Articles

Alkylamino Derivatives of 4-Aminomethylpyridine as Inhibitors of Copper-Containing Amine Oxidases

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The first substratelike, reversible inhibitors of different copper amine oxidases (CAOs) with IC_{50} (M) as low as 2.0×10^{-8} corresponding to derivatives of 4-aminomethylpyridine with alkoxy (**1a**−**d**), alkylthio (**2a**,**b**), and alkylamino (**3a**−**e**, **4a**−**j**) groups in the positions 3 and 5 have been prepared and studied. The inhibitors **1a**−**d** are active on benzylamine oxidase and semicarbazide-sensitive amine oxidase and are very selective with respect to diamine oxidase, lysyl oxidase, and monoamine oxidases. The inhibitors **2a**,**b** are selective for benzylamine oxidase whereas **2a** is also a new type of good substrate of diamine oxidase. The inhibitors **3a**−**e** and **4a**−**j** are substratelike, reversible, nonselective inhibitors of various CAOs including pea seedling amine oxidase and *Hansenula polymorpha* amine oxidase, whose enzymatic sites are known from X-ray structure determinations. The inhibitors **3b**,**c** and **4b**,**c** are excellent substratelike tools for studies correlating CAOs that afford crystals suitable for X-ray structure determinations with CAOs from mammals.

Introduction

Copper-containing amine oxidases (CAOs) are widely distributed in prokaryotes and eukaryotes where they catalyze the oxidative deamination of primary amines containing the CH_2NH_2 group to the corresponding aldehyde with formation of ammonia and hydrogen peroxide. CAOs constitute a family of enzymes, often present in the same organism, very effective on different substrates, but poorly selective as their widespread moderate activity shows. The progress on the knowledge of such enzymes regarding important points such as physiological role, nature of the cofactor, mechanism of the enzymatic reaction, and role of the copper has received increased attention in the last years with the X-ray structure determination of crystalline CAOs from various sources such as *Escherichia coli* (ECAO),[1] pea seedling (PSAO),[2] *Arthrobacter globiformis* (AGAO),[3] *Hansenula polymorpha* (HPAO),[4] and of a covalent complex ECAO−inhibitor[5] useful for confirming some aspects of the proposed ping-pong mechanism of the enzymatic reaction.[6] Such a mechanism is divided into a first reductive half-reaction involving reduction of the cofactor 2,4,5-trihydroxyphenylalanine quinone (TPQ) to aminoquinol and oxidation of the amine to the aldehyde and a second oxidative half-reaction involving oxidation of aminoquinol to TPQ with consumption of oxygen and release of hydrogen peroxide and ammonia. The mechanism implies the initial formation of a Schiff base between the substrate amino group and the quinone carbonyl group in position 5 of the cofactor, followed by the abstraction of a proton from the CH_2 next to the NH_2 group, catalyzed by the carboxylate of Asp383 in ECAO or Asp300 in PSAO.[7] In substantial agreement with the proposed mechanism, in the structure of the complex between the nonsubstratelike, nonselective, 2-hydrazinopyridine inhibitor and ECAO,[5] the inhibitor exploits its $NHNH_2$ system for the formation of an imine double bond between the NH_2 and the C-5 carbonyl group of TPQ and of a hydrogen bond between the NH and the protein carboxylate Asp383.

CAO inhibitors containing the CH_2NH_2 function have not received proper attention for many years.[8] Our contribution in this field has been the synthesis of substratelike, reversible CAO inhibitors, very active on porcine serum benzylamine oxidase (BAO) and tissular semicarbazide sensitive amine oxidase (SSAO) and highly selective with respect to porcine kidney diamine oxidase (DAO) and porcine aorta lysyl oxidase (LO) as well as mitochondrial monoamine oxidase (MAO) A and B. They are a series of 2,6-dialkoxybenzylamines[9] and 3,5-diethoxy-4-aminomethylpyridine,[10] the pyridine derivative being more selective and less toxic.

With this work, we explore the effect on the inhibitory activity toward different CAOs of previously prepared alkoxy, alkylthio, and alkylamino disubstituted derivatives of the 4-aminomethylpyridine dihydrochloride (**12**)

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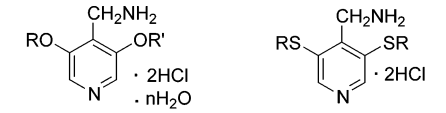
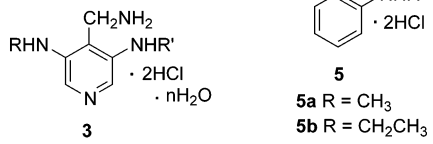
Chart 1



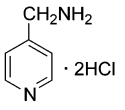
1a R = R' = CH_2CH_3, n=0
1b R = R' = $(CH_2)_3OH$, n=3
1c R = R' = $(CH_2)_4OH$, n=0
1d R = CH_2CH_3, R' = $CH_2C_6H_5$, n=0

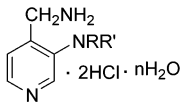
2a R = CH_2CH_3
2b R = t-Bu



5a R = CH_3
5b R = CH_2CH_3

3a R = H, R'= CH_3, n=2
3b R = R' = CH_3, n=1
3c R = R' = CH_2CH_3, n=0
3d R = R' = $CH(CH_3)_2$, n=0
3e R = R' = $(CH_2)_3CH_3$, n=2





4a R = R' = H, n=0
4b R = H, R' = CH_3, n=0
4c R = H, R' = CH_2CH_3, n=0
4d R = H, R' = $CH(CH_3)_2$, n=0
4e R = H, R' = cyclopropyl, n=1.5
4f R = H, R' = cyclopentyl, n=0.5
4g R = H, R' = cyclohexyl, n=1
4h R = H, R' = cycloheptyl, n=1
4i R = H, R' = cyclohexylmethyl, n=1
4j R = R' = $-(CH_2)_5-$, n=0.5

