Journal of Medicinal Chemistry

Article

Cite This: J. Med. Chem. XXXX, XXX, XXX−XXX

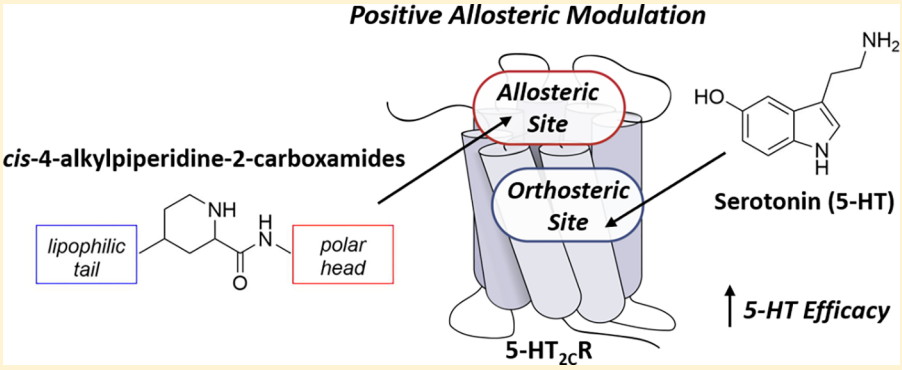
pubs.acs.org/jmc

Design, Synthesis, and Characterization of 4-Undecylpiperidine-2-carboxamides as Positive Allosteric Modulators of the Serotonin (5-HT) 5-HT$_{2C}$ Receptor

Christopher T. Wild,[†,‡,∥] Joanna M. Miszkiel,[†,∥] Eric A. Wold,[†,‡,∥] Claudia A. Soto,[†] Chunyong Ding,[†,‡] Rachel M. Hartley,[†] Mark A. White,[§] Noelle C. Anastasio,*[,†,‡] Kathryn A. Cunningham,*[,†,‡] and Jia Zhou*[,†,‡]

[†]Center for Addiction Research, [‡]Department of Pharmacology and Toxicology, and [§]Sealy Center for Structural Biology and Molecular Biophysics, University of Texas Medical Branch, Galveston, Texas 77555, United States

Supporting Information



ABSTRACT: An impaired signaling capacity of the serotonin (5-HT) 5-HT$_{2C}$ receptor (5-HT$_{2C}$R) has been implicated in the neurobehavioral processes that promote relapse vulnerability in cocaine use disorder (CUD). Restoration of the diminished 5-HT$_{2C}$R signaling through positive allosteric modulation presents a novel therapeutic approach. Several new molecules with the 4-alkylpiperidine-2-carboxamide scaffold were designed, synthesized, and pharmacologically evaluated, leading to the discovery of selective 5-HT$_{2C}$R positive allosteric modulators (PAMs). Compound **16** (CYD-1-79) potentiated 5-HT-evoked intracellular calcium release in cells stably expressing the human 5-HT$_{2C}$R but not the 5-HT$_{2A}$R cells. A topographically distinct allosteric site was identified based on the newly solved 5-HT$_{2C}$R structure. Compound **16** modulated 5-HT$_{2C}$R-mediated spontaneous ambulation, partially substituted for the training dose of the 5-HT$_{2C}$R agonist WAY163909, synergized with a low dose of WAY163909 to substitute fully for the stimulus effects of WAY163909, and attenuated relapse vulnerability as assessed in a rodent self-administration model, indicating its therapeutic promise for CUD.

INTRODUCTION

The United States is reeling from a public health crisis driven by the misuse of psychoactive drugs, the pervasive trajectory to substance use disorders (SUDs), and the limited access to efficacious treatment strategies. A 61% increase in adults initiating cocaine use coupled with the highest cocaine overdoses reported since 2006 reveal alarming trends[1] and signal the potential for a resurgence in the incidence of cocaine use disorder (CUD), an acquired brain disorder marked by habitual cocaine-seeking despite adverse consequences. Core challenges in recovering from CUD include the risk for relapse promoted in part by the attentional orientation toward motivationally relevant, drug-associated stimuli (cue reactivity) that support drug-seeking during abstinence.[2] New, effective, and accessible modalities for the treatment of CUD are needed to combat relapse risk to alleviate the chronic psychological and physical burdens of this disorder.

Dysfunctional serotonin (5-hydroxytryptamine; 5-HT) neurotransmission contributes to the neurobiological states that precipitate relapse events in CUD and is an important target in the quest to develop effective medications for CUD.[2,3] Serotonin is involved in essential brain functions as diverse as appetite, cognition, reward, and motor control.[4] The actions of 5-HT are transduced by 14 receptor subtypes, which are classified into seven receptor families based upon the conjunction of genetic and molecular structure, intracellular transduction mechanisms, and pharmacological criteria. The 5-HT$_2$ receptor (5-HT$_2$R) subtype family of 5-HT receptors is composed of three G protein-coupled receptors (GPCRs), 5-HT$_{2A}$R, 5-HT$_{2B}$R, and 5-HT$_{2C}$R, that share a high level of

Special Issue: Allosteric Modulators

Received: March 12, 2018
Published: April 5, 2018

DOI: 10.1021/acs.jmedchem.8b00401
J. Med. Chem. XXXX, XXX, XXX−XXX

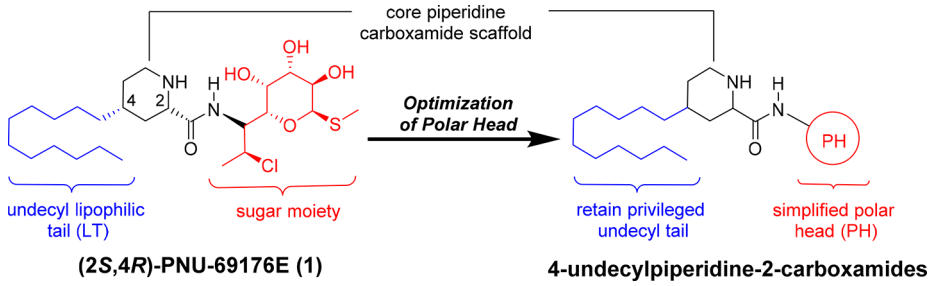


Figure 1. Initial design strategy for novel 5-HT_{2C}R positive allosteric modulators based on **1**.

amino acid sequence homology within transmembrane regions.[5] In particular, hypofunctional 5-HT_{2C}R neurotransmission is implicated as a core mediator of relapse vulnerability, and selective 5-HT_{2C}R agonists are efficacious to blunt cocaine cue reactivity as well as the rewarding effects of cocaine in preclinical models[2,3,6−12] but lack abuse liability on their own.[13−15] However, achieving receptor subtype selectivity versus the highly homologous 5-HT_{2A}R and 5-HT_{2B}R is an essential requisite for the successful development of 5-HT_{2C}R ligands because activation of the related 5-$HT_{2A/2B}$R systems can result in significant adverse CNS (5-HT_{2A}R) and cardiovascular (5-HT_{2B}R) effects.[16] Despite advances in the past decade,[17,18] attaining high 5-HT_{2C}R selectivity remains a challenge yet to be solved. For instance, dexfenfluramine is a 5-HT_{2C}R agonist that was previously approved for antiobesity therapy. However, this drug was later withdrawn from the market because of side effects caused by its potent agonist activity at the 5-HT_{2B}R.[16] Lorcaserin, a conformationally restricted analogue of dexfenfluramine, is a 5-HT_{2C}R agonist that has recently been approved by the FDA for treatment of obesity. However, lorcaserin is classified as a Schedule IV drug due to potential side effects, and like most other 5-HT_{2C}R agonists, its selectivity for 5-HT_{2C}R (K_i = 15 nM) is only modest with a 7.5-fold selectivity over the 5-HT_{2A}R (112 nM) and 11.6-fold selectivity versus the 5-HT_{2B}R (174 nM).[19]

Most therapeutic compounds that target GPCRs interact at the orthosteric site, which accommodates the endogenous ligand, to control downstream intracellular signaling. However, with an increasing emphasis on cellular functional screens, a growing number of allosteric modulators of GPCRs have been discovered and are under investigation as potential medications. These small molecules do not bind to the orthosteric ligand site but instead act at a topographically distinct allosteric site on the receptor and either potentiate or inhibit the binding or signaling of an orthosteric ligand. There are theoretical reasons that allosteric ligands may be preferred therapeutics, including the prospects for increased subtype selectivity, avoidance of receptor desensitization, better control of physiological systems, and upper ceiling effects, as well as separate control of affinity and efficacy of orthosteric ligands.[20] In recent years, multiple allosteric modulators of GPCRs have been developed and predicted to have robust effects in a variety of CNS disorders.[21−23] The recent preclinical indications of efficacy, coupled with the launch of cinacalcet[24] and maraviroc[25] as the first marketed GPCR allosteric modulators, provide strong validation of the clinical utility of both positive and negative allosteric modulators, respectively. The validated proof of concept is fueling the discovery of highly selective ligands for other GPCRs that have been previously intractable. Therefore, allosteric modulators of 5-HT_{2C}R present a novel pharmacological strategy to fine-tune binding or signaling in response to endogenous 5-HT or synthetic ligands in a site- and event-specific manner. Such a strategy may address issues concerning receptor subtype selectivity, toxicity, desensitization, and long-term changes in receptor up or down regulation that orthosteric ligands may promote.[26]

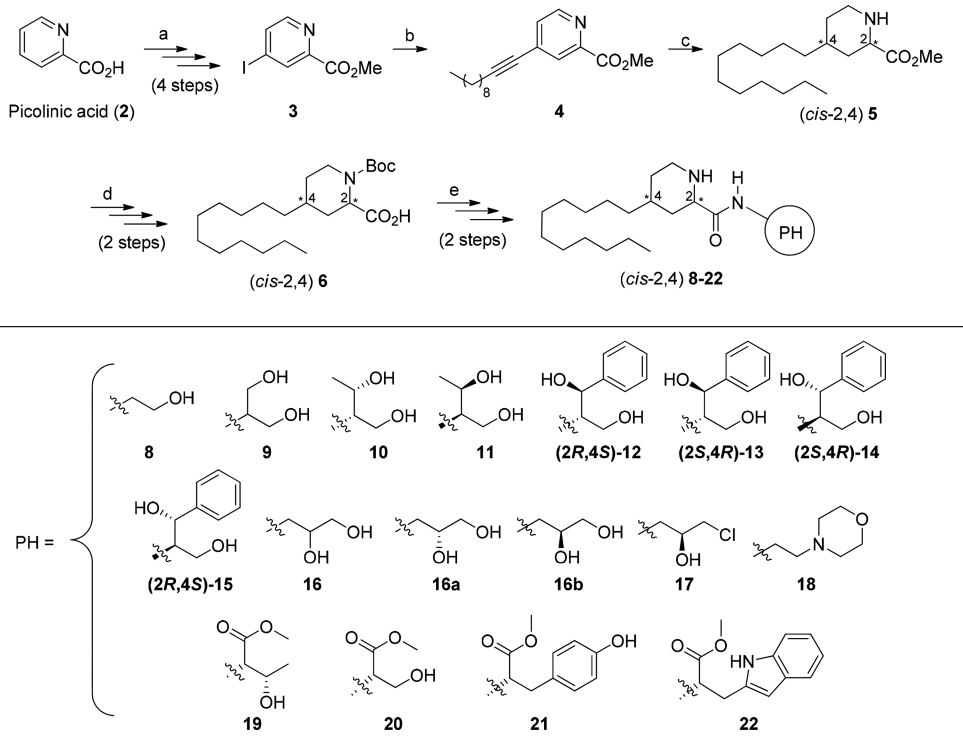
An analogue of the antibiotic lincomycin, PNU-69176E (**1**, Figure 1), was identified via a chemical library screen as a 5-HT_{2C}R positive allosteric modulator (PAM).[27] Nevertheless, little effort has been made on structural modifications of chemical lead **1** to optimize its PAM activity, and thus the relevant structure−activity relationship (SAR) studies remain sparse. Structurally, **1** consists of two moieties directly attached to the piperidine-2-carboxamide core scaffold, an undecyl lipophilic tail (LT) and a polar head (PH) moiety including a complex α-D-galactopyranoside, neither of which are optimal for displaying drug-like properties such as cLogP, tPSA, and bioavailability [see Supporting Information (SI), Table S1]. We have established the only reported method to readily access **1** and its diastereomer and demonstrated that **1** exhibits a profile of 5-HT_{2C}R PAM activity with no intrinsic agonist activity at the 5-HT_{2C}R or 5-HT_{2A}R.[28] While the lead 5-HT_{2C}R PAM recently described was shown to suppress locomotor activity, food intake, and body weight gain,[29] the impact of 5-HT_{2C}R PAMs remains to be explored with *in vivo* models to uncover interaction with exogenous 5-HT_{2C}R stimulation and to determine efficacy to suppress relapse vulnerability in CUD. These combined observations clearly justify our structural optimization campaign to further explore the SAR of structural modifications of **1** and develop new 5-HT_{2C}R putative PAMs with an enhanced pharmacological profile for the treatment of CUD. Herein, we describe our efforts for the rational design, chemical synthesis, and pharmacological evaluation of novel, simplified 5-HT_{2C}R PAMs based on **1** as the chemical lead. The identification of small molecule 5-HT_{2C}R PAMs affords novel chemical probes for elucidating the neurobiology of 5-HT_{2C}R signaling and new opportunities for proof of concept studies to develop pharmacotherapies for CUD arising from deficient 5-HT_{2C}R signaling capacity.

RESULTS AND DISCUSSION

Chemistry. Very little is known about allosteric modulation of the 5-HT_{2C}R, and the binding site of **1** remains unclear. In our drug design strategy, we speculate that **1** consists of three possible moieties important for binding, including the previously described PH and LT, and the N−H of the core piperidine, as depicted in Figure 1. This hypothesis was formulated initially on the basis of the available pharmacophore-directed homology modeling of the human 5-HT_{2C}R[30,31] and the limited SAR studies on **1** that showed that shorter alkyl chains (methyl to *n*-hexyl groups) or the undecyl derivatives

DOI: 10.1021/acs.jmedchem.8b00401
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 1. Synthesis of PNU-69176E Analogs with Simplified Polar Heads (PH)[a]



[a]Reagents and conditions: (a) see ref 28; (b) 1-undecyne, CuI, $Pd(PPh_3)_2Cl_2$, Et_3N, rt, 12 h, 94%; (c) H_2 (60 psi), PtO_2, HCl/MeOH/H_2O, rt, 16 h, 95%; (d) (i) $(Boc)_2O$, Et_3N, MeOH, rt, 16 h, 90%; (ii) LiOH, THF/H_2O (v/v = 2:1), rt, 48 h, 85%; (e) (i) amino alcohols, HBTU, DIPEA, DMF, rt, 16 h; (ii) TFA, CH_2Cl_2, rt, 63−88% (two steps).

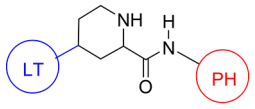
with the headgroup of *N*-phenyl, *N*,*N*-diethyl, *N*-[2-(dimethylamino)ethyl]-*N*-methyl, or *N*-(4-acetoamido-1-naphthylsulfonyl) in place of the specific polar sugar moiety failed to exert positive allosteric modulation on 5-HT_{2C}R.[27] This suggests that the PH and LT are likely important pharmacophore groups required for allosteric modulation. Considering the poor drug-like properties of **1**, our initial effort was directed to simplify and optimize the PH. The N−H of the piperidine was kept intact so that the target compounds could form HCl salts for improved aqueous solubility.

We first designed a series of new analogues to optimize the PH by keeping the *n*-undecyl LT intact while replacing the sugar moiety of **1** with a variety of simple amino alcohols (Scheme 1). These simplified PHs structurally resemble fragments of the polar α-D-galactopyranoside moiety of **1**, which presumably undergo selective binding via hydrogen bonding or dipole−dipole interactions near the membrane surface. These new analogues, with simplified and readily accessible PHs, have enhanced drug-like properties (Table S1) and development potential. According to our previously established synthetic route,[28] a four-step protocol affords **3**, which undergoes Sonogashira coupling with 1-undecyne, resulting in the alkynylated picolinic derivative **4**. Metal-catalyzed hydrogenation readily provided the (*cis*-2,4)-piperidyl carboxylate **5**. Boc-protection and hydrolysis of **5** provided **6** as the key intermediate in high yield on the multigram scale (68% yield from **3**). Direct deprotection of **6** afforded the parent amino acid **7**. With **6** in hand, amidation coupling with a variety of amino alcohols using HBTU/DIPEA followed by Boc-deprotection with TFA generated a series of novel derivatives **8**−**22** (Table 1) with diverse, simplified PHs in 63−88% yields (2 steps, Scheme 1). It was noted that the diastereomers **12**−**15** could be separated through preparative TLC (PTLC) when the phenyl amino alcohols were employed as the PHs. The other compounds were inseparable enantiomeric or diastereomeric mixtures of isomers by TLC.

