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Identification of Multiple 5-HT$_4$ Partial Agonist Clinical Candidates for the Treatment of Alzheimer's Disease

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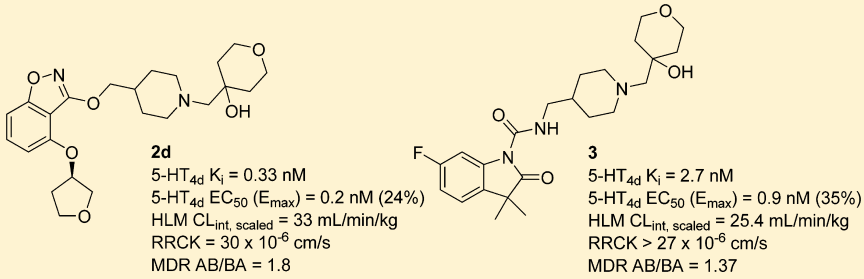
Supporting Information



ABSTRACT: The cognitive impairments observed in Alzheimer's disease (AD) are in part a consequence of reduced acetylcholine (ACh) levels resulting from a loss of cholinergic neurons. Preclinically, serotonin 4 receptor (5-HT$_4$) agonists are reported to modulate cholinergic function and therefore may provide a new mechanistic approach for treating cognitive deficits associated with AD. Herein we communicate the design and synthesis of potent, selective, and brain penetrant 5-HT$_4$ agonists. The overall goal of the medicinal chemistry strategy was identification of structurally diverse clinical candidates with varying intrinsic activities. The exposure−response relationships between binding affinity, intrinsic activity, receptor occupancy, drug exposure, and pharmacodynamic activity in relevant preclinical models of AD were utilized as key selection criteria for advancing compounds. On the basis of their excellent balance of pharmacokinetic attributes and safety, two lead 5-HT$_4$ partial agonist candidates **2d** and **3** were chosen for clinical development.

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia and is characterized by cognitive decline, primarily affecting memory.[1] The societal impact of caring for people with AD and dementia is enormous. In 2011, family members and unpaid caregivers provided 17 billion hours of care in the U.S. and worldwide costs for 2009 were estimated at $422 billion, highlighting the need for more effective therapies.[2,3] The exact cause of AD is not known; however, some of the earliest pathology that occurs involves loss of acetylcholine (ACh) releasing cholinergic neurons in brain regions critical to cognitive function, such as the frontal cortex and hippocampus.[4,5] Currently, AD treatment centers on the use of acetylcholinesterase inhibitors that nonselectively increase ACh throughout the brain. An approach that targets the cholinergic circuitry within specific brain regions involved in cognition might provide improved therapeutic relief.

5-HT$_4$ receptors are G-protein-coupled receptors (GPCRs) positively coupled to adenylate cyclase and are found in brain areas that are critical for cognitive function, including the cortex and hippocampus.[6] Preclinical evidence indicates that 5-HT$_4$ receptor agonists increase Ach-release in these areas.[7,8] In various behavioral models, pharmacological induction of memory deficits with anticholinergic drugs (e.g., atropine and scopolamine) has been used to simulate the cognitive impairments associated with the loss of cholinergic function in AD, and 5-HT$_4$ agonists have been shown to reverse these deficits.[9,10] On the basis of these reports, 5-HT$_4$ receptor agonists might provide a new mechanistic approach for providing symptomatic relief in AD.

A 5-HT$_4$ agonist that is suitable for clinical development must be potent and highly selective. Early generation 5-HT$_4$ agonists, such as tegaserod (**I**) and cisapride (**II**), although clinically effective in the treatment of gastrointestinal motility disorders, caused serious adverse events that were likely related to off-target pharmacology, a property that has severely restricted their use (Figure 1).[11] Second generation agonists such as prucalopride (**III**) were recently approved in Europe and Canada for the treatment of constipation. From the perspective of treating central nervous systems (CNS) indications, a major limitation of these early generation 5-

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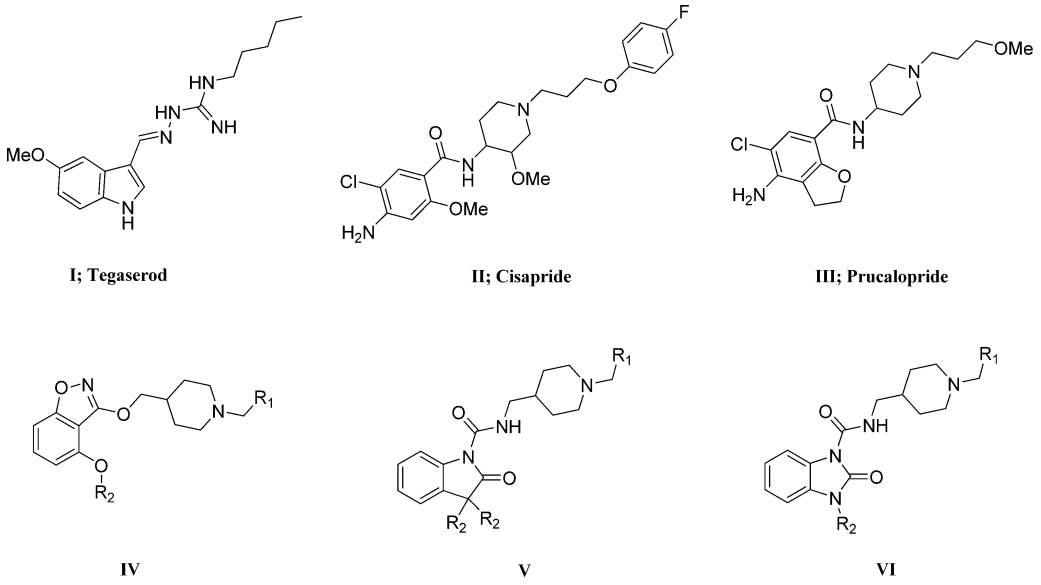
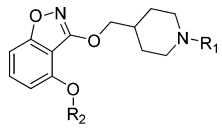
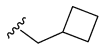
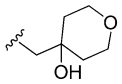


Figure 1. Literature 5-HT$_4$ agonists (**I**–**III**) and lead chemical series (**IV**–**VI**).

Table 1. 5-HT$_{4d}$ Binding, Functional Activity, Passive Permeability, and in Vitro Human Microsomal Clearances of Analogues 1a–f[e]



Compound	R_1	R_2	5-HT$_{4d}$ K_i[a]	5-HT$_{4d}$ EC_{50} (E_{max})[b]	HLM $CL_{int,\ scaled}$ (% inh CYP_{2D6})[c]	RRCK Papp AB (10^{-6} cm/s)[d]
1a	*n*-Bu		0.27 nM	0.8 nM (53%)	77 mL/min/kg (91%)	2.1
1b	*n*-Bu		0.74 nM	1.2 nM (81%)	77 mL/min/kg (79%)	2.4
1c	*n*-Bu		0.97 nM	1.1 nM (89%)	ND (93%)	ND
1d	*n*-Bu		0.29 nM	1.2 nM (73%)	70 mL/min/kg (87%)	0.31
1e	*n*-Bu		5.2 nM	6.3 nM (95%)	ND (93%)	ND
1f			0.36 nM	0.98 nM (85%)	72 mL/min/kg (54%)	3.8

[a]Binding K_i values (geometric mean; at least n = 3 measurements) were determined in HEK293 cells stably expressing the human 5-HT$_{4d}$ isoform using [^{3}H]GR113808 as the radioligand . [b]Functional activity was assessed as the EC_{50} and E_{max} values for stimulation of cAMP production in HEK293 cells stably expressing the human 5-HT$_{4d}$ receptor isoform. EC_{50} values (geometric mean; at least n = 3 measurements) represent the concentration producing 50% of the maximal cAMP response. E_{max} values (arithmetic mean) represent the maximal effect on cAMP production expressed as a percentage of a maximal response of 5-HT (a full agonist). [c]Predicted hepatic apparent clearance (h-$CL_{int,scaled}$) from human liver microsomal stability assay using well-stirred approach. CYP_{2D6} inhibition was obtained by measuring inhibition of dextromethorphan at 3 μM substrate concentration. [d]RRCK cells with low transporter activity were isolated from Madin–Darby canine kidney cells and were used to estimate intrinsic absorptive permeability.[17] [e]ND: not determined.

HT$_4$ agonists was poor brain penetration likely due to P-glycoprotein (Pgp) mediated efflux.[12]

An additional concern is that prolonged exposure of GPCRs to an agonist can result in receptor desensitization, thereby limiting the agent's therapeutic usefulness. Agonist-induced desensitization is often dependent on the degree of intrinsic activity; while weak partial agonists minimize the potential for desensitization,[12] they can in fact act as functional antagonists when low receptor numbers are present. Therefore, it is critical to identify compounds with appropriate intrinsic activities.

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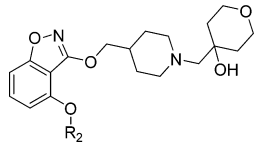
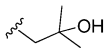
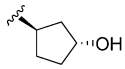
Table 2. 5-HT_4 Binding, Functional Activity, in Vitro Human Microsomal Clearance, and P-gp Liability of Analogues 2a−d



Compound	R_2	clogP	5-HT_{4d} K_i[a]	5-HT_{4d} EC_{50} (E_{max})[b]	HLM $CL_{int\ scaled}$ (% inh CYP_{2D6})[c]	RRCK P_{app} AB (10^{-6} cm/s)[d]	MDR Er[e]
2a		1.6	3.6 nM	1.7 nM (50%)	38 mL/min/kg (21%)	35	1.6
2b		2.5	0.50 nM	0.40 nM (28%)	56 mL/min/kg (37%)	15	2.2
2c		1.6	4.5 nM	1.9 nM (85%)	60 mL/min/kg (30%)	32	1.4
2d		1.7	0.15 nM	0.2 nM (24%)	33 mL/min/kg (13%)	30	1.8

[a]Binding K_i values (geometric mean; at least $n = 3$ measurements) were determined in HEK293 cells stably expressing the human 5-HT_{4d} isoform using [^{3}H]GR113808 as the radioligand. [b]Functional activity was assessed as the EC_{50} and E_{max} values for stimulation of cAMP production in HEK293 cells stably expressing the human 5-HT_{4d} receptor isoform. EC_{50} values (geometric mean; at least $n = 3$ measurements) represent the concentration producing 50% of the maximal cAMP response. E_{max} values (arithmetic mean) represent the maximal effect on cAMP production expressed as a percentage of a maximal response of 5-HT (a full agonist). [c]Predicted hepatic apparent clearance (h-$CL_{int,scaled}$) in HLM, CYP_{2D6} inhibition was obtained by measuring inhibition of dextromethorphan at 3 μM substrate concentration. [d]RRCK cells with low transporter activity were isolated from Madin−Darby canine kidney cells and were used to estimate intrinsic absorptive permeability.[17] [e]MDR efflux ratio (MDR Er) from an MDR1-transfected MDCK cell line represents the ratio of permeability, P_{app}(BA)/P_{app}(AB).

On the basis of this information, our criteria for the selection of a desirable CNS candidate were high binding affinity (<10 nM), excellent off-target selectivity (>100×), low intrinsic activity (<50%), no significant barriers to CNS penetration, and an acceptable safety (tolerability and cardiovascular) profile. As part of a longstanding effort to discover $5HT_4$ agonists for the treatment of gastrointestinal (GI) disorders, a diverse set of chemical series possessing excellent oral drug properties had been identified. Since 5-HT_4 receptor activation in the GI is peripherally mediated, optimization of physicochemical properties for CNS indications was not a key design element of the previous program. To improve our chances of identifying compounds with excellent CNS penetration, the known chemical series were triaged to select cores with a reduced number of hydrogen bond donors (≤1), low molecular weight (<400), and weakly basic amine centers (pK_a < 8.5).[13] From this analysis, multiple chemotypes emerged possessing the above CNS criteria, as represented by benzisoxazole **IV**, oxindole **V**, and benzimidazolidinone **VI** (Figure 1).[14−16] We postulated that the R_1 and R_2 positions in each chemical series **IV**−**VI** would provide enabled and flexible handles to rapidly modulate intrinsic activity and ADME properties. Herein we disclose the SAR, pharmacological, pharmacodynamic, and safety profiles of the lead clinical candidates derived from these starting points.

RESULTS AND DISCUSSION

Our efforts to identify viable CNS development candidates began by exploring alkyl and cycloalkyl ether substituents (OR_2) off the benzisoxazole ring while maintaining an *n*-butyl substituent on the piperidine (examples **1a**−**e**, Table 1; see Chemistry section for synthesis). Initial in vitro binding studies were conducted in a HEK293 cell line expressing the 5-HT_{4d} isoform of the human 5-HT_4 receptor. Analogues **1a**−**e** with cycloalkyl and branched alkyl R_2 substituents showed high binding and functional activity, with a range of E_{max} values from 50% to 95%. SAR at the R_2 position of the benzisoxazole indicated that the molecular shape and size of this group might provide a means to modulate intrinsic activity. For example, replacement of the cyclopentyl group (**1b**) with cyclobutyl (**1a**) resulted in a reduction in E_{max} from 81% to 53%. This series of compounds demonstrated high clearance from human liver microsomes (HLM) and low passive permeability in the RRCK cell line, likely due to the high lipophilicity (ClogP > 5) of analogues **1a**−**e**.[17] To test whether replacing the piperidine substituent (R_1) was a viable strategy to introduce polarity, the *n*-butyl side chain of compound **1d** was modified to afford hydroxyl-THP analogue **1f**. Despite the overall reduction in ClogP (5.5−3.5) and modified pK_a, analogue **1f** demonstrated high intrinsic clearance in HLM and significant inhibition of CYP_{2D6} at a single substrate concentration of 3 μM.

