Full Paper

Synthesis and Functional Characterization of Imbutamine Analogs as Histamine H_3 and H_4 Receptor Ligands

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Imbutamine (4-(1*H*-imidazol-4-yl)butanamine) is a potent histamine H_3 (H_3R) and H_4 receptor (H_4R) agonist (EC_{50} values: 3 and 66 nM, respectively). Aiming at improved selectivity for the H_4R, the imidazole ring in imbutamine was methyl-substituted or replaced by various differently substituted heterocycles (1,2,3-triazoles, 1,2,4-triazoles, pyridines, pyrimidines) as potential bioisosteres. Investigations in [^{35}S]GTPγS binding assays using membranes of Sf9 insect cells expressing the respective human histamine receptor subtype revealed only very weak activity of most of the synthesized hetarylalkylamines at both receptors. By contrast, the introduction of substituents at the 4-imidazolyl ring was most effective regarding H_4R selectivity. This holds for methyl substitution in position 2 and, especially, in position 5. 5-Methylimbutamine (H_4R: $EC_{50} = 59$ nM, $\alpha = 0.8$) was equipotent with imbutamine at the hH_4R, but revealed about 16-fold selectivity for the hH_4R compared to the hH_3R (EC_{50} 980 nM, $\alpha = 0.36$), whereas imbutamine preferred the hH_3R. The functional activities were in agreement with radioligand binding data. The results support the hypothesis that, by analogy with histamine, methyl substitution in histamine homologs offers a way to shift the selectivity in favor of the H_4R.

Keywords: Bioisosteres / H_3 receptor / H_4 receptor / Histamine / Imbutamine

Received: August 20, 2013; Revised: September 11, 2013; Accepted: September 12, 2013

DOI 10.1002/ardp.201300316

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Introduction

Since the discovery of the human histamine H_4 receptor (hH_4R) in the years 2000 and 2001 [1–7], this new member of the histamine receptor family [8] has been considered a potential drug target. The predominant expression of the H_4R on various cells of the immune system like eosinophils, T-lymphocytes, dendritic cells, mast cells, and basophils [1, 4, 5, 9–13], and results of *in vitro* and *in vivo* studies suggest that the H_4R plays a crucial role in immunological and inflammatory processes [14–16]. Hence, the H_4R is considered a promising drug target for the treatment of inflammatory and immunological diseases like allergic rhinitis, rheumatoid arthritis, bronchial asthma, and pruritus [17–19]. Nevertheless, recent reports on partial agonistic effects of the standard H_4R antagonist JNJ 7777120 [20] at certain H_4R species orthologs [21] *in vitro* and alternative (β-arrestin-mediated) signaling [22] suggest that ligand effects *in vivo* should be interpreted with caution [23, 24]. Therefore, both selective antagonists and agonists are required as pharmacological tools to further explore the role of the H_4R [25].

The H_4R shares highest sequence similarity with the histamine H_3 receptor (H_3R). Therefore, it is not surprising that the H_4R is targeted by various imidazole-containing H_3R ligands [1, 4–6]. Histamine (**1**) and its homologs homohistamine (**2**) and imbutamine (**3**) are agonists with similar hH_3R and hH_4R affinity, whereas the higher homolog impentamine (**4**) is an almost full hH_3R agonist but shows no agonistic activity at the hH_4R [26, 27]. Therefore, aiming at H_4R agonists with a further improved selectivity profile, especially compared to the H_3R, by analogy with a successful approach to H_2R agonists [28, 29], the bioisosteric replacement of the 1*H*-imidazol-4-yl ring was considered a promising approach

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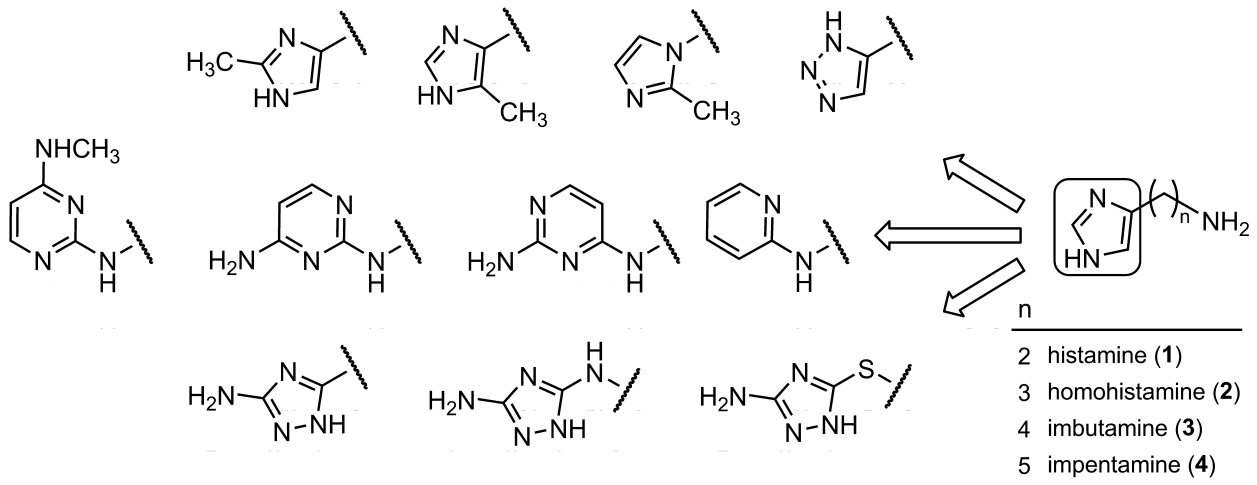


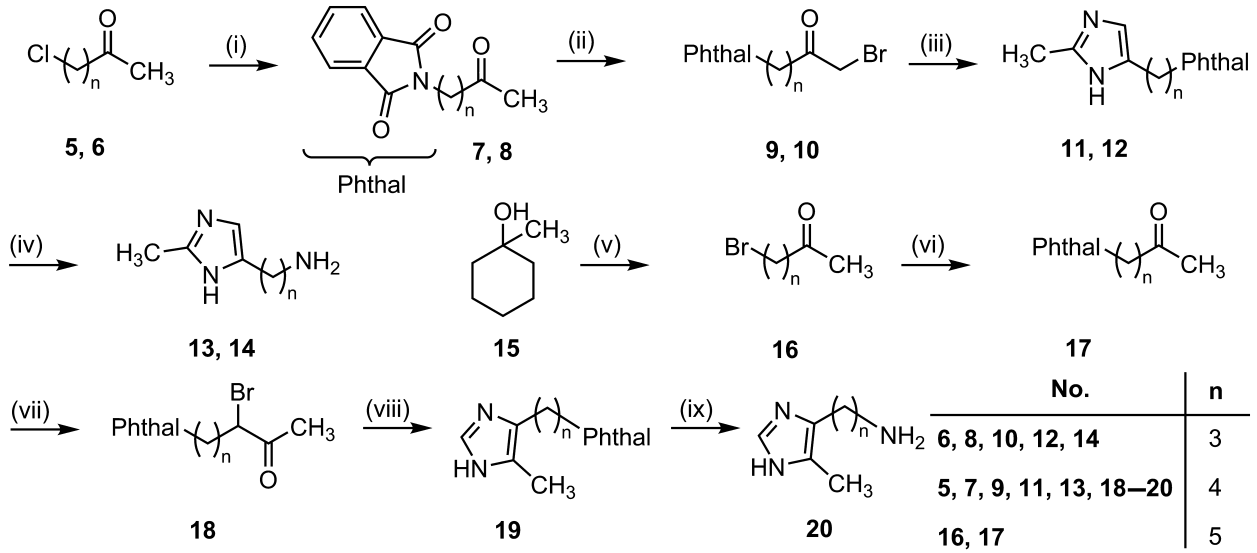
Figure 1. Replacement of the imidazole ring by potential bioisosteres in histamine analogs.

(Fig. 1). In the present study, a series of hetarylalkylamines related to imbutamine (**3**), bearing various heterocycles instead of the imidazol-4-yl ring, were synthesized and investigated on recombinant human histamine receptor subtypes. In addition, methyl substitution at the imidazole ring was performed, since a 5-methyl group in histamine (cf. 5-methylhistamine, often also referred to as 4-methylhistamine) has been shown to confer H_4R selectivity [26]. In the present study, the attention was turned to heterocycles that have already been employed in the development of HR ligands, especially for the preparation of hetaryl analogs of histamine [30–32]. Furthermore, we tried to cover a wide range of physico-chemical properties, with emphasis on different basicity, and focused, in a first approach, on prototypical compounds that were easily accessible.

