Stereochemical Diversity-Oriented Conformational Restriction Strategy. Development of Potent Histamine H_3 and/or H_4 Receptor Antagonists with an Imidazolylcyclopropane Structure

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The stereochemical diversity-oriented conformational restriction strategy can be an efficient method for developing specific ligands for drug target proteins, especially in cases where neither the bioactive conformation nor the pharmacophore is known. To develop potent H_3 and H_4 receptor antagonists, a series of conformationally restricted analogues of histamine with a chiral *cis*- or *trans*-cyclopropane structure were designed on the basis of this strategy. These target compounds with stereochemical diversity were synthesized from the versatile chiral cyclopropane units (1*S*,2*R*)- and (1*R*,2*R*)-2-(*tert*-butyldiphenylsilyloxy)-methyl-1-formylcyclopropane (**6** and **7**, respectively) or their enantiomers *ent*-**6** and *ent*-**7**. Pharmacological profiles of these conformationally restricted analogues were shown to be different depending on the cyclopropane backbones. Among the analogues, (1*R*,2*S*)-2-[2-(4-chlorobenzylamino)ethyl]-1-(1*H*-imidazol-4-yl)cyclopropane (**11a**) with the (1*R*)-*trans*-cyclopropane structure has remarkable antagonistic activity to both the H_3 (K_i = 8.4 nM) and H_4 (K_i = 7.6 nM) receptors. The enantiomer of **11a**, i.e., *ent*-**11a**, with the (1*S*)-*trans*-cyclopropane structure turned out to be a highly potent and selective H_3 receptor antagonist with a K_i of 3.6 nM. Conversely, (1*R*,2*R*)-2-[(4-chlorobenzylamino)methyl]-1-(1*H*-imidazol-4-yl)cyclopropane (**10a**) with the (1*R*)-*trans* structure was selective for the H_4 receptor (K_i = 118 nM) compared to the H_3 receptor (K_i > 10^3 nM). Thus, a variety of compounds with different pharmacological profiles have been developed. These results show that when the structure of the target protein is unknown, the stereochemical diversity-oriented approach can be a powerful strategy in medicinal chemical studies.

Introduction

Considerable effort has been devoted toward developing new methods for drug discovery. In particular, the process of identifying therapeutic agents targeting G-protein-coupled receptors (GPCRs) has generated much interest, since GPCRs are considered to be major targets for drug development.[1] Indeed, it is estimated that over 50% of all modern drugs are targeted at GPCRs.[1a] However, because of the membranous nature of these proteins and their very low natural abundance, structural analysis of GPCRs is, not surprisingly, difficult. In fact, the only the high-resolution structure of a GPCR currently available is that of bovine rhodopsin.[1b] One obvious drawback in drug development targeting GPCRs is therefore a lack of structural data on the proteins. Thus, a method for effectively identifying compounds targeting GPCRs, which does not involve the structural data, would be highly useful in drug development. Consequently, we have devised a stereochemical diversity-oriented conformational restriction strategy to develop compounds that bind selectively to target proteins of unknown structure such as GPCRs. On the basis of this approach, we planned to develop histamine H_3 and H_4 selective antagonists.

Homeostatic processes related to the neurotransmitter histamine (Figure 1) are mediated by at least four receptor subtypes termed H_1, H_2, H_3, and H_4 receptors.[2] In recent years, much attention has been focused on the H_3 receptor, which is a newly cloned G_i-protein-coupled receptor distributed mainly in the central nervous system.[3] Homology analysis of the H_3 receptor demonstrated that it is significantly different from the previously cloned H_1 and H_2 receptors.[3c,d] Antagonists to the H_3 receptor are considered to be potential drugs for various diseases such as Alzheimer's disease, attention-deficit/hyperactivity disorder (ADHD), schizophrenia, depression, dementia, and epilepsy.[3b] There have been attempts to develop H_3 receptor antagonists, and throughout these studies, potent H_3 receptor antagonists have been reported,[3] some of which are shown in Figure 1.

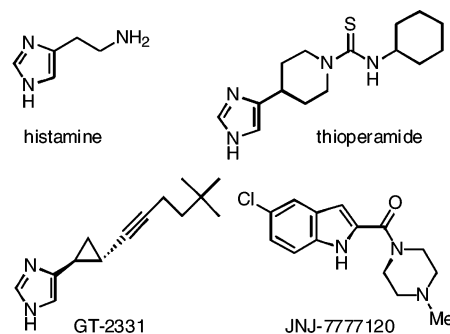

Figure 1. Structures of histamine and some H_3 and H_4 receptor antagonists.

In 2000 and 2001, another histamine receptor subtype, the H_4 receptor, was cloned by several groups and identified as a G-protein-coupled receptor.[4] The H_4 receptor is expressed in immunocytes, such as eosinophils or mast cells, and chemotaxis of these cells via histamine has been shown to be triggered through the H_4 receptor activation.[5] Accordingly, H_4 receptor antagonists may be effectively used in new therapeutic modalities for the treatment of allergic diseases.[4]

The bioactive conformations of histamine for the H_3 receptor and especially for the H_4 receptor are unclear. Furthermore, only

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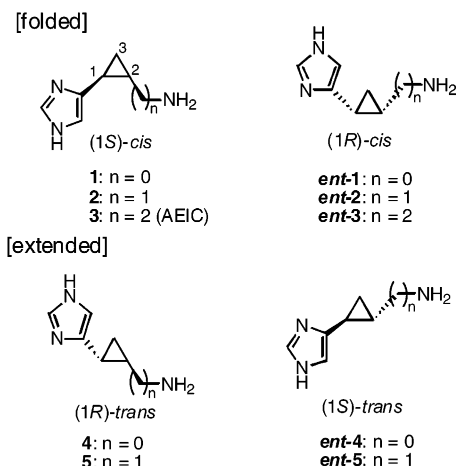


Figure 2. Conformationally restricted analogues of histamine with stereochemical diversity.

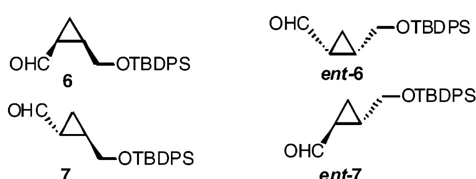


Figure 3. Chiral cyclopropane units.

a few H_4 receptor antagonists have been reported,[5,6] and the pharmacophore for the H_4 receptor antagonist has not yet been established. In such cases, the stereochemical diversity-oriented conformational restriction strategy may be effective. We recently synthesized a series of conformationally restricted analogues of histamine, which, as shown in Figure 2, were designed by this strategy using the four chiral cyclopropane units with different stereochemistries, the structures of which are shown in Figure 3.[7] In these conformationally restricted analogues, the imidazole and the amino groups, which are essential for activating the H_3 receptor, are located in a variety of spatial arrangements due to the conformational restriction. Consequently, a series of these analogues can be stereochemically diverse. Some of these analogues were shown to be potent H_3 receptor agonists, and in particular, AEIC (**3**) with the (1*S*)-*cis*-cyclopropane structure was identified as a highly potent and selective H_3 receptor agonist.[7b]

With these results in mind, we investigated the functional conversion of these H_3 receptor agonists with a cyclopropane ring into antagonists by their structural modification and thereby successfully developed potent H_3 and/or H_4 receptor selective antagonists via the stereochemical diversity-oriented conformational restriction strategy using the versatile chiral cyclopropane units. In this report, we describe the design, synthesis, and pharmacological effects of these H_3 and/or H_4 receptor antagonists.

Results and Discussion

Design of the Antagonists by the Stereochemical Diversity-Oriented Conformational Restriction Strategy. Most neurotransmitters, including histamine, are conformationally flexible because of their common "aromatic ring$-$C(sp^3)$-$C(sp^3)$-$N" backbone, and accordingly, they can assume a variety of conformations, which may make it possible for them to bind to different proteins, i.e., receptor subtypes, via different conformations.[8] Therefore, conformational restriction of neurotransmitters may improve the specific binding to one of the receptor subtypes. In conformationally restricted analogues highly selectively bound to the target receptor, the functional groups essential for the receptor binding must assume a special arrangement superimposing on the bioactive conformation, in which these functional groups effectively interact with certain amino acid residues in the binding pocket of the receptor. The major problem in designing conformationally restricted analogues specific for a receptor subtype is that the conformation of the conformationally flexible compound that binds to the target subtype, i.e., the bioactive conformation, is usually unknown. This is mainly because structural analysis of membrane-bound proteins, such as GPCRs, is tremendously difficult,[1] compared with that of proteins soluble in blood or cytosol. We therefore propose a strategy for designing conformationally restricted analogues based on stereochemical diversity, where the versatile cyclopropane units (Figure 3) are effectively used as the key tool, as described below.

In the design of conformationally restricted analogues, it is essential that the analogues should be as similar as possible to the parent compound in size, shape, and molecular weight.[9] Because of its characteristic rigid and small structural feature, a cyclopropane ring is likely to be effective in rigidly restricting the conformation of a molecule, leaving intact the chemical and physical properties of the lead compounds.[10,11] In fact, there have been reports of cyclopropane-based conformationally restricted analogues of histamine,[12,13] such as the H_3 receptor antagonist GT-2331 with the (1*S*)-*trans*-cyclopropane structure[13b] (Figure 1), indicating that the cyclopropane ring might be effective in restricting the bioactive conformation of the histamine.

As described above, we previously designed and synthesized a series of conformationally restricted analogues of histamine with stereochemical diversity and identified the desired highly selective H_3 receptor agonist AEIC (**3**) with the (1*S*)-*cis*-cyclopropane structure. These results demonstrated that the stereochemical diversity-based approach is indeed effective and prompted us to develop H_3 and/or H_4 receptor antagonists by this approach.

