Influence of Bulky Substituents on Histamine H_3 Receptor Agonist/Antagonist Properties†

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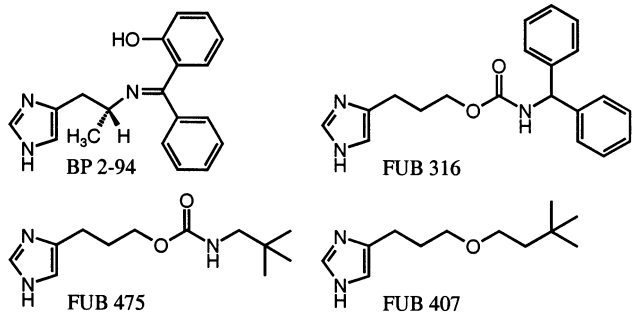
Novel derivatives of 3-(1*H*-imidazol-4-yl)propanol were designed on the basis of lead compounds belonging to the carbamate or ether series possessing (partial) agonist properties on screening assays of the histamine H_3 receptor. One pair of enantiomers in the series of α-methyl-branched chiral carbamates was stereoselectively prepared in high optical yields. Enantiomeric purity was checked by Mosher amide derivatives of precursors and capillary electrophoresis of the final compounds with trimethyl-*β*-cyclodextrin as chiral selector, and was determined to be ≥95%. The novel compounds were investigated in various histamine H_3 receptor assays in vitro and in vivo. Some compounds displayed partial agonist activity on synaptosomes of rat brain cortex, whereas others exhibited antagonist properties only. Selected compounds were investigated in [^{125}I]iodoproxyfan binding studies on the human histamine H_3 receptor and showed high affinity in the nanomolar concentration range. Under in vivo conditions after oral administration to mice, some of the compounds exhibited partial or full agonist activity in the brain at low dosages. The (*S*)-enantiomer of one pair of chiral carbamates (**9**) proved to be the eutomer; thus, the (*S*)-enantiomer was selected for further pharmacological studies. In a peripheral in vivo test model in rats, measuring the level of inhibition of capsaicin-induced plasma extravasation, (*S*)-**9** again proved its high oral agonist potency with full intrinsic activity (ED_{50} values of 0.07−0.1 mg/kg depending on tissue).

Introduction

In 1983, the histamine H_3 receptor was first described pharmacologically as an inhibitory autoreceptor on histaminergic neurons in the central nervous system.[3] Many highly selective and potent histamine H_3 receptor agonists and antagonists have been developed for pharmacological characterization, e.g., radioligands,[4] but also for therapeutical implications, e.g., BP 2-94,[5,6] GT-2331[7] (cipralisant, formerly Perceptin), and ciproxifan.[8,9] In 1999, the human histamine H_3 receptor cDNA was discovered by Lovenberg et al.[10] Binding properties and functional investigations with known histamine H_3 receptor ligands were performed. In following experiments, species differences between human and rat H_3 receptors were observed[11,12] as well as high constitutive activity of both H_3 receptors.[13−15] Therapeutic targets for histamine H_3 receptor agonists have been proposed in neurogenic inflammation (e.g., airways and urinary bladder), migraine and sleep disorders.[16] In the search for novel histamine H_3 receptor agonists, which lack a basic moiety in the side chain of the molecule, thus improving pharmacokinetic properties such as resorption and penetration of the blood−brain barrier, carbamate and ether derivatives of 3-(1*H*-imidazol-4-yl)-propanol with high agonist in vivo potencies have been described.[17,18] Derived from FUB 316[18] (carbamate), FUB 407[17] (ether), and FUB 475[17] (carbamate) as lead compounds (Chart 1), new aliphatic ethers and carbamates were designed as potential histamine H_3 receptor agonists and pharmacologically described in this study. As one of the novel carbamate racemates [(*R*/*S*)-**9**] exhibited interesting pharmacological behavior in stand-

Chart 1



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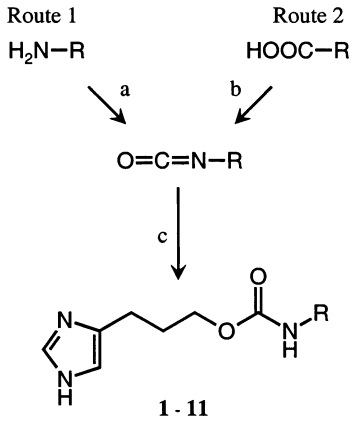
Scheme 1. Synthesis of Carbamates **1**−**11**[a]



[a] (a) $ClCOOCCl_3$, charcoal (cat.), ethyl acetate, reflux for 4−5 h; (b) DPPA, Et_3N, dioxane, reflux for 30 min; (c) 3-(1*H*-imidazol-4-yl)propanol·HCl,[21] acetonitrile, reflux for 4−5 h.

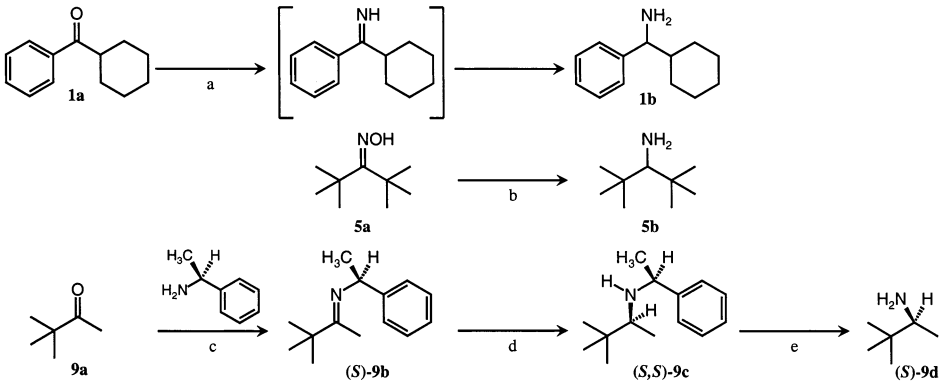
ard in vitro and in vivo screening assays, its enantiomers were synthesized stereoselectively. Enantiomeric purity of the enantiomers was determined by capillary electrophoresis (CE) by a method developed for structurally similar optically active compounds.[19,20] For selected compounds, additional pharmacological investigations were performed such as [^{125}I]iodoproxyfan binding studies on the human H_3 receptor, stably transfected into CHO-K1 cells as well as peripheral in vivo agonist activity based on a capsaicin-induced plasma extravasation assay in rats after oral treatment, and a receptor profile.

Chemistry

3-(1*H*-Imidazol-4-yl)propanol in its unprotected and trityl-protected form was synthesized as described previously from urocanic acid.[21] Carbamates (**1**−**5** and **7**−**11**) were prepared from appropriate amines by reaction of the amine with diphosgene, forming an intermediate isocyanate, which was isolated from the reaction mixture by careful distillation. The isocyanate that was obtained was then added to 3-(1*H*-imidazol-4-yl)-propanol·HCl (Scheme 1, route 1). 2,2,3,3-Tetramethylcyclopropyl carbamate **6** was synthesized from 2,2,3,3-tetramethylcarboxylic acid by a modified Curtius reaction using diphenyl phosphorazidate (DPPA) under basic conditions,[22] leading to an intermediate carboxylic acid azide, which reacts by nitrogen liberation to the corresponding isocyanate. The alcoholic component for building up the carbamate, 3-(1*H*-imidazol-4-yl)propanol·HCl, was then added to this one-pot two-step reaction (Scheme 1, route 2). Amines needed for carbamate synthesis by route 1 (Scheme 1) were either commercially available [**4a**, **8a**, **9a**, **11a**, (*R*)-**11a**, and (*S*)-**11a**] or synthesized from appropriate ketones/oximes as outlined in Scheme 2. Amines **1b**−**3b** were synthesized by reductive amination as shown for **1b** (Scheme 2). In the presence of ammonium acetate, as a buffer and nitrogen donor, ketones preferably react to imines, which are rapidly reduced by $NaBH_3CN$ as described by Borch et al.[23] Competing direct reduction of ketones was avoided by properly controlling the pH with ammonium acetate, as ketones are reduced at lower pH than imines. The sterically highly hindered di-*tert*-butyl ketoxime **5a** was commercially available and reduced to amine **5b** with $TiCl_4/NaBH_4$.[24]

