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Investigation of the Histamine H3 Receptor Binding Site. Design and Synthesis of Hybrid Agonists with a Lipophilic Side Chain

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As a part of our search for novel histamine H3 receptor agonists, we designed and synthesized hybrid compounds in which the lipophilic (4′-alkylphenylthio)ethyl moiety of a novel H3 receptor agonist, 4-(2-(4′-*tert*-butylphenylthio)ethyl)-1*H*-imidazole (**1**), was incorporated into N^{α}-methylhistamine, immepip, and immethridine derivatives. These hybrid compounds were expected to interact concurrently with the histamine-binding site and a putative hydrophobic region in the H3 receptor. Among them, piperidine- and pyridine-type derivatives displayed partial agonist activity, and (*S*)-4-(1-(1*H*-imidazol-4-yl)-2-(4-(trifluoromethyl)phenylthio)ethyl)piperidine (**36**) was identified as a potent H3 agonist. We performed computational docking studies to examine the binding mode of the agonists. The results indicated that immepip interacts with the key residues, Asp114 and Glu206, in a different manner from histamine. The binding mode of **36** to these residues is similar to that of immepip, and the lipophilic tail of **36** has an additional interaction with a hydrophobic region in transmembrane helix 6 of the receptor. These results indicated that **36** served as a useful tool for studies on receptor−agonist interactions and drug design.

Introduction

Histamine receptors (H1−H4[a]) are involved in various biological pathways in both central and peripheral systems. Among these receptors, H3 receptor was discovered as an inhibitory autoreceptor by Arrang et al. in 1983, using classic pharmacological approaches.[1] This receptor regulates the synthesis and release of histamine in the central nervous system (CNS).[2,3] It behaves as a presynaptic heteroreceptor, modulating the release of several important neurotransmitters, including serotonin,[4] noradrenaline,[5] dopamine,[6] and acetylcholine.[7] Specific ligands for the H3 receptor are expected to have potential for treating various CNS disorders.[8,9] We recently reported a novel H3 receptor agonist **1** that possesses a lipophilic (4′-*tert*-butylphenylthio)ethyl moiety at the 4 position of the imidazole ring (Figure 1).[10] Compound **1** exhibited high affinity and potent agonistic activity for the H3 receptor and showed a good pharmacokinetic profile following oral administration in rodents. It also showed a remarkable in vivo efficacy in the resident−intruder aggression model, an atypical antidepressant effective animal model of anxiety.[10] We expect that H3 agonists would offer an effective therapeutic option for the treatment of some forms of anxiety disorders in which commonly prescribed selective serotonin reuptake inhibitors (SSRIs) have not exerted satisfactory therapeutic efficacy.[9]

It has been suggested that the pharmacophore of general H3 agonists, such as histamine, N^{α}-methylhistamine **2**,[1] immepip **3**,[11] and imetit **4**,[12] consists of an imidazole ring and a terminal basic amine[13] (Figure 1). The physiologically protonated imidazole or the basic amine of these compounds is supposed to interact with the aspartate residue (Asp114) of the H3 receptor in transmembrane helix 3 (TM3), which is highly conserved in G-protein-coupled receptors (GPCRs) for biogenic neurotransmitter amines.[14,15] Site-directed mutagenesis and a modeling study of histamine indicated that the interaction between the protonated N-atom of agonists and the glutamate residue (Glu206) in TM5 plays a key role in agonist-induced activation of the H3 receptor.[15] Interestingly, H3 agonists of another type, possessing a less basic side chain or highly lipophilic moiety in place of the terminal basic nitrogen atom, have been discovered, as represented by immethridine **5**, UCL 1470 **8**, and our compound **1**.[10,13,16−21] We have found that the introduction of an alkylated benzene ring to the terminal nitrogen atom of histamine, 4-(2-(*N*-(alkylphenyl)amino)ethyl)-1*H*-imidazole derivatives, partially maintained the agonistic activity of histamine.[10] Noticeably, para CF_3 and para *t*-Bu substituents on the benzene ring improved the H3 receptor affinity, and the alteration of its aminoethyl spacer part to the thioether of **1** increased the agonistic efficacy. On the contrary, the SAR study of **8** revealed that the para CF_3 or CN on the benzene ring of 4-(4-(alkylphenoxy)-butyl)-1*H*-imidazole derivatives dramatically decreased the agonistic efficacy and provided antagonists, though meta CF_3 substitution yielded a full agonist.[19] It seems that the subtle structural divergence of the lipophilic moieties of these ligands greatly impacts their agonistic activities. They presumably activate the H3 receptor through hydrophobic interaction between their lipophilic moiety and an unidentified hydrophobic region of the receptor. We considered that hybridizing the pharmacophore components of these different types of H3 agonists would

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[a] Abbreviations: CADD, computer-assisted computational drug design; CHO, Chinese hamster ovary; CNS, central nervous system; CYP, cytochrome P450; DIBAL-H, diisobutylaluminum hydride; ECL, extracellular loop; GPCRs, G-protein-coupled receptors; H1, histamine type 1; H2, histamine type 2; H3, histamine type 3; H4, histamine type 4; ia, intrinsic activity; TM, transmembrane helix; RAMH, (*R*)-α-methylhistamine; [^{35}S]GTPγS, [^{35}S]guanosine 5′-[γ-thio]triphosphate; SSRIs, selective serotonin reuptake inhibitors.

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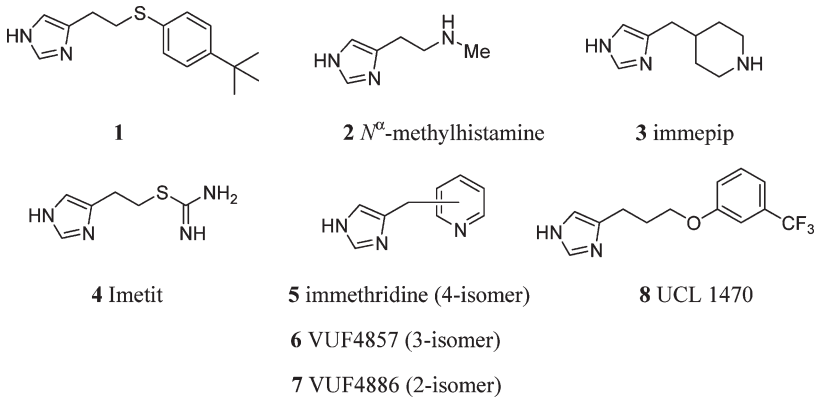


Figure 1. H3 receptor agonists.

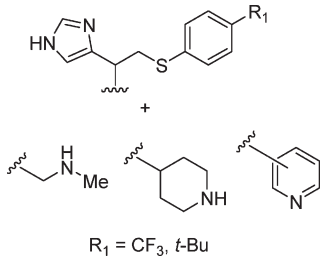


Figure 2. Target hybrid molecules.

afford a novel H3 agonist and provide information about the recognition of agonist structure by the H3 receptor.

Herein we report the design, synthesis, and evaluation of novel H3 receptor agonists that interact with both the histamine-binding site and a hydrophobic region of the H3 receptor. We also performed computational docking of one of the new agonists, **36**, with the homology-modeled human H3 receptor in order to examine the binding mode.

Compound Design

H3 agonists, such as histamine, **2**, and **3**, which possess a basic moiety in the side chain, are physiologically protonated and thought to activate the receptor through interaction with both the aspartate residue (Asp114) in TM 3 and glutamate (Glu206) in TM 5.[14,15] The less basic pyridine derivative **5** (the pK_a value of the pyridine in **5** was 5.53, calculated with SPARC online calculator[22,23]) activates the receptor equipotently with **3**, though the binding mode of **5** has not been established.[16] We anticipated that introduction of the lipophilic part of **1**, ethylthio-(4-alkyl)-phenyl, into these agonists would generate novel compounds that would have an additional interaction with a hydrophobic region of the H3 receptor. To test this idea, we planned to introduce the liphophilic part of **1** into three types of agonist: **2**, **3**, and immethridine derivatives **5**, VUF4857 **6**, and VUF4886 **7**.[16] The lipophilic side chains were introduced to the α-position of the side chain attached to imidazole ring of the agonists (Figure 2).

Chemistry

The synthesis of **15** and **16** is outlined in Scheme 1. 2-(1-Tosyl-1*H*-imidazol-4-yl)acetate methyl ester **9**[24] was converted to a Mannich intermediate using Eschenmoser's salt.[25] Then treatment with MeI gave α-methylene ester **10** via elimination of an ammonium salt.[26] Michael addition of substituted thiophenol to methylene product **10** under basic conditions gave thioether derivatives **11a**,**b**. The methyl ester of compounds **11a**,**b** was reduced with DIBAL-H and $NaBH_4$ to give alcohols **12a**,**b**, which were treated with NsNHBoc under Mitsunobu conditions[27] to provide Boc-protected Ns-amides **13a**,**b**. Selected deprotection of Boc group and subsequent methylation gave *N*-methyl Ns-amides **14a**,**b**. Deprotection of Ns group using 4-*t*-Bu-PhSH and Cs_2CO_3 provided the target compounds **15** and **16**. 4-*t*-Bu-PhSH was used instead of unsubstituted thiophenol because of its reduced odor.

The synthesis of piperidine derivatives **23** and **24** is summarized in Scheme 2. Dehydrative condensation between 4-cyanomethylimidazole **17** and *N*-Boc-4-piperidone **18** under basic conditions gave olefin **19**, which was reduced by catalytic hydrogenation. Protection of imidazole provided **20**. Hydrolysis of cyanide and reduction of the generated carboxylic group with BH_3 gave alcohol **22**. This alcohol was converted to the mesylate, then coupled with substituted sodium thiophenates (generated from sodium hydride and substituted thiophenols) to give thioethers. Exposure of the thioethers to acidic conditions gave **23** and **24**.

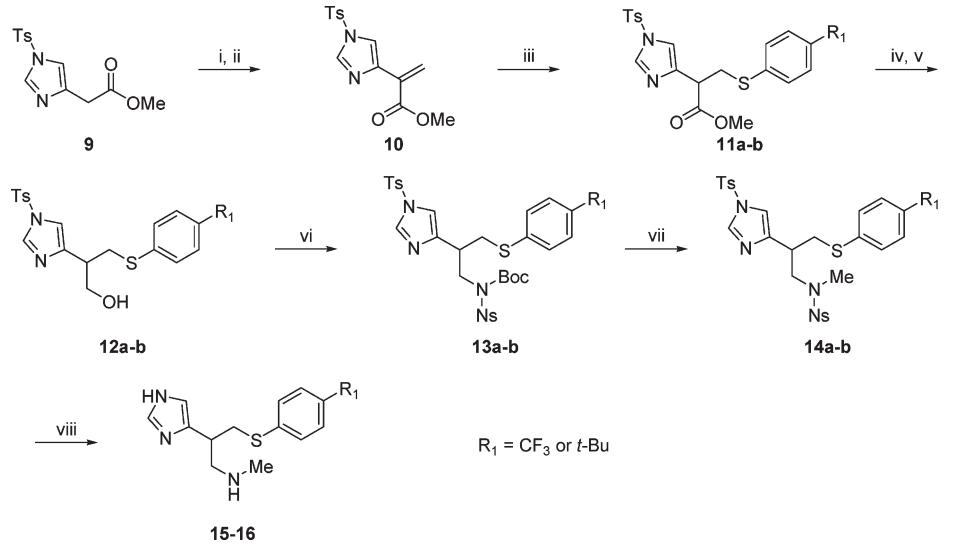
The synthesis of pyridine derivatives **30**−**35** was started from the reported alcohols **25a**−**c**[16] (Scheme 3). The alcohols **25a**−**c** were oxidized with MnO_2 to give ketones, which were converted to epoxides **26a**−**c** under Corey−Chaykovsky epoxidation conditions.[28] Reduction of epoxides **26a**−**c** by catalytic hydrogenation provided alcohols **27a**−**c**. These alcohols **27a**−**c** were converted to the thioethers as described above, and treatment with 5 N HCl afforded the desired pyridine derivatives **30**−**35**.

