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Preliminary communication

Synthesis and histamine H_1-receptor antagonist activity of 4-(diphenylmethyl)-1-piperazine derivatives with a terminal heteroaryl or cycloalkyl amide fragment

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Summary — New 4-(diphenylmethyl)-1-piperazine derivatives with a terminal heteroaryl or cycloalkyl amide fragment were synthesized and evaluated for their antihistaminic, anticholinergic and antiallergic activities. Tested compounds were found to be moderate to potent in vitro (guinea-pig ileum) histamine H_1-receptor antagonists. Derivatives with a four methylene chain (**1e–1h**) were as potent in vivo (capillary permeability in rats) as cetirizine; the heteroaryl derivatives **1e** and **1h** were found to be the most active agents. These compounds displayed only weak in vitro (guinea-pig ileum) muscarinic M_3-receptor antagonist activity. Compounds **1e** and **1g** were about 100 times more potent than ketotifen in preventing the compound 48/80-induced histamine release from rat peritoneal mast cells. Derivatives **1e**, **1f** and **1h** did not modify the spontaneous motor activity in rats at 100 mg/kg po. Compound **1e** has been selected for further studies.

4-(diphenylmethyl)-1-piperazine derivative / H_1-antihistaminic activity / anticholinergic activity / antiallergic activity / CNS effect

Introduction

Since the discovery of the first histamine H_1-receptor antagonist more than 50 years ago, many different chemical structures have been found to exhibit H_1-receptor antagonist activity [1]. Before 1982, more than 40 compounds belonging to the so-called 'first generation' antihistamines were marketed [2]. Although these 'older' antihistamines have been used in the treatment of allergic rhinitis and urticaria for many years, they are characterized by a lack of specificity. Because of their lipophilicity, they induce marked sedation, CNS depressant and anticholinergic effects. The advent of relatively nonsedating antihistamines a decade ago opened a new era [3]. Unlike the older compounds, 'second generation' antihistamines do not cross the blood–brain barrier and are also highly selective for H_1-receptors. Several potent and selective antihistamine drugs, belonging to both first and second generations, possess a 4-(diphenylmethyl)-piperazine moiety, eg, cyclizine, oxatomide, hydroxyzine, cetirizine and AL-3264 (fig 1) [4]. Cetirizine is an excellent example of the second generation histamine H_1-receptor antagonists, because it combines potency, negligible CNS depressant effects and a weak anticholinergic activity [5].

The aim of the present study was to develop a series of 4-(diphenylmethyl)piperazine derivatives with a terminal heteroaryl or cycloalkyl amide fragment **1a–h** as selective H_1-receptor antagonists devoid of anticholinergic activity and CNS side effects.

Chemistry

Compounds **1a–h** were prepared following two standard procedures: either by reaction of *N*-aminoalkyl-4-(diphenylmethyl)-1-piperazine **2** with appropriate acid chlorides **3** in the presence of triethylamine, according to conditions reported earlier [6, 7], or by coupling reactions of acids **4** with amines **2**, using carbonyldiimidazole in DMF (scheme 1). Amines **2** were prepared from 1-(diphenylmethyl)piperazine according to previously reported methods [8]. Physical data are presented in table I.

Pharmacology

All compounds were initially assayed in vitro and in vivo for their ability to inhibit the effects of histamine on guinea-pig ileum and rat skin respectively. Only

