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NEW INHIBITORS OF HISTAMINE-*N*-METHYLTRANSFERASE

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Abstract— A variety of histamine agonists, antagonists and their analogs were tested for their ability to inhibit histamine-*N*-methyltransferase. In earlier studies, we had shown that the enzyme is inhibited noncompetitively by the histamine H_2-receptor agonist, Dimaprit, and we had suggested that the enzyme possessed an inhibitory site with an affinity for both Dimaprit and histamine. The most potent inhibitors had, in addition to a side chain ammonium group, a thiourea or imidazole group which, like the imidazole group of histamine, is capable of prototropic tautomerism. The H_1-receptor agonists, 2-pyridyl-β-ethylamine and 2-thiazolyl-β-ethylamine, which do not undergo prototropic tautomerism, were only weakly inhibitory. There was no correlation, however, between pharmacological and inhibitory activity. The most potent inhibitor ($K_i \sim 10^{-6}$ M) was a Dimaprit analog, SKF 91488, which has no histamine agonist activity. SKF 91488, therefore, may be especially useful in studies of histamine metabolism.

The enzyme histamine-*N*-methyltransferase (EC 2.1.1.8) is inhibited by H_1-[1–4] and H_2-[4–6] histamine receptor antagonists, by various side chain and ring methylated derivatives of histamine [3, 6, 7], and, in concentrations of 10^{-5} M and above, by histamine itself [2]. A number of antimalarial drugs are also potent inhibitors of the enzyme [2, 3, 8]. The inhibition is reported to be competitive in the case of the H_1-receptor antagonists [2–4], side chain methylated derivatives [6] and antimalarial drugs [2, 3] and competitive, noncompetitive, or uncompetitive in the case of the ring methylated derivatives [3, 6]. Recently, we have shown that the thiourea derivative Dimaprit, which is a highly selective H_2-receptor agonist [9], is a noncompetitive inhibitor of histamine-*N*-methyltransferase [10]. We have proposed that Dimaprit and histamine act through an inhibitory site on the enzyme [10].

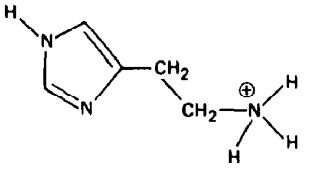
None of the compounds described to date are ideal inhibitors, since they either have intrinsic pharmacological activity or are weakly active *in vivo*. In this study, we have tested a variety of histamine and Dimaprit analogs for their ability to inhibit histamine-*N*-methyltransferase. Compounds which possess a side chain ammonium group and a group that undergoes prototropic tautomerism, i.e. imidazole or a thiourea moiety, were noncompetitive inhibitors of the enzyme. The most potent inhibitor, SKF 91488, is of particular interest because this compound is devoid of agonist activity [11].

METHODS

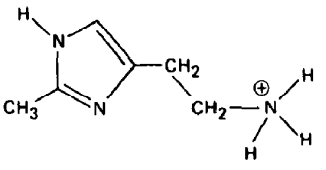
Radiochemicals were obtained from Amersham Searle Corp., Arlington Heights, IL, and New England Nuclear Corp., Boston, MA. Side chain labeled histamine, 4-(5)-(β-aminoethyl[β-^{3}H]) imidazole ([β-^{3}H]-histamine) was prepared from side chain labeled [β-^{3}H]-L-histidine as described previously [12]. Hyamine hydroxide (10 M) was purchased from New England Nuclear Corp.; 1-methyl-4-(β-aminoethyl) imidazole dihydrochloride, or N^τ-methylhistamine according to the nomenclature of Black and Ganellin [13], was obtained from CalBiochem, San Diego, CA; histamine dihydrochloride, L-histidine and pyridoxal-5-phosphate from Sigma Chemical Co., St. Louis, MO; and chlorpheniramine from K & K Laboratories, Inc., Plainview, NY. Burimamide, metiamide, Dimaprit, its analogs, and other histamine receptor agonsts were gifts from Drs. W. A. M. Duncan and C. R. Ganellin, Smith Kline & French Laboratories, Welwyn Garden City, Hertfordshire, England.