Enantiomer Separation and Determination of Absolute Configuration. The enantiomers of **23** were separated by preparative liquid chromatography on a Chiralpak AD-H 20 mm × 250 mm column with a solution of 0.1% diethylamine−MeOH. The eluate was analyzed by means of HPLC on a AD-H 4.6 mm × 150 mm column with the same solution (flow rate, 1 mL/min). The retention times of the obtained enantiomers (*S*)-**36** and (*R*)-**37** were 4.07 and 6.14 min, respectively, under the conditions used. Crystals of each enantiomer were obtained as the 1 TsOH salt from isopropanol−Et_2O using the vapor phase diffusion technique.[29] The absolute configuration of each enantiomer was identified by X-ray crystallography (Supporting Information pp S3 and S4).

Pharmacology

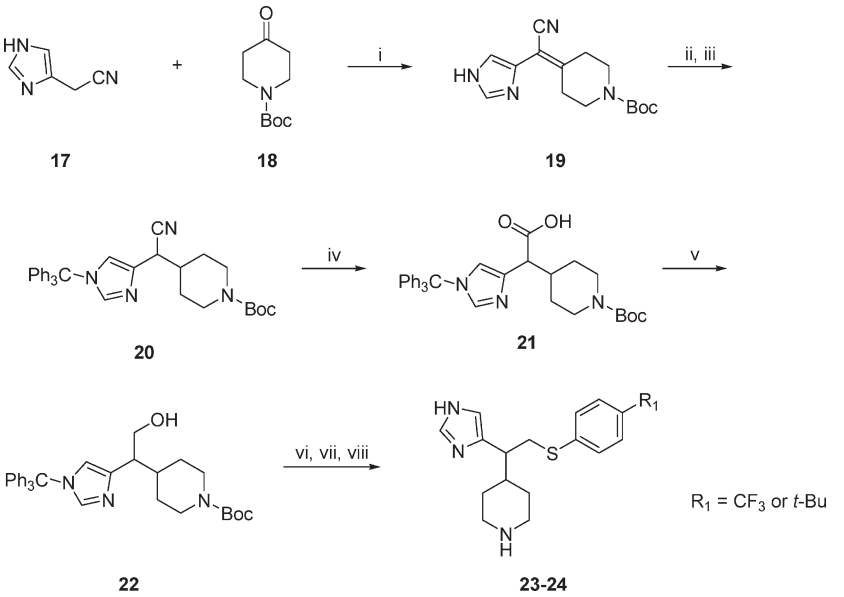
Binding Affinity and Functional Activity at Human Histamine H3 Receptor. Affinity of the synthesized compounds for the human histamine H3 receptor was measured in terms of displacement of [^{3}H]-(*R*)-α-methylhistamine binding to membranes of CHO cells expressing the human H3 receptor.[30] Functional activities of these ligands were measured by means of [^{35}S]GTPγS binding assay to membranes of CHO cells expressing the human H3 receptor.[31] Intrinsic activity (ia) is the

Scheme 1. Synthetic Pathway for **15** and **16**[a]



[a] Reagents and conditions: (i) LiHMDS, $H_2C{=}N^+Me_2I^-$, THF, 0 °C to room temp; ii) MeI, MeOH, room temp; (iii) 4-R_1-PhSH, *t*-BuOK, THF, room temp; (iv) DIBAL-H, CH_2Cl_2, −78 to 0 °C; (v) $NaBH_4$, MeOH, room temp; (vi) NsNHBoc, DEAD, PPh_3, THF, room temp; (vii) TFA−H_2O, room temp, evap, then K_2CO_3, MeI, DMF, room temp; (viii) 4-*t*-Bu-PhSH, Cs_2CO_3, CH_3CN, room temp.

Scheme 2. Synthetic Pathway for **23** and **24**[a]



[a] Reagents and conditions: (i) KOH, MeOH, 60 °C; (ii) Pd/C, H_2, EtOAc, room temp; (iii) Ph_3CCl, Et_3N, CH_2Cl_2, room temp; (iv) KOH aq, 2-ethoxyethanol, 100 °C; (v) BH_3·THF, THF, room temp; (vi) $MeSO_2Cl$, Et_3N, CH_2Cl_2, 0 °C; (vii) 4-R_1-PhSH, NaH, DMF, 50 °C; (viii) 4 N HCl−dioxane, 90 °C.

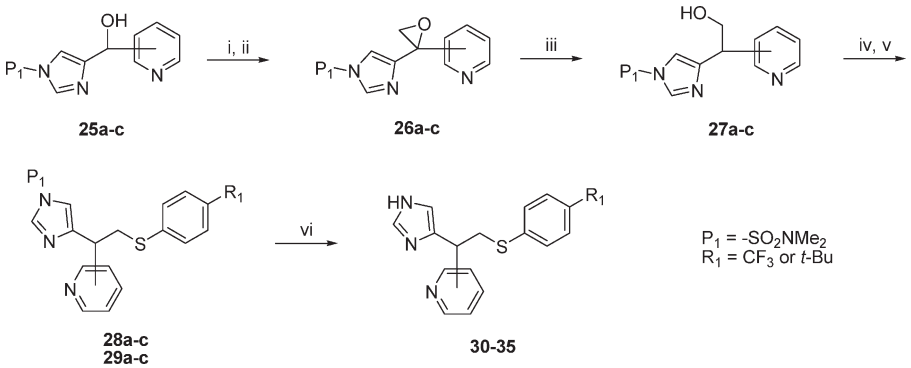
ratio of the maximum response to each ligand to the maximum response to (*R*)-α-methylhistamine (RAMH).

Cytochrome P450 (CYP) Inhibitory Activity. Inhibitory activity of synthesized compounds toward CYPs was measured as the concentration giving 50% inhibition (IC_{50}) of the metabolism of CYP-specific substrates (CYP1A2, 3-cyano-7-ethoxycoumarin; CYP2C19, dibenzylfluorescein; CYP2D6, 3-[2-(*N*, *N*-diethylamino)ethyl]-7-methoxy-4-methylcoumarin; CYP3A4, 7-benzyloxy-4-(trifluoromethyl)coumarin) (Supporting Information p S5).

Computational Chemistry

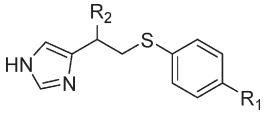
Modeling. The crystal structure of ligand-free GPCR opsin obtained at low pH has been reported and is thought to represent an activated form of the receptor.[32] In this study, we used this structure as the template for constructing activated form of human H3 receptor. Receptor model construction and docking simulation were performed with the Discovery Studio 2.5 from Accelrys, Inc. (San Diego, CA). Molecular mechanics calculations were performed using DS CHARMm module and using the CHARMm force field. The human H3 receptor model was developed using homology modeling techniques in the DS MODELLER module using the crystal structure of bovine opsin as a template (Protein Data Bank code 3CAP).[32] The sequence of the human histamine H3 receptor was taken from GenBank (accession code BAB17030). The sequence alignment between human H3 receptor and bovine opsin was performed based on the results published by Baldwin et al.[33] In docking simulations, ligands with the most basic moiety were manually placed in the active site to interact with Glu206 in TM5 and/or

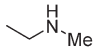
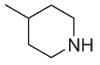
Scheme 3. Synthetic Pathway for **30**−**35**[a]



[a] Reagents and conditions: (i) MnO_2, 1,2-dichloroethane, 50 °C; (ii) $Me_3S^+I^-$, NaH, DMSO, 50 °C; (iii) Pd/C, H_2, EtOAc−AcOH, room temp; (iv) $MeSO_2Cl$, Et_3N, CH_2Cl_2, 2 °C; (v) 4-R_1-PhSH, NaH, DMF, room temp; (vi) 5 N HCl aq, 80 °C.

Table 1. Affinity and Functional Activity at Human H3 Receptor of the Synthesized Compounds



			Human histamine H3 receptor		
			Ki[a] (nM)	EC_{50} (nM)	ia[c](%)
compounds	R_1	R_2		± SEM[b]	± SEM[b]
15	CF_3		>1000	>1000	-
16	*t*-Bu		>1000	>1000	-
23	CF_3		18	38 ± 6	76 ± 3
24	*t*-Bu		530	540 ± 180	65 ± 3
30	CF_3		75	48 ± 6	95 ± 1
31	*t*-Bu		16	15 ± 2	86 ± 2
32	CF_3		120	67 ± 9	73 ± 3
33	*t*-Bu		64	22 ± 9	91 ± 6
34	CF_3		200	140 ± 14	57 ± 2
35	*t*-Bu		130	140 ± 28	62 ± 4
1	*t*-Bu	H	0.43	0.75 ± 0.10	76 ± 1
3			0.30	0.67 ± 0.10	82 ± 2
7			1800	1300 ± 220	59 ± 6
6			76	140 ± 14	84 ± 3
5			0.77	1.1 ± 0.2	94 ± 2

[a] Mean of two experiments. [b] Results are presented as the mean ± SEM of at least three independent experiments. [c] Intrinsic activity, being the ratio of the maximum response to each ligand to the maximum response to RAMH.

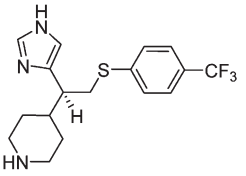
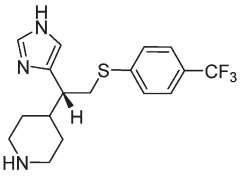
Asp114 in TM3 and minimized in the receptor model together with surrounding amino acid residues.

Results and Discussion

The affinity and functional activity at human H3 receptor of the newly synthesized compounds are presented in Table 1.

The original agonist **1**, **3**, and **5** exhibited high affinity and agonistic activity (K_i = 0.43 nM, EC_{50} = 0.75 nM, and ia = 76%; K_i = 0.30 nM, EC_{50} = 0.67 nM, and ia = 82%; K_i = 0.77 nM, EC_{50} = 1.1 nM, and ia = 94%; respectively). *N*-Methyl derivatives **15** and **16** showed no affinity for the receptor. In contrast, piperidine and pyridine derivatives (**23**,**24** and

Table 2. Affinity and Functional Activity of the Two Enantiomers of **23** at Human H3 Receptor

		Human histamine H3 receptor		
		Ki (nM)	EC_{50} (nM)	ia[b](%)
compounds	structure	± SEM[a]	± SEM[a]	± SEM[a]
(*S*)-**36**		14 ± 1	23 ± 2	77 ± 1
(*R*)-**37**		180 ± 27	74 ± 6	63 ± 1

[a] Results are presented as the mean ± SEM of at least three independent experiments. [b] Intrinsic activity, being the ratio of the maximum response of each ligand to the maximum response of RAMH.