Despite the significant reduction in lipophilicity for compound **1f** relative to **1d**, the similarly high microsomal intrinsic clearance suggested that further reductions in ClogP were required as a key design criteria. To this end, the isobutyl side chain (R_2) was replaced with more polar groups such as analogues **2a**−**d** (Table 2; see Chemistry section for synthesis). The tertiary carbinol **2a** exhibited excellent potency and functional activity (E_{max} = 50%) with reduced clearance and low CYP_{2D6} liability. In addition, the compound showed high passive permeability and no predicted P-gp mediated efflux as measured by MDR efflux ratio in MDCK-MDR1 cell lines.[18] Further modification of the R_2 group led to cyclopentyl carbinol **2b** and tetrahydropyran **2c** which exhibited poor

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metabolic stability. Interestingly, the degree of intrinsic activity appeared to correlate with the size of the R_2 group as demonstrated by tetrahydropyran **2c** which is now a full agonist relative to 5-HT (E_{max} = 85%), in the 5-HT_{4d} functional assay. Reduction of the ring size from tetrahydropyran to tetrahydrofuran provided (4-{4-[(*R*)-(tetrahydrofuran-3-yl)-oxy]benzo[*d*]isoxazol-3-yloxymethyl}piperidin-1-ylmethyl)-tetrahydropyran-4-ol (**2d**, PF-04995274), a potent (5-HT_{4d} K_i = 0.15 nM), partial agonist (E_{max} = 24%) with low clearance and no predicted efflux from Pgp.[16]

To understand the brain penetration of analogue **2d**, a single dose was subcutaneously (sc) administered to rats and time points over a range of 0−4 h were studied. Brain tissue, plasma, and CSF were collected and analyzed for total drug exposure levels using LC−MS/MS. Protein binding values were also determined using equilibrium dialysis to calculate free drug exposures in plasma and brain as previously described.[19] As shown in Table 3, compound **2d** exhibited excellent brain penetration, as measured by unbound brain to plasma ratio ($C_{u,b}/C_{u,p}$) of 0.7 and CSF to unbound plasma ratio (CSF/$C_{u,p}$) of 0.5 for AUC_{0-4h}.

Table 3. Neuropharmacokinetic Parameters of Lead 5HT_4 Partial Agonists in Rats[a]

parameter	2d	3	4
$C_{u,b}$ (AUC_{0-4h}, nM·h)	706	2922	44.3
$C_{u,p}$ (AUC_{0-4h}, nM·h)	1054	1669	27.6
CSF (AUC_{0-4h}, nM·h)	519	1381	ND
$C_{u,b}/C_{u,p}$	0.7	1.8	1.6
CSF/$C_{u,b}$	1.4	2.1	ND
CSF/$C_{u,p}$	0.5	0.8	ND

[a]Ratios are derived from respective matrices by measuring brain, plasma, and CSF concentrations over 0−4 h followed by calculating AUC_{0-4h} in Winnonlin (Pharsight, California) for 2−4 animals; $C_{b,u}$ = total brain concentration *$f_{u,b}$ for each compound; $C_{p,u}$ = total plasma concentration*$f_{u,p}$ for each compound; ND: not determined. CSF: cerebrospinal fluid.

The excellent brain penetration, low partial agonism, and high binding affinity for compound **2d** led us to consider that additional chemical matter containing the hydroxyl tetrahydropyran R_1 group might provide similar alignment of CNS properties.[13] To this end, piperidine−THP containing analogues in the oxindole series (**V**) and imidazolidinone series (**VI**) were selected for additional profiling (Figure 1).

The key compounds from the oxindole and imidazolidione series were analogue 6-fluoro-3,3-dimethyl-2-oxo-2,3-dihydroindole-1-carboxylic acid [1-(4-hydroxytetrahydropyran-4-ylmethyl)piperidin-4-ylmethyl]amide (**3**, PF-03382792) and 6-fluoro-*N*-({1-[(4-hydroxytetrahydro-2*H*-pyran-4-yl)methyl]-piperidin-4-yl}methyl)-3-isopropyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-1-carboxamide (**4**, PF-01352968).[14,15] Analogues **3** and **4** were highly potent in binding and functional assays with low predicted Pgp efflux (Figure 2). Assessment of brain penetration for **3** ($C_{u,b}/C_{u,p}$ = 1.8) and **4** ($C_{u,b}/C_{u,p}$ = 1.6) in rats, as shown in Table 3, confirmed excellent brain penetration properties for both analogues.

To increase our understanding around the 5-HT_4 isoform and tissue selectivity, in vitro binding studies were conducted using cells lines expressing the 4a, 4b, 4d, and 4e isoforms of the human 5-HT_4 receptor as well as brain membranes prepared from rat, dog, and monkey striatal tissue (Table 4).

Table 4. In Vitro Binding Studies Performed Using Transfected Cells and Native Tissue[a]

			binding K_i (nM)		
tissue	receptor		2d	3	4
HEK293	human	4a	0.36	4.6	4.4
HEK293	human	4b	0.46	6.8	5.5
HEK293	human	4d	0.15	2.7	1.7
CHO	human	4e	0.32	6.3	4.9
striatal membranes	rat		0.30	3.9	1.5
striatal membranes	dog		0.30	4.8	3.8
striatal membranes	monkey		0.27	8.9	1.9

[a]Binding studies were conducted using human 5-HT_4 receptor isoforms stably expressed in cells lines or native receptors in brain membranes prepared from striatal tissue. K_i values are expressed as the geometric mean of three to six determinations. HEK is human embryonic kidney. CHO is Chinese hamster ovary.

Compound **2d** displayed subnanomolar affinity for the various human receptor isoforms (K_i of 0.15−0.46 nM) and showed similar potencies in native tissue preparations (K_i of 0.27−0.30 nM). Analogues **3** and **4** were approximately 10-fold less potent than **2d**, with K_i values in the nanomolar range. The binding potencies for all three compounds were generally consistent across species.

The ability of compounds **2d**, **3**, and **4** to increase cAMP production across multiple 5-HT_4 isoforms and consistently between human and rats was next examined (Table 5).

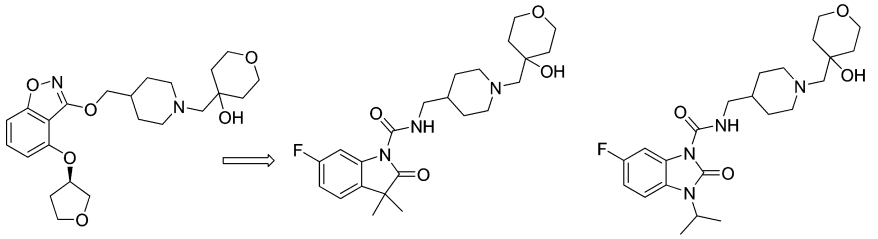

Figure 2. Identification of higher intrinsic activity 5-HT_4 agonists.

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Table 5. Cyclic AMP Production in Cell Lines Expressing Human and Rat 5-HT$_4$ Receptor Isoforms[a]

		EC_{50}, nM (E_{max}, %)		
species	isoform	**2d**	**3**	**4**
human	4a	0.47 (33)	0.8 (53)	0.38 (89)
	4b	0.36 (6)	1.3 (40)	1.20 (61)
	4d	0.37 (15)	0.9 (35)	1.13 (88)
	4e	0.26 (7)	1.2 (21)	0.98 (58)
rat	4l	0.65 (22)	1.52 (46)	0.96 (105)
	4s	0.59 (29)	1.15 (63)	0.90 (106)
	4e	0.62 (15)	2.15 (37)	1.59 (104)

[a]In vitro studies comparing the compounds' abilities to stimulate cAMP production were conducted in HEK293 cells transiently expressing human and rat receptor isoforms. EC_{50} and E_{max} values are expressed as geometric and arithmetic means, respectively. Mean values are based on three to four determinations. EC_{50}, 50% of the maximal compound response, values are reported in nM. E_{max}, maximal effect on cAMP production, is expressed as a percentage of the response observed with 1 μM 5-HT, a full agonist.

Compound **2d** displayed high functional potencies with EC_{50} values ranging from 0.26 to 0.65 nM across the human and rat receptor isoforms, closely matching its binding affinity. For compounds **3** and **4**, EC_{50} values ranged from 0.38 to 2.15 nM and were 2- to 10-fold lower than their respective binding K_i values. For each compound, E_{max} values showed some variation across the receptor isoforms tested. With respect to intrinsic activity levels across compounds, the rank order for E_{max} values was **2d** < **3** < **4** for each receptor isoform.

In vivo RO was determined as previously described using [^{3}H]-(1-methylpiperidin-4-yl)methyl-8-amino-7-chloro-2,3-dihydro-1,4-benzodioxine-5-carboxylate-([^{3}H]SB207145).[12] One hour following subcutaneous administration, **2d**, **3**, and **4** were all shown to have penetrated the brain and bind to striatal 5-HT$_4$ receptors in a dose-dependent manner (Figure 3 A). Whole brain tissue was collected from the animals tested for in vivo RO and drug exposures measured (see Supporting Information Tables 1−3). Free brain drug concentrations ($C_{b,u}$) were calculated by incorporation of free fractions in brain to total brain exposures measured using LC−MS/MS. Plots of $C_{b,u}$ versus % RO were fitted to a simple E_{max} model (eq 1) using NONMEM, via the naive-pooled approach.[20]

$$\%\ \text{receptor occupancy} = E_0 + \frac{E_{max} \times \text{concentration}^{\gamma}}{EC_{50}{}^{\gamma} + \text{concentration}^{\gamma}} \quad (1)$$

The relationship between $C_{b,u}$ vs RO is shown for compounds **2d** (Figure 3B), **3** (Figure 3C), and **4** (Figure 3D). The simple E_{max} model generally described the observed data (Figure 3). By use of the model, free brain EC_{50} and E_{max} values were determined for **2d** (0.48 nM, 15%), **3** (19.7 nM, 53%), and **4** (10.9 nM, 86%). Relative to the 5-HT$_{4d}$ binding K_i values (0.33 nM, 2.7 nM and 1.7 nM), these in vivo binding values were respectively 1.45-, 7.3-, and 6.4-fold higher. We have previously observed differences between in vitro and in vivo derived K_i values for the high intrinsic activity of the 5-HT$_4$ full agonist prucalopride (11-fold).[12] Since the degree of separation observed between in vitro and in vivo derived K_i values appears to generally correlate with the 5-HT$_4$ agonism, this difference might be due to the degree of interaction occurring with high- and low-affinity binding states, which have been described for 5-HT$_4$ receptors.[12,21] The free brain % E_{max} values determined using the model showed the same rank order of effect that was observed for E_{max} values determined in vitro for 5-HT$_{4d}$ receptors (**2d**, 24%; **3**, 35%; **4**, 86%).

The isolated rat esophagus has been used previously to test the functional response of 5-HT$_4$ receptor ligands at native receptors in intact tissue.[22] 5-HT$_4$ receptors are also located on enteric neurons in the gastrointestinal tract, and agonist activation of these receptors produces an increase in gastrointestinal transit by modulating contraction and relaxation.[23,24] Functional activities of compounds **2d**, **3**, and **4**, were compared to the high intrinsic activity agonist prucalopride (**III**) and to the endogenous full agonist 5-HT for their ability

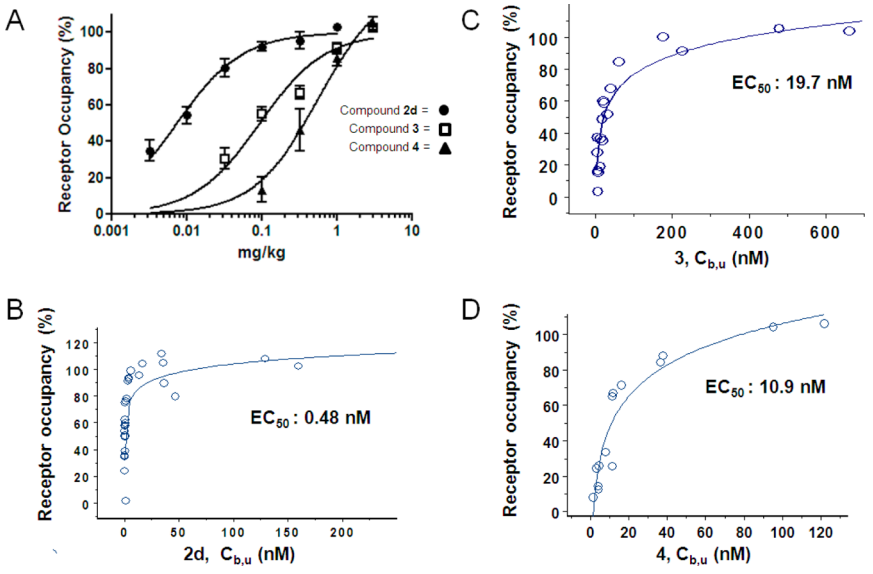

Figure 3. Compound **2d**, compound **3**, and compound **4** (A) displaced radioligand binding to rat striatal membranes. Data shown are the mean ± SEM for RO and the mean ± SD for exposure, combined from two to three experiments; n = 4−8 animals. Free brain exposure measured from individual animals ($C_{b,u}$) vs RO data (blue circles) were fitted to an E_{max} model, with resulting predicted values. Open circles represent observed data, whereas the blue solid line represents the simulated trials for compound **2d** (B), compound **3** (C), and compound **4** (D).