The following preparations of enzymes were used. The source of histidine decarboxylase activity (EC 4.1.1.22) was a soluble extract of rat gastric mucosa. Rats were given pentagastin, 1 mg/kg s.c., to induce histidine decarboxylase activity and then were killed 2 hr later. The glandular portion of the stomach was removed and washed free of bacteria [14]. A 1 to 10 homogenate of the tissue was prepared in 0.1 M sodium phosphate buffer, pH 6.8. The homogenate was centrifuged at 18,000 *g* for 15 min to remove particulate matter and nonspecific decarboxylase activity [15]. The supernatant extract was stored at −20°. Diamine oxidase (diamine, O_2, oxidoreductase (deaminating), EC 1.4.3.6) was prepared in a similar manner from rat ileum [12]. Histamine-*N*-methyltransferase was obtained from two sources, guinea pig brain and rat kidney. The enzyme was prepared from soluble extracts of these tissues by ammonium sulfate fractionation procedures described previously [16]. The preparation from guinea pig brain contained 11.2 mg protein/ml and that from kidney, 21.7 mg protein/ml. The enzyme from kidney was diluted fifty times with sodium phosphate buffer (0.1 M, pH 7.9) before use.

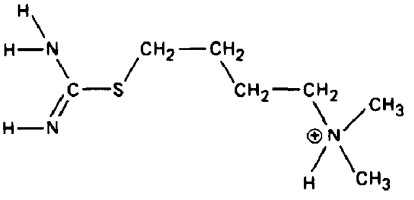
Histidine decarboxylase activity was assayed by the measurement of $^{14}CO_2$ release from L-histidine-1-[carboxyl-^{14}C] [17], DAO activity by the measure-



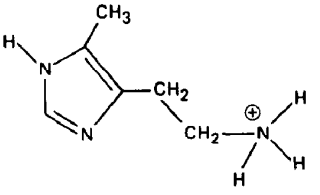
Histamine



2-Methylhistamine



SK&F Cpd. 91488



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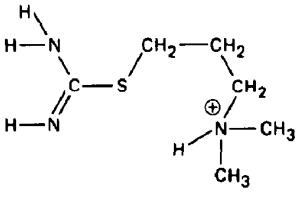
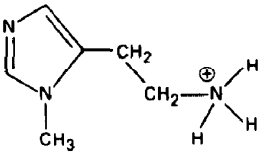
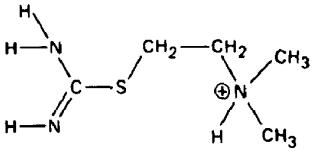


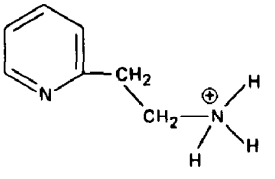
Dimaprit



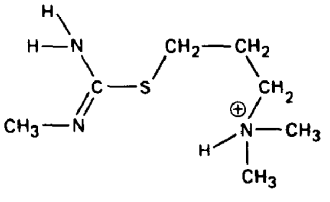
N^{τ} or 1-Methylhistamine



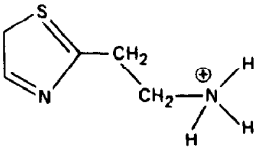
SK&F Cpd. 91487



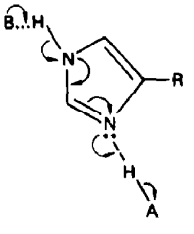
2-Pyridylethylamine



SK&F Cpd. 92054



2-Thiazolylethylamine



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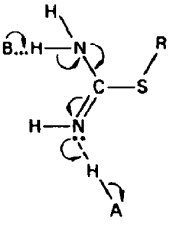

II

Table 1. Inhibition of rat kidney histamine-*N*-methyltransferase by histamine receptor agonists, antagonists and related compounds

