

A Multivalent Approach to the Design and Discovery of Orally Efficacious 5-HT₄ Receptor Agonists

R. Murray McKinnell,^{*,†} Scott R. Armstrong,[‡] David T. Beattie,[‡] Seok-Ki Choi,[†] Paul R. Fatheree,[†] Roland A. L. Gendron,[†] Adam Goldblum,[†] Patrick P. Humphrey,[†] Daniel D. Long,[†] Daniel G. Marquess,[†] J. P. Shaw,[§] Jacqueline A. M. Smith,^{||} S. Derek Turner,[†] and Ross G. Vickery^{||}

[†]Department of Medicinal Chemistry and [‡]Department of Pharmacology and [§]Department of Drug Metabolism and Pharmacokinetics and ^{||}Department of Molecular and Cellular Biology, Theravance, Inc., 901 Gateway Boulevard, South San Francisco, California 94080

Received June 16, 2009

5-HT₄ receptor agonists such as tegaserod have demonstrated efficacy in the treatment of constipation predominant irritable bowel syndrome (IBS-C), a highly prevalent disorder characterized by chronic constipation and impairment of intestinal propulsion, abdominal bloating, and pain. The 5-HT₄ receptor binding site can accommodate functionally and sterically diverse groups attached to the amine nitrogen atom of common ligands, occupying what may be termed a “secondary” binding site. Using a multivalent approach to lead discovery, we have investigated how varying the position and nature of the secondary binding group can be used as a strategy to achieve the desired 5-HT₄ agonist pharmacological profile. During this study, we discovered the ability of amine-based secondary binding groups to impart exceptional gains in the binding affinity, selectivity, and functional potency of 5-HT₄ agonists. Optimization of the leads generated by this approach afforded compound **26**, a selective, orally efficacious 5-HT₄ agonist for the potential treatment of gastrointestinal motility-related disorders.

Introduction

Serotonin (5-hydroxytryptamine, 5-HT^a) mediates its diverse pharmacological functions via activation of at least seven 5-HT receptor families (5-HT₁–5-HT₇, with multiple subtypes for the majority), differentiated on the basis of their location, structure, function, and transductional pathways.^{1,2} In mammals, approximately 95% of 5-HT in the body is localized in the gastrointestinal tract, primarily in enterochromaffin cells. The 5-HT₄ receptor was discovered in cultured mouse embryonic colliculi neuronal cells,³ and its expression has since been demonstrated in a range of human tissues, including the brain,⁴ gut,⁵ heart,⁶ adrenal cortex,⁷ and bladder.⁸ The 5-HT₄ receptor belongs to the superfamily of seven-transmembrane G protein-coupled receptors (GPCRs), which, together with its potential role in many central and peripherally mediated disorders (irritable bowel syndrome,⁹ gastroparesis,¹⁰ Alzheimer's disease,¹¹ arrhythmia¹²), has made it an attractive target for drug discovery.

Of relevance to the treatment of gastrointestinal disorders was the initial observation by Craig and Clark that the chloroaniline class of 5-HT₄ agonists such as Renzapride (Figure 1) activates the peristaltic reflex of the guinea pig

ileum and colon.¹³ Subsequently, the role of 5-HT₄ agonist activity in promoting gastrointestinal motility has become more fully appreciated. For example, activation of 5-HT₄ receptors on motoneurons and interneurons within the gut wall is associated with facilitation of cholinergic and nonadrenergic, noncholinergic neurotransmission. Release of acetylcholine, substance P, and calcitonin gene-related peptide results in the coordinated propulsion of contents along the gastrointestinal tract.¹⁴ Additionally, 5-HT₄ receptors are expressed on smooth muscle cells; their activation results in, for example, esophageal relaxation in rats¹⁵ and inhibition of human colonic circular muscle contractile activity.¹⁶ Furthermore, activation of 5-HT₄ receptors on enteric neurons or enterocytes promotes fluid secretion in the gastrointestinal tract in a variety of species, including humans.¹⁷

Consequently, much effort has been devoted to the discovery and development of selective 5-HT₄ receptor agonists for the treatment of chronic constipation and constipation-predominant irritable bowel syndrome (IBS-C), a highly prevalent disorder characterized by impairment of intestinal propulsion, abdominal bloating, and discomfort.¹⁸ Cisapride, a 5-HT₄ receptor agonist (albeit with significant activity at other 5-HT receptor subtypes), was frequently prescribed for this indication but was withdrawn in 2000 following its association with an increased risk of mortality as a consequence of inhibition of the human ether-à-go-go-related gene (hERG) potassium ion channel.¹⁹ Prucalopride, a highly selective 5-HT₄ agonist, was shown in several clinical trials to significantly increase the frequency of bowel movements, improve stool consistency, and accelerate colonic transit in constipated patients.^{10,20} Prucalopride was recently licensed by Movetis from Janssen with the objective of gaining registration in Europe.²¹ Tegaserod, an indole-based 5-HT₄

*To whom correspondence should be addressed. Phone: +1-650-808-6039. Fax: +1-650-808-6120. E-mail: mmckinnell@theravance.com.

^aAbbreviations: IBS-C, constipation predominant irritable bowel syndrome; 5-HT, 5-hydroxytryptamine; GPCR, G-protein coupled receptor; hERG, human ether-à-go-go-related gene; HEK, human embryonic kidney; GR113808, 1-methyl-1*H*-indole-3-carboxylic acid 1-(2-methanesulfonylamino-ethyl)-piperidin-4-ylmethyl ester; GR65630, 3-(4-methyl-1*H*-imidazol-5-yl)-1-(1-methyl-1*H*-indol-3-yl)-1-propanone; cAMP, cyclic adenosine monophosphate; SAR, structure–activity relationship; CHO, Chinese hamster ovary; RLM, rat liver microsome; AUC, area under the curve; *F*, oral bioavailability.

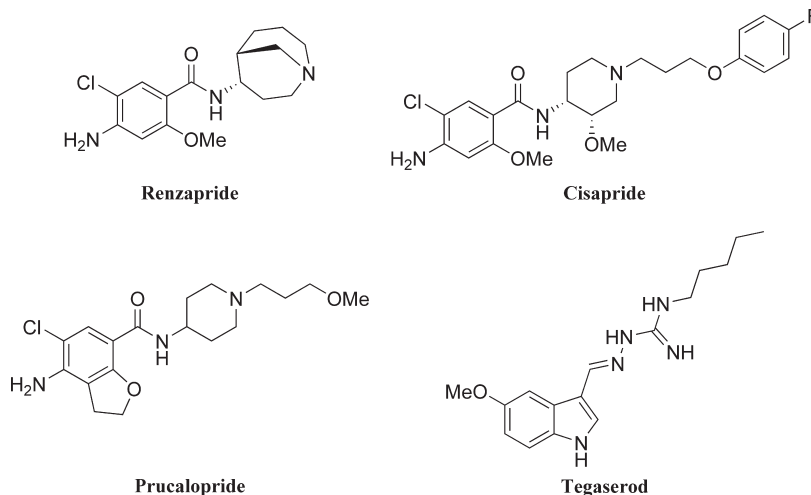


Figure 1. 5-HT₄ agonists.

receptor agonist, was approved in the United States for IBS-C and chronic constipation and has been shown to reduce total colonic transit time in healthy subjects²² as well as improving multiple symptoms in patients with IBS-C.²³ Tegaserod has an oral bioavailability of 10% in humans,²⁴ is dosed twice-daily,²⁵ and is subject to a significant negative food effect on its oral absorption.²³ Tegaserod also lacks selectivity for the 5-HT₄ receptor, having significant affinity for a number of other 5-HT receptor subtypes (i.e., 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2B}, and 5-HT₇).²⁶ Recently, tegaserod was removed from the U.S. market on the basis of a possible association with ischemic cardiovascular events.²⁷

Herein, we describe the application of a multivalent approach to the discovery and optimization of potent, selective, and orally bioavailable 5-HT₄ agonists for the treatment of gastrointestinal disorders characterized by reduced motility.

Chemical Strategy

Multivalent interactions have been defined as those instances where a ligand has two or more exclusive binding domains that can bind to two or more distinct sites either on the same receptor or on two distinct receptors.^{28,29} This mode of binding can improve the ligands association/dissociation equilibrium at a particular receptor and also allow the ligand to better differentiate between two or more closely related receptors. Thus, a multivalent approach, when applied to the design of bioactive molecules, can afford compounds with enhanced potency and selectivity for the desired target. In the most simple case, a molecule can consist of two distinct binding groups ("primary" and "secondary"), which bind to proximal primary and secondary binding sites, respectively. In the case of 5-HT₄ receptor ligands, the primary binding group may be regarded as an appropriately substituted aromatic system connected through a coplanar amide bond to a conformationally restricted amine. Suitable aromatic systems include 4-amino-5-chloro-2-methoxy benzoic acid,³⁰ *N*-alkyl quinolones,³¹ and *N*-alkyl indazoles,³² among others. Multiple conformationally restricted amines have been employed, such as tropane,³² quinuclidine,³³ and pyrrolizidine.³⁴

In marked contrast to the majority of 5-HT₃ ligands, several 5-HT₄ ligands have demonstrated an ability to accommodate voluminous substituents attached to the amine nitrogen atom, occupying what may be described as a secondary binding site (Figure 2).³⁴ While much effort has focused on the optimization

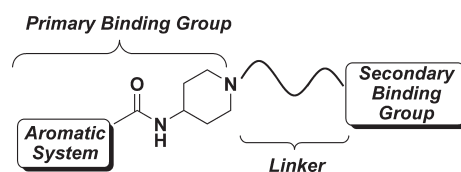


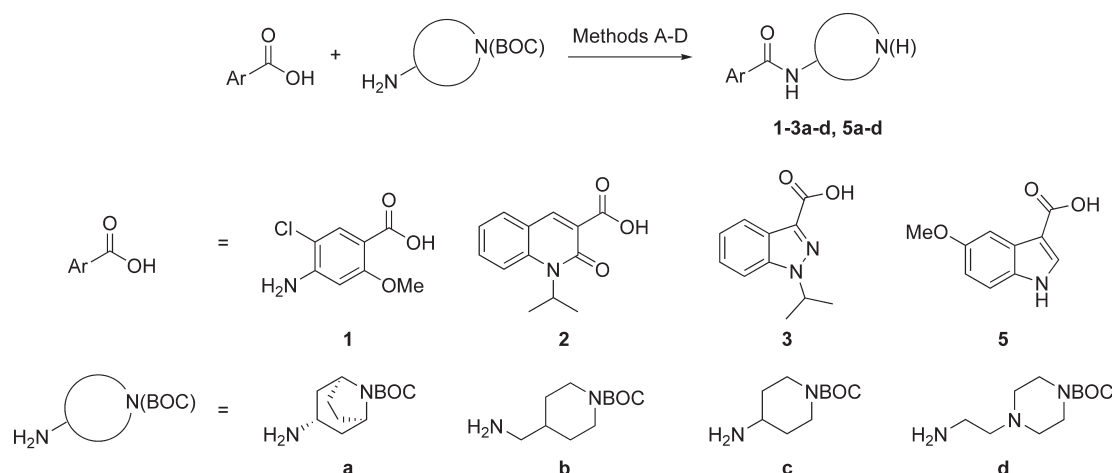
Figure 2. 5-HT₄ agonist pharmacophore.

of 5-HT₄ agonist primary binding groups,^{35,36} relatively few studies have focused on variation of the secondary binding group as a means to achieve the desired pharmacokinetic and pharmacological profile for an orally bioavailable prokinetic agent.^{37,38} In light of this, a multivalent approach to the lead discovery efforts was adopted in which functionally diverse secondary binding groups were attached via linkers of variable length to selected 5-HT₄ agonist primary binding groups, with the goal of enhancing their potency, oral pharmacokinetics, and selectivity over the 5-HT₃ receptor (5-HT₃ antagonism is known to reduce gastrointestinal motility in man and may partially attenuate the clinical benefit of 5-HT₄ agonists).³⁹ In addition, secondary binding groups, which resulted in minimal inhibition of the hERG potassium ion channel, were targeted, as this would potentially minimize the risk of the cardiac arrhythmia issues associated with cisapride.

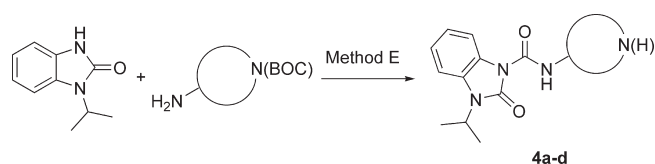
Chemistry

Synthesis of Primary Binding Groups. For the synthesis of primary binding groups **1–3a–d** and **5a–d**, the requisite aromatic systems were prepared using literature procedures and coupled to commercially available BOC-protected diamines using one of several standard amide bond-forming methods, followed by removal of the BOC group using trifluoroacetic acid (Scheme 1). In the case of benzimidazolones **4a–d**, 1-isopropyl-1,3-dihydro-2*H*-benzimidazol-2-one was first converted to an activated carbamate using *p*-nitrophenylchloroformate then treated with the appropriate amine (Scheme 2).

