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Original article

Histamine analogues. 33rd communication: 2-phenylhistamines with high histamine H_1-agonistic activity

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Summary — 2-Phenylhistamines with various substituents at the phenyl ring were synthesized and the influence of substitution in *ortho*, *meta* or *para* position on histamine H_1-agonistic activity was investigated. Compounds with high activity occurred in the *meta* phenyl series. Increased activity in the guinea-pig ileum assay was achieved by monosubstitution with halogen on the phenyl ring. 2-[2-(3-Fluorophenyl)-4-imidazolyl]ethanamine (**12**) is the most potent highly selective H_1-agonist known so far, showing 87% relative potency compared with histamine and full efficacy at the H_1-receptor. Relative activities of the 3-chloro analogue (**11**) and the 3-methyl analogue (**13**) are 81% and 29%, respectively. Histamine H_2-activity could not be detected. 2-Phenylhistamines are available *via* reaction of the corresponding benzimidates with 2-oxo-4-phthalimido-1-butyl acetate (**6**) in liquid ammonia.

Résumé — Analogues de l'histamine. 33e Communication: 2-phénylhistamines, puissants agonistes histamine-H_1. *Une série de 2-phénylhistamines avec différents substituants sur le groupe phényle a été synthétisée et l'influence des positions* ortho, méta *ou* para *sur l'activité agoniste-H_1 a été examinée sur l'iléon de cobaye. Des composés à l'activité forte apparaissent dans la série à substituant en* méta *sur le phényle. Un accroissement d'activité est observé pour la monosubstitution par un halogène sur le noyau phényle. La 2-[2(3-fluorophényl)-4-imidazolyl]éthanamine] (**12**) est le plus fort agoniste-H_1 sélectif et possède 87% de l'activité de l'histamine avec une sélectivité totale pour le récepteur-H_1. Aucune activité agoniste-H_2 n'est observée. Les 2-phénylhistamines sont accessibles par réaction des benzimidates correspondants avec le 2-oxo-4-phtalimidobutylacétate (**6**) dans l'ammoniaque.*

histamine H_1-receptor / H_1-agonists / 2-phenylhistamines

Introduction

Many efforts have been made [1, 2] to synthesize selectively acting potent histamine H_1-agonists but only a small number of compounds with reasonable potencies have been reported. By chemical modification of the histamine molecule it could be demonstrated that the imidazole nucleus is not essential for H_1-agonistic activity [3]. Among the series of heterocyclic histamine analogues 2-(2-thiazolyl)ethanamine and 2-(2-pyridyl)ethanamine (table I) have become significant pharmacological tools for the selective stimulation of H_1-receptors though they are less potent than histamine. Furthermore, pronounced H_1-selectivity, unfortunately again accompanied with a decrease of potency, was observed for 2-alkyl, 2-aryl and 2-(3-pyridyl) substituted histamines [5] (table I). 2-Phenylhistamine [5] possesses about 13% of the potency of histamine and full intrinsic activity at the guinea-pig ileum. Substitution of the phenyl moiety [6] resulted in a further decrease of potency with the exception of 2-(3-methoxyphenyl)histamine that shows about 32% of the activity of histamine at H_1-receptors.

Table I. Relative potency of histamine H_1-agonists at the guinea-pig ileum.

Histamine H_1-agonists	Rel. Potency [%]
Histamine	100
2-(2-Thiazolyl)ethanamine	20-32 [3]; 11 [7]
2-(2-Pyridyl)ethanamine	6 [3]
2-Methylhistamine	16 [4,5]
2-Phenylhistamine	13 [5]
2-(3-Methoxyphenyl)histamine	32 [6]
2-(3-Pyridyl)histamine	13 [5]

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Since only a limited series of substituted 2-phenylhistamines have been investigated so far [6], the aim of the present study was to gain further information about structure-activity relationships concerning the phenyl substitution pattern and obtain even more potent selective H_1-agonists.

Recently, in the field of H_1-receptor research, interest has been focussed on H_1-receptor mediated positive inotropic effects [8, 9] and vasodilatation following release of endothelium derived relaxing factor (EDRF) which is initiated by H_1-stimulation [10-14]. As pointed out above, the repertoire of tools for histamine receptors is lacking potent and selective H_1-agonists.

