alogs were potent antihyperlipidemic agents in mice by suppressing both cholesterol and triglyceride levels. The probable site of action of the boron analogs is in the early synthesis of lipids.

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# Inhibition of Histidine Decarboxylase by Imidazole Derivatives

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**Abstract** □ A series of 4-imidazolylmethyl arylsulfides, sulfoxides, and sulfones and two carbon isosteres of 4-imidazolylmethylphenyl sulfide (I) were synthesized and tested for inhibition of histidine decarboxylase from rat stomach. None of these analogs of I met the criterion of a potent and specific inhibitor of histidine decarboxylase.

**Keyphrases** □ Imidazoles, 4-substituted—synthesis and evaluation for inhibition of histidine decarboxylase □ Histidine decarboxylase—inhibition, synthesis and evaluation of 4-substituted imidazoles □ Structure–activity relationships—synthesis and evaluation of 4-substituted imidazoles for inhibition of histidine decarboxylase

Due to the implication of histamine in many physiological processes (1, 2), there has been interest in regulating its *de novo* biosynthesis by inhibiting histidine decarboxylase (EC 4.1.1.22) (3–7). A specific and potent inhibitor of this enzyme would be important as a new type of antihistamine and as a useful research tool. The authors previously reported on some derivatives of histidine as candidate inhibitors of histidine decarboxylase from rat stomach (3, 4). This paper summarizes the synthesis and evaluation of some 4-substituted imidazoles as inhibitors of this pharmacologically important enzyme.

## DISCUSSION

The imidazolyl sulfides in Table I were prepared by reaction of the appropriate 4-imidazolylalkyl chloride with a mercaptan by a modification of a literature method (7, 8). Oxidation of I or II with hydrogen peroxide gave the sulfoxides VI and VIII or sulfones VII and IX. If the reactions were heated, partial decomposition occurred and the yields were diminished. Application of the Wittig reaction to 4-(*N*-triphenylmethyl)imidazolecarboxaldehyde (4), followed by removal of the trityl group and catalytic hydrogenation, gave IV and V.

Inhibition of histidine decarboxylase was measured as described previously (3). The inhibition obtained with I and II was in close agreement with reported values (7). Varying the nature of the bridge between the imidazole and phenyl rings resulted in a loss in inhibition relative to I (II–IX in Table II). Insertion of nitrogen in the phenyl ring (X and XI) also was deleterious to activity. Substitution of a methyl group on the bridge (XII) gave some enhancement in inhibition with an $I_{50}$ of $4.7 \pm 0.3 \times 10^{-5}$ *M*. Extending the chain length (XIII and XIV) resulted in a loss in inhibition.

In conclusion, several alterations in the structure of I were made, but none of the analogs met the criteria of a potent and specific inhibitor of histidine decarboxylase.

## EXPERIMENTAL[1]

Each analytical sample had spectral data compatible with its assigned structure and moved as a single spot on TLC. The analytical samples gave combustion values for carbon, hydrogen, and nitrogen within 0.4% of the theoretical values.

**4-Imidazolylmethylphenyl Sulfide Hydrochloride (I)**—*Method A*—To an ice bath-cooled solution of 3.24 g (60.0 mmoles) of sodium methoxide in 100 ml of ethanol was added 6.15 ml (60.0 mmoles) of thiophenol, followed by the addition of 4.45 g (29.0 mmoles) of 4-chloromethylimidazole hydrochloride (8, 9) in 25 ml of ethanol. After 1 hr, the ice bath was removed, and the reaction was stirred at ambient temperature for several hours. The mixture was filtered, and the filtrates were spin evaporated *in vacuo*.

The residue was dissolved in 10% HCl and extracted with ether to remove the excess thiol. The aqueous solution was adjusted to pH 8–9 with 1 *M* NaOH and extracted with ether. The combined extracts were washed with water and brine and then dried. Spin evaporation gave an oil, which was converted to the solid hydrochloride and recrystallized.

*Method B*—The reaction was carried out with an equimolar quantity of mercaptan. The reaction mixture was spin evaporated to give a residue. This residue was dissolved in chloroform, washed with water, dried, and reevaporated prior to hydrochloride formation.

