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Il Farmaco 58 (2003) 891–899

IL FARMACO

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The role of HB-donor groups in the heterocyclic polar fragment of H_3-antagonists. I. Synthesis and biological assays

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Received 5 December 2002; accepted 20 February 2003

Abstract

It has been recently reported that compounds composed of an imidazole connected through an alkyl spacer to a 2-aminobenzimidazole showed high affinity towards the H_3-receptor. The guanidine fragment of the 2-aminobenzimidazole is probably involved in hydrogen bond interactions at the binding site, and is referred to as the 'polar fragment'. In the present work, starting from 2-aminobenzimidazole derivatives with a di-methylene spacer **1** ($pK_i = 7.25$) or a tri-methylene one **2** ($pK_i = 8.90$), we investigated the importance of the hydrogen bond (HB) donor groups at the polar fragment in the interaction with the H_3-receptor. The replacement of 2-aminobenzimidazoles with different moieties [2-aminobenzothiazole, **3**, **4**; 2-thiobenzimidazole, **5**, **6**; 2-thiobenzothiazole, **7**, **8**; 2-thio-4-phenyl- or 2-thio-5-phenyl-*N*-methylimidazoles, **9–12**] highlighted the effect of the polar group basicity on the optimal length of the alkyl chain: longer spacers were preferred with polar groups of moderate basicity whereas, in the presence of neutral polar groups, the best affinity values were obtained with di-methylene chains. Moreover, N-methylation at the 2-aminobenzimidazole moiety **13**–**16** revealed different behaviour for compounds having different spacer lengths. In fact, methylation of the exocyclic NH group maintained high affinity for the tri-methylene 2-aminobenzimidazole derivative, while a drop in affinity was observed for the annular N-methylation. An opposite trend characterised di-methylene derivatives. These observed SAR suggest that, within this class of compounds, the number of HB-donor groups can be lowered while maintaining high receptor affinity. Since the presence of HB-donor groups strongly affects brain access, this observation could be useful to design and prepare new H_3-antagonists.

Keywords: Histamine; H_3-receptor antagonists; 2-Aminobenzimidazole; Polar group

1. Introduction

Histamine is a neurotransmitter that exerts its pharmacological actions by interacting with four histamine receptors (H_1, H_2, H_3 [1] and H_4 [2,3]).

The histamine H_3-receptor is a presynaptically located autoreceptor [4] in the central nervous system (CNS) and in the peripheral nervous system (PNS) of many species; it regulates the synthesis and release of histamine by a negative feedback mechanism [5,6]. On non-histaminergic neurons, H_3-heteroreceptors modulate the release of several neurotransmitters, such as glutamate, acetylcholine, noradrenaline, dopamine and serotonin [7–12].

H_3-receptor modulation could have a therapeutic use for the treatment of cognitive disorders, including attention deficit hyperactivity disorder (ADHD), Alzheimer's disease, obesity and schizophrenia [13–15].

In recent years, new developments have been made in histamine H_3-receptor research, starting from the first

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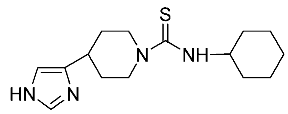
doi:10.1016/S0014-827X(03)00147-2



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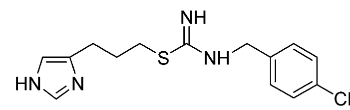

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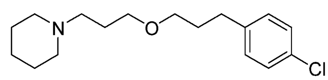

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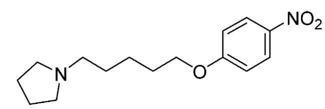

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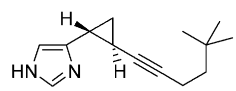

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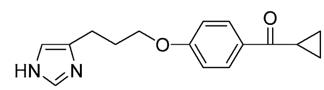

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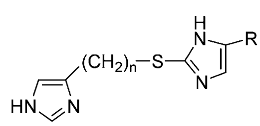

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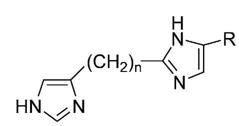


2-alkylimidazole derivatives

Chart 1. Histamine H_3-receptor antagonists.

selective H_3-receptor antagonist thioperamide [16] (see Chart 1) and the other reference antagonist clobenpropit [17] (see Chart 1).