30−**35**, respectively) showed moderate to high affinity and agonistic activity. Of the piperidine derivatives, para-CF_3-substituted compound **23** had higher binding affinity and agonistic activity (K_i = 18 nM, EC_{50} = 38 nM, and ia = 76%) than the para *t*-Bu substituted compound **24** (K_i = 530 nM, EC_{50} = 540 nM, and ia =65%). As for the pyridine derivatives, the bulky para *t*-Bu substituent was somewhat preferable to CF_3. The substituent effect in pyridine-type compounds seems to be different from that in piperidine-type compounds. Among pyridines with the same substituent on the benzene ring, the rank order of binding affinity and agonistic efficacy was 2-pyridyl derivatives (**30** and **31**) > 3-pyridyl derivatives (**32** and **33**) > 4-pyridyl derivatives (**34** and **35**). Intriguingly, the original agonist **7**, **6**, and **5** showed the reverse order of binding affinity and agonistic efficacy: 4-pyridyl (**5**) > 3-pyridyl (**6**) > 2-pyridyl (**7**).[16] The binding mode of the hybrid pyridine derivatives in the receptor is therefore likely to be different from that of the original **5** derivatives. Among the pyridine derivatives, **31** showed the highest binding affinity and EC_{50} (K_i = 16 nM, EC_{50} = 15 nM). The 2-pyridyl derivatives **30** and **31** and 3-pyridyl derivative **33** possessed comparatively high intrinsic activity (95%, 86%, and 91%, respectively).

Neither the H3 receptor affinity nor agonistic efficacy of the hybridized compounds (**23**, **24** and **30**−**35**) was superior to those of the original agonists **1**, **3**, and **5**. This result indicates that the newly introduced each substructure did not contribute to the efficiency of the ligands.[34] The branched structure of the hybrid agonists is thought to cause steric hindrance in the H3 receptor binding site, which might reduce the biding affinity and agonistic potency. The piperidine and pyridine derivatives possess rigid ring structure and might serve to keep the basic moiety and the lipophilic moiety in appropriate locations to interact with the human H3 receptor. The amino side chain of *N*-methyl derivatives is shorter and more flexible than the piperidine and the pyridine moiety. These structural features of *N*-methyl derivatives would not be suitable to make optimal interaction with the H3 receptor.

The binding affinity and functional activity at human H3 receptor of the enantiomers (*S*)-**36** and (*R*)-**37** are shown in Table 2. The (*S*)-enantiomer **36** exhibited higher affinity and functional activity than the (*R*)-enantiomer **37**. In particular, the binding affinity of (*S*)-**36** exceeded that of (*R*)-**37** by a factor of over 13-fold. This result indicates that the human H3 receptor strictly recognizes the enantiomeric structure of ligands for interaction with the three-point binding site.

In order to examine further the binding mode of the agonists, we performed docking studies of histamine, **3**, (*S*)-**36**, and **1** into the active site of the homology-modeled human histamine H3 receptor, which was built based on the crystal structure of bovine opsin (Figures 3 and 4). On the basis of the previous report,[15] the extended histamine molecule showed interaction between the imidazole moiety and Glu206 in TM 5 and between the basic nitrogen and Asp114 in TM3 (Figure 3A). These interactions are considered to be critical for receptor activation. Additionally, the imidazole ring was located in a hydrophobic pocket near Glu206, consisting of aromatic amino acid residues (Tyr115 in TM3, Tyr189 in extracellular loop 2 (ECL2), Trp371 in TM6, and Tyr374 in TM6). Although there is space between the imidazole ring and the hydrophobic pocket, this hydrophobic interaction should stabilize histamine binding in the active site. In the case of **3**, the same binding orientation, with imidazole proximate to Glu206 and the basic nitrogen proximate to Asp114, results in steric repulsion between the piperidine ring of **3** and Leu401 in TM7 (Figure 3B). This steric repulsion pushes **3** toward Tyr115 (Figure 3D), destabilizing the binding. On the other hand, **3** was more stably docked into the receptor in a reverse binding mode (Figure 3C), in which imidazole interacts with Asp114 and basic nitrogen interacts with Glu206. In this case, the piperidine ring was tightly fitted into the hydrophobic pocket formed by the aromatic amino acid residues, resulting in a favorable hydrophobic interaction. Thus **3**, which is conformationally restricted by the piperidine ring, appears to bind to the homology-modeled H3 receptor in the reverse orientation compared with histamine. Although this result should be verified by means of experimental studies, such as site-directed mutagenesis, the calculated binding modes of histamine and **3** in the receptor appear plausible.

(*S*)-**36** bound to the H3 receptor through imidazole−Asp114 interaction and basic nitrogen−Glu206 interaction (Figure 4). The lipophilic side chain of (*S*)-**36** extended toward

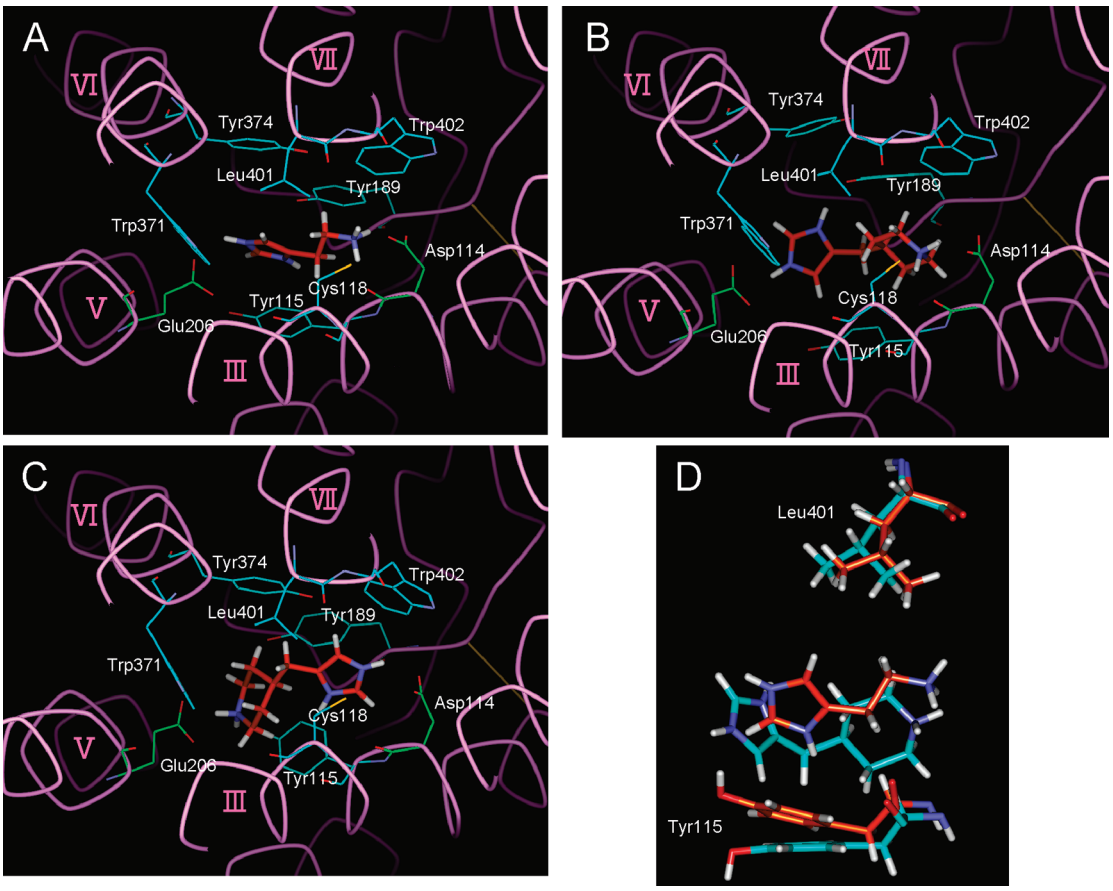


Figure 3. Homology models of human histamine H3 receptor. (A) Modeled binding mode of histamine in the receptor binding site. The imidazole ring interacts with Glu206, and the basic nitrogen interacts with Asp114. (B) Modeled binding mode of **3** in the receptor binding site (unstable model). (C) Modeled binding mode of **3** in the receptor binding site (stable model). The imidazole ring interacts with Asp114, and the basic nitrogen interacts with Glu206. (D) Overlay of the unstable immepip binding model (blue) and histamine binding model (red). The steric repulsion between the piperidine ring and Leu401 pushed **3** toward Tyr115. Only ligands, Leu401 and Try115 are depicted.

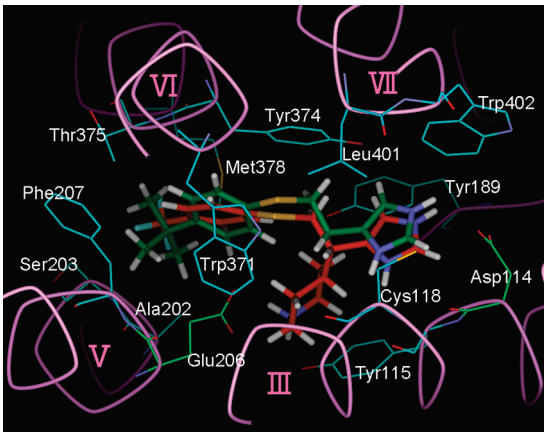


Figure 4. Modeled binding mode of **36** (red) and **1** (green) in the human H3 receptor binding site from the docking model with **36** is depicted. The terminal lipohilic moieties of ligands interact with the hydrophobic region in TM6.

a hydrophobic region comprised Trp371, Tyr374, Thr375, and Met378 in TM 6, which is one of the key segments for GPCR activation. When bovine rhodopsin is compared with active bovine opsin, the whole TM6 region of opsin is moved slightly away from the center bundle and the cytoplasmic segment in TM6 is tilted outward so that it runs almost parallel to TM5.[32] These structural changes are associated with the active GPCR state. Thus, hydrophobic interaction between the liphophilic side chain of (*S*)-**36** and the hydrophobic region in TM6 is speculated to participate in the receptor activation. However, our model indicated that the bulky branched (*S*)-**36** developed some steric repulsion in the H3 receptor binding site. This might reduce the biding affinity and agonistic potency of (*S*)-**36**, compared to **3**. The (*R*)-enantiomer **37** is supposed to bind to the receptor as same way as (*S*)-**36**, but the lipophilic tail of (*R*)-**37** is thought to be more distant from the hydrophobic region in TM6 compared with that of (*S*)-**36**, and this unfavorable orientation should reduce the binding affinity to the receptor. Both the lipophilic tail and the piperidine ring of (*S*)-**36** have the same direction in the receptor, and the lipophilic tail is pinched between the piperidine ring and the hydrophobic region in TM6. In this constrained binding mode, bulky substituents at the 4-position of the benzene ring is likely to cause steric repulsion between the hydrophobic region in TM6, and this is presumably the reason why the binding affinity of *t*-Bu-substituted **24** is declined.

