New 2-Piperazinylbenzimidazole Derivatives as 5-HT_3 Antagonists. Synthesis and Pharmacological Evaluation

Aurelio Orjales,* Ramón Mosquera, Luis Labeaga, and Rosa Rodes

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

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A series of 2-piperazinylbenzimidazole derivatives were prepared and evaluated as 5-HT_3 receptor antagonists. Their 5-HT_3 receptor affinities were evaluated by radioligand binding assays, and their abilities to inhibit the 5-HT-induced Bezold−Jarisch reflex in anesthetized rats were determined. Compound **7e** (lerisetron, pK_i = 9.2) exhibited higher affinity for the 5-HT_3 receptor than did tropisetron and granisetron, while compound **7q** (pK_i = 7.5) had very low affinity for this receptor, showing that substitution on the N_1 atom of the benzimidazole ring is essential for affinity and activity. The effect of substitution on the aromatic ring of benzimidazole by several substituents in different positions is also discussed. A strong correlation between the 5-HT_3 antagonistic activity of the studied compounds and the position of substitution on the aromatic ring was established. Thus, while the 4-methoxy derivative **7m** showed weak affinity for the 5-HT_3 receptor (pK_i = 6.7), the 7-methoxy derivative **7n** exhibited the highest affinity (pK_i = 9.4). Compounds **7e** and **7n** are now under further investigation as drugs for the treatment of nausea and emesis evoked by cancer chemotherapy and radiation.

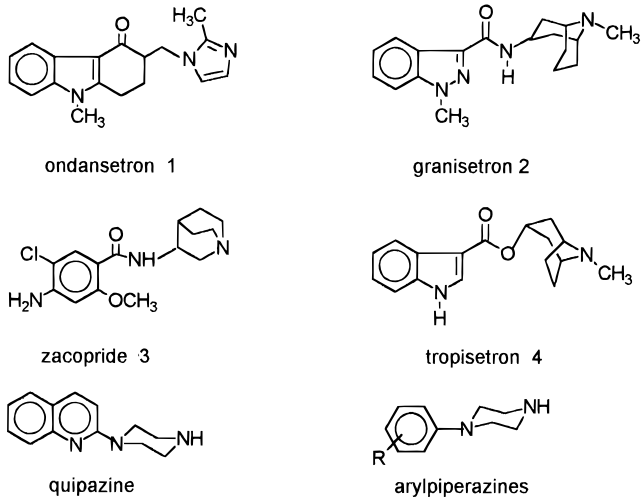
Introduction

Since its discovery in the late 1940s[1] 5-HT has been implicated in a wide variety of physiological roles in both the central nervous system (CNS) and periphery. Recent molecular studies and cloning strategies have identified seven major subclasses of 5-HT receptor, classified as 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7.[2−5] Over the past few years, special attention has been paid to the 5-HT_3 receptor, and intensive efforts have been made toward the discovery of selective 5-HT_3 receptor antagonists.[6,7] Ondansetron **1**,[8] granisetron **2**,[9] zacopride **3**,[10] and tropisetron **4**[11] are representatives of 5-HT_3 antagonists (Chart 1), and they have been shown to be highly effective in the control of nausea and emesis evoked by citotoxic drugs such as cisplatin.[12,13] Some of them have already been marketed for this therapeutic indication. Moreover, animal behavior and binding studies on animal and human brain tissues have suggested a role for these 5-HT_3 antagonists within the CNS,[14] and they may be effective in the treatment of migraine,[15] schizophrenia,[16] and anxiety.[17] We started these studies with the aim of developing potent and long-acting 5-HT_3 antagonists.

On the basis of the structures of known ligands, several studies have been conducted to define the structural requirements for the 5-HT_3 antagonistic activity. Three structural features were shown to contribute to 5-HT_3 receptor binding:[18] (1) an aromatic ring, (2) a basic nitrogen atom, and (3) a linking acyl functional group.

Although most of the 5-HT_3 antagonists so far described present these structural requirements, there are several other examples of 5-HT_3 radioligands not having the acyl group which bind with high affinity for the 5-HT_3 receptor,[19−21] thus showing that the acyl group is not a necessary requirement. Instead, a hydrogen-bonding interaction with the receptor[22] appears to play an important role in the affinity. During the course of our investigation on a series of 2-piperazinylbenzimidazole, the 1-(phenylmethyl)-2-piperazinyl derivative **7e** (lerisetron)[23] was found to possess high-affinity binding for the 5-HT_3 receptors as well as a potent ability to inhibit the 5-HT-evoked reflex bradycardia [von Bezold−Jarisch (B−J) reflex] in urethane-anesthetized rats. Afterward, the equivalent *N*-methyl compound of **7e** has been published.[24] In this paper we report the synthesis and initial pharmacological evaluation of compound **7e** and a series of piperazinylbenzimidazole derivatives as a novel and potent class of 5-HT_3 antagonists. These compounds do not have any acyl group in their structure, and position 4 of the piperazine moiety is always unsubstituted.

