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Brief Article

Role of Hydrophobic Substituents on the Terminal Nitrogen of Histamine in Receptor Binding and Agonist Activity: Development of an Orally Active Histamine Type 3 Receptor Agonist and Evaluation of Its Antistress Activity in Mice

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The terminal nitrogen atom of histamine was modified with lipophilic substituents to investigate the structure−activity relationship of histamine type 3 receptor (H3R) agonists. The introduction of an alkylated benzene rings maintained or increased the H3R binding affinity. The most potent compound, 4-(2-(4-*tert*-butylphenylthio)ethyl)-1*H*-imidazole, possessed in vivo agonistic activity, decreasing brain N^{τ}-methylhistamine levels in mice after oral administration. It also exhibited antistress activity in the mouse resident−intruder test.

Introduction

The histamine type 3 receptor (H3R[a]) was found in 1983 by Arrang and colleagues.[1] It acts not only as a presynaptic autoreceptor, inhibiting the synthesis and release of histamine in the brain, but also as a heteroreceptor modulating the release of other neurotransmitters in both the central and peripheral nervous systems.[2−5] Human H3R cDNA was identified in 1999 by Lovenberg and co-workers.[6] The H3R also exhibits constitutive G-protein-coupled receptor (GPCR) activity.[7,8] The H3R can signal independently of any agonist, and careful classification of agonist, pure antagonist, and inverse agonist is required in the study of H3R ligands. Many selective H3R ligands have been identified, and some H3R inverse agonists are in clinical trial for the treatment of cognitive disorders, dementia, or narcolepsy.[9−11] On the other hand, therapeutic application of H3R agonists,[12,13] has been limited because potent H3R agonists, such as (*R*)-α-methylhistamine (**1**) and imetit (**2**), are highly hydrophilic and show poor oral absorption or brain penatration[14,15] (Figure 1).

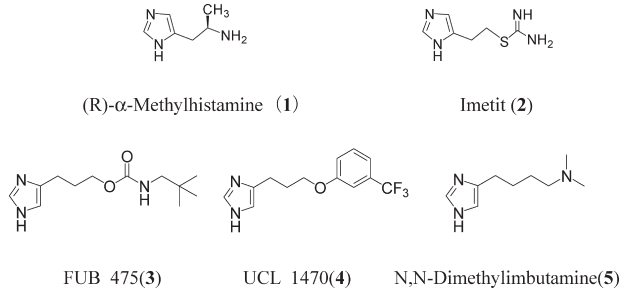

Figure 1

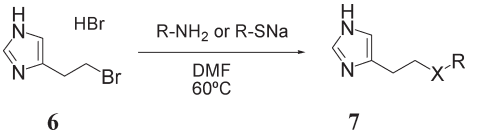

Figure 2

Recently, more lipophilic H3R agonists, such as FUB 475 (**3**)[16], UCL 1470 (**4**)[17], and *N*,*N*-dimethylimbutamine (**5**)[18], have been reported. All of them have a 4-alkylated imidazole ring and a terminal lipophilic moiety with or without a nitrogen atom. Compounds **3** and **4** showed H3R agonistic activity in vivo following oral administration. However, the structure−activity relationship of H3R agonists is still poorly understood. Modifications tend to change the agonistic activity to antagonistic or inverse agonistic activity, even if the binding affinity is maintained. It appears that the H3R strictly recognizes the hydrophobic part of ligands and may require a conformational change for signaling. We considered that modifying the corresponding part of the endogenous agonist histamine on the terminal nitrogen atom might afford orally available H3R agonists and provide information about the recognition of lipophilic structure by H3R. The aim of our study is, therefore, to find novel, orally active H3R agonists by modifying histamine with lipophilic substituents. The introduction of an alkylated benzene ring at the terminal nitrogen atom recovered or increased the agonistic activity toward human H3R. The most potent compound showed in vivo agonistic activity in mice, modulating the brain N^{τ}-methylhistamine level, and also showed antistress activity in the mouse resident−intruder test.

