Benzimidazole Derivatives. 2. Synthesis and Structure−Activity Relationships of New Azabicyclic Benzimidazole-4-carboxylic Acid Derivatives with Affinity for Serotoninergic 5-HT_3 Receptors

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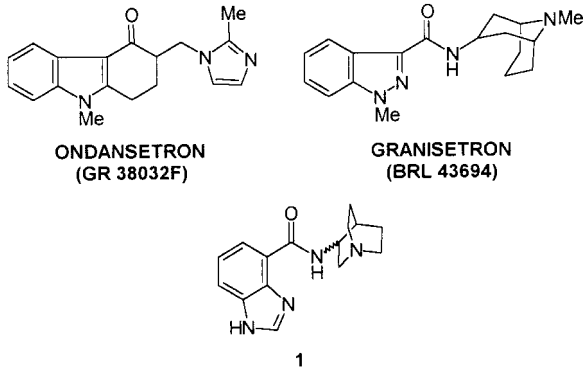
A new series of azabicyclic benzimidazole-4-carboxamides **2**−**21** and -carboxylates **22**−**30** were synthesized and evaluated for binding affinity at serotoninergic 5-HT_3 and 5-HT_4 receptors in the CNS. Most of the synthesized compounds exhibited high or very high affinity for the 5-HT_3 binding site and low to no significant affinity for the 5-HT_4 receptor. SAR observations indicated that a halogen atom at the 6-position and a nitro group at the 7-position of the benzimidazole ring is the best substitution pattern for 5-HT_3 affinity and 5-HT_3/5-HT_4 selectivity, as well as no substitution in this ring. (*S*)-(−)-*N*-(Quinuclidin-3-yl)benzimidazole-4-carboxamides **2**, **8**, and **14** bound at central 5-HT_3 sites with high affinity (K_i = 2.6, 0.13, and 1.7 nM, respectively) and excellent selectivity over serotonin 5-HT_4 and 5-HT_{1A} receptors (K_i > 1000−10000 nM). Furthermore, these new 5-HT_3 receptor ligands were pharmacologically characterized as potent and selective 5-HT_3 antagonists in the isolated guinea pig ileum (pA_2 = 9.6, 9.9, and 9.1, respectively).

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter involved in a wide variety of pharmacological effects in several peripheral as well as central nervous tissues.[1−7] Seven 5-HT receptor classes (5-HT_{1-7}), including 15 different subtypes (A, B, ...), have so far been identified.[8−12] Most of them belong to the G-protein-linked receptor superfamily involving in their action a second-messenger system such as cAMP or diacylglycerol and inositolphosphates. The 5-HT_3 subtype, on the other hand, has a special place because it is a ligand-gated cation channel receptor[13,14] and is present within the central and peripheral nervous systems.[15] In recent years, there has been considerable interest in the search for new specific 5-HT_3 ligands due to their implication in various (patho)physiological processes[16,17] and their potential applications in therapy. Indeed, 5-HT_3 receptor antagonists—ondansetron, granisetron (Chart 1)—are used clinically as agents to prevent the emesis which is a frequent, severe, and sometimes incapacitating syndrome associated with anticancer chemotherapy.[18,19] Further possible therapeutic indications in the treatment of central nervous system disorders such as anxiety, schizophrenia, drug abuse and withdrawal, anorectic responses, and cognitive disfunctions are the subject of intense investigations.[16,20−23]

As multiple known 5-HT_3 receptor antagonists—tropisetron, zacopride—also exhibit activity in the 5-HT_4 subtype, significant effort has been made toward the development of potent and selective 5-HT_3 receptor ligands. In the course of a program aimed at the discovery of new 5-HT_3 and 5-HT_4 receptor agents, we have recently reported a comparative receptor mapping of both serotoninergic binding sites.[24] This computer-aided conformational analysis has allowed us to confirm the generally accepted three-component pharmacophore for 5-HT_3 receptor antagonists as well as to propose a steric model for 5-HT_4 receptor recognition. This study led to the design of a new structural class of selective 5-HT_3 and 5-HT_4 receptor ligands,[25−27] which are benzimidazole-4-carboxylic acid derivatives. Among them, compound **1** was identified as a potent and selective 5-HT_3 receptor ligand [K_i (5-HT_3) = 3.7 nM, K_i (5-HT_4) > 1000 nM].[26] In this paper, we report the synthesis of a new series of azabicyclic benzimidazole-4-carboxamides **2**−**21** and carboxylates **22**−**30** related to lead

Chart 1



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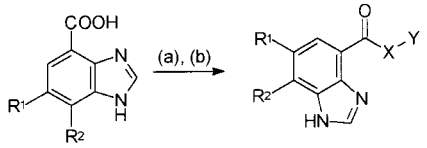
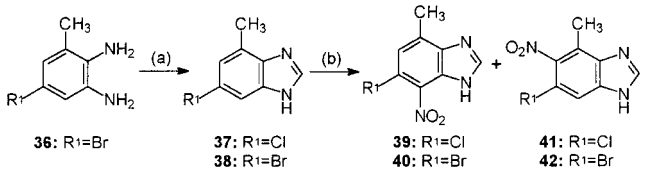
Scheme 1[a]



31: R1=R2=H
32: R1=Cl, R2=H
33: R1=Br, R2=H
34: R1=Cl, R2=NO_2
35: R1=Br, R2=NO_2

[a] Regents and conditions: (a) CDI, DMF, 40 °C, 1 h; (b) Y-NH_2 or Y-OH, DBU, DMF, 50 °C, 20−24 h.

Scheme 2[a]



36: R1=Br
37: R1=Cl
38: R1=Br
39: R1=Cl
40: R1=Br
41: R1=Cl
42: R1=Br
33: R1=Br, R2=H
34: R1=Cl, R2=NO_2
35: R1=Br, R2=NO_2

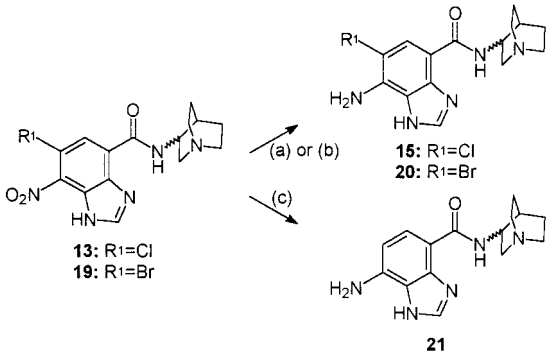
[a] Reagents and conditions: (a) HCOOH, H_2O, 100 °C, 5 h; (b) HNO_3/H_2SO_4, 5 °C, 2 h; (c) flash chromatography; (d) $KMnO_4$, 0.5 N NaOH, 100 °C, 5 h.

compound **1** (Chart 1) and also their affinities for 5-HT_3 and 5-HT_4 receptors obtained by radioligand binding assays. In these analogues we have analyzed the influence of different substituents at the benzimidazole ring, different azabicycloalkanes as amino moieties, and the stereogenic center of the quinuclidine system on the affinity for 5-HT_3 receptors and selectivity over 5-HT_4 sites, with respect to unsubstituted lead compound **1**. Three analogues with interesting 5-HT_3 affinity and selectivity were evaluated for 5-HT_3 antagonist activity, by analyzing their ability to inhibit the contractions induced by 5-HT in the isolated guinea pig ileum.

Chemistry

The general procedure for the preparation of target compounds **2**−**14**, **16**−**19**, and **22**−**30** is shown in Scheme 1. Starting benzimidazolecarboxylic acids **31**−**35** were suitably activated with 1,1′-carbonyldiimidazole (CDI), and the corresponding imidazolides were subsequently coupled with the appropriate 3-substituted quinuclidine, tropane, or granatane amines and alcohols in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in *N*,*N*-dimethylformamide (DMF) solutions, to afford the desired amides and esters. In analogy with 6-chlorobenzimidazole-4-carboxylic acid (**32**),[27] acid **33** was obtained by condensation of 2-amino-5-bromo-3-methylaniline (**36**) with formic acid and subsequent oxidation of 6-bromo-4-methylbenzimidazole (**38**) with potassium permanganate (Scheme 2). 7-Nitrobenzimidazole-4-carboxylic acids **34** and **35** were prepared as illustrated in Scheme 2. Nitration of 4-methylbenzimidazoles **37** and **38** provided the desired 7-nitro derivatives **39** and **40**, along with a minor amount (30%) of their 5-nitro isomers **41** and **42**, which were separated by flash chromatography. The benzimidazole substitution pattern was assigned using steady state 1D-NOE experiments through irradiation of the methyl substituent. Irradiation of the CH_3 signal caused NOE enhancement of the aromatic proton signal at δ 7.35 ppm for **39** and no effect of any signal for **41**, indicating that the former isomer has an aromatic proton situated at an *ortho* position with respect to the methyl group, while **41** has a substituent (the nitro group) in this position. Similar results were obtained for **40** and **42**. Derivatives **39** and **40** were converted into their carboxylic acids **34** and **35**, respectively, by oxidation with potassium permanganate. 7-Aminocarboxamides **15, 20**, and **21** were prepared from their corresponding 7-nitro derivatives (Scheme 3). Thus, **13** was subjected to catalytic hydrogenation over Raney nickel in ethanol to afford **15**. The treatment of **19** with hydrazine and Raney nickel yielded **20**, while its reduction with hydrazine and palladium on carbon led to the heterolytic reduction of the C−Br bond to give **21**. As for intermediate amines and alcohols, quinuclidine derivatives were commercially available, and tropane and granatane derivatives were synthesized according to previously described methods.

