Inhibition of Copper-Dependent Amine Oxidases by Some Hydrazides of Pyrrol-1-ylbenzoic and Pyrrol-1-ylphenylacetic Acids

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Some hydrazides of pyrrol-1-ylbenzoic and pyrrol-1-ylphenylacetic acids were prepared, and their effect on copper-dependent amine oxidases (Cu-AOs) and FAD monoamine oxidases (MAOs) activities was tested. The compounds were not substrates for Cu-AO enzymes but acted as noncompetitive inhibitors. Hydrazides of pyrrol-1-ylphenylacetic acids were highly specific for plasma amine oxidase (K_i = 0.5–1 μM). In contrast, all the hydrazides were weak inhibitors of MAO activity. Incubation with the hydrazide derivatives led to irreversible inactivation of Cu-AOs. Therefore, the inhibition implied two distinct steps. The first one consisted of the rapid formation of the enzyme–inhibitor complex and was reversed by dialysis. In the second step, the complex was irreversibly transformed, probably by the formation of a Schiff base between the hydrazide and the prosthetic carbonyl group of the enzyme.

The oxidative deamination of biologically important amines is brought about by two different classes of enzymes. The parameter of distinction is the nature of the prosthetic group, i.e., a flavin moiety or a copper ion.

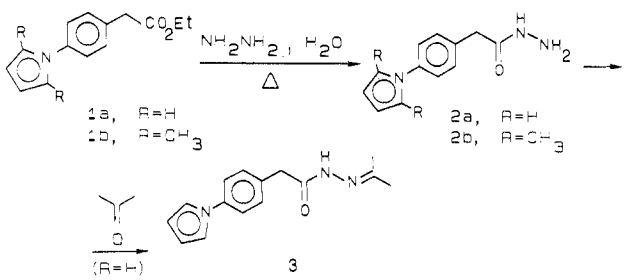
To the FAD-amine oxidases belongs the mitochondrial monoamine oxidase (MAO)(E.C. 1.4.3.4). This enzyme, present in two catalytically distinguishable subforms, MAO-A and MAO-B,[1] plays a very important role in the metabolism of neurotransmitters (dopamine, norepinephrine, 5-hydroxytryptamine, tyramine). Numerous inhibitors of MAO activity are described in the literature, and some of them have clinical importance in the treatment of psychiatric diseases.[2]

Copper-dependent amine oxidases (Cu-AOs) contain cupric copper and a second organic cofactor, tightly bound to the enzyme, capable of strong interaction with carbonyl reagents. The nature of the organic prosthetic group has been debated for many years. It was first proposed that it might be pyridoxal phosphate.[3,4] Recent chromatographic and spectroscopic evidence allows identification as pyrroloquinoline quinone (PQQ), a cofactor previously found in a number of prokaryotic oxidoreductases.[5,6]

On the basis of the substrate specificity and of the origin of the enzyme, the Cu-AOs are classified as plasma amine oxidase (AO)(E.C. 1.4.3.6), diamine oxidase (DAO)(E.C. 1.4.3.6), and lysyl oxidase (E.C. 1.4.3.13). Plasma amine oxidase acts on primary monoamines. Diamine oxidase, present both in tissues and fluids, oxidizes preferentially histamine and diamines like putrescine: histamine plays an important function in gastric secretion and in allergic phenomena;[7] putrescine is a precursor of spermine and spermidine, involved in cellular differentiation and proliferation, both in normal and neoplastic tissues.[8] Lysyl oxidase initiates cross-linkage formation in collagen and elastin by converting the ε-amino group of lysine or hydroxylysine into aldehydes.

Cu-AO activities are altered in several common physiopathological conditions. Diamine oxidase activity significantly increases in the plasma during pregnancy,[9] in the presence of certain tumors,[10-12] in intestinal ischemia,[13] in anaphylactic shock,[14] and after heparin administration.[15] The inhibition of DAO activity in semen causes a decrease of motility and survival of spermatozoa.[16] Lysyl oxidase and collagenase activities increase in chronic liver damage,[17,18] causing liver fibrosis.[19]