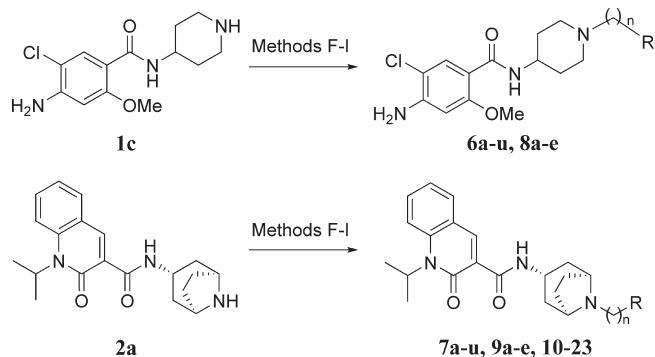
Attachment of Linkers and Secondary Binding Groups. Compounds **6a–l** and **7a–l** were furnished by simple *N*-alkylation of compounds **1c** and **2a**, respectively, using commercially available alkyl bromides and potassium iodide in dimethylformamide (method F, Scheme 3). Compounds that contained amines as secondary binding groups (**6m–u**, **7m–u**, **8a–e**, **9a–e**, and **10–23**) were synthesized by one of

Scheme 1^a

^a Reagents and conditions: Method A: (a) CDI, DMF; (b) TFA, DCM. Method B (a) EDC, *i*Pr₂NEt, DMF; (b) TFA, DCM. Method C: (a) EDC, HOBT, *i*Pr₂NEt, DMF; (b) TFA, DCM. Method D: (a) SOCl₂, NaOH, PhMe, H₂O (b) TFA, DCM.

Scheme 2^a

^a Reagents and conditions: mMethod E: (a) NaH, THF; (b) *p*-NO₂C₆H₄OCOC₂H₅, THF; (c) TFA, DCM.

Scheme 3^a

^a Reagents and conditions: Method F: (a) Br(CH₂)_nR, KI, NaHCO₃, DMF, H₂O. Method G: (a) R₁R₂NH, Br(CH₂)_nBr, KI, NaHCO₃, DMF, H₂O. Method H: (a) R₁R₂NH, Br(CH₂)_nBr, *i*Pr₂NEt, MeOH. Method I: (a) Br(CH₂)_nOH, Et₃N, MeCN; (b) PySO₃, *i*Pr₂NEt, DMSO, CH₂Cl₂; (c) R₁R₂NH, NaBH(OAc)₃, MeOH.

three methods. In methods G and H, an equimolar mixture of the primary binding group and secondary binding group was reacted with 1 equiv of alkyl dibromide. The desired products could easily be separated from the homodimeric byproduct by preparative HPLC. In method I, compounds **1c** and **2a** were first alkylated with commercially available bromoalkylalcohols. The primary alcohol was then oxidized by sulfur trioxide–pyridine complex to the aldehyde, which then underwent smooth reductive amination using the required amine and sodium triacetoxyborohydride.

Compounds **24–26** were made by treatment of piperazine *N*-methylsulfonamide with racemic or enantiopure epichlorohydrin to form an intermediate chloro-alcohol, which upon

treatment with sodium hydroxide underwent ring closure to form 1-methylsulfonyl-4-(oxiranymethyl)piperazine. This was then treated with compound **2a** to afford the desired products (Scheme 4).

Results and Discussion

Selection of Primary Binding Groups. In this multivalent approach, the first goal was to identify suitable primary binding groups from which to rapidly access and explore the putative secondary binding site. As such, a systematic *in vitro* evaluation of the relative binding affinity (*pK_i*), functional potency (*pEC₅₀*), and intrinsic activity (IA) of a range of primary binding groups at the human recombinant 5-HT₄ receptor was initiated. The choice of aromatic systems encompassed chloroaniline (**1**), quinolone (**2**), indazole (**3**), benzimidazolinone (**4**), and indole (**5**) ring systems, coupled through amide bonds to four representative amines {(3-amino-8-methyl-8-azabicyclo[3.2.1]octane (**a**), 4-(aminomethyl)piperidine (**b**), 4-aminopiperidine (**c**), and 4-(2-aminomethyl)piperazine (**d**)}.

As can be seen from Table 1, a wide range of *in vitro* profiles was observed. In general, these simple primary binding groups had higher binding affinity for the 5-HT₃ receptor than the 5-HT₄ receptor. Several compounds (**3b**, **5c**, **5d**) were antagonists at the 5-HT₄ receptor and were removed from further consideration. Of the remaining compounds, those with intrinsic activity greater than 90% were prioritized. Several compounds met these criteria (**1c–d**, **2a–c**, **3a**, **4a–d**) and will be the subject of future publications. This publication will focus on the elaboration compounds **1c** and **2a**, which were adequately representative of major chloroaniline and *N*-alkylheteroaromatic classes of 5-HT₄ agonist.

Exploring the Secondary Binding Site. To rapidly assess the electronic, steric, and positional preferences of the putative secondary binding site, we designed a selection of secondary binding groups which encompassed neutral, acidic, and basic functionality and which were capable of varying degrees of hydrogen bonding and aromatic interactions. These were linked to the secondary amine of the primary binding group through simple alkyl chains of two, five, and eight carbon lengths (Table 2), which allowed evaluation of the role of distance between the primary and secondary binding groups.

Scheme 4^a

^a Reagents and conditions: (a) *rac*-epichlorohydrin (**24**) or (*R*)-epichlorohydrin, (**25**) or (*S*)-epichlorohydrin (**26**), EtOH; (b) NaOH; (c) **2a**, *rac*-1-methylsulfonyl-4-(oxiranylmethyl)piperazine (**24**), or (*R*)-1-methylsulfonyl-4-(oxiranylmethyl)piperazine (**25**), or (*S*)-1-methylsulfonyl-4-(oxiranylmethyl)piperazine (**26**), EtOH, 80 °C.

Table 1. 5-HT₄ Agonist Primary Binding Groups

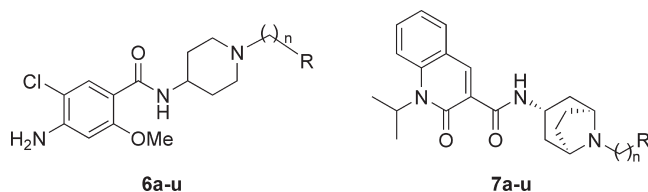
R	Compound Number	Method	5-HT ₄ pK _i ^a	5-HT ₃ K _i /5-HT ₄ K _i	5-HT ₄ pEC ₅₀ ^c	5-HT ₄ I.A. ^d
	1a	A	6.8	0.2	7.0	57
	1b	B	6.5	0.12	6.4	81
	1c	A	5.8	0.095	6.4	>100
	1d	C	5.9	1.1	6.3	>100
	2a	D	6.6	0.85	7.9	>100
	2b	D	6.1	1.2	7.0	>100
	2c	D	5.5	0.14	6.8	>100
	3a	A	7.2	0.092	7.4	94
	3b	B	8.1	3	1.0	0
	3c	A	6.4	0.52	6.7	50
	4a	E	6.5	0.013	7.8	>100
	4b	E	7.4	0.21	7.8	92
	4c	E	5.8	0.019	6.5	>100
	4d	E	6.9	1	8.1	>100
	5a	C	5.5	1.4	5.6	77
	5b	C	5.4	1.1	5.2	42
	5c	C	5.5	6.7	1.0	0
	5d	C	6.5	25	1.0	0

^a 5-HT₄ pK_i values were determined using [³H]-GR113808 in HEK-293 cells stably transfected with human 5-HT_{4(c)} receptor cDNA; 5-HT₃ pK_i values were determined with [³H]-GR65630 in HEK-293 cells stably transfected with human 5-HT_{3A} receptor cDNA. ^b Selectivity for the 5-HT₄ receptor subtype with respect to the 5-HT₃ receptor subtype was calculated as the ratio K_i(5-HT_{3A})/K_i(5-HT_{4(c)}). ^c pEC₅₀ determined using whole-cell cAMP accumulation studies in HEK-293 cells stably transfected with the human recombinant 5-HT_{4(c)} receptor splice variant. ^d Maximum compound-evoked response (minus basal) was expressed as a percentage of the maximum response evoked by 5-HT.

Because selectivity over 5-HT₃ was considered essential, affinity for this receptor was included in the primary screen together with functional potency and intrinsic activity at the 5-HT₄ receptor.

Attaching a simple alkyl as a secondary binding group in both the chloroaniline and quinolone series (**6a–c**, **7a–c**) had the expected orthogonal effect on binding affinity for the 5-HT₃ and 5-HT₄ receptors, with the resultant improvement in selectivity in favor of 5-HT₄, and this effect was of greater

magnitude with the longer C5 and C8 chains. Functional potencies revealed the first contrasting SAR between the two series, with the alkyl chain imparting an approximately 10-fold gain in the chloroanilines **6a–c** compared to a 0.5 log unit reduction observed with the quinolones. Intrinsic activity was essentially unchanged in both series. A more polar secondary binding group (alcohols **6d–f**, **7d–f**) had no significant effect on the 5-HT₄ pK_i or pEC₅₀ relative to the simple alkyl chain, indicating a lack of significant interactions of

Table 2. 5-HT₄ Agonist Secondary Binding Group Scan

Compound	<i>n</i>	Synthetic Procedure	R	5-HT ₄ pK _i ^a	5-HT ₃ K _i / 5-HT ₄ K _i ^b	5-HT ₄ pEC ₅₀ ^c	5-HT ₄ I.A. ^d
1c	-	-	-	5.8	0.095	6.4	>100
2a	-	-	-	6.6	0.85	7.9	>100
6	a	2	F	6.0	4.2	7.1	>100
	b	5	F	7.0	33	7.6	>100
	c	8	F	7.1	23	7.4	>100
	d	2	F	6.9	19	7.5	96
7	a	2	F	7.2	890	7.4	>100
	b	5	F	7.2	150	7.4	>100
	c	8	F	6.7	1.9	7.2	>100
	d	2	F	7.2	110	7.8	>100
6	e	5	F	7.1	29	7.8	>100
	f	8	F	6.9	3.2	7.5	>100
	g	2	F	7.2	460	7.6	>100
	h	5	F	7.3	1100	7.8	>100
7	i	8	F	5.1	13	6.2	94
	j	2	F	7.7	120	8.3	>100
	k	5	F	7.4	40	8.5	89
	l	8	F	6.0	55	7.1	>100
6	m	2	F	7.8	1200	7.6	>100
	n	5	F	7.5	120	7.3	>100
	o	8	F	4.6	1.5	5.8	>100
	p	2	F	5.2	0.15	6.4	93
7	q	5	F	4.8	4.3	6.0	>100
	r	8	F	6.0	49	8.2	>100
	s	2	F	5.8	21	7.9	>100
	t	5	F	7.2	230	7.7	>100
6	u	8	F	6.6	3.9	7.0	>100
	v	2	H	8.0	990	8.3	>100
	w	5	H	8.5	2000	8.5	>100
	x	8	H	7.1	720	8.0	>100
7	y	2	H	8.3	8100	9	>100
	z	5	H	8.5	9900	8.9	99
	aa	8	H	6.9	21	7.7	97
	ab	2	H	7.4	280	7.9	>100
6	ac	5	H	7.8	330	7.4	>100
	ad	8	H	7.2	450	8.2	>100
	ae	2	H	7.8	2800	8.9	>100
	af	5	H	7.8	2100	8.5	>100
7	ag	8	H	7.4	290	7.8	>100
	ah	2	H	8.8	12000	8.9	>100
	ai	5	H	8.9	2800	9.1	>100
	aj	8	H	7.4	480	8.9	92
6	ak	2	G	8.5	6000	9.2	>100
	al	5	G	8.5	2300	9.0	>100
	am	8	G				
	an		G				

^a 5-HT₄ pK_i values were determined using [³H]-GR113808 in HEK-293 cells stably transfected with human 5-HT_{4(c)} receptor cDNA; 5-HT₃ pK_i values were determined with [³H]-GR65630 in HEK-293 cells stably transfected with human 5-HT_{3A} receptor cDNA. ^b Selectivity for the 5-HT₄ receptor subtype with respect to the 5-HT₃ receptor subtype was calculated as the ratio K_i(5-HT_{3A})/K_i(5-HT_{4(c)}). ^c pEC₅₀ determined using whole-cell cAMP accumulation studies in HEK-293 cells stably transfected with the human recombinant 5-HT_{4(c)} receptor splice variant. ^d Maximum compound-evoked response (minus basal) was expressed as a percentage of the maximum response evoked by 5-HT.

