A Chemical Switch for the Modulation of the Functional Activity of Higher Homologues of Histamine on the Human Histamine H_3 Receptor: Effect of Various Substitutions at the Primary Amino Function

Marinella Govoni, Herman D. Lim, Dris El-Atmioui, Wiro M. P. B. Menge, Henk Timmerman, Remko A. Bakker, Rob Leurs, and Iwan J. P. De Esch*

Leiden/Amsterdam Center for Drug Research, Department of Pharmacochemistry, Vrije Universiteit Amsterdam, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands

Received May 8, 2005

In an effort to establish the structural requirements for agonism, neutral antagonism, and inverse agonism at the human histamine H_3 receptor (H_3R) we have prepared a series of higher homologues of histamine in which the terminal nitrogen of the side chain has been either mono- or disubstituted with several aliphatic, alicyclic, and aromatic moieties or incorporated in cyclic systems. The novel ligands have been pharmacologically investigated in vitro for their affinities on the human H_3R and H_4R subtypes by radioligand displacement experiments and for their intrinsic H_3R activities via a CRE-mediated β-galactosidase reporter gene assay. Subtle changes of the substitution pattern at the side chain nitrogen alter enormously the pharmacological activity of the ligands, resulting in a series of compounds with a wide spectrum of pharmacological activities. Among the several neutral H_3R antagonists identified within this series, compounds **2b** and **2h** display an H_3R affinity in the low nanomolar concentration range (pK_i values of 8.1 and 8.4, respectively). A very potent and selective H_3R agonist (**1l**, $pEC_{50} = 8.9$, $\alpha = 0.94$) and a very potent, though not highly selective, H_3R inverse agonist (**2k**, $pIC_{50} = 8.9$, $\alpha = -0.97$) have been identified as well.

Introduction

The biogenic amine histamine exerts its multiple biological activities through the activation of at least four distinct histamine receptors, i.e., H_1, H_2, H_3, and H_4 receptors,[1−4] all belonging to the superfamily of heptahelical (7TM) G-protein coupled receptors.

The histamine H_3 receptor (H_3R) was discovered in 1983 by Arrang and co-workers as a presynaptic autoreceptor,[5] and the gene was successively cloned in 1999 by Lovenberg and colleagues.[3] Tissue distribution analysis indicated that the expression of the receptor is predominantly restricted to the brain.[6] The H_3R does not only mediate the inhibition of synthesis and release of histamine from histaminergic neurons via a negative feedback loop,[7,8] but also exerts modulatory effects on other neurotransmitter systems, e.g. the cholinergic,[9,10] dopaminergic,[11] noradrenergic,[12] and serotoninergic[13] systems, in both the central and peripheral nervous systems. It has been recognized that the H_3R is a potential therapeutic target.[14−16] H_3R antagonists have been proposed to be potential drugs for the treatment of several CNS disorders, such as attention-deficit hyperactivity disorder (ADHD),[17,18] Alzheimer's disease,[19] epilepsy,[20−22] schizophrenia,[22,23] and obesity,[24−26] whereas the therapeutic potential of H_3R agonists has been shown for myocardial ischemia,[27] inflammatory[28] and gastric acid related diseases,[29] migraine, and sleep disorders.[30]

The constitutive activity of several 7TM receptor systems has been investigated within the past decade.[31−36] For a long time it was not known whether this phenomenon existed only in cells overexpressing (mutant) GPCRs or also occurred in vivo. Constitutive activity has been shown recently for both the histamine H_1 and H_2 receptors.[37,38] Our group reported that the therapeutically important H_1 and H_2 receptor antagonists act, in fact, as inverse agonists, but we also identified neutral antagonists for both receptors.[37,39] Histaminergic neurotransmission in rodent brain was shown to be regulated by constitutively active H_3Rs in vitro as well as in vivo,[40] and our group also demonstrated that the human and rat H_3Rs stably expressed in SK-N-MC cells[3,6] show a high level of constitutive activity.[41,42] This led to the identification of several standard H_3R antagonists (thioperamide and clobenpropit) as inverse agonists in this cell system. Moreover, burimamide and impentamine (Figure 1), previously identified as H_3R antagonists,[5,43,44] behave as H_3R agonists at both recombinant H_3Rs (human and rat). We also showed that in a small series of impentamine analogues we were able to manipulate the intrinsic activity (α) by substitution of the amino group.[41]

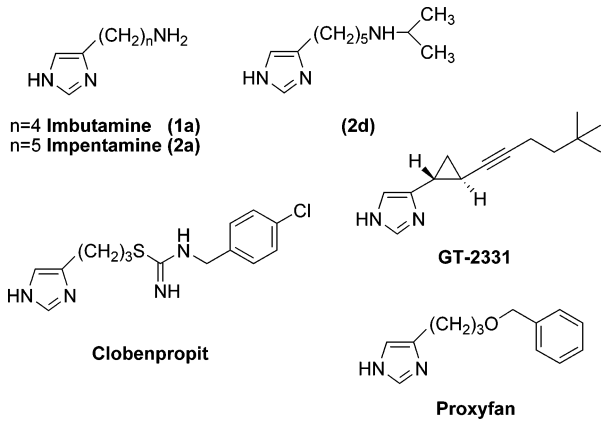


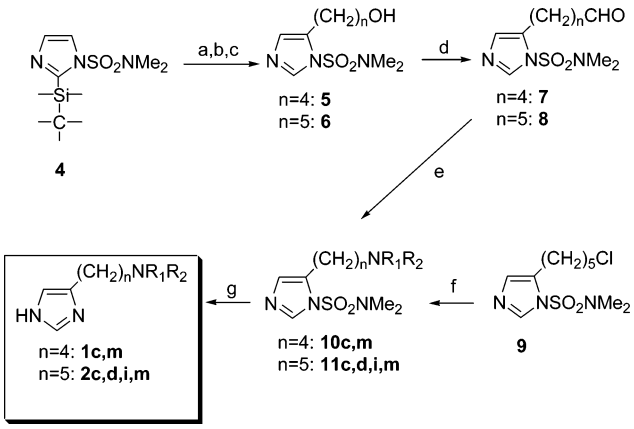
Figure 1. Structures of 4-[1*H*-imidazol-4-yl]butylamine (imbutamine, **1a**), 5-[1*H*-imidazol-4-yl]pentylamine (impentamine, **2a**), [5-(1*H*-imidazol-4-yl)pentyl]isopropylamine (**2d**), clobenpropit, GT-2331 (cipralisant, Perceptin), and proxyfan.

In an attempt to establish a correlation between the structure of the ligands and their functional activity at the human H_3R,

* Author for correspondence. Tel: 31−20−597600. Fax: 31−20−5987610. E-mail: i.de.esch@few.vu.nl.

