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Synthesis, biological activity, QSAR and QSPR study of 2-aminobenzimidazole derivatives as potent H_3-antagonists

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Abstract—We report the design, synthesis, QSPR and QSAR of a new class of H_3-antagonists, having a 2-aminobenzimidazole moiety connected to the 4(5) position of an imidazole ring through di- or tri-methylene chains. Eleven substituents, selected by experimental design to obtain broad and non-correlated variation in their lipophilic, electronic and steric properties, were introduced at the 5(6) position of the benzimidazole nucleus. The compounds were tested for their H_3-receptor affinity, by displacement of [^{3}H]-(*R*)-α-methylhistamine ([^{3}H]-RAMHA) binding to rat brain membranes (pK_i), for intrinsic activity, evaluating their effect on [^{35}S]GTPγS binding to rat brain membranes, and for H_3-antagonist potency, on electrically stimulated guinea-pig ileum (pK_B). The pK_i values of the derivatives with longer chain (**5a**–**k**) ranged over 2 orders of magnitude, with the 5(6)-methoxy derivative **5d** endowed with sub-nanomolar affinity ($pK_i = 9.37$). The series having two methylene groups in the chain spacer (**4a**–**k**), showing a small variation in affinity, revealed to be somewhat insensitive to ring substitution. Lipophilicity (log *P*) and basicity (pK_a) of the newly synthesized compounds were measured and related to receptor affinity in a QSAR study. Multiple regression analysis (MRA) showed an approximate parabolic dependence of pK_i on log *P*, while an additional electronic effect of the substituents on benzimidazole tautomerism is suspected.

1. Introduction

Histamine acts as a neurotransmitter in the peripheral and central nervous system (CNS), exerting its pharmacological actions through the activation of distinct receptor subtypes, named H_1, H_2, H_3 and H_4,[1,2] belonging to the family of G-protein coupled receptors. The recent cloning of the gene encoding the human H_3-receptor, discovered in 1983 by Arrang et al.,[3] has shown a 93% identity with rat H_3-receptor.[4] The H_3-receptor can be located both presynaptically and postsynaptically, acting both as an autoreceptor and as a heteroreceptor. Histamine H_3-receptor antagonists increase the synthesis and the release of histamine, blocking a negative feed-back mechanism,[3,5,6] and the release of several other neurotransmitters, such as acetylcholine, noradrenaline, dopamine and serotonin.[7–10] The high density of H_3-receptors in the CNS suggests that H_3-antagonists could be useful for the treatment of central disorders in which an enhancement of neurotransmitter release is required, such as diseases involving impaired cognitive functions.[11,12] In the last few years a large number of potent H_3-receptor antagonists have been reported in the literature. Most of them contain the classical structure consisting of an imidazole ring connected by a spacer to a polar group, which is attached to a lipophilic ending group.[13] This general formula can be recognized in many derivatives (see Chart 1), having different polar groups, such as thioureas (e.g., thioperamide[14]), isothioureas (e.g., clobenpropit[15] and iodophenpropit[16]), guanidines (**1**),[17] carbamates (**2**),[18] sulfonamides (**3**,**4**).[19] In some series of H_3-antagonists, the polar group is represented by a

Keywords: Histamine; H_3-receptor antagonists; 2-aminobenzimidazole; QSAR.
Abbreviations: Et_2O; diethyl ether; EtOH; ethanol; Acet; acetone; DMSO; dimethyl sulfoxide.
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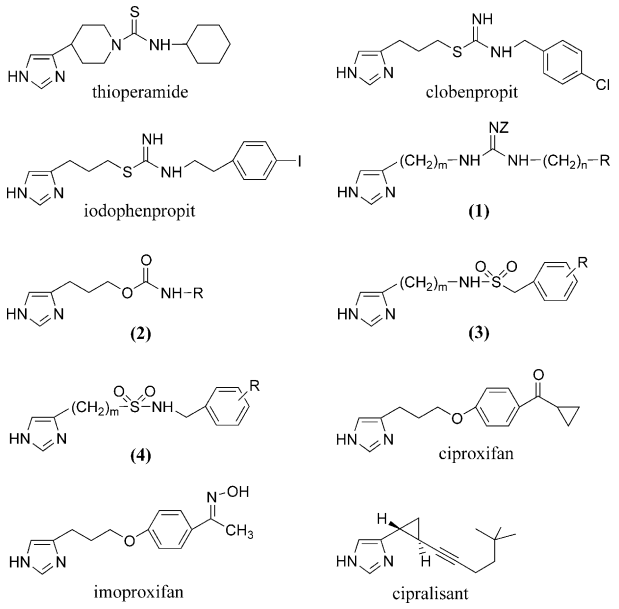


Chart 1. Classical histamine H_3-receptor antagonists.

heterocyclic nucleus (Chart 2), such as oxadiazole (**5**),[20] pyridine (**6**),[21] thiazole or benzothiazole (**7**),[22,23] or imidazole (**8**).[24,25] More recently, several classes of non classical H_3-antagonists, lacking the imidazole ring, have also been described in the literature.[26–28]

In spite of the large number of known potent classical H_3-antagonists, the role of the polar group still has to be fully understood. In fact, H_3-antagonists such as ciproxifan[29] and imoproxifan[30] (Chart 1), having a phenolic ether fragment, endowed with high lipophilicity and low propensity to accept hydrogen bonds, have displayed high affinity for the H_3-receptor and high in vivo potency. Moreover, the alkyne derivative cipralisant[31] and some H_3-antagonists recently described are devoid of a polar group.[32]

Referring to the classical antagonists, we started from the hypothesis that they could be grouped into two classes, depending on polar group basicity: compounds having a neutral polar group (e.g., thioperamide), and those having a strongly basic one, mainly positively charged at physiological pH (e.g., clobenpropit). In principle, a strong basicity should be detrimental for in vivo potency, because of reduced access to the CNS. Nevertheless, during our past investigations on H_3-receptor antagonists having different heterocycles as polar groups (e.g., 2-alkylthioimidazole[22,24] and 2-alkylimidazole[25], Chart 2), we observed that a moderate increase in basicity led to an improvement in affinity for compounds with longer chain spacers, thus revealing a dependence of the optimal length of the alkyl chain on the polar group basicity. These observations also indicated that an intermediate basicity of the polar fragment could yield H_3-antagonists with high affinity values, while maintaining a significant unionized fraction at physiological pH, useful for drug distribution.

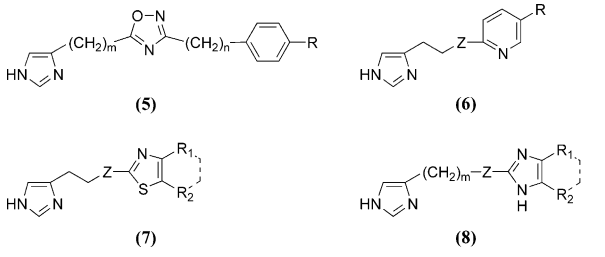


Chart 2. Histamine H_3-receptor antagonists with heterocyclic polar groups.

On the basis of these considerations, we designed and synthesized the series of H_3-antagonists reported in this work, choosing the 2-aminobenzimidazole fragment as a moderately basic polar group, also including in its structure a lipophilic ending domain. Two series of 5(6)-substituted 2-[ω-[4(5)-imidazolyl]alkyl]amino]benzimidazole derivatives (Tables 1 and 2), characterized by a di- or a tri-methylene alkyl chain, respectively, were thus designed, synthesized and tested. Physico-chemical properties, which could be relevant for receptor affinity, that is, log *P* and pK_a, were also measured and these properties were employed for QSAR and quantitative structure–property relationship (QSPR) analysis.

2. Design

We applied an experimental design to modulate the basicity of the new compounds by introducing, on the 5(6) position of the benzimidazole nucleus, substituents with different electronic features. The pK_a values of a series of 5(6)-substituted 2-aminobenzimidazoles had been shown to be well correlated to the average of σ_m and σ_p (referred to as σ_{m-p} henceforth), accounting for benzimidazole tautomerism,[33] which was then employed as a descriptor of substituent electronic effect.

In order to build QSAR models by multiple regression analysis (MRA), the variation in the electronic properties had to be as non-correlated as possible to that in the lipophilic and steric ones. The substituent selection was therefore made starting from a factorial design on a variable space defined by the physico-chemical parameters σ_{m-p}, π and MR, taken from the list of Skagerberg et al.[34] An arbitrary pre-selection was based on the supposed synthetic accessibility of the final compounds.

The first set of 8 substituents (**a**–**h** in Table 1 and Table 2) allowed for a good exploration of the property space, although some correlation between π and MR ($r = 0.44$) resulted from the fact that the experimental domain was limited.

