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Benzimidazole Derivatives. Part 1: Synthesis and Structure–Activity Relationships of New Benzimidazole-4-carboxamides and Carboxylates as Potent and Selective 5-HT_4 Receptor Antagonists

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Abstract—New benzimidazole-4-carboxamides **1**–**16** and -carboxylates **17**–**26** were synthesized and evaluated for binding affinity at serotonergic 5-HT_4 and 5-HT_3 receptors in the CNS. Most of the synthesized compounds exhibited moderate-to-very high affinity (in many cases subnanomolar) for the 5-HT_4 binding site and no significant affinity for the 5-HT_3 receptor. SAR observations and structural analyses (molecular modeling, INSIGHT II) indicated that the presence of a voluminous substituent in the basic nitrogen atom of the amino moiety and a distance of ca. 8.0 Å from this nitrogen to the aromatic ring are of great importance for high affinity and selectivity for 5-HT_4 receptors. These results confirm our recently proposed model for recognition by the 5-HT_4 binding site. Amides **12**–**15** and esters **24** and **25** bound at central 5-HT_4 sites with very high affinity (K_i = 0.11–2.9 nM) and excellent selectivity over serotonin 5-HT_3, 5-HT_{2A}, and 5-HT_{1A} receptors (K_i > 1000–10,000 nM). Analogues **12** (K_i(5-HT_4) = 0.32 nM), **13** (K_i(5-HT_4) = 0.11 nM), **14** (K_i(5-HT_4) = 0.29 nM) and **15** (K_i(5-HT_4) = 0.54 nM) were pharmacologically characterized as selective 5-HT_4 antagonists in the isolated guinea pig ileum (pA_2 = 7.6, 7.9, 8.2 and 7.9, respectively), with a potency comparable to the 5-HT_4 receptor antagonist RS 39604 (pA_2 = 8.2). The benzimidazole-4-carboxylic acid derivatives described in this paper represent a novel class of potent and selective 5-HT_4 receptor antagonists. In particular, compounds **12**–**15** could be interesting pharmacological tools for the understanding of the role of 5-HT_4 receptors.

Introduction

Since the discovery of the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) in 1948 the knowledge about its role in (patho)physiological processes, both peripherally and centrally, is steadily growing.[1–7] Seven 5-HT receptor classes (5-$HT_{1–7}$) including 15 different subtypes have been identified in recent years.[8–12] There is considerable interest in the 5-HT_4 receptor[13,14] which has been recently cloned,[15,16] showing that it is a member of the G-protein-coupled receptor family and confirming that it is linked positively to adenylyl cyclase in the central nervous system (CNS).[17] The presence of 5-HT_4 receptors which are supposed to facilitate release of acetylcholine has been shown in the CNS, and thus 5-HT_4 receptor agonists may have a role in improving cognitive function.[18] To date, considerable progress has been made in the localization of 5-HT_4 receptors and they have also been found in several animal and human peripheral tissues, such as the gastrointestinal tract,[19–21] the heart,[22] and the bladder,[23] where they mediate a variety of pharmacological responses. Thus, in addition to the clinical use of 5-HT_4 receptor agonists—cisapride and renzapride—as prokinetic agents in the treatment of gastrointestinal motility disorders,[24] a number of potential therapeutic indications for antagonists of this receptor are currently under investigation, including IBS (irritable bowel syndrome),[25] arrhythmias[26] and micturition disturbances.[27,28] In many cases, the clinical

Key words: Serotonin; 5-HT_4 antagonist; benzimidazole; pharmacophore.
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use of currently available drugs acting at the 5-HT_4 receptor has been hampered by their lack of selectivity, and significant effort has been made towards the development of potent and specific 5-HT_4 receptor antagonists,[29] such as SDZ 205-557, GR 113808, SB 204070, and RS 39604.

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 serotonergic binding sites.[30] This computer-aided conformational analysis has allowed us to propose a steric model for 5-HT_4 receptor recognition, which offers structural insights to aid the design of novel selective 5-HT_4 antagonists. This model consists of an aromatic moiety, a coplanar carbonyl group with the oxygen situated at ca. 3.6 Å from the centroid of the aromatic ring, a nitrogen atom situated at ca. 8.0 Å from this centroid and ca. 5.4 Å from the oxygen of the carbonyl group, and a voluminous substituent in the basic amino framework of the molecule (Fig. 1). On the basis of these results, in this paper we have designed a new series of benzimidazole-4-carboxylic acid derivatives and analyzed the influence of some structural variations of the different pharmacophore elements on the affinity for the 5-HT_4 receptor. Herein, we describe the synthesis of **1**–**26**, the affinity for 5-HT_4 receptors and the selectivity over other serotonergic receptors (5-HT_3, 5-HT_{2A}, and 5-HT_{1A}), obtained by radioligand binding assays. Four analogues with interesting 5-HT_4 binding properties were also evaluated for 5-HT_4 antagonist activity, by analyzing their ability to inhibit the contractions induced by 5-HT in the isolated guinea pig ileum.

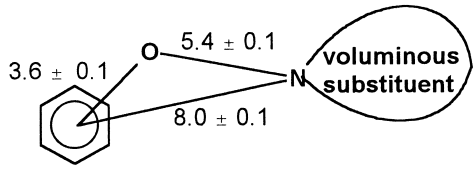
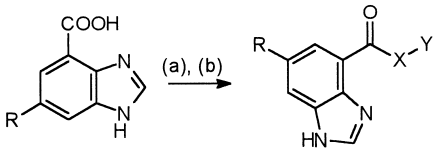


Figure 1. Proposed pharmacophore model for 5-HT_4 receptor antagonists.

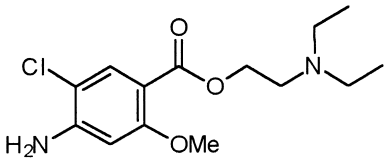


Scheme 1. Reagents and conditions: (a) CDI, DMF, 40°C, 1 h; (b) Y-NH_2 or Y-OH, DBU, DMF, 50°C, 20–24 h.

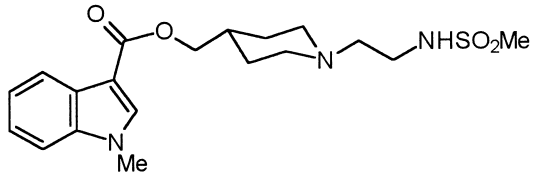
Chemistry

Target amides and esters **1**–**26** were synthesized as described in Scheme 1, by activation of benzimidazole-4-carboxylic acid (**27**) or 6-chlorobenzimidazole-4-carboxylic acid (**28**) with 1,1′-carbonyldiimidazole (CDI), and subsequent coupling of the imidazolides with the appropriate dialkylaminoalkyl or piperidine amines and alcohols in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in *N*,*N*-dimethylformamide (DMF) solutions. Acid **28** was obtained by condensation of 2-amino-5-chloro-3-methylaniline (**29**) with formic acid and subsequent oxidation of 6-chloro-4-methylbenzimidazole (**30**) with potassium permanganate (Scheme 2). The (1-alkyl-4-piperidyl)methylamines **31**–**34** were prepared from 4-piperidylmethylamine, by reaction with the appropriate halide (1-bromobutane, 1-bromo-2-methylpropane, 1-chloropentane or *N*-(2-chloroethyl)methanesulfonamide) in dry acetonitrile (Scheme 3). In all cases, small amounts of the dialkylated compound and starting material were observed in the reaction crudes. The rest of the amines and alcohols were commercially available or prepared by previously described methods.

