New Esters of 4-Amino-5-chloro-2-methoxybenzoic Acid as Potent Agonists and Antagonists for 5-HT$_4$ Receptors

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A number of benzoates derived from 4-amino-5-chloro-2-methoxybenzoic acid and substituted 1-piperidineethanol were synthesized and found to be potent 5-HT$_4$ receptor agonists in the electrically-stimulated myenteric plexus and longitudinal muscle of the guinea pig ileum and the rat esophagus muscle. Monosubstitution of the piperidine ring with Me, OH, NH-Ac, or $CONH_2$ groups gave compounds equipotent to **7a** (ML 10302), a 5-HT$_4$ receptor agonist previously reported to have nanomolar affinity. **7a**,**k** were as potent as serotonin (5-HT) but had maximal responses which were only 60−80% of that of 5-HT, suggesting a partial agonist profile for these compounds. Binding assays were performed with [^{3}H]GR 113808 in the rat striatum, and several of these compounds were found to have nanomolar affinity for 5-HT$_4$ receptors (**7a**, $K_i = 1.07 \pm 0.5$ nM; **7k**, $K_i = 1.0 \pm 0.3$ nM). The introduction of two methyl groups on the piperidine ring brought about a dramatic change in the pharmacological profile of 2-[(*cis*- and *trans*-3,5-dimethylpiperidinyl)ethyl]-4-amino-5-chloro-2-methoxybenzoate, **7g**,**h**. **7g** ($K_i = 0.26 \pm 0.06$ nM) inhibited the relaxant action of 5-HT in the rat esophagus muscle with a pA_2 value of 8.6. The advantage of the ester function was demonstrated by comparing the activity of several such compounds at 5-HT$_4$ receptors with those of the corresponding amidic derivatives. This difference was less marked when the basic moiety was sterically constrained as in the quinuclidine and tropane moieties. Structural analyses of **7a**,**g** were performed by determining their X-ray crystal structures and by molecular modeling (SYBYL). A relatively limited number of minimum energy conformers was found for both compounds. They were characterized by the *cis* folded conformation of the ethyl chain and by the orientation of the lone pair of the nitrogen atom pointing out of the molecule as seen in conformationally-constrained benzamides such as zacopride and renzapride. A hypothetical model for the 5-HT$_4$ receptor with two sites for the binding of agonist and antagonist molecules was proposed.

Recent data on the cloning of the 5-HT$_4$ receptor[1,2] showed that it is a member of the G-protein-coupled receptor family and confirmed the early report of Dumuis[3] on the existence of a serotoninergic receptor linked positively to adenylyl cyclase in the central nervous system (CNS). This receptor has been shown to be similar to that discovered by Clarke[4a] in the gastrointestinal (GI) system where it was shown to be responsible for the stimulation of gut motility by 5-HT. Stimulation of these receptors provided a mechanism to explain the pharmacological action of gastrokinetic drugs such as cisapride, renzapride, and zacopride which enhance GI propulsion and can correct intestinal motility disorders.[5] In the isolated guinea pig ileum, Clarke[4a] showed that 5-HT$_4$ receptors were implicated in the first phase of the biphasic concentration curve to 5-HT, while 5-HT$_3$ receptors mediated the second phase. This finding explained the initial confusion surrounding the role of 5-HT$_3$ receptor antagonists[4b] in the GI tract because a number of these compounds were also demonstrated[6] to be 5-HT$_4$ receptor agonists. It was shown that the action of these receptors was mediated by the cholinergic system and that they were neuronally located, either directly on cholinergic nerves or upstream.[7] To date, 5-HT$_4$ receptors have been found in several peripheral tissues: rat esophagus,[4] colon,[8] and urinary bladder[9] where they are implicated in the contraction or relaxation of smooth muscles, and they have also been identified in porcine[10] and human[11] atria where they mediate an increase in contractile force. Moreover, 5-HT$_4$ receptors have been found in rat, guinea pig, and human brain[12] with high receptor concentrations in the striatum and substantia nigra. Considerable progress has been made in the localization of 5-HT$_4$ receptors and in the study of their functions with the development of potent antagonists such as SDZ 205-557 (**1**),[13] SB 204070 (**2**),[14] SB 207710 (**3**),[15a] GR 113808 (**4**),[16] and carbazimidamide derivatives,[17c] in particular which have been used as radiolabeled ligands.[12a,15b,c] More recently, SB 207266 (**5**), an amidic derivative of a tricyclic indole moiety, was reported as the first potent, selective 5-HT$_4$ receptor antagonist active orally.[18a]

Thus, the mechanism of action of the gastric prokinetic drugs has been determined, and a number of them have been found to be 5-HT$_4$ receptor agonists.[6] Many of these compounds are members of the generic benzamide family, derived from metoclopramide,[19] a drug well-known for gastric prokinetic activity. These compounds, such as renzapride,[20] BRL 24682 (4-amino-5-chloro-2-methoxy-*N*-(*endo*-8-methylazabicyclo[3.2.1]octan-3-yl)benzamide),[21] and zacopride,[22] are amides of 4-amino-5-chloro-2-methoxybenzoic acid and possess a relatively rigid basic structure. These structural characteristics are in common with those of the 5-HT$_3$

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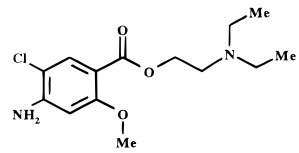
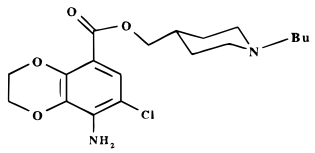
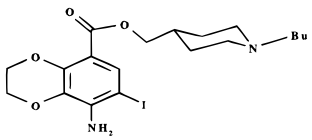
Chart 1



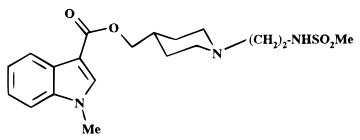
1 (SDZ 205-557)



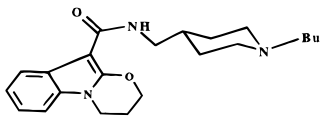
2 (SB 204070)



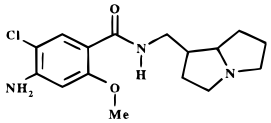
3 (SB 207710)



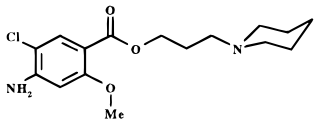
4 (GR 113808)



5 (SB 207266)

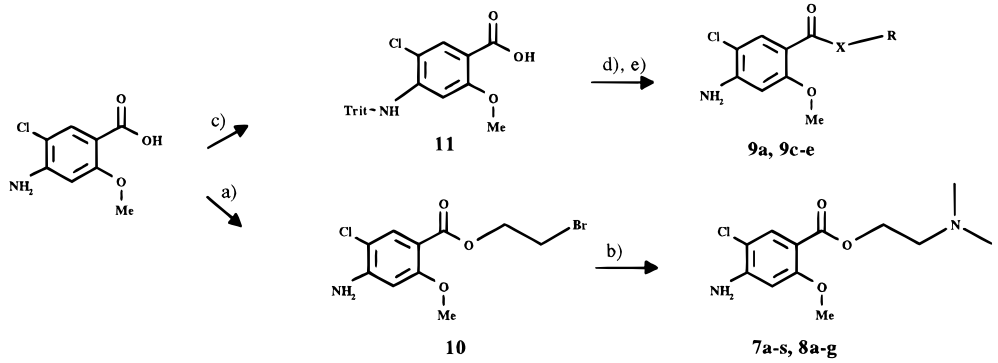


6 (SC-53116)



15 (RS 23597-190)

Scheme 1[a]



[a] (a) 1,2-Dibromoethane, THF, reflux, DBU; (b) amine, K_2CO_3, DMF, rt or 50 °C (method A) or amine, toluene, reflux (method B); (c) trityl chloride, CH_2Cl_2, pyridine; (d) CDI, THF; (e) alcohol, DBU, THF, reflux.

receptor antagonists and explain why almost all of these compounds are active at both receptor types. However, it has been clearly demonstrated that 5-HT$_3$ receptor antagonist activity is not implicated in the gastrokinetic effect of these drugs as several selective 5-HT$_3$ receptor antagonists are devoid of gastrokinetic properties.[23] The search for selective 5-HT$_4$ receptor agonists equipotent to serotonin is an important goal to develop new compounds to correct intestinal motility disorders.[24] Moreover, the presence of 5-HT$_4$ receptors which are supposed to facilitate the release of acetylcholine has been shown in the CNS, and thus 5-HT$_4$ receptor agonists may have a role in improving cognitive function.[12b,25]

In recent years, several reports have described new compounds with 5-HT$_4$ receptor agonist activity possessing better selectivity than the previous compounds. Thus SC-53116 (**6**),[26] the quaternized butyl derivative of renzapride,[27] and a macrocyclic compound[28] were the first reported benzamides which surpassed the selectivity of the other benzamides for this receptor. ML 10302 (**7a**), a compound structurally closely related to **1**, was also shown by us to be a selective 5-HT$_4$ receptor agonist[29] with nanomolar affinity in the electrically-evoked contraction model in the guinea pig ileum, while 5-HT$_4$ receptor antagonist properties have been reported previously.[30,31] More recently, several ketone derivatives[32] and some potent carbazimidamines[17a,b] were reported as selective and metabolically-stable 5-HT$_4$ receptor agonists.

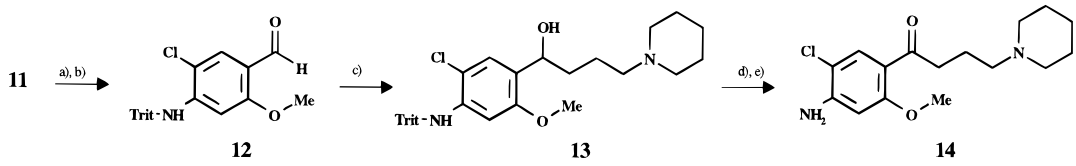
In our early approach to the design of new 5-HT$_4$ receptor agonists or antagonists, we were interested in the dramatic variation of the pharmacological activity observed with **1** compared to metoclopramide, due only to the substitution of the amidic function by an ester function. It is well-known[33] that the amidic function of the benzamides is implicated in an intramolecular hydrogen bond with the *o*-methoxy group, and it was considered worthwhile to examine the influence of this structural property using different reference benzamides. Preliminary reports[29a,34] from our laboratory demonstrated that the affinities of the corresponding esters for 5-HT$_3$ receptors were little affected with regard to those of the amidic derivatives, while an increase in affinity for 5-HT$_4$ receptors was generally observed. Moreover, it seemed that the magnitude of the variation was related to the flexibility of the basic framework.

We present, herein, structural variations[35] carried out with compounds **7** and **8** and the surprising data concerning the development of potent antagonist molecules designed using small structural variations on the piperidine ring. Some comparisons between the biological activities of the esters and the corresponding amides (compounds **9a**−**h**) are also presented.

Chemistry

Compounds **7** and **8** were synthesized according to the pathway described in Scheme 1. 4-Amino-5-chloro-2-methoxybenzoic acid was transformed to the 2-bromoethyl ester **10** by the reaction between the acid and 1,2-dibromoethane in THF in the presence of DBU (1,8-diazabicyclo[5.4.0]undec-7-ene). Compound **10** was then condensed with appropriate piperidines or cyclic amines

Scheme 2[a]



[a] (a) $LiAlH_4$, THF; (b) CH_2Cl_2, MnO_2, 24 h, rt; (c) Mg, THF, 1-(3-chloropropyl)piperidine, 60 °C, 24 h; (d) CH_2Cl_2, MnO_2, 40−50 °C, 7 days; (e) acetone, HCl, rt.

in DMF in the presence of K_2CO_3 (method A) or in toluene at reflux (method B) to provide compounds **7a**−**s** and **8a**−**g**, respectively. The amines were commercially available or prepared by previously-described methods. The esters derived from tropine (compound **9e**), (*R*)- and (*S*)-3-quinuclidinol (compounds **9c**,**d**), and 2-(dimethylamino)ethanol (compound **9a**) were prepared from 5-chloro-2-methoxy-4-(tritylamino)benzoic acid (**11**) (Scheme 1) which was synthesized by the reaction between trityl chloride and the acid in the presence of pyridine. The acid obtained was activated with 1,1′-carbonyldiimidazole in THF to give the imidazolide which was isolated and reacted with alcohol by heating in THF in the presence of DBU to provide compounds **9a**,**c**−**e**. The derivatives of 3,5-dimethylpiperidine were obtained as a 4:1 mixture of the *cis* (compound **7g**) and *trans* (compound **7h**) isomers which were easily separated by column chromatography and characterized by their ^1H NMR spectra. The (+)- and (−)-3-hydroxypiperidine derivatives (compounds **7k**,**l**) were synthesized from (+)- and (−)-3-hydroxypiperidine which was resolved according to a process already reported.[36]

The amides reported in Table 3 (compounds **9b**,**f**−**h**) were synthesized from 4-amino-5-chloro-2-methoxybenzoic acid[37] using the mixed anhydride method. In the course of this work, it was deemed worthwhile to prepare the bioisosteric ketone **14** to compare its biological activity with that of **7a**. The synthesis of **14** was carried out according to the method presented in Scheme 2: the tritylated acid **11** was reduced to the corresponding alcohol and oxidized by MnO_2 to the aldehyde **12**. Condensation with 2-(1-piperidinyl)ethylmagnesium chloride provided the alcohol **13** which was oxidized by MnO_2 to the ketone **14**.

