Synthesis and Structure–Activity Relationships of a New Series of Benzimidazoles as H_1-Antihistaminic Agents

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New 2-(4-(4-azolylbutyl)piperazinyl)-, 2-(4-(4-azolylbutyl)piperazinylmethyl)-, 2-(4-(4-azolylbutyl)homopiperazinyl)- and 2-(4-(4-azolylbutyl)homopiperazinylmethyl)benzimidazoles were synthesized, characterized and tested for *in vitro* and *in vivo* H_1-antihistaminic activity. Structure–activity relationships implied that the best antihistaminic activity required the simultaneous presence of a homopiperazinylbenzimidazole system (or a methylene linker between the benzimidazole and the piperazine rings) and an unsubstituted pyrazole ring. 1-(2-Ethoxyethyl)-2-{4-[4-(pyrazol-1-yl)butyl]homopiperazin-1-yl}benzimidazole (17), as its dimaleate salt, has been chosen for further development.

Key words H_1-antihistaminic drug; azolyl-butyl-piperazinyl (or homopiperazinyl)benzimidazole; synthesis; structure–activity relationship

Although several classical histamine H_1 receptor antagonists are very potent *in vitro*, the clinical usefulness of these compounds has been limited due to unwanted side-effects. An effective H_1 antihistaminic agent which would be free from sedative and anticholinergic activity is an attractive target for drug research.[1]

General screening of a series of anxiolytics azolylalkyl(piperazinyl)pyrimidines synthesized in our laboratories[2] showed that compound **1** possessed weak H_1 antagonism *in vitro* against histamine-induced contractions of the guinea pig ileum. This observation led us to design and synthesize two series of compounds (Fig. 1): the diphenylmethyl series I[3] and the benzimidazolyl series II.[4,5] Preliminary accounts of the synthesis and activity studies on these series of compounds have been previously reported.[6,7]

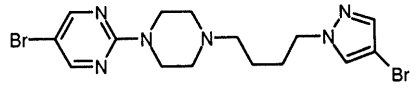
Quantitative structure–activity relationships and molecular modelling studies performed on both classical and non-classical H_1-antagonists have been reviewed and discussed.[8] Recently,[9] we have reported a pharmacophoric analysis for classical and non-classical H_1-antagonists including the benzimidazolyl series II.

In this paper, we describe the synthesis and structure–activity relationships of new 2-(4-(4-azolylbutyl)-piperazinyl)-, 2-(4-(4-azolylbutyl)piperazinylmethyl)-, 2-(4-(4-azolylbutyl)homopiperazinyl)- and 2-(4-(4-azolylbutyl)homopiperazinylmethyl)benzimidazoles, II. Novel, potent and nonsedative antihistaminic agents were found, and compound **17** was selected for further evaluation.

Chemistry and Structure–Activity Relationships The target compounds II (Table 1) were obtained in two related ways. The synthetic routes are outlined in Chart 1. The desired compounds **2**—**8**, **10**—**12**, **14**—**18** and **20** were prepared either by alkylating III with 1-(4-chlorobutyl)-azole IV (method A), or by reacting V with the corresponding azole VI (method B). The carboxylic acids **9**, **13**, **19** and **21** were prepared by hydrolysis of the corresponding ethylesters **8**, **12**, **18** and **20** (method C). The azoniaspiro compounds V were synthesized by reacting III with 1,4-dibromobutane (method D). Dimaleate or difumarate salts were prepared in ethanol or 2-propanol and recrystallized from the same solvent (method E). 1-(2-Ethoxyethyl)-2-(piperazin-1-yl)- and 1-(2-ethoxyethyl)-2-(homopiperazin-1-yl)benzimidazoles III ($n=0$), used as starting materials for the no-alkyl bridged derivatives **11**—**13** and **17**—**19** (Table 1), were prepared as described previously.[10] The corresponding methylene-bridged ($n=1$) derivatives III used to obtain compounds **2**—**10**, **14**—**16** and **20**—**21** (Table 1) were prepared according to reported procedures.[11]

The structures of the prepared compounds, some characteristic salts, ^{1}H-NMR data, as well as the yield and method of preparation for the new compounds **2**—**21** are shown in Table 1 and ^{13}C-NMR chemical shifts are displayed in Table 2. The new compounds were tested for H_1-antihistaminic activity *in vitro* (guinea pig ileum and H_1 receptor binding) and *in vivo* (protection of rats from compound 48/80-induced lethality). The results are listed in Table 3.

The main structural feature of the new compounds (Table 1) is the presence of a benzimidazole ring and a heteroaromatic ring both linked to a basic nitrogen through a carbon chain. The benzimidazole ring is present in only two of the antihistaminic H_1 compounds on the market: The non-classical histamine H_1 receptor antagonist astemizole[12] (1-benzylbenzimidazole derivative),



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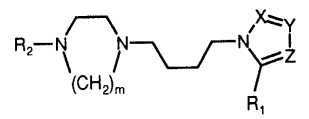

I: $R_2 = Ph_2CH$- II: $R_2 =$

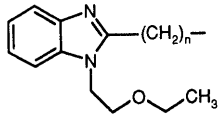

Fig. 1.

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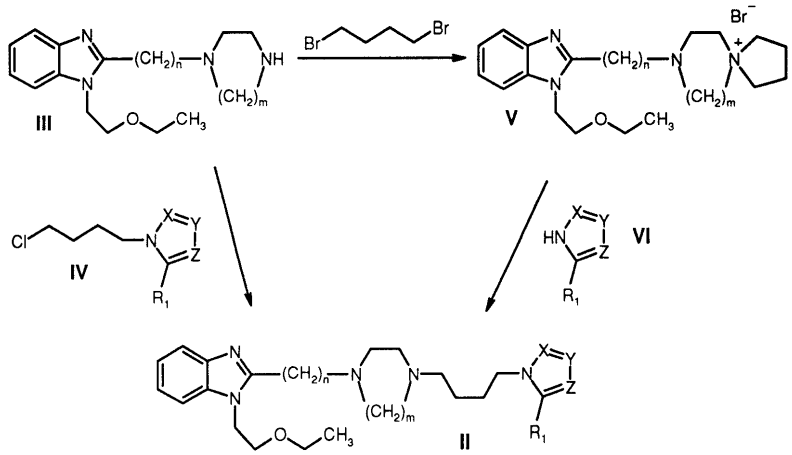


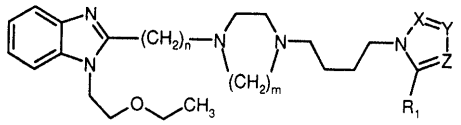
Chart 1. Synthetic Pathways for Compounds II (**2**—**21**, Table 1)

and emedastine,[13] which has a single aromatic unit. The metabolites of emedastine,[13b] quantitative structure–activity relationships of H_1-antihistaminic benzimidazole derivatives,[13c] and bioisosteric imidazo [4,5-*b*]-pyridines with H_1-antihistaminic activity[13d] have also been described.

