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Bioorganic & Medicinal Chemistry 14 (2006) 1413–1424
Bioorganic & Medicinal Chemistry

Synthesis and biological evaluation of new non-imidazole H_3-receptor antagonists of the 2-aminobenzimidazole series

Mirko Rivara,[a] Valentina Zuliani,[a] Giuseppe Cocconcelli,[a] Giovanni Morini,[a] Mara Comini,[a] Silvia Rivara,[a] Marco Mor,[a,*] Fabrizio Bordi,[a] Elisabetta Barocelli,[b] Vigilio Ballabeni,[b] Simona Bertoni[b] and Pier Vincenzo Plazzi[a]

[a]*Dipartimento Farmaceutico, Università degli Studi di Parma, Parco Area delle Scienze 27/A, I-43100 Parma, Italy*
[b]*Dipartimento di Scienze Farmacologiche, Biologiche e Chimiche Applicate, Università degli Studi di Parma, Parco Area delle Scienze 27/A, I-43100 Parma, Italy*

Received 26 July 2005; revised 21 September 2005; accepted 27 September 2005
Available online 2 November 2005

Abstract—A novel series of non-imidazole H_3-receptor antagonists was developed, by chemical modification of a potent lead H_3-antagonist composed by an imidazole ring connected through an alkyl spacer to a 2-aminobenzimidazole moiety (e.g., 2-[[3-[4(5)-imidazolyl]propyl]amino]benzimidazole), previously reported by our research group. We investigated whether the removal of the imidazole ring could allow retaining high affinity for the H_3-receptor, thanks to the interactions undertaken by the 2-aminobenzimidazole moiety at the binding site. The imidazole ring of the lead was replaced by a basic piperidine or by a lipophilic *p*-chlorophenoxy substituent, modulating the spacer length from three to eight methylene groups; moreover, the substituents were moved to the 5(6) position of the benzimidazole nucleus. Within both the 2-alkylaminobenzimidazole series and the 5(6)-alkoxy-2-aminobenzimidazole one, the greatest H_3-receptor affinity was obtained for the piperidine-substituted compounds, while the presence of the *p*-chlorophenoxy group resulted in a drop in affinity. The optimal chain length was different in the two series. Even if the new compounds did not reach the high receptor affinity shown by the imidazole-containing lead compound, it was possible to get good H_3-antagonist potencies with 2-aminobenzimidazoles having a tertiary amino group at appropriate distance.

1. Introduction

Histamine is a biogenic amine implicated in a wide range of physiological processes through the activation of four G-protein coupled receptors (H_1, H_2, H_3, and H_4).[1] The postsynaptic H_1- and H_2-receptors are mainly involved in the control of allergic responses and gastric acid secretion, respectively, while the more recently described H_4-receptor seems to be implicated in inflammatory processes.[2,3] The third receptor subtype (H_3) is mainly located presynaptically on histaminergic neurons in the central nervous system (CNS), where it downregulates histamine synthesis and release acting as an autoreceptor.[4,5] Thus, H_3-receptor antagonists inhibit this negative feedback mechanism, increasing the concentration of the amine in the CNS.[6] Moreover, this receptor acts as a heteroreceptor on non-histaminergic neurons in the brain and in periphery, inhibiting the release of other neurotransmitters (e.g., acetylcholine, noradrenaline, dopamine, and serotonin)[7–10] and of neuropeptides.[11] Several different histamine H_3-receptor isoforms have been characterized in rats, guinea-pigs, and humans, and they have been shown to exhibit distinct pharmacological profiles.[12,13] The high density of H_3-receptors found in the CNS[14] has suggested potential therapeutic applications of histamine H_3-antagonists for several CNS diseases, such as attention-deficit hyperactivity disorders (ADHD),[15] Alzheimer's disease,[16] obesity and control of food intake,[17,18] epilepsy,[19–21] and schizophrenia.[22]

Many H_3-antagonists belong to the class of imidazole-containing compounds.[23,24] It is now well established that the presence of the imidazole ring might cause pharmacological liabilities. In fact, the coordination of this heterocycle with the heme moiety of cytochrome P450

Keywords: Histamine; H_3-receptor antagonists; 2-Aminobenzimidazole; Non-imidazole.
* Corresponding author. Tel.: +39 0521 905062; fax: +39 0521 905006; e-mail: marco.mor@unipr.it

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doi:10.1016/j.bmc.2005.09.063

isoenzymes can lead to drug–drug interaction and metabolic interferences.[25]

With the aim of obtaining new H_3-antagonists with improved pharmacokinetic properties and enhanced access to the CNS, several different classes of H_3-antagonists, lacking the imidazole ring, have been synthesized. Some examples of the most recently reported non-imidazole H_3-receptor antagonists are depicted in Figure 1.[26–37] These non-imidazole H_3-antagonists are usually characterized by the presence of an aminoalkyl moiety, although with different chain lengths and an overall structure. The absence of the imidazole ring does not prevent these compounds from displaying a high potency at the H_3-receptor, since many compounds are active at nanomolar concentrations. Moreover, some of these compounds have demonstrated high activity not only in vitro, but also in vivo.[38–41]

Several attempts to develop new non-imidazole compounds were based on the bioisosteric replacement of the imidazole moiety of well-known and very potent H_3-receptor antagonists.[42–44] This replacement has often led to a drop in H_3-antagonist potency but, in some cases, in vitro affinity values were maintained (e.g., FUB 649 and UCL 2190, obtained from FUB 181 and ciproxifan, respectively, Fig. 2).[44] Following this

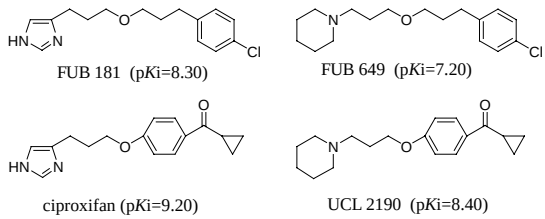


Figure 2. Non-imidazole H_3-receptor antagonists obtained by bioisosteric replacement of the imidazole moiety and their parent compounds.

approach, we synthesized new non-imidazole H_3-receptor antagonists starting from a series of imidazole-containing 2-aminobenzimidazole derivatives, previously described by us,[45] which afforded a lead structure (Fig. 3), endowed with high affinity for the H_3-receptor ($pK_i = 8.90$, rat brain membranes and $pK_i = 8.31$, human H_3-receptor). In this series, the 2-aminobenzimidazole fragment and its 5(6)-methoxy derivative behaved as weak bases ($pK_a = 6.2–6.7$), thus being able

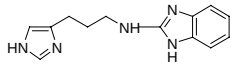


Figure 3. Lead structure.