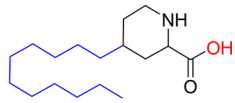
To further explore the SAR of the LT attached to the core piperidinyl scaffold, replacement of the long alkyl chain with other saturated moieties (cyclohexyl, cyclohexylethyl or 4-methylcyclohexylethyl) were investigated. The 2-amino-1-phenyl-1,3-propanediol PHs consistently resulted in separable isomers when coupled with the piperidine scaffold bearing an undecyl tail; therefore, these polar heads were retained with the cyclohexyl tails with the hope that the isomers would again be separable by TLC. As shown in Scheme 2, Suzuki coupling of **3** with phenylboronic acid or Sonogashira coupling of **3** with ethynylbenzene or 1-ethynyl-4-methylbenzene afforded requisite substituted picolinic derivatives **23**−**25**. Metal-catalyzed hydrogenation of compounds on a Parr hydrogenator at 60 psi in the presence of 0.4 equiv of PtO_2 and 1 equiv of 37% aq HCl readily provided compounds **26**−**28**. Boc-protection and hydrolysis of **26**−**28** provided **29**−**31** as the key intermediates in high yield on the multigram scale. Amidation coupling with (1*S*,2*S*)- or (1*R*,2*R*)-(−)-2-amino-1-phenyl-1,3-propanediol followed by removal of Boc groups with TFA furnished a series of novel derivatives **32**−**37** with compact LTs in 83−86% yields (two steps). As previously observed, each amidation coupling reaction also produced a pair of diastereoisomers, which could be separated by PTLC after the Boc-deprotection. It was our expectation that the compact LTs would result in final compounds that could form crystals suitable for X-ray diffraction. To our delight, two of the six compounds were crystallized and characterized. As shown in Figure 2, the absolute stereochemistry of compounds **32** and **35** was

DOI: 10.1021/acs.jmedchem.8b00401
J. Med. Chem. XXXX, XXX, XXX−XXX

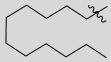
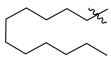
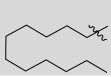
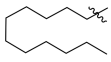
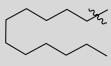
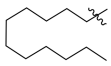
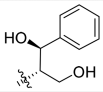
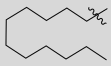
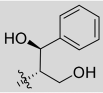
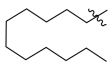
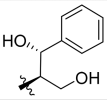
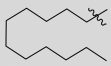
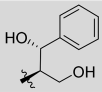
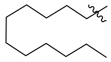
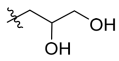
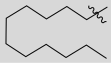
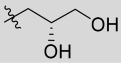
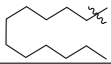
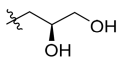
Table 1. Effects of 4-Alkylpiperdine-2-carboxamides (1 nM) on 5-HT-Induced Ca_i^{2+} Release in h5-HT_{2C}R-CHO Cells

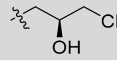
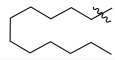
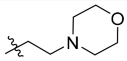
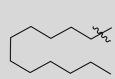
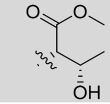
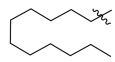
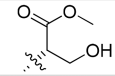
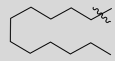
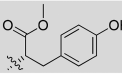
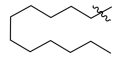
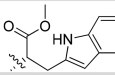
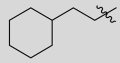
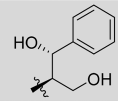
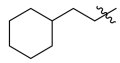


8-22, 32-37



7

Compd	LT	PH	Emax RFU (%5-HT)[a]
5-HT[c]	-	-	100.0%
7		-COOH	109.2 ± 4.0% p=0.0837
8			107.7 ± 4.9% p=0.1984
9			107.6 ± 2.2%* 0.0243
10			111.7 ± 5.6% p=0.0909
11			111.1 ± 6.6% p=0.1673
(2*R*,4*S*)-12			102.1 ± 3.3% p=0.5900
(2*S*,4*R*)-13			109.2 ± 8.1% p=0.3100
(2*S*,4*R*)-14			85.1 ± 3.2%* p=0.0187
(2*R*,4*S*)-15			93.9 ± 2.8% p=0.1177
16			123.2 ± 4.1%* p=0.0005
16a			105.8 ± 2.8% p=0.0710
16b			113.7 ± 3.4%* p=0.0035

Compd	LT	PH	Emax RFU (%5-HT)[a]
17			114.4 ± 9.0% p=0.1690
18			107.1 ± 2.6% p=0.0536
19			101.8 ±4.4% p=0.7052
20			99.6 ± 7.4% p=0.9611
21			104.2 ± 10.2% p=0.7031
22			102.1 ± 10.5% p=0.8528
(2*S*,4*R*)-32			96.0 ± 1.9% p=0.1698
(2*R*,4*S*)-33			89.6 ± 4.8% p=0.1161
(2*S*,4*R*)-34			106.9 ± 6.5% p=0.3477
(2*R*,4*S*)-35			116.2 ± 7.9% p=0.0759
(2*S*,4*R*)-36			104.9 ± 4.1% p=0.3031
(2*R*,4*S*)-37			104.1 ± 4.3% p=0.3907

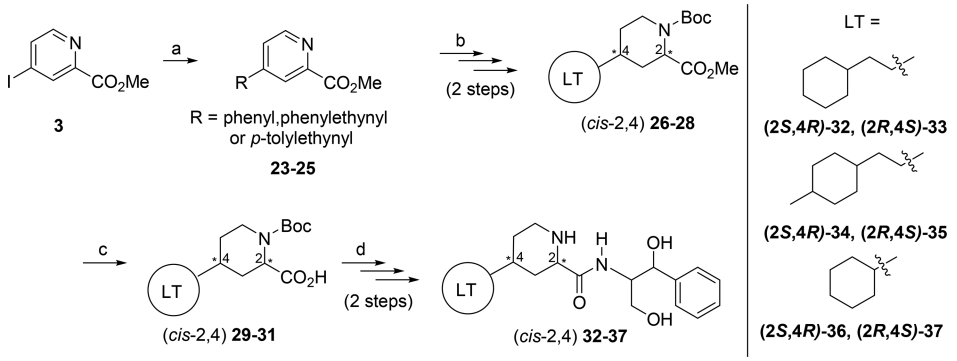
[a]Maximum 5-HT-induced Ca_i^{2+} release (E_{max}) in the presence of 1 nM of test compound; the screen utilized concentrations of 5-HT (vehicle, 10^{-11} to 10^{-6} M) to establish the E_{max} of 5-HT in the presence of the test compound as described in Figure S2. * indicates $p < 0.05$. The E_{max} for the test compound plus 5-HT was normalized to the E_{max} for 5-HT alone. Subsequent post hoc comparisons between means for E_{max} were made using an unpaired *t* test with Welch's correction (GraphPad Prism). All statistical analyses were conducted with an experiment-wise error rate of $\alpha = 0.05$.

unambiguously determined by their X-ray crystallography analyses (Table S2, Figure S1), from which the stereochemistry of the other diastereoisomers could also be deduced as described in our previous work.[28]

***In Vitro* Ca_i^{2+} Release Assay in h5-HT_{2C}R-CHO Cells.** The best-characterized intracellular pathway of 5-HT_{2C}R signaling is the activation of phospholipase Cβ (PLCβ) via $G_{\alpha q/11}$ proteins followed by the production of inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG), leading to increased Ca_i^{2+} release from intracellular stores.[32] Therefore, *in vitro* functional characterization of these synthetic compounds can be determined by utilizing a fluorescence-based, live cell Ca_i^{2+} release assay to measure activation of the 5-HT_{2C}R signaling pathway. The Ca_i^{2+} levels can be regarded as a primary readout, as has been utilized in a multitude of 5-HT_{2C}R drug discovery projects.[33] Our assay was conducted in Chinese hamster ovary (CHO) cells stably transfected with the human 5-HT_{2C}R (unedited INI isoform; h5-HT_{2C}R-CHO cells).[28,34,35] The maximum capacity of 5-HT to promote Ca_i^{2+} release (E_{max}) was established and set to 100% response. All compounds are reported relative to the E_{max} of 5-HT. Previous titration studies with compound **1** revealed an upward shift of 5-HT-evoked Ca_i^{2+} release at the concentration effective to evoke 20% response (EC_{20}), while 1 nM of **1** produced a leftward shift of the 5-HT-evoked Ca_i^{2+} response curve.[28] Therefore, all compounds were initially screened at 1 nM for

DOI: 10.1021/acs.jmedchem.8b00401
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 2. Synthesis of PNU-69176E Analogs with Compact Lipophilic Tails (LT)[a]



[a]Reagents and conditions: (a) Suzuki coupling, (i) phenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, EtOH/H_2O/PhMe = 2:1:1, reflux; (ii) H_2SO_4, methanol, reflux, 98% (two steps); or Sonogashira coupling, ethynylbenzene or 1-ethynyl-4-methylbenzene, CuI, $Pd(PPh_3)_2Cl_2$, Et_3N, rt, 12 h, 88% to 97%; (b) (i) H_2 (60 psi), PtO_2, HCl/MeOH/H_2O, rt, 16 h, 97−98%; (ii) $(Boc)_2O$, Et_3N, MeOH, rt, 16 h, 96−98%; (c) LiOH, THF/H_2O (v/v = 2:1), rt, 48 h, quantitative yield; (d) (i) amino alcohols, HBTU, DIPEA, DMF, rt, 16 h; (ii) TFA, CH_2Cl_2, rt, 83−86% (two steps).

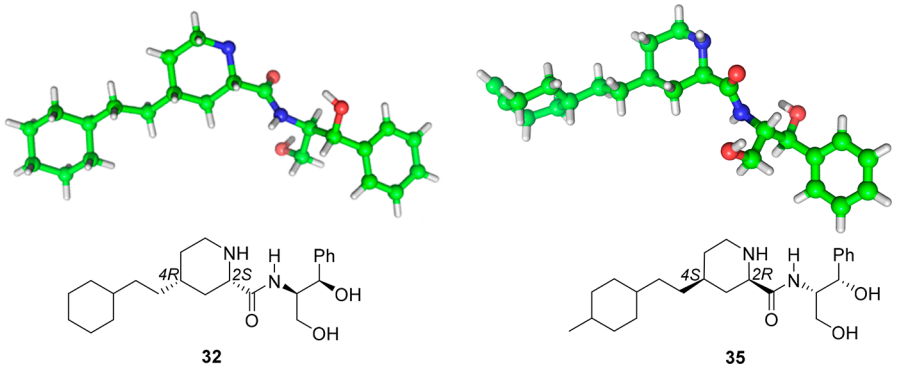


Figure 2. Determination of absolute configuration of chiral carbons 2 and 4 of the piperdinyl ring. X-ray crystallography structures of analogues (2*S*,4*R*)-**32** and (2*R*,4*S*)-**35**.

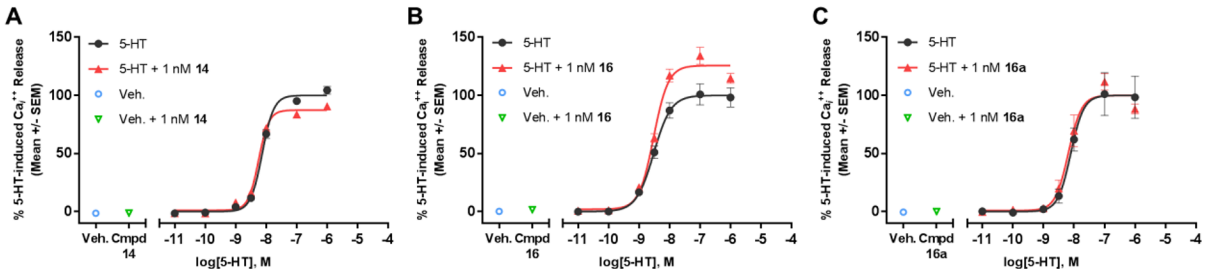


Figure 3. Effects of select compounds on Ca_i^{2+} release in live h5-HT_{2C}R-CHO cells in the absence (black circles) and in the presence of the test compound (red triangles) against the concentration−response curve for 5-HT, vehicle (blue circle), or vehicle in the presence of test compound (green triangle). (A) Compound **14** (1 nM), representative example of a potential NAM; (B) compound **16** (1 nM), representative example of a potential PAM; (C) compound **16a** (1 nM), representative example of an inactive compound. The maximum 5-HT-induced Ca_i^{2+} release in the absence of the test compounds was set as 100%. The E_{max} of 5-HT in the presence of the test compounds are listed in Table 1. Concentration−response curves for remaining compounds are in Figure S2.

their ability to enhance 5-HT-induced Ca_i^{2+} release evoked by increasing concentrations of 5-HT in h5-HT_{2C}R-CHO cells (Figure S2). The cells were pretreated for 15 min with each test compound to establish an absence of intrinsic agonist activity in each assay. The analysis of novel compounds on maximum 5-HT-induced Ca_i^{2+} release in h5-HT_{2C}R-CHO cells is summarized in Table 1.

None of the 24 new analogs screened exhibited intrinsic efficacy at 1 nM to induce Ca_i^{2+} release in h5-HT_{2C}R-CHO cells in the absence of 5-HT (Figure S2). Compound **7**, the deprotected amino acid of the key intermediate **6** (Scheme 1), exhibited no significant PAM activity (Table 1). Topographical analysis of **1** revealed 2- or 3-carbon long chains to the nearest heteroatom (O and Cl) in the sugar-like PH. Therefore, the selection of PHs emphasized the incorporation of −OH(s) two carbons away from the amide linker. The coupling of ethanolamine with the key intermediate afforded **8**. Similar to **7**, no significant PAM activity with **8** was observed (Table 1). Incorporation of two −OH groups (a 1,3-propandiol fragment, **9**) provided a negligible increase in 5-HT-induced Ca_i^{2+} release, though statistically significant. Various analogues that retained the 1,3-propanediol fragment with the addition of a methyl or

DOI: 10.1021/acs.jmedchem.8b00401
J. Med. Chem. XXXX, XXX, XXX−XXX

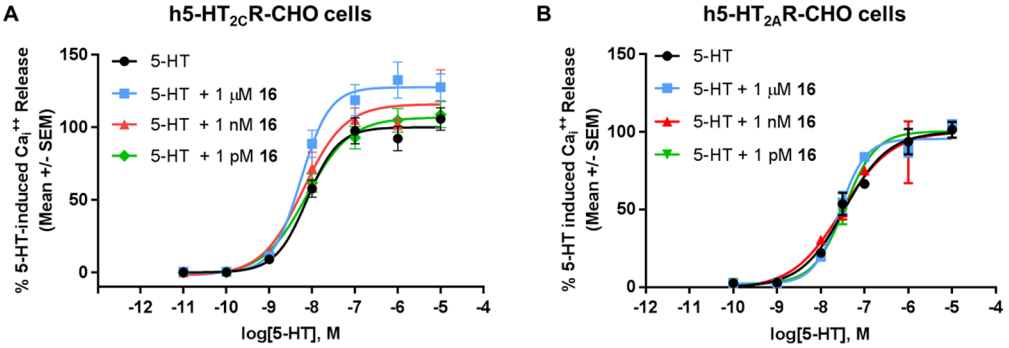


Figure 4. Effects of **16** on Ca_i^{2+} release in live h5-HT_{2C}R-CHO cells (A) and h5-HT_{2A}R-CHO cells (B) against the concentration−response curve for 5-HT. (A) Compound **16** (1 pM, 1 nM, 1 μM) demonstrates an upward shift in h5-HT_{2C}R-CHO cells. (B) Compound **16** (1 pM, 1 nM, 1 μM) does not alter 5-HT-induced signaling in h5-HT_{2A}R-CHO cells. The maximum 5-HT-induced Ca_i^{2+} release in the absence of the test compounds was set as 100%.

phenyl group (**10**−**15**) did not provide for the desired PAM activity (Table 1). However, compound **14**, which was a separable isomer with the (2*S*,4*R*) configuration at the piperidine ring and the (1*R*,2*R*) configuration for the PH moiety, displayed significant activity consistent as a negative allosteric modulator (NAM, Figure 3A, Table 1). Next, the use of an additional carbon spacer between the amide linker and the most distal −OH was explored through the incorporation of a 1,2-propanediol fragment (**16**). The glycol moiety proved fruitful. Compound **16** displayed significant PAM activity and increased the 5-HT-evoked Ca_i^{2+} release ∼23% above the 5-HT E_{max} value (Figure 3B). The enantiomerically pure versions of the PH of **16** were incorporated into the scaffold and were tested and showed a stereochemical preference for PAM activity. Compound **16a**, the *R*-variant at the PH, lacked appreciable PAM activity (Figure 3C), while the *S*-configuration (**16b**), like **16**, displayed significant PAM activity (Table 1). Incorporation of a terminal −Cl abolished statistically significant activity (**17**). Additional attempts to incorporate various hydrogen bond donor/acceptor moieties as PHs did not produce compounds with PAM activity [*N*-ethyl morpholino (**18**), methyl esters of threonine (**19**), serine (**20**), tyrosine (**21**), and tryptophan (**22**)]. The lactate dehydrogenase assay was employed to predict the cytotoxicity of compounds at 0.1 nM and 1 μM; none of the tested compounds exhibited values in this assay indicative of cellular toxicity (Table S3).

Compound **16** promoted an upward shift of 5-HT-evoked Ca_i^{2+} release at multiple concentrations (Figure 4A) without a leftward shift, an indication that **16** promotes improved 5-HT efficacy but did not impact 5-HT potency at the 5-HT_{2C}R (Figure 4A). To determine the selectivity of **16** for allosteric modulation of the 5-HT_{2C}R over the 5-HT_{2A}R, multiple concentrations of **16** were employed to assess 5-HT-evoked Ca_i^{2+} release in h5-HT_{2A}R-CHO cells (Figure 4B).[28,34] Compound **16** produced no change in the 5-HT E_{max} or EC_{50} at the 5-HT_{2A}R (Figure 4B). These data provide the conceptual framework for the continued development of selective PAMs of the 5-HT_{2C}R given the ability of **16** to affect 5-HT efficacy at the 5-HT_{2C}R (Figure 4A), but not the 5-HT_{2A}R (Figure 4B).

The compounds synthesized according to Scheme 2 retained the 1-phenyl-1,3-propandiol PH in an effort to produce separable isomers to further explore the impact of changes made to the LT. Preserving the PH used in the potential NAM **14**, which produced a decrease in 5-HT-mediated Ca_i^{2+} release, while changing the LT from the undecyl tail to an ethylcyclohexyl moiety, afforded a NAM that promoted a slight but significant decrease in 5-HT-induced Ca_i^{2+} release (**32**, 96.0% ± 1.9%). All other attempts to utilize various LTs failed to produce any compounds with allosteric effects (**34**−**37**). Exploration of the remaining chemical space, in particular with regard to the LT, will be reported in due course.

Molecular Docking to 5-HT_{2C}R. To explore a potential binding mode and provide additional lines of evidence that the described compounds are allosteric modulators, molecular docking using the Schrödinger Drug Discovery Suite was employed to identify the allosteric binding site relative to the orthosteric binding site (see SI for details on docking protocol). Until recently, the only solved structures of serotonin receptors were the 5-HT_{1B}R and 5-HT_{2B}R.[36−38] However, the very recently disclosed 5-HT_{2C}R structure has afforded the opportunity to conduct the first allosteric-5-HT_{2C}R docking study.[39] With compound **16b** displaying positive allosteric modulation, molecular docking using the active state of the 5-HT_{2C}R was used to search the extracellular portion of the receptor for a suitable allosteric site.