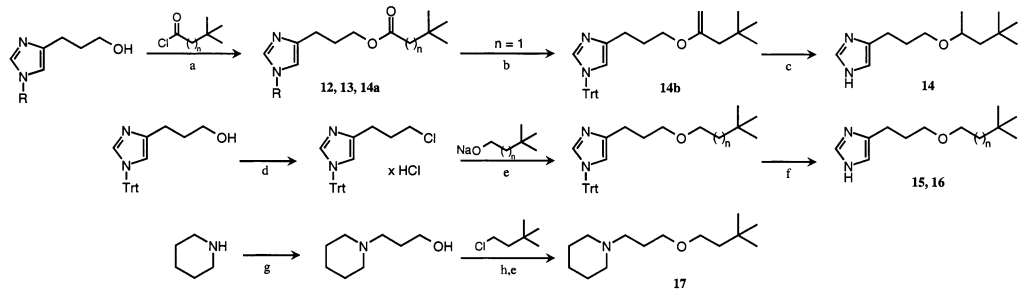
Chiral amines (*R*)-**9d** and (*S*)-**9d** were synthesized by a stereoselective three-step reaction procedure shown in Scheme 2 for (*S*)-**9d**, first described by Charles et al.[25] However, the optimized reaction protocol developed by Moss et al.[26] was applied, leading to the high enantiomeric purity of the optically active products by working at low temperature for the formation of the Schiff base and mild reaction conditions for the reduction to the secondary amine. The prochiral ketone pinacolone (**9a**) is transformed to the corresponding (*E*)-Schiff base with an enantiomerically pure amine, e.g., (*S*)-1-phenylethan-1-amine, leading to (*S*)-**9b**. By reduction of (*S*)-**9b** under mild conditions, a diastereomeric secondary amine (*S*,*S*)-**9c** is formed, its chirality determined by the chiral auxiliary amine used for the initial formation of the Schiff base [e.g., (*S*)-1-phenylethan-1-amine, Scheme 2]. Purification of the diastereomeric amine (*S*,*S*)-**9c** from traces of the (*R*,*S*)-diastereomer was easily achieved by column chromatography and repeated recrystallization. Subsequent liberation of the enantiomerically pure primary amine (*S*)-**9d** was performed by debenzylation according to mild standard deprotection methods (Scheme 2). Racemic amines **7d** and **10d** were prepared by the same procedure, using (*RS*)-1-phenylethan-1-amine and benzylamine, respectively. Enantiomeric purity of the stereoselectively synthesized amines (*S*)- and (*R*)-**9d** was checked by preparing the corresponding Mosher amide derivatives[27] and determining the diastereomeric ratios by ^{1}H NMR spectroscopy as well as HPLC (de ≥ 95%).

Scheme 2. Synthesis of Amines[a]



[a] (a) $NaBH_3CN$, NH_4CH_3COO, MeOH, rt, 196 h; (b) $NaBH_4/TiCl_4$, 1,2-dimethoxyethane, 0 °C → rt, 12 h; (c) Et_3N, $TiCl_4$, CH_2Cl_2, 0 °C → reflux for 2 h; (d) $NaBH_4$, EtOH, −78 °C → −25 °C, 2 h; (e) $Pd(OH)_2$, H_2 (1 bar), MeOH, rt, 3 h.

Scheme 3. Synthesis of Esters (**12** and **13**) and Ethers **14**−**17**[a]



[a] (a) R = H for **12** and **13**, R = triphenylmethyl for **14a**, 4-(dimethylamino)pyridine, pyridine, rt, 12 h; (b) Tebbe's reagent,[30] THF, 0 °C, 1 h; (c) Pd/C, H_2 (1 bar), MeOH, 48 h; (d) $SOCl_2$, THF, 0 °C → rt, 2 h; (e) 15-crown-5 (cat.), $(C_4H_9)_4NI$ (cat.), toluene, reflux for 12 h; (f) 2 N HCl/THF (20:30), 2 h, reflux; (g) 3-chloropropan-1-ol, KI (cat.), EtOH, reflux, 12 h; (h) NaH (60% in mineral oil), toluene, rt, 12 h.

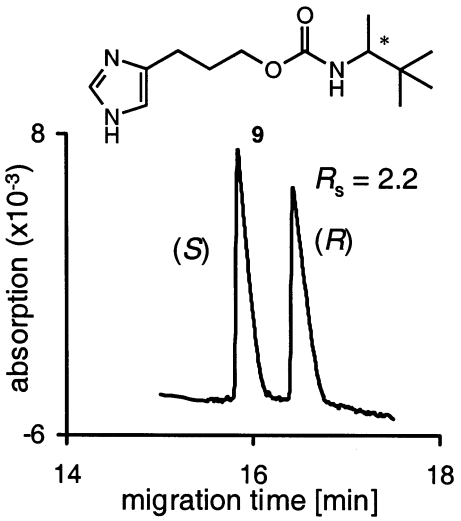


Figure 1. Separation of enantiomers of **9** by CE. Background electrolyte: 20 mM TM-β-CD, 150 mM H_3PO_4, pH 2.7 (triethylamine), 25 °C, sample concentration of 2.5 mg/mL, and injection time of 2 s.

Final carbamates (*S*)-**9** and (*R*)-**9** were then synthesized as shown in Scheme 1, route 1, although, to increase reaction yields, a solution of phosgene in toluene was used for the formation of the isocyanate. This change in protocol was necessary because the intermediate isocyanate that was obtained had a low boiling point which is close to that of diphosgene. Thus, problems occurred in the fractional distillation of remaining traces of diphosgene. Thus, advantage was taken of the low boiling point of phosgene, which was removed from the reaction vessel with nitrogen and securely inactivated with aqueous KOH and NH_3 solutions through which it was passed. Piperidine analogue **18** was likewise synthesized from (*S*)-**9d** and 3-piperidinopropan-1-ol.[28] Enantiomeric purities of final carbamates (*S*)-**9** and (*R*)-**9** were determined by capillary electrophoresis (CE). The development of the CE method has been described by Sasse et al. for comparable histamine H_3 receptor ligands.[19,20] Trimethyl-β-cyclodextrin (TM-β-CD) as a chiral selector was able to achieve baseline separation with a resolution (R_s) of 2.2 for (*R*/*S*)-**9** (Figure 1). The migration order of the enantiomers was determined by spiking experiments. Enantiomeric purity of (*S*)-**9** and (*R*)-**9** was determined to be as high as ≥95% (ee).

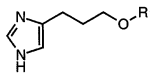
Esters **12** and **13** were prepared by standard methods according to Einhorn[29] from deprotected 3-(1*H*-imidazol-4-yl)propanol·HCl and 3,3-dimethylbutanoic acid chloride (Scheme 3). For subsequent reaction to **14**, the triphenylmethyl-protected analogue of **13** was prepared (**14a**). Methylenation of ester **14a** was carried out with Tebbe's reagent [(μ-chloro)(μ-methylene)bis(cyclopentadienyl)(dimethylaluminum)titanium] under inert conditions in THF.[30] The triphenylmethyl-protected derivative of **12** did not react, which might be due to steric hindrance of the *tert*-butyl group. Isolation of substituted vinyl ether **14a** was achieved; however, deprotection of the imidazole ring by mild acid hydrolysis under different conditions resulted in degradation to the deprotected starting material, 3-(1*H*-imidazol-4-yl)propanol. Upon hydrogenation under mild conditions, a deprotection of the imidazole and hydrogenation of the double bond was achieved which led to α-methyl-branched ether **14** (Scheme 3).

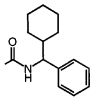
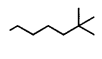
Ethers **15**−**17** were synthesized by classical Williamson ether synthesis[31] (Scheme 3). To achieve higher reaction yields, 3-(1-triphenylmethyl-1*H*-imidazol-4-yl)-propanol was transformed into the corresponding alkyl chloride to which freshly prepared sodium alkyl alcoholate was added (Scheme 3). Alcoholate precursors of **15** and **16** were synthesized from 1-chloro-3,3-dimethylbutane by elongation of the alkyl chain via the corresponding nitrile and malonic acid ester, respectively, according to standard methods. 3-Piperidinopropan-1-ol was prepared from piperidine and 3-chloropropan-1-ol as described previously[28,32] and transformed into its sodium alcoholate to which 1-chloro-3,3-dimethylbutane was added (Scheme 3).

Pharmacological Results and Discussion

Functional in Vitro Assays. Many of the novel compounds were tested on two functional in vitro models for histamine H_3 receptor activity in different species. Modulated release of [^{3}H]histamine from K^+-evoked depolarized synaptosomes of rat cerebral cortex[33] and concentration-dependent inhibition of electrically evoked twitches of guinea pig ileum by (*R*)-α-methylhistamine in the presence of an antagonist were used as screening assays.[34,35] All novel imidazole-containing carbamates (**1**−**11**) are highly potent histamine H_3 receptor antagonists in the guinea pig ileum assay (Table 1). Most of the carbamates exhibit K_B values between 10 and 25 nM, except for **1**, which is less potent, and for dicyclopropylmethyl carbamate **3**, which is more potent (1 nM), but does not seem to be a pure competitive antagonist as the slope of the Schild plot was observed to be significantly less than 1 (not shown). Ester compounds with a *tert*-butyl moiety (**12** and **13**) are far less potent than the corresponding carbamates, which is in agree-

