Novel Partial Agonists for the Histamine H_3 Receptor with High in Vitro and in Vivo Activity†

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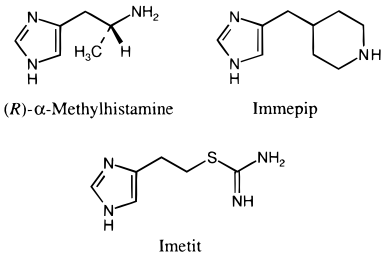
Novel branched *N*-alkylcarbamates and aliphatic ethers derived from 3-(1*H*-imidazol-4-yl)-propanol were prepared. The compounds were investigated on two functional histamine H_3-receptor assays. Some compounds displayed partial agonist activity on synaptosomes of rat brain cortex but behaved as pure competitive antagonists on the guinea pig ileum. Under in vivo conditions after po application to mice, some of the compounds showed partial or full agonist activity. Highest in vivo potency was found for the 3,3-dimethylbutyl ether **10** (ED_{50} = 0.29 mg/kg, full intrinsic activity). These novel agonists are structurally diverse from classical aminergic histamine H_3-receptor agonists (e.g., (*R*)-α-methylhistamine, imetit) as they lack a basic moiety in the side chain, which is supposed to be important for the activation of the receptor protein. The selectivity for the histamine H_3 receptor was proven by determination of H_1- and H_2-receptor activity on functional assays of the guinea pig.

Introduction

In 1983, Arrang et al. discovered a third histamine receptor subtype (H_3), which is located presynaptically and inhibits synthesis and release of histamine.[2] Additionally, the histamine H_3 receptor plays an important role as a heteroreceptor in the regulation of the release of other neurotransmitters.[3] Therapeutic targets of histamine H_3-receptor antagonists and agonists have been reviewed extensively.[4]

Selected agonists for the histamine H_3 receptor are shown in Chart 1. These typical H_3-receptor agonists, (*R*)-α-methylhistamine,[5] immepip,[6] and imetit,[7,8] are structurally related to histamine inasmuch as they consist of an imidazole ring, an alkyl spacer, and a second basic moiety. Under physiological conditions the isothiourea or the aliphatic amino group is protonated and presumably interacts with a carboxylate group at the receptor.[7] This ionic interaction seemed to be essential for activation of the receptor. All classical H_3-receptor agonists have this structural feature in common. One disadvantage of these highly hydrophilic compounds is their poor penetration of the blood−brain barrier. To overcome this problem azomethine prodrugs of (*R*)-α-methylhistamine have been developed.[9] Impentamine, a homologue of histamine, was reported to display agonist or antagonist properties on the histamine H_3 receptor depending on the test model.[10] Iodoproxyfan showed partial agonist activity on H_3-receptor functional models of guinea pig ileum[11] and mouse brain cortex,[11a] whereas purely antagonist behavior on rat synaptosomes was observed.[12] Iodoproxyfan and analogoues were the first non-aminergic compounds showing partial agonism at H_3 receptors.[11a]

Chart 1



Recently, we have described novel carbamates as antagonists at the histamine H_3 receptor, e.g., **1** and **3** (Table 1).[13] These carbamates are derivatives of 3-(1*H*-imidazol-4-yl)propanol with alkyl- or methyl-branched chains on the nitrogen of the carbamate functionality. Novel compounds have been designed and prepared with higher degrees of substitution on the *N*-alkyl chain (Table 1, **2** and **4**−**7**). These bulkier residues in some cases led to a different and unexpected pharmacological behavior on histamine H_3 receptors, as some of these compounds showed partial or full agonist action in vitro and/or in vivo. Similar compounds with an ether moiety instead of the carbamate group were also prepared and tested (Table 1, **8**−**11**).

Chemistry

3-(1*H*-Imidazol-4-yl)propanol[12] was the key intermediate for the novel compounds. This synthon was conveniently synthesized from urocanic acid and obtained in its trityl-protected and deprotected form.[12] Carbamates **1** and **3** have been described previously,[13] with the synthetic route analogous to that of compound

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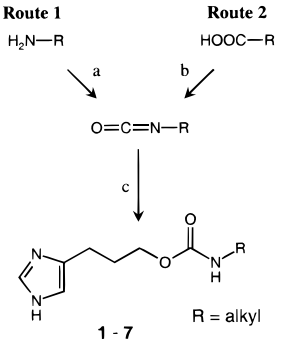
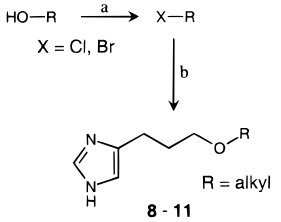
Scheme 1[a]



[a] (a) $ClCOOCCl_3$, charcoal (cat.), ethyl acetate, 4−5 h reflux; (b) DPPA, Et_3N, dioxane, 30 min reflux; (c) 3-(1*H*-imidazol-4-yl)propanol·HCl,[12] acetonitrile, 4−5 h reflux.

