J. Pharm. Pharmacol. 1995, 47: 324–328
Received May 5, 1994
Accepted December 7, 1994

Irreversible Inhibition of Rat Liver Mitochondrial MAO A and MAO B by Enantiomers of Deprenyl and α-Methylpargyline

J. B. ROBINSON, R. BOCCHINFUSO AND AMIRA KHALIL

Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada

Abstract

Using rat liver mitochondrial monoamine oxidase (MAO) A and MAO B, the possible influence of stereochemical factors upon the irreversible inhibition by propargylamine derivatives has been studied using the enantiomers of deprenyl and of α-methylpargyline.

Whether studying the inhibition of MAO A or MAO B, little difference was found among enantiomeric pairs in the first-order rate constant (k_2) for formation of the enzyme inhibitor adduct. Similarly, and with the exception of (*S*)-D-(+)-deprenyl ($k_2 = 0$ or an extremely low value at MAO A), the computed value of k_2 for the individual enantiomers showed little variation between MAO A and MAO B.

These results suggest that inhibitor selectivity towards a particular form of the enzyme is determined predominantly at the competitive phase of the inhibition.

The identification and differentiation of the different forms of the enzyme monoamine oxidase (MAO) (monoamine: O_2 oxido-reductase (deaminating) (flavin-containing) (E.C. 1.4.3.4.)) within a tissue or crude enzyme preparation depends upon the use of selective substrates and inhibitors. Thus, MAO A-catalysed oxidative deamination of 5-hydroxytryptamine (5-HT) (MAO A-selective substrate) is inhibited by clorgyline (approximately 10^{-8} M) (Johnston 1968) and MAO B-catalysed oxidative deamination of β-phenylethylamine or benzylamine (MAO B-selective substrates) is inhibited by (*R*)-L-(−)-deprenyl (approximately 10^{-8} M) (Yang & Neff 1973).

Both clorgyline and (−)-deprenyl are derivatives of *N*-methylpropargylamine and, although highly selective towards different forms of MAO, show, from a chemical standpoint, an identical mechanism of inhibition, i.e. formation of a covalent bond at N5 of the reduced FAD moiety which is itself covalently bound to the protein (Maycock et al 1976; Salach et al 1979; Yu 1981; Bach et al 1988). Such inhibitors are regarded as suicide inhibitors and the inhibition mechanism can be represented in the reaction below, in which the initially formed reversible enzyme-inhibitor complex (E.I) reacts further to form the irreversible covalent enzyme-inhibitor adduct (E-I):

$$[E] + [I] \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [E.I] \xrightarrow{k_2} [E\text{-}I]$$

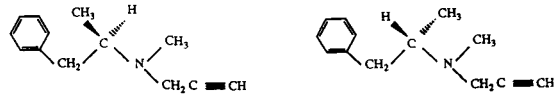
where $K_i = k_{-1}/k_1$.

Based on such a model, selectivity towards a particular form of the enzyme may derive from differences in the initial affinity of the inhibitor towards the different forms as expressed by the competitive K_i value of the inhibitor, differences in the rate of formation of the irreversible enzyme-inhibitor adduct E-I as expressed by k_2 or a combination of the two effects (Tipton & Mantle 1981). In this context, the selectivity of pargyline (*N*-methyl-*N*-propargylbenzylamine) towards MAO B resides in the greater affinity of the inhibitor towards MAO B ($K_{i\,MAOB} = 0{\cdot}5\,\mu M$; $K_{i\,MAOA} = 13\,\mu M$) rather than in the subsequent rate of formation of the enzyme-inhibitor adduct ($k_2 = 0{\cdot}20\,min^{-1}$ at 30°C at both MAO A and MAO B sites). Among the more selective inhibitors, e.g. clorgyline and (−)-deprenyl, it appears that both differences in affinity (K_i) and differences in the rate of irreversible inactivation (k_2) are contributing factors in the selectivity towards a particular form of the enzyme (Tipton & Mantle 1981; Fowler et al 1982).