Variable ranges were as follows: σ_{m-p}, −0.41 (NH_2) to 0.75 (NO_2); π, −1.27 ($CONHCH_3$) to 2.13 (n-C_4H_9); MR, 1.03 (H) to 19.69 (n-C_4H_9). Correlation coefficients were as follows: π-σ_{m-p},-0.02; π-MR, 0.44; σ_{m-p}-MR, 0.15.

To further explore the effect of the electronic properties on receptor affinity, new compounds were synthesized, having small electron-donor (CH_3) or withdrawing (CF_3) groups, or a methylenedioxy fragment (O–CH_2–O).

3. Chemistry

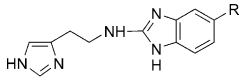
The 2-aminobenzimidazole derivatives described in this paper were prepared following a general synthetic route represented in Scheme 1.

The 4-substituted *o*-phenylendiamines (**1**), commercially available or prepared according to different literature methods,[35–41] were cyclized to 5(6)-substituted 2-mercaptobenzimidazoles (**2**) (intermediates **2a–d** and **2i** were purchased). 5(6)-Substituted 2-benzimidazolesulphonic acids (**3**) were synthesized, according to Sorba,[42] by oxidation from the 2-mercaptobenzimidazole derivatives **2**, and then condensed with the appropriate 4(5)-(ω-aminoalkyl)imidazole[43] to give the desired products **4a–g**, **4i–k**, **5a–g** and **5i–k**. The final compounds **4h** and **5h** were prepared by reduction of the nitro derivatives **4c** and **5c**, respectively. The carboxyethyl substituent on the intermediate **2f** was hydrolized to COOH during the oxidation, and it was treated with abs EtOH saturated with gaseous HCl to obtain **3f**.

4. Pharmacology

H_3-Receptor affinity of the newly synthesized compounds was measured by displacement of [^{3}H]-(*R*)-α-methylhistamine ([^{3}H]RAMHA) bound to rat cerebral

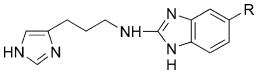
Table 1. Histamine H_3-receptor affinity (pK_i), antagonist potency (pK_B), partition and distribution coefficients (log *P* and log $D_{7.4}$) and dissociation constants (pK_a) of compounds **4a–k**



Compd	R	pK_i[a]	pK_B[b]	log *P*[c]	log $D_{7.4}$	p$K_{a,1}$[d]	p$K_{a,2}$[d]
4a	H	7.25±0.05[e]	7.33±0.20[f]	1.49±0.02	1.09±0.01	6.20	7.40
4b	Cl	7.82±0.07	7.98±0.14	2.57±0.04	2.31±0.04	5.76	7.16
4c	NO_2	7.73±0.06	8.42±0.24	1.83±0.01	1.81±0.01	4.76	6.96
4d	OCH_3	7.78±0.06	8.05±0.17	1.43±0.01	1.02±0.01	6.20	7.42
4e	*n*-C_4H_9	7.24±0.16	7.84±0.25	3.64±0.03	3.30±0.07	6.27	7.57
4f	$COOC_2H_5$	7.24±0.12	7.41±0.05	2.39±0.07	2.28±0.03	5.50	7.09
4g	$CONHCH_3$	7.10±0.16	7.68±0.20	0.48±0.01	0.34±0.01	5.67	7.20
4h	NH_2[g]	7.30±0.14	7.71±0.22	0.01±0.03	−0.56±0.01	6.54	7.75
4i	CH_3	7.16±0.10	7.42±0.15	1.93±0.01	1.49±0.01	6.27	7.54
4j	CF_3	7.83±0.05	7.32±0.09	3.01±0.01	2.82±0.01	5.49	7.07
4k	O–CH_2–O	6.91±0.06	7.47±0.12	1.44±0.04	1.12±0.01	6.19	7.40

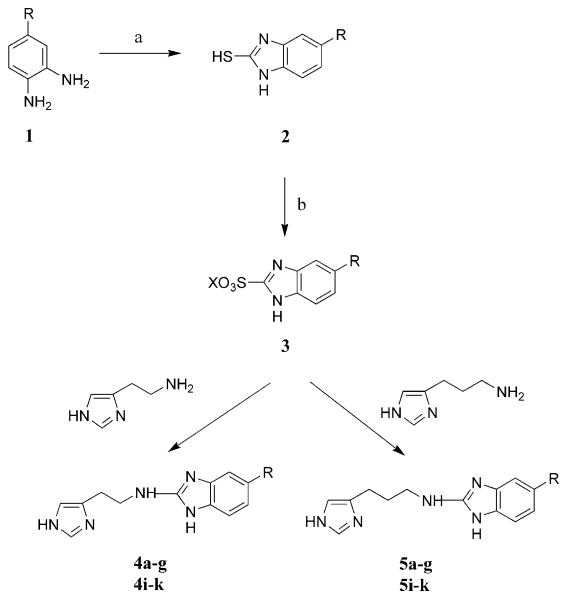
[a] Inhibition of [^{3}H]RAMHA binding to rat brain membranes.
[b] Inhibition of RAMHA-induced effects on guinea-pig isolated ileum. pK_B Values obtained according to Furchgott's method.[55]
[c] For the neutral species.
[d] The values of p$K_{a,1}$ were mainly attributed to the protonation of the 2-aminobenzimidazole nucleus (see text); s.d. was around 0.01 ($n=3$).
[e] pK_i versus [^{3}H]NAMHA = 7.14±0.05.[22]
[f] pA_2 Value.[23]
[g] For the amino group p$K_{a,3}$ = 4.16.

Table 2. Histamine H_3-receptor affinity (pK_i), antagonist potency (pK_B), partition and distribution coefficients (log *P* and log $D_{7.4}$) and dissociation constants (pK_a) of compounds **5a–k**



Compd	R	pK_i[a]	pK_B[b]	log *P*[c]	log $D_{7.4}$	p$K_{a,1}$[d]	p$K_{a,2}$[d]
5a	H	8.90±0.05	9.46±0.57	1.78±0.01	1.11±0.01	6.70	7.65
5b	Cl	8.95±0.03	8.53±0.11	2.84±0.08	2.62±0.08	6.20	7.44
5c	NO_2	8.68±0.12	8.32±0.09	2.22±0.01	2.09±0.02	5.10	7.34
5d	OCH_3	9.37±0.28	8.34±0.16	1.77±0.01	1.14±0.04	6.65	7.65
5e	*n*-C_4H_9	8.06±0.18	7.38±0.12	3.93[e]	N.A.[f]	6.70	7.78
5f	$COOC_2H_5$	8.31±0.18	7.48±0.12	2.64±0.03	2.43±0.02	5.94	7.40
5g	$CONHCH_3$	7.57±0.19	8.53±0.49	0.80±0.01	0.47±0.01	6.04	7.41
5h	NH_2[g]	7.33±0.09	7.91±0.15	0.17±0.01	−0.64±0.04	6.82	7.85
5i	CH_3	8.52±0.06	8.18±0.08	2.26±0.01	1.56±0.01	6.76	7.75
5j	CF_3	8.56±0.13	8.39±0.14	3.31±0.01	3.03±0.03	5.92	7.41
5k	O–CH_2–O	8.46±0.17	8.19±0.13	1.89±0.10	1.21±0.01	6.68	7.67

[a] Inhibition of [^{3}H]RAMHA binding to rat brain membranes.
[b] Inhibition of RAMHA-induced effects on guinea-pig isolated ileum. pK_B Values obtained according to Furchgott's method.[55]
[c] For the neutral species.
[d] The values of p$K_{a,1}$ were mainly attributed to the protonation of the 2-aminobenzimidazole nucleus (see text); s.d. was around 0.01 ($n=3$).
[e] Estimated by adding the contribution of *n*-C_4H_9 in series **4** to the log *P* of **5a**.
[f] N.A., Not Available.
[g] For the amino group p$K_{a,3}$ = 4.27.



Scheme 1. Reagents: (a) CS_2, EtOH, KOH or $C_2H_5OCS_2K$, EtOH; (b) 10% NaOH, 30% H_2O_2 or 10% NaOH, $KMnO_4$. Note: Compounds **4h** and **5h** were prepared by reduction of the nitro derivatives **4c** and **5c**, respectively.

cortex synaptosomes. Histamine H_3-receptor antagonist potency was evaluated on electrically stimulated guinea-pig ileum, by inhibition of RAMHA-induced responses.[44]

The ability of compounds **5a–k** to influence [^{35}S]GTPγS binding to rat cerebral cortex synaptosomes was also tested, to evaluate their intrinsic activity on this tissue. In the experimental conditions employed, the agonist RAMHA effectively stimulated [^{35}S]GTPγS binding, which, conversely, was unaffected by thioperamide and clobenpropit.

