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Fluorinated phenylcyclopropylamines. Part 3: Inhibition of monoamine oxidase A and B

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Abstract—Fluorinated phenylcyclopropylamines and alkylamines were examined as inhibitors of recombinant human liver monoamine oxidase A (MAO A) and B (MAO B). For a series of *trans*- and *cis*-2-fluoro-2-phenylcyclopropylamine analogues, the presence of fluorine attached to a cyclopropane ring was found to result in an increase in inhibitory activity towards both MAO A and B. In addition, *p*-substitution of electron-withdrawing groups such as Cl and F in the aromatic ring of the *trans*-isomers increased the inhibition of both enzymes. (1*S*,2*S*)-2-Fluoro-2-phenylcyclopropylamine was a more potent inhibitor of both MAO A and B than was the (1*R*,2*R*)-enantiomer, indicating that the presence of fluorine has no influence on the enantioselectivity of MAO inhibition, since a similar effect of stereochemistry has been reported for tranylcypromine. Interestingly, fluorination at the 2-position of 1-phenycyclopropylamine, which is known as a selective inhibitor of MAO B relative to MAO A, reversed the selectivity and resulted in a potent inhibitor selective for MAO A. All inhibitors showed time- and concentration-dependent inhibition for both enzymes, with the exception of *trans*-2-fluoro-2-phenylcyclopropyl ethylamine, which acts as a competitive and reversible MAO A selective inhibitor.

1. Introduction

Monoamine oxidases, widely existing in mammals, plants, and both prokaryotic and eukaryotic microorganisms, catalyze the oxidation of amines to aldehydes. They have been classified into two groups, copper- (EC: 1.4.3.6) and flavin- (EC: 1.4.3.4) containing amine oxidases.[1] Copper-containing amine oxidases require copper and an organic co-factor, for example 2,4,5-trihydroxyphenylalanine quinone, for activity and are strongly inhibited by semicarbazide.[2] Flavin-containing amine oxidases exist in two forms, namely MAO A and MAO B. Both subtypes are characterized by specific substrates and inhibitors. MAO A has a higher affinity for serotonin (5-hydroxytryptamine) and norepinephrine and is more sensitive to inhibition by clorgyline, whereas MAO B preferentially deaminates phenylethylamine and benzylamine and is sensitive to low concentrations of deprenyl.[3] Dopamine, tyramine, and tryptamine are common substrates for both MAOs. MAO A and B are composed of 527 and 520 amino acids, respectively, and have a 70% amino acid identity.[4] Each isoenzyme has a flavin cofactor covalently linked to a cysteine residue in the active center.[5] For the MAO catalysis, two models, one involving an iminium cation radical mechanism and the other a polar nucleophilic mechanism, have been proposed on the basis of the work with purified enzymes and chemical model systems.[6]

Due to the importance of MAO in the metabolism of monoamine neurotransmitters, both reversible and irreversible inhibitors of MAO have been used clinically in the treatment of neurological disorders, including such as depressive illness. The MAO B inhibitor, L-deprenyl, is administrated to potentiate L-dopa therapy in the treatment of Parkinson's patients as well as to provide neuroprotective effects in patients exhibiting

Keywords: Monoamine oxidase; Fluorinated phenylcyclopropylamine; Flavin; Inhibitor.

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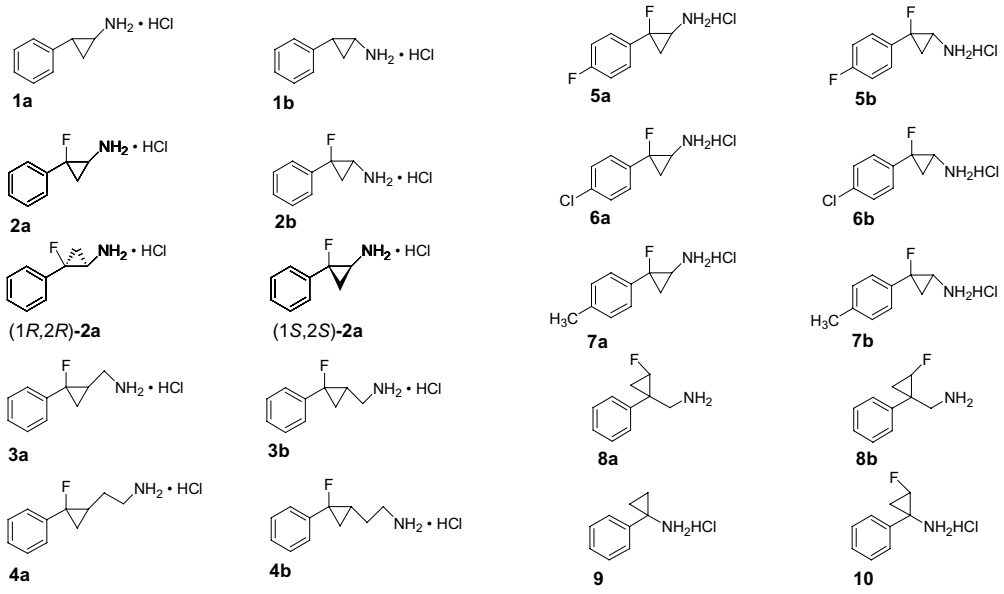