Such differences in K_i and k_2 reflect structural differences in both the inhibitor and the respective active sites of MAO A and MAO B. However, with certain inhibitors there exists the further possibility of stereochemical differences influencing the affinity and inactivation rates of such compounds, and contributing towards the stereoselectivity of an inhibitor towards a particular form of the enzyme. In this context, the enantiomers of deprenyl (*N*-methyl-*N*-propargyl-1-phenyl-2-aminopropane) (I) and α-methylpargyline (*N*-methyl-*N*-propargyl-1-phenylethylamine) (II) show stereochemical differences in their affinities towards MAO A and MAO B (Robinson 1985a, b). Differences have also been demonstrated in both the affinity and inactivation rate of enantiomeric and diastereomeric allenes when reacting with purified MAO B (Smith et al 1988).

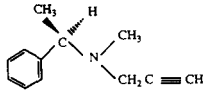
The present work reports the use of enantiomers of deprenyl (Ia and Ib) and α-methylpargyline (IIa and IIb) (Scheme 1) as probes of the influence of stereochemistry upon the irreversible phase of the inactivation of MAO A and MAO B.

Correspondence: J. B. Robinson, Faculty of Pharmacy, University of Toronto, 19 Russell Street, Toronto, Ontario, M5S 1A1, Canada.

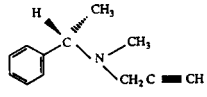


Ia
L-(*R*)-(-)-Deprenyl

Ib
D-(*S*)-(+)-Deprenyl



IIa
(*R*)-(+)-α-Methylpargyline



IIb
(*S*)-(-)-α-Methylpargyline

SCHEME 1.

Materials and Methods

Livers of male Sprague-Dawley rats were used as the enzyme source and preparations of MAO A and MAO B were prepared by incubation of the crude mitochondrial enzyme preparation with (−)-deprenyl and clorgyline, respectively (Fowler & Tipton 1981; Ali & Robinson 1991). The washed suspension of each form of the enzyme was stored at −20°C until required.

To solutions of the inhibitor prepared in phosphate buffer (10 mM, pH 7·4) (2 mL) was added enzyme preparation (500 μL) and the mixture was incubated at 35°C. Aliquots (50 μL) were removed at fixed time intervals up to 30 min (up to 15 min in the case of the more concentrated solutions) and immediately added to ^{14}C-labelled MAO B substrate solution (350 μL) (phenylethylamine, approximately 20 μM in phosphate buffer, 10 mM, pH 7·4), and incubated at 35°C for 3 min. The enzyme-catalysed reaction was stopped by addition of 2 M HCl (200 μL) and the metabolites were extracted and counted as previously reported (Robinson 1985a).

Similar studies employing the MAO A enzyme preparation used inhibitor solution (1 mL) and enzyme preparation (1 mL) and the residual enzyme activity was determined using ^{14}C-labelled 5-HT as substrate (350 μL; 100 μM).

All determinations were performed in duplicate and at least two independent experiments were performed, each employing 4–5 different concentrations of inhibitor.

Kinetic data were interpreted using the method developed by Kitz & Wilson (1962) (see also Tipton 1989). The apparent first-order rate constant for loss of enzymatic activity (k_{obs}) was obtained by linear regression of a plot of log (% residual activity) against incubation time (gradient = $-k_{obs}/2{\cdot}303$) and linear regression of a plot of $1/k_{obs}$ against 1/[I] yielded K_i/k_2 (gradient). A typical set of experimental data is presented in Fig. 1 and the computed results for the enantiomers studied are presented in Table 1.