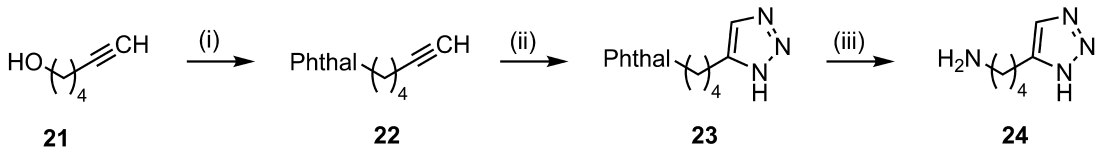
Results and discussion

Chemistry

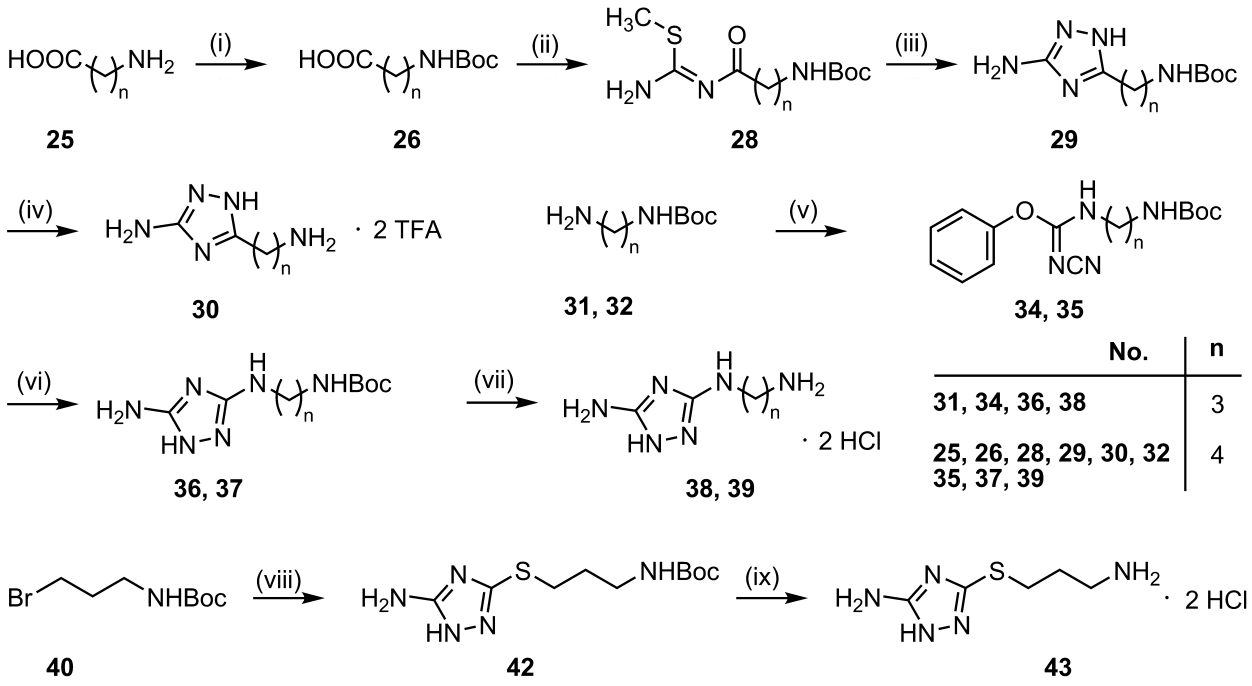
The investigated amines were synthesized as depicted in Schemes 1–4. The ω-(2- or 5-methyl-1*H*-imidazole-4-yl)alkan-1-amines **13**, **14**, and **20** (Scheme 1) were prepared by analogy



Scheme 1. Synthesis of the (2- or 5-methyl-1*H*-imidazol-4-yl)alkan-1-amines **13**, **14**, and **20**. Reagents and conditions: (i) K-phthalimide (1 eq), K_2CO_3 (1.5 eq), KI (cat.), DMF, 12 h, 100°C; (ii) Br_2 (1 eq), urea (1 eq), MeOH, 24 h, rt; (iii) acetamidinium chloride (4 eq), K_2CO_3 (4 eq), DMF, 48 h, 75°C; (iv) $N_2H_4 \cdot H_2O$ (6 eq), EtOH, 2 h, reflux → overnight, rt; (v) Br_2 (5 eq), K_2CO_3 (6.1 eq), $CHCl_3$, 5 h, 0°C; (vi) K-phthalimide (1 eq), K_2CO_3 (1.5 eq), KI (cat.), DMF, 12 h, 100°C; (vii) Br_2 (1 eq), glacial acetic acid, 1.5 h, 10°C → rt; (viii) formamide, 12 h, 170°C; (ix) $N_2H_4 \cdot H_2O$ (6 eq), EtOH, 2 h, reflux → overnight, rt.



Scheme 2. Synthesis of 4-(1*H*-1,2,3-triazol-5-yl)butan-1-amine **24**. Reagents and conditions: (i) phthalimide (1.1 eq), PPh_3 (1.1 eq), DIAD (1.1 eq), THF, overnight, 0°C → rt; (ii) $TMSN_3$ (2 eq), CuI (cat.), DMF/MeOH 9:1 v/v, microwave, 12 h, 100°C; (iii) $N_2H_4 \cdot H_2O$ (6 eq), EtOH, 2 h, reflux → overnight, rt.



Scheme 3. Synthesis of the 2-amino-1,2,4-triazole derivatives **30**, **38**, **39**, and **43**. Reagents and conditions: (i) Boc_2O (1.1 eq), 1 M NaOH, dioxane/water 2:1 v/v, overnight, 0°C → rt; (ii) (a) TBTU (1.2 eq), DIPEA (2 eq), DCM, 30 min, rt; (b) *S*-methylisothiuronium iodide (**27**) (1 eq), DIPEA (3 eq), DCM, overnight, rt; (iii) $N_2H_4 \cdot H_2O$ (30 eq), NEt_3 (32.5 eq), EtOH, overnight, reflux; (iv) TFA 50%, MeOH, overnight, rt; (v) diphenyl cyanocarbonimidate (**33**) (1 eq), 2-propanol, 2 h, rt; (vi) $N_2H_4 \cdot H_2O$ (1.5 eq), MeOH, 24 h, rt; (vii) 6 M HCl in 2-propanol, EtOAc/MeOH 6:1 v/v, overnight, rt; (viii) 5-amino-4*H*-1,2,4-triazole-3-thiol (**41**) (0.8 eq), 1 M NaOH, EtOH, 3 h, reflux; (ix) 6 M HCl in 2-propanol, EtOAc/MeOH 6:1 v/v, overnight, rt.

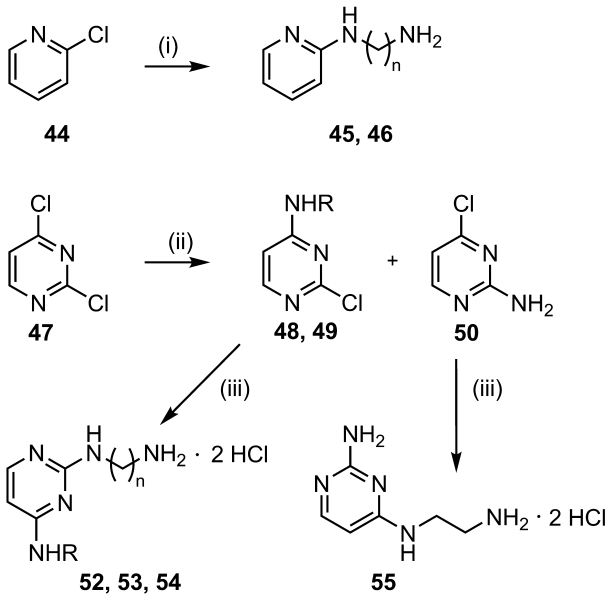
with a pathway for the preparation of homohistamine [33]. The ω-phthalimidoalkan-2-ones (**7**, **8**, and **17**) were obtained from the reaction of the corresponding ω-chloroalkan-2-one (**5** and **6**) or 7-bromoheptan-2-one (**16**) with potassium phthalimide [34]. 7-Bromoheptan-2-one (**16**) was prepared from 1-methylcyclohexanol (**15**) in a *retro-Barbier* reaction [35]. Regioselective bromination of **7** and **8** was achieved in methanol as solvent in the presence of urea according to Zav'yalov and Kravchenko [36]. For the regioselective bromination of **17**, glacial acetic acid was used as solvent according to Black et al. [37]. Cyclization of acetamidine with the α-bromoketone **9** or **10** in a *Bredereck* synthesis [38] gave the imidazoles **11** and **12**, which were converted to **13** and **14** by hydrazinolysis of the phthalimide group. For the synthesis of **19**, formamide was used instead of acetamidine, again followed by hydrazinolysis to yield **20**.

The preparation of 4-(1*H*-1,2,3-triazol-5-yl)butan-1-amine **24** (Scheme 2) started from hex-5-yn-1-ol (**21**), which was converted to the corresponding 2-(hex-5-yn-1-yl)isoindoline-1,3-dione (**22**) under *Mitsunobu* conditions [39]. 2-[4-(1*H*-1,2,3-Triazol-5-yl)butyl]isoindoline-1,3-dione (**23**) was synthesized, using *click-chemistry* according to Jin et al. [40], and subsequently converted to the amine **24** with the help of hydrazine.

The *N*-Boc protected 5-aminopentanoic acid (**26**) was coupled to **27**, as described by Adang et al. [41], to yield **28**. Cyclization of **28** with hydrazine and deprotection under acidic conditions gave 5-(4-aminobutyl)-1*H*-1,2,4-triazol-3-amine (**30**) (Scheme 3).

The 3,5-diamino-1,2,4-triazole derivatives **38** and **39** were synthesized using diphenyl cyanocarbonimidate (**33**) as a synthon [42, 43]. Reaction of mono-protected diaminoalkanes **31** and **32** with **33** and subsequent ring closure with hydrazine yielded the 3,5-diaminotriazoles **36** and **37**. Deprotection under acidic conditions gave the hydrochlorides of **38** and **39**. Substitution of *tert*-butyl 3-bromopropylcarbamate (**40**) with 5-amino-4*H*-1,2,4-triazole-3-thiol (**41**) and deprotection under acidic conditions gave **43** [44].

The aminopyridine derivatives **45** and **46** [45] (Scheme 4) were prepared by reaction of the pertinent alkanediamine and 2-chloropyridine (**44**) under microwave irradiation. The diaminopyrimidines **52**–**55** were prepared from 2,4-dichloropyrimidine (**47**). Heating with ammonia under microwave irradiation yielded the isomers **48** and **50**, which can be easily separated by flash chromatography. Compound **49** was synthesized from **47** using methylamine instead of ammonia. The introduction of the second amino substituent was performed in a similar manner, using the respective Boc-protected alkanediamine (**31** or **51**). Subsequent deprotection under acidic conditions gave the hydrochlorides of **52**–**55**.



No.	45	46	48	49	52	53	54
R	–	–	H	Me	H	H	Me
n	3	4	–	–	2	3	2

Scheme 4. Synthesis of aminopyridines **45**, **46** and diaminopyrimidines **52**, **53**, **54**, and **55**. Reagents and conditions: (i) alkanediamine (6 eq), pyridine (1.3 eq), microwave, 30 min, 190°C; (ii) for **48** and **50**: 7 M NH_3 in MeOH (1.5 eq), DIPEA (2 eq), EtOAc/THF 3:1 v/v, microwave, 10 min, 100°C, separation of isomers by flash chromatography; for **49**: 40% methylamine in H_2O (1 eq), DIPEA (1.5 eq), EtOAc/MeOH 1:1 v/v, 36 h, rt; (iii) (a) for **52**, **54**, **55**: *tert*-butyl 2-aminoethylcarbamate (**51**) (1 eq), DIPEA (1.5 eq), EtOH, microwave, 30 min, 120°C, (b) acetyl chloride (3 eq), MeOH, 4 h, 0°C → rt; for **53**: **31** (1 eq), DIPEA (1.5 eq), EtOH, microwave, 30 min, 120°C, (b) acetyl chloride (3 eq), MeOH, 4 h, 0°C → rt.

