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Investigation of the Bioactive Conformation of Histamine H_3 Receptor Antagonists by the Cyclopropylic Strain-Based Conformational Restriction Strategy

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We previously identified the highly potent histamine H_3 receptor antagonists (1*R*,2*S*)-2-[2-(4-chlorobenzylamino)ethyl]-1-(1*H*-imidazol-4-yl)cyclopropane (**1**) and its enantiomer ***ent*-1**. Although the conformations of **1** and ***ent*-1** are restricted by the central cyclopropane ring, the 2-(4-chlorobenzylamino)ethyl side chain essential for the H_3 receptor binding may somewhat freely rotate. To investigate the bioactive conformation, the 1′-ethyl-substituted derivatives **2a** and **2b** and their enantiomers ***ent*-2a** and ***ent*-2b** were designed as side chain conformation-restricted analogues of **1** and ***ent*-1**, based on the cyclopropylic strain. These compounds were synthesized, and their analysis by NMR and calculations suggested that the side chain moiety was effectively restricted in a *syn*-form or an *anti*-form by the cyclopropylic strain as expected. Pharmacological evaluation and docking simulation showed that the bioactive conformations of **1** and ***ent*-1** appear to be the *syn*-form and the *anti*-form, respectively. Thus, the cyclopropylic strain can be effectively used for conformational restriction of the side chain moiety of cyclopropane compounds.

Introduction

Attention has been focused on the histamine H_3 receptor, which is a G-protein-coupled receptor (GPCR[a]) distributed mainly in the central nervous system.[1] 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, obesity, and epilepsy.[1b,d,e] As a result, attempts to develop H_3 receptor antagonists have led to the identification of potent H_3 receptor ligands,[1b−f] some of which are shown in Figure 1.

GPCRs are considered to be major targets for drug development.[2] Indeed, it is estimated that over 50% of all modern drugs are targeted at GPCRs.[2a] However, because of the membranous nature of these proteins and their very low natural abundance, structural analysis of GPCRs is difficult. In fact, until the most recent resolution of the adrenergic β_2 receptor structures,[3] the only high-resolution structure of a GPCR available had been that of bovine rhodopsin.[2b] One obvious drawback in drug development targeting GPCRs is therefore poor structural data on these proteins.

Conformational restriction of neurotransmitters may improve the specific binding to one of the receptor subtypes.[4] In conformationally restricted analogues highly selectively bound to the target receptor, the functional groups essential for the receptor binding must assume a special arrangement superimposed 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 lead compound that binds to the target subtype, i.e., the bioactive conformation, is often unknown. This is mainly because structural analysis of membrane-bound proteins is tremendously difficult,[2] compared with that of proteins soluble in blood or cytosol. Thus, a method for effectively identifying compounds targeting GPCRs, which do not involve 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.[5] In this strategy, the versatile chiral cyclopropane units with different stereochemistries (Figure 2)[5a] are effectively used as the key tool for the design and synthesis of a series of conformationally restricted analogues with stereochemical diversity.[5]

On the basis of this stereochemical diversity-oriented strategy, we recently designed and synthesized a series of conformationally restricted analogues of histamine, as shown in Figure 2, with different stereochemistries.[5] In these analogues, the imidazole

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[a] Abbreviations: ADHD, attention-deficit/hyperactivity disorder; EBNA, Epstein−Barr virus nuclear antigen-1; GPCR, G-protein-coupled receptor; GPCRDB, G-protein-coupled receptors data bank; IFD, induced fit docking; OPLS-AA, optimized potentials for liquid simulations-all-atom; PGME, phenylglycine methyl ester; XP, extra-precision.

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and the amino groups are located in a variety of spatial arrangements because of the conformational restriction. Some of these analogues were shown to be potent H_3 receptor ligands, and a conformationally restricted analogue **1** with a (1*R*)-*trans*-cyclopropane structure and its enantiomer ***ent*-1** (Figure 3) were identified as highly potent H_3 receptor antagonists.[5c]

With these results in hand, we thought that, based on the structures of **1** and ***ent*-1**, identification of the bioactive conformation for the H_3 receptor antagonists might be possible. If indeed this could be accomplished, the information obtained would be useful in designing further effective compounds. Although the conformation of **1** is restricted by the (1*R*)-*trans*-cyclopropane structure, the spatial arrangement of the basic nitrogen of the side chain, which seems to be essential for activity, would be somewhat flexible. Therefore, we decided to restrict the side chain conformation by the cyclopropylic strain-based method,[6,7] a detail of which is described below, to identify the bioactive conformation. During our study, X-ray crystallographic structures of adrenergic β_2 receptors were reported,[3] and as a consequence, the H_3 receptor model using the structural data of a β_2 receptor was constructed and used for investigating the binding conformation of the conformationally restricted analogues.

In this report, we describe the design, synthesis, pharmacological effects, and receptor modeling studies of the cyclopropylic strain-based conformationally restricted analogues **2a** and **2b** and their enantiomers ***ent*-2a** and ***ent*-2b** (Figure 3) for the identification of their bioactive conformations.

Results and Discussion

Cyclopropylic Strain-Based Design of the Conformationally Restricted Analogues. Because of its small and rigid ring structure, cyclopropane is effective in restricting the conformation of a molecule without changing the chemical and physical properties of the lead compound.[8] A characteristic structural feature of cyclopropane is that cis-oriented adjacent substituents on the ring exert significant mutual steric repulsion because they are fixed in the eclipsed orientation, which we previously termed "cyclopropylic strain".[7] Consequently, conformation of the substituents on a cyclopropane can be restricted so that the steric repulsion due to the strain is minimal, as indicated in Figure 4.

Considering that the basic amino function of the H_3 receptor antagonist **1** is likely to be important for the H_3 receptor binding, the conformation of the side chain would significantly affect the activity of the compound. While the cyclopropyl-C1′ (C2−C1′) bond may freely rotate somewhat, the rotation can be restricted by the cyclopropylic strain. Therefore, the two conformers **A** (*syn*, the C-3 of the cyclopropane "up"/the benzylaminomethyl "up") and **C** (*anti*, the C-3 of the cyclopropane "up"/the benzylaminomethyl "down") would be preferable to conformer

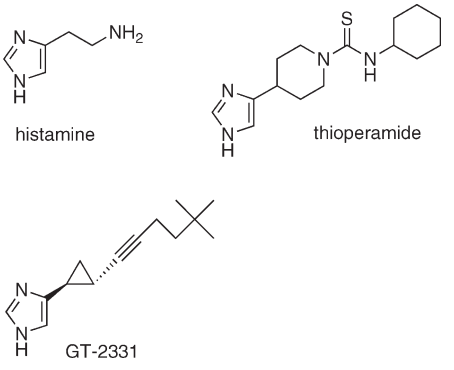


Figure 1. Histamine and representative H_3 receptor ligands.