Biological Methods

The assessment of the affinity of the compounds for 5-HT_4 receptors was performed using radioligand binding assays with [^3H]GR 113808 and rat striatal membranes.[38] The existence of 5-HT_4 receptors has been clearly demonstrated in the CNS, and there are data to show that the 5-HT_4 receptors in the brain are identical with those in the periphery, in particular, those in the intestine.[25] Consequently, binding assays in the brain constitute a valuable test to select compounds which will be active in the GI tract. Previously, several 5-HT_4 receptor agonists and antagonists have also been described as potent 5-HT_3 receptor antagonists, and consequently, it was mandatory to evaluate the selectivity for both receptors. For this purpose, the most active compounds selected as ligands for 5-HT_4 receptors were evaluated in 5-HT_3 receptor binding assays using [^3H]-BRL 43694[39] and rat posterior cortex membranes.

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 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 which gave a 50% increase in the response to electrical stimulation (EC_{50}, nM) with regard to its maximum. Antagonist activity (IC_{50}, nM) was calculated as the concentration which produced a 50% reduction of the 5-HT-induced contractions.

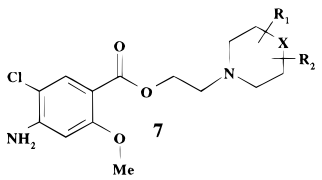
5-HT_4 receptors are also present in the rat esophagus where they mediate the muscle relaxation.[40] Several compounds were evaluated for their ability to relax carbachol-contracted rat esophageal muscularis mucosae. 5-HT_4 receptor agonist activity (EC_{50}) and the pA_2 value of several antagonist compounds were evaluated from the concentration−effect curves according to previously-reported methods.[41]

Biological Results and Discussion

The results of the compounds tested are reported in Tables 1−3. The values for 5-HT_4 receptor activation of the compounds were compared to those of the reference compounds: BIMU 8 (*endo*-*N*-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-2-oxo-3-(prop-2-yl)-1*H*-benzimidazole-1-carboxamide),[3b] **1**, **3**, and RS 23597-190 (**15**).[42]

The data from the binding assays for the piperidine derivatives **7** presented in Table 1 highlighted their potent affinity for 5-HT_4 receptors since a number of compounds, regardless of the nature of the substituent on the heterocycle, possessed nanomolar affinity for these receptors and were much more potent than the previously-described amides derived from 4-amino-5-chloro-2-methoxybenzoic acid, such as zacopride, renzapride, and **6**. The structural differences to the benzamide references compounds reside in the presence of the ester function and in the totally flexible basic moieties. Compound **7a** (ML 10302), the 5-HT_4 receptor agonist activity of which was reported previously,[29] is structurally closely related to **14**, which has been described as a potent 5-HT_4 receptor antagonist.[42] However, **7a** was clearly more potent in the binding assays, indicating that the distance between the benzene ring and the basic nitrogen is a crucial structural parameter in this class of compounds for recognition by the 5-HT_4 receptor. Examination of the results in Table 1 showed that affinity for this receptor is unrelated to the nature of the substituent in the monosubstituted piperidine because groups as different as Me, OH, NHAc, $CONH_2$, and Bz on the 4 position gave compounds **7b**,**i**,**m**−**o** which were approximately equipotent. The affinity was not much affected by the substituent position (compounds **7c**,**d**,**j**); on the other hand, modification of the basicity of the nitrogen atom (compound

Table 1. Pharmacological Activity of the Benzoates **7** at 5-HT$_4$ Receptors



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compd	R_1	R_2	X	binding assays K_i, nM,[a] 5-HT$_4$	functional activity at 5-HT$_4$ receptors: EC_{50}, nM[b]	functional activity at 5-HT$_4$ receptors: IC_{50}, nM[c]
7a	H	H	CH_2	1.07 ± 0.5	4 [3.7−4.3] (80%)	ne[d]
7b	4-Me	H	CH_2	4.08 ± 0.5	3 [2−5] (57%)	ne
7c	3-Me	H	CH_2	0.93 ± 0.05	15 [9−23] (54%)	ne
7d	2-Me	H	CH_2	7.8 ± 0.6	6 [5−8] (82%)	ne
7e	2-Me	6-Me	CH_2	39.5 ± 7.2	NT[e]	NT
7f	3-Me	3-Me	CH_2	1.72 ± 0.33	I [f]	150
7g	3-Me	5-Me, *cis*	CH_2	0.26 ± 0.06	I	11 [9−14]
7h	3-Me	5-Me, *trans*	CH_2	2.32 ± 0.6	I	13 [8−16]
7i	4-OH	H	CH_2	1.42 ± 0.4	14 [13−17] (72%)	ne
7j	3-OH (±)	H	CH_2	4.8 ± 0.5	5 [4−7] (64%)	ne
7k	3-OH (+)	H	CH_2	1 ± 0.3	4 [3−5] (62%)	
7l	3-OH (−)	H	CH_2	2 ± 0.2	10 [8−13] (61%)	
7m	4-NHAc	H	CH_2	1.05 ± 0.36	13 [10−17] (59%)	ne
7n	4-CONH2	H	CH_2	1.43 ± 0.32	19 [16−24] (65%)	ne
7o	4-Bz	H	CH_2	7.64 ± 0.38	I	100 [75−150]
7p	H	H	O	35.6 ± 3.1	113 [87−146] (53%)	I
7q	3-Me	5-Me, *cis*	O	6.28 ± 0.32	I	I
7r	3-Me	5-Me, *trans*	O	NT	I	300
7s	H	H	N-Ph	NT	I	100 [70−165]
5-HT				187 ± 33	5 [3−8] (100%)	I
BIMU 8				257 ± 43	43 [29−63] (86%)	I
1 (SDZ 205557)				5.5 ± 0.7	I	77 [55−130]
4 (GR 113808)				0.15 ± 0.03	I	13 [9−18]
15 (RS 23597-190)				28.1 ± 6.9	30 [17−49] (52%)	ne

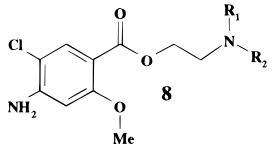
[a] [^{3}H]GR 113808 was used as the radioligand in rat striatal membranes, nonspecific binding was determined with **7a** (10 μM), and K_i ± SEM values were determined from the Cheng−Prussof equation. [b] The agonist activity was assessed as the concentration which gave a 50% increase in the response to electrical stimulation (EC_{50}) with regard to the maximum in the guinea pig ileum. The activity is expressed as 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.

7p) produced a clear decrease in affinity. Introduction of an additional substitution such as a methyl group had a favorable effect with the *cis*-3,5-dimethylpiperidine derivative (compound **7g**), while the *trans*-2,6-dimethyl-substituted compound **7e** was less active.

In the isolated guinea pig ileum preparation, the monosubstituted piperidine derivatives (compounds **7a**−**d**,**i**−**n**,**p**) displayed an agonist profile. They were equipotent to 5-HT and more potent than the reference compounds such as BIMU 8[25] (Table 1), zacopride,[25] renzapride,[20] and **6**.[26] However, their efficacy as agonists was found to be 60−80% of that of 5-HT, suggesting a partial agonist profile. Maximal contraction of the guinea pig ileum produced by compound **7a** was 80% of that of 5-HT, and it was the most potent compound tested. The agonist profiles of compounds **7a**,**i**,**j** were confirmed by evaluating their activity at 5-HT$_4$ receptors in the rat esophagus preparation. The potencies of **7a** (EC_{50} = 2.4 nM (80%)), **7i** (EC_{50} = 3 nM (56%)), and **7j** (EC_{50} = 2.7 nM (67%)) were shown to be comparable to that of 5-HT (EC_{50} = 2.3 nM (100%)); nevertheless, these compounds were again apparent partial agonists in this system. **1** acted as a competitive antagonist in this preparation with slopes not significantly different from unity and had pA_2 values of 7.7 ± 0.1 and 7.6 ± 0.2 when it was evaluated against 5-HT and compound **7a**, respectively. **15**, reported to be a 5-HT$_4$ receptor antagonist[42] in the esophageal muscularis mucosae preparation, was found, unexpectedly, to have partial agonist activity (EC_{50} = 30 nM (52%)) in the electrically-stimulated longitudinal muscle of the guinea pig ileum (Table 1).

The apparent partial agonist activity of compound **7a** contrasted markedly with the antagonist profile of **1** which had an IC_{50} (±SE) value of 77 ± 8 nM in the guinea pig ileum and 57 ± 10 nM in rat esophagus using 5-HT as the agonist. These two molecules are structurally similar, indicating, doubtless, a key role of the basic framework in the pharmacological profile for the 5-HT$_4$ receptor. As has already been suggested, a suitable orientation of the lone pair of the basic nitrogen atom[29a] seems to be required for receptor stimulation. This orientation depends upon the conformational flexibility and steric hindrance of the basic chain. It is possible that the 1-piperidineethyl chain of compounds derived from **7a** adopts a markedly favorable conformation in the receptor site where the basic nitrogen atom occupies the correct orientation to stimulate the receptor. The steric hindrance of the less conformationally-restricted chain of **1** could disturb this orientation and induce a change in the pharmacological profile without marked modification of the affinity. The antagonist activity of the 3,5-dimethylpiperidine derivatives seemed to confirm this hypothesis. They were isolated as the *cis* and *trans* isomers (compounds **7g**,**h**) and found to act as potent antagonists of 5-HT-mediated contractions in the guinea pig ileum preparation (IC_{50} = 11 and 13 nM, respectively). Competitive antagonist activity was con-

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



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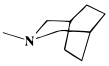
Compd	NR_1R_2	Binding assays *Ki*, nM[a], 5-HT_4	Functional activity at EC_{50}, nM[b]	5-HT_4 receptors IC_{50}, nM[c]
8a		5.35± 0.31	40 [27-59] (50%)	~200
8b		0.92 ± 0.03	4.4 [3.8-5.2] (59%)	n.e[d]
8c		9.1 ± 0.6	29 [23-38] (65%)	n.e
8d		6.2 ± 0.29	17 [14-21] (76%)	ne
8e		6.03 ± 0.3	5.3 [4-8] (78%)	~20
8f		5.45 ± 0.35	7.8 [5-12] (61%)	ne
8g		9.3 ± 0.3	40 [32-51] (63%)	ne

[a−d] See footnotes to Table 1.

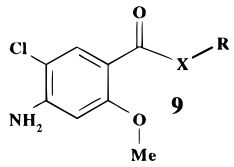
firmed in the rat tunica muscularis mucosae preparation where the compounds inhibited the relaxant activity of 5-HT with pA_2 values of 8.6 and 8.2, respectively, confirming the superiority of the *cis* derivative. **7g** (K_i = 0.26 ± 0.06 nM) was found to be equipotent to **4** (K_i = 0.15 ± 0.03 nM) in the binding assays and the guinea pig ileum preparation (IC_{50} = 13 nM). The 3,3′-dimethyl derivative (compound **7f**) was also found to be an antagonist, although its antagonist potency was much lower than its affinity for 5-HT_4 receptors (IC_{50} = 150 nM, K_i = 1.72 nM).

Several structural variations of compound **7** were also produced to evaluate the influence of the size and the nature of the cyclic amine on the pharmacological profile, and these results are reported in Table 2. It was noteworthy that all compounds had high affinity for 5-HT_4 receptors and behaved as agonists while possessing marked structural differences such as the size of the ring (compounds **8a**−**c**), the existence of a bicyclic structure (compounds **8f**,**g**), or the presence of one charge on the basic nitrogen atom (compounds **8d**,**e**). The introduction of a conformational constraint in the aza ring with an ethylene bridge (compounds **8f**,**g**) did not affect the profile and potency of the pharmacological activity, which were similar to those of the parent compound. Moreover, the quaternary compounds **8d**,**e**, which possess a totally-rigid basic framework, were found to be agonists for 5-HT_4 receptors with good efficacy (EC_{50} = 17 nM (76%), and 5.3 nM (78%), respectively). These results agree with the report of King[27] which showed the favorable role of the quaternary nitrogen atom on the 5-HT_4 receptor agonist activity of renzapride.

