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Piperidine-containing histamine H_3-receptor antagonists of the carbamate series: variation of the spacer length

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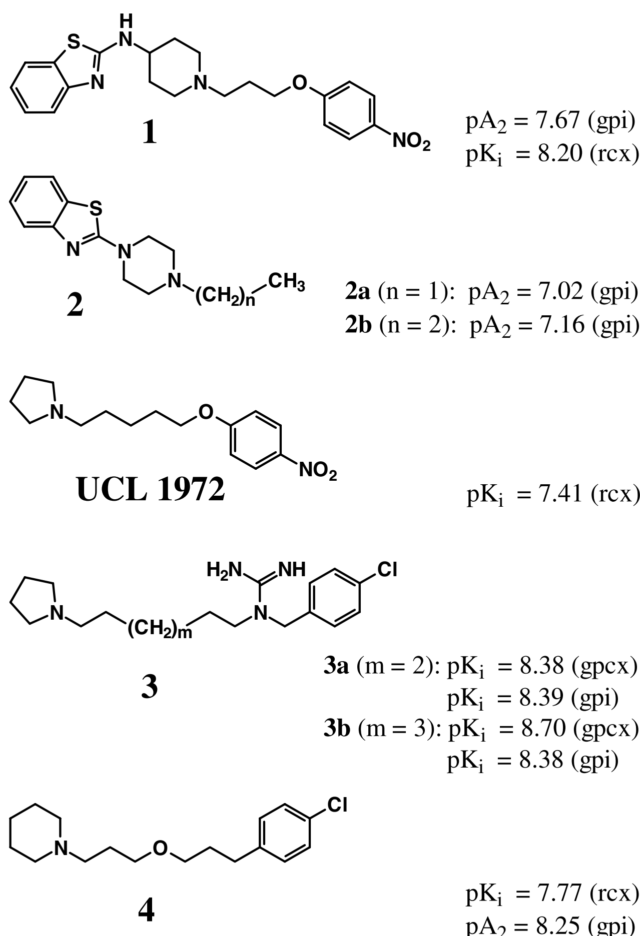
Ten carbamate derivatives have been prepared from appropriate isocyanates and ω-piperidino-1-alkanols. All compounds belong to the new generation of non-imidazole histamine H_3-receptor ligands which may have beneficial pharmacokinetic properties compared with the classical imidazole-containing H_3-receptor antagonists. The carbamates were evaluated *in vitro* for antagonist activity at guinea-pig (gp) H_3, H_2, H_1, and M_3 receptors, respectively. They displayed moderate affinity for H_3 receptors (pA_2 5.8–7.0 in the gp ileum assay) as well as low to moderate selectivities vis-à-vis H_2 (gp atrium), H_1 (gp ileum), and M_3 (gp ileum) receptors. A typical member of this series is 7-piperidino-1-heptyl *N*-(4-phenyl-1-butyl)carbamate (**17**) with pA_2 values of 7.02 (H_3), 5.92 (H_1), and 6.38 (M_3), respectively, and a pD'_2 value of 5.46 (H_2).

1. Introduction

Histamine is an important physiological amine that acts as a chemical messenger to exert numerous functions in central and peripheral tissues. These effects are mediated through at least three pharmacologically distinct subtypes of receptors, i.e., the H_1, H_2, and H_3 receptors, which are all members of the G-protein-coupled receptor family [1]. Some time ago, human histamine H_3-receptor cDNA became available [2]. Increasing pharmacological evidence implying the existence of additional types of histamine receptors [3, 4] were the prerequisite for further studies which have led to the molecular cloning of a novel type of histamine receptor named GPRv53 or H_4 receptor [5]. The potential therapeutic utility of ligands for histamine H_3 and H_4 receptors has renewed interest in the field of histamine research.
Histamine H_3 receptors are either presynaptic autoreceptors, which regulate the synthesis and release of histamine from histaminergic neurones, or they work as heteroreceptors, which control the release of noradrenaline, serotonin, acetylcholine, and dopamine from the respective neuronal tissues [6, 7]. Therapeutic targets of histamine H_3-receptor agonists might be, e.g., neurogenic airway inflammation, migraine, and sleep disorders [8]. H_3-Receptor antagonists increasing the histamine level in brain might be useful in the therapy of neurodegenerative diseases, e.g., dementia, epilepsy, Morbus Alzheimer, and schizophrenia [8, 9].
So far, the majority of potent histamine H_3-receptor ligands contain an imidazole moiety separated from a lipophilic part by a suitable chain, which usually possesses a polar linkage [10]. However, the design of non-imidazole histamine H_3-receptor antagonists seems to be attractive. The imidazole ring (strong H-bond acceptor and donor) may cause reduced brain penetration [11]. In addition, the possible interaction of the imidazole nucleus with cytochrome P_{450} may also confer potentially disadvantageous pharmacokinetic and metabolic *in vivo* properties [12]. The search for non-imidazole histamine H_3-receptor ligands has been taken up by several research groups [13–21]. First attempts to replace the imidazole ring of potent H_3-receptor antagonists by other nitrogen-containing heterocyclic nuclei failed [13–15]. A different approach has been reported using sabeluzole, a low affinity H_3-receptor antagonist, as a chemical lead [22]. Some active benzothiazole derivatives have been discovered, of which **1** and **2** are the most potent [16–18]. A systematic structure-activity study of tertiary amine derivatives furnished compounds active not only *in vitro* but also *in vivo*, with UCL 1972 being the most prominent [19]. Based on the low-affinity histamine H_2-receptor agonist and H_3-receptor ligand dimaprit, a detailed structure-activity survey



Non-imidazole histamine H_3-receptor antagonists [16–21] (gpcx, guinea-pig cortex; gpi, guinea-pig ileum; rcx, rat cortex)