this group with the secondary binding site. When the steric bulk of the secondary binding group was increased considerably (phthalimides **6g–i**, **7g–i**), there was a sharp drop in binding affinity in both series at the shortest linker length (**6g**, **7g**), due perhaps to inductive electron withdrawal from the amine of the primary binding group by the phthalimide or a limited amount of space available in this region of the receptor. At longer linker lengths, higher 5-HT₄ binding affinities and potencies were observed only with the chloroanilines (**6h–i**). Acidic secondary binding groups (sulfonic acids **6j–l**, **7j–l**), were not well tolerated in either series and led to a sharp reduction in binding affinity at both 5-HT₃ and

the 5-HT₄ receptors. Introduction of piperidine as a basic secondary binding group afforded a significant increase in the 5-HT₄ pK_i in both the chloroaniline (**6m–o**) and quinolone (**7m–o**) series relative to the simple alkyl chain derivatives. This effect was much more pronounced with the C5 and C8 linkers. Functional potency also benefited from the introduction of this second positively charged group, and for the first time an improvement in this property in the quinolone series was observed. Reducing the basicity of the secondary binding group by replacing the piperidine (calculated pK_a = 10.6) with a piperazinesulfonamide (calculated pK_a = 8.8, compounds **6p–r**, **7p–r**) appeared to

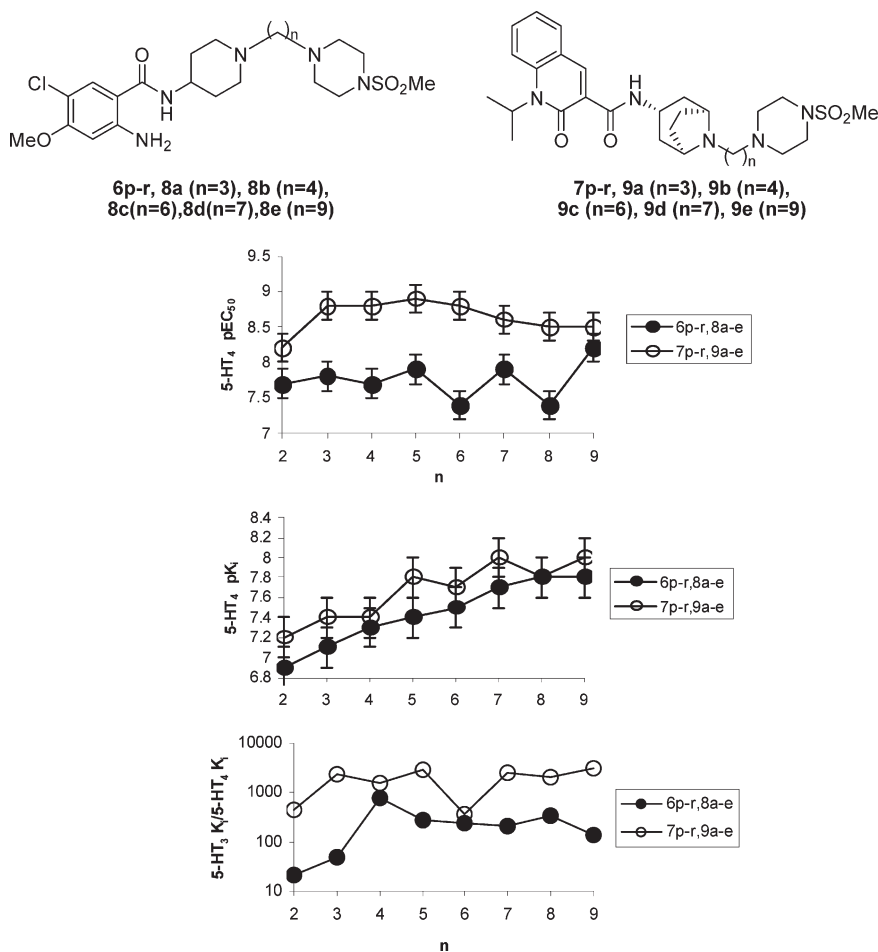


Figure 3. Effect of linker length on 5-HT₄ pK_i, pEC₅₀, and selectivity vs 5-HT₃.

partially negate this beneficial effect in both the C5 and C8 series, although this could also be due to negative steric interactions related to the increased bulk of the methylsulfonamide group. However, excellent binding affinity and potency could be restored by reverting to a bulky, strongly basic methyltryptamine system (calculated pK_a = 9.9, **6s–u**, **7s–u**), suggesting that the pK_a of the secondary binding group was the key factor in the observed improvements and that the positively charged secondary binding group was highly preferred in this region of the receptor.

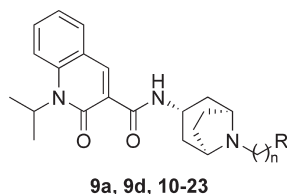
To more fully understand the positional preference of the positively charged secondary binding groups, the in vitro profile of the full set of linker lengths (C2 through C9) was examined using a representative piperazinesulfonamide (Figure 3). With the chloroaniline- and quinolone-piperazinesulfonamide systems (**6p–r**, **8a–e**, and **7p–r**, **9a–e**, respectively), the 5-HT₄ binding affinity generally increased as the distance between the primary and secondary binding groups increased, but functional potency remained essentially constant for all linker lengths. With respect to selectivity over the 5-HT₃ receptor, the quinolone-based agonists were superior to the chloroanilines at all linker lengths except C6, which had unusually high 5-HT₃ binding affinity.

Lead Optimization

Although at this juncture the ability of the amine-based secondary binding groups to consistently elevate the in vitro potency of 5-HT₄ agonists to desirable levels had been demonstrated, the study of the role of linker length had not afforded

a clear focal point for optimization efforts. Thus, attention focused on other “drug-likeness” features of these compounds, specifically permeability in the Caco-2 assay and hERG potassium ion channel inhibition. In light of the higher pK_i, selectivity, and pEC₅₀ observed with quinolone-based agonists, efforts were concentrated on this series. The set of amine-based secondary binding groups was expanded to cover a wide spectrum of calculated pK_as (Table 3), and these were attached via linkers of representative lengths (C3 and C7).

In general, the expanded set of amines followed the SAR trends described earlier for linker length, with 5-HT₄ pK_i increasing as the distance between the primary and secondary binding groups increased. Functional potency had a more complex relationship with linker length, at times increasing (**10**, **11**), remaining unchanged (**14**, **15**), or even declining (**18**, **19**) within a given series as the linker length increased. With respect to basicity, increasing the calculated pK_a of the tropane and secondary binding group (pK_{a1} and pK_{a2}, respectively), either by increasing the separation between them (to avoid inductive electron withdrawal) or employing a more basic secondary binding group (*N*-phenylpiperazine **18** vs morpholine **14**), generally afforded an increase in binding affinity and selectivity. This improvement was maintained with more basic cyclic amines **20** and **22** although the acyclic amine **16** did not follow this trend. Functional potency was independent of the pK_a of the secondary binding group, with excellent potencies being attainable even with relatively low pK_a secondary binding groups (**11**). Permeability (as measured by the Caco-2 assay) was low when highly flexible (**16**,

Table 3. Varying the pK_a of the Secondary Binding Group

Cmpd	<i>n</i>	Synthetic Proced.	R	Calc pK_a 1 ^a	Calc pK_a 2 ^b	5-HT ₄ pK_i ^c	5-HT ₃ K_i / 5-HT ₄ K_i ^d	5-HT ₄ pEC_{50} ^e	5-HT ₄ I.A. ^f	Caco-2 K _p (1×10 ⁻⁶ cm/s)	hERG % Inhibition ^g
10	3	G		8.7	6.0	7.3	1500	8.4	>100	2	12
11	7	G		9.7	8.0	8.7	20000	9.4	>100	9	7
12	3	G		8.7	6.3	7.4	1300	8.6	>100	31	75
13	7	G		9.7	8.3	8.1	810	8.5	>100	14	87
9a	3	G		8.7	6.8	7.4	2300	8.8	>100	20	38
9d	7	I		9.7	8.8	8.0	2600	8.6	>100	22	46
14	3	H		8.7	6.9	7.4	750	8.6	>100	22	80
15	7	I		9.7	8.8	8.7	1600	8.6	>100	28	34
16	3	G		8.7	7.7	6.7	210	8.0	>100	4	6
17	7	I		9.7	9.7	8.3	6500	9.2	96	3	12
18	3	H		9.0	7.7	8.1	1000	9.1	>100	22	90
19	7	H		10	9.7	8.4	5300	8.1	>100	20	97
20	3	H		9.4	7.7	8.2	9200	8.7	>100	24	39
21	7	H		10.4	9.7	8.6	10000	9.1	90	23	9
22	3	G		9.4	7.7	8.3	11000	9.0	>100	3	14
23	7	G		10.4	9.7	8.6	3100	9.2	98	2	5

^a Calculated pK_a of the tropane group. pK_a was calculated with the pKalc 3.2 prediction module of Pallas system (CompuDrug Chemistry, Ltd., Hungary). ^b Calculated pK_a of the secondary binding group amine. ^c 5-HT₄ pK_i values were determined using [³H]-GR113808 in HEK-293 cells stably transfected with human 5-HT_{4(c)} receptor cDNA; 5-HT₃ pK_i values were determined with [³H]-GR65630 in HEK-293 cells stably transfected with human 5-HT_{3A} receptor cDNA. ^d Selectivity for the 5-HT₄ receptor subtype with respect to the 5-HT₃ receptor subtype was calculated as the ratio $K_i(5-HT_{3A})/K_i(5-HT_{4(c)})$. ^e pEC_{50} determined using whole-cell cAMP accumulation studies in HEK-293 cells stably transfected with the human recombinant 5-HT_{4(c)} receptor splice variant. ^f Maximum compound-evoked response (minus basal) expressed as a percentage of the maximum response evoked by 5-HT. ^g hERG inhibition determined at a concentration of 3 μ M using Chinese hamster ovary (CHO) cells stably expressing hERG potassium channels.

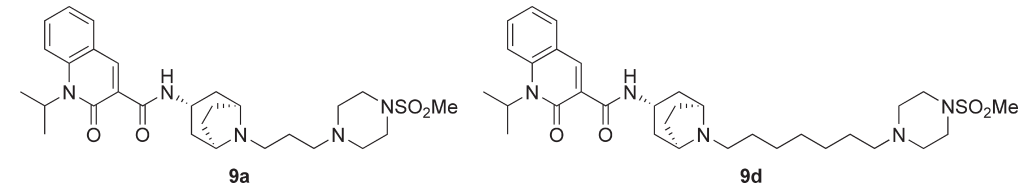
17) or H-bond donating secondary binding groups (**10**, **11**, **22**, **23**) were employed. However, with cyclic secondary binding groups, permeability was generally good, even with C7 linkers (**9d**, **15**) and highly basic amines (**18**, **20**).

Inhibition of the hERG channel was highly dependent on the nature of the secondary binding group, in some cases being consistently low (prolines **10**, **11**) or high (phenylpiperazines **18**, **19**) and having no clear relationship to calculated pK_a . When piperidine was employed as the secondary binding group, increasing the linker length successfully reduced hERG inhibition (**20**, **21**), while in the case of the piperazinesulfonamides (**9a**, **9d**), the opposite was true. Overall, hERG inhibition had a highly unpredictable relationship with linker length and the nature of the secondary binding group.

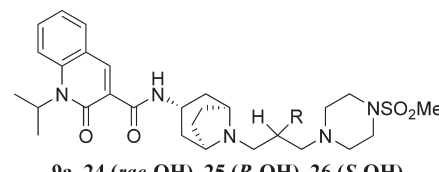
On the basis of the promising potency, good in vitro permeability and moderate hERG inhibition observed with compounds **9a** and **9d**, they were advanced into a rat oral pharmacokinetic study in order to establish the relationship between their in vitro permeability and metabolism and their in vivo pharmacokinetics.

The rat in vivo oral pharmacokinetic data for compounds **9a** and **9d** are shown in Table 4. Although both compounds had similar permeability in the Caco-2 assay and **9a** was less stable in the rat liver microsome (RLM) assay, the in vivo oral exposure of **9a** was superior to that of the C7 analogue **9d** as well as tegaserod. Thus, optimization efforts were focused on the C3 linked derivative **9a**, and in particular, modifications to the linker domain, through which improvements in the 5-HT₄ receptor binding affinity and reduced hERG inhibition were sought.

