N^G-Acylated Imidazolylpropylguanidines as Potent Histamine H_4 Receptor Agonists: Selectivity by Variation of the N^G-Substituent▽

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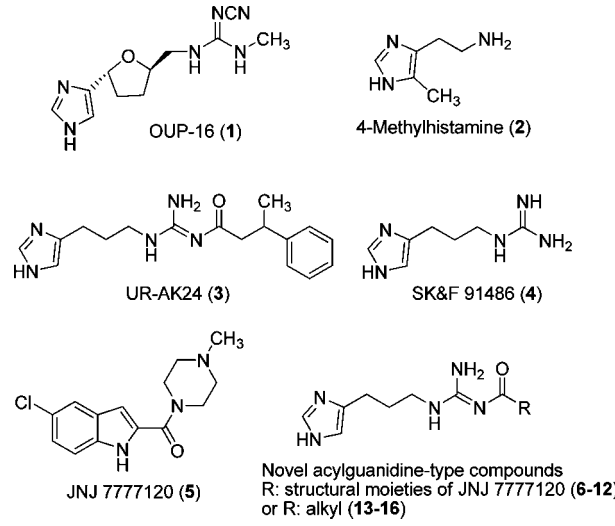
3-(1*H*-Imidazol-4-yl)propylguanidine (SK&F 91486, **4**) was identified as a potent partial agonist at the human histamine H_3 receptor (hH_3R) and human histamine H_4 receptor (hH_4R). With the aim to increase selectivity for the hH_4R, the guanidine group in **4** was acylated. N^1-Acetyl-N^2-[3-(1*H*-imidazol-4-yl)propyl]guanidine (UR-PI288, **13**) was a potent full agonist at the hH_4R ($pEC_{50} = 8.31$; $\alpha = 1.00$), possessing more than 1000- and 100-fold selectivity relative to the hH_1R and hH_2R, respectively, and possessing only low intrinsic activity ($\alpha = 0.27$) at the hH_3R.

Introduction

The biogenic amine histamine mediates its effects via four histamine receptor subtypes, termed H_1, H_2, H_3, and H_4 receptors (H_1R, H_2R, H_3R, and H_4R, respectively), all belonging to the superfamily of G-protein-coupled receptors (GPCRs[a]).[1] The H_4R is the most recently identified member of the family of histamine receptors and was identified in 2000 and 2001, when several research groups identified and cloned the gene encoding the hH_4R based on its high degree of sequence homology with the hH_3R. This rather high degree of homology (37% at the protein level) explains the high affinity of many H_3R ligands for the hH_4R, in particular imidazole-containing compounds such as thioperamide [4-(1*H*-imidazol-4-yl)piperidin-1-yl)(piperidin-1-yl)methanethione].[2] The hH_4R is mainly expressed on hematopoietic cells like mast cells, eosinophils, dendritic cells, T lymphocytes, and monocytes and seems to play a crucial role in inflammatory and immunological processes (for recent reviews, see refs 3 and 4).

To further study the (patho)physiological functions of the receptor, selective ligands—including agonists—are required as pharmacological tools. At present, only a few selective H_4R agonists like **1** (OUP-16) or **2** (4-methylhistamine) are available (Chart 1).[2,3,5] Among a series of N^G-acylated imidazolylpropylguanidines[6−8] originally developed by our group as potent H_2R agonists, several compounds were serendipitously found to be even more potent at the hH_3R and the hH_4R [for example, **3** (UR-AK24) (Chart 1)].[7,8] Most of the investigated compounds displayed high intrinsic activity at the hH_4R but low intrinsic activity at the hH_3R. Subsequent evaluation of the parent structure, 3-(1*H*-imidazol-4-yl)propylguanidine [**4** (SK&F 91486) (Chart 1)], a weak partial H_2R agonist,[9] surprisingly revealed this compound as a highly potent partial agonist at the hH_3R and hH_4R (Table 1).