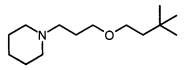
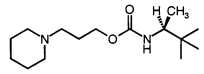
Table 1. Structures, Chemical Data, and Pharmacological Results of Screening for Histamine H_3 Receptor Agonist and Antagonist Activity in Vitro and in Vivo



					in vitro					in vivo	
				mp[a]	K_i[b] (nM)	K_B[c] (nM)	K_i[d] (nM)	EC_{50}[e] (nM)		ED_{50}[f] (mg/kg)	
no.	R	formula	M_r	(°C)		$\bar{x} \pm s_{\bar{x}}$	$\bar{x} \pm s_{\bar{x}}$	$\bar{x} \pm s_{\bar{x}}$	i.a.[g]	$\bar{x} \pm s_{\bar{x}}$	i.a.[g]
1		$C_{20}H_{27}N_3O_2 \cdot C_4H_4O_4 \cdot 0.75H_2O$	471.0	136		123 ± 25	56 ± 14		-	15 ± 2	-
2		$C_{17}H_{21}N_3O_2 \cdot 0.8C_2H_2O_4$	371.4	124		20 ± 2	44 ± 9		-	≈10	-
3		$C_{14}H_{21}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	383.9	136	8.2	1.05[h]	11 ± 2		-	1.1 ± 0.3	-
4		$C_{14}H_{25}N_3O_2 \cdot C_2H_2O_4$	357.4	144		12 ± 5				>10	-
5		$C_{16}H_{29}N_3O_2 \cdot C_2H_2O_4$	385.5	179	77	13 ± 2				≈ 15	-
6		$C_{14}H_{23}N_3O_2 \cdot C_4H_4O_4$	381.4	127		14 ± 1				≥ 10[i]	-
7		$C_{12}H_{19}N_3O_2 \cdot C_2H_2O_4 \cdot 0.5H_2O$	336.4	128	20	18 ± 1				6.6 ± 1.7	-
8		$C_{12}H_{21}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	333.9	136		22 ± 3				0.51 ± 0.38	0.6
9		$C_{13}H_{23}N_3O_2 \cdot C_2H_2O_4 \cdot 0.25H_2O$	347.9	123		15 ± 2	13 ± 1	n.c.[i]	≈ 0.2	0.31 ± 0.06	1.0
(*R*)-**9**					6.7	7 ± 1	13 ± 1	n.c.[i]	≈ 0.1	0.75 ± 0.34	0.45
(*S*)-**9**					14	13 ± 1	10 ± 2	46 ± 35	0.45	0.27 ± 0.10	1.0
10		$C_{13}H_{21}N_3O_2 \cdot C_2H_2O_4 \cdot 0.25H_2O$	345.9	131	6.0	18 ± 2	5.1 ± 1.0	60 ± 27	0.25	0.20 ± 0.07	1.0
11		$C_{15}H_{25}N_3O_2 \cdot C_4H_4O_4 \cdot 0.5H_2O$	404.5	118	16	18 ± 3				4.0 ± 0.4	-
(*R*)-**11**					9.7	20 ± 1				8.6 ± 2.1	
(*S*)-**11**					37	13 ± 1				3.3 ± 1.0	-
12		$C_{11}H_{18}N_2O_2 \cdot C_2H_2O_4$	300.3	145			1733 ± 108		-	>10	-
13		$C_{12}H_{20}N_2O_2 \cdot C_2H_2O_4$	314.3	166		294 ± 62				>10	-
14		$C_{13}H_{24}N_2O \cdot 0.85C_2H_2O_4$	300.9	182	119	65 ± 16				3.8 ± 1.3	-
15		$C_{13}H_{24}N_2O \cdot C_4H_4O_4$	340.4	135	5.4	24 ± 3				0.13 ± 0.07	0.6
16		$C_{14}H_{26}N_2O \cdot 0.8C_2H_2O_4$	310.4	184	14	44 ± 4				1.4 ± 0.4	-
FUB 407[k]					11	42 ± 7	10 ± 3	45 ± 10	0.55	0.29 ± 0.17	1.0
Imetit[l]						6.6[m] (i.a. = 1.0)		2.8 ± 0.7[l]	1.0	1.0 ± 0.3[l]	1.0

[a] Crystallization solvent, Et_2O/EtOH. [b] [^{125}I]Iodoproxyfan binding assay on the human H_3 receptor.[12] [c] Functional H_3 receptor assay on guinea pig ileum.[34,35] [d] Functional H_3 receptor assay on synaptosomes of rat cerebral cortex, compounds tested as antagonists.[33] [e] Functional H_3 receptor assay on synaptosomes of rat cerebral cortex, compounds tested as agonists.[33] [f] Central H_3 receptor screening after oral administration as a methylcellulose suspension to mice.[33] [g] i.a. = intrinsic activity (histamine i.a. = 1.0). [h] The 95% confidence interval, 0.58−1.91 nM; slope of the Schild plot, 0.85 ± 0.05 ($P < 0.01$). [i] At 30 mg/kg, toxicity signs appeared. [j] n.c. = cannot be calculated due to a low i.a. [k] From ref 17. [l] From ref 33. [m] EC_{50} value.[35a]

Table 2. Piperidine Replacement of the Imidazole Heterocycle[e]

no.	structure	formula	M_r	mp[a] (°C)	K_B[b] (nM) $\bar{x} \pm s_{\bar{x}}$	K_i[c] (nM) $\bar{x} \pm s_{\bar{x}}$	ED_{50}[d] (mg/kg) $\bar{x} \pm s_{\bar{x}}$
17		$C_{14}H_{29}NO \cdot 1.1C_2H_2O_4$	326.4	143	308 ± 45	286 ± 69	≈30
18		$C_{15}H_{30}N_2O_2 \cdot C_2H_2O_4 \cdot 0.25H_20$	365.0	148	5000 ± 1600		>10

[a] Crystallization solvent, Et_2O/EtOH. [b] Functional H_3 receptor assay on guinea pig ileum.[34,35] [c] Functional H_3 receptor assay on synaptosomes of rat cerebral cortex.[33] [d] Central H_3 receptor screening after oral administration as a methylcellulose suspension to mice.[33] [e] Structures, chemical data, and pharmacological results of screening for histamine H_3 receptor antagonist activity in vitro and in vivo.

ment with previous results.[21] Furthermore, imidazole replacement of (*S*)-**9** by piperidine (**18**) also led to an enormous drop in histamine H_3 receptor potency on the guinea pig assay (from 13 to 5000 nM, Table 2). In some other classes of compounds, a similar structural replacement of the imidazole heterocycle maintained antagonist histamine H_3 receptor potency.[32] Aliphatic ethers (**14**−**16**) structurally derived from lead FUB 407[17] (Chart 1, Table 1, $K_B = 42$ nM) showed similar potencies in the ileal preparation of guinea pig, with **14** being slightly less potent, **15** being more potent, and **16** being equipotent (cf. Table 1). In agreement with the observation on carbamates, **17**, the piperidine analogue of FUB 407, did not retain H_3 receptor potency (Table 2).

Some compounds were tested in a second functional model on the modulated release of [^{3}H]histamine from K^+-evoked depolarized synaptosomes of rat cerebral cortex[33] to assess histamine H_3 receptor potency and to investigate their potential agonist properties (Table 1). In previous studies on structurally related antagonists, partial agonism of some carbamates and ethers could be observed using this functional model, e.g., FUB 316,[18] FUB 407,[17] and FUB 475,[17] whereas the same compounds did not induce a depression of contraction of electrically evoked twitches of guinea pig ileum, but shifted the concentration−response curve of (*R*)-α-methylhistamine competitively to the right, thus displaying histamine H_3 receptor antagonist properties. A similar behavior was found for some of the new compounds described here. Whereas compounds **1**−**3** behave as antagonists in the [^{3}H]histamine release model on rat synaptosomes, partial agonist behavior was observed for racemate **9**, but due to low intrinsic activity ($\alpha \approx 0.2$), an exact EC_{50} value could not be calculated (Table 1). Structurally related to the *tert*-butyl derivative **9** is the cyclopropylmethyl derivative **10**, which also showed partial agonist properties on rat synaptosomes. Thus, the nature of the bulky alkyl group determines whether antagonist or agonist properties are displayed. These differences will be important in following theoretical studies on models of receptor activation. For general histamine H_3 receptor potency, the imidazole moiety seems to be very important within this class of compounds, as the piperidine analogue of FUB 407 is clearly less potent (Table 2, FUB 407 → **17**).

Binding Assay on Human H_3 Receptors. [^{125}I]Iodoproxyfan binding was performed for selected compounds in CHO-K1 cells stably transfected with the full-length coding sequence of the hH_3 receptor, as described previously.[12] All compounds that were investigated (**3**, **5**, **7**, **9**−**11**, and **14**−**16**) showed good to high affinities for the human histamine H_3 receptor, although some differences from values obtained from functional studies on guinea pig and rat were observed. However, this effect was not surprising as binding studies with the rat H_3 receptor in comparison to the human counterpart revealed distinct pharmacology for certain compounds, with binding affinities being equipotent or in favor of either hH_3 or rH_3 receptors.[11,12] The most important compounds in the study presented here, the enantiomers of **9**, showed similar potencies both in in vitro binding and in functional assays (Table 1).

In Vivo Assay on Swiss Mice. All novel compounds were screened for modulating effects on N^τ-methylhistamine levels in the brain cortex of Swiss mice after oral treatment (Tables 1 and 2). Compounds **1**, **2**, and **4**−**6** enhanced N^τ-methylhistamine levels with weak antagonist potency only. However, dicyclopropylmethyl derivative **3** showed antagonist behavior equipotent to that of thioperamide (ED_{50} = 1 mg/kg, po).[36] Some α-methyl-branched compounds revealed agonist behavior with full or partial intrinsic activities (**8**−**10**), whereas compounds with a cyclopropyl (**7**) or cyclohexyl (**11**) moiety showed antagonist behavior and were only moderately potent. Isopropyl (**8**), *tert*-butyl (**9**), and cyclopropylmethyl derivatives (**10**) were able to decrease N^τ-methylhistamine levels, thus revealing agonist action with high potency ($ED_{50} \leq 0.5$ mg/kg, po). In this in vivo assay, carbamates **9**, (*S*)-**9**, and **10** displayed full agonism.

