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Fluorinated phenylcyclopropylamines
Part 6. Effects of electron withdrawing or donating aryl substituents on the inhibition of tyramine oxidase from *Arthrobacter* sp. by diastereomeric 2-aryl-2-fluoro-cyclopropylamines

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ABSTRACT

Diastereomeric arylcyclopropylamines substituted with fluorine in the 2-position and with electron donating or electron withdrawing groups at the aromatic ring were evaluated as inhibitors of microbial tyramine oxidase. The *trans*-isomers were consistently more potent inhibitors of the enzyme than the *cis*-isomers. Electron donating substituents increased the potency of tyramine oxidase inhibition, while electron withdrawing substituents decreased the activity. The results obtained are discussed in terms of pK_a and log *D* values of the inhibitors as well as the mechanism of action of tranylcypromines and the geometry of the active site of the enzyme.

1. Introduction

Tyramine oxidase belongs to the class of monoamine oxidases designated as copper amine oxidases (CAO), which contain one or more copper ions in the active center. These enzymes (EC 1.4.3.6) are also called semicarbazide sensitive amine oxidases (SSAO) because they can be inactivated by carbonyl reagents such as semicarbazide. There is currently much interest in CAOs due to their association with pathological conditions of several diseases. For example, CAO-catalyzed oxidation of exogenous amine substrates or endogenous neurotransmitters is thought to contribute to damage to nerve cells, tissues, capillary vessel and other similar problems that are associated with diabetes mellitus types I and II [1]. This has been ascribed to abnormally high activities of CAOs which leads to an excessive formation of toxic metabolites such as hydrogen peroxide and ammonia [2]. Other enzymes, including MAO A and B, have similar roles of oxidative deamination. However, abnormal functioning of these enzymes is associated with other diseases such as depressions (MAO A) or Parkinson's and Alzheimer's diseases (MAO B) [3,4]. Since the latter enzymes are flavin-dependent they have a different mechanism of action. They also have different biodistributions. Despite these differences, inhibitors targeted to a particular amine oxidase may show cross-reactivities with other types of monoamine oxidases. This is a major cause of various side effects elicited by drugs of this class that are in clinical use.

The problems associated with side effects can be exemplified by the behavior of tranylcypromine (**1**) (Fig. 1). Recent reports also discussed MAO inhibitors as useful agents against neurodegenerative disorders such as Parkinson's or Alzheimer's diseases [5]. However, **1** has little selectivity and also inhibits MAO A, B and CAOs [6]. To target the inhibition of MAO B for the treatment of above diseases, the non-selective inhibitor, tranylcypromine, can lead to side effects, including the well known "cheese" effect caused by inhibition of MAO A. Moreover, to prevent damage to nerve cells, tissues, capillary vessel and other similar problems that are associated with diabetes mellitus types I and II [1], clinical use of non-selective CAO inhibitors may result in MAO A inhibition which can result in cheese effect. In this work we have explored the influence of fluorine substitution at the cyclopropane ring and of substituents at the aromatic ring on the activity and selectivity in analogues of tranylcypromine. The goal was to develop inhibitors

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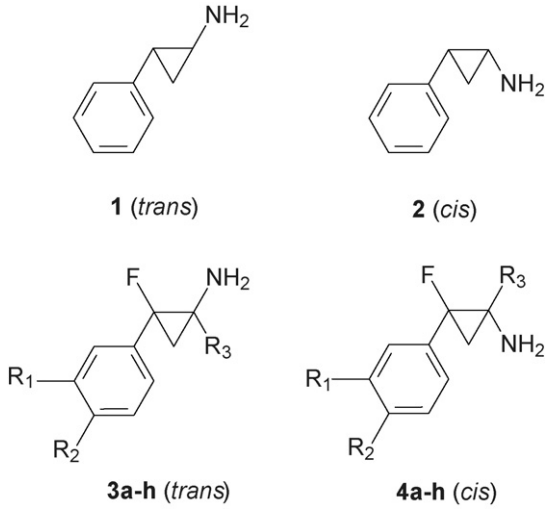

Fig. 1. Investigated compounds.

with higher potency and better selectivity than the parent compound as useful tools for understanding all roles and function of CAOs and as valuable substances for treatment of various diseases [7]. We have reported previous results in this series that revealed influences of fluorine substitution on both selectivity and potency [6]. In this report we present further studies on the influence of electron withdrawing and donating groups on the inhibition of microbial tyramine oxidase from *Arthrobacter* sp. by fluorinated arylcyclopropylamines. Previous data and new results are also reviewed and analyzed with respect to physico-chemical properties, including pK_a and lipophilicity (log *D*).

