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Original article

Ring-substituted histaprodifen analogues as partial agonists for histamine H_1 receptors: synthesis and structure–activity relationships[#]

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Abstract – Thirteen racemic benzene ring-substituted analogues of histaprodifen (**8a**; 2-[2-(3,3-diphenylpropyl)-1*H*-imidazol-4-yl]ethanamine), a novel lead for potent and selective histamine H_1-receptor agonists, have been prepared from substituted 4,4-diphenylbutyronitriles **5** via cyclization of the corresponding methyl butyrimidates **6** with 2-oxo-4-phthalimido-1-butyl acetate in liquid ammonia, followed by deprotection. Nitriles **5** were accessible by alkylation of either substituted diphenylmethanes with 3-bromopropionitrile or diethyl malonate with substituted 1-chloro-diphenylmethanes and subsequent standard reactions. The title compounds **8** displayed partial agonism on contractile H_1 receptors of the guinea-pig ileum (E_{max} = 2–98% relative to histamine) and, compared with the endogenous agonist, were endowed with agonist potencies of 4–92%. The *meta* fluorinated (**8c**) and *meta* chlorinated (**8f**) analogues showed the highest relative potency in this series (95% confidence limits 85–99% and 78–102%), but did not exceed the value of the lead **8a** (99–124%). Compound **8c** (2-[2-[3-(3-fluorophenyl)-3-phenylpropyl]-1*H*-imidazol-4-yl]ethanamine) was a partial agonist at contractile H_1 receptors of the guinea-pig aorta (relative potency 154% vs. 100% for histamine) and at relaxation-mediating endothelial H_1 receptors of the rat aorta (relative potency 556% vs. 100% for histamine) and matched with the functional behaviour of **8a**. Agonism observed for each compound was sensitive to blockade by the selective H_1-receptor antagonist mepyramine ($pA_2 \approx 9$ (guinea-pig) and $pA_2 \approx 8$ (rat aorta)). All histaprodifen analogues **8** stimulated neither histaminergic H_2/H_3 nor cholinergic M_3 receptors. They displayed only low to moderate affinity for these sites (H_2: $pD'_2 < 5$; H_3/M_3: $pA_2 < 6$). With regard to the substitution pattern on the benzene ring, there was no correlation between the histaprodifen series and the corresponding derivatives of another selective H_1-receptor agonist, viz. 2-phenylhistamine. © 2000 Éditions scientifiques et médicales Elsevier SAS

guinea-pig aortic H_1 receptor / H_1-receptor agonists / histamine H_1 receptor / histaprodifen / rat aortic H_1 receptor

1. Introduction

The important role of the biogenic amine histamine (*figure 1*) in causing a large number of physiological and pathophysiological effects via interaction with histamine H_1, H_2 and H_3 receptors has been well established [2]. Over the years highly potent and selective antagonists for the three histamine receptor subtypes have become available, as well as highly potent agonist ligands for H_2 and H_3 receptors [2]. By contrast, efforts of several groups to produce selective H_1-receptor agonists endowed with potency superior to histamine have failed for a long time [3–5]. The first selective compounds, which drew even with histamine, became available when the aromatic portion of 2-phenylhistamine (*figure 1*), a moderately active but selective H_1-receptor agonist [6, 7], was substituted systematically in the *meta* position, preferentially by halogen or halogen-containing groups [7, 8]. The relative potency of the most prominent member of this series of primary amines, 2-(3-trifluoromethylphenyl)-histamine (*figure 1*) [7], was recently improved by N^{α}-methylation of the side-chain nitrogen, leading to the secondary amine N^{α}-methyl-2-(3-trifluoromethylphenyl)-histamine (*figure 1*) which acts as a full H_1-receptor agonist on the guinea-pig ileum and displays a relative potency of 174% compared with histamine [9].

A structurally divergent family of H_1-receptor agonists have been discovered in the late nineties after replacing the 2-phenyl substituent of the imidazole nucleus by a

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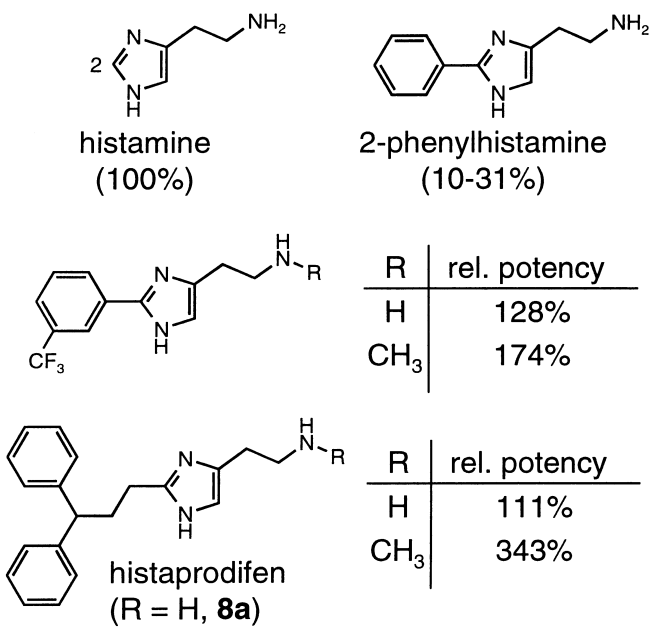

Figure 1. Structure of histamine and prominent H_1-receptor agonists.

3,3-diphenylpropyl moiety [10, 11]. Prototypic members of this series are histaprodifen and its N^{α}-methylated derivative (*figure 1*) which possess 101–528% and 343–2 825% relative potency, respectively, in several functional H_1-receptor assays [11]. The aim of the present study was to evaluate whether structure–activity relationships of substituted histaprodifen derivatives are congruent with those of the 2-phenylhistamine familiy, as far as the substitution pattern of the benzene rings is concerned. Provided that both series were in agreement with each other, this would allow histaprodifen analogues with enhanced H_1-receptor agonist potency to be obtained. For the sake of a more convenient synthetic access to a larger series of compounds, only racemic primary amines, i.e., analogues of histaprodifen, were synthesized and evaluated in functional in vitro receptor assays.

2. Chemistry

Ring-substituted congeners **8b**–**o** of histaprodifen (**8a**) were synthesized by cyclocondensation of a substituted α-hydroxyketone with the appropriate methyl imidates **6b**–**o** in liquid ammonia under pressure [12] (*figure 2*). The nitriles **5b**–**o** required for the acid-catalysed formation of the methyl imidates **6b**–**o** were either prepared by direct alkylation of a potassium amide/liquid ammonia-solution of diphenylmethanes **2b**, **h**, **i** and **n** with 3-bromopropionitrile (method A), or by alkylation of diethyl malonate with substituted chlorodiphenylmethanes **3c**–**g** and **j**–**m**. The alkylated diethyl malonates were decarboxylated, subsequently reduced and chlorinated to give the 3,3-diphenyl-1-chloropropanes **4c**–**g** and **j**–**m**, which afforded the desired nitriles by Kolbe nitrile synthesis (method B). Compound **4o** was prepared from commercially available 3,3-di-(4-fluorophenyl)propanoic acid by reduction to the corresponding propanol with lithium aluminum hydride, followed by chlorination with thionylchloride. The addition of dry methanol to the nitriles **5b**–**o** led to the methyl imidates **6b**–**o** upon treatment with an excess of thionylchloride. The synthesis of the substituted, side-chain protected histaprodifen derivatives **7b**–**o** was performed by cyclocondensation of **6b**–**o** in liquid ammonia with 2-oxo-4-phthalimido-1-butyl acetate which was available in four steps from butin-1,4-diol [8]. After deprotection by acidic hydrolysis or hydrazinolysis, the target compounds **8b**–**o** were purified by chromatography and crystallized as dihydrogen maleates (*table I*). Histaprodifen (**8a**) was available according to method A using commercially available diphenylmethane (**2a**) [11].