Scheme 3[a]



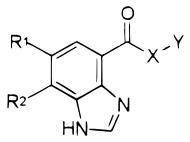
13: R1=Cl
19: R1=Br
15: R1=Cl
20: R1=Br
21

[a] Reagents and conditions: (a) R^1 = Cl: H_2/Ni-Ra, EtOH, rt, 2 h; (b) R^1 = Br: NH_2NH_2, Ni-Ra, EtOH, Δ, 15 min; (c) NH_2NH_2, Pd(C), EtOH, Δ, 30 min.

All new compounds (Table 1) were characterized by IR and NMR spectroscopy and gave satisfactory combustion analyses (C, H, N). The unambiguous assignment of all bicyclic proton and carbon resonances was achieved by a combined use of 1D ^1H and ^{13}C NMR, 2D NMR techniques (COSY and $^1H-^{13}C$ correlation spectra), and double-resonance experiments.

A structural analysis of our 3′-substituted tropanes and granatanes was performed by means of NMR spectroscopy and molecular mechanics calculations. ^1H NMR spectra of esters **23**, **25**, and **29** show H3′ as a triple triplet (septet-like) with coupling constant (*J*) of ca. 10.5 and 6.5 Hz, and the signal of the bridgehead protons H1′/H5′ is a broad triplet (J = ca. 3.5 Hz), indicating that they are *exo*-isomers in a chair conformation. Thus, *exo*-granatane derivatives adopt a chair−chair conformation (Figure 1a). In the spectra of tropane esters **24** and **28**, the signals of H3′ and H1′/H5′ are both triplets or broad triplets (J = 5.1 and ca. 3 Hz, respectively), while in the case of granatane carboxylates **26** and **30** the spectra show a triple triplet (quintet-like) with J = 6.9 and 6.0 Hz for the H3′ signal and a

Table 1. In Vitro Binding Data[a]



					$K_i \pm$ SEM (nM)	
compd	R^1	R^2	X	Y[b]	5-HT_3 [^{3}H]LY 278584	5-HT_4 [^{3}H]GR 113808
1	H	H	NH	Q	3.7 ± 0.1	>1000
2	H	H	NH	(*S*)-Q	2.6 ± 0.3	>1000
3	H	H	NH	(*R*)-Q	38.3 ± 0.6	>10000
4	H	H	NH	*exo*-T	118 ± 1	>10000
5	H	H	NH	*exo*-G	230 ± 1	>10000
6	H	H	NH	*endo*-G	6.1 ± 0.8	>1000
7	Cl	H	NH	Q	0.29 ± 0.08	168 ± 6
8	Cl	H	NH	(*S*)-Q	0.13 ± 0.02	>10000
9	Cl	H	NH	(*R*)-Q	0.76 ± 0.14	205 ± 8
10	Cl	H	NH	*exo*-T	16.6 ± 1.6	319 ± 75
11	Cl	H	NH	*exo*-G	83.0 ± 12.1	>1000
12	Cl	H	NH	*endo*-G	154 ± 24	167 ± 32
13	Cl	NO_2	NH	Q	5.2 ± 0.6	>1000
14	Cl	NO_2	NH	(*S*)-Q	1.7 ± 0.2	>1000
15	Cl	NH_2	NH	Q	0.24 ± 0.01	187 ± 5
16	Br	H	NH	Q	0.29 ± 0.01	168 ± 6
17	Br	H	NH	(*S*)-Q	0.28 ± 0.03	38.3 ± 5.0
18	Br	H	NH	(*R*)-Q	3.4 ± 0.1	270 ± 19
19	Br	NO_2	NH	Q	8.8 ± 0.1	>10000
20	Br	NH_2	NH	Q	0.42 ± 0.02	227 ± 3
21	H	NH_2	NH	Q	2.4 ±0.1	779 ± 105
22	H	H	O	Q	185 ± 3	>10000
23	H	H	O	*exo*-T	>1000	>10000
24	H	H	O	*endo*-T	>1000	>10000
25	H	H	O	*exo*-G	678 ± 120	>10000
26	H	H	O	*endo*-G	129 ± 1	>10000
27	Cl	H	O	Q	9.3 ± 1.0	440 ± 87
28	Cl	H	O	*endo*-T	61.2 ± 12.4	356 ± 33
29	Cl	H	O	*exo*-G	88.7 ± 1.1	>1000
30	Cl	H	O	*endo*-G	4.9 ± 0.8	>1000
	ondansetron				0.77 ± 0.01	>1000
	granisetron				3.9 ± 0.1	>1000

[a] K_i values are means ± SEM of two to four assays, performed in triplicate. Inhibition curves were analyzed by a computer-assisted curve-fitting program (Prism GraphPad), and K_i values were determined from the Cheng−Prusoff equation. [b] Q: quinuclidin-3-yl (1-azabicyclo[2.2.2]oct-3-yl). T: tropan-3-yl (8-methyl-8-azabicyclo[3.2.1]oct-3-yl). G: granatan-3-yl (9-methyl-9-azabicyclo[3.3.1]non-3-yl).

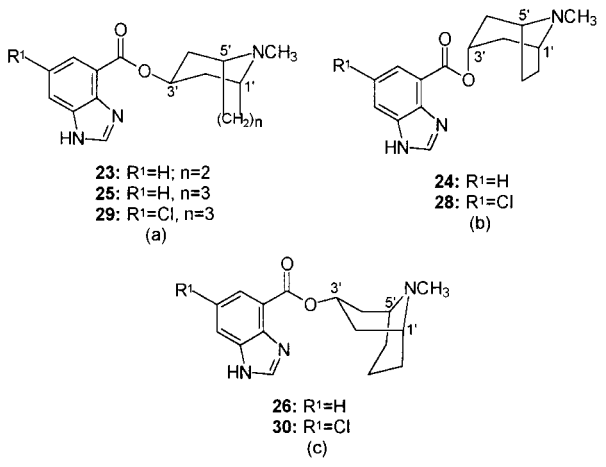


Figure 1. (a) In *exo*-tropane and -granatane esters the disubstituted ring adopts a chair conformation. (b) In *endo*-tropane esters the piperidine ring adopts a chair conformation. (c) *Endo*-granatane esters adopt a boat−chair conformation.

broad doublet (J = ca. 9 Hz) for the H1′/H5′ signal. These different patterns for H3′ and H1′/H5′ signals suggest that the piperidine ring of *endo*-tropane esters **24** and **28** are in a chair conformation (Figure 1b), but in the case of *endo*-granatane derivatives **26** and **30** the disubstituted ring has a boat conformation (Figure 1c). Thus, *endo*-granatane derivatives adopt a boat-chair conformation.

To obtain additional support, *endo*-esters **24** and **26** were subjected to computer-aided conformational analysis with INSIGHT II software 97.0 using the Builder, Discover, and Analysis programs. According to the calculated lowest energy conformations, the piperidine ring of *endo*-tropane ester **24** adopts a chair conformation, whereas *endo*-granatane ester **26** is in a boat−chair conformation. On the other hand, it is known that the vicinal coupling constants are very useful magnetic parameters for the conformational analysis of bicyclic systems owing to their strong dependence on the dihedral angles. By that, the coupling parameters of H3′ were empirically estimated for the computed disubstituted ring in chair and boat conformations of **24** and **26** by using the equation proposed by Altona[28] and compared with those deduced from the analysis of the spectra (Table 2). The experimental values are consistent with those calculated for the chair conformation of tropane **24** and the boat−chair conformation of granatane **26**. In the case of the carboxamides, H3′ signals were complicated multiplets due to couplings with amide protons. Nevertheless, H1′/H5′ signals of amides **6** and **12** showed the same pattern as those of ester **26**, indicating the same boat−chair conformation.