Chemistry. 4-(2-Bromoethyl)imidazole (**6**) was prepared according to Bloemhoff and Kerling.[19] Alkyl or aryl amines were introduced by heating the appropriate derivative with **6** in DMF. To obtain the thioether derivative, 4-*tert*-butylthiophenol was treated with NaH at 60 °C in DMF, then

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[a] Abbreviations: H3R, histamine type 3 receptor; cDNA, cloned DNA; GPCR, G-protein-coupled receptor; DMF, *N*,*N*-dimethylformamide; NaH, sodium hydride; [^{35}S]GTPγ[S], [^{35}S]guanosine 5′-[γ-thio]-triphosphate; CHO cell, Chinese hamster ovary cell; CYP, cytochrome P450; mRNA, mRNA; HPA, hypothalamic-pituitary-adrenal; CRF, corticotropin releasing factor.

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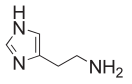
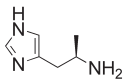
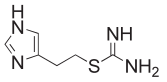
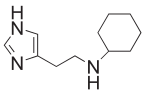
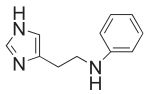
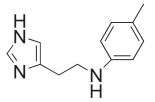
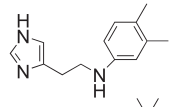
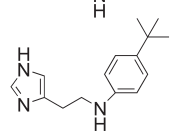
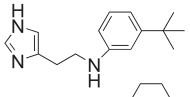
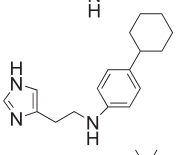
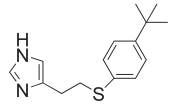
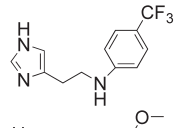
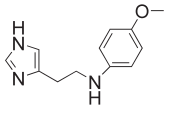
Table 1. In Vitro Binding and Functional Assay in Human H3R

compound		human H3R Ki(nM)[a]	human [35S]GTP[S] EC_{50}(nM)[a]	intrinsic activity (%)[a,b]
Histamine		5.2 ± 0.5	640 ± 20	110 ± 16
1		0.55 ± 0.09	2.8 ± 0.6	100 ± 3
2		0.57 ± 0.02	2.4 ± 0.6	91 ± 5
7a		1600 ± 50	3400 ± 100	31 ± 2
7b		56 ± 3	320 ± 70	42 ± 2
7c		14 ± 1	88 ± 10	49 ± 2
7d		9.9 ± 1	25 ± 6	59 ± 2
7e		0.89 ± 0.10	1.6 ± 0.5	70 ± 2
7f		2.2 ± 0.4	4.6 ± 2	48 ± 1
7g		4.1 ± 0.4	NA[c]	NA[c]
7h		0.17 ± 0.02	0.75 ± 0.10	77 ± 1
7i		1.1 ± 0.10	19 ± 4	56 ± 1
7j		24 ± 2	65 ± 6	56 ± 3

[a] Each result represents the mean ± SEM ($n = 3$). [b] Percent ratio to the response obtained from **1**. [c] Not active.

4-(2-bromoethyl)imidazole was added to the reaction mixture, and further heating for 16 h afforded the coupling compound **7h** (Figure 2).

Pharmacology. In Vitro Human H3R Studies. The affinity (pK_i) values and the functional activities were determined using CHO cells stably expressing the human H3R.[20,21] The procedures are described in Supporting Information.

In Vivo Mouse Studies. The N^{τ}-methylhistamine level in the brain of mice was measured following oral administration of the test compounds.[22,23] The mouse resident−intruder test was performed according to Sanchez and Hyttel.[24] The procedures are described in Supporting Information.

Results and Discussion

The results of examination of H3R binding and agonist activity of the synthesized compounds are presented in Table 1. Compared with the standard compounds **1** and **2**, introduction of a cyclohexane ring resulted in loss of agonistic activity (**7a**). Intriguingly, the change of a cyclohexane ring (**7a**) to a benzene ring (**7b**) clearly improved the binding affinity

Table 2. N^{τ}-Methylhistamine Level (ng/g Tissue) in the Brain of Mice Following Oral Administration[a]

compd	control	1 mg/kg	5 mg/kg	30 mg/kg
2	315 ± 20	239 ± 19	133 ± 14	98 ± 11[b]
7h	315 ± 20	298 ± 38	92 ± 7[b]	96 ± 8[b]

[a] Each result represents the mean ± SEM of five animals. [b] $p < 0.01$ vs vehicle control.