Current H_3-antagonists can be divided into two main classes: imidazole and non-imidazole derivatives. Although some progress has been made in the last few years in the non-imidazole class (e.g. FUB 649 [18], UCL 1972 [19], see Chart 1), the most potent and most studied H_3-antagonists belong to the imidazole class (e.g. Perceptin™ [20,21], ciproxifan [22], see Chart 1), including compounds characterised by the classical structure consisting of an imidazole ring connected by a spacer to a polar group (e.g. guanidines [23], carbamates [24,25], sulfonamides [26–28] or heterocyclic rings), which is attached to a lipophilic ending group [29].

A further classification of the imidazole H_3-antagonists was based on the basicity of the polar group, in compounds charged at physiological pH (e.g. clobenpropit) and in neutral ones (e.g. thioperamide).

During our past investigations on H_3-antagonists, we have focused our attention on imidazole derivatives having heterocyclic rings as polar groups, with the purpose of clarifying the interaction between this fragment and the H_3-receptor.

Different ways of interaction with the H_3-receptor have been hypothesised for neutral and moderately basic heterocycles, respectively. In fact, considering series of H_3-antagonists having a 2-alkylthioimidazole polar group (see Chart 1) [30,31] (not protonated at physiological pH) and compounds with higher basicity, such as 2-alkylimidazole derivatives (see Chart 1) [32], we observed that the basicity of the polar group affects the optimal length of the alkyl chain connecting this fragment to the imidazole ring. In the 2-alkylthioimidazole derivatives, a better affinity was measured in the presence of shorter chains (di-methylene), whereas an increase in basicity led to an improvement in affinity for compounds with longer chain spacers (tri-methylene) [32].

On the basis of these considerations, we have synthesised a new class of H_3-antagonists having a 2-aminobenzimidazole polar group (moderately basic) and a tri-methylene spacer, with the aim of comparing it with its shorter homologous **1** (2-[2-[imidazol-4(5)-yl]ethylamino]benzimidazole; $pK_i = 7.25$), previously reported by us [31,33]. We have thus obtained a new H_3-receptor antagonist with high affinity **2** ($pK_i = 8.90$ [34,35]), useful as a lead for the new study reported in this work, regarding the interaction between the heterocyclic polar group and the H_3-receptor.

In fact, considering the remarkable improvement in affinity obtained for the 2-aminobenzimidazole derivative having a tri-methylene chain, compared to its shorter homologous **1**, it is possible to assume that, in longer derivatives, there is an optimal arrangement of the imidazole ring and the moderately basic polar group, which could establish important interactions with the H_3-receptor.

The compounds reported in this work were synthesised and tested to investigate the importance of the hydrogen bond (HB) donor NH groups of the aminobenzimidazole moiety both in di-methylene derivatives and in longer ones, to find out if these two classes of compounds interact with different mode of binding at the H_3-receptor.

Moreover, the presence of free NH groups could affect compound distribution [36], and thus it might be more difficult for these derivatives to cross the blood brain barrier and to reach the CNS, the site of the proposed therapeutic action for the H_3-receptor antagonists. Therefore, in this work we also evaluated which polar fragments are effectively required for the interaction with the H_3-receptor.

Derivatives with di- and tri-methylene spacers and 2-aminobenzothiazole, **3**, **4**; 2-thiobenzimidazole, **5**, **6**; 2-thiobenzothiazole, **7**, **8** moieties were thus synthesised, together with 2-thio-*N*-methylimidazoles substituted with a phenyl ring in different positions of the heterocyclic polar group **9**–**12**. We also prepared 2-aminobenzimidazole derivatives substituted with a methyl group on the NH of the alkyl chain **13**, **14** or on the benzimidazole annular NH **15**, **16**, to obtain new compounds where the 2-aminobenzimidazole moiety conserved its moderate basic properties, but where the hydrogen donor groups were alternately masked.

2. Pharmacology

H_3-receptor affinity of the newly synthesised compounds was measured by displacement of [^{3}H]-(R)-α-methylhistamine ([^{3}H]-RAMHA) bound to rat cerebral cortex synaptosomes. Histamine H_3-receptor antagonist potency was evaluated on electrically stimulated guinea-pig ileum, by inhibition of RAMHA-induced responses [37].

