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2-Substituted histamines with G-protein-stimulatory activity †

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Summary — The cationic-amphiphilic 2-substituted histamines, 2-(3-chlorophenyl)histamine (2-[2-(3-chlorophenyl)-1*H*-imidazol-4-yl]ethanamine) and 2-(2-cyclohexylethyl)histamine, activate pertussis toxin-sensitive guanine nucleotide-binding proteins (G-proteins) of the G_i-subfamily by a receptor-independent mechanism. We studied structure–activity relationships of 2-substituted histamine derivatives for this G-protein activation using six known and 12 newly synthesized compounds. Elongation of the alkyl chain between imidazole and the ring system enhanced the potency and efficiency of substances in activating high-affinity GTP hydrolysis, *ie* the enzymatic activity of G-protein α-subunits, in membranes of HL-60 leukemic cells. Cyclopentyl-, cyclohexyl- and norbornyl-substituted histamines were more effective and potent than phenyl-substituted histamines in mediating G-protein activation in HL-60 membranes and in activating reconstituted bovine brain G_i/G_o-proteins. Our data show that the chain length and the type of ring system are important determinants for receptor-independent G-protein activation by 2-substituted histamines. With respect to histamine H_1-receptors, most of the substances studied displayed weak antagonistic activity.

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

Introduction

Some 2-substituted histamines are histamine H_1-receptor agonists such as 2-(3-chlorophenyl)histamine [1] and H_1-receptor antagonists such as 2-(2-cyclohexylethyl)histamine [2]. 2-Substituted histamines are cationic-amphiphilic substances, *ie* they possess a basic domain (aminoethylimidazole) and a lipophilic domain (phenyl or cyclohexyl residue) (table I). Interestingly, like other cationic-amphiphilic substances, such as local anesthetics, β-adrenoceptor antagonists and the wasp venom, mastoparan [3, 4], 2-substituted histamines possess the ability to activate pertussis toxin-sensitive guanine nucleotide-binding proteins (G-proteins) in a receptor-independent manner [5, 6]. These findings prompted us to study structure–activity relationships of 2-substituted histamines for receptor-independent G-protein activation. We studied the effects of six known compounds ((**1–3**)-**h** and (**5–7**)-**h**) [2, 7] and 12 newly synthesized compounds ((**4**, **8–18**)-**h**) (structural formulae shown in table I) on high-affinity GTPase, *ie* the enzymatic activity of G-protein α-subunits, in membranes of HL-60 leukemic cells and reconstituted bovine brain G_i/G_o-proteins.

Results

Chemistry

Retrosynthetic analysis of histamines with substituents in the 2-position of the imidazole ring gives two possible routes for synthesis, *ie* either alkylation of an all-*N*-protected histamine or the construction of the imidazole ring. A suitable route for the latter is the condensation of imidoesters with α-hydroxyketones in ammonia [8]. Therefore, we used 1-acetoxy-4*N*-phthalimidobutan-2-one **19** [9] as a synthon, bearing the C_2-unit of the imidazole as well as the protected 2-aminoethyl side-chain. This compound is easily available in four steps from but-2-yne-1,4-diol [10]. The C_1-unit of the imidazole is represented by the sp^2-carbon of carboxylic acid imidomethylesters (**4**, **8–18**)-**f** generated from the corresponding nitrile [11] with the desired cycloalkylalkyl residue. Condensation of both compounds in liquid ammonia leads to

†Dedicated to Prof Dr Fritz Sauter, Chair of Organic Chemistry at the Technical University Vienna, on the occasion of his 65th birthday.