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to relax carbamylcholine contracted rat esophagus (Figure 4A).[25] In comparison to 5-HT (10 μM), **2d** (10 μM) and **3** (10 μM) produced incomplete relaxations that were 21% and 22% of the response observed with 5-HT, respectively, and were indicative of partial agonism (Figure 4A). The relaxation observed in response to **4** (10 μM) was reflective of its higher intrinsic acticvity in that it produced a relaxation that was approximately 70% of that observed with 5-HT (Figure 4A). Intrinsic activity levels for prucalopride reportedly range from 52% to 77% in cell lines expressing the 4l, 4s, and 4e isoforms of the rat 5-HT$_4$ receptor.[12] Under the test conditions used in this study, prucalopride (10 μM) produced a relaxation that was equivalent to the full agonist 5-HT (Figure 4A).

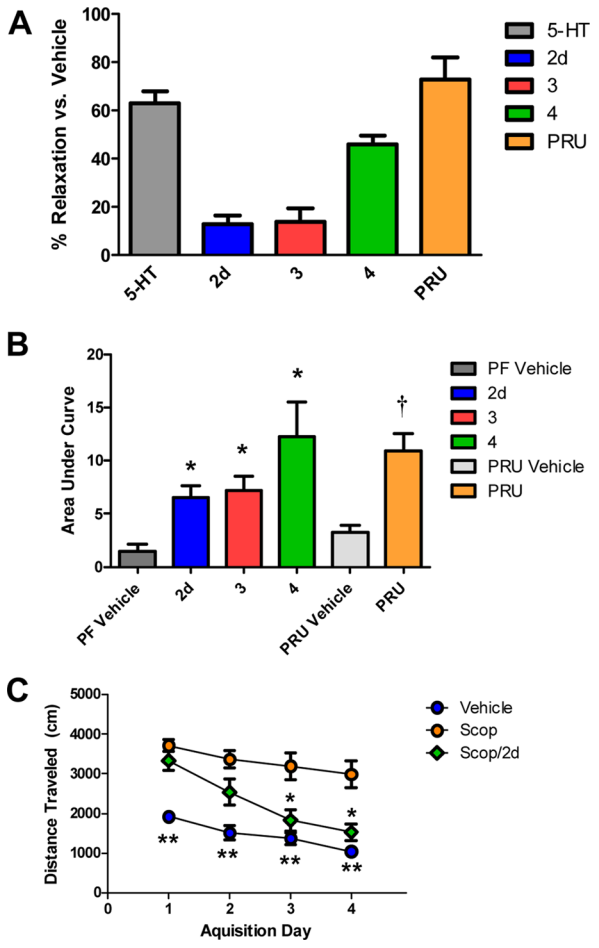

Figure 4. (A) 5-HT, compound **2d**, compound **3**, compound **4**, and prucalopride (PRU, **III**) relaxed carbamylcholine-contracted rat esophagus in a manner that was consistent with the compounds' intrinsic activity level. Data are the mean ± SEM (n = 4–17) and represent the percent relaxation compared to vehicle. (B) Compounds **2d**, **3**, **4**, and prucalopride increased ACh release in the rat prefrontal cortex. Data are expressed as the mean ± SEM of the AUC as a fraction of baseline (five to seven animals per group): unpaired t tests, (*) $P < 0.05$ vs PF-compound vehicle, (†) $P < 0.05$ vs PRU vehicle. (C) Compound **2d** reversed scopolamine-induced learning deficits in the rat Morris water maze. The distance traveled to find the platform during acquisition testing was used as the test parameter. Data were analyzed by two-way ANOVA (days × treatment) followed by Dunnett's multiple comparison test: (*) $P < 0.05$ or (**) $P < 0.01$ versus scopolamine, n = 12 animals per group. PRU is prucalopride. Scop is scopolamine.

Previous studies have shown that activation of 5-HT$_4$ receptors increases brain ACh levels in rats.[7,8] Microdialysis studies were conducted in conscious rats to examine the effects of **2d**, **3**, or **4** on brain ACh release in the prefrontal cortex. Figure 4B shows the overall ACh response to compound treatment as a fraction of baseline, expressed as the increase in the area under the effect curve (AUC) for the time period 3 h post-treatment. Compound **2d** (1 mg/kg), **3** (5 mg/kg), and **4** (5 mg/kg) all produced moderate increases in cortical ACh that were similar in magnitude to that observed with a single dose of prucalopride (10 mg/kg, Figure 4B). On the basis of our previous results, this dose of prucalopride would be expected to produce a maximal ACh response and occupy approximately 50% of the 5-HT$_4$ receptors at 1 h postdose.[12] The % RO required by GPCR agonist ligands for efficacy is generally dependent on the degree of intrinsic activity, with full agonists demonstrating in vivo activity at lower levels of RO than that required by partial agonists and antagonists.[26] Previously we have shown this to be the case for the 5-HT$_4$ partial agonists prucalopride (E_{max} = 77%) and 4-hydroxy-7-isopropyl-6-oxo-*N*-(3-piperidin-1-ylpropyl)-6,7-dihydrothieno[2,3-*b*]pyridine-5-carboxamide PRX-03140 (E_{max} = 33%) in microdialysis studies, where maximal ACh increases were observed at <40% RO and >85% RO, respectively.[12] This learning was extrapolated to ACh release studies for **2d**, **3**, and **4** tested at 1 or 5 mg/kg. By application of the E_{max} model described above and neuro-PK studies described in the previous section, free brain concentrations and thereby in vivo RO in rats were simulated for all three compounds at the doses tested for the ACh release. It was confirmed that at 1 and/or 5 mg/kg the % RO achieved for all three compounds was greater than 90% at 0–4 h, postdose (see Supporting Information Table 1–3 for simulated output of these doses in rats). At the same or similar receptor occupancies, the magnitude of ACh release was dependent on intrinsic activity of the compounds tested, as demonstrated by microdialysis data (Figure 4B).

5-HT$_4$ agonists have been reported to reverse behavioral deficits caused by treatment with anticholinergic agents.[10,27] The Morris water maze (MWM) is a classical behavioral testing paradigm that examines effects on spatial learning.[28] The cholinergic system appears to play an integral role in spatial learning as treatment with anticholinergic agents (e.g., scopolamine) can cause significant learning impairments in this task. Compound **2d** was tested for its ability to reverse scopolamine-induced learning deficits in the rat MWM (Figure 4C). Prior to compound testing, the ability to reverse scopolamine-induced learning deficits in this assay was verified using donepezil, a cholinesterase inhibitor, as a positive control. Results indicate that scopolamine caused a significant learning impairment during acquisition testing on days 1–4. Pretreatment with **2d** (0.032 mg/kg) significantly reversed the scopolamine deficits on acquisition days 3 and 4. This dose of **2d** achieved >90% RO in rats as shown above and in the Supporting Information.

To predict human pharmacokinetic properties and clinical doses, studies were conducted to project the metabolic clearance, oral bioavailability, and plasma elimination half-life in humans. Initial in vivo clearance studies in rat and dog suggested that hepatic clearance (Cl_b) mediated by P450 was the primary route of metabolism (Table 6). Therefore, blood clearance in humans was projected by application of the well-stirred model and scaling NADPH-dependent intrinsic clearances observed in HLMs, via the substrate depletion

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Table 6. Summary of Preclinical Pharmacokinetics and Predicted Human PK for Lead $5HT_4$ Partial Agonists[a]

parameter	2d	3	4
predicted Cl_b from HHEP (HLM) (mL min^{-1} kg^{-1})	11 (8.8)	ND (10.0)	10.4 (7.9)
predicted F (%)	55.8	50.0	60.5
Vd_{ss} (L/kg)	6.0	4.3	6.5
predicted plasma elimination $t_{1/2}$ (h)	~10	12	10
rat (dog) iv Cl_b (mL min^{-1} kg^{-1})	81.8 (21.2)	104.5 (12.4)	12.6 (40)
rat (dog) F (%)	40 (47)	100 (51)	40 (100)
RLM (DLM) in vitro Cl_b (mL min^{-1} kg^{-1})	48.9 (27.9)	42.7 (35.4)	45.2 (<5.6)

[a]Hepatic blood clearance from HLM was scaled using a well-stirred method by incorporation of free fraction in plasma (f_{up}), free fraction in microsomes ($f_{u,mics}$), and blood to plasma partitioning ($K_{b/p}$) to intrinsic clearance (Cl_{int}, not shown above). Blood clearance from hepatocytes was scaled using a well-stirred model but without incorporation of binding factors, whereas in vivo clearance (Cl_b) was scaled by incorporation of $K_{b/p}$ into total plasma clearance during iv PK single dose studies.[29]

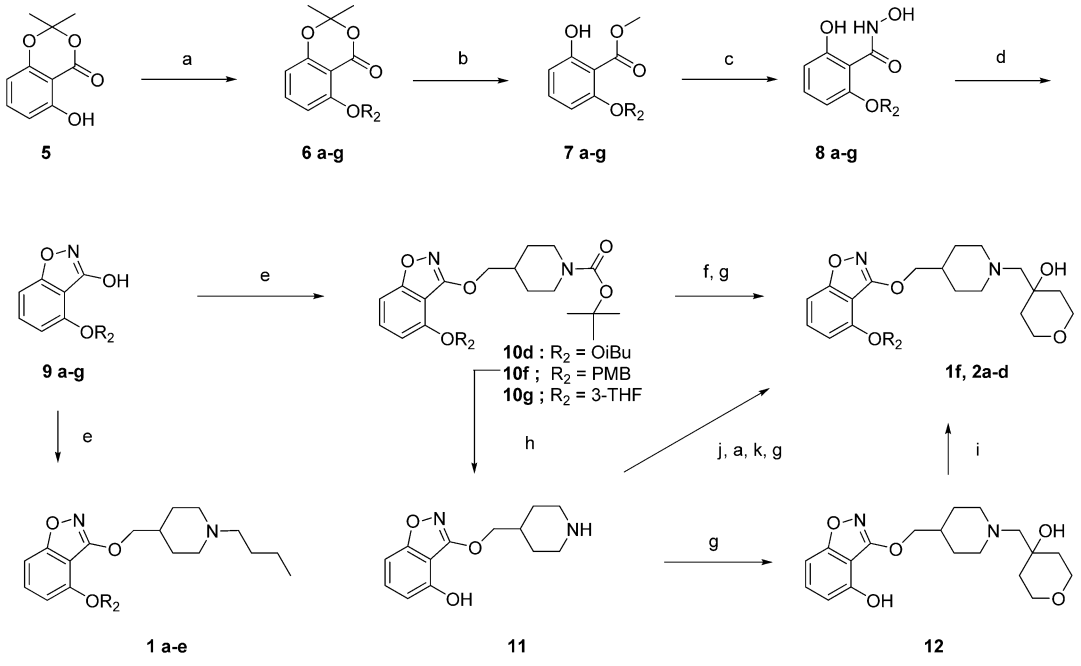
method.[19] The predicted Cl_b values from HLM for **2d**, **3**, and **4** were 8.83, 10.0, and 7.9 mL min^{-1} kg^{-1}, respectively. The oral bioavailability was calculated to be 55.8%, 50.0%, and 60.5%, respectively, with the assumption that hepatic elimination was the primary clearance mechanism. The projected elimination half-lives for **2d**, **3**, and **4** were ~10−12 h using a moderate volume of distribution (VD_{SS}) of ~4.3−6 L/kg from single species allometry and in silico methods. Using the C_{ss} equation, dose = $(C_{eff}*\tau*Cl_p)/F$, where total plasma C_{eff} was predicted to correspond to 50−70% RO in humans, the compounds were predicted to have low daily doses of <30 mg using a q.d. dosing regimen to maintain steady state plasma concentration at the 5-HT_{4d} in vitro binding K_i.

With the primary design objectives for a CNS drug candidate satisfied, the nonclinical safety profiles of **2d**, **3**, and **4** were assessed in repeat dose toxicology studies up to 1 month in duration in rats and dogs, genetic toxicology assays, and safety pharmacology studies (see Supporting Information Table 4). The safety profile of these compounds supported continued development and identified the following toxicology as neurological (convulsions), cardiovascular (increase in systolic blood pressure), and gastrointestinal (emesis, decreased food intake and body weight, and/or mucosal injury in small and large intestine). The neurological and gastrointestinal findings occurred at high multiples of the exposures predicted to be efficacious (>900×). The non-dose-related increase in systolic blood pressure in dogs was limited to the first dose and was not observed after subsequent doses of **3** and **4** in the same animal. These findings are also consistent with broad selectivity panel data for **2d**, **3**, and **4** including excellent selectivity against other 5-HT receptor families (see Supporting Information Table 5).

CHEMISTRY

The syntheses of analogues **1a−f** and **2a−d** (Tables 1 and 2) are illustrated in Scheme 1. Mitsunobu couplings between compound **5** and the appropriate alcohol afforded intermediates **6a−g**.[30] Methanolysis provided esters **7a−g**, which were converted to their hydroxamic acid derivatives **8a−f** by treatment with hydroxylamine sulfate and potassium carbonate. Substituted hydroxylbenzisoxazoles **9** were prepared by cyclization of hydroxamic acids using carbonyldiimidazole (CDI). Mitsunobu coupling of **9a−e** with (1-butylpiperidin-4-yl)methanol afforded the *n*-butyl-substituted piperidine analogues **1a−e**. Alternatively, treatment of **9d** (R = isobutyl) or

Scheme 1[a]



[a]Reagents and conditions: (a) R_2OH, diethyl azodicarboxylate, PPh_3, THF; (b) K_2CO_3, MeOH; (c) $(NH_2OH)_2$−H_2SO_4 (5 equiv), K_2CO_3, water, MeOH; (d) CDI, THF, reflux; (e) 4-hydroxymethyl-*N*-BOC-piperidine or (1-butylpiperidin-4-yl)methanol, diethyl azodicarboxylate, PPh_3, THF; (f) HCl, ether; (g) 1,6-dioxa-spiro[2.5]octane, diisopropylethylamine, EtOH, reflux; (h) thioanisole, *o*-cresol, TFA; (i) NaOH, 2,2-dimethyloxirane, EtOH, 50 °C; (j) BOC_2O, TEA, THF; (k) HCl (conc), MeOH.

