

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

Patrick Igel,[†] Erich Schneider,[‡] David Schnell,[‡] Sigurd Elz,[†] Roland Seifert,[§] and Armin Buschauer^{*,†}

Department of Pharmaceutical/Medicinal Chemistry and Department of Pharmacology and Toxicology, Faculty of Chemistry and Pharmacy, University of Regensburg, Universitätsstrasse 31, D-93053 Regensburg, Germany, and Institute of Pharmacology, Medical School of Hannover, D-30625 Hannover, Germany

Received October 18, 2008

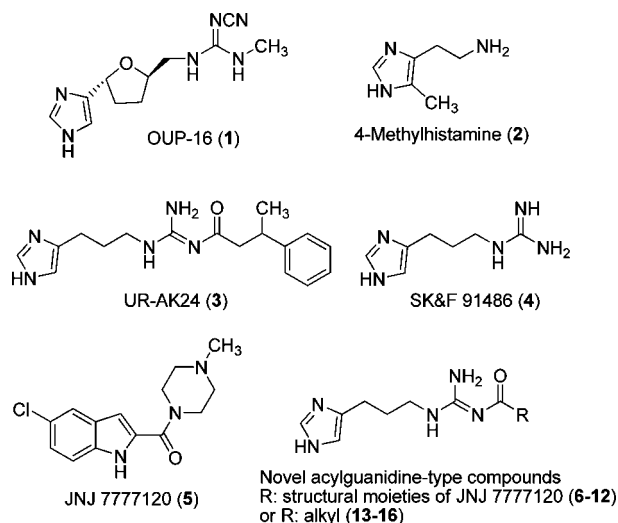
3-(1*H*-Imidazol-4-yl)propylguanidine (SK&F 91486, **4**) was identified as a potent partial agonist at the human histamine H_3 receptor (hH₃R) and human histamine H_4 receptor (hH₄R). With the aim to increase selectivity for the hH₄R, the guanidine group in **4** was acylated. N^1 -Acetyl- N^2 -[3-(1*H*-imidazol-4-yl)propyl]guanidine (UR-PI288, **13**) was a potent full agonist at the hH₄R (pEC₅₀ = 8.31; α = 1.00), possessing more than 1000- and 100-fold selectivity relative to the hH₁R and hH₂R, respectively, and possessing only low intrinsic activity (α = 0.27) at the hH₃R.

Introduction

The biogenic amine histamine mediates its effects via four histamine receptor subtypes, termed H₁, H₂, H₃, and H₄ receptors (H₁R, H₂R, H₃R, and H₄R, respectively), all belonging to the superfamily of G-protein-coupled receptors (GPCRs^a).¹ The H₄R 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 hH₄R based on its high degree of sequence homology with the hH₃R. This rather high degree of homology (37% at the protein level) explains the high affinity of many H₃R ligands for the hH₄R, in particular imidazole-containing compounds such as thioperamide [4-(1*H*-imidazol-4-yl)piperidin-1-yl](piperidin-1-yl)methanethione].² The hH₄R 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 H₄R 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 H₂R agonists, several compounds were serendipitously found to be even more potent at the hH₃R and the hH₄R [for example, **3**

Chart 1. Structures of Selective H₄R Agonists **1**⁵ and **2**,² Imidazolylpropylguanidines **3**⁷ and **4**,⁹ Selective H₄R Antagonist **5**,¹⁰ and Novel N^G -Acylated Imidazolylpropylguanidines **6–16** Presented in This Study



(UR-AK24) (Chart 1)].^{7,8} Most of the investigated compounds displayed high intrinsic activity at the hH₄R but low intrinsic activity at the hH₃R. Subsequent evaluation of the parent structure, 3-(1*H*-imidazol-4-yl)propylguanidine [**4** (SK&F 91486) (Chart 1)], a weak partial H₂R agonist,⁹ surprisingly revealed this compound as a highly potent partial agonist at the hH₃R and hH₄R (Table 1).