Compound **1**, the source compound of this series, showed *in vitro* guinea pig ileum activity similar to that of diphenhydramine, but unfortunately did not protect rats from compound 48/80-induced lethality. Replacement of the 5-bromo-2-pyrimidinyl group in **1** with a 1-ethoxyethyl-2-benzimidazolyl group leads to compound **11**, which showed a slight increase in *in vitro* activity and an *in vivo* potency nine times greater than that of diphenhydramine. The addition of a methylene linker in **6** maintained the *in vitro* activity and the *in vivo* potency was increased by a factor of seven with respect to **11**. As for the 4-bromopyrazolyl derivatives **6** and **11**, a 4-carboxyl substituent (**9**, **13**) also showed higher activity in the compound with a methylene linker **9**. Replacement of the substituent in the 4-position of pyrazole with a carboxyethyl group (**8**) or sulfonic acid radical (**7**) leads to a considerable decrease in antihistaminic activity, especially *in vivo*. However the unsubstituted pyrazole derivative **2** showed good *in vitro* activity and gave the best result in protection of rats from compound 48/80-induced lethality. Concerning homopiperazinyl derivatives, the best profile was observed for the unsubstituted pyrazole compounds **17** and **14**, and for the unsubstituted pyrrole **15**. On the other hand, pyrrole and triazole derivatives containing a piperazine group (**3**, **4**) showed moderate *in vitro* activity but high *in vivo* potency, while imidazole derivatives **5**, **10** and **16** exhibited considerably weaker *in vivo* activity. With respect to binding to the H_1 histamine receptor, compounds **2**, **14**, **15** and **17**, with the highest *in vivo* potency, showed also high affinity for the receptor. In contrast, sulfonic and carboxylic acid derivatives **7**, **9**, **13**, **19** and **21** showed very low affinity for H_1 histamine receptor, and in addition showed non competitive antagonism.

In order to study the sedative effect of the new compounds, they were administered to rats at 80 mg/kg (i.p.) and the behavior of the animals was observed, following the standards described in the test of Irwin.[14] We did not observe any sedative effect of our compounds at this dose. This lack of sedative effects of the most active antihistaminic compounds listed in Table 3 prompted us to measure experimentally their distribution coefficients ($\log D$) at pH 4 or at physiological pH (7.4) for amphoteric compounds. The partition coefficients of the neutral species ($\log P$) in octanol/water and in cyclohexane/water could then be calculated on the basis of the experimental $\log D$ and pK_a values (Table 4).[15–17] It has previously been shown that the difference in the partition coefficients ($\log P$) calculated in octanol and in an aliphatic alkane such as cyclohexane or dodecane ($(\log P = \log P_{oct} - \log P_{alk})$ can be correlated with the brain-penetrating ability of histamine H_2 antagonists.[15] More recently,[16] the partition coefficient $\log D_{oct}$, at the physiological pH 7.4 has been proposed as a physicochemical parameter correlated with the lack of side effects of histamine H_1 antagonists. As can be seen in Table 4, the most active antihistaminic compounds of our series have $\log D_{oct}$ in the range 2.6—3.5 or a negative value, and $\Delta \log P$ values in the range 2.0—2.7. According to the above studies,[15,16] these values would predict, as was confirmed experimentally, a hindered brain penetration for the compounds in our series. More sedating antihistaminics such as diphenhydramine or mepyramine have $\log D_{oct}$ values at pH 7.4 between 1 and 2, and $\Delta \log P$ values lower than 1, while less sedating antihistaminics like astemizole or terfenadine present $\Delta \log P$ values similar to those observed for our non-sedating compounds. Compounds **3** and **15**, containing a pyrrole as the heteroaromatic ring, tend to have comparatively higher $\log D_{oct}$ and lower $\Delta \log P$ values than compounds **2**, **14**, and **17**, containing a pyrazole heteroaromatic ring, which showed the best antihistaminic profile. In summary, the best antihistaminic activity was seen in the simultaneous presence of: a) a methylene linker between the benzimidazole and the piperazine rings, or a homopiperazine ring without a linker as in compound **17**; and b) an unsubstituted pyrazole as the preferred heteroaromatic ring. Compound **17**, as its dimaleate salt, has been chosen for further development because of its high H_1-antihistaminic activity and its lack of side-effects.

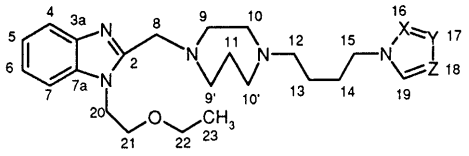
Table 1. Physicochemical Data for Azolyl-butyl-piperazinylbenzimidazole Derivatives (II)