2. Results and discussion

2.1. Inhibition of copper-containing tyramine oxidase by fluorinated arylcyclopropylamines

Both diastereomers of aryl substituted 2-aryl-2-fluorocyclopropylamines **3** and **4** (Fig. 1) were examined as inhibitors of microbial tyramine oxidase from *Arthrobacter* sp. and compared to the non-fluorinated parents **1** and **2** (Fig. 1). The effects of both electron withdrawing (F, Cl, CF_3) and electron donating (Me, MeO) substituents were studied. Synthesis of these analogues is described in our previous paper as is their activity as inhibitors of MAO A and MAO B [7].

The *para*-CF_3 substituted *trans*-2-fluoro-2-phenylcyclopropylamine (**3d**) was a competitive inhibitor of CAO as expected from the previous assay results of similar analogues (Table 1) [8–11]. The IC_{50} value of this compound (3.6 μM) was comparable to the non-substituted **3a** and the *para*-chloro-substituted **3b** analogues (Table 1, Fig. 2). No inhibition was observed with the *cis*-isomer **4d** in the micromolar range (Fig. 3). As shown previously, the *para*-methyl analogue **3e** (*trans*) was 10 times more potent than the non-substituted **3a** and 100 times more potent than tranylcypromine (**1**). In the *cis*-series, the *para*-methyl analogue **4e** (*cis*) likewise was more potent than the unsubstituted **4a** (*cis*). The *para*-OMe substituted *trans*-analogue **3f** was a more potent inhibitor (0.8 μM), but this compound showed a weak time- and concentration-dependent inhibition of tyramine oxidase (Fig. 4A). The *cis*-isomer **4f** showed modest inhibition for tyramine oxidase (Table 1 and Fig. 4B). The dimethoxy-substituted *trans*-analogue **3g** was a good inhibitor and showed a weak time- and concentration-dependent inhibition (Table 1 and Fig. 4C). Neither

Table 1
IC_{50} values and types of inhibition of known and new inhibitors of tyramine oxidase

R^1	R^2	R^3	Compound	IC_{50} [μM] (type of inhibition)[a]
H	H	H	**1**[b]	35 ± 6 (comp.)
			2[b]	33 ± 1 (irr.)
H	H	H	**3a**[b]	3.6 ± 1.5 (comp.)
			4a[b]	190 ± 90 (part. irr.)
H	Cl	H	**3b**[b]	3.7 ± 0.3 (comp.)
			4b[b]	89 ± 25 (part. irr.)
H	F	H	**3c**[b]	8.1 ± 1.6 (comp.)
			4c[b]	75 ± 12 (part. irr.)
H	CF_3	H	**3d**	3.6 ± 0.1 (comp.)
			4d	–[c](–)[d]
H	Me	H	**3e**[b]	0.39 ± 0.17 (comp.)
			4e[b]	51 ± 5 (–)[d]
H	OMe	H	**3f**	0.82 ± 0.0 (part. irr.)
			4f	17 ± 6 (irr.)
OMe	OMe	H	**3g**	2.2 ± 0.1 (part. irr.)
			4g	176 ± 4 (–)[d]
H	H	Ph	**3h**[e]	–[c](–)[d]
			4h[e]	–[c](–)[d]

[a] irr.: irreversible, comp.: competitive.
[b] The corresponding data were taken from the literature [6].
[c] No inhibition was observed in micromolar range.
[d] Could not be determined.
[e] **3h** (*cis*) and **4h** (*trans*): *cis* and *trans* refers to the arrangement of the two phenyl rings.

of the diphenyl analogues (**3h**, **4h**) showed detectable inhibition of tyramine oxidase.

The K_i values of competitive inhibitors, **1** and **3a**–**3e**, were calculated from the data of Lineweaver–Burk plot as listed in Table 2.