Scheme 2[a]



[a] (a) 48% HBr, H_2SO_4 (concd), 1.5 h reflux; (b) i, Na 3-(1-(triphenylmethyl)-1*H*-imidazol-4-yl)propanolate, 15-crown-5 (cat.), $(C_4H_9)_4NI$ (cat.), toluene, 12 h reflux, ii, 2 N HCl/THF (20/30), 2 h reflux.

6 and following route 1 in Scheme 1. Compounds **2** and **7** were prepared from commercially available isocyanates (Scheme 1, route 1). Starting with the carboxylic acids, **4** and **5** were synthesized via a modified Curtius reaction using diphenyl phosphorazidate (DPPA) (Scheme 1, route 2).[14]

Ethers **8**−**11** were prepared by classical Williamson synthesis[15] from 3-(1-(triphenylmethyl)-1*H*-imidazol-4-yl)propanolate and corresponding alkyl halides with subsequent deprotection of the products (Scheme 2).

Pharmacology

Compounds were tested on three pharmacological models for the histamine H_3 receptor. The functional in vitro assay on rat cerebral cortex synaptosomes with K^+-evoked depolarization-induced release of [^{3}H]histamine was performed according to Garbarg et al.[7] Another functional in vitro assay was performed on guinea pig ileum measuring the concentration-dependent inhibition of electrically evoked twitches by (*R*)-α-methylhistamine in the presence of the antagonist.[16,17] Agonist measurements recorded concentration−response curves of the new compounds instead of (*R*)-α-methylhistamine. In vivo testing was performed after po administration of the compounds to Swiss mice. The increased (antagonist) or decreased (agonist) level of the main histamine metabolite, N^τ-methylhistamine, in cerebral cortex was measured.[7] The compounds were also screened for H_1- and H_2-receptor activity on functional models on ileum and right atrium of guinea pigs.[18]

Results and Discussion

The structures of the novel compounds and the results of pharmacological testing on histamine H_3-receptor assays are presented in Table 1. On guinea pig ileum all compounds showed moderate to high antagonist potency. In the series of carbamates, compounds with short *N*-alkyl chains (**1** and **2**) were slightly less active than the homologous compounds **3**−**6** and the multibranched compound **7**. In the ether series the same pattern of increasing antagonist potency on the guinea pig ileum with increasing chain length was observed (**8** → **9** → **10** → **11**). Compound **11** showed the highest antagonist potency (K_B = 13 nM) on this functional model. No agonist activity was observed.

Surprisingly, some of the compounds (**4**, **9**−**11**) displayed partial agonist activity on the rat synaptosome model, and under in vivo conditions two compounds (**9**, **10**) showed agonist action with full intrinsic activity at very low doses. Partial agonism was observed in vivo for **2**, **4**, **5**, and **11**, whereas only weak partial agonism (**4**, **11**) or antagonism (**2**, **5**) in vitro was displayed. Due to low intrinsic activities, the EC_{50} values could not be calculated in every case (**4**, **11**).

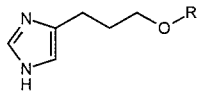
In general the intrinsic activities of compounds found in vitro on the synaptosomal preparation were lower than those of the same compounds evaluated from modulation of N^τ-methylhistamine levels in brain in vivo. Thus compound **9** with an intrinsic activity of 0.22 behaved as full agonist in vivo. This apparent difference in receptor reserve may appear surprising inasmuch as both tests evaluate the influence of H_3-autoreceptor stimulation upon endogenous histamine release. However, whereas the in vitro response is selectively mediated by receptors on nerve terminals, in vivo response may also involve somato-dentritic autoreceptors detected autoradiographically[19] controlling the firing rate of histaminergic neurons. In addition, the character of the response in vitro depends on the parameters of the depolarizing stimulus.[2] Here release from synaptosomes was monitored after a continuous 30 mM K^+ stimulus, whereas physiological release in vivo results from trains of impulses, i.e., intermittent depolarizations, which may result in different receptor reserves in the two models.

