2,3-Dihydro-2-oxo-1*H*-benzimidazole-1-carboxamides with Selective Affinity for the 5-HT$_4$ Receptor: Synthesis and Structure−Affinity and Structure−Activity Relationships of a New Series of Partial Agonist and Antagonist Derivatives

Inés Tapia, Luisa Alonso-Cires, Pedro Luis López-Tudanca, Ramón Mosquera, Luis Labeaga, Ana Innerárity, and Aurelio Orjales*

Departamento de Investigación, FAES, S.A., Máximo Aguirre 14, 48940 Leioa, Vizcaya, Spain

Received February 22, 1999

A series of 2,3-dihydro-2-oxo-1*H*-benzimidazole-1-carboxamide derivatives bearing a piperazine moiety was synthesized. Their in vitro 5-HT$_4$, 5-HT$_3$, and D$_2$ receptors affinities were evaluated by radioligand binding assay. For selected compounds functional studies at the 5-HT$_4$ receptor were made by using precontracted (by carbachol) preparations of rat esophageal tunica muscularis mucosae (TMM). The influence of the 3-substituent of the benzimidazole ring, the 4-substituent of the piperazine moiety, and the alkylene spacer was studied. Compounds with an ethyl or a cyclopropyl substituent in the 3-position of the benzimidazole ring showed moderate to high affinity (K_i = 6.7−75.4 nM) for the 5-HT$_4$ receptor with selectivity over 5-HT$_3$ and D$_2$ receptors and moderate antagonist activity (pK_b = 6.19−7.73). Compounds with an isopropyl substituent in the 3-benzimidazole position exhibited moderate and selective 5-HT$_4$ affinity ($K_i \geq$ 38.9 nM) and a partial agonist activity (**5a**, i.a. = 0.94) higher than that of the reference compound BIMU 8 (i.a. = 0.70). This reversal of the pharmacological activity due only to a small structural difference might confirm the existence of two binding sites on the 5-HT$_4$ receptor. In the alkylene spacer, a two-methylene chain is favorable to optimize the affinity and the antagonist or the partial agonist activity. In the ethyl and cyclopropyl series, 5-HT$_4$ antagonist activity seems to be unrelated to the size of the 4-substituent of the piperazine moiety, whereas a methyl group is optimal for high partial agonist activity in the isopropyl series; however, the presence of a butyl substituent is a favorable pattern for 5-HT$_4$ antagonism and even causes a reversal of the pharmacological profile in the isopropyl series (**5h**, pK_b = 7.94). *N*-Butyl quaternization of **5a** led to an improvement in affinity for the 5-HT$_4$ receptor and mantained the high partial agonist activity (**5r**, K_i = 66.3 nM, i.a. = 0.93).

Introduction

The 5-HT$_4$ receptor was discovered in 1987, and shortly later it was shown that a series of gastrointestinal prokinetic benzamide derivatives (metoclopramide, renzapride, cisapride, etc.) act as agonists at the 5-HT$_4$ receptor.[1−3] Since then, the 5-HT$_4$ receptor has been located in the central nervous system (CNS) (limbic system, hippocampo−habenulo−interpeduncular pathway, striato−nigro−tectal pathway) and peripheral tissues (gastrointestinal tract, heart, adrenal gland, urinary bladder).[4−7] Considerable progress has been made with the development of specific radioligands, such as [^{3}H]GR 113808 and [^{125}I]SB 207710.[8,9] The 5-HT$_4$ receptor was cloned from rat brain in 1995, and it was found that it exists in two isoforms (5-HT$_{4S}$ and 5-HT$_{4L}$) that differ in the length and sequence of their carboxy termini.[10,11] Recently, the molecular structure and functional characterization of four splice variants of the human 5-HT$_4$ receptor have been described.[12]

To date, several 5-HT$_4$ receptor antagonists and agonists have been discovered.[13,14] The chemical structures of the 5-HT$_4$ antagonists belong to five different classes including indolecarboxylates, benzoates, aryl ketones, imidazolopyridines, and benzimidazolones. On the other hand, there are six distinct classes of 5-HT$_4$ agonists including indoles, benzamides, benzoates, aryl ketones, 8-naphthalimides, and benzimidazolones. Some of the most known 5-HT$_4$ ligands are included in Figures 1 and 2.

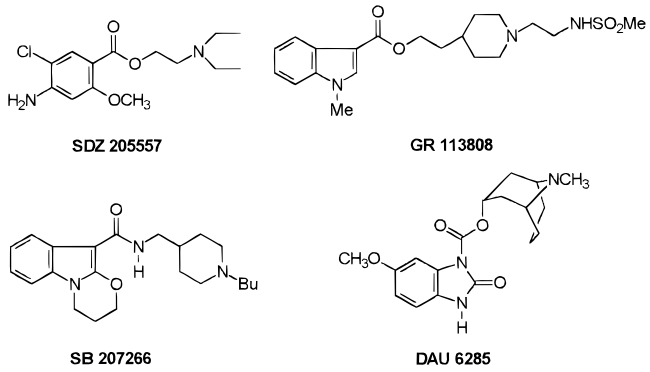


Figure 1. 5-HT$_4$ receptor antagonists.

Recently, three-dimensional maps of the 5-HT$_4$ agonist and antagonist recognition sites have been proposed.[14−16] The structural features that define the 5-HT$_4$ pharmacophore can be regarded as an aromatic moiety, a coplanar carbonyl function, and a basic nitrogen atom; distances between pharmacophoric elements and their spatial orientations have been determined. For both 5-HT$_4$ receptor agonist and antagonist recognition sites, the existence of a hydrophobic pocket which would

10.1021/jm981098j CCC: $18.00
Published on Web 07/02/1999

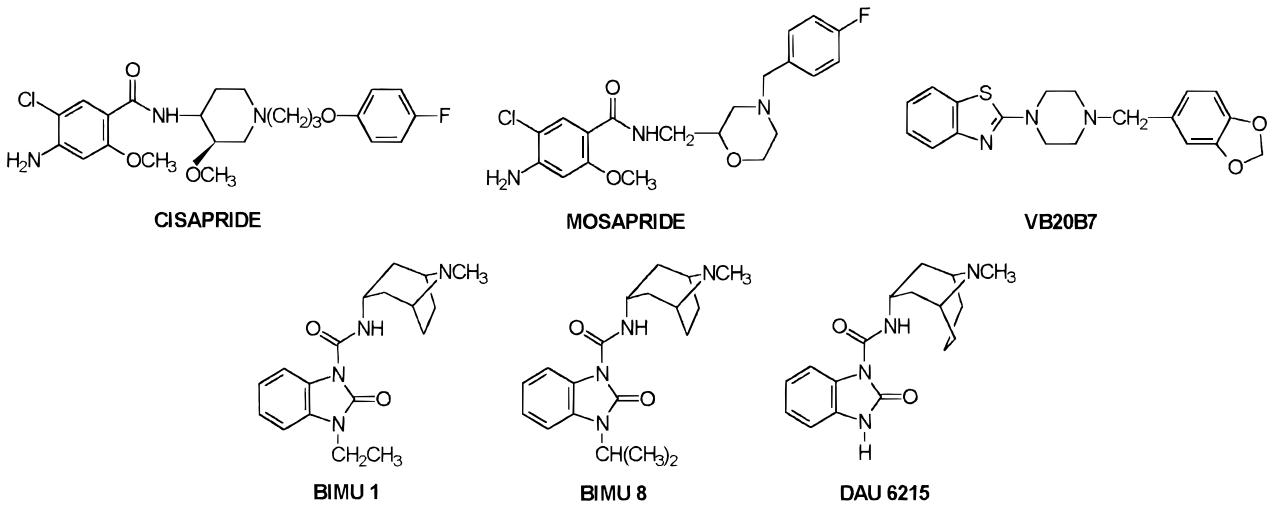


Figure 2. 5-HT_4 receptor agonists and DAU 6215 (5-HT_3 receptor antagonist).

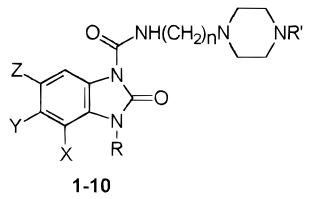


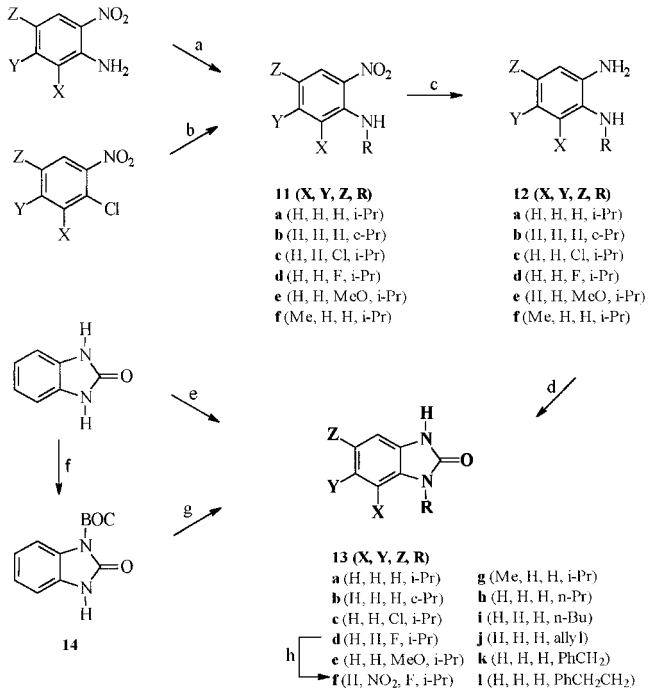
Figure 3. 2,3-Dihydro-2-oxo-1*H*-benzimidazole-1-carboxamides **1**−**10**.

interact with the voluminous substituents of the basic nitrogen of active ligands is accepted. On the other hand, the existence of two binding sites at the 5-HT_4 receptor has been suggested to explain the agonist and antagonist activities of compounds with structural similarity.[17]

With regard to benzimidazolone derivatives, it has been reported that members of this class of compounds interact with both 5-HT_3 and 5-HT_4 receptors with different profiles of agonist or antagonist activity and are devoid of affinity for the dopamine receptors.[18,19] Among them, DAU 6215 (Figure 2) is a selective 5-HT_3 receptor antagonist, whereas affinities of DAU 6285 (Figure 1) for 5-HT_3 and 5-HT_4 receptors are of the same order of magnitude and it behaves as a competitive 5-HT_4 antagonist.[20] In addition, BIMU 1 and BIMU 8 (Figure 2) combine 5-HT_3 antagonism and 5-HT_4 agonism.[21−23]

Although the 5-HT_4 agonism or antagonism of some benzimidazolones is well-known, very little has been published about their 5-HT_4 receptor affinity and their structure−affinity relationships.[24] It is possible that the combination of 5-HT_3 antagonism and 5-HT_4 agonism will have advantages over a selective 5-HT_4 agonist action in some areas.[25] However, selective 5-HT_4 receptor ligands in this series could provide a useful tool to investigate the pharmacology of the 5-HT_4 receptor and to develop new therapies for the treatment of CNS disorders (cognition, emotional and motor functional control) and other peripherical disorders (irritable bowel syndrome, arrhythmia, urinary disturbance). Our aim was to find new benzimidazolones with affinity for the 5-HT_4 receptor and selectivity versus 5-HT_3 and D_2 receptors. In the present paper, we report the synthesis and the binding profile at 5-HT_4, 5-HT_3, and D_2 receptors of a series of 2,3-dihydro-2-oxo-1*H*-benzimidazole-1-carboxamides including a piperazine moiety, **1**−**10** (Figure 3). We have studied the influence on the 5-HT_4 receptor affinity of each of these elements: the 3-substituent of the benzimidazolone ring, the 4-substituent of the piperazine moiety, and the alkylene spacer. In addition the functional activity at 5-HT_4 receptors has been also evaluated. Preliminary results in structure−affinity and structure−activity relationships are presented.