**4-Imidazolylmethylphenyl Sulfone Hydrochloride (VII): *Method C***—A solution of 3.13 g (13.8 mmoles) of I and 15 ml of 30% aqueous hydrogen peroxide in 20 ml of acetic acid was stirred at ambient temperature for 20 hr. The reaction was poured over 200 ml of ether containing 10 ml of hydrogen chloride-saturated ethanol, and the mixture was stirred vigorously to induce the oil to solidify. The solvent was decanted from the semisolid, a moderate amount of ethanol was added, and the mixture was stirred until a crystalline solid was formed. The product was collected, washed with ether, and recrystallized.

**1-(4-Imidazolyl)-2-phenylethylene Hydrochloride (IV)**—To a

[1] Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected.

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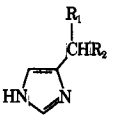
**Table I—4-Substituted Imidazoles**



| Compound | $R_1$ | $R_2$ | Method | Yield, % | Melting Point | Formula | Analysis, % | Calc. | Found |
|---|---|---|---|---|---|---|---|---|---|
| I | H | $SC_6H_5$ | A | 77 | 124–125°[a,b] | $C_{10}H_{10}N_2S$·HCl | C | 53.0 | 53.2 |
| | | | | | | | H | 4.89 | 4.91 |
| | | | | | | | N | 12.4 | 12.5 |
| II | H | $SCH_2C_6H_5$ | A | 57[a] | 143–144°[c] | $C_{11}H_{12}N_2S$·HCl | C | 54.9 | 54.6 |
| | | | | | | | H | 5.44 | 5.46 |
| | | | | | | | N | 11.6 | 11.8 |
| III | H | $CH_2SC_6H_5$ | A[d] | 73 | 108–109°[e] | $C_{11}H_{12}N_2S$·HCl | C | 54.9 | 54.5 |
| | | | | | | | H | 5.44 | 5.45 |
| | | | | | | | N | 11.6 | 11.7 |
| IV | | $=CHC_6H_5$ | Exp. | 48 | 98–110°[a] | $C_{11}H_{10}N_2$·HCl | C | 63.9 | 63.7 |
| | | | | | | | H | 5.37 | 5.37 |
| | | | | | | | N | 13.6 | 13.5 |
| V | H | $CH_2C_6H_5$ | Exp. | 99 | 147–149°[a] | $C_{11}H_{12}N_2$·HCl | C | 63.3 | 63.4 |
| | | | | | | | H | 6.28 | 6.22 |
| | | | | | | | N | 13.4 | 13.8 |
| VI | H | $S(O)C_6H_5$ | C[f] | 78[a] | 155–157° | $C_{10}H_{10}N_2OS$·HCl | C | 49.5 | 49.8 |
| | | | | | | | H | 4.57 | 4.59 |
| | | | | | | | N | 11.5 | 11.5 |
| VII | H | $S(O)_2C_6H_5$ | C | 69[g] | 217–225° | $C_{10}H_{10}N_2O_2S$·HCl | C | 46.4 | 46.5 |
| | | | | | | | H | 4.29 | 4.35 |
| | | | | | | | N | 10.8 | 11.0 |
| VIII | H | $S(O)CH_2C_6H_5$ | C[f] | 86 | 181–182°[a] | $C_{11}H_{12}N_2OS$·HCl | C | 51.5 | 51.6 |
| | | | | | | | H | 5.10 | 5.24 |
| | | | | | | | N | 10.9 | 11.0 |
| IX | H | $S(O)_2CH_2C_6H_5$ | C | 72[g] | 228–233° eff. | $C_{11}H_{12}N_2O_2S$·HCl | C | 48.4 | 48.3 |
| | | | | | | | H | 4.80 | 4.97 |
| | | | | | | | N | 10.3 | 10.5 |
| X | H | *S*-Pyridin-2-yl | B | 52 | 218–219° dec.[a] | $C_9H_9N_3S$·2HCl | C | 40.9 | 41.0 |
| | | | | | | | H | 4.20 | 4.29 |
| | | | | | | | N | 15.9 | 15.8 |
| XI | H | *S*-Pyrimidin-2-yl | B | 48 | 147–149° eff.[a] | $C_8H_8N_4S$·HCl·$H_2O$ | C | 38.9 | 39.0 |
| | | | | | | | H | 4.49 | 4.49 |
| | | | | | | | N | 22.7 | 22.4 |
| XII | $CH_3$ | $SC_6H_5$ | A[h] | 52[a] | 124–126° | $C_{11}H_{12}N_2S$·HCl | C | 54.9 | 54.6 |
| | | | | | | | H | 5.44 | 5.54 |
| | | | | | | | N | 11.6 | 11.6 |
| XIII | $CH_2CH_3$ | $SC_6H_5$ | A[h,i] | 46 | 71–72°[j] | $C_{12}H_{14}N_2S$ | C | 66.0 | 66.0 |
| | | | | | | | H | 6.46 | 6.49 |
| | | | | | | | N | 12.8 | 12.9 |
| XIV | $(CH_2)_5CH_3$ | $SC_6H_5$ | A[h,k] | 29 | 64–65°[j] | $C_{16}H_{22}N_2S$ | C | 70.0 | 69.6 |
| | | | | | | | H | 8.08 | 8.15 |
| | | | | | | | N | 10.2 | 10.6 |

