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A Multivalent Approach to the Design and Discovery of Orally Efficacious 5-HT$_4$ 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

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5-HT$_4$ 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-HT$_4$ 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-HT$_4$ 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-HT$_4$ agonists. Optimization of the leads generated by this approach afforded compound **26**, a selective, orally efficacious 5-HT$_4$ 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-HT$_1$−5-HT$_7$, 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-HT$_4$ 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-HT$_4$ 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] arrhythmia[12]), 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-HT$_4$ agonists such as Renzapride (Figure 1) activates the peristaltic reflex of the guinea pig ileum and colon.[13] Subsequently, the role of 5-HT$_4$ agonist activity in promoting gastrointestinal motility has become more fully appreciated. For example, activation of 5-HT$_4$ receptors on motorneurons 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-HT$_4$ receptors are expressed on smooth muscle cells; their activation results in, for example, esophageal relaxation in rats[15] and inhibition of human colonic circular muscle contractile activity.[16] Furthermore, activation of 5-HT$_4$ 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-HT$_4$ 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-HT$_4$ 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-HT$_4$ 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-HT$_4$

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

[a] Abbreviations: 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.

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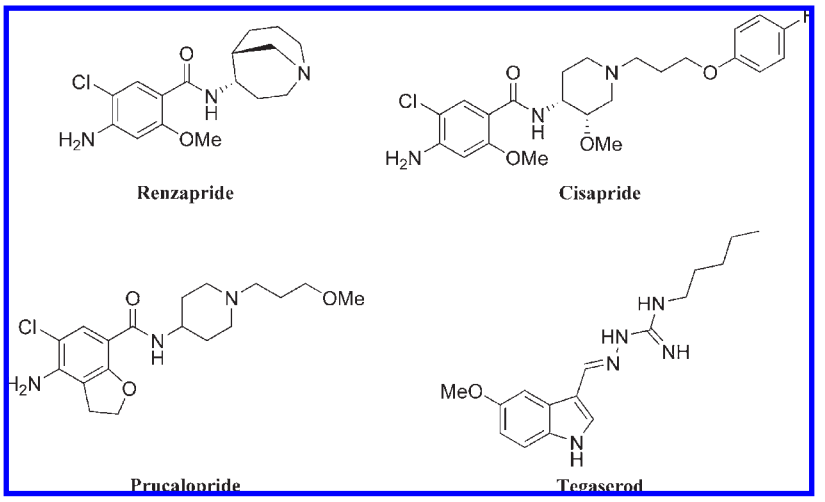


Figure 1. 5-HT_4 agonists.

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 subjects[22] 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-HT_4 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-HT_7).[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-HT_4 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-HT_4 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-HT_3 ligands, several 5-HT_4 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

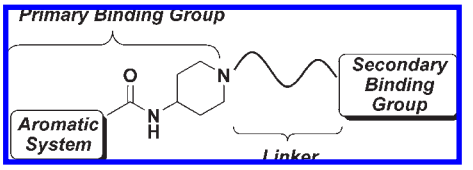


Figure 2. 5-HT_4 agonist pharmacophore.