Conversion of an agonist of a receptor into the corresponding antagonists is one of the major objectives in medicinal chemistry. It has been recognized that hydrophobic interactions play a crucial role in the binding of many ligands to their target biomolecules.[14] Introduction of a hydrophobic group to lead compounds sometimes improves the binding affinity because of its interactions with hydrophobic amino acid residues of the target protein located near the binding site.[14,15] Such derivatizations sometimes bring functional inversion of an agonist into the corresponding antagonist to the same receptor, which is well-known, for example, in the development of H_1 histaminergic[15a] and adrenergic[15b] receptor antagonists. And H_3 receptor antagonists having a hydrophobic group on a histamine-related structure are also known.[3] On the basis of these results, we designed a series of cyclopropane-based conformationally restricted analogues of histamine as potential histamine H_3 receptor antagonists. In their structures, a hydrophobic cyclohexylmethyl or 4-chlorobenzyl group is attached to the amino group of the cyclopropane-based conformationally restricted histamine analogues, which were previously designed as H_3 receptor agonists, as shown in Figure 4. This series of conformationally restricted analogues has stereochemical diversity; i.e., the key imidazole and hydrophobic groups are located in various spatial arrangements because of the chiral *cis*- and *trans*-cyclopropane backbones.

Furthermore, we expected that H_4 receptor selective antagonists might also be found by this approach. The H_4 receptor

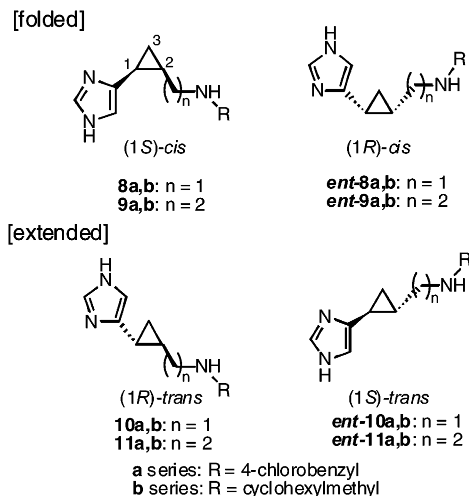


Figure 4. Target compounds designed as H_3 and/or H_4 receptor antagonists with stereochemical diversity.

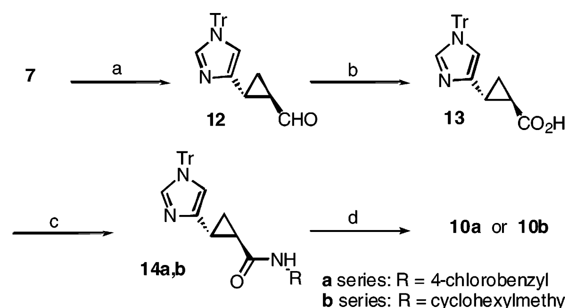
has significant sequence homology (about 40%) to the H_3 receptor cDNA.[4] It is noteworthy that the H_3 and the H_4 receptors share about 60% sequence identity in their transmembrane regions,[4] which would make it difficult to develop H_4 receptor selective ligands. In fact, only a few H_4 receptor selective ligands have been known.[6] However, because of the stereochemical diversity of the conformationally restricted analogues designed, some of these analogues might assume a conformation superimposed on the bioactive conformation of histamine for the H_4 receptor binding.

Chemistry. 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.[10,11,16,17] Nevertheless, a drawback in the cyclopropane-based conformational restriction is that stereoselective synthesis of cyclopropane derivatives with a desired stereochemistry is troublesome. To address this problem, we developed chiral units for cyclopropane-based conformational restriction, which were composed of four stereoisomeric cyclopropane derivatives bearing two adjacent carbon substituents in a *cis* or a *trans* relationship, namely, **6** and **7** and their enantiomers *ent*-**6** and *ent*-**7**, as shown in Figure 3.[7] These units, which are generally useful for synthesizing various compounds having an asymmetric *cis*- or *trans*-cyclopropane structure, were employed as the key intermediates in this study.

The synthesis of the "extended"-type target compounds **10a** and **10b** with the (1*R*)-*trans*-cyclopropane structure is shown in Scheme 1. *N*-Tritylimidazolylcyclopropanecarbaldehyde (**12**), prepared from the chiral unit **7**, was oxidized by the usual method to the carboxylic acid **13**,[7b] which was then condensed with 4-chlorobenzylamine or cyclohexylmethylamine with EDC/DMAP to give the corresponding carboxamide **14a** or **14b**, respectively. Reductive treatment of the amides **14a** and **14b** with BH_3 under reflux in THF, followed by acidic detritylation, produced the desired (1*R*)-*trans* analogues **10a** and **10b**.

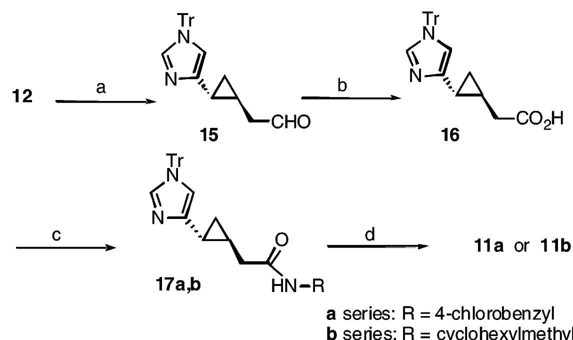
The one-carbon-elongated "extended" analogues **11a** and **11b** with the (1*R*)-*trans*-cyclopropane structure were synthesized as shown in Scheme 2. Wittig reaction of the (1*R*)-*trans*-aldehyde **12** with $MeOCH_2PPh_3Cl$/NaHMDS in THF, followed by acidic hydrolysis, gave the one-carbon-elongated aldehyde **15**. Oxidation of the aldehyde **15** produced the carboxylic acid **16**, which was converted into the (1*R*)-*trans* analogues **11a** and **11b** by the procedure described above.

Scheme 1[a]



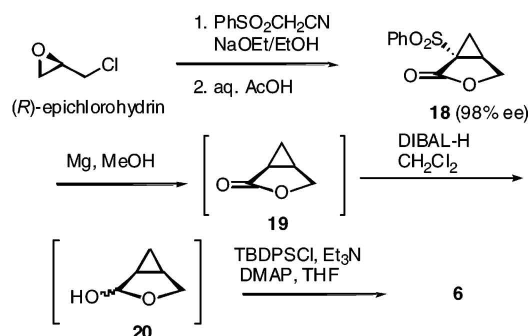
[a] Conditions: (a) ref 7a; (b) ref 7b; (c) RNH_2, EDC, DMAP, CH_2Cl_2/DMF, room temp, 89% (**14a**), 90% (**14b**); (d) (1) BH_3, THF, reflux, (2) HCl, aqueous EtOH, reflux, 94% (**10a**), 85% (**10b**).

Scheme 2[a]



[a] Conditions: (a) (1) $MeOCH_2PPh_3$,Cl, NaHMDS, THF, 0 °C, (2) HCl, aqueous acetone, room temp, 90%; (b) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, aqueous *t*-BuOH, 81%; (c) RNH_2, EDC, DMAP, CH_2Cl_2/DMF, room temp, 67% (**17a**), 80% (**17b**); (d) (1) BH_3, THF, reflux, (2) HCl, aqueous EtOH, reflux, 93% (**11a**), 93% (**11b**).

Scheme 3

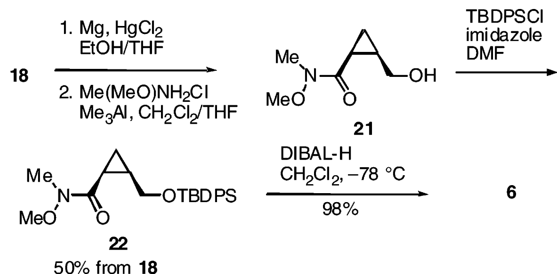


The "extended"-type enantiomers *ent*-**10a**, *ent*-**10b**, *ent*-**11a**, and *ent*-**11b** with the (1*S*)-*trans*-cyclopropane structure were similarly synthesized from the *trans* unit *ent*-**7**.

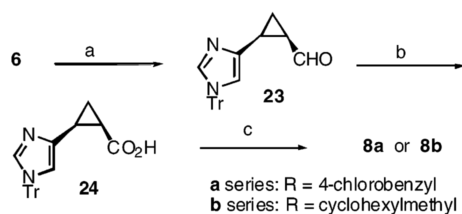
We previously synthesized the *cis* unit **6** from the readily available (*R*)-epichlorohydrin via the key intermediate **18** as summarized in Scheme 3.[7a] The corresponding enantiomer *ent*-**6** was similarly synthesized from (*S*)-epichlorohydrin. During these studies, we have sometimes encountered problems in the preparation of the *cis* units **6** and *ent*-**6**. In this procedure, the lactone **18** was successively treated with Mg in MeOH, DIBAL-H in CH_2Cl_2, and TBDPSCl/DMAP/Et_3N in THF without purifying the intermediates **19** and **20**, and the yield of the *cis* unit **6** was sometimes low, particularly in large-scale experiments. This might be caused, at least to some extent, by the volatility of the intermediates **19** and **20**.

Therefore, we set out to develop an alternative method for preparing the *cis* units **6** and *ent*-**6** via Weinreb amide **21**, as shown in Scheme 4. Reductive treatment of **18** with Mg in the

Scheme 4

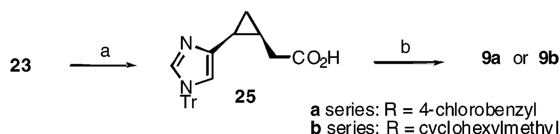


Scheme 5[a]



[a] Conditions: (a) ref 7a; (b) ref 7b; (c) (1) RNH_2, EDC, DMAP, HOBt, DMF, room temp, (2) BH_3, THF, reflux, (3) HCl, aqueous EtOH, reflux, 64% (**8a**), 62% (**8b**).