Table 3. In Vitro and in Vivo Rat Pharmacokinetic Parameters for **7h**

in vitro[a]	in vivo[b] (2 mg/kg, iv)			in vivo[b] (5 mg/kg, po)		
S9 metabolic activity (pmol/min/mg protein)	Cl_{tot} (L/h/kg)	V_{dss} (L/kg)	$t_{1/2}$ (h)	AUC_{0-inf} (mg·h/mL)	BA (%)	brain/plasma ratio[c]
13.0 ± 0.9	2.8	3.4	1.0	0.99	53.5	6.0

[a] Mean ± SEM, $n = 3$. [b] $n = 2$. [c] Ratio at 1 h after administration.

(K_i = 1600−56 nM). 4-Methylation of the aromatic ring of **7b** increased the H3R agonist activity (**7c**, EC_{50} = 88 nM). A 3,4-dimethylbenzene ring resulted in a moderate improvement of H3R affinity (**7d**). Optimization of the para substitution of the aromatic ring for agonistic activity led to the *tert*-butyl moiety; the 4-*tert*-butyl compound exhibited excellent activity (**7e**, K_i = 0.89 nM, EC_{50} = 1.6 nM, ia = 70%). The 3-*tert*-butyl compound (**7f**) was inferior to the 4-*tert*-butyl derivative. The introduction of a bulkier alkyl group, a cyclohexane ring, resulted in loss of agonistic activity, even though high affinity was retained (**7g**, K_i = 4.1 nM). The 4-*tert*-butyl phenyl thioether compound **7h** showed the most potent H3R agonist activity (K_i = 0.17 nM, EC_{50} = 0.75 nM, ia = 77%) among the compounds examined. The 4-trifluoromethyl (**7i**) and 4-methoxy (**7j**) compounds had higher potency but similar efficacy in the functional assay compared with the 4-methyl derivative **7c**. These results indicate that the combination of a benzene ring and an alkyl substituent of appropriate bulkiness is important for agonist activity of terminally nitrogen-modified histamine.

The most potent compound **7h** was further studied in vivo. In the N^{τ}-methylhistamine formation assay in mouse brain, **7h** induced a significant reduction of N^{τ}-methylhistamine level following oral administration. The response at 5 and 30 mg/kg was similar to that in the case of the full H3R agonist **2** (Table 2). Compound **7h** is more lipophilic than **2** and therefore may cross the blood−brain barrier more effectively. However, the dose−response relationship in terms of N^{τ}-methylhistamine level was almost the same for the two compounds, and their binding affinities were also similar. In mice, the aromatic ring and thioether structure of **7h** could cause this compound to be metabolized faster than **2**, which has an isothiourea structure.[15] **7h** showed acceptable profiles in rat pharmacokinetic tests (Table 3).

H3R agonists are expected to be candidate drugs to improve migraine, nociception, and sleep disorder.[25] We are interested in the antistress effect of H3R agonists, because in some animal stress models, an increase of brain histamine release has been recognized.[26] We have already found that **1** significantly reduced isolation-induced vocalizations in guinea pig pups and isolation-induced aggressive behavior in mice in the resident−intruder test.[27] In the mouse resident−intruder test, treatment with **7h** at 30 mg/kg also significantly reduced the duration of attacks on the intruder (Table 4).