Pharmacology

The synthesized compounds described above were investigated for agonism and antagonism at hH_4R and hH_3R subtypes in [^{35}S]GTPγS binding assays using membrane preparations of Sf9 insect cells co-expressing the hH_4R plus $G\alpha_{i2}$ plus $G\beta_1\gamma_2$ or co-expressing the hH_3R plus $G\alpha_{i2}$ plus $G\beta_1\gamma_2$. Moreover, the reference H_4R agonist 5-methylhistamine (**56**) [46], 2-methylimidazole-1-pentanamine (**57**) [47], and 2-[(5-methylimidazol-4-yl)methylsulfanyl]ethanamine (**58**) [48], a thia-analog of **20**, known as a building block for the preparation of cimetidine, were included (Fig. 2). In addition, the hH_3R and hH_4R binding data of selected compounds were determined on the corresponding Sf9 membrane preparations. In the following, agonistic potencies are expressed as EC_{50} values. Intrinsic activities (α) refer to the maximal response induced by the standard agonist histamine. Compounds identified to be inactive as agonists ($\alpha < 0.1$ or negative values, respectively, determined in the agonist mode; cf. Table 1) were investigated in the antagonist mode. The corresponding K_B values of neutral antagonists and inverse agonists (Table 1) were determined from the concentration-dependent inhibition of the histamine-induced increase in [^{35}S]GTPγS binding. Binding data represent K_i values determined by displacement of [^{3}H]N^α-methylhistamine (hH_3R) or [^{3}H]histamine (hH_4R) (Table 2).

Prior to the discovery of the H_3R and H_4R, in search for the structural determinants of H_1R or H_2R agonism, numerous derivatives and heterocyclic analogs of histamine were investigated. Although even minor structural modifications of the endogenous ligand led to less active compounds at the H_1R and H_2R, these studies revealed possible bioisosteric replacements of the imidazole ring [30]. Decades later, numerous histamine analogs proved to be potent agonists at the H_3R and the H_4R [26]. For instance, 5-methylhistamine, used by Black et al. as a weak agonist preferring the H_2R

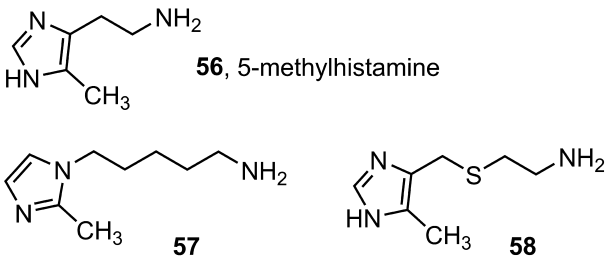


Figure 2. Structures of amines **56**–**58**.

Table 1. Potencies and efficacies of the synthesized amines at the hH_3R and hH_4R in the [^{35}S]GTPγS assay.[a)]

	hH_3R			hH_4R		
Compound	**EC_{50} or (K_B, nM)**	**α**	**N**	**EC_{50} or (K_B, nM)**	**α**	**N**
1, Histamine	$23.2 \pm 7.2^{b,c}$	1.00	2	$10.2 \pm 1.8^{d,e}$	1.00	4
2, Homohistamine	103 ± 63^{f}	0.81 ± 0.06	3	19.3 ± 2.2^{g}	0.77 ± 0.06	3
3, Imbutamine	3.13 ± 1.1^{h}	0.82 ± 0.13	3	66.1 ± 24.6^{i}	0.69 ± 0.07	4
4, Impentamine	$(57.6 \pm 28.8)^{j}$	0.13 ± 0.02	2	(n.d.)	-0.01 ± 0.02	3
13	>10,000	0.45 ± 0.04	2	346 ± 7	0.39 ± 0.05	2
14	(>10,000)	0.09 ± 0.04	2	1700 ± 186	0.39 ± 0.01	2
20	980	0.36 ± 0.11	2	59.4 ± 20.2^{k}	0.80 ± 0.08	5
24	>10,000	0.36 ± 0.04	3	>10,000	0.20 ± 0.04	3
30	Inactive	n.d.	2	(>10,000)	-0.15 ± 0.03	2
38	Inactive	n.d.	2	(>10,000)	-0.30 ± 0.1	2
39	Inactive	n.d.	2	(>10,000)	-0.11 ± 0.09	2
43	Inactive	n.d.	2	Inactive	n.d.	2
45	(2790 ± 164)	-0.51 ± 0.09	2	(>10,000)	-0.22 ± 0.08	2
46	(1430 ± 164)	-0.50 ± 0.05	2	(>10,000)	-0.22 ± 0.09	2
52	Inactive	n.d.	2	Inactive	n.d.	3
53	(>10,000)	-0.28 ± 0.14	2	Inactive	n.d.	2
54	(>10,000)	-0.13 ± 0.11	2	inactive	n.d.	2
55	(91 ± 1)	-0.16 ± 0.03	2	500 ± 150	0.87 ± 0.04	3
56, 5-Me-histamine	$12{,}400 \pm 3130$	0.70 ± 0.11	3	70.3 ± 44.1^{k}	0.98 ± 0.03	3
57	(2460 ± 58)	-0.97 ± 0.12	3	Inactive	n.d.	2
58	751 ± 303	0.49 ± 0.05	3	1590 ± 1300	0.70 ± 0.01	3

[a)] Functional [^{35}S]GTPγS binding assays using membrane preparations of Sf9 cells expressing the $hH_3R + G\alpha_{i2} + G\beta_1\gamma_2$ or the $hH_4R + G\alpha_{i2} + G\beta_1\gamma_2$. *N* gives the number of independent experiments performed in duplicate. The intrinsic activity (α, maximal response) of histamine was set to 1.00 and α values of other compounds were referred to this value. α values of neutral antagonists and inverse agonists were determined at 10 μM. n.d., no agonistic activity detected. The K_B values of neutral antagonists and inverse agonists were determined in the antagonist mode versus histamine (100 nM) as the agonist. [b)][49]: EC_{50} 25 nM. [c)][26]: EC_{50} 13 nM. [d)][49]: EC_{50} 12 nM. [e)][26]: EC_{50} 11 nM. [f)][26]: EC_{50} 40 nM. [g)][26]: EC_{50} 200 nM. [h)][26]: EC_{50} 0.6 nM. [i)][49]: EC_{50} 32 nM. [j)][26]: EC_{50} 4 nM. [k)][49]: EC_{50} 70 nM. Data from the literature: [b,d,k)]EC_{50} values determined in the steady-state GTPase assay on membranes of Sf9 cells expressing the human hH_3R or hH_4R [49], [e–h,j)]EC_{50} values determined from the inhibition of 1 μM forskolin-induced CRE-β-galactosidase activity in SK-N-MC/hH_3 or SK-N-MC/hH_4 cells [26].

compared to the H_1R to pharmacologically define the H_2R, turned out to be a potent agonist at the human H_4R [26]. Extension of the carbon chain between imidazole and amino group from two carbon atoms (histamine, **1**) was tolerated up to four methylene groups (imbutamine, **3**), and the incorporation of imbutamine as a building block proved to be highly efficient to increase the potency of cyanoguanidine-type H_4R agonists [49, 50].

Stimulated by these previous results we substituted the imidazole ring in imbutamine with a methyl group at different positions and prepared several structural analogs. The introduction of a 2-methyl substitution at the imidazole ring

Table 2. Binding data of selected compounds at the hH_3R and hH_4R.[a)]

	hH_3R		hH_4R	
Compound	**K_i (nM)**	**N**	**K_i (nM)**	**N**
1, Histamine	18.3 ± 3.5	3	12.7 ± 1.5	3
2, Homohistamine	94.4 ± 0.4	2	31.8 ± 11.3	3
3, Imbutamine	4.27 ± 0.8	2	12.7 ± 0.8	2
4, Impentamine	36.9 ± 7.0	2	627 ± 63	3
20, 5-Me-imbutamine	639 ± 227	2	68.8 ± 14.5	3
56, 5-Me-histamine	9940 ± 1400	2	28.5 ± 4.4	3
58	1670	1	1460	1

[a)] Determined on membrane preparations of Sf9 insect cells expressing the $hH_3R + G\alpha_{i2} + G\beta_1\gamma_2$ or the $hH_4R + G\alpha_{i2} + G\beta_1\gamma_2$, the $hH_3R + G\alpha_{i2} + G\beta_1\gamma_2$; radioligands: for hH_3R: [^{3}H]N^{α}-methylhistamine (3 nM), for hH_4R: [^{3}H]histamine (10 nM). *N* gives the number of independent experiments performed in duplicate.

in **4** decreased potency and efficacy at the H_4R and, much more pronounced, at the H_3R. Compound **13** was a partial agonist at the H_4R with an EC_{50} of 346 nM. The same tendency became obvious for the 2-methyl analog of homohistamine (**14**, H_4R: $EC_{50} = 1700$ nM). Hence, both compounds showed a changed selectivity profile and preferred the H_4R compared to the respective parent compounds (**2** and **3**). This is in accordance with data for 2-methylhistamine [26]. Thus, regardless of lowering the potency, substituents at position 2 of the imidazole ring can be taken into consideration to increase the selectivity of imidazole-type H_4R agonists. A methyl substitution in position 5 of the imidazole ring in **3** did not markedly influence the potency and the intrinsic activity at the H_4R, but slightly reduced the intrinsic activity ($EC_{50} = 59$ nM, $\alpha = 0.8$). At the H_3R a 300-fold decrease in activity and partial agonism ($\alpha = 0.36$) was observed for compound **20**.

The replacement of the imidazole in **3** by the less basic 1,2,3-triazole ring (**24**) decreased potency and intrinsic activity in the [^{35}S]GTPγS assay and yielded a very weakly active partial agonist at both receptor subtypes. A drastic decrease in potency compared to the imidazole series was also observed for the 3-amino-1,2,4-triazole derivatives **30**, **38**, **39**, and **43**. At the H_4R very weak inverse agonism was detected for **30**, **38**, and **39**. Compound **43** was inactive (up to 100 μM, the highest concentration tested). The results suggest that the basicity of the heterocycle should be comparable to that of imidazole to obtain histamine H_4R activity. At the H_3R, neither agonistic nor antagonistic activity was detectable for **30**, **38**–**39**, and **43** in the [^{35}S]GTPγS assay. The two pyridine derivatives **45** and **46** were weak inverse agonists at both receptor subtypes. The K_B values for antagonism at the H_3R were in the low micromolar range, and the concentration–response curves revealed inverse agonism ($\alpha = -0.5$). The antagonist activities at the H_4R were even lower. The spatial demand of the six-membered ring and again the lower basicity of the heterocycle might contribute to this dramatic decrease in potency.