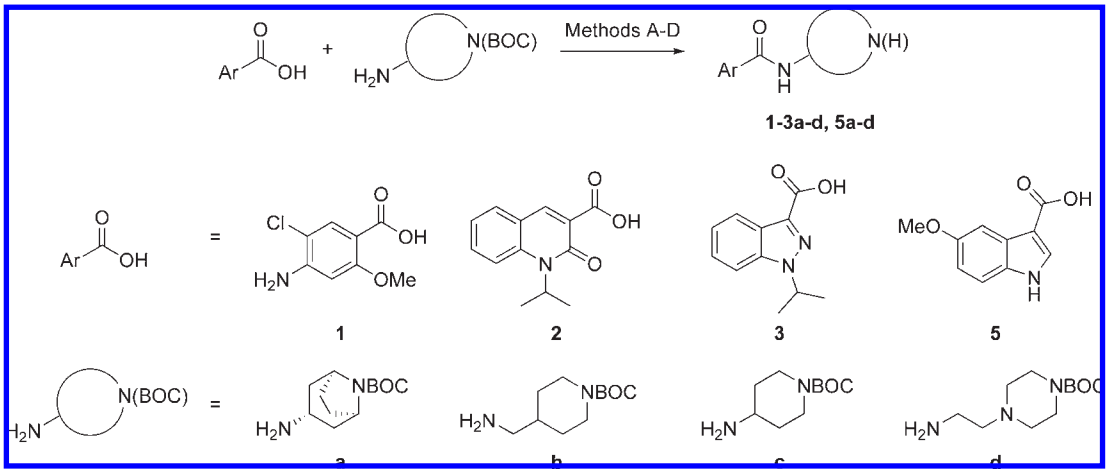
of 5-HT_4 agonist primary binding groups,[35,36] relatively few studies have focused on variation of the secondary binding group as a means to achieve the desired pharmacokinetic and pharmacological profile for an orally bioavailable prokinetic agent.[37,38] In light of this, a multivalent approach to the lead discovery efforts was adopted in which functionally diverse secondary binding groups were attached via linkers of variable length to selected 5-HT_4 agonist primary binding groups, with the goal of enhancing their potency, oral pharmacokinetics, and selectivity over the 5-HT_3 receptor (5-HT_3 antagonism is known to reduce gastrointestinal motility in man and may partially attenuate the clinical benefit of 5-HT_4 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).

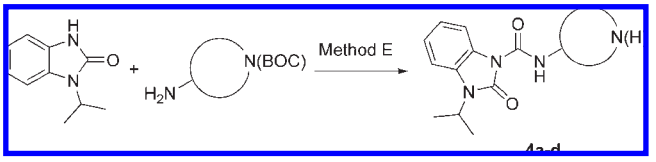
Attachment of Linkers and Secondary Binding Groups. Compounds **6a**−**l** and **7a**−**l** were furnished by simple *N*-alkylation of compounds **1c** and **2a**, respectively, using commercially available alkyl bromides and potassium iodide in dimethylformamide (method F, Scheme 3). Compounds that 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]



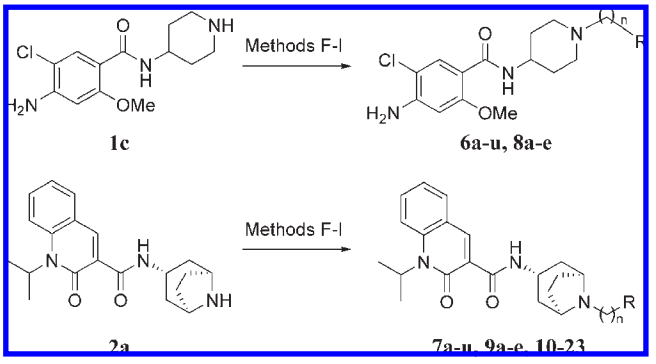
[a] Reagents and conditions: Method A: (a) CDI, DMF; (b) TFA, DCM. Method B (a) EDC, iPr_2NEt, DMF; (b) TFA, DCM. Method C: (a) EDC, HOBt, iPr_2NEt, DMF; (b) TFA, DCM. Method D: (a) $SOCl_2$, NaOH, PhMe, H_2O (b) TFA, DCM.

Scheme 2[a]



[a] Reagents and conditions: mMethod E: (a) NaH, THF; (b) *p*-$NO_2C_4H_4OCOCl$, THF; (c) TFA, DCM.