Figure 5A depicts a global view of the 5-HT_{2C}R and the docked endogenous ligand 5-HT (pink space-fill) in the orthosteric site engaging residues consistent with previous reports based on site-directed mutagenesis studies.[41] The red cluster overlay of **16b** (red wire-representation of top five poses using Schrödinger Glide scoring function) highlighted a possible binding pocket for the proposed PAM as being topologically distinct from the orthosteric site.

The allosteric site (Figure 5A) is near extracellular loop 2 (EL2) accommodating the PH of the proposed PAM, an observation also made when describing similar binding modes of other class A GPCRs.[42] Figure 5B depicts the 1,2-diol PH moiety with the *S*-configuration engaging in a bidentate, H-bonding interaction with the backbone carbonyl L209 of EL2, a residue conserved in the 5-HT_2R subfamily. Additionally, the ionizable N atom of the piperidine ring engages in an H-bond with the −OH side chain of S334 of transmembrane helix VI (TMH VI), which is not present in either the 5-HT_{2A}R or 5-HT_{2B}R, thus suggesting a predicted molecular basis for the 5-HT_{2C}R subtype functional selectivity over the 5-HT_{2A}R as described in our cell-based studies. The predicted bridging effect of **16b** between EL2 and TMH VI may result in a drawing in of the extracellular portion of TMH VI and, thereby, promote the outward shift of the intracellular portion of TMH VI, consistent with reports that this helical movement is

DOI: 10.1021/acs.jmedchem.8b00401
J. Med. Chem. XXXX, XXX, XXX−XXX

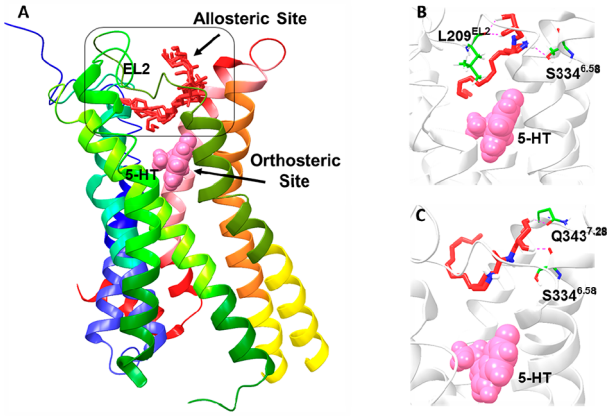


Figure 5. Binding poses of (A) global view of 5-HT_{2C}R (PDB 6BQG) with 5-HT (pink space-fill representation) at the orthosteric site and **16b** (red stick representation overlay of top five poses) at the proposed allosteric site; (B) representative pose of **16b** (red stick representation) engaged with residues of proposed allosteric site (superscript designation represents Ballesteros–Weinstein notation);[40] (C) representative pose of **16a** (red stick representation).

associated with the active state of the receptor.[39,43,44] Compound **16a**, containing the 1,2-diol PH with the *R*-configuration, does not form the same EL2-to-TMH VI bridge (Figure 5C), providing a potential explanation as to why **16a** is inactive in the described cell-based assay.

***In Vitro* Assessment of Off-target Effects of Compound 16.** Preliminary data from a broad-panel assay generously provided by the National Institute of Mental Health (NIMH) Psychoactive Drug Screening Program (PDSP)[45] showed that the most promising PAM, compound **16** assessed at 10 μM displayed no significant ability to displace binding to a variety of related receptors (Table 2). Specifically, at 10 μM, **16** did not displace binding of radioligands to 5-HT receptors (5-HT_{1A}R, 5-HT_{1B}R, 5-HT_{1D}R, 5-HT_{1E}R, 5-HT_{2A}R, 5-HT_{2B}R, 5-HT_{2C}R, 5-HT_3R, 5-HT_{5A}R, 5-HT_6R, 5-HT_7R), dopamine receptors (D_1, D_2, D_4, D_5), or monoamine transporters [5-HT transporter (SERT) and norepinephrine transporter (NET)]. Compound **16** (10 μM) shows weak to no displacement of binding to other evaluated receptors and monoamine transporters (Table S4). Compound **16** (10 μM) did display significant inhibition (defined as greater than 50%) of radioligand binding at the dopamine transporter (DAT; 66.3%), dopamine D_3 receptor (71.7%), or α_{2A} and α_{2B} receptors (85.1% and 80.7%, respectively). However, the concentration (10 μM) tested in the radioligand displacement assay is significantly higher than the concentrations of **16** (1 pM, 1 nM, 1 μM) screened for activity in the functional assay. Therefore, in total, compound **16** appears to be promising with regard to displaying minimal off-target effects, a commonly postulated advantage of allosteric modulators of GPCRs.[20] Moreover, **16** showed little to no interaction with the orthosteric sites of the tested 5-HT receptor subtypes.

***In Vivo* Pharmacokinetic (PK) Profile of Compound 16.** The PK profile of **16** was assessed in a pilot study to investigate whether this class of molecules exhibits favorable drug-like properties. The PK parameters of **16** were evaluated in rats after single iv (5 mg/kg) or po (10 mg/kg) administration. As summarized in Table 3, Compound **16** displays a decent half-life ($T_{1/2}$) after administration of 5 mg/kg iv ($t_{1/2}$ = 6.59 ± 0.26 h) or 10 mg/kg po (5.82 ± 0.37 h). The time of maximum concentration (T_{max}) after 10 mg/kg po was 3.3 ± 0.58 h. The plasma clearance (CL) of **16** was 5.37 ± 0.61 L h^{-1} kg^{-1} after 5 mg/kg iv, while the volume of distribution plasma distribution (V_{ss}) was 35.07 ± 4.66 L/kg. Compound **16** displayed a maximum serum concentration (C_{max}) of 68.1 ± 6.8 ng/mL after 10 mg/kg po, while **16** exhibited moderate plasma exposure (AUC_{0-inf}) after administration of 5 mg/kg iv (939 ± 108 ng·h·mL^{-1}) or 10 mg/kg po (737 ± 56 ng·h·mL^{-1}). Decent oral bioavailability (F) was observed (10 mg/kg po; 39.1%). We also calculated the central nervous system (CNS) multiparameter optimization (MPO) value for compound **16** to be 4.5; collective scores range from 0 to 6 with higher MPO scores predicted to be more desirable for CNS medication candidates (Table S5).[46] Based upon these data, compound **16** was selected for subsequent evaluation of *in vivo* efficacy.

Table 2. Displacement of Radioligand Binding by Compound 16 (10 μM) in a Broad Panel of Receptors and Transporters[a]

receptor or transporter	radioligand	% inhib (10 μM)[b]	K_i (μM)[c]
5-HT_{1A}	[^{3}H]-8-OH-DPAT	25.7	[e]
5-HT_{1B}	[^{3}H]-GR125743	−18.1	[e]
5-HT_{1D}	[^{3}H]-GR125743	5.5	[e]
5-HT_{1E}	[^{3}H]-5-HT	0.7	[e]
5-HT_{2A}	[^{3}H]-ketanserin	37.1[d]	[e]
5-HT_{2B}	[^{3}H]-LSD	15.1[d]	[e]
5-HT_{2C}	[^{3}H]-mesulergine	9.8[d]	[e]
5-HT_3	[^{3}H]-LY278584	27.0	[e]
5-HT_{5A}	[^{3}H]-LSD	17.2	[e]
5-HT_6	[^{3}H]-LSD	18.1	[e]
5-HT_7	[^{3}H]-LSD	26.8	[e]
D_1	[^{3}H]-SCH23390	29.3	[e]
D_2	[^{3}H]-*N*-methylspiperone	28.8	[e]
D_3	[^{3}H]-*N*-methylspiperone	71.4	4.7
D_4	[^{3}H]-*N*-methylspiperone	−14.2	[e]
D_5	[^{3}H]-SCH23390	32.5	[e]
DAT	[^{3}H]-WIN35428	66.3	1.8
SERT	[^{3}H]-citalopram	24.8	[e]

[a]National Institute of Mental Health (NIMH) Psychoactive Drug Screening Program (PDSP).[45] Receptor binding profiles were generously provided by NIMH PDSP, Contract no. HHSN-271-2013-00017-C. The PDSP is directed by Bryan L. Roth, M.D., Ph.D., at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscoll at NIMH, Bethesda MD, USA. [b]Data represent mean % inhibition (n = 4) for compound **16** assessed for displacement of binding at targets. Inhibition of binding >50% at 10 μM of compound **16** resulted in determination of K_i. [c]Data represent K_i (μM) values obtained from nonlinear regression of radioligand competition isotherms. K_i values are calculated from best fit IC_{50} values using the Cheng–Prusoff equation. [d]Data for 5-HT_2 receptor subtypes represent the average of the mean % inhibition (n = 8) for compound **16**. [e]Not tested.

Effects of Compound 16 on Spontaneous Locomotor Activity. Our candidate 5-HT_{2C}R PAM could be expected to enhance the effects of endogenous (5-HT) or exogenous 5-HT_{2C}R agonists *in vivo*. Selective and nonselective agonists of the 5-HT_{2C}R induce dose-dependent suppression of spontaneous locomotor activity in rodents, which is reversed by selective 5-HT_{2C}R antagonists.[9,47,48] In the present experiment, we tested the hypothesis that **16** would evoke a modest

DOI: 10.1021/acs.jmedchem.8b00401
J. Med. Chem. XXXX, XXX, XXX–XXX

Table 3. *In Vivo* Pharmacokinetic Profile of Compound 16[a]

dose (mg/kg)	$T_{1/2}$ (h)	T_{max} (h)	CL (L h^{-1} kg^{-1})	V_{ss} (L/kg)	C_{max} (ng/mL)	AUC_{0-inf} (ng·h·mL^{-1})	F (%)
5, iv	6.59 ± 0.26	[b]	5.37 ± 0.61	35.07 ± 4.66		939 ± 108	
10, po	5.82 ± 0.37	3.3 ± 0.58	[b]	[b]	68.1 ± 6.8	737 ± 56	39.1

[a]Values are the average of three runs. Vehicle, DMSO/Tween 80/saline (v/v 1:3:6). $T_{1/2}$, half-life; T_{max}, time of maximum concentration; CL, plasma clearance; V_{ss}, volume of distribution; C_{max}, maximum concentration; AUC_{0-inf}, area under the plasma concentration−time curve; F, oral bioavailability. [b]Not determined.

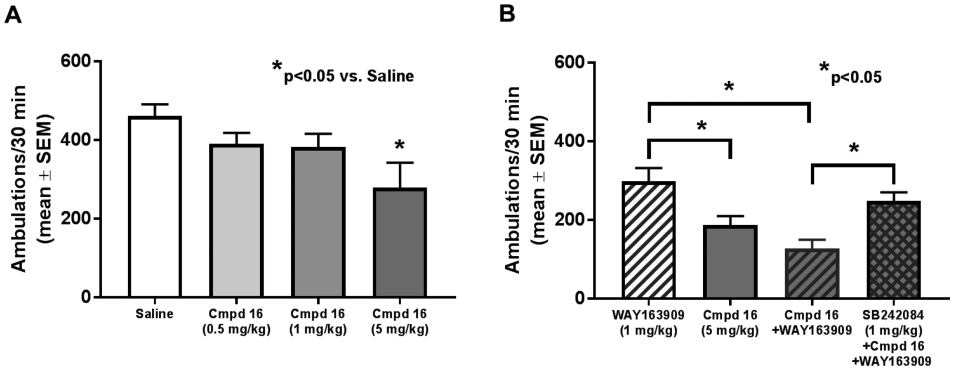


Figure 6. Effects of compound **16** on spontaneous locomotor activity. (A) Groups of rats (n = 7−8/group) naive to the activity monitors were injected with a single dose of saline or compound **16** (0.5, 1, or 5 mg/kg; ip) immediately prior to the start of locomotor assessment. (B) Groups of rats (n = 9−10/group) naive to the activity monitors were injected with a single dose of the 5-HT_{2C}R antagonist SB242084 (1 mg/kg; ip; 15 min pretreatment) and the 5-HT_{2C}R agonist WAY163909 (1 mg/kg; ip) in combination with compound **16** (5 mg/kg; ip) immediately prior to the start of locomotor assessment. The mean total ambulations (±SEM) in 30 min are plotted. *p < 0.05.

suppression of motor activity in adult male Sprague−Dawley rats similar to the observations made for a recently evaluated candidate 5-HT_{2C}R PAM.[29] We assessed the effects of compound **16** (0.5, 1, or 5 mg/kg ip) on spontaneous locomotor activity over a 90 min session. As the effects of compound **16** were confined to the initial 30 min of the session, Figure 6 presents ambulations totaled for that 30 min. A main effect of compound **16** was observed for mean total ambulations (±SEM) [$F_{(3,25)}$ = 3.44, p = 0.03]. *A priori* comparisons revealed that 5 mg/kg of compound **16** significantly reduced mean total ambulations (±SEM) vs saline (p < 0.05; Figure 6A). A main effect of compound **16** treatment was also observed for mean total vertical activity (±SEM) in 30 min [$F_{(3,25)}$ = 9.14, p = 0.0003]. *A priori* comparisons revealed that 5 mg/kg of compound **16** significantly reduced mean total vertical activity (±SEM) vs saline (p < 0.05; data not shown). Ambulation and vertical activity are often positively correlated; however, these measures are actually independent of one another, can be pharmacologically dissociated, and appear to be mediated by different neural circuitry.[49−52]

Figure 6B illustrates the allosteric effects of compound **16** (5 mg/kg) in combination with the selective 5-HT_{2C}R orthosteric agonist WAY163909 (1 mg/kg) on spontaneous locomotor activity in the presence or absence of the 5-HT_{2C}R antagonist SB242084 (1 mg/kg). Pretreatment with SB242084 inhibits WAY163909-evoked suppression of ambulations, but not vertical activity (data not shown). A main effect of treatment was observed for ambulations totaled across the 30 min session [$F_{(3,34)}$ = 8.25, p = 0.0003]. *A priori* comparisons revealed that the combination of compound **16** plus WAY163909 reduced total ambulations versus WAY163909 alone (p < 0.05; Figure 6B); this effect was prevented by SB242084 (p < 0.05; Figure 6B). A main effect of treatment was observed for vertical activity totaled across the 30 min session [$F_{(3,34)}$ = 19.19, p < 0.0001]; *a priori* comparisons revealed that the combination of compound **16** plus WAY163909 reduced vertical activity versus WAY163909 alone (p < 0.05; data not shown); this effect was only marginally prevented by SB242084 pretreatment (p < 0.05; data not shown).

These data demonstrate that the 5-HT_{2C}R is engaged in the effects of compound **16** to suppress motor activity in the presence of exogenous 5-HT_{2C}R stimulation with WAY163909. Furthermore, compound **16** may augment endogenous 5-HT_{2C}R signaling *in vivo* to suppress spontaneous ambulations, as might be expected of a PAM.

Effects of Compound 16 in a WAY163909 vs Saline Drug Discrimination Assay. We employed the drug discrimination assay to explore the impact of compound **16** in the context of endogenous or exogenous 5-HT_{2C}R stimulation. The drug discrimination assay is widely recognized as one of the major methods in neuropharmacology and drug discovery.[53−55] This assay has face validity for modeling the subjective effects of drugs that penetrate the blood−brain barrier as well as to assess the influence of the novel compounds on the interoceptive effects of receptor-selective agonists or other ligands, including allosteric modulators.[56−59] Drug discrimination procedures have been extensively used to characterize the neuropharmacological profile of novel small molecules as well as 5-HT_2R subtype agonists, including 5-HT_{2C}R agonists (e.g., *m*-chlorophenylpiperazine, Ro 60-0175).[60−63] Here, we trained rats to discriminate 0.75 mg/kg of the selective 5-HT_{2C}R agonist WAY163909 from saline in a two-lever drug discrimination protocol. The mean number of sessions required to meet the criterion for acquisition of the WAY163909 (0.75 mg/kg, ip; 15 min pretreatment) versus saline discrimination was 44 training sessions (range 39−57). During dose−response tests, WAY163909 (0.125−1.0 mg/kg, ip) elicited a dose-dependent increase in WAY163909-appropriate responding whereas saline resulted in <10% WAY163909-appropriate responding (Figure 7A, closed

DOI: 10.1021/acs.jmedchem.8b00401
J. Med. Chem. XXXX, XXX, XXX−XXX

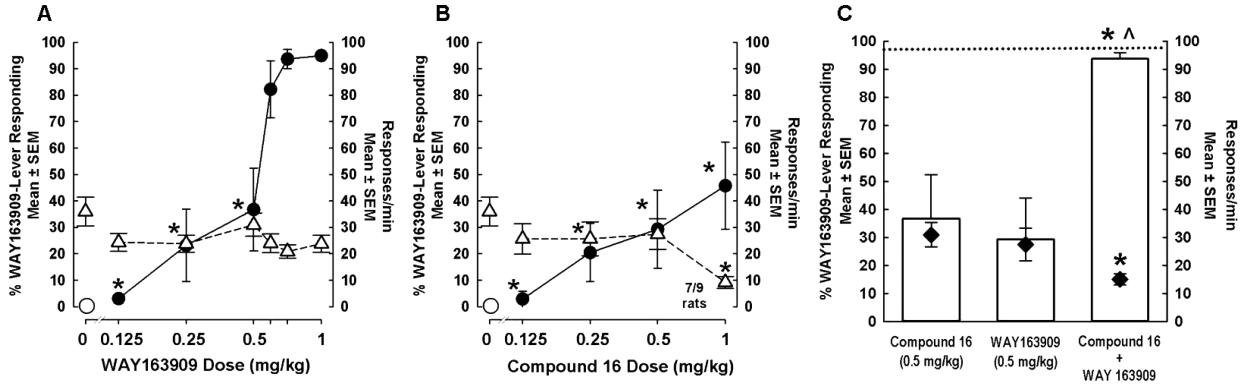


Figure 7. Dose−response relationship of WAY163909, compound **16**, and the combination in a WAY163909 vs saline drug discrimination assay. The dose−response curves (n = 7−9/dose) for (A) WAY163909 (ip) and (B) compound **16** (ip) are presented. Closed circles (solid lines) denote the mean percentage of WAY163909-appropriate responding (±SEM; left axis), and open triangles (dotted lines) denote the mean responses/min (±SEM; right axis). *p < 0.05 vs 0.75 mg/kg WAY163909. (C) The results of substitution tests in which WAY163909 alone or in combination with compound **16** are presented. The dotted line represents the % WAY163909-lever response observed for the training dose of WAY163909 (0.75 mg/kg). *p < 0.05 vs 0.5 mg/kg of compound **16**; p < 0.05 vs 0.5 mg/kg of WAY163909.

circles). Greater than 95% WAY163909-appropriate responding was observed at the training dose (0.75 mg/kg, ip). Response rates were stable across all test doses of WAY163909 (Figure 7A, open triangles). The time course of WAY163909-appropriate responding at 0.75 mg/kg (Figure S3) indicated that the stimulus effects of the training dose of WAY163909 (0.75 mg/kg, ip) are maximal at a 15 min pretreatment time and decline until 120 min at which time rats cannot discriminate this dose of WAY163909 (Figure S3). The dose predicted to elicit 50% of WAY163909-lever responding (ED_{50}) in rats is 0.53 mg/kg.