Results and Discussion

The inhibition of MAO by derivatives of *N*-methylpropargylamine (suicide inhibitors) is characterized by a competitive inhibitory phase preceding a time-dependent irreversible inhibition. The analysis originally developed by Kitz & Wilson (1962) (for discussion see Tipton (1989)) is commonly employed for such systems. Thus, provided formation of the catalytically inactive covalent adduct [E-I] is slow relative to the breakdown of the enzyme inhibitor complex [E.I] ($k_{-1} \gg k_2$) and that [I] > [E] then a quasi-equilibrium will exist and the rate of formation of the irreversible adduct can be expressed as:

$$\frac{d[E-I]}{dt} = k_2[E.I] = k_2\frac{(E_t - [E\text{-}I])}{1+\frac{K_i}{[I]}} \qquad (1)$$

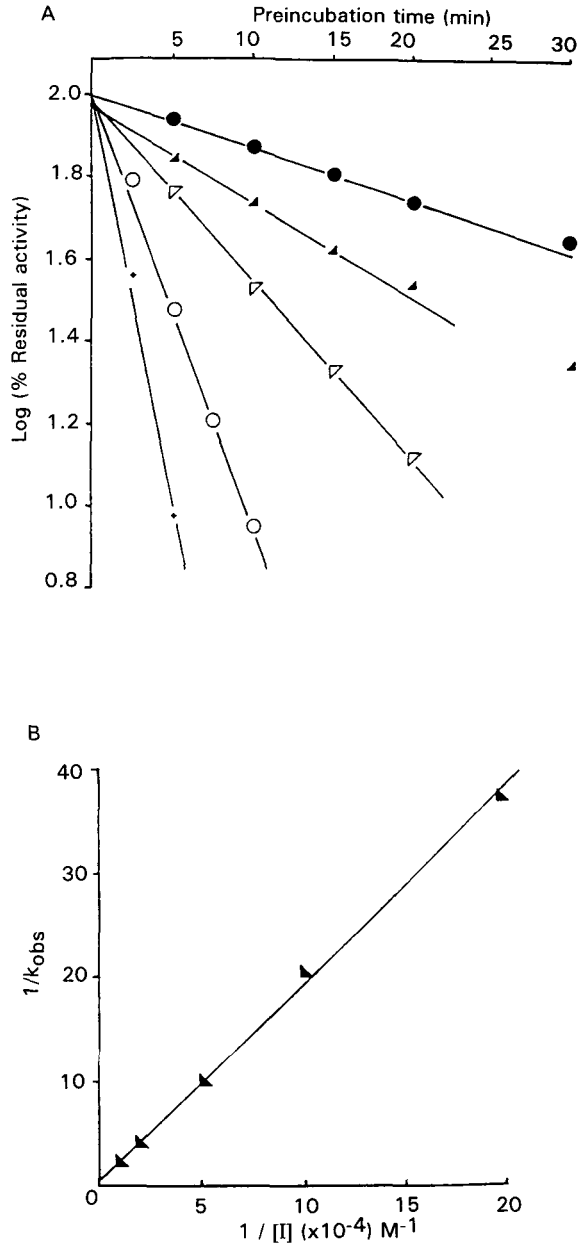


FIG. 1. Time-dependent inhibition of MAO A by (*S*)-(−)-α-methylpargyline (typical data set from one experiment). A. Plot of log (% residual activity) against incubation time. All points are the mean of duplicate determinations. Concentration of (*S*)-(−)-methylpargyline: 5·06 μM (●); 10·12 μM (▲); 20·24 μM (△); 50·6 μM (○); 101·2 μM (+). B. Plot of $1/k_{obs}$ (from panel A) against 1/[I]. The line is that of best fit by linear regression.

Table 1. Kinetic constants for the time-dependent inhibition of MAO A and MAO B by the enantiomers of deprenyl and α-methylpargyline.