No.	n	m	X	Y	Z	R_1	Salt mp (°C)	Method (% yield)[a]	Formula[b]	^{1}H-NMR (δ, $CDCl_3$)
2	1	2	N	CH	CH	H	122[c]	A (90) B (35)	$C_{31}H_{42}N_6O_9$[c]	1.12 (t, 3H); 1.50 (br, 2H); 1.85 (br, 2H); 2.45 (br, 10H); 3.41 (q, 2H); 3.75 (t, 2H); 3.87 (s, 2H); 4.14 (t, 2H); 4.50 (t, 2H); 6.22 (m, 1H); 7.19—7.47 (br, 5H); 7.70 (br, 1H)
3	1	2	CH	CH	CH	H	138—142[d]	A (65)	$C_{32}H_{43}N_5O_9$[d]	1.1 (t, 3H); 1.70 (br, 4H); 2.69 (br, 10H); 3.40 (q, 2H); 3.55—3.90 (m, 6H); 4.46 (t, 2H); 6.12 (m, 2H); 6.62 (m, 2H); 7.29 (br, 3H); 7.70 (br, 1H)
4	1	2	N	CH	N	H	Oil[g]	B (46)	$C_{22}H_{33}N_7O \cdot 2H_2O$	1.13 (t, 3H); 1.46 (br, 2H); 1.90 (br, 2H); 2.45 (br, 10H); 3.42 (q, 2H); 3.76 (t, 2H); 3.88 (s, 2H); 4.20 (t, 2H); 4.52 (t, 2H); 7.30 (br, 3H); 7.71 (br, 1H); 7.94 (s, 1H); 8.06 (s, 1H)
5	1	2	CH	N	CH	H	Oil[g]	B (37)	$C_{23}H_{34}N_6O \cdot 3H_2O$	1.12 (t, 3H); 1.50—1.80 (br, 4H); 2.35 (br, 10H); 3.40 (q, 2H); 3.75 (m, 6H); 4.35 (t, 2H); 6.9 (s, 1H); 7.0 (s, 1H); 7.18—7.45 (br, 4H); 7.70 (br, 1H)
6	1	2	N	CH	C–Br	H	Oil[g]	B (30)	$C_{23}H_{33}BrN_6O \cdot H_2O$	1.12 (t, 3H); 1.46 (br, 2H); 1.81 (br, 2H); 2.45 (br, 10H); 3.41 (q, 2H); 3.81 (m, 4H); 4.08 (t, 2H); 4.50 (t, 2H); 7.33 (br, 5H); 7.69 (br, 1H)
7	1	2	N	CH	C–SO_3H	H	Oil[g]	B (40)	$C_{23}H_{34}N_6O_4S \cdot 5H_2O$	[e]1.0 (t, 3H); 2.11 (br, 4H); 3.67 (br, 16H); 4.37 (t, 2H); 4.65 (t, 2H); 7.4—8.0 (br, 6H)
8	1	2	N	CH	C–CO_2Et	H	114—117[c]	B (69)	$C_{34}H_{46}N_6O_{11}$[c]	1.12 (t, 3H); 1.45 (m, 5H); 1.90 (br, 2H); 2.44 (br, 10H); 3.41 (q, 2H); 3.60—3.86 (m, 4H); 4.0—4.3 (m, 4H); 4.50 (t, 2H); 7.28 (br, 3H); 7.60—7.80 (m, 3H)
9	1	2	N	CH	C–CO_2H	H	164—166[f]	C (81)	$C_{28}H_{38}N_6O_7$[f]	1.10 (t, 3H); 1.58 (br, 2H); 1.86 (br, 2H); 2.68 (br, 10H); 3.38 (q, 2H); 3.73 (t, 2H); 3.89 (s, 2H); 4.15 (t, 2H); 4.48 (t, 2H); 7.27 (br, 3H); 7.84 (br, 3H)
10	1	2	CH	N	C–Cl	Cl	123—130[d]	B (43)	$C_{31}H_{40}Cl_2N_6O_9$[d]	1.12 (t, 3H); 1.55 (br, 2H); 1.80 (br, 2H); 2.1—2.6 (br, 10H); 3.41 (q, 2H); 3.86 (m, 6H); 4.49 (t, 2H); 7.29 (br, 4H); 7.75 (br, 1H)
11	0	2	N	CH	C–Br	H	137—139[c]	B (66)	$C_{30}H_{39}BrN_6O_9$[c]	1.13 (t, 3H); 1.60 (br, 2H); 1.87 (m, 2H); 2.55 (t, 2H); 2.76 (br, 4H); 3.44 (br, 6H); 3.81 (t, 2H); 4.12 (dt, 4H); 7.12—7.61 (m, 6H)
12	0	2	N	CH	C–CO_2Et	H	Oil[g]	B (81)	$C_{25}H_{36}N_6O_3 \cdot 3H_2O$	1.1 (t, 3H); 1.3 (t, 3H); 1.8 (br, 4H); 2.15—2.7 (br, 6H); 3.25 (br, 6H); 3.7 (t, 2H); 3.8—4.3 (m, 6H); 7.2 (br, 3H); 7.50 (br, 1H); 7.80 (s, 2H)
