Synthesis and Histamine H_1 Receptor Agonist Activity of a Series of 2-Phenylhistamines, 2-Heteroarylhistamines,† and Analogues‡

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Received November 21, 1994®

New histamine derivatives characterized by a (substituted) aryl, heteroaryl, benzyl, or heteroarylmethyl substituent in the C2 position of the imidazole ring have been prepared from appropriate imidates or amidines, respectively, and 2-oxo-4-phthalimido-1-butyl acetate (1). The compounds were screened as potential H_1 receptor agonists on the isolated guinea pig ileum. The 3-halogenated 2-phenylhistamines (halogen = Br (**35**) and I (**36**)) were equipotent with histamine, while 2-(3-(trifluoromethyl)phenyl)histamine (2-[2-(3-(trifluoromethyl)phenyl)-1*H*-imidazol-4-yl]ethanamine (**39**)) was significantly more potent than histamine (**39**: pD_2 = 6.81, relative activity = 128%). The 2-substituted histamine analogues were partial H_1 receptor agonists on the endothelium-denuded isolated guinea pig aorta with pEC_{50} values generally smaller than observed on the guinea pig ileum, but the rank order of potency was found to be similar. The contractile effects on guinea pig ileum and aorta, respectively, could be blocked concentration-dependently by the H_1 receptor antagonist mepyramine, yielding K_B values for mepyramine in the nanomolar range. *In vitro* compounds **35** and **39** bound to [^{3}H]mepyramine-labeled guinea pig cerebellar membranes with a pK_i of 6.1 and 5.9, respectively. However, upon iv administration, **35** (3–100 mg/kg) and **39** (3–300 mg/kg) failed to inhibit the binding of [^{3}H]mepyramine to mouse cerebral cortex *in vivo*, thereby indicating that these histamine derivatives are not able to penetrate the blood–brain barrier. In functional *in vitro* studies on histamine H_2, H_3, and other neurotransmitter receptors the selectivity of **39** was found to be 2138 (H_1:H_2), >64 (H_1:H_3), 1000 (H_1:M_3), 105 (H_1:α_1), 708 (H_1:β_1), and 71 (H_1:5HT_{2A}). Thus compound **39** is the most potent and selective H_1 receptor agonist reported so far. These results make *meta*- substituted 2-phenylhistamines, especially 2-(3-(trifluoromethyl)phenyl)- and 2-(3-bromophenyl)histamine (**39** and **35**, respectively) valuable experimental tools for the selective stimulation of histamine H_1 receptors and the study of H_1 receptor-mediated functions.

Introduction

Histamine H_1 receptor agonists have gained significant importance for the investigation of H_1 receptor mediated effects, such as neuroregulation of arousal,[1–3] allergy, modulation of cardiovascular parameters,[4–6] and release of endothelium derived relaxing factor (identified as nitric oxide).[4,7,8] The study of these topics has always been hampered by the lack of subtype selectivity or potency of the H_1 receptor agonists used (*viz.* histamine, 2-(2-thiazolyl)ethanamine (2-TEA), and *N*-methyl-(2-pyridyl)ethanamine (betahistine), respectively) (Table 1). The use of the endogenous ligand histamine often requires the concomitant presence of H_2 and H_3 receptor blockers, while the "selective" H_1 receptor agonist 2-TEA also stimulates H_2 receptors with 2.2% relative activity compared with histamine.[9] Betahistine lacks potency (Table 1) and blocks H_3 receptors with a K_i of 7 μM.[9,10] On historical grounds SK&F's work on methylated histamines justifies special mention.[11,12] The methylation of the C2 position of histamine led to a compound with selective H_1 receptor agonist activity, although lower than histamine[11] (Table 1), while C5-substituted histamines were H_2 receptor agonists.[11–14] Dziuron and Schunack[15] showed that an aromatic system on C2 of the imidazole nucleus led to selective H_1 receptor agonists, while arylalkyl or cycloalkyl substituents decreased the activity. Interest was refocused on this class of H_1 receptor agonists when *meta*-substituted 2-phenylhistamines were identified as potent and selective H_1 receptor agonists,[16,17] the 3-Cl and 3-F derivatives being full agonists and about as active as histamine on the guinea pig ileum[16] (Table 1). However, the inositol phospholipid hydrolysis in DDT_1-MF-2 cells was stimulated only to a submaximal degree, indicating partial agonist properties of 2-phenylhistamines.[18]

On the basis of these results, we report the synthesis, *in vitro* evaluation, and structure–activity relationships

Table 1. Relative Activity of Some Histamine H_1 Receptor Agonists on the Guinea Pig Ileum

H_1 receptor agonist	rel. act. (%)
histamine	100
2-(2-thiazolyl)ethanamine (2-TEA)	20–33[a]
N-methyl-(2-pyridyl)ethanamine (betahistine)	8,[b] 9[c]
2-methylhistamine	15–18[a,d,e]
2-phenylhistamine	10,[f] 13[d]
2-(3-methoxyphenyl)histamine	40[f]
2-(3-fluorophenyl)histamine	87,[g] 85[c]
2-(3-chlorophenyl)histamine	81,[g] 96[c]

[a] Reference 12. [b] Reference 10. [c] Unpublished results from our laboratory employing experimental conditions described in this paper. [d] Reference 15. [e] Reference 11. [f] Reference 17. [g] Reference 16.

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† Nomenclature of substituted histamine derivatives is based on the method of Black and Ganellin.[50]

‡ Presented in part: XIIth International Symposium on Medicinal Chemistry (AbstractP-126.A), Basel, October 13–17, 1992.

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® Abstract published in *Advance ACS Abstracts*, March 15, 1995.

0022-2623/95/1838-1287$09.00/0

Table 2. Preparation and Properties of Imidate Salts $RC(=NH)OCH_3 \cdot HCl$ and $RC(=NH)OC_2H_5 \cdot HBF_4$[a]