Pharmacology

Target compounds **2**−**30** were assessed for in vitro affinity at serotoninergic 5-HT_3 and 5-HT_4 receptors by radioligand binding assays, using [^{3}H]LY 278584 in rat cerebral cortex membranes[29] and [^{3}H]GR 113808 in rat striatum membranes,[30] respectively. The inhibition constant K_i was defined from the IC_{50} by the Cheng−Prusoff equation[31] (Table 1). Three highly active 5-HT_3 ligands were also evaluated for in vitro affinity at 5-HT_{1A} receptors with [^{3}H]-8-OH-DPAT in rat cerebral cortex membranes[32] and for 5-HT_3 biological activity in the isolated guinea pig ileum (Table 3). It is well-known that concentrations higher than 10^{-6} M of 5-HT induce contractile responses in the nonstimulated guinea pig ileum by activation of 5-HT_3 receptors, whereas lower concentrations activate 5-HT_4 receptors.[33−41] To test the antagonist activity of the new compounds, their capacity to inhibit the contractions induced by 5-HT in the myenteric plexus-longitudinal muscle strip preparations, isolated from guinea-pig ileum, was evaluated and the pA_2 values were determined following the equation described by Furchgott[42] using one appropriate concentration of the antagonists. The concentration ratios of 5-HT, in the presence or absence of antagonists, reflect the rightward displacement of the concentration effect measured at the point where the force of the contraction induced by 5-HT was 50% of the maximal control value. The 5-HT_3 receptor antagonists ondansetron and granisetron were used as reference compounds. The effects of the antagonists were also tested on the contraction induced by low concentrations of 5-HT ($<10^{-6}$ M), in order to discard activity on 5-HT_4 receptors.

Table 2. Experimental Coupling Constants and Calculated Values (in Hz) of H3′ for Compounds **24** and **26** with the Disubstituted Ring in Chair and Boat Conformations

	chair[a]		boat[a]		exp[b]	
compd	*J*(H3′−H2′ax)	*J*(H3′−H2′eq)	*J*(H3′−H2′ax)	*J*(H3′−H2′eq)	*J*(H3′−H2′ax)	*J*(H3′−H2′eq)
24	5.04	1.93	7.80	7.85	5.1	
26	6.10	1.50	7.55	7.98	6.0	6.9

[a] Coupling constants values calculated from Altona's equation for chair and boat conformations in the disubstituted ring obtained by molecular mechanics calculations. [b] Experimental coupling constants measured from ^{1}H NMR spectra in $CDCl_3$ solutions.

Table 3. Pharmacological Activity at 5-HT_3 Receptors in Isolated Guinea Pig Ileum

compd	5-HT ED_{50}, M[a]	pA_2[b]	N[c]
control	8.8×10^{-7}		10
ondansetron	4.3×10^{-5}	9.9	6
granisetron	7×10^{-6}	9.2	7
14	6.8×10^{-6}	9.1	7
8	1.7×10^{-5}	9.9	6
2	3.3×10^{-5}	9.6	6

[a] Concentration of 5-HT required to produce a 50% of maximal contraction induced by 5-HT through 5-HT_3 receptors evaluated in the absence (control) and in the presence of tested compounds (5×10^{-9} M). [b] Single-point analysis using 5×10^{-9} M concentration of the antagonists ($pA_2 = -\log([B]/\text{concentration ratio} - 1)$; [B] = concentration of the antagonist). [c] Number of experiments.

Results and Discussion

The results of the tested compounds are reported in Tables 1 and 3. The values for 5-HT_3 activation of the compounds were compared to those of the reference compounds: ondansetron (GR 38032F) and granisetron (BRL 43694).

Most of the synthesized compounds exhibit high or very high affinity for the 5-HT_3 receptor and low to no significant affinity for the 5-HT_4 binding site. An examination of the 5-HT_3 binding data presented in Table 1 shows the following:

(a) In general, the carboxamides are more potent at 5-HT_3 receptors than their corresponding carboxylates; only ester **30** ($K_i = 4.9$ nM) displayed ca. 30-fold higher affinity than its directly related amide **12** ($K_i = 154$ nM).

(b) With respect to the aromatic moiety, the introduction of a chloro or bromo atom at the 6-position and/or a nitro or amino group at the 7-position of the benzimidazole ring is tolerated for binding to 5-HT_3 sites. Thus, *N*-(quinuclidin-3-yl)benzimidazole-4-carboxamides **1** ($R^1 = R^2 = H$), **7** ($R^1 = Cl$, $R^2 = H$), **13** ($R^1 = Cl$, $R^2 = NO_2$), **15** ($R^1 = Cl$, $R^2 = NH_2$), **16** ($R^1 = Br$, $R^2 = H$), **19** ($R^1 = Br$, $R^2 = NO_2$), **20** ($R^1 = Br$, $R^2 = NH_2$), and **21** ($R^1 = H$, $R^2 = NH_2$) display nanomolar or subnanomolar affinity for the 5-HT_3 receptor ($K_i = 0.24-8.8$ nM). Regarding 5-HT_3/5-HT_4 selectivity, unsubstituted compounds (e.g., **1, 2**) and derivatives with $R^1 = Cl, Br$, $R^2 = NO_2$ (**13, 14**, and **19**) show high selectivity. Most analogues with $R^1 = Cl, Br$, $R^2 = H$ (e.g., **7** and **16**) and $R^1 = H, Cl, Br$, $R^2 = NH_2$ (**15**, **20**, and **21**) are moderately active at 5-HT_4 receptors, and consequently they show lower selectivity for the 5-HT_3 receptor. A remarkable exception is compound **8** ($R^1 = Cl$, $R^2 = H$), which displays the best selectivity ratio (5-HT_3: $K_i = 0.13$ nM, 5-HT_4: $K_i > 10000$ nM).

(c) Concerning the azabicyclic amino moiety (Y), quinuclidine derivatives are significantly more potent than compounds with tropane or granatane systems; only amide **6** and ester **30** (Y = *endo*-granatan-3-yl) display high 5-HT_3 affinity ($K_i = 6.1$ and 4.9 nM, respectively) and selectivity over 5-HT_4 receptors ($K_i >$ 1000 nM). In agreement with the literature studies,[40,43] compounds with *endo*-granatanes are more potent than their corresponding *exo*-analogues; e.g., **6** ($K_i = 6.1$ nM) exhibits 38-fold more affinity than **5** ($K_i = 230$ nM) and **30** ($K_i = 4.9$ nM) is 18-fold more potent than **29** ($K_i =$ 88.7 nM). The only exception is compound **12** ($K_i = 154$ nM), which displays slightly lower affinity than its *exo*-counterpart **11** ($K_i = 83.0$ nM). For all quinuclidine derivatives *S*-enantiomers are more potent than the corresponding *R*-isomers, but they display an affinity for the 5-HT_3 receptor similar to the racemic compounds. For example, racemic bromo derivative **16** and its *S*-enantiomer **17** are equipotent ($K_i = 0.29$ and 0.28 nM, respectively) and both exhibit higher affinity than the *R*-isomer **18** ($K_i = 3.4$ nM); the same trend is observed for unsubstituted compound **1** ($K_i = 3.7$ nM), its *S*-enantiomer **2** ($K_i = 2.6$ nM), and the *R*-isomer **3** ($K_i = 38.3$ nM).

Of special interest were the (*S*)-quinuclidin-3-yl derivatives **2** ($R^1 = R^2 = H$, X = NH), **8** ($R^1 = Cl$, $R^2 = H$, X = NH), and **14** ($R^1 = Cl$, $R^2 = NO_2$, X = NH), since they were found to be potent 5-HT_3 ligands ($K_i = 2.6$, 0.13, and 1.7 nM, respectively) devoid of affinity at 5-HT_4 and 5-HT_{1A} receptors ($K_i > 1000-10000$ nM). For this reason, they were selected for the evaluation of their 5-HT_3 receptor activity in the guinea pig ileum. All the tested compounds inhibited the concentrations induced by 10^{-6} to 10^{-5} M 5-HT without modification of the effect of low doses (5×10^{-8} to 5×10^{-7} M) of 5-HT. From these data it can be concluded that at the tested doses (10^{-9} to 10^{-8} M) **2**, **8**, and **14** selectively antagonize the effect of 5-HT mediated through 5-HT_3 receptors without significant differences vs reference compounds. Table 3 shows pA_2, determined following the equation described by Furchgott[42] using the appropriate concentration (5×10^{-9} M) of the antagonists, and ED_{50} values. When the effect of the tested compounds on the contractions mediated through 5-HT_4 receptors ([5-HT] < 10^{-6} M) was analyzed, no differences were found (Figure 2).

To discard effects involving other receptors present in these tissues, the effect of **2, 8**, and **14** (5×10^{-9} M) on the contractions induced by acetylcholine ($10^{-6}-10^{-5}$ M), substance P ($10^{-7}-5 \times 10^{-6}$ M), histamine ($10^{-6}-5 \times 10^{-6}$ M), and bradykinin ($5 \times 10^{-7}-5 \times 10^{-6}$ M) or by electrical stimulation (single square waves: 0.3 Hz, 2 ms and supramaximal voltage) was tested. None of the compounds induced significant modifications in the contractile responses, and did not display agonist activity in the guinea pig ileum when administered alone. These data confirm the selectivity of the effect observed on contractions mediated through 5-HT_3 receptors.