Compound	Activity at histamine receptors*	Inhibition of histamine-*N*-methyltransferase Type of inhibition	K_i† (μM)
Thiourea derivatives			
SKF 91488	Inactive	Noncompetitive	0.9–1.6‡§
Dimaprit	Agonist, H_2-receptors	Noncompetitive	8§
SKF 91487	Inactive	Noncompetitive	15
SKF 92054	Inactive	Noncompetitive	30
Thiourea	Inactive		>1000
Dimethylaminopropanol	Inactive		>1000
Imidazole derivatives			
2-Methylhistamine	Agonist, H_1-receptors	Uncompetitive	14‖
4-Methylhistamine	Agonist, H_2-receptors	Uncompetitive	18‖
N^{τ}-methylhistamine	Inactive	Uncompetitive	300
Imidazole acetic acid	Inactive		>1000
Heteroaromatic derivatives			
2-Pyridyl-β-ethylamine	Agonist, H_1-receptors		300
2-Thiazolyl-β-ethylamine	Agonist, H_1-receptors		~100
H_1-receptor antagonists			
Chlorpheniramine	Antagonist, H_1-receptors	Competitive¶	1.8
Promethazine	Antagonist, H_1-receptors	Competitive¶	10
H_2-receptor antagonists			
Metiamide	Antagonist, H_2-receptors	Noncompetitive	60
Burimamide	Antagonist, H_2-receptors	Noncompetitive	300

* Refs. 2, 11, 40 and 41.

† Values were determined by the method of Dixon [20]. For each determination, five concentrations of inhibitor were tested at histamine concentrations of 0.25, 0.5 and 1.0 μM (see text).

‡ Range of values for three separate determinations.

§ Comparable values obtained with histamine-*N*-methyltransferase from guinea pig brain were 3 μM for SKF 91488 and 7–9 μM for Dimaprit.

‖ Concentration required for 50 per cent inhibition of enzyme at 1 μM histamine. The inhibition was less at lower histamine concentrations (see text).

¶ Taylor and Snyder [2] report competitive inhibition and a K_i of 1.8 and 9 μM, respectively, for chlorpheniramine and promethazine.

ment of tritium release from [β-^{3}H]-histamine [18] and histamine-*N*-methyltransferase activity by the measurement of the rate of formation of [^{14}C]-methylhistamine from histamine in the presence of *S*-adenosyl-L-methionine[^{14}C-methyl] [19]; final substrate concentrations in these assays were: L-histidine, 2.5×10^{-4} M, and histamine, 7.5×10^{-8} and 5×10^{-7} M respectively. In the more detailed kinetic studies with histamine-*N*-methyltransferase, the concentration of histamine was varied by the addition of unlabeled compound. The concentration of *S*-adenosyl-L-methionine[^{14}C-methyl] (5×10^{-6} M) was left unchanged.

The procedure of Dixon [20] was used to determine the "apparent" K_i value for the different inhibitors. The lines were plotted by the method of least squares with the aid of a Cannon SX computer. The histamine concentrations chosen for study, 0.25, 0.5 and 1 μM, were below those expected to inhibit the enzyme and were within the range of those found in tissue fluids and blood under normal and pathological conditions (Refs. 19 and 21, and unpublished data). All values are the mean of duplicate determinations.

RESULTS

The structures of some of the compounds tested are shown in Fig. 1. The Dimaprit analog, S-[4-(dimethylaminobutyl)]isothiourea (SKF 91488), was the most potent inhibitor. This compound inhibited histamine-*N*-methyltransferase from guinea pig brain and rat kidney equally well (Table 1). Compounds of shorter length, i.e. compound SKF 91487 (2-carbon chain length) and Dimaprit (3-carbon chain length), or those having a methylated thiourea moiety, for example compound SKF 92054, were less inhibitory (Table 1). Thiourea and dimethylaminopropanol, which were selected as analogs of the structural fragments of Dimaprit, were weakly inhibitory or inactive (Table 1).

