Biochemical Pharmacology, Vol. 31, No. 7, pp. 1251–1255, 1982.
Printed in Great Britain.

0006-2952/82/071251-05 $03.00/0
Pergamon Press Ltd.

INHIBITION OF RAT LIVER MONOAMINE OXIDASE BY α-METHYL- AND *N*-PROPARGYL-AMINE DERIVATIVES

KEITH F. TIPTON, JOHN M. MCCRODDEN, ASHER S. KALIR* and MOUSSA B. H. YOUDIM†
Department of Biochemistry, Trinity College, Dublin 2, Ireland, *Israel Institute of Biology, Ness Ziona, Israel, and †School of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

(*Received* 1 *July* 1981; *accepted* 23 *October* 1981)

Abstract—The inhibition of rat liver monoamine oxidase by a number of *N*-propargyl and α-methyl amine derivatives has been examined. The results indicate that α-methyl-substituted primary and secondary amine derivatives tend to show selectivity as reversible inhibitors towards the A-form of the enzyme. The structural features that result in selectivity in irreversible inhibitors are less easy to define and substitution of an *N*-propargyl group into a compound that is a selective reversible inhibitor of monoamine oxidase will not necessarily result in retention of that selectivity. Replacement of the acetylenic group in a B-selective irreversible inhibitor by an ethylenic group resulted in a compound that was a reversible inhibitor showing slight selectivity for the A-form of the enzyme.

The discovery of the presence in many tissues of two forms of monoamine oxidase (monoamine:O_2 oxidoreductase (deaminating) (flavin-containing) EC 1.4.3.4) that differ in their inhibitor sensitivity and substrate specificities (see [1, 2] for reviews) has led to a resurgence of interest in the pharmacological actions of its inhibitors (see [3] for review). The A-form of the enzyme has been shown to oxidise serotonin preferentially and to be more sensitive to inhibition by clorgyline [4] whereas the B-form, which has greater activity towards benzylamine and 2-phenethylamine, is more sensitive to inhibition by deprenyl [5]. Dopamine and tyramine have been shown to be substrates for both forms of the enzyme although the specificities of the two forms may not be identical in all organs and species [6]. Clorgyline has been reported to be an effective antidepressant drug [7–9] whereas the efficacy of deprenyl in this respect is controversial [10–12]. The latter compound has, however, been shown to be of value in the treatment of Parkinson's disease [13–15].

Clorgyline and deprenyl are both *N*-propargyl amine derivatives which have been shown to inhibit the enzyme irreversibly by reacting with the enzyme-bound flavin group [16, 17]. A number of reversible inhibitors of the enzyme have also been reported to exhibit selectivity (see, e.g., refs 2, 6) and α-methyl-substituted amines, such as amphetamine, α-methylbenzylamine and α-methyltryptamine, have been shown to be selective inhibitors of the A-form [18].

Although a relatively large amount of data have accumulated on the properties and effects of selective inhibitors it has not yet been possible to formulate any detailed and consistent structure–activity relationships that might be of value in their design. As part of a study on the structural features involved in selectivity and in order to identify compounds that might be worth further pharmacological studies we have examined the *in vitro* inhibition of rat liver monoamine oxidase by a number of *N*-propargyl and α-methyl-substituted amine derivatives. The relative potencies of some of these compounds as inhibitors of rat brain monoamine oxidase have recently been reported [19], but these studies did not involve any detailed analysis of the kinetics of the reversible inhibitors.

MATERIALS AND METHODS

Rat liver mitochondria from 3–5 animals, prepared by the method of Hawkins [20], were pooled and suspended in 10 mM sodium phosphate buffer, pH 7.2, containing 0.25 M sucrose and stored at −20° until use. 1-^{14}C-Labelled serotonin creatinine sulphate and 2-phenethylamine hydrochloride were obtained from the Radiochemical Centre (Amersham, Bucks, U.K.). The AGN series of inhibitors were kind gifts from Aspro-Nicholas Research Ltd. (Australia). The series of inhibitors designated by the letter K were synthesised by Kalir *et al.* [19, 21, 22] and Abbott 21855 was a kind gift from Abbott Laboratories Ltd (North Chicago, IL). All the inhibitors were supplied as their hydrochloride salts. All other materials were standard laboratory chemicals and were of analytical reagent grade whenever possible.