Results

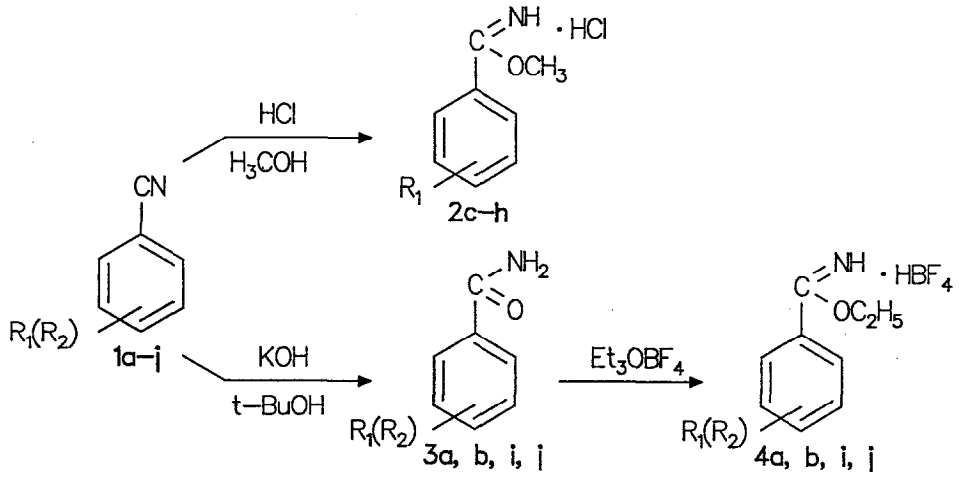
Chemistry

All 2-substituted histamine derivatives were prepared using the general methods I or II (scheme 2). The benzonitriles (**1a–j**) as starting materials were purchased from commercial sources. The appropriate benzimidate salts were directly obtained according to Pinner [15] by reaction of the nitrile and anhydrous methanol in the presence of hydrogen chloride (**2c–h**) or by converting them into benzamides [16] (**3a, b, i, j**) followed by O-alkylation with triethyloxonium tetrafluoroborate in methylene chloride described by Weintraub [17] (**4a, b, i, j**) (scheme 1).

In order to avoid poor overall yields as a result of gradual build-up of the histamine side chain [18] it was intended to obtain 2-(2-phenyl-4-imidazolyl)ethanamines *via* reaction of an imidate salt with a bifunctional synthon. Two starting compounds were tested for their suitability (scheme 2): method I represents the reaction of benzimidates with 2-oxo-4-phthalimido-1-butyl acetate (**6**) [19] synthesized in accordance with the Michael reaction from 2-oxo-3-buten-1-yl acetate [20] and phthalimide in triethylamine and DMF. 2-Bromo-4-phthalimidobutyraldehyde (**7**) used in method II was available by bromination of 4-phthalimidobutyraldehyde [21] in acetic acid. The polar products, obtained after reaction of the benzimidate salts (except for **19**), of formamidine acetate and for **14**, of 4-aminobenzamidine·2HCl (**5**) with **6** or **7** in liquid ammonia in an autoclave, had to be worked up in a skilful manner [22]. The crude dark brown oily amines were purified by precipitation of the picrate in the case of **19**, or by derivatisation with N-ethoxycarbonylphthalimide in ethanol. The corresponding phthalimide could be separated by column chromatography on silicagel. Subsequent hydrazinolysis led to the pure amines **8–18**. Comparing method I and II, the first one has the advantage that **6** is conveniently available and the storage of this solid is without problems, although only fair yields are attainable. The disadvantage of method II is not only that the synthon **7** is instable and has to be prepared freshly each time but also that yields in the subsequent step are poor. Under mild conditions, only 5% of compound **13** were isolated. Independent of the chosen method (I or II) the isolation of the primary amines is rather tedious.

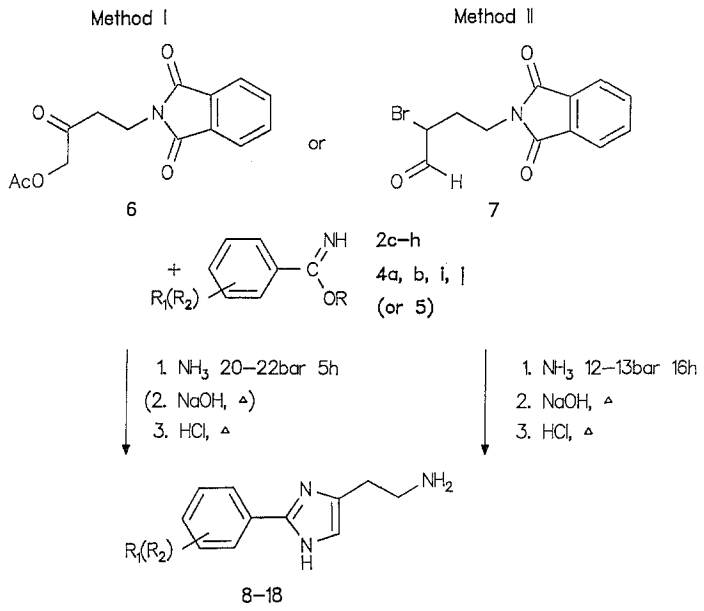
Pharmacology

The novel compounds were screened for H_1-agonistic activity according to a modified procedure of Len-



	R_1	R_2
a	2–Cl	
b	2–F	
c	3–NH_2	
d	3–Cl	
e	3–F	
f	3–CH_3	
g	4–$N(CH_3)_2$	
h	4–F	
i	2–F	3–F
j	3–F	5–F

Scheme 1. Synthesis of benzimidate salts.

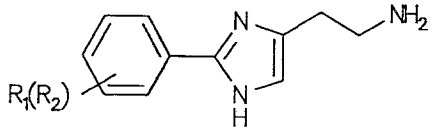


Scheme 2. Synthesis of 2-phenylhistamines.

nartz *et al* [23] on the isolated guinea-pig ileum. The pharmacological results are summarized in table II.