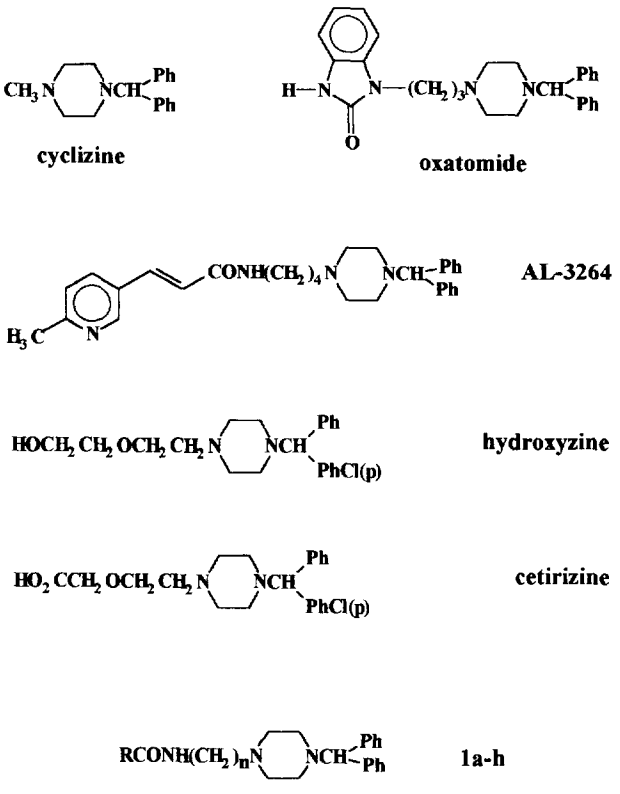


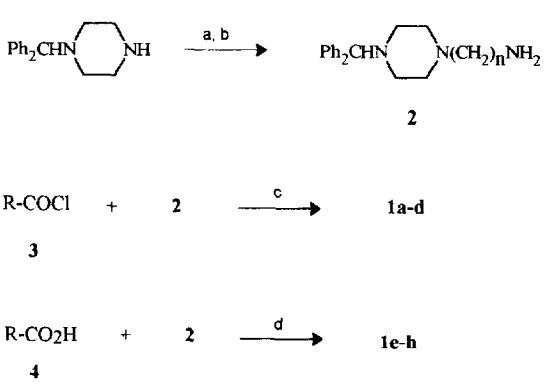
Fig 1. Histamine H_1-receptor antagonists and compounds **1a–h**.

compounds with ED_{50} values < 10 mg/kg po were selected, and their antiallergic, anticholinergic and CNS effects were determined using standard methods and appropriate reference drugs.

Results

The in vitro antihistaminic activity data reported in table I indicate that all derivatives **1a–h** and the reference drug cetirizine exhibit moderate to potent ability to antagonize (in a concentration-dependent manner) the histamine H_1-receptors mediated response, with K_b values ranging from 7.6 nmol/L (**1f**) to 50.7 nmol/L (**1b**). All derivatives **1a–h** and cetirizine exhibited 100% inhibition at 1 μmol/L. The K_b values for compounds **1c–e** and **1g** were not statistically different to that of cetirizine.

As reported in table I the resulting skin wheal, due to an increase in capillary permeability caused by intradermal injection of histamine, was not significantly inhibited by compounds **1a–d** at the highest administered dose (10 mg/kg po). Derivatives **1e–h**



Scheme 1. Synthesis of **1a–h**. a. $Cl(CH_2)_{n-1}CN$, CH_3CN, K_2CO_3, 3 h, 60 °C; b. $LiAlH_4$, Et_2O, 20 h, rt; c. Et_3N, THF, 1 h, rt; d. CDI, DMF, 20 h, rt.

exhibited a strong ability to inhibit (in a dose-dependent manner) the histamine-induced cutaneous reaction. Estimated ED_{50} values for these compounds ranged from 1.8–5 mg/kg po. On comparison with cetirizine (ED_{50} = 2.86 mg/kg), derivatives **1e–h** showed a similar potency.

The in vitro anticholinergic activity data reported in table II show that derivatives **1e–h** and cetirizine inhibit the acetylcholine-induced contractions only at concentrations near to or higher than 50 nmol/L. Their K_b values were 350–4000 times higher than that of the reference drug atropine (0.15 nmol/L). Compounds **1e** (K_b = 602.4 nmol/L) and cetirizine (K_b = 581.1 nmol/L) showed the lowest in vitro anticholinergic activity.

The data reported in table III show the concentrations of the tested drugs at which maximal inhibition of histamine release was recorded. Derivatives **1e** and **1g** significantly inhibited the histamine release at 1.6 μmol/L (72 and 65% respectively). The reference drug ketotifen exhibited similar potency to **1e** and **1g** at a 100-fold higher concentration (160 μmol/L). Compound **1f** did not significantly inhibit the histamine release and derivative **1h**, at a concentration higher than 0.16 μmol/L, showed a stimulant releasing effect per se.

According to the data reported in table IV, derivatives **1e**, **1f**, **1h** and cetirizine did not significantly modify the spontaneous motor activity in rats at a dose of 100 mg/kg po. Compound **1g**, at the same dose, significantly diminished all the measured parameters.

Discussion

All compounds **1a–h** showed moderate to potent ability to antagonize the histamine H_1-receptor in guinea-

Table I. Physical data and H_1-antihistamine activity of compounds **1a–h**.