13	0	2	N	CH	C–CO_2H	H	145—150[g]	C (85)	$C_{23}H_{32}N_6O_3 \cdot 2H_2O$[g]	1.14 (t, 3H); 1.59 (br, 2H); 1.90 (br, 2H); 2.4—2.8 (m, 6H); 3.3—3.6 (br, 6H); 3.83 (t, 2H); 4.17 (dt, 4H); 4.88 (s, 1H); 7.13—7.31 (br, 3H); 7.55 (br, 1H); 7.92 (br, 2H)
14	1	3	N	CH	CH	H	99—102[d]	A (47)	$C_{32}H_{44}N_6O_9$[d]	1.11 (t, 3H); 1.41 (br, 2H); 1.79 (br, 4H); 2.57 (t, 2H); 2.78 (br, 8H); 3.39 (q, H); 3.75 (t, 2H); 3.96 (s, 2H); 4.12 (t, 2H); 4.55 (t, 2H); 6.22 (m, 1H); 7.2—7.47 (br, 5H); 7.68 (br, 1H)
15	1	3	CH	CH	CH	H	117—120[d]	A (56)	$C_{33}H_{45}N_5O_9$[d]	1.11 (t, 3H); 1.47 (br, 2H); 1.82 (br, 4H); 2.48 (t, 2H); 2.73 (br, 8H); 3.39 (q, 2H); 3.75 (t, 2H); 3.87 (t, 2H); 3.97 (s, 2H); 4.53 (t, 2H); 6.11 (m, 2H); 6.63 (m, 2H); 7.25 (br, 3H); 7.67 (br, 1H)
16	1	3	C–CH_3	N	C–Cl	Cl	Oil[g]	B (36)	$C_{25}H_{36}Cl_2N_6O \cdot 3H_2O$	1.11 (t, 3H); 1.5—1.9 (br, 6H); 2.36 (s, 3H); 2.5—2.9 (br, 10H); 3.39 (q, 2H); 3.7—3.9 (dt, 4H); 3.99 (s, 2H); 4.54 (t, 2H); 7.26 (br, 3H); 7.68 (br, 1H)
17	0	3	N	CH	CH	H	102—105[c]	A (90)	$C_{31}H_{42}N_6O_9$[c]	1.13 (t, 3H); 1.46 (br, 2H); 1.95 (br, 4H); 2.52 (t, 2H); 2.79 (m, 4H); 3.34—3.81 (m, 8H); 4.05—4.19 (dt, 4H); 6.20 (m, 1H); 7.0—7.5 (m, 6H)
18	0	3	N	CH	C–CO_2Et	H	Oil[g]	B (33)	$C_{26}H_{38}N_6O_3 \cdot 3H_2O$	1.13 (t, 3H); 1.33 (t, 3H); 1.93 (br, 6H); 2.6 (t, 2H); 2.8 (br, 4H); 3.35—3.82 (m, 8H); 4.07—4.4 (m, 6H); 7.1—7.25 (m, 3H); 7.5 (br, 1H); 7.85 (s, 2H)
19	0	3	N	CH	C–CO_2H	H	>300[g]	C (87)	$C_{24}H_{34}N_6O_3$[g]	[h]0.93 (t, 3H); 1.3—2.0 (br, 6H); 2.6 (br, 2H); 2.95 (br, 4H); 3.17-3.62 (m, 8H); 4.11 (m, 4H); 7.1 (br, 3H); 7.4 (br, 1H); 7.87 (s, 1H); 7.97 (s, 1H)
20	1	3	N	CH	C–CO_2Et	H	Oil[g]	B (25)	$C_{27}H_{40}N_6O_3 \cdot 3H_2O$	1.11 (t, 3H); 1.33 (t, 3H); 1.45 (br, 2H); 1.75—1.90 (br, 4H); 2.49 (t, 2H); 2.6—2.8 (br, 6H); 3.40 (q, 2H); 3.75 (t, 2H); 3.96 (s, 2H); 4.12 (t, 2H); 4.27 (q, 2H); 4.50 (t, 2H); 7.2 (m, 2H); 7.37 (br, 1H); 7.70 (br, 1H); 7.87 (s, 2H)
21	1	3	N	CH	C–CO_2H	H	Oil[g]	C (85)	$C_{25}H_{36}N_6O_3 \cdot H_2O$	[h]1.05 (t, 3H); 1.65 (br, 2H); 1.90 (br, 2H); 2.03 (br, 2H); 2.82 (t, 2H); 2.96 (m, 2H); 3.1 (m, 2H); 3.25—3.40 (br, 6H); 3.75 (t, 2H); 4.05 (s, 2H); 4.17 (t, 2H); 4.60 (t, 2H); 7.27 (m, 2H); 7.5—7.6 (m, 2H); 7.80 (s, 1H); 7.90 (s, 1H)

