Article

Conformational Restriction of Histamine with a Rigid Bicyclo[3.1.0]hexane Scaffold Provided Selective H3 Receptor Ligands

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Abstract: We designed and synthesized conformationally rigid histamine analogues with a bicyclo[3.1.0]hexane scaffold. All the compounds were selectively bound to the H3 receptor subtype over the H4 receptor subtype. Notably, compound 7 showed potent binding affinity and over 100-fold selectivity for the H3 receptors (Ki = 5.6 nM for H3 and 602 nM for H4). These results suggest that the conformationally rigid bicyclo[3.1.0]hexane structure can be a useful scaffold for developing potent ligands selective for the target biomolecules.

Keywords: histamine; H3 receptor; conformational restriction; selective ligands

1. Introduction

Histamine (1, Figure 1), a monoamine chemical mediator, acts on four different receptor subtypes, H1, H2, H3, and H4 receptors, which are members of the G protein-coupled receptor superfamily. In early the 1980s, the histamine H3 receptor (H3R) was identified as a presynaptic autoreceptor in rats [1], and the human H3R was cloned in 1999 [2]. The H3R, which is mainly expressed in the central nervous system (CNS), not only acts as an autoreceptor to regulate the central levels of histamine but also acts as a heteroreceptor to regulate the synthesis and release of other neurotransmitters, such as noradrenaline, acetylcholine, dopamine, and serotonin, and it is involved in various physiological processes, including memory function, cognition, anxiety, pain, food intake, and body temperature regulation. Thus, H3R activity inhibition could be a potential treatment for various CNS-related disorders, such as attention-deficit hyperactivity disorder, Alzheimer’s disease, narcolepsy, Parkinson’s disease, schizophrenia, and obesity. Much effort has been devoted to developing potent and selective H3R inhibitors (agonists/inverse agonists) [3–5], and pitolisant (2, Figure 1) was recently authorized in Europe as a medicine for treating narcolepsy with or without cataplexy [6]. Further research is needed to better understand the functions of the histaminergic system in CNS-related disorders and to advance useful H3 antagonists into clinical trials and the market [7].
which are fixed in an eclipse orientation on the cyclopropane ring. The stable conformation is where the

we developed a three-dimensional structural diversity-oriented strategy based on the characteristic

Therefore, one restricted structure of these isomers may be analogous to the bioactive form of the parent

using MacroModel 8.6 showed that the

smallest group on the C3 position orients inward to the cyclopropane ring. Conformational analysis

cis- and trans-configured substituents, which are the two most stable conformations

orient to the side opposite and same to the cyclopropane ring, respectively, due to the C2–C3 bond

2.1. Design

The development of efficient H3-selective antagonists is often complicated by the high affinity

of H3 antagonists for the H4 receptor (H4R) because the structures of the two receptor subtypes

are presumed to be analogous due to their highly homologous gene sequences (58% identity in the transmembrane regions) [8,9]. However, precise three-dimensional structural information of H3R and H4R is unavailable, because their X-ray crystal structures have not been reported. To efficiently develop bioactive ligands, even if the structural information of the target proteins is unknown, we developed a three-dimensional structural diversity-oriented strategy based on the characteristic structural properties of chiral cyclopropane, which is the rigid and smallest carbocyclic structure [10]. Introducing cyclopropane into the conformationally flexible acyclic backbone of a parent compound can restrict the conformation of the molecule in either its trans- or cis-form. That is, four stereoisomer derivatives, including the enantiomers, can be produced from one parent ligand, in which the functional groups essential for the binding to a target protein can be restricted in different spatial arrangements. Therefore, one restricted structure of these isomers may be analogous to the bioactive form of the parent ligand, resulting in the discovery of a lead compound that is superior to the prototype ligand in its target selectivity and/or binding potency [11–15]. Based on this strategy, we previously designed and synthesized a series of cyclopropane-based conformationally restricted histamine analogues having an imidazolyccyclopropane scaffold and identified 3 and its enantiomer ent-3 as potent H3 antagonists ($K_i$: 3.6 nM and 8.4 nM for the H3R, respectively, shown in Figure 1) [16]. These antagonists have high binding affinities for the H3R; however, the selectivity between H3R and H4R is insufficient with selectivity ratios for H4/H3 of 10.3 (3) and 0.9 (ent-3). Thus, in this study, to obtain more highly selective ligands for H3R, we adopted bicyclo[3.1.0]hexane as a more rigid scaffold for newly designed conformationally restricted histamine analogues [17].