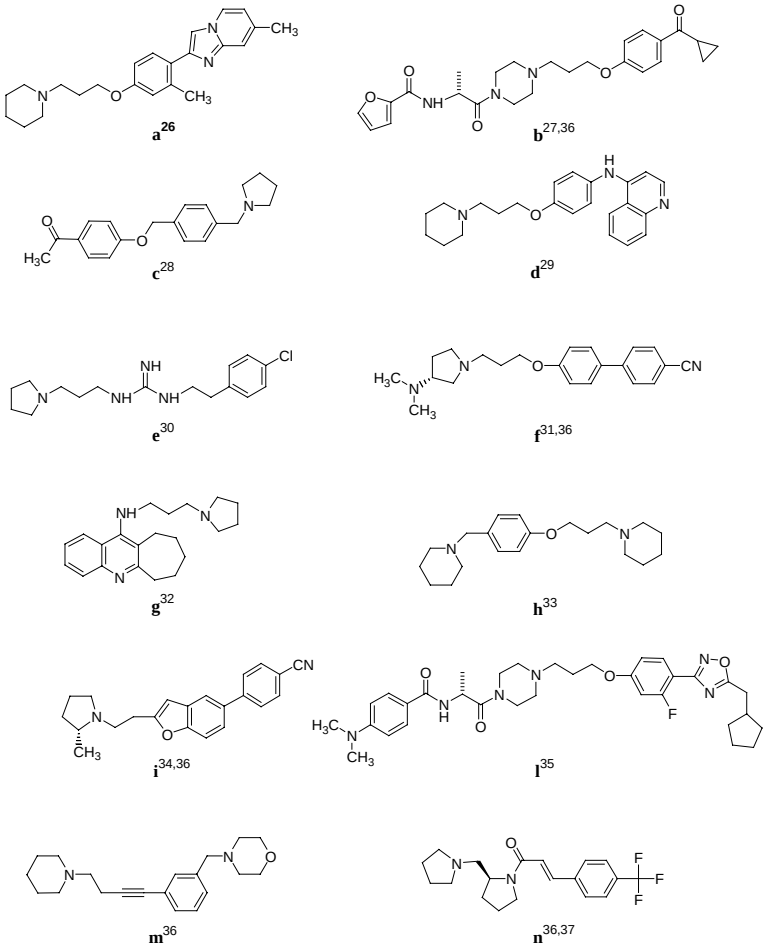


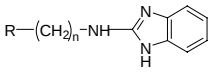
Figure 1. Non-imidazole H_3-receptor antagonists.

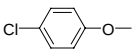
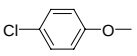
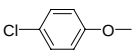
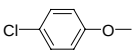
to interact with the H_3-receptor binding site in their protonated or, more probably, neutral form.[46] We retained the 2-aminobenzimidazole fragment which, according to our previous results,[47] is the heterocyclic polar fragment conferring the highest potency at the H_3-receptor, and we replaced the imidazole ring, to investigate if it were possible to maintain high affinity for the H_3-receptor. Following the hypothesis that the 2-aminobenzimidazole moiety would constitute a critical anchor group at the H_3-receptor binding site, additional interactions were supposed for a side chain with a basic or a lipophilic ending group, introduced at different positions of the 2-aminobenzimidazole nucleus. Potent non-imidazole H_3-receptor antagonists, characterized by the presence of a piperidine or a phenoxyalkylamine moiety, have been reported.[36,48] Thus, starting from the lead structure reported in Figure 3, the imidazole ring was replaced by a basic piperidine or by a lipophilic *p*-chlorophenoxy ring, and the chain length was also modulated, preparing compounds with three to eight methylene linkers (Table 1, **1**–**9**). In fact, although the replacement of the imidazol-4(5)-yl ring by suitable moieties has been successfully applied to different classes of imidazole-containing H_3-antagonists, different structure–activity relationships have been observed in the two series. The side chain was also moved to the 5(6) position of the 2-aminobenzimidazole moiety (Table 2, **10**–**15**), to test a different relative disposition of the 2-aminobenzimidazole nucleus and the basic piperidine or the lipophilic *p*-chlorophenoxy group.

2. Chemistry

The 2-aminobenzimidazole derivatives described in this paper were prepared following various synthetic routes, as outlined in Schemes 1–3.

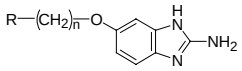
Table 1. Histamine H_3-receptor affinity (pK_i) and antagonist potency (pK_B) of compounds **1**–**9**

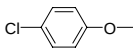
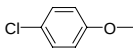
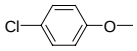


Compound	*n*	R	Human H_3 pK_i[a]	Rat H_3 pK_i[b]	Guinea-pig H_3 pK_B[c]
1	3		5.56 ± 0.16	5.65 ± 0.08	nd[d]
2	5		7.16 ± 0.01	6.62 ± 0.01	7.07 ± 0.16
3	6		7.48 ± 0.02	7.62 ± 0.05	7.62 ± 0.07
4	7		7.43 ± 0.11	6.90 ± 0.05	7.50 ± 0.07
5	8		6.82 ± 0.06	7.27 ± 0.15	7.52 ± 0.10
6	4		6.03 ± 0.16	[e]	[f]
7	5		5.98 ± 0.09	[e]	[f]
8	6		6.02 ± 0.02	[e]	[f]
9	7		6.25 ± 0.03	5.66 ± 0.19	6.64 ± 0.01
Thioperamide			7.28 ± 0.15	8.59 ± 0.05[g]	9.04 ± 0.14[h]

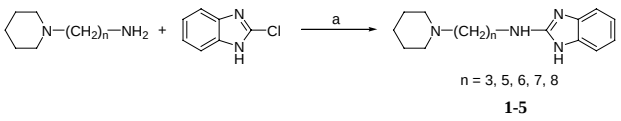
[a] Inhibition of [^{3}H]RAMHA binding to GPCR97-transfected SK-N-MC cells stably expressing the human histamine H_3-receptor.
[b] Inhibition of [^{3}H]RAMHA binding to rat brain membranes.
[c] Inhibition of RAMHA-induced effects on guinea-pig isolated ileum. pK_B values obtained according to Furchgott's method.[57]
[d] Not determined.
[e] Inactive until 30 μM.
[f] Inactive until 10 μM.
[g] Ref. 58.
[h] Ref. 59.

Table 2. Histamine H_3-receptor affinity (pK_i) and antagonist potency (pK_B) of compounds **10**–**15**



Compound	*n*	R	Human H_3 pK_i[a]	Rat H_3 pK_i[b]	Guinea-pig pK_B[c]
10	4		7.53 ± 0.08	7.31 ± 0.25	7.38 ± 0.13
11	5		7.00 ± 0.12	6.92 ± 0.04	8.03 ± 0.13
12	6		7.14 ± 0.03	6.54 ± 0.14	7.48 ± 0.07
13	3		5.46 ± 0.07	5.22 ± 0.13	[e]
14	4		5.28 ± 0.09	[d]	[e]
15	5		5.70 ± 0.14	5.37 ± 0.09	[e]
Thioperamide			7.28 ± 0.15	8.59 ± 0.05[f]	9.04 ± 0.14[g]

[a] Inhibition of [^{3}H]RAMHA binding to GPCR97-transfected SK-N-MC cells stably expressing the human histamine H_3-receptor.
[b] Inhibition of [^{3}H]RAMHA binding to rat brain membranes.
[c] Inhibition of RAMHA-induced effects on guinea-pig isolated ileum. pK_B values obtained according to Furchgott's method.[57]
[d] Inactive until 30 μM.
[e] Inactive until 10 μM.
[f] Ref. 58.
[g] Ref. 59.



Scheme 1. Synthesis of compounds **1**–**5**. Conditions: (a) isoamyl alcohol, reflux.

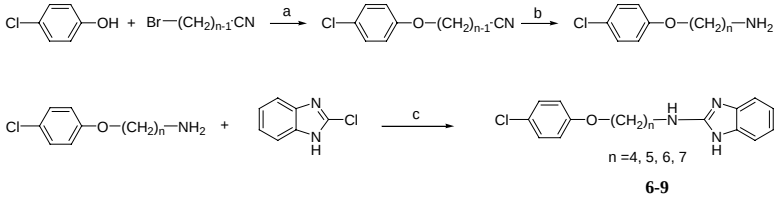
The 2-[ω-(piperidin-1-yl)alkylamino]-1*H*-benzimidazole derivatives (**1**–**5**) were synthesized by condensation of the corresponding ω-piperidin-1-yl-alkylamine[29,44,49,50] with 2-chlorobenzimidazole, according to Scheme 1.

The 2-[ω-(*p*-chlorophenoxy)alkylamino]-1*H*-benzimidazole derivatives (**6**–**9**) were obtained starting from 4-chlorophenol and the appropriate commercially available ω-bromoalkyl cyanide. The ω-(4-chlorophenoxy) alkyl cyanide derivatives were then reduced and condensed with 2-chlorobenzimidazole, to give the desired products (Scheme 2).