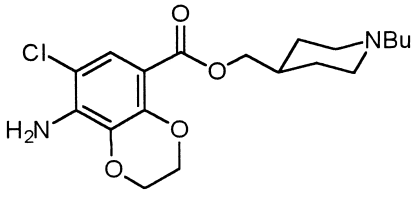
All new compounds (Table 1) were characterized by IR and ^1H and ^{13}C NMR spectroscopy, and gave satisfactory combustion analyses (C, H, N). COSY and HETCOR experiments of several compounds were carried



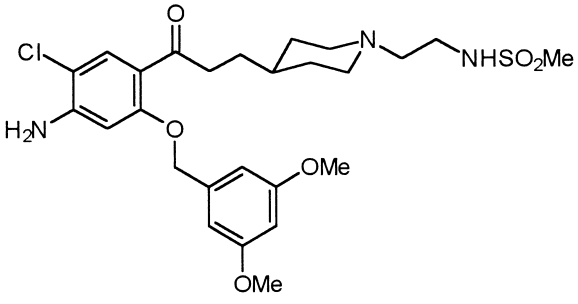
SDZ 205-557



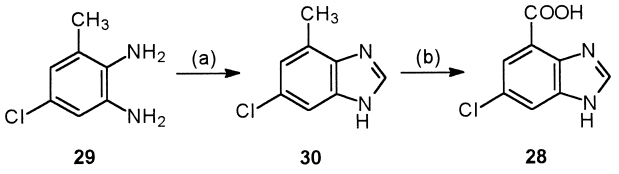
GR 113808



SB 204070

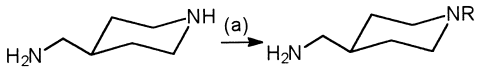


RS 39604



29 30 28

Scheme 2. Reagents and conditions: (a) HCOOH, H_2O, 100°C, 6 h; (b) $KMnO_4$, NaOH 0.5 N, 100°C, 5 h.



31: R=Bu
32: R=Bu^i
33: R=Pent
34: R=$(CH_2)_2NHSO_2Me$

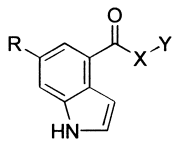
Scheme 3. Reagents and conditions: (a) RX (BuBr, *i*-BuBr, $CH_3(CH_2)_4Cl$, $Cl(CH_2)_2NHSO_2Me$), CH_3CN, 0°C to rt, overnight.

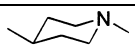
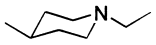
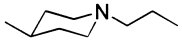
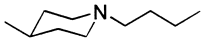
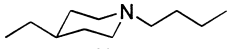
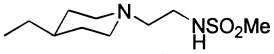
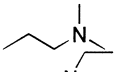
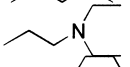
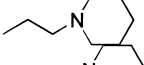
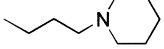
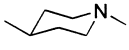
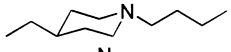
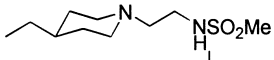
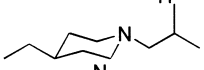
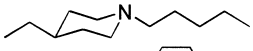
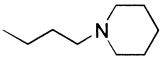
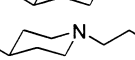
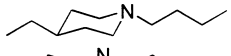
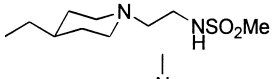
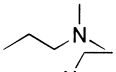
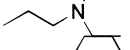
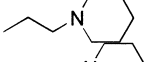
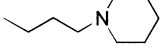
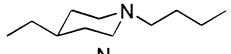
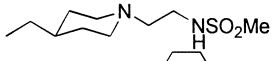
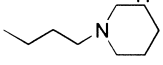
out in order to assign all the protons and carbons of these new structures.

Pharmacology

Final compounds were assessed for in vitro affinity at 5-HT_4 receptors by radioligand binding assays, using [^{3}H]GR 113808 in rat striatum membranes[31] (Table 1). As several 5-HT_4 receptor agonists and antagonists have also been described as potent 5-HT_3 receptor antagonists, it was mandatory to evaluate the selectivity of all active compounds over this receptor, using radioligand binding assays with [^{3}H]LY 278584 in rat cerebral cortex membranes[32] (Table 1). Six highly active 5-HT_4 ligands were also evaluated for in vitro affinity at other serotonergic receptors by using the following specific radioligands and tissue sources: 5-HT_{2A}, [^{3}H]ketanserin, rat frontal cerebral cortex,[33] 5-HT_{1A}, [^{3}H]-8-OH-DPAT, rat cerebral cortex.[34] The inhibition constant K_i was defined from the IC_{50} using the Cheng–Prusoff equation.[35] Additionally, four active and selective analogues in the 5-HT_4 binding profile have been evaluated for 5-HT_4 biological activity in the isolated guinea pig ileum. It is well known that concentrations lower than 10^{-6} M of 5-HT induce contractile responses in the non-stimulated guinea pig ileum by activation of 5-HT_4 receptors,[19,36–40] and these contractions are antagonized by selective 5-HT_4 antagonists,[41] whereas higher concentrations activate 5-HT_3 receptors.[19,42] In order to test the antagonist activity of the new compounds, non-cumulative concentration–response curves of 5-HT (10^{-8} to 3×10^{-7} M) were constructed in the presence and absence of the tested compounds (10^{-6} and 10^{-7} M). Then, the ED_{50} of 5-HT in the presence and absence of the antagonists was calculated as the concentration of 5-HT required to produce 50% of maximal contraction induced by 5-HT through 5-HT_4 receptors. The effect of 3×10^{-7} M 5-HT in control tissues was accepted as the maximal response (100%) mediated by 5-HT_4 receptors.[19] The pA_2 values were determined following the equation described by Furchgott,[43] using an appropriate concentration of the antagonists (Table 2); the concentration-ratios of 5-HT,

Table 1. Binding affinity of amides **1**–**16** and esters **17**–**26** at 5-HT_3 and 5-HT_4 receptors[a]



Compd	R	X	Y	$K_i \pm$ SEM (nM) 5-HT_4 [^{3}H]GR 113808	5-HT_3 [^{3}H]LY 278584
1	H	NH		719 ± 58	145 ± 10
2	H	NH		> 1000	< 1000
3	H	NH		> 1000	> 10,000
4	H	NH		290 ± 54	198 ± 16
5	H	NH		13.7 ± 0.9	> 10,000
6	H	NH		11.4 ± 3.0	> 1000
7	H	NH		> 1000	> 1000
8	H	NH		> 1000	> 1000
9	H	NH		157 ± 28	290 ± 54
10	H	NH		499 ± 95	> 10,000
11	Cl	NH		54.0 ± 2.8	29.5 ± 4.4
12	Cl	NH		0.32 ± 0.07	> 1000
13	Cl	NH		0.11 ± 0.03	> 1000
14	Cl	NH		0.29 ± 0.04	> 1000
15	Cl	NH		0.54 ± 0.10	> 1000
16	Cl	NH		9.1 ± 1.3	> 1000
17	H	O		> 10,000	> 1000
18	H	O		24.6 ± 0.5	> 1000
19	H	O		26.1 ± 0.3	> 1000
20	H	O		> 1000	> 10,000
21	H	O		> 10,000	> 10,000
22	H	O		> 1000	> 1000
23	H	O		470 ± 35	> 10,000
24	Cl	O		2.9 ± 0.4	> 10,000
25	Cl	O		2.3 ± 1.1	> 1000
26	Cl	O		15.1 ± 0.3	> 10,000
			SDZ 205-557	10.7 ± 1.2	155
			RS 39604	3.9 ± 0.2	> 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.

Table 2. Pharmacological activity at 5-HT_4 receptors in isolated guinea pig ileum

Compd	5-HT ED_{50}, M[a]		pA_2[b]	N[c]
	R1	R2		
Control	—	—	—	6
12	1.3×10^{-8}	6.8×10^{-8}	7.6	7
13	2.5×10^{-8}	2.4×10^{-7}	7.9	5
14	1.1×10^{-8}	1.8×10^{-7}	8.2	6
15	1.7×10^{-8}	1.4×10^{-7}	7.9	6
RS 39604	1.5×10^{-8}	2.9×10^{-7}	8.2	6

[a] Concentration of 5-HT required to produce a 50% of maximal contraction induced by 5-HT to 5-HT_4 receptors evaluated in the absence (R1) and in the presence (R2) of tested compounds.
[b] Single point analysis using 10^{-7} M concentration of the antagonists ($pA_2 = -\log([B]/\text{concentration ratio} - 1$; [B] = concentration of the antagonist).
[c] Number of experiments.

in the presence and absence of the 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_4 receptor antagonist RS 39604 was used as reference compound. The effects of the antagonists were also tested on the contraction induced by 10^{-6} and 3×10^{-6} M 5-HT, in order to discard activity on 5-HT_3 receptors.

Molecular Modelling

A structural analysis of compounds **4**, **5**, **9**, and **10** was carried out with a Silicon Graphics INDIGO2 computer and the multifaceted software package INSIGHT II,[44] using the Builder, Discover and Analysis programs. The molecules were built de novo in their protonated forms, which is believed to be the bioactive form, using the Builder module of INSIGHT II. Their geometry was optimized by using the Optimize option of this module, which combines three different minimization algorithms through the DISCOVER program (steepest descent, conjugate gradients, and the BFGS algorithm). A systematic conformational search was then performed on each compound, allowing rotatable bonds to rotate with a 15° stepwise increment of the dihedral angles. Lowest energy conformation(s) were identified through the analysis module of INSIGHT II, and the distances and dihedral angles between the pharmacophoric elements were measured (Table 3).