Scheme 6[a]



[a] Conditions: (a) (1) $MeOCH_2PPh_3Cl$, NaHMDS, THF, 0 °C, (2) HCl, aqueous acetone/THF/CH_2Cl_2, (3) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, aqueous *t*-BuOH, 83%; (b) (1) RNH_2, EDC, DMAP, CH_2Cl_2, room temp, (2) BH_3, THF, reflux, (3) HCl, aqueous EtOH, reflux, 72% (**9a**), 68% (**9b**).

presence of a catalytic amount of $HgCl_2$ in EtOH/THF formed the lactone intermediate **19**, which, without purification, was treated with $Me(MeO)NH_2Cl$ and Me_3Al in CH_2Cl_2/THF to give the crude Weinreb amide **21**. The hydroxyl of **21** was subsequently protected with a TBDPS group to give the pure **22** in 50% yield from **18**, after silica gel column chromatography. The desired *cis* unit **6** was readily obtained from **22** by DIBAL-H reduction. Employing this procedure, we obtained the unit **6** reproducibly. The enantiomeric *cis* unit *ent*-**6** was similarly prepared from *ent*-**18**.

As shown in Schemes 5 and 6, the "folded" conformationally restricted analogues **8a**, **8b**, **9a**, and **9b** with the (1*S*)-*cis*-cyclopropane structure and also their enantiomers *ent*-**8a**, *ent*-**8b**, *ent*-**9a**, and *ent*-**9b** were synthesized from the *cis* units **6** and *ent*-**6**, respectively, by a procedure similar to that for the synthesis of the corresponding "extended" analogues mentioned above.

These synthetic studies showed that the chiral cyclopropane units **6** and **7** and their enantiomers *ent*-**6** and *ent*-**7** are useful as versatile intermediates for synthesizing a series of compounds having an asymmetric *cis*- or *trans*-cyclopropane structure in the stereochemical diversity-oriented conformational restriction studies.

Pharmacological Effects. Binding affinities of the conformationally restricted analogues for the human H_3 receptor subtype using $[^3H]N^{\alpha}$-methylhistamine and also for the human H_4 receptor subtype using $[^3H]$histamine were investigated.

Binding affinities of the compounds for the H_3 subtype receptor are summarized in Table 1. Although the *cis* analogues **8b**, *ent*-**8a**, *ent*-**8b**, *ent*-**9b**, and the *trans* analogue **10a** did not show any significant binding to the receptor (K_i > 1000 nM), it was evident that the other 11 analogues inhibited the specific binding of $[^3H]N^{\alpha}$-methylhistamine to the H_3 receptor in a concentration-dependent manner, as shown in Figure 5a. In these compounds, the (1*S*)-*cis* analogues **9a** and **9b**, the (1*R*)-*cis* analogue *ent*-**9a**, the (1*R*)-*trans* analogues **10b**, **11a**, and **11b**, and the (1*S*)-*trans* analogues *ent*-**11a** and *ent*-**11b** had remarkable activity (K_i < 50 nM), which were more potent than the well-known H_3 receptor antagonist thioperamide (K_i = 51.1 nM). In order of the binding affinity, these compounds ranked as follows: *ent*-**11a** > *ent*-**11b** > **11a** > *ent*-**9a** > **9b** > **10b**, **11b** > **9a**. Among them, the three *trans* analogues **11a**, *ent*-**11a**, and *ent*-**11b** had a significant nanomolar K_i.

The binding affinity of the conformationally restricted analogues for the human H_4 receptor subtype is also summarized in Table 1. The three *cis* analogues **9a**, **9b**, and *ent*-**9a** and all of the eight *trans* analogues inhibited the specific binding of $[^3H]$histamine to the H_4 subtype receptor in a concentration-dependent manner (Figure 5b). The three *cis* analogues had moderate binding affinities for the receptor (100 nM < K_i < 200 nM). On the other hand, in the *trans* series, the (1*R*)-analogues **11a** and **11b** and the (1*S*)-analogue *ent*-**11a** showed remarkable binding affinity for the receptor, with **11a** being the most potent (K_i = 7.6 ± 0.4 nM).

The compounds, which showed binding affinity (K_i < 1000 nM) for the H_3 and/or H_4 receptors in the above-mentioned evaluations, were selected for the next evaluation using luciferase reporter gene assay.[4a] The human histamine receptor subtypes were individually expressed in 293-EBNA cells according to the previously reported method,[4a,7b] and the function of the compounds on these receptors expressed on the cells was investigated.

We first tested the function of these compounds on the H_3 and H_4 subtypes. None of the compounds showed any agonistic activity to either of the two subtypes at 10^{-5} M. On the other hand, these compounds functioned as antagonists to both the H_3 and H_4 subtypes; at 10^{-4} M, agonistic activity of histamine was completely or almost completely inhibited in all cases, as shown in Table 1 (inhibition, %).

The function of the three *trans* analogues **11a**, *ent*-**11a**, and *ent*-**11b**, which had a low nanomolar K_i for the H_3 and/or H_4 receptors on all four histamine receptor subtypes, was investigated in detail. As shown in Table 2, none of the three compounds functioned as agonists for the H_3 and H_4 subtypes or for the H_1 and H_2 subtypes. These three compounds were identified as H_3 and H_4 subtype selective antagonists, in accordance with the results of the above receptor binding experiments. Compounds **11a** and *ent*-**11a** also showed only a very weak antagonist activity to the H_1 subtype.

Discussion. All of the conformationally restricted analogues having a cyclopropane ring were efficiently synthesized from the chiral cyclopropane units **6** and **7** and their enantiomers *ent*-**6** and *ent*-**7**. Thus, these units are shown to be very useful synthetic intermediates for preparing various conformationally restricted chiral cyclopropane analogues of stereochemical diversity.

As described above, we have developed some highly potent H_3 and/or H_4 receptor antagonists based on the stereochemical diversity-based approach. These results are of vital importance with respect to conversion of agonists into antagonists, which is one of the major objectives in medicinal chemistry today.

As shown in Table 1, the pharmacological effect is well-related to the backbone structure of the compounds. For example, most of the *cis* analogues bind to the H_3 receptor more effectively than to the H_4 receptor. As for the carbon chain length (*n*) between the cyclopropane and the basic nitrogen atom,

Table 1. Effects of Compounds on the Human H_3 and H_4 Receptor Subtypes[a]

compd	configuration	*n*	H_3 K_i, nM	H_3 inhibition,[b] %	H_4 K_i, nM	H_4 inhibition,[b] %	selectivity index $K_i(H_3)/K_i(H_4)$
8a	(1*S*)-*cis*	1	88.0 ± 14.3	82	>10³	NT	<0.088
8b	(1*S*)-*cis*	1	>10³	NT	>10³	NT	
9a	(1*S*)-*cis*	2	43.7 ± 9.3	>100	130 ± 21	>100	0.34
9b	(1*S*)-*cis*	2	20.5 ± 1.4	87	177 ± 33	>100	0.12
ent-**8a**	(1*R*)-*cis*	1	>10³	NT	>10³	NT	
ent-**8b**	(1*R*)-*cis*	1	>10³	NT	>10³	NT	
ent-**9a**	(1*R*)-*cis*	2	13.9 ± 4.7	86	103 ± 2	86	0.14
ent-**9b**	(1*R*)-*cis*	2	>10³	NT	>10³	NT	
10a	(1*R*)-*trans*	1	>10³	NT	118 ± 27	100	>8.5
10b	(1*R*)-*trans*	1	34.4 ± 6.1	99	143 ± 48	97	0.24
11a	(1*R*)-*trans*	2	8.4 ± 1.5	100	7.6 ± 0.4	>100	1.1
11b	(1*R*)-*trans*	2	35.0 ± 13.8	99	31.2 ± 4.0	98	1.1
ent-**10a**	(1*S*)-*trans*	1	203 ± 40	96	115 ± 29	>100	1.8
ent-**10b**	(1*S*)-*trans*	1	289 ± 5.4	97	90.4 ± 13	83	3.2
ent-**11a**	(1*S*)-*trans*	2	3.6 ± 0.4	>100	37.2 ± 2.7	>100	0.097
ent-**11b**	(1*S*)-*trans*	2	5.3 ± 0.7	100	127 ± 21	>100	0.042
thioperamide			51.1 ± 3.8	98	124 ± 14	90	0.41

[a] Assay was carried out with cell membranes expressing human H_3 or H_4 receptor subtype ($n = 3$). [b] Inhibitory effect of compound (10^{-4} M) on the agonistic activity of histamine (10^{-6} M).

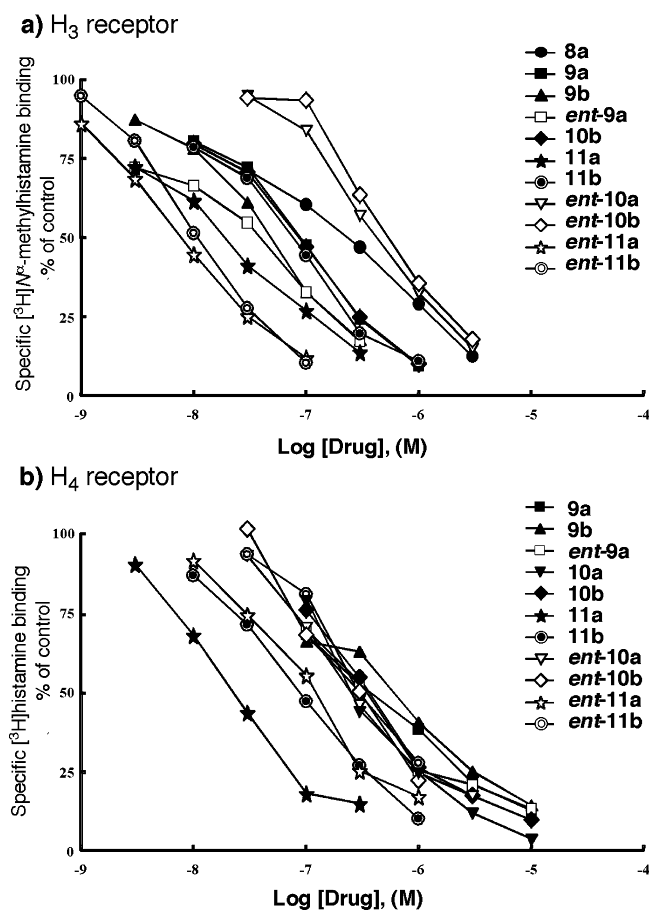


Figure 5. Effects of compounds on specific binding of [^{3}H]N^{α}-methylhistamine to the human H_3 receptor subtype (a) and of [^{3}H]-histamine to the human H_4 receptor subtype (b).

two-carbon-type compounds ($n = 2$) are generally more potent than the corresponding one-carbon-type compounds ($n = 1$) with the same configuration. When the compounds have the same backbone structure, the pharmacological property can be analogous; e.g., both **9a** and **9b** with the (1*S*)-*cis* structure ($n = 2$) show similar significant H_3 antagonistic activity (**9a**, $K_i = 43.7$ nM; **9b**, $K_i = 20.5$ nM) and moderate H_4 antagonistic activity (**9a**, $K_i = 130$ nM; **9b**, $K_i = 177$ nM), and *ent*-**8a** and *ent*-**8b** with the same (1*R*)-*cis* strucure ($n = 1$) are inactive to both the H_3 and H_4 receptors.

