ACS Chemical Neuroscience

Letter

Synthesis and structure-activity relationships of tools based on WAY163909, a 5-HT2c receptor agonist

Ying-Chu Chen, Rachel M. Hartley, Noelle C. Anastasio, Kathryn A. Cunningham, and Scott R. Gilbertson

ACS Chem. Neurosci., **Just Accepted Manuscript** • DOI: 10.1021/acschemneuro.6b00439 • Publication Date (Web): 17 Apr 2017

ACS Publications

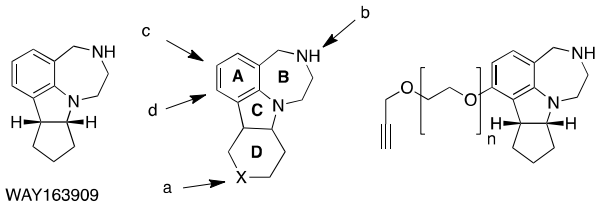
ACS Chemical Neuroscience is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Synthesis and structure activity relationships of tools based on WAY163909, a 5-HT$_{2C}$ receptor agonist

Ying-Chu Chen[1], Rachel M. Hartley[2], Noelle C. Anastasio[2,3], Kathryn A. Cunningham[2,3], and Scott R. Gilbertson[1,2]

[1]Department of Chemistry, University of Houston, Houston TX [2]Center for Addiction Research, [3]Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX

Abstract



The development of probe molecules that can be used to investigate G protein-coupled receptor (GPCR) pharmacology, trafficking and relationship with other GPCRs is an important and growing area of research. Here, we report the synthesis of analogs of the known selective serotonin (5-HT) 5-HT$_{2C}$ receptor (5-HT$_{2C}$R) agonist WAY163909 which were designed to allow for the attachment of a second ligand, signaling or reporter molecules as well as immobilization agents to the parent molecule with the maintenance of agonist activity. This goal was accomplished by the synthesis of novel molecules in which sites **a-d** were modified and resulting compounds were analyzed pharmacologically *in vitro*.

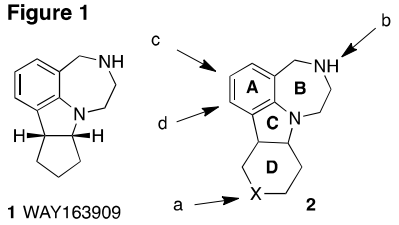
Keywords

Serotonin, 5-HT$_{2C}$ receptor agonist, WAY163909 derivatives

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) receptors are implicated in a wide variety of physiological functions in both the central and peripheral nervous systems. We are focused on the development of tool compounds to study two 5-HT GPCRs, the 5-HT$_{2A}$ receptor (5-HT$_{2A}$R) and 5-HT$_{2C}$R. These receptors exhibit an oppositional regulatory role over multiple behaviors such that selective 5-HT$_{2A}$R antagonists and 5-HT$_{2C}$R agonists exert similar, and synergistic effects, upon behavioral outcomes in preclinical studies.[1-2] Furthermore, the 5-HT$_{2A}$R and 5-HT$_{2C}$R are proposed to form and/or function within both homo- and/or heteromeric protein complexes.[3-12] The functional and/or physiological impact of these types of multimeric receptors are not fully understood and the development of tool molecules to biochemically and/or pharmacologically distinguish between the types of oligomeric receptor complexes is an important goal. Thus, we have initiated a program to develop ligands capable of being linked to other molecules, including a second ligand, a reporter molecule (e.g., fluorescent dyes) or immobilization agents (e.g., biotin). To accomplish this goal, it is critical to identify locations on known 5-HT$_2$R ligands that will allow for linking such groups without alteration of ligand binding and/or functional activity. Our initial paper reported the synthesis and *in vitro* pharmacology of a homobivalent 5-HT$_{2A}$R antagonist.[13] Here, we report the synthesis and structure-activity relationships (SAR) for derivatives of the selective 5-HT$_{2C}$R agonist WAY163909 (**1**)[1, 14-27] which can be used to attach tethers [e.g., polyethylene glycol (PEG)] to link these molecules to various other partners. Versions of the basic structure were synthesized in which locations around the molecule, sites **a**-**d** (**2**), were modified to determine the viability of these locations as points for the attachment of a necessary linker.