The newly synthesized pyridine derivatives are thought to bind to the receptor similarly to (*S*)-**36**. In this case, the pyridine ring is close to Glu206 in the binding site of the receptor. The nitrogen atom of the pyridine ring has a weak negative charge because the pyridine nitrogen is not likely to be protonated under physiological conditions. (The calculated pK_a values[22,23] of the pyridine in 2-pyridyl **31**, 3-pyridyl **33**, and 4-pyridyl **35** were 3.15, 4.56, and 5.32, respectively.) In the case of the 4-pyridine derivatives, the

Table 3. Binding Affinity of Selected Compounds for Human Histamine H1, H2, H3, and H4 Receptors

	human histamine receptor binding (K_i, nM)					
compd	H3	H1[a]	H2[a]	H4[a]	H1(K_i) / H3(K_i)	H4(K_i) / H3(K_i)
31	16	> 10000	> 10000	840	> 620	53
33	64	> 10000	> 10000	360	> 150	5.6
34	200	540	> 10000	17	2.7	0.085
(*S*)-**36**	14	280	> 10000	1300	20	93
(*R*)-**37**	180	48	> 10000	310	0.27	1.7

[a] Mean of two experiments.

pyridine nitrogen atom is located close to the deprotonated carboxylic acid of Glu206. This may result in weak electrostatic repulsion, which would disfavor binding. Consequently, the binding affinity of **34** and **35** is inferior to that of other pyridine derivatives. Compared to the piperidine derivatives, the pyridine ring has a flat conformation, which is thought to alleviate steric repulsion involving the lipophilic tail. This may be the reason why *t*-Bu on the benzene ring interacts with the hydrophobic region in TM6 more favorably than does CF_3.

In the case of *N*-methyl derivatives **15** and **16**, steric repulsion would be generated between their lipophilic side chains and TM3 if they take the similar binding mode of histamine, in which the imidazoles and the *N*-methylamines are placed proximate to Glu206 and Asp114, respectively. **15** and **16** were able to bind to the receptor without generating such repulsion if they took the binding mode of (*S*)-**36**. However, in this mode of binding, the somewhat short *N*-methylamino moieties cannot form effective three point interaction (Figure S3, see Supporting Information p S7), interaction with Glu206, Asp114, and some hydrophobic region in the receptor. Our model suggested that this might be one of the fundamental reasons for the loss of affinity of the *N*-methyl derivatives to the H3 receptor.

In the docking model, **1** bound to the H3 receptor through the interaction of imidazole with Asp114, and the peripheral lipophilic tail with the hydrophobic region in TM6 (Figure 4). Although **1** did not interact with Glu206, its molecular flexibility may allow it to take a suitable conformation for strong hydrophobic interaction between the lipophilic tail and the hydrophobic region in TM6 without steric repulsion, resulting in high binding affinity for the receptor. This two-point interaction appears to be sufficient to induce receptor activation and is consistent with the hypothesis of De Esch et al.[21] On the basis of a CADD (computer-assisted computational drug design) approach, they predicted that a conformationally constrained agonist, which contained imidazole as the only basic moiety and oxazoline as the lipophilic moiety, would interact with Asp114 through the imidazole moiety and with a putative hydrophobic pocket via the peripheral lipophilic group. The results of our docking study suggest that the hydrophobic region comprising Trp371, Tyr374, Thr375, and Met378 in TM6 adjacent to the histamine-binding site has a key role in the receptor activation induced by agonists such as (*S*)-**36** and **1**.

The binding profiles of selected compounds with other human histamine receptor congeners, H1, H2, and H4, were next examined (Table 3). None of the evaluated compounds showed affinity for human H2 receptor. Among the pyridine derivatives, 2-pyridyl derivative **31** exhibited the highest selectivity for the H3 receptor. The 3-pyridyl derivative **33** did not show affinity for the H1 receptor but possessed moderate affinity for the H4 receptor, resulting in low selectivity for the H3 receptor (H4(K_i)/H3(K_i) = 5.6). The 4-pyridyl derivative **34** showed relatively high affinity for the H4 receptor (H1(K_i)/H3(K_i) = 2.7, H4(K_i)/H3(K_i) = 0.085). Of the enantiomers (*S*)-**36** and (*R*)-**37**, (*S*)-**36** exhibited good selectivity for the H3 receptor (H1(K_i)/H3(K_i) = 20, H4(K_i)/H3(K_i) = 93). (*R*)-**37** had moderate affinity for the H1 and H4 receptors. The position of the basic N atom and the enantiomeric configuration appear to be important determinants of selectivity.

Table 4. CYP-Inhibitory Activity of Selected Compounds

	CYP IC_{50}[a] (μM)			
compd	1A2	2C19	2D6	3A4
23	> 94	12	9.5	17
24	> 100	1.4	3.6	6.5
31	5.1	< 0.010	0.82	0.27
34	8.6	0.055	4.3	0.35
1	0.68	0.0085	0.68	0.47

[a] Mean of two experiments.

Imidazole-based drug candidates may have the potential to inhibit the function of cytochrome P450 (CYP) isoforms, owing to imidazole nitrogen complexation to heme iron in the active site of the enzyme.[35] Since these enzymes play roles in the metabolism of many medicines, inhibitors of CYPs may cause drug−drug interactions by reducing or blocking the clearance of coadministered medicines.[36,37] Therefore, we tested selected compounds for inhibitory activity toward important CYP isoforms (Table 4). The lead compound **1** had an unfavorable profile of CYP inhibition. Compounds **31** and **34**, which contain a pyridine ring, also exhibited strong CYP-inhibitory ability, especially toward CYP2C19 and CYP3A4. In contrast, the piperidine derivatives **23** and **24** showed weaker inhibition. Reducing the lipophilicity by introducing a piperidine side chain has been reported to suppress CYP-inhibitory activity of H3 antagonists.[38] The IC_{50} values of **23** for CYPs are in the range from 9.5 to > 94 μM, differing by > 500-fold from the K_i value for human H3 receptor. Compound **23** and its enantiomers (**36** and **37**) seem unlikely to be involved in CYP-mediated drug−drug interactions. Unfortunately, **23** showed no oral bioavailability at preliminary pharmacokinetic profile study in mice. Parallel artificial membrane permeation assay[39] of **23** indicated that it had very low membrane permeability (data not shown), which is possibly due to the polar nature of piperidine moiety of **23**. This should be considered in further compound design.

Conclusion

Novel hybrid compounds, possessing an imidazole ring, a basic moiety, and a lipophilic moiety, were designed and synthesized as candidate H3 receptor ligands. Among them, piperidine and pyridine derivatives showed partial agonist activity at human H3 receptor, and (*S*)-**36** was identified as a potent H3 agonist. To examine the binding mode of the agonists, computational homology-modeled docking studies with the human H3 receptor were performed. The results suggested that **3** and (*S*)-**36** bind to the histamine H3 receptor through interactions between imidazole and Asp114 in TM3 and between the basic nitrogen and Glu206 in TM5, whereas histamine binds in a reverse orientation through interactions between the imidazole and Glu206 in TM5 and between the basic nitrogen and Asp114 in TM3. The lipophilic tail of (*S*)-**36** may have a hydrophobic interaction with the hydrophobic region in TM6, comprising Trp371, Tyr374, Thr375, and Met378. Hydrophobic interaction with TM6 is considered to play an important role in receptor activation.

From the viewpoint of clinical potential, the piperidine derivative (*S*)-**36** exhibited high selectivity for H3 receptor

over other histamine receptor congeners and weak CYP-inhibitory activity (as examined with the racemate **23**). These characteristics suggest that (*S*)-**36** is not only a useful tool for probing the receptor active site but also a good lead compound for developing human H3 receptor agonists with therapeutic potential for treating anxiety disorders.

Experimental Section

NMR spectra were obtained on JEOL GX-400 FT-NMR spectrometers. The following abbreviations were used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad. Mass spectra were measured with Hitachi M-80B, Agilent HP-5989A, and JEOL JMS-700 instruments. Elemental analysis data were obtained on a VarioEL (Elementar). Compounds **1**, **3**, **5**, **6**, and **7** were synthesized using procedures earlier described.[10,11,16] Column chromatography was carried out using silica gel 60N 40−50 μm (Kanto Chemical) or NH-chromatography silica gel 100−200 mesh (Fulisilysia Chemical) or Sephadex LH-20 (Amersham Biosciences). Thin layer chromatography was performed on silica gel 60 F254 plates (Merck) or NH TLC plates (Fulisilysia Chemical). All final compounds have a purity of >95% (see Supporting Information for details). Profiling of binding affinity for receptors other than H3R was done by MDS Pharma Services.

Chemistry. Methyl 2-(1-Tosyl-1*H*-imidazol-4-yl)-3-(4-(trifluoromethyl)phenylthio)propanoate (11a). 11a was synthesized as a colorless oil, using the procedure for **11b** from **9** and 4-trifluoromethylbenzenethiol (25%). ^{1}H NMR ($CDCl_3$) δ 7.91 (d, 1H, J = 1.2 Hz), 7.83 (ddd, 2H, J = 1.9, 1.9, 8.5 Hz), 7.48 (d, 2H, J = 8.3 Hz), 7.37 (d, 2H, J = 8.3 Hz), 7.35 (d, 2H, J = 8.5 Hz), 7.21 (m, 1H), 3.87 (dd, 1H, J = 6.6, 8.3 Hz), 3.69 (s, 3H), 3.52 (dd, 1H, J = 8.3, 13.6 Hz), 3.43 (dd, 1H, J = 6.6, 13.6 Hz), 2.45 (s, 3H); MS (FAB+) m/z 485 $(M + H)^+$.