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

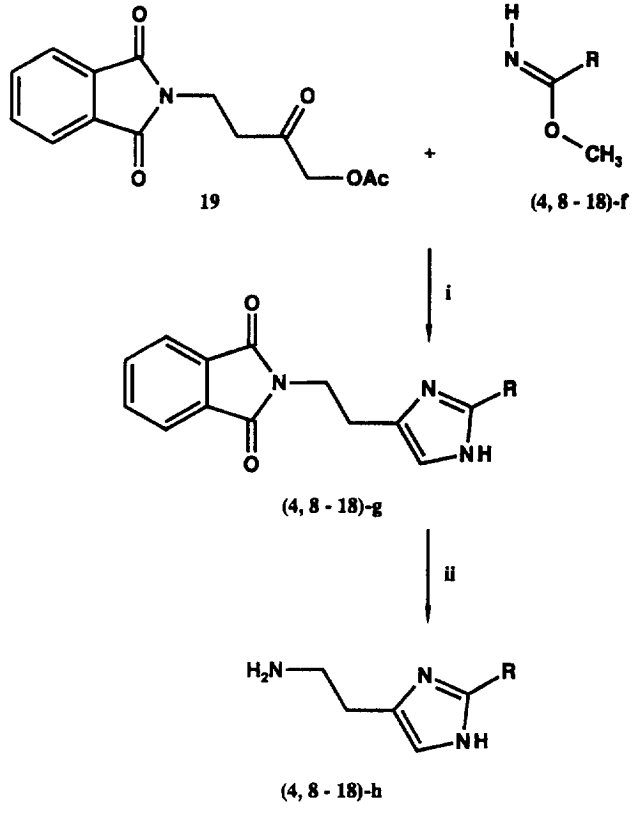
Compound	R =	H_1-Receptor agonist rel.act. [%]	H_1-Receptor antagonist -log K_B	GTPase Stimulation efficiency [%]	GTPase Stimulation pEC_{50}
1-h		31		36	3.5
2-h		1		49	3.2
3-h		3		74	3.4
4-h			5.0	90	3.7
5-h			5.8	42	
6-h			5.7	66	3.5
7-h			5.3	100	4.0
8-h			4.3	110	4.5
9-h			5.2	120	5.0
10-h		7		75	3.4
11-h			5.0	122	4.1
12-h		<1		80	3.4
13-h			4.1	100	4.0
14-h			5.1	130	4.7
15-h			5.0	104	4.5
16-h			5.2	104	4.7
17-h			5.4	112	4.7
18-h			4.8	112	5.0

H_1-receptors. The values of the relative activities (rel act), *ie* potency compared to histamine, of compounds **(1–3)-h** at H_1-receptors in the guinea-pig ileum were taken from the literature [1, 2, 7]; –log K_B values of compounds **(5–7)-h** were taken from Dziuron and Schunack [2]. The rel act and –log K_B values of the other compounds were determined as described in the *Experimental protocols*. *GTP hydrolysis.* GTP hydrolysis in HL-60 membranes was determined in the presence of compounds **(1–18)-h** at concentrations ranging from 1 μM to 3 mM as described in the *Experimental protocols*. The efficiency of substances is defined as the maximal increase in GTP hydrolysis above basal activity. The SD values of the data was generally < 5% of the means. Basal GTP hydrolysis in HL-60 membranes was 18.4 ± 0.6 pmol mg^{-1} min^{-1}.

the substituted and protected histamine derivative **(4, 8–18)-g** (scheme 1). Removal of the protecting group with boiling hydrochloric acid and extraction of the alkalized solution gives the free histamine **(4, 8–18)-h** which is purified by chromatography first and then by recrystallization of the corresponding bishydrogen maleate **(4, 8–18)-i** to obtain an analytically pure and storable product.

Pharmacology

Table I summarizes the effects of compounds **(1–18)-h** at the H_1-receptors of the guinea-pig ileum and on G-protein activation in HL-60 membranes. Compound **1-h** is an H_1-receptor agonist of about one third of histamine potency, while compounds **2-h, 3-h, 10-h** and **12-h** show much lower agonistic activity than compound **1-h** at H_1-receptors. By analogy to compounds **(5–7)-h** [2], other cyclohexyl derivatives (compounds **(8, 9, 15–18)-h**) are weak H_1-receptor antagonists as well. Cyclopentyl and norbornyl derivatives with an ethyl, propyl or butyl chain (compounds **(11, 13** and **14)-h**) are also weak H_1-receptor antagonists.



Scheme 1. Reagents: i) liquid ammonia; ii) 20% HCl, then NaOH.

With regard to GTP hydrolysis in HL-60 membranes, all substances studied showed stimulatory effects. Preliminary investigations showed that activation of GTP hydrolysis by 2-substituted histamines was inhibited by pretreatment with pertussis toxin ([2] and data not shown), indicative of activation of G_i-proteins.