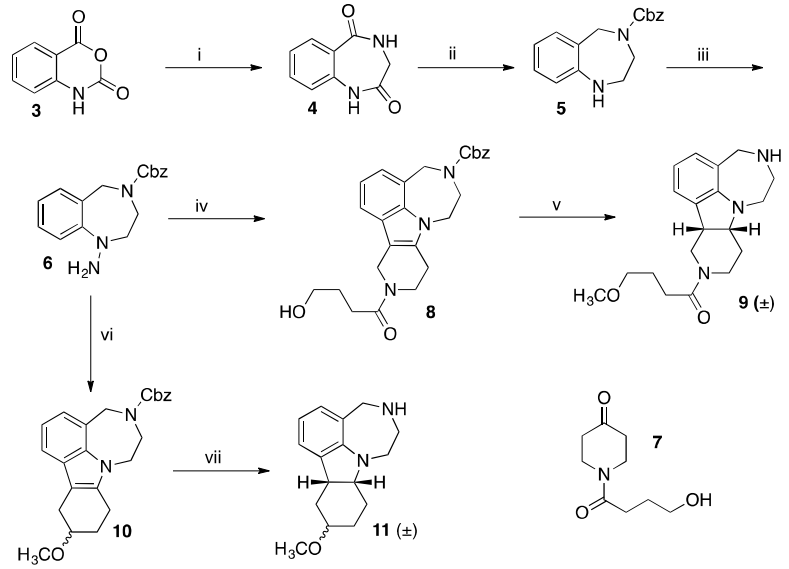
Figure 1



Results and Discussion

The initial step was to identify a site on WAY163909 capable of modification without significant loss of activity. This work can present challenges as the derivatives not only must bind to the 5-HT_{2C}R orthosteric site but, for some studies, the ligands should also retain their agonist activity. The SAR data from the original work on these structures indicated that ring D (**Figure 1 cpd 2**) was rather insensitive to different sizes, so a synthetic plan was selected to allow for the synthesis of derivatives of ring D at a late stage in the route.[27] The synthetic approach taken was a modified version of the published route (**Scheme 1**)[28] selected because ring D is formed late in the synthesis by a Fischer indole reaction, using a ketone as its source. Given the variety of ketones available, this provides access to a number of different substituted D rings. For the first example, 1-(benzyloxycarbonyl)-4-piperidinone (**7**) was reacted with hydrazine **6** to provide **8** in a 64% yield. The hydrazine **6** was obtained from isatoic anhydride (**3**) and glycine followed by reduction of the diamide, selective acetylation of the diamine, reaction with sodium nitrite, and reduction with titanium tetrachloride and magnesium. After formation of the indole, the hydroxyl group was converted to a methyl ether with sodium hydride and methyl iodide. This was followed by reduction of the double bond in **8** with sodium borohydride and trifluoroacetic acid and then hydrogenolysis of the Cbz group to give the desired product, **9**. The piperidinone was selected so the amine could be used as a handle for the attachment of linkers. Additionally, 4-(methoxy) cyclohexanone was used in this sequence to provide **11** with an ether to serve as a protected alcohol.

