Cyclopropane-Based Conformational Restriction of Histamine. (1*S*,2*S*)-2-(2-Aminoethyl)-1-(1*H*-imidazol-4-yl)cyclopropane, a Highly Selective Agonist for the Histamine H_3 Receptor, Having a *cis*-Cyclopropane Structure

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A series of cyclopropane-based conformationally restricted analogues of histamine, the "folded" cis-analogues, i.e., (1*S*,2*R*)-2-(aminomethyl)-1-(1*H*-imidazol-4-yl)cyclopropane (**11**), (1*S*,2*S*)-2-(2-aminoethyl)-1-(1*H*-imidazol-4-yl)cyclopropane (**13**), and their enantiomers *ent*-**11** and *ent*-**13**, and the "extended" trans-analogues, i.e., (1*R*,*2R*)-2-(aminomethyl)-1-(1*H*-imidazol-4-yl)cyclopropane (**12**) and its enantiomer *ent*-**12**, were designed as histamine H_3 receptor agonists. These target compounds were synthesized from the versatile chiral cyclopropane units, (1*S*,2*R*)- and (1*R*,2*R*)-2-(*tert*-butyldiphenylsilyloxy)methyl-1-formylcyclopropane (**14** and **15**, respectively) or their enantiomers *ent*-**14** and *ent*-**15**. Among the conformationally restricted analogues, the "folded" analogue **13** (AEIC) having the *cis*-cyclopropane structure was identified as a potent H_3 receptor agonist, which showed a significant binding affinity (K_i = 1.31 ± 0.16 nM) and had an agonist effect (EC_{50} value of 10 ± 3 nM) on the receptor. This compound owes its importance to being the first highly selective H_3 receptor agonist to have virtually no effect on the H_4 subtype receptor. These studies showed that the *cis*-cyclopropane structure is very effective in the conformational restriction of histamine to improve the specific binding to the histamine H_3 receptor.

Introduction

A variety of homeostatic processes, such as sleeping and wakefulness, eating and drinking, learning and memory, or neuroendocrine, are known to be related to the neurotransmitter histamine (**1**, Figure 1).[1] The effects of histamine are mediated by at least four receptor subtypes termed H_1, H_2, H_3, and recently discovered H_4 receptors, and the agonists and antagonists specific to each one of these subtypes can be used as drugs as well as pharmacological tools.[1]

Recently, much attention has been focused on the H_3 receptor,[2] a G_i protein-coupled receptor, which was cloned by Lovenberg and co-workers in 1999.[2c] Homology analysis of the H_3 receptor showed it to be significantly different from the previously cloned H_1 and H_2 receptors.[2c,d] The H_3 receptors are located presynaptically and inhibit synthesis and release of histamine.[3] They are also known to play an important role as heteroreceptors in the regulation of the release of several other important neurotransmitters.[4] It has been recognized that the H_3 receptor is a potential therapeutic target.[5] Agonists to the H_3 receptor are considered to be potential drugs for the treatment of sleep disorders, migraines, asthma, inflammation, and ulcers.[5a] On the other hand, the H_3 receptor antagonists may be useful therapeutically for Alzheimer's disease, attention-deficit hyperactive disorder (ADHD), schizophrenia, depression, dementia, and epilepsy.[5b]

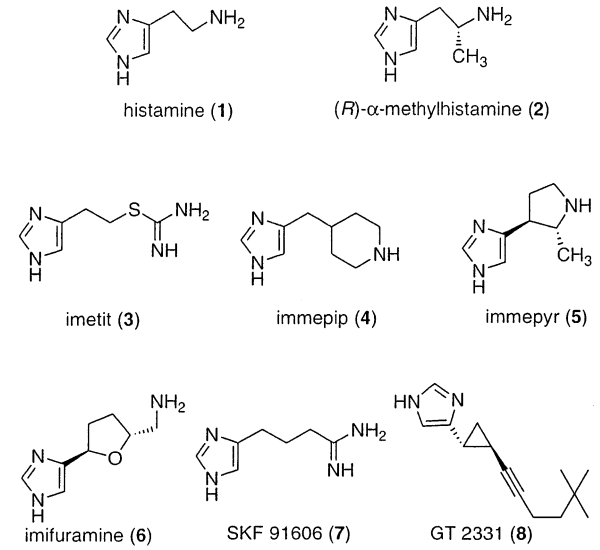

Figure 1. Histamine and H_3 receptor ligands.

In 2000 and 2001, another histamine receptor subtype, i.e., the H_4 receptor, was cloned by several groups and identified as a G protein-coupled receptor.[6] The H_4 receptor has significant sequence homology (about 40%) to the H_3 receptor cDNA and seems to couple with G_i protein like that of the H_3 receptor.[6f] It is noteworthy that the H_3 and the H_4 receptors share about 60% sequence identity in their transmembrane regions,[6d] which suggests that the two receptors possibly have a

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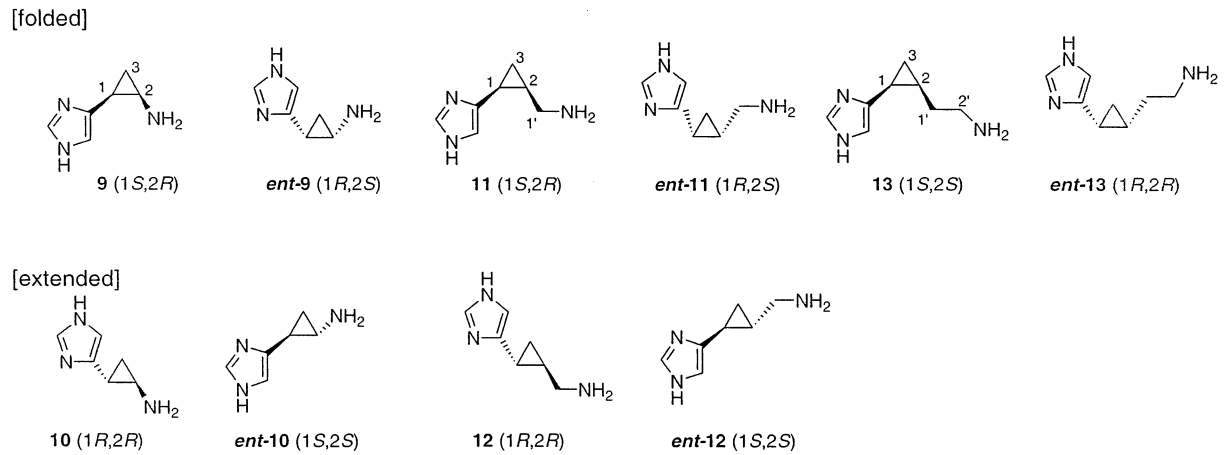


Figure 2. The cyclopropane-based conformationally restricted analogues of histamine.

similar mode of ligand recognition. In fact, previous H_3 agonists, such as (*R*)-α-methylhistamine (**2**), imetit (**3**), and immepip (**4**), which had been considered to be highly selective H_3 receptor agonists before the discovery of the H_4 receptor, were disclosed to bind not only to the H_3 receptor but also interestingly to the H_4 receptor.[6b−d,f,g] Consequently, development of highly selective H_3 receptor agonists, which are completely or virtually lacking in affinity for the H_4 receptor, is required. This potentially new class of highly selective H_3 selective agonists would not only be very useful as tools for pharmacological studies but would also be important as leads for the development of new drugs.