Results and Discussion

The results of the tested compounds are reported in Tables 1 and 2. The values for 5-HT_4 activation of the compounds were compared to those of the reference compounds: SDZ 205-557[45] and RS 39604.[38]

The data from the binding assays for the synthesized compounds presented in Table 1 highlighted their high affinity for 5-HT_4 receptors since a number of them exhibited nanomolar or subnanomolar affinity for these

Table 3. Energy and structural parameters of lowest energy conformers of analyzed compounds

Compd	Energy (kcal mol^{-1})	Ar–O[a] (Å)	Ar–N[b] (Å)	O–N[c] (Å)	h[d] (Å)
4	97.88	3.74	7.82	4.38	0.07
5	135.34	3.74	8.48	5.62	3.55
9	150.11	3.75	7.49	4.48	1.42
10	128.04	3.74	8.34	5.20	2.56

[a] Distance between the centroid of the aromatic ring and the oxygen of the carbonyl group.
[b] Distance between the centroid of the aromatic ring and the basic nitrogen of the amine.
[c] Distance between the oxygen of the carbonyl group and the basic nitrogen of the amine.
[d] Deviation of the basic nitrogen with respect to the aromatic ring.

binding sites. Furthermore, most of the compounds displayed no significant affinity for the 5-HT_3 receptor (K_i > 1000–10,000 nM) demonstrating their high 5-HT_4/5-HT_3 selectivity. An examination of the binding data presented in Table 1 shows the following.

a. With respect to the aromatic moiety the introduction of a chloro atom at the 6-position of the benzimidazole ring induces a notable increase in potency at 5-HT_4 receptors. Thus, 6-chloro substituted analogues are one or two orders of magnitude more potent than their directly related non-substituted derivatives (e.g. K_i (**6**) = 11.4 nM versus K_i (**13**) = 0.11 nM; K_i (**19**) = 26.1 nM versus K_i (**25**) = 2.3 nM). The influence of the 6-chloro substitution in the benzimidazole moiety represents a structural difference between the 5-HT_4 receptor ligands of this class and the related conformationally restricted 5-HT_3 receptor ligands, where such a general trend was not observed.[46]

b. The affinity for the 5-HT_4 receptor is not much affected by the nature of the acyl group; carboxamides display slightly higher affinity than their corresponding carboxylates, though no significant difference is observed and both show the same order of magnitude for their potency. The only exception was observed in esters **17** and **22** which were inactive while their corresponding amides **1** and **9** exhibited moderate affinity.

c. In regard to the basic amino moiety of the molecule, the presence of at least one methylene unit between the acyl group and the 4-substituted piperidine ring is necessary for selective binding at 5-HT_4 sites over 5-HT_3 receptors. Thus, analogues **1**–**4**, **11** and **17** (no methylene unit) were either inactive or non-selective in the 5-HT_4 receptor. In the case of dialkylaminoalkyl and 1-alkylpiperidine derivatives we can observe that only 1-propylpiperidine chain leads to selective compounds at 5-HT_4 receptors (e.g. **16**: K_i(5-HT_4) = 9.1 nM, K_i(5-HT_3) > 1000 nM; **26**: K_i(5-HT_4) = 15.1 nM, K_i(5-HT_3) > 10,000 nM).

In order to understand the influence of the methylene chain length in 5-HT_4 affinity and selectivity, a structural analysis of compounds **4, 5, 9,** and **10** was carried out, and the structural parameters of lowest energy

conformers are given in Table 3. We can observe that the distance between the aromatic ring and the basic nitrogen atom (Ar–N) is the key structural parameter. In non-selective analogues **4** (K_i(5-HT_4) = 290 nM, K_i(5-HT_3) = 198 nM) and **9** (K_i(5-HT_4) = 157 nM, K_i(5-HT_3) = 290 nM) the distance is 7.82 Å and 7.49 Å, respectively, whereas in selective compounds **5** (K_i(5-HT_4) = 13.7 nM, K_i(5-HT_3) > 10,000 nM) and **10** (K_i(5-HT_4) = 499 nM, K_i(5-HT_3) > 10,000 nM) the nitrogen atom is situated at a further distance from the aromatic ring (8.48 and 8.34 Å, respectively). These structural data are in agreement with our recently proposed hypothesis for 5-HT_4 affinity and 5-HT_4/5-HT_3 selectivity,[30] in which we postulated a distance of ca. 8.0 Å between the aromatic ring and the basic nitrogen atom for the 5-HT_4 antagonist pharmacophore and a distance of ca. 7.5 Å for the 5-HT_3 antagonist model.

On the other hand, analogues with a substituent in the nitrogen atom less voluminous than a butyl group are devoid of affinity at the 5-HT_4 receptor, except compound **11** (K_i = 54.0 nM) probably due to the presence of a chloro atom at the 6-position of the benzimidazole ring, since non-substituted analogue **1** shows a clear drop in binding affinity (K_i = 719 nM).

These results confirm that the presence of a voluminous substituent in the basic nitrogen atom of the amino moiety and the distance from this nitrogen to the aromatic ring are of great importance for high affinity and selectivity for 5-HT_4 receptors, as we postulated in our recently reported model for the 5-HT_4 binding site.[30] The crucial influence of this distance has also been recently reported by Langlois et al.[47] for recognition of esters of 4-amino-5-chloro-2-methoxybenzoic acid by 5-HT_4 receptors. When both requirements are verified the nature of the *N*-substituent does not seem to have influence on 5-HT_4 potency, since compounds **12** (K_i = 0.32 nM), **13** (K_i = 0.11 nM), **14** (K_i = 0.29 nM) and **15** (K_i = 0.54 nM) were approximately equipotent. The most potent 5-HT_4 ligands of the series **12**–**15**, **24**, and **25** were devoid of affinity at 5-HT_{1A} and 5-HT_{2A} sites (K_i > 1000–10,000 nM), confirming their excellent selectivity for the 5-HT_4 receptor.

Derivatives **12**–**15** were evaluated for 5-HT_4 receptor activity in the guinea pig ileum. When administered alone, they did not display agonist activity at the tested doses (10^{-6} and 10^{-7} M). They produced a selective inhibition of the 5-HT induced contractile response mediated by 5-HT_4 receptors without modification of the contractions mediated through 5-HT_3 receptors: interestingly, even when the contractions induced by low doses of 5-HT (< 10^{-6} M) were 100% blocked, the contractions involving 5-HT_3 receptors ([5-HT] > 10^{-6} M) were not modified by none of the new tested compounds. This difference in the effect of these compounds on contractions involving 5-HT_4 or 5-HT_3 receptors confirms, doubtless, their selectivity for the 5-HT_4 receptor. The addition of derivatives **12**–**15** or RS 39604 induced a parallel rightward shift of the 5-HT_4-depending responses (Fig. 2), and consequently the 5-HT ED_{50} decreased. The pA_2 values were quite similar for the five tested

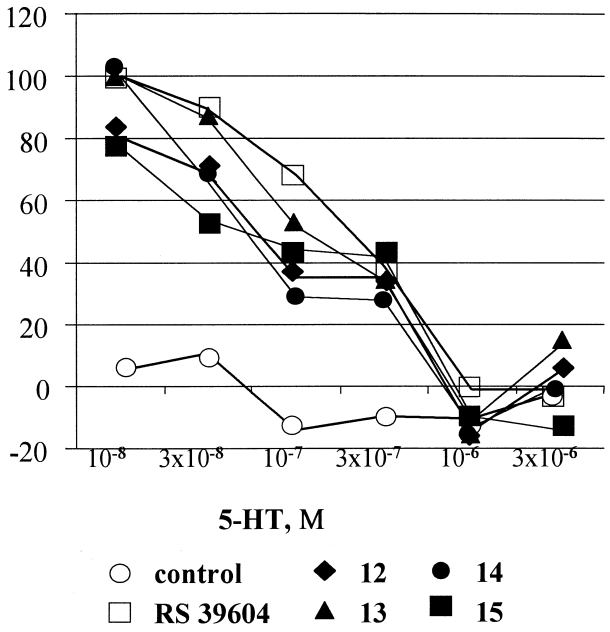


Figure 2. Each point shows the mean (the SEM are omitted for clarity) of the percentage of inhibition of the contractile responses induced by second administration of 5-HT versus the first administration in the absence of drug (control), or in the presence of 0.1 μM RS 39604 as reference 5-HT_4 antagonist, or in the presence of the same concentrations (0.1 μM) of derivatives **12**–**15** (n = 5).

compounds (Table 2) suggesting an equipotent effect of **12**–**15** to the 5-HT_4 receptor antagonist RS 39604.