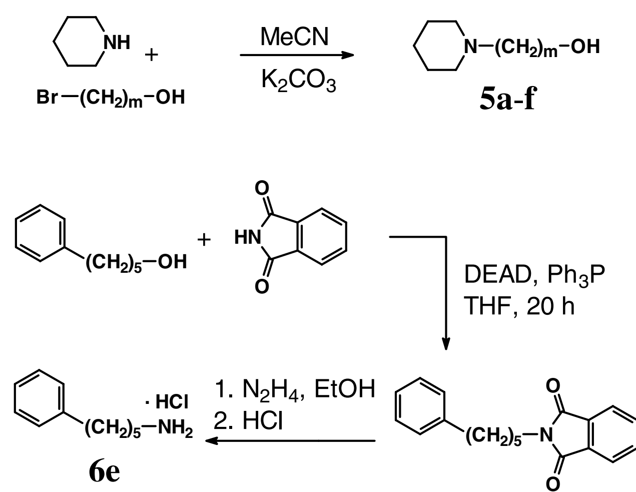
revealed that pyrrolidine-guanidine derivatives **3** exhibit high affinity for the histamine H_3 receptor [20]. Replacement of the imidazole ring in the most potent histamine H_3-receptor antagonists by a piperidine moiety was successful in some classes of compounds and led to the antagonist **4** which is endowed with high *in vitro* as well as *in vivo* potency [21].
Compound **4** was chosen as the lead because it is one of the most active non-imidazole H_3-receptor antagonists discovered up to date and allows a lot of structural modifications. Since the number of piperidine analogues investigated until now is very limited [19, 21], it seemed worthwhile to examine systematically piperidine-containing potential H_3-receptor antagonists related to **4**. According to the general construction pattern of histamine H_3-receptor antagonists [9], a series of easily accessible carbamates was designed containing a benzene ring apart from the piperidine moiety. In this series the length of the spacer (3–8 membered carbon chain) connecting the polar carbamate group to piperidine, and the length of the spacer (1–5 membered carbon chain) between phenyl group and urethane functionality were varied. For the preliminary screening of the potential ligands a simple and rapid functional *in vitro* assay on isolated segments of the guinea-pig ileum [23] was selected. Moreover, the new compounds were screened for activity at histamine H_1, H_2, and muscarine M_3 receptors in functional tests on isolated guinea-pig preparations [24, 25].

2. Investigations, results, and discussion

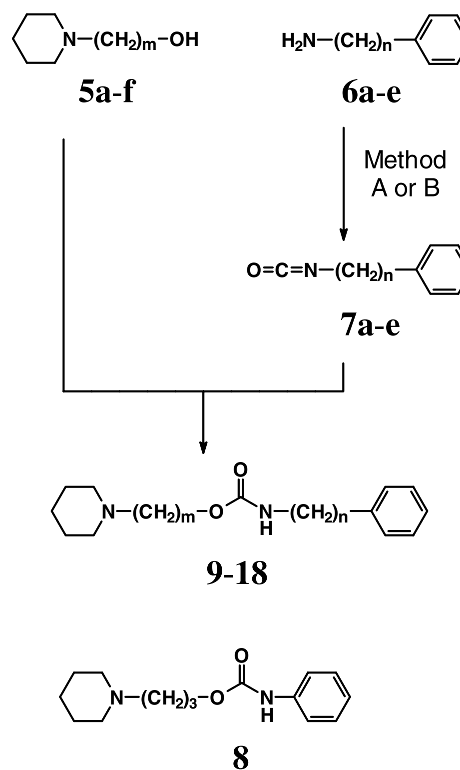
2.1. Chemistry

The target carbamates were prepared from the appropriate ω-piperidino-1-alkanols **5a–f** and isocyanates **7a–e**. The alcohols **5a–f** were obtained by alkylation of piperidine with the corresponding ω-bromo-1-alkanols in acetonitrile in the presence of potassium carbonate (Scheme 1) [26]. Amines **6a–e** were used as starting material for the isocyanates **7a–e**. Apart from **6e** all amines were commercially available. 5-Phenyl-1-pentanamine hydrochloride (**6e**) was obtained from 5-phenyl-1-pentanol by a Mitsunobu-type reaction (Scheme 1) [27, 28]. The required isocyanates **7a–e** were synthesized by refluxing the corresponding amines with an excess of diphosgene (Method A, Scheme 2). The isocyanates were immediately reacted with alcohols **5a–f** yielding the desired carbamates **9–13**, **16**, and **18**. For the preparation of **14**, **15**, and **17**, di-*tert*-butyl dicarbonate in the presence of 4-(dimethylamino)pyridine was used to connect the amines with the appropriate ω-piperidino-1-alkanols [29] (Method B, Scheme 2).

Scheme 1: Synthesis of starting material 5a–f and 6e



Scheme 2: Synthesis of carbamates 9–18 and structure of compound 8 [21]

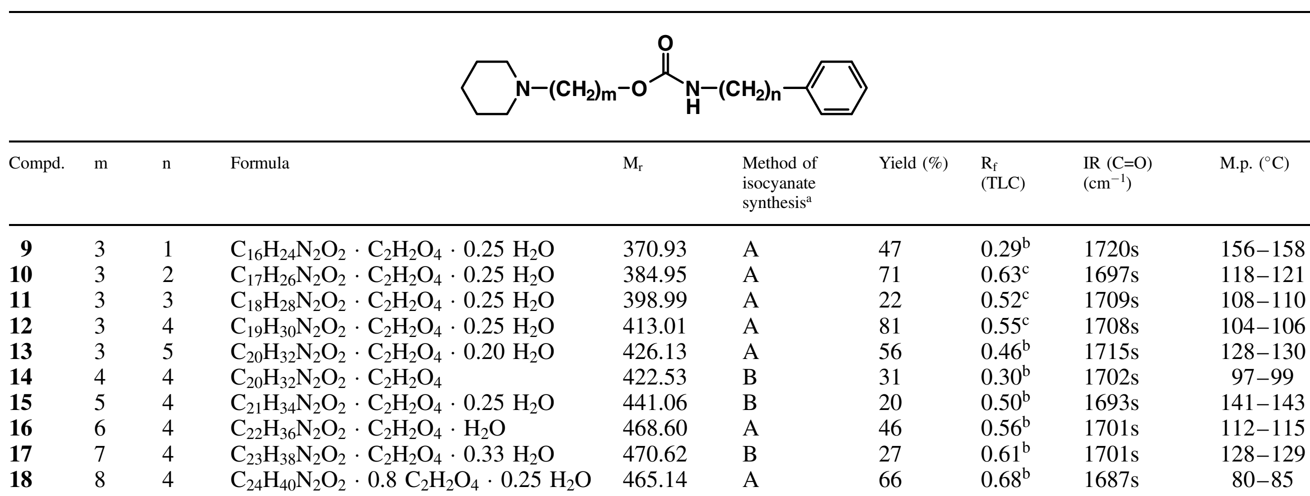


Compounds **5b–f** and **9–18** were purified by column chromatography. The carbamates **9–18** were isolated as hydrogen oxalates, their purity was checked by TLC, and their structures were confirmed by standard spectral techniques (^{1}H NMR, MS, IR) and elemental analysis. Some preparative and physicochemical properties of the carbamates **9–18** are presented in Table 1.

2.2. Pharmacological results and discussion

At first the novel compounds **9–18** were tested *in vitro* for potential antagonism at muscarine M_3 receptors. Then their interaction with peripheral histamine H_3 receptors was studied. Field stimulation of isolated guinea-pig longitudinal ileal muscle with adhering myenteric plexus results in a neuronal release of acetylcholine which activates muscarine M_3 receptors on the smooth muscle, thus leading to contraction. Stimulation of neuronal H_3 heteroreceptors by (*R*)-α-methylhistamine, a potent and selective H_3-receptor agonist, functionally antagonizes the electrically evoked contraction. Blockade of postsynaptic M_3 receptors on the ileal muscle would also result in an inhibition of the contraction without interfering with the H_3 receptor but mimicking the effect of an H_3 agonist. In order to avoid such a conflict, the potential H_3-receptor antagonists had to be tested at concentrations that did not block M_3 receptors. Therefore, all compounds were routinely checked for M_3-receptor affinity, expressed as $pA_2(M_3)$ value (Table 2 and 3). Their antagonism of (*R*)-α-methylhistamine-evoked effects was studied at concentrations that were equal to or preferentially below $0.5 \cdot 10^{-pA_2(M_3)}$ mol/l.