no.	R	formula	method	mp (°C)	yield (%)
2	2-thienyl	$C_6H_7NOS \cdot HCl$	B	180–181[b]	92
3	2-furyl	$C_6H_7NO_2 \cdot HCl$	B	140–141[c]	92
4	2-pyrrolyl	$C_6H_8N_2O \cdot HCl$	B	117–118	82
5	3-thienyl	$C_6H_7NOS \cdot HCl$	A	159–160[d]	65
6	5-bromothien-3-yl	$C_6H_6BrNOS \cdot HCl$	A	121–122	72
7	2-thienylmethyl	$C_7H_9NOS \cdot HCl$	A	*e*	94
8	3-thienylmethyl	$C_7H_9NOS \cdot HCl$	A	98–99	99
9	5-bromopyridin-3-yl	$C_7H_7BrN_2O \cdot HCl$	C	*e*	99
11	3-bromophenyl	$C_8H_8BrNO \cdot HCl$	A	156–157	93
12	3-iodophenyl	$C_8H_8INO \cdot HCl$	A	181–182	96
13	3-methylphenyl	$C_9H_{11}NO \cdot HCl$[f]	C	86–88	96
14	2-(trifluoromethyl)phenyl	$C_{10}H_{10}F_3NO \cdot HBF_4$	*g*	124–125	82
15	3-(trifluoromethyl)phenyl	$C_9H_8F_3NO \cdot HCl$	A	117	80
16	4-(trifluoromethyl)phenyl	$C_9H_8F_3NO \cdot HCl$	C	190	97
17	3-(trifluoromethoxy)phenyl	$C_9H_8F_3NO_2 \cdot HCl$	C	99	95
18	2-(trifluoromethyl)benzyl	$C_{10}H_{10}F_3NO \cdot HCl$[h]	C	153–154	93
19	3-(trifluoromethyl)benzyl	$C_{10}H_{10}F_3NO \cdot HCl$	C	108[e]	96
20	4-(trifluoromethyl)benzyl	$C_{10}H_{10}F_8NO \cdot HCl$	C	135–138[e]	95
21	2-chlorobenzyl	$C_9H_{10}ClNO \cdot HCl$	B	*e*	96
22	3-chlorobenzyl	$C_9H_{10}ClNO \cdot HCl$	B	*e*	96
23	2-naphthyl	$C_{12}H_{11}NO \cdot HCl$	C	209[i]	85
24	9-phenanthryl	$C_{17}H_{15}NO \cdot HBF_4$	*g*	150–153	30

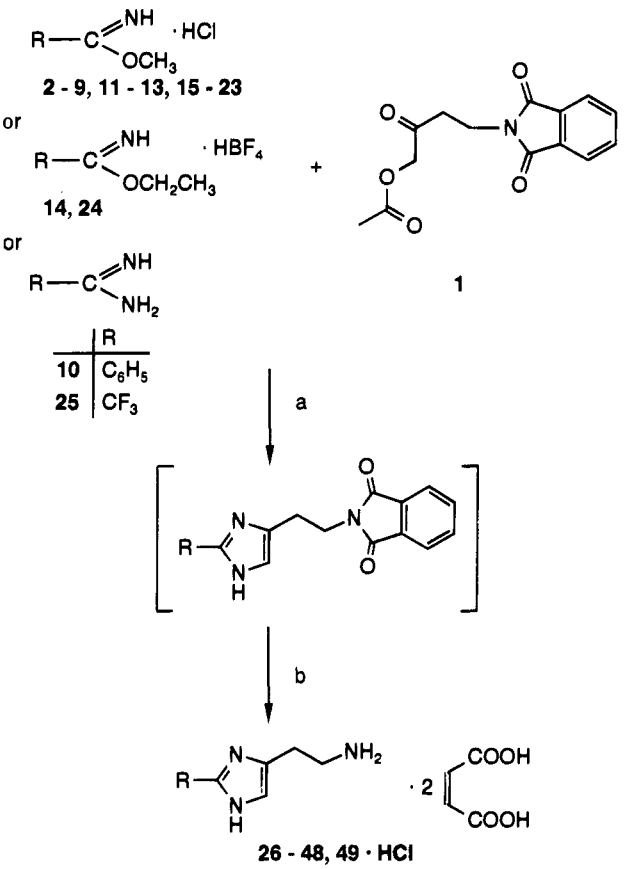
[a] All compounds were analyzed for C, H, and N and are within ±0.4% of the theoretical values unless otherwise indicated. They were characterized through ^{1}H-NMR, IR (KBr), and EI-MS (70 eV). Eight compounds have been published but not fully characterized in the literature: **9** (ref 40); **11** and **15** (ref 41); **12** (ref 42); **13** (ref 43); **14** (ref 44); **16** (ref 45); **23** (ref 46). [b] 173 °C (ref 47). [c] Oil (ref 48). [d] 110 °C (ref 34). [e] Processed without further characterization. [f] Anal. C: calcd, 58.2; found, 57.5; N: calcd, 7.55; found, 8.08. [g] According to ref 21. [h] Anal. C: calcd, 47.4; found, 46.8. [i] 193–194 °C (ref 46).

of further 2-substituted histamines with prominent H_1 receptor-stimulating properties. Two compounds of the series (**35** and **39**) have already been shown to influence cardiovascular parameters in the pithed rat.[6] The most potent derivative (**39**) is able to increase waking in the rat upon intracerebroventricular administration.[3] Halogenated compounds of this series have also been found to interact with pertussis toxin-sensitive G-proteins in HL-60 cells[19] which makes them promising candidates for the study of receptor independent direct interactions with G-proteins.

Chemistry

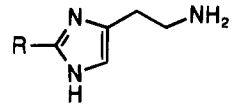
The nitriles were directly converted to methyl imidate hydrochlorides (Table 2, **2**–**9**, **11**–**13**, and **15**–**23**) by the Pinner reaction in the presence of dry hydrogen chloride and equimolar amounts of anhydrous methanol in diethylether or in excess anhydrous methanol, respectively (methods A–C). The Pinner reaction was not applicable to nitriles with high steric demand (2-(trifluoromethyl)benzonitrile and phenanthrene-9-carbonitrile). In these cases ethyl imidate salts (Table 2, **14** and **24**) were prepared by alkylation of the amides[20] with triethyloxonium tetrafluoroborate in methylene chloride described by Weintraub.[21] Commercially available amidines (**10** and **25**) or the imidates (**2**–**9** and **11**–**24**) were cyclized with 2-oxo-4-phthalimido-1-butyl acetate (**1**)[16,22] in liquid ammonia to yield the crude phthalimidoethyl-substituted imidazoles (Scheme 1). Acidic hydrolysis with aqueous hydrogen chloride led to the crude brown amines. Derivatization with *N*-(ethoxycarbonyl)phthalimide (method D) in ethanol and column chromatographic purification yielded the corresponding phthalimides. Subsequent hydrazinolysis, separation of the amines by preparative rotatory chromatography, and crystallization with maleic acid led to the title compounds (Table 3). Alternatively, the free amines were purified chromatographically immediately after acidic hydrolysis and converted to hydrogen maleates (method E). At present, in our hands the cyclization using **1** represents the most effective route for the synthesis of 2-substituted (aryl/heteroaryl) histamine derivatives although the yields are poor.

Scheme 1. Synthesis of 2-Substituted Histamines[a]



[a] For R, see also Tables 2 and 3. Reagents: (a) liquid NH_3, 20–25 bar; (b) method D: 6 N HCl (reflux), *N*-(ethoxycarbonyl)phthalimide/EtOH, column chromatography, N_2H_4/1 N HCl, centrifugal chromatography, maleic acid; method E: 6 N HCl (reflux), centrifugal chromatography, maleic acid (HCl for **49**).

