The enzyme-activated irreversible inhibition of type-B monoamine oxidase by 3-{4-[(3-chlorophenyl)methoxy]phenyl}-5-[(methylamino)methyl]-2-oxazolidinone methanesulphonate (compound MD 780236) and the enzyme-catalysed oxidation of this compound as competing reactions

Keith F. TIPTON,* Christopher J. FOWLER,*†‡ John M. MCCRODDEN* and Margherita STROLIN BENEDETTI†

*Department of Biochemistry, Trinity College, Dublin 2, Ireland, and †Centre de la Recherche Delalande, F-92500 Rueil-Malmaison, France

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3-{4-[(3-Chlorophenyl)methoxy]phenyl}-5-[(methylamino)methyl]-2-oxazolidinone methanesulphonate (compound MD 780236) is a selective inhibitor of the B-form of monoamine oxidase. Inhibition involves an initial non-covalent interaction between enzyme and inhibitor followed by a time-dependent process resulting in irreversible inhibition. The initial, reversible, phase of inhibition was found to be competitive with respect to phenethylamine and 5-hydroxytryptamine, and a comparison of the K_i values indicated the affinity of the inhibitor for the B-form of the enzyme to be some 7-fold greater than its affinity for the A-form. This selectivity was considerably enhanced by preincubation of the enzyme and inhibitor. Time courses showed that complete inhibition was not achieved under conditions where the inhibitor concentration was over 100-fold greater than that of the enzyme. Assay of the activity of monoamine oxidase by determining the release of hydrogen peroxide fluorometrically showed compound MD 780236 to be a substrate for, as well as an inhibitor of, monoamine oxidase, and kinetic analysis revealed that the rate of product formation was some 530-fold greater than that of the process leading to irreversible inhibition of the B-form of the enzyme.

The activity of the mitochondrial enzyme monoamine oxidase [monoamine: oxygen oxidoreductase (deaminating) (flavin-containing), EC 1.4.3.4] exists in two forms, one of which (the A-form) is sensitive to inhibition by low concentrations of the acetylenic inhibitor clorgyline whereas the other (the B-form) is sensitive to inhibition by deprenyl (Johnston, 1968; Knoll & Magyar, 1972). These two forms also differ in their substrate specificities. Although such selectivity is not absolute (for discussion see Tipton *et al.*, 1982), at relatively low concentrations 5-hydroxytryptamine is a substrate essentially for the A-form alone whereas only the B-form has significant activity towards phenethylamine.

The compound 3-{4-[(3-chlorophenyl)methoxy]phenyl}-5-[(methylamino)methyl]-2-oxazolidinone methanesulphonate (compound MD 780236) shown in Fig. 1 has been found to be a monoamine oxidase inhibitor with selectivity towards the B-form of the enzyme, but its behaviour *in vivo* has been found to differ from that *in vitro* (Strolin Benedetti *et al.*,

1982). Inhibition by this compound *in vitro* was found to be irreversible, whereas the inhibition that resulted from administration of the compound *in vivo* was found mainly to be reversible. The present paper reports the results of studies designed to assess the mechanism of the inhibitory process *in vitro* with the aim of understanding the factors that might account for this behaviour.

Materials

The radioactive substrates 5-hydroxy[*side-chain*-2- 14 C]tryptamine creatinine sulphate and [*Et*-1- 14 C]-phenethylamine hydrochloride were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Compound MD 780236 and its acid (compound MD 240098) and alcohol (compound MD 760548) derivatives (see Fig. 1) were synthesized at the Department of Chemistry, Centre de Recherche Delalande. Stock solutions of compound MD 780236 were dissolved in water and stored frozen until use. Stock solutions of compounds MD 780548 and MD 240098 were made up in 50% dimethyl sulphoxide and then diluted with the appropriate

‡ Present address: Astra Läkemedel AB, S-15185 Södertälje, Sweden.

Fig. 1. Structures of 3-{4-[(3-chlorophenyl)methoxy]-phenyl}-5-[(methylamino)methyl]-2-oxazolidinone methanesulphonate (compound MD 780236) and its acid (compound MD 240098) and alcohol (compound MD 760548) derivatives

buffer to give the desired concentration. At the concentration used in the experiments reported below dimethyl sulphoxide did not affect the activity of monoamine oxidase.