3. Pharmacology

Histaprodifen (**8a**) and the new histaprodifen analogues **8b**–**o** were routinely screened on the guinea-pig ileum preparation to characterize functional H_1-receptor potency and the relative maximum effect (formerly termed 'intrinsic activity' [13]), compared with the standard agonist histamine. Direct or indirect muscarinic effects on the ileal muscle were excluded by the presence of atropine (100 nM) during all experiments. For compounds which produced contraction under these conditions, the susceptibility of the contractile effect to blockade by the competitive H_1-receptor antagonist mepyramine was verified. Histaprodifen (**8a**) itself has been shown to produce a concentration-dependent rightward shift of the histamine concentration–effect curve, when the compound is incubated for a limited period, just like a partial agonist [11]. From this experiment the affinity of **8a** was estimated as pK_P which is defined as the negative decadic logarithm of the dissociation constant of a partial-agonist/receptor complex [14, 15]. Similar experiments with **8b**–**o** allowed the estimation of the affinity for the majority of the new compounds. Compound **8c**, which appeared to be the most interesting analogue of the study, was additionally characterized by two other assays predictive of H_1-receptor agonism, viz. the contraction of guinea-pig aorta [11, 16] and the

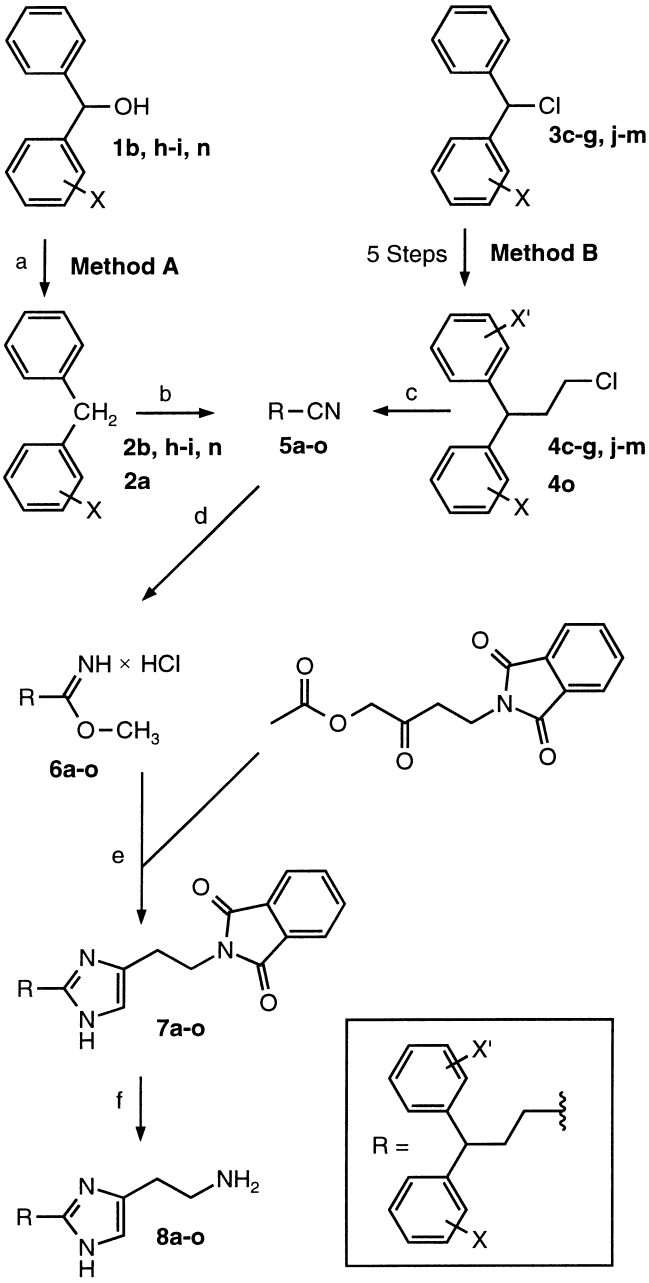

Figure 2. Synthesis of histaprodifen (**8a**) and substituted analogues **8b–o**: (a) $LiAlH_4$, $AlCl_3$, Et_2O, Δ; (b) BrC_2H_4CN, THF/Et_2O, KNH_2, liq. NH_3; (c) KCN, KI, DMSO, Δ; (d) MeOH, $SOCl_2$, –30 °C; (e) liq. NH_3, 60 °C; (f) 6 N HCl, Δ, or N_2H_4, MeOH. The 5 steps leading from **3c–g** and **j–m** to **4c–g**, **j–m** and **o** are described in detail in the Experimental protocols (Method B). For X and X′, see *tables I* and *II*.

NO-mediated relaxation of precontracted rat aorta [11, 17]. Finally, a functional selectivity profile vis-à-vis to H_2, H_3 and M_3 receptors was determined for some of the compounds. In the case of **8c**, this profiling was extended to other monoaminergic receptor interactions.

4. Results and discussion

4.1. In vitro H_1-receptor pharmacology

In the presence of the muscarinic receptor antagonist atropine (100 nM), histaprodifen analogues **8b–o** displayed partial agonism on the H_1 receptor of the guinea-pig ileum reaching maximum effects in the range of 2–98% relative to histamine (*table II*). Compared with histamine (relative potency 100%) and histaprodifen (**8a**, 111% [11]), the potencies of the new agonists were lower (4–52%) or equivalent in the case of the *meta* fluoro (**8c**, 92%, *figure 3*, upper panel) and the *meta* chloro (**8f**, 89%) analogue. The concentration–effect curves of all partial agonists, which reached more than 30% of the maximal histamine contraction, were shifted to the right by nanomolar concentrations of the competitive H_1-receptor antagonist mepyramine and allowed the calculation of nanomolar affinities for this antagonist (pA_2 range 8.85–9.37, *table III*), which is in good agreement with literature data [2]. The contractions evoked by the histaprodifen analogues, either in the absence or particularly in the presence of mepyramine, developed less rapidly compared with histamine, which may be indicative of slower binding kinetics. The contractile effect of partial agonists which produced less than 30% contraction (**8j**, **m** and **o**) was abolished by 100 nM mepyramine. Therefore, the ileal contraction evoked by the title compounds in the presence of 100 nM atropine was specifically mediated by H_1 receptors. Earlier studies on H_1-receptor agonists of the 2-phenylhistamine [9] and the histaprodifen series [11] have presented evidence that a reasonable affinity estimate, viz. the pK_P value [14, 15], may be deduced from functional experiments on the guinea-pig ileum by construction of a second histamine curve in the presence of the other agonist. This was even possible for 'full' agonists of both series (e.g., **8a** [11]) after allowing the contraction to fade time-dependently to a lower level. Following the same experimental protocol, pK_P values could be calculated for eleven of the new histaprodifen analogues (*table II*; *figure 3*, lower panel for **8c**). These data run parallel to the rank order of agonist potency, although pK_P values are approximately 0.6 logarithmic units smaller than the corresponding pEC_{50} values (for correlation of both parameters see *figure 4*).

Table I. Preparative and analytical data of histaprodifens **8b–o**.[a]

Compound	X	X′	Formula	Molecular mass	M.p. (°C)	Method	$^{+}$FAB spectra, m/z (rel. intensity)
8b	2-F	H	$C_{20}H_{22}FN_3 \cdot 2C_4H_4O_4$	555.6	142–143	A	324 (100), 307 (10)
8c	3-F	H	$C_{20}H_{22}FN_3 \cdot 2C_4H_4O_4 \cdot 0.25H_2O$	559.1	147–149	B	324 (100), 307 (19)
8d	4-F	H	$C_{20}H_{22}FN_3 \cdot 2C_4H_4O_4$	555.6	147–149	B	324 (100), 307 (13)
8e	2-Cl	H	$C_{20}H_{22}ClN_3 \cdot 2C_4H_4O_4$	527.1	137–139	B	340 (100), 323 (12)
8f	3-Cl	H	$C_{20}H_{22}ClN_3 \cdot 2C_4H_4O_4$	527.1	143–144	B	340 (100), 323 (10)
8g	4-Cl	H	$C_{20}H_{22}ClN_3 \cdot 2C_4H_4O_4$	527.1	134–136	B	340 (100), 323 (8)
8h	2-Br	H	$C_{20}H_{22}BrN_3 \cdot 2C_4H_4O_4$	616.5	132–134	A	386 (93), 384 (100)
8i	3-Br	H	$C_{20}H_{22}BrN_3 \cdot 2C_4H_4O_4$	616.5	138–139	A	386 (94), 384 (100)
8j	4-Br	H	$C_{20}H_{22}BrN_3 \cdot 2C_4H_4O_4$	616.5	144–145	B	386 (98), 384 (100)
8k	2-CH_3	H	$C_{21}H_{25}N_3 \cdot 2C_4H_4O_4$	551.6	137–139	B	320 (100), 303 (6)
8l	3-CH_3	H	$C_{21}H_{25}N_3 \cdot 2C_4H_4O_4$	551.6	143–145	B	320 (100), 303 (11)
8m	4-CH_3	H	$C_{21}H_{25}N_3 \cdot 2C_4H_4O_4$	551.6	141–142	B	320 (100), 303 (9)
8n	3-CF_3	H	$C_{21}H_{22}F_3N_3 \cdot 2C_4H_4O_4 \cdot 0.5H_2O$	614.6	133–134	A	374 (100), 345 (8)
8o	4-F	4-F	$C_{20}H_{21}F_2N_3 \cdot 2C_4H_4O_4$	573.6	128–129	B	342 (100), 325 (11)

[a] For structure see *figure 2*. All compounds gave satisfactory analyses (C, H, N) and spectra.