Table 1: Structure, preparative and physicochemical data of compounds 9–18



Compd.	m	n	Formula	M_r	Method of isocyanate synthesis[a]	Yield (%)	R_f (TLC)	IR (C=O) (cm^{-1})	M.p. (°C)
9	3	1	$C_{16}H_{24}N_2O_2 \cdot C_2H_2O_4 \cdot 0.25\ H_2O$	370.93	A	47	0.29[b]	1720s	156–158
10	3	2	$C_{17}H_{26}N_2O_2 \cdot C_2H_2O_4 \cdot 0.25\ H_2O$	384.95	A	71	0.63[c]	1697s	118–121
11	3	3	$C_{18}H_{28}N_2O_2 \cdot C_2H_2O_4 \cdot 0.25\ H_2O$	398.99	A	22	0.52[c]	1709s	108–110
12	3	4	$C_{19}H_{30}N_2O_2 \cdot C_2H_2O_4 \cdot 0.25\ H_2O$	413.01	A	81	0.55[c]	1708s	104–106
13	3	5	$C_{20}H_{32}N_2O_2 \cdot C_2H_2O_4 \cdot 0.20\ H_2O$	426.13	A	56	0.46[b]	1715s	128–130
14	4	4	$C_{20}H_{32}N_2O_2 \cdot C_2H_2O_4$	422.53	B	31	0.30[b]	1702s	97–99
15	5	4	$C_{21}H_{34}N_2O_2 \cdot C_2H_2O_4 \cdot 0.25\ H_2O$	441.06	B	20	0.50[b]	1693s	141–143
16	6	4	$C_{22}H_{36}N_2O_2 \cdot C_2H_2O_4 \cdot H_2O$	468.60	A	46	0.56[b]	1701s	112–115
17	7	4	$C_{23}H_{38}N_2O_2 \cdot C_2H_2O_4 \cdot 0.33\ H_2O$	470.62	B	27	0.61[b]	1701s	128–129
18	8	4	$C_{24}H_{40}N_2O_2 \cdot 0.8\ C_2H_2O_4 \cdot 0.25\ H_2O$	465.14	A	66	0.68[b]	1687s	80–85

[a] For methods A and B, see 3.1.2. [b] $CHCl_3$: 25% aqueous NH_3 (10 : 1). [c] $CHCl_3$: Me_2CO (30 : 70, NH_3-saturated)

The results for the 3-piperidino-1-propanol derivatives **9–13** are presented in Table 2. For comparison the previously described compound **8** [21] was also included (for structure see Scheme 2). The results for 4-phenyl-1-butanamine derivatives **12** and **14–18** are given in Table 3. All compounds investigated (except **18**) possess measurable, albeit moderate affinity for guinea-pig histamine H_3 receptors (pA_2 5.8–7.0) but they are substantially less potent than the lead **4** ($pA_2 = 8.25$ [21]). Receptor affinity is virtually independent of the spacer length, at least within the range studied for n and m. Only two compounds (**9** and **10**) are significantly less potent than some of the others. The highest mean pA_2 value is found for the 7-piperidino-1-heptanol derivative **17** while for the higher homologue **18** a reliable measurement could not be performed due to the comparably high antimuscarinic activity of the compound ($pA_2 = 6.6$). As a conclusion, a systematic influence of both spacer lengths on H_3-receptor affinity could not be verified. A part of this result is in line with data obtained for a series of analogous carbamate derivatives of 3-(4-imidazolyl)propanol where the distance between carbamate nitrogen and phenyl group (n = 0–3 methylene units) had virtually no impact on H_3-receptor affinity [30].

With regard to the potent lead compound, the comparison of selected members of the present series with the ether **4** is of certain interest. Apparently, neither **11** (relative affinity $^1/_{105}$) which possesses the same combination of spacers as **4** (two $(CH_2)_3$ units), nor **9** (relative affinity $^1/_{263}$) which displays a chain of seven non-hydrogen atoms connecting piperidine nitrogen with the aromatic residue, fit the ligand binding site of the H_3 receptor in a favourable manner. This observation may be caused by the relative rigidity of the planar –O–CO–NH– unit compared to a flexible ether linkage ($-CH_2-O-CH_2-$). On the other hand, increasing one or both spacer lengths does not significantly compensate this bad fit which indicates that additional but unknown reasons may be responsible for this drop of affinity. The loss of the chlorine substituent present in **4** would also explain only a minor reduction of affinity since the hydrogen analogue of **4** still displays approximately 60% of the affinity measured for **4** ($pA_2 = 8.05$ [21]). Although planar and polar functional groups, e.g., carbamate or guanidine, have been identified as compatible with high H_3-receptor affinity in imidazole-containing H_3-receptor antagonists [9, 28, 30], this feature is obviously not valid in the series of non-imidazole piperidine-containing antagonists presented in this study.

Table 2: Antagonist activity of 8–13 at histamine H_3 and muscarine M_3 receptors

Compound	H_3^a pA_2 (95% conf. lim.)	M_3^b $pA_2 \pm$ SEM
8[c]	6.18 (5.89–6.47)	4.70 ± 0.08
9	5.88 (5.69–6.07)	4.63 ± 0.07
10	5.76 (5.34–6.06)	4.64 ± 0.06
11	6.28 (6.22–6.34)	4.85 ± 0.03
12	6.39 (6.20–6.55)	5.16 ± 0.04
13	6.47 (6.13–6.75)	5.49 ± 0.02

[a] N = 6. [b] N = 4–12. [c] [21]

Table 3: Antagonist activity of 12 and 14–18 at histamine H_3 and muscarine M_3 receptors

Compd.	H_3^a pA_2 (95% conf. lim.)	M_3^b $pA_2 \pm$ SEM
12	6.39 (6.20–6.55)	5.16 ± 0.04
14	6.28 (6.10–6.43)	5.66 ± 0.06
15	6.48 (6.23–6.67)	5.96 ± 0.05
16	6.41 (5.59–6.78)	6.29 ± 0.04
17	7.02 (6.67–7.30)	6.38 ± 0.03
18	<6.9[c]	6.61 ± 0.03

[a] N = 6. [b] N = 4–12. [c] The corrected rightward shift induced by 0.1 μM **18** was 0.08 (95% conf. lim. −0.09 up to 0.24). The upper limit would correspond to a pA_2 value of 6.87