Among the phenyl derivatives (compounds **(1–4)-h**), elongation of the alkyl chain connecting imidazole with the ring system resulted in an increase in efficiency of substances in supporting G-protein activation. As regards cyclohexyl derivatives (compounds **(6–9)-h**), we observed an increase in efficiency and potency of substances in stimulating GTPase. The same holds for cyclopentyl derivatives (compounds **10-h** and **11-h**) and norbornyl derivatives (compounds **(12–14)-h**). Branching of the alkyl chain at various positions had little effect on G-protein-stimulatory activity (compare compounds **8-h** and **9-h** with compounds **(15–18)-h**). When the type of ring system is considered, the efficiency and potency of cyclopentyl derivatives was higher than that of the cyclohexyl derivatives and the cyclohexyl was more efficient than a phenyl ring (compare compounds **4-h**, **8-h** and **11-h** (substances with a propyl chain)). Comparing compounds **2-h**, **6-h** and **10-h** (substances with a methyl chain), an increase in efficiency is apparent. As regards cyclohexyl and norbornyl rings, their efficiency and potency in stimulating GTP hydrolysis is similar (compare **7-h** and **13-h** (substances with an ethyl chain) and **9-h** and **14-h** (substances with a butyl chain)).

In order to confirm the notion that 2-substituted histamines act in a receptor-independent manner [5, 6], we studied the effects of compounds **9-h**, **14-h**, and **18-h** on GTP hydrolysis by reconstituted bovine brain G_i/G_o-proteins. In accordance with the data obtained with other 2-substituted histamines [6], compounds **9-h**, **14-h**, and **18-h** were found to be stimulatory (fig 1). In the reconstituted system, the compounds were less potent than in HL-60 membranes (compare table I with fig 1). This difference in potency has also been observed for other receptor-independent G-protein activators and may be due to differences in the association of G-proteins with phospholipids between the two systems [3, 6]. However, as is the case for HL-60 membranes, a phenyl ring is less potent and effective than a cyclohexyl or norbornyl ring in mediating activation of reconstituted G-proteins (compare **4-h** with **9-h**, **14-h** and **18-h**).

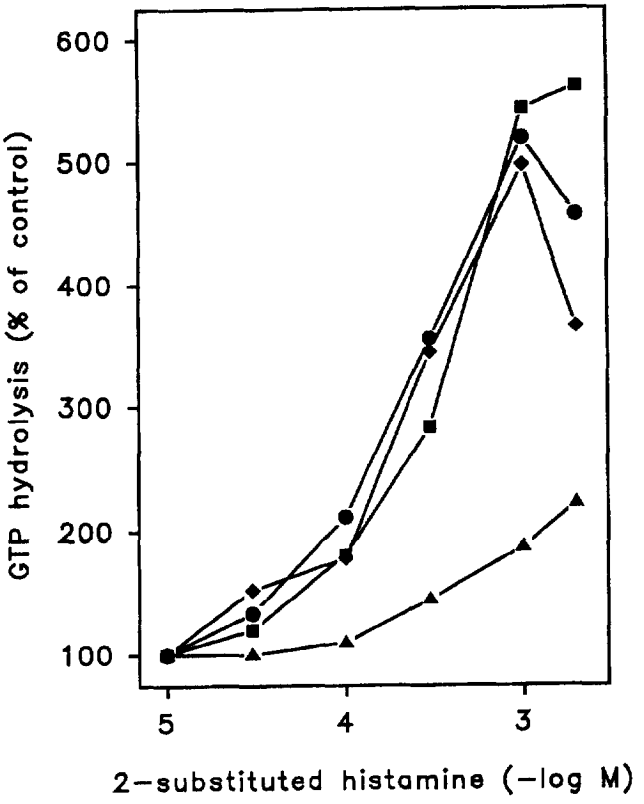


Fig 1. Concentration–response curves of the stimulatory effect of compounds **4-h** (▲), **9-h** (■), **14-h** (◆) and **18-h** (●) on GTP hydrolysis by reconstituted G_i/G_o-proteins. GTPase activity was determined as described in the *Experimental protocols*. The SD values of the data were generally < 5% of the means. Basal GTP turnover of G_i/G_o-proteins was 0.108 ± 0.005 min^{-1} (means ± SD, $n = 3$).

Conclusions

Lipophilicity may be important for receptor-independent G-protein activation by 2-substituted histamines because an increase in the length of the alkyl chain of substances enhanced their efficiency and potency. With respect to local anesthetics, β-adrenoceptor antagonists and mastoparan-derived peptides, lipophilicity is also an important determinant for their G-protein-activating properties [3, 4]. A detailed comparison of the receptor-independent G-protein activations induced by 2-substituted histamines and mastoparan has been presented elsewhere [6]. By analogy to receptor-independent G-protein activation, hydrophobic amino acids in the third intracellular loop of receptor molecules are essential for β-adrenoceptor-mediated activation of the stimulatory G-protein of adenylyl cyclase, G_s [12].