To develop the new class of highly H_3 selective agonists mentioned above, we have designed and synthesized the conformationally restricted analogues of histamine, **9**−**13** and their enantiomers *ent*-**9**−**13**, having a cis- or a *trans*-cyclopropane ring. Among the newly synthesized compounds, (1*S*,2*S*)-2-(2-aminoethyl)-1-(1*H*-imidazol-4-yl)cyclopropane (**13**) having the *cis*-cyclopropane structure was identified as a highly H_3-selective agonist. In this report, we describe the design, synthesis, and pharmacological effects of these conformationally restricted analogues of histamine.

Results and Discussion

Design of the Conformationally Restricted Analogues of Histamine. Histamine, like other neurotransmitters, is conformationally flexible due to the "aromatic ring−C(sp^3)−C(sp^3)−N" backbone. Consequently, histamine can assume a variety of conformations, which may make it possible for it to bind to several receptor subtypes via different conformations.[7] For example, the conformation binding to the H_3 receptor, i.e., the bioactive conformation for the H_3 subtype, may be different from those for the H_1, H_2, and/or H_4 subtype receptors. Similarly the bioactive conformation for the H_4 subtype may be different from those for the other receptor subtypes. Therefore, because conformational restriction of histamine may improve the specific binding to one of the receptor subtypes, we planned to develop novel H_3 selective agonists by restricting the conformation of histamine.

The previous structure−activity relationship studies on the H_3 receptor agonists showed that (1) the 4-alkylimidazole moiety of the histamine molecule is an essential partial structure for H_3 receptor agonism, which is common to all potent H_3 receptor agonists known so far;[5] and (2) introduction of a chiral center into the histamine side chain can bring about the selective and potent agonism to the H_3 subtype,[5] which is typically observed in the eutomers (*R*)-α-methylhistamine (**2**),[8a] immepyr (**5**),[8b] and imifuramine (**6**).[8c] These findings suggest that the spatial arrangement of the imidazole ring and the basic amino nitrogen atom is a determining factor in the potency of the H_3 subtype agonists. Thus, we speculated that suitable conformational restriction of histamine may be effective in developing highly selective H_3 subtype agonists.

In the design of conformationally restricted analogues,[9] it is essential that the conformationally restricted analogues should be as similar as possible to the parent compound in size, shape, and molecular weight.[9a] Conformationally restricted analogues have usually been designed and synthesized by introducing often bulky cyclic moieties into the lead compounds. Consequently, the chemical and physical properties of these analogues can be quite different from those of the original leads. Because of its characteristic structural feature, a cyclopropane ring is likely to be effective in restricting the conformation of a molecule without changing the chemical and physical properties of the lead compounds.[10] In fact, cyclopropanes have already been successfully used to restrict the bioactive conformations of neurotransmitters, amino acids, peptides and nucleosides.[10] We also devised a new method for restricting the conformation of cyclopropane derivatives based on the fact that adjacent substituents on the ring exert mutual steric repulsion because of their eclipsed conformation to one another.[11a] This method has been successfully used in the design of NMDA (*N*-methyl-D-aspartic acid) receptor antagonists.[11]

De Esch and co-workers designed the four stereoisomers of 2-amino-1-(1*H*-imidazol-4-yl)cyclopropane, i.e., the "folded" cis-isomer **9** and its enantiomer *ent*-**9** and the "extended" trans-isomer **10** and its enantiomer *ent*-**10** (Figure 2), in which the basic amino group was directly attached to the cyclopropane ring.[12] Although they failed to synthesize the "folded" **9** and *ent*-**9**, the synthesis of the "extended" trans-analogues **10** and *ent*-**10**, via optical resolution of a racemic intermediate, was accomplished, and *ent*-**10** was identified as a potent H_3 agonist.[12] Furthermore, a very potent H_3-receptor antagonist GT-2331 (**8**, Figure 1), having an "extended"

trans-cyclopropylimidazole structure, was reported by Ali and co-workers.[13] These studies suggest that the *trans*-cyclopropane ring is likely to be effective in restricting the bioactive conformation of the histamine H_3 receptor. However, potency of the *cis*-cyclopropane ring as a template for the conformational restriction of histamine is unknown.

On the other hand, SKF91606 (**7**),[14] having its two-carbon-elongated structure (imidazole-C−C−C−C−N) compared with that of histamine (imidazole-C−C−N), is the strongest H_3-receptor agonist reported to date. Such carbon-elongation in SKF 91606 may relate to its strong affinity for the H_3 receptor subtype.

On the basis of these findings and considerations, we designed the histamine analogues **11**, *ent*-**11**, **12**, and *ent*-**12** (Figure 2), conformationally restricted by the *cis*- or the *trans*-cyclopropane ring, which have the carbon-elongated "imidazole−C(1)−C(2)−C(1′)−N" and/or the "imidazole−C(1)−C(2)−C(3)−C(1′)−N" structure. In these conformationally restricted analogues the spatial relationship between the imidazole ring and the basic nitrogen of the cis-substituted **11** and *ent*-**11** are restricted in the "folded" form, while those of the *trans*-isomers **12** and *ent*-**12** are in the "extended" form. The conformationally restricted analogues **9**, *ent*-**9**, **10**, and *ent*-**10** previously designed by De Esch and co-workers described above were also included as synthetic targets in this study to compare their biological activities with those of the newly designed compounds. We were especially interested in the pharmacological effect of the unknown "folded" **9** and *ent*-**9**.

Since, as described below, the "folded" analogue **11** with its (1*S*,2*R*)-configuration proved to be a very potent and selective H_3 receptor agonist, the further carbon-elongated "folded" analogue **13** with the same stereochemistry as for **11** and its enantiomer *ent*-**13** were also designed as other synthetic targets.

Investigation of the effects of these compounds on the histamine receptors may clarify the three-dimensional structure−activity relationship of the conformationally restricted analogues of histamine having a cyclopropane ring.

Chemistry. Chiral cyclopropanes are not only useful for restricting the conformation of biologically active compounds in order to improve the activity, but are also important as key fragments in many natural products.[15] Therefore, considerable effort has been devoted to developing practical methods for preparing chiral cyclopropanes, including enantioselective cyclopropanations, chemical or enzymatic optical resolutions, and transformations from chiral synthons.[10,11,15−17]

We most recently developed the four types of chiral cyclopropane units bearing two adjacent substituents in a cis or a trans relationship, namely **14** and **15**, and their enantiomers *ent*-**14** and *ent*-**15**, as shown in Figure 3.[16] These are versatile intermediates for synthesizing various biologically important compounds having an asymmetric cyclopropane structure, such as the target conformationally restricted analogues in this study. Thus, the cis and trans units **14** and **15** were converted into the corresponding imidazole derivatives **16** and **20**, from which the conformationally restricted analogues **11** and **12** were synthesized. The enantiomers *ent*-**11** and *ent*-**12** were likewise synthesized from *ent*-**14** and *ent*-**15**, respectively (Scheme 1).[16]