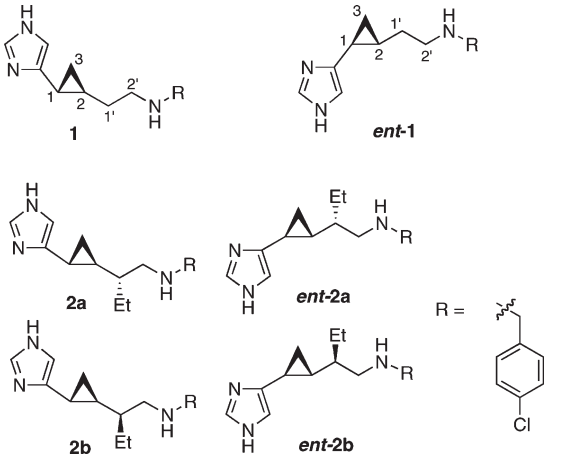


Figure 3. Previously synthesized H_3 receptor antagonists **1** and ***ent*-1** and their side chain conformationally restricted analogues newly designed.

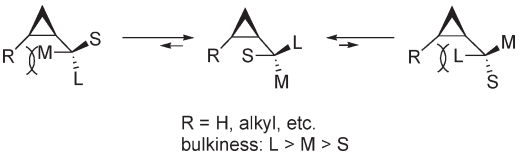


Figure 4. Cyclopropylic strain-based conformational restriction.

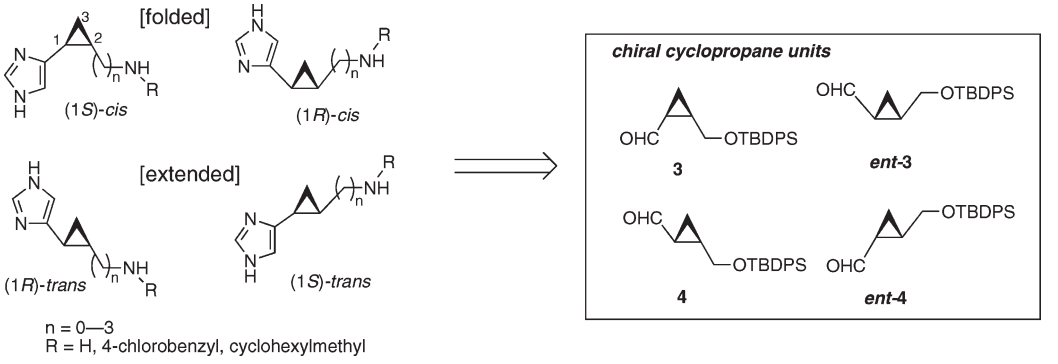


Figure 2. A series of conformationally restricted analogues of histamine with stereochemical diversity synthesized from the chiral cyclopropane units.

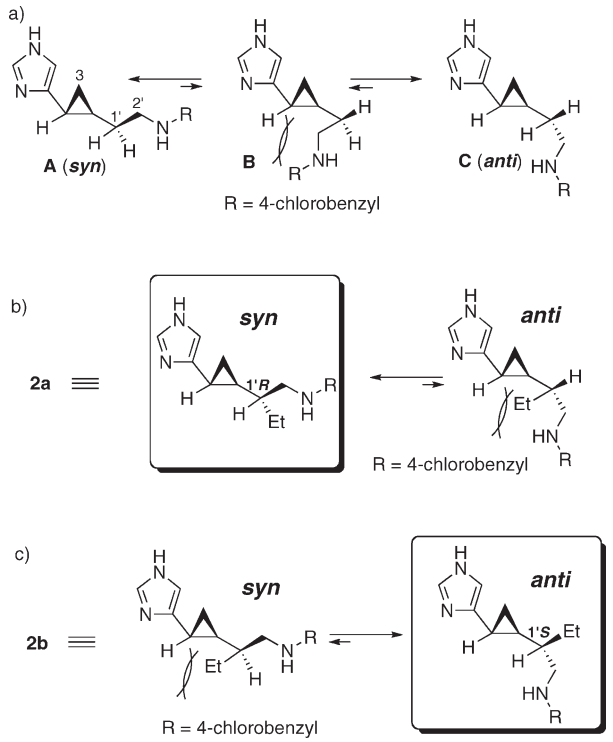


Figure 5. Presumed stable conformations of **1** (a), **2a** (b), and **2b** (c).

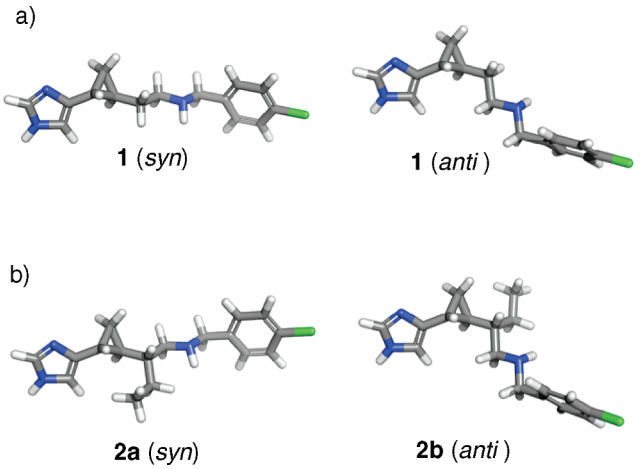
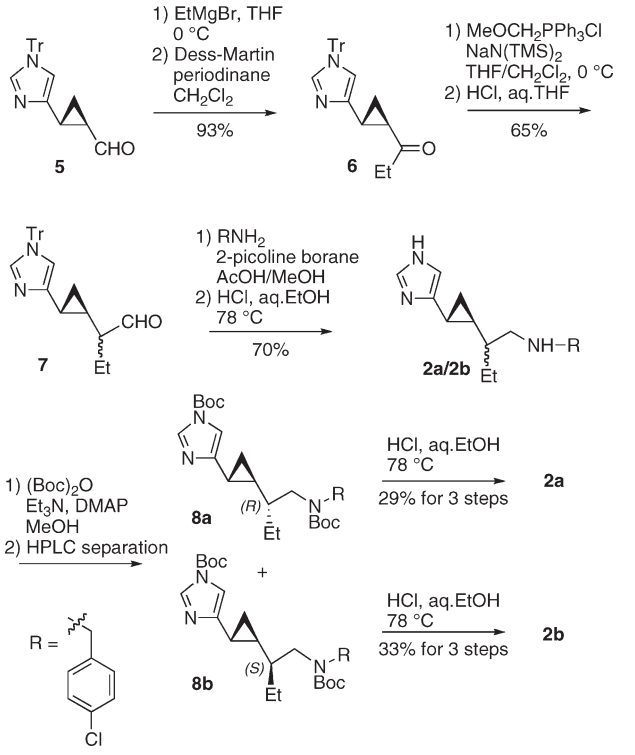


Figure 6. Stable structures of the conformationally restricted analogues in the gas phase obtained by the conformational search program in MacroModel: (a) the *syn*-conformer and the *anti*-conformer for **1**; (b) the *syn*-conformer for **2a** and the *anti*-conformer for **2b**.

B in compound **1** because of the significant steric repulsion for the adjacent cis-proton in conformer **B**, as shown in Figure 5a.