Compound **8c** (‘*meta* fluorohistaprodifen’) was selected for further characterization due to its equipotency with **8a** in terms of relative potency, maximum effect, and affinity (*table II*). Compound **8c** contracted guinea-pig aortic segments in the absence of endothelium in a mepyramine-sensitive fashion (*figure 5*, upper panel). The affinity of mepyramine was also found to be in the lower nanomolar range. The guinea-pig aorta was less sensitive to H_1-receptor agonists, especially to the full agonist histamine, which confirms earlier observations [7, 9, 11]. Relative to histamine, the potency of **8c** was increased by a factor of 1.7 and matched with the potency of **8a** [11]. The attenuated relative maximum contraction evoked by **8c** typifies the partial agonist

Table II. Contraction of guinea-pig ileal whole segments by histamine and histaprodifens **8a–o** in the presence of 0.1 μM atropine.

	H_1-receptor agonism						antagonism vs. histamine			
Compound[a]	X	X′	n[b]	$pEC_{50} \pm SEM$	relative potency	95% confidence limits	$E_{max} \pm SEM$	n[b]	pK_P[c] ± SEM	c[d] [μM] (t [min])
8a [11]	H	H	34	6.74 ± 0.02	111	99–124	100	12	6.04 ± 0.05	3–30 ([e])
8b	2-F	H	10	5.66 ± 0.06	9	7–12	86 ± 2	10	4.83 ± 0.05	10–20 (3)
8c	3-F	H	13	6.66 ± 0.015	92[f]	85–99	98 ± 1[f]	10	6.22 ± 0.08	3–10 (10)
8d	4-F	H	6	6.33 ± 0.10	43	23–79	85 ± 3	6	5.58 ± 0.11	10 (5)
8e	2-Cl	H	6	6.18 ± 0.06	30	22–42	89 ± 2	–	n.d.	–
8f	3-Cl	H	10	6.65 ± 0.03	89[g]	78–102	93 ± 1[h]	–	n.d.	–
8g	4-Cl	H	8	5.72 ± 0.05	10	8–14	58 ± 4	4	5.28 ± 0.04	5 (0.5)
8h	2-Br	H	11	6.31 ± 0.05	41	32–52	86 ± 2	5	5.37 ± 0.10	10 (10)
8i	3-Br	H	8	6.42 ± 0.03	52	43–62	87 ± 2	7	5.52 ± 0.07	30 (3)
8j	4-Br	H	9	5.61 ± 0.09	8	5–13	12 ± 2	9	5.43 ± 0.02	20–30 (0.5)
8k	2-CH_3	H	8	6.25 ± 0.04	35	28–44	82 ± 5	22	5.73 ± 0.04[i]	1–30 (5)
8l	3-CH_3	H	6	5.99 ± 0.06	20	14–27	88 ± 3	6	5.33 ± 0.07	10 (10)
8m	4-CH_3	H	18	n.d.	n.d.	–	2 ± 1	10	5.67 ± 0.05	10–30 (0.5)
8n	3-CF_3	H	6	5.27 ± 0.05	4	3–5	62 ± 4	5	4.88 ± 0.12	20 (0.5)
8o	F	F	3	5.52 ± 0.09	7	3–17	23 ± 9	–	n.d.	–
histamine	–	–	> 100	6.70 ± 0.02	100	–	100	–	–	–

[a] For structure see *figure 2*. [b] Number of experiments. [c] Negative logarithm of the partial-agonist/receptor dissociation constant K_P [14, 15]. [d] Concentration of partial agonist. Incubation time given in parentheses. [e] Incubation time 10 min (3 and 10 μM) or 3 min (30 μM). [f] Significantly different from 100 ($P < 0.05$). [g] Not significantly different from 100 ($P > 0.05$). [h] Significantly different from 100 ($P < 0.001$). [i] Calculated from a Kaumann-Marano plot [15], with slope constrained to unity (0.99 ± 0.07, $P > 0.50$). n.d. not determined.

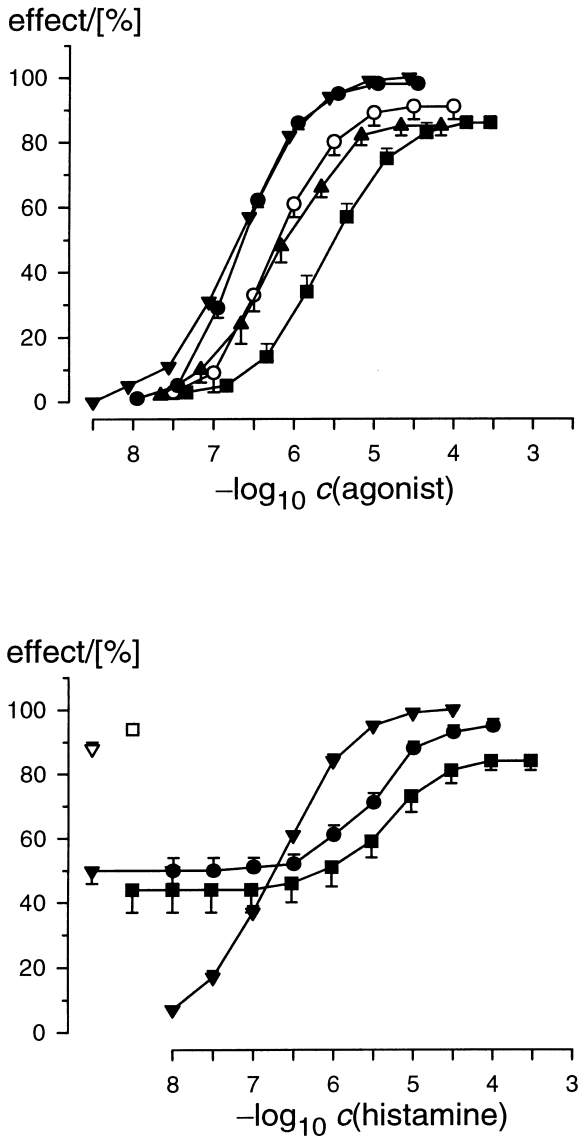

Figure 3. Upper panel: contraction of guinea-pig ileum induced by histamine (▼, $n > 10$), *meta* fluorohistaprodifen (**8c**) in the absence (●, $n = 13$, $E_{max} = 98 \pm 1\%$) and presence (○, $n = 3$, $91 \pm 4\%$) of the competitive H_1-receptor antagonist mepyramine (1 nM, 15 min, $pA_2 = 9.15 \pm 0.03$), **8d** (▲, $n = 6$, $85 \pm 3\%$), and **8b** (■, $n = 10$, $86 \pm 2\%$) in the absence of mepyramine. Lower panel: contraction of guinea-pig ileal segments induced by histamine in the absence (▼, $n = 10$) and presence of **8c** (●, 3 μM, $95 \pm 2\%$, $n = 5$; and ■, 10 μM, $84 \pm 3\%$, $n = 5$). Open symbols represent the maximum contraction elicited by **8c** ($88 \pm 2\%$ (▽) and $94 \pm 1\%$ (□)) which faded to $50 \pm 4\%$ (▼) and $44 \pm 7\%$ (■) during an equilibration period of 10 min. For **8c** the affinity parameter pK_P [14, 15] was calculated as 6.22 ± 0.08 ($n = 10$).

Table III. Mepyramine antagonism of ileal contractions evoked by histamine and histaprodifens **8a–o**.

