CHEMMEDCHEM

DOI: 10.1002/cmdc.200800296

N^G-Acylated Aminothiazolylpropylguanidines as Potent and Selective Histamine H_2 Receptor Agonists

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The bioisosteric replacement of the guanidino group in arpromidine-like histamine H_2 receptor (H_2R) agonists by an acylguanidine moiety is a useful approach to obtain potent H_2R agonists with improved oral bioavailability and blood–brain barrier penetration. Unfortunately, the selectivity of such N^G-acylated imidazolylpropylguanidines for the H_2R is poor, in particular versus histamine H_3 (H_3R) and H_4 receptors (H_4R). This drawback appears to depend on the "privileged" imidazolylpropylguanidine structure. The 2-amino-4-methylthiazol-5-yl moiety is a bioisostere of the imidazole ring in the moderately potent H_2R-selective histamine analogue amthamine. This approach was successfully applied to acylguanidine-type H_2R agonists. The aminothiazoles are nearly equipotent to the corresponding imidazoles as H_2R agonists. Compared with histamine, the potency is increased up to 40-fold on the guinea pig right atrium, and up to 125- and 280-fold in GTPase assays with human and guinea pig H_2R–$G_{s\alpha S}$ fusion proteins expressed in Sf9 insect cells, respectively. Docking studies on H_2R models support the hypothesis that 2-aminothiazolyl and imidazolyl derivatives interact with H_2Rs as bioisosteres. In contrast to the imidazoles, the aminothiazoles are devoid of agonistic or relevant antagonistic effects on H_1, H_3, and H_4 receptors. Moreover, unlike amthamine, the 4-methyl group does not significantly contribute to the H_2R agonism of N^G-acylated 2-amino-4-methylthiazol-5-ylpropylguanidines.

Introduction

Recently, we reported on the synthesis and structure–activity relationships (SAR) of N^G-acylated imidazolylpropylguanidines (Figure 1), a novel class of histamine H_2 receptor (H_2R) agonists with decreased basicity and therefore improved pharmacokinetic properties relative to the corresponding strongly basic N^G-alkylated analogues.[1] However, depending on the substitution pattern, these compounds are also more or less active on other histamine receptors, in particular histamine H_3 (H_3R) and H_4 receptors (H_4R). H_3R affinity is very often found in compounds having an imidazol-4-yl moiety, which is also present in numerous highly potent and selective histamine H_3R ligands. Therefore, the bioisosteric replacement of the imidazole ring is the key to improve the selectivity for H_2R over H_3R.

Amthamine (AMT) (Figure 1), a thiazole analogue of histamine (HIS) and a cyclic analogue of dimaprit (DIM), is a full histamine H_2R agonist and exhibits a slightly higher potency than histamine (HIS) at the isolated guinea pig right atrium.[2] Moreover, amthamine is devoid of histamine H_1R, H_3R, and H_4R stimulatory activities at relevant concentrations.[3–5] Accordingly, the common imidazole ring of arpromidine-type compounds was bioisosterically replaced by a 2-amino-4-methylthiazole group without affecting the H_2R agonistic activity, but resulting in increased selectivity for H_2R over H_3R.[6,7]

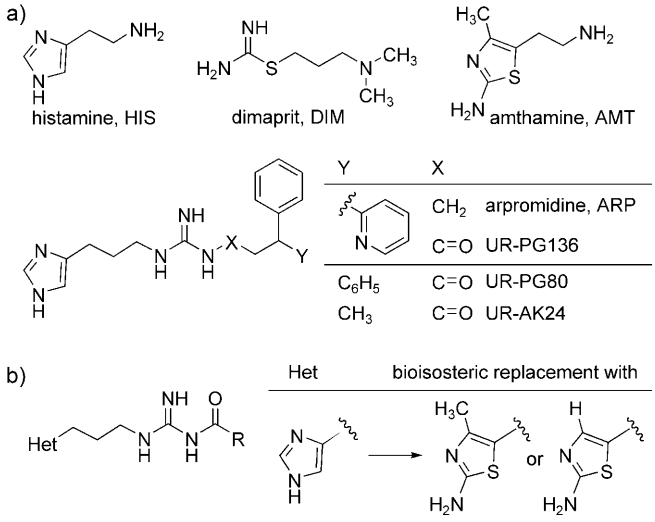

Figure 1. a) Structures of histamine and histamine H_2 receptor agonists including arpromidine and related prototypical acylguanidine-type H_2R agonists ("oxo-arpromidine" and UR-AK). b) Structural modifications of acylated imidazolylpropylguanidines resulting in the title compounds.

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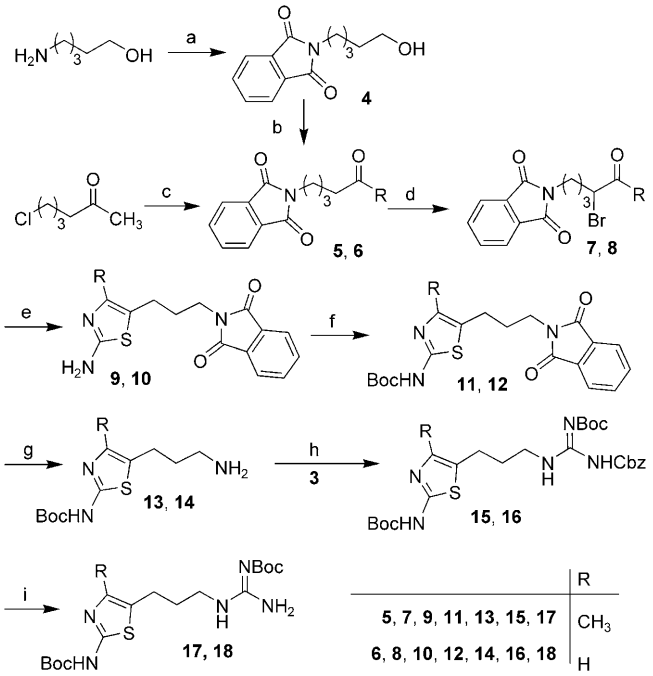
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.200800296.

In the 1970s, methyl groups were introduced at different positions of the imidazole ring and side chain to study the SAR of histamine.[8,9] 4(5)-Methylhistamine was the first H_2R selective agonist described at that time when only H_1R and H_2R were known. Meanwhile, 4(5)-methylhistamine turned out to be a high-affinity H_4R agonist.[5] As, by analogy with the SAR of the natural ligand, the introduction of a 4-methyl substituent in 2-amino-5-(2-aminoethyl)thiazole led to an increase in agonistic activity at the guinea pig right atrium,[2] the H_2R selectivity of amthamine may be attributed to this methyl group.

Herein we report on the synthesis and the H_2R agonistic potency of N^G-acylated 2-amino-4-methylthiazol-5-ylpropylguanidines which are structurally derived from recently reported N^G-acyl-imidazolylpropylguanidines. Furthermore, a set of analogues lacking the 4-methyl substituent at the thiazole ring was prepared to investigate the contribution of the 4-methyl group to histaminergic activity. The aim of the bioisosteric replacement was to improve the selectivity mainly versus H_3R and H_4R. Therefore, the receptor selectivity was studied in GTPase assays on the four recombinantly expressed human histamine receptors.