Table 3. Preparation, Physicochemical Properties and Contractile Effects of Histamine Analogues on the Guinea Pig Ileum



						H_1 receptor agonism				antagonism by mepyramine	
no.	R	formula[a]	method	mp (°C)	yield[b] (%)	N[c]	ia[d]	pD_2[e]	rel. act. (%)	pK_B[f]	slope[g]
26	2-thienyl	$C_9H_{11}N_3S\cdot 2C_4H_4O_4$	D	147–148	16	22	1.0	6.36	46	8.93[h]	1.07
27	2-furyl	$C_9H_{11}N_3O\cdot 2C_4H_4O_4$	D	149–150	6	10	1.0	6.37	47	9.16	
28	2-pyrrolyl	$C_9H_{12}N_4\cdot 2C_4H_4O_4$	D	139–141	4	12	0.93[i]	5.86	14	9.03	
29	3-thienyl	$C_9H_{11}N_3S\cdot 2C_4H_4O_4$	D	154	3	17	1.0	6.52	66	9.13[h]	1.01
30	5-bromothien-3-yl	$C_9H_{10}BrN_3S\cdot 2C_4H_4O_4$	D	145–146	4	8	1.0	6.11	26	9.04	
31	2-thienylmethyl	$C_{10}H_{13}N_3S\cdot 2C_4H_4O_4$	D	142	7	9	1.0	5.09	3	8.92	
32	3-thienylmethyl	$C_{10}H_{13}N_3S\cdot 2C_4H_4O_4$	D	139	8	7	1.0	5.26	4	8.97	
33	5-bromopyridin-3-yl	$C_{10}H_{11}BrN_4\cdot 2C_4H_4O_4$[j]	E	161–162	1	16	1.0	6.54	70	9.03	
34	phenyl	$C_{11}H_{13}N_3\cdot 2C_4H_4O_4$	D	164–165	8	9	1.0	6.20	31[k]	8.87	
35	3-bromophenyl	$C_{11}H_{12}BrN_3\cdot 2C_4H_4O_4$	D	168–169	9	18	1.0	6.75	112	9.17[l]	0.98
36	3-iodophenyl	$C_{11}H_{12}BrN_4\cdot 2C_4H_4O_4$	D	170–171	7	19	1.0	6.68	96	9.28[h]	0.90
37	3-methylphenyl	$C_{12}H_{15}N_3\cdot 2C_4H_4O_4$	D	164–165[m]	5	18	1.0	5.87	15[n]	9.04	
38	2-(trifluoromethyl)phenyl	$C_{12}H_{12}F_3N_3\cdot 2C_4H_4O_4$	E	170–172	1	4	0.26	4.47	<1	*o*	
39	3-(trifluoromethyl)phenyl	$C_{12}H_{12}F_3N_3\cdot 2C_4H_4O_4$	D	153–154	4	21	1.0	6.81[p]	128	9.14[h]	0.92
40	4-(trifluoromethyl)phenyl	$C_{12}H_{12}F_3N_3\cdot 2C_4H_4O_4$	E	175–176	6	4	0.24	4.60	<1	*o*	
41	3-(trifluoromethoxy)phenyl	$C_{12}H_{12}F_3N_3O\cdot 2C_4H_4O_4$	E	150–151	8	12	1.0	6.43	54	8.92	
42	2-(trifluoromethyl)benzyl	$C_{13}H_{14}F_3N_3\cdot 2C_4H_4O_4$	E	135–136	7	4	0.0	(pK_B = 3.55)[q,r]			
43	3-(trifluoromethyl)benzyl	$C_{13}H_{14}F_3N_3\cdot 2C_4H_4O_4$	E	155–157	15	4	0.28	4.57	<1	*o*	
44	4-(trifluoromethyl)benzyl	$C_{13}H_{14}F_3N_3\cdot 2C_4H_4O_4$	E	166–167	4	4	0.0	(pK_B= 4.53)[q,s]			
45	2-chlorobenzyl	$C_{12}H_{14}ClN_3\cdot 2C_4H_4O_4$	E	146–147	13	7	0.54	5.11	3	8.91[t]	
46	3-chlorobenzyl	$C_{12}H_{14}ClN_3\cdot 2C_4H_4O_4$	E	148	10	7	0.76	4.88	2	8.81[t]	
47	2-naphthyl	$C_{15}H_{15}N_3\cdot 2C_4H_4O_4$	E	178–180	6	8	0.24	4.85	1	*o*	
48	9-phenanthryl	$C_{19}H_{17}N_3\cdot 2C_4H_4O_4\cdot {}^1/_2H_2O$[u]	E	156–157	2	4	0.0	(pK_B= 4.73)[s,v]			
49	trifluoromethyl	$C_6H_8F_3N_3\cdot 2HCl$[w]	E	214–216	2	10	1.0	5.57	7	9.19[t]	
	histamine					>240	1.0	6.70[x]	100	9.09[l]	0.97
	2-TEA					40	1.0	6.36	45[y]	9.17[h]	1.01

[a] All compounds were analyzed for C, H, and N and are within ±0.4% of the theoretical values unless otherwise indicated. They were characterized through ^{1}H-NMR, IR (KBr), and $^+$FAB-MS ((Xe, DMSO/glycerol) or (Xe, DMSO/3-NO_2-$C_6H_4CH_2OH$) only for **30** and **39**). [b] Yield related to amount of imidate salt or amidine used. [c] N number of agonist experiments. [d] Intrinsic activity (ref 35) is given as 1.0 if the mean was not significantly different from 1.0 for histamine (paired t test; $P > 0.05$). Otherwise SEM was 0.01–0.08. [e] Reference 35. SEM was 0.02–0.11. [f] Reference 35. Measured for a single mepyramine concentration (10 nM; $N = 3–5$; SEM 0.04–0.09) unless otherwise indicated. Some entries represent pA_2. [g] Slope of Schild plot regression (ref 24) was not significantly different from unity, except for **36** ($P < 0.05$). [h] pA_2 value (ref 24; SEM 0.10–0.20; $N = 12–16$). [i] SEM 0.02; significantly different from 1.0 (paired t test; $P < 0.05$). [j] Anal. C: calcd, 43.3; found, 42.7. [k] 10% (ref 17); 13% (ref 15). [l] pA_2 value (ref 24; SEM 0.10; $N = 24–29$). [m] 163 °C (ref 49). [n] 30% (ref 16); 13% (ref 17). [o] Effect could be antagonized with 1 μM mepyramine added to the bath solution when a plateau was reached. [p] Significantly more potent than histamine (paired t test; $P < 0.001$). [q] Antagonism measured at 300 μM. [r] Maximum unaffected. [s] Maximum reduced to 50–60%. [t] SEM 0.07–0.20 ($N = 2–3$), measured for 3 nM mepyramine. [u] Anal. N: calcd, 7.95; found, 8.44. [v] Antagonism measured at 30 μM. [w] Anal. C: calcd, 28.6; found, 29.2. [x] SD 0.24. [y] 20–33% (ref 12).