Scheme I



These reports suggest that the development of very specific inhibitors of Cu-AOs devoid of inhibitory effects on FAD-MAOs would be useful in evaluating the role of Cu-AOs in such disease states. In fact, specific inhibitors of Cu-AOs may have therapeutic use as antifibrotic, antihypertensive, and antitumor chemotherapeutic agents. Furthermore, they may be conceivably used in contra-

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Table I. Chemical and Physical Properties of the Compounds 2–4, 7, and 8

compd	formula	anal.[a]	mp, °C	bp °C (mmHg)	recrystn solvent[b]	yield, %
2a	$C_{12}H_{13}N_3O$	C, H, N	175–178		A	79
2b	$C_{14}H_{17}N_3O$	C, H, N	199–201		A	78
3	$C_{15}H_{17}N_3O$	C, H, N	187–189		A	84
4a	$C_{12}H_{11}NO_2$	C, H, N		115–117 (0.05)		65
4b	$C_{11}H_{11}NO$	C, H, N	66–67		B	73
4c	$C_{11}H_{11}BrN$	C, H, Br, N	47–50		A	95
4d	$C_{12}H_{10}N_2$	C, H, N		148–150 (0.06)		65
4e	$C_{12}H_{11}NO_2$	C, H, N	142–145		B	71
4f	$C_{13}H_{13}NO_2$	C, H, N		132–134 (0.05)		81
4g	$C_{12}H_{13}N_3O$	C, H, N	153–154		A	75
7a	$C_{12}H_{11}NO_2$	C, H, N		85–90 (0.5)[c]		75
7b	$C_{13}H_{13}NO_2$	C, H, N	73–74[d]		A	80
8a	$C_{11}H_{11}N_3O$	C, H, N	121–123[e]		C	90
8b	$C_{11}H_{11}N_3O$	C, H, N	123–125		A	58
8c	$C_{11}H_{11}N_3O$	C, H, N	180–181		A	77

[a] Elements shown analysed correctly to within ±0.4% of the calculated values. [b] Recrystallization solvents: A, ethanol; B, benzene–cyclohexane; C, chloroform. [c] Literature[31] bp 83–85 °C (0.15 mmHg). [d] Literature[32] mp 74.5–75.0 °C. [e] Literature[33] mp 123–125 °C.

ception.

Carbonyl group reagents, like hydrazines and hydrazides, have been reported to inhibit amine oxidases. However, the compounds studied until now exert inhibition on both MAO and DAO, as shown by comparative studies of Bardsley et al.[20,21] Recently it has been reported that hydralazine inhibits lysyl oxidase in vitro[22] and that isoniazid inhibits lysyl oxidase in vivo.[23] Also, unsaturated amines, like allylamine derivatives, were found to be specific and potent irreversible inhibitors of aorta copper amine oxidase.[24,25]

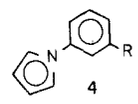
In a search for new potent and specific inhibitors of copper amine oxidases some hydrazides of pyrrol-1-ylbenzoic and pyrrol-1-ylphenylacetic acids have been synthesized. The compounds, in fact, due to the presence of the pyrrole and phenyl moieties, can be structurally regarded as a simplified modification of the indole portion of some amine oxidase substrates and inhibitors, e.g., tryptamine,[26] etryptamine,[27] harmala alkaloids, and other carbolines.[28]

Bovine plasma amine oxidase and human placenta diamine oxidase have been used as models of Cu-AO enzymes to test the inhibitory effect. Activity studies have been performed on rat brain mitochondrial MAO and human platelet MAO to check the inhibition specificity (in rat brain mitochondria the concentration of MAO-A and -B are similar, while human platelets contain only the type B enzyme). Isoniazid has been used as a reference compound.

Chemistry. The synthesis of 4-pyrrol-1-ylphenylacetic acid hydrazide (**2a**) was accomplished by the action of hydrazine hydrate on the ethyl ester of 4-pyrrol-1-ylphenylacetic acid (**1a**).[29] With 4-(2,5-dimethylpyrrol-1-yl)phenylacetic acid ethyl ester (**1b**)[29] as starting material, a similar reaction furnished the hydrazide **2b**. Reaction of **2a** with acetone afforded the corresponding hydrazone **3** (Scheme I).