2.2. Inhibition mechanism of tyramine oxidase

Tyramine oxidase from *Arthrobacter* sp. is a copper-containing topaquinone-dependent amine oxidase. This had been sold by Sigma as a flavin-dependent enzyme, although we and others have carried out experiments that characterize it as a copper-dependent amine oxidase (CAO). Thus it will be considered as such in the following discussion [11–13].

The enzymatic oxidative deamination of amines by tyramine oxidase follows a ping-pong mechanism consisting of two hemi reactions, one being an oxidation and the other being a reduction

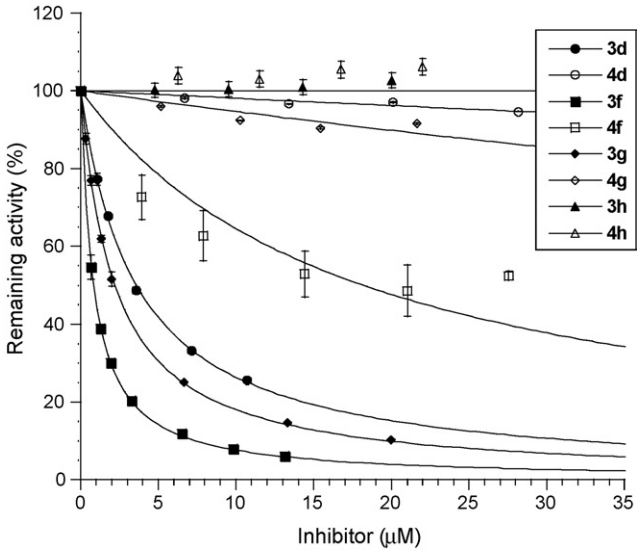

Fig. 2. Effect of concentration of fluorinated phenylcyclopropylamines on tyramine oxidase activity.

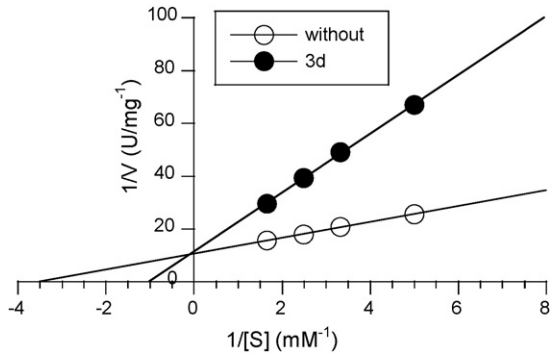

Fig. 3. Lineweaver–Burk plot for inactivation of tyramine oxidase by *trans*-2-fluoro-2-(4-trifluoromethyphenyl)cyclopropylamine (**3d**). The benzylamine oxidation was monitored as described in Section 4 in the presence (0.0035 mM) and in the absence of compound **3d**. The data were collected after the inhibition by **3d** was checked not to be time- and concentration-dependent.