Chart 1. Structures of Selective H_4R Agonists **1**[5] and **2**,[2] Imidazolylpropylguanidines **3**[7] and **4**,[9] Selective H_4R Antagonist **5**,[10] and Novel N^G-Acylated Imidazolylpropylguanidines **6**−**16** Presented in This Study



Starting from this model compound, we sought to explore the potential to develop more selective hH_4R agonists by acylation of the guanidine group in **4**. One strategy was to introduce acyl residues containing structural motifs of the potent and selective hH_4R antagonist **5** (Chart 1).[10] The resulting hybrid molecules (**6**−**12**) consisting of an H_4R agonistic and antagonistic moiety could be favorable for hH_4R selectivity and activity, provided that the acyl residue can additionally interact with the binding site of **5** (JNJ 7777120). The second approach focused on the size of the acyl residues with respect to selectivity for the H_4R over the H_2R. The imidazolylpropylguanidine portion of the N^G-acylated imidazolylpropylguanidines is considered to be responsible for H_2R agonistic activity, whereas

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▽ Dedicated to Prof. Dr. Gottfried Märkl, Regensburg, on the occasion of his 80th birthday.

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[a] Abbreviations: gpH_1R, guinea pig H_1R; gpH_2R, guinea pig H_2R; GPCR, G-protein-coupled receptor; $G_{\beta 1\gamma 2}$, G-protein β_1 and γ_2 subunit; $G_{\alpha i}$, α subunit of the G_i-protein; HMBC, heteronuclear multiple-bond correlation; hH_1R, hH_2R, hH_3R, and hH_4R, human histamine receptor subtypes H_{1-4}, respectively; $hH_2R-G_{s\alpha S}$, fusion protein of the hH_2R and the short splice variant of $G_{s\alpha}$; hH_4R−RGS19, fusion protein of the hH_4R and RGS19; N^G, guanidine nitrogen; NOESY, nuclear Overhauser enhancement spectroscopy; RGS, regulator of G-protein signaling proteins; TM, transmembrane domain of a GPCR.

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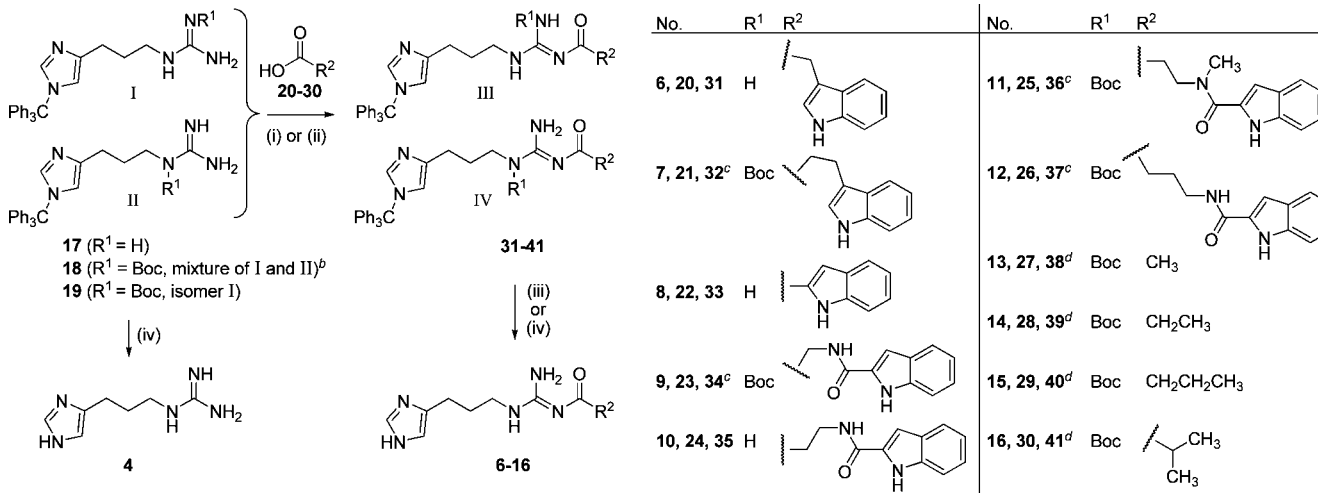
Table 1. Activities of the Prepared Compounds at the hH_1R, hH_2R, hH_3R, and hH_4R in the Steady-State GTPase Assay[a]