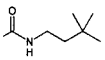
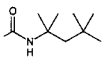
When tested as antagonists on rat synaptosomes, the compounds showed higher potency compared to the antagonist action observed on guinea pig ileum.

Small structural changes in the side chain of the new compounds influence the pharmacological behavior to a large extent. Changing from an isopropyl (**1**) to *tert*-butyl (**2**) on the *N*-residue of the carbamates leads to a transition from weak antagonism to partial agonism in vivo. The same change is found for **3** and **4** which are homologues of **1** and **2** with an isobutyl (**3**) and neopentyl (**4**) group. Compound **4** is a partial agonist with the same range of intrinsic activity but with higher in vivo potency than **2**. Further elongation of the *N*-alkyl chain (**4** → **6**) or introduction of more methyl groups (**7**) led to a loss of agonist activity. Compound **6** showed pronounced antagonist potency, whereas **7** was inactive under in vivo conditions. A loss in intrinsic activity but increase in potency was observed for **5**.

Structure−activity relationships in the group of ethers are comparable to those of the carbamates. Compound **8** possessing an isobutyl residue showed antagonist potency in vitro as well as in vivo. Increasing chain length, leading to the isopentyl derivative **9**, converted the antagonist mode of action of **8** to partial agonism

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



					in vitro				in vivo	
				mp[a]	K_B[b] (nM)	K_i[c] (nM)	EC_{50}[c] (nM)		ED_{50}[d] (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.[e]	$\bar{x} \pm s_{\bar{x}}$	i.a.[e]
1[f]		$C_{10}H_{17}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	331.8	113	91 ± 10	82 ± 16		-	15 ± 5	-
2		$C_{11}H_{19}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	345.9	108	60 ± 10			-	0.9 ± 0.1	~ 0.7
3[f]		$C_{11}H_{19}N_3O_2 \cdot C_4H_4O_4$	341.4	105	41 ± 5	30 ± 10		-	≥ 10	-
4		$C_{12}H_{21}N_3O_2 \cdot C_2H_2O_4 \cdot 0.5H_2O$	338.4	132	40 ± 4	23 ± 4	n.c.[g]	~ 0.15	0.48 ± 0.15	~ 0.7
5		$C_{12}H_{21}N_3O_2 \cdot C_2H_2O_4$	329.4	130	46 ± 6			-	~ 0.2	~ 0.4
6		$C_{13}H_{23}N_3O_2 \cdot C_4H_4O_4 \cdot 0.5H_2O$	378.4	91	30 ± 4			-	2.8 ± 0.6	-
7		$C_{15}H_{27}N_3O_2 \cdot C_2H_2O_4$	371.4	164	28 ± 3	42 ± 13		-	> 10	-
8		$C_{10}H_{18}N_2O \cdot C_4H_4O_4$	298.3	82	228 ± 56	139 ± 56		-	0.74 ± 0.12	-
9		$C_{11}H_{20}N_2O \cdot C_4H_4O_4$	312.4	74	93 ± 20	14 ± 4	128 ± 86	~ 0.22	0.51 ± 0.26	1.0
10		$C_{12}H_{22}N_2O \cdot C_4H_4O_4$	326.4	91	42 ± 7	10 ± 3	45 ± 10	0.55	0.29 ± 0.17	1.0
11		$C_{12}H_{22}N_2O \cdot C_4H_4O_4$	326.4	86	13 ± 3	9.4 ± 1.6	n.c.[g]	~ 0.25	0.15 ± 0.08	0.6
Imetit[h]					6.6[i] (i.a. = 1.0)		2.8 ± 0.7[k]	1.0	1.0 ± 0.3[k]	1.0

[a] Crystallization solvent: Et_2O/EtOH. [b] Functional H_3-receptor assay on guinea pig ileum.[16,17] [c] Functional H_3-receptor assay on synaptosomes of rat cerebral cortex.[7] [d] Central H_3-receptor screening with po administration as a methylcellulose suspension to mice.[7] [e] i.a., intrinsic activity. [f] Reference 13. [g] n.c., not calculable due to low intrinsic activity. [h] References 7, 8. [i] EC_{50} value.[17] [k] Reference 7.

in vitro on rat synaptosomes and full agonism in vivo. It should be noted that the antagonist potency of **8** under in vivo conditions exceeds that of the reference antagonist thioperamide[20] (ED_{50} = 1 mg/kg). Terminal addition of another methyl group, leading to the 3,3-dimethylbutyl derivative **10**, increased in vivo potency and maintained full agonism. Of all the new compounds, **10** showed highest agonist affinity and highest intrinsic activity in vitro on rat synaptosomes. Increasing the chain length by one more methylene group (**11**) decreased intrinsic activity while maintaining high potency in vivo. In vitro, only slight partial agonism could be observed, not allowing exact quantification.

