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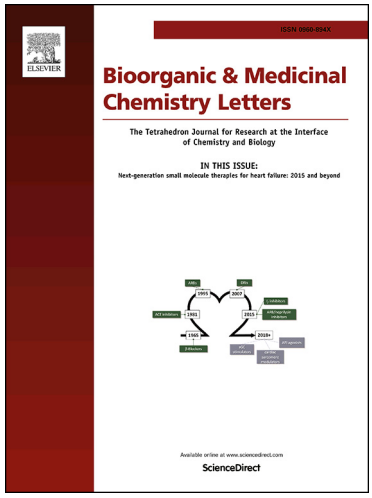

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Design and Synthesis of Histamine H_3/H_4 Receptor Ligands with a Cyclopropane Scaffold

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Abstract

We previously designed and synthesized a series of histamine analogues with an imidazolylcyclopropane scaffold and identified potent non-selective antagonists for histamine H_3 and H_4 receptor subtypes. In this study, to develop H_4 selective ligands, we newly designed and synthesized cyclopropane-based derivatives having an indole, benzimidazole, or piperazine structure, which are components of representative H_4 selective antagonists such as JNJ7777120 and JNJ10191584. Among the synthesized derivatives, imidazolylcyclopropanes **12** and **13** conjugated with a benzimidazole showed binding affinity to the H_3 and H_4 receptors comparable to that of a well-known non-selective H_3/H_4 antagonist, thioperamide. These results suggest that the binding modes of the cyclopropane-based H_3/H_4 ligands in the H_4 receptor can be different from those of the indole/benzimidazole-piperazine derivatives.

Keywords

histamine; GPCR; H_3 receptor; H_4 receptor; antagonist; cyclopropane

Highlights

- A new series of cyclopropane-based histamine analogs were designed and synthesized.
- Compounds **12** and **13** bound to both the H_3 and H_4 receptors.
- Their affinities were comparable to a well-known H_3/H_4 antagonist, thioperamide.

Histamine (**1**, Fig. 1), one of the neurotransmitters, functions through four different histamine receptor subtypes in the whole body. These receptor subtypes, which belong to seven-transmembrane G protein-coupled receptors (GPCRs), are classified into H_1, H_2, H_3, and H_4 receptors. Among them, the H_4 receptor (H_4R) cloned in early the 2000s is mainly expressed in various immune system cells such as eosinophils, dendritic cells, mast cells, and leukocytes.[1] The H_4R is also involved in cutaneous tissues and central nervous system.[2] Since indole- or benzimidazole-piperazine derivatives, JNJ7777120 (**2**) and JNJ10191584 (**3**) shown in Fig. 1, were identified as the first non-imidazole H_4R selective antagonists through high-throughput screening,[3] various physiological functions of the H_4R have been elucidated, and the H_4R has been expected as a therapeutic target for autoimmune and inflammatory diseases.[4] In fact, for example, an H_4R selective antagonist, JNJ38518168 (**4**), advanced to Phase II in the clinical trial as a remedy for asthma and rheumatoid arthritis. Additionally, H_4R antagonists have shown synergistic effects with H_1 receptor (H_1R) antagonists in the treatment of allergic diseases including atopic dermatitis.[5] Thus, H_4R selective antagonists can be high potential therapeutic candidates, although there are no ligands as approved medicines yet.

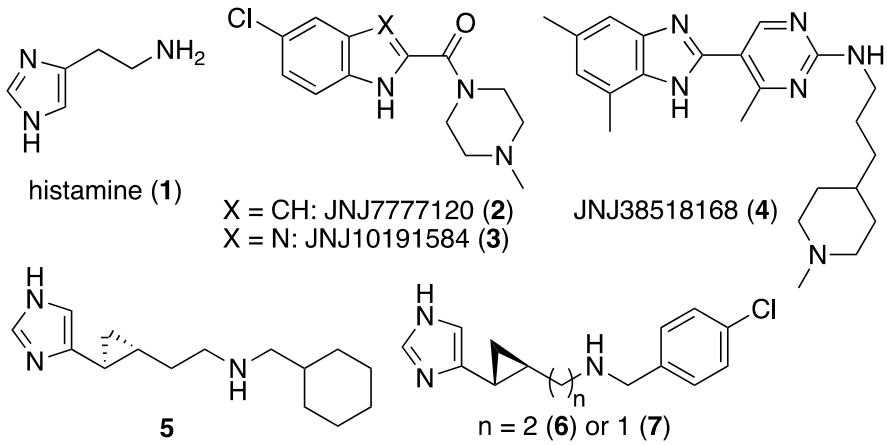


Figure 1. Chemical structures of histamine (**1**), representative indole/benzimidazole-piperazine type of H_4 receptor antagonists (**2**–**4**), and imidazolylcyclopropane H_3 and/or H_4 antagonists (**5**–**7**).