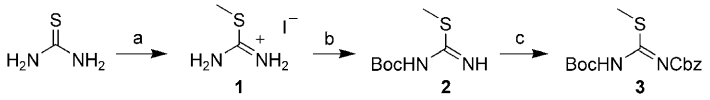


Scheme 2. General procedure for the preparation of the Boc-protected aminothiazolylpropylguanidines **17** and **18**. Reagents and conditions: a) phthalic anhydride (1 equiv), 3 h, 80–100 °C; b) $(COCl)_2$ (1.25 equiv), DMSO (2.65 equiv), NEt_3 (5.5 equiv), CH_2Cl_2 (anhyd), −50 °C, 45 min; c) phthalimide (0.5 equiv), K_2CO_3 (0.75 equiv), DMF, 24 h, 80 °C; d) Br_2 (1 equiv), dioxane, CH_2Cl_2 (anhyd), 1 h, room temperature; e) thiourea (1 equiv), DMF, 3 h, 100 °C; f) $(Boc)_2O$ (1.08 equiv), NEt_3 (1.16 equiv), DMAP (cat.), $CHCl_3$, overnight, room temperature; g) $N_2H_4{\cdot}H_2O$ (5 equiv), EtOH, overnight, room temperature; h) **3** (1 equiv), $HgCl_2$ (2 equiv), NEt_3 (3 equiv), CH_2Cl_2 (anhyd), 48 h, room temperature; i) H_2, Pd/C (10%), MeOH/THF (1:1), 8 bar, 3–4 days, room temperature.

Results and Discussion

Synthesis

The preparation of the title compounds was performed by analogy with the procedure developed for the N^G-acylation of protected imidazolylpropylguanidines.[1] The isothiourea **3**[10–12] (Scheme 1) proved to be an appropriate guanidinylating reagent to obtain Boc-guanidines for coupling to carboxylic acids. The required building blocks, *tert*-butyl-5-(3-aminopropyl)-4-methylthiazol-2-ylcarbamate **13** and *tert*-butyl-5-(3-aminopropyl)thiazol-2-ylcarbamate **14** (Scheme 2) were synthesised in the main according to a previously reported procedure.[2] After treating the amines **13** and **14** with **3** and hydrogenolytic cleavage of the Cbz protecting group, the Boc-protected aminothiazolylpropylguanidines **17** and **18** were obtained in good yields (Scheme 2).



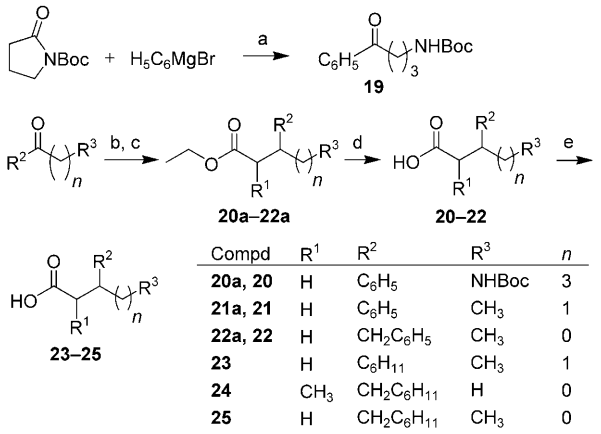
Scheme 1. Synthesis of *N*-*tert*-butoxycarbonyl-*N*′-benzyloxycarbonyl-*S*-methylisothiourea (**3**). Reagents and conditions: a) MeI (1 equiv), MeOH, 1 h, reflux; b) $(Boc)_2O$ (1 equiv), NEt_3 (1 equiv), CH_2Cl_2 (anhyd), overnight, room temperature; c) CbzOSu (1 equiv), CH_2Cl_2 (anhyd), 20 h, room temperature.

The required carboxylic acids were mainly synthesised from commercially available or synthesised ketones according to standard procedures (Scheme 3). To include an aminoalkyl-substituted building block to accommodate optional side chain modifications of H_2R agonists, ketone **19** was prepared as a starting material by selective ring opening of *N*-Boc-butyrolactam with phenylmagnesium bromide as described.[13] The carboxylic acids **20**–**22** were obtained by the Horner–Wadsworth–Emmons reaction with triethyl phosphonoacetate, hydrogenation, and ester hydrolysis. The cyclohexylalkanoic acids **23**–**25** were prepared from the corresponding synthesised (compounds **21**–**22**) or commercially available phenylalkanoic acids by hydrogenation of the benzene ring with Rh/Al_2O_3 or Rh/C as catalyst and AcOH as solvent.

The protected guanidines **17** and **18** were treated with the pertinent carboxylic acids using standard coupling reagents (EDAC, HOBt, and DIEA) to yield the N^G-acylated di-Boc-protected aminothiazolylpropylguanidines **26**–**42a** (Scheme 4). Both Boc groups can be removed by treating with TFA in CH_2Cl_2 to obtain the N^G-acylated 2-aminothiazolylpropylguanidines **26**–**42**.

Pharmacology

All compounds were examined for histamine H_2R agonism on the isolated spontaneously beating guinea pig right atrium (positive chronotropic response) as a pharmacological standard model for the functional characterisation of H_2R ligands[15] (Table 1) and in the GTPase assay on hH_2R–$G_{s\alpha S}$ and gpH_2R–$G_{s\alpha S}$ fusion proteins expressed in Sf9 insect cell membranes[16] (Table 2). To study the receptor selectivity, representative com-



Compd	R^1	R^2	R^3	n
20a, 20	H	C_6H_5	NHBoc	3
21a, 21	H	C_6H_5	CH_3	1
22a, 22	H	$CH_2C_6H_5$	CH_3	0
23	H	C_6H_{11}	CH_3	1
24	CH_3	$CH_2C_6H_{11}$	H	0
25	H	$CH_2C_6H_{11}$	CH_3	0

Scheme 3. Synthesis of the building blocks **19**–**25**. Reagents and conditions: a) THF (anhyd), 1 h, −78 °C, room temperature; b) NaH (60% dispersion in mineral oil) (1.56 equiv), triethyl phosphonoacetate (1.4–1.5 equiv), THF (anhyd), 24 h, reflux; c) H_2, Pd/C (10%) (cat.), EtOH, 24 h, room temperature; d) 20% NaOH (aq), 2–3 h, reflux; e) H_2, Rh/C or Rh/Al_2O_3 (cat.), AcOH, 8 bar, 48 h, room temperature.

pounds were investigated in GTPase assays on human histamine H_1, H_3, and H_4 receptors (Table 3).

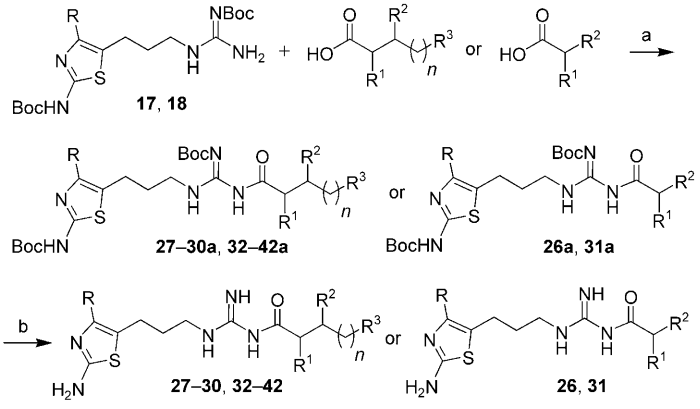
Histamine H_2R agonism on the guinea pig right atrium

The synthesised compounds were partial to nearly full agonists at the spontaneously beating guinea pig right atrium with moderately (up to a factor of four) lower potencies than those

Table 1. Histamine H_2 receptor agonism on the guinea pig right atrium.