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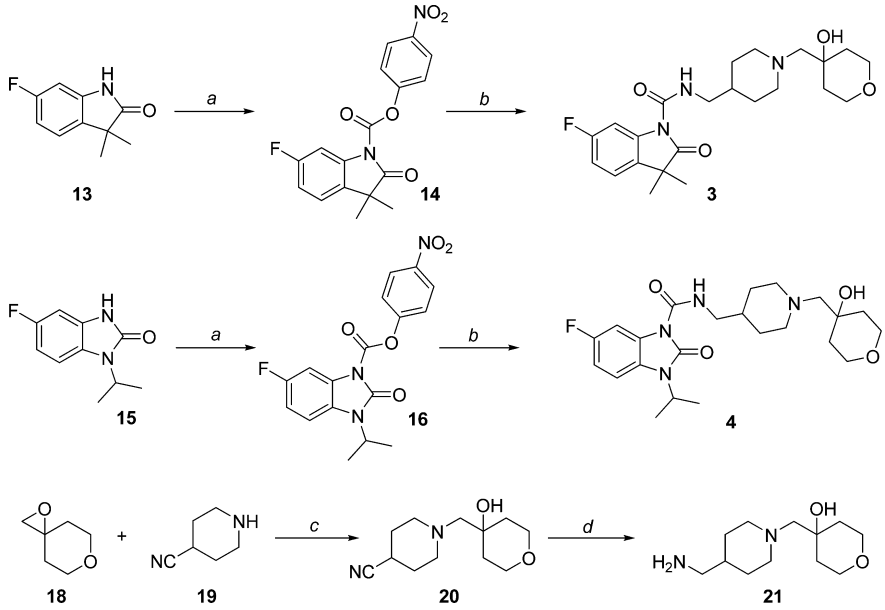
Scheme 2[a]



[a]Reagents and conditions: (a) 4-nitrophenyl chloroformate, triethylamine, toluene, 0 °C, 10 min; (b) compound **21**, triethylamine, 2-Me-tetrahydrofuran, 5 h, rt; (c) MeOH, 55 °C, 5 h; (d) tetrahydrofuran, sponge Ni, H_2, 30 psi, 12 h.

9g (R = (*R*)-tetrahydrofuran-3-yl) with 4-hydroxymethyl-*N*-BOC-piperidine yielded piperidines **10d** and **10g**. Removal of the BOC protecting group and epoxide opening with 1,6-dioxa-spiro[2.5]octane in refluxing ethanol afforded analogues **1f** and **2d**. An alternative synthetic sequence was developed allowing for the late stage incorporation of various R_2 groups using compound **10f**, where R_2 was now an appropriate protecting group such as *p*-methoxybenzyl (PMB). Deprotection of **10f** with thioanisole provided compound **11**. Compound **2a** was prepared from **11** by installation of the R_2 ether following BOC protection and epoxide opening on the piperidine with dimethyloxirane in the presence of NaOH. By use of methods described above, compound **11** was converted into piperidine **12**. Mitsunobu coupling then afforded the cyclopentyl analogue **2b**. By use of analogous procedures, tetrahydropyranyl analogue **2c** was prepared.

Compounds **3** and **4** were readily prepared via the route outlined in Scheme 2. Activation of oxindole **13** or imidazolidinone **15** with 4-nitrophenyl chloroformate afforded intermediates **14** and **16**.[31,32] Treatment of the carbamate intermediates with amine **21** provided final analogues **3** and **4**. The synthesis of amine **21** begins with spiro-epoxide **18** which undergoes ring opening with piperidine **19** followed by reduction of the nitrile group using hydrogenolysis.[14]

CONCLUSION

We have identified three highly potent, selective, and brain penetrant $5HT_4$ partial agonists that possess a wide range of intrinsic activities with ADME properties suitable for further development. In vivo RO studies demonstrated a clear relationship between the binding affinity, free brain exposure, and RO levels. All three compounds increased ACh release in the rat prefrontal cortex, a finding that is consistent with previous reports. Since these studies were conducted under conditions of high RO (>90%), additional testing is required to further examine the relationships between intrinsic activity, receptor occupancy, and ACh release. 5-HT_4 agonists have been reported to reverse learning deficits induced by cholinergic agents. In this report we have demonstrated that the partial agonist **2d** reverses scopolamine-induced learning deficits in the rat MWM, thus providing further evidence that the cognitive effects of these compounds are at least partly mediated via the cholinergic system. A safety assessment of these compounds revealed large therapeutic margins between efficacious concentrations and safety findings. On the basis of the excellent balance of pharmacokinetic attributes and safety, the two lead 5-HT_4 partial agonist candidates **2d** and **3** were selected for further clinical development.

EXPERIMENTAL SECTION

Biology. Animal Care. All animal studies were conducted in accordance with animal use protocols approved by Pfizer's local institutional animal care and use committee and in accordance with the guidelines for the care and use of laboratory animals.[33] Male Sprague–Dawley rats, used for all studies unless otherwise noted, were obtained from Charles River Laboratories (Raleigh, NC). Animals were housed on a 12 h light/dark cycle with free access to food and water and were allowed to acclimate for at least 5 days after arrival. For radioligand administration during in vivo RO studies, rats were purchased with preimplanted jugular vein catheters.

In Vitro Binding to Native 5-HT_4 Receptors in Brain Membranes. Binding assays on brain membranes prepared from rat, dog, and monkey striatal tissue were performed as previously described.[12] Briefly, striatal tissue was homogenized in 50 mM Tris buffer (pH 7.4 at 4 °C) and then centrifuged at 40000*g* for 10 min. The resulting pellet was resuspended in Tris buffer, incubated at 37 °C for 10 min to eliminate endogenous serotonin, then centrifuged again at 40000*g* for 10 min. The final membrane pellet was resuspended in 50 mM HEPES assay buffer (pH 7.4) containing 2 mM $MgCl_2$. Binding assays were conducted in 96-well plates in a total assay volume of 250 μL containing 225–250 μg of membrane protein and 0.5 nM tritiated 1-methyl-1*H*-indole-3-carboxylic acid, (1-{2-[(methylsulfonyl)amino]ethyl}piperidin-4-yl)methyl ester ([^{3}H]-GR113808), a 5-HT_4 receptor antagonist. Nonspecific binding was determined in the presence of 10 μM (1-{2-[(methylsulfonyl)amino]-ethyl}piperidin-4-yl)methyl 5-fluoro-2-methoxy-1*H*-indole-3-carboxylate (GR125487), a 5-HT_4 receptor antagonist. Test compounds

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were prepared as 10× stocks in 50 mM Tris buffer (pH 7.6) containing 10% dimethyl sulfoxide and assayed in duplicate at each test concentration. After a 30 min incubation period at 37 °C, the samples were filtered over Whatman GF/B filters and rinsed with ice-cold 50 mM Tris buffer (pH 7.4). Membrane-bound [^{3}H]GR113808 content was determined by liquid scintillation counting. IC_{50} values (concentration at which 50% inhibition of specific binding occurs) were calculated by linear regression of the concentration response data. K_i values were calculated according to the Cheng−Prusoff equation, $K_i = IC_{50}/(1 + (L/K_d))$, where L is the radioligand concentration and the K_d is the dissociation constant for the radioligand (determined previously by saturation binding analysis).

In Vitro Binding in Cell Lines Stably Expressing 5HT$_4$ Receptors. Membranes from CHO or HEK293 cells transfected and stably expressing the human **4a**, **4b**, **4d**, or **4e** receptor isoforms were prepared from frozen cell paste by homogenization in 50 mM Tris buffer (pH 7.4, 4 °C) containing 2 mM $MgCl_2$, followed by centrifugation at 40000*g* for 10 min. The resulting pellet was resuspended in 50 mM HEPES assay buffer (pH 7.4) containing 2 mM $MgCl_2$. Appropriate tissue levels for binding studies were based on the B_{max} and K_d values for each isoform which were established by saturation binding analysis. Binding studies were conducted in 96-well plates in a total volume of 250 μL containing 2−25 μg of membrane protein. [^{3}H]GR113808 was added to a final concentration of 0.1 nM for membranes expressing the **4a**, **4b**, and **4e** isoforms or 0.2 nM for the **4d** membranes. Nonspecific binding was determined in the presence of 10 μM GR125487. Compound preparation, assay procedures, and data analysis were performed as described for binding to native receptors in brain membranes.

Cyclic AMP Production in Cell Lines Expressing 5-HT$_4$ Receptors. The ability to increase cAMP production in cell lines expressing human and rat 5-HT$_4$ isoforms was used to establish in vitro functional potency and intrinsic activity levels. Increases in cAMP were determined according to methods previously described.[12] For general SAR, screening was conducted using HEK293 cells that were stably transfected with the human 5-HT$_{4d}$ receptor isoform. For functional comparisons at the human 4a, 4b, 4d, or 4e as well as the rat 4s, 4l, or 4e isoforms (DNA 2.0, Menlo Park, CA), HEK293 cells were transduced to transiently express receptors using a BacMam expression system (Invitrogen, Carlsbad, CA). For transient expression, cells were incubated at 37 °C for 24 h post-transduction, then used immediately or stored frozen for later use. Test compounds were prepared in 100% DMSO and diluted to 2× their final concentration in phosphate-buffered saline containing 1 mM isobutylmethylxanthine and 10 μM HEPES buffer. Assays were performed in 384-well plates, and each compound was tested in triplicate or quadruplicate. Test compound (5 μL) was added to each well, and the assay was initiated by the addition of 5 μL of cell suspension containing either 8000 or 4000 cells for the human or rat isoforms, respectively. Following a 30 min incubation at 37 °C, the assay was terminated and cAMP levels were determined using a Dynamic 2 homogeneous time-resolved fluoresence cAMP assay kit from Cisbio (Medford, MA). The maximal effects on cAMP production (E_{max}) for each compound were expressed as the percentage of the response produced by 1 μM 5-HT, a full agonist. EC_{50} values represent the concentration that yields 50% of the E_{max}. E_{max} and EC_{50} values were calculated using nonlinear least-squares curve fitting of the concentration response data using a four-parameter sigmoidal fit.

In Vivo Receptor Binding. In vivo binding experiments were performed as described.[12] Briefly, rats (~200 g at use, n = 4/group) were subcutaneously administered **2d** (0.0032−1 mg/kg), **3** (0.032−3.2 mg/kg), or **4** (0.1−3 mg/kg). Forty five minutes later, rats were administered a 1 mL/kg intravenous (iv) injection of [^{3}H]-(1-methylpiperidin-4-yl)methyl-8-amino-7-chloro-2,3-dihydro-1,4-benzodioxine-5-carboxylate (100 μCi/mL, 20−25 μCi/rat) via a jugular vein cannula. After a further 15 min, rats were euthanized by live decapitation and brains removed. Trunk blood was reserved to obtain plasma for compound exposure analysis. Striatum (from all subjects) and cerebellum (from vehicle control group only, to define nonspecific binding) were rapidly dissected, homogenized, filtered, and counted using a LS6500 scintillation counter (Beckman Coulter). The rest of the brain was frozen on dry ice and reserved for compound exposure analysis. Curve fit analyses were performed using the equation "one-site binding (hyperbola)" from GraphPad Prism 5 software (GraphPad Software Inc., Santa Clara, CA).

Rat Esophagus Assay. Male Sprague−Dawley rats were euthanized by exsanguination under isoflurane anesthesia, and the esophagus in the area of the thoracic aorta was quickly removed and cleared of any extra tissue. The esophagus was cut through the middle, laid flat, and then cut into two strips. Each strip was then suspended in 4 mL of tissue baths containing Krebs−Henseleit physiological salt solution of the following composition (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4 \cdot H_2O$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; dextrose, 10. The solution was maintained at 37 °C and continuously aerated with 95% O_2 and 5% CO_2. The force of isometric contraction was measured using Grass model FT03C force transducers connected to AD Instruments bridge amplifiers. Data were collected using the AD Instruments Chart data acquisition and analysis system (Unit 13, 22 Lexington Drive, Bella Vista, New South Wales 2153, Australia). After an equilibration period of 90 min at a resting tension of 1 g, tissues were exposed to 1 μM carbamylcholine to cause contraction. After 7 mins or when the tissue was no longer increasing the force of contraction, solvent, 5-HT, and **2d**, **3**, or **4** were added to a final concentration of 10 μM and the tissues were allowed to relax for 30 min. The greatest point of relaxation within the first 5 min after adding the compound was used in calculations.

Acetylcholine (ACh) Determination in Rats. Microdialysis was performed in conscious rats, and ACh levels were determined by online HPLC as previously described.[12] Briefly, microdialysis guide cannulas were implanted into the prefrontal cortex of male Sprague−Dawley rats (300−350 g), and on the day after implantation microdialysis probes were inserted and perfused with artificial cerebrospinal fluid overnight. On the day of testing, 100 nM neostigmine was added to the perfusion fluid. Microdialysis samples were collected at 15 min intervals and analyzed for ACh content by online HPLC using a modification of the Bioanalytical Systems (BAS; West Lafayette, IN) acetylcholine−choline assay kit (BAS MF-8910). After baseline stabilization, animals were given compounds subcutaneously at the indicated dose and ACh levels were monitored for at least 3 h postdose. The overall ACh response was quantified as the increase in the area under the curve (AUC) for the 0−3 h period postdose. Statistical analyses were performed using GraphPad Prism 5 software (GraphPad Software Inc., Santa Clara, CA). Chromatography data were collected and analyzed using EZChrom Elite software (Agilent Technologies, Inc., Santa Clara, CA).

