Br. J. Pharmac. (1981), **73**, 55–64

SELECTIVE ACETYLENIC 'SUICIDE' AND REVERSIBLE INHIBITORS OF MONOAMINE OXIDASE TYPES A AND B

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1 A number of aromatic-*N*-propargyl (acetylenic) compounds and indoleamines were tested for their inhibitory action on monoamine oxidase (MAO) type A and type B using the substrates 5-hydroxytryptamine (5-HT), β-phenylethylamine (PEA) and dopamine.

2 Structure activity studies with aromatic-*N*-propragyl (acetylenic) derivatives have shown that MAO inhibitory potency is least dependent on the aromatic portion of the compounds. *N*-methylated propargyl derivatives are the most active and replacement of the methyl group with a higher alkyl or aromatic group results in significant reduction of activity. The triple bond in the *N*-propargyl portion is absolutely essential for activity and must be β-to the nitrogen. It is the acetylenic group that gives these compounds their irreversible MAO inhibitory property.

3 The present study has indicated that since the acetylenic compounds resemble the enzyme substrates the distance between the aromatic ring and the *N*-propargyl terminal is crucial in designating the type A or type B MAO inhibitory property. For MAO type A inhibition, a distance equivalent to at least three carbon units is required, while for the inhibition of the B type enzyme this distance can be 1 or 2 carbon units.

4 The compounds AGN-1133 and AGN-1135 show most promise in Parkinson's disease or as anti-depressants because of their irreversible selective type B MAO inhibition *in vitro* and *in vivo*.

5 A number of indoleamine derivatives were found to be reversible selective type A inhibitors.

Introduction

Although the differentiation of central nervous system and peripheral tissue mitochondrial monoamine oxidase (MAO) into types A and B has serious limitations (Fowler, Callingham, Mantle & Tipton, 1978), it is a useful model for the biochemical characterization of the enzyme active site and its selective inhibitors (Salach, Detmer & Youdim, 1979). There is abundant evidence that MAO inhibitors benefit certain types of depression (Pare, 1976; Youdim & Paykel, 1981) and B type inhibitors can act as adjuncts to L-DOPA therapy of Parkinson's disease (Birkmayer, Riederer, Youdim & Linauer, 1975; Birkmayer, Riederer, Ambrozi & Youdim, 1977; Birkmayer & Yahr, 1978). Of the irreversible MAO inhibitors so far described, clorgyline and deprenyl are best known for their selective inactivation of MAO type A and type B respectively (Johnston, 1968; Knoll & Magyar, 1972). In addition to their MAO inhibitory action, most irreversible MAO inhibitors have pharmacological side-effects on the sympathetic nervous system; of particular importance is their ability to potentiate the effects of indirectly acting sympathomimetic amines, e.g. tyramine, either derived from decarboxylation of tyrosine or of dietary origin. This has been the limiting factor in the use of MAO inhibitors. However, deprenyl is unique among MAO inhibitors, in that it does not potentiate the sympathomimetic action of tyramine in the isolated preparations (Knoll, 1976; Finberg, Sabbagh & Youdim, 1980; Finberg, Tenne & Youdim, 1981) *in vivo* and in rats and cats as well as in man (Knoll, Ecsery, Magyar & Satory, 1978; Elsworth, Glover, Reynolds, Sandler, Lees, Phuapradit, Shaw, Stern & Kumar, 1978; Finberg *et al.*, 1980, 1981).

The successful use of the MAO-B inhibitor, deprenyl, as an adjunct to L-DOPA in the treatment of Parkinson's disease or depressive illness (Birkmayer *et al.*, 1975, 1977; Yahr, 1978; Mendlewicz & Youdim, 1978; Mann & Gershon, 1980) without the occurrence of untoward side-effects encountered with other MAO inhibitors, together with the consideration that human brain MAO is mainly of type B (Squires, 1972; Youdim, 1977; Gover, Sandler, Owen & Riley, 1977; Riederer, Youdim, Rausch, Birkmayer, Jellinger & Seemann, 1978) has led us to search for other selective MAO type B inhibitors using 5-hydroxytryptamine and phenylethylamine as selective substrates of MAO type A and type B respectively (Johnston, 1968; Yang & Neff, 1973).

0007–1188/81/050055–010 $01.00

Methods

Animals

Wistar male rats of between 200–250 g were used for experiments. The rats were killed by decapitation, the brains and livers rapidly removed and placed in ice-cold (4°C) 0.3 M sucrose solution.

Preparation of mitochondrial monoamine oxidase

Brain mitochondrial MAO was prepared according to the method described by Youdim (1975). The isolated mitochondria were dispersed in ice-cold (4°C) 0.3 M sucrose solution to give a preparation corresponding to 10% (w/v) of brain tissue; 5 ml samples were placed in plastic vials and frozen at −20°C for future use.

Liver mitochondria (5% w/v) were prepared by the procedure of Hawkins (1952) and kept at −20°C.

Assay of monoamine oxidase

MAO type A and type B activity (Johnston, 1968; Yang & Neff, 1973) was estimated with [^{14}C]-5-hydroxytryptamine and [^{14}C]-phenylethylamine respectively as substrates by the radioassay technique of Tipton & Youdim (1976). The substrate concentrations were at least equivalent to the *Km* of the enzyme for the particular amine (Houslay & Tipton, 1974). Protein determinations were performed by the colorimetric procedure of Lowry, Rosebrough, Farr & Randall (1951) with bovine serum albumin as standard.

