Brief Articles

A Selective Human H_4-Receptor Agonist: (−)-2-Cyano-1-methyl-3-{(2*R*,5*R*)-5-[1*H*-imidazol-4(5)-yl]tetrahydrofuran-2-yl}methylguanidine

Takeshi Hashimoto,[‖] Shinya Harusawa,[‡] Lisa Araki,[‡] Obbe P. Zuiderveld,[†] Martine J. Smit,[†] Tomonari Imazu,[‡] Seiichiroh Takashima,[‡] Yumiko Yamamoto,[‖] Yasuhiko Sakamoto,[§] Takushi Kurihara,[‡] Rob Leurs,[†] Remko A. Bakker,[†] and Atsushi Yamatodani*

Department of Bioinformatics, Graduate School of Allied Health Sciences, Faculty of Medicine, Osaka University, Osaka 565-0871, Japan, Leiden/Amsterdam Center for Drug Research (LACDR), Division of Medicinal Chemistry, Department of Pharmacology, Faculty of Chemistry, Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands, Department of Synthetic Organic Chemistry, Osaka University of Pharmaceutical Sciences, 4−20−1 Nasahara, Takatsuki, Osaka 569-1094, Japan, and R&D Division, Azwell, Inc., 2−24−3, Sho, Ibaraki, Osaka 567-0806, Japan

Received January 2, 2003

A series of 16 compounds related to chiral 4(5)-(5-aminomethyltetrahydrofuran-2-yl)imidazoles (**1**) have been designed, synthesized, and examined in vitro by radioligand displacement studies and functional assays for both the human H_3- and H_4-receptors expressed in SK-N-MC cells. Among them, the (2*S*,5*S*)-isomer **1d** of amino compounds showed approximately 300-fold higher selectivity at the H_3-receptor than the H_4-receptor. On the other hand, (2*R*,5*S*)- and (2*R*,5*R*)-cyanoguanidines **3b** and **3c**, in which the amino group of the compounds **1b** and **1c** was substituted by the cyanoguanidino moiety, bound to the H_4-receptor with a pEC_{50} value of 6.65 and 7.11, respectively, and had >40-fold selectivities over the H_3-receptor. As such, **3b** and **3c** are the first selective H_4 receptor agonists.

Introduction

A new histamine receptor, H_4-receptor, was discovered by several groups in the quest for new G-protein-coupled receptors (GPCR).[1−6] The overall amino acid sequence showed approximately 37% homology between H_3- and H_4-receptors. However, the distribution of the human H_4-receptor mRNA was entirely different from that of the H_3-receptor.[1,6−8] Although little is known about its function in vivo, its expression in the bone marrow and eosinophils may elucidate a potential new role for histamine in the regulation of hemopoietic and immune functions.[1,3] To investigate the possible physiological function of the receptor, a specific ligand is required. Yet, most H_3-receptor ligands are active at the H_4-receptor as well. For example, the classical "selective" H_3-receptor agonist, (*R*)-α-methylhistamine shows H_4-agonistic activity, and thioperamide, the H_3-antagonist prototype, has moderate affinities for the H_4-receptor.[1] No ligands have so far been reported that can selectively target the human H_4-receptor.

In our previous study, we synthesized the respective four stereoisomers of the tetrahydrofurans (THFs) and dihydrofurans (DHFs) containing imidazole and examined their H_3-receptor pharmacology using the in vivo microdialysis method in rats. These compounds spatially arrange the imidazole and aminomethyl groups across the THF and DHF rings (Figure 1). Among them, only (+)-4(5)-[(2*R*,5*R*)-5-aminomethyltetrahydrofuran-2-yl]-