Introduction of a racemic alcohol (compound **24**, Table 5) to the central carbon of the C3 linker provided a convenient functional group handle with which to explore this domain. However, this simple modification itself led to a minor improvement in affinity and a significant reduction in hERG inhibition. When the single enantiomers **25** and **26** were isolated, the (*S*)-enantiomer was found to have significantly better affinity, selectivity over 5-HT₃, and functional potency relative to the (*R*)-enantiomer. With respect to selectivity over other 5-HT receptors, compound **26** displayed less than 50%

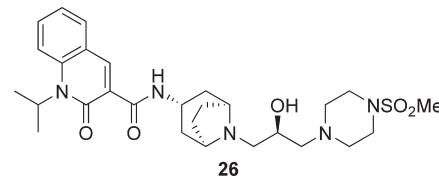
Table 4. Oral Pharmacokinetics of Compounds **9a** and **9d** in Rat


compd	Caco-2 K _p (1×10^{-6} cm/s)	RLM $t_{1/2}$ (min)	AUC _(0–t) ($\mu\text{g} \cdot \text{h/mL}$) ^a	C _{max} ($\mu\text{g/mL}$)	$t_{1/2}$ (h)	F% ^b
9a	20	41	0.183	0.034	7.9	38
9d	22	> 90	0.083	0.010	20.3	20
tegaserod	11	> 90	0.021	0.006	1.8	5

^a Pharmacokinetic properties were evaluated in male Sprague–Dawley rats dosed with test compounds via oral gavage (PO) at a dose of 5 mg/kg. $n = 3$.^b F = oral bioavailability, %.**Table 5.** In Vitro Data for Compounds **9a**, **24–26**


compd	general procedure	R	5-HT ₄ pK _i ^a	5-HT ₃ K _i /5-HT ₄ K _i ^b	5-HT ₄ pEC ₅₀ ^c	5-HT ₄ IA ^d	Caco-2 K _p (1×10^{-6} cm/s)	hERG % Inhibition ^e
9a	G	H	7.4	2300	8.8	> 100	20	38
24	J	OH	7.7	2400	9.1	> 100	19	10
25	J	(R)–OH	7.3	1300	8.9	> 100	20	6
26	J	(S)–OH	7.9	7400	9.4	94	14	4

^a 5-HT₄ pK_i values were determined using [³H]-GR113808 in HEK-293 cells stably transfected with human 5-HT_{4(c)} receptor cDNA; 5-HT₃ pK_i values were determined with [³H]-GR65630 in HEK-293 cells stably transfected with human 5-HT_{3A} receptor cDNA. ^b Selectivity for the 5-HT₄ receptor subtype with respect to the 5-HT₃ receptor subtype was calculated as the ratio K_i(5-HT_{3A})/K_i(5-HT_{4(c)}). ^c pEC₅₀ determined using whole-cell cAMP accumulation studies in HEK-293 cells stably transfected with the human recombinant 5-HT_{4(c)} receptor splice variant. ^d Maximum compound-evoked response (minus basal) was expressed as a percentage of the maximum response evoked by 5-HT. ^e hERG inhibition determined at a concentration of 3 μM using Chinese hamster ovary (CHO) cells stably expressing hERG potassium channels.

Table 6. Pharmacokinetics of Compound **26** in Rat and Dog


compd	rat					dog	
	RLM $t_{1/2}$ (min)	AUC _(0–t) ($\mu\text{g} \cdot \text{h/mL}$) ^a	C _{max} ($\mu\text{g/mL}$)	$t_{1/2}$ (h)	F% ^b	C _{max} ($\mu\text{g/mL}$)	F%
26	> 90	0.20	0.031	5.2	20	0.32	63

^a Pharmacokinetic properties were evaluated in male Sprague–Dawley rats dosed with test compounds via oral gavage (PO) at a dose of 5 mg/kg. $n = 3$.^b F = oral bioavailability, %. ^c Pharmacokinetic properties were evaluated in male Beagle dogs dosed with test compounds via oral gavage (PO) at a dose of 2 mg/kg. $n = 3$.

inhibition of 5-HT_{1A,C,D}, 5-HT_{2A,B,C}, 5-HT_{5A}, 5-HT₆, and 5-HT₇ at a concentration of 10 μM .

The oral pharmacokinetics of **26** in rats and dogs are shown in Table 6. Introduction of the hydrophilic hydroxyl group reduced the oral bioavailability in the rat relative to **9a**, perhaps due to lower permeability, although bioavailability in the dog was excellent.

In Vitro/In Vivo Pharmacology of Compound 26. Compound **26** produced a concentration-dependent contraction of the guinea pig isolated colon longitudinal muscle/

myenteric plexus preparation. The potency of **26** (pEC₅₀ = 8.6) was superior to that of 5-HT (pEC₅₀ = 6.9) and similar to tegaserod (pEC₅₀ = 8.8). Compound **26** had an intrinsic activity greater than that of tegaserod (63% vs 44% of the 5-HT maximum, respectively). Considering the wealth of literature demonstrating that 5-HT₄ receptor activation results in contraction of this smooth muscle preparation^{40,41} and the presence of antagonists of 5-HT₁, 5-HT₂ and 5-HT₃ receptors, in this study, the observed activity of **26** was concluded to represent 5-HT₄ receptor activation.

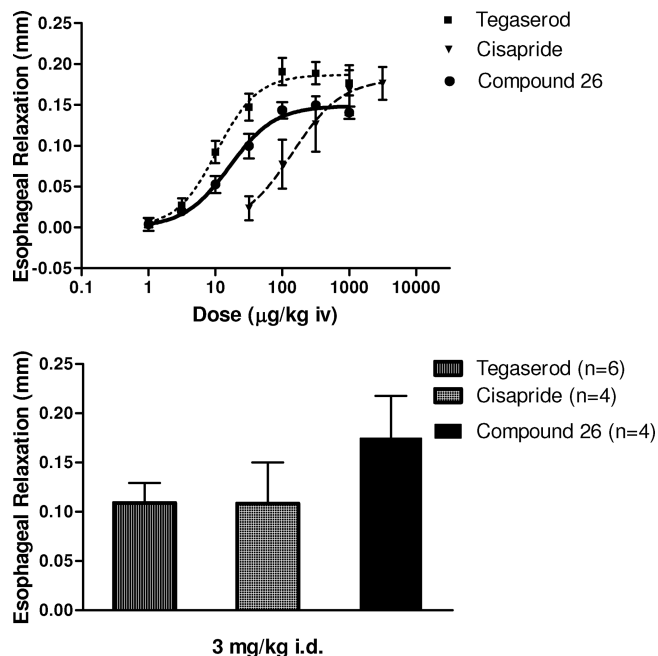


Figure 4. Relaxation of rat esophagus following intravenous and intraduodenal administration of compound **26**.

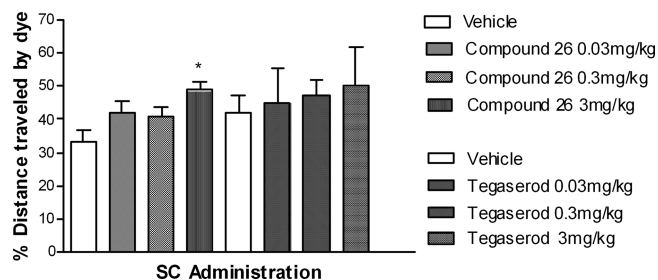


Figure 5. Guinea pig colonic transit following subcutaneous dosing of compound **26**.

To characterize the *in vivo* activity of **26**, digital sonomicrometry was used to monitor 5-HT₄ receptor-mediated relaxation of the rat esophagus.⁴² This method provided a novel and sensitive means to demonstrate 5-HT₄ receptor agonist-mediated changes in endogenous esophageal tone in anesthetized rats. Compound **26** produced a dose-dependent, 5-HT₄ receptor-mediated relaxation of the esophagus in anesthetized rats, following intravenous or intraduodenal administration (Figure 4). Following intravenous administration, the rank order of potencies was tegaserod > **26** > cisapride. The mean ED₅₀ values for the relaxant response mediated by intravenous **26**, tegaserod and cisapride were 16, 10.6, and 142 $\mu\text{g/kg}$ respectively. Following intraduodenal administration at a dose of 3 mg/kg, the degree of relaxation of the rat esophagus afforded by **26** was superior to that afforded by tegaserod and cisapride.

In the guinea pig colonic transit model, **26** (3 mg/kg sc) produced a statistically significant increase in colonic transit of carmine red dye relative to vehicle treated animals.⁴³ Following subcutaneous administration, prokinetic activity was evident when the distance traveled by the dye was measured 60 min later or when the time for excretion of the first fecal pellet containing the marker was recorded (Figure 5). In this assay, **26** appeared to be similar in potency

to tegaserod, although tegaserod did not reach statistical significance at the doses tested.

Conclusions

Applying a multivalent approach to lead discovery led to conceptualization of the 5-HT₄ agonist pharmacophore as consisting of "primary" and "secondary" binding groups and investigation of how the secondary binding group could be used to modulate the profile of fixed primary binding groups. A diverse selection of secondary binding groups was screened by linking them to chloroaniline-piperidine **1c** and quinolone-tropane **2a**, which demonstrated the distinct ability of amine-based secondary binding groups to improve the 5-HT₄ receptor binding affinity and selectivity. A study of the role of linker length using a representative amine revealed that 5-HT₄ binding affinity increased as the linker length was increased, although functional potency did not improve significantly after the linker exceeded three carbons in length. Focusing on the quinolone-tropane series, the influence of the pK_a of the secondary binding group was investigated, and higher pK_as were found to enhance the binding affinities in the C3 linked compounds. *In vitro* permeability and hERG inhibition data were also heavily dependent on the nature of the secondary binding group. The need to balance potency with satisfactory oral pharmacokinetics led to a focus on 5-HT₄ agonists containing weakly basic secondary binding groups and short linkers. Compound **9a** was selected for optimization, and introduction of a chiral alcohol to the linker (compound **26**) resulted in a significant improvement in binding affinity, selectivity over 5-HT₃, and functional potency as well as a reduction in hERG inhibition. In animal models, **26** produced a dose-dependent, 5-HT₄ receptor-mediated relaxation of the esophagus in rats and colonic prokinetic activity comparable to tegaserod in guinea pigs. On the basis of these data, compound **26** was selected for advanced efficacy and safety evaluation.

Experimental Section

Chemistry. Unless noted otherwise, starting materials and solvents were purchased from commercial suppliers and used without further purification. Reactions were run under nitrogen atmosphere, unless noted otherwise. Progress of reaction mixtures was monitored by analytical high performance liquid chromatography (HPLC) and mass spectrometry, the details of which are given below and separately in specific examples of reactions. Reaction mixtures were worked up as described specifically in each reaction and routinely purified by preparative HPLC: a general protocol is described below. Test compounds with >95% purity, assessed by analytical HPLC (solvent A = 98% water/2% MeCN/1.0 mL/L TFA; solvent B = 90% MeCN/10% water/1.0 mL/L TFA), were submitted for biological evaluation. Melting points were determined on a TA Instruments' Q100 differential scanning calorimeter. Characterization of reaction products was routinely performed by ¹H and ¹³C NMR spectrometry in deuterated solvents (CD₃OD, DMSO-*d*₆) acquired under standard parameters using a Varian Gemini 2000 instrument (400 or 300 MHz) or Bruker UltraShield^{plus} NMR spectrometer (600 MHz). Chiral purity was determined on a Beckman P/ACE MDQ capillary electrophoresis system employing a 50 μM \times 60 cm fused silica capillary and 25 μM triethylammonium phosphate/10% w/v highly sulfated γ -cyclodextrin (Beckman) buffer system. Mass spectrometric identification of compounds was performed using standard electrospray ionization methods (ESMS) with a Perkin-Elmer SCIEX API 150 EX. High resolution mass

spectrometry was performed using standard electrospray ionization methods (ESMS) with an Applied Biosystems/MDS SCIEX QSTAR.

General Procedure for Compounds 1a, 1c, 3a, 3c (Method A): 4-Amino-5-chloro-2-methoxy-*N*-piperidin-4-yl-benzamide·TFA (1c). 4-Amino-5-chloro-2-methoxy-benzoic acid (20.1 g, 0.1 mol) was dissolved in DMF (200 mL) and CDI (1 equiv) was added. The solution was stirred at room temperature for 4 h, then 4-amino-piperidine-1-carboxylic acid *tert*-butyl ester (1 equiv) was added. The reaction was stirred for 16 h at room temperature, the solvent evaporated, and the residue partitioned between EtOAc (500 mL) and brine. The organic phase was separated and washed (2 × 200 mL 0.2 M H₃PO₄/brine and 1 M NaOH/brine). The organic phase was dried (MgSO₄), evaporated, and the residue purified by flash chromatography (SiO₂) to afford 4-(4-amino-5-chloro-2-methoxy-benzoylamino)-piperidine-1-carboxylic acid *tert*-butyl ester (14.6 g). This material was dissolved in CH₂Cl₂ (25 mL) and anisole (10 mL), cooled to 0 °C, and TFA (25 mL) was added. After 10 min at 0 °C, the solution was allowed to warm to room temperature and stirred for 1 h. The solvent was evaporated, and the residue added to Et₂O (50 mL). The resultant precipitate was filtered and washed with Et₂O (500 mL) to afford a beige solid (12.0 g). This crude material was purified by preparative reversed-phase HPLC and the pure fractions lyophilized to afford **1c** as a white powder (1.97 g, 5%); 100% purity by HPLC (2–90% MeCN/H₂O over 6 min; *t*_R 2.26 min). ¹H NMR (DMSO-*d*₆) δ 1.68 (m, 2H), 2.03 (m, 2H), 3.03 (m, 2H), 3.25 (m, 2H), 3.80 (s, 3H), 3.99 (m, 1H), 5.80 (br s, 2H), 6.48 (s, 1H), 7.60 (s, 1H), 7.76 (d, *J* = 7.2, 1H), 8.65 (br s, 1H), 8.86 (br s, 1H). ¹³C NMR (400 MHz, DMSO-*d*₆) δ 28.7, 42.5, 44.2, 56.4, 98.0, 109.3, 111.0, 131.7, 148.9, 157.7, 163.8. MS *m/z*: 285.3 (M + H⁺).