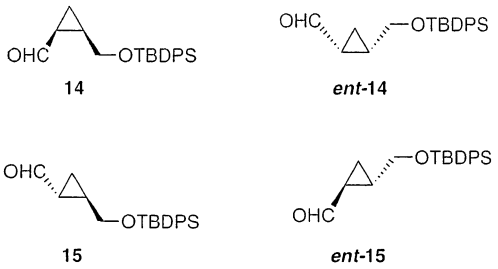
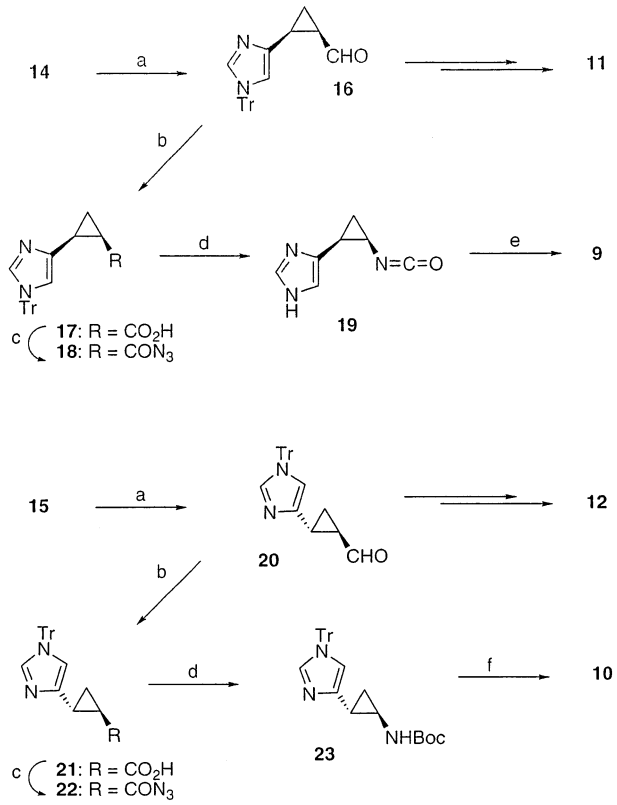


Figure 3. The versatile chiral cyclopropane units.

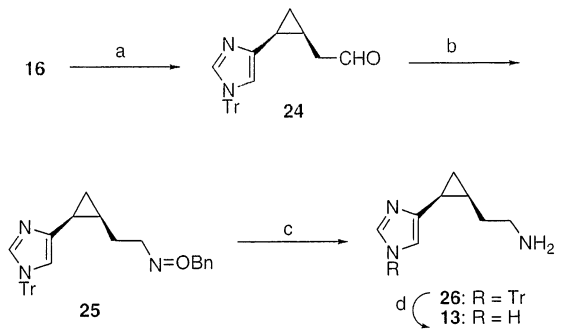
Scheme 1[a]



[a] Conditions: (a) (1) $TsCH_2NC$, NaCN, EtOH, (2) NH_3, EtOH, (3) TrCl, Et_3N, CH_2Cl_2, (4) TBAF, THF, (5) Swern ox. (ref 16); (b) $NaClO_2$, NaH_2PO_4, aq acetone, rt, 89% (**17**), 89% (**21**); (c) $(PhO)_2PON_3$, Et_3N, CH_2Cl_2, rt, 80% (**18**), 74% (**22**); (d) *t*-BuOH, reflux, 96% (**19**), 48% (**23**); (e) 6 N HCl, reflux, 92%; (f) HCl, aq MeOH, reflux, 98%.

As mentioned above, De Esch and co-workers successfully synthesized the "extended" analogues **10** and *ent*-**10** via optical resolution of a racemic intermediate.[12] They also tried various approaches to the synthesis of the corresponding "folded" cis-isomer **9** and its enantiomer *ent*-**9**, but these were unsuccessful,[12] which suggests that the synthesis of the conformationally restricted analogues of histamine having the *cis*-cyclopropane structure would be rather difficult. However, we thought that the chiral imidazolylcyclopropanecarbaldehydes **16** and **20** and their enantiomers *ent*-**16** and *ent*-**20** should be effective intermediates not only for synthesizing the trans-analogues **10** and *ent*-**10** but also synthesizing for the *cis*-analogues **9** and *ent*-**9**. Since the basic amino group is directly attached to the cyclopropane ring in these target compounds, a one-carbon contraction was required for their synthesis, which

Scheme 2[a]



[a] Conditions: (a) (1) $MeOCH_2PPh_3Cl$, NaHMDS, THF, 0 °C, (2) HCl, aq MeOH, rt, 76%; (b) $BnONH_2$·HCl, MS 4A, THF, rt, quant; (c) $LiAlH_4$, THF, rt, 97%; (d) HCl, aq MeOH, reflux, 99%.

seemed possible via the Curtius rearrangement. Oxidation of the *cis*-imidazolylcyclopropanecarbaldehyde **16** with $NaClO_2$ in aqueous acetone gave the cyclopropanecarboxylic acid **17**, which was then treated with $(PhO)_2PON_3/Et_3N$ in CH_2Cl_2 to produce the corresponding acid azide **18**. When the acid azide **18** was heated in *t*-BuOH under reflux, the expected Curtius rearrangement and simultaneous removal of the trityl group occurred to give the one-carbon-contracted isocyanate **19**. Acidic hydrolysis of the isocyanate moiety of **19** by heating it in 6 N HCl under reflux furnished the target folded analogue **9** as a hydrochloride. From the *trans*-imidazolylcyclopropanecarbaldehyde **20**, the *trans*-acid azide **22** was prepared according to a similar procedure as that for the cis-series. Heating the *trans*-acid azide **22** in *t*-BuOH under the same conditions as those for the *cis*-**18** resulted in producing the Curtius-rearranged Boc-amino derivative **23** without removing the *N*-trityl group. Treatment of **23** with HCl in aqueous MeOH gave the "extended" (1*R*,2*R*)-conformationally restricted analogue **10**. The spectral data of the synthesized **10** were in accord with those reported previously.[12] The enantiomers *ent*-**9** and *ent*-**10** were also effectively synthesized from *ent*-**16** and *ent*-**20**, respectively.

Synthesis of the one-carbon-elongated "folded" analogues **13** and *ent*-**13** was next investigated (Scheme 2). The Wittig reaction of the *cis*-aldehyde **16** with $MeOCH_2PPh_3Cl$/NaHMDS in THF followed by acidic treatment of the product gave the corresponding homologous aldehyde **24**. After conversion of **24** into the benzyloxime **25**, reduction with $LiAlH_4$ afforded the amine **26**. The *N*-trityl group was finally removed by the usual acidic treatment with aqueous HCl to afford the one-carbon-elongated analogue **13**. Similarly, its enantiomer *ent*-**13** was synthesized from *ent*-**16**.

These results showed that the chiral cyclopropane units **14** and **15**, and their enantiomers *ent*-**14** and *ent*-**15**, are useful as versatile intermediates for synthesizing various compounds having asymmetric a *cis*- or a *trans*-cyclopropane structure.

Binding Affinities for Histamine Receptor Subtypes. The binding affinity of the cyclopropane-based conformationally restricted analogues of histamine for the H_3 subtype of rat brains was evaluated using [^{3}H]-N^{α}-methylhistamine as a radio ligand.[18] The results are shown in Figure 4 and Table 1. All of the newly synthesized analogues inhibited the specific binding of [^{3}H]N^{α}-methylhistamine to the H_3 subtype receptor of

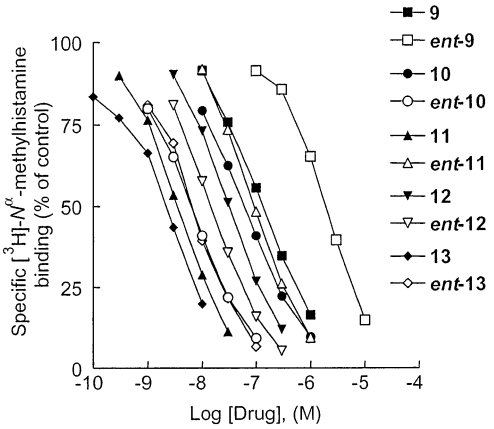


Figure 4. Effects of compounds on specific binding of [^{3}H]-N^{α}-methylhistamine.