A strict relationship was not observed between binding affinity (K_i, Table 1) and potency (pA_2, Table 2) of the new antagonists. This lack of close correlation between binding affinity and potency of drugs in functional in vivo or in vitro tests is not unusual,[40,42,47,48] and differences are generally attributed to 5-HT receptor differences in the tissues or tests used. In this study, rat striatum membranes were used for the binding assays and guinea pig ileum tissues were used to assess the antagonist potency.

Conclusion

The benzimidazole-4-carboxamides and -carboxylates of piperidine derivatives described in this paper represent a novel class of potent and selective 5-HT_4 receptor antagonists in the CNS and in the guinea pig ileum. Structure–activity relationships and structural analyses have shown that the presence of a voluminous substituent in the basic nitrogen atom of the amino moiety and a distance of ca. 8.0 Å from the aromatic ring to this nitrogen are both required for high affinity and selectivity for 5-HT_4 receptors. These results confirm our recently proposed model for recognition by the 5-HT_4 binding site. Four compounds of the series (**12**–**15**) displayed subnanomolar affinity at central 5-HT_4 receptors, high selectivity over other serotonin receptors (5-HT_3, 5-HT_{2A}, and 5-HT_{1A}) and potent 5-HT_4 antagonist activity in the guinea pig ileum, comparable to the 5-HT_4 receptor antagonist RS 39604. Consequently, these novel 5-HT_4 antagonists could be

interesting pharmacological tools for the understanding of the role of 5-HT_4 receptors.

Experimental

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), quint (quintet), sext (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. Low resolution mass spectra were recorded by direct injection on a HP 5989 instrument using the electronic impact technique with an ionization energy of 70 eV. Peaks are expressed in *m*/*z*, and molecular and base peaks are indicated as (M) and (100), respectively. 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. 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,[49] 2-amino-5-chloro-3-methylaniline,[50] 4-amino-1-methylpiperidine,[51] 4-amino-1-ethylpiperidine,[52] 4-amino-1-propylpiperidine,[52] 4-amino-1-butylpiperidine,[52] 3-piperidinopropylamine,[53] 1-methyl-4-piperidinol,[54] (1-butyl-4-piperidyl)methanol,[55] *N*-[2-(4-hydroxymethyl-1-piperidyl)ethyl]-methanesulfonamide,[56] and 3-piperidinopropanol.[57]

Spectral data of all described compounds were consistent with the proposed structures; for series **31**–**34** and **1**–**26**, assignments are included for compounds **1**, **11**, **17**, **24**, and **31** as examples.

6-Chlorobenzimidazole-4-carboxylic acid (28). A solution of **29** (3.3 g, 21 mmol) and formic acid (2.9 g, 60 mmol) in water (20 mL) was refluxed for 6 h (TLC). The reaction mixture was cooled and a cold 1 N aqueous solution of KOH was added. 6-Chloro-4-methylbenzimidazole **30** (3.4 g, 98%) was recrystallized from chloroform; mp 172–173°C. ^1H NMR (Me_2SO-d_6) δ 2.54 (s, 3H, CH_3), 7.07 (s, 1H, H_5), 7.50 (s, 1H, H_7), 8.29 (s, 1H, H_2), 12.70 (br s, 1H, NH); ^{13}C NMR (Me_2SO-d_6) δ 16.6 (CH_3), 112.5 (C_7), 122.0 (C_5), 125.9, 126.8 (C_4, C_6), 136.8 (C_{3a}, C_{7a}), 142.9 (C_2).

To a solution of crude **30** (2.2 g, 13 mmol) in 0.5 N aqueous solution of NaOH (130 mL) warmed in a steam bath were added five portions of 2.1 g (15 mmol) of potassium permanganate, every hour. After the last addition, the reaction mixture was refluxed for 2 more hours (TLC), hot-filtered and the solvent was evaporated under reduced pressure. The solid was purified by column chromatography ($CHCl_3$:MeOH, from 9:1 to 1:1) to yield 0.9 g (30%) of **28** which was recrystallized from diluted hydrochloric acid; mp >300°C. ^1H NMR (Me_2SO-d_6) δ 7.94 (d, J=1.9, 1H, H_7), 8.17 (d, J=1.9, 1H, H_5), 9.46 (s, 1H, H_2); ^{13}C NMR (Me_2SO-d_6) δ 118.6 (C_4), 119.8 (C_7), 126.7 (C_5), 128.9, 129.0 (C_{3a}, C_6), 135.2 (C_{7a}), 143.8 (C_2), 164.5 (COOH).

General procedure for the synthesis of (1-alkyl-4-piperidyl)methylamines (31–34). To an ice-cold solution of 4-piperidylmethylamine (5 g, 44 mmol) in dry CH_3CN (45 mL) was added dropwise a solution of the appropriate halide (1-bromobutane, 1-bromo-2-methylpropane, 1-chloropentane, or *N*-(2-chloroethyl)methanesulfonamide) (35 mmol) in CH_3CN (35 mL). The reaction mixture was allowed to warm to room temperature and stirred overnight. Then a 4% aq sol of NaOH was added dropwise (200 mL), the mixture was extracted with $CHCl_3$ (3×200 mL), and the combined organic layers were washed with brine and dried over Na_2SO_4. After evaporation of the solvent, the crude oil was purified by column chromatography to afford the corresponding alkylated amine as a pure compound. In all cases, small amounts of the dialkylated compound (5–20%) and starting material (5–10%) were observed in the ^1H NMR spectra of the reaction crudes.

(1-Butyl-4-piperidyl)methylamine (31). Yield 3.8 g (51%); chromatography $CHCl_3$:MeOH:NH_3, from 9:1:0.1 to 9:4:0.1; bp 45°C (0.3 mm Hg); ^1H NMR ($CDCl_3$) δ 0.82 (t, J=7.2, 3H, CH_3), 1.08–1.27 (m, 5H, CH_2CH_3, H_4, H_{3ax}, H_{5ax}), 1.39 (quint, J=7.5, 2H, NCH_2CH_2), 1.62 (d, J=9.6, 2H, H_{3eq}, H_{5eq}), 1.80 (t, J=11.7, 2H, H_{2ax}, H_{6ax}), 1.99 (br s, 2H, NH_2), 2.18–2.23 (m, 2H, NCH_2), 2.48 (d, J=6.0, 2H, CH_2NH_2), 2.86 (d, J=12.0, H_{2eq}, H_{6eq}); ^{13}C NMR ($CDCl_3$) δ 14.1 (CH_3), 21.0 (CH_2CH_3), 29.2 (NCH_2CH_2), 29.9 (C_3, C_5), 39.3 (C_4), 48.0 (CH_2NH_2), 53.8 (C_2, C_6), 59.0 (NCH_2).

(1-*iso*-Butyl-4-piperidyl)methylamine (32). Yield 4.7 g (63%); chromatography $CHCl_3$:MeOH:NH_3, from 9:1:0.1 to 8:2:0.1; mp 106–108°C (MeOH/Et_2O); ^1H NMR ($CDCl_3$) δ 0.88 (d, J=6.6, 6H), 1.15–1.30 (m, 3H), 1.63–1.80 (m, 5H), 1.83 (tm, J=10.5, 2H), 2.05 (d, J=7.3, 2H), 2.56 (dm, J=5.6, 2H), 2.87 (dm, J=11.6, 2H); ^{13}C NMR ($CDCl_3$) δ 20.9, 25.3, 29.7, 39.2, 47.9, 53.9, 67.2.

(1-Pentyl-4-piperidyl)methylamine (33). Yield 4.4 g (54%); chromatography $CHCl_3$:MeOH:NH_3, from 9:1:0.1 to 8:2:0.1; mp 98–100°C (MeOH:Et_2O); ^1H NMR ($CDCl_3$)

δ 0.77 (t, J=6.7, 3H), 1.09–1.21 (m, 7H), 1.37 (m, 2H), 1.59 (dm, J=9.5, 2H), 1.76 (tm, J=11.1, 2H), 1.88 (br s, 2H), 2.16 (m, 2H), 2.45 (dm, J=4.8, 2H), 2.82 (dm, J=11.9, 2H); ^{13}C NMR ($CDCl_3$) δ 14.0, 22.6, 26.7, 29.9, 39.0, 47.8, 53.7, 59.2.