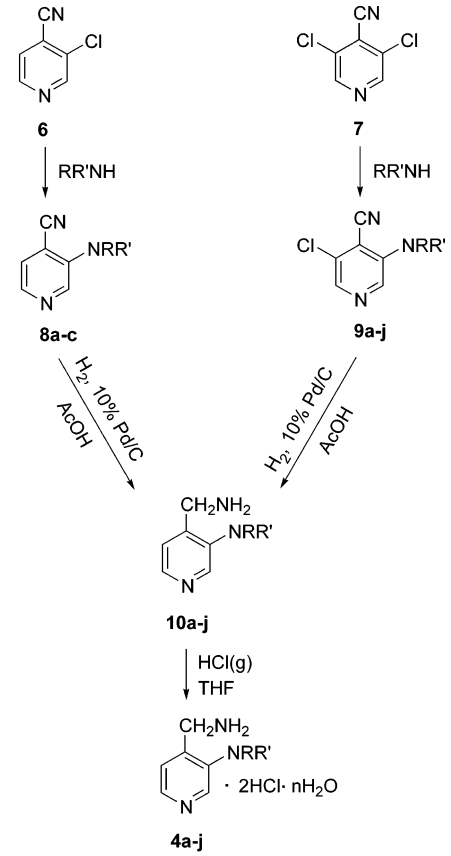
(**1**−**3**, Chart 1) and of totally new alkylamino monosubstituted derivatives of **12** (**4**). Two benzylamine derivatives (**5**) have been prepared for comparison purposes aimed to ascertain the importance of the pyridine ring. MAO enzymes have been tested for recording their possible sensitivity to the same products.

Chemistry

The new 3-amino- and 3-alkylamino-4-aminomethylpyridine derivatives in the form of stable dihydrochlorides **4a**−**j** (Chart 1) were synthesized starting from 3-chloro-4-pyridinecarbonitrile (**6**)[11] or 3,5-dichloro-4-pyridinecarbonitrile (**7**)[12] through the initial nucleophilic substitution of a chlorine atom with ammonia or amines, in analogy to a previous report.[13] The catalytic reduction of the cyano group, which followed the substitution, also performed the hydrogenolysis of the residual C−Cl bond in the intermediates **9** (Scheme 1). The compounds **4a**−**c** were prepared from both **6** and **7** to compare the two procedures. Because the reagent **6**, obtained according to a known method,[11] needed laborious crystallizations to remove the 2-chloro isomer, **7** has to be considered as a more satisfactory starting material.

The use of ethanol in place of glacial acetic acid in the catalytic hydrogenation of the pyridinecarbonitriles **8a**−**c** and **9a**−**j** afforded incomplete transformations of the reagents even with prolonged reaction times. The

Scheme 1



4a R = R' = H, n=0
4b R = H, R' = CH_3, n=0
4c R = H, R' = CH_2CH_3, n=0
4d R = H, R' = $CH(CH_3)_2$, n=0
4e R = H, R' = cyclopropyl, n=1.5
4f R = H, R' = cyclopentyl, n=0.5
4g R = H, R' = cyclohexyl, n=1
4h R = H, R' = cycloheptyl, n=1
4i R = H, R' = cyclohexylmethyl, n=1
4j R = R' = $-(CH_2)_5-$, n=0.5

free bases **10a**−**j** owing to their low stability were identified by GC-MS and immediately transformed into the corresponding dihydrochlorides **4a**−**j**, which were crystallized, some in the form of hydrates.

It is to be noted that both monoaminated (**4**) and bisaminated (**3**) derivatives afford dihydrochlorides such as the progenitor **12**, probably by protonating the aminomethyl group and ring nitrogen, whose positive charge reduces the basicity of the amino or alkylamino groups in the positions 3 and 5 of the aromatic ring.[14]

Scheme 2 represents the synthesis of benzylamines **11a**,**b** and their transformation into the corresponding dihydrochlorides **5a**,**b**.

Results and Discussion

Before the inhibitory activity of all of the products containing the skeleton of **12** on different CAOs such as DAO from porcine kidney, BAO from porcine serum, PSAO, HPAO, and also rat liver MAO A and B was assayed, **12** was tested as a substrate of the same enzymes (Table 1). The deamination of **12** was modest with DAO and MAO A and B, barely detectable with PSAO, and remarkable with BAO and HPAO.

Table 2 compares the inhibitory activity of **1**−**5** for the various enzymes and for DAO, BAO, PSAO, and

Scheme 2

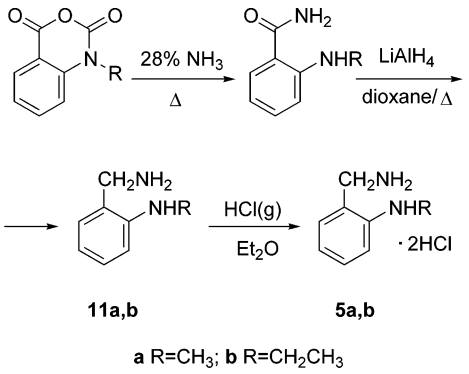


Table 1. Substrate Activity of 1 mM **12** as Percentage of the Activity of the Best Substrate (1 mM) for Various Amine Oxidases[a]

DAO a	BAO b	MAO A c	MAO B d	PSAO a	HPAO d
8%	87%	3%	4%	<0.1%	45%

[a] Enzymes: DAO = diamine oxidase of porcine kidney; BAO = benzylamine oxidase of porcine serum; MAO A and B = monoamine oxidases of rat liver; PSAO = pea seedling amine oxidase; HPAO = Hansenula polymorpha amine oxidase. Substrates: a = putrescine; b = benzylamine; c = 5-hydroxytryptamine; d = β-phenylethylamine.

HPAO assigns the letter s to the compounds that behave as substrates showing an oxidation rate higher than the 5% of that of the best substrate (putrescine for DAO and PSAO, benzylamine for BAO, and β-phenylethylamine for HPAO) or the letter n if their oxidation rate is lower than the 5% or is under the limit of the experimental detectability.

With **1a**−**d** corresponding to the introduction of alkoxy groups of different length and structure in the positions 3 and 5 of **12**, we have found the first substratelike, fully reversible, very active inhibitors of BAO, selective with respect to DAO, LO, and also MAO A and B. They are weak substrates of DAO, never above 15% of the rate of putrescine.