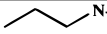
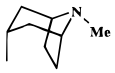
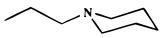
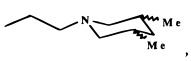
The presence of an ester function in the pharmacologically-active molecules could be an important drawback for their use as drugs because of the putative short half-lives of such compounds. Initially, the advantage of the presence of the ester function over that of the amidic group was demonstrated by the superior activity of **1** with regard to metoclopramide. It seemed to us worthwhile to compare the pharmacological activity at 5-HT_4 receptors of a number of amidic derivatives and the corresponding esters in order to understand the influence of this latter function. Examination of the results reported in Table 3 showed in all cases, except for BRL 24682, the superiority of the ester derivatives over the amidic derivatives and that the difference in the pharmacological activities depended essentially upon the structure of the basic framework. Thus, we observed a dramatic drop in the affinity of the amidic compounds derived from 1-piperidinoethylamine (compounds **9f**−**h**) and *N*,*N*-dimethyl- and *N*,*N*-diethylethylenediamines (compounds **9b** and metoclopramide) for 5-HT_4 receptors. In particular, compound **9h**, the amidic analogue of the potent 5-HT_4 receptor antagonists **7g**,**h**, was 500-fold less potent than the ester derivatives in the binding assays and possessed partial agonist activity in the micromolar range in the ileum preparation. On the other hand, the effect was less marked for derivatives with a conformationally-constrained basic moiety such as quinuclidine or tropane. Thus the *R* and *S* enantiomers of zacopride, a well-known 5-HT_4 receptor full agonist,[43] had potencies similar to those of the esters (compounds **9c**,**d**) derived from the *R* and *S* enantiomers of 3-quinuclidinol, while BRL 24682 and the corresponding ester **9e** had similar affinity for 5-HT_4 receptors. These data confirm the results reported by Gaster[18a] which showed the superiority of the indolic esters of (1-butyl-4-piperidinyl)-methanol over the corresponding amides. On the other hand, high potency was retained with the tricyclic amidic derivative **5** which was demonstrated to be a potent and selective 5-HT_4 receptor antagonist with oral activity.[18b]

The advantages of compounds such as **7a**,**b**,**d**,**i**−**k** reside in their equipotency to 5-HT in the *in vitro* preparations and their selectivity for 5-HT_4 receptors. The results reported in Table 4 show the low affinity of these compounds for 5-HT_3 receptors and confirm their advantage over the 5-HT_4 receptor agonist benzamides which are also potent 5-HT_3 receptor antagonists. It is well-known that this latter receptor type is implicated to the same extent as 5-HT_4 receptors in mediating intestinal contractions, and the 5-HT_3 receptor antagonist properties of the benzamides could be an important drawback for the development of gastrokinetic drugs. On the other hand, the presence of an ester function in compound **7a** and its derivatives does not explain the drop in affinity for 5-HT_3 receptors because we have previously shown that the corresponding esters of a number of 5-HT_3 receptor antagonists were equipotent to the amidic derivatives at 5-HT_3 receptors.[34] In the course of this study, we prepared the ketone **14**, the bioisosteric derivative of the ester **7a** recently described by Clarke,[32] to show the influence of the oxygen atom on the pharmacological activity with regard to the benzoate derivative. However, we saw a marked decrease in the affinity of this compound for 5-HT_4 receptors (K_i = 103 ± 65 nM) and in its pharmacological activity on the guinea pig ileum (EC_{50} = 115 nM, 60% of the maximal effect of 5-HT). These data confirm the importance of the ester function in the benzoate derivatives of 1-piperidineethanol for recognition by 5-HT_4 receptors, although several aromatic ketones, analogues of the benzoate esters of 4-amino-5-chloro-2-methoxy-

Table 3. Pharmacological Activity of the Derivatives **9** at 5-HT$_4$ Receptors



9

Compd	R	X	Binding assays, 5-HT$_4$ K_i, nM[a],	Functional activity at 5-HT$_4$ receptors EC_{50}, nM[b]	IC_{50}, nM[c]
9a		O	47 ± 37	180 [150-220] (60%)	~600
9b	" "	NH	> 1000	NT[e]	NT
9c	(R) 3-quinuclidinyl	O	488 ± 63	110 [80-140] (75%)	~30
9d	(S) 3-quinuclidinyl	O	91.4 ± 6	153 [134-174] (54%)	~450
9e		O	64.3 ± 6	27 [20-36] (41%)	ne[d]
9f		NH	135 ± 25	>1000	>1000
9g	" "	N-Me	>1000	>1000	>1000
9h	 *cis, trans*	NH	103 ± 52	~930 (80%)	ne
1 (SDZ 205-557)			5.3 ± 0.7	It[f]	77[55-130]
Metoclopramide			> 1000	5.7 ± 0.1 (pEC_{50})g	NT
(S) Zacopride			383 ± 64	77 [65-91] (100%)	ne
(R) Zacopride			1510 ± 420	166 [142-195] (84%)	ne
BRL 24682			48.6 ± 5.6	NT	NT

[a–f] See footnotes to Table 1. [g] See ref 8.

Table 4. Affinity of Compounds **7a**,**c**,**g**,**h**,**j** for 5-HT$_3$ Receptors[a]

compd	binding assays 5-HT$_3$, K_i, nM
7a	730 ± 75
7c	>1000
7g	>1000
7h	>1000
7j	>1000

[a] [^{3}H]BRL-43694 was used as the radioligand, and the binding assays were carried out using rat posterior cortex (30 min, −25 °C); nonspecific binding was determined with GR 38032F (10 μM).

benzoic acid but possesing a different basic chain, have been reported to be 5-HT$_4$ receptor agonists[32] or antagonists[44] in the rat esophageal muscularis mucosae preparation. In particular, a series of derivatives of 3-(4-piperidinyl)propiophenone, derived from the benzoates described by Gaster,[14a] were shown to be potent partial agonists at these receptors. In this case, substitution of the oxygen atom by a methylene group reversed the pharmacological profile without decreasing the affinity.

Structural and Conformational Analysis

In order to understand the dramatic change from an agonist to an antagonist profile of the molecule **7a** by the addition of two methyl groups on the piperidine ring (compound **7g**), we carried out structural analyses to examine the structural properties of both molecules. The orientation of the phenyl ring was found to be markedly different between the X-ray crystal structures of the hydrochloride salt forms of the agonist **7a** and the antagonist **7g** (Figure 1). The methoxy group of **7g** was found to be in the *syn* configuration with regard to the carbonyl function, while it was in the *anti* position in compound **7a** as has been observed for orthopramide derivatives. However, it was coplanar with the aromatic ring in both compounds. On the other hand, both compounds had a similar orientation of the piperidine ring with regard to the benzoate moiety. This was due to the folded conformation of the ethyl chains of compounds **7a**,**g**, the values of the torsion angles τ_1 = C(20)−O(7)−C(19)−C(18) and τ_2 = O(7)−C(19)−C(18)−N(1) being 289° and 260° for **7a** and 179° and 62° for **7g**, respectively. Consequently, the molecules possessed a folded shape with the hydrogen atom on the quaternary nitrogen atom pointing toward the outside of the molecule as seen in benzamides such as zacopride, renzapride, and **6**. Moreover, the distance between the oxygen atom O(21) of the carbonyl group and the protonated nitrogen atom N(1), which appears to be an important structural parameter of the 5-HT$_4$ receptor pharmacophore, was 4.80 Å in **7a**, close to that of the two minimum energy conformers of zacopride (5.1 and 4.79 Å) reported previously.[45] These data confirm the structurally-closely-related pharmacophores of the 5-HT$_3$ and 5-HT$_4$ receptor antagonists and agonists, respectively. However, the marked structural similarity between **7a**,**g**, which possess opposite pharmacological profiles, was intriguing. The only structural difference between compounds **7a**,**g** resided in the change of the orientation of the aromatic ring which brought about the different configuration of the *o*-methoxy group with regard to that of the carbonyl function and the difference in the spatial position of the chlorine atom. However, these data cannot satisfactorily explain the reversal of the pharmacological profile. To date, no structural data have been reported on 5-HT$_4$ receptor antagonists, but

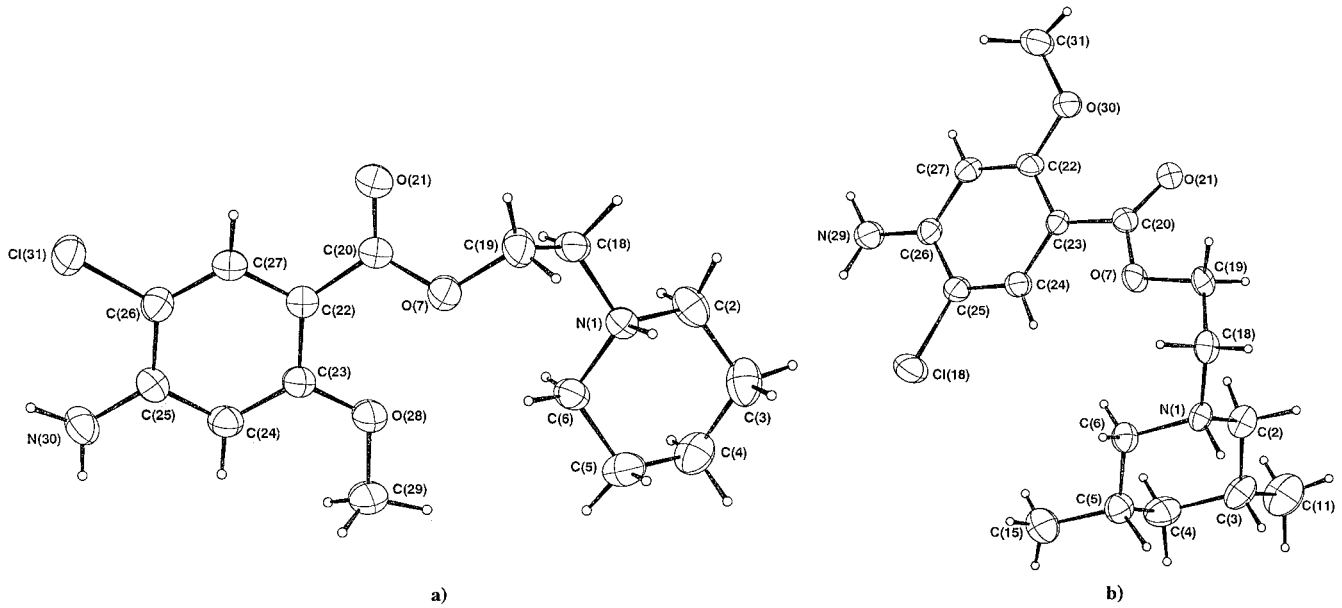


Figure 1. ORTEP drawing of compounds **7a** (a) and **7g** (b).

Table 5. Values for the Torsion Angles τ_1 and τ_2 and Energies of the Minimum Energy Conformers of Compounds **7a**,**g**

compd	conformer	τ_1, deg	τ_2, deg	energy, kcal mol^{-1}
7a	1	280	300	−5.36
	2	280	180	−5.33
	3	60	60	−4.54
	4	80	180	−5.43
	5	180	60	−6.05
	6	180	180	−6.37
7g	1	280	180	−4.81
	2	300	300	−5.01
	3	300	180	−4.33
	4	60	60	−5.53
	5	80	180	−4.55
	6	180	60	−5.38
	7	180	300	−3. 47
	8	180	180	−5.54

it is likely that potent compounds such as **2** and **4** have an extended conformation because the large substituents on positions 1 and 4 of the piperidine ring prefer to occupy diequatorial stereochemistry.

The structural analyses of **7a**,**g** were performed with SYBYL software 6.03 using the Random Search and Grid Search programs, and the values of the minimum energy conformers are presented in Table 5. This study showed that there was a relatively limited number of minimun energy conformers, such as the extended conformers 6 and 8 ($\tau_1 = 180°$, $\tau_2 = 180°$) and those resulting from the *cis*-folded conformation of the ethyl chain; on the other hand, it did not show essential structural differences between compounds **7a**,**g**.

Therefore, we assume that the additional methyl of **7g** and, consequently, the steric hindrance are implicated in the differences in the pharmacological profiles. Thus, one of the *cis*-staggered conformers of **7a** may represent the active form at the binding site of the 5-HT_4 receptor, producing an agonist response. This would be due essentially to the particular orientation of the lone pair which is directed outside the molecule as has been observed for the 5-HT_4 receptor agonist benzamides such as zacopride and renzapride. For compound **7g,** it is probable that the folded conformations cannot occupy the agonist site because the presence of the methyl groups on the piperidine ring hampers the binding to the receptor site by steric hindrance. The presence of both methyl groups seems to be essential for antagonist activity because the monomethylpiperidine derivatives, such as compounds **7b**−**d**, are potent agonists in the pharmacological assays.