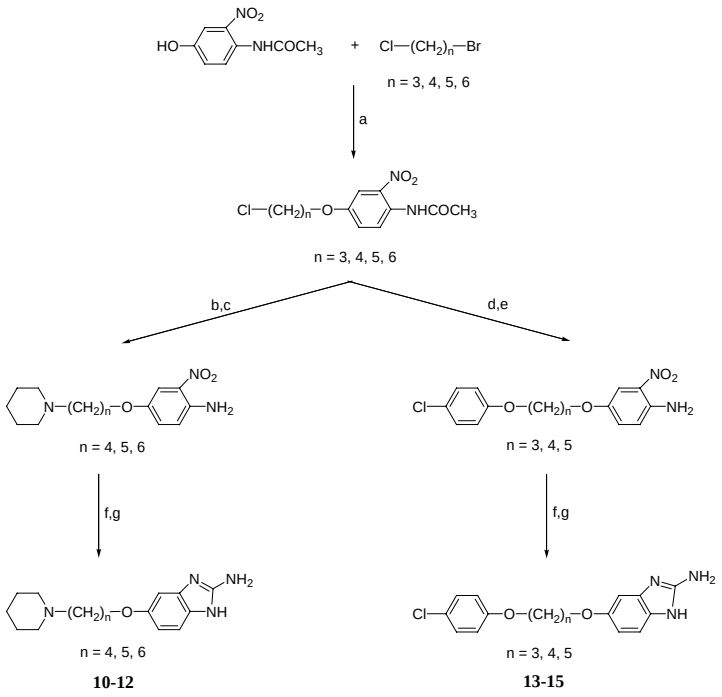
The synthetic procedure followed for the 2-aminobenzimidazole derivatives substituted in 5(6) position (**10**–**15**) is represented in Scheme 3. The final products were obtained starting from the common intermediates *N*-[4-(ω-chloroalkoxy)-2-nitrophenyl]acetamides, which were treated with piperidine (**10**–**12**) or 4-chlorophenol (**13**–**15**). The 4-substituted-2-nitrophenylacetamide derivatives so obtained were hydrolyzed, reduced, and treated with BrCN to give the final products.

3. Pharmacology

H_3-receptor affinity of the newly synthesized compounds was measured by displacement of [^{3}H]-(*R*)-α-methylhistamine ([^{3}H]RAMHA) from GPCR97-transfected



Scheme 2. Synthesis of compounds **6**–**9**. Reagents and conditions: (a) K_2CO_3, acetone, reflux; (b) $LiAlH_4$, THF, rt; (c) isoamyl alcohol, reflux.



Scheme 3. Synthesis of compounds **10**–**15**. Reagents and conditions: (a) Na_2CO_3, KI, DMF, 50 °C; (b) piperidine, 50 °C; (c) NaOH, reflux; (d) 4-chlorophenol, K_2CO_3, KI, DMF, 50 °C; (e) KOH, ethanol, rt; (f) H_2, 10% Pd/C, ethanol, rt; (g) BrCN, rt.

SK-N-MC cells stably expressing the human histamine H_3-receptor[51] and from rat cerebral cortex membranes.[52] Histamine H_3-receptor antagonist potency was evaluated on electrically stimulated guinea-pig ileum, by inhibition of RAMHA-induced responses.[53]

4. Results and discussion

Affinity data (pK_i) for human H_3-receptor and for rat brain histamine H_3-receptor and H_3-antagonist potency (pK_B) on guinea-pig ileum for the newly synthesized 2-alkylaminobenzimidazole derivatives having a piperidine or a *p*-chlorophenoxy substituent (**1**–**9**) are reported in Table 1. Table 2 reports the biological data for the 5(6)-alkoxy-2-aminobenzimidazoles, substituted with the piperidine or the *p*-chlorophenoxy group in the 5(6) position (**10**–**15**). The most evident result is the different behavior observed for compounds having a basic or a lipophilic group replacing the imidazole ring of the lead 2-[[3-[4(5)-imidazolyl]propyl]amino]benzimidazole (Fig. 3), for both aminobenzimidazoles substituted on the amino-group in the 2 position and the 5(6)-alkoxy derivatives. The piperidine derivatives acted as competitive H_3-antagonists on guinea-pig ileum and showed medium to quite good affinity values for human histamine H_3-receptors and for rat cerebral H_3-receptors. On the contrary, the *p*-chlorophenoxy derivatives were inactive or endowed with very low pK_i values; moreover, no competitive antagonism was detected for these compounds, with the only exception of the weakly potent compound **9**. In particular, within the 2-alkylaminobenzimidazole series (**1**–**9**), the highest affinity values were observed for the hexamethylene (**3**) and the heptamethylene (**4**) derivatives. As mentioned above, the replacement of the basic piperidine group with the lipophilic *p*-chlorophenoxy fragment resulted in a fifteen to thirty times drop in affinity at the human H_3 receptor; this suggests a direct interaction of the basic moiety with the H_3-receptor binding site. The highest pK_i value was observed for compound **3**, both at human H_3 receptor and at rat H_3 receptor, and it was also the most potent in the H_3 functional assay, although with a pK_B value comparable to those of compounds **4** and **5**. Within the series of 5(6)-alkoxy-2-aminobenzimidazoles, the highest potency was observed for the piperidine derivative having a four methylene spacer (**10**) and, again, an evident decrease in affinity was observed for the lipophilic *p*-chlorophenoxy derivatives. The piperidine derivatives **10**–**12** showed an apparent modest selectivity for the human H_3-receptor.

While significantly different affinity values were observed for thioperamide between human and rat receptors, our compounds showed only minor differences ($\leqslant 0.6$). Higher discrepancies were observed with the functional potency data on guinea-pig ileum (e.g., for compound **11**), which can be due to species-specificity or to the different nature of the pharmacological tests.

It can be concluded that the introduction of the basic piperidine substituent led to a quite good affinity for the H_3-receptor, given an appropriate distance from the 2-aminobenzimidazole nucleus. In fact, the optimal length of the alkyl spacer depended on the position of substitution: six methylene groups for the 2 position (**3**) and a tetramethylene chain for the 5(6) position (**10**). On the other hand, the most potent compounds of the two series **3** and **10**, although structurally different, can be easily superposed (Fig. 4). They can place

Figure 4. Superposition of compounds **3** (white carbons) and **10** (yellow carbons) obtained from energy minimized structures by a rigid fit superposition of nitrogen atoms.

their polar groups in a similar disposition, suggesting a common interaction with the H_3-receptor binding site; this hypothesis is supported by the similar potencies of the two compounds.

The compounds described here were also evaluated for H_3-receptor selectivity with respect to the H_1- and H_2-receptors on guinea-pig ileum and on guinea-pig atria, respectively.[45] They did not exhibit any remarkable inhibition of H_1 and H_2 mediated responses up to 1 μM concentration.

5. Conclusions

A novel series of non-imidazole H_3-antagonists was developed, obtained from chemical modification of a very potent imidazole H_3-receptor antagonist, characterized by a 2-aminobenzimidazole heterocyclic polar fragment (Fig. 3). When the imidazole ring was replaced by a lipophilic *p*-chlorophenoxy moiety (**6**–**9** and **13**–**15**), a remarkable decrease in affinity was observed, probably due to the inability of this lipophilic fragment to undertake a good interaction at the H_3 binding site. On the contrary, it was possible to obtain good H_3-antagonist binding affinities for 2-aminobenzimidazoles carrying a basic piperidine substituent at appropriate distance, although this novel structural class of non-imidazole derivatives did not reach the high receptor affinity shown by the lead imidazole derivative. No substantial differences between human and rat histamine H_3-receptor binding data were observed for these series of compounds. The results obtained suggest the series of piperidine-substituted 2-aminobenzimidazole H_3-receptor antagonists as an interesting subject for further investigations.