Figure 1. Compounds used in this study.

pre-Parkinson's Syndrome.[7] MAO B inhibitors are also currently in clinical trials for the treatment of Alzheimer's disease, because an increased level of MAO B was detected in plaque-associated astrocytes of brains from Alzheimer's patients.[8] Moclobemide, the first reversible inhibitor of MAO A, has also been used as an antidepressant.[9]

Cyclopropylamines make up an important class of MAO inhibitors.[10] A commercially important example that has important clinical use for treatment of certain depressive illnesses is tranylcypromine (TCP) (**1a**) (Parnate®, Jatrosom® N), an irreversible inhibitor of both MAO A and B. Another cyclopropylamine analogue, 1-phenylcyclopropylamine, is also a potent MAO inhibitor. The cyclopropylamines have been shown to inactivate MAO by attachment of a ring-opened reactive intermediate to a flavin cofactor or a cysteine residue in the active site.

Despite proven clinical effectiveness, there are certain problems associated with utilization of these inhibitors for clinical practice. For example, after the ingestion of tyramine containing foods, such as cheese and red wine, patients who are treated with nonselective MAO inhibitors can suffer from a severe hypertensive crisis. This so-called 'cheese effect' is caused by the increase of tyramine concentration associated with the inhibition of MAO A.[11]

In the development of more potent and selective inhibitors, the introduction of fluorine has been a frequently used strategy, owing to the well-documented ability of fluorine to alter biological activity of bioactive compounds.[12] An example of the successful use of this approach is the development of 2-(3,4-dimethoxyphenyl)-3-fluoroallyl amine as a potent and selective MAO B inhibitor with a B/A selectivity of about 100.[13] Interestingly, the selectivity for MAO A and B depend on the nature of aromatic ring substitution of 2-phenyl-3-fluoroallyl amine.[13]

We have recently synthesized two series of compounds featuring both the cyclopropyl ring and fluorine substitution as key structural elements. These are 2-fluoro-1-phenylcyclopropylamine and (2-fluoro-1-phenylcyclopropyl)methylamines,[14] as well as 2-fluoro-2-phenylcyclopropylamines and (2-fluoro-2-phenylcyclopropyl)-alkylamines,[14] and phenyl ring-substituted derivatives.[15] We have reported their activities as inhibitors of microbial tyramine oxidase, a copper-containing monoamine oxidase. In these reports, we described the effects of fluorine substitution at cyclopropane ring and aromatic ring substitution on copper-containing tyramine oxidase.[14,15]

We now have investigated the inhibitory activity of the fluorinated phenylcyclopropylamines shown in Figure 1 towards MAO A and B. In this report, we discuss the influence of the fluorine substitution at the cyclopropane ring and *p*-substitution of the phenylcyclopropylamine on the selectivity for these MAO isoforms.

2. Results

2.1. Inhibition potency of MAO A and B by 2-fluoro-2-phenylcyclopropylamines and (2-fluoro-2-phenylcyclopropyl)alkylamines

The inhibition of MAO A and B as a function of concentrations of 2-fluoro-2-phenylcyclopropylamines was investigated (Fig. 2) and IC_{50} values (inhibitor concentration at 50% remaining activity) were determined graphically from the inhibition curves obtained. The IC_{50} values are summarized in Table 1. As already known, MAO A and B were strongly and selectively inhibited by clorgyline and (*R*)-(−)-deprenyl, respectively. *trans*-2-Phenylcyclopropylamine, (tranylcypromine, **1a**) also inhibited both MAO A and B at similar inhibitor concentration. The inhibition of MAO A by the *cis*-isomer **1b** of this compound was 1.8-times

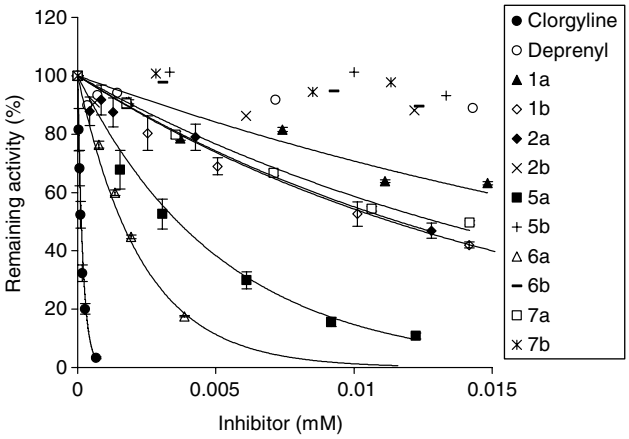


Figure 2. Effect of the concentration of clorgyline, (*R*)-(−)-deprenyl, phenylcyclopropylamines (**1a** and **1b**), 2-fluoro-2-phenylcyclopropylamines (**2a**,**b**, **5a**,**b**, **6a**,**b**, and **7a**,**b**) on the inhibition of MAO A.

stronger than by the *trans*-isomer, while the potency of inhibition of MAO B was the same for the two isomers.