The existence of two binding sites on the 5-HT_4 receptor (Figure 2) is therefore suggested to explain the agonist and antagonist activities of **7a**,**g**, respectively. Thus, 5-HT_4 receptor agonist or antagonist molecules in the energetically-stable-extended conformation would bind with high affinity to the first site. The agonist effect is mediated by the propensity of the molecule to adopt the folded conformation in the binding site, bringing about a conformational rearrangement of the ligand−receptor complex and the binding of the molecule in another conformation to the second site (Figure 2). In the course of this process, G-protein decoupling would occur, mediating the agonist effect. Any structural feature such as steric hindrance of the ligand would block this process and, consequently, block activation of the receptor. This hypothesis could explain the antagonist effect of compound **7g** which would be hampered by the two methyl groups on the piperidine ring from adopting the folded conformation and thus could only bind to the first site. The high antagonist potency of compounds such as **2** and **4** could be due to their inability to occupy a folded conformation with a suitable orientation of the nitrogen atom. On the other hand, we suggest that 5-HT_4 receptor agonists such as benzamides, with the tied-back basic cyclic structure, may bind directly to the second site, and thus their full agonist properties result from their inability to occupy the first site.

Conclusion

The data presented here demonstrate the potential of monosubstituted 1-piperidinoethyl esters derived from 4-amino-5-chloro-2-methoxybenzoates to act as potent agonists for 5-HT_4 receptors in the CNS, guinea pig ileum, and rat esophagus. The presence of two methyl groups with *cis* stereochemistry on positions 3 and 5 of the piperidine ring increased the affinity for

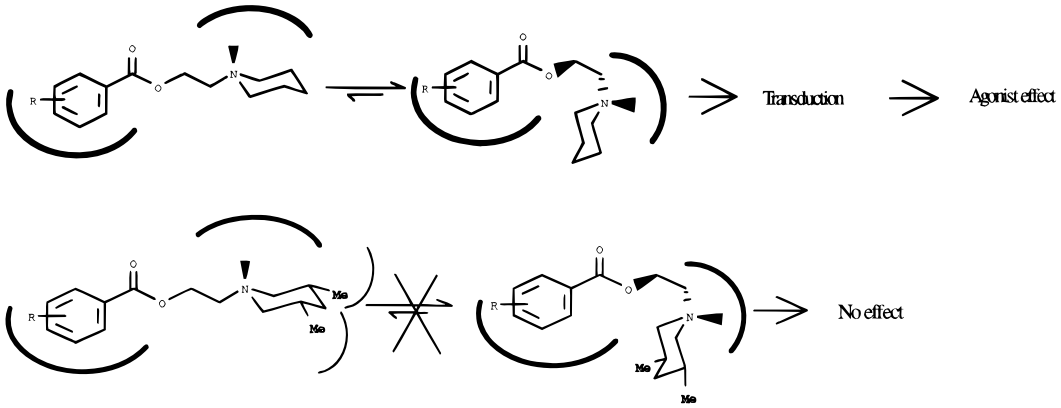


Figure 2. Hypothetical model of the binding of 5-HT$_4$ receptor ligands to the receptor site to explain the agonist and antagonist activities of the 1-piperidineethanol benzoates **7a**,**g**, respectively.

5-HT$_4$ receptors and induced a dramatic change of the pharmacological profile as compound **7g** is a potent antagonist for this receptor. The pharmacological activities of several esters and the corresponding amides at 5-HT$_4$ receptors were compared, and as has been reported previously, the role of the ester function in increasing the potency and selectivity for the 5-HT$_4$ receptor was confirmed. This point was particularly marked with the esters of 2-piperidinoethanol which were 100-fold more active than the corresponding amides. **7a**,**k**,**g**, with nanomolar affinity, constitute interesting pharmacological tools for the understanding of the role of 5-HT$_4$ receptors. Comparison of the structural analyses of **7a**,**g** did not provide an explanation for the reversal of the pharmacological profile and suggested the involvement of steric hindrance. The 5-HT$_4$ receptor agonist activity of these compounds could be explained by a dynamic process whereby the first phase is the recognition and binding of the molecule to the receptor followed by a conformational rearrangement of the molecule and the receptor site, mediating the coupling of G-proteins and thus the agonist activity. Compounds such as **7g** possess high affinity for the first binding site but are unable to occupy the second site because of the steric hindrance of the methyl groups, thus resulting in an antagonist action at 5-HT$_4$ receptors. The possibility of an equilibrium between the two sites could explain the partial agonist profile of the piperidine derivatives, and further experiments are in progress to test this hypothesis.

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, trityl chloride, the mixture of *cis*- and *trans*-3,5-dimethylpiperidine, 4-benzylpiperidine, hexamethylenimine, 1,2,3,6-tetrahydropyridine, *cis*-2,6-dimethylpiperidine, the mixture of *cis*- and *trans*-2,6-dimethylmorpholine, 4-methylpiperidine, 3-methylpiperidine, 2-methylpiperidine, 3,3-dimethylpiperidine, 4-hydroxypiperidine, 3-hydroxypiperidine, 4-piperidinecarboxamide, quinuclidine, 3-azabicyclo[3.2.2]nonane, *N*,*N*-dimethylethylenediamine, morpholine, pyrrolidine, 1-(3-chloropropyl)piperidine, and MnO_2 were purchased from Aldrich (France).

4-Acetamidopiperidine was synthesized from 4-amino-1-benzylpiperidine[46] by acetylation and debenzylation; 8-azabicyclo[3.2.1]octane was prepared from *N*-benzyltropinone (Janssen) by the Wolf−Kischner reduction[47] followed by debenzylation. (*R*)- and (*S*)-3-quinuclidinol were prepared according to the previously-reported process.[48]

***N*-Methyl-2-(1-piperidinyl)ethylamine.** A solution of 1-(2-chloroethyl)piperidine hydrochloride (20 g, 100 mmol) in distilled water (30 mL) was added to a stirred solution of 40% aqueous methylamine (77.4 mL) for 20 min. The mixture became warm during the addition. After 30 min, the reaction mixture was made basic by the addition of 37 g of NaOH pellets, and the compound was separated as a yellow oil. The cooled solution was extracted with ether (4 × 50 mL), and the combined organic layers were evaporated *in vacuo* to afford the product as a pale yellow oil. The oil was distilled to give 9 g (58%) of a colorless oil: bp 54−58 °C (1 mmHg); ^{1}H NMR ($CDCl_3$) δ 2.5 (t, 2H), 2.45−2.2 (m, 9H), 1.65−1.25 (m, 6H).

2-Bromoethyl 4-Amino-5-chloro-2-methoxybenzoate (10). A mixture of 4-amino-5-chloro-2-methoxybenzoic acid (20.1 g, 100 mmol) in 1,2-dibromoethane (100 mL) and dry THF (200 mL) was heated to reflux. Then 15.2 g (100 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was added dropwise over 2 h, and the resulting mixture was maintained under reflux for 3 h. The cooled mixture was filtered, and the filtrate was concentrated *in vacuo*. The residue was diluted with CH_2Cl_2 (500 mL), washed with water, and dried over $MgSO_4$. The solution was filtered through a short column of silica gel and eluted with CH_2Cl_2. Evaporation of the combined eluates and recrystallization of the solid residue from Et_2O/pentane gave **10** (22 g, 71%): mp 137 °C; R_f 0.63 (CH_2Cl_2/MeOH, 95:5); ^{1}H NMR ($CDCl_3$) δ 7.8 (s, 1H, ArH), 6.22 (s, 1H, ArH), 4.46 (t, J = 6 Hz, 2H, OCH_2), 4.45 (s, 2H, NH_2), 3.78 (s, 3H, OCH_3), 3.54 (t, J = 6 Hz, 2H, CH_2Br). Anal. ($C_{10}H_{11}NO_3BrCl$).

5-Chloro-2-methoxy-4-(tritylamino)benzoic Acid (11). Trityl chloride (33.3 g, 119 mmol) in dry CH_2Cl_2 (60 mL) was added portionwise at 0 °C to a mixture of 20 g (99 mmol) of 4-amino-5-chloro-2-methoxybenzoic acid in 100 mL of pyridine. The mixture was stirred overnight at room temperature, and the solvent was evaporated. The residue was taken up in CH_2Cl_2 and washed several times with water. The organic layer was dried over $MgSO_4$, and the solvent was evaporated. The solid was recrystallized from a mixture of CH_2Cl_2/EtOH to yield a white solid 38 g (86%): mp 214 °C; R_f 0.58 (CH_2Cl_2/MeOH, 90:10); ^{1}H NMR ($CDCl_3$) δ 8.02 (s, 1H, ArH), 7.4−7.18

(m, 15H, tritylH), 6.34 (br s, 1H, NH), 5.65 (s, 1H, ArH), 3.29 (s, 3H, OCH_3). Anal. ($C_{27}H_{22}NO_3Cl$).

General Method A: Preparation of Compounds 7a,g,h, m,o,p,s and 8a−c,f. 2-Piperidinoethyl 4-amino-5-chloro-2-methoxybenzoate Hydrochloride (7a). A mixture of compound **10** (1.54 g, 5 mmol), piperidine (0.85 g, 10 mmol), and K_2CO_3 (1.38 g, 10 mmol) in dry DMF (20 mL) was stirred for 18 h at room temperature. The resulting precipitate was filtered off, and the filtrate was concentrated *in vacuo*. The residue was dissolved in ethyl acetate (100 mL), washed with water, and dried over $MgSO_4$. The resulting solution was then treated with 3 N HCl ethyl acetate solution to afford the crude hydrochloride salt **7a** (1.4 g, 80%) which was recrystallized from *i*-PrOH/*i*-Pr_2O: mp >200 °C; R_f 0.72 (CH_2Cl_2/MeOH/NH_4-OH, 99:0.9:0.1); ^{1}H NMR (CD_3OD) δ 7.8 (s, 1H, ArH), 6.5 (s, 1H, ArH), 4.6−4.5 (m, 2H, OCH_2), 3.7 (s, 3H, OCH_3), 3.75−2.9 (m, 6H), 2.1−1.4; ^{13}C NMR (CD_3OD) δ 166.17 (C═O), 162.29 (C_2), 152.20 (C_4), 134.65 (C_6), 110.71−107.67 (C_1−C_5), 98.88 (C_3), 59.78, 57.27, 56.64, 55.19, 24.41, 22.71. Anal. ($C_{15}H_{21}N_2O_3Cl$·HCl).

2-(*cis*-3,5-Dimethylpiperidino)ethyl 4-Amino-5-chloro-2-methoxybenzoate (7g) and 2-(*trans*-3,5-Dimethylpiperidino)ethyl 4-Amino-5-chloro-2-methoxybenzoate (7h). As described for the preparation of **7a**, compound **10** (3.08 g, 10 mmol) was reacted with the commercially-available mixture of *cis*- and *trans*-3,5-dimethylpiperidine (2.26 g, 20 mmol) in dry DMF at 50 °C for 5 h. After evaporation of the solvent, the residue was dissolved in a minimum amount of methylene chloride and chromatographed on a column of silica gel with a mixture of Et_2O/CH_2Cl_2/MeOH (7:3:0.1); the *trans* isomer **7h** (0.5 g, 15%) was eluted first followed by the *cis* isomer **7g** (1.7 g, 50%).

7g: mp 115 °C; R_f 0.2 (Et_2O/CH_2Cl_2/MeOH, 7:3:0.1); ^{1}H NMR ($CDCl_3$) δ 7.8 (s, 1H, ArH), 6.25 (s, 1H, ArH), 4.39 (s, 2H, NH_2), 4.35 (t, J = 6.3 Hz, 2H, OCH_2), 3.81 (s, 3H, OCH_3), 2.88 (m, $2H_{eq}$, 2,6-piperidinyl H), 2.71 (t, J = 6.3 Hz, 2H, CH_2N), 1.71−1.52 (m, 5H), 0.84 (d, J = 5.8 Hz, 6H, CH_3 × 2), 0.52 (m, $1H_{ax}$, 4-piperidinyl H); ^{13}C NMR ($CDCl_3$) δ 164.5 (C═O), 160.2 (C_2), 147.9 (C_4), 133.09 (C_6), 109.69−109.32 (C_1−C_5), 98.01 (C_3), 61.96, 61.77, 56.82, 55.85 (OCH_3), 41.87, 30.96, 19.51 (CH_3 × 2). Anal. ($C_{17}H_{25}N_2O_3Cl$).

7h: mp 114−115 °C; R_f 0.3 (Et_2O/CH_2Cl_2/MeOH, 7:3:0.1); ^{1}H NMR ($CDCl_3$) δ 7.82 (s, 1H, ArH), 6.28 (s, 1H, ArH), 4.44 (s, 2H, NH_2), 4.33 (t, J = 6.3 Hz, 2H, OCH_2), 3.84 (s, 3H, OCH_3), 2.64 (m, 2H, CH_2N), 2.48 (m, $2H_{eq}$, 2,6-piperidinyl H), 2.14 (m, $2H_{ax}$, 2,6-piperidinyl H), 1.99 (m, 2H, 3,5-piperidinyl H), 1.26 (t, J = 5.8 Hz, 2H, 4-piperidinyl H), 0.94 (d, J = 6.9 Hz, 6H, CH_3 × 2); ^{13}C NMR ($CDCl_3$) δ 164.48 (C═O), 160.16 (C_2), 147.84 (C_4), 133.17 (C_6), 109.77−109.64 (C_1−C_5), 98.17 (C_3), 61.96, 61.17, 57.07, 55.92 (OCH_3), 38.85, 27.34, 19.07 (CH_3 × 2). Anal. ($C_{17}H_{25}N_2O_3Cl$).