Biological Results and Discussion

Most of the new histamine derivatives exert a contractile effect on isolated guinea pig ileal segments (Table 3). 2-Heteroarylhistamines (**26–30** and **33**) and *meta*-substituted 2-phenylhistamines (**35-37**, **39** and **41**) are full agonists compared with histamine. Isosteric replacement of phenyl (**34**: relative activity (rel. act. 31%) by heteroaromatic substituents hardly affects the H_1 receptor agonist activity. 2-(2-Thienyl)histamine (**26**: rel. act. 46%) and 2-(2-furyl)histamine (**27**: rel. act. 47%) are more effective than the 2-pyrrolyl analogue **28** (rel. act. 14%), while 2-(3-thienyl)histamine (**29**: rel. act. 66%) is significantly more potent than the 2-thienyl isomer **26**. Apparently, increasing the distance between imidazole and thienyl ring by one methylene group attenuates the H_1 receptor activity by at least 1 order of magnitude without affecting the intrinsic activity. *Meta*-substituted, halogenated 2-phenylhistamines **35**, **36**, **39**, and **41** (rel. act. 54–128%) are of special interest due to their high potency compared with histamine. This series, including the 3-fluoro and 3-chloro analogues,[16] represents the most interesting set of experimental H_1 receptor agonists to date. Among the *meta*-halogenated compounds, the differences are marginal and would be hard to verify statistically. However, the 3-bromo derivative **35** represents the apparent optimum. The 3-trifluoromethyl derivative **39** is the first H_1 receptor agonist reported to be significantly more potent than histamine on the guinea pig ileum (**39**: rel. act. 128%; $P < 0.001$; paired t-test). Two position isomers of **39** (**38** and **40**) dramatically lose intrinsic activity and efficacy. Benzylic analogues of **39** and 2-(3-chlorophenyl)histamine,[16] respectively, are only weak partial agonists (**43**, **45**, and **46**) or possess negligible H_1 receptor antagonist properties (**42** and **44**). This finding and similar reports for isomers of 3-fluoro and 3-chloro analogues[16] emphasize the prominent role of the *meta* position of 2-phenylhistamines. The importance of this binding area of the H_1 receptor is also reflected by structure–activity relationships observed for thienyl and pyridyl isosters of 2-phenylhistamine. The sulfur atom of the 3-thienyl analogue **29** (rel. act. 66%) virtually corresponds to the *m*-phenyl position rather than the sulfur of the 2-thienyl analogue **26** (rel. act. 46%). A similar rank order has been reported for 2-(pyridyl)-substituted histamines[15] where the pyridine

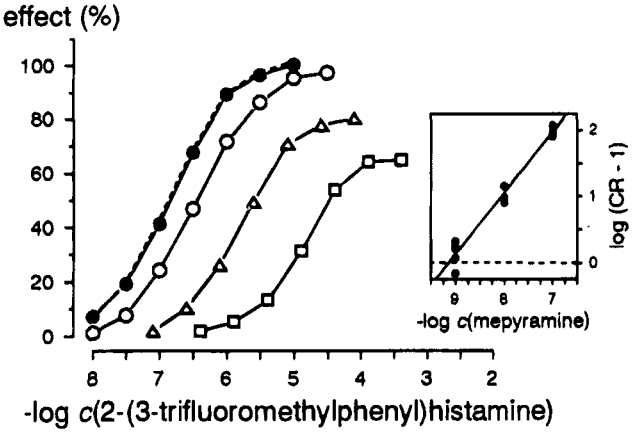


Figure 1. Contractile response of guinea pig ileal whole segments to increasing concentrations of 2-(3-(trifluoromethyl)-phenyl)histamine (**39**) in the absence (●; $N = 21$) and presence of the H_1 receptor antagonist mepyramine: 1 nM (○; $N = 5$), 10 nM (△; $N = 5$), and 100 nM (□; $N = 5$). *Inset*: The corresponding Schild plot regression for mepyramine yields a slope ± SD of 0.92 ± 0.04 and a pA_2 ± SEM value of 9.14 ± 0.04. The dashed curve was obtained for untreated controls ($N = 6$). Error bars are omitted for sake of clarity.

nitrogen of the most potent congener, 2-(3-pyridyl)-histamine,[15] occupies a "*meta*" position. Interestingly, the agonist potency of this compound is enhanced by introduction of a bromo substituent in the other *meta* position (rel. act. 13%[15] *versus* 70% (**33**)), as it is observed in the phenyl series (rel. act. 31% (**34**) *versus* 112% (**35**)) but not in the 3-thienyl series (rel. act. 66% (**29**) *versus* 26% (**30**))!

Bulkier aromatic substituents in the C2 position of the imidazole ring, *e.g.*, 2-naphthyl (**47**) or 9-phenanthryl (**48**), are favorable neither for intrinsic activity nor for binding *per se*. 2-(Trifluoromethyl)histamine (**49**), a fluorinated analogue of the H_1 selective agonist 2-methylhistamine,[11,12] turns out to be a full agonist on the ileum with reduced potency (rel. act. 7% *versus* 15–18%[11,12,15]). The agonist potencies of 2-(2-thiazolyl)-ethanamine (2-TEA)[12] and compounds **34** and **37**[15,17] measured in this study deviate moderately from already published data which may be explained by different experimental conditions employed in these studies. Especially the use of 1 μM atropine in the guinea pig ileum assay[12] is crucial since atropine competitively blocks ileal H_1 receptors with a K_B of 0.8 μM[23] and thus shifts the concentration–effect curves of H_1 receptor agonists to the right. In order to avoid this influence, we used a concentration of atropin essentially below its K_B value (0.1 μM).

The H_1 receptor-mediated nature of the ileum contraction has been verified by competition experiments with the H_1 receptor antagonist mepyramine yielding pK_B or pA_2 values in the expected range around 9 (8.81–9.28, Table 3). A typical set of concentration–effect curves including an affinity determination for mepyramine according to Schild[24] is given in Figure 1. Typically the maximum response for agonists is significantly reduced in the presence of increasing concentrations of mepyramine. This pseudoirreversible antagonism may be a result of a "hemi-equilibrium" state[25] between agonist and antagonist and indicates slow kinetics of the drugs involved and a low receptor reserve for the new agonists.