2.1.1. Effect of fluorine introduction to cyclopropyl ring on the potency of inhibition of MAO A and B. The introduction of fluorine at the 2-position of tranylcypromine (**1a**) (*trans*-series) increased the potency of inhibition of both MAO A and B, but only modestly (Table 1, Figs. 2 and 3a). Thus, the inhibition of MAO A and MAO B by compound **2a** was 1.7- and 3-times higher, respectively, than inhibition by the nonfluorinated compound, tranylcypromine (**1a**). In contrast, the fluorine-containing

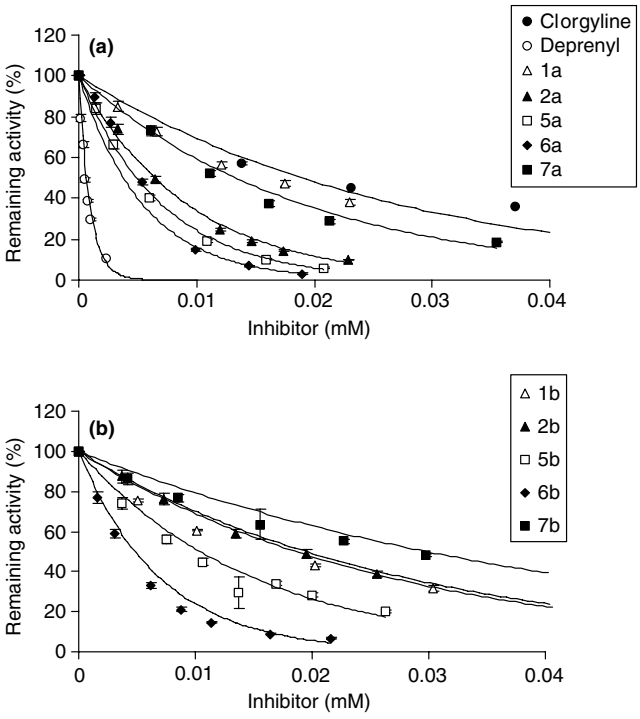


Figure 3. Effect of the concentration of clorgyline, (*R*)-(−)-deprenyl, and 2-fluoro-2-phenylcyclopropylamines (*trans*-series (a), *cis*-series (b)) on the inhibition of MAO B. In this figure, *trans* and *cis* mean the relative configuration of amino chain to aromatic ring.

cis-isomer **2b** was slightly less active than **1b** as an inhibitor of MAO A, and was of comparable activity as an inhibitor of MOA B (Table 1, Fig. 3b).

Table 1. IC_{50} values and inhibition type for 2-fluoro-2-phenylcyclopropylamines and alkylamines

Compound	Isomer type[a]	MAO A		MAO B	
		IC_{50} (mM)	Inhibition type[b]	IC_{50} (mM)	Inhibition type[b]
1a	*trans*	0.020 ± 0.000	Irreversible	0.019 ± 0.000	Irreversible
1b	*cis*	0.011 ± 0.001	Irreversible	0.019 ± 0.001	Irreversible
2a	*trans*	0.012 ± 0.001	Irreversible	0.0064 ± 0.0001	Irreversible
2b	*cis*	0.065 ± 0.042	Irreversible	0.019 ± 0.001	Irreversible
3a	*trans*	Ni[c]	Nd[d]	Ni[c]	Nd[d]
3b	*cis*	Ni[c]	Nd[d]	Ni[c]	Nd[d]
4a	*trans*	0.041 ± 0.002	Competitive	0.19 ± 0.01	Nd[d]
4b	*cis*	Ni[c]	Nd[d]	Ni[c]	Nd[d]
5a	*trans*	0.0036 ± 0.0002	Irreversible	0.0049 ± 0.0001	Irreversible
5b	*cis*	0.27 ± 0.07	Irreversible	0.010 ± 0.000	Irreversible
6a	*trans*	0.0016 ± 0.0000	Irreversible	0.0037 ± 0.0001	Irreversible
6b	*cis*	0.089 ± 0.009	Irreversible	0.0048 ± 0.0001	Irreversible
7a	*trans*	0.013 ± 0.000	Irreversible	0.013 ± 0.000	Irreversible
7b	*cis*	0.23 ± 0.12	Irreversible	0.030 ± 0.001	Irreversible
Deprenyl[e]	—	Ni[c]	Nd[d]	0.0006 ± 0.0001	Nd[d]
Clorgyline	—	0.00013 ± 0.00001	Nd[d]	0.025 ± 0.0012	Nd[d]

[a] Relative configuration of aromatic ring and amine-containing side chain.