Amino-substituted pyrimidines are known as core structures of numerous high-affinity H_4R antagonists [32, 51], including those compounds that entered clinical trials (e.g., ZPL-38937887; for a recent review, cf. [52]). To explore the diaminopyrimidine moiety as a replacement of the imidazole ring in histamine analogs, we synthesized the aminoalkylpyrimidine-2,4-diamines **52**–**55**. The three N^2-substituted derivatives **52**–**54** were inactive or only very weakly active at the H_4R and H_3R. Interestingly, the N^4-substituted derivative **55** was a rather potent H_3R antagonist ($K_B = 91$ nM, $\alpha = -0.16$) with some selectivity over the H_4R ($EC_{50} = 500$ nM, $\alpha = 0.87$).

Compound **57**, a methyl-substituted structural isomer of impentamine (**4**), was inactive at the H_4R but revealed inverse agonism at the H_3R ($\alpha = -0.97$) with a K_B value of 2460 nM. According to Yao et al. the imidazole N–H in histamine is essential to form a hydrogen bond, which is necessary for H_3R and H_4R activation [53, 54]. This is not possible in the case of compound **57**. Furthermore, as impentamine is devoid of H_4R agonistic properties as well, the spacer length of five methylene groups obviously prevents optimal orientation of the ligand in the binding pocket of the H_4R. Interestingly, compound **58**, the thia-analog of compound **20**, was equipotent with **20** at the H_3R, but proved to be about 30 times less potent as an H_4R agonist.

The functional data are in accordance with the affinities determined for selected compounds (**1**–**4**, **20**, **56**, and **58**) at the hH_3R and the hH_4R (Table 2).

Essentially, the results are in agreement with data from previous studies on imidazole-type H_4R agonists, indicating that both ethylamine and butylamine side chains are equivalent substructures. The search for bioisosteres of the imidazole ring was not successful in terms of H_4R agonism. Obviously, the basicity of the heterocycle, the H-bond donor and acceptor properties, and the spatial demand should be comparable to those of imidazole. By analogy with histamine, methyl substitution at the 4-imidazolyl ring resulted in a preference for the H_4R. This holds for methyl substitution in position 2 and, especially, in position 5 (cf. **56** vs. **1**, **14** vs. **2**, **20** vs. **3**). 5-Methylimbutamine (**20**) was the most potent and selective H_4R agonist among the ring-substituted higher homologs of histamine.

In conclusion, the search for bioisosteres of the imidazole ring in H_4R agonists related to histamine remains challenging. A different scaffold as in oxime-type agonists derived from H_4R antagonists with an indole or benzimidazole moiety, e.g., JNJ 28610244 [55], may offer an alternative approach to H_4R selectivity. Moreover, simple structures as that of *S*-(2-guanidinoethyl)isothiourea (VUF 8430) [56] suggest that a heterocyclic core is not required at all to obtain potent and selective H_4R agonists.

Experimental

Chemistry

Commercial reagents and chemicals were purchased from Acros Organics (Geel, Belgium), IRIS Biotech GmbH (Marktredwitz, Germany), Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany), Merck KGaA (Darmstadt, Germany), Sigma–Aldrich Chemie GmbH (Munich, Germany), TCI Europe (Zwijndrecht, Belgium) and used without further purification. Deuterated solvents for NMR spectroscopy were from Deutero GmbH (Kastellaun, Germany). 5-(2-Methyl-1*H*-imidazol-1-yl)pentan-1-amine (**57**) was a gift from Dr. Birgit Striegl, Department of Pharmaceutical/Medicinal Chemistry I, University of Regensburg. All solvents were of analytical grade or distilled prior to use. Millipore water was used throughout for the preparation of buffers and HPLC eluents. If moisture-free conditions were required, reactions were performed in dried glassware under inert atmosphere (argon or nitrogen). Anhydrous DMF was purchased from Sigma–Aldrich Chemie GmbH and stored over 3 Å molecular sieves. Flash chromatography was performed in glass columns on silica gel

(Merck silica gel 60, 40–63 μm). Automated flash chromatography was performed on a Varian IntelliFlash 310 using pre-packed Varian Superflash columns (Varian, Darmstadt, Germany). Reactions were monitored by TLC on aluminum plates coated with silica gel (Merck silica gel 60 F_{254}, thickness 0.2 mm). The compounds were detected by UV light (254 nm), a 0.3% solution of ninhydrine in *n*-butanol (amines), a 1.0% solution of Fast Blue B salt (imidazole containing compounds) in EtOH/H_2O = 30:70 v/v or iodine staining. All melting points are uncorrected and were measured on a Büchi 530 (Büchi GmbH, Essen, Germany) apparatus. Lyophilization was done with a Christ alpha 2-4 LD equipped with a vacuubrand RZ 6 rotary vane vacuum pump. Microwave-assisted reactions were performed on an Initiator 2.0 synthesizer (Biotage, Uppsala, Sweden).

Nuclear magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) were recorded with Bruker Avance 300 (^{1}H: 300.1 MHz, ^{13}C: 75.5 MHz), Avance 400 (^{1}H: 400.1 MHz, ^{13}C: 100.6 MHz), or Bruker Avance 600 (^{1}H: 600.1 MHz, ^{13}C: 150.9 MHz) NMR spectrometers (Bruker BioSpin GmbH, Rheinstetten, Germany). Chemical shifts are given in δ (ppm) relative to external standards. Abbreviations for the multiplicities of the signals: s (singlet), d (doublet), t (triplet), m (multiplet), brs (broad singlet), and combinations thereof. The multiplicity of carbon atoms (^{13}C NMR) was determined by DEPT 135 (distortionless enhancement by polarization transfer): "+" primary and tertiary carbon atom (positive DEPT 135 signal), "−" secondary carbon atom (negative DEPT 135 signal), "C_{quat}" quaternary carbon atom. In certain cases, 2D-NMR techniques (COSY, HMQC, HSQC, HMBC, and NOESY) were used to assign ^{1}H and ^{13}C chemical shifts. Mass spectra (MS) were recorded on a Finnigan MAT 95 (EI-MS 70 eV, HR-MS), Finnigan SSQ 710A (CI-MS (NH_3)), and on a Finnigan ThermoQuest TSQ 7000 (ES-MS) spectrometer. The peak-intensity in % relative to the strongest signal is indicated in parenthesis. Elemental analysis (C, H, N, Heraeus Elementar Vario EL III) was performed by the Analytical Department of the University Regensburg and are within ±0.4% unless otherwise noted. The purity of tested compounds was >95% as determined by HPLC.

For the synthesis and the experimental data of compounds **7**–**10**, **16**, **17**, **22**, **26**, **27**, **31**, **32**, **40**, **45**, **46**, and **48**–**51**, conditions of preparative and analytical HPLC, HPLC purity data, and elemental analyses cf. Supporting Information.

*2-[4-(2-Methyl-1H-imidazol-4-yl)butyl]isoindoline-1,3-dione (**11**)*

A mixture of **9** (5 g, 15.4 mmol), acetamidine hydrochloride (5.8 g, 61.7 mmol), and K_2CO_3 (8.5 g, 61.7 mmol) in 50 mL DMF was stirred for 72 h at 50°C. After evaporation of the solvent, the residue was taken up in DCM and extracted with saturated $NaHCO_3$. Removal of the solvent *in vacuo* and flash chromatography (DCM/MeOH 95:5–85:15 v/v) yielded a yellow oil (0.84 g, 20%); ^{1}H NMR (300 MHz, CD_3OD): δ [ppm] = 1.60 (m, 4H, Phthal-CH_2–C***H***$_2$–C***H***$_2$), 2.27 (s, 3H, C***H***$_3$), 2.50 (t, 2H, ^3J = 7.1 Hz, Phthal-$(CH_2)_3$–C***H***$_2$), 3.58 (t, 2H, ^3J = 6.7 Hz, Phthal-C***H***$_2$), 6.58 (s, 1H, Im-***H***-5), 7.67 (m, 4H, Phthal-***H***). ^{13}C NMR (75 MHz, CD_3OD): δ [ppm] = 13.68 (+, ***C***H_3), 27.08 (−, ***C***H_2), 27.81 (−, ***C***H_2), 29.21 (−, ***C***H_2), 38.68 (−, ***C***H_2), 117.45 (+, Im-***C***-5), 124.10 (+, Phthal-***C***-4,7), 133.26 (C_{quat}, Phthal-***C***-3a,7a), 135.33 (+, Phthal-***C***-5,6), 137.13 (C_{quat}, Im-***C***-4), 145.18 (C_{quat}, Im-***C***-2), 169.68 (C_{quat}, 2 Phthal-***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m*/*z* (%): 284 (100) $[M+H]^+$. $C_{16}H_{17}N_3O_2$ (283.33).

*2-[3-(2-Methyl-1H-imidazol-4-yl)propyl]isoindoline-1,3-dione (**12**) [57]*

A mixture of **10** (8.0 g, 25.8 mmol), acetamidine hydrochloride (9.8 g, 103.2 mmol), and K_2CO_3 (14.3 g, 103.2 mmol) in 50 mL DMF was stirred at 75°C for 48 h. The solvent was evaporated, the residue taken up in DCM and extracted with saturated $NaHCO_3$. Evaporation of the solvent and flash chromatography (DCM/MeOH 100:0–90:10 v/v) gave a yellow oil (1.0 g, 15%); ^{1}H NMR (300 MHz, CD_3OD): δ [ppm] = 1.93 (m, 2H, Phthal-CH_2–C***H***$_2$), 1.98 (s, 3H, C***H***$_3$), 2.56 (t, 2H, ^3J = 7.2 Hz, Phthal-$(CH_2)_2$–C***H***$_2$), 3.70 (t, 2H, ^3J = 6.9 Hz, Phthal-C***H***$_2$), 6.66 (s, 1H, Im-***H***-5), 7.75–7.99 (m, 4H, Phthal-***H***). ^{13}C NMR (75 MHz, CD_3OD): δ [ppm] = 13.21 (+, ***C***H_3), 25.11 (−, ***C***H_2), 29.02 (−, ***C***H_2), 38.49 (−, ***C***H_2), 116.98 (+, Im-***C***-5), 124.05 (+, Phthal-***C***-4,7), 133.39 (C_{quat}, Phthal-***C***-3a,7a), 135.33 (+, Phthal-***C***-5,6), 136.75 (C_{quat}, Im-***C***-4), 145.26 (C_{quat}, Im-***C***-2), 169.87 (C_{quat}, 2 Phthal-***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m*/*z* (%): 270 (100) $[M+H]^+$. $C_{15}H_{15}N_3O_2$ (269.30).