These biological results indicate that these compounds are new potent and selective 5-HT_3 receptor antagonists.

A number of pharmacophore models for antagonists binding to the 5-HT_3 receptor have been presented[44-47]

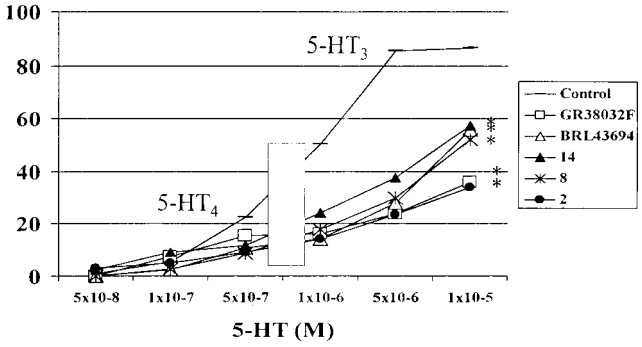


Figure 2. Each point shows the mean (the SEM are omitted for clarity) of the contraction induced by 5-HT in the second dose−response curve, expressed as the percentage of the maximal response reached in the first dose−response curve, in the absence (control) and in the presence of ondansetron or granisetron (5×10^{-9} M) as reference 5-HT_3 antagonists or of the same concentration of derivatives **2**, **8**, and **14** ($n \geq 6$). The asterisk (*) indicates the difference vs control value ($p < 0.05$, two ways ANOVA test); it refers to the second half of the graph.

and are in general agreement as to which pharmacophoric elements are important for significant binding affinity. In our search for new 5-HT_3 receptor antagonists, we recently reported a computer-aided conformational analysis[24] that has allowed us to confirm the generally accepted three-component pharmacophore for 5-HT_3 receptor antagonists. In this study, we also performed a receptor mapping of the 5-HT_3 binding site, which defines the essential volume occupied by this receptor. The synthesized benzimidazole-4-carboxylic acid derivatives represent a new structural class of potent and selective 5-HT_3 ligands that fit the 5-HT_3 receptor antagonist pharmacophore and the 5-HT_3 receptor essential volume. In these compounds, the 4-substituted benzimidazole ring represents a novel aromatic moiety where a hydrogen bond between a benzimidazole nitrogen atom and the hydrogen of the amide group, in the case of carboxamides, or between the oxygen of the ester group and the hydrogen of the benzimidazole ring, in the case of carboxylates, is likely to exist. On the other hand, a related derivative synthesized in our laboratory ((±)-*N*-(1-azabicyclo[2.2.2]-oct-3-yl)-1,3-dihydro-2*H*-benzimidazole-2-one-4-carboxamide) has been demonstrated as inactive at the 5-HT_3 receptor, despite fitting the 5-HT_3 antagonist pharmacophore and the 5-HT_3 receptor essential volume.[48] In this case, the inactivity could be due to the absence of a hydrogen bond in the molecule. This result supports the hypothesis that this hydrogen bond may block the acyl group in a pseudo aromatic cycle, and this is recognized as an important point for a better interaction of 5-HT_3 antagonists with the receptor.[16]

Conclusion

In the present paper, we have synthesized a new series of azabicyclic benzimidazole-4-carboxamides and -carboxylates with affinity and selectivity for serotoninergic 5-HT_3 receptors. The novel benzimidazoles are an enrichment to the SAR of the 5-HT_3 field. Three compounds of the series (**2**, **8**, and **14**) displayed very high affinity at central 5-HT_3 receptors, high selectivity over other serotonin receptors (5-HT_4 and 5-HT_{1A}), and potent 5-HT_3 antagonist activity in the guinea pig ileum, comparable to the 5-HT_3 receptor antagonists ondansetron and granisetron. Consequently, these new 5-HT_3 antagonists could be potential substrates to be developed in different pharmacological areas, since the 5-HT_3 antagonists have been reported to display a wide range of nonantiemetic activities (control of anxiety, psychosis, nociception, drug abuse and withdrawal, cognitive impairments, and anorectic responses). These therapeutic options are currently under investigation.

Experimental Section

Chemistry. Melting points (uncorrected) were determined on a Gallenkamp electrothermal apparatus. Infrared (IR) spectra were obtained on a Perkin-Elmer 781 infrared spectrophotometer; the frequencies are expressed in cm^{-1}. ^1H and ^{13}C NMR spectra were recorded on a Varian VXR-300S or Bruker 300-AM instrument at 300 and 75 MHz, respectively, or on a Bruker 250-AM spectrometer at 250 and 62.5 MHz, respectively. Chemical shifts (δ) are expressed in parts per million relative to internal tetramethylsilane, coupling constants (J) are in hertz (Hz). The following abbreviations are used to describe peak patterns when appropriate: s (singlet), d (doublet), t (triplet), q (quartet), qt (quintet), sx (sextet), m (multiplet), br (broad). Elemental analyses (C, H, N) were determined at the UCM's analysis services and were within ±0.4% of the theoretical values. Analytical thin-layer chromatography (TLC) was run on Merck silica gel plates (Kieselgel 60 F-254) with detection by UV light, iodine, acidic vanillin solution, or 10% phosphomolybdic acid solution in ethanol. For flash chromatography, Merck silica gel type 60 (size 230−400 mesh) was used. The enantiomeric purities of the (*R*)- and (*S*)-quinuclidine derivatives, determined by chiral HPLC, were confirmed to be more than 98% ee. Unless stated otherwise, all starting materials and reagents were high-grade commercial products purchased from Aldrich, Fluka, or Merck. All solvents were distilled prior to use. Dry DMF was obtained by stirring with CaH_2 followed by distillation under argon.

The following intermediates were synthesized according to literature procedures: benzimidazole-4-carboxylic acid (**31**),[49] 6-chlorobenzimidazole-4-carboxylic acid (**32**),[27] 2-amino-5-bromo-3-methylaniline (**36**),[50] 6-chloro-4-methylbenzimidazole (**37**),[27] *exo*-8-methyl-8-azabicyclo[3.2.1]oct-3-ylamine,[51] *exo*-9-methyl-9-azabicyclo[3.3.1]non-3-ylamine,[51] *endo*-9-methyl-9-azabicyclo[3.3.1]non-3-ylamine,[52] *exo*-8-methyl-8-azabicyclo-[3.2.1]octan-3-ol,[53] *exo*-9-methyl-9-azabicyclo[3.3.1]nonan-3-ol,[54] and *endo*-9-methyl-9-azabicyclo[3.3.1]nonan-3-ol.[55]

General Procedure for the Synthesis of Benzimidazole-4-carboxamides and Benzimidazole-4-carboxylates (2−14, 16−19, and 22−30). To a solution of acids **31**−**35** (5 mmol) in dry DMF (5 mL) under an argon atmosphere was added 1,1′-carbonyldiimidazole (CDI, 811 mg, 5 mmol). The mixture was stirred at 40 °C for 1 h, then a solution of the appropriate amine or alcohol (6−10 mmol) and 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU, 761 mg, 5 mmol) in DMF (6−10 mL) was added dropwise, and the reaction mixture was stirred at 50 °C for 20−24 h. The solvent was removed under reduced pressure, and the crude was taken up in $CHCl_3$ (50 mL) and washed with water (20 mL) and 20% aqueous K_2CO_3 (20 mL). The organic layer was dried over Na_2SO_4 or $MgSO_4$ and evaporated to afford the crude product, which was purified by column chromatography and recrystallization from the appropriate solvents.

(±)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)benzimidazole-4-carboxamide (1): yield 0.9 g (35%); chromatography $CHCl_3$/MeOH, from 9:1 to 7:3; mp 196−198 °C ($CHCl_3$/MeOH); ^1H NMR (Me_2SO-d_6) δ 1.39−1.46 (m, 1H, H5′b or H8′b), 1.58−1.63 (m, 2H, 2H5′ or 2H8′), 1.85−1.92 (m, 2H, H4′, H5′a or H8′a), 2.54−2.56 (m, 1H, H2′b), 2.70 (t, J = 7.8, 2H, 2H6′ or 2H7′), 2.75−2.83 (m, 2H, 2H6′ or 2H7′), 3.26 (dd, J = 13.5, 9.3, 1H, H2′a), 4.03−4.05 (m, 1H, H3′), 7.33 (t, J = 7.5, 1H, H6), 7.74 (dd, J = 7.8, 0.9, 1H, H7), 7.86 (dd, J = 7.5, 0.9, 1H, H5), 8.47 (s, 1H, H2), 10.13 (br s, 1H, CONH); ^{13}C NMR (Me_2SO-d_6) δ 20.2, 25.4 (C5′, C8′), 25.7 (C4′), 46.1, 46.9 (C6′, C7′),

46.3 (C3′), 56.1 (C2′), 116.0 (C7), 122.1 (C4), 122.2 (C5, C6), 134.3 (C7a), 139.6 (C3a), 143.0 (C2), 164.4 (CONH). Anal. ($C_{15}H_{18}N_4O$) C, H, N.