***N*-[2-(4-Aminomethyl-1-piperidyl)ethyl]methanesulfonamide (34).** Yield 5.1 g (49%); chromatography $CHCl_3$:MeOH:NH_3, from 9:4:0.2 to 8:2:1; mp 111–113°C (MeOH:Et_2O); ^{1}H NMR ($CDCl_3$) δ 1.20–1.26 (m, 3H), 1.72 (dm, J=10.5, 2H), 2.00 (td, J=11.7, 2.2, 2H), 2.51 (t, J=6.1, 2H), 2.57 (d, J=6.3, 2H), 2.88 (dm, J=11.5, 2H), 2.92 (s, 3H), 3.19 (t, J=5.6, 2H); ^{13}C NMR ($CDCl_3$) δ 29.5, 38.4, 39.5, 39.6, 47.3, 53.0, 56.9.

General procedure for the synthesis of benzimidazole-4-carboxamides and benzimidazole-4-carboxylates (1–26). To a solution of acid **27** or **28** (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% aq 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-Methyl-4-piperidyl)benzimidazole-4-carboxamide (1).** Yield 413 mg (32%); mp 122–124°C (water); ^{1}H NMR (Me_2SO-d_6) δ 1.51–1.61 (m, 2H, $H_{3'ax}$, $H_{5'ax}$), 1.91 (d, J=10.2, 2H, $H_{3'eq}$, $H_{5'eq}$), 2.09 (t, J=10.2, 2H, $H_{2'ax}$, $H_{6'ax}$), 2.17 (s, 3H, CH_3), 2.69 (d, J=10.8, 2H, $H_{2'eq}$, $H_{6'eq}$), 3.85–3.88 (m, 1H, $H_{4'}$), 7.33 (t, J=8.1, 1H, H_6), 7.75 (d, J=8.1, 1H, H_7), 7.86 (d, J=7.2, 1H, H_5), 8.46 (s, 1H, H_2), 9.80 (br s, 1H, CONH); ^{13}C NMR (Me_2SO-d_6) δ 31.9 ($C_{3'}$, $C_{5'}$), 46.0 ($C_{4'}$), 46.2 (CH_3), 53.9 ($C_{2'}$, C6′), 116.5 (C_7), 122.0 (C_4), 122.1, 122.2 (C_5, C_6), 135.2 (C_{7a}), 139.5 (C_{3a}), 143.3 (C_2), 164.3 (CONH). Anal. ($C_{14}H_{18}N_4O$) C, H, N.

***N*-(1-Ethyl-4-piperidyl)benzimidazole-4-carboxamide (2).** Yield 537 mg (37%); chromatography AcOEt:EtOH, from 9:1 to 4:1; mp 126–128°C (water); ^{1}H NMR (Me_2SO-d_6) δ 1.01 (t, J=7.2, 3H), 1.57 (qd, J=12.3, 3.3, 2H), 1.94–1.98 (m, 2H), 2.11 (t, J=10.2, 2H), 2.34 (q, J=7.2, 2H), 2.79 (d, J=11.4, 2H), 3.91–3.94 (m, 2H), 7.35 (t, J=7.8, 1H), 7.77 (dd, J=8.1, 1.2, 1H), 7.89 (dd, J=7.5, 1.2, 1H), 8.49 (s, 1H), 9.80 (br s, 1H); ^{13}C NMR (Me_2SO-d_6) δ 12.2, 31.8, 46.0, 51.2, 51.6, 116.0, 121.9, 122.1, 134.1, 139.1, 142.8, 163.9. Anal. ($C_{15}H_{20}N_4O{\cdot}H_2O$) C, H, N.

***N*-(1-Propyl-4-piperidyl)benzimidazole-4-carboxamide (3).** Yield 573 mg (40%); chromatography $CHCl_3$:MeOH, 9:1; mp 119–121°C (acetone:Et_2O); ^{1}H NMR (Me_2SO-d_6) δ 0.86 (t, J=7.5, 3H), 1.44 (sext, J=7.5, 2H), 1.58 (q, J=9.9, 2H), 1.95 (d, J=10.5, 2H), 2.14 (br t, 2H), 2.26 (t, J=7.8, 2H), 2.79 (d, J=10.2, 2H), 3.92–3.94 (m, 1H), 7.35 (t, J=8.1, 1H), 7.77 (d, J=8.1, 1H), 7.89 (d, J=7.5, 1H), 8.49 (s, 1H), 9.97 (br s, 1H), 13.00 (br s, 1H); ^{13}C NMR (Me_2SO-d_6) δ 11.8, 19.6, 31.7, 45.9, 51.6, 59.8, 115.3, 121.9, 122.2, 133.5, 140.3, 142.8, 163.8. Anal. ($C_{16}H_{22}N_4O$) C, H, N.

***N*-(1-Butyl-4-piperidyl)benzimidazole-4-carboxamide (4).** Yield 661 mg (44%); chromatography from AcOEt to AcOEt:EtOH, 9.5:0.5; mp 94–97°C (acetone:water); ^{1}H NMR (Me_2SO-d_6) δ 0.88 (t, J=7.2, 3H), 1.28 (sext, J=7.2, 2H), 1.41 (quint, J=7.2, 2H), 1.56 (q, J=9.3, 2H), 1.94 (d, J=10.2, 2H), 2.11 (t, J=10.5, 2H), 2.27 (t, J=7.2, 2H), 2.77 (d, J=11.1, 2H), 3.91–3.93 (m, 1H), 7.35 (t, J=7.8, 1H), 7.77 (d, J=7.8, 1H), 7.88 (d, J=7.5, 1H), 8.48 (s, 1H), 9.81 (br s, 1H); ^{13}C NMR (Me_2SO-d_6) δ 13.9, 20.1, 28.7, 31.8, 46.0, 51.7, 57.6, 116.1, 122.0, 122.1, 134.8, 139.4, 142.8, 163.9. Anal. ($C_{17}H_{24}N_4O$) C, H, N.

***N*-[(1-Butyl-4-piperidyl)methyl]benzimidazole-4-carboxamide (5).** Yield 770 mg (49%); chromatography AcOEt:EtOH, 9:1; mp 122–125°C (AcOEt); ^{1}H NMR (Me_2SO-d_6) δ 0.86 (t, J=7.2, 3H), 1.24–1.38 (m, 6H), 1.55 (m, 1H), 1.70 (d, J=12.0, 2H), 1.83 (t, J=10.8, 2H), 2.23 (t, J=7.2, 2H), 2.85 (d, J=10.5, 2H), 3.34 (br t, 2H), 7.35 (t, J=7.5, 1H), 7.77 (d, J=7.8, 1H), 7.89 (d, J=6.9, 1H), 8.48 (s, 1H), 9.92 (br s, 1H), 12.88 (br s, 1H); ^{13}C NMR (Me_2SO-d_6) δ 14.0, 20.3, 28.7, 29.9, 36.3, 44.5, 53.2, 57.9, 116.0, 122.2, 122.3, 134.8, 139.6, 143.0, 164.8. Anal. ($C_{18}H_{26}N_4O$) C, H, N.

***N*-[[1-[2-[(Methylsulfonyl)amino]ethyl]-4-piperidyl]methyl]benzimidazole-4-carboxamide (6).** Yield 1.1 g (59%); chromatography from $CHCl_3$:MeOH, 9:1 to $CHCl_3$:MeOH:NH_3, 8:2:1; mp 67–69°C; ^{1}H NMR ($CDCl_3$) δ 1.39 (qm, J=9.1, 2H), 1.71–1.81 (m, 1H, $H_{4'}$), 1.83 (d, J=12.1, 2H), 2.05 (td, J=11.9, 2.4, 2H), 2.53 (t, J=5.9, 2H), 2.89 (dm, J=11.9, 2H), 2.97 (s, 3H), 3.19 (t, J=5.5, 2H), 3.50 (t, J=5.5, 2H), 7.38 (t, J=7.9, 1H), 7.68 (d, J=7.9, 1H), 8.06 (d, J=7.5, 1H), 8.16 (s, 1H), 10.01 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 29.8, 36.0, 39.7, 39.8, 44.9, 53.0, 58.0, 116.0, 122.0, 122.5, 122.9, 134.3, 139.7, 141.3, 166.4. Anal. ($C_{17}H_{25}N_5O_3S$) C, H, N.