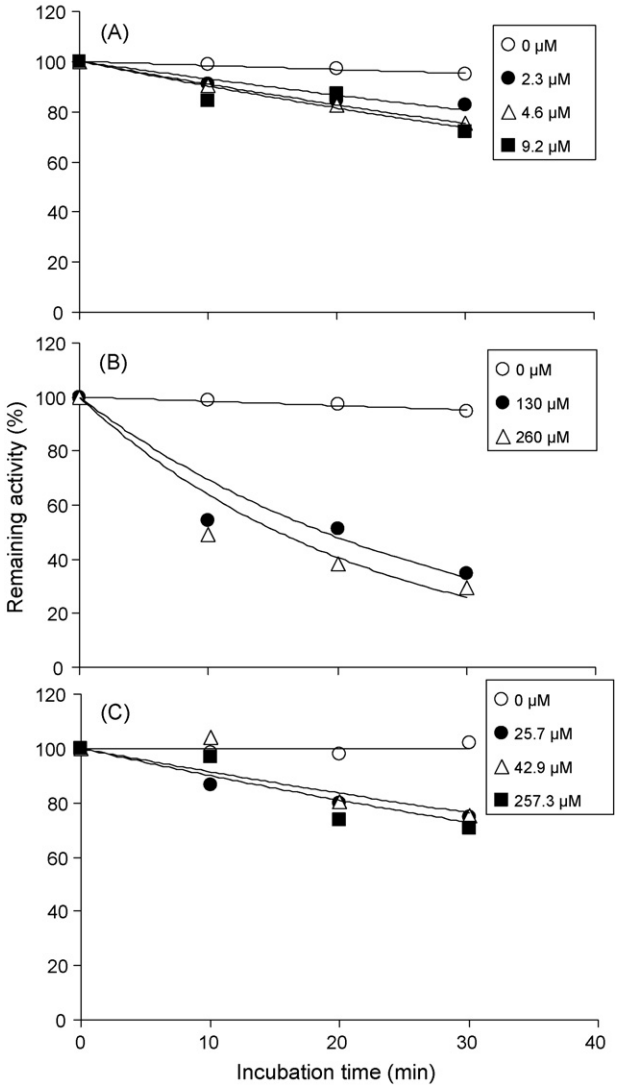

Fig. 4. Time- and concentration-dependent inactivation of tyramine oxidase by 2-fluoro-2-(*para*-methoxyphenyl)cyclopropylamine HCl (A, *trans*-isomer (**3f**); B, *cis*-isomer (**4f**)) and 2-fluoro-2-(3,4-dimethoxyphenyl)cyclopropylamine HCl (C, **3g**).

Table 2
Kinetic parameters of *trans*-arylcyclopropylamines **1** and **3** for tyramine oxidase

R^1	R^2	R^3	Compound	k_{cat} (s^{-1})	K_i (μM)
H	H	H	**1**	0.11 ± 0.02	17 ± 3
H	H	H	**3a**	0.12 ± 0.02	0.83 ± 0.01
H	Cl	H	**3b**	0.11 ± 0.00	0.91 ± 0.01
H	F	H	**3c**	0.12 ± 0.01	3.8 ± 1.4
H	CF_3	H	**3d**	0.11 ± 0.02	1.3 ± 0.3
H	Me	H	**3e**	0.12 ± 0.01	0.16 ± 0.01

[14,15]. In the first step of the reductive hemi reaction the amine attacks the C(5) of TPQ and forms the Schiff's base **II** (see Fig. 5) via a hemiaminal. A channel with a negatively charged entrance has been identified that facilitates attraction of the substrate as the ammonium species [16]. This channel becomes more hydrophobic as it approaches the active site to give an environment that would favor the more hydrophobic free amine [16,17]. Nucleophilic attack of the amine at C(5) would follow the normal general acid-catalyzed process for nucleophilic addition to a carbonyl group [18]. This attack occurs exclusively at C(5) because the other possible positions, C(2) and C(4), have lower electrophilicities due to resonance stabilization and because they are in a less favorable orientation towards the substrate. As the reaction proceeds, an active site aspartate residue abstracts a proton from the C_α atom leading to tautomerization and aromatization of the quinone ring to give the intermediate iminium ion **III**. For the elimination of water during the formation of **II** a proton is donated from an amino acid residue to give the product as an iminium ion. Subsequent hydrolysis releases an aldehyde and the cofactor as amino resorcinol (TPQ_{red}). In the oxidative hemi reaction TPQ_{red} can be reoxidized to TPQ catalyzed by copper in the presence of oxygen with formation of ammonia and hydrogen peroxide.

2.3. Inhibitors of tyramine oxidase.

Inhibitors of CAO have been developed that possess a C=C double bond or a halogen substituent in the β-position of the amine function. These compounds exhibit a highly electrophilic center in intermediate **III** that can form a covalent bond with an amino acid residue in the active site [19–22].

The cyclopropane ring of tranylcypromine (**1**) can be seen as a mimic of the double bond and this relationship could contribute to the ability of **1** to function as an inhibitor of various CAOs [22]. A crystal structure of the adduct formed between **1** and CAO from *Escherichia coli* (ECAO) shows that, although racemic **1** was used, only the (1*S*,2*R*)-enantiomer was found in the active site (see Fig. 6). Steric constraints thus permit only this enantiomer to fit into the active site and form a Schiff's base with C(5) of TPQ [23]. In this arrangement the proton at the C_α atom points away from the aspartate residue and therefore cannot be abstracted. This is in agreement with the favored pro-(*S*) deprotonation during oxidation of primary amines with ECAO [22]. Since the formation of the Schiff's base is a reversible step (1*S*,2*R*)-(+)-**1** is a reversible, competitive inhibitor of CAO. For CAOs from other sources the geometry of the active site could be different, so that no or even pro-(*R*) selectivity might be observed in some cases [22].