Among the compounds, **11a** with the (1*R*)-*trans* structure ($n = 2$) has remarkable antagonistic activity to both the H_3 ($K_i = 8.4$ nM) and H_4 ($K_i = 7.6$ nM) subtypes. While the indole derivative JNJ-7777120[6] (Figure 1) was most recently developed as a highly potent H_4 receptor antagonist based on the HTS approach, **11a** is the first highly potent H_4 receptor antagonist with low nanomolar K_i developed by this kind of rational design approach.

The enantiomeric analogues of **11a**, i.e., *ent*-**11a** and *ent*-**11b** with the (1*S*)-*trans* structure, are highly potent and selective H_3 receptor antagonists with K_i values of 3.6 nM (*ent*-**11a**) and 5.3 nM (*ent*-**11b**), respectively. Conversely, compound **10a** with the (1*R*)-*trans* structure ($n = 1$) is selectively active to the H_4 receptor ($K_i = 118$ nM) compared to the H_3 receptor ($K_i > 10^3$ nM). To our knowledge, the *trans* analogue **10a** is the first H_4 subtype selective antagonist designed rationally on the basis of the structure of the natural ligand histamine.

Although the corresponding enantiomer of **10a**, i.e., *ent*-**10a**, is also active to the H_4 receptor ($K_i = 115$ nM) as **10a**, it is not selective to the H_4 receptor, of which the K_i value for the H_3 receptor is 203 nM. It is worth noting that, as exemplified by **10a**/*ent*-**10a** and above-mentioned **11a**/*ent*-**11a**, most of the pairs of the enantiomers in this series were not in a usual relationship between a eutomer and a distomer, but each enantiomer had a characteristic pharmacological feature.

When the compounds have the same structure except for the hydrophobic moiety [4-chlorobenzyl (**a** series) or cyclohexylmethyl (**b** series) group], in some cases, such as **9a**/**9b** and *ent*-**10a**/*ent*-**10b**, they show analogous activities. However, in the other cases, the hydrophobic moiety clearly affected the pharmacological profiles of compounds. For example, *ent*-**9a** with a 4-chlorobenzyl group is a potent antagonist to both the H_3 and the H_4 receptors, while *ent*-**9b** with a cyclohexylmethyl group is inactive to both receptors; both *ent*-**11a** with a 4-chlorobenzyl group and *ent*-**11b** with a cyclohexylmethyl group are the H_3 receptor-selective antagonists with low nanomolar K_i, while *ent*-**11b** is more selective to the H_3 receptor than *ent*-**11a**.

In the previous study, we showed that conformational restriction by the *cis*-cyclopropane structure was much more effective than the *trans* for developing potent and selective H_3 receptor agonists. On the other hand, most of highly potent compounds identified as H_3 and/or H_4 receptor antagonists in this study have the *trans*-cyclopropane structure.

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

compd	agonist EC_{50}, nM				antagonist IC_{50},[b] nM			
	H_1	H_2	H_3	H_4	H_1	H_2	H_3	H_4
11a	$>10^4$	$>10^4$	$>10^4$	$>10^4$	6800 ± 500	$>10^4$	17 ± 3	110 ± 20
ent-**11a**	$>10^4$	$>10^4$	$>10^4$	$>10^4$	8500 ± 200	$>10^4$	8.5 ± 3	860 ± 50
ent-**11b**	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	37 ± 13	1500 ± 100
histamine	45 ± 2	650 ± 60	88 ± 6	1800 ± 700				
thioperamide							16 ± 1	680 ± 30

[a] Assay was carried out by the luciferase reporter gene method with the 293-EBNA cells expressing human H_1, H_2, H_3, or H_4 receptor subtype ($n = 3$). [b] 50% inhibitory concentration of compound on the agonistic activity of histamine at 10^{-6} M.

The eutomer of the cyclopropane-based H_3 receptor antagonist GT-2331 was recently identified as the (1*S*)-*trans* isomer (Figure 1), which is 10 times more active than the distomer with the (1*R*)-*trans* structure.[13b] However, this study demonstrated that the (1*R*)-*trans*-cyclopropane structure can also be an effective backbone to develop highly potent H_3-receptor antagonists such as **11a**.

As described, using this stereochemical diversity-oriented approach, we have developed a variety of compounds with different pharmacological profiles. Each of these compounds can be a lead for developing further active and/or selective H_3 or H_4 receptor antagonists. It should be noted that this approach can work without structural data of the target protein. These results clearly show that subtle changes in stereochemical restriction can affect pharmacological profiles of compounds and that a systematic study by the stereochemical diversity-oriented conformational restriction strategy allows exhaustive investigation of the bioactive conformation of compounds to develop the desired ligands for binding selectively to the target protein.

Conclusion

A series of conformationally restricted analogues of histamine with a chiral *cis*- or *trans*-cyclopropane structure were designed and synthesized on the basis of the stereochemical diversity-oriented strategy. Pharmacological profiles of these analogues were shown to be different depending on the structure of the cyclopropane backbones, as expected, and potent H_3 and/or H_4 receptor antagonists with a low nanomolar K_i were successfully identified. Particularly when the structure of the target protein is unknown, the stereochemical diversity-oriented approach can be a powerful strategy in medicinal chemical studies.

Experimental Section

Chemical shifts are reported in ppm downfield from tetramethylsilane. Thin-layer chromatography was done on Merck coated plate $60F_{254}$. Silica gel chromatography was done on silica gel 5715 (Merck) or NH silica gel (Chromatorex, Fuji Silysia Chemical). Reactions were carried out under an argon atmosphere.

(1*R*,2*R*)-*N*-(4-Chlorobenzyl)-1-(1-triphenylmethyl-1*H*-imidazol-4-yl)-2-cyclopropanecarboxamide (14a). A mixture of **13**[7b] (97 mg, 0.25 mmol), 4-chlorobenzylamine (93 μL, 0.75 mmol), EDC (144 mg, 0.75 mmol), and DMAP (12 mg, 0.10 mmol) in CH_2Cl_2/DMF (1:1, 1.0 mL) was stirred at room temperature overnight. The resulting mixture was partitioned between AcOEt and aqueous HCl (1 M), and the organic layer was washed with aqueous saturated $NaHCO_3$ and brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (66% AcOEt in hexane) to give **14a** (107 mg, 83%) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.31 (1 H, m, H-3a), 1.48 (1 H, m, H-3b), 1.87 (1 H, m, H-1), 2.39 (1 H, m, H-2), 4.40 (2 H, dd, 4-ClPhCH_2−, $J = 5.8, 5.8$ Hz), 6.23 (1 H, t, NH, $J = 5.8$ Hz), 6.66 (1 H, d, imidazole-CH, $J = 1.2$ Hz), 7.10−7.13 (6 H, m, aromatic), 7.19−7.23 (4 H, m, aromatic), 7.27 (1 H, s, imidazole-CH), 7.31−7.34 (9 H, m, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.3, 18.9, 24.4, 43.1, 75.2, 118.1, 127.9, 128.6, 129.0, 129.6, 132.9, 137.0, 138.2, 139.8, 142.2, 172.2; HRMS (FAB) calcd for $C_{33}H_{29}ClN_3O$ 518.1999, found 518.2017 ((M + H)$^+$).

(1*R*,2*R*)-*N*-Cyclohexylmethyl-1-(1-triphenylmethyl-1*H*-imidazol-4-yl)-2-cyclopropanecarboxamide (14b). Compound **14b** (110 mg, 90%, white solid) was prepared from **13** (99 mg, 0.25 mmol) as described for the preparation of **14a** using cyclohexylmethylamine instead of 4-cholorobenzylamine: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.91 (2 H, m, cyclohexyl), 1.11−1.25 (3 H, m, cyclohexyl), 1.26 (1 H, m, H-3a), 1.44 (2 H, m, H-3b and cyclohexyl), 1.63−1.72 (5 H, m, cyclohexyl), 1.83 (1 H, m, H-1), 2.35 (1 H, m, H-2), 3.06 (1 H, m, −NC*Ha*Hb−), 3.14 (1 H, m, −NCHa*Hb*−), 5.84 (1 H, t, NH, $J = 5.8$ Hz), 6.66 (1 H, d, imidazole-CH, $J = 1.2$ Hz), 7.11−7.14 (6 H, m, aromatic), 7.27 (1 H, s, imidazole-CH), 7.31−7.34 (9 H, m, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.9, 18.6, 24.3, 25.8, 26.4, 30.8, 38.0, 46.0, 75.2, 118.1, 128.0, 129.7, 130.0, 138.3, 140.2, 142.4, 172.3; HRMS (FAB) calcd for $C_{33}H_{36}N_3O$ 490.2858, found 490.2867 ((M + H)$^+$).