The majority of the synthesized 2-phenylhistamines possesses significant histamine H_1-agonistic activity. For compounds **10**, **13** and **14** the affinity was about one order of potency greater than recently reported [24]. Obviously the introduction of a methyl-, chloro- or fluoro-substituent in *meta* position of the phenyl ring (**11–13**) achieves a large increase of the histaminergic effect. These compounds have full intrinsic activity and the pD_2-values [25, 26] range from 6.32 to 6.79, this means a relative potency of 29.5 to 87.1% compared with histamine. Substituents in *para* position produce weaker H_1-agonistic activity (**14**, **16**) and *ortho* residues (**8**, **9**) lead to a decrease of the H_1-agonistic potency below the range of the unsubstituted parent molecule 2-phenylhistamine. The potency of the *meta* fluoro derivative **12** is diminished by the introduction of a second fluoro substituent in the 2 and 5 position, respectively. However, complete loss of intrinsic activity as reported for the 3,4-dichloro compound [24] was not observed. Neither H_1-agonistic nor H_1-antagonistic activity is observed for

Table II. Structures, formulas and results of the pharmacological screening on the isolated guinea-pig ileum for 2-phenylhistamines.



cpd.	$R_1(R_2)$	formula[a]	method[b]	mp [°C]	yield [%]	H_1 (guinea-pig ileum) intrinsic activity	pD_2	nd	rel.potency [%]
8	2-chloro	$C_{11}H_{12}ClN_3 \cdot 2C_4H_4O_4$	I	160	8	0.8	4.90	2	1.1
9	2-fluoro	$C_{11}H_{12}FN_3 \cdot 2C_4H_4O_4$	I	170	18	0.9	5.20	2	2.2
10	3-amino	$C_{11}H_{14}N_4 \cdot 2C_4H_4O_4$	I	156	22	1.0	5.71±0.20[e]	10	7.3
11	3-chloro	$C_{11}H_{12}ClN_3 \cdot 2C_4H_4O_4$	I	158	15	1.0	6.76±0.18	9	81.3
12	3-fluoro	$C_{11}H_{12}FN_3 \cdot 2C_4H_4O_4$	I	167	32	1.0	6.79±0.13	9	87.1
13	3-methyl	$C_{12}H_{15}N_3 \cdot 2C_4H_4O_4$	II	163	5	1.0	6.32±0.06[f]	6	29.5
14	4-amino	$C_{11}H_{14}N_4 \cdot 2C_4H_4O_4$	I	170	30	1.0[g]	5.27±0.19[h]	6	2.6
15	4-dimethyl-amino	$C_{13}H_{18}N_4 \cdot 2C_4H_4O_4$	I	174	19	0	0	2	0
16	4-fluoro	$C_{11}H_{12}FN_3 \cdot 2C_4H_4O_4$	I	174	21	1.0	6.00	2	14.1
17	2,3-difluoro	$C_{11}H_{11}F_2N_3 \cdot 2C_4H_4O_4$	I	169	15	0.7	5.40	2	3.6
18	3,5-difluoro	$C_{11}H_{11}F_2N_3 \cdot 2C_4H_4O_4$	I	163	19	0.9	5.94±0.17	7	12.3
19	histamine	$C_5H_9N_3 \cdot 2HCl$	I	244	51	1.0	6.85[i]	>20	100

[a]All compounds were analyzed for C, H, N. [b]Method refers to general procedure in the Experimental Section. [c]No attempts were made to optimize yields. [d]Number of determinations. [e]4.9 reported [24]. [f]4.9 reported [24]. [g]0.7 reported [24]. [h]4.4 reported [24]. [i]6.7 reported [24].

compound **15**. Highest H_1-agonistic potency on the ileum was found for the *meta*-halogenated derivatives **11** and **12**. Concentration-response curves in comparison with histamine are presented in figures 1–2. Statistically significant differences were not observed when 4 consecutive curves were recorded for **11** on the same organ preparation. The same was found for **12**. Both agonists could easily be washed out after maximal stimulation. The H_1-selectivity of **11** and **12** was examined *versus* different concentrations of diphenhydramine [27] (10^{-8} M to 10^{-6} M) and atropine (10^{-8} M to 10^{-7} M). The range of concentrations for atropine was chosen as mentioned above to avoid an H_1-antagonistic contribution (pA_2 for atropine: 8.91 *versus* acetylcholine and 5.91 *versus* histamine [28]). The rightward shift of the concentration-response curves of **11** and **12** produced by diphenhydramine was analyzed according to Schild [29] (figs 3, 4 and table III). The pA_2-value lies around 8 and is in good agreement with the literature value [27]. However the slope for the fluoro derivative **12** is significantly less than unity thus indicating non-competitive antagonism, while the interaction **11** - diphenhydramine is competitive (slope – 0.94). Atropine did not shift the concentration-response curve of **12**, but moved the curve of **11** by ≈ 0.28 log units to the right in a concentration independent manner (Student's *t*-test $P = 0.05$).