2.4. Inhibition by fluorinated analogues of tranylcypromine

The inhibition of tyramine oxidase from *Arthrobacter* sp. by both isomers of tranylcypromine **1** (*trans*) and **2** (*cis*) is in the same order of magnitude as the inhibition of MAO A and B, giving IC_{50} values of 33 μM and 35 μM, respectively, without preferences for any configuration. As an approach to find analogues of **1** and **2** with improved activity and selectivity, we examined 2-arylcyclopro-

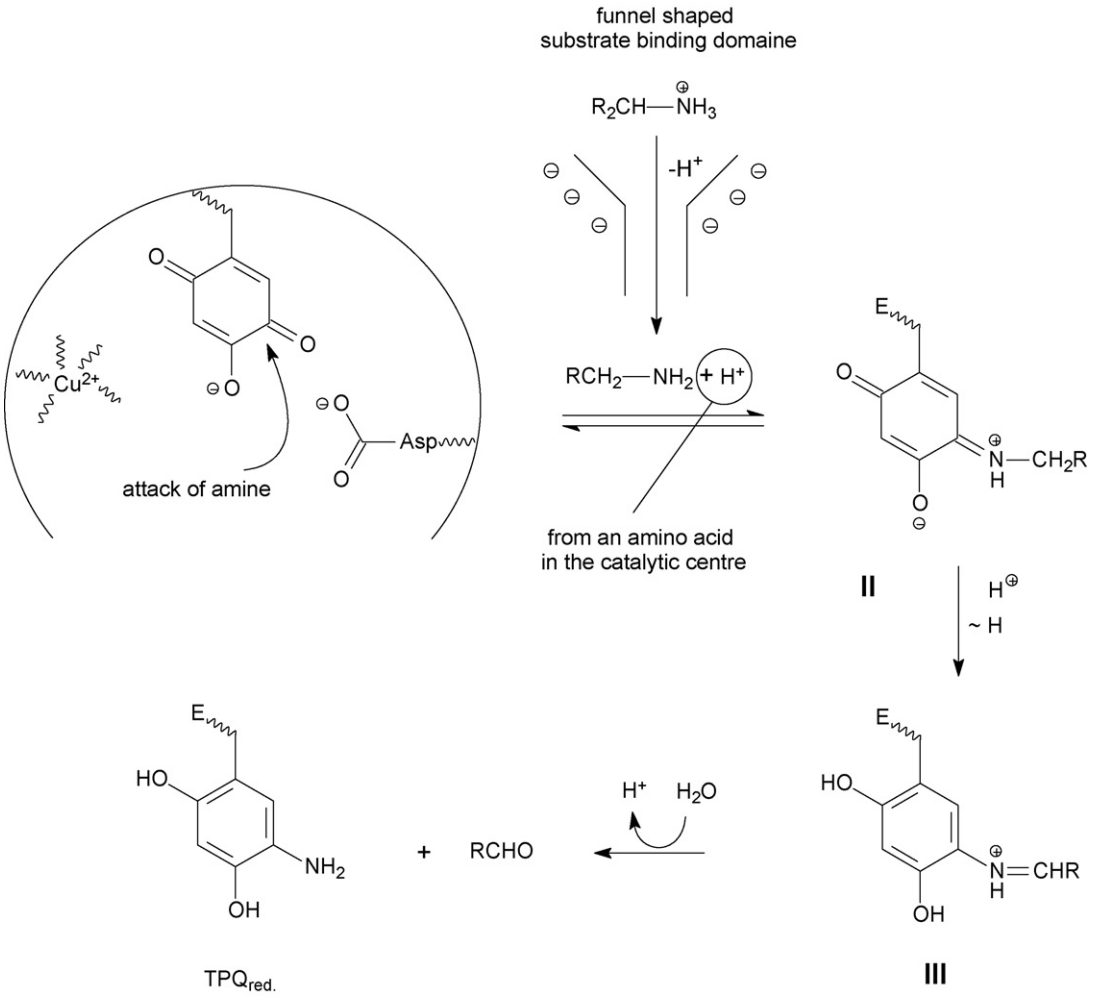

Fig. 5. Reductive reaction of the catalysis by TPQ–CAO, E: enzyme.

pylamines with modified electronic and geometric characteristics bearing electron donating or electron withdrawing substituents of different size at the aromatic ring and a fluorine substituent at the three-membered ring.