Chart 1



Chemistry

All new compounds are shown in Table 1, and general synthetic procedures used for their preparation are illustrated in Schemes 1−3. 2-(1-Piperazinyl)benzimid-

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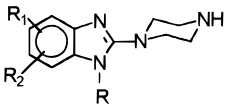
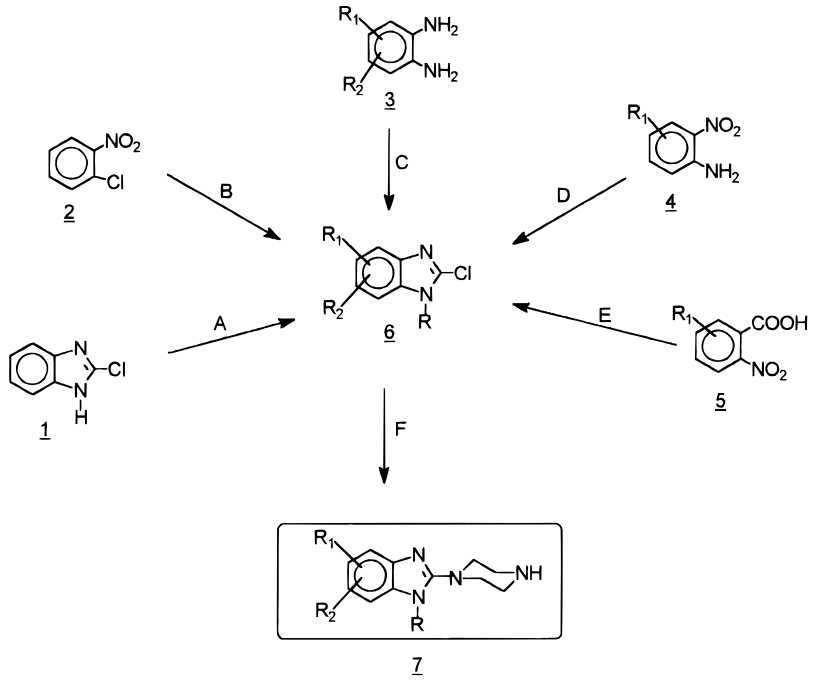
Table 1. 2-(1-Piperazinyl)benzimidazole Derivatives **7**



compd	R	R_1	R_2	formula[a]	method	% yield	mp, °C[b]
7a	CH_3	H	H	$C_{12}H_{16}N_4$	A, F	92	80−2
7b	CH_2CH_3	H	H	$C_{13}H_{18}N_4$	A, F	95	oil
7c	$(CH_2)_2CH_3$	H	H	$C_{14}H_{20}N_4$	A, F	90	175−7
7d	cyclo-C_3H_5	H	H	$C_{14}H_{18}N_4$	B, F	65	56−8
7e	CH_2Ph	H	H	$C_{18}H_{20}N_4$	A, F	98	130−2
7f	CH_2Ph	(5)F	H	$C_{18}H_{19}FN_4$	D, F	82	108−10
7g	CH_2Ph	(5)Cl	H	$C_{18}H_{19}ClN_4$	D, F	47	125−7
7h	CH_2Ph	(5)CH_3	H	$C_{19}H_{22}N_4$	D, F	50	106−8
7i	CH_2Ph	(5)CH_3O	H	$C_{19}H_{22}N_4O$	D, F	70	91−3
7j	CH_2Ph	(5)OH	H	$C_{18}H_{20}N_4O$	*d*	63	100−2
7k	CH_2Ph	(6)CH_3O	H	$C_{19}H_{22}N_4O$	E, F	54	102−4
7l[c]	CH_2Ph	(6)OH	H	$C_{18}H_{20}N_4O$ BrH	*d*	50	>245
7m	CH_2Ph	(4)CH_3O	H	$C_{19}H_{22}N_4O$	D, F	68	125−6
7n	CH_2Ph	(7)CH_3O	H	$C_{19}H_{22}N_4O$	E, F	85	101−4
7o	CH_2Ph	(5)CH_3	(6)CH_3	$C_{20}H_{24}N_4$	C, F	62	191−3
7p	CH_2Ph	(5)Cl	(6)Cl	$C_{18}H_{18}Cl_2N_4$	C, F	47	160−2
7q	H	H	H	$C_{11}H_{14}N_4$	F	42	>200

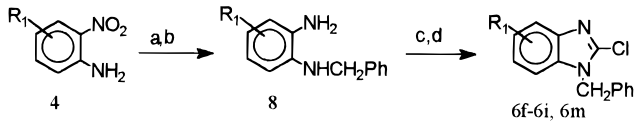
[a] Analyses for C, H, N. [b] Recrystallization solvent: hexane. [c] Bromohydrate. [d] Obtained from **7i** and **7k** (see the Experimental Section).

Scheme 1[a]



[a] Reagents: (A) RX, NaH, DMF, rt, 1 h; (B) (i) cyclopropylamine, 150 °C; (ii) H_2, Pd/C, EtOH; (iii) urea, Δ; (iv) $POCl_3$/HCl, Δ; (C) (i) urea, 3 h, 130 °C; (ii) $BrCH_2Ph$, K_2CO_3, DMF; (iii) $POCl_3$/HCl, 150 °C, 3 h; (D and E) (see Scheme 2 and 3); (F) piperazine, 150 °C.

Scheme 2[a]

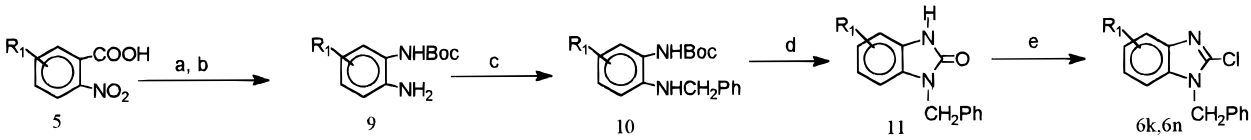


[a] Reagents: (a) $BrCH_2Ph$, K_2CO_3, DMF; (b) H_2, Pd/C, THF; (c) urea, Δ; (d) $POCl_3$/HCl, Δ.

azole derivatives **7** were obtained from 2-chlorobenzimidazole derivatives **6** by nucleophilic substitution of the chlorine atom by piperazine following a standard procedure.[25] 2-Chlorobenzimidazole derivatives **6** were prepared through different routes of synthesis (see Scheme 1).