5. Results and discussion

Biological data relating to the affinity (pK_i) for rat brain H_3-receptor and to H_3-antagonist potency (pK_B) on guinea-pig ileum, for the 2-aminobenzimidazole derivatives having two (**4a–k**) and three (**5a–k**) methylene groups in the chain spacer, are reported in Tables 1 and 2, respectively. In these tables the results of ionization (pK_a) and lipophilicity (log P and log $D_{7.4}$) measurements are also reported. The compounds belonging to the more active class, **5a–k**, did not significantly stimulate, nor inhibited, [^{35}S]GTPγS binding to rat cerebral cortex synaptosomes at concentrations able to displace [^{3}H]RAMHA from rat brain H_3-receptors (0.01nM–1μM), thus confirming their antagonist behavior on this tissue (Fig. 1).

5.1. Ionization, lipophilicity and QSPR

Investigating the ionization of a series of 5(6)-substituted 2-aminobenzimidazole derivatives, Serafino et al.[33] reported pK_a values in the range 4.5–6.8 for the 2-aminobenzimidazole moiety, slightly influenced by the presence of a second basic group, but strictly correlated

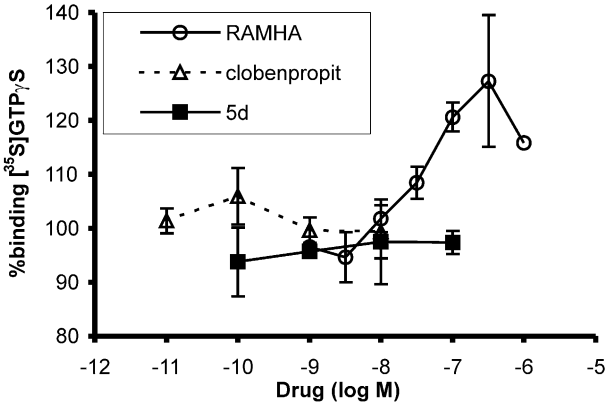


Figure 1. Effect of (*R*)-α-methylhistamine (RAMHA), clobenpropit and **5d** on specific [^{35}S]GTPγS binding to rat cerebral membranes. Error bars represent standard errors of the means.

to σ_{m-p}. The compounds described in the present work have two basic groups, the imidazole ring and the 2-aminobenzimidazole nucleus. The experimentally determined (macroscopic) constants, p$K_{a,i}$, are a composite function of the microscopic ones, but tend to approximate them when the two ionizable centers have sizeable differences in basicity.[45]

Titration curves for the compounds tested revealed two sites of protonation (three for the NH_2 derivatives **4h** and **5h**, see Table 1 and 2). Although complex equilibria exist in solution, the p$K_{a,1}$ was mainly attributed to the protonation of the 2-aminobenzimidazole fragment, as it is strongly influenced by the electronic effect of the substituent in the 5(6) position, and because its values ($4.7 < pK_{a,1} < 6.9$) approach those observed by Serafino et al.[33] The best Hammett equation was obtained employing σ_{m-p} for compounds **4a–k** (eq 1), while for the longer chain derivatives **5a–k** statistically equivalent models could be obtained from σ_{m-p} (eq 2) and σ_m (not reported). The average of σ_m and σ_p seems therefore to encode a sufficiently accurate balance of inductive and resonance effects to describe the influence on protonation constants of the 2-aminobenzimidazole nucleus.

For compounds **4a–k**:

$$pK_{a,1} = -1.43(\pm 0.11)\sigma_{m-p} + 6.08(\pm 0.04) \quad (1)$$

$$n = 11;\ r^2 = 0.950;\ s = 0.13;\ F = 158.4;$$

$$q^2 = 0.887;\ SDEP = 0.17$$

For compounds **5a–k**:

$$pK_{a,1} = -1.46(\pm 0.16)\sigma_{m-p} + 6.51(\pm 0.06) \quad (2)$$

$$n = 11;\ r^2 = 0.910;\ s = 0.17;\ F = 87.2;$$

$$q^2 = 0.798;\ SDEP = 0.23$$

The substituent constants employed for the above-mentioned eq 1 and 2, and for the regression models reported below, are shown in Table 3. For common

Table 3. Substituent constants[a] employed for experimental design, or in QSAR and QSPR models

Compd	Substituent	π	MR	σ_m	σ_p	σ_{m-p}[b]
a	H	0.00	1.03	0.00	0.00	0.000
b	Cl	0.71	6.03	0.37	0.23	0.300
c	NO_2	−0.28	7.36	0.71	0.78	0.745
d	OCH_3	−0.02	7.87	0.12	−0.27	−0.075
e	n-C_4H_9	2.13	19.69	−0.08	−0.16	−0.120
f	$COOC_2H_5$	0.51	17.47	0.37	0.45	0.410
g	$CONHCH_3$	−1.27	14.57	0.35	0.36	0.355
h	NH_2	−1.23	5.42	−0.16	−0.66	−0.410
i	CH_3	0.56	5.65	−0.07	−0.17	−0.120
j	CF_3	0.88	5.02	0.43	0.54	0.485
k	O-CH_2-O	−0.05 [c]	9.00[c]	−0.16[c]	−0.16[c]	−0.160

[a] Taken or calculated from Ref. 34, unless differently indicated.
[b] $\sigma_{m-p} = (\sigma_m + \sigma_p)/2$.
[c] From Ref. 47.

substituents, identical values of the substituent constants were reported in the compilation of Skagerberg[34] and in that of van de Waterbeemd and Testa;[46] the constants values for the methylenedioxy group (**k**) were obtained from the Hansch data bank.[47]

log *P* Experimental values of the neutral species in the two-phase *n*-octanol/water system were correlated to the π values of the substituents, showing unexpectedly high residuals ($r^2 = 0.921$, $s = 0.31$ for **4a–k**; $r^2 = 0.890$, $s = 0.33$ for **5a–k**). Nevertheless, significant improvements in the fitting were obtained by introducing the electronic constant $\sigma_{m\text{-}p}$, as shown by eqs 3 and 4 (for **4a–k** and **5a–k**, respectively).

$$\log P = 1.04(\pm 0.03)\pi + 0.82(\pm 0.08)\sigma_{m-p} + 1.55(\pm 0.03) \quad (3)$$

$$n = 11;\ r^2 = 0.995;\ s = 0.08;\ F = 812.4;\ q^2 = 0.990;\ SDEP = 0.10;\ r(\pi/\sigma_{m-p}) = 0.039$$

$$\log P = 1.06(\pm 0.05)\pi + 0.85(\pm 0.11)\sigma_{m-p} + 1.86(\pm 0.04) \quad (4)$$

$$n = 10;\ r^2 = 0.989;\ s = 0.11;\ F = 306.2;\ q^2 = 0.981;\ SDEP = 0.10;\ r(\pi/\sigma_{m-p}) = 0.278$$

In these equations, the coefficients of the two variables are practically the same for the two subsets, indicating an influence of the electronic features of the 5(6) substituent on the partition of the neutral species which is independent of chain length. All the residuals were lower than 0.2, with the exception of **5k**, being 0.22. The effect of ionization can be ruled out, the log *P* being measured at high pH values, where a plateau was reached in the log *D*/pH profiles, and where the fraction of ionized species, calculated from the pK_a values, was negligible. The fact that the measured log *P* values correctly refer to the unionized species is further confirmed by the observation that experimental log $D_{7.4}$ values were in good agreement with those calculated from log *P*, p$K_{a,1}$ and p$K_{a,2}$, according to the formula: log D = log P- log $[1 + 10^{(pKa,2-pH)} + 10^{(pKa,1+pKa,2-2pH)}]$:[48] for the available values of the 19 diprotic bases reported in Tables 1 and 2 (i.e., **4a–g, 4i–k**, **5a–d**, **5f**, **5g** and **5i–k**), we observed a mean difference between measured and calculated log $D_{7.4}$ of 0.03, and a mean absolute difference of 0.15.

Moreover, the formation of ion pairs was prevented by the use of a zwitterionic buffer (see Experimental). Therefore, the dependence of log *P* on σ_{m-p} should reflect an interesting, but as yet unexplained, effect of charge distribution through the 2-aminobenzimidazole nucleus on its interaction with one or both of the two solvents employed in partition experiments. In fact, compounds with electron-withdrawing substituents appear more lipophilic than expected from the relative π values.