Scheme 1[a]



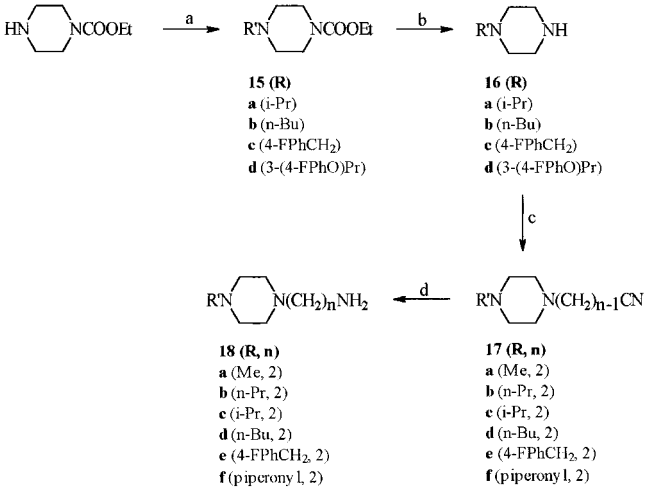
[a] Reagents: (a) i, 2,2-dimethoxypropane, TFA, ii, H_3B−pyridine; (b) cyclopropylamine; (c) H_2, 10% Pd/C, or Zn, 1 N NaOH, EtOH; (d) CDI, THF; (e) HNa, DMF, RI; (f) HNa, DMF, (^{t}BOC)$_2$O; (g) RBr, K_2CO_3, DMF; (h) HNO_3, Ac_2O.

Chemistry

2,3-Dihydro-2-oxo-1*H*-benzimidazole-1-carboxamides **1**−**10** were prepared by using 2,3-dihydro-2-oxo-1*H*-benzimidazoles **13** and appropriate aminoalkylpiperazines **18** as key intermediates (Schemes 1−3).

According to Scheme 1 reductive amination of commercially available 2-nitroanilines by using 2,2-dimeth-

Scheme 2[a]



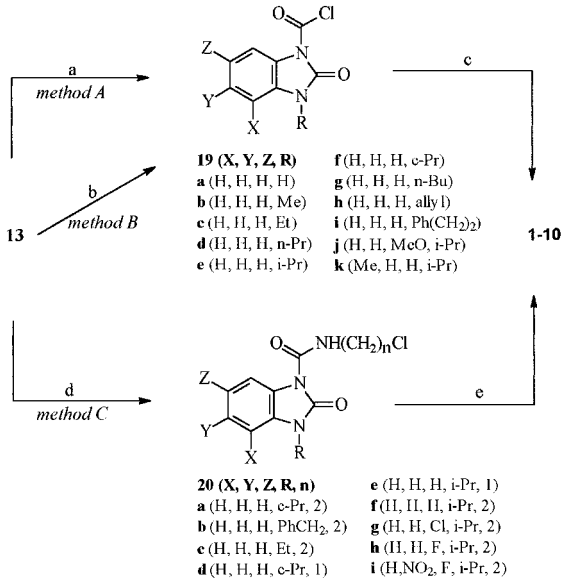
[a] Reagents: (a) K_2CO_3, CH_3CN, R′Br; (b) 48% HBr; (c) K_2CO_3, CH_3CN, $Cl(CH_2)_{n-1}CN$; (d) H_4LiAl, Et_2O.

oxypropane/trifluoroacetic acid and a boron−pyridine complex yielded the isopropyl derivatives **11** (R = *i*-Pr). Cyclopropyl derivatives **11** (R = *c*-Pr) were prepared following a previously described route:[26] 2-chloronitrobenzene was heated with cyclopropylamine in a closed vessel to afford *N*-cyclopropyl-2-nitroaniline **11b** (X, Y, Z = H; R = *c*-Pr). Compounds **11** (R = *i*-Pr, *c*-Pr) were hydrogenated over palladium/carbon to give *o*-phenylenediamines **12** (R = *i*-Pr, *c*-Pr) which, by cyclization with *N*,*N*′-carbonyldiimidazole, gave 1-alkyl-2,3-dihydro-2-oxo-1*H*-benzimidazoles **13** (R = *i*-Pr, *c*-Pr). Alkylation of commercially available 2,3-dihydro-2-oxo-1*H*-benzimidazole with sodium hydride and the corresponding alkyl halide provided a mixture of mono- and dialkylated derivatives; isolation of monoalkylated derivatives **13** (R = *n*-Pr, *n*-Bu, allyl, $PhCH_2$) was accomplished by flash chromatography. Treatment of 2,3-dihydro-2-oxo-1*H*-benzimidazole with di-*tert*-butyl dicarbonate according to reported conditions[27] yielded the alkoxycarbonyl-protected derivatives **14**, which were alkylated with an alkyl halide; the spontaneous elimination of the protecting group BOC provided derivatives **13** (R = $PhCH_2CH_2$).

Aminoalkylpiperazines **18** were prepared following standard procedures[28] (Scheme 2). Alkylation of *N*-carboxyethylpiperazine with a bromoalkane in acetonitrile in the presence of potassium carbonate afforded the corresponding derivatives **15**, which, after hydrolysis with 48% HBr, yielded the alkylpiperazines **16**; by alkylation with chloroalkylnitriles in acetonitrile and potassium carbonate, compounds **16** were converted to **17**, which, after reduction with lithium aluminum hydride in ether, provided the amines **18**.

2,3-Dihydro-2-oxo-1*H*-benzimidazole-1-carboxamides **1**−**10** were prepared through different routes of synthesis (Scheme 3). Chlorocarbonyl derivatives **19** were obtained according to previously reported conditions by employing trichloromethyl chloroformate in the presence of activated charcoal[18] (method A) or by reaction with sodium hydride and phosgene[29] (method B). Coupling with substituted piperazines **18** yielded 2,3-dihydro-2-oxo-1*H*-benzimidazole-1-carboxamides **1**−**10**. Reaction of 2,3-dihydro-2-oxo-1*H*-benzimidazoles **13** with chloroalkyl isocyanates[30] provided chloroalkyl

Scheme 3[a]



[a] Reagents: (a) $ClCO_2CCl_3$, THF, charcoal; (b) 60% HNa, THF, phosgene; (c) **18**, TEA, THF; (d) $Cl(CH_2)_nNCO$, toluene; (e) K_2CO_3, toluene, KI, **16**, or TEA, CH_3CN, KI, **16**.

derivatives **20** (method C), which were converted into the 2,3-dihydro-2-oxo-1*H*-benzimidazole-1-carboxamides **1**−**10** by reaction with substituted piperazines **16**.

Pharmacology

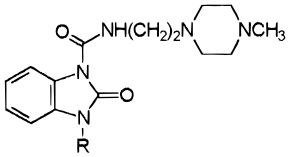
The new benzimidazolone derivatives were initially evaluated for in vitro 5-HT_4 receptor affinity by radioligand binding assay. For each compound the ability to displace the specific ligand [^{3}H]GR 113808 from 5-HT_4 receptors of guinea pig striatum was determined.[31] Concentrations required to inhibit 50% of radioligand specific binding (IC_{50}) and K_i values were calculated from two separate competition experiments with samples in triplicate, using 10−12 different concentrations of displacer. Compounds with moderate to high affinity were selected for functional studies at the 5-HT_4 receptor by using rat isolated thoracic esophageal tunica muscularis mucosae (TMM) precontracted by carbachol.[32,33]

In agonism studies, responses to the cumulative addition of 5-HT or agonist were expressed as percentage relaxation of carbachol-induced tone. For each agonist, the negative logarithm of the molar concentration that relaxated 50% relative to their individual maximun effect (pEC_{50}) was calculated. The intrinsic activity (i.a.) versus 5-HT was determined comparing the maximun relaxation obtained at the concentration of 1 or 3 μM in the same TMM preparation.

In antagonism studies, the effect of the antagonists on 5-HT pEC_{50} values was registered, and the values were compared by using the unpaired Student's *t*-test. A *p* value of 0.05 or less was considered statistically significant. The apparent affinity (pK_b) for antagonists against 5-HT was determined.

For each compound, the ability to displace the specific ligands [^{3}H]LY 278584 and [^{3}H]raclopride from 5-HT_3 sites of rat entorhinal cortex or dopamine D_2 sites of rat striatum, respectively, was also determined.[34,35] No functional studies were carried out with the compounds that exhibited affinity at these receptors.

Table 1. 2,3-Dihydro-2-oxo-1*H*-benzimidazole-1-carboxamides



compd	R	synthetic method	mp, °C	formula	5-HT$_4$ binding K_i (nM) ± SE[a]	5-HT$_3$ binding IC_{50} (nM)	5-HT$_4$ antagonism pK_b ± SE[a]	5-HT$_4$ agonism i.a.
1	H	A	188−190	$C_{15}H_{21}N_5O_2$	≃1000[b]	>1000	<6.0	0.35
2	Me	A	87−90	$C_{16}H_{23}N_5O_2$	305.8 ± 20.8	>1000	<6.0	0.42
3a	Et	A	194−196[c]	$C_{17}H_{25}N_5O_2 \cdot 2C_4H_4O_4$	75.4 ± 19.9	>1000	6.79 ± 0.12	<0.30
4	*n*-Pr	A	205−208[c]	$C_{18}H_{27}N_5O_2$	252.9 ± 54.4	>1000	<6.0	0.48
5a	*i*-Pr	A	126−128	$C_{18}H_{27}N_5O_2$	91.1 ± 10.8	>1000	<6.0	0.94
6a	*c*-Pr	C	104−108	$C_{18}H_{25}N_5O_2$	10.2 ± 3.2	>1000	7.24 ± 0.18	0.36
7	*n*-Bu	A	202−204[c]	$C_{19}H_{29}N_5O_2 \cdot 2C_4H_4O_4$	>1000[b]	>1000	<6.0	<0.30
8	allyl	A	198−201[c]	$C_{18}H_{25}N_5O_2 \cdot 2C_4H_4O_4$	>1000[b]	>1000	<6.0	0.63
9	$PhCH_2$	C	110−113	$C_{22}H_{27}N_5O_2$	>1000[b]	>1000	<6.0	<0.30
10	$Ph(CH_2)_2$	A	212 dec[d]	$C_{23}H_{29}N_5O_2 \cdot 2HCl$	>1000[b]	>1000	<6.0	<0.30
BIMU 8					63.9 ± 14.8	0.5 ± 0.1[e]	<6.0	0.70
GR 113808					0.11 ± 0.01	>1000	9.49 ± 0.06	NT

[a] Standard error. [b] IC_{50} (nM). [c] Fumarate. [d] Hydrochloride. [e] K_i (nM).

