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α-Trifluoromethylhistamine: A Mechanism-Based Inhibitor of Mammalian Histidine Decarboxylase

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α-Trifluoromethylhistamine (**1**), proposed as a suicide inhibitor of histidine decarboxylase, has been prepared from β-trifluoromethyl-β-alanine. Histidine decarboxylase from hamster placenta is inhibited in a time-dependent manner by **1**; however, the adduct formed between inhibitor and enzyme is labile. **1** inhibits stomach histidine decarboxylase activity *in vivo* in rats, but has no antisecretory effect in the pyloric-ligated stomach of the mouse.

Histamine is recognized as an important mediator of such physiological processes as allergy and gastric acid secretion. As a consequence, specific antagonists for H_1 receptors which mediate the former response (*1, 2*), and for H_2 receptors for the latter (*3*) have been developed as highly successful drug classes. In contrast to the intense activity in the receptor antagonist area, little has been achieved until recently (*4–6*) in controlling the biosynthesis of histamine by inhibition of histidine decarboxylase, the pyridoxal phosphate-dependent enzyme responsible for the conversion of histidine to histamine.

Reversible inhibitors such as α-methylhistidine lack potency (*7*). We felt that a specific irreversible inhibitor would be necessary to elicit useful *in vivo* effects. One such inhibitor, α-fluoromethylhistidine, which is of the "suicide" type (*8*) has been reported to inactivate brain histidine decarboxylase *in vivo* after i.p. administration (*9*). Both α-chloromethylhistidine (*6*) and α-fluoromethylhistidine (*5*) have been reported to effect a dose-dependent decrease of stomach histidine decarboxylase activity.

α-Fluoromethylhistidine and α-chloromethylhistidine are analogs of histidine, the substrate for enzymatic decarboxylation. However, it has become apparent that pyridoxal phosphate-dependent α-amino acid decarboxylases are also susceptible to irreversible inhibition by acetylenic (*10, 11*) and mono- (*4*) and difluoromethyl analogs (*12*) of the normal product of enzymatic decarboxylation. The absolute stereochemistry of the inactivation process suggests that this can be a consequence of the microscopic reversibility principle (*10, 11, 13*) or of a transamination mode (*14*), depending on the enzyme. While α-mono and α-difluoromethyl putrescine are suicide inactivators of ornithine decarboxylase (*12*), the α-

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trifluoromethyl analog is not (*15*). Similarly, γ-trifluoromethyl GABA does not inactivate GABA-transaminase (*16*).

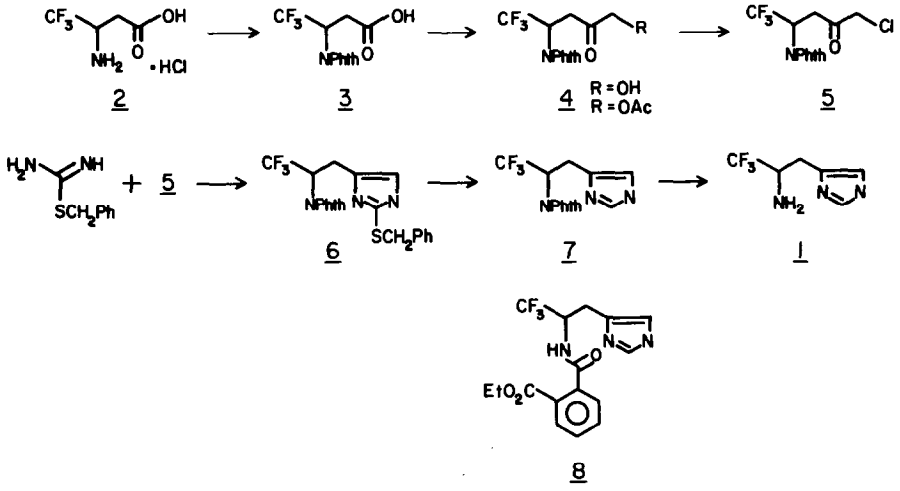
On the other hand, trifluoroalanine is an efficient inactivator of alanine racemase (*17*) and of γ-cystathionase (*18*) while 3,3,3-trifluoro-2-aminoisobutyrate is a suicide inhibitor of the pyruvate-dependent α-dialkylaminoacid transaminase from *Pseudomonas cepacia* (*19*). α-Trifluoromethylhistamine (**1**) thus appeared to be of interest as a synthetic target as a potential suicide inhibitor of histidine decarboxylase not only to provide further information as to the utility of trifluorinated compounds, but also as a potential means to control histamine formation *in vivo*.