A priori one might expect fluorine substitution to lead to lower inhibitory activity, since ammonium groups are preferentially recognized in the initial approach of the substrate to the active site and since fluorine substitution will lower pK_a values, as discussed above. Additionally, the fluorinated cyclopropyl free amine should be a weaker nucleophile and react more slowly with the enzyme to form the inhibitory TPQ-adduct. This is consistent with the weaker inhibitory activity observed with **4a** (*cis*). However, **3a** (*trans*) was found to be a better inhibitor, having a 10-times lower IC_{50} value compared to **1**. An explanation might be the ability of C–F-bonds to coordinate metal-ions [24]. In some cases, inactivation of CAOs by copper chelating agents was observed [25,26]. In the case of *trans*-fluoro-tranylcypromines (*cis*-arrangement of fluorine and the amino group) a chelation might occur with the copper ion that is situated in the active site of tyramine oxidase. This is in agreement with the significantly higher activities of the corresponding *trans*-isomers. But there is another difference between the isomers: the type of inhibition. Whereas the *cis*-isomers are irreversible inhibitors, the *trans*-compounds are competitive ones. Consequently the two isomers interact with the enzyme in different ways. However, the inhibition mechanism for *trans*- and *cis*-isomers is still unclear. A Schiff base might be produced reversibly with the cyclopropylamine that cannot undergo proton abstraction from the *trans*-isomers. The formation of a Schiff base complex of tranylcypromine and *E. coli* amine oxidase has already been observed by X-ray analysis [23]. Indeed, tranylcypromine (**1**), like **3a**, is also a competitive inhibitor. Another similarity between the inhibition by **1** and **3a** is seen in the fact that the efficacy of **1** is present only in the (1*S*,2*R*)-enantiomer. Since fluorine changes the stereochemical priorities of substituents in a molecule, this

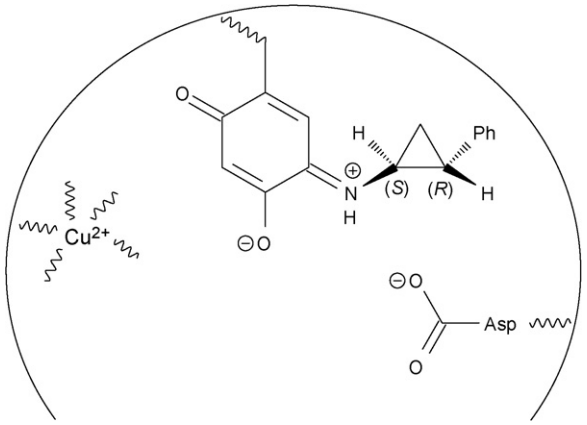

Fig. 6. Schiff's base adduct of (+)-*trans*-tranylcypromine (**1**) and TPQ [23].

Table 3
Physical–chemical properties of known and new inhibitors of tyramine oxidase

R^1	R^2	R^3	Compound	pK_a[a]	Log *D* (lipo class)[b]
H	H	H	**1**[c]	8.50	–[d]
			2[c]	8.47	1.41 (medium)
H	H	H	**3a**[c]	7.35	1.53 (medium)
			4a[c]	6.98	1.78 (medium)
H	Cl	H	**3b**[c]	7.19	2.23 (medium)
			4b[c]	6.81	2.66 (high)
H	F	H	**3c**[c]	7.31	1.60 (medium)
			4c[c]	6.88	2.14 (medium)
H	CF_3	H	**3d**	7.00	2.66 (high)
			4d	6.60	3.13 (high)
H	Me	H	**3e**[c]	7.41	1.83 (medium)
			4e[c]	7.04	–[e]
H	OMe	H	**3f**	7.50	–[d]
			4f	–[e]	–[d]
OMe	OMe	H	**3g**	7.40	0.58 (low)
			4g	–[e]	–[d]
H	H	Ph	**3h**[f]	7.20	2.39 (medium)
			4h[f]	5.80	–[d]