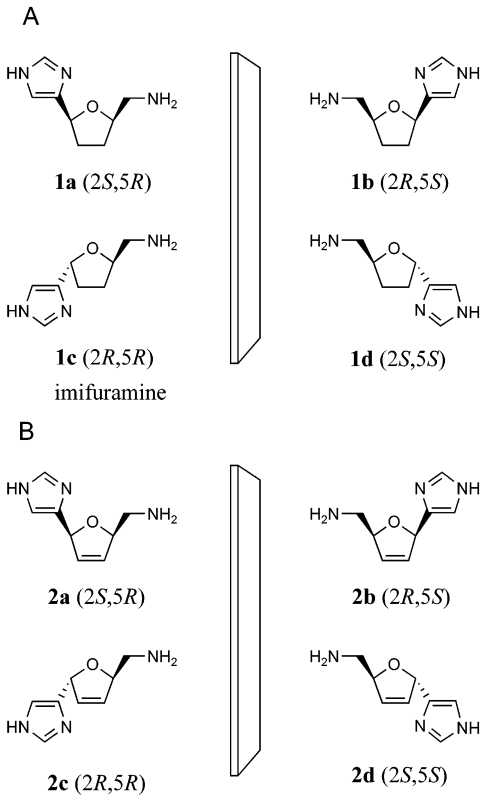

Figure 1.

* To whom correspondence should be addressed. Tel: +81−6−6879−2562, Fax: +81−6−6879−2562, E-mail: yamatoda@sahs.med.osaka-u.ac.jp.
[‖] Osaka University.
[†] Leiden/Amsterdam Center for Drug Research (LACDR).
[‡] Osaka University of Pharmaceutical Sciences.
[§] Azwell, Inc.

10.1021/jm0300025 CCC: $25.00
Published on Web 05/30/2003

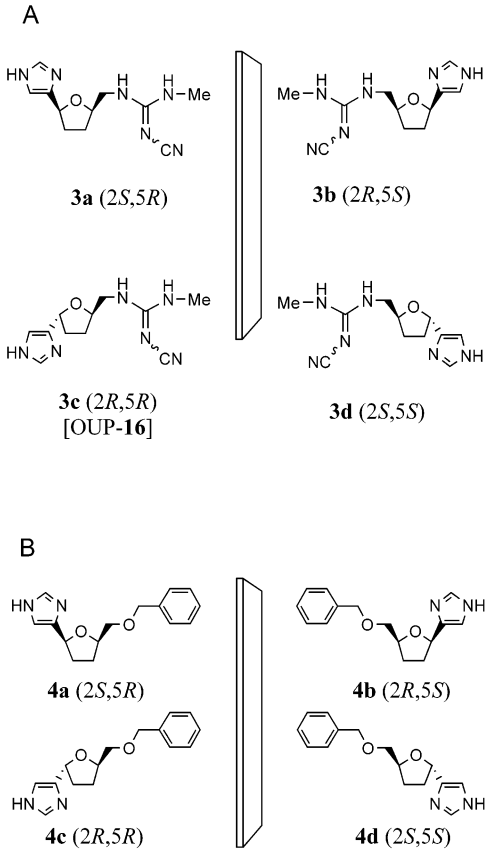

Figure 2.

imidazole (imifuramine, **1c**) was the eutomer exhibiting H_3-agonistic activity.[9,10]

In this study, we examined the binding affinity and functional activity for the human H_3- (hH_3-) and H_4- (hH_4-) receptors of a total of 16 tetrahydrofuranylimidazoles: THFs (Figure 1A), DHFs (Figure 1B), and newly synthesized cyanoguanidines (Figure 2A) and benzyl ethers (Figure 2B).

Results

The competitive binding affinities (pK_i value) for the hH_3-receptor of the DHFs (**2a**−**d**) and cyanoguanidines (**3a**−**d**) were significantly lower than that of the THFs (**1a**−**d**) and benzyl ethers (**4a**−**d**) (Table 1). The pK_i values of **1c**, **1d**, **4b**, and **4c** for the binding to the hH_3-receptor were 10-fold higher than that of the other compounds, and were 6.64 ± 0.12, 6.66 ± 0.14, 6.48 ± 0.16, and 6.61 ± 0.14, respectively. The trans-isomers (**1c**, **1d**, **2c**, and **2d**) of amino compounds (THFs and DHFs) exhibited about 10-fold higher affinity than their cis-isomers (**1a**, **1b**, **2a**, and **2b**). In a functional H_3-receptor assay, the compounds **1a**, **1b**, **1c**, **1d**, **2b**, **2c**, **4b**, and **4c** acted as full agonists ($0.9 < \alpha < 1.0$, Table 1). Among them, **1c** had the highest agonistic activity.