Compound **16** alone did not stimulate Ca_i^{2+} release in live h5-HT_{2C}R-CHO cells, indicating an inability to act as a 5-HT_{2C}R agonist *in vitro*; therefore, we hypothesized that compound **16** would not substitute fully for the full 5-HT_{2C}R agonist WAY163909 in rats trained to discriminate WAY163909 from saline. Compound **16** (0.125−0.5 mg/kg; ip) evoked a maximum of 45% ± 20% (SEM) WAY163909-appropriate responding at a dose of 1 mg/kg (Figure 7B, closed circles). All doses of **16** elicited WAY163909-lever responding that was significantly different than the training dose (0.75 mg/kg, p < 0.05); this lack of substitution of **16** suggests a dissociation between the stimulus effects of the full 5-HT_{2C}R agonist WAY163909 and compound **16**. Response rates (Figure 7B, open triangles) were stable across the lowest doses of compound **16** (0.125−0.5 mg/kg). At the highest dose of compound **16** tested (1 mg/kg), seven out of the nine rats tested completed the lever press response criterion; the response rate for these rats was significantly decreased relative to the training dose of WAY163909 (p < 0.05). While this observation may reflect motor suppression evoked by **16**, the maximal dose of **16** (1 mg/kg) employed in drug discrimination studies was not associated with suppressed ambulations (Figure 6A). In general, responding in an operant task is relatively more sensitive than is locomotor activity, and it is important to note that modifications in response rates are dissociable from response choice.[60,64]

The observation that compound **16** partially substituted for WAY163909 may not be unexpected given that the effects of endogenous 5-HT could be enhanced by a 5-HT_{2C}R PAM. Coupled with data that compound **16** does not have intrinsic activity in the h5-HT_{2C}R-CHO assay, these data support the contention that compound **16** does not exhibit appreciable efficacy as a 5-HT_{2C}R agonist and are consistent with other findings regarding the discriminative stimulus effects of allosteric modulators.[65]

We also postulated that a 5-HT_{2C}R PAM would enhance the effects of the 5-HT_{2C}R agonist WAY163909 to evoke its discriminative stimulus effects. To further probe this premise, rats were injected with low doses of WAY163909 (0.5 mg/kg) in combination with compound **16** (0.5 mg/kg; Figure 7C); each evokes ∼30% WAY163909-appropriate responding with rates of ∼30 responses/min. Interestingly, the coadministration of 0.5 mg/kg of WAY163909 *plus* 0.5 mg/kg of compound **16** synergized to evoke a full substitution for WAY163909 (∼95% WAY163909-lever responding). The response rate seen with the combination (15 ± 2.0 responses/min) did not differ from 0.5 mg/kg of WAY163909 (31.0 ± 4.4 responses/min), but was significantly lower than 0.5 mg/kg of compound **16** (27.4 ± 5.8 responses/min) administered alone (p < 0.05). Taken together, these data suggest that compound **16** acts *in vivo* to augment 5-HT_{2C}R agonist-mediated signaling *in vivo*.

Effects of Compound 16 on Cocaine Cue Reactivity. We employed a rat model of cocaine self-administration to establish the efficacy of compound **16** to suppress relapse vulnerability assessed by responding for the discrete cue complex paired with cocaine intake (cue reactivity).[8,9,12,66,67] Selective 5-HT_{2C}R agonists effectively suppress cocaine cue reactivity and cocaine intake in the self-administration paradigm,[9,12,47,68,69] with no evidence of abuse liability on their own.[13−15] Relapse during abstinence from cocaine use is a major hurdle for the successful treatment of CUD; an effective pharmacotherapy that improves inhibitory control in the context of exposure to cocaine-associated cues would represent a first-in-class drug useful for those suffering from CUD.[2,3]

The self-administration assay has the greatest face validity for modeling human drug-taking and cue-reactivity and for therapeutic drug discovery. Cocaine self-administration training consisted of 14 daily 180 min sessions during which rats were trained to lever press for cocaine infusions (0.75 mg/kg per 0.1 mL infusion, iv).[6,8,9,11] Schedule completions on the active lever resulted in delivery of a cocaine infusion paired simultaneously with illumination of the house and stimulus lights and activation of the infusion pump (discrete cue complex paired with cocaine delivery); inactive lever presses produced no scheduled consequences. Following stable acquisition, rats were

DOI: 10.1021/acs.jmedchem.8b00401
J. Med. Chem. XXXX, XXX, XXX−XXX

reintroduced to the self-administration chambers 24 h later and assayed in a cue reactivity test session comprised of two sequential components. The first component (10 min) evaluated whether rats would exhibit differential levels of lever presses when placed in the context in the absence of the discrete cue complex. The second component (60 min) was signaled by a single, nonresponse contingent delivery of the discrete cue complex presented immediately at the termination of the first component.[6]

We assessed the effects of compound **16** (1 mg/kg; ip) on cocaine cue reactivity across both components of the test session. A main effect of treatment [$(F_{(1,14)} = 7.28, p = 0.0173$], time [$(F_{(13,182)} = 82.07, p < 0.0001$], and a treatment × time interaction [$(F_{(13,182)} = 4.58, p < 0.0001$] for previously active lever presses was observed. Previously active lever presses reinforced by the discrete cue complex were significantly lower following pretreatment with **16** vs saline (Figure 8; $p < 0.05$);

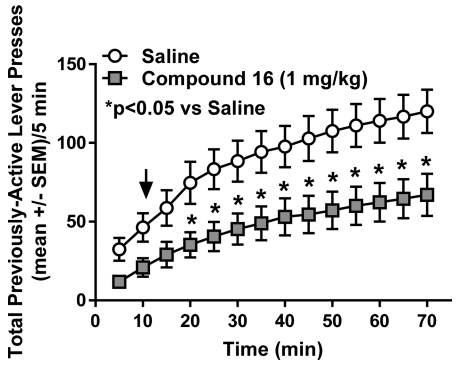


Figure 8. Effects of compound **16** on cocaine cue reactivity in rats (n = 7/treatment group). Mean (±SEM) previously active lever are presented for the cue reactivity test session following administration for saline or compound **16**. The first component of the cue reactivity session (initial 10 min) evaluated lever presses in the absence of the discrete cue complex. The second component was signaled by the single delivery of the discrete cue complex (denoted by arrow). Presses on the previously active lever in the 60 min second component were reinforced by the discrete cue complex; inactive lever presses were recorded but produced no scheduled consequences. Rats were injected with saline (1 mL/kg, ip) or compound **16** (1 mg/kg; ip) 15 min prior to a cocaine cue reactivity test session. Compound **16** suppressed cue reactivity vs saline (*$p < 0.05$).

compound **16** did not significantly alter inactive lever presses vs saline (data not shown). These *in vivo* data provide the first reported *in vivo* evidence that a putative 5-HT_{2C}R PAM suppresses a key facet of relapse vulnerability in CUD.

CONCLUSIONS

A series of structurally simplified analogues have been rationally designed and synthesized based on the 5-HT_{2C}R PAM **1**. The allosteric modulatory SAR of the 5-HT_{2C}R was explored using a fluorescence-based, 5-HT-evoked Ca_i^{2+} release assay. The stereochemistry of the synthesized compounds was secured by X-ray crystallographic analysis of compounds **32** and **35**. Simplification of the PH of **1** is tolerable or even favorable for potentiation of 5-HT_{2C}R-evoked Ca_i^{2+} release. None of the compounds exhibited 5-HT_{2C}R agonist efficacy when tested alone, and **16** (CYD-1-79) potentiated 5-HT-evoked Ca_i^{2+} release in h5-HT_{2C}R-CHO cells with receptor subtype selectivity versus the 5-HT_{2A}R. Radioligand binding results suggest that these compounds do not bind to the orthosteric site of the 5-HT_{2C}R; a topographically distinct site was identified by our molecular docking analyses. Compound **16** exhibited a favorable overall pharmacokinetic profile such as decent oral bioavailability and half-life, and a respectable CNS MPO value (4.5 out of 6). Compound **16** evoked a modulation of 5-HT_{2C}R-mediated spontaneous ambulations, partially substituted for the training dose of WAY163909, synergized with a low dose of WAY163909 to substitute fully for the stimulus effects of this selective 5-HT_{2C}R agonist, and attenuated cocaine cue reactivity in rats. Our current knowledge of the selectivity and specificity of compound **16** is driving our future studies to develop drug-like 5-HT_{2C}R PAMs with constrained off-target effects.

Our success in the chemical design, synthesis, and pharmacological evaluation of these new molecules opens new doors to a greater understanding of 5-HT_{2C}R allosteric modulation and the development of novel pharmacotherapeutics for CUD characterized by deficient 5-HT_{2C}R signaling capacity.[2,3] To the best of our knowledge, this report includes the only *in vivo* investigation of a 5-HT_{2C}R PAM in a proof-of-concept study toward the treatment of CUD and is the only study that proposes allosteric binding sites for candidate PAMs on the 5-HT_{2C}R. As impaired signaling capacity of the 5-HT_{2C}R may contribute to the neurobehavioral processes that underlie chronic health issues such as depression, impulsivity disorders, obesity, and schizophrenia, the full potential for novel molecules with actions as selective 5-HT_{2C}R PAMs remains to be elucidated. Continued medicinal chemistry efforts toward structural optimization, in particular at the LT, and *in vitro* and *in vivo* cellular evaluations are underway.

EXPERIMENTAL SECTION

Chemistry. *General.* All commercially available starting materials and solvents were reagent grade and used without further purification. Reactions were performed under a nitrogen atmosphere in dry glassware with magnetic stirring. Preparative column chromatography was performed using silica gel 60, particle size 0.063−0.200 mm (70−230 mesh, flash). Analytical TLC was carried out employing silica gel 60 F254 plates (Merck, Darmstadt). Visualization of the developed chromatograms was performed with detection by UV (254 nm). NMR spectra were recorded on a Bruker-600 (^{1}H, 600 MHz; ^{13}C, 150 MHz) spectrometer or Bruker-300 (^{1}H, 300 MHz; ^{13}C, 75 MHz). ^{1}H and ^{13}C NMR spectra were recorded with TMS as an internal reference. Chemical shifts were expressed in ppm, and J values were given in Hz. High-resolution mass spectra (HRMS) were obtained from Thermo Fisher LTQ Orbitrap Elite mass spectrometer. Parameters include the following: Nano ESI spray voltage was 1.8 kV; capillary temperature was 275 °C, and the resolution was 60 000; ionization was achieved by positive mode. Melting points were measured on a Thermo Scientific electrothermal digital melting point apparatus and were uncorrected. Purity of final compounds was determined by analytical HPLC, which was carried out on a Shimadzu HPLC system (model CBM-20A LC-20AD SPD-20A UV/vis). HPLC analysis conditions were as follows: Waters μBondapak C18 (300 mm × 3.9 mm); flow rate 0.5 mL/min; UV detection at 270 and 254 nm; linear gradient from 30% acetonitrile in water (0.1% TFA) to 100% acetonitrile (0.1% TFA) in 20 min followed by 30 min of the last-named solvent. All newly synthesized compounds have been fully characterized with ^{1}H NMR, ^{13}C NMR, HRMS, and HPLC analyses to ensure a purity >95% prior to the biological evaluations.

*(cis-2,4)-4-Undecylpiperidine-2-carboxylic acid (**7**).* Compound **6** (54 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (1 mL). TFA (0.25 mL) was added to the solution at rt. The reaction mixture was stirred for 2 h at rt, and the reaction was judged complete by TLC. The reaction mixture was then concentrated under reduced pressure to afford an oily , which was partitioned between CH_2Cl_2 (15 mL) and a saturated

DOI: 10.1021/acs.jmedchem.8b00401
J. Med. Chem. XXXX, XXX, XXX−XXX

solution of $NaHCO_3$ (5 mL). The organic layer was treated with anhydrous Na_2SO_4 (5 mL), filtered, and concentrated under reduced pressure. The crude mixture was loaded on a PTLC plate (hexanes/EtOAc; 5:1) which afforded **7** (22.5 mg, 40%) as a white solid; mp 185−187 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.55 (s, 1H), 8.77 (s, 1H), 3.44 (t, J = 12.4 Hz, 2H), 3.01−2.76 (m, 1H), 2.34 (d, J = 13.2 Hz, 1H), 1.81 (d, J = 11.5 Hz, 1H), 1.64−1.42 (m, 2H), 1.30 (s, 21H), 0.93 (t, J = 6.7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.12, 59.64, 43.72, 36.17, 35.05, 33.50, 31.91, 29.68, 29.64, 29.62, 29.34, 28.70, 26.48, 22.68, 14.09. HRMS (ESI) calcd for $C_{17}H_{33}NO_2$ $[M + H]^+$ 284.2584; found 284.2580.

*cis-N-(2-Hydroxyethyl)-4-undecylpiperidine-2-carboxamide (**8**).* To a solution of **6** (70 mg, 0.18 mmol) and 2-aminoethanol (11 mg, 0.18 mmol) in 4 mL of DMF was added HBTU (89 mg, 0.23 mmol) and DIPEA (58 mg, 0.45 mmol). The resulting mixture was stirred at room temperature for 16 h. The DMF was removed under vacuum to give a brown oily residue, which was partitioned between CH_2Cl_2 (50 mL) and 10% citric aqueous solution (10 mL). The organic layer was separated and washed with saturated aqueous $NaHCO_3$ (10 mL). After drying over anhydrous Na_2SO_4, the solvent was removed under vacuum to give an oily residue. This residue was purified with a silica gel column (5% MeOH in CH_2Cl_2) and afforded the Boc-protected amide (58 mg, 75%). The amide (58 mg, 0.13 mmol) was dissolved in CH_2Cl_2 (1 mL), followed by the addition of TFA (250 μL). The resulting mixture was stirred at room temperature. After 2 h, TLC showed that the starting material had disappeared. The solvent was removed under vacuum to give an oily residue. The residue was partitioned between CH_2Cl_2 (30 mL) and saturated $NaHCO_3$ aqueous solution (10 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated to give an oily residue. This residue was purified with a silica gel column (eluting with 10% MeOH in CH_2Cl_2), affording **8** (34 mg, 73%) as a colorless amorphous gel. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.54 (d, 1H, J = 4.8 Hz), 4.11 (br s, 2H), 3.71 (s, 2H), 3.46 (d, 2H, J = 10.2 Hz), 3.32 (m, 1H), 3.24 (d, 1H, J = 12.0 Hz), 2.78 (t, 1H, J = 11.4 Hz), 2.09 (d, 1H, J = 12.6 Hz), 1.73 (d, 1H, J = 13.2 Hz), 1.48 (br s, 1H), 1.25 (m, 22H), 0.88 (t, 3H, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.5, 61.2, 60.0, 45.1, 42.2, 36.8, 36.0, 35.3, 31.9, 31.4, 29.8, 29.7, 29.6, 29.4 (4C), 26.4, 22.7, 14.1. HRMS Calcd for $C_{19}H_{38}N_2O_2$: $[M + H]^+$ 327.3006; found 327.3010.

*cis-N-(1,3-Dihydroxypropan-2-yl)-4-undecylpiperidine-2-carboxamide (**9**).* Compound **9** (50 mg) was prepared in 76% yield (2 steps) by a procedure similar to that used to prepare compound **8**. The title compound was obtained as a colorless amorphous gel. ^{1}H NMR (600 MHz, CD_3OD) δ ^{1}H NMR (600 MHz, $CDCl_3$ + CD_3OD) δ 3.89 (t, 1H, J = 4.2 Hz), 3.66 (m, 4H), 3.15 (m, 1H), 2.62 (m, 1H), 1.99 (d, 1H, J = 12.0 Hz), 1.71 (d, 1H, J = 12.6 Hz), 1.43 (m, 1H), 1.26 (m, 20H), 1.02 (m, 2H), 0.88 (t, 3H, J = 6.6 Hz). ^{13}C NMR (150 MHz, $CDCl_3$ + CD_3OD) δ 174.5, 60.9, 60.1, 52.1, 45.1, 36.7, 36.4, 35.6, 32.1, 31.5 (2C), 29.4, 29.3 (3C), 29.0 (2C), 26.0, 22.3, 13.5. HRMS Calcd for $C_{20}H_{40}N_2O_3$: $[M + H]^+$ 357.3112; found 357.3119.

*cis-N-((2S,3S)-1,3-Dihydroxybutan-2-yl)-4-undecylpiperidine-2-carboxamide (**10**).* Compound **10** (48 mg) was prepared in 71% yield (2 steps) by a procedure similar to that used to prepare compound **8**. The title compound was obtained as a colorless amorphous gel. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.59 (m, 1H), 4.78 (br s, 3H), 4.06 (m, 1H), 3.74 (m, 3H), 3.5 (m, 1H), 3.22 (m, 1H), 2.73 (q, 1H, J = 13.8 Hz), 2.06 (m, 1H), 1.74 (t, 1H, J = 13.8 Hz), 1.47 (m, 1H), 1.25 (m, 19H), 1.15 (m, 5H), 0.87 (t, 3H, J = 7.2 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 173.7, 172.9, 67.6, 67.4, 63.4, 60.4, 59.7, 55.5, 45.3, 44.8, 36.8, 36.2, 36.0, 35.6, 35.2, 31.9, 31.3, 30.9, 29.8, 29.6, 29.4, 26.5, 26.4, 22.7, 20.4, 14.1. HRMS Calcd for $C_{21}H_{42}N_2O_3$: $[M + H]^+$ 371.3274; found 371.3270.