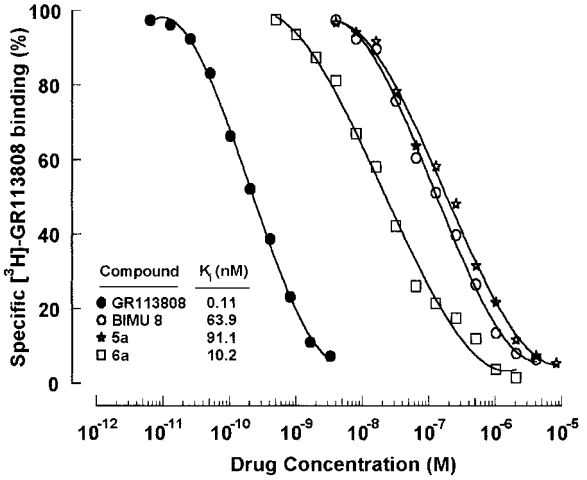


Figure 4. Competition for [^{3}H]GR 113808 binding sites in guinea pig striatum by compounds **5a**, **6a**, GR 113808, and BIMU 8.

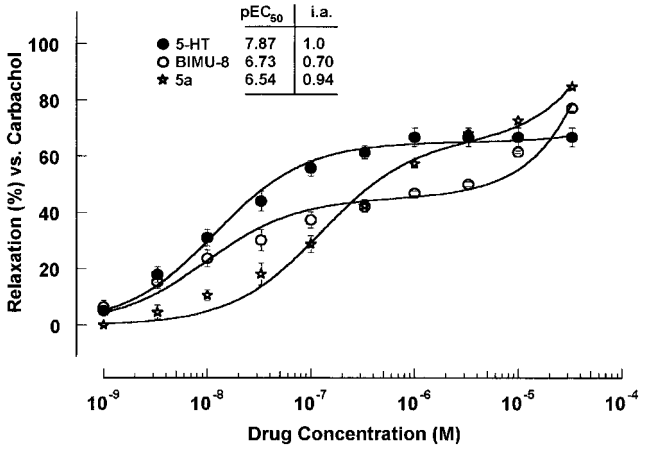


Figure 5. Concentration−relaxation curves to 5-HT, BIMU 8, and compound **5a** in rat isolated TMM. Values are mean, with SEM, $n = 2-3$.

Results and Discussion

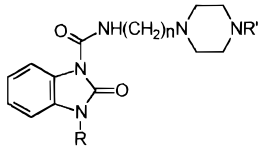
Pharmacological results are reported in Tables 1−4.

To study the effect of the 3-substituent of the benzimidazolone ring and because of the synthetic availability, we fixed the 4-methylpiperazine moiety and a two-methylene spacer as our starting point. Examination of the results in Table 1 showed that the nature of the substituent in the 3-position of the benzimidazolone ring was critical for 5-HT$_4$ receptor affinity. Only compounds with ethyl (**3a**, $K_i = 75.4$ nM), isopropyl (**5a**, $K_i = 91.1$ nM), and cyclopropyl (**6a**, $K_i = 10.2$ nM) substituents showed moderate to high affinity for the 5-HT$_4$ receptor, and **6a** was even more potent than the reference benzimidazolone BIMU 8 ($K_i = 63.9$ nM), although it showed weaker affinity than GR 113808 ($K_i = 0.11$ nM) (Figure 4). Replacement by other small groups such as hydrogen (**1**, $IC_{50} \simeq 1\ \mu$M), methyl (**2**, $K_i = 305.8$ nM), or propyl (**4**, $K_i = 252.9$ nM) led to a large decrease in affinity. In addition, the introduction of bulkier groups, e.g., butyl (**7**), benzyl (**9**), phenylethyl (**10**), severely reduced 5-HT$_4$ receptor affinity ($IC_{50} > 1\ \mu$M).

All compounds showed a remarkable improvement in selectivity versus 5-HT$_3$ receptor ($IC_{50} > 1\ \mu$M) with respect to the reference compound BIMU 8. It can therefore be concluded that in the benzimidazolone series replacement of the rigid tropane ring (BIMU 8 and BIMU 1) by a flexible piperazine moiety significantly reduced or abolished 5-HT$_3$ affinity while retained the 5-HT$_4$ affinity. No compound displayed significant affinity for the D_2 receptor ($IC_{50} > 1\ \mu$M, data not shown).

The 5-HT$_4$ receptor activity was also dependent on the substituent at the 3-position of the benzimidazolone ring. Ethyl and cyclopropyl derivatives **3a** and **6a** showed moderate antagonist activity (p$K_b = 6.79$ and 7.24, respectively). However, isopropyl derivative **5a** and BIMU 8 acted as partial agonists, showing a classical biphasic concentration−relaxation curve with pEC_{50} values of 6.54 and 6.73 and with an intrinsic activity of 0.94 and 0.70, respectively (Figure 5). At concentrations higher than 1 μM, both compounds showed 5-HT$_4$-independent relaxation effects, probably due to a muscarinic cholinoceptor blockade.[33] The 5-HT$_4$ antagonist GR 113808 induced a parallel, dextral shift of the concentration−relaxation curves for 5-HT, **5a**, and BIMU 8 in the rat TMM with pK_b values from 9.1 to 9.5 (data not shown). The observed change on the pharmacological profile of benzimidazolones **1**−**10** sug-

Table 2. 2,3-Dihydro-2-oxo-1*H*-benzimidazole-1-carboxamides: 3-Ethyl and 3-Cyclopropyl Derivatives



compd	R	*n*	R′	synthetic method	mp, °C	formula	5-HT$_4$ binding K_i (nM) ± SE[a]	5-HT$_3$ binding IC_{50} (nM)	5-HT$_4$ antagonism pK_b ± SE[a]	5-HT$_4$ agonism i.a.
3a	Et	2	Me	A	194−196[b]	$C_{17}H_{27}N_5O_2 \cdot 2C_4H_4O_4$	75.4 ± 19.9	>1000	6.79 ± 0.12	<0.30
3b	Et	2	Et	C	90−93	$C_{18}H_{27}N_5O_2$	17.9 ± 1.9	>1000	6.89 ± 0.14	<0.30
3c	Et	2	*n*-Pr	A	97−100	$C_{19}H_{29}N_5O_2$	16.9 ± 2.0	>1000	7.63 ± 0.12	<0.30
3d	Et	2	*i*-Pr	C	208−210[b]	$C_{19}H_{29}N_5O_2 \cdot 2C_4H_4O_4$	25.2 ± 2.5	>1000	7.42 ± 0.10	<0.30
3e	Et	2	*n*-Bu	A	203−205[b]	$C_{20}H_{31}N_5O_2 \cdot 2C_4H_4O_4$	34.1 ± 2.0	>1000	7.73 ± 0.18	<0.30
6a	*c*-Pr	2	Me	C	104−108	$C_{18}H_{25}N_5O_2$	10.2 ± 3.2	>1000	7.24 ± 0.18	0.36
6b	*c*-Pr	2	*n*-Pr	C	62−64	$C_{20}H_{29}N_5O_2$	8.2 ± 1.0	>1000	7.46 ± 0.16	0.41
6c	*c*-Pr	2	*i*-Pr	B	oil	$C_{20}H_{29}N_5O_2$	6.7 ± 0.4	>1000	7.38 ± 0.15	<0.30
6d	*c*-Pr	2	*n*-Bu	B	oil	$C_{21}H_{31}N_5O_2$	13.3 ± 0.9	>1000	7.51 ± 0.20	0.46
6e	*c*-Pr	1	Me	C	86−88	$C_{17}H_{23}N_5O_2$	49.4 ± 8.2	>1000	6.19 ± 0.10	0.53
6f	*c*-Pr	3	Me	B	>250[c]	$C_{19}H_{27}N_5O_2 \cdot 2HCl$	39.7 ± 7.4	>1000	6.40 ± 0.09	0.46

[a] Standard error. [b] Fumarate. [c] Hydrochloride.

gests that the mode of binding might not be identical through the series. A similar dramatic variation in the 5-HT$_4$ pharmacological activity due only to a small structural modification has been previously reported for benzoate derivatives; as a consequence, a hypothetical model for the 5-HT$_4$ receptor with two sites for the binding of agonist and antagonist molecules was proposed.[17]

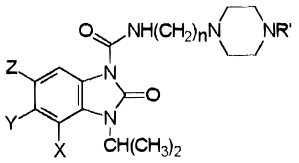
These findings led us to evaluate a series of 3-ethyl- and 3-cyclopropylbenzimidazolone derivatives (Table 2). According to previously proposed 5-HT$_4$ receptor models, a fairly large lipophilic region is located near the basic nitrogen recognition sites, and increasing the size and lipophilic character of the side chain may result in increased affinity.[16] The first modification, therefore, was to increase the size of the 4-substituent of the piperazine moiety. Replacement of the methyl group in compound **3a** (K_i = 75.4 nM) by ethyl or propyl groups (**3b**, K_i = 17.9 nM; **3c**, K_i = 16.9 nM, respectively) led to an increased affinity for the 5-HT$_4$ receptor. However, the introduction of an isopropyl or a butyl group (**3d**, K_i = 25.2 nM; **3e**, K_i = 34.1 nM, respectively) did not improve the affinity of **3b**. In the cyclopropyl series the replacement of the methyl group in **6a** (K_i = 10.2 nM) by a propyl (**6b**, K_i = 8.2 nM), isopropyl (**6c**, K_i = 6.7 nM), or butyl (**6d**, K_i = 13.3 nM) group did not produce a significant change on the affinity for the 5-HT$_4$ receptor. These data also showed that a cyclopropyl substituent in the 3-position of the benzimidazolone ring is better than an ethyl group for 5-HT$_4$ receptor affinity. On the other side, these structural modifications had divergent and slight influence on 5-HT$_4$ antagonist activity. Although butyl derivatives **3e** and **6d** displayed moderate 5-HT$_4$ affinity, they showed the highest 5-HT$_4$ antagonist values (pK_b = 7.73, 7.51 respectively). This result agrees with the hypothesized positive steric interaction between the *n*-butyl group and the hydrophobic pocket of the 5-HT$_4$ receptor in compounds with 5-HT$_4$ antagonist activity.[16] Besides, propyl derivatives **3c** and **6b** (pK_b = 7.63 and 7.46) did not show remarkable differences on the antagonist activity with respect to the related isopropyl derivatives **3d** and **6c** (pK_b = 7.42 and 7.38) and methyl analogues **3a** and **6a** (pK_b = 6.79 and 7.24). Moreover, improvement on the affinity did not always imply a correlative improvement on the 5-HT$_4$ antagonism, e.g., compare **3b** and **3e** or **6c** and **6d**. Since those compounds were approximately equipotent, antagonist activity for the 5-HT$_4$ receptor seems to be unrelated to the size of the 4-substituent of the piperazine moiety in the ethyl and cyclopropyl series.

The modification of the alkylene spacer was the next step to be studied. The shortening of the two-methylene chain of derivative **6a** led to a decrease in affinity (**6e**, K_i = 49.4 nM) and also reduced the antagonist activity (pK_b = 6.19). On the other hand, the lengthening to a three-methylene chain caused a similar reduction in both affinity and antagonist activity (**6f**, K_i = 39.7 nM, pK_b = 6.40). Consequently, in this series a chain length of two methylenes seem to be optimal for high affinity and antagonist activity at the 5-HT$_4$ receptor site.

Similar structural modifications were also carried out on the 3-isopropyl derivative **5a** (Table 3). As a general trend a significant reduction in 5-HT$_4$ receptor affinity with regard to the corresponding 3-ethyl and 3-cyclopropyl derivatives was observed, with a partial agonist profile comparable to that of the reference compound BIMU 8 (K_i = 63.9 nM, i.a. = 0.70). Surprisingly, butyl derivative **5h** (K_i = 38.9 nM, pK_b = 7.94) showed the most potent 5-HT$_4$ antagonist activity in our series, which confirms the key role of the butyl group in the antagonist profile for the 5-HT$_4$ receptor.

