**Introduction**

The discovery that inhibitors of monoamine oxidase (EC 1.4.3.4.; MAO) are antidepressants (see [1] for a review) has resulted in the synthesis of large numbers of inhibitors, several of which have proved to be valuable in clinical use. Such work was given added impetus when it was recognized that there are two monoamine oxidases in most mammalian tissues, MAO-A and MAO-B, with different substrate and inhibitor specificities. 5-Hydroxytryptamine (5-HT; also known as serotonin) is a preferred substrate for MAO-A and the trace amine 2-phenethylamine is a preferred substrate for MAO-B. Both enzymes from human brain catalyse the oxidation of dopamine, noradrenaline and tyramine (see [2,3] for reviews). Inhibitors of MAO-A have been shown to be effective antidepressants (see [1]), whereas inhibitors of MAO-B appear to be of value in the treatment of Parkinson’s disease, either in combination with l-dopa [4] or, perhaps, alone [5]. It has also been suggested that at least some inhibitors of MAO-B may be capable of either protecting or rescuing neurons from potentially lethal damage (see [6,7]).

A particular problem with the use of MAO inhibitors as antidepressants has been that they can give rise to strong hypertensive responses following the ingestion of some foods and beverages. This was shown to be a result of the relatively high concentrations of amines, often tyramine, in the ingested material (see [1,8–10]). Because some cheeses are particularly rich in tyramine, this effect has become known as the ‘cheese reaction’. However, many other foods and beverages contain amines which can interact in this way, and because of the nature of some of the foods involved and the lack of any legislation controlling the tyramine contents, the quantities present can be extremely variable (see [8–10]). Although tyramine is a good substrate for both forms of the enzyme, it is only those inhibitors that affect MAO-A that give rise to this reaction. This is because MAO-A predominates in the human intestine and stomach, which are the first lines of defence against ingested amines (see [11]). Since the crystal structures of the MAOs have yet to be determined, information for the design of more effective inhibitors has to be inferred from the structures and behaviour of known inhibitory compounds. Some of the factors that may be of value in such work are considered below.

**Selective irreversible inhibitors**

Many of the early MAO inhibitory antidepressants were hydrazine or cyclopropylamine derivatives which showed little or no discrimination between the two MAOs [1]. The acetylenic MAO inhibitors clorgyline and (−)-deprenyl (see Figure 1), however, exhibit high degrees of selectivity towards MAO-A and MAO-B respectively. They have been found to act as mechanism-based inhibitors of MAO: the compound first forms a non-covalent complex with the active site of the enzyme and subsequent reaction within that complex leads to the generation of a reactive species which reacts with the enzyme to form the irreversibly inhibited species. Inhibitors of this type can show a high degree of specificity towards a target enzyme because the generation of the effective inhibitory

![Figure 1](image-url)

**Abbreviations used:** MAO, monoamine oxidase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

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species from an essentially unreactive compound involves the catalytic function of the enzyme.

The interaction between MAO (E) and the mechanism-based inhibitor (I) can be represented by the following minimum reaction mechanism:

\[ E + I \xrightarrow{k_+} E \cdot I \xrightarrow{k_-} E \cdot I \]

where \( E \cdot I \) represents the non-covalent complex, analogous to the reversible complex formed between an enzyme and a competitive inhibitor or an enzyme-substrate complex, and \( E \cdot I \) represents the covalent adduct between enzyme and inhibitor. The potency of inhibition will be governed by two factors, the affinity of the inhibitor for non-covalent binding to the enzyme and the rate of reaction within this complex to form the irreversibly inhibited species. Thus the degree of selectivity of an inhibitor towards one or more of the MAOs will depend on the relative magnitudes of these two processes. Studies with a series of acetylenic amine derivatives \([12-14]\) have shown that the degree of selectivity exhibited by these compounds towards MAO-A or MAO-B can derive either from a greater affinity for non-covalent binding to that form of the enzyme or from a faster rate of reaction within the non-covalent complex to give the irreversibly inhibited species. A combination of both these factors will determine the overall selectivity of an inhibitor, although in several cases these two processes conflict, rather than reinforce, each other.