3. Chemistry

The compounds listed in Table 1 were prepared following various synthetic routes. The synthesis of compounds **1**, **2**, **3**, **5**, **7** has been described by us in previous papers [31,35], while the synthesis of compound **10** has been described in a patent [38], with a different synthetic route.

According to Scheme 1, 2-[3-[imidazol-4(5)-yl]propylamino]benzothiazole (**4**) was synthesised by condensation of 3-[imidazol-4(5)-yl]propylamine [39] with 2-chlorobenzothiazole.

Compounds **13** and **14** were prepared from the corresponding *N*-[ω-[imidazol-4(5)-yl]alkyl]methylamine, by condensation with 2-chlorobenzimidazole ($n = 2$) and with 2-benzimidazolesulphonic acid [40] ($n = 3$), while compounds **15** and **16** were synthesised from *N*-methyl-2-chlorobenzimidazole and the appropriate ω-[imidazol-4(5)-yl]alkylamine.

According to Scheme 2, compounds **6** and **8** were prepared by condensation of 4(5)-(3-chloropropyl)imidazole [41,42] with 2-mercaptobenzimidazole and 2-mercaptobenzothiazole, respectively.

Compounds **9**–**12** were obtained by condensation of 4(5)-(2-chloroethyl)imidazole [43] and 4(5)-(3-chloropropyl)imidazole with 1-methyl-4-phenyl-imidazoline-2-thione [44] and 1-methyl-5-phenyl-imidazoline-2-thione [44].

4. Experimental procedures

4.1. Chemistry

Melting points were not corrected, and were determined with a Büchi instrument (Tottoli) and with Gallenkamp melting point apparatus. The final compounds were analysed 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. The ^{1}H NMR spectra are reported in order: multiplicity, number and type of protons and J values (Hz). Abbreviations are the following: Im, imidazolyl; Bzim, benzimidazolyl; Bzth, benzothiazolyl. Mass spectra were recorded using a Finnigan MAT SSQ 710 instrument, and IR spectra were recorded using a JASCO FT/IR 300E 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 and MN Kiesegel 60, 25–40 μm, Macherey-Nagel); the eluents were mixtures of CH_2Cl_2/CH_3OH at various volume ratios. When indicated, gaseous NH_3 was added to the methanolic phase to obtain a 5% w/w solution.

Abbreviations for solvents are the following: Et_2O, diethyl ether; EtOH, ethanol; DMSO, dimethyl sulfoxide; DMF, *N*,*N*-dimethyl formamide; *i*BuOH, isobutanol.

4.1.1. 2-[3-[Imidazol-4(5)-yl]propylamino]benzothiazole (4)

A mixture of 7.5 mmol (0.94 g) of 3-[imidazol-4(5)-yl]propylamine and 8.3 mmol (1.41 g) of 2-chlorobenzothiazole was heated at 130 °C for 19 h. The residue was then treated with a saturated aq. sodium bicarbonate solution and extracted with ethyl acetate. The

Table 1
Yields and characteristic data of final products

Comp.	Yield (%)	Crystallisation Solvent	M.p. (°C) [a]	Analysis
4	25	abs EtOH/Et_2O	286–289	$C_{13}H_{14}N_4S\cdot 2HCl$
6	70	EtOH/H_2O	172–174 [b]	$C_{13}H_{14}N_4S$
8	26	abs EtOH/Et_2O	165–168	$C_{13}H_{13}N_3S_2\cdot HCl\cdot H_2O$
9	52	abs EtOH/Et_2O	147–148	$C_{15}H_{16}N_4S\cdot 2HCl\cdot 1/2H_2O$
10	66	abs EtOH/Et_2O	147–149	$C_{16}H_{18}N_4S\cdot C_2H_2O_4$
11	58	abs EtOH/Et_2O	198–199	$C_{15}H_{16}N_4S\cdot 2HCl\cdot H_2O$
12	64	abs EtOH/Et_2O	180–181	$C_{16}H_{18}N_4S\cdot 2HCl\cdot 3/2H_2O$
13	42	abs EtOH/Et_2O	303–307	$C_{13}H_{15}N_5\cdot 2HCl$
14	26	*i*BuOH/Et_2O	234–236	$C_{14}H_{17}N_5\cdot 2HCl\cdot H_2O$
15	46	abs EtOH	299–300	$C_{13}H_{15}N_5\cdot 2HCl$
16	27	abs EtOH/Et_2O	269–272	$C_{14}H_{17}N_5\cdot 2HCl\cdot H_2O$

[a] The melting points refer to the analysed salts, unless otherwise indicated.
[b] The melting point refers to the analysed free base.

organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by column chromatography (SiO_2, CH_2Cl_2/$CH_3OH(NH_3)$ = 15:1).