Table 4: Antagonist activity of 9–18 at histamine H_2 and H_1 receptors

Compd.	H_2^a $pD'_2 \pm$ SEM[c]	H_1^b $pA_2 \pm$ SEM
9	4.17 ± 0.17	4.41 ± 0.05
10	5.38 ± 0.15[d]	4.78 ± 0.05
11	4.56 ± 0.33	5.15 ± 0.03
12	6.13 ± 0.22	5.53 ± 0.04
13	5.69 ± 0.13	5.80 ± 0.03
14	5.64 ± 0.12	5.78 ± 0.06
15	5.74 ± 0.13	5.80 ± 0.06
16	5.88 ± 0.03	5.78 ± 0.04
17	5.46 ± 0.13	5.92 ± 0.19
18	5.45 ± 0.13	6.12 ± 0.05

[a] N = 2. [b] N = 4–12. [c] Corresponds to the range of observed values. [d] pA_2 value

While the affinity for M_3 receptors is positively correlated with spacer length and thus with lipophilicity in both subsets (Tables 2 and 3), clear-cut structure-activity relationships cannot be deduced from the data set for H_1 and H_2 receptors, respectively (Table 4). At best, a slight increase of H_1-receptor affinity is observed with increasing distance between carbamate nitrogen and phenyl group (**9–13**). With regard to the family of histamine receptors, the carbamates **9–18** display moderate affinity for guinea-pig H_3 receptors and, all in all, only moderate selectivity versus H_1 and H_2 receptors, too.

3. Experimental

3.1. Chemistry

Melting points were determined on a Mel-Temp II apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker DPX 400 Avance (400 MHz) spectrometer. Chemical shifts are expressed in ppm downfield from internal Me_4Si as reference. ^{1}H NMR Data are reported in the following order: multiplicity (br, broad; def, deformed; s, singlet; t, triplet; qu, quintet; m, multiplet); approximate coupling constants J in Hertz; number of protons; *, exchangeable by D_2O; Pip, piperidine; Ph, phenyl. MS were obtained on an EI-MS Finnigan MAT CH7A. IR spectra were recorded with a Perkin-Elmer 1420 Ratio-Recording or a Perkin-Elmer 297 spectral photometer from KBr discs (s, strong). Elemental analyses (C, H, N) were measured on a Perkin-Elmer 240 B or a Perkin-Elmer 240 C instrument and were within ±0.4% of theoretical values for all final compounds. CC was performed using silica gel 60 (0.063–0.20 mm; Merck). TLC was carried out using silica gel F_{254} plates (Merck). The spots were visualized with Dragendorff's reagent or by UV absorption at 254 nm.

3.1.1. Starting materials

3.1.1.1. Synthesis of ω-piperidino-1-alkanols **5a–f**

All piperidino alcohols have been described in the literature (**5b**, **c** [31]; **5d** [32]; **5e** [33]; **5f** [34]). In the present study, **5a–f** were prepared according to ref. [19], but except **5a**, they were purified by CC as outlined below.

3.1.1.1.1. 3-Piperidino-1-propanol (**5a**)

Compound **5a** was obtained as described in ref. [19]. B.p. 110–130 °$C_{100–120\ mm\ Hg}$ (b.p. 125 °$C_{30\ mm\ Hg}$ [26]), yield 76%; ^{1}H NMR ([D$_6$]DMSO): δ = 4.49 (s, 1 H, OH*), 3.42 (t, J = 6.3, 2 H, C*H*$_2$OH), 2.13–2.40 (m, 6 H, Pip–2,6–H and Pip–CH$_2$), 1.55 (qu, J = 6.5, 2 H, Pip–CH$_2$–C*H*$_2$), 1.41–1.51 (m, 4 H, Pip–3,5–H), 1.37 (def qu, 2 H, Pip–4–H).

3.1.1.1.2. 4-Piperidino-1-butanol (**5b**)

Compound **5b** was prepared according to the synthesis of **5a** but was purified by CC. [eluent $CHCl_3$: MeOH : 25% aqueous NH_3 (90 : 10 : 6 drops per 100 ml)], yield 24%; ^{1}H NMR ([D$_6$]DMSO): δ = 4.62 (br s, 1 H, OH*), 3.37 (t, J = 6.0, 2 H, C*H*$_2$OH), 2.08–2.39 (m, 6 H, Pip–2,6–H and Pip–CH$_2$), 1.40–1.50 (m, 8 H, Pip–CH$_2$–(C*H*$_2$)$_2$ and Pip–3,5–H), 1.37 (m, 2 H, Pip–4–H).

3.1.1.1.3. 5-Piperidino-1-pentanol (**5c**)

Compound **5c** was prepared according to the synthesis of **5a** but was purified by CC [eluent $CHCl_3$: MeOH : 25% aqueous NH_3 (95 : 5 : 1.5)], yield 79%; ^{1}H NMR ([D$_6$]DMSO): δ = 4.32 (s, 1 H, OH*), 3.35 (t, J = 6.3, 2 H, C*H*$_2$OH), 2.25 (br, 2 H, Pip–CH$_2$), 2.14–2.19 (m, 4 H, Pip–2,6–H), 1.32–1.49 (m, 8 H, Pip–CH$_2$–C*H*$_2$ and Pip–3,5–H and Pip–(CH$_2$)$_3$–C*H*$_2$), 1.20–1.29 (m, 4 H, Pip–4–H and Pip–(CH$_2$)$_2$–C*H*$_2$).

3.1.1.1.4. 6-Piperidino-1-hexanol (**5d**)

Compound **5d** was prepared according to the synthesis of **5a** but was purified by CC [eluent Me_2CO : 25% aqueous NH_3 (100 : 6 drops per 100 ml)], yield 70%. For analysis, **5d** hydrochloride was precipitated from ether saturated with dry HCl: white needles (MeCN), m.p. 171–174 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 10.28 (br s, 1 H, Pip-NH^{+}*), 4.39 (t, J = 4.9, 1 H, OH*), 3.33–3.39 (m, 4 H, Pip–2,6–H), 2.88–2.95 (m, 2 H, C*H*$_2$OH), 2.72–2.84 (m, 2 H, Pip–CH$_2$), 1.66–1.84 (m, 8 H, Pip–CH$_2$–C*H*$_2$ and Pip–(CH$_2$)$_4$–C*H*$_2$ and Pip–3,5–H), 1.28–1.42 (m, 6 H, Pip–4–H and Pip–(CH$_2$)$_2$–(C*H*$_2$)$_2$).