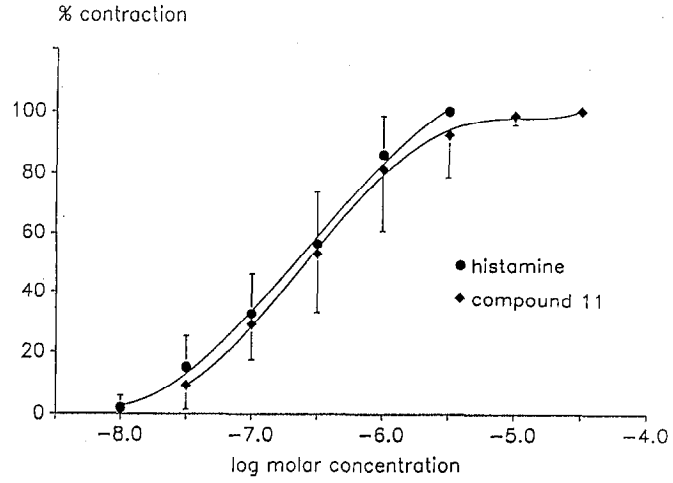


Fig 1. Cumulative concentration-response curves of 2-[2-(3-chlorophenyl)-4-imidazolyl]ethanamine (**11**) and histamine as the reference substance.

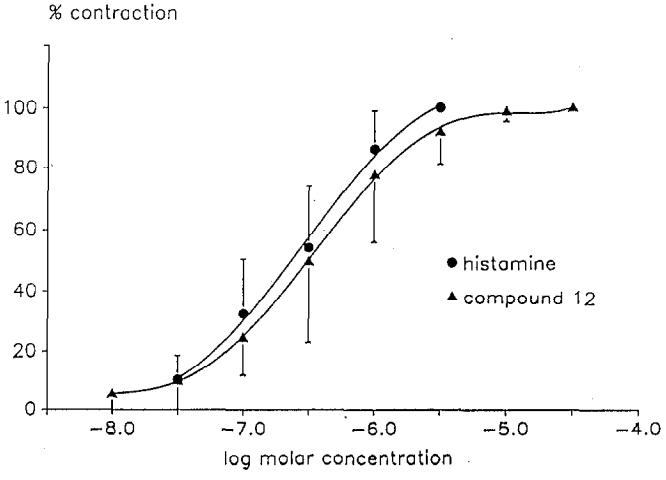


Fig 2. Cumulative concentration-response curves of 2-[2-(3-fluorophenyl)-4-imidazolyl]ethanamine (**12**) and histamine as the reference substance.

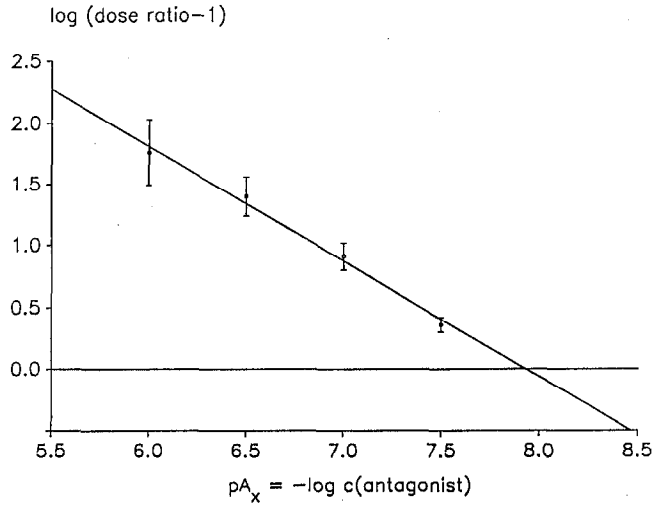


Fig 3. Schild plot for diphenhydramine, 2-[2-(3-chlorophenyl)-4-imidazolyl]ethanamine (**11**) used as agonist.

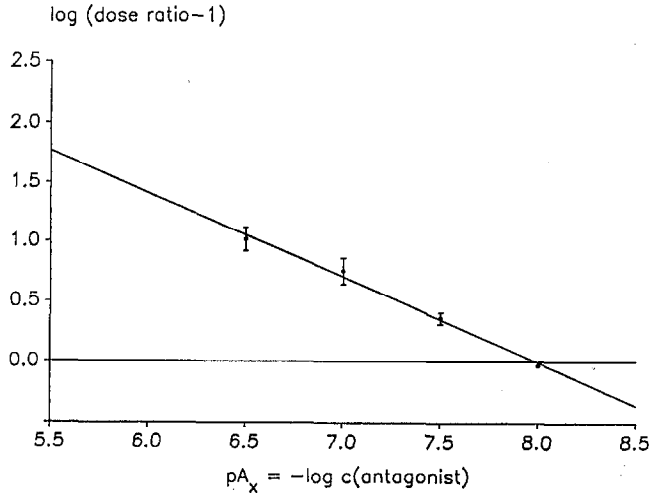


Fig 4. Schild plot for diphenhydramine, 2-[2-(3-fluorophenyl)-4-imidazolyl]ethanamine (**12**) used as agonist.