The compound **1a** shows a low toxicity (Table 3), which further decreases going to **1b** through **1c** in accordance with the increasing hydrophilicity of the molecule and the increasing difficulty in crossing the brain barrier. Compound **1a** is 100% reversible and suitable for in vitro and in vivo experiments also under oral administration.[10]

The compounds **1b**,**c**, chosen to increase the hydrophilicity of the inhibitor, can be suitable also for the preparation of interesting glycoconjugates or polymeric systems, properly exploiting one of the two OH groups. In this respect, the benzyl-substituted **1d** represents a model for a polymerizable molecule having a styryl in place of the phenyl group.

The inhibitor **1a** tested toward PSAO and HPAO confirmed its high selectivity for BAO.

The replacement of the alkoxy with linear or branched alkylthio groups (compounds **2a**,**b**) still gives reversible inhibitors of BAO, very selective with respect to DAO and also MAO, but unexpectedly with **2a**, we have found a totally new type of good substrate of DAO (45% of the putrescine), much better than the progenitor **12** (8%), thus indicating an active role of the sulfur atoms. The *tert*-butylthio groups of **2b** still favor the inhibition of BAO, but their bulkiness makes the sulfur atoms unable to influence the reactivity of **2b** as substrate of DAO through the formation of coordinate bonds.

The replacement of the alkylthio with alkylamino groups (compounds **3a**−**e**) has the very important consequence of causing the loss of selectivity in the

Table 2. IC_{50}(M) Values of the Inhibitors of **1**−**5** Series for Different Amine Oxidases[a,b]

compd	DAO a	BAO b	LO c	MAO A d	MAO B e	PSAO a	HPAO e
1a	$>1.0 \times 10^{-3}$ s	1.7×10^{-7} n	$>1.0 \times 10^{-3}$ n	$>1.6 \times 10^{-3}$	$>1.0 \times 10^{-3}$	3.3×10^{-4} n	$>1.0 \times 10^{-3}$ n
1b	$>1.0 \times 10^{-3}$ n	3.2×10^{-6} n	1.0×10^{-4} n	$>1.6 \times 10^{-3}$	$>1.0 \times 10^{-3}$		
1c	$>1.0 \times 10^{-3}$ s	1.5×10^{-7} n	1.0×10^{-4} n		$>1.0 \times 10^{-3}$		
1d	$>1.0 \times 10^{-3}$ n	5.0×10^{-6} n	$>1.0 \times 10^{-3}$ n	6.3×10^{-4}	1.0×10^{-3}		
2a	1.0×10^{-3} s	1.3×10^{-7} n			$>1.0 \times 10^{-3}$		
2b	$>1.0 \times 10^{-3}$ n	1.6×10^{-6} n			$>1.0 \times 10^{-3}$		
3a	1.2×10^{-7} n	1.7×10^{-6} n		1.7×10^{-4}	$>1.7 \times 10^{-4}$		
3b	6.1×10^{-8} n	4.4×10^{-7} s		4.4×10^{-5}	2.5×10^{-4}	1.7×10^{-5} s	1.0×10^{-4} n
3c	2.5×10^{-6} n	2.0×10^{-8} n		6.3×10^{-5}	3.0×10^{-4}	5.5×10^{-5} n	3.5×10^{-4} n
3d	1.0×10^{-6} n	$>1.0 \times 10^{-7}$ n					
3e	1.0×10^{-5} n	3.5×10^{-4} n		1.0×10^{-5}	2.0×10^{-5}		
4a	3.0×10^{-7} s	1.6×10^{-5} n		$>2.0 \times 10^{-3}$	$>2.0 \times 10^{-3}$		
4b	1.6×10^{-7} n	8.0×10^{-6} s		4.2×10^{-4}	$>1.7 \times 10^{-4}$	7.1×10^{-7} n	5.3×10^{-5} n
4c	5.0×10^{-7} n	2.0×10^{-6} s		3.0×10^{-4}	$>1.7 \times 10^{-4}$	9.3×10^{-6} n	8.7×10^{-5} n
4d	1.6×10^{-5} s	1.0×10^{-4} s		2.2×10^{-3}	2.0×10^{-3}		$>1.0 \times 10^{-3}$ s
4e	5.0×10^{-6} n	$>1.0 \times 10^{-4}$ n		$>1.0 \times 10^{-4}$	9.0×10^{-5}		$>1.0 \times 10^{-3}$ s
4f	1.9×10^{-5} n	1.6×10^{-5} n[c]		$>1.0 \times 10^{-4}$	$>1.0 \times 10^{-4}$		7.0×10^{-4} n
4 g	1.6×10^{-5} n	5.6×10^{-6} n[c]		$>1.0 \times 10^{-4}$	$>1.0 \times 10^{-4}$		$>1.0 \times 10^{-3}$ s
4h	1.8×10^{-5} n	7.9×10^{-5} n[c]		1.5×10^{-4}	$>1.0 \times 10^{-4}$	1.0×10^{-3} s	$>3.0 \times 10^{-4}$ n
4i	5.0×10^{-5} n	5.6×10^{-6} n[c]		9.5×10^{-5}	7.0×10^{-5}	8.9×10^{-4} s	5.4×10^{-4} n
4j	$>1.0 \times 10^{-4}$ n	1.0×10^{-4} n[c]		$>1.0 \times 10^{-4}$	$>1.0 \times 10^{-4}$		$>1.0 \times 10^{-3}$ s
5a	$>1.0 \times 10^{-4}$ n	$>1.0 \times 10^{-4}$ s[c]			$>1.0 \times 10^{-4}$	$>1.0 \times 10^{-4}$ n	7.5×10^{-4} n
5b	$>1.0 \times 10^{-4}$ n	$>1.0 \times 10^{-4}$ s[c]			$>1.0 \times 10^{-4}$	$>1.0 \times 10^{-4}$ n	1.5×10^{-3} n

[a] The values of IC_{50} (M) are the means obtained from five concentrations of each inhibitor studied in duplicate at a saturating concentration (1 mM) of the substrate. The variability of the measures averaged 7% for DAO, BAO, LO, and HPAO, 6% for MAO A and B, and 3% for PSAO. [b] Enzymes: DAO = diamine oxidase of porcine kidney; BAO = benzylamine oxidase of porcine serum; LO = lysyl oxidase of porcine aorta; MAO A and B = monoamine oxidases of rat liver; PSAO = pea seedling amine oxidase; HPAO = Hansenula polymorpha amine oxidase. Best substrates: a = putrescine; b = benzylamine; c = protein-bound lysine; d = 5-hydroxytryptamine; e = β-phenylethylamine. s = the compound is substrate with an oxidation rate above 5% of that of the best substrate; n = the compound shows an oxidation rate under 5% of that of the best substrate or it is a nonsubstrate in the limit of the experimental detectability. [c] Data obtained with porcine serum from ICN.