3.1.1.1.5. 7-Piperidino-1-heptanol (**5e**)

Compound **5e** was prepared according to the synthesis of **5a** but was purified by CC [eluent CH_2Cl_2 : MeOH : 25% aqueous NH_3 (97 : 3 : 2)], yield 55%. For analysis, **5e** hydrochloride was precipitated from ether saturated with dry HCl: white needles (MeCN), m.p. 145–148 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 9.80 (br s, 1 H, Pip-NH^{+}*), 4.36 (s, 1 H, OH*), 3.37–3.40 (m, 4 H, Pip–2,6–H), 2.93–2.98 (m, 2 H, C*H*$_2$OH), 2.77–2.85 (m, 2 H, Pip–CH$_2$), 1.69–1.76 (m, 4 H, Pip–CH$_2$–C*H*$_2$ and Pip–(CH$_2$)$_5$–C*H*$_2$), 1.59–1.69 (m, 4 H, Pip–3,5–H), 1.32–1.48 (m, 2 H, Pip–4–H), 1.21–1.32 (m, 6 H, Pip–(CH$_2$)$_2$–(C*H*$_2$)$_3$).

3.1.1.1.6. 8-Piperidino-1-octanol (**5f**)

Compound **5f** was prepared according to the synthesis of **5a** but was purified by CC [eluent CH_2Cl_2 : Me_2CO : 25% aqueous NH_3 (30 : 70 : 6 drops per 100 ml)], yield 60%. For analysis, **5f** hydrochloride was precipitated from ether saturated with dry HCl: white needles (MeCN), m.p. 173–176 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 10.29 (br s, 1 H, Pip–NH^{+}*), 4.34 (s, 1 H, OH*), 3.33–3.38 (m, 4 H, Pip–2,6–H), 2.91–2.98 (m, 2 H, C*H*$_2$OH), 2.75–2.84 (m, 2 H, Pip–CH$_2$), 1.67–1.80 (m, 6 H, Pip–3,5–H and Pip–(CH$_2$)$_6$–C*H*$_2$), 1.34–1.41 (m, 4 H, Pip–4–H and Pip–CH$_2$–C*H*$_2$), 1.14–1.32 (m, 8 H, Pip–(CH$_2$)$_2$–(C*H*$_2$)$_4$).

3.1.1.2. Synthesis of 5-phenyl-1-pentanamine (**6e**)

Compound **6e** was prepared from 5-phenyl-1-pentanol in a Mitsunobu protocol-adapted Gabriel synthesis [27, 28]. The corresponding *N*-alkylphtalimide was transferred into the desired amine by hydrazinolysis, and **6e** was isolated as hydrochloride: m.p. 136–140 °C (dec.); ^{1}H NMR (CDCl$_3$): δ = 8.28 (br, NH$_3^{+}$*), 7.24–7.30 (m, 2 H, Ph–3,5–H), 7.13–7.21 (m, 3 H, Ph–2,4,6–H), 2.97 (m, 2 H, NH$_3^+$–C*H*$_2$), 2.60 (t, J = 7.8, 2 H, Ph–C*H*$_2$), 1.80 (qu, J = 7.8, 2 H, NH$_3^+$–CH$_2$–C*H*$_2$), 1.64 (qu, J = 7.8, 2 H, Ph–CH$_2$–C*H*$_2$), 1.43 (qu, J = 7.2, 2 H, Ph–(CH$_2$)$_2$–C*H*$_2$).

3.1.2. Synthesis of carbamates **9–18**

General procedure – method A: A solution of trichloromethyl chloroformate (0.37 ml, 3 mmol) and a catalytic amount of charcoal in 20 ml of dry ethyl acetate were mixed at room temperature for 15 min. After heating up to 50 °C, the appropriate amine **6** (2.5 mmol) in 15 ml of dry ethyl acetate was added rapidly. The reaction mixture was heated to reflux for 5 h. Then the black solution was cooled, filtered, and the solvent was evaporated under reduced pressure. The freshly prepared isocyanate was redissolved in 30 ml of dry MeCN, and the respective piperidino alcohol **5** (2.5 mmol) in 15 ml of dry MeCN was added. The solution was refluxed for 5–18 h (controlled by TLC) and concentrated in vacuo. The residue was purified by CC. The pure fractions were concentrated in vacuo, dried and the carbamates were crystallized as hydrogen oxalates from EtOH/Et$_2$O.
General procedure – method B: To a solution of di-*tert*-butyl dicarbonate (0.92 g, 4.2 mmol) and 4-dimethylaminopyridine (0.37 g, 3 mmol) in 20 ml of dry MeCN, the appropriate amine **6** (3 mmol) was added in one portion. After stirring for 20–30 min at room temperature, the respective alcohol **5** (4.2 mmol) was added. The reaction mixture was heated to reflux for 8–9 h. After removing of the solvent, the residue was purified by CC. The pure fractions were concentrated in vacuo, dried and the carbamates were crystallized as hydrogen oxalates from EtOH/Et$_2$O.

3.1.2.1. 3-Piperidino-1-propyl *N*-benzylcarbamate hydrogen oxalate (**9**)

From 0.27 g (2.5 mmol) of benzylamine (**6a**) and 0.36 g (2.5 mmol) of 3-piperidino-1-propanol (**5a**). Purification by CC [eluent CH_2Cl_2 : MeOH : MeOH saturated with gaseous NH_3 (90 : 10 : 6 drops per 100 ml)]; ^{1}H NMR ([D$_6$]DMSO): δ = 7.70 (t, J = 5.9, 1 H, CONH*), 7.30–7.34 (m, 2 H, Ph–3,5–H), 7.22–7.26 (m, 3 H, Ph–2,4,6–H), 4.18 (d, J = 6.1, 2 H, Ph–CH$_2$), 4.02 (t, J = 6.2, 2 H, CH$_2$O), 2.81–2.97 (m, 6 H, Pip–2,6–H and Pip–CH$_2$), 1.94 (def. qu, 2 H, Pip–CH$_2$–C*H*$_2$), 1.61–1.76 (m, 4 H, Pip–3,5–H), 1.44 (br, 2 H, Pip–4–H); MS (70 eV): m/z (%) = 276 ([M$^{\cdot}$]$^+$, 3), 142 (2), 132 (3), 99 (7), 98 (100), 91 (7), 84 (7), 55 (5), 41 (7).

3.1.2.2. 3-Piperidino-1-propyl *N*-(2-phenyl-1-ethyl)carbamate hydrogen oxalate (**10**)

From 0.30 g (2.5 mmol) of 2-phenethylamine (**6b**) and 0.36 g (2.5 mmol) of 3-piperidino-1-propanol (**5a**). Purification by CC [eluent CH_2Cl_2 : Me_2CO : MeOH saturated with gaseous NH_3 (60 : 40 : 6 drops per 100 ml)]; ^{1}H NMR ([D$_6$]DMSO): δ = 7.27–7.31 (m, 2 H, Ph–3,5–H), 7.19–7.24 (m, 4 H, Ph–2,4,6–H and CONH*), 3.98 (t, J = 6.1, 2 H, CH$_2$O), 3.18–3.23 (m, 2 H, CONH–C*H*$_2$), 3.05 (m, 4 H, Pip–2,6–H), 2.94–2.98 (m, 2 H, Pip–CH$_2$), 1.92 (qu, J = 7.4, 2 H, Pip–CH$_2$–C*H*$_2$), 1.69–1.72 (m, 4 H, Pip–3,5–H), 1.51 (br, 2 H, Pip–4–H); MS (70 eV): m/z (%) = 290 ([M$^{\cdot}$]$^+$, 3), 142 (3), 132 (3), 99 (7), 98 (100), 91 (5), 84 (9), 55 (4), 41 (7).