*4-(2-Methyl-1H-imidazol-4-yl)butan-1-amine (**13**)*

A solution of **11** (0.8 g, 2.8 mmol) and hydrazine monohydrate (0.7 mL, 14.1 mmol, 5 eq) in EtOH (50 mL) was refluxed for 2 h and cooled to room temperature overnight. The precipitate was filtered off and washed with cold ethanol. The filtrate was concentrated *in vacuo* and subjected to flash chromatography (DCM/MeOH/32% $NH_{3(aq)}$ 80:18:2 v/v/v) to yield a yellow oil (0.31 g, 72%); ^{1}H NMR (300 MHz, CD_3OD): δ [ppm] = 1.53 (m, 2H, C***H***$_2$), 1.62 (m, 2H, C***H***$_2$), 2.29 (s, 3H, C***H***$_3$), 2.52 (t, 2H, ^3J = 7.6 Hz, C***H***$_2$), 2.68 (t, 2H, ^3J = 7.0 Hz, C***H***$_2$), 6.58 (s, 1H, Im-***H***-5). ^{13}C NMR (75 MHz, CD_3OD): δ [ppm] = 13.51 (+, ***C***H_3), 27.35 (−, ***C***H_2), 27.82 (−, ***C***H_2), 32.47 (−, ***C***H_2), 42.05 (−, ***C***H_2–NH_2), 117.25 (+, Im-***C***-5), 137.59 (C_{quat}, Im-***C***-4), 145.10 (C_{quat}, Im-***C***-2). HRMS (EI-MS) calcd. for $C_8H_{15}N_3$ $[M^{+\bullet}]$ 153.1266; found 153.1262. Anal. ($C_8H_{15}N_3 \cdot 0.85\ H_2O$) C, H, N. $C_8H_{15}N_3$ (153.22).

*3-(2-Methyl-1H-imidazol-4-yl)propan-1-amine (**14**) [58]*

A solution of **12** (0.5 g, 1.9 mmol) and hydrazine monohydrate (0.54 mL, 11.1 mmol, 5 eq) in EtOH (20 mL) was refluxed for 2 h and cooled to room temperature overnight. The precipitate was filtered off and washed with cold ethanol. The filtrate was concentrated *in vacuo* and subjected to flash chromatography (DCM/7 M NH_3 in MeOH 90:10–70:30 v/v), yielding a yellow oil (0.21 g, 79%); ^{1}H NMR (300 MHz, CD_3OD): δ [ppm] = 1.75 (m, 2H, C***H***$_2$), 2.29 (s, 3H, C***H***$_3$), 2.54 (t, 2H, ^3J = 7.5 Hz, C***H***$_2$), 2.64 (t, 2H, ^3J = 7.2 Hz, C***H***$_2$), 6.59 (s, 1H, Im-***H***-5). ^{13}C NMR (75 MHz, CD_3OD): δ [ppm] = 13.54 (+, ***C***H_3), 25.02 (−, ***C***H_2), 33.43 (−, ***C***H_2), 41.99 (−, ***C***H_2–NH_2), 117.19 (+, Im-***C***-5), 137.43 (C_{quat}, Im-***C***-4), 145.20 (C_{quat}, Im-***C***-2). HRMS (EI-MS) calcd. for $C_7H_{13}N_3$ $[M^{+\bullet}]$ 139.1109; found 139.1113. Anal. ($C_7H_{13}N_3 \cdot 0.5H_2O \cdot 0.4CH_3OH$) C, H, N. $C_7H_{13}N_3$ (139.20).

*2-(5-Bromo-6-oxoheptyl)isoindoline-1,3-dione (**18**)*

Compound **17** (14.0 g, 54.0 mmol) was dissolved in 150 mL glacial acetic acid. After the addition of bromine (2.7 g, 52.0 mmol) at ~10°C, the mixture was allowed to warm to room temperature and stirred for 1.5 h. Subsequently the solution was poured in 1 L of ice-cold water and extracted with $CHCl_3$. The organic layer was evaporated and the residue was crystallized from 100 mL MeOH. The precipitated product was filtered, washed with MeOH (10 mL), and dried *in vacuo* to yield a white solid (11.3 g, 62%);

m.p. 82°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.51 (m, 2H, C***H***$_2$), 1.71 (m, 2H, C***H***$_2$), 2.00 (m, 2H, C***H***$_2$), 2.34 (s, 2H, C***H***$_3$), 3.68 (t, 2H, ^3J = 7.1 Hz, Phthal-C***H***$_2$), 4.21 (t, 1H, ^3J = 6.9 Hz, C***H***–Br), 7.70 (m, 2H, Phthal-***H***), 7.82 (m, 2H, Phthal-***H***). ^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm] = 24.55 (–, ***C***H$_2$), 26.36 (+, ***C***H$_3$), 27.92 (–, ***C***H$_2$), 32.79 (–, ***C***H$_2$), 37.47 (–, ***C***H$_2$), 53.86 (+, ***C***H–Br), 123.25 (+, Phthal-***C***-4,7), 132.05 (C$_{quat}$, Phthal-***C***-3a,7a), 133.99 (+, Phthal-***C***-5,6), 168.39 (C$_{quat}$, 2 Phthal-***C***=O), 201.81 (C$_{quat}$, ***C***=O). CI-MS (NH_3) *m/z* (%): 355, 357 (50) [M+NH$_4$]$^+$, 277 (100) [M−Br+H+NH$_4$]$^+$. Anal. ($C_{15}H_{16}BrNO_3$) C, H, N. $C_{15}H_{16}BrNO_3$ (338.20).

*2-[4-(5-Methyl-1H-imidazol-4-yl)butyl]isoindoline-1,3-dione (**19**)*

Compound **18** (1.0 g, 3.0 mmol) was dissolved in formamide (30 mL) and the mixture was stirred for 12 h at 170°C. The mixture was taken up in 100 mL saturated $NaHCO_3$ and extracted with 3 × 80 mL $CHCl_3$. The organic layer was dried over $MgSO_4$ and evaporated. Flash chromatography (DCM/MeOH 95:5 v/v) yielded a yellow solid (0.58 g, 68%); m.p. 115°C. ^{1}H NMR (300 MHz, CD_3OD): δ [ppm] = 1.62 (m, 4H, Phthal-CH$_2$–C***H***$_2$–C***H***$_2$), 2.13 (s, 3H, C***H***$_3$), 2.55 (t, 2H, ^3J = 6.9 Hz, Phthal-(CH$_2$)$_3$–C***H***$_2$), 3.66 (t, 2H, ^3J = 6.7 Hz, Phthal-C***H***$_2$), 7.44 (s, 1H, Im-***H***-2), 7.80–8.04 (m, 4H, Phthal-***H***). ^{13}C NMR (75 MHz, CD_3OD): δ [ppm] = 10.42 (+, ***C***H$_3$), 25.53 (–, ***C***H$_2$), 28.09 (–, ***C***H$_2$), 28.96 (–, ***C***H$_2$), 38.57 (–, ***C***H$_2$), 124.10 (+, Phthal-***C***-4,7), 127.32 (C$_{quat}$, Im-***C***), 131.71 (C$_{quat}$, Im-***C***), 133.40 (C$_{quat}$, Phthal-***C***-3a,7a), 133.94 (+, Im-***C***-2), 135.37 (+, Phthal-***C***-5,6), 169.90 (C$_{quat}$, 2 Phthal-***C***=O). ES-MS (DCM/MeOH + NH$_4$OAc) *m/z* (%): 284 (100) [M+H]$^+$. $C_{16}H_{17}N_3O_2$ (283.33).

*4-(5-Methyl-1H-imidazol-4-yl)butan-1-amine (**20**) [48]*

A solution of **19** (2.5 g, 8.8 mmol) and hydrazine monohydrate (2.14 mL, 44.1 mmol, 5 eq) in EtOH (50 mL) was refluxed for 2 h and cooled to room temperature overnight. The precipitate was filtered off and washed with cold ethanol. The filtrate was concentrated *in vacuo* and directly subjected to flash chromatography (Superflash SF15-30g, RP C18 pre-packed column, H_2O/MeCN 100:0–80:20 v/v) yielding a yellow oil (1.1 g, 82%); m.p. (dihydrochloride) 215°C (AcOH) ([48]: 206–208°C). ^{1}H NMR (300 MHz, CD_3OD): δ [ppm] = 1.71 (m, 4H, C***H***$_2$), 2.29 (s, 3H, C***H***$_3$), 2.72 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$), 2.97 (t, 2H, ^3J = 7.2 Hz, C***H***$_2$), 8.53 (s, 1H, Im-***H***-2). ^{13}C NMR (75 MHz, CD_3OD): δ [ppm] = 9.16 (+, ***C***H$_3$), 24.07 (–, ***C***H$_2$), 27.12 (–, ***C***H$_2$), 27.86 (–, ***C***H$_2$), 40.45 (–, ***C***H$_2$–NH$_2$), 126.63 (C$_{quat}$, Im-***C***), 130.08 (C$_{quat}$, Im-***C***-4), 133.19 (+, Im-***C***-2). HRMS (EI-MS) calcd. for $C_8H_{15}N_3$ [M$^{+\bullet}$] 153.1266; found 153.1265.