Starting from this model compound, we sought to explore the potential to develop more selective hH₄R agonists by acylation of the guanidine group in **4**. One strategy was to introduce acyl residues containing structural motifs of the potent and selective hH₄R antagonist **5** (Chart 1).¹⁰ The resulting hybrid molecules (**6–12**) consisting of an H₄R agonistic and antagonistic moiety could be favorable for hH₄R 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 H₄R over the H₂R. The imidazolylpropylguanidine portion of the N^G -acylated imidazolylpropylguanidines is considered to be responsible for H₂R agonistic activity, whereas

* To whom correspondence should be addressed. Phone: +49-941 9434827. Fax: +49-941 9434820. E-mail: armin.buschauer@chemie.uni-regensburg.de.

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

[†] Department of Pharmaceutical/Medicinal Chemistry, Faculty of Chemistry and Pharmacy, University of Regensburg.

[‡] Department of Pharmacology and Toxicology, Faculty of Chemistry and Pharmacy, University of Regensburg.

[§] Medical School of Hannover.

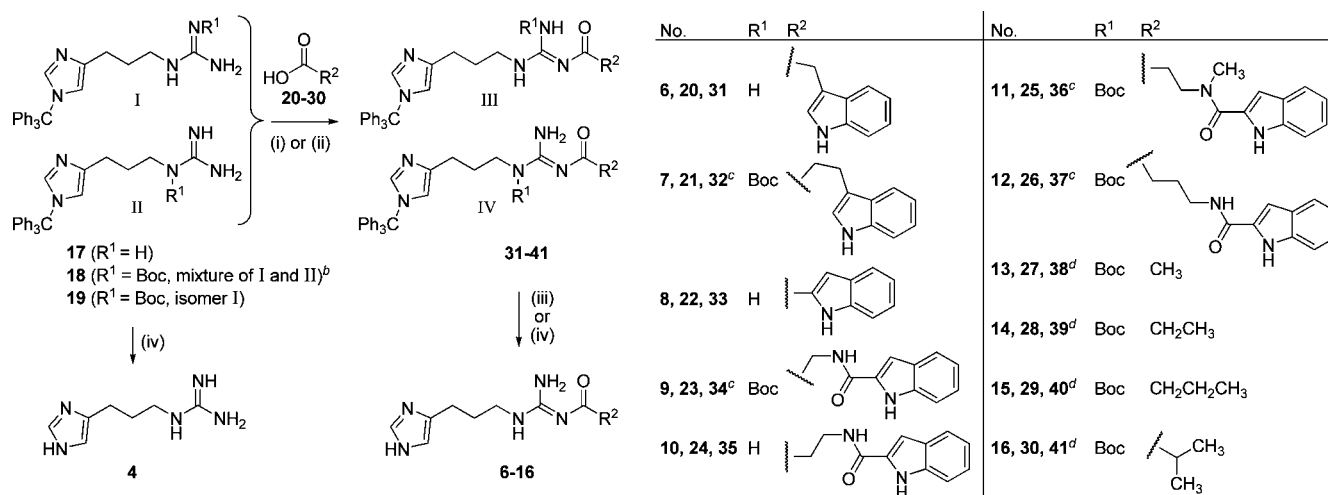
^a Abbreviations: gpH₁R, guinea pig H₁R; gpH₂R, guinea pig H₂R; GPCR, G-protein-coupled receptor; G_{β1γ2}, G-protein β_1 and γ_2 subunit; G_{ai}, α subunit of the G_i-protein; HMBC, heteronuclear multiple-bond correlation; hH₁R, hH₂R, hH₃R, and hH₄R, human histamine receptor subtypes H_{1–4}, respectively; hH₂R-G_{soS}, fusion protein of the hH₂R and the short splice variant of G_{soS}; hH₄R-RGS19, fusion protein of the hH₄R and RGS19; N^G , guanidine nitrogen; NOESY, nuclear Overhauser enhancement spectroscopy; RGS, regulator of G-protein signaling proteins; TM, transmembrane domain of a GPCR.