6. 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 on a ThermoQuest (Italia) FlashEA 1112 Elemental Analyzer, 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; signals were characterized as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), and m (multiplet) br s (broad signal). Abbreviations are the following: Bzim, benzimidazolyl; Ph, phenyl. 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, gaseous NH_3 was added to the methanolic phase to obtain a 5% w/w solution. Yields and characteristic data of the final compounds are described in Table 3.

Table 3. Yields and characteristic data of the final compounds

Compound	Yield (%)	Crystallization solvent	Mp (°C)
1	81	abs EtOH/Et_2O	177–178[a]
2	75	CCl_4/$CHCl_3$	130–132[b]
3	73	Et_2O/petroleum ether	153–155[b]
4	81	CCl_4/$CHCl_3$	151–152[b]
5	79	Et_2O/petroleum ether	145–147[b]
6	78	AcOEt/CCl_4	173–174[b]
7	80	AcOEt	127–129[b]
8	85	AcOEt	184–185[b]
9	78	AcOEt	144–145[b]
10	30	abs EtOH/Et_2O	192–193[c]
11	28	abs EtOH/Et_2O	220–222[d]
12	28	abs EtOH	215–217[e]
13	54	EtOH/H_2O	167–169[b]
14	52	EtOH/H_2O	205–207[b]
15	52	EtOH/H_2O	156–157[b]

[a] Dioxalate.
[b] Free base.
[c] Dihydrochloride·1.5H_2O.
[d] Dihydrochloride·1/2C_2H_5OH.
[e] Dihydrochloride·H_2O.

6.1. General method of preparation of 2-[ω-(piperidin-1-yl)alkylamino]-1*H*-benzimidazole derivatives (1–5)

A solution of 4.4 mmol of 2-chlorobenzimidazole and 8.8 mmol of the appropriate ω-piperidin-1-yl-alkylamine in 2.0 ml of isoamyl alcohol was heated at 130 °C for 16 h. The crude products were purified by column chromatography (SiO_2, CH_2Cl_2:$CH_3OH(NH_3)$ = 9:1).

6.1.1. 2-[3-(Piperidin-1-yl)propylamino]-1*H*-benzimidazole dioxalate (1·2$C_2H_2O_4$). ^{1}H NMR (DMSO-d_6) δ 1.53–1.55 (m, 2H, CH_2), 1.72–1.74 (m, 4H, CH_2), 2.00 (m, 2H, CH_2), 3.08–3.13 (m, 6H, CH_2), 3.46 (t, J = 6.3 Hz, 2H, CH_2), 7.09–7.14 (m, 2H, Bzim), 7.30–7.34 (m, 2H, Bzim). MS (CI) 259 $[M+1]^+$. Anal. calcd for $C_{15}H_{22}N_4$·2$C_2H_2O_4$: C, 52.05; H, 5.98; N, 12.78. Found: C, 52.30; H, 6.28; N, 12.57.

6.1.2. 2-[5-(Piperidin-1-yl)pentylamino]-1*H*-benzimidazole (2). ^{1}H NMR (DMSO-d_6) δ 1.21–1.31 (m, 2H, CH_2), 1.38–1.47 (m, 4H, CH_2), 1.49–1.60 (m, 6H, CH_2), 2.20 (t, J = 7.6 Hz, 2H, CH_2), 2.30–2.35 (m, 4H, CH_2), 3.36 (t, J = 7.0 Hz, 2H, CH_2), 6.98–7.04 (m, 2H, Bzim), 7.23–7.27 (m, 2H, Bzim). MS (EI) 286 $[M^+]$.

Anal. calcd for $C_{17}H_{26}N_4$: C, 71.28; H, 9.15; N, 19.56. Found: C, 70.91; H, 8.99; N, 19.16.

6.1.3. 2-[6-(Piperidin-1-yl)hexylamino]-1*H*-benzimidazole (3). ^{1}H NMR ($CDCl_3$) δ 1.17–1.62 (m, 14H, CH_2), 2.21 (t, J = 7.6 Hz, 2H, CH_2), 2.32–2.37 (m, 4H, CH_2), 3.33 (t, J = 6.9 Hz, 2H, CH_2), 6.98–7.01 (m, 2H, Bzim), 7.23–7.26 (m, 2H, Bzim). MS (CI) 301 $[M+1]^+$. Anal. calcd for $C_{18}H_{28}N_4$: C, 71.95; H, 9.39; N, 18.65. Found: C, 71.70; H, 9.40; N, 18.31.

6.1.4. 2-[7-(Piperidin-1-yl)heptylamino]-1*H*-benzimidazole (4). ^{1}H NMR ($CDCl_3$) δ 1.12–1.32 (m, 6H, CH_2), 1.40–1.63 (m, 10H, CH_2), 2.24 (t, J = 7.6 Hz, 2H, CH_2), 2.34–2.40 (m, 4H, CH_2), 3.37 (t, J = 6.9 Hz, 2H, CH_2), 6.99–7.04 (m, 2H, Bzim), 7.23–7.28 (m, 2H, Bzim). MS (CI) 315 $[M+1]^+$. Anal. calcd for $C_{19}H_{30}N_4$: C, 72.56; H, 9.62; N, 17.82. Found: C, 72.53; H, 9.61; N, 17.70.

6.1.5. 2-[8-(Piperidin-1-yl)octylamino]-1*H*-benzimidazole (5). ^{1}H NMR ($CDCl_3$) δ 1.12–1.28 (m, 6H, CH_2), 1.40–1.64 (m, 12H, CH_2), 2.27 (t, J = 7.5 Hz, 2H, CH_2), 2.34–2.42 (m, 4H, CH_2), 3.38 (t, J = 6.8 Hz, 2H, CH_2), 6.99–7.05 (m, 2H, Bzim), 7.24–7.30 (m, 2H, Bzim). MS (CI) 329 $[M+1]^+$. Anal. calcd for $C_{20}H_{32}N_4$: C, 73.12; H, 9.82; N, 17.06. Found: C, 72.79; H, 9.75; N, 16.70.

6.2. General method of preparation of ω-(4-chlorophenoxy)alkyl cyanide derivatives

A mixture of 50 mmol of 4-chlorophenol, 40 mmol of the appropriate commercially available ω-bromoalkyl cyanide, and 40 mmol K_2CO_3 in 10.0 ml CH_3COCH_3 was refluxed under stirring for 24 h and the solid residue was then filtered off. Removal of the solvent in vacuo gave the crude products, which were purified by column chromatography (SiO_2, CH_2Cl_2:CH_3OH = 99:1) and characterized by ^{1}H NMR and mass spectra.

6.2.1. 4-(4-Chlorophenoxy)butyronitrile. 98%, ^{1}H NMR (DMSO-d_6) δ 1.97–2.06 (m, 2H, CH_2), 2.64 (t, J = 7.0 Hz, 2H, CH_2), 4.02 (t, J = 6.2 Hz, 2H, CH_2), 6.93–6.99 (m, 2H, Ph), 7.29–7.34 (m, 2H, Ph). MS (EI) 195 $[M^+]$.

6.2.2. 5-(4-Chlorophenoxy)pentanenitrile. 96%, ^{1}H NMR (DMSO-d_6) δ 1.67–1.81 (m, 4H, CH_2), 2.55 (t, J = 6.8 Hz, 2H, CH_2), 3.97 (t, J = 6.2 Hz, 2H, CH_2), 6.91–6.96 (m, 2H, Ph), 7.28–7.33 (m, 2H, Ph). MS (EI) 209 $[M^+]$.

6.2.3. 6-(4-Chlorophenoxy)hexanenitrile. 99%, ^{1}H NMR (DMSO-d_6) δ 1.44–1.77 (m, 6H, CH_2), 2.50 (t, J = 7.0 Hz, 2H, CH_2), 3.95 (t, J = 6.4 Hz, 2H, CH_2), 6.91–6.97 (m, 2H, Ph), 7.27–7.33 (m, 2H, Ph). MS (EI) 223 $[M^+]$.