*2-[4-(1H-1,2,3-Triazol-5-yl)butyl]isoindoline-1,3-dione (**23**)*

To a solution of **22** (1.5 g, 6.6 mmol) in DMF/MeOH (10 mL, 9:1 v/v), azidotrimethylsilane (1.73 mL, 13.2 mmol) and CuI (0.63 g, 0.33 mmol) were added [40]. The mixture was stirred under microwave irradiation at 100°C for 12 h and then cooled to room temperature. The solvents were evaporated and the residue was taken up in 50 mL EtOAc. The solution was filtered through a short pad of silica gel and washed with 3 × 30 mL water. Flash chromatography (DCM/MeOH 95:5 v/v) yielded a yellow solid (1.5 g, 84%); m.p. 113–114°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.73 (m, 4H, 2 C***H***$_2$), 2.79 (m, 2H, C***H***$_2$-triazol), 3.71 (t, 2H, ^3J = 6.4 Hz, C***H***$_2$-Phthal), 7.51 (s, 1H, Triazol-***H***), 7.70 (m, 2H, Phthal-***H***), 7.80 (m, 2H, Phthal-***H***). ^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm] = 24.38 (–, ***C***H$_2$), 26.44 (–, ***C***H$_2$), 28.04 (–, ***C***H$_2$), 37.52 (–, ***C***H$_2$-Phthal), 123.26 (+, Phthal-***C***-4,7), 132.04 (C$_{quat}$, Phthal-***C***-3a,7a), 133.99 (+, Phthal-***C***-5,6), 134.29 (+, Triazol-***C***), 141.76 (C$_{quat}$, Triazol-***C***), 168.55 (C$_{quat}$, 2 ***C***=O). ES-MS (DCM/MeOH + NH$_4$OAc) *m/z* (%): 271 (90) [M+H]$^+$, 312 (100) [M+H+MeCN]$^+$. $C_{14}H_{14}N_4O_2$ (270.29).

*4-(1H-1,2,3-Triazol-5-yl)butan-1-amine (**24**)*

A solution of **23** (4.0 g, 14.8 mmol) and hydrazine monohydrate (3.6 mL, 75 mmol, 5 eq) in EtOH (50 mL) was refluxed for 1.5 h and cooled to room temperature overnight. The precipitate was filtered off and washed with cold ethanol. The filtrate was concentrated *in vacuo* and the residue taken up in 30 mL water. Lyophilization yielded a white solid (2.0 g, 97%); m.p. 119–120°C. ^{1}H NMR (300 MHz, CD_3OD): δ [ppm] = 1.58 (m, 2H, C***H***$_2$), 1.72 (m, 2H, C***H***$_2$), 2.74 (m, 4H, 2 C***H***$_2$), 7.52 (s, 1H, Triazol-***H***). ^{13}C NMR (75 MHz, CD_3OD): δ [ppm] = 25.13 (–, ***C***H$_2$), 27.54 (–, ***C***H$_2$), 30.25 (–, ***C***H$_2$), 41.18 (–, ***C***H$_2$–NH$_2$), 129.14 (+, triazol-***C***), 145.47 (C$_{quat}$, triazol-***C***). ES-MS (DCM/MeOH + NH$_4$OAc) *m/z* (%): 141 (100) [M+H]$^+$, 182 (25) [M+H+MeCN]$^+$. HRMS (EI-MS) calcd. for $C_6H_{12}N_4$ [M$^{+\bullet}$] 140.1062; found 140.1058. Anal. ($C_6H_{12}N_4$) C, H, N. $C_6H_{12}N_4$ (140.19).

*tert-Butyl 5-[imino(methylsulfanyl)methylamino]-5-oxopentylcarbamate (**28**)*

TBTU (8.8 g, 28 mmol, 1.2 eq) was added to a solution of **26** (5.0 g, 23 mmol, 1 eq) and DIPEA (8.1 mL, 46 mmol, 2 eq) in DCM (50 mL) and the mixture was stirred for 30 min. Subsequently a solution of **27** (5.0 g, 23 mmol, 1 eq) and DIPEA (12.2 mL, 69 mmol, 3 eq) in DCM (20 mL) was slowly added. The mixture was stirred overnight. After adding 50 mL ice-cold water the mixture was extracted with 3 × 30 mL DCM. The combined organic layers were dried over $MgSO_4$ and the solvent was removed *in vacuo*. Flash chromatography (PE/EtOAc 60:40 v/v) yielded a yellow oil (3.0 g, 45%); ^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.44 (s, 9H, Boc), 1.52 (m, 2H, C***H***$_2$), 1.68 (m, 2H, C***H***$_2$), 2.45 (m, 2H, C***H***$_2$), 2.48 (s, 3H, S-C***H***$_3$), 3.12 (m, 2H, C***H***$_2$-NHBoc). ^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm] = 13.26 (+, ***C***H$_3$), 22.45 (–, ***C***H$_2$), 28.39 (+, C(***C***H$_3$)$_3$), 29.50 (–, ***C***H$_2$), 39.23 (–, ***C***H$_2$), 40.12 (–, ***C***H$_2$), 78.37 (C$_{quat}$, ***C***(CH$_3$)$_3$), 156.08 (C$_{quat}$, ***C***=O), 165.72 (C$_{quat}$, C–S), 182.49 (C$_{quat}$, ***C***=O). CI-MS (NH_3) *m/z* (%): 290 (100) [M+H]$^+$. $C_{12}H_{23}N_3O_3S$ (289.39).

*tert-Butyl 4-(3-amino-1H-1,2,4-triazol-5-yl)-butylcarbamate (**29**)*

A solution of **28** (3.6 g, 12.4 mmol), hydrazine monohydrate (18 mL, 374 mmol), and NEt_3 (56 mL, 404 mmol) in ethanol (150 mL) was heated at reflux overnight. The solvent was evaporated and subjected to flash chromatography (DCM/MeOH 95:5 v/v) yielding a white oily solid (0.7 g, 22%); ^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.44 (s, 9H, Boc), 1.54 (m, 2H, C***H***$_2$), 1.72 (m, 2H, C***H***$_2$), 2.69 (m, 2H, C***H***$_2$), 3.14 (m, 2H, C***H***$_2$-NHBoc). ^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm] = 28.71 (–, ***C***H$_2$), 30.66 (–, ***C***H$_2$), 32.12 (+, C(***C***H$_3$)$_3$), 32.98 (–, ***C***H$_2$), 43.74 (–, ***C***H$_2$), 83.10 (C$_{quat}$, ***C***(CH$_3$)$_3$), 160.70 (C$_{quat}$, ***C***=O), 162.91 (C$_{quat}$, triazol-***C***), 171.27 (C$_{quat}$, triazol-***C***). ES-MS (DCM/MeOH + NH$_4$OAc) *m/z* (%): 256 (100) [M+H]$^+$. $C_{11}H_{21}N_5O_2$ (255.32).

*5-(4-Aminobutyl)-1H-1,2,4-triazol-3-amine (**30**) [59]*

Compound **29** (0.7 g, 2.9 mmol) was dissolved in 5 mL MeOH and 5 mL TFA was added. The mixture was stirred overnight and the solvents were removed *in vacuo*. The crude product was purified by preparative HPLC to yield a white solid (0.59 g, 53%, trifluoroacetate); m.p. 135–137°C. ^{1}H NMR (300 MHz, CD_3OD,

trifluoroacetate): δ [ppm] = 1.77 (m, 2H, 2 C***H***$_2$), 2.73 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$), 2.96 (t, 2H, ^3J = 7.0 Hz, C***H***$_2$). ^{13}C NMR (75 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 24.40 (−, ***C***H$_2$), 25.81 (−, ***C***H$_2$), 27.74 (−, ***C***H$_2$), 40.21 (−, ***C***H$_2$–NH$_2$), 152.25 (C$_{quat}$, triazol-***C***), 153.33 (C$_{quat}$, triazol-***C***), 163.32 (C$_{quat}$, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m*/*z* (%): 156 (45) $[M+H]^+$, 161 (100) $[M+2H+4MeCN]^{2+}$. HRMS (EI-MS) calcd. for $C_6H_{13}N_5$ $[M^{+\bullet}]$ 155.1171; found 155.1172. Anal. ($C_6H_{13}N_5$·1.9 TFA·0.4 H_2O) C, H, N. $C_6H_{13}N_5$·2 TFA (383.25).

*General procedure for the preparation of the isoureas (**34** and **35**)*

A solution of the pertinent amine (1 eq) and diphenyl cyanocarbonimidate (**33**, 1 eq) in 2-propanol was stirred for 2 h. After evaporation of the solvent, the product was crystallized from Et_2O.

*tert-Butyl 3-[(cyanoimino)(phenoxy)methylamino]-propylcarbamate (**34**)*

A solution of **31** (1.5 g, 8.4 mmol) and **33** (2.0 g, 8.4 mmol) in 2-propanol (50 mL) was stirred for 2 h. Flash chromatography (PE/EtOAc 60:40–40:60 v/v) yielded a white solid (2.26 g, 85%); m.p. 113°C (Et_2O). ^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.43 (s, 9H, Boc), 1.80 (m, 2H, C***H***$_2$), 3.24 (m, 2H, C***H***$_2$), 3.46 (m, 2H, C***H***$_2$), 7.08 (m, 2H, Ph-***H***), 7.26 (m, 1H, Ph-***H***-4), 7.40 (m, 2H, Ph-***H***). ^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm] = 28.39 (+, C(***C***H$_3$)$_3$), 29.87 (−, ***C***H$_2$), 37.33 (−, ***C***H$_2$–N), 39.65 (−, ***C***H$_2$–N), 79.48 (C$_{quat}$, ***C***(CH$_3$)$_3$), 115.88 (C$_{quat}$, ***C***N), 121.49 (+, 2 Ph-***C***), 126.62 (+, Ph-***C***-4), 129.52 (+, 2 Ph-***C***), 151.07 (C$_{quat}$, Ph-***C***-1), 156.33 (C$_{quat}$, ***C***=O), 163.90 (C$_{quat}$, ***C***=N). ES-MS (DCM/MeOH+NH_4OAc) *m*/*z* (%): 319 (65) $[M+H]^+$, 637 (100) $[2M+]^+$, 654 (90) $[2M+NH_4]^+$. Anal. ($C_{16}H_{22}N_4O_3$) C, H, N. $C_{16}H_{22}N_4O_3$ (318.37).

