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Short communication

2-Alkyl-substituted histamines and hydroxyethylimidazoles with G-protein-stimulatory activity

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Summary — Cationic-amphiphilic 2-substituted histamines activate pertussis toxin-sensitive guanine nucleotide-binding proteins (G-proteins) by a receptor-independent mechanism. From our recent studies it became apparent that lipophilicity is an important determinant for this G-protein activation, but the influence of basicity remained unknown. We prepared seven novel 2-alkyl-substituted histamines and five novel 2-alkyl-substituted hydroxyethylimidazoles and studied their effects on high-affinity guanosine triphosphate (GTP) hydrolysis in membranes of the human leukemia cell line, HL-60. 2-Octylhistamine was found to be the most effective GTPase activator among 2-substituted histamines presently available (150% stimulation above basal), and 2-tetradecylhistamine is the most potent substance in this regard (pEC_{50} = 5.9). Branching of the alkyl chain and the introduction of an ether group adversely affected GTPase activation. Compared to a phenyl ring, a bulky adamantyl sphere enhanced G-protein-stimulatory activity. In the case of 2-(3-bromophenyl)histamine, 2-adamantylhistamine and 2-(3-phenylpropyl)histamine, replacement of the aminoethyl group by a hydroxyethyl group at the imidazole greatly reduced GTPase-activating properties, pointing to the importance of the basic domain in the activation process. Unexpectedly, however, in the case of a very lipophilic substituent (heptadecyl chain) the exchange of the aminoethyl group by a hydroxyethyl group had no substantial inhibitory effect, indicating that the presence of a primary amine is not a *conditio sine qua non* for a substance being a receptor-independent G-protein activator. Concerning histamine H_1-receptors the newly prepared compounds proved to be weak antagonists.

G-protein / GTPase / 2-substituted histamine / 2-substituted hydroxyethylimidazole / histamine H_1-receptor

Introduction

Besides their well known agonistic or antagonistic activity at histamine H_1-receptors [1–4], 2-substituted histamines possess the ability to activate pertussis toxin-sensitive heterotrimeric regulatory guanine nucleotide-binding proteins (G-proteins) in a receptor-independent manner [5–7]. G-proteins play a central role in transmembrane signal transduction from heptahelical receptors to cellular effector systems [8]. The human leukemia cell line HL-60 has been shown to be a particularly useful system for the analysis of receptor-independent G-protein activation by 2-substituted histamines [5–7].

2-Substituted histamines are cationic-amphiphilic substances, ie, they possess a basic domain (aminoethylimidazole) and a lipophilic domain (eg, a (substituted) phenyl or cyclohexyl residue) [1–7]. The relatively simple structures of 2-substituted histamines, which can be modified in a logical manner, render them a suitable starting point for the systematic analysis of the as yet incompletely understood structure–activity relationships of receptor-independent G-protein activators and the development of potent and selective compounds of this novel class of drugs. To this end, we have shown that substitution at the phenyl ring with bulky and lipophilic halogens increases G-protein-stimulatory activity as assessed by measurement of high-affinity guanosine triphosphate (GTP) hydrolysis (reflecting the enzymatic activity of G-protein α-subunits) [6]. Moreover, replacement of the planar phenyl ring by non-planar ring systems (cyclohexane or norbornane) increases G-protein-stimulatory activity [6, 7]. Furthermore, elongation of the alkyl chain connecting the ring system with aminoethylimidazole enhances GTPase-activating properties [6, 7]. The most potent 2-substituted histamines with GTPase-activating properties currently available are

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cyclohexylbutyl-substituted derivatives (pEC_{50} = 5.0) (compounds **9-h** and **18-h** in reference [7]).

Although it is generally assumed that both the basic and the lipophilic domain are required for receptor-independent G-protein activation by cationic-amphiphilic substances [5–7, 9, 10], the influence of the basic domain has been less well studied than that of the lipophilic domain. In the case of 2-substituted histamines, this question can readily be addressed by comparing the effects of 2-substituted histamines with those of the corresponding 2-substituted hydroxyethylimidazoles on G-protein activation.

Our present study had three major aims. First, we wished to learn more about the importance of lipophilicity of 2-substituted histamines for their receptor-independent G-protein activation. Second, we hoped to obtain more potent GTPase activators than those presently available. Third, we intended to shed light on the role of the basic domain of 2-substituted histamines for their G-protein-stimulatory activity. Therefore, we prepared seven novel alkyl-substituted histamines (**1–6** and **9**: structural formulae shown in table I) and five novel 2-substituted hydroxyethylimidazoles (**10–14**: structural formulae shown in table I), and studied their effects on high-affinity GTP hydrolysis in HL-60 membranes. The effects of the novel compounds on G-protein activation are discussed in relation to the effects of recently studied substances [6, 7].