As shown in Table 2, **1c**, **3b**, **3c**, and **4b** competed for [^{3}H] histamine binding to the hH_4-receptor with pK_i values of 6.05 ± 0.04, 6.65 ± 0.06, 6.90 ± 0.17, and 6.36 ± 0.11, respectively. Moreover, the six compounds, **1a**, **3a**, **3b**, **3c**, **3d**, and **4b**, all showed agonist properties with high intrinsic activities ($0.9 < \alpha < 1.0$). Among them, **3c** most potently inhibited the 1 μM forskolin-stimulated responses with an apparent pEC_{50} value of 7.11 ± 0.05 (Table 2).

Table 1. pEC_{50} Values and Affinity Values of Tetrahydrofuranylimidazoles for the Human H_3-Receptor[a]

		functional activity		
compound	config	pEC_{50}	efficacy (α)	binding p*K*i
histamine		8.39 ± 0.06	1.00	7.47 ± 0.11
(*R*)-α-methyl-histamine		9.91 ± 0.05	0.85 ± 0.05	
1a	2*S*,5*R*	6.09 ± 0.05	0.95 ± 0.04	5.77 ± 0.03
1b	2*R*,5*S*	6.11 ± 0.08	1.06 ± 0.04	5.69 ± 0.05
1c [imifuramine]	2*R*,5*R*	7.35 ± 0.07	1.04 ± 0.05	6.64 ± 0.12
1d	2*S*,5*S*	6.98 ± 0.05	0.91 ± 0.04	6.66 ± 0.14
2a	2*S*,5*R*	<4		4.70 ± 0.20
2b	2*R*,5*S*	4.57 ± 0.15	0.93 ± 0.06	4.94 ± 0.08
2c	2*R*,5*R*	6.57 ± 0.10	1.00 ± 0.05	5.99 ± 0.10
2d	2*S*,5*S*	5.55 ± 0.13	0.75 ± 0.06	5.60 ± 0.09
3a	2*S*,5*R*	NE	<0.1	5.09 ± 0.08
3b	2*R*,5*S*	4.99 ± 0.08	0.43 ± 0.03	5.15 ± 0.17
3c [OUP-**16**]	2*R*,5*R*	5.50 ± 0.08	0.79 ± 0.06	5.66 ± 0.09
3d	2*S*,5*S*	<4		4.73 ± 0.13
4a	2*S*,5*R*	5.01 ± 0.07	0.86 ± 0.04	5.16 ± 0.19
4b	2*R*,5*S*	6.72 ± 0.15	1.06 ± 0.05	6.48 ± 0.16
4c	2*R*,5*R*	7.04 ± 0.10	0.97 ± 0.04	6.61 ± 0.14
4d	2*S*,5*S*	5.02 ± 0.11	0.66 ± 0.05	4.98 ± 0.12

[a] The pEC_{50} values were determined by the inhibition of the forskolin-stimulated (1 μM) cAMP production, expressing the human H_3-receptor. All values shown are means ± SEM of at least four experiments. H_3-receptor competition binding was performed using [^{3}H] N^{α}-methylhistamine (1 nM).

Table 2. pEC_{50} Values and Affinity Values of Tetrahydrofuranylimidazoles for the Human H_4-Receptor[a]