(1*S*,2*S*)-*N*-(4-Chlorobenzyl)-1-(1-triphenylmethyl-1*H*-imidazol-4-yl)-2-cyclopropanecarboxamide (*ent*-14a). Compound *ent*-**14a** (120 mg, 78%, white solid) was prepared from *ent*-**13**[7b] (125 mg, 0.316 mmol) as described for the preparation of **14a**: HRMS (FAB) calcd for $C_{33}H_{29}ClN_3O$ 518.1999, found 518.2003 ((M + H)$^+$).

(1*S*,2*S*)-*N*-Cyclohexylmethyl-1-(1-triphenylmethyl-1*H*-imidazol-4-yl)-2-cyclopropanecarboxamide (*ent*-14b). Compound *ent*-**14b** (98 mg, 77%, white solid) was prepared from *ent*-**13** (103 mg, 0.26 mmol) as described for the preparation of **14b**: HRMS (FAB) calcd for $C_{33}H_{36}N_3O$ 490.2858, found 490.2860 ((M + H)$^+$).

(1*R*,2*R*)-*trans*-2-(4-Chlorobenzylamino)methyl-1-(1*H*-imidazol-4-yl)cyclopropane Dihydrochloride (10a). To a solution of **14a** (102 mg, 0.197 mmol) in THF (1.4 mL) was added BH_3·THF complex (1.07 M in THF, 0.560 mL, 6.00 mmol) at 0 °C, and then the reaction mixture was heated under reflux for 12 h. After the mixture was cooled to room temperature, aqueous HCl (4 M) was added, and the resulting mixture was stirred at 65 °C for 3 h and then diluted with EtOH and MeOH. The solvent was removed, and the residue was coevaporated with EtOH and MeOH. The residue was partitioned between aqueous NaOH (2 M) and Et_2O/CH_2Cl_2, and the organic layer was washed with brine, dried (Na_2SO_4), and evaporated. A solution of the residue in EtOH (1.0 mL)/aqueous HCl (2 M, 1.0 mL) was stirred at 78 °C for 2 h, and then the solvent was evaporated. The residue was partitioned between aqueous NaOH (2 M) and Et_2O/CH_2Cl_2, and the organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by NH silica gel column chromatography (0−10% MeOH in $CHCl_3$) to give the free amine of **10a**. The amine was dissolved in aqueous HCl (4 M), and the solvent was then evaporated. The residue was triturated with Et_2O to give **10a** as dihydrochloride (62 mg, 94%, light-yellow solid): $[\alpha]^{19.7}_D$ −51.1 (*c* 1.01, MeOH); ^{1}H NMR (270 MHz, CD_3OD) δ 1.26 (2 H, m, H-3a and H-3b), 1.65 (1 H, m, H-2), 2.21 (1 H, m, H-1), 3.19 (2 H, d, −CH_2NH−, $J = 7.3$ Hz), 4.27 (2 H, s, 4-ClPhCH_2−), 7.37 (1 H, s, imidazole-CH), 7.47 (2 H, d, aromatic, $J = 8.4$ Hz), 7.60 (2 H, d, aromatic, $J = 8.4$ Hz), 8.79 (1 H, d, imidazole-CH, $J = 1.3$ Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 13.4, 13.5, 18.1, 51.4, 51.8, 116.4, 130.2, 131.2, 132.9, 134.6, 135.6, 136.6; HRMS (EI) calcd for $C_{14}H_{16}ClN_3$ 261.1033, found 261.1035 (M$^+$). Anal. ($C_{14}H_{18}Cl_3N_3$·0.5H_2O) C, H, N.

(1*R*,2*R*)-*trans*-2-(Cyclohexylmethylamino)methyl-1-(1*H*-imidazol-4yl)cyclopropane Dihydrochloride (10b). Compound **10b** (53 mg, 89%, light brown solid) was prepared from **14b** (95 mg, 0.19 mmol) as described for the preparation of **10a**: $[\alpha]^{20.5}_D$ −53.0 (*c* 1.02, MeOH); ^1H NMR (270 MHz, CD_3OD) δ 1.07 (2 H, m, H-3a and H-3b), 1.20−1.40 (5 H, m, cyclohexyl), 1.63 (1 H, m, H-2), 1.70−1.89 (6 H, m, cyclohexyl), 2.18 (1 H, m, H-1), 2.92 (2 H, d, $-NCH_2C_6H_{11}$, *J* = 7.0 Hz), 3.01 (2 H, dd, −C*Ha*HbN−, *J* = 13.4, 7.3 Hz), 3.17 (2 H, dd, −CHa*Hb*N−, *J* = 12.9, 7.3 Hz), 7.36 (1 H, s, imidazole-CH), 8.79 (1 H, d, imidazole-CH, *J* = 1.2 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 13.3, 13.6, 18.1, 26.6, 27.1, 31.6, 36.4, 52.5, 55.0, 116.5, 134.6, 135.4; HRMS (EI) calcd for $C_{14}H_{23}N_3$ 233.1892, found 233.1892 (M^+). Anal. ($C_{14}H_{23}N_3$·0.1H_2O) C, H, N.

(1*S*,2*S*)-*trans*-2-(4-Chlorobenzylamino)methyl-1-(1*H*-imidazol-4-yl)cyclopropane Dihydrochloride (*ent*-10a). Compound *ent*-**10a** (55 mg, 78%, white solid) was prepared from *ent*-**14a** (109 mg, 0.210 mmol) as described for the preparation of **10a**: $[\alpha]^{19.3}_D$ +52.2 (*c* 0.82, MeOH); HRMS (EI) calcd for $C_{14}H_{16}ClN_3$ 261.1033, found 261.1034 (M^+). Anal. ($C_{14}H_{18}Cl_3N_3$) C, H, N.

(1*S*,2*S*)-*trans*-2-(Cyclohexylmethylamino)methyl-1-(1*H*-imidazol-4-yl)cyclopropane Dihydrochloride (*ent*-10b). Compound *ent*-**10b** (45 mg, 81%, white solid) was prepared from *ent*-**14b** (90 mg, 0.18 mmol) as described for the preparation of **10a**: $[\alpha]^{19.8}_D$ +57.7 (*c* 0.41, MeOH); HRMS (EI) calcd for $C_{14}H_{23}N_3$ 233.1892, found 233.1891 (M^+). Anal. ($C_{14}H_{25}Cl_2N_3$·0.5H_2O) C, H, N.

(1*R*,2*S*)-2-Formylmethyl-1-(1-triphenylmethyl-1*H*-imidazol-4-yl)cyclopropane (15). To a suspension of $MeOCH_2PPh_3Cl$ (1.10 g, 3.20 mmol) in THF (15 mL) was added NaHMDS (1.0 M in THF, 2.80 mL, 2.80 mmol) at 0 °C, and the mixture was stirred at the same temperature for 15 min. To the resulting solution was added a solution of **12**[7b] (530 mg, 1.40 mmol) in THF (5 mL) at 0 °C, and the reaction mixture was stirred at the same temperature for 3 h. After addition of aqueous saturated NH_4Cl, the solvent was evaporated, and the residue was partitioned between AcOEt and aqueous NH_4Cl. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (25−30% AcOEt in hexane) to give the enol ether product as a light-yellow solid. To a solution of the product in acetone (10 mL) was added aqueous HCl (12 M, 5 mL), and the mixture was vigorously stirred at room temperature for 5 s. Immediately, the mixture was poured into aqueous saturated $NaHCO_3$ (100 mL), and the resulting solution was extracted with AcOEt. The organic layer was washed with aqueous saturated $NaHCO_3$ and brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (50% AcOEt in hexane) to give **15** (492 mg, 90%) as a white solid: $[\alpha]^{26}_D$ −47.6 (*c* 1.58, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 0.74 (1 H, m, H-3a), 1.09 (1 H, m, H-3b), 1.41 (1 H, m, H-2), 1.68 (1 H, m, H-1), 2.28 (1 H, m, −C*Ha*HbCHO), 2.56 (1 H, m, −CHa*Hb*CHO), 6.56 (1 H, s, imidazole-CH), 7.12−7.14 (6 H, m, aromatic), 7.29 (1 H, s, imidazole-CH), 7.31−7.33 (9 H, m, aromatic), 9.81 (1 H, dd, −CHO, *J* = 2.1, 2.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.6, 14.3, 16.2, 47.8, 75.1, 116.8, 127.9, 127.9, 129.7, 138.3, 141.8, 142.4, 202.9; LRMS (FAB) *m*/*z* 393 ($(M + H)^+$); HRMS (FAB) calcd for $C_{27}H_{25}N_2O$ 393.1967, found 393.1946 ($(M + H)^+$).

(1*S*,2*R*)-2-Formylmethyl-1-(1-triphenylmethyl-1*H*-imidazol-4-yl)cyclopropane (*ent*-15). Compound *ent*-**15** (301 mg, 72%, white solid) was prepared from *ent*-**12** (404 mg, 1.07 mmol) as described for the preparation of **15**: $[\alpha]^{25}_D$ +47.3 (*c* 1.12, $CHCl_3$); LRMS (EI) *m*/*z* 392 (M^+); HRMS (EI) calcd for $C_{27}H_{24}N_2O$ 392.1889, found 392.1891 (M^+).