In addition to an enhancement of lipophilicity, an increasing length of the connecting chain increases the flexibility of the molecule and the distance between the basic domain (aminoethylimidazole) and the lipophilic domain (phenyl, cyclohexyl, cyclopentyl or

norbornyl rings). These functional consequences of chain elongation may facilitate the interaction of 2-substituted histamines with G-proteins. Future studies will have to answer the question of what the optimal chain length for receptor-independent G-protein activation by 2-substituted histamines is.

The results obtained with derivatives possessing various ring systems cannot be interpreted in a straightforward manner. Specifically, the cyclopentyl ring is the smallest ring system studied, but it is very effective in mediating G-protein activation. However, more bulky ring systems, *ie* cyclohexyl and norbonyl rings, are also effective. These findings clearly indicate that additional studies with substances possessing other ring systems have to be performed for better understanding of the structure–activity relationships of 2-substituted histamines for G-protein activation.

Taken together, the relatively simple structure of 2-substituted histamines, which can be modified in a logical manner, may render them a suitable starting point for the systematic analysis of the as yet poorly understood structure–activity relationships of receptor-independent G-protein activators and the development of potent and selective compounds of this novel class of drugs.

Experimental protocols

Chemistry

Melting points are uncorrected and were determined using a Büchi 512 Dr Tottoli apparatus. ^{1}H-NMR and ^{13}C-NMR spectra (table II) 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 case of $^+$FAB spectra, a Finnigan MATCH5DF (xenon, DMSO/glycerol). Chromatographic separation was done by column chromatography using silica gel (230–400 mesh) with $CHCl_3$/methanol/triethylamine (25:10:1) as eluent.

*General procedure for chain elongation by alkylation of malonester (**9, 14, 16, 18**)-**d**, 15-**b** and 17-**b***

Fifty millimoles of NaH and 5 mmol of NaI were suspended in 60 ml of abs DMF, 50 mmol of methylmalonic acid diethylester, or, in the case of **9-a** and **14-a** 60 mmol of malonic acid diethylester, dissolved in 25 ml of abs DMF was added dropwise while stirring in a N_2 atmosphere. The temperature was maintained below 40°C by gently cooling. After 15 min stirring, a solution of the alkyl bromide (**9**, **14–18**)-**a** (50 mmol) in 20 ml abs DMF was added dropwise. After 30 min, the mixture was heated to 65°C for 2 h and 90°C for 1 h while stirring was continued. Cooling to ambient temperature and pouring into 400 ml of distilled water was followed by fivefold extraction with 100 ml of ether each. The combined organic solutions were washed twice with brine, the solvent was distilled off and the oily residue was transferred to a solution consisting of 10 g KOH in 30 ml water and 20 ml ethanol. The mixture was heated to reflux under vigorous stirring for 36 h, cooled in an ice bath and acidified with concentrated hydrochloric acid. The precipitate was filtered off and transferred to a 250 ml round-bottomed flask. Decarboxylation was achieved by heating to 270°C *in vacuo*. The generated carboxylic acid (**9**, **14–18**)-**b** distilled off and was purified by repeated distillation. The acid was reduced to the alcohol (**9**, **14–18**)-**c** with $LiAlH_4$ in refluxing ether and subsequently converted to the cycloalkylalkylbromide (**9**, **14–18**)-**d** by stirring with hydrobromic acid (47%) and catalytic amounts of sulfuric acid and heating to reflux for 3 h. Purification by distillation; yield 55–74%.

*General procedure for nitrile synthesis from bromides (**8–14**, **16**, **18**)-**e***

Twenty millimoles of a cycloalkylalkylbromide were added to a solution of 22 mmol sodium cyanide and 1.0 mmol NaI in 50 ml of DMSO. The reaction mixture was stirred for 1 h at ambient temperature, 1 h at 60°C and 3 h at 95°C. After cooling, the mixture was poured into 300 ml distilled water, and the nitrile was isolated by fivefold extraction with 50 ml ether each, washing the combined organic layers twice with 50 ml brine, drying with $CaCl_2$ and distillation *in vacuo* after removal of solvent; yield 86–98%.