[b] Inhibition type was determined by the observation of time- and concentration-dependent inactivation of MAO A and B in the presence of the inhibitor. The incubation of MAO B with an inhibitor was carried out at 4 °C in 0.1 mL of 0.1 M potassium phosphate (pH 7.2) containing 11.2 μg of enzyme, 6% of dimethylsulfoxide and different concentration of an inhibitor. Aliquots (20 μL) were taken out periodically from the mixture, and diluted with 0.68 mL of assay solution. The increase of absorbance at 250 nm was monitored as described in the Experimental section. The conditions of MAO incubation are described in the legend of Figure 6.

[c] Inhibition was not observed at mM scale.

[d] Not determined.

[e] (*R*)-(−)-form.

2.1.2. Effect of aryl-ring substitution on the potency of inhibition of MAO A. Introduction of *p*-substituents in the aromatic ring of the fluorinated compounds (**2a** and **2b**) significantly influenced the potencies of inhibition of both MAO A and B.

The presence of an electron-withdrawing substituent in the *p*-position in the *trans*-series, compounds **5a** (-F) and **6a** (-Cl), increased the inhibition of MAO A relative to the unsubstituted **2a** by a factor of 3.3 and 7.5, respectively (Fig. 2 and Table 1). In contrast, little effect on the inhibitory potency was observed for the methyl substituted derivative **7a** (+I substituent) (Table 1). The rank order of potency of the *trans*-oriented compounds as inhibitors of MAO A is **6a** > **5a** > **7a** = **2a** > **1a**, with **6a** being about 12 times more potent than tranylcypromine (**1a**).

In the *cis*-series introduction of a fluorine at the cyclopropane ring caused a lower activity by a factor of 6 against MAO A (Table 1, compounds **1b**, **2b**) and *p*-substitution in the phenyl ring makes the compounds even less active (Table 1, compounds **5b**, **6b**, **7b**, Fig. 2). The rank order of potency for the *cis*-oriented compounds as inhibitors of MAO A is **1b** > **2b** > **6b** > **7b** > **5b**, with **5b** being some 20 times less potent than *cis*-tranylcypromine (**1b**).

In the *trans*-series, substitution of electron withdrawing groups (Cl, F) on the aromatic ring leads to compounds that are more potent inhibitors of both MAO A and MAO B, showing a modest increase in MAO A selectivity.

2.1.3. Effect of aryl-ring substitution on the potency of inhibition of MAO B. As mentioned above, introduction of fluorine in the 2-position in the *trans*-series increased the activity against MAO B of compound **2a** compared to **1a** by a factor of 3. Electron withdrawing groups in *p*-position led to a modest increase in activity, with **5a** and **6a** having potencies 1.3 and 1.7 greater than the potency of **2a**. The introduction of a methyl group (compound **7a**) led to a lower activity by a factor of 2. The rank order of potency of the *trans*-compounds as inhibitors of MAO B was **6a** > **5a** > **2a** > **7a** > **1a**.

In the *cis*-series, introduction of fluorine on the cyclopropane ring of **1a** to give compound **2b** had no measurable effect on the potency of inhibition of MAO B. Introduction of *p*-substituents led to slightly higher activity, with **5b** and **6b** having potencies 1.9 and 4 times greater, respectively, than the potency of **2b**. In contrast, the *p*-methyl derivative **7b** was 1.6 times less active than **2b** (Table 1, Fig. 3b). Thus, the rank order of potencies in the *cis*-series as inhibitors of MAO B was **6b** > **5b** > **2b** = **1b** > **7b**.

2.1.4. Substituent effects on MAO B/MAO A selectivities. Tranylcypromine itself has no selectivity in inhibition potency towards MAO A or MAO B. Introduction of fluorine in the 2-position of the cyclopropane ring led to modest 2-fold MAO B selectivity along with an increased potency for both isoenzymes. However, introduction of electron-withdrawing groups resulted in MAO A selectivity, with the most active compound **6a** showing selectivity ratio for MAO A to MAO B of 2.3:1. This A selectivity was smaller for **5a**, and the methyl substituted compound **7a** had no selectivity. In contrast, the fluorinated *cis*-compounds were shown to have significant MAO B selectivity, with MAO B to MAO A ratios of 27:1 for **5b**, 19:1 for **6b**, and 8:1 for **7b**. The combination of the introduction of fluorine at the 2-position and electron-withdrawing groups (Cl and F) in the aromatic ring was effective on improving MAO B selectivity as well as increasing inhibitory potency. Thus, the *p*-chloro-compound **6b** was four times more potent than the parent nonselective *cis*-tranylcypromine **1b** as an inhibitor of MAO B, and showed a 19:1 MAO B selectivity.

2.1.5. Inhibition by higher homologues. The *trans*- and *cis*-(2-fluoro-2-phenylcyclopropyl)methylamines (**3a**,**b**) did not show any inhibitory activity toward either MAO A or B in millimolar scale (Fig. 4). The nonfluorinated compounds were found to be good substrates, however without irreversible inhibitory effect on the enzyme.[16] The *cis*-(2-fluoro-2-phenylcyclopropyl)ethylamine (**4b**) also showed no inhibition against either enzyme. However, the *trans*-isomer **4a** of the ethylamine was a weak inhibitor for both enzymes (Fig. 4), but showed a 1:5 selectivity for MAO A (Table 1).