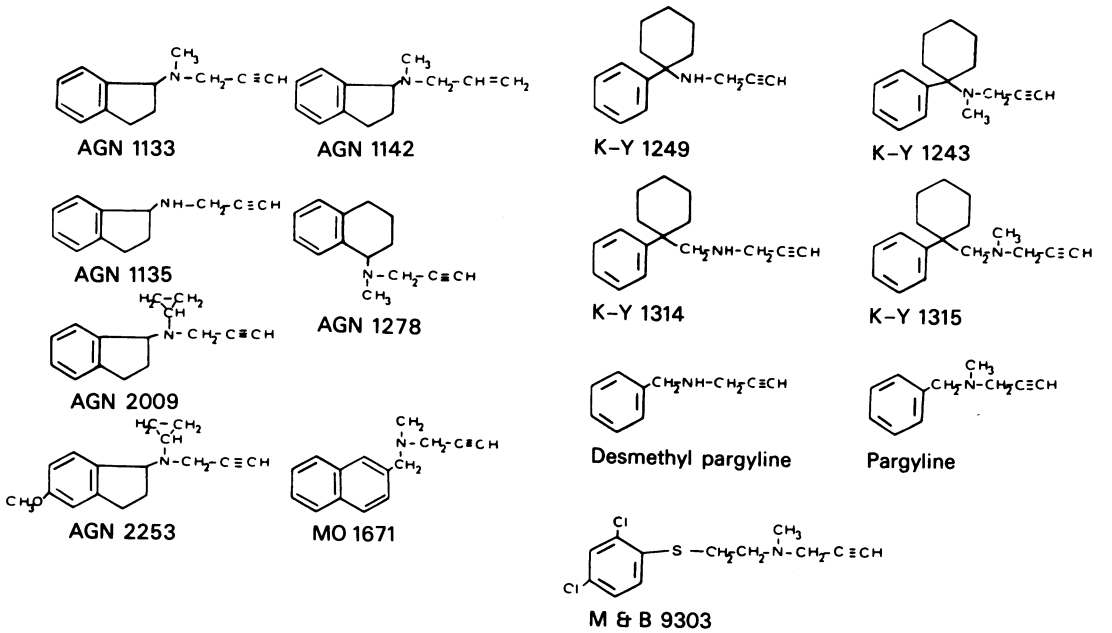
Materials

The following radioactive amines were used: [2-^{14}C]-5-hydroxytryptamine creatinine sulphate (54 mCi/mmol); [1-^{14}C]-dopamine hydrochloride (48 mCi/mmol); [2-^{14}C]-tyramine hydrochloride (41 mCi/mmol) (all from the Radiochemical Centre, Amersham); [1-^{14}C] β-phenylethylamine hydrochloride (48 mCi/mmol) from New England Nuclear, Boston. The unlabelled substrates were purchased from Sigma. The gifts of (1) deprenyl-hydrochloride (Prof. J. Knoll, Budapest); clorgyline hydrochloride and M & B 9303 (May & Baker, Dagenham); *N*-propargyl-l-amino indane hydrochloride (AGN 1135); *N*-methyl-*N*-propargyl-l-amino indane hydrochloride (AGN 1133); *N*-allyl-*N*-methyl-l-amino indane hydrochloride (AGN 1142); *N*-methyl-*N*-propartyl-l-amino- 1; 2, 3, 4-tetrahydronaphthalene hydrochloride (AGN 1278); *N*-cyclopropyl-methyl-l-aminoindane hydrogen oxalate (AGN 1517) and *N*-cyclopropyl-*N*-propargyl-l-aminoindane hydrochloride (AGN 2009); *N*-cyclopropyl-*N*-propargyl-5-methoxy-l-aminoindane hydrochloride (AGN 2253) (Aspro Nicholas, Australia) are gratefully acknowledged. We also thank Abbott Laboratories (Chicago, U.S.A.) for the samples of pargyline, *m*-bromo-pargyline hydrochloride (MO 1588), desmethylpargyline and *N*-methyl-*N*-propargyl-quinoline hydrochloride (MO 1671). The sample of Lilly 51641 and Eli Lilly and Co. (Indianopolis, U.S.A.) is gratefully acknowledged. All other reagents were of highest available purity and were purchased from Sigma (Israel). The following compounds were synthesized by Professer Kalir: K-502, 3-(3′-isopropylaminobutyl) indole hydrochloride; K-511, 6-fluoro-α-methyltryptamine hydrochloride (Kalir & Balderman, 1968); K-622, 4-trifluoromethyl-α-methyl-tryptamine bimalmate (Kalir, Pelah & Balderman, 1967); Ka, N-ethyl-α-methyltryptamine hydrochloride (Kalir & Szara, 1966); Kb, N-isopropyl-α-methyltryptamine hydrochloride (Kalir & Szara, 1966); Kc, 3-(3′-ethylaminobutyl) indole hydrochloride (Kalir & Szara, 1966) and Kd, 5-fluoro-α-methyltryptamine hydrochloride (Kalir & Szara, 1963). The compounds K-Y 1243, *N*-methyl-*N*-propartyl-l-phenyl-cyclohexylamine hydrochloride; K-Y 1249, *N*-propargyl-l-phenyl-cyclohexylamine hydrochloride; K-Y 1314, *N*-propargyl-l-phenylcyclohexyl-methylamine hydrochloride were synthesized from a phencyclidine derivative (Kalir & Youdim, unpublished). The compound K-Y 1349, *N*-propartyl-imipramine hydrochloride, was synthesized from desimipramine (Kalir & Youdim, unpublished).

Results

Acetylenic suicide irreversible inhibitors of monoamine oxidase

In vitro *studies* It is already known that *N*-propargylamine compounds such as pargyline, clorgyline, deprenyl and *N*, *N*-dimethylpropargylamine .are acetylenic 'suicide' irreversible inhibitors of MAO. They inactivate the enzyme by virtue of their covalent binding to the N-5 of FAD, the enzyme co-factor (Maycock, Abeles, Salach & Singer, 1976; Youdim, 1976; Salach *et al.*, 1979). We have examined the MAO inhibitory action of a number of acetylenic compounds with structural resemblance to pargyline (Figure 1). In order to organize the structural changes in some orderly fashion we have broken down the acetylenic inhibitor molecule into four components, A, B, C and D (Figure 2). Structural modifications of the acetylenic inhibitor molecule were investigated as indicated in Figure 2. Portion A is the least specific of the four groups. The presence of an aromatic ring renders such compounds as clorgyline, deprenyl, pargyline, des-methylpargyline and M & B 9303 very active inhibitors. The latter compound is a selective inhibitor of MAO type A and like clorgyline has



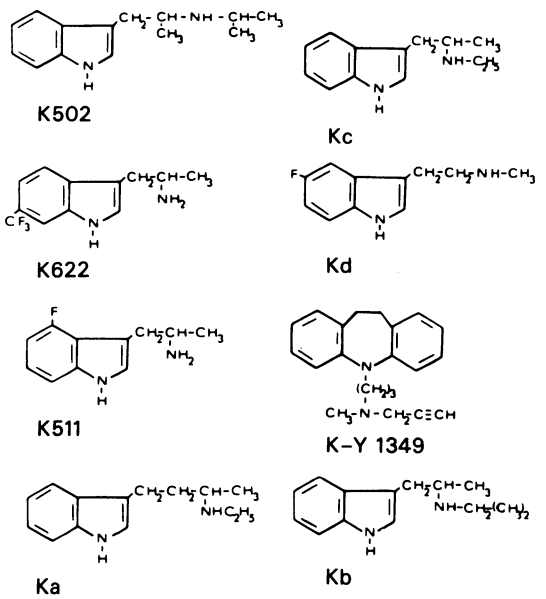


Figure 1 Structures of irreversible acetylenic and reversible inhibitors of mitochondrial monoamine oxidase.