In design of bioactive molecules, three-dimensional structure information of the target protein is significantly beneficial. However, the X-ray crystal structure analysis of the H_4R has not succeeded. Although there are various reports of homology modeling of the H_4R based on the crystal structure of the bovine rhodopsin,[6] the adrenergic GPCR,[7] or the H_1R,[8] the estimated binding modes of histamine and H_4R ligands are somewhat different depending on the models.[9] Therefore, the precise binding modes are still unclear.

To efficiently develop bioactive ligands even if the structural information of the target protein is unknown, we have presented a three-dimensional structural diversity-oriented strategy based on structural properties of a chiral cyclopropane.[10] Our strategy has allowed us to design and synthesize a series of cyclopropane-based conformationally restricted histamine analogues having imidazolylcyclopropane scaffold.[11] Through the studies, we successfully identified a selective and potent antagonist **5** for the H_3 receptor (H_3R) (H_3, K_i = 5.3 nM; H_4, K_i = 127 nM) and a non-selective potent antagonist **6** for the H_3R and the H_4R (H_3, K_i = 8.4 nM; H_4, K_i = 7.6 nM) shown in Fig. 1.[11b] However, as for H_4R selectivity, even the most potent H_4R-selective compound **7** in the series of the imidazolylcyclopropane derivatives, the selectivity and potency were moderate (H_3, K_i > 1,000 nM; H_4, K_i = 118 nM). Thus, in this study, we aimed to convert the potent H_3/H_4 dual antagonist **6** and the moderate H_4R-selective antagonist **7** into a highly H_4R-selective ligand. The indole/benzimidazole-piperazine derivatives have two nitrogen-containing ring moieties, an indole/benzimidazole (***A***) and piperazine (***B***), in their structures (Fig. 2). We thought that these moieties, ***A*** or ***B***, might make the molecules highly H_4R-selective. The imidazolylcyclopropane derivatives also have two nitrogen-containing moieties, an imidazole (***C***) and benzylamine (***D***), which correspond to the ***A*** or ***B***. Thus, we have designed cyclopropane derivatives **8**–**13** as H_4 selective antagonists, which have the ***A*** or ***B*** moiety instead of the ***C*** or ***D*** in the imidazolylcyclopropane derivatives (Fig. 2). This molecular design might improve the H_4R selectivity of cyclopropane-based derivatives to give an insight into the binding modes of the cyclopropane-based compounds and the indole/benzimidazole-piperazine derivatives to the H_4R.