*cis-N-((2R,3R)-1,3-Dihydroxybutan-2-yl)-4-undecylpiperidine-2-carboxamide (**11**).* Compound **11** (52 mg) was prepared in 77% yield (2 steps) by a procedure similar to that used to prepare compound **8**. The title compound was obtained as a colorless amorphous gel. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.27 (m, 1H), 4.10 (m, 1H), 3.77 (m, 6H), 3.31 (dd, 1H, J = 2.4 Hz, 12.0 Hz), 3.25 (dd, 1H, J = 2.4 Hz, 11.4 Hz), 3.15 (m, 1H), 2.65 (t, 1H, J = 12.6 Hz), 2.05 (m, 1H), 1.68 (m, 1H), 1.43 (m, 1H), 1.25 (m, 19H), 1.17 (m, 3H), 1.08 (m, 2H), 0.88 (t, 3H, J = 7.2 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 174.8, 174.2, 67.8, 67.7, 63.7, 61.0, 60.4, 54.9, 45.7, 45.3, 37.0, 36.9, 36.0, 35.7, 32.2, 31.9, 29.9, 29.7, 29.4, 26.5, 26.4, 22.7, 20.5, 20.4, 14.1. HRMS Calcd for $C_{21}H_{42}N_2O_3$: $[M + H]^+$ 371.3274; found 371.3258.

*(2R,4S)-N-((1S,2S)-1,3-Dihydroxy-1-phenylpropan-2-yl)-4-undecylpiperidine-2-carboxamide ((2R,4S)-**12**) and (2S,4R)-N-((1S,2S)-1,3-Dihydroxy-1-phenylpropan-2-yl)-4-undecylpiperidine-2-carboxamide ((2S,4R)-**13**).* Compounds (2*R*,4*S*)-**12** (14 mg, 35%) and (2*S*,4*R*)-**13** (15 mg, 35%) were prepared (2 steps) by a procedure similar to that used to prepare compound **8**. These two isomers could be separated by preparative TLC as colorless amorphous gels. (2*R*,4*S*)-**12**. ^{1}H NMR (600 MHz, $CDCl_3$ + CD_3OD) δ 7.37 (m, 2H), 7.32 (t, 2H, J = 7.2 Hz), 7.24 (t, 1H, J = 6.6 Hz), 4.98 (m, 1H), 4.03 (d, 1H, J = 4.2 Hz), 3.69 (m, 1H), 3.62 (m, 1H), 3.10 (d, 2H, J = 11.4 Hz), 2.60 (t, 1H, J = 12.0 Hz), 1.78 (d, 1H, J = 12.6 Hz), 1.67 (d, 1H, J = 12.6 Hz), 1.33 (m, 1H), 1.26 (m, 20H), 1.18 (m, 1H), 1.01 (m, 1H), 0.88 (t, 3H, J = 6.6 Hz), 0.75 (t, 1H, J = 12.6 Hz). ^{13}C NMR (150 MHz, $CDCl_3$ + CD_3OD) δ 174.7, 141.2, 128.0 (2C), 127.3, 125.8 (2C), 72.3, 62.1, 60.2, 56.2, 45.2, 36.7, 36.1, 35.5, 31.9, 31.7, 29.6, 29.5 (2C), 29.4 (2C), 29.1, 26.1, 22.5, 13.8. HRMS Calcd for $C_{26}H_{44}N_2O_3$: $[M + H]^+$ 433.3425; found 433.3415. (2*S*,4*R*)-**13**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41 (m, 1H), 7.38 (m, 1H), 7.31 (t, 2H, J = 7.2 Hz), 7.25 (m, 1H), 5.06 (s, 1H), 4.08 (m, 1H), 3.80 (m, 4H), 3.22 (d, 1H, J = 10.8), 3.03 (d, 1H, J = 12.0 Hz), 2.54 (t, 1H, J = 12.0 Hz), 1.77 (d, 1H, J = 12.0 Hz), 1.61 (d, 1H, J = 12.6 Hz), 1.27 (m, 20H), 1.13 (m, 2H), 0.95 (m, 1H), 0.88 (t, 3H, J = 6.6 Hz), 0.81 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.6, 141.4, 128.2 (2C), 127.4, 125.8 (2C), 73.3, 63.2, 60.0, 56.4, 44.9, 36.7, 36.2, 35.2, 31.8, 31.6, 29.7, 29.6 (3C), 29.3 (2C), 26.2, 22.6, 14.0. HRMS Calcd for $C_{26}H_{44}N_2O_3$: $[M + H]^+$ 433.3425; found 433.3427.

*(2S,4R)-N-((1R,2R)-1,3-Dihydroxy-1-phenylpropan-2-yl)-4-undecylpiperidine-2-carboxamide ((2S,4R)-**14**) and (2R,4S)-N-((1R,2R)-1,3-Dihydroxy-1-phenylpropan-2-yl)-4-undecylpiperidine-2-carboxamide ((2R,4S)-**15**).* Compounds (2*S*,4*R*)-**14** (35 mg, 43%) and (2*R*,4*S*)-**15** (37 mg, 45%) were prepared (2 steps) by a procedure similar to that used to prepare compound **8**. These two isomers could be separated by preparative TLC as colorless amorphous gels. (2*S*,4*R*)-**14**. ^{1}H NMR (600 MHz, $CDCl_3$ + CD_3OD) δ 7.67 (br s, 1H), 7.40 (d, 2H, J = 7.8 Hz), 7.31 (t, 2H, J = 7.8 Hz), 1.24 (t, 1H, J = 7.8 Hz), 4.94 (d, 1H, J = 4.8 Hz), 4.10 (m, 1H), 3.67 (dd, 1H, J = 6.0 Hz, 11.4 Hz), 3.52 (dd, 1H, J = 6.0 Hz, 11.4 Hz), 3.17 (d, 1H, J = 12.6 Hz), 2.71 (m, 1H), 1.93 (d, 1H, J = 13.2 Hz), 1.75 (d, 1H, J = 13.8 Hz), 1.46 (br s, 1H), 1.28 (s, 20H), 1.14 (m, 1H), 0.97 (q, 1H, J = 12.6 Hz), 0.89 (t, 3H, J = 7.2 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 172.9, 141.8, 128.0 (2C), 127.3, 126.0 (2C), 71.7, 61.4, 59.5, 56.7, 44.5, 36.5, 35.5, 35.1, 31.7, 30.6, 29.6 (4C), 29.4, 29.1, 26.1, 22.4, 13.5. HRMS Calcd for $C_{26}H_{44}N_2O_3$: $[M + H]^+$ 433.3425; found 433.3427. (2*R*,4*S*)-**15**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40 (d, 1H, J = 8.4 Hz), 7.35 (d, 2H, J = 7.2 Hz), 7.28 (m, 2H), 7.21 (t, 1H, J = 7.8 Hz), 5.02 (d, 1H, J = 3.0 Hz), 4.52 (br s, 2H), 4.08 (m, 1H), 3.77 (m, 1H), 3.71 (m, 1H), 3.13 (dd, 1H, J = 1.8 Hz, 12.0 Hz), 2.94 (d, 1H, J = 11.4 Hz), 2.43 (m, 1H), 1.71 (d, 1H, J = 12.6 Hz), 1.56 (d, 1H, J = 12.0 Hz), 1.26 (m, 20H), 0.99 (m, 2H), 0.88 (t, 3H, J = 7.2 Hz), 0.73 (q, 1H, J = 12.0 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 173.8, 141.7, 128.2 (2C), 127.4, 125.9 (2C), 72.9, 63.0, 60.0, 56.3, 44.9, 36.8, 36.4, 35.4, 31.9, 31.8, 29.8, 29.7 (4C), 29.4, 26.3, 22.7, 14.1. HRMS Calcd for $C_{26}H_{44}N_2O_3$: $[M + H]^+$ 433.3425; found 433.3427.

*cis-4-Undecyl-piperidine-2-carboxylic Acid (2,3-Dihydroxypropyl)amide (**16**).* Compound **16** (45 mg) was prepared in 68% yield (2 steps) by a procedure similar to that used to prepare compound **8**. The title compound was obtained as a colorless amorphous gel. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (m, 1H), 3.82 (m, 4H), 3.50 (m, 3H), 3.36 (m, 1H), 3.29 (d, 1H, J = 10.5 Hz), 3.15 (m, 1H), 2.66 (t, 1H, J = 11.1 Hz), 2.03 (m, 1H), 1.69 (d, 1H, J = 12.9 Hz), 1.42 (m, 1H), 1.25 (br s, 20 H), 1.02 (m, 2H), 0.88 (t, 3H, J = 6.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.2, 70.9 (2C), 63.8, 63.7, 60.4, 45.6, 42.0, 37.0, 36.7, 35.8, 32.2, 31.9, 29.8, 29.7, 29.4, 26.5, 22.7, 14.1. HRMS Calcd for $C_{20}H_{40}N_2O_3$: $[M + H]^+$ 357.3112; found 357.3116.

DOI: 10.1021/acs.jmedchem.8b00401
J. Med. Chem. XXXX, XXX, XXX−XXX

*cis-N-((R)-2,3-Dihydroxypropyl)-4-undecylpiperidine-2-carboxamide (**17**).* Compound **17** (54 mg) was prepared in 80% yield (2 steps) by a procedure similar to that used to prepare compound **8**. The title compound was obtained as a colorless amorphous gel. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (m, 1H), 3.99 (br s, 3H), 3.77 (d, 1H, J = 2.1 Hz), 3.56 (m, 1H), 3.50 (m, 1H), 3.35 (s, 1H), 3.33 (m, 1H), 3.29 (d, 1H, J = 5.4 Hz), 3.15 (d, 1H, J = 5.4 Hz), 2.66 (t, 1H, J = 6.0 Hz), 2.02 (d, 1H, J = 5.7 Hz), 1.69 (d, 1H, J = 6.3 Hz), 1.43 (br s, 1H), 1.25 (br s, 20 H), 1.04 (m, 2H), 0.88 (t, 3H, J = 3.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.0, 70.8, 63.8 (2C), 60.4, 45.5, 42.0, 37.0, 36.7, 35.7, 32.1, 31.9, 29.9, 29.7, 29.4, 26.5, 22.7, 14.1. HRMS Calcd for $C_{20}H_{40}N_2O_3$: $[M + H]^+$ 357.3112; found 357.3113.

*cis-N-((S)-2,3-Dihydroxypropyl)-4-undecylpiperidine-2-carboxamide (**18**).* Compound **18** (44 mg) was prepared in 67% yield (2 steps) by a procedure similar to that used to prepare compound **8**. The title compound was obtained as a colorless amorphous gel. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (m, 1H), 5.77 (br s, 3H), 3.82 (s, 2H), 3.52 (m, 4H), 3.20 (dd, 1H, J = 3.0 Hz), 2.89 (m, 1H), 2.12 (d, 1H, J = 4.5 Hz), 1.79 (d, 1H, J = 5.1 Hz), 1.57 (br s, 1H), 1.36 (m, 1H), 1.25 (br s, 22H), 0.88 (t, 3H, J = 3.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.6, 70.8, 70.6, 63.7, 59.1, 44.6, 42.3, 36.5, 34.7, 32.0, 29.9, 29.7, 29.4, 26.5, 22.7, 14.1. HRMS Calcd for $C_{20}H_{40}N_2O_3$: $[M + H]^+$ 357.3112; found 357.3115.

*cis-N-((S)-3-Chloro-2-hydroxypropyl)-4-undecylpiperidine-2-carboxamide (**19**).* Compound **19** (43 mg) was prepared in 63% yield (2 steps) by a procedure similar to that used to prepare compound **8**. The title compound was obtained as a colorless amorphous gel. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (br s, 1H), 3.89 (br s, 3H), 3.53 (m, 3H), 3.36 (m, 2H), 3.16 (m, 1H), 2.69 (t, 1H, J = 11.4 Hz), 2.07 (m, 1H), 1.70 (m, 1H), 1.43 (m, 1H), 1.26 (br s, 20H), 1.06 (m, 2H), 0.88 (t, 3H, J = 6.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.2, 70.7, 70.5, 60.4, 46.4 (2C), 45.6, 45.5, 43.0, 42.9, 36.9, 36.6, 35.7, 35.6, 32.0, 31.9, 29.8, 29.7, 29.6, 29.4, 26.4, 22.7, 14.1. HRMS Calcd for $C_{20}H_{39}ClN_2O_2$: $[M + H]^+$ 375.2773; found 375.2776.

*cis-N-(3-Morpholinopropyl)-4-undecylpiperidine-2-carboxamide (**20**).* Compound **20** (55 mg) was prepared in 74% yield (2 steps) by a procedure similar to that used to prepare compound **8**. The title compound was obtained as a colorless amorphous gel. ^{1}H NMR (600 MHz, CD_3OD) δ 4.05 (d, 2H, J = 12.6 Hz), 3.79 (m, 3H), 3.48 (d, 2H, J = 10.8 Hz), 3.37 (m, 2H), 3.22 (m, 5H), 2.98 (m, 1H), 2.21 (d, 1H, J = 13.2 Hz), 1.94 (m, 3H), 1.66 (br s, 1H), 1.25 (m, 22H), 0.84 (t, 3H, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.2, 63.8, 58.0, 54.8, 52.1, 51.9, 43.6, 36.2, 36.1, 35.8, 33.9, 33.4, 31.7, 29.5 (2C), 29.4 (2C), 29.2, 28.0, 26.2, 23.7, 22.4, 13.2. HRMS Calcd for $C_{24}H_{47}N_3O_2$: $[M + H]^+$ 396.3585; found 396.3588.

*Methyl((cis-2,4)-4-undecylpiperidine-2-carbonyl)-L-allothreoninate (**21**).* Compound **21** (14 mg) was prepared as a whitish wax in 87.5% yield (2 steps) by a procedure similar to that used to prepare compound **8**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (t, J = 9.8 Hz, 1H), 4.58 (td, J = 9.1, 2.6 Hz, 1H), 4.44–4.30 (m, 1H), 3.78 (d, J = 1.1 Hz, 3H), 3.30 (dd, J = 11.6, 2.7 Hz, 1H), 3.22–3.11 (m, 1H), 2.68 (tt, J = 11.6, 2.6 Hz, 1H), 2.45 (s, 3H), 2.11 (d, J = 13.4 Hz, 1H), 1.70 (d, J = 12.9 Hz, 1H), 1.52–1.37 (m, 1H), 1.27 (s, 22H), 1.11–0.98 (m, 2H), 0.89 (t, J = 6.7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.76, 171.48, 67.99, 67.80, 60.91, 60.71, 57.07, 56.89, 52.54, 45.86, 45.70, 36.98, 36.64, 35.95, 32.52, 32.34, 31.92, 29.81, 29.68, 29.64, 29.35, 26.45, 22.69, 20.05, 14.12. HRMS (ESI) calcd for $C_{22}H_{42}N_2O_4$ $[M + H]^+$ 399.3217; found 399.3208.

*Methyl((cis-2,4)-4-undecylpiperidine-2-carbonyl)-L-serinate (**22**).* Compound **22** (16 mg) was prepared as a whitish wax in 89.0% yield (2 steps) by a procedure similar to that used to prepare compound **8**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (dd, J = 20.8, 7.7 Hz, 1H), 4.70–4.58 (m, 1H), 4.07–3.83 (m, 2H), 3.79 (s, 3H), 3.25 (d, J = 11.5 Hz, 1H), 3.15 (d, J = 12.1 Hz, 1H), 2.83 (s, 2H), 2.66 (t, J = 12.2 Hz, 1H), 2.07 (t, J = 10.4 Hz, 1H), 1.69 (d, J = 13.0 Hz, 1H), 1.41 (s, 1H), 1.27 (s, 20H), 1.14–0.94 (m, 2H), 0.89 (t, J = 6.7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.61, 174.21, 170.98, 170.92, 62.86, 60.64, 54.48, 54.45, 52.67, 45.88, 45.65, 37.03, 36.99, 36.77, 36.54, 35.99, 35.85, 32.57, 32.51, 31.92, 29.81, 29.68, 29.64, 29.35, 26.45, 26.40, 22.69, 14.12. HRMS (ESI) calcd for $C_{21}H_{45}N_2O_3$ $[M + H]^+$ 385.3061; found 385.3058.

*Methyl((cis-2,4)-4-undecylpiperidine-2-carbonyl)-L-tyrosinate (**23**).* Compound **23** (32 mg) was prepared as a white solid in 86.0% yield (2 steps) by a procedure similar to that used to prepare compound **8**; mp 129.0–129.6 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.07 (dd, J = 36.9, 8.4 Hz, 1H), 6.95 (dd, J = 8.4, 3.6 Hz, 2H), 6.67 (dd, J = 13.6, 8.5 Hz, 2H), 4.91–4.75 (m, 1H), 3.74 (d, J = 1.8 Hz, 3H), 3.24–3.03 (m, 3H), 2.95 (td, J = 14.2, 7.0 Hz, 1H), 2.61 (ddd, J = 12.1, 9.8, 5.6 Hz, 1H), 1.99 (t, J = 14.3 Hz, 1H), 1.74–1.55 (m, 1H), 1.27 (s, 22H), 1.10–0.93 (m, 3H), 0.89 (t, J = 6.7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.38, 173.99, 172.24, 172.20, 155.80, 130.34, 130.17, 126.93, 126.70, 115.66, 115.61, 60.52, 60.39, 53.01, 52.94, 52.40, 45.79, 45.46, 37.22, 36.95, 36.91, 36.65, 35.90, 35.75, 32.37, 32.29, 31.92, 29.81, 29.68, 29.66, 29.36, 26.43, 26.37, 22.69, 14.13. HRMS (ESI) calcd for $C_{27}H_{44}N_2O_4$ $[M + H]^+$ 461.3374; found 461.3362.