2. Results

2.1. Design

We assumed that the reason for the low H4/H3 selectivity of 3 and ent-3 was the flexibility of the aminoalkyl side chain in the compounds. Although the backbone conformation is restricted to the trans-configuration, there are two conformers, anti- and syn-form, whose aminoalkyl chains orient to the side opposite and same to the cyclopropane ring, respectively, due to the C2–C3 bond rotation (Figure 2a). This bond rotation is limited by the “cyclopropyl strain” that causes a sizable steric repulsion similar to the 1,3-allylic strain between the two adjacent cis-configured substituents, which are fixed in an eclipse orientation on the cyclopropane ring. The stable conformation is where the smallest group on the C3 position orients inward to the cyclopropane ring. Conformational analysis using MacroModel 8.6 showed that the anti- and syn-forms of 3 are the two most stable conformations ($\Delta E = 0.3$ kcal/mol), whereas the A-form of 3 is extremely unstable due to the cyclopropyl strain ($\Delta E > 3$ kcal/mol, relative potential energy from the global minimum; Figure 2a).
The biological evaluation for H3 anti depending on the configuration at the cyclopropane adjacent carbon. On the other hand, instead of utilizing the cyclopropylic strain due to the cyclopropyl strain \[10\]. Accordingly, depending on the configuration at the C3 position, the conformation of the compounds can be restricted to the anti-form in 4 and the syn-form in 5. The biological evaluation for H3R demonstrated that the anti-restricted 4 exhibited high binding affinity for H3R \(K_i = 6.7 \text{nM}\) comparable to 3; on the other hand, the binding affinity of syn-restricted 5 for H3R \(K_i = 63.0 \text{nM}\) was more than 15-fold lower than that of 3. These results showed that the conformational restriction of the side chain to the anti- or syn-form affects the binding affinity to the target protein. Unfortunately, however, further biological assay showed that 4 and 5 not only bound to H3R but also to H4R and were non-selective for the two receptor subtypes (selectivity ratios for H4/H3 of 1.2–1.3, Table 1).

![Figure 2. (a) The anti-, syn- and A-forms in 3; (b) The conformationally restricted histamine analogues 4 and 5 with cyclopropyl strain. R indicates a 4-chlorobenzyl group.](image)

We designed and synthesized the C3-ethyl-substituted derivatives 4 and 5, as side-chain conformation-restricted analogues of 3 (Figure 2b) \[18\]. Introducing an ethyl group into the C3 position of 3 prevents the rotation of the side aminoalkyl-chain moiety to effectively restrict the conformation due to the cyclopropyl strain \[10\]. Accordingly, depending on the configuration at the C3 position, the conformation of the compounds can be restricted to the anti-form in 4 and the syn-form in 5.

![Table 1. Binding affinities of the compounds for the human H3 and H4 receptor subtypes.\(^a\)](table)

| Compound | Form | H3, Ki (nM) | H4, Ki (nM) | Selectivity Ratio (H4/H3) |
|----------|------|-------------|-------------|--------------------------|
| 6        | anti | 31.6 ± 8.2  | 501 ± 201   | 16                       |
| 7        | syn  | 5.6 ± 1.6   | 602 ± 343   | 108                      |
| ent-6    | anti | 21.9 ± 6.0  | 416 ± 155   | 19                       |
| ent-7    | syn  | 14.8 ± 3.8  | 69.2 ± 21   | 4.7                      |
| 3        | –    | 3.6 ± 0.4   | 37.2 ± 2.7  | 10                       |
| ent-3    | –    | 8.4 ± 1.5   | 7.6 ± 0.4   | 0.9                      |
| 4        | anti | 6.7 ± 0.4   | 9.0 ± 1.0   | 1.3                      |
| 5        | syn  | 63.0 ± 6.1  | 76.5 ± 14   | 1.2                      |
| Thioperamide | – | 51 ± 3.8 | 124 ± 14 | 2.4                      |

\(^a\) Data are expressed as means ± SEM \((n = 3–5)\). \(^b,c\) Data were taken from \[16\] (b) and \[18\] (c).