*General procedure for nitrile synthesis from carboxylic acids 15-**e**, 17-**e***

Fifteen millimoles cycloalkylalkylcarboxylic acid **15-b** or **17-b** and 35 mmol thionylchloride were stirred at room temperature for 12 h, heated to reflux for 2 h and the excess thionylchloride was distilled off. The remaining acyl chloride was diluted with 30 ml of ether and added dropwise to a vigorously stirred concentrated aqueous solution of ammonia at 0°C. The precipitated amide was filtered off, washed twice with water and with ether and dried thoroughly. The amide, twice the mass of phosphorus pentoxide and the fourfold mass of sand were mixed intensively in a mortar and was than transferred to a distillation apparatus. The mixture was heated slowly to 250°C *in vacuo*. The generated nitrile **15-e** or **17-e** distilled off and was purified by repeated distillation; yields **15-e** 84 and **17-c** 69%.

*General procedure for synthesis of imidomethylester hydrochlorides (**4**, **8–18**)-**f***

Ten millimoles of the nitrile were dissolved in twice the volume of abs methanol and 20 mmol thionylchloride were added dropwise. After 3 d at 4°C 10 ml hexane were added and the mixture was evaporated to dryness. The residue was taken up in hexanes, filtered and washed with hexanes. The crude imidomethylester hydrochlorides were used for the next step without further purification; yield 15–62%.

*General procedure for synthesis of histamines with substituents in the 2-position of the imidazole (**4**, **8–18**)-**h***

Ten millimoles of cycloalkylalkylcarboxylic acid imidomethylester hydrochloride (**4**, **8–18**)-**f** and 10 mmol 1-acetoxy-4*N*-phthalimidobutan-2-one **19** were added to 50 ml liquid ammonia and the mixture was stirred at ambient temperature in an autoclave for 18 h. Thereafter, stirring was continued for 6 h at 60°C (pressure rising to 25 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 made strongly basic

Table II. Analytical data of 2-substituted histamines.

