The Serotonin 5-HT$_4$ Receptor. 1. Design of a New Class of Agonists and Receptor Map of the Agonist Recognition Site

Karl-Heinz Buchheit, Rainer Gamse, Rudolf Giger, Daniel Hoyer, Francois Klein, Edgar Klöppner, Hans-Jürgen Pfannkuche, and Henri Mattes*

Preclinical Research, Sandoz Pharma Limited, CH-4002 Basel, Switzerland

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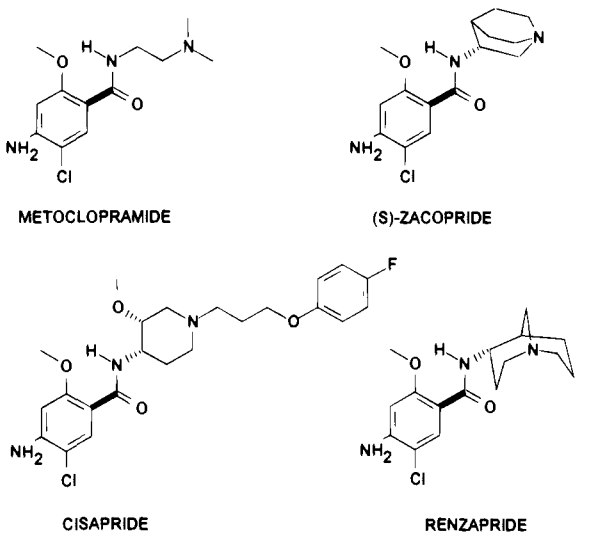
The design and synthesis of a new class of potent and selective 5-HT$_4$ receptor agonists containing an indole nucleus linked to a carbazimidamide are presented. A conformational study of the 5-HT$_4$ receptor agonists serotonin and zacopride led to the identification of an initial pharmacophore and to the definition of a three-dimensional map of the 5-HT$_4$ agonist recognition site. **1**, a representative member of our new class of 5-HT$_4$ receptor agonists, incorporates all reference structural features and matched perfectly with these models. **1** is a highly potent, full agonist at 5-HT$_4$ receptors present in the isolated electrically stimulated guinea pig ileum preparation, with a pD_2 value of 8.8, displaying selectivity (ranging from 40- to over 10 000-fold) versus other members of the serotonin receptor family.

Substantial biochemical, pharmacological, and histochemical evidence has been accumulated over the last 35 years in support for a role of serotonin (5-HT) as a neurotransmitter, neuromodulator, and hormone.[1] The largest amount of 5-HT in the body is found in the gut and more precisely in the enterochromaffin (EC) cells of the gastrointestinal mucosa.[2] Its role in the gut is complicated by the presence of multiple 5-HT receptor subtypes.[3,4] The inhibitory 5-HT$_{1A}$[5] receptor is present on enteric neurones. The 5-HT$_{2A}$ receptor, mediating contractions, has been identified on smooth muscle.[6] A ligand-gated ion channel termed 5-HT$_3$ has been found on ganglia.[7] Finally, the excitatory 5-HT$_4$[8] receptor is located on neurones,[9] smooth muscle cells,[10] and secretory cells.[11]

We report here the design and the pharmacological evaluation of a new, selective 5-HT$_4$ receptor agonist as well as a receptor map[12] of the 5-HT$_4$ agonist recognition site. This program was initiated, several years ago, by the observation that the activity of prokinetic agents of the benzamide class (cisapride, metoclopramide, zacopride, renzapride) (Chart 1) can be attributed to agonism at the 5-HT$_4$ receptor.[8,13] Another class of compounds derived from benzimidazolones and exemplified by BIMU-8[14] was later shown to act as agonists at this 5-HT$_4$ receptor. On the other hand, no potent and selective 5-HT$_4$ receptor antagonist was known at that time.