'Schizophrenic substrates'

Since mechanism-based inhibitors behave like substrates in binding to the active site of the enzyme and being converted to the reactive species through a process involving the normal catalytic process of the enzyme, it is perhaps not surprising that it is possible for the reactive species to break down to form product. In such cases, the formation of product and the mechanism-based inhibition of the enzyme will be competing reactions according to the general scheme:

\[ E + I \xrightarrow{k_+} E \cdot I \xrightarrow{k_-} E \cdot I^* \xrightarrow{k_{1-2}} E \cdot I \]

\[ E + \text{Products} \]

where \( E \cdot I^* \) represents an activated complex. In the case of the inhibition of MAO by clorgyline and \((-\)-deprenyl, there is no detectable formation of products during the inhibitory process. However, the inhibitors phenelzine and MD 780236 (see Figure 2) have both been shown also to be substrates for the enzyme as well as mechanism-based inhibitors \([15,16]\). Furthermore, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and milacemide, which are both substrates for the enzyme (see Figure 2), are also time-dependent inhibitors of MAO-B \([17,18]\).

Clearly the behaviour of such 'schizophrenic substrates' that act as mechanism-based irreversible inhibitors as well as substrates of MAO may be complicated by the products of the reaction having physiological effects that are different from those of the starting material. In the case of MPTP, it is the final product of the MAO-catalysed reaction, 1-methyl-4-phenylpyridinium (MPP\(^+\)), that is the effective dopaminergic neurotoxin that gives rise to a condition resembling Parkinson's disease (for review, see \([19]\)), whereas the product of milacemide oxidation by MAO-B has been suggested to be important for its anticonvulsant properties \([20]\, \text{but see}\ [21]\).

The partition ratio, which corresponds to the number of moles of product formed per mole of enzyme at complete inhibition \((k_{1-2}/k_{-2})\), provides a convenient measure of the relative potencies of a compound as an enzyme substrate and inhibitor. Although the substrate specificities of the MAOs appear to be quite similar in many species that have been examined, there appear to be important differences in their behaviour with some of these 'schizophrenic substrates'. Thus the partition ratio of MAO-B from rat liver with MPTP is ~10-fold higher than with the enzyme from ox liver \([22]\). Furthermore, MAO-B from rat liver has been shown to be much more sensitive than the enzyme from ox liver to time-dependent inhibition by
milacemide, and there are also differences in the kinetic parameters of these enzymes and those from rodents in the oxidation of, and inhibition by, a number of derivatives of milacemide (E. M. O'Brien, P. Dostert and K. F. Tipton, unpublished work). Since rodents have been used as models to assess the anticonvulsant behaviour of milacemide (see [21]), such results indicate that it cannot be assumed that such compounds will behave in the same way in the human.

Some primary amine substrates, such as 2-phenethylamine, appear also to act as time-dependent inhibitors of the enzyme, but in these cases it appears that the inhibition is slowly reversible [23].