Yield and characteristic data of this compound are reported in Table 1.

^{1}H NMR (DMSO-d_6) **4** (dihydrochloride) δ 1.96 (m, 2H, CH_2), δ 2.75 (t, 2H, CH_2, $J = 7.5$), δ 3.46 (t, 2H, CH_2, $J = 7.3$), δ 7.23 (m, 1H, Bzth), δ 7.36 (s, 1H, Im-5-H), δ 7.40 (dd, 1H, Bzth, $J = 7.3$, 1.0), δ 7.47 (dd, 1H, Bzth, $J = 7.4$, 0.9), δ 7.76 (d, 1H, Bzth, $J = 7.5$), δ 8.80 (s, 1H, Im-2-H).

*4.1.2. 2-[3-[Imidazol-4(5)-yl]propylthio]benzimidazole (**6**)*

A mixture of equimolar ratios of 4(5)-(3-chloropropyl)imidazole, 2-mercaptobenzimidazole and 4% NaOH were stirred at 40 °C for 2 h to give a solid. This product was then purified by column chromatography (SiO_2, CH_2Cl_2/$CH_3OH(NH_3)$ = 9:1).

Yield and characteristic data of this compound are reported in Table 1.

^{1}H NMR (DMSO-d_6) **6** (free base): δ 2.00 (m, 2H, CH_2), δ 2.64 (t, 2H, CH_2, $J = 7.3$), δ 3.29 (t, 2H, CH_2, $J = 7.2$), δ 6.80 (s, 1H, Im-5-H), δ 7.09–7.11 (m, 2H, Bzim), δ 7.41–7.44 (m, 2H, Bzim), δ 7.54 (s, 1H, Im-2-H).

*4.1.3. 2-[3-[Imidazol-4(5)-yl]propylthio]benzothiazole (**8**)*

To a boiling solution of 73.3 mmol (12.27 g) of 2-mercaptobenzothiazole in 24.45 ml of 20% NaOH and 24.45 ml of EtOH, was added a solution of 48.9 mmol (7.07 g) of 4(5)-(3-chloropropyl)imidazole in 30.5 ml of EtOH. The reaction mixture was refluxed under stirring for 2 h 30 min; the solvent was then evaporated under reduced pressure. The solid residue was dissolved in water and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to give the crude product as an oil. The compound was then purified by column chromatography (SiO_2, CH_2Cl_2/$CH_3OH(NH_3)$ = 15:1).

Yield and characteristic data of this compound are reported in Table 1.

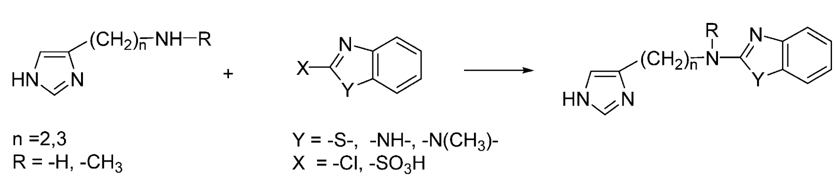
^{1}H NMR (DMSO-d_6) **8** (hydrochloride): δ 2.15 (m, 2H, CH_2), δ 2.83 (t, 2H, CH_2, $J = 7.3$), δ 3.39 (t, 2H, CH_2, $J = 7.3$), δ 7.34 (m, 1H, Bzth), δ 7.45 (m, 1H, Bzth), δ 7.49 (s, 1H, Im-5-H), δ 7.83 (d, 1H, Bzth, $J = 8.1$), δ 8.00 (dd, 1H, Bzth, $J = 8.0$, 1.1), δ 9.02 (s, 1H, Im-2-H).