General Procedure for Compounds 1b, 3b (Method B): 1-Isopropyl-1*H*-indazole-3-carboxylic Acid (Piperidin-4-ylmethyl)-amide·TFA (1b). 1-Isopropylindazole-3-carboxylic acid (5.0 g, 24.6 mmol) and *tert*-butyl 4-(aminomethyl)piperidinecarboxylate (5.2 g, 1 equiv) were dissolved in anhydrous DMF (30 mL). The solution was stirred in an ice bath and diisopropylethylamine (1.5 equiv) was added, followed by *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (1.5 equiv) and washed with an additional 20 mL of DMF. After 10 min, the ice bath was removed and the reaction was stirred at room temperature for 16 h. The reaction was quenched with water and extracted with EtOAc (100 mL). The organic extract was washed with saturated KHSO₄ (100 mL), 1N NaOH (100 mL), and brine (100 mL) and then dried (MgSO₄), filtered, and evaporated to afford 4-[[1-(isopropyl-1*H*-indazole-3-carbonyl)-amino]-methyl]-piperidine-1-carboxylic acid *tert*-butyl ester as a colorless oil (4.52 g). A dichloromethane (20 mL) solution of 4-[[1-(isopropyl-1*H*-indazole-3-carbonyl)-amino]-methyl]-piperidine-1-carboxylic acid *tert*-butyl ester (0.484 g, 1.2 mmol) was cooled in an ice bath. TFA (20 mL) was added dropwise and stirring continued for one hour. The solution was then evaporated and the residue triturated with Et₂O (60 mL) by stirring at room temperature overnight. The solid was collected by filtration to give **1b** as a white solid (0.47 g); 98% purity by HPLC (2–90% MeCN/H₂O over 6 min; *t*_R 2.72 min). ¹H NMR (DMSO-*d*₆) δ 1.37 (m, 2H), 1.49 (d, *J* = 6.6 Hz, 6H), 1.85 (m, 3H), 2.83 (m, 2H), 3.23 (m, 4H), 5.00 (sept, *J* = 6.6 Hz, 1H), 7.20 (t, *J* = 7.2 Hz, 1H), 7.39 (t, *J* = 6.6 Hz, 1H), 7.73 (d, *J* = 8.6 Hz, 1H), 8.15 (d, *J* = 8.2 Hz, 1H), 8.35 (t, *J* = 6.3 Hz, 1H), 8.60 (br s, 1H), 8.95 (br s, 1H). ¹³C NMR (400 MHz, DMSO-*d*₆) δ 22.5, 27.1, 34.5, 43.6, 43.9, 50.9, 110.9, 116.3, 119.3, 122.5, 122.9, 126.9, 137.4, 140.2, 163.0. MS *m/z*: 301.3 (M + H⁺). HRMS calcd for C₁₇H₂₄N₄O, 301.2028; found, 301.2032.

General Procedure for Compounds 1d, 5a–d (Method C): 5-Methoxy-1*H*-indole-3-carboxylic Acid (Piperidin-4-ylmethyl)-amide·TFA (5b). 5-Methoxy-1*H*-indole-3-carboxylic acid (0.50 g, 2.6 mmol) and *tert*-butyl 4-(aminomethyl)piperidinecarboxylate (0.60 g, 1.05 equiv) were dissolved in DMF (5 mL).

1-Hydroxybenzotriazole (1 equiv) and *N*-(3-dimethylamino-propyl)-*N'*-ethylcarbodiimide hydrochloride (1 equiv) were added, and the reaction was allowed to stir overnight at room temperature. Diisopropylethylamine (3 equiv) was added, and the reaction was allowed to stir at room temperature for 4 h, at which point the reaction was still incomplete. A further 1 equiv of *tert*-butyl 4-(aminomethyl)piperidinecarboxylate was added portionwise until the reaction was complete. The solution was diluted with EtOAc (30 mL) and washed with saturated NH₄Cl (30 mL), followed by 1 M NaOH (30 mL) and then brine (30 mL). The organics were dried (MgSO₄) and the solvent evaporated to afford 4-[[5-methoxy-1*H*-indole-3-carbonyl)-amino]-methyl]-piperidine-1-carboxylic acid *tert*-butyl ester (0.9 g). This compound was dissolved in CH₂Cl₂ (20 mL) and cooled to 0 °C. TFA (20 mL) was added dropwise, and the solution was stirred for 0.5 h. The solvent was evaporated and the residue triturated with Et₂O and filtered to afford a white solid. This crude material was purified by preparative reversed-phase HPLC and the pure fractions lyophilized to afford **5b** as a white powder (135 mg, 13%); 93% purity by HPLC (2–90% MeCN/H₂O over 6 min; *t*_R 1.99 min). ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.32 (m, 2H), 1.80 (m, 2H), 2.85 (m, 2H), 3.15 (t, *J* = 6.1 Hz, 1H), 3.28 (m, 2H), 3.73 (s, 3H), 6.75 (dd, *J* = 2.5, 8.8, 1H), 7.28 (d, *J* = 8.8, 1H), 7.65 (d, *J* = 2.5, 1H), 8.01 (m, 1H), 11.51 (d, *J* = 2.5, 1H). ¹³C NMR (400 MHz, DMSO-*d*₆) δ 27.1, 34.7, 43.6, 43.9, 55.8, 103.2, 110.8, 112.6, 113.1, 127.5, 128.7, 131.8, 154.9, 165.6. MS *m/z*: 288.4 (M + H⁺). HRMS calcd for C₁₆H₂₂N₃O₂, 288.1712; found, 288.1703.

2-Amino-5-chloro-4-methoxy-*N*-(2-piperazin-1-yl-ethyl)-benzamide·TFA (1d). Compound **1d** was prepared in the same manner as **5b** using 4-amino-5-chloro-2-methoxybenzoic acid and 4-*N*-(2-aminoethyl)-1-*N*-Boc-piperazine; 99% purity by HPLC (2–30% MeCN/H₂O over 4 min; *t*_R 1.32 min). ¹H NMR (400 MHz, DMSO-*d*₆) δ 3.16 (m, 2H), 3.22–3.40 (br s, 10H), 3.56 (m, 2H), 3.81 (s, 3H), 6.47 (s, 1H), 7.69 (s, 1H). ¹³C NMR (400 MHz, DMSO-*d*₆) δ 35.2, 40.8, 49.6, 56.4, 98.0, 109.1, 110.4, 132.4, 149.5, 158.2, 165.1. MS *m/z*: 313.2 (M + H⁺). HRMS calcd for C₁₄H₂₂ClN₄O₂, 313.1431; found, 313.1433.

5-Methoxy-1*H*-indole-3-carboxylic Acid (8-Aza-bicyclo[3.2.1]oct-3-yl)-amide·TFA (5a). Compound **5a** was prepared in the same manner as **5b** using 5-methoxy-1*H*-indole-3-carboxylic acid and 4(1*S*,3*R*,5*R*)-3-amino-8-azabicyclo[3.2.1]octane-8-carboxylic acid *tert*-butyl ester; 99% purity by HPLC (2–90% MeCN/H₂O over 6 min; *t*_R 2.06 min). ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.50 (m, 2H), 1.73 (m, 4H), 1.86 (m, 2H), 3.26 (m, 3H), 3.49 (s, 3H), 6.29 (m, 1H), 6.85 (m, 1H), 7.12 (s, 1H), 7.18 (s, 1H), 7.56 (m, 1H), 8.33 (br s, 1H), 8.48 (br s, 1H), 11.13 (s, 1H). ¹³C NMR (400 MHz, DMSO-*d*₆) δ 25.1, 31.9, 40.4, 53.2, 55.0, 102.2, 110.0, 112.1, 112.5, 126.7, 128.7, 131.1, 154.3, 165.7. MS *m/z*: 300.8 (M + H⁺). HRMS calcd for C₁₇H₂₂N₃O₂, 300.1712; found, 300.1721.

5-Methoxy-1*H*-indole-3-carboxylic Acid Piperidin-4-ylamide·TFA (5c). Compound **5c** was prepared in the same manner as **5b** using 5-methoxy-1*H*-indole-3-carboxylic acid and 4-amino-1-*N*-Boc-piperidine; 90% purity by HPLC (2–90% MeCN/H₂O over 6 min; *t*_R 2.23 min). ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.69 (m, 2H), 1.98 (m, 2H), 2.99 (m, 2H), 3.33 (m, 2H), 3.72 (s, 3H), 4.02 (m, 1H), 6.75 (dd, *J* = 2.6, 8.8 Hz, 1H), 7.28 (d, *J* = 8.8 Hz, 1H), 7.63 (d, *J* = 2.5 Hz, 1H), 7.86 (d, *J* = 7.2 Hz, 1H), 8.03 (d, *J* = 3.1 Hz, 1H), 8.62–8.74 (br s, 1H), 8.80–8.90 (br s, 1H), 11.52 (s, 1H). ¹³C NMR (400 MHz, DMSO-*d*₆) δ 29.3, 43.0, 44.2, 55.8, 103.3, 110.5, 112.6, 113.1, 127.7, 129.0, 131.8, 155.0, 165.1. MS *m/z*: 274.6 (M + H⁺). HRMS calcd for C₁₅H₁₉N₃O₂, 274.1566; found, 274.1556.

5-Methoxy-1*H*-indole-3-carboxylic Acid (2-Piperazin-1-yl-ethyl)-amide·TFA (5d). Compound **5d** was prepared in the same manner as **5b** using 5-methoxy-1*H*-indole-3-carboxylic acid and 4-*N*-(2-aminoethyl)-1-*N*-Boc-piperazine; 90% purity by HPLC (2–90% MeCN/H₂O over 6 min; *t*_R 2.03 min). ¹H NMR (400 MHz, DMSO-*d*₆) δ 3.25 (m, 2H), 3.30–3.50 (br m, 10H), 3.58

(m, 2H), 3.73 (s, 3H), 6.76 (dd, $J = 2.5, 8.8$ Hz, 1H), 7.30 (d, $J = 8.8$ Hz, 1H), 7.64 (d, $J = 2.5$ Hz, 1H), 7.96 (d, $J = 2.9$ Hz, 1H), 8.18 (t, $J = 5.3$ Hz, 1H), 11.59 (d, $J = 2.3$ Hz, 1H). ^{13}C NMR (400 MHz, DMSO- d_6) δ 34.4, 41.1, 49.1, 55.8, 103.1, 110.3, 112.7, 113.2, 127.4, 129.1, 131.8, 155.1, 166.1. MS m/z : 303.6 ($M + H^+$). HRMS Calcd for $\text{C}_{16}\text{H}_{23}\text{N}_4\text{O}_2$, 303.1821; found, 303.1818.