(*S*)-(−)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)benzimidazole-4-carboxamide (2): yield 1.1 g (80%); chromatography $CHCl_3$/MeOH, from 9.5:0.5 to 9:1; mp 234−236 °C (AcOEt); $[\alpha]^{25}_D$ = −25.6 (*c* = 1, MeOH).

(*R*)-(+)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)benzimidazole-4-carboxamide (3): yield 1.1 g (84%); chromatography $CHCl_3$/MeOH, from 9.5:0.5 to 9:1; mp 247−248 °C (water); $[\alpha]^{25}_D$ = +26.3 (*c* = 1, MeOH).

***N*-(*exo*-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl)benzimidazole-4-carboxamide (4):** yield 1.2 g (42%); chromatography $CHCl_3$/MeOH, 1:1; mp 233−235 °C ($CHCl_3$/Et_2O); ^{1}H NMR (Me_2SO-d_6) δ 1.57−1.64 (m, 4H, H2′ax, H4′ax, H6′b, H7′b), 1.75−1.80 (m, 2H, H2′eq, H4′eq), 1.94−1.97 (m, 2H, H6′a, H7′a), 2.18 (s, 3H, CH_3), 3.09 (br t, 2H, H1′, H5′), 4.13−4.26 (m, 1H, H3′), 7.30 (t, *J* = 7.8, 1H), 7.73 (d, *J* = 7.8, 1H), 7.82 (d, *J* = 7.8, 1H), 8.40 (s, 1H), 9.54 (d, *J* = 5.4, 1H); ^{13}C NMR (Me_2SO-d_6) δ 26.2 (C6′, C7′), 37.5 (C2′, C4′), 39.4 (CH_3), 40.6 (C3′), 60.2 (C1′, C5′), 116.6, 121.9, 122.0, 122.2, 135.4, 139.0, 143.1, 164.1. Anal. ($C_{16}H_{20}N_4O$) C, H, N.

***N*-(*exo*-9-Methyl-9-azabicyclo[3.3.1]non-3-yl)benzimidazole-4-carboxamide (5):** yield 1.9 g (64%); chromatography $CHCl_3$/MeOH, from 9:1 to 1:1; mp 203−205 °C (acetone); ^{1}H NMR (Me_2SO-d_6) δ 1.71−2.16 (m, 10H, 2H2′, 2H4′, 2H6′, 2H7′, 2H8′), 2.75 (s, 3H, CH_3), 3.32 (br t, 2H, H1′, H5′), 4.76−4.84 (m, 1H, H3′), 7.35 (t, *J* = 7.5, 1H), 7.79 (d, *J* = 7.6, 1H), 7.88 (d, *J* = 7.4, 1H), 8.50 (s, 1H), 9.81 (br s, 1H), 13.18 (br s, 1H); ^{13}C NMR (Me_2SO-d_6) δ 18.4 (C7′), 25.0 (C6′, C8′), 32.4 (C2′, C4′), 38.7 (C3′), 41.2 (CH_3), 53.7 (C1′, C5′), 115.0, 122.1, 122.2, 134.8, 139.1, 142.9, 164.1. Anal. ($C_{17}H_{22}N_4O$) C, H, N.

***N*-(*endo*-9-Methyl-9-azabicyclo[3.3.1]non-3-yl)benzimidazole-4-carboxamide (6):** yield 1.5 g (50%); chromatography $CHCl_3$/MeOH, 9:1; mp 240−242 °C (MeOH/AcOEt); ^{1}H NMR (Me_2SO-d_6) δ 0.95 (d, *J* = 11.7, 2H, H6′eq, H8′eq), 1.37 (t, *J* = 11.7, 2H, H2′b, H4′b), 1.46 (d, *J* = 11.1, 1H, H7′eq), 1.89−2.03 (m, 3H, H6′ax, H7′ax, H8′ax), 2.34−2.42 (m, 5H, CH_3, H2′a, H4′a), 3.01 (br d, *J* = 10.5, 2H, H1′, H5′), 4.34−4.45 (m, 1H, H3′), 7.34 (t, *J* = 7.5, 1H), 7.67 (d, *J* = 7.8, 1H), 7.88 (d, *J* = 7.5, 1H), 8.47 (s, 1H), 9.73 (br s, 1H), 12.80 (br s, 1H); ^{13}C NMR (Me_2SO-d_6) δ 13.9 (C7′), 24.0 (C6′, C8′), 33.0 (C2′, C4′), 39.4 (CH_3), 40.6 (C3′), 50.4 (C1′, C5′), 116.3, 121.6, 121.7, 121.9, 135.2, 138.8, 142.8, 163.9. Anal. ($C_{17}H_{22}N_4O$) C, H, N.

(±)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-6-chlorobenzimidazole-4-carboxamide (7): yield 1.2 g (39%); mp 264−266 °C (water); ^{1}H NMR (Me_2SO-d_6) δ 1.45−1.51 (m, 1H), 1.61−1.67 (m, 2H), 1.83−1.87 (m, 1H), 1.94 (sx, *J* = 3.0, 1H), 2.58 (dd, *J* = 13.8, 4.5, 1H), 2.74 (t, *J* = 7.8, 2H), 2.78−2.87 (m, 2H), 3.29 (dd, *J* = 13.5, 9.6, 1H), 4.06−4.10 (m, 1H), 7.84 (d, *J* = 2.1, 1H, H5), 7.86 (d, *J* = 2.1, 1H, H7), 8.53 (s, 1H, H2), 9.96 (d, *J* = 6.3, 1H, CONH); ^{13}C NMR (Me_2SO-d_6) δ 20.1, 25.3, 25.6, 46.0, 46.8, 46.5, 55.7, 116.0 (C7), 121.7 (C5), 122.7, 126.5 (C4, C6), 136.2, 137.8 (C3a, C7a), 144.5 (C_2), 163.2 (CONH). Anal. ($C_{15}H_{17}ClN_4O$) C, H, N.

(*S*)-(−)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-6-chlorobenzimidazole-4-carboxamide (8): yield 1.2 g (80%); chromatography $CHCl_3$/MeOH, from 9.5:0.5 to 9:1; mp 273−274 °C (d) (water); $[\alpha]^{25}_D$ = −27.7 (*c* = 1, MeOH).

(*R*)-(+)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-6-chlorobenzimidazole-4-carboxamide (9): yield 1.0 g (68%); chromatography $CHCl_3$/MeOH, from 9.5:0.5 to 9:1; mp 272−273 °C (d) (water); $[\alpha]^{25}_D$ = +26.9 (*c* = 1, MeOH).

***N*-(*exo*-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl)-6-chlorobenzimidazole-4-carboxamide (10):** yield 1.0 g (31%); chromatography $CHCl_3$/MeOH, from 9:1 to 8:2; mp 255−257 °C (water); ^{1}H NMR (Me_2SO-d_6) δ 1.60−1.67 (m, 4H), 1.75−1.81 (m, 2H), 1.97−2.00 (m, 2H), 2.22 (s, 3H), 3.13 (br t, 2H), 4.14−4.25 (m, 1H), 7.79 (s, 1H), 7.83 (s, 1H), 8.45 (s, 1H), 9.38 (d, *J* = 5.2, 1H); ^{13}C NMR (Me_2SO-d_6) δ 26.0, 37.0, 39.1, 40.7, 60.1, 116.0, 121.6, 122.2, 126.3, 136.4, 138.0, 144.5, 162.8. Anal. ($C_{16}H_{19}ClN_4O{\cdot}H_2O$) C, H, N.

***N*-(*exo*-9-Methyl-9-azabicyclo[3.3.1]non-3-yl)-6-chlorobenzimidazole-4-carboxamide (11):** yield 1.3 g (39%); chromatography $CHCl_3$/MeOH, from 7:3 to 3:7; mp 198−200 °C (d) (acetone); ^{1}H NMR (Me_2SO-d_6) δ 1.64−2.08 (m, 10H), 2.66 (s, 3H), 3.20 (br t, 2H), 4.73−4.77 (m, 1H), 7.79 (d, *J* = 1.5, 1H), 7.83 (d, *J* = 1.5, 1H), 8.44 (s, 1H), 9.50 (br s, 1H), 12.50 (br s, 1H); ^{13}C NMR (Me_2SO-d_6) δ 18.7, 25.0, 32.4, 39.1, 41.7, 53.5, 116.6, 122.2, 122.9, 126.9, 136.6, 137.9, 144.5, 163.0. Anal. ($C_{17}H_{21}ClN_4O$) C, H, N.