a) Yields not optimized except for compounds **2** (method A) and **17**. *b*) Satisfactory analytical data (±0.4% for C, H, N) were obtained for new compounds. *c*) Dimaleate. *d*) Difumarate. *e*) Solvent: DMSO-d_6. *f*) Monomaleate. *g*) Base. *h*) Solvent: D_2O.

Table 2. ^{13}C-NMR (δ, $CDCl_3$) Data for Azolyl-butyl-piperazinylbenzimidazole Derivatives (II)



	2	3	4	5	6	7[a]	8	9	10	11	13[b]	14	15	16	17	19[c]	20	21[b]
C_2	151.5	151.4	151.5	151.4	151.5	151.5	151.0	151.2	151.5	158.0	156.7	152.1	152.3	152.0	159.1	161.2	152.4	153.7
C_{3a}	142.2	142.1	142.7	142.1	142.2	141.9	142.0	141.8	142.3	141.4	140.3	141.9	142.2	141.9	141.7	143.3	142.2	142.6
C_4	119.6	119.4	119.5	119.4	119.5	118.8	119.2	119.5	119.6	119.1	116.7	119.2	119.5	119.0	117.3	123.1	119.6	119.5
C_5	121.8	121.6	121.7	121.7	121.7	121.4	121.5	122.0	121.6	121.1	121.2	121.6	121.7	121.3	120.4	123.8	121.8	122.2
C_6	122.4	122.2	122.4	122.3	122.3	122.2	122.1	122.6	122.4	121.6	121.4	122.2	122.3	122.0	121.4	124.7	122.4	123.5
C_7	109.5	109.5	109.5	109.5	109.5	110.5	109.4	109.6	109.6	109.3	109.2	109.4	109.5	109.3	108.9	112.6	109.6	111.5
C_{7a}	135.7	135.6	135.7	135.6	135.7	135.8	135.5	135.4	135.8	135.1	134.0	135.4	135.7	135.3	135.5	137.5	135.7	136.6
C_8	55.5	55.4	55.6	55.5	55.5	51.9	55.3	54.9	55.6	—	—	55.1	55.4	55.1	—	—	55.4	55.5
C_9	53.0	52.9	53.0	53.0	53.0	52.3	52.9	52.3	53.1	52.8	49.2	54.2	54.5	54.2	54.7	56.6	54.5	54.4
$C_{9'}$	53.0	52.9	53.0	53.0	53.0	52.3	52.9	52.3	53.1	52.8	49.2	54.6	54.9	54.6	55.8	57.0	55.0	55.6
C_{10}	53.0	52.9	53.0	53.0	53.0	52.3	52.9	52.3	53.1	52.6	49.2	53.5	53.9	53.6	51.8	53.7	54.0	51.1
$C_{10'}$	53.0	52.9	53.0	53.0	53.0	52.3	52.9	52.3	53.1	52.6	49.2	53.6	54.2	54.0	53.0	43.0	54.4	52.2
C_{11}	—	—	—	—	—	—	—	—	—	—	—	26.5	27.0	26.8	28.2	28.2	27.9	28.4
C_{12}	59.7	57.6	57.5	57.5	57.6	56.3	57.4	56.7	59.4	57.7	56.6	57.2	57.5	56.9	57.2	58.9	57.4	57.9
C_{13}	23.7	23.7	23.6	23.6	23.7	21.1	23.4	22.5	23.6	23.6	21.9	23.7	24.4	23.9	24.5	24.8	24.3	22.9
C_{14}	28.3	29.2	27.7	28.8	28.1	27.2	27.7	27.7	27.9	28.1	29.1	27.9	29.2	27.1	28.2	29.9	27.1	25.7
C_{15}	51.8	49.2	49.4	46.7	52.5	43.6	52.1	51.8	46.9	50.8	51.1	51.4	49.3	44.3	51.8	51.7	52.4	52.2
C_{16}	—	120.1	—	136.8	—	—	—	—	134.0	—	—	—	120.3	141.8	—	—	—	—
C_{17}	139.0	107.7	151.5	—	139.4	138.5	140.5	141.0	—	139.5	139.8	138.7	107.9	—	138.8	143.0	140.9	142.0
C_{18}	105.2	107.7	—	129.2	92.5	115.1	114.6	117.5	126.5	92.6	117.7	105.0	107.9	123.5	105.0	128.9	114.9	124.1
C_{19}	128.8	120.1	142.7	118.6	128.9	132.5	132.0	132.5	113.0	129.0	132.1	128.7	120.3	111.2	128.5	135.6	132.3	133.7
C_{20}	44.0	43.8	44.0	43.9	43.9	47.6	43.7	44.0	44.0	44.3	43.8	43.8	44.0	43.5	44.7	47.0	44.0	45.2
C_{21}	69.0	68.8	69.0	68.9	68.9	68.9	68.8	68.9	69.0	68.1	67.3	68.7	69.0	68.6	68.1	69.9	69.0	70.0
C_{22}	66.7	66.5	66.7	66.6	66.7	65.9	66.4	66.7	66.8	66.7	66.0	66.5	66.7	66.3	66.7	69.3	66.7	67.7
C_{23}	15.1	14.9	15.1	15.0	15.0	15.1	14.8	15.0	15.1	15.0	14.0	14.8	15.0	14.7	14.9	17.0	15.1	15.4
CO_2H								166.9			167.0					173.6		170.5
CH_3														13.5				
CO_2Et							167.6										163	

a) DMSO-d_6. *b*) MeOH-d_4. *c*) D_2O.

Table 3. Antihistaminic Activity of the Synthesized Compounds

Compd.	*In vitro*				*In vivo*
	Guinea pig ileum		Binding		
	pA_2	pD'_2	% Inhibn. (0.05 mM)	K_i (nM)	ED_{50} (mg/kg, i.p.)
1	7.8				48
2	9.0	8.0	79.6	8.0	0.02
3	9.0	7.6	77.8	61.5	0.05
4	8.3	7.7	32.3		0.04
5	9.2	8.2	75.2		0.13
6	9.1	8.3	62.9		0.09
7			5.5		2.6
8	8.4	7.8	61.8		0.15
9		7.2	20.3	68.4	0.06
10	8.5	7.5	82.3		0.66
11		8.0	92.9		0.63
13		6.2	21.3		0.84
14	8.7	7.4	89.8	3.9	0.04
15	9.0	8.4	95.0	3.2	0.03
16	9.0	8.0	80.1		0.16
17	9.5	8.4	93.2	1.8	0.03
19		6.2	0.3		0.2
21		6.3	10.5		0.04
DPH[a]	7.6		47.7	18.0	5.4
MEP[b]	8.7		>90	1.6[e]	5.4
AST[c]	8.6	7.8	77.5	7.3	0.2
TER[d]	7.8	6.5	9.5	115	1.3

a) DPH: Diphenhydramine. *b*) MEP: Mepyramine. *c*) AST: Astemizole. *d*) TER: Terfenadine. *e*) K_d (nM).

Table 4. Ionization Constants[a] and Partition Coefficients[b] of Selected Compounds

Compd.	pK_a	log *D* (oct/7.4)[c]	log *P* (oct)	log *P* (cy_6)	Δ log P[d]
2	7.9; 4.8	2.64	3.26	0.57	2.69
3[e]		3.50	4.12	2.08	2.04
9	8.0; 4.9; 3.8	−0.86[l]			
14	9.2; 4.9	2.79	4.61	2.13	2.48
15[f]		3.39	5.20	2.88	2.32
17	8.5; 4.9	2.85	3.99	1.40	2.59
19	8.5; 5.1; 3.7	−0.59[l]			
21	9.1; 4.8; 4.4	−0.79[l]			
DPH[g]	8.6[k]	1.21	2.44	2.33	0.11
MEP[h]	8.9; 4.1[k]	1.95	3.46	2.93	0.53
AST[i]	6.7; 5.4	3.48	3.56	0.95	2.61
TER[j]	8.6	4.46	6.69	2.63	3.06

a) Measured by potentiometric titration unless otherwise mentioned. *b*) Calculated from the distribution coefficients in octanol/water and cyclohexane/water systems. *c*) Calculated distribution coefficient in octanol/water at pH 7.4 unless otherwise mentioned. *d*) $\log P_{oct} - \log P_{cy6}$. *e*) Insoluble. pK_a of **2** was used for log *P* calculations. *f*) Insoluble. pK_a of **14** was used for log *P* calculations. *g*) Diphenhydramine. *h*) Mepyramine. *i*) Astemizole. Values are taken from reference 16. *j*) Terfenadine. Values are taken from reference 16. *k*) Spectrophotometric determination. *l*) Distribution coefficient in octanol/water measured at pH 7.4.