Monoamine oxidase activity was assayed by the method of Otsuka and Kobayashi [23] extracting the deaminated products into toluene: ethyl acetate (1:1, v/v) containing 0.6 per cent (w/v) PPO (2,5-diphenyloxazole) [24]. Time-courses of the reaction were determined to ensure that in all cases the fixed times used for the assays corresponded to the linear, initial-rate, period of the reaction. Assays were carried out at 30° in 100 mM sodium phosphate buffer, pH 7.2. Irreversible inhibitors were preincubated with the enzyme preparation for 30 min at 30° before the activity was assayed either with 200 μM serotonin or 100 μM 2-phenethylamine. In studies with reversible inhibitors, assays were carried out over a range of substrate concentrations in the presence of several

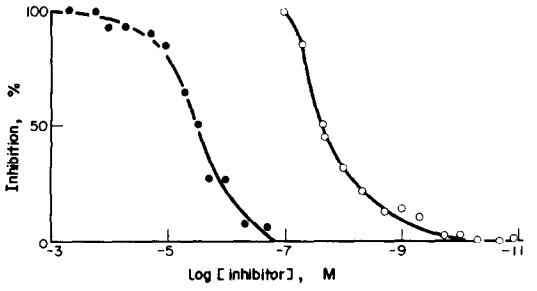


Fig. 1. Irreversible inhibition of rat liver monoamine oxidase by Abbott 21855. The enzyme preparation was incubated at 30° for 30 min with the indicated concentration of the inhibitor before activity was assayed with either serotonin (○) or 2-phenethylamine (●). Other details of the experimental procedures used are described in the text.

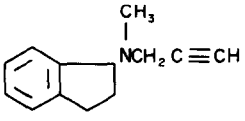
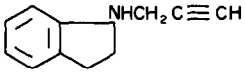
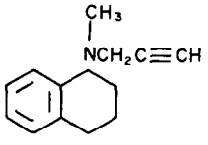
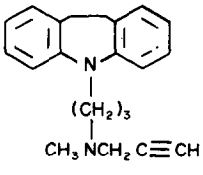
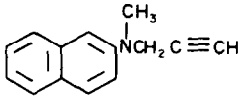
fixed concentrations of the inhibitor without any preincubation of enzyme and inhibitor. The data obtained were plotted as double-reciprocal plots and the K_i values were determined from replots of the slopes of the lines obtained against the inhibitor concentration (see Fig. 2). The results from studies with irreversible inhibitors are given as pI_{50} values, which are defined as the negative logarithm (base 10) of the inhibitor concentration that causes 50% inhibition of enzyme activity under these conditions. Values were obtained from duplicate or triplicate determinations which differed by less than 5 per cent at the pI_{50} value.

In order to assess whether inhibition was reversible in each case a sample of the mitochondrial preparation was incubated with the inhibitor, at a concentration sufficient to cause more than 70% inhibition, for 30 min at 30°. The mitochondria were then sedimented in an Ole Dich minifuge and the pellet was washed twice, by resuspension and centrifugation with 10 mM sodium phosphate buffer, before it was finally resuspended in that buffer and assayed. Control samples of mitochondria were carried through an identical procedure with the inhibitor solution being replaced by an equal volume of distilled water. Failure to recover activity after the washing procedure was taken as indicating that the inhibition was irreversible.