Table 3. Intraperitoneal LD_{50} of **1a**−**c** in *Mus musculus*

compound	LD_{50} mg/kg
1a	342
1b	>600
1c	>500

reversible inhibition of different CAOs. Compounds **3a**−**e** are similarly active on both BAO and DAO. The enzymes MAO A and B are always moderately inhibited. The highest inhibitory activity is attained for DAO with **3b** and for BAO with **3c**. The fact that **3a**, containing an unsubstituted NH_2 in the position 3, is a little less active than **3b**, more active than **3c** for DAO, and less active than **3b** and **3c** for BAO provides evidence that the inhibition is essentially determined by the coordinative effect of the nitrogen atoms in the positions 3 and 5, even though the activity increase for BAO going from **3b** to **3c** shows that for this enzyme a certain hindrance of the alkyl chain can bring a significant contribution.

The inhibitors **3b**,**c**, in accordance with their nonselective character for DAO and BAO, tested toward PSAO and HPAO showed a good inhibitory activity for the first and modest for the second enzyme, with **3b** being more active than **3c**.

The introduction of only one alkylamino group in the position 3 of **12** (**4a**−**j**) still produces nonselective, reversible inhibitors for CAOs, whereas MAOs are poorly or moderately inhibited. The highest inhibitory activity is obtained for DAO with **4b** and for BAO with **4c** containing a methylamino or ethylamino group, respectively, in analogy with the observations reported for **3b** and **3c**.

By examination of the inhibitory activity for DAO and BAO of **4a**−**j** where the amino group at position 3 is unsubstituted (**4a**), or substituted with linear, branched, or cyclic alkyl moieties (**4b**−**j**), it appears evident that the bioactivity strongly depends on the coordinative effect of the nitrogen atom at the position 3. The alkyl moiety, which transforms the amino group from primary (**4a**) into secondary (**4b**−**i**) or tertiary (**4j**), can enhance the bioactivity only if it is a methyl for DAO and BAO (**4b**) or possesses an adequate shape for BAO (**4c**,**f**,**g**,**i**).

A comparison between **3b** and **4b** for DAO and **3c** and **4c** for BAO shows that two alkylamino groups (**3b**,**c**) are more effective than one (**4b**,**c**), probably because the double substitution provides the inhibitor with two equivalent faces for the interaction with the enzymatic site. The positive effect of the double substitution is greater for BAO, for which **4c** is also a modest substrate.

The nonselective inhibitors for DAO and BAO **4b**,**c**, tested toward PSAO and HPAO, proved to be better than **3b**,**c**, attaining a very good activity for PSAO and good for HPAO, **4b** being more active than **4c** for both the enzymes.

To ascertain if inhibitors of the series **4** with large alkylamino groups in the position 3 could show some selective activity for different CAOs, **4d**,**j** were tested toward PSAO and HPAO obtaining only modest values of inhibitory activity.

Because very good selective inhibitors of BAO having the structure of 2,6-dialkoxybenzylamines are known,[9] it seemed right to prepare the monoalkylamino benzylamines **5a**,**b** analogous to the nonselective inhibitors **4b**,**c** and test them with the main enzymes considered in this work. The replacement of the pyridine with the benzene ring caused a generalized collapse of the inhibitory activity and stimulated a careful examination of the kinetics of the enzymatic oxidations with DAO and BAO of the substrates **12** and benzylamine, characterized by a significant substrate-inhibition effect. The kinetic parameters, determined by the Michaelis−Menten equation, are reported in Table 4. The observed K_m values concerning the reactions performed with **12** always are smaller than those with benzylamine, indicating a greater affinity of **12** for either DAO or BAO, thus assigning to the pyridine ring an interaction with both the enzymes stronger than that of the benzene ring.

Table 4. Kinetic Parameters of the Reactions of DAO and BAO with **12** and Benzylamine Determined by the Michaelis−Menten Equation

reagents	V_{max} nmol mL^{-1} h^{-1}	K_m μmol
DAO + **12**	21 ± 0.9	4 ± 0.1
DAO + benzylamine	22 ± 0.3	557 ± 39
BAO[a] + **12**	1247 ± 169	16 ± 0.7
BAO[a] + benzylamine	1653 ± 39	124 ± 17

[a] Porcine serum from ICN.

Inhibitors active as weak substrates were investigated for the effect of the preincubation time on the inhibitory activity, and they showed a decrease of activity with the preincubation time, in agreement with what was found in a careful study of the mechanism of the inhibition accomplished using **1a** and a crystalline preparation of the porcine serum benzylamine oxidase.[10] Such study indicated that kinetically **1a** appears to be a mixed-competitive site-directed inhibitor, which increases the K_m and decreases the V_m, but it can appear as a pure noncompetitive inhibitor if it is preincubated at a concentration lower than that of the enzyme. The inhibitor **1a** forms with the quinone in the active site of BAO a substrate Schiff base, which probably proceeds to a product Schiff base endowed with good stability that sluggishly hydrolyzes to produce the aldehyde derived from **1a** and the aminoquinol which regenerates the oxidized enzyme by oxidation with molecular oxygen. In fact when the molar ratio **1a**/BAO is higher than 1 in the presence of a saturating concentration of benzylamine, the enzyme is totally inhibited excluding the possibility of transimination, but gradually the inhibition reverts, and benzylamine is oxidized at a rate that is proportional to the freed enzyme.