Scheme 3[a]



[a] Reagents and conditions: Method F: (a) $Br(CH_2)_nR$, KI, $NaHCO_3$, DMF, H_2O. Method G: (a) R_1R_2NH, $Br(CH_2)_nBr$, KI, $NaHCO_3$, DMF, H_2O. Method H: (a) R_1R_2NH, $Br(CH_2)_nBr$, iPr_2NEt, MeOH. Method I: (a) $Br(CH_2)_nOH$, Et_3N, MeCN; (b) $PySO_3$, iPr_2NEt, DMSO, CH_2Cl_2; (c) R_1R_2NH, $NaBH(OAc)_3$, 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-(oxiranylmethyl)piperazine. This was then treated with compound **2a** to afford the desired products (Scheme 4).

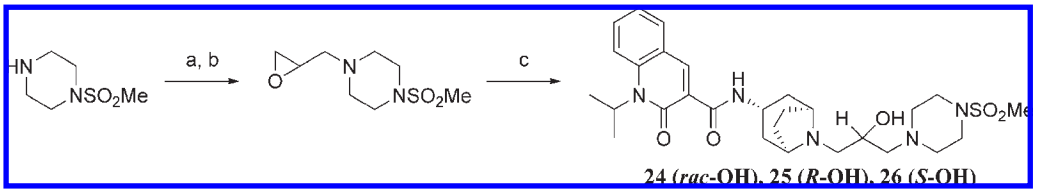
Results and Discussion

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 (pEC_{50}), and intrinsic activity (IA) of a range of primary binding groups at the human recombinant 5-HT_4 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-aminoethyl)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-HT_3 receptor than the 5-HT_4 receptor. Several compounds (**3b**, **5c**, **5d**) were antagonists at the 5-HT_4 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-HT_4 agonist.

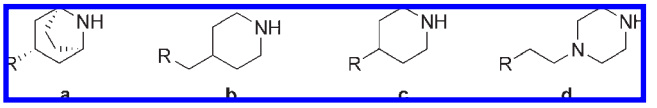
Exploring the Secondary Binding Site. To rapidly assess the electronic, steric, and positional preferences of the putative secondary binding site, we designed a selection of secondary binding groups which encompassed neutral, acidic, and basic functionality and which were capable of varying degrees of 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.

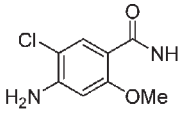
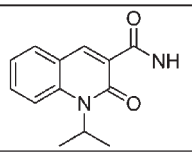
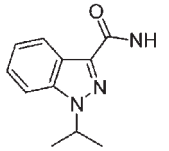
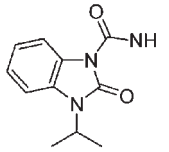
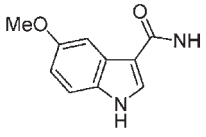
Scheme 4[a]



[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-HT$_4$ Agonist Primary Binding Groups



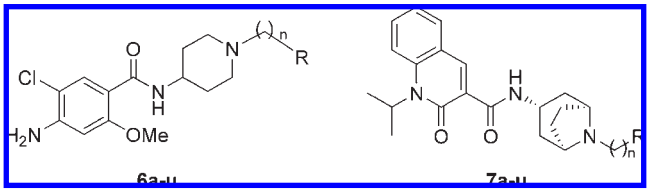
R	Compound Number	Method	5-HT$_4$ pK_i[a]	5-HT$_3$ K_i/ 5-HT$_4$ K_i	5-HT$_4$ pEC$_{50}$[c]	5-HT$_4$ I.A.[d]
	**1a**	A	6.8	0.2	7.0	57
	1b	B	6.5	0.12	6.4	81
	1c	A	5.8	0.095	6.4	>100
	1d	C	5.9	1.1	6.3	>100
	**2a**	D	6.6	0.85	7.9	>100
	2b	D	6.1	1.2	7.0	>100
	2c	D	5.5	0.14	6.8	>100
	**3a**	A	7.2	0.092	7.4	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