Clorgyline hydrochloride was a gift from May and Baker, Dagenham, Essex, U.K. L-Deprenyl hydrochloride and compound J-508 [*N*-methyl-*N*-propargyl-L-aminointhane hydrochloride (compound AGN 1133; compound Su-11739)] were gifts from Professor J. Knoll, Department of Pharmacology, Semmelweis University of Medicine, Budapest, Hungary. Peroxidase was obtained from Boehringer Corp., Lewes, E. Sussex, U.K., and homovanillic acid from Calbiochem-Behring Corp., Bishop's Stortford, Herts., U.K.

Methods

Male Wistar rats (body weight 150–300 g) were killed by a blow to the head, and the livers were rapidly removed, blotted on filter paper and homogenized in 5 vol. (v/w) of ice-cold sucrose buffer (0.25 M-sucrose in 10 mM-potassium phosphate buffer, pH 7.2) in a Dounce homogenizer. Homogenates were combined so that each preparation was derived from two, or more, livers. The homogenates were centrifuged at 600 *g* for 10 min at 4°C to remove nuclei and cell debris, and the supernatant was centrifuged at 15000 *g* for 10 min to sediment the mitochondrial fraction. The pellet was washed by resuspension in sucrose buffer followed by centrifugation at 15000 *g* for 10 min. The pellet thus obtained was resuspended in sucrose buffer to give a protein concentration of about 5 mg/ml, and was stored frozen until used for assay.

Monoamine oxidase activity was determined radiochemically by the method of Otsuka &

Kobayashi (1964) as modified by Fowler & Tipton (1981), with 5-hydroxytryptamine and phenethylamine as substrates for monoamine oxidase A-form and B-form respectively. The results were corrected for the efficiencies of extraction of the deaminated metabolites into the organic layer so that activities could be expressed as nmol/min per mg of protein (Fowler & Orelund, 1980). Activities were also determined fluorimetrically by following the production of H_2O_2 by coupling it to peroxidase-catalysed oxidation of homovanillic acid (Tipton, 1969), a Perkin-Elmer MPF 44B fluorimeter connected to a Rikadenki chart recorder being used. All assays were performed at 37°C in 100 mM-potassium phosphate buffer, pH 7.2, and under free equilibrium with air. Protein, concentration was determined by the method of Markwell *et al.* (1978), with bovine serum albumin as the protein standard.

Results and discussion

Initial experiments showed that preincubation of monoamine oxidase and compound MD 780236 was necessary in order to achieve full inhibition. In the absence of such preincubation the concentration of inhibitor necessary to give 50% inhibition (IC_{50}) of the oxidation of phenethylamine was 1.9 μM , but this value fell to 0.05 μM (Table 1) after the enzyme and inhibitor had been preincubated for 90 min at 37°C. The time-dependence of the inhibition of the activity towards 5-hydroxytryptamine was less marked, with IC_{50} values of 6.9 μM and 4.4 μM being obtained before and after 90 min preincubation respectively. Values similar to those obtained after preincubation have been reported for the inhibition of rat brain monoamine oxidase activities after 60 min preincubation (Strolin Benedetti *et al.*, 1982).

Table 1. *Characteristics of the inhibition of monoamine oxidase A-form and B-form by compound MD 780236*
 K_m and K_i values were calculated from the data drawn in Fig. 1. IC_{50} values were calculated from plots of percentage inhibition of monoamine oxidase activity against concentration of compound MD 780236 (four to six inhibitor concentrations used), with 20 μM -phenethylamine and 200 μM -5-hydroxytryptamine as substrates for B-form and A-form enzyme respectively. K_i values were also determined from the IC_{50} and K_m values by the method of Cheng & Prusoff (1973). Values are given as means $\pm$ S.E.M. for determinations in three mitochondrial preparations of rat liver.

Substrate	...Phenethylamine	5-Hydroxytryptamine
K_m of monoamine oxidase towards substrate (μM)	16 $\pm$ 1.4	150 $\pm$ 40
IC_{50} values (μM)		
No preincubation	1.9 $\pm$ 0.3	6.9 $\pm$ 0.3
90 min preincubation	0.055 $\pm$ 0.005	4.4 $\pm$ 0.6
K_i values (μM) for initial inhibition		
From slope replots	0.54 $\pm$ 0.10	3.7 $\pm$ 0.2
From IC_{50} and K_m	0.85 $\pm$ 0.17	2.8 $\pm$ 0.3

The initial phase of inhibition of each of the two forms of monoamine oxidase was investigated by starting the reaction by the addition of enzyme to the substrate/inhibitor mixture. Short incubation times (2 min for phenethylamine and 5 min for 5-hydroxytryptamine) were used to ensure that no significant irreversible inhibition occurred during the assay period. Inhibition was found to be competitive with respect to both substrates (Fig. 2), and K_i values of 0.54 μM and 3.7 μM towards phenethylamine and 5-hydroxytryptamine respectively were determined. As shown in Table 1, similar K_i values were calculated from the IC_{50} values, determined in the absence of preincubation, by the method of Cheng & Prusoff (1973).

