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(*Z*)-2-(2,4-Dichlorophenoxy)methyl-3-fluoroallylamine (MDL 72638): a clorgyline analogue with surprising selectivity for monoamine oxidase type B

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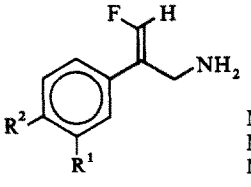
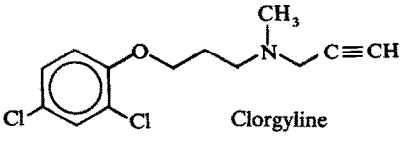
We have recently reported [1–4] that derivatives of 2-aryl-3-fluoroallylamine and 2-arylalkyl-3-fluoroallylamine are potent enzyme-activated inhibitors of monoamine oxidase (MAO; EC 1.4.3.4). These substances generally show selectivity for the B-form of the enzyme. For example the 3,4-dimethoxyphenethylamine derivative MDL 72145 is approximately 100-fold selective in this respect. A few examples, notably the *meta*-tyramine derivative MDL 72392 and the dopamine analogue MDL 72394, nevertheless, did show modest selectivity (up to 10-fold) for the A-form of the enzyme. In general, the selectivity displayed by these inhibitors reflects the substance specificities of the corresponding phenethylamine derivatives [2].

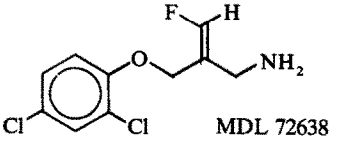


MDL 72145 $R^1 = R^2 = OCH_3$
MDL 72392 $R^1 = OH$; $R^2 = H$
MDL 72394 $R^1 = R^2 = OH$

Of the many studies aimed at developing an understanding of the structure–activity relationship regarding substrate and inhibitor specificities for one form of the enzyme or the other, the most accepted conclusion to date is that selectivity is related in part to the distance between the aromatic ring and the nitrogen atom [5, 6]. The type A selectivity of clorgyline has been attributed to the 4-atom linkage between the dichlorobenzene ring and the nitrogen atom. Since clorgyline is probably the most selective type A, irreversible inhibitor known, we decided to prepare a structurally related fluoroallylamine in an attempt to improve the modest A-selectivity seen in this series. Thus, the analogue MDL 72638 [(*Z*)-2-(2,4-dichlorophenoxy)methyl-3-fluoroallylamine] has been synthesized [7]. Although the exact conformation of clorgyline in the active site of MAO is not known, the conformational flexibility of both substances is similar so that, whatever the aromatic ring–nitrogen atom distance clorgyline adopts, MDL 72638 can more or less adopt the same. The average aromatic ring–nitrogen distance for the two molecules existing in a variety of conformations would appear to be very similar from an examination of Dreiding models.



Clorgyline



MDL 72638

We report in this communication that, despite this close structural relationship to clorgyline, MDL 72638 is a potent, very selective inhibitor of the B form of MAO.

Methods and results

When partially purified rat brain mitochondrial MAO was incubated [4] with varying concentrations of MDL 72638, time-dependent inhibition was observed. With either 5-hydroxytryptamine (5-HT; Type A) or phenethylamine (PEA; Type B) as substrate, pseudo first order kinetics were observed for at least three half-lives. The minimum half-life (τ_{50}) at saturating conditions and the apparent dissociation constant (K_i), calculated according to the method of Kitz and Wilson [8], were 8.9 min and 1.75 μM and 2.9 min and 0.088 μM for the A and B forms of MAO, respectively (Fig. 1 and Table 1). The selectivity for the B form of the enzyme, approximated from the ratio of concentrations of MDL 72638 required to inhibit both forms of the enzyme at the same rate, was found to be 100.