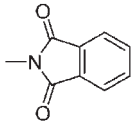
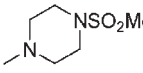
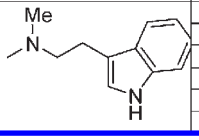
[a] 5-HT$_4$ pK_i values were determined using [^{3}H]-GR113808 in HEK-293 cells stably transfected with human 5-HT$_{4(c)}$ receptor cDNA; 5-HT$_3$ pK_i values were determined with [^{3}H]-GR65630 in HEK-293 cells stably transfected with human 5-HT$_{3A}$ receptor cDNA. [b] Selectivity for the 5-HT$_4$ receptor subtype with respect to the 5-HT$_3$ receptor subtype was calculated as the ratio K_i(5-HT$_{3A}$)/K_i(5-HT$_{4(c)}$). [c] 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. [d] Maximum compound-evoked response (minus basal) was expressed as a percentage of the maximum response evoked by 5-HT.

Because selectivity over 5-HT$_3$ was considered essential, affinity for this receptor was included in the primary screen together with functional potency and intrinsic activity at the 5-HT$_4$ 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-HT$_3$ and 5-HT$_4$ receptors, with the resultant improvement in selectivity in favor of 5-HT$_4$, 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-HT$_4$ pK_i or pEC$_{50}$ relative to the simple alkyl chain, indicating a lack of significant interactions of

Table 2. 5-HT$_4$ Agonist Secondary Binding Group Scan



Compound		*n*	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	—H	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
7	**a**	2	F		6.9	19	7.5	96
	b	5	F		7.2	890	7.4	>100
	c	8	F		7.2	150	7.4	>100
6	**d**	2	F	—OH	6.7	1.9	7.2	>100
	e	5	F		7.2	110	7.8	>100
	f	8	F		7.1	29	7.8	>100
7	**d**	2	F		6.9	3.2	7.5	>100
	e	5	F		7.2	460	7.6	>100
	f	8	F		7.3	1100	7.8	>100
6	**g**	2	F		5.1	13	6.2	94
	h	5	F		7.7	120	8.3	>100
	i	8	F		7.4	40	8.5	89
7	**g**	2	F		6.0	55	7.1	>100
	h	5	F		7.8	1200	7.6	>100
	i	8	F		7.5	120	7.3	>100
6	**j**	2	F		4.6	1.5	5.8	>100
	k	5	F		5.2	0.15	6.4	93
	l	8	F		4.8	4.3	6.0	>100
7	**j**	2	F		6.0	49	8.2	>100
	k	5	F		5.8	21	7.9	>100
	l	8	F		7.2	230	7.7	>100
6	**m**	2	H		6.6	3.9	7.0	>100
	n	5	I		8.0	990	8.3	>100
	o	8	H		8.5	2000	8.5	>100
7	**m**	2	H		7.1	720	8.0	>100
	n	5	I		8.3	8100	9	>100
	o	8	II		8.5	9900	8.9	99
6	**p**	2	H		6.9	21	7.7	97
	q	5	I		7.4	280	7.9	>100
	r	8	H		7.8	330	7.4	>100
7	**p**	2	H		7.2	450	8.2	>100
	q	5	I		7.8	2800	8.9	>100
	r	8	H		7.8	2100	8.5	>100
6	**s**	2	H		7.4	290	7.8	>100
	t	5	I		8.8	12000	8.9	>100
	u	8	I		8.9	2800	9.1	>100
7	**s**	2	G		7.4	480	8.9	92
	t	5	I		8.5	6000	9.2	>100
	u	8	H		8.5	2300	9.0	>100

[a] 5-HT$_4$ pK_i values were determined using [^{3}H]-GR113808 in HEK-293 cells stably transfected with human 5-HT$_{4(c)}$ receptor cDNA; 5-HT$_3$ pK_i values were determined with [^{3}H]-GR65630 in HEK-293 cells stably transfected with human 5-HT$_{3A}$ receptor cDNA. [b] Selectivity for the 5-HT$_4$ receptor subtype with respect to the 5-HT$_3$ receptor subtype was calculated as the ratio K_i(5-HT$_{3A}$)/K_i(5-HT$_{4(c)}$). [c] 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. [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-HT$_4$ 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-HT$_3$ and the 5-HT$_4$ receptors. Introduction of piperidine as a basic secondary binding group afforded a significant increase in the 5-HT$_4$ 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