In a second approach the histaminomimetic properties

Table 4. Contractile Effects of Selected H_1 Receptor Agonists on the Guinea Pig Aorta

	H_1 receptor agonism (second curve[a])				antagonism by mepyramine
no.	N[b]	ia[c]	pEC_{50}[d]	rel. act. (%)	pK_B[e]
26	8	0.50	5.00	12	8.83
27	9	0.47	5.27	23	9.28
28	8	0.74	4.99	12	9.27
29	8	0.43	5.21	19	9.01
30	9	0.60	4.94	11	8.63
31	4	0.45	3.83	0.8	*f*
32	4	0.40	3.89	0.9	*f*
34	9	0.86	5.20	19	9.10
35	16	0.64	5.60	48	8.90
36	13	0.49	5.59	47	9.13
39	19	0.80	5.89	94[g]	9.04[h]
45	4	0.38	3.24	0.2	*f*
46	4	0.51	3.54	0.4	*f*
histamine	18	1.06	5.94[i]	105	9.11[h]
2-TEA	8	1.02	5.17	18	9.02[h]

[a] First curve for histamine. [b] N number of agonist experiments. [c] Intrinsic activity (ref 35; SEM 0.01–0.12). [d] SEM 0.03–0.18. [e] Reference 35. Measured for a single mepyramine concentration (3 or 10 nM; $N = 4–8$; SEM 0.03–0.14). Some entries represent pA_2. [f] Effect could be antagonized with 1 μM mepyramine added to the bath solution when a plateau was reached. [g] Not significantly different from histamine (paired t test; $P > 0.05$). [h] pA_2 (ref 24; $N = 12–34$; SEM 0.04–0.24). Slope not significantly different from unity (SD 0.02–0.07). [i] First curve for histamine ($N = 18$; $pEC_{50} = 5.92 \pm 0.03$; rel. act. = 100%).

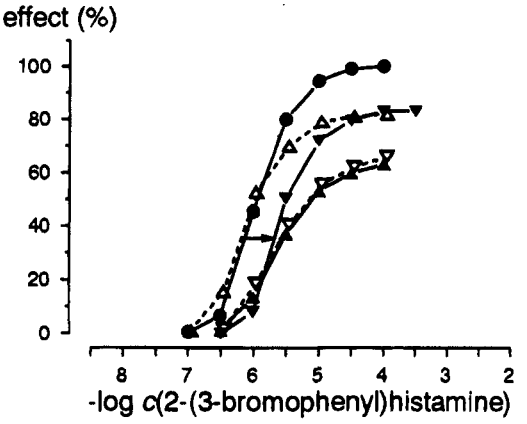


Figure 2. Contractile response of guinea pig aortic rings to increasing concentrations of histamine (●, first curve) and 2-(3-bromophenyl)histamine (**35**) in control organs (dashed lines: ▽, second curve; △, third curve, $N = 8$) and in preparations before (▲, second curve; $N = 8$) and after 30 min treatment with 3 nM mepyramine (▼, third curve; $N = 8$). In control organs the third curve was shifted to the left (ΔpEC_{50} ± SEM = 0.48 ± 0.04) and the maximum effect was increased from 66 ± 1% (▽) to 82 ± 1% (△). In the mepyramine-treated group, the maximum effect was 63 ± 1% (▲) *versus* 83 ± 2% (▼). Considering the monitored sensitization, a pK_B ± SEM value of 8.90 ± 0.04 was calculated for mepyramine ($N = 8$). Error bars are omitted for sake of clarity.

of some of the title compounds have been demonstrated in a vascular H_1 receptor assay.[26] Histamine, 2-TEA, and selected histamine analogues contract isolated endothelium-denuded guinea pig aortic rings with pEC_{50} values generally smaller than on the ileum (Table 4), indicating a less effective coupling of the aortic H_1 receptor with postreceptor-located intracellular mechanisms. Except histamine and 2-TEA, all compounds which are full agonists on the ileum are partial agonists on the aorta (intrinsic activity 0.38–0.86). A typical example (compound **35**) is illustrated in Figure 2. However, the rank order of potency is similar, and a

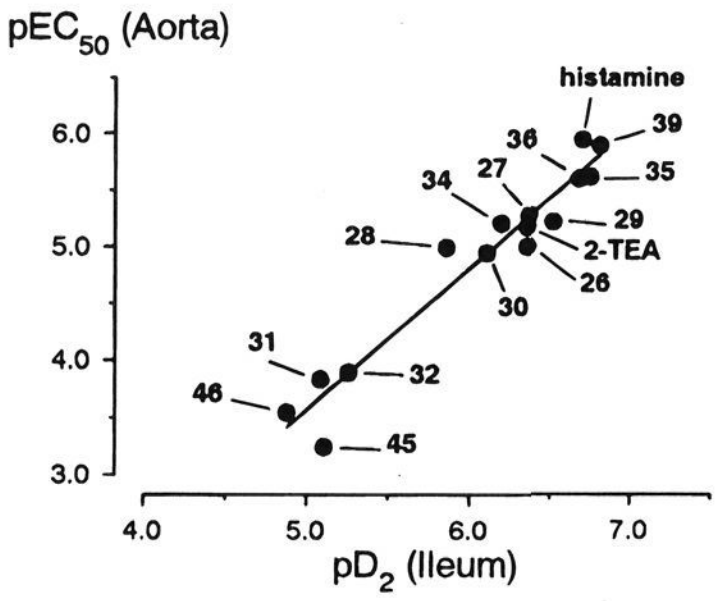


Figure 3. Linear relationship between pD_2 and pEC_{50} values determined for histamine, 2-(2-thiazolyl)ethanamine (2-TEA), and 13 H_1 receptor agonists on the guinea pig ileum (abscissa) and aorta (ordinate), respectively. The linear regression yields an equation of pEC_{50}(aorta) = 1.23pD_2(ileum) − 2.57 with a correlation coefficient of $r = 0.9695$ ($N = 15$; $P < 0.001$). Error bars are omitted for sake clarity.

Table 5. *In Vivo* Binding of H_1 Receptor Agonists Measured by Binding of [^{3}H]Mepyramine to Mouse Cerebral Cortex[a]

treatment			[^{3}H]mepyramine binding
agent no.	dose (mg/kg)	N[b]	(% control ± SEM)
35	3	5	106 ± 4
	10	5	98 ± 10
	30	5	98 ± 4
	100	4	98 ± 10
39	3	5	101 ± 5
	10	5	122 ± 7
	30	5	98 ± 4
	100	3	112 ± 13
	300	3	129 ± 11

[a] For details, see the Experimental Section. [b] N, number of mice treated.

linear relationship exists between ileum and aorta data (Figure 3). The contractile effects can be antagonized by injection of mepyramine (1 μM) when the maximum is reached, or by shifting the concentration−effect curves to the right by suitable concentrations of mepyramine (2−50 nM; for details see Table 4, Figure 2, and the Experimental Section). As a conclusion, the guinea pig aorta assay allows to detect partial agonist properties of novel H_1 receptor agonists.