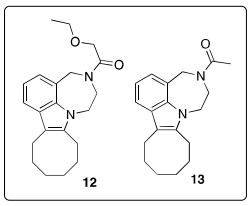
Scheme 1



Reagents and conditions: (i) a. 6N NaOH, 100 ˚C, 12 h, b. glycine, 6N NaOH, H_2O, reflux, 12 h, then L-tartaic acid, 100 ˚C, 2 h, 86%; (ii) a. LAH, THF, 24 h, 93%; b. CbzCl, Et_3N, CH_2Cl_2, rt, 18 h, 88%; (iii) a. $NaNO_2$, 1,4-dioxane, b. 1N HCl, rt, 24 h, 98%; c. $TiCl_4$, Mg, CH_2Cl_2, Et_2O, rt, 0.75 h, 97% crude product; (iv) ketone **8**, acetic acid, reflux, 18 h, 64%; (v) a. NaH, CH_3I, DMF, 0 ˚C-rt 16h; b. $NaBH_4$, TFA, 18 h, rt, 78%; c. 10% Pd/C, H_2, EtOH, rt, 48 h, 88%; (vi) 4-(methoxy)cyclohexanone, acetic acid, reflux, 16 h, 45%; (vii) a. $NaBH_4$, TFA, 18 h, rt, 70%; b. 10% Pd/C, H_2, EtOH, rt, 48 h 42%.

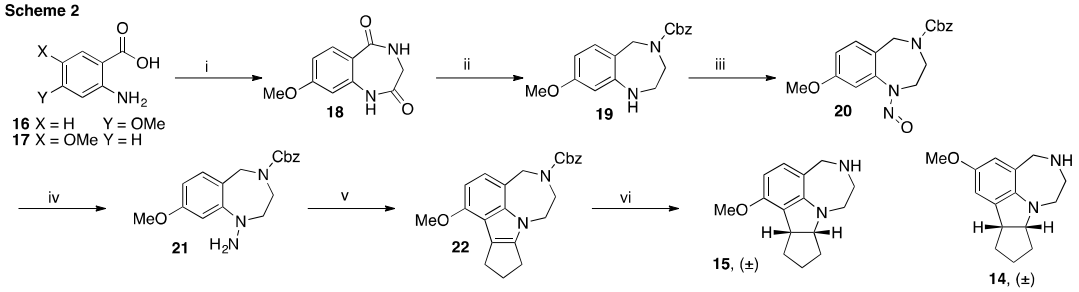
The ability of the molecules to act as agonists to induce 5-HT_{2C}R-mediated intracellular calcium (Ca_i^{++}) release[13, 29-31] was conducted in a U2OS cell line stably expressing the human 5-HT_{2C}R. While the potency for the novel molecules was lower relative to 5-HT or the parent WAY163909, the derivatives maintained full efficacy (**Table 1** and **Supporting Information Figure 1**). However, since the potency of the first set of synthesized derivatives (**9, 11, 24, 25** and **26**) was in the range of 1-10 μM, significantly reduced relative to 5-HT (**23**) and WAY163909 (**1**) (**Table 1**), the decision was made to examine other attachment points.

Figure 2



An alternative location investigated for the attachment of a tether to the WAY163909 scaffold was the secondary amine (site **b,** cpd **2**). The necessary molecules were synthesized by the route outlined in **Scheme 1**. The two versions examined (**Figure 2**; **12** and **13**) both demonstrated reduced potency (EC_{50} >10 μM) in the Ca_i^{++} assay (data not shown).

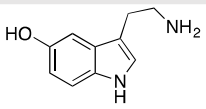
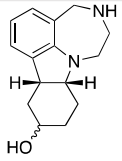
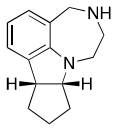
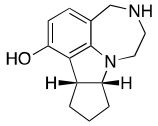
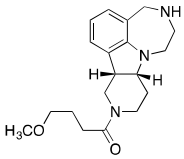
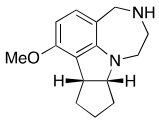
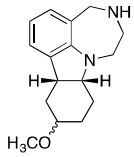
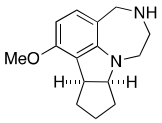
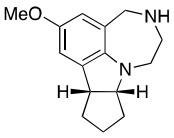
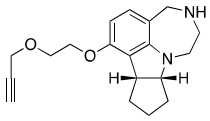
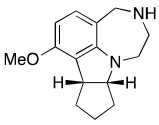
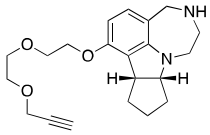
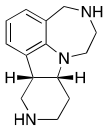
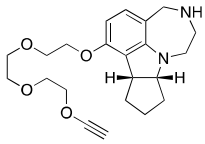
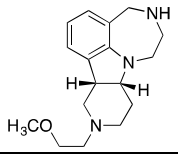
Scheme 2