*Methyl((cis-2,4)-4-undecylpiperidine-2-carbonyl)-L-tryptophanate (**24**).* Compound **21** (43 mg) was prepared as a white solid in 83% yield (2 steps) by a procedure similar to that used to prepare compound **8**; mp 112.6–113.8 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, J = 13.5 Hz, 1H), 7.56 (dd, J = 7.6, 3.3 Hz, 1H), 7.35 (d, J = 7.9 Hz, 1H), 7.15 (ddd, J = 14.8, 10.9, 6.9 Hz, 3H), 6.97 (s, 1H), 4.96 (td, J = 10.1, 5.7 Hz, 1H), 3.71 (d, J = 5.6 Hz, 3H), 3.43–3.23 (m, 2H), 3.12 (dt, J = 11.5, 3.1 Hz, 1H), 3.05–2.92 (m, 1H), 2.62–2.46 (m, 1H), 2.08–1.93 (m, 1H), 1.59 (d, J = 11.0 Hz, 2H), 1.29 (s, 20H), 1.21–1.11 (m, 2H), 1.06–0.82 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.22, 174.10, 172.49, 172.42, 136.15, 136.12, 127.73, 127.70, 122.81, 122.72, 122.11, 119.47, 119.39, 118.69, 118.47, 111.42, 111.32, 110.12, 110.03, 60.66, 60.61, 52.61, 52.52, 52.34, 45.77, 37.05, 36.97, 36.74, 36.71, 36.01, 35.94, 32.65, 32.46, 31.94, 29.85, 29.82, 29.66, 29.37, 27.71, 27.57, 26.44, 22.71, 14.14. HRMS (ESI) calcd for $C_{29}H_{45}N_3O_3$ $[M + H]^+$ 484.3534; found 484.3517.

*(2R,4S)-4-(2-Cyclohexylethyl)-N-((1R,2R)-1,3-dihydroxy-1-phenylpropan-2-yl)piperidine-2-carboxamide ((2R,4S)-**35**) and (2S,4R)-4-(2-Cyclohexylethyl)-N-((1R,2R)-1,3-dihydroxy-1-phenylpropan-2-yl)-piperidine-2-carboxamide ((2S,4R)-**34**).* Compounds (2*R*,4*S*)-**35** (53 mg, 44%) and (2*S*,4*R*)-**34** (50 mg, 42%) were prepared from **33** in 2 steps by a procedure similar to that used to prepare compound **8**. These two isomers could be separated by preparative TLC as colorless amorphous gels. Compound **35**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77 (d, 1H, J = 6.0 Hz), 7.32 (d, 2H, J = 7.2 Hz), 7.26 (m, 2H), 7.19 (t, 1H, J = 7.2 Hz), 5.50 (br s, 2H), 4.97 (d, 1H), 4.12 (d, 1H, J = 3.0 Hz), 3.74 (m, 2H), 3.39 (d, 1H, J = 11.4 Hz), 3.03 (d, 1H, J = 9.0 Hz), 2.50 (m, 1H), 1.69 (m, 6H), 1.58 (d, 1H, J = 9.6 Hz), 1.19 (m, 10H), 0.97 (m, 1H), 0.86 (m, 2H), 0.74 (q, 1H, J = 12.0 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 172.3, 141.5, 128.1 (2C), 127.3, 125.8 (2C), 72.6, 62.7, 59.2, 56.5, 44.3, 37.8, 35.2, 34.9, 34.0, 33.7, 33.4 (2C), 30.3, 26.7, 26.4 (2C). HRMS Calcd for $C_{23}H_{36}N_2O_3$: $[M + H]^+$ 389.2799; found 389.2802. Compound **34**. ^{1}H NMR (600 MHz, $CDCl_3$ + CD_3OD) δ 7.69 (br s, 1H), 7.39 (d, 2H, J = 7.8 Hz), 7.31 (t, 2H, J = 7.8 Hz), 7.23 (t, 1H, J = 7.8 Hz), 4.97 (d, 1H, J = 4.2 Hz), 4.09 (m, 1H), 3.70 (m, 1H), 3.5 (m, 1H), 3.15 (dd, 1H, J = 2.4 Hz, 12.0 Hz), 3.11 (d, 1H, J = 12.0 Hz), 2.63 (m, 1H), 1.80 (m, 1H), 1.69 (m, 7H), 1.20 (m, 10H), 0.89 (m, 1H), 0.82 (q, 1H, J = 12.6 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 174.5, 141.9, 127.9 (2C), 127.2, 125.9 (2C), 71.5, 61.5, 60.1, 56.2, 45.0, 37.7, 36.3, 35.8, 33.9, 33.2 (2C), 31.7, 29.3, 26.5, 26.2 (2C). HRMS Calcd for $C_{23}H_{36}N_2O_3$: $[M + H]^+$ 389.2799; found 389.2802.

*(2S,4S)-N-((1S,2S)-1,3-Dihydroxy-1-phenylpropan-2-yl)-4-(2-(4-methylcyclohexyl)ethyl)piperidine-2-carboxamide ((2R,4S)-**36**) and (2S,4R)-N-((1R,2S)-1,3-Dihydroxy-1-phenylpropan-2-yl)-4-(2-(4-methylcyclohexyl)ethyl)piperidine-2-carboxamide ((2S,4R)-**37**).* Compounds (2*R*,4*S*)-**36** (40 mg, 41%) and (2*S*,4*R*)-**37** (42 mg, 43%) were prepared from **33** in 2 steps by a procedure similar to that used to prepare compound **8**. These two isomers could be separated by preparative TLC as colorless amorphous gels. Compound **36**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (m, 5H), 4.99 (d, 1H, J = 7.2 Hz), 4.08 (m, 1H), 3.70 (m, 1H), 3.62 (m, 1H), 3.34 (m, 1H), 3.09 (m, 2H), 2.61 (m, 1H), 1.46 (m, 4H), 1.27 (m, 13H), 0.89 (m, 3H), 0.76 (q, 1H, J = 12.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.6, 142.2,

DOI: 10.1021/acs.jmedchem.8b00401
J. Med. Chem. XXXX, XXX, XXX–XXX

128.5, 127.8, 126.4, 72.4, 62.4, 60.9, 56.9, 45.8, 38.0, 37.0, 36.5, 35.7, 35.1, 34.7, 33.8, 33.7, 33.3, 32.7, 31.1 (2C), 30.7, 29.2, 29.1, 22.8, 20.4. HRMS Calcd for $C_{24}H_{38}N_2O_3$: $[M + H]^+$ 403.2955; found 403.2955. Compound **37**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.31 (m, 5H), 7.22 (t, 1H, J = 7.2 Hz), 5.03 (d, 1H, J = 3.6 Hz), 4.08 (m, 1H), 3.93 (br s, 3H), 3.78 (m, 1H), 3.72 (m, 1H), 3.13 (dd, 1H, J = 2.4 Hz, 12.0 Hz), 2.99 (d, 1H, J = 10.8 Hz), 2.48 (m, 1H), 1.74 (m, 1H), 1.67 (m, 1H), 1.60 (m, 2H), 1.44 (m, 3H), 1.20 (m, 10H), 0.91 (d, 3H, J = 6.6 Hz), 0.87 (m, 2H), 0.78 (q, 1H, J = 12.6 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 174.0, 141.6, 128.2, 127.4, 125.9, 73.1, 63.1, 60.2, 56.4, 50.5, 45.1, 37.5, 36.4, 35.7, 35.3, 34.6, 34.2, 34.0, 33.3, 32.9, 32.0, 30.8, 30.1, 28.7, 22.6, 20.2. HRMS Calcd for $C_{24}H_{38}N_2O_3$: $[M + H]^+$ 403.2955; found 403.2953.

*(2S,4R)-4-Cyclohexyl-N-((1R,2R)-1,3-dihydroxy-1-phenylpropan-2-yl)piperidine-2-carboxamide (**38**) and (2R,4S)-4-Cyclohexyl-N-((1R,2R)-1,3-dihydroxy-1-phenylpropan-2-yl)piperidine-2-carboxamide (**39**).* Compounds **38** (70 mg, 40%) and **39** (74 mg, 43%) were prepared from **33** (2 steps) by a procedure similar to that used to prepare compound **8**. These two isomers could be separated by preparative TLC as colorless amorphous gel. Compound **38**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.48 (d, 1H, J = 8.4 Hz), 7.38 (d, 2H, J = 7.2 Hz), 7.28 (m, 2H), 7.22 (t, 1H, J = 7.8 Hz), 4.98 (d, 1H, J = 4.8 Hz), 4.30 (br s, 3H), 4.08 (m, 1H), 3.70 (m, 1H), 3.62 (m, 1H), 3.11 (dd, 1H, J = 2.4 Hz, 12.0 Hz), 2.97 (m, 1H), 2.46 (m, 1H), 1.72 (m, 2H), 1.59 (m, 4H), 1.13 (m, 5H), 0.99 (m, 2H), 0.84 (m, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 174.5, 141.7, 128.2 (2C), 127.4, 126.2 (2C), 72.9, 62.7, 61.0, 56.6, 45.5, 42.7, 41.0, 33.3, 29.8, 28.6 (2C), 26.6, 26.5 (2C). HRMS Calcd for $C_{21}H_{32}N_2O_3$: $[M + H]^+$ 361.2486; found 361.2488. Compound **39**. ^{1}H NMR (600 MHz, $CDCl_3$+CD_3OD) δ 7.48 (s, 1H), 7.37 (d, 2H, J = 7.2 Hz), 7.30 (m, 2H), 7.23 (t, 1H, J = 7.2 Hz), 4.99 (d, 1H, J = 3.6 Hz), 4.10 (m, 1H), 3.72 (m, 1H), 3.62 (m, 1H), 3.13 (m, 2H), 2.58 (dt, 1H, J = 3.0 Hz, 12.6 Hz), 1.75 (m, 3H), 1.66 (m, 4H), 1.15 (m, 6H), 0.93 (m, 2H), 0.87 (q, 1H, J = 12.0 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 173.9, 141.7, 128.0 (2C), 127.2, 125.8 (2C), 71.9, 62.0, 60.0, 56.3, 45.1, 42.7, 41.0, 33.5, 29.8, 29.7, 28.7, 26.5, 26.4 (2C). HRMS Calcd for $C_{21}H_{32}N_2O_3$: $[M + H]^+$ 361.2486; found 361.2487.

***In Vitro* Pharmacology.** *Intracellular Calcium (Ca_i^{2+}) Release Assay in Live h5-HT_2R-CHO Cells.* Chinese hamster ovary (CHO) cells stably transfected with human unedited (INI) h5-HT_{2C}R (h5-HT_{2C}R-CHO cells) or the human 5-HT_{2A}R (h5-HT_{2A}R-CHO cells) were a generous gift of Drs. Kelly A. Berg and William P. Clarke (University of Texas Health Science Center, San Antonio). Cells were grown at 37 °C, 5% CO_2, and 85% relative humidity environment in GlutaMax-MEM medium (Invitrogen, Carlsbad CA) containing 5% fetal bovine serum (Atlanta Biologicals, Atlanta GA) and 100 μg/mL hygromycin (Mediatech, Manassas VA) and were passaged when they reached 80% confluence. The Ca_i^{2+} release assay was performed according to our recent publications.[9,28,70] Briefly, cells (150 μL; passages 6−16) were plated in serum-replete medium at a density of 14 000−16 000 (FlexStation 3; Molecular Devices) or 30 000 cells/well (FLIPR^{TETRA}; Molecular Devices) in black-wall 96-well culture plates with optically clear flat bottoms. To ensure even plating of cells, the source reservoir was frequently agitated or triturated, and plates were maintained on a rotary shaker at low speed for 20 min after plating and returned to the incubator overnight. Approximately 24 h following plating, the medium was replaced with serum-free (SF) GlutaMax-MEM medium supplemented with 20 nM to 100 μM putrescine (Sigma-Aldrich, St. Louis, MO), 20 nM to 100 μM progesterone (Sigma-Aldrich), and 1:100 ITS (1000 mg/L human recombinant insulin, 550 mg/L human recombinant transferrin, 0.67 mg/L selenious acid; Corning Inc., Corning, NY) (SF+ medium). Following a 3 h incubation, SF+ medium was replaced with 40 μL of Hank's balanced saline solution (HBSS; without $CaCl_2$ or $MgCl_2$, pH 7.4) plus 40 μL of Calcium 4 dye solution (FLIPR No-wash kit, Molecular Devices, Sunnyvale, CA, part no. R8142) supplemented with 2.5 mM of water-soluble probenicid (Sigma) to inhibit extracellular transport of the dye. Plates were incubated for 60 min at 37 °C followed by 15 min at room temperature in the dark. Drug dilutions were prepared at 5× final concentration in 1× HBSS; delivery of compound (20 μL/well) was followed 15 min later by 5-HT (10 pM to 10 μM; 25 μL/well). A baseline was established for each well before addition of the test compound and again before addition of 5-HT. The fluorescence read after compound addition was used to establish potential intrinsic agonist activity of the compounds. The fluorescence read following the addition of 5-HT was used to assess allosteric modulation of 5-HT-evoked Ca_i^{2+} release. Fluorescence was measured using a FlexStation 3 (Molecular Devices) or FLIPR^{TETRA} (130 gain, 60% intensity, 0.3 s exposure). For the FlexStation 3, a 17 s baseline was established before addition of compounds following which fluorescence was recorded every 1.7 s for 240 s. Maximum peak height was determined by the SoftMax software (Pro 5.4.5) for each well. For the FLIPR^{TETRA}, a 10 s baseline was established before addition of compounds following which fluorescence was recorded every 1 s for 120 s following compound or for 360 s following 5-HT. Maximum peak height was determined by ScreenWorks 4.0 software for each well. After the final readings, cells were fixed in 2% paraformaldehyde (Sigma) overnight. The maximum 5-HT-induced Ca_i^{2+} release (E_{max}) in the presence of test compound was determined using 4-parameter nonlinear regression analysis (GraphPad Prism 7.04) and calculated from at least three biological replicates, each conducted in technical triplicates. The E_{max} for the test compound plus 5-HT was normalized to the E_{max} for 5-HT alone. Subsequent post hoc comparisons between means for E_{max} were made using an unpaired t test with Welch's correction (GraphPad Prism). All statistical analyses were conducted with an experiment-wise error rate of α = 0.05.

***In Vivo* Pharmacokinetics and Behavior.** *Pharmacokinetic Analysis.* Male Sprague−Dawley rats were randomly assigned into control and treatment groups (n = 3/group). Rats in the control group were injected with vehicle [DMSO/Tween 80/saline (v/v 1:3:6)], and rats in the treatment groups were administered compound **16** at 5 mg/kg iv or 10 mg/kg po. Blood samples (0.3 mL) were collected from the retro-orbital sinus vein before dosing and at 5 min and 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 7.0, 9.0, and 24 h postdosing for iv administration and 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 7.0, 9.0, and 24 h postdosing for po administration. Blood samples were placed in heparinized tubes and immediately centrifuged at 12 000g for 5 min at 4 °C. All samples were stored at −20 °C until analysis.

The pharmacokinetic parameters of compound **16** were calculated according to a noncompartmental model using WinNonlin (Pharsight Corporation, ver 5.3, Mountain View, CA, USA). The peak concentration (C_{max}) and time of peak concentration (T_{max}) were directly obtained by visual inspection of the plasma concentration−time profile. The elimination rate constant (λ) was obtained by the least-squares fitted terminal log−linear portion of the slope of the plasma concentration−time profile. The elimination half-life ($T_{1/2}$) was evaluated according to 0.693/λ. The area under the plasma concentration−time curve from 0 to time t (AUC_{0-t}) was evaluated using the linear trapezoidal rule and further extrapolated to infinity (AUC_{0-inf}) according to the following equation: $AUC_{0-inf} = AUC_{0-t} + C_{last}/\lambda$. The pharmacokinetic parameters were presented as mean ± SD.

*Effects of Compound **16** on Spontaneous Locomotor Activity. Animals.* A total of 67 male Sprague−Dawley rats (Harlan, Inc., Indianapolis, IN) weighing 225−325 g at the start of the experiments were used. Rats were housed two per cage and allowed to acclimate for 5−7 days in a colony room at a constant temperature (21−23 °C) and humidity (45−50%) on a 12 h light−dark cycle (lights on 0700−1900 h). Food and water were available *ad libitum*. Rats were handled for 1 week prior to the start of behavioral testing. All experiments were carried out in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (2011) and with the approval of the UTMB Institutional Animal Care and Use Committee. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to *in vivo* techniques, when available.

Drugs. WAY163909 [(7b-*R*,10a-*R*)-1,2,3,4,8,9,10,10a-octahydro-7b*H*-cyclopenta[*b*][1,4]diazepino[6,7,1*hi*]indole] was a gift from Pfizer, Inc. (New York, NY) and dissolved in 0.9% NaCl (vehicle

DOI: 10.1021/acs.jmedchem.8b00401
J. Med. Chem. XXXX, XXX, XXX−XXX

employed for comparison to WAY163909). SB242084 [6-chloro-5-methyl-1-[[2-(2-methylpyrid-3-yloxy)pyrid-5-yl]carbamoyl]indoline dihydrochloride; Sigma Chemical Co., St. Louis, MO, USA] was dissolved in saline containing 10 mmol/L citric acid (Sigma Chemical Co.) and 8% 2-hydroxypropyl-β-cyclodextrin (Trappsol Hydroxpropyl Beta Cyclodextrin, pharmaceutical grade, Cyclodextrin Technologies Development Inc., High Springs, FL, USA) with the final pH of the solution adjusted to 5.6. SB242084, WAY163909, and compound **16** were injected intraperitoneally (ip) at a volume of 1 mL/kg.

Locomotor Activity Assessments. Locomotor activity was monitored and quantified under low light conditions using a modified open field activity system (San Diego Instruments, San Diego, CA) according to previous publications with minor modifications.[47] Clear plexiglass chambers (40 × 40 × 40 cm^3) were surrounded by a 4 × 4 photobeam matrix positioned 4 cm from the chamber floor. Total ambulations were quantified as the sum of consecutive photobeam breaks that occurred within the inner central 16 × 16 cm^2 perimeter and in the surrounding outer peripheral 16 × 16 cm^2 perimeter of the activity monitor. Vertical activity counts (rearing) were quantified as the sum of breaks in the upper row of 16 photobeams positioned 16 cm from the monitor floor.

(i) A between-subjects design was employed to study the efficacy of compound **16** to impact spontaneous motor activity. Rats (n = 29) were administered vehicle (saline, 1 mL/kg, ip) or compound **16** (0.5, 1, or 5 mg/kg, ip) immediately prior to placement in activity monitors on the test day; ambulations and vertical activity counts were monitored in 5 min bins for 90 min.

(ii) A between-subjects design was used to investigate the selectivity of compound **16** to allosterically modulate WAY163909-mediated suppression of spontaneous motor activity. Rats (n = 38) were pretreated with SB242084 (1 mg/kg, ip) or vehicle (8% 2-hydroxypropyl-β-cyclodextrin) 15 min prior to ip administration of WAY163909 (1 mg/kg) plus compound **16** (5 mg/kg) and immediately placed into activity monitors on the test day; ambulations and vertical activity counts were monitored in 5 min bins for 90 min.

