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Selective inhibitors of monoamine oxidases A and B

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Monoamine oxidase (MAO) amine-oxygen oxidoreductase (deaminating) (flavin-containing) EC1.4.3.4 occurs in two forms, A and B, which differ in substrate specificity and in sensitivity to some inhibitors. Prop-2-ynylamines are irreversible MAO inhibitors (MAOIs) [1] and two such compounds, *N*-3-(2,4-dichlorophenoxy)propyl-*N*-methylprop-2-ynylamine (clorgyline) and (−)-*N*-methyl-*N*-isopropylprop-2-ynylamine (deprenyl), inhibit the A and B forms, respectively [2, 3]. Substrates for MAO A include 5-hydroxytryptamine (5HT) and noradrenaline (NA), whereas MAO B will oxidize, for example, benzylamine (BZA) and 2-phenylethylamine (PEA). Some amines are substrates for both forms of MAO (e.g. tyramine). MAOIs have been used to treat certain categories of depressive illness and the knowledge that both forms of the enzyme are present in brain [4] has focused interest on MAOIs which might selectively block the metabolism of a single amine or small group of amines. Most clinically useful MAOIs were developed before the discovery of the two forms of the enzyme. Their clinical use has been tempered by side-effects, notably hypertension, which has been associated with concomitant ingestion of amine-containing foods, particularly some types of cheese. The pressor effects have usually been attributed to a failure to metabolize dietary amines when MAO action is blocked, but recently it has been suggested that MAO inhibition and this cheese effect may be dissociable [5–7]. If so, this could remove a major objection to the use of MAOIs in humans. Use of MAO B inhibitors as an adjunct to L-dopa therapy in Parkinson's disease has been suggested as a means of raising dopamine concentration in brain, because this amine has been found to be a substrate for MAO B in human brain [8]. Deprenyl is reported to be useful in this respect [9], though recent evidence suggests that it is metabolized to amphetamine, a known indirect dopamine agonist [10, 11]. Because selective MAOIs will inhibit their non-preferred form of MAO as their concentration is increased, problems can arise in therapy as well as in pharmacological and biochemical studies of the enzyme. The biochemical differences between, and physiological significance of, the two forms of MAO are still unclear. Numerous suggestions, from the idea of two different proteins to different mitochondrial environments for the A and B forms, have been offered [12–17]. We are studying the structural determinants of A/B specificity in inhibitors. This may help to improve understanding of the differences in binding sites of the two forms of MAO which in turn may lead to better therapeutic agents.

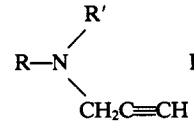
The propynylamines used in this study were prepared in this laboratory and their identity and purity established by infra-red and elemental analysis. Compounds **8** and **10** (Table 1) were obtained as their hydrogen oxalate salts, the remainder as HCl salts. Mitochondria, used as a source of MAO, were prepared as previously described [18]. Protein was measured by the method of Lowry *et al.* [19]. Radioassay of MAO, using commercial ^{14}C-labelled substrates, was carried out as formerly [18], as were experiments of time-dependent and concentration-dependent inhibition of MAO [18]. For some of the inhibitors (compounds **1**, **3**, **6**, and **7**), the effects of dialysis were examined, following treatment with inhibitor. Samples of the enzyme preparation were incubated with inhibitor at the concentration used in the time-dependent study, for a period of 45 min at 30°. Their activity was then measured and compared to controls (no inhibitor). Identical samples (3 ml) of inhibited and uninhibited enzyme were then placed inside Visking membrane and dialysed separately against 3 × 1500 ml changes of assay buffer for a total of 24 hr at 4°. Duplicate 0.95 ml samples were then assayed for MAO activity. The dialysis failed to restore MAO activity in inhibited samples. Occasionally it produced a small increase or decrease in the activity of control samples, but in no case was there any significant restoration of activity in inhibited samples when compared to the changes in controls.

Data from studies of increasing inhibitor concentration (range = 10^{-11}–10^{-3} M) on MAO activity were plotted in the form -log I against % inhibition. Selective inhibition was indicated by differences in I_{50} values when comparing selective substrates. Values for all the inhibitors, taken from the curves obtained, appear in Table 1. Results for time-dependent inhibition were plotted as pre-incubation times against log (% remaining MAO activity). These were linear, as expected for an irreversible inhibitor when its concentration is much greater than that of the enzyme [20], except where significant zero-time inhibition was observed (compounds **1**, **3**, and **6**). Values for $t_{1/2}$ (time for 50% inhibition to occur) appear in Table 1. When significant selectivity was obtained, inhibition of the non-preferred form of MAO was inevitably poor. In these cases and in those mentioned above, $t_{1/2}$ values were obtained as minimum or maximum times (see Table 1) from plots of time vs % inhibition (by extrapolation in the case of weak inhibition).