Inhibitor	MAO A			MAO B		
	K_m (5-HT) = 42·8 (± 7·8) μM			K_m (phenylethylamine = 3·54 (± 0·33) μM		
	K_i/k_2 (×10⁶)	K_i (μM)*	k_2 (min⁻¹)	K_i/k_2 (×10⁶)	K_i (μM)*	k_2 (min⁻¹)
(*R*)-L-(−)-Deprenyl	58·7 ± 2 (n = 14)	76 ± 2·9	1·295 ± 0·07	0·38 ± 0·02 (n = 10)	0·325 ± 0·02	0·86 ± 0·06
(*S*)-*D*-(+)-Deprenyl	—	14 ± 0·4	—	10·7 ± 1·1 (n = 8)	8·1 ± 0·034	0·76 ± 0·08
(*R*)-(+)-α-Methylpargyline	11·6 ± 0·27 (n = 9)	35·1 ± 1	3·025 ± 0·12	0·131 ± 0·02 (n = 10)	0·193 ± 0·02	1·47 ± 0·26
(*S*)-(−)-α-Methylpargyline	342 ± 36 (n = 9)	385 ± 5·8	1·125 ± 0·12	76·8 ± 2·4 (n = 10)	104 ± 5·8	1·35 ± 0·09

n = number of data points (concentrations) employed in the correlation. *Data from Robinson (1985a,b).

where E_t = total enzyme concentration. Integration of equation 1 leads to:

$$\ln\frac{(E_t - [E\text{-}I])}{E_t} = k_{obs} \cdot t \qquad (2)$$

where $k_{obs} = k_2/(1 + K_i/[I])$.

Thus, a plot of log residual enzymatic activity against preincubation time, t, is linear with gradient $-k_{obs}/2{\cdot}303$, and the values of k_2 and K_i can normally be obtained from a double reciprocal plot of $1/k_{obs}$ against $1/[I]$.

$$\frac{1}{k_{obs}} = \frac{K_i}{k_2[I]} + \frac{1}{k_2} \qquad (3)$$

In measuring the residual enzymatic activity, it is necessary to both halt the formation of the covalent enzyme inhibitor adduct (E-I) and to reverse any E.I complex present. This is accomplished by dilution of an aliquot of the enzyme/inhibitor incubation mixture with a relatively high concentration of substrate and allowing the enzyme-catalysed reaction to proceed for only a short time. Since relatively high concentrations of substrate (phenylethylamine or 5-HT) were employed, any possible lack of substrate specificity towards a particular form of the enzyme under such conditions was prevented in the present work by the use of mitochondrial enzyme preparations in which the undesirable form of the enzyme had been previously inhibited by the use of clorgyline (MAO B preparation) or L-deprenyl (MAO A preparation).

Within the present work, only (*S*)-D-(+)-deprenyl when reacting with MAO A failed to demonstrate an irreversible component in its inhibition. All other compounds when reacting with either MAO A or MAO B produced a first-order loss of enzymatic activity with respect to time (Fig. 1A). However, while plots of k_{obs} vs [I] would normally be expected to be hyperbolic ($k_{obs} = k_2/(1 + K_i/[I])$) such was only clearly seen in the plots generated from the inhibition of MAO-A with (*R*)-L-(−)-deprenyl. It should be noted however, that, because of the fast rates of irreversible inhibition encountered in this study, few of the compounds were studied at concentrations exceeding their competitive K_i value.

While the double reciprocal plots ($1/k_{obs}$ vs $1/[I]$) should lead theoretically to values of both k_2 and K_i calculated from the values of the intercept and gradient, respectively, only the gradient (K_i/k_2) was reasonably reproducible, reflecting again the relatively high affinity of many of the compounds coupled with the high values of k_2. The first-order rate constant for the irreversible phase of the inhibition was, therefore, more conveniently calculated from the gradient of the double reciprocal plots and employing the competitive K_i values previously obtained from studies in which the conditions had been controlled to eliminate the irreversible inhibition (Robinson 1985a,b).