Methyl 3-(4-*tert*-Butylphenylthio)-2-(1-tosyl-1*H*-imidazol-4-yl)propanoate (11b). LiHMDS (1.6 M in THF, 1.30 mL, 2.08 mmol) was added to a solution of 2-(tosyl-1*H*-imidazol-4-yl)-acetate methyl ester **9** (460 mg, 1.56 mmol) in THF (7.8 mL) at 0 °C, and the mixture was stirred at the same temperature for 30 min. To the mixture was added *N*,*N*-dimethylmethyleneammonium iodide (580 mg, 3.13 mmol) at 0 °C, and the mixture was stirred for 20 min, then at room temperature for 10 min. The reaction mixture was poured into brine (30 mL) and extracted with $CHCl_3$ (50 mL × 3). The organic layer was dried (Na_2SO_4) and evaporated. The residue was dissolved in MeOH (6 mL), and MeI (0.90 mL, 14.4 mmol) was added. After being stirred at room temperature for 1 h, the reaction mixture was poured into brine (30 mL) and extracted with EtOAc (50 mL). The organic layer was dried (Na_2SO_4), evaporated, and the residue was dissolved in THF (7 mL). To the mixture was added *t*-BuOK (18 mg, 0.16 mmol) and 4-*tert*-butylbenzenethiol (0.26 mL, 1.56 mmol) successively, and the mixture was stirred at room temperature for 10 min. The reaction mixture was diluted with EtOAc (50 mL) and filtered by a short pad of Celite. The filtrate was evaporated and purified with SiO_2 thin layer chromatography (EtOAc/hexane = 2:3) to give **11b** as a colorless oil (199 mg, 27%). ^{1}H NMR ($CDCl_3$) δ 7.89 (d, 1H, J = 1.2 Hz), 7.82 (d, 2H, J = 8.3 Hz), 7.37 (d, 2H, J = 8.3 Hz), 7.25 (d, 4H, J = 1.4 Hz), 7.19 (m, 1H), 3.84 (dd, 1H, J = 6.2, 8.5 Hz), 3.67 (s, 3H), 3.42 (dd, 1H, J = 8.5, 13.4 Hz), 3.42 (dd, 1H, J = 6.2, 13.4 Hz), 2.43 (s, 3H), 1.30 (s, 9H); MS (FAB+) m/z 473 $(M + H)^+$.

2-(1-Tosyl-1*H*-imidazol-4-yl)-3-(4-trifluoromethylphenylthio)-1-propanol (12a). 12a was synthesized as a colorless oil, using the procedure for **12b** from **11a** (68%). ^{1}H NMR ($CDCl_3$) δ 7.92 (d, 1H, J = 1.2 Hz), 7.83 (d, 2H, J = 8.3 Hz), 7.45 (d, 2H, J = 8.5 Hz), 7.35 (d, 2H, J = 8.3), 7.27 (d, 2H, J = 8.0), 7.14 (m, 1H), 3.90 (m, 2H), 3.34 (dd, 1H, J = 7.1, 13.2 Hz), 3.28 (dd, 1H, J = 7.3, 13.2 Hz), 3.19 (br s, 1H), 3.00 (m, 1H), 2.44 (s, 3H); MS (FAB+) m/z 457 $(M + H)^+$.

3-(4-*tert*-Butylphenylthio)-2-(1-tosyl-1*H*-imidazol-4-yl)-1-propanol (12b). To a solution of **11b** (199 mg. 0.42 mmol) in CH_2Cl_2 (4.2 mL) was added DIBAL-H (1.0 M in toluene, 1.30 mL, 1.30 mmol) at −78 °C. The reaction mixture was stirred at the same temperature for 30 min, then warmed to 2 °C and stirred for 15 min. The reaction was quenched with 1 N aqueous HCl (20 mL), and the mixture was diluted with EtOAc (50 mL) and stirred at room temperature for 10 min. The residue was partitioned, and the obtained organic layer was washed with 1 N aqueous HCl (20 mL), saturated aqueous $NaHCO_3$ (20 mL), and brine (20 mL), successively, and dried (Na_2SO_4). After concentration of the mixture under reduced pressure, the residue was dissolved in MeOH (4 mL)−THF (2 mL). To the mixture was added $NaBH_4$ (16 mg, 0.42 mmol) at 0 °C, and then the mixture was stirred at room temperature overnight. The reaction was quenched with saturated aqueous NH_4Cl (5 mL), and the mixture was poured into brine (20 mL). The mixture was extracted with EtOAc (50 mL), and the organic layer was dried (Na_2SO_4), evaporated, and purified with SiO_2 column chromatography (EtOAc/hexane = 1:1, then 3:2) to give **12b** as a colorless oil (134 mg, 71%). ^{1}H NMR ($CDCl_3$) δ 7.90 (d, 1H, J = 1.2 Hz), 7.83 (d, 2H, J = 8.5 Hz), 7.35 (d, 2H, J = 8.5 Hz), 7.29−7.23 (m, 4H), 7.14 (m, 1H), 3.90 (m, 2H), 3.18 (dd, 1H, J = 7.3, 13.0 Hz), 3.15 (dd, 1H, J = 7.0, 13.0 Hz), 2.99−2.93 (m, 2H), 2.44 (s, 3H), 1.32 (s, 9H); MS (FAB+) m/z 445 $(M + H)^+$.

***tert*-Butyl (2-Nitrophenyl)sulfonyl(2-(1-tosyl-1*H*-imidazol-4-yl)-3-(4-(trifluoromethyl)phenylthio)propyl)carbamate (13a). 13a** was synthesized as a colorless oil, using the procedure for **13b** from **12a** (99%). ^{1}H NMR ($CDCl_3$) δ 8.25 (m, 1H), 7.92 (d, 1H, J = 1.2 Hz), 7.82 (d, 2H, J = 8.5 Hz), 7.80−7.72 (m, 3H), 7.45 (d, 2H, J = 8.8 Hz), 7.36 (d, 2H, J = 8.0 Hz), 7.30 (d, 2H, J = 8.0 Hz), 7.18 (d, 1H, J = 1.2 Hz), 4.13 (m, 1H), 4.02 (dd, 1H, J = 6.1, 14.4 Hz), 3.42 (m, 1H), 3.37−3.32 (m, 2H), 2.43 (s, 3H), 1.24 (s, 9H); MS (FAB+) m/z 741 $(M + H)^+$.

***tert*-Butyl (3-(4-*tert*-Butylphenylthio)-2-(1-tosyl-1*H*-imidazol-4-yl)propyl)-(2-nitrophenylsulfonyl)carbamate (13b).** To a solution of **12b** (133 mg, 0.30 mmol), PPh_3 (118 mg, 0.45 mmol), and *tert*-butyl 2-nitrophenylsulfonylcarbamate (109 mg, 0.36 mmol) was added DEAD (2.2 M in toluene, 0.20 mL, 0.44 mmol), and the mixture was stirred at room temperature for 10 min, then 50 °C for 20 min. After the evaporation of the solvent, the resulting residue was purified with SiO_2 column chromatography (EtOAc/hexane = 2:3) to give **13b** as a colorless oil (133 mg, 61%). ^{1}H NMR ($CDCl_3$) δ 8.22 (m, 1H), 7.89 (d, 1H, J = 1.0 Hz), 7.80 (d, 2H, J = 8.3 Hz), 7.74−7.69 (m, 3H), 7.34 (d, 2H, J = 8.3 Hz), 7.26−7.18 (m, 5H), 4.09 (m, 1H), 4.06 (dd, 1H, J = 7.3, 14.6 Hz), 3.36−3.23 (m, 3H), 2.42 (s, 3H), 1.31 (s, 9H), 1.27 (s, 9H); MS (FAB+) m/z 729 $(M + H)^+$.

***N*-Methyl-*N*-3-(4-trifluoromethylphenylthio)-2-(1-tosyl-1*H*-imidazol-4-yl)propyl-2-nitrobenzensulfonamide (14a). 14a** was synthesized as a colorless oil, using the procedure for **14b** from **13a** (86%). ^{1}H NMR ($CDCl_3$) δ 7.94−7.80 (m, 2H), 7.80 (d, 2H, J = 7.2 Hz), 7.69−7.63 (m, 2H), 7.57 (m, 1H), 7.44 (d, 2H, J = 7.8 Hz), 7.35 (d, 2H, J = 7.8 Hz), 7.29 (d, 2H, J = 7.3 Hz), 7.19 (s, 1H), 3.73 (dd, 1H, J = 5.1, 14.0 Hz), 3.43 (dd, 1H, J = 6.1, 14.0 Hz), 3.31 (m, 1H), 3.29−3.21 (m, 2H), 2.69 (s, 3H), 2.43 (s, 3H); MS (FAB+) m/z 655 $(M + H)^+$.

***N*-3-(4-*tert*-Butylphenylthio)-2-(1-tosyl-1*H*-imidazol-4-yl)propyl-*N*-methyl-2-nitrobenzensulfonamide (14b).** A solution of **13b** (132 mg, 0.18 mmol) in TFA (1.8 mL)−H_2O (10 μL) was stirred at room temperature for 2.5 h. After concentration of the solvent, the residue was dissolved in DMF (1.8 mL). To the solution was added K_2CO_3 (50 mg, 0.36 mmol) and MeI (17 μL, 0.27 mmol), successively, and the mixture was stirred at room temperature overnight. Then the reaction mixture was poured into H_2O (20 mL) and extracted with EtOAc (50 mL × 2). The organic layer was washed with brine, dried (Na_2SO_4), evaporated, and purified with SiO_2 thin layer chromatography (EtOAc/hexane = 1:1) to give **14b** as a colorless oil (88 mg, 77%). ^{1}H NMR ($CDCl_3$) δ 7.89 (dd, 1H, J = 1.7, 7.6 Hz), 7.89 (s, 1H), 7.88 (d, 2H, J = 8.8 Hz), 7.65 (dd, 1H, J = 1.7, 7.6 Hz), 7.62 (dd, 1H, J = 1.7, 7.4 Hz), 7.56 (dd, 1H, J = 1.7, 7.4 Hz),

7.34 (d, 2H, J = 8.8 Hz), 7.27 (ddd, 2H, J = 2.2, 2.2, 8.6 Hz), 7.19 (ddd, 2H, J = 2.2, 2.2, 8.6 Hz), 7.15 (d, 1H, J = 1.2 Hz), 3.66 (m, 1H), 3.43 (m, 1H), 3.19−3.13 (m, 3H), 2.64 (s, 3H), 2.43 (s, 3H), 1.24 (s, 9H); MS (FAB+) m/z 643 $(M + H)^+$.

2-(1*H*-Imidazol-4-yl)-*N*-methyl-3-(4-(trifluoromethyl)phenylthio)propane-1-amine (15). 15 was synthesized as a colorless oil, using the procedure for **16** from **14a** (12%). ^{1}H NMR ($CDCL_3$) δ 7.58 (d, 1H, J = 1.0 Hz), 7.48 (d, 2H, J = 8.3 Hz), 7.34 (d, 2H, J = 8.3 Hz), 6.88 (s, 1H), 3.37 (dd, 1H, J = 7.0, 12.9 Hz), 3.28 (dd, 1H, J = 6.6, 12.9 Hz), 3.16 (m, 1H), 2.98 (d, 2H, J = 6.3 Hz), 2.43 (s, 3H); MS (ESI+) m/z 316 $(M + H)^+$; HRMS (FAB+) calcd for $C_{14}H_{17}N_3F_3S$ $(M + H)^+$ 316.1095, found 316.1101.