	hH_1R			hH_2R			hH_3R			hH_4R		
compound	pEC_{50} (pK_B)	α	*n*	pEC_{50}	α	*n*	pEC_{50} (pK_B)	α	*n*	pEC_{50} (pK_B)	α	*n*
histamine	6.72[6]	1.00		5.92[6]	1.00		7.60 ± 0.05	1.00	3	7.92 ± 0.09	1.00	8
thioperamide	–	–		–	–		7.01 ± 0.08	−0.71 ± 0.06	5	6.96 ± 0.06	−0.95 ± 0.07	6
3[6]	(<4.90)	0.35[7]		7.17[7]	0.87[7]		8.60[8]	0.24[8]		7.82[8]	0.84[8]	
4	(<5.00)	nd[b]	2	5.59 ± 0.01	0.66 ± 0.02	3	8.12 ± 0.10	0.69 ± 0.04	3	8.09 ± 0.04	0.83 ± 0.01	3
6	(5.68 ± 0.13)	0.00 ± 0.08	2	7.44 ± 0.05	0.81 ± 0.02	2	(7.51 ± 0.12)	−0.15 ± 0.02	2	7.72 ± 0.14	0.70 ± 0.12	3
7	(5.40 ± 0.00)	0.09 ± 0.02	2	7.09 ± 0.03	0.77 ± 0.02	2	(7.57 ± 0.15)	−0.18 ± 0.02	2	8.15 ± 0.17	0.45 ± 0.08	3
8	5.77 ± 0.03	0.42 ± 0.00	2	6.54 ± 0.02	0.87 ± 0.00	2	(6.96 ± 0.04)	−0.38 ± 0.01	2	(6.43 ± 0.02)	0.21 ± 0.03	2
9	nd[b]	nd[b]		6.02 ± 0.16	0.51 ± 0.01	3	7.80 ± 0.00	0.45 ± 0.03	2	7.55 ± 0.20	0.71 ± 0.05	3
10	nd[b]	nd[b]		7.08 ± 0.00	0.33 ± 0.04	2	(7.17 ± 0.04)	0.10 ± 0.02	2	7.48 ± 0.12	0.37 ± 0.02	2
11	nd[b]	nd[b]		6.46 ± 0.07	0.68 ± 0.04	3	7.96 ± 0.08	0.30 ± 0.01	2	6.82 ± 0.07	0.52 ± 0.06	4
12	(4.96 ± 0.01)	0.16 ± 0.08	2	7.52 ± 0.11	0.51 ± 0.01	2	(7.17 ± 0.06)	−0.18 ± 0.02	2	(7.02 ± 0.05)	−0.06 ± 0.14	2
13	4.89 ± 0.03	0.24 ± 0.02	2	6.14 ± 0.04	0.76 ± 0.02	2	8.44 ± 0.14	0.27 ± 0.02	3	8.31 ± 0.04	1.00 ± 0.02	3
14	5.46 ± 0.15	0.30 ± 0.01	2	6.43 ± 0.03	0.83 ± 0.08	2	8.80 ± 0.06	0.39 ± 0.03	3	8.52 ± 0.04	0.90 ± 0.02	3
15	5.66 ± 0.06	0.33 ± 0.03	2	6.85 ± 0.03	0.77 ± 0.02	2	8.80 ± 0.07	0.37 ± 0.00	3	8.59 ± 0.02	0.96 ± 0.03	3
16	5.59 ± 0.04	0.35 ± 0.01	2	6.96 ± 0.14	0.86 ± 0.01	2	8.85 ± 0.18	0.26 ± 0.02	3	8.43 ± 0.02	0.94 ± 0.04	3