Morris Water Maze Testing. Compound **2d** was dissolved in 20% hydroxylpropyl-β-cyclodextrin (HPCD), and scopolamine was dissolved in saline. At 60 min prior to place acquisition testing on days 1−4 in the water maze, rats received HPCD vehicle or **2d** (0.032 mg/kg, sc). At 30 min prior to testing, rats received a second injection with scopolamine (0.32 mg/kg, sc). The total pretreatment time for **2d** was 60 min. All doses were corrected for salt content.

Prescreening Procedure. Male Wistar rats, 160−180 g at arrival, were acclimated for 5−7 days prior to testing and given free access to food and water. Sixty rats (12 per group) were selected prior to compound testing based on the following prescreening procedure: A circular tank was filled with water (24 ± 1 °C) to a height of 30−35 cm. A 12 cm^2 visible escape platform, which was exposed 1 cm above the surface of the water, was placed in the center of the pool. Each rat was placed in the water facing the pool wall at one of four release points (north, south, east and west quadrants) and allowed to swim a maximum of 60 s in order to find the visible platform. When successful, the rat was allowed a 30 s rest period on the platform. If unsuccessful within the allotted time period, the rat was given a score of 60 s and then hand-guided to the platform and also allowed the 30 s rest period. A total of four trials were conducted for each rat, one from each release point. Rats were selected for compound testing in the MWM based on the criteria that animals found the visible platform twice out of four trials within the 60 s cutoff time.

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Place Acquisition Testing. A circular tank was filled with water to a level of 1 cm above a platform, which was located in one of four quadrants, midway between the center of the pool and the outer rim. White nontoxic paint was added to the water to obscure the platform. Animals were gently placed in the tank facing the pool wall and released from starting points within one of four quadrants. The animals were allowed to swim in the pool for a maximum of 90 s per trial. When the rat reached the platform, it was allowed to remain on it for 30 s. If an animal did not find the hidden platform within the 90 s, then the experimenter gently guided it there by hand. The animals were allowed to remain on the platform for 30 s before being returned to the home cage, or a holding cage, for a brief period (1−2 min) before the next trial. Animals received a total of four trials per day for 4 consecutive days. The location of the platform remained the same for all tests. The distance traversed to reach the hidden platform was recorded using a video monitoring system. For the hidden platform test, distances traveled across the four trials for each rat were averaged for each day. These results were then analyzed across the 4 days of testing. Two-way (days × treatment) repeated measures ANOVAs followed by Dunnett's multiple comparisons were used to compare performance between groups for the acquisition trials

Measurement of Fraction Unbound in Brain. The unbound fraction of each compound was determined in brain tissue homogenate using a 96-well equilibrium dialysis method as previously described with the following exceptions.[19] Brain homogenates were prepared from freshly harvested rat brains following dilution with a 4-fold volume of phosphate buffer and spiked with 1 μM compound. The homogenates were dialyzed against an equal volume (150 μL) of phosphate buffer at 37 °C for 6 h. Following the incubation, equal volumes (50 μL) of brain homogenate and buffer samples were collected and mixed with 50 μL of buffer or control homogenate, respectively, for preparation of mixed matrix samples. All samples were then precipitated with internal standard in acetonitrile (200 μL), vortexed, and centrifuged. Supernatants were analyzed using an LC−MS/MS assay. A dilution factor of 5 was applied to the calculation of brain fraction unbound.

Chemistry. General Methods. Solvents and reagents were of reagent grade and were used as supplied by the manufacturer. All reactions were run under a N_2 atmosphere. Organic extracts were routinely dried over anhydrous Na_2SO_4. Concentration refers to rotary evaporation under reduced pressure. Chromatography refers to flash chromatography using Merck silica gel 60 (230−400 mesh) columns or Redisep Rf disposable columns on a CombiFlash Companion system. All target compounds were analyzed using ultrahigh performance liquid chromatography/UV/evaporative light scattering detection coupled to time-of-flight mass spectrometry (UHPLC/UV/ELSD/TOFMS). UHPLC analysis was performed on a Waters ACQUITY UHPLC system (Waters, Milford, MA), which was equipped with a binary solvent delivery manager, column manager, and sample manager coupled to ELSD and UV detectors (Waters, Milford, MA). Detection was performed on a Waters LCT premier XE mass spectrometer (Waters, Milford, MA). The instrument was fitted with an Acquity BEH (bridged ethane hybrid) C18 column (30 mm × 2.1 mm, 1.7 μm particle size, Waters, Milford, MA) operated at 60 °C. Unless otherwise noted, all tested compounds were found to be ≥95% pure by this method. NMR spectra were recorded as designated on either a 270 MHz JEOL JNM-LA 270 spectrometer or a 400 MHz Varian instrument. Chemical shifts (δ) are quoted in ppm relative to tetramethylsilane (TMS); all coupling constants (J) are given in Hz. High resolution electrospray ionization mass spectrometry was carried out using a Bruker (USA) Daltronics BioApex II with a 7 T superconducting magnet and an analytical ESI source.

(4-{4-[(*R*)-(Tetrahydrofuran-3-yl)oxy]benzo[*d*]isoxazol-3-yloxymethyl}piperidin-1-ylmethyl)tetrahydropyran-4-ol (2d). Mitsunobu Coupling, Representative Procedure for Step a, Scheme 1. 2,2-Dimethyl-5-[(*R*)-(tetrahydrofuran-3-yl)oxy]-benzo[1,3]dioxin-4-one (6g). Diethyl azodicarboxylate (130 g, 0.750 mol) was added in a dropwise fashion to a mixture of 5-hydroxy-2,2-dimethylbenzo[1,3]dioxin-4-one[30] **5** (100 g, 0.510 mol), triphenylphosphine (196.5 g, 0.750 mol), and (*S*)-tetrahydrofuran-3-ol (44.0 g, 0.500 mol) in 600 mL of anhydrous THF. The resulting mixture was stirred at room temperature for 18 h. The solvent was removed under reduced pressure and the crude material was purified on a silica gel flash column, eluting with petroleum ether/ethyl acetate (15:1 → 3:1). An amount of 86.0 g (65% yield) of product was isolated as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.67 (s, 6H), 2.30 (m, 2H), 4.2 (m,4H), 4.97 (m, 1H), 6.49 (d, J = 8.4, 1H), 6.51 (d, J = 8.4, 1H), 7.39 (t, J = 8.4, 1H).

Methanolysis, Representative Procedure for Step b, Scheme 1. Methyl 2-Hydroxy-6-[(*R*)-(tetrahydrofuran-3-yl)oxy]-benzoate (7g). Potassium carbonate (135 g, 0.980 mol) was added to a solution of 2,2-dimethyl-5-[(*R*)-(tetrahydrofuran-3-yl)oxy]benzo-[1,3]dioxin-4-one (86.0 g, 0.330 mol) in 1000 mL of methanol. The mixture was stirred at room temperature for 2 h, then concentrated in vacuo. The residue was dissolved in ethyl acetate and washed with aqueous ammonium chloride solution. The organic layer was dried (Na_2SO_4) and concentrated to afford 72.0 g of the product as a yellow solid (92% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.20 (m, 2H), 3.99 (s, 3H), 4.80 (m, 4H), 4.94 (m, 1H), 6.31 (dd, J = 8.4, 0.8, 1H), 6.59 (dd, J = 8.4, 0.8, 1H), 7.30 (t, J = 8.4, 1H).

Methyl 2-(Cyclobutyloxy)-6-hydroxybenzoate (7a). **7a** was prepared in a similar manner, telescoping the two steps described above utilizing cyclobutanol as a replacement for (*S*)-tetrahydrofuran-3-ol. Compound **6a** was carried on without purification or characterization. Quantitative yield. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.70−1.90 (m, 2H), 2.17−2.25 (m, 2H), 2.41−2.51 (m, 2H), 4.63−4.68 (m, 1H), 6.24 (d, J = 8.4, 1H), 6.70 (d, J = 8.4, 1H), 7.26 (dd, J = 8.4, 8.4, 1H), 11.45 (s, 1H).

Methyl 2-(cyclopentyloxy)-6-hydroxybenzoate (7b). **7b** was prepared in a similar manner, telescoping the two steps described above utilizing cyclopentanol as a replacement for (*S*)-tetrahydrofuran-3-ol. Compound **6b** was carried on without purification or characterization. Quantitative yield. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.66−2.10 (m, 8H), 3.91 (s, 3H), 4.76−4.84 (m, 1H), 6.41 (d, J = 8.6, 1H), 6.54 (d, J = 8.2, 1H), 7.28 (dd, J = 8.6, 8.2, 1H).

Methyl 2-(Cyclobutylmethoxy)-6-hydroxybenzoate (7c). **7c** was prepared in a similar manner, telescoping the two steps described above utilizing cyclobutylmethanol as a replacement for (*S*)-tetrahydrofuran-3-ol. Compound **6c** was carried on without purification or characterization. Quantitative yield. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.86−2.11 (m, 6H), 2.72−2.82 (m, 1H), 3.93 (d, J = 5.6, 2H), 3.95 (s, 3H), 6.39 (d, J = 8.4, 1H), 6.55 (d, J = 8.4, 1H), 7.30 (dd, J = 8.4, 8.4, 1H), 11.46 (s, 1H).

Methyl 2-Hydroxy-6-(2-methylpropoxy)benzoate (7d). **7d** was prepared in a similar manner, telescoping the two steps described above utilizing 2-methylpropan-1-ol as a replacement for (*S*)-tetrahydrofuran-3-ol. Compound **6d** was carried on without purification or characterization. 82% yield. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.06 (d, J = 6.8, 6H), 2.07−2.17 (m, 1H), 3.75 (d, J = 6.3, 1H), 3.94 (s, 3H), 6.38 (d, J = 8.2, 1H), 6.56 (d, J = 8.4, 1H), 7.30 (dd, J = 8.4, 8.2, 1H).

Methyl 2-(2,2-Dimethylpropoxy)-6-hydroxybenzoate (7e). **7e** was prepared in a similar manner, telescoping the two steps described above utilizing 2,2-dimethylpropan-1-ol as a replacement for (*S*)-tetrahydrofuran-3-ol. Compound **6e** was carried on without purification or characterization. 99% yield. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.06 (s, 9H), 3.62 (s, 2H), 3.95 (s, 3H), 6.36 (d, J = 8.4, 1H), 6.56 (d, J = 8.2, 1H), 7.30 (dd, J = 8.4, 8.2, 1H).

Methyl 2-Hydroxy-6-[(4-methoxybenzyl)oxy]benzoate (7f). **7f** was prepared in a similar manner, telescoping the two steps described above utilizing (4-methoxyphenyl)methanol as a replacement for (*S*)-tetrahydrofuran-3-ol. Compound **6f** was carried on without purification or characterization. 79% yield. ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.80 (s, 3H), 3.87 (s, 3H), 5.05 (s, 2H), 6.49 (d, J = 8.2, 1H), 6.60 (d, J = 8.2, 1H), 6.91 (d, J = 8.5, 2H), 7.32 (dd, J = 8.2, 8.2, 1H), 8.51 (d, J = 7.4, 2H), 11.48 (s, 1H).

Hydroxamic Acid Formation, Representative Procedure for Step c, Scheme 1. 2,*N*-Dihydroxy-6-[(*R*)-(tetrahydrofuran-3-yl)oxy]benzamide (8g). Potassium carbonate (121 g, 0.867 mol) was added portionwise to a solution of hydroxylamine sulfate (120 g,

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0.732 mol) in 360 mL of water at 0 °C. After the mixture was stirred for 30 min, sodium sulfite (3.74 g, 0.029 mol) and a solution of methyl 2-hydroxy-6-[(*R*)-(tetrahydrofuran-3-yl)oxy]benzoate (**7g**) (35.0 g, 0.146 mol) in 360 mL of methanol were added and the mixture was stirred at 50 °C for 30 h. Methanol was removed from the cooled reaction mixture under reduced pressure, and the resulting aqueous layer was acidified with 2 N HCl. The aqueous layer was extracted with EtOAc and the organic layer was dried (Na_2SO_4) and concentrated to afford 25.0 g (76% yield) of the product as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00 (m, 1H), 2.15 (m, 1H), 3.80 (m, 4H), 5.05 (m, 1H), 6.48 (d, J = 8, 1H), 6.49 (d, J = 8, 1H), 7.19 (t, J = 8, 1H), 10.41 (br s, 1H), 11.49 (br s, 1H). MS (APCI) m/z = 239 (M + 1).

2-(Cyclobutyloxy)-*N*,6-dihydroxybenzamide (8a). **8a** was prepared from compound **7a** following the general procedure described above. 95% yield. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.70−2.04 (m, 2H), 2.20−2.32 (m, 2H), 2.48−2.58 (m, 2H), 4.74−4.79 (m, 1H), 6.25 (d, J = 8.2, 1H), 6.61 (d, J = 8.4, 1H), 7.26 (dd, J = 8.4, 8.2, 1H), 10.57 (br s 1H), 12.70 (s, 1H).