Table III. H_1-antagonistic activity of diphenhydramine.

	Schild plot [29]		
agonist	pA_2	slope	r
histamine	8.14 [27]		
11	7.93	-0.94±0.06	-0.996
12	8.00	-0.71±0.04	-0.994

11 and **12** were additionally assayed for H_2-agonistic activity on the isolated spontaneously beating guinea-pig right atrium and showed some chronotropic and inotropic stimulatory effects in concentrations between 10^{-5} and 10^{-4}M. By registrating cumulative concentration-response curves an increase of the effects was observed immediately after addition of **11** or **12** followed by a quick decrease. These effects were not sensitive to the presence of the H_2-antagonist cimetidine (10^{-5}M) and could not be abolished with the ß-blocker metoprolol (10^{-5}M). Thus, the effect seems to be neither histamine nor noradrenaline mediated but of another nature.

Preliminary experiments indicate only very weak interactions of the parent molecule 2-phenylhistamine with the cerebral H_3-receptor (JC Schwartz, personal communication).

Conclusions

2-[2-(3-Fluorophenyl)-4-imidazolyl]ethanamine (**12**) and its chloro analogue **11** are the most potent highly selective H_1-agonists known so far, showing 80–90% relative potency compared with histamine and full efficacy at the ileal H_1-receptor. Histamine H_2-activity could not be detected. An examination of all known pharmacological data of 2-phenylhistamines [5, 6, 24] reveals that there is no simple correlation between lipophilicity (π), electronical influence (σ) or sterical effects (E_s-constants [30]) and stimulation of H_1-receptors. In the limited series of *ortho* halogenated compounds none of the substituents are favourable for H_1-activity. Among the *para* substituted derivatives only the fluoro and the hydroxy compound reveal more than 10% potency while the chloro analogue has been reported as a weak H_1-antagonist [6, 24]. The conclusion might be that *para* substituents bulkier than hydroxy or 3,4-disubstitution bulkier than difluoro drastically reduce the H_1-agonistic effect. On the other hand, a variety of substituents at the *meta* position is tolerated independent of their electronic properties (3-NO_2, 3-F, but also 3-OCH_3, 3-CH_3). Interestingly, in the series of pyridyl substituted histamines 2-[2-(3-pyridyl)-4-imidazolyl]ethanamine [5] unites maximal potency and full intrinsic activity. For this compound and for 3-F, 3-OH, 3-OCH_3 and 3-NH_2 phenylhistamines, H-bonding at the active site of the H_1-receptor might be involved while this is unlikely in the case of the 3-Cl and the 3-CH_3 compounds. Though **11** and **12** appear to be equipotent, their molecular mode of action is probably different as indicated by the Schild plot slopes. Further pharmacological evaluations in the *meta* substituted phenyl series as well as the pyridyl series are necessary to decide whether this group of H_1-agonists has to be divided into 2 subgroups.

Experimental protocols

Chemistry

Melting points were determined on a Büchi 512 melting point apparatus and are uncorrected. IR spectra were measured on a Perkin Elmer 1420 Spectrophotometer. ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 solution on a Bruker AC 300 Spectrometer with TMS as an internal reference. Chemical shifts relative to TMS are reported in parts per million (δ). Analytical results for compounds indicated by the molecular formula are within ± 0.4% of the theoretical values. The stationary phase used for column chromatography was Baker silicagel type 0253 (0.05-0.2 mm). Methylene chloride / methanol/ ammonia mixtures were used as eluants.

Method I

30 mmol benzimidate salt (or amidine salt) and 30 mmol 2-oxo-4-phthalimido-1-butyl acetate (**6**) were mixed with 150 ml of liquid ammonia. The autoclave was sealed and stirred overnight at room temperature and then heated for 5 h up to 60°C (22 bar). After evaporation of ammonia the residue was precipitated in water. 6 N NaOH was added to bring the liquid phase to pH > 13. Refluxing for 2 h, evaporation *in vacuo* to dryness, taking up in ethanol (250 ml), short boiling, filtering off and removing the solvent was followed by addition of 250 ml of 6 N HCl and refluxing for 6 h. The solution was then allowed to cool and phthalic acid removed by filtration. The filtrate was evaporated, re-dissolved in water and filtered. The acidic layer was washed 3 times with CH_2Cl_2 and then made strongly basic with NaOH. The desired product was extracted 6 times into methylene chloride / isopropanol (3:1), each 100 ml. The organic layer was concentrated under reduced pressure and afterwards the syrup was dissolved in ethanol and treated slowly with about 15 mmol of N-ethoxycarbonylphthalimide. The crude phthalimide was column chromatographed on silicagel (400 g) with an ammonia saturated methanol (7%)-methylene chloride (93%) eluant. Subsequent hydrazinolysis afforded the amine. In some cases, the pale yellow oils had to be separated from minor impurities with a short column (40 g of silicagel; ammonia saturated methanol (10%)-methylene chloride (90%) as the eluant). The base was treated with maleinic acid to form a salt which was recrystallized once or twice.

