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N^{α}-ALKYLATED DERIVATIVES OF 2-PHENYLHISTAMINES: SYNTHESIS AND *IN VITRO* ACTIVITY OF POTENT HISTAMINE H_1-RECEPTOR AGONISTS

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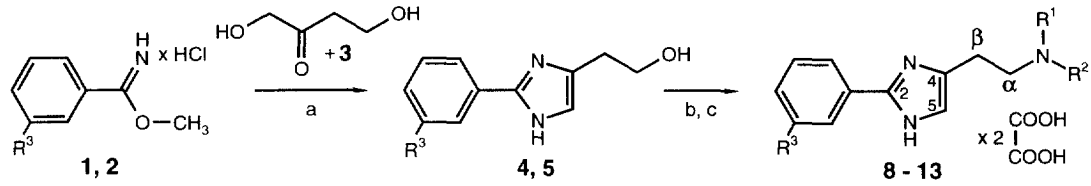
Abstract. New potent N^{α}-alkylated histamine H_1-receptor agonists have been prepared and functionally evaluated for partial agonist potency and selectivity. N^{α}-Methyl-2-(3-trifluoromethylphenyl)histamine contracts ileal segments and aortic rings of guinea-pig with a relative potency of 174% (95% confid. lim. 161 - 188%) and 217% (164 - 287%), respectively (histamine: 100%) and is the most potent H_1 receptor agonist described so far.

During the last two decades numerous histamine H_1-receptor mediated effects have been described[1] which emphasize the important physiological and pathophysiological role of histamine. For example, histamine acting through H_1-receptors is effective as an endogenous anticonvulsant,[2] increases wakefulness,[3] inhibits appetite,[4] modulates pain,[5,6] and regulates intestinal transit.[7] Additionally, H_1-receptor stimulatory properties of histamine are responsible for cardiovascular parameters such as blood pressure,[8] inotropic effects,[9] and occurrence of coronary spasms.[10] Research in the above-mentioned fields has always been hampered by the lack of highly potent and subtype-selective H_1-receptor agonists. Since the discovery of the class of 2-substituted histamines[11] by *Dziuron* and *Schunack* in 1975,[12] several derivatives with improved potency and selectivity, predominantly *meta*-substituted 2-phenylhistamine derivatives have been synthesized.[13,14] The most prominent members of this series, 2-(3-chlorophenyl)histamine, 2-(3-trifluoromethylphenyl)histamine (**6**) and 2-(3-bromophenyl)histamine (**7**) are full agonists and at least equipotent with histamine in the guinea-pig ileum assay (96%, 128%, and 112% relative potency *vs.* 100% for histamine)[14] whereas they display partial agonist activity in the isolated guinea-pig aorta and other preparations.[13-16] Guided by the finding of *Hepp* and *Schunack*[17] that in a series of N^{α}-substituted 2-methylhistamines, *di-* and *mono*-methylated congeners maintained H_1-receptor agonist potency or even possessed threefold activity compared with the primary amine, we intended to synthesize a new series of secondary and tertiary amines derived from **6** and **7**. The aim of the present study was to develop more potent analogues in order to acquire additional information about structure-activity relationships of histamine-type H_1-receptor agonists.

Scheme 1 outlines the general synthetic approach used to prepare N^{α}-substituted 2-phenylhistamines. Cyclization of benzimidates **1** and **2** with 2-oxobutane-1,4-diol (**3**) in liquid ammonia afforded imidazole ethanols **4** and **5**. Imidates **1** and **2** were obtained by acid-catalyzed addition of methanol to the respective benzonitriles.[18] Diol **3** was prepared by mercury(II)-catalyzed addition of water to butine-1,4-diol.[19] Imidazole ethanols **4** and **5** were converted to chloroethanes which yielded N^{α}-substituted 2-phenylhistamines **8** - **13** upon treatment with excess amine. The target compounds were characterized as dihydrogen oxalates (Table 1). The synthesis of primary amines **6** and **7** has been reported elsewhere.[14]

Scheme 1. Synthesis of N^{α}-substituted 2-phenylhistamines **8 - 13**.



Experimental conditions: (a) liq. NH_3 (6 - 8 bar, ambient temp., 12 h; then 25 - 27 bar, 60 °C, 6 h), column chromatography (silica gel, CH_2Cl_2/MeOH/triethylamine 90+9+1), yield 77% (R^3 = CF_3), 16% (R^3 = Br); (b) $SOCl_2$, Δ, 4 h, column chromatography (silica gel, CH_2Cl_2/MeOH 9+1), yield 36% (R^3 = CF_3), 74% (R^3 = Br); (c) excess NHR^1R^2, aqu. EtOH (90/10), KI, K_2CO_3, Δ, 4 - 6 h, rotatory chromatography (silica gel containing gypsum, CH_2Cl_2/MeOH 9+1, NH_3-saturated), excess oxalic acid (crystallization from EtOH/Et_2O). For yields and mp see Table 1.