3.1.2.3. 3-Piperidino-1-propyl *N*-(3-phenyl-1-propyl)carbamate hydrogen oxalate (**11**)

From 0.34 g (2.5 mmol) of 3-phenyl-1-propanamine (**6c**) and 0.36 g (2.5 mmol) of 3-piperidino-1-propanol (**5a**). Purification by CC [eluent CH_2Cl_2 : Me_2CO : MeOH saturated with gaseous NH_3 (30 : 70 : 6 drops per 100 ml)]; ^{1}H NMR ([D$_6$]DMSO): δ = 7.26–7.29 (m, 2 H, Ph–3,5–H),

7.15–7.20 (m, 4 H, Ph–2,4,6–H and CONH*), 3.99 (t, J = 6.3, 2 H, CH_2O), 3.04 (br, 2 H, CONH–CH_2), 2.95–3.01 (m, 6 H, Pip–2,6–H and Pip–CH_2), 2.57 (t, J = 7.7, 2 H, Ph–CH_2), 1.93 (qu, J = 7.4, 2 H, Pip–CH_2–CH_2), 1.66–1.73 (m, 6 H, Pip–3,5–H and Ph–CH_2–CH_2), 1.51 (br, 2 H, Pip-4-H); MS (70 eV): m/z (%) = 304 ([M˙]+, 3), 142 (3), 132 (3), 99 (7), 98 (100), 91 (6), 84 (9), 55 (5), 41 (8).

3.1.2.4. 3-Piperidino-1-propyl *N*-(4-phenyl-1-butyl)carbamate hydrogen oxalate (**12**)

From 0.37 g (2.5 mmol) of 4-phenyl-1-butanamine (**6d**) and 0.36 g (2.5 mmol) of 3-piperidino-1-propanol (**5a**). Purification by CC [eluent CH_2Cl_2 : Me_2CO : MeOH saturated with gaseous NH_3 (30 : 70 : 6 drops per 100 ml)]; ^{1}H NMR ([D_6]DMSO): δ = 7.23–7.28 (m, 2 H, Ph–3,5–H), 7.12–7.18 (m, 4 H, Ph–2,4,6–H and CONH*), 3.95 (t, J = 6.0, 2 H, CH_2O), 2.93–3.01 (m, 8 H, CONH–CH_2, Pip–2,6–H and Pip–CH_2), 2.55 (t, J = 7.1, 2 H, Ph–CH_2), 1.91 (def qu, 2 H, Pip–CH_2–CH_2), 1.58–1.68 (m, 4 H, Pip–3,5–H and CONH–CH_2–CH_2), 1.48–1.56 (m, 4 H, Pip–3,5–H and Ph–CH_2–CH_2), 1.39 (qu, J = 7.4, 2 H, Pip–4–H); MS (70 eV): m/z (%) = 318 ([M˙]+, 4), 142 (3), 99 (7), 98 (100), 91 (7), 84 (9), 55 (3), 41 (4).

3.1.2.5. 3-Piperidino-1-propyl *N*-(5-phenyl-1-pentyl)carbamate hydrogen oxalate (**13**)

From 0.41 g (2.5 mmol) of 5-phenyl-1-pentanamine (**6e**) and 0.36 g (2.5 mmol) of 3-piperidino-1-propanol (**5a**). Purification by CC [eluent CH_2Cl_2 : MeOH : MeOH saturated with gaseous NH_3 (98.5 : 1.5 : 3 drops per 100 ml)]; ^{1}H NMR ([D_6]DMSO): δ = 7.25–7.29 (m, 2 H, Ph–3,5–H), 7.15–7.20 (m, 3 H, Ph–2,4,6–H), 7.11 (t, J = 5.2, 1 H, CONH*), 3.97 (t, J = 6.2, 2 H, CH_2O), 3.06 (br, 2 H, CONH–CH_2), 2.93–3.00 (m, 6 H, Pip–2,6–H and Pip–CH_2), 2.55 (t, J = 7.7, 2 H, Ph–CH_2), 1.92 (def qu, 2 H, Pip–CH_2–CH_2), 1.65–1.75 (m, 4 H, Pip–3,5–H), 1.52–1.59 (m, 4 H, CONH–CH_2–CH_2 and Pip–4–H), 1.42 (qu, J = 7.4, 2 H, Ph–CH_2–CH_2), 1.26 (qu, J = 7.9, 2 H, Ph–$(CH_2)_2$–CH_2); MS (70 eV): m/z (%) = 332 ([M˙]+, 3), 142 (3), 99 (8), 98 (100), 91 (8), 84 (8), 55 (5), 41 (7).

3.1.2.6. 4-Piperidino-1-butyl *N*-(4-phenyl-1-butyl)carbamate hydrogen oxalate (**14**)

From 0.45 g (3 mmol) of 4-phenyl-1-butanamine (**6d**) and 0.66 g (4.2 mmol) of 4-piperidino-1-butanol (**5b**). Purification by CC [eluent CH_2Cl_2 : *i*-PrOH : MeOH saturated with gaseous NH_3 (85 : 15 : 1.5)]; ^{1}H NMR ([D_6]DMSO): δ = 7.25–7.29 (m, 2 H, Ph–3,5–H), 7.16–7.19 (m, 3 H, Ph–2,4,6–H), 7.08 (t, J = 5.4, 1 H, CONH*), 3.93 (t, J = 6.3, 2 H, CH_2O), 3.05 (br, 2 H, CONH–CH_2), 2.94–3.01 (m, 6 H, Pip–2,6–H and Pip–CH_2), 2.56 (t, J = 7.6, 2 H, Ph–CH_2), 1.62–1.72 (m, 6 H, Pip–CH_2–CH_2 and CONH–CH_2–CH_2 and O–CH_2–CH_2), 1.51–1.58 (m, 6 H, Pip–3,5–H and Ph–CH_2–CH_2), 1.40 (qu, J = 7.3, 2 H, Pip–4–H); MS (70 eV): m/z (%) = 332 ([M˙]+, 3), 156 (2), 140 (6), 132 (3), 99 (7), 98 (100), 91 (7), 84 (4), 55 (6), 45 (6), 41 (8).