Agonist[a]	n[b]	$pA_2 \pm$ SEM	E_{max} (agonist) ± SEM	c[c] [μM]
8a [11]	17	9.11 ± 0.04[d]	see [11]	1–30
8b	3	9.11 ± 0.05	50 ± 16	1
8c	3	9.15 ± 0.03	91 ± 4	1
8d	4	9.37 ± 0.10	73 ± 3	1
			31 ± 17	3
8e	3	9.24 ± 0.04	72 ± 6	1
8f	4	8.93 ± 0.14	78 ± 6	1
			51 ± 6	3
8g	3	9.13 ± 0.10	34 ± 2	1
8h	3	8.88 ± 0.04	69 ± 8	1
8i	4	9.20 ± 0.07	79 ± 5	1
8j	3	n.d.	0	100
8k	4	8.95 ± 0.14	63 ± 8	1
8l	4	8.85 ± 0.12	47 ± 4	3
8m	–	n.d.	–	–
8n	3	9.18 ± 0.12	14 ± 5	1
8o	3	n.d.	0	100
histamine	29	9.07 ± 0.03[e]	see [11]	0.3–100

[a] For structure see *figure 2* and *table I*. [b] Number of experiments. [c] Concentration of mepyramine. [d] Schild plot slope 1 (0.90 ± 0.06, $P > 0.05$). [e] Schild plot slope 1 (0.97 ± 0.04, $P > 0.20$). n.d. not determined.

character of all histaprodifens studied so far in this preparation [11]. Interestingly, the pEC_{50} of **8c** and reported histaprodifens [11] was close to the pK_P value determined on the guinea-pig ileum (e.g., 5.80 ± 0.05 vs. 6.22 ± 0.08 for **8c**), which indicates that fractional receptor occupation and biological response run parallel in the aorta assay.

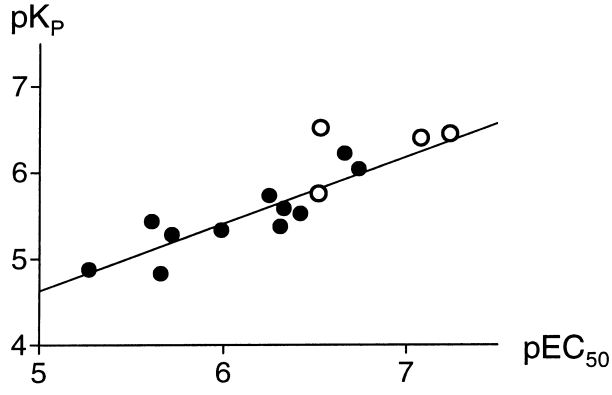

Figure 4. Correlation of agonist potency (abscissa: pEC_{50}) and receptor affinity (ordinate: pK_P value) of 15 histaprodifen analogues on the guinea-pig ileum. Data are from this study (●) or taken from [11] (○). r^2 was 0.7782 ($P < 0.001$).

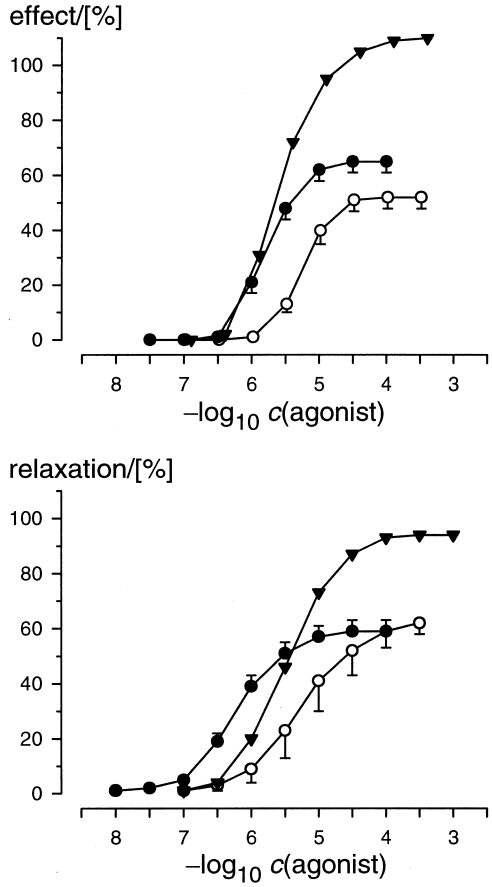

Figure 5. Upper panel: contraction of endothelium-denuded guinea-pig aortic rings by histamine (▼, n = 11, E_{max} = 110 ± 1%) and **8c** (●, n = 12, 65 ± 4%) in the absence of antagonist, and by **8c** in the presence (○, n = 5, 52 ± 4%) of mepyramine (5 nM, 30 min, pA_2 = 8.76 ± 0.07). Only second curves are shown. The maximum relative to the second histamine curve (▼) was 59 ± 3% (●) and 47 ± 4% (○). Relative potency of **8c** was 154% (126–188) compared with 100% for histamine (P < 0.001). From a Schild plot analysis [19] for mepyramine (1–1 000 nM), a pA_2 of 9.11 ± 0.02 (n = 34, slope constrained to unity) was determined using histamine as the agonist [11] (data not shown). Lower panel: Relaxation of rat aortic rings with intact endothelium relative to the relaxation induced by carbachol (0.3–1 mM). Histamine (▼, n = 6) and **8c** (●, n = 10) relaxed rings precontracted with 15.8 nM U46619 (a thromboxane A_2-receptor agonist) by 94 ± 3 and 59 ± 4%, respectively. Incubation of mepyramine (100 nM, 75 min) produced a rightward shift of the concentration–relaxation curves of histamine (n = 8, not shown) and **8c** (○, n = 4). pA_2 values for mepyramine were 8.00 ± 0.07 (vs. histamine) and 8.02 ± 0.17 (vs. **8c**). In accordance with literature [2] the affinity of mepyramine for the rat H_1 receptor is one order of magnitude lower than for H_1 receptors of other species. The relative maximum effect of **8c** was 68 ± 3%, and relative potency was 556% (449–689) compared with 100% for histamine. Only one curve was established with each preparation.

Precontracted rat aortic rings with intact endothelium were relaxed to a submaximal extent by increasing concentrations of **8c** in a mepyramine-sensitive manner. While **8c** was five times as potent as histamine, the affinity of mepyramine was reduced by one order of magnitude, which confirms literature values for mepyramine in rat tissues [2, 11] (*figure 5*, lower panel).

4.2. Structure–activity relationships with regard to H_1 receptors

Concerning the substituent position, the potency pattern of **8b**–**m** was heterogeneous. Relative agonist potencies followed the rank order *meta* > *para* > *ortho* (X = F; **8b**–**d**), *meta* > *ortho* > *para* (X = Cl; **8e**–**g**), or *ortho* ≥ *meta* > *para* (X = Br, CH_3; **8h**–**m**). Obviously, smaller electronegative atoms are favoured in the *meta* position (**8c** and **8f**), while in the *ortho* position less electronegative substituents are beneficial for optimum potency. Except for **8d**, a *para* substitution was accompanied by a prominent loss of contractile potency (E_{max} = 2–58%). The relative maximum effect decreased rather homogeneously in the order *meta* > *ortho* > *para*. The introduction of a second *para* fluoro substituent was detrimental for both agonist potency and maximum effect (**8o**). The structure–activity relationships of histaprodifens differ from those of the 2-phenylhistamine series (*figure 6*). *Meta* fluorohistaprodifen (**8c**) and its chloro analogue **8f** were equipotent with the unsubstituted lead **8a**, while *meta* halogenated 2-phenylhistamines were significantly more potent than 2-phenylhistamine [7]. Another key compound which supported this dissimilarity, was *meta* trifluoromethylhistaprodifen (**8n**) which was 32 times less potent than 2-(3-trifluoromethylphenyl)histamine [7]. With regard to the relative maximum effect, the 2-phenylhistamine analogues of *para* substituted histaprodifens **8d**, **8g** and **8m** also displayed significantly reduced values compared with the respective *meta* substituted congener.

Based on molecular dynamics simulations, binding models for histamine and unsubstituted histaprodifens have been proposed recently [11]. The cited study has revealed essential differences in the putative binding mode of histamine and **8a**. Both phenyl rings of **8a** fill out a lipophilic receptor pocket so that no space is available for substituents much larger than hydrogen. In the light of the fact that in the 2-phenylhistamine series, *meta* substituents such as iodine or bromine result in a three-fold increase of potency [7], it would appear that the orientation of 2-phenylhistamines relative to the H_1 receptor is substantially different from the position adopted by histaprodifens.