Compound	$pEC_{50} \pm SEM$[a]	Pot_{rel} [%][b]	$E_{max} \pm SEM$ [%][c]
HIS	6.00 ± 0.02	100	100
AMT[d]	6.21 ± 0.09	162	95 ± 2
ARP[e]	8.01 ± 0.10	10200	100
UR-PG136[f]	7.47 ± 0.12	2930	100 ± 1
UR-PG80[f]	7.55 ± 0.09	3530	85 ± 3
UR-AK24[f]	7.80 ± 0.07	6350	99 ± 2
26, UR-BIT23	6.22 ± 0.01	165	82 ± 3
27, UR-BIT24	6.72 ± 0.04	528	78 ± 2
28, UR-PG267	7.31 ± 0.17	2060	89 ± 3
29, UR-PG278	7.27 ± 0.11	1850	71 ± 4
30, UR-AK466	7.55 ± 0.03	3530	97 ± 2
31, UR-BIT29	6.61 ± 0.07	405	86 ± 2
32, UR-PG283	7.16 ± 0.06	1450	74 ± 8
33, UR-AK423	6.75 ± 0.10	566	65 ± 3
34, UR-AK420	7.25 ± 0.12	1760	70 ± 3
35, UR-AK421	6.81 ± 0.18	650	49 ± 7
36, UR-AK471	7.18 ± 0.04	1520	92 ± 2
37, UR-AK470	7.55 ± 0.10	3510	92 ± 4
38, UR-AK478	7.15 ± 0.06	1400	65 ± 6
39, UR-AK469	7.54 ± 0.08	3470	74 ± 5
40, UR-AK472	7.27 ± 0.12	1850	84 ± 5
41, UR-AK479	7.05 ± 0.11	1120	73 ± 2
42, UR-AK477	7.61 ± 0.12	4070	74 ± 4

[a] pEC_{50} values were calculated from the mean shift ΔpEC_{50} of the agonist curve relative to the histamine reference curve by the equation: $pEC_{50} = 6.00 + 0.13 + \Delta pEC_{50}$; summand 0.13 represents the mean desensitisation observed for control organs when two successive curves for histamine were performed (0.13 ± 0.02, $n = 16$); the SEM given for pEC_{50} is the SEM calculated for ΔpEC_{50} for 3–7 experiments. [b] Potency relative to histamine. [c] Efficacy, maximal response, relative to the maximal increase in heart rate induced by 30 μM histamine. [d] Data from Ref. [17]. [e] Data from Ref. [18]. [f] Data from Ref. [1].



	R	R^1	R^2	R^3	n		R	R^1	R^2	R^3	n
26	CH_3	H	C_6H_5	–	–	**35**	CH_3	H	CH_3	C_6H_{11}	1
27	CH_3	H	H	C_6H_5	0	**36**	H	H	H	C_6H_5	0
28[c]	CH_3	H	CH_3	C_6H_5	0	**37**	H	H	CH_3	C_6H_5	0
29	CH_3	H	C_6H_5	C_6H_5	0	**38**	H	H	CH_2CH_3	C_6H_5	0
30	CH_3	H	$(CH_2)_3NH_2$	C_6H_5	0	**39**	H	H	C_6H_5	C_6H_5	0
31	CH_3	H	C_6H_{11}	–	–	**40**	H	H	CH_3	C_6H_{11}	0
32[c]	CH_3	H	CH_3	C_6H_{11}	0	**41**	H	CH_3	H	C_6H_{11}	0
33	CH_3	CH_3	H	C_6H_{11}	0	**42**	H	H	CH_2CH_3	C_6H_{11}	0
34	CH_3	H	CH_2CH_3	C_6H_{11}	0						

Scheme 4. General procedure for the coupling of carboxylic acids with aminothiazolylpropylguanidine building blocks. Reagents and conditions: a) EDAC (1 equiv), HOBt (1 equiv), DIEA (1 equiv), CH_2Cl_2 (anhyd), 24 h, room temperature; b) 20% TFA, CH_2Cl_2 (anhyd), 3–5 h, room temperature. [c] A different synthetic pathway.[14]

of the corresponding imidazole analogues, for comparison, **28** versus UR AK24, and **29** (UR-PG278) versus UR-PG80, respectively. The most potent H_2R agonists exceed the potency of histamine about 40 times. Except for compound **42** (versus **38**) the cyclohexyl-substituted compounds are somewhat less potent than the corresponding phenylalkanoylguanidines. 2-Aminothiazolylpropylguanidines lacking the 4-methyl substituent (**36**–**42**) consistently show slightly higher potencies and in some cases also higher efficacies than their methylated analogues, for example, **37** versus **28**, **39** versus **29**, **41** versus **33**, and **42** versus **34**). The positive chronotropic effect of **26**–**42** was susceptible to blockade by the H_2R antagonist cimetidine (10–100 μM). Typical competition experiments are shown for **30** (Figure 2).

Agonism at human and guinea pig H_2Rs in the GTPase assay

Similar to the results from the guinea pig atrium, all investigated compounds were moderate to potent partial or full agonists in the GTPase assay at hH_2R–$G_{s\alpha s}$ and gpH_2R–$G_{s\alpha s}$ fusion proteins expressed in Sf9 cell membranes (Table 2). Compared with the guinea

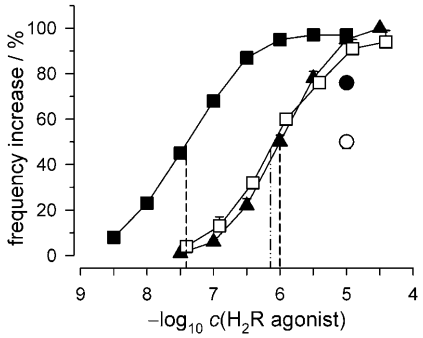


Figure 2. Concentration–response curves on the guinea pig right atrium. Histamine (▲, $pEC_{50} = 6.00 \pm 0.06$, $n = 4$), **30** alone (■, $pEC_{50} = 7.42 \pm 0.03$, relative potency 3530% (95% confidence limits 2900–4310), $E_{max} = 97 \pm 2\%$, $n = 4$) and **30** (□) in the presence of the H_2R antagonist cimetidine (10 μM, pre-incubation for 30 min, $pA_2 = 6.24 \pm 0.12$, $n = 2$). Addition of cimetidine (30 μM and 100 μM, incubation for 60 min each) led to a fading of the maximum response induced by **30** (10 μM, ■) to 76 ± 2% (●) and 50 ± 4% (○) ($n = 4$ each). Also from these experiments, affinity of cimetidine was estimated to be $pA_2 = 6.32 \pm 0.08$ and 6.40 ± 0.05, respectively ($n = 4$ each) by measuring the horizontal distance of ● and ○ relative to the agonist curve (■).

pig atrium, the potencies of the aminothiazoles were higher at the recombinant gpH_2R, with highest potencies in the range of 200–300 times that of histamine (for example, **32** and **40**). The structure–activity relationships derived from both assays and the order of potencies are essentially in good agreement. In accordance with the results for alkylated and acylated imidazolylpropylguanidines[16,19,20] (for example, ARP, UR-PG136, UR-PG80, and UR-AK24 included in Table 2; for structures, see Figure 1), the aminothiazole analogues exhibit higher potencies and efficacies at gpH_2R–$G_{s\alpha S}$ than at hH_2R–$G_{s\alpha S}$ (Figure 3).

Generally, 3,3-disubstitution of the alkanoyl residue was favourable. However, a methyl substituent in the α-position was also tolerated (**33**, **41**). The exchange of a phenyl against a cyclohexyl ring resulted in similar or slightly higher potencies and efficacies at hH_2R and gpH_2R (**28** versus **32**, **37** versus **40**). The ratio of EC_{50} values (EC_{50} hH_2R–$G_{s\alpha S}$/EC_{50} gpH_2R–$G_{s\alpha S}$) was highest for compound **30** (UR-AK466) with a free amino group in the side chain. This compound exhibited moderate agonistic activity at hH_2R–$G_{s\alpha S}$ ($EC_{50} = 150$ nM), whereas it was about 17 times more potent at gpH_2R–$G_{s\alpha S}$ ($EC_{50} = 8.9$ nM) (Table 2, Figure 3).

The unmethylated compounds show similar or slightly increased efficacies and similar potencies at gpH_2R–$G_{s\alpha S}$ and hH_2R–$G_{s\alpha S}$ relative to the 2-amino-4-methylthiazoles. Thus, the introduction of a methyl group at position 4 of the thiazole ring does not generally increase the agonistic activity of the N^G-acylguanidines, that is, the influence of the ring methylation on the H_2R agonistic potency is dependent on the side chain. In contrast to the thiazolylethylamine amthamine,[2] the methyl group neither enhances the agonistic activity for N^G-acylated thiazolylpropylguanidines in the GTPase assay nor at the guinea pig right atrium.