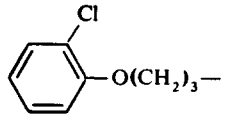
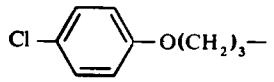
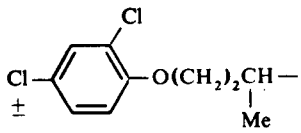
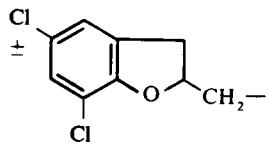
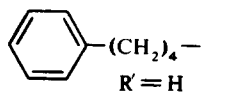
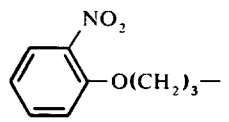
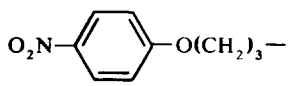
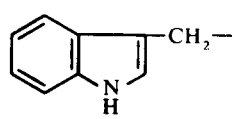
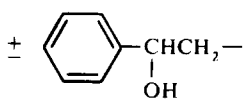
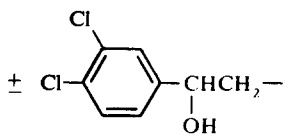
Propynylamines usually inhibit MAO by covalent bond formation at position 5 of the isoalloxazine ring of the flavin [20]. The initial interaction is by reversible binding at the active site which is competitive with substrate, followed by the irreversible phase [21, 22]. These steps are kinetically distinguishable, as recently shown for clorgyline and deprenyl [22]. The inhibitors examined here are time-dependent and the inhibition is not relieved by dialysis. It seems reasonable to assume that they inhibit the enzyme as other propynylamines do. No attempt was made to examine separately the reversible and irreversible steps referred to, so the results simply provide an overall picture of the relative efficacy of the compounds as irreversible inhibitors. However, as indicated, some showed measurable zero-time inhibition. The simplest explanation for this, assuming that inhibitor and substrate compete for the active site of MAO, is that the affinity of the inhibitor for the enzyme is such that even in the presence of substrate the inhibitor is able to bind to the enzyme to a measurable extent. This explanation has been verified for clorgyline and deprenyl [23].

The structures of the compounds were based upon those of existing MAOIs and upon substrates. The results throw some light on criteria for selective inhibition of MAO. Compounds **1** and **2** were attempts to assess the relative importance of the *ortho* and *para* chlorine atoms of clorgyline in its selectivity. Both compounds showed a strong preference for MAO A but neither is an improvement on clorgyline. Nevertheless, there are differences between them, the *ortho* compound being the better time-dependent inhibitor, with an indication that a substituent in this position gives better A/B discrimination. This is even more

Table 1. Inhibition of MAO by some propynylamines



R' = Me, unless stated otherwise

Compound		$t_{\frac{1}{2}}$ (min) for A and B substrates at the indicated [I] (M)			I_{50} values (M) with A or B substrate (pre-inc. time = 15 min)	
		[I] (M)	5HT	PEA	5HT	PEA
1		10^{-8}	<1	>60	10^{-8}	$>10^{-3}$
2		10^{-8}	22	>60*	5×10^{-9}	6×10^{-5}
3		10^{-8}	2.5	>60*	10^{-11}	5×10^{-6}
4		10^{-6}	40	>60	4×10^{-6}	10^{-5}†
5		10^{-5}	74	70	10^{-4}	10^{-4}
6		10^{-8}	2	>90*	$<10^{-9}$	$>10^{-4}$
7		10^{-6}	15	>60*	10^{-6}	2×10^{-5}
8		10^{-6}	12	3	10^{-6}	2×10^{-7}
9		5×10^{-5}	45	11†	$>10^{-4}$	2×10^{-5}†
10		5×10^{-5}	14	30	5×10^{-5}	10^{-5}

* Less than 5% inhibition in this time.
† Substrate = BZA.

