Arylcarbamate Derivatives of 1-Piperidineethanol as Potent Ligands for 5-HT_4 Receptors

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A series of carbamate derivatives (**7**) of 2-(1-piperidinyl)ethyl 4-amino-5-chloro-2-methoxybenzoates, which have been described as potent agonists and antagonists of 5-HT_4 receptors, were synthesized. They were evaluated using radioligand binding assays with [^{3}H]GR 113808, a 5-HT_4 receptor selective ligand, in the rat striatum and the electrically stimulated myenteric plexus longitudinal muscle of the guinea pig. In contrast to the previously described ester derivatives, a drop in the affinity for 5-HT_4 receptors was observed and the compounds were inactive as agonists in the guinea pig ileum preparation. Unexpectedly, the *ortho*-substituted carbamates **8b**,**c** (R′ = H, RO = MeO or EtO, R″ = H) had nanomolar affinity for 5-HT_4 receptors (K_i = 8.9 ± 0.5 and 2.6 ± 0.4 nM, respectively). As reported previously, the *cis*- or *trans*-3,5-dimethyl substitution of piperidine (**8n**,**o**) was particularly favorable (K_i = 1.1 ± 0.6 nM for both isomers). **8c** is an antagonist equipotent to the 5-HT_4 receptor antagonist SDZ 205-557 (**1**).

Stimulation of 5-HT_4 receptors is responsible for the gastrokinetic effects of drugs such as the benzamides.[1−3] In the guinea pig[4b] they are located on the ileal enteric nerve where they mediate the release of acetylcholine, thus bringing about the first phase of the muscle contraction−concentration curve to 5-HT. The effect of stimulation of 5-HT_4 receptors seems to be species-dependent as very different results have been obtained in the rat where 5-HT_4 receptor stimulation in the rat ileum induced relaxation via an atropine-insensitive mechanism, suggesting a localization[1] on the smooth muscle. On the other hand, in man 5-HT_4 receptor-mediated effects have not been observed in the ileum. However 5-HT_4 receptors are present in the colon[1] where they appear to mediate a direct effect on the smooth muscle, inducing muscle relaxation. Several studies have shown the presence of 5-HT_4 receptors in the stomach of a variety of species, such as the guinea pig, rat, dog, and man,[1] where they mediate an increase in gastric emptying. 5-HT_4 receptors are also responsible for the relaxation of the esophagus muscle in the rat.[4a]

Considerable progress has been made in the localization and study of the functions of 5-HT_4 receptors with the development of potent and selective antagonists such as SDZ 205-557 (**1**),[5] SB 204070A (**2**),[6,7] SB207266 (**3**),[8] and GR 113808 (**4**)[9] and, in particular, by the use of a number of them as radiolabeled ligands.[7,9,10]

Research into selective 5-HT_4 receptor agonists as gastrointestinal (GI) prokinetic drugs for the treatment of motility disorders such as gastroesophageal reflux, functional dyspepsia, and constipation has received considerable interest recently. Many of the compounds described as possessing these properties, such as cisapride, renzapride, SC-53116 (**5**), BRL 24682 (**6**), and zacopride, are members of the generic benzamide family being amides of 4-amino-5-chloro-2-methoxybenzoic acid,[1] which appears to be an important structural requirement for 5-HT_4 receptor agonist activity.

Recently, a new family of esters, derived from 2-(1-piperidinyl) 4-amino-5-chloro-2-methoxybenzoate,[11] were described by us, as potent selective ligands for 5-HT_4 receptors. Almost all of these derivatives were potent agonists for this receptor, although it was shown that the introduction of a 3,5-dimethylpiperidine moiety in the basic chain brought about a dramatic change in the pharmacological profile, as 2-(3,5-dimethylpiperidino)-ethyl 4-amino-5-chloro-2-methoxybenzoate[12] was demonstrated to be a potent 5-HT_4 receptor antagonist. However, the presence of the ester function is an important drawback for their administration *in vivo* due to their putative short half-lives. To overcome this problem, a series of carbamates (**7**) derived from the esters previously described were synthesized, several structural variations of the aromatic moiety were carried out (compounds **8**), and the role of the oxygen atom in the basic chain was evaluated with the synthesis of the amidic derivative **9**.

Chemistry

The carbamates **7**, structurally closely related to benzoates derived from 4-amino-5-chloro-2-methoxybenzoic acid, were prepared according to the synthetic pathway described in Scheme 1. 5-Chloro-*o*-anisidine was acetylated and transformed into the corresponding nitro amine derivative **10** according to the process described by Hadley.[13] It was treated with phosgene in a toluene solution to give 5-chloro-2-methoxy-4-nitrophenyl isocyanate (**11**) which reacted with the different amino alcohols, easily synthesized by the reaction of 2-bromoethanol and the secondary amines in the presence of NaOH, to give compounds **12**. The nitro group of compounds **12** was reduced into the amino derivatives **7a**−**g** by the reaction of $Na_2S_2O_4$ in a MeOH/H_2O[14] mixture with a moderate yield. Compounds were isolated as hydrochloride or oxalate salts.

Mono- or disubstituted carbamates **8** were prepared from the isocyanates **13** which were either commercially

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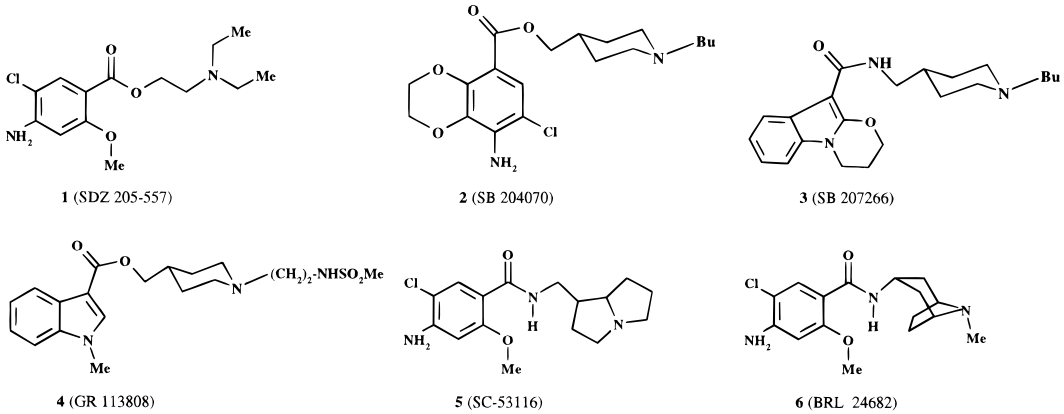
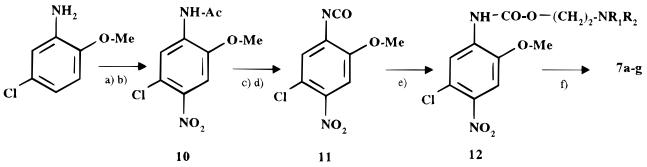
Chart 1