Experimental

General Methods Unless otherwise noted, materials were obtained from commercial sources and used without further purification. All melting points were determined on a Bausch & Lomb apparatus and are uncorrected. Infrared (IR) spectra were determined as a film or in KBr with a Nicolet FT-IR 5DXC spectrophotometer. Proton and ^{13}C magnetic resonance spectra were recorded with either a Bruker AM-100 spectrometer operating at 100 MHz and 25 MHz, respectively, or a Varian Unity 300 spectrometer operating at 300 MHz and 75 MHz respectively. Chemical shifts are expressed in ppm (δ) relative to internal tetramethylsilane. Mass spectra were obtained with a Finnigan Mat TSQ-70 mass spectrometer. The IR, NMR, and mass spectral data of all compounds were consistent with the assigned structures. Elemental analyses were obtained for the new compounds reported. Carbon, hydrogen, and nitrogen analyses were within 0.4% of theoretical values. All organic phases were dried over anhydrous $MgSO_4$ and evaporated *in vacuo* with a Büchi rotatory evaporator under reduced pressure. Chromatography was done using the medium-pressure flash method on Merck Silica gel 60 (230—400 mesh ASTM).

Representative preparative procedures are outlined below.

Method A. 1-(2-Ethoxyethyl)-2-{4-[4-(pyrazol-1-yl)butyl]piperazin-1-ylmethyl}benzimidazole, 2 A mixture of 1-(2-ethoxyethyl)-2-(1-piperazinylmethyl)-1*H*-benzimidazole (III, $n=1$, $m=2$)[11] (10 g, 35 mmol), potassium carbonate (14.4 g, 104 mmol), potassium iodide (5.2 g, 35 mmol) and 1-(4-chlorobutyl)pyrazole (5.5 g, 35 mmol) in ethyl acetate (100 ml) was heated under reflux for 24 h. Then, 1-(4-chlorobutyl)pyrazole (2.8 g, 17 mmol) was added and heating was continued for five additional days. The mixture was cooled and filtered, and the filtrate was evaporated to dryness. The residue was redissolved in toluene (80 ml) and the solution was washed with water (pH 10). The organic layer was treated with activated charcoal and evaporated to dryness to obtain the title compound (12.8 g, 90% yield) as an oil.

Method B. 1-(2-Ethoxyethyl)-2-{4-[4-(pyrazol-1-yl)butyl]piperazin-1-ylmethyl}benzimidazole, 2 A mixture of V ($n=1$, $m=2$) (5 g, 11.8 mmol), pyrazole (0.9 g, 13.2 mmol) and potassium carbonate (3 g, 21.7 mmol) in dimethylformamide (60 ml) was refluxed for 12 h. The mixture was cooled, filtered and evaporated to dryness. The residue was dissolved in chloroform, and the organic solution was washed with water, dried over sodium sulfate, filtered and evaporated. The residue was purified by column chromatography through silica gel (elution with chloroform–methanol 9 : 1) to afford the title compound (1.7 g, 35%) as an oil.

Method C. 1-(2-Ethoxyethy)-2-{4-[4-(4-carboxypyrazol-1-yl)butyl]piperazin-1-ylmethyl}benzimidazole, 9 A solution of compound **8** (3.15 g, 6.5 mmol) in ethanol (25 ml) and 10% NaOH (25 ml) was stirred overnight at room temperature. The mixture was neutralized and evaporated to dryness. The dry residue was extracted with 2-propanol at 40 °C, and the solvent was evaporated to yield the title compound, **9** (2.4 g, 81%) as an hygroscopic, non crystalline solid.

Method D. 8-[1-(2-Ethoxyethyl)benzimidazol-2-ylmethyl]-8-aza-5-azoniaspiro[4,5]decane Bromide (V, $n=1$, $m=2$) A mixture of 1-(2-ethoxyethyl)-2-(1-piperazinylmethyl)-1*H*-benzimidazole (III, $n=1$, $m=2$) (1.5 g, 5.2 mmol), 1,4-dibromobutane (1.41 g, 6.5 mmol) and potassium carbonate (0.72 g, 5.2 mmol) in chloroform (50 ml) was refluxed for 16 h. The mixture was cooled, filtered and evaporated to dryness to yield the title compound (2.1 g, 95%) as a crude oil, which was used in the next reaction (method B) without further purification. IR (film): 3030—2450, 1530, 1475, 1350, 1120, 760 cm^{-1}. ^1H-NMR ($CDCl_3$): δ 1.05 (t, 3H), 2.25 (m, 4H), 3.25—4.15 (m, 18H), 4.45 (t, 2H), 7.27 (m, 3H), 7.60 (m, 1H).

Method E. 1-(2-Ethoxyethyl)-2-{4-[4-(pyrazol-1-yl)butyl]piperazin-1-ylmethyl}benzimidazole Dimaleate, 2.2$C_4H_4O_4$ A solution of maleic acid (0.74 g, 6.4 mmol) in 2-propanol (4 ml) was added to a solution of **2** (1.3 g, 3.2 mmol) in 2-propanol (4 ml) at 40—50 °C. The obtained solution was allowed to crystallize to yield the title compound (1.7 g, 76%), mp 122 °C. *Anal.* Calcd for $C_{31}H_{42}N_6O_9$: C, 57.93; H, 6.59; N, 13.08. Found: C, 57.82; H, 6.68; N, 13.05. ^1H-NMR (DMSO-d_6) δ: 1.01 (t, 3H); 1.57 (quint., 2H); 1.79 (quint., 2H); 2.70 (br, 4H); 3.08 (t, 2H); 3.20 (br, 4H); 3.37 (q, 2H); 3.71 (t, 2H); 3.97 (s, 2H); 4.14 (t, 2H); 4.51 (t, 2H); 6.15 (s, 4H); 6.23 (dd, 1H); 7.26 (m, 2H); 7.43 (d, 1H); 7.62 (m, 2H); 7.71 (d, 1H). ^{13}C-NMR (DMSO-d_6) δ: (see Table 2 for numbering), 15.1 (C-23), 23.3 (C-13), 28.0 (C-14), 43.5 (C-20), 51.0 (C-15), 52.8 (C-9), 53.0 (C-10), 54.9 (C-8), 57.2 (C-12), 65.8 (C-22), 68.7 (C-21), 104.9 (C-18), 110.5 (C-7), 118.9 (C-4), 121.4 (C-5), 122.1 (C-6), 129.6 (C-19), 135.9 (C-7a), 138.4 (C-17), 142.0 (C-3a), 151.6 (C-2).