QSAR. The comparison of the pK_i values with the pK_B ones shows that, while all the compounds tested are potent H_3-antagonists, there is poor correlation between the two series of biological data, at least within the two classes of compounds ($r = 0.51$ for **4a–k**; $r = 0.41$ for **5a–k**). No statistically sound QSAR model was observed, for the two subsets **4a–k** and **5a–k**, relating pK_B on guinea-pig ileum to the experimentally derived properties reported in Tables 1 and 2, or to the substituent constants reported in Table 3, by linear or multi-linear regression analysis. For the first subset, this could be due to limited variation of potency (the standard deviation of the pK_B column being only 0.43), compared to the uncertainty of the individual pK_B values. Moreover, most of the variation in the antagonist potency of compounds **5a–k** is due to the high pK_B value of the parent compound, **5a**.

On the other hand, more significant structure–activity relationships were observed for the competitive binding (pK_i) data on rat brain. First of all, we observed that compounds having three methylene groups in the chain spacer (**5a–k**) showed very high affinity for rat brain H_3-receptor and pK_i variation spanning two orders of magnitude. In contrast, the corresponding di-methylene chain derivatives (**4a–k**) showed lower pK_i values and a limited influence of substitution on receptor affinity.

A QSAR study was undertaken for the series **5a–k**, investigating the correlation between the affinity values and a multivariate description of physico-chemical properties and substituent constants. Traditional descriptors of substituent electronic (σ_m, σ_p, σ_{m-p}, F, R) and steric (MR, L, B_1, B_5, S_b) effects[46] were used together with the experimental parameters; the square of the lipophilicity (log P^2) was included to consider a second-degree effect. Given the small number of compounds, MRA was limited to models with no more than two variables.

Surprisingly, no MRA model resulted definitely acceptable from a statistic point of view. The best one

obtained described a parabolic dependence of pK_i on experimental log P (eq 5 and Fig. 2):

$$pK_i = 1.51(\pm 0.37)\log P - 0.31(\pm 0.09)\log P^2 + 6.96(\pm 0.38) \quad (5)$$

$$n = 11;\ \ r^2 = 0.682;\ \ s = 0.38;\ \ F = 8.6;$$
$$q^2 = 0.478;\ \ SDEP = 0.41$$

This model seems to indicate that a suitable range of lipophilicity (around log P 2.40) could be required for optimal pK_i values. No additional steric or electronic parameter could improve the fitting of the model obtained with experimental lipophilicity.

The same data were also submitted to PLS analysis:[49] starting from a matrix including the variables log P (expanded to the squared term), p$K_{a,1}$, σ_m, σ_p and MR, the best q^2 (0.45) was observed for a two-latent variable model. The first latent variable, explaining 60% of pK_i variation, was mainly related to lipophilicity (with a positive coefficient for log P, and a negative one for its squared term), and the second one, explaining a further 18% (total r^2 was therefore 0.78) was dominated by the electronic variables. The pseudo coefficients for the original variables, calculated from their weights on the two latent ones, were:

$$pK_i = 1.40\log P - 0.28\log P^2 + 0.14pK_{a,1} + 0.07\sigma_m - 0.03\sigma_p - 0.04MR + 4.48 \quad (6)$$

This model confirmed the marginal influence of the electronic variables on affinity variation. Even if QSAR analysis did not lead to conclusive deductions, as a tentative explanation of the SAR observed, we concluded that the 2-aminobenzimidazole moiety seems to interact with the receptor in its neutral state, as suggested by the lack of correlation between pK_i and basicity, and that the influence of the 5(6) substituents on receptor affinity could be related to their lipophilicity (see Fig. 2), even if their precise role remains unexplained.

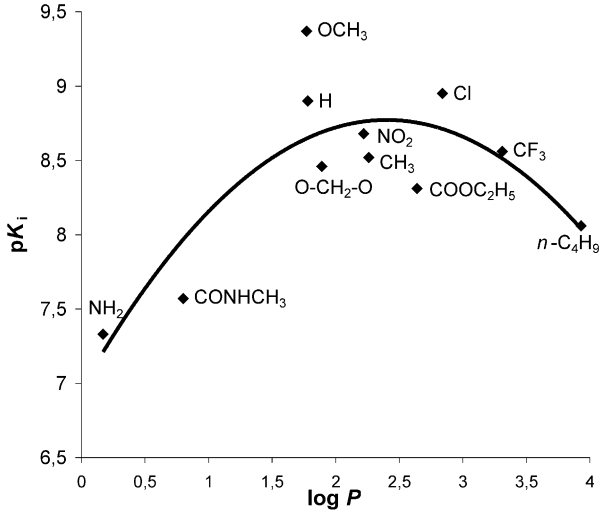


Figure 2. pK_i versus log P for compounds **5a–k**.

However, the hypothesis that the benzimidazole nucleus can be strongly involved in receptor binding is supported by the fact that longer chain derivatives (**5a–k**) showed both higher affinity than the shorter ones (**4a–k**), and higher sensitivity to the effect of 5(6) substitution, revealing more specific interactions. This could be due to strict geometrical requirements for simultaneous interaction at the imidazole and 2-aminobenzimidazole binding sites at the H_3-receptor, which should be fulfilled by the three-methylene derivatives only.

6. Conclusions

2-Aminobenzimidazole derivatives represent a new class of H_3-receptor antagonists, having a polar group (i.e., the 2-aminobenzimidazole itself) of intermediate basicity, compared with those of the classical antagonists thioperamide and clobenpropit, yet able to strongly interact with its binding site at rat H_3-receptor. The introduction of substituents at the benzimidazole 5(6) position exerts the expected influence on compound basicity, and an interesting effect on lipophilicity, also dependent on their electronic properties.

Compounds with an additional imidazole ring and a three-methylene chain (**5a–k**) showed very high affinity for rat brain H_3-receptor, with pK_i variations depending on the properties of the substituents at the 5(6) position of the benzimidazole moiety. An exploration of the substituent space led to the 5(6)-methoxy derivative (**5d**), having sub-nanomolar affinity for rat brain receptor; a dependence of pK_i from experimental lipophilicity indicated an optimal log P value around 2.40. Although compounds **5a–k** also showed high potency as H_3-antagonists on guinea-pig ileum, this pharmacological test revealed a structure–activity profile slightly different from that observed for the displacement of [^{3}H]RAMHA from rat brain membranes; this difference is as yet unexplained. The observed inter-species differences, recently reported in the literature, in receptor binding of H_3-antagonists could explain these differences, and indicate that caution is necessary in the extrapolation of SAR profiles from one species to another.

Compounds with shorter chain (**4a–k**) had lower H_3-receptor affinity and antagonist potency, and showed a different SAR profile, suggesting non-optimal interaction with the two putative binding regions, one for the imidazole nucleus and the other for the 2-aminobenzimidazole one.

Compound **5a** represents a lead structure, endowed with high affinity for the H_3-receptor, which could be considered as a pharmacological tool, or further optimized for in vivo administration.

7. Experimental

Melting points were not corrected and were determined with a Büchi instrument (Tottoli) and with Gallenkamp

melting point apparatus. The final compounds were analyzed for C, H and N. The percentages we found were within ±0.4% of the theoretical values. The ^{1}H NMR spectra were recorded on a Bruker 300 spectrometer (300 MHz); chemical shifts (δ scale) are reported in parts per million (ppm) relative to the central peak of the solvent. ^{1}H NMR spectra are reported in order: multiplicity, approximate coupling constant (*J* value) in hertz (Hz) and number of protons. Abbreviations are the following: Im, imidazolyl; Bz, benzimidazolyl. Mass spectra were recorded using a Finnigan MAT SSQ 710 instrument. Reactions were monitored by TLC, on Kieselgel 60 F 254 (DC-Alufolien, Merck). Final compounds and intermediates were purified by chromatography on preparative Gilson MPLC, using a SiO_2 column (LiChroprep, Si 60, 25–40 μm, Merck); the eluents were mixtures of CH_2Cl_2/CH_3OH at various volume ratios. When indicated, the methanolic phase was saturated with gaseous NH_3.

Yields, reaction times and characteristic data of the final compounds are described in Table 4.

7.1. General method of preparation of 5(6)-substituted 2-mercaptobenzimidazole derivatives (2)

5(6)-Substituted 2-mercaptobenzimidazole derivatives (**2e–g**, **2j–k**[50,51]) were prepared starting from the corresponding 4-substituted *o*-phenylendiamine with carbon disulphide and aqueous potassium hydroxide in 95% ethanol, following the general method described by Rao.[52] 5(6)-Trifluoromethyl-2-mercaptobenzimidazole (**2j**) was synthesized, according to Ref. 50, using potassium ethyl xanthogenate.