Receptors of the 5-HT$_4$ type have been identified both peripherally and centrally.[9,15] In the periphery, the pharmacology of the 5-HT$_4$ receptor has been characterized by using the electrically stimulated[9] or nonstimulated[18] guinea pig ileum longitudinal muscle preparation, the recently developed tunica muscularis mucosae preparation of rat esophagus,[10] and the guinea pig ascending[16] and distal colon longitudinal muscle-myenteric plexus preparations.[17] Agonists at the 5-HT$_4$ receptors cause contractions of the guinea pig ileum and colon mediated by activation of the cholinergic system, enhancements of "twitch" responses in electrically stimulated guinea pig ileum, and relaxation of the muscularis mucosae preparation. They have also been implicated in certain cardiac effects[19] and in the release of corticotropin-releasing factor (CRF).[20] In the central nervous system (CNS), 5-HT$_4$ agonists have been shown to activate adenylate cyclase[15] and induce an increase in EEG energy.[21] The clinical use of currently available drugs (benzamide class) acting at this receptor has been hampered by their modest potency, efficacy, and/or lack of selectivity. The design of potent and selective ligands for the 5-HT$_4$ receptor could therefore open the door to new therapies for gastrointestinal as well as central nervous diseases.

Chart 1



Drug Design

Due to its potent agonism at the 5-HT$_4$ receptor (vide infra), serotonin appeared as a promising starting point for the design of new ligands for this receptor. The strategy used followed three main principles. We desired to conformationally restrict the alkylamine side chain to produce agents more selective and potent than serotonin. We also wanted to replace the primary amine with a basic function displaying increased stability toward metabolic degradation, and finally we intended

* Author for correspondence.
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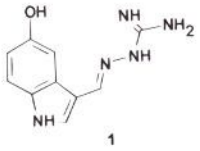
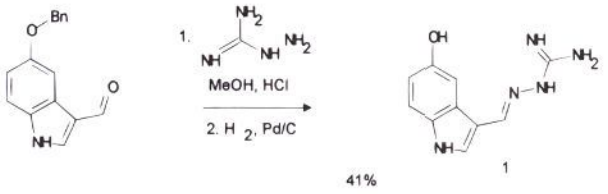
Chart 2. Structure of **1**



Scheme 1. Preparation of **1**



to implement high polarity in order to avoid penetration in the central nervous system. Among several candidates, the carbazimidamide **1**[22] (Chart 2) emerged as an interesting lead.

Chemistry. Our effort toward the synthesis of **1** is outlined in Scheme 1. Condensation of 5-(benzyloxy)-1*H*-indole-3-carboxaldehyde with aminoguanidine under acidic conditions in methanol followed by cleavage of the benzyl group afforded compound **1** in 41% overall yield.

Receptor Mapping

The strategy used has been described previously[12] and consisted of the following steps: (a) definition of the features of a common pharmacophore, (b) search for low-energy conformations, (c) superposition of these conformations via their common pharmacophore points, and (d) mapping of the recognition site. The basis of this study was laid by establishing the 5-HT$_4$ receptor agonist properties of standard compounds, namely, zacopride, cisapride, and renzapride (representing the benzamide class), and 5-HT. All compounds are agonists at the 5-HT$_4$ receptor and are thus anticipated to bind at the same site of the receptor.

Definition of the Pharmacophore. As can be seen in Chart 1, zacopride and serotonin display fairly different structures. A structure–activity relationship (SAR) based on different derivatives of both compounds led us to define three elements as the basic pharmacophore, namely, an aromatic ring, a basic nitrogen, and a hydrogen-bond donor–acceptor function (phenol or ether). The spatial relationship between these elements was then built using the SYBYL molecular modeling package (Tripos) in the following way.

Two conformational isomers of zacopride (Chart 3) were built, and the lowest energy conformers were searched with MAXIMIN. These two conformers differ in the torsion angle around the highlighted bond, one having a possible H-bond between the ether oxygen and the amide NH group. Both structures can adopt different conformations by modifying the torsion angles of their rotatable bonds. These bonds were moved freely in increments of 5° using the SEARCH module, and the internal energy for each conformation was calculated. At the same time the distances O_1-N_2, N_3-N_2, and N_2-center of the aromatic ring were registered (Chart 3, Table 1). Low-energy conformations of renzapride and cisapride were similarly determined, and the obtained distance maps were within the range reported for zacopride.