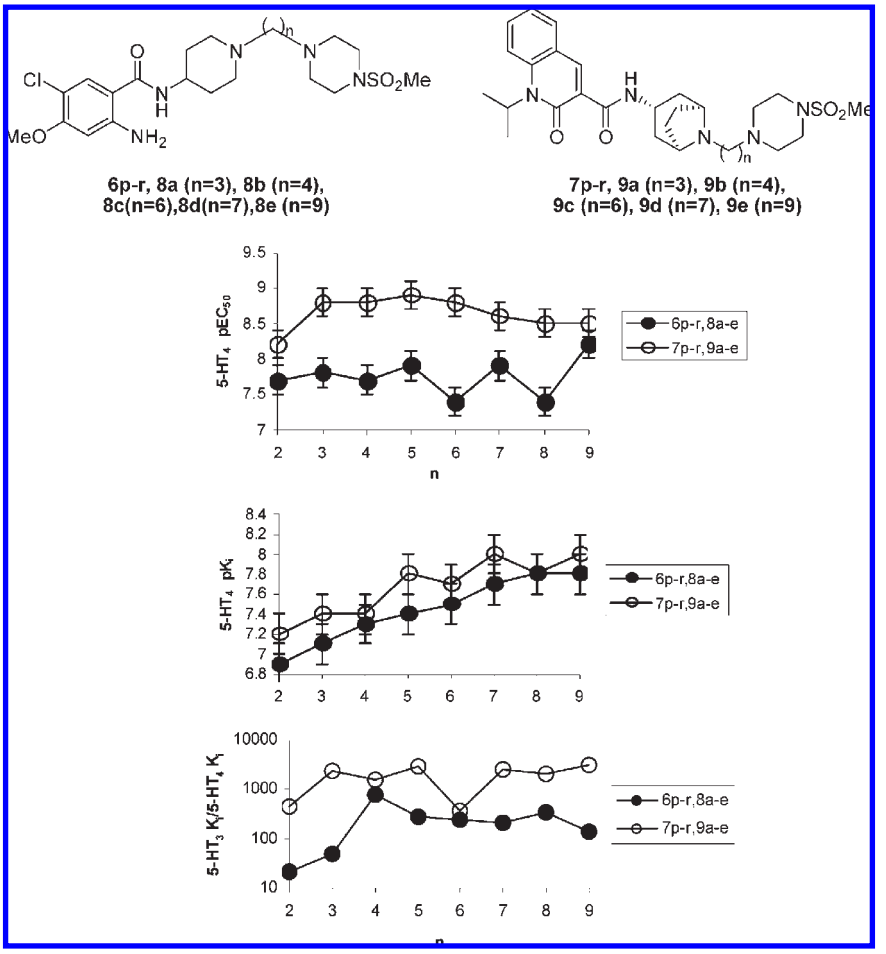


Figure 3. Effect of linker length on 5-HT_4 pK_i, pEC_{50}, and selectivity vs 5-HT_3.

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.

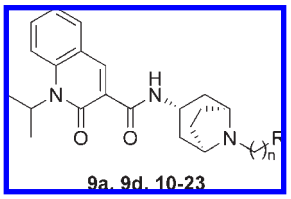
To more fully understand the positional preference of the positively charged secondary binding groups, the in vitro profile of the full set of linker lengths (C2 through C9) was examined using a representative piperazinesulfonamide (Figure 3). With the chloroaniline- and quinolone-piperazinesulfonamide systems (**6p**−**r**, **8a**−**e**, and **7p**−**r**, **9a**−**e**, respectively), the 5-HT_4 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-HT_3 receptor, the quinolone-based agonists were superior to the chloroanilines at all linker lengths except C6, which had unusually high 5-HT_3 binding affinity.