10.1021/jm0504353 CCC: $33.50
Published on Web 03/22/2006

Scheme 1. Synthesis of Compounds **1c**,**m** and **2c**,**d**,**i**,**m**[a]



[a] Reagents: (a) *n*-BuLi, THF, −70 °C; (b) 2-(*ω*-iodoalkyloxy)tetrahydropyran; (c) HCl, rt; (d) Swern oxidation; (e) R_1R_2-amine, AcOH, $NaBH(OAc)_3$, DCE, rt (method B, reductive amination); (f) R_1R_2-amine, 100 °C (method A); (g) 30% HBr or 37% HCl, reflux (method D). R_1R_2-amines are given in Tables 1 and 2.

we report here the synthesis, affinity, and intrinsic H_3R activity of a variety of analogues of imbutamine and impentamine (**1a** and **2a**, Figure 1) in which the primary amino function has been mono- or disubstituted with several aliphatic and aromatic moieties. All the ligands were additionally screened for their affinities at the human histamine H_4 receptor (H_4R) subtype in order to evaluate H_3 versus H_4 receptor selectivity of the compounds.

Chemistry

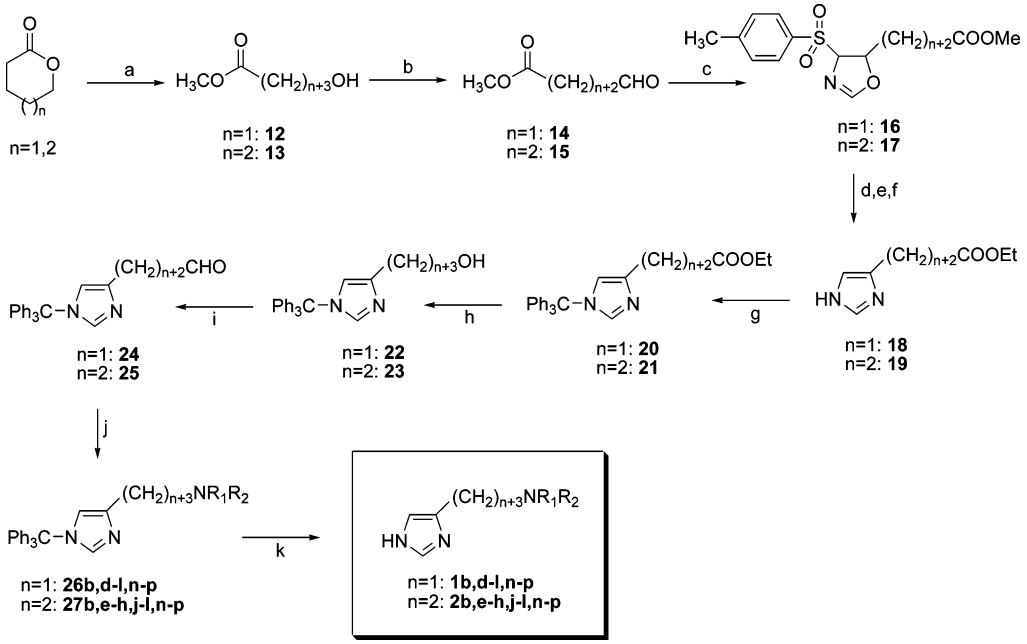
Aminoalkylimidazoles **1c**,**m** and **2c**,**m** were prepared by lithiation of a suitable 1,2-diprotected imidazole **4**[45] and subsequent treatment with 2-(*ω*-iodoalkyloxy)tetrahydropyrans to give compounds **5** and **6** in moderate yields (35−40%, Scheme 1). The *ω*-hydroxy group of **5** and **6** was converted into an aldehyde via a Swern oxidation[46,47] followed by reductive amination[48] with the appropriate amine in the presence of sodium triacetoxyborohydride and cleavage of the dimethylsulfamoyl protecting group to give compounds **1c**,**m** and **2c**,**m** in moderate to good yield (44−70%). Alternatively, the *ω*-chloro group of **9**[44] was converted either into an isopropylamino group or into a cyclohexylamino group via heating with isopropylamine and cyclohexylamine, respectively, to give compounds **11d**,**i**. Deprotection yielded compounds **2d**,**i**.

Aminoalkylimidazoles **1b**,**d**−**l**,**n**−**p** and **2b**,**e**−**h**,**j**−**l**,**n**−**p** were prepared according to the synthetic pathway shown in Scheme 2. Methanolysis of *δ*-valerolactone and *ϵ*-caprolactone followed by Swern oxidation of esters **12** and **13** afforded aldehydes **14** and **15**, which were readily converted into imidazoles using TosMIC chemistry.[49] The intermediate 4-tosyloxazolines **16** and **17** were treated with ammonia in ethanol under pressure, affording a mixture of amide and methyl and ethyl ester which were converted into the carboxylic acid via acidic hydrolysis and then re-esterified to give esters **18** and **19** in good yields (82% and 51%, respectively). Protection of the imidazole ring with a trityl group, followed by reduction of esters **20** and **21** with lithium aluminum hydride and Swern oxidation of the alcohols **22** and **23**, yielded the key intermediates **24** and **25** (49% and 70% yield). Reductive amination with a suitable amine and subsequent deprotection afforded the final compounds **1b**,**d**−**l**,**n**−**p** and **2b**,**e**−**h**,**j**−**l**,**n**−**p**, in variable isolated yields (17−95%).

Pharmacology

Radioligand Displacement Studies. Homogenates of SK-N-MC cells, stably expressing either the human H_3R[3] or the human H_4R,[50] were used for determining ligand affinities for the H_3R and H_4R, respectively, as previously described.[51] Cell homogenates of H_3R-expressing cells (475 ± 32 fmol/mg of protein) were incubated for 40 min at 25 °C with 0.9−1.1 nM [^{3}H]-N^{α}-methylhistamine (82 Ci/mmol) in 50 mM sodium phosphate buffer (pH 7.4) with or without competing ligands; cell homogenates of H_4R-expressing cells (620 ± 44 fmol/mg of protein) were incubated for 60 min at 37 °C with 9−11 nM [^{3}H]histamine (23.2 Ci/mmol) in 50 mM Tris HCl (pH 7.4),

Scheme 2. Synthesis of Compounds **1b**,**d**−**l**,**n**−**p** and **2b**,**e**−**h**,**j**−**l**,**n**−**p**[a]



[a] Reagents: (a) MeOH, H_2SO_4, reflux; (b) Swern oxidation; (c) TosMIC, NaCN, abs EtOH; (d) NH_3/EtOH, 120−140 °C, 10 atm; (e) HCl, reflux; (f) EtOH, H_2SO_4, reflux; (g) triphenylmethyl chloride, triethylamine, DCM, rt; (h) $LiAlH_4$, THF, rt; (i) Swern oxidation; (j) R_1R_2-amine, AcOH, $NaBH(OAc)_3$, DCE, rt (method B, reductive amination); (k) 1 M HCl or 1 M HBr, reflux (method C). R_1R_2-amines are given in Tables 1 and 2.

with or without competing ligands. Incubations were terminated by rapid dilution and subsequent filtration over Whatman GF/C filters pretreated with 0.3% polyethyleneimine and the residue was subsequently washed using ice-cold wash buffer (H_3R: 25 mM Tris HCl, 145 mM NaCl, pH 7.4 at 4 °C; H_4R: 50 mM Tris HCl, pH 7.4 at 4 °C). The radioactivity retained on the filters was measured by liquid scintillation counting. Nonspecific binding was determined with 1 μM clobenpropit as competing ligand. Competition isotherms were evaluated by a nonlinear, least squares curve-fitting using GraphPad Prism (GraphPad Software, Inc., San Diego, CA).