(1*R*,2*S*)-1-(1-Triphenylmethyl-1*H*-imidazol-4-yl)-2-cyclopropaneacetic Acid (16). To a solution of **15** (255 mg, 0.650 mmol) in *t*-BuOH (4.8 mL) were added H_2O (1.3 mL), NaH_2PO_4·2H_2O (101 mg, 0.650 mmol), 2-methyl-2-butene (309 μL, 2.90 mmol), and $NaClO_2$ (80%, 257 mg, 2.30 mmol), and the resulting mixture was stirred at room temperature for 2 h. The reaction mixture was acidified with aqueous HCl (1 M), where the pH of the resulting solution was about 2. The solution was extracted with $CHCl_3$, and the organic layer was washed with H_2O and brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (0−6% MeOH in $CHCl_3$) to give **16** (214 mg, 81%) as a white solid: $[\alpha]^{20.9}_D$ −26.5 (*c* 1.02, $CHCl_3$/MeOH = 9/1); ^1H NMR (270 MHz, $CDCl_3$) δ 0.75 (1 H, m, H-3a), 0.98 (1 H, m, H-3b), 1.48 (1 H, m, H-2), 1.80 (1 H, m, H-1), 2.19−2.41 (2 H, m, $-CH_2COOH$), 6.44 (1 H, s, imidazole-CH), 7.09−7.12 (6 H, m, aromatic), 7.31−7.34 (9 H, m, aromatic), 7.46 (1 H, s, imidazole-CH); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$ = 3/2) δ 13.4, 15.8, 16.6, 38.3, 75.2, 116.7, 127.7, 127.7, 129.3, 137.5, 141.5, 141.8, 175.1; HRMS (FAB) calcd for $C_{27}H_{25}N_2O_2$ 409.1916, found 409.1917 ($(M + H)^+$).

(1*S*,2*R*)-1-(1-Triphenylmethyl-1*H*-imidazol-4-yl)-2-cyclopropaneacetic Acid (*ent*-16). Compound *ent*-**16** (258 mg, 98%, white solid) was prepared from *ent*-**15** (254 mg, 0.647 mmol) as described for the preparation of **16**, using THF/*t*-BuOH (3:2) instead of *t*-BuOH as the reaction solvent: $[\alpha]^{20.9}_D$ +27.8 (*c* 0.97, $CHCl_3$/MeOH = 9/1); HRMS (FAB) calcd for $C_{27}H_{25}N_2O_2$ 409.1916, found 409.1920 ($(M + H)^+$).

(1*R*,2*S*)-*N*-(4-Chlorobenzyl)-1-(1-triphenylmethyl-1*H*-imidazol-4-yl)-2-cyclopropanacetamide (17a). Compound **17a** (86 mg, 67%, white solid) was prepared from **16** (100 mg, 0.24 mmol) as described for the preparation of **14a** using CH_2Cl_2 as the reaction solvent: ^1H NMR (400 MHz, $CDCl_3$) δ 0.74 (1 H, m, H-3a), 1.03 (1 H, m, H-3b), 1.20 (1 H, m, H-2), 1.66 (1 H, m, H-1), 2.20 (1 H, dd, −C*Ha*HbCO−, *J* = 17.2, 8.6 Hz), 2.53 (1 H, dd, −CHa*H*-*b*CO−, *J* = 17.2, 6.6 Hz), 4.44 (2 H, d, 4-ClPhCH_2−, *J* = 5.9 Hz), 6.54 (1 H, s, imidazole-CH), 7.08−7.11 (6 H, m, aromatic), 7.22 (5 H, m, imidazole-CH and aromatic), 7.30−7.34 (9 H, m, aromatic); LRMS (FAB) *m*/*z* 532 ($(M + H)^+$); HRMS (FAB) calcd for $C_{34}H_{31}ClN_3O$ 532.2156, found 532.2139 ($(M + H)^+$).

(1*R*,2*S*)-*N*-(Cyclohexylmethyl)-1-(1-triphenylmethyl-1*H*-imidazol-4-yl)-2-cyclopropaneacetamide (17b). Compound **17b** (99 mg, 80%, white solid) was prepared from **16** (102 mg, 0.25 mmol) as described for the preparation of **17a** using cyclohexylmethylamine instead of 4-chlorobenzylamine: ^1H NMR (270 MHz, $CDCl_3$) δ 0.75 (1 H, m, H-3a), 0.91 (2 H, m, cyclohexyl), 1.06 (1 H, m, H-3b), 1.11−1.29 (4 H, m, cyclohexyl), 1.48 (1 H, m, H-2), 1.63−1.72 (6 H, m, H-1 and cyclohexyl), 2.22 (1 H, dd, −C*Ha*H-bCO−, *J* = 17.2, 8.6 Hz), 2.42 (1 H, dd, −CHa*Hb*CO−, *J* = 17.2, 6.6 Hz), 3.12 (2 H, t, $-NCH_2-$), 6.58 (1 H, d, imidazole-CH, *J* = 1.3 Hz), 6.67 (1 H, br s, NH), 7.10−7.14 (6 H, m, aromatic), 7.29 (1 H, d, imidazole-CH, *J* = 1.3 Hz), 7.32−7.35 (9 H, m, aromatic); HRMS (FAB) calcd for $C_{34}H_{38}N_3O$ 504.3015, found 504.2999 ($(M + H)^+$).

(1*S*,2*R*)-*N*-(4-Chlorobenzyl)-1-(1-triphenylmethyl-1*H*-imidazol-4-yl)-2-cyclopropaneacetamide (*ent*-17a). Compound *ent*-**17a** (100 mg, 78%, white solid) was prepared from *ent*-**16** (98 mg, 0.24 mmol) as described for the preparation of **17a**: HRMS (FAB) calcd for $C_{34}H_{31}ClN_3O$ 532.2156, found 532.2148 ($(M + H)^+$).

(1*S*,2*R*)-*N*-(Cyclohexylmethyl)-1-(1-triphenylmethyl-1*H*-imidazol-4-yl)-2-cyclopropaneacetamide (*ent*-17b). Compound *ent*-**17b** (96 mg, 80%, white solid) was prepared from *ent*-**16** (98 mg, 0.24 mmol) as described for the preparation of **17b**: HRMS (FAB) calcd for $C_{34}H_{38}N_3O$ 504.3015, found 504.3017 ($(M + H)^+$).

(1*R*,2*S*)-*trans*-2-[2-(4-Chlorobenzylamino)ethyl]-1-(1*H*-imidazol-4-yl)cyclopropane Dihydrochloride (11a). Compound **11a** (50 mg, 93%, white solid) was prepared from **17a** (83 mg, 0.16 mmol) as described for the preparation of **10a**: $[\alpha]^{19.6}_D$ −56.5 (*c* 1.03, MeOH); ^1H NMR (270 MHz, CD_3OD) δ 0.99−1.14 (2 H, m, H-3a and H-3b), 1.25−1.34 (1 H, m, H-2), 1.74−1.95 (3 H, m, H-1 and $-CH_2CH_2N-$), 3.24 (2 H, m, $-CH_2CH_2N-$), 4.25 (2 H, s, 4-ClPhCH_2−), 7.29 (1 H, s, imidazole-CH), 7.45−7.49 (2 H, m, aromatic), 7.55−7.59 (2 H, m, aromatic), 8.76 (1 H, d, imidazole-CH, *J* = 1.4 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 13.3, 14.3, 19.6, 30.9, 48.1, 51.6, 115.7, 130.2, 131.2, 132.7, 132.8, 134.3, 136.6; HRMS (EI) calcd for $C_{15}H_{18}ClN_3$ 275.1189, found 275.1187 (M^+). Anal. ($C_{15}H_{20}Cl_3N_3$) C, H, N.

(1*R*,2*S*)-*trans*-2-[2-(Cyclohexylmethylamino)ethyl]-1-(1*H*-imidazol-4-yl)cyclopropane Dihydrochloride (11b). Compound **11b** (44 mg, 93%, white solid) was prepared from **17b** (76 mg, 0.15 mmol) as described for the preparation of **10a**: $[\alpha]^{19.7}_D$ −57.2 (*c*

0.97, MeOH); ^{1}H NMR (270 MHz, CD_3OD) δ 1.00−1.12 (4 H, m), 1.22−1.38 (4 H, m), 1.70−1.93 (9 H, m), 2.88 (2 H, d, J = 7.0 Hz), 3.13−3.18 (2 H, m), 7.28 (1 H, s, imidazole-CH), 8.74 (1 H, d, imidazole-CH, J = 1.2 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 13.2, 14.2, 19.7, 26.6, 27.0, 30.7, 31.6, 36.4, 55.1, 115.8, 134.4, 136.9; HRMS (EI) calcd for $C_{15}H_{25}N_3$ 247.2048, found 247.2047 (M^+). Anal. ($C_{15}H_{27}Cl_2N_3$·$0.5H_2O$) C, H, N.

(1*S*,2*R*)-*trans*-2-[2-(4-Chlorobenzylamino)ethyl]-1-(1*H*-imidazol-4-yl)cyclopropane Dihydrochloride (*ent*-11a). Compound *ent*-**11a** (50 mg, 75%, white solid) was prepared from *ent*-**17a** (99 mg, 0.19 mmol) as described for the preparation of **10a**: $[\alpha]^{19.8}_D$ +55.6 (*c* 0.96, MeOH); HRMS (EI) calcd for $C_{15}H_{18}ClN_3$ 275.1189, found 275.1191 (M^+). Anal. ($C_{15}H_{20}Cl_3N_3$) C, H, N.

(1*S*,2*R*)-*trans*-2-[2-(Cyclohexylmethylamino)ethyl]-1-(1*H*-imidazol-4-yl)cyclopropane Dihydrochloride (*ent*-11b). Compound *ent*-**11b** (46 mg, 0.14 mmol, 80%, white solid) was prepared from *ent*-**17b** (90 mg, 0.18 mmol) as described for the preparation of **10a**: $[\alpha]^{20.1}_D$ +58.5 (*c* 0.99, MeOH); HRMS (EI) calcd for $C_{15}H_{25}N_3$ 247.2048, found 247.2049 (M^+). Anal. ($C_{15}H_{27}Cl_2N_3$·$0.5H_2O$) C, H, N.