Results

Chemistry

The condensation of α-hydroxyketones with activated derivatives of carboxylic acids is one of the most useful methods for synthesis of imidazoles [11]. This route tolerates a large variety of substituents on the carbons of the five-membered ring. The desired groups at the 2-, 4-, or 5-positions can easily be introduced into the condensation step. An alternative route to the substituted imidazole is the alkylation of the N-protected heterocycle by subsequent lithiation and alkylation steps [12].

The substitution pattern required consisted of a very lipophilic residue at the 2-position and a 2-aminoethyl or 2-hydroxyethyl group at the imidazole C-4. The synthesis of the compounds investigated in the communication is outlined in scheme 1. The α-hydroxyketone unit with the desired functionalized ethyl side chain is accessible from 1,4-butynediol by mercury-catalyzed addition of water [13]. This was used as the hydroxy-terminated synthon. Subsequent reaction with acetic acid anhydride and phthalimide generates the protected N-terminated C-4 unit [7, 14]. We used imidomethyl esters for the C-1 unit of the imidazole carrying the lipophilic residue. These were obtained as hydrochlorides by the action of HCl in methanol on the corresponding aliphatic or araliphatic nitriles.

In liquid ammonia as solvent and reagent these units condensed to the hydroxyethyl derivatives **10–14** and, after acid-catalyzed deprotection, the aminoethyl derivatives **1–9**. The hydroxyethylimidazole could be converted to the histamine derivatives by reacting with thionyl chloride followed by ammonia (scheme 1). The latter route is the more reasonable when both compounds are to be investigated.

Pharmacology

Table I summarizes the effects of compounds **1–14** at H_1-receptors in the guinea pig ileum and on high-affinity GTPase activation in HL-60 membranes. All of the newly synthesized compounds (**1–6**, **9–14**) turned out to be weak H_1-receptor antagonists. Of the substances studied, compound **3** was the most potent H_1-receptor antagonist. Interestingly the influence on H_1-receptor antagonism of an aminoethyl or hydroxyethyl group at the imidazole ring depended on the alkyl chain length at the 2-position. Specifically, in the case of a heptadecyl chain (**5** and **11**), the amino group increased H_1-antagonist activity whereas in the case of a tetradecyl chain the opposite was true (**4** and **10**). When 2-(3-bromophenyl)histamine **8** is considered, substitution of the amino group by a hydroxyl group (**12**) resulted in conversion of potent H_1-receptor agonism into weak H_1-receptor antagonism.

The novel 2-alkyl-substituted histamines showed stimulatory effects on high-affinity GTP hydrolysis in HL-60 membranes. Among all 2-substituted histamines studied so far, 2-octylhistamine **2** was the most efficacious substance, ie, it increased GTP hydrolysis by 150% above basal. Elongation (**3–5**) of the alkyl chain reduced efficacy as did branching of the alkyl chain (**1**). Considering the potency of the substances, compound **4** was the most active derivative. Shortening of the alkyl chain by six carbon atoms (**2**) reduced potency by more than one order of magnitude. Elongation of the alkyl chain by three carbon atoms (**5**) slightly reduced potency. The introduction of an ether function diminished potency about tenfold and had little effect on efficacy (compare **3** and **6**).

Compared to a phenyl ring (compound **1-h** in reference [7]), the more bulky adamantyl group (**9**) moderately increased potency and efficacy of the substance to activate GTPase. The data obtained with the adamantyl derivative fit well to those obtained with cyclopentyl, cyclohexyl, and norbornyl deriva-

Table I. Effects of various 2-substituted histamines and hydroxyethylimidazoles at histamine H_1-receptors in the guinea-pig ileum and on high-affinity GTP hydrolysis in HL-60 membranes.

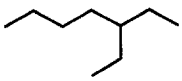
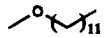
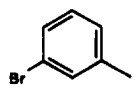
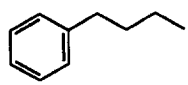
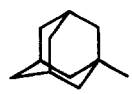
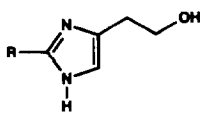
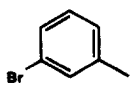
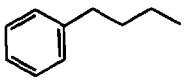
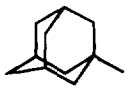
Compound		*H_1-Receptor*		*GTPase Stimulation*	
R =		*Agonist rel act (%)*	*Antagonist –log K_B*	*Efficacy (%)*	*pEC_{50}*
1			4.1	110	4.0
2			5.0	150	4.5
3			6.1	95	5.8
4			4.7	60	5.9
5			5.2	96	5.5
6			5.1	98	4.9
7		112		80	3.7
8			5.0	90	3.7
9			4.0	55	4.0
R =					
10			5.7	80	–
11			3.6	64	5.4
12			3.9	0	–