Unsubstituted aromatic derivatives **6a**−**c** and **6e** were synthesized according to procedure A by N-alkylation of the sodium salt of 2-chlorobenzimidazole with the appropriate alkyl halide. Cyclopropyl derivative **6d** was prepared following an alternative route (procedure B) because of the known reluctance of cyclopropyl halides to undergo nucleophilic substitution. Thus, 2-chloronitrobenzene was heated with cyclopropylamine in a closed vessel to afford *N*-cyclopropyl-2-nitroaniline, which was hydrogenated over palladium/carbon, cyclized with urea, and halogenated with phosphorous oxychloride and hydrochloric acid under pressure to yield 2-chloro-1-cyclopropyl-1*H*-benzimidazole (**6d**). Compound **7q** was obtained by reaction of 2-chlorobenzimidazole with piperazine.[25]

Scheme 3[a]



[a] Reagents: (a) (i) $SOCl_2$; (ii) NaN_3; (iii) *t*-BuOH; (b) H_2, Pd/C, EtOH; (c) $BrCH_2Ph$, K_2CO_3, CH_3CN; (d) K_2CO_3, DMF, Δ; (e) $POCl_3$/HCl, Δ.

Disubstituted aromatic derivatives **6o** and **6p** were synthesized according to procedure C by cyclization of *o*-phenylenediamines with urea and N-benzylation and halogenation with phosphorous oxychloride and hydrochloric acid under pressure. Monosubstituted aromatic derivatives having the substituent at the 4- or 5-position were synthesized according to procedure D (Scheme 2): 2-nitroanilines **4** were benzylated with benzyl bromide/potassium carbonate, hydrogenated over palladium/carbon, cyclized with urea, and halogenated to the corresponding 2-chlorobenzimidazole **6f**−**i** and **6m**.

Compounds having aromatic substitution at the 6- or 7-position were prepared according procedure E as illustrated in Scheme 3; regioselectively monoprotected 1,2-diaminobenzene derivatives **9** were obtained in an indirect way from 2-nitrobenzoic acids **5** through a Curtius rearrangement in the presence of *t*-BuOH followed by hydrogenation over palladium/carbon. These diamines were benzylated and converted into the corresponding benzimidazolones **11** by intramolecular cyclization in the presence of K_2CO_3. Conventional treatment of **11** with $POCl_3$/HCl yielded the chloro derivatives **6k** and **6n**. Hydroxylated compounds **7j** and **7l** were prepared from the corresponding methoxylated compounds **7i** and **7k** by heating to reflux with hydrobromic acid in acetic acid.

Pharmacological Results and Discussion

The new 2-piperazinylbenzimidazole derivatives **7** were initially evaluated for *in vitro* 5-HT_3 receptor affinity by radioligand binding assay. For each compound, the ability to displace the specific ligand [^{3}H]LY278584 from 5-HT_3 sites of rat entorhinal cortex was determined. The antagonistic activity at 5-HT_3 receptors was performed by evaluating the inhibition of the B−J reflex evoked by 5-HT in urethane-anesthetized rats. None of the tested compounds *per se* evoked bradycardia in anesthetized rats at doses up to 100 μg/kg iv. A binding profile for the main 5-HT receptors and the dopamine D_2 receptor was performed by radioligand binding assays using specific ligands at different brain areas from rats or guinea pigs. 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. The intravenous effective doses to inhibit 50% of B−J reflex (ED_{50}) were estimated from dose−response curves based on measurements obtained from five rats/dose.

The pK_i and ED_{50} values for the new compounds **7** are summarized in Table 2 (data for tropisetron and granisetron are also included in this table for comparison). Their affinities for other serotonin receptors (5-HT_{1A}, 5-HT_{2A}, 5-HT_4) and the dopamine D_2 are shown in Table 3.

The presence of the piperazine moiety in the 2-position of benzimidazole is necessary to 5-HT_3 receptor affinity, and replacement of the piperazine by other amines as piperidine, morpholine, pyrrolidine, arylpiperazine, and *N*-methylaniline accounts for a complete loss of activity (ED_{50} > 100 μg/kg iv in the inhibition of B−J reflex). Because of that these compounds were not selected for further studies.

Table 2. 5-HT_3 Receptor Binding Data[a] and *in Vivo* Receptor Antagonism

compd	binding pK_i (±SE)[b]	B−J reflex inhibition ED_{50}[c] [95% + CL][d]
7a	7.3 (±0.1)	5.3 [4.1−7.5]
7b	7.1 (±0.1)	7.7 [6.2−9.0]
7c	8.0 (±0.2)	6.5 [5.6−7.8]
7d	8.8 (±0.1)	2.6 [1.7−3.8]
7e	9.2 (±0.3)	2.0 [1.4−2.9]
7f	9.5 (±0.1)	1.1 [0.7−1.6]
7g	9.5 (±0.2)	2.5 [1.2−3.5]
7h	8.8(±0.1)	1.2 [0.8−1.8]
7i	7.2 (±0.1)	>100
7j	9.5 (±0.2)	1.3 [0.5−2.0]
7k	8.4 (±0.1)	26.8 [22.5−32.6]
7l	9.0 (±0.1)	1.0 [0.4−1.7]
7m	6.7 (±0.1)	>100
7n	9.4 (±0.2)	0.7 [0.4−1.2]
7o	8.7 (±0.1)	4.9 [3.8−6.0]
7p	8.5 (±0.2)	16.6 [13.1−20.5]
7q	7.5 (±0.1)	>100
4, tropisetron	8.8 (±0.2)	1.7 [1.0−2.7]
2, granisetron	8.7 (±0.1)	0.7 [0.5−0.9]

[a] Displacement of [^{3}H]-LY278584. [b] Standard error. [c] ED_{50} from dose−effect curves. Values in μg/kg iv. [d] 95% confidence limits.