Scheme 1[a]



[a] (a) AcCl, CH_2Cl_2, rt; (b) HNO_3, H_2SO_4, AcOH; (c) NaOH, EtOH/H_2O, reflux; (d) 20% toluene solution of $COCl_2$, CH_2Cl_2, 0 °C; (e) $R_2R_1N(CH_2)_2OH$, 0 °C, reflux; (f) $Na_2S_2O_4$, MeOH/H_2O.

available or prepared from the corresponding anilino derivatives by reaction with phosgene. Their condensation (Scheme 2) with the corresponding amino alcohols in THF under reflux gave compounds **8a**−**i,m**−**o**. **8j**−**l** were obtained by the reduction of the corresponding nitro derivatives **8p**−**r** by $Na_2S_2O_4$.[14] The derivatives of 3,5-dimethylpiperidine (compounds **7g**, **8m**−**o**) were obtained as a 4:1 mixture of *cis* and *trans* compounds, respectively, which were separated by column chromatography and characterized by their ^{1}H-NMR spectra.

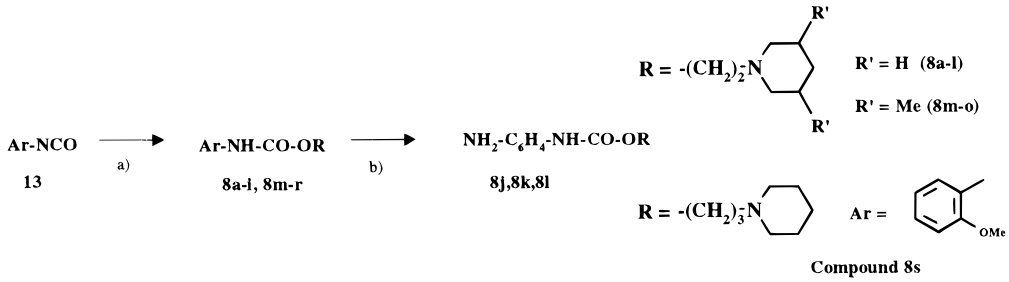
Amide **9b** was prepared from 5-chloro-2-methoxy-4-nitroaniline (Scheme 3) which was condensed with 4-chlorobutyryl chloride to give the chloro amide **15**. The reaction of **15** (Ar = 5-chloro-2-methoxy-4-nitrophenyl) and piperidine gave a mixture of 1-(5-chloro-2-methoxy-4-nitrophenyl)-2-pyrrolidone by a cyclization reaction and the amide **9a** which was isolated with a poor yield and reduced by $SnCl_2$[13] in an HCl mixture to the amino derivative **9b**. In contrast, **9c** was prepared directly from *o*-anisidine with an excellent yield.

Biological Methods

Assessment of the affinity of the compounds for 5-HT_4 receptors was performed using radioligand binding assays with [^{3}H]GR 113808 and rat striatal membranes.[15] The existence of 5-HT_4 receptors has been clearly demonstrated in the central nervous system (CNS), and these receptors have been shown to be indentical to those in the periphery, in particular, those in the intestine.[16] Consequently, binding assays in the brain constitute a valuable test to select compounds which will be active in the GI tract.

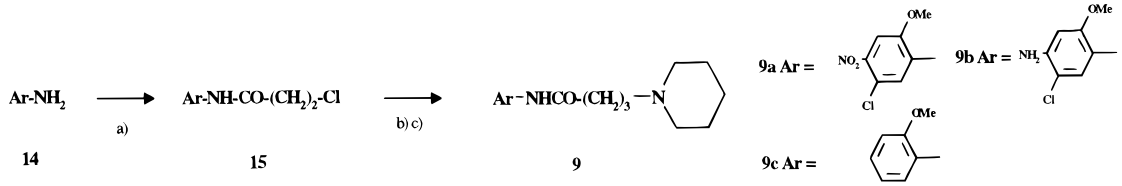
The facilitatory role of 5-HT_4 receptors in the peristaltic reflex was demonstrated by Craig and Clarke[4] who found that both 5-HT_3 and 5-HT_4 receptor agonists induced peristalsis in the guinea pig isolated ileum maintained at a subthreshold intraluminal pressure. The 5-HT_4 receptor agonist activity of the tested compounds was measured in the electrically stimulated myenteric plexus and longitudinal muscle of the guinea pig ileum, and it was assessed as the concentration (EC_{50}, nM) which gave a 50% increase in the response

Scheme 2[a]



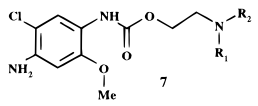
[a] (a) ROH, 0 °C, reflux; (b) $Na_2S_2O_4$, MeOH/H_2O, only with the compounds **8p**−**r**.

Scheme 3[a]



[a] (a) ClCO-$(CH_2)_3$-Cl, benzene, rt; (b) piperidine, toluene, reflux for 4 h or piperidine, NaI, rt for 2 days; (c) 1-(3-(1-piperidinyl)propanecarboxamido)-4-nitro-5-chloro-2-methoxybenzene, $SnCl_2$, HCl.