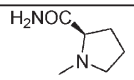
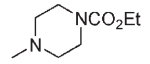
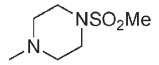
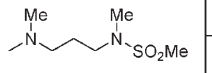
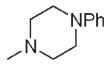
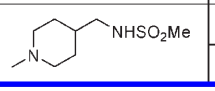
Lead Optimization

Although at this juncture the ability of the amine-based secondary binding groups to consistently elevate the in vitro potency of 5-HT_4 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 pEC_{50} 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-HT_4 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	*n*	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 Kp (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 p*K*alc 3.2 prediction module of Pallas system (CompuDrug Chemistry, Ltd., Hungary). [b] Calculated pK_a of the secondary binding group amine [c] 5-HT$_4$ pK_i values were determined using [^{3}H]-GR113808 in HEK-293 cells stably transfected with human 5-HT$_{4(c)}$ receptor cDNA; 5-HT$_3$ pK_i values were determined with [^{3}H]-GR65630 in HEK-293 cells stably transfected with human 5-HT$_{3A}$ receptor cDNA. [d] Selectivity for the 5-HT$_4$ receptor subtype with respect to the 5-HT$_3$ 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 μM 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**).

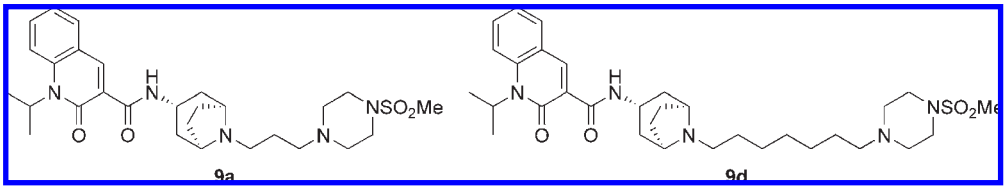
Inhibition of the hERG channel was highly dependent on the nature of the secondary binding group, in some cases being consistently low (prolines **10**, **11**) or high (phenylpiperazines **18**, **19**) and having no clear relationship to calculated pK_a. When piperidine was employed as the secondary 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-HT$_4$ 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-HT$_3$, 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

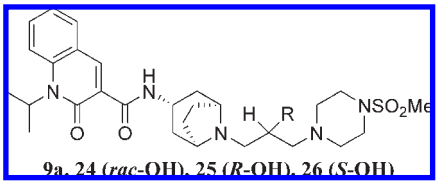


9a **9d**

compd	Caco-2 *Kp* (1×10^{-6} cm/s)	RLM $t_{1/2}$ (min)	$AUC_{(0-t)}$ (μg·h/mL)[a]	C_{max} (μg/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**

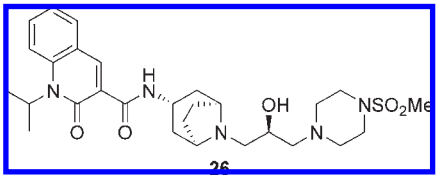


9a, **24** (*rac*-OH), **25** (*R*-OH), **26** (*S*-OH)

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 *Kp* (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-HT_4 pK_i values were determined using [^{3}H]-GR113808 in HEK-293 cells stably transfected with human 5-$HT_{4(c)}$ receptor cDNA; 5-HT_3 pK_i values were determined with [^{3}H]-GR65630 in HEK-293 cells stably transfected with human 5-HT_{3A} receptor cDNA. [b] Selectivity for the 5-HT_4 receptor subtype with respect to the 5-HT_3 receptor subtype was calculated as the ratio K_i(5-HT_{3A})/K_i(5-$HT_{4(c)}$). [c] 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. [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



26

	rat					dog	
compd	RLM $t_{1/2}$ (min)	$AUC_{(0-t)}$ (μg·h/mL)[a]	C_{max} (μg/mL)	$t_{1/2}$ (h)	*F*%[b]	C_{max} (μg/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-HT_7 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** (pEC_{50} = 8.6) was superior to that of 5-HT (pEC_{50} = 6.9) and similar to tegaserod (pEC_{50} = 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-HT_4 receptor activation results in contraction of this smooth muscle preparation[40,41] and the presence of antagonists of 5-HT_1, 5-HT_2 and 5-HT_3 receptors, in this study, the observed activity of **26** was concluded to represent 5-HT_4 receptor activation.