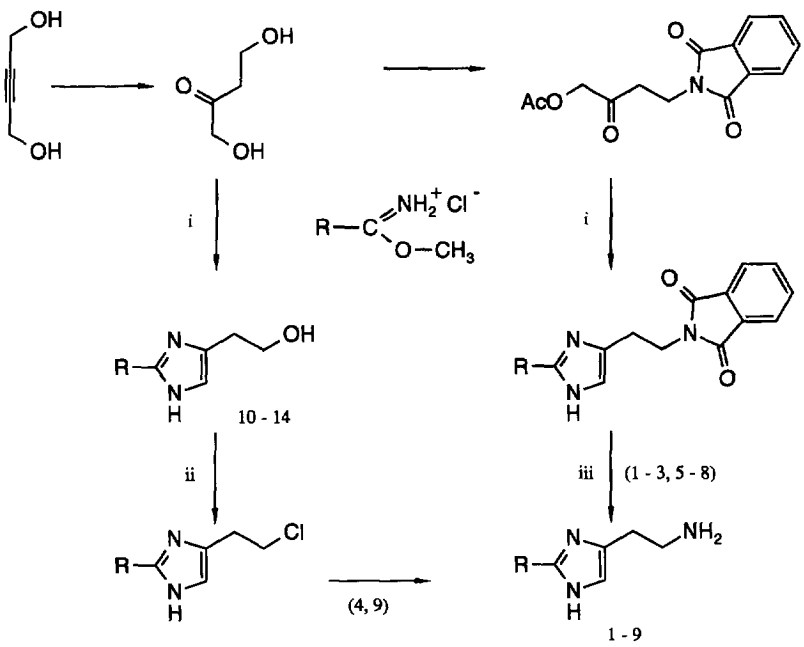
Table I. *(Continued.)*

Compound	*H_1-Receptor*		*GTPase Stimulation*	
	Agonist rel act (%)	*Antagonist –log K_B*	*Efficacy (%)*	*pEC_{50}*
13 		4.3	0	–
14 		4.6	15	–

H_1-receptors: The value of the relative activity (rel act), ie, potency compared to histamine, of compound **7** at H_1-receptors in the guinea-pig ileum was taken from reference [3]. The –log K_B value of compound **8** was taken from Detert et al [7]. The –log K_B values of the other compounds ($N = 2$) were determined as described in the *Experimental protocols*.
GTP hydrolysis: GTP hydrolysis in HL-60 membranes was determined in the presence of compounds **1–14** at concentrations ranging from 0.1 μM – 3 mM as described in the *Experimental protocols*. The efficacy of substances is defined as the maximal increase in GTP hydrolysis above basal activity. When no saturation of the concentration–response curves could be obtained this is indicated by –. In such cases only the efficacy of the substance is given. The SD values of the data were generally <5% of the means. Basal GTP hydrolysis in HL-60 membranes was 18.4 ± 0.6 pmol/mg/min.

tives. These ring systems also are more bulky than a phenyl ring and increase the GTPase-activating properties of a substance (compounds **5-h**, **10-h**, and **12-h** in reference [7]). Compared to cyclopentyl, cyclohexyl, and norbornyl groups, the adamantyl group results in a small increase in potency, but the efficacy tends to decrease.

In order to study the importance of the basic aminoethyl group at the imidazole ring for GTPase stimulation we compared the effects of aminoethyl-



Scheme 1. Reagents: i) liquid ammonia, ii) HCl in ethanol, then $SOCl_2$, iii) 20% HCl, then NaOH.

and hydroxyethyl-substituted substances on GTP hydrolysis. In the case of compounds **7**, **8**, and **9**, exchange of the aminoethyl group by a hydroxyethyl group resulted in subtotal to total loss of stimulatory activity (compare with compounds **12**, **13** and **14** respectively). Tetradecyl chain substitution of the aminoethyl group by a hydroxyethyl group increased efficacy and decreased potency (compare **4** and **10**). When a heptadecyl chain is considered the introduction of a hydroxyethyl group had a moderate inhibitory effect on efficacy and no effect on potency (compare **5** and **11**).

Conclusions

In our present study we report the synthesis of the most efficacious and potent receptor-independent GTPase activators in the class of 2-substituted histamines. Until this study 2-norbornylbutylhistamine (compound **14-h** in reference [7]) was known to be the most efficacious substance (130% stimulation above basal) of this class of drugs. 2-Octylhistamine (**2**) surpasses this activity by 20% (150% stimulation above basal). With respect to potency the tetradecyl-substituted derivative **4** was found to be the most active compound. Its potency is almost one order of magnitude higher than that of 2-cyclohexylbutylhistamine (compound **9-h** in reference [7]) which was hitherto the most potent substance available. Additionally we found that branching of the alkyl chain (compare **1** and **2**) and the introduction of an ether function (compare **3** and **6**) had inhibitory effects on the efficacy and/or potency of substances. Taken together our data show that a straight alkyl chain is an important structural determinant for obtaining potent and effective GTPase activators within the class of 2-substituted histamines. One can imagine that a straight alkyl chain facilitates the insertion of the substances into the plasma membrane and thereby G-protein activation.