		functional activity		
compound	config	pEC_{50}	efficacy (α)	pK_i
histamine		7.68 ± 0.05	1.00	
(*R*)-α-methylhistamine		6.26 ± 0.07	1.01 ± 0.01	
1a	2*S*,5*R*	5.12 ± 0.05	1.02 ± 0.07	5.19 ± 0.07
1b	2*R*,5*S*	5.26 ± 0.07	0.88 ± 0.06	5.60 ± 0.10
1c [imifuramine]	2*R*,5*R*	5.70 ± 0.05	0.70 ± 0.01	6.05 ± 0.04
1d	2*S*,5*S*	4.51 ± 0.01	0.60 ± 0.04	4.89 ± 0.06
2a	2*S*,5*R*	4.12 ± 0.08	0.71 ± 0.05	4.92 ± 0.16
2b	2*R*,5*S*	4.39 ± 0.04	0.74 ± 0.04	4.66 ± 0.12
2c	2*R*,5*R*	4.62 ± 0.08	0.76 ± 0.04	4.93 ± 0.03
2d	2*S*,5*S*	4.07 ± 0.20	0.85 ± 0.11	<4
3a	2*S*,5*R*	5.12 ± 0.06	1.07 ± 0.01	5.09 ± 0.07
3b	2*R*,5*S*	6.65 ± 0.03	1.01 ± 0.01	6.65 ± 0.06
3c [OUP-**16**]	2*R*,5*R*	7.11 ± 0.05	0.99 ± 0.01	6.90 ± 0.17
3d	2*S*,5*S*	4.67 ± 0.03	1.06 ± 0.02	4.69 ± 0.12
4a	2*S*,5*R*	4.94 ± 0.03	0.82 ± 0.02	4.87 ± 0.07
4b	2*R*,5*S*	6.87 ± 0.05	0.91 ± 0.02	6.36 ± 0.11
4c	2*R*,5*R*	6.13 ± 0.10	0.46 ± 0.02	5.98 ± 0.12
4d	2*S*,5*S*	4.89 ± 0.08	0.49 ± 0.05	4.67 ± 0.05

[a] The pEC_{50} values were determined by the inhibition of the forskolin-stimulated (1 μM) cAMP production, expressing the human H_4-receptor. All values shown are means ± SEM of at least four experiments. H_4-receptor competition binding was performed using [^{3}H] histamine (10 nM).

As shown in Figure 3, **1c**, **1d**, **2c**, **2d**, **3b**, and **3c** exhibited receptor selectivity for either the hH_3- or hH_4-receptor. Amino compounds **1c**, **1d**, **2c**, and **2d** showed selective H_3-agonistic activity, which was approximately 45-, 300-, 89-, and 30-fold higher than for the H_4-receptor, respectively. In contrast, the cyanoguanidine analogues **3b** and **3c** exhibited full agonistic activities at the H_4-receptor with 45- and 41-fold higher potency than at the H_3-receptor, respectively.

Conclusions

The substitution of an amino group of tetrahydrofuranylimidazoles with a cyanoguanidine moiety led to a decrease in the agonistic activity at the H_3-receptor and an increase in the H_4-receptor selectivity. The **3b**

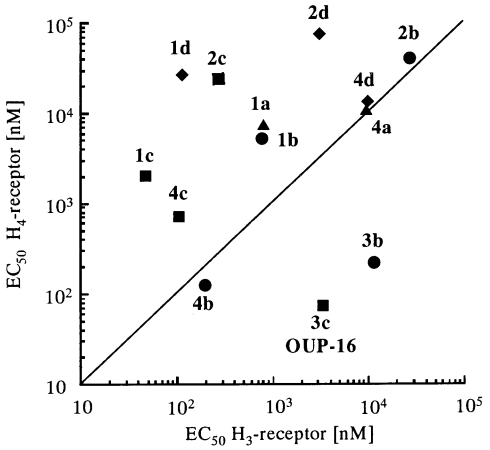

Figure 3. Relation of the functional activity between the H_3- and H_4-receptors. Data for tetrahydrofuranylimidazoles in Tables 1 and 2 are plotted as H_3 EC_{50} values (ordinate, Table 1) versus H_4 EC_{50} values (abscissa, Table 2). Compound numbers correspond with those in Tables 1 and 2. ●, (2*R*,5*S*); ▲, (2*S*,5*R*); ■, (2*R*,5*R*); ◆, (2*S*,5*S*).

and **3c** [OUP-**16**], having the 2*R*-configuration,[11] were highly selective compounds at the H_4-receptor. On the other hand, **1d**, having the 2*S*,5*S*-configuration, behaved as the most selective H_3-receptor agonist in our series. The present results suggest that the stereochemistry of the tetrahydrofuranylimidazoles is useful for the investigation of selective ligands for hH_3- and hH_4-receptors and that the 2*R*-configured isomers of cyanoguanidines exhibit a high agonistic activity for the H_4-receptor.