Conclusions

With **1a**−**d**, we have found the first substratelike, very active, nontoxic, fully reversible, useful for oral administration inhibitors selective for BAO.

With **2a**,**b**, we have found a new type of substratelike, reversible inhibitors selective for BAO and also a totally new type of good substrate for DAO.

With **3b**,**c** and **4b**,**c**, we have found the first nonselective inhibitors of CAOs, very active on DAO, BAO, and also PSAO and HPAO that, contrarily to hydrazine derivatives, are substratelike and fully reversible. The inhibition activity of **3b**,**c** and **4b**,**c** decreases going from DAO to HPAO through PSAO, and for each enzyme it decreases going from methylamino to ethylamino substituents, i.e., from **3b** to **3c** and from **4b** to **4c**, in

accordance with an active site of DAO having similarities mostly with that of PSAO, within the enzymes whose structures are known. As a result, PSAO is expected to be a reasonable model for DAO useful for structural studies in the presence of substratelike inhibitors. In agreement with the observed peculiar sensitivity of BAO to steric effects, the inhibition of such an enzyme increases from **3b** to **3c** and from **4b** to **4c**, with an opposite trend with respect to PSAO and HPAO, therefore these enzymes are not fully satisfactory models for BAO. Nevertheless, HPAO resembles BAO in preferring primary monoamine substrates, as confirmed by the noticeable activity of both HPAO and BAO toward **12** (Table 1).

The inhibition mechanism of the prepared inhibitors deduced from kinetic data foresees the formation of a stable Schiff base that sluggishly hydrolyzes producing the aldehyde derived from the inhibitor and allowing the regeneration of the enzyme.

The nonselective inhibitors of the series **3** and **4**, for which a plurality of factors such as the coordinative effects of the nitrogen atoms of the amino or alkylamino groups ortho to the aminomethyl function, the significant interaction of the pyridine ring with the enzyme, and also the steric effects in the case of BAO contribute in stabilizing the enzyme−inhibitor complex, appear to be suitable for comparative studies of CAOs from different sources. Interesting insights regarding the enzymatic and inhibition reactions and useful structural indications for devising new selective inhibitors of various CAOs in mammals are expected to be obtained from new X-ray structure determinations of CAO complexes with the best substratelike inhibitors **3b**,**c** and **4b**,**c**.

Experimental Section

Pure BAO from porcine serum was prepared by the method of Buffoni and Blaschko[15] and used in all the applications unless otherwise stated. Porcine serum with BAO specific activity 1492 ± 90 nmol mL^{-1} h^{-1} was obtained from ICN (Irvine, CA). DAO from porcine kidney, with specific activity 100 ± 8.9 nmol mL^{-1} h^{-1}, was obtained from Sigma. LO from porcine aorta was prepared according to Buffoni and Raimondi.[16] Tritiated protein-bound lysine was prepared according to Melet et al.[17] Homogeneate of pea seedling was prepared from 8 days old seedling, homogeneized 1:8 in 0.1 M phosphate buffer pH 7.4, centrifuged at 2000*g* for 20 min, filtered on paper, and further centrifuged at 3000*g* for 30 min (PSAO specific activity 100.1 ± 8.7 nmol mL^{-1} min^{-1}). *Hansenula polymorpha* wild-type NCYC-495 was incubated for 24 h in a medium containing 2% glucose, 1 μM $CuSO_4$, 1.7% yeast nitrogen base without ammonium sulfate and amino acids (Y 1251, Sigma), and 0.25% methylamine hydrochloride, then it was centrifuged four times at 1500*g* for 5 min. The precipitate, stored at −80 °C, was suspended in 10 mL of 0.1 M phosphate buffer pH 7.4, homogeneized, and centrifuged at 2500*g* for 30 min (HPAO specific activity 54.3 ± 0.2 nmol mL^{-1} h^{-1}). Mitochondria were obtained from rat liver by homogeneization 1:10 in 0.01 M phosphate buffer pH 7.4 containing 0.25 M sucrose and sequential centrifugation at 1000*g* for 20 min and 10 000*g* for 20 min (specific activity MAO A 43.4 ± 2.5, MAO B 12.5 ± 0.7 nmol mL^{-1} min^{-1}).

The ^{14}C-substrates such as benzylamine, β-phenylethylamine, putrescine, and 5-hydroxytryptamine were obtained from Amersham Biosciences. All of the other reagents for biological assays were analytical reagent grade from either BDH, Merck, or Sigma.

The enzymatic activities of MAO and HPAO were assayed using ^{14}C-substrates as described by Pino et al.[18] The enzymatic activities of DAO, BAO, and PSAO, as well as the evaluations of the various products as substrate of all of the considered enzymes, were obtained by measuring the production of H_2O_2 with the method of 4-aminoantipyrine.[19] The protein content was assayed by the method of Lowry et al.[20] The inhibition reversibility was ordinarily ascertained by incubating the enzyme with the inhibitor and determining the specific activity of the mixture after progressive dilutions in the presence of constant substrate concentration. The reversibility of the BAO inhibitor **1a** was measured from the recovered enzyme activity after exhaustive dialysis against 0.07 M phosphate buffer pH 7.4. The acute toxicity (LD_{50}) was studied in *Mus musculus*, Swiss white. Tests were carried out by intraperitoneal administration of five scaled doses to 10 animals for each dose. The toxic symptomatology began after 15−20 min and was characterized by tonic-clonic convulsions followed by respiratory block. The survived animals, observed for 48 h, did not show apparent symptoms.