(1*S*,2*R*)-*cis*-2-(*tert*-Butyldiphenylsilyloxymethyl)-*N*-methoxy-*N*-methyl-1-cyclopropanecarboxamide (22). To a solution of **18**[7a] (477 mg, 2.00 mmol) in EtOH/THF (3:1, 10 mL) was added Mg powder (146 mg, 6.00 mmol) and $HgCl_2$ (10 mg), and the reaction mixture was stirred at room temperature for 5 h. The reaction mixture was poured into aqueous HCl (4 M), and the resulting mixture was extracted with CH_2Cl_2. The organic layer was washed with aqueous saturated $NaHCO_3$ and brine and dried (Na_2SO_4). The resulting solution was distilled at atmospheric pressure to give the crude desulfonylated product as the residue. To a suspension of *N*,*O*-dimethylhydroxylamine hydrochloride (585 mg, 6.00 mmol) in dry THF (3.0 mL) was added $AlMe_3$ (15% in hexane, 2.88 mL, 6.00 mmol) at 0 °C, and the mixture was stirred at room temperature for 30 min. To this reaction mixture was added a solution of the above residue in CH_2Cl_2 (3.0 mL) at room temperature, and the reaction mixture was stirred at the same temperature for 5 h. To the resulting mixture was added slowly a solution of TBDPSCl (1.56 mL, 6.00 mmmol) and imidazole (816 mg, 12.0 mmol) in DMF (4.0 mL), and the mixture was stirred at room temperature for 30 h. After addition of MeOH, the solvent was evaporated, and the residue was partitioned between AcOEt and HCl (1 M). The organic layer was washed with aqueous saturated $NaHCO_3$ and brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (5−15% AcOEt in hexane) to give **22** (395 mg, 50%) as a colorless amorphous solid: $[\alpha]^{21.8}_D$ −22.0 (*c* 0.95, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.87−0.95 (1 H, m, H-3a), 1.02 (9 H, s, $-C(CH_3)_3$), 1.02−1.11 (1 H, m, H-3b), 1.54−1.68 (1 H, m, H-2), 2.33 (1 H, m, H-1), 3.23 (3 H, s, $-NCH_3$), 3.69 (1 H, dd, −C*Ha*HbOTBDPS, J = 10.6, 9.2 Hz), 3.78 (3 H, s, $-OCH_3$), 3.92 (1 H, dd, −CHa*Hb*OTBDPS, J = 10.6, 5.3 Hz), 7.33−7.43 (6 H, m, aromatic), 7.63−7.70 (4 H, m, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.6, 15.3, 19.2, 23.5, 26.9, 32.7, 61.6, 62.3, 127.5, 129.4, 133.8, 134.0, 135.4, 173.4; LRMS (EI) *m/z* 397 (M^+); HRMS (EI) calcd for $C_{23}H_{31}NO_3Si$ 397.2073, found 397.2074 (M^+).

(1*R*,2*S*)-*cis*-2-(*tert*-Butyldiphenylsilyloxymethyl)-*N*-methoxy-*N*-methyl-1-cyclopropanecarboxamide (*ent*-22). Compound *ent*-**22** (356 mg, 45%, colorless amorphous solid) was prepared from *ent*-**18** (477 mg, 2.00 mmol) as described for the preparation of **22**: $[\alpha]^{21.5}_D$ +23.4 (*c* 1.00, $CHCl_3$); LRMS (EI) *m/z* 397 (M^+); HRMS (EI) calcd for $C_{23}H_{31}NO_3Si$ 397.2073, found 397.2074 (M^+).

(1*S*,2*R*)-2-(*tert*-Butyldiphenylsilyloxy)methyl-1-formylcyclopropane (6). To a solution of **22** (398 mg, 1.00 mmol) in CH_2Cl_2 (8.0 mL) was added DIBAL-H (0.95 M in hexane, 1.10 mL, 1.05 mmol) at −78 °C, and the mixture was stirred at the same temperature for 1 h. After addition of MeOH and then brine, the mixture was stirred at room temperature for 1 h and filtered with Celite. The filtrate was evaporated, and the residue was purified by silica gel column chromatography (10% AcOEt in hexane) to give **6** (330 mg, 98%) as a white solid, the ^{1}H NMR data of which were in accord with those reported previously.[7a]

(1*R*,2*S*)-2-(*tert*-Butyldiphenylsilyloxy)methyl-1-formylcyclopropane (*ent*-6). Compound *ent*-**6** (757 mg, 95%, white solid) was prepared from *ent*-**22** (937 mg, 2.36 mmol) as described for the preparation of **6**, the ^{1}H NMR data of which were in accord with those reported previously.[7a]

(1*S*,2*R*)-*cis*-2-(4-Chlorobenzylamino)methyl-1-(1*H*-imidazol-4-yl)cyclopropane Dihydrochloride (8a). A mixture of **24**[7b] (130 mg, 0.330 mmol), 4-chlorobenzylamine (124 μL, 1.00 mmol), EDC (192 mg, 1.00 mmol), HOBt (135 mg, 1.00 mmol), and DMAP (12 mg, 0.10 mmol) in DMF (2 mL) was stirred at room temperature overnight. The resulting mixture was partitioned between AcOEt and aqueous HCl (1 M), and the organic layer was washed with aqueous saturated $NaHCO_3$ and brine, dried (Na_2SO_4), and evaporated. To a solution of the residue in dry THF was added BH_3·THF complex (1.07 M in THF, 0.93 mL, 1.0 mmol) at 0 °C, and then the reaction mixture was heated under reflux for 12 h. After the mixture was cooled to room temperature, aqueous HCl (4 M) was added, and the resulting mixture was stirred at 65 °C for 3 h and then diluted with EtOH and MeOH. The solvent was then evaporated, and the residue was coevaporated with EtOH and MeOH (five times). The residue was partitioned between aqueous NaOH (2 M) and Et_2O, and the organic layer was washed with brine, dried (Na_2SO_4), and evaporated. A solution of the residue in EtOH (1 mL)/aqueous HCl (2 M, 1 mL) was stirred at 78 °C for 2 h, and then the mixture was concentrated in vacuo to remove EtOH. The resulting mixture was partitioned between aqueous NaOH (2 M) and Et_2O, and the organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by NH silica gel column chromatography (0−10% MeOH in $CHCl_3$) to give the **8a** as a free amine. The amine was dissolved in aqueous HCl (4 M), and the solvent was then evaporated. The residue was triturated with Et_2O to give **8a** as dihydrochloride (55 mg, 64%, white solid): $[\alpha]^{19.7}_D$ −54.0 (*c* 1.02, MeOH); ^{1}H NMR (270 MHz, CD_3OD) δ 1.20 (1 H, m, H-3a), 1.47 (1 H, m, H-3b), 1.70 (1 H, m, H-2), 2.36 (1 H, m, H-1), 2.55 (1 H, dd, −C*Ha*HbN−, J = 13.0, 9.9 Hz), 3.27 (1 H, dd, −CHa*Hb*N−, J = 13.0, 5.1 Hz), 4.19 (2 H, s, 4-ClPhCH_2−), 7.42−7.54 (5 H, m, imidazole-CH and aromatic), 8.84 (1 H, d, imidazole-CH, J = 1.3 Hz); ^{13}C NMR (67.5 MHz, CD_3OD) δ 11.5, 12.1, 15.9, 51.7, 118.8, 130.6, 131.6, 132.6, 133.1, 135 7, 137.1; HRMS (EI) calcd for $C_{14}H_{16}ClN_3$ 261.1033, found 261.1032 (M^+). Anal. ($C_{14}H_{18}Cl_3N_3$) C, H, N.

(1*S*,2*R*)-*cis*-2-(Cyclohexylmethylamino)methyl-1-(1*H*-imidazol-4-yl)cyclopropane Dihydrochloride (8b). Compound **8b** (62 mg, 62%, white solid) was prepared from **24** (130 mg, 0.330 mmol) as described for the preparation of **8a** using cyclohexylmethylamine instead of 4-chlorobenzylamine: $[\alpha]^{19.8}_D$ −47.1 (*c* 0.96, MeOH); ^{1}H NMR (270 MHz, CD_3OD) δ 0.96−1.04 (2 H, m), 1.17−1.39 (4 H, m), 1.47 (1 H, m), 1.64−1.85 (7 H, m), 2.35 (1 H, m, H-1), 2.45 (1 H, dd, −C*Ha*HbN−, J = 13.1, 9.9 Hz), 2.83 (2 H, d, $-NCH_2C_6H_{11}$, J = 6.7 Hz), 3.23 (1 H, dd, −CHa*Hb*N−, J = 13.1, 4.8 Hz), 7.44 (1 H, s, imidazole-CH), 8.86 (1 H, d, imidazole-CH, J = 1.2 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 11.2, 11.7, 15.6, 26.6, 27.0, 31.5, 36.4, 54.8, 118.4, 132.2, 135.3; HRMS (EI) calcd for $C_{14}H_{23}N_3$ 233.1892, found 233.1891 (M^+). Anal. ($C_{14}H_{25}Cl_2N_3$) C, H, N.

(1*R*,2*S*)-*cis*-2-(4-Chlorobenzylamino)methyl-1-(1*H*-imidazol-4-yl)cyclopropane Dihydrochloride (*ent*-8a). Compound *ent*-**8a** (45 mg, 59%, white solid) was prepared from *ent*-**24** (100 mg, 0.23 mmol) as described for the preparation of **8a**: $[\alpha]^{20.2}_D$ +55.2 (*c* 1.00, MeOH); HRMS (EI) calcd for $C_{14}H_{16}ClN_3$ 261.1033, found 261.1030 (M^+). Anal. ($C_{14}H_{18}Cl_3N_3$) C, H, N.

(1*R*,2*S*)-*cis*-2-(Cyclohexylmethylamino)methyl-1-(1*H*-imidazol-4-yl)cyclopropane Dihydrochloride (*ent*-8b). Compound *ent*-**8b** (52 mg, 53%, white solid) was prepared from *ent*-**24** (125 mg, 0.33 mmol) as described for the preparation of **8b**: $[\alpha]^{20.2}_D$ +47.7 (*c* 0.99, MeOH); HRMS (EI) calcd for $C_{14}H_{23}N_3$ 233.1892, found 233.1892 (M^+). Anal. ($C_{14}H_{25}Cl_2N_3$·$0.5H_2O$) C, H, N.