Table 1. Pharmacological Activity of the Carbamates **7** at 5-HT_4 Receptors



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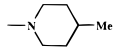
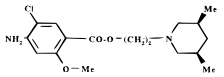
Compd	NR_1R_2	Binding assays K_i, nM[a], 5-HT_4	Functional activity at 5-HT_4 receptors EC_{50}, nM[b]	IC_{50}, nM[c]
7a		39 ± 7.2	>1000	ne[d]
7b		125 ± 67	~200 (23%)	ne
7c		14.5 ± 6.4	100 (30%)	207[176-244]
7d		150 ± 68	300 (39%)	1000
7e		10.2 ± 5.5	NT[e]	NT
7f		25.1 ± 4.8	~60 (25%)	200
7g		2.4 ± 0.5	>1000	180
		0.26 ± 0.05	I[f]	11 [9-14]
GR 113808 (**4**)		0.15 ± 0.03	I	13 [9-18]
ML 10302		1.07 ± 0.5	4 [3.7-4.3] (78%)	ne
SDZ 205-557 (**1**)		5.5 ± 0.7	I	77 [55-130]

[a] [^{3}H]GR 113808 was used as the radioligand in the rat striatum; K_i ± SEM values were determined from the Cheng–Prussof equation. [b] The agonist activity was assessed as the concentration which gave a 50% increase in the response to electrical stimulation (EC_{50}) in the guinea pig ileum and is expressed as a percent of the maximum 5-HT response; 95% confidence limits are in brackets. [c] The antagonist activity (IC_{50}) was calculated as the concentration which produced a 50% reduction in the response to 5-HT in the guinea pig ileum; 95% confidence limits are in brackets. [d] ne = not capable of evaluation. [e] NT = not tested. [f] I = inactive up to 10^{-5} M.

to electrical stimulation with regard to its maximum and related to the maximal effect of 5-HT (100%). Antagonist activity (IC_{50}, nM) was calculated as the concentration which produced a 50% reduction in the submaximal (80%) of 5-HT-induced contractions. The activity of the compounds was compared to those of the reference compounds: ML 10302 (2-(1-piperidinyl)ethyl 4-amino-5-chloro-2-methoxybenzoate),[11] 2-(*cis*-3,5-dimethylpiperidino)ethyl 4-amino-5-chloro-2-methoxybenzoate,[12] a recently described potent antagonist, and compounds **1** and **4**.

Results and Discussion

The results in Table 1 show that the carbamates **7** are not as good ligands for 5-HT_4 receptors as the corresponding esters described previously;[11b] in particular, **7a** (K_i = 39 nM) was clearly less potent than ML 10302. However, as had already been noted,[12] an increase in the affinity for the receptor was observed following methyl substitution, particularly with the methylpiperidine derivatives **7e**–**g**. The favorable role of the 3,5-dimethyl substitution was again noted for **7g** which had nanomolar affinity. No compound displayed significant agonist activity by increasing the response to electrical stimulation in the guinea pig ileum. Only compounds **7c**,**f**,**g** produced a moderate inhibition of the 5-HT response in the guinea pig ileum. These data show that, in contrast to the esters, the carbamate function is less favorable for recognition by the 5-HT_4 receptor and the basic moiety does not influence the pharmacological profile.

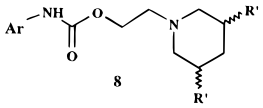
In the benzamide series derived from 4-amino-5-chloro-2-methoxybenzoic acid, no clear structure–activity relationships have been described for the role of aromatic substituents on pharmacological activity. Preliminary studies on ML 10302 showed that such substituents were essential for maintaining affinity for 5-HT_4 receptors, as a large drop in the affinity value was observed with any mono- or disubstituted benzoate esters of 2-piperidinoethanol.[17] Different results were obtained with the monosubstituted carbamates **8**. The data reported in Table 2 show the favorable influence of monosubstitution by MeO or EtO in the *ortho* position (compounds **8b**,**c**,**f**,**m**,**n**), while substitution in the *meta* or *para* position brought about a large decrease in the biological activity (compounds **8d**,**e)**. The presence of the amino function was particularly harmful and could explain the decrease in activity observed with the carbamates **7** with regard to the corresponding esters. Only methoxy or ethoxy substitution in the ortho position had a favorable effect, as NH_2, Cl, CN,[18] and NO_2[18] substitutions produced inactive compounds (K_i > 1000 nM) in the binding assays. On the other hand, the length of the basic chain is an important structural parameter in recognition by the binding site because the carbamate **8s** (K_i = 81 nM), derived from 3-(1-piperidinyl)propanol, was 1 order of magnitude less potent than the corresponding compound **8b** (K_i = 8.9 nM). As observed previously, the introduction of 3,5-dimethylpiperidine was favorable, and compounds **8m**–**o** had nanomolar affinity, the effect of the *cis* or *trans* stereochemistry not being very marked.

In contrast to the esters, no compound displayed agonist activity in the functional assay for 5-HT_4 receptors. Thus, compounds **8b**,**c**,**f** were antagonists in the guinea pig ileum, and **8c** was equipotent to **1**. An increase in the inhibitory effect was not observed with the 3,5-dimethyl derivatives (compounds **8m**–**o**) which were equipotent to or less potent than compound **8c**.

In a previous paper,[12] we emphasized the important role of the oxygen atom of the basic chain in the recognition of the 5-HT_4 receptor binding site by the benzoate series. This finding was confirmed by the results obtained with the amidic derivatives **9b**,**c** which were weak ligands for 5-HT_4 receptors (K_i > 1000 nM).

In summary, the present study has shown that the carbamate derivatives of 1-piperidineethanol and 2-(3,5-dimethylpiperidino)ethanol are potent ligands for 5-HT_4 receptors. It emphasized the importance of the aromatic substituents and, particularly, of *o*-methoxy or *o*-ethoxy substituents for recognition by the 5-HT_4 receptor. The results demonstrated, unexpectedly, the unfavorable role of the amino group in the 4-amino-5-chloro-2-methoxybenzoic acid pharmacophore. Unlike the corresponding esters, no potent agonist was found with an unsubstituted piperidine ring, suggesting the inability of such molecules to occupy the putative agonist site suggested previously.[12] Several investigations are in course to understand the cause of the weak functional activity of these compounds on the GI receptors and their potent affinity for central 5-HT_4 receptors.