Melting points were determined on Electrothermal apparatus and are uncorrected. ^1H NMR spectra were obtained on Bruker WM-300 or AcP-300 spectrometers. Chemical shifts are reported on the δ scale and are referred to tetramethylsilane. Mass spectra were recorded on Hewlett-Packard GC-MSD 5972 instrument. IR spectra were recorded on 1000 PC FT-IR Paragon spectrometer. Microanalyses for C, H, N, and Cl were performed at the Microanalysis Service of the Department of Chemistry, University of Calabria and were within ±0.4% of the theoretical values. 4-Aminomethylpyridine, reagents, and solvents were purchased from Aldrich. Compounds **1a**−**d**,[12] **2a**,**b**,[21] **3a**−**e**,[13] **6**,[11] **7**,[12] **9a**−**d**,[13] **11a**,[22] and *N*-ethylisatoic anhydride[23] were prepared according to known procedures. 4-Aminomethylpyridine dihydrochloride (**12**) was obtained from the corresponding commercial amine as described for **10a**−**j**.

General Procedure for the Reaction of 3-Chloro-4-pyridinecarbonitrile (6) and 3,5-Dichloro-4-pyridinecarbonitrile (7) with Amines or Ammonia. Compound **6** or **7** (2.0 mmol) and excess of the proper amine or ammonia used as solvent were introduced at −50 °C under nitrogen into a 50−100 mL pressure resistant glass vial with a Rotaflo stopcock and allowed to react in thermostatic bath at 80 °C (room temperature for the synthesis of **9e**). The reaction times were determined by checking with GC-MS the increase of the product. The mixture, after evaporation at reduced pressure of all the volatiles, treatment with water, and extraction with chloroform, was dried over anhydrous Na_2SO_4. After removal of the solvent the crude products **8a**−**c** and **9a**−**j** were purified by column chromatography on Merck neutral alumina grade I using a mixture hexane/chloroform 1:1 as eluent and characterized. Compounds **8a**[24] and **9a**−**d**[13] showed results identical to the known ones. Products (including **8a** prepared according to Scheme 1), starting nitriles, reaction times, yields, melting points, IR, ^1H NMR, and GC-MS data are listed below.

3-Amino-4-pyridinecarbonitrile (8a). 6; 54 days; 14%; 137−138 °C (lit.[24] 137−138 °C). IR (KBr): 3398, 2225, 1561, 808, 593 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 4.52 (br s, 2H); 7.24 (d, 1H, J = 5.5 Hz); 8.04 (d, 1H, J = 5.5 Hz); 8.28 (s, 1H). GC-MS (EI) *m/z*: 119 (M^+, 100%).

3-Methylamino-4-pyridinecarbonitrile (8b). 6; 24 h; 46%; 143−145 °C. IR (KBr): 3279, 2219, 1571, 805, 593 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 3.02 (d, 3H, J = 5.5 Hz); 4.58 (br s, 1H); 7.21 (d, 1H, J = 5.0 Hz); 8.00 (d, 1H, J = 5.0 Hz); 8.20 (s, 1H). GC-MS (EI) *m/z*: 133 (M^+, 100%). Anal. ($C_7H_7N_3$): C, H, N.

3-Ethylamino-4-pyridinecarbonitrile (8c). 6; 24 h; 54%; 84−86 °C. IR (KBr): 3213, 2219, 1569, 809, 593 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.35 (t, 3H, J = 7.2 Hz); 3.36 (dq, 2H, J_1 = 7.2 Hz, J_2 = 5.3 Hz); 4.44 (br s, 1H); 7.22 (d, 1H, J = 5.3 Hz); 7.99 (d, 1H, J = 5.3 Hz); 8.21 (s, 1H). GC-MS (EI) *m/z*: 147 (M^+, 33%); 132 (100%). Anal. ($C_8H_9N_3$): C, H, N.

3-Cyclopropylamino-5-chloro-4-pyridinecarbonitrile (9e). 7; 53 h; 67%; 109−110 °C. IR (KBr): 3343, 2229, 1559, 862, 585 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.68 (m, 2H); 0.94 (m, 2H);

2.61 (m, 1H); 5.09 (br s, 1H); 8.04 (s, 1H); 8.48 (s, 1H). GC-MS (EI) *m/z*: 193 (M^+, 68%); 192 (100%). Anal. ($C_9H_8N_3Cl$): C, H, N, Cl.

3-Cyclopentylamino-5-chloro-4-pyridinecarbonitrile (9f). 7; 36 h; 48%; 119−120 °C. IR (KBr): 3239, 2223, 1559, 846, 589 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.56 (m, 2H); 1.75 (m, 4H); 2.12 (m, 2H); 3.95 (m, 1H); 4.60 (br s, 1H); 7.96 (s, 1H); 8.10 (s, 1H). GC-MS (EI) *m/z*: 221 (M^+, 40%); 192 (100%). Anal. ($C_{11}H_{12}N_3Cl$): C, H, N, Cl.

3-Cyclohexylamino-5-chloro-4-pyridinecarbonitrile (9g). 7; 24 h; 59%; 124−125 °C. IR (KBr): 3341, 2231, 1560, 844, 587 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.34 (m, 5H); 1.69 (m, 1H); 1.82 (m, 2H); 2.07 (m, 2H); 3.47 (m, 1H); 4.53 (br d, 1H, J = 7.4 Hz); 7.93 (s, 1H); 8.08 (s, 1H). GC-MS (EI) *m/z*: 235 (M^+, 33%); 192 (100%). Anal. ($C_{12}H_{14}N_3Cl$): C, H, N, Cl.

3-Cycloheptylamino-5-chloro-4-pyridinecarbonitrile (9h). 7; 24 h; 74%; 83−84 °C. IR (KBr): 3355, 2227, 1561, 847, 586 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.64 (m, 11H); 2.04 (m, 1H); 3.64 (m, 1H); 4.57 (br d, 1H, J = 7.4 Hz); 7.94 (s, 1H); 8.01 (s, 1H). GC-MS (EI) *m/z*: 249 (M^+, 23%); 192 (100%). Anal. ($C_{13}H_{16}N_3Cl$): C, H, N, Cl.

3-Cyclohexylmethylamino-5-chloro-4-pyridinecarbonitrile (9i). 7; 24 h; 49%; 149−150 °C. IR (KBr): 3346, 2231, 1566, 847, 585 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.01 (m, 2H); 1.23 (m, 3H); 1.73 (m, 6H); 3.14 (m, 2H); 4.75 (br s, 1H); 7.96 (s, 1H); 8.07 (s, 1H). GC-MS (EI) *m/z*: 249 (M^+, 19%); 166 (100%). Anal. ($C_{13}H_{16}N_3Cl$): C, H, N, Cl.