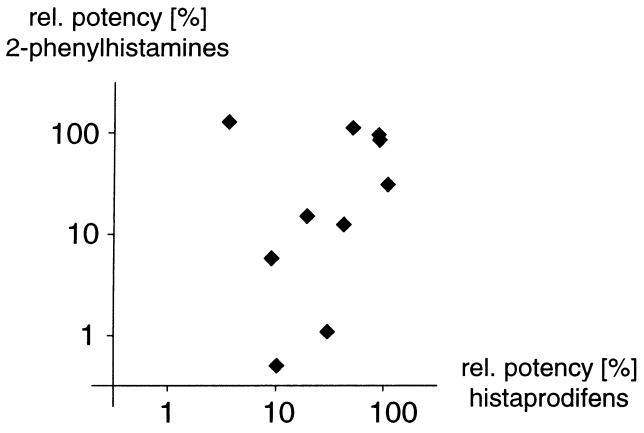

Figure 6. Plot of relative agonist potencies of 2-phenylhistamine and nine phenyl-ring mono-substituted analogues (ordinate, data from [4, 7, 8]) versus relative agonist potencies of histaprodifen (**8a**) and the nine correspondingly mono-substituted histaprodifens (abscissa, data from [11] and this study). Substitution included 2-F, 3-F, 4-F, 2-Cl, 3-Cl, 4-Cl, 3-Br, 3-CH_3 and 3-CF_3 groups. Data were measured on the guinea-pig ileum. No significant correlation was observed (r^2 = 0.095, $P > 0.39$). Therefore, it is unlikely that structure–activity relationships of 2-phenylhistamines and histaprodifenes run parallel.

4.3. Functional receptor selectivity of histaprodifen analogues

As far as other members of the family of histaminergic receptors are concerned, the new histaprodifen analogues lacked agonist potency and possessed low or moderate affinity for H_2 and H_3 receptors, respectively (*table IV*). All compounds studied on the spontaneously beating guinea-pig right atrium (H_2 receptors) depressed concentration–frequency curves of histamine with low potency (pD_2' = 4.3–4.9). When studied as potential antagonists of (*R*)-α-methylhistamine on the field-stimulated longitudinal muscle of the guinea-pig ileum, histaprodifens with small or medium-sized *ortho* substituents (**8b**, **8e** and **8k**) elicited a significant rightward displacement of the concentration–relaxation curve (pA_2 = 5.6–5.8), while the others were inactive, at least at the concentrations studied. It was not possible to characterize the potential H_3-receptor antagonism in full detail since at higher concentrations the histaprodifen analogues blocked the muscarinic M_3 receptors of the preparation and disturbed the electrically evoked, acetylcholine-mediated contraction of the tissue by themselves. Moreover, *meta* fluorohistaprodifen (**8c**), as the most interesting congener of **8a**, was subjected to a broader spectrum of functional receptor assays (see *table IV*, footnote e) and, all in all, displayed the same affinity profile as **8a** [11].

5. Conclusions

Histaprodifen (**8a**, 2-(3,3-diphenylpropyl)histamine, *figure 1*) is the prototype of a new familiy of potent and selective histamine H_1-receptor agonists which offers a wide array of structural modifications to gain more insight into structure–activity relationships of H_1-histaminergic agonists and into the underlying molecular principles of H_1-receptor activation [11]. The presented investigation conclusively demonstrates that an optimization of agonist potency in this series is not attainable by

Table IV. Functionally determined antagonist affinity of histaprodifens **8a–i**, **k** and **l** for histamine H_2, H_3 and muscarinic M_3 receptors.

Compound[a]			H_2 (guinea-pig right atrium)			H_3 (guinea-pig ileum)			M_3 (guinea-pig ileum)		
No.	X	X′	n[b]	$pD'_2 \pm$ range	c[d]	n[b]	pA_2 (95 % conf. lim.)[c]	c[d]	n[b]	$pA_2 \pm$ SEM[c]	c[d]
8a [11]	H	H	2	4.46 ± 0.08	20	5	5.51 (5.00–5.80)	2	11	5.55 ± 0.04	3–30
8b	2-F	H	2	4.68 ± 0.10	20	5	5.64 (5.50–5.75)	2	10	5.54 ± 0.04	3–30
8c[e]	3-F	H	2	4.47 ± 0.16	20	4	5.52 (5.10–5.78)	2	12	5.63 ± 0.10	3–10
8d	4-F	H	2	4.37 ± 0.01	30	3	< 5.7	2	8	5.42 ± 0.07	3–10
8e	2-Cl	H	2	4.77 ± 0.01	20	5	5.66 (5.53–5.77)	1	12	5.74 ± 0.05	1–10
8f	3-Cl	H	3	4.74 ± 0.15	10–30	5	< 6.0	1	8	5.82 ± 0.11	3–10
8g	4-Cl	H	2	4.50 ± 0.07	20	5	< 6.0	1	6	5.62 ± 0.08	3–10
8h	2-Br	H	2	4.94 ± 0.03	16–20	4	< 6.0	1	11	5.75 ± 0.05	1–10
8i	3-Br	H	2	4.31 ± 0.02	20	–	n.d.		–	n.d.	–
8k	2-CH_3	H	2	4.47 ± 0.18	20	4	5.84 (5.59–6.03)	1	8	5.74 ± 0.07	1–10
8l	3-CH_3	H	3	4.76 ± 0.34	10–20	3	< 5.7	2	8	5.43 ± 0.07	3–10

[a] For structure see *figure 2*. [b] Number of experiments. [c] Apparent pA_2 (see Experimental protocols). [d] Concentration(s) of **8** [μM]. [e] Further affinities of **8c**: β_1: pD_2' < 4.5 (n = 2); α_{1D}: pA_2 = 5.77 ± 0.12 (n = 5); 5-HT_{1B}: pA_2 = 5.09 ± 0.15 (n = 4); 5-HT_{2A}: pA_2 = 5.33 ± 0.04 (n = 8); 5-HT_3: pA_2 < 5.5 (n = 6); 5-HT_4: pA_2 < 5.5 (n = 3). For assays see Experimental protocols. n.d. not determined.

substitution of the aromatic rings of **8a**. At best a *meta* fluoro substituent, as present in **8c** ('*meta* fluorohistaprodifen'), is tolerated without loss of H_1-receptor activity compared with **8a**. Partial H_1-receptor agonism is exhibited when **8c** is studied as a constrictor of guinea-pig aorta and as a relaxant agent on the precontracted rat aorta, respectively. With regard to receptor selectivity, compound **8c** also perfectly matches with **8a**. Contrary to results in the 2-phenylhistamine series [3, 7, 8], the desired increase of agonist potency after introduction of a *meta* halogen or trifluoromethyl substituent in **8a** is not observed. Hence it follows that structure–activity relationships in both series are different. Therefore, it will be necessary to focus on the modification of other parts of the histaprodifen molecule to increase H_1-receptor potency.

6. Experimental protocols

6.1. Chemistry

6.1.1. General procedures

Melting points were determined on an Electrothermal IA 9000 digital apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer 1420 spectrometer. ^{1}H-NMR spectra were recorded on a Bruker Avance-TM-DPX 400 (400 MHz) spectrometer. Chemical shifts are given in ppm downfield from TMS as internal reference. ^{1}H-NMR data are reported in the order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; *, exchangeable by D_2O), approximate coupling constant *J*, number of protons, and location of protons (Im, imidazole; Ph, phenyl; Mal, maleic acid). ^{13}C-NMR spectra are presented accordingly. Mass spectra were recorded using a Finnigan MAT CH7A (70 eV, EI spectra) or a Finnigan MAT CH5DF ($^+$FAB spectra). Elemental analyses (C, H, N, Vario EL) were within ± 0.4% of the theoretical values unless otherwise indicated. Yields were not optimized. Chromatographic separation was achieved by column chromatography using silica gel 60 (Merck No. 9285, 230–400 mesh). Preparative rotatory layer chromatography was performed using a Chromatotron 7924T (Harrison Research, CA, USA) and glass rotors coated with 4 mm layers of silica gel 60 PF_{254} containing gypsum (Merck).