RESULTS AND DISCUSSION

The structures and pI_{50} values for compounds that were found to be irreversible inhibitors of rat liver monoamine oxidase are shown in Table 1 and a representative inhibition curve, obtained with the inhibition Abbott 21855 is shown in Fig. 1. All the irreversible inhibitors were *N*-propargyl amine derivatives and thus contained the acetylenic group

Table 1. Irreversible inhibitors of monoamine oxidase

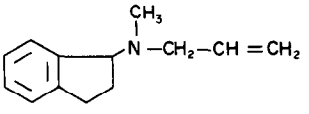
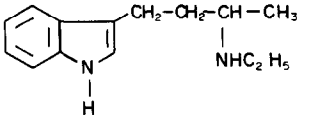
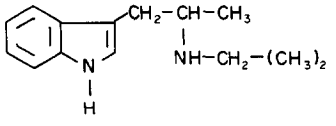
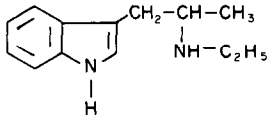
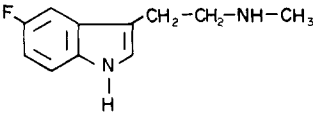
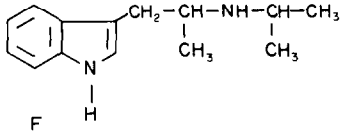
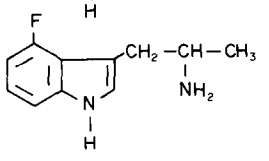
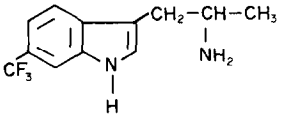
Inhibitor	Structure	pI_{50} Serotonin	pI_{50} 2 - Phenethylamine
AGN 1133		7.1	7.8
AGN 1135		5.7	7.7
AGN 1278		7.3	8.0
K 1349		~ 3.8	No significant inhibition at 10^{-4} M
Abbott 21855		7.7	5.5

which has been shown, with other compounds, to form a covalent adduct with the flavin component of monoamine oxidase [16, 17]. The compound AGN 1133 has previously been described by Huebner *et al.* [25], who referred to it as SU-11739, and by Knoll *et al.* [26], who termed it J-508. It has been shown to be a selective inhibitor of the B-form of monoamine oxidase both *in vivo* and *in vitro* [26–29]. In agreement with the results of Finberg *et al.* [27, 28] both AGN 1133 and AGN 1135 are selective inhibitors of the B-form of the enzyme, although the presence of the *N*-methyl group in the former compound results in a reduced degree of selectivity. Despite the relatively poor selectivity of AGN 1133, it has been used successfully to determine the concentrations of active sites in the B-form of monoamine oxidase *in vitro* [30, 31].

The compound AGN 1278 (also known as J-512 [26]) resembles AGN 1133 except that a saturated six-membered ring bears the propargyl-containing side-chain. This structural difference can be seen to cause little change in the selectivity of inhibition. Unsaturation of that six-membered ring and a changed position of the substituent in Abbott 21855 results in a change in the selectivity towards the A-form of the enzyme.

K 1349 is a poor inhibitor of either form of monoamine oxidase but it does have a greater potency towards the A-form of the enzyme. It may be regarded as being an *N*-propargyl analogue of desipramine from which it was synthesised [19]. That compound has been shown to be a B-selective reversible inhibitor of monoamine oxidase [32] and thus it appears that such substitution of a selective

Table 2. Reversible inhibitors of monoamine oxidase

Inhibitor	Structure	Ki (μM) Serotonin	Ki (μM) 2-Phenethylamine
AGN 1142		150	300
K_a		1	60
K_b		3.4	1,000
K_c		0.75	240
K_d		0.8	180
K 502		18	90
K 511		~ 0.1	No significant inhibition at 450 μM
K 622		5	19