3-(4-*tert*-Butylphenylthio)-2-(1*H*-imidazol-4-yl)-*N*-methylpropane-1-amine (16). To a solution of **14b** (88 mg, 0.14 mmol) in CH_3CN (1.4 mL) was added Cs_2CO_3 (67 mg, 0.21 mmol) and 4-*tert*-butylbenzenethiol (28 μL, 0.17 mmol). Then the mixture was stirred at room temperature for 20 min. After additional adding of Cs_2CO_3 (67 mg, 0.21 mmol) and 4-*tert*-butylbenzenethiol (28 μL, 0.17 mmol), the reaction mixture was stirred for 1.5 h. The solvent was evaporated and the resulting residue was purified with NH thin layer chromatography (MeOH:$CHCl_3$ = 1:19) to give **16** as a colorless oil (7 mg, 17%). ^{1}H NMR ($CDCL_3$) δ 7.55 (s, 1H), 7.33−7.23 (m, 4H), 6.87 (s, 1H), 3.28 (dd, 1H, J = 6.1, 12.4 Hz), 3.17−3.07 (m, 2H), 2.96−2.91 (m, 2H), 2.40 (s, 3H), 1.29 (s, 9H); MS (FAB+) m/z 303 $(M)^+$; HRMS (FAB+) calcd for $C_{17}H_{26}N_3S$ $(M + H)^+$ 304.1847, found 304.1852.

***tert*-Butyl 4-(Cyano(1*H*-imidazol-4-yl)methylene)piperidine-1-carboxylate (19).** To a solution of KOH (1.06 g, 18.9 mmol) in MeOH (6.3 mL) was added 1-(*tert*-butoxycarbonyl)-4-piperidone **18** (1.25 g, 6.27 mmol) and 4-cyanomethylimidazole **17** (0.67 g, 6.26 mmol), and the mixture was stirred at 60 °C for 3 h. The reaction mixture was poured into H_2O (50 mL) and extracted with EtOAc (100 mL × 2). The combined organic layer was washed with brine (50 mL), dried (Na_2SO_4), and evaporated. The resulting residue was purified with SiO_2 column chromatography (EtOAc/hexane = 1:1, then EtOAc) to give **19** as a white solid (1.25 g, 68%). ^{1}H NMR ($CDCl_3$) δ 7.67 (s, 1H), 7.23 (s, 1H), 3.61−3.58 (m, 2H), 3.53−3.50 (m, 2H), 3.09 (m, 2H), 2.76 (t, 2H, J = 5.8 Hz), 1.48 (s, 9H); MS (FAB+) m/z 289 $(M + H)^+$.

***tert*-Butyl 4-(Cyano(1-trityl-1*H*-imidazol-4-yl)methyl)piperidine-1-carboxylate (20).** A suspension of **19** (1.42 g, 4.92 mmol) and 10% Pd/C (1.35 g) in EtOAc (40 mL) was stirred at room temperature under H_2 atmosphere for 20 h. The reaction mixture was filtered with a pad of Celite, and the obtained filtrate was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (40 mL), and to the solution was added Et_3N (1.4 mL, 10.0 mmol) and TrCl (1.80 g, 6.46 mmol). The mixture was stirred at room temperature for 2 h, and the reaction was quenched with H_2O (5 mL). The resulting mixture was poured into brine (50 mL) and extracted with EtOAc (100 mL × 2). The combined organic layer was dried (Na_2SO_4), evaporated, and purified with SiO_2 column chromatography (EtOAc/hexane = 2:3, then 1:1) to give **20** as a white solid (2.75 g, quant). ^{1}H NMR ($CDCl_3$) δ 7.42 (d, 1H, J = 1.4 Hz), 7.36−7.33 (m, 9H), 7.14−7.11 (m, 6H), 6.83 (s, 1H), 4.15−4.10 (m, 2H), 3.76 (m, 1H), 2.66 (m, 2H), 2.15 (m, 1H), 1.66−1.63 (m, 2H), 1.45 (s, 9H), 1.40−1.38 (m, 2H); MS (ESI+) m/z 533 $(M + H)^+$.

2-(1-(*tert*-Butoxycarbonyl)piperidin-4-yl)-2-(1-trityl-1*H*-imidazol-4-yl)acetic Acid (21). A solution of **20** (2.75 g, 5.17 mmol) in 2-ethoxyethanol (5 mL) was added to the 40% KOH aqueous solution (20 mL), and the mixture was stirred at 100 °C overnight. The cooling reaction mixture was adjusted to pH 6.5 with 1 N aqueous HCl and then diluted with $CHCl_3$ (50 mL). The mixture was filtered with a pad of Celite, and the filtrate was extracted with $CHCl_3$ (50 mL × 3). The organic layer was dried (Na_2SO_4), evaporated, and purified with SiO_2 column chromatography (MeOH/$CHCl_3$ = 1:19 to 1:5) to give **21** as a white solid (1.88 g, 66%). ^{1}H NMR ($CDCl_3$) δ 7.30 (m, 10H), 7.00 (m, 6H), 6.56 (s, 1H), 3.91 (m, 2H), 3.13 (m, 1H), 2.35 (m, 1H), 1.94 (m, 2H), 1.43 (s, 9H), 1.24 (m, 2H), 0.80 (m, 2H); MS (ESI+) m/z 552 $(M + H)^+$.

1-(*tert*-Butoxycarbonyl)-4-(2-hydroxy-(1-trityl-1*H*-imidazol-4-yl)ethyl)piperidine (22). To a solution of **21** (4.14 g, 7.50 mmol) in THF (75 mL) was added BH_3·THF (1.2 M in THF, 25.0 mL, 30.0 mmol) at 0 °C, and the mixture was then warmed to room temperature. After the mixture was stirred at room temperature for 2 h, the reaction was carefully quenched with H_2O (10 mL). The resulting mixture was poured into brine (100 mL) and extracted with EtOAc (200 mL × 3). The combined organic layer was dried (Na_2SO_4) and evaporated. The residue was dissolved in THF (20 mL) and treated with *N*,*N*-dimethylethylendiamine (2.5 mL, 23.2 mmol). The generated precipitate was filtered off, and the filtrate was evaporated. The residue was purified with SiO_2 column chromatography (EtOAc/hexane = 2:3 then 3:2) to give **22** as a white solid (2.65 g, 65%). ^{1}H NMR ($CDCl_3$) δ 7.78 (d, 1H, J = 1.4 Hz), 7.40−7.33 (m, 9H), 7.11−7.08 (m, 6H), 6.61 (s, 1H), 4.14−4.04 (m, 2H), 3.86−3.83 (m, 2H), 3.21 (m, 1H), 2.63−2.54 (m, 2H), 1.80−1.77 (m, 2H), 1.45 (s, 9H), 1.36 (m, 1H), 1.18−1.15 (m, 2H); MS (ESI+) m/z 538 $(M + H)^+$.

4-(1-(1*H*-Imidazol-4-yl)-2-(4-(trifluoromethyl)phenylthio)ethyl)-piperidine Hydrochloride Salt (23). 23 was synthesized as a colorless syrup, using the procedure for **24** from **22** and 4-trifluoromethylbenzenethiol (57% in three steps, 2 HCl salt). ^{1}H NMR (CD_3OD) δ 8.28 (d, 1H, J = 1.5 Hz), 7.37 (d, 2H, J = 8.3 Hz), 7.19 (d, 2H, J = 8.3 Hz), 7.07 (d, 1H, J = 1.5 Hz), 3.39 (m, 1H), 3.31 (m, 1H), 3.28−3.10 (m, 2H), 2.92 (m, 1H), 2.81−2.73 (m, 2H), 1.99−1.90 (m, 2H), 1.50 (m, 1H), 1.30 (m, 1H), 1.16 (m, 1H); MS (ESI+) m/z 356 $(M + H)^+$; HRMS (FAB+) calcd for $C_{17}H_{21}N_3F_3S$ $(M + H)^+$ 356.1408, found 356.1409.

4-(2-(4-*tert*-Butylphenylthio)-1-(1*H*-imidazol-4-yl)ethyl)piperidin Hydrochloride Salt (24). To a solution of **22** (153 mg, 0.28 mmol) in CH_2Cl_2 (2.8 mL) was added Et_3N (0.12 mL, 0.86 mmol) and MsCl (54 μL, 0.70 mmol) at 0 °C, and the mixture was stirred at the same temperature for 30 min. The reaction was quenched with H_2O (1 mL), and the resulting mixture was poured into brine (20 mL). The mixture was extracted with EtOAc (50 mL), and the organic layer was dried (Na_2SO_4) and evaporated to give the crude mesylate.

To a solution of 4-*tert*-butylbenzenethiol (96 μL, 0.57 mmol) in DMF (2 mL) was added NaH (60 wt %, 23 mg, 0.57 mmol), and the mixture was stirred at room temperature for 20 min. To the mixture was added the above mesylate in DMF (2 mL), and the mixture was stirred at room temperature for 20 min, then 50 °C for 2 h. The reaction mixture was poured into saturated aqueous NH_4Cl (20 mL) and extracted with $CHCl_3$ (50 mL × 3 times). The combined organic layer was dried (Na_2SO_4), evaporated, and purified with short SiO_2 column chromatography (EtOAc/hexane = 1:9, then 1:4) to give the thioether. This obtained thioether was used the next step without further purification.

A mixture of above thioether in 4 N HCl-dioxane solution (1.5 mL) was stirred at room temperature for 10 min, then at 60 °C for 1 h. After additional adding of 4 N HCl−dioxane solution (1.5 mL), the reaction mixture was stirred at 90 °C for 1 h. The solvent was evaporated, and the obtained residue was purified with LH-20 (MeOH/$CHCl_3$ = 1:1) to give **24** as a colorless syrup (46 mg, 71% in three steps, 2 HCl salt). ^{1}H NMR (CD_3OD) δ 8.21 (s, 1H), 7.19 (d, 2H, J = 8.2 Hz), 7.08 (d, 2H, J = 8.2 Hz), 7.07 (s, 1H), 3.30−3.24 (m, 2H), 3.19 (m, 1H), 3.06 (dd, 1H, J = 10.4, 14.0 Hz), 2.89−2.70 (m, 3H), 1.95−1.82 (m, 2H), 1.46 (m, 1H), 1.30−1.12 (m, 2H), 1.10 (s, 9H); MS (ESI+) m/z 344 $(M + H)^+$; HRMS (FAB+) calcd for $C_{20}H_{30}N_3S$ $(M + H)^+$ 344.2160, found 344.2162.

***N*,*N*-Dimethyl-4-(2-(pyridin-2-yl)oxirane-2-yl)-1*H*-imidazole-1-sulfonamide (26a). 26a** was synthesized as a white solid, using the procedure for **26c** from 4-(hydroxyl(pyridine-2-yl)methyl)-*N*,*N*-dimethyl-1*H*-imidazole-1-sulfoneamide **25a** (12% in two steps). ^{1}H NMR ($CDCl_3$) δ 8.62 (m, 1H), 7.87 (d, 1H, J = 1.2 Hz), 7.77 (ddd, 1H, J = 1.2, 8.0, 8.0 Hz), 7.57 (d, 1H, J = 8.0 Hz), 7.36 (d, 1H, J = 1.2 Hz), 7.29 (m, 1H), 3.78 (d, 1H, J = 6.0 Hz), 3.27 (d, 1H, J = 6.0 Hz), 2.86 (s, 6H); MS (ESI+) m/z 295 $(M + H)^+$.

