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Synthesis, SAR and selectivity of 2-acyl- and 2-cyano-1-hetarylalkyl-guanidines at the four histamine receptor subtypes: a bioisosteric approach†

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In the search for potential bioisosteres of the 4-imidazolyl ring in acylguanidines (*e.g.* UR-AK24), known to possess affinity to several histamine receptor subtypes (H_xR, $x = 1–4$), and cyanoguanidine-type H_4R agonists (*e.g.* UR-PI376), the contribution of various heterocycles to agonism, antagonism and HR subtype selectivity was studied (recombinant human $H_{1,2,3,4}Rs$, isolated guinea pig organs (H_1R, H_2R)). While minor structural modifications of UR-PI376 analogues were not tolerated regarding H_4R agonism, in the case of the acylguanidines, a 1,2,4-triazole ring shifted the selectivity toward the H_2R.

Introduction

The physiological and pathophysiological effects of the biogenic amine histamine are mediated through four receptor subtypes, referred to as H_1, H_2, H_3 and H_4 receptors (H_1R, H_2R, H_3R, and H_4R), all belonging to class A of G protein coupled receptors.[1–4] H_1R and H_2R antagonists have been used for decades in the treatment of allergic conditions and as antiulcer drugs, respectively. The H_3R is mainly expressed in the brain and is considered a promising drug target, for instance, for the treatment of attention-deficit hyperactivity disorder, Alzheimer's disease, Parkinson's disease, sleep disorders or obesity.[5] The H_4R was discovered by several groups based on its high sequence homology with the H_3R.[6–12] The expression on hematopoietic cells such as mast cells, basophils, eosinophils, T-cells and dendritic cells suggests a role of the H_4R in the regulation of immune responses and inflammation.[13–15] Therefore, the H_4R is considered a potential drug target for the treatment of diseases like allergic rhinitis, rheumatoid arthritis, bronchial asthma and pruritus.[16–18] Recent reports on β-arrestin-mediated signalling[19–21] of H_4R ligands and partial agonistic effects of the standard H_4R antagonist JNJ-7777120 (ref. 22) at certain H_4R species orthologs suggest that the interpretation of ligand effects *in vivo* in terms of agonism or antagonism should be interpreted with caution.[19,23] Hence, both selective antagonists and agonists are required as pharmacological tools to further explore the role of the H_4R.[15,24]

Guanidine-type compounds like arpromidine and analogues represent highly potent H_2R agonists.[25,26] Drawbacks due to the strongly basic guanidine, *e.g.* very low oral bioavailability and lack of penetration across the blood brain barrier, were eliminated by replacing the guanidine group with a considerably less basic acylguanidine moiety (Fig. 1, UR-AK24 (**1**)).[27–29] However, these N^G-acylated imidazolylpropylguanidines developed as H_2R agonists lacked selectivity toward the hH_3R and hH_4R.[30] By analogy with the H_2R agonist amthamine (**2**),[31] the replacement

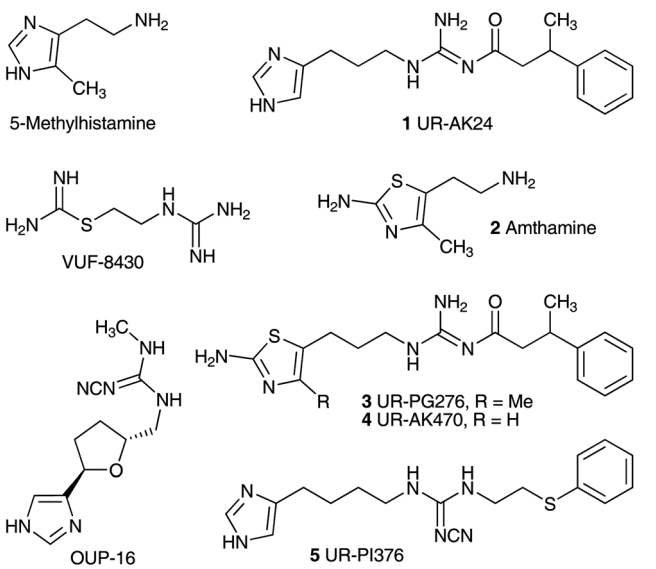

Fig. 1 Structures of the selective H_4R agonists 5-methylhistamine, VUF8430 and OUP-16, the N^G-acylated imidazolylpropylguanidine UR-AK24 (**1**), which is active at the H_2R, H_3R and H_4R, the H_2R agonist amthamine (**2**), related potent and selective acylguanidine-type H_2R agonists **3** and **4** and the potent and selective H_4R agonist UR-PI376 (**5**).

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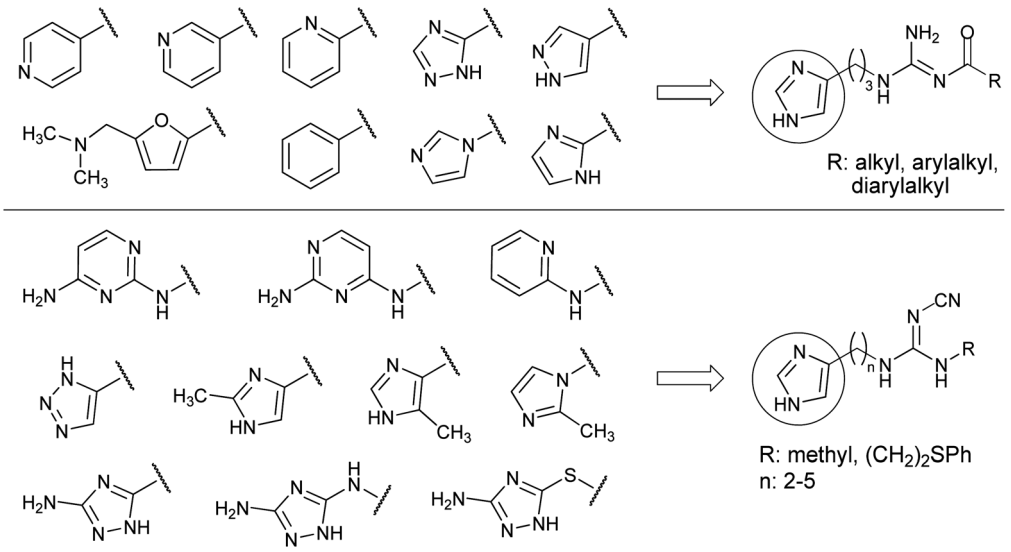


Fig. 2 Imidazole replacement in acylguanidine-type non-selective H_2R agonists and in cyanoguanidine-type H_4R agonists. Overview of the introduced aromatic rings.

of the imidazole ring in acylguanidine-type ligands by a 2-amino-thiazole ring, resulted in selective H_2R agonists (**3** and **4**).[27,32,33] In contrast potent selective agonists for the hH_4R were obtained by replacing the basic acylguanidine group with a cyanoguanidine moiety as in UR-PI376 (**5**).[34] The highest potency resided in imidazolylalkylcyanoguanidines with a tetramethylene linker, connecting the imidazole and the cyanoguanidine moiety. Unlike hH_4R agonists such as 5-methylhistamine,[35] VUF-8430 (ref. 36) or OUP-16 (ref. 37) compound **5** is devoid of agonistic activities at hHR subtypes other than the hH_4R.[34]

The previous results suggest that the bioisosteric approach harbours the potential of further increasing the preference or the selectivity of hetarylalkylguanidines for a certain HR subtype. In the present work various heterocycles were introduced to replace the 1*H*-imidazol-4-yl ring (Fig. 2). Special attention was paid to substructures known from early studies on hetaryl analogues of histamine.[38,39] These structural modifications were combined with an acylguanidine or a cyanoguanidine moiety as less basic or non-basic guanidine replacements.