Taking these results into account, we planned to obtain highly selective ligands for H3R by adopting an alternative strategy that utilizes a rigid fused-ring structure as a scaffold (Figure 3). Cyclopropane-based conformationally restricted ligands have two side-chain orientations, anti and syn, as described above. The introduction of a “branch” substituent on the cyclopropane adjacent carbon in the side-chain allows the orientation to be restricted to anti or syn due to the cyclopropyl strain, depending on the configuration at the cyclopropane adjacent carbon. On the other hand, instead of utilizing the cyclopropyl strain, the side-chain orientation can be restricted to anti or syn by introducing a “splint” structure between the cyclopropane and the side chain to form a rigid bicyclic structure. Both of these different conformational restriction strategies can generate the anti- and syn-restricted cyclopropane derivatives. However, even if they are the same anti- or syn-restricted
analogs, the difference in the branch and splint units introduced to regulate the conformation may cause different biological profiles. Therefore, in this study, we newly designed conformationally restricted histamine analogues 6, 7, and their enantiomers ent-6, ent-7 with a bicyclo[3.1.0]hexane scaffold that can restrict the spatial arrangement of the imidazole and the aminoalkyl chain (Figure 4). The aminoalkyl chains of 6 and 7 were restricted to anti or syn, respectively, because the splint “two-carbon unit” forming the bicyclic backbone can lock the C2–C3 bond rotation. We previously used the bicyclo[3.1.0]hexane structure as a backbone for conformationally restricted analogues of γ-aminobutyric acid (GABA) and reported the first potent and selective inhibitor of BGT-1, which is a GABA transporter subtype [19]. Thus, we expected that the conformationally restricted histamine analogues with the bicyclo[3.1.0]hexane scaffold would show selectivity for H₃R, in contrast to the previous cyclopropyl strain-based conformationally restricted analogues.

Figure 3. The two strategies to conformationally restrict the cyclopropane-based analogues to the anti- or syn-form by the introduction of a “branch” substituent inducing the cyclopropyl strain or a “splint” moiety forming the bicyclic structure.

Figure 4. The conformationally restricted histamine analogues 6 and 7 with a bicyclo[3.1.0]hexane backbone, and their enantiomers ent-6 and ent-7. R indicates a 4-chlorobenzyl group.

2.2. Chemistry

The diastereomerically pure bicyclo[3.1.0]hexane 12 and 13 with all of the asymmetric centers in target compounds 6 and 7, respectively, were synthesized from ketone 8 [19], which was prepared from (S)-epichlorohydrin, as shown in Scheme 1. After removing the O-silyl protecting group of 8, one-carbon addition to 9 was conducted by Wittig reaction to give olefin 10 as an E/Z mixture. The treatment of 10 with p-toluenesulfonyl acid in MeOH under reflux conditions afforded dimethyl acetal 11 as a diastereomeric mixture. Oxidation of the hydroxyl group of 11 and final separation by silica gel column chromatography gave the aldehyde isomers 12 and 13, respectively.
The conformationally restricted histamine analogue 6 was synthesized from 12 (Scheme 2). The imidazole ring was constructed by treating 12 with tosylmethyl isocyanide, followed by saturated ethanolic ammonia [20]. The resulting unpurified imidazole product was further treated with trityl chloride to afford N-tritylimidazolylcyclopropane 14 in 69% overall yield. Removal of the dimethyl acetal group under acidic conditions followed by reductive amination with 4-chlorobenzyl amine gave the desired secondary amine, which was protected by a Boc group for easy purification. Finally, acidic treatment of the protected amine simultaneously removed the N-Boc and N-Tr groups to provide the desired compound 6. The diastereomer 7 was similarly prepared from 13 (Scheme S1). Configurations of the C3 position in 6 and 7 were determined based on the nuclear Overhauser effect (NOE) difference spectra (Figure S1). In addition, enantiomers ent-6 and ent-7 were synthesized by the same procedure from ent-8, which was prepared from (R)-epichlorohydrin (Scheme S2).

Scheme 2. Reagents and conditions. (a) (1) TsCH2NC, NaOEt, EtOH, 0 °C; (2) sat. NH3/EtOH, 125 °C, sealed tube; (3) TrCl, Et3N, CH2Cl2, 69% in 3 steps; (b) (1) HCO2H, hexane (2) 4-chlorobenzyl amine, NaBH(OAc)3, MS4Å, CH2Cl2; (3) Boc2O, Et3N, DMAP, MeOH; (4) aq. HCl, EtOH, reflux, 49% in 4 steps.

2.3. Biological Evaluation

The binding affinities of 6, 7, ent-6, and ent-7 for the human H3R subtype with [3H]Nα-methylhistamine and the human H4R subtype with [3H]histamine were evaluated using cell membranes expressing human H3R or H4R according to the previously reported method, and the results are summarized in Table 1. These conformationally locked compounds, 6, 7, ent-6, and ent-7 had potent binding affinities for H3R, although their Ki values were slightly larger than those of their parent compounds 3 or ent-3 [Ki: 6 (31.6 nM), 7 (5.6 nM) versus 3 (3.6 nM) and ent-6 (21.9 nM) versus ent-3 (8.4 nM)]. On the other hand, their Ki values for H4R were much larger than those of the parents [Ki: 6 (501 nM), 7 (602 nM) versus 3 (37.2 nM) and ent-6 (416 nM), ent-7 (69.2 nM) versus ent-3 (7.6 nM)]. Thus, compared to the parents, the bicyclo compounds exhibited clearly improved selectivity for H3R. Notably, 7 showed highly potent binding affinity and more than 100-fold selectivity for H3R over the H4R.
3. Discussion