Discussion

The difference in MAO inhibitory selectivity between (*Z*) - 2 - (2,4 - dichlorphenoxy)methyl - 3 - fluoroallylamine (MDL 72638) and clorgyline is quite remarkable considering the close structural similarity between the two compounds. The K_i values of MDL 72638 for MAO A and B, respectively, are 30 times higher and 660 times lower than those of clorgyline [9]. Obviously, the relationship between the aromatic ring and nitrogen atom is in itself not a dominant feature in bestowing selectivity for one form of the enzyme or the other, at least when inhibitors from different structural classes of compounds are concerned. Within a particular series of inhibitors, however, this factor may still play a role. An explanation for the observed disparity in selectivity in the present case cannot be attempted with confidence until the mechanism of inactivation of MAO by MDL 72638 and related compounds has been fully elaborated. At first sight, there would appear to be three possible explanations. If both MDL 72638 and clorgyline were, after enzymic activation, to alkylate the same enzyme or cofactor group the selectivity could be attributed to a difference in the binding step to the two enzyme forms. This situation would be similar to that of clorgyline and L-deprenyl which are structurally related

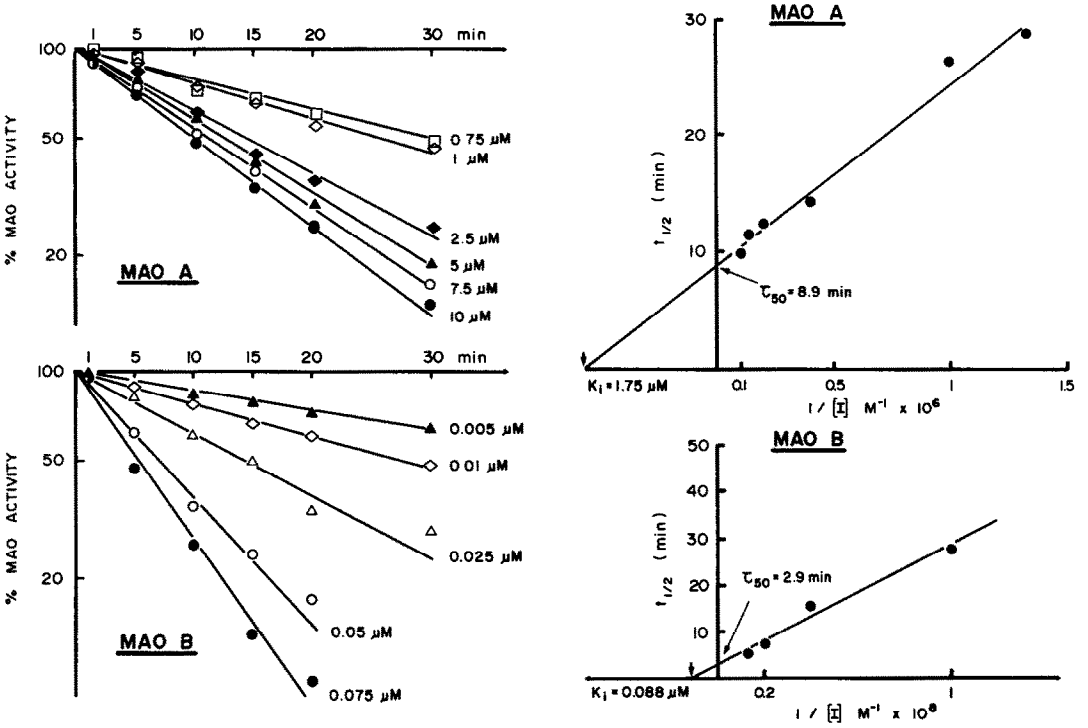


Fig. 1. Time-dependent inhibition of rat-brain mitochondrial MAO by MDL 72638 at 37°. Various concentrations of MDL 72638 were incubated for different times according to the published procedure [4]. Remaining enzyme activity was determined with either [^{14}C]-5HT for MAO type A or [^{14}C]-PEA for type B. The plot of t_i against 1/[I] and calculation of the τ_o and K_i values followed the description of Kitz and Wilson [8].