Com-pound	Formula (Molecular weight)	Yield [%] (mp [°C])	Mass spectra (FAB^+) m/e	IR Spectra (in KBr)	^{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
4-i	$C_{22}H_{22}N_3O_8$ (461.453)	25 (131)	230	3414 m, 3018 s, 2922 s, 1578 vs, 1494 vs, 1382 s, 1363 s, 1191 m, 1080m, 1012 m, 862 s	7.90 bs, 2 H; 7.31 m, 3 H; 7.22 m, 3 H; 6.07 s, 4 H (Mal); 3.11 t, J = 7.4 Hz, 2 H, CH_2-1; 2.88, 2.86 each: t, J = 8.2 Hz, 2 H (et); 2.65 t, J = 7.5 Hz, 2 H, CH_2-3; 2.01 qui, J = 7.4 Hz, 2 H, CH_2-2	167.2 (Mal), 147.1 (Ph), 140.8 (Ph), 135.6 (Mal), 128.8 (Ph), 128.3 (Im), 128.3(Ph), 126.0 (Ph), 115.9 (Im), 37.4, 34.2, 289.3, 25.1, 22.7
8-i	$C_{22}H_{33}N_3O_8$ x 1/2 H_2O (467,458)	8 (144)	236	3416 w, 3030 s, 2918 vs, 2847 s, 170 m, 1644 s, 1572 vs, 1483 vs, 1383 s, 1358 s, 1193 m, 1091 m, 989 m, 864 s, 704 m	7.9 bs, 1 H, N-H (Im); 7.31 s, 1 H, H-C-5 (Im); 6.05 s, 4 H, (Mal); 3.09 t, 2 H, 2.86 t, 2 H, A_2X_2-system, J = 7.2 Hz, (et); 2.78 t, J = 7.8 Hz, 2 H, H_2C-1 propylen; 1.66 m, 7 H; 1.18 m, 6 H; 0.85 m, 2 H	167.2 (Mal), 147.4 C-2 (Im), 135.6 (Mal), 138.9 C-4 (Im), 115.3 C-5 (Im), 37.4, 36.4, 36.0, 32.5, 26.0, 25.6, 25.6, 24.1, 22.7
9-i	$C_{23}H_{35}N_3O_8$ (481,533)	27 (153)	250	3416 s, 2916 vs, 2847 s, 1567 vs, 1498 s, 1383 m, 1364 m, 1192 w, 1005 w, 863 m, 712 w	7.95 bs, 1 H, N-H; 7.33 s, 1 H, H-C-5 (Im); 6.07 s, 4 H (Mal); 3.11 t, J = 7.4 Hz, 2 H (et); 2.90 t, J = 7.4 Hz, 2 H (et); 2.83 t, J = 7.7 Hz, 2 H, CH_2-1 (bu); 1.65 m, 7 H; 1.33 m, 2 H; 1.17 m, 6 H; 0.85 m, 2 H	167.2 (Mal), 147.5 (Im), 135.6 (Mal), 128.6 (Im), 115.9 (Im), 37.4, 36.7, 36.2, 32.7 2C, 27.0, 26.1, 25.8 2C, 25.5, 25.4, 25.3, 22.7
10-i	$C_{19}H_{27}N_3O_8$ (425,40)	4 (150)	194	3417 s, 2949 vs, 1574 vs, 1497 vs, 1384 vs, 1363 vs, 1190 s, 1085 s, 991 s, 863 s, 797 w, 709 m	7.9 bs 2 H; 7.32 s, 1H, H-C-4 (Im); 6.05 s, 4 H (Mal); 3.40 bs, 4 H; 7.09, 2.88 each: t, J = 7.4 Hz, 2 H (et);2.80 d, J = 7.6 Hz, 2 H (methylen); 2.20 sept., J = 7.6 Hz, 1 H, C-H-1 (cyclopentyl); 1.60 m, 6 H; 1.19 m, 2 H	167.3 (Mal); 146.9 C-2 (Im); 135.7 (Mal); 128.5 C-4 (Im); 116.0 C-5 (Im); 38.1, 37.3 (et); 31.5 2 C, 30.9, 24.3 2 C, 22.6
11-i	$C_{21}H_{31}N_3O_8$ (453,479)	33 (138)	222	3412 s, 2939 s, 1706 m, 1622 s, 1574 vs, 1490 s, 1363 s, 1191 m, 864 s,	8.05 vbs, 1 H; 7.36 s, 1H, H-C-5 (Im); 6.08 s, 4 H, (Mal); 3.14, 2.92 each: t, J = 7.3 Hz, 2 H (et); 2.85 t, J = 7.7 Hz, 2 H, 1.70 m, 5 H, 1.55 m, 4 H; 1.30 m, 2 H, 1.06 m, 2 H	167.3 (Mal); 147.4 (C-2 Im); 135.6 (Mal); 128.4 (C-4 Im), 115.9 (C-5 Im), 37.3, 34.6, 32.0 (2C), 26.0, 25.4, 24.6 (2C), 22.6
12-i	$C_{20}H_{27}N_3O_8$ (437.436)	2 (165)	206	3440 m, 3150 m, 3050 m, 2950 vs, 2890 s, 2750 m, 1750 w, 1590 vs, 1445 vs, 1397 vs, 1384 vs, 1200 m, 1100 m, 1000 m, 875 s	7.95 bs, 2 H A,B; 7.4 1 H A; 7.33 1 H, B , H-C-4 (Im); 6.05 s, 4 H Mal.; 3.4 m, 1 H A, H-C-2 (Nb); 3.18.m + 2.92 m, 2 H A + 2 H B each, (et); 3.01 m, 1 H B, H-C-2 (Nb); 2.61 s, 1 H A; 2.47 s, 1 H B, 2.38 m, 1 H A,B. 1.99 tt, J = 12 Hz, J´= 2 Hz, 1 H A; 1.80 m, 1 H A,B; 1.58 - 1.10 m, 5 H A, 7 H B; 0.92 m, 1 H A	167.8 (Mal); 151.3, 149.6 (C-2 Im); 136.2 (Mal); 131.8, 131.7 (C-4, Im); 118.8, 118.7 (C-5, Im); 42.0, 41.4, 39.3, 38.3, 37.7, 37.6, 36.5, 36.2, 35.9, 35.3, 32.0, 29.0, 28.9, 28.2, 23.8, 23.0, 22.9