The meta isomer of **2a** has been prepared by starting from the methyl ester of 3-pyrrol-1-ylphenylacetic acid (**4f**). This ester has been synthesized as follows. Condensation of the ethyl ester of 3-aminobenzoic acid with 2,5-dimethoxytetrahydrofuran in glacial acetic acid furnished methyl 3-pyrrol-1-ylbenzoate (**4a**), which was reduced by the action of lithium aluminum hydride to 3-pyrrol-1-ylbenzyl alcohol (**4b**). Treatment of **4b** with phosphorus tribromide afforded the bromide **4c**, which was then transformed into the nitrile **4d** by reaction with sodium cyanide. Alkaline hydrolysis of the nitrile **4d** gave 3-pyrrol-1-ylphenylacetic acid (**4e**), which was converted to the corresponding methyl ester **4f** by treatment with

Chart I



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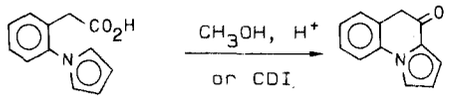
4a. CO_2Me 4e. CH_2CO_2H

4b. CH_2OH 4f. CH_2CO_2Me

4c. CH_2Br 4g. $CH_2CONHNH_2$

4d. CH_2CN

Scheme II



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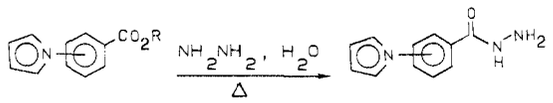
Scheme III



7a, o-isomer R=CH_3 8a, o-isomer

4a, m-isomer R=CH_3 8b, m-isomer

7c, p-isomer R=C_2H_5 8c, p-isomer

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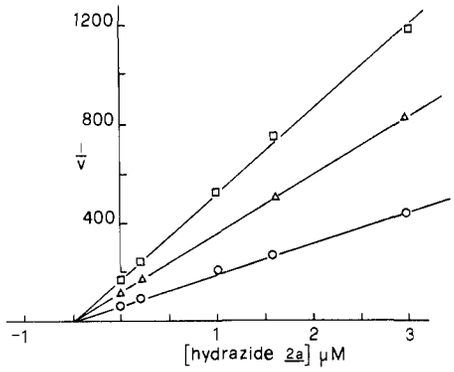

Figure 1. Dixon plot for inhibition of plasma amine oxidase by 4-pyrrol-1-ylphenylacetic acid hydrazide. The reaction mixture (1 mL) contained 36 μg (0.2 μM) of bovine plasma amine oxidase in 0.1 M phosphate buffer, pH 7.2, and the following amounts of benzylamine: (○) 0.5 mM, (△) 2.5 mM, (□) 5 mM. V was expressed as micromoles of benzaldehyde formed × minutes^{-1} at 37 °C.

methanol in the presence of concentrated sulfuric acid (Chart I).

Reaction of **4f** with hydrazine hydrate at reflux in ethanol afforded the required hydrazide **4g**.

Efforts to synthesize the ortho isomer of **2a** were unsuccessful because of difficulties encountered in preparing the ethyl ester of 2-pyrrol-1-ylphenylacetic acid (**5**).[30] In fact, when the acid **5** was refluxed with methanol and concentrated sulfuric acid, intramolecular cyclization occurred, leading to the known pyrrolo[1,2-*a*]quinolone **6**[30] as the only detectable product. The same reaction took place when *N*,*N*-carbonyldiimidazole (CDI) was used as a condensing agent (Scheme II).

In an attempt to establish some structure–activity relationships, compounds **2a**, **2b**, **3**, and **4g** were compared as amine oxidase inhibitors with the *o*-, *m*-, and *p*-pyrrol-1-ylbenzoic acid hydrazides **8a–c** prepared from the reaction between the corresponding alkyl ester of *o*-, *m*-, and *p*-pyrrol-1-ylbenzoic acid and hydrazine hydrate (Scheme III, Table I).