***N*-(*endo*-9-Methyl-9-azabicyclo[3.3.1]non-3-yl)-6-chlorobenzimidazole-4-carboxamide (12):** yield 0.9 g (27%); chromatography AcOEt/EtOH, from 9:1 to 2:8; mp >300 °C ($CHCl_3$); ^{1}H NMR (Me_2SO-d_6) δ 0.96 (d, *J* = 12.9, 2H), 1.36 (t, *J* = 12.6, 2H), 1.47 (dm, *J* = 10.9, 1H), 1.90−2.00 (m, 3H), 2.31−2.40 (m, 2H), 2.42 (s, 3H), 3.01 (br d, *J* = 9.9, 2H), 4.29−4.41 (m, 1H), 7.74 (d, *J* = 0.9, 1H), 7.77 (d, *J* = 0.9, 1H), 8.35 (s, 1H), 9.62 (br s, 1H); ^{13}C NMR (Me_2SO-d_6) δ 14.2, 23.2, 33.0, 39.6, 40.8, 50.6, 116.6, 122.2, 122.9, 126.9, 136.6, 137.9, 144.7, 163.1. Anal. ($C_{17}H_{21}ClN_4O$) C, H, N.

(±)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-6-chloro-7-nitrobenzimidazole-4-carboxamide (13): yield 0.8 g (46%); chromatography $CHCl_3$/MeOH, 9:1; mp 265−266 °C (AcOEt); ^{1}H NMR (Me_2SO-d_6) δ 1.69−1.74 (m, 1H), 1.79−1.83 (m, 2H), 2.04−2.06 (m, 1H), 2.13 (sx, *J* = 2.7, 1H), 2.94 (dd, *J* = 13.8, 4.2, 1H), 3.03 (t, *J* = 7.8, 2H), 3.10−3.15 (m, 2H), 3.55 (dd, *J* = 13.2, 9.0, 1H), 4.23−4.25 (m, 1H), 7.80 (s, 1H, H5), 8.29 (s, 1H, H2), 10.33 (d, *J* = 6.3, 1H, CONH); ^{13}C NMR (Me_2SO-d_6) δ 18.8, 23.4, 24.9, 45.5, 45.7, 46.3, 54.0, 114.5 (C6), 119.9 (C5), 122.5 (C4), 137.0, 138.2, 142.0 (C3a, C7, C7a), 152.3 (C2), 163.6 (CONH). Anal. ($C_{15}H_{16}ClN_5O_3$) C, H, N.

(*S*)-(−)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-6-chloro-7-nitrobenzimidazole-4-carboxamide (14): yield 1.4 g (79%); chromatography $CHCl_3$/MeOH, from 9.5:0.5 to 9:1; mp 187−189 °C ($CHCl_3$/Et_2O); $[\alpha]^{25}_D$ = −15.3 (*c* = 1, MeOH).

(±)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-6-bromobenzimidazole-4-carboxamide (16): yield 1.4 g (80%); chromatography $CHCl_3$/MeOH, from 9.5:0.5 to 9:1; mp 259−260 °C ($CHCl_3$/Et_2O); ^{1}H NMR (Me_2SO-d_6) δ 1.45−1.51 (m, 1H), 1.61−1.65 (m, 2H), 1.84−1.86 (m, 1H), 1.92 (sx, *J* = 2.7, 1H), 2.53−2.58 (m, 1H), 2.72 (t, *J* = 7.5, 2H), 2.77−2.84 (m, 2H), 3.27 (dd, *J* = 13.8, 9.6, 1H), 4.02−4.06 (m, 1H), 7.93 (d, *J* = 1.5, 1H, H5), 7.98 (d, *J* = 1.8, 1H, H7), 8.49 (s, 1H, H2), 9.93 (br s, 1H, CONH); ^{13}C NMR (Me_2SO-d_6) δ 20.2, 25.7, 25.4, 46.1, 46.9, 46.6, 55.7, 114.2 (C6), 119.0 (C7), 123.0 (C4), 124.4 (C5), 136.9, 138.2 (C3a, C7a), 144.4 (C2), 163.2 (CONH). Anal. ($C_{15}H_{17}BrN_4O$) C, H, N.

(*S*)-(−)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-6-bromobenzimidazole-4-carboxamide (17): yield 1.4 g (80%); chromatography $CHCl_3$/MeOH, from 9.5:0.5 to 9:1; mp 292−293 °C (d) (MeOH); $[\alpha]^{25}_D$ = −24.9 (*c* = 1, MeOH).

(*R*)-(+)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-6-bromobenzimidazole-4-carboxamide (18): yield 1.4 g (81%); chromatography $CHCl_3$/MeOH, from 9.5:0.5 to 9:1; mp 259−260 °C (d) ($CHCl_3$); $[\alpha]^{25}_D$ = +24.2 (*c* = 1, MeOH).

(±)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-6-bromo-7-nitrobenzimidazole-4-carboxamide (19): yield 1.6 g (82%); chromatography $CHCl_3$/MeOH, from 9.5:0.5 to 9:1; mp 196−197 °C (AcOEt); ^{1}H NMR (Me_2SO-d_6) δ 1.69−1.74 (m, 1H), 1.81−1.87 (m, 2H), 2.06−2.09 (m, 1H), 2.16 (sx, *J* = 3.0, 1H), 2.96 (dd, *J* = 13.5, 4.5, 1H), 3.05 (t, *J* = 7.8, 2H), 3.11−3.18 (m, 2H), 3.57 (ddd, *J* = 13.2, 9.6, 2.1, 1H), 4.24−4.26 (m, 1H), 7.96 (s, 1H, H5), 8.28 (s, 1H, H2), 10.29 (br s, 1H, CONH); ^{13}C NMR (Me_2SO-d_6) δ 18.8, 23.4, 24.9, 45.5, 45.6, 46.2, 54.0, 101.4 (C6), 122.5 (C4, C5), 137.5, 140.2, 142.5 (C3a, C7, C7a), 152.3 (C2), 163.5 (CONH). Anal. ($C_{15}H_{16}BrN_5O_3$) C, H, N.

(±)-(1-Azabicyclo[2.2.2]oct-3-yl) benzimidazole-4-carboxylate (22): yield 2.1 g (77%); mp 210−212 °C ($CHCl_3$/Et_2O); ^{1}H NMR ($CDCl_3$) δ 1.48−2.08 (m, 4H, 2H5′, 2H8′), 2.21 (sx, *J* = 3.3, 1H, H4′), 2.79−3.04 (m, 5H, H2′b, 2H6′, 2H7′), 3.42 (ddd, *J* = 14.7, 8.4, 1.5, 1H, H2′a), 5.10−5.12 (m, 1H, H3′), 7.35 (t, *J* = 7.8, 1H, H6), 7.97 (dd, *J* = 7.5, 0.9, 1H, H5 or H7), 8.06 (dd, *J* = 7.8, 0.9, 1H, H7 or H5), 8.18 (s, 1H, H2), 11.20 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 19.8, 24.6 (C5′, C8′), 25.4 (C4′), 45.6, 47.4 (C6′, C7′), 55.6 (C2′), 72.3 (C3′), 114.1 (C4),

121.7 (C6), 124.9, 125.5 (C5, C7), 133.5 (C3a), 141.8 (C2), 143.7 (C7a), 166.2 (COO). Anal. ($C_{15}H_{17}N_3O_2$) C, H, N.

(*exo*-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl) benzimidazole-4-carboxylate (23): yield 0.7 g (28%); chromatography $CHCl_3$/MeOH, 1:1; mp 164−166 °C ($CHCl_3$/Et_2O); ^{1}H NMR ($CDCl_3$) δ 1.65−1.70 (m, 2H), 1.90−1.96 (m, 4H), 2.01−2.06 (m, 2H), 2.29 (s, 3H), 3.22 (br t, J = 3.3, 2H), 5.27 (tt, J = 10.5, 6.5, 1H), 7.25 (t, J = 7.8, 1H), 7.85 (dd, J = 7.5, 0.9, 1H), 7.95 (dd, J = 8.1, 0.9, 1H), 8.07 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 26.3, 36.0, 38.8, 60.2, 68.0, 114.0, 121.6, 125.1, 125.2, 132.9, 141.4, 143.4, 165.7. Anal. ($C_{16}H_{19}N_3O_2$) C, H, N.

(*endo*-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl) benzimidazole-4-carboxylate (24): yield 1.6 g (56%); mp 205−206 °C ($CHCl_3$/Et_2O); ^{1}H NMR ($CDCl_3$) δ 1.86 (d, J = 14.1, 2H, H2′eq, H4′eq), 2.10 (m, 4H, 2H6′, 2H7′), 2.24 (dt, J = 14.7, 4.2, 2H, H2′ax, H4′ax), 2.30 (s, 3H, CH_3), 3.16 (br t, J = 3.0, 2H, H1′, H5′), 5.31 (t, J = 5.1, 1H, H3′), 7.33 (t, J = 8.1, 1H), 7.86 (d, J = 7.5, 1H), 8.03 (d, J = 8.1, 1H), 8.15 (s, 1H), 10.90 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 25.9 (C6′, C7′), 37.0 (C2′, C4′), 40.7 (CH_3), 59.9 (C1′, C5′), 68.6 (C3′), 114.6, 121.9, 124.6, 125.5, 133.7, 141.8, 143.8, 166.1. Anal. ($C_{16}H_{19}N_3O_2$) C, H, N.

