Fluorinated Phenylcyclopropylamines as Inhibitors of Monoamine Oxidases

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1. Introduction

Monoamine oxidases, prevalent 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-containing amine oxidases (EC 1.4.3.4).[1]

Flavin-linked mitochondrial monoamine oxidases (MAO) are subdivided further into two catalytically distinguishable subtypes with different substrate selectivities: MAO A and MAO B. They are crucial for the regulation of physiological amine levels throughout the organism.[2]

Copper-containing amine oxidases (CAO) also have important and diverse functions in prokaryotes, including roles in nutrient metabolism. More recently, the importance of CAO in eukaryotes for different physiological processes, such as wound healing, detoxification of amines, cell growth, signaling, apoptosis,[3] and glucose uptake,[4] was demonstrated. They constitute a wide family of enzymes covering bovine and sheep plasma amine oxidase, lysil oxidase, diamine oxidase, and a tissue-bound oxidase.

Monoamine oxidases are important pharmacological and medicinal targets because of their diverse physiological roles. Selective inhibitors for different enzyme subtypes have been intensively studied. A commercially important example is tranylcypromine (**1a**) (*trans*-2-phenylcyclopropylamine, TCP, Parnate, Jatrosom N), which is an irreversible inhibitor of both MAO A and B. Additionally, **1a** is a potent reversible inhibitor of copper-containing amine oxidases.[5]

Tranylcypromine has an important clinical use as a treatment for certain depressive illnesses. Recent studies also discussed MAO inhibitors as useful agents against neurodegenerative disorders such as Parkinson's or Alzheimer's diseases.[6] Despite impressive clinical successes, clinical use of tranylcypromine (**1a**) and other MAO inhibitors is limited by various problems. For example, patients treated with nonselective MAO inhibitors can suffer a severe hypertensive crisis after the ingestion of tyramine-containing foods, such as cheese and red wine. This so-called "cheese effect" is caused by the increase of tyramine concentration associated with the inhibition of MAO A.[7]

There has been extensive research directed toward the development of more potent and selective MAO inhibitors. In the work reviewed here, the pharmacological activity of **1a** in combination with its undesired side effects prompted us to regard this molecule as the starting point for a lead-optimization process. The modifications incorporated our current interests in the chemistry and pharmacology of fluorinated compounds, interests that had recently focused on cyclopropylamines and amino acids. The goals of this optimization included increased inhibitory activity and an improved pharmacokinetic profile and selectivity. The fundamental strategy consisted of incorporation of a fluorine substituent onto the cyclopropyl moiety.

The incorporation of fluorine into bioactive compounds is known to be a very effective method for the enhancement of the physiological properties of such molecules.[8] The modifications are caused by the unique properties of fluorine. As the most electronegative element, this substituent influences the acidity/basicity of neighboring groups such as NH_2.[9] Also, the lipophilicity of molecules is influenced.[8d] A fluorine substituent can mimic some properties of a hydroxy group because of its isoelectronic properties. In fact, we recently found indications both from X-ray studies of crystalline derivatives[10] and from high-level quantum chemical calculations[11] that a fluorine atom attached to a cyclopropyl group can act as a hydrogen-bond acceptor.[12] Another important factor comes from the observation that fluorinated cyclopropanes possess an increased ring-strain energy.[13] Since ring-opening reactions are important for the inhibition mechanism of certain aminocyclopropanes,[14] fluorine substitution could be expected to enhance the inhibitory activity of these substances.

For the optimization of the pharmacological profile we selectively modified the structure of tranylcypromine (**1a**) by substitution on the cyclopropyl ring with a single fluorine atom. A series of tranylcypromine analogues with different substituent patterns and stereochemistry, such as **11a–d** or **38** and its fluorinated analogue **30**, were synthesized and their activities as inhibitors of monoamine oxidases were investigated and compared to other inhibitors.[15–17]

We herein summarize our recent results concerning the inhibitory activity of these fluorinated arylcyclopropylamines.

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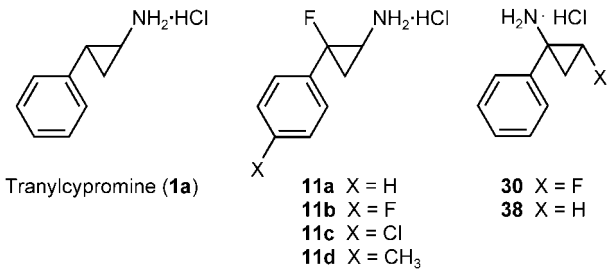


These results demonstrate that certain analogues possess very high activity as inhibitors of MAO A and B as well as of tyramine oxidase, a copper-dependent amine oxidase. Data for the pharmacokinetic profiles of the analogues as well as mechanistic reasons for the strong influence of the fluorine substituent on the activity are discussed.

2. Synthesis of Fluorinated Arylcyclopropylamines and (Arylcyclopropyl)alkylamines

Regioisomeric fluorinated phenylcyclopropylamines were obtained by using two different methods of cyclopropanation of the corresponding fluorinated alkenes.[18, 19a]

2-Aryl-2-fluorocyclopropylamines and (2-fluoro-2-phenylcyclopropyl)alkylamines

2-Aryl-2-fluorocyclopropylamines **11a–d** and **12a–d** were synthesized[15] according to the sequence shown in Scheme 1 by using transition-metal-catalyzed cyclopropanation of 1-fluorostyrenes **4a–d** as the key-step.[18]

Enantiopure *trans*-2-fluoro-2-phenylcyclopropylamines[16a] (1*S*,2*S*)-**11a** and (1*R*,2*R*)-**11a** were synthesized via (1*S*,2*S*)-(−)-2-fluoro-2-phenylcyclopropanecarboxylic acid ((1*S*,2*S*)-**7a**) and its enantiomer (1*R*,2*R*)-**7a** (Scheme 2).[16b] These acids were obtained by separation of the corresponding diasteromeric (*S*)-1-phenylethylamides followed by hydrolysis of *N*-nitrosamides prepared according to a method invented by White[20a] and further developed by others.[20b]

The diastereopure racemic carboxylic acids **7a** and **8a** also served as starting materials for the preparation of homologous fluorinated alkylamines[15] as shown in Scheme 3.

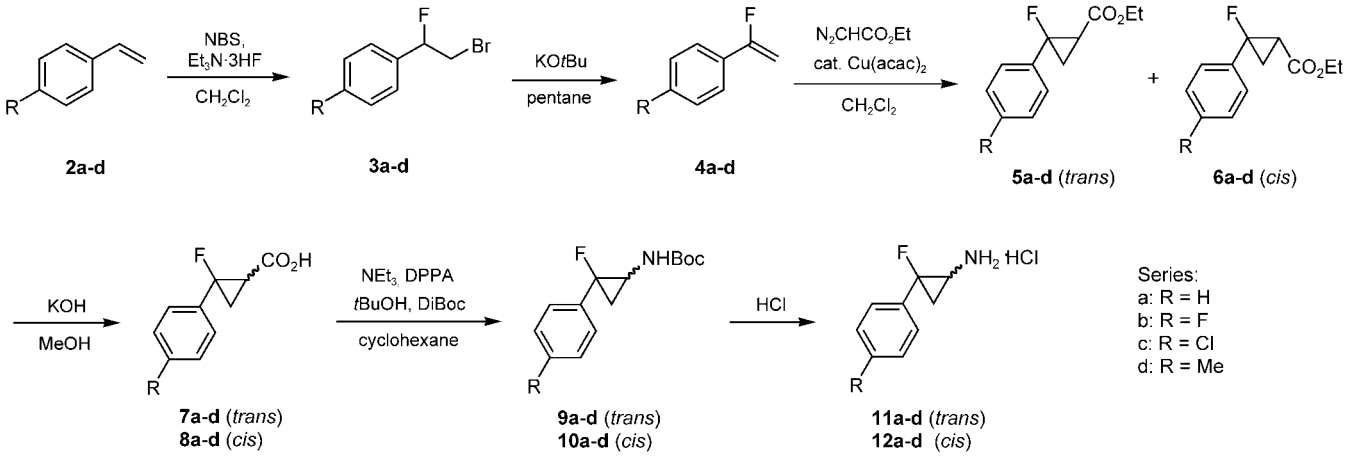
Applying classical homologation techniques, the diastereopure ethylamines **23a** and **24a** were prepared (Scheme 4).[15]