13-i	$C_{22}H_{31}N_3O_8$ (465.49)	14 (141)	234	3415 w, 2943 vs, 2867 s, 2735 m, 1645 s, 1572 vs, 1483 vs, 1469 vs, 1383 s, 1357 vs, 1195 s, 1091 s, 1000 s, 863 s, 718 s	7.80 bs; 7.30 s, 1 H, H-C-5 (Im); 6.05 s, 4 H (Mal); 3.40 bs; 3.09 t, J= 7.3 Hz, 2 H, (et); 2.87 t, J= 7.3 Hz, 2 H, (et); 2.78 t, J= 7.8 Hz, 2 H; 2.19 s, 1 H; 1.96 s, 1 H; 1.65 m, 1 H, H-C-2 (Nb); 1.45 - 1,27 m, 6 H; 1.09 - 1.02 m, 4 H	167.2 (Mal); 147.5 C-2 (Im), 135.6 (Mal), 128.7, 115.8, 40.9, 40.1, 37.4, 37.2, 35.9, 34.8, 33.7, 29.4, 28.2, 23.9, 22.7
14-i	$C_{24}H_{35}N_3O_8$ x 1/4 H_2O (498.084)	41 (152)	262	3419 m, 2941vs, 2864 s, 1700 m, 1619 s, 1575 vs, 1483 vs, 1358 s, 1208 m, 1091 m, 986 m, 865 s, 699 m	7.95 bs, 1 H, N-H (Im); 7.37 s, 1 H, H-C-5 (Im); 6.08 s, 4 H (Mal); 3.12 t, J = 7.2 Hz, 2 H (et); 2.92 t, J = 7.1 Hz, 2 H (et); 2.84 t, J = 7.6 Hz, 2 H, H_2C-1 (bu); 2.15 s, 1 H; 1.91 s, 1 H; 1.67 m, 2 H, H_2C-2 (bu); 1.50 - 0.90 m, 13 H	167.2 (Mal); 147.5 C-2 (Im); 135.5 (Mal); 128.4 C-4 (Im), 116.0 C-4 Im; 41.4, 37.7, 37.3, 35.9 (2C), 35.6, 34.8, 29.6, 28.3, 26.8, 26.5, 25.3, 22.6
15-i	$C_{23}H_{35}N_3O_8$ (481.533)	24 (131)	250	3416 m, 2919 vs, 2847 s, 1573 vs, 1483 vs, 1383 vs, 1360 vs, 1195 m, 1090 m, 991 m, 865 s, 797 w, 698 m	7.9 bs, 1 H, N-H; 7.34 s, 1H, H-C-4 (Im); 6.06 s, 4 H (Mal); 3.3 bs, 2 H, NH_2; 3.11, 2.89 each: t, J = 7,4 Hz, 2 H, (et); 3.01 sext., J = 7,1 Hz, 1H, H-C-2 (bu); 1.65 m, 7 H; 1.28 d, J = 7 Hz, 3 H, CH_3; 1.14 m, 5 H; 1.02 m, 1 H; 0.83 m, 3 H	167.2 (Mal), 151.4 C-2 (Im), 135.6 (Mal), 128.8 C-4 (Im), 115.7 C-5 (Im), 37.4, 36.7 CH, 34.0, 32.6 (2 C), 32.1, 31.8 CH, 26.0, 25.7 (2 C), 22.8, 18.3 CH_3
16-i	$C_{23}H_{35}N_3O_8$ x 1/2 H_2O (490,541)	31 (126)	250	3416 m, 2919 vs, 2848 s, 1575 vs, 1482 vs, 1356 s, 1198 m, 1092 m, 989 m, 865 s, 700 m	7.9 bs 1 H; 7.39 s, 1 H, H-C-5 (Im); 6.09 s, 4 H (Mal); 3.12, 2.92 each t, J = 7 Hz, 2 H (et); 2.81 dd, ^{2}J = 16 Hz, ^{3}J = 5.5 Hz, 1 H, H-CH-1 (propyl); 2.61 dd, ^{2}J = 16 Hz, ^{3}J = 9 Hz, 1 H, HC-H-1 (propyl); 2.02 m, 1 H, H-C-2 (propyl); 1.67 m, 5 H; 1.18 m, 6 H; 0.84 m + d, J_d = 6.3 Hz, 5 H	167.0 (Mal); 146.4 C-2 (Im); 135.5 (Mal); 129.1 C-4 (Im); 115.7 C-5 (Im); 43.8, 37.2, 33.9, 33.0, 32.9, 32.2, 29.0, 25.9, 25.5, 25.4, 22.6, 19.1
17-i	$C_{24}H_{37}N_3O_8$ (495.56)	27 (142)	264	3412 m, 2915 vs, 2847 s, 1576 vs, 1571 vs, 1749 s, 1506 vs, 1501 vs, 1383 s, 1363 s, 1191 m, 1092 m, 1002 m, 873 s, 862 s	7.94 bs, 1 H; 7.37 s, 1 H, H-C-4 (Im); 6.07 s, 4 H, H-C-2,3 (Mal); 3.13, 2.91 each: t, 2 H, J = 7.3 Hz, (et); 3.08 m, 1 H, H-C-2 (pentyl); 1.61 m, 7 H; 1.39 d, J = 7.0 Hz, 3 H, CH_3; 1.13 m, 8 H; 0.83 m, 2 H	167.2 (Mal); 151.2 (C-2, Im); 135.6 (Mal), 128.8 (C-4 Im), 116.0 (C-5 Im), 37.3, 36.6, 36.3, 34.9, 32.7, 32.6, 31.5, 26.0, 25.7 (2 C), 23.5, 22.6; 18.2 (CH_3)
18-i	$C_{24}H_{32}N_3O_8$ (495.560)	31 (160)	264	3416 w, 2915 vs, 2847 s, 1701 m, 1617 s, 1573 vs, 1483 vs, 1468 vs, 1384 s, 1351 s, 1209 m, 1193 m, 1081 m, 863 s	7.85 bs, 1 H; 7.32 s, 1 H, H-C-5 (Im), 6.04 s, 4 H (Mal); 3.09 t, J = 7.5 Hz, 2 H, 2.86 t, J = 7.4 Hz, 2 H, (et); 2.80 dd, ^{3}J = 4 Hz, ^{2}J = 14.5 Hz, 1 H, H-CH-1 2.63 t, ^{3}J = 8.3 Hz, ^{2}J = 14.6 Hz, 1H, HC-H-1; 1.86 m, 1 H, 1.64 m, 5 H; 1.19 m, 8 H; 0.83 m, 5 H	167.2 (Mal), 146.5 (Im), 135.6 (Mal), 128.9 (Im), 115.8 (Im), 37.4, 37.0, 33.8, 33.0, 32.9, 32.8, 32.6, 32.4, 26.1, 25.7, 22.8, 19.0