Table 2. Pharmacological Activity of the Carbamates **8** at 5-HT_4 Receptors



8

compd	Ar	R′	binding assays K_i, nM,[a] 5-HT_4	functional activity at 5-HT_4 receptors	
				EC_{50}, nM[b]	IC_{50}, nM[c]
8a	C_6H_5	H	31.7 ± 7.5	>1000	~1000
8b	2-$OMeC_6H_4$	H	8.9 ± 0.5	~100 (14%)	~300
8c	2-$OEtC_6H_4$	H	2.6 ± 0.4	>1000	62 [40−95]
8d	3-$OMeC_6H_4$	H	469 ± 44	NT[d]	NT
8e	4-$OMeC_6H_4$	H	>1000	NT	NT
8f	2-OMe-5-ClC_6H_3	H	4.2 ± 0.5	~1000 (14%)	30 (60%)
8g	2-ClC_6H_4	H	>1000	NT	NT
8h	3-ClC_6H_4	H	60.8 ± 5.1	>1000	>1000
8i	4-ClC_6H_4	H	728 ± 63	NT	NT
8j	2-$NH_2C_6H_4$	H	>1000	NT	NT
8k	3-$NH_2C_6H_4$	H	>1000	NT	NT
8l	4-$NH_2C_6H_4$	H	>1000	NT	NT
8m	2-$OMeC_6H_4$	Me, *cis*	2.6 ± 0.52	>1000	160 (60%)
8n	2-$OEtC_6H_4$	Me, *cis*	1.05 ± 0.68	>1000	30 (70%)
8o	2-$OEtC_6H_4$	Me, *trans*	1.16 ± 0.6	>1000	100

[a−d] See corresponding footnotes in Table 1.

Experimental Section

Chemistry. Melting points were determined on a Mettler FP 61 melting point apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AC 200 spectrometer at 200 and 50 MHz, respectively. Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane as the internal standard, and signals are quoted as s (singlet), ds (dedoublet singlet), d (doublet), dd (dedoublet doublet), t (triplet), dt (dedoublet triplet), q (quartet), br s (broad singlet), or m (multiplet). Elemental analyses were performed at the CNRS's analysis services in Châtenay-Malabry (France) and were within 0.4% of the theoretical values unless otherwise noted.

Materials. Tetrahydrofuran (THF) was distilled from sodium/benzophenone. Acetonitrile, dimethylformamide, toluene, and the usual solvents were purchased from SDS (Paris, France). Column chromatography was performed on Merck silica gel 60 (70/230 mesh). Thin-layer chromatography was done on silica gel 60F-254 (0.26 mm thickness) plates.

4-Amino-5-chloro-2-methoxybenzoic acid, 2-bromoethanol, the mixture of *cis*- and *trans*-3,5-dimethylpiperidine, hexamethylenimine, 4-methylpiperidine, 3-methylpiperidine, 2-methylpiperidine, pyrrolidine, 1-piperidineethanol, 1-pyrrolidineethanol, and *N*,*N*-diethylaminoethanol were purchased from Aldrich (France). 2-(Hexamethyleneimino)ethanol, 2-(*cis*,*trans*-3,5-dimethylpiperidino)ethanol, 2-(4-methylpiperidino)ethanol, 2-(3-methylpiperidino)ethanol, and 2-(2-methylpiperidino)ethanol were prepared from 2-bromoethanol according to the process already reported.[19] *N*-Acetyl-5-chloro-4-nitro-2-anisidine was synthesized from 5-chloro-2-anisidine according to the process reported by Hadley.[13]

5-Chloro-4-nitro-2-anisidine. *N*-Acetyl-5-chloro-4-nitro-2-anisidine was refluxed with stirring for 2 h in a solution of 10% NaOH in an EtOH/H_2O mixture (7:3) to give, after evaporation and extraction with CH_2Cl_2, 5-chloro-4-nitro-2-anisidine which was recrystallized in an acetone/AcOEt mixture (68%) as yellow spangles: ^{1}H NMR ($CDCl_3$) δ 7.57 (s, 1H), 6.69 (s, 1H), 4.53 (m, 2H), 3.91 (s, 3H).

General Method for the Preparation of Carbamates 7a−g and 8j−l. 5-Chloro-4-amino-2-anisidine or another appropriate aniline (5 mmol) was added to a solution of phosgene (2.74 mL, 20% in toluene) in anhydrous CH_2Cl_2 (20 mL) at 0 °C under argon. The solution was stirred for 15 min, and NEt_3 (1.6 mL, 11.5 mmol) was added. After 15 min, a solution of the amino alcohol (1.3 mL, 10 mmol) in anhydrous CH_2Cl_2 (5 mL) was added dropwise at 0 °C. The reaction mixture was stirred for 10 min and refluxed overnight. The solvent was evaporated under reduced pressure, and the residue was taken up with 10% aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on a column of silica gel with a CH_2Cl_2/MeOH mixture (9:1) to give a crude product which was reduced to the amino derivative according to the following procedure. It was dissolved in a mixture of MeOH (30 mL), H_2O (25 mL), and 1.92 g of $Na_2S_2O_4$, and the solution was stirred for 4 h at room temperature (**7d−f**, **8j−l**) or at 60 °C (**7a**,**b**,**g**). The solvent was removed by distillation *in vacuo*, and the residue was taken up with brine and extracted with CH_2Cl_2 (4 × 25 mL). The aqueous layer was made alkaline with a 1 N NaOH aqueous solution and extracted again with CH_2Cl_2 (4 × 25 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to give the amino derivative which was transformed into the hydrochloride salt with a 2 N HCl methanol solution or the oxalate salt by treatment with oxalic acid in acetone. The salt was recrystallized according to the usual procedure.

General Method for the Preparation of Carbamates 8b−e,g−i,n−s. These compounds were prepared from the corresponding anilines according to the previous procedure except for the nitro reduction step and were transformed into the hydrochloride or oxalic salt according to classical methods.