[a] Steady-state GTPase activity in Sf9 membranes expressing the hH_1R plus RGS4, hH_2R-$G_{s\alpha S}$, hH_3R plus $G_{i\alpha 2}$ plus $G_{\beta 1\gamma 2}$ plus RGS4 or hH_4R-RGS19 fusion protein plus $G_{i\alpha 2}$ plus $G_{\beta 1\gamma 2}$ determined as described in Pharmacological methods in the Supporting Information. [b] Not determined.

Scheme 1. Synthesis of the N^G-Acylated Imidazolylpropylguanidines **6**−**16** and **4**a



[a] Reagents and conditions: (i) for **31**, **33**, and **35**, CDI (1.2 equiv), NaH (60% dispersion in mineral oil) (2 equiv), THF, 5 h, room temperature; (ii) for **32**, **34**, and **36**−**41**, EDC·HCl (1.2 equiv), DMAP (1.1 equiv), DCM, 24 h, 0 °C → room temperature; (iii) for **31**, **33**, and **35**, TFA (20%), DCM, 5 h, room temperature; (iv) for **4**, **32**, **34**, and **36**−**41**, 1 M HCl, 30 min, reflux. [b] Compare to Scheme 2 in the *Supporting Information*. [c] Mixture of III and IV. [d] Isomer III.

the N^G-acyl residue is assumed to contribute to H_2R affinity.[8] However, the endogenous ligand histamine, lacking additional affinity-conferring residues, is ∼50 times more potent at the hH_3R and the hH_4R than at the hH_2R (Table 1). As bulky N^G-acyl groups may be unnecessary or unfavorable for obtaining highly potent compounds at the hH_3R and hH_4R, acylation of **4** with small alkanoyl groups (**13**−**16**) was explored to switch selectivity toward the hH_3R and hH_4R and to gain more insight into the structure−activity relationships of the N^G-acylated imidazolylpropylguanidines at the distinct histamine receptor subtypes.

Chemistry

The N^G-acylated imidazolylpropylguanidines **31**, **33**, and **35** were synthesized by acylation of the guanidine base **17** with CDI-activated carboxylic acids (compounds **20**, **22**, **24**) according to the method described by Ghorai et al. (Scheme 1).[8] However, preparation of the acylguanidines via this route showed drawbacks. The synthesis of **34** failed since under the strongly basic conditions (NaH) required for the deprotonation of the guanidine moiety an intramolecular cyclization of the carboxylic acid **23** occurred (acylation of the indole nitrogen giving a six-membered ring; analyzed by mass spectrometry). Therefore, a modified synthetic pathway was employed. To avoid the strongly basic conditions, instead of the free guanidine ($pK_a \approx 13$),[11] Boc-protected derivative ($pK_a \approx 5$)[11] **18** or **19** (**18**, mixture of isomers I and II; **19**, single isomer depending on the synthetic procedure employed for the preparation; cf. the Supporting Information) was used,[12] yielding the trityl- and Boc-protected intermediates **32**, **34**, and **36**−**41**. This procedure can be performed in a one-pot reaction without preactivation of the carboxylic acids. Moreover, the isolation and purification of the less polar and less basic 2-fold acylated intermediates were facilitated. Fewer side reactions were observed, and excessive acylation of the guanidine moiety was prevented. Nevertheless, as marked cleavage of one acyl group could be detected by NMR after storage in solution for few days, intermediates **32**, **34**, and **36**−**41** should be deprotected in a timely manner. Detritylation of the imidazole ring was routinely performed in a mixture of 20% TFA in dichloromethane (**6**, **8**, and **10**).[8] However, under these conditions, the deprotection of compound **35** gave **10** in only 3% yield and produced a large number of side products, whereas refluxing in hydrochloric acid resulted in 10−20 times higher yields. In contrast to the TFA/

dichloromethane mixture, side reactions were reduced in aqueous medium, presumably because the intermediate trityl cation was trapped and precipitated as triphenylmethanol. This procedure was applied for the preparation of N^G-acylated imidazolylpropylguanidines **7**, **9**, and **11**−**16** and for **4**.[9]