Table 1. Affinity of Compounds for the Rat H_3 Receptor Subtype[a]

compd	K_i, nM
9	91.2 ± 6.5
ent-**9**	1380 ± 140
10	42.2 ± 3.7
ent-**10**	4.30 ± 0.63
11	2.50 ± 0.38
ent-**11**	64.7 ± 6.8
12	22.6 ± 3.6
ent-**12**	10.1 ± 0.6
13 (AEIC)	1.31 ± 0.16
ent-**13**	4.78 ± 0.02
thioperamide	2.65 ± 0.76
(*R*)-α-methylhistamine (**2**)	0.88 ± 0.06

[a] Assay was carried out with rat brains using [^{3}H]N^{α}-methylhistamine ($n = 3$).

rat brains in a concentration-dependent manner (0.1 nM to 10 μM), as shown in Figure 4.[19] In order of the binding affinity, the compounds ranked as follows: **13** > **11** > *ent*-**10**, *ent*-**13** > *ent*-**12** > **12** > **10** > *ent*-**11** > **9** ≫ *ent*-**9**. Among the compounds, **13**, **11**, *ent*-**10**, and *ent*-**13** had a significant nM order K_i value. Compound **13** [(1*S*,2*S*)-2-(2-aminoethyl)-1-(1*H*-imidazol-4-yl)cyclopropane, AEIC] showed the strongest binding affinity ($K_i = 1.31 \pm 0.16$ nM) in this series of compounds, which is comparable to that of (*R*)-α-methylhistamine[8a] (**2**, $K_i = 0.88 \pm 0.06$ nM) and is more potent than the well-known H_3 receptor antagonist thioperamide[5b] ($K_i = 2.65 \pm 0.76$ nM).

The binding affinities of the compounds **13** (AEIC), which strongly bind to the rat H_3 receptor, for human H_1, H_2, and H_3 receptor subtypes were next evaluated using [^{3}H]pyrilamine (H_1), [^{3}H]tiotidine (H_2), and [^{3}H]-N^{α}-methylhistamine (H_3) as radioligands.[6b] AEIC showed a significant binding affinity for the human H_3 receptor with the K_i values of 1.5 nM, which is similar to that for the rat brain H_3 receptor described above. On the other hand, AEIC had any effect on the specific bindings of [^{3}H]pyrilamine to the human H_1 receptor and [^{3}H]-tiotidine to the human H_2 receptor at concentrations up to 10 μM.

Functional Effects on Histamine Receptor Subtypes. The functional potencies of the compounds on the human H_1, H_2, H_3, and H_4 receptor subtypes were assessed using luciferase reporter gene assay.[6a] The four human histamine receptor subtypes were individually expressed in 293-EBNA cells according to the previously reported method,[6a] which were used in the evaluation

Table 2. Functional Effects of Compounds on Human Histamine Receptor Subtypes

	functional activity (EC_{50} nM)[a]			
compd	H_1	H_2	H_3	H_4
9	$>10^4$	$>10^4$	2330 ± 1520	$>10^4$
ent-**9**	$>10^4$	$>10^4$	$>10^4$	$>10^4$
10	$>10^4$	2500 ± 2190	724 ± 360	$>10^4$
ent-**10**	864 ± 349	696 ± 218	42 ± 19	$>10^4$
11	$>10^4$	$>10^4$	96 ± 54	$>10^4$
ent-**11**	$>10^4$	$>10^4$	$>10^4$	$>10^4$
12	$>10^4$	$>10^4$	$>10^4$	$>10^4$
ent-**12**	$>10^4$	$>10^4$	$>10^4$	$>10^4$
13 (AEIC)	$>10^4$	$>10^4$	10 ± 3	$>10^4$
ent-**13**	$>10^4$	$>10^4$	93 ± 5	1410 ± 37
histamine (**1**)	13 ± 1	463 ± 25	77 ± 51	75 ± 21
imetit (**3**)	-	-	1.9 ± 0.4	51 ± 22

[a] Assay was carried out by luciferase reporter gene method with the 293-EBNA cells expressing human H_1, H_2, H_3, or H_4 receptor subtype ($n = 3$).

of the compounds. The EC_{50} values of the conformationally restricted analogues, as well as histamine, are summarized in Table 2.

All the conformationally restricted analogues, except for *ent*-**10**, were inactive or showed only a very weak agonist activity ($EC_{50} > 10^3$ nM) to the H_1 and the H_2 subtype receptors, as expected from the results of the above binding experiments, while *ent*-**10** showed a moderate agonist effect on the H_1 ($EC_{50} = 864 \pm 349$ nM) and the H_2 ($EC_{50} = 696 \pm 218$ nM) subtypes. Although *ent*-**9**, *ent*-**11**, **12**, and *ent*-**12** were also inactive to the H_3 subtype ($EC_{50} > 10^4$ nM), the other conformationally restricted analogues caused evident activation of the H_3 receptor with the ranking of **13** > *ent*-**10** > *ent*-**13**, **11** > **10** > **9**, which was roughly in accord with their binding affinities summarized in Table 1. In particular, compound **13** (AEIC) showed an excellent EC_{50} value of 10 ± 3 nM. On the other hand, none of the compounds, except for *ent*-**13** ($EC_{50} = 1410 \pm 37$ nM), showed any agonist effect on the H_4 subtype ($EC_{50} > 10^4$ nM). None of the conformationally restricted analogues showed an antagonist effect on any of the four histamine receptor subtypes (data not shown).

These results demonstrated that ***ent*-10**, **11**, **13**, and *ent*-**13** were selective H_3 receptor agonists. It is worth noting that the conformationally restricted analogue **13** (AEIC) was an agonist of the H_3 receptor with a significant EC_{50} value of 10 ± 3 nM, lower than that of endogenous ligand histamine ($EC_{50} = 76.7 \pm 51$ nM), while it was inactive or almost inactive[20] to the other three histamine subtype receptors.

Discussion. All of the target conformationally restricted analogues having a cyclopropane ring were efficiently synthesized from the chiral cyclopropane units. These results proved that the chiral cyclopropane units, **14** and **15**, and their enantiomers *ent*-**14** and *ent*-**15**, are very useful synthetic intermediates for preparing various conformationally restricted chiral cyclopropane analogues of biologically active compounds.