2-Fluoro-1-phenylcyclopropylamine and (2-fluoro-1-phenylcyclopropyl)methylamines

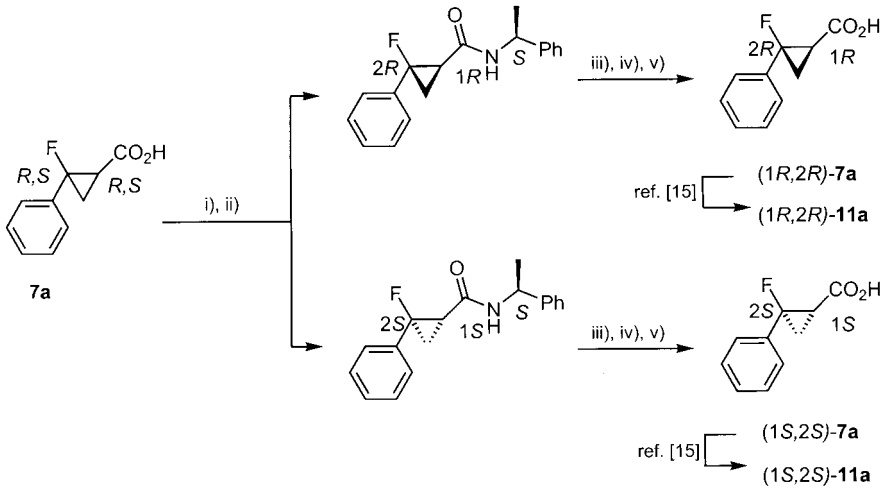
2-Fluoro-1-phenylcyclopropylamine and -methylamines, the regioisomeric series, were synthesized by using cyclopropanation of ethyl 3-fluoro-2-phenylacrylates **25** and **26** with diazomethane as the key step (Scheme 5).[15, 19]

3. Physicochemical Data for 2-Aryl-2-fluorocyclopropylamines

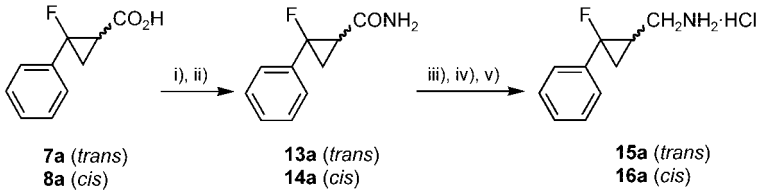
Different qualitative models have been developed for the identification of potential drug molecules, for example, Lipinski and co-workers' rule-of-five included four criteria: molecular mass <500 Daltons, octanol/water partition coefficient <5, number of hydrogen-bond donors <5, and number of hydrogen-bond acceptors <10.[21] Muegge described how specific combinations of side chains would allow identification of pharmacophores.[22] In addition to intrinsic inhibitory activity, pharmacokinetic properties are among the most important aspects of a lead-optimization process.[23] Pharmacokinetic factors such as adsorption, distribution, metabolism, and excretion (ADME) are strongly related to oral bioavailability and metabolic half-life.[24] An analysis of the main reasons for attrition in drug development revealed that 39% of all failures are attributed to poor pharmacokinetics.[25] Different models have been developed for the prediction of these important properties. Besides Lipinski and co-workers' rule-of-five, Veber et al. defined a sum of hydrogen-bond donors and acceptors ≤ 12 and rotatable bonds ≤ 10 as qualitative criteria for good oral bioavailabili-



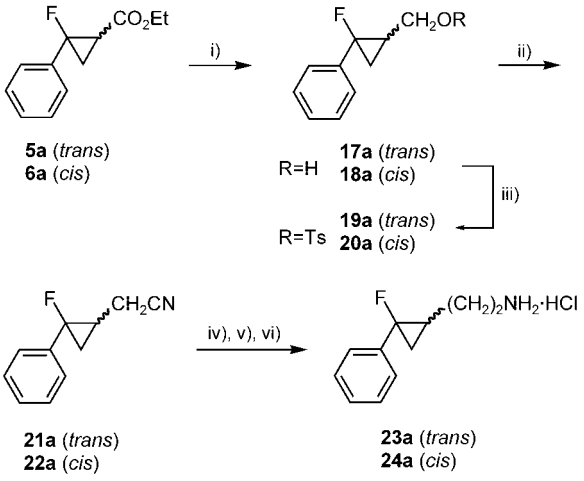
Scheme 1. *Synthesis of diastereopure 2-aryl-2-fluoro-cyclopropylamine hydrochlorides. acac = acetylacetone, Boc = tert-butoxycarbonyl, DiBoc = di-tert-butyl carbonate, DPPA = diphenylphosphoryl azide, NBS = N-bromosuccinimide.*



Scheme 2. *Synthesis of enantiopure* trans*-2-fluoro-2-phenylcyclopropylamines (1S,2S)-**11 a** and (1R,2R)-**11 a**. i) (S)-PhCH(NH$_2$)CH$_3$, N,N′-dicyclohexylcarbodiimide, cat. 4-dimethylaminopyridine, CH$_2$Cl$_2$, RT, 20 h; ii) Chromatography; iii) NaNO$_2$, HOAc, Ac$_2$O, 4 °C, 18 h; iv) Dioxane, Δ, 20 h; v) KOH/MeOH, RT.*



Scheme 3. *Synthesis of diastereopure 2-fluoro-2-phenylcyclopropylmethylamine hydrochlorides. i) SOCl$_2$; ii) NH$_4$OH; iii) BH$_3$·THF; iv) H$_3$O$^+$; v) HCl(g)*



Scheme 4. *Synthesis of diastereopure 2-fluoro-2-phenylcylcopropylethylamine hydrochlorides. i) LiAlH$_4$, Et$_2$O; ii) NaCN,* N,N*-dimethylformamide; iii) TsCl, NEt$_3$, CH$_2$Cl$_2$; iv) BH$_3$·THF, Et$_2$O; v) H$_3$O$^+$; vi) HCl(g). Ts = tosyl = toluene-4-sulfonyl.*

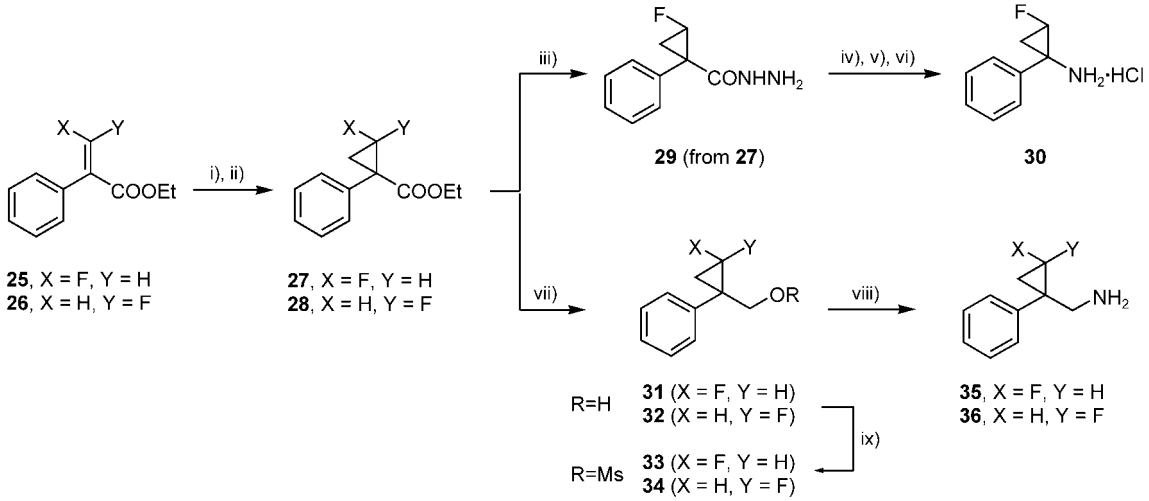
ty.[26] Clearly, the synthesized 2-aryl-2-fluorocyclopropylamines **11 a**–**d** and **12 a**–**d** as well as the homologous alkylamines met all these criteria. For a more precise quantification of the pharmacokinetic properties, physicochemical values such as pK_a value, lipophilicity, permeation, and solubility are very useful. Since fluorine substitution is known to influence these properties,[8d,27] examination of the physicochemical properties of the fluorinated cyclopropylamines became important[28] for further optimization of the pharmacological behavior of compounds derived from the lead compound tranylcypromine (**1 a**; Table 1).

First, pK_a values were measured, since the ionization state at physiological pH values will affect solubility, adsorption, and reactivity. Fuller and Molloy reported that, for aliphatic amines such as amphetamine, β,β-difluorination leads to a significant decrease in the basicity of the amino group, as would be expected.[29] The influence of fluorine on the acidity of neighboring groups was also described for other substrates.[9]

Similarly, for tranylcypromine the introduction of a fluorine substituent in the *trans*-2 position caused a decreased basicity of the amino group by more than one order of magnitude: pK_a = 8.47 for tranylcypromine (**1 a**) relative to pK_a = 7.35 for *trans*-2-fluoro-2-phenylcylopropylamine (**11 a**). Also, a direct influence of the substituent in the *para* position was observed: Whereas an electron-donating methyl group slightly increased the basicity (pK_a = 7.41 for **11 d**), lower pK_a values were found for compounds bearing the electron-withdrawing *para*-fluoro (**11 b**, pK_a = 7.31) and *para*-chloro (**11 c**, pK_a = 7.19) substituents.