Our present data raise important questions about future directions of development of receptor-independent G-protein activators. Clearly compound **2**, bearing an octyl chain, is the most efficacious substance among the 2-substituted histamines presently available, but it is far from being the most potent one (see table I) [6, 7]. Conversely, compound **4**, with a tetradecyl chain, is the most potent but several substances are more efficacious (see table I) [6, 7]. These data show that efficacy and potency of a receptor-independent G-protein activator can be dissociated from one another and are independently determined drug parameters. Our findings also show that an increase in lipophilicity per se does not lead to an increase in substance activity in general. On the whole efficacy and potency have different lipophilicity optima. However, the situation may in fact be far more complicated, as the initial concentration of the compounds in the test tube could differ from the concentrations in the lipidic neighbourhood of the G-protein, which is unknown.

If one intends to identify specific binding sites of cationic-amphiphilic substances at G-protein subunits one evidently needs a substance with high affinity (which is presumably reflected by high potency to activate GTPase). However, of what importance in this respect is the efficacy of a substance to activate GTPase? Is low, intermediate or high efficacy of GTPase activation a desired aim of drug development? In this context we note that 2-cyclohexylhistamine possesses a high efficacy for activating GTPase in HL-60 membranes, but in intact cells the compound is ineffective in increasing free cytosolic Ca^{2+} concentration [6]. We were also surprised to observe that, in contrast to GTPase activation in HL-60 membranes, cyclohexylethylhistamine is only poorly effective in stimulating binding of the stable GTP analogue [^{35}S]-guanosine 5'-*O*-[3-thio]triphosphate in this system (Hagelüken and Seifert, unpublished results). The latter findings and the dissociation of potency and efficacy of 2-substituted histamines in activating GTPase raise the intriguing possibility that there is more than one site of interaction of 2-substituted histamines with G-proteins. The prototype of peptidic receptor-independent G-protein activators, mastoparan, is also assumed to interact with G-proteins at multiple sites [15].

Our findings that compounds **12**, **13** and **14** showed smaller stimulatory effects on GTPase than compounds **7**, **8** and **9**, respectively, were not surprising, and are in accordance with the view generally held that basic domains are required for a cationic-amphiphilic substance to be a receptor-independent G-protein activator [5–7, 9, 10]. However, compounds **10** and **11**, which do not have primary amine groups, showed unexpectedly high GTPase-activating properties in comparison to the aminoethyl group-containing derivatives (compare with compounds **4** and **5** respectively). Interestingly, compounds **4**, **5**, **10** and **11** bear long and lipophilic alkyl chains. Thus, it appears that, provided a substance possesses a high degree of lipophilicity, an aliphatic amino group is not needed any more for G-protein activation. These data clearly show that a primary amino group is not a *conditio sine qua non* for a given compound displaying G-protein-stimulatory activity. These findings have important implications for the further search for potent and selective G-protein activators. So far pharmacological studies with naturally occurring and synthetic substances have focused on compounds containing multiple basic groups [9, 10].

Table II. Analytical data of 2-substituted histamines and hydroxyethylimidazoles.