Previous studies, employing L-(−)-deprenyl and clorgyline (Tipton & Mantle 1981; Fowler et al 1982) have reported a dependence of k_2 upon the inhibitor concentration, a phenomenon ascribed mainly to nonspecific binding of the inhibitor leading to a significant depletion in the free inhibitor concentration. While it was necessary to employ higher protein concentrations (approximately threefold) in the MAO A studies than in the MAO B studies, the inhibitor concentrations employed in the MAO A studies were, in all instances, greater than those employed in the MAO B studies and reflected the known differences in affinity of these compounds for the respective binding sites. Thus, while the concentration of inhibitor undoubtedly exceeds the enzyme concentration, no indication of dependence of k_2 upon inhibitor concentration was obtained, i.e. calculations using the equation $k_{obs} = k_2/(1 + K_i/[I])$ gave reasonably consistent values of k_2 over the employed inhibitor concentration range. It would appear that use of preparations of MAO in which one of the forms of the enzyme has been previously irreversibly inhibited by use of clorgyline or L-(−)-deprenyl, provides an enzyme preparation in which nonspecific binding has been partially reduced.

In comparing the data generated among the enantiomeric pairs, the absence of any clear stereochemical influence upon values of k_2 is immediately apparent. Further, the values of k_2 determined for the individual enantiomers when reacting with MAO A or MAO B are all very similar. Such data, although obtained from studies upon relatively few compounds, would indicate that selectivity towards a particular form of MAO among the propargylamine-type inhibitors is mainly governed by the initial affinity of the inhibitor for the enzyme active site. The absence of any stereochemical influence upon the values of k_2 determined for the enantiomeric propargylamines is consistent with the free-radical mechanism proposed for both the enzyme-catalysed oxida-