6.2.4. 7-(4-Chlorophenoxy)heptanenitrile. 98%, ^{1}H NMR (DMSO-d_6) δ 1.39–1.44 (m, 4H, CH_2), 1.52–1.61 (m, 2H, CH_2), 1.65–1.74 (m, 2H, CH_2), 2.47 (t, J = 7.2 Hz, 2H, CH_2), 3.94 (t, J = 6.4 Hz, 2H, CH_2), 6.91–6.96 (m, 2H, Ph), 7.27–7.32 (m, 2H, Ph). MS (CI) 238 $[M+1]^+$.

6.3. General method of preparation of ω-(4-chlorophenoxy)alkylamine derivatives

A solution of 20 mmol of the appropriate ω-(4-chlorophenoxy)alkyl cyanide in 20.0 ml of dry THF was added dropwise to a suspension of 100 mmol $LiAlH_4$ in 40.0 ml of dry THF, kept in an ice bath and under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 30 min, then the excess of $LiAlH_4$ was decomposed by successive addition of 2.0 ml of a mixture water:THF = 1:1 and 20.0 ml of 10% w/v NaOH. The cake was filtered off and washed with THF, and the combined organic filtrates were evaporated in vacuo to dryness. The crude products were then purified by column chromatography [SiO_2, CH_2Cl_2:CH_3OH (NH_3) = 9:1.5] and characterized by ^{1}H NMR and mass spectra.

6.3.1. 4-(4-Chlorophenoxy)butylamine. 46%, ^{1}H NMR ($CDCl_3$) δ 1.54–1.64 (m, 2H, CH_2), 1.74–1.83 (m, 2H, CH_2), 2.74 (t, J = 6.9 Hz, 2H, CH_2), 3.91 (t, J = 6.5 Hz, 2H, CH_2), 6.75–6.81 (m, 2H, Ph), 7.16–7.21 (m, 2H, Ph). MS (EI) 199 $[M^+]$.

6.3.2. 5-(4-Chlorophenoxy)pentylamine. 54%, ^{1}H NMR ($CDCl_3$) δ 1.43–1.50 (m, 2H, CH_2), 1.70–1.80 (m, 4H, CH_2), 2.68 (t, J = 6.5 Hz, 2H, CH_2), 3.88 (t, J = 6.3 Hz, 2H, CH_2), 6.72–6.81 (m, 2H, Ph), 7.14–7.20 (m, 2H, Ph). MS (EI) 213 $[M^+]$.

6.3.3. 6-(4-Chlorophenoxy)hexylamine. 49%, ^{1}H NMR ($CDCl_3$) δ 1.28–1.48 (m, 6H, CH_2), 1.68–1.77 (m, 2H, CH_2), 2.65 (t, J = 6.7 Hz, 2H, CH_2), 3.86 (t, J = 6.4 Hz, 2H, CH_2), 6.72–6.78 (m, 2H, Ph), 7.14–7.19 (m, 2H, Ph). MS (EI) 227 $[M^+]$.

6.3.4. 7-(4-Chlorophenoxy)heptylamine. 46%, ^{1}H NMR ($CDCl_3$) δ 1.30–1.48 (m, 8H, CH_2), 1.68–1.78 (m, 2H, CH_2), 2.65 (t, J = 6.7 Hz, 2H, CH_2), 3.87 (t, J = 6.7 Hz, 2H, CH_2), 6.75–6.80 (m, 2H, Ph), 7.15–7.20 (m, 2H, Ph). MS (EI) 241 $[M^+]$.

6.4. General method of preparation of 2-[ω-(*p*-chlorophenoxy)alkylamino]-1*H*-benzimidazole derivatives (6–9)

A solution of 5.0 mmol of 2-chlorobenzimidazole and 10.0 mmol of the appropriate ω-(4-chlorophenoxy)alkylamine in 2.0 ml of isoamyl alcohol was heated at 130 °C for 16 h. The crude products were purified by column chromatography (SiO_2, CH_2Cl_2:CH_3OH = 9:1).

6.4.1. 2-[4-(*p*-Chlorophenoxy)butylamino]-1*H*-benzimidazole (6). ^{1}H NMR ($CDCl_3$) δ 1.82–1.88 (m, 4H, CH_2), 3.50 (t, J = 6.6 Hz, 2H, CH_2), 3.95 (t, J = 5.9 Hz, 2H, CH_2), 6.77–6.81 (m, 2H, Ph), 7.04–7.09 (m, 2H, Bzim), 7.19–7.24 (m, 2H, Ph), 7.24–7.29 (m, 2H, Bzim). MS (EI) 315 $[M^+]$. Anal. calcd for $C_{17}H_{18}N_3OCl$: C, 64.65; H, 5.75; N, 13.31. Found: C, 64.27; H, 5.64; N, 12.94.

6.4.2. 2-[5-(*p*-Chlorophenoxy)pentylamino]-1*H*-benzimidazole (7). ^{1}H NMR ($CDCl_3$) δ 1.43–1.52 (m, 2H, CH_2), 1.58–1.65 (m, 2H, CH_2), 1.69–1.79 (m, 2H, CH_2), 3.28

(q, J = 6.4 Hz, 2H, CH_2), 3.96 (t, J = 6.2 Hz, 2H, CH_2), 6.52 (br s, 1H, NH), 6.81–6.87 (m, 2H, Bzim), 6.91–6.96 (m, 2H, Ph), 7.07–7.12 (m, 2H, Bzim), 7.26–7.32 (m, 2H, Ph). MS (EI) 329 [M^+]. Anal. calcd for $C_{18}H_{20}N_3OCl$: C, 65.54; H, 6.11; N, 12.74. Found: C, 65.85; H, 6.12; N, 12.40.

6.4.3. 2-[6-(*p*-Chlorophenoxy)hexylamino]-1*H*-benzimidazole (8). ^{1}H NMR (DMSO-d_6) δ 1.34–1.48 (m, 4H, CH_2), 1.54–1.63 (m, 2H, CH_2), 1.67–1.76 (m, 2H, CH_2), 3.26 (q, J = 7.2 Hz, 2H, CH_2), 3.95 (t, J = 6.3 Hz, 2H, CH_2), 6.50 (br s, 1H, NH), 6.81–6.87 (m, 2H, Bzim), 6.90–6.95 (m, 2H, Ph), 7.07–7.12 (m, 2H, Bzim), 7.26–7.31 (m, 2H, Ph). MS (EI) 343 [M^+]. Anal. calcd for $C_{19}H_{22}N_3OCl$: C, 66.36; H, 6.45; N, 12.22. Found: C, 66.11; H, 6.40; N, 11.99.

6.4.4. 2-[7-(*p*-Chlorophenoxy)heptylamino]-1*H*-benzimidazole (9). ^{1}H NMR (DMSO-d_6) δ 1.25–1.45 (m, 6H, CH_2), 1.52–1.62 (m, 2H, CH_2), 1.65–1.74 (m, 2H, CH_2), 3.24 (q, J = 6.8 Hz, 2H, CH_2), 3.94 (t, J = 6.4 Hz, 2H, CH_2), 6.46 (br s, 1H, NH), 6.82–6.85 (m, 2H, Bzim), 6.90–6.95 (m, 2H, Ph), 7.08–7.11 (m, 2H, Bzim), 7.26–7.31 (m, 2H, Ph). MS (EI) 357 [M^+]. Anal. calcd for $C_{20}H_{24}N_3OCl$: C, 67.12; H, 6.76; N, 11.74. Found: C, 67.27; H, 6.78; N, 11.67.