Replacement in derivative **5a** of the methyl substituent at the 4-position of the piperazine by ethyl (**5e**, K_i = 103.6 nM, i.a. = 0.87), propyl (**5f**, K_i = 87.3 nM, i.a. = 0.68), or isopropyl (**5g**, K_i = 89.8 nM, i.a. = 0.66) substituents retained affinity for the 5-HT$_4$ receptor and caused a reduction in partial agonist activity. 4-Fluorophenoxypropyl, 4-fluorophenylmethyl, and piperonyl groups have shown to be excellent substituents for 5-HT$_4$ ligands with agonist activity (Figure 2). The introduction of these substituents at the 4-position of the piperazine led to **5i** (K_i = 375 nM, i.a. = 0.78), **5j** (K_i = 262 nM, i.a. = 0.78), and **5k** (IC_{50} > 1 μM, i.a. = 0.30) derivatives with reduced affinity for the 5-HT$_4$ receptor and a decrease in the partial agonist activity. These data suggest the existence of steric limitations around the 4-substituent of the piperazine moiety for 5-HT$_4$ agonism and that a methyl group seems to be optimal in terms of both affinity and agonist activity at the 5-HT$_4$ receptor site.

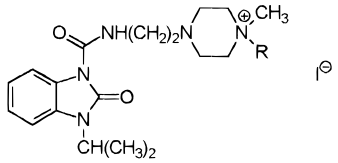
Table 3. 2,3-Dihydro-2-oxo-1*H*-benzimidazole-1-carboxamides: 3-Isopropyl Derivatives



compd	X	Y	Z	*n*	R′	synthetic method	mp, °C	formula	5-HT$_4$ binding K_i (nM) ± SE[a]	5-HT$_3$ binding IC$_{50}$ (nM)	5-HT$_4$ antagonism pK_b ± SE[a]	5-HT$_4$ agonism i.a.
5a	H	H	H	2	Me	A	126−128	$C_{18}H_{27}N_5O_2$	91.1 ± 10.8	>1000	<6.0	0.94
5b	H	H	H	0	Me	A	141−143	$C_{16}H_{23}N_5O_2$	>1000[b]	200.8[c]	<6.0	<0.30
5c	H	H	H	1	Me	C	71−73	$C_{17}H_{25}N_5O_2$	>1000[b]	309.0[c]	<6.0	<0.30
5d	H	H	H	3	Me	A	>240[d]	$C_{19}H_{29}N_5O_2$·2HCl	>1000[b]	>1000	<6.0	<0.30
5e	H	H	H	2	Et	C	190−193[e]	$C_{19}H_{29}N_5O_2$·2$C_4H_4O_4$	103.6 ± 7.8	>1000	6.62 ± 0.13	0.87
5f	H	H	H	2	*n*-Pr	C	202−205[e]	$C_{20}H_{31}N_5O_2$·2$C_4H_4O_4$	87.3 ± 10.3	>1000	6.89 ± 0.19	0.68
5g	H	H	H	2	*i*-Pr	C	210−212[e]	$C_{20}H_{31}N_5O_2$·2$C_4H_4O_4$	89.8 ± 6.0	>1000	<6.0	0.66
5h	H	H	H	2	*n*-Bu	A	210−212[e]	$C_{21}H_{33}N_5O_2$·2$C_4H_4O_4$	38.9 ± 3.6	>1000	7.94 ± 0.16	<0.30
5i	H	H	H	2	4-FPhO(CH$_2$)$_3$	B	90−94	$C_{26}H_{34}FN_5O_3$	375.0 ± 23.7	>1000	6.11 ± 0.15	0.78
5j	H	H	H	2	4-FPhCH$_2$	A	206−208[e]	$C_{24}H_{30}FN_5O_2$·2$C_4H_4O_4$	262.0 ± 17.9	>1000	<6.0	0.78
5k	H	H	H	2	piperonyl	C	218 dec[e]	$C_{25}H_{31}N_5O_4$·2$C_4H_4O_4$	>1000[b]	>1000	<6.0	0.30
5l	H	H	Cl	2	Me	C	134−137	$C_{18}H_{26}ClN_5O_2$	>1000[b]	>1000	<6.0	0.70
5m	H	H	F	2	Me	C	130−134	$C_{18}H_{26}FN_5O_2$	108.4 ± 11.0	>1000	6.88 ± 0.11	0.67
5n	H	H	MeO	2	Me	B	109−111	$C_{19}H_{29}N_5O_3$	>1000[b]	>1000	<6.0	<0.30
5o	H	NO_2	F	2	Me	C	198−201	$C_{18}H_{25}FN_6O_4$	>1000[b]	>1000	<6.0	<0.30
5p	H	NH_2	F	2	Me	*f*	84 dec	$C_{18}H_{27}FN_6O_2$	>1000[b]	>1000	<6.0	<0.30
5q	Me	H	H	2	Me	B	82−84	$C_{19}H_{29}N_5O_2$	216.4 ± 15.3	73.9[c]	<6.0	<0.30

[a] Standard error. [b] IC$_{50}$ (nM). [c] K_i (nM). [d] Hydrochloride. [e] Fumarate. [f] Reduction of **5o** with Zn/1 N NaOH.

Table 4. 2,3-Dihydro-2-oxo-1*H*-benzimidazole-1-carboxamides: Quaternary Derivatives



compd	R	synthetic method	mp, °C	formula	5-HT$_4$ binding K_i (nM) ± SE	5-HT$_3$ binding IC$_{50}$ (nM)	5-HT$_4$ antagonism pK_b ± SE	5-HT$_4$ agonism i.a.
5r	*n*-Bu	D	170−172	$C_{22}H_{36}IN_5O_2$	66.3 ± 4.6	>1000	6.0 ± 0.23	0.93
5s	Me	D	60 dec	$C_{19}H_{30}IN_5O_2$	68.5 ± 26.8	>1000	<6.0	0.75
5t	allyl	E	177−179	$C_{21}H_{32}IN_5O_2$	56.3 ± 6.00	>1000	6.28 ± 0.17	<0.30

Modifications of the spacer in the parent compound **5a** led to derivatives **5b**, **5c**, and **5d** with a remarkable decrease in the 5-HT$_4$ affinity (IC$_{50}$ > 1 μM) and slight reduction in the 5-HT$_4$ versus 5-HT$_3$ receptor selectivity. As observed previously for ethyl and cyclopropyl derivatives, the length of the alkylene chain is an important structural feature in recognition by the 5-HT$_4$ receptor binding site. A two-methylene spacer is also optimal for high 5-HT$_4$ agonist activity.

Introduction of substituents on the benzene ring had a negative effect on 5-HT$_4$ receptor affinity, except for derivative **5m** (K_i = 108.4 nM) which has a fluorine in the 6-position. The structurally related benzimidazolone DAU 6285 has a methoxy group in the 6-position and behaves as a 5-HT$_4$ antagonist.[20] A similar substitution in this series led to derivative **5n** (IC$_{50}$ > 1 μM) with a remarkable reduction in the affinity for the 5-HT$_4$ receptor. It can be concluded that the 5-HT$_4$ receptor can only accommodate small substituents in the position of the benzene ring in our series. Therefore, it seems that the electronic distribution within the benzimidazole ring has a small effect on the 5-HT$_4$ receptor affinity, whereas steric hindrance might play a significant role.

It has been reported that increasing the polarity by *n*-butyl quaternization of renzapride increases the 5-HT$_4$ receptor agonist potency.[36] Several quaternary derivatives of **5a** were prepared (Table 4). All of them showed higher affinity for the 5-HT$_4$ receptor (K_i = 56.3−68.5 nM) than **5a**, but while the butyl derivative **5r** (i.a. = 0.93) retained the partial agonist activity, the methyl derivative **5s** (i.a. = 0.75) showed a significant decrease in the partial agonist activity and the allyl derivative **5t** (i.a. < 0.30) was only weakly active as a 5-HT$_4$ agonist.

Conclusion

1. The 2,3-dihydro-2-oxo-1*H*-benzimidazole-1-carboxamides including a piperazine moiety described in this paper represent a novel class of ligands at the 5-HT$_4$ receptor with selectivity over 5-HT$_3$ and D$_2$ receptors.

2. The 3-substituent at the benzimidazolone ring is a determinant structural feature for both binding and pharmacological profile. 3-Ethyl and 3-cyclopropyl derivatives showed high affinity (**6c**, K_i = 6.7 nM) and moderate antagonist activity (**3e**, pK_b = 7.73), whereas 3-isopropyl analogues exhibited moderate affinity and high partial agonist activity (**5a**, K_i = 91.1 nM, i.a. = 0.94). The reversal of the pharmacological activity due only to a small structural difference might confirm the existence of two binding sites at the 5-HT$_4$ receptor.

3. The length of the alkylene spacer is an important structural feature in the recognition by the 5-HT$_4$

receptor binding site. A chain length of two methylenes is optimal for high affinity and also for good antagonist or agonist activity.

4. In the ethyl and cyclopropyl series, 5-HT_4 antagonist activity seems to be unrelated to the nature of the 4-substituent of the piperazine moiety. However, the presence of a butyl substituent is a favorable pattern for 5-HT_4 antagonism and even in the 5-HT_4 agonist series (isopropyl derivatives) caused a reversal of the pharmacological profile (**5h**, $pK_b = 7.94$).

5. Derivative **5a** showed a partial agonist activity greater than that of BIMU 8 and exhibited better selectivity versus 5-HT_3 and D_2 receptors.

6. *N*-Butyl quaternization of **5a** led to **5r** ($K_i = 66.3$, i.a. = 0.93) with an improvement in affinity for the 5-HT_4 receptor and a similar partial agonist activity.

Experimental Section

Chemistry. Flash column chromatography was performed on silica gel, particle size 60 Å, mesh = 230−400 (Merck). Melting points were determined in open capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. Elemental analyses are within ±0.4% of the theoretical values. IR spectra were taken on a Perkin-Elmer 1310 instrument on KBr plates. ^1H and ^{13}C NMR were recorded on a Bruker AC-200 spectrometer; chemical shifts δ are reported in parts per million (ppm) downfield from tetramethylsilane (TMS), which was used as an internal standard. Spectral data are consistent with assigned structures.

***N*-(1-Methylethyl)-2-nitroaniline, 11a.** 2-Nitroaniline (40.0 g, 0.29 mol), 2,2-dimethoxypropane (54.5 g, 0.52 mol), and TFA (41.5 g, 0.29 mol) were dissolved in toluene (600 mL) and stirred at room temperature for 1 h. A boron−pyridine complex (26.6 g, 0.29 mol) was slowly added. The reaction mixture was stirred for 20 h. The solvent was evaporated in vacuo, and the residue was taken up into water and extracted with CH_2Cl_2. The organic extract was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by flash chromatography on silica gel using hexane and ethyl acetate (95/5) to give **11a** as an orange oil (44.0 g, 84%). ^1H NMR ($CDCl_3$): δ 8.19 (1H, d); 7.41 (1H, t); 6.83 (1H, d); 6.49 (1H, t); 3.81 (1H, m); 1.28 (6H, d). ^{13}C NMR ($CDCl_3$): δ 144.7, 136.0, 131.6, 127.0, 114.8, 114.0, 43.8, 22.6.