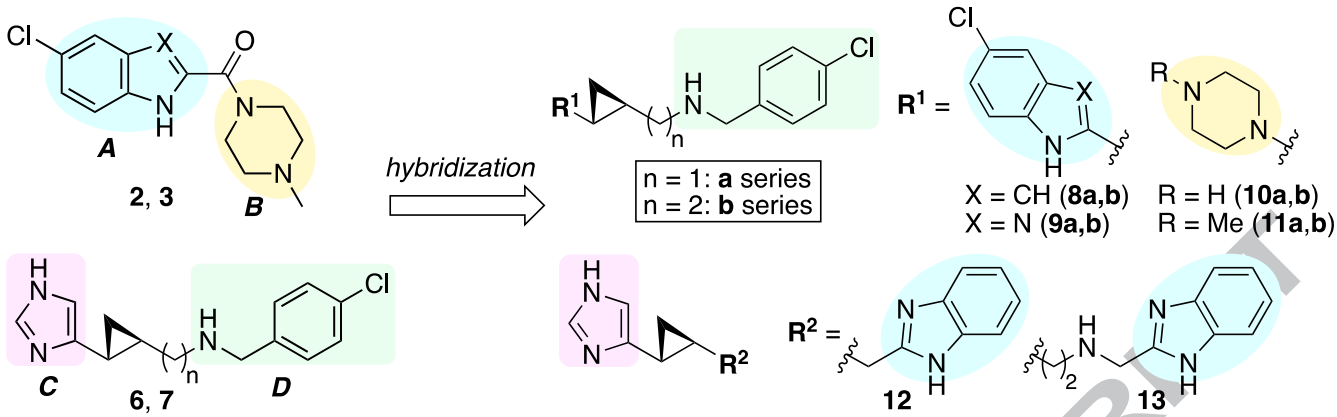
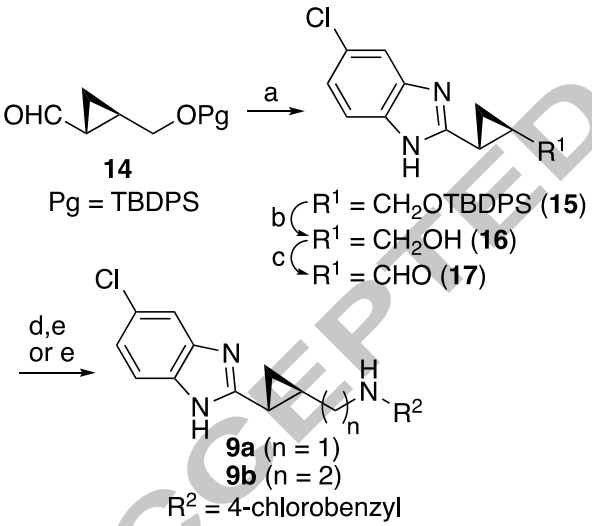


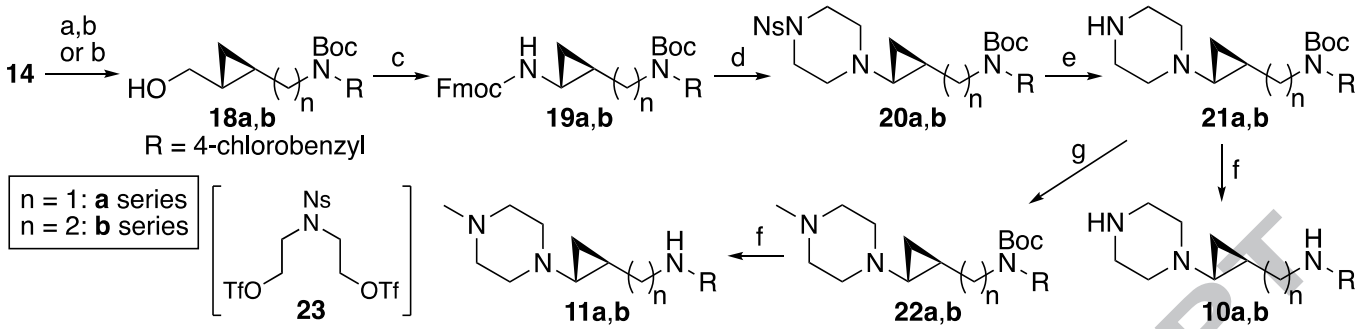
Figure 2. Design of cyclopropane derivatives **8**–**13** by hybridization of the imidazolylcyclopropane derivative with the indole/benzimidazole-piperazine derivative.

All of the designed compounds **8**–**13** was synthesized from *trans*-cyclopropane aldehyde **14**, which was prepared following a procedure we reported previously.[12] Treatment of **14** with 4-chlorobenzene-1,2-diamine in pyridine constructed benzimidazole structure of **15**, which was subjected to deprotection and subsequent oxidation conditions to give aldehyde **17** (Scheme 1). Reductive amination of **17** with 4-chlorobenzylamine afforded **9a**. Wittig reaction of **17** using $MeOCH_2PPh_3Cl$ and NaHMDS, followed by acidic hydrolysis and subsequent reductive amination provided **9b**. Indole derivatives **8a** and **8b** were synthesized using a method we reported.[13]