Remarkably, the configuration of the substituents attached to the three-membered ring had a strong influence on the pK_a value of the fluorinated tranylcypromine derivatives. Since *trans*- and *cis*-2-phenylcyclopropylamine had nearly identical pK_a values of 8.47 (**1 a**) and 8.50 (**1 b**), the lower basicity (by about 0.4 orders of magnitude) of the fluorinated *cis*-configured amines **12 a**–**d** relative to the corresponding *trans* derivatives **11 a**–**d** (Table 1) must be due to the relative stereochemistry of the amino group and the fluorine substituent. The nature of this effect is still under investigation. As expected, the alkylamines **15 a** and **23 a** had higher pK_a values than tranylcypromine (**1 a**).

Additionally, permeability resulting from passive diffusion was recorded by using a parallel artificial-membrane permeation assay (PAMPA).[30] The permeation rate determined by this method corresponds to the transcellular permeation potential, which is important for the absorption of drugs.[30] Tranylcypromine (**1 a**) itself, as well as the fluorinated derivatives **11 a**–**d** and **12 a**–**d**, is characterized by high permeability. Introduction of a fluorine substituent in position 2 increased permeability,



Scheme 5. *Synthesis of diastereopure 2-fluoro-1-phenyl-cyclopropylamine and -methylamine hydrochlorides. i) CH_2N_2; ii) hν, Et_2O; iii) NH_2NH_2, EtOH; iv) HCl, $NaNO_2$, Et_2O; v) tBuOH; vi) 3 N HCl, EtOAc; vii) DIBAL, toluene; viii) NH_3, THF; ix) MsCl, NEt_3, CH_2Cl_2. DIBAL = diisobutylaluminum hydride, Ms = mesyl = methane sulfonyl.*

Table 1. *Physicochemical data for 2-aryl-2-fluorocyclopropylamines and -alkylamines.*

Compound	Isomer type	NH_2/F relation	R	M_W	pK_a[a]	P[b] [10^{-6} cm s^{-1}]	log D[c]
1a	*trans*	–	H	169.7	8.47	1.30 ± 0.02	n.d.[d]
1b	*cis*	–	H	169.7	8.50	2.27 ± 0.23	1.41
11a	*trans*	*cis*	H	187.7	7.35	3.48 ± 0.01	1.53
12a	*cis*	*trans*	H	187.7	6.98	7.83 ± 0.69	1.78
11b	*trans*	*cis*	F	205.6	7.31	5.12 ± 0.28	1.60
12b	*cis*	*trans*	F	205.6	6.88	4.00 ± 0.53	2.14
11c	*trans*	*cis*	Cl	222.1	7.19	2.90 ± 0.00	2.23
12c	*cis*	*trans*	Cl	222.1	6.81	1.28 ± 0.01	2.66
11d	*trans*	*cis*	Me	201.7	7.41	2.58 ± 0.04	1.83
12d	*cis*	*trans*	Me	201.7	7.04	n.d.[d]	n.d.[d]
15a	*trans*	*cis*	H	201.7	9.76	0.00	–
23a	*trans*	*cis*	H	215.7	9.95	0.83 ± 0.17	precipitated

[a] Ionization constants (pK_a) were determined pH-metrically in 0.1 M KNO_3 at 21 °C. [b] Permeability was determined at pH 6.5 by using the PAMPA PSR4p method.[30] [c] Log D values were measured from the partition coefficients for 1-octanol/0.05 N NaOH + 5 %/vol dimethylsulfoxide at pH 7.4. [d] n.d. = not determined.

as can be seen by comparison of the values for *trans*-configured compounds **11a–d** with that for **1a**. Interestingly, for derivatives bearing electron-withdrawing *para* substituents, higher permeation rates were found for the *trans*-configured amines **11b** and **11c** than for **12b** and **12c**, whereas the highest value was recorded for unsubstituted **12a** in the *cis* configuration. In general, the type of substituent in the *para* position had a significant influence on permeability. In particular, introduction of an additional fluorine atom in this position increased permeability values, as observed for **11b** and **12b**. Significantly lower rates were found for alkylamines **15a** and **23a**.

The lipophilicity of compounds, which is important for pharmacokinetics in cell systems, can be expressed as the partition coefficient (log P) in an octanol/water system. Since lipophilic substances preferentially permeate membranes, this value is an important indicator for oral absorption as well.[24] From the literature it is known that the introduction of fluorine has an important influence on the lipophilicity of substrates.[8d] Analysis of distribution coefficients (log D) revealed that all the investigated phenylcyclopropylamines belong to a class of compounds with medium lipophilicity.[31] Introduction of a fluorine substituent in position 2 of the cyclopropane ring led to increased lipophilicity, as observed for **12a** relative to **1b**. The *para* substituent also influenced this value. Higher lipophilicities were observed for all *para*-substituted derivatives than for 2-fluorotranylcypromines **11a** or **12a.** Compounds **11c** and **12c**, with a *para*-chloro substituent, were the most lipophilic derivatives in this group.

4. Inhibition of a Copper-Containing Amine Oxidase by Fluorinated Arylcyclopropylamines

As already described, copper-containing amine oxidases (CAO) form a diverse group of enzymes. Different studies have shown that CAO contain covalently bound quinones as organic cofactors.[32] An important example of this class of cofactors is 2,4,5-trihydroxyphenylalanine quinone (TPQ).[33] Recently, the crystal structures of CAO from different species have been reported and the mechanism for the oxidation of amines by these enzymes has been discussed in detail.[32] This process is important for different biological processes in prokaryotes and eukaryotes.[34,35] Stimulation of CAO correlates with an increased glucose uptake.[36] CAO is reported to be important in relation to several diseases, such as Alzheimer's disease, cerebral autosomal dominant arteriopathy with subcortical infarcts,

and leukoencephalopathy, vascular plaques linked to congestive heart failure.[37,38] In addition, toxic metabolites of CAO-catalyzed deamination have been described.[35]

These diverse physiological functions demonstrate the potential therapeutic use of selective inhibitors of this class of enzymes. In general, copper-containing amine oxidases are strongly inhibited by compounds such as semicarbazide.[39] Additionally, 3-haloallylamines, such as 2-(3,4-dimethoxyphenyl)-3-fluoroallylamine (**37**), are also effective irreversible inhibitors. Recently, Saysell et al. described (1*S*,2*R*)-(+)-tranylcypromine ((1*S*,2*R*)-**1a**) as a reversible inhibitor of *Escherichia coli* CAO.[5a] Crystallographic studies revealed the formation of a Schiff's base adduct of this inhibitor with the O5 position of the organic cofactor 2,4,5-trihydroxyphenylalanine quinone as the site of substrate binding.

With these results in mind, the influence of monofluorination of the cyclopropyl moiety on the inhibitory activity of tranylcypromines was investigated. For these studies commercially available tyramine oxidase from *Arthrobacter* sp. (EC 1.4.3.4) was used; this enzyme is sold by Sigma as a flavin-containing amine oxidase. However, Wouters et al. characterized this enzyme as a copper-containing amine oxidase on the basis of spectral characteristics, molecular weight, and inhibition profile.[40] We independently confirmed that the tyramine oxidase from *Arthrobacter* sp. was a copper/TPQ-type amine oxidase.[15,41]

IC_{50} values (inhibitor concentration at 50% remaining activity) were determined for regio- and stereoisomeric fluorinated arylcyclopropylamines and phenylalkylamines (Table 2). Tranylcypromine (**1a**) itself was confirmed to be a competitive inhibitor. Introduction of a fluorine substituent in position 2 caused a dramatic change in the inhibition: Compound **11a** with *cis* arrangement of the fluorine substituent and the amino group has an IC_{50} value ten times lower than that of tranylcypromine (**1a**). In contrast, the corresponding diastereomer **12a** with a *trans* arrangement of the two substituents was five times *less* active than **1a**.[15] Moreover, a strong influence of the absolute configuration on the activity of *trans*-2-fluoro-2-phenylcyclopropylamine was observed. Whereas (1*S*,2*S*)-**11a** strongly inhibited the tyramine oxidase ($IC_{50} = 2.3$ μM), the opposite enantiomer (1*R*,2*R*)-**11a** did not show any activity at millimolar concentrations.[16]

Substituents in the *para* position of the aromatic ring also had a significant effect on the IC_{50} values. In the *trans* series, electron-withdrawing substituents decreased inhibitory activity, while the electron-donating methyl group led to a sevenfold higher inhibitory activity than that of **11a**. As described before, the *cis* isomers **12a**–**d** were one to two orders of magnitude less active than the *trans* compounds **11a**–**d**. Kinetic analysis demonstrated clearly that the inhibition of tyramine oxidase by **11a**–**d** was competitive. For the *cis* series, compound **1b** showed time- and concentration-dependent inhibition against tyramine oxidase. Irreversibility was also observed for compounds **12a**–**d**; however, the inhibitory activity was lowered by the introduction of a fluorine substituent.