Table 4. Yields, reaction times and characteristic data of the final compounds **4a–k** and **5a–k**

Compd	Reaction Time (min)	Yield (%)	Crystallization solvent	mp (°C)
4a[a]	60	65	95% EtOH/Et_2O	223–225[b]
4b	240	25	abs EtOH/Et_2O	261–264[c]
4c	45	25	abs EtOH/Et_2O	267–269[c]
4d	240	26	abs EtOH/Et_2O	196–198[b] (dec.)
4e	240	30	abs EtOH/Et_2O	204–206[b] (dec.)
4f	240	22	abs EtOH/Et_2O	253–255[c]
4g	240	22	abs EtOH/Et_2O	260–262[b] (dec.)
4h	—	90[d]	abs EtOH/Et_2O	299–302[e] (dec.)
4i	60	20	abs EtOH/Et_2O	257–260[c]
4j	60	21	abs EtOH/Et_2O	243–246[c]
4k	240	18	abs EtOH/Et_2O	247–249[c]
5a	60	22	abs EtOH	196–198[f]
5b	240	25	abs EtOH/Acet	241–244[c]
5c	45	25	abs EtOH	246–247[c]
5d	85	21	abs EtOH	196–200[g]
5e	240	22	abs EtOH/Acet	183–185[f]
5f	240	22	95% EtOH	237–240[h]
5g	60	19	abs EtOH/Et_2O	194–197[i] (dec.)
5h	—	90[d]	abs EtOH/Acet	296–298[j]
5i	60	19	abs EtOH/Et_2O	260–262[c]
5j	60	17	abs EtOH/Acet	174–177[f]
5k	60	17	abs EtOH/Acet	188–191[f]

[a] From Ref. 24.
[b] Dihydrochloride·1/2H_2O.
[c] Dihydrochloride.
[d] Reduction from nitroderivatives **4c** and **5c**.
[e] Trihydrochloride·1/2C_2H_5OH.
[f] Dioxalate.
[g] Difumarate.
[h] Oxalate.
[i] Dihydrochloride·H_2O.
[j] Trihydrochloride.

7.2. 5(6)-*n*-Butyl-2-mercaptobenzimidazole (2e)

80%, mp 230–233 °C, ^{1}H NMR (DMSO-d_6) δ 0.88 (t, 3H, CH_3), 1.28 (m, 2H, CH_2), 1.53 (m, 2H, CH_2), 2.60 (t, 2H, CH_2), 6.93 (m, 2H, Bz-H), 7.03 (d, 1H, Bz-H).

7.3. 5(6)-Carboxyethyl-2-mercaptobenzimidazole (2f)

80%, mp 281–283 °C, ^{1}H NMR (DMSO-d_6) δ 1.31 (t, 3H, CH_3), 4.40 (m, 2H, CH_2), 7.22 (d, J = 8.3 Hz, 1H, Bz-H), 7.66 (s, 1H, Bz-H), 7.76 (d, J = 8.3 Hz, 1H, Bz-H).

7.4. 5(6)-*N*-Methylcarbamoyl-2-mercaptobenzimidazole (2g)

80%, 336–340 °C, ^{1}H NMR (DMSO-d_6) δ 2.77 (d, 3H, CH_3), 7.16 (d, J = 8.2 Hz, 1H, Bz-H), 7.62 (s, 1H, Bz-H), 7.64 (d, J = 8.3 Hz, 1H, Bz-H), 8.40 (m, 1H, NH).

7.5. General method of preparation of 5(6)-substituted 2-benzimidazolesulphonic acid derivatives (3)

5(6)-Substituted 2-benzimidazolesulphonic acid derivatives (**3a–b**,[42] **3d**,[42] **3e–g**, **3i**,[42] **3j–k**, sodium salt for **3c**[42]) were prepared starting from the corresponding 5(6)-substituted 2-mercaptobenzimidazole with 10% sodium hydroxide and 30% hydrogen peroxide ($KMnO_4$ for **3c**) following the methods reported by Sorba.[42] The carboxyethyl derivative **3f** was prepared by treating 5(6)-carboxy-2-benzimidazolesulphonic acid (12 mmol, 3g) with abs ethanol (20 mL) saturated with gaseous HCl. The reaction mixture was stirred at 100 °C for 3 h, under dry nitrogen. The solvent was then evaporated under reduced pressure and the crude product crystallized from abs EtOH/Et_2O.

7.6. 5(6)-*n*-Butyl-2-benzimidazolesulphonic acid (3e)

68%, 335–338 °C, ^{1}H NMR (DMSO-d_6) δ 0.89 (t, 3H, CH_3), 1.31 (m, 2H, CH_2), 1.59 (m, 2H, CH_2), 2.76 (t, 2H, CH_2), 7.43 (d, 1H, Bz-H), 7.48 (s, 1H, Bz-H), 7.61 (d, 1H, Bz-H).

7.7. 5(6)-Carboxyethyl-2-benzimidazolesulphonic acid (3f)

89%, 324–327 °C, ^{1}H NMR (DMSO-d_6) δ 1.34 (t, 3H, CH_3), 4.37 (m, 2H, CH_2), 7.79 (d, J = 8.8 Hz, 1H, Bz-H), 8.10 (dd, J = 8.7 Hz and J = 1.6 Hz, 1H, Bz-H), 8.21 (d, 1H, Bz-H).

7.8. 5(6)-*N*-Methylcarbamoyl-2-benzimidazolesulphonic acid (3g)

71%, >340 °C, ^{1}H NMR (DMSO-d_6) δ 2.81 (d, 3H, CH_3), 7.75 (d, J = 8.7 Hz, 1H, Bz-H), 8.04 (dd, J = 8.7

Hz and $J = 0.9$ Hz, 1H, Bz-H), 8.17 (d, 1H, Bz-H), 8.70 (m, 1H, NH).

7.9. 5(6)-Trifluoromethyl-2-benzimidazolesulphonic acid (3j)

62%, 301–303 °C, ^{1}H NMR (DMSO-d_6) δ 7.78 (d, $J = 8.5$ Hz, 1H, Bz-H), 7.87 (d, $J = 8.5$ Hz, 1H, Bz-H), 7.98 (s, 1H, Bz-H).

7.10. 5,6-Methylenedioxy-2-benzimidazolesulphonic acid (3k)

70%, >340 °C, ^{1}H NMR (DMSO-d_6) δ 6.17 (s, 2H, CH_2), 7.13 (s, 2H, Bz-H).

7.11. 5(6)-Carboxy-2-benzimidazolesulphonic acid

68%, 343–345 °C, ^{1}H NMR (DMSO-d_6) δ 7.78 (d, $J = 8.6$ Hz, 1H, Bz-H), 8.10 (dd, $J = 8.7$ Hz and $J = 1.1$ Hz, 1H, Bz-H), 8.21 (d, 1H, Bz-H).

7.12. General method of condensation of 5(6)-substituted 2-benzimidazolesulphonic acids with 4(5)-(ω-aminoalkyl)-imidazole (4a,[22] 4b–g, 4i–k, 5a–g, 5i–k)

A mixture of the 5(6)-substituted 2-benzimidazolesulphonic acid (10 mmol) (**3a–g, 3i–k**) and the appropriate 4(5)-(ω-alkylamino)imidazole (15 mmol) was heated at 130 °C for the period of time reported in Table 4. The residue was then treated with a saturated aqueous sodium bicarbonate solution and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The crude products were purified by column chromatography (SiO_2, **4a**,**5a** CH_2Cl_2:$CH_3OH(NH_3)$ = 10:1; **4g**,**5g** CH_2Cl_2:$CH_3OH(NH_3)$ = 5:1; **4c**,**5c** CH_2Cl_2:$CH_3OH(NH_3)$ = 20:1; **4b**,**4d**, **4e**,**4f**,**4i**,**4j**,**4k**,**5b**,**5d**,**5e**,**5f**,**5i**,**5j**,**5k** CH_2Cl_2:$CH_3OH(NH_3)$ = 15:1).

7.13. Method of preparation of the 5(6)-aminoderivatives (4h,5h)

The 5(6)-aminoderivatives **4h** and **5h** were obtained by hydrogenation (50 psi) of the nitro compounds **4c** and **5c** in Parr apparatus with Pd/C (10%), in abs EtOH, at room temperature, for **3h**.