Table 1. Physico-chemical data of dihydrogen oxalates of substituted histamine derivatives **8 - 13**.[a]

compound	R^1	R^2	R^3	mp [°C] (decomp.)	yield [%]	formula	mol. mass	*m/z* $[M+H^+]$
8	CH_3	H	CF_3	202 - 204	50	$C_{13}H_{14}F_3N_3 \cdot 2\ C_2H_2O_4$	449.3	270
9	CH_3	CH_3	CF_3	204 - 206	47	$C_{14}H_{16}F_3N_3 \cdot 2\ C_2H_2O_4$	451.4	284
10	C_2H_5	H	CF_3	223 - 225	52	$C_{14}H_{16}F_3N_3 \cdot 2\ C_2H_2O_4$	451.4	284
11	c-C_3H_5[b]	H	CF_3	200 - 201	50	$C_{15}H_{16}F_3N_3 \cdot 2\ C_2H_2O_4$	475.4	296
12	CH_3	H	Br	206 - 207	19	$C_{12}H_{14}BrN_3 \cdot 2\ C_2H_2O_4$	460.2	280, 282
13	CH_3	CH_3	Br	200 - 204	16	$C_{13}H_{16}BrN_3 \cdot 2\ C_2H_2O_4$	474.3	294, 296

[a] For structure see Scheme 1. All compounds gave satisfactory elemental analyses (C, H, N within ±0.4% of theoretical values), IR (KBr), $^+$FAB (Xe, DMSO/glycerol) and ^{1}H-NMR spectra (see note 21 for **8** and **12**). [b] Cyclopropyl.

Phenylhistamines **6** - **13** were studied functionally for interaction with H_1-receptors of guinea-pig ileum and aorta (Table 2 and 3), and with H_2-, H_3- and cholinergic M_3-receptors of guinea-pig, respectively (Table 3). The most potent derivative (**8**) was also examined with regard to selectivity *vs.* other neurotransmitter receptors in functional assays (Figure 1). All histamine analogues exhibited partial H_1-receptor agonism in the guinea-pig ileum preparation. Vasoconstrictory properties were observed when the more potent compounds (**6** - **9**, **12**, **13**) were studied on the endothelium denuded guinea-pig aorta (Figure 2). All contractile effects were antagonized by nanomolar concentrations of mepyramine, a selective and competitive H_1-receptor antagonist. The fact that for all agonists studied, pA_2 values for mepyramine close to 9.0 were calculated in both assays, conclusively shows that a pure H_1-receptor-mediated mechanism is involved. Furthermore, the concentration-effect curve of histamine was shifted to the right in a concentration-dependent manner, when partial agonists were incubated as competitors for histamine at the H_1-receptor site (Figure 3). This dextral shift allowed the calculation of micromolar affinity values (pK_P) for **6** - **13** in the ileum assay due to the existence of a high amount of spare receptors in this preparation.[20] In radioligand binding studies, similar pK_i values for **6** and **7** have been determined in guinea-pig cerebellar membranes (5.92 and 6.10).[14] The lower pEC_{50} value for the full agonist histamine (5.92 *vs.* 6.70) as well as the diminished relative maximum effects of partial agonists in the aorta preparation compared with the ileum model reflect the less efficient coupling of the aortic H_1-receptor to intracellular signal transduction.

Table 2. Interaction of compounds **6** - **13** with histamine H_1-receptors of guinea-pig ileum.[a]

				agonism[b]			affinity[c]	mepyramine antagonism[d]	
no.	R^1	R^2	R^3	$E_{max} \pm SE$	rel. pot. (95% c. l.)		$pK_P \pm SE$[e]	$pA_2 \pm SE$	$E_{max} \pm SE$[f]
histamine				100	100	-	-	9.07 ± 0.03[g]	100
6[h]	H	H	CF_3	100	128	(120 - 136)[i]	6.23 ± 0.07	9.05 ± 0.05[g]	97/80/65[j]
7[h]	H	H	Br	100	112	(95 - 133)	5.97 ± 0.07	9.13 ± 0.03[g]	97 - 61[k]
8	CH_3	H	CF_3	97 ± 1	174	(161 - 188)[i]	6.47 ± 0.04	9.04 ± 0.06	77 ± 2
9	CH_3	CH_3	CF_3	88 ± 2	95	(80 - 111)	6.39 ± 0.05	8.92 ± 0.08	67 ± 3
10	C_2H_5	H	CF_3	63 ± 3	30	(26 - 35)	5.68 ± 0.05	8.98 ± 0.11	40 ± 7
11	c-C_3H_5[l]	H	CF_3	36 ± 2	28	(22 - 35)	6.12 ± 0.03	9.13 ± 0.11	20 ± 2
12	CH_3	H	Br	95 ± 1	109	(92 - 128)	6.17 ± 0.08	9.13 ± 0.09	71 ± 6
13	CH_3	CH_3	Br	81 ± 3	87	(60 - 126)	6.20 ± 0.07	9.15 ± 0.18	58 ± 4