3-(1-Piperidinyl)-5-chloro-4-pyridinecarbonitrile (9j). 7; 1 h; 91%; 55−56 °C. IR (KBr): 2230, 1554, 863, 583 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.66 (m, 2H); 1.79 (m, 4H); 3.34 (m, 4H); 8.20 (s, 1H); 8.27 (s, 1H). GC-MS (EI) *m/z*: 221 (M^+, 66%); 220 (100%). Anal. ($C_{11}H_{12}N_3Cl$): C, H, N, Cl.

General Procedure for the Reduction of Nitriles 8a−c and 9a−j to Amines 10a−j and Their Transformation into Dihydrochlorides 4a−j. A 0.3 M solution of **8a−c** or **9a−j** in glacial acetic acid was treated with H_2 at 1.5 atm in the presence of 10% Pd on activated charcoal (3.5 g per mmol of nitrile) for 1−16 h. The slurry was then filtered, and the filtrate was concentrated at reduced pressure, treated with 1 N aqueous NaOH, extracted with chloroform, and dried over anhydrous Na_2SO_4. After removal of the solvent at reduced pressure, the crude amines **10a−j** were dissolved in anhydrous THF and saturated with gaseous HCl to afford the dihydrochlorides **4a−j**, which were crystallized from 95% ethanol and characterized. Products, starting nitriles, yields, melting points, IR, ^1H NMR data, and GC-MS of the free bases are listed below.

3-Amino-4-aminomethylpyridine Dihydrochloride (4a). 8a; 72%; 279 °C (dec). IR (KBr): 3472, 3289, 1531, 795, 604 cm^{-1}. ^1H NMR (CD_3OD) δ: 4.28 (s, 2H); 7.78 (d, 1H, J = 5.8 Hz); 8.08 (d, 1H, J = 5.8 Hz); 8.20 (s, 1H). GC-MS (EI) *m/z*: 123 (M^+, 100%). Anal. ($C_6H_{11}N_3Cl_2$): C, H, N, Cl.

3-Methylamino-4-aminomethylpyridine Dihydrochloride (4b). 8b; 60%; 256 °C (dec). IR (KBr): 3286, 1551, 801, 592 cm^{-1}. ^1H NMR (CD_3OD) δ: 2.97 (s, 3H); 4.26 (s, 2H); 7.76 (d, 1H, J = 5.5 Hz); 8.13 (d, 1H, J = 5.5 Hz); 8.18 (s, 1H). GC-MS (EI) *m/z*: 137 (M^+, 43%), 119 (100%). Anal. ($C_7H_{13}N_3Cl_2$): C, H, N, Cl.

3-Ethylamino-4-aminomethylpyridine Dihydrochloride (4c). 8c; 65%; 217 °C (dec). IR (KBr): 3362, 3294, 1569, 816, 550 cm^{-1}. ^1H NMR (CD_3OD) δ: 1.35 (t, 3H, J = 7.3 Hz); 3.33 (q, 2H, J = 7.3 Hz); 4.27 (s, 2H); 7.71 (d, 1H, J = 5.3 Hz); 8.11 (d, 1H, J = 5.3 Hz); 8.15 (s, 1H). GC-MS (EI) *m/z*: 151 (M^+, 29%), 119 (100%). Anal. ($C_8H_{15}N_3Cl_2$): C, H, N, Cl.

3-[(1-Methylethyl)amino]-4-aminomethylpyridine Dihydrochloride (4d). 9d; 70%; 197 °C (dec). IR (KBr): 3279, 1562, 806, 524 cm^{-1}. ^1H NMR (CD_3OD) δ: 1.29 (d, 6H, J = 6.4 Hz); 3.79 (sept, 1H, J = 6.4 Hz); 4.11 (s, 2H); 7.22 (d, 1H, J = 5.0 Hz); 7.88 (d, 1H, J = 5.0 Hz); 8.03 (s, 1H). GC-MS (EI) *m/z*: 165 (M^+, 38%), 133 (100%). Anal.($C_9H_{17}N_3Cl_2$): C, H, N, Cl.

3-Cyclopropylamino-4-aminomethylpyridine Dihydrochloride Sesquihydrate (4e). 9e; 47%; 254 °C (dec). IR (KBr): 3412, 3288, 1554, 812, 578 cm^{-1}. ^1H NMR (CD_3OD) δ: 0.69 (m, 2H); 0.94 (m, 2H); 2.59 (m, 1H); 4.27 (s, 2H); 7.83 (m, 1H); 8.19 (m, 1H); 8.46 (s, 1H). GC-MS (EI) *m/z*: 163 (M^+, 36%), 145 (100%). Anal.($C_9H_{18}N_3Cl_2O_{1.5}$): C, H, N, Cl.

3-Cyclopentylamino-4-aminomethylpyridine Dihydrochloride Hemihydrate (4f). 9f; 79%; 258 °C (dec). IR (KBr): 3543, 3312, 1545, 804, 578 cm^{-1}. ^1H NMR (CD_3OD) δ: 1.69 (m, 4H); 1.84 (m, 2H); 2.15 (m, 2H); 3.94 (m, 1H); 4.33 (s, 2H); 7.80 (d, 1H, J = 5.9 Hz); 8.12 (d, 1H, J = 5.9 Hz); 8.19 (s, 1H). GC-MS (EI) *m/z*: 191 (M^+, 33%), 122 (100%). Anal. ($C_{11}H_{20}N_3Cl_2O_{0.5}$): C, H, N, Cl.