6.1.2. Synthesis of diphenylbutyronitriles **5** *from diphenylmethanols* **1** *via diphenylmethanes* **2** *(method A)*

6.1.2.1. Diphenylmethanes **2b**, **h–i** *and* **n**

General procedure:

A solution of anhydrous $AlCl_3$ in Et_2O (50 mL) was added to a suspension of $LiAlH_4$ (1.5 g, 40 mmol) in dry Et_2O (100 mL). This suspension was stirred for 30 min before a solution of the respective diphenylmethanol (**1b**, **h–i** or **n**, 31 mmol) dissolved in Et_2O (50 mL) was added dropwise. After heating to reflux for 12 h, a mixture of Et_2O/MeOH (10 mL each) was added carefully under cooling in an ice-bath. After hydrolysis with 1 N HCl (100 mL) the product was separated by extraction with Et_2O (3 × 50 mL). After removal of the solvent, the pure diphenylmethane was obtained as a colourless oil after chromatographic purification using petrolether/CH_2Cl_2 (9:1) as eluent.

(3-Bromophenyl)phenylmethane (**2i**). Yield: 6.6 g (86%). ^{1}H-NMR ($CDCl_3$) δ 7.39–7.13 (m, 9H, 9 Ph-H), 3.98 (s, 2H, CH_2). EI-MS *m/z* 248 ($M^{+\bullet}$, 26), 246 ($M^{+\bullet}$, 31), 168 (36), 167 (100), 165 (37), 152 (22). IR (KBr, cm^{-1}) 2 910 m, 1 567 st, 1 474 st, 1 071 st, 850 m. Anal. $C_{13}H_{11}Br$ (C, H, N).

6.1.2.2. Diphenylbutyronitriles **5a**, **b**, **h–i** *and* **n**

General procedure:

A solution of the respective diphenylmethane (**2a**, **b**, **h–i** or **n**) (68 mmol) in a mixture of dry Et_2O/THF (25 mL each) was added dropwise over 15 min to a stirred suspension of potassium (3.2 g, 82 mmol) and catalytic amounts of $Fe(NO_3)_3$ in liquid NH_3 (100 mL, –70 °C). The deep red suspension was stirred for 30 min and a solution of 3-bromopropionitrile (9.4 g, 70 mmol) in dry Et_2O/THF (20 mL) was added. After the deep red colour had disappeared, NH_3 was allowed to evaporate. The organic layer was separated and the residue extracted twice with Et_2O (40 mL). The combined extracts were washed with 0.1 N HCl (2 × 50 mL), water (100 mL), brine (50 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the resulting colourless oil purified by column chromatography using CH_2Cl_2/petrolether (40/60) as eluent.

4-(3-Bromophenyl)-4-phenylbutyronitrile (**5i**). Yield: 7.9 g (39%). ^{1}H-NMR ($CDCl_3$) δ 7.41–7.15 (m, 9H, 9 Ph-H), 4.06 (t, *J* = 7.9 Hz, 1H, C*H*CH_2), 2.43–2.37 (m, 4H, CH_2CH_2CN). EI-MS *m/z* 301 ($M^{+\bullet}$, 15), 299 ($M^{+\bullet}$, 15), 247 (59), 245 (63), 168 (89), 167 (100), 166 (78), 165 (75). IR (KBr, cm^{-1}) 2 246 m (CN), 1 592 m, 1 567 m, 1 495 m, 1 473 m, 1 452 m, 1 424 m, 1 217 st, 1 075 st. Anal. $C_{16}H_{14}BrN$ (C, H, N).

6.1.3. Synthesis of diphenylbutyronitriles **5** *from diethylmalonates (method B)*

6.1.3.1. 1-Chloro-3,3-diphenylpropanes **4c–g** *and* **j–m**

General procedure:

A solution of diethyl malonate (8.0 g, 50 mmol) in dry DMF (30 mL) was added to a suspension of NaH (2.0 g, 50 mmol) and catalytic amounts of NaI in dry DMF

(60 mL). When the formation of hydrogen had ceased, a solution of the respective chloro-diphenylmethane (**3c**–**g** or **j**–**m**) (40 mmol) in dry DMF (30 mL) was slowly added. After stirring for 2 h at 60 °C, the mixture was heated to 90 °C for an additional 36 h, cooled, and poured into water (300 mL). The mixture was extracted with Et_2O (3 × 70 mL), the organic solvents were removed by evaporation, and the residue was dissolved in a solution of KOH (10.0 g) in water (30 mL) and EtOH (20 mL), and refluxed for 12 h. After cooling to ambient temperature, by-products were separated by extraction with Et_2O (50 mL). The aqueous layer was diluted with 3 N NaOH (100 mL) and extracted with Et_2O (2 × 50 mL). The organic layer was discarded and the aqueous phase acidified with 6 N H_2SO_4. After extraction with Et_2O (3 × 70 mL) and removal of the solvent under reduced pressure the 2-(diphenylmethyl)malonic acid was obtained as a pale yellow solid. The malonic acid derivative (34 mmol) was converted into the corresponding 3,3-diphenylpropanoic acid by decarboxylation at 180 °C in vacuo (10 min). After cooling, the residue was dissolved in a mixture of 3 N NaOH (100 mL) and MeOH (50 mL) and extracted twice with a mixture of petrolether/Et_2O (1:1). The aqueous layer was acidified (6 N H_2SO_4) and the product extracted with Et_2O (3 × 70 mL). After removal of the solvent under reduced pressure, the substituted propionic acid was obtained as a pale brown solid which was pure enough for the next step.

The above described propionic acid derivative or commercially available 3,3-di-(4-fluorophenyl)propionic acid (33 mmol) was subsequently converted into the corresponding alcohol upon treatment with $LiAlH_4$ in dry Et_2O according to standard methods, and purified by column chromatography (CH_2Cl_2/MeOH (9/1)). The substituted 3,3-diphenyl-1-propanol (24 mmol) was then converted into the corresponding 1-chloro-3,3-diphenylpropane (**4c**–**g** and **j**–**m**) by reaction with thionylchloride (10 mL) and a catalytic amount of 4-DMAP in CH_2Cl_2 (20 mL), followed by column chromatography using petrolether/CH_2Cl_2 (8:2) as eluent.

1-Chloro-3-(3-fluorophenyl)-3-phenylpropane (**4c**):

2-((3-Fluorophenyl)phenylmethyl)malonic acid. Yield: 5.2 g (45%). M.p. 164 °C (dec.). ^{1}H-NMR ($CDCl_3$) 7.80 (br*, 2H, 2 COO*H*), 7.35–7.16 (m, 6H, 5 Ph-H, F-Ph-5-H), 7.02 (d, J = 7.8 Hz, 1H, F-Ph-6-H), 6.93 (d, $^3J_{H–F}$ = 9.8 Hz, 1H, F-Ph-2-H), 6.98–6.86 (ddd, $^3J_{H–H}$ = $^3J_{H–F}$ = 8.4 Hz, $^4J_{H–H}$ = 2.3 Hz, 1H, F-Ph-4-H), 4.64 and 4.28 (2d, J = 12.2 Hz, 2H, CHCH). EI-MS *m/z* 288 ($M^{+\bullet}$, 8), 270 (12), 242 (34), 224 (18), 185 (100), 166 (45). IR (KBr, cm^{-1}) 2 692 w, 1 723 st, 1 593 st, 1 490 st, 1 408 st, 1 108 m. Anal. $C_{16}H_{13}FO_4$ (not available).

3-(3-Fluorophenyl)-3-phenylpropionic acid. Yield: 8.2 g (77%). M.p. 107 °C. ^{1}H-NMR (DMSO-d_6) 12.14 (br*, 1H, COO*H*), 7.36–7.16 (m, 8H, 5 Ph-H, 3 F-Ph-H), 6.98 (ddd, $^3J_{H–F}$ = $^3J_{H–H}$ = 8.5 Hz, $^4J_{H–H}$ = 2.1 Hz, 1H, F-Ph-4-H), 4.44 (t, J = 8.0 Hz, 1H, C*H*CH_2), 3.04 (m, 2H, CHC*H*$_2$). EI-MS *m/z* 244 ($M^{+\bullet}$, 42), 198 (22), 185 (100), 165 (19). IR (KBr, cm^{-1}) 3 423 br, 1 712 st, 1 589 m, 1 487 st, 1 290 st. Anal. $C_{15}H_{13}FO_2$ (C, H, N).