General Method for the Preparation of Carbamates 8a,f,m. To a stirred solution of commercial isocyanate (10 mmol) in anhydrous CH_2Cl_2 was added dropwise the corresponding amino alcohol (20 mmol). The reaction mixture was refluxed for 1.5 h. After evaporation of the solvent *in vacuo*, the residue was chromatographed on a column of silica gel with a CH_2Cl_2/MeOH mixture (9:1), and the compound was transformed into the hydrochloride salt with a 2 N HCl methanol solution or the oxalate salt by treatment with oxalic acid in acetone. The salt was recrystallized according to the usual procedure.

2-Piperidinoethyl *N*-(4-amino-5-chloro-2-methoxyphenyl)carbamate (7a): hydrochloride salt (11%) from *i*-Pr_2O/MeOH; mp 196−198 °C dec; ^{1}H NMR (CD_3OD) δ 8.12 (s, 1H), 7.03 (s, 1H), 4.53 (m, 2H), 3.92 (s, 3H), 3.64 (m, 2H), 3.47 (m, 2H), 3.03 (m, 2H), 2.1−1.4 (m, 6H). Anal. ($C_{15}H_{22}ClN_3O_3$·HCl·3H_2O).

2-Pyrrolidinoethyl *N*-(4-amino-5-chloro-2-methoxyphenyl)carbamate (7b): hydrochloride salt (5%) from *i*-Pr_2O/MeOH; mp 180 °C; ^{1}H NMR (CD_3OD) δ 7.62 (s, 1H), 6.56 (s, 1H), 4.46 (m, 2H), 3.84 (s, 3H), 3.56 (m, 2H), 3.47 (m, 4H), 2.35−2 (m, 4H). Anal. ($C_{14}H_{20}ClN_3O_3$·HCl).

2-(Hexamethyleneimino)ethyl *N*-(4-amino-5-chloro-2-methoxyphenyl)carbamate (7c): oxalate salt (10%) from *i*-Pr_2O/MeOH; mp 174−176 °C; ^{1}H NMR (CD_3OD) δ 7.57 (s,

1H), 6.51 (s, 1H), 4.46 (m, 2H), 3.92 (s, 3H), 3.79 (m, 2H), 3.49 (m, 2H), 3.34 (m, 4H), 1.92 (m, 4H), 1.73 (m, 4H). Anal. ($C_{16}H_{24}ClN_3O_3 \cdot C_2H_2O_4$).

2-(Diethylamino)ethyl *N*-(4-amino-5-chloro-2-methoxyphenyl)carbamate (7d): oxalate salt (28%) from *i*-Pr_2O/MeOH; mp 146−148 °C; ^{1}H NMR (CD_3OD) δ 7.60 (s, 1H), 6.57 (s, 1H), 4.51 (m, 2H), 3.85 (s, 3H), 3.54 (m, 2H), 3.37 (m, 4H), 1.4 (m, 6H). Anal. ($C_{14}H_{22}ClN_3O_3 \cdot C_2H_2O_4$).

2-(3-Methylpiperidino)ethyl *N*-(4-amino-5-chloro-2-methoxyphenyl)carbamate (7e): oxalate salt (9%) from *i*-Pr_2O/MeOH; mp 166−168 °C; ^{1}H NMR (CD_3OD) δ 7.56 (s, 1H), 6.51 (s, 1H), 4.47 (m, 2H), 3.79 (s, 3H), 3.7−3.45 (m, 2H), 3.42 (m, 2H), 3−2.8 (m, 1H), 2.5−2.72 (m, 1H), 2.1−1.7 (m, 4H), 1.35−1.05 (m, 1H), 0.99 (d, 3H). Anal. ($C_{16}H_{24}ClN_3O_3 \cdot C_2H_2O_4$).

2-(4-Methylpiperidino)ethyl *N*-(4-amino-5-chloro-2-methoxyphenyl)carbamate (7f): oxalate salt (30%) from *i*-Pr_2O/MeOH; mp 160 °C; ^{1}H NMR (CD_3OD) δ 7.58 (s, 1H), 6.52 (s, 1H), 4.46 (m, 2H), 3.8 (s, 3H), 3.75−3.52 (m, 2H), 3.52−3.35 (m, 2H), 3.15−2.9 (m, 2H), 2.05−1.8 (m, 2H), 1.8−1.35 (m, 3H), 1.02 (d, 3H). Anal. ($C_{16}H_{24}ClN_3O_3 \cdot C_2H_2O_4 \cdot 1.5H_2O$).

2-(*cis*-3,5-Dimethylpiperidino)ethyl *N*-(4-amino-5-chloro-2-methoxyphenyl)carbamate (7g): oxalate salt (24%) from *i*-Pr_2O/MeOH; mp 136−138 °C; ^{1}H NMR (CD_3OD) δ 7.57 (s, 1H), 6.51 (s, 1H), 4.48 (m, 2H), 3.79 (s, 3H), 3.65−3.4 (m, 4H), 2.56 (m, 2H), 2.1−1.8 (m, 3H), 1.05−0.8 (m, 7H). Anal. ($C_{17}H_{26}ClN_3O_3 \cdot C_2H_2O_4.H_2O$).

2-Piperidinoethyl *N*-phenylcarbamate (8a): hydrochloride salt (28%) from *i*-Pr_2O/MeOH; mp 216−218 °C; ^{1}H NMR (CD_3OD) δ 8.6 (s, 1H), 7.48 (m, 2H), 7.28 (m, 2H), 7.04 (m, 1H), 4.58 (m, 2H), 3.27 (m, 2H), 4−1.3 (m, 10H). Anal. ($C_{14}H_{21}N_2O_2 \cdot HCl$).

2-Piperidinoethyl *N*-(2-methoxyphenyl)carbamate (8b): oxalate salt (38%) from *i*-Pr_2O/MeOH; mp 180−182 °C; ^{1}H NMR (CD_3OD) δ 7.83 (m, 1H), 7.2−6.85 (m, 3H), 4.5 (m, 2H), 3.87 (s, 3H), 3.6−3 (m, 6H), 2−1.55 (m, 6H). Anal. ($C_{15}H_{22}N_2O_3 \cdot C_2H_2O_4$).