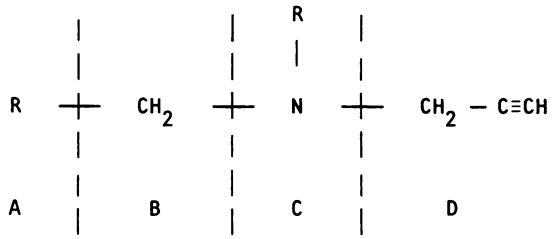


Figure 2 Basic structure of irreversible acetylenic monoamine oxidase inhibitor.

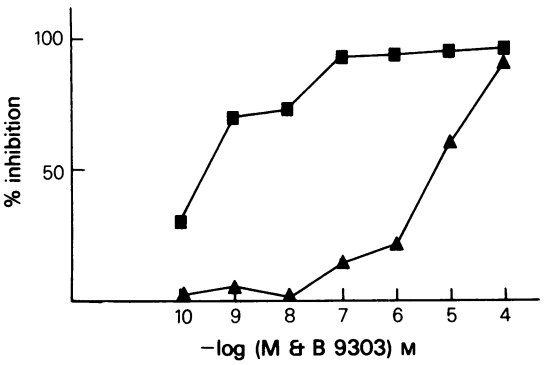


Figure 3 Inhibition by M & B 9303 of rat brain mitochondrial monoamine oxidase (MAO) *in vitro* with [^{14}C]-5-hydroxytryptamine (■) and [^{14}C]-phenylethylamine (▲) as substrates of MAO type A and type B respectively. The enzyme preparations were preincubated with the inhibitor for 20 min before introduction of the substrates (Tipton & Youdim, 1976).

chlorine in the ring, while the former three are MAO-B-type inactivators (Table 1 and Figures 3 and 4). The replacement of portions A and B with an indane moiety as with AGN 133 and AGN 1135 does not modify the inhibitory potency or selectivity for the B type enzyme with respect to pargyline. In comparison, substitution of indane with phencyclidine or methylphencyclidine derivatives (K 1243, K 1249, K 1314 and K 1315), resulted in the loss of inhibitory activity. This diminished inhibitory activity was not due merely to addition of weight since compounds with tetrahydronaphthyl ring (AGN 1278) or quinolyl ring (MO 1671) are very active inhibitors (Table 1). The rationale for the synthesis of phencyclidines was two fold: firstly, to see whether these compounds had MAO inhibitory activity and secondly, whether introduction of a six membered ring at position 'B' of the basic acetylenic compound structure would modify MAO inhibitory selectivity. The poor MAO inhibitory activity of phencyclidines derivatives (K-Y, 1243, K-Y 1249; K-Y 1314; K-Y 1315) may be due to the presence of six membered ring in position B (Table 1 and Figure 1), which prevents their binding to the enzyme. However, the *N*-desmethylated derivative, K-Y 1315, was slightly selective for MAO-B.

We have not examined variations in Group B, i.e. the distance between the aromatic ring and the propargyl group, but Knoll *et al.* (1978) have reported extensively on this. They pointed out that substitution at this point may be very important in designating type B inhibitory activity. Up to 2-carbon chain compounds may have specificity for MAO-B inhibition while with more than 2-carbons, the inhibitory potency is lost. However, these authors used only PEA (type B substrate) to measure MAO activity with compounds having more than 2-carbon unit chain at this position. This point is rather important since in clorgyline, M & B 9393, MO 1671 and K-Y 1349 the distance between the aromatic ring and nitrogen group is longer than 2-carbon units and these inhibitors are potent inactivators of MAO-A (Table 1 and Figure 3). We now have further evidence that this is so since acetylenic indoleamine derivatives synthesized by us are potent irreversible MAO-A inhibitors (Kalir, Finberg & Youdim, unpublished observations).

In position C the limitations for MAO inhibitor activity are quite strict with -NH- and -N-methyl comprising the only active amine portions found. These are well illustrated with compounds AGN 1133 and AGN 1135, pargyline and des-methylpargyline and K 1314 and K 1315. *N*-methylated derivatives are the most active. Replacing the hydrogen or methyl group with higher alkyl, cyclopropyl group we found a reduction of activity as best illustrated with the compound AGN 2009. Introduction of alkoxy at position 4 of the indane moiety in AGN 2009 resulted in restoration of MAO inhibitory activity (see AGN 2253, Figure 1 and Table 1). However, selectivity for MAO-B as shown by AGN 1133 and AGN 1135 is lost. A similar result was reported by Swett, Martin, Taylor, Everett, Wykes & Gladish (1963) in a study of structure activity relationships with pargyline.

Requirements in Group D are again quite stringent. The triple bond is essential and it must be β to the nitrogen for inhibition of the enzyme. Replacing the propnyl radical with alkene as in AGN 1142 results in a loss of inhibitory activity (Table 1).

In vivo *studies* The structure-activity studies discussed in the previous section suggested to us that compounds AGN 1133 and AGN 1135 showed most promise as selective MAO-B inhibitors as reflected in the activity-structure dose relationship (Table 1 and Figure 4) studies. Their *in vivo* action on liver and brain MAO was investigated.