6.5. General method of preparation of *N*-[4-(ω-chloroalkoxy)-2-nitrophenyl]acetamide derivatives

A mixture of 20 mmol of *N*-(4-hydroxy-2-nitrophenyl)acetamide,[54] 30 mmol of the appropriate commercially available 1-bromo-ω-chloroalkane, 40 mmol Na_2CO_3, and a catalytic amount of KI in 40 ml DMF was stirred at 50 °C for 24 h. The solvent was then evaporated under reduced pressure, the residue was dissolved in H_2O, and the product was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The crude products were crystallized from 50% EtOH.

6.5.1. *N*-[4-(3-Chloropropoxy)-2-nitrophenyl]acetamide. 95%, mp 80–84 °C, ^{1}H NMR (DMSO-d_6) δ 2.01 (t, 3H, CH_3), 2.13–2.27 (m, 2H, CH_2), 3.79 (t, J = 6.5 Hz, 2H, CH_2), 4.16 (t, J = 6.0 Hz, 2H, CH_2), 7.30 (dd, J = 9.0 Hz and 2.7 Hz, 1H, Ph), 7.45–7.48 (m, 2H, Ph), 10.02 (s, 1H, NH). MS (CI) 273 [M+1]$^+$.

6.5.2. *N*-[4-(4-Chlorobutoxy)-2-nitrophenyl]acetamide. 90%, mp 76–78 °C, ^{1}H NMR (DMSO-d_6) δ 1.82–1.96 (m, 4H, CH_2), 2.00 (t, 3H, CH_3), 3.71 (t, J = 6.2 Hz, 2H, CH_2), 4.08 (t, J = 6.2 Hz, 2H, CH_2), 7.26 (dd, J = 8.9 Hz and 2.8 Hz, 1H, Ph), 7.42–7.44 (m, 2H, Ph), 10.04 (s, 1H, NH). MS (CI) 287 [M+1]$^+$.

6.5.3. *N*-[4-(5-Chloropentyloxy)-2-nitrophenyl]acetamide. 80%, mp 76–79 °C, ^{1}H NMR (DMSO-d_6) δ 1.48–1.60 (m, 2H, CH_2), 1.70–1.89 (m, 4H, CH_2), 2.00 (t, 3H, CH_3), 3.65 (t, J = 6.5 Hz, 2H, CH_2), 4.04 (t, J = 6.5 Hz, 2H, CH_2), 7.27 (dd, J = 9.0 Hz and 2.4 Hz, 1H, Ph), 7.41–7.44 (m, 2H, Ph), 10.04 (s, 1H, NH). MS (CI) 301 [M+1]$^+$.

6.5.4. *N*-[4-(6-Chlorohexyloxy)-2-nitrophenyl]acetamide. 80%, mp 89–90 °C, ^{1}H NMR (DMSO-d_6) δ 1.48–1.60 (m, 2H, CH_2), 1.70–1.89 (m, 4H, CH_2), 2.00 (t, 3H, CH_3), 3.65 (t, J = 6.5 Hz, 2H, CH_2), 4.04 (t, J = 6.5 Hz, 2H, CH_2), 7.27 (dd, J = 9.0 Hz and 2.4 Hz, 1H, Ph), 7.41–7.44 (m, 2H, Ph), 10.04 (s, 1H, NH). MS (CI) 315 [M+1]$^+$.

6.6. General method of preparation of *N*-[2-nitro-4-(ω-piperidin-1-yl-alkoxy)phenyl]acetamide derivatives

A mixture of 8.0 mmol of the appropriate *N*-[4-(ω-chloroalkoxy)-2-nitrophenyl]acetamide in 80 mmol piperidine was stirred at 50 °C for 5 h. The solvent was then evaporated under reduced pressure and the crude products were directly used in the successive reaction without purification.

6.7. General method of preparation of *N*-{4-[ω-(4-chlorophenoxy)alkoxy]-2-nitrophenyl}acetamide derivatives

To a solution of 10 mmol of the appropriate *N*-[4-(ω-chloroalkoxy)-2-nitrophenyl]acetamide in 20 ml DMF were added 20 mmol K_2CO_3, 10 mmol of 4-chlorophenol, and a catalytic amount of KI. The reaction mixture was stirred at 50 °C for 48 h and then the solvent was evaporated under reduced pressure. The residue was dissolved in H_2O, the product was extracted with CH_2Cl_2, and the organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The crude products were washed with CH_3OH and crystallized from 95% EtOH.

6.7.1. *N*-{4-[3-(4-Chlorophenoxy)propoxy]-2-nitrophenyl}acetamide. 90%, mp 129–131 °C, ^{1}H NMR (DMSO-d_6) δ 2.01 (t, 3H, CH_3), 2.13–2.22 (m, 2H, CH_2), 4.12 (t, J = 6.2 Hz, 2H, CH_2), 4.20 (t, J = 6.2 Hz, 2H, CH_2), 6.95–7.00 (m, 2H, Ph), 7.28–7.34 (m, 3H, Ph), 7.45 (d, J = 9.0 Hz, 1H, Ph), 7.46 (d, J = 2.7 Hz, 1H, Ph), 10.01 (s, 1H, NH). MS (CI) 365 [M+1]$^+$.

6.7.2. *N*-{4-[4-(4-Chlorophenoxy)butoxy]-2-nitrophenyl}acetamide. 90%, mp 129–130 °C, ^{1}H NMR (DMSO-d_6) δ 1.85–1.87 (m, 4H, CH_2), 2.01 (t, 3H, CH_3), 4.03 (t, J = 5.7 Hz, 2H, CH_2), 4.11 (t, J = 6.0 Hz, 2H, CH_2), 6.92–6.98 (m, 2H, Ph), 7.26–7.32 (m, 3H, Ph), 7.43 (d, J = 2.4 Hz, 1H, Ph), 7.46 (d, J = 9.3 Hz, 1H, Ph), 9.99 (s, 1H, NH). MS (CI) 379 [M+1]$^+$.

6.7.3. *N*-{4-[5-(4-Chlorophenoxy)pentyloxy]-2-nitrophenyl}acetamide. 90%, mp 130–132 °C, ^{1}H NMR (DMSO-d_6) δ 1.50–1.60 (m, 2H, CH_2), 1.72–1.83 (m, 4H, CH_2), 2.01 (t, 3H, CH_3), 3.98 (t, J = 6.4 Hz, 2H, CH_2), 4.06 (t, J = 6.2 Hz, 2H, CH_2), 6.92–6.97 (m, 2H, Ph), 7.25–7.33 (m, 3H, Ph), 7.43 (d, J = 3.6 Hz, 1H, Ph), 7.45 (d, J = 9.0 Hz, 1H, Ph), 9.99 (s, 1H, NH). MS (EI) 392 [M^+].

6.8. General method of preparation of 2-nitro-4-(ω-piperidin-1-yl-alkoxy)phenylamine derivatives

A mixture of 8.0 mmol of the appropriate *N*-[2-nitro-4-(ω-piperidin-1-yl-alkoxy)phenyl]acetamide in 29.0 ml of 4 N NaOH was refluxed under stirring for 2 h.

The residue was then poured into a mixture of ice/water and the resulting precipitate was collected by filtration. The crude products were crystallized from 50% EtOH.

6.8.1. 2-Nitro-4-(4-piperidin-1-yl-butoxy)phenylamine. 82%, mp 80–81 °C, ^{1}H NMR (DMSO-d_6) δ 1.33–1.40 (m, 2H, CH_2), 1.43–1.58 (m, 6H, CH_2), 1.64–1.73 (m, 2H, CH_2), 2.23–2.30 (m, 6H, CH_2), 3.92 (t, J = 6.3 Hz, 2H, CH_2), 6.98 (t, J = 9.3 Hz, 1H, Ph), 7.15 (dd, J = 9.1 Hz and 2.7 Hz, 1H, Ph), 7.21 (s, 2H, NH_2), 7.35 (d, J = 2.7 Hz, 1H, Ph). MS (CI) 294 $[M+1]^+$.