Comparison of the most stable conformations of 7 and the syn-form in 3 that were obtained by the MacroModel calculations revealed that the imidazole and amino moieties, which are essential for binding to the receptors, are superimposable; however, the splint “two-carbon unit” introduced for forming the bicyclo backbone of 7 is rather bulky and protrudes from the backbone of 3 (Figure 5). Therefore, the higher H3R selectivity of the bicyclo compounds than that of the parents (Table 1) suggest that the splint unit in 6 and 7 caused a steric repulsion in the binding mode for H4R, resulting in their low affinity for H4R. In other words, there seems to be a difference in the three-dimensional structures between H3R and H4R around the imidazole binding area in their orthosteric sites.

![Figure 5](image)

The syn-restricted 7 with the bicyclo scaffold displayed high selectivity for H3R, although the similarly syn-restricted 5 with a branched ethyl group for the cyclopropylic strain was non-selective for H3R and H4R (Table 1). Compared with their three-dimensional structures, the most stable conformations of 5 and 7 calculated by MacroModel are superimposable at the essential imidazole and amino functions (Figure 6). However, the “branch” and “splint” substituents, i.e., the ethyl group introduced to restrict the conformation by the cyclopropylic strain in 5 and the “two-carbon unit” moiety to form the bicyclo scaffold in 7, are positioned differently in space. In addition, in the most stable conformations of anti-restricted 4 and 6, the imidazole and amino functions are superimposable, but the branch and splint moieties are differently positioned (Figure S3). These results indicate that even when restricted to the same anti- or syn-form, the “branch” or “splint” moieties that were introduced to restrict the conformation may interact with the target receptor or undergo steric repulsion due to the difference in the spatial arrangement of the moieties, potentially causing the different subtype selectivity. For example, among these four compounds, 4–7, only 4 had a high binding affinity for H4R ($K_1 = 9.0$ nM, Table 1).

![Figure 6](image)

The rigid bicyclo[3.1.0]hexane scaffold was effectively used for the conformational restriction of cyclopropane side chains to improve the H3R/H4R-selectivity, similar to the previous results in the development of a selective inhibitor for the GABA transporter BGT-1 subtype [19]. On the other hand, the cyclopropylic strain-based conformational restriction strategy has successfully provided a variety of compounds of biological interest, such as NMDA receptor antagonists [21], proteasome inhibitors [22], melanocortin receptor antagonists [23], and membrane-permeable cyclic peptides [24]. Thus, the cyclopropylic strain-based conformational restriction strategy has successfully provided a variety of compounds of biological interest, such as NMDA receptor antagonists [21], proteasome inhibitors [22], melanocortin receptor antagonists [23], and membrane-permeable cyclic peptides [24].
inhibitors [22], melanocortin receptor antagonists [23], and membrane-permeable cyclic peptides [24]. Thus, the two conformational restriction methods using cyclopropane as the key structure effectively complement each other in medicinal chemistry studies for developing potent and selective ligands for various target proteins.

4. Materials and Methods

4.1. Synthesis

All $^1$H-NMR and $^{13}$C-NMR spectra were recorded on a JEOL JNM-AL-400, JEOL JMM-ECX-400P, or JEOL JMM-ECA-500 spectrometer. $^1$H-NMR chemical shifts are reported as δ values in ppm relative to tetramethylsilane (0.00 ppm) when CDCl$_3$ was used as the solvent, or a solvent residual peak (CD$_2$H-OD: 3.31 ppm) when CD$_2$OD was used as the solvent. Coupling constants (J) are reported in Hz, and multiplicity is indicated as follows: s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), brs (broad singlet). $^{13}$C-NMR chemical shifts are reported as δ values in ppm relative to deuterated solvent (CDCl$_3$: 77.0 ppm or CD$_2$OD: 49.0 ppm). All mass spectra were obtained on a JEOL JMS-T100LC spectrometer. Elemental analysis was performed with a Yanaco CHN Corder MT-6 or J-Science MICRO CODER JM10 analyzer. Optical rotations were measured with a JASCO P-1030 digital polarimeter. All non-aqueous reactions were carried out under argon atmosphere in anhydrous deuterated solvent (CDCl$_3$ or CD$_2$OD).