Wistar male rats (weight 200–250 g) were injected

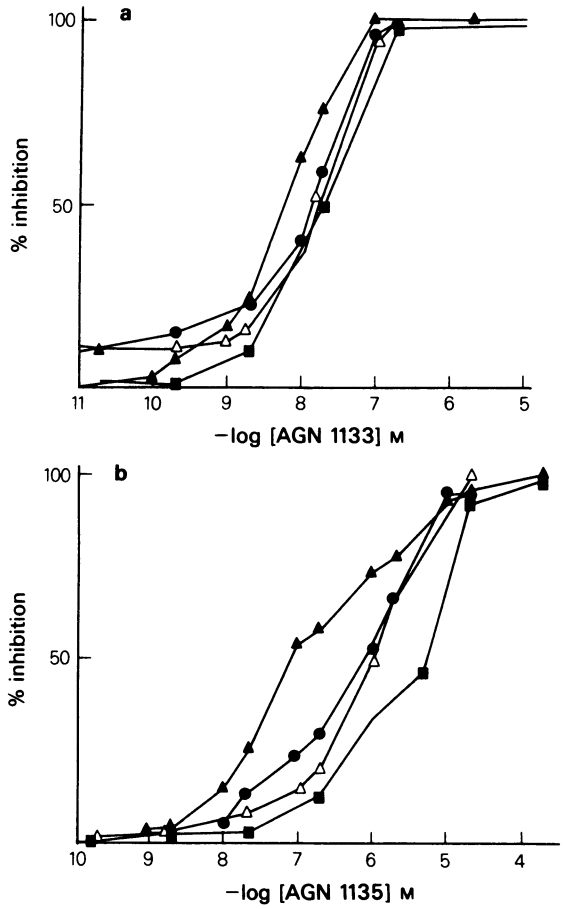


Figure 4 *In vitro* inhibition of rat brain mitochondrial monoamine oxidase (MAO) by (a) AGN 1133 and (b) AGN 1135 with [^{14}C]-5-hydroxytryptamine; [^{14}C]-phenylethlamine (■) [^{14}C]-tyramine (▲) and [^{14}C]-dopamine (△) as substrates. The enzyme preparations were preincubated with the inhibitor for 20 min before the addition of the substrates (Tipton & Youdim, 1976).

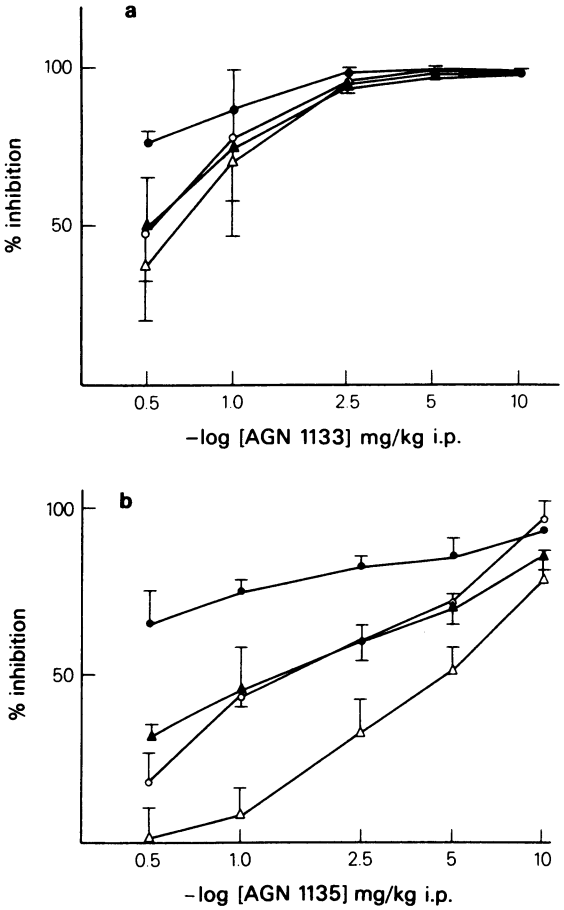


Figure 5 The *in vivo* effect of (a) AGN 1133 and (b) AGN 1135 on rat brain mitochondrial monoamine oxidase (MAO) activity with [^{14}C]-phenylethylamine [^{14}C]-5-hydroxytryptamine (△); [^{14}C]-tyramine (▲) and [^{14}C]-dopamine (○) as substrates. Brain mitochondrial MAO was prepared according to the method described by Youdim (1975) from control rats and from rats pretreated intraperitoneally with the MAO inhibitors 2 h before they were killed. The results are the mean from six individual animals; vertical lines show s.e. mean.

intraperitoneally with varying doses of the inhibitors and 2 h later they were killed by decapitation. Brains and liver were removed and MAO activity was estimated in mitochondrial preparations with [^{14}C]-dopamine, [^{14}C]-tyramine, [^{14}C]-5-HT and [^{14}C]-PEA as substrates (Tipton & Youdim, 1976). Both compounds are potent irreversible inhibitors of brain and liver MAO (Figures 5 and 6). Furthermore, they reflected the *in vitro* selectivity for MAO-B. This selectivity was more pronounced with AGN 1135 in the brain and liver than with AGN 1133 (Figures 5 and 6). At a dose of 1.0 mg/kg, AGN 1135 inactivated brain MAO-B by 80% and MAO-A by 20%, while AGN 1133 showed poorer selectivity. At 2.5 mg/kg (i.p.) the selectivity for inhibition of MAO-B was retained by AGN 1135 but lost with AGN 1133. The inhibition of tyramine and dopamine deamination was midway between that of 5-HT and PEA for both compounds (Figures 5 and 6).

Reversible inhibitors

Table 2 shows the effect of a variety of α and N substituted indoleamines on the oxidative deamination of 5-HT and PEA by rat brain mitochondrial preparation *in vitro*. With the exception of K-502 and

Table 1 Irreversible inhibition of brain mitochondrial monoamine oxidase (MAO) by acetylenic 'suicide' inactivators

Inhibitor	IC_{50} *5-HT*	IC_{50} *PEA*	*MAO inhibitory selectivity*
AGN 1133	1.6×10^{-8}	5.0×10^{-9}	B
AGN 1135	2.0×10^{-6}	8.0×10^{-8}	B
AGN 1142	$>10^{-4}$	$>10^{-4}$	—
AGN 1278	2.2×10^{-9}	6.3×10^{-10}	B
AGN 2009	1.6×10^{-4}	5.0×10^{-5}	B
AGN 2253	10^{-7}	1.6×10^{-7}	A–B
Pargyline	1.6×10^{-6}	2.2×10^{-8}	B
Des-methylpargyline	4.5×10^{-5}	6.0×10^{-7}	B
Deprenyl	8.0×10^{-7}	5.0×10^{-8}	B
M & B 9303	3.2×10^{-10}	5.6×10^{-6}	A
Clorgyline	2.0×10^{-10}	9.0×10^{-6}	A
MO 1671	1.5×10^{-9}	7.7×10^{-8}	A
K-Y 1243	$>10^{-4}$	$>10^{-4}$	—
K-Y 1249	$>10^{-4}$	$>10^{-4}$	—
K-Y 1314	$>10^{-4}$	$>10^{-4}$	—
K-Y 1315	1.1×10^{-5}	3.5×10^{-6}	B
K-Y 1349	2.3×10^{-6}	$>10^{-4}$	A
LY 51641	3.5×10^{-8}	5.0×10^{-7}	A