6.8.2. 2-Nitro-4-(5-piperidin-1-yl-pentyloxy)phenylamine. 80%, mp 81–83 °C, ^{1}H NMR (DMSO-d_6) δ 1.29–1.50 (m, 10H, CH_2), 1.63–1.70 (m, 2H, CH_2), 2.17–2.25 (m, 6H, CH_2), 3.89 (t, J = 6.4 Hz, 2H, CH_2), 6.98 (t, J = 9.2 Hz, 1H, Ph), 7.14 (dd, J = 9.2 Hz and 2.9 Hz, 1H, Ph), 7.21 (s, 2H, NH_2), 7.34 (d, J = 2.8 Hz, 1H, Ph). MS (EI) 307 $[M^+]$.

6.8.3. 2-Nitro-4-(6-piperidin-1-yl-hexyloxy)phenylamine. 85%, mp 73–74 °C, ^{1}H NMR (DMSO-d_6) δ 1.28–1.47 (m, 12H, CH_2), 1.63–1.72 (m, 2H, CH_2), 2.16–2.26 (m, 6H, CH_2), 3.89 (t, J = 6.3 Hz, 2H, CH_2), 6.98 (t, J = 9.0 Hz, 1H, Ph), 7.15 (dd, J = 9.6 Hz and 2.7 Hz, 1H, Ph), 7.26 (s, 2H, NH_2), 7.34 (d, J = 3.0 Hz, 1H, Ph). MS (CI) 322 $[M+1]^+$.

6.9. General method of preparation of 4-[ω-(4-chlorophenoxy)alkoxy]-2-nitrophenylamine derivatives

To a solution of 10 mmol KOH in 10 ml of absolute EtOH was added 10 mmol of the appropriate *N*-{4-[ω-(4-chlorophenoxy)alkoxy]-2-nitrophenyl}acetamide. The reaction mixture was stirred at room temperature for 5 h, then the solvent was evaporated under reduced pressure and the residue was extracted with ethyl acetate. The organic layer was washed with water, dried over Na_2SO_4, and evaporated under reduced pressure. The crude products were used in the successive reaction without further purification.

6.9.1. 4-[3-(4-Chlorophenoxy)propoxy]-2-nitrophenylamine. 86%, mp 98–101 °C, ^{1}H NMR (DMSO-d_6) δ 2.09–2.19 (m, 2H, CH_2), 4.05–4.14 (m, 4H, CH_2), 6.96–7.01 (m, 3H, Ph), 7.16 (dd, J = 9.1 Hz and 3.1 Hz, 1H, Ph), 7.22 (s, 2H, NH_2), 7.29–7.33 (m, 2H, Ph), 7.39 (d, J = 2.7 Hz, 1H, Ph). MS (EI) 322 $[M^+]$.

6.9.2. 4-[4-(4-Chlorophenoxy)butoxy]-2-nitrophenylamine. 90%, mp 109–111 °C, ^{1}H NMR (DMSO-d_6) δ 1.83–1.85 (m, 4H, CH_2), 3.96–4.04 (m, 4H, CH_2), 6.94–7.00 (m, 3H, Ph), 7.15 (dd, J = 9.3 Hz and 2.7 Hz, 1H, Ph), 7.21 (s, 2H, NH_2), 7.28–7.32 (m, 2H, Ph), 7.37 (d, J = 3.0, 1H, Ph). MS (CI) 337 $[M+1]^+$.

6.9.3. 4-[5-(4-Chlorophenoxy)pentyloxy]-2-nitrophenylamine. 87%, mp 114–116 °C, ^{1}H NMR (DMSO-d_6) δ 1.49–1.59 (m, 2H, CH_2), 1.70–1.81 (m, 4H, CH_2), 3.91–3.99 (m, 4H, CH_2), 6.91–6.96 (m, 2H, Ph), 6.99 (d, J = 9.0 Hz, 1H, Ph), 7.15 (dd, J = 8.8 Hz and 2.8 Hz, 1H, Ph), 7.21 (s, 2H, NH_2), 7.27–7.32 (m, 2H, Ph), 7.36 (d, J = 2.7 Hz, 1H, Ph). MS (CI) 351 $[M+1]^+$.

6.10. General method of preparation of 4-(ω-piperidin-1-yl-alkoxy)-1,2-phenylendiamine derivatives and of 4-[ω-(4-chlorophenoxy)alkoxy]-1,2-phenylendiamine derivatives

A solution of the appropriate 2-nitrophenylamine derivative (10 mmol) in 40 ml of absolute EtOH was hydrogenated using a Parr apparatus with Pd/C (10%) at room temperature, for 5 h. The products obtained after filtration of the catalyst were used in the successive reaction without further purification.

6.11. General method of preparation of 5(6)-[ω-(piperidin-1-yl)alkoxy]-2-aminobenzimidazole derivatives (10–12)

A cyanogen bromide solution (5.0 M in acetonitrile, 1.57 ml) was added to an ice-cooled solution (0 °C) of 6.56 mmol of the appropriate 4-(ω-piperidin-1-yl-alkoxy)-1,2-phenylenediamine in 13.35 ml H_2O. The reaction mixture was stirred at room temperature for 12 h, then it was cooled again and acidified with an ethanol solution saturated with gaseous HCl. The solvent was evaporated under reduced pressure and the residue was extracted with abs EtOH. The crude products so obtained were again changed into the free bases before starting purification by column chromatography (SiO_2, CH_2Cl_2:$CH_3OH(NH_3)$ = 10:1).

6.11.1. 5(6)-[4-(Piperidin-1-yl)butoxy]-2-aminobenzimidazole (10·2HCl·1.5H$_2$O). ^{1}H NMR (DMSO-d_6) δ 1.48–2.08 (m, 12H, CH_2), 3.01–3.16 (m, 4H, CH_2), 3.98 (t, J = 5.4 Hz, 2H, CH_2), 6.74 (d, J = 8.7 Hz, 1H, Bzim), 6.89 (s, 1H, Bzim), 7.18 (d, J = 8.7 Hz, 1H, Bzim). MS (CI) 289 $[M+1]^+$. Anal. calcd for $C_{16}H_{24}N_4O$·2HCl·1.5H_2O: C, 49.48; H, 7.53; N, 14.43. Found: C, 49.24; H, 7.21; N, 14.33.

6.11.2. 5(6)-[5-(Piperidin-1-yl)pentyloxy]-2-aminobenzimidazole (11·2HCl·1/2C$_2$H$_5$OH). ^{1}H NMR (DMSO-d_6) δ 1.38–1.76 (m, 12H, CH_2), 2.76–2.84 (m, 2H, CH_2), 2.94–2.99 (m, 2H, CH_2), 3.35–3.39 (m, 2H, CH_2), 3.94 (t, J = 6.2 Hz, 2H, CH_2), 6.80 (d, J = 8.5 Hz and 2.6 Hz, 1H, Bzim), 6.92 (d, J = 1.8 Hz, 1H, Bzim), 7.24 (d, J = 8.7 Hz, 1H, Bzim). MS (EI) 302 $[M^+]$. Anal. calcd for $C_{17}H_{26}N_4O$·2HCl·1/2C_2H_5OH: C, 54.26; H, 7.84; N, 14.06. Found: C, 54.13; H, 7.54; N, 14.44.