*4.1.4. N-methyl-2-[2-[imidazol-4(5)-yl]ethylthio]-4-phenyl-imidazole (**9**)*

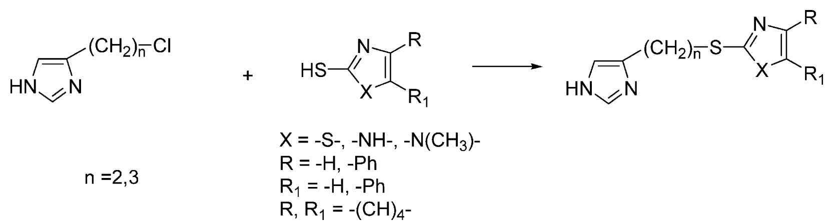
A mixture of equimolar ratios of 4(5)-(2-chloroethyl)imidazole, 1-methyl-4-phenyl-imidazoline-2-thione and EtONa in the minimum amount of DMSO was stirred, at room temperature (r.t.), for 24 h to give a product that was purified by column chromatography (SiO_2, CH_2Cl_2/$CH_3OH(NH_3)$ = 12:1).

Yield and characteristic data of this compound are reported in Table 1.

^{1}H NMR (DMSO-d_6) **9** (dihydrochloride) δ 3.06 (t, 2H, CH_2, $J = 7.4$), δ 3.70 (t, 2H, CH_2, $J = 7.4$), δ 3.80 (s, 3H, CH_3), δ 7.40–7.51 (m, 3H, Ph), δ 7.57 (s, 1H,



Scheme 1. Synthesis of compounds **4**, **13**–**16**.



Scheme 2. Synthesis of compounds **6**, **8**–**12**.

Im-5-H), δ 7.96 (dd, 2H, Ph, $J = 7.2, 1.4$), δ 8.28 (s, 1H, Im-5-H), δ 9.02 (s, 1H, Im-2-H).

*4.1.5. N-methyl-2-[3-[imidazol-4(5)-yl]propylthio]-4-phenyl-imidazole (**10**)*

A mixture of equimolar ratios of K_2CO_3 and of 1-methyl-4-phenyl-imidazoline-2-thione in the minimum amount of DMF was stirred at 80 °C for 15 min; a solution of 4(5)-(3-chloropropyl)imidazole in the minimum amount of DMF was then added and the reaction mixture was kept at 80 °C, under magnetic stirring, for 1 h 45 min. The residue was dissolved in H_2O and the product was extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by column chromatography (SiO_2, CH_2Cl_2/$CH_3OH(NH_3)$ = 9:1).

Yield and characteristic data of this compound are reported in Table 1.

^{1}H NMR (DMSO-d_6) **10** (oxalate) δ 1.97 (m, 2H, CH_2), δ 2.71 (t, 2H, CH_2, $J = 7.2$), δ 3.09 (t, 2H, CH_2, $J = 7.1$), δ 3.61 (s, 3H, CH_3), δ 7.12 (s, 1H, Im-5-H), δ 7.18 (t, 1H, Ph, $J = 7.7$), δ 7.34 (t, 2H, Ph, $J = 7.7$), δ 7.68 (d, 2H, Ph, $J = 8.0$), δ 7.70 (s, 1H, Im-5-H), δ 8.28 (s, 1H, Im-2-H).

*4.1.6. N-methyl-2-[ω-[imidazol-4(5)-yl]alkylthio]-5-phenyl-imidazole Δ(**11**, **12**)*

A mixture of equimolar ratios of the appropriate 4(5)-(ω-chloroalkyl)imidazole, 1-methyl-5-phenyl-imidazoline-2-thione and EtONa in the minimum amount of DMSO was stirred, at r.t., for 72 h. The products were then purified by column chromatography (SiO_2, **11**, **12** CH_2Cl_2/$CH_3OH(NH_3)$ = 12:1).

Yields and characteristic data of these compounds are reported in Table 1.

^{1}H NMR (DMSO-d_6) **11** (dihydrochloride) δ 3.08 (t, 2H, CH_2, $J = 6.9$), δ 3.67 (t, 2H, CH_2, $J = 6.9$), δ 3.72 (s, 3H, CH_3), δ 7.51–7.63 (m, 6H, Ph and Im-4-H), δ 7.88 (s, 1H, Im-5-H), δ 9.03 (s, 1H, Im-2-H).

^{1}H NMR ($CDCl_3$) **12** (dihydrochloride) δ 2.20 (m, 2H, CH_2), δ 3.02 (t, 2H, CH_2, $J = 7.1$), δ 3.47 (t, 2H, CH_2, $J = 7.0$), δ 3.76 (s, 3H, CH_3), δ 7.21 (s, 1H, Im-4-H), δ 7.26 (s, 1H, Im-5-H), δ 7.45–7.53 (m, 5H, Ph), δ 8.47 (s, 1H, Im-2-H).