Looking at the homologues of **11a**, the *trans*-methylamine **15a** is about three orders of magnitude less active than **11a** and the *trans*-ethylamine **23a** is about two orders of magnitude less active than **11a**. The corresponding *cis* isomers **16a** and **24a** did not show any inhibition at the millimolar scale (Table 2).[16]

In addition, the inhibitory activities of regioisomeric 2-fluoro-1-phenylcyclopropylamines and -methylamine were studied to examine the importance of the position of the fluorine substituent (Table 3). In contrast to **11a** the regioisomeric 2-fluoro-1-phenylcyclopropylamine (**30**) is not an inhibitor for tyramine oxidase from *Arthrobacter* sp. at the millimolar scale. However, the cyclopropylmethylamine **35** shows high activity. Since the electronic properties of a double bond and a cyclopropane ring are considered similar,[42] **35** can be regarded as a cyclopropyl analogue of 2-(3,4-dimethoxyphenyl)-3-fluoroallyl amine (**37**). Nonetheless, **37** was described as an irreversible inhibitor of CAO,[43] whereas **35** proved to be a reversible and noncompetitive inhibitor. Again, inhibition by compound **36**, the *cis* isomer of **35**, was very low. Further kinetic studies characterized **35** as a noncompetitive inhibitor, thereby suggesting the existence of a separate binding site. Finally, hydrazide **29** was found to be an irreversible inhibitor of the enzyme, with threefold lower activity than semicarbazide, the classic inhibitor of CAO. Similar to semicarbazide, hydrazide **29** may also react with the quinone moiety.

Table 2. *IC_{50} values and inhibition type for 2-fluoro-2-phenylcyclopropylamines and -methylamines.*

Compound	Isomer type	NH_2/F relation	R	IC_{50} [μM]	Inhibition type
1a	*trans*	–	H	35 ± 6	competitive
1b	*cis*	–	H	33 ± 1	irreversible
11a	*trans*	*cis*	H	3.6 ± 1.5	competitive
(1*R*,2*R*)-**11a**	*trans*	*cis*	H	n.i.[a]	competitive
(1*S*,2*S*)-**11a**	*trans*	*cis*	H	2.3 ± 0.1	competitive
12a	*cis*	*trans*	H	190 ± 90	partially irreversible[b]
11b	*trans*	*cis*	F	8.1 ± 1.6	competitive
11c	*trans*	*cis*	Cl	3.7 ± 0.3	competitive
11d	*trans*	*cis*	Me	0.39 ± 0.17	competitive
15a	*trans*	*cis*	H	1240 ± 60	noncompetitive
23a	*trans*	*cis*	H	490 ± 90	competitive

[a] n.i. = no inhibition detected at mM concentrations. [b] The term "partially irreversible" is used when time-dependent inhibition but no clear concentration-dependent inhibition was observed.

Table 3. *IC_{50} values and inhibition type for 2-fluoro-1-phenylcyclopropylamines and -methylamines.*

Compound	NH_2/F relation	IC_{50} [μM]	Inhibition type
29	*trans*	20 ± 6	irreversible
30	*trans*	n.i.[a]	n.d.
35	*trans*	12 ± 1	noncompetitive
36	*cis*	660 ± 210	n.d.
semicarbazide	–	6.7 ± 0.2	n.d.

[a] n.i. = no inhibition detected at 0.1 mM concentrations.

From these results, structure–activity relationships for fluorinated arylcyclopropylamines and (phenylcyclopropyl)alkylamines could be deduced: 1) A fluorine substituent in position 2 of the cyclopropyl group increases inhibitory activity; 2) for potent inhibition the fluorine substituent and the amino group must be in a *cis* configuration; 3) only the (1*S*,2*S*) enantiomer shows activity as an inhibitor; 4) the amino group must be directly linked to the cyclopropyl group; 5) electron-donating substituents in *para* position increase inhibition, while electron-withdrawing groups lower the activity.

For the explanation of these results, we considered the mechanism for the oxidation of amines by copper/TPQ amine oxidase from *A. globiformis*.[44] It was demonstrated that the organic cofactor TPQ forms a covalent zwitterionic adduct (**I**) with the amine substrate as a Schiff's base in an initial oxidative deamination step (Scheme 6).[32e] For tranylcypromine (**1 a**) the formation of an analogous adduct for *E. coli* amine oxidase was described.[5a]

The first step in the formation of Schiff's base **I** is a nucleophilic attack of the amino group of the substrate on the carbonyl group of TPQ. Since 2-fluoro-2-arylcyclopropylamines in general are characterized by a lower basicity than the non-fluorinated tranylcypromine (**1 a**; Table 1), this initial step should be disfavored for the fluorinated derivatives. However, at the pH value of 7.2 used for these inhibition studies, the amount of free amine was increased by this effect. Since a higher concentration of free amine should accelerate the rate of formation of **I**, this factor might explain the increased activity. Analogously, the electronic effects of the *para* substituent on the amino group can be related to the effects on inhibitory activity. Thus, a significant correlation of the pK_a values and the inhibitory activity was found for these fluorinated arylcyclopropylamines (Figure 1). In particular, for the methyl-substituted compound **11 d** increased basicity of the amino group is accompanied by very high activity. In contrast, decreased inhibition and basicity compared to **11 a** (unsubstituted) were observed for **11 b** (*para*-fluoro) and **11 c** (*para*-chloro) because of electron withdrawing substituents. Thus, two effects seem to be operating: the intrinsic effect of the fluorine atom attached at the cyclopropane ring and its influence on the pK_a value of the amino group and on the lipophilicity of the compound.

Whereas the electronic influence of the *para* substituents is consistent with effects on the pK_a values and correlates well with the inhibitory activities, the strong dependence of activity on the stereochemistry of fluorine substitution remains to be explained. Moreover, an additional inhibition mechanism for the fluorinated derivatives in contrast to tranylcypromine (**1 a**) has to be considered. As already mentioned, CAO enzymes are efficiently inhibited by chelating compounds such as triethylenetetramine.[45] There are reports in the literature that C–F bonds can also coordinate metal ions.[46] Based on these observations, we believe that chelation of the copper ion of CAO by the fluorinated arylcyclopropylamines might be involved in the inhibition. The formation of such a five-membered chelate incorporating the fluorine and amine moieties would only be possible for the *trans*-configured isomers bearing the fluorine substituent and the amino group in a *cis* configuration. The lower activity found for the diastereomers with a *trans* arrangement of these substituents further supports this assumption (Scheme 7). Also, such chelation is absent in both of the non-fluorinated tranylcypromines **1 a** and **1 b**, a fact that could explain the almost equal inhibitory activities of these isomers. No activity on a millimolar scale was observed for compound **30**, which has a *trans* configuration of the two substituents.

Again, the electronic influence of the *para* substituents on the basicity of the amino group correlates very well with this model of chelation. The amino group of the *trans*-configured, methyl-substituted derivative **11 d** should have the strongest donor ability towards the copper center, characterized by the

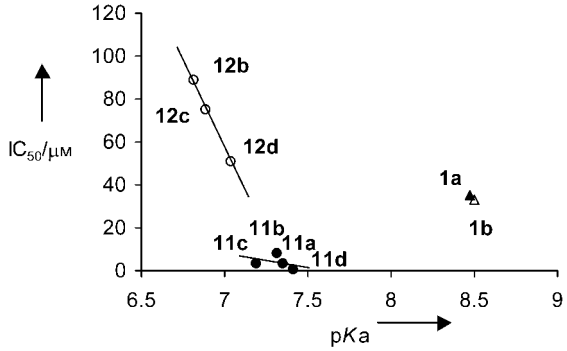
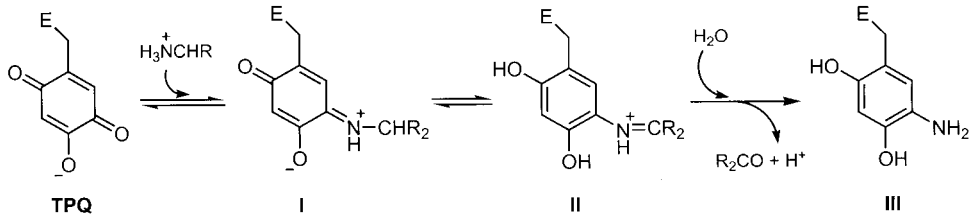
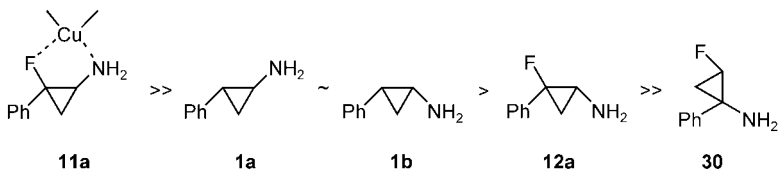


Figure 1. *Correlation of pK_a values of 2-aryl-2-fluorocyclopropylamines with IC_{50} values.*



Scheme 6. *Mechanism of the oxidation of amines by CAO, adapted from the literature.[32d] E = CH(NH$_2$)(CO$_2$H).*



Scheme 7. *Activity of different phenylcyclopropylamines.*

highest pK_a value in this series. In contrast, higher basicity but decreased inhibition was found for the homologous *trans*-2-fluoro-2-phenylcyclopropylalkylamines **15a** and **23a**. In these cases the formation of a chelate might also be disfavored, but more importantly, these compounds do not belong to the cyclopropylamines and different properties could be expected.