SYNTHESIS

The synthesis of **1** (Scheme 1) begins with conversion of the hydrochloride of the known amino acid **2** (*20*) to the phthalimide **3** in 75% yield via phthalic anhydride fusion. The acid chloride derived from **3** by treatment with Cl_2CHOCH_3 (*21*) smoothly acylated tris(trimethylsiloxy)ethylene in the presence of $SnCl_4$ (*22*). Aqueous acid treatment liberated the oily hydroxyketone **4a** in 75% yield from **3.** Alcohol **4a** was converted in 87% yield to the chloride **5** (CH_3SO_2Cl, Et_3N, CH_2Cl_2) which, on treatment with *S*-benzylisothiourea (NaI, Na_2CO_3, DMF), afforded imidazole **6** in 90% yield. Desulfurization of **6** with Raney nickel afforded **7.** Ester-amide acid **8** invariably appeared as a byproduct in larger-scale reactions. Hydrolysis of **7** (and **8**) gave **1** as the dihydrochloride in 95% yield.

ENZYME INHIBITORY ACTIVITY

α-Trifluoromethylhistamine inhibited hamster placental histidine decarboxylase



SCHEME 1

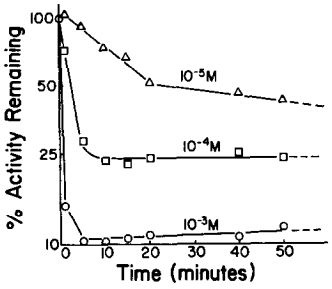


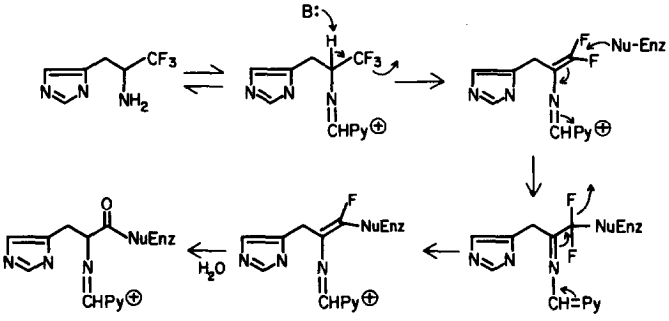
FIGURE 1

in vitro in a time- and concentration-dependent manner. The inhibition process did not obey pseudo-first-order kinetics (Fig. 1). Using a 60-min assay period immediately after diluting the 10^{-4} *M* preincubate 100-fold, the inhibition was found to reach a limiting value of 75% within 10 min. If, however, the assay time was decreased to 30 min the observed inhibition increased to 95% at the same preincubation concentration. On the other hand, when the 10^{-4} *M* preincubate was kept for 60 min after dilution and then assayed for 30 min, the inhibition was only 49%. These results suggested that the reaction between histidine decarboxylase and α-trifluoromethylhistamine formed an adduct that was labile. In fact, dialysis of inhibited enzyme (against 0.05 *M* KP_i, pH 7, containing 10^{-5} *M* pyridoxal phosphate) resulted in complete recovery of enzymatic activity. The rate of recovery of enzymatic activity was determined after separating free inhibitor from the enzyme by Sephadex G-25 chromatography, and was found to follow a first-order process with a $t_{1/2}$ of 1.3 hr (data not given).