Of the imadazole derivatives tested, 2- and 4-

Fig. 1. Structures of some of thiourea and imidazole derivatives used in the present studies. The SKF compounds, Dimaprit and the 2- and 4-methylhistamines are capable of prototropic tautomerism (illustrated for imidazole (I) and isothiourea (II) derivatives in the bottom of the figure) and are moderate to strong inhibitors of histamine-*N*-methyltransferase (Table 1). 2-Pyridylethylamine and 2-thiazolylethylamine cannot undergo prototropic tautomerism and are very weak inhibitors of the enzyme. Compounds with no side chain ammonium group, e.g. imidazole acetic acid and thiourea (not shown), are also weak or inactive as inhibitors of the enzyme. The reaction product N^{τ}-methylhistamine (see Ref. 13 for nomenclature) is a weak inhibitor, as discussed in the text.

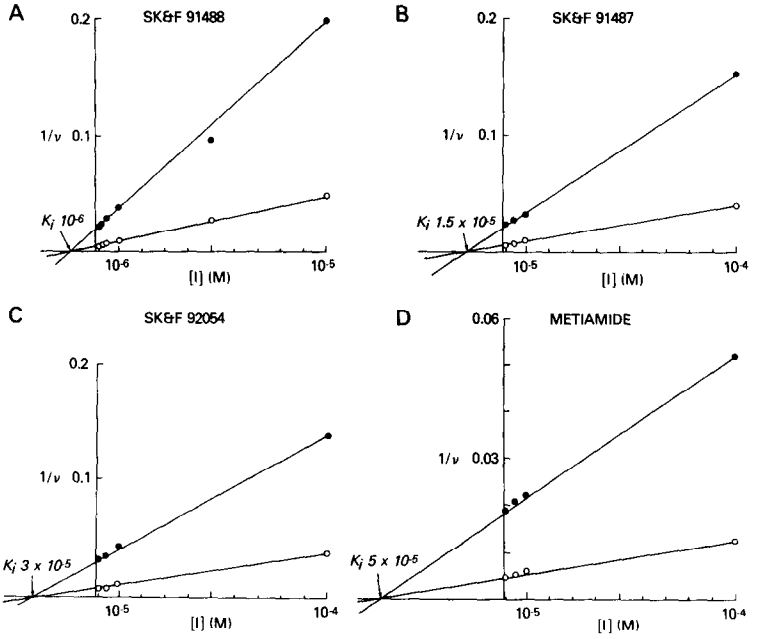

Fig. 2. Examples of noncompetitive inhibition of histamine-*N*-methyltransferase by various compounds as plotted by the method of Dixon [20]. In these experiments histamine concentrations of 0.25 (●) and 0.5 μM (○) were used. The source of histamine-*N*-methyltransferase was rat kidney.

methylhistamine were about one-tenth as active as SKF 91488. The histamine metabolite, N^{τ}-methylhistamine, was a weak inhibitor and another metabolite, imidazole acetic acid, was without activity (Table 1).

Two H_1-histamine receptor agonists, 2-pyridyl-β-ethylamine and 2-thiazolyl-β-ethylamine, were very weak inhibitors of the enzyme. The two histamine H_2 receptor antagonists, burimamide and metiamide, were also weak inhibitors of the enzyme, although metiamide was somewhat more active than burimamide (Table 1).

The inhibition by Dimaprit and its analogs was noncompetitive as was the inhibition by metiamide and burimamide (Fig. 2). In contrast, the inhibition by the H_1-receptor antagonists was competitive (Table 1). The inhibition produced by the methylated derivatives of histamine was neither competitive nor noncompetitive, but it was influenced by the concentration of substrate. The apparent K_i for 2-methylhistamine, for example, was 1.4×10^{-5} M in the presence of 10^{-6} M histamine and 4×10^{-5} M in the presence of 2.5×10^{-7} M histamine. Comparable values for 4-methylhistamine were 1.8 and 5.8×10^{-5} M. Because of the crude nature of the enzyme preparation and the complication introduced by substrate inhibition at higher histamine concentrations, more detailed studies of the kinetics of inhibition were not attempted.