2-(Cyclopentyloxy)-*N*,6-dihydroxybenzamide (8b). **8b** was prepared from compound **7b** following the general procedure described above. 78% yield. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.76−2.11 (m, 8H), 4.90−5.00 (m, 1H), 6.40 (d, J = 8.6, 1H), 6.60 (d, J = 8.4, 1H), 7.26 (dd, J = 8.6, 8.4, 1H), 10.58 (br s, 1H), 12.7 (s, 1H).

2-(Cyclobutylmethoxy)-*N*,6-dihydroxybenzamide (8c). **8c** was prepared from compound **7c** following the general procedure described above. An inseparable mixture was isolated and utilized in the next step.

***N*,2-Dihydroxy-6-(2-methylpropoxy)benzamide (8d).** **8d** was prepared from compound **7d**. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.09 (d, J = 6.4, 6H), 2.10−2.26 (m, 1H), 3.93 (d, J = 7.85, 2H), 6.40 (d, J = 7.6, 1H), 6.63 (d, J = 8.4, 1H), 7.26 (dd, J = 8.4, 7.6, 1H), 10.57 (br s, 1H), 12.71 (s, 1H).

2-(2,2-Dimethylpropoxy)-*N*,6-dihydroxybenzamide (8e). **8e** was prepared from compound **7e** following the general procedure described above. Quantitative yield. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.15 (s, 9H), 3.85 (s, 2H), 6.41 (d, J = 8.2 Hz, 1H), 6.64 (d, J = 7.6 Hz, 1H), 7.28 (dd, J = 8.2, 7.3 Hz, 1H), 12.69 (s, 1H).

***N*,2-Dihydroxy-6-[(4-methoxybenzyl)oxy]benzamide (8f).** **8f** was prepared from compound **7f** following the general procedure described above. 95% yield. ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.81 (s, 3H), 5.07 (s, 2H), 6.50 (d, J = 8.4, 1H), 6.65 (d, J = 8.4, 1H), 6.96 (d, J = 8.7, 2H), 7.26−7.37 (m, 3H), 10.42 (br s, 1H), 12.77 (br s, 1H).

Cyclization, Representative Procedure for Step d, Scheme 1. 4-[(*R*)-(Tetrahydrofuran-3-yl)oxy]benzo[*d*]isoxazol-3-ol (9g). A solution of 2,*N*-dihydroxy-6-[(*R*)-(tetrahydrofuran-3-yl)oxy]-benzamide (25.0 g, 0.105 mol) in 250 mL of THF was heated to 50 °C. Carbonyldiimidazole was added portionwise, and the resulting mixture was stirred at 50 °C for 14 h. After the mixture was cooled to room temperature, 100 mL of 2 N HCl was added and the aqueous layer was extracted with ethyl acetate. The combined organic layers were then extracted three times with 10% aqueous potassium carbonate. The aqueous extracts were washed with ethyl acetate and then acidified to pH 2−3 with 2 N HCl. The acidified aqueous layer was extracted with ethyl acetate. The ethyl acetate extracts were washed with brine, dried (Na_2SO_4), and concentrated to afford 20.0 g of product as a yellow solid (43% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.20 (m, 2H), 3.89 (m, 1H), 4.01 (m, 3H), 5.05 (m, 1H), 6.48 (d, J = 7.6, 1H), 6.92 (d, J = 7.6, 1H), 7.37 (t, J = 7.6, 1H). MS (APCI) m/z = 222 (M + 1).

4-(Cyclobutyloxy)-1,2-benzoxazol-3-ol (9a). **9a** was prepared from compound **8a** in the manner described above. 25% yield. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.69−1.87 (m, 1H) 1.90−2.04 (m, 2H), 2.25−2.40 (m, 2H), 2.48−2.59 (m, 2H), 4.77−4.87 (m, 1H), 6.47 (d, J = 8.1, 1H), 7.12 (d, J = 8.4, 1H), 7.41 (dd, J = 8.4, 8.1, 1H).

4-(Cyclopentyloxy)-1,2-benzoxazol-3-ol (9b). **9b** was prepared from compound **8b** in the manner described above. 46% yield. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.67−1.88 (m, 1H), 1.98−2.05 (m, 7H), 4.94−4.97 (m, 1H), 6.60 (d, J = 8.1, 1H), 6.94 (d, J = 8.4, 1H), 7.43 (dd, J = 8.4, 8.1, 1H).

4-(Cyclobutylmethoxy)-1,2-benzoxazol-3-ol (9c). **9c** was prepared from compound **8c** in the manner described above. 24% yield. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.95−1.98 (m, 4H), 2.16−2.20 (m, 2H), 2.86−2.91 (m, 1H), 4.13 (d, J = 6.4, 2H), 6.61 (d, J = 7.9, 1H), 6.96 (d, J = 8.4, 1H), 7.45 (dd, J = 6.4, 7.9, 1H).

4-(2-Methylpropoxy)-1,2-benzoxazol-3-ol (9d). **9d** was prepared from compound **8d** in the manner described above. 42% yield. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.04 (d, J = 6.8, 6H), 2.05−2.20 (m, 1H), 3.84 (d, J = 6.6, 2H), 6.71 (d, J = 8.4, 1H), 6.84 (d, J = 8.1, 1H), 7.06 (dd, J = 8.1, 8.4, 1H).

4-(2,2-Dimethylpropoxy)-1,2-benzoxazol-3-ol (9e). **9e** was prepared from compound **8e** in the manner described above. 53% yield. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.12 (s, 9H), 3.76 (s, 2H), 6.59 (d, J = 8.1, 1H), 6.95 (d, J = 8.4, 1H), 7.41 (dd, J = 8.4, 8.1, 1H).

4-[(4-Methoxybenzyl)oxy]-1,2-benzoxazol-3-ol (9f). **9f** was prepared from compound **8f** in the manner described above. 63% yield. ^{1}H NMR (270 MHz, DMSO-d_6) δ 4.69 (s, 3H), 5.18 (s, 2H), 6.85 (d, J = 8.2, 1H), 6.96 (d, J = 8.5, 2H), 7.06 (d, J = 8.4, 1H), 7.43 (d, J = 8.9, 2H), 7.48 (dd, J = 8.4, 8.2, 1H).

Mitsunobu Coupling, Representative Procedure for Step e, Scheme 1. *tert*-Butyl 4-{4-[(*R*)-(Tetrahydrofuran-3-yl)oxy]-benzo[*d*]isoxazol-3-yloxymethyl}piperidine-1-carboxylate (10g). Diethyl azodicarboxylate (15.6 g, 0.09 mol) was added to a mixture of 4-[(*R*)-(tetrahydrofuran-3-yl)oxy]benzo[*d*]isoxazol-3-ol, **9g** (10 g, 0.045 mol), *tert*-butyl 4-hydroxymethylpiperidine-1-carboxylate (11.6 g, 0.054 mol), and triphenylphosphine (23.5 g, 0.090 mol) in 300 mL of tetrahydrofuran. After the addition was complete, the mixture was heated at reflux for 18 h. After concentration in vacuo, the crude product was purified on a silica gel flash column, eluting with petroleum ether/ethyl acetate (15:1 → 5:1) to afford 22.0 g of the product as an oil. 51% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (m, 2H), 1.39 (s, 9H), 1.76 (m, 2H), 1.99 (m, 1H), 2.15 (m, 2H), 2.70 (br t, J = 11.6, 2H), 3.95 (m, 4H), 4.13 (m, 2H), 4.34 (d, J = 6.4, 2H), 4.98 (m, 1H), 6.43 (d, J = 8, 1H), 6.93 (d, J = 8, 1H), 7.31 (t, J = 8, 1H). MS (APCI) m/z = 319 [(M + 1) − 99 (loss of BOC)].

Designated compounds were isolated as the tosylate salts, which were prepared by dissolving the free bases in ethyl acetate and adding 0.95 equiv of *p*-toluenesulfonic acid. The resulting white solids were filtered and dried.

3-[(1-Butylpiperidin-4-yl)methoxy]-4-(cyclobutyloxy)-1,2-benzoxazole (1a). **1a** was prepared in the manner described above, utilizing compound **9a** rather than **9g** and (1-butylpiperidin-4-yl)methanol in place of *tert*-butyl 4-hydroxymethylpiperidine-1-carboxylate. 65% yield (tosylate salt). ^{1}H NMR (400 MHz, MeOH-d_4) δ 0.97 (t, J = 7.2, 3H), 1.39 (dq, J = 14.8, 7.4, 2H), 1.51−1.92 (m, 6H), 2.06−2.20 (m, 4H), 2.23−2.38 (m, 4H), 2.43−2.58 (m, 2H), 2.90−3.17 (m, 4H), 3.63 (d, J = 10.9, 2H), 4.27 (d, J = 5.5, 2H), 4.67−4.83 (m, 1H), 6.55 (d, J = 8.2, 1H), 6.96 (d, J = 8.6, 1H), 7.19 (d, J = 7.8, 2H), 7.42 (dd, J = 8.6, 8.2, 1H), 7.67 (d, J = 8.2, 2H). HRMS calcd for $C_{21}H_{31}N_2O_3$ (M + 1): 359.2329. Found: 359.2326.

3-[(1-Butylpiperidin-4-yl)methoxy]-4-(cyclopentyloxy)-1,2-benzoxazole (1b). **1b** was prepared in the manner described above, utilizing compound **9b** rather than **9g** and (1-butylpiperidin-4-yl)methanol in place of *tert*-butyl 4-hydroxymethylpiperidine-1-carboxylate. 41% yield. ^{1}H NMR (400 MHz, MeOH-d_4) δ 0.98 (t, J = 7.4, 3H), 1.40 (dq, J = 14.8, 7.4, 2H), 1.53−2.03 (m, 12H), 2.16 (d, J = 14.1, 2H), 2.26 (br s, 1H), 2.95−3.16 (m, 4H), 3.63 (d, J = 10.9, 2H), 4.26 (d, J = 6.2, 2H), 4.94 (tt, J = 5.5, 2.5, 1H), 6.69 (d, J = 8.2, 1H), 6.94 (d, J = 8.2, 1H), 7.43 (dd, J = 8.2, 1H). HRMS calcd for $C_{22}H_{33}N_2O_3$ (M + 1): 373.2486. Found: 373.2481.

3-[(1-Butylpiperidin-4-yl)methoxy]-4-(cyclobutylmethoxy)-1,2-benzoxazole (1c). **1c** was prepared in the manner described above, utilizing compound **9c** rather than **9g** and (1-butylpiperidin-4-yl)methanol in place of *tert*-butyl 4-hydroxymethylpiperidine-1-carboxylate. 55% yield (tosylate salt). ^{1}H NMR (400 MHz, MeOH-d_4) δ 0.97 (t, J = 7.4, 3H), 1.39 (dq, J = 15.0, 7.37, 2H), 1.50−1.79 (m, 3H), 1.87−2.40 (m, 8H), 2.72−2.87 (m, 2H), 2.90−3.18 (m, 4H), 3.62 (d, J = 10.2, 2H), 4.04 (d, J = 5.9, 2H), 4.25 (d, J = 5.1, 2H), 6.70 (d, J = 7.8, 1H), 6.97 (d, J = 8.2, 1H), 7.19 (d, J = 7.8, 2H), 7.45 (dd, J

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= 8.2, 7.8, 1H), 7.67 (d, J = 8.2, 2H). HRMS calcd for $C_{22}H_{33}N_2O_3$ (M + 1): 373.2486. Found: 373.2485.

3-[(1-Butylpiperidin-4-yl)methoxy]-4-(2-methylpropoxy)-1,2-benzoxazole (1d). **1d** was prepared in the manner described above, utilizing compound **9d** rather than **9g** and (1-butylpiperidin-4-yl)methanol in place of *tert*-butyl 4-hydroxymethyl-piperidine-1-carboxylate. 54% yield (tosylate salt). ^{1}H NMR (400 MHz, MeOH-d_4) δ 0.97 (t, J = 7.2, 3H), 1.05 (d, J = 6.6, 6H), 1.39 (dq, J = 14.9, 7.5, 2H), 1.51−1.77 (m, 4H), 2.00−2.37 (m, 7H). 2.91−3.15 (m, 4H), 3.62 (d, J = 9.8, 2H), 3.86 (d, J = 6.2, 2H), 4.25 (d, J = 5.5, 2H), 6.61−6.75 (m, 1H), 6.96 (d, J = 8.2, 1H), 7.19 (d, J = 7.8, 2H), 7.44 (t, J = 8.2, 1H), 7.67 (d, J = 8.2 Hz, 2H). HRMS calcd for $C_{21}H_{33}N_2O_3$ (M + 1): 361.2486. Found: 361.2483.

3-[(1-Butylpiperidin-4-yl)methoxy]-4-(2,2-dimethylpropoxy)-1,2-benzoxazole (1e). **1e** was prepared in the manner described above, utilizing compound **9e** rather than **9g** and (1-butylpiperidin-4-yl)methanol in place of *tert*-butyl 4-hydroxymethylpiperidine-1-carboxylate. 58% yield (tosylate salt). ^{1}H NMR (400 MHz, MeOH-d_4) δ 0.97 (t, J = 7.2, 3H), 1.03−1.11 (m, 9H), 1.31−1.46 (m, 2H), 1.70 (dt, J = 7.9, 4.05, 5H), 2.32 (s, 5H), 2.89−3.16 (m, 4H), 3.54−3.69 (m, 2H), 3.74 (s, 2H), 4.25 (d, J = 6.2, 2H), 6.62−6.72 (m, 1H), 6.96 (d, J = 8.2, 1H), 7.19 (d, J = 8.2, 2H), 7.45 (t, J = 8.2, 1H), 7.67 (d, J = 8.2, 2H). HRMS calcd for $C_{22}H_{35}N_2O_3$ (M + 1): 375.2642. Found: 375.2638.