***N*,*N*-Dimethyl-4-(2-(pyridin-3-yl)oxirane-2-yl)-1*H*-imidazole-1-sulfonamide (26b). 26b** was synthesized as a white solid, using the procedure for **26c** from 4-(hydroxyl(pyridine-3-yl)methyl)-*N*,*N*-dimethyl-1*H*-imidazole-1-sulfoneamide **25b** (12% in two steps). ^{1}H NMR ($CDCl_3$) δ 8.78 (dd, 1H, J = 0.7, 2.2 Hz), 8.62 (dd, 1H, J = 1.7, 4.8 Hz), 7.89−7.85 (m, 2H), 7.35 (ddd, 1H, J = 1.0, 4.8, 8.0 Hz), 7.08 (d, 1H, J = 1.4 Hz), 3.78 (d, 1H, J = 5.8 Hz), 3.14 (d, 1H, J = 5.8 Hz), 2.86 (s, 6H); MS (ESI+) m/z 295 $(M + H)^+$.

***N*,*N*-Dimethyl-4-(2-(pyridin-4-yl)oxirane-2-yl)-1*H*-imidazole-1-sulfonamide (26c).** To a solution of 4-(hydroxyl(pyridine-4-yl)-methyl)-*N*,*N*-dimethyl-1*H*-imidazole-1-sulfoneamide **25c** (351 mg, 1.24 mmol) in 1,2-dichloroethane (12 mL) was added MnO_2 (650 mg, 7.47 mmol), and the mixture was stirred at 50 °C for 1.5 h. To the mixture was added MnO_2 (110 mg, 1.26 mmol) additionally, and the mixture was stirred at the same temperature for 30 min. The reaction mixture was filtrated by a pad of Celite, and the filtrate was concentrated under reduced pressure. The residue was purified with short SiO_2 column chromatography (EtOAc) to give the ketone. The obtained ketone was used for the next reaction without further purification.

To a solution of trimethylsulfonium iodide (415 mg, 1.88 mmol) in DMSO (3.5 mL) was added NaH (60 wt % in mineral oil, 73 mg, 1.85 mmol), and the mixture was stirred at room temperature for 10 min. To the mixture was added above ketone in DMSO (3 mL), and the mixture was stirred at room temperature for 30 min, then at 50 °C for 20 min. The reaction mixture was poured into brine (50 mL) and extracted with EtOAc (100 mL). The organic layer was dried (Na_2SO_4), evaporated, and purified with SiO_2 column chromatography ($MeOH/CHCl_3$ = 1:19) to give **26c** as a white solid (282 mg, 77% in two steps). ^{1}H NMR ($CDCl_3$) δ 8.64 (dd, 2H, J = 1.7, 4.6 Hz), 7.89 (d, 1H, J = 1.2 Hz), 7.46 (dd, 2H, J = 1.7, 4.6 Hz), 7.14 (d, 1H, J = 1.2 Hz), 3.78 (d, 1H, J = 5.8 Hz), 3.07 (d, 1H, J = 5.8 Hz), 2.87 (s, 6H); MS (FAB+) m/z 295 $(M + H)^+$.

4-(2-Hydroxy-1-(pyridine-2-yl)ethy)-*N*,*N*-dimethyl-1*H*-imidazole-1-sulfonamide (27a). 27a was synthesized as a colorless oil, using the procedure for **27c** from **26a** (52%). ^{1}H NMR ($CDCl_3$) δ 8.54 (ddd, 1H, J = 0.7, 1.7, 4.8 Hz), 7.85 (d, 1H, J = 1.4 Hz), 7.67 (ddd, 1H, J = 1.7, 7.6, 7.6 Hz), 7.25 (d, 1H, J = 7.6 Hz), 7.21 (ddd, 1H, J = 1.2, 4.8, 7.6 Hz), 7.06 (s, 1H), 4.29−4.22 (m, 3H), 2.85 (s, 6H); MS (ESI+) m/z 297 $(M + H)^+$.

4-(2-Hydroxy-1-(pyridine-3-yl)ethy)-*N*,*N*-dimethyl-1*H*-imidazole-1-sulfonamide (27b). 27b was synthesized as a colorless oil, using the procedure for **27c** from **26b** (42%). ^{1}H NMR ($CDCl_3$) δ 8.55 (d, 1H, J=2.2 Hz), 8.53 (dd, 1H, J=1.7, 4.8 Hz), 7.89 (d, 1H, J=1.4 Hz), 7.67 (ddd, 1H, J=1.9, 1.9, 7.8 Hz), 7.28 (dd, 1H, J = 4.8, 7.8 Hz), 6.89 (s, 1H), 4.16 (m, 1H), 4.05 (m, 1H), 3.54 (m, 1H), 2.85 (s, 6H); MS (EI+) m/z 296 $(M)^+$.

4-(2-Hydroxy-1-(pyridine-4-yl)ethy)-*N*,*N*-dimethyl-1*H*-imidazole-1-sulfonamide (27c). A suspension of **26c** (47 mg, 0.16 mmol) and 10% Pd/C (568 mg) in EtOAc (1.6 mL)−AcOH (80 μL) was stirred at room temperature under H_2 atmosphere for 45 min. The reaction mixture was filtered by a pad of Celite, and the filtrate was evaporated. The residue was purified with SiO_2 thin layer chromatography ($MeOH/CHCl_3$ = 1:9) to give **27c** as a white solid (40 mg, 84%). ^{1}H NMR ($CDCl_3$) δ 8.56 (dd, 2H, J = 1.7, 4.6 Hz), 7.90 (d, 1H, J = 1.2 Hz), 7.25 (dd, 2H, J = 1.7, 4.6 Hz), 6.94 (dd, 1H, J = 1.0, 1.0 Hz), 4.20−4.11 (m, 2H), 4.06 (m, 1H), 2.88 (s, 6H); MS (FAB+) m/z 297 $(M + H)^+$.

***N*,*N*-Dimethyl-4-(1-(pyridine-2-yl)-2-(4-trifluoromethylphenylthio)ethyl)-1*H*-imidazole-1-sulfonamide (28a). 28a** was synthesized as a colorless oil, using the procedure for **29b** from **27a** and 4-trifluoromethylbenzenethiol (77% in two steps). ^{1}H NMR ($CDCl_3$) δ 8.58 (ddd, 1H, J = 0.7, 1.7, 4.6 Hz), 7.84 (d, 1H, J = 1.2 Hz), 7.63 (ddd, 1H, J = 1.7, 7.8, 7.8 Hz), 7.49 (d, 2H, J = 8.5 Hz), 7.37 (d, 2H, J = 8.5 Hz), 7.26 (m, 1H), 7.17 (m, 1H), 7.04 (s, 1H), 4.36 (t, 1H, J = 7.6 Hz), 3.78 (d, 2H, J = 7.6H), 2.80 (s, 6H); MS (FAB+) m/z 457 $(M + H)^+$.

***N*,*N*-Dimethyl-4-(1-(pyridine-3-yl)-2-(4-trifluoromethylphenylthio)ethyl)-1*H*-imidazole-1-sulfonamide (28b). 28b** was synthesized as a colorless oil, using the procedure for **29b** from **27b** and 4-trifluoromethylbenzenethiol (63% in two steps). ^{1}H NMR ($CDCl_3$) δ 8.55 (s, 1H) 8.53 (dd, 1H, J = 1.5, 4.9 Hz), 7.85 (d, 1H, J = 1.2 Hz), 7.74 (m, 1H), 7.50 (d, 2H, J = 8.8), 7.35 (d, 2H, J = 8.8), 7.25 (m, 1H), 6.97 (d, 1H, J = 0.7 Hz), 4.17 (t, 1H, J = 7.6 Hz), 3.84 (dd, 1H, J = 7.6, 13.2 Hz), 3.47 (dd, 1H, J = 7.6, 13.2 Hz), 2.82 (s, 6H); MS (FAB+) m/z 457 $(M + H)^+$.

***N*,*N*-Dimethyl-4-(1-(pyridine-4-yl)-2-(4-trifluoromethylphenylthio)ethyl)-1*H*-imidazole-1-sulfonamide (28c). 28c** was synthesized as a colorless oil, using the procedure for **29b** from **27c** and 4-trifluoromethylbenzenethiol (84% in two steps). ^{1}H NMR ($CDCl_3$) δ 8.57 (dd, 2H, J = 1.7, 4.6 Hz), 7.85 (d, 1H, J = 1.2 Hz), 7.51 (d, 2H, J = 8.2 Hz), 7.35 (d, 2H, J = 8.2 Hz), 7.27 (dd, 2H, J = 1.7, 4.6 Hz), 6.99 (s, 1H), 4.13 (t, 1H, J = 7.6 Hz), 3.80 (dd, 1H, J = 7.6, 13.2 Hz), 3.48 (dd, 1H, J = 7.6, 13.2 Hz), 2.82 (s, 6H); MS (EI+) m/z 456 $(M)^+$.

4-(2-(4-*tert*-Butylphenylthio)-1-(pyridine-2-yl)ethyl)-*N*,*N*-dimethyl-1*H*-imidazole-1-sulfonamide (29a). 29a was synthesized as a colorless oil, using the procedure for **29b** from **27a** and 4-*tert*-butylbenzenethiol (64% in two steps). ^{1}H NMR ($CDCl_3$) δ 8.55 (ddd, 1H, J = 0.7, 1.7, 4.6 Hz), 7.81 (d, 1H, J = 1.2 Hz), 7.63 (ddd, 1H, J = 1.9, 7.8, 7.8 Hz), 7.30−7.25 (m, 5H), 7.15 (ddd, 1H, J = 0.9, 4.8, 7.8 Hz), 7.07 (dd, 1H, J = 0.7, 1.2 Hz), 4.33 (t, 1H, J = 7.6 Hz), 3.68 (d, 2H, J = 7.6H), 2.51 (s, 6H), 1.28 (s, 9H); MS (FAB+) m/z 445 $(M + H)^+$.

4-(2-(4-*tert*-Butylphenylthio)-1-(pyridine-3-yl)ethyl)-*N*,*N*-dimethyl-1*H*-imidazole-1-sulfonamide (29b). To a solution of **27b** (74 mg, 0.25 mmol) in CH_2Cl_2 (2.5 mL) was added Et_3N (87 μL, 0.62 mmol) and MsCl (23 μL, 0.30 mmol) at 0 °C, and the mixture was stirred at the same temperature for 10 min. The reaction was quenched with H_2O (3 mL), and the resulting mixture was poured into brine (20 mL). The mixture was extracted with $CHCl_3$ (50 mL × 3), and the combined organic layer was dried (Na_2SO_4) and evaporated to give the crud mesylate.

To a solution of 4-*tert*-butylbenzenethiol (0.12 mL, 0.71 mmol) in DMF (2 mL) was added NaH (60 wt %, 30 mg, 0.75 mmol), and the mixture was stirred at room temperature for 20 min. To the mixture was added the above mesylate in DMF (2 mL), and the mixture was stirred at room temperature overnight. The reaction mixture was poured into saturated aqueous NH_4Cl (20 mL) and extracted with $CHCl_3$ (50 mL × 3). The combined organic layer was dried (Na_2SO_4), evaporated, and purified with SiO_2 thin layer chromatography ($MeOH/CHCl_3$ = 1:19) to give **29b** as a colorless oil (93 mg, 84% in two steps). ^{1}H NMR ($CDCl_3$) δ 8.52 (d, 1H, J = 1.9 Hz), 8.49 (dd, 1H, J = 1.6, 4.8 Hz), 7.82 (d, 1H, J = 1.2 Hz), 7.70 (ddd, 1H, J = 1.9,1.9, 8.0 Hz), 7.31−7.00 (m, 5H), 7.00 (s, 1H), 4.16 (t, 1H, J = 7.6 Hz), 3.72 (dd, 1H, J = 7.6, 13.2 Hz), 3.40 (dd, 1H, J = 7.6, 13.2 Hz), 2.83 (s, 6H), 1.27 (s, 9H); MS (FAB+) m/z 445 $(M + H)^+$.