Compound	*Formula (molecular weight)*	*Yield (%) (mp (°C))*	*Mass spectra (FAB+) m/e*	*IR Spectra (in KBr) (cm^{-1})*	*^{1}H-NMR (δ in ppm) TMS as internal standard, DMSO-d_6 as solvent*	*^{13}C-NMR (δ in ppm) TMS as internal standard, DMSO-d_6 as solvent*
1	$C_{13}H_{25}N_3 \times 2C_4H_4O_4$ (455.495)	24 (142)	224 100%, 207 22%	3421 m, 3007 s, 2952 vs, 2923 vs, 1580 vs, 1571 vs, 1527 vs, 1506 vs, 1455 vs, 1383 vs, 1191 s, 872 s, 862 s	7.93 bs, 1H, NH; 7.37 s, 1H, H-C-5 (Im); 6.07 s, 4H, (Mal); 3.12, 2.91 each: (t, J = 7.3 Hz, 2H) (et); 2.76 d, J = 7.3 Hz, 2H, H_2C-1 (*i*-oct); 1.76 m, 1H, H-C-2 (*i*-oct); 1.24 m, 8H, CH_2 (*i*-oct); 0.83 m, 6H, CH_3	173.3 (Mal); 146.8 C-2 (Im); 135.7 (Mal); 128.9 C-4 (Im); 115.9 C-5 (Im); 38.0, 37.4, 31.8, 29.9, 27.8, 25.1, 22.8, 22.2, 19.4 (7 CH_2, 1CH); 13.8, 10.2 CH_3
2	$C_{13}H_{25}N_3 \times 2C_4H_4O_4$ (455.495)	38 (156)	224 100%, 207 24%	3418 s, 2921 vs, 2854 s, 1577 vs, 1483 vs, 1363 s	7.85 bs, 1H, NH; 7.33 s, 1H, H-C-5 (Im); 6.06 s, 4 H (Mal); 3.35 bs, 4H; 3.11 t, 2H, J = 7.0 Hz; 2.89 t, 2H, J = 7.6 Hz; 2.82 t, J = 7.6 Hz, 2H; 1.68 m, 2H; 1.27 m, 10H; 0.87 t, J = 6.9 Hz, 2H	167.1 (Mal); 147.4 C-2 (Im); 135.6 (Mal); 129.5 C-4 (Im); 115.8 C-5 (Im); 37.4, 31.1, 28.3, 28.2, 26.7, 25.4, 22.8, 22.0, 21.8 CH2; 13.8 CH_3
3	$C_{17}H_{33}N_3 \times 2C_4H_4O_4$ (511.603)	43 (137)	280 100%, 263 29%	3414 m, 2917 vs, 2849 s, 1578 vs, 1499 vs, 1382 m, 1364 m	7.83 bs, 1H, NH; 7.29 s, 1H, H-C-5 (Im); 6.05 s, 4H (Mal); 3.08 t, J = 7.1 Hz, 2H; 2.86 t, 2H, J = 7.5 Hz; 2.79 t, J = 7.4 Hz, 2H; 1.66 m, 2H, H_2C-2; 1.24 m, 18H; 0.86 t, J = 6.9 Hz, 3H, CH_3	167.2 (Mal); 147.4 C-2 (Im); 135.6 (Mal); 128.7 C-4 (Im); 115.8 C-5 (Im); 37.4, 31.2 (2C), 28.9 (2C), 28.7, 28.6, 28.4, 28.2, 26.7, 25.4, 22.7, 22.0, 19.9 (2C), CH_2; 13.9 CH_3
4	$C_{19}H_{37}N_3 \times 2C_4H_4O_4$ (539.657)	12 (132)	308 63%, 41 100%	3423 m, 2924 s, 2854 m, 1581 vs, 1500 vs, 1367 s, 1193 m, 1087 m, 1006 m, 868 m	7.86 bs, 1H, NH; 7.31 s, 1H, H-C-5 (Im); 6.05 s, 4H (Mal); 3.09 t, J = 7.0 Hz, 2H; 2.87 t, J = 7.3 Hz, 2 H; 2.81 t, J = 7.7 Hz, 2H; 1.66 m, 2H; 1.23 m, 22H; 0.88 t, J = 6.8 Hz, 3H	167.3 (Mal); 147.4 C-2 (Im); 135.9 (Mal); 128.7 C-4 (Im); 115.8 C-5 (Im); 37.4, 31.2 2C, 29.0 2C, 28.7, 28.6 2C, 28.4, 28.2, 26.8, 25.4, 22.7, 22.0 2C, CH_2; 13.9 CH_3
5	$C_{22}H_{43}N_3 \times 2C_4H_4O_4$ (581.738)	52 (138)	350 23%, 322 40%	3422 vs, 2918 s, 2850 m, 1626 s, 1579 s, 1485 s, 1364 m	7.85 bs, 2H, NH; 7.29 s, 1H (Im); 6.05 s, 4H, (Mal); 3.09 t, J = 7.4 Hz, 2H; 2.87 t, J = 7.4 Hz, 2H; 2.80 t, J = 7.7 Hz; 1.67 qui, 2H; 1.24 m, 28H; 0.85 t, J = 6.6 Hz, 3H, CH_3	167.3 (Mal); 147.7 C-2 (Im); 135.7 (Mal); 128.4 C-4 (Im); 115.3 C-5 (Im); 37.8, 31.2, 29.0 5C, 28.8, 28.6, 28.3, 28.2, 27.1, 25.8, 22.7, 22.0, 19.8, CH_2; 13.9 CH_3
6	$C_{17}H_{31}N_3O \times 2C_4H_4O_4$ (525.587)	21 (115)	296 100%, 282 21%, 267 8%	3360 s, 2910 s, 2847 m, 1722 m, 1638 s, 1570 vs, 1495 s, 1370 m, 1350 m, 1180 m, 1010 w, 860 m	7.32 s, 1H, H-C-4 (Im); 6.06 s, 4H (Mal); 3.57 s, 3H, OCH_3; 3.10, 3.29 each: (t, J = 7.3 Hz, 2H) (et); 2.81 t, J = 7.7 Hz, 2H, CH_2-11; 2.29 t, J = 7.2, 2H, CH_2-1; 1.67, 1.50 each: (qui, 2H, CH_2-2,10); 1.25 m, 14H	167.1 (Mal); 147.4 C-2 (Im); 135.6 (Mal); 128.7 C-4 (Im); 115.7 C-5 (Im); 60.6 H_2C-O; 57-7 H_3C-O; 37.4, 32.4, 30.0, 28.9, 28.9, 28.8, 28.7, 28.3, 26.7, 25.5, 25.4, 22.7
6a	$C_{12}H_{23}NO$ (197.316)	84 (39)	(EI) 197 1%, 55 100%	3429 m, 2918 vs, 2850 s, 2244 m, 1463 m, 1054 s, 721 m	($CDCl_3$) 3.64 m, 2H, CH_2-12; 2.19 t, J = 7.1 Hz, 2H, CH_2-2; 1.88–1.45 m, 4H; 1.43–1.22 m, 15H	
6b	$C_{13}H_{25}NO$ (211.343)	87	(EI) 211 1%, 195 15%, 45 100 %		($CDCl_3$) 3.40 t, J = 6.6 Hz, 2H, CH_2-12; 3.38 s, 3H, CH_3; 2.36 t, J = 8.1 Hz, 2H, CH_2-2; 1.64 qui, J = 7.1 Hz, 2H; 1.58 m, 2H; 1.44 m, 2H; 1.36 m, 12H	
9	$C_{15}H_{23}N_3 \times 2C_4H_4O_4 \times$ 1/2 H_2O (486.53)	10 (137)	246 100%, 135 18%	3426 m, 3033 m, 2914 s, 2855 m, 1695 w, 1579 s, 1478 s, 1359 s, 1199 w, 1004 w, 866 m	7.88 bs, 2H; 7.37 s, 1H, H-C-5 (Im); 6.09 s, 4H (Mal); 3.18 t, J = 6.8 Hz, 2H; 2.92 t, J = 6.9 Hz, 2H, (et); 2.12 s, 3H, (Ad); 2.01 s, 6H (Ad), 1.78 s 6 H (Ad)	167.2 (Mal); 153.8 C-2 (Im); 135.6 (Mal); 128.8 C-4 (Im); 115.8 C-5 (Im); 39.5, 37.4, 35.4, 34.1, 27.1, 22.7