In vivo α-trifluoromethylhistamine at a dose of 100 mg/kg i.p. in rats inhibited stomach histidine decarboxylase activity by 80% 1 hr after administration. Three hours after administration the enzyme was still 70% inhibited. Despite this inhibition of histidine decarboxylase activity, it was noted in a separate experiment that **1** has no antisecretory effect up to 200 mg/kg (p.o. administration) in the pyloric-ligated stomach of the mouse (*23*). α-Fluoromethylhistidine, which does form a stable adduct with histidine decarboxylase, also has no effect on gastric acid secretion despite prolonged enzyme inhibitory effect *in vivo* (*5*). As higher doses of **1** proved toxic further studies were terminated.

DISCUSSION

A putative mechanism for the inhibition of histidine decarboxylase by α-trifluoromethylhistamine (**1**) is shown in Scheme 2. It is presumed that the normal Schiff's base formation should occur. Then, as a consequence of microscopic reversibility (*10, 11, 13*) or via transamination, (*14*) the proton α to the nitrogen function would be abstracted. Such carbanion formation would induce β-elimination of fluoride ion leading to formation of a Michael acceptor. Addition of some enzyme nucleophile would then lead to a situation wherein transformed inhibitor is covalently linked to the protein. Further transformations, however, could take



SCHEME 2

place, with the additional loss of fluoride ion eventually leading to an acylated enzyme. Deacylation could then be the mechanism by which inhibited enzyme regenerates on standing or on dialysis. By way of precedent, when γ-cystathionase is inactivated by trifluoroalanine, three fluoride ions are released per mole of inactivator which becomes covalently bonded to the enzyme via an acyl linkage (*18*). While the possibility of deacylation might appear to suggest an inherent weakness in the use of trifluorinated amines as suicide inhibitors, this is clearly not the case, as evidenced by the stable adducts formed from trifluoroalanine (*17*) and trifluoro-2-aminoisobutyrate (*19*). In contrast, the adduct formed between difluoroalanine and *Escherichia coli* B alanine racemase is labile (*17*). Similarly, 5-trifluoromethyl-2′-deoxyuridylate also forms an unstable adduct as a result of it's reaction with thymidylate synthetase. In this case the possibility of an acylated enzyme intermediate has also been invoked (*24*). α-Trifluoromethylamines have now been described as inactive (*15*) and active (*17*) as irreversible inhibitors and, in the present case, as leading to a temporarily inactivated enzyme. It seems that empiricism still persists even with a design approach which is considered among the most rational (*8*).

EXPERIMENTAL PROCEDURES

Melting points were determined in a Thomas–Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were obtained in KBr discs (solids) or as a film (liquids) using a Perkin–Elmer 337 instrument. NMR spectra were obtained on a Varian EM 360 instrument. Elemental analyses were determined by the Analytical Chemistry Department of Merrell Dow and were within ±0.4% of the theoretical value.

Materials used for *in vitro* and *in vivo* experiments were Sephadex G-25 fine, Pharmacia; L-[1-^{14}C]histidine (NEC493) and OmniFluor, New England Nuclear; Sprague–Dawley rats; Charles River Syrian hamsters; Bradford protein reagent, Bio-Rad. All other reagents were analytical grade commercially available.

1,3-Dihydro-1,3-dioxo-β-(trifluoromethyl)-2H-isoindole-2-propanoic acid (**3**). The amino acid hydrochloride **2** (100 g, 0.52 mol) and phthalic acid (77 g, 0.52 mol)

were ground together in a mortar, then fused until evolution of H_2O and HCl ceased. On cooling, the brown mass was dissolved in chloroform and acetone, then treated with charcoal and filtered. The filtrate was concentrated, whereupon crystallization occurred to afford **3** (110.8 g, 75%), mp 153.5–155°C.