4-(2-(4-*tert*-Butylphenylthio)-1-(pyridine-4-yl)ethyl)-*N*,*N*-dimethyl-1*H*-imidazole-1-sulfonamide (29c). 29c was synthesized as a colorless oil, using the procedure for **29b** from **27c** and 4-*tert*-butylbenzenethiol (7% in two steps). ^{1}H NMR ($CDCl_3$) δ 8. 54 (m, 2H), 7.82 (d, 1H, J = 1.5 Hz), 7.32−7.23 (m, 6H), 7.01 (m, 1H), 4.10 (t, 1H, J = 7.6 Hz), 3.67 (dd, 1H, J = 7.8, 13.4 Hz), 3.50 (dd, 1H, J = 7.8, 13.4 Hz), 2.85 (s, 6H), 1.31 (s, 9H); MS (EI+) m/z 444 $(M)^+$.

2-(1-(1*H*-Imidazole-4-yl)-2-(4-trifluoromeethylphenylthio)-ethyl)pyridine Hydrochloride Salt (30). A solution of **28a** (79 mg, 0.18 mmol) in 5 N aqueous HCl (1 mL) was stirred at 80 °C for 4 h. The solvent was evaporated, and the obtained residue was purified with LH-20 ($MeOH/CHCl_3$ = 1:1) to give **30** as a colorless solid (53 mg, 70%, 2 HCl salt), mp 129 °C. ^{1}H NMR (CD_3OD) δ 8.98 (s, 1H), 8.80 (d, 1H, J = 5.6 Hz), 8.5 (m, 1H), 8.06 (m, 1H), 7.95 (m, 1H), 7.81 (s, 1H), 7.56 (d, 2H, J = 8.5 Hz), 7.51 (d, 2H, J = 8.5 Hz), 5.03 (m, 1H), 4.06−4.03 (m, 2H); MS (ESI+) m/z 350 $(M + H)^+$; HRMS (FAB+) calcd for $C_{17}H_{15}N_3F_3S$ $(M + H)^+$ 350.0939, found 350.0940.

2-(2-(4-*tert*-Butylphenylthio)-1-(1*H*-imidazol-4-yl)ethyl)pyridine (31). 31 was synthesized as a colorless oil, using the procedure for **33** from **29a** (80%). ^{1}H NMR ($CDCL_3$) δ 8.55(ddd, 1H, J =

0.7, 1.7, 4.8 Hz), 7.63 (ddd, 1H, J = 1.9, 7.8, 7.8 Hz), 7.55 (d, 1H, J = 0.7 Hz), 7.30−7.23 (m, 5H), 7.14 (ddd, 1H, J = 1.2, 4.8, 7.3 Hz), 6.89 (s, 1H), 4.35 (t, 1H, J = 6.8 Hz), 3.65−3.48 (m, 2H), 1.29 (s, 9H); MS (FAB+) m/z 338 $(M + H)^+$; HRMS (FAB+) calcd for $C_{20}H_{24}N_3S$ $(M + H)^+$ 338.1691, found 338.1687.

3-(1-(1*H*-Imidazole-4-yl)-2-(4-trifluoromeethylphenylthio)ethyl)-pyridine (32). 32 was synthesized as a colorless oil, using the procedure for **33** from **28b** (81%). ^{1}H NMR ($CDCL_3$) δ 8.53 (d, 1H, J = 2.2 Hz), 8.46 (dd, 1H, J = 1.7, 4.6 Hz), 7.70 (m, 1H), 7.60 (d, 1H, J = 1.2 Hz), 7.48 (d, 2H, J = 8.8 Hz), 7.33 (d, 2H, J = 8.8 Hz), 7.23 (m, 1H), 6.78 (s, 1H), 4.23 (dd, 1H, J = 7.6, 7.6 Hz), 3.86 (dd, 1H, 7.0, 13.0 Hz), 3.48 (dd, 1H, 7.0, 13.0 Hz); MS (FAB+) m/z 350 $(M + H)^+$; HRMS (FAB+) calcd for $C_{17}H_{15}N_3F_3S$ $(M + H)^+$ 350.0939, found 350.0929.

3-(2-(4-*tert*-Butylphenylthio)-1-(1*H*-imidazol-4-yl)ethyl)pyridine (33). A solution of **29b** (93 mg, 0.21 mmol) in 5 N aqueous HCl (1.5 mL) was stirred at 80 °C for 20 h. The reaction mixture was basified with 5 N aqueous NaOH and diluted with saturated aqueous K_2CO_3 (20 mL) and brine (10 mL). The mixture was extracted with $CHCl_3$ (30 mL × 3), and the combined organic layer was dried (Na_2SO_4), evaporated, and purified by NH thin layer chromatography (MeOH/$CHCl_3$ = 1:9) to give **33** as a white solid (59 mg, 83%), mp 103 °C. ^{1}H NMR ($CDCl_3$) δ 8.47 (d, 1H, J = 1.7 Hz), 8.43 (dd, 1H, J = 1.7, 4.8 Hz), 7.66 (d, 1H, J = 8.0 Hz), 7.56 (s, 1H), 7.30−7.19 (m, 6H), 6.78 (s, 1H), 4.19 (t, 1H, J = 7.6 Hz), 3.76−3.69 (m, 2H), 1.27 (s, 9H); MS (FAB+) m/z 338 $(M + H)^+$; HRMS (FAB+) calcd for $C_{20}H_{24}N_3S$ $(M + H)^+$ 338.1691, found 338.1686.

4-(1-(1*H*-Imidazole-4-yl)-2-(4-trifluoromeethylphenylthio)ethyl)-pyridine (34). 34 was synthesized as a colorless oil, using the procedure for **33** from **28c** (18%). ^{1}H NMR ($CDCL_3$) δ 8.52 (dd, 2H, J = 1.6, 3.0 Hz), 7.62 (s, 1H), 7.49 (d, 2H, J = 8.2 Hz), 7.35 (d, 2H, J = 8.2 Hz), 7.28 (dd, 2H, J = 1.6, 3.0 Hz), 6.79 (s, 1H), 4.20 (t, 1H, J = 7.6 Hz), 3.84 (dd, 1H, J = 7.6, 13.0 Hz), 3.50 (dd, 1H, J = 7.6, 13.0 Hz); MS (EI+) m/z 349 $(M)^+$; HRMS (FAB+) calcd for $C_{17}H_{15}N_3F_3S$ $(M + H)^+$ 350.0939, found 350.0946.

4-(2-(4-*tert*-Butylphenylthio)-1-(1*H*-imidazol-4-yl)ethyl)pyridine Hydrochloride Salt (35). 35 was synthesized as a colorless syrup, using the procedure for **30** from **29c** (52%, 2 HCl salt). ^{1}H NMR (CD_3OD) δ 8.87 (d, 1H, J = 1.2 Hz), 8.77 (d, 2H, J = 6.6 Hz), 8.02 (d, 2H, J = 6.6 Hz), 7.68 (s, 1H), 7.32 (d, 2H, J = 6.0 Hz), 7.25 (d, 2H, J = 6.0 Hz), 4.73 (t, 1H, J = 7.6 Hz), 3.78 (dd, 1H, J = 7.6, 14.0 Hz), 3.71 (dd, 1H, J = 7.6, 14.0 Hz), 1.26 (s, 9H); MS (FAB+) m/z 338 $(M + H)^+$; HRMS (FAB+) calcd for $C_{20}H_{24}N_3S$ $(M + H)^+$ 338.1691, found 338.1687.

(*S*)-4-(1-(1*H*-Imidazol-4-yl)-2-(4-(trifluoromethyl)phenylthio)-ethyl)piperidine (36) and (*R*)-4-(1-(1*H*-Imidazol-4-yl)-2-(4-(trifluoromethyl)phenylthio)ethyl)piperidine (37). The enantiomers of **23** were separated by preparative liquid chromatography using a Chiralpak AD-H (DAICEL) 20 mm × 250 mm (0.1% diethylamine−MeOH). **36** (white foam) 99.5% ee; $[\alpha]_D$ −22.5 (c 0.26, $CHCl_3$); HRMS (FAB+) calcd for $C_{17}H_{21}N_3F_3S$ $(M + H)^+$ 356.1408, found 356.1406. Anal. Calcd for $C_{17}H_{20}F_3N_3S\cdot H_2O$: C, 54.68; H, 5.94; N, 11.25. Found: C, 54.67; H, 6.03; N, 11.17. **37** (white foam) 98.5% ee; $[\alpha]_D$ +20.4 (c 0.25, $CHCl_3$); HRMS (FAB+) calcd for $C_{17}H_{21}N_3F_3S$ $(M + H)^+$ 356.1408, found 356.1409. Anal. Calcd for $C_{17}H_{20}F_3N_3S\cdot H_2O$: C, 54.68; H, 5.94; N, 11.25. Found: C, 54.54; H, 5.85; N, 11.13.

Pharmacology. H3 Receptor Binding Assay. Membranes prepared from CHO cells stably expressing human recombinant histamine H3 receptors were incubated with [^{3}H]-(*R*)-α-methylhistamine (3 nM, GE Healthcare Bio-Sciences) and test compounds in a buffer containing 50 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, and 0.04% BSA at 25 °C for 1 h. Nonspecific binding was assessed with (*R*)-α-methylhistamine (1 μM, Sigma-Aldrich). Radioligand binding was terminated by filtering through GF/B filters (PerkinElmer Life Sciences), and the amount of bound radiolabel was determined by liquid scintillation counting.

[^{35}S]GTPγS Binding Assay. Membranes prepared from CHO cells stably expressing human recombinant histamine H3 receptors were incubated with [^{35}S]GTPγS (200 pM, GE Healthcare Bio-Sciences) and test compounds alone or in the presence of (*R*)-α-methylhistamine (10 nM, Sigma-Aldrich) in a buffer containing 50 mM Tris-HCl (pH 7.4), 100 mM NaCl, 3 mM $MgCl_2$, 0.2 mM EDTA, 1 mM DTT, and 10 μM GDP at 25 °C for 1 h. Radioligand binding was terminated by filtering through GF/B filters, and the amount of bound radiolabel was determined by liquid scintillation counting.

Supporting Information Available: HPLC analytical data for **15**, **16**, **23**, **24**, and **30**−**37**; crystal structure determination of compounds **36** and **37**; experimental details of in vitro human CYP inhibition test; modeled binding mode of **16** in the human H3 receptor binding site. This material is available free of charge via the Internet at http://pubs.acs.org.

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