Results and discussion

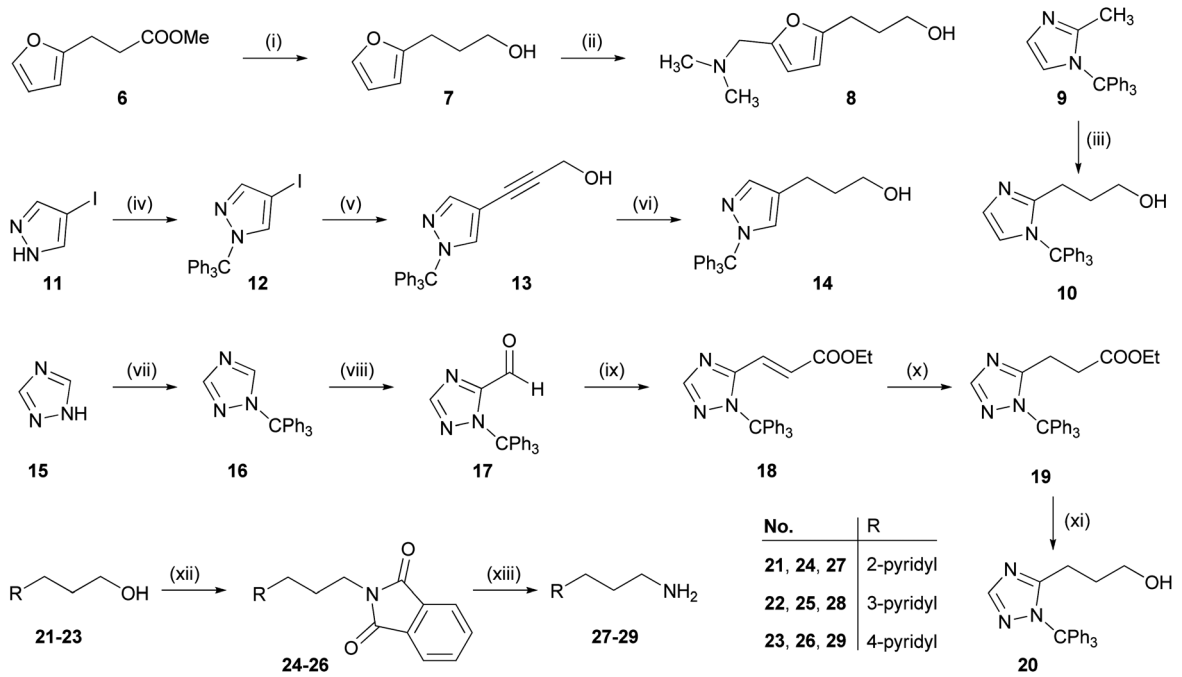
Chemistry

Synthesis of the acylguanidines. The amines and alcohols required for the preparation of the arylpropylguanidines **44–52** were synthesized as depicted in Scheme 1. Reduction of **6** with $LiAlH_4$ followed by aminomethylation in a Mannich reaction[40] gave the furan analog **8**. The imidazolylpropanol **10** was prepared by deprotonation of the methyl group in **9** with *n*-BuLi in THF and treatment with oxirane as an electrophile.[41] Introduction of a three-membered carbon chain to the pyrazole core was performed by C–C coupling of the trityl-protected iodopyrazole **11** with propargyl alcohol under Sonogashira conditions[42] using $Pd(PPh_3)_2Cl_2$ and CuI as catalysts. Hydrogenation over Pd/C (10%) provided the pyrazolylpropanol **14**. The triazolylpropanol **20** was obtained in five steps starting from 1*H*-1,2,4-triazole (**15**). After trityl-protection of **15**,[43,44] **16** was treated with *n*-BuLi and DMF in THF to afford the aldehyde **17**.[45] Elongation of the side chain by two carbon atoms was carried out *via* the Horner–Wadsworth–Emmons reaction employing triethyl phosphonoacetate.[46] Subsequent hydrogenation of the C=C double bond and reduction of the ethyl ester yielded **20**. Conversion of the pyridylpropanols **21–23** to the corresponding phthalimides **24–26** under Mitsunobu conditions[47] followed by hydrazinolysis gave the pyridylpropylamines **27–29**.[48]

The arylpropylguanidines **44–52** were synthesized starting from the corresponding arylpropyl alcohols **8**, **10**, **14** and **20** or arylpropylamines **27–31** (Scheme 2). Conversion of the alcohol to the di-Cbz-protected guanidines **35–38** was accomplished under Mitsunobu conditions[47] by analogy with the procedure described by Feichtinger *et al.*[49] The arylpropylamines **27–31** were treated with the triflyl-di-Cbz-protected guanidine **34** (ref. 49) to give the di-Cbz protected arylpropylguanidines **39–43**. Finally, the arylpropylguanidines **44–52** were obtained by hydrogenolytic cleavage of the Cbz groups. The N^G-acylated arylpropylguanidines were prepared as outlined in Scheme 2. Coupling of the CDI-activated carboxylic acids[50,51] **53–56** to the arylpropylguanidines **44–52** gave the acylguanidines **57–78**.[27] Tritylated heterocycles were deprotected under acidic conditions yielding **79–86**.