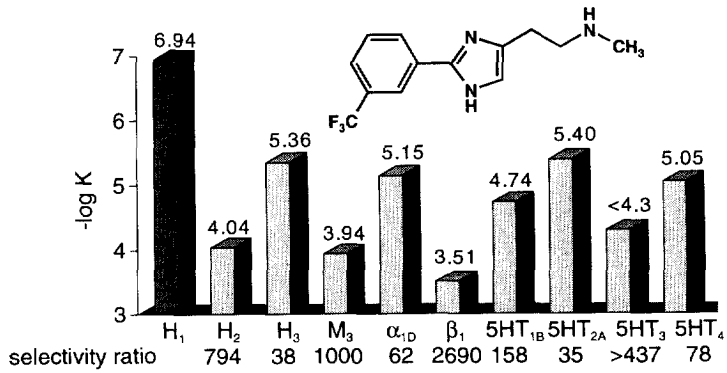
[a] Experimental conditions: whole segments (1.5 cm), preload 5 mN, isotonic registration, *Tyrode* solution with 0.1 µM atropine (37 °C), 3 priming doses of histamine (1 + 10 µM), first cumulative curve for histamine (0.01 - 30 µM), 10 min after washout second curve for partial agonist, finally third curve for histamine in the presence of partial agonist (determination of pK_P value). [b] n = 7 - 21. [c] n = 6 - 9 (for **8** see Figure 3). Partial agonist conc. was 30 µM (**6**, **7**, **11**) or 100 µM (**9**, **10**, **12**, **13**). Incubation time 10 min except for **10** (2 min) and **11** (1 min) to avoid a complete breakdown of the contractility of the preparations. [d] Incubation time 10 min. Mepyramine conc. 1 (**10**, **11**, n = 4), 3 (**9**, **12**, **13**, n = 4), 10 (**8**, n = 5), 0.3 - 100 (histamine , n = 29), 1 - 100 (**6**, n = 21), and 1 - 300 nM (**7**, n = 24). [e] Negative logarithm of the dissociation constant of the receptor/partial agonist complex.[20] [f] E_{max} of (partial) agonist in the presence of mepyramine. [g] pA_2 from *Schild* plot[22] constrained to unity. [h] Data for agonism from ref. 14. [i] More potent than histamine ($P < 0.001$). [j] For 1, 10, and 100 nM mepyramine. [k] For 1 - 300 nM mepyramine. [l] Cyclopropyl.

Table 3. Interaction of compounds **6** - **13** with histamine (H_1 - H_3) and M_3-acetylcholine receptors of guinea-pig.

	H_1 (aorta)[a]					H_2[b]	H_3[c]	M_3[d]
	agonism[e]			mepyramine antagonism[f]		(atrium)	(ileum)	(ileum)
no.	$E_{max} \pm SE$	rel. pot. (95% c. l.)		$pA_2 \pm SE$	$E_{max} \pm SE$[g]	pD'_2[h]	pA_2	pA_2
hist.	100	100	-	9.11 ± 0.02[i]	100	-	-	n. d.
6[j]	80 ± 3	94	(77 - 114)	9.01 ± 0.04[i]		3.48	5.21	3.97
7[j]	64 ± 1	48	(41 - 56)	8.90 ± 0.04		3.37	5.20	3.81
8	59 ± 4	217	(164 - 287)[k]	9.08 ± 0.14	66 ± 3	4.04	5.36	3.94
9	54 ± 5	169	(130 - 221)[k]	8.85 ± 0.04	64 ± 4	4.56	5.95	4.57
10	n. d.	n. d.		n. d.	n. d.	4.55	5.63	4.16
11	n. d.	n. d.		n. d.	n. d.	4.36	n. d.	n. d.
12	76 ± 2	43	(26 - 70)	9.01 ± 0.03	75 ± 6	3.86[l]	4.93	3.95
13	58 ± 4	31	(23 - 43)	8.94 ± 0.16	49 ± 6	4.74	5.84	4.19