In vivo, no effect was observed for esters **12** and **13**. Aliphatic ethers **14** and **16** showed antagonist potency, but **15**, the higher homologue of FUB 407, showed extraordinarily high in vivo potency with partial intrinsic activity ($ED_{50} = 0.13$ mg/kg, po, ia = 0.6). Meanwhile, FUB 407 has proven its high histamine H_3 receptor agonist activity in further functional in vitro experiments regarding [^{35}S]GTPγ[S] binding and electrically evoked [^{3}H]noradrenaline overflow from mouse cortex membranes.[37] Recent in vivo experiments in rats demonstrated that FUB 407 possesses gastroprotective properties on gastric mucosal lesions induced by HCl by increasing the level of histamine H_3 receptor-dependent mucus secretion and intracellular mucus content.[38]

Taken together, these results verify the high interest in this group of compounds. From a structural point of view, it can be concluded that a bulky group within a distinct distance and specific three-dimensional orientation for sterically rigidified structures such as carbamates is an essential prerequisite in this class of imidazole compounds for agonist activity. It must also be stressed that the piperidine analogues **17** and **18** do not show

Table 3. Capsaicin-Induced Plasma Extravasation in Rats after Oral Treatment

	BP 2-94[a]		(*S*)-**9**	
tissue	ED_{50} (mg/kg)	E_{max} (%)	ED_{50} (mg/kg)	E_{max} (%)
conjunctiva	0.40 ± 0.20	65 ± 6	0.10 ± 0.05	67 ± 6
trachea	0.30 ± 0.10	55 ± 5	0.07 ± 0.05	67 ± 8
esophagus	1.00 ± 0.40	65 ± 5	0.10 ± 0.04	68 ± 6
urinary bladder	0.20 ± 0.03	63 ± 2	0.07 ± 0.05	81 ± 10

[a] From ref 5.

any agonist behavior. They are inactive or only weakly active in vivo in mice as expected from in vitro studies.

Enantiomers (*R*)- and (*S*)-9. Stereoisomers of (*R*/*S*)-**9** were synthesized because of the interesting pharmacological behavior of the racemate to investigate the role of the steric demands of the bulky aliphatic group, which seems to be responsible for activation of the receptor protein within this class of compounds. Stereochemical effects of H_3 receptor agonists have been observed previously in the series of compounds structurally related to histamine.[39]

Compounds (*R*)- and (*S*)-**9** clearly show differences in pharmacological behavior. In the in vitro assay on rat synaptosomes, the (*R*)-distomer still retained weak agonist properties, whereas the (*S*)-enantiomer proved to be the eutomer with increased intrinsic activity and a low EC_{50} value. When tested as antagonists, the enantiomers differed only slightly. However, the (*R*)-enantiomer displayed slightly higher antagonist potency on the guinea pig ileum model, pointing out differences within the two functional assays, as described previously.[17,40] [^{125}I]Iodoproxyfan binding experiments with the human histamine H_3 receptor revealed an affinity similar to that observed on the guinea pig ileum assay (Table 1). Under in vivo conditions, the (*S*)-eutomer showed higher agonist activity than the (*R*)-enantiomer which, in addition, has lost full intrinsic activity. Thus, (*S*)-**9** seemed to be a promising candidate for further development.

Since H_3 receptors are not found exclusively in the brain, but also control neurotransmission in the periphery, (*S*)-**9** was also investigated on a model of inflammatory processes. Peripheral in vivo potency was investigated by the inhibition of capsaicin-induced plasma protein extravasation in rat tissues after oral administration of (*S*)-**9**.[5] The inhibitory effect of the agonist was determined in various peripheral tissues, e.g., conjunctiva, trachea, esophagus, and urinary bladder, by measuring the amount of extravasated Evans Blue dye. Compared to the promising prodrug BP 2-94, which is already in clinical trials, (*S*)-**9** induced at least the same or even increased the maximal level of inhibition in all tissues investigated with an ED_{50} up to 10-fold lower than that of BP 2-94 (Table 3). Since BP 2-94 is a prodrug, compound (*S*)-**9** has the advantage of acting directly without having to undergo the complex prodrug/drug pharmacokinetics involved in drug liberation.

Receptor Profile of Selected Compounds. The receptor profile of selected compounds regarding histamine H_1 and H_2 receptors was determined on functional models of the guinea pig ileum[41] (Table 4). Compounds **1**−**16** showed high selectivity for the histamine H_3 receptor, with a 2−4 orders of magnitude difference versus H_1 and H_2 receptors. Even compound **17**, which is a weak histamine H_3 receptor antagonist, showed some H_3 receptor preference over H_1 or H_2 receptors. For racemate **9**, additional selectivity was determined regarding various serotonergic[42,43] (5-HT_{2A}, 5-HT_3, and 5-HT_4), adrenergic[41,42] (α_{1D} and $\beta_{1/2}$), and muscarinic[42] (M_3) receptors (Figure 2). Compound **9** proved to be highly selective, being at least 250 times more potent on histamine H_3 than on any other aminergic receptor that was tested.

Table 4. Receptor Profile Regarding Histamine Receptor Subtypes

	H_3		H_2	H_1
no.	pK_i[a]	pA_2[b]	pD'_2[c]	pA_2[d]
1	7.3	7.0	4.6	5.1
2	7.4	7.7	4.2	4.5
3	8.0	9.0	4.0	<4.3
4		8.0	3.6	4.0[e]
5		7.9	4.7	4.9
6		7.9	4.1	3.6[e]
7		7.7	<4.0	5.7
8		7.7	<4.0	<4.0
9	7.9	7.9	3.7	<4.5
10	8.3	7.8	<4.0[f]	3.8
(*R*)-**11**		7.7	4.2	4.3
(*S*)-**11**		7.9	4.1	<4.0
14		7.3	4.3	4.3
15		7.6	4.9	4.7
16		7.4	4.5	5.0
17	6.5	6.5	4.7	4.6
18		5.4	4.5[f]	4.4

[a] H_3 receptor assay on synaptosomes of rat cerebral cortex.[33] [b] H_3 receptor assay on guinea pig ileum.[34,35] [c] H_2 receptor assay on guinea pig atrium.[41] [d] H_1 receptor assay on guinea pig ileum.[41] [e] pD'_2 value. [f] pA_2 value.

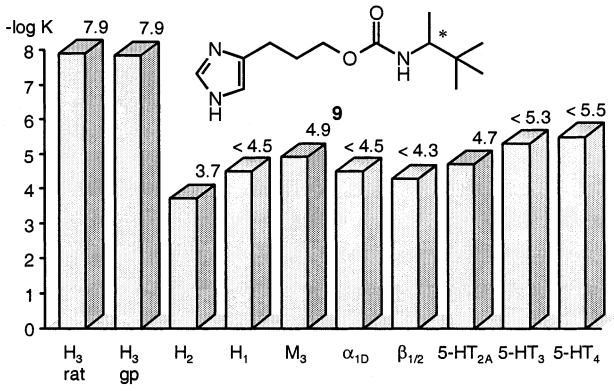


Figure 2. Receptor profile of racemic **9**.

Conclusions

Novel derivatives of 3-(1*H*-imidazol-4-yl)propanol belonging to the carbamate, ester, or ether series were prepared on the basis of lead compounds possessing (partial) agonist properties on models for histamine H_3 receptor activation. The compounds were investigated on various histamine H_3 receptor assays in vitro and in vivo. Some compounds displayed partial agonist activity on synaptosomes of rat brain cortex, but behaved as pure competitive antagonists on the guinea pig ileum. [^{125}I]Iodoproxyfan binding studies on the human histamine H_3 receptor provided high affinities in the nanomolar concentration range. Under in vivo conditions after oral administration to mice, some compounds showed partial or full agonist activities with ED_{50} values of <1 mg/kg. An exchange of the imidazole ring with a

piperidine moiety was not tolerated. Selectivity of the novel compounds for the histamine H_3 receptor was established by determination of histamine H_1 and H_2 receptor activities in functional assays on guinea pigs. Additionally, for carbamate **9** a receptor profile was conducted regarding other neurotransmitter receptors. The (*S*)-enantiomer of one pair of chiral carbamates (**9**) proved to be the eutomer, with pharmacological agonist activity superior to that of the (*R*)-distomer, which displayed equal or slightly higher potency when tested as an antagonist or on the human H_3 receptor binding assay. Thus, the stereochemical conformation of the branched bulky side chain of the carbamate seems to be highly important for induction of a change in receptor conformation, which is necessary for activation of a functional response in this receptor class. Thus, H_3 receptors might be a good target for principal investigations on molecular studies for activation of G protein-coupled receptors.

The (*S*)-enantiomer of **9** was selected for further pharmacological studies. In a peripheral in vivo test model in rats, measuring the level of inhibition of capsaicin-induced plasma extravasation, (*S*)-**9** showed 3−10-fold higher agonist activity than the prodrug agonist BP 2-94. The promising data for (*S*)-**9** shown in this study give rise to the hope for a potential followup candidate for clinical development.