Reversible inhibitors
A variety of compounds have been shown to be more or less selective inhibitors of one of the MAOs (see [1,2,24]). With several MAO substrates, the substitution of a hydrogen by a methyl group at the carbon in the α position with respect to the nitrogen atom has resulted in the formation of MAO-A-selective reversible inhibitors [2,25,26]. Studies with selectivity deuterated substrates have shown that the reaction catalysed by both forms of MAO involves the abstraction of the pro-(R)-hydrogen from the α-carbon of primary amine substrates [27]. The (S) enantiomer of amphetamine, in which the methyl group occupies the position of the non-abstracted hydron in the parent substrate (2-phenethylamine), has been shown to be a more potent inhibitor of MAO-A than the (R) enantiomer, there being little difference between the potencies of the two enantiomers as inhibitors of MAO-B [2]. However, the (R) enantiomer of α-methylbenzylamine, in which the methyl group is in the position of the abstracted hydron in benzylamine, was reported to be a more potent inhibitor of both MAO-A and -B from rat brain than the (S) enantiomer but the enantiomers of α-methylbenzylamine were reported to have similar Ki values as inhibitors of MAO-B from ox liver [2]. Smith et al. [28] showed that the enantiomeric preference of a series of α-substituted aryalkylamines as reversible inhibitors of MAO-B is variable; some of the compounds studied showed little difference between the (R) and (S) enantiomers, and (R)-1,2,3,4-tetrahydro-1-naphthylamine was shown to be 150-fold more potent than the (S) enantiomer. In the case of the MAO-B selective substrate milacemide, the α-methyl derivative shows little selectivity as a competitive inhibitor [21]. Stereoselective effects of substitution have also been reported for oxazolidinone derivatives as inhibitors of MAO, with the greatest potency and selectivity being seen when the methyl group of the α-carbon occupied the position of the non-abstracted hydron (see [2,29]).

As with substrates, appropriate substitution of a methyl group in acetylenic inhibitors may prevent the transformation that leads to irreversible inhibition. Substitution of an α-methyl group into clorgyline, to yield the (R)+(+) and (S)(−) enantiomers of N-[(−)-3-(2,4-dichlorophenoxy)propyl]-N-methyl-3-butyln-2-amine (3-methylclorgyline) (see Figure 1), resulted in reversible competitive inhibitors of MAO-A and -B. Thus the α-methyl substitution did not prevent non-covalent binding of the inhibitors to the active sites, but it did prevent these compounds from reacting within that complex to form covalent adducts with the enzymes. The substitution of the methyl group resulted in a decreased affinity, compared with clorgyline, for non-covalent binding to MAO-A, with the (S) enantiomer having the lower affinity. In contrast, the substitutions increased the affinity of the (S) enantiomer for MAO-B. These effects resulted in the high selectivity of the parent compound, clorgyline, towards MAO-A being lost. The (R) enantiomer showed a small degree of selectivity towards the A-form of the enzyme, whereas the (S) enantiomer had a higher affinity for the B-form [30].

Thus it appears that resolution of the enantiomers of inhibitors bearing an α-methyl substitution may be of value in increasing potency and selectivity, but that the effects with a new compound cannot yet be predicted with certainty.

Choice of inhibitor type
In the case of reversible inhibitors that are competitive with respect to the amine substrate, increasing concentrations of tyramine should displace the inhibitor from the enzyme, allowing oxidative deamination to occur (see [11]). Thus, the 'cheese reaction' will be much less with such inhibitors than with their irreversible counterparts. Such a conclusion is supported, for example, by studies on the pressor response to oral tyramine of patients treated with
the competitive MAO-A inhibitors brofaromine, moclobemide and befloxetine [31,32].

The effects of reversible inhibitors are of shorter duration than those of the irreversible type. The rate of recovery from the effects of a reversible inhibitor will be dependent on the rate at which it is eliminated from the tissues, since removal of the free drug will result in it dissociating from the enzyme. For example, the rates of recovery of MAO-A activity in the rat after a single dose of brofaromine correspond to half-lives \( t_{1/2} \) of \( \sim 12 \) and \( 8 \) h in liver and brain respectively [33]. In contrast, the rates of recovery from the effects of an irreversible inhibitor will depend on the rate of turnover of the enzyme itself. This has been shown to vary between species and tissues (for reviews, see [1–3]). MAO-A and -B in rat liver have \( t_{1/2} \) in the range 2.5–3.5 days, whereas the \( t_{1/2} \) in rat brain was found to be \( \sim 10–13 \) days. In rat intestine, rates of turnover of the two forms of MAO were found to be different, with the \( t_{1/2} \) values of 2.2 and 7.5 days being reported for MAO-A and -B respectively, and in heart from the same species the \( t_{1/2} \) of \( \sim 30 \) days has been reported for the primate (baboon) brain enzyme [34].