Chart 3

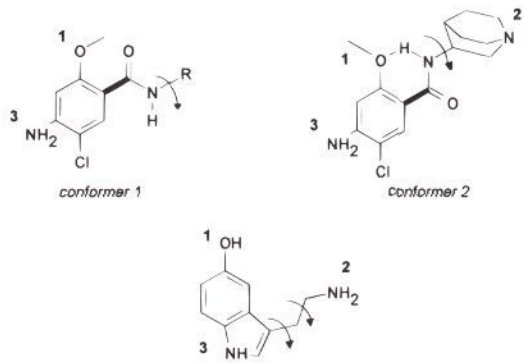


Table 1. Relative Energies and Distance Map[a]

	distances			
	O_1-N_2[b]	N_2-center[c]	N_2-N_3[d]	E[e]
zacopride (conf1)	6.8–7.4	7.1–7.4	9.8–10.1	2.6
zacopride (conf2)	5.4–5.9	7.1–7.5	9.8–10.1	1.2
serotonin	6.3–8.2	5.6–6.6	5.6–5.9	4.3

[a] Distances are given in angstroms. [b] Distance of O_1-N_2. [c] Distance of N_2-center of the aromatic ring. [d] Distance of N_1-N(aniline or indole). [e] Energy differences between the lowest and highest energy conformer in kcal/mol; conf: conformation.

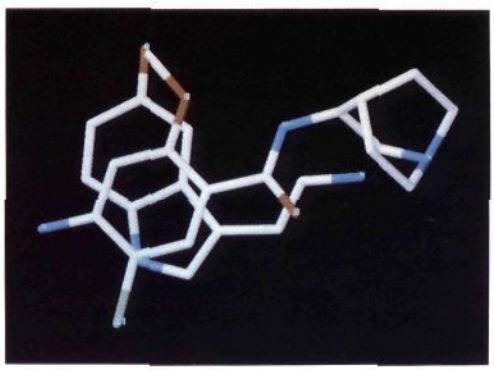


Figure 1. Superposition of zacopride and serotonin.

A *crystallographic structure of serotonin*[23] was utilized for further modeling. Low-energy conformations of serotonin were determined as described above using the SEARCH module to generate a distance map and an energy profile for all rotatable bonds (Table 1).

A comparison of the distance maps generated for serotonin and zacopride, as a prototype of the benzamide class, showed clearly that the distances from the center of the aromatic ring to N_2 and from N_2 to N_3 were much longer in zacopride (respectively 7.1–7.4 and 9.8–10.1 Å) than in the lower energy conformers of serotonin (respectively 5.6–6.6 and 5.6–5.9 Å). This observation led us to conclude that both compound classes were binding in a different mode to the 5-HT$_4$ receptor.

We then tried to overlap all low-energy conformers of both molecules paying attention to the vectors defined by the nitrogen lone pair. On the basis of energetic values and lone pair vector alignments, the best overlap is shown in Figure 1. The basic nitrogen of serotonin and that of zacopride are 2.0 Å apart in that overlap. The distance between the oxygens of a carboxylic acid being 2.3 Å, we hypothesized that the molecules were binding to different mesomeric forms or different conformations of a carboxylic acid, the putative counterpart. The position of the amino substituent of zacopride enables hydrogen bonding with the same H-bond ac-

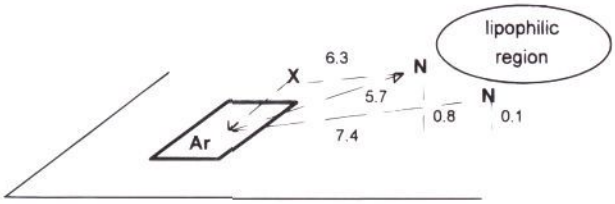


Figure 2. Pharmacophore of the 5-HT_4 agonist recognition site. Distances are expressed in angstroms. X represents a H-bond donor group.

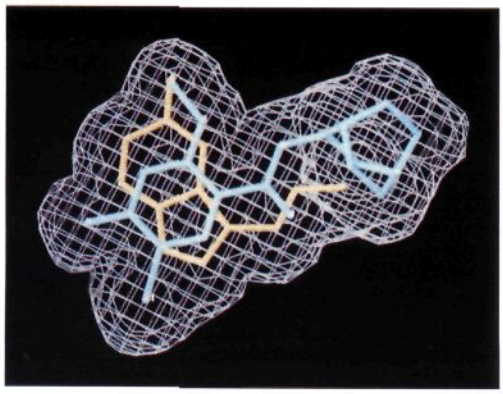


Figure 3. Accessible volume depicted in wireframe (white) for agonists in the 5-HT_4 recognition site. Serotonin (orange) and zacopride (cyan) are represented.

ceptor potentially binding the indole NH of serotonin. The fact that no function in zacopride could be matched with the 5-OH group of 5-HT was accounted for the lower affinity of zacopride for the 5-HT_4 receptor.