[a] Recrystallized from ethanol–ether. [b] Lit. (7) mp 124–125°. [c] Lit. (7) mp 142–143°. [d] For the preparation of the starting 4-(2-chloroethyl)imidazole, see Refs. 11 and 12. [e] Recrystallized from acetonitrile. [f] Only a 10% molar excess of 30% hydrogen peroxide was used. [g] Recrystallized from ethanol. [h] For preparation of the starting chloroalkylimidazoles, see Ref. 4. [i] Purified as oxalic acid salt prior to neutralization. [j] Recrystallized from ethyl acetate–hexane. [k] Purified by column chromatography on Florisil as free base.

stirred mixture of 6.66 g (17.1 mmoles) of benzyltriphenylphosphonium chloride (10) and 5.70 g (16.8 mmoles) of 4-(*N*-triphenylmethyl)imidazolecarboxaldehyde (4) in 150 ml of ethanol was added 1.30 g (2.41 mmoles) of sodium methoxide in 20 ml of ethanol. After 23 hr at ambient temperature, the reaction was diluted with 400 ml of water and extracted with three 75-ml portions of chloroform. The combined extracts were washed with 50 ml of water and 50 ml of brine, dried, and spin evaporated *in vacuo*.

The residual syrup was dissolved in a minimum of hot ethyl acetate and introduced on a column of magnesium silicates[2] (65 g, 31 cm × 2.4 cm) in hexane. After the column had cooled, it was eluted with hexane. The first 200 ml of eluate, which contained the triphenylphosphine oxide free product, was spin evaporated *in vacuo* to give a syrup. This syrup was triturated under hexane to give a white solid (60% yield, 4.10 g) of 1-[4-(*N*-triphenylmethyl)imidazolyl]-2-phenylethylene (XV) as a mixture of *cis*- and *trans*-isomers, which was used without further purification.

A solution of 4.05 g (10.1 mmoles) of XV, 20 ml of tetrahydrofuran, and 30 ml of 6 *M* HCl was heated on a steam bath for 3 hr. The reaction was cooled, spin evaporated *in vacuo*, and diluted with 50 ml of water. The solids were removed by filtration and washed with water. The combined filtrates and washes were spin evaporated *in vacuo* to give an oil, which crystallized under ether. The compound was a mixture of the *cis*- and *trans*-isomers and appeared as two spots on TLC in chloroform–ethanol (9:1) on silica gel plates.

**4-(2-Phenethyl)imidazole Hydrochloride (V)**—A mixture of 1.05 g (5.08 mmoles) of IV, 100 ml of ethanol, and 100 mg of 10% palladium-on-carbon was shaken in the presence of hydrogen at 2–3 atm for 0.5 hr.

**Table II—Inhibition of Histidine Decarboxylase[a]**

| Compound | Inhibition at $10^{-4}$ *M*, % |
|---|---|
| I | 69 |
| II | 40 |
| III | 35 |
| IV | 21 |
| V | 5 |
| VI | 7 |
| VII | 43 |
| VIII | 7 |
| IX | 6 |
| X | 34 |
| XI | 6 |
| XII | 84 |
| XIII | 54 |
| XIV | 31 |

[a] The enzyme from rat stomach was assayed with 0.24 m*M* [*carboxyl*-$^{14}$C]-L-histidine as previously described (3).

[2] Florisil.

The reaction was filtered and spin evaporated *in vacuo* to give white crystals. These crystals were collected, washed with ether, and recrystallized.