The (1*S*,2*S*)-trans analogue *ent*-**10**, the amino function of which directly bonded to the cyclopropane ring, was previously shown to be a potent H_3 agonist, which was stronger than its enantiomer, the (1*R*,2*R*)-trans analogue **10**.[12] However, *ent*-**10** also had a moderate effect on the H_1 and the H_2 subtypes.[12] These previous results on the trans-analogues **10** and *ent*-**10** by De Esch and co-workers were supported by this study.[21] We synthesized anew the corresponding *cis*-isomers **9** and *ent*-**9** and clarified that their binding and functional effects on the H_3 receptor were relatively weak compared with the trans-analogues **10** and *ent*-**10**. [22]

Pharmacological evaluation of the conformationally restricted analogues **11**, *ent*-**11**, **12**, and *ent*-**12**, which have the carbon-elongated "imidazole−C(1)−C(2)−C(1′)−N" and/or the "imidazole−C(1)−C(2)−C(3)−C(1′)−N" structure, showed that the *cis*-(1*S*,2*R*) analogue **11** was a potent agonist of the H_3 receptor. Although the potency of **11** was somewhat weaker than *ent*-**10** in the functional assay (Table 2), it was much more selective to the H_3 subtype compared with *ent*-**10**. Thus, since the one-carbon elongation resulted in improvement of the H_3-agonist potency in the *cis*-analogues, we synthesized and evaluated the further carbon-elongated *cis*-analogue **13** (AEIC) having the same stereochemistry as that of **11** and its enantiomer *ent*-**13**. Consequently, AEIC (**13**) was identified as the most potent and selective H_3 receptor agonist in this series of compounds, which showed that the *cis*-cyclopropane structure is very effective in the conformational restriction of histamine to improve the specific binding to the histamine H_3 receptor. This compound owes its importance to being the first highly selective H_3 receptor agonist with virtually no effect on the H_4 subtype receptor, while the previous H_3 antagonists have been shown to clearly affect the H_4 subtype.[6b−d,f,g] AEIC (**13**) can be an efficient lead for clinically useful H_3 receptor agonists and would also be useful as a pharmacological tool for various studies on histaminergic systems.

These results of the cyclopropane-based conformationally restricted analogues of histamine as H_3 subtype receptor agonists showed that, in both the cis- and the trans-series, the 1*S*-enantiomers **9**, *ent*-**10**, **11**, *ent*-**12**, and **13** were more potent than the corresponding 1*R*-enantiomers *ent*-**9**, **10**, *ent*-**11**, **12**, and *ent*-**13**, respectively. The 1*S*-structure proved to be essential for the strong binding to the H_3 receptor.

Previous studies on GT2331 (**8**, Figure 1)[13] as well as on *ent*-**10**[12] have demonstrated that the trans-cyclopropane structure was very effective in the conformational restriction of histamine for improving affinity for the H_3 receptor. This study proved that conformational restriction of histamine by the *cis*-cyclopropane structure may be much more effective than the trans-one for the development of potent and selective H_3 receptor ligands.

The results of **9**, *ent*-**9**, **10**, and *ent*-**10** suggest that the bioactive conformation of histamine for the H_3 receptor subtype seems to be the "extended" form. However, the highly selective H_3 agonist potency of the *trans*-analogues **11** and AEIC (**13**) contradicts the "folded" bioactive conformation. The bioactive conformation may be between the "folded" and the "extended" forms. Further studies are needed to confirm this supposition.

Conclusion. A series of cyclopropane-based conformationally restricted analogues of histamine were designed as novel H_3 receptor agonists and were synthesized from the versatile chiral cyclopropane units **14**, **15**, and their enantiomers *ent*-**14** and *ent*-**15**. Among the compounds, (1*S*,2*S*)-2-(2-aminoethyl)-1-(1*H*-imidazol-4-

yl)cyclopropane (**13**, AEIC) was identified as the first highly selective H_3 receptor agonist. These results showed that the *cis*-cyclopropane structure is very effective in the conformational restriction of histamine to improve the specific binding to the histamine H_3 receptor, and also that the cyclopropane-based conformational restriction strategy is very effective in medicinal chemical studies for improving the potency of compounds.

Experimental Section

Chemical shifts are reported in ppm downfield from TMS. All of the ^{1}H NMR assignments described were in agreement with COSY spectra. Thin-layer chromatography was performed on Merck coated plate $60F_{254}$. Silica gel chromatography was performed on Merck silica gel 5715. Reactions were carried out under an argon atmosphere.

(1*S*,2*R*)-1-(1-Triphenylmethyl-1*H*-imidazol-4-yl)cyclopropane-2-carboxylic Acid (17). A mixture of **16**[16] (151 mg, 0.40 mmol), $NaClO_2$ (128 mg, 1.4 mmol), and $NaH_2PO_4 \cdot 2H_2O$ (64 mg, 0.40 mmol) in acetone/CH_2Cl_2/H_2O (4:2:1, 28 mL) was stirred at room temperature for 12 h. The solvent was evaporated, and the residue was partitioned between H_2O and $CHCl_3$. The organic layer was washed with brine, dried (Na_2SO_4), evaporated, and purified by column chromatography (silica gel; $CHCl_3$/MeOH, 1:9) to give **17** as a white solid (141 mg, 89%, white solid): $[\alpha]^{21}_D$ +51.4 (*c* 1.59, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.52 (1 H, m, H-3a), 1.56 (1 H, m, H-3b), 2.13 (1 H, m, H-1), 2.23 (1 H, m, H-2), 6.77 (1 H, s, CH═), 7.12−7.13 (6 H, m, aromatic), 7.35−7.37 (9 H, m, aromatic), 7.40 (1 H, s, CH═); ^{13}C NMR (125 MHz, $CDCl_3$) δ 15.04 (C-3), 23.53 (C-1), 29.36 (C-2), 75.89 (*C*Ph_3), 119.85, 128.20, 128.31, 129.69, 137.08, 137.32, 141.72 (aromatic), 174.78 (C-1′); LR-MS (FAB) *m*/*z* 395 [(M + H)$^+$]; HR-MS (FAB) calcd for $C_{26}H_{23}N_2O_2$ 395.1759; found 395.1736 [(M + H)$^+$]. Anal. ($C_{26}H_{22}N_2O_2 \cdot 2H_2O$) C, H, N.

(1*R*,2*S*)-1-(1-Triphenylmethyl-1*H*-imidazol-4-yl)cyclopropane-2-carboxylic Acid (*ent*-17). *ent*-**17** (131 mg, 83%, white solid) was prepared from *ent*-**16**[16] (151 mg, 0.40 mmol) as described for preparing **17**: $[\alpha]^{22}_D$ −50.3 (*c* 1.11, $CHCl_3$); LR-MS (FAB) *m*/*z* 395 [(M + H)$^+$]. Anal. ($C_{26}H_{22}N_2O_2 \cdot 2H_2O$) C, H, N.

(1*R*,2*R*)-1-(1-Triphenylmethyl-1*H*-imidazol-4-yl)cyclopropane-2-carboxylic Acid (21). Compound **21** (35 mg, 89%, white crystals) was prepared from **20**[16] (38 mg, 0.10 mmol) as described for preparing **17**: mp (EtOH) 132−133 °C; $[\alpha]^{20}_D$ −75.1 (*c* 1.10, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$:CD_3OD/1:2) δ 1.25 (1 H, m, H-3a), 1.35 (1 H, m, H-3b), 1.79 (1 H, m, H-1), 2.28 (1 H, m, H-2), 6.63 (1 H, s, CH═), 7.03−7.04 (6 H, m, aromatic), 7.25−7.28 (10 H, m, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$:CD_3OD/1:2) δ 14.77 (C-3), 21.49 (C-1), 28.48 (C-2), 75.92 (*C*Ph_3), 117.15, 127.68, 127.75, 129.55, 136.88, 137.06, 140.39 (aromatic), 175.52 (C-1′); HR-MS (FAB) calcd for $C_{26}H_{23}N_2O_2$ 395.1759; found 395.1734 ((M + H)$^+$]; Anal. ($C_{26}H_{22}N_2O_2 \cdot 2H_2O$) C, H, N.