IC_{50}s were calculated graphically from the inhibition curves obtained with inhibitor concentrations ranging from 10^{-4} to 10^{-10} M. All inhibitors were incubated with rat brain mitochondrial preparation for 20 min before the addition of the radiolabelled substrates. The results are the mean of 2–4 determinations.

K-622 all indoleamines tested were selective reversible inhibitors of MAO type A. 5-Fluoro (Kd) and 6-fluoro-α-methyl tryptamine (K-511) were the most potent inhibitors of MAO in these series of indoles. However, 4-trifluoromethyl-α-methyltryptamine (K-622) was devoid of strong inhibitory activity. The results are not unexpected since α-methyl-amines (Biel, 1970) are reversible inhibitors of MAO. These compounds were chosen because introduction of propargyl moiety at the nitrogen atom does not alter their MAO-A inhibitory selectivity and these compounds are irreversible (Kalir, Youdim & Finberg, unpublished observations).

Discussion

The limiting factor in the use of MAO inhibitors as anti-depressants or as adjuncts to L-DOPA therapy of Parkinson's disease has been their pressor potentiating effect of indirectly acting sympathomimetic amines, e.g. tyramine on the sympathetic nervous system (Youdim, 1977). Knoll & Magyar (1972) and Knoll (1976) found that deprenyl, an irreversible 'suicide' acetylenic inhibitor of MAO type B, is lacking in this property. Indeed, studies in human volunteers challenged with high doses of oral tyramine (Elsworth *et al.*, 1978) or L-DOPA after receiving deprenyl confirm their findings (Birkmayer *et al.*, 1975; 1977; Birkmayer & Yahr, 1978). It is not known whether this is an intrinsic property of deprenyl or whether other MAO-B inhibitors share this property. In the search for other MAO type A and type B inhibitors with similar pharmacological action towards tryamine to that exhibited by deprenyl, various compounds with structural similarity to pargyline were studied. These studies allow us to speculate about the structure-activity relationship of irreversible acetylenic inhibitors and the active sites of MAO type A and type B. The requirement of an acetylenic moiety β to the nitrogen of the propargyl portion of the indane derivative inhibitors is absolute. This portion is not responsible for the selectivity of inhibitors since it has been shown that the acetylenic inhibitors pargyline, clorgyline and deprenyl bind through their acetylenic group to the N-5 position of the isoalloxazine moiety of the FAD, the MAO-A and B co-factor (Salach *et al.*, 1979). The results in Table 1 further show that substitution at the -N- position with an alkyl moiety (e.g. methyl), not only increases the inhibitor potency, but also modifies its selectivity for enzyme forms. This is well illustrated with compounds AGN 1133, AGN 1135, pargyline, desmethylpargyline, K 1314 and K 1315. A closer look at the structure of compounds examined (Figure 1) indicates that the distance between the aromatic ring and the -*N*-pro-

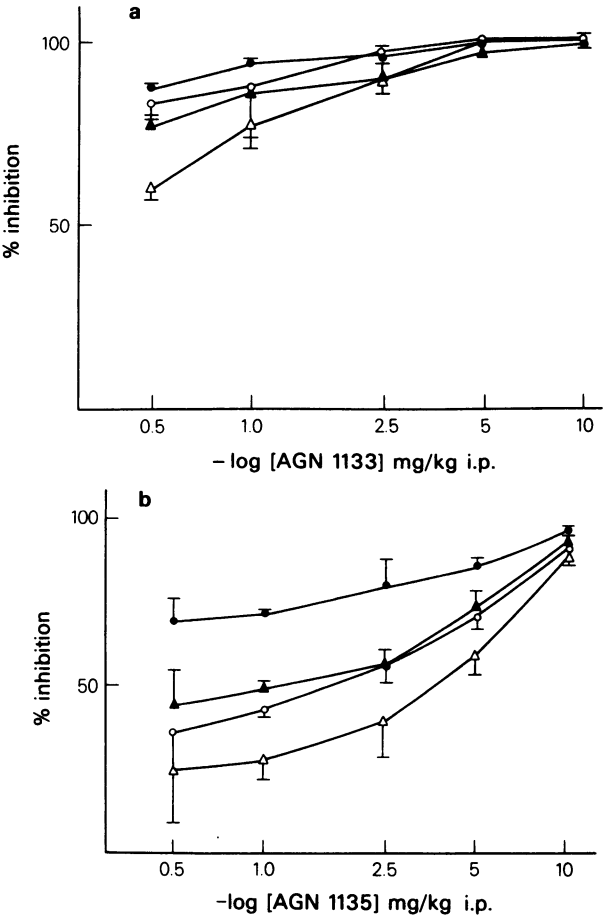


Figure 6 The *in vivo* effect of (a) AGN 1133 and (b) AGN 1135 on rat liver mitochondrial monoamine oxidase (MAO) activity with [^{14}C]-phenylethylamine (●) [^{14}C]-5-hydroxtryptamine (△); [^{14}C]-tyramine (▲) and [^{14}C]-dopamine (○) as substrates. Liver mitochondrial MAO was prepared according to the method of Hawkins (1952). The results are the mean from six individual animals; vertical lines show s.e. mean. For further details see Figure 5.

pargyl group (Figure 2) may be crucial in determining the selectivity of the inhibitors for the two enzyme forms. Compounds having a distance equivalent to 1 or 2 carbon units between the aromatic ring and -N-propargyl exhibit selectivity for the MAO type B as shown by Knoll *et al.* (1978). This is best illustrated with the compounds AGN 1133, AGN 1135, AGN 1278, pargyline, des-methylpargyline, deprenyl and K 1315 (Table 1) and U-1424 (Knoll *et al.*, 1978). Swett *et al.* (1963) observed that by changing the length of the side chain (position B in Figure 2) in pargyline, MAO inhibitory activity was modified. However, they used only 5-HT as the substrate for MAO and thus could not distinguish between inhibition of MAO-A and B.