In addition to inhibiting histamine-*N*-methyltransferase, SKF 91488 and Dimaprit were weak noncompetitive inhibitors of diamine oxidase. Neither compound inhibited histidine decarboxylase (Table 2).*

* Preliminary studies show that another methyltransferase enzyme, bovine pineal hydroxindole-*O*-methyltransferase, is not inhibited by SKF 91488.

DISCUSSION

The two major routes of inactivation of histamine in mammalian species are methylation and deamina-

Table 2. Per cent inhibition of histamine-metabolizing enzymes by SKF 91488*

Drug concn (M)	Histidine decarboxylase (rat gastric mucosa)	Diamine oxidase (rat ileum)	Histamine-*N*-methyltransferase (guinea pig brain)
10^{-7}			10
2.5×10^{-7}			21
5×10^{-7}			34
10^{-6}	4 (0)	3 (1)	52 (7)
5×10^{-6}	9	10	82
10^{-5}	5 (0)	22 (3)	90 (56)
10^{-4}	0 (0)	67 (14)	99 (89)
10^{-3}	12 (2)	95 (70)	100 (90)
2.5×10^{-3}	— (1)	— (81)	— (95)

* Values are mean of two assays. Values in parentheses indicate inhibition by Dimaprit.

tion [24–26] and most tissues appear to have the capacity of carrying out one or both of these reactions [27–30]. The two enzymes responsible for these reactions, histamine-*N*-methyltransferase and diamine oxidase, differ in their distribution in the body. Diamine oxidase is localized in certain tissues, such as placenta, small intestine, kidney and thymus [31–33], whereas histamine-*N*-methyltransferase appears to be more uniformly distributed throughout the body [34]. In some tissues, for example, the acid-secreting region of the gastric mucosa, histamine-*N*-methyltransferase is the only histamine-catabolizing enzyme present.* The availability of selective inhibitors of histamine-*N*-methyltransferase would greatly facilitate studies of histamine turnover and release, especially when used in combination with aminoguanidine, a selective and potent inhibitor of diamine oxidase *in vitro* [18, 35] and *in vivo* [22. 32, 36].

The mechanism by which histamine is methylated is uncertain. Kinetic studies have been carried out with partially purified (6- to 300-fold purification) preparations of the enzyme and have yielded conflicting data [2, 3, 7]. Thithapandha and Cohn [3], for example, reported that the K_m value for histamine was influenced by the concentrations of co-reactant *S*-adenosyl-L-methionine, and vice versa, and that N^{τ}-methylhistamine, a reaction product, inhibited the enzyme competitively with respect to *S*-adenosyl-methionine and noncompetitively with respect to histamine. The kinetics of these reactions were consistent with a Ping–Pong type of reaction. The authors concluded that the enzyme interacted first with *S*-adenosyl-L-methionine to give a methylated enzyme, and the methylated enzyme then reacted with histamine to yield N^{τ}-methylhistamine [3]. Attempts to isolate a methylated enzyme were unsuccessful [3]. In contrast, Taylor and Snyder [2] reported that the affinity of histamine for the enzyme was not influenced by the concentration of *S*-adenosyl-L-methionine and suggested that histamine and *S*-adenosyl-L-methionine added sequentially to the enzyme, i.e. to form a ternary complex [2]. Gustafsson and Forshell [7] found no evidence for methylation or demethylation of the enzyme, and they concluded that the methyl group was transferred directly from *S*-adenosyl-L-methionine to histamine without the formation of an intermediate methylated enzyme. An additional complication in the interpretation of the data is that multiple forms of the enzyme have been demonstrated by starch gel electrophoresis [23] and by comparison of kinetic parameters [37]. These forms have not been isolated or characterized.

All studies to date indicate that the enzyme has a high degree of substrate specificity [3, 7, 34]. Only minor alteration of the histamine molecule will lead to loss of an ability of the compound to act as substrate, although many of the analogs tested retained weak inhibitory activity [7].