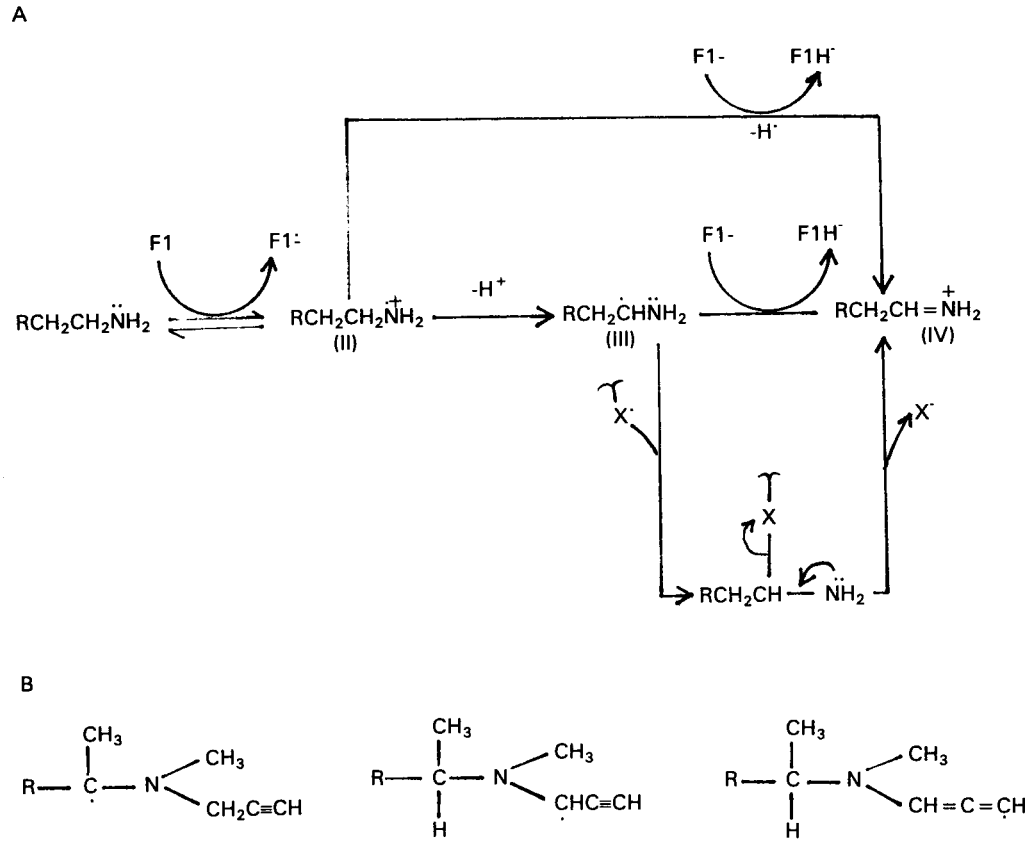


FIG. 2. A. Proposed mechanism of MAO-catalysed oxidative deamination of amine substrates via two one-electron transfer stages (Silverman et al 1980, 1993). B. Potential radical species generated from MAO-catalysed oxidation of propargylamine-based suicide inhibitors.

tive deamination reaction with substrates and the suicide-inhibition reaction with cyclopropylamine type inhibitors (Fig. 2A) (Silverman et al 1980; Richards & Burger 1986). While the initial one-electron transfer to the flavin moiety leads to a radical cation (II), subsequent reaction of this species may proceed by several different routes to the iminium ion intermediate (IV) before hydrolysis to yield the product aldehyde. Thus, if the rapid proton loss from the radical cation intermediate is predominantly enzyme mediated, it will occur at the benzylic carbon atom (α-methylpargyline series) or the α-carbon of the phenylethylamine chain (deprenyl series) leading to a common planar (racemic) radical intermediate, which then equilibrates with the active inhibitory species, an allene radical (Fig. 2B). Evidence has recently been presented indicating that proton loss from a radical cation species to form an α-amino radical (III) does occur in the inhibition of MAO by aminomethylcubane derivatives (Silverman et al 1993).

As mentioned above, only the (*S*)-D-(+)-deprenyl enantiomer when inhibiting MAO A failed to give any indication of time-dependent inhibition, despite the use of, in this instance, a very broad inhibitor concentration ($0{\cdot}025 - 7{\cdot}5 \times K_i$). No rational explanation for this observation can be presented at present. The known greater psychostimulant activity (Taylor & Snyder 1970) of the (*S*)-D-(+) enantiomers of amphetamine and methamphetamine relative to the (*R*)-L-(−)-enantiomers (metabolites of (±)-deprenyl) (Reynolds et al 1978; Maurer & Kraemer 1992) limits the clinical use of deprenyl enantiomers to that of the (*R*)-L-enantiomer only, but such may not be the case for in-vitro experimentation. Indeed, when only blockade of MAO B sites in an enzyme preparation known to contain both MAO A and MAO B is required, the present results would suggest that use of (*R*)-L-(−)-deprenyl offers no advantage over the use of (±)-deprenyl.

References

Ali, A., Robinson, J. B. (1991) Synthesis, biological evaluation and quantitative structure activity relationship analysis of nuclear-substituted pargylines as competitive inhibitors of MAO-A and MAO-B. J. Pharm. Pharmacol. 43: 750–757

Bach, C. W., Lam, N. C., Johnson, D. L., Abell, C. W., Bembenek, M. E., Kwan, S.-W., Seeburg, P. H., Shih, J. C. (1988) cDNA cloning of human liver monoamine oxidase A & B: molecular basis of differences in enzymatic properties. Proc. Natl. Acad. Sci. 85: 4934–4938

Fowler, C. J., Tipton, K. F. (1981) Concentration dependence of the oxidation of tyramine by the two forms of mitochondrial monoamine oxidase. Biochem. Pharmacol. 30: 3329–3332

Fowler, C. J., Mantle, T. J., Tipton, K. F. (1982) The nature of the inhibition of rat liver monoamine oxidase types A and B by the acetylenic inhibitors clorgyline, l-deprenyl and pargyline. Biochem. Pharmacol. 31: 3555–3561