Table 3. Displacement of Binding to 5-HT_{1A}, 5-HT_{2A}, and 5-HT_4 Serotonin Receptors and to D_2 Dopamine Receptors by Compounds of Table 1 and Reference Drugs

	pK_i (±SE)			
compd	5-HT_{1A}	5-HT_{2A}	5-HT_4	D_2
7a−**p**	<6[a]	<6[a]	<6[a]	<6[a]
8-OH-DPAT	8.9 (±0.2)			
ritanserin		9.2 (±0.3)		
SB-203186			9.0 (±0.3)	
haloperidol				9.2 (±0.2)

[a] pIC_{50}: negative logarithm of the molar concentration required to inhibit 50% of radioligand specific binding (IC_{50}).

From results shown in Table 2 the following conclusions can be reached:

(a) N_1-substituted benzimidazoles **7a**−**e** show higher affinities than the parent N_1-unsubstituted compound **7q**; moreover, compounds with more voluminous substituent (cyclopropyl and phenylmethyl) are the most active. Therefore, the compound 1-(phenylmethyl)-2-piperazinyl-1*H*-benzimidazole (**7e**) (pK_i = 9.2, ED_{50} = 2.0 μg/kg iv) shows higher affinity for the 5-HT_3 receptor than do granisetron and tropisetron (Figure 1a).

(b) The effect of substitution on the aromatic ring of 1-(phenylmethyl)-2-piperazinyl-1*H*-benzimidazole (**7e**) was studied. Introduction of a fluorine atom or a hydroxy group in the 5-position led to compounds **7f** (pK_i = 9.5) and **7j** (pK_i = 9.5) with higher affinity for the 5-HT_3 receptor and greater ability to inhibit the B−J

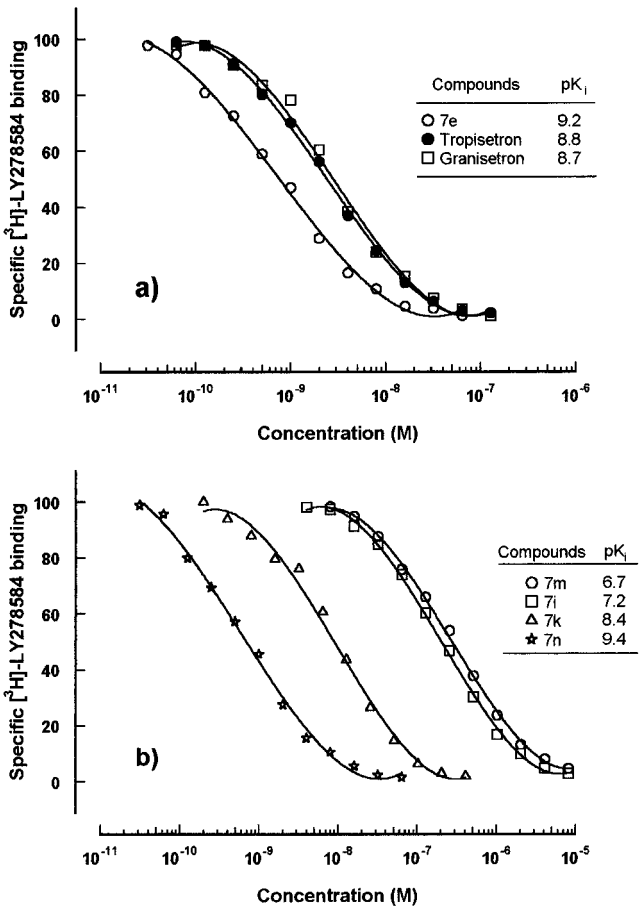


Figure 1. Competition for [^{3}H]LY278584 binding sites in rat entorhinal cortex by (a) compound **7e**, tropisetron, and granisetron and (b) methoxy derivatives **7m**, **7i**, **7k**, and **7n**.

reflex (ED_{50} = 1.1 and 1.3 μg/kg iv) than that of the unsubstituted compound **7e**. The importance of methoxy substitution in 5-HT$_3$ antagonists[26] prompted us to synthesize the 5-methoxy derivative **7i** (pK_i = 7.2). This modification unexpectedly decreased by 100-fold the affinity for the 5-HT$_3$ receptor over **7e**, and the ED_{50} was higher than 100 μg/kg iv in B−J reflex inhibition. Consequently we sought to study the effect on the affinity and the activity of the methoxy group in the different positions of the aromatic ring of compound **7e**. Thus, introduction of a methoxy group in the 7-position led to compound **7n** (pK_i = 9.4, ED_{50} = 0.7 μg/kg iv) with greater affinity for the 5-HT$_3$ receptor and higher B−J reflex inhibition than compound **7e**. These pK_i and ED_{50} values gradually decreased upon introduction of the same substituent in the remaining positions of the ring according to the sequence 7 > 6 ≫ 5 > 4 (Figure 1b). It can be concluded that 5-HT$_3$ antagonistic activity of the studied compounds are more directly related to the different substitution position than to the nature of the substituent.

(c) Disubstituted compounds 5,6-dimethyl **7o** (pK_i = 8.7, ED_{50} = 4.9 μg/kg iv) and 5,6-dichloro **7p** (pK_i = 8.5, ED_{50} = 16.6 μg/kg iv) showed good affinity, but ED_{50} values were higher than those for monosubstituted compounds **7h** (pK_i = 8.8, ED_{50} = 1.2 μg/kg iv) and **7g** (pK_i = 9.5, ED_{50} = 2.5 μg/kg iv).