clearly illustrated in the corresponding nitro derivatives (compounds **6** and **7**) where the *ortho* isomer shows much better selectivity than its *para* analogue. Also, when tested using TYR as substrate the latter showed no plateau effect [2], confirming the closeness of the I_{50} values obtained for A and B enzymes. Further studies may reveal whether this *ortho/para* phenomenon is a general one in this type of inhibitor. Perhaps size or polarity should be considered in this context. There are large differences between nitro and chloro substituents for both these variables, the dipole moments, for example, of nitro- and chloro-benzenes being 3.9 and 1.57 Debye units, respectively [24]. The effect of nitro groups in selectivity has already been observed [18]. Introduction of an alpha-methyl group into the clorgyline structure (compound **3**) does not noticeably affect selectivity for MAO A suggesting that in the B-selective inhibitors deprenyl and *n*-propargyl amphetamine the presence of this group, though apparently important, does not of itself confer the observed selectivity. The effects of such chain-branching and consequent chirality in MAOIs appear complex and have recently been commented upon [25]. Compound **4** was an attempt to rigidify the side-chain of clorgyline. The fall in selectivity shown by this dihydrobenzofuran, as compared to clorgyline, suggests that restricting the distance between the aromatic ring and the N atom is inimical to A-selectivity. Of course, compound **4** may not be the ideal clorgyline analogue for this kind of comparison. It has been argued [26] that a side-chain of three or four atoms attached to an aromatic substituent is necessary for selectivity towards MAO A, and the contrast between alpha-methyl clorgyline and compound **4** accords with this. However, though a separation of this order between ring and N atom may be a necessary condition for A-selectivity, it is by no means sufficient as can be seen from the results obtained with 4-phenyl-*N*-prop-2-ynyl-*n*-butylamine (compound **5**). We have previously reported that this inhibitor is non-selective to MAO, based upon its effects on oxidation of TYR [27]. The compound has now been re-examined using the selective substrates 5HT and BZA but no selective action was observed. The distances between the benzene ring and N atom, with the chain fully extended, has been estimated, using molecular models, for clorgyline and the phenyl butylamine derivative. These are 6.1 and 6.3 Å, respectively. It is clear that features in addition to chain length are important for A-selectivity.

It was thought that compounds **9** and **10** might differ in selectivity because although 2-phenylethanolamine is a substrate for MAO B [28, 29], substituted derivatives such as noradrenaline are oxidized by MAO A. However, neither compound showed marked selectivity and their I_{50} values point to their low potency, suggesting that inhibitors based upon phenylethanolamine may be of little value, despite their relationship to endogenous substrates. It is interesting that the hydrophobic substituents in the dichloro analogue do not confer A-selectivity because it has been suggested that the difference between MAO A and B may be due to their having different lipid environments in the mitochondrion [30], which, it has been argued, is consistent with selective actions of clorgyline and deprenyl, since MAO A has been assumed to exist in a more hydrophobic environment than MAO B [16, 31]. Though there seems to be a good correlation between hydrophobicity and potency of propynylamines as MAO inhibitors, this does not necessarily confer selectivity [27, 32]. Kalir *et al.* [26] have recently shown that propynylamines carrying an indole ring are selective inhibitors of MAO A. The only indole examined in the present study (compound **8**) does not appear to be strongly selective, though it is a reasonably good inhibitor of MAO (I_{50} approximately 10^{-6} M).

It seems that a good starting point for an inhibitor of MAO A may be phenoxyalkylamine with four atoms in the side-chain and a substituent in the *ortho* position. It may be that such compounds are A-selective because their overall structure is too bulky to allow effective interaction with MAO B. If so, development of better specific inhibitors of MAO B could be difficult, on the assumption that a molecule compact enough to bind to MAO B could as readily be accommodated by MAO A. This oversimplification ignores deprenyl but simple affinity of an irreversible inhibitor for an enzyme is only one factor influencing potency. It has been shown that for the reversible binding step clorgyline has a much greater affinity for MAO A than for MAO B, K_i values being 0.012 and 65 μM, respectively, sufficiently different to account for the observed selectivity [23]. For deprenyl, although its K_i with MAO B was less than with MAO A, the difference was much less than in the case of clorgyline, suggesting that factors other than simple affinity may be involved in selectivity. If so, more detailed investigations of the inhibitory process in propynylamines might yield further information about the basis of selectivity.

In summary, a number of propynylamines have been examined as selective inhibitors of the A and B forms or MAO. Derivatives of clorgyline were in general A-specific, though this selectivity is influenced both by the nature and position of ring substituents. The effects of length of side chain were also considered in selectivity, the conclusion being that though important, other factors are involved. The alpha-methyl analogue of clorgyline was also found to be strongly A-selective, indicating that chain-branching at this point, as seen in a number of MAO B inhibitors of the propynylamine type, is not uniquely associated with irreversible inhibitors for MAO B.