***(exo*-9-Methyl-9-azabicyclo[3.3.1]non-3-yl) benzimidazole-4-carboxylate (25):** yield 1.2 g (40%); chromatography $CHCl_3$/MeOH, 1:1; mp 195−197 °C (acetone/Et_2O); ^{1}H NMR ($CDCl_3$) δ 1.52 (d, J = 13.5, 2H), 1.69−1.77 (m, 2H), 1.96−2.10 (m, 4H), 2.26 (td, J = 10.5, 6.0, 2H), 2.59 (s, 3H), 3.08 (br t, J = 4.5, 2H), 5.83 (tt, J = 10.5, 6.6, 1H), 7.30 (t, J = 8.1, 1H), 7.92 (dd, J = 8.1, 0.9, 1H), 8.00 (dd, J = 7.8, 1.2, 1H), 8.13 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 19.4, 26.3, 31.7, 40.2, 53.5, 68.8, 114.2, 121.5, 125.0, 125.2, 133.1, 141.6, 143.5, 165.7. Anal. ($C_{17}H_{21}N_3O_2$) C, H, N.

(*endo*-9-Methyl-9-azabicyclo[3.3.1]non-3-yl) benzimidazole-4-carboxylate (26): yield 1.2 g (40%); chromatography $CHCl_3$/MeOH, 9:1; mp 198−200 °C ($CHCl_3$/Et_2O); ^{1}H NMR ($CDCl_3$) δ 1.25 (dd, J = 13.5, 4.2, 2H), 1.55 (dt, J = 13.8, 5.1, 1H), 1.66 (ddd, J = 14.7, 6.0, 1.8, 2H), 2.01 (tt, J = 13.5, 5.1, 2H), 2.30 (qt, J = 13.8, 5.1, 1H), 2.52 (s, 3H), 2.59 (dd, J = 14.7, 6.6, 2H), 3.04 (br d, J = 8.4, 2H), 5.49 (tt, J = 6.9, 6.0, 1H), 7.33 (t, J = 8.1, 1H), 7.94 (d, J = 7.5, 1H), 8.04 (d, J = 8.1, 1H), 8.22 (s, 1H), 11.10 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.4, 24.7, 31.0, 40.3, 51.9, 67.7, 114.3, 121.5, 124.6, 125.2, 133.4, 141.7, 143.6, 163.7. Anal. ($C_{17}H_{21}N_3O_2$) C, H, N.

(±)-(1-Azabicyclo[2.2.2]oct-3-yl) 6-chlorobenzimidazole-4-carboxylate (27): yield 1.2 g (39%); chromatography $CHCl_3$/MeOH, 9:1; mp 201−203 °C (Et_2O/hexane); ^{1}H NMR (Me_2SO-d_6) δ 1.39−1.93 (m, 4H), 2.15 (sx, J = 3.0, 1H), 2.69−2.97 (m, 5H), 3.26 (ddd, J = 14.7, 8.4, 1.5, 1H), 5.03−5.07 (m, 1H), 7.83 (d, J = 2.1, 1H, H7 or H5), 8.08 (d, J = 2.1, 1H, H7 or H5), 8.41 (s, 1H, H2), 12.75 (br s, 1H, NH); ^{13}C NMR (Me_2SO-d_6) δ 19.3, 24.1, 25.0, 45.9, 46.7, 54.7, 72.7, 115.2 (C4), 123.4 (C5, C7), 125.3 (C6), 132.0 (C3a), 144.2 (C7a), 145.6 (C2), 163.9 (COO). Anal. ($C_{15}H_{16}ClN_3O_2$) C, H, N.

(*endo*-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl) 6-chlorobenzimidazole-4-carboxylate (28): yield 0.8 g (25%); chromatography $CHCl_3$/MeOH, 9:1; mp 248−250 °C ($CHCl_3$/Et_2O); ^{1}H NMR (Me_2SO-d_6) δ 2.01 (d, J = 15.3, 2H), 2.15 (m, 4H), 2.39 (m, 2H), 2.46 (s, 3H), 3.48 (br t, J = 3.1, 2H), 5.24 (br t, J = 5.1, 1H), 7.73 (s, 1H), 8.08 (s, 1H), 8.39 (s, 1H), 12.90 (br s, 1H); ^{13}C NMR (Me_2SO-d_6) δ 24.7, 34.6, 38.7, 59.8, 67.4, 114.8, 123.0, 125.3, 132.0, 144.2, 145.6, 163.7. Anal. ($C_{16}H_{18}ClN_3O_2$) C, H, N.

***(exo*-9-Methyl-9-azabicyclo[3.3.1]non-3-yl) 6-chlorobenzimidazole-4-carboxylate (29):** yield 1.3 g (39%); chromatography $CHCl_3$/MeOH, 9:1; mp 195−197 °C ($CHCl_3$/MeOH); ^{1}H NMR (MeSO-d_6) δ 1.49 (d, J = 13.5, 2H), 1.64−1.70 (m, 2H), 1.84−1.95 (m, 4H), 2.15 (td, J = 11.1, 6.0, 2H), 2.47 (s, 3H), 2.95 (br t, J = 3.5, 2H), 5.81 (tt, J = 10.1, 6.0, 1H), 7.79 (d, J = 1.5, 1H), 8.06 (d, J = 1.5, 1H), 8.38 (s, 1H), 12.62 (br s, 1H); ^{13}C NMR (MeSO-d_6) δ 19.9, 26.8, 30.6, 40.2, 53.1, 70.0, 115.4, 123.5, 125.3, 131.9, 144.1, 145.5, 163.7. Anal. ($C_{17}H_{20}ClN_3O_2$) C, H, N.

(*endo*-9-Methyl-9-azabicyclo[3.3.1]non-3-yl) 6-chlorobenzimidazole-4-carboxylate (30): yield 0.8 g (24%); chromatography $CHCl_3$/MeOH, 9:1; mp 240−242 °C (acetone/Et_2O); ^{1}H NMR (Me_2SO-d_6) δ 1.15 (d, J = 12.6, 2H), 1.48 (dm, J = 13.5, 1H), 1.70 (ddd, J = 13.8, 7.5, 1.8, 2H), 1.92 (tt, J = 13.2, 4.5, 2H), 2.23 (qt, J = 13.2, 5.1, 1H), 2.41 (s, 3H), 2.49 (dd, J = 14.1, 5.1, 2H), 3.00 (br d, J = 8.7, 2H), 5.38 (tt, J = 6.9, 6.0, 1H), 7.79 (d, J = 2.1, 1H), 8.06 (d, J = 1.5, 1H), 8.40 (s, 1H), 12.66 (br s, 1H); ^{13}C NMR (Me_2SO-d_6) δ 14.2, 24.2, 30.8, 40.3, 50.9, 68.2, 115.3, 123.6, 123.9, 125.4, 131.5, 144.8, 145.6, 163.7. Anal. ($C_{17}H_{20}ClN_3O_2$) C, H, N.

(±)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-7-amino-6-chlorobenzimidazole-4-carboxamide (15). A solution of **13** (1.0 g, 3 mmol) in ethanol (150 mL) was hydrogenated over Raney nickel at 50 psi and room temperature for 2 h (TLC). The catalyst was removed by filtration over Celite, and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography ($CHCl_3$/MeOH/NH_3, 8:1.5:0.5) to yield 0.25 g (26%) of **15**; mp 273−274 °C (MeOH/Et_2O); ^{1}H NMR (Me_2SO-d_6) δ 1.41−1.51 (m, 1H), 1.61−1.70 (m, 2H), 1.82−1.88 (m, 1H), 1.91−1.93 (m, 1H), 2.71−2.93 (m, 5H), 3.41 (m, 1H), 4.05 (m, 1H), 6.11 (s, 2H, NH_2), 7.76 (br s, 1H, H5), 8.41 (br s, 1H, H2), 9.90 (br s, 1H, CONH), 12.37 (br s, 1H, NH); ^{13}C NMR (Me_2SO-d_6) δ 20.1, 25.4, 25.8, 46.3, 46.2, 46.9, 55.3, 108.1 (C4, C6), 124.0 (C5), 141.6 (C2), 164.3 (CONH). Anal. ($C_{15}H_{18}ClN_5O$) C, H, N.