Table 2 Inhibition of brain mitochondrial monoamine oxidase (MAO) by indoleamine derivatives

	IC_{50}		*MAO inhibitory selectivity*
Inhibitor	*5-HT*	*PEA*	
K-502	$>10^{-4}$	$>10^{-4}$	—
K-511	4.5×10^{-7}	$>10^{-4}$	A
K-622	$>10^{-4}$	$>10^{-4}$	—
Ka	5.0×10^{-6}	$>10^{-4}$	A
Kb	4.5×10^{-6}	$>10^{-4}$	A
Kc	1.0×10^{-6}	$>10^{-4}$	A
Kd	1.0×10^{-6}	$>10^{-4}$	A

IC_{50}s were calculated as indicated in Table 1 and are the mean of at least two separate determinations.

Knoll *et al.* (1978) have suggested that the active site of MAO-A is adapted for the attachment of the 5-hydroxyindole ring as in 5-HT and the indole group is pre-requisite for the selective inhibition of MAO-A. Although this may be the case with 5-HT and the selective MAO type A reversible inhibitors such as the indoleamine derivatives (Table 2) and tetrahydro-β-carbolines (Bucholtz & Boggan, 1976; Oppenheim, Youdim, Goldstein & Hefez, 1978; Youdim & Oppenheim, 1981), it is difficult to reconcile the results with the structure of clorgyline, M & B 9303, MO 1671, K-Y 1349 (Table 1) and Lilly 51461 (Fuller, Slater & Mill, 1979). A simpler explanation may be that for the MAO type A active site to be occupied with an inhibitor selectivity, the distance between the aromatic portion and nitrogen atom must be equivalent to at least 3 carbon bond units. This hypothesis is well illustrated with the reversible inhibitors, α and N substituted (Table 2) tryptamines and tetrahydro-β-carbolines (Oppenheim *et al.*, 1978) and the irreversible inhibitors, clorgyline, M & B 9303, MO 1671 and K-Y 1349 (Table 1), Lilly 51461 and Lilly 54930 (Fuller *et al.*, 1979). The latter two compounds are selective irreversible inhibitors of type A MAO having a *N*-cyclopropylamine structure in which O-CH_2-CH_2-moieties occupy the distance between the aromatic ring and *N*-cyclopropylamine. The position that phenoxy occupies in these compounds is similar to that in clorgyline. The presence of phenoxy *per se* does not play a crucial role in the inhibitor selectivity since replacement of this oxygen by sulphur as in M & B 9303 and its absence in K-Y 1349 does not modify the drug's inhibitory selectivity for type A MAO (see Figure 7).

It can safely be said that the differences in the inhibitor sensitivity of MAO type A and type B do not reside in the mode of covalent attachment of (a) selective inhibitors to the flavin prosthetic group or (b) the flavin prosthetic group to the MAO apo-

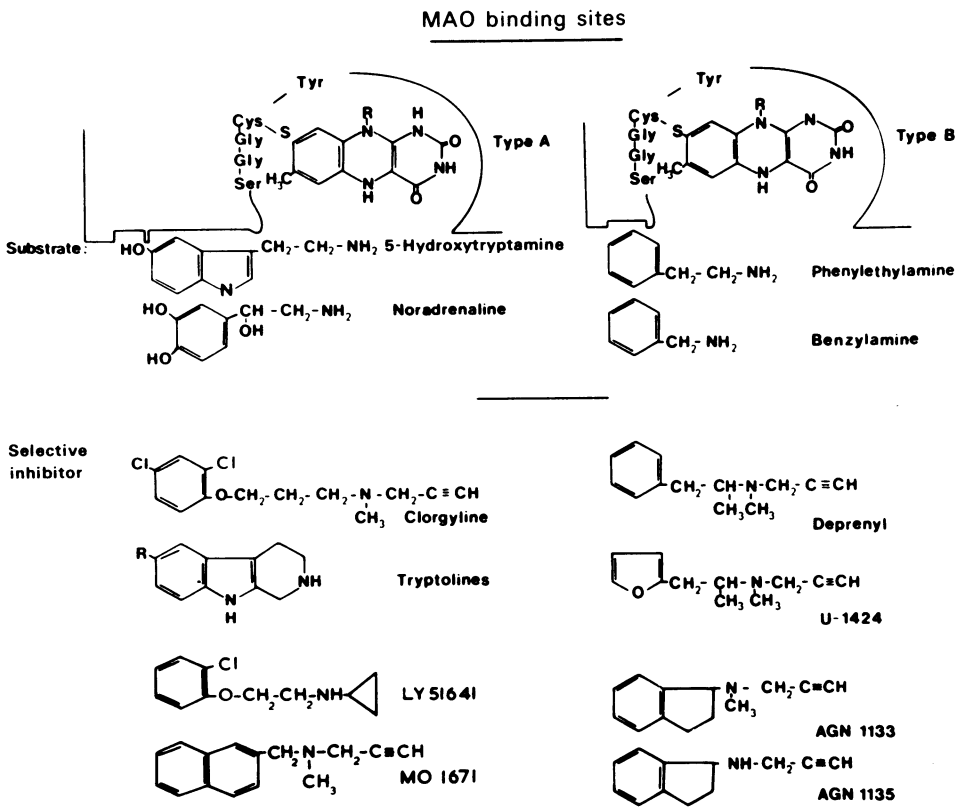


Figure 7 The schematic representation of the active sites of monoamine oxidase (MAO) type A and B, the binding sites of their substrates and selective inhibitors. This scheme is constructed from the data obtained in the present study, those of Houslay & Tipton (1974), Oppenheim *et al.* (1978), Knoll (1979), Salach *et al.* (1979) and Youdim & Finberg (1981).

enzyme. In both enzyme forms the flavin is covalently bound through its 8-α-position through the thioether of cysteine (Salach *et al.*, 1979; Salach & Detmer, 1979). Selective A (clorgyline) and selective B (deprenyl) inhibitors bind to the N-5 position of FAD (Figure 7) (Salach *et al.*, 1979). The selectivity of inhibitors may thus reside in the protein configuration at the vicinity of the active site to which the aromatic portion of inhibitor may attach itself noncavalently. One explanation as to why the selectivity of MAO type B inhibitors for the B enzyme is not as pronounced as that of MAO-A inhibitors for MAO type A (see Youdim & Finberg, 1981, and Tables 1 and 2) is that the access of MAO-A inhibitors to the smaller MAO-B active site may be more restricted than that of the MAO-B inhibitors to the MAO-A active site (Figure 7).