3-(3-Fluorophenyl)-3-phenyl-1-propanol [18]. Yield: 5.9 g (77%). Colourless oil. ^{1}H-NMR ($CDCl_3$) 7.31–7.18 (m, 6H, 5 Ph-H, F-Ph-5-H), 7.03 (d, J = 7.8 Hz, 1H, F-Ph-6-H), 6.99–6.94 (m, 1H, F-Ph-2-H), 6.87 (ddd, $^3J_{H–H}$ = $^3J_{H–F}$ = 8.2 Hz, $^4J_{H–H}$ = 2.5 Hz, 1H, F-Ph-4-H), 4.15 (t, J = 7.9 Hz, 1H, C*H*CH_2), 3.60 (t, J = 6.4 Hz, 2H, C*H*$_2$OH), 2.29 (dt, J = 6.6 Hz, 2H, CHC*H*$_2$CH$_2$), 1.31 (br*, 1H, OH). EI-MS *m/z* 230 ($M^{+\bullet}$, 27), 212 (51), 185 (100), 165 (25). IR (KBr, cm^{-1}) 3 339 br, 1 612 m, 1 589 m, 1 487 m, 1 447 m, 1 246 m, 1 033 m. Anal. $C_{15}H_{15}FO$ (C, H, N).

1-Chloro-3-(3-fluorophenyl)-3-phenylpropane (**4c**). Yield: 5.1 g (86%). Colourless oil. ^{1}H-NMR ($CDCl_3$) δ 7.32–7.19 (m, 6H, 5 Ph-H, F-Ph-5-H), 7.03 (d, J = 7.6 Hz, 1H, F-Ph-6-H), 6.94 (d, $^3J_{H–F}$ = 10.1 Hz, 1H, F-Ph-2-H), 6.89 (ddd, $^3J_{H–H}$ = $^3J_{H–F}$ = 8.4 Hz, $^4J_{H–H}$ = 2.3 Hz, 1H, F-Ph-4-H), 4.22 (t, J = 7.8 Hz, 1H, C*H*CH_2), 3.44 (t, J = 6.5 Hz, 2H, CH_2Cl), 2.47 (dt, J = 7.0 Hz, 2H, CHC*H*$_2$). EI-MS *m/z* 248 ($M^{+\bullet}$, 13), 185 (100), 171 (6), 166 (28). IR (KBr, cm^{-1}) 1 612 m, 1 589 st, 1 488 st, 1 447 st. Anal. $C_{15}H_{14}ClF$ (C, H, N).

6.1.3.2. Diphenylbutyronitriles **5c**–**g**, **j**–**m** *and* **o**

General procedure:

The above described 1-chloropropanes **4c**–**g**, **j**–**m** and **o** (21 mmol), potassium cyanide (1.4 g, 22 mmol) and a catalytic amount of KI were dissolved in DMSO (50 mL) and stirred for 1 h at ambient temperature, 1 h at 65 °C, and 3 h at 95 °C. After cooling, the mixture was diluted with water (500 mL), and the butyronitrile was isolated as a colourless liquid by extraction with Et_2O (3 × 50 mL), followed by column chromatography using CH_2Cl_2/petrolether (1:1) as eluent.

4-(3-Fluorophenyl)-4-phenylbutyronitrile (**5c**):

Colourless liquid. Yield: 4.5 g (92%). ^{1}H-NMR ($CDCl_3$) 7.34–7.20 (m, 6H, 5 Ph-H, F-Ph-5-H), 7.03 (d, J = 7.8 Hz, 1H, F-Ph-6-H), 6.93–6.87 (m, 2H, F-Ph-2-H, F-Ph-4-H), 4.06 (t, J = 7.9 Hz, 1H, C*H*CH_2), 2.37 (dt, J = 7.2 Hz, 2H, C*H*$_2$CH$_2$CN), 2.27 (t, J = 7.6 Hz, 2H, CH_2CN). ^{13}C-NMR ($CDCl_3$) 163.1 (d, $^1J_{C–F}$ = 246.8 Hz, F-Ph-$C_{(3)}$), 145.4 (d, $^3J_{C–F}$ = 6.7 Hz, F-Ph-$C_{(1)}$), 142.0 (Ph-$C_{(1)}$), 130.3 (d, $^3J_{C–F}$ = 8.0 Hz, F-Ph-$C_{(5)}$), 129.0, 127.7, 127.1 (5 Ph-$C_{(2–6)}$), 123.4 (d, $^4J_{C–F}$ = 2.7 Hz, F-Ph-$C_{(6)}$), 119.1 (CN), 114.5 (d, $^2J_{C–F}$ = 21.4 Hz,

F-Ph-$C_{(2)}$), 113.8 (d, $^2J_{C–F}$ = 20.9 Hz, F-Ph-$C_{(4)}$), 49.5 (Ph-*C*H-Ph), 30.1 (*C*$H_2$$CH_2$CN), 15.7 ($CH_2$*C*$H_2$CN). EI-MS *m/z* 239 ($M^{+\bullet}$, 20), 185 (100), 170 (7), 165 (36). IR (KBr, cm^{-1}) 3 027 m, 2 936 m, 2 246 m (CN), 1 590 st, 1 490 st, 1 449 st, 1 425 w. Anal. $C_{16}H_{14}FN$ (C, H, N).

6.1.4. Synthesis of methyl butyrimidates **6b–o**

6.1.4.1. General procedure

A solution of the respective butyronitriles **5b–o** (17 mmol) in dry MeOH (30 mL) was chilled to –30 °C. After stirring for 5 min, thionylchloride (3.0 mL) was added to the solution. After 7 days in a freezer the solvent was removed in vacuo. The bulk was pure enough for the subsequent cyclization and was therefore used without further purification.

*6.1.4.2. Methyl 4-(3-fluorophenyl)-4-phenylbutyrimidate hydrochloride (***6c***)*

Colourless semisolid material. Yield: 4.8 g (83%). $^+$FAB-MS (Xe/3-NO_2-benzyl-OH) *m/z* 272 ([M + H]$^+$, 100), 258 (3), 241 (3), 185 (3), 185 (53). Anal. $C_{17}H_{18}FNO{\cdot}HCl$ (not available).

6.1.5. Synthesis of histaprodifens **8b–o**

6.1.5.1. General procedure

Equimolar amounts of 2-oxo-4-phthalimido-1-butyl acetate (4.2 g, 15 mmol) and the respective methyl butyrimidate **6b–o** (15 mmol) were dissolved in liquid NH_3 (150 mL) in an autoclave (1 000 mL, Kotter, Germany). After stirring for 12 h at room temperature the mixture was heated to 60 °C (24–26 bar) for 6 h. After the evaporation of NH_3, the crude amines **8b–o** were obtained after deprotection by acidic hydrolysis with 6 N HCl under reflux for 6 h. After alkalization with excess 3 N NaOH, the substituted histaprodifens were extracted with 3 portions of CH_2Cl_2/MeOH (9:1). Alternatively, the protecting phthaloyl group was cleaved by refluxing the crude mixture containing **7b–o** in a mixture of MeOH (50 mL) and hydrazine hydrate (1.0 g, 20 mmol) for 3 h. After removal of the solvent, the free bases were extracted with CH_2Cl_2/MeOH (3 × 50 mL). For all compounds, rotatory chromatography (CH_2Cl_2/MeOH (10/1), NH_3-saturated) afforded the purified histaprodifen bases **8b–o** which were crystallized as dihydrogen maleates (*table I*).