(1S,5S)-1-Hydroxy(bicyclo[3.1.0]hexan-4-one (9). The mixture of 8 (983 mg, 2.70 mmol) and 3HF•Et$_3$N (2.2 mL, 6.99 mmol) in THF (25 mL) was stirred at room temperature (rt) for 3 days. The solvent was evaporated, and the residue was purified by flash silica gel column chromatography (hexane/AcOEt, 1/1-0/1) to give 9 (307 mg, 2.43 mmol, 90%) as a colorless liquid. [α]$^D=$+4.1° (c 0.90, CHCl$_3$); $^1$H-NMR (400 MHz, CDCl$_3$) δ 5.85 (1 H, d, J = 11.6 Hz, –CHaHbOH), 3.66 (1 H, d, J = 11.6 Hz, –CHaHbOH), 2.22–2.09 (4 H, m, H-2, H-3), 1.78 (1 H, dd, J = 9.1, 3.2 Hz, H-5), 1.33 (1 H, dd, J = 9.1, 5.0 Hz, H-6a), 1.19 (1 H, dd, J = 5.0, 3.2 Hz, H-6b); $^{13}$C-NMR (100 MHz, CDCl$_3$) δ 124.98, 64.67, 35.92, 33.00, 31.99, 23.83, 17.55; HRMS (ESI) calcld for C$_7$H$_{10}$NaO$_2$ 149.0573, found 149.0573 [(M + Na)$^+$].

(1S,5S)-1-Hydroxymethyl-4-methoxymethylenebicyclo[3.1.0]hexane (10, E/Z-mixture). A suspension of methoxymethyltriphenylphosphonium chloride (2.96 g, 8.52 mmol) in THF (20 mL) was stirred at 0°C for 3 days. After the addition of sat. aq. NH$_4$Cl, the reaction mixture was concentrated in reduced pressure, and the residue was partitioned between AcOEt and sat. aq. NH$_4$Cl. The organic layer was washed with brine, dried (Na$_2$SO$_4$), and evaporated. The residue was purified by silica gel column chromatography (hexane/AcOEt, 50/1) to give 10 (338 mg, 2.19 mmol, 90%, E/Z-mixture, 1/0.8) as a pale yellow oil. [α]$^D=$+49.6° (c 1.00, CHCl$_3$); HRMS (ESI) calcld for C$_9$H$_{14}$NaO$_2$ 177.0886, found 177.0887 [(M + Na)$^+$]; Major isomer, $^1$H-NMR (400 MHz, CDCl$_3$) δ 5.85 (1 H, s, MeOCH=–C–), 3.62 (2 H, s, –CH$_2$OH), 3.55 (3 H, s, CH$_2$O–), 3.02 (1 H, brs, OH), 2.12 (1 H, m, H-3a), 1.97 (1 H, dd, J = 8.1, 3.6 Hz, H-5), 1.93–1.78 (3 H, m, H-2, H-3b), 0.81 (1 H, dd, J = 8.1, 4.5 Hz, H-6a), 0.74 (1 H, m, H-6b); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 137.57, 121.41, 67.07, 59.14, 33.11, 28.16, 24.60, 22.69, 14.27; Minor isomer, $^1$H-NMR (400 MHz, CDCl$_3$) δ 5.92 (1 H, s, MeOCH=–C–), 3.62 (2 H, s, –CH$_2$OH), 3.53 (3 H, s, CH$_2$O–), 3.02 (1 H, brs, OH), 2.55 (1 H, m, H-3a), 1.93–1.77 (3 H, m, H-2, H-3b), 1.61 (1 H, dd, J = 8.1, 3.6 Hz, H-5), 0.73 (1 H, m, H-6a), 0.61 (1 H, m, H-6b).