2.2. Inhibition potency of MAO A and B by 2-fluoro-1-phenylcyclopropylamine and (2-fluoro-1-phenylcyclopropyl)methylamines

1-Phenylcyclopropylamine (**9**) is known to be a selective inhibitor of MAO B,[10c] as confirmed in this study (Table 2 and Fig. 5). In contrast to the results we observed with the 2-fluoro-2-phenylcyclopropylamine series, the introduction of fluorine at the 2-position (compound **10**) resulted in a marked decrease in potency of MAO B inhibition (Fig. 5). In contrast, this fluorinated com-

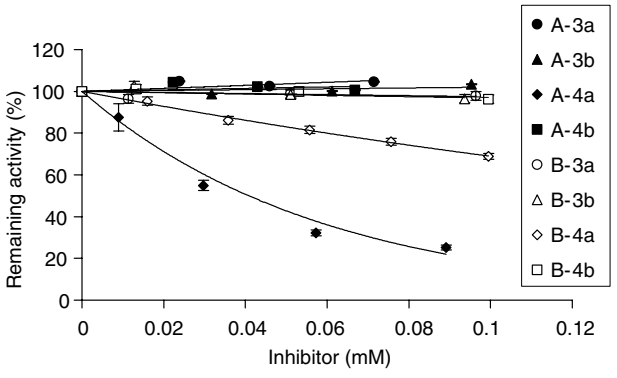


Figure 4. Effect of the concentration of 2-fluoro-2-phenylcyclopropyl methylamines (**3a** and **3b**) and ethylamines (**4a** and **4b**) on the inhibition of MAO A and B.

Table 2. IC_{50} values and inhibition type for 2-fluoro-1-phenylcyclopropylamine and methylamines

Compound	Isomer type[a]	MAOA		MAOB	
		IC_{50} (mM)	Inhibition type[b]	IC_{50} (mM)	Inhibition type[b]
8a	*trans*	0.32 ± 0.01	Nd[d]	0.024 ± 0.001	Irreversible
8b	*cis*	Ni[c]	Nd[d]	0.48 ± 0.23	Nd[d]
9	—	0.73 ± 0.15	Nd[d]	0.19 ± 0.02	Irreversible
10	*trans*	0.0031 ± 0.0001	Irreversible	0.42 ± 0.19	Nd[d]
Moclobemide[e]	—	0.0061	Reversible	>1	Reversible
Moclobemide[f]	—	0.0076[g]	Reversible	0.078[g]	Reversible

[a] Relative configuration of fluorine and amine-containing side chain.
[b] Inhibition type was determined as described in the note of Table 1.
[c] Inhibition was not observed at mM scale.
[d] Not determined.
[e] In vitro, rat brain homogenate. Data from Ref. 27.
[f] Ex vivo, rat brain homogenate. Data from Ref. 27.
[g] ED_{50} mmol/kg p.o.

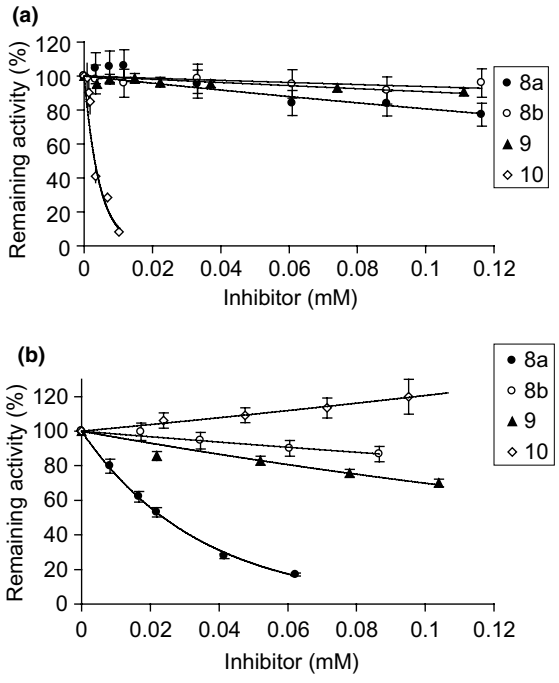


Figure 5. Effect of the concentration of 2-fluoro-1-phenylcyclopropyl methylamines (**8a** and **8b**), 1-phenylcyclopropylamine (**9**) and 2-fluoro-1-phenylcyclopropylamine (**10**) on the inhibition of MAO A (a) and B (b).

pound **10** showed excellent inhibitory activity for MAO A. The combined decrease in MAO B activity and increase in MAO A activity results in a dramatic MAO A versus MAO B selectivity of 135:1 (Table 2). The MAO A versus MAO B ratio of parent 1-phenylcyclopropyl amine (**9**) was 0.26:1. Thus, there was a shift to MAO A selectivity by over 500-fold due to introduction of fluorine.

The inhibitory potency of *trans*-(2-fluoro-1-phenylcyclopropyl)methylamine (**8a**) for MAO B was comparable to that of tranylcypromine, but was 16 time less potent as an inhibitor of MAO A. Thus, in contrast to tranylcypromine, **8a** shows significant MAO B selectivity.