Statistical Analyses. Locomotor activity data are presented as mean total ambulations (±SEM) summed in 30 min. The main effect of treatment on total ambulations or vertical activity was analyzed with a one-way analysis of variance using the GLM procedure (SAS version 9.4 for Windows). Subsequent *a priori* comparisons to vehicle were made with Dunnett's procedure (experiment i) or a Bonferroni multiple comparisons test (experiment ii). All statistical analyses were conducted with an experiment-wise error rate of α = 0.05.

*Effects of Compound **16** in a WAY163909 versus Saline Drug Discrimination Assay. Animals.* Male Sprague−Dawley rats (n = 9; Harlan, Inc.) weighing 300−325 g at the beginning of the experiment were housed two per cage in a temperature- (21−23 °C) and humidity- controlled (45−50%) environment; lighting was maintained under a 12 h light−dark cycle (0700−1900 h). Rats were maintained at 80−90% of their free-feeding weights by restricting access to water. Rats received water during daily training sessions (5−6 mL/rat/session), in the afternoon several hours after training (20 min), and over the weekend (36 h). Experiments were conducted during the light phase of the light−dark cycle (between 0900 and 1200 h) and were carried out in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and with the approval of the Institutional Animal Care and Use Committee at University of Texas Medical Branch.

Drug Discrimination Procedures. Standard two-lever, water-reinforced drug discrimination procedures were used (Med Associates, St. Albans, USA). Each chamber was equipped with a water-filled dispenser mounted equidistantly between two retractable response levers on the wall and housed in a light- and sound-proof cubicle. Illumination came from a 28-V house light; ventilation and masking noise were provided by a ventilation fan. A computer with Med-PC IV software was used to run programs and record all experimental events.

Rats were trained to discriminate an injection of WAY163909 (0.75 mg/kg; 1.0 mL/kg, ip) from saline (1.0 mL/kg, ip) administered 15 min before start of training sessions (see Supporting Information for WAY163909 time course). Daily sessions lasted 15 min and were conducted Monday through Friday. During the phase of errorless training, only the stimulus-appropriate (drug or saline) lever was present. Training began under a fixed ratio 1 (FR1) schedule of water reinforcement, and the FR requirement was incremented until all animals were responding reliably under an FR20 schedule for each experimental condition. For half of the rats, left lever responses were reinforced after WAY163909 administration, whereas right lever responses were reinforced after saline administration; conditions were reversed for the remaining animals. During this phase of training, WAY163909 and saline were administered irregularly with the restriction that neither condition prevailed for more than three consecutive sessions. After responding stabilized, both levers were presented simultaneously during 15 min training sessions. The rats were required to respond on the stimulus-appropriate (correct) lever to obtain water reinforcement. There were no programmed consequences for responding on the incorrect lever. This phase of training continued until the performance of all rats attained criterion (defined as mean accuracies of at least 80% stimulus-appropriate responding for ten consecutive sessions).

Test sessions were initiated and conducted once or twice per week following attainment of criterion. Training sessions were run during the intervening days to maintain discrimination accuracy. Rats were required to maintain accuracies of at least 80% correct for saline and WAY163909 maintenance sessions, which immediately preceded a test. During test sessions, animals were placed in the chambers and, upon completion of 20 responses on either lever, a single reinforcer was delivered and the house lights were turned off. The rat was removed from the chamber, returned to the colony, and allowed free access to water for 15 min beginning 15−30 min after the end of each test. The test sessions were terminated after 15 min if the rats did not complete 20 responses on either lever; only data from rats that accomplished the FR20 during test sessions within 15 min were employed in data analysis.

Time course, substitution and combination tests were conducted. To determine the time course of WAY163909, rats were pretreated with WAY163909 (0.75 mg/kg, ip) 15, 30, 60, or 120 min prior to placement into the chambers. In substitution tests, rats were administered WAY163909 (0.125, 0.25, 0.5, 0.75, or 1.0 mg/kg, ip), compound **16** (0.125, 0.25, 0.5, 1.0 mg/kg, ip), or saline 15 min prior to test. In combination tests, rats were tested for lever selection following simultaneous ip administration of compound **16** (0.5 mg/kg) and WAY163909 (0.5 mg/kg) and placed in the operant chamber 15 min later. The doses of WAY163909 and compound **16** employed were based upon the results of the substitution tests as producing <50% drug-appropriate responding. Full substitution was defined as ≥80% drug-appropriate responding and not statistically different from the training drug, and partial substitution as ≥40% and <80% drug-appropriate responding.

Statistical Analyses. Accuracy was defined as the percentage of correct responses to total responses before the delivery of the first reinforcer. During test sessions, performance was expressed as the percentage of drug-lever responses to total responses upon completion of an FR20 on either lever. The response rate (responses per minute) was calculated as the total number of responses emitted before completion of the first FR20 divided by the number of minutes taken to complete the first ratio. For compound **16** dose−response tests, Student's t-test for repeated measures was used to compare the percentage of drug-lever responding and response rate during test sessions with the corresponding values for the previous saline session (substitution tests). A two-way ANOVA for repeated measures was employed to analyze the effects of pretreatment with compound **16** (factor 1) and WAY163909 dose (factor 2; SAS for Windows, version 9.4). *A priori* comparisons were calculated to compare the effects of each dose of WAY163909 in the presence versus absence of compound **16** using a Bonferonni-corrected test. Log-probit analyses were used to estimate the dose of WAY163909 predicted to elicit 50%

DOI: 10.1021/acs.jmedchem.8b00401
J. Med. Chem. XXXX, XXX, XXX−XXX

WAY163909-appropriate responding (ED_{50}).[48] Only data from animals that completed the FR20 during the test sessions were used. All statistical analyses were conducted with an experiment-wise error rate of $\alpha = 0.05$.

Effects of Compound ***16*** *on Cocaine Cue Reactivity. Animals.* Male, outbred Sprague−Dawley rats ($n = 17$; Harlan, Houston, TX) weighing 250−275 g upon arrival were housed two per cage under a 12-h light−dark cycle with controlled temperature (21−23 °C) and humidity (40−50%). Animals were acclimated for 7 days to the colony room prior to the start of handling and experimental procedures. All experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (2011) and with the University of Texas Medical Branch Institutional Animal Care and Use Committee approval.

Drugs. (−)-Cocaine (National Institute on Drug Abuse, Research Triangle Park, NC) was dissolved in 0.9% NaCl.

Cocaine Self-Administration and Cue Reactivity Analyses. Cocaine self-administration studies employed standard operant conditioning chambers (Med-Associates, Inc., St. Albans, VT, USA) housed in ventilated, sound-attenuating cubicles with fans (Med-Associates, Inc.). Each chamber was outfitted with two retractable response levers, a stimulus light above each response lever, a houselight opposite the levers, and a magazine-type pellet dispenser. The cocaine infusions were delivered via syringes attached to infusion pumps (Med Associates, Inc.) located outside the cubicles. The infusion pumps were connected to liquid swivels (Instech, Plymouth Meeting, PA, USA) that were fastened to the catheters via polyethylene 20 tubing encased inside a metal spring leash (Plastics One, Roanoke, VA).

Rats were anesthetized (8.6 mg/kg of xylazine, 1.5 mg/kg of acepromazine, 43 mg/kg of ketamine in bacteriostatic saline) and implanted with intravenous catheters with back mounts and allowed to recover for 5−7 days.[6,8,9,47] Catheter patency was maintained by daily flushes with a solution of 0.1 mL of bacteriostatic saline containing heparin sodium (10 U/mL; American Pharmaceutical Partners, East Schaumburg, IL), streptokinase (0.67 mg/mL; Sigma Chemical), and ticarcillin disodium (66.67 mg/mL; Research Products International, Mt. Prospect, IL) immediately following daily cocaine self-administration sessions.

Cocaine self-administration training consisted of 14 daily 180 min sessions during which rats were trained to lever press for cocaine infusions (0.75 mg/kg per 0.1 mL infusion).[6,8,9,47] Schedule completions on the active lever resulted in delivery of a cocaine infusion over a 6 s period paired simultaneously with illumination of the house and stimulus lights and activation of the infusion pump (discrete cue complex paired with cocaine delivery); inactive lever presses produced no scheduled consequences. Following reinforcer delivery, the stimulus light as well as the infusion pump were inactivated; the house light remained on for an additional 20 s to indicate a timeout period during which lever presses had no scheduled consequences. Rats were trained on a FR1 schedule of reinforcement and progressed to an FR5 schedule after achieving seven infusions per hour with less than 10% variability for three consecutive days. Upon achieving stability on the FR5 schedule (less than 10% variability for a minimum of three consecutive days), rats were pseudorandomly assigned to either vehicle or compound **16** and returned to their home cages for the appropriate time period. Rats that exhibited diminished catheter patency were excluded from analysis ($n = 3$).

Rats were reintroduced to the self-administration chambers 24 h after the last self-administration session and assayed in a test session comprised of two sequential components. In the first component, responses on either lever were recorded, but no discrete cues (e.g., stimulus light, pump) were present nor delivered during the 10 min period. The second component was signaled by the single delivery of the discrete cue complex presented at the termination of the first 10 min component. To assess cocaine cue reactivity, presses on the previously active lever in the 60 min second component were reinforced by the discrete cue complex on an FR1; inactive lever presses were recorded but produced no scheduled consequences.[6] Rats received an injection of vehicle (0.9% NaCl, 1 mL/kg, ip) or compound **16** (1.0 mg/kg, ip) 15 min prior to the cue reactivity session.

Statistical Analyses. The data from the self-administration and cue reactivity phases were analyzed separately. For the cue reactivity tests, a two-way ANOVA for repeated measures was employed to analyze the effects of treatment with compound **16** (factor 1) and time (factor 2; SAS for Windows, version 9.4) on previously active or inactive lever presses. *A priori* comparisons were calculated to compare the effects of compound **16** at each time point using Fishers LSD test. All statistical analyses were conducted with an experiment-wise error rate of a $\alpha = 0.05$.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.8b00401.

Calculated physicochemical properties, radioligand binding data, cytotoxicity data, additional cellular assay data, CNS-MPO values, docking protocol, drug discrimination time course study, ^{1}H and ^{13}C NMR spectra for the compounds described in this paper, X-ray data for compounds **32** and **35** (PDF)

Molecular formula strings (CSV)

■ AUTHOR INFORMATION

Corresponding Authors

*Tel: (409) 772-9748. Fax: (409) 772-9648. E-mail: jizhou@utmb.edu (J. Zhou).

*Tel: (409)-772-9629. Fax: (409)-772-7050. E-mail: kcunning@utmb.edu (K. A. Cunningham).

*Tel: (409)-772-9656. Fax: (409)-772-7050. E-mail: ncanasta@utmb.edu (N. C. Anastasio).

ORCID

Eric A. Wold: 0000-0001-6434-7562

Claudia A. Soto: 0000-0002-5214-9136

Noelle C. Anastasio: 0000-0001-5579-4213

Kathryn A. Cunningham: 0000-0002-4257-1739

Jia Zhou: 0000-0002-2811-1090

Author Contributions

‖C.T.W., J.M.M., and E.A.W. contributed equally to this work. J.Z., K.A.C., and N.C.A. developed the concepts and approaches as well as supervised the work. C.T.W., E.A.W., and C.D. synthesized, purified, and characterized the reported compounds. J.M.M., C.A.S., and R.M.H. performed and analyzed the *in vitro* cellular studies. N.C.A. and K.A.C. analyzed the *in vitro* and *in vivo* studies. C.T.W., N.C.A., K.A.C., and J.Z., wrote the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by Grants R21 MH093844 (J.Z./K.A.C.), R01 DA038446 (J.Z./K.A.C.), K05 DA020087 (K.A.C.), P30 DA28821 (K.A.C.), T32 DA07287 (C.T.W. and E.A.W.), F31 DA038922 (C.T.W.), and F31 DA045511 (E.A.W.) from the National Institutes of Health, R.A. Welch Foundation Chemistry and Biology Collaborative Grant from Gulf Coast Consortia (GCC) for Chemical Genomics, a training fellowship from the Keck Center for Interdisciplinary Bioscience Training of the GCC (NIGMS Grant T32 GM089657-03, C.T.W.), Sealy and Smith Foundation grant

DOI: 10.1021/acs.jmedchem.8b00401
J. Med. Chem. XXXX, XXX, XXX−XXX

(to the Sealy Center for Structural Biology and Molecular Biophysics), John Sealy Memorial Endowment Fund, and the Center for Addiction Research at UTMB. We thank Lawrence C. Sowers, Jacob A. Theruvathu, and Tianzhi Wang for the NMR spectroscopy assistance, Robert G. Fox, Gongliang Zhang, and Sonja J. Stutz for performing and analyzing *in vivo* studies, Claudia M. Crawford, Carrie E. McAllister, and Konrad Pazdrak for conducting *in vitro* assays, and Marcy B. Jordan for helpful discussions during the development of this project. Receptor binding profiles and agonist functional data was generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract no. HHSN-271-2013-00017-C (NIMH PDSP). The NIMH PDSP is directed by Bryan L. Roth at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscoll at NIMH, Bethesda MD, USA.

■ ABBREVIATIONS USED

5-HT, serotonin; 5-HT_{2A}R, 5-HT_{2A} receptor; 5-HT_{2B}R, 5-HT_{2B} receptor; 5-HT_{2C}R, 5-HT_{2C} receptor; CNS, central nervous system; GPCR, G protein-coupled receptor; FDA, U.S. Food and Drug Administration; PAM, positive allosteric modulator; NAM, negative allosteric modulator; SAR, structure−activity relationship; tPSA, total polar surface area; HBTU, *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate; DIPEA, *N*,*N*-diisopropylethylamine; TFA, trifluoroacetic acid; PTLC, preparative thin layer chromatographic; PLCβ, phospholipase Cβ; IP3, inositol-1,4,5-trisphosphate; DAG, diacylglycerol; CHO, Chinese hamster ovary; CCR5, C−C chemokine receptor type 5; PLD, phospholipase D; PK, pharmacokinetics; PD, pharmacodynamics; ADMET, absorption, distribution, metabolism, excretion, and toxicity; EL, extracellular loop; TMH, transmembrane helix; MPO, multiparameter optimization; TMS, tetramethylsilane; DMF, dimethylformamide

■ REFERENCES

(1) Hughes, A.; Williams, M. R.; Lipari, R. N.; Van Horn, S. State Estimates of Past Year Cocaine Use among Young Adults: 2014 and 2015; *The CBHSQ Report of 2016*; Center for Behavioral Health Statistics and Quality, Substance Abuse and Mental Health Services Administration, U.S. Government Printing Office: Rockville, MD, 2016.

(2) Cunningham, K. A.; Anastasio, N. C. Serotonin at the nexus of impulsivity and cue reactivity in cocaine addiction. *Neuropharmacology* **2014**, *76* (Pt B), 460−478.

(3) Howell, L. L.; Cunningham, K. A. Serotonin 5-HT2 receptor interactions with dopamine function: implications for therapeutics in cocaine use disorder. *Pharmacol. Rev.* **2015**, *67*, 176−197.

(4) Berger, M.; Gray, J. A.; Roth, B. L. The expanded biology of serotonin. *Annu. Rev. Med.* **2009**, *60*, 355−366.

(5) Roth, B. L.; Willins, D. L.; Kristiansen, K.; Kroeze, W. K. 5-Hydroxytryptamine2-family receptors (5-hydroxytryptamine2A, 5-hydroxytryptamine2B, 5-hydroxytryptamine2C): where structure meets function. *Pharmacol. Ther.* **1998**, *79*, 231−257.

(6) Anastasio, N. C.; Liu, S.; Maili, L.; Swinford, S. E.; Lane, S. D.; Fox, R. G.; Hamon, S. C.; Nielsen, D. A.; Cunningham, K. A.; Moeller, F. G. Variation within the serotonin (5-HT) 5-HT(2)C receptor system aligns with vulnerability to cocaine cue reactivity. *Transl. Psychiatry* **2014**, *4*, e369.

(7) Anastasio, N. C.; Stutz, S. J.; Fink, L. H.; Swinford-Jackson, S. E.; Sears, R. M.; DiLeone, R. J.; Rice, K. C.; Moeller, F. G.; Cunningham, K. A. Serotonin (5-HT) 5-HT2A receptor (5-HT2AR):5-HT2CR imbalance in medial prefrontal cortex associates with motor impulsivity. *ACS Chem. Neurosci.* **2015**, *6*, 1248−1258.

(8) Anastasio, N. C.; Stutz, S. J.; Fox, R. G.; Sears, R. M.; Emeson, R. B.; DiLeone, R. J.; O'Neil, R. T.; Fink, L. H.; Li, D.; Green, T. A.; Moeller, F. G.; Cunningham, K. A. Functional status of the serotonin 5-HT2C receptor (5-HT2CR) drives interlocked phenotypes that precipitate relapse-like behaviors in cocaine dependence. *Neuropsychopharmacology* **2014**, *39*, 360−372.

(9) Cunningham, K. A.; Anastasio, N. C.; Fox, R. G.; Stutz, S. J.; Bubar, M. J.; Swinford, S. E.; Watson, C. S.; Gilbertson, S. R.; Rice, K. C.; Rosenzweig-Lipson, S.; Moeller, F. G. Synergism between a serotonin 5-HT2A receptor (5-HT2AR) antagonist and 5-HT2CR agonist suggests new pharmacotherapeutics for cocaine addiction. *ACS Chem. Neurosci.* **2013**, *4*, 110−121.

(10) Cunningham, K. A.; Bubar, M. J.; Anastasio, N. C. The serotonin 5-HT2C receptor in medial prefrontal cortex exerts rheostatic control over the motivational salience of cocaine-associated cues: new observations from preclinical animal research. *Neuropsychopharmacology* **2010**, *35*, 2319−2321.

(11) Cunningham, K. A.; Fox, R. G.; Anastasio, N. C.; Bubar, M. J.; Stutz, S. J.; Moeller, F. G.; Gilbertson, S. R.; Rosenzweig-Lipson, S. Selective serotonin 5-HT(2C) receptor activation suppresses the reinforcing efficacy of cocaine and sucrose but differentially affects the incentive-salience value of cocaine- vs. sucrose-associated cues. *Neuropharmacology* **2011**, *61*, 513−523.