Biological Activity and Discussion

Effect of Hydrazides of Pyrrol-1-ylbenzoic and Pyrrol-1-ylphenylacetic Acids on Copper-Dependent Amine Oxidase Activity. Hydrazides of pyrrol-1-ylbenzoic and pyrrol-1-ylphenylacetic acids are not substrates for plasma amine oxidase and placental diamine oxidase. Instead, they act as noncompetitive inhibitors for both copper enzymes (Figure 1). The apparent K_i values of each compound at zero time of incubation, as determined by the Dixon plot, are summarized in Table II. Hydrazides of pyrrol-1-ylbenzoic acids are the least effective inhibitors. Their K_i's range from a value of 80 μM for the para derivative **8c** to 300 μM for the ortho derivative **8a**. Similar values are obtained for placental diamine oxidase. The hydrazides of phenylacetic acids are more potent inhibitors. In this case, the inhibitory power exerted on plasma amine oxidase is 1–2 orders of magnitude greater than that exerted on placental diamine oxidase. Also, the hydrazone **3** acts as an inhibitor but with a more complex pattern (Figure 2).

Table II. K_i Values of Complexes between Pyrrol-1-ylbenzoic Acid or Pyrrol-1-ylphenylacetic Acid Hydrazides and Copper-Dependent Amine Oxidase[a]

inhibitor	K_i, μM	
	bovine plasma amine oxidase	human placenta diamine oxidase
2a	0.5	10
2b	10.0	
3	0.8	10
4g	0.7	100
8a	300	250
8b	85	110
8c	80	100

[a] The incubation mixture contained 36 μg/mL plasma amine oxidase or 47 μg/mL (0.2 μM) placental diamine oxidase in 0.1 M phosphate buffer, pH 7.2. Other experimental conditions are described in Figure 1 (standard deviations within 10%).

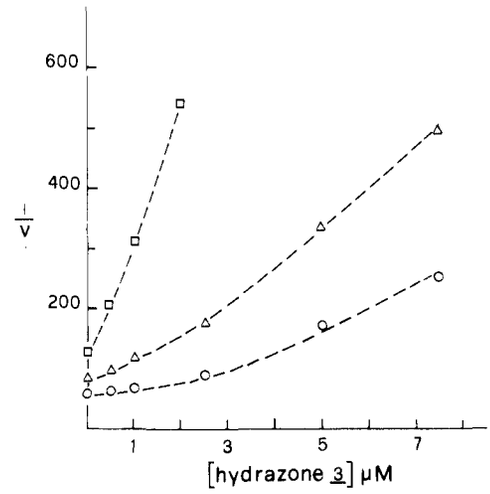

Figure 2. Dixon plot for inhibition of plasma amine oxidase by hydrazone **3**. Experimental conditions were the same as reported in Figure 1.

As the extrapolated value of K_i (Table II) and I_{50} (Table III) are similar to those calculated for the hydrazide of 4-pyrrol-1-ylphenylacetic acid, it could be possible that the hydrazone is enzymatically hydrolyzed to the corresponding hydrazide and that the inhibition is the result of the activity of both forms, which present different affinities.

As shown in Table III, the inhibitions is time dependent, leading to irreversible inactivation of the enzyme. With regard to hydrazides of phenylacetic acids, exhaustive dialysis against phosphate buffer removes inhibition at zero time but is not able to restore activity after 24 h of incubation. Moreover, these treatments are not able to restore significant enzymatic activity after inhibition by pyrrol-1-ylbenzoic acid hydrazides. Similar results are obtained with both copper amine oxidases. However, in the case of placental diamine oxidase, the reactivation process is difficult to quantify as incubation leads to enzyme denaturation. Typical experiments are reported in Figure 3.

Effects of Hydrazides of Pyrrol-1-ylbenzoic and Pyrrol-1-ylphenylacetic Acids on Monoamine Oxidase. Time-dependent inhibition of rat brain mitochondrial and human platelet MAO by hydrazides is shown in Table IV. In this case, slight inhibition was observed only at inhibitor concentration of 100 μM and after 2 h of incubation at 37 °C.

Conclusion

The present data indicate that hydrazides of pyrrol-1-ylphenylacetic acids are highly specific and potent irre-

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Table III. Effect of Hydrazides of Pyrrol-1-ylbenzoic and Pyrrol-1-ylphenylacetic Acids on Plasma Amine Oxidase and Placental Diamine Oxidase[a]

	I_{50}, μM					
	plasma amine oxidase: time of incubation, min				placental diamine oxidase: time of incubation, min	
inhibitor	0	15	30	60	0	30
2a	1	0.1	0.1	0.1	10	10
2b	20	10	10	10	100	50
3	1	0.5	0.1	0.1	50	10
4g	0.5	0.1	0.1	0.1	50	10
8a	300	300	300	300	100	100
8b	100	50	10	10	100	100
8c	65	10	5	5	200	50
isoniazid	100	10	10	5		

[a] The incubation mixture contained 0.1 μM plasma amine oxidase or placental diamine oxidase in 0.1 M phosphate buffer, pH 7.2. Time-dependent inhibition was determined at 37 °C as described in the Experimental Section. Data are the results of two experiments done in duplicate (standard deviations within 10%).