Colorimetric cAMP Assay. Colorimetric cAMP assays were performed as previously described.[51] Briefly, SK-N-MC cells stably expressing the human H_3R[3] and a cyclic AMP responsive element (CRE)-β-galactosidase reporter gene were grown overnight in 96-well plates before the assay. To start the assay, the cells were incubated for 6 h with 1 μM forskolin and respective ligands in a humidified incubator at 37 °C. Thereafter, the medium was aspirated and cells were incubated overnight at room temperature with 100 μL of assay buffer [100 mM NaH_2PO_4, 100 mM Na_2HPO_4, pH 8, 2 mM $MgSO_4$, 0.1 mM $MnCl_2$, 0.5% Triton, 40 mM β-mercaptoethanol, and 4 mM *o*-nitrophenyl-β-D-galactopyranoside (ONPG)]. The absorbance at 405 nm was determined by using a Victor[2] plate reader (Perkin-Elmer).

Analytical Methods. Protein levels were determined spectrophotometrically by a Packard Argus 400 microplate reader according to the method of Bradford,[52] using bovine serum albumin as a standard. All data shown are expressed as a mean ± SEM.

Results and Discussion

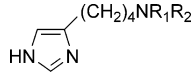
A previous study from our group identified compound **2d** (Figure 1 and Table 2), an impentamine analogue with an isopropyl group at the amino group of the side chain, as a neutral H_3R antagonist[41] in SK-N-MC cells stably transfected with the human H_3R.[3] In the same study it was also shown that the modification of the primary amino group of the H_3R agonist impentamine, via substitution or incorporation in a piperidine ring, dramatically affected the pharmacological activity of the ligands, resulting in a series of compounds spanning the whole spectrum of pharmacological activities.

The amine function of impentamine is thought to interact with the aspartate residue Asp[114] in transmembrane domain 3, which is highly conserved in the family of biogenic amine receptors.[53,54]

In view of the fact that this series of ligands may be of great help to understand the mechanism of receptor (in)activation, we have prepared a series of imbutamine and impentamine analogues in which the primary amino function has been mono- or disubstituted with either a variety of aliphatic (**1b**−**g**,**l**,**m**, **2b**−**g**,**l**,**m**), alicyclic (**1h**−**j**, **2h**−**j**), or aromatic moieties (**1k**, **2k**) or incorporated in cyclic systems (**1n**−**p**, **2n**−**p**).

As shown in Table 1, substitution of the N^{α}-position of imbutamine (**1a**) caused in general a decrease in the H_3R affinity, with the exception of compounds **1f**, bearing an isobutyl substituent, and N^{α},N^{α}-dimethylimbutamine (**1l**), in which the terminal amino function is dimethylated. With a pEC_{50} of 8.9, **1l** is the most potent ligand of this series and it displays a nearly full agonistic intrinsic activity ($\alpha = 0.94$) on the human H_3R. This behavior has already been observed in the case of histamine, where mono- or dimethylation of the amino function of the side chain results in agonists that are more active than histamine at the H_3R.[5,55] Surprisingly, the dimethylated analogue of impentamine, **2l** (Table 2), shows instead reduced affinity and potency, with a pK_i value of 7.8 and a pEC_{50} value of 7.5, as well as a diminished intrinsic H_3R activity, resulting in partial H_3R agonism ($\alpha = 0.68$). It must be noted, however, that the parent compound **2a**, opposite to what was observed in our previous study, where it behaved as a nearly full agonist ($\alpha = 0.9$),[41] is now found to be also a partial agonist ($\alpha = 0.71$), with almost the same intrinsic activity as **2l**. A discussion of these differences is described below. A further increase of the steric hindrance on the primary amine, as achieved via introduction of a diethylamino group, is generally not well-tolerated, as shown by the decreased H_3R affinities of compounds **1m** and **2m**. Parallel to the decrease in the H_3R affinity, a diminished H_3R intrinsic activity is observed; in fact, both ligands are neutral H_3R antagonists.

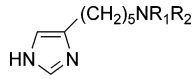
Table 1. Chemical Structures and Pharmacological Properties of Compounds **1a**−**p** for the Human H_3 (hH_3R) and H_4 (hH_4R) Receptors[a]



			hH_3R			hH_4R:
compd	R_1	R_2	pK_i[b]	pEC_{50}[b]	α[b,c]	pK_i
1a	H	H	8.4 ± 0.1	8.3 ± 0.1	0.98 ± 0.02	8.0 ± 0.1
1b	H	Et	7.6 ± 0.1	7.6 ± 0.1	0.73 ± 0.10	7.0 ± 0.1
1c	H	n-Pr	8.1 ± 0.1	7.8 ± 0.1	0.45 ± 0.07	7.3 ± 0.1
1d	H	i-Pr	7.1 ± 0.1	−[d]	0.10 ± 0.08	6.7 ± 0.1
1e	H	n-Bu	8.2 ± 0.1	8.3 ± 0.1	0.55 ± 0.09	7.2 ± 0.1
1f	H	i-Bu	8.5 ± 0.1	8.1 ± 0.1[e]	−0.14 ± 0.10	7.1 ± 0.1
1g	H	t-Bu	6.2 ± 0.1	−[d]	0.01 ± 0.06	6.1 ± 0.1
1h	H	cyclopentyl	7.4 ± 0.1	−[f]	−[d]	7.4 ± 0.1
1i	H	cyclohexyl	7.5 ± 0.1	−[f]	−[d]	7.0 ± 0.1
1j	H	cycloheptyl	7.4 ± 0.1	−[d]	0.00 ± 0.05	7.3 ± 0.0
1k		p-Cl-benzyl	7.8 ± 0.1	−[f]	−[d]	7.7 ± 0.1
1l	Me	Me	8.5 ± 0.1	8.9 ± 0.1	0.94 ± 0.04	6.1 ± 0.1
1m	Et	Et	7.0 ± 0.1	−[d]	0.07 ± 0.08	6.5 ± 0.1
1n		$-(CH_2)_4-$	7.4 ± 0.1	7.4 ± 0.1	0.47 ± 0.11	6.3 ± 0.1
1o		$-(CH_2)_5-$	7.7 ± 0.1	7.5 ± 0.1	0.64 ± 0.04	6.4 ± 0.2
1p		$-(CH_2)_6-$	7.4 ± 0.1	7.3 ± 0.2	0.35 ± 0.07	6.3 ± 0.1

[a] The values are expressed as means ± SEM of separate experiments, each performed in triplicate, unless indicated otherwise. [b] $N > 3$. [c] α = intrinsic activity value. [d] Could not be estimated. [e] pIC_{50} value. [f] Biphasic behavior (partial agonism up to 1 μM, non-H_3R-mediated effects at 10 and 100 μM).