Since the copper center of the enzyme is involved in the activation of a water molecule responsible for the protonation of the C2 oxygen atom of **I**[32] as well as in the activation of molecular oxygen for the regeneration of TPQ[47] chelation could prevent both mechanisms. As a result, compound **II** (Scheme 6) would not be formed; this would explain reversible inhibition. However, inhibition of other CAO enzymes with other types of copper chelators, such as triethylenetetramine or *o*-phenanthroline, has been shown to be noncompetitive in nature. This would be expected if the inhibition is occurring at a site separate from the binding site.[45]

Alternatively, the interaction of fluorine with an aspartate residue of the enzyme could be responsible for the increased activity found for the fluorinated amines. It has been postulated that, during the catalytic cycle, this aspartate residue in the active site of CAO, accepts a proton from the amine substrate during oxidation.[5a, 32c, 32e] The formation of a hydrogen bond between the fluorine atom and the amino group would influence this proton transfer. Recently, we demonstrated that fluorine substituents in several monofluorinated cyclopropanes can act as acceptors for intra- and intermolecular hydrogen bonds to H–X bonds (X = C, N) in the crystalline state.[10,12] Moreover, quantum chemical calculations on the interaction energies of fluorocyclopropane dimers in the gas phase reveal weak hydrogen bridges of C–F moieties to acidic hydrogen atoms in H–C bonds.[11]

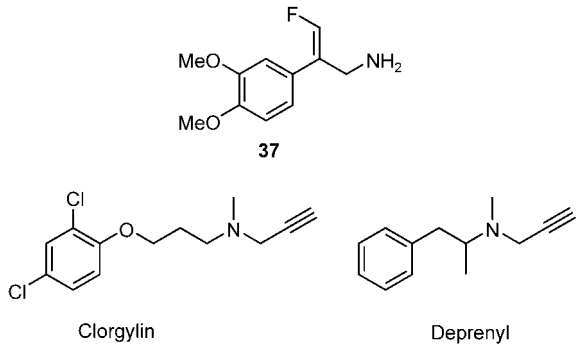
Shepard and co-workers have previously reported that, with a series of inhibitors linked to the TPQ cofactor, one enantiomer was preferentially processed.[5b,48] Analogous results were found for enantiopure amines (1*R*,2*R*)- and (1*S*,2*S*)-**11a**. Saysell et al. reported that only the (1*S*,2*R*)-(+)-enantiomer of tranylcypromine inhibits *E. coli* CAO.[5a] Enantioselectivity of tranylcypromine (**1a**) was discussed in the inhibition of *A. globiformis*.[5b] These authors suggested steric exclusion of (1*R*,2*S*)-(−)-tranylcypromine from the active site of CAO as an important factor. The results of our studies with tyramine oxidase from *Arthrobacter* sp. are consistent with the enantioselectivity of inhibition observed in earlier studies.

A more precise understanding of the molecular mechanism of the potent inhibitory activity of 2-aryl-2-fluorocyclopropylamines will clearly require more detailed studies. In particular, the coordination ability of these derivatives to copper ions in combination with molecular modeling studies should give useful information.

5. Inhibition of Flavin-Dependent Amine Oxidases MAO A and B by Fluorinated Arylcyclopropylamines

As mentioned above, flavin-containing amine oxidases exist in two isoforms: MAO A and MAO B. In vivo, these enzymes catalyze the oxidative deamination of specific biogenic amines. MAO A has a high affinity for serotonin (5-hydroxytryptamine) and norepinephrine, whereas MAO B preferentially deaminates phenylethylamine and benzylamine.[49] Dopamine, tyramine, and tryptamine are common substrates for both types of MAO. Structurally, both enzymes are closely related, with a 70% amino acid identity.[50] Each isoenzyme has a flavin cofactor covalently linked to a cysteine residue in the active center.[51–53] The crystal structure of human MAO B was recently reported by Binda et al.[54] For the MAO catalysis, two models, involving an iminium cation radical mechanism or a polar nucleophilic mechanism, have been proposed on the basis of work with purified enzymes and chemical model systems.[55]

Due to their role in the metabolism of monoamine neurotransmitters, hundreds of inhibitors of MAO have been developed and used clinically in the treatment of neurological disorders. Selective inhibitors have also been described. Whereas MAO A is more sensitive to inhibition by clorgyline, MAO B is sensitive to low concentrations of deprenyl (Scheme 8).[49] L-

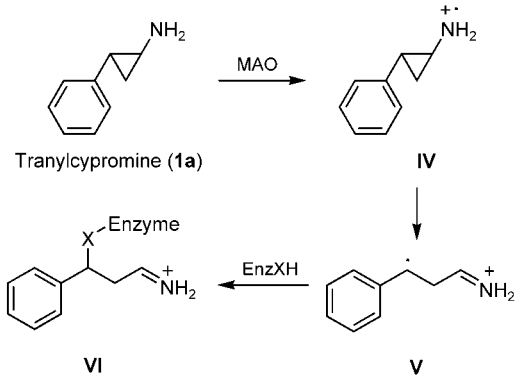


Scheme 8. *Known selective MAO inhibitors.*

Deprenyl is clinically administrated to potentiate L-DOPA therapy in the treatment of Parkinson's disease patients, as well as to provide neuroprotective effects in patients exhibiting pre-Parkinson's syndrome.[56] MAO B inhibitors are also currently in clinical trials for the treatment of Alzheimer's disease, because increased levels of MAO B have been demonstrated in plaque-associated astrocytes of brains from Alzheimer's disease patients.[57] Moclobemide, the first reversible inhibitor of MAO A, has also been used as an antidepressant.[58] A search for MAO B selective inhibitors that would be free of the well-documented "cheese effect" described above produced a series of potent 3-haloallylamines.[59] For example, 2-(3,4-dimethoxyphenyl)-3-fluoroallylamine (**37**), showed an MAO B/A selectivity of about 100.

Cyclopropylamines are also frequently used as MAO inhibitors. Tranylcypromine (**1a**) is known as an irreversible inhibitor of MAO without selectivity for MAO A or B. The inhibitory potency of tranylcypromine and its *para*-fluoro or *para*-methoxy derivatives on MAO A and MAO B in rat brains was investigated, with the results showing that the substituted compounds are slightly stronger inhibitors for both isoforms of the enzyme.[60]

The regioisomer of tranylcypromine (**1 a**), 1-phenylcyclopropylamine (**38**), is also a MAO inhibitor. Silverman and Zieske proposed a mechanism for the inhibition of MAO by **1 a** that involves initial single-electron transfer from the amine nitrogen atom to the oxidized flavin cofactor to form radical cation **IV** (Scheme 9). A similar mechanism was proposed for the inactivation of monoamine oxidase by 1-phenylcyclopropylamine.[61]



Scheme 9. *Possible mechanism for the inhibition of MAO by tranylcypromine (**1 a**), adapted from the literature.*[59]

After ring opening of **IV** to **V** followed by covalent attachment of this radical to a flavin cofactor or a cysteine residue in the active site, **VI** is formed. Hydrolysis and elimination of the enzyme from **VI** liberates cinnamylaldehyde.[62] This mechanism was also studied by Binda et al. who identified a tranylcypromine–MAO B covalent adduct formed between the ring-opened cyclopropyl moiety and the C4a position of the flavin in the X-ray crystal structure of MAO B.[54] The tranylcypromine–MAO B adduct formation is also consistent with the mechanism of inhibition for the quinone-mediated oxidative cleavage of cyclopropylamines.[63]

A facile strategy to increase the activity of analogues of tranylcypromine (**1 a**) would be to enhance this ring-opening tendency. In the literature it is well documented that fluorine substituents increase the ring strain of cyclopropanes.[13] Therefore, we expected fluorination of the cyclopropyl ring of **1 a** would be an interesting strategy to increase the activity of this class of inhibitors. In fact, analysis of the inhibition of human liver mitochondrial-outer-membrane monoamine oxidases MAO A and B by regiosomeric fluorinated arylcyclopropylamines and alkylamines revealed a significant influence of the halogen substituent on the IC_{50} values (Table 4).

Introduction of fluorine at position 2 of tranylcypromine (**1 a**) to give **11 a** slightly increased the inhibitory activity for both MAO A and B. Fluorination of the *cis* isomer **1 b** to give **12 a** resulted in no change in the inhibition of MAO B and resulted in 5.9-times lower activity against MAO A relative to **1 b**.