Since the selectivities of MAO inhibitors are not absolute, care is necessary to ensure that the cumulative effects of irreversible inhibitors do not lead to loss of selectivity. However, in the case of \((\sim)\)-deprenyl it appears that the correct dosage will result in effective inhibition of MAO-B without any significant effects on MAO-A over extended periods of treatment (see [6]).

There have been very few useful reversible MAO-B-selective inhibitors reported in the literature. Studies on the possible metabolites of the MAO-A inhibitor moclobemide have revealed Ro 19-6327 (Lazeberamide) to be a potent and highly selective reversible inhibitor of MAO-B [35]. However, since the use of irreversible inhibitors of this enzyme, such as \((\sim)\)-deprenyl, appears to be without undue adverse side-effects or dietary interactions [6], the advantage of reversible inhibitors is not so well established.

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Introduction

Monoamine oxidase (MAO) occurs in two forms, A and B, and is a tightly bound component of the outer membrane of mitochondria. The two forms differ in substrate selectivity, although some substrates are oxidized by both forms. In man, MAO-A metabolizes primarily noradrenaline and 5-hydroxytryptamine, whereas MAO-B preferentially oxidizes 3,4-dihydroxyphenethylamine (dopamine) [1]. Inhibitors of MAO-B have been used as adjuncts to the treatment of Parkinson’s disease with L-dopa [2,3]. For instance, the MAO-B inhibitor l-deprenyl, used in combination with l-dopa and a peripheral DOPA decarboxylase inhibitor like benserazide or carbidopa, significantly prolongs the life expectancy of Parkinsonian patients [4,5]. Relevant to this observation is the finding that l-deprenyl administration in mice and monkeys blocks the neurotoxic effects of L-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) which produces a Parkinson’s-like syndrome in man [6]. An MPTP-like neurotoxin might be the cause of idiopathic Parkinson’s disease [7] and more recent studies showed that l-deprenyl monotherapy delayed the onset of disability in Parkinsonian patients [8,9].

Materials and methods

Chemicals

The following compounds were purchased: [7-14C]benzylamine hydrochloride (55 mCi/mmol) and 5-hydroxy[side-chain-2-14C]tryptamine creatinine sulphate (57 mCi/mmol) (Amersham Int.); β[ethyl-1-14C]phenylethylamine hydrochloride (50 mCi/mmol) (NEN Research Products); benzylamine (distilled) (Aldrich); β-phenylethylamine hydrochloride, 5-hydroxytryptamine creatinine sulphate, tyramine hydrochloride, MPTP hydrochloride, 2-mercaptoethanol, putrescine dihydrochloride and hog kidney diamine oxidase (Sigma); horseradish peroxidase (Boehringer Mannheim) and homovanillic acid (HVA; Calbiochem).

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Biochemical and pharmacological evaluation of 2,4-difluorobenzylidimethylsilylmethanamine, a new highly selective inhibitor of monoamine oxidase-B

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In man and animals, l-deprenyl produces amphetamine-like side effects [10] and its selectivity for MAO-B versus MAO-A is limited [11]. In order to avoid the ‘cheese effect’, a facilitated hypertensive response to tyramine due to inhibition of MAO-A, the search for more selective inhibitors of MAO-B has been continued [12-14].

Recently, we explored the potential of a series of benzylidimethylsilylmethanamines as selective enzyme-activated irreversible inhibitors of rat brain MAO-B in vitro [14]. Here we show that such compounds, and more particularly 2,4-difluorobenzylidimethylsilylmethanamine, not only have an extreme selectivity for MAO-B relative to a number of other amine oxidases in vitro but also are effective in vivo without apparent side effects.

Abbreviations used: DOPAC, 3,4-dihydroxyphenylacetic acid; HR, heart rate; HVA, homovanillic acid; i.p. intraperitoneal; i.v. intravenous; MAO, monoamine oxidase; MAP, mean arterial pressure; MPTP, L-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

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