Compound	*R*	*n*	*Molecular formula*[a]	*Mp*[b,c] *(°C)*	*Yield (%)*	*H_1-Antihistamine activity*	
						K_b[d] *(nmol/L)*	*ED_{50}*[e] *(mg/kg po)*
1a		2	$C_{24}H_{27}N_3OS$	149–151	82	22.7	> 10
						(20.5–25.0)[f]	
1b		2	$C_{24}H_{28}N_4O$	152–154	75	50.7	> 10
						(43.1–58.3)	
1c		2	$C_{25}H_{28}N_4O$	140–142	79	15.5	> 10
						(12.6–18.3)	
1d		3	$C_{26}H_{30}N_4O$	101–103	85	12.8	> 10
						(10.9–14.8)	
1e		4	$C_{27}H_{32}N_4O$	105–107	77	16.3	1.79
						(14.0–18.7)	(0.70–3.66)[f]
1f		4	$C_{32}H_{44}N_3O$	115–117	71	7.6	4.80
						(6.3–8.9)	(0.41–7.18)
1g		4	$C_{29}H_{39}N_3O$	90–92	72	21.9	2.06
						(17.7–26.0)	(1.10–3.85)
1h		4	$C_{29}H_{37}N_3O$	104–106	74	28.9	1.82
						(23.2–34.5)	(0.61–5.40)
Cetirizine						15.0	2.86
						(12.4–17.6)	(0.91–4.82)

[a]Elemental analyses were within ±0.4% of the theoretical values. [b]References [6,7]. [c]Recrystallization solvent: isopropyl ether/ethanol. [d]Inhibition of histamine-induced contractions of guinea-pig ileum. [e]Effect on histamine-induced capillary permeability increase in rats. [f]95% confidence interval.

pig ileum. Compounds **1c** (n = 2, K_b = 15.5 nmol/L), **1d** (n = 3, K_b = 12.8 nmol/L) and **1e** (n = 4, K_b = 16.3 nmol/L) showed similar results in the in vitro assay for histamine H_1-receptor antagonism. Therefore, it seems that the alkyl chain length has no significant influence on the in vitro activity. On the other hand, a comparison of the K_b values for compounds of the same chain length, ie, **1e** (K_b = 16.3 nmol/L), **1f** (K_b = 7.6 nmol/L), **1g** (K_b = 21.9 nmol/L) and **1h** (K_b = 28.9 nmol/L), suggests that replacement of the heteroaryl moiety by a cycloalkyl group does not always lead to the same modification of the in vitro activity. Further results from isolated guinea-pig ileum have demonstrated that compound **1e** shows features of a non-competitive histamine H_1-receptor antagonist (data not shown).

In the in vivo assay for antihistaminic activity (capillary permeability in rats), derivatives with a two- or three-carbon chain (**1a–d**) showed ED_{50} values higher than 10 mg/kg po. In contrast, the potencies of the four methylene chain compounds (**1e–h**) to reduce the histamine-induced increase on cutaneous capillary permeability in rats (ED_{50} values 1.79–4.80 mg/kg po) were similar to that of cetirizine (ED_{50} = 2.86 mg/kg po, $p > 0.05$).

The antagonistic effect on the muscarinic M_3-receptor in guinea-pig ileum was evaluated only for compounds with higher oral H_1-antihistaminic activity (**1e–h**). Evidence for the relative specificity of the antihistaminic effect of these compounds was provided by the finding that all derivatives **1e–h** had a weak to moderate effect on responses of the guinea-

Table II. Anticholinergic activity of compounds **1e–h**.

Compound	*In vitro*[a] K_b *(nmol/L)*	K_b $(M_3)/K_b$ (H_1) *ratio*
1e	602.4 (400.0–804.8)[b]	36.96
1f	54.0 (36.6–71.3)	7.10
1g	145.5 (114.7–176.4)	6.64
1h	60.7 (33.7–87.7)	2.10
Cetirizine	585.1 (538.7–631.3)	39.01
Atropine	0.15 (0.12–0.19)	–

[a]Inhibition of acetylcholine-induced contractions of guinea-pig ileum. [b]95% confidence interval.

Table III. Antiallergic activity of compounds **1e–h**[a].