Synthesis of the cyanoguanidines. The amines **92–106** required for the preparation of the cyanoguanidines **107–133** were synthesized as recently reported.[52] The synthesis of **107–133** was accomplished by analogy with a previously described procedure (Scheme 3).[34] Diphenyl cyanocarbonimidate (**87**)[53] and the primary amines **88–89** gave the isourea intermediates **90–91**, which were treated with **92–106** in acetonitrile in a microwave oven to yield **107–133**.[54]

Pharmacological results and discussion. The acylguanidines **57–86** were investigated for histamine receptor agonism or



6 **7** **8** **9** **10** **11** **12** **13** **14** **15** **16** **17** **18** **19** **20** **21-23** **24-26** **27-29**

Scheme 1 Synthesis of the arylpropyl alcohols **8**, **10**, **14** and **20**, and the pyridylpropylamines **27–29**. *Reagents and conditions*: (i) $LiAlH_4$ (2 eq.), Et_2O, overnight, 0 °C → rt; (ii) $NH(CH_3)_2 \cdot HCl$ (1.6 eq.), $(CH_2O)_n$ (1.6 eq.), EtOH, overnight, reflux; (iii) oxirane (5 eq.), *n*-BuLi (1.1 eq.), THF, overnight, −78 °C → rt; (iv) TrCl (1 eq.), NEt_3 (1.2 eq.), DCM, 12 h, 0 °C → rt; (v) $Pd(PPh_3)_2Cl_2$ (0.03 eq.), CuI (0.05 eq.), DIPA (4.5 eq.), propargyl alcohol (1.1 eq.), DMF, 48 h, −15 °C → rt; (vi) H_2, Pd/C (10%) (cat.), MeOH, overnight, rt; (vii) TrCl (1 eq.), NEt_3 (1 eq.), DCM, overnight, rt; (viii) TMEDA (1 eq.), *n*-BuLi (1.1 eq.), DMF (0.9 eq.), THF, 12 h, −78 °C; (ix) triethyl phosphonoacetate (1.2 eq.), NaH (60% dispersion in mineral oil) (1.2 eq.), THF, overnight, rt; (x) H_2, Pd/C (10%) (cat.), EtOH/THF, overnight, rt; (xi) $LiAlH_4$ (2 eq.), THF, 2 h, 0 °C → reflux; (xii) phthalimide (1.1 eq.), PPh_3(1.1 eq.), DIAD (1.1 eq.), THF, overnight, 0 °C → rt. (xiii) $N_2H_4 \cdot H_2O$ (6 eq.), EtOH, overnight, rt.

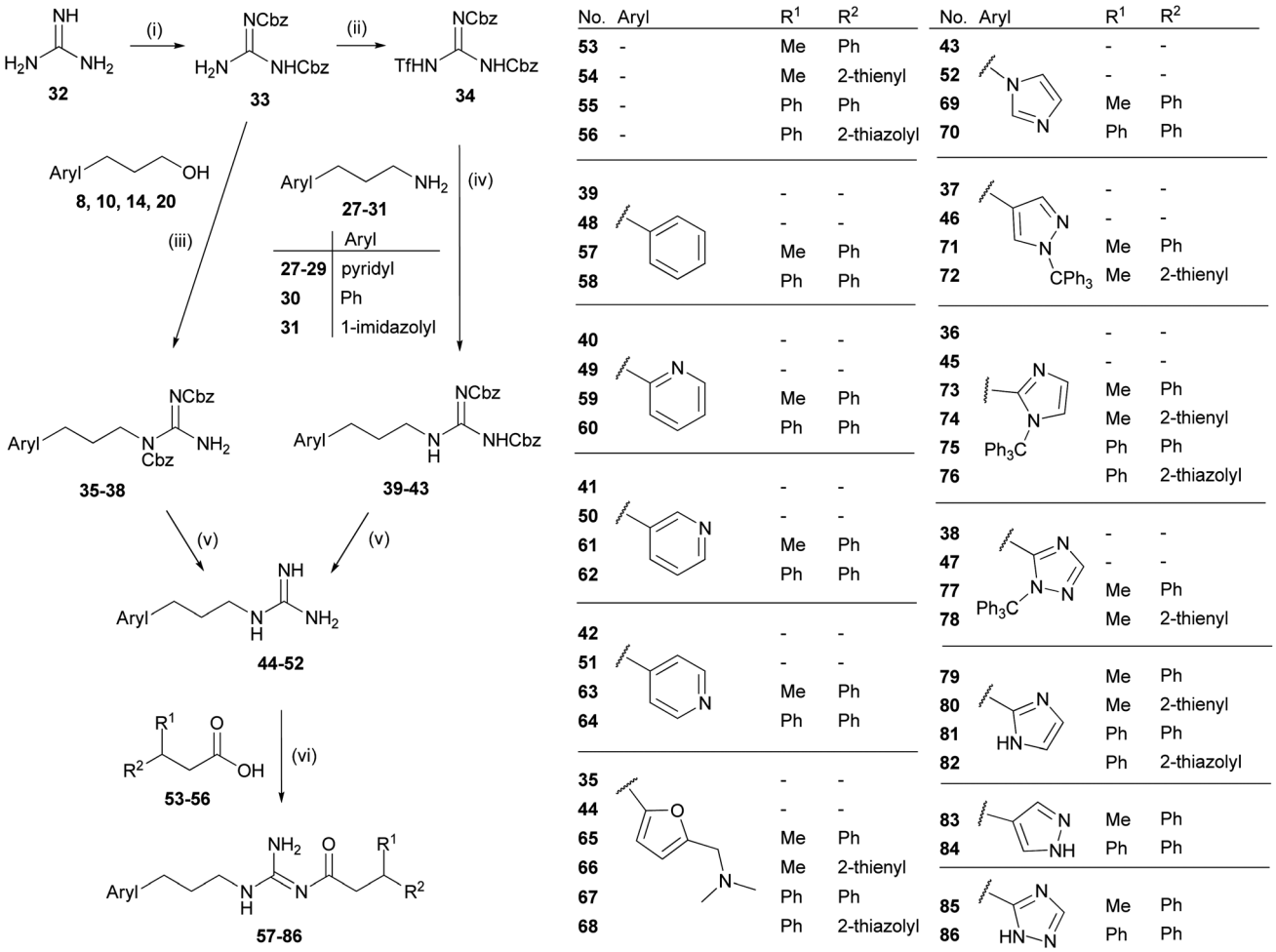
antagonism in steady-state GTPase assays using [^{32}P] or [^{33}P] radiolabeled GTP. These experiments were performed using membrane preparations of Sf9 insect cells expressing the following proteins: hH_1R plus regulator of G protein signalling 4 (RGS4), hH_2R-Gsα_S fusion protein, hH_3R plus Gα_{i2} plus G$\beta_1\gamma_2$ plus RGS4 or hH_4R-RGS19 fusion protein plus Gα_{i2} plus G$\beta_1\gamma_2$ (Table 1).[27,55,56] Selected compounds were additionally investigated for H_1R and H_2R activity at the guinea pig (gp) ileum and the spontaneously beating guinea pig right atrium, respectively (Table 2). The cyanoguanidines **107–133** were investigated at the hH_1R as described above and at the other three HR subtypes in [^{35}S]GTPγS binding assays using membrane preparations of Sf9 cell expressing the hH_2R-Gsα_S fusion protein, the hH_3R plus Gα_{i2} plus G$\beta_1\gamma_2$ or the hH_4R plus Gα_{i2} plus G$\beta_1\gamma_2$ (Table 3).[57,58] In the following agonistic potencies are expressed as pEC_{50} ($-\log EC_{50}$) values. Intrinsic activities (α) refer to the maximal response induced by the standard agonist histamine. Compounds identified to be inactive as agonists ($\alpha < 0.1$ or negative values, respectively, determined in the agonist mode) were investigated in the antagonist mode. The pK_B values of neutral antagonists and inverse agonists were determined from the concentration-dependent inhibition of the histamine-induced increase in [^{35}S]GTPγS binding or [γ^{32}P]GTP ([γ^{33}P]-GTP) hydrolysis, respectively.