Anal. ($C_{12}H_8F_3NO_4$) C, H, N.

2-[4-Chloro-3-oxo-1-(trifluoromethyl)butyl]-1,3-dihydro-2H-isoindole-1,3-dione (**5**). A solution of the acid **3** (110.8 g, 0.39 mol) and dichloromethyl methyl ether (175 ml, 1.9 mol) was heated at reflux for 1 hr, then cooled, concentrated, and diluted with chloroform. Solid K_2CO_3 was added, the mixture was filtered, and the filtrate was concentrated to afford the acid chloride, which was used without further purification.

To the acid chloride derived from **3** was added tris(trimethylsiloxy)ethylene (*18*) (237.7 g, 0.8 mol) and $SnCl_4$ (0.5 ml). The mixture was warmed until it became homogeneous and then was cooled. The mixture was then treated with 0.5 *N* HCl (200 ml) in dioxane (500 ml), heated on the steam bath for 20 min, then cooled and concentrated. The alcohol **4a** was isolated by ether extraction and used directly. A sample was converted to the acetate **4b** for characterization.

Anal. ($C_{15}H_{12}F_3NO_5$) C, H, N.

The crude alcohol **4a** was dissolved in CH_2Cl_2 (500 ml), cooled to 0°C, and treated with methane sulfonyl chloride (61 ml, 0.78 mol), followed by NEt_3 (109 ml, 0.78 mol). The mixture was stirred at room temperature for 6 hr then filtered and concentrated. Ether was added and the solution was washed with H_2O, 1 *N* HCl and brine, then dried ($MgSO_4$) and concentrated. The residue was purified by HPLC using 40% EtOAc–hexane as eluant, followed by recrystallization from ethanol to afford the chloride **5** (108.4 g, 87%), mp 78–80°C.

Anal. ($C_{13}H_9ClF_3NO_3$) C, H, N, Cl.

1,3-Dihydro-2-[2-[2-[(phenylmethyl)thio]-1H-imidazol-4-yl]-1-(trifluoromethyl)ethyl]-2H-isoindole-1,3-dione (**6**). A mixture of the chloride **5** (18.3 g, 57.4 mmol), NaI (2.5 g, 17.2 mmol), and Na_2CO_3 (30.4 g, 287 mmol) in DMF (dimethylformamide) (115 ml) was cooled to 0°C, and *S*-benzylisothiourea hydrochloride (13.9 g, 68.9 mmol) was added. The mixture was heated to 80°C, then cooled, and H_2O (5 ml) was added. The DMF was distilled off using an aspirator and the product (24.3 g, 98% crude) was isolated by ethyl acetate extraction. Recrystallization from ethanol afforded pure **6**, mp 171–172.5°C.

Anal. ($C_{21}H_{16}F_3N_3O_2S$) C, H, N, S.

1,3-Dihydro-2-[2-(1H-imidazol-4-yl)-1-(trifluoromethyl)ethyl]-2H-isoindole-1,3-dione (**7**). A mixture of the thioester **6** (21.3 g, 49.4 mmol); Raney Ni, which had been prepared from Ni/Al alloy (200 g); H_2O (960 ml); and NaOH (256 g) in ethanol (600 ml) was heated at reflux for 2 hr, then filtered. The filter cake was washed with hot ethanol and the combined filtrates were concentrated. Flash chromatography using 5% CH_3OH–CH_2Cl_2 as eluant gave the imide **7** (3.9 g), the amide **8** (1.1 g), and a mixture of **7** and **8** (1.0 g). The imide **7** was recrystallized from 50% aqueous ethanol to give the pure sample, mp 181–182.5°C.

Anal. ($C_{14}H_{10}F_3NO_3$) C, H, N.