Table 1. Kinetic constants for the inhibition of MAO Type A and Type B by MDL 72638 and clorgyline

Inhibitor	5-HT		PEA	
	K_i(μM)	τ_{50}(min)	K_i(μM)	τ_{50}(min)
MDL 72638	1.75	8.9	0.088	2.9
Clorgyline*	0.054	<0.9	58	13

* Taken from reference [9].

propargylamines, one a potent type A inhibitor and the other a B inhibitor. Both compounds have been reported [10] to inactivate MAO by covalently binding to the N-5 atom of the flavin cofactor. If MDL 72638 also attaches itself to N-5 then this substance and L-deprenyl would be expected to bind in a similar fashion to the active site of MAO in contrast to that of clorgyline. Conversely, the inhibitors could bind to the enzyme in the same way but alkylate different enzyme or cofactor residues. Finally, mechanistic differences may well occur in both the mode of binding and the point of attachment to the enzyme or cofactor. The latter two possibilities may prove to be difficult to differentiate experimentally.

At least three other attachment sites between the activated inhibitor and MAO apart from N-5 have been reported. Krantz and Lipkowitz implied [11] that *N*-2,3-butadienyl-*N*-benzylmethylamine inactivates MAO by covalent modification of the flavin nucleus at a site distinct from N-5. Secondly, it is well established [12] that phenylhydrazine inhibition of MAO leads to a covalent adduct involving C-4a. Finally, a thiol residue has been implicated in the mechanism of action of *trans*-phenylcyclopropylamine [13, 4]. Unfortunately, none of the inhibitors used in these studies, apart from the propargylamines, are sufficiently selective for either the A or B form of MAO to indicate what relevence the site of alkylation has to selectivity.

We are currently undertaking a detailed structure–activity relationship study with our fluorallylamine inhibitors and known selective propargylamine inhibitors to try and evaluate the nature of the binding and covalent modification processes in the hope that this will shed some light on which factors are critical in influencing the selectivity of an inhibitor for one form of MAO or the other.

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Uptake of α-difluoromethylornithine by *Trypanosoma brucei brucei*

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α-Difluoromethylornithine (DFMO), a catalytic irreversible inhibitor of ornithine decarboxylase (ODC) [1], has been shown to rapidly deplete the intracellular polyamines putrescine and spermidine in *Trypanosoma brucei brucei* and to inhibit trypanosomal DNA synthesis and proliferation [2], as it does in a number of rapidly proliferating cell types [3]. Administration of DFMO effects cures of African trypanosomiasis in both experimental murine infections [4] and human sleeping sickness [5]. Trypanosomal proliferation *in vitro* is inhibited completely by concentrations of DFMO as low as 50 μM (unpublished observations), whereas rat hepatoma tissue culture cells (HTC cells) continue to grow, albeit at a much reduced rate, in the presence of 5 mM DFMO [6].

Presently, there is not an adequate explanation as to why trypanosomes appear to be uniquely sensitive to the polyamine-depleting effects of DFMO as compared to other cells. Trypanosomal ODC has approximately the same sensitivity to DFMO ($K_i = 130$ μM) [7] as does mammalian ODC ($K_i = 39$ μM) [8]. However, preliminary studies suggested that DFMO may be transported into the trypanosome by an active, energy-dependent system [2]. This was an intriguing hypothesis since selective, active uptake of DFMO by trypanosomes might partially explain the great sensitivity of the organisms to the drug. Since DFMO is a basic amino acid analogue and active transport of basic amino acids has been described for mammalian cells [9] as well as for trypanosomes [10], it was possible that DFMO uptake was mediated by one of these transport systems in the trypanosome. Other workers had already shown this not to be the case for mammalian cells [11–13]. We have now investigated more rigorously the uptake of DFMO by *T. b. brucei* and have concluded that DFMO enters the parasite by passive diffusion.