(d) Hydroxy-substituted compounds exhibited higher affinities than those of methoxy substituted ones. Thus **7i** (pK_i = 7.2, ED_{50} > 100 μg/kg iv) had a lower affinity, and the related hydroxy derivative **7j** (pK_i = 9.5, ED_{50} = 1.3 μg/kg iv) showed the highest affinity in the series. The same trend was observed for compounds **7k** (pK_i = 8.4, ED_{50} = 26.8 μg/kg iv) and **7l** (pK_i = 9.0, ED_{50} = 1.0 μg/kg iv).

In order to establish the receptor selectivity of the compounds **7** for the 5-HT$_3$ receptor, their affinities for other receptors, namely 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_4$, and D_2, were also evaluated. Results are summarized in Table 3. These 2-piperazinylbenzimidazole derivatives represent a new class of high-affinity ligands for the 5-HT$_3$ receptor and exhibit potent activity in the B−J reflex inhibition. **7e** and **7n** are now under further investigation as drugs for the control of nausea and emesis evoked by citotoxic drugs such as cisplatin.

Experimental Section

Chemistry. Each compound was characterized by elemental analysis and their IR, ^{1}H-NMR, and ^{13}C-NMR spectra. IR spectra were recorded on a Perkin-Elmer 1310 instrument on KBr plates; the frequencies are expressed in cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker AC-200; chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS), which was used as internal standard. Coupling constants are given in hertz. Melting points were determined in open capillaries on a Büchi SMP-20 apparatus and are uncorrected. Flash column chromatography were performed with silica gel, particle size 60 Å, mesh = 230−400 (Merck). Elemental analyses were within 0.4% of the theoretical values.

2-Chloro-1-(phenylmethyl)-1*H*-benzimidazole (6e). Procedure A. This procedure illustrates the general method of preparation of compounds **6a**−**c** and **6e**.

Sodium hydride (60% in oil, 0.44 g, 11 mmol) was portionwise added to an ice-cooled solution of 2-chlorobenzimidazole (1.52 g, 10 mmol) in 10 mL of DMF, and the mixture was stirred at room temperature for 1 h. Benzyl bromide (1.18 mL, 10 mmol) was added, and stirring was continued for 1 h. The reaction mixture was diluted with water, and the formed precipitate was collected by filtration and purified by recrystallization from hexane to give 2.18 g (9 mmol, 90%) of a white solid: mp 106−108 °C; ^{1}H NMR ($CDCl_3$) δ 5.4 (s, 2H), 7.2−7.4 (m, 8H), 7.9 (m, 1H).

2-Chloro-1-cyclopropyl-1*H*-benzimidazole (6d). Procedure B. (a) *N*-Cyclopropyl-2-nitroaniline. A mixture of 2-chloronitrobenzene (3.14 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 a mixture of hexane and CH_2Cl_2 (7:3) as eluent to yield after solvent evaporation 2.15 g (12 mmol, 60%) of an orange oil: ^{1}H NMR ($DCCl_3$) δ 0.6−0.7 (m, 2H), 0.8−1 (m, 2H), 2.6 (m, 1H), 6.7 (m, 1H), 7.3 (2d, 1H), 7.4−7.6 (m, 2H), 8.2 (2d, 1H); ^{13}C NMR ($CDCl_3$) δ 7.74, 24.36, 115.23, 126.39, 135.83, 146, 164.

(b) 1,2-Diamino-*N*-cyclopropylbenzene. A solution of *N*-cyclopropyl-2-nitroaniline (2.15 g, 12 mmol) in EtOH (100 mL) was hydrogenated over 10% palladium/carbon (0.5 g) at 3 atm for 4 h. The catalyst was filtered off and the filtrate evaporated in vacuo to give 1.54 g (10.44 mmol, 87%) of the desired product: ^{1}H NMR ($CDCl_3$) δ 0.5 (m, 2H), 0.7−0.8 (m, 2H), 2.4 (m, 1H), 3.4 (m, NH), 6.6−6.7 (m, 2H), 6.8 (m, 1H), 7.1 (d, 1H).

(c) 2-Chloro-1-cyclopropyl-1*H*-benzimidazole (6d). A mixture of 1,2-diamino-*N*-cyclopropylbenzene (1.55 g, 10.49 mmol) and urea (0.66 g, 11 mmol) was stirred at 220 °C for 45 min. The reaction mixture was diluted with water and extracted with $CHCl_3$ and the extract washed with water and dried over Na_2SO_4. The solvent was evaporated in vacuo and the residue triturated with ether to yield 0.72 g (4.16 mmol, 40%) of a brown solid, which was heated in a 25 mL high-

pressure vessel with $POCl_3$ (7 mL) and HCl (2 drops) at 150 °C for 3 h. The reaction mixture was poured onto ice−water, neutralized with 50% NaOH, and extracted with CH_2Cl_2. The extract was washed with water, dried over Na_2SO_4, and concentrated to yield 0.6 g (3.12 mmol, 75%) of the product as a brown oil: ^{1}H NMR ($CDCl_3$) δ 1.2 (m, 4H), 3.2 (m, 1H), 7.2 (m, 2H), 7.4 (m, 1H), 7.7 (m, 1H).

2-Chloro-5,6-dimethyl-1-(phenylmethyl)-1*H*-benzimidazole (6o). Procedure C. This procedure illustrates also the preparation of compound **6p**. A mixture of 4,5-dimethyl-1,2-diaminobenzene (3.4 g, 25 mmol) and urea (1.5 g, 25 mmol) was stirred at 150 °C for 30 min. The reaction mixture was diluted with water and 10% NaOH (10 mL), and the precipitate was collected by filtration to give 3.5 g (21.8 mmol, 87.5%) of a brown solid. This product was heated in a high-pressure vessel with $POCl_3$ (30 mL) and HCl (6 drops) at 150 °C for 3 h. The reaction mixture was poured onto ice−water and treated with 50% NaOH, and the precipitate was collected by filtration to give 3.53 g of 5,6-dimethyl-2-chlorobenzimidazole (19.6 mmol, 90%) as a brown solid.