Table 2. Chemical Structures and Pharmacological Properties of Compounds **2a**−**p** for the Human H_3 (hH_3R) and H_4 (hH_4R) Receptors[a]



			hH_3R			hH_4R:
compd	R_1	R_2	pK_i[b]	pEC_{50}[b]	α[b,c]	pKi
2a	H	H	8.3 ± 0.1	8.3 ± 0.1	0.71 ± 0.05	6.6 ± 0.1
2b	H	Et	8.1 ± 0.1	−[d]	0.11 ± 0.09	6.5 ± 0.1
2c	H	n-Pr	8.3 ± 0.1	8.0 ± 0.1	0.44 ± 0.11	6.8 ± 0.1
2d	H	i-Pr	7.9 ± 0.1	7.4 ± 0.1[e]	−0.55 ± 0.11	6.9 ± 0.1
2e	H	n-Bu	8.4 ± 0.1	8.3 ± 0.1	0.55 ± 0.09	6.7 ± 0.1
2f	H	i-Bu	8.8 ± 0.1	8.1 ± 0.2	0.35 ± 0.07	6.9 ± 0.1
2g	H	t-Bu	7.4 ± 0.1	−[d]	0.04 ± 0.05	7.2 ± 0.1
2h	H	cyclopentyl	8.4 ± 0.1	−[d]	0.02 ± 0.08	7.4 ± 0.1
2i	H	cyclohexyl	8.0 ± 0.1	7.9 ± 0.1[e]	−0.51 ± 0.12	7.4 ± 0.1
2j	H	cycloheptyl	8.5 ± 0.1	8.0 ± 0.1[e]	−0.65 ± 0.03	7.3 ± 0.1
2k		p-Cl-benzyl	8.6 ± 0.1	8.9 ± 0.2[e]	−0.97 ± 0.01	7.2 ± 0.2
2l	Me	Me	7.8 ± 0.2	7.5 ± 0.2	0.68 ± 0.08	6.3 ± 0.1
2m	Et	Et	7.2 ± 0.1	−[d]	0.06 ± 0.02	6.2 ± 0.1
2n		$-(CH_2)_4-$	7.5 ± 0.1	7.5 ± 0.1	0.32 ± 0.07	6.1 ± 0.1
2o		$-(CH_2)_5-$	8.1 ± 0.1	7.7 ± 0.1	0.46 ± 0.08	6.5 ± 0.1
2p		$-(CH_2)_6-$	7.8 ± 0.1	7.3 ± 0.2	0.22 ± 0.07	6.8 ± 0.1

[a] The values are expressed as means ± SEM of separate experiments, each performed in triplicate, unless indicated otherwise. [b] $N > 3$. [c] α = intrinsic activity value. [d] Could not be estimated. [e] pIC_{50} value.

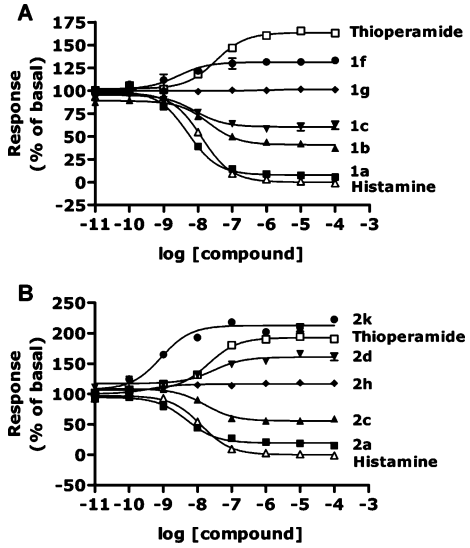


Figure 2. The effects of several selected compounds on the basal signaling of the H_3R, as measured by CRE-mediated β-galactosidase reporter gene assay in forskolin-stimulated (1 μM) SK-N-MC cells. (A) Dose−response curves of the full H_3R agonist **1a** (■, imbutamine), the partial H_3R agonists **1b** (▲) and **1c** (▼), the neutral H_3R antagonist **1g** (◆), and the partial inverse H_3R agonists **1f** (●) (see also Table 1). (B) Dose−response curves of the partial H_3R agonists **2a** (■, impentamine) and **2c** (▲), the neutral H_3R antagonist **2h** (◆), the partial inverse H_3R agonist **2d** (▼), and the full inverse H_3R agonist **2k** (●) (see also Table 2). Representative dose−response curves of the full H_3R agonist histamine (△) and of the partial inverse H_3R agonist thioperamide (□) are shown (A and B). Data are normalized to the basal signaling observed in the assay (set to 100%).

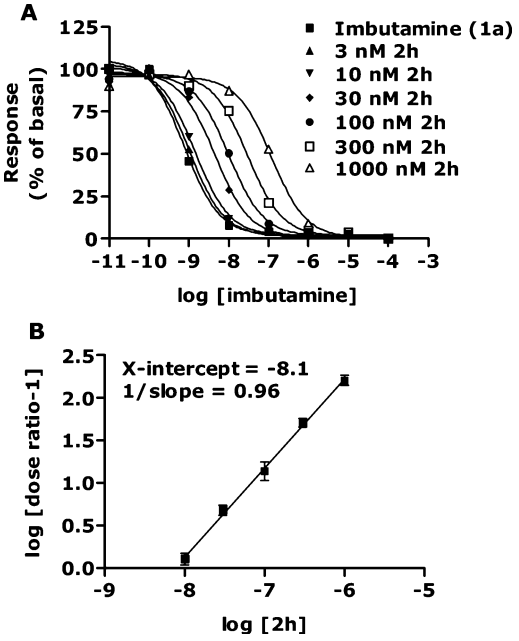


Figure 3. (A) Rightward shift of concentration−response curves of imbutamine (**1a**) in the presence of increasing concentrations of N^{α}-cyclopentylimpentamine (**2h**) determined by the inhibition of the CRE-stimulated β-galactosidase transcription in SK-N-MC cells stably expressing the human H_3R. (B) The log of the (dose ratio − 1) at each concentration of antagonist was plotted versus the log of **2h** concentration. The pA_2 value was determined from the *x*-intercept on the linear Schild regression plot and was in this particular experiment 8.1. The data points represent means ± SEM of triplicate determinations from one representative experiment.

In the impentamine series shown in Table 2, compounds **2c**, **2e**, **2f**, **2h**, **2j**, and **2k** display an H_3R affinity comparable to that of the parent compound **2a** or slightly increased.