3.1.2.7. 5-Piperidino-1-pentyl *N*-(4-phenyl-1-butyl)carbamate hydrogen oxalate (**15**)

From 0.45 g (3 mmol) of 4-phenyl-1-butanamine (**6d**) and 0.59 g (4.2 mmol) of 5-piperidino-1-pentanol (**5c**). Purification by CC [eluent $CHCl_3$: EtOH : MeOH saturated with gaseous NH_3 (99 : 1 : 1.5)]; ^{1}H NMR ([D_6]DMSO): δ = 7.25–7.29 (m, 2 H, Ph–3,5–H), 7.15–7.18 (m, 3 H, Ph–2,4,6–H), 7.04 (def t, 1 H, CONH*), 3.92 (t, J = 6.4, 2 H, CH_2O), 3.53 (br, 2 H, CONH–CH_2), 2.92–3.01 (m, 6 H, Pip–2,6–H and Pip–CH_2), 2.56 (t, J = 7.7, 2 H, Ph–CH_2), 1.62–1.75 (m, 4 H, Pip–CH_2–CH_2 and O–CH_2–CH_2), 1.59–1.66 (m, 2 H, CONH–CH_2–CH_2), 1.46–1.59 (m, 6 H, Pip–3,5–H and Ph–CH_2–CH_2), 1.40 (qu, J = 7.5, 2 H, Pip–4–H), 1.30 (qu, J = 7.7, 2 H, Pip–$(CH_2)_2$–CH_2); MS (70 eV): m/z (%) = 346 ([M˙]+, 4), 154 (7), 99 (7), 98 (100), 91 (8), 84 (3), 55 (4), 45 (4), 41 (6).

3.1.2.8. 6-Piperidino-1-hexyl *N*-(4-phenyl-1-butyl)carbamate hydrogen oxalate (**16**)

From 0.37 g (2.5 mmol) of 4-phenyl-1-butanamine (**6d**) and 0.46 g (2.5 mmol) of 6-piperidino-1-hexanol (**5d**). Purification by CC [eluent CH_2Cl_2 : Me_2CO : MeOH saturated with gaseous NH_3 (30 : 70 : 6 drops per 100 ml)]; ^{1}H NMR ([D_6]DMSO): δ = 7.25–7.29 (m, 2 H, Ph–3,5–H), 7.15–7.19 (m, 3 H, Ph–2,4,6–H), 7.06 (t, J = 5.4, 1 H, CONH*), 3.91 (t, J = 6.5, 2 H, CH_2O), 3.08 (br, 2 H, CONH–CH_2), 2.72–3.00 (m, 6 H, Pip–2,6–H and Pip–CH_2), 2.56 (t, J = 7.6, 2 H, Ph–CH_2), 1.68–1.84 (m, 4 H, Pip–CH_2–CH_2 and O–CH_2–CH_2), 1.45–1.68 (m, 8 H, CONH–CH_2–CH_2 and Pip-3,5-H and Ph–CH_2–CH_2), 1.39 (qu, J = 6.9, 2 H, Pip–4–H), 1.18–1.32 (m, 4 H, Pip–$(CH_2)_2$–$(CH_2)_2$); MS (70 eV): m/z (%) = 360 ([M˙]+, 3), 168 (4), 99 (7), 98 (100), 91 (5), 85 (6), 55 (5), 45 (3), 41 (5).

3.1.2.9. 7-Piperidino-1-heptyl *N*-(4-phenyl-1-butyl)carbamate hydrogen oxalate (**17**)

From 0.22 g (1.5 mmol) of 4-phenyl-1-butanamine (**6d**) and 0.45 g (2.1 mmol) of 7-piperidino-1-heptanol (**5e**). Purification by CC [eluent $CHCl_3$: EtOH : MeOH saturated with gaseous NH_3 (99 : 1 : 1.5)]; ^{1}H NMR ([D_6]DMSO): δ = 7.25–7.29 (m, 2 H, Ph–3,5–H), 7.14–7.19 (m, 3 H, Ph–2,4,6–H), 7.03 (def t, 1 H, CONH*), 3.91 (t, J = 6.6, 2 H, CH_2O), 3.07 (br, 2 H, CONH–CH_2), 2.85-3.02 (m, 6 H, Pip–2,6–H and Pip–CH_2), 2.56 (t, J = 7.7, 2 H, Ph–CH_2), 1.64–1.75 (m, 4 H, Pip–CH_2–CH_2 and O–CH_2–CH_2), 1.56–1.64 (m, 2 H, CONH–CH_2–CH_2), 1.46–1.56 (m, 6 H, Pip–3,5–H and Ph–CH_2–CH_2), 1.40 (qu, J = 7.2, 2 H, Pip–4–H), 1.14–1.34 (m, 6 H, Pip–$(CH_2)_2$–$(CH_2)_3$); MS (70 eV): m/z (%) = 374 ([M˙]+, 2), 142 (2), 99 (7), 98 (100), 91 (3), 84 (6), 55 (4), 45 (4), 41 (5).

3.1.2.10. 8-Piperidino-1-octyl *N*-(4-phenyl-1-butyl)carbamate hydrogen oxalate (**18**)

From 0.37 g (2.5 mmol) of 4-phenyl-1-butanamine (**6d**) and 0.63 g (2.5 mmol) of 8-piperidino-1-octanol (**5f**). Purification by CC [eluent $CHCl_3$: Me_2CO : MeOH saturated with gaseous NH_3 (70 : 30 : 6 drops per 100 ml)]; ^{1}H NMR ([D_6]DMSO): δ = 7.25–7.29 (m, 2 H, Ph-3,5-H), 7.14–7.19 (m, 3 H, Ph–2,4,6–H), 7.03 (def t, 1 H, CONH*), 3.91 (t, J = 6.6, 2 H, CH_2O), 2.88–3.02 (m, 6 H, Pip–2,6–H and Pip–CH_2), 2.81 (br, 2 H, CONH–CH_2), 2.56 (t, J = 7.6, 2 H, Ph–CH_2), 1.70–1.82 (m, 4 H, Pip–CH_2–CH_2 and O–CH_2–CH_2), 1.59–1.70 (m, 2 H, CONH–CH_2–CH_2), 1.46–1.56 (m, 6 H, Pip–3,5–H and Ph–CH_2–CH_2), 1.40 (qu, J = 7.3, 2 H, Pip–4–H), 1.19–1.33 (m, 6 H, Pip–$(CH_2)_2$–$(CH_2)_4$); MS (70 eV): m/z (%) = 388 ([M˙]+, 3), 196 (2), 99 (7), 98 (100), 91 (6), 84 (3), 55 (6), 41 (5).