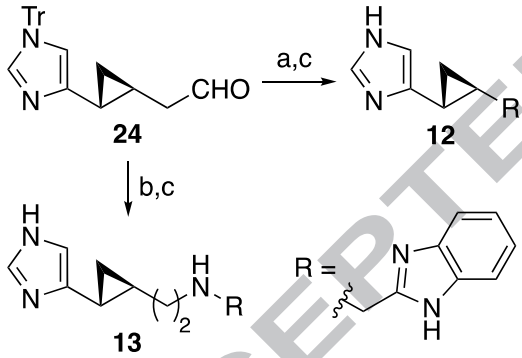
Scheme 1. Reagents and conditions. (a) 4-chlorobenzene-1,2-diamine, pyridine, reflux, 73%; (b) TBAF, THF, 87%; (c) Dess–Martin periodinane, CH_2Cl_2, quant.; (d) (1) $MeOCH_2PPh_3Cl$, NaHMDS, THF, 0 °C; (2) aq. HCl, THF, 0 °C; (e) 4-chlorobenzylamine, $NaBH(OAc)_3$, CH_2Cl_2, MS4A, 76% (**9a**), 42% (**9b**) for 3 steps.

Reductive amination of **14** with 4-chlorobenzylamine and subsequent protecting group manipulation gave **18a** (Scheme 2). Stepwise Dess–Martin and Pinnick oxidations of **18a** and the subsequent conversion to the corresponding acid azides, followed by Curtius rearrangement provided **19a**. After removal of the Fmoc group of **19a**, a piperazine structure was constructed with ditriflate **23**[14] to afford **20a**, which was treated with PhSH to give **21a**. Removal of the Boc group of **21a** gave **10a**. *N*-methylation of **21a** using formaldehyde and subsequent deprotection of the amino group provided **11a**. Wittig reaction of **14** with $MeOCH_2PPh_3Cl$/NaHMDS, followed by acidic treatment gave the corresponding one-carbon elongated aldehyde, which was subjected to the same procedure described above produced **10b** and **11b**.



Scheme 2. Reagents and conditions. (a) (1) $MeOCH_2PPh_3Cl$, NaHMDS, THF, 0 °C; (2) aq. HCl, THF, 0 °C; (b) (1) 4-chlorobenzylamine, $NaBH(OAc)_3$, CH_2Cl_2, MS4A; (2) Boc_2O, Et_3N, DMAP, MeOH; (3) TBAF, THF, 73% (**18a**) for 3 steps, 43% (**18b**) for 5 steps; (c) (1) Dess–Martin periodinane, pyridine, CH_2Cl_2; (2) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, aq. *t*BuOH; (3) $(PhO)_2P(O)N_3$, Et_3N, CH_2Cl_2; (4) 9-fluorenylmethanol, toluene, 80 °C, 67% (**19a**) and 84% (**19b**) for 4 steps; (d) (1) K_2CO_3, MeOH; (2) **23**, K_2CO_3, CH_3CN, 60 °C, 74% (**20a**) and 89% (**20b**) for 2 steps; (e) PhSH, K_2CO_3, CH_3CN, 72% (**21a**), 69% (**21b**); (f) aq. HCl, EtOH, reflux, quant. (**10a**), quant. (**10b**), quant. (**11a**), quant. (**11b**); (g) aq. HCHO, $NaBH_3CN$, AcOH, MeOH, 85% (**22a**), 56% (**22b**).

Treatment of **24**, which was prepared from **14**,[12] with benzene-1,2-diamine in pyridine to construct a benzimidazole structure and subsequent removal of the trityl group afforded **12** (Scheme 3). Reductive amination of **24** with 2-benzimidazolylmethylamine, which was prepared from benzene-1,2-diamine and glycine,[15] followed by acidic hydrolysis gave **13**.



Scheme 3. Reagents and conditions. (a) benzene-1,2-diamine, pyridine; (b) RNH_2, $NaBH(OAc)_3$, CH_2Cl_2, MS4A; (c) aq. HCl, EtOH, reflux, 80% (**12**) and 47% (**13**) for 2 steps, respectively.

Binding affinities of **8**–**13** for the human H_3R subtype with [^{3}H]N^{α}-methylhistamine and the human H_4R subtype with [^{3}H]histamine were evaluated using cell membranes expressing the human H_3R or H_4R according to the reported method[11b] (Table 1). Compounds **8**–**11**, which have an indole, benzimidazole, or piperazine structure instead of the imidazole of parent compounds **6** and **7**, failed to show any significant binding to both the H_3R and H_4R (K_i > 1,000 nM). These results indicate that the bulkier heterocycles, indole and benzimidazole, than imidazole might cause a steric repulsion in the binding to the H_3R and H_4R. The non-planar six-membered ring structure, piperazine, also might be sterically repulsive in the binding and might not be able to form appropriate hydrogen-bonds between the receptors due to the different position of nitrogen from that of imidazole. These results suggest that, when a series of *trans*-cyclopropane-based histamine analogues bind to the H_3R or H_4R, the space accommodating the imidazole moiety seems to be limited.