Johnston, J. P. (1968) Some observations upon a new inhibitor of monoamine oxidase in brain tissue. Biochem. Pharmacol. 17: 1285–1297

Kitz, R., Wilson, I. R. (1962) Esters of methanesulfonic acid as irreversible inhibitors of acetylcholinesterase. J. Biol. Chem. 237: 3245–3249

Maurer, H. H., Kraemer, T. (1992) Toxicological detection of selegiline and its metabolites in urine using fluorescence polarization immunoassay (FPIA) and gas chromatography-mass spectrometry (GC-MS) and differentiation by enantioselective GC-MS of the intake of selegiline from abuse of methamphetamine or amphetamine. Arch. Toxicol. 66: 675–678

Maycock, A. L., Abeles, R. H., Salach, J. I., Singer, T. P. (1976) The structure of the covalent adduct formed by the interaction of 3-dimethylamino-1-propyne and the flavin of mitochondrial monoamine oxidase. Biochemistry 115: 114–125

Reynolds, G. P., Elsworth, J. D., Blau, K., Sandler, M., Lees, A. J., Stern, G. M. (1978) Deprenyl is metabolised to methamphetamine and amphetamine in man. Br. J. Clin. Pharmacol. 6: 542–544

Richards, L. E., Burger, A. (1986) Mechanism-based inhibitors of monoamine oxidase. Progr. Drug Res. 30: 205–219

Robinson, J. B. (1985a) Stereoselectivity and isoenzyme selectivity of monoamine oxidase inhibitors: enantiomers of amphetamine, *N*-methylamphetamine and deprenyl. Biochem. Pharmacol. 34: 4105–4108

Robinson, J. B. (1985b) Stereoselectivity and isoenzyme selectivity of monoamine oxidase inhibitors: enantiomers of α-methylbenzylamine and other *N*-alkyl derivatives. J. Pharm. Pharmacol. 37: 72P

Salach, J. I., Detmer, K., Youdim, M. B. H. (1979) Reaction of bovine and rat liver monoamine oxidase with ^{14}C-clorgyline and ^{14}C-deprenyl. Mol. Pharmacol. 16: 234–241

Silverman, R. B., Hoffman, S. J., Catus, W. B. (1980) A mechanism for mitochondrial monoamine oxidase catalysed amine oxidation. J. Am. Chem. Soc. 102: 7126–7128

Silverman, R. B., Zhou, J. P., Eaton, P. E. (1993) Inactivation of monoamine oxidase by (aminomethyl)cubane. First evidence for an α-amino radical during enzyme catalysis. J. Am. Chem. Soc. 115: 8841–8842

Smith, R., White, R. L., Krantz, A. (1988) Stereoisomers of allenic amines as inactivators of monoamine oxidase type B. Stereochemical probes of the active site. J. Med. Chem. 31: 1558–1566

Taylor, K. M., Snyder, S. H. (1970) Amphetamine: differentiation by d- and l-isomers of behaviour involving brain norepinephrine and dopamine. Science 168: 1487–1489

Tipton, K. F. (1989) Mechanism-based inhibitors. In: Sandler, M., Smith, H. J. (eds) Design of Enzyme Inhibitors as Drugs. Oxford University Press, Oxford, pp 70–93

Tipton, K. F., Mantle, T. J. (1981) The inhibition of rat liver monoamine oxidase by clorgyline and deprenyl. In: Youdim, M. B. H., Paykel, E. S. (eds) Monoamine Oxidase Inhibitors—The State of the Art. John Wiley and Sons Ltd, New York, pp 3–15

Yang, H.-Y. T, Neff, N. H. (1973) Phenylethylamine, a specific substrate for type B monoamine oxidase in brain. J. Pharmacol. Exp. Ther. 187: 365–371

Yu, P. H. (1981) Studies on the pargyline-binding site of different types of monoamine oxidase. Can. J. Biochem. 59: 30–37