*4.1.7. 2-[N-methyl-N-[2-[imidazol-4(5)-yl]ethyl]-amino]benzimidazole (**13**)*

A solution of 9.0 mmol (1.13 g) of *N*-[2-[imidazol-4(5)-yl]ethyl]methylamine and 4.5 mmol (0.69 g) of 2-chlorobenzimidazole in 9 ml of isoamyl alcohol was heated at 130 °C for 16 h. The solvent was evaporated under reduced pressure. The residue was then treated with a saturated aq. sodium bicarbonate solution and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by column chromatography (SiO_2, CH_2Cl_2/$CH_3OH(NH_3)$ = 30:1).

Yield and characteristic data of this compound are reported in Table 1.

^{1}H NMR (DMSO-d_6) **13** (dihydrochloride) δ 3.11 (t, 2H, CH_2, $J = 7.4$), δ 3.24 (s, 3H, CH_3), δ 3.95 (t, 2H, CH_2, $J = 7.4$), δ 7.22–7.28 (m, 2H, Bzim), δ 7.40–7.46 (m, 2H, Bzim), δ 7.60 (s, 1H, Im-5-H), δ 9.05 (s, 1H, Im-2-H).

*4.1.8. 2-[N-methyl-N-[3-[imidazol-4(5)-yl]propyl]amino]benzimidazole (**14**)*

A mixture of 9.0 mmol (1.25 g) of *N*-[3-[imidazol-4(5)-yl]propyl]methylamine and 6.0 mmol (1.19 g) of 2-benzimidazolesulphonic acid was heated at 160 °C for 3 h. The residue was then treated with a saturated aq. sodium bicarbonate solution and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by column chromatography (SiO_2, CH_2Cl_2/$CH_3OH(NH_3)$ = 40:1).

Yield and characteristic data of this compound are reported in Table 1.

^{1}H NMR (DMSO-d_6) **14** (dihydrochloride) δ 2.05 (m, 2H, CH_2), δ 2.79 (t, 2H, CH_2, $J = 7.2$), δ 3.27 (s, 3H, CH_3), δ 3.72 (t, 2H, CH_2, $J = 7.2$), δ 7.21–7.24 (m, 2H, Bzim), δ 7.40–7.44 (m, 3H, Bzim and Im-5-H), δ 9.01 (s, 1H, Im-2-H).

*4.1.9. N-methyl-2-[ω-[imidazol-4(5)-yl]alkylamino]-benzimidazoles (**15**–**16**)*

A solution of 4.2 mmol of *N*-methyl-2-chlorobenzimidazole and 8.4 mmol of the appropriate ω-[imidazol-4(5)-yl]alkylamine in 4 ml of isoamyl alcohol was heated at 135 °C for 18 h. The solvent was evaporated under reduced pressure. The residue was then treated with a saturated aq. 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, **15** $CH_2Cl_2/CH_3OH(NH_3) = 30:1$; **16** $CH_2Cl_2/CH_3OH(NH_3) = 20:1$).

Yields and characteristic data of these compounds are reported in Table 1.

^{1}H NMR (DMSO-d_6) **15** (dihydrochloride) δ 3.07 (t, 2H, CH_2, $J = 6.5$), δ 3.65 (s, 3H, CH_3), δ 3.80 (t, 2H, CH_2, $J = 6.5$), δ 7.28–7.31 (m, 2H, Bzim), δ 7.45–7.53 (m, 2H, Bzim), δ 7.62 (s, 1H, Im-5-H), δ 9.04 (s, 1H, Im-2-H).

^{1}H NMR (DMSO-d_6) **16** (dihydrochloride) δ 2.04 (m, 2H, CH_2), δ 2.81 (t, 2H, CH_2, $J = 7.5$), δ 3.51 (t, 2H, CH_2, $J = 7.2$), δ 3.68 (s, 3H, CH_3), δ 7.26–7.30 (m, 2H, Bzim), δ 7.41–7.52 (m, 3H, Bzim and Im-5-H), δ 9.00 (s, 1H, Im-2-H).