As with tyramine oxidase, the type of *para* substituent attached to the aromatic ring influenced the IC_{50} values. However, in marked contrast to the results obtained for tyramine oxidase, the presence of an electron-withdrawing substituent in the *trans* series increased the inhibitory activity for MAO A, while the methyl group did not change the inhibitory potency relative to unsubstituted **11 a** (Table 4). With MAO B the introduction of electron-withdrawing *para* substituents caused an even greater increase in inhibition. The *para*-chloro derivative **11 c** was the most active compound and was a compound selective for MAO A (2.3:1) in this series.

In contrast to the results with MAO A (and CAO), the *cis* series of *para*-substituted 2-fluorinated analogues of tranylcypromine showed strong inhibition of MAO B. This results in a selectivity for MAO B versus MAO A of 27:1 for **12 b**, 19:1 for **12 c**, and 8:1 for **12 d**.

In literature reports, the increase of inhibitory ability of *para*-substituted tranylcypromine analogues was explained by higher levels in the brain than the parent compound due to blocking of this position for metabolic *para*-hydroxylation in vivo.[60e] The results of our experiments, particularly the opposite action of either *para*-fluorine and -chlorine or *para*-methyl substituents, respectively, show that there is a strong electronic influence of the different *para* substituents on the intrinsic activity as well. We are currently extending our investigations to explore this issue further.

The strong effect of stereochemistry on the inhibition of MAO A and B was also demonstrated for enantiopure 2-fluoro-2-phenylcyclopropylamines (1*R*,2*R*)-**11 a** and (1*S*,2*S*)-**11 a**. Analo-

Table 4. *IC_{50} values and inhibition type for 2-fluoro-2-arylcyclopropylamines and -alkylamines.*

Compound	Isomer type[a]	R	MAO A		MAO B	
			IC_{50} [μM]	inhibition type	IC_{50} [μM]	inhibition type
1 a	*trans*	H	20 ± 0	irreversible	19 ± 0	irreversible
1 b	*cis*	H	11 ± 1	irreversible	19 ± 1	irreversible
11 a	*trans*	H	12 ± 1	irreversible	6.4 ± 0.1	irreversible
12 a	*cis*	H	65 ± 42	irreversible	19 ± 1	irreversible
11 b	*trans*	F	3.6 ± 0.2	irreversible	4.9 ± 0.1	irreversible
12 b	*cis*	F	270 ± 70	irreversible	10 ± 0	irreversible
11 c	*trans*	Cl	1.6 ± 0	irreversible	3.7 ± 0.1	irreversible
12 c	*cis*	Cl	89 ± 9	irreversible	4.8 ± 0.1	irreversible
11 d	*trans*	Me	13 ± 0	irreversible	13 ± 0	irreversible
12 d	*cis*	Me	230 ± 120	irreversible	30 ± 1	irreversible
15 a	*trans*	H	n.i.[b]	n.d.	n.i.[b]	n.d.
23 a	*trans*	H	41 ± 2	competitive	190 ± 10	n.d.
deprenyl[c]	–	–	n.i.[b]	n.d.	0.6 ± 0.1	n.d.
clorgyline	–	–	0.13 ± 0.01	n.d.	25 ± 1.2	n.d.

[a] Relative configuration of aromatic ring and amine-containing side chain. [b] n.i. = no inhibition detected at mM concentrations. [c] (*R*)-(−)-form.

gous to our results with tyramine oxidase,[15] enantiomer (1*S*,2*S*)-**11 a** was an excellent inhibitor for both MAO A and B, whereas the other enantiomer had low activity. For the homologous alkylamines, only low inhibitory activity toward either MAO A or B was found.

We also investigated the inhibitory activity of 2-fluoro-1-phenylcyclopropylamine and (2-fluoro-1-phenyl)methylamines. Both *trans*-(2-fluoro-1-phenylcyclopropyl)methylamine (**35**) and its *cis* isomer **36** showed quite low activity, but significant MAO B selectivity. Remarkably, the 2-fluoro analogue **30** of 1-phenylcyclopropylamine (**38**) was an excellent inhibitor of MAO A. In contrast, nonfluorinated **38** is known to be a weak and moderately selective (A:B = 0.26:1) inhibitor of MAO B.[64] Thus, introduction of a fluorine substituent in position 2, *trans* to the amino group, caused a dramatic reversal of isoform selectivity to give A versus B selectivity of 135:1.

The fluorinated arylcyclopropylamines caused a time- and concentration-dependent inhibition of MAO, a result indicating that the inhibition type was irreversible (Tables 4 and 5). This is in contrast to the inhibition of tyramine oxidase where reversible inhibition was observed.

Table 5. *IC_{50} values and inhibition type for 2-fluoro-1-phenylcyclopropylamine and (2-fluoro-1-phenylcyclopropyl)methylamines.*

Compound	Isomer type[a]	MAO A		MAO B	
		IC_{50} [μM]	inhibition type	IC_{50} [μM]	inhibition type
35	*trans*	320 ± 10	n.d.	24 ± 1	irreversible
36	*cis*	n.i.	n.d.	480 ± 230	n.d.
30	*trans*	3.1 ± 0.1	irreversible	420 ± 190	n.d.
38	–	730 ± 150	n.d.	190 ± 20	irreversible

[a] Relative configuration of the fluorine substituent and amine-containing side chain. [b] n.i. = no inhibition detected at mM concentrations.

As already described for tranylcypromine (**1 a**), irreversible opening of the cyclopropane ring is considered to be a crucial step in the inhibition of MAO A and B. As we had expected, fluorination of the cyclopropyl ring led to increased activity. Increased ring strain in the three-membered ring which favors ring-opening processes is one possible explanation for this result and provides a reasonable explanation for the increased potency of inhibition of MAOs by **11 a**. At this time, it is not clear how the inhibition potency is affected by *para* substitution of fluorinated *trans*-2-phenylcyclopropylamine. The correlation of the IC_{50} values with the Hammett parameters, as shown in Figure 2, indicates that *para* electron-withdrawing groups, such as Cl and F, increase potency whereas *para* electron-donating groups, such as CH_3, decrease potency. In addition, the position of the fluorine substituent on the cyclopropane ring was demonstrated to have an important effect on the MAO A/B selectivity.

Interestingly, inhibition studies with mono- and difluorinated phenethylamines revealed that consecutive fluorination leads to decreased inhibitory activity against MAO as a result of

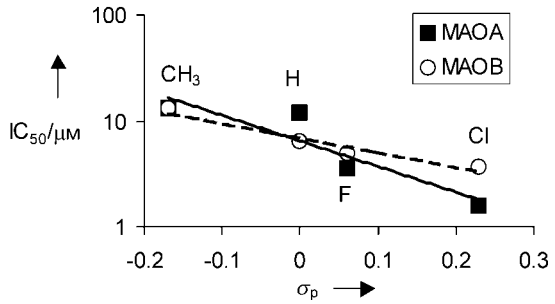


Figure 2. *Correlation of IC_{50} values of the inhibitors with the Hammett parameters (σ_p) of the* para *substituents. The parameters of the substituents were taken from ref. [65].*

lower pK_a values of the amino group.[29] Whereas we also observed lower basicity for 2-aryl-2-fluorocyclopropylamines, higher inhibition activity was observed compared to nonfluorinated **1 a**. This suggests that effects other than changes in basicity, such as an increase in cyclopropyl ring strain, are important.

Recently, structural differences of human MAO A and B were discussed that were considered to be responsible for substrate and inhibitor selectivity of these enzymes.[66] For the binding of the inhibitors to these enzymes, differences in lipophilicity could be important. In our case, we observed a dramatic reversal of isoform selectivity by introduction of fluorine in position 2 of 1-phenylcyclopropylamine. At this time we have no explanation for why fluorine substitution reverses the MAO selectivity. However, it is possible that the presence of fluorine would affect the interaction of the inhibitor with key amino acid residues that play important roles in catalysis and/or in binding at the MAO active site. It should be noted that physicochemical data reveal that the introduction of a fluorine atom, as well as the type of substituent in *para* position, influenced the log *D* values in the 2-phenylcyclopropylamine series. The physicochemical data of 1-phenylcyclopropylamine analogues have not been obtained yet.

6. Summary and Outlook

In summarizing these results we conclude that the introduction of a fluorine substituent on the three-membered ring of tranylcypromine (**1 a**) improved inhibitory activity against amine oxidases such as copper-dependent tyramine oxidase (CAO) and flavin-dependent human liver mitochondrial-outer-membrane monoamine oxidases A and B (MAO A and MAO B). Physicochemical data such as pK_a values, permeability, and log *D* values revealed an interesting pharmacokinetic profile for diastereopure 2-aryl-2-fluorocyclopropylamines that is strongly influenced by the fluorine substituent, the regiochemistry of incorporation, and the relative and absolute configuration of substituents on the three-membered ring. *para* Substituents in the aromatic ring were shown to have a weak influence on these properties. Results of the biological studies demonstrated that both substitution with fluorine and cyclopropane stereochemisty have a strong influence on the effectiveness of

inhibition. However, a definitive understanding of the mechanism(s) leading to the increased activity will require further investigation. To that end, synthesis of additional analogues of fluorinated phenylcyclopropylamines is ongoing. In addition to providing mechanistic insight, we feel this may lead to even more potent inhibitors.