n. d. not determined. [a] Conditions:[14] Endothelium-denuded aortic segments (3 - 4 mm), initial tension 10 mN, isometric registration, modified *Krebs* solution (37 °C), 3 priming doses of histamine (10 µM), first cumulative curve for histamine (0.1 - 300 µM), 30 min after washout second curve for partial agonist in the absence or presence of mepyramine. [b] See ref. 23 (n = 2 - 3, range ±0.05 - ±0.23, 20 - 300 µM in the presence of 0.3 µM mepyramine). [c] See ref. 24 (n = 5 - 6, *SE* 0.04 - 0.12, 15.8 - 50 µM in the presence of 2 µM mepyramine). [d] See ref. 25 (n = 4 - 8, *SE* 0.05 - 0.15, 30 - 100 µM in the presence of 1 µM mepyramine). [e] n = 7 - 19. [f] Incubation time 30 min. Mepyramine conc. 1 - 1000 (histamine, n = 34), 2 - 50 (**6**, n = 12), 3 (**7**, n = 8), and 5 nM (**8**, **9**, **12**, **13**, n = 4). [g] E_{max} of (partial) agonist in the presence of mepyramine. For **6**, **7** see ref. 14. [h] For pD'_2 see ref. 26. [i] pA_2 from *Schild* plot[22] constrained to unity. [j] Data for H_1, H_2, and M_3 activity from ref. 14. [k] More potent than histamine ($P < 0.001$ (**8**) and $P < 0.01$ (**9**)). [l] Transient positive inotropic and chronotropic effect (EC_{50} *ca.* 10 µM, E_{max} = 27 - 39% of histamine) but not sensitive to cimetidine (30 µM).

Figure 1. Functional *in-vitro* selectivity of **8** was determined on guinea-pig right atrium (H_2, β_1),[23] ileum (H_3,[24] M_3,[25] 5-HT_3[27]), and iliac artery (5-HT_{1B}),[28] and on rat aorta (α_{1D}),[25] tail artery (5-HT_{2A}),[25] and oesophagus (5-HT_4),[27] respectively. Compound **8** displayed no agonist effects at all except on histamine H_1-receptors. Similar antagonist activity patterns have been obtained for **6** and **7**.[14]



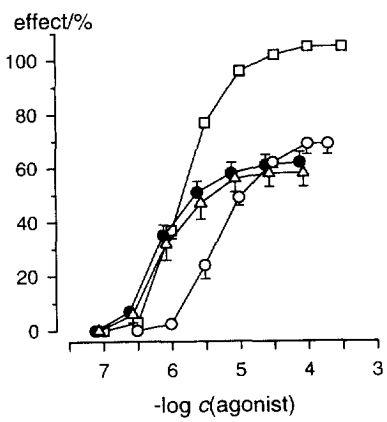


Figure 2. Partial H_1-receptor agonist activity of **8** (●, $n = 7$, $E_{max} = 62 \pm 4$ % of first histamine curve) and **9** (Δ, $n = 8$, 58 ± 5 %) on the endothelium-denuded guinea-pig aorta, compared with histamine controls (□, $n = 8$, 105 ± 1 %). Only second curves are shown. The contractile effect of **8** was antagonized by 5 nM mepyramine (○, $n = 4$, 69 ± 4 %) yielding a pA_2 of 9.08 ± 0.14. For further data see Table 3. Error bars indicate *SE* of the arithmetic mean.