Receptor Map. A three-dimensional map of the 5-HT_4 agonist recognition site is proposed in Figure 2. This model defines the pharmacophore as a basic nitrogen which is either 5.7 or 7.4 Å from the center of an aromatic group and respectively 0.8 or 0.1 Å below (or above) the plane defined by this group. High affinity is given by a hydrogen-bond donor group, located in the plane defined by the aromatic ring, placed at 6.3 or 7.6 Å from the basic nitrogen. An important secondary lipophilic binding site, able to accommodate the large N-substituent of cisapride or the bicyclic ring of renzapride and zacopride, is located near the basic nitrogen recognition site.

A van der Waals surface was created corresponding to the envelope of the superimposed agonists in their possibly active conformations. The volume thus created defined the space accessible in theory to agonists in the 5-HT_4 recognition site (Figure 3). This volume does not include the large lipophilic region needed to accommodate the N-substituent of cisapride. This region clearly represents an important secondary binding site whose clear definition needs the study of a larger compound collection.[24]

Superposition of 1 with the Receptor Map. According to our model, one aromatic ring as well as a basic nitrogen was needed for affinity and a phenol or hydrogen-bond donor group was required for high affinity. Figure 4 highlights the perfect match of **1** with these reference structural features. The compound moreover incorporates two potential binding groups, embedded in a guanidine function, near the respective positions of the basic nitrogen of zacopride and serotonin

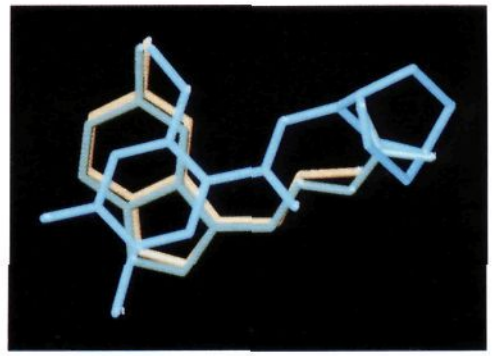


Figure 4. Superposition of **1** (green) with zacopride (cyan) and serotonin (orange).

Table 2. 5-HT_4 Receptor Agonist Activities of **1** and Reference Substances[a]

	pD_2 (SEM)	intrinsic activity[b]
serotonin	8.4 (0.1)	1.0
metoclopramide	5.6 (0.1)	1.0
cisapride	6.0 (0.1)	0.7
(*S*)-zacopride	6.8 (0.1)	1.9
1[c]	8.8 (0.2)	1.5

[a] Assay conditions: ability of compounds to enhance the "twitch" response in field-stimulated guinea pig ileum preparations (see the Experimental Section for further details). [b] Relative to serotonin = 1.0. [c] Direct comparison of potency values (compound **1**, pD_2 = 8.83 ± 0.35, vs 5-HT, pD_2 = 8.34 ± 0.33) via Student's unpaired *t*-test revealed a *p*-value of 0.07 in parallel experimental determinations (n = 3).

and could therefore interact in a twin nitrogen–twin oxygen fashion with the putative carboxylic acid counterpart.

Results and Discussion

5-HT_4 Receptor-Mediated Activity. Serotonin 5-HT_4 receptor agonism was measured in the electrically stimulated longitudinal muscle preparations of the guinea pig ileum. 5-HT causes an augmentation of contractions of the guinea pig ileum induced by field stimulation through activation of the 5-HT_4 receptor. 5-HT is a potent agonist in this assay, exhibiting a pD_2 value of 8.4 in the field-stimulated preparation (see Table 2). Moderate to weak activities were found for the substituted benzamides cisapride (pD_2 = 6.0), metoclopramide (pD_2 = 5.6), and (*S*)-zacopride (pD_2 = 6.8). We found that compound **1** is a very potent agonist in these models, exhibiting a pD_2 value of 8.8 with an efficacy of 1.5 as compared to serotonin in the field-stimulated preparation. It is thus 2.5 times more potent than 5-HT in this assay. Recently a substituted pyrrolizidine (SC-53116)[25] has been reported to surpass the potency and selectivity of other benzamides as 5-HT_4 receptor agonists. This compound, however, only approaches the potency of 5-HT in the rat esophagus in vitro preparation. Since 5-HT, metoclopramide, and (*S*)-zacopride display potencies[25,26] in the rat esophagus comparable with their potencies in the field-stimulated guinea pig ileum preparation, one could assume that compound **1** is at least 5 times more potent than SC-53116 acting as a 5-HT_4 receptor agonist.