Experimental Section

Chemistry. General Procedures. Melting points were determined on an Electrothermal IA 9000 digital or a Büchi 512 melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker DPX 400 Avance (400 MHz) spectrometer. Chemical shifts are expressed in parts per million downfield from internal Me_4Si as a reference. ^{1}H NMR data are reported in the following order: multiplicity (br, broad; s, singlet; d, doublet; dd, doublet of a doublet; dt, doublet of a triplet; t, triplet; and m, multiplet), approximate coupling constants in hertz, and the number of protons (*) that can be exchanged by D_2O (Im, 4-imidazolyl; Ph, phenyl; Cyhexl, cyclohexyl; Mal, maleic acid). Optical rotations were determined on a Perkin-Elmer 241 MC polarimeter. Elemental analyses (C, H, N) were measured on a Perkin-Elmer 240 B or Perkin-Elmer 240 C instrument and were within ±0.4% of theoretical values for all compounds. Preparative, centrifugally accelerated, rotatory chromatography was performed using a Chromatotron 7924T (Harrison Research) and glass rotors with 4 mm layers of silica gel 60 PF_{254} containing gypsum (Merck). Column chromatography was carried out using silica gel 63−200 μm (Macherey, Nagel & Co.). "NH_3-solution" means an aqueous solution of ammonia (w = 25%). Thin-layer chromatography (TLC) was performed on silica gel PF_{254} plates (Merck). Spectral data are shown only for parent compounds which were obtained by different reactions or methods [**1**, **5**, **6**, (*S*)-**9**, **12**, and **14**−**17**].

(*RS*)-*N*-[(Cyclohexyl)phenylmethyl] 3-(1*H*-Imidazol-4-yl)propyl Carbamate (1). Cyclohexylphenylmethanone (**1a**) (2.8 g, 15 mmol), sodium cyanoboranate (0.63 g, 10 mmol), and freshly sublimed ammonium acetate (11.6 g, 150 mmol) were dissolved in 50 mL of dry MeOH and stirred under a nitrogen atmosphere at room temperature for 1 week until no further reaction was observed as monitored by TLC. The reaction was quenched by addition of 20 mL of 6 N HCl; the white precipitate was filtered, and the filtrate was extracted with ethyl acetate. The aqueous phase was basified with 6 N NaOH and extracted with ethyl acetate. The organic phase was dried with Na_2SO_4, the solvent evaporated under reduced pressure, and the yellow oily residue crystallized as a hydrochloride salt from MeOH/Et_2O to afford cyclohexylphenylmethanamine (**1b**):[44] yield 28%; mp 276 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 8.46* (s, 3H, $NH_3^{\oplus}$), 7.43−7.35 (m, 5H, 5Ph-H), 3.96 (d, J = 8.4 Hz, 1H, C*H*$NH_3^{\oplus}$), 1.93−0.74 (m, 11H, 11Cyhexl-H). Anal. ($C_{13}H_{19}N$·HCl) C, H, N. To a solution of trichloromethyl chloroformate (0.6 g, 3 mmol) and a catalytic amount of activated charcoal in 20 mL of dry ethyl acetate was rapidly added **1b** hydrochloride (0.6 g, 2.5 mmol). The reaction mixture was heated to reflux for 4 h, and then the black solution was cooled and filtered and the solvent evaporated carefully under reduced pressure. The freshly prepared isocyanate was dissolved in 20 mL of dry acetonitrile and added to 3-(1*H*-imidazol-4-yl)-propanol hydrochloride[21] (0.4 g, 2.5 mmol) in 10 mL of dry acetonitrile. The solution was refluxed for 5 h and concentrated in vacuo. The residue was purified by rotatory chromatography [eluent, CH_2Cl_2/MeOH (gradient from 100:1 to 10:1), ammonia atmosphere]. Separation was controlled by TLC. The product (**1**) was obtained as a colorless oil and crystallized as a salt of maleic acid from Et_2O/EtOH: yield 27%; mp 136 °C; ^{1}H NMR (Me_2SO-d_6) δ 8.81 (s, 1H, Im-2-H), 7.64* (d, J = 9.1 Hz, 1H, CONH), 7.33−7.05 (m, 6H, 5Ph-H + Im-5-H), 6.03 (s, 2H, Mal), 4.19 (m, 1H, NHC*H*), 3.90 (t, J = 6.8 Hz, 2H, CH_2O), 2.64 (t, J = 7.4 Hz, 2H, Im-CH_2), 1.85−0.78 (m, 13H, Im-CH_2CH_2 + 11Cyhexl-H). Anal. ($C_{20}H_{27}N_3O_2$·$C_4H_4O_4$·$^3/_4H_2O$) C, H, N.

3-(1*H*-Imidazol-4-yl)propyl *N*-[3-(2,2,4,4-Tetramethylpentyl)] Carbamate (5). To a solution of 2,2,4,4-tetramethylpentanone oxime (**5a**) (1.9 g, 12 mmol) in 15 mL of 1,2-dimethoxyethane was added sodium boranate (1.9 g, 50 mmol). $TiCl_4$ (2.8 mL, 25 mmol) was added slowly under an argon atmosphere at 0 °C. The mixture was warmed to room temperature and stirred for 12 h. One hundred milliliters of cold water was added, and the solution was basified with ammonia and extracted with ethyl acetate. The organic layer was washed with a saturated solution of NaCl, dried over Na_2SO_4, and evaporated under reduced pressure. 2,2,4,4-Tetramethylpentan-3-amine[45] (**5b**) was crystallized as a hydrochloride salt from EtOH/Et_2O: yield 58%; mp 234 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 7.72 (s, 3H, $NH_3^{\oplus}$), 2.70 (d, J = 5.2 Hz, 1H, CH), 1.08 {s, 18H, $[C(CH_3)_3]_2$}. Anal. ($C_9H_{21}N$·HCl·$^1/_8H_2O$) C, H, N. Carbamate **5** was synthesized as described for **1** with **5b** and crystallized as a salt of oxalic acid from Et_2O/EtOH: yield 24%; mp 179 °C; ^{1}H NMR (Me_2SO-d_6) δ 8.32 (m, 1H, Im-2-H), 7.11 (s, 1H, Im-5-H), 6.97* (d, J = 10.7 Hz, 1H, CONH), 3.98 (t, J = 6.6 Hz, 2H, CH_2O), 3.19 (d, J = 10.7 Hz, 1H, CH), 2.64 (t, J = 7.5 Hz, 2H, Im-CH_2), 1.89 (m, 2H, Im-CH_2CH_2), 0.97 {s, 18H, $[C(CH_3)_3]_2$}. Anal. ($C_{16}H_{29}N_3O_2$·$C_2H_2O_4$) C, H, N.

3-(1*H*-Imidazol-4-yl)propyl *N*-(2,2,3,3-Tetramethylcyclopropyl) Carbamate (6). A mixture of 2,2,3,3-tetramethylcyclopropylcarboxylic acid (1.0 g, 7.5 mmol), triethylamine (1.4 mL, 10 mmol), diphenylphosphoryl azide (2.1 g, 7.5 mmol), and 3-(1*H*-imidazol-4-yl)propanol hydrochloride[21] (0.8 g, 5 mmol) was refluxed for 16 h in 30 mL of dry dioxane. The solvent was removed under reduced pressure, and the residue was dissolved in ethyl acetate and extracted with a saturated solution of K_2CO_3. After concentration of the combined organic fractions, the residue was purified by column chromatography [eluent, CH_2Cl_2/MeOH (10:1), ammonia atmosphere]. The combined pure fractions were concentrated, dried, and crystallized as a salt of maleic acid from Et_2O/EtOH: yield 30%; mp 127 °C; ^{1}H NMR (Me_2SO-d_6) δ 8.85 (s, 1H, Im-2-H), 7.38 (s, 1H, Im-5-H), 6.91* (s, 1H, CONH), 6.04 (s, 1H, Mal), 3.97 (t, J = 6.4 Hz, 2H, CH_2O), 2.68 (m, 2H, Im-CH_2), 1.91−1.85 (m, 3H, Im-CH_2CH_2 and CH), 1.02 [s, 6H, $C(CH_3)_2$], 0.88 [s, 6H, $C(CH_3)_2$]. Anal. ($C_{14}H_{23}N_3O_2$·$C_4H_4O_4$) C, H, N.