Table IV. Effect of Hydrazides of Pyrrol-1-ylbenzoic and Pyrrol-1-ylphenylacetic Acids on Brain Mitochondrial and Platelet MAO Activity[a]

	inhibition, %						
	brain mitochondria: inhibitor concentration, μM			platelets: inhibitor concentration, μM			
inhibitor	100	10	1	100	10	1	incubation time, min
2a	6	0	0	0	0	0	0
	9	4	2	13	4	0	120
2b	2	0	0	10	0	0	0
	3	0	0	4	3	0	120
3	0	0	0	0	0	0	0
	18	12	12	14	7	4	120
4g	3	2	0	0	0	0	0
	15	3	0	4	3	2	120
8a	0	0	0	0	0	0	0
	6	5	3	23	17	5	120
8b	0	0	0	0	0	0	0
	8	5	0	2	0	0	120
8c	0	0	0	0	0	0	0
	18	16	2	7	2	0	120
isoniazid	19	14	12				0
	22	16	16				120

[a] The incubation mixture contained mitochondria or platelet suspension in 0.1 M phosphate buffer, pH 7.2, at 37 °C. Data are the results of three experiments done in duplicate (standard deviations within 15%).

versible inactivators of copper-dependent amine oxidases, especially of plasma amine oxidase. In fact, comparison with the inhibitory effect exerted by isoniazid, which has been suggested as an antifibrotic agent,[23] shows that all hydrazides tested are potent inhibitors of Cu-AO and weak inhibitors of MAO activity. Furthermore, hydrazides of pyrrol-1-ylphenylacetic acids also selectively inhibit copper-dependent amine oxidases, having greater affinity for plasma amine oxidase. The results of this study also clearly indicate some structure–activity relationships. The length of the side chain is important because the presence of one methylene group increases the affinity of inhibitors for amine oxidase by 1–2 orders of magnitude. Also important is the point of attachment of the pyrrole ring to the phenyl moiety, since the inhibitory potency progressively decreases from para to meta and ortho isomers. This effect is evident for pyrrol-1-ylbenzoic acid hydrazides, while it is less relevant for pyrrol-1-ylphenylacetic acid derivatives. The presence of methyl groups in the pyrrole ring also produces a drastic decrease in potency.

As previously reported for other carbonyl group reagents,[24] the inhibition of copper-dependent amine oxidases by hydrazides is time dependent and implies two distinct steps. The first is fast and consists of the formation of a reversible complex between enzyme and inhibitor. In the second step, the complex is irreversibly transformed into a new species, involving the formation of a Schiff base.

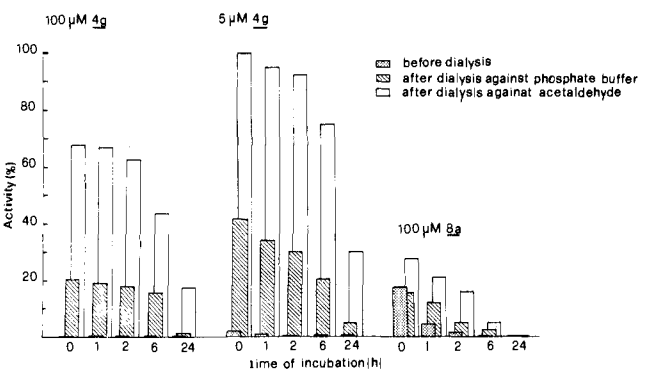

Figure 3. Reactivation of inhibitor-treated plasma amine oxidase after dialysis against buffer or acetaldehyde. The incubation mixture contained 0.20 μM plasma amine oxidase and 100 or 5 μM 3-pyrrol-1-ylphenylacetic acid hydrazide or 100 μM 4-pyrrol-1-ylbenzoic acid hydrazide in 0.1 M phosphate buffer, pH 7.2. After incubation for the required length of time at 25 °C, the mixture was dialyzed exhaustively against phosphate buffer or 5 mM acetaldehyde. Restored activity was expressed as percent of control. Other procedures are described in the Experimental Section (standard deviations within 15%).