(±)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-7-amino-6-bromobenzimidazole-4-carboxamide (20). To a solution of **19** (0.6 g, 1.5 mmol) in ethanol (46 mL) warmed at 60 °C were added Raney nickel and 80% hydrazine hydrate (0.12 mL, 2 mmol). The reaction mixture was refluxed for 15 min (TLC) and filtered through Celite. The solvent was removed under reduced pressure, and the residue was purified by column chromatography ($CHCl_3$/MeOH/NH_3, 8:2:0.2) to yield 0.3 g (56%) of **20**: mp 278−279 °C (toluene); ^{1}H NMR (Me_2SO-d_6) δ 1.40−1.51 (m, 1H), 1.61−1.65 (m, 2H), 1.83−1.87 (m, 1H), 1.90−1.92 (m, 1H), 2.58−2.64 (m, 1H), 2.72−2.75 (m, 2H), 2.82−2.87 (m, 2H), 3.21−3.28 (m, 1H), 4.02 (m, 1H), 6.04 (s, 2H, NH_2), 7.92 (s, 1H, H5), 8.28 (br s, 1H, H2), 9.78 (br s, 1H, CONH); ^{13}C NMR (Me_2SO-d_6) δ 20.1, 25.4, 25.8, 46.2, 46.9, 46.4, 55.4, 97.4, 110.4 (C4), 126.8 (C5, C7a), 137.2 (C3a, C7), 141.5 (C2), 164.3 (CONH). Anal. ($C_{15}H_{18}BrN_5O$) C, H, N.

(±)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-7-aminobenzimidazole-4-carboxamide (21). A solution of **19** (0.6 g, 1.5 mmol) in ethanol (46 mL) was warmed to 60 °C. Then Pd(C) (186 mg, 1 mmol) and 80% hydrazine hydrate (0.18 mL, 3 mmol) were added, and the mixture was refluxed for 30 min (TLC). The reaction was treated as in **15** to yield 0.35 g (81%) of **21**: mp 200−201 °C (MeOH/Et_2O); ^{1}H NMR (Me_2SO-d_6) δ 1.80−2.02 (m, 3H), 2.12−2.17 (m, 1H), 2.24−2.27 (m, 1H), 3.09−3.50 (m, 5H), 3.76 (m, 1H), 4.37 (m, 1H), 6.11 (s, 2H, NH_2), 6.48 (s, 1H, H6), 7.71 (s, 1H, H5), 8.38 (br s, 1H, H2), 10.10 (br s, 1H, CONH), 12.85 (br s, 1H, NH); ^{13}C NMR (Me_2SO-d_6) δ 17.4, 21.5, 24.5, 43.9, 45.0, 45.5, 52.9, 104.5 (C6), 124.8 (C5), 140.4 (C2), 165.9 (CONH). Anal. ($C_{15}H_{19}N_5O$) C, H, N.

Radioligand Binding Assays. For all receptor binding assays, male Sprague−Dawley rats (*Rattus norvegicus albinus*), weighing 180−200 g, were killed by decapitation and the brains rapidly removed and dissected. Tissues were stored at −80 °C for subsequent use and homogenized on a Polytron PT-10 homogenizer. Membrane suspensions were centrifuged on a Beckman J2-HS instrument.

1. 5-HT$_{1A}$ Receptor. Binding assays were performed by a modification of the procedure previously described by Clark et al.[32] The cerebral cortex was homogenized in 10 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25 °C) and centrifuged at 28000*g* for 15 min. The membrane pellet was washed twice by resuspension and centrifugation. After the third wash the resuspended pellet was incubated at 37 °C for 10 min. Membranes were then collected by centrifugation, and the final pellet was resuspended in 50 mM Tris-HCl, 5 mM $MgSO_4$, and 0.5 mM EDTA buffer (pH 7.4 at 25 °C). Fractions of the final membrane suspension (about 1 mg of protein) were incubated at 37 °C for 15 min with 0.6 nM [^{3}H]-8-OH-DPAT (8-hydroxy-2-(dipropylamino)tetralin) (133 Ci/mmol), in the presence or absence of the competing drug (1 μM), in a final volume of 1.1 mL of assay buffer (50 mM Tris-HCl, 10 nM

clonidine, and 30 nM prazosin, pH 7.4 at 25 °C). Nonspecific binding was determined with 10 μM 5-HT and represented less than 15% of the total binding.

2. 5-HT$_3$ Receptor. Binding assays were performed according to the procedure previously described by Wong et al.[29] The cerebral cortex was homogenized in 9 volumes of 0.32 M sucrose and centrifuged at 1000*g* for 10 min. The supernatant was centrifuged at 17000*g* for 20 min. The membrane pellet was washed twice by resuspension in 60 volumes of 50 mM Tris-HCl buffer (pH 7.4 at 25 °C) and was centrifuged at 48000*g* for 10 min. After the second wash the resuspended pellet was incubated at 37 °C for 10 min and centrifuged at 48000*g* for 10 min. Membranes were resuspended in 2.75 volumes of assay buffer (50 mM Tris-HCl, 10 μM pargyline, 0.6 mM ascorbic acid, and 5 mM $CaCl_2$, pH 7.4 at 25 °C). Fractions of 100 μL of the final membrane suspension (about 2 mg/mL of protein) were incubated at 25 °C for 30 min with 0.7 nM [^{3}H]LY 278584 (83 Ci/mmol), in the presence or absence of six concentrations of the competing drug, in a final volume of 2 mL of assay buffer. Nonspecific binding was determined with 10 μM 5-HT and represented less than 25% of the total binding.

3. 5-HT$_4$ Receptor. Binding assays were performed according to the procedure previously described by Grossman et al.[30] The striatum was homogenized in 15 volumes of ice-cold 50 mM HEPES buffer (pH 7.4 at 4 °C) and centrifuged at 48000*g* for 10 min. The pellet was resuspended in 4.5 mL of assay buffer (50 mM HEPES, pH 7.4 at 4 °C). Fractions of 100 μL of the final membrane suspension were incubated at 37 °C for 30 min with 0.1 nM [^{3}H]GR 113808 (85 Ci/mmol), in the presence or absence of six concentrations of the competing drug, in a final volume of 1 mL of assay buffer. Nonspecific binding was determined with 30 μM 5-HT and represented less than 35% of the total binding.

For all binding assays, competing drug, nonspecific, total, and radioligand bindings were defined in triplicate. Incubation was terminated by rapid vacuum filtration through Whatman GF/B filters, presoaked in 0.05% poly(ethylenimine), using a Brandel cell harvester. The filters were then washed (twice with 4 mL of ice-cold 50 mM Tris-HCl, pH 7.4 at 25 °C, for 5-HT$_{1A}$ and 5-HT$_3$ receptor binding assays, and once with 4 mL of ice-cold 50 mM HEPES, pH 7.4 at 4 °C, for 5-HT$_4$ receptor binding assays) and dried. The filters were placed in poly(ethylene) vials to which were added 4 mL of a scintillation cocktail (Aquasol), and the radioactivity bound to the filters was measured by liquid scintillation spectrometry. The data were analyzed by an iterative curve-fitting procedure (program Prism, Graph Pad), which provided IC_{50}, K_i, and r^2 values for test compounds, K_i values being calculated from the Cheng and Prusoff equation.[31] The protein concentrations of the rat cerebral cortex and the rat striatum were determined by the method of Lowry,[56] using bovine serum albumin as the standard.

5-HT$_4$ Receptor Activity in the Isolated Guinea Pig Ileum. Tissues were obtained from adult guinea pigs (300−400 g). Portions of ileum were dissected 1.5 cm proximal to the ileocecal junction and myenteric plexus-longitudinal muscle strips were obtained from guinea pig ileum, as previously described by Buchheit et al.,[33] and mounted in an organ bath containing Krebs solution at 37 °C and gassed with 5% CO_2/95% O_2. Methysergide was added at a concentration of 10^{-6} M as described by Eglen et al.,[34] in order to exclude any potential effects on 5-HT$_1$ or 5-HT$_2$ receptors. Ileal responses were measured by determining changes in isometric tension recorded with force transducers connected to an Omniscribe polygraph.

Two 5-HT (5×10^{-8} to 10^{-5} M) concentration−response curves were constructed, in a noncumulative manner, in each tissue. Compounds **2**, **8**, and **14**, ondansetron, granisetron, or saline solution (control tissues) were added 30 min before the second curves were carried out. Contractions were evoked every 5 min by addition of the separate doses of 5-HT, and the bathing solution was replaced before addition of the antagonists. The effect of 5-HT has been expressed as a percentage of the maximal contraction reached during the first curve. Then, the ED_{50} of 5-HT, in the presence and absence of the antagonists, and pA_2 values were calculated for the second phase of the curve ([5-HT] $\geq 10^{-6}$ M).

The effect of **2**, **8**, and **14** (5×10^{-9} M) on the contractions induced by acetylcholine (10^{-6}−10^{-5} M), substance P (10^{-7}−5×10^{-6} M), histamine (10^{-6}−5×10^{-6} M), and bradykinin (5×10^{-7}−5×10^{-6} M) or by electrical stimulation (single square waves: 0.3 Hz, 2 ms, and supramaximal voltage) was also tested.

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Supporting Information Available: Synthetic procedures and characterization data for new intermediate compounds **33**−**35** and **38**−**40**. This material is available free of charge via the Internet at http://pubs.acs.org.

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