(1*S*,2*S*)-1-(1-Triphenylmethyl-1*H*-imidazol-4-yl)-2-cyclopropaneacetic Acid (25). To a suspension of $MeOCH_2PPh_3Cl$ (1.10 g, 3.20 mmol) in THF (9 mL) was added NaHMDS (1.0 M in THF, 3.00 mL, 3.00 mmol) at 0 °C, and the mixture was stirred at the

same temperature for 15 min. To the resulting solution was added a solution of **23**[7a] (398 mg, 1.05 mmol) in THF (3 mL) at 0 °C, and the reaction mixture was stirred at the same temperature for 3 h. After addition of aqueous saturated NH_4Cl, the solvent was evaporated, and the residue was partitioned between AcOEt and aqueous NH_4Cl. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (25−30% AcOEt in hexane) to give the enol ether compound (418 mg) as a light-yellow solid. To a solution of the product in acetone/THF/CH_2Cl_2 (2:1:1, 20 mL) was added aqueous HCl (12 M, 10 mL), and the mixture was vigorously stirred at room temperature for 5 s. Immediately, the mixture was poured into aqueous saturated $NaHCO_3$ (150 mL), and the resulting solution was extracted with AcOEt. The organic layer was washed with aqueous saturated $NaHCO_3$ and brine, dried (Na_2SO_4), and evaporated. To a solution of the residue in *t*-BuOH (7.8 mL) were added H_2O (2.2 mL), $NaH_2PO_4 \cdot 2H_2O$ (156 mg, 1.00 mmol), 2-methyl-2-butene (477 μL, 4.50 mmol), and $NaClO_2$ (80%, 396 mg, 3.50 mmol), and the resulting mixture was stirred at room temperature for 30 min. The reaction mixture was cooled to 0 °C and acidified with aqueous HCl (1 M), where the pH of the resulting solution was about 2. The solution was extracted with $CHCl_3$, and the organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (0−5% MeOH in $CHCl_3$) to give **25** (354 mg, 83%) as a white solid: $[\alpha]^{21.1}_{D}$ +63.0 (*c* 1.03, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 0.52 (1 H, m, H-3a), 1.13 (1 H, m, H-3b), 1.49 (1 H, m, H-2), 1.94 (1 H, dd, −C*Ha*HbCOOH, J = 11.2, 3.3 Hz), 2.06 (2 H, m, H-1), 2.70 (1H, dd, −CHa*Hb*COOH, J = 13.9, 11.2 Hz), 6.70 (1 H, s, imidazole-CH), 7.09−7.12 (6 H, m, aromatic), 7.36−7.39 (9 H, m, aromatic), 7.72 (1 H, s, imidazole-CH), 8.99 (1 H, br s, −COOH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.6, 12.2, 14.7, 35.9, 77.2, 121.1, 128.4, 128.6, 129.5, 136.4, 136.7, 140.8; LRMS (FAB) *m/z* 409 ((M + H)$^+$); HRMS (FAB) calcd for $C_{27}H_{25}N_2O_2$ 409.1916, found 409.1906 ((M + H)$^+$).

(1*R*,2*R*)-1-(1-Triphenylmethyl-1*H*-imidazol-4-yl)-2-cyclopropaneacetic Acid (*ent*-25). Compound *ent*-**25** (256 mg, 78%, white solid) was prepared from *ent*-**23** (305 mg, 0.806 mmol) as described for the preparation of **25**: $[\alpha]^{21.0}_{D}$ −61.9 (*c* 0.99, $CHCl_3$); LRMS (EI) *m/z* 408 (M^+); HRMS (EI) calcd for $C_{27}H_{24}N_2O_2$ 408.1838, found 408.1840 (M^+).

(1*S*,2*S*)-*cis*-2-[2-(4-Chlorobenzylamino)ethyl]-1-(1*H*-imidazol-4-yl)cyclopropane Dihydrochloride (9a). Compound **9a** (44 mg, 72%, white solid) was prepared from **25** (72 mg, 0.18 mmol) as described for the preparation of **8a** with a minor modification. In this case, the condensation with 4-chlorobenzylamine was carried out without using HOBt in CH_2Cl_2 as the reaction solvent: $[\alpha]^{20.3}_{D}$ −26.5 (*c* 0.98, MeOH); ^1H NMR (270 MHz, CD_3OD) δ 0.87 (1 H, m, H-3a), 1.26−1.39 (2 H, m, H-2 and H-3b), 1.71 (1 H, m, −C*H₂*CH₂N−), 2.16 (1 H, m, H-1), 3.09 (2 H, m, −CH₂C*H₂*N−), 4.18 (2 H, s, 4-ClPhC*H₂*−), 7.35 (1 H, s, imidazole-CH), 7.44−7.52 (4 H, m, aromatic), 8.81 (1 H, d, imidazole-CH, J = 1.5 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 11.0, 11.5, 17.0, 26.5, 48.1, 51.6, 118.0, 130.2, 131.2, 132.7, 133.4, 134.8, 134.9, 136.6; HRMS (EI) calcd for $C_{15}H_{18}ClN_3$ 275.1189, found 275.1187 (M^+). Anal. ($C_{15}H_{20}Cl_3N_3$) C, H, N.

(1*S*,2*S*)-*cis*-2-[2-(Cyclohexylmethylamino)ethyl]-1-(1*H*-imidazol-4-yl)cyclopropane Dihydrochloride (9b). Compound **9b** (46 mg, 68%, light-yellow solid) was prepared from **25** (87 mg, 0.21 mmol) as described for the preparation of **9a** using cyclohexylmethylamine instead of 4-chlorobenzylamine: $[\alpha]^{19.7}_{D}$ −26.0 (*c* 1.00, MeOH); ^1H NMR (270 MHz, CD_3OD) δ 0.89 (1 H, m), 0.99−1.06 (2 H, m), 1.21−1.39 (6 H, m), 1.68−1.81 (7 H, m), 2.16 (1 H, m, H-1), 2.82 (2 H, d, −NC*H₂*C₆H₁₁, J = 6.7 Hz), 3.03 (2 H, m, −CH₂C*H₂*N−), 7.37 (1 H, s, imidazole-CH), 8.83 (1 H, s, imidazole-CH); ^{13}C NMR (100 MHz, CD_3OD) δ 10.9, 11.5, 17.0, 26.4, 26.6, 27.0, 31.5, 36.4, 55.0, 118.0, 133.5, 134.9; HRMS (EI) calcd for $C_{15}H_{25}N_3$ 247.2048, found 247.2050 (M^+). Anal. ($C_{15}H_{27}Cl_2N_3$) C, H, N.

(1*R*,2*R*)-*cis*-2-[2-(4-Chlorobenzylamino)ethyl]-1-(1*H*-imidazol-4-yl)cyclopropane Dihydrochloride (*ent*-9a). Compound *ent*-**9a** (41 mg, 48%, light-brown solid) was prepared from *ent*-**25** (100 mg, 0.245 mmol) as described for the preparation of **9a**: $[\alpha]^{20.4}_{D}$ +27.1 (*c* 1.01, MeOH); HRMS (EI) calcd for $C_{15}H_{18}ClN_3$ 275.1189, found 275.1189 (M^+). Anal. ($C_{15}H_{20}Cl_3N_3$) C, H, N.

(1*R*,2*R*)-*cis*-2-[2-(Cyclohexylmethylamino)ethyl]-1-(1*H*-imidazol-4-yl)cyclopropane Dihydrochloride (*ent*-9b). Compound *ent*-**9b** (22 mg, 26%, light-brown solid) was prepared from *ent*-**25** (107 mg, 0.264 mmol) as described for the preparation of **9b**: $[\alpha]^{19.7}_{D}$ +23.5 (*c* 0.53, MeOH); HRMS (EI) calcd for $C_{15}H_{25}N_3$ 247.2048, found 247.2045 (M^+). Anal. ($C_{15}H_{27}Cl_2N_3 \cdot 0.5H_2O$) C, H, N.

Binding Assay with Human Histamine Receptors. The membrane preparations of the Chinese hamster ovary cells, which expressed recombinant human histamine H_3 or H_4 receptors, were purchased from Euroscreen (Brussels, Belgium). The binding assay of the H_3 and H_4 receptors was performed using [^{3}H]N^α-methylhistamine (Perkin-Elmer, Boston, MA) and [^{3}H]histamine (Perkin-Elmer), respectively. Briefly, the membrane preparations (7.5−15 μg of protein) were incubated with different concentrations of [^{3}H]-N^α-methylhistamine (0.1−3 nM) and of [^{3}H]histamine (1−30 nM) for 30 min at 25 °C in 50 mM Tris/5 mM $MgCl_2$ buffer (pH 7.4). The reaction was terminated by rapid filtration (Cell Harvester, Brandel Co., Gaithersburg, MD) through Whatman GF/B glass fiber filters presoaked for 2 h in 0.5% polyethyleneimine, and the filters were rinsed three times with an ice-cold buffer (2 mL). Membrane-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. The apparent dissociation constants (K_d) for each radioligand was determined by nonlinear regression analysis of the curve generated by plotting specific binding concentration vs concentration of the radioligand with GraphPad Prism (GraphPad Software, San Diego, CA) using a one-site binding curve equation. The ability of each compound to inhibit the specific binding of [^{3}H]N^α-methylhistamine (1.5 nM) and [^{3}H]histamine (25 nM) was estimated by IC_{50} values, which are the molar concentrations of unlabeled drugs necessary for displacing 50% of specific binding (estimated by log probit analysis). The inhibition constant, K_i, 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 the mean ± SE (n = 3).

Luciferase Reporter Gene Assay. The reporter gene assay was performed according to the method described previously.[4a,7b]

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Supporting Information Available: Optical purities of compounds analyzed by chiral HPLC and detailed combustion analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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