The conformation of **1** was analyzed by molecular mechanics calculations with MacroModel (Schrödinger, LLC). As a result, as shown in Figure 6a, two significantly stable structures were obtained, which correspond to the *syn*- and the *anti*-conformers in Figure 5a, respectively. The two conformers are nearly equally stable, while the *anti*-conformer is only 0.31 kcal/mol more stable than the *syn*-conformer. Thus, the results of calculations are in accord with the above hypothesis on the conformation of **1**, which suggests that the bioactive conformation may be analogous to either the *syn*-conformer or the *anti*-conformer.

Scheme 1



We designed the 1′-ethyl-substituted derivatives **2a** and **2b** (Figure 3) as side chain conformation-restricted analogues of **1**. Introducing an ethyl group into the α-position of the amino function of **1** would prevent the rotation of the side chain moiety by restricting the conformation due to the cyclopropylic strain, i.e., the steric repulsion for the adjacent eclipsed proton. Accordingly, depending on the configuration at the C1′ position, the conformation of the compounds can be restricted; the *syn*-conformer would be quite stable in **2a** of the 1′*R*-configuration (Figure 5b); conversely, the *anti*-conformer would be stable in **2b** of the 1′*S*-configuration (Figure 5c).

Thus, while cyclopropane is very effective for conformational restriction of conformationally flexible lead compounds, the cyclopropylic strain-based conformational restriction makes the more precise conformational restriction of cyclopropane compounds possible, especially in the side chain moiety.

Synthesis. Although much effort has been devoted to developing practical methods for preparing chiral cyclopropanes, synthesis of cyclopropane derivatives with the desired stereochemistry is often troublesome.[9] The chiral cyclopropane units (Figure 2), which are composed of four stereoisomeric cyclopropane derivatives bearing two adjacent carbon substituents in a cis or a trans relationship, are useful for the synthesis of various cyclopropane compounds, particularly for those with stereochemical diversity.[5]

As summarized in Scheme 1, the target compounds **2a** and **2b** were synthesized from an imidazolylcylcopropanecarboxaldehyde **5** with the (1*R*,2*R*)-structure, which was prepared from the unit **4** by our previous method.[5a] Treatment of **5** with EtMgBr in THF gave a diastereomeric mixture of the addition products, the Dess−Martin oxidation of which afforded the corresponding ketone **6**. Wittig reaction of **6** with $MeOCH_2PPh_3Cl/NaN(TMS)_2$ followed by acidic treatment gave the

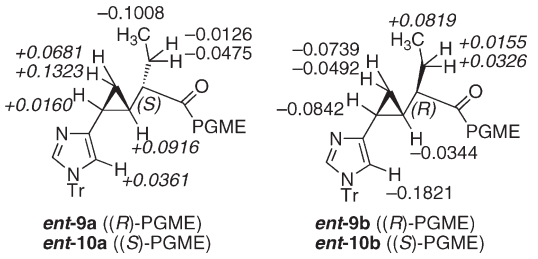


Figure 7. Determination of the 1′-configurations based on the Δδ values (DMSO-d_6, 500 MHz) of the (*R*)- and (*S*)-PGME amides.

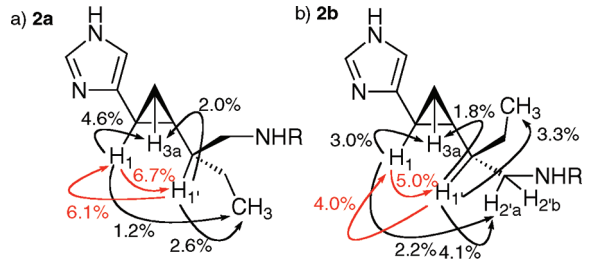


Figure 8. NOE data of **2a** and **2b** in D_2O.

aldehyde **7** as an inseparable diastereomeric mixture (dr, 1/1.1). Reductive amination of **7** with 4-chlorobenzylamine and 2-picoline borane in AcOH/MeOH and subsequent acidic removal of the trityl group of the product gave the desired cyclopropylic strain-based conformationally restricted analogues **2a** and **2b** as a diastereomeric mixture. Although the diastereomers were inseparable at this stage, they were successfully separated by HPLC after protection of the imidazole and amino nitrogens with Boc groups to give the diastereomerically pure **8a** and **8b**, respectively. Acidic removal of the Boc groups of **8a** and **8b** afforded the target (1′*R*)-product **2a** and the (1′*S*)-product **2b**, respectively. The enantiomers ***ent*-2a** and ***ent*-2b** were similarly synthesized from ***ent*-5**.

The 1′-configurations of the cyclopropylic strain-based conformationally restricted analogues synthesized were determined by the phenylglycine methyl ester (PGME) method[10] by converting diastereomerically pure ***ent*-7a** and ***ent*-7b** into the corresponding (*R*)-PGME amides ***ent*-9a** and ***ent*-9b** and (*S*)-PGME amides ***ent*-10a** and ***ent*-10b**,[11] respectively, as shown in Figure 7.

Conformational Analysis by NMR and Calculations. Stable structures of the conformationally restricted analogues **2a** and **2b** were investigated by NOE experiments (Figure 8). Irradiations of H-1′ of **2a** or **2b** gave NOEs with both H-1 and H-3a oriented cis to H-1. Especially significant NOEs were observed between H-1′ and H-1 in both **2a** and **2b**, which show that the side chain conformation of the two compounds seems to be actually restricted by the cyclopropylic strain. When H-1 on the cyclopropane ring of the (1′*R*)-diastereomer **2a** was irradiated, an NOE with the terminal methyl protons of the ethyl group was observed to suggest that it is restricted to the *syn*-form as expected. On the other hand, during irradiation of H-1 of the (1′*S*)-diastereomer **2b**, an NOE was observed with the methylene proton H-2′a adjacent to the basic nitrogen to demonstrate that it is in the *anti*-form, as expected.

The conformations of **2a** and **2b** were also examined by calculations with MacroModel. As shown in Figure 6b, the most stable structures obtained by the calculations were the *syn*-form for **2a** and the *anti*-form for **2b**, respectively.

Table 1. Effects of Compounds on the Human H_3 Receptor Subtype[a]

compd	configuration	conformation	inhibition (%)[b]	K_i (nM)
2a	(1*R*)-*trans*-(1′*R*)	*syn*	95	19.8 ± 2.8
2b	(1*R*)-*trans*-(1′*S*)	*anti*	73	129 ± 6.0
1	(1*R*)-*trans*	*syn*/*anti*	100	8.4 ± 1.5[c]
***ent*-2a**	(1*S*)-*trans*-(1′*S*)	*syn*	96	63.0 ± 6.1
***ent*-2b**	(1*S*)-*trans*-(1′*R*)	*anti*	88	6.7 ± 0.4
***ent*-1**	(1*S*)-*trans*	*syn*/*anti*	100	3.6 ± 0.4[c]
thioperamide			98	51.1 ± 3.8[c]

[a] Assays were carried out with 293-EBNA cells or cell membranes expressing the human H_3 receptor subtype. [b] Inhibitory effect of compound (10^{-4} M) on the agonistic activity of histamine (10^{-6} M). [c] Data were taken from ref 5c.

The calculated energy barriers for the rotation of the C2−C1′ bond between the *syn*-form and the *anti*-form are significant, which are 5.22 kcal/mol for **2a** and 5.55 kcal/mol for **2b**, respectively.