***N*-[2-(*N*,*N*-Dimethylamino)ethyl]benzimidazole-4-carboxamide (7).** Yield 464 mg (40%); chromatography CH_2Cl_2:MeOH, from 9:1 to 7:3; mp 79–81°C ($CHCl_3$:Et_2O); ^{1}H NMR (Me_2SO-d_6) δ 2.24 (s, 6H), 2.51 (t, J=6.6, 2H), 3.55 (q, J=6.0, 2H), 7.36 (t, J=7.5, 1H), 7.78 (d, J=8.1, 1H), 7.90 (d, J=7.5, 1H), 8.47 (s, 1H), 9.77 (br s, 1H); ^{13}C NMR (Me_2SO-d_6) δ 36.9, 45.1, 58.3, 116.2, 121.9, 122.1, 134.9, 139.1, 142.8, 164.7. Anal. ($C_{12}H_{16}N_4O$) C, H, N.

***N*-[2-(*N*,*N*-Diethylamino)ethyl]benzimidazole-4-carboxamide (8).** Yield 625 mg (48%); chromatography CH_2Cl_2:MeOH, from 9:1 to 4:1; ^{1}H NMR (Me_2SO-d_6) δ 1.00 (t, J=7.2, 6H), 2.57 (q, J=7.2, 4H), 2.65 (t, J=6.3, 2H), 3.50 (q, J=6.0, 2H), 7.34 (t, J=8.1, 1H), 7.76 (d, J=8.1, 1H), 7.92 (d, J=7.5, 1H), 8.45 (s, 1H), 9.83 (br s, 1H); ^{13}C NMR (Me_2SO-d_6) δ 11.7, 37.2, 46.6, 51.5, 115.8, 121.9, 122.0, 134.7, 138.9, 142.7, 164.7. Anal. ($C_{14}H_{20}N_4O$) C, H, N.

***N*-(2-Piperidinoethyl)benzimidazole-4-carboxamide (9).** Yield 408 mg (30%); chromatography AcOEt:EtOH, 9:1; ^{1}H NMR (Me_2SO-d_6) δ 1.38–1.41 (m, 2H), 1.53 (quint, J = 5.4, 4H), 2.42 (br t, 4H), 2.50 (t, J = 6.9, 2H), 3.53 (q, J = 6.9, 2H), 7.34 (t, J = 7.8, 1H), 7.75 (d, J = 7.8, 1H), 7.87 (d, J = 7.8, 1H), 8.44 (s, 1H), 9.91 (br s, 1H), 12.90 (br s, 1H); ^{13}C NMR (Me_2SO-d_6) δ 24.2, 25.7, 36.7, 54.1, 57.7, 116.2, 122.1, 122.2, 135.0, 139.1, 142.9, 164.8. Anal. ($C_{15}H_{20}N_4O$) C, H, N.

***N*-(3-Piperidinopropyl)benzimidazole-4-carboxamide (10).** Yield 601 mg (42%); chromatography AcOEt:EtOH, 9:1; mp 161–164°C (acetone); ^{1}H NMR (Me_2SO-d_6) δ 1.35–1.37 (m, 2H), 1.46–1.48 (m, 4H), 1.73 (quint, J = 6.6, 2H), 2.31–2.36 (m, 6H), 3.45 (q, J = 6.3, 2H), 7.35 (t, J = 7.8, 1H), 7.77 (d, J = 7.8, 1H), 7.88 (d, J = 7.5, 1H), 8.45 (s, 1H), 9.74 (br s, 1H); ^{13}C NMR (Me_2SO-d_6) δ 24.3, 25.7, 26.7, 37.4, 54.2, 56.3, 116.4, 122.0, 122.1, 135.1, 139.2, 142.9, 164.8. Anal. ($C_{16}H_{22}N_4O$) C, H, N.

***N*-(1-Methyl-4-piperidyl)-6-chlorobenzimidazole-4-carboxamide (11).** Yield 483 mg (33%); chromatography $CHCl_3$:MeOH, 9:1; mp 238–239°C (acetone:AcOEt); ^{1}H NMR (Me_2SO-d_6) δ 1.57 (q, J = 9.9, 2H, $H_{3'ax}$, $H_{5'ax}$), 1.92 (d, J = 10.2, 2H, $H_{3'eq}$, $H_{5'eq}$), 2.09 (t, J = 11.7, 2H, $H_{2'ax}$, $H_{6'ax}$), 2.18 (s, 3H, CH_3), 2.71 (d, J = 11.1, 2H, $H_{2'eq}$, $H_{6'eq}$), 3.86–3.88 (m, 1H, $H_{4'}$), 7.82 (d, J = 1.5, 1H, H_5), 7.84 (d, J = 1.8, 1H, H_7), 8.50 (s, 1H, H_2), 9.58 (br s, 1H, CONH); ^{13}C NMR (Me_2SO-d_6) δ 31.5 ($C_{3'}$, $C_{5'}$), 45.7 ($C_{4'}$), 45.9 (CH_3), 53.7 ($C_{2'}$, $C_{6'}$), 116.2 (C_7), 121.8 (C_5), 122.6, 126.5 (C_4, C_6), 136.2, 137.5 (C_{3a}, C_{7a}), 144.3 (C_2), 162.7 (CONH). Anal. ($C_{14}H_{17}ClN_4O$) C, H, N.

***N*-[(1-Butyl-4-piperidyl)methyl]-6-chlorobenzimidazole-4-carboxamide (12).** Yield 752 mg (43%); chromatography $CHCl_3$:MeOH, 1:9; mp 184–186°C (toluene); ^{1}H NMR (CDCl3) δ 0.87 (t, J = 7.1, 3H), 1.29 (sext, J = 7.5, 2H), 1.49–1.59 (m, 3H), 1.64 (qm, J = 12.7, 2H), 1.78–1.89 (m, 1H), 1.87 (dm, J = 12.3, 2H), 2.18 (t, J = 11.5, 2H), 2.48–2.53 (m, 2H), 3.16 (dm, J = 11.9, 2H), 3.54 (t, J = 5.2, 2H), 7.56 (s, 1H), 7.98 (s, 1H), 8.00 (s, 1H), 9.85 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.8, 20.7, 28.0, 28.9, 35.7, 44.4, 53.2, 115.0, 123.1, 123.5, 128.6, 134.4, 138.9, 142.0, 165.1; MS 348 (M), 305 (100) 262, 179, 151, 138, 110, 55. Anal. ($C_{18}H_{25}ClN_4O$) C, H, N.

***N*-[[1-[2-[(Methylsulfonyl)amino]ethyl]-4-piperidyl]methyl]-6-chlorobenzimidazole-4-carboxamide (13).** Yield 1.1 g (52%); chromatography $CHCl_3$:MeOH, from 9:1 to 3:1; mp 85–87°C; ^{1}H NMR ($CDCl_3$) δ 1.39 (qm, J = 11.4, 2H), 1.71–1.82 (m, 1H), 1.80 (d, J = 13.5, 2H), 2.03 (t, J = 11.5, 2H), 2.52 (t, J = 5.5, 2H), 2.87 (d, J = 11.1, 2H), 2.97 (s, 3H), 3.18 (t, J = 5.5, 2H), 3.48 (t, J = 5.9, 2H), 7.67 (s, 1H), 8.02 (s, 1H), 8.15 (s, 1H), 10.01 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 29.9, 36.1, 39.7, 40.0, 45.1, 53.1, 56.7, 115.2, 123.7, 138.4, 141.9, 165.1. Anal. ($C_{17}H_{24}ClN_5O_3S$) C, H, N.

***N*-[(1-*iso*-Butyl-4-piperidyl)methyl]-6-chlorobenzimidazole-4-carboxamide (14).** Yield 872 mg (50%); chromatography $CHCl_3$:MeOH, 10:1; mp 184–186°C (toluene); ^{1}H NMR ($CDCl_3$) δ 0.86 (d, J = 6.3, 6H), 1.42–1.58 (m, 2H), 1.73–1.87 (m, 5H), 1.98 (tm, J = 13.5, 2H), 2.14 (d, J = 8.9, 2H), 2.99 (dm, J = 14.4, 2H), 3.52 (t, J = 7.4, 2H), 7.59 (s, 1H), 8.02 (s, 2H), 9.93 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.1, 25.4, 29.5, 36.2, 44.9, 53.8, 67.1, 115.1, 123.5, 128.7, 133.5, 139.1, 141.9, 165.1. Anal. ($C_{18}H_{25}ClN_4O$) C, H, N.