(*S*)-3-(1*H*-Imidazol-4-yl)propyl *N*-(1,2,2-Trimethylpropyl) Carbamate [(*S*)-9]. (*S*)-1-Phenylethan-1-amine (6.1 g, 50 mmol) and triethylamine (25.3 g, 250 mmol) were dissolved in dry CH_2Cl_2. The solution was cooled to 0 °C, and $TiCl_4$ (4.0 g, 21 mmol) was added dropwise through a septum under a nitrogen atmosphere. The reaction mixture was then heated to reflux, and pinacolone (**9a**) (4.2 g, 42 mmol) was added; the mixture was refluxed for an additional 2 h and stirred at room temperature overnight. Then, 50 mL of Et_2O was added to the ice-cooled mixture, and the white precipitate was filtered repeatedly until a clear yellow filtrate was obtained, which was evaporated. The dried yellow oil, (*S*)-1-phenylethyl-1-

(1,2,2-trimethylpropane)imine[25] [(*S*)-**9b**], was stored under nitrogen at 4 °C until it was further needed: yield 90%; ^{1}H NMR ($CDCl_3$) δ 7.41−7.17 (m, 5H, 5Ph-H, $CDCl_3$), 4.57 (q, J = 6.5 Hz, 0.84 × 1H, *trans*-CH), 4.28 (q, J = 6.6 Hz, 0.16 × 1H, *cis*-CH), 1.79 (s, 3H, $NCCH_3$), 1.35 (d, J = 6.6 Hz, 3H, $CHCH_3$), 1.12 [s, 9H, $C(CH_3)_3$]. Anal. ($C_{14}H_{21}N$). The imine (*S*)-**9b** (6.1 g, 30 mmol) was dissolved in 50 mL of dry EtOH and cooled to −78 °C. $NaBH_4$ (0.56 g, 15 mmol) was added, and the reaction mixture was stirred for 30 min at −78 °C. It was then allowed to warm to −20 °C, and the reaction was quenched by the dropwise addition of 30 mL of 6 N HCl. EtOH was then evaporated under reduced pressure, and the aqueous residue was alkalized with K_2CO_3 and extracted with ethyl acetate. The product was purified by column chromatography [eluent, ethyl acetate, NH_3-solution (100:3)], and n(*S*,*S*)-*N*-(1,2,2-trimethylpropyl) phenylethyl-1-amine α-methylbenzylamine [(*S*,*S*)-**9c**] was crystallized as a hydrochloride salt from Et_2O/EtOH and recrystallized once: yield 76%; mp 222 °C; $[\alpha]^{23}_D$ −8.45 (*c* 1.0, EtOH); ^{1}H NMR (Me_2SO-d_6) δ 8.92* and 7.90* (two br s, 2H, $NH_2^{\oplus}$), (br s, 1H, $NH^{\oplus}$), 7.72−7.40 (m, 5H, 5Ph-H), 4.39 (m, 1H, CHPh), 2.93 [m, 1H, $CHC(CH_3)_3$], 1.68 (d, J = 6.8 Hz, 3H, $CHCH_3Ph$), 1.08 [d, J = 6.7 Hz, 3H, $CHCH_3C(CH_3)_3$], 0.98 [s, 9H, $C(CH_3)_3$]. Anal. ($C_{14}H_{23}N$·HCl) C, H, N. (*S*,*S*)-**9c** (3.6 g, 15 mmol) was dissolved in 50 mL of MeOH, and 250 mg of $Pd(OH)_2$/C was added. The mixture was hydrogenated for 48 h at 1 bar until H_2 consumation ceased. The catalyst was filtered, methanolic HCl added, and the solvent evaporated under reduced pressure. The residue was taken up in MeOH, and (*S*)-1,2,2-trimethylpropylamine (*S*)-**9d** was crystallized as a hydrochloride salt after addition of Et_2O: yield 83%. mp 256 °C; $[\alpha]^{23}_D$ 2.8 (*c* 4.0, MeOH); ^{1}H NMR (Me_2SO-d_6) δ 7.91* (s, 3H, $NH3^{\oplus}$), 2.94 (q, J = 6.7 Hz, 1H, CH), 1.13 (d, J = 6.8 Hz, 3H, CH_3), 0.92 [s, 9H, $C(CH_3)_3$]. Anal. ($C_6H_{15}N$·HCl) C, H, N. (*S*)-**9d** (2.1 g, 15 mmol) was suspended in 30 mL of dry toluene and the mixture cooled to 0 °C. Then, phosgene (20% solution in toluene, 15 mL, 30 mmol) was added rapidly through a septum and the mixture heated to 60 °C for 3 h. The solution was then cooled to room temperature, and nitrogen was bubbled through for 1 h to remove the excess of phosgene. This solution was then added to 3-(1*H*-imidazol-4-yl)propanol hydrochloride[21] (1.6 g, 10 mmol), dissolved in 2 mL of DMF, and heated to 80 °C for 12 h. MeOH was added, the mixture concentrated on a rotatory evaporator, and the residue purified by rotatory chromatography [eluent, CH_2Cl_2/MeOH (gradient from 100:1 to 10:1), ammonia atmosphere]. Separation was controlled by TLC. The product (*S*)-**9** was obtained as a colorless oil and crystallized as a salt of oxalic acid from Et_2O/EtOH: yield 35%; $[\alpha]^{23}_D$ 9.66 (*c* 0.5, methanol); mp 122 °C; ^{1}H NMR (Me_2SO-d_6) δ 8.48 (s, 1H, Im-2-H), 7.20 (s, 1H, Im-5-H), 6.88* (d, J = 9.4 Hz, 1H, CONH), 3.95 (t, J = 6.5 Hz, 2H, CH_2O), 3.36 (m, 1H, CH), 2.64 (t, J = 7.5 Hz, 2H, Im-CH_2), 1.88 (m, 2H, Im-CH_2CH_2), 0.97 (d, J = 6.9 Hz, 3H, CH_3), 0.82 [s, 9H, $C(CH_3)_3$]. Anal. ($C_{13}H_{23}N_3O_2$·$C_2H_2O_4$·$^1/_4H_2O$) C, H, N.

3-(1*H*-Imidazol-4-yl)propyl 2,2-Dimethylpropionate (12). 3-(1*H*-Imidazol-4-yl)propanol hydrochloride[21] (0.8 g, 5 mmol) and a catalytic amount of 4-(dimethylamino)pyridine were dissolved in 30 mL of pyridine. 2,2-Dimethylpropionic acid chloride (0.6 g, 5 mmol) was added through a septum, and then the mixture was stirred for 12 h at room temperature. The solvent was evaporated under reduced pressure, and the residue was taken up in ethyl acetate and washed with saturated K_2CO_3 and NaCl solutions. The crude product was then purified by column chromatography [eluent, CH_2Cl_2/MeOH (10:1, ammonia atmosphere)], and the pure fractions were evaporated, dried, and crystallized as a salt of oxalic acid from Et_2O/EtOH: yield 50%; mp 145 °C; ^{1}H NMR (CD_3OD) δ 8.74 (s, 1H, Im-2-H), 7.32 (s, 1H, Im-5-H), 4.12 (t, J = 6.2 Hz, 2H, CH_2O), 2.82 (t, J = 7.8 Hz, 2H, Im-CH_2), 2.04 (m, 2H, Im-CH_2CH_2), 1.19 [s, 9H, $C(CH_3)_3$]. Anal. ($C_{11}H_{18}N_2O_2$·$C_2H_2O_4$) C, H, N.

(*RS*)-3-(1*H*-Imidazol-4-yl)propyl 1,3,3-Trimethylbutyl Ether (14). Synthesis of the trityl-protected ester {3-[1-(triphenylmethyl)-1*H*-imidazol-4-yl]propyl 3,3-dimethylbutyrate, **14a**} precursor was performed as described for **12** with 3,3-dimethylbutyric acid chloride (0.67 g, 5 mmol) and 3-[1-(triphenylmethyl)-1*H*-imidazol-4-yl]propanol (1.8 g, 5 mmol). The crude product was purified by column chromatography [eluent, ethyl acetate/NH_3 solution (100:3)] and crystallized at 4 °C: yield 95%; ^{1}H NMR (Me_2SO-d_6) δ 7.42−7.35 (m, 10H, 10Ph-H), 7.26 (s, 1H, Im-2-H), 7.08 (m, 5H, 5Ph-H), 6.59 (s, 1H, Im-5-H), 3.97 (t, J = 6.5 Hz, 2H, CH_2O), 2.50 (t, J = 7.2 Hz, 2H, Im-CH_2 partially covered by DMSO), 2.14 (s, 2H, $COCH_2$), 1.83 (m, 2H, Im-CH_2CH_2), 0.94 [s, 9H, $C(CH_3)_3$]. Anal. ($C_{31}H_{34}N_2O_2$) C, H, N. Ester **14a** (0.9 g, 2 mmol) was dissolved in 10 mL of freshly dried THF under an argon atmosphere. Tebbe's reagent[46] (0.5 M solution in toluene, 4.4 mL, 2.2 mmol) was added slowly through a septum. The mixture was stirred for 1 h at 0 °C. Then 2 mL of 2 N NaOH was added carefully to stop the reaction. The blue precipitate was filtered and the orange filtrate concentrated under reduced pressure. The residue was taken up in ethyl acetate/Et_2O (1:1) and washed with 0.2 N NaOH. The crude product was then purified by column chromatography [eluent, ethyl acetate/NH_3 solution (100:3)], and 3-[1-(triphenylmethyl)-1*H*-imidazol-4-yl]propyl 2-(4,4-dimethylpent-1-enyl) ether **14b** was obtained as a light yellow oil: yield 60%; ^{1}H NMR (Me_2SO-d_6) δ 7.37−7.13 (m, 16H, 15Ph-H + Im-2-H), 6.61 (s, 1H, Im-5-H), 3.86 (s, 1H, =C*H*H), 3.75 (s, 1H, =CH*H*), 3.55 (t, J = 6.1 Hz, 2H, CH_2O), 2.63 (t, J = 7.3 Hz, 2H, Im-CH_2), 1.93 [m, 4H, Im-CH_2CH_2 + $CH_2C(CH_3)_3$], 0.88 [s, 9H, $C(CH_3)_3$]. Anal. ($C_{32}H_{36}N_2O$) C, H, N. Trityl-protected enyl ether **14b** (0.6 g, 1.2 mmol) and Pd/C (60 mg) were dissolved in 20 mL of methanol and hydrogenated at 1 bar for 48 h. After filtration, the crude product was purified by rotary chromatography [eluent, CH_2Cl_2/MeOH (10:1), ammonia atmosphere] and then crystallized as a salt of oxalic acid from Et_2O/EtOH and recrystallized once: yield 95%; mp 182 °C; ^{1}H NMR (CD_3OD) δ 8.50 (s, 1H, Im-2-H), 7.17 (s, 1H, Im-5-H), 3.57 (dt, ^2J = 9.2 Hz, ^3J = 6.1 Hz, 1H, CH*H*O), 3.51 (m, 1H, CH), 3.36 (dt, ^2J = 9.2 Hz, ^3J = 6.1 Hz, 1H, C*H*HO), 2.77 (2t, J = 7.5 Hz, 2H, Im-CH_2), 1.90 (m, 2H, Im-CH_2CH_2), 1.48 [dd, ^2J = 14.5 Hz, ^3J = 7.6 Hz, 1H, CH*H*$(CH_3)_3$], 1.23 [dd, ^2J = 14.5 Hz, ^3J = 3.2 Hz, 1H, C*H*H$(CH_3)_3$], 1.11 (d, J = 6.1 Hz, 3H, CH_3), 0.93 [s, 9H, $C(CH_3)_3$]. Anal. ($C_{13}H_{24}N_2O$·$0.85C_2H_2O_4$) C, H, N.