Although the inhibition of the activity towards 5-hydroxytryptamine appeared to be strictly competitive at all inhibitor concentrations used, Fig. 2(b) shows that the line of the double-reciprocal plot obtained in the absence of inhibitor intersected the reciprocal velocity axis below the point of intersection of the other lines. Similar results have been reported for the reversible phase of inhibition of the activity of monoamine oxidase A-form by (–)-deprenyl and pargyline, and interpreted as being due

Fig. 2. *Double-reciprocal plots of the inhibition by compound MD 780236 of (a) phenethylamine oxidation and (b) 5-hydroxytryptamine oxidation by mitochondrial monoamine oxidase activity*

In (a) the samples were assayed without prior preincubation in the absence (●) and in the presence of 0.02 μM - (▲), 0.5 μM - (△), 1.0 μM - (▼) and 2 μM - (○) compound MD 780236. In (b) the samples were assayed without prior preincubation in the absence (●) and in the presence of 5 μM - (△), 10 μM - (▼) and 15 μM - (○) compound MD 780236. All values are means for determinations in three mitochondrial fractions, set to an assay protein concentration of 0.63 mg/ml. Inserts: secondary plots of the slopes (calculated by linear-regression analysis) against inhibitor concentration.

Fig. 3. Time courses of the inhibition by compound MD 780236 of monoamine oxidase B-form (a) and A-form (b)

Substrates and inhibitor concentrations used were: (a) phenethylamine (20 μM) as substrate, preincubated with 20 nM- (●), 50 nM- (▽), 150 nM- (▲) and 200 nM- (○) compound MD 780236; (b) 5-hydroxytryptamine (200 μM) as substrate, preincubated with 2 μM- (●), 5 μM- (▽), 20 μM- (▲) and 50 μM- (○) compound MD 780236. Percentage inhibition of activity is expressed with respect to samples preincubated for the same length of time with distilled water. Points are means $\pm$ S.E.R. for determinations in three or more mitochondrial preparations. Preincubation protein concentrations were set to 0.63 mg/ml.

to the B-form of the enzyme having significant activity towards high concentrations of 5-hydroxytryptamine (Tipton & Mantle, 1981; Fowler *et al.*, 1982). From this intercept effect the maximum

velocity of monoamine oxidase B-form towards 5-hydroxytryptamine can be calculated to be about 18% of the total, in reasonable agreement with the value of 11% determined by a more direct approach (Tipton *et al.*, 1982).

It can be seen from the values in Table 1 that in the reversible phase of the inhibition compound MD 780236 exhibits a relatively small degree of selectivity towards the B-form of the enzyme (K_i for B-form/ K_i for A-form ratio 1:6.9), whereas a much greater selectivity is evident after incubation of the enzyme and inhibitor for 90 min (IC_{50} for B-form/ IC_{50} for A-form ratio 1:80). These results suggest that, as has been observed for the inhibition of monoamine oxidase by (–)-deprenyl (Fowler *et al.*, 1982), the difference in affinities of the two forms for reversible interaction with the inhibitor must be further enhanced by large differences in the rates of reaction within the non-covalent complex to produce to irreversibly inhibited species. This suggests that compound MD 780236 acts as an enzyme-activated ('suicide' or k_{cat}) irreversible inhibitor of monoamine oxidase, as has been shown to be the case for the propargylamine derivatives (see, e.g., Abeles & Maycock, 1976; Fowler *et al.*, 1982).

The time-dependent inhibition of the monoamine oxidase activities by compound MD 780236 was studied by preincubating the enzyme for various times before assaying the activity towards 5-hydroxytryptamine or phenethylamine. As shown in Fig. 3(a) the inhibition of monoamine oxidase B-form activity showed a marked time-dependence, with inhibition reaching completion in less than 20 min. After this time, dilution experiments showed the inhibition to be essentially irreversible (Table 2). The time-dependence of the inhibition of the A-form of the enzyme was much less marked (Fig. 3b), and, as shown in Table 2, the irreversible component of the inhibition after 30 min preincubation was considerably smaller.