In a study of selective serotonin reuptake inhibitors, we had obtained evidence that fluvoxamine might exhibit therapeutic activity through blocking excessive release of corticosterone in rats.[28] Overactivation of the hypothalamic-pituitary-adrenal (HPA) axis, such as elevation of plasma cortisol, was observed in patients with major depression, and this change was normalized in response to therapy.[29] Corticotropin releasing factor (CRF) is an upstream factor in the HPA axis, and CRF type I receptor antagonists have been studied as novel antidepressant drug candidates.[30,31] It was reported that central administration of histamine increased CRF mRNA in rats.[32] H3R agonists might show antistress activity by the functional modulation of CRF neurons via inhibition of histamine release in the central nervous system and through control of the HPA axis.

Table 4. Duration of Attacks (s) in Mouse Resident−Intruder Test[a]

compd	control	3 mg/kg	7.5 mg/kg	30 mg/kg
fluvoxamine	54.5 ± 6.7		30.7 ± 6.0	19.9 ± 3.5[b]
7h	54.5 ± 6.7	42.9 ± 9.5		16.3 ± 6.8[b]

[a] Each result represents the mean ± SEM of eight animals. [b] $p < 0.01$ vs vehicle control. The test compound was administered po.

In regards to the profile of activity toward human cloned histamine receptor subtypes, **7h** showed good selectivity over H_1 and H_2 receptors ($K_i > 1\ \mu M$) but displayed similar binding affinity for the H_4 receptor (K_i = 1.1 nM, $K_i(H_4)/K_i(H_3)$ = 6.5). Some conventional H3R agonists, such as **1** and **2**, exhibit binding affinity for both H_3 and H_4 receptors, presumably because the newly identified H_4 receptor has a high sequence homology to H3R (54% in the transmembrane domains in humans).[33] Because the H_4 receptor is not expressed in the brains of rodents,[34] the result obtained with **7h** in the mouse resident-intruder test should not be related to H_4 receptor activity. One of the cyclopropane-based conformationally restricted histamine analogues has been reported to show increased H3R selectivity.[35] Recently 4-benzyl-1*H*-imidazoles were also reported as novel H3R agonists.[36] We have set out to design and synthesize further H3R agonists, aiming to improve the selectivity between H_3 and H_4 receptors by introducing conformational restrictions. Compound **7h** showed high selectivity against other aminergic receptors ($K_i > 1\ \mu M$: adrenergic $\alpha_1, \beta_1, \beta_2$; muscarinic M_1, M_3; serotonergic 5-HT_{1A}, 5-HT_{2A}, 5-HT_3; dopaminergic D_1, D_2, D_3). As for the lead compound for drug development, **7h** exhibited an unfavorable profile in the inhibition of CYP enzymes (CYP1A2, CYP2C19, CYP2D6, and CYP3A4; IC_{50} values 680, 8.5, 680, 470 nM, respectively). This also should be considered in further compound design.[37]

Conclusion

Chemical modification of the terminal amine of histamine afforded novel H3R agonists containing an aromatic ring with a hydrophobic substituent on the para and/or meta position. The introduction of a cyclohexyl group (**7g**) resulted in loss of agonistic activity despite retention of affinity for H3R. The most potent compound **7h** showed H3R agonistic activity and antistress activity in mice in vivo.

On the basis of our structure−activity data and reported findings,[36] we hypothesize that two binding sites, a terminal heteroatom recognition site and a hydrophobic pocket, are involved in the binding of agonists to H3R, and they are located in close proximity. Compounds such as **7e** and **7h** could interact with both sites. Further studies with conformationally restricted H3R ligands might clarify the spatial relationship of these sites.

Experimental Section

^{1}H and ^{13}C NMR spectra were obtained on JEOL LA-400 FT-NMR spectrometers. Mass spectra were recorded on Hitachi M-80B and JEOL JMS-700 instruments. Elemental analysis data were obtained on a VarioEL (Elementar), and purity over 95% was confirmed for all synthesized compounds. Histamine, **1**, and **2** were commercial products.

Profiling of binding affinity for receptors other than H3R was done by MDS Pharma Services. All biological results are expressed as mean ± SEM Statistical analysis was performed with the SAS statistical package (SAS Institute Japan, Tokyo, Japan). The statistical significance of differences among multiple groups was tested by one-way analysis of variance followed by Dunnett's multiple comparison test. A two-tailed Student's *t* test was used to evaluate differences between two experimental groups. Differences were considered significant at $p < 0.05$. Animal care was performed according to the protocols reviewed by the Ethical Committee for Animal Experiment in Meiji Seika Pharmaceutical Research Center.