***N*-Cyclopropyl-2-nitroaniline, 11b.** A mixture of 2-chloronitrobenzene (3.1 g, 20 mmol) and cyclopropylamine (3.5 mL, 50 mmol) was placed in a high-pressure vessel and heated at 100 °C for 24 h. Then the reactor was opened, the reaction mixture was diluted with water and extracted with CH_2Cl_2, and the extract was washed with water and dried over Na_2SO_4. The solvent was evaporated in vacuo, and the residue was purified by flash chromatography using hexane and CH_2Cl_2 (7/3) as eluent to give **11b** as an orange oil (2.1 g, 60%). ^1H NMR ($CDCl_3$): δ 8.20 (1H, d); 7.61−7.42 (2H, m); 7.30 (1H, dd); 6.72 (1H, m); 2.60 (1H, m); 0.81−1.01 (2H, m); 0.60−0.72 (2H, m). ^{13}C NMR ($CDCl_3$): δ 164.0, 146.0, 135.8, 126.4, 115.8, 115.2, 24.4, 7.7.

2-[(1-Methylethyl)amino]aniline, 12a. A solution of **11a** (2.3 g, 12.7 mmol) in EtOH (50 mL) was hydrogenated over 10% palladium/carbon (0.5 g) at 3 atm and 20 °C for 16 h. The catalyst was filtered off and the filtrate evaporated in vacuo to give **12a** (1.6 g, 83%). ^1H NMR ($CDCl_3$): δ 6.71−6.62 (4H, m); 3.61 (1H, m); 3.13 (3H, br s); 1.20 (6H, d). ^{13}C NMR ($CDCl_3$): δ 136.7, 134.4, 120.6, 118.4, 116.7, 112.9, 44.3, 23.1.

5-Chloro-2-[(1-methylethyl)amino]aniline, 12c. A suspension of **11c** (20.0 g, 93 mmol) in EtOH (400 mL) and 1 N NaOH (300 mL) was stirred vigorously and heated until the solution boiled gently. The bath was removed, and zinc dust (60.8 g, 930 mmol) was added in several portions frequently enough to keep the solution boiling. After refluxing for 1 h, the mixture was filtered and the filtrate was poured into water (100 mL) and extracted with CH_2Cl_2. The organic phase was dried with $MgSO_4$ and evaporated in vacuo to give **12c** (17.0 g, 99%). ^1H NMR ($CDCl_3$): δ 6.78 (1H, br d); 6.65 (1H, br s); 6.57 (1H, d); 3.52 (1H, m); 3.38 (3H, br s); 1.22 (6H, d). ^{13}C NMR ($CDCl_3$): δ 135.9, 135.2, 122.9, 119.8, 116.2, 113.7, 44.3, 22.9.

1,3-Dihydro-1-(1-methylethyl)-2*H*-benzimidazol-2-one, 13a. A solution of **12a** (29.6 g, 0.19 mol) and *N*,*N*′-carbonyldiimidazole (32.1 g, 0.19 mol) in dry THF (400 mL) was stirred at room temperature for 20 h and then evaporated.The residue was taken up in water and extracted with CH_2Cl_2. The dried organic phase was evaporated, and the residue was purified by flash chromatography using CH_2Cl_2 and methanol (98/2) as eluent to give a product that was triturated with hexane and filtered to yield **13a** as pale-yellow solid (16.1 g, 46%). Mp: 125−127 °C. ^1H NMR ($CDCl_3$): δ 7.22−7.15 (4H, m); 4.78 (1H, m); 1.58 (6H, d). ^{13}C NMR ($CDCl_3$): δ 155.4, 128.9, 128.3, 121.0, 120.8, 109.9, 109.2, 44.6, 20.2.

1,3-Dihydro-5-fluoro-1-(1-methylethyl)-6-nitro-2*H*-benzimidazol-2-one, 13f. The compound **13d** (11.0 g, 56.7 mmol) was suspended in acetic anhydride (75 mL) and cooled in an ice bath. To this suspension was added dropwise 22.5 mL of a mixture of nitric acid and acetic anhydride (1/2). The reaction mixture was stirred for 3 min before being poured into ice−water. The solid was filtered, washed with water and cold Et_2O, and dried to afford **13f** (11.8 g, 87%). ^1H NMR ($CDCl_3$): δ 11.20 (1H, br s); 7.62 (1H, d); 6.80 (1H,d); 4.58 (1H, m); 1.41 (6H, d). ^{13}C NMR ($CDCl_3$): δ 154.7, 152.7 (d, $J = 245$ Hz), 134.7 (d, $J = 12$ Hz), 130.2 (d, $J = 7$ Hz), 124.9, 104.9, 98.5 (d, $J = 27$ Hz), 45.0, 19.9.

1,3-Dihydro-1-propyl-2*H*-benzimidazol-2-one, 13h. HNa (2.0 g, 50 mmol, 60% in mineral oil) was added portionwise to a stirred solution of 1,3-dihydro-2*H*-benzimidazol-2-one (6.7 g, 50 mmol) in dry DMF (50 mL). After 1 h, a solution of propyl iodide (4.9 g, 50 mmol) in dry DMF (20 mL) was added dropwise and the mixture stirred at room temperature for 24 h.The solvent was removed in vacuo, and the residue was taken up into water and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, the solvent was evaporated to dryness, and the residue was chromatographed on a column of silica gel using a mixture of dicloromethane and methanol (98/2) as eluent to separate **13h** (55%). Mp: 97−9 °C. ^1H NMR ($CDCl_3$): δ 10.38 (1H, br s); 7.20−6.94 (4H, m); 3.90 (2H, t); 1.83 (2H, m); 1.02 (3H, t). ^{13}C NMR ($CDCl_3$): δ 155.8, 130.4, 128.0, 121.3, 121.1, 109.6, 107.8, 42.4, 21.7, 11.3.

2,3-Dihydro-2-oxo-1*H*-benzimidazole-1-carboxylic Acid, 1,1-Dimethylethyl Ester, 14. HNa (1.6 g, 41 mmol, 60% in mineral oil) was added portionwise to a stirred solution of 1,3-dihydro-2*H*-benzimidazol-2-one (5.0 g, 37.2 mmol) in dry DMF (100 mL) under an atmosphere of argon. After 1.5 h, a solution of di-*tert*-butyl dicarbonate (8.1 g, 37.2 mmol) in dry DMF (20 mL) was added dropwise and the mixture stirred at room temperature for 24 h. The solvent was evaporated and the residue diluted with saturated NH_4Cl solution and extracted with EtOAc. The residue was purified by flash chromatography using hexane and EtOAc (7/3) as eluent to give **14** as a white solid (6.6 g, 76%). Mp: >250 °C. ^1H NMR ($CDCl_3$): δ 10.44 (1H, s); 7.69 (1H, d); 7.18−7.06 (3H, m); 1.68 (9H, s). ^{13}C NMR ($CDCl_3$): δ 153.4, 148.6, 127.4, 126.8, 124.1, 122.0, 114.4, 110.0, 85.0, 28.1.

1,3-Dihydro-1-(2-phenylethyl)-2*H*-benzimidazol-2-one, 13l. A mixture of **14** (2.3 g, 10 mmol), 2-phenylethyl bromide (1.8 g, 10 mmol), K_2CO_3 (4.1 g, 30 mmol), and DMF (40 mL) was stirred at reflux for 24 h. The mixture was concentrated in vacuo and the residue extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, and the solvent was evaporated in vacuo. The residue was purified by flash chromatography using a mixture of hexane and ethyl acetate (7/3) as eluent to yield **13l** (90%). Mp: 142−144 °C. ^1H NMR (DMSO-d_6): δ 10.40 (1H, br s); 7.18−7.01 (4H, m); 7.01−6.81 (5H, m); 4.18 (2H, t); 3.14 (2H, t). ^{13}C NMR (DMSO-d_6): δ 155.6, 138.2,130.1, 128.8, 128.6, 128.0, 126.6, 121.4, 121.1, 109.7, 107.7, 42.4, 34.7.

Ethyl 4-(1-Methylethyl)piperazine-1-carboxylate, 15a. A mixture of 1-ethoxycarbonylpiperazine (7.9 g, 50 mmol), isopropyl iodide (5 mL, 50 mmol), K_2CO_3 (8.3 g, 60 mmol), and

CH_3CN (100 mL) was refluxed for 24 h. The solvent was evaporated in vacuo, and the residue was poured onto water and extracted with dichloromethane. The organic phase was dried over Na_2SO_4 and the solvent evaporated in vacuo to yield **15a** as an oil (98%). ^{1}H NMR ($CDCl_3$): δ 4.12 (2H, q); 3.48 (4H, m); 2.70 (1H, m); 2.46 (4H, m); 1.25 (3H, t); 1.04 (6H, d). ^{13}C NMR ($CDCl_3$): δ 154.8, 61.1, 54.5, 48.3, 44.0, 18.3, 14.6.

1-(1-Methylethyl)piperazine 16a. A solution of **15a** (9.8 g, 49 mmol) and 45% HBr (200 mL) was heated at reflux for 3 h. The reaction mixture was cooled, 50% NaOH solution was added until pH > 7, and the mixture was extracted with a solution of CH_3Cl and *i*-PrOH (4/1). The organic phase was dried over Na_2SO_4, and the solvent was evaporated in vacuo to yield **16a** (90%) as an oil. ^{1}H NMR ($CDCl_3$): δ 2.90 (4H, m); 2.63 (1H, m); 2.48 (4H, m); 1.21 (1H, br s); 1.04 (6H, d). ^{13}C NMR ($CDCl_3$): δ 54.8, 49.9, 46.4, 18.3.

1-Butylpiperazine (Hydrobromide), 16b. A mixture of **15b** (1.0 g, 4.6 mmol) and 45% HBr (30 mL) was heated at reflux for 1 h. The solvent was evaporated and the residue triturated with Et_2O and filtered to give **16b** (1.4 g, 98%). Mp: >240 °C. ^{1}H NMR (DMSO-d_6 + D_2O): δ 4.01 (5H, m); 3.41 (6H, m); 3.12 (2H, m); 1.60 (2H, m); 1.28 (2H, m); 0.86 (3H, t). ^{13}C NMR (DMSO-d_6 + D_2O): δ 56.6, 48.4, 40.7, 25.6, 19.8, 14.0.

1-(4-Fluorophenylmethyl)piperazine, 16c. The compound was prepared according to the process reported for **16b**.The hydrobromide was dissolved in a solution of 10% NaOH, extracted with CH_2Cl_2, and dried over Na_2SO_4. Evaporation of solvent gave **16c** as an oil.Yield: 99%. ^{1}H NMR ($CDCl_3$): δ 7.30−6.98 (4H, m); 3.45 (2H, s); 2.86 (4H, m); 2.38 (4H, m); 1.80 (1H, s). ^{13}C NMR ($CDCl_3$): δ 161.7 (d, J = 243 Hz), 133.6 (d, J = 3 Hz), 130.4 (d, J = 7 Hz), 114.7 (d, J = 21 Hz), 62.6, 54.2, 45.9.

(4-Methylpiperazin-1-yl)acetonitrile, 17a. A suspension of 1-methylpiperazine (9.0 g, 90 mmol), acetonitrile (60 mL), K_2CO_3 (60.0 g, 430.0 mmol), and 2-chloroacetonitrile (8.2 g, 100.0 mmol) was stirred at room temperature for 20 h. Et_2O (100 mL) was added, and the suspension was filtered. The filtrate was concentrated in vacuo to give **17a** as a yellow solid (12.0 g, 96%). Mp: 50−52 °C. ^{1}H NMR ($CDCl_3$): δ 3.40 (2H, s); 2.63 (4H, m); 2.44 (4H, m); 2.25 (3H, s). ^{13}C NMR ($CDCl_3$): δ 114.5, 54.3, 51.5, 45.6, 45.5.