Acylguanidines 57–86 (Tables 1 and 2). When the imidazole ring in acylguanidines such as **1** was replaced by a phenyl ring (**57** and **58**), the agonistic potencies at the $hH_{2,3,4}Rs$ dramatically decreased. However, in terms of antagonism at the hH_2R, these compounds ($pK_B = 5.89$) were comparable to the H_2R antagonists cimetidine or ranitidine.[59]

Replacing the imidazole ring in **1** with a 2-pyridyl ring resulted in an hH_2R partial agonist (**59**) with a potency comparable to that of the endogenous ligand histamine ($pEC_{50} = 6.08$, $E_{max} = 0.30$). At the hH_1R, this compound also behaved as a weak partial agonist. This is in agreement with data for the 2-pyridyl analogue of histamine, betahistine, which is a weak agonist at the hH_1R and hH_2R.[56,59] The bulky diphenylpropanoyl residue in **60** was not tolerated in terms of agonistic potency. At the hH_3R and hH_4R, **59** and **60** were almost inactive. The 3- and 4-pyridyl analogues **61–64** displayed moderate antagonism at the hH_2R and negligible activities at the other HR subtypes.

Replacement of the imidazole ring by the 5-[(dimethylamino)-methyl]furan-2-yl group, reminiscent of the H_2R antagonist ranitidine, afforded hH_2R antagonists (**65–68**): all prepared compounds turned out to be superior to ranitidine ($pK_B \sim 6.10$)[59] at the hH_2R, with the highest antagonist activity exhibited by the diphenylpropanoylguanidine **67** ($pK_B = 7.03$). **65–68** were weak inverse agonists at the hH_3R and almost inactive at the hH_1R and hH_4R.

Apart from other heterocycles, isomers of the 1*H*-imidazol-4-yl ring were investigated. The 1*H*-imidazol-1-yl isomer (**69**) was comparable with UR-AK24 (**1**) regarding intrinsic activity (E_{max}: 0.77 *vs.* 0.84) at the hH_4R, but the potency was 65-fold lower



No.	Aryl	R^1	R^2
53	-	Me	Ph
54	-	Me	2-thienyl
55	-	Ph	Ph
56	-	Ph	2-thiazolyl
39		-	-
48		-	-
57		Me	Ph
58		Ph	Ph
40		-	-
49		-	-
59		Me	Ph
60		Ph	Ph
41		-	-
50		-	-
61		Me	Ph
62		Ph	Ph
42		-	-
51		-	-
63		Me	Ph
64		Ph	Ph
35		-	-
44		-	-
65		Me	Ph
66		Me	2-thienyl
67		Ph	Ph
68		Ph	2-thiazolyl

No.	Aryl	R^1	R^2
43		-	-
52		-	-
69		Me	Ph
70		Ph	Ph
37		-	-
46		-	-
71		Me	Ph
72		Me	2-thienyl
36		-	-
45		-	-
73		Me	Ph
74		Me	2-thienyl
75		Ph	Ph
76		Ph	2-thiazolyl
38		-	-
47		-	-
77		Me	Ph
78		Me	2-thienyl
79		Me	Ph
80		Me	2-thienyl
81		Ph	Ph
82		Ph	2-thiazolyl
83		Me	Ph
84		Ph	Ph
85		Me	Ph
86		Ph	Ph

Scheme 2 Synthesis of the N^G-acylated arylpropylguanidines **57–86**. *Reagents and conditions*: (i) benzyl chloroformate (3 eq.), NaOH (5 eq.), H_2O/DCM, 20 h, 0 °C; (ii) Tf_2O (1 eq.), NaH (60% dispersion in mineral oil) (2 eq.), chlorobenzene, overnight, −45 °C → rt; (iii) PPh_3 (1.5 eq.), DIAD (1.5 eq.), THF, overnight, 0 °C → rt; (iv) NEt_3 (1 eq.), DCM, overnight, rt; (v) H_2, Pd/C (10%) (cat.), MeOH, 3 h, rt; (vi) (a) CDI (1.2 eq.), NaH (60% dispersion in mineral oil) (2 eq.), THF, 5 h, rt; (b) for trityl-protected intermediates **71–78**: TFA (20%), DCM, 5 h, rt.

(pEC_{50}: 6 *vs.* 7.82). The activities (**69**) at the other HRs were negligible ($pK_B < 5$). However, the results for **69** suggest that, in contrast to other HR subtypes, an imidazole–NH group is obviously dispensible, though it is crucial to obtain highly potent hH_4R agonists. As obvious from the diphenylpropanoyl analogue **70**, which is almost inactive at the hH_4R, the hH_4R agonism strongly depends on the constitution of the acyl residue.

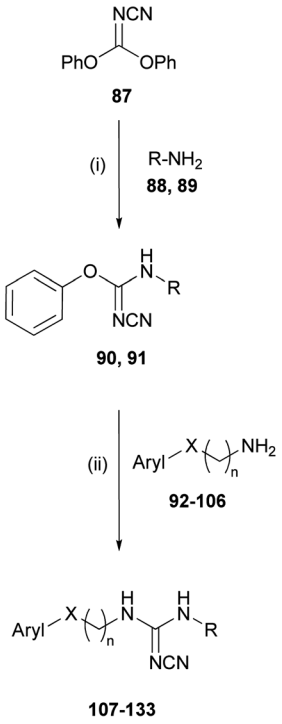
For the 1*H*-imidazol-2-yl isomers **79–82** similar pharmacological activities at the hH_4R were observed as for the 1*H*-imidazol-1-yl isomers **69** and **70**. Both 3-arylbutanoylguanidines (**79** and **80**) exhibited moderate partial agonistic potencies and low intrinsic activities. Similar to the isomer **70**, diarylpropanoyl residues (**81** and **82**) abolished agonism at the hH_4R. At the other HR subtypes, **79–82** were very weak antagonists or inverse agonists.