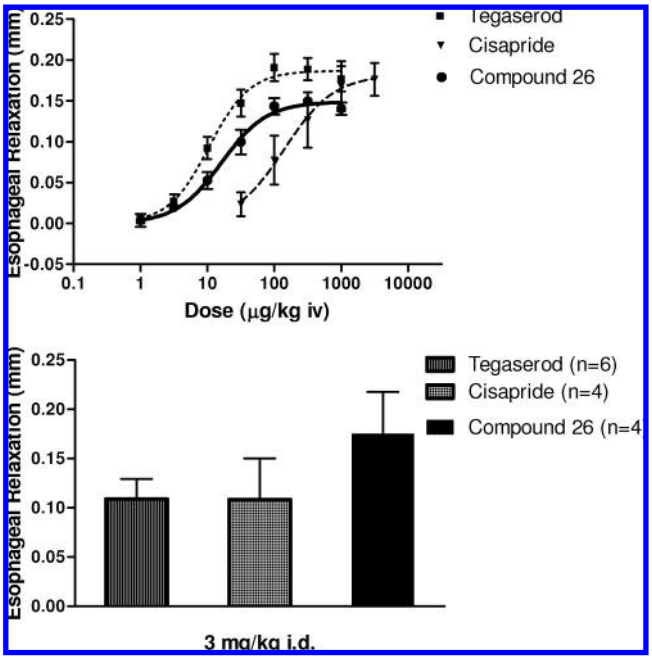


Figure 4. Relaxation of rat esophagus following intravenous and intraduodenal administration of compound **26**.

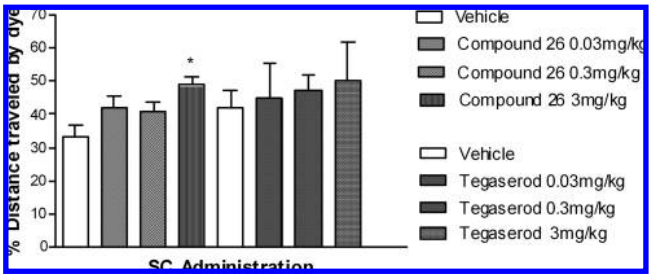


Figure 5. Guinea pig colonic transit following subcutaneous dosing of compound **26**.