Imetit has been described as a highly potent and selective histamine H_3-receptor agonist with full intrinsic activity in vitro as well as in vivo (Table 1).[7,8] Imetit and other classical H_3-receptor agonists (Chart 1) exist as monocations under physiological conditions due to their second basic moiety. This monocationic moiety is presumed to interact with acidic amino acids of the receptor protein inducing a change in receptor conformation, thus leading to agonist activity.[21] The novel compounds are devoid of a comparable structural feature. Most probably an interaction with a lipophilic pocket with distinct steric demands could cause a similar change in receptor conformation leading to activation. The transition from antagonist to agonist responses and vice versa is caused by only small structural changes (**1** → **2**, **3** → **4**). Activation of the receptor only occurs when very distinct lipophilic and steric demands are fulfilled. The extent of partial agonism depends very much on the receptor tissue system. The diversity of pharmacologic H_3-receptor action with different compounds needs further investigation.

Selectivity of the novel compounds with regard to other histamine receptors is presented in Table 2. Most compounds are highly selective for the histamine H_3

Table 2. Antagonist Activity at Histamine Receptor Subtypes Determined on Functional Models

	H_3		H_2	H_1
no.	pK_i[a]	pK_B[b]	pK_B[c]	pK_B[d]
1[e]	7.09	7.04	<4.3	<4.0
2	n.d.[f]	7.22	<4.0[g]	<4.0[g]
3[e]	7.52	7.39	<4.0[g]	<4.5
4	7.64	7.40	3.2[g]	3.5[g]
5	n.d.[f]	7.34	3.5[g]	4.8
6	n.d.[f]	7.53	4.3[g]	4.2
7	7.38	7.58	4.3[g]	6.0
8	6.86	6.64	n.d.[f]	n.d.[g]
9	7.85	7.03	3.5	4.5
10	8.00	7.38	<4.0	4.5
11	8.03	7.89	4.6	5.0

[a] H_3-Receptor assay on synaptosomes of rat cerebral cortex;[7] for standard errors see Table 1. [b] H_3-Receptor assay on guinea pig ileum;[16,17] for SEM see Table 1. [c] H_2-Receptor test on guinea pig atrium;[18] SEM ± 0.2. [d] H_1-Receptor test on guinea pig ileum;[18] SEM < 0.1. [e] Reference 13. [f] n.d., not determined. [g] pD'_2 value.

receptor with pK_B values below 4.5 at H_1 and H_2 receptors. Compounds **7** and **11** displayed moderate activity for the H_1 receptor. Except for **7**, all tested compounds showed higher histamine H_3-receptor antagonist activity by at least 2.5 log units compared to H_1 or H_2 receptors, proving the high selectivity of the new compounds for histamine H_3 receptors.

Conclusions

Activation of histamine H_3 receptors was achieved in vitro and in vivo with some of the novel compounds of the carbamate and the ether classes. These new agonists are structurally diverse from classical histamine H_3-receptor agonists as they do not possess a basic moiety in the side chain of the molecule. Hence they do not exist as monocations under physiological conditions.

These compounds behave as partial agonists or antagonists in two tests in vitro but as potent full agonists in vivo (**9**, **10**) on an index of histaminergic neuron activity suggesting that they constitute not only interesting pharmacological tools but also promising therapeutic agents with central activity.

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 Brucker DPX 400 Avance (400 MHz) spectrometer. Chemical shifts are expressed in ppm downfield from internal Me_4Si as reference. ^{1}H NMR data are reported in the following order: multiplicity (br, broad; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; *, exchangeable by D_2O; Im, imidazole; Mal, maleic acid), number of protons, and approximate coupling constants in hertz (Hz). Mass spectra were obtained on an EI-MS Finnigan MAT CH7A. Spectral data of parent compounds are shown only for **2**, **4**, **6**, and **8** which were obtained by different methods. Elemental analyses (C, H, N) were measured on Perkin-Elmer 240 B or Perkin-Elmer 240 C instruments and were within ±0.4% of the theoretical values. 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.). Thin-layer chromatography (TLC) was performed on silica gel PF_{254} plates (Merck); the spots were visualized with Dragendorff's reagent, fast blue salt BB, or by UV absorption at 254 nm.