(1*S*,2*S*)-1-(1-Triphenylmethyl-1*H*-imidazol-4-yl)cyclopropane-2-carboxylic Acid (*ent*-21). *ent*-**21** (65 mg, 82%, white crystals) was prepared from *ent*-**20**[16] (76 mg, 0.20 mmol) as described for preparing **17**: mp (EtOH) 130−132 °C; $[\alpha]^{20}_D$ +73.2 (*c* 1.20, $CHCl_3$); LR-MS (FAB) *m*/*z* 395 [(M + H)$^+$]; Anal. ($C_{26}H_{22}N_2O_2 \cdot 2H_2O$) C, H, N.

(1*S*,2*R*)-2-Azidocarbonyl-1-(1-triphenylmethyl-1*H*-imidazol-4-yl)cyclopropane (18). A mixture of **17** (55 mg, 0.14 mmol), diphenylphosphoryl azide (120 mg, 0.56 mmol), and Et_3N (40 μL, 0.28 mmol) in CH_2Cl_2 (2 mL) was stirred at room temperature for 5 h. The reaction mixture was partitioned between H_2O and $CHCl_3$. The organic layer was washed with brine, dried (Na_2SO_4), evaporated, and purified by column chromatography (silica gel; AcOEt/hexane, 1:3 then 1:2) to give **18** as a white solid (47 mg, 80%): $[\alpha]^{21}_D$ +50.3 (*c* 2.40, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.38 (1 H, m, H-3a), 1.58 (1 H, m, H-3b), 2.04 (1 H, m, H-1), 2.63 (1 H, m, H-2), 6.62 (1 H, s, CH═), 7.11−7.15 (6 H, m, aromatic), 7.32 (1 H, s, CH═), 7.33−7.34 (9 H, m, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.11 (C-3), 22.46 (C-2), 23.96 (C-1), 75.28 (*C*Ph_3), 120.43, 127.96, 128.04, 129.82, 136.10, 138.05, 142.34 (aromatic), 176.62 (C-1′); LR-MS (FAB) *m*/*z* 420 [(M + H)$^+$]. Anal. ($C_{26}H_{21}N_5O$) C, H, N.

(1*R*,2*S*)-2-Azidocarbonyl-1-(1-triphenylmethyl-1*H*-imidazol-4-yl)cyclopropane (*ent*-18). *ent*-**18** (46 mg, 78%, white solid) was prepared from *ent*-**17** (55 mg, 0.14 mmol) as described for preparing **18**: $[\alpha]^{21}_D$ −51.2 (*c* 3.75, $CHCl_3$); LR-MS (FAB) *m*/*z* 420 [(M + H)$^+$]. Anal. ($C_{26}H_{21}N_5O$) C, H, N.

(1*R*,2*R*)-2-Azidocarbonyl-1-(1-triphenylmethyl-1*H*-imidazol-4-yl)cyclopropane (22). Compound **22** (140 mg, 74%, white solid) was prepared from **21** (178 mg, 0.45 mmol) as described for preparing **18**: $[\alpha]^{21}_D$ −205.3 (*c* 1.51, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.58−1.61 (2 H, m, H-3), 2.05 (1 H, m, H-1), 2.51 (1 H, m, H-2), 6.66 (1 H, d, CH═, *J* = 1.3 Hz), 7.10−7.14 (6 H, m, aromatic), 7.29 (1 H, d, CH═, *J* = 0.9 Hz), 7.33−7.34 (9 H, m, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$) δ 15.68 (C-3), 22.72 (C-1), 25.59 (C-2), 75.21 (*C*Ph_3), 118.34, 128.00, 128.02, 129.67, 138.54, 138.75, 142.21 (aromatic), 179.79 (C-1′); LR-MS (FAB) *m*/*z* 420 [(M + H)$^+$]. Anal. ($C_{26}H_{21}N_5O$) C, H, N.

(1*S*,2*S*)-2-Azidocarbonyl-1-(1-triphenylmethyl-1*H*-imidazol-4-yl)cyclopropane (*ent*-22). *ent*-**22** (143 mg, 76%, white solid) was prepared from *ent*-**21** (178 mg, 0.45 mmol) as described for preparing **18**: $[\alpha]^{21}_D$ +203.5 (*c* 1.23, $CHCl_3$); LR-MS (FAB) *m*/*z* 420 [(M + H)$^+$]. Anal. ($C_{26}H_{21}N_5O$) C, H, N.

(1*S*,2*R*)-1-(1*H*-Imidazol-4-yl)-2-aminocyclopropane Dihydrochloride (9). A solution of **18** (59 mg, 0.14 mmol) in *t*-BuOH (2 mL) was heated under reflux for 2 h. The reaction mixture was evaporated and purified by column chromatography (silica gel; $CHCl_3$/MeOH, 1:19) to give (1*S*,2*R*)-1-(1*H*-imidazol-4-yl)cyclopropane-2-isocyanate (**19**) as a white solid (20 mg, 96%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.53 (1 H, m, H-3a), 1.36 (1 H, m, H-3b), 2.41 (1 H, m, H-1), 3.23 (1 H, m, H-2), 6.98 (1 H, s, CH═), 7.28 (1 H, s, CH═). Compound **19** was immediately used for the next reaction. A solution of **19** (15 mg, 100 μmol) in 6 N HCl (1 mL) was heated under reflux for 5 h. The solvent was evaporated, and then the residue was treated with Et_2O/EtOH to give a white solid of **9** as dihydrochloride (18 mg, 92%): $[\alpha]^{20}_D$ +9.5 (*c* 0.33, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ 1.57 (1 H, m, H-3a), 1.77 (1 H, m, H-3b), 2.61 (1 H, m, H-1), 3.25 (1 H, m, H-2), 7.70 (1 H, s, CH═), 9.08 (1 H, s, CH═); ^{13}C NMR (125 MHz, $CDCl_3$) δ 10.27 (C-1), 10.99 (C-3), 29.06 (C-2) 119.73, 129.04, 136.28 (imidazole C); HR-MS (EI) calcd for $C_6H_{10}N_3$ 124.0875; found 124.0880 [(M + H)$^+$). Anal. ($C_6H_{11}Cl_2N_3 \cdot 2H_2O$) C, H, N.

(1*R*,2*S*)-1-(1*H*-Imidazol-4-yl)-2-aminocyclopropane Dihydrochloride (*ent*-9). *ent*-**19** (20 mg, 95%), white solid) was prepared from *ent*-**18** (59 mg, 0.14 mmol) as described for preparing **19** and was immediately used for the next reaction. *ent*-**9** (20 mg, 90%, white solid) was prepared from *ent*-**19** (15 mg, 100 μmol) as described for preparing **9**: $[\alpha]^{23}_D$ −10.2 (*c* 0.61, CH_3OH); LR-MS (EI) *m*/*z* 124 [(M + H)$^+$). Anal. ($C_6H_{11}Cl_2N_3 \cdot 2H_2O$) C, H, N.