The compounds so far described show that with a slight modification in their structure, the biochemical and pharmacological action of MAO inhibitors can be altered. The acetylenic compounds, AGN 1133 and AGN 1135, which are conformationally restricted analogues of pargyline are of great interest. Both compounds are selective irreversible inactivators of type B MAO; However, AGN 1135 (the *N*-desmethylated derivative) shows greater selectivity *in vitro* as well *in vivo*. The differences in their pharmacological properties are even greater (see Finberg *et al.*, 1981); while AGN 1133 potentiates the effects of tyramine in the rat isolated vas deferens and *in vivo*, AGN 1135 possesses a tyramine antagonistic effect, similar to that described for deprenyl. However, unlike deprenyl (Green & Youdim, 1975), high doses of AGN 1135 (10 mg/kg) do not produce CNS or cardiovascular effects (Finberg *et al.*, 1981). The elimination of the latter side effect may be related to the presence of the indane moiety in the structure of AGN 1133 and AGN 1135, since deprenyl is synthesized from its parent molecule, methamphetamine (Knoll *et al.*, 1978), and is metabolized to methamphetamine and amphetamine *in vivo* (Reynolds, Elsworth, Blau, Sandler, Lees & Stern, 1978).

This work was supported by a grant from the National Council for Research and Development, Prime Minister's Office (Jerusalem) and Technion Research and Development (Haifa).

References

BIEL, J. (1970). Structure-activity relationships of amphetamine and derivatives. In *Amphetamines and Related Compounds*. ed. Costa, E. & Garattini, S. pp. 3–20. New York: Raven Press.

BIRKMAYER, W. & YAHR, M. (1978). *Deprenyl, an Inhibitor of MAO Type B in the Treatment of Parkinsonism*. Wien: Springer-Verlag.

BIRKMAYER, W., RIEDERER, P., AMBROZI, L. & YOUDIM, M.B.H. (1977). Implications of combined treatment with "Madopar" and L-deprenyl in Parkinson's disese: A long term study. *Lancet*, **i**, 439–443.

BIRKMAYER, W., RIEDERER, P., YOUDIM, M.B.H. & LINAUER, W. (1975). Potentiation of the anti-akinetic effect of L-dopa treatment by an inhibitor of MAO-B, deprenil. *J. Neural Transm.*, **36**, 303–326.

BUCHHOLTZ, N.S. & BOGGAN, W. (1976). Effect of tetrahydro-β-carboline on monoamine oxidase and serotonin uptake in mouse brain. *Biochem. Pharmac.*, **25**, 1271–1274.

ELSWORTH, J.D., GLOVER, V., REYNOLDS, G.P., SANDLER, M., LEES, A.J., PHUAPRADIT, P., SHAW, K.M., STERN, G.M. & KULMAR, P. (1978). Deprenyl administration in man: A selective MAO-B inhibitor without the 'cheese' effect. *Psychopharmacologia*, **57**, 33–38.

FINBERG, J.P., SABBAGH, A. & YOUDIM, M.B.H. (1980). Pharmacology of selective acetylenic 'suicide inhibitors' of monoamine oxidase. In *Enzymes and Neurotransmitters in Mental Disease*. ed. Usdin, E. Sourkes, T.L. & Youdim, M.B.H. pp. 205–219. Chichester: Wiley.

FINBERG, J.P.M., TENNE, M. & YOUDIM, M.B.H. (1981). Tyramine antagonistic properties of AGN 1135—an irreversible inhibitor of monoamine oxidase type B. *Br. J. Pharmac.*, **73**, 65–74.

FOWLER, C.J., CALLINGHAM, B.A., MANTLE, T.J. & TIPTON, K.F. (1978). Monoamine oxidase A and B: a useful concept. *Biochem. Pharmac.*, **27**, 97–101.

FULLER, R.W., SLATER, I.H. & MILL, J. (1979). The development of N-cyclopropylarylalkylamines as monoamine oxidase inhibitors. In *Monoamine Oxidase; Structure, Function and Altered Functions*, Ed. Singer, T.P., Von Korf, R.W. & Murphy, D.L. pp. 317–324. New York: Academic Press.

GLOVER, V., SANDLER, M., OWEN, F. & RILEY, G.J. (1977). Dopamine is a monoamine oxidase-B substrate in man. *Nature*, **265**, 80–81.

GREEN, A.R. & YOUDIM, M.B.H. (1975). Effects of monoamine oxidase inhibition by clorgyline, deprenil and tranylcypramine on 5-hydroxytryptamine concentrations in rat brain and hyperactivity following subsequent tryptophan administration. *Br. J. Pharmac.*, **55**, 415–422.

HAWKINS, J. (1952). Subcellular fractionation of mitochondrial monoamine oxidase. *Biochem. J.*, **52**, 577–562.

HOUSLAY, M.D. & TIPTON, K.F. (1974). A kinetic evaluation of monoamine oxidase activity in rat liver mitochondrial outer membrane. *Biochem. J.*, **139**, 645–652.

JOHNSTON, J.P. (1968). Some observation upon a new inhibitor of monoamine oxidase in brain tissue. *Biochem. Pharmac.*, **17**, 1285–1297.

KALIR, A.S. & BALDERMAN, D. (1968). Improved synthesis of fluoroindoles. *Israel J. Chem.*, **6**, 927–932.

KALIR, A.S. & SZARA, S. (1963). Synthesis and pharmacological activity of fluoroinated tryptamine derivatives. *J. med. Chem.*, **6**, 716–719.

KALIR, A.S. & SZARA, S. (1966). Synthesis and pharmacological activity of alkylated tryptamines. *J. med. Chem.*, **9**, 341–344.

KALIR, A.S., PELAH, Z. & BALDERMAN, D. (1967). 6-trifluoromethyl-tryptamines. *Israel J. Chem.*, **5**, 101–106.