Acetaldehyde acts as a competing carbonyl group and brings about recovery of enzyme activity.

Further experiments are in progress to evaluate the pharmacological behavior of compounds **2a** and **4g**.

Experimental Section

Melting points were determined with a Fisher-Johns apparatus and are uncorrected. IR spectra (Nujol mulls) were recorded on a Perkin-Elmer 297 spectrophotometer. Microanalyses were performed by A. Pietrogrande, Padova, Italy.

Preparation of Hydrazides. A mixture of the alkyl ester of the appropriate acid (0.015 mol), 10 mL of hydrazine hydrate, and 10 mL of ethanol was heated at reflux for 3 h. After cooling, the precipitated hydrazide was collected and recrystallized from a suitable solvent (see Table I for **2a**, **2b**, **4g**, **8a**, **8b**, and **8c**).

Methyl 3-Pyrrol-1-ylbenzoate (4a). 2,5-Dimethoxytetrahydrofuran (16.0 g, 0.12 mol) was added to methyl 3-aminobenzoate (15.1 g, 0.1 mol) in 100 mL of glacial acetic acid, and the mixture was heated at reflux for 30 min. After removal of solvent, the oily residue was chromatographed on silica gel, eluting with benzene. The collected eluates, after evaporation of the solvent, furnished 16.4 g of crude **4a**, which was distilled under reduced pressure.

3-Pyrrol-1-ylbenzyl Alcohol (4b). A solution of **4a** (30.0 g, 0.15 mol) in 150 mL of anhydrous ethyl ether was added dropwise with stirring to a suspension of 7.6 g of lithium aluminum hydride in 200 mL of the same solvent. After the addition was complete, the mixture was heated at reflux for 3 h. Addition of crushed ice to destroy the excess of hydride followed by filtration and separation of the ether phase gave a solution, which was dried over anhydrous sodium sulfate. Evaporation of this solution furnished **4b**.

3-Pyrrol-1-ylbenzyl Bromide (4c). Phosphorus tribromide (14.0 g, 0.05 mol) in 60 mL of anhydrous ethyl ether was added to a solution of **4b** (10.0 g, 0.1 mol) in the same solvent (300 mL), and the mixture was heated at reflux for 4 h. After addition of 30 mL of absolute ethanol, the mixture was refluxed for an additional hour, then was cooled, washed with diluted sodium carbonate and with water, and then dried over sodium sulfate. Evaporation of the solvent furnished **4c** as an oil, which slowly solidified.

3-Pyrrol-1-ylbenzeneethanenitrile (4d). A solution of **4c** (22.1 g, 0.094 mol) in 180 mL of ethanol was added to a stirred solution of sodium cyanide (5.5 g, 0.112 mol) in 70 mL of water. The mixture was heated at reflux for 3 h, then treated with crushed ice (500 g), and extracted with chloroform. The organic layer was separated, dried over anhydrous sodium sulfate, and then evaporated in vacuo. The residual oil was distilled under reduced pressure to afford **4d**.

3-Pyrrol-1-ylphenylacetic Acid (4e). A mixture of **4d** (10.0 g, 0.055 mol), potassium hydroxide (16 g), and 60 mL of ethylene glycol was heated at 160 °C for 6 h. The solution was treated with crushed ice (300 g) and acidified by adding 30 mL of concentrated hydrochloric acid. The precipitate was filtered, dried, and recrystallized to give **4e**.

Methyl 3-Pyrrol-1-ylphenylacetate (4f). A solution of **4e** (2.0 g, 0.01 mol) in 50 mL of anhydrous methanol containing 0.5 mL of concentrated sulfuric acid was heated at reflux for 12 h. Evaporation of solvent and treatment with crushed ice (100 g) furnished an oil, which was extracted with ethyl ether. The extracts were washed with water, dried over anhydrous sodium sulfate, and evaporated to give the oily **4f**, which was purified by distillation under reduced pressure.