Experimental Section

SK-N-MC cells expressing the hH_3-[12] or hH_4-receptor[1] were maintained in Eagle's minimal essential medium (BioWhittaker, Verviers, Belgium) supplemented with 10% fetal calf serum (Integro, Zaandam, The Netherlands), 50 IU/mL penicillin, nonessential amino acids solution, 2 mM L-glutamine, 50 μg/mL streptomycin, and 50 μg/mL sodium pyruvate (Invitrogen, Breda, The Netherlands) under the selection of 600 μg/mL G_{418} disulfate (Calbiochem, Amsterdam, The Netherlands) at 37 °C in a humidified atmosphere of 5% CO_2 in air by the method described by Wieland et al.[13] Cells were detached from the dishes with 0.05% trypsin−EDTA (Invitrogen).

SK-N-MC cells stably expressing the hH_3-[12] or hH_4-receptors[1] were grown overnight in 96-well plates before the assay. To start the assay, the cells were incubated for 6 h with 1 μM forskolin (Sigma-Aldrich, St. Louis, MO) and respective tetrahydrofuranylimidazoles at 37 °C. Thereafter, the medium was aspirated, and cells were incubated overnight in a refrigerator with 100 μL of assay buffer (100 mM NaH_2PO_4, 100 mM Na_2HPO_4, pH 8, 2 mM $MgSO_4$, 0.1 mM $MnCl_2$, 0.5% Triton, and 40 mM β-mercaptoethanol).[13] Transcription of β-galactosidase was determined by 4 mM *o*-nitrophenyl-β-D-galactopyranoside. Absorbance was quantified on a microplate reader at 420 nm.

The binding affinity of the hH_3- and hH_4-receptors was determined with 1.0 nM [^{3}H] N^{α}-methylhistamine (82 Ci/mmol, PerkinElmer Life Sciences, Zaventem, Belgium) and 10 nM [^{3}H] histamine (23.3 Ci/mmol, PerkinElmer Life Sciences), respectively. The cell pellets were harvested, washed, and homogenized in incubation buffer (50 mM Na_2HPO_4, pH 7.4). The cell homogenates (hH_3-: 131 ± 11 fmol/mg of protein, hH_4-: 166 ± 26 fmol/mg of protein) were incubated for 1 h at 37°C with each radioligand in incubation buffer, pH 7.4, with or without competing ligands. Then, the membranes were filtered through the GF/C filters pretreated with 0.3% polyethylenimine. The filter was washed three times with ice-cold washing buffer (hH_3-: 25 mM Tris HCl, 145 mM NaCl, pH 7.4, hH_4-: 50 mM Tris HCl, pH 7.4) and the radioactivity was determined by liquid scintillation counting. Nonspecific binding was defined with 1 μM thioperamide as the competing ligand.

Protein concentrations were determined spectrophotometrically by a Packard Argus 400 Microplate Reader using the Bradford reagent,[14] with bovine serum albumin as a standard.

The value of pK_i and pEC_{50} was obtained by fitting these data to a sigmoidal relation using GraphPad Prism (GraphPad Software, San Diego, CA). The intrinsic activities were calculated in comparison with the effects of histamine (100 μM).

Acknowledgment. We are grateful to Dr. Timothy Lovenberg (The R.W. Johnson Pharmaceutical Research Institute) for generously providing the SK-N-MC cells expressing either human histamine H_3- or H_4-receptors. This study was supported in part by a grant to T.K. from the Ministry of Education, Culture, Sports, Science and Technology of Japan (no. 11672127).

Supporting Information Available: Synthetic procedures, spectral data, and Scheme 1 for compounds **3a**−**d** and **4a**−**d**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Liu, C.; Ma, X. J.; Jiang, X.; Wilson, S. J.; Hofstra, C. L.; Blevitt, J.; Pyati, J.; Li, X.; Chai, W.; Carruthers, N.; Lovenberg, T. W. Cloning and pharmacological characterization of a fourth histamine receptor (H_4) expressed in bone marrow. *Mol. Pharmacol.* **2001**, *59*, 420−426.

(2) Nakamura, T.; Itadani, H.; Hidaka, Y.; Ohta, M.; Tanaka, K. Molecular cloning and characterization of a new human histamine receptor, *HH4R*. *Biochem. Biophys. Res. Commun.* **2000**, *279*, 615−620.