Selectivity. Many previously reported 5-HT_4 receptor agonists, based on a benzamide nucleus, possess strong 5-HT_3, 5-HT_2, dopamine, and other monoamine receptor binding properties. (*S*)-Zacopride exhibits strong interaction (K_i = 9.5 nM) at the 5-HT_3 receptor

Table 3. Receptor Profiles of Compound 1, Metoclopramide, Cisapride and (*S*)-Zacopride (Potencies[a] are expressed as pD_2 values (5-HT$_4$ agonism); receptor binding results[b] are given as pK_D values (mean ± SEM); additionally, relative receptor potencies/affinities as compared to the compound's 5-HT$_4$ receptor potencies (=1.0) are indicated in italic/bold)

	1	metoclopramide	cisapride	(*S*)-zacopride
5-HT$_4$	8.8 ± 0.2	5.6 ± 0.1	6.0 ± 0.1	6.8 ± 0.1
	1	***1***	***1***	***1***
5-HT$_{1A}$	7.2 ± 0.1	5.1 ± 0.1	5.7 ± 0.1	<5
	0.03	***0.32***	***0.50***	***0.02***
5-HT$_{2A}$	6.6 ± 0.2	5.4 ± 0.2	8.1 ± 0.1	<5
	0.007	***0.63***	***125.9***	***<0.02***
5-HT$_3$	<5[c]	6.7 ± 0.1	7.2 ± 0.1	9.5 ± 0.2
	<0.001	***12.58***	***15.85***	***501.2***
D$_1$	5.4 ± 0.1	<5	5.2 ± 0.1	<5
	<0.001	***<0.25***	***0.16***	***<0.02***
D$_2$	<5	7.1 ± 0.1	6.5 ± 0.1	<5
	<0.001	***31.6***	***3.16***	***<0.02***
α_1	5.5 ± 0.1	<5[d]	7.5[d]	<5
	<0.001	***<0.25***	***31.62***	***<0.02***

[a] 5-HT$_4$ receptor agonism was determined in the guinea pig ileum assay.[27] [b] Tissues and [^{3}H]radioligands used in binding assays:[28] 5-HT$_{1A}$ (pig cortex, 8-OH-DPAT), 5-HT$_{2A}$ (rat cortex, ketanserin), 5-HT$_3$ (mouse N1E-115, tropisetron), D$_1$ (calf striatum, SCH 23390), D$_2$ (calf striatum, spiperone), and α_1 (calf cortex, prazosin). [c] pA_2 value determined in a functional 5-HT$_3$ receptor assay (guinea pig ileum). [d] cf. ref 25.

subtype (see Table 3). Cisapride, on the other hand, interacts with 5-HT$_2$ (K_i = 7.2 nM) and α-adrenergic (K_i = 30 nM) receptors. Metoclopramide finally shows interactions with the 5-HT$_3$ receptor subtype (K_i = 200 nM) as well as with the D$_2$ receptor subtype (K_i = 80 nM). We therefore investigated the binding profile of our new 5-HT$_4$ receptor agonist, in comparison with the standard compounds, in various known receptor binding assays.

Compound **1** exhibited a moderate affinity (K_i = 63 nM) for 5-HT$_{1A}$ receptors while showing only weak binding affinities in all other radioligand-binding assays (K_i's ranging from 250 to over 10 000 nM). Compound **1** thus represents a fairly selective 5-HT$_4$ receptor agonist.

Conclusion

We report here the design of one of the most potent and selective serotonin 5-HT$_4$ receptor agonists known to date as well as a three-dimensional map of the 5-HT$_4$ agonist recognition site. Derivatives of compound **1** have been synthesized, and they also displayed the expected activity with increased potency and selectivity for the serotonin 5-HT$_4$ receptor subtype. Synthesis as well as detailed analyses of structure–activity relationships will be described soon.

Experimental Section

Computational Analyses. Molecular modeling was performed using the SYBYL 5.5 software package (Tripos Assoc., St. Louis, MO) on a VAX 8530 workstation. Zacopride and **1** were built within SYBYL, and bond angles and lengths were optimized with the MAXIMIN program. The atomic coordinates from serotonin, taken from X-ray data, were optimized using MAXIMIN. Hydrogen atoms were included during the optimization process. Atomic point charges were calculated by using the Gasteiger–Hueckel method. A cutoff of 8 Å and a minimum energy change of 0.00001 kcal were used as the termination criteria for minimizations. Conformational analyses were performed by employing the SEARCH option within SYBYL. The defined rotatable bonds were rotated with a stepwise increment of 5°. The various energy minima available to each molecule as well as selected distances were recorded. Low-energy conformers were then overlapped, by using all the possible reference pairs, with the FIT command of SYBYL.