1-Isopropylidene-[2-(4-pyrrol-1-ylphenyl)acetyl]hydrazine (3). A solution of **2a** (1.07 g, 0.005 mol) in 75 mL of acetone was refluxed for 1 h. Evaporation of solvent furnished the required **3**.

Biological Methods. Preparation and Assay of Amine Oxidases. Benzylamine, kynuramine, and pargyline were purchased from Sigma; aminoguanidine was purchased from Merck, and *p*-(dimethylamino)benzylamine was prepared according to Bardsley et al.[35]

Bovine plasma amine oxidase was prepared according to Turini et al.[36] The enzyme was homogeneous by the criteria of disk electrophoresis. Enzymic activity was determined both spectrophotometrically by monitoring at 250 nm the production of benzaldehyde (E = 12.500 M^{-1} cm^{-1})[37] and polarographically by determination of oxygen uptake. Human placental diamine oxidase was purified according to Crabbe et al.[38] The DAO activity was obtained according to Bardsley et al.[35] by monitoring at 250 nm the production of *p*-[(dimethylamino)methyl]benzaldehyde (E = 10.400 M^{-1} cm^{-1}).

Rat brain mitochondria were isolated according to Achee et al.[39] by differential centrifugation of the tissue homogenates in 0.25 M sucrose, pH 7.4. Platelet-rich plasma from healthy donors was obtained by centrifugation at 190*g*. The platelets were pelleted at 27000*g* in plastic tubes. The platelet pellet was suspended in isotonic saline and sonicated for 1 min in a 100-W MSE ultrasonic disintegrator. Mitochondrial and platelet MAO activity was determined by a fluorimetric method with kynuramine as substrate.[40]

All the enzyme preparations were assayed in the presence of specific inhibitors to exclude the presence of contaminating activities. Pargyline (1 mM) and 1 mM aminoguanidine were employed as inhibitors of, respectively, mitochondrial type A and B MAOs and copper-dependent AOs. The results demonstrated that no amine oxidizing activity other than that expected was present in each preparation.

The protein concentration was measured by the biuret method of Goa.[41] The enzyme activity was expressed as moles of substrate oxidized × (milligrams of protein)$^{-1}$ × minutes^{-1}. Purified plasma amine oxidase and placental diamine oxidase had specific activity of 0.44 μmol × mg^{-1} × min^{-1} and 0.12 μmol × mg^{-1} × min^{-1}, respectively, at 37 °C.

Spectrophotometric analyses were performed with a Beckman Model 26 instrument. Fluorimetric measurements were recorded with an Aminco-Bowman spectrophotofluorometer. Oxygen uptake was measured with a YSI Oxigraph Model 53 instrument.

Calculation of Inhibitory Activity Parameters (K_i and I_{50}) for Hydrazide Derivative–Amine Oxidase Complexes. The inhibitory activity is expressed by I_{50}, i.e., the inhibitor concentration that diminished the enzyme activity by 50%. Dixon plots were used to estimate the equilibrium constant (K_i) for the hydrazide–enzyme complex.

Possible time-dependent inhibition was checked by the following procedure. Inhibitor was added at zero time, then after incubation for the required length of time, a saturating amount of substrate (3 mM benzylamine or *p*-[(dimethylamino)-methyl]benzylamine, 1 mM kynuramine) was added, and the change in absorbance or in fluorescence was recorded.

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Registry No. **1a**, 22106-49-6; **1b**, 85209-63-8; **2a**, 112596-34-6; **2a** (meta isomer), 112596-35-7; **2b**, 112575-81-2; **3**, 112575-82-3; **4a**, 83140-93-6; **4b**, 83140-94-7; **4c**, 112596-36-8; **4d**, 112575-86-7; **4e**, 112575-87-8; **4f**, 112575-85-6; **7a**, 10333-67-2; **7b**, 5044-37-1; **8a**, 31739-63-6; **8b**, 112575-83-4; **8c**, 112575-84-5; AO, 9059-11-4; MAO, 9001-66-5; DAO, 9001-53-0; hydrazine hydrate, 7803-57-8; acetone, 67-64-1; ethyl 3-aminobenzoate, 582-33-2; 2,5-dimethoxytetrahydrofuran, 696-59-3; isoniazid, 54-85-3.

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