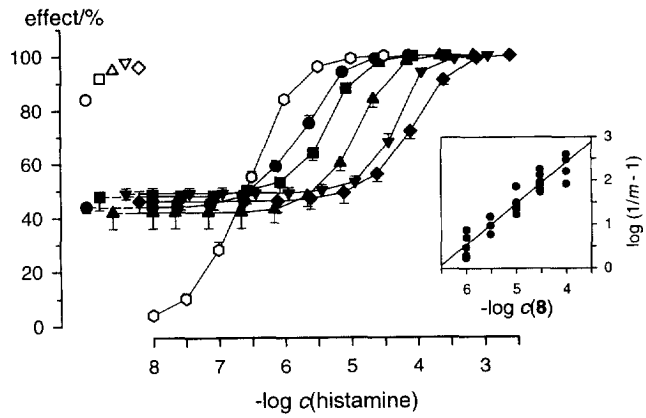


Figure 3. Determination of H_1-receptor affinity of **8** on the guinea-pig ileum. Contractions elicited by 1, 3, 10, 30, and 100 μM **8** (84 ± 3, 92 ± 1, 95 ± 2, 98 ± 1, 96 ± 1 %, open symbols) faded to 44 ± 2, 48 ± 3, 42 ± 6, 49 ± 2, 46 ± 4 % (closed symbols) within 10 min. Afterwards, without washing, histamine concentration-effect curves in the presence of 1 (●, $n = 5$), 3 (■, $n = 4$), 10 (▲, $n = 5$), 30 (▼, $n = 10$), and 100 μM **8** (◆, $n = 5$) were shifted to the right compared with the first histamine curve (open hexagons). All curves were normalized to 100% histamine response. *Inset: Kaumann-Marano* plot for the calculation of partial agonist affinity (pK_P).[29] Data were approximated by a straight line of unity slope ($m = 0.94 \pm 0.06$, not significantly different from 1 ($P > 0.20$)) and yielded $pK_P = 6.47 \pm 0.04$ (95% confidence limits 6.39 - 6.55, $n = 29$).

Considering the results of both H_1-receptor assays allows to conclude that the *meta*-trifluoromethyl substitution is more favourable for agonist potency than *meta*-bromo (Table 2 and 3). This fact is also reflected by the higher affinity values measured on the ileum (pK_P 6.23 - 6.47 *vs.* 5.97 - 6.20, Table 2). While brominated

phenylhistamines **7**, **12**, and **13** are equipotent with histamine on the ileum, their counterparts in the trifluoromethyl series exceed histamine in potency, except **9** on the ileum, and **6** on the aorta preparation, respectively. With regard to the degree of amine nitrogen substitution, the rank order of potency is *secondary* > *tertiary* > *primary* amine in either assay for trifluoromethyl derivatives **6**, **8**, and **9** while brominated agonists **7**, **12**, and **13** are equipotent in both *in-vitro* systems. Potency and intrinsic activity of the secondary amine **8** are attenuated when the methyl substituent is replaced by more space-demanding groups such as ethyl (**10**) or cyclopropyl (**11**), indicating that the fit of bulkier side-chain residues may be sterically hindered. This trend is in agreement with data for N^{α}-substituted histamines.[17] As far as intrinsic activity (E_{max}) is concerned, CF_3- and Br-substituted derivatives display the same rank order (*primary* > *secondary* > *tertiary* amine) on the ileal preparation while their rank orders differ in the vascular system.

The search for potent and selective histamine H_1-receptor agonists has been a quite fruitless task for several decades. With the advent of 2-phenylhistamines[12-14] compounds have been devised that are nearly *as potent as* or at least *equipotent with* the biogenic amine, *vic.* **6**.[14] The present *in-vitro* study conclusively demonstrates that the secondary amine **8** (N^{α}-methyl-2-(3-trifluoromethylphenyl)histamine) is the most potent histamine H_1-receptor agonist reported so far in the literature. The H_1-receptor agonist potency of **8** is about 200% compared with histamine while the compound is devoid of agonist properties on several neurotransmitter receptor subtypes including histamine H_2- and H_3-receptors. The antagonist activity at these sites is negligible, or moderate in some cases (Figure 1). Therefore, **8** may become an attractive tool for research into physiology and pathophysiology of histamine H_1-receptor-mediated functions.

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21. ^{1}H-NMR (DMSO-d_6) for compound **8** (salt): δ [ppm] 8.79 (broad, exchangeable with D_2O, 2 H, NH_2^+), 8.26 (s, 1 H, ph-2-H), 8.22 (dd, ^3J = 4 Hz, 1 H, ph-5-H), 7.69 (2 d, ^3J = 4 Hz, 2 H, ph-4-H, ph-6-H), 7.14 (s, 1 H, imi-5-H), 3.21 (t, ^3J = 7.5 Hz, 2 H, $CH_2CH_2NH_2^+CH_3$), 2.91 (t, ^3J = 7.5 Hz, 2 H, $CH_2CH_2NH_2^+CH_3$), 2.62 (s, 3 H, CH_3); for compound **12** (salt): δ [ppm] 8.81 (broad, exchangeable with D_2O, 2 H, NH_2^+), 8.18 (s, 1 H, ph-2-H), 7.99 (d, ^3J = 8 Hz, 1 H, ph-6-H), 7.59 (d, ^3J = 8 Hz, 1 H, 1 ph-4-H), 7.47 (dd, ^3J = 8 Hz, 1 H, ph-5-H), 7.17 (s, 1 H, imi-5-H), 3.27 (t, ^3J = 7.4 Hz, 2 H, $CH_2CH_2NH_2^+CH_3$), 2.96 (t, ^3J = 7.4 Hz, 2 H, $CH_2CH_2NH_2^+CH_3$), 2.69 (s, 3 H, CH_3).
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