(1S,5R)-4-Dimethoxymethyl-1-hydroxymethylbicyclo[3.1.0]hexane (11, diastereomixture). The mixture of 10 (20 mg, 0.13 mmol) and p-toluenesulfonic acid monohydrate (5.0 mg, 0.026 mmol) in MeOH (2 mL) was stirred under reflux conditions for 3 h. After the addition of sat. aq. NaHCO$_3$ at 0°C, the resulting mixture was concentrated in reduced pressure. The residue was partitioned between AcOEt and sat. aq.
NaHCO₃. The organic layer was washed with brine, dried (Na₂SO₄), and evaporated. The residue was purified by flash silica gel column chromatography (hexane/AcOEt, 4/1–0/1) to give 11 (20 mg, 0.11 mmol, 85%, diastereomixture, 1/0.8) as a pale yellow liquid. [α]₀°D = +54.1° (c 1.18, CHCl₃); HRMS (ESI) calcd for C₁₀H₁₂NaO₅ 209.1148, found 209.1149 [(M + Na)⁺]. Major diastereomer, ¹H-NMR (400 MHz, CDCl₃) δ 4.10 (1 H, d, J = 9.0 Hz, –CH(CH(OMe))₂), 3.74 (1 H, d, J = 11.4 Hz, –CH₂(OMe)), 3.45 (1 H, d, J = 11.4 Hz, –CH₂(OH)), 3.39 (3 H, s, –OCH₃), 3.37 (3 H, s, –OCH₃), 2.24 (1 H, m, H-4), 1.93 (1 H, m, H-3a), 1.82 (1 H, m, H-2a), 1.77 (1 H, m, H-2b), 1.36 (1 H, m, H-3b), 1.18 (1 H, m, H-5), 0.58 (1 H, m, H-6a), 0.45 (1 H, m, H-6b); ¹³C NMR (100 MHz, CDCl₃) δ 107.66, 67.42, 52.99, 52.70, 42.27, 30.72, 28.70, 23.70, 22.74, 8.77; Minor diastereomer, ¹H-NMR (400 MHz, CDCl₃) δ 4.12 (1 H, d, J = 6.7 Hz, –CH(CH(OMe))₂), 3.64–3.57 (2 H, s, –CH₂OH), 3.39 (3 H, s, –OCH₃), 3.31 (3 H, s, CH₂O–), 2.50 (1 H, m, H-4), 1.82 (1 H, m, H-3a), 1.71–1.62 (2 H, m, H-2a), 1.18 (1 H, m, H-5), 1.02 (1 H, m, H-3b), 0.58 (1 H, m, H-6a), 0.41 (1 H, m, H-6b); ¹³C NMR (100 MHz, CDCl₃) δ 107.15, 67.19, 54.19, 53.15, 42.71, 31.13, 27.19, 23.49, 22.54, 11.85.

(1S,4S,5R)-4-Dimethoxymethyl-1-formylbicyclo[3.1.0]hexane (12, anti) and (1S,4R,5R)-4-Dimethoxymethyl-1-formylbicyclo[3.1.0]hexane (13, syn). To a solution of 11 (152 mg, 0.816 mmol) in DMSO (9.0 mL) were added Et₃N (0.349 mL, 2.46 mmol) and pyridine–SO₃ complex (262 mg, 1.64 mmol), and the mixture was stirred at rt for 3 h. After the addition of sat. aq. NH₄Cl, the mixture was partitioned between AcOEt and sat. aq. NH₄Cl. The organic layer was washed with brine, dried (Na₂SO₄), and evaporated. The residue was purified by flash silica gel column chromatography (hexane/AcOEt, 8/1) to give 12 (43 mg, 0.23 mmol, 29%, anti, less polar) as a colorless oil and 13 (78 mg, 0.43 mmol, 52%, syn, more polar) as a colorless oil. ¹H-NMR (400 MHz, CDCl₃) δ 8.90 (1 H, s, CHO), 3.98 (1 H, d, J = 8.2 Hz, –CH(CH(OMe))₂), 3.28 (3 H, s, –OCH₃), 3.26 (3 H, s, –OCH₃), 2.26 (1 H, dd, J = 8.2, 7.7 Hz, H-4), 2.16 (1 H, m, H-3a), 1.96 (1 H, dd, J = 9.1, 5.4 Hz, H-5), 1.73 (1 H, dd, J = 14.0, 8.6 Hz, H-2a), 1.64 (1 H, dd, J = 13.1, 8.6 Hz, H-5b), 1.40–1.29 (2 H, m, H-3b, H-6a), 1.02 (1 H, dd, J = 5.4, 5.4 Hz, H-6b); ¹³C NMR (100 MHz, CDCl₃) δ 200.19, 105.39, 53.41, 53.34, 42.03, 41.73, 29.44, 23.06, 22.81, 15.68; HRMS (ESI) calcd for C₁₀H₁₆NaO₃ 207.0992, found 207.0992 [(M + Na)⁺]; ¹H-NMR (400 MHz, CDCl₃) δ 8.87 (1 H, s, CHO), 4.10 (1 H, d, J = 7.7 Hz, –CH(CH(OMe))₂), 3.32 (3 H, s, –OCH₃), 3.26 (3 H, s, –OCH₃), 2.48 (1 H, m, H-4), 2.23 (1 H, m, H-3a), 1.90 (1 H, m, H-5), 1.76–1.66 (2 H, m, H-2), 1.26 (1 H, dd, J = 8.6, 5.9 Hz, H-6a), 1.16 (1 H, dd, J = 5.9, 5.4 Hz, H-6a), 1.05 (1 H, m, H-3b); ¹³C NMR (100 MHz, CDCl₃) δ 200.12, 106.86, 53.32, 52.81, 41.57, 41.26, 28.71, 23.97, 22.99, 13.24; HRMS (ESI) calcd for C₁₀H₁₆NaO₃ 207.0992, found 207.0993 [(M + Na)⁺].