2.3. Time- and concentration-dependent inhibition experiments

To obtain information on the mechanism of inhibition of MAO A and B by these inhibitors, time- and concentration-dependent inhibition experiments were carried out using the previously described method by Kitz and Wilson.[17] Due to instability of MAO, especially the A type, the incubations of MAOs with inhibitors were carried out at 4 °C.

The addition of compound **10** to the mixture containing MAO A resulted in time- and concentration-dependent inactivation (Fig. 6). However, it should be noted that the relative activity did not show an exponential decrease at each concentration of inhibitor. One reason for this could be attributed to instability of compound **10** under the assay conditions.

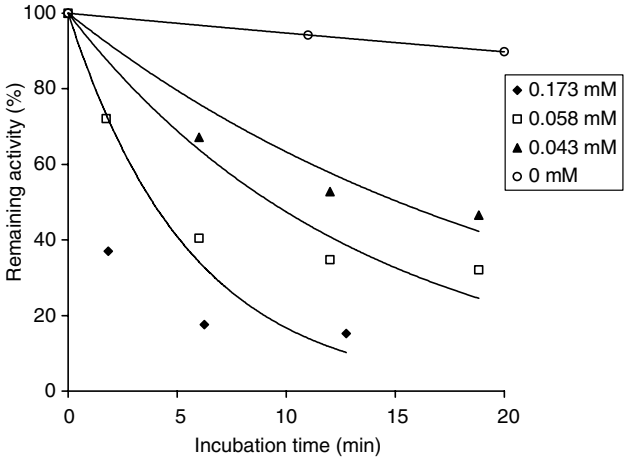


Figure 6. Time- and concentration-dependent inactivation of MAO A by 2-fluoro-phenylcyclopropylamine (**10**). The incubation of MAO A with compound **10** was carried out at 4 °C in 0.1 mL of 50 mM potassium phosphate (pH 7.2) containing 0.5% of Triton X-100 (reduced), 19.1 μg of enzyme, 6% of dimethylsulfoxide and different concentration of inhibitor. Aliquots (20 μL) were taken out periodically from the mixture, and diluted with 0.68 mL of assay solution. The increase of absorbance at 316 nm was monitored as described in Experimental section.

With the exception of compound **4a**, the other inhibitors caused a time- and concentration-dependent inhibition of MAO, indicating that the inhibition was irreversible (Tables 1 and 2). Kinetic analysis of MAO A in the presence of the compound **4a** showed that this inhibition was competitive (Fig. 7).

2.4. Enantioselectivity in the inhibition of MAO A and B

To investigate the effects of absolute stereochemistry in the inhibition of MAO A and B, the two enantiomers of 2-fluoro-2-phenylcyclopropylamine ((1*R*,2*R*)-**2a** and (1*S*,2*S*)-**2a**) were examined as inhibitors. Similar to our results with tyramine oxidase,[15] clear differences in inhibitory potencies were found with each enzyme (Fig. 8). Thus, the (1*S*,2*S*)-**2a** was an excellent inhibitor for both MAO A and B, in contrast to the inactivity seen with (1*R*,2*R*)-**2a**.

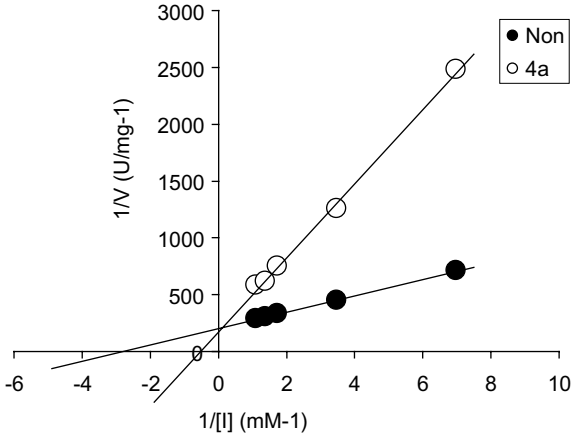


Figure 7. Lineweaver–Burk plot for the inactivation of MAO A by *trans*-(2-fluoro-2-phenylcyclopropyl)ethylamine (**4a**). The kynuramine oxidation was monitored as described in Experimental section in the presence (0.0298 mM) and absence of compound **4a**. In the preliminary experiment, no time- and concentration-dependent inhibition by **4a** was observed.