(1*R*,2*R*)-2-(*t*-Butyloxycarbonylamino)-1-(1-triphenylmethyl-1*H*-imidazol-4-yl)cyclopropane (23). A solution of **22** (138 mg, 0.33 mmol) in *t*-BuOH (2 mL) was heated under reflux for 5 h. The reaction mixture was evaporated and purified by column chromatography (silica gel; $CHCl_3$/MeOH, 1:19) to give **23** as a white solid (74 mg, 48%): $[\alpha]^{20}_D$ −54.3 (*c* 1.31, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.02 (1 H, m, H-3a), 1.20 (1 H, m, H-3b), 1.42 (9 H, s, (CH_3)$_3$C), 1.93 (1 H, m, H-1), 2.76 (1 H, m, H-2), 6.57 (1 H, s, CH═), 7.11−7.13 (6 H, m, aromatic), 7.28 (1 H, s, CH═), 7.31−7.34 (9 H, m, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$) δ 15.54 (C-3), 28.38 ((CH_3)$_3$C), 31.41 (C-2), 75.14 (*C*Ph_3), 79.38 (C-1), 117.13, 127.95, 127.96, 129.76, 138.31, 140.67, 142.47 (aromatic), 156.32 (C═O); LR-MS (FAB) *m*/*z* 466 [(M + H)$^+$]. Anal. ($C_{30}H_{31}N_3O_2$) C, H, N.

(1*S*,2*S*)-2-(*t*-Butyloxycarbonylamino)-1-(1-triphenylmethyl-1*H*-imidazol-4-yl)cyclopropane (*ent*-23). *ent*-**23** (77 mg, 50%, white solid) was prepared from *ent*-**22** (138 mg, 0.33

mmol) as described for preparing **23**: $[\alpha]^{22}_D$ +53.5 (*c* 1.04, $CHCl_3$); LR-MS (FAB) *m/z* 466 [(M + H)$^+$]. Anal. ($C_{30}H_{31}N_3O_2$) C, H, N.

(1*R*,2*R*)-1-(1*H*-Imidazol-4-yl)-2-aminocyclopropane Dihydrochloride (10). A solution of **23** (23 mg, 50 μmol) in MeOH (2 mL) and 3 N HCl (1 mL) was heated under reflux for 3 h. The solvent was evaporated, and then residue was treated with Et_2O/EtOH to give a white solid of **10** as a dihydrochloride (10 mg, 98%): ^{1}H NMR (500 MHz, D_2O) δ 1.46 (1 H, m, H-3a), 1.59 (1 H, m, H-3b), 2.54 (1 H, m, H-1), 3.07 (1 H, m, H-2), 7.29 (1 H, s, CH═), 8.61 (1 H, s, CH═); LR-MS (EI) *m/z* 123 (M$^+$). Anal. ($C_6H_{11}Cl_2N_3$) C, H, N. $[\alpha]^{25}_{546}$ +52.3 (dihydrobromide prepared by treating the dihydrochloride successively with Diaion-PK-312 (OH^-) and Diaion-WA-30 (Br^-) resins, *c* 0.68, H_2O) [lit.[12] $[\alpha]^{30}_{546}$ +54.4 (*c* 0.35, H_2O)]

(1*S*,2*S*)-1-(1*H*-Imidazol-4-yl)-2-aminocyclopropane Dihydrochloride (*ent*-10). *ent*-**10** (10 mg, 98%, white solid) was prepared from *ent*-**23** (23 mg, 50 μmol) as described for preparing **10**: LR-MS (EI) *m/z* 123 (M$^+$). Anal. ($C_6H_{11}Cl_2N_3$) C, H, N. $[\alpha]^{25}_{546}$ −55.1 (dihydrobromide prepared by treating the dihydrochloride successively with Diaion-PK-312 (OH^-) and Diaion-WA-30 (Br^-) resins), *c* 1.28, H_2O] [lit.[12] $[\alpha]^{30}_{546}$ −54.2 (*c* 0.35, H_2O)].

(1*S*,2*S*)-2-Formylmethyl-1-(1-triphenylmethyl-1*H*-imidazol-4-yl)cyclopropane (24). To a suspension of $MeOCH_2PPh_3Cl$ (120 mg, 0.35 mmol) in THF (2 mL) was added NaHMDS (1.0 M in THF, 0.30 mL, 0.30 mmol) dropwise at 0 °C, and the resulting mixture was stirred at the same temperature for 5 min. To a solution of **16** (38 mg, 0.10 mmol) in THF (2 mL) was added dropwise the prepared bright red solution of the Wittig reagent, and the resulting mixture was stirred at the same temperature for 30 min. The reaction was quenched with saturated aqueous NH_4Cl, and the solvent was evaporated. The residue was partitioned between H_2O and $CHCl_3$, and the organic layer was washed with brine, dried (Na_2SO_4), evaporated, and purified by column chromatography (silica gel; hexane/AcOEt, 1:1) to give the corresponding Wittig reaction product as a white solid (32 mg). To a solution of the product (32 mg) in acetone (2 mL) was added 12 N HCl (1 mL), and the resulting solution was immediately added to a saturated aqueous $NaHCO_3$ solution. After concentration of the mixture under reduced pressure (for removing acetone), the resulting aqueous mixture was partitioned between H_2O and $CHCl_3$. The organic layer was washed with brine, dried (Na_2SO_4), evaporated, and purified by column chromatography (silica gel; hexane/AcOEt, 1:1 then $CHCl_3$/MeOH, 10:1) to give **24** as a yellow oil (30 mg, 76%): $[\alpha]^{23}_D$ +17.9 (*c* 1.02, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.79 (1 H, m, H-3a), 1.10 (1 H, m, H-3b), 1.30 (1 H, m, H-2), 2.10 (1 H, m, H-1), 2.35 (2 H, m, H-1′), 6.58 (1 H, s, CH═), 7.12−7.14 (6 H, m, aromatic), 7.32−7.33 (10 H, m, aromatic), 9.66 (1 H, br s, H-2′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 9.85 (C-3), 12.06 (C-1), 13.58 (C-2), 42.87 (C-1′), 75.14 (*C*Ph_3), 119.52, 127.97, 127.99, 129.65, 138., 139.23, 142.41 (aromatic), 202.94 (C-2′); LR-MS (FAB) *m/z* 393 [(M + H)$^+$]; Anal. ($C_{27}H_{24}N_2O$) C, H, N.

(1*R*,2*R*)-2-Formylmethyl-1-(1-triphenylmethyl-1*H*-imidazol-4-yl)cyclopropane (*ent*-24). *ent*-**24** (27 mg, 70%, yellow oil) was prepared from *ent*-**16** (38 mg, 0.10 mmol) as described for preparing **24**: $[\alpha]^{23}_D$ −17.6 (*c* 1.32, $CHCl_3$); LR-MS (FAB) *m/z* 393 [(M + H)$^+$]. Anal. ($C_{27}H_{24}N_2O$) C, H, N.