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REFERENCES

1. L. R. Swett, W. B. Martin, J. D. Taylor, G. M. Everett, A. A. Wykes and Y. C. Gladish, *Ann. N.Y. Acad. Sci.* **107**, 891 (1963).
2. J. P. Johnston, *Biochem. Pharmac.* **17**, 1285 (1968).
3. J. Knoll and K. Magyar, *Adv. Biochem. Psychopharmac.* **5**, 393 (1972)
4. D. W. R. Hall, B. W. Logan and G. H. Parsons, *Biochem. Pharmac.* **18**, 1447 (1969).
5. M. Sandler, V. Glover, J. D. Elsworth, R. Lewinsohn and M. A. Reveley, in *Monoamine Oxidase: Structure, Function and Altered Functions* (Eds. T. P. Singer, R. W. von Korff and D. L. Murphy), p. 447. Academic Press, New York (1979).
6. M. Sandler, *Psychol. Med.* **11**, 455 (1981).
7. T. J. Van de Merwe, C. B. M. Pare, V. Glover and M. Sandler, in *Monoamine Oxidase and its Selective Inhibitors* (Eds. H. Beckmann and P. Riederer), p. 189. S. Karger, Basel (1982).
8. V. Glover and M. Sandler, *Nature, Lond.* **265**, 80 (1977).
9. M. B. H. Youdim, P. Riederer, W. Birkmayer and J. Mendlewicz, in *Monoamine Oxidase: Structure, Function and Altered Functions* (Eds. T. P. Singer, R. W. von Korff and D. L. Murphy), p. 477. Academic Press, New York (1979).
10. G. P. Reynolds, P. Riederer and M. Sandler, *Biochem. Soc. Trans.* **7**, 143 (1979).
11. S. R. Philips, *J. Pharm. Pharmac.* **33**, 739 (1981).

12. R. McCauley and E. Racker, *Molec. cell. Biochem.* **1**, 73 (1973).
13. R. M. Cawthon, J. E. Pintar, F. P. Haseltine and X. O. Breakefield, *J. Neurochem.* **37**, 363 (1981).
14. T. J. Mantle, K. Wilson and R. F. Long, *Biochem. Pharmac.* **24**, 2039 (1975).
15. I. S. Severina, in *Monoamine Oxidase: Structure, Function and Altered Functions* (Eds. T. P. Singer, R. W. von Korff and D. L. Murphy), p. 169. Academic Press, New York (1979).
16. M. D. Housley and K. F. Tipton, *Biochem. J.* **135**, 173 (1973).
17. S. M. Russell, J. Davey and R. J. Mayer, in *Monoamine Oxidase: Structure, Function and Altered Functions* (Eds. T. P. Singer, R. W. von Korff and D. L. Murphy), p. 265. Academic Press, New York (1979).
18. C. H. Williams, *Biochem. Pharmac.* **31**, 2305 (1982).
19. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
20. M. Dixon, E. C. Webb, C. J. R. Thorne and K. F. Tipton, in *Enzymes*, 3rd Edn, p. 369. Longman, London (1979).
21. A. Maycock, R. H. Abeles, J. I. Salach and T. P. Singer, *Biochemistry* **15**, 114 (1976).
22. C. M. McEwen, G. Sasaki and D. C. Jones, *Biochemistry* **8**, 3963 (1969).
23. K. F. Tipton and T. J. Mantle, in *Monoamine Oxidase Inhibitors—The State of the Art* (Eds. M. B. H. Youdim and E. S. Paykel), p. 3. Wiley, London (1981).
24. G. W. C. Kaye and T. H. Laby, in *Tables of Physical and Chemical Constants*, 11th Edn, p. 143. Longmans, Green, London (1956).
25. C. H. Williams, *J. Pharm. Pharmac.* **34**, 386 (1982).
26. A. Kalir, A. Sabbagh and M. B. H. Youdim, *Br. J. Pharmac.* **73**, 55 (1981).
27. C. H. Williams and J. Lawson, *Biochem. Pharmac.* **23**, 629 (1974).
28. C. H. Williams, *Biochem. Soc. Trans.* **5**, 1770 (1977).
29. D. J. Edwards, *Life Sci.* **23**, 1201 (1978).
30. I. S. Severina and T. M. Sheremet'evskaya, *Biokhimiya* **34**, 125 (1969).
31. M. D. Houslay, *J. Pharm. Pharmac.* **29**, 664 (1977).
32. C. H. Williams and J. Lawson, *Biochem. Pharmac.* **24**, 1889 (1975).