Table 1. Activities of the Prepared Compounds at the hH₁R, hH₂R, hH₃R, and hH₄R in the Steady-State GTPase Assay^a

compound	hH ₁ R			hH ₂ R			hH ₃ R			hH ₄ R		
	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 hH₁R plus RGS4, hH₂R-G_{αs}, hH₃R plus G_{αi2} plus G_{β1γ2} plus RGS4 or hH₄R-RGS19 fusion protein plus G_{αi2} plus G_{β1γ2} determined as described in Pharmacological methods in the Supporting Information. ^b Not determined.

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



^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 H₂R affinity.⁸ However, the endogenous ligand histamine, lacking additional affinity-conferring residues, is ~50 times more potent at the hH₃R and the hH₄R than at the hH₂R (Table 1). As bulky N^G-acyl groups may be unnecessary or unfavorable for obtaining highly potent compounds at the hH₃R and hH₄R, acylation of **4** with small alkanoyl groups (**13–16**) was explored to switch selectivity toward the hH₃R and hH₄R and to gain more insight into the structure–activity relationships of the N^G-acylated imidazolypropylguanidines at the distinct histamine receptor subtypes.

Chemistry

The N^G-acylated imidazolypropylguanidines **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 ≈ 13),¹¹ Boc-protected derivative (pK_a ≈ 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 imidazolypropylguanidines **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 imidazolypropylguanidines **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 hH₁R with RGS4 (regulator of G-protein signaling 4), the hH₂R–G_{sas} fusion protein, the hH₃R with G_{ia2}, G_{β1γ2}, and RGS4, or the hH₄R–RGS19 fusion protein with G_{ia2} and G_{β1γ2} (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 gpH₁R and gpH₂R, respectively (see the Supporting Information).

Reference acylguanidine **3** was similar in potency to histamine at the hH₄R,⁸ had high affinity but low intrinsic activity at the hH₃R, was ~35 times more potent than histamine at the hH₂R, and was a weak antagonist at the hH₁R. **4**, which is considered an essential moiety for agonistic activity of such acylguanidine-type H₂R agonists,⁸ was approximately half as potent as histamine at the hH₂R and acted as a partial agonist (pEC₅₀ = 5.59; α = 0.66). This is in accordance with previous findings at the guinea pig right atrium (H₂R).⁹ At the hH₃R and hH₄R, **4** exhibited similar pEC₅₀ values of ≈8.1 and intrinsic activities of ≈0.7–0.8, whereas the compound was almost inactive at the hH₁R. These results suggest that the imidazolypropylguanidine moiety is a suitable agonist structure not just at the hH₂R, but also at the hH₃R and hH₄R.

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 hH₄R 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 hH₄R 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 hH₄R 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 hH₂R and thereby produced a diminished level of discrimination between the hH₂R and hH₄R. Intrinsic activity at the hH₃R was abolished. In compounds **8–12**, indole-2-carboxylic acid is attached to the imidazolypropylguanidine 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 H₄R 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 hH₁R (pEC₅₀ = 5.77; α = 0.42). At the hH₂R, **8** was ~10 times more potent than **4** and exhibited slightly elevated intrinsic activity (pEC₅₀ = 6.54; α = 0.87). In contrast, at the hH₃R and hH₄R, **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 hH₂R activity; compared to that of **8**, the agonistic activity dropped remarkably (pEC₅₀ = 6.02; α = 0.51). At the hH₃R and hH₄R, **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 hH₂R by factors of 10 (pEC₅₀ = 7.08) and 30 (pEC₅₀ = 7.52), respectively. Evidently, the increased flexibility of the higher homologues favored high hH₂R activity. The hH₃R and hH₄R affinities remained largely unaffected, whereas the intrinsic activities at these receptors were significantly reduced. At all hH_xR, especially at the hH₂R, *N*-methylation of the amide nitrogen (**11**) increased intrinsic activities, but the affinities at the hH₂R and hH₄R (pEC₅₀ values of 6.46 and 6.82, respectively) were reduced, suggesting that the amide NH group contributes to hH₂R and hH₄R binding, although steric factors may also play a role. In contrast, at the hH₃R, **11** was more active as a partial agonist than unmethylated analogue **10**. Taken together, the hybrid approach, combining hH₄R agonistic (**4**) and antagonistic (**5**) structural elements, was unsuccessful with respect to improving hH₄R selectivity.