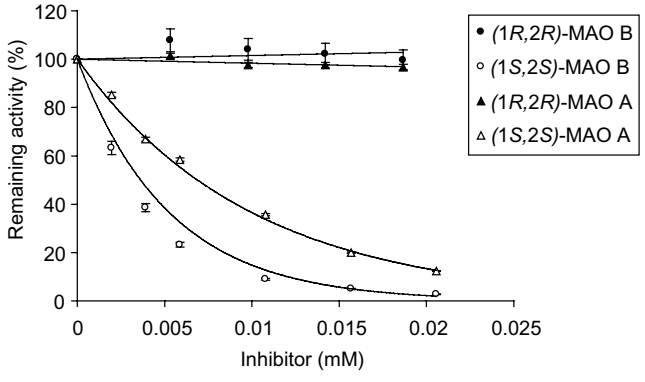
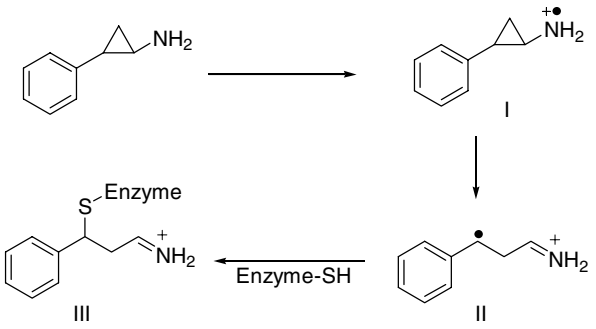


Figure 8. Effect of the concentration of 2-fluoro-2-phenylcyclopropylamine enantiomers ((1*R*,2*R*)-**2a** and (1*S*,2*S*)-**2a**) on the inhibition of MAO A and B.



Scheme 1.

3. Discussion

In discussing the effects of fluorine substitution in these two series of cyclopropyl amines, it is instructive to consider information that is known about the mechanism of inactivation by similar nonfluorinated species. Silverman has proposed that the cyclopropyl ring-opened intermediate **II** is produced via an initial single-electron transfer from the amine nitrogen of tranylcypromine (**1a**) to the oxidized flavin cofactor to form the nitrogen centered radical cation **I**, which opens to the carbon centered radical **II**. This intermediate reacts with an SH group of the enzyme to give **III** (Scheme 1).[10b,18]

The mechanistic pathway for MAO inhibition by cyclopropyl amines is an area of current interest and further details undoubtedly will be forthcoming. However, the rapid opening of the cyclopropane ring seems to be a key step for the inhibition. This step is also consistent with the irreversible inhibition found for tranylcypromine. Fluorine substitution is known to increase the ring strain in cyclopropyl rings,[19] so it can be expected that introduction of fluorine at the 2-position of tranylcypromine (**1a**) would result in a more rapid opening of cyclopropane ring of the fluoro analogue **2a**.

Facilitated ring opening would provide a reasonable explanation for the increased potency of inhibition of MAO by **2a**. At this time, it is not clear why the inhibition potency toward MAOs increased by the further *p*-aromatic substitution of fluorinated *trans*-2-phenylcyclopropylamine. However, the IC_{50} values correlate with the Hammett parameter rather than with the hydrophobicity or steric parameter of the substituents (Fig. 9). These facts suggest that electron-withdrawing groups, such as Cl and F, at the *para*-position might have a more positive effect on the increase of inhibitory than the introduction of the electron-donating group, $-CH_3$.

Other factors may also contribute to the different activities of these compounds, including relative stabilities of intermediary radicals as influenced by ring substituents. We are unable to estimate the importance of such factors presently. We note that an increased in vivo activity of *trans*-(4-fluorophenyl)cyclopropylamine compared to tranylcypromine has been reported. This

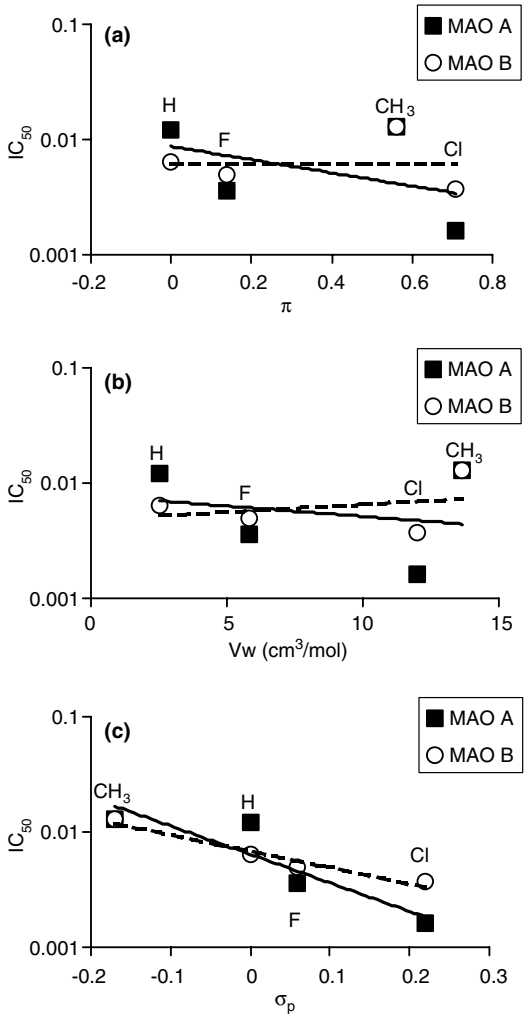


Figure 9. Correlation of IC_{50} values of the inhibitors with hydrophobicity (a), van der Waals volume (b) and the Hammett parameter (c) of the *p*-aromatic substituent. The solid and the broken lines are fitted for MAO A and B data, respectively. The parameters of the substituents were taken from Ref. 28–30.