3-Cyclohexylamino-4-aminomethylpyridine Dihydrochloride Monohydrate (4g). 9g; 76%; 246 °C (dec). IR (KBr): 3437, 3326, 1545, 807, 578 cm^{-1}. ^1H NMR (CD_3OD) δ: 1.46 (m, 5H); 1.73 (m, 1H); 1.85 (m, 2H); 2.08 (m, 2H); 3.48 (m, 1H); 4.31 (s, 2H); 7.78 (d, 1H, J = 6.1 Hz); 8.08 (d, 1H, J = 6.1 Hz); 8.22 (s, 1H). GC-MS (EI) *m/z*: 205 (M^+, 15%), 122 (100%). Anal. ($C_{12}H_{23}N_3Cl_2O$): C, H, N, Cl.

3-Cycloheptylamino-4-aminomethylpyridine Dihydrochloride Monohydrate (4h). 9h; 47%; 249 °C (dec). IR (KBr): 3425, 1550, 799, 547 cm^{-1}. ^1H NMR (CD_3OD) δ: 1.69 (m, 11H); 2.03 (m, 1H); 3.31 (m, 1H); 4.28 (s, 2H); 7.78 (d, 1H, J = 5.5 Hz); 8.08 (s, 1H); 8.09 (d, 1H, J = 5.5 Hz). GC-MS (EI) *m/z*: 219 (M^+, 35%), 122 (100%). Anal. ($C_{13}H_{25}N_3Cl_2O$): C, H, N, Cl.

3-Cyclohexylmethylamino-4-aminomethylpyridine Dihydrochloride Monohydrate (4i). 9i; 48%; 242 °C (dec). IR (KBr): 3382, 3308, 1551, 809, 553 cm^{-1}. ^1H NMR (CD_3OD) δ: 1.49 (m, 11H); 3.15 (d, 2H, J = 7.8 Hz); 4.28 (s, 2H); 7.76 (d, 1H, J = 5.5 Hz); 8.08 (d, 1H, J = 5.5 Hz); 8.14 (s, 1H). GC-MS (EI) *m/z*: 219 (M^+, 19%), 119 (100%). Anal. ($C_{13}H_{25}N_3Cl_2O$): C, H, N, Cl.

3-(1-Piperidinyl)-4-aminomethylpyridine Dihydrochloride Hemihydrate (4j). 9j; 59%; 245 °C (dec). IR (KBr): 3449, 1520, 820, 559 cm^{-1}. ^1H NMR (CD_3OD) δ: 1.70 (m, 2H); 1.82 (m, 4H); 3.07 (m, 4H); 4.50 (s, 2H); 8.09 (d, 1H, J = 6.1 Hz); 8.63 (d, 1H, J = 6.1 Hz); 8.65 (s, 1H). GC-MS (EI) *m/z*: 191 (M^+, 24%), 173 (100%). Anal. ($C_{11}H_{20}N_3Cl_2O_{0.5}$): C, H, N, Cl.

2-Methylaminobenzylamine Dihydrochloride (5a). According to Coyne and Cusic,[22] the commercial *N*-methylisatoic anhydride was transformed into 2-methylaminobenzamide. (Yield 80%; mp 169−171 °C (95% ethanol). IR (KBr): 3401, 3357, 3172, 1660, 1617, 747 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 2.84 (d, 3H, J = 5.1 Hz); 6.53 (m, 1H); 6.67 (dd, 1H, J_1 = 7.6 Hz, J_2 = 0.9 Hz); 6.97−7.53 (very br, NH_2); 7.30 (m, 1H); 7.63 (dd, 1H, J_1 = 7.9 Hz, J_2 = 1.5 Hz); 8.09 (br s, NH). GC-MS (EI) *m/z*: 150 (M^+, 100%), 133 (49%).) This was subsequently transformed into 2-methylaminobenzylamine (**11a**). (Oil; yield 91%. IR (film): 3367, 3330, 1607, 1587, 1519, 1470, 749 cm^{-1}. GC-MS (EI) *m/z*: 136 (M^+, 100%), 118 (91%).) The compound **11a** (4.0 mmol) was converted into its dihydrochloride by dissolution in dry ethyl ether (50 mL) and saturation with gaseous HCl. The crude product was crystallized to give white crystals of **5a**. (Yield 89%; mp 205 °C (95% ethanol) (lit.[25] 208−210 °C). IR (KBr): 3008−2589 (broad), 1591, 1500, 1475, 1457 cm^{-1}. ^1H NMR (CD_3OD) δ: 3.13 (s, 3H); 4.39 (s, 2H); 4.95 (br s, NH_2^+ + NH_3^+); 7.52−7.74 (m, 4H). Anal. ($C_8H_{14}N_2Cl_2$): C, H, N, Cl.)

2-Ethylaminobenzylamine Dihydrochloride (5b). The *N*-ethylisatoic anhydride, prepared from isatoic anhydride according to Hardtmann,[23] was transformed as described above into 2-ethylaminobenzamide. (Yield 20%; mp 127−128 °C (95% ethanol). IR (KBr): 3404, 3185, 1642, 1619, 748 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.29 (t, 3H, J = 7.2 Hz); 3.20 (q, 2H, J = 7.2 Hz); 5.78 (br s, NH_2); 6.56 (m, 1H); 6.69 (dd, 1H, J = 7.9 Hz); 7.34 (m, 2H); 7.70 (br s, NH). GC-MS (EI) *m/z*: 164 (M^+, 60%), 132 (100%).) This was subsequently transformed into 2-ethylaminobenzylamine (**11b**). (Oil; yield 98%. IR (film): 3374, 3301, 1606, 1588, 1519, 1471, 749 cm^{-1}. GC-MS (EI) *m/z*: 150 (M^+, 54%), 118 (100%).) And this was finally transformed into white crystals of **5b**. (Yield 66%; mp 185−188 °C (propanol/95% ethanol 1:1). IR (KBr): 3038−2645 (br), 1592, 1519, 1505, 1473 cm^{-1}. ^1H NMR (CD_3OD) δ: 1.46 (t, 3H, J = 7.3 Hz); 3.53 (q,

2H, $J = 7.3$ Hz); 4.45 (s, 2H); 4.99 (br s, $NH_2^+ + NH_3^+$); 7.64 (m, 3H); 7.78 (m, 1H). Anal. ($C_9H_{16}N_2Cl_2$): C, H, N, Cl.)

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Supporting Information Available: Elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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