4.2. Pharmacology

4.2.1. Binding assays

Rat (Wistar) brain membranes were incubated for 30 min with [^{3}H]-RAMHA 0.05 nM and the compounds studied (1 nM–10 μM), in Tris–HCl 50 mM, pH 7.4, NaCl 50 mM, EDTA 0.5 mM and rapidly filtered under vacuum. Specific binding was defined as the binding inhibited by thioperamide 10 μM, and the pK_i values were calculated from the inhibition curves of the compounds tested versus 0.5 nM [^{3}H]-RAMHA according to Cheng and Prusoff's equation [45].

4.2.2. 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. The preparation was then equilibrated for 60 min under 1 g of resting tension, with replacement of fresh solution every 15 min. Single electrical pulses were delivered to the tissue at 0.1 Hz frequency and 1 ms duration from a stimulator (LACE Elettronica model ES-3, Ospedaletto PI, Italy) with submaximal voltage (1.5–3.0 V). Cumulative concentration-response curves for the inhibition of electrically stimulated contractions were determined for the H_3 selective agonist RAMHA (1 nM–1 μM). The tissues were allowed to equilibrate with the compounds under study (1 nM–10 μM) for 30 min before the generation of concentration-response curves to the agonist. pK_B values were determined according to Furchgott's equation [46]:

$$pK_B = \log([E']/[E] - 1) - \log[B]$$

where [E′] and [E] are the concentrations of the agonist producing the half-maximum effect in the presence and absence, respectively of the antagonist; [B] is the concentration of the antagonist.

5. Results and discussion

H_3-receptor affinity (pK_i) and antagonist potency (pK_B) for the compounds examined in this work are reported in Table 2.

Biological data of some compounds have been already published in previous papers, as indicated in the footnotes of Table 2. Nevertheless, for some of these H_3-antagonists, the pK_i values are slightly different from those previously reported, due to the fact that these products have been re-evaluated (e.g. **1**, **3**, **5**, **7**), using a different labelled ligand ([^{3}H]-(R)-α-methylhistamine instead of [^{3}H]-N^{α}-methylhistamine). The syntheses of the compounds reported in Table 1 are new, even if the affinity and potency values of compounds **6** and **8** have been previously reported.

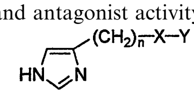
Most of the compounds tested behaved as competitive H_3-antagonists on guinea-pig ileum, and showed medium to high affinity for rat cerebral H_3-receptor. Some differences were observed between binding affinities in rat cerebral cortex membranes (pK_i) and potencies in guinea-pig ileum (pK_B); they are probably due to the different biological procedures, to the different tissues and, mostly, to the interspecies histamine H_3-receptor heterogeneity, which has been observed for a different class of compounds [47].

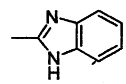
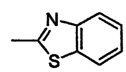
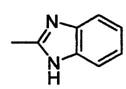
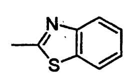
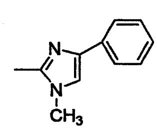
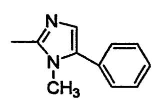
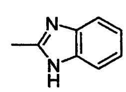
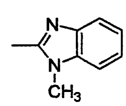
Considering the biological data reported in Table 2, it is possible to observe an influence of the polar group basicity on the optimal length of the chain spacer, as referred to in a previous paper [32] and as observed for the clobenpropit-like isothiourea derivatives [17].

Based on the reported pK_a values for reference structures, it can be inferred that the 2-aminobenzothiazole (p$K_a = 4.23$ [48]), 2-thiobenzimidazole (p$K_a = 2.60$ [49]), 2-thiobenzothiazole (p$K_a = 3.08$ [49]) and 2-thio-4(5)-phenylimidazole (p$K_a = 4.28$ [30]) fragments should be mainly neutral at physiological pH, while for the 2-aminobenzimidazoles (pK_a values ranging from 6.35 to 6.54 were observed for series of 2-alkylaminobenzimidazole derivatives [40,50]), a significant fraction of protonation can be expected. As for H_3-receptor affinity, shorter chains are preferred in the presence of neutral polar groups, as in compounds **3**–**8**, whereas an increase in the polar group basicity, as in the 2-aminobenzimidazoles **1** and **2** leads to a better affinity value for the tri-methylene chain compound. This behaviour was maintained by the *N*-methylimidazoles **9**–**12**, irrespective of the relative position of the methyl and the phenyl groups.