Table 2. Agonist efficacies and potencies at hH_2R–$G_{s\alpha S}$ and gpH_2R–$G_{s\alpha S}$ expressed in Sf9 cell membranes.[a]

Compound	hH_2R–$G_{s\alpha S}$			gpH_2R–$G_{s\alpha S}$			EC_{50} hH_2R–$G_{s\alpha S}$/EC_{50} gpH_2R–$G_{s\alpha S}$
	$E_{max} \pm$ SEM [%]	EC_{50} [nM]	Pot_{rel} [%]	$E_{max} \pm$ SEM [%]	EC_{50} [nM]	Pot_{rel} [%]	
HIS[16]	1.00	1260 ± 250	100	1.00	1200 ± 240	100	1.05
DIM[16]	0.85 ± 0.07	1940 ± 70	65	0.93 ± 0.04	1200 ± 210	100	1.62
AMT[16]	0.90 ± 0.06	450 ± 40	280	1.04 ± 0.05	440 ± 40	271	1.02
ARP[16]	0.79 ± 0.07	190 ± 40	659	1.02 ± 0.04	70 ± 10	1600	2.71
UR-PG136[19]	0.73 ± 0.03	420 ± 90	290	0.93 ± 0.04	45 ± 4	2700	9.21
UR-PG80[19]	0.69 ± 0.09	78 ± 42	1500	0.93 ± 0.32	6 ± 1	19000	12.1
UR-AK24[20]	0.87 ± 0.01	67 ± 2	1800	1.03 ± 0.06	12 ± 1	10000	5.58
26	0.68 ± 0.02	95.8 ± 5.9	1315	0.79 ± 0.01	27.9 ± 17.7	4301	3.43
27	0.79 ± 0.02	22.2 ± 5.1	5676	0.76 ± 0.02	7.5 ± 0.9	16000	2.96
28	0.78 ± 0.05	35.3 ± 12.7	3569	0.93 ± 0.05	17.5 ± 4.9	6857	2.02
29	0.47 ± 0.03	88.4 ± 9.5	1425	0.86 ± 0.02	7.3 ± 3.3	16438	12.1
30	0.66 ± 0.04	150.6 ± 15.7	837	1.03 ± 0.11	8.9 ± 5.6	13483	16.9
31	0.71 ± 0.09	49.7 ± 4.0	2535	0.83 ± 0.09	19.0 ± 9.2	6316	2.61
32	0.66 ± 0.10	10.2 ± 1.1	12353	0.87 ± 0.06	5.3 ± 1.9	22642	1.92
33	0.62 ± 0.02	15.1 ± 5.3	8344	0.54 ± 0.12	8.4 ± 3.6	14286	1.79
34	0.64 ± 0.02	14.9 ± 0.44	8456	0.80 ± 0.03	6.3 ± 0.8	19048	2.36
35	0.42 ± 0.03	24.6 ± 1.4	5122	0.62 ± 0.04	17.1 ± 6.0	7017	1.44
36	0.82 ± 0.02	23.8 ± 1.9	5294	0.80 ± 0.19	11.1 ± 4.2	10811	2.14
37	0.81 ± 0.07	29.8 ± 9.6	4228	0.84 ± 0.04	6.9 ± 1.9	17391	4.32
38	0.67 ± 0.05	31.1 ± 6.8	4051	0.87 ± 0.20	25.2 ± 11.5	4762	1.23
39	0.66 ± 0.05	54.4 ± 21.2	2316	0.88 ± 0.06	9.1 ± 3.4	13187	5.98
40	0.73 ± 0.07	12.0 ± 3.6	10500	0.94 ± 0.04	4.3 ± 1.1	27907	2.79
41	0.72 ± 0.07	23.4 ± 8.4	5385	0.79 ± 0.11	10.9 ± 1.4	11009	2.15
42	0.68 ± 0.05	13.6 ± 3.4	9265	0.88 ± 0.06	9.4 ± 5.0	12766	1.45

[a] Steady-state GTPase activity in Sf9 membranes expressing hH_2R–$G_{s\alpha S}$ and gpH_2R–$G_{s\alpha S}$ was determined as described.[16] Reaction mixtures contained ligands at concentrations from 1 nM to 10 μM as appropriate to generate saturated concentration–response curves. Data were analysed by nonlinear regression and were best fit to sigmoidal concentration–response curves. Typical basal GTPase activities ranged between ~0.5 and 2.5 pmol mg^{-1} min^{-1}, and activities stimulated by histamine (100 μM) ranged between ~2 and 13 pmol mg^{-1} min^{-1}. The efficacy (E_{max}) of histamine was determined by nonlinear regression and was set at 1.0. The E_{max} values of other agonists were normalised to this value. Data shown are means ± SEM of 2–3 experiments, each performed in duplicate. The relative potency of histamine was set to 100, and the potencies of other agonists were normalised to this value.

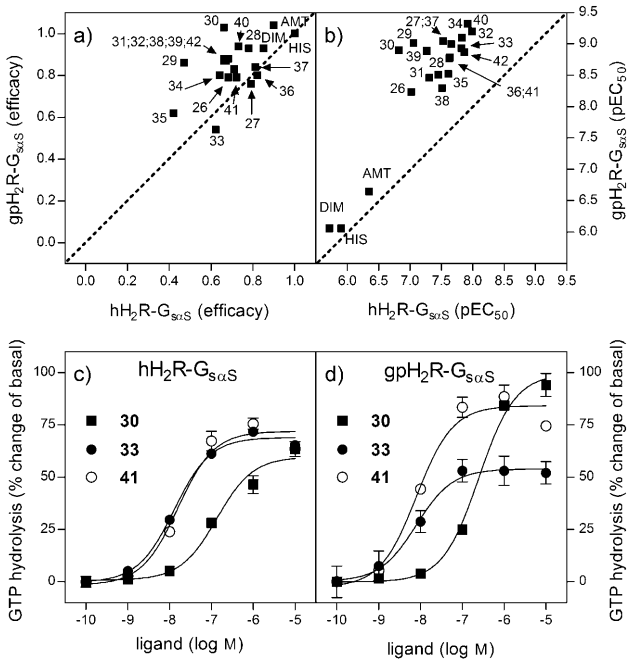


Figure 3. Efficacies and potencies of ligands at hH_2R–$G_{s\alpha S}$ in comparison with gpH_2R–$G_{s\alpha S}$ as determined in the steady-state GTPase assay. The dotted lines represent the line of identity. a) Relation between the efficacies of compounds **26**–**42**, histamine (HIS), amthamine (AMT), and dimaprit (DIM) at hH_2R–$G_{s\alpha S}$ versus gpH_2R–$G_{s\alpha S}$. b) Relation between the potencies of compounds **26**–**42**, HIS, AMT, and DIM at hH_2R–$G_{s\alpha S}$ versus gpH_2R–$G_{s\alpha S}$. Effects of **30**, **33**, and **41** on the GTPase activity ±SEM of representative experiments performed in duplicate in membranes expressing c) hH_2R–$G_{s\alpha S}$ and d) gpH_2R–$G_{s\alpha S}$. Data are expressed as percentage change in GTPase activity induced by the ligands relative to the GTPase activity stimulated by histamine (100 μM).