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Keywords: aminocyclopropanes · fluorine · inhibitors · oxidases · structure–activity relationships

[1] T. P. Singer, R. W. Von Korff, D. L. Murphy in *Monoamine Oxidase: Structure, Function and Altered Functions*, Academic Press, New York, **1979**.
[2] a) A. Mai, M. Artico, M. Esposito, G. Sbardella, S. Massa, O. Befani, P. Turini, V. Giovannini, B. Mondovi, *J. Med. Chem.* **2002**, *45*, 1180 – 1183, and references therein; b) C. J. Fowler, S. B. Ross, *Med. Res. Rev.* **1984**, *4*, 323 – 358, and references therein.
[3] a) J. P. Klinman, *Chem. Rev.* **1996**, *96*, 2541 – 2561, and references therein; b) P. H. Yu, S. Wright, E. H. Fan, Z.-R. Lun, D. Gubisne-Haberle, *Biochem. Biophys. Acta* **2003**, *1647*, 193 – 199, and references therein.
[4] A. Zorzano, A. Abella, L. Marti, C. Carpene, M. Palacin, X. Testar, *Biochem. Biophys. Acta* **2003**, *1647*, 3 – 9, and references therein.
[5] a) C. G. Saysell, W. S. Tambyrajah, J. M. Murray, C. M. Wilmot, S. E. V. Phillips, M. J. McPherson, P. F. Knowels, *Biochem. J.* **2002**, *365*, 809 – 816; b) E. M. Shepard, H. Heather, G. A. Juda, D. M. Dooley, *Biochim. Biophys. Acta* **2003**, *1647*, 252 – 259.
[6] P. Foley, M. Gerlach, M. B. H. Youdin, P. Riederer, *Parkinsonism Rel. Disord.* **2000**, *6*, 25 – 47.
[7] a) D. G. Folks, *J. Clin. Psychopharmacol.* **1983**, *3*, 249 – 252; b) M. B. Youdim, J. P. Finberg, *Mod. Probl. Pharmacopsychiatry* **1983**, *19*, 63 – 74.
[8] Reviews: a) K. L. Kirk in *Selective Fluorination in Organic and Bioorganic Chemistry* (Ed.: J. T. Welch), ACS Symposium Series 456, American Chemical Society, Washington, DC, **1991**, pp. 136 – 155; b) R. Filler in *Organofluorine Compounds in Medicinal Chemistry and Biomedical Applications* (Eds.: R. Filler, Y. Kobashi, L. M. Yagupolskii), Elsevier, Amsterdam, **1993**, 1 – 22; c) M. Schlosser in *Enantiocontrolled Synthesis of Fluoro-Organic Compounds. Stereochemical Challenges and Biomedicinal Targets* (Ed.: V. A. Soloshonok), Wiley, Chichester, **1999**, pp. 613 – 659; d) B. E. Smart, *J. Fluorine Chem.* **2001**, *109*, 3 – 11.
[9] M. Schlosser, *Angew. Chem.* **1998**, *110*, 1538 – 1556; *Angew. Chem. Int. Ed.* **1998**, *37*, 1496 – 1513.
[10] G. Haufe, T. C. Rosen, O. G. J. Meyer, R. Fröhlich, K. Rissanen, unpublished results.
[11] I. Hyla-Kryspin, G. Haufe, S. Grimme, *Chem. Eur. J.* **2004**, *10*, 3411 – 3422.
[12] G. Haufe, T. C. Rosen, O. G. J. Meyer, R. Fröhlich, K. Rissanen, *J. Fluorine Chem.* **2002**, *114*, 189 – 198.
[13] W. R. Dolbier, M. A. Battiste, *Chem. Rev.* **2003**, *103*, 1071 – 1098, and references therein.
[14] G. B. Baker, L. J. Urichuk, K. F. McKenna, S. H. Kennedy, *Cell Mol. Neurobiol.* **1999**, *19*, 411 – 426.
[15] S. Yoshida, O. G. J. Meyer, T. C. Rosen, G. Haufe, S. Ye, M. J. Sloan, K. L. Kirk, *J. Med. Chem.* **2004**, *47*, 1796 – 1806.
[16] a) The designation *cis* or *trans* is used to assign the relative configuration of the aryl group relative to the substituent in the vicinal position in order to allow comparison with the parent tranylcypromine; b) T. C. Rosen, S. Yoshida, R. Fröhlich, K. L. Kirk, G. Haufe, unpublished results.
[17] S. Yoshida, T. C. Rosen, O. G. J. Meyer, S. Ye, M. J. Sloan, G. Haufe, K. L. Kirk, *Bioorg. Med. Chem.* **2004**, *12*, 2645 – 2652.
[18] O. G. J. Meyer, R. Fröhlich, G. Haufe, *Synthesis* **2000**, 1479 – 1490.
[19] a) M. J. Sloan, K. L. Kirk, *Tetrahedron Lett.* **1997**, *38*, 1677 – 1680; b) M. J. Sloan, K. L. Kirk, P. Skolnick, I.-H. Kim, R. Layer, *Proceedings: NOBCChE*, **2000**, *27*, 89 – 100.
[20] a) E. H. White, *J. Am. Chem. Soc.* **1955**, *77*, 6008 – 6010; b) D. T. Glatzhofer, R. R. Roy, K. N. Cossey, *Org. Lett.* **2002**, *4*, 2349 – 2352, and references therein.
[21] a) C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney, *Adv. Drug Delivery Rev.* **1997**, *23*, 3 – 25; b) C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney, *Adv. Drug Delivery Rev.* **2001**, *46*, 3 – 26.
[22] I. Muegge, *Chem. Eur. J.* **2002**, *8*, 1976 – 1981.
[23] G. Wess, M. Urmann, B. Sickenberger, *Angew. Chem.* **2001**, *113*, 3443 – 3453, *Angew. Chem. Int. Ed.* **2001**, *40*, 3341 – 3350.
[24] H. van de Waterbeemd, E. Gifford, *Nat. Rev. Drug Discovery* **2003**, *2*, 192 – 204, and references therein.
[25] T. Kennedy, *Drug Discovery Today* **1997**, *2*, 436 – 444.
[26] D. F. Veber, S. R. Johnson, H.-Y. Cheng, B. R. Smith, K. W. Ward, K. D. Kopple, *J. Med. Chem.* **2002**, *45*, 2615 – 2623.
[27] B. E. Smart, *Characteristics of C–F Systems in Organofluorine Chemistry. Principles and Commercial Applications* (Eds.: R. E. Banks, B. E. Smart, J. C. Tatlow), Plenum Press, New York, **1994**, pp. 57 – 88.
[28] These measurements were done in cooperation with Prof. K. Müller, Hoffmann La Roche AG, Basel, Switzerland.
[29] R. W. Fuller, B. B. Molloy in *Biochemistry involving carbon–fluorine bonds* (Ed.: R. Filler), ACS Symposium Series 28, American Chemical Society, Washington, DC, **1976**, pp. 77 – 98.
[30] M. Kansy, F. Senner, K. Gubernator, *J. Med. Chem.* **1998**, *41*, 1007 – 1010.
[31] Classification of lipophilicity: $\log D \leq -1$ very low, $\log D \leq 1$ low, $\log D \leq 2.5$ medium, $\log D \leq 4$ high, $\log D > 4$ very high. The distribution coefficient ($\log D$) assigns the ratio of the concentration of compound in the lipid phase to the concentration of all species in the aqueous phase at a given pH value, while the partition coefficient ($\log P$) refers to the ratio of un-ionized compound in each phase. R. A. Scherrer, S. M. Howard, *J. Med. Chem.* **1977**, *20*, 53 – 58.
[32] a) M. R. Parsons, M. A. Convery, C. M. Wilmot, K. D. S. Yadav, V. Blakeley, A. S. Corner, S. E. V. Phillips, M. J. McPherson, P. F. Knowles, *Structure* **1995**, *3*, 1171 – 1184; b) M. C. J. Wilce, D. M. Dooley, H. C. Freeman, J. M. Guss, H. Matsunami, W. S. McIntire, C. E. Ruggiero, K. Tanizawa, H. Yamaguchi, *Biochemistry* **1997**, *36*, 16116 – 16133; c) R. Li, J. P. Klinman, F. S. Mathews, *Structure* **1998**, *6*, 293 – 307; d) V. Kumar, D. M. Dooley, H. C. Freeman, J. M. Guss, I. Harvey, M. A. McGuirl, M. C. J. Wilce, V. M. Zubak, *Structure* **1996**, *4*, 943 – 955; e) M. Mure, S. A. Mills, J. P. Klinman, *Biochemistry* **2002**, *41*, 9269 – 9278.
[33] S. M. Janes, D. Mu, D. Wemmer, A. J. Smith, S. Kaur, D. Maltby, A. L. Burlingame, J. P. Klinman, *Science* **1990**, *248*, 981 – 987; b) S. M. Janes, J. P. Klinman, *Biochemistry* **1991**, *30*, 4599 – 4605; c) R. A. Cooper, P. F. Knowles, D. E. Brown; M. A. McGuire, D. M. Dooley, *Biochem. J.* **1992**, *288*, 337 – 340; d) J. P. Klinman, *Biochem. Biophys. Acta* **2003**, *1647*, 131 – 137.
[34] J. Salaün, *Top. Curr. Chem.* **2000**, *207*, 1 – 67.
[35] P. H. Yu, S. Wright, E. H. Fan, Z.-R. Lun, D. Gubisne-Haberle, *Biochem. Biophys. Acta* **2003**, *1647*, 193 – 199, and references therein.
[36] A. Zorzano, A. Abella, L. Marti, C. Carpene, M. Palacin, X. Testar, *Biochem. Biophys. Acta* **2003**, *1647*, 3 – 9, and references therein.
[37] I. Ferrer, J. M. Lizcano, M. Hernández, M. Unzeta, *Neurosci. Lett.* **2002**, *321*, 21 – 24.
[38] a) R. Lewinsohn, *Brazilian J. Med. Biol. Res.* **1984**, *17*, 223 – 256; b) J. M. Lizcano, E. Escrich, T. Ribalta, J. Muntane, M. Unzeta, *Biochem. Pharmacol.* **1995**, *42*, 263 – 269.
[39] F. Boomsma, U. S. Bhaggoe, A. M. B. van der Houwen, A. H. van den Meiracker, *Biochem. Biophys. Acta* **2003**, *1647*, 48 – 54, and references therein.
[40] J. Wouters, P. Perpete, P. Hayen, N. Anceau, F. Durant, *Biochem. Mol. Biol. Int.* **1994**, *32*, 737 – 743.