increase was discussed in terms of increased lipophilicity or, alternatively, by the blocking of metabolic hydroxylation of the *p*-position.[20]

We demonstrated that (1*S*,2*S*)-**2a**, but not (1*R*,2*R*)-**2a**, is a potent MAO inhibitor. This shows that fluorination at the 2-position of 2-phenylcyclopropylamine has no influence on the enantiopreference of MAO inhibition. It has been shown previously that the (1*S*,2*R*) configurated *trans*-2-phenylcyclopropylamine, which has the same geometry as does (1*S*,2*S*)-*trans*-2-fluoro-2phenylcyclopropylamine ((1*S*,2*S*)-**2a**), is a more potent MAO inhibitor than the (1*R*,2*S*) isomer.[10a,21]

A dramatic reversal of isoform selectivity was found by introduction of fluorine in the 2-position of 1-phenylcyclopropylamine. This substitution converts the MAO B selective parent to the potent MAO A selective **10**. It is not clear yet why the fluorine substitution of 1-phenylcyclopropylamine reverses the MAO selectivity. In any case, we feel that this dramatic reversal of selectivity caused by substitution of a single fluorine is worthy of further investigation. As part of our plans to that end, we are preparing the more difficultly accessible *cis* isomer of **10**, as well as the pure enantiomers of both *cis*- and *trans*-2-fluoro-1-phenylcyclopropylamine, and phenyl ring-substituted analogues.

Among the inhibitors used in this study, the only competitive inhibitor for either MAO A or B was *trans*-(2-fluoro-2-phenylcyclopropyl)ethylamine (**4a**). The structure–activity relationship study in the oxidation of phenethylamine analogues by recombinant human liver MAO A showed 3-phenylpropylamine to be oxidized 2.5-fold more slowly than phenethylamine, and 4-phenylbutylamine was not a substrate but was a good competitive inhibitor for MAO A.[22] Further investigation is needed to understand the mechanism of inhibition by compound **4a**. However, it should be noted that the side chain length of compound **4a** is the same as that of 4-phenylbutylamine.

We are currently synthesizing other analogues of fluorinated phenylcyclopropylamines to understand the effects of fluorine substitution on the inhibition of MAO and to develop more potent inhibitors. We will report these results in due course.

4. Experimental section

4.1. Inhibitors

The fluorinated phenylcyclopropylamines and alkylamines used in this study were synthesized as previously reported.[14,15]

4.2. Enzyme assay

Stock solutions of the human liver mitochondrial outer membrane monoamine oxidases A and B, expressed in the methylotrophic yeast *Pichia pastoris*[23,24] were kindly provided by Professor Dale E. Edmondson's laboratory, Departments of Biochemistry and Chemistry, Emory University, Atlanta, Georgia, USA.

Prior to use, the enzyme stock solution was passed through a gel-filtration column (PD 10 desalting column, Amersham Biosciences) pre-equilibrated with 50 mM K phosphate (pH 7.2) containing 0.8% octylglucoside. The activity of MAO A was measured spectrometrically at 25 °C by the modified method of Li et al.[24] using 0.7 mL of standard reaction mixture containing 1 mM kynuramine hydrobromide, 50 mM potassium phosphate buffer (pH 7.2), 0.5% Triton X100 (reduced), 6% dimethylsulfoxide and MAO A. The reaction was monitored at 316 nm, which is the maximum absorption wavelength of 4-hydroxyquinoline. The enzyme activity was calculated by using 12,300 $M^{-1}\,cm^{-1}$ as the extinction coefficient of 4-hydroxyquinoline at 316 nm. One unit of the enzyme oxidizes

1 μmol of kynuramine to 4-hydroxyquinoline per 1 min. The activity of MAO B was also measured spectrometrically at 25 °C by the modified method of Houslay and Tipton[25] using 0.7 mL of standard reaction mixture containing 1 mM benzylamine, 0.1 M potassium phosphate buffer (pH 7.2), 6% dimethylsulfoxide and MAO B. The reaction was monitored at 250 nm, which is the maximum absorption wavelength of benzaldehyde. The enzyme activity was calculated by using 13,800 $M^{-1} cm^{-1}$ as extinction coefficient of benzylamine at 250 nm. One unit of the enzyme oxidizes 1 μmol of benzylamine to benzaldehyde per 1 min. Protein concentration was determined by the method of Bradford[26] using bovine serum albumin as a standard. Each inhibitor was dissolved in DMSO and diluted with the same solvent to give the appropriate concentration. The solution was immediately divided into several vials and wrapped with aluminum foil. These vials were stocked in an ice bath until used for inhibition experiments. Inhibition experiments were carried out as follows: varying concentrations of inhibitor were added to the reaction mixture described above (without substrate), and allowed to stand for 10 min at 10 °C. The reaction was started by the addition of substrate stock, and the time course of the absorption increase of the reaction product was monitored as described above.

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