(1S,4S,5R)-4-Dimethoxymethyl-1-(1-triphenylmethyl-1H-imidazol-4-yl)bicyclo[3.1.0]hexane (14). To a suspension of 12 (43 mg, 0.23 mmol) and tosylmethylisocyanide (50 mg, 0.26 mmol) in EtOH (2.3 mL) was added NaOEt (ca. 20% solution in EtOH, 23 μL, 0.053 mmol) at 0 °C, and the mixture was stirred at the same temperature for 3 h. To the reaction mixture was added a saturated NH₃ solution in EtOH (6 mL) at 0 °C, and the resulting mixture was heated at 125 °C for 20 h in a sealed tube. After being cooled to rt, the reaction mixture was concentrated in reduced pressure. The residue was dissolved in CH₂Cl₂ (3.2 mL), and to the solution were added Et₃N (96 μL, 0.69 mmol) and TrCl (128 mg, 0.84 mmol). The resulting mixture was stirred at rt for 5 h. After the addition of MeOH, the mixture was partitioned between AcOEt and sat. aq. NaHCO₃. The organic layer was washed with brine, dried (Na₂SO₄), and evaporated. The residue was purified by flash silica gel column chromatography (hexane/AcOEt, 10/1–3/1–1/1) to give 14 (75 mg, 0.12 mmol, 69% in 3 steps) as a pale yellow oil. ¹H-NMR (400 MHz, CDCl₃) δ 7.32–7.31 (10 H, m, aromatic), 7.15–7.13 (6 H, m, aromatic), 6.54 (1 H, s, imidazole-5), 4.15 (1 H, d, J = 8.1 Hz, –CH(CH(OMe))₂), 3.32 (3 H, s, –OCH₃), 3.30 (3 H, s, –OCH₃), 2.30 (1 H, dd, J = 8.1, 7.6 Hz, H-4), 2.02 (1 H, m, H-3a), 1.85 (1 H, dd, J = 12.1, 8.3 Hz, H-2a), 1.72 (1 H, dd, J = 13.9, 8.3 Hz, H-2b), 1.63 (1 H, m, H-5), 1.39 (1 H, m, H-3b), 1.15 (1 H, dd, J = 8.1, 4.5 Hz, H-6a), 0.72 (1 H, dd, J = 4.5, 4.5 Hz, H-6b); ¹³C NMR (100 MHz, CDCl₃) δ 144.6, 142.4, 138.2, 130.0, 129.7, 127.9, 116.3, 106.1, 75.0, 53.5, 52.8, 43.7, 29.0, 27.9, 27.0, 22.9, 14.6; HRMS (ESI) calcd for C₃₁H₃₂N₂Na₂O₂ 487.2356, found 487.2368 [(M + Na)⁺].
(1S,4S,5R)-4-[N-(4-Chlorobenzyl)aminomethyl]-1-(1H-imidazol-4-yl)bicyclo[3.1.0]hexane dihydrochloride (6•2HCl). To a suspension of 14 (49 mg, 0.105 mmol) in hexane (0.20 mL) was added formic acid (0.80 mL), and the mixture was stirred at rt for 3 h. After the addition of sat. aq. NaHCO₃ at 0 °C, the mixture was partitioned between AcOEt and sat. aq. NaHCO₃. The organic layer was washed with brine, dried (Na₂SO₄), and evaporated. The residue was dissolved in CH₂Cl₂ (3.5 mL), and to the solution was added 4-chlorobenzylamine (0.038 mL, 0.32 mmol), molecular sieves 4Å (powder, 40 mg), and sodium NaBH(OAc)₃ (27 mg, 0.13 mmol), and the mixture was stirred at rt for 15 h. After the addition of sat. aq. NaHCO₃, the mixture was partitioned between AcOEt and sat. aq. NaHCO₃. The organic layer was washed with brine, dried (Na₂SO₄), and evaporated. The residue was passed through silica gel column chromatography (CHCl₃/MeOH, 1/0–50/1–9/1) to give a corresponding secondary amine. To a solution of the amine in MeOH (1.1 mL) were added Et₃N (0.088 mL, 0.63 mmol), N,N-dimethyl-4-aminopyridine (DMAP, 3 mg, 0.025 mmol), and Boc₂O (0.113 mL, 0.54 mmol), and the mixture was stirred at rt for 5 h. The reaction mixture was concentrated in reduced pressure, and the residue was purified by flash silica gel column chromatography (hexane/AcOEt, 15/1–2/1) to give a corresponding N-Boc-amine (33 mg, 0.051 mmol) as a pale yellow oil. To a solution of N-Boc-amine (33 mg) in EtOH (1.1 mL) was added aq. HCl (12 M, 0.4 mL), and the mixture was stirred under reflux conditions for 3 h. After being cooled to rt, the reaction mixture was concentrated in reduced pressure. The residue was partitioned between aq. HCl (2 M) and CH₂Cl₂. The aqueous layer was neutralized by aq. NaOH (2 M) and partitioned between aq. NaOH (2 M) and CH₂Cl₂. The organic layer was washed with brine, dried (Na₂SO₄), and evaporated. The residue was purified by NH silica gel column chromatography (CHCl₃/MeOH, 1/0–50/1–9/1) to give 6 as a free amine. 6 was dissolved in a solution of HCl in MeOH (2 M), and the mixture was concentrated in reduced pressure. The residue was triturated with Et₂O to give 6•2HCl (19 mg, 0.051 mmol, 49% in 4 steps) as a hygroscopic white solid. [α]D²⁹ = +16.0° (c 0.66, CH₃OH); ¹H-NMR (400 MHz, CD₂OD) δ 8.81 (1 H, s, imidazole-2), 7.62–7.61 (2 H, d, J = 8.0 Hz, aromatic), 7.47–7.43 (3 H, m, aromatic and imidazole-5), 4.28 (2 H, s, benzyl), 3.19 (1 H, m, –CHCHaHbN–), 3.11 (1 H, m, –CHCHaHbN–), 2.54 (1 H, m, H-4), 2.23–2.08 (2 H, m, H-2a, and H-3a), 1.86 (1 H, m, H-5), 1.78 (1 H, m, H-2b), 1.66 (1 H, m, H-3b), 1.13 (2 H, m, H-6); ¹³C NMR (100 MHz, CD₂OD) δ 138.04, 136.66, 134.60, 133.13, 131.22, 130.25, 116.54, 52.43, 52.11, 39.05, 30.29, 29.85, 25.57, 24.42, 15.19; HRMS (ESI) calcd for C₁₇H₂₁N₃ClO 302.1419, found 302.1420 [(M + H)]⁺; Anal. Calcd for C₁₇H₂₁N₃Cl•2HCl: C, 53.96; H, 5.89; N, 11.11. Found: C, 53.90; H, 5.85; N, 11.07.