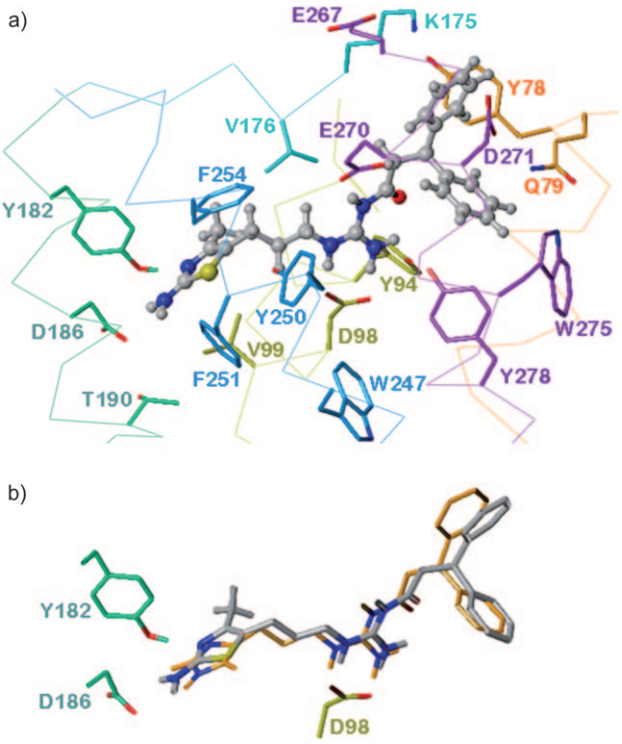


Figure 4. a) Model of the gpH_2R binding site for compound **29** with illustration of side chains and Cα atoms of all amino acids within 3 Å of the ligand and, additionally, the putative toggle switch Trp 247. The backbone trace and the amino acid residues are individually coloured: TM2 orange, TM3 yellow, E2 cyan, TM5 green–blue, TM6 blue, TM7 purple. The ligand is shown in ball-and-stick representation, with C and H atoms in grey and all other atoms coloured by atom type. b) Alignment of compounds **29** (C and essential H atoms grey) and UR-PG80 (C and essential H atoms orange) resulting from superposition of the minimised ligand–gpH_2R complexes. Additionally, amino acids contained in polar key interactions with the ligands are drawn (colour code same as for part a).

Docking of bioisosteres on H_2R models

Does the clear similarity of imidazolyl and 2-aminothiazolyl groups impart similar binding at H_2Rs, that is, are compounds from both structural classes really bioisosteres? To suggest binding modes of imidazolylpropylguanidines, homology models of H_2Rs have been derived[1] by using the recently described crystal structures of the β_2-adrenoceptor as templates.[21,22] Figure 4 is based on the docking of two representative analogues, namely the diphenylpropanoyl derivatives UR-PG80 and **29** (UR-PG278), on a model of the gpH_2R.

Figure 4a represents the suggested binding mode of compound **29** as derived from docking poses of histamine and arpromidine derivatives,[1] indicating that the imidazolylpropylguanidine moiety binds to H_2R in a manner similar to that of histamine. According to studies with H_2R mutants, an ionic interaction of the protonated amino or guanidino group with Asp 98$^{3.32}$ occurs[23] (the superscripts represent the generic numbering scheme of amino acids in transmembrane domains (TMs) 1–7 proposed by Ballesteros and Weinstein[24]). The second and third site of the widely accepted three-point model for biogenic amine/GPCR interaction could principally be formed by the couples Asp 186$^{5.42}$/Thr 190$^{5.46}$[23] or Tyr 182$^{5.38}$/Asp 186$^{5.42}$.[25]

Although docking in the first mode is generally possible as well, the pose in Figure 4 reflects the Tyr 182/Asp 186 mode of binding. This assumption is in agreement with a pH-dependent model of H_2R activation that suggests subsequent tautomerisation of the imidazole ring into the N^{π}-H form caused by neutralisation of histamine upon binding and accompanied by proton transfers from Tyr 182 to N^{π} and from N^{τ} to Asp 186, respectively.[24] 2-Aminothiazoles show a similar type of tautomerisation with the imino form being clearly favoured in polar medium.[26] Also interactions of nontautomeric agonists with H_2Rs are compatible with this model. Thus, the 2-aminothiazolyl group forms two H bonds with Tyr 182$^{5.38}$ (N3 as acceptor) and Asp 186$^{5.42}$ (2-NH_2 as donor), respectively. The thiazolylpropyl side chain additionally fits into a pocket consisting of Val 176 (E2), Tyr 250$^{6.51}$, Phe 251$^{6.52}$, and Phe 254$^{6.55}$. The 4-methyl group approaches Thr 95$^{3.29}$, Val 176, and Val 178 (E2). These hydrophobic interactions could potentially be affinity increasing, but also decrease the flexibility of the ligand necessary for optimal fit to TM5 and TM6 and/or hamper the transition into the active receptor conformation. That the agonistic potencies and some efficacies at the guinea pig atrium are slightly higher in the case of the unmethylated derivatives is therefore not in contrast to the model based on the inactive state.

The previous models[1] predict that N^1-propanoyl and N^1-propyl chains may bind in close superposition and that an in-

teraction of the acyl oxygen with a particular amino acid is rather unlikely. The suggested site for the first phenyl group of compound **29** consists of Gln 79$^{2.65}$, Leu 274$^{7.39}$, Trp 275$^{7.40}$, and Tyr 278$^{7.43}$. The second phenyl moiety projects upwards into the extracellular region of the gpH_2R and is in contact with Tyr 78$^{2.64}$, Asp 271$^{7.36}$, Lys 175 (E2), and Glu 267$^{7.32}$. Indications for interactions with Asp 271 and Lys 175 also result from the potency increasing effect of electron attracting substituents in *para* position.[1]

Strictly speaking, the "bioisostere hypothesis" implies identical interactions which cannot be concluded if 2-aminothiazolyl and imidazolyl analogues are aligned according to the structural formulas, simply because the corresponding N=C-NH fragments are in different positions. However, by superposition of the minimised ligand–gpH_2R complexes, it becomes clear that compounds **29** and UR-PG80 exhibit the same binding mode (see Figure 4b). The putative bioactive conformations are tight fitting, and the key interactions identical. In particular, the bidentate H bonds with the Tyr 182/Asp 186 couple in TM5 can be formed in both cases as the analogous donor and acceptor functions, respectively, are also in close superposition. In conclusion, the docking approaches support the hypothesis that 2-aminothiazolyl- and imidazolylpropylguanidines interact with H_2Rs as bioisosteres.

Table 3. Agonist/antagonist activities on recombinant human histamine H_1, H_3, and H_4 receptors in GTPase assays.[a]

Compound	K_B [nM]		
	hH_1R	hH_3R	hH_4R
30	>10000	>1000	>1000
33	>1000	>1000	>1000
34	>1000	>10000	>1000
35	>1000	>1000	>1000
36	>10000	>10000	>10000
37	>10000	>10000	>10000
38	>1000	>10000	>10000
39	>10000	>10000	>1000
40	>10000	>1000	>1000
41	>1000	>10000	>1000
42	>1000	>10000	>1000
UR-PG80[b]	2980	17.1	EC_{50} = 8.6 nM (efficacy: 0.76)
UR-AK24[b]	14300	EC_{50} = 2.45 nM (efficacy 0.25)	EC_{50} = 15.3 nM (efficacy: 0.84)

[a] Steady-state GTPase activity determined on membrane preparations of Sf9 insect cells expressing hH_1R (co-expressed with RGS4), hH_3R (co-expressed with $G\alpha_i$, $G\beta_1\gamma_2$, and RGS4), or hH_4R–RGS19 fusion protein co-expressed with $G\alpha_{i2}$ and $G\beta_1\gamma_2$.[1,27] Typical basal GTPase activities ranged between ~1.5 and 2.5 pmol mg^{-1} min^{-1}, and activities stimulated by histamine (10 μM) ranged between ~3.5 and 4.5 pmol mg^{-1} min^{-1}. Data shown are means of 2–4 experiments, each performed in duplicate. Reaction mixtures contained histamine (100 nM) and ligands at concentrations from 0.1 nM to 1 mM. [b] Data from Ref. [1].