Method II

31.2 mmol benzimidate salt, 31.2 mmol 2-bromo-4-phthalimidobutyraldehyde (**7**) and 150 ml of liquid ammonia were mixed. The autoclave was sealed and immediately heated, the pressure rose up to 12-13 bar and stirring was continued for 16 h. For further workup see Method I.

Representative examples of the reactions referred to in scheme 1 or scheme 2 are given below.

*2-Oxo-4-phthalimido-1-butyl acetate (**6**)*
132.4 g (0.9 mmol) phthalimide was dissolved under heating and stirring in 126 ml of triethylamine and dry DMF (380 ml) 38.4 g (0.3 mol) 2-oxo-3-buten-1-yl acetate [20] was added and the reaction mixture was stirred for 1.5 h at 90-100°C. The solvent was removed *in vacuo* and the crystalline residue was suspended in $CHCl_3$ (600 ml), stirred overnight and filtered off. The filtrate was washed 15 times with 0.2 N-NaOH (each 200 ml) and twice with water (each 100 ml). The organic phase was evaporated to dryness and the solid was triturated with ether, filtered and washed with ether to give 47.9 g (58%) of **6**. An analytical sample was obtained upon recrystallization from ether / diisopropylether, mp 139-140°C (mp [19] 133-134°C (water)); ^{1}H NMR ($CDCl_3$) δ 7.86-7.71 (m, 4H, ArH), 4.67 (s, 2H, O-CH_2), 4.00 (t, J = 7.3 Hz, 2H, N-CH_2), 2.89 (t, J = 7.3 Hz, 2H, CO-CH_2-CH_2), 2.16 (s, 3H, CH_3). Anal ($C_{14}H_{13}NO_5$) C, H, N.

*2-Bromo-4-phthalimidobutyraldehyde (**7**)*
To a solution of 7.5 g (34.5 mmol) 4-phthalimidobutyraldehyde [21] in 84 ml of acetic acid 5.3 g bromine (33.2 mmol) was added dropwise at 10°C. The reaction mixture was stirred at room temperature under exclusion of light. After discoloration it was stirred again for 30 min and then poured into an emulsion of ice water (1 l) and $CHCl_3$ (330 ml). The phases were separated and the water layer extracted again with $CHCl_3$. The combined extracts were washed with water and concentrated under reduced pressure (bath temperature below 25°C) and yielded 9.6 g (94%) clear viscous oil that was immediately processed further. ^{1}H NMR ($CDCl_3$) δ 9.48 (d, J = 1.5 Hz, 1H, CHO), 7.89-7.73 (m, 4H, ArH), 4.38-4.33 (dt, J_1 = 1.5 Hz, J_2 = 7.0 Hz, *H*CBr-CHO), 3.92-3.87 (t, 2H, N-CH_2), 2.63-2.18 (m, CH_2-CHBr).

*3-Fluorobenzenecarboximidic acid methyl ester (**2e**)*
Dry hydrogen chloride was passed at – 5 to 0°C into a solution of 5.0 g (41.3 mmol) 3-fluoro-benzonitrile (**1e**) in 150 ml of absolute methanol until saturation. The flask was tightly stoppered and the solution was allowed to stand in a freezer for 4 d. The solvent was then removed *in vacuo* (bath temperature below 25°C) to give 6.5 g (83%) of a semi-solid residue. This material was used for the next step without further purification.

*2-[2-(3-Fluorophenyl)-4-imidazolyl]ethanamine (**12**)*
Compound **12** was obtained *via* the general method I and recrystallized from dry ethanol-ether. ^{1}H NMR (DMSO-d6) δ 7.81 (br, 3H, exch/D_2O, NH_3+), 7.76 (m, 2H, ArH), 7.54 (m, 1H, ArH), 7.22 (m, 1H, ArH), 7.19 (s, 1H, ArH), 6.14 (s, 4H, Mal), 3.12 (br, 2H, CH_2-N), 2.86 (t, J = 7.1 Hz, 2H, CH_2-Im).

*2-[2-(3-Aminophenyl)-4-imidazolyl]ethanamine (**10**)*
Compound **10** was obtained *via* the general method I and recrystallized from dry ethanol-ether. ^{1}H NMR (DMSO-d6) δ 7.84 (br, 3H, exch/D_2O, NH_3+), 7.32 (s, 1H, ArH), 7.19 (dd, J = 7.8/7.8, 1H, ArH), 7.05 (m, 2H, ArH), 6.71 (m, 1H, ArH), 6.09 (s, 4H, Mal), 3.43 (br, 2H, exch/D_2O, NH_2), 3.15 (br, 2H, CH_2-N), 2.91 (t, J = 7.1 Hz, 2H, CH_2-Im).