2-(4-Acetamidopiperidino)ethyl 4-Amino-5-chloro-2-methoxybenzoate (7m). As described for the preparation of **7a**, compound **10** was reacted with 4-acetamidopiperidine. Purification by chromatography on silica gel (CH_2Cl_2/MeOH, 7:1) and recrystallization from AcOEt/hexane gave **7m** (1.1 g, 60%): mp 135 °C; R_f 0.35 (CH_2Cl_2/MeOH, 85:15); ^{1}H NMR ($CDCl_3$) δ 7.80 (s, 1H, ArH), 6.27 (s, 1H, ArH), 5.36 (d, 1H, NH), 4.48 (s, 2H, NH_2), 4.34 (t, J = 6 Hz, 2H, OCH_2), 3.75 (s and m, 4H, OCH_3, 4-piperidinyl H), 2.92 (m, $2H_{eq}$, 2,6-piperidinyl H), 2.72 (t, J = 6 Hz, 2H, CH_2N), 2.24 (m, $2H_{ax}$, 2,6-piperidinyl H), 1.96 (s, 3H, CH_3), 1.9 (m, $2H_{eq}$, 3,5-piperidinyl H), 1.45 (m, $2H_{ax}$, 3,5-piperidinyl H); ^{13}C NMR ($CDCl_3$) δ 169.4 (CONH), 164.5 (CO_2), 160.2 (C_2), 147.9 (C_4), 133.3 (C_6), 109.9−109.6 (C_1−C_5), 98.2 (C_3), 62.2, 56.7, 56.0, 46.4, 52.6, 32.2, 23.5 (CH_3). Anal. ($C_{17}H_{24}N_3O_4Cl$).

2-(4-Benzylpiperidino)ethyl 4-Amino-5-chloro-2-methoxybenzoate Hydrochloride (7o). As described for the preparation of **7a**, compound **10** (1.23 g, 4 mmol) was reacted with 4-benzylpiperidine (0.7 g, 4.0 mmol) in dry DMF to give **7o**, transformed into the hydrochoride salt and recrystallized from MeOH/AcOEt/hexane: 1.2 g (68%); mp 170 °C; R_f 0.33 (CH_2Cl_2/MeOH/NH_3(aq), 95:5:0.1); ^{1}H NMR (CD_3OD) δ 7.86 (s, 1H, ArH), 7.37−7.22 (m, 5H, PhH), 6.52 (s, 1H, ArH), 4.60 (t, J = 5 Hz, 2H, OCH_2), 3.85 (s, 3H, OCH_3), 3.71 (m, 2H), 3.55 (t, J = 5 Hz, 2H, CH_2N), 3.12 (m, 2H), 2.69 (d, J = 7 Hz, 2H, CH_2Ph), 1.97 (m, 3H), 1.58 (m, 2H). Anal. ($C_{22}H_{27}N_2O_3$-Cl·HCl).

2-Morpholinoethyl 4-Amino-5-chloro-2-methoxybenzoate (7p). As described previously for **7a**, compound **10** (1.23 g, 4 mmol) was reacted with morpholine in dry DMF to give **7p** (58%): mp 80 °C; ^{1}H NMR ($CDCl_3$) δ 7.9 (s, 1H, ArH), 6.35 (s, 1H, ArH), 4.5 (s, 2H, NH_2), 4.42 (t, J = 5 Hz, 2H, OCH_2), 3.85 (s, 3H, OCH_3), 3.75 (m, 4H, CH_2OCH_2), 2.8 (t, J = 5 Hz, 2H, NCH_2), 2.6 (m, 4H, CH_2NCH_2). Anal. ($C_{14}H_{19}N_2O_4$-Cl·0.5H_2O).

2-(4-Phenylpiperazino)ethyl 4-Amino-5-chloro-2-methoxybenzoate Hydrochloride (7s). As described previously for **7a**, compound **10** (1.23 g, 4 mmol) was reacted with 4-phenylpiperazine (0.65 g, 4.0 mmol) in dry DMF to give **7s**, transformed into the hydrochloride and recrystallized from the MeOH/AcOEt/hexane mixture (1.0 g, 59%): mp 224 °C; R_f 0.44 (CH_2Cl_2/MeOH/NH_3(aq), 95:5:0.1); ^{1}H NMR (CD_3OD) δ 7.91 (s, 1H, ArH), 7.34 (m, 2H, PhH), 7.09 (m, 2H, PhH), 6.99 (m, 1H, PhH), 6.54 (s, 1H, ArH), 4.68 (t, J = 5 Hz, 2H, OCH_2), 3.89 (s, 3H, OCH_3), 3.7−3.3 (m, 10H). Anal. ($C_{20}H_{24}N_3O_3Cl$·-HCl).

2-Pyrrolidinoethyl 4-Amino-5-chloro-2-methoxybenzoate (8a). As described for the preparation of **7a**, compound **10** (1.23 g, 4 mmol) was reacted with pyrrolidine to give **8a** as an oil: ^{1}H NMR ($CDCl_3$) δ 7.81 (s, 1H, ArH), 6.27 (s, 1H, ArH), 4.37 (t, 2H, J = 6.1 Hz, 2H, OCH_2), 3.82 (s, 3H, OCH_3), 2.83 (t, 2H, NCH_2, J = 6.1 Hz), 2.7−2.5 (m, 4H), 1.9−1.65 (m, 4H). Anal. ($C_{14}H_{19}N_2O_3Cl$·0.5H_2O).

7-(Hexamethyleneimino)ethyl 4-Amino-5-chloro-2-methoxybenzoate Hydrochloride (8b). As described for the preparation of **7a**, compound **10** (1.54 g, 5 mmol) was reacted with hexamethylenimine (0.5 g, 5.0 mmol) in dry DMF to give **8b**, transformed into the hydrochoride salt and recrystallized from MeOH/AcOEt (1.1 g, 86%): mp 140 °C; R_f 0.44 (CH_2Cl_2/MeOH/NH_4OH, 99:1:0.1); ^{1}H NMR (CD_3OD) δ 7.65 (s, 1H, ArH), 6.34 (s, 1H, ArH), 4.5 (t, J = 5 Hz, 2H, OCH_2), 3.67 (s, 3H, OCH_3), 3.44 (t, J = 5 Hz, 2H, CH_2N), 3.21 (m, 4H), 1.88 (m, 4H), 1.73 (m, 4H). Anal. ($C_{16}H_{23}N_2O_3Cl$·HCl·0.5H_2O).

2-(1,2,3,6-Tetrahydropyridino)ethyl 4-Amino-5-chloro-2-methoxybenzoate (8c). As described for the preparation of **7a**, compound **10** was reacted with 1,2,3,6-tetrahydropyridine to give **8c** as a white solid (yield 10%) after recrystallization from hexane/AcOEt: mp 110 °C; R_f 0.37 (CH_2Cl_2/MeOH, 95:5); ^{1}H NMR ($CDCl_3$) δ 7.82 (s, 1H, ArH), 6.27 (s, 1H, ArH), 5.8−5.55 (m, 2H, CH═CH), 4.44 (br s, 2H, NH_2), 4.40 (t, J = 6.2 Hz, 2H, OCH_2), 3.84 (s, 3H, OCH_3), 3.14−3.07 (m, 2H), 2.83 (t, J = 6.2 Hz, 2H, CH_2N), 2.68 (t, J = 5.6 Hz, 2H), 2.2−2.1 (m, 2H). Anal. ($C_{15}H_{19}N_2O_3Cl$).

2-(3-Azabicyclo[3.2.2]nonan-3-yl)ethyl 4-Amino-5-chloro-2-methoxybenzoate Hydrochloride (8f). It was prepared according to method A from compound **10** (2.45 g, 8.0 mmol) and 3-azabicyclo[3.2.2]nonane (1.0 g, 8.0 mmol) in dry DMF, transformed into the hydrochloride salt, and recrystallized from *i*-PrOH/*i*-Pr_2O: 2.2 g (78%); mp 224 °C; R_f 0.63 (CH_2Cl_2/MeOH/NH_4OH, 99:1:0.1); ^{1}H NMR (CD_3OD) δ 7.60 (s, 1H, ArH), 6.35 (s, 1H, ArH), 4.54 (t, J = 5 Hz, 2H, OCH_2), 3.65 (s, 3H, OCH_3), 3.40 (t, J = 5 Hz, 2H, CH_2N), 3.7−3.0 (m, 4H), 2.1 (br, 2H), 1.9−1.6 (m, 8H). Anal. ($C_{18}H_{25}N_2O_3Cl$·HCl).

General Method B: Preparation of Compounds 7b−f,i−l,n,q−r and 8d,e,g. 2-(*cis*-2,6-Dimethylmorpholino)-ethyl 4-Amino-5-chloro-2-methoxybenzoate Hydrochloride (7q) and 2-(*trans*-2,6-Dimethylmorpholino)ethyl 4-Amino-5-chloro-2-methoxybenzoate Hydrochloride (7r). A mixture of compound **10** (1.54 g, 5 mmol) and the commercially-available *cis*,*trans*-2,6-dimethylmorpholine (1.5 g, 13 mmol) in dry toluene (20 mL) was heated to reflux for 5 h. After evaporation of the toluene, the residue was dissolved in CH_2Cl_2 (100 mL). The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated. The mixture of *cis* and *trans* isomers was separated by flash chromatography on silica gel and eluted with CH_2Cl_2/Et_2O/MeOH (5:5:0.1) to give **7r** (0.4 g, 23%) and **7q** (0.85 g, 50%). Compounds **7q**,**r** were converted into HCl salts by a 4 N HCl ether solution.

7q: mp 224 °C; R_f 0.4 (Et_2O/CH_2Cl_2/MeOH, 7:3:0.1); ^{1}H NMR ($CDCl_3$) δ 7.8 (s, 1H, ArH), 6.25 (s, 1H, ArH), 4.52 (s, 2H, NH_2), 4.37 (t, J = 6.3 Hz, 2H, OCH_2), 3,8 (s, 3H, OCH_3), 3.68 (m, 2H, 2,6-morpholinyl H), 2.80 (m, $2H_{eq}$, 3,5-morpholinyl H), 2.7 (t, J = 6.3 Hz, 2H, CH_2N), 1.86 (m, $2H_{ax}$, 3,5-morpholinyl H), 1.15 (d, J = 6.4 Hz, 3H, $CH_3 \times 2$); ^{13}C NMR ($CDCl_3$) δ 164.38 (C=O), 160.17 (C_2), 147.80 (C_4), 133.18 (C_6), 109.78, 109.36, 98.09 (C_3), 71.54, 61.63, 59.59, 56.60, 55.94 (OCH_3), 19.03 ($CH_3 \times 2$). Anal. ($C_{16}H_{23}N_2O_4Cl \cdot HCl \cdot 0.5H_2O$).

7r: mp >170 °C; R_f 0.5 (Et_2O/CH_2Cl_2/MeOH, 7:3:0.1); ^{1}H NMR ($CDCl_3$) δ 7.77 (s, 1H, ArH), 6.26 (s, 1H, ArH), 4.59 (s, 2H, NH_2), 4.30 (t, J = 6.3 Hz, 2H, OCH_2), 3.97 (m, 2H, 2,6-morpholinyl H), 3.77 (s, 3H, OCH_3), 2.64−2.50 (m, 4H), 2.21 (m, 2H), 1.28 (d, J = 6.4 Hz, 6H, $CH_3 \times 2$); ^{13}C NMR ($CDCl_3$) δ 164.38 (C=O), 160.15 (C_2), 147.95 (C_4), 133.10 (C_6), 109.71−109.36 (C_1−C_5), 98.10 (C_3), 66.53, 61.45, 58.78, 56.83, 55.89 (OCH_3), 18.01 ($CH_3 \times 2$). Anal. ($C_{16}H_{23}N_2O_4Cl \cdot HCl \cdot 0.5H_2O$).