In theory, acylation of the Boc-protected guanidine is possible at the nitrogen adjacent to the carbon chain or at the unsubstituted nitrogen. However, two-dimensional NMR experiments (HMBC and NOESY) with compound **15**, synthesized from building block **19**, i.e., isomer I, bearing the Boc group at the terminal nitrogen, confirmed the acylation at the designated third guanidine nitrogen. The preparation of imidazolylpropylguanidines **17**−**19**, guanidinylation reagents **49**−**52**, and carboxylic acids **23**−**26** is reported in the Supporting Information.

Results and Discussion

The synthesized compounds were investigated for agonism and antagonism at the four human histamine receptor subtypes in steady-state GTPase assays using membrane preparations of Sf9 insect cells expressing the hH_1R with RGS4 (regulator of G-protein signaling 4), the $hH_2R-G_{s\alpha S}$ fusion protein, the hH_3R with $G_{i\alpha 2}$, $G_{\beta 1\gamma 2}$, and RGS4, or the $hH_4R-RGS19$ fusion protein with $G_{i\alpha 2}$ and $G_{\beta 1\gamma 2}$ (Table 1).[8] A major advantage of the test systems applied in this study is that for any given H_xR subtype, we used an identical readout, namely steady-state GTP hydrolysis. This is a very proximal readout in G-protein-mediated signal transduction, reducing bias of agonist evaluation usually introduced by downstream measurements of second-messenger generation, changes in cell function, or gene transcription. Thus, the receptor profiles described herein for acylguanidines reflect true differences in pharmacology and not differences in readout between various receptors. In addition, selected compounds were investigated at the guinea pig ileum and the guinea pig right atrium for activity at the gpH_1R and gpH_2R, respectively (see the Supporting Information).

Reference acylguanidine **3** was similar in potency to histamine at the hH_4R,[8] had high affinity but low intrinsic activity at the hH_3R, was ∼35 times more potent than histamine at the hH_2R, and was a weak antagonist at the hH_1R. **4**, which is considered an essential moiety for agonistic activity of such acylguanidine-type H_2R agonists,[8] was approximately half as potent as histamine at the hH_2R and acted as a partial agonist (pEC_{50} = 5.59; α = 0.66). This is in accordance with previous findings at the guinea pig right atrium (H_2R).[9] At the hH_3R and hH_4R, **4** exhibited similar pEC_{50} values of ≈8.1 and intrinsic activities of ≈0.7−0.8, whereas the compound was almost inactive at the hH_1R. These results suggest that the imidazolylpropylguanidine moiety is a suitable agonist structure not just at the hH_2R, but also at the hH_3R and hH_4R.