Sodium hydride (60% in oil, 0.93 g, 21.6 mmol) was added portionwise to an ice-cooled solution of 5,6-dimethyl-2-chlorobenzimidazole (3.53 g, 19.6 mmol) in DMF (30 mL), and the mixture was stirred at room temperature for 1 h. Benzyl bromide (2.43 mL, 20 mmol) was added and stirring continued for 1 h. The reaction mixture was diluted with water and the precipitate collected by filtration and purified by flash chromatography using a mixture of MeOH and CH_2Cl_2 (2:98) as eluent to yield after evaporation of the solvent 2.97 g (10.78 mmol, 50%) of a yellow solid: mp 178−180 °C; ^{1}H NMR ($CDCl_3$) δ 2.3 (2s, 6H), 5.4 (s, 2H), 7−7.5 (m, 7H).

2-Chloro-5-fluoro-1-(phenylmethyl)-1*H*-benzimidazole (6f). Procedure D. This procedure illustrates the general method for preparation of compounds **6f**−**i** and **6m**.

(a) 2-Amino-4-fluoro-*N*-(phenylmethyl)aniline (8f). Benzyl bromide (3.16 mL, 26 mmol) was added dropwise to a suspension of 4-fluoro-2-nitrobenzenamine (3.2 g, 20 mmol) and K_2CO_3 powder (3.6 g, 26 mmol) in DMF (30 mL), and the resulting mixture was maintained at 110−120 °C for 4 h. The reaction mixture was diluted with water and extracted with CH_2Cl_2. The extract was washed with water and dried over Na_2SO_4, the solvent was evaporated in vacuo, and the residue was dissolved in THF (70 mL) and hydrogenated at 24−25 °C over 10% Pd/C (0.8 g) for 3 h at 3 atm. The catalyst was removed by filtration and the filtrate evaporated. The remaining crude oil was purified by flash chromatography using a mixture of AcOEt and hexane (5:1) as eluent to yield, after solvent evaporation, 2.65 g (12.3 mmol, 75%) of the desired product: ^{1}H NMR ($CDCl_3$) δ 3.4 (broad, NH), 4.2 (s, 2H), 6.4−6.6 (cluster, 3H), 7.3−7.4 (cluster, 5 H).

Compounds **8g**, **8h**, **8i**, and **8m** were prepared following a similar procedure.

(b) 2-Chloro-5-fluoro-1-(phenylmethyl)-1*H*-benzimidazole (6f). A mixture of **8f** (2.65 g, 12.30 mmol) and urea (0.78 g, 13 mmol) was stirred at 220 °C for 0.5 h. The reaction mixture was diluted with water and extracted with $CHCl_3$; the extract was washed with water and dried over Na_2SO_4. The solvent was evaporated in vacuo and the residue triturated with hexane to yield, after filtration, 2.42 g (10 mmol, 77%) of 5-fluoro-1-(phenylmethyl)-1*H*-benzimidazol-2-one. This product was heated in a 25 mL high-pressure vessel with $POCl_3$ (4 mL) and HCl (3 drops) at 150 °C for 3 h, and the reaction mixture was poured onto ice−water, neutralized with 50% NaOH, and extracted with CH_2Cl_2. The extract was washed with water, dried over Na_2SO_4, and concentrated to yield a brown solid: 1.56 g (6 mmol, 60%): ^{1}H NMR ($CDCl_3$) δ 5.4 (s 2H), 7−7.4 (cluster, 8H).

Compounds **6g**−**i** and **6m** were prepared by the procedure described above.

2-Chloro-7-methoxy-1-(phenylmethyl)-1*H*-benzimidazole (6n). Procedure E. This procedure illustrates the general method for the preparation of compounds **6n** and **6k**.

(a) *tert*-Butyl *N*-(2-Amino-3-methoxyphenyl)carbamate (9n). A mixture of 2-nitro-3-methoxybenzoic acid (3.94 g, 20 mmol), thionyl chloride (2.2 mL, 30 mmol), and DMF (4 drops) in toluene (20 mL) was refluxed for 0.5 h. The solvent was evaporated in vacuo, and the residue was dissolved in acetone (20 mL). The solution was added dropwise to an ice-cooled solution of NaN_3 (2 g, 30 mmol) in water (20 mL), and stirring was continued for 1 h. The reaction mixture was diluted with water and the precipitate collected by filtration and dried. A mixture of the crude azide and *tert*-butyl alcohol (20 mL) was gradually warmed and then refluxed for 10 min. After evaporation of the solvent in vacuo, the residue was purified by flash chromatography using a mixture of AcOEt and hexane (5:1) as eluent to yield, after solvent evaporation, 3.16 g (12 mmol, 60%) of *tert*-butyl *N*-(2-nitro-3-methoxyphenyl)carbamate as a pale yellow solid: mp 99−100 °C; ^{1}H NMR ($CDCl_3$) δ 1.50 (s, 9H), 3.89 (s, 3H), 6.7 (dd, 1H, J = 0.83 and 8.43), 7.38 (t, 1H, J = 8.43), 7.6 (broad, NH), 7.76 (dd, 1H, J = 0.83 and 8.43); IR (KBr) 3900, 1720, 1600 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 152.67, 151.96, 132.89, 131.85, 113.16, 106.55, 81.64, 56.54, 28.07.