Abbreviations used in the table: Im: imidazole, Mal: maleic acid, et: ethylene ($ImCH_2CH_2NH_2$), Nb: norbornyl, bu: butyl, Ph: phenyl.

with an excess of a concentrated solution of sodium hydroxide. The substituted histamine (**4, 8–18**)-**h** was extracted with 12 portions of 40 ml each of a mixture of chloroform and 2-propanol (4:1). The combined organic layers were washed twice with brine containing some sodium hydroxide.

The solvent was evaporated and the residue purified by column chromatography on silica gel using chloroform/methanol/triethylamine (25:10:1) as an eluent. The free histamine (**4, 8–18**)-**h** was dissolved in abs ethanol, and a concentrated solution of 2.5 mol equivalents 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 (**4, 8–18**)-**i** crystallized upon standing and were recrystallized twice from ethanol by adding ether; yield 2–41%. Synthesis and analytical data of compounds **1-h**, **2-h**, **3-h**, **5-h**, **6-h** and **7-h** have been reported in the literature [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 [14] in the continuous presence of 0.1 μM atropine. Organs were allowed to stabilize for 80 min, during which time they were stimulated 3 times with 1 μM histamine. Each preparation was used to obtain a cumulative curve with histamine first (0.01–10 μM) and 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, the antagonist added (0.03–0.1 mM) and after 15 min a second curve with increasing concentration of histamine [15].

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 [16].

Purification and reconstitution of G_i/G_o-proteins

A mixture of heterotrimeric G_i/G_o-proteins was purified from bovine brain membranes as described [16]. The preparation of G_i/G_o-proteins (purity > 90%) contained predominantly G_{o1}, significant amounts of G_{o2} and another as yet unidentified G_o-subtype ('G_{o3}') as well as G_{i1} and G_{i2} and traces of G_{i3}. G-proteins (25–30 pmol) were reconstituted into azolectine-containing phospholipid vesicles by chromatography onto a G50 column. A detailed protocol of the reconstitution procedure will be published elsewhere. Association of G_i/G_o-proteins to liposomes was confirmed by [^{35}S]guanosine 5'-*O*-[3-thio]triphosphate binding [17]. Pooled fractions were then used for measurement of GTP hydrolysis.

GTPase assay

GTP hydrolysis was determined as described [5]. For determination of GTP hydrolysis in membranes, reaction mixtures (100 μl) contained 3.0–7.0 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 15 min at 25°C. For determination of the GTPase activity of reconstituted G-proteins, reaction mixtures (100 μl) contained 0.4–0.6 pmol of G_i/G_o-proteins, 50 nM [γ-^{32}P]GTP, 1.0 mM $MgCl_2$, 0.8 mM EDTA and 30 mM NaCl. The other assay conditions were as described above.

Miscellaneous

Protein was determined according to Lowry *et al* [18]. [γ-^{32}P]GTP was prepared as described previously [19].

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