3-(1*H*-Imidazol-4-yl)propyl *N*-*tert*-Butylcarbamate (2). *tert*-Butyl isocyanate (6 mmol, 0.6 g) was added to a solution of 3-(1*H*-imidazol-4-yl)propanol·HCl[12] (5 mmol, 0.8 g) in 30 mL of dry acetonitrile under nitrogen atmosphere and refluxed for 4 h. The solvent was removed under reduced pressure and the reaction mixture purified by rotatory chromatography [eluent: $CHCl_3$/MeOH (gradient from 95/5 to 90/10), ammonia atmosphere]. The combined fractions were concentrated, dried, and crystallized as hydrogen maleate: ^{1}H NMR (Me_2SO-d_6) δ 8.89 (s, 1H, Im-2-H), 7.40 (s, 1H, Im-5-H), 6.85 (s, 1H, CONH*), 6.05 (s, 2H, Mal), 3.93 (t, J = 6.3 Hz, 2H, CH_2−O), 2.68 (t, J = 7.6 Hz, 2H, Im-CH_2), 1.89 (m, 2H, Im-CH_2−CH_2), 1.21 (s, 9H, $(CH_3)_3$); MS *m*/*z* 225 ($M^{\bullet+}$, 12), 108 (85), 95 (100), 81 (77), 72 (29), 54 (30), 45 (25), 26 (42). Anal. ($C_{11}H_{19}N_3O_2$·$C_4H_4O_4$·0.25H_2O) C, H, N.

3-(1*H*-Imidazol-4-yl)propyl *N*-Neopentylcarbamate (4). A mixture of 3,3-dimethylbutanecarboxylic acid (5 mmol, 0.6 g), triethylamine (5 mmol, 0.5 g), and diphenyl phosphorazidate (5 mmol, 1.4 g) was stirred for 45 min in 30 mL of dry acetonitrile at room temperature. The reaction mixture was then heated to reflux for 30 min. Then 3-(1*H*-imidazol-4-yl)-propanol·HCl[12] (5 mmol, 0.8 g) was added and the solution again refluxed for 4−5 h. The solvent was removed under reduced pressure and the residue was dissolved in Et_2O and extracted with a saturated solution of K_2CO_3 and $NaHCO_3$. After concentration of the combined organic fractions the residue was purified by rotatory chromatography [eluent: $CHCl_3$/MeOH (gradient from 95/5 to 90/10), ammonia atmosphere]. The combined fractions were concentrated, dried, and crystallized as hydrogen oxalate: ^{1}H NMR (Me_2SO-d_6) δ 8.63 (s, 1H, Im-2-H), 7.27 (s, 1H, Im-5-H), 7.12 (m, 1H, CONH*), 3.97 (t, J = 6.4 Hz, 2H, CH_2−O), 2.79 (d, 2H, J = 6.3 Hz, NH−CH_2), 2.68 (t, J = 7.5 Hz, 2H, Im-CH_2), 1.89 (m, 2H, Im-CH_2−CH_2), 0.82 (s, 9H, $(CH_3)_3$); MS *m*/*z* 239 ($M^{\bullet+}$, 17), 108 (100), 95 (62), 81 (78), 54 (11), 45 (63). Anal. ($C_{12}H_{21}N_3O_2$·$C_2H_2O_4$·0.5H_2O) C, H, N.