Most of the new acylguanidines (**6**−**16**) listed in Table 1, except compounds **8** and **12**, which had negligible intrinsic activities if any, proved to be hH_4R agonists achieving low nanomolar activity. However, the two groups of compounds, **6**−**12** and **13**−**16**, differed in particular in terms of intrinsic activity and hH_4R selectivity. In compounds **6**−**12**, the guanidine group of **4** was acylated with an indole-3-alkanoyl or indole-2-carbonyl residue reminiscent of the core structure of selective hH_4R antagonist **5**.[10] Compared to that of **4**, the acylation of the guanidine group with indole-3-acetic or -propanoic acid (**6** or **7**, respectively) substantially increased agonistic activity at the hH_2R and thereby produced a diminished level of discrimination between the hH_2R and hH_4R. Intrinsic activity at the hH_3R was abolished. In compounds **8**−**12**, indole-2-carboxylic acid is attached to the imidazolylpropylguanidine portion either directly (**8**) or via amino acid linkers (**9**−**12**). The amino acid spacers were considered as "ring-opened versions" of the piperazine ring in **5**, and the basic tertiary amine, which is regarded as crucial for H_4R affinity of **5**,[13] may be mimicked by the acylguanidine group. Direct attachment of the indole-2-carboxylic acid (**8**) significantly changed the pharmacological profile at H_xRs relative to reference compound **4**. Compound **8** exhibited moderate partial agonism at hH_1R (pEC_{50} = 5.77; α = 0.42). At the hH_2R, **8** was ∼10 times more potent than **4** and exhibited slightly elevated intrinsic activity (pEC_{50} = 6.54; α = 0.87). In contrast, at the hH_3R and hH_4R, **8** was >10 times less potent and the intrinsic activity substantially decreased. The incorporation of a glycine spacer (**9**) was not tolerated in terms of hH_2R activity; compared to that of **8**, the agonstic activity dropped remarkably (pEC_{50} = 6.02; α = 0.51). At the hH_3R and hH_4R, **9** was more active than compound **8** and displayed partial agonistic activity at both receptors. Replacing the glycine spacer with β-alanine (**10**) and 4-aminobutyric acid (**12**) increased activity at the hH_2R by factors of 10 (pEC_{50} = 7.08) and 30 (pEC_{50} = 7.52), respectively. Evidently, the increased flexibility of the higher homologues favored high hH_2R activity. The hH_3R and hH_4R affinities remained largely unaffected, whereas the intrinsic activities at these receptors were significantly reduced. At all hHRs, especially at the hH_2R, *N*-methylation of the amide nitrogen (**11**) increased intrinsic activities, but the affinities at the hH_2R and hH_4R (pEC_{50} values of 6.46 and 6.82, respectively) were reduced, suggesting that the amide NH group contributes to hH_2R and hH_4R binding, although steric factors may also play a role. In contrast, at the hH_3R, **11** was more active as a partial agonist than unmethylated analogue **10**. Taken together, the hybrid approach, combining hH_4R agonistic (**4**) and antagonistic (**5**) structural elements, was unsuccessful with respect to improving hH_4R selectivity.

The second approach to increasing selectivity for the hH_4R, in particular over that of the hH_2R, was focused on the introduction of small N^G-alkanoyl residues instead of the larger arylalkanoyl groups found to be useful in H_2R agonists.[8] This idea was stimulated by the fact that the natural agonist, histamine, is 100 times more active at the hH_4R than at the hH_2R (pEC_{50} values of 7.92 and 5.92, respectively). Obviously, additional affinity-conferring substituents are not required to achieve low nanomolar hH_4R activities. Acetylation of the guanidine group yielded **13** (UR-PI288) and provided a moderate increase in agonistic activity (pEC_{50} = 6.14; α = 0.76) relative to that of **4** at the hH_2R. As expected, the activities of the acylguanidines at the hH_2R further increased (pEC_{50} = 6.14 → pEC_{50} = 6.96) with the extension of the alkanoyl residue (**14**, UR-PI294, for the preparation and characterization of tritiated **14** cf. ref 14; **15**, UR-PI295; **16**, UR-PI287), whereas the intrinsic activities remained unaffected. However, with the small N^G-alkanoyl residues in **13**−**16**, the hH_2R agonistic activity was kept considerably lower than with larger arylalkanoyl groups.[8] The N^G-alkanoylguanidines **13**−**16** were very weak partial agonists at the hH_1R. With regard to the hH_3R, acetylation of the guanidine group in **4** slightly increased affinity but substantially decreased intrinsic activity (pEC_{50} = 8.44; α = 0.27). Likewise, substitution of the guanidine group with larger alkanoyl residues was poorly tolerated in terms of hH_3R intrinsic activity. Obviously, acylation of the guanidine group modifies the interaction of the imidazolylpropylguanidine moiety with the hH_3R in a manner that impedes the stabilization of an active receptor conformation. Contrary to the structure−activity relationships at the hH_3R, the alkanoyl residue was beneficial for the activation of the hH_4R. The N^G-alkanoyl imidazolyl-

propylguanidines **13**−**16** were highly active (almost) full agonists at the hH_4R (pEC_{50} = 8.31−8.59; α = 0.90−1.00). This demonstrates that acylation of the guanidine group in **4** is a successful way to shift the agonistic activity to the hH_4R at the expense of hH_3R agonism.