Thus, the results obtained confirmed a different behaviour of the two classes of imidazole H_3-antagonists

Table 2
H_3-receptor affinity (pK_i on rat cerebral cortex membranes) and antagonist activity (pK_B on guinea-pig ileum) of tested compounds



Comp.	n	pK_i [a]	pK_B [a]	X	Y	Comp.	n	pK_i [a]	pK_B [a]
1	2	7.25±0.05	7.33±0.20 [b]	NH		**2**	3	8.90±0.05 [c]	9.46±0.57 [c]
3	2	8.13±0.19	7.24±0.12 [b]	NH		**4**	3	7.89±0.14	7.58±0.06
5	2	7.72±0.05	7.57±0.28 [b]	S		**6**	3	7.47±0.06	7.51±0.29 [b]
7	2	8.53±0.06 [c]	7.43±0.21 [c]	S		**8**	3	7.08±0.08 [c]	7.51±0.16 [c]
9	2	8.03±0.09	8.43±0.17	S		**10**	3	7.72±0.15	7.48±0.12
11	2	7.16±0.07	6.53±0.20 [d]	S		**12**	3	6.87±0.08	N.C. [e]
13	2	6.26±0.17	N.C. [f]	N-CH3		**14**	3	8.60±0.09	9.18±0.12
15	2	7.71±0.16	7.57±0.12	NH		**16**	3	6.83±0.11	7.30±0.12

[a] The data are reported as mean ± SEM of four observations.
[b] Ref. [33].
[c] Ref. [34].
[d] Non-competitive antagonism. pD_2' value calculated according to Van Rossum's equation [51].
[e] Non-competitive antagonism was observed at 300 nM.
[f] Non-competitive antagonism was observed at 10 nM.

(basic and neutral at physiological pH) in the H_3-receptor binding.

Moreover, we investigated the importance of the two NH groups of the polar fragment (annular and exocyclic) of the 2-aminobenzimidazole derivatives **1** and **2**, considering the great difference in affinity observed for these two compounds. For this purpose we prepared and tested the four *N*-methyl derivatives **13**–**16**.

The *N*-methyl-benzimidazole derivative with ethylene chain 15 showed a pK_i value slightly higher than the unsubstituted 2-aminobenzimidazole with di-methylene alkyl chain **1**, whereas the corresponding compound having the methyl group on the exocyclic NH (13) showed a slight drop in affinity.

Considering the corresponding *N*-methyl derivatives with longer chain spacers **14** and **16**, an opposite trend was observed. In fact, the affinity of compound **16** (*N*-methyl-benzimidazole with tri-methylene chain) dropped by more than two orders of magnitude, compared to compound **2**, and potency data on guinea-pig ileum (pK_B) confirmed the affinity trend measured on rat brain membranes. It was observed, however, that the exocyclic N-methylation 14 does not modify the biological activity of the lead.

These results seem to indicate that the contribution of the HB groups of the benzimidazole nucleus depends, in the moderately basic 2-aminobenzimidazole derivatives, on the distance between the polar group and the imidazole ring.

Analogously, comparing the 2-thio-*N*-methylimidazole derivatives substituted with a phenyl ring and having a di-methylene chain **9**, **11** with the corresponding 2-[2-[imidazol-4(5)-yl]ethylthio]-4(5)-phenyl-imidazole ($pK_i = 7.83$ versus [^{3}H]-NAMHA), previously reported by us [31], it is possible to observe a similar behaviour. In fact, especially when the imidazole polar group is substituted with a phenyl ring in the 4-position 9, the compound results as being insensitive to the NH annular masking, maintaining the pK_i value observed for the unmethylated derivative.

Since the presence in the molecule of polar acceptor or donor hydrogen-bonding groups is a limit to the crossing of the blood brain barrier and thus to access to the brain, the determination of those effectively important for the interaction with the H_3-receptor is important for the modulation of the 2-aminobenzimidazole moiety; the aim is to obtain compounds able to cross lipophilic barriers, maintaining the characteristics necessary for the interaction with the H_3-receptor. With respect to this aspect, compound **14** represents an improvement over compound **2**, because of the masking of an unnecessary HB-donor group.

Acknowledgements

Financial support from the Italian MIUR is gratefully acknowledged. We are grateful to the Centro Interfacoltà Misure of the University of Parma for instrumentation placed at our disposal.

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