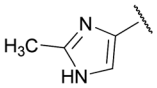
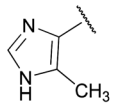
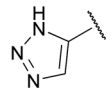
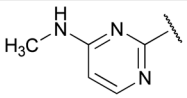
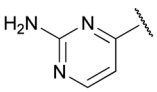
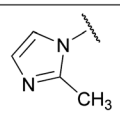
The results for the imidazole isomers suggest that, regarding agonism and compared to the other HR subtypes, the hH_4R tolerates some modifications in the arrangement of side chains and nitrogen atoms in the heterocycle.

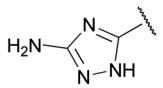
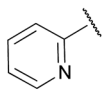
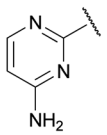
Exchange of the imidazole ring in UR-AK24 by a 1*H*-pyrazol-4-yl ring (**83**) resulted in a moderate decrease in potency and efficacy at the hH_2R ($pEC_{50} = 6.62$, $E_{max} = 0.44$). Interestingly, this compound was virtually inactive at all other HRs, suggesting the pyrazole ring to be an appropriate bioisostere of the imidazole ring to shift the receptor subtype selectivity toward the hH_2R. However, the bulky diphenylpropanoyl residue in **84** was deleterious for agonistic activity at the hH_2R.

Compared to the pyrazole **83**, the 1*H*-1,2,4-triazol-3-yl analogue **85** was more efficacious, but slightly less potent at the hH_2R ($pEC_{50} = 6.13$, $E_{max} = 0.66$). In contrast to the pyrazole **84**, the triazole analog **86** with a diphenylpropanoyl moiety was an hH_2R partial agonist with slightly higher potency than **85** ($pEC_{50} = 6.39$, $E_{max} = 0.42$). This suggests that the acylguanidines containing a triazole or a pyrazole ring can adopt different binding modes at the hH_2R. Like the pyrazoles **83** and **84**, the triazoles **85** and **86** were almost inactive at the other HRs.

The basicity of pyrazole ($pK_a \approx 3$)[60] and triazole ($pK_a \approx 3$)[60] is considerably lower than that of imidazole ($pK_a \approx 7$).[60] This may



No.	Aryl	n	X	R
88	-	-	-	CH_3
89				$(CH_2)_2$-S-Ph
90				CH_3
91				$(CH_2)_2$-S-Ph
92				-
107		3	CH_2	CH_3
108				$(CH_2)_2$-S-Ph
93				-
109		2	CH_2	CH_3
110				$(CH_2)_2$-S-Ph
94				-
111		3	CH_2	CH_3
112				$(CH_2)_2$-S-Ph
95				-
113		3	CH_2	CH_3
114				$(CH_2)_2$-S-Ph
96		2	NH	-
97		2	NH	-
115				$(CH_2)_2$-S-Ph
98				-
116		4	CH_2	CH_3
117				$(CH_2)_2$-S-Ph

No.	Aryl	n	X	R
99				-
118		3	CH_2	$-CH_3$
119				$-(CH_2)_2$-S-Ph
100				-
120		3	NH	$-CH_3$
121				$-(CH_2)_2$-S-Ph
101				-
122		4	NH	$-CH_3$
123				$-(CH_2)_2$-S-Ph
102				-
124		3	S	$-CH_3$
125				$-(CH_2)_2$-S-Ph
103				-
126		3	NH	$-CH_3$
127				$-(CH_2)_2$-S-Ph
104				-
128		4	NH	$-CH_3$
129				$-(CH_2)_2$-S-Ph
105				-
130		2	NH	$-CH_3$
131				$-(CH_2)_2$-S-Ph
106				-
132		3	NH	$-CH_3$
133				$-(CH_2)_2$-S-Ph

Scheme 3 Synthesis of the cyanoguanidines **107–133**. *Reagents and conditions*: (i) 2-propanol, 1 h, rt; (ii) MeCN, microwave 150 °C, 15 min.

be interpreted as a hint that the presence of a heterocycle, which is positively charged at physiological pH value, is not required for hH_2R activation. Moreover, the modification of the acyl residue in triazolylalkylguanidines obviously harbours the potential of increasing H_2R selectivity.

The results from isolated guinea pig organs were essentially in line with the data gained from recombinant human H_1 and H_2 receptors, but, in general, the guinea pig receptors proved to be more sensitive than the human orthologs. At the guinea pig ileum the investigated N^G-acylated arylpropylguanidines (Table 2) were moderate H_1R antagonists. Like at the hH_2R, introduction of a phenyl (**58**), 3-pyridyl (**61**), 4-pyridyl (**63**), 5-[(dimethylamino)-methyl]furan-2-yl (**65**), 1*H*-imidazol-1-yl (**69**) and 1*H*-imidazol-2-yl moiety (**79**) resulted in a loss of agonistic efficacy at the gpH_2R relative to compound **1** and yielded weak gpH_2R antagonists. Remarkably, the H_2R antagonist activity of compound **65** was comparable to that of cimetidine at the guinea pig right atrium.[27]

In accordance with the results at the hH_2R, the exchange of the 1*H*-imidazol-4-yl ring in UR-AK24 (**1**) by a 2-pyridyl ring (**59**) resulted in a gpH_2R partial agonist with lower potency and efficacy than **1** ($E_{max} = 0.26$). This tendency is reminiscent of the close histamine analogues 2-(2-pyridyl)ethanamine and betahistine, which likewise display weak partial agonism at the guinea pig right atrium.[61] Replacing the 1*H*-imidazol-4-yl by a 1*H*-pyrazol-4-yl ring (**83** and **84**) resulted in compounds with retained gpH_2R partial agonistic activity. However, relative to UR-AK24 (**1**), the potency decreased by about one order of magnitude. In contrast to **83** and **84**, the analogues bearing a 1*H*-1,2,4-triazol-3-yl ring (**85**, **86**) and UR-AK24 were equiefficacious, though 6-fold less potent at the guinea pig right atrium. These findings support the hypothesis that the 1*H*-1,2,4-triazol-3-yl ring is a potential bioisostere of the 1*H*-imidazol-4-yl moiety with respect to gpH_2R affinity.

Cyanoguanidines 107–133 (Table 3). The investigation of the synthesized cyanoguanidines for functional activity at the hH_4R revealed a high sensitivity even towards minor structural modifications and corroborated, in this respect, previous results.[34] All cyanoguanidines bearing heterocycles other than imidazole revealed only negligible partial agonism or antagonism, or were even inactive at all four histamine receptor subtypes. The phenylthioethyl substituted aminopyrimidine derivative **115** was the only compound with inverse agonistic activity in the lower micromolar range ($pK_B \sim 5.5$) at both, the hH_4R and the hH_3R.