7.14. 2-[2-[4(5)-Imidazolyl]ethyl]amino]benzimidazole dihydrochloride (4a·2HCl)

^{1}H NMR (DMSO-d_6) δ 3.06 (t, 2H, CH_2-Im), 3.78 (m, 2H, CH_2-N), 7.19–7.24 (m, 2H, Bz-H), 7.37–7.43 (m, 2H, Bz-H), 7.59 (s, 1H, Im-5-H), 9.06 (s, 1H, Im-2-H), 9.38 (t, 1H, NH). MS (EI) 227 [M^+]. Anal. ($C_{12}H_{13}N_5$·2HCl·1/2H_2O) C, H, N.

7.15. 5(6)-Chloro-2-[2-[4(5)-imidazolyl]ethyl]amino]benzimidazole dihydrochloride (4b·2HCl)

^{1}H NMR (DMSO-d_6) δ 3.05 (t, 2H, CH_2-Im), 3.74 (t, 2H, CH_2-N), 7.26 (dd, $J = 8.6$ Hz and $J = 1.8$ Hz, 1H, Bz-H), 7.39 (d, $J = 8.6$ Hz, 1H, Bz-H), 7.43 (d, $J = 1.8$ Hz, 1H, Bz-H), 7.58 (s, 1H, Im-5-H), 9.04 (s, 1H, Im-2-H). MS (CI) 262 [$M + 1$]$^+$. Anal. ($C_{12}H_{12}N_5Cl$·2HCl) C, H, N.

7.16. 5(6)-Nitro-2-[2-[4(5)-imidazolyl]ethyl]amino]benzimidazole dihydrochloride (4c·2HCl)

^{1}H NMR (DMSO-d_6) δ 3.08 (t, 2H, CH_2-Im), 3.80 (t, 2H, CH_2-N), 7.58 (d, 1H, Bz-H), 7.60 (s, 1H, Im-5-H), 8.13 (dd, 1H, Bz-H), 8.17 (d, 1H, Bz-H), 9.05 (s, 1H, Im-2-H). MS (CI) 273 [$M + 1$]$^+$. Anal. ($C_{12}H_{12}N_6O_2$·2HCl) C, H, N.

7.17. 5(6)-Methoxy-2-[2-[4(5)-imidazolyl]ethyl]amino]-benzimidazole dihydrochloride (4d·2HCl)

^{1}H NMR (DMSO-d_6) δ 3.04 (t, 2H, CH_2-Im), 3.73 (t, 2H, CH_2-N), 3.77 (s, 3H, CH_3), 6.82 (dd, $J = 8.7$ Hz and $J = 2.4$ Hz, 1H, Bz-H), 6.94 (d, $J = 2.4$ Hz, 1H, Bz-H), 7.29 (d, $J = 8.7$ Hz, 1H, Bz-H), 7.58 (s, 1H, Im-5-H), 9.05 (s, 1H, Im-2-H). MS (EI) 257 [M^+]. Anal. ($C_{13}H_{15}N_5O$·2HCl·1/2H_2O) C, H, N.

7.18. 5(6)-*n*-Butyl-2-[2-[4(5)-imidazolyl]ethyl]amino]benzimidazole dihydrochloride (4e·2HCl)

^{1}H NMR (DMSO-d_6) δ 0.91 (t, 3H, CH_3), 1.23–1.36 (m, 2H, CH_2), 1.50–1.60 (m, 2H, CH_2), 2.50 (t, 2H, CH_2), 2.61 (t, 2H, CH_2-Im), 3.43 (t, 2H, CH_2-N), 6.92 (d, $J = 7.9$ Hz, 1H, Bz-H), 7.12 (s, 1H, Bz-H), 7.20 (d, $J = 7.9$ Hz, 1H, Bz-H), 7.22 (s, 1H, Im-5-H), 8.57 (s, 1H, Im-2-H). MS (EI) 283 [M^+]. Anal. ($C_{16}H_{21}N_5$·2HCl·1/2H_2O) C, H, N.

7.19. 5(6)-Carboxyethyl-2-[2-[4(5)-imidazolyl]ethyl]amino]-benzimidazole dihydrochloride (4f·2HCl)

^{1}H NMR (DMSO-d_6) δ 1.33 (t, 3H, CH_3), 3.07 (t, 2H, CH_2-Im), 3.82 (t, 2H, CH_2-N), 4.32 (m, 2H, CH_2), 7.49 (d, $J = 8.4$ Hz, 1H, Bz-H), 7.60 (s, 1H, Im-5-H), 7.86 (dd, $J = 7.9$ Hz and $J = 1.3$ Hz, 1H, Bz-H), 7.94 (d, $J = 1.3$ Hz, 1H, Bz-H), 9.05 (s, 1H, Im-2-H). MS (CI) 300 [$M + 1$]$^+$. Anal. ($C_{15}H_{17}N_5O_2$·2HCl) C, H, N.

7.20. 5(6)-*N*-Methylcarbamoyl-2-[2-[4(5)-imidazolyl]ethyl]-amino]benzimidazole dihydrochloride (4g·2HCl)

^{1}H NMR (DMSO-d_6) δ 2.78 (s, 3H, CH_3), 3.06 (t, 2H, CH_2-Im), 3.80 (t, 2H, CH_2-N), 7.43 (d, $J = 8.3$ Hz, 1H, Bz-H), 7.60 (s, 1H, Im-5-H), 7.75 (dd, $J = 8.4$ Hz and $J = 1.5$ Hz, 1H, Bz-H), 7.87 (d, $J = 1.5$ Hz, 1H, Bz-H), 9.05 (s, 1H, Im-2-H). MS (CI) 285 [$M + 1$]$^+$. Anal. ($C_{14}H_{16}N_6O$·2HCl·1/2H_2O) C, H, N.

7.21. 5(6)-Amino-2-[2-[4(5)-imidazolyl]ethyl]amino]benzimidazole trihydrochloride (4h·3HCl)

^{1}H NMR (DMSO-d_6) δ 3.06 (t, 2H, CH_2-Im), 3.77 (m, 2H, CH_2-N), 7.17 (dd, 1H, Bz-H), 7.42 (s, 1H, Bz-H), 7.45 (d, 1H, Bz-H), 7.59 (s, 1H, Im-5-H), 9.05 (s, 1H, Im-2-H), 9.53 (t, 1H, NH). MS (CI) 287 [$M + 1$]$^+$. Anal. ($C_{13}H_{14}N_6O_2$·3HCl·1/2C_2H_5OH) C, H, N.

7.22. 5(6)-Methyl-2-[2-[4(5)-imidazolyl]ethyl]amino]benzimidazole dihydrochloride (4i·2HCl)

^{1}H NMR (DMSO-d_6) δ 2.36 (s, 3H, CH_3), 3.05 (t, 2H, CH_2-Im), 3.76 (m, 2H, CH_2-N), 7.03 (d, J = 8.2 Hz, 1H, Bz-H), 7.20 (s, 1H, Bz-H), 7.27 (d, J = 8.1 Hz, 1H, Bz-H), 7.59 (s, 1H, Im-5-H), 9.05 (s, 1H, Im-2-H), 9.27 (t, 1H, NH). MS (CI) 242 $[M+1]^+$. Anal. ($C_{13}H_{15}N_5$·2HCl) C, H, N.

7.23. 5(6)-Trifluoromethyl-2-[2-[4(5)-imidazolyl]ethyl]-amino]benzimidazole dihydrochloride (4j·2HCl)

^{1}H NMR (DMSO-d_6) δ 3.07 (t, 2H, CH_2-Im), 3.80 (m, 2H, CH_2-N), 7.58–7.61 (m, 3H, Bz-H), 7.67 (s, 1H, Im-5-H), 9.07 (s, 1H, Im-2-H), 9.76 (t, 1H, NH). MS (CI) 296 $[M+1]^+$. Anal. ($C_{13}H_{12}N_5F_3$·2HCl) C, H, N.

7.24. 5,6-Methylenedioxy-2-[2-[4(5)-imidazolyl]ethyl]-amino]benzimidazole dihydrochloride (4k·2HCl)

^{1}H NMR (DMSO-d_6) δ 3.01 (t, 2H, CH_2-Im), 3.67 (m, 2H, CH_2-N), 6.04 (s, 2H, CH_2), 7.01 (s, 2H, Bz-H), 7.55 (s, 1H, Im-5-H), 8.93 (t, 1H, NH), 9.02 (s, 1H, Im-2-H). MS (CI) 272 $[M+1]^+$. Anal. ($C_{13}H_{13}N_5O_2$·2HCl) C, H, N.