Thus, these conformational analyses suggested that the cyclopropylic strain-based conformational restriction seems to work effectively in **2a** and **2b**, and therefore, pharmacological evaluations of these compounds would help to identify the bioactive conformation.

Pharmacological Effects. Effects of compounds on the H_3 receptor were investigated by luciferase reporter gene assay. The human histamine receptor subtypes were individually expressed in 293-Epstein−Barr virus nuclear antigen-1 (EBNA) cells according to the previously reported method,[5b] and the function of the compounds on these receptors expressed on the cells was evaluated. None of the newly synthesized compounds **2a**, **2b**, ***ent*-2a**, and ***ent*-2b** showed any agonistic activity to the H_3 receptor at 10^{-5} M (data not shown). On the other hand, all of these compounds inhibited the agonistic effect of histamine to show that they are antagonists of the H_3 receptor as are the parent compounds **1** and ***ent*-1**, as shown in Table 1.

Binding affinities of compounds **2a**, **2b**, ***ent*-2a**, and ***ent*-2b** for the human H_3 receptor subtype using [^{3}H]N^{α}-methylhistamine[5c] were next investigated and were compared with those of their parent compounds **1** and ***ent*-1** (Table 1). In this system, the well-known H_3 receptor antagonist thioperamide showed a K_i value of 51.1 nM, and compounds **1** and ***ent*-1** displayed much higher binding affinity for the human H_3 receptor as shown by the K_i values of 8.4 and 3.6 nM, respectively.

Although compound **2a**, which is restricted in the *syn*-conformation, showed remarkable binding affinity for the receptor with a K_i value of 19.8 nM, compound **2b**, restricted in the *anti*-conformation, showed more than 15-fold reduction of potency (K_i = 129 nM), compared with the parent compound **1**. On the other hand, of the enantiomers ***ent*-2a** and ***ent*-2b**, ***ent*-2b** (K_i = 6.7 nM), which is restricted in the *anti*-conformation, showed 10-fold higher binding affinity for the human H_3 receptor than the ***ent*-2a** (K_i = 63 nM), which is restricted in the *syn*-conformation.

Docking Simulation by Homology Modeling. The above conformational analysis and pharmacological results showed that in the diastereomeric pair of **2a** and **2b**, the *syn*-restricted **2a** was more potent than the *anti*-restricted **2b**, while in their enantiomers ***ent*-2a** and ***ent*-2b**, the *anti*-restricted ***ent*-2b** was more potent than the *syn*-restricted ***ent*-2a**. In order to understand the discrepancy in the conformation−activity relationship between **2a**/**2b** and ***ent*-2a**/***ent*-2b**, we planned to perform a docking simulation with a

homology modeling of the H_3 receptor to investigate the binding mode of these cyclopropylic strain-based conformationally restricted analogues in the active site of the H_3 receptor.

Previous studies showed that homology models of H_3 receptor are useful for providing structural insight into the ligand binding mechanism, QSAR analysis, and in silico drug discovery.[13] Furthermore, most recent resolutions of the ligand-binding adrenergic β_2 receptor structures[3] give us a chance to generate more accurate three-dimensional models for target GPCRs with a ligand using homology modeling and docking simulation.[14]

Thus, in this study, a three-dimensional model of the H_3 receptor was constructed on the basis of a structural template from the crystal structure of the human β_2-adrenergic GPCR recently reported by Cherezov and co-workers,[3a] and docking simulations of the compounds into the H_3 receptor model were performed with ligand and receptor flexibility.

We constructed a homology model based on the conformationally restricted analogue ***ent*-2b**, which is the most potent H_3 receptor ligand in this series of the cyclopropane-based conformationally restricted analogues. Using this model, we performed docking simulation with a series of the cyclopropane-based conformationally restricted H_3-receptor ligands having stereochemical diversity (16 compounds) synthesized previously[5c,12] and also with the four cyclopropylic strain-based conformationally restricted analogues **2a**, **2b**, ***ent*-2a**, and ***ent*-2b** synthesized in this study. Correlations between the calculated binding score and the pK_i were examined. As a result, as shown in Figure 9, a reliable correlation ($R^2 = 0.41$) between binding score and pK_i was obtained. Consequently, the ***ent*-2b**-bound H_3 receptor model was also used for further studies.

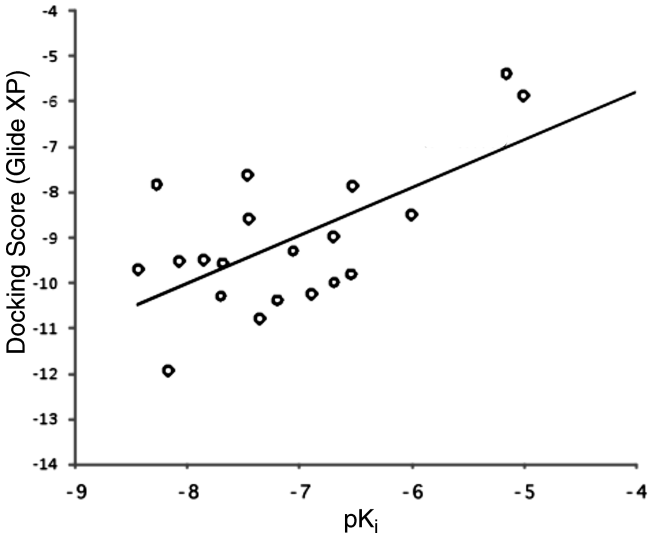


Figure 9. Plot of binding score calculated by Glide extraprecision (XP) based on ***ent*-2b**-bound H_3 receptor model versus experimental binding affinity pK_i for 20 conformational restriction analogues. The coefficient of determination, R^2, between binding score and pK_i was 0.41 for 20 conformational restriction analogues.

In order to understand the binding modes of the newly synthesized cyclopropylic strain-based conformationally restricted analogues to the H_3 receptor, docking simulations of **2a** and **2b** and their enantiomers ***ent*-2b** and ***ent*-2b** were carried out by using the ***ent*-2b**-bound model. Figure 10 shows the proposed binding modes of the potent H_3 antagonists **2a** and ***ent*-2b** to the homology model of the H_3 receptor obtained by the simulation. These compounds are accommodated in the active site concavity formed by TM2, TM3, TM5, TM6, and TM7. The H_3 receptor-binding conformations of **2a** and ***ent*-2b** are the *syn*-form and the *anti*-form, respectively, as shown in Figures 10 and 11, which are in accord with the stable forms proposed by their conformational analysis by NMR and the calculations described above.