For the most potent compounds, **35** and **39**, affinity estimates have been confirmed by *in vitro* binding experiments. Histamine inhibits [^{3}H]mepyramine binding to H_1 receptors from guinea pig cerebellar membranes with a K_i ± SEM value of 39 ± 1 μM. Compounds **35** and **39** are approximately 40 times more potent than histamine on this binding test (K_i ± SEM 0.8 ± 0.03 μM (**35**); 1.2 ± 0.08 μM (**39**)). Such a difference might suggest that they are partial agonists, an interpretation which agrees well with aforementioned data showing these compounds to be full agonists in a system containing many spare receptors like guinea pig ileum[27] but partial agonists on guinea pig aorta.

When administered systemically to mice at doses up to 100 mg/kg for **35** and 300 mg/kg for **39**, these compounds do not inhibit the *in vivo* specific binding of [^{3}H]mepyramine in the mouse cerebral cortex (Table 5). Because this test has been shown to reflect the occupancy of H_1 receptors in the brain of living animals,[28]

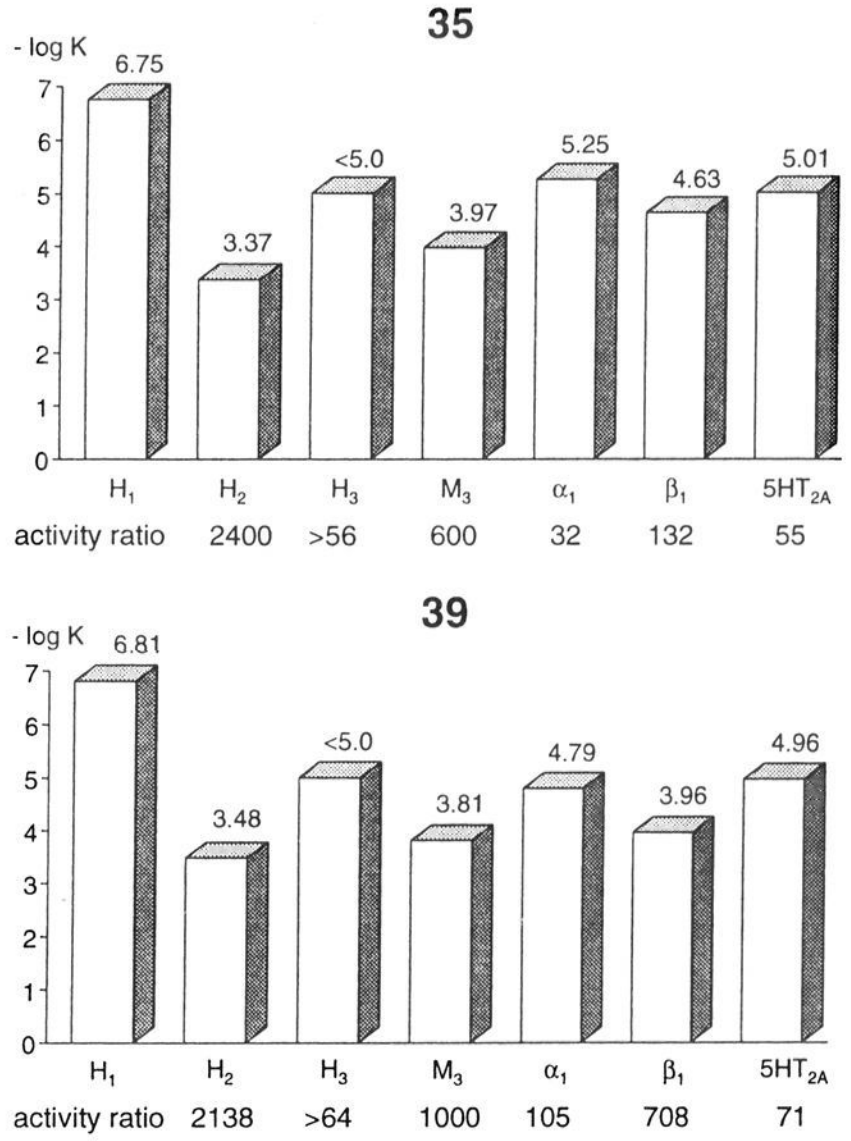


Figure 4. Functional receptor selectivity of 2-(3-bromophenyl)histamine (**35**, upper panel) and 2-(3-(trifluoromethyl)-phenyl)histamine (**39**, lower panel).

Table 6. Histamine H_2 Receptor Activity of Selected H_1 Receptor Agonists on the Isolated Guinea Pig Atrium[a]

no.	H_2 agonism ia	H_2 antagonism pD'_2	functional H_1:H_2 selectivity
26	(0.25 ± 0.14)[b]	<3.0	>2290
27	(0.36 ± 0.03)[b]	<3.0	>2344
29	(0.13 ± 0.05)[b]	<3.0	>3311
34	(0.20 ± 0.06)[b]	<3.0	>1585
35	0.0	3.37 ± 0.07	2400
36	0.0	3.76 ± 0.09	832
39	0.0	3.48 ± 0.12	2138
49	0.55 ± 0.08[c,d]		525
histamine	1.0[e]		5

[a] N, number of experiments: 2−3. $N = 3$: ± SEM. $N = 2$: range given. [b] Transient positive chronotropic and inotropic effects were observed at 10−100 μM which were neither cimetidine nor metoprolol-sensitive. [c] Relative activity compared with histamine: 0.07 ± 0.01%. [d] Estimated pK_B for cimetidine (3 μM) was 6.20 ± 0.09 ($N = 3$). [e] pD_2 = 6.0 ($N > 50$).

it is likely that these compounds do not reach central H_1 receptors following systemic administration. However, Monti et al.[3] have clearly demonstrated the H_1 receptor-mediated effects of **39** on the waking state in rats using the intracerebroventricular route of administration.

For the most potent agonists, **35** and **39**, a receptor affinity profile has been established in various functional receptor assays (Figure 4). Activity ratios are in the range of 32−2400. Considerably high values for several agonists are observed with regard to the selectivity H_1:H_2 (Table 6). Except 2-(trifluoromethyl)histamine (**49**) which is a cimetidine-sensitive partial H_2 receptor agonist, none of the H_1 receptor agonists

studied produces specific H_2 receptor agonism on the guinea pig atrium.