2-Piperidinoethyl *N*-(2-ethoxyphenyl)carbamate (8c): oxalate salt (46%) from *i*-Pr_2O/MeOH; mp 295 °C; ^{1}H NMR (CD_3OD) δ 7.83 (m, 1H), 7.1−6.8 (m, 3H), 4.49 (m, 2H), 4.1 (q, 2H), 3.44 (m, 2H), 3.45−3.1 (m, 4H), 2−1.5 (m, 6H), 1.42 (t, 3H). Anal. ($C_{16}H_{24}N_2O_3 \cdot C_2H_2O_4$).

2-Piperidinoethyl *N*-(3-methoxyphenyl)carbamate (8d): hydrochloride salt (43%) from *i*-Pr_2O/MeOH; mp 182 °C; ^{1}H NMR (CD_3OD) δ 7.19 (m, 1H), 7.13 (s, 1H), 6.97 (m, 1H), 6.61 (m, 1H), 4.5 (m, 2H), 3.76 (s, 3H), 3.46 (m, 2H), 3.9−2.8 (m, 4H), 2.1−1.55 (m, 6H). Anal. ($C_{15}H_{22}N_2O_3 \cdot HCl$).

2-Piperidinoethyl *N*-(4-methoxyphenyl)carbamate (8e): oxalate salt (56%) from *i*-Pr_2O/MeOH; mp 160 °C; ^{1}H NMR (CD_3OD) δ 7.33 (d, 2H), 6.84 (d, 2H), 4.45 (m, 2H), 3.75 (s, 3H), 3.41 (m, 2H), 3.7−2.9 (m, 4H), 2−1.45 (m, 6H). Anal. ($C_{15}H_{22}N_2O_3 \cdot C_2O_4H_2$).

2-Piperidinoethyl *N*-(5-chloro-2-methoxyphenyl)carbamate (8f): oxalate salt (47%) from *i*-Pr_2O/MeOH; mp 226−228 °C; ^{1}H NMR (CD_3OD) δ 7.96 (d, 1H), 7.05 (dd, 1H), 6.96 (d, 1H), 4.51 (m, 2H), 3.87 (s, 3H), 3.44 (m, 2H), 3.5−3.15 (m, 4H), 1.96−1.6 (m, 6H). Anal. ($C_{15}H_{21}ClN_2O_3 \cdot C_2O_4H_2$).

2-Piperidinoethyl *N*-(2-chlorophenyl)carbamate (8g): hydrochloride salt (85%) from *i*-Pr_2O/EtOH; mp >220 °C dec; ^{1}H NMR (CD_3OD) δ 7.85 (m, 1H), 7.4 (m, 1H), 7.3 (m, 1H), 7.1 (m, 1H), 4.5 (m, 2H), 3.3 (m, 2H), 3.15 (m, 4H), 2.3 (m, 4H), 1.65 (m, 2H). Anal. ($C_{14}H_{19}ClN_2O_2 \cdot HCl$).

2-Piperidinoethyl *N*-(3-chlorophenyl)carbamate (8h): hydrochloride salt (84%) from *i*-Pr_2O/MeOH; mp 222 °C; ^{1}H NMR (CD_3OD) δ 7.6 (m, 1H), 7.31 (m, 1H), 7.25 (t, 1H), 7 (m, 1H), 4.5 (m, 2H), 3.45 (m, 2H), 3.9−2.7 (m, 4H), 2.1−1.4 (m, 6H). Anal. ($C_{14}H_{19}ClN_2O_2 \cdot HCl$).

2-Piperidinoethyl *N*-(4-chlorophenyl)carbamate (8i): hydrochloride salt (72%) from *i*-Pr_2O/EtOH; mp 214 °C; ^{1}H NMR (CD_3OD) δ 7.45 (d, 2H), 7.25 (d, 2H), 4.5 (m, 2H), 3.48 (m, 2H), 3.9−2.9 (m, 4H), 2.1−1.4 (m, 6H). Anal. ($C_{14}H_{19}ClN_2O_2 \cdot HCl$).

2-Piperidinoethyl *N*-(2-aminophenyl)carbamate (8j): hydrochloride salt (77%) from *i*-Pr_2O/MeOH; mp 172 °C; ^{1}H NMR (CD_3OD) δ 7.15 (m, 1H), 7 (m, 1H), 6.82 (m, 1H), 6.69 (m, 1H), 4.46 (m, 2H), 3.6−3 (m, 6H), 2.1−1.5 (m, 6H). Anal. ($C_{14}H_{21}N_3O_2 \cdot HCl$).

2-Piperidinoethyl *N*-(3-aminophenyl)carbamate (8k): hydrochloride salt (88%) from *i*-Pr_2O/MeOH; mp 230 °C; ^{1}H NMR (DMSO) δ 10.8 (m, 1H), 10.16 (s, 1H), 7.59 (s, 1H), 7.42 (m, 1H), 7.35 (m, 1H), 7 (m, 1H), 4.5 (m, 2H), 3.46 (m, 2H), 3.35 (m, 2H), 2.97 (m, 2H), 2−1.5 (m, 5H), 1.3 (m, 1H). Anal. ($C_{14}H_{21}N_3O_2 \cdot 2HCl$).

2-Piperidinoethyl *N*-(4-aminophenyl)carbamate (8l): hydrochloride salt (34%) from *i*-Pr_2O/MeOH; mp >230 °C; ^{1}H NMR (DMSO) δ 10.55 (s, 1H), 10.06 (s, 1H), 7.55 (d, 2H), 7.31 (d, 2H), 4.48 (m, 2H), 3.46 (m, 2H), 3.34 (m, 2H), 2.95 (m, 2H), 2−1.6 (m, 5H), 1.77 (m, 1H). Anal. ($C_{14}H_{21}N_3O_2 \cdot 2HCl$).