KNOLL, J. (1976). Analysis of the pharmacological effects of selective monoamine oxidase inhibitors. In *Monoamine Oxidase and Its Inhibition*. Ciba Foundation Symposium, No. 39. ed. Wolstenholme, G.E.W. & Knight, J. pp. 135–162. Amsterdam: Elsevier.

KNOLL, J. (1979). (−) Deprenyl—the MAO inhibitor without the 'cheese' effect. *Trends in Neurosciences*, **2**, 111–114.

KNOLL, J. & MAGYAR, K. (1972). Some puzzling pharmacological effects of monoamine oxidase inhibitors. *Adv. Biochem. Psychopharmac.*, **5**, 393–408.

KNOLL, J., ECSERY, Z., MAGYAR, K. & SATORY, E. (1978). Novel (−) deprenyl-derived selective inhibitors of B-type monoamine oxidase. The relation of structure to their action. *Biochem. Pharmac.*, **27**, 1739–1747.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the folin phenol reagent. *J. biol. Chem.*, **193**, 265–275.

MANN, J. & GERSHON, S. (1980). *L*-Deprenyl, a selective monoamine oxidase type B inhibitor in endogenous depression. *Life Sci.*, **26**, 877–882.

MAYCOCK, A.L., ABELES, R.H., SALACH, J.I. & SINGER, T.P. (1976). The action of acetylenic inhibitors on mitochondrial monoamine oxidase: Structure of the flavin site in the inhibited enzyme. In *Monoamine Oxidase and Its Inhibition*. Ciba Foundation Symposium, No. 39. ed. Wolstenholme, G.E.W. & Knight, J. pp. 33–47. Amsterdam: Elsevier.

MENDLEWICZ, J. & YOUDIM, M.B.H. (1978). Antidepressant potentiation of 5-hydroxytryptophan by L-deprenyl, an MAO "type B" inhibitor. *J. Neural Transm.*, **43**, 279–286.

OPPENHEIM, B., YOUDIM, M.B.H., GOLDSTEIN, S. & HEFEZ, A. (1978). Human platelets as a neuronal model for study of the pharmacological activity of tryptolines and neuroleptics. *Israel J. med. Sci.*, **14**, 1096–1097.

PARE, C.M.B. (1976). Introduction to clinical aspects of monoamine oxidase inhibitors in the treatment of depression. In *Monoamine Oxidase and Its Inhibition*. Ciba Foundation Symposium, No. 39, ed. Wolstenholme, G.E.W. & Knight, J. pp. 271–280. Amsterdam: Elsevier.

REYNOLDS, G.P., ELSWORTH, J.D., BLAU, K., SANDLER, M., LEES, A.J. & STERN, G.M. (1978). Deprenyl is metabolized to methamphetamine and amphetamine in man. *Br. J. clin. Pharmac.*, **6**, 542–544.

RIEDERER, P., YOUDIM, M.B.H., RAUSCH, W.D., BIRKMAYER, W., JELLINGER, K. & SEEMANN, D. (1978). On the mode of action of L-deprenyl in the human central nervous system. *J. Neural Transm.*, **43**, 217–226.

SALACH, J.I. & DETMER, K. (1980). Chemical characterization of monoamine oxidase A from human placental mitochondria. In *Monoamine Oxidase; Structure, Function and Altered Functions*. ed. Singer, T.P., Von Korf, R.W. & Murphy, D.L. pp. 121–128. New York: Academic Press.

SALACH, J.I., DETMER, K. & YOUDIM, M.B.H. (1979). The reaction of bovine and rat liver monoamine oxidase with ^{14}(C)-clorgyline and ^{14}(C)-deprenyl. *Mol. Pharmac.*, **16**, 234–241.

SQUIRES, R.F. (1972). Multiple forms of monoamine oxidase in intact mitochondria as characterized by selective inhibitors and thermal stability: a comparison of eight mammalian tissues. *Adv. Biochem. Psychopharmac.*, **5**, 355–370.

SWETT, L.R., MARTIN, W.B., TAYLOR, J.D., EVERETT, G.M., WYKES, A.A. & GLADISH, Y.C. (1963). Structure-activity relations in the pargyline series. *Ann. N.Y. Acad. Sci.*, **107**, 891–899.

TIPTON, K.F. & YOUDIM, M.B.H. (1976). Assay of monoamine oxidase. In *Monoamine Oxidase and Its Inhibition*. ed. Wolstenholme, G.E.W. & KNIGHT, J. Ciba Foundation Symposium, No. 39. pp. 393–403. Amsterdam, Elsevier.

YAHR, M.D. (1978). Overview of present day treatment of Parkinson's disease. *J. Neural Transm.*, **43**, 227–238.

YANG, H-Y. & NEFF, N.H. (1973). β-phenylethylamine: a specific substrate for type B monoamine oxidase of brain. *J. Pharmac. exp. Ther.*, **187**, 363–371.

YOUDIM, M.B.H. (1975). Assay and purification of brain monoamine oxidase. In *Research Methods in Neurochemistry*. ed. Marks, N. & Rodnight, R.R. pp. 167–210. New York: Plenum.

YOUDIM, M.B.H. (1976). Rat liver mitochondrial monoamine oxidase – an iron requiring flavorprotein. In *Flavins and Flavoproteins*. ed. Singer, T.P. pp. 593–604. Amsterdam: Elsevier.

YOUDIM, M.B.H. (1977). Tyramine and psychiatric disorders. In *Neuroregulators and Psychiatric Disorders*. ed. Usdin, E., Hamburg, D. & Barch, J. pp. 57–67. New York: Oxford University Press.

YOUDIM, M.B.H. & FINBERG, J. (1981). The sites of action of selective monoamine oxidase inhibitors. In *Drug Receptors in the Central Nervous System*. ed. Littauer, L. & Dudai, J. Chichester: Wiley (in press).

YOUDIM, M.B.H. & OPPENHEIM, B. (1981). The effect of 1, 2, 3, 4-tetrahydro-β-carbolines on monoamine metabolism in the human platelet and platelet aggregation. *Neuroscience* (in press).

YOUDIM, M.B.H. & PAYKEL, E.S. (1981). *Monoamine Oxidase Inhibitors: The State of the Art*. Chichester: Wiley.

(Received June 13, 1980. Revised October 23, 1980)