Partition Coefficient Measurements Distribution coefficients ($\log D$) were measured by a conventional shaken-flask technique at 37 °C ± 1.[18-20] The concentrations of the compounds in the aqueous and organic phases were determined spectrophotometrically (Hewlett-Packard UV HP8452A spectrophotometer). In most experiments, 0.1 M phosphate buffer adjusted at pH 4 was used as the aqueous phase. For amphoteric compounds, 0.1 M phosphate buffer adjusted to pH 7.4 was used as the aqueous phase, and 0.025 M phosphate buffer at pH 10 was used for diphenhydramine. Each phase was saturated with the other at 37 °C ± 1. The test tubes were shaken mechanically for 1 h at 37 °C ± 1. Stability of the solutions was confirmed. Partition coefficients ($\log P$)[15-17] of the neutral species were calculated from the following equations:

$$\log P = \log D + \log\{1 + 10^{(pK_a - pH)}\} \text{ (for monoprotic compounds)}$$

$$\log P = \log D + \log\{1 + 10^{(pK_2 - pH)} + 10^{(pK_1 + pK_2 - 2pH)}\} \text{ (for diprotic compounds)}$$

All the partition coefficients are the mean of at least two determinations.

pK_a Measurements Potentiometric titrations were performed with a Metrohm 665 Dosimat and with a Crison 2002 pH meter, under a nitrogen atmosphere, using a solute concentration of approximately 10^{-3} M in 0.2 M KCl and 0.1 M KOH in 0.1 M KCl as the titrant. The temperature of the titration cell was maintained at 25 °C ± 0.1. The pK_a values were calculated from the titration curves using the equation: $\bar{j} = \{H_t - [H^+] + [OH^-]\}/At$,[21] where $\bar{j}$ is defined as the formation function that give us an evaluation of the average number of protons linked to the substance, H_t is the total acidity, $[H^+]$ is the free hydrogen ion concentration, $[OH^-] = K_w[H^+]^{-1}$, and A_t is the compound concentration. After the calculation of $\bar{j}$ for each point, $\bar{j}/[H^+]$ pairs of values were adjusted to the models[22]:

$$\bar{j} = \beta_1[H^+]/\{1 + \beta_1[H^+]\}, \text{ where } \beta_1 = 1/K_1 \text{ (for monoprotic compounds)}$$

$$\bar{j} = \{\beta_1[H^+] + 2\beta_2[H^+]^2\}/\{1 + \beta_1[H^+] + \beta_2[H^+]^2\},$$

where $\beta_1 = 1/K_2$ and $\beta_2 = 1/K_1K_2$ (for diprotic compounds)

$$\bar{j} = \{\beta_1[H^+] + 2\beta_2[H^+]^2 + 3\beta_3[H^+]^3\}/\{1 + \beta_1[H^+] + \beta_2[H^+]^2 + \beta_3[H^+]^3\}, \text{ where } \beta_1 = 1/K_3;\ \beta_2 = 1/K_2 \cdot K_3 \text{ and } \beta_3 = 1/K_1K_2K_3$$

(for amphoteric compounds)

Spectrophotometric determinations were performed as described previously.[23] The spectral measurements were carried out with a Hewlett-Packard UV HP8452A spectrophotometer and the pH values were measured with a Crison 2002 pH meter (temperature was maintained at 25 °C ± 0.1). The pH of the acidified solution, at constant ionic strength of 0.2 M, was varied by adding 0.1 M KOH in 0.1 M KCl. Optimum wavelengths were chosen for each compound where the absorbance of the species varied significantly, and the change of absorbance with pH, at the selected wavelength, was monitored.

The pK_a values were determined from the following equations[24]:

$$A_T = (A_M[H^+] + A_AK_1)/(K_1 + [H^+]) \text{ (for monoprotic compunds)}$$

$$A_T = (A_C[H^+]^2 + A_MK_1[H^+] + A_AK_1K_2)/([H^+]^2 + K_1[H^+] + K_1K_2) \text{ (for diprotic compounds),}$$

where A_T is the measured absorbance, A_C is the absorbance of the cation, A_M is the absorbance of the neutral species, A_A is the absorbance of the anion, and K_1 and K_2 denote the dissociation constants. The pK_a values were calculated by fitting these equations to the experimental data.[22]

All the dissociation constants in the potentiometric and spectrophotometric determinations are the mean of at least two determinations.

H_1-Antihistaminic Activity. Inhibition of Histamine-Induced Contraction of Guinea Pig Ileum According to Magnus,[25] segments of preterminal ileum, about 2 cm long, were excised from male guinea pigs and rapidly suspended in a 100 ml organ bath containing Tyrode's solution, maintained at 37 °C and gassed with air, under an initial load of 1 g. After 15 min stabilization time non-cumulative dose–response curves to histamine (10^{-8}—3×10^{-6} M) were obtained by adding graded concentrations of histamine every minute to the bath. The contractile responses to histamine were measured with an isotonic transducer (HP 7DCDT-1000, Hewlett Packard). The pD'_2 (negative logarithm of the

molar concentration of antagonist which causes a depression to 50% of the maximum response of agonist) or pA_2 (negative logarithm of the molar concentration of antagonist which causes a shift of agonist activity by a factor of 2) values for noncompetitive or competitive antagonism, respectively, were calculated according to Van Rossum.[26]

H_1 Histamine Receptor Binding Assay Male Dunkin-Hartley guinea pigs weighing 250—400 g were used to evaluate *in vitro* [^{3}H]mepyramine binding to the H_1 histamine receptor in cerebral cortex according to a minor modification of the reported method.[27] The incubation was carried out at 25 °C for 30 min. Radioactivity was determined with a Formula 989 (DuPont) liquid scintillation analyzer. Specific binding of [^{3}H]mepyramine was estimated as the difference between radioactivity bound in the absence and in the presence of triprolidine. All competition and saturation binding data were analyzed by non-linear iterative curve-fitting procedures by use of the computer programme EBDA-LIGAND,[28] and K_i values were calculated from IC_{50} values.[29]

***In Vivo* Compound 48/80-Induced Lethality in Rats** The assay was performed in male rats of the Wistar strain (weighing 200—250 g) according to the method of Niemegeers *et al.*[30] The antihistaminic activity was studied by determining the protection of animals from lethality induced by compound 48/80.[31] Compounds **1**—**21** were administered (i.p.) to rats 60 min before the intravenous challenge with compound 48/80 (0.5 mg/kg, i.v.). The protective activity is defined as the survival of the rats 240 min after the injection of 48/80. The activity of new compounds was studied at several doses in order to determine ED_{50}.