The second approach to increasing selectivity for the hH₄R, in particular over that of the hH₂R, was focused on the introduction of small *N*^G-alkanoyl residues instead of the larger arylalkanoyl groups found to be useful in H₂R agonists.⁸ This idea was stimulated by the fact that the natural agonist, histamine, is 100 times more active at the hH₄R than at the hH₂R (pEC₅₀ values of 7.92 and 5.92, respectively). Obviously, additional affinity-conferring substituents are not required to achieve low nanomolar hH₄R activities. Acetylation of the guanidine group yielded **13** (UR-PI288) and provided a moderate increase in agonistic activity (pEC₅₀ = 6.14; α = 0.76) relative to that of **4** at the hH₂R. As expected, the activities of the acylguanidines at the hH₂R further increased (pEC₅₀ = 6.14 → pEC₅₀ = 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 hH₂R 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 hH₁R. With regard to the hH₃R, acetylation of the guanidine group in **4** slightly increased affinity but substantially decreased intrinsic activity (pEC₅₀ = 8.44; α = 0.27). Likewise, substitution of the guanidine group with larger alkanoyl residues was poorly tolerated in terms of hH₃R intrinsic activity. Obviously, acylation of the guanidine group modifies the interaction of the imidazolypropylguanidine moiety with the hH₃R in a manner that impedes the stabilization of an active receptor conformation. Contrary to the structure–activity relationships at the hH₃R, the alkanoyl residue was beneficial for the activation of the hH₄R. The *N*^G-alkanoyl imidazoly-

propylguanidines **13–16** were highly active (almost) full agonists at the hH₄R (pEC₅₀ = 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 hH₄R at the expense of hH₃R agonism.

Summary and Conclusion

Starting from the potent nonselective acylguanidine-type hH₄R agonist **3**, which was initially designed and synthesized as an H₂R agonist,⁸ we identified parent compound **4** (weak partial agonist at the H₂R⁹) as a highly active hH₃R and hH₄R partial agonist. With the aim of increasing the selectivity of the acylguanidine-type compounds for the hH₄R, 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 hH₄R 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 hH₄R activity was not substantially improved. An explanation therefore may be provided by an hH₄R 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 H₂R,^{8,15} histamine and N^G-acylated imidazolylpropylguanidines also predominantly interact with identical amino acid residues at the hH₄R, 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 hH₂R also increased, confirming the importance of the acyl group as an affinity-conferring moiety at the hH₂R. At the hH₃R, acylation drastically lowered efficacy, whereas the same compounds turned out to be highly potent full (or nearly full) hH₄R agonists. Thus, although the imidazolylpropylguanidine portion is capable of stimulating the hH₂R, hH₃R, and hH₄R, selectivity can be achieved by appropriate N^G-acylation.

hH₄R agonists **13–16** are among the most active hH₄R agonists reported to date and may become useful experimental tools in addition to previously described H₄R ligands like selective hH₄R agonist **2**² or selective H₄R antagonist **5**¹⁰ to analyze the as yet incompletely understood (patho)physiological functions of the H₄R. In most immune cells like mast cells or eosinophils, where the H₄R is mainly located, H₃Rs are not expressed.^{4,16,17} Therefore, on the basis of the >100-fold selectivity relative to the hH₁R and hH₂R subtypes, N^G-alkanoylimidazolylpropylguanidines like **13** may also be useful for investigating the function of the hH₄R in such native cells devoid of the hH₃R. Moreover, these potent hH₄R agonists may be suitable pharmacological probes for desensitization studies with the hH₄R.