Table II. *(Continued.)*

Compound	*Formula (molecular weight) (°C))*	*Yield (%) (mp m/e*	*Mass spectra (FAB+)*	*IR Spectra (in KBr) (cm^{-1})*	*^{1}H-NMR (δ in ppm) TMS as internal standard, DMSO-d_6 as solvent*	*^{13}C-NMR (δ in ppm) TMS as internal standard, DMSO-d_6 as solvent*
10	$C_{19}H_{36}N_2O$ (308.497)	28 (63)	309 100%, 291 8%, 41 63%	3428 s, 2920 vs, 2852 m, 1633 w, 1465 w, 1048 w, 722 vw	($CDCl_3$) 6.67 s, 1H, H-C-5 (Im); 3.87 t, J = 5.7 Hz, 2H, H_2C-OH; 2.77 t, J = 5.6 Hz, 2H, H_2C-C-4 (Im); 2.66 t, J = 6.6 Hz, 2H, H_2C-C-2 (Im); 1.69 qui, J = 7.4 Hz, 2H; 1.25 m, 22H; 0.88 t, J = 6.6 Hz, 3H	146.9 C-2 (Im); 133.1 C-4 (Im); 116.4 C-5 (Im), 61.0 , 31.3 2C, 30.5, 29.1 3C, 29.0, 28.8 3C, 28.7, 28.1, 22.1 2C, 13.9
11	$C_{22}H_{42}N_2O \times 1/4H_2O$ (355.078)	63 (77)	(EI) 351 100%, 323 78%	3304 m, 2950 s, 2912 vs, 2846 vs, 1598 vw, 1465 s, 1055 m	($CDCl_3$) 6.65 s, 1H, H-C-5 (Im); 3.85 t, J = 5.8 Hz, 2H, CH_2OH; 2.78 t, J = 5.8 Hz, 2H; 2.65 t, J = 7.8 Hz, 2H; 1.68 qui, 2H; 1.25 m, 28H; 0.88 t, J = 6.6 Hz, 3H, CH_3	($CDCl_3$) 149.1, 136.0, 116.4, 63.0, 32.6, 30.4–30.0 14C; 29.3, 29.2, 27.5, 23.3, 14.8 CH_3
12	$C_{11}H_{11}BrN_2O$ (267.113)	71 (114)	(EI) 187 7%, M-Br+; 58 100%	(neat) 3150 vs, 2955 vs, 2870 vs; 1583 s, 1565 s, 1465 vs, 1446 vs, 1047 vs, 7878 vs	($CDCl_3$) 7.96 s, 1H, H-C-2 (Ph); 7.73 d, J = 7.7 Hz, 1H, H-C-4 (Ph); 7.42 d, J = 7.9 Hz, 1H, H-C-6 (Ph); 7.22 "t", J = 7.9 Hz, 1H, H-C-5 (Ph); 6.87 s, 1H, H-C-5 (Im); 3.92 t, J = 5.7 Hz, 2H, CH_2OH; 4.0–3.2, bs, 2H, OH, NH; 2.86 t, J = 5.7 Hz, 2H, CH_2	142.9, 136.3, 132.9, 130.8, 130.1, 126.9, 123.3, 122.0, 120.4, 60.6, 30.3
13	$C_{14}H_{18}N_2O$ (230.298)	83 (oil)	(EI) 230 3%; 126 100%	(neat) 3103 vs, 3017 vs, 2932 vs, 2855 vs, 1599 m, 1578 m, 1451 vs, 1048 s, 747 s, 699 s	($CDCl_3$) 7.25–7.13 m, 2H; 7.11–7.08 m, 3H, 6.9–6.4 bs, 2H; 6.60 s, 1H, H-C-5 (Im); 3.81 t, J = 5.9 Hz, 2H, CH_2-OH; 2.73 t, J = 5.9 Hz, 2H, CH_2-2; 2.63, 2.60. each: (t, J = 7.8 Hz, 2H) CH_2-1,3 (prop); 1.98 qui, J = 7.8 Hz, 2H, $CH2$-$_2$ (prop)	($CDCl_3$) 147.8, 141.6, 134.7, 128.4 3C; 125.9 2C; 116.3, 62.1, 35.3, 29.9, 29.6, 27.9
14	$C_{15}H_{22}N_2O$ (246.341)	49 (168)	247 100%, 229 12%	3417 s, 3246 m, 2906 vs, 2851 s, 1633 w, 1577 w, 1426 m, 1367 w, 1053 m	($CDCl_3$) 6.67 s, 1H, H-C-5 (Im); 3.86 t, J = 5.6 Hz, 2H, H_2C-OH; 2.78 t, J = 5.6 Hz, 2H, H_2C-Im; 1.98 m, 3H; 1.97 m, 6H; 1.75 m, 6H	154.2 C-2 (Im); 136.9 C-4 (Im); 111.3 C-5 (Im); 61.9 COH; 41.2 3C H_2C (Ad); 36.2 3C H_2C (Ad); 34.0 C (Ad); 32.0 H_2C-Im; 27.8 3C HC (Ad)