4.2. Biological Assay

The protocol of the biological assay was according to the previous report [16]. The membrane preparations of Chinese hamster ovary (CHO) cells, which expressed recombinant human histamine H₃ or H₄ receptors, were purchased from Euroscreen (Brussels, Belgium). The binding assay of the H₃ and H₄ receptors was performed using [³H]Nα-methylhistamine (Perkin-Elmer, Boston, MA) and [³H]histamine (Perkin-Elmer, respectively. Briefly, the membrane preparations (7.5–15 µg protein) were incubated with different concentrations of [³H]Nα-methylhistamine (0.1–3 nM) and of [³H]histamine (1–9 nM) for 30 min at 25 °C in 50 mM Tris/5 mM MgCl₂ 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 (K₄) for each radioligand was determined by nonlinear regression analysis of the curve generated by plotting specific binding concentration versus 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 [³H]Nα-methylhistamine (1.5 nM) and [³H]histamine (25 nM) was estimated by...
IC\textsubscript{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, \( K_i = \text{IC}_{50}/(1 + L/K_d) \), where \( L \) equals the concentration of each radioligand. The data were presented as mean $\pm$ SE (n = 3–5).

5. Conclusions

The imidazolyl bicyclo[3.1.0]hexane derivatives as conformationally restricted histamine analogues designed and synthesized in this study showed binding affinity selective for \( H_3 \)R over \( H_4 \)R, while the parent imidazolylcyclopropane derivatives showed no selectivity. Notably, compound 7 exhibited over 100-fold \( H_3 \)R selectivity. The findings of the present study suggest that the imidazolyl bicyclo[3.1.0]hexane structure is a more useful scaffold than the imidazolylcyclopropane structure for the development of selective \( H_3 \) receptor ligands. Using both conformationally restricted structures, bicyclo[3.1.0]hexane and cyclopropane with cyclopropylic strain, could allow us to produce more bioactive compounds for structurally unknown proteins, e.g., histamine \( H_3 \) and \( H_4 \) receptors, effectively.

**Supplementary Materials:** The followings are available online: Figures S1–S3; Schemes S1 and S2; Synthetic procedures and characterization of compounds 7, \textit{ent}-6, and \textit{ent}-7; \textit{H}-NMR spectra of compounds 6, 7, \textit{ent}-6, and \textit{ent}-7.

**Author Contributions:** M.W., T.K. and S.S. conceived this work, designed the compounds, performed the synthesis, and analyzed the data; Y.I. and S.Y. performed the biological assays and analyzed the data; M.W. and S.S. wrote the paper. All authors have read and agreed to the published version of the manuscript.

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**Sample Availability:** Samples of the compounds are not available from the authors.