α-Trifluoromethylhistamine dihydrochloride (*1*). The imide **7** (508 mg) in methanol (5 ml) and concentrated HCl (5 ml) was heated at reflux for 24 hr then concen-

trated. Further methanol (5 ml) and concentrated HCl (5 ml) were added and the mixture was heated for 4 hr. On cooling the mixture was filtered and the filtrate was concentrated. The residue was recrystallized from ethanol to afford **1** (300 mg), mp 241–245°.

Anal. ($C_6H_{10}Cl_2F_3N_3$) C, H, N, Cl.

Assay of Time-Dependent Inhibition of Histidine Decarboxylase Activity in Vitro

Histidine decarboxylase was partially purified using hamster placenta at the 16th day of gestation as the source and following Procedure A of Leinweber (*25*). Enzymatic activity was determined with a $^{14}CO_2$ trapping method (*26*) using L-[1-^{14}C]histidine as substrate. The specific activity of the final $(NH_4)_2SO_4$ fraction was 0.55 μmol mg^{-1} hr^{-1}. This is to be compared with a reported specific activity of 0.76 μmol mg^{-1} hr^{-1} for a preparation from fetal rat liver (*27*), and of 55.4 μmol mg^{-1} hr^{-1} from rat stomach (*28*). Assuming similar kinetic properties for the enzyme from hamster placenta and rat stomach, this preparation would be approximately 1% pure. 1 unit is defined as 1 μmol hr^{-1} at 37°C using 0.5 m*M* L-histidine at pH 7. Protein was determined by the method of Bradford (*29*) using bovine serum albumin as the standard.

To test for irreversible inhibition *in vitro*, hamster placental histidine decarboxylase was incubated with **1** and, at selected times, aliquots were removed and assayed for residual activity (*26*). A typical incubate consisted of 50 μl of enzyme (0.5 units) in a total volume of 100 μl 5 $\times$ 10^{-2} *M* potassium phosphate buffer, pH 7, containing 10^{-5} *M* pyridoxal phosphate and 0–10^{-3} *M* **1**. It was incubated at 37°C; at the indicated times a 10-μl aliquot was withdrawn and added to a glass scintillation vial closed with a serum cap and containing 1 ml of assay buffer consisting of 0.1 μCi L-histidine (2 mCi/mmol), 50 μmol potassium phosphate, pH 7, and 0.01 μmol pyridoxal phosphate.

Assays were routinely run for 60 min at 37°C, at which time they were stopped by the addition of 500 μl of 45% trichloroacetic acid. Released $^{14}CO_2$ was quantitatively absorbed onto 1-cm^2 pieces of filter paper which had previously been spotted with 50 μl hyamine hydroxide and were suspended from the serum cap with a hook. After 30 min the filter papers were removed and added to 10 ml of scintillation cocktail (Toluene–methanol 4/1, v/v, containing Omnifluor, 4 g/liter) and counted in a Beckman LS330 liquid scintillation counter.

Measurement of Histidine Decarboxylase Activity ex Vivo

Histidine decarboxylase activity of the glandular stomach of male Sprague–Dawley rats (150–200 g) was induced by refeeding for 1½ hr after 24 hr starvation. Enzyme activity peaked between 4 and 6 hr after refeeding. Histidine decarboxylase was determined *ex vivo* 1–4 hr after administration of 0–100 mg/kg **1** i.p. (5 hr after refeeding). The rats were killed with CO_2, and their stomachs were removed rapidly, opened along the greater curvature, rinsed with isotonic saline, and frozen on dry ice and stored at −20°C. The glandular portion of the stomach was homogenized with a Tekmar Tissuemizer in 2 vol of 0.1 *M* potassium phosphate

buffer, pH 7, containing 10^{-5} *M* pyridoxal phosphate and centrifuged at 12,000*g* for 30 min, and the supernatant was assayed for histidine decarboxylase activity (*26*). Each sample consisted of 100 μl tissue extract and 10 μl L-[1-^{14}C]histidine (0.1 μCi, 0.2 mCi/mmol) in a volume of 110 μl and was assayed at 37°C for 1 hr as in the *in vitro* assays.

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