(4-Propylpiperazin-1-yl)acetonitrile, 17b. A mixture of propylpiperazine dihydrobromide (10.0 g, 34.4 mmol), acetonitrile (100 mL), K_2CO_3 (15.2 g, 110.2 mmol), and chloroacetonitrile (2.8 g, 37.7 mmol) was stirred at reflux for 5 h. The solvent was evaporated, and the residue was taken up into water and extracted with Cl_2CH_2. The organic extract was dried over Na_2SO_4 and concentrated in vacuo to give **17b** as an oil (4.8 g, 83%). ^{1}H NMR ($CDCl_3$): δ 3.55 (2H, s); 2.66−2.26 (10H, m); 1.51 (2H, m); 0.90 (3H, t). ^{13}C NMR ($CDCl_3$): δ 114.5, 60.0, 52.3, 51.5, 45.6, 19.7, 11.6.

2-(4-Methylpiperazin-1-yl)ethylamine, 18a. To a stirred suspension of H_4LiAl (3.3 g) in dry Et_2O (100 mL), cooled at 0 °C, was slowly added a solution of **17a** (11.2 g, 80.4 mmol) in dry Et_2O (150 mL) and dry THF (150 mL). The mixture was stirred at room temperature for 24 h, cooled in an ice bath, and hydrolyzed with a 20% NaOH solution (50 mL). After stirring for 20 min, the mixture was filtered and the solvent evaporated. The residue was dissolved in Et_2O and dried over Na_2SO_4. The solvent was evaporated to dryness to give **18a** as an oil (11.5 g, 99%). ^{1}H NMR ($CDCl_3$): δ 2.80 (2H, t); 2.45 (10H, m); 2.25 (3H, s); 1.61 (2H, br s). ^{13}C NMR ($CDCl_3$): δ 60.9, 54.9, 52.9, 45.8, 38.5.

2,3-Dihydro-*N*-[2-(4-methylpiperazin-1-yl)ethyl]-2-oxo-1*H*-benzimidazole-1-carboxamide, 1 (Method A). Trichloromethyl chloroformate (5.5 g, 27.8 mmol) dissolved in dry THF (10 mL) was added dropwise to a stirred suspension of 1,3-dihydro-1*H*-benzimidazol-2-one (5.0 g, 37.2 mmol), activated charcoal (0.08 g), and dry THF (100 mL). The reaction mixture was refluxed for 8 h; then heating was removed while stirring was continued 15 h more. The solid was removed by filtration. Evaporation of the solvent led to a crude material which was triturated with Et_2O to give 2,3-dihydro-2-oxo-1*H*-benzimidazole-1-carbonyl chloride (**19a**) (5.5 g). The solid was added to a solution of 2-(4-methylpiperazin-1-yl)ethylamine (**18a**) (2.8 g, 20.0 mmol) and triethylamine (2.8 g, 28.0 mmol) in dry THF (60 mL) and was stirred at room temperature for 24 h. The solvent was evaporated, and the residue was taken up into water and extracted with Cl_2CH_2. The organic extract was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography using CH_2Cl_2 and MeOH (9/1 to 3/1) as eluent to give **1** (2.4 g, 41%). Mp: 188−190 °C. ^{1}H NMR ($CDCl_3$): δ 8.18 (1H, m); 7.15 (3H, m); 3.48 (2H, t); 2.65−2.41 (10H, m); 2.25 (3H, s). ^{13}C NMR ($CDCl_3$): δ 154.1, 151.9, 127.4, 123.5, 122.2, 114.9, 109.1, 56.4, 54.5, 52.2, 45.3, 36.5.

3-Cyclopropyl-2,3-dihydro-*N*-[2-[4-(1-methylethyl)piperazin-1-yl]ethyl]-2-oxo-1*H*-benzimidazole-1-carboxamide, 6c (Method B). 1-Cyclopropyl-1,3-dihydro-2*H*-benzimidazol-2-one (**13b**) (2.0 g, 11.5 mmol) was added to a suspension of HNa (60% mineral oil, 0.9 g, 23.0 mmol) in dry THF (30 mL). The mixture was stirred 15 min before adding a solution of phosgene in toluene (1.93 M) (23.8 mL, 46.0 mmol), and the stirring was continued for 1 h. The resulting mixture was filtered through Celite and concentrated (**19f**). The residue was dissolved in dry THF (50 mL), and 2-[4-(1-methylethyl)-piperazin-1-yl]ethylamine (**18c**) (2.0 g, 11.6 mmol) and triethylamine (4 mL) were added. The solution was stirred at room temperature for 18 h. The solvent was evaporated, and the residue was taken up into water and extracted with Cl_2CH_2. The organic extract was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography using CH_2Cl_2 and MeOH (95/5 to 9/1) as eluent to give **6c** as an oil (1.8 g, 42%). ^{1}H NMR ($CDCl_3$): δ 8.92 (1H, br s); 8.21 (1H, m); 7.20 (3H, m); 3.59 (2H, q); 2.90 (1H, m); 2.60 (11H, m); 1.11 (10H, m). ^{13}C NMR ($CDCl_3$): δ 153.6, 151.7, 129.6, 126.3, 123.4, 122.7, 115.1, 108.5, 56.9, 54.4, 53.3, 48.6, 37.0, 22.4, 18.6, 6.0.

3-Cyclopropyl-2,3-dihydro-*N*-[2-(4-methylpiperazin-1-yl)ethyl]-2-oxo-1*H*-benzimidazole-1-carboxamide, 6a (Method C). 1-Cyclopropyl-1,3-dihydro-2*H*-benzimidazol-2-one (**13b**) (4.0 g, 23.0 mmol) was reflux with 2-chloroethyl isocyanate (2.9 g, 27.5 mmol) in toluene (50 mL) for 20 h. The mixture was cooled and the solid filtered and washed with hexane to give *N*-(2-chloroethyl)-3-cyclopropyl-2,3-dihydro-2-oxo-1*H*-benzimidazole-1-carboxamide (**20a**) (4.7 g, 73%). Mp: 152−153 °C. ^{1}H NMR ($CDCl_3$): δ 9.20 (1H, br s); 8.19 (1H, m); 7.30−7.17 (3H, m); 3.74 (4H, m); 2.93 (1H, m); 1.28−0.95 (4H, m). ^{13}C NMR ($CDCl_3$): δ 153.6, 151.8, 129.6, 126.1, 123.7, 122.9, 115.1, 108.7, 43.2, 41.6, 22.5, 6.0.

A mixture of 1-methylpiperazine (0.8 g, 8.6 mmol), toluene (50 mL), **20a** (2.0 g, 7.1 mmol), K_2CO_3 (1.2 g, 8.6 mmol), and a pinch of KI was stirred at reflux for 17 h. The solvent was evaporated, and the residue was taken up into water and extracted with Cl_2CH_2. The organic extract was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography using CH_2Cl_2 and MeOH (9/1 to 3/1) as eluent to give **6a** (1.0 g, 41%). Mp: 104−108 °C. ^{1}H NMR ($CDCl_3$): δ 8.91 (1H, br s); 8.19 (1H, d); 7.20 (3H, m); 3.43 (2H, q); 2.80 (1H, m), 2.58 (10H, m); 2.22 (3H, s); 1.24−0.82 (4H, m). ^{13}C NMR ($CDCl_3$): δ 153.2, 151.4, 129.3, 125.9, 123.1, 122.3, 114.7, 108.3, 56.5, 54.6, 52.5, 45.5, 36.7, 22.2, 5.8.

3-Cyclopropyl-2,3-dihydro-*N*-[(4-methylpiperazin-1-yl)methyl]-2-oxo-1*H*-benzimidazole-1-carboxamide, 6e. 1-Cyclopropyl-1,3-dihydro-2*H*-benzimidazol-2-one (**13b**) (3.0 g, 17.2 mmol) was heated at 60 °C with chloromethyl isocyanate (1.9 g, 1.8 mL) in toluene (30 mL) for 24 h. The solvent was evaporated, and the residue was triturated with Et_2O to give *N*-(chloromethyl)-3-cyclopropyl-2,3-dihydro-2-oxo-1*H*-benzimidazole-1-carboxamide (**20d**) (4.0 g, 87%). Mp: 202−204 °C. ^{1}H NMR (DMSO-d_6): δ 9.23 (1H, br s); 8.16 (1H, d); 7.38−7.20 (3H, m); 4.79 (2H, d); 2.94 (1H, m); 1.21−0.82 (4H, m). ^{13}C NMR (DMSO-d_6): δ 152.8, 151.0, 129.9, 125.8, 123.6, 122.2, 114.1, 109.1, 63.4, 22.4, 5.7.

A mixture of 1-methylpiperazine (0.56 g, 5.6 mmol), acetonitrile (25 mL), **20d** (1.0 g, 3.7 mmol), triethylamine (0.37 g, 3.7 mmol), and a pinch of KI was stirred at room temperature

for 10 min. The solvent was evaporated, and the residue was taken up into water and extracted with Cl_2CH_2. The organic extract was dried over Na_2SO_4 and concentrated in vacuo. The residue was triturated with Et_2O to give **6e** (1.0 g, 81%). Mp: 86−88 °C. ^{1}H NMR ($CDCl_3$): δ 9.20 (1H, br s); 8.19 (1H, m); 7.21 (3H, m); 4.37 (2H, d); 2.91 (1H, m), 2.71 (4H, m); 2.49 (4H, m); 2.28 (3H, s); 1.10 (4H, m). ^{13}C NMR ($CDCl_3$): δ 153.4, 152.0, 129.4, 126.0, 123.5, 122.6, 115.0, 108.5, 61.3, 54.6, 49.1, 45.6, 22.3, 5.8.

5-Amino-2,3-dihydro-6-fluoro-3-(1-methylethyl)-*N*-[2-(4-methylpiperazin-1-yl)ethyl]-2-oxo-1*H*-benzimidazole-1-carboxamide, 5p. A suspension of **5o** (1.2 g, 3 mmol) in THF (75 mL) and 1 N NaOH (75 mL) was stirred vigorously and heated until the solution boiled gently. The bath was removed, and zinc dust (3.0 g, 45 mmol) was added in several portions frequently enough to keep the solution boiling. The mixture was refluxed for 1 h and filtered, and the filtrate was poured into water (50 mL) and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and evaporated in vacuo to give **5p** (0.95 g, 82%). Mp: 84 °C dec. ^{1}H NMR ($CDCl_3$): δ 6.78 (1H, d); 6.61 (1H, d); 5.63 (1H, br t); 4.60 (1H, m); 3.95 (2H, t); 3.61 (2H, br s); 3.45 (2H, q); 3.31 (4H, t); 2.30 (4H, t); 2.23 (3H, s); 1.41 (6H, d). ^{13}C NMR ($CDCl_3$): δ 157.6, 154.3, 147.5 (d, J = 230 Hz), 128.9 (d, J = 14 Hz), 124.0, 121.3 (d, J = 11 Hz), 98.4 (d, J = 3 Hz), 96.5 (d, J = 26 Hz), 54.4, 45.8, 44.8, 43.2, 40.5, 40.3, 19.9.