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 Na₂SO₄. The solvent was evaporated and the crude product purified by flash chromatography.

N¹-Acetyl-N²-(tert-butoxycarbonyl)-N³-[3-(1-trityl-1H-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 (CHCl₃/MeOH, 97.5/2.5, v/v) yielded a colorless oil (360 mg, 65%): ¹H NMR (300 MHz, CDCl₃) 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 + NH₄OAc) *m/z* (%) 552 (100) [M + H]⁺. C₃₃H₃₇N₅O₃ (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.

N¹-Acetyl-N²-[3-(1H-imidazol-4-yl)propyl]guanidinium Ditri-fluoroacetate (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%): ¹H NMR (300 MHz, D₂O, 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); ¹³C NMR (75 MHz, D₂O, 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 C₉H₁₅N₅O [M⁺] 209.1277, found 209.1275. C₉H₁₅N₅O·2TFA (437.30).

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

Acknowledgment. We are grateful to Mrs. Kerstin Fisch, Mrs. Karin Schadendorf, Mrs. Gertraud Wilberg, Mrs. Christine Braun, and Mrs. Kerstin Röhl for expert technical assistance. This work was supported by the Graduate Training Program (Graduiertenkolleg) GRK 760, “Medicinal Chemistry: Molecular Recognition-Ligand-Receptor Interactions”, of the Deutsche Forschungsgemeinschaft.