*2-{2-[4-(N,N-Dimethylamino)phenyl]-4-imidazolyl}ethanamine (**15**)*
Compound **15** was obtained *via* the general method I and recrystallized from dry ethanol-ether. ^{1}H NMR (DMSO-d6) δ 7.80 (br, 3H, exch/D_2O, NH_3+), 7.77 (m, 2H, ArH), 7.38 (s, 1H, ArH), 6.87 (m, 2H, ArH), 6.13 (s, 4H, Mal), 3.17 (br, 2H, CH_2-N), 3.02 (s, 6H, $(H_3C)_2N$), 2.94 (t, J = 7.1 Hz, 2H, CH_2-Im).

*2-[2-(4-Fluorophenyl)-4-imidazolyl]ethanamine (**16**)*
Compound **16** was obtained *via* the general method I and recrystallized from dry ethanol-ether. ^{1}H NMR (DMSO-d6) δ 7.97 (m, 2H, ArH), 7.84 (br, 3H, exch/D_2O, NH_3+), 7.41 (m, 2H, ArH), 7.24 (s, 1H, ArH), 6.13 (s, 4H, Mal), 3.14 (br, 2H, CH_2-N), 2.88 (t, J = 7.1 Hz, 2H, CH_2-Im).

*2-[2-(3-Chlorophenyl)-4-imidazolyl]ethanamine (**11**)*
After ring formation and evaporation of ammonia, the residue was treated with 300 ml of 6 N HCl and refluxed for 6 h. Further purification followed the described method I. Recrystallization from dry ethanol-ether. ^{1}H NMR (DMSO-d6) δ 8.00 (m, 1H, ArH), 7.89 (m, 1H, ArH), 7.82 (br, 3H, exch/D_2O, NH_3+), 7.48 (m, 2H, ArH), 7.19 (s, 1H, ArH), 6.14 (s, 4H, Mal), 3.12 (br, 2H, CH_2-N), 2.86 (t, J = 7.2 Hz, 2H, CH_2-Im).

*2-[2-(2-Chlorophenyl)-4-imidazolyl]ethanamine (**8**)*
Compound **8** was prepared from **4a** as described for the synthesis of **11**. ^{1}H NMR (DMSO-d6) δ 7.81 (br, 3H, exch/D_2O, NH_3+), 7.78 (m, 1H, ArH), 7.59 (m, 1H, ArH), 7.47 (m, 2H, ArH), 7.23 (s, 1H, ArH), 6.13 (s, 4H, Mal), 3.13 (br, 2H, CH_2-N), 2.88 (t, J = 7.3 Hz, 2H, CH_2-Im).

*2-[2-(2-Fluorophenyl)-4-imidazolyl]ethanamine (**9**)*
Compound **9** was prepared from **4b** as described for the synthesis of **11**. ^{1}H NMR (DMSO-d6) δ 7.99 (m, 1H, ArH), 7.79 (br, 3H, exch/D_2O, NH_3+), 7.45-7.30 (m, 3H, ArH), 7.18 (s, 1H, ArH), 6.13 (s, 4H, Mal), 3.12 (br, 2H, CH_2-N), 2.87 (t, J = 7.2 Hz, 2H, CH_2-Im).

*2-[2-(4-Aminophenyl)-4-imidazolyl]ethanamine (**14**)*
Compound **14** was synthesized from 4-aminobenzamidine• 2HCl (**5**) and **6** in liquid ammonia following method I. The crude phthalimide was obtained after extraction into EtOAc. Hydrazinolysis and column chromatography (40 g silicagel, ammonia saturated methanol (12%)-methylene chloride (88%) as eluant) led to the pure amine **14**. Treatment with maleinic acid and recrystallization from dry ethanol-ether led to yellow crystals. ^{1}H NMR (DMSO-d6) δ 7.87 (br, 3H, exch/D_2O, NH_3+), 7.63 (m, 2H, ArH), 7.36 (s, 1H, ArH), 6.68 (m, 2H, ArH), 6.06 (s, 4H, Mal), 3.42 (br, 2H, exch/D_2O, NH_2), 3.15 (br, 2H, CH_2-N), 2.92 (t, J = 7.0 Hz, 2H, CH_2-Im).

*3,5-Difluorobenzamide (**3j**)*
A solution of 9.9 g (71 mmol) 3,5-difluorobenzonitrile (**1j**) in t-BuOH (100 ml) was heated under reflux with 20 g of finely powdered KOH for 1 hour. The cold suspension was treated with 200 ml of an aqueous sodium chloride solution (5%) and extracted 3 times with $CHCl_3$ and the organic layer dried over Na_2SO_4. After evaporation the solid was washed with cold petroleum ether and gave 8.4 g (75%) white crystals, mp 146°C; ^{1}H NMR ($CDCl_3$) δ 7.35-7.32 (m, 2H, ArH), 7.03-6.96 (m, 1H, ArH), 6.29-5.58 (br, 2H, $CONH_2$). Anal ($C_7H_5F_2NO$) C, H, N.