Upon comparing imbutamine and impentamine derivatives, it can be appreciated that, with the exception of the parent compounds (**1a**, **2a**) and those bearing a dimethyl substitution (**1l**, **2l**), the H_3R affinities are higher for the impentamine analogues. Alkylation in the N^{α}-position of **1a** and **2a** with longer alkyl groups, such as ethyl, *n*-propyl, or *n*-butyl, has only limited effect on the H_3R affinity in both series (**1b**,**c**,**e** and **2b**,**c**,**e**), while ramification to isopropyl (**1d**, **2d**) or *tert*-butyl (**1g**, **2g**) is detrimental for H_3R affinity (see Tables 1 and 2). Alkylation of the histamine side-chain terminal nitrogen with bulky groups leads to a loss of H_3R intrinsic activity, generating partial agonists[56] or antagonists.[57] Similarly, alkylation of imbutamine and impentamine with the above-mentioned residues, e.g. ethyl, *n*-propyl, isopropyl, *n*-butyl, and *tert*-butyl, causes a decrease in the intrinsic activity of the compounds (Figure 2), therefore generating partial agonists (**1b**,**c**,**e** and **2c**,**e**) or neutral antagonists (**1d**,**g** and **2b**,**g**, Tables 1 and 2). Remarkably, compound **2d** (Table 2), also bearing an isopropyl moiety, which was identified as a neutral antagonist ($\alpha = -0.10 \pm 0.10$) in our previous study,[41] in the present research behaves as a partial inverse agonist ($\alpha = -0.55$). However, a different assay system has been used in the current study that is more sensitive than the classical cAMP measurements used in the previous study.[41] Thus, enhancement of the van der Waals volume of the N^{α}-position diminishes agonist properties and increases antagonistic activity, but no definite relation between the substituting alkyl residue and the intrinsic H_3R activity of the compound can be derived. Surprisingly, ramification to isobutyl (**1f** and **2f**) generates compounds with higher H_3R affinity; in fact, compound **2f** (Table 2), with a pK_i of 8.8, exhibits the highest H_3R affinity among this series of ligands. Unfortunately, no definite correlation between the alkyl residue and the intrinsic H_3R activity could be observed. In fact, **1f** shows partial inverse agonism ($\alpha = -0.14$, Table 1), whereas **2f** is a partial agonist ($\alpha = 0.35$, Table 2).

Substitution with alicyclic residues (cyclopentyl, cyclohexyl or cycloheptyl) exerts a different behavior for imbutamine and impentamine derivatives; in the former, alkylation with either of the moieties consistently produces a 10-fold decrease in the H_3R affinity (**1h**−**j**, Table 1), whereas in the latter H_3R affinities are not significantly altered (**2h**−**j**, Table 2). As already seen in case of alkylation with aliphatic residues, insertion of alicyclic residues results in a loss of agonistic activity compared to the unsubstituted compounds; in fact, **1j** and **2h** are neutral antagonists, whereas **2i** and **2j** are partial inverse agonists.

In view of its nanomolar affinity for the H_3R (p$K_i = 8.4$) and lack of intrinsic H_3R activity ($\alpha = 0.02 \pm 0.08$), N^{α}-cyclopentylimpentamine (**2h**, Table 2) can be considered an interesting pharmacological tool. As depicted in Figure 3, ligand **2h** effectively antagonizes the response to the full agonist imbutamine (**1a**, Figure 3), yielding a pA_2 value of 8.1.

Ligands **1h** and **1i** (Table 1), respectively alkylated with a cyclopentyl and a cyclohexyl moiety, are also showing a diminished H_3R agonistic activity; in fact, they behave as partial agonists at concentrations up to 1 μM, but they turn into apparent (partial) inverse agonists at higher concentrations (10 and 100 μM). This "biphasic" behavior is probably due to non-H_3R-mediated effects at high concentrations that interfere with an accurate estimation of the intrinsic H_3R activity of the aforementioned compounds in our cell-based assay. Further experiments showed indeed that responses to **1h** and **1i** in SK-N-MC cells transfected with the human H_3R could not be antagonized by the neutral H_3R antagonist **2h** (data not shown).

Introduction of para-substituted phenyl rings has proven to be a successful strategy to improve affinity and/or potency of the compounds, as seen with the very potent H_3R antagonist clobenpropit (Figure 1).[1,58] However, introduction of a *p*-chlorobenzyl moiety on the primary amino function of **1a** and **2a**, yielding ligands **1k** and **2k** (Tables 1 and 2), results in an increased affinity only in the case of the impentamine derivative **2k** (about 4-fold more active than the parent compound **2a**, Table 2). The potency of ligand **2k** is also enhanced ($pEC_{50} = 8.9$), but remarkably a huge decrease in the intrinsic H_3R activity of the compound can be observed as well, leading to the only full inverse agonist ($\alpha = -0.97$) identified among the ligands investigated in this study.

Incorporation of the N^{α}-amino group of histamine into a pyrrolidine nucleus led to a compound with reduced potency and a maximal response that was only 56% with respect to histamine, implying that this compound is a partial H_3R agonist.[59] Similarly, incorporation of the amino group of imbutamine and impentamine in a pyrrolidine (**1n**, **2n**), piperidine (**1o**, **2o**), or azepane (**1p**, **2p**) ring generates ligands with reduced potency and intrinsic H_3R activity (Tables 1 and 2), all the compounds being partial agonists with α values ranging from 0.22 (**2p**) to 0.64 (**1o**). Ligand **2o** (Table 2), a partial agonist in this study, displaying an α value of 0.46, was previously found to behave as a full agonist ($\alpha = 1.00$).[41] Parallel to a diminished H_3R potency and intrinsic activity, these ligands also show H_3R affinities that are decreased by 4−10-fold, excluding compound **2o**, for which the decrease in affinity was less marked (pK_i of 8.1 vs a pK_i value of 8.3 for **2a**). The only apparent relation between the structures of the compounds and their activities is that affinity and potency values are slightly higher for the impentamine derivatives **2n**−**p** (Table 2) than for ligands **1n**−**p** (Table 1), while the opposite behavior could be observed for the intrinsic activity, compounds **1n**−**p** displaying higher partial agonistic activity over **2n**−**p**.

When compared to our previous study,[41] three of the six compounds analyzed in that research showed a different intrinsic H_3R activity. More precisely, impentamine (**2a**) and N^{α}-pyrrolidine-impentamine (**2o**) behaved in the present study as partial agonists (α values respectively 0.78 and 0.46, Table 2) whereas they were identified as full agonists ($\alpha = 0.9$ and 1.00) in the previous one. N^{α}-isopropylimpentamine (**2d**), earlier recognized as a neutral antagonist, now behaved as a partial inverse agonist ($\alpha = -0.55$, Table 2). The other three (**2i,k,l**, Table 2) showed instead values comparable to those formerly measured.

However, it is important to point out that impentamine was initially recognized as an H_3R antagonist[44] and subsequently proved to act also as a partial agonist, depending on the test system.[60]

More recently, Fox and co-workers[61] have reported the partial agonistic behavior of GT-2331 (cipralisant, Perceptin, Figure 1), so far presumed to be an H_3R antagonist.[62] Furthermore, Gbahou et al.[63] identified proxyfan (Figure 1) as a protean H_3 receptor ligand with the ability to display agonism, neutral antagonism, and inverse agonism, depending on the test system. Thus, the complex pharmacodynamic profile of the discussed compounds may be caused by different assay and species differences. The distribution of distinct receptor active states or alternative coupling routes in different cells or tissue may also play a role.