Compound	*Concentration (μmol/L)*	*Histamine release (%) (mean ± SEM)*	*Maximal inhibition (% vs vehicle)*
Vehicle	–	84.43 ± 4.82	–
1e	1.6	23.91 ± 4.04	71.7[c]
1f	1.6	80.00 ± 1.20	5.2
1g	1.6	29.27 ± 4.70	65.3[c]
1h	0.16[d]	57.17 ± 6.03	32.3[b]
Ketotifen	160	31.10 ± 2.97	63.2[c]

[a]Effect on compound 48/80-induced histamine release from rat peritoneal mast cells. [b]$P < 0.05$. [c]$P < 0.01$. [d]At higher concentrations it had a stimulant-releasing property.

pig ileum to acetylcholine ($K_b > 50$ nmol/L) compared with the reference drug atropine ($K_b = 0.15$ nmol/L). Moreover, it seems that the anticholinergic activity became relevant only at a concentration some 2–40 times higher than that for the same range of antihistaminic activity (guinea-pig ileum). Besides, the structurally related cetirizine is known to be devoid of in vitro anticholinergic activity [5]. Derivative **1e** presented the lowest muscarinic M_3-antagonism, with a $K_b(M_3) / K_b(H_1)$ ratio greater than 35. This ratio was 39 for the reference drug cetirizine.

Many antihistamines, including ketotifen, are able to inhibit in vitro histamine release from mast cells. However, this effect is not related to histamine H_1-receptor antagonism but to a direct interaction of the drug with the mast cell membrane [9]. Moreover, the concentration of antihistamine required to achieve such an in vitro mast cell-stabilizing effect is usually far in excess of that used therapeutically. In order to examine the antiallergic activity of compounds **1e–h**, their action on compound 48/80-induced histamine release from rat peritoneal mast cells was evaluated. At 1.6 μmol/L, compound **1f** showed a weak and not significant inhibition, while **1e** and **1g** potently inhibited the histamine release (72 and 65% respectively). The reference drug ketotifen markedly inhibited (63%) the histamine release only at 160 μmol/L. Thus, compounds **1e** and **1g** were about 100 times more effective than ketotifen in preventing the histamine release induced by compound 48/80. Compound **1h** showed a stimulant-releasing effect per se at concentrations higher than 0.16 μmol/L.

With regard to possible effects on the central nervous system (CNS) it was shown that derivatives **1e**, **1f** and **1h** did not significantly modify the spontaneous motor activity in rats at 100 mg/kg po. Moreover, compound **1g**, at the same dose, was the unique agent that showed significant inhibitory CNS effects.

Table IV. Effect of compounds **1e–h** on spontaneous motor activiy in rats.

Compound	*Dose (mg/kg po)*	*Horizontal activity*[a]	*Vertical activity*[a]	*Stereotipy counts*[a]
Vehicle	–	6011 ± 406	325 ± 34	3008 ± 178
1e	100	6431 ± 285 (7)	344 ± 62 (6)	3338 ± 273 (11)
1f	100	6582 ± 620 (9)	441 ± 80 (35)	3129 ± 252 (4)
1g	100	2932 ± 291 (–51)[b]	80 ± 19 (–75)[b]	1384 ± 124 (–54)[b]
1h	100	4946 ± 343 (–17)	233 ± 34 (–28)	2439 ± 179 (–19)
Cetirizine	100	7133 ± 505 (19)	478 ± 82 (47)	3410 ± 251 (13)

[a]Results are expressed as mean of counts ± SEM. In parentheses: percentage of modification versus vehicle values. [b]$P < 0.01$.

The structurally related cetirizine exerts slight but not significant stimulant effects ($P > 0.05$) at 100 mg/kg po. The penetration of compound **1e** into the CNS requires further investigation because the lack of sedative effects in an antihistaminic compound may also be due to its poor penetration into the brain.

It may be concluded that, in these preliminary pharmacological studies, compound **1e**, a novel 4-(diphenylmethyl)-1-piperazine derivative with a heteroaryl amide fragment, has been demonstrated to be an excellent histamine H_1-receptor antagonist, highly active when orally administered, with antiallergic properties and without anticholinergic and sedative CNS side effects. Thus, compound **1e** has been selected for further pharmacological and toxicological studies.

Experimental protocols

Chemistry

Flash column chromatography was performed with silica gel, particle size 60 Å, mesh = 230–400 (Merck). Melting points were determined in open capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. Elemental analyses were within ±0.4% of the theoretical values. Infrared spectra were measured on a Perkin-Elmer 1310 instrument on KBr plates. ^{1}H- and ^{13}C-NMR were recorded on a Bruker AC-200; chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS), which was used as an internal standard. Spectral data are consistent with assigned structures.