*tert-Butyl 4-[(cyanoimino)(phenoxy)methylamino]-butylcarbamate (**35**)*

A solution of **32** (1.58 g, 8.4 mmol) and **33** (2.0 g, 8.4 mmol) in 2-propanol (50 mL) was stirred for 2 h. Flash chromatography (PE/EtOAc 60:40–40:60 v/v) yielded a white solid (2.24 g, 80%); m.p. 99°C (Et_2O). ^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.42 (s, 9H, Boc), 1.56 (m, 2H, C***H***$_2$), 1.65 (m, 2H, C***H***$_2$), 3.14 (m, 2H, C***H***$_2$), 3.41 (m, 2H, C***H***$_2$), 7.07 (m, 2H, Ph-***H***), 7.26 (m, 1H, Ph-***H***-4), 7.40 (m, 2H, Ph-***H***). ^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm] = 26.71 (−, ***C***H$_2$), 27.21 (−, ***C***H$_2$), 28.41 (+, C(***C***H$_3$)$_3$), 39.98 (−, ***C***H$_2$–N), 42.22 (−, ***C***H$_2$–N), 79.26 (C$_{quat}$, ***C***(CH$_3$)$_3$), 115.87 (C$_{quat}$, ***C***N), 121.50 (+, 2 Ph-***C***), 126.57 (+, Ph-***C***-4), 129.55 (+, 2 Ph-***C***), 151.09 (C$_{quat}$, Ph-***C***-1), 156.07 (C$_{quat}$, ***C***=O), 163.94 (C$_{quat}$, ***C***=ON). ES-MS (DCM/MeOH + NH_4OAc) *m*/*z* (%): 333 (70) $[M+H]^+$, 665 (80) $[2M+]^+$, 682 (100) $[2M+NH_4]^+$. Anal. ($C_{17}H_{24}N_4O_3$) C, H, N. $C_{17}H_{24}N_4O_3$ (332.40).

*General procedure for the preparation of 3,5-diamino-triazoles (**36** and **37**)*

Hydrazine monohydrate was added to a solution of the isourea in 40 mL MeOH and the mixture was stirred at room temperature for 24 h. The solvent was evaporated, the residue was taken up in 30 mL water and 50 mL DCM was added. The precipitated product was filtered off and washed with water and DCM.

*tert-Butyl 3-(5-amino-1H-1,2,4-triazol-3-ylamino)-propylcarbamate (**36**)*

The title compound was prepared from **34** (2.2 g, 6.9 mmol) and hydrazine monohydrate (0.5 mL, 10 mmol) in MeOH (40 mL) according to the general procedure yielding a white solid (1.25 g, 71%); m.p. 84–85°C. ^{1}H NMR (300 MHz, CD_3OD): δ [ppm] = 1.43 (s, 9H, Boc), 1.70 (m, 2H, C***H***$_2$), 3.11 (t, 2H, ^3J = 6.7 Hz, C***H***$_2$-NHBoc), 3.16 (t, 2H, ^3J = 6.8 Hz, C***H***$_2$–NH). ^{13}C NMR (75 MHz, CD_3OD): δ [ppm] = 28.80 (+, C(***C***H$_3$)$_3$), 30.97 (−, ***C***H$_2$), 38.77 (−, ***C***H$_2$–N), 41.39 (−, ***C***H$_2$–N), 79.97 (C$_{quat}$, ***C***(CH$_3$)$_3$), 158.68 (C$_{quat}$, ***C***=OO). ES-MS (DCM/MeOH + NH_4OAc) *m*/*z* (%): 257 (100) $[M+H]^+$. Anal. ($C_{10}H_{20}N_6O_2$) C, H, N. $C_{10}H_{20}N_6O_2$ (256.30).

*tert-Butyl 4-(5-amino-1H-1,2,4-triazol-3-ylamino)-butylcarbamate (**37**)*

The title compound was prepared from **35** (2.3 g, 6.9 mmol) and hydrazine monohydrate (0.5 mL, 10 mmol) in MeOH (40 mL) according to the general procedure yielding a white solid (1.6 g, 86%); m.p. 138°C. ^{1}H NMR (300 MHz, CD_3OD): δ [ppm] = 1.42 (s, 9H, Boc), 1.53 (m, 4H, C***H***$_2$), 3.05 (t, 2H, ^3J = 6.6 Hz, C***H***$_2$-NHBoc), 3.13 (t, 2H, ^3J = 6.4 Hz, C***H***$_2$–NH). ^{13}C NMR (75 MHz, CD_3OD): δ [ppm] = 28.08 (−, ***C***H$_2$), 28.40 (−, ***C***H$_2$), 28.83 (+, C(***C***H$_3$)$_3$), 41.09 (−, ***C***H$_2$–N), 43.80 (−, ***C***H$_2$–N), 79.88 (C$_{quat}$, ***C***(CH$_3$)$_3$), 158.59 (C$_{quat}$, ***C***=OO). ES-MS (DCM/MeOH + NH_4OAc) *m*/*z* (%): 271 (100) $[M+H]^+$. Anal. ($C_{11}H_{22}N_6O_2$) C, H, N. $C_{11}H_{22}N_6O_2$ (270.33).

*General procedure for the preparation of N^3-aminoalkyl-1H-1,2,4-triazole-3,5-diamines (**38** and **39**)*

Thirty milliliters of 6 M HCl in 2-propanol was added to a solution of the Boc-protected precursor in 30 mL EtOAc and 5 mL MeOH. The mixture was stirred overnight; the precipitate was filtered off, washed, and dried *in vacuo*.

*N^3-(3-Aminopropyl)-1H-1,2,4-triazole-3,5-diamine (**38**)*

Compound **38** was prepared from **36** (1.2 g, 4.7 mmol) according to the general procedure yielding a white solid (0.95 g, 72%); m.p. 117–118°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm] = 1.83 (m, 2H, C***H***$_2$), 2.81 (m, 2H, C***H***$_2$), 3.23 (t, 2H, ^3J = 6.6 Hz, C***H***$_2$), 7.68 (brs, 1H, N***H***), 8.22 (brs, 3H, N***H***), 10.58 (brs, 4H, N***H***). ^{13}C NMR (75 MHz, DMSO-d_6): δ [ppm] = 26.17 (−, ***C***H$_2$), 36.31 (−, ***C***H$_2$–N), 39.15 (−, ***C***H$_2$–N), 151.55 (C$_{quat}$, 2 triazol-***C***). HRMS (EI-MS) calcd. for $C_5H_{12}N_6$ $[M^{+\bullet}]$ 156.1123; found 156.1126. Anal. ($C_5H_{12}N_6$·2.5 HCl·0.3 H_2O) C, H, N. $C_5H_{12}N_6$·3 HCl (265.57).

*N^3-(4-Aminobutyl)-1H-1,2,4-triazole-3,5-diamine (**39**)*

Compound **39** was prepared from **37** (1.6 g, 5.9 mmol) according to the general procedure yielding a white solid (1.6 g, 96%); m.p. 120–121°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm] = 1.57 (m, 4H, 2 C***H***$_2$), 2.75 (m, 2H, C***H***$_2$), 3.14 (t, 2H, ^3J = 6.4 Hz, C***H***$_2$), 7.72 (brs, 1H, N***H***), 8.19 (brs, 3H, N***H***), 11.31 (brs, 4H, N***H***). ^{13}C NMR (75 MHz, DMSO-d_6): δ [ppm] = 24.09 (−, ***C***H$_2$), 25.52 (−, ***C***H$_2$), 38.14 (−, ***C***H$_2$–N), 41.50 (−, ***C***H$_2$–N), 151.70 (C$_{quat}$, 2 triazol-***C***). HRMS (EI-MS) calcd. for $C_6H_{14}N_6$ $[M^{+\bullet}]$ 170.1280; found 170.1279. Anal. ($C_6H_{14}N_6 \cdot 2.5HCl \cdot 0.5H_2O$) C, H, N. $C_6H_{14}N_6 \cdot 3HCl$ (279.60).

*tert-Butyl 3-(5-amino-1H-1,2,4-triazol-3-ylsulfanyl)-propylcarbamate (**42**)*

A solution of **40** (3.0 g, 12.6 mmol) in 50 mL EtOH was added to a mixture of 12.6 mL 1 M NaOH and 5-amino-4*H*-1,2,4-triazole-3-thiol (**41**) (1.22 g, 10.5 mmol). The solution was refluxed for 3 h, concentrated *in vacuo*, diluted with 10 mL water, and extracted with 3 × 100 mL DCM. The organic layer was dried over $MgSO_4$ and the solvent was removed *in vacuo*. Flash chromatography

(DCM/MeOH 95:5 v/v) yielded a colorless foam-like solid (2.25 g, 78%); ^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.41 (s, 9H, Boc), 1.84 (m, 2H, C***H***$_2$), 3.04 (t, 2H, ^3J = 6.0 Hz, C***H***$_2$–S), 3.19 (m, 2H, C***H***$_2$–N). ^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm] = 28.47 (+, C(***C***H$_3$)$_3$), 29.55 (−, ***C***H$_2$), 30.08 (−, ***C***H$_2$–S), 39.03 (−, ***C***H$_2$–N), 79.48 (C_{quat}, ***C***(CH$_3$)$_3$), 155.50 (C_{quat}, triazol-***C***), 156.46 (C_{quat}, ***C***=OO), 158.24 (C_{quat}, Triazol-***C***-3). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 271 (100) $[M+H]^+$. Anal. ($C_{10}H_{19}N_5O_2S$) C, H, N. $C_{10}H_{19}N_5O_2S$ (273.36).

*3-(3-Aminopropylsulfanyl)-1H-1,2,4-triazol-5-amine (**43**)*

Thirty milliliters of 6 M HCl in 2-propanol was added to a solution of **42** (2.2 g, 8 mmol) in 30 mL EtOAc and 5 mL MeOH. The mixture was stirred over-night; the precipitate was filtered off, washed, and dried *in vacuo*. The crude product was recrystallized from EtOH to yield a white solid (1.67 g, 85%); m.p. 137°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm] = 1.96 (m, 2H, C***H***$_2$), 2.85 (m, 2H, C***H***$_2$), 3.19 (t, 2H, ^3J = 7.0 Hz, S–C***H***$_2$), 8.28 (brs, 5H, N***H***), 13.22 (brs, 2H, N***H***). ^{13}C NMR (75 MHz, DMSO-d_6): δ [ppm] = 26.60 (−, ***C***H$_2$), 28.33 (−, ***C***H$_2$–S), 37.08 (−, ***C***H$_2$–N), 146.91 (C_{quat}, triazol-***C***-5), 152.19 (C_{quat}, triazol-***C***-3). HRMS (EI-MS) calcd. for $C_5H_{11}N_5S$ $[M^{+\bullet}]$ 173.0735; found 173.0734. Anal. ($C_5H_{11}N_5S \cdot 1.9HCl$) C, H, N. $C_5H_{11}N_5S \cdot 2HCl$ (246.16).