4-Methoxyethylimidazole.[19] A solution of 4-butyne-1,4-diol (8.0 g, 93 mmol) in methanol (50 mL) was added to a well-stirred mixture of mercury(II) sulfate (1.7 g), conc H_2SO_4 (1.4 g), water (10 mL), and methanol (50 mL) at room temperature. The reaction mixture was cooled in a water bath, and the temperature was kept below 35 °C. After the addition was completed, the gray solution was stirred at room temperature for 16 h and then neutralized with Na_2CO_3. The resultant precipitate was filtered off, and the filtrate was added to a solution of $Cu(OAc)_2$ (116 g) and formaldehyde (36 mL) in NH_3 aq (580 mL) and water (580 mL). The mixture was heated at 100 °C for 2 h and stirred at room temperature for 16 h. The resultant precipitate was collected by filtration and suspended in 4 N acetic acid (300 mL). A solution of potassium hexacyanoferrate (50 g) in water (150 mL) was added to the suspension, and the mixture was stirred at room temperature for 2 h. Insoluble material was filtered off. The water layer was concentrated, and 5 N NaOH aq was added to adjust the pH to 9. The solution was extracted with *n*-butanol (100 mL × 3). The organic layer was dried over $MgSO_4$ and concentrated to afford crude 4-methoxyethylimidazole (7.4 g, 59 mmol) as a brown solid.

4-(2-Bromoethyl)imidazole (6).[19] Crude 4-methoxyethylimidazole (1.0 g, 7.9 mmol) was dissolved in 48% HBr aq (25 mL). The reaction mixture was heated at 120 °C for 60 h in a sealed tube. After cooling, the solution was concentrated in vacuo. The resultant residue was recrystallized from *n*-butanol/ethyl acetate/hexane to afford **6** (480 mg, 1.9 mmol) as colorless plates (23.7%).

2-(2-(4-*tert*-Butylphenylthio)ethyl)-1*H*-imidazole (7h). 4-*tert*-Butylthiophenol (585 mg, 3.5 mmol) was dissolved in DMF (10 mL). NaH (84 mg) was added, and the reaction mixture was heated at 80 °C for 0.5 h. Then **6** (450 mg, 1.8 mmol) was added and the reaction mixture was stirred for 16 h at 80 °C. The reaction was quenched with ethyl acetate (50 mL), and the whole was washed with satd $NaHCO_3$ aq (20 mL). The organic layer was washed with water (20 mL) twice, dried with $MgSO_4$, and concentrated. The residue was taken up in 5 N HCl aq (1 mL), and the solution was evaporated. The residual brown oil was slowly solidified with ether and recrystallized from methanol/ether to afford **7h** (417 mg, 1.4 mmol HCl salt) as a light-brown powder (79.5%); mp 97 °C. ^{1}H NMR (400 MHz, CD_3OD) δ: 1.37 (9H, s, *t*-butyl H), 3.07 (2H, t, J = 7.3 Hz, methylene H), 3.30 (2H, t, J = 7.3 Hz, methylene H), 7.35−7.45 (4H, m, benzene ring H), 8.08 (1H, s, imidazole ring H), 8.83 (1H, s, imidazole ring H). MS (FAB+) m/z: 261 ($M^+ + 1$). Anal. Calcd for $C_{15}H_{20}N_2S$ HCl·$^2/_3H_2O$: C, 58.33; H, 7.29; N, 9.07. Found: C, 58.28; H, 7.16; N, 9.00.

Supporting Information Available: Synthesis of **7a**−**7g**, **7i**, and **7j**. Experimental details of receptor binding assay, [^{35}S]GTPγS binding assay, measurement of N$^{\tau}$-methylhistamine level in mouse brain, mouse resident−intruder test, and pharmacokinetic tests in rats. This material is available free of charge via the Internet at http://pubs.acs.org.

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