A solution of the above compound (3 g, 11 mmol) in EtOH (150 mL) was hydrogenated (3 atm) over 10% palladium/carbon (0.5 g) at room temperature for 3 h. The catalyst was filtered off and the filtrate evaporated in vacuo to give 2.64 g (11 mmol, 100%) of the desired product as a white solid: mp 110−112 °C; ^{1}H NMR ($CDCl_3$) δ 1.5 (s, 9H), 3.89 (s, 3H), 3.89 (s, NH), 6.35 (broad, NH), 6.65 (d, 1H), 6.75 (t, 1H), 6.9 (d, 1H); IR (KBr) 3350, 1680 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 153.66, 148.64, 129.50, 125.17, 118.36, 116.56, 107.14, 80.4, 57.75, 28.28.

Compound **9k** was prepared by the procedure described above.

(b) *tert*-Butyl *N*-[3-Methoxy-2-[(phenylmethyl)amino]-phenyl]carbamate (10n). To a stirred mixture of **9n** (2.15 g, 9 mmol), K_2CO_3 (1.38 g, 10 mmol), and CH_3CN (30 mL) was added dropwise benzyl bromide (1.19 mL, 10 mmol). The reaction mixture was stirred at room temperature for 7 h. After evaporation of the solvent, the residue was diluted with water and extracted with AcOEt. The extract was washed with water and dried over Na_2SO_4. The solution was concentrated in vacuo, and the residue was triturated with hexane to yield 1.55 g (4.7 mmol, 52%) of a white solid: mp 86−88 °C; ^{1}H NMR ($CDCl_3$) δ 1.5 (s, 9H), 3.7 (s, 3H), 4.0 (s, 2H), 6.5 (d, 1H), 7.0 (t, 1H), 7.4 (m, 5H), 7.5 (broad, NH), 7.7 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 153.67, 153.06, 139.92, 134.37, 128.45, 127.25, 125.80, 124.54, 111.46, 104.94, 80.04, 55.63, 28.34.

Compound **10k** was prepared following a similar procedure.

(c) 7-Methoxy-1-(phenylmethyl)-1*H*-benzimidazol-2-one (11n). A mixture of **10n** (1.55 g, 4.7 mmol) and K_2CO_3 (0.69 g, 5 mmol) in DMF (20 mL) was heated at 130 °C for 4 h. The reaction mixture was poured onto ice−water and filtered to yield 1.42 g (4.5 mmol, 95%) of **11n** as a yellowish solid: ^{1}H NMR ($CDCl_3$) δ 3.8 (s, 3H), 5.3 (s, 2H), 6.6 (d, 1H), 6.7 (d, 1H), 7 (t, 1H), 7.2−7.4 (broad, 5H), 10.1 (s, NH); ^{13}C NMR ($CDCl_3$) δ 155.11, 144.57, 138.41, 129.26, 127.92, 127.09, 126.68, 121.39, 118.19, 104.11, 102.85, 55.3, 45.43.

Compound **11k** was prepared following a similar procedure.

(d) 2-Chloro-7-methoxy-1-(phenylmethyl)-1*H*-benzimidazole (6n). A mixture of **11n** (2.11 g, 8.3 mmol), $POCl_3$ (30 mL), and concentrated HCl (6 drops) was heated in a high-pressure vessel at 150 °C for 3 h. The reaction mixture was poured onto ice−water and extracted with CH_2Cl_2. The organic layer was washed with water, dried over Na_2SO_4, and concentrated in vacuo to yield 1.8 g (6.6 mmol, 80%) of a brown solid: mp 111−113 °C; ^{1}H NMR ($CDCl_3$) δ 3.9 (s, 3H), 5.6 (s, 2H), 6.7 (d, 1H), 7.3 (m, 7H).

Compound **6k** was prepared by the procedure described above.

1-(Phenylmethyl)-2-piperazinyl-1*H*-benzimidazole (7e). General Procedure for the Preparation of 2-(1-Piperazinyl)benzimidazole 7 (Table 1). Procedure F. A mixture of **6e** (2.42 g, 10 mmol) and piperazine (4.3 g, 50 mmol) was heated at 150−160 °C for 30 min. The reaction mixture was poured onto water, and then 10% HCl was added to acidify the mixture, which was washed twice with $CHCl_3$. Then a solution of 10% NaOH was added to basify the mixture, followed by extraction with $CHCl_3$. The organic layer was washed with water and dried over Na_2SO_4. The solvent was evaporated under reduced pressure, and the residue was

purified by recrystallization from hexane to give the desired product **7e** (2.77 g, 9.5 mmol, 95%) as a white solid: mp 129−130 °C; ^{1}H NMR ($CDCl_3$) δ 2.2 (broad, 1H), 2.5 (m, 4H), 2.6 (m, 4H), 4.6 (s, 2H), 7−7.4 (m, 8H), 7.7 (m, 1H).

5-Hydroxy-1-(phenylmethyl)-2-piperazinyl-1*H*-benzimidazole (7j). A mixture of **7i** (1.21 g, 4 mmol), 20 mL of HBr (48%), and 10 mL of acetic acid was stirred at 150 °C for 3 h. After evaporation of the solvent, the residue was treated with 10% Na_2CO_3 solution until pH = 10 and extracted with CH_2Cl_2. The organic layer was washed with water and dried over Na_2SO_4. The solution was concentrated in vacuo to give 0.74 g (2.4 mmol, 60%) of the desired product as a yellow solid: mp 100−102 °C; ^{1}H NMR (DMSO-d_6) δ 2.9 (m, 4H), 3.2 (m, 4H), 5.2 (s, 2H), 6.5 (m, 1H), 6.9 (m, 2H), 7.1−7.4 (m, 5H), 9.0 (broad, OH).

Compound **7l** was prepared by a similar procedure.