General procedure for the synthesis of compounds ***1***

N-[2-[4-(Diphenylmethyl)-1-piperazinyl]ethyl]-3-thiophenecarboxamide ***1a***. To a solution of 3-thiophenecarboxylic acid (1.3 g, 10 mmol) in DMF (40 mL), 1,1'-carbonyldiimidazol (1.8 g, 11 mmol) was added. After the reaction mixture was stirred at room temperature for 1 h, *N*-(2-aminoethyl)-4-(diphenylmethyl)-1-piperazine (3.2 g, 11 mmol) was added. Stirring was continued overnight and then the solvent was evaporated. The residue was poured into water (30 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was dried (Na_2SO_4) and the solvent evaporated under reduced pressure to yield 3.8 g of an oil, which was purified by silica-gel column chromatography, using a mixture of CH_2Cl_2/MeOH (9:1) as eluent, to yield 3.3 g (82%) of the desired product. ^{1}H-NMR ($CDCl_3$): 2.45–2.63 (cluster, 10H, NCH_2), 3.49 (q, 2H, $CONHCH_2$), 4.22 (s, 1H, CH), 6.94 (t br, 1H, CONH), 7.11–7.42 (cluster, 10H, phenyl, 2H, thiophene), 7.85 (m, 1H, thiophene). ^{13}C NMR ($CDCl_3$): 35.89, 51.65, 53.03, 56.30, 76.12, 126.07, 126.20, 126.94, 127.79, 128.04, 128.46, 137.57, 142.51, 162.98.

N-[2-[4-(Diphenylmethyl)-1-piperazinyl]ethyl]-2-pyrrolecarboxamide ***1b***. ^{1}H-NMR ($CDCl_3$): 2.43–2.69 (cluster, 10H, NCH_2), 3.47 (q, 2H, $CONHCH_2$), 4.20 (s, 1H, CH), 6.18 (m, 1H, pyrrole), 6.53 (m, 1H, pyrrole), 6.68 (t br, 1H, CONH), 6.86 (m, 1H, pyrrole), 7.12–7.42 (cluster, 10H, arom), 10.07 (s br, 1H, NH). ^{13}C NMR ($CDCl_3$): 35.74, 51.88, 53.09, 56.48, 76.20, 108.83, 109.47, 121.36, 126.05, 126.89, 127.82, 128.43, 142.63, 161.22.

N-[2-[4-(Diphenylmethyl)-1-piperazinyl]ethyl]-3-pyridinecarboxamide ***1c***. ^{1}H-NMR ($CDCl_3$): 2.44–2.62 (cluster, 10H, NCH_2), 3.51 (q, 2H, $CONHCH_2$), 4.22 (s, 1H, CH), 7.06 (t br, 1H, CONH), 7.12–7.43 (cluster, 10H, phenyl, 1H, pyridine), 8.10 (m, 1H, pyridine), 8.69 (m, 1H, pyridine), 8.95 (m, 1H, pyridine). ^{13}C NMR ($CDCl_3$): 36.24, 51.87, 52.97, 55.92, 76.14, 123.40, 126.87, 127.77, 128.40, 130.20, 135.06, 142.55, 147.77, 151.97, 165.25.

N-[3-[4-(Diphenylmethyl)-1-piperazinyl]propyl]-3-pyridinecarboxamide ***1d***. ^{1}H-NMR ($CDCl_3$): 1.77 (m, 2H, CCH_2C), 2.25–2.61 (cluster, 10H, NCH_2), 3.55 (q, 2H, $CONHCH_2$), 4.21 (s, 1H, CH), 7.12–7.42 (cluster, 10H, phenyl, 1H, pyridine), 8.11 (m, 1H, pyridine), 8.61 (t br, 1H, CONH), 8.74 (m, 1H, pyridine), 9.02 (m, 1H, pyridine). ^{13}C NMR($CDCl_3$): 23.80, 41.07, 51.86, 53.61, 58.43, 76.10, 123.26, 126.95, 127.84, 128.46, 130.44, 135.04, 142.46, 148.22, 151.92, 165.38.