Summary and Conclusion

Starting from the potent nonselective acylguanidine-type hH_4R agonist **3**, which was initially designed and synthesized as an H_2R agonist,[8] we identified parent compound **4** (weak partial agonist at the H_2R[9]) as a highly active hH_3R and hH_4R partial agonist. With the aim of increasing the selectivity of the acylguanidine-type compounds for the hH_4R, two distinct strategies were explored.

In the first approach, the guanidine group in **4** was acylated with indolealkanoic and indole-2-carboxylic acid moieties as structural motifs derived from the selective, high-affinity hH_4R antagonist **5**. Depending on the residues and amino acid spacers, compounds with varying activities (GTPase assay) at the different histamine receptor subtypes were obtained. Clearly, the compounds containing the indole substructure were not suitable for conferring additional affinity by interaction with the binding site of **5** as hH_4R activity was not substantially improved. An explanation therefore may be provided by an hH_4R homology model, suggesting that both histamine and **5** mainly interact with Asp-94 of TM3 and Glu-182 of TM5.[13] Presumably, as previously described for the H_2R,[8,15] histamine and N^G-acylated imidazolylpropylguanidines also predominantly interact with identical amino acid residues at the hH_4R, resulting in overlapping binding sites of the imidazolylpropylguanidine group and **5**.

In the second approach, small alkanoyl residues were attached to **4** (**13**−**16**). With the increasing size of the alkanoyl residues, activity at the hH_2R also increased, confirming the importance of the acyl group as an affinity-conferring moiety at the hH_2R. At the hH_3R, acylation drastically lowered efficacy, whereas the same compounds turned out to be highly potent full (or nearly full) hH_4R agonists. Thus, although the imidazolylpropylguanidine portion is capable of stimulating the hH_2R, hH_3R, and hH_4R, selectivity can be achieved by appropriate N^G-acylation.

hH_4R agonists **13**−**16** are among the most active hH_4R agonists reported to date and may become useful experimental tools in addition to previously described H_4R ligands like selective hH_4R agonist **2**[2] or selective H_4R antagonist **5**[10] to analyze the as yet incompletely understood (patho)physiological functions of the H_4R. In most immune cells like mast cells or eosinophils, where the H_4R is mainly located, H_3Rs are not expressed.[4,16,17] Therefore, on the basis of the >100-fold selectivity relative to the hH_1R and hH_2R subtypes, N^G-alkanoylimidazolylpropylguanidines like **13** may also be useful for investigating the function of the hH_4R in such native cells devoid of the hH_3R. Moreover, these potent hH_4R agonists may be suitable pharmacological probes for desensitization studies with the hH_4R.

Altogether, as previously observed, from the medicinal chemistry perspective, the imidazolylpropylguanidine scaffold may be considered a "privileged structure" providing ligands for several histamine receptor subtypes.[8] However, as demonstrated in this study, activities and receptor selectivities of the imidazolylpropylguanidines can be obtained by variation of the N^G-acyl substituent.

Experimental Section

Chemical Synthesis and Analytical Methods. See the Supporting Information. The purity of all pharmacologically investigated compounds was >95% as determined by RP-HPLC.

Preparation of the Boc/Trityl-Protected N^G-Acylated Imidazolylpropylguanidines (32, 34, and 36−41). General Procedure A. To a solution of the pertinent carboxylic acid (1 equiv) and Boc-protected guanidine **18** or **19** (1 equiv) in DCM (20 mL) were added EDC·HCl (1.2 equiv) and DMAP (1.1 equiv) at 0 °C. After being stirred for 4 h at 0 °C, the solution was allowed to warm to ambient temperature and stirred for an additional 20 h. DCM (20 mL) was added, and the organic phase was washed with water and brine and dried over Na_2SO_4. The solvent was evaporated and the crude product purified by flash chromatography.