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References and Notes

1) Leurs R., van der Goot H., Timmerman H., *Adv. Drug Res.*, **20**, 217—304 (1991).
2) Colombo A., Frigola J., Parés J., Eur. Pat. EP 382 637 [*Chem. Abstr.*; **114**, 122 411*c* (1991)].
3) Cuberes M. R., Frigola J., Parés J., Eur. Pat. EP 468 885 [*Chem. Abstr.*, **116**, 214 497*e* (1992)].
4) Cuberes M. R., Frigola J., Parés J., Eur. Pat. EP 468 884 [*Chem. Abstr.*, **116**, 235 655*v* (1992)].
5) Cuberes M. R., Frigola J., Parés J., Eur. Pat. EP 507 696 [*Chem. Abstr.*, **118**, 124 568*r* (1993)].
6) Contijoch M., Cuberes R., Parés J., Frigola J., Abstracts of papers, VII Congreso de la Sociedad Española de Química Terapéutica, Jaca, Spain, September 1991, Abstr. No. P-64.
7) Gutiérrez B., Fisas M. A., Dordal A., Fort M., Cuberes R., Frigola J., Farré A. J., Abstracts of papers, I Congreso Ibero-Americano de Farmacologí, Benalmádena, Spain, September 1992, Abstr. No. 339.
8) ter Laak A. M., van Drooge M. J., Timmerman H., Donné-op den Kelder G. M., *Quant. Struct.-Act. Relat.*, **11**, 348—363 (1992).
9) Quintana J., Contijoch M., Cuberes R., Frigola J., "QSAR and Molecular Modelling: Concepts, Computational Tools and Biological Applications," ed. by Sanz F., Giraldo J., Manaut F., J. R. Prous Science Publishers, Barcelona, 1995, pp. 282—288.
10) Iemura R., Kawashima T., Fukuda T., Ito K., Tsukamoto G., *J. Med. Chem.*, **29**, 1178—1183 (1986).
11) Iemura R., Kawashima T., Fukuda T., Ito K., Tsukamoto G., *J. Heterocyc. Chem.*, **24**, 31—37 (1987).
12) Anonymous, *Drugs of Fut.*, **7**, 10—12 (1982).
13) *a*) Anonymous, *Drugs of Fut.*, **15**, 527—528 (1990); *b*) Iemura R., Hori M., Ohtaka H., *Chem. Pharm. Bull.*, **37**, 962—966 (1989); *c*) Iemura R., Ohtaka H., *ibid.*, **37**, 967—972 (1989); *d*) Iemura R., Hori M., Saito T., Ohtaka H., *ibid.*, **37**, 2723—2726 (1989).
14) Irwin S., *Science*, **136**, 123—128 (1962).
15) Young R. C., Mitchell R. C., Brown T. H., Ganellin C. R., Griffiths R., Jones M., Rana K. K., Saunders D., Smith I. R., Sore N. E., Wilks T. J., *J. Med. Chem.*, **31**, 656—671 (1988).
16) ter Laak A. M., Tsai R. S., Donné-op den Kelder G. M., Carrupt P.-A., Testa B., Timmerman H., *Eur. J. Pharm. Sci.*, **2**, 373—384 (1994).
17) Von Geldern T. W., Hoffman D. J., Kester J. A., Nellans H. N., Dayton B. D., Calzadilla S. V., Marsh K. C., Hernández L., Chiou W., Dixon D. B., Wu-Wong J. R., Opgenorth T. J., *J. Med. Chem.*, **39**, 982—991 (1996).
18) OCDE guide for the analysis of chemical products, May 12th, 1981.
19) Dearden J. C., Bresnen G. M., *Quant. Struct.-Act. Relat.*, **7**, 133—144 (1988).
20) Leo A., Hansch C., Elkins D., *Chem. Rev.*, **71**, 525—616 (1971).
21) Rossotti H., "The study of ionic equilibria. An Introduction," Longman, London and New York, 1978.
22) Table Curve, Automated Curve Fitting Software, Jandel Scientific.
23) Frigola J., Torrens A., Castrillo J. A., Mas J., Vañó D., Berrocal J.M., Calvet C., Salgado L., Redondo J., Garcia-Granda S., Valentí E., Quintana J. R., *J. Med. Chem.*, **37**, 4195—4210 (1994).
24) Ross D. L., *Int. J. Pharm.*, **63**, 237—250 (1990).
25) Magnus R., *Pflugers Arch.*, **102**, 123—151 (1904).
26) Van Rossum J. M., *Arch. Int. Pharmacodyn.*, **143**, 299—330 (1963).
27) Ahn H. S., Barnett A., *Eur. J. Pharmacol.*, **127**, 153—155 (1986).
28) McPherson G. A., *Computer Prog. Biomed.*, **17**, 107—114 (1983).
29) Cheng Y. C., Prusoff W. M., *Biochem. Pharmacol.*, **22**, 3099—3108 (1973).
30) Niemegeers C. J. E., Awouters F., Van Nueten J. M., De Nollin S., Janssen P. A. J., *Arch. Int. Pharmacodyn.*, **234**, 164—176 (1978).
31) Baltzly R., Buck J. S., De Beer E. J., Webb F. J., *J. Amer. Chem. Soc.*, **71**, 1301—1305 (1949).