[41] a) S. M. Janes, J. P. Klinman, *Biochemistry* **1991**, *30*, 4599 – 4605; b) R. Matsuzaki, T. Fukui, H. Sato, Y. Ozaki, K. Tanizawa, *FEBS Lett.* **1994**, *351*, 360 – 364; c) R. A. Cooper, P. F. Knowles, D. E. Brown, M. A. McGuirl, D. M. Dooley, *Biochem. J.* **1992**, *288*, 337 – 340; d) S. M. Janes, M. M. Palcic, C. H. Scaman, A. J. Smith, D. E. Brown, D. M. Dooley, M. Mure, J. P. Klinman, *Biochemistry* **1992**, *31*, 12147 – 12154.

[42] a) K. B. Wiberg in *The Chemistry of the Cyclopropyl Group, Vol. 1* (Ed.: Z. Rappoport), Wiley, Chichester, **1987**, pp. 1 – 26; b) D. Cremer, E. Kralla, K. J. Szabo in *The Chemistry of the Cyclopropyl Group, Vol. 2* (Ed.: Z. Rappoport), Wiley, Chichester, **1995**, pp. 43 – 137; c) K. B. Wiberg in *Methods of Organic Chemistry, Houben-Weyl, Vol. E17a* (Ed.: A. de Meijere), Thieme, Stuttgart, **1997**, pp. 1 – 27.

[43] G. A. Lyles, C. M. S. Marshall, I. A. McDonald, P. Bey, M. G. Palfreyman, *Biochem. Pharmacol.* **1987**, *36*, 2847 – 2853.

[44] S. Hirota, T Iwamoto, S. Kishishita, T. Okajima, O. Yamauchi, K. Tanizawa, *Biochemistry* **2001**, *40*, 15789 – 15796.

[45] L. Zajoncová, I. Frébort, L. Luhová, M. Šebela, P. Galuszka, P. Pec, *Biochem. Mol. Biol. Int.* **1999**, *47*, 47 – 61.

[46] a) J. L. Kiplinger, T. G. Richmond, C. E. Osterberg, *Chem. Rev.* **1994**, *94*, 373 – 431; b) H. Plenio, *Chem. Rev.* **1997**, *97*, 3363 – 3384.

[47] R. Prabhakar, P. E. M. Siegbahn, *J. Phys. Chem. B* **2003**, *107*, 3944 – 3953, and references therein.

[48] E. M. Shepard, J. Smith, B. O. Elmore, J. A. Kuchar, L. M. Sayre, D. M. Dooley, *Eur. J. Biochem.* **2002**, *269*, 3645 – 3658.

[49] a) J. P. Johnston, *Biochem. Pharmacol.* **1968**, *17*, 1285; b) J. Knoll, K. Magyar, *Adv. Biochem. Psychopharmacol.* **1972**, *5*, 393 – 408.

[50] A. W. Bach, N. C. Lan, D. L. Johnson, C. W. Abell, M. E. Bembenek, S. W. Kwan, P. D. Seeburg, J. C. Shih, *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 4934 – 4938.

[51] J. Wouters, *Curr. Med. Chem.* **1998**, *5*, 137 – 162.

[52] E. B. Kearney, J. I. Salach, W. H. Walker, R. L. Seng, W. Kenny, E. Zeszotek, T. P. Singer, *Eur. J. Biochem.* **1971**, *24*, 321 – 327.

[53] a) P. H. Yu, *Can. J. Biochem.* **1981**, *59*, 30 – 37; b) H. F. Wu, K. Chen, J. C. Shih, *Mol. Pharmacol.* **1993**, *43*, 888 – 893.

[54] a) C. Binda, P. Newton-Vinson, F. Hubalek, D. E. Edmondson, A. Mattevi, *Nat. Struct. Biol.* **2002**, *9*, 22 – 26; b) C. Binda, M. Li, F. Hubalek, N. Restelli, D. E. Edmondson, A. Mattevi, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 9750 – 9755; c) C. Binda, F. Hubálek, M. Li, Y. Herzig, J. Sterling, D. E. Edmondson, A. Mattevi, *J. Med. Chem.* **2004**, *47*, 1767 – 1774.

[55] a) M. C. Walker, D. E. Edmondson, *Biochemistry* **1994**, *33*, 7088 – 7098; b) J. R. Miller, D. E. Edmondson, *Biochemistry* **1999**, *38*, 13670 – 13683; c) R. B. Silverman, S. J. Hoffman, W. B. Catus, *J. Am. Chem. Soc.* **1980**, *102*, 7126 – 7128; d) J. M. Kim, M. A. Bogdan, P. S. Mariano, *J. Am. Chem. Soc.* **1993**, *115*, 10591 – 10595.

[56] A. M. Cesura, A. Pletscher, *Prog. Drug Res.* **1992**, *38*, 171 – 297.

[57] J. Saura, J. M. Luque, A. M. Cesura, M. Da Prada, V. Chan-Palay, G. Huber, J. Loffler, J. G. Richards, *Neuroscience* **1994**, *62*, 15 – 30.

[58] R. Amrein, J. R. Martin, A. M. Cameron, *Adv. Neurol.* **1999**, *80*, 509 – 519.

[59] M. G. Palfreyman, P. Bey, A. Sjoerdsman, *Essays Biochem.* **1987**, *23*, 28 – 81.

[60] a) R. T. Coutts, T. S. Rao, G. B. Baker, R. G. Micetich, T. W. E. Hall, *Cell. Mol. Neurobiol.* **1987**, *7*, 271 – 289; b) A. J. Greenshaw, A. J. Nazarali, T. S. Rao, G. B. Baker, R. T. Coutts, *Eur. J. Pharmacol.* **1988**, *154*, 67 – 72; c) A. J. Greenshaw, T. S. Rao, A. J. Nazarali, G. B. Baker, R. T. Coutts, *Biol. Psychiatry* **1989**, *25*, 1014 – 1020; d) R. L. Sherry, G. B. Baker, R. T. Coutts, *Biol. Psychiatry* **1990**, *28*, 539 – 543; e) R. L. Sherry, G. B. Baker, R. T. Coutts, D. D. Mousseau, *J. Neural. Transm.* **1990**, *Suppl. 32*, 107 – 112.

[61] R. B. Silverman, P. A. Zieske, *Biochemistry* **1985**, *24*, 2128 – 2138.

[62] R. B. Silverman, *J. Biol. Chem.* **1983**, *258*, 4766 – 4769.

[63] L. M. Sayre, M. P. Singh, P. B. Kokil, F. Wang, *J. Org. Chem.* **1991**, *56*, 1353 – 1355.

[64] R. B. Silverman, C. K. Hiebert, *Biochemistry* **1988**, *27*, 8448 – 8453.

[65] S. H. Pine, *Organic Chemistry*, 5th ed., McGraw–Hill Book Company, New York, **1987**, p. 634.

[66] R. M. Geha, I. Rebrin, K. Chen, J. C. Shih, *J. Biol. Chem.* **2001**, *276*, 9877 – 9882.

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