Although inhibition of the B-form of the enzyme appeared to have reached completion in about 30 min, this did not result in full inhibition of enzyme activity at concentrations of compound MD 780236 as high as 200 nM. Such behaviour might be expected if the inhibitor concentration was less than that of the enzyme; however, in the present case this cannot be the explanation, since the concentration of monoamine oxidase B-form in the rat liver mitochondrial preparations used in this work was found to be about 20.5 pmol/mg of protein (see Table 4), which would correspond to a final molarity of 13 nM under the conditions used here. In addition, if the enzyme concentration were greater than that of the inhibitor, a graph of the percentage inhibition obtained after long incubation times against the inhibitor concentration should approximate to a straight line, with the concentration of inhibitor

Table 2. *Reversibility of inhibition of monoamine oxidase A-form and B-form by compound MD 780236*

Samples were preincubated with inhibitor for 30 min at 37°C and then either assayed directly for monoamine oxidase activity ('Before washing') or washed (by centrifugation of 1 ml of the mixture to sediment the mitochondria followed by resuspension in 1 ml of 100mM-potassium phosphate buffer, pH 7.2) three times before assay for enzyme activity ('After three washings'). Such a procedure gives an effective dilution of the inhibitor of more than 800 000 : 1. The preincubation protein concentration was 0.31 mg/ml. Data represent means ($\pm$ S.E.R. where appropriate) of determinations in different mitochondrial preparations of the percentage inhibition of B-form enzyme (towards 20 μ M-phenethylamine) and A-form enzyme (towards 100 μ M-5-hydroxytryptamine) with respect to samples preincubated for the same time (and then treated in the same way) with distilled water.

Concn. of compound MD 780236	No. of samples	Inhibition of monoamine oxidase activity (%)	
		Before washing	After three washings
B-form enzyme			
30nM	3	26 ± 3	17 ± 9
60nM	3	47 ± 3	35 ± 9
100nM	1	56	33
A-form enzyme			
5 μM	4	44 ± 3	14 ± 7
10 μM	1	61	24
20 μM	4	82 ± 3	37 ± 12

giving 50% inhibition corresponding to one-half of the enzyme concentration (see, e.g., Fowler & Callingham, 1979. Fowler *et al.*, 1980). Such a graph for the inhibition of monoamine oxidase B-form by compound MD 780236, shown in Fig. 4, is clearly non-linear.

The possibility that the failure of excess inhibitor to cause complete inhibition of the B-form of the enzyme could be due to non-specific binding removing substantial proportions from the system was ruled out by the results of the experiments shown in Table 3, where the inhibitor was preincubated with a sample of mitochondria that had been pretreated with a mixture of clorgyline plus deprenyl (0.3 μ M of each) to inhibit all the monoamine oxidase activity (see Tipton *et al.*, 1982) before the ability of this mixture to inhibit the activity of an equal amount of untreated mitochondria was determined. The results clearly show that there was no significant decrease in the inhibitory potency due to this preincubation, indicating that a non-specific binding of the inhibitor does not occur to any significant extent. The results

Fig. 4. *Inhibition by compound MD 780236 of monoamine oxidase B-form activity (towards 20 μ M-phenethylamine) after 90 min preincubation*

Percentage inhibition of activity is expressed with respect to samples preincubated for the same length of time with distilled water. Each point represents the mean $\pm$ S.E.R. for determinations in three mitochondrial preparations. Preincubation protein concentrations were set to 0.63 mg/ml.

shown in Table 3 also indicate that destruction of the inhibitor by an enzyme other than monoamine oxidase does not occur to any significant extent under the conditions used here.

The failure of the inhibitor to cause complete inhibition is thus likely to be due to substantial metabolism of this compound by the action of monoamine oxidase itself. Two possible mechanisms whereby such degradation could occur have been considered in connection with the inhibition of other enzymes. A model in which the covalently bound enzyme-inhibitor complex is unstable and slowly decomposes to regenerate the free enzyme plus a modified form of the inhibitor according to the mechanism:

where $E \cdot I$ and $E-I$ represent the non-covalent and covalently bound enzyme-inhibitor complexes respectively, and P represents the modified form of the inhibitor, has, for example, been shown to describe the inhibition, of acetylcholinesterase by methylcarbamates (see, e.g., O'Brien, 1968). In the present case, however, such a mechanism cannot account for the inhibition kinetics observed, since no significant recovery of the activity of the irreversibly inhibited B-form of the enzyme occurred during prolonged dialysis (Strolin Benedetti *et al.*, 1982) or after repeated centrifugation and washing (Table 2).