In the obtained binding models shown in Figure 10, the NH of the imidazole ring of both compounds likewise serves as a hydrogen donor and forms a hydrogen bond with an oxygen atom of the side chain carboxyl group of Glu206. Furthermore, the protonated amine in both **2a** and ***ent*-2b** forms a similar salt bridge with Asp114 in TM3, and the 4-chlorobenzyl group in **2a** and ***ent*-2b** is observed to make a $\pi-\pi$ interaction with the indole ring of Trp110 in TM3. Thus, the special positioning of the imidazole moiety and the 4-chlorobenzylamino moiety in **2a** and ***ent*-2b**, which are likely to be essential for their H_3 receptor binding, is analogous in the active site, and therefore **2a** and ***ent*-2b** may have a common pharmacophore. As shown in Figure 11a, the imidazole and 4-chlorobenzylamino moieties of **2a** and ***ent*-2b** can be superimposed, where the two cyclopropane rings orient oppositely, i.e., "up" in **2a** and "down" in ***ent*-2b**, respectively. This would explain why **2a** and ***ent*-2b** have a similar potent antagonistic effect on the H_3 receptor, even though the two compounds are conformationally restricted

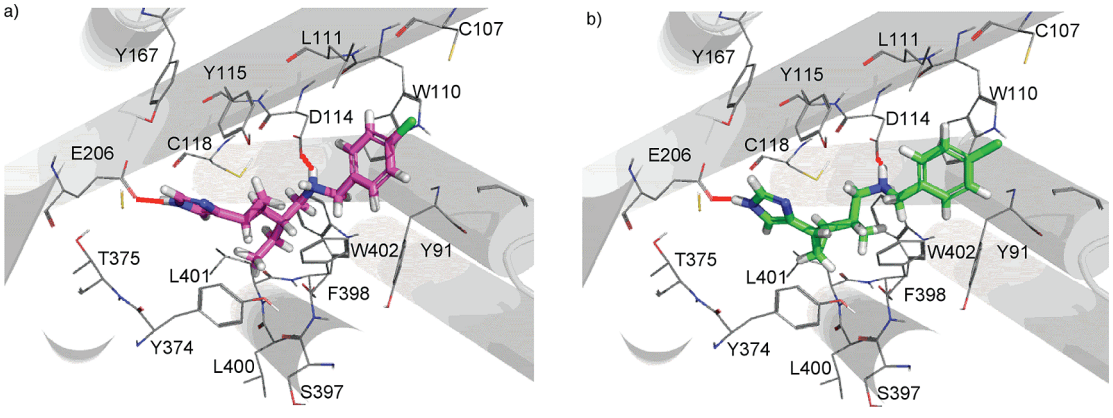


Figure 10. Proposed models for **2a** (a) and ***ent*-2b** (b) binding to the homology model of the H_3 receptor from docking simulation. Receptor residues around the compounds within 4 Å are shown in line representation. Carbon atoms of **2a** and ***ent*-2b** are shown in magenta and green, respectively. All nonpolar hydrogen atoms of receptor residues are omitted for clarify. Hydrogen bonding and salt bridge to side chain carboxyl group of Glu206 and Asp114 are depicted by red dots.

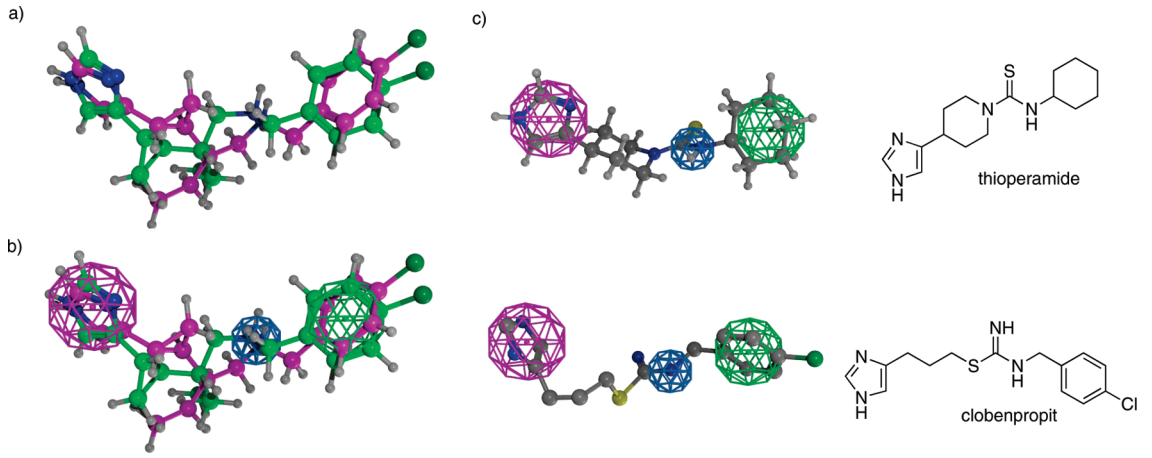


Figure 11. Superimposition of the structures of **2a** (magenta) and ***ent*-2b** (green) binding to the homology model of the H_3 receptor (a). Three-feature pharmacophore model generated for **2a** and ***ent*-2b** using MOE: hydrogen bond acceptor/donor (magenta feature), hydrogen bond donor/cationic atom (blue feature), and aromatic ring center/hydrophobic region (green feature) (b). Known H_3 receptor ligands are mapped onto the pharmacophore model obtained from **2a** and ***ent*-2b** (c).

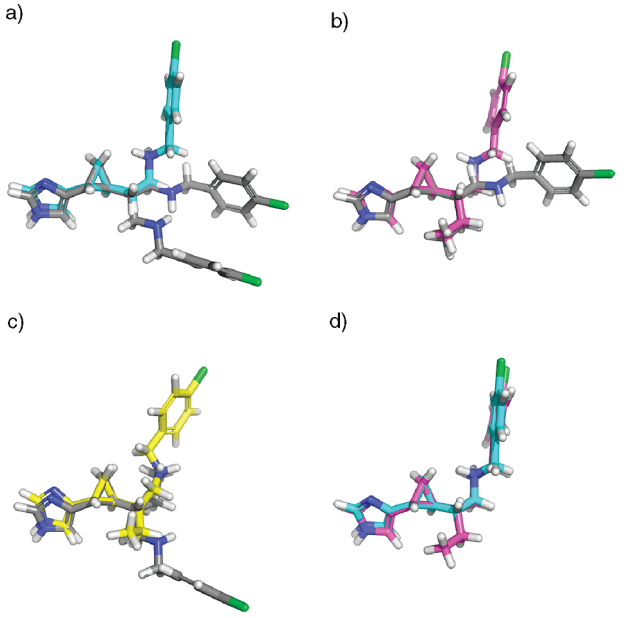


Figure 12. Comparison of conformational changes between the stable forms shown in Figure 6 and the bioactive forms proposed by the H_3 receptor-bound model. Shown are the two stable conformations and the bioactive conformations for **1** (a), the stable and the bioactive conformations for **2a** (b) and **2b** (c), and superimposition of the bioactive conformations of **1** and **2a** (d). Carbon atoms of stable conformations for all compounds are shown in gray, and carbon atoms of the bioactive conformations for **1**, **2a**, and **2b** are shown in blue, magenta, and yellow, respectively. All compounds are aligned on the cyclopropane ring.

differently, i.e., the *syn*-form and the *anti*-form, respectively, by the cyclopropylic strain. Figure 11b shows a common pharmacophore model for **2a** and ***ent*-2b**, and the model effectively fitted in known H_3 receptor ligands,[1] as shown in Figure 11c.