1-Butyl-4-[2-[2,3-dihydro-3-(1-methylethyl)-2-oxobenzimidazol-1-yloxoamino]ethyl]-1-methylpiperazonium Iodide, 5r. (Method D). A solution of *N*-[2-(4-butylpiperazin-1-yl)ethyl]-2,3-dihydro-3-(1-methylethyl)-2-oxo-1*H*-benzimidazole-1-carboxamide (**5h**) (1.2 g, 3.1 mmol) and methyl iodide (2 mL, 32.0 mmol) in THF (30 mL) was stirred at room temperature for 20 h. The solvent was removed, and the residue was taken up into water and extracted with Cl_2CH_2. The organic extract was dried over Na_2SO_4 and concentrated in vacuo to give **5r** as a solid (1.1 g, 67%). Mp: 170−172 °C. ^{1}H NMR ($CDCl_3$): δ 9.12 (1H, br s); 8.21 (1H, m); 7.19 (3H, m); 4.70 (1H, m); 3.79 (4H, m); 3.61 (4H, m); 3.42 (3H, s); 3.05 (4H, m); 2.84 (2H, t); 1.84 (2H, m); 1.58 (8H, m); 1.03 (3H, t). ^{13}C NMR ($CDCl_3$): δ 152.5, 151.8, 127.5, 126.6, 123.3, 122.2, 115.1, 108.9, 60.6, 55.5, 47.5, 46.1, 45.3, 36.4, 23.9, 19.8, 19.5, 13.7.

1-Allyl-4-[2-[2,3-dihydro-3-(1-methylethyl)-2-oxobenzimidazol-1-yloxoamino]ethyl]-1-methylpiperazonium Iodide, 5t (Method E). To a cooled solution of 2,3-dihydro-3-(1-methylethyl)-*N*-[2-(4-methylpiperazin-1-yl)ethyl]-2-oxo-1*H*-benzimidazole-1-carboxamide (**5a**) (1.0 g, 2.9 mmol) in DMF (12 mL) was added allyl iodide (2.4 g, 14.3 mmol). The mixture was stirred at room temperature for 2 h. The solvent was evaporated and the residue purified by flash chromatography using CH_2Cl_2 and MeOH (9/1) as eluent to give **5t** as a white solid (1.0 g, 67%). Mp: 177−179 °C. ^{1}H NMR ($CDCl_3$): δ 9.08 (1H, br s); 8.25 (1H, m); 7.18 (3H, m); 4.18−5.77 (3H, m); 4.70 (1H, m); 4.51 (2H, d); 3.63 (6H, m); 3.44 (3H, s); 3.04 (4H, m); 2.83 (2H, t); 1.57 (6H, d). ^{13}C NMR ($CDCl_3$): δ 152.4, 151.8, 131.7, 127.2, 126.6, 123.3, 122.5, 115.4, 109.2, 65.8, 60.0, 54.8, 46.1, 45.0, 44.7, 35.1, 19.8.

Pharmacological Methods. 1. 5-HT$_4$ Receptor Binding Assay.[31] Adult male Dunkin−Hartley guinea pigs weighing 350−400 g were used. Animals were killed by decapitation. The whole brain with the exception of the brainstem and cerebellum was quickly removed, and the various areas were dissected, weighed, and immediately frozen at −70 °C. Striatum used for the binding experiments was homogenized with an Ultra-Turrax homogenizer (setting 5 for 20 s) in 15 volumes of ice-cold 50 mM Hepes buffer (pH 7.4) and centrifuged at 48000*g* for 20 min (4 °C). The resulting pellet was resuspended in 10 volumes of ice-cold 50 mM Hepes buffer (pH 7.4) and was stored at −70 °C until use. At time of experiment, the membranes were diluted in the same ice-cold buffer (final dilution 1:80, wt/vol). Competition assays were performed in triplicate, in a final volume of 1 mL. To each assay tube were added the following: 0.1 mL of the displacer drug concentration (0.1 mL of vehicle if no competing drug was added) and 0.1 mL of [^{3}H]GR 113808 (Amersham, 60−85 Ci/mmol) in buffer (final concentration 0.1 nM). Nonspecific binding was determined using 10 μM cold 5-HT. Binding experiment was initiated by addition of 0.8 mL of membrane suspension (800−900 μg of protein). After incubation for 30 min at 25 °C the reaction was stopped for vacuum filtration through Whatman GF/B glass pretreated filters (1% poly(ethylenimine) in 50 mM Hepes buffer), using a Brandel cell harvester. Filters were placed in scintillation poly(ethylene) vials (with 5 mL of scintillation cocktail) and equilibrated, and filter-retained radioactivity was measured in a liquid scintillation counter (Kontron Beta V). IC_{50} and K_i values were calculated using the computer program EBDA (McPherson).[37]

2. 5-HT$_3$ Receptor Binding Assay.[34] Adult male Wistar rats weighing 220−280 g were used. Animals were killed by decapitation. The whole brain with the exception of the brainstem and cerebellum was quickly removed, and the various areas were dissected, weighed, and immediately frozen at −70 °C. Enthorinal cortex used for the binding experiments was homogenized with an Ultra-Turrax homogenizer (setting 5 for 20 s) in 10 volumes of ice-cold 0.32 M sucrose buffer and centrifuged at 1000*g* for 10 min (4 °C), and the supernatant was recentrifuged at 17000*g* for 20 min (4 °C). The resulting pellet was resuspended in 50 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4), incubated at 37 °C for 10 min, and then centrifuged three times more at 48000*g* for 10 min (4 °C). The final pellet was resuspended in 10 volumes of ice-cold 50 mM Tris-HCl buffer containing 5 mM $CaCl_2$ and 0.1% ascorbate and was stored at −70 °C until use. At time of experiment, the membranes were diluted in the same ice-cold buffer with 10 μM pargyline (final dilution 1:40, wt/vol). Competition assays were performed in triplicate, in a final volume of 1 mL. To each assay tube were added the following: 0.1 mL of the displacer drug concentration (0.1 mL of vehicle if no competing drug was added) and 0.1 mL of [^{3}H]LY 278584 (Amersham, 60−85 Ci/mmol) in buffer (final concentration 2 nM). Nonspecific binding was determined using 10 μM cold 5-HT. Binding experiment was initiated by addition of 0.8 mL of membrane suspension (500−600 μg of protein). After incubation for 30 min at 25 °C the reaction was stopped for vacuum filtration through Whatman GF/B glass pretreated filters (1% poly(ethylenimine) in 50 mM Tris-HCl buffer), using a Brandel cell harvester, followed by two washes with 5 mL of ice-cold 50 mM Tris-HCl (pH 7.4) buffer. Filters were placed in scintillation poly(ethylene) vials (with 5 mL of scintillation cocktail) and equilibrated, and filter-retained radioactivity was measured in a liquid scintillation counter (Kontron Beta V). IC_{50} and K_i values were calculated using the computer program EBDA (McPherson).[37]

3. D$_2$ Receptor Binding Assay.[35] Adult male Wistar rats weighing 220−280 g were used. Animals were killed by decapitation. The whole brain with the exception of the brainstem and cerebellum was quickly removed, and the various areas were dissected, weighed, and immediately frozen at −70 °C. Striatum used for the binding experiments was homogenized with an Ultra-Turrax homogenizer (setting 5 for 20 s) in 50 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.7), centrifuged at 48000*g* for 10 min (4 °C). The resulting pellet was resuspended in 50 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.7), incubated at 37 °C for 10 min, and then centrifuged once more at 48000*g* for 10 min (4 °C). The resulting pellet was resuspended in 10 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4) and was stored at −70 °C until use. At time of experiment, the membranes were diluted in the same ice-cold buffer with 10 μM pargyline (final dilution 1:150, wt/vol). Competition assays were performed in triplicate, in a final volume of 1 mL. To each assay tube were added the following: 0.1 mL of the displacer drug concentration (0.1 mL of vehicle if no competing drug was added) and 0.1 mL of [^{3}H]-raclopride (NEN, 60−87 Ci/mmol) in buffer (final concentration 1 nM). Nonspecific binding was determined using 1 μM cold (+)-butaclamol. Binding experiment was initiated by addition of 0.8 mL of membrane suspension (300−400 μg of protein). After incubation for 60 min at 25 °C the reaction was stopped

for vacuum filtration through Whatman GF/B glass filters, using a Brandel cell harvester, followed by two washes with 5 mL of ice-cold 50 mM Tris-HCl (pH 7.7) buffer. Filters were placed in scintillation poly(ethylene) vials (with 5 mL of scintillation cocktail) and equilibrated, and filter-retained radioactivity was measured in a liquid scintillation counter (Kontron Beta V). IC_{50} and K_i values were calculated using the computer program EBDA (McPherson).[37]

4. Rat Isolated Esophageal Tunica Muscularis Mucosae (TMM).[32,33] Male Wistar rats (250−300 g) were killed by a blow on the head, and a 2-cm segment of intrathoracic esophagus proximal to the diaphragm was removed and placed in standard Krebs-Henseleit (K-H) solution (37 °C, pH 7.4). The external muscularis propria containing the outer longitudinal and circular muscle layers of the esophagus was carefully removed to isolate the inner smooth muscle of TMM. The TMM preparations were suspended longitudinally in a 20-mL tissue bath containing K-H solution at 37 °C, pH 7.4, and continuously aerated with carbogen (95% O_2/5% CO_2). This solution routinely contained 1 μM ketanserin, 10 μM zimelidine, 30 μM cocaine, and 100 μM pargyline. Tissues were placed under an initial tension of 0.5 g, equilibrated for 60 min by washing every 15 min before starting the experiment. Responses were isometrically recorded using a F30 transducer coupled to a two-channel chart recorder (Hugo Sachs Elektronik, Freiburg, Germany). The TMM preparations were contracted by addition of a submaximal concentration of carbachol (3 μM).

In agonism studies, upon establishment of a stable contraction, two concentration−effect curves (relaxation) were constructed in the same TMM in a cumulative fashion: the first for 5-HT itself and the second, 90 min later, for either 5-HT (in time control experiments) or a test agonist. Responses to the cumulative addition of agonist were expressed as percentage relaxation of carbachol-induced tone. For each agonist, two or three separate TMM preparations were used and the concentration−effect curves obtained were fitted to calculate the negative logarithm of the molar concentration of the agonist that relaxated 50% relative to their individual maximun effect (pEC_{50}). The intrinsic activity (i.a.) versus 5-HT was calculated comparing the maximun relaxation obtained at the concentration 1 or 3 μM in the same TMM preparation. To confirm that the agonist effect was 5-HT_4 receptor-dependent, another series with TMM preparations was used in which two concentration−effect curves were constructed: the first to agonist itself and the second, 90 min later, in the presence of 10 nM GR 113808 added 30 min before the first concentration of agonist. Apparent affinity (pK_b) for GR 113808 against all agonists was determined from concentration ratios by the equation: $pK_b = \log(X - 1) + 8$, where X is the concentration ratio and 8 is the negative logarithm of the GR 113808 concentration (10 nM). The effect of this 5-HT_4 antagonist on pEC_{50} value was registered and then compared by using the unpaired Student's t-test. A p value of 0.05 or less was considered statistically significant.

In antagonist studies, two concentration−effect curves were constructed in the same TMM preparation: the first for 5-HT itself and the second, 90 min later, in the presence of a single concentration (0.1 or 1 μM) of antagonist added 30 min before the first concentration of 5-HT. For each antagonist, two or three separate TMM preparations were used. Apparent affinity (pK_b) for antagonists against 5-HT were determined from concentration ratios by the equation: $pK_b = \log(X - 1) - \log[A]$, where X is the concentration ratio and [A] is the used antagonist concentration (mol/L). The method assumes a competitive interaction. The effect of the antagonists on pEC_{50} values was registered, and the values were compared by using the unpaired Student's t-test. A p value of 0.05 or less was considered statistically significant.