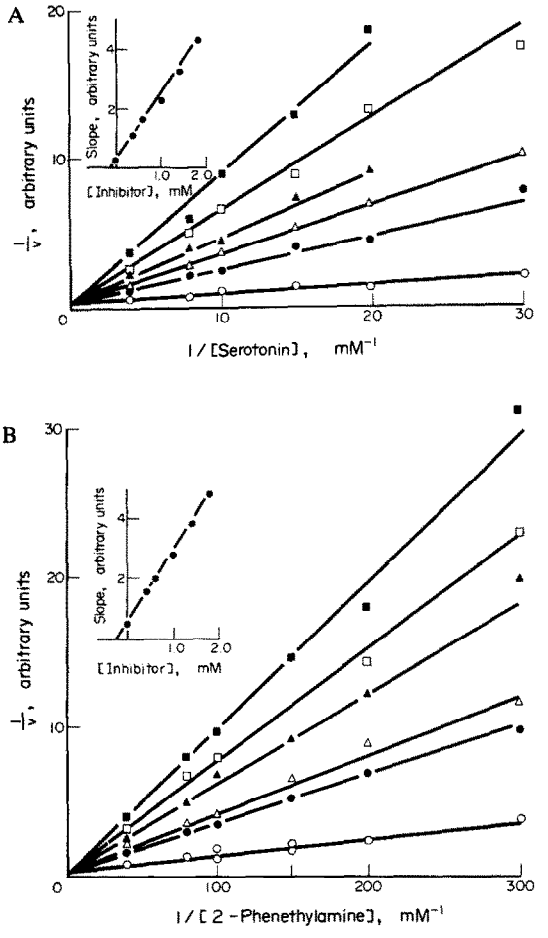


Fig. 2. Reversible inhibition of rat liver monoamine oxidase by AGN 1142. Results are presented as double-reciprocal plots and the insets represent replots of the slopes of the lines obtained against the inhibitor concentration. (A) Activities determined with serotonin. (B) Activities determined with 2-phenethylamine. In both cases the inhibitor concentrations used were 0 (○), 0.4 mM (●), 0.6 mM (△), 1.0 mM (▲), 1.4 mM (□) and 1.8 mM (■). Experimental details are given in the text.

reversible inhibitor cannot necessarily be expected to result in an irreversible inhibitor with the same selectivity.

Studies on the kinetics of inhibition of monoamine oxidase by clorgyline and deprenyl have shown the reaction to involve the initial reversible formation of a noncovalent enzyme–inhibitor complex, with subsequent reaction occurring, within this complex, to form the irreversibly inhibited enzyme [33]. These studies have also indicated that selectivity may result from differences in the affinities of the two forms for reversible combination with the inhibitor, from differences in the irreversible rates of reaction to form the covalent complex, or from a combination of both of these factors. This complexity could cause difficulties in attempts to define relationships between inhibitor structure and selective potency. It has recently been suggested that the distance between the ring system and the *N*-propargyl terminal group of the side-chain may be crucial in determining the selectivity of irreversible inhibition [19]. Detailed comparative kinetic studies of the factors involved in the selectivity of the compounds shown in Table 1 and the selective potency of structurally-related reversible inhibitors would be of value in obtaining a better understanding of the factors involved in selectivity.

The structures and K_i-values for compounds that were found to be reversible inhibitors of rat liver monoamine oxidase are shown in Table 2 and the inhibition pattern obtained with AGN 1142 is shown in Fig. 2. In all cases inhibition was found to be competitive both towards 2-phenethylamine and serotonin. The compounds, K_a, K_b, K_c, K 502, K 622 and K 511 were all α-methyl-substituted derivatives of primary or seconday amines and, as has been shown for other amines bearing an α-methyl group [18], they were all found to be selective for the A-form of monoamine oxidase. K_i values of 1250 μM and 0.5 μM have previously been reported for the inhibition of the A- and B-forms, respectively, of rat liver monoamine oxidase by α-methyl tryptamine [18] and thus the values for K_b and K_c shown in Table 2 suggest that substitution of the amino group does not greatly affect the selectivity of this compound. Compounds K_d, K 511 and K 622 contain substituted fluorine atoms and may be of value for nuclear magnetic resonance studies. A comparison of the inhibitor constants for K_a and K_c indicates that the introduction of an extra methylene group into the side-chain of α-methyl, *N*-ethyltryptamine results in only a small change in its selectivity.