Conclusions

In the series of substituted 2-phenylhistamines[16] and their heteroaromatic analogues,[15] we have identified full H_1 receptor agonists equipotent with histamine in the guinea pig ileum which behave as partial agonists on the guinea pig aorta. 2-(3-(Trifluoromethyl)phenyl)-histamine (**39**) is the first and only H_1 receptor agonist reported to date that is significantly more potent than histamine in the ileum assay and possesses a satisfactory selectivity profile. These features make **39** a promising tool for the experimental characterization of H_1 receptor-mediated biological functions. Preliminary results concerning *quantitative* structure–activity relationships of this class of H_1 receptor agonists have been presented recently.[29]

Experimental Section

Chemistry. General Procedures. Melting points were determined on a Büchi 510 apparatus and are uncorrected. For all compounds, ^{1}H-NMR spectra were recorded on a Bruker AC 300 (300 MHz) or a Bruker WM 250 (250 MHz) spectrometer. Chemical shifts are expressed in ppm downfield from internal TMS. ^{1}H-NMR data are reported in order: multiplicity, number of protons, and approximate coupling constants in hertz. IR spectra (KBr) were measured on a Perkin-Elmer 1420 spectrophotometer. $^+$FAB-MS spectra were obtained on a Finnigan MAT CH5DF, EI-MS spectra on a Kratos MS 25 Rf and a Varian MAT CH7. Elemental analyses (C, H, N) for novel compounds were within ±0.4% of the theoretical values unless otherwise indicated. Yields are not optimized. 3-Iodobenzonitrile and 5-bromothiophene-3-carbonitrile were prepared from the corresponding acids[30] according to the general procedure reported by Hulkenberg and Troost.[31] Thiophene-3-carbonitrile was synthesized from 3-bromothiophene with copper(I) cyanide[32] and pyrrole-2-carbonitrile by a modified Vilsmeier–Haack synthesis from pyrrole.[33] The other nitriles were commercially available.

Methyl Imidate Hydrochlorides (2–9, 11–13, 15–23). Method A. A solution of the nitrile (25–137 mmol) in dry Et_2O and the equimolar amount of MeOH was saturated (30 min) with dry HCl at 0–5 °C. After 2–5 days in a freezer, the solvent was distilled *in vacuo*. For analysis the imidate salt was recrystallized from dry MeOH/Et_2O. The bulk was pure enough for subsequent cyclization.

Method B. A solution of the nitrile (53–86 mmol) in excess dry MeOH was saturated (45 min) with dry HCl at 0–5 °C. For the next steps, see method A.

Method C. The nitrile (10–93 mmol) was converted as described under method B. After 2–3 weeks the imidate salt was isolated according to method A.

Methyl Thiophene-3-carboximidate Hydrochloride (5). A solution of thiophene-3-carbonitrile (15.0 g, 137.5 mmol) in dry Et_2O (40 mL) and dry MeOH (4.4 g, 137.5 mmol) was converted by method A (yield: 15.8 g, 65%): mp 159–160 °C (lit.[34] mp 110 °C); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 12.51, 11.81 (br, exchangeable by D_2O, 2H, NH_2^+), 9.08 (d, 1H, J = 1.7, thiophene-H), 7.97 (dd, 1H, J^1 = 1.1, J^2 = 4.1, thiophene-H), 7.45 (dd, 1H, J^1 = 2.2, J^2 = 3.0, thiophene-H), 4.51 (s, 3H, CH_3); EI-MS (70 eV) m/z 141 (M^+, 23), 127 (16), 124 (30), 111 (39), 110 ([M − MeO]$^+$, 100), 96 ($C_5H_4S^+$, 17), 83 ([110 − HCN]$^+$, 20), 45 (20), 39 (53), 38 (18), 36 (58). Anal. (C_6H_7NOS·HCl) C, H, N.

Methyl 3-(Trifluoromethyl)benzimidate Hydrochloride (15). 3-(Trifluoromethyl)benzonitrile (15.0 g, 87.7 mmol) in dry Et_2O (100 mL) and dry MeOH (2.8 g, 87.7 mmol) was obtained *via* method A (yield: 16.9 g, 80%): mp 117 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.65 (d, 1H, J = 7.8, aryl-H), 8.45 (s, 1H, aryl-H), 7.98 (d, 1H, J = 7.7, aryl-H), 7.79 (m, 1H, aryl-H), 4.59 (s, 3H, CH_3); EI-MS (70 eV) m/z 203 (M^+, 8), 202 (78), 173 (22), 172 ([M − MeO]$^+$, 100), 145 ([172 − HCN]$^+$, 27), 134 ([M − CF_3]$^+$, 3). Anal. ($C_9H_8F_3NO$·HCl) C, H, N.

2-Substituted Histamines. Method D. In an autoclave (1000 mL, Kotter, Germany) were dissolved equimolar amounts of imidate salt or amidine (18–86 mmol) and 2-oxo-4-phthalimido-1-butyl acetate (1) in liquid NH_3 (150 mL), and the mixture was stirred overnight at ambient temperature. Then the temperature was raised to 60 °C (20–25 bar) for 6 h. After evaporation of NH_3, the residue was added to 6 N HCl (250 mL) and heated under reflux for 4–6 h. Phthalic acid was separated by filtration. The filtrate was washed with CH_2Cl_2 (3 × 100 mL) and alkalized with 3 N NaOH. The crude amine was extracted with CH_2Cl_2/*i*-PrOH (3:1). The organic phase was evaporated and dissolved in EtOH, and the equivalent amount of *N*-(ethoxycarbonyl)phthalimide was added. The phthalimidoethyl-substituted imidazole was isolated by column chromatography (Baker silica gel type 0253 (0.05–0.2 mm), CH_2Cl_2/MeOH (90:10, NH_3 saturated) as eluent) or by crystallization. The phthalimides were not characterized. Hydrazinolysis in 1 N HCl and purification with a Chromatotron (Harrison Research, model 7924, 4 mm plates, silica gel 60 PF_{254} containing gypsum (Merck), CH_2Cl_2/MeOH (90:10, NH_3 saturated) as eluent) gave the pure amine. With maleic acid the base was crystallized from absolute EtOH/Et_2O as dihydrogen maleate.

Method E. The imidate salts were converted as described under method D. After acidic hydrolysis the crude amine was purified with a Chromatotron (CH_2Cl_2/MeOH (90:10, NH_3 saturated) as eluent). Crystallization with maleic acid from absolute EtOH/Et_2O afforded the dihydrogen maleate.

2-[2-(3-Thienyl)-1*H*-imidazol-4-yl]ethanamine Dihydrogen Maleate (29). Compound **5** (6.7 g, 37.7 mmol) and 2-oxo-4-phthalimido-1-butyl acetate (1, 10.4 g, 37.7 mmol) were cyclized *via* method D (yield: 0.5 g, 3%): mp 154 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.09 (d, 1H, J = 1.8, thiophene-H), 7.84 (br, exchangeable by D_2O, 3H, NH_3^+), 7.75 (dd, 1H, J^1 = 2.1, J^2 = 2.9, thiophene-H), 7.63 (dd, 1H, J^1 = 1.1, J^2 = 3.9, thiophene-H), 7.28 (s, 1H, imidazole-5-H), 6.10 (s, 4H, maleate-H), 3.14 (br, 2H, N-CH_2), 2.89 (t, 2H, J = 7.1, imidazole-CH_2); $^+$FAB-MS (Xe, DMSO/glycerol) m/z 194 ([M + H]$^+$, 100), 177 (M^+ − NH_2, 19), 165 ([M + H]$^+$ − CH_3N, 15), 79 (13), 57 (18). Anal. ($C_9H_{11}N_3S$·2$C_4H_4O_4$) C, H, N.