***N*-[(1-Pentyl-4-piperidyl)methyl]-6-chlorobenzimidazole-4-carboxamide (15).** Yield 835 mg (46%); chromatography $CHCl_3$:MeOH, from 8:1 to 4:1; mp 176–178°C (toluene); ^{1}H NMR ($CDCl_3$) δ 0.86 (t, J = 6.3, 3H), 1.24–1.31 (m, 4H), 1.50–1.68 (m, 4H), 1.77–1.89 (m, 1H), 1.87 (d, J = 12.7, 2H), 2.16 (t, J = 11.9, 2H), 2.45–2.50 (m, 2H), 3.15 (d, J = 11.5, 2H), 3.57 (t, J = 5.2, 2H), 7.56 (s, 1H), 8.00 (s, 2H), 9.86 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.9, 22.4, 25.8, 29.1, 29.7, 35.8, 44.4, 53.3, 58.8, 114.7, 123.6, 128.7, 134.3, 138.8, 141.9, 165.0. Anal. ($C_{19}H_{27}ClN_4O$) C, H, N.

***N*-(3-Piperidinopropyl)-6-chlorobenzimidazole-4-carboxamide (16).** Yield 850 mg (53%); chromatography $CHCl_3$:MeOH, from 1:1 to 1:4; mp 208–210°C (MeOH); ^{1}H NMR (DMSO-d_6) δ 1.42–1.48 (m, 2H), 1.54–1.80 (m, 4H), 1.85 (quint, J = 6.9, 2H), 2.46–2.51 (m, 6H), 7.86 (d, J = 1.9, 1H), 7.90 (d, J = 2.2, 1H), 8.51 (s, 1H), 9.62 (br s, 1H); ^{13}C NMR (DMSO-d_6/CF_3CO_2H) δ 23.1, 24.7, 25.9, 37.9, 56.2, 116.4, 121.7, 122.5, 126.5, 137.5, 138.1, 144.3, 163.5; MS 320 (M), 230, 205, 183, 152, 108, 98 (100), 60. Anal. ($C_{16}H_{21}ClN_4O$) C, H, N.

1-Methyl-4-piperidyl benzimidazole-4-carboxylate (17). Yield 583 mg (45%); chromatography $CHCl_3$:MeOH, 9:1; mp 163–165°C (water); ^{1}H NMR (Me_2SO-d_6) δ 1.86–1.96 (m, 4H, $2H_{3'}$, $2H_{5'}$), 2.21–2.25 (m, 5H, CH_3, $H_{2'ax}$, $H_{6'ax}$), 2.65–2.68 (m, 2H, $H_{2'eq}$, $H_{6'eq}$), 5.04 (tt, J = 8.1, 3.9, 1H, $H_{4'}$), 7.34 (t, J = 7.5, 1H, H_6), 7.88 (d, J = 7.5, 1H, H_5/H_7), 7.99 (d, J = 8.1, 1H, H_5/H_7), 8.32 (s, 1H, H_2), 12.48 (br s, 1H, NH); ^{13}C NMR (Me_2SO-d_6) δ 30.6 ($C_{3'}$, $C_{5'}$), 46.0 (CH_3), 52.6 ($C_{2'}$, $C_{6'}$), 70.6 ($C_{4'}$), 114.2 (C_4), 121.2 (C_6), 124.4, 124.8 (C_5, C_7), 132.7 (C_{3a}), 144.0 (C_2), 144.4 (C_{7a}), 164.8 (COO). Anal. ($C_{14}H_{17}N_3O_2$) C, H, N.

(1-Butyl-4-piperidyl)methyl benzimidazole-4-carboxylate (18). Yield 788 mg (50%); chromatography AcOEt:EtOH, 9:1; mp 159–161°C (acetone); ^{1}H NMR ($CDCl_3$) δ 0.87 (t, J = 7.2, 3H), 1.27 (sext, J = 7.4, 2H), 1.39–1.50 (m, 4H), 1.75–1.80 (m, 3H), 1.90 (t, J = 11.6, 2H), 2.26-2.32 (m, 2H), 2.95 (d, J = 11.4, 2H), 4.23 (d, J = 5.9, 2H), 7.29 (t, J = 7.8, 1H), 7.92 (d, J = 7.6, 1H), 8.00 (d, J = 8.0, 1H), 8.14 (s, 1H), 10.83 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.2, 21.0, 29.1, 29.3, 35.7, 53.5, 59.0, 69.6, 114.1, 121.8, 125.2, 125.5, 143.8, 166.6. Anal. ($C_{18}H_{25}N_3O_2$) C, H, N.

[1-[2-[(Methylsulfonyl)amino]ethyl]-4-piperidyl]methyl benzimidazole-4-carboxylate (19). Yield 942 mg (49%); chromatography $CHCl_3$:MeOH, from 10:1 to 4:1; mp 134–136°C (toluene); ^{1}H NMR ($CDCl_3$) δ 1.42 (qd, J = 11.9, 3.2, 2H), 1.68 (m, 1H), 1.84 (d, J = 12.6, 2H), 2.08 (td, J = 10.3, 2.4, 2H), 2.55 (t, J = 5.9, 2H), 2.91

(dm, $J=11.5$, 2H), 2.98 (s, 3H), 3.21 (t, $J=5.5$, 2H), 4.28 (d, $J=5.9$, 2H), 5.01 (br s, 1H), 7.36 (t, $J=7.5$, 1H), 7.96 (d, $J=7.5$, 1H), 8.06 (d, $J=7.9$, 1H), 8.17 (s, 1H), 10.50 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 28.9, 35.4, 39.6, 40.0, 52.9, 56.7, 69.1, 113.7, 121.7, 124.9, 125.5, 133.3, 141.4, 143.6, 166.4. Anal. ($C_{17}H_{24}N_4O_4S$) C, H, N.

2-(*N,N*-Dimethylamino)ethyl benzimidazole-4-carboxylate (20). Yield 595 mg (51%); mp 132.5–134.5°C ($CHCl_3$:Et_2O); ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 6H), 2.69 (t, $J=5.5$, 2H), 4.43 (t, $J=5.5$, 2H), 7.26 (t, $J=7.8$, 1H), 7.86 (dd, $J=7.6$, 0.8, 1H), 7.96 (dd, $J=8.0$, 0.9, 1H), 8.02 (s, 1H), 12.30 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 44.9, 57.5, 61.0, 115.0, 121.7, 125.0, 125.5, 133.9, 142.1, 144.0, 165.6. Anal. ($C_{12}H_{15}N_3O_2$) C, H, N.

2-(*N,N*-Diethylamino)ethyl benzimidazole-4-carboxylate (21). Yield 747 mg (57%), chromatography $CHCl_3$:MeOH, 9:1; mp 139–141°C ($CHCl_3$:Et_2O); ^{1}H NMR ($CDCl_3$) δ 1.06 (t, $J=7.2$, 6H), 2.67 (q, $J=7.2$, 4H), 2.81 (t, $J=6.0$, 2H), 4.43 (t, $J=5.7$, 2H), 7.26 (t, $J=7.8$, 1H), 7.87 (dd, $J=7.8$, 0.9, 1H), 7.96 (dd, $J=8.1$, 0.9, 1H), 8.01 (s, 1H), 12.34 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 11.0, 46.0, 51.1, 60.6, 114.7, 121.5, 124.8, 125.2, 133.6, 141.6, 143.8, 165.3. Anal. ($C_{14}H_{19}N_3O_2$) C, H, N.

2-Piperidinoethyl benzimidazole-4-carboxylate (22). Yield 711 mg (52%); chromatography AcOEt:EtOH, 9.5:0.5; mp 148–150°C (AcOEt); ^{1}H NMR ($CDCl_3$) δ 1.50–1.52 (m, 2H), 1.63 (quint, $J=5.1$, 4H), 2.51 (m, 4H), 2.68 (t, $J=5.7$, 2H), 4.43 (t, $J=5.7$, 2H), 7.26 (td, $J=8.1$, 1.2, 1H), 7.86 (d, $J=7.5$, 1H), 7.96 (d, $J=8.1$, 1H), 8.05 (s, 1H), 12.40 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 24.2, 26.2, 54.1, 57.1, 59.9, 115.1, 121.8, 125.1, 125.6, 133.8, 141.9, 144.2, 165.4. Anal. ($C_{15}H_{19}N_3O_2$) C, H, N.