Table 1 Potencies and efficacies of the prepared acylguanidines **57–86** at hH_1R, hH_2R, hH_3R and hH_4R in the steady-state GTPase assay[a,b]

	hH_1R		hH_2R		hH_3R		hH_4R	
Compound	pEC_{50} or (pK_B)	*N*	pEC_{50} or (pK_B)	*N*	pEC_{50} or (pK_B)	*N*	pEC_{50} or (pK_B)	*N*
Histamine	6.72 ± 0.02 (ref. 28) *α*: 1.00		5.92 ± 0.11 (ref. 28) *a*: 1.00		7.60 ± 0.05 *a*: 1.00	3	7.92 ± 0.11 *a*: 1.00	8
UR-AK24 (**1**)	(<5)[27] —		7.17 (ref. 29) *α*: 0.87		8.60 ± 0.11 *a*: 0.24 ± 0.02	2	7.82 ± 0.01 *a*: 0.84 ± 0.06	2
Thioperamide	n.d.		n.d.		(7.01 ± 0.08) *a*: −0.71 ± 0.6	5	(6.96 ± 0.06) *a*: −0.95 ± 0.07	6
57	(5.05 ± 0.06) *a*: −0.02 ± 0.05	2	(5.89 ± 0.04) *a*: −0.10 ± 0.00	2	(<5) *a*: −0.19 ± 0.03	2	(<5) n.d.	2
58	(5.26 ± 0.03) *a*: −0.01 ± 0.01	2	(5.89 ± 0.08) *a*: −0.12 ± 0.01	2	(5.10 ± 0.0) *a*: −0.24 ± 0.01	2	(<5) n.d.	2
59	(5.34 ± 0.01) *a*: 0.21 ± 0.03	2	6.08 ± 0.11 *a*: 0.30 ± 0.01	3	(<5) *a*: −0.17 ± 0.01	2	(<5) n.d.	2
60	(5.41 ± 0.02) *a*: 0.10 ± 0.02	2	(5.68 ± 0.01) *a*: −0.00 ± 0.03	2	(5.35 ± 0.04) *a*: −0.44 ± 0.01	2	(<5) n.d.	2
61	(<5) n.d.	2	(5.27 ± 0.0) *a*: −0.11 ± 0.05	2	(<5) *a*: −0.01 ± 0.01	2	(<5) n.d.	2
62	(5.04 ± 0.04) *a*: 0.06 ± 0.04	2	(6.14 ± 0.08) *a*: −0.16 ± 0.05	2	(<5) *a*: −0.14 ± 0.01	2	(<5) n.d.	2
63	(<5) *a*: 0.12 ± 0.06	2	(5.54 ± 0.01) *a*: 0.00 ± 0.09	2	(<5) *a*: −0.07 ± 0.06	2	(<5) n.d.	2
64	(<5) *a*: 0.08 ± 0.07	2	(6.14 ± 0.01) *a*: −0.12 ± 0.06	2	(5.06 ± 0.01) *a*: −0.08 ± 0.07	2	(<5) n.d.	2
65	(<5) *a*: 0.04 ± 0.01	2	(6.52 ± 0.03) *a*: −0.12 ± 0.02	3	(5.92 ± 0.01) *a*: −0.64 ± 0.00	2	(<5) n.d.	2
66	n.d.		(6.51 ± 0.12) *a*: −0.12 ± 0.02	2	(5.77 ± 0.04) *a*: −0.73 ± 0.01	2	(<5) *a*: −0.14 ± 0.05	2
67	(5.11 ± 0.01) *a*: −0.02 ± 0.00	2	(7.03 ± 0.13 *a*: −0.16 ± 0.02	2	(5.89 ± 0.07) *a*: −0.65 ± 0.01	2	(<5) *a*: −0.16 ± 0.09	2
68	n.d.		(6.13 ± 0.12) *a*: −0.13 ± 0.02	2	(5.51 ± 0.03) *a*: −0.62 ± 0.04	2	(<5) n.d.	2
69	(<5) n.d.	2	(<5) n.d.	2	(<5) *a*: −0.05 ± 0.03	2	6.00 ± 0.13 *a*: 0.77 ± 0.01	2
70	(<5) *a*: 0.01 ± 0.01	2	(5.82 ± 0.03) *a*: −0.07 ± 0.02	2	(<5) *a*: −0.07 ± 0.02	2	(<5) n.d.	2
79	(<5) *a*: 0.09 ± 0.06	2	(5.30 ± 0.02) *a*: 0.03 ± 0.03	2	(5.41 ± 0.11) *a*: −0.32 ± 0.02	2	5.54 ± 0.19 *a*: 0.35 ± 0.01	2
80	n.d.		(5.26 ± 0.14) *a*: 0.04 ± 0.00	2	(5.35 ± 0.14) *a*: −0.46 ± 0.02	2	6.11 ± 0.12 *a*: 0.50 ± 0.04	3
81	(5.07 ± 0.09) *a*: 0.04 ± 0.01	2	(6.13 ± 0.12) *a*: −0.04 ± 0.06	2	(5.47 ± 0.04) *a*: −0.33 ± 0.00	2	(5.85 ± 0.15) *a*: −0.08 ± 0.19	2
82	n.d.		(5.19 ± 0.11) *a*: −0.00 ± 0.00	2	(5.28 ± 0.04) *a*: −0.46 ± 0.01	2	(<5) n.d.	3
83	(<5) n.d.	4	6.62 ± 0.11 *a*: 0.44 ± 0.01	2	(<5) *a*: 0.04 ± 0.01	2	(<5) n.d.	2
84	(5.06 ± 0.03) *a*: 0.06 ± 0.02	2	(5.42 ± 0.09) *a*: 0.08 ± 0.04	2	(<5) *a*: −0.02 ± 0.01	2	(<5) n.d.	2
85	(<5) n.d.	2	6.13 ± 0.03 *a*: 0.66 ± 0.02	3	(<5) *a*: −0.03 ± 0.00	2	Inactive	2
86	(<5) *a*: 0.06 ± 0.02	2	6.39 ± 0.01 *a*: 0.42 ± 0.01	2	(<5) *a*: −0.03 ± 0.02	2	Inactive	3

[a] Steady-state GTPase activity in Sf9 insect cell membranes expressing the hH_1R + RGS4, hH_2R-$Gs\alpha_S$ fusion protein, hH_3R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ + RGS4 or hH_4R-RGS19 fusion protein + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ was determined as described in the ESI. *N* gives the number of independent experiments performed in duplicate. [b] n.d.: not determined.