7.25. 2-[3-[4(5)-Imidazolyl]propyl]amino]benzimidazole (5a)

^{1}H NMR (DMSO-d_6) δ 1.86–1.89 (m, 2H, CH_2), 2.57 (t, 2H, CH_2-Im), 3.32 (t, 2H, CH_2-N), 6.77 (s, 1H, Im-5-H), 6.85–6.90 (m, 2H, Bz-H), 7.12–7.16 (m, 2H, Bz-H), 7.54 (s, 1H, Im-2-H). MS (EI) 241 $[M^+]$. Anal. ($C_{13}H_{15}N_5$·2$C_2H_2O_4$) C, H, N.

7.26. 5(6)-Chloro-2-[3-[4(5)-imidazolyl]propyl]amino]benzimidazole dihydrochloride (5b·2HCl)

^{1}H NMR (DMSO-d_6) δ 1.99–2.02 (m, 2H, CH_2), 2.80 (t, 2H, CH_2-Im), 3.48 (t, 2H, CH_2-N), 7.23 (dd, J = 8.5 Hz and J = 1.8 Hz, 1H, Bz-H), 7.38 (d, J = 8.5 Hz, 1H, Bz-H), 7.42 (d, J = 1.8 Hz, 1H, Bz-H), 7.47 (s, 1H, Im-5-H), 9.01 (s, 1H, Im-2-H). MS (CI) 276 $[M+1]^+$. Anal. ($C_{13}H_{14}N_5Cl$·2HCl) C, H, N.

7.27. 5(6)-Nitro-2-[3-[4(5)-imidazolyl]propyl]amino]benzimidazole dihydrochloride (5c·2HCl)

^{1}H NMR (DMSO-d_6) δ 1.99–2.02 (m, 2H, CH_2), 2.79 (t, 2H, CH_2-Im), 3.49 (t, 2H, CH_2-N), 7.49 (d, 1H, Bz-H), 7.52 (s, 1H, Im-5-H), 8.10 (d, 1H, Bz-H), 8.13 (s, 1H, Bz-H), 9.00 (s, 1H, Im-2-H). MS (EI) 286 $[M^+]$. Anal. ($C_{13}H_{14}N_6O_2$·2HCl) C, H, N.

7.28. 5(6)-Methoxy-2-[3-[4(5)-imidazolyl]propyl]amino]-benzimidazole (5d)

^{1}H NMR (DMSO-d_6) δ 1.86–1.89 (m, 2H, CH_2), 2.58 (t, 2H, CH_2-Im), 3.30 (t, 2H, CH_2-N), 3.70 (s, 3H, CH_3), 6.52 (dd, J = 8.1 Hz and J = 2.2 Hz, 1H, Bz-H), 6.76 (d, J = 2.2 Hz, 1H, Bz-H), 6.81 (s, 1H, Im-5-H), 7.04 (d, J = 8.5 Hz, 1H, Bz-H), 7.65 (s, 1H, Im-2-H). MS (EI) 271 $[M^+]$. Anal. ($C_{14}H_{17}N_5O$·2$C_4H_4O_4$) C, H, N.

7.29. 5(6)-*n*-Butyl-2-[3-[4(5)-imidazolyl]propyl]amino]benzimidazole dioxalate (5e·2$C_2H_2O_4$)

^{1}H NMR (DMSO-d_6) δ 0.89 (t, 3H, CH_3), 1.23–1.35 (m, 2H, CH_2), 1.50–1.60 (m, 2H, CH_2), 1.89–1.98 (m, 2H, CH_2), 2.63 (t, 2H, CH_2), 2.72 (t, 2H, CH_2-Im), 3.41 (t, 2H, CH_2-N), 7.00 (dd, 1H, Bz-H), 7.16 (d, 1H, Bz-H), 7.24–7.26 (m, 2H, Bz-H, Im-5-H), 8.53 (s, 1H, Im-2-H). MS (CI) 298 $[M+1]^+$. Anal. ($C_{17}H_{23}N_5$·2$C_2H_2O_4$) C, H, N.

7.30. 5(6)-Carboxyethyl-2-[3-[4(5)-imidazolyl]propyl]-amino]benzimidazole oxalate (5f·$C_2H_2O_4$)

^{1}H NMR (DMSO-d_6) δ 1.31 (t, 3H, CH_3), 1.92–1.95 (m, 2H, CH_2), 2.72 (t, 2H, CH_2-Im), 3.38 (t, 2H, CH_2-N), 4.28 (m, 2H, CH_2), 7.25 (d, J = 8.3 Hz, 1H, Bz-H), 7.36 (s, 1H, Im-5-H), 7.65 (dd, J = 8.3 Hz and J = 1.6 Hz, 1H, Bz-H), 7.77 (d, J = 1.4 Hz, 1H, Bz-H), 8.75 (s, 1H, Im-2-H). MS (CI) 314 $[M+1]^+$. Anal. ($C_{16}H_{19}N_5O_2$·$C_2H_2O_4$) C, H, N.

7.31. 5(6)-*N*-Methylcarbamoyl-2-[3-[4(5)-imidazolyl]propyl]amino]benzimidazole dihydrochloride (5g·2HCl)

^{1}H NMR (DMSO-d_6) δ 1.99–2.03 (m, 2H, CH_2), 2.78 (s, 3H, CH_3), 2.80 (t, 2H, CH_2-Im), 3.30 (t, 2H, CH_2-N), 7.41 (d, 1H, Bz-H), 7.46 (s, 1H, Im-5-H), 7.73 (dd, 1H, Bz-H), 7.83 (s, 1H, Bz-H), 8.98 (s, 1H, Im-2-H). MS (CI) 299 $[M+1]^+$. Anal. ($C_{15}H_{18}N_6O$·2HCl·H_2O) C, H, N.

7.32. 5(6)-Amino-2-[3-[4(5)-imidazolyl]propyl]amino]benzimidazole trihydrochloride (5h·3HCl)

^{1}H NMR (DMSO-d_6) δ 2.00–2.03 (m, 2H, CH_2), 2.80 (t, 2H, CH_2-Im), 3.48 (t, 2H, CH_2-N), 7.18 (dd, J = 8.5 Hz, 1H, Bz-H), 7.43 (d, J = 8.5 Hz, 1H, Bz-H), 7.47–7.49 (m, 2H, Bz-H, Im-5-H), 9.01 (s, 1H, Im-2-H). MS (CI) 257 $[M+1]^+$. Anal. ($C_{13}H_{16}N_6$·3HCl) C, H, N.

7.33. 5(6)-Methyl-2-[3-[4(5)-imidazolyl]propyl]amino]-benzimidazole dihydrochloride (5i·2HCl)

^{1}H NMR (DMSO-d_6) δ 1.99–2.03 (m, 2H, CH_2), 2.36 (s, 3H, CH_3), 2.79 (t, 2H, CH_2-Im), 3.47 (m, 2H, CH_2-N), 7.01 (dd, 1H, Bz-H), 7.19 (s, 1H, Im-5-H), 7.26 (d, 1H, Bz-H), 7.47 (d, 1H, Bz-H), 9.01 (s, 1H, Im-2-H), 9.27 (t, 1H, NH). MS (CI) 256 $[M+1]^+$. Anal. ($C_{14}H_{17}N_5$·2HCl) C, H, N.

7.34. 5(6)-Trifluoromethyl-2-[3-[4(5)-imidazolyl]propyl]-amino]benzimidazole dioxalate (5j·2$C_2H_2O_4$)

^{1}H NMR (DMSO-d_6) δ 1.90–1.94 (m, 2H, CH_2), 2.72 (t, 2H, CH_2-Im), 3.36 (t, 2H, CH_2-N), 7.25 (dd, 1H, Bz-H), 7.32 (d, 1H, Bz-H), 7.37 (d, 1H, Bz-H), 7.46 (s, 1H, Im-5-H), 8.80 (s, 1H, Im-2-H). MS (CI) 310 $[M+1]^+$. Anal. ($C_{14}H_{14}N_5F_3$·2$C_2H_2O_4$) C, H, N.

7.35. 5,6-Methylenedioxy-2-[3-[4(5)-imidazolyl]propyl]-amino]benzimidazole dioxalate (5k·2$C_2H_2O_4$)

^{1}H NMR (DMSO-d_6) δ 1.90–1.94 (m, 2H, CH_2), 2.72 (t, 2H, CH_2-Im), 3.32 (m, 2H, CH_2-N), 5.97 (s, 2H, CH_2), 6.96 (s, 2H, Bz-H), 7.30 (s, 1H, Im-5-H), 8.72 (s, 1H,

Im-2-H). MS (EI) 285 [M^+]. Anal. ($C_{14}H_{15}N_5O_2 \cdot 2\text{-}C_2H_2O_4$) C, H, N.