Acknowledgment. This work was supported in part by the Ministry of Industry and Energy of Spain and the Department of Industry, Agriculture and Fishery of the Basque Government.

Supporting Information Available: Yields, melting points, and spectral data (^1H and ^{13}C NMR) for compounds **2**−**20**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Shenker, A.; Maayani, S.; Weinstein, H.; Green, J. P. Pharmacological characterization of two 5-hydroxytryptamine receptors coupled to adenylate cyclase in guinea pig hippocampal membranes. *Mol. Pharmacol.* **1987**, *31*, 357−367.
(2) Dumuis, A.; Bouhelal, R.; Sebben, M.; Cory, R.; Bockaert, J. A non classical 5-hydroxytryptamine receptor positively coupled with adenylate cyclase in the central nervous system. *Mol. Pharmacol.* **1988**, *34*, 880−887.
(3) Dumuis, A.; Sebben, M.; Bockaert, J. The gastrointestinal prokinetic benzamide derivatives are agonists at the non classical 5-HT receptor (5-HT_4) positively coupled to adenylate cyclase in neurons. *Naunyn Schmiedeberg's Arch Pharmacol.* **1989**, *340*, 403−410.
(4) Ford, A. P. D. W.; Clarke, D. E. The 5-HT_4 receptor. *Med. Res. Rev.* **1993**, *13 (6)*, 633−662.
(5) Eglen, R. M.; Wong, E. H. F.; Dumuis, A.; Bockaert, J. Central 5-HT_4 receptors. *Trends Pharmacol. Sci.* **1995**, *16*, 391−398.
(6) Barnes, J. M.; Barnes, N. M. Central 5-HT_4 receptors. Targets for new clinical opportunities? *Curr. Res. Serotonin* **1998**, *3 (2)*, 49−63.
(7) Hegde, S.; Eglen, R. Peripheral 5-HT_4 receptors. *FASEB J.* **1996**, *10 (12)*, 1398−1407.
(8) Grossman, C. J.; Kilpatrick, G. J.; Bunce, K. T. Development of a radioligand binding assay for 5-HT_4 receptors in guinea-pig and rat brian. *Br. J. Pharmacol.* **1993**, *109*, 618−624.
(9) Brown, A. M.; Young, T. J.; Patch, T. L.; Cheung, C. W. [^{125}I]-SB207710, a potent, selective radioligand for the 5-HT_4 receptors. *Br. J. Pharmacol.* **1993**, *110*, 10P.
(10) Gerald, C.; Adham, N.; Kao, H.-T.; Olsen, M.; Laz, T. M.; Schechter, L. E.; Bard, J. A.; Vaysse, P. J. J.; Hartig, P. R.; Branchek, T. A.; Weinshank, R. L. The 5-HT_4 receptor: molecular cloning and pharmacological characterization of two splice variants. *EMBO J.* **1995**, *14*, 2806−2815.
(11) Claeysen, S.; Faye, P.; Sebben, M.; Lemaire, S.; Bockaert, J.; Dumuis, A. Cloning and expression of human 5-HT_{4S} receptors. Effect of receptor density on their coupling to adenylyl cyclase. *Neuropharmacol. Neurotoxicol.* **1997**, *8*, 3189−3196.
(12) Blondel, O.; Gastineau, M.; Dahmoune, Y.; Langlois, M.; Fischmeister, R. Cloning, expression, and pharmacology of four human 5-hydroxytryptamine$_4$ receptor isoforms produced by alternative splicing in the carboxyl terminus. *J. Neurochem.* **1998**, *70 (6)*, 2252−2261.
(13) Bockaert, J.; Fagni, L.; Dumuis, A. 5-HT_4 receptors: an update. In *Handbook of Experimental Pharmacology, vol. 129. Serotoninergic neurons and 5-HT receptors in the CNS*; Baumgarten, H. G., Göthert, M., Eds.; Springer-Verlag: Berlin, Heidelberg, 1997; pp 439−474.
(14) Gaster, L. M.; King, F. D. Serotonin 5-HT_3 and 5-HT_4 receptor antagonists. *Med. Res. Rev.* **1997**, *17 (2)*, 163−214.
(15) Buchheit, K.-H.; Gamse, R.; Giger, R.; Hoyer, D.; Klein, F.; Klöppner, E.; Pfannkuche, H.-J.; Mattes, H. The serotonin 5-HT_4 receptor.1. Design of a new class of agonists and receptor map of the agonist recognition site. *J. Med. Chem.* **1995**, *38*, 2326−2330.
(16) López-Rodríguez, M. L.; Morcillo, M. J.; Benhamú, B.; Rosado, M. L. Comparative receptor mapping of serotonergic 5-HT_3 and 5-HT_4 binding sites. *J. Comput.-Aided Mol. Des.* **1997**, *11*, 589−599.
(17) Yang, D.; Soulier, J.-L.; Sicsic, S.; Mathé-Allainmat, M.; Brémont, B.; Croci, T.; Cardamone, R.; Aureggi, G.; Langlois, M. New esters of 4-amino-5-chloro-2-methoxybenzoic acid as potent agonists and antagonists for 5-HT_4 receptors. *J. Med. Chem.* **1997**, *40*, 608−621.
(18) Turconi, M.; Nicola, M.; Quintero, M. G.; Maiocchi, L.; Micheletti, R.; Giraldo, E.; Donetti, A. Synthesis of a new class of 2,3-dihydro-2-oxo-1*H*-benzimidazole-1-carboxylic acid derivatives as highly potent 5-HT_3 receptor antagonists. *J. Med. Chem.* **1990**, *33*, 2101−2108.
(19) Turconi, M.; Schiantarelli, P.; Borsini, F.; Rizzi, C. A.; Ladinsky, H.; Donetti, A. Azabicycloalkyl benzimidazolones: interaction with serotonergic 5-HT_3 and 5-HT_4 receptors and potential therapeutic implications. *Drugs Future* **1991**, *16 (11)*, 1011−1026.
(20) Schiavone, A.; Giraldo, E.; Giudici, L.; Turconi, M.; Sagrada, A. Dau 6285: a novel antagonist at the putative 5-HT_4 receptor. *Life Sci.* **1992**, *52*, 583−592.
(21) Dumuis, A.; Sebben, M.; Monferini, E.; Nicola, M.; Turconi, M.; Ladinsky, H.; Bockaert, J. Azabycicloalkyl benzimidazolone derivatives as a novel class of potent agonists at the 5-HT_4 receptor positively coupled to adenylate cyclase in brain. *Naunyn Schmiedeberg's Arch. Pharmacol.* **1991**, *343*, 245−251.

(22) Baxter, G. S.; Clarke, D. E. Benzimidazolone derivatives act as 5-HT_4 receptor ligands in rat oesophagus. *Eur. J. Pharmacol.* **1992**, *212*, 225−229.
(23) Rizzi, C. A.; Coccini, T.; Onori, L.; Manzo, L.; Tonini, M. Benzimidazolone derivatives: a new class of 5-hydroxytryptamine$_4$ receptor agonists with prokinetic and acetylcholine releasing properties in the guinea pig ileum. *J. Pharmacol. Exp. Ther.* **1992**, *261 (2)*, 412−419.
(24) Schaus, J. M.; Thompson, D. C.; Bloomquist, W. E.; Susemichel, A. D.; Calligaro, D. O.; Cohen, M. L. Synthesis and structure−activity relationships of potent and orally active 5-HT_4 receptor antagonists: indazole and benzimidazolone derivatives. *J. Med. Chem.* **1998**, *41*, 1943−1955.
(25) Tonini, M.; Candura, S. M.; Messori, E.; Rizzi, C. A. Therapeutic potential of drug with mixed 5-HT_4 agonist/5-HT_3 antagonist action in the control of emesis. *Pharmacol. Res.* **1995**, *31 (5)*, 257−260.
(26) Orjales, A.; Mosquera, R.; Labeaga, L.; Rodes, R. New 2-piperazinylbenzimidazole derivatives as 5-HT_3 antagonists. Synthesis and pharmacological evaluation. *J. Med. Chem.* **1997**, *40*, 586−593.
(27) Meanwell, N. A.; Sit, Y. S.; Gao, J.; Wong, H. S.; Gao, Q.; St. Laurent, D. R.; Balasubramanian, N. Regioespecific functionalization of 1,3-dihydro-2H-benzimidazol-2-one and structurally related cyclic ureas. *J. Org. Chem.* **1995**, *60*, 1565−1582.
(28) Orjales, A.; Alonso-Cires, L.; Labeaga, L.; Corcóstegui, R. New (2-methoxyphenyl)piperazine derivatives as 5-HT_{1A} receptor ligands with reduced α_1-adrenergic activity. Synthesis and structure-affinity relationships. *J. Med. Chem.* **1995**, *38*, 1273−1277.
(29) Flynn, D. L.; Moormann, A. E. Benzimidazole compounds. Patent WO 94/00449, 1994.
(30) Zára-Kaczián, E.; Hazai, L.; Deák, G.; György, L.; Sohár, P. Synthesis of 1-aryl-1,4-dihydro-3(2H)-isoquinolinones with piperazine ring in the side chain having potential antiserotoninergic activity. *Acta Chim. Hung.* **1990**, *127 (4)*, 607−627.
(31) Grossman, C. J.; Kilpatrick, G. J.; Bunce, K. T. Development of a radioligand binding assay for the 5-HT_4 receptors in guinea-pig and rat brain. *Br. J. Pharmacol.* **1993**, *109*, 618−624.
(32) Baxter, G. S.; Craig, D. A.; Clarke, D. E. 5-Hydroxytryptamine$_4$ receptors mediate relaxation of the rat oesophageal tunica muscularis mucosae. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1991**, *343*, 439−446.
(33) Waikar, M. V.; Hegde, S. S.; Ford, A. P. D. W.; Clarke, D. E. Pharmacological analyses of endo-6-methoxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl-2,3-dihydro-2-oxo-1*H*-benzimidazole-1-carboxylate hydrochloride (DAU 6285) at the 5-hydroxytryptamine$_4$ receptor in the tunica muscularis mucosae of rat esophagus and ileum of guinea pig: role of endogenous 5-hydroxytryptamine. *J. Pharmacol. Exp. Ther.* **1993**, *264*, 654−661.
(34) Wong, D. T.; Robertson, D. W.; Reid, L. R. Specific [^{3}H]-LY278584 binding to 5-HT_3 recognition sites in rat cerebral cortex. *Eur. J. Pharmacol.* **1989**, *166*, 107−110.
(35) Kohler, C.; Hall, H.; Ogren, S. O.; Gawell, L. Specific in vitro and in vivo binding of [^{3}H]-Raclopride. A potent substituted benzamide drug with high affinity for dopamine D_2 receptors in the rat brain. *Biochem. Pharmacol.* **1985**, *34*, 2251−2259.
(36) Baxter, G. S.; Boyland, P.; Gaster, L. M.; King, F. D. Quaternised renzapride as a potent and selective 5-HT_4 receptor agonist. *Bioorg. Med. Chem. Lett.* **1993**, *3 (4)*, 633−634.
(37) McPherson, G. A. A personal computer-based approach to the analysis of radioligand binding experiments. *Comput. Prog. Biomed.* **1983**, *17*, 107−114.

JM981098J