The compound K_d does not bear an α-methyl substituent. Although it is a relatively potent reversible inhibitor of the enzyme it shows selectivity towards the A-form under the test conditions employed. It is possible that it may also be a substrate for the enzyme (see, e.g., [34]) and further studies will be necessary to investigate this. The reversible inhibitor AGN 1142 differs from the irreversible inhibitor AGN 1133 only in containing an ethylenic rather than an acetylenic group. The observation that it shows very little selectivity, with slight preference for the A-form, whereas AGN 1133 is selective for the B-form, might suggest that the selectivity of the latter compound depends on the rate of irreversible reaction rather than on the affinity for non-covalent binding. An alternative explanation might be that the acetylenic group itself is actually important in the non-covalent interactions. Detailed studies of the kinetics of irreversible inhibition by AGN 1133 would be necessary to indicate which may be the case.

In conclusion the results presented here support the view that the presence of an α-methyl substituent tends to lead to A-selective reversible inhibition. The structural features that result in selectivity in irreversible inhibitors are difficult to define and it would appear that substitution of a propargyl group onto a selective reversible inhibitor will not necessarily result in retention of that selectivity.

Several of the compounds investigated here show relatively high degrees of selectivity and might prove to be of value *in vivo* studies. In view of the recent indications that selective reversible inhibitors of the A-form of monoamine oxidase may be of particular

value as antidepressants (see, e.g., [35, 36]), pharmacological studies with inhibitors such as K 511 could be of particular interest.

Acknowledgements—K.F.T. and J.M.M. are grateful to the St. Patrick's Hospital–Trinity College Research Fund and A.K. and M.B.Y.H. are grateful to the Israel Council for Research and Development, the Prime Minister's Office, Jerusalem, for the support of research grants. We would also like to thank Miss A. Sabbagh for her highly skilled technical assistance.