N-[4-[4-(Diphenylmethyl)-1-piperazinyl]butyl]-3-pyridinecarboxamide ***1e***. ^{1}H-NMR ($CDCl_3$): 1.63 (m, 4H, CCH_2C), 2.35–2.46 (cluster, 10H, NCH_2), 3.45 (q, 2H, $CONHCH_2$), 4.12 (s, 1H, CH), 7.11–7.40 (cluster, 10H, phenyl, 1H, pyridine, 1H, CONH), 8.07 (m, 1H, pyridine), 8.69 (m, 1H, pyridine), 8.94 (m, 1H, pyridine). ^{13}C-NMR ($CDCl_3$): 24.40, 27.34, 39.97, 51.60, 53.39, 57.85, 76.13, 123.38, 126.88, 127.83, 128.41, 130.81, 135.08, 142.57, 147.99, 151.94, 165.38.

N-[4-[4-(Diphenylmethyl)-1-piperazinyl]butyl]-1-adamantanecarboxamide ***1f***. ^{1}H-NMR ($CDCl_3$): 1.45–2.02 (cluster, 19H, adamantane, CCH_2C), 2.30–2.43 (cluster, 10H, NCH_2), 3.22 (q, 2H, $CONHCH_2$), 4.20 (s, 1H, CH), 7.16–7.43 (cluster, 10H, arom, 1H, CONH). ^{13}C NMR ($CDCl_3$): 24.25, 27.62, 28.13, 36.52, 39.09, 39.30, 40.53, 51.86, 53.50, 58.13, 76.28, 126.88, 127.92, 128.43, 142.73, 165.25.

N-[4-[4-(Diphenylmethyl)-1-piperazinyl]butyl]-2-norbonanecarboxamide (mixture of isomers) ***1g***. ^{1}H-NMR ($CDCl_3$: 1.10–1.61 (cluster, 12H, CCH_2C), 1.85 (m, 1H, CH, norbornane), 2.05 (m, 1H, CH, norbornane), 2.27–2.50 (cluster, 10H, NCH_2, 1H, CH, norbornane), 3.22 (q, 2H, $CONHCH_2$), 4.26 (s, 1H, CH), 6.09 (t br, 1H, CONH), 7.12–7.42 (cluster, 10H, arom). ^{13}C-NMR ($CDCl_3$): 23.86, 27.41, 28.62, 29.80, 34.29, 35.84, 36.42, 39.01, 41.58, 47.98, 51.42, 53.19, 57.67, 76.10, 126.91, 127.82, 128.43, 142.42, 175.67.

N-[4-[4-(Diphenylmethyl)-1-piperazinyl]butyl]-5-norbornene-2-carboxamide (mixture of isomers) ***1h***. ^{1}H-NMR ($CDCl_3$): 1.21–1.98 (cluster, 8H, CCH_2C), 2.39–2.79 (cluster, 10H, NCH_2), 2.84 (m, 1H, CH, norbornene), 2.89 (m, 1H, CH, norbornene), 3.09 (m, 1H, CH, norbornene), 3.22 (q, 2H, $CONHCH_2$), 4.22 (s, 1H, CH), 5.75 (t br, 1H, CONH), 5.95 (m, 1H, CH=CH), 6.09–6.25 (cluster, 1H, CH=CH), 7.12–7.42 (cluster, 10H, arom). ^{13}C NMR ($CDCl_3$): 23.97 (24.13), 27.49 (27.54), 29.85 (30.43), 39.06 (39.24), 41.53 (42.68), 44.72 (44.74), 46.24 (46.27), 47.28 (49.96), 51.56, 53.32, 57.79 (57.87) 126.92, 127.86, 128.45, 132.21 (135.98), 137.62 (138.23), 142.55, 174.10 (175.45).

Pharmacology

Adult male Dunkin–Hartley guinea-pigs (250–300 g) and Wistar rats (180–250 g) were used. Before use, animals were housed in rooms at 18–20 °C with a 12-h light/dark cycle and relative humidity of 55–60%, and allowed food and water ad libitum. They were distributed at random into treated and control groups, and were fasted 17 h before the experiment.

Histamine dihydrochloride (H-7250), acetylcholine chloride (A-6625), atropine sulphate (A-0257), compound 48/80 (C-4257), ketotifen (K-2628) and Percoll (P-1644) were purchased from Sigma Chemicals, Madrid, Evan's blue (3169) and formamide (9684) from Merck, Madrid, and cetirizine from Zyrtec™

(UCB Pharma). All other chemicals were used in reagent grade and purchased from standard commercial sources.