2-(4-Methylpiperidino)ethyl 4-Amino-5-chloro-2-methoxybenzoate (7b). It was prepared according to method B from compound **10** and 4-methylpiperidine in dry CH_3CN. Recrystallization from AcOEt/hexane afforded **7b** (1.2 g, 73%): mp 123 °C; R_f 0.49 (CH_2Cl_2/MeOH/NH_3 (aq), 9:1:0.1); ^{1}H NMR ($CDCl_3$) δ 7.73 (s, 1H, ArH), 6.20 (s, 1H, ArH), 4.47 (s, 2H, NH_2), 4.30 (t, J = 6.2 Hz, 2H, OCH_2), 3.75 (s, 3H, OCH_3), 2.85 (m, 2H), 2.65 (t, J = 6.2 Hz, 2H, CH_2N), 2.01 (m, 2H), 1.52 (m, 2H), 1.2−1.1 (m, 3H), 0.85 (d, J = 6 Hz, 3H, CH_3). Anal. ($C_{16}H_{23}N_2O_3Cl$).

2-(3-Methylpiperidino)ethyl 4-Amino-5-chloro-2-methoxybenzoate Hydrochloride (7c). It was prepared according to method B from compound **10** (0.92 g, 3 mmol) and 3-methylpiperidine (0.74 g, 7.5 mmol) to give crude compound **7c** as a colorless foam, which was converted into the HCl salt by a 4 N HCl ether solution. Recrystallization from *i*-PrOH/*i*-Pr_2O gave **7c** (0.7 g, 64%): mp 205 °C; R_f 0.5 (CH_2Cl_2/MeOH/NH_3(aq), 9:1:0.1); ^{1}H NMR ($CDCl_3$) δ 7.68 (s, 1H, ArH), 6.15 (s, 1H, ArH), 4.4 (s, 2H, NH_2), 4.35 (t, J = 6 Hz, 2H, OCH_2), 3.72 (s, 3H, OCH_3), 2.85 (m, 2H), 2.68 (t, J = 6 Hz, 2H, CH_2N), 1.8 (m, 1H), 1.7−1.5 (m, 5H), 0.65−0.9 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 164.36 (C=O), 160.12 (C_2), 148.14 (C_4), 133.08 (C_6), 109.67, 108.84, 97.96 (C_3), 61.74, 61.23, 56.86, 55.85 (OCH_3), 53.91, 32.31, 30.57, 24.92, 19.94 (CH_3). Anal. ($C_{16}H_{23}N_2O_3Cl \cdot HCl$).

2-(2-Methylpiperidino)ethyl 4-Amino-5-chloro-2-methoxybenzoate Hydrochloride (7d). It was prepared according to method B from compound **10** (0.92 g, 3 mmol) and 2-methylpiperidine (0.74 g, 7.5 mmol) as a colorless foam, which was converted into the HCl salt by a 4 N HCl ether solution. Recrystallization from *i*-PrOH/*i*-Pr_2O gave **7d** (0.9 g, 80%): mp 185 °C; R_f 0.47 (CH_2Cl_2/MeOH/NH_4OH, 9:1:0.1); ^{1}H NMR ($CDCl_3$) δ 7.8 (s, 1H, ArH), 6.25 (s, 1H, ArH), 4.47 (s, 2H, NH_2), 4.35 (t, J = 6.2 Hz, 2H, OCH_2), 3.82 (s, 3H, OCH_3), 3.0−2.7 (m, 3H), 2.35 (m, 2H), 1.6 (m, 4H), 1.25 (m, 2H), 1.1 (d, J = 6 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 164.48 (C=O), 160.25 (C_2), 147.71 (C_4), 133.31 (C_6), 109.87, 109.46, 98.18 (C_3), 61.77, 56.02−55.96, 53.29, 52.06, 34.64, 26.12, 24.03, 19.56 (CH_3). Anal. ($C_{16}H_{23}N_2O_3Cl \cdot HCl$).

2-(*cis*-2,6-Dimethylpiperidino)ethyl 4-Amino-5-chloro-2-methoxybenzoate (7e). It was prepared from compound **10** (0.92 g, 3 mmol) and 2,6-dimethylpiperidine (1.0 g, 9 mmol) as a colorless foam. It was triturated with Et_2O and recrystallized from AcOEt/Hexane to give **7e** (0.4 g, 39%): mp 109−111 °C; R_f 0.3 (CH_2Cl_2/MeOH/NH_4OH, 9:1:0.1); ^{1}H NMR ($CDCl_3$) δ 7.8 (s, 1H, ArH), 6.25 (s, 1H, ArH), 4.47 (s, 2H, NH_2), 4.25 (t, J = 6 Hz, 2H, OCH_2), 3.8 (s, 3H, OCH_3), 3.0 (t, J = 6 Hz, 2H, CH_2N), 2.5 (m, 2H, 2,6-piperidinyl H), 1.5−1.7 (m, 3H), 1.4−1.2 (m, 3H), 1.15 (d, J = 6 Hz, 6H, $CH_3 \times 2$); ^{13}C NMR ($CDCl_3$) δ 164.42 (C=O), 160.31 (C_2), 147.75 (C_4), 133.26 (C_6), 109.81, 109.4, 98.13 (C_3), 61.8, 56.72, 56.01 (OCH_3), 46.33, 34.44, 24.59, 21.55 (CH_3). Anal. ($C_{17}H_{25}N_2O_3Cl$).

2-(3,3-Dimethylpiperidino)ethyl 4-Amino-5-chloro-2-methoxybenzoate Hydrochloride (7f). It was prepared according to method B from compound **10** and 3,3-dimethylpiperidine in toluene and transformed into the hydrochloride salt (1.5 g, 80%): mp 260 °C dec; R_f 0.5 (CH_2Cl_2/MeOH/NH_4OH, 9:1:0.1); ^{1}H NMR (DMSO) δ 10.3 (s, 1H, HCl), 7.65 (s, 1H, ArH), 6.48 (s, 1H, ArH), 6.26 (s, 2H, NH_2), 4.55 (m, 2H, OCH_2), 3.68 (s, 3H, OCH_3), 3.5−3.1 (m, 4H), 2.9−2.7 (m, 2H), 2.0−1.6 (m, 2H), 1.5−1.1 (m, 2H), 1.1 (s, 3H, CH_3), 0.90 (s, 3H, CH_3). Anal. ($C_{17}H_{25}N_2O_3Cl \cdot HCl \cdot 0.5H_2O$).

2-(4-Hydroxylpiperidino)ethyl 4-Amino-5-chloro-2-methoxybenzoate Hydrochloride (7i). It was prepared according to method B from compound **10** (1.54 g, 5 mmol) and 4-hydroxypiperidine (1.26 g, 12.5 mmol) and transformed into the hydrochloride salt which was recrystallized from the *i*-PrOH/$CHCl_3$ mixture (1.2 g, 66%): mp 130−150 °C; R_f 0.35 (CH_2Cl_2/MeOH/NH_4OH, 9:1:0.1); ^{1}H NMR (CD_3OD) δ 7.75 (s, 1H, ArH), 6.4 (s, 1H, ArH), 4.5 (t, J = 5 Hz, 2H, OCH_2), 3.75 (s, 3H, OCH_3), 3.7−3.6 (m, 1H), 3.5 (t, J = 5 Hz, 2H, CH_2N), 3.5−3.0 (m, 4H), 1.88 (m, 4H), 1.73 (m, 4H). Anal. ($C_{15}H_{21}N_2O_4Cl \cdot HCl \cdot H_2O$).

2-(3-Hydroxylpiperidino)ethyl 4-Amino-5-chloro-2-methoxybenzoate Hydrochloride (7j). It was prepared according to method B from compound **10** (1.54 g, 5 mmol) and 3-hydroxypiperidine as a colorless foam, which was then converted into the HCl salt by a 4 N HCl ether solution and recrystallized from a EtOH/$CHCl_3$ mixture to give 1.0 g (55%) of **7j**: mp 130−150 °C; R_f 0.31 (CH_2Cl_2/MeOH/NH_4OH, 9:1:0.1); ^{1}H NMR (DMSO) δ 7.62 (s, 1H, ArH), 6.48 (s, 1H, ArH), 6.19 (s, 2H, NH_2), 4.61 (d, 1H, OH), 4.23 (m, 2H, OCH_2), 3.77 (s, 3H, OCH_3), 3.58−3.31 (m, 1H, CH), 2.92 (m, 1H), 2.75 (m, 1H), 2.63 (m, 2H, NCH_2), 2.10−1.72 (m, 3H), 1.72−1.56 (m, 3H), 1.56−1.25 (m, 1H), 1.25−0.93 (m, 1H). Anal. ($C_{15}H_{21}N_2O_4Cl \cdot HCl \cdot H_2O$).

2-(3-(*S*)-Hydroxylpiperidino)ethyl 4-Amino-5-chloro-2-methoxybenzoate Hydrochloride (7k). It was prepared according to method B from compound **10** (1.6 g, 5.3 mmol) and 3-(*S*)-hydroxypiperidine (1.3g, 12.9 mmol) and then purified by chromatography on silica gel (CH_2Cl_2/MeOH, 9:1). The residue was further purified through the formation of the hydrochloride salt. It was treated with base to give **7k** (0.54 g, 30%): mp 95−98 °C; $[\alpha]^{20} = +3°$ (c = 1, MeOH); ^{1}H NMR (DMSO) δ 7.60 (s, 1H, ArH), 6.47 (s, 1H, ArH), 6.20 (s, 2H, NH_2), 4.66 (d, 1H, OH), 4.25 (m, 2H, OCH_2), 3.78 (s, 3H, OCH_3), 3.63−3.28 (m, 1H, CH), 2.94 (m, 1H), 2.77 (m, 1H), 2.66 (m, 2H, NCH_2), 2.09−1.72 (m, 3H), 1.72−1.53 (m, 3H), 1.53−125 (m, 1H), 1.25−0.91 (m, 1H). Anal. ($C_{15}H_{21}N_2O_4Cl \cdot HCl \cdot H_2O$).

2-(3-(*R*)-Hydroxylpiperidino)ethyl 4-Amino-5-chloro-2-methoxybenzoate Hydrochloride (7l). It was prepared according to method B from compound **10** (1.6 g, 5.2 mmol) and 3-(*R*)-hydroxypiperidine (1.3 g, 12.9 mmol) and then purified by chromatography on silica gel (CH_2Cl_2/MeOH, 9:1). The residue was further purified through the formation of the hydrochloride salt. It was treated with base to give **7l** (0.54 g, 30%): mp 95−98 °C; $[\alpha]^{20} = -3.5°$ (c = 1, MeOH); ^{1}H NMR (DMSO) δ 7.60 (s, 1H, ArH), 6.47 (s, 1H, ArH), 6.21 (s, 2H, NH_2), 4.66 (d, 1H, OH), 4.25 (m, 2H, OCH_2), 3.78 (s, 3H, OCH_3), 3.60−3.34 (m, 1H, CH), 2.94 (m, 1H), 2.78 (m, 1H), 2.66 (m, 2H, NCH_2), 2.09−1.72 (m, 3H), 1.72−1.55 (m, 3H), 1.55−1.31 (m, 1H), 1.25−0.97 (m, 1H). Anal. ($C_{15}H_{21}N_2O_4Cl \cdot HCl \cdot 0.5H_2O$).

2-(4-Carboxamidopiperidino)ethyl 4-Amino-5-chloro-2-methoxybenzoate (7n). It was prepared according to method B from compound **10** (1.0 g, 3.2 mmol) and 4-piperidinecarboxamide (0.82 g, 6.4 mmol) in dry DMF at 50 °C for 5 h. Purification by flash chromatography (CH_2Cl_2/MeOH, 9:1) and recrystallization from $CHCl_3$/hexane yielded **7n** (0.8 g, 71%): mp 137 °C; R_f 0.4 (CH_2Cl_2/MeOH, 8:2); ^{1}H NMR ($CDCl_3$) δ 7.79 (s, 1H, ArH), 6.27 (s, 1H, ArH), 5.61 (s, 2H, $CONH_2$), 4.51 (s, 2H, NH_2), 4.34 (t, J = 6.0 Hz, OCH_2), 3.82 (s, 3H, OCH_3), 3.01 (m, 2H), 2.72 (t, J = 6.0 Hz, 2H, CH_2N), 2.18−2.06 (m, 3H), 1.9−1.6 (m, 4H); ^{13}C NMR (CD_3OD) δ 175.7 (CONH), 169.0 (CO_2), 164.5 (C_2), 154.0 (C_4), 136.7 (C_6), 112.9−111 (C_1−C_5), 101.3 (C_3), 65.2, 60.5−58.9, 57.0, 45.8, 32.1. Anal. ($C_{16}H_{22}N_3O_4Cl$).

[2-[(4-Amino-5-chloro-2-methoxybenzoyl)oxy]ethyl]pyridinium Bromide (8d). A solution of compound **10** (0.62 g, 2.0 mmol) in pyridine (20 mL) was refluxed for 5 h; 20 mL of ether was added to the cooled reaction mixture, and the crude compound **8d** was collected by filtration. Recrystallization from EtOH/*i*-Pr_2O gave **8d** (0.6 g, 78%) as a white solid: mp 223 °C; ^{1}H NMR (D_2O) δ 8.75 (m, 2H, pyridinyl H), 8.35 (m, 1H, pyridinyl H), 7.83 (m, 2H, pyridinyl H), 7.01 (s, 1H, ArH),

6.0 (s, 1H, ArH), 4.7 (m, 2H), 4.4 (m, 2H), 3.4 (s, 3H, OCH_3). Anal. ($C_{15}H_{16}N_2O_3ClBr$).