Reagents and conditions: (i) a. triphosgene, THF, rt, 18 h, 97%; b. glycine, 6N NaOH, H_2O, reflux, 12 h, then L-tartaric acid, reflux, 2 h, 77%; (ii) a. LAH, THF, reflux, 48 h, 98%; b. benzyl chloroformate, Et_3N, CH_2Cl_2, rt, 18 h, 77%; (iii) $NaNO_2$, 1N HCl, H_2O, 1,4-dioxane, rt, 15 min, 99%; (iv) $TiCl_4$, Mg, CH_2Cl_2, diethyl ether, rt, 15 min, 99% crude product; (v) cyclopentanone, *p*-TSA, toluene, reflux, 3 h, 48% over steps iv and v; (vi) a. $NaBH_4$, TFA, rt, 10 min, 99%; b. 10% Pd/C, H_2, MeOH, rt, 3 h, 99%. Steps for **14** are the same as **15**. Yields and details are reported in the supporting information.

We reasoned that a methoxy group would be a good representation of the polyether type connection ultimately desired to link this molecule to others. Thus, versions with a methoxy group at locations **c** and **d** were synthesized (**Scheme 2**). The two derivatives were synthesized using two different starting materials, 2-amino-4-methoxy benzoic acid (**16**) or 2-amino-5-methoxy-benzoic acid (**17**). Reaction of **16** with triphosgene provided the benzoxazanone**,** which was reacted with glycine to form the bisamide (**18**). Exhaustive reduction of both amides then provided the diamine, which was selectively protected as the Cbz carbamate (**19**). Formation of the nitroso compound (**20**) followed by reduction with titanium tetrachloride and magnesium metal provided the hydrazine (**21**) necessary for the Fischer indole synthesis step. Reduction of the double bond (**22**) with sodium borohydride in the presence of trifluoroacetic acid and hydrogenolysis of Cbz carbamate afforded **15**. Compound **14** was obtained by the same route with comparable yields.

Table 1. Effects of WAY163909 derivatives on intracellular calcium release in h5-HT_{2C}R-U2OS cells

Cpd #	Structure	EC_{50} (nM)[a] (95% C.I.)	E_{max}[b,c]	Cpd #	Structure	EC_{50} (nM)[a] (95% C.I.)	E_{max}[b,c]
23		0.44 (0.1-0.8)	100	**26 (±)**		>1 μM	ND
1 (±)		18.4 (16-21)	100	**31 (±)**		122.6 (92-153)	95.3 ± 2.3
9 (±)		>10 μM	ND	**32**		1.3 (0.5-2.0)	101 ± 7.3
11 (±)		>1 μM	ND	**33**		72.9 (34-112)	94.9 ± 6.4
14 (±)		1487 (810-2163)	97.6 ± 2.7	**40 (±)**		53.4 (26-80)	96.5 ± 2.2
15 (±)		2.2 (1.3-3.1)	103 ± 3.8	**41 (±)**		236.7 (37-437)	96.8 ± 5.2
24 (±)		>1 μM	ND	**42 (±)**		647.3 (271-1024)	94 ± 2.5
25 (±)		>10 μM	ND				