***N*^{1}-Acetyl-*N*^{2}-(*tert*-butoxycarbonyl)-*N*^{3}-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (38).** The title compound was prepared from acetic acid **27** (60 mg, 1.0 mmol), **19** (510 mg, 1.0 mmol), EDC·HCl (230 mg, 1.2 mmol), and DMAP (134 mg, 1.1 mmol) according to general procedure A. Purification by flash chromatography ($CHCl_3$/MeOH, 97.5/2.5, v/v) yielded a colorless oil (360 mg, 65%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (s, 9H), 1.85−1.99 (m, 2H), 2.16 (s, 3H), 2.59 (t, 2H, ^3J = 7.6 Hz), 3.39−3.49 (m, 2H), 6.53 (d, 1H, ^4J = 1.3 Hz), 7.08−7.17 (m, 6H), 7.29−7.39 (m, 10H), 8.96 (t, 1H, ^3J = 5.2 Hz), 12.41 (brs, 1H); ES-MS (DCM/MeOH + NH_4OAc) *m*/*z* (%) 552 (100) $[M + H]^+$. $C_{33}H_{37}N_5O_3$ (551.68).

Compounds **32**, **34**, **36**, **37**, and **39**−**41** were prepared by analogy (see the Supporting Information).

Preparation of N^G-Acylated Imidazolylpropylguanidines 7, 9, and 11−16. General Procedure B. The pertinent Boc-trityl-protected N^G-acylated imidazolylpropylguanidine was refluxed for 30 min in HCl (1 M, 20 mL). After the precipitated trityl alcohol had been removed by filtration, the solvent was removed in vacuo. Purification of the crude product was performed by preparative HPLC. The solvent was removed by lyophilization, and the compounds were obtained as trifluoroacetates.

***N*^{1}-Acetyl-*N*^{2}-[3-(1*H*-imidazol-4-yl)propyl]guanidinium Ditrifluoroacetate (13).** The title compound was prepared from **38** (350 mg, 0.63 mmol) according to general procedure B. Purification by preparative HPLC [MeCN/0.1% TFA (aqueous), 4/96] yielded a colorless semisolid compound (150 mg, 54%): ^{1}H NMR (300 MHz, D_2O, trifluoroacetate) δ 1.84−1.97 (m, 2H), 2.08 (s, 3H), 2.69 (t, 2H, ^3J = 7.6 Hz), 3.26 (t, 2H, ^3J = 6.9 Hz), 7.12 (d, 1H, ^4J = 1.3 Hz), 8.46 (d, 1H, ^4J = 1.3 Hz); ^{13}C NMR (75 MHz, D_2O, trifluoroacetate) δ 21.06, 23.69, 26.04, 40.38, 115.48, 132.44, 133.03, 152.98, 174.73; IR (cm^{-1}) 3139, 3035, 2854, 1662, 1629, 1179, 1124; HRMS (EI-MS) calcd for $C_9H_{15}N_5O$ [$M^{+\bullet}$] 209.1277, found 209.1275. $C_9H_{15}N_5O$·2TFA (437.30).

Compounds **7**, **9**, **11**, **12**, and **14**−**16** were prepared by analogy (see the Supporting Information).

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Supporting Information Available: Synthesis and analytical data of **4**, **6**−**12**, **14**−**16**, **23**−**26**, **31**−**37**, **39**−**41**, **43**−**52**, **56**, and **58**−**61**; HPLC purity data of target compounds **6**−**16**; pharmacological activities of **6**, **7**, and **11**−**16** at the guinea pig ileum (gpH_1R) and guinea pig right atrium (gpH_2R); and pharmacological methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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