General Procedure for Compounds 2a–d (Method D): 1-Isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic Acid [(1*S*,3*R*,5*R*)-8-Azabicyclo[3.2.1]oct-3-yl]amide·TFA (2a). To a stirred suspension of 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (80 g, 0.35 mol) in toluene (600 mL) was added thionyl chloride (36.6 mL, 1.5 equiv). The mixture was stirred at 95 °C for 2 h then allowed to cool to rt and then added over 0.5 h to a vigorously stirred biphasic solution of (1*S*,3*R*,5*R*)-3-amino-8-azabicyclo[3.2.1]octane-8-carboxylic acid *tert*-butyl ester (78.2 g, 1 equiv) and sodium hydroxide (69.2 g, 5 equiv) in toluene/water (1:1) (1 L) at 0 °C. After 1 h, the layers were allowed to separate, and the organic phase was concentrated under reduced pressure. The aqueous phase was washed with EtOAc (1 L), and the organic extract was used to dissolve the concentrated organic residue. This solution was washed with 1 M H_3PO_4 (500 mL), saturated NaHCO_3 (500 mL), and brine (500 mL), dried (MgSO_4), filtered, and concentrated under reduced pressure to afford (1*S*,3*R*,5*R*)-3-[(3-isopropyl-2-oxo-2,3-dihydro-benzimidazole-1-carbonyl)-amino]-8-aza-bicyclo[3.2.1]octane-8-carboxylic acid *tert*-butyl ester (127.9 g, 84%) as a yellow solid. This intermediate was dissolved in CH_2Cl_2 (600 mL) and cooled to 0 °C. TFA (300 mL) was added, and the reaction mixture was warmed to room temperature and stirred for 1 h then evaporated. The oily brown residue was then poured into a vigorously stirred solution of Et_2O (3 L) and a solid precipitate formed immediately. The suspension was stirred at rt for 16 h and the solid collected by filtration and washed with Et_2O to afford **2a** (131.7 g, 86% over two steps) as a light-yellow solid; 98% purity by HPLC (2–90% MeCN/ H_2O over 6 min; t_R 3.01 min). ^1H NMR (DMSO- d_6) δ 1.56 (d, $J = 6.8$ Hz, 6H), 1.93 (d, $J = 15.2$ Hz, 2H), 2.06–2.31 (m, 6H), 3.32–3.46 (br s, 3H), 4.00 (bs, 2H), 4.16 (m, 1H), 7.34 (t, $J = 7.6$ Hz, 1H), 7.72 (td, $J = 1.6, 7.2$ Hz, 1H), 7.87 (d, $J = 8.8$ Hz, 1H), 8.00 (dd, $J = 1.6, 8.0$ Hz, 1H), 8.70 (br s, 1H), 8.78 (s, 1H), 8.85 (br s, 1H), 10.43 (d, $J = 7.2$ Hz, 1H). ^{13}C NMR (400 MHz, DMSO- d_6) δ 19.9, 25.9, 33.6, 39.9, 53.8, 116.2, 119.1, 120.6, 121.8, 123.4, 131.9, 133.4, 143.8, 162.5, 162.9. MS m/z : 340.4 ($M + H^+$). HRMS calcd for $\text{C}_{20}\text{H}_{25}\text{N}_3\text{O}_2$, 340.2025; found, 340.2034.

1-Isopropyl-2-oxo-1,2-dihydro-quinoline-3-carboxylic Acid (Piperidin-4-ylmethyl)-amide (2b). Compound **2b** was prepared in the same manner as **2a** using 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid and *tert*-butyl 4-(aminomethyl)-piperidinecarboxylate; 95% purity by HPLC (2–90% MeCN/ H_2O over 6 min; t_R 2.95 min). ^1H NMR (400 MHz, DMSO- d_6) δ 1.36 (q, $J = 10.4$ Hz, 2H), 1.55 (d, $J = 6.8$ Hz, 6H), 1.78–1.85 (br m, 3H), 2.84 (q, $J = 10.0$ Hz, 2H), 3.27 (t, $J = 6$ Hz, 4H), 3.53 (bs, 1H), 7.32 (t, $J = 7.2$ Hz, 1H), 7.70 (t, $J = 7.6$ Hz, 1H), 7.84 (d, $J = 8.8$ Hz, 1H), 7.95 (d, $J = 8.0$ Hz, 1H), 8.50 (br s, 1H), 8.75 (br s, 1H), 8.86 (bs, 1H), 9.79 (t, $J = 6.0$ Hz, 1H). ^{13}C NMR (400 MHz, DMSO- d_6) δ 19.9, 27.0, 34.4, 43.5, 44.2, 119.3, 120.6, 122.2, 122.3, 123.4, 130.4, 131.9, 133.4, 143.8, 162.5, 163.6. MS m/z : 328.6 ($M + H^+$). HRMS calcd for $\text{C}_{19}\text{H}_{26}\text{N}_3\text{O}_2$, 328.2025; found, 328.2013.

1-Isopropyl-2-oxo-1,2-dihydro-quinoline-3-carboxylic Acid Piperidin-4-ylamide (2c). Compound **2c** was prepared in the same manner as **2a** using 1-isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid and 4-amino-1-Boc-piperidine; 98% purity by HPLC (2–90% MeCN/ H_2O over 6 min; t_R 2.86 min). ^1H NMR (400 MHz, DMSO- d_6) δ 1.55 (d, $J = 6.8$ Hz, 6H), 1.68 (q, $J = 10.6$ Hz, 2H), 2.07 (d, $J = 10.9$ Hz, 2H), 3.05 (t, $J = 11.9$ Hz, 2H), 3.28 (d, $J = 12.7$ Hz, 2H), 4.07 (m, 1H), 4.02 (m, 1H), 7.32 (t, $J = 7.4$ Hz, 1H), 7.70 (t, $J = 7.4$ Hz, 1H), 7.84 (d, $J = 8.8$ Hz, 1H), 7.95 (d, $J = 6.8$ Hz, 1H), 8.75 (s, 1H), 9.80 (d, $J = 7.2$ Hz, 1H).

^{13}C NMR (400 MHz, DMSO- d_6) δ 19.9, 29.0, 34.9, 42.8, 44.5, 116.3, 119.3, 120.6, 122.0, 123.4, 132.0, 133.5, 143.9, 162.5, 162.9. MS m/z : 314.6 ($M + H^+$). HRMS calcd for $\text{C}_{18}\text{H}_{24}\text{N}_3\text{O}_2$, 314.1869; found, 314.1864.

General Procedure for Compounds 4a–d (Method E): *N*-(1*S*,3*R*,5*R*)-3-Isopropyl-2-oxo-2,3-dihydrobenzimidazole-1-carboxylic Acid (8-Azabicyclo[3.2.1]oct-3-yl)amide·TFA (4a). To a suspension of sodium hydride (9.25 g; 1.5 equiv, 60% dispersion in mineral oil) in dry THF (1 L) in an ice bath was added 1-isopropyl-1,3-dihydro-2*H*-benzimidazol-2-one (27.2 g, 154.2 mmol) in THF (50 mL). The mixture was stirred at 0–5 °C for 0.5 h, and then 4-nitrophenyl chloroformate (34.2 g, 170 mmol) in THF (50 mL) was added. The mixture was stirred at rt for 16 h. To this solution was added (1*S*,3*R*,5*R*)-3-amino-8-azabicyclo[3.2.1]octane-8-carboxylic acid *tert*-butyl ester (36.7 g, 1.05 equiv) in THF (50 mL). The mixture was stirred for 12 h and then warmed to 75 °C for 3 h. The mixture allowed to cool, evaporated, and the residue dissolved in CH_2Cl_2 (1 L). The solution was washed with 1 M H_3PO_4 (500 mL) followed by saturated NaHCO_3 (500 mL). The solvent was evaporated and the intermediate dissolved in CH_2Cl_2 (200 mL), cooled to 0 °C, and TFA (200 mL) was added. The mixture was stirred for 0.5 h at 5 °C and then for 1 h at rt. After evaporation of the mixture, Et_2O (500 mL) was added to the oily residue. The resulting precipitate was collected, rinsed with Et_2O , and dried in vacuo, to provide **4a** as a white solid (47 g, 71% over 2 steps); 98% purity by HPLC (2–90% MeCN/ H_2O over 6 min; t_R = 3.05 min). ^1H NMR (DMSO- d_6) δ 1.45 (d, $J = 6.8$ Hz, 6H), 1.93 (d, $J = 15.5$ Hz, 2H), 2.05–2.18 (m, 4H), 2.75 (m, 2H), 3.98 (br s, 1H), 4.07 (q, $J = 6.5$ Hz, 1H), 4.69 (sep, $J = 6.8$ Hz, 1H), 7.11 (td, $J = 1.1, 7.8$ Hz, 1H), 7.18 (td, $J = 1.4, 7.6$ Hz, 1H), 7.40 (d, $J = 7.4$ Hz, 1H), 8.04 (dd, $J = 1.1, 8.0$ Hz, 1H), 8.98 (br s, 1H), 9.12 (br s, 1H), 9.30 (d, $J = 7.0$). ^{13}C NMR (400 MHz, DMSO- d_6) δ 20.0, 25.8, 33.5, 40.7, 45.6, 53.7, 110.2, 114.9, 122.5, 124.1, 126.8, 128.1, 151.0, 152.9. MS m/z : 329.6 ($M + H^+$). HRMS calcd for $\text{C}_{18}\text{H}_{24}\text{N}_4\text{O}_2$, 329.1978; found, 329.1985.

3-Isopropyl-2-oxo-2,3-dihydro-benzimidazole-1-carboxylic Acid Piperidin-4-ylamide (4c). Compound **4c** was prepared in the same manner as **4a** using 3-isopropyl-2-oxo-2,3-dihydro-benzimidazole-1-carboxylic acid and 4-amino-1-Boc-piperidine. 99% purity by HPLC (2–90% MeCN/ H_2O over 6 min; t_R = 2.98 min). ^1H NMR (400 MHz, DMSO- d_6) δ 1.45 (d, $J = 7.1$ Hz, 6H), 1.65 (m, 2H), 2.07 (dd, $J = 3.1, 13.7$ Hz, 2H), 3.04 (q, $J = 11.0$ Hz, 2H), 3.28 (d, $J = 12.9$ Hz, 2H), 3.97 (m, 1H), 4.63 (m, 1H), 7.13 (t, $J = 7.8$ Hz, 1H), 7.18 (t, $J = 7.6$ Hz, 1H), 7.40 (d, $J = 7.4$ Hz, 1H), 8.02 (d, $J = 7.8$ Hz, 1H), 8.64 (br s, 1H), 8.78 (d, $J = 7.2$ Hz, 1H), 8.91 (br s, 1H). ^{13}C NMR (400 MHz, DMSO- d_6) δ 20.0, 28.9, 42.7, 45.1, 45.7, 110.2, 114.9, 122.6, 124.2, 126.8, 128.4, 151.0, 152.7. MS m/z : 303.6 ($M + H^+$). HRMS calcd for $\text{C}_{16}\text{H}_{23}\text{N}_4\text{O}_2$, 303.1821; found, 303.1811.

3-Isopropyl-2-oxo-2,3-dihydro-benzimidazole-1-carboxylic Acid (2-Piperazin-1-yl-ethyl)-amide (4d). Compound **4d** was prepared in the same manner as **4a** using 3-isopropyl-2-oxo-2,3-dihydro-benzimidazole-1-carboxylic acid and 4-*N*-(2-aminoethyl)-1-*N*-Boc-piperazine; 99% purity by HPLC (2–90% MeCN/ H_2O over 6 min; t_R = 2.62 min). ^1H NMR (400 MHz, DMSO- d_6) δ 1.45 (d, $J = 7.0$ Hz, 6H), 3.15 (m, 2H), 3.20–3.40 (br m, 12H), 3.60 (m, 2H), 4.61 (s, $J = 7.0$ Hz, 1H), 7.12 (t, $J = 7.6$ Hz, 1H), 7.18 (t, $J = 7.8$ Hz, 1H), 7.38 (d, $J = 7.4$ Hz, 1H), 8.04 (d, $J = 8.0$ Hz, 1H), 8.92 (t, $J = 5.7$ Hz, 1H). ^{13}C NMR (400 MHz, DMSO- d_6) δ 20.0, 35.5, 41.1, 45.6, 49.2, 56.0, 110.0, 115.0, 122.5, 124.1, 126.8, 128.5, 152.2, 152.4. MS m/z : 332.4 ($M + H^+$). HRMS calcd for $\text{C}_{17}\text{H}_{25}\text{N}_5\text{O}_2$, 332.2087; found, 332.2103.

General Procedure for Compounds 6a–l, 7a–l (Method F): 1-Isopropyl-2-oxo-1,2-dihydro-quinoline-3-carboxylic Acid [(1*R*,3*R*,5*S*)-8-[(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-pentyl]-8-azabicyclo[3.2.1]oct-3-yl]amide·TFA (7h). Compound **2a** (200 mg, 0.44 mmol) and *N*-(5-bromopentyl)phthalimide (1.2 equiv) were dissolved in DMF (3 mL) and H_2O (0.5 mL). NaHCO_3 (3 equiv) and KI (1.2 equiv) were added, and the reaction was stirred at

90 °C for 16 h. The mixture was allowed to cool, then partitioned between EtOAc (20 mL) and H₂O (10 mL). The organic phase was evaporated and the residue purified by preparative reverse-phase HPLC (2–90% MeCN/H₂O over 50 min) to afford **7h** as a white solid (55 mg, 19%); 97% purity by HPLC (2–90% MeCN/H₂O over 6 min; t_R = 3.78 min). ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.30 (m, 2H), 1.56 (d, J = 6.8 Hz, 6H), 1.67 (m, 3H), 2.02 (d, J = 15.2 Hz, 1H), 2.25 (m, 3H), 2.45 (m, 3H), 2.92 (m, 1H), 3.57 (t, J = 6.8 Hz, 2H), 3.98 (br s, 1H), 4.19 (br m, 6H), 7.33 (t, J = 7.2 Hz, 1H), 7.71 (td, J = 1.6, 5.6 Hz, 1H), 7.85 (m, 5H), 7.97 (dd, J = 1.6, 8.0 Hz, 1H), 8.77 (s, 1H), 9.52 (br m, 1H), 10.44 (d, J = 7.2 Hz, 1H). ¹³C NMR (400 MHz, DMSO-*d*₆) δ 19.9, 23.9, 24.1, 24.4, 28.2, 34.9, 37.8, 39.6, 49.3, 51.4, 61.0, 120.6, 121.1, 121.8, 123.5, 123.7, 131.3, 132.0, 132.2, 133.5, 135.1, 143.8, 162.6, 162.9, 168.7. MS m/z : 555.4 (M + H⁺). HRMS calcd for C₃₃H₃₈N₄O₄, 555.2972; found, 555.2950.