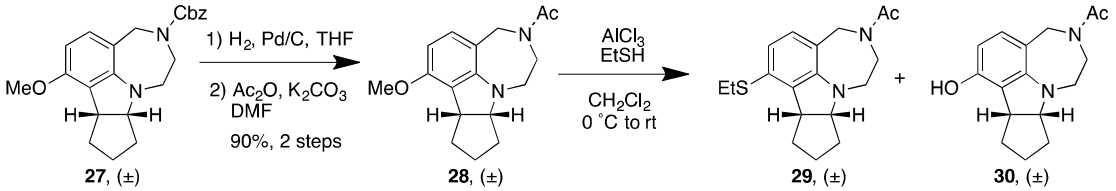
[a] EC_{50} is presented as the mean; 95% confidence interval (CI) is presented as the range

[b] E_{max} is presented as mean ± SEM; % Ca_i^{++} release of compounds (except 5-HT) normalized to 1 μM of WAY163909 (**1**)

[c] ND = not determined

The product from 2-amino-5-methoxy-benzoic acid, (**14**) proved to be less potent (EC_{50} ~1.5 μM) relative to WAY163909 (EC_{50}~18.4 nM), but exhibited efficacy (E_{max} ~97%) similar to that observed at 1 μM of WAY163909 (**Supporting Information Figure 2**). The product with the methoxy group in the 4-position (**15**) demonstrated an increase in potency (EC_{50} ~2.2 nM) relative to WAY163909 with no change in efficacy (E_{max} ~103%; **Supporting Information, Figure 2**). Thus, this small change in moving the methoxy group over one carbon (**14** versus **15**) resulted in a significant change in the potency, but not the efficacy, of the derivative.

Scheme 3



27, (±) **28**, (±) **29**, (±) **30**, (±)

Table 2. Deprotection of the methyl ether

Equivalents of $AlCl_3$	Equivalents of EtSH	Time (hr)	29:30	Isolated Yield
4 added 2X	24 X 2	48	1:1.3	36%
1.5 added 2X	20 X 2	24	NA	No reaction
4*	4	18	1:25	95%
4	2	18	1:120	97%
6	6	18	1:16	87%

Starting material and $AlCl_3$ where premixed for 1 hour at 0 ˚C

The next step in the process was the deprotection of the methoxy group to provide a phenol, which could then be connected to a PEG linker. Initial attempts to remove the methyl group resulted in loss of the Cbz group. A wide variety of reactions were tested but either no reaction, or decomposition with no significant identifiable products were observed. Details of the various reaction conditions are presented in the Supporting Information. Consequently, the Cbz group was exchanged for an acetate. Through careful control of the reaction conditions, the methoxy group could be cleaved with aluminum chloride and ethanethiol in methylene chloride. Initially, the free phenol product (**30**) was obtained as a mixture with the ethylthioether (**29**). After optimization of the reaction conditions, the desired product (**30**) was obtained in up to 97% isolated yield (**Table 2**).

Given the previous demonstration that WAY163909 exhibits differential pharmacological properties, the two enantiomers of compound **15** were resolved.[25] The originally reported resolution of WAY163909 was performed by synthesizing the diastereomeric salt with dibenzoyl-L-tartaric acid. However, in the case of molecule **15**, this method failed to provide the desired separation. A variety of chiral acids were tested with a number of different solvents. Success was ultimately achieved with di-*p*-toluoyl-L-tartaric acid in isopropanol. The absolute stereochemistry was determined by X-ray diffraction of both enantiomers after separation. The potencies of the two enantiomers of compound **15** (**32** and **33**) are considerably different (EC_{50}~1.3 nM and ~72.9 nM, respectively), but both retained full efficacy (E_{max}~100% and 95%, respectively) (**Table 1**; **Figure 3**). The two enantiomers (**32** and **33**) were submitted to CEREP (www.crep.fr) to determine receptor selectivity to displace binding to the 5-HT_{2A}R, 5-HT_{2B}R and 5-HT_{2C}R. Compounds were tested at a single concentration of 10 μM to determine their ability to displace [^{125}I]-(±)-2,5-dimethoxy-4-iodoamphetamine ([^{125}I]-(±)-DOI) binding according to their standard assay protocols. The (R,R)-enantiomer (**32**) displaced ~85-100% of specific binding at 5-HT_{2A}R, 5-HT_{2B}R and 5-HT_{2C}R, indicating a lack of selectivity across these three receptors (Table 3). However, the (S,S)-enantiomer (**33**) was selective for displacing ~91% of [^{125}I]-(±)-DOI binding to the 5-HT_{2C}R, with ~5-20% displacement of binding to the 5-HT_{2A}R or 5-HT_{2B}R (**Table 3**).