Syntheses. Melting points were determined with a Buchi 535 apparatus (capillary method) and are uncorrected. ^{1}H NMR spectra were recorded on a AM-360 Bruker spectrometer. Flash chromatography columns were run on silica gel (60 mesh). Analyses indicated by symbols were within 0.4% of the theoretical values.

3-[[5-(Benzyloxy)-1*H*-indol-3-yl]methylene]carbazimidamide Hydrochloride. Aminoguanidine hydrogen carbonate (2.8 g, 0.02 mol) was added portionwise to a solution of 5-(benzyloxy)-1*H*-indole-3-carbaldehyde (5.0 g, 0.02 mol; from Lancaster) in MeOH (100 mL). The solution was acidified (pH 3–4) with concentrated HCl (5 mL). After 2 h at reflux, the mixture was evaporated. The residue was taken up in MeOH (50 mL) and treated with a solution of etheral HCl (15 mL). The crystals were filtrated off and recrystallized from MeOH/Et_2O to yield the title compound (4.75 g, 69%): mp 260–262 °C; ^{1}H NMR (DMSO-d_6) δ 5.2 (s, 2H, OCH_2), 6.9–7.9 (m, 13H, ArH + NH), 8.3 (s, 1H, CHN), 11.7 (br s, 2H, NH); MS *m/e* 307 (M^+). Anal. ($C_{17}H_{17}N_5O$·HCl) C, H, N, Cl.

3-[(5-Hydroxy-1*H*-indol-3-yl)methylene]carbazimidamide Hydrochloride (1). Pd/C (200 mg) was added to a solution of 3-[[5-(benzyloxy)-1*H*-indol-3-yl]methylene]carbazimidamide (1.5 g, 0.005 mol) in MeOH (50 mL). The mixture was hydrogenated at room temperature for 24 h. The catalyst was filtrated off, and the solvent was evaporated. The residue was treated with a methanolic HCl solution. The solvent was evaporated, and the residue was crystallized from EtOH/hexane to yield **1** (710 mg, 59%): mp 245–247 °C; ^{1}H NMR (DMSO-d_6) δ 5.2–5.8 (m, 5H, NH), 6.6 (d, J = 9 Hz, 1H, HC6), 7.15 (d, J = 9 Hz, 1H, HC7), 7.4 (s, 1H, HC2), 7.6 (s, 1H, HC4), 8.2 (s, 1H, CHN), 11.0 (br s, 1H, NH); MS *m/e* 217 (M^+). Anal. ($C_{10}H_{11}N_5O$·HCl) C, H, N, Cl.

Biological Activities. 5-HT$_3$ Receptor Antagonism. The guinea pig longitudinal muscle with adhering 'plexus myentericus' was prepared as described before.[27] Small strips (2 cm) of the preparation were mounted in an organ bath containing tyrode solution at 37 °C and bubbled with 5% CO_2 in O_2. The tyrode solution contained 0.1 μM methylsergide. 5-HT$_3$ receptor-mediated contractions were measured isotonically, and concentration–response curves were recorded in a noncumulative fashion. When compound **1** was tested against serotonin, a 10 min preincubation procedure was performed.

5-HT$_4$ Receptor Agonism. Longitudinal muscle strips from guinea pig ileum were prepared and maintained as described previously.[27] The tyrode solution contained 0.1 μM methylsergide. "Twitch" responses (rapid contractions lasting 2–3 s) were evoked using square wave pulses (0.1 Hz, 2 ms pulse duration) delivered from a Grass S48 stimulator via platinum electrodes situated on either side of a muscle strip. After obtaining a stable submaximal response, concentration–response curves using putative 5-HT$_4$ receptor agonists were constructed in a noncumulative fashion.

Binding Experiments. The radioligand-binding experiments have been performed as previously described.[28] Radioligands used in the binding assays were 8-OH-DPAT (pig cortex), ketanserin (rat cortex), tropisetron (mouse N1E-115), SCH 23390 (calf striatum), spiperone (calf striatum), and prazosin (calf cortex) for 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_3$, D$_1$, D$_2$, and α_1 receptors, respectively.

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