***tert*-Butyl 4-({[4-(2-Methylpropoxy)-1,2-benzoxazol-3-yl]-oxy}methyl)piperidine-1-carboxylate (10d).** **10d** was prepared in the manner described above utilizing compound **9d** rather than compound **9g**. 71% yield. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.07 (d, J = 6.8, 6H), 1.23−1.47 (m, 12H), 1.82−1.87 (m, 2H), 2.04−2.19 (m, 2H), 2.71− 2.76 (m, 2H), 3.84 (d, J = 6.4, 2H), 4.27 (d, J = 6.3, 2H), 6.55 (d, J = 7.9, 1H), 6.97 (d, J = 8.6, 1H), 7.39 (dd J = 8.6, 7.9, 1H).

***tert*-Butyl 4-[({4-[(4-Methoxybenzyl)oxy]-1,2-benzoxazol-3-yl}oxy)methyl]piperidine-1-carboxylate (10f).** **10f** was prepared in the manner described above utilizing compound **9f** rather than compound **9g**. 68% yield ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.23−1.32 (m, 2H), 1.47 (s, 9H), 1.78−1.82 (m, 2H), 1.98−2.14 (m, 1H), 2.66−2.76 (m, 2H), 3.84 (s, 2H), 4.05−4.20 (m, 2H), 4.24 (d, J = 6.9, 2H), 6.67 (d, J = 7.9, 1H), 6.92 (d, J = 8.7, 2H), 7.02 (d, J = 8.4, 1H), 7.38−7.44 (m, 3H).

Representative Procedure for Step f, Scheme 1. BOC Deprotection, 3-(Piperidin-4-ylmethoxy)-4-[(*R*)-(tetrahydrofuran-3-yl)oxy]benzo-[*d*]isoxazole. A 0 °C solution of *tert*-butyl 4-{4-[(*R*)-(tetrahydrofuran-3-yl)oxy]benzo-[*d*]isoxazol-3-yloxymethyl}-piperidine-1-carboxylate (**10g**) in 500 mL of ether was treated with a saturated solution of HCl (g) in 200 mL of ether. After addition was complete, the mixture was warmed to room temperature and stirred for 16 h. The reaction mixture was filtered. The white solid was washed with ethyl acetate followed by ether and dried to yield 15.0 g (81% yield) of the desired product as a white solid. ^{1}H NMR (400 MHz, MeOH-d_4) δ 1.51−1.69 (m, 2H), 2.04−2.19 (m, 3H), 2.22−2.37 (m, 2H), 2.99−3.14 (m, 2H), 3.40−3.51 (m, 2H), 3.85−4.02 (m, 4H), 4.25−4.31 (m, 2H), 5.17 (td, J = 3.7, 1.6, 1H), 6.72 (d, J = 8.0, 1H), 7.01 (d, J = 8.6, 1H), 7.47 (t, J = 8.2, 1H). MS (APCI) m/z = 319 (M + 1).

4-(2-Methylpropoxy)-3-(piperidin-4-ylmethoxy)-1,2-benzoxazole. The compound was prepared according to the general procedure described above utilizing compound **10d** as starting material. 75% yield. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.05 (d, J = 6.8, 6H), 1.75−1.88 (m, 2H), 2.04−2.16 (m, 3H), 2.90−2.98 (m, 2H), 3.55−3.60 (m, 2H), 3.85 (d, J = 6.3, 2H), 4.32 (d, J = 7.1, 2H), 6.56 (d, J = 7.9, 1H), 6.98 (d, J = 8.4, 1H), 7.40 (dd, J = 8.4, 7.9, 1H).

Epoxide Opening, Representative Procedure for Step g, Scheme 1. (4-{4-[(*R*)-(Tetrahydrofuran-3-yl)oxy]benzo[*d*]-isoxazol-3-yloxymethyl}piperidin-1-ylmethyl)-tetrahydropyran-4-ol (2d). 1,6-Dioxaspiro[2.5]octane (9.70 g, 0.084 mol) and triethylamine (8.60 g, 0.084 mol) were added to a solution of 3-(piperidin-4-ylmethoxy)-4-[(*R*)-(tetrahydrofuran-3-yl)-oxy]benzo[*d*]isoxazole (15.0 g, 0.042 mol) in 200 mL of methanol. The resulting solution was heated at reflux for 18 h. The cooled mixture was concentrated, and ethyl acetate and water were added to the residue. The layers were separated, and the organic extracts were washed with brine, dried (Na_2SO_4), and concentrated to provide 17.0 g of crude product as a yellow oil. The crude material was purified by preparative HPLC to afford 10.0 g of the desired product as a white solid. (50% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41−1.63 (m, 6H), 1.71−1.81 (m, 2H), 1.81−1.94 (m, 1H), 2.17−2.26 (m, 2H), 2.33 (s, 2H), 2.4 (td, J = 11.7, 2.3, 2H), 2.92 (d, J = 11.8, 2H), 3.46 (s, 1H), 3.71−3.84 (m, 4H), 3.91−4.10 (m, 4H), 4.24 (d, J = 5.9, 2H), 5.03−5.08 (m, 1H), 6.50 (d, J = 8.2, 1H), 7.00 (d, J = 8.2, 1H), 7.38 (t, J = 8.2, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.11, 33.10, 35.20, 36.92, 36.96, 56.15, 63.93, 67.14, 67.46, 68.27, 72.94, 74.06, 78.37, 103.17, 105.15, 131.71, 152.71, 166.02, 166.28. Elemental anal. calcd: C 63.87, H 7.46, N 6.48. Found: C 63.91, H 7.78, N 6.48. Optical rotation: +5.6° (c 0.0104 g/mL, MeOH). Melting point 92.6 °C.

4-{[4-({[4-(2-Methylpropoxy)-1,2-benzoxazol-3-yl]oxy}-methyl)piperidin-1-yl]methyl}tetrahydro-2*H*-pyran-4-ol (1f). **1f** was synthesized in the manner described above from 4-(2-methylpropoxy)-3-(piperidin-4-ylmethoxy)-1,2-benzoxazole. 68% yield. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.17 (d, J = 6.6, 6H), 1.44−1.65 (m, 7H), 1.80−1.90 (m, 3H), 2.11−2.20 (m, 1H), 2.34 (s, 2H), 2.42 (t, J = 10.2, 2H), 2.92 (d, J = 11.4, 2H), 3.77−3.87 (m, 6H), 4.27 (d, J = 5.9, 2H), 6.56 (d, J = 8.1, 1H), 6.97 (d, J = 8.2, 1H), 7.38 (dd, J = 8.2, 8.1, 1H). HRMS calcd for $C_{23}H_{35}N_2O_5$ (M + 1): 419.2450. Found: 419.2544.

4-{[4-({[4-(2-Hydroxy-2-methylpropoxy)-1,2-benzoxazol-3-yl]oxy}methyl)piperidin-1-yl]methyl}tetrahydro-2*H*-pyran-4-ol (2a). **2a** was prepared from compound **10f** in three steps as depicted below.

(1). 3-(Piperidin-4-ylmethoxy)-1,2-benzoxazol-4-ol (11). *tert*-Butyl 4-[({4-[(4-methoxybenzyl)oxy]-1,2-benzoxazol-3-yl}oxy)-methyl]piperidine-1-carboxylate (**10f**) (2.29 g, 4.89 mmol) was combined with thioanisole (6.0 mL, 46.0 mmol) and *o*-cresol (5.30 g, 49.0 mmol). Trifluoroacetic acid (3 mL) was added. After the mixture was stirred at room temperature for 2 h, 50 mL of hexane was added and the solvent was decanted. After two additional hexane washes, 50 mL of diethyl ether was added. After the mixture was stirred for 30 min, a white solid was filtered and air-dried to afford 1.82 g of the trifluoroacetic acid salt of the title compound as a white solid. ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.45−1.53 (m, 2H), 1.91−1.96 (m, 2H), 2.10−2.30 m, 1H), 2.91−2.96 (m, 2H), 3.45−3.47 (m, 2H), 4.25 (d, J = 6.3, 2H), 6.65 (d, J = 7.7, 1H), 6.95 (d, J = 8.4, 1H), 7.38 (dd, J = 8.4, 7.7, 1H), 8.61 (br s, 1H), 10.73 (s, 1H).

(2). 3-({1-[(4-Hydroxytetrahydro-2*H*-pyran-4-yl)methyl]-piperidin-4-yl}methoxy)-1,2-benz-oxazol-4-ol (12). **12** was prepared from compound **11** in the manner described in step g above. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.47−1.65 (m, 7H), 1.79−1.84 (m, 2H), 1.97−2.04 (m, 1H), 2.34−2.45 (m, 2H), 2.90−2.95 (m, 2H), 3.77−3.96 (m. 5H), 4.35 (d, J = 6.8, 2H), 6.66 (d, J = 7.9, 1H), 6.97 (d, J = 8.5, 1H), 7.41 (dd, J = 8.5, 7.9, 1H).

(3). Step i, Scheme 1. 4-{[4-({[4-(2-Hydroxy-2-methylpropoxy)-1,2-benzoxazol-3-yl]oxy}methyl)piperidin-1-yl]methyl}-tetrahydro-2*H*-pyran-4-ol (2a). Compound **12** (109 mg, 0.3 mmol) was stirred with 0.5 mL of 2 N NaOH (1.00 mmol) and 2 mL of ethanol at 50 °C for 20 h. 2,2-Dimethyloxirane (139 mg, 1.93 mmol) was then added, and the mixture was stirred at 50 °C for an additional 20 h. After cooling, the reaction mixture was diluted with ethyl acetate and washed with brine. The organic layer was dried (Na_2SO_4) and concentrated to an oil. The crude product was purified on an alumina column to afford 89.8 mg of the title compound as a white solid in 80% yield. ^{1}H NMR (400 MHz, MeOH-d_4) δ 1.35 (s, 6H), 1.41−1.59 (m, 4H), 1.68 (ddd, J = 13.6, 11.4, 5.1, 2H), 1.79 (d, J = 12.1, 2H), 2.21−2.37 (m, 4H), 2.96 (d, J = 11.3, 2H), 3.61−3.80 (m, 5H), 3.87 (s, 2H), 4.19 (d, J = 5.9, 2H), 6.69 (d, J = 8.2, 1H), 6.97 (d, J = 8.6, 1H), 7.44 (dd, J = 8.6, 8.2, 1H). HRMS calcd for $C_{23}H_{35}N_2O$ (M + 1): 435.2490. Found: 435.2486.

4-[(4-{[(4-{[(1*R*,3*R*)-3-Hydroxycyclopentyl]oxy}-1,2-benzoxazol-3-yl)oxy]methyl}piperidin-1-yl)methyl]tetrahydro-2*H*-pyran-4-ol (2b). **2b** was prepared from compound **11** in four steps as shown below.

(1). Step j, Scheme 1. *tert*-Butyl 4-{[(4-Hydroxy-1,2-benzoxazol-3-yl)oxy]methyl}piperidine-1-carboxylate. Triethylamine (2.10 mL, 15.0 mmol) was added to a solution of compound **11**

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(1.82 g, 5 mmol) in 15 mL of THF. Di-*tert*-butyl dicarbonate (1.17 g, 5.36 mmol) was added, and the reaction mixture was stirred at room temperature. After 18 h, the mixture was diluted with ethyl acetate and washed three times with brine. The organic layer was dried ($MgSO_4$) and concentrated to afford 1.77 g of the desired product in quantitative yield. ^{1}H NMR (270 MHz,$CDCl_3$) δ 1.26−1.56 (m, 13H), 1.75−1.86 (m, 2H), 2.05−2.15 (m, 1H), 2.65−2.83 (m, 2H), 4.34 (d, J = 6.6, 2H), 6.67 (d, J = 7.9, 1H), 6.95 (d, J = 8.4, 1H), 7.35 (dd, J = 8.4, 7.4, 1H).

(2). *tert*-Butyl 4-{[(4-{[(*1R,3R*)-3-Hydroxycyclopentyl]oxy}-1,2-benzoxazol-3-yl)oxy]methyl}piperidine-1-carboxylate. The compound was prepared according to the representative procedure for step e, substituting *tert*-butyl 4-{[(4-hydroxy-1,2-benzoxazol-3-yl)oxy]-methyl}piperidine-1-carboxylate for **9g** and (1*S*,3*S*)-cyclopentane-1,3-diol for *tert*-butyl 4-hydroxymethylpiperidine-1-carboxylate. Quantitative yield. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.43 (s, 9H), 1.67−2.22 (s, 10H), 2.73−3.01 (m, 3H), 4.08−4.37 (m, 5H), 5.05−5.09 (m, 1H), 6.61 (d, J = 7.9, 1H), 7.00 (d, J = 8.4, 1H), 7.41 (dd, J = 8.4, 7.9, 1H).

(3). Representative Procedure for Step k, Scheme 1. (1*R*,3*R*)-3-{[3-(Piperidin-4-ylmethoxy)-1,2-benzoxazol-4-yl]oxy}-cyclopentanol. *tert*-Butyl 4-{[(4-{[(1*R*,3*R*)-3-hydroxycyclopentyl]-oxy}-1,2-benzoxazol-3-yl)oxy]methyl}piperidine-1-carboxylate (175 mg, 0.405 mmol) was treated with a solution of 200 μL of concentrated HCl in 1.8 mL of methanol. After the mixture was stirred for 3 h at room temperature, the solvent was evaporated to afford the title compound as the HCl salt. 60% yield. ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.54−1.99 (m, 10H), 2.18−2.39 (m, 1H), 2.51−2.93 (m, 2H), 3.28−3.39 (m, 2H), 4.02−4.24 (m, 3H), 4.85−4.90 (m 1H), 6.75 (d, J = 8.1, 1H), 7.04 (d, J = 8.4, 1H), 7.51 (dd, J = 8.4, 8.1, 1H), 9.05 (br s, 1H).