We next examined the possible conformational differences between the stable form and the bioactive form of the compounds, which could significantly affect the binding affinity, based on the proposed receptor-bound model. In Figure 12, the stable conformations of **1** and its conformationally restricted analogues **2a** and **2b** based on NMR and calculation analysis are superimposed on their receptor-bound (bioactive) conformations by the receptor modeling simulations. As shown in Figure 12a, the bioactive conformation of **1** is in accord with the *syn*-form of the two stable *syn*- and *anti*-conformations. Figure 12b shows that the stable *syn*-form of **2a** is almost identical with the bioactive conformation in **2a**, which would make it highly potent. On the other hand, in **2b**, the bioactive conformation is the *syn*-form similar to **1** and **2a**, while **2b** itself is stable in the *anti*-form (Figure 12c). Thus, because of the entropic cost for the conformational change from the stable *anti*-form into the bioactive *syn*-form in its binding to the H_3 receptor, the binding affinity of **2b** for the H_3 receptor is significantly decreased. In Figure 12d, the receptor-bound conformations of **1** and **2a** were superimposed, showing that their bioactive conformations are the same. These results suggested that the cyclopropylic strain-based conformational restriction worked effectively as expected.

As described, we identified the potent H_3 receptor antagonists **1** and ***ent*-1** by the stereochemical diversity-oriented conformational restriction method with chiral cyclopropane units as shown in Figure 2. Their bioactive conformations and a pharmacophore model were further elaborated by the cyclopropylic strain-based conformational restriction method. These results showed that the combinational use of the stereochemical diversity-oriented and the cyclopropylic strain-based conformational restriction methods seems to be an effective strategy for developing significantly active compounds and also for identifying their bioactive conformations, especially in cases where the structural data of the target biomolecule are lacking or poorly documented. In these studies, simulations with homology modeling of the target biomolecule can be effective. This is due to the fact that a series of cyclopropane analogues and also cyclopropane strain-based conformationally restricted analogues are suitable for validating the homology models, since these consist of compounds having diversity not only in their conformation, i.e., three-dimensional structure, but also in their binding affinity for the target.

Conclusion

In order to clarify the bioactive conformation of the previously developed H_3 receptor antagonists **1** and ***ent*-1**, the 1′-ethyl-substituted derivatives **2a** and **2b** and their enantiomers

***ent*-2a** and ***ent*-2b** were designed as side chain conformation-restricted analogues of **1** and ***ent*-1**, based on the cyclopropylic strain, and were synthesized from the versatile chiral cyclopropane units. Conformational analysis, pharmacological evaluation, and docking simulation of the compounds showed that the bioactive conformations of **1** and ***ent*-1** seem to be the *syn*-form and the *anti*-form, respectively. On the basis of these results, a common pharmacophore for the compounds was obtained. These results suggest that the cyclopropylic strain-based strategy can be effectively used for precise conformational restriction of the side chain moiety and bioactive conformation analysis of cyclopropane compounds.

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 Ltd.). Reactions were carried out under an argon atmosphere. Estimated purity of all of the final compounds by combustional analysis was always at least 95%.

(1*R*,2*R*)-2-(1-Oxopropyl)-1-(1-triphenylmethyl-1*H*-imidazol-4-yl)cyclopropane (6). To a solution of **5**[5a] (238 mg, 0.629 mmol) in THF (5.0 mL) was added EtMgBr (0.91 M in THF, 830 μL, 0.755 mmol) at 0 °C, and the mixture was stirred at the same temperature for 1 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. To a solution of the residue in CH_2Cl_2 (5 mL) was added Dess−Martin periodinane (320 mg, 0.755 mmol) at room temperature, and the mixture was stirred at the same temperature for 1 h. To the reaction mixture was added a mixture of aqueous saturated $NaHCO_3$ and aqueous saturated $Na_2S_2O_3$ (3/1, 12 mL) at room temperature, and the resulting mixture was vigorously stirred at the same temperature for 10 min. The resulting solution was extracted with AcOEt, and the organic layer was washed with aqueous saturated $NaHCO_3$, brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (20−33% AcOEt in hexane) to give **6** (239 mg, 93%) as a white amorphous solid: $[\alpha]^{19}_D$ −217.3 (*c* 0.86, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.07 (3 H, t, J = 7.3 Hz, C$\underline{H}_3$CH$_2$-), 1.44 (1 H, m, H-3a), 1.50 (1 H, m, H-3b), 2.38 (2 H, m, H-1 and H-2), 2.61 (2 H, q, J = 7.3 Hz, CH$_3$C$\underline{H}_2$-), 6.64 (1 H, d, J = 1.2 Hz, imidazolyl), 7.11−7.14 (6 H, m, aromatic), 7.28 (1 H, s, imidazolyl), 7.32−7.34 (9 H, m, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.96, 18.0, 22.5, 30.1, 37.0, 75.2, 118.0, 127.9, 129.7, 138.4, 138.4, 139.9, 142.2, 209.9; LRMS (EI) *m*/*z* 406 (M^+); HRMS (EI) calcd for $C_{28}H_{26}N_2O$ 406.2045, found 406.2050 (M^+). Anal. ($C_{28}H_{26}N_2O$) C, H, N.

(1*R*,2*R*)-2-(1-Formylpropyl)-1-(1-triphenylmethyl-1*H*-imidazol-4-yl)cyclopropane (7). To a suspension of $MeOCH_2PPh_3Cl$ (608 mg, 1.77 mmol) in THF (5.0 mL) was added NaN(Si(CH$_3$)$_3$)$_2$ (~1.9 M in THF, 800 μL, 1.52 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 **6** (206 mg, 0.507 mmol) in CH_2Cl_2 (2.0 mL) at 0 °C, and the reaction mixture was stirred at the same temperature for 2 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 (30% AcOEt in hexane) to give the enol ether product (151 mg) as a light-yellow solid. To a solution of the product in THF (10 mL) was added aqueous HCl (12 M, 5.0 mL), and the mixture was vigorously stirred at room temperature for 10 s. Immediately, the mixture was poured into aqueous saturated $NaHCO_3$ (100 mL). Then the resulting solution was extracted with AcOEt. The organic layer was washed with aqueous saturated $NaHCO_3$, brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography (25−40% AcOEt in hexane) to give **7** (diastereomixture, 138 mg, 65%) as a yellow amorphous solid: HRMS (EI) calcd for $C_{29}H_{28}N_2O$ 420.2202, found 420.2200 (M^+).