Table 3. Effects of preincubation with clorgyline-plus-(−)-deprenyl-treated mitochondria on the inhibitory potency of compound MD 780236 towards monoamine oxidase B-form

Samples of compound MD 780236 at the indicated concentrations were incubated in 100 mM-phosphate buffer, pH 7.2, with either mitochondria (0.018 mg of protein) that had been treated with 0.3 μM-clorgyline plus 0.3 μM-(−)-deprenyl to inhibit the monoamine oxidase activity (see Tipton *et al.*, 1982), or with an equal volume of the buffer, for 30 min at 37°C. Fresh untreated mitochondria were then added, and incubation was continued for another 30 min before the activity was determined with phenethylamine as the substrate. Values are the means (± S.E.R.) of four separate determinations.

Concn. of inhibitor (nM)	Preincubation medium	Inhibition (%)
50	Monoamine oxidase-inhibited mitochondria	28 ± 7
50	Buffer	34 ± 4
150	Monoamine oxidase-inhibited mitochondria	68 ± 3
150	Buffer	71 ± 5

An alternative model that would account for the observed time courses is one in which inhibitor breakdown and irreversible inhibition represent alternative pathways. The kinetic behaviour of such a mechanism, which may be represented by the scheme:

has been considered in detail by Waley (1980), who showed that the rate of inhibition will be given by the equation:

$$\frac{d(\ln[E-I])}{dt} = \frac{k' \cdot i}{K' + i} \quad (3)$$

where i represents the inhibitor concentration, $k' = (k_{+2}k_{+4})/(k_{+2} + k_{+3} + k_{+4})$ and represents the catalytic constant for the overall flux, and K' , which corresponds to the Michaelis constant of the system, is given by the relationship:

$$K' = \left(\frac{k_{-1} + k_{+2}}{k_{+1}} \right) \left(\frac{k_{+3} + k_{+4}}{k_{+2} + k_{+3} + k_{+4}} \right)$$

Such a mechanism predicts that compound MD 780236 should be a substrate for monoamine oxidase, and this was studied by determining the formation of H_2O_2 , which is produced stoichiometrically with amine oxidation (see, e.g., Tipton, 1969), during reaction between the enzyme and

Fig. 5. Time course of the liberation of H_2O_2 by the interaction of monoamine oxidase B-form with compound MD 780236

H_2O_2 formation was followed at 37°C and at pH 7.2 as described in the text. Clorgyline-treated mitochondria were used as the enzyme source (1.45 pmol of monoamine oxidase B-form and the initial inhibitor concentration was 500 nM. At the point indicated by A, a further sample of inhibitor was added to increase the concentration by a further 500 nM, at point B a portion of enzyme equivalent to that originally present was added, and at point C benzylamine was added to give a concentration of 500 μM. The activity of the enzyme before incubation with compound MD 780236 corresponded to 7.7 nmol of H_2O_2 produced/min per mg of protein with benzylamine as substrate.

inhibitor. Fig. 5 shows the time courses of the reaction when monoamine oxidase B-form was incubated with 500 nM-compound MD 780236. The observation that product formation ceases rather than tends to a constant rate, long before the substrate has all been exhausted, is consistent with the mechanism represented by eqn. (2), rather than that of eqn. (1), being followed. Under conditions where the inhibitor is present in amounts that result in complete inhibition, the ratio of the amount of product formed to the enzyme concentration should be constant and correspond to the ratio k_{+3}/k_{+4} (the partition ratio) (see Waley, 1980). The results obtained at a number of different, saturating, inhibitor concentrations are shown in Table 4, and correspond to a partition ratio of 530 ± 16 (mean ± S.E.M.). From the half-time of the inhibitory process determined at a series of different initial inhibitor concentrations values of k' and K' of $0.3 \pm 0.01 \text{ min}^{-1}$ and $0.36 \pm 0.03 \text{ μM}$ could be calculated according to the equation:

$$\text{Half-time} = \frac{1}{k'} \left[\frac{\left(\frac{K'}{i} \right) \cdot \ln \left(2 - \frac{r \cdot e}{i} \right)}{1 - \frac{r \cdot e}{i}} + \ln 2 \right]$$