3-(1*H*-Imidazol-4-yl)propyl 4,4-Dimethylpentyl Ether (15). 3,3-Dimethylbutyl chloride (**15a**) (4.3 g, 36 mmol) was added dropwise to a solution of KCN (2.9 g, 45 mmol) and a catalytic amount of KI in 50 mL of dry DMSO and heated to 95 °C for 12 h. The solution was poured into 300 mL of H_2O after being cooled to room temperature and extracted with Et_2O. The organic phase was washed with a saturated solution of NaCl, dried over Na_2SO_4, and concentrated. 3,3-Dimethylbutane nitrile[47] (**15b**) was obtained as a colorless oil: yield 68%; ^{1}H NMR (Me_2SO-d_6) δ 2.50 (t, J = 8.0 Hz, 2H, CH_2CN), 1.51 (t, J = 7.9 Hz, 2H, CH_2CH_2CN), 0.86 [s, 9H, $C(CH_3)_3$]. Anal. ($C_7H_{13}N$) C, H, N. Nitrile **15b** (2.7 g, 24 mmol) and KOH (20 g) were refluxed for 12 h in EtOH/H_2O (1:1). After the solution was cooled to room temperature, EtOH was distilled off, and the aqueous residue was washed with Et_2O. The aqueous solution was then carefully acidified with 2 N HCl and extracted with Et_2O. The organic phase was washed with a saturated NaCl solution, dried over Na_2SO_4, and concentrated. 4,4-Dimethylpentanoic acid[47] (**15c**) was obtained as a colorless oil: yield 63%; ^{1}H NMR (Me_2SO-d_6) δ 11.95* (s, 1H, COOH), 2.15 (t, J = 8.2 Hz, 2H, CH_2COOH), 1.43 (t, J = 8.3 Hz, 2H, CH_2CH_2COOH), 0.86 [s, 9H, $C(CH_3)_3$]. Anal. ($C_7H_{14}O_2$) C, H, N. The carboxylic acid **15c** (2 g, 15 mmol), dissolved in 20 mL of Et_2O, was carefully added dropwise to a suspension of $LiAlH_4$ (0.65 g, 17 mmol) in 50 mL of Et_2O at 0 °C in an argon atmosphere. The reaction mixture was heated to reflux for 12 h. Excess $LiAlH_4$ was destroyed by addition of EtOH and hydrolyzed by addition of 2 N HCl. The organic solvents were evaporated, and the aqueous residue was extracted with Et_2O, washed with saturated solutions of K_2CO_3 and NaCl, dried over Na_2SO_4, and concentrated. 4,4-Dimethylpentan-1-ol (**15d**)[48] was obtained as a colorless oil: yield 76%. ^{1}H NMR (Me_2SO-d_6) δ 4.35* (t, J = 5.2 Hz, 1H, OH), 3.38 (m, 2H,

CH_2OH), 1.37 (m, 2H, CH_2CH_2OH), 1.15 [m, 2H, $CH_2(CH_3)_3$], 0.86 [s, 9H, $C(CH_3)_3$]. Anal. ($C_7H_{16}O$) C, H, N. A mixture of 4,4-dimethylpentan-1-ol (**15d**) (0.7 g, 5 mmol) and NaH (60%) (0.2 g, 5 mmol) was stirred in 10 mL of dry DMSO for 6 h at room temperature under an argon atmosphere. Then 3-[1-(triphenylmethyl)-1*H*-imidazol-4-yl]propyl chloride (0.6 g, 2 mmol), a catalytic amount of tetrabutylammonium iodide, and 15-crown-5 were added, and then the mixture was heated to 80 °C for 24 h. After the solution was cooled to room temperature, 100 mL of H_2O was added and the product extracted with Et_2O and purified by column chromatography [eluent, ethyl acetate/NH_3 solution (100:3)]. The pure fractions were concentrated in vacuo, redissolved in 2 N HCl/THF (20:30), and heated at 70 °C for 2 h. After removal of the organic solvent under reduced pressure, the aqueous suspension was filtered and extracted with Et_2O. The aqueous solution was basified (NaOH) and extracted with Et_2O. The organic layer was dried and concentrated in vacuo. The oily residue was purified by column chromatography [eluent, CH_2Cl_2/MeOH (10:1), ammonia atmosphere]. The colorless oil was crystallized as a salt of oxalic acid from EtOH/Et_2O and recrystallized once: yield 10%; mp 180 °C; ^{1}H NMR (Me_2SO-d_6) δ 8.39 (s, 1H, Im-2-H), 7.14 (s, 1H, Im-5-H), 3.35 (m, 4H, CH_2OCH_2), 2.62 (t, J = 7.5 Hz, 2H, Im-CH_2), 1.81 (br s, 2H, Im-CH_2CH_2), 1.44 [m, 2H, $CH_2CH_2C(CH_3)_3$], 1.16 [m, 2H, $CH_2(CH_3)_3$], 0.86 [s, 9H, $C(CH_3)_3$]. Anal. ($C_{13}H_{24}N_2O\cdot0.8C_2H_2O_4$) C, H, N.

3-(1*H*-Imidazol-4-yl)propyl 5,5-Dimethylhexyl Ether (16). To a solution of NaH (60% in mineral oil) (2.4 g, 60 mmol) and a catalytic amount of KI in dry DMF was added malonic acid diethyl ester (9.6 g, 60 mmol) dropwise at 0 °C under a nitrogen atmosphere. After the reaction vessel had been warmed to room temperature, 3,3-dimethylbutyl chloride (6.0 g, 50 mmol) was added and then heated to 80 °C for 12 h. The cooled solution was poured into 300 mL of H_2O and extracted with ethyl acetate/Et_2O (1:1). The organic phase was dried with Na_2SO_4 and concentrated. The product was suspended in 200 mL of H_2O/EtOH (1:1); 40 g of KOH was added, and the mixture was refluxed for 12 h. EtOH was evaporated under reduced pressure and the aqueous residue extracted with Et_2O. The organic phase was dried with Na_2SO_4 and concentrated. 3,3-Dimethylbutylmalonic acid (**16b**) was crystallized from EtOH: yield 60% (two steps); mp 128 °C; ^{1}H NMR (Me_2SO-d_6) δ 12.60* (s, 2H, 2COOH), 3.11 (t, J = 7.4 Hz, 1H, CH), 1.67 (m, 2H, $CHCH_2$), 1.14 [m, 2H, $CH_2C(CH_3)_3$], 0.86 [s, 9H, $C(CH_3)_3$]. Anal. ($C_9H_{16}O_4$) C, H, N. Compound **16b** (5.5 g, 29 mmol) was heated to 150 °C for 3 h to decarboxylate it. The cooled reaction product was dissolved in 2 N NaOH and washed with Et_2O. The aqueous phase was acidified with 2 N HCl and extracted with ethyl acetate/Et_2O (1:1). The organic phase was dried with Na_2SO_4 and concentrated. 5,5-Dimethylhexanoic acid (**16c**)[49] crystallized at 4 °C: yield 36%; mp 36 °C; ^{1}H NMR (Me_2SO-d_6) δ 11.96* (s, 1H, COOH), 2.17 (t, J = 7.3 Hz, 2H, CH_2COOH), 1.45 (m, 2H, CH_2CH_2COOH), 1.15 [m, 2H, $CH_2C(CH_3)_3$], 0.86 [s, 9H, $C(CH_3)_3$]. Anal. ($C_9H_{16}O_4$) C, H, N. Carboxylic acid (**16c**) was treated as described for **15c**, to obtain 5,5-dimethylhexan-1-ol (**16d**):[48] yield 57%; ^{1}H NMR (Me_2SO-d_6) δ 4.31* (t, J = 5.1 Hz, 1H, OH), 3.38 (m, 2H, CH_2OH), 1.38 (m, 2H, CH_2CH_2OH), 1.28−1.12 [m, 4H, $(CH_2)_2(CH_3)_3$], 0.85 [s, 9H, $C(CH_3)_3$]. Anal. ($C_8H_{18}O$) C, H, N. Alcohol **16d** was treated as described for **15d**, to obtain 3-(1*H*-imidazol-4-yl)propyl 5,5-dimethylhexyl ether (**16**) as a salt of oxalic acid: yield 25%; mp 184 °C; ^{1}H NMR (Me_2SO-d_6) δ 8.38 (s, 1H, Im-2-H), 7.13 (s, 1H, Im-5-H), 3.35 (m, 4H, CH_2OCH_2), 2.62 (t, J = 7.6 Hz, 2H, Im-CH_2), 1.81 (s, 2H, Im-CH_2CH_2), 1.46 [m, 2H, $CH_2(CH_2)_2C(CH_3)_3$], 1.20 [m, 4H, $CH_2CH_2(CH_3)_3$], 0.85 [s, 9H, $C(CH_3)_3$]. Anal. ($C_{14}H_{26}N_2O\cdot0.8C_2H_2O_4$) C, H, N.