REFERENCES

1. M. D. Houslay, K. F. Tipton and M. B. H. Youdim, *Life Sci.* **19**, 467 (1976).
2. F. M. Achee, S. Gabay and K. F. Tipton, *Prog. Neurobiol.* **8**, 325 (1977).
3. M. B. H. Youdim and E. S. Paykel (Eds), *Monoamine Oxidase Inhibitors. The State of the Art.* John Wiley and Sons, Chichester (1981).
4. J. P. Johnston, *Biochem. Pharmac.* **17**, 1285 (1968).
5. J. Knoll and K. Magyar. *Adv. Biochem. Psychopharmac.* **5**, 393 (1972).
6. C. J. Fowler, B.A. Callingham, T. J. Mantle and K. F. Tipton, *Biochem. Pharmac.* **27**, 97 (1978).
7. J. A. Herd, *Clin. Trials* **6**, 219 (1969).
8. D. Wheatley, *Br. J. Psychiat.* **17**, 573 (1970).
9. D. L. Murphy, S. Lipper, D. Pickar, D. Jimerson, R. M. Cohen, N. A. Garrick, I. S. Alterman and I. C. Campbell, in *Monoamine Oxidase Inhibitors. The State of the Art* (Eds. M. B. H. Youdim and E. S. Paykel), p. 189. John Wiley and Sons, Chichester (1981).
10. M. Mendis, C. M. B. Pare, M. Sandler, V. Glover and G. Stern, in *Monoamine Oxidase Inhibitors. The State of the Art* (Eds. M. B. H. Youdim and E. S. Paykel), p. 171. John Wiley and Sons, Chichester (1981).
11. J. Mendlewicz and M. B. H. Youdim, in *Monoamine Oxidase Inhibitors. The State of the Art* (Eds. M. B. H. Youdim and E. S. Paykel), p. 177. John Wiley and Sons, Chichester (1981).
12. J. Mann and S. Gershon, *Life Sci.* **26**, 877 (1980).
13. W. Birkmayer, P. Riederer, M. B. H. Youdim and W. Linauer. *J. Neural Transm.* **35**, 303 (1975).
14. W. Birkmeyer, P. Riederer, L. Ambrozi and M. B. H. Youdim. *Lancet* **ii**, 439 (1977).
15. M. Yahr, *J. Neural Transm.* **43**, 227 (1978).
16. C. M. McEwen, G. Sasaki and D. C. Jones, *Biochemistry* **8**, 3963 (1969).
17. T. P. Singer, in *Monoamine Oxidase: Structure, Function and Altered Functions* (Eds. T. P. Singer, R. W. Von Korff and D. L. Murphy), p. 7. Academic Press, New York (1979).
18. T. J. Mantle, K. F. Tipton and N. J. Garrett, *Biochem. Pharmac.* **25**, 2073 (1976).
19. A. S. Kalir, A. Sabbagh and M. B. H. Youdim, *Br. J. Pharmac.* **73**, 55 (1981).
20. J. Hawkins, *Biochem. J.* **50**, 577 (1952).
21. A. S. Kalir and D. Balderman, *Israel J. Chem.* **6**, 927 (1968).
22. A. S. Kalir and S. Szara, *J. med. Chem.* **9**, 341 (1966).
23. S. Otsuka and Y. Kobayashi, *Biochem. Pharmac.* **13**, 995 (1964).
24. K. F. Tipton and M. B. H. Youdim, *Ciba Found. Symp.* **39**, 393 (1976).
25. C. F. Huebner, E. M. Donoghue, A. J. Plummer and P. A. Furness, *J. med. Chem.* **9**, 830 (1966).
26. J. Knoll, Z. Ecsery, K. Magyar and E. Satory, *Biochem. Pharmac.* **27**, 1739 (1978).
27. J. P. M. Finberg, A. Sabbagh and M. B. H. Youdim, in *Enzymes and Neurotransmitters in Mental Disease* (Eds. E. Usdin, T. L. Sourkes and M. B. H. Youdim), p. 205. John Wiley and Sons, New York (1980).
28. J. P. M. Finberg, M. Tenne and M. B. H. Youdim, in *Monoamine Oxidase Inhibitors. The State of the Art* (Eds. M. B. H. Youdim and E. S. Paykel), p. 31, John Wiley and Sons, Chichester (1981).
29. B. Eksted, K. Magyar and J. Knoll, *Biochem. Pharmac.* **28**, 919 (1979).
30. C. J. Fowler, A. Wiberg, L. Oreland and B. Winblad, *Neurochem. Res.* **5**, 697 (1980).
31. C. J. Fowler and A. Wiberg, *Naunyn-Schmiedeberg's Arch. Pharmac.* **313**, 77 (1980).
32. J. A. Roth and C. N. Gillis, *Biochem. Pharmac.* **23**, 1138 (1974).
33. 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. John Wiley and Sons, Chichester (1981).
34. K. F. Tipton in *Handbook of Physiology*, Section 7, Vol. 2 (Eds. A. D. Smith and H. Blaschko), p. 667. American Physiological Society, Washington (1975).
35. M. Strolin Benedetti, J-P. Kan and P. E. Keane, in *Monoamine Oxidase: Structure, Function and Altered Functions* (Eds. T. P. Singer, R. W. Von Korff and D. L. Murphy), p. 335. Academic Press, New York (1979).
36. S-O. Ogren, A-L. Ask, A-C. Holm, L. Florvall, L-O. Lindbom, J. Lundström and S. B. Ross, in *Monoamine Oxidase Inhibitors. The State of the Art* (Eds. M. B. H. Youdim and E. S. Paykel), p. 103. John Wiley and Sons, Chichester (1981).