2-[2-(3-(Trifluoromethyl)phenyl)-1*H*-imidazol-4-yl]-ethanamine Dihydrogen Maleate (39). Compound **39** was obtained *via* method D from **15** (10.0 g, 41.7 mmol) and 1 (11.5 g, 41.7 mmol) (yield: 0.9 g, 4%): mp 153–154 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.27 (s, 1H, aryl-H), 8.21 (d, 1H, J = 4.4, aryl-H), 7.79 (br, exchangeable by D_2O, 3H, NH_3^+), 7.72 (s, 2H, aryl-H), 7.19 (s, 1H, imidazole-5-H), 6.20 (s, 4H, maleate-H), 3.12 (br, 2H, N-CH_2), 2.68 (t, 2H, J = 7.3, imidazole-CH_2); $^+$FAB-MS (Xe, DMSO/3-$NO_2C_6H_4CH_2OH$) m/z 256 ([M + H]$^+$, 100), 239 (M^+ − NH_2, 13), 79 (24). Anal. ($C_{12}H_{12}F_3N_3$·2$C_4H_4O_4$) C, H, N.

Pharmacology. General Procedures. Pharmacological parameters were calculated as geometric means from graphically analyzed sets of individual concentration–effect curves. For the characterization of agonists pEC_{50}, pD_2[35] and ia (intrinsic activity[35]) were determined. Antagonists were characterized by pD'_2[35], pK_B,[35] or pA_2.[24] Binding affinities from radioligand competition experiments were characterized by K_i values.[36] Significant differences of means were discerned by Fisher's F test and Student's t test.

H_1 Receptor Assay on the Guinea Pig Ileum. Guinea pigs of either sex were stunned and exsanguinated. The ileum was removed, and whole segments (1.5 cm in length) were mounted isotonically (preload 5 mN) in 20 mL organ baths filled with Tyrode solution containing atropine (0.1 μM). The solution was gassed with 95% O_2/5% CO_2 and warmed to 37 °C. During a stabilization period of *ca.* 80 min the organs were stimulated three times with histamine (1 μM). Each preparation was used to establish a cumulative curve with histamine (0.01–10 μM) followed by two curves with the respective H_1 receptor agonist. In competition experiments, mepyramine (1–300 nM) was incubated for 10–15 min before the last curve. In the absence of mepyramine, the means of the second and third curves were superimposable for each agonist (N = 3–6).

H_1 Receptor Assay on the Guinea Pig Aorta. The protocol of Dodel and Borchard[26] was modified as follows: Endothelium-denuded 4–6 mm rings of guinea pig thoracic aorta were pushed over two L-shaped stainless steel hooks and mounted isometrically (preload 10 mN) in a modified Krebs-Henseleit solution (37 °C) gassed with 95% O_2/5% CO_2. During an equilibration period of 130 min, organs were stimulated three times with 10 μM histamine. Cumulative concentration–response curves were recorded in the presence of 30 μM corticosterone, 100 μM cimetidine, 0.1 μM (*R,S*)-propranolol, and phentolamine. The first curve was established with histamine (0.1–100 μM), the second and third with the respective H_1 receptor agonist. In competition experiments *versus* mepyramine (1–1000 nM, 30 min incubation), at least one ring served as time control. The dextral shift observed in the presence of mepyramine was corrected individually using the sensitization measured for the control rings.

H_1 Receptor Binding *in Vitro*. Male Hartley guinea pigs (200–300 g) were sacrificed by rapid decapitation, and the cerebellum was homogenized in 100 volumes of cold 50 mM Na_2HPO_4/KH_2PO_4 buffer pH 7.5. After centrifugation at 20000*g* for 10 min, the pellets were resuspended in phosphate buffer for [^{3}H]mepyramine binding. Membranes (100 μg of protein) were incubated with 1 nM [^{3}H]mepyramine for 30 min at 25 °C in a final volume of 0.5 mL. Incubations were stopped by addition of cold buffer followed by rapid filtration through glass-fiber filters (GF/B). Radioactivity retained on the filters was measured by liquid scintillation spectrometry. Specific binding was defined as radioactivity bound after subtracting nonspecific binding determined in the presence of 0.2 μM mianserin. Hill numbers (n_H) were not significantly different from unity.

H_1 Receptor Binding *in Vivo*. Experiments were performed according to Arrang et al.[10] The number of mice for determination of total and nonspecific binding was 10 and 9, respectively.

H_2 Receptor Assay on the Spontaneously Beating Guinea Pig Right Atrium. The right atrium was set up isometrically under a resting force of 5 mN similar to the method described by Black et al.[37] (*R,S*)-Metoprolol (300 nM) was present in the organ bath throughout the experiment. For the analysis of potential H_2 receptor agonism, the compounds were added cumulatively (1–2000 μM). For **49**, a pK_B estimate for cimetidine was calculated from the decrease of heart rate observed after injection of cimetidine (3 μM) into the maximum.

H_3 Receptor Assay on the Guinea Pig Ileum (Longitudinal Muscle with Adhering Myenteric Plexus). Experiments were performed according to Schlicker et al.[38]

M_3 Receptor Assay on the Guinea Pig Ileum. The assay was performed as described for the H_1 receptor assay on the ileum in the absence of atropine, using carbachol (0.003–10 μM) as the agonist. The initial H_1-mediated contraction observed for **35** and **39** was allowed to return to base line before application of carbachol.

β_1 Receptor Assay on the Guinea Pig Atrium. The assay was performed as described for the H_2 receptor assay in the absence of (*R,S*)-metoprolol, using (*R,S*)-isoprenaline (0.1–100 nM) as the agonist.

α_1 and 5HT$_{2A}$ Receptor Assay on the Rat Aorta and Tail Artery, Respectively. Experiments were carried out as described by Pertz and Eich[39] in the presence of mepyramine (α_1, 0.3 μM; 5HT$_{2A}$, 0.2 μM).

Acknowledgment. This project is supported by the Verband der Chemischen Industrie, Fonds der Chemie (Frankfurt/Main, Germany). The authors acknowledge the contributions of Hannelore Lambrecht and Inge Walther to the pharmacological experiments and the advisory comments of Richard Dodel and Ulrich Borchard (University of Düsseldorf, Germany). Dijana Topic and Thomas Rudolf are thanked for their skillful technical assistance.

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JM940784P