Methods

Trypanosomes. Trypanosoma brucei brucei (EATRO 110) was maintained by syringe passage in male Sprague–Dawley rats (250–300 g). Trypanosomes were harvested from blood obtained by cardiac puncture from 72-hr infected rats and separated from blood elements on DEAE-cellulose columns [14] using a solution of 90 mM Tris·HCl (pH 7.8) containing 50 mM NaCl and 2% (w/v) glucose for equilibration and elution of the columns.

Mammalian cell culture. Hepatoma tissue culture (HTC) cells were maintained in spinner cultures using Swims G67 medium containing 2 mM glutamine, 100 μg/ml streptomycin, 100 units/ml penicillin and 10% newborn calf serum.

DFMO uptake. Purified trypanosomes were washed twice with either Eagle's Minimum Essential Medium (MEM), containing Earle's salts, supplemented with non-essential amino acids, 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, 0.2% glucose, 10% fetal bovine serum and 30 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) (pH 7.5) or with 70 mM phosphate buffer (pH 8.0) containing 43 mM NaCl, 1% glucose, 50 units/ml penicillin, 50 μg/ml streptomycin and 1% bovine serum albumin (PSG-BSA), and then suspended in the respective wash buffer in preparation for uptake studies. HTC cells were washed twice with fresh growth medium and then suspended in the same medium for uptake incubations. Trypanosomes or HTC cells were then incubated with continuous shaking with [^{14}C]DFMO (usually 3.3 μCi/ml) or nonradioactive DFMO at 37°. Incubations were terminated by rapid centrifugation (1 min) of the trypanosomes or HTC cells and rapid washing with an ice-cold solution of 10 mM Na_2HPO_4, 3 mM KH_2PO_4 and 0.125 M NaCl (pH 7.2) after which the sedimented cells were extracted overnight with small volumes of 0.4 M perchloric acid. Cell debris was sedimented and the perchloric acid-soluble supernatant fraction was analyzed for [^{14}C]DFMO or nonradioactive DFMO by high performance liquid chromatography (HPLC) coupled to a radioactive flow detector or a fluorometer respectively. Uptake of DFMO was linear for greater than 60 min for both trypanosomes and HTC cells. Each uptake experiment was repeated at least twice with similar results. The data shown in the tables and figures are from single representative experiments.

Determination of DFMO. DFMO was separated by ion-exchange chromatography using a modification of a published procedure [15]. Separation was achieved on a Whatman Partisil-SCX 10 column (250 mm × 4.6 mm i.d.) using a two-step gradient which was formed by mixing 0.2 M acetic acid (Buffer A) and 0.2 M sodium acetate, pH 4.5 (Buffer B). The first gradient step consisted of a change from 6% Buffer B to 15% Buffer B in 15 min followed by the second gradient step which changed Buffer B from 15% to 100% in 12 min. After the gradient was completed the column was returned to initial conditions in 0.5 min and equilibrated for 10 min before the next run. In each instance where a percentage of Buffer B is given, Buffer A accounted for the remaining percentage of the flow. Flow rate was 1.0 ml/min. Detection of [^{14}C]DFMO was accomplished using a model IC Radioactive Flow Detector (Radiomatic Instruments) with a scintillant flow rate of 3 ml/min. Counting efficiency for [^{14}C]DFMO was 35–40%. In some experiments intracellular DFMO was measured using a fluorescence detector (Kratos Analytical Instruments) after post-column derivitization with *o*-pthaldialdehyde [15, 16].

Chemicals. [5-^{14}C]Difluoromethylornithine (60 mCi/mmole) was from Amersham; culture media were from the Grand Island Biological Co.; and ornithine, lysine and arginine from Sigma. Nonradioactive α-DFMO (DL-α-difluoromethylornithine hydrochloride monohydrate, MDL 71,782) and DL-α-monofluoromethylornithine hydrochloride monohydrate were synthesized at Merrell Dow Research Institute [1, 8].