Selectivity for human H_2R versus hH_1R, hH_3R, and hH_4R

To study the histamine receptor selectivity, representative compounds were examined in GTPase assays using membrane preparations of Sf9 insect cells expressing human H_1, H_3, and H_4 receptors (Table 3). Specifically, compounds were examined for H_1R, H_3R, and H_4R agonism and antagonism, respectively. In addition, selected compounds (**28**, **29**, **32**) were investigated for H_3R antagonism on the electrically stimulated guinea pig ileum. In contrast to N^G-acylated imidazolylpropylguanidines[1] which are moderate to potent H_3R antagonists with pA_2 values of approximately eight,[1,14] the aminothiazole analogues were inactive on the guinea pig ileum at concentrations up to 500 nM (data not shown).

In the GTPase assays on hH_1R, hH_3R, and hH_4R, the investigated aminothiazolylpropylguanidines showed no agonistic effect. Instead, very weak, if any, antagonistic activity was detected at high concentrations. This is particularly important with respect to selectivity for H_2R over H_3R and H_4R, as these data confirm the working hypothesis that the aminothiazole and the imidazole moiety are bioisosteric groups at the H_2R but not at the H_3R and the H_4R.

Conclusions

The replacement of the imidazole by an aminothiazole ring in N^G-acylated imidazolylpropylguanidines resulted in approximately the same agonistic activity at the human and guinea pig H_2 receptor. Whereas compounds of the imidazole series are very potent H_3R antagonists and H_4R agonists, respectively, the N^G-acylated 2-aminothiazol-5-ylpropylguanidines are highly selective for the H_2R. Supported by docking studies, the imidazoles and aminothiazoles interact with H_2Rs as bioisosteres. The potency of derivatives lacking the 4-methyl group indicates that, in contrast to amthamine, this substitution neither enhances the agonistic activity for N^G-acylated compounds in the GTPase assay nor at the guinea pig right atrium. In summary, the study presents a successful bioisosteric approach to the development of highly potent and selective H_2R agonists.

Experimental Section

General conditions

Commercially available reagents were purchased from Acros Organics (Belgium), Lancaster Synthesis GmbH (Germany), Sigma–Aldrich Chemie GmbH (Germany), Alfa Aesar GmbH & Co KG (Germany), or Merck (Germany), and used as received. Where indicated, reactions were carried out under a dry, oxygen-free argon atmosphere. All solvents used were of analytical grade or distilled before use. THF and Et_2O were distilled over Na, CH_2Cl_2 was predried over $CaCl_2$ or distilled from P_4O_{10} and stored under argon atmosphere over molecular sieves 3 Å. Column chromatography was carried out using Merck silica gel Geduran 60 (0.063–0.200) and Merck silica gel 60 (0.040–0.063) for flash column chromatography. Reactions were monitored by thin-layer chromatography (TLC) on Merck silica gel 60 F_{254} aluminum sheets, and spots were visualised with UV light at 254 nm. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 300 spectrometer with perdeuterated solvents. Chemical shifts (δ) are given in parts per million (ppm) with reference to the chemical shift of the residual protic solvent relative to tetramethylsilane (δ = 0 ppm). Multiplicities were specified with the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad signal) as well as combina-

tions thereof. The multiplicity of carbon atoms (^{13}C NMR) were determined by DEPT 135 and DEPT 90 (distortionless enhancement by polarisation transfer): "+" primary and tertiary carbon atoms (positive DEPT 135 signal), "−" secondary carbon atom (negative DEPT 135 signal), "quat" quaternary carbon atom. IR spectra were measured on a Bruker Tensor 27 spectrometer equipped with an ATR (attenuated total reflection) unit from Harrick Scientific Products Inc. (Ossining, NY, USA). MS analysis was performed on a Finnigan MAT 95, a Finnigan SSQ 710A, and on a Finnigan ThermoQuest TSQ 7000 spectrometer. Melting points (mp) were measured on a Büchi 530 electrically heated copper block apparatus using an open capillary and are uncorrected. The Department of Microanalysis, University of Regensburg, carried out elemental analysis. Compounds were dried in vacuo at room temperature or with heating up to 50 °C for at least 24 h prior to submission for elemental analysis. Preparative HPLC was performed with a pump model K-1800 (Knauer, Berlin, Germany), the column was Eurosphere-100 (250× 32 mm) (Knauer), which was attached to the UV detector model K-2000 (Knauer). UV detection was performed at 254 and 210, or 220 nm. The temperature was 25 °C and the flow rate 37 mL min^{-1}. The mobile phase was 0.1% TFA in millipore purified H_2O and MeCN. Analytical HPLC was performed on a system from Thermo Separation Products equipped with an SN400 controller, P4000 pump, an AS3000 autosampler, and a Spectra Focus UV/Vis detector. Stationary phase was a Eurosphere-100 C_{18} (250×4.0, 5 μm) column (Knauer) thermostated at 30 °C. As mobile phase, gradients of MeCN/TFA (0.02 or 0.05% aq) were used (flow rate = 0.7 mL min^{-1}). Absorbance was detected at 210 nm. t_0 (Eurosphere-100 C_{18}) = 3.318 min; $k' = (t_R - t_0)/t_0$. For a detailed description of the preparation of compounds **1**–**14**, **19**, **20a**–**22a**, **20**–**25**, **26a**–**33a**, **35a**–**41a**, **26**–**33**, and **35**–**41**, as well as HPLC purity data for the acylguanidines **26**–**33**, **35**–**41**, **35**–**41**, and pharmacological methods, see the Supporting Information.

Preparation of the building blocks 17 and 18

General procedure for the guanidinylation reaction: NEt_3 (3 equiv) was added to a suspension of **13** or **14** (1 equiv), **3** (1 equiv), and $HgCl_2$ (2 equiv) in CH_2Cl_2 (anhyd) and stirred at ambient temperature for 48 h. Subsequently, EtOAc was added and the precipitate filtered over Celite. The crude product was purified by flash chromatography (PE/EtOAc 80:20 *v/v*).

***tert*-Butyl-5-[3-(*N*-benzyloxycarbonyl-*N*′-*tert*-butyloxycarbonyl-guanidino)propyl]-4-methylthiazol-2-ylcarbamate (15):** Prepared from **13** (4.08 g, 15 mmol), **3** (4.88 g, 15 mmol), $HgCl_2$ (8.2 g, 30 mmol), and NEt_3 (4.55 g, 6.24 mL, 45 mmol) in 500 mL CH_2Cl_2 (anhyd) and 500 mL EtOAc according to the general procedure yielding **15** as a colourless foamlike solid (6.3 g, 77%); mp: 114–116 °C; ^{1}H NMR ($CDCl_3$): δ = 11.35 (br s, 1H, N*H*), 8.47 (t, 1H, ^3J = 5.3 Hz, CH_2NH), 7.34 (m, 5H, Ar-*H*), 5.13 (s, 2H, CH_2-Ar), 3.46 (m, 2H, CH_2NH), 2.69 (t, 2H, ^3J = 7.6 Hz, Thiaz-5-CH_2), 2.20 (s, 3H, Thiaz-4-CH_3), 1.88 (m, 2H, Thiaz-5-CH_2CH_2), 1.52 (s, 9H, C(CH_3)$_3$), 1.48 ppm (s, 9H, C(CH_3)$_3$); ESMS (CH_2Cl_2/MeOH + NH_4OAc) *m/z* (%): 548 ([*M*+H]$^+$, 100); $C_{26}H_{37}N_5O_6S$ (547.67).