To perform in vitro assays, all tested compounds were dissolved in 0.1 mol/L aqueous tartaric acid. Atropine and ketotifen were dissolved in physiological saline solution. For in vivo experiments, tested compounds and the reference drug were suspended in 0.25% (w/v) xanthan gum aqueous solution and administered orally by gastric gavage.

For statistical evaluation of parametric data, the Student's unpaired *t*-test was used. For statistical significance, $P < 0.05$ level was required.

Histamine- and acetylcholine-induced contractions of isolated guinea-pig ileum

Fragments of distal ileum (2–3 cm length) were obtained from male guinea pigs (after cervical dislocation and exsanguination) and vertically suspended from isotonic lever transducers (HSE Type B 368) under 1.2 g resting tension in a 20-mL Hoeschst-type organ bath (Hugo Sachs Elektronik, Fribourg, Germany) containing Tyrode solution at 37 °C and oxygenated with carbogen (95% O_2, 5% CO_2). The physiological solution was changed several times during a 45–60 min equilibration period before addition of the drugs. Contractions were recorded using a Watanabe 3101 polygraph. For each ileum fragment, two initial histamine or acetylcholine cumulative concentration–response curves were obtained to calculate EC_{50} and submaximal concentrations. After washing, the preparation was allowed to equilibrate for 30 min. Then, submaximal concentrations of histamine (1 μmol/L) or acetylcholine (0.3 μmol/L) were added at 15 min intervals until a constant response was obtained. This contraction (in mm) was taken as the 100% value (control value). Different concentrations of the tested drugs (one per preparation, in triplicate) were added to the bath 30 min before the addition of the submaximal concentration of the agonist. The decrease in the height of the contraction was expressed as percentage inhibition upon comparison with the control value. Linear regression analysis was performed to determine the concentration of drugs which elicited 50% inhibition of the control value (IC_{50}). IC_{50} values were then converted into K_b values using equations derived from the Cheng–Prusoff equation for analysing antagonist inhibition curves in functional experiments [10, 11].

Histamine-induced capillary permeability increase in rats

Animals (ten per dose) were administered orally, by gastric gavage, a wide range of doses of the tested drugs (0.3–10 mg/kg). One hour later under light ether anaesthesia, histamine (5 μg in 0.1 mL saline solution) was injected intradermally at two sites on the shaved back skin of the rats. Immediately Evan's blue (EB) (25 mg/kg) dissolved in saline was injected into the tail vein. Thirty minutes later the animals were sacrificed in a CO_2 atmosphere and the pieces of skin with the blue spot (wheal) were excised [12]. The dye (EB) of each skin piece was extracted by immersion in formamide (5 mL) and heated for 48 h at 55–60 °C [13]. EB was measured spectrophotometrically at 620 nm and expressed as percentage of inhibition upon comparison with the control group. ED_{50} values were estimated graphically.

Compound 48/80-induced histamine release from rat peritoneal mast cells

Mast cells were collected from mixed peritoneal cell suspensions of eight female rats and purified (purity higher than 95%) by centrifugation on a Percoll gradient as previously described [14]. Aliquots of mast cells suspension were preincubated with tested drugs or vehicle (1 mL final volume) in quintuplicate at 37 °C for 10 min prior to stimulation. Drugs were added over a wide range of concentrations (0.016–160 μmol/L) in a 0.1 mL volume. Histamine release was then stimulated by the addition of compound 48/80 (10 μg/mL final concentration) and incubation (37 °C) was continued for 10 min. The secretion was stopped by cooling the tubes in an ice-cold bath (20 min) and centrifugation at 4000 *g*. Histamine was determined fluorimetrically both in supernatants and cell pellets [15]. Histamine release was expressed as a percentage of the total found in the cells and supernatants. All values were corrected for the spontaneous release (below 10%) occurring in the absence of the releasing agent.

Spontaneous motor activity in rats

Animals (eight per group) were administered orally, by gastric gavage, 100 mg/kg of tested drugs or vehicle and placed individually in the cages of a Digiscan Actimeter (Omnitech Electronics, Columbus, Ohio). Spontaneous motor activity was measured within 4 h, selecting horizontal and vertical activities and stereotipy counts as the main parameters to evaluate [16]. Results were expressed as percentage variation related to the vehicle-treated group.

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