(12) Swinford-Jackson, S. E.; Anastasio, N. C.; Fox, R. G.; Stutz, S. J.; Cunningham, K. A. Incubation of cocaine cue reactivity associates with neuroadaptations in the cortical serotonin (5-HT) 5-HT2C receptor (5-HT2CR) system. *Neuroscience* **2016**, *324*, 50−61.

(13) Shram, M. J. Evaluation of the abuse potential of lorcaserin, a serotonin 2C (5-HT2C) receptor agonist, in recreational polydrug users. *Clin. Pharmacol. Ther.* **2011**, *89*, 683−692.

(14) Smith, S. R. Lorcaserin (APD356), a selective 5-HT(2C) agonist, reduces body weight in obese men and women. *Obesity* **2009**, *17*, 494−503.

(15) Smith, S. R.; et al. Multicenter, placebo-controlled trial of lorcaserin for weight management. *N. Engl. J. Med.* **2010**, *363*, 245−256.

(16) Hutcheson, J. D.; Setola, V.; Roth, B. L.; Merryman, W. D. Serotonin receptors and heart valve disease—It was meant 2B. *Pharmacol. Ther.* **2011**, *132*, 146−157.

(17) Lee, J.; Jung, M. E.; Lee, J. 5-HT2C receptor modulators: a patent survey. *Expert Opin. Ther. Pat.* **2010**, *20*, 1429−1455.

(18) Chen, Y.-C.; Hartley, R. M.; Anastasio, N. C.; Cunningham, K. A.; Gilbertson, S. R. Synthesis and structure−activity relationships of tool compounds based on WAY163909, a 5-HT2C receptor agonist. *ACS Chem. Neurosci.* **2017**, *8*, 1004−1010.

(19) Thomsen, W. J.; Grottick, A. J.; Menzaghi, F.; Reyes-Saldana, H.; Espitia, S.; Yuskin, D.; Whelan, K.; Martin, M.; Morgan, M.; Chen, W.; Al-Shamma, H.; Smith, B.; Chalmers, D.; Behan, D. Lorcaserin, a novel selective human 5-hydroxytryptamine 2C agonist: in vitro and in vivo pharmacological characterization. *J. Pharmacol. Exp. Ther.* **2008**, *325*, 577−587.

(20) Wild, C.; Cunningham, K. A.; Zhou, J. Allosteric modulation of G protein-coupled receptors: an emerging approach of drug discovery. *Austin J. Pharmacol. Ther.* **2014**, *2*, 1101.

(21) Melancon, B. J.; Hopkins, C. R.; Wood, M. R.; Emmitte, K. A.; Niswender, C. M.; Christopoulos, A.; Conn, P. J.; Lindsley, C. W. Allosteric modulation of seven transmembrane spanning receptors: theory, practice, and opportunities for central nervous system drug discovery. *J. Med. Chem.* **2012**, *55*, 1445−1464.

(22) Nickols, H. H.; Conn, P. J. Development of allosteric modulators of GPCRs for treatment of CNS disorders. *Neurobiol. Dis.* **2014**, *61*, 55−71.

(23) van der Westhuizen, E. T.; Valant, C.; Sexton, P. M.; Christopoulos, A. Endogenous allosteric modulators of G protein−coupled receptors. *J. Pharmacol. Exp. Ther.* **2015**, *353*, 246−260.

(24) Harrington, P. E.; Fotsch, C. Calcium sensing receptor activators: calcimimetics. *Curr. Med. Chem.* **2007**, *14*, 3027−3034.

(25) Dorr, P.; Westby, M.; Dobbs, S.; Griffin, P.; Irvine, B.; Macartney, M.; Mori, J.; Rickett, G.; Smith-Burchnell, C.; Napier, C.;

DOI: 10.1021/acs.jmedchem.8b00401
J. Med. Chem. XXXX, XXX, XXX−XXX

Webster, R.; Armour, D.; Price, D.; Stammen, B.; Wood, A.; Perros, M. Maraviroc (UK-427,857), a potent, orally bioavailable, and selective small-molecule inhibitor of chemokine receptor CCR5 with broad-spectrum anti-human immunodeficiency virus type 1 activity. *Antimicrob. Agents Chemother.* **2005**, *49*, 4721−4732.
(26) Conn, P. J.; Christopoulos, A.; Lindsley, C. W. Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. *Nat. Rev. Drug Discovery* **2009**, *8*, 41−54.
(27) Im, W. B.; Chio, C. L.; Alberts, G. L.; Dinh, D. M. Positive allosteric modulator of the human 5-HT 2C receptor. *Mol. Pharmacol.* **2003**, *64*, 78−84.
(28) Ding, C.; Bremer, N. M.; Smith, T. D.; Seitz, P. K.; Anastasio, N. C.; Cunningham, K. A.; Zhou, J. Exploration of synthetic approaches and pharmacological evaluation of PNU-69176E and its stereoisomer as 5-HT2C receptor allosteric modulators. *ACS Chem. Neurosci.* **2012**, *3*, 538−545.
(29) García-Cárceles, J.; Decara, J. M.; Vázquez-Villa, H.; Rodríguez, R.; Codesido, E.; Cruces, J.; Brea, J.; Loza, M. I.; Alén, F.; Botta, J.; McCormick, P. J.; Ballesteros, J. A.; Benhamú, B.; Rodríguez de Fonseca, F.; López-Rodríguez, M. L. A positive allosteric modulator of the serotonin 5-HT2C receptor for obesity. *J. Med. Chem.* **2017**, *60*, 9575−9584.
(30) Farce, A.; Dilly, S.; Yous, S.; Berthelot, P.; Chavatte, P. Homology modelling of the serotoninergic 5-HT2c receptor. *J. Enzyme Inhib. Med. Chem.* **2006**, *21*, 285−292.
(31) Zuo, Z.; et al. Pharmacophore-directed homology modeling and molecular dynamics simulation of G protein-coupled receptor: study of possible binding modes of 5-HT2C receptor agonists. *Acta Biochim. Biophys. Sin.* **2007**, *39*, 413−422.
(32) Berg, K. A.; Clarke, W. P.; Cunningham, K. A.; Spampinato, U. Fine-tuning serotonin2c receptor function in the brain: Molecular and functional implications. *Neuropharmacology* **2008**, *55*, 969−976.
(33) Cho, S. J.; Jensen, N. H.; Kurome, T.; Kadari, S.; Manzano, M. L.; Malberg, J. E.; Caldarone, B.; Roth, B. L.; Kozikowski, A. P. Selective 5-hydroxytryptamine 2C receptor aonists derived from the lead compound tranylcypromine: identification of drugs with antidepressant-like action. *J. Med. Chem.* **2009**, *52*, 1885−1902.
(34) Berg, K. A.; Clarke, W. P.; Sailstad, C.; Saltzman, A.; Maayani, S. Signal transduction differences between 5-hydroxytryptamine type 2A and type 2C receptor systems. *Mol. Pharmacol.* **1994**, *46*, 477−484.
(35) Anastasio, N. C.; Gilbertson, S. R.; Bubar, M. J.; Agarkov, A.; Stutz, S. J.; Jeng, Y.; Bremer, N. M.; Smith, T. D.; Fox, R. G.; Swinford, S. E.; Seitz, P. K.; Charendoff, M. N.; Craft, J. W., Jr.; Laezza, F. M.; Watson, C. S.; Briggs, J. M.; Cunningham, K. A. Peptide inhibitors disrupt the serotonin 5-HT2C receptor interaction with phosphatase and tensin homolog to allosterically modulate cellular signaling and behavior. *J. Neurosci.* **2013**, *33*, 1615−1630.
(36) Wang, C.; Jiang, Y.; Ma, J.; Wu, H.; Wacker, D.; Katritch, V.; Han, G. W.; Liu, W.; Huang, X.-P.; Vardy, E.; McCorvy, J. D.; Gao, X.; Zhou, E.; Melcher, K.; Zhang, C.; Bai, F.; Yang, H.; Yang, L.; Jiang, H.; Roth, B. L.; Cherezov, V.; Stevens, R. C.; Xu, H. E. Structural basis for molecular recognition at serotonin receptors. *Science* **2013**, *340*, 610−614.
(37) Wacker, D.; Wang, C.; Katritch, V.; Han, G. W.; Huang, X.-P.; Vardy, E.; McCorvy, J. D.; Jiang, Y.; Chu, M.; Siu, F. Y.; Liu, W.; Xu, H. E.; Cherezov, V.; Roth, B. L.; Stevens, R. C. Structural features for functional selectivity at serotonin receptors. *Science* **2013**, *340*, 615−619.
(38) Wacker, D.; Wang, S.; McCorvy, J. D.; Betz, R. M.; Venkatakrishnan, A. J.; Levit, A.; Lansu, K.; Schools, Z. L.; Che, T.; Nichols, D. E.; Shoichet, B. K.; Dror, R. O.; Roth, B. L. Crystal structure of an LSD-bound human serotonin receptor. *Cell* **2017**, *168*, 377−389.
(39) Peng, Y.; McCorvy, J. D.; Harpsøe, K.; Lansu, K.; Yuan, S.; Popov, P.; Qu, L.; Pu, M.; Che, T.; Nikolajsen, L. F.; Huang, X.-P.; Wu, Y.; Shen, L.; Bjørn-Yoshimoto, W. E.; Ding, K.; Wacker, D.; Han, G. W.; Cheng, J.; Katritch, V.; Jensen, A. A.; Hanson, M. A.; Zhao, S.; Gloriam, D. E.; Roth, B. L.; Stevens, R. C.; Liu, Z.-J. 5-HT2C receptor structures reveal the structural basis of GPCR polypharmacology. *Cell* **2018**, *172*, 719−730.
(40) Ballesteros, J. A.; Weinstein, H. Integrated methods for the construction of three-dimensional models and computational probing of structure-function relations in G protein-coupled receptors. In *Methods in Neuroscience*; Sealfon, S. C., Ed.; Academic Press: 1995; Vol. *25*, Chapter 19, pp 366−428.
(41) Manivet, P.; Schneider, B. t.; Smith, J. C.; Choi, D.-S.; Maroteaux, L.; Kellermann, O.; Launay, J.-M. The serotonin binding site of human and murine 5-HT2B receptors: molecular modeling and site-directed mutagenesis. *J. Biol. Chem.* **2002**, *277*, 17170−17178.
(42) Ma, L.; Seager, M. A.; Wittmann, M.; Jacobson, M.; Bickel, D.; Burno, M.; Jones, K.; Graufelds, V. K.; Xu, G.; Pearson, M.; McCampbell, A.; Gaspar, R.; Shughrue, P.; Danziger, A.; Regan, C.; Flick, R.; Pascarella, D.; Garson, S.; Doran, S.; Kreatsoulas, C.; Veng, L.; Lindsley, C. W.; Shipe, W.; Kuduk, S.; Sur, C.; Kinney, G.; Seabrook, G. R.; Ray, W. J. Selective activation of the M(1) muscarinic acetylcholine receptor achieved by allosteric potentiation. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 15950−15955.
(43) Venkatakrishnan, A. J.; Deupi, X.; Lebon, G.; Tate, C. G.; Schertler, G. F.; Babu, M. M. Molecular signatures of G-protein-coupled receptors. *Nature* **2013**, *494*, 185.
(44) Preininger, A. M.; Meiler, J.; Hamm, H. E. Conformational flexibility and structural dynamics in GPCR-mediated G protein activation: a perspective. *J. Mol. Biol.* **2013**, *425*, 2288−2298.
(45) Besnard, J.; Ruda, G. F.; Setola, V.; Abecassis, K.; Rodriguiz, R. M.; Huang, X. P.; Norval, S.; Sassano, M. F.; Shin, A. I.; Webster, L. A.; Simeons, F. R.; Stojanovski, L.; Prat, A.; Seidah, N. G.; Constam, D. B.; Bickerton, G. R.; Read, K. D.; Wetsel, W. C.; Gilbert, I. H.; Roth, B. L.; Hopkins, A. L. Automated design of ligands to polypharmacological profiles. *Nature* **2012**, *492*, 215−220.
(46) Wager, T. T.; Hou, X.; Verhoest, P. R.; Villalobos, A. Moving beyond rules: the development of a central nervous system multiparameter optimization (CNS MPO) approach to enable alignment of druglike properties. *ACS Chem. Neurosci.* **2010**, *1*, 435−449.
(47) Cunningham, K. A.; Fox, R. G.; Anastasio, N. C.; Bubar, M. J.; Stutz, S. J.; Moeller, F. G.; Gilbertson, S. R.; Rosenzweig-Lipson, S. Selective serotonin 5-HT(2C) receptor activation suppresses the reinforcing efficacy of cocaine and sucrose but differentially affects the incentive-salience value of cocaine- vs. sucrose-associated cues. *Neuropharmacology* **2011**, *61*, 513−523.
(48) Halberstadt, A. L.; van der Heijden, I.; Ruderman, M. A.; Risbrough, V. B.; Gingrich, J. A.; Geyer, M. A.; Powell, S. B. 5-HT(2A) and 5-HT(2C) receptors exert opposing effects on locomotor activity in mice. *Neuropsychopharmacology* **2009**, *34*, 1958−1967.
(49) Jackson, D. M.; et al. A functional effect of dopamine in the nucleus accumbens and in some other dopamine-rich parts of the rat brain. *Psychopharmacologia* **1975**, *45*, 139−149.
(50) Lever, C.; et al. Rearing on hind legs, environmental novelty, and the hippocampal formation. *Rev. Neurosci. (Berlin, Ger.)* **2006**, *17*, 111−133.
(51) Walsh, R. N.; Cummins, R. A. The Open-Field Test: a critical review. *Psychol. bull.* **1976**, *83*, 482−504.
(52) Wu, J.; et al. Bimodal effects of MK-801 on locomotion and stereotypy in C57BL/6 mice. *Psychopharmacology (Berlin, Ger.)* **2005**, *177*, 256−263.
(53) Colpaert, F. C. Drug discrimination in neurobiology. *Pharmacol., Biochem. Behav.* **1999**, *64*, 337−345.
(54) Appel, J. B.; et al. The use of drug discrimination procedures to characterize hallucinogenic drug actions. *Psychopharmacol. Bull.* **1986**, *22*, 959−967.
(55) Negus, S. S.; Banks, M. L. Pharmacokinetic—pharmacodynamic (PKPD) analysis with drug discrimination. *Curr. Top. Behav. Neurosci.* **2016**, DOI: 10.1007/7854_2016_36.
(56) Filip, M. Hyperlocomotive and discriminative stimulus effects of cocaine are under the control of serotonin(2C) (5-HT(2C)) receptors in rat prefrontal cortex. *J. Pharmacol. Exp. Ther.* **2003**, *306*, 734−743.

DOI: 10.1021/acs.jmedchem.8b00401
J. Med. Chem. XXXX, XXX, XXX−XXX

(57) McCreary, A. C.; et al. Discriminative stimulus properties of (/-)-fenfluramine: the role of 5-HT2 receptor subtypes. *Behav. Neurosci.* **2003**, *117*, 212−221.
(58) De La Garza, R.; Callahan, P. M.; Cunningham, K. A.; et al. Detailed investigations of 5-HT3 compounds in a drug discrimination model. *Pharmacol., Biochem. Behav.* **1996**, *54*, 533−540.
(59) Cunningham, K. A.; et al. Discriminative stimulus properties of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT): implications for understanding the actions of novel anxiolytics. *Eur. J. Pharmacol.* **1987**, *138*, 29−36.
(60) Callahan, P. M.; Cunningham, K. A. Modulation of the discriminative stimulus properties of cocaine by 5-HT1B and 5-HT2C receptors. *J. Pharmacol. Exp. Ther.* **1995**, *274*, 1414−1424.
(61) Callahan, P. M.; Cunningham, K. A. Involvement of 5-HT2C receptors in mediating the discriminative stimulus properties of m-chlorophenylpiperazine (mCPP). *Eur. J. Pharmacol.* **1994**, *257*, 27−38.
(62) Cunningham, K. A. Discriminative stimulus properties of clonidine: substitution by ergot derivatives. *Eur. J. Pharmacol.* **1985**, *119*, 225−229.
(63) Frankel, P. S.; Cunningham, K. A. m-Chlorophenylpiperazine (mCPP) modulates the discriminative stimulus effects of cocaine through actions at the 5-HT2C receptor. *Behav. Neurosci.* **2004**, *118*, 157−162.
(64) Callahan, P. M.; Cunningham, K. A. Modulation of the discriminative stimulus properties of cocaine: comparison of the effects of fluoxetine with 5-HT1A and 5-HT1B receptor agonists. *Neuropharmacology* **1997**, *36*, 373−381.
(65) Glennon, R. A.; Young, R. Drug Discrimination and Development of Novel Agents and Pharmacological Tools. In *Drug Discrimination: Applications to Medicinal Chemistry and Drug Discovery*; Glennon, R. A., Young, R., Eds.; John Wiley and Sons: Hoboken, NJ, 2011; pp 217−238.
(66) Neelakantan, H.; et al. Lorcaserin suppresses oxycodone self-administration and relapse vulnerability in rats. *ACS Chem. Neurosci.* **2017**, *8*, 1065−1073.
(67) Miller, W. R.; et al. PPARγ agonism attenuates cocaine cue reactivity. *Addict. Biol.* **2018**, *23*, 55−68.
(68) Grottick, A. J.; Fletcher, P. J.; Higgins, G. A. Studies to investigate the role of 5-HT2C receptors on cocaine- and food-maintained behavior. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 1183−1191.
(69) Neisewander, J. L.; Acosta, J. I. Stimulation of 5-HT2C receptors attenuates cue and cocaine-primed reinstatement of cocaine-seeking behavior in rats. *Behav. Pharmacol.* **2007**, *18*, 791−800.
(70) Seitz, P. K.; Bremer, N. M.; McGinnis, A.; Cunningham, K. A.; Watson, C. S. Quantitative changes in intracellular calcium and extracellular-regulated kinase activation measured in parallel in CHO cells stably expressing serotonin (5-HT) 5-HT2A or 5-HT2C receptors. *BMC Neurosci.* **2012**, *13*, 25.

DOI: 10.1021/acs.jmedchem.8b00401
J. Med. Chem. XXXX, XXX, XXX−XXX