Recently, another histamine receptor subtype, the H_4R, was cloned by several groups and identified as a G-protein coupled receptor.[4,50,64−68] The H_4R has significant sequence homology (about 40%) to the H_3R cDNA,[67] and it shares about 60% sequence identity with the H_3R in the transmembrane regions,[50] suggesting that the two receptors might have a similar mode of ligand recognition. Although the development of selective H_3R ligands is not the primary goal of this study, the synthesized compounds were additionally screened for their affinity for the human H_4R in order to investigate the H_3/H_4 receptor selectivity of the synthesized compounds. The affinity of most of the compounds is lower for the human H_4R than for the human H_3R (Tables 1 and 2). Impentamine (**2a**) and its ethyl (**2b**), *n*-butyl (**2e**), and isobutyl (**2f**) derivatives show a high selectivity for the H_3R over H_4R, the difference in affinity between the two receptors ranging between 1.6 and 1.9 log units (Table 2), but the best H_3R selectivity among the synthesized compounds is displayed by N^{α},N^{α}-dimethylimbutamine **1l** (Table 1), with 2.4 log units difference between the two receptors' affinities ($pK_i = 8.5$ on H_3R versus 6.1 on H_4R). Excluding ligands **1a**,**d**,**g**,**h**,**j**,**k** (Table 1) and **2g** (Table 2), which have almost the same affinity for both receptors and therefore lack H_3/H_4 receptor selectivity, all the other compounds of the series bear a selectivity that ranges between 0.5 and 1.5 log units for the H_3 over the H_4 receptor (Tables 1 and 2).

Conclusions

The synthesis of a series of imbutamine and impentamine analogues in which the primary amino function has been mono- or disubstituted with several aliphatic, alicyclic, and aromatic moieties, as well as incorporated in cyclic systems, has been reported in this study. The novel ligands were pharmacologically investigated in vitro for their affinities on the human H_3R and H_4R subtypes and for their intrinsic H_3R activities.

Modification of the primary amine function of **1a** and **2a** dramatically affects the H_3R binding and functional properties of the compounds. Subtle changes in the substitution of the side chain nitrogen have great influence on the H_3R pharmacological activities of the ligands: agonists, antagonists, and inverse agonists are in fact present in this series of compounds. Previously, we had indeed proposed that the side chain amine function of impentamine (**2a**) interacts with the aspartate residue Asp^{114} in transmembrane domain 3.[53] We hypothesized that rotameric changes of the side chain of this amino acid residue could influence the agonistic properties of the interacting ligands.[41]

However, substitution with the same moiety on the two parent compounds (**1a** and **2a**) yields ligands with different structure−activity relationships (SAR) at the H_3R, depending on the length of the side chain. This is possibly due to the existence of diverse binding pockets and a different set of receptor site points available for interaction with derivatives of **1a** and **2a** that are very much dependent on the distance between the basic nitrogen of the side chain and the imidazole nucleus, as well as on the kind of substituting moiety present on the aforementioned basic nitrogen. Our group already proposed that the differences in SAR observed for the lipophilic tails of different classes of H_3R antagonists could be explained by the existence of two distinct lipophilic pockets available for antagonist binding.[53] The obtained qualitative model for the H_3R revealed a molecular determinant explaining intrinsic activity, as the conformation of the aspartic acid residue differs according to whether it is binding to agonists or antagonists.[53]

Several neutral H_3R antagonists (**1d,g,j,m** and **2b,g,h,m**) have been identified within this series, but also a very potent and selective H_3R agonist (**1l**) and a very potent, though not highly selective, H_3R inverse agonist (**2k**). It has proven very difficult

to draw SAR among these series of ligands, since there does not seem to be a clear correlation between the modifications applied to the side chain nitrogen and the different H_3R affinities and functional activities. Very small modifications can lead to dramatic changes in the affinity as well as in the functional activity of the generated ligands. In this perspective, the side chain nitrogen can be seen as a very subtle chemical switch for the modulation of the functional activity of higher homologues of histamine on the human H_3R.

The target compounds were additionally investigated for their affinities on the human H_4R subtype in order to check selectivity, which led to the identification of N^{α},N^{α}-dimethylimbutamine (**1l**), showing an H_3R/H_4R affinity ratio of 2.4 log units, as a potent and selective agonist for the human H_3R.

The H_3R is one of the few GPCRs that has been shown to modulate important physiological processes by means of its constitutive activity.[40] This underlines not only the impact of assay setup and species differences when evaluating the behavior of ligands on the histamine H_3R but also the usefulness of the compounds presented in this study as pharmacological tools. In view of the many potential therapeutic applications of H_3R antagonists,[16,73,74] the availability of high-affinity neutral H_3R antagonists such as N^{α}-ethylimpentamine **2b** and N^{α}-cyclopentylimpentamine **2h** as well as of the potent inverse H_3R agonist N^{α}-*p*-chlorobenzylimpentamine **2k** may help in unraveling the effects and clinical applications of inverse H_3R agonists or neutral H_3R antagonists.

Experimental Section

General Procedures. Reagents were obtained from commercial suppliers and used without further purification. Solvents used were either AR or HPLC grade. Dry THF and dichloromethane were freshly distilled from $LiAlH_4$ and CaH_2, respectively. Compounds **1a** and **2a** were obtained from our own laboratory stock.[44] The 2-(ω-iodoalkyloxy)tetrahydropyrans were prepared by refluxing the corresponding 2-(ω-chloroalkyloxy)tetrahydropyrans[69] with 1.1 equiv of sodium iodide in acetone and purified by distillation.[70,71] Melting points were measured on an Electrothermal IA 9200 apparatus. ^{1}H NMR spectra were recorded on a Bruker AC-200 (200 MHz) spectrometer. Chemical shifts are given in ppm using the residual undeuterated solvent as reference. Elemental analyses were performed by the Mikroanalytisches Labor Pascher, Remagen-Bandorf, Germany. Flash chromatography was performed on J.T. Baker Kieselgel 60. All reactions were performed under an atmosphere of dry nitrogen.

5(4)-(4-Hydroxybutyl)imidazole-1-sulfonic Acid Dimethylamide (5). 2-(*tert*-Butyldimethylsilanyl)imidazole-1-sulfonic acid dimethylamide **4**[45] (8.59 g, 0.030 mol) was dissolved in THF (100 mL) and cooled to −70 °C. *n*-Butyllithium in hexane (20.35 mL, 0.033 mol) was added dropwise at −65 °C. After 30 min, a solution of 2-(4-iodobutyloxy)tetrahydropyran (8.42 g, 0.030 mol) in THF was added dropwise and the mixture was gradually allowed to warm to room temperature overnight. The solution was acidified with HCl (6 M) and stirred for 3 h, and then the solvents were removed in vacuo. The residue was diluted with water, basified with K_2CO_3, and extracted with dichloromethane. The organic layers were combined, washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The product was purified by flash chromatography by gradually switching the eluent from ethyl acetate to ethyl acetate/methanol (9/1) to afford 2.94 g (40%) of product as a mixture of the 4- and 5-isomers (10:90). ^{1}H NMR ($CDCl_3$): δ 1.70 (m, 4H, central CH_2's 4- + 5-isomer), 1.93 (s, 1H, O*H*, 4- + 5-isomer), 2.60 + 2.75 (t, 2H, imidazole-5(4)-CH_2, 4- + 5-isomer), 2.80 + 2.85 (s, 6H, N(CH_3)$_2$, 4- + 5-isomer), 3.65 (t, 2H, CH_2OH 4- + 5-isomer), 6.80 + 6.92 (s, 1H, imidazole-4(5)H, 4- + 5-isomer), 7.80 + 7.85 (s, 1H, imidazole-2H, 4- + 5-isomer).