General Procedure for Compounds 7s, 9a, 9c, 9e, 10–13, 16, 22, 23 (Method G): 4-(3-((1*S*,3*R*,5*R*)-3-[(1-Isopropyl-2-oxo-1,2-dihydro-quinoline-3-carbonyl)-amino]-8-aza-bicyclo[3.2.1]oct-8-yl)-propyl)-piperazine-1-carboxylic Acid Ethyl Ester (**13**). Compound **2a** (4.01 g) and 1-piperazine carboxylic acid ethyl ester (1 equiv) were dissolved in DMF (10 mL). A solution of KI (1 equiv) and NaHCO₃ (5 equiv) in water (5 mL) was added followed by 1,3-dibromopropane (1 equiv). The mixture was stirred at 85 °C for 16 h, allowed to cool, and then partitioned between CH₂Cl₂ (10 mL) and H₂O (10 mL). The organic phase was evaporated, and the residue purified by preparative reverse-phase HPLC (2–90% MeCN/H₂O over 50 min) to afford **13** as a white solid (2.7 mg, 5%); 98% purity by HPLC (2–90% MeCN/H₂O over 6 min; t_R = 2.92 min). ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.16 (t, J = 7.2 Hz, 3H), 1.56 (d, J = 6.9 Hz, 6H), 2.03 (br m, 4H), 2.24 (m, 4H), 2.46 (m, 4H), 2.55–3.80 (br m, 8H), 4.04 (m, 4H), 4.17 (m, 1H), 7.30 (t, 1H, J = 7.2), 7.68 (t, 1H, J = 7.2), 7.80 (d, 1H, J = 8.4), 7.91 (d, 1H, J = 7.2), 8.72 (s, 1H), 10.5 (s, 1H). ¹³C NMR (600 MHz, DMSO-*d*₆) δ 14.7, 19.4, 19.5, 23.7, 34.5, 40.7, 48.5, 51.0, 53.1, 61.0, 61.8, 116.1, 118.0, 120.3, 123.2, 131.7, 133.5, 143.5, 154.8, 162.5, 162.6. MS m/z : 538.4 (M + H⁺). HRMS calcd for C₃₀H₄₄N₅O₄, 538.3393; found, 538.3393.

General Procedure for Compounds 6m, 6o, 6p, 6r, 6s, 7m, 7o, 7p, 7r, 7u, 8a, 8c, 8e, 14, 18–21 (Method H): 1-Isopropyl-2-oxo-1,2-dihydro-quinoline-3-carboxylic Acid ((1*S*,3*R*,5*R*)-8-[2-(4-methanesulfonyl-piperazin-1-yl)-ethyl]-8-aza-bicyclo[3.2.1]oct-3-yl)-amide·TFA (**9d**). Compound **2a** (527 mg, 0.10 mmol) and 1-methanesulfonylpiperazine (1 equiv) were dissolved in MeOH (1 mL). DIPEA (3 equiv) was added, followed by 1,3-dibromopropane (1 equiv). The solution was stirred at 65 °C overnight and then allowed to cool. The solvent was evaporated, and the residue purified by reverse-phase HPLC to afford **9d** as a white solid (1.2 mg, 2%); 99% purity by HPLC (2–90% MeCN/H₂O over 6 min; t_R = 2.91 min). ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.56 (d, J = 7.0 Hz, 6H), 1.98 (m, 2H), 2.20–2.35 (m, 4H), 2.43 (m, 1H), 2.46 (m, 2H), 2.82 (m, 4H), 2.89 (s, 3H), 3.00 (m, 2H), 3.15–3.33 (br m, 6H), 4.08 (m, 2H), 4.18 (q, J = 6.5 Hz, 1H), 7.33 (t, J = 7.6 Hz, 1H), 7.71 (td, J = 1.8, 7.3 Hz, 1H), 7.87 (d, J = 8.8 Hz, 5H), 7.99 (dd, J = 1.6, 7.8 Hz, 1H), 8.78 (s, 1H), 10.44 (d, J = 7.2 Hz, 1H). MS m/z : 530.4 (M + H⁺). HRMS calcd for C₂₇H₄₀N₅O₄S, 530.2801; found, 530.2817.

General Procedure for Compounds 6n, 7n, 6q, 7q, 6t, 6u, 7t, 8b, 8d, 9b, 9d, 15, 17 (Method I): 4-Amino-5-chloro-*N*-[1-[7-(4-methanesulfonyl-piperazin-1-yl)-heptyl]-piperidin-4-yl]-2-methoxy-benzamide·2TFA (**8d**). Compound **1c** (5.69 g, 11.1 mmol) and 7-bromo-1-heptanol (1.9 equiv) were dissolved in MeCN (35 mL). Et₃N (3.9 equiv) was added, and the reaction was stirred at 65 °C for 16 h. The reaction was then diluted with EtOAc (200 mL), washed (saturated NaHCO₃ then brine), and evaporated to afford 4-amino-5-chloro-*N*-[1-[7-(hydroxy-heptyl)-piperidin-4-yl]-2-methoxy-benzamide as a clear oil (4.51 g). The oil was dissolved in CH₂Cl₂ (70 mL) and DMSO (25 mL) and cooled to 0 °C. DIPEA (3 equiv) was added, followed by sulfur trioxide pyridine complex (2.5 equiv). The

solution was stirred at 0 °C for 0.5 h and then diluted with CH₂Cl₂ (250 mL). The solution was washed (saturated NaHCO₃ then brine), dried (MgSO₄), and evaporated to afford 4-amino-5-chloro-2-methoxy-*N*-[1-(7-oxo-heptyl)-piperidin-4-yl]-benzamide (3.94 g). A portion of this crude intermediate (198 mg, 0.5 mmol) and 1-methanesulfonylpiperazine (1.5 equiv) were dissolved in MeOH (3 mL). Sodium triacetoxyborohydride (1.1 equiv) was added, and the solution was stirred at room temperature for 0.5 h. The solvent was evaporated and the residue purified by reversed-phase HPLC to afford **8d** as a white solid (62 mg, 16%); 95% purity by HPLC (2–90% MeCN/H₂O over 6 min; t_R = 2.47 min). ¹³C NMR (600 MHz, DMSO-*d*₆) δ 23.4, 23.6, 26.0, 26.1, 28.3, 29.3, 35.4, 42.9, 44.7, 50.9, 51.4, 56.0, 56.2, 56.4, 98.0, 109.4, 110.6, 131.8, 149.0, 157.9, 164.1. MS m/z : 544.8 (M + H⁺). HRMS calcd for C₂₅H₄₃ClN₅O₄S, 544.2724; found, 544.2718.

General Procedure for Compounds 24–26 (Method J): 1-Isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic Acid ((1*S*,3*R*,5*R*)-8-[(*S*)-2-Hydroxy-3-(4-methanesulfonylpiperazin-1-yl)-propyl]-8-azabicyclo[3.2.1]oct-3-yl)amide (**26**). (a). (*S*)-1-methylsulfonyl-4-(oxiranylmethyl)piperazine. To a stirred solution of piperazine *N*-methylsulfonamide (87.3 g, 0.53 mol) in ethanol (1.3 L) at room temperature was added (*S*)-epichlorohydrin (48.0 mL, 1.1 equiv). The reaction mixture was stirred for 18 h, and the white solid precipitate that formed was collected by filtration and washed with ethanol to afford (*S*)-1-chloro-3-(4-methylsulfonyl-1-piperazinyl)-2-propanol (107.8 g) as a white solid. This material was dissolved in THF/H₂O (4:1, 1500 mL) at 0 °C, and NaOH (22.15 g, 1.15 equiv) was added with vigorous stirring. The reaction mixture was stirred for 1.5 h, and the layers were separated. The organic layer was evaporated and the residue dissolved in CH₂Cl₂ (1500 mL) and washed with a mixture of the previously separated aqueous layer and 1 M NaOH (500 mL). The organic layer was further washed with 1 M NaOH (500 mL) and brine (500 mL), dried (MgSO₄), filtered, and evaporated to yield a white solid. This solid was recrystallized from EtOAc/hexanes (800 mL) to yield (*S*)-1-methylsulfonyl-4-(oxiranylmethyl)piperazine as a white solid (43.3 g, 37%). ¹H NMR (DMSO-*d*₆) δ 2.22 (m, 1H), 2.45–2.60 (m, 5H), 2.69–2.75 (m, 2H), 2.87 (s, 3H), 3.02 (m, 1H), 3.11 (m, 4H). MS m/z : 221.3 (M + H⁺)⁺.

(b). 1-Isopropyl-2-oxo-1,2-dihydroquinoline-3-carboxylic Acid ((1*S*,3*R*,5*R*)-8-[(*S*)-2-Hydroxy-3-(4-methanesulfonylpiperazin-1-yl)propyl]-8-azabicyclo[3.2.1]oct-3-yl)amide. Compound **2a** (100 g, 0.3 mol) and (*S*)-1-methylsulfonyl-4-(oxiranylmethyl)piperazine (69.4 g, 1.05 equiv) were mixed in EtOH (980 mL) and the reaction mixture stirred at 80 °C for 18 h. The reaction mixture was cooled to room temperature and evaporated. The foamy solid was suspended in MeCN/H₂O (860 mL/940 mL) and sonicated until it became homogeneous. The solution was filtered while hot, and the filtrate was allowed to cool to 5 °C. Crystals were formed and collected by filtration yielded **26** (122 g, 73%) as a white crystalline solid, mp 108 °C; 98% purity by HPLC (2–40% MeCN/H₂O over 6 min; t_R = 2.84 min); optical purity > 99% by chiral CE. ¹H NMR (CD₃OD, 400 MHz) δ (ppm) 1.68 (d, J = 6.8 Hz, 6H), 1.72 (br d, J = 14.5 Hz, 2H), 2.10 (br s, 4H), 2.28 (m, 2H), 2.36 (m, 1H), 2.42 (m, 1H), 2.47–2.53 (m, 2H), 2.65 (m, 4H), 2.85 (s, 3H), 3.24 (t, J = 4.9 Hz, 4H), 3.29 (br s, 1H), 3.36 (br s, 1H), 3.86 (m, 1H), 4.19 (t, J = 6.6 Hz, 1H), 5.50 (br s, 1H), 7.34 (t, J = 7.2 Hz, 1H), 7.72 (td, J = 1.5, 7.2 Hz, 1H), 7.83 (td, J = 1.4, 7.6 Hz, 1H), 8.72 (s, 1H). ¹³C NMR (400 MHz, DMSO-*d*₆) δ 19.9, 26.2, 26.4, 34.2, 36.8, 46.2, 53.3, 57.6, 59.1, 59.5, 62.7, 67.9, 115.9, 120.6, 121.9, 123.3, 131.9, 133.2, 140.0, 143.6, 162.0, 162.8. MS m/z : 560.5 (M + H⁺)⁺. HRMS calcd for C₂₈H₄₂N₅O₅S, 560.2907; found, 560.2907.

Acknowledgment. We greatly appreciate the contributions of Drs. John Jacobsen, Uwe Klein, and Zhengtian Gu to this manuscript.