[2-(4-Amino-5-chloro-2-methoxybenzoxy)ethyl]quinuclidinium Bromide (8e). A solution of compound **10** (1.24 g, 4.0 mmol) and quinuclidine (0.44 g, 4.0 mmol) in THF (25 mL) was refluxed for 24 h. The solution was cooled and filtered to give **8e** (1.4 g, 71%) after recrystallization from EtOH/*i*-Pr_2O: mp >260 °C; ^{1}H NMR (CD_3OD) δ 7.64 (s, 1H, ArH), 6.38 (s, 1H, ArH), 4.54 (m, 2H, OCH_2), 3.7 (s, 3H, OCH_3), 3.54 (m, 8H), 2.08 (m, 1H), 1.95 (m, 6H). Anal. ($C_{17}H_{24}N_2O_3ClBr$).

2-(8-Azabicyclo[3.2.1]octan-8-yl)ethyl 4-Amino-5-chloro-2-methoxybenzoate (8g). It was prepared according to method B from compound **10** and 8-azabicyclo[3.2.1]octane. The crude product was purified by chromatography on silica gel (CH_2Cl_2/MeOH, 85:15) and by recrystallization from an AcOEt/hexane mixture to give **8g** (1.0 g, 59%): mp 122 °C; R_f 0.46 (CH_2Cl_2/MeOH, 85:15); ^{1}H NMR ($CDCl_3$) δ 8.00 (s, 1H, ArH), 6.46 (s, 1H, ArH), 4.79 (s, 2H, NH_2), 4.53 (t, J = 6.4 Hz, 2H, OCH_2), 3.99 (s, 3H, OCH_3), 3.45 (m, 2H), 2.89 (t, J = 6.4 Hz, 2H, CH_2N), 2.2−1.5 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 164.5 (C=O), 160.1 (C_2), 147.8 (C_4), 133.1 (C_6), 109.7−109.6 (C_1−C_5), 98.1 (C_3), 64.0, 60.2, 55.9 (OCH_3), 51.1, 30.5, 26.3, 16.4. Anal. ($C_{17}H_{23}N_2O_3Cl$).

(*R*)-1-Azabicyclo[2.2.2]octan-3-yl 4-Amino-5-chloro-2-methoxybenzoate (9c). It was prepared from 5-chloro-4-(tritylamino)-2-methoxybenzoic acid imidazolide which was synthesized according to the following process: Carbonyldiimidazole (3.2 g, 20 mmol) in dry THF (40 mL) was added under an inert atmosphere to a solution of the benzoic acid derivative **11** (4.44 g, 10 mmol) in 200 mL of dry THF. The reaction mixture was refluxed for 3 h, and the solvent was removed *in vacuo*. The residue was taken up with ether and filtrated to give 3.77 g (74%) of product as a white powder: ^{1}H NMR ($CDCl_3$) δ 7.73 (s, 1H, ArH), 7.28 (s, 1H), 7.24 (s, 15H, tritylH), 7.21 (s, 1H), 6.95 (s, 1H), 6.21 (br s, 1H, NH), 5.60 (s, 1H, ArH), 2.98 (s, 3H, OCH_3).

(*R*)-3-Quinuclidinol (0.13 g, 1 mmol) and DBU (0.15 mL, 1 mmol) were added dropwise to a solution of 0.51 g (1 mmol) of the previous compound in dry THF. The reaction mixture was refluxed overnight. The solvent was removed *in vacuo*, and the residue was taken up in CH_2Cl_2 and washed with water. The organic layer was dried over $MgSO_4$ and evaporated to dryness to give a solid which was purified by chromatography on silica gel and eluted with a $CHCl_3$/MeOH (95:5) mixture to give the pure *R*-tritylated benzoate derivative (64%). The ester (0.8 g, 1.44 mmol) was dissolved in pure $CHCl_3$, and 2 equiv of concentrated HCl was added. After stirring for 2 h at 25 °C, the solvent was removed *in vacuo* and the corresponding amino derivative was purified on a silica gel column and eluted with $CHCl_3$/MeOH (80:20) to give the pure hydrochloride salt **9c** (60%): mp 244 °C; TLC R_f (on silica gel) 0.28 (CH_2Cl_2/MeOH, 80:20); $[\alpha]_{546} = -36.8°$ (c = 1.0, MeOH); ^{1}H NMR (CD_3-OD) δ 7.91 (s, 1H, ArH), 6.64 (s, 1H, ArH), 5.31 (m, 1H), 4.18 (m, 1H), 3.98 (s, 3H, OCH_3), 3.6−3.4 (m, 5H), 2.6−2.1 (m, 5H). Anal. ($C_{15}H_{19}N_2O_3Cl$·HCl).

(*S*)-1-Azabicyclo[2.2.2]octan-3-yl 4-Amino-5-chloro-2-methoxybenzoate (9d). 9d was prepared from (*S*)-3-quinuclidinol (1 mmol) as described for **9c.** After the column chromatography purification, the pure hydrochloride salt was obtained (59%): mp 240 °C; $[\alpha]_{546} = +40.6°$ (c = 1.0, MeOH); ^{1}H NMR (CD_3OD) δ 7.91 (s, 1H, ArH), 6.64 (s, 1H, ArH), 5.31 (m, 1H), 4.18 (m, 1H), 3.98 (s, 3H, OCH_3), 3.6−3.4 (m, 5H), 2.6−2.1 (m, 5H). Anal. ($C_{15}H_{19}N_2O_3Cl$·HCl).

2-(Dimethylamino)ethyl 4-Amino-5-chloro-2-methoxybenzoate (9a). The compound was prepared according to the previous process reported for **9c** from 2-(dimethylamino)-ethanol and the benzoic acid **11**. It was isolated as a hydrochloride salt after recrystallization from the *i*-Pr_2O/MeOH mixture (global yield 13%): mp 230 °C; ^{1}H NMR ($CDCl_3$) δ 7.82 (s, 1H, ArH), 6.47 (s, 1H, ArH), 4.54 (m, 2H, OCH_2), 3.8 (s, 3H, OCH_3), 3.49 (m, 2H, N-CH_2), 2.94 (s, 6H, $N(CH_3)_2$). Anal. ($C_{12}H_{18}N_2O_3Cl$·$2H_2O$).

8-Methyl-8-azabicyclo[3.2.1]octan-3-yl 4-Amino-5-chloro-2-methoxybenzoate (9e). It was prepared according to the previous process reported for **9c** from tropine and the benzoic acid **11** with modifications. BuLi in THF at −10 °C was used in place of DBU. Compound **9e** was isolated as a hydrochloride salt after recrystallization from the MeOH/*i*-Pr_2O mixture (global yield 19%): mp >260 °C; ^{1}H NMR (CD_3OD) δ 7.71 (s, 1H, ArH), 6.51 (s, 1H, ArH), 5.21 (m, 1H), 4−3.85 (m, 2H), 3.82 (s, 3H, OCH_3), 2.82 (s, 3H, N-CH_3), 2.65−2.3 (m, 6H), 2.27−2.07 (m, 2H). Anal. ($C_{16}H_{21}N_2O_3Cl$·HCl·H_2O).

4-Amino-5-chloro-2-methoxy-*N*-methyl-*N*-(7-piperidinoethyl)benzamide Hydrochloride (9g). Triethylamine (1 g, 9.9 mmol) was added to a suspension of 4-amino-5-chloro-2-methoxybenzoic acid (2 g, 9.9 mmol) in dry THF (100 mL). The mixture was heated to dissolve the reactants and cooled to between −5 and −10 °C. Ethyl chloroformate (1.07 g, 9.9 mmol) was slowly added, and the mixture was stirred for 30 min. A solution of *N*-methyl-2-(1-piperidinyl)ethylamine (1.54 g, 9.9 mmol) in dry CH_2Cl_2 (5 mL) was added slowly, the temperature was allowed to rise to 25 °C, and the mixture was left stirring overnight. After evaporation of the solvent, the residue was taken up with water and extracted with CH_2Cl_2. The organic layers were washed with NaOH (4%) and brine and then dried over Na_2SO_4 and evaporated to afford a pale yellow oil which was purified by column chromatography on silica gel by eluting with CH_2Cl_2/MeOH (90:10) to afford 0.62 g (19%) of the free base. Treatment of the base with a 2 N HCl/MeOH solution and precipitation by *i*-Pr_2O give a solid which was recrystallized from the *i*-Pr_2O/MeOH mixture to yield 0.38 g (11%) of the hydrochloride salt: mp 254−256 °C; R_f 0.098 (CH_2Cl_2/MeOH, 90:10); ^{1}H NMR (CD_3OD) δ 7.19 (s, 1H, ArH), 6.53 (s, 1H, ArH), 3.87 (3.87, J = 6 Hz, 3H), 3.8 (s, 3H, OCH_3), 3.68 (m, 2H), 3.37 (t, J = 6 Hz, 3H), 3.06 (m, 2H), 2.96 (s, 3H, N-CH_3), 2.1−1.4 (m, 6H). Anal. ($C_{16}H_{24}O_2ClN_3$·HCl·$0.5H_2O$).

4-Amino-5-chloro-*N*-[2-(dimethylamino)ethyl]-2-methoxybenzamide Hydrochloride (9b). This compound was synthesized from *N*,*N*-dimethylethylenediamine (0.88 g, 0.01 mol) according to the previous method described for **9g**. The free base was treated with a solution of HCl (3 N) in AcOEt to give **9b** as a hydrochloride salt (2.3 g, 77%): mp 220 °C; ^{1}H NMR (CD_3OD) δ 7.8 (s, 1H, ArH), 6.65 (s, 1H, ArH), 3.8 (s, 3H, OCH_3), 3.6 (t, J = 5.7 Hz, 2H), 3.2 (t, J = 5.7 Hz, 2H), 2.8 (s, 6H, CMe_2). Anal. ($C_{12}H_{18}N_3O_2Cl$·HCl).

4-Amino-5-chloro-*N*-[2-(3,5-dimethylpiperidino)ethyl]-2-methoxybenzamide (9h). A mixture of 2-bromoethylamine hydrobromide (2.05 g, 10 mmol), the commercially-available mixture of *cis*- and *trans*-3,5-dimethylpiperidine (2.26 g, 20 mmol), and K_2CO_3 (1.38 g, 10 mmol) in MeOH (20 mL) was stirred for 3 h at room temperature. After evaporation of MeOH, the residue was dissolved in CH_2Cl_2 and water. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the solvent under reduced pressure afforded *cis,trans*-1-(2-aminoethyl)-3,5-dimethylpiperidine as a colorless oil, which was then diluted with THF. This solution was added dropwise to a mixture of Et_3N (1.01 g, 10 mmol), $ClCO_2Et$ (1.08 g, 10 mmol), and 4-amino-5-chloro-2-methoxybenzoic acid (2.01 g, 10 mmol) in THF (50 mL) at 0 °C for 30 min. The reaction mixture was warmed to room temperature and stirred for 4 h. The solvent was removed, and the residue was partitioned between aqueous AcOEt and Na_2CO_3 solutions. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was recrystallized from ether to yield **9h** (0.8 g, 24%) as a mixture of the *cis*/*trans* isomers (70:30): mp 178−179 °C; ^{1}H NMR ($CDCl_3$) δ 8.2 (m, 1H, CONH), 8.1 (s, 1H, ArH), 6.2 (s, 1H, ArH), 4.4 (s, 2H, NH_2), 3.8 (s, 3H, OCH_3), 3.5 (m, 2H, $NHCH_2$), 2.8 (m, 1.4H_{eq}, *cis*-2,6-piperidinyl H), 2.55−2.35 (m, 2H, CH_2N), 2.3−1.2 (m, 6H), 0.95 (d, J = 6 Hz, 1.8H, *trans*-dimethyl), 0.85 (d, J = 6 Hz, 4.2H, *cis*-dimethyl), 0.55 (m, 0.7H_{ax}, 4-piperidinyl H); ^{13}C NMR ($CDCl_3$) δ 164.6 (C=O), 157.8 (C_2), 146.9 (C_4), 133.2 (C_6), 112.9−111.6 (C_1−C_5), 98.1 (C_3), 61.6 and 61.1, 57.4 and 57.0, 56.2 (OCH_3), 42.5, 39.4, 37.0, 31.7 and 27.7, 19.8 and 19.4. Anal. ($C_{17}H_{26}N_3O_2Cl$).