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 (gpH₁R) and guinea pig right atrium (gpH₂R); and pharmacological methods. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Hill, S. J.; Ganellin, C. R.; Timmerman, H.; Schwartz, J.-C.; Shankley, N. P.; Young, J. M.; Schunack, W.; Levi, R.; Haas, H. L. International union of pharmacology. XIII. Classification of histamine receptors. *Pharmacol. Rev.* **1997**, *49*, 253–278.
- (2) Lim, H. D.; van Rijn, R. M.; Ling, P.; Bakker, R. A.; Thurmond, R. L.; Leurs, R. Evaluation of histamine H₁-, H₂-, and H₃-receptor ligands at the human histamine H₄ receptor: Identification of 4-methylhistamine as the first potent and selective H₄ receptor agonist. *J. Pharmacol. Exp. Ther.* **2005**, *314*, 1310–1321.
- (3) Lim, H. D.; Smits, R. A.; Leurs, R.; De Esch, I. J. The emerging role of the histamine H₄ receptor in anti-inflammatory therapy. *Curr. Top. Med. Chem.* **2006**, *6*, 1365–1373.
- (4) Thurmond, R. L.; Gelfand, E. W.; Dunford, P. J. The role of histamine H₁ and H₄ receptors in allergic inflammation: The search for new antihistamines. *Nat. Rev. Drug Discovery* **2008**, *7*, 41–53.
- (5) Hashimoto, T.; Harusawa, S.; Araki, L.; Zuiderveld, O. P.; Smit, M. J.; Imazu, T.; Takashima, S.; Yamamoto, Y.; Sakamoto, Y.; Kurihara, T.; Leurs, R.; Bakker, R. A.; Yamatodani, A. A selective human H₄-receptor agonist: (–)-2-Cyano-1-methyl-3-[(2*R*,5*R*)-5-[(1*H*-imidazol-4(5)-yl]tetrahydrofuran-2-yl)methyl]guanidine. *J. Med. Chem.* **2003**, *46*, 3162–3165.
- (6) Xie, S.-X.; Ghorai, P.; Ye, Q.-Z.; Buschauer, A.; Seifert, R. Probing ligand-specific histamine H₁- and H₂-receptor conformations with *N*^G-acylated imidazolylpropylguanidines. *J. Pharmacol. Exp. Ther.* **2006**, *317*, 139–146.
- (7) Xie, S.-X.; Kraus, A.; Ghorai, P.; Ye, Q.-Z.; Elz, S.; Buschauer, A.; Seifert, R. *N*¹-(3-Cyclohexylbutanoyl)-*N*²-[3-[(1*H*-imidazol-4-yl)propyl]guanidine (UR-AK57), a potent partial agonist for the human histamine H₁- and H₂-receptors. *J. Pharmacol. Exp. Ther.* **2006**, *317*, 1262–1268.
- (8) Ghorai, P.; Kraus, A.; Keller, M.; Götte, C.; Igel, P.; Schneider, E.; Schnell, D.; Bernhardt, G.; Dove, S.; Zabel, M.; Elz, S.; Seifert, R.; Buschauer, A. Acylguanidines as bioisosteres of guanidines: *N*^G-Acylated imidazolylpropylguanidines, a new class of histamine H₂ receptor agonists. *J. Med. Chem.* **2008**, *51*, 7193–7204.
- (9) Parsons, M. E.; Blakemore, R. C.; Durant, G. J.; Ganellin, C. R.; Rasmussen, A. C. 3-[4(5)-Imidazolyl]propylguanidine (SK&F 91486): A partial agonist at histamine H₂-receptors. *Agents Actions* **1975**, *5*, 464.
- (10) Jablonowski, J. A.; Grice, C. A.; Chai, W.; Dvorak, C. A.; Venable, J. D.; Kwok, A. K.; Ly, K. S.; Wei, J.; Baker, S. M.; Desai, P. J.; Jiang, W.; Wilson, S. J.; Thurmond, R. L.; Karlsson, L.; Edwards, J. P.; Lovenberg, T. W.; Carruthers, N. I. The first potent and selective non-imidazole human histamine H₄ receptor antagonists. *J. Med. Chem.* **2003**, *46*, 3957–3960.
- (11) The p*K*_a value was calculated with ACD Labs 9.0, Advanced Chemistry Development, Toronto, ON.
- (12) Schneider, E.; Keller, M.; Brennauer, A.; Hoefelschweiger, B. K.; Gross, D.; Wolfbeis, O. S.; Bernhardt, G.; Buschauer, A. Synthesis and characterization of the first fluorescent nonpeptide NPY Y₁ receptor antagonist. *ChemBioChem* **2007**, *8*, 1981–1988.
- (13) Kiss, R.; Noszál, B.; Rácz, Á.; Falus, A.; Erős, D.; Keseru, G. M. Binding mode analysis and enrichment studies on homology models of the human histamine H₄ receptor. *Eur. J. Med. Chem.* **2008**, *43*, 1059–1070.
- (14) Igel, P.; Schnell, D.; Bernhardt, G.; Seifert, R.; Buschauer, A. Tritium-labeled *N*¹-[3-[(1*H*-imidazol-4-yl)propyl]-*N*²-propionyl]guanidine ([³H]UR-PI294), a high-affinity histamine H₃ and H₄ receptor radioligand. *ChemMedChem* **2009**, *4*, 225–231.
- (15) Nederkoorn, P. H. J.; van Lenthe, J. H.; van der Goot, H.; Donné-Op den Kelder, G. M.; Timmermann, H. The agonistic binding site at the histamine H₂ receptor. I. Theoretical investigations of histamine binding to an oligopeptide mimicking a part of the fifth transmembrane α-helix. *J. Comput.-Aided Mol. Des.* **1996**, *10*, 461–478.
- (16) Ling, P.; Ngo, K.; Nguyen, S.; Thurmond, R. L.; Edwards, J. P.; Karlsson, L.; Fung-Leung, W.-P. Histamine H₄ receptor mediates eosinophil chemotaxis with cell shape change and adhesion molecule upregulation. *Br. J. Pharmacol.* **2004**, *142*, 161–171.
- (17) Lippert, U.; Artuc, M.; Grutzkau, A.; Babina, M.; Guhl, S.; Haase, I.; Blaschke, V.; Zachmann, K.; Knosalla, M.; Middel, P.; Kruger-Krasagakis, S.; Henz, B. M. Human skin mast cells express H₂ and H₄, but not H₃ receptors. *J. Invest. Dermatol.* **2003**, *123*, 116–123.

JM9000693