As expected, the compounds **107–112**, bearing a methyl substituted imidazole ring, showed some activity at the H_4R. The 2-methylimidazole derivatives **107** and **108** with a tetramethylene chain connecting imidazole and cyanoguanidine were weak inverse agonists at the H_4R, devoid of noteworthy activity at the other HR subtypes. As reported previously, the phenylthioethyl residue confers higher potency at the H_4R compared to a methyl substitution. Reducing the spacer chain length to three carbon atoms provides the hH_4R partial agonists **109** and **110** with pEC_{50} values around 6.3 and no

Table 2 Pharmacological activities of selected compounds at the guinea pig ileum (gpH_1R) and the guinea pig right atrium (gpH_2R)

	gpH_1R		gpH_2R	
Compound	pA_2	N^a	$pEC_{50}{}^b/(pA_2)/[pD_2']^c/\alpha^d$	N^a
Histamine	—	—	6.00 ± 0.10 *a*: 1.00 ± 0.02	>50
UR-AK24 (**1**)[27]	5.87 ± 0.14	4	7.80 ± 0.07 *a*: 0.99 ± 0.02	4
UR-PG276 (**3**)	n.d.		(<4.5) [< 4.5]	2
59	n.d.		6.71 ± 0.04 *a*: 0.26 ± 0.03	3
61	n.d.		(<4.5) [4.22 ± 0.04]	2
63	n.d.		(4.72 ± 0.34) [4.54 ± 0.03]	2
65	5.52 ± 0.06	18	(6.28 ± 0.13) *a*: 0[e]	2
69	5.95 ± 0.05	18	(<4.5) [4.16 ± 0.05] *a*: 0[f]	2
79	5.63 ± 0.04	18	(4.90 ± 0.16) [4.44 ± 0.15] *a*: <10[g]	2
83	5.42 ± 0.10	15	6.33 ± 0.07 *a*: 0.54 ± 0.03	3
84	5.59 ± 0.09	17	6.44 ± 0.11 *a*: 0.41 ± 0.05	3
85	5.83 ± 0.07	16	7.00 ± 0.07[h] *a*: 1.00 ± 0.02	3
86	5.79 ± 0.04	16	6.69 ± 0.02 *a*: 0.83 ± 0.06	3

[a] Number of experiments. [b] pEC_{50} was calculated from the mean shift ΔpEC_{50} of the agonist curve relative to the histamine reference curve by equation: $pEC_{50} = 6.00 + 0.13 + \Delta pEC_{50}$. Summand 0.13 represents the mean desensitization observed for control organs when two successive curves for histamine were performed (0.13 ± 0.02, $N = 16$). The SEM given for pEC_{50} is the SEM calculated for ΔpEC_{50}. [c] pD_2' values given in brackets for compounds producing a significant, concentration-dependent reduction of the maximal response of histamine. [d] Efficacy, maximal response, relative to the maximal increase in heart rate induced by the reference compound histamine. [e] E_{max} (histamine) at 10 μM: 0.68 ± 0.01. [f] E_{max} (histamine) at 30 μM: 0.69 ± 0.03. [g] E_{max} (histamine) at 30 μM: 0.54 ± 0.09. [h] pA_2 of cimetidine (10 μM, $N = 2$): 6.32 ± 0.06. For experimental details, *cf.* ESI.

agonistic activity at the other three histamine receptors. This is in agreement with the results for the amines **92** and **93**.[52] Compound **111**, the carba analogue of cimetidine,[61] was a weak partial agonist at the hH_4R ($pEC_{50} = 5.44$) and the hH_3R ($pEC_{50} = 5.97$) and showed only very weak antagonistic properties at the hH_1R and at the hH_2R. This is in accordance with data reported for H_2R antagonism at guinea pig right atrium and the rat uterus, where **111** was inferior to cimetidine by a factor of 6 to 10.[61] The phenylthioethyl cyanoguanidine **112** was 15 times more potent as an hH_4R agonist than the methyl cyanoguanidine **111**. With a pEC_{50} value of 6.61 at the H_4R, the 5-methyl analogue of UR-PI376, compound **112**, showed a more than 10-fold selectivity for the H_4R over the H_3R and the other HR subtypes. Nevertheless, none of the investigated hetarylalkylcyanoguanidines was superior to UR-PI376 in terms of H_4R agonistic potency or receptor subtype selectivity.

Conclusions

In summary, for most of the investigated acylguanidines the replacement of the 1*H*-imidazol-4-yl ring with isomers or other heterocycles resulted in considerably reduced potency and efficacy at the hH_2R, hH_3R and hH_4R. This underlines the substantial contribution of an appropriate arrangement of the nitrogen atoms in the heterocycle for binding to the H_2R, H_3R and H_4R and for stabilizing an active conformation of the H_2R and H_4R. Strikingly, the imidazol-1-yl-propylguanidine derivative **69** displayed a comparable maximal response as its isomer, reference compound UR-AK24 (**1**), at the H_4R subtype, although, the potency was about 50-fold lower. As these acylguanidines were poorly active at the other HR subtypes and the hH_4R activity largely depended on the type of acyl residue, further modifications in this moiety appear promising with respect to the development of more potent and selective hH_4R agonists.

Introduction of a 2-pyridyl (**59** and **60**), a 1*H*-pyrazol-4-yl (**83** and **84**) or a 1*H*-1,2,4-triazol-3-yl (**85** and **86**) ring provided compounds exhibiting partial to full agonist activity at the hH_2R and gpH_2R. Except for the 2-pyridyl analogues, these compounds had negligible activities at the other hHR subtypes. In particular, the triazole ring was identified as a promising bioisostere for the imidazole ring with respect to H_2R activity. At the gpH_2R, the N^G-acylated triazolylpropylguanidines (**85** and **86**) were equiefficacious to UR-AK24, but devoid of activities at the other hHR subtypes, suggesting the 1*H*-1,2,4-triazol-3-yl ring as a potential bioisostere for the design of H_2R selective ligands. 2-Aminothiazole analogues of acylguanidine-type H_2R ligands are described[33,63] as highly selective agonists with higher potencies compared to the triazole analogs. However, compared to the aminothiazoles, the triazole ring is considered as relatively stable against enzymatic oxidation.[64] This may offer an alternative to improve the drug-like properties.

The cyanoguanidines derived from OUP-16 and UR-PI376 revealed high sensitivity against both, replacement of the heterocycle and minor structural modifications such as methyl-substitution of the imidazole ring. None of the analogues showed improved potency and/or H_4R selectivity compared to UR-PI376. Except for the heterocycle, the cyanoguanidines are devoid of basic groups. Since previous studies revealed higher potency of H_4R agonists with retained basicity in the central structural motif, *e.g.* acylguanidines, a combination of bioisosteric replacement of both, imidazole ring and cyanoguanidine moiety, should be considered in future ligand design.

In conclusion, the presented data suggest alternative bioisosteric approaches, including the synthesis and pharmacological evaluation of additional heterocyclic analogues of known histamine receptor ligands, with respect to retained/increased potency, improved receptor subtype selectivity and drug-like properties.