To characterize the in vivo activity of **26**, digital sonomicrometry was used to monitor 5-HT_4 receptor-mediated relaxation of the rat esophagus.[42] This method provided a novel and sensitive means to demonstrate 5-HT_4 receptor agonist-mediated changes in endogenous esophageal tone in anesthetized rats. Compound **26** produced a dose-dependent, 5-HT_4 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 ED_{50} values for the relaxant response mediated by intravenous **26**, tegaserod and cisapride were 16, 10.6, and 142 μg/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-HT_4 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-HT_4 receptor binding affinity and selectivity. A study of the role of linker length using a representative amine revealed that 5-HT_4 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-HT_4 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-HT_3, and functional potency as well as a reduction in hERG inhibition. In animal models, **26** produced a dose-dependent, 5-HT_4 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 ^{1}H and ^{13}C NMR spectrometry in deuterated solvents (CD_3OD, 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 × 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 H_3PO_4/brine and 1 M NaOH/brine). The organic phase was dried ($MgSO_4$), evaporated, and the residue purified by flash chromatography (SiO_2) 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 CH_2Cl_2 (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 Et_2O (50 mL). The resultant precipitate was filtered and washed with Et_2O (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/H_2O over 6 min; t_R 2.26 min). ^{1}H 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). ^{13}C 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 diisopropyethylamine (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 $KHSO_4$ (100 mL), 1N NaOH (100 mL), and brine (100 mL) and then dried ($MgSO_4$), 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 Et_2O (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/H_2O over 6 min; t_R 2.72 min). ^{1}H 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). ^{13}C 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 $C_{17}H_{24}N_4O$, 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-dimethylaminopropyl)-*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 NH_4Cl (30 mL), followed by 1 M NaOH (30 mL) and then brine (30 mL). The organics were dried ($MgSO_4$) 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 CH_2Cl_2 (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 Et_2O 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/H_2O over 6 min; t_R 1.99 min). ^{1}H 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). ^{13}C 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 $C_{16}H_{22}N_3O_2$, 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/H_2O over 4 min; t_R 1.32 min). ^{1}H 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). ^{13}C 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 $C_{14}H_{22}ClN_4O_2$, 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/H_2O over 6 min; t_R 2.06 min). ^{1}H 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). ^{13}C 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 $C_{17}H_{22}N_3O_2$, 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-Boc-piperidine; 90% purity by HPLC (2−90% MeCN/H_2O over 6 min; t_R 2.23 min). ^{1}H 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). ^{13}C 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 $C_{15}H_{19}N_3O_2$, 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/H_2O over 6 min; t_R 2.03 min). ^{1}H 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 $C_{16}H_{23}N_4O_2$, 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). ^{1}H 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 $C_{20}H_{25}N_3O_2$, 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-dihydro-quinoline-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). ^{1}H 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 $C_{19}H_{26}N_3O_2$, 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). ^{1}H 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 $C_{18}H_{24}N_3O_2$, 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-dihydrobenzoimidazole-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 rt 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). ^{1}H 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 $C_{18}H_{24}N_4O_2$, 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). ^{1}H 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 $C_{16}H_{23}N_4O_2$, 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). ^{1}H 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 $C_{17}H_{25}N_5O_2$, 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-[5-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-pentyl]-8-aza-bicyclo[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 H_2O (10 mL). The organic phase was evaporated and the residue purified by preparative reverse-phase HPLC (2−90% MeCN/H_2O over 50 min) to afford **7h** as a white solid (55 mg, 19%); 97% purity by HPLC (2−90% MeCN/H_2O over 6 min; t_R = 3.78 min). ^{1}H 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). ^{13}C 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 $C_{33}H_{38}N_4O_4$, 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 $NaHCO_3$ (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 CH_2Cl_2 (10 mL) and H_2O (10 mL). The organic phase was evaporated, and the residue purified by preparative reverse-phase HPLC (2−90% MeCN/H_2O over 50 min) to afford **13** as a white solid (2.7 mg, 5%); 98% purity by HPLC (2−90% MeCN/H_2O over 6 min; t_R = 2.92 min). ^{1}H 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). ^{13}C 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 $C_{30}H_{44}N_5O_4$, 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/H_2O over 6 min; t_R = 2.91 min). ^{1}H 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 $C_{27}H_{40}N_5O_4S$, 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). Et_3N (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 $NaHCO_3$ 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 CH_2Cl_2 (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 CH_2Cl_2 (250 mL). The solution was washed (saturated $NaHCO_3$ then brine), dried ($MgSO_4$), 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/H_2O over 6 min; t_R = 2.47 min). ^{13}C 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 $C_{25}H_{43}ClN_5O_4S$, 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/H_2O (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 CH_2Cl_2 (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 ($MgSO_4$), filtered, and exaporated 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%). ^{1}H 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/H_2O (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/H_2O over 6 min; t_R = 2.84 min); optical purity >99% by chiral CE. ^{1}H NMR (CD_3OD, 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). ^{13}C 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 $C_{28}H_{42}N_5O_5S$, 560.2907; found, 560.2907.

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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.

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