2-(*cis*-3,5-Dimethylpiperidino)ethyl *N*-(2-methoxyphenyl)carbamate (8m): oxalate salt (22%) from *i*-Pr_2O/EtOH; mp 188−190 °C; ^{1}H NMR (CD_3OD) δ 7.83 (m, 1H), 7.1−6.8 (m, 3H), 4.51 (m, 2H), 3.86 (s, 3H), 3.56 (m, 2H), 3.45 (m, 2H), 2.54 (t, 2H), 2.15−1.75 (m, 3H), 0.98 (d, 6H), 0.86 (m, 1H). Anal. ($C_{17}H_{26}N_2O_3 \cdot C_2H_2O_4$).

2-(*cis*-3,5-Dimethylpiperidino)ethyl *N*-(2-ethoxyphenyl)carbamate (8n): oxalate salt (30%) from *i*-Pr_2O/MeOH; mp 164 °C; ^{1}H NMR (CD_3OD) δ 7.82 (m, 1H), 7.09−6.84 (m, 3H), 4.51 (m, 2H), 4.11 (q, 2H), 3.53 (m, 2H), 3.46 (m, 2H), 2.55 (t, 2H), 2.1−1.75 (m, 3H), 1.42 (t, 3H), 0.99 (d, 6H), 0.86 (m, 1H). Anal. ($C_{18}H_{28}N_2O_3 \cdot C_2H_2O_4$).

2-(*trans*-3,5-Dimethylpiperidino)ethyl *N*-(2-ethoxyphenyl)carbamate (8o): oxalate salt (6%) from *i*-Pr_2O/MeOH; mp 174−176 °C; ^{1}H NMR (CD_3OD) δ 7.81 (m, 1H), 7.1−6.8 (m, 3H), 4.69−4.4 (m, 2H), 4.11 (q, 2H), 3.5−3.4 (m, 2H), 3.4−2.9 (m, 2H), 2.35−2.1 (m, 2H), 1.53 (m, 2H), 1.42 (t, 3H), 1.08 (d, 6H). Anal. ($C_{18}H_{28}N_2O_3 \cdot C_2O_4H_2$).

2-Piperidinoethyl *N*-(2-nitrophenyl)carbamate (8p): hydrochloride salt (83%) from *i*-Pr_2O/EtOH; mp 164 °C; ^{1}H NMR (CD_3OD) δ 8.2 (m, 1H), 8.12 (m, 1H), 7.68 (m, 1H), 7.27 (m, 1H), 4.58 (m, 2H), 3.51 (m, 2H), 3.8−2.8 (m, 4H), 2.2−1.4 (m, 6H). Anal. ($C_{14}H_{19}N_3O_4 \cdot HCl$).

2-Piperidinoethyl *N*-(3-nitrophenyl)carbamate (8q): hydrochloride salt (61%) from *i*-Pr_2O/MeOH; mp >210 °C; ^{1}H NMR (CD_3OD) δ 8.49 (m, 1H), 7.87 (m, 1H), 7.75 (m, 1H), 7.51 (m, 1H), 4.56 (m, 2H), 3.49 (m, 2H), 3.9−2.9 (m, 4H), 2.2−1.4 (m, 6H). Anal. ($C_{14}H_{19}N_3O_4 \cdot HCl$).

2-Piperidinoethyl *N*-(4-nitrophenyl)carbamate (8r): base (71%) from petroleum ether; mp 104 °C; ^{1}H NMR ($CDCl_3$) δ 8.15 (d, 2H), 7.5 (d, 2H), 7.45 (s, 1H), 4.43 (t, 2H), 2.65 (t, 2H), 2.45 (m, 4H), 1.7−1.3 (m, 6H). Anal. ($C_{14}H_{19}N_3O_4$).

3-Piperidinopropyl *N*-(2-methoxyphenyl)carbamate (8s): oxalate salt (34%) from *i*-Pr_2O/MeOH; mp 170 °C; ^{1}H NMR (CD_3OD) δ 7.82 (d, 1H), 6.81−7.1 (m, 3H), 4.22 (m, 2H), 3.86 (s, 3H), 3.8−3.35 (m, 2H), 3.27−3.15 (m, 2H), 3.15−2.6 (m, 2H), 2.3−2.23 (m, 2H), 1.6−1.4 (m, 6H). Anal. ($C_{16}H_{24}N_2O_3 \cdot C_2H_2O_4$).

1-(3-(1-Piperidinyl)propanecarboxamido)-4-amino-5-chloro-2-methoxybenzene (9b). 4-Chlorobutyryl chloride (0.7 g, 5 mmol) in 10 mL of benzene was added to 1 g (5 mmol) of 5-chloro-4-nitro-2-anisidine in 20 mL of benzene at room temperature, and the solution was stirred for 30 min. Then 50 mL of a saturated aqueous solution of Na_2CO_3 was introduced, and the mixture was stirred for 15 min. The organic solution was separated, washed with a 1 N HCl aqueous solution and water, and dried over $MgSO_4$. The solvent was evaporated to give 1 g of chloroamide **15** (65%) which was added to a mixture of 4 mL of piperidine and toluene and refluxed for 4 h. The precipitate of piperidine hydrochloride was filtered, and the organic solution was evaporated. The crude product was dissolved in AcOEt, and the addition of 3 N HCl gave **9a** hydrochloride (0.5 g) which was isolated by filtration as an orange powder. The evaporation of the filtrate provided 0.6 g of 1-(5-chloro-2-methoxy-4-nitrophenyl)-2-pyrrolidone as the product of the cyclization reaction; 0.5 g of **9a** hydrochloride was dissolved in a mixture of HOAc and concentrated HCl and stirred in the presence of $SnCl_2$ overnight. Then, it was poured onto ice, basified, and extracted by EtOAc. The organic solution was dried over $MgSO_4$ and evaporated. The crude material was transformed into the hydrochloride salt and recrystallized from a *i*-Pr_2O/MeOH mixture to give 150 mg of **9b** (33%): mp 190−200 °C;

^{1}H NMR (CD_3OD) δ 8.4 (s, 1H), 7.1 (s, 1H), 3.55 (m, 2H), 3.45 (s, 3H), 3.15 (m, 2H), 2.95 (m, 2H), 2.65 (m, 2H), 2.2−1.6 (m, 7H), 1.55 (m, 1H). Anal. ($C_{16}H_{24}ClN_3O_2 \cdot 2HCl \cdot 2H_2O$).