Our knowledge of the possible modes of inhibition of histamine-*N*-methyltransferase is also incomplete. The compounds that inhibit the enzyme competitively are of such diverse structures that it is difficult to visualize the structural features that are responsible for the inhibition [3]. These compounds act, presumably, by binding reversibly to, or near, the active site of the enzyme.

Both reaction products, N^{τ}-methylhistamine [3, 7] and *S*-adenosyl-L-homocysteine [38, 39], inhibit histamine-*N*-methyltransferase. Although the mechanism of inhibition by N^{τ}-methylhistamine is in dispute, *S*-adenosyl-L-homocysteine appears to inhibit the enzyme through competition with the co-substrate *S*-adenosyl-L-methionine. The inhibition is reported to be noncompetitive [3] or uncompetitive [6] between N^{τ}-methylhistamine and histamine non-competitive between *S*-adenosyl-L-homocysteine and histamine [38, 39], and competitive between *S*-adenosyl-L-homocysteine and *S*-adenosyl-L-methionine [38, 39].

Other modes of inhibition are possible. The enzyme is inhibited by sulfhydryl reagents, and this inhibition is prevented by the addition of reduced glutathione [7]. Histamine-*N*-methyltransferase, like diamine oxidase, is inhibited by histamine in concentrations greater than 10^{-5} M [2]. Low concentrations of the H_1-receptor antagonists appear to enhance the enzyme activity, and this enhancement is thought to be due to reversal of substrate inhibition [2, 4].† If, as we have suggested, inhibitory sites exist on the enzyme, other histamine and Dimaprit analogs might be equally capable of inhibiting the enzyme, and the present studies suggest that this might be the case.

The above studies provide some indication as to the structural features required for noncompetitive inhibition of the enzyme. Compounds which do not undergo prototropic tautomerism, such as 2-pyridylethylamine or 2-thiazolylethylamine (see Ref. 40), are weak inhibitors, whereas all potent noncompetitive inhibitors of the enzyme possess an imidazole or isothiourea group which can undergo prototropic tautomerism [11, 40]. Metiamide, whose electronic and tautomeric characteristics more closely resemble those of histamine than do those of burimamide [41], is a stronger inhibitor of the enzyme than burimamide, although both of these compounds are relatively weak inhibitors. Compounds without a side chain ammonium, thiourea or cyanoguanidine group, as for example imidazole acetic acid and thiourea, are devoid of inhibitory activity.

It could be argued that the above features are similar to those required for interaction with the H_2-histamine receptor [41]. However, the distance between the tuatomeric and ammonium groups does not appear to be as crucial for interaction with histamine-*N*-methyltransferase as it is for interaction with the H_2-receptor. SKF 91487, Dimaprit and SKF 91488, which have different chain lengths, are all inhibitory, but of these compounds only Dimaprit has agonist activity [11]. The lack of agonist activity in SKF 91488 may make this a useful compound in studies of histamine metabolism and function.

The interaction of the methylhistamines with histamine-*N*-methyltransferase is complex. The 2- and 4-methylhistamines are reported to be uncompetitive and competitive inhibitors respectively [6]. In the present study, it was found that the

* A. Soll and M. A. Beaven, unpublished data.

† In higher concentrations, the H_1-receptor antagonists are competitive inhibitors, as mentioned in the text.

inhibitory activity of 2- and 4-methylhistamine was greater when histamine concentrations were increased. It is possible that the methylhistamines have several modes of action, but this question requires reinvestigation with purified preparations of enzyme.

Note added to proof. Since the completion of this study, Dr. C. R. Ganellin has synthesized and sent to us two compounds, *S*-propyl-isothiourea and *S*-methyl-isothiourea, which as he pointed out are more analogous to the isothiourea moiety of the Dimaprit compounds than thiourea. Although both compounds were weak inhibitors (K_i; *S*-propyl-isothiourea, 200 μM, and *S*-methyl-isothiourea, 600 μM), like the parent compounds, they were non-competitive inhibitors of rat kidney histamine-*N*-methyltransferase. Studies in mice have shown also that methylation of histamine is inhibited *in vivo* by SKF 91488 in doses of 100–500 mg/kg.

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