*6.1.5.2. 2-[2-[3-(3-Fluorophenyl)-3-phenylpropyl]-1H-imidazol-4-yl]ethanamine (***8c***)*

Yield: 0.68 g (8%). M.p. 147–149 °C. ^{1}H-NMR (CD_3OD) δ 7.33–7.27 (m, 5H, 5 Ph-H), 7.21–7.17 (m, 2H, F-Ph-5-H, Im-5-H), 7.11 (d, $^3J_{H–H}$ = 7.9 Hz, 1H, F-Ph-6-H), 7.05–7.01 (m, 1H, F-Ph-2-H), 6.90 (ddd, $^3J_{H–H}$ = $^3J_{H–F}$ = 8.4 Hz, $^4J_{H–H}$ = 2.2 Hz, 1H, F-Ph-4-H), 6.25 (s, 4H, 4 Mal-H), 4.04 (t, *J* = 7.8 Hz, 1H, C*H*CH_2), 3.27 (t, *J* = 7.5 Hz, 2H, CH_2CH_2N), 3.03 (t, *J* = 7.5 Hz, 2H, $Im_{(C4)}$-CH_2), 2.90 (t, *J* = 8.0 Hz, 2H, $Im_{(C2)}$-CH_2), 2.59–2.53 (m, 2H, CHCH_2). ^{13}C-NMR (CD_3OD) δ 170.9 (4 COOH), 164.4 (d, $^1J_{C–F}$ = 244.6 Hz, F-Ph-$C_{(3)}$) 149.2 (Im-$C_{(2)}$), 148.1 (d, $^3J_{C–F}$ = 6.8 Hz, F-Ph-$C_{(1)}$), 144.3 (Ph-$C_{(1)}$), 136.6 (4 *C*HCOOH), 131.4 (d, $^3J_{C–F}$ = 8.2 Hz, F-Ph-$C_{(5)}$), 130.0 (Im-$C_{(4)}$), 129.9 and 128.8 (4 Ph-$C_{(2,3,5,6)}$), 127.9 (Ph-$C_{(4)}$), 124.7 (d, $^4J_{C–F}$ = 2.7 Hz, F-Ph-$C_{(6)}$), 117.6 (Im-$C_{(5)}$), 115.4 (d, $^2J_{C–F}$ = 21.6 Hz, F-Ph-$C_{(2)}$), 114.3 (d, $^2J_{C–F}$ = 21.1 Hz, F-Ph-$C_{(4)}$), 51.8 (Ph-*C*H-Ph), 39.2 (CH_2NH_2), 33.6 (CH*C*$H_2$$CH_2$), 25.8 (Im-$C_{(2)}$-*C*$H_2$), 23.9 (Im-$C_{(4)}$-*C*$H_2$). $^+$FAB-MS (Xe/glycerol) *m/z* 324 ([M + H]$^+$, 100), 307 (19), 295 (17). IR (KBr, cm^{-1}) 3 419 m, 1 619 st, 1 583 st, 1 486 st, 1 358 m, 867 m. Anal. $C_{20}H_{22}FN_3{\cdot}2\ C_4H_4O_4{\cdot}0.25\ H_2O$ (C, H, N).

6.2. Pharmacology

6.2.1. Data handling and pharmacological parameters

Data are presented as mean ± standard error (SEM or SE) or with 95% confidence limits. Graphical data (*figures 3* and *5*) are given as mean ± SEM (SEM does not appear when smaller than symbol). Significant differences of means or the difference between a mean and a constant value ($P < 0.05$) were discerned by Student's *t* test. Agonists were characterized by relative potency compared with histamine, calculated as the antilog of (pEC_{50}(agonist) – pEC_{50}(histamine)). The first concentration–effect curve (CEC) for histamine served as an internal reference when two CECs were performed on each preparation. The daily mean of untreated histamine control organs served as reference when only one CEC per organ was established. E_{max} [%] of the new agonists (formerly termed 'intrinsic activity' [13]) was calculated relative to histamine. The negative logarithm of the dissociation constant of the partial-agonist/receptor complex, pK_P, was calculated by a weighted linear regression procedure from individual sets of equi-effective concentrations of histamine in the absence and presence of a maximal or supramaximal concentration of the partial agonist [14, 15]. The affinity of the H_1-receptor antagonist mepyramine was calculated as pA_2 from experiments with histamine or the new agonists in the absence or presence of suitable concentrations of mepyramine. Apparent pA_2 values were obtained from experiments with a single mepyramine concentration according to the equation $pA_2 = -\log_{10} c(\text{mepyramine}) + \log_{10}(r - 1)$, where *r* is the ratio of agonist concentrations in the absence and

presence of mepyramine that elicit 50% of the respective maximum effect [13]. When a set of at least three different mepyramine concentrations was studied, full pA_2 values were calculated from Arunlakshana-Schild plots after constraining the Schild plot slope to unity [19]. Antagonist potencies of the new compounds at selected neurotransmitter receptor subtypes were calculated as apparent pA_2 values or as pD'_2 values [13], when only depression of CECs instead of rightward displacement was observed.

6.2.2. Histamine H_1-receptor assay on the isolated guinea-pig ileum

Guinea-pigs of either sex were stunned by a blow on the head and exsanguinated. The ileum was removed and whole segments (2.0–2.5 cm) were mounted isotonically (preload 0.5 g) at 37 °C in Tyrode's solution, aerated with 95% O_2/5% CO_2, in the continuous presence of 0.1 μM atropine, a concentration not affecting H_1 receptors [7, 20]. During an equilibration period of ca. 80 min the organs were stimulated three times with histamine (1 and 10 μM) followed by washout. Each preparation was used to establish a cumulative concentration–effect curve for histamine (0.01–30 μM) followed by a second curve for a new agonist in the absence or presence of mepyramine (1–100 nM, incubation time 10–15 min). The pEC_{50} difference was not corrected since two successive curves for histamine were superimposable (n = 10). For the determination of pK_P, the partial agonist was not washed out and incubated for 0.5–10 min (*table II*). A final cumulative curve for histamine was then constructed.

6.2.3. Histamine H_1-receptor assay on the isolated guinea-pig aorta

Thoracic aortae of sacrificed guinea-pigs were quickly removed and cleared of connective tissue [7, 11, 16]. Rings of 2–4 mm length were cut and rolled with a pair of tweezers to damage the endothelium. Organs were mounted isometrically (initial tension 10 mN) by means of two L-shaped stainless steel hooks in a modified Krebs-Henseleit solution (37 °C, gassed with 95% O_2/5% CO_2, 1.80 mM Ca^{2+}, 1.20 mM Mg^{2+}). During an equilibration period of ca. 130 min, organs were stimulated three times with histamine (10 μM) followed by washout. Cumulative concentration–effect curves for histamine (0.1–300 μM) followed by a second curve for histamine or a new agonist in the absence or presence of mepyramine (1–1 000 nM, incubation time 30 min) were established in the presence of cimetidine, corticosterone, cocaine (30 μM each), prazosin, yohimbine (0.3 μM each), and propranolol (0.1 μM). The pEC_{50} difference was corrected using the sensitivity change monitored by untreated histamine control preparations.

6.2.4. Histamine H_1-receptor assay on the isolated rat aorta

Male Wistar rats were stunned (CO_2) and decapitated. The thoracic portion of the aorta was rapidly removed, rinsed, and cleared of connective tissue [11, 17]. Rings of 2–4 mm length were cut and set up isometrically (initial tension 10 mN) by means of two L-shaped stainless steel hooks in a modified Krebs-Henseleit solution (37 °C, gassed with 95% O_2/5% CO_2, 1.25 mM Ca^{2+}, 1.20 mM Mg^{2+}). After an equilibration period of ca. 100 min, vascular rings were contracted with a submaximal concentration of U46619, a TP (thromboxane-A_2)-receptor agonist (15.8 nM) in the presence of prazosin (100 nM). When the effect had plateaued (usually after 45 min), a cumulative concentration–relaxation curve was established for histamine (0.1–1 000 μM) or a new agonist in the absence or presence of mepyramine (100 nM, total incubation time approximately 75 min). When the agonist had elicited its maximum effect, a final relaxation was induced by addition of the M_3-receptor agonist carbachol (300–1 000 μM).

6.2.5. Antagonist activity of title compounds at histamine H_2, H_3 and other neurotransmitter receptors

In vitro experiments were performed according to published protocols for the following functional receptor assays: histaminergic H_2 receptors of the spontaneously beating guinea-pig right atrium (in the presence of 0.3 μM propranolol and 1 μM mepyramine) [20], histaminergic H_3 receptors of field-stimulated guinea-pig ileal longitudinal muscle with adhering plexus myentericus (in the presence of 1 μM mepyramine) [21], muscarinic M_3 receptors of guinea-pig whole ileal segments (in the presence of 1 μM mepyramine) [20], adrenergic α_{1D} receptors of rat thoracic aorta and β_1 receptors of spontaneously beating guinea-pig right atrium (in the presence of 1 μM mepyramine) [20], serotonergic 5-HT_{1B} receptors of guinea-pig arteria iliaca (in the presence of 1 μM mepyramine) [20], 5-HT_{2A} receptors of rat tail artery [20], 5-HT_3 receptors of quiescent guinea-pig ileal longitudinal muscle with adhering plexus myentericus (in the presence of 1 μM mepyramine) [22], and 5-HT_4 receptors of rat oesophagal tunica muscularis mucosae [22], respectively. For the new compounds, agonist effects were not observed in all non-H_1-receptor assays.

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