ± 2
		liver	89 ± 1	90 ± 1	89 ± 1	86 ± 1	38 ± 4	25 ± 4	24 ± 3
	5-HT	brain	11 ± 2	14 ± 3	12 ± 2	9 ± 2	0 ± 2	1 ± 1	3 ± 2
		liver	22 ± 5	16 ± 4	24 ± 3	20 ± 3	4 ± 3	0 ± 4	1 ± 4

s.e. = standard error of the percentage of inhibition.
PEA = 12 μM (brain) and 75 μM (liver); 5-HT = 480 μM (brain) and 555 μM (liver).

The % inhibition of MAO from the ex-vivo experiments are presented in Tables 3, 4 and 5. As shown in Table 3, MD 780236 still inhibits MAO-B activity 24 h after administration (31 and 25% inhibition in brain and liver respectively). This residual inhibition decreases slowly in brain and more rapidly in liver, probably due to the faster turnover of the enzyme in this tissue (Felner & Waldmeier 1979; Della Corte & Tipton 1980). Interestingly, the *R*-enantiomer of MD 780236, MD 240928, does not show any residual inhibitory activity 24 h after administration whereas the other, MD 240931, appears to be responsible for the residual inhibitory activity observed with MD 780236. The higher specificity of MD 240928 observed in-vitro in brain tissue is also maintained in these experiments, both in brain and liver tissues. The hydrated aldehyde (MD 240233) behaves in ex-vivo experiments as a short-acting inhibitor with practically no residual inhibitory activity from 24 h on (Table 4). The acid derivative, MD 240098, at the dose administered, is devoid of any inhibitory properties of MAO-B (Table 4). The racemic alcohol, MD 760548, behaves as a short-acting MAO-B inhibitor with no residual inhibitory activity at 24 h and with moderate selectivity towards the B-form of the enzyme (Table 5), confirming the in-vitro results. The greater selectivity but weaker MAO-B inhibitory activity of the *S*-enantiomer (MD 240926) observed in in-vitro conditions is confirmed in the ex-vivo experiments (Table 5).

The racemic amine, MD 780236, has been shown in-vitro in both rat brain and liver to act as an irreversible inhibitor of MAO-B (Strolin Benedetti et al 1982a; Tipton et al 1982c); therefore the two enantiomers might also be thought to be irreversible inhibitors under the same conditions. However, the experiments shown in Table 3 indicate that a considerable proportion of the inhibition found ex-vivo is reversible in nature, and even that one of the enantiomers, MD 240928, gives no residual inhibitory activity at 24 h.

Table 4. Time course of brain MAO-B inhibition ex-vivo after acute administration of MD 240233 and MD 240098 in the rat.

Inhibitor (5 mg kg^{-1} oral)	% Inhibition MAO (PEA) ± s.e. Time after administration (h) 1	2	4	8	24	48
MD 240233	60 ± 3	70 ± 2	63 ± 2	53 ± 3	10 ± 4	5 ± 3
MD 240098	5 ± 3	−2 ± 3	−3 ± 3	−3 ± 2	−3 ± 3	−2 ± 3

s.e. and PEA, see Table 3.

After administration of MD 780236 to the rat both the alcohol and acid derivatives have been found as metabolites (Strolin Benedetti & Dow 1983) indicating that MD 780236 can be deaminated in-vivo. Studies with monoamine oxidase preparations from rat brain and liver (Strolin Benedetti & Dow 1983; Tipton et al 1982c) have, indeed, shown that this compound acts as both a substrate and suicide inhibitor of monoamine oxidase-B, whereas with the A-form of the enzyme its role as a substrate predominates. Competition between the two forms

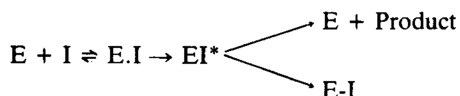
Table 5. Time course of brain MAO inhibition ex-vivo after acute administration of MD 760548 and its enantiomers in the rat.

Inhibitor (5 mg kg^{-1} oral)	Substrate	% Inhibition MAO ± s.e.					
		Time after administration (h)					
		0·5	1	2	4	8	24
MD 760548	PEA	79 ± 1	78 ± 1	79 ± 1	68 ± 1	47 ± 3	1 ± 2
	5-HT	21 ± 3	18 ± 2	20 ± 2	9 ± 1	0 ± 2	2 ± 2
MD 240926	PEA	47 ± 3	47 ± 2	43 ± 2	30 ± 3	5 ± 3	−5 ± 3
	5-HT	3 ± 3	3 ± 2	2 ± 2	3 ± 3	0 ± 2	−2 ± 2
MD 240929	PEA	82 ± 1	82 ± 1	85 ± 1	76 ± 1	58 ± 2	8 ± 2
	5-HT	20 ± 2	19 ± 2	24 ± 1	16 ± 1	6 ± 2	−1 ± 2

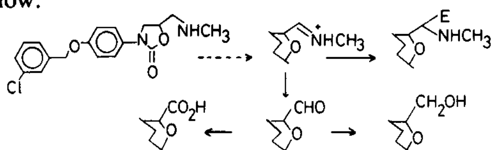
s.e., PEA and 5-HT, see Table 3.

of the enzyme for the inhibitor will clearly determine the extent of irreversible inhibition of the B-form that will be observed in-vivo but the degree of reversible inhibition encountered will be further affected if the inhibitory alcoholic metabolite can accumulate in the vicinity of the enzyme.

The reaction pathway for the reaction of MD 780236 with monoamine oxidase-B that has been proposed (Strolin Benedetti et al 1982b) involves the formation of an intermediate complex (EI*) that can either break down to give products or react with the enzyme to form a stable covalently-bound adduct according to the general scheme:



where I represents MD 780236, and E.I and E-I are the non-covalent and covalently-bound enzyme-inhibitor complexes respectively. Since the oxidation of other primary and secondary amines by monoamine oxidase produces the corresponding aldehydes it is reasonable to assume that the immediate product of the reaction with MD 780236 as a substrate is the corresponding aldehyde which can then be further metabolized by the aldehyde reductases and dehydrogenases (see e.g. Tipton et al 1977) to form the alcohol and acid derivatives. The nature of the intermediate EI* is uncertain, but, by analogy to the oxidation of other substrates it may be an imine, which could then either react with a group on the enzyme surface or be released, perhaps after hydrolysis. A possible scheme for this is shown below:



The failure of the *R*-enantiomer of MD 780236 (MD 240928) to show any significant irreversible inhibition in the ex-vivo experiments could result from its failure to react with monoamine oxidase-B beyond the stage of the initial non-covalent complex, from a slower rate of reaction to form the irreversibly inhibited species, or perhaps from a more rapid rate of destruction catalysed by monoamine oxidase-A. More detailed studies will be required to choose between these alternatives.

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