4-Amino-5-chloro-*N*-(2-piperidinoethyl)-2-methoxybenzamide (9f). This compound was synthesized from 2-piperidinoethylamine and 4-amino-5-chloro-2-methoxybenzoic acid according to the process described for **9g** and isolated as an amorphous solid (0.6 g, 27%): ^{1}H NMR ($CDCl_3$) δ 8.2 (br s, 1H, CONH), 8.08 (s, 1H, ArH), 6.3 (s, 1H, ArH), 4.4 (s, 2H,

NH_2), 3.85 (s, 3H, OCH_3), 3.5 (m, 2H, $NHCH_2$), 2.48 (t, $J = 6$ Hz, 2H), 2.4 (m, 4H, CH_2N), 1.35−1.65 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 164.32 (C=O), 157.6 (C_2), 146.48 (C_4), 133.02 (C_6), 113.04−111.54 (C_1−C_5), 97.97 (C_3), 57.25, 56.01, 54.28, 36.66, 26.31, 24.48. Anal. ($C_{15}H_{22}N_3O_2Cl$).

5-Chloro-2-methoxy-4-(tritylamino)benzaldehyde (12). **11** (14 g, 32 mmol) in anhydrous THF (150 mL) was added dropwise to a magnetically-stirred suspension of $LiAlH_4$ (1.63 g, 43 mmol) in anhydrous THF (60 mL) cooled with an ice bath. After the end of the addition, the mixture was left at room temperature for 4 h. The excess of $LiAlH_4$ was quenched by the successive dropwise additions of 1.63 mL of water, 1.63 mL of 15% aqueous NaOH, 1.5 mL of water, and solid $MgSO_4$. The solution was filtered through Celite, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on neutral alumina by eluting successively with CH_2Cl_2 and CH_2Cl_2/MeOH (95:5) to yield 8.09 g (59%) of 5-chloro-2-methoxy-4-(tritylamino)benzyl alcohol as a white foaming solid: R_f 0.62 (CH_2Cl_2/MeOH, 95:5); ^{1}H NMR ($CDCl_3$) δ 7.4−7.18 (m, 15H, tritylH), 7.09 (s, 1H, ArH), 5.82 (br s, 1H, NH), 5.67(s, 1H, ArH), 4.42 (d, 2H, CH_2OH), 3.14 (s, 3H, OCH_3), 1.97 (t, 1H, OH). Anal. ($C_{27}H_{24}NO_2Cl$).

MnO_2 (1.4 g, 16.2 mmol) was added to a solution of the alcohol prepared above (0.7 g, 1.62 mmol) in anhydrous CH_2Cl_2 (20 mL). The reaction mixture was stirred at room temperature for 24 h. Then the suspension was filtered through Celite and the filtrate concentrated *in vacuo*. The yellow foamy solid obtained was recrystallized from hexane/CH_2Cl_2 to give 0.59 g (86%) of **12** as white-yellow prisms: mp 198 °C; R_f 0.8 (CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 10 (s, 1H, CHO), 7.73 (s, 1H, ArH), 7.4−7.18 (m, 15H, tritylH), 6.4 (br s, 1H, NH), 5.58 (s, 1H, ArH), 3.17 (s, 3H, OCH_3). Anal. ($C_{27}H_{22}NO_2Cl$).

1-[5-Chloro-2-methoxy-4-(tritylamino)phenyl]-4-piperidinobutanol (13). Magnesium turnings (0.34 g, 14 mmol) were covered with 3 mL of dry THF under an Ar atmosphere. Iodine crystals and 1 or 2 drops of ethyl bromide were added. As soon as the reaction had started, the suspension was heated at 60 °C and 1-(3-chloropropyl)piperidine (2.27 g, 14 mmol) in 3 mL of dry THF was added dropwise to maintain gentle reflux. When the addition was over, the reaction mixture was refluxed with stirring for 30 min and cooled to 0 °C. Compound **12** (3 g, 6.97 mmol) was added portionwise, and the mixture was heated at 60 °C for 24 h. The reaction mixture was hydrolyzed by a solution of 16.5 g of ammonium chloride in 40 mL of water. The organic layer was separated, the aqueous phase was extracted with ether (3 × 60 mL), and the combined extracts were dried over $MgSO_4$. After concentration *in vacuo*, the residue was purified by column chromatography on neutral alumina with AcOEt as the eluant to give **13** as a foamy white solid (1.7 g, 44%): R_f (neutral alumina) 0.43 (AcOEt); ^{1}H NMR ($CDCl_3$) δ 7.4−7.18 (m, 16H, ArH, tritylH), 5.7 (br s, 1H, NH), 5.61 (s, 1H, ArH), 4.7−4.6 (m, 1H, ArCHO), 3.08 (s, 3H, OCH_3), 2.7−2.6 (m, 6H), 1.9−1.3 (m, 10H). Anal. ($C_{35}H_{39}N_2O_2Cl\cdot 0.5H_2O$).

1-(5-Chloro-2-methoxy-4-aminophenyl)-4-piperidinobutanone (14). MnO_2 (2.6 g, 30 mmol) was added to the previous alcohol **13** (1.7 g, 3 mmol) in 50 mL of dry CH_2Cl_2. The mixture was stirred overnight at room temperature and heated at 40−50 °C for 7 days in the presence of an additional quantity of MnO_2 (2.6 g). The mixture was filtered through Celite and purified on neutral alumina using AcOEt as the eluant to yield a yellow-orange solid (1.6 g). The compound was dissolved in a mixture of 40 mL of dry acetone with 2.1 equiv of 12.4 N HCl and stirred at room temperature for 2 h. After evaporation of the solvent, the ketone was converted into the hydrochloride salt with a 2 N HCl/MeOH solution and precipitated by the addition of *i*-Pr_2O. It was recrystallized from *i*-Pr_2O/MeOH to afford 0.26 g (25%) of a clear pink solid: mp 242−244 °C; R_f 0.074 (CH_2Cl_2/MeOH, 90:10); ^{1}H NMR (CD_3OD) δ 7.64 (s, 1H, ArH), 6.37 (s, 1H, ArH), 3.78 (s, 3H, OCH_3), 3.4−2.8 (m, 6H), 2.05−1.86 (m, 2H, $ArCOCH_2$), 1.86−1.4 (m, 6H); ^{13}C NMR (CD_3OD) δ 198.07 (C=O), 162.06, 152.06, 132.608, 117.3, 111.77, 98.03, 58.09, 56.19 (OCH_3), 54.37, 41.048, 24.35, 22.76, 20.12; IR (cm^{-1}, CH_2Cl_2) 1620 (C=O); MS (CI, NH_3) *m/z* 312 (MH^+) (free base, M = 311). Anal. ($C_{16}H_{23}N_2O_2Cl\cdot HCl\cdot 0.25H_2O$).

Conformational Studies. Conformational studies were performed using the SYBYL 6.03 and 6.1 programs of Tripos. Energies were calculated using the Tripos force field and MOPAC charges. Current minimizations were performed using a gradient termination of 0.05 kcal mol^{-1}. Conformational random search studies were performed using 1000 cycles and an energy cutoff of 30 kcal mol^{-1}. Conformational grid searches were performed with rotational steps of 20°.

X-ray Crystallography. A selected crystal was set up on a Nonius CAD4 automatic diffractometer. Unit cell dimensions with estimated standard deviations were obtained from least-squares refinements of the setting angles of 25 well-centered reflections. Two standard reflections were monitored periodically and showed no change during data collection. Computations were performed using CRYSTALS (University of Oxford, U.K.). The structures were solved by a direct method using the SHELX 86 (University of Göttingen, Germany) program and successive Fourier maps. For compounds **7a**,**g**, all the hydrogen atoms could be found on difference maps. Non-hydrogen atoms were not refined, and they were given overall isotropic thermal parameters. Full matrix least-squares refinements were carried out by minimizing the function $\sum w(|F_o| - |F_c|)^2$, where F_o and F_c are the observed and calculated structure factors. Unit weight was used. Models reached convergence with $R = \sum(||F_o| - |F_c||)/\sum|F_o|$ and $R_w = [\sum w(|F_o| - |F_c|)^2/\sum w(F_o)^2]^{1/2}$ having values of 0.054 and 0.052, respectively, for **7a** and values of 0.044 and 0.046, respectively, for **7g**. Criteria for a satisfactory complete analysis were the ratios of the rms shift to standard deviation being less than 0.1 and no significant features in the final difference map.

7a: $C_{15}H_{21}N_2O_3Cl\cdot HCl$, MW = 349.26, crystals are parallelepiped and colorless and belong to the monoclinic system, space group *P*21/*c*; cell parameters, $a = 12.490$ Å, $b = 10.805$ Å, $c = 12.838$ Å; $\beta = 92.53°$, $V = 1731$ Å^3, $d = 1.34$ g/cm^3.

7g: $C_{17}H_{25}N_2O_3Cl\cdot HCl$, MW = 377.31, crystals are parallelepiped and colorless and belong to the monoclinic system, space group *P*21/*c*; cell parameters, $a = 6.887$ Å, $b = 19.926$ Å, $c = 14.219$ Å; $\beta = 96.52°$, $V = 1948$ Å^3, $d = 1.29$ g/cm^3.

Tables of atomic coordinates, bond distances, and bond angles are available from the authors.

Pharmacological Methods. 1. 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.

2. 5-HT$_3$ Receptors. Membranes were prepared from rat posterior cortex according to the procedure described by Hall.[49] [^{3}H]BRL 43694 (61 Ci/mmol) was purchased from NEN Research Products. GR 38032F was a generous gift from Glaxo (U.K.). All other chemicals and reagents were commercially available from Sigma. The binding of 1.2 nM [^{3}H]-BRL 43694 ($K_D = 1.5$ nM, $B_{max} = 30$ fmol/mg of protein for 5-HT$_3$ receptors) was measured using membranes (100 μL aliquots equivalent to 0.95 mg of protein) suspended in a final volume of 0.5 mL of 50 mM Hepes buffer, pH 8.4, and incubated at 25 °C for 30 min; 7−11 concentrations of each drug were used in triplicate. Nonspecific binding was determined by the addition of 10 μM GR 38032F in duplicate and represented less than 50% of the total binding. Total binding was defined in quadruplicate.

3. 5-HT$_4$ Receptors. Membranes were prepared from rat striatum and olfactory tubercules which were pooled separately and stored at −80 °C. Tissues were thawed at 0 °C and homogenized in 15 vol 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 vol of Hepes 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 described[12a] previously with modifications. The binding of [^{3}H]-GR 113808 (85 Ci/mmol; purchased from 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) and incubated at 25 °C for 30 min. Seven concentrations of each drug were used, and the assay was done in triplicate. Nonspecific binding was defined with 10 μM **7a** 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 poly(ethylene) 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.

4. Protein Estimation. The protein concentration of the rat posterior cortex membranes was determined by the method of Lowry[50] using bovine serum albumin as the standard. The protein concentrations of the rat striatum and olfactory tubercle membrane preparations were determined by the method of Bradford.[51]

5. 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). According to Craig and Clarke,[4a] segments (2 cm long, 10 cm from the ileocecal 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 1.5 ms) were recorded by means of an isotonic transducer connected to a polygraph (Gemini, Basile, Italy). The preparations were subjected to 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 concentration−effect curves to 5-HT (contact time about 60 s) were obtained. After two fully reproducible cumulative concentration−effect curves to 5-HT, a concentration−effect curve to the 5-HT_4 receptor agonist under test was carried out (contact time 60−120 s) and related to the maximal effect of 5-HT (100%). In antagonist studies, the antagonist was added 30 min before the last agonist concentration−effect curve, several concentrations of antagonists were tested, and the IC_{50} value was calculated.

Rat Esophageal Tunica Muscularis Mucosae. Tissues were obtained from male Crl (CD) Br rats (225−250 g). According to Baxter and Clarke,[4a] the rat esophageal segment (2 cm of intrathoracic esophagus, proximal to the diaphragm) was removed and the outer muscle layers were carefully dissected away. The segment, i.e., the tunica muscularis mucosae, was mounted, under 1 g of tension, in a tissue bath containing a warm (37 °C), aerated (95% O_2, 5% CO_2) Krebs solution, and contractions were recorded by means of an isotonic transducer connected to a polygraph. After 60 min of equilibration, tissues were incubated with 10^{-4} M pargyline (30 min). In order to reach a steady state of tonus, preparations were contracted with cumulative submaximal concentrations of carbachol (10^{-6}−4×10^{-6} M). In control experiments, three fully reproducible contractions to carbachol and then three concentration−effect curves to 5-HT (inhibition of carbachol contractions) were carried out (contact time about 60 s). After two reproductible cumulative concentration−effect curves to 5-HT, a concentration−effect curve to the 5-HT_4 receptor agonist under test (contact time 60−120 s) was carried out cumulatively and related to the maximal effect of 5-HT (100%). In antagonist studies, the compound was added 30 min before the last agonist concentration−effect curve.

The pA_2 values, as defined by Arunlakshana and Schild,[41a] were obtained from the plot (Cr − 1) vs negative log of the antagonist concentration. The computer analyses were done as described by Tallarida and Murray.[41b]

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