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# Effects of Chronic Emetine Treatment on Mitochondrial Function


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**Abstract** □ Emetine has long been recognized as a cardiotoxic drug, but its mechanism remains unknown. Since many studies reported an effect of emetine on cardiac metabolism, permanent damage to the heart could result from such treatment. To investigate this action of emetine in relation to the cardiotoxicity seen after a therapeutic regimen, New Zealand albino rabbits were given 2 mg/kg/day ip of emetine for 9 days. Heart and liver mitochondrial metabolism was assessed polarographically using pyruvate, pyruvate plus malate, or succinate as the substrate. Heart mitochondrial metabolism was reduced in emetine-treated rabbits in comparison to the normal controls. However, the metabolic activity of the pair-fed control group was equally reduced. Only the liver mitochondrial metabolism of the pair-fed control group was reduced in relation to the normal controls, while the liver mitochondrial metabolism of emetine-treated rabbits remained unchanged. These data indicated that the reduction in heart mitochondrial metabolism resulted from the inanition induced by the chronic emetine treatment and not from a primary effect of the drug.



**Keyphrases** □ Emetine—effects of chronic treatment on heart and liver mitochondrial metabolism □ Mitochondrial function—effects of chronic emetine treatment □ Cardiotoxicity—effects of chronic emetine treatment on mitochondrial function


Emetine remains important in the treatment of amebiasis. Its role, however, has been reduced to an alternative drug choice, primarily because of its numerous side effects, the most serious affecting the cardiovascular system. Many studies reported adverse effects of high concentrations of emetine on cardiac metabolism (1–4), although the mechanism of this cardiotoxicity remains unknown.

## BACKGROUND

In a series of experiments, rats injected with ~2 mg/kg/day of emetine for 14–17 days showed a reduction in heart homogenate respiration with butyrate, β-hydroxybutyrate, citrate, α-ketoglutarate, malate, pyruvate, or lactate as the substrate (5–7). Since no effects were observed on the liver homogenate respiration, Appelt and Heim (5–7) concluded that emetine appeared to be selectively toxic to the heart. However, they reported that animals chronically treated with emetine reduced their food intake and lost weight. Since pair-fed controls were not included in their study, their conclusion must be reevaluated.

Few studies investigated the action of emetine on mitochondrial metabolism, but two reports indicated that therapeutic doses of emetine may affect heart mitochondria. Brink *et al.* (8) found a reduction in oxygen uptake and [$^{14}$C]carbon dioxide formation from glucose, pyruvate, and palmitate. If these effects are not secondary to the reduced contractility observed, a reduction in heart mitochondrial metabolism must be postulated. Pearce *et al.* (9) described the mitochondrion as the primary structure in the cardiac cell to be affected morphologically in response to therapeutic doses of emetine in dogs.

Since metabolic changes in the heart could indicate cardiac damage, further study of emetine is important in the overall evaluation of the cardiotoxicity seen clinically. Thus, heart mitochondrial metabolism was assessed in rabbits treated with therapeutic doses of emetine. Since emetine is toxic to the heart yet exerts a major therapeutic effect in the liver, liver mitochondrial metabolism was studied for comparison with the heart.

## EXPERIMENTAL

Male New Zealand albino rabbits were given 2 mg/kg/day ip of emetine hydrochloride for 9 days. Since emetine-treated animals ate less, pair-fed controls were included to exclude effects due to inanition. Normal controls were fed *ad libitum*, and water was freely available to all groups.

Heart mitochondria were prepared as described previously (10). After removal of the heart, a piece of ventricle was removed and prepared for electron microscopy according to techniques outlined by Hayat (11). Rabbit liver mitochondria were prepared similarly. The homogenization and suspending media consisted of 0.25 *M* sucrose plus 1 m*M* ethylenediaminetetraacetic acid (pH 7.2).

Heart and liver mitochondrial metabolism was studied polarographically with an oxygraph. Pyruvate (5 m*M*), pyruvate (5 m*M*) plus malate (0.1 m*M*), and succinate (5 m*M*) were the substrates. The reaction medium for the heart mitochondrial studies contained 0.25 *M* mannitol, 10 m*M* KCl, 10 m*M* tris(hydroxymethyl)aminomethane hydrochloride, 0.1 m*M* ethylenediaminetetraacetic acid, and 5 m*M* phosphate buffer (pH 7.2). The reaction medium for the liver studies consisted of 0.25 *M* sucrose, 0.1 m*M* KCl, and 10 m*M* tris(hydroxymethyl)aminomethane phosphate buffer (pH. 7.2). Small volumes of concentrated adenine diphosphate and magnesium chloride were added to reach final concen-


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