5(4)-(4-Oxobutyl)imidazole-1-sulfonic Acid Dimethylamide (7). A solution of oxalyl chloride (2.27 g, 0.018 mol) in anhydrous dichloromethane (DCM) (50 mL) was cooled to −60 °C. A solution of DMSO (2.79 g, 0.036 mol) in anhydrous dichloromethane (10 mL) was added dropwise. After stirring for 10 min, a solution of **5** (2.94 g, 0.012 mol) in dichloromethane (15 mL) was added dropwise. After additional stirring for 15 min, triethylamine (9.03 g, 0.089 mol) was added. The mixture was allowed to warm to room temperature and poured into water. The aqueous layer was extracted with dichloromethane, and the combined organic layers were washed successively with dilute sodium carbonate (5%), water, and brine and then dried ($MgSO_4$) and concentrated in vacuo to yield 2.72 g (93%) of product as a mixture of the 4- and 5-isomers (12.5:87.5). ^{1}H NMR ($CDCl_3$): δ 1.95 (m, 4H, central CH_2's, 4- + 5-isomer), 2.50 (m, 2H, CH_2CHO 4- + 5-isomer), 2.70 (t, 2H, imidazole-5(4)-CH_2, 4- + 5-isomer), 2.78 + 2.82 (s, 6H, N(CH_3)$_2$, 4- + 5-isomer), 6.78 + 6.90 (s, 1H, imidazole-4(5)H, 4- + 5-isomer), 7.75 + 7.80 (s, 1H, imidazole-2H, 4- + 5-isomer), 9.70 + 9.75 (t, 1H, C*H*O 4- + 5-isomer).

5-Hydroxypentanoic Acid Methyl Ester (12). δ-Valerolactone (47.17 g, 0.47 mol) in methanol (300 mL) and concentrated H_2SO_4 (4 mL) was heated to reflux for 16 h. The mixture was cooled to room temperature and the solvent was removed in vacuo. The residue was dissolved in dichloromethane, washed three times with a saturated solution of $NaHCO_3$, dried over $MgSO_4$, and evaporated to dryness, yielding 46.91 g (76%) of product. ^{1}H NMR ($CDCl_3$): δ 1.48−1.80 (m, 4H, central CH_2's), 2.30 (m, 2H, CH_2COOCH$_3$), 3.58 (m, 3H, CH_2OH + CH_3OCO).

4-[4-(Toluene-4-sulfonyl)-4,5-dihydrooxazol-5-yl]butyric Acid Methyl Ester (16). To a stirred suspension of tosyl methyl isocyanide (TosMIC) (49.68 g, 0.25 mol) and **14** (33.15 g, 0.25 mol) in absolute ethanol (800 mL) was added finely powdered sodium cyanide (1.24 g, 0.025 mol). The yellow-orange suspension was stirred for 30 min, filtered, and washed with hexane/ether (1:1), to give 40.06 g (49%) of product. ^{1}H NMR ($CDCl_3$): δ 1.75 (m, 4H, 2 × CH_2), 2.36 (m, 2H, 1 × CH_2), 2.46 (s, 3H, *p*-CH_3), 3.55 (s, 3H, CH_3OCO), 4.79 (d, 1H, oxazoline-4H), 5.05 (q, 1H, oxazoline-5H), 6.95 (s, 1H, oxazoline-2H), 7.35 (d, 2H, 2,6-phenyl-H), 7.80 (d, 2H, 3,5-phenyl-H).

4-(1*H*-Imidazol-4-yl)butyric Acid Ethyl Ester (18). In a resealable pressure tube, a solution of **16** in absolute ethanol saturated with ammonia was heated between 120 and 140 °C. After 20 h the reaction mixture was cooled to room temperature and the solvent was removed in vacuo. The residue (45.67 g) was dissolved in HCl (37%, 300 mL) and heated to reflux for 16 h. The mixture was cooled to room temperature, evaporated to dryness, dissolved in water, washed with ether, then concentrated in vacuo. The residue in ethanol (300 mL) and concentrated H_2SO_4 (ca. 3 mL) was heated to reflux. After 16 h the mixture was cooled to room temperature and evaporated to dryness. The residue was dissolved in water and washed with ether, and then the water layer was basified with $NaHCO_3$ and extracted with dichloromethane. The combined organic extracts were dried over $MgSO_4$ and evaporated in vacuo, yielding 18.46 g (82%) of product. ^{1}H NMR ($CDCl_3$): δ 1.20 (t, 3H, CH_3CH$_2$OCO), 1.95 (m, 2H, central CH_2), 2.32 (t, 2H, CH_2-COO), 2.62 (t, 2H, imidazole-CH_2), 4.05 (q, 2H, CH$_3$CH_2OCO), 6.78 (s, 1H, imidazole-5H), 7.65 (s, 1H, imidazole-2H), 10.08 (s, 1H, imidazole-N*H*).

4-(1-Trityl-1*H*-imidazol-4-yl)butyric Acid Ethyl Ester (20). Compound **18** (15.20 g, 0.083 mol) was dissolved in dichloromethane (200 mL). Triphenylmethyl chloride (23.03 g, 0.083 mol) in dichloromethane (200 mL) was added dropwise, followed by triethylamine (17.18 mL, 0.12 mol). The reaction mixture was stirred at room temperature overnight, and then it was washed successively with an aqueous solution of K_2CO_3 (1 M) and water. The organic layer was dried over $MgSO_4$ and then concentrated under reduced pressure to give 34.22 g (97%) of product. ^{1}H NMR ($CDCl_3$): δ 1.20 (t, 3H, CH_3CH$_2$OCO), 1.95 (m, 2H, central CH_2), 2.30 (t, 2H, CH_2COO), 2.55 (t, 2H, imidazole-CH_2), 4.05 (q, 2H, CH$_3$CH_2OCO), 6.50 (s, 1H, imidazole-5H), 7.00−7.38 (m, 16H, imidazole-2H + Ph$_3$C).