Supporting Information Available: Biological assay details, and characterization data on compounds **6a–u**, **7a–u**, **8a–e**, **9a–e**, **10–24**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Saxena, P. R. Serotonin receptors: subtypes, functional responses and therapeutic relevance. *Pharmacol. Ther.* **1995**, 339–368.
- (2) Hoyer, D.; Clarke, D. E.; Fozard, J. R.; Hartig, P. R.; Martin, G. R.; Mylecharane, E. J.; Saxena, P. R.; Humphrey, P. P. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol. Rev.* **1994**, 157–203.
- (3) Dumuis, A.; Bouhelal, R.; Sebben, M.; Cory, R.; Bockaert, J. A nonclassical 5-hydroxytryptamine receptor positively coupled with adenylate cyclase in the central nervous system. *Mol. Pharmacol.* **1988**, 34, 880–887.
- (4) Reynolds, G. P.; Mason, S. L.; Meldrum, A.; De Kecker, S.; Parnes, H.; Eglen, R. M.; Wong, E. H. 5-Hydroxy-tryptamine (5-HT)₄ receptors in post mortem human brain tissue: distribution, pharmacology and effects of neurodegenerative diseases. *Br. J. Pharmacol.* **1995**, 114, 993–998.
- (5) Hegde, S. S.; Eglen, R. M. Peripheral 5-HT₄ receptors. *FASEB J.* **1996**, 10, 1398–1407.
- (6) Kaumann, A. J. Blockade of human atrial 5-HT₄ receptors by GR 113808. *Br. J. Pharmacol.* **1993**, 110, 1172–1174.
- (7) Lefebvre, H.; Contesse, V.; Delarue, C.; Vaudry, H.; Kuhn, J. M. Serotonergic regulation of adrenocortical function. *Hormone Metab. Res.* **1998**, 30, 398–403.
- (8) Candura, S. M.; Messori, E.; Franceschetti, G. P.; D'Agostino, G.; Vicini, D.; Tagliani, M.; Tonini, M. Neural 5-HT₄ receptors in the human isolated detrusor muscle: effects of indole, benzimidazole and substituted benzamide agonists and antagonists. *Br. J. Pharmacol.* **1996**, 118, 1965–1970.
- (9) (a) Sanger, G. J. 5-Hydroxytryptamine and functional bowel disorders. *Neurogastroenterol. Motil.* **1996**, 8, 319–331. (b) Tonini, M.; Fabio, P. Drugs acting on serotonin receptors for the treatment of functional GI disorders. *Dig. Dis.* **2006**, 24, 59–69.
- (10) Bouras, E. P.; Camilleri, M.; Burton, D. D.; McKinzie, S. Selective stimulation of colonic transit by the benzofuran 5-HT₄ agonist, prucalopride, in healthy humans. *Gut* **1999**, 44, 682–686.
- (11) Maillet, M.; Robert, S. J.; Lezoualc'h, F. New Insights into Serotonin 5-HT₄ Receptors: A Novel Therapeutic Target for Alzheimer's Disease? *Curr. Alzheimer Res.* **2004**, 1, 79–85.
- (12) Kaumann, A. J. Do human atrial 5-HT₄ receptors mediate arrhythmias? *Trends Pharmacol. Sci.* **1994**, 15, 451–455.
- (13) Clarke, D. E.; Baxter, G. S.; Young, H.; Craig, D. A. Pharmacological properties of the putative 5HT₄ receptor in guinea pig ileum and rat oesophagus: role in peristalsis. In *Serotonin: Molecular Biology, Receptors and Functional Effects*; Fozard, J. R., Saxena, P. R., Eds.; Birkhäuser Verlag: Basel, Switzerland, 1991; pp 232–242.
- (14) Jin, J. G.; Foxx-Orenstein, A. E.; Grider, J. R. Propulsion in guinea pig colon induced by 5-hydroxytryptamine (HT) via 5-HT₄ and 5-HT₃ receptors. *J. Pharmacol. Exp. Ther.* **1999**, 288, 93–97.
- (15) Brijer, M. R.; Bosmans, J. P.; Van Dale, P.; Jurzak, M.; Heylen, L.; Leysen, J. E.; Prins, N. H.; Schuurkes, J. A. J. The in vitro pharmacological profile of prucalopride, a novel enterokinetic compound. *Eur. J. Pharmacol.* **2001**, 423, 71–83.
- (16) Hillier, K.; Tam, F. S. F.; Bunce, K. T.; Grossman, C. Inhibition of motility induced by the activation of 5-HT₁ and 5-HT₄ receptor types in isolated human colon smooth muscle. *Br. J. Pharmacol.* **1994**, 112 (Proc. Suppl. May), 102P.
- (17) Hansen, M. B.; Skadhauge, E. Signal transduction pathways for serotonin as an intestinal secretagogue. *Comp. Biochem. Physiol.* **1997**, 118, 283–290.
- (18) Scarpignato, C.; Pelosini, I. Management of the irritable bowel syndrome: novel approaches to the pharmacology of gut motility. *Can. J. Gastroenterol.* **1999**, 13, 50A–65A.
- (19) Rampe, D.; Roy, M.-L.; Dennis, A.; Brown, A. M. A mechanism for the proarrhythmic effects of cisapride (Propulsid): high affinity blockade of the human cardiac potassium channel HERG. *FEBS Lett.* **1997**, 417, 28–32.
- (20) Sloots, C. E. J.; Poen, A. C.; Kerstens, R.; Stevens, M.; De Pauw, M.; Van Oene, J. C.; Meuwissen, S. G. M.; Felt-Bersma, R. J. F. Effects of prucalopride on colonic transit, anorectal function and bowel habits in patients with chronic constipation. *Aliment. Pharmacol. Ther.* **2002**, 16, 759–767.
- (21) Movetis files RESOLOR with EMEA for the indication of chronic constipation. Movetis Press Release, 29 May 2008.
- (22) Degen, L.; Matzinger, D.; Merz, M.; Appel-Dingemanse, S.; Osborne, S.; Luchinger, S.; Bertold, R.; Maecke, H.; Beglinger, C. Tegaserod, a 5-HT₄ receptor partial agonist, accelerates gastric emptying and gastrointestinal transit in healthy male subjects. *Aliment. Pharmacol. Ther.* **2001**, 15, 1745–1751.
- (23) Muller-Lissner, S. A.; Fumagalli, I.; Bardhan, K. D.; Pace, F.; Pecher, E.; Nault, B.; Ruegg, P. Tegaserod, a 5-HT₄ receptor partial agonist, relieves symptoms in irritable bowel syndrome patients with abdominal pain, bloating and constipation. *Aliment. Pharmacol. Ther.* **2001**, 15, 1655–1666.
- (24) Clinical pharmacokinetics of tegaserod, a serotonin 5-HT₄ receptor partial agonist with promotile activity. Appel-Dingemanse, S. *Clin. Pharmacokinet.* **2002**, 41, 1021–1042.
- (25) Zelnorm prescribing information.
- (26) Beattie, D. T.; Smith, J. A.; Marquess, D.; Vickery, R. G.; Armstrong, S. R.; Pulido-Rios, T.; McCullough, J. L.; Sandlund, C.; Richardson, C.; Mai, N.; Humphrey, P. P. A. The 5-HT₄ receptor agonist, tegaserod, is a potent 5-HT_{2B} receptor antagonist in vitro and in vivo. *Br. J. Pharmacol.* **2004**, 143, 549–560.
- (27) *Public Health Advisory*, March 30, 2007; U.S. Food and Drug Administration: Silver Spring, MD, 2007.
- (28) Mammen, M.; Choi, S.-K.; Whitesides, G. M. Polyvalent interactions in biological systems: implications for design and use of multivalent ligands and inhibitors. *Angew. Chem., Int. Ed.* **1998**, 37, 2754–2794.
- (29) Wright, D.; Usher, L. Multivalent binding in the design of bioactive compounds. *Curr. Org. Chem.* **2001**, 5, 1107–1131.
- (30) Kato, S.; Morie, T.; Hino, K.; Kon, T.; Naruto, S.; Yoshida, N.; Karasawa, T.; Matsumoto, J. Novel benzamides as selective and potent gastric prokinetic agents. 1. Synthesis and structure-activity relationships of N-[(2-morpholinyl)alkyl]benzamides. *J. Med. Chem.* **1990**, 33, 1406–1413.
- (31) Suzuki, M.; Ohuchi, Y.; Asanuma, H.; Kaneko, T.; Yokomori, S.; Ito, C.; isobe, Y.; Muramatsu, M. C. Synthesis and Evaluation of Novel 2-Oxo-1,2-dihydro-3-quinolinecarboxamide Derivatives as Serotonin 5-HT₄ Receptor Agonists. *Chem. Pharm. Bull.* **2000**, 48, 2003–2008.
- (32) Schaus, J. M.; Thompson, D. C.; Bloomquist, W. E.; Susemichel, A. D.; Calligaro, D. O.; Cohen, M. L. Synthesis and Structure–Activity Relationships of Potent and Orally Active 5-HT₄ Receptor Antagonists: Indazole and Benzimidazole Derivatives. *J. Med. Chem.* **1998**, 41, 1943–1955.
- (33) King, F. D.; Hadley, M. S.; Joiner, K. T.; Martin, R. T.; Sanger, G. J.; Smith, D. M.; Smith, G. E.; Smith, P.; Turner, D. H.; Watts, E. A. Substituted benzamides with conformationally restricted side chains. 5. Azabicyclo[x.y.z] derivatives as 5-HT₄ receptor agonists and gastric motility stimulants. *J. Med. Chem.* **1993**, 36, 683–689.
- (34) (a) Lopez-Rodriguez, M. L.; Benhamu, B.; Morcillo, M. J.; Murcia, M.; Viso, A.; Campillo, M.; Pardob, L. 5-HT₄ receptor antagonists: structure–affinity relationships and ligand–receptor interactions. *Curr. Top. Med. Chem.* **2002**, 2, 625–641. (b) Lopez-Rodriguez, M. L.; Morcillo, M. J.; Benhamu, B.; Rosado, M. L. Comparative receptor mapping of serotonergic 5-HT₃ and 5-HT₄ binding sites. *J. Comput.-Aided Mol. Des.* **1997**, 11, 589–599. (c) Rivail, L.; Giner, M.; Gastineau, M.; Berthouze, M.; Soulier, J.-L.; Fischmeister, R.; Bezoualc'h, Maigret, B.; Sicsic, S.; Berque-Bestel, I. New insights into the human 5-HT₄ receptor binding site: exploration of a hydrophobic pocket. *Br. J. Pharmacol.* **2004**, 143, 361–370. (d) Lopez-Rodriguez, M. L.; Morcillo, M. J.; Benhamu, B.; Olivella, M.; Campillo, M.; Pardo, L. Computational model of the complex between GR113808 and the 5-HT₄ receptor guided by site-directed mutagenesis and the crystal structure of rhodopsin. *J. Comput.-Aided Mol. Des.* **2001**, 15, 1025–1033.
- (35) Becker, D. P.; Flynn, D. L.; Moormann, A. E.; Nosal, R.; Villamil, C. I.; Loeffler, R.; Gullikson, G. W.; Moummi, C.; Yang, D. C. Pyrrolizidine esters and amides as 5-HT₄ receptor agonists and antagonists. *J. Med. Chem.* **2006**, 49, 1125–1139.
- (36) Katsuhiko, I.; Koji, K.; Tsuguo, I.; Takanobu, K.; Hideo, T.; Shuji, S.; Noriko, S.; Keiichi, H.; Takeshi, K. Synthesis and pharmacological evaluation of carboxamide derivatives as selective serotonergic 5-HT₄ receptor agonists. *Eur. J. Med. Chem.* **1999**, 34, 977–989.
- (37) Harada, H.; Yamazaki, H.; Toyotomi, Y.; Tateishi, H.; Mine, Y.; Yoshida, N.; Kato, S. Novel N-[1-(1-substituted 4-piperidinyl)methyl]-4-piperidinylbenzamides as potent colonic prokinetic agents. *Bioorg. Med. Chem. Lett.* **2002**, 12, 967–970.
- (38) Katsuhiko, I.; Hideo, T.; Hidetoshi, H.; Shuji, S.; Kiyoshi, A.; Masatake, F.; Noriko, S.; Keiichi, H.; Takeshi, K. Synthesis and pharmacological properties of novel benzamide derivatives acting as ligands to the 5-hydroxytryptamine 4 (5-HT₄) receptor. *Eur. J. Med. Chem.* **1999**, 34, 1101–1108.
- (39) Coremans, G. Cilansetron: A novel, high-affinity 5-HT₃ receptor antagonist for irritable bowel syndrome with diarrhea predominance. *Therapy* **2005**, 2, 559–567.

- (40) Briejer, M. R.; Bosmans, J. P.; Van Daele, P.; Jurzak, M.; Heylen, L.; Leysen, J. E.; Prins, N. H.; Schuurkes, J. A. J. The in vitro pharmacological profile of prucalopride, a novel enterokinetic compound. *Eur. J. Pharmacol.* **2001**, *423*, 71–83.
- (41) Wardle, K. A.; Sanger, G. J. The guinea pig distal colon—a sensitive preparation for the investigation of 5-HT₄ receptor mediated contractions. *Br. J. Pharmacol.* **1993**, *110*, 1593–1599.
- (42) Adelson, D. W.; Million, M Tracking the moveable feast: sonomicrometry and gastrointestinal motility. *News Physiol. Sci.* **2004**, *19*, 27–32.
- (43) Beattie, D. T.; Armstrong, S. R.; Shaw, J. P.; Marquess, D.; Sandlund, C.; Smith, J. A.; Taylor, J. A.; Humphrey, P. P. The in vivo gastrointestinal activity of TD-5108, a selective 5-HT₄ receptor agonist with high intrinsic activity. *Naunyn Schmiedeberg's Arch. Pharmacol.* **2008**, *378*, 139–47.