Abbreviations: Im: imidazole, Mal: maleic acid, et: ethylene ($ImCH_2CH_2NH_2$), prop: propyl, Ph: phenyl, Ad: adamant-1-yl, *i*-oct: 2-ethylhexyl, v: very, w: weak, m: medium, s: strong (IR) or singlet (NMR), b: broad, d: doublet, t: triplet, q: quartet, qui: quintet.

Experimental protocols

Chemistry

Melting points are uncorrected and were determined using a Büchi 512 Dr Tottoli apparatus. IR spectra were recorded on a Perkin-Elmer 298 and 1420 spectrometer; the compounds were prepared as KBr pellets or as a film on NaCl plates. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker WC 300 spectrometer with tetramethylsilane as internal standard and $CDCl_3$ and DMSO-d_6 as solvents. Elemental analyses were performed on Perkin-Elmer 240 B and Perkin-Elmer 240 C instruments. Analyses indicated by the symbols of the elements were within ±0.4% of the theoretical values. Mass spectra were recorded using Finnigan MAT CH7A (70 eV), Finnigan MAT 711 (80 eV), Kratos MS 25 RF (70 eV) or, in the case of $^+$FAB spectra, Finnigan MATCH5DF (xenon, DMSO/glycerol) instruments. Chromatographic separation was achieved by column chromatography using silica gel (230-400 mesh) with $CHCl_3$/methanol/ triethylamine (25:10:1) as eluent.

The nitriles for the synthesis of **1–4**, **8**, **10** and **13** were prepared via the sequence alcohol→bromide→nitrile; the general procedures are reported in an earlier communication [7]. The synthesis of **7** and **8** are reported in the literature [7, 3]. 12-Methoxydodecanoic nitrile **6a** was prepared from 11-bromoundecan-1-ol by substitution of bromine with cyanide according to general procedure [7] and reacting the 12-hydroxydodecanoic nitrile **6b** with NaH/CH_3I. Compounds **5**, **12** and **14** were synthesized from commercially-available nitriles. The nitriles were directly converted to imidomethyl esters with dry hydrogen chloride in anhydrous methanol [7].

*12-Methoxydodecanoic nitrile **6a***
The hydroxynitrile **6b** (5.95 g, 30 mmol) was dissolved in 25 mL of DMF and 0.72 g of NaH was added. After 20 min stirring at ambient temperature, iodomethane (5.6 g, 40 mmol) was added, the mixture was stirred overnight and heated to 50 °C for 2 h. The cooled solution was poured into water (100 mL), extracted with ether (4 × 30 mL), the pooled extracts were washed with brine (2 × 50 mL), dried with $MgSO_4$, filtered through a pad of silica gel, concentrated and used without further purification. Yield: 87%.

*General procedure for the synthesis of histamines with substituents in the 2-position of the imidazole (**1, 2, 3, 5, 6, 8**)*
The corresponding carboxylic acid imidomethylester hydrochloride (10 mmol) and 1-acetoxy-4*N*-phthalimidobutan-2-one (10 mmol) were dissolved in 50 mL liquid ammonia, and the mixture was stirred at ambient temperature in an autoclave for 18 h then for 6 h at 60 °C (pressure rising to 30 bar). After cooling the solvent was evaporated, and the resulting dark yellow residue was added to 100 mL of 20% hydrochloric acid and stirred and heated to reflux for 6 h. The cooled mixture was rendered strongly basic with an excess of a concentrated solution of NaOH. The substituted histamine was extracted with portions (12 × 40 mL) of a mixture of chloroform and 2-propanol (4:1). The combined organic layers were washed twice with alkalinized (NaOH, pH 10) brine. The solvent was evaporated and the residue purified by column chromatography on silica gel using $CHCl_3$/methanol/triethylamine (25:10:1) as eluent. The free histamine was dissolved in abs ethanol, and a concentrated solution of 2.5 equiv of maleic acid in abs ethanol was added. This mixture was warmed to 40 °C, and abs ether was added dropwise while stirring until the solution became slightly turbid. The bishydrogenmaleates crystallized upon standing and were recrystallized twice from ethanol by adding ether. Yield: 21–52%.