[a] pK_a values were determined titrametrically in 0.1 M KNO_3 at 21 °C.
[b] Log *D* values were determined from the partition coefficient for 1-octanol/0.05N NaOH + 5 vol.% DMSO at pH 7.4.
[c] The corresponding data were taken from the literature [6].
[d] Was not determined.
[e] Could not be determined.
[f] **3h** (*cis*) and **4h** (*trans*): *cis* and *trans* refers to the arrangement of the two phenyl rings.

matches the (1*S*,2*S*)-enantiomer of **3a** which we have previously shown to be the active isomer [10].

From the above considerations, increased basicity and higher nucleophilicity brought about by introduction of an electron donating group (EDG) in the *para*-position of the phenyl ring should have a positive effect on chelation (if present) and the velocity of the reaction towards TQP. In fact the activity of the *para*-methyl analogue (**3e**, *trans*) was significantly enhanced to give an IC_{50} value of 0.39 μM. Similar pK_a values were also found for the *trans*-configured *para*-methoxy-(**3f**) and the dimethoxy compound (**3g**). However, compound **3f** was found to be two times less active as an inhibitor of tyramine oxidase than was **3e**. However, **3f** was determined to be a partially irreversible inhibitor. Furthermore, the partially irreversible inhibitor **3g** with a pK_a value of 7.40, was shown to be three times less potent than **3f**. Thus, the influence of the substituents cannot be directly associated with the pK_a value. Further interaction with other groups in the active site that contribute or diminish formation of the Schiff's base intermediate may also be important. In fact similar IC_{50} values were observed for electron withdrawing substituted **3b**, **3c** and **3d** (*trans*). For compounds **3h** (*cis*) and **4h** (*trans*) no inhibition activity was found in the micromolar range. Whether steric interactions are the reason for that is not known yet. There appears to be no obvious correlation between activity (Table 2) and lipophilicity (Table 3).

The different catalytic mechanisms of bacterial, plant and mammalian CAOs were discussed recently [27]. We plan to investigate the inhibition of mammalian CAO by these fluorinated cyclopropylamine analogues in future work.

3. Conclusions

Earlier results had suggested that potent and selective inhibitors of CAO could be based on 2-aryl-2-fluoro analogues of tranylcypromine **1**. In particular, the *para*-methyl derivative **3e** (*trans*) was some 100-fold more potent than **1**. Based on these results we have prepared additional analogues, including those with more effective electron donating groups (e.g. OMe, di-OMe) (**3f**,**g** **4f**,**g**) as well as further examples having electron withdrawing groups (CF_3) (**3d**,**4d**). No improvement in inhibitory potency was seen relative to the *para*-methyl analogue **3e** (*trans*).

4. Experimental

4.1. Syntheses of 2-aryl-2-fluorocyclopropylamines

The investigated 2-aryl-2-fluorocyclopropylamines were synthesized as previously reported [7].

4.2. Enzyme assay

Tyramine oxidase was purchased from Sigma and dissolved in 25 mM potassium phosphate buffer (pH 7.2). The enzyme activity was measured spectrophotometrically at 30 °C as previously reported using 0.7 mL of standard reaction mixture containing 0.6 mM benzylamine, 0.1 M potassium phosphate buffer (pH 7.2), 6% dimethylsulfoxide and tyramine oxidase [8–10]. The reaction was monitored at 250 nm, the maximum absorption wavelength of benzaldehyde. The enzyme activity was calculated by using 13,800 M^{-1} cm^{-1} as the extinction coefficient of benzaldehyde at 250 nm. One unit of the enzyme oxidizes 1 μmol of benzylamine to benzaldehyde per minute. The protein concentration was determined by the method of Bradford using bovine serum albumin as a standard [28].

4.3. Inhibition experiments

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 the inhibitor were added to the reaction mixture described in Section 4.2 (without substrate), and allowed to stand for 10 min at 25 °C. The reaction was started by addition of the substrate stock, and the time course of the absorption increase of the reaction product was monitored as described in Section 4.2. Control experiments were carried out by using the reaction mixture omitting both benzylamine and tyramine oxidase from the standard assay solution.

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