4-(1-Trityl-1*H*-imidazol-4-yl)butan-1-ol (22). A suspension of **20** (34.22 g, 0.081 mol) in THF (200 mL) was added, with efficient

stirring, to an ice-cold suspension of $LiAlH_4$ (3.06 g, 0.081 mol) in THF (50 mL). The reaction mixture was stirred for 15 min at room temperature. Next, water (5 mL), followed by saturated K_2CO_3 solution (5 mL), was added dropwise. The suspension was filtered and concentrated in vacuo to afford 25.81 g (84%) of product. ^{1}H NMR ($CDCl_3$): δ 1.50−1.78 (m, 4H, central CH_2's), 2.50 (t, 2H, imidazole-CH_2), 3.58 (t, 2H, CH_2OH), 6.50 (s, 1H, imidazole-5H), 7.00−7.40 (m, 16H, imidazole-2H + Ph_3C).

General Procedures for the Synthesis of the Protected Aminoalkylimidazoles 10c,m, 11c,d,i,m, 26b,d−l,n−p, and 27b,e−h,j−l,n−p. Method A. One equivalent of **9**[72] was mixed with 3 equiv of the appropriate amine and heated at 100 °C. After 48 h, the excess amine was evaporated under reduced pressure and the residue purified by flash chromatography (starting with ethyl acetate/methanol (9/1) as eluent and gradually switching to methanol and methanol/ammonia (95/5) for elution of the product).

Method B (Reductive Amination). One equivalent of the required aldehyde (**7**, **8**, **24**, or **25**) was stirred for 10 min with the appropriate amine (3 equiv in the case of primary amines, 1 equiv in the case of secondary amines) and 1 equiv of acetic acid in dichloroethane (DCE) at room temperature. Then 1.5 equiv of sodium triacetoxyborohydryde was added and the mixture stirred overnight. Water was added and the reaction stirred for 15 min. Dichloroethane was removed in vacuo, and the residue was basified with K_2CO_3 and extracted with chloroform. The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was then purified by flash chromatography as described in method A.

5(4)-(4-Propylaminobutyl)imidazole-1-sulfonic Acid Dimethylamide (10c). Method B with aldehyde **7** and *n*-propylamine yielded 0.49 g (30%) as a mixture of 4- and 5-isomers (9:91). ^{1}H NMR ($CDCl_3$): δ 0.82 (t, 3H, CH_3 4- + 5-isomer), 1.30−1.72 (m, 6H, $CH_2CH_2CH_3$ 4- and 5-isomer + central CH_2's, 4- and 5-isomer), 2.40−2.60 (m, 4H, CH_2NHCH_2 4- + 5-isomer), 2.68 (t, 2H, imidazole-5(4)-CH_2, 4- + 5-isomer), 2.78 + 2.82 (s, 6H, N(CH_3)$_2$, 4- + 5-isomer), 6.78 + 6.88 (s, 1H, imidazole-4(5)H, 4- + 5-isomer), 7.72 + 7.78 (s, 1H, imidazole-2H, 4- + 5-isomer).

5(4)-(5-Isopropylaminopentyl)imidazole-1-sulfonic Acid Dimethylamide (11d). Method A with compound **9** and isopropylamine yielded 1.50 g (36%). ^{1}H NMR ($CDCl_3$): δ 1.44−1.53 (m, 10H, (CH_3)$_2$CH + 2 × CH_2), 1.57−1.75 (m, 2H, 1 × CH_2), 1.81−2.01 (m, 2H, 1 × CH_2), 2.68 (t, 2H, imidazole-5(4)-CH_2), 2.82−2.92 (m, 9H, CH_2NH + N(CH_3)$_2$ + CH(CH_3)$_2$), 6.79 (s, 1H, imidazole-4(5)H), 7.82 (s, 1H, imidazole-2H).

General Procedures for the Deprotection of the Final Aminoalkylimidazoles 1b−p and 2b−p. Method C (Removal of the Trityl Group). One equivalent of the protected aminoalkylimidazole was mixed with 10 equiv of hydrochloric acid (1 N) (**1b**,**d**−**l**,**n**−**p** and **2b**,**e**−**h**,**k**,**l**,**n**−**p**) or hydrobromic acid (1 N) (**2j**) and the mixture was heated under reflux. After 2 h the mixture was cooled, washed with diethyl ether, and concentrated in vacuo. The salt was either washed with diethyl ether, recrystallized from ethanol/ethyl acetate or ethanol/diethyl ether, or converted into the oxalic acid or L-(+)-tartaric acid salt according to the following procedure. The residue was dissolved in water, basified with K_2CO_3, and extracted with $CHCl_3$. The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. The free base was dissolved in ethyl acetate with a few drops of methanol and then added dropwise to a solution of 2 equiv of oxalic acid dihydrate or L-(+)-tartaric acid in ethyl acetate/methanol. The precipitate was collected by filtration.

Method D (Removal of the Dimethylsulfamoyl Group). The same procedure as described in method A was adopted, but 30% HBr (**1c**,**m** and **2c**,**d**,**i**,**m**) was used and the mixture was refluxed for 16 h. The dihydrobromic acid salt was either washed with diethyl ether, recrystallized from ethanol/ethyl acetate or ethanol/ diethyl ether, or converted into the oxalic acid or tartaric acid salt as described above.

Ethyl[4-(1*H*-imidazol-4-yl)butyl]amine Oxalic Acid Salt (1b). Method C yielded 0.011 g (20%) of a white powder. Mp: 135−137 °C. ^{1}H NMR (D_2O): δ 1.20 (t, 3H, CH_3CH$_2$), 1.58−1.85 (m, 4H, central CH_2's), 2.70 (m, 2H, imidazole-CH_2), 2.90−3.18 (m, 4H, CH_2NHCH_2), 7.20 (s, 1H, imidazole-5H), 8.55 (s, imidazole-2H). Anal. ($C_9H_{17}N_3 \cdot 2C_2H_2O_4$) C, H, N.

[4-(1*H*-Imidazol-4-yl)butyl]propylamine Oxalic Acid Salt (1c). Method D yielded 0.46 g (77%) of a white powder. Mp: 156−158 °C. ^{1}H NMR (D_2O): δ 0.90 (t, 3H, CH_3CH$_2$CH$_2$), 1.50−1.80 (m, 6H, CH$_3$CH_2CH$_2$ + central CH_2's), 2.72 (t, 2H, imidazole-CH_2), 2.85−3.15 (m, 4H, CH_2NHCH_2), 7.19 (s, 1H, imidazole-5H), 8.50 (s, imidazole-2H). Anal. ($C_{10}H_{19}N_3 \cdot 1.5C_2H_2O_4 \cdot 1.5H_2O$) C, H, N.

Acknowledgment. The authors thank Dr. T. Lovenberg, Johnson & Johnson Pharmaceutical Research and Development, San Diego, CA, for the cells stably expressing the human histamine H_3 and H_4 receptors.

Supporting Information Available: Elemental analyses for all compounds, experimental details, data for compounds **6**, **8**, **13**−**15**, **17**, **19**, **21**, **23**−**25**, **10m**, **11c**,**i**,**m**, **26b**,**d**−**l**, **26n**−**p**, **27b**,**e**−**h**, **27j**−**l**,**n**−**p**, **1d**−**p**, **2b**−**p**, and ^{1}H NMR and LC−MS spectra from **1f** and **2d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM0504353