3-Piperidinopropyl benzimidazole-4-carboxylate (23). Yield 661 mg (46%); chromatography AcOEt:EtOH, 9:1; mp 153–155°C (acetone:Et_2O); ^{1}H NMR ($CDCl_3$) δ 1.43–1.49 (m, 2H), 1.59 (quint, $J=5.7$, 4H), 1.99 (quint, $J=6.6$, 2H), 2.41 (br t, 4H), 2.49 (t, $J=6.6$, 2H), 4.42 (t, $J=6.3$, 2H), 7.31 (t, $J=7.8$, 1H), 7.94 (d, $J=7.5$, 1H), 8.01 (d, $J=7.8$, 1H), 8.15 (s, 1H), 11.30 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 24.1, 25.4, 25.7, 54.6, 56.4, 64.2, 113.9, 121.5, 125.1, 125.2, 133.0, 141.7, 143.7, 166.1. Anal. ($C_{16}H_{21}N_3O_2$) C, H, N.

(1-Butyl-4-piperidyl)methyl 6-chlorobenzimidazole-4-carboxylate (24). Yield 1.0 g (60%); chromatography $CHCl_3$:MeOH, 8:1; mp 165–167°C (acetone); ^{1}H NMR ($CDCl_3$) δ 0.90 (t, $J=7.5$, 3H, CH_3), 1.30 (sext, $J=7.2$, 2H, CH_2CH_3), 1.45–1.59 (m, 4H, NCH_2CH_2, $H_{3'ax}$, $H_{5'ax}$), 1.79–1.88 (m, 1H, $H_{4'}$), 1.81 (d, $J=11.9$, 2H, $H_{3'eq}$, $H_{5'eq}$), 1.99 (t, $J=11.5$, 2H, $H_{2'ax}$, $H_{6'ax}$), 2.34–2.39 (m, 2H, NCH_2), 3.03 (d, $J=11.5$, 2H, $H_{2'eq}$, $H_{6'eq}$), 4.26 (d, $J=5.9$, 2H, $COOCH_2$), 7.89 (s, 1H, H_5/H_7), 7.99 (s, 1H, H_5/H_7), 8.17 (s, 1H, H_2), 10.80 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 14.0 (CH_3), 20.8 (CH_2CH_3), 28.8 ($C_{3'}$, $C_{5'}$), 29.0 (NCH_2CH_2), 35.4 ($C_{4'}$), 53.3 ($C_{2'}$, $C_{6'}$), 58.7 (NCH_2), 69.8 ($COOCH_2$), 114.5 (C_4), 125.0 (C_5, C_7), 127.4 (C_6), 131.9 (C_{3a}), 142.8 (C_2), 144.5 (C_{7a}), 165.4 (COO). Anal. ($C_{18}H_{24}ClN_3O_2$) C, H, N.

[1-[2-[(Methylsulfonyl)amino]ethyl]-4-piperidyl]methyl 6-chlorobenzimidazole-4-carboxylate (25). Yield 871 mg (42%); chromatography $CHCl_3$:MeOH, 10:1; mp 137–139°C (toluene); ^{1}H NMR ($CDCl_3$) δ 1.40 (qd, $J=11.5$, 3.2, 2H), 1.73–1.92 (m, 3H), 2.09 (td, $J=11.9$, 2.0, 2H), 2.56 (t, $J=5.9$, 2H), 2.93 (dm, $J=11.1$, 2H), 2.99 (s, 3H), 3.22 (t, $J=5.5$, 2H), 4.28 (d, $J=6.3$, 2H), 5.03 (br s, 1H), 7.91 (d, $J=2.0$, 1H), 8.03 (d, $J=2.0$, 1H), 8.19 (s, 1H), 10.58 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 29.0, 35.4, 39.9, 40.2, 53.4, 57.1, 69.7, 114.7, 125.0, 127.4, 132.2, 143.4, 144.6, 165.4. Anal. ($C_{17}H_{23}ClN_4O_4S$) C, H, N.

3-Piperidinopropyl 6-chlorobenzimidazole-4-carboxylate (26). Yield 756 mg (47%); chromatography $CHCl_3$:MeOH, 9:1; mp 158–160°C (acetone); ^{1}H NMR ($CDCl_3$) δ 1.13–1.40 (m, 4H), 1.51–1.59 (m, 4H), 1.97 (quint, $J=6.3$, 2H), 2.43 (m, 4H), 2.49 (t, $J=6.7$, 2H), 4.32 (t, $J=5.9$, 2H), 7.77 (s, 1H), 7.84 (s, 1H), 8.12 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 23.7, 24.9, 25.2, 54.3, 55.7, 64.1, 114.6, 124.4, 125.1, 127.1, 131.5, 143.6, 144.5, 164.8; MS 321 (M), 210, 179, 151, 124, 98 (100), 55. Anal. ($C_{16}H_{20}ClN_3O_2$) C, H, N.

Pharmacology

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.

5-HT$_{1A}$ receptor. Binding assays were performed by a modification of the procedure previously described by Clark et al.[34] 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 28,000 *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.

5-HT$_{2A}$ receptor. Binding assays were performed according to the procedure previously described by Titeler et al.[33] The frontal cortex was homogenized in 60 volumes of ice-cold 50 mM Tris–HCl, 0.5 mM Na_2EDTA, and 10 mM $MgSO_4$ buffer (pH 7.4 at 37°C), and centrifuged at 30,000 *g* for 15 min. The membrane pellet was resuspended and incubated at 37°C for 15 min. After centrifuging at 30,000 *g* for 15 min, the

membranes were washed twice by resuspension and centrifugation, and the final pellet was resuspended in assay buffer (50 mM Tris–HCl, 0.5 mM Na_2EDTA, 10 mM $MgSO_4$, 0.1% ascorbic acid, and 10 μM pargyline, pH 7.4 at 25°C). Fractions of the final membrane suspension (about 0.5 mg of protein) were incubated at 37°C for 15 min with 0.4 nM [^{3}H]ketanserin (77 Ci/mmol), in the presence or absence of the competing drug (1 μM), in a final volume of 2 mL of assay buffer. Nonspecific binding was determined with 1 μM cinanserin and represented less than 20% of the total binding.

5-HT$_3$ receptor. Binding assays were performed according to the procedure previously described by Wong et al.[32] 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 17,000 *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 centrifugation at 48,000 *g* for 10 min. After the second wash the resuspended pellet was incubated at 37°C for 10 min, and centrifuged at 48,000 *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 30% of the total binding.

5-HT$_4$ receptor. Binding assays were performed according to the procedure previously described by Grossman et al.[31] The striatum was homogenized in 15 volumes of ice-cold 50 mM HEPES buffer (pH 7.4 at 4°C) and centrifuged at 48,000 *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 [^{2}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 40% 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$_{1A}$, 5-HT$_{2A}$, 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.[35] The protein concentrations of the rat cerebral cortex and the rat striatum were determined by the method of Lowry,[58] 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). According to Eglen et al.,[19] portions of ileum were dissected 1.5 cm distal to ileocecal junction and whole segments of 2 cm long were gently flushed with Krebs solution and suspended, under 1 g tension, in an organ bath containing 5 mL of Krebs solution at 32°C and gassed with 95% O_2:5% CO_2. Methysergide was added at a concentration of 10^{-6} M, as described by Smith et al.,[59] in order to exclude any potential effects of 5-HT$_1$ or 5-HT$_2$ stimulation.

Ileal responses were measured by determining changes in isometric tension recorded with force transducers connected to an Omniscribe polygraph. In all experiments tissues were exposed to 50 mM KCl (3 min) to obtain an estimate of the maximal size of contraction of the preparation. After 15 min of stabilization concentration–response curves to 5-HT were constructed in a non-cumulative fashion (10^{-8} to 3×10^{-6} M) with an agonist exposure period of 30 s on a 5 min dose cycle. After the final 5-HT exposure the bathing solution was replaced and the ileum was incubated during a period of 60 min with an antagonist and, subsequently, the concentration–response curve was reconstructed. One dose of one antagonist was tested in each ileal segment. Parallel control studies were undertaken to discard changes in sensitivity of the tissues to 5-HT.

Biphasic concentration–response curves were obtained and ED_{50} for 5-HT and pA_2 for tested antagonists were calculated for the first phase of the curve ([5-HT] $< 10^{-6}$ M) considering 100% the contraction induced by 3×10^{-7} M 5-HT in control curves.

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