(4). 4-[(4-{[(4-{[(1*R*,3*R*)-3-Hydroxycyclopentyl]oxy}-1,2-benzoxazol-3-yl)oxy]methyl}piperidin-1-yl)methyl]tetrahydro-2*H*-pyran-4-ol (2b). **2b** was prepared according to the general procedure for step g substituting (1*R*,3*R*)-3-{[3-(piperidin-4-ylmethoxy)-1,2-benzoxazol-4-yl]oxy}cyclopentanol for 3-(piperidin-4-ylmethoxy)-4-[(*R*)-(tetrahydrofuran-3-yl)oxy]benzo[*d*]isoxazole. 63% yield. ^{1}H NMR (400 MHz, MeOH-d_4) δ 1.38−1.60 (m, 4H), 1.62−2.11 (m, 10H), 2.24−2.46 (m, 5H), 2.97 (d, J = 10.9, 2H), 3.62−3.79 (m, 4H), 4.18 (d, J = 6.2, 2H), 4.27 (dd, J = 11.2, 5.5, 1H), 4.90 (d, J = 3.1, 1H), 6.65 (d, J = 7.8, 1H), 6.94 (d, J = 8.2, 1H), 7.43 (dd, J = 8.4, 7.8, 1H). HRMS calcd for $C_{24}H_{35}N_2O_6$ (M + 1): 447.2490. Found: 447.2488.

4-{[4-({[4-(Tetrahydro-2*H*-pyran-4-yloxy)-1,2-benzoxazol-3-yl]oxy}methyl)piperidin-1-yl]methyl}tetrahydro-2*H*-pyran-4-ol (2c). **2c** was prepared in three steps from *tert*-butyl 4[(4-hydroxybenzo[*d*]isoxazol-3-yloxy)methyl]piperidine-1-carboxylate as described below.

(1). *tert*-Butyl 4-({[4-(Tetrahydro-2*H*-pyran-4-yloxy)-1,2-benzoxazol-3-yl]oxy}methyl)piper-idine-1-carboxylate. The compound was synthesized according to the general procedure in step a, substituting *tert*-butyl 4-[(4-hydroxybenzo[*d*]isoxazol-3-yloxy)-methyl]piperidine-1-carboxylate for 5-hydroxy-2,2-dimethylbenzo-[1,3]dioxin-4-one and tetrahydro-2*H*-pyran-4-ol for (*S*)-tetrahydrofuran-3-ol. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.25−1.45 (m, 2H), 1.47 (s, 9H), 1.75−1.93 (m, 4H), 1.94−2.20 (m, 2H), 2.60−2.90 (m, 2H), 3.54−3.71 (m, 2H), 3.85−4.25 (m, 3H), 4.28 (d, J = 6.2, 2H), 4.62−4.76 (m, 1H), 6.61 (d, J = 8.4, 1H), 7.00 (d, J = 8.4, 1H), 7.39 (dd, J = 8.4, 1H).

(2). 3-(Piperidin-4-ylmethoxy)-4-(tetrahydro-2*H*-pyran-4-yloxy)-1,2-benzoxazole. The compound was prepared according to the representative procedure of step k, substituting *tert*-butyl 4-({[4-(tetrahydro-2*H*-pyran-4-yloxy)-1,2-benzoxazol-3-yl]oxy}methyl)-piperidine-1-carboxylate for *tert*-butyl 4-{[(4-{[(1*R*,3*R*)-3-hydroxycyclopentyl]oxy}-1,2-benzoxazol-3-yl)oxy]methyl}piperidine-1-carboxylate. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.75−1.95 (m, 4H), 1.99−2.37 (m, 5H), 2.85−3.05 (m, 2H), 3.50−3.71 (m, 4H), 3.91−4.05 (m, 2H), 4.34 (d, J = 6.9, 2H), 4.65−4.76 (m, 1H), 6.62 (d, J = 8.4, 1H), 7.00 (d, J = 8.4, 1H), 7.40 (dd, J = 8.4, 8.4, 1H).

(3). 4-{[4-({[4-(Tetrahydro-2*H*-pyran-4-yloxy)-1,2-benzoxazol-3-yl]oxy}methyl)piperidin-1-yl]methyl}tetrahydro-2*H*-pyran-4-ol (2c). **2c** was prepared according to the general procedure for step g, substituting 3-(piperidin-4-ylmethoxy)-4-(tetrahydro-2*H*-pyran-4-yloxy)-1,2-benzoxazole for 3-(piperidin-4-ylmethoxy)-4-[(*R*)-(tetrahydrofuran-3-yl)oxy]benzo[*d*]isoxazole. ^{1}H NMR (400 MHz, MeOH-d_4) δ 1.40−1.61 (m, 5H), 1.63−1.73 (m, 3H), 1.73−1.89 (m, 6H), 1.98−2.07 (m, 3H), 2.25−2.37 (m, 5H), 2.97 (d, J = 11.3, 2H), 3.57−3.79 (m, 7H), 3.97 (ddd, J = 11.3, 7.8, 3.1, 3H), 4.20 (d, J = 6.2, 2H), 4.76−4.80 (m, 1H), 6.76 (d, J = 8.2, 1H), 6.96 (d, J = 8.6, 1H), 7.43 (dd, J = 8.6, 8.2, 1H). HRMS calcd for $C_{24}H_{35}N_2O_6$ (M + 1): 447.2490. Found: 447.2487.

Representative Procedure for Step a. 4-Nitrophenyl 6-Fluoro-3,3-dimethyl-2-oxo-2,3-dihydroindole-1-carboxylate (14). Triethylamine (34.0 g, 330 mmol) was added to a solution of 6-fluoro-3,3-dimethyl-1,3-dihydroindol-2-one[31] (10.0 g, 56.0 mmol) and 4-nitrophenyl chloroformate (22.0 g, 220 mmol) in toluene (150 mL). The resulting reaction mixture was heated at reflux for 18 h, then cooled to room temperature and concentrated under reduced pressure. Cold methanol was added and the mixture was filtered, washed with additional cold methanol, and dried to afford 15.0 g of the title compound as a white solid (79% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51 (s, 6H), 7.00−6.90 (m, 1H), 7.26−7.16 (m, 1H), 7.56−7.49 (m, 2H), 7.76−7.71 (m, 1H), 8.36−8.33 (m, 2H).

Representative Procedure for Step b. 6-Fluoro-3,3-dimethyl-2-oxo-2,3-dihydroindole-1-carboxylic Acid [1-(4-Hydroxytetrahydropyran-4-ylmethyl)piperidin-4-ylmethyl]amide (3). Triethylamine (26 g, 256 mmol) was added to 4-(4-aminomethylpiperidin-1-ylmethyl)tetrahydropyran-4-ol hydrochloride (**20**)[15] in 300 mL of dichloromethane. After the mixture was stirred for 5 min, 4-nitrophenyl 6-fluoro-3,3-dimethyl-2-oxo-2,3-dihydroindole-1-carboxylate **14** (15 g, 44 mmol) was added and the reaction mixture was stirred at room temperature for 16 h. The mixture was concentrated under reduced pressure and the crude material was purified on a silica gel flash column, eluting with petroleum ether/ethyl acetate (50:1 → 3:1) to yield the desired product as a white solid (13 g, 69% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25−1.37 (m, 2H), 1.40 (s, 6H), 1.45−1.47 (m, 1H), 1.50−1.63 (m, 4H), 1.72 (d, J = 12.7, 2H), 2.30 (s, 2H), 2.34 (td, J = 11.8, 2.34, 1H), 8.63 (t, J = 5.6, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.3, 30.7, 35.7, 37.3, 45.2, 45.6, 56.3, 64.2, 67.6, 68.4, 105.1, 105.4, 111.3, 111.6, 122.8, 122.9, 163.8, 184.2. Elemental anal. calcd: C 63.72, H 7.44, N 9.69. Found: C 63.70, H 7.68, N 9.73. Melting point 155.1 °C.

6-Fluoro-*N*-({1-[(4-hydroxytetrahydro-2*H*-pyran-4-yl)-methyl]piperidin-4-yl}methyl)-3-isopropyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-1-carboxamide (4). Analogue **4** was prepared over two steps in an analogous fashion to compound **3** above utilizing 5-fluoro-1-isopropyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one[14] (**15**) as starting material.

(1). 4-Nitrophenyl 6-Fluoro-3-isopropyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-1-carboxy-late (16). **16** was prepared using the procedure from step l, substituting 5-fluoro-1-isopropyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one (**15**) for 6-fluoro-3,3-dimethyl-1,3-dihydroindol-2-one. 72% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.57 (d, J = 7.0, 6H), 4.68 (dt, J = 14.0, 7.0, 1H), 6.94−7.01 (m, 1H), 7.03−7.10 (m, 1H), 7.47−7.53 (m, 2H), 7.73 (dd, J = 9.0, 2.5, 1H), 8.30−8.38 (m, 2H).

(2). 6-Fluoro-*N*-({1-[(4-hydroxytetrahydro-2*H*-pyran-4-yl)-methyl]piperidin-4-yl}methyl)-3-isopropyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-1-carboxamide (4). **4** was prepared according to the procedure for step m, substituting 4-nitrophenyl 6-fluoro-3-isopropyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-1-carboxylate (**16**) for 4-nitrophenyl 6-fluoro-3,3-dimethyl-2-oxo-2,3-dihydroindole-1-carboxylate (**14**). 89% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23−1.65 (m, 15H), 1.73 (d, J = 12.5, 2H), 2.26−2.39 (m, 4H), 2.87 (d, J = 11.7, 2H), 3.29 (t, J = 6.2, 2H), 3.42 (br s, 1H), 3.68−3.83 (m, 4H), 4.66 (spt, J = 7.0, 1H), 6.89 (td, J = 8.9, 2.6, 1H), 7.04 (dd, J = 8.8, 4.5, 1H), 8.03 (dd, J = 9.6, 2.7, 1H), 8.87 (t, J = 5.8, 1H). Elemental anal. calcd: C 61.59, H 7.42, N 12.49. Found: C 61.38, H 7.52, N 12.31. Melting point 112.4 °C.

1-[(4-Hydroxytetrahydro-2*H*-pyran-4-yl)methyl]piperidine-4-carbonitrile (20). To a mixture of cyanopiperidine (148.7 g, 1.35 mol) in methanol (800 mL) was charged epoxide (167.3 g, 1.47 mol) as a solution in methanol (200 mL). The resulting mixture was heated to 55 °C for 5 h and then concentrated to an oil, which was crystallized

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from heptane (1 L), filtered, and dried on the filter to afford 283 g of 4-(4-hydroxytetrahydropyran-4-ylmethyl)cyclohexanecarbonitrile as a white solid. ^{1}H NMR (CD_3OD, 400 MHz) δ 3.77−3.64 (m, 4H), 2.77−2.73 (m, 3H), 2.50−2.46 (m, 2H), 2.31 (s, 2H), 1.94−1.87 (m, 2H), 1.83−1.75 (m, 2H), 1.68−1.61 (m, 2H), 1.49−1.48 (m, 2H). ^{13}C NMR (CD_3OD, 100 MHz) δ 122.0, 69.1, 68.2, 63.6, 53.8, 35.6, 29.0, 25.6.

4-(4-Aminomethylpiperidin-1-ylmethyl)tetrahydropyran-4-ol Maleate Acid (21). A solution of nitrile **20** in THF (3.36 L) was charged with sponge nickel (JM type A, 1.67 mol, 98.00 g) under nitrogen. The mixture was hydrogenated at 30 psi for 12 h and was then filtered and treated with a solution of maleic acid (145 g, 1.25 mol) in 400 mL of THF over 1 h. The mixture was stirred for 2 h and the resulting solids were collected by filtration, rinsed with THF (500 mL), and dried on the filter for 12 h to afford 415 g of 4-(4-aminomethylpiperidin-1-ylmethyl)tetrahydropyran-4-ol, maleate salt as a white solid, mp 135−137 °C. ^{1}H NMR (CD_3OD, 400 MHz) δ 6.22 (s, 2H), 3.78−3.67 (m, 4H), 3.08 (d, J = 11.6, 2H), 2.83 (d, J = 7.1, 2H), 2.45 (s, 2H), 2.39 (t, J = 11.6, 2H), 1.74−1.60 (m, 5H), 1.53−1.38 (m, 4H). ^{13}C NMR (CD_3OD, 100 MHz) δ 170.0, 135.1, 68.7, 68.0, 63.5, 55.4, 44.5, 35.8, 33.8, 29.0.

■ ASSOCIATED CONTENT

Supporting Information

Measured and predicted brain concentrations, summary of toxicology results, and Cerep profile for **2d**, **3**, and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

5-HT, serotonin; 5-HT$_4$, serotonin receptor subtype 4; ACh, acetylcholine; AD, Alzheimer's disease; AUC, area under the curve; cAMP, cyclic adenosine monophosphate; CHO, Chinese hamster ovary; CNS, central nervous system; CSF, cerebrospinal fluid; EC_{50}, concentration giving a 50% response; E_{max}, maximal effect; GPCR, G-protein-coupled receptor; HEK, human embryonic kidney; RRCK, HLM, human liver microsome; K_i, constant for inhibition of binding; MDCK, Madin−Darby canine kidney; MWM, Morris water maze; Pgp, P-gycloprotein; RO, receptor occupancy; SEM, standard error of the mean; sc, subcutaneous; THP, tetrahydropyran

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