6.11.3. 5(6)-[6-(Piperidin-1-yl)hexyloxy]-2-aminobenzimidazole (12·2HCl·H$_2$O). ^{1}H NMR (DMSO-d_6) δ 1.02–1.49 (m, 6H, CH_2), 1.65–1.76 (m, 10H, CH_2), 2.75–2.83 (m, 2H, CH_2), 2.92–2.98 (m, 2H, CH_2), 3.95 (t, J = 6.3 Hz, 2H, CH_2), 6.79 (dd, J = 8.7 Hz and 2.1 Hz, 1H, Bzim), 6.92 (d, J = 2.1 Hz, 1H, Bzim), 7.23 (d, J = 8.7 Hz, 1H, Bzim). MS (CI) 317 $[M+1]^+$. Anal. calcd for $C_{18}H_{28}N_4O$·2HCl·H_2O: C, 53.06; H, 7.92; N, 13.75. Found: C, 53.41; H, 7.95; N, 13.36.

6.12. General method of preparation of 5(6)-[ω-(*p*-chlorophenoxy)alkoxy]-2-aminobenzimidazole derivatives (13–15)

A cyanogen bromide solution (5.0 M in acetonitrile, 0.58 ml) was added to an ice-cooled solution (0 °C) of 2.38 mmol of the appropriate 4-[ω-(4-chlorophen-

oxy)alkoxy]-1,2-phenylendiamine in 8.0 ml of absolute EtOH. The reaction mixture was stirred at room temperature for 2 h, then the solvent was evaporated under reduced pressure and the residue was extracted with abs EtOH. The solvent was evaporated in vacuo again and the crude products so obtained were washed with CH_2Cl_2.

6.12.1. 5(6)-[3-(*p*-Chlorophenoxy)propoxy]-2-aminobenzimidazole (13). ^{1}H NMR (DMSO-d_6) δ 2.08–2.17 (m, 2H, CH_2), 4.05 (t, J = 6.2 Hz, 2H, CH_2), 4.13 (t, J = 6.4 Hz, 2H, CH_2), 6.45 (dd, J = 8.4 Hz and 2.1 Hz, 1H, Bzim), 6.71 (d, J = 2.1 Hz, 1H, Bzim), 6.93–7.00 (m, 3H, Bzim and Ph), 7.28–7.34 (m, 2H, Ph). MS (EI) 317 [M^+]. Anal. calcd for $C_{16}H_{16}N_3O_2Cl$: C, 60.47; H, 5.08; N, 13.22. Found: C, 60.72; H, 5.04; N, 12.97.

6.12.2. 5(6)-[4-(*p*-Chlorophenoxy)butoxy]-2-aminobenzimidazole (14). ^{1}H NMR (DMSO-d_6) δ 1.70–1.92 (m, 4H, CH_2), 3.95 (t, J = 5.7 Hz, 2H, CH_2), 4.02 (t, J = 5.7 Hz, 2H, CH_2), 6.46 (dd, J = 8.7 Hz and 2.4 Hz, 1H, Bzim), 6.70 (d, J = 2.4 Hz, 1H, Bzim), 6.40–6.98 (m, 3H, Bzim and Ph), 7.27–7.33 (m, 2H, Ph). MS (EI) 331 [M^+]. Anal. calcd for $C_{17}H_{18}N_3O_2Cl$: C, 61.53; H, 5.47; N, 12.67. Found: C, 61.51; H, 5.48; N, 12.37.

6.12.3. 5(6)-[5-(*p*-Chlorophenoxy)pentyloxy]-2-aminobenzimidazole (15). ^{1}H NMR (DMSO-d_6) δ 1.50–1.60 (m, 2H, CH_2), 1.70–1.81 (m, 4H, CH_2), 3.91 (t, J = 6.4 Hz, 2H, CH_2), 3.97 (t, J = 6.4 Hz, 2H, CH_2), 6.44 (dd, J = 8.5 Hz and 2.4 Hz, 1H, Bzim), 6.69 (d, J = 2.4 Hz, 1H, Bzim), 6.92–6.97 (m, 3H, Bzim and Ph), 7.27–7.33 (m, 2H, Ph). MS (EI) 345 [M^+]. Anal. calcd for $C_{18}H_{20}N_3O_2Cl$: C, 62.51; H, 5.83; N, 12.15. Found: C, 62.74; H, 5.77; N, 11.87.

6.13. Pharmacology

6.13.1. Human H_3 binding assay. Homogenates of GPCR97-transfected SK-N-MC cells were used for determining affinity values of the new compounds at the H_3-receptor, in radioligand displacement studies according to the method described by Lovenberg et al.[51] SK-N-MC cells in confluent culture plates were harvested using a cell scraper and centrifuged for 5 min at 1100 rpm. Pellets, either fresh or stored at −80 °C until the moment of use, were mechanically homogenized with Potter–Elvhejem in 20 mM Tris–HCl/0.5 mM EDTA. Supernatants from a 2000 rpm spin (10 min) were collected and re-centrifuged at 10,000 rpm for 30 min. Pellets were rehomogenized in 50 mM Tris–HCl/5 mM EDTA (pH 7.4). Membranes were incubated for 60 min at room temperature with 0.5 nM [^{3}H]-(*R*)-α-methylhistamine (30.0 Ci/mmol, Amersham Bioscience) in 50 mM Tris–HCl/5 mM EDTA (pH 7.4) with or without competing ligands. Incubation was terminated by rapid filtration over Millipore AAWP 0.8 μm filters followed by two washes with ice-cold buffer (50 mM Tris–HCl/5 mM EDTA). Retained radioactivity was determined by liquid scintillation counting. Non-specific binding was defined with 10 μM histamine as competing ligand. pIC_{50} values were estimated from the displacement curves of the tested compounds (0.01 nM–100 μM) versus [^{3}H]-(*R*)-α-methylhistamine (K_d in human recombinant H_3 expressing cells = 0.15 nM and K_d on rat brain = 0.40 nM)[51,55] and converted to pK_i values according to Cheng and Prusoff's equation.[56]

6.13.2. Cell culture. GPCR97-transfected SK-N-MC cells, a human neuroblastoma cell line stably expressing the human histamine H_3-receptor, were grown in 75 cm^2 culture flasks at 37 °C in a humidified atmosphere with 5% CO_2 in Eagle's minimal essential medium, supplemented with 10% v/v fetal calf serum, 1% non-essential amino acids, 1% penicillin–streptomycin, 1% L-glutamine, and 1% disodium pyruvate in the presence of 300 μg/ml G418. Cells were used for experiments when they reached about 70–80% confluence.

6.13.3. Rat H_3 binding assays. Rat (Wistar) cerebral cortex membranes were incubated for 30 min with [^{3}H]RAMHA 0.5 nM and the compounds under study (1 nM–30 μM), in Tris–HCl 50 mM, pH 7.4, NaCl 50 mM, and EDTA 0.5 mM, then rapidly filtered (Millipore AAWP 0.8 μm) under vacuum and rinsed with ice-cold buffer. Specific binding was defined as the binding inhibited by thioperamide 10 μM, and the pIC_{50} values were estimated from the displacement curves of the compounds tested versus [^{3}H]RAMHA bound to cerebral membranes.[52] pK_i values were calculated according to Cheng and Prusoff's equation.[56]

6.13.4. 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–1 μM). The tissues were allowed to equilibrate with the compounds under study (1 nM–10 μM) for 30 min before the generation of a second concentration–response curve to the agonist. pK_B values ('apparent pA_2') were determined according to Furchgott's equation.[57]

Antagonistic activities of compounds at H_1- and H_2-receptors were determined in isolated guinea-pig ileum contracted by histamine and in isolated guinea-pig atria stimulated by H_2-agonist dimaprit, respectively.[53]

Acknowledgments

Financial support from Italian MIUR is gratefully acknowledged. We are grateful to the Centro Interfacoltà Misure of the University of Parma for providing the NMR and MS instrumentation. The authors thank Dr. Massimo Coruzzi and Mr. Fabio Panni for technical assistance. The authors thank Dr. Timothy W. Loven-

berg, and Dr. Sandy J. Wilson, Johnson & Johnson Pharmaceutical Research and Development, San Diego, CA, for the cells stably expressing the human histamine H_3-receptor.

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