***tert*-Butyl-5-[3-(*N*-benzyloxycarbonyl-*N*′-*tert*-butyloxycarbonyl-guanidino)propyl]thiazol-2-ylcarbamate (16):** Prepared from **14** (4 g, 15.5 mmol), **3** (5 g, 15.5 mmol), $HgCl_2$ (8.42 g, 31 mmol), and NEt_3 (6.4 mL, 46.5 mmol) in 500 mL CH_2Cl_2 (anhyd) and 500 mL EtOAc according to the general procedure yielding **16** as a colourless foamlike solid (8.48 g, 100%); mp: 140–142 °C; ^{1}H NMR ($CDCl_3$): δ = 11.35 (s, 1H, N*H*), 8.47 (t, 1H, ^3J = 5.4 Hz, CH_2NH), 7.34 (m, 5H, Ar-*H*), 7.05 (s, 1H, Thiaz-4-*H*), 5.13 (s, 2H, CH_2-Ph), 3.47 (m, 2H, CH_2NH), 2.77 (t, 2H, ^3J = 7.5 Hz, Thiaz-5-CH_2), 1.92 (m, 2H, Thiaz-5-CH_2CH_2), 1.55 (s, 9H, C(CH_3)$_3$), 1.48 ppm (s, 9H, C(CH_3)$_3$); ESMS (CH_2Cl_2/MeOH + NH_4OAc) *m/z* (%): 534 ([*M*+H]$^+$, 100); $C_{25}H_{35}N_5O_6S$ (533.64).

General procedure for the hydrogenolytic cleavage of Cbz groups. Pd/C (10%) was added to a solution of **15** or **16** in a mixture of THF/MeOH (1:1) and hydrogenated at 8 bar for 3–4 days. The catalyst was removed by filtration over Celite and washed with MeOH. The solvent was removed in vacuo.

***tert*-Butyl-5-[3-(*N*-*tert*-butoxycarbonylguanidino)propyl]-4-methylthiazol-2-ylcarbamate (17).** Prepared from **15** (5.8 g, 10.6 mmol) and 6 g of Pd/C (10%) in a mixture of 160 mL THF/MeOH (1:1) according to the general procedure yielding **17** as a colourless foamlike solid (4.38 g, 100%); mp: 113 °C; ^{1}H NMR (CD_3OD): δ = 3.23 (t, 2H, ^3J = 6.9 Hz, CH_2NH), 2.75 (t, 2H, ^3J = 7.5 Hz, Thiaz-5-CH_2), 2.17 (s, 3H, Thiaz-4-CH_3), 1.86 (m, 2H, Thiaz-5-CH_2CH_2), 1.52 (s, 9H, C(CH_3)$_3$), 1.47 ppm (s, 9H, C(CH_3)$_3$); ESMS (CH_2Cl_2/MeOH + NH_4OAc) *m/z* (%): 414 ([*M*+H]$^+$, 100); $C_{18}H_{31}N_5O_4S$ (413.53).

***tert*-Butyl-5-[3-(*N*-*tert*-butoxycarbonylguanidino)propyl]thiazol-2-ylcarbamate (18):** The title compound was prepared from **16** (5.8 g, 10.6 mmol) and 6 g of Pd/C (10%) in a mixture of 160 mL THF/MeOH (1:1) according to the general procedure yielding **18** as a colourless foamlike solid (3.39 g, 75%); mp: 122–124 °C; ^{1}H NMR ($CDCl_3$): δ = 7.03 (s, 1H, Thiaz-4-*H*), 3.26 (t, 2H, ^3J = 6.9 Hz, CH_2NH), 2.84 (t, 2H, ^3J = 7.2 Hz, Thiaz-5-CH_2), 1.95 (m, 2H, Thiaz-5-CH_2CH_2), 1.55 (s, 9H, C(CH_3)$_3$), 1.47 ppm (s, 9H, C(CH_3)$_3$); ESMS (CH_2Cl_2/MeOH + NH_4OAc) *m/z* (%): 400 ([*M*+H]$^+$, 100). $C_{17}H_{29}N_5O_4S$ (399.50).

Preparation of Boc-protected aminothiazolylpropylguanidines 34a and 42a

General procedure. DIEA (1 equiv) under argon was added to a solution of carboxylic acid (1 equiv), EDAC (1 equiv), and HOBt-monohydrate (1 equiv) in CH_2Cl_2 (anhyd) and stirred for 15 min. To this mixture a solution of **17** or **18** (1 equiv), respectively, in CH_2Cl_2 (anhyd) was added and stirred over night at room temperature. The solvent was removed under reduced pressure and EtOAc and H_2O were added to the resulting crude mixture. The organic phase was separated and the aqueous phase extracted twice with EtOAc. After drying over $MgSO_4$, the solvent was removed in vacuo. The crude product was purified by flash chromatography (PE/EtOAc 80:20 *v/v*) unless otherwise indicated.

***tert*-Butyl-5-[3-[*N*-*tert*-butoxycarbonyl-*N*′-(3-cyclohexylpentanoyl)guanidino]propyl]-4-methylthiazol-2-ylcarbamate (34a):** Prepared from 3-cyclohexylpentanoic acid (**23**) (180 mg, 1 mmol), EDAC (190 mg, 1 mmol), HOBt-monohydrate (150 mg, 1 mmol), DIEA (0.17 mL, 1 mmol) in 5 mL CH_2Cl_2 (anhyd), and **17** (410 mg, 1 mmol) in 5 mL CH_2Cl_2 (anhyd) according to the general procedure yielding **34a** as a pale yellow foamlike solid (420 mg, 83%); ^{1}H NMR ($CDCl_3$): δ = 12.41 (s, 1H, N*H*), 9.05 (t, 1H, ^3J = 5.2 Hz, CH_2NH), 3.45 (m, 2H, CH_2NH), 2.70 (t, 2H, ^3J = 7.5 Hz, Thiaz-5-CH_2), 2.41 (dd, 1H, ^3J = 5.9 Hz, ^2J = 15.4 Hz, COC*H*H), 2.21 (m, 3H, COCH*H*, Thiaz-4-CH_3), 1.87 (m, 2H, Thiaz-5-CH_2CH_2), 1.68 (m, 6H, *c*Hex-C*H*, *c*Hex-CH_2), 1.52 (s, 9H, C(CH_3)$_3$), 1.50 (s, 9H, C(CH_3)$_3$), 1.34–1.03 (m, 8H, *c*Hex-CH_2, CH_2CH_3, CH_2CH), 0.89 ppm (t, 3H, ^3J = 7.4 Hz, CH_3); ESMS (CH_2Cl_2/MeOH + NH_4OAc) *m/z* (%): 580 ([*M*+H]$^+$, 100); $C_{29}H_{49}N_5O_5S$ (579.79).

***tert*-Butyl-5-[3-[*N*-*tert*-butoxycarbonyl-*N*′-(3-cyclohexylpentanoyl)guanidino]-propyl]thiazol-2-ylcarbamate (42a):** Prepared from 3-cyclohexylpentanoic acid (**23**) (80 mg, 0.5 mmol), EDAC

(95 mg, 0.5 mmol), HOBt-monohydrate (800 mg, 0.5 mmol), DIEA (0.09 mL, 0.5 mmol) in 2.5 mL CH_2Cl_2 (anhyd), and **18** (200 mg, 0.5 mmol) in 2.5 mL CH_2Cl_2 (anhyd) according to the general procedure (PE/EtOAc 70:30 *v/v*) yielding **42a** as a colourless foamlike solid (80 mg, 28%); ^{1}H NMR ($CDCl_3$): $\delta = 12.41$ (s, 1H, N*H*), 9.06 (t, 1H, $^3J = 5.1$ Hz, CH_2NH), 7.05 (s, 1H, Thiaz-4-*H*), 3.48 (m, 2H, CH_2NH), 2.79 (t, 2H, $^3J = 7.4$ Hz, Thiaz-5-CH_2), 2.42 (dd, 1H, $^3J = 5.9$ Hz, $^2J = 15.5$ Hz, COC*H*H), 2.21 (dd, 1H, $^3J = 7.7$ Hz, $^2J = 15.4$ Hz, COCH*H*), 1.92 (m, 2H, Thiaz-5-CH_2CH_2), 1.75–1.60 (m, 6H, *c*Hex-CH_2, CH_2CH, *c*Hex-C*H*), 1.31–1.05 1.56 (s, 9H, C(CH_3)$_3$), 1.50 (s, 9H, C-(CH_3)$_3$), (m, 8H, *c*Hex-CH_2, CH_2CH_3), 0.89 ppm (t, 3H, $^3J = 7.5$ Hz, CH_3); CIMS (NH_3) *m/z* (%): 566 ([*M*+H]$^+$, 100); $C_{28}H_{47}N_5O_5S$ (565.76).