(1*R*,2*S*)-2-[1-Ethyl-2-(4-chlorobenzylamino)ethyl]-1-(1*H*-imidazol-4-yl)cyclopropane (2a/2b). To a solution of **7** (112 mg, 0.266 mmol) and 4-chlorobenzylamine (35 μL, 0.28 mmol) in MeOH/AcOH (10/1, 2.2 mL) was added 2-picolineborane (30 mg, 0.28 mmol) at room temperature, and the mixture was stirred at the same temperature for 12 h. After evaporation of the solvent, a solution of the residue in aqueous HCl (4 M, 4.0 mL) was stirred at 0 °C for 20 min, and then the mixture was neutralized with Na_2CO_3. The mixture was partitioned between CH_2Cl_2 and aqueous saturated $NaHCO_3$, and the organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by neutral silica gel column chromatography (0−10% MeOH in $CHCl_3$) to give amine product (diastereomixture, 108 mg) as a light-yellow amorphous solid. To a solution of amine (108 mg) in EtOH (1.0 mL) was added aqueous HCl (2 M, 1.0 mL), and the resulting solution was stirred at 78 °C for 2 h, and then the solvent was evaporated. The residue was partitioned between aqueous HCl (1 M) and CH_2Cl_2, and the aqueousueous layer was neutralized with aqueous NaOH (2 M). The resulting solution was extracted with Et_2O, and the organic layer was washed with H_2O, brine, dried (Na_2SO_4), and evaporated. The residue was purified by NH silica gel column chromatography (0−5% MeOH in $CHCl_3$) to give the diastereomixture of **2a** and **2b** (56 mg, 70%) as a colorless amorphous solid: HRMS (EI) calcd for $C_{17}H_{22}ClN_3$ 303.1502, found 303.1500 (M^+).

(1*R*,2*S*)-2-[(1*R*)-1-Ethyl-2-(4-chlorobenzylamino)ethyl]-1-(1*H*-imidazol-4-yl)cyclopropane (2a) and (1*R*,2*S*)-2-[(1*S*)-1-Ethyl-2-(4-chlorobenzylamino)ethyl]-1-(1*H*-imidazol-4-yl)cyclopropane (2b). A solution of the diastereomixture of **2a** and **2b** (56 mg, 0.15 mmol), Et_3N (83 μL, 0.60 mmol), DMAP (1.8 mg, 0.015 mmol), and $(Boc)_2O$ (130 mg, 0.60 mmol) in MeOH (1 mL) was stirred at room temperature for 16 h. After evaporation of the solvent, the residue was partitioned between AcOEt and H_2O, and the organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was separated by HPLC (28% AcOEt in hexane, 13 mL/min, room temperature, 253 nm) with Mightysil Si 60 (0.25 cm × 20 cm, Kanto Chemical Co.) to give **8a** (24 mg, a colorless amorphous solid) and **8b** (26 mg, a colorless amorphous solid). Each compound was dissolved in EtOH (1.5 mL)/aqueous HCl (4 M, 0.5 mL), and the mixture was stirred at 78 °C for 2 h. After the mixture was concentrated and dried in vacuo, the residue was purified by NH silica gel column chromatography (0−10% MeOH in $CHCl_3$) to give **2a** (12 mg, 29% for three steps, a colorless amorphous solid) or **2b** (14 mg, 33% for three steps, a colorless amorphous solid) as a free amine.

2a: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.76 (1 H, m, H-3a), 0.87−0.96 (6 H, m, H-3b and H-1′ and H-2 and C$\underline{H}_3$CH$_2$-), 1.45 (1 H, m, CH$_3$C$\underline{H}_2$-), 1.54 (1 H, m, CH$_3$C$\underline{H}_2$-), 1.64 (1 H, m, H-1), 2.65 (2 H, dd, J = 5.0, 12.0 Hz, H-2′), 3.77 (2 H, s, -C$\underline{H}_2$Ph), 6.63 (1 H, s, imidazolyl), 7.27 (4 H, dd, J = 8.0, 8.6 Hz, aromatic), 7.48 (1 H, s, imidazolyl); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.0, 13.1, 14.6, 24.7, 26.3, 44.8, 53.8, 53.8, 128.7, 129.6, 132.8, 134.4, 139.2; HRMS (EI) calcd for $C_{17}H_{22}ClN_3$ 303.1502, found 303.1501 (M^+).

The free amine **2a** (12 mg) was dissolved in aqueous HCl (4 M), and the solvent was then evaporated. The residue was triturated with Et_2O to give **2a** dihydrochloride (15 mg, a white amorphous solid): $[\alpha]^{21}_D$ −20.5 (*c* 0.81, MeOH); ^{1}H NMR (400 MHz, D_2O) δ 0.89 (3 H, t, J = 7.5 Hz, C$\underline{H}_3$CH$_2$-), 0.93 (1 H, m, H-3a), 0.98−1.06 (2 H, m, H-2 and H-3b), 1.22 (1 H, m, H-1′), 1.54 (2 H, m, CH$_3$C$\underline{H}_2$-), 1.88 (1 H, m, H-1), 3.11 (2 H, dd, J = 1.8, 7.1 Hz, H-2′), 4.20 (1 H, d, J = 13.6 Hz, -C$\underline{H}_2$Ph), 4.26 (1 H, d, J = 13.6 Hz,

-C$\underline{H}_2$Ph), 7.10 (1 H, s, imidazolyl), 7.46 (4 H, dd, J = 8.6, 8.6 Hz, aromatic), 8.44 (1 H, d, J = 1.4 Hz, imidazolyl); LRMS (EI) m/z 303 ((M − 2HCl)$^+$). Anal. ($C_{17}H_{24}Cl_3N_3$) C, H, N.

2b: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.72 (1 H, m, H-3a), 0.81 (1 H, m, H-3b), 0.93−0.99 (5 H, m, H-1′ and H-2 and C$\underline{H}_3$CH$_2$-), 1.47 (2 H, m, CH$_3$C$\underline{H}_2$-), 1.64 (1 H, m, H-1), 2.64 (1 H, dd, J = 7.4, 11.4 Hz, H-2′), 2.75 (1 H, dd, J = 5.7, 11.4 Hz, H-2′), 3.74 (1 H, d, J = 13.3 Hz, -C$\underline{H}_2$Ph), 3.78 (1 H, d, J = 13.3 Hz, -C$\underline{H}_2$Ph), 6.67 (1 H, s, imidazolyl), 7.22 (2 H, d, J = 8.6 Hz, aromatic), 7.26 (2 H, d, J = 8.6 Hz, aromatic), 7.44 (1 H, s, imidazolyl); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.8, 12.5, 14.6, 24.9, 26.4, 44.8, 53.8, 54.4, 128.8, 129.7, 132.9, 134.5, 139.1; LRMS (EI) m/z 303 (M$^+$); HRMS (EI) calcd for $C_{17}H_{22}ClN_3$ 303.1502, found 303.1509 (M$^+$).

The free amine **2b** (14 mg) was dissolved in aqueous HCl (4 M), and the solvent was then evaporated. The residue was triturated with Et_2O to give **2b** dihydrochloride (17 mg, a white amorphous solid): $[\alpha]^{21}_D$ −64.5 (c 1.23, MeOH); ^{1}H NMR (400 MHz, D_2O) δ 0.92 (3 H, t, J = 7.2 Hz, C$\underline{H}_3$CH$_2$-), 1.05 (2 H, m, H-3), 1.09 (1 H, m, H-2), 1.23 (1 H, m, H-1′), 1.43−1.60 (2 H, m, CH$_3$C$\underline{H}_2$-), 1.74 (1 H, m, H-1), 3.12 (1 H, dd, J = 7.4, 13.1 Hz, H-2a′), 3.19 (1 H, dd, J = 5.0, 13.1 Hz, H-2b′), 4.13 (1 H, d, J = 13.6 Hz, -C$\underline{H}_2$Ph), 4.26 (1 H, d, J = 13.6 Hz, -C$\underline{H}_2$Ph), 6.97 (1 H, s, imidazolyl), 7.39 (4 H, s, aromatic), 8.46 (1 H, d, J = 1.4 Hz, imidazolyl); LRMS (EI) m/z 303 ((M−2HCl)$^+$). Anal. ($C_{17}H_{24}Cl_3N_3$) C, H, N.