3-(1*H*-Imidazol-4-yl)propyl *N*-(3,3-Dimethylbutyl)carbamate (6). To a solution of trichloromethyl chloroformate (6 mmol, 1.2 g) and a catalytic amount of activated charcoal in 20 mL of dry ethyl acetate was added 3,3-dimethylbutylamine (5 mmol, 0.5 g) rapidly. The reaction mixture was heated to reflux for 4−5 h, the black solution was cooled and filtered, and the solvent was evaporated carefully under reduced pressure. The freshly prepared isocyanate was dissolved in 20 mL of dry acetonitrile and added to a mixture of 3-(1*H*-imidazol-4-yl)propanol·HCl[12] (5 mmol, 0.8 g) in 10 mL of dry acetonitrile. The solution was refluxed for 4−5 h and concentrated in vacuo. The residue was purified by rotatory chromatography [eluent: CH_2Cl_2/MeOH (gradient from 99/1 to 90/10), ammonia atmosphere]. The product was obtained as a colorless oil and crystallized as hydrogen maleate in Et_2O/EtOH: ^{1}H NMR (Me_2SO-d_6) δ 8.83 (s, 1H, Im-2-H), 7.37 (s, 1H, Im-5-H), 6.98 (m, 1H, CONH*), 6.04 (s, 2H, Mal), 3.96 (t, J = 6.4 Hz, 2H, CH_2−O), 2.97 (m, 2H, NH−CH_2), 2.67 (t, J = 7.4 Hz, 2H, Im-CH_2), 1.89 (m, 2H, Im-CH_2−CH_2), 1.32 (m, 2H, NH−CH_2−CH_2), 0.88 (s, 9H, $(CH_3)_3$); MS *m*/*z* 253 ($M^{\bullet+}$, 14), 109 (42), 108 (100), 107 (33), 95 (72), 82 (30), 81 (58), 80 (11), 57 (11), 54 (11), 43 (12), 41 (21). Anal. ($C_{13}H_{23}N_3O_2$·$C_4H_4O_4$·0.5H_2O) C, H, N.

3-(1*H*-Imidazol-4-yl)propyl 2-Methylpropyl Ether (8). A mixture of 3-(1-(triphenylmethyl)-1*H*-imidazol-4-yl)propanol[12] (5 mmol, 1.84 g) and NaH (60%) (6 mmol, 0.24 g) was

stirred in 10 mL of dry toluene for 12 h. Then a catalytic amount of tetrabutylammonium iodide, 15-crown-5, and 2-methylpropyl bromide (8 mmol, 1.1 g) were added and then heated to 80 °C for 12 h. The reaction was monitored by TLC (solvent: ethyl acetate, ammonia atmosphere). Excess NaH was destroyed by addition of EtOH; the solution was then concentrated in vacuo, redissolved in 2 N HCl/THF (20/30), and heated to reflux for 1 h. The organic solvent was removed under reduced pressure; the aqueous suspension was filtered and extracted with Et_2O. The aqueous solution was basified (ammonia) 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/NH_3 (95/4/1)]. The colorless oil was crystallized as hydrogen maleate in EtOH/Et_2O: ^{1}H NMR (Me_2SO-d_6) δ 8.88 (s, 1H, Im-2-H), 7.39 (s, 1H, Im-5-H), 6.04 (s, 2H, Mal), 3.38 (t, J = 6.2 Hz, 2H, CH_2−O), 3.12 (m, 2H, CH_2−CH), 2.67 (t, J = 7.6 Hz, 2H, Im-CH_2), 1.86 (m, 2H, Im-CH_2−CH_2), 1.81−1.72 (m, 1H, CH), 0.84 (d, J = 6.7 Hz, 6H, $(CH_3)_2$); MS m/z 182 ($M^{\bullet+}$, 82), 125 (23), 10 (15), 109 (38), 108 (48), 107 (19), 95 (80), 82 (100), 81 (67), 72 (19), 57 (14), 54 (16). Anal. ($C_{10}H_{18}N_2O\cdot C_4H_4O_4$) C, H, N.

Pharmacology. General Methods. 1. Histamine H_3-Receptor Assay on Synaptosomes of Rat Cerebral Cortex. Compounds were tested at least in triplicate 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.[7]

2. Histamine H_3-Receptor 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 ileum segments induced by (*R*)-α-methylhistamine in the presence of the antagonist as described previously.[17,22] Agonist activity was recorded by concentration−response curves of the tested compound instead of (*R*)-α-methylhistamine. Each experiment was performed at least in triplicate.

3. Histamine H_3-Receptor Potency in Vivo in Mouse. In vivo testing was performed at least in triplicate after po administrationof the compounds to Swiss mice as described by Garbarg et al.[7] Increased or decreased brain histamine turnover was assessed by measuring an increased or decreased level of the main metabolite of histamine, N^{τ}-methylhistamine.[7]

4. In Vitro Screening at Other Histamine Receptors. Compounds were screened for histamine H_2-receptor activity on the isolated spontaneously beating guinea pig right atrium as well as for H_1-receptor activity on the isolated guinea pig ileum by standard methods described by Hirschfeld et al.[18] Each pharmacological test was performed at least in triplicate, but the exact type of interaction had not been determined in each case.

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