(3) Morse, K. L.; Behan, J.; Laz, T. M.; West, R. E.; Greenfeder, S. A.; Anthes, J. C.; Umland, S.; Wan, Y.; Hipkin, R. W.; Gonsiorek, W.; Shin, N.; Gustafson, E. L.; Qiao, X.; Wang, S.; Hedrick, J. A.; Greene, J.; Bayne, M.; Monsma, F. J. Cloning and characterization of a novel human histamine receptor. *J. Pharmacol. Exp. Ther.* **2001**, *296*, 1058−1066.

(4) Zhu, Y.; Michalovich, D.; Wu, H. L.; Tan, K. B.; Dytko, G. M.; Mannan, I. J.; Boyce, R.; Alston, J.; Tierney, L. A.; Li, X.; Herrity, N. C.; Vawter, L.; Sarau, H. M.; Ames, R. S.; Davenport, C. M.; Hieble, J. P.; Wilson, S.; Bergsma, D. J.; Fitzgerald, L. R. Cloning, expression, and pharmacological characterization of a novel human histamine receptor. *Mol. Pharmacol.* **2001**, *59*, 434−441.

(5) Nguyen, T.; Shapiro, D. A.; George, S. R.; Setola, V.; Lee, D. K.; Cheng, R.; Rauser, L.; Lee, S. P.; Lynch, K. R.; Roth, B. L.; O'Dowd, B. F. Discovery of a novel member of the histamine receptor family. *Mol. Pharmacol.* **2001**, *59*, 427−433.

(6) Oda, T.; Morikawa, N.; Saito, Y.; Masuho, Y.; Matsumoto, S. Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. *J. Biol. Chem.* **2000**, *275*, 36781−36786.

(7) Liu, C.; Wilson, S. J.; Kuei, C.; Lovenberg, T. W. Comparison of human, mouse, rat, and guinea pig histamine H_4 receptors reveals substantial pharmacological species variation. *J. Pharmacol. Exp. Ther.* **2001**, *299*, 121−130.

(8) Coge, F.; Guenin, S. P.; Rique, H.; Boutin, J. A.; Galizzi, J. P. Structure and expression of the human histamine H_4-receptor gene. *Biochem. Biophys. Res. Commun.* **2001**, *284*, 301−309.

(9) Harusawa, S.; Imazu T.; Takashima S.; Araki L.; Ohishi H.; Kurihara T.; Sakamoto Y.; Yamamoto Y.; Yamatodani A. Synthesis 4(5)-[5-(Aminomethyl)-tetrahydrofuran-2-yl- or 5-(Aminomethyl)-2,5-dihydrofuran-2-yl]imidazoles by efficient use of a PhSe Group: Application to Novel Histamine H_3−Ligands. *J. Org. Chem.* **1999**, *64*, 8608−8615.

(10) Harusawa, S.; Imazu, T.; Takashima S.; Araki, L.; Ohishi, H.; Kurihara, T.; Yamamoto, Y. and Yamatodani, A.: Synthesis of Imifuramine and its stereoisomers exhibiting histamine H_3-agonistic activity. *Tetrahedron Lett.* **1999**, *40*, 2561−2564.

(11) In case of cyanoguanidines (A) in Figure 2, the carbon bonding to imidazole is numbered as the 2-position of the THF ring for convenience in order to clarify the mutual configurational relationships among the compounds in Figures 1 and 2. The numbering system of THF in the title compounds (OUP-16) is properly employed.

(12) Lovenberg, T. W.; Roland, B. L.; Wilson, S. J.; Jiang, X.; Pyati, J.; Huvar, A.; Jackson, M. R.; Erlander, M. G. Cloning and functional expression of the human histamine H_3 receptor. *Mol. Pharmacol.* **1999**, *55*, 1101−1107.

(13) Wieland, K.; Bongers, G.; Yamamoto, Y.; Hashimoto, T.; Yamatodani, A.; Menge, W. M.; Timmerman, H.; Lovenberg, T. W.; Leurs, R. Constitutive activity of histamine H_3 receptors stably expressed in SK-N-MC cells: display of agonism and inverse agonism by H_3 antagonists. *J. Pharmacol. Exp. Ther.* **2001**, *299*: 908−914.

(14) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.

JM0300025