Preparation of the deprotected acylguanidines 34 and 42

General procedure: TFA (20%) was added to a solution of the protected acylguanidine in CH_2Cl_2 (anhyd) and the mixture was stirred at ambient temperature until the Boc groups were removed (3–5 h). Subsequently, the solvent was removed in vacuo and the residue was purified by preparative RP HPLC. All compounds were obtained as trifluoroacetates.

***N*-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-*N′*-(3-cyclohexylpentanoyl)guanidine (34):** Prepared from **34a** (400 mg, 0.65 mmol) in 10 mL CH_2Cl_2 (anhyd) and 2 mL TFA according to the general procedure yielding **34** as a pale yellow oil (320 mg, 76%). ^{1}H NMR (CD_3OD): $\delta = 3.35$ (t, 2H, $^3J = 6.9$ Hz, CH_2NH), 2.71 (t, 2H, $^3J = 7.6$ Hz, Thiaz-5-CH_2), 2.50 (dd, 1H, $^3J = 6.2$ Hz, $^2J = 15.6$ Hz, COC*H*H), 2.32 (dd, 1H, $^3J = 7.5$ Hz, $^2J = 15.6$ Hz, COCH*H*), 2.18 (s, 3H, Thiaz-4-CH_3), 1.91 (m, 2H, Thiaz-5-CH_2CH_2), 1.68 (m, 6H, *c*Hex-C*H*, *c*Hex-CH_2), 1.39–1.07 (m, 8H, *c*Hex-CH_2, CH_2CH_3, CH_2CH), 0.89 ppm (t, 3H, $^3J = 7.4$ Hz, CH_3); ^{13}C NMR (CD_3OD): $\delta = 177.82$ (quat. *C*=O), 170.38 (quat. Thiaz-*C*-2), 155.32 (quat. *C*=NH), 132.59 (quat. Thiaz-*C*-4), 118.41 (quat. Thiaz-*C*-5), 42.99 (−, $COCH_2$), 41.59 (+, *c*Hex-*C*H), 41.45 (−, CH_2NH), 39.71 (+, CH_2CH), 31.18 (−, *c*Hex-CH_2), 30.35 (−, *c*Hex-CH_2), 29.72 (−, Thiaz-5-CH_2CH_2), 27.91 (−, *c*Hex-CH_2), 27.89 (−, *c*Hex-CH_2), 27.79 (−, *c*Hex-CH_2), 24.75 (−, CH_2CH_3), 23.60 (−, Thiaz-5-CH_2), 12.05 (+, CH_3), 11.44 ppm (+, Thiaz-CH_3); IR (neat): $\nu =$ 3091w, 2929w, 2853w, 1662m, 1181s, 1137s cm^{-1}; HREIMS: *m/z* for ($C_{19}H_{33}N_5OS$) calcd: 379.24050, found: 379.24061; prep. HPLC: MeCN/TFA (0.1% aq) (25:75→40:60); anal. HPLC: gradient mode: 0 min: MeCN/TFA (0.05% aq) 10:90, 20 min: 60:40, 20–23 min: 95:5, −33 min: 95:5, $k' = 4.45$ min ($t_R = 18.08$ min), purity = 100%; $C_{19}H_{33}N_5OS{\cdot}2$TFA (607.54).

***N*-[3-(2-Aminothiazol-5-yl)propyl]-*N′*-(3-cyclohexylpentanoyl)-guanidine (42):** Prepared from **42a** (80 mg, 0.14 mmol) in 5 mL CH_2Cl_2 (anhyd) and 1 mL TFA according to the general procedure yielding **42** as a colourless oil (70 mg, 84%). ^{1}H NMR (CD_3OD): $\delta =$ 7.01 (s, 1H, Thiaz-4-*H*), 3.37 (t, 2H, $^3J = 7.0$ Hz, CH_2NH), 2.77 (t, 2H, $^3J = 7.5$ Hz, Thiaz-5-CH_2), 2.50 (dd, 1H, $^3J = 6.1$ Hz, $^2J = 15.7$ Hz, COC*H*H), 2.32 (dd, 1H, $^3J = 7.5$ Hz, $^2J = 15.7$ Hz, COCH*H*), 1.96 (m, 2H, Thiaz-5-CH_2CH_2), 1.69 (m, 6H, *c*Hex-CH_2, CH_2CH, *c*Hex-C*H*), 1.19 (m, 8H, *c*Hex-CH_2, CH_2CH_3), 0.89 ppm (t, 3H, $^3J = 7.4$ Hz, CH_3); ^{13}C NMR (CD_3OD): $\delta = 177.73$ (quat. *C*=O), 170.60 (quat. Thiaz-*C*-2), 155.06 (quat. *C*=NH), 126.35 (quat. Thiaz-*C*-5), 123.36 (+, Thiaz-*C*-4), 42.94 (+, *c*Hex-*C*H), 41.52 (−, Thiaz-5-CH_2), 41.41 (+, CH_2CH), 39.71 (−, $COCH_2$), 31.19 (−, CH_2NH), 30.34 (−, *c*Hex-CH_2), 29.51 (−, *c*Hex-CH_2), 27.88 (−, 2 *c*Hex-CH_2), 27.79 (−, Thiaz-5-CH_2CH_2), 24.89 (−, *c*Hex-CH_2), 24.75 (−, CH_2CH_3), 12.05 ppm (+, CH_3); IR (neat): $\nu =$ 3092w, 2927w, 2854w, 1662m, 1182s, 1133s cm^{-1}; HREIMS: *m/z* for ($C_{18}H_{31}N_5OS$) calcd: 365.22493, found: 365.22445; prep. HPLC: MeCN/TFA (0.1% aq) (30:70→50:50); anal. HPLC: gradient mode: 0 min: MeCN/TFA (0.05% aq) 10:90, 20 min: 60:40, 20–23 min: 95:5, −33 min: 95:5, $k' = 4.32$ min ($t_R = 17.64$ min), purity = 100%; $C_{18}H_{31}N_5OS{\cdot}2$TFA (593.51).

Molecular modelling

A roughly minimised model of the gpH_2R, based on the PDB file 2RH1 of the β_2-adrenoceptor crystal structure,[21] served as source for the docking of representative ligands (for details of the model generation, see Ref. [1]). Compounds **29** and UR-PG80 were manually docked into the model in extended, energetically favourable conformations, considering results of previous in vitro mutagenesis and theoretical studies.[1,16,23,25,28,29] The complexes were first minimised with the Amber-FF99 force field[30] (ligands fixed and provided with Gasteiger–Hückel charges, distant dependent dielectric constant of 4, 20 cycles steepest descent, then Powell algorithm) down to an RMS gradient of less than 0.1 kcal mol^{-1} Å^{-1}. The final minimisations considered the ligands and a region of 6 Å around (combined organic-protein MMFF94 force field[31] and charges, distant dependent dielectric constant of 1, Powell method, RMS gradient criterion 0.05 kcal mol^{-1} Å^{-1}). The RMS deviation of the minimised models (with compounds **29** and UR-PG80, respectively) amounts to 0.22 Å. All calculations were performed with SYBYL 7.3 (Tripos, St. Louis, MO, USA) on an SGI Octane workstation.

Acknowledgements

We thank Kerstin Fisch, Gertraud Wilberg, Karin Schadendorf, Christine Braun, and Kerstin Röhrl for expert technical assistance. This work was supported by Deutsche Forschungsgemeinschaft Graduate Training Program (Graduiertenkolleg) GRK 760, "Medicinal Chemistry: Molecular Recognition—Ligand–Receptor Interactions".

Keywords: acylguanidines · aminothiazoles · GTPase · medicinal chemistry · receptors · structure–activity relationships

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Received: September 11, 2008
Revised: October 16, 2008
Published online on December 15, 2008