Table 4. Product formed at complete inhibition of monoamine oxidase B-form by compound MD 780236

The amount of H_2O_2 formed after complete inhibition were determined with three different mitochondrial preparations that had been pretreated for 1 h at 37°C with $0.3\ \mu\text{M}$ -clorgyline to inhibit the activity due to monoamine oxidase A-form (see Tipton *et al.*, 1982). The concentration of monoamine oxidase B-form in the preparations were determined by titrating the activity against the inhibitor compound J-508 as described by Fowler *et al.* (1980). By this technique a mean concentration of $20.5\ \text{pmol/mg}$ of protein was found. The partition ratios were then calculated as (product formed)/(enzyme concentration) in the incubation medium (see Waley, 1980).

Concn. of inhibitor (μM)	Product formed (nmol)	Partition ratio (k_{+3}/k_{+4})
0.5	0.79, 0.68	545, 471
50	0.79	545
100	0.85	586
250	0.68	471
500	0.74, 0.75, 0.86	510, 517, 593

where e and i are the initial enzyme and inhibitor concentrations respectively and r is the partition ratio k_{+3}/k_{+4} (see Waley, 1980).

By using a mitochondrial preparation that had been incubated for 1 h at 37°C with $0.3\ \mu\text{M}$ -(-)-deprenyl to inhibit the B-form of the enzyme (see Tipton *et al.*, 1982), compound MD 780236 was found also to be a substrate for the A-form of monoamine oxidase. The progress curves of the reaction showed relatively little curvature, in agreement with the low efficiency of this compound as a time-dependent irreversible inhibitor of that form. The initial rate of the reaction was shown to be proportional to enzyme concentration, and K_m and V_{max} values of about $1.1\ \mu\text{M}$ and $4\ \text{nmol/min per mg}$ of protein were calculated from half-reciprocal (Hanes) plots of the dependence of rates on substrate concentration (Fig. 6). This K_m value is quite similar to the K_i value of $3.7\ \mu\text{M}$ determined for compound MD 780236 as a reversible inhibitor of 5-hydroxytryptamine oxidation.

In systems containing only the B-form of monoamine oxidase, the extent of irreversible inhibition obtained will be governed by its concentration and the ratio of the rate constants for breakdown and irreversible inhibition (the partition ratio, k_{+3}/k_{+4}). The behaviour of systems containing both forms of the enzyme will be further complicated by the activity of the A-form towards this compound.

The nature of the product formed by the action of monoamine oxidase is not revealed by the present studies. However, both the acidic and alcoholic metabolites of compound MD 780236 have been

Fig. 6. Half-reciprocal plot for the oxidation of compound MD 780236 by monoamine oxidase A-form. Activity was determined by monitoring the production of H_2O_2 fluorimetrically at 37°C with the use of mitochondrial preparations that had been treated with $0.3\ \mu\text{M}$ -(-)-deprenyl to inhibit the activity of the B-form enzyme. The assay protein concentration was $0.06\ \text{mg/ml}$. Each point represents the mean of two separate determinations with different mitochondrial preparations.

shown to be formed in rat after the administration of the inhibitor *in vivo* (Strolin Benedetti & Dow, 1982). Thus it is probable that degradation of compound MD 780236 involves a similar process to that involved in the oxidation of other primary and secondary amines, with the formation of the corresponding aldehyde, which can then be either further oxidized by aldehyde dehydrogenase to give the acid or reduced by an aldehyde reductase to give the alcohol. The acid derivative (compound MD 240098) was found to be a poor inhibitor of monoamine oxidase B-form activity (assayed with $20\ \mu\text{M}$ -phenethylamine): after 60 min preincubation of enzyme and $1\ \mu\text{M}$ inhibitor at 37°C there was no significant inhibition, and increasing the inhibitor concentration to $3\ \mu\text{M}$ resulted in $17 \pm 2\%$ inhibition (mean $\pm$ S.E.R.). The alcohol derivative (compound MD 760548) was a considerably more potent inhibitor, giving $31 \pm 2\%$ inhibition at a concentration of $30\ \text{nM}$ and $90 \pm 4\%$ at $300\ \text{nM}$ under the same conditions. Thus under conditions where metabolism of compound MD 780236 occurs any accumulation of the alcoholic metabolite will itself result in some inhibition of the enzyme.

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