7.36. Lipophilicity

log *P* And log $D_{7.4}$ values were determined by the shake-flask technique. The two-phase system consisted of mutually saturated *n*-octanol and aqueous buffers 0.05M CAPS (3-cyclohexylamino-1-propanesulfonic acid) pH 11 for log *P* and 0.05M MOPS [3-(*N*-morpholino)propanesulfonic acid] pH 7.4 for log *D*. Ionic strength was adjusted at 0.15M with KCl.

After partitioning, samples were centrifuged at 3000 rpm for 10 min, each of the two phases was manually separated and diluted with analytical grade CH_3OH before injection in HPLC apparatus equipped with UV detector (Gilson); the volume injected was 20 μL per run. The column was a Spherisorb CN-S10, 250×4.6 mm (Waters). Mobile phases were mixtures of analytical CH_3CN and KH_2PO_4 0.01 M (pH between 5.0 and 7.4). log *P* And log $D_{7.4}$ values were calculated from the ratio of the mean peak areas in the two phases, correcting for instrumental attenuation and dilution; they are the means of at least four different measurements.

7.37. Dissociation constants

pK_a Values were measured using an automatic potentiometric apparatus with automatic burette addition of KOH of known normality (0.1 N). 0.04–0.05 mmol Of the compound (salt) were dissolved in CO_2-free water inside a jacketed vessel, keeping the temperature at 25 ± 0.1 °C. Air was displaced with a slow stream of N_2. Ionic strength was kept fixed at 0.15 M by the addition of KCl. The standard potential E_0 and the pK_w values were determined from titrations of fixed volumes of HCl of known concentration with KOH, determining the neutralization point with the Gran method. The dissociation constant values are the means of at least three different titrations with a mean s.d. = ±0.01 and were refined by employing the SUPERQUAD program.[53]

7.38. Pharmacology. Binding assays

Rat (Wistar) brain membranes were incubated for 30 min with [^{3}H]RAMHA 0.5 nM and the compounds under study (1 nM–10μM), in Tris–HCl 50 mM, pH 7.4, NaCl 50 mM, EDTA 0.5 mM, then rapidly filtered (AAWP 0.8μM) under vacuum and rinsed with ice-cold buffer. Specific binding was defined as the binding inhibited by thioperamide 10μM, and the IC_{50} values were estimated from the displacement curves of the compounds tested versus [^{3}H]RAMHA bound to cerebral membranes.[22] pK_i Values were calculated according to Cheng and Prusoff's equation.[54]

7.39. Functional assays

Portions of guinea-pig ileum were mounted on a coaxial platinum electrode assembly in a 10 mL water-jacketed organ-bath containing Krebs–Henseleit solution aerated with 95%O_2:5%CO_2 and maintained at 37 °C. Single electrical pulses were delivered to the tissue (0.1 Hz, 1 ms, 1.5–3.0 V) from a stimulator (LACE Elettronica model ES-3, Ospedaletto PI, Italy). Cumulative concentration-response curves for the inhibition of electrically stimulated contractions were determined for the H_3 selective agonist RAMHA (1 nM–1μM). The tissues were allowed to equilibrate with the compounds under study (1 nM–10μM) for 30 min before the generation of concentration-response curves to the agonist. pK_B Values ('apparent pA_2') were determined according to Furchgott's equation.[55]

7.40. [^{35}S]GTPγS binding assay

[^{35}S]GTPγS binding assays were performed according to Nickel et al.[56] with modifications. Cerebral cortex and striatum from female Wistar rats (150–200g) were homogenized (Potter–Elvehjem) in 6 volumes of ice-cold membrane buffer (Tris–HCl 50 mM, $MgCl_2$ 3 mM, EGTA 1 mM, pH 7.4) and centrifuged twice at 14,500 rpm for 15 min. The final pellet, resuspended and homogenized in 6 volumes of assay buffer (Tris–HCl 50 mM, $MgCl_2$ 3 mM, EGTA 0.2 mM, NaCl 100 mM, pH 7.7), was then preincubated for 10 min at 30 °C with adenosine deaminase (0.6 U/mL) to remove endogenous adenosine.[57,58] Membranes in aliquots of 0.5 mL (50 μg protein) were incubated for 60 min at 30 °C with 0.05nM [^{35}S]GTPγS and, when required, with the different drugs tested, in a final volume of 1 mL of assay buffer containing 100μM GDP. The non specific binding was determined using GTPγS (20μM). Incubations were terminated by rapid filtration through Whatman GF/B glass filters. Filters were washed three times with 3 mL of ice-cold Tris–HCl 50 mM buffer, pH 7.7, and bound radioactivity was measured by liquid scintillation spectrometry. Protein concentration was assayed applying Bradford's method.[59] Following incubation with 0.05 nM [^{35}S]GTPγS, specific binding to rat brain membranes gave 1500–3000 d.p.m.; non specific binding was 15–20% of total binding. Specific binding was significantly increased (about 27%) by RAMHA (EC_{50} = 34.67 nM, maximal effect at 300 nM), which had been reported to behave as a full agonist in H_3-receptor mediated stimulation of [^{35}S]GTPγS binding in cerebral cortex rat membranes.[60] The drugs under study were used at concentrations ranging from 0.01 nM to 1 μM. The H_3-antagonists thioperamide and clobenpropit did not significantly affect [^{35}S]GTPγS binding in the range 0.01–100 nM, while they abolished the effect of 100 nM RAMHA at the concentrations of 100 and 10 nM, respectively.

7.41. QSAR

Multiple regression analysis calculations were performed with an Excel (Microsoft Co., version 97) spreadsheet, employing the built-in statistical functions and automated macro procedures. For each subset of compounds, and for the dependent variables employed, MRA models were calculated with one or two variables simultaneously included; all the possible combinations were tested. Partial least squares (PLS) analysis was performed, after scaling of the variables to unit

Elemental analysis data (found data within ±0.4% of theoretical values)

Compd.	C calc.	C found	H calc.	H found	N calc.	N found
4a	C = 46.61	C = 46.57	H = 5.21	H = 4.88	N = 22.65	N = 22.80
4b	C = 43.07	C = 43.27	H = 4.22	H = 4.18	N = 20.93	N = 20.84
4c	C = 41.75	C = 41.80	H = 4.09	H = 4.14	N = 24.35	N = 24.23
4d	C = 46.02	C = 46.28	H = 5.35	H = 5.54	N = 20.65	N = 20.28
4e	C = 52.60	C = 52.83	H = 6.62	H = 6.79	N = 19.17	N = 19.08
4f	C = 48.39	C = 48.36	H = 5.14	H = 5.22	N = 18.81	N = 18.59
4g	C = 45.91	C = 45.71	H = 5.23	H = 5.24	N = 22.95	N = 22.84
4h	C = 41.67	C = 41.34	H = 5.38	H = 5.02	N = 22.43	N = 22.76
4i	C = 49.69	C = 49.73	H = 5.45	H = 5.52	N = 22.29	N = 22.12
4j	C = 42.40	C = 42.45	H = 3.83	H = 3.82	N = 19.02	N = 18.98
4k	C = 45.36	C = 45.10	H = 4.39	H = 4.39	N = 20.35	N = 20.10
5a	C = 48.46	C = 48.50	H = 4.55	H = 4.93	N = 16.62	N = 16.85
5b	C = 44.78	C = 44.56	H = 4.63	H = 4.62	N = 20.08	N = 19.84
5c	C = 43.46	C = 43.10	H = 4.49	H = 4.60	N = 23.40	N = 23.07
5d	C = 52.48	C = 52.48	H = 5.00	H = 5.13	N = 13.91	N = 13.60
5e	C = 52.82	C = 52.72	H = 5.70	H = 5.83	N = 14.67	N = 14.31
5f	C = 53.59	C = 53.66	H = 5.24	H = 5.32	N = 17.36	N = 17.09
5g	C = 46.28	C = 46.66	H = 5.70	H = 5.96	N = 21.59	N = 21.34
5h	C = 42.69	C = 43.00	H = 5.24	H = 5.51	N = 22.98	N = 22.59
5i	C = 51.22	C = 51.12	H = 5.83	H = 6.01	N = 21.34	N = 21.20
5j	C = 44.17	C = 44.12	H = 3.71	H = 3.85	N = 14.31	N = 14.34
5k	C = 46.45	C = 46.40	H = 4.12	H = 4.20	N = 15.05	N = 14.79

variance, with the program Simca 6.0;[61] the lipophilicity variable (log *P*) was squared, in order to include parabolic effects. Standard deviation of the errors in prediction (*SDEP*), and the relative predictivity parameter, q^2, were calculated by cross-validation, omitting one compound at a time from the set, according to the leave-one-out technique (LOO).[62]

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