Pharmacological Methods. 5-HT$_{1A}$ Receptor Binding Assay.[27] Adult male Wistar rats weighing 220−280 g were used. Animals were killed by decapitation, and the whole brain with the exception of the brainstem and cerebellum was quickly removed and the various areas dissected, weighed, and immediately frozen at −70 °C. The cerebral cortex used for the binding experiments was homogenized with an Ultra-Turrax (setting 5 for 20 s) in 10 volumes of ice-cold 0.32 M sucrose buffer, centrifuged at 900*g* for 10 min (4 °C), and the supernatant was recentrifuged at 4800*g* for 25 min (4 °C). The resulting pellet was resuspended in 10 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.5), incubated at 37 °C for 15 min, and then centrifuged once more at 48000*g* for 25 min (4 °C). The final pellet was resuspended in 4 volumes of ice-cold 50 mM Tris-HCl buffer containing 4 mM $CaCl_2$ and 0.1% ascorbate and was stored at −70 °C until use. At the time of the experiment, the membranes were diluted in the same ice-cold buffer with 10 μM pargyline (final dilution 1:28, 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 vehicule if no competing drug was added) and 0.1 mL of [^{3}H]-8-OH-DPAT (NEN, 148−163 Ci/mmol) in buffer (final concentration 1.5 nM). Nonspecific binding was determined using 10 μM cold 5-HT. The binding experiment was initiated by addition of 0.8 mL of a membrane suspension (700−800 μg of protein). After incubation for 30 min at 37 °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 7.5) buffer. Filters were placed in scintillating polyethylene 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 by using the computer program EBDA (McPherson).[28]

5-HT$_{2A}$ Receptor Binding Assay.[29] Adult male Wistar rats weighing 220−280 g were used. Animals were killed by decapitation, the whole brain with the exception of the brain stem and cerebellum was quickly removed, and the various areas were dissected, weighed, and immediately frozen at −70 °C. Prefrontal cortex used for the binding experiments were homogenized with an Ultra-Turrax (setting 5 for 20 s) in 10 volumes of ice-cold 0.25 M sucrose buffer and centrifuged at 1080*g* for 10 min (4 °C), and the supernatant was recentrifuged at 35000*g* for 10 min (4 °C). The resulting pellet was resuspended in 40 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.7) and then centrifuged once more at 3500*g* for 10 min (4 °C). The final pellet was resuspended in 10 volumes of ice-cold 50 mM Tris-HCl buffer and was stored at −70 °C until use. At the time of the experiment, the membranes were diluted in the same ice-cold buffer (final dilution 1:60, 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 in Tris-HCl buffer with 10% ethanol (0.1 mL of Tris-ethanol buffer in no competing drug was added) and 0.1 mL of [^{3}H]ketanserin (NEN, 60−90 Ci/mmol) in Tris-ethanol buffer (final concentration 0.8 nM). Nonspecific binding was determined using 1 μM cold methysergide. Binding experiment was initiated by addition of 0.8 mL of membrane suspension (400−500 μg of protein). After incubation for 15 min at 37 °C the reaction was stopped for vacuum filtration through Whatman GF/B glass pretreated filters (1% polyethylenimine 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 7.7) buffer. Filters were placed in scintillating polyethylene vials (with 5 mL 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).[28]

5-HT$_3$ Receptor Binding Assay.[30] Adult male Wistar rats weighing 220−280 g were used. Animals were killed by decapitation, the whole brain with the exception of the brain stem and cerebellum was quickly removed, and the various areas were dissected, weighed, and immediately frozen at −70 °C. The enthorinal cortex used for the binding experiments was homogenized with an Ultra-Turrax (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 more times 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 the 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 vehicule if no competing drug was added) and 0.1 mL of [^{3}H]LY278584 (Amersham, 60−85 Ci/mmol) in buffer (final concentration 2 nM). Nonspecific binding was determined using 10 μM cold 5-HT. The 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% polyethylenimine 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 scintillating polyethylene vials (with 5 mL 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).[28]

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. The striatum used for the binding experiments was homogenized with an Ultra-Turrax (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 vehicule 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% polyethylenimine in 50 mM Hepes buffer), using a Brandel cell harvester. Filters were placed in scintillating polyethylene 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).[28]

D_2 Receptor Binding Assay.[32] Adult male Wistar rats weighing 220−280 g were used. Animals were killed by decapitation, the whole brain with the exception of the brain stem 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 (setting 5 for 20 s) in 50 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.7) and 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 the 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 vehicule 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. The 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 scintillating polyethylene 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).[28]

Inhibition of the Bezold−Jarisch Reflex. Adult male Wistar rats weighing 220−300 g and fasted for 18 h were used. Rats were anesthetized with urethane (1.25 g/kg ip) and placed on a heating table (Hugo Sachs Elektronik, Freiburg, Germany) to maintain body temperature at 37 °C. The trachea and right jugular vein were cannulated to facilitate respiration and drug administration, respectively. The carotid artery was also cannulated and connected to a Goul Statham P23Db pressure transducer to record blood pressure. The heart rate was measured using the blood pressure signal and a cardiotachometer coupler and recorded on a Graphtec Linearcorder WR3101 (Hugo Sachs Elektronik, Freiburg, Germany). The B−J reflex was evoked by rapid bolus iv injection of 5-HT (30 μg/kg). When 5-HT-induced bradycardia (65−75% fall of heart rate from control heart rate) returned to pretreatment levels (within 5 min), either antagonist or saline were administered (1 mL/kg), and 5-HT-induced bradycardia was elicited again 5 min later. Drugs were prepared daily and were dissolved in distilled water or 0.1 M tartaric acid, with subsequent dilutions in physiological saline. ED_{50} were calculated from three to five doses (four or five rats per dose) by a linear-regression analysis.

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Supporting Information Available: Yields, melting points, and spectral data (^{1}H NMR) for compounds **7a**−**d** and **7f**−**k** (2 pages). Ordering information is given on any current masthead page.

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