(1*S*,2*S*)-2-(1-Oxopropyl)-1-(1-triphenylmethyl-1*H*-imidazol-4-yl)cyclopropane (*ent*-6). Compound ***ent*-6** (173 mg, 85%, a white amorphous solid) was prepared from ***ent*-5** (190 mg, 0.50 mmol) as described for the preparation of **6**: $[\alpha]^{20}_D$ +232.1 (c 1.03, $CHCl_3$); HRMS (EI) calcd for $C_{28}H_{26}N_2O$ 406.2045, found 406.2044 (M$^+$). Anal. ($C_{28}H_{26}N_2O$) C, H, N.

(1*S*,2*S*)-2-(1-Formylpropyl)-1-(1-triphenylmethyl-1*H*-imidazol-4-yl)cyclopropane (*ent*-7). Compound ***ent*-7** (94 mg, 60%, a white amorphous solid) was prepared from ***ent*-6** (152 mg, 0.37 mmol) as described for the preparation of **7**: HRMS (EI) calcd for $C_{29}H_{28}N_2O$ 420.2202, found 420.2220 (M$^+$).

(1*S*,2*R*)-2-[(1*S*)-1-Ethyl-2-(4-chlorobenzylamino)ethyl]-1-(1*H*-imidazol-4-yl)cyclopropane (*ent*-2a). Compound ***ent*-2a** (10 mg, 30%, a white amorphous solid) was prepared from ***ent*-8** (35 mg, 0.11 mmol) as described for the preparation of **2a**: HRMS (EI) calcd for $C_{17}H_{22}ClN_3$ 303.1502, found 303.1500 (M$^+$). The free amine ***ent*-2a** (10 mg) was dissolved in aqueous HCl (4 M), and the solvent was then evaporated. The residue was triturated with Et_2O to give ***ent*-2a** dihydrochloride (13 mg, a white amorphous solid): $[\alpha]^{21}_D$ +19.9 (c 0.62, MeOH); LRMS (EI) m/z 303 ((M−2HCl)$^+$). Anal. ($C_{17}H_{24}Cl_3N_3$) C, H, N.

(1*S*,2*R*)-2-[(1*R*)-1-Ethyl-2-(4-chlorobenzylamino)ethyl]-1-(1*H*-imidazol-4-yl)cyclopropane (*ent*-2b). Compound ***ent*-2b** (10 mg, 30%, a white amorphous solid) was prepared from ***ent*-8** (35 mg, 0.11 mmol) as described for the preparation of **2b**: LRMS (EI) m/z 303 (M$^+$); HRMS (EI) calcd for $C_{17}H_{22}ClN_3$ 303.1502, found 303.1500 (M$^+$). The free amine ***ent*-2b** (10 mg) was dissolved in aqueous HCl (4 M), and the solvent was then evaporated. The residue was triturated with Et_2O to give ***ent*-2b** dihydrochloride (13 mg, a white amorphous solid): $[\alpha]^{21}_D$ +63.2 (c 1.11, MeOH); LRMS (EI) m/z 303 ((M−2HCl)$^+$). Anal. ($C_{17}H_{24}Cl_3N_3$) C, H, N.

Binding Assay with Human Histamine Receptors. The assay was performed according to the method described previously.[5c]

Luciferase Reporter Gene Assay. The assay was performed according to the method described previously.[5b]

Homology Modeling of the H_3 Receptor. The histamine H_3 receptor sequence was aligned with the human β_2-adrenergic receptor sequence[3a] and 40 representative sequences of class A rhodopsin-like amine families (G-protein-coupled receptors data bank (GPCRDB): http://www.gpcr.org/7tm/) using the CLUSTAL W (version 1.8) multiple alignment program.[15] The alignment was refined manually on the basis of the compatibility of the amino acid position with the corresponding structure of the β_2-adrenergic receptor. A three-dimensional model of H_3 receptor was constructed using a homology modeling approach incorporated in the program SegMod[16] of GeneMine.[17] Because it is very long and predicted to be disordered, the third intracellular loop was truncated by the five residues leading out of the helix V and the five residues leading into helix VI. The second extracellular loop was modeled without aligning them with those of the β_2-adrenergic receptor.

Docking Simulation. Initial coordinates of all compounds were constructed using the Molecular Builder module in Maestro (Schrödinger LLC.). Energy minimization of all compounds was performed using the optimized potentials for liquid simulations-all-atom (OPLS-AA) force field in the LigPrep in the Maestro (Schrödinger LLC.). The homology model of H_3 receptor was refined for docking simulations using the Protein Preparation Wizard Script within Maestro. This protein preparation procedure involves optimization of contacts by changing hydroxyl group orientations, flipping of Asn and Gln side chains, and selecting His tautomeric states, followed by constrained energy refinement using the OPLS-AA force field. Docking of the compounds into the H_3 receptor model utilized three main steps that take into account several levels of structural flexibility and scoring criteria: (1) molecular modeling of compound bound H_3 receptor model by docking the ***ent*-2b** molecule, considering both ligand and receptor flexibility, (2) rigid receptor docking of 20 cyclopropane-based conformational restriction analogues[5c] into the active site of ***ent*-2b** compound bound H_3 receptor model from the previous step, (3) rescoring according to the calculated binding score by Glide extraprecision (XP) score (Schrödinger LLC).

Following are the details of each step. In order to account for both compound and receptor flexibility in the first step, the Glide "Induced Fit Docking (IFD)" protocol (Schrödinger LLC.) was utilized, followed by iteratively combining rigid receptor docking (Glide) and protein remodeling by side chain searching and minimization (Prime) techniques. Hydrogen-bonding constraints between the side chain COO$^-$ group of Asp114 and Glu206 were introduced because this hydrogen-bonding formation is highly conserved in almost all known complexes of histamine receptor subfamily bound to histamine and to a wide variety of inhibitors. In the protein remodeling stage, all residues within a 14.0 Å radius of each initial docked compound were refined using Prime. Compound was then redocked into the refined receptor structure using Glide in the standard precision (SP) mode. All of the docked structures were then ranked according to GlideScore. After modeling of the compound−H_3 receptor complex using the IFD protocol, grid generation and rigid receptor docking of the 20 cyclopropane-based conformational restriction analogues using Glide (SP mode) were carried out, using the hydrogen bonding constraint to connect the side chain COO$^-$ group of Asp114 and Glu206. The best orientation for each docked compound was rescored according to its binding score, which was calculated using the Glide XP Score (Schrödinger LLC).

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Supporting Information Available: Synthesis of PGME amides ***ent*-9a**, ***ent*-9b**, ***ent*-10a**, and ***ent*-10b**; the experimental details by which the determination of the 1′-configurations of ***ent*-2a** and ***ent*-2b** by the PGME method was effected; the structures and pK_i values of the H_3 receptor antagonists used for the docking simulations; and elemental analysis data of the final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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