(1*S*,2*S*)-2-(2-Aminoethyl)-1-(1-triphenylmethyl-1*H*-imidazol-4-yl)cyclopropane (26). A solution of **24** (39 mg, 0.10 mmol), *O*-benzylhydroxylamine hydrochloride (32 mg, 0.20 mmol), and molecular sieves 4A (10 mg) in THF (3 mL) was stirred at room temperature for 5 h. After filtration of the resulting mixture with Florisil, the filtrate was evaporated and purified by column chromatography (silica gel; AcOEt/hexane, 1:1) to give the corresponding benzyloxime **25** as a white solid (*E*/*Z* mixture, 49 mg, quant). To a solution of **25** (49 mg) in THF (2 mL) was added $LiAlH_4$ (1.0 M in THF, 0.30 mL, 0.30 mmol), and the mixture was stirred at room temperature for 1 h. The reaction was quenched with MeOH, and then the solvent was evaporated. The residue was partitioned between $CHCl_3$ and H_2O, and the organic layer was washed with saturated aqueous NH_4Cl and brine, dried (Na_2SO_4), evaporated, and purified by column chromatography (silica gel; MeOH/$CHCl_3$, 1:9 then 1:4) to give **26** as a white solid (38 mg, 97%): $[\alpha]^{24}_D$ +17.1 (*c* 0.28, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.26 (1 H, m, H-3a), 0.98 (1 H, m, H-3b), 1.07 (1 H, m, H-1′a), 1.23−1.32 (2 H, m, H-1′b and H-2), 2.03 (1 H, m, H-1), 3.16−3.26 (2 H, m, H-2′), 6.62 (1 H, d, CH═), 7.09−7.11 (6 H, m, aromatic), 7.34−7.37 (10 H, m, aromatic), 9.11 (2 H, br s, $-NH_2$); ^{13}C NMR (125 MHz, CD_3OD) δ 10.22 (C-3), 13.46 (C-2), 13.62 (C-1), 26.10 (C-1′), 38.94 (C-2′), 75.56 (*C*Ph_3), 121.89, 128.15, 128.17, 129.67, 137.74, 139.49, 142.08 (aromatic); HR-MS (FAB) calcd for $C_{27}H_{28}N_3$ 394.2283; found 394.2298 [(M + H)$^+$].

(1*R*,2*R*)-2-(2-Aminoethyl)-1-(1-triphenylmethyl-1*H*-imidazol-4-yl)cyclopropane (*ent*-26). *ent*-**26** (37 mg, 95%, white solid) was prepared from *ent*-**24** (39 mg, 0.10 mmol) as described for preparing **26**: $[\alpha]^{24}_D$ −17.4 (*c* 0.48, MeOH); LR-MS (FAB) *m/z* 394 [(M + H)$^+$].

(1*S*,2*S*)-2-(2-Aminoethyl)-1-(1*H*-imidazol-4-yl)cyclopropane Dihydrochloride (13). Compound **13** as a dihydrochloride (13 mg, 99%, white solid) was prepared from **26** (24 mg, 60 μmol) as described for preparing **10**: $[\alpha]^{22}_D$ −35.1 (*c* 0.32, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 0.87 (1 H, m, H-3a), 1.23−1.39 (3 H, m, H-3b and H-1′), 1.67 (1 H, m, H-2), 2.16 (1 H, m, H-1), 2.92−3.03 (2 H, m, H-2′), 7.35 (1 H, s, CH═), 8.83 (1 H, d, CH═, *J* = 1.1 Hz); ^{13}C NMR (125 MHz, CD_3OD) δ 10.91 (C-3), 11.27 (C-1), 16.78 (C-2), 27.75 (C-1′), 40.28 (C-2′), 118.06, 133.70, 135.01 (imidazole C); HR-MS (EI) calcd for $C_8H_{13}N_3$ 151.1109; found 151.1101 (M$^+$). Anal. ($C_8H_{15}Cl_2N_3\cdot 2H_2O$) C, H, N.

(1*R*,2*R*)-2-(2-Aminoethyl)-1-(1*H*-imidazol-4-yl)cyclopropane Dihydrochloride (*ent*-13). *ent*-**13** was prepared from *ent*-**26** (24 mg, 60 μmol) as described for preparing **10**: $[\alpha]^{17}_D$ +36.1 (*c* 0.22, MeOH); LR-MS (EI) *m/z* 151 (M$^+$). Anal. ($C_8H_{15}Cl_2N_3\cdot 2H_2O$) C, H, N.

Binding Assay of Rat Histamine H_3 Receptor. Male Sprague−Dawley rats at 8 to 10 weeks of age (Japan SLC Inc., Shizuoka, Japan) were housed three or four per cage in the laboratory with free access to food and water and maintained on a 12 h dark/light cycle in a room with controlled temperature (24 ± 1 °C) and humidity (55 ± 5%). Rats were killed by taking the blood from the descending aorta under light anesthesia with Et_2O. The brain tissues (minus cerebellum) were removed and used for the measurements of H_3 receptor subtype. The binding assay was performed by using [^{3}H]N^α-methylhistamine (DuPont-NEN), as described previously.[18] Briefly, the brain homogenates (800 μg) were incubated with different concentrations of [^{3}H]N^α-methylhistamine (0.1−3.0 nM) for 1 h at 25 °C in 50 mM Na_2HPO_4/KH_2PO_4 buffer (pH 7.5). The reaction was terminated by rapid filtration (Cell Harvester, Brandel Co., Gaithersburg, MD) through Whatman GF/B glass fiber filters, and the filters were rinsed three times with an ice-cold buffer (2 mL). Tissue-bound radioactivity was extracted from filters overnight in scintillation fluid (toluene, 2 L; Triton X-100, 1 L; 2,5-diphenyloxazole, 15 g; 1,4-bis[2-(5-phenyloxazolyl)]benzene, 0.3 g) and determined in a liquid scintillation counter. The specific binding of each radioligand was determined experimentally from the difference between counts in the presence of 10 μM thioperamide. Analysis of the binding data was performed as described previously.[23,24] The apparent dissociation constants (K_d) for [^{3}H]N^α-methylhistamine were estimated by Rosenthal analysis of the saturation data. The ability of each compound to inhibit the specific binding of [^{3}H]N^α-methylhistamine (0.25 nM) was estimated by IC_{50} values, which are the molar concentrations of the unlabeled drug necessary for displacing 50% of specific binding (estimated by log probit analysis). The inhibition constant, *K* value, was calculated from the equation, $K_i = IC_{50}/(1 + L/K_d)$, where L equals concentration of each radioligand. The data were presented as mean ± SE.

Binding Assay of Human Histamine Receptors. The stable CHO cell lines expressing H_1, H_2, and H_3 receptors are established by transfection of the plasmids harboring human H_3 and H_4 cDNA as described previously.[6b] The binding of the

compounds to the membrane fractions were evaluated using [^{3}H]pyrilamine (H_1), [^{3}H]tiotidine (H_2), and [^{3}H]N^α-methylhistamine (H_3) (Perkin-Elmer) as radioligands, respectively.

Luciferase Reporter Assay. Reporter gene assay was performed according to the method described previously.[6a] Briefly, 3×10^4 cells of 293-EBNA (Invitrogen) were harvested on collagen-coated 48-well plates for 24 h. An H_1-expression plasmid and a pSRE-Luc (Stratagene) or an H_2-expression plasmid and pCRE-Luc (Stratagene) were cotransfected using Fugene 6 (Roche Diagnostics) according to the manufacturer's recommendations. An expression plasmid for $G_{\alpha q/i}$, chimera G_α protein of $G_{\alpha q}$ and $G_{\alpha i}$, was constructed[25] and cotransfected with an H_3 or H_4-expression plasmid and a pSRE-Luc. The following day, the cells were treated with compounds for 5 h and laid on ice. Intracellular luciferase activity in aliquots from each lysate was measured using a model ML3000 luminometer (Dynatech laboratories).

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