3,3-Dimethylbutyl 3-Piperidinopropyl Ether (17). 3-Piperidinopropanol (1.43 g, 10 mmol) and 3,3-dimethylbutyl chloride (0.6 g, 5 mmol) were treated as described for **15**. The colorless oil was crystallized as a salt of oxalic acid from EtOH/Et_2O and recrystallized once: yield 16%; mp 143 °C; ^{1}H NMR (Me_2SO-d_6) δ 3.40 (m, 4H, CH_2OCH_2), 3.00 (m, 6H, Pip-CH_2 + 4Pip-2,6-H), 1.82 (m, 2H, Pip-CH_2CH_2), 1.71 (m, 4H, 4Pip-3,5-H), 1.52 (s, 2H, 2Pip-4-H), 1.43 [t, J = 7.3 Hz, 2H, $CH_2C(CH_3)_3$], 0.89 [s, 9H, $C(CH_3)_3$]. Anal. ($C_{14}H_{29}NO\cdot1.1C_2H_2O_4$) C, H, N.

Capillary Electrophoresis (CE). CE separations were carried out using an automated CE apparatus (Beckman P/ACE 2100): Software Gold 7.11, capillary 57 cm × 50 μm i.d. (Grohm), 50 cm to the detector according to Sasse et al.;[20] UV detection at 200 nm; voltage of 25 kV; capillary temperature thermostated at 25.0 °C; sample injection for 2 s with pressure (5 bar). The composition of the background electrolyte (BGE) was as follows: 150 mM phosphate buffer [aqueous H_3PO_4 (85% w/w)] adjusted to pH 2.7 with triethanolamine and 20 mM trimethyl-β-cyclodextrin (Fluka) and I = 65 μA. At the beginning of each working day, the capillary was rinsed for 15 min with 0.1 N NaOH, for 5 min with H_2O, and for 5 min with BGE. Between each run, the capillary was rinsed for 1 min with 0.1 N NaOH and for 3 min with BGE. Each experiment was performed at least in triplicate.

General Pharmacology Methods

Histamine H_3 Receptor Antagonist Activity on Guinea Pig Ileum. For selected compounds, H_3 receptor activity was measured by concentration-dependent inhibition of electrically evoked twitches of isolated guinea pig ileal segments induced by (*R*)-α-methylhistamine in the absence and presence of the antagonist according to Ligneau et al.[35b] In brief, longitudinal muscle strips were prepared from the small intestine, 20−50 cm proximal to the ileocaecal valve. The muscle strips were mounted between two platinum electrodes (4 mm apart) in 20 mL of Krebs buffer, containing 1 μM mepyramine, connected to an isometric transducer, continuously gassed with oxygen containing 5% CO_2 at 37 °C. After equilibration of the muscle segments for 1 h with washing every 10 min, they were stimulated continuously with rectangular pulses of 15 V and 0.5 ms duration at a frequency of 0.1 Hz. After stimulation for 30 min, a cumulative concentration−response curve was recorded. Subsequently, the preparations were thoroughly washed twice every 10 min without stimulation. The antagonist was incubated for 20−30 min before redetermination of the (*R*)-α-methylhistamine concentration−response curve.[34,35] After addition of the investigated compounds, no depression of contraction, e.g., agonist activity, was observed. Each experiment was performed at least in triplicate.

Histamine H_3 Receptor Assay on Synaptosomes of Rat Cerebral Cortex. Compounds were tested for their H_3 receptor agonist and antagonist activity in an assay with K^+-evoked depolarization-induced release of [^{3}H]histamine from rat synaptosomes according to Garbarg et al.[33] A synaptosomal fraction from rat cerebral cortex prepared according to the method of Whittaker[50] was preincubated for 30 min with L-[^{3}H]histidine (0.4 μM) at 37 °C in a modified Krebs-Ringer solution. The synaptosomes were washed extensively, resuspended in fresh 2 mM K^+ Krebs-Ringer medium, and incubated for 2 min with 2 or 30 mM K^+ (final concentration). Antagonists and 1 μM histamine or the agonist in increasing concentrations were added 5 min before the depolarization stimulus. Incubation was stopped by rapid centrifugation, and [^{3}H]histamine levels were determined after purification by liquid scintillation spectrometry.[33] K_i values were determined according to the Cheng−Prussoff equation.[51] The data that are presented are given as mean values with the standard error of the mean (SEM) each for a minimum of three separate determinations.

[^{125}I]Iodoproxyfan Binding Assay on Stably Transfected CHO-K1 Cells. CHO-K1 cells were transfected with pCIneo-hH_3 plasmids using SuperFect reagent (Quiagen). Stable transfectants were selected with 2 mg/mL G418 and tested for [^{125}I]iodoproxyfan binding.[35b] Positive clones were maintained in the presence of 1 mg/mL G418. For [^{125}I]iodoproxyfan binding experiments, cells were harvested, washed, and homogenized (Polytron) in ice-cold binding buffer [50 mM Na_2HPO_4/KH_2PO_4 (pH 6.8)]. The binding assay was performed as described previously.[12,35b] Briefly, aliquots of membrane suspensions (5−15 μg of protein) were incubated at 25 °C for

60 min with 22−25 pM [^{125}I]iodoproxyfan alone or together with competing drugs (final volume of 200 μL). The level of nonspecific binding was determined using imetit (1 μM). Each experiment was performed at least in triplicate.

Histamine H_3 Receptor Antagonist Potency in Vivo in Mice. In vivo testing was performed after peroral administration of the antagonist to Swiss mice as described by Garbarg et al.[33] Brain histamine turnover was assessed by measuring the level of the main metabolite of histamine, N^{τ}-methylhistamine. Mice were fasted for 24 h before po treatment. Animals were decapitated 90 min after treatment, and the brain was dissected out and homogenized in 10 volumes of ice-cold perchloric acid (0.4 M). The N^{τ}-methylhistamine level was measured with a radioimmunoassay.[52] By treatment with 3 mg of ciproxifan/kg or 10 mg of imetit/kg, the maximum and minimum N^{τ}-methylhistamine levels were obtained and related to the level reached with the administered drug. The ED_{50} value was calculated as the mean with the SEM.[53]

Capsaicin-Induced Plasma Extravasation. Male Wistar rats were orally administered BP 2-94 or (*S*)-**9** in increasing doses or vehicle. After 90 min, they were anesthetized with pentobarbital (6 mg/kg, ip) and after an additional 30 min were treated with capsaicin (90 μg/kg, iv) and Evans Blue dye (30 mg/kg, iv) or vehicle. Five minutes after capsaicin treatment, rats were perfused with saline to remove the intravascular dye. Tissues were dissected out and analyzed for extravasated Evans Blue dye by measuring absorption at 630 nm after dye extraction.[5]

In Vitro Screening at Other Histamine Receptors. Selected compounds were screened for histamine H_2 receptor potency on the isolated spontaneously beating guinea pig right atrium as well as for H_1 receptor potency on the isolated guinea pig ileum by standard methods described by Hirschfeld et al.[41] Each pharmacological test was performed at least in triplicate. The values that are given represent means.

Muscarinic M_3 Receptor Assay on Guinea Pig Ileum. The procedure that was used was that described by Pertz and Elz.[42]

Adrenergic α_{1D} Receptor Assay on Rat Aorta. The procedure that was used was that described by Hirschfeld et al.[41]

Adrenergic $\beta_{1/2}$ Receptor Assay on Guinea Pig Right Atrium. The procedure that was used was that described by Pertz and Elz.[43]

Serotonergic 5-HT_{2A} Receptor Assay on Rat Tail Artery. The procedure that was used was that described by Pertz and Elz.[42]

Serotonergic 5-HT_3 Receptor Assay on Guinea Pig Ileum. The procedure that was used was that described by Elz and Keller.[42]

Serotonergic 5-HT_4 Receptor Assay on Rat Esophagus. The procedure that was used was that described by Elz and Keller.[43]

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