*3,5-Difluorobenzenecarboximidic acid ethyl ester (**4j**)*
To a stirred suspension of 6.0 g (38.2 mmol) **3j** in 60 ml of dry methylene chloride 38.2 mmol Et_3OBF_4 (1 M commercial solution in CH_2Cl_2) was added dropwise. After a few min a clear solution resulted. The mixture was stirred overnight at

room temperature and most of the product precipitated during the reaction. 150 ml of dry ether was added and the stoppered flask was kept in a freezer for 2 h. The solid was filtered off and dried *in vacuo*, yield 6.2 g (60%). An analytical sample was obtained by recrystallization from cold methylene chloride-ether, mp 167°C; IR (KBr) 1634 (C=N), 1102 cm^{-1} (BF_4–). Anal ($C_9H_{10}BF_6NO$) C, H, N.

*2-[2-(3.5-Difluorophenyl)-4-imidazolyl]ethanamine (**18**)*
Compound **18** was prepared from **4j** as described for the synthesis of **11**. Recrystallization from dry ethanol-ether. ^1H NMR (DMSO-d6) δ 7.81 (br, 3H, exch/D_2O, NH_3+), 7.63 (m, 2H, ArH), 7.24 (m, 1H, ArH), 7.19 (s, 1H, ArH), 6.15 (s, 4H, Mal), 3.11 (br, 2H, CH_2-N), 2.85 (t, J = 7.2 Hz, 2H, CH_2-Im).

*2-[2-(2.3-Difluorophenyl)-4-imidazolyl]ethanamine (**17**)*
Compound **17** was prepared from **4i** as described for the synthesis of **11**. ^1H NMR (DMSO-d6) δ 7.80 (br, 3H, exch/D_2O, NH_3+), 7.76 (m, 1H, ArH), 7.50-7.30 (m, 2H, ArH), 7.18 (s, 1H, ArH), 6.15 (s, 4H, Mal), 3.12 (br, 2H, CH_2-N), 2.86 (t, J = 7.3 Hz, 2H, CH_2-Im).

*2-(4-Imidazolyl)ethanamine histamine (**19**)*
Compound **19** was prepared from formamidine acetate and **6** according to that described in method I. After refluxing in 300 ml of 6 N HCl for 6 h the mixture was cooled, filtered off and washed 3 times with CH_2Cl_2. The acidic layer was evaporated to dryness and treated with water and sodium carbonate to bring the pH to 8.5-9. The solution was washed with methylene chloride and poured into 1.3 l of a warm aqueous picric acid solution (1.2% (m/m)). The precipitated solid was filtered at room temperature. Repeated concentration of the mother liquor was necessary to improve the yield. The combined crystalline fractions were recrystallized from water to give 10.0 g (59%) of pure picrate mp 240°C (mp [31] 239°C). The picrate was converted into the hydrochloride.

*2-[2-(3-Methylphenyl)-4-imidazolyl]ethanamine (**13**)*
Compound **13** was synthesized according to method II. ^1H NMR (DMSO-d6) δ 7.83 (br, 3H, exch/D_2O, NH_3+), 7.74 (m, 2H, ArH), 7.42 (m, 1H, ArH), 7.29-7.27 (m, 2H, ArH), 6.11 (s, 4H, Mal), 3.15 (br, 2H, CH_2-N), 2.89 (t, J = 7.2 Hz, 2H, CH_2-Im), 2.39 (s, 3H, CH_3).

Pharmacology

H_1-Agonism on the isolated guinea-pig ileum
Ileum strips of about 1.5 cm from guinea pigs (350-500 g) of either sex were placed in a 20 ml organ bath and after 5 min loaded with 0.5 g (Tyrode solution gassed with carbogen, 37°C). The organs were allowed to stabilize for 20 min, then the tissue was stimulated with 1 μM histamine. After 10 min of repose and control of the tension, the procedure was repeated twice. After 10 min, 2 control concentration-response curves of histamine were recorded isotonically (cumulative technique as described by [23, 25]). The tissue was washed thoroughly and 10 min later the cumulative curve of the agonist (**8**–**18**) was registered. H_1-Receptor selectivity was verified in a first approach by addition of 10^{-6}M of diphenhydramine at the maximal response. After that the organ was replaced by a fresh one. Intrinsic activity and pD_2-value of each agonist was determined according to [23, 25].

Antagonistic effect of diphenhydramine or atropine on the isolated guinea-pig ileum
Ileum strips were prepared and stimulated 3 times with 1 μM of histamine as described above. After 10 min of repose a cumulative concentration-response curve of the agonist was recorded isotonically. After washing out and 10 min rest the antagonist was added to the bath fluid and allowed to distribute during 5 min. Subsequently, a second cumulative curve of the agonist was recorded. This procedure was repeated with up to 2 higher concentrations of antagonist. pA_2-values and slopes were determined according to Arunlakshana and Schild [29].

H_2-Agonism on the spontaneously beating guinea-pig atrium
Guinea-pigs of either sex (350-500 g) were sacrificed by a blow on the head. Right atria were rapidly removed, attached to a tissue holder in a 20 ml organ bath (32.5°C) containing McEwens solution [32] and gassed with carbogen. After 30-40 min of equilibration, concentration response curves were recorded isometrically (cumulative technique) as described by [23, 25], using histamine as the reference substance.

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