Table 3 Potencies and efficacies of the cyanoguanidines **107–133** at the hHR subtypes in the [^{35}S]GTPγS assay[a] or the steady-state [^{32}P]GTPase assay[b,c]

	hH_1R		hH_2R		hH_3R		hH_4R	
Compound	pEC_{50} or (pK_B)	*N*	pEC_{50} or (pK_B)	*N*	EC_{50} or (pK_B)	*N*	EC_{50} or (pK_B)	*N*
Histamine	6.72 ± 0.02 (ref. 28) α: 1.00		5.92 ± 0.11 (ref. 28) *a*: 1.00		7.89 ± 0.07 *a*: 1.00	3	7.96 ± 0.12 *a*: 1.00	5
UR-PI376 (**5**)	(<5)[34] α: 0.07		(<5)[34] α: 0.08		(6.14 ± 0.02) *a*: −0.52 ± 0.05	2	7.43 ± 0.04 *a*: 0.88 ± 0.08	3
Cimetidine	n.d.		(5.77 ± 0.11)[62] *a*: −0.08 ± 0.01		n.d.		(<5)[35]	
107	(<5) α: 0.01 ± 0.03	2	(<5) *a*: 0.04 ± 0.02	2	(<5) *a*: −0.13 ± 0.08	2	(<5) *a*: −0.23 ± 0.03	2
108	(<5.30) *a*: −0.01 ± 0.03	2	4.79 ± 0.01 *a*: −0.05 ± 0.02	2	(<5) *a*: −0.47 ± 0.04	2	(6.21 ± 0.04) *a*: −0.24 ± 0.11	2
109	Inactive	2	(<5) *a*: −0.02 ± 0.01	2	(<5) *a*: −0.52 ± 0.04	2	6.26 ± 0.02 *a*: 0.74 ± 0.02	2
110	(<5.30) *a*: 0.03 ± 0.05	2	(<5) *a*: −0.05 ± 0.02	2	(<5) *a*: −1.11 ± 0.03	2	6.33 ± 0.03 *a*: 0.40 ± 0.07	2
111	(<5) *a*: −0.03 ± 0.02	2	(<5) *a*: 0.04 ± 0.01	2	5.97 ± 0.04 *a*: 0.23 ± 0.03	2	5.44 ± 0.04 *a*: 0.60 ± 0.17	2
112	(<5.30) *a*: −0.03 ± 0.01	2	(5.36 ± 0.04) *a*: −0.06 ± 0.02	2	(5.50 ± 0.02) *a*: −0.65 ± 0.02	2	6.61 ± 0.0 *a*: 0.37 ± 0.1	2
113	Inactive	2	Inactive	2	Inactive	2	Inactive	2
114	(<5) *a*: −0.02 ± 0.01	2	(<5) *a*: −0.04 ± 0.0	2	Inactive	2	Inactive	2
115	(<5) *a*: −0.02 ± 0.04	2	(<5) *a*: −0.07 ± 0.01	2	(5.54 ± 0.01) *a*: −1.03 ± 0.01	2	(5.43 ± 0.09) *a*: −0.83 ± 0.06	2
116	(<5) *a*: −0.01 ± 0.03	2	(<5) *a*: −0.07 ± 0.01	2	(<5) *a*: −0.26 ± 0.03	2	Inactive *a*: 0.06 ± 0.03	2
117	(<5) *a*: 0.03 ± 0.01	2	(<5) *a*: −0.08 ± 0.00	2	(<5) *a*: −0.42 ± 0.06	2	(<5) *a*: −0.05 ± 0.04	2
118	Inactive	2	<5 *a*: 0.31 ± 0.02	2	Inactive	2	Inactive	2
119	(<5) *a*: 0.01 ± 0.04	2	(<5) *a*: 0.03 ± 0.02	2	Inactive	2	Inactive	2
120	Inactive	2	(<5) *a*: −0.03 ± 0.0	2	Inactive	2	Inactive	2
121	Inactive	2	(<5) *a*: −0.01 ± 0.04	2	Inactive	2	Inactive	2
122	Inactive	2	Inactive	2	Inactive	2	Inactive	2
123	Inactive	2	(<5) *a*: −0.09 ± 0.01	2	Inactive	2	Inactive	2
124	Inactive	2	(<5) *a*: −0.02 ± 0.0	2	Inactive	2	Inactive	2
125	Inactive	2	(<5) *a*: −0.05 ± 0.00	2	Inactive	2	Inactive	2
126	Inactive	2	Inactive	2	(<5) *a*: −0.03 ± 0.04	2	(<5) *a*: −0.13 ± 0.05	2
127	(<5) *a*: 0.02 ± 0.03	2	(<5) *a*: −0.12 ± 0.02	2	(<5) *a*: −0.21 ± 0.02	2	(<5) *a*: −0.10 ± 0.01	2
128	(<5) *a*: 0.03 ± 0.03	2	Inactive	2	(<5) *a*: −0.10 ± 0.03	2	(<5) *a*: −0.11 ± 0.03	2
129	(<5) *a*: −0.01 ± 0.01	2	(<5) *a*: −0.13 ± 0.01	2	(<5) *a*: −0.36 ± 0.04	2	(<5) *a*: −0.34 ± 0.01	2
130	Inactive	2	Inactive	2	(<5) *a*: −0.16 ± 0.02	2	(<5) *a*: −0.11 ± 0.03	2
131	(<5) *a*: 0.01 ± 0.03	2	(<5) *a*: −0.08 ± 0.02	2	(<5) *a*: −1.43 ± 0.14	2	Inactive	2
132	(<5) *a*: 0.03 ± 0.02	2	Inactive	2	(<5) *a*: −0.24 ± 0.05	2	Inactive	2

Table 3 (*Contd.*)

	hH_1R		hH_2R		hH_3R		hH_4R	
Compound	pEC_{50} or (pK_B)	*N*	pEC_{50} or (pK_B)	*N*	EC_{50} or (pK_B)	*N*	EC_{50} or (pK_B)	*N*
133	(<5) *a*: 0.01 ± 0.02	2	(<5) *a*: −0.11 ± 0.01	2	(5.14 ± 0.01) *a*: −0.71 ± 0.03	2	(<5) *a*: −0.09 ± 0.05	2

[a] Functional [^{35}S]GTPγS binding assay with membrane preparations of Sf9 cells expressing the hH_3R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ or the hH_4R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ or the hH_2R-$Gs\alpha_s$ fusion protein were performed as described in the ESI. [b] Steady-state GTPase activity in Sf9 cell membranes expressing the hH_1R + RGS4 was determined as described under Pharmacological methods. [c] Reaction mixtures contained ligands at a concentration range from 1 nM to 1 mM as appropriate to generate saturated concentration/response curves. *N* gives the number of independent experiments performed in duplicate. The intrinsic activity (α) of histamine was set to 1.00 and α values of other compounds were referred to this value. The α values of neutral antagonists and inverse agonists were determined at a concentration of 10 μM. The pK_B values of neutral antagonists and inverse agonists were determined in the antagonist mode *versus* histamine as the agonist.

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