On the other hand, imidazolylcyclopropane derivatives **12** and **13** having a 2-substituted benzimidazolyl group showed moderate binding affinities for both the H_3R and H_4R. Replacement of 4-chlororbenzylamino group of **7** with

a benzimidazolyl group (**12**) led to increase the affinity for the H_3R more than 5-fold (K_i = 186 nM for **12**; > 1,000 nM for **7**), whereas the replacement did not significantly affect the H_4R affinity (K_i = 295 nM for **12**; 118 nM for **7**). Notably, the K_i values of **13**, which have a benzimidazolyl group instead of 4-chlorophenyl group of **6**, to the H_3R (52 nM) and the H_4R (166 nM) were comparable to those of a well-known non-selective H_3/H_4 antagonist, thioperamide (H_3, 51 nM; H_4, 124 nM; its structure is shown in Fig. S1), although their values were bigger than those of **6** (H_3, 8.4 nM; H_4, 7.6 nM). The indole/benzimidazole-piperazine derivatives **2** and **3** are the highly H_4R selective antagonists; however, this series of *trans*-cyclopropane derivatives **8**–**13** with an indole, benzimidazole, or piperazine structure did not show the H_4R selectivity.

Table 1. Binding affinities of the compounds for the human H_3 and H_4 receptor subtypes[a]

Compound	H_3, K_i (nM)	H_4, K_i (nM)
8a	>1,000	>1,000
8b	>1,000	>1,000
9a	>1,000	>1,000
9b	>1,000	>1,000
10a	>1,000	>1,000
10b	>1,000	>1,000
11a	>1,000	>1,000
11b	>1,000	>1,000
12	186 ± 32	295 ± 49
13	52 ± 8.2	166 ± 23
6[b]	8.4 ± 1.5	7.6 ± 0.4
7[b]	>1,000	118 ± 27
thioperamide[b]	51 ± 3.8	124 ± 14

[a] Data are expressed as means ± SEM (n = 3). [b] Data were taken from ref. 11b.

In summary, to develop newly H_4R selective ligands, we hybridized our cyclopropane-based conformationally restricted histamine analogues with indole/benzimidazole-piperazine derivatives, which are representative H_4 selective antagonists. Whereas most of the synthesized derivatives showed no binding affinities for both the H_3R and H_4R, compound **13** showed the H_3/H_4 binding affinities comparable to thioperamide. The present study suggests that the binding modes of the cyclopropane-based ligands in the H_4R might be entirely different from those of the indole/benzimidazole-piperazine derivatives.

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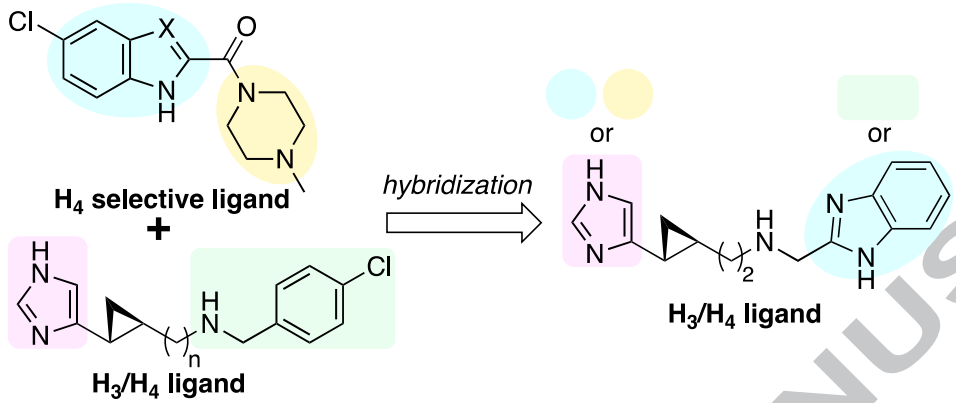

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