*General procedure for the preparation of the N-(ω-aminoalkyl)pyrimidine-2,4-diamines (**52–55**)*

The respective chloropyrimidine (1 eq) was dissolved in 10 mL ethanol. The pertinent mono-Boc-protected diamine (1 eq) and DIPEA (1.5 eq) were added and the mixture was heated under microwave irradiation at 120°C for 30 min. The reaction mixture was diluted with EtOAc, and washed with water and brine; the organic layer was dried over $MgSO_4$ and evaporated. Flash chromatography (DCM/MeOH 90:10 v/v) gave the Boc-protected precursors, which were taken up in 10 mL methanol. Acetylchloride (3 eq) was slowly added at 0°C and the mixture was stirred at room temperature for 4 h. Evaporation of the solvent and crystallization from MeOH/EtOAc yielded **52–55** as hydrochlorides.

*N^2-(2-Aminoethyl)pyrimidine-2,4-diamine dihydrochloride (**52**) [60]*

Compound **52** was prepared from **48** (0.155 g, 1.2 mmol) and **51** (0.192 g, 1.2 mmol) according to the general procedure. Yield 0.13 g (49%) white solid, m.p. 228–230°C (dec.) ^{1}H NMR (300 MHz, CD_3OD): δ [ppm] = 3.22 (t, 2H, ^3J = 5.7 Hz, C***H***$_2$–N), 3.74 (t, 2H, ^3J = 5.7 Hz, C***H***$_2$–N), 6.17 (d, 1H, ^3J = 7.2 Hz, Py-***H***-5), 7.69 (d, 1H, ^3J = 7.2 Hz, Py-***H***-6). ^{13}C NMR (75 MHz, CD_3OD): δ [ppm] = 39.70 (−, ***C***H$_2$–N), 40.48 (−, ***C***H$_2$–N), 98.67 (+, Py-***C***-5), 143.08 (+, Py-***C***-6), 155.81 (C_{quat}, Py-***C***), 166.88 (C_{quat}, Py-***C***). HRMS (EI-MS) calcd. for $C_6H_{11}N_5$ $[M^{+\bullet}]$ 153.1014; found 153.1016. Anal. ($C_6H_{11}N_5 \cdot 2HCl$) C, H, N. $C_6H_{11}N_5 \cdot 2HCl$ (226.11).

*N^2-(3-Aminopropyl)pyrimidine-2,4-diamine (**53**)*

Compound **53** was prepared from **48** (0.155 g, 1.2 mmol) and **31** (0.209 g, 1.2 mmol) according to the general procedure. Yield: 0.13 g (44%) white solid, m.p. 193–195°C (dec.) ^{1}H NMR (300 MHz, CD_3OD): δ [ppm] = 1.99 (m, 2H, C***H***$_2$), 3.02 (m, 2H, C***H***$_2$–N), 3.53 (t, 2H, ^3J = 6.5 Hz, C***H***$_2$–N), 6.12 (d, 1H, ^3J = 7.2 Hz, Py-***H***-5), 7.64 (d, 1H, ^3J = 7.3 Hz, Py-***H***-6). ^{13}C NMR (75 MHz, CD_3OD): δ [ppm] = 28.36 (−, ***C***H$_2$), 38.35 (−, ***C***H$_2$–N), 38.81 (−, ***C***H$_2$–N), 98.33 (+, Py-***C***-5), 142.89 (+, Py-***C***-6), 155.50 (C_{quat}, Py-***C***), 166.85 (C_{quat}, Py-***C***). HRMS (EI-MS) calcd. for $C_7H_{13}N_5$ $[M^{+\bullet}]$ 167.1171; found 167.1172. Anal. ($C_7H_{13}N_5 \cdot 2.5HCl \cdot 0.8H_2O$) C, H, N. $C_7H_{13}N_5 \cdot 2HCl$ (240.13).

*N^2-(2-Aminoethyl)-N^4-methylpyrimidine-2,4-diamine (**54**)*

Compound **54** was prepared from **49** (0.215 g, 1.5 mmol) and **51** (0.24 g, 1.5 mmol) according to the general procedure. Crystallization yielded a pale green solid (0.2 g, 55%), m.p. 217–219°C (dec.) ^{1}H NMR (300 MHz, CD_3OD): δ [ppm] = 3.03 (s, 3H, C***H***$_3$), 3.25 (t, 2H, ^3J = 6.0 Hz, C***H***$_2$–N), 3.79 (t, 2H, ^3J = 6.0 Hz, C***H***$_2$–N), 6.14 (d, 1H, ^3J = 7.3 Hz, Py-***H***-5), 7.58 (d, 1H, ^3J = 7.3 Hz, Py-***H***-6). ^{13}C NMR (75 MHz, CD_3OD): δ [ppm] = 28.21 (+, ***C***H$_3$), 39.68 (−, ***C***H$_2$–N), 40.01 (−, ***C***H$_2$–N), 99.59 (+, Py-***C***-5), 141.19 (+, Py-***C***-6), 155.73 (C_{quat}, Py-***C***), 164.77 (C_{quat}, Py-***C***). HRMS (EI-MS) calcd. for $C_7H_{13}N_5$ $[M^{+\bullet}]$ 167.1171; found 167.1170. Anal. ($C_7H_{13}N_5 \cdot 2HCl$) C, H, N. $C_7H_{13}N_5 \cdot 2HCl$ (240.13).

*N^4-(2-Aminoethyl)pyrimidine-2,4-diamine (**55**) [61]*

Compound **55** was prepared from **50** (0.155 g, 1.2 mmol) and **51** (0.192 g, 1.2 mmol) according to the general procedure. Yield 0.16 g (60%) white solid, m.p. 239–241°C (dec.) ^{1}H NMR (300 MHz, CD_3OD): δ [ppm] = 3.22 (t, 2H, ^3J = 5.9 Hz, C***H***$_2$–N), 3.77 (t, 2H, ^3J = 5.9 Hz, C***H***$_2$–N), 6.20 (d, 1H, ^3J = 7.3 Hz, Py-***H***-5), 7.64 (d, 1H, ^3J = 7.3 Hz, Py-***H***-6). ^{13}C NMR (75 MHz, CD_3OD): δ [ppm] = 39.54 (−, ***C***H$_2$–N), 40.22 (−, ***C***H$_2$–N), 99.83 (+, Py-***C***-5), 141.85 (+, Py-***C***-6), 157.05 (C_{quat}, Py-***C***), 165.71 (C_{quat}, Py-***C***). HRMS (EI-MS) calcd. for $C_6H_{11}N_5$ $[M^{+\bullet}]$ 153.1014; found 153.1016. Anal. ($C_6H_{11}N_5 \cdot 2HCl$) C, H, N. $C_6H_{11}N_5 \cdot 2HCl$ (226.11).

Pharmacology

[^{35}S]GTPγS binding assays on hH$_3$R and hH$_4$R

[^{35}S]GTPγS binding assays were performed as previously described [49, 62, 63]. For details cf. Supporting Information. All data are presented as mean of *N* independent experiments ± SEM. Agonist potencies were given as EC_{50} values (molar concentration of the agonist causing 50% of the maximal response). Maximal responses (intrinsic activities) were expressed as α values. The α value of histamine was set to 1.00, α values of other compounds were referred to this value. IC_{50} values were converted to K_B values using the Cheng–Prussoff equation [64]. EC_{50} and K_B values from the functional GTPγS assays were analyzed by nonlinear regression and best fit to sigmoidal dose–response curves (GraphPad Prism 5.0 software, San Diego, CA).

Histamine H$_3$ and H$_4$ radioligand binding assays on hH$_3$R and hH$_4$R

Competition binding experiments were performed on membrane preparations of Sf9 insect cell expressing the $hH_3R + G\alpha_{i2} + G\beta_1\gamma_2$ + RGS4 or the $hH_4R + G\alpha_{i2} + G\beta_1\gamma_2$. General procedures for the generation of recombinant baculoviruses, culture of Sf9 cells, and membrane preparation have been described elsewhere [65, 66]. The respective membranes were thawed and sedimented by centrifugation at 4°C and 13,000*g* for 10 min. Membranes were resuspended in binding buffer (12.5 mM $MgCl_2$, 1 mM EDTA, and 75 mM Tris/HCl, pH 7.4). Each tube (total volume 100 µL) contained 40 µg (hH_3R) or 60 µg (hH_4R) of membrane protein and increasing concentrations of unlabeled ligands. Radioligands: H_3R [^{3}H]N^α-methylhistamine (Hartmann Analytic, Braunschweig, Germany), specific activity 85.3 Ci/mmol, K_d = 8.6 nM, c = 3 nM,

nonspecific binding determined in the presence of 10 μM of thioperamide (R&D Systems, Wiesbaden, Germany); H_4R: [^{3}H]histamine (Hartmann Analytic), specific activity 25 Ci/mmol, $K_d = 15.9$ nM, $c = 10$ nM, nonspecific binding determined in the presence of 10 μM of histamine (Sigma, Deisenhofen, Germany). The membrane–ligand mixtures were incubated for 60 min at RT and shaken at 300 rpm. Filtration through 0.3% polyethyleneimine-pretreated glass microfiber filters (Whatman GF/B, Maidstone, UK) using a Brandel 96 sample harvester (Brandel, Gaithersburg, MD) separated unbound from membrane-associated radioligand. After three washing steps with binding buffer, filter pieces for each well were punched and transferred into untapped 96-well sample plates 1450-401 (Perkin Elmer, Rodgau, Germany). Each well was supplemented with 200 μL of scintillation cocktail (Rotiscint Eco plus, Roth, Karlsruhe, Germany) and incubated in the dark. Radioactivity was measured with a Micro Beta2 1450 scintillation counter (Perkin Elmer, Rodgau, Germany).

Protein concentration was determined by the method of Lowry using bovine serum albumin as standard [67]. Data analysis of the resulting competition curves was accomplished by nonlinear regression analysis using the algorithms in PRISM GraphPad Software (GraphPad Prism 5.0 software). K_i values were derived from the corresponding EC_{50} data utilizing the equation of Cheng and Prusoff [64].

The authors are grateful to Mrs. Maria Beer-Krön and Mrs. Gertraud Wilberg 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 and by ESF COST Action BM0806, "Recent advances in histamine receptor H_4R research".

The authors have declared no conflict of interest.

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