1-(3-(1-Piperidinyl)propanecarboxamido)-2-methoxybenzene (9c). A 3.4 mL aliquot (30 mmol) of 4-chlorobutyryl chloride in 50 mL of benzene was added to 4.9 g (40 mmol) of 2-anisidine in 100 mL of benzene at room temperature, and the solution was stirred for 30 min. Then 50 mL of a saturated Na_2CO_3 aqueous solution was added, and the mixture was stirred for 15 min. The organic solution was separated, washed with a 1 N HCl aqueous solution and water, and dried over $MgSO_4$; 6.8 g of crude product was obtained after the evaporation of the solvent. It was added to 10 mL of piperidine and stirred at room temperature in the presence of 0.1 g of NaI for 2 days. The piperidine was evaporated, and the residue was taken up in ether, a 10% Na_2CO_3 aqueous solution, and water, and dried over $MgSO_4$. After evaporation of the solvent, the crude compound was transformed into the hydrochloride salt with 3 N HCl/AcOEt solution and recrystallized from an AcOEt/EtOH mixture (7 g, 74.6%): mp >220 °C; ^{1}H NMR (CD_3OD) δ 7.9 (m, 1H), 7.15 (m, 1H), 7 (m, 1H), 6.9 (m, 1H), 3.85 (s, 3H), 3.1 (m, 4H), 3 (m, 2H), 2.6 (m, 2H), 2.05 (m, 2H), 1.8 (m, 4H), 1.62 (m, 2H). Anal. ($C_{16}H_{24}N_2O_2 \cdot HCl$).

Pharmacological Methods. 1. 5-HT$_4$ Receptor Binding Assays. Male Sprague−Dawley rats from Janvier Laboratories (France) were used. Animals were housed at 22 ± 1 °C, with 55% humidity, on a 12 h light−dark cycle with free access to food and water for 4 days before the experiments.

Membranes were prepared from rat striatum and olfactory tubercules which were pooled separately and stored at −80 °C. Tissues were thawed at 0 °C, homogenized in 15 volumes of ice-cold 50 mM Hepes (pH 7.4) using a Polytron homogenizer, and then centrifuged once at 40000*g* at 4 °C for 15 min. The resulting pellet was resuspended in 5 volumes of Hepes buffer to a final concentration of 4−5 mg of protein/mL. Membrane aliquots of 2.6 mL were kept frozen at −80 °C for subsequent use.

Binding assays were performed according to the method previously described[12] with modifications. The binding of [^{3}H]-GR 113808 (85 Ci/mmol; Amersham) was measured using membranes (50 μL aliquots, equivalent to 0.1−0.2 mg of protein) suspended in a final volume of 0.5 mL of 50 mM Hepes buffer (pH 7.4). Seven concentrations of each drug were used, and the assay was done in triplicate. Nonspecific binding was defined with 10 μM ML 10302 in duplicate and represented less than 10% of the total binding. Total binding was defined in quadruplicate.

For each assay the bound radioactivity was separated by vacuum filtration through Whatman GF/B glass filters presoaked in 0.1% poly(ethylenimine) using a Brandel Cell harvester. The filters were then washed twice with 5 mL of 50 mM Tris-HCl (pH 8.4) at room temperature and dried. The filters were placed in polyethylene vials to which was added 4 mL of a scintillation cocktail (Beckman, Ready-Safe), and after equilibration, the radioactivity was determined using liquid scintillation spectrometry. The data were analyzed by a computer-assisted curve-fitting program in Lotus 1.2.3. to provide IC_{50}, K_i, and r^2 values, K_i values being calculated from the Cheng−Prussof equation.

2. Protein Estimation. The protein concentrations of the rat striatum and olfactory tubercule membrane preparations were determined by the method of Bradford.[20]

3. 5-HT$_4$ Receptor Activity: Myenteric Plexus and Longitudinal Muscle Preparation from Guinea Pig Ileum. Tissues were obtained from male Crl (HA) SPF guinea pigs (400−600 g) housed as described previously. According to Craig and Clarke,[4a] segments (2 cm long, 10 cm from the ileo-cecal junction) of myenteric plexus and longitudinal muscle from the ileum were carefully dissected and mounted in a 20 mL organ bath containing a warm (37 °C), aerated (95% O_2, 5% CO_2) Krebs solution. The tissue was stretched with a 0.5 g weight, and contractions elicited by transmural electrical stimulation (0.2 Hz, pulse duration of 1.5 ms) were recorded by means of an isotonic transducer connected to a polygraph (Gemini, Basile, Italy). The preparations were subjected to a supramaximal voltage stimulation. After stabilization and incubation with 10^{-7} M phenoxybenzamine, the voltage was reduced to obtain contractions 50% of those elicited by supramaximal stimulation. In control experiments, at least three fully reproducible cumulative concentration−effect curves to 5-HT (3×10^{-10}−10^{-7} M, contact time of about 60 s) were obtained. After two fully reproducible cumulative concentration−effect curves to 5-HT, a cumulative concentration-effect curve to the 5-HT$_4$ receptor agonist under test was carried out (contact time of 60−120 s). The agonist activity (EC_{50}, nM) was assessed as the concentration which gave a 50% increase in the response to the electrical stimulation with regard to the maximum, and the results are expressed as a percentage of the maximal effect of 5-HT (100%) found in the second reference curve. In antagonist studies, the compound under test was added 30 min before the last cumulative concentration−effect curve to 5-HT (3×10^{-10}−10^{-5} M). Several concentrations ($n \geq 3$) of antagonist were tested, and the IC_{50} value was calculated as the concentration producing a 50% reduction of the submaximal (80%) 5-HT contraction. The submaximal (80%) 5-HT concentration (3×10^{-8} M) was calculated on the second reference cumulative concentration−response curve.

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