*General procedure for the synthesis of 4-(2-hydroxyethyl)-imidazoles with substituents in the 2-position (**10–14**)*
The corresponding substituted carboxylic acid imidomethylester hydrochloride (10 mmol) and 1,4-dihydroxbutane-2-one (15 mmol) were added to 50 mL of liquid ammonia, and the mixture was stirred at ambient temperature in an autoclave for 18 h. Thereafter, stirring was continued for 8 h at 40 °C (pressure rising to 17 bar). After cooling the solvent was evaporated and the resulting light yellow residue was dissolved in dichloromethane. The solution was washed with water and brine and dried over Na_2SO_4. The solvent was distilled off and the oily residue purified by column chromatography using silica gel and dichloromethane/triethylamine (50:1) as eluent. The solution was concentrated and the substituted imidazolylethanol crystallized upon standing. Yield: 28–83 %.

*General procedure for the conversion of 4-(2-hydroxyethyl)-imidazoles with substituents in the 2-position to substituted histamines (**4, 9**)*
The imidazolyl ethanol (5 mmol) was dissolved in ethanol, and 10 mL of a concentrated solution of hydrogen chloride in ethanol was added. The solvent was distilled off, a second portion of ethanolic HCl was added, and after 10 min of stirring the ethanol was evaporated. The oily residue was stirred at 0 °C, and thionyl chloride (4 mL) was added dropwise. After 18 h at ambient temperature the mixture was warmed to 40 °C for 1 h. The excess of thionyl chloride was evaporated off, and the chloroethyl imidazole derivative was added to 60 mL of liquid ammonia. The mixture was stirred in an autoclave for 24 h at room temperature and for 8 h at 40 °C. After evaporation of the ammonia the residue was dissolved in dichloromethane, the solution washed with brine, dried over Na_2SO_4 and concentrated. Purification was achieved by chromatography on silica gel using dichloromethane/methanol/triethylamine (25:10:1) as eluent. The substituted histamines were converted to their bishydrogenmaleates as described above. Yield: 10–12 %.

Pharmacology

H_1-receptor assay on guinea-pig ileum
Contractile responses of whole ileal segments were measured isotonically (load 5 mN) under standard conditions [16] in the continuous presence of 0.1 μM atropine. Organs were allowed to stabilize for 80 min, during which time they were stimulated three times with 1 μM histamine. Each preparation was used to obtain a cumulative concentration–response curve with histamine first (0.01–10 μM) then two curves with the respective H_1-agonist. In competition experiments organs were incubated with mepyramine (1–300 nM) for 10–15 min before obtaining the last curve. For antagonists, a curve with histamine was measured first, then the antagonist (0.03–0.1 mM) was added and a second curve with increasing concentration of histamine was determined after 15 min [17].

Cell culture and membrane preparation
HL-60 cells were grown in suspension culture in RPMI-1640 medium supplemented with 10% (v/v) horse serum, 1% (v/v) non-essential amino acids, 2 mM L-glutamine, 50 U/mL penicillin and 50 μg/mL streptomycin in a humidified atmosphere with 7% CO_2 at 37 °C and were differentiated towards neutrophil-like cells with dibutyryl cAMP (0.2 mM) for 48 h [5]. HL-60-membranes were prepared as described previously [18].

GTPase assay

GTP hydrolysis was determined as described previously [5]. Briefly, reaction mixtures (100 µL) contained 2.5–5.0 µg of membrane protein/tube, 0.5 µM [γ-^{32}P]GTP (0.1 µCi/tube), 0.5 mM $MgCl_2$, 0.1 mM EGTA, 0.1 mM ATP, 1 mM adenosine 5'-[β,γ-imido]triphosphate, 5 mM creatine phosphate, 40 µg creatine kinase, 1 mM dithiothreitol and 0.2% (w/v) bovine serum albumin in 50 mM triethanolamine/HCl, pH 7.4, and 2-substituted histamines at various concentrations. Reactions were conducted for 30 min at 25 °C.

Miscellaneous

Protein was determined according to Lowry et al [19]. [γ-^{32}P]GTP was prepared as described previously [20]. Stock solutions of 2-substituted histamine derivatives were prepared at a concentration of 10 mM in distilled water (**1–9** and **14**) or in 10% DMSO (**10–13**). The maximum final DMSO concentration in the GTPase assay was 1% (v/v). DMSO up to 1% (v/v) had no effect on basal GTPase activity (data not shown).

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