3.2. Pharmacology

3.2.1. Guinea-pig ileal longitudinal muscle (preparation with myenteric plexus): H_3 receptors

Strips of guinea-pig ileal longitudinal muscle with adhering myenteric plexus, approximately 2 cm in length and proximal to the ileocaecal junction, were prepared as previously described [35]. The strips were mounted isometrically under a tension of approximately 7.5 ± 2.0 mN in 20-ml organ baths filled with a modified Krebs-Henseleit solution of composition (mM): NaCl 117.9, KCl 5.6, $CaCl_2$ 2.5, $MgSO_4$ 1.2, NaH_2PO_4 1.3, $NaHCO_3$ 25.0, D-glucose 5.5, and choline chloride 0.001, aerated with 95% O_2/5% CO_2 (V/V) and kept at 37 °C. Mepyramine (1 μM) was present throughout the experiment to block ileal H_1 receptors.
After an equilibration period of 1 h with washings every 10 min, the preparations were stimulated for 30 min with rectangular pulses of 15 V and 0.5 ms at a frequency of 0.1 Hz. Viability of the muscle strips was monitored by addition of the H_3-receptor agonist (*R*)-α-methylhistamine (100 nM), which caused a relaxation of the twitch response of more than 50% up to 100%. After wash-out, reequilibration and 30 min field-stimulation, a cumulative concentration-response curve for (*R*)-α-methylhistamine (1–1000 nM) was constructed. Subsequently, the preparations were washed intensively and reequilibrated for 20–30 min in the absence of the antagonist under study. During the incubation period the strips were stimulated continuously for 30 min. Finally a second concentration-effect curve for (*R*)-α-methylhistamine was obtained.
The rightward displacement of the curve for the H_3 agonist evoked by the antagonist under study was corrected with the mean shift monitored by daily control preparations in the absence of antagonist. For the reference H_3-receptor antagonist thioperamide (10–300 nM), a pA_2 value of 8.69 ± 0.05 (Schild plot slope unity: 0.93 ± 0.09, N = 20) was obtained.

3.2.2. Guinea-pig right atrium (spontaneously beating): H_2 receptors

Guinea-pigs of either sex were stunned and exsanguinated. The heart was quickly removed and the right atrium set up isometrically under an initial resting force of 5.0 ± 0.5 mN in a modified Krebs-Henseleit solution (32.5 °C) of composition (mM): NaCl 118.1, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.6, KH_2PO_4 1.2, $NaHCO_3$ 25.0, D-glucose 5.0, and sodium pyruvate 2.0. The solution was gassed with 95% O_2/5% CO_2 (V/V) and contained (±)-propranolol (0.3 μM) to block β-adrenoceptors.
After 30 min of continuous washing and an equilibration period of 15 min, a concentration-frequency curve for histamine (0.1–30 μM) was constructed cumulatively, followed by at least 20 min wash-out. Antagonists were incubated for 30 min before a second curve for histamine was obtained.
Usually the depression of the second concentration-frequency curve for histamine was used to calculate the antagonist affinity parameter pD'_2 (see 3.2.5.). When the rightward shift between first and second curve for histamine was used to calculate a pA_2 value, the shift was lowered by 0.14 logarithmic units because in a set of 8 control atria in the absence of H_2-receptor antagonist, a significant rightward shift of 0.14 ± 0.02 (95% confidence limits 0.09–0.19) was found. In this control group the maximum

effect of histamine in the second curve amounted to 100 ± 1% compared with the first curve. For the reference H_2-receptor antagonist cimetidine (1–100 μM), a pA_2 value of 6.00 ± 0.06 (Schild plot slope 0.85 ± 0.04, N = 16) was obtained.

3.2.3. Guinea-pig ileum (whole segments): H_1 receptors

Whole segments of ileum, 1.5–2.0 cm in length, were mounted isotonically (preload 0.5 g) in Tyrode solution (37 °C) of composition (mM): NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, NaH_2PO_4 0.4, $NaHCO_3$ 11.9, and D-glucose 5.1. The solution was aerated with 95% O_2/5% CO_2 (V/V) and contained atropine (0.1 μM) to block ileal M_3 receptors.
During an equilibration period of 80 min the organs were primed three times with histamine (1 and 10 μM every time). Up to four cumulative concentration-effect curves for histamine (0.01–30 μM for the first curve) were determined in the absence and presence of potential antagonists, which were incubated for 5–10 min.
Control experiments in the absence of antagonist revealed that four successive concentration-effect curves for histamine were superimposable (data not shown). For the reference H_1-receptor antagonist mepyramine (0.3–100 nM), a pA_2 value of 9.07 ± 0.03 (Schild plot slope unity: 0.97 ± 0.04, N = 29) was obtained.

3.2.4. Guinea-pig ileum (whole segments): M_3 receptors

Whole segments of ileum were set up as described for H_1-receptor experiments (3.2.3.) in the absence of atropine. During an equilibration period of at least 80 min the organs were primed at least three times with carbachol (1 μM). Up to four cumulative concentration-effect curves for carbachol (0.003–10 μM for the first curve) were constructed in the absence and presence of potential antagonists which were incubated for 5–10 min.
Control experiments in the absence of antagonist revealed that four successive concentration-effect curves for carbachol were superimposable (data not shown). For the reference M_3-receptor antagonist atropine (6.3–2000 nM), a pA_2 value of 8.81 ± 0.03 (Schild plot slope unity: 1.05 ± 0.03, N = 30) was obtained. A virtually identical affinity for atropine was found when the longitudinal muscle preparation of the H_3-receptor assay was used for M_3 experiments (Elz and Pertz, unpublished).

3.2.5. Pharmacological parameters

Results are expressed as mean ± standard error (SEM or SE) unless otherwise indicated. Antagonist affinity was calculated as apparent pA_2 value according to eq. (1) when only one or two antagonist concentrations were used ([c] = mol/l, r is the ratio of agonist concentrations in the presence and absence of antagonist, that elicit 50% of the respective maximum effect) [36]. Full pA_2 values were calculated according to the method of Schild [37] when a set of different antagonist concentrations over at least 1.5 logarithmic units was studied. For compounds evoking a low corrected rightward shift (ΔpEC_{50}) of the (*R*)-α-methylhistamine curve in the H_3-receptor assay, the mean ΔpEC_{50} was checked for significance versus zero (single sample t-test, $P < 0.05$ considered as significant). 95% Confidence limits λ, i.e., λ_1 and λ_2, for this mean ΔpEC_{50} were then transformed into confidence limits for pA_2 according to eq. (2). Non-competitive antagonists in the H_2-receptor assay were characterized by their pD'_2 value according to eq. (3) where E_{max} is the relative maximum response of histamine in the presence of antagonist [38].

$$pA_2 = -\log_{10} c(\text{antagonist}) + \log_{10} (r - 1) \quad (1)$$

$$pA_2(\text{limit } \lambda_i) = -\log_{10} c(\text{antagonist}) + \log_{10} (10^{\lambda_i} - 1) \quad (2)$$

$$pD'_2 = -\log_{10} c(\text{antagonist}) + \log_{10} ((100/E_{max}) - 1) \quad (3)$$

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