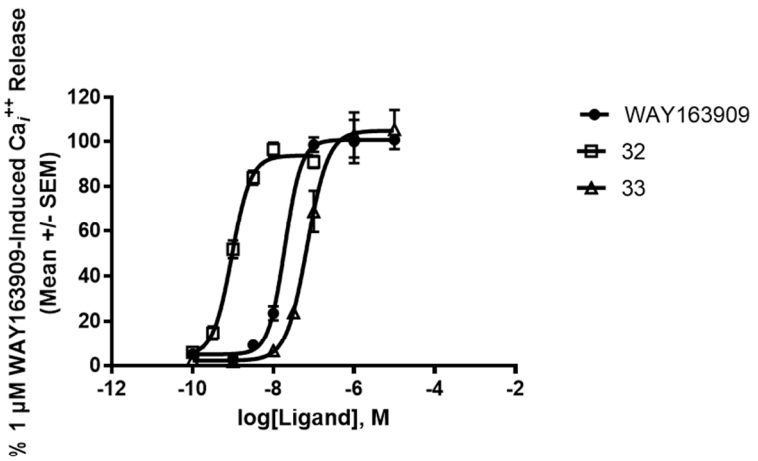
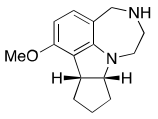
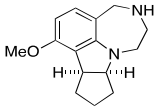
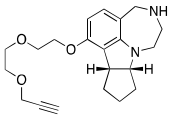


Figure 3. Representative intracellular calcium release response of WAY163909 derivatives at human 5-HT_{2C}R compared with WAY163909. The E_{max} and EC_{50} of these compounds are listed in Table 1.

Table 3. Selectivity profile of WAY163909 derivatives at 5-HT_2R

Cpd #	Structure	5-HT_{2A}R	5-HT_{2B}R	5-HT_{2C}R
32		**86.5**	**98.6**	**100.2**
33		**22.8**	**4.8**	**91.5**
41 (±)		**67.1**	**67.7**	**99.6**

Compounds were submitted to CEREP to determine receptor selectivity at the 5-HT_2 Compounds were tested at a single concentration of 10 µM in duplicate to determine their ability to displace [^{125}I] (+/-) DOI. Data are presented as mean % inhibition of control specific binding for compound tested at each receptor subtype. Significant inhibition is considered >50%; 25-50% inhibition is indicative of weak to moderate effects.

The next step in the process was the attachment of a molecule that can be used to link either two WAY163909 derivatives together or attach another molecule. This was accomplished by reaction of the free phenol (**30**) with the alkyl tosylates **34-36** in DMF with sodium hydride as base (**Scheme 4**). In all three cases investigated, the alkylation of the phenol proceeded in excellent to good yield. The length of the alkyne tether proportionately impacted the potency of the WAY163909 derivatives with little to no effect on efficacy (**40-42, Table 1; Figure 4**). In CEREP assays, **41(±)** (10 µM) displaced 100% of [^{125}I]-(±)-DOI binding to the 5-HT_{2C}R, and ~70% of binding to the 5-HT_{2A}R and 5-HT_{2B}R (**Table 3**). It should be noted that **41(±)** is a racemic mixture of both enantiomers. Given the observation that in the case of **32** and **33** one enantiomer (**33**) is much more selective for the 5-HT_{2C}R versus the other (**32**), it is completely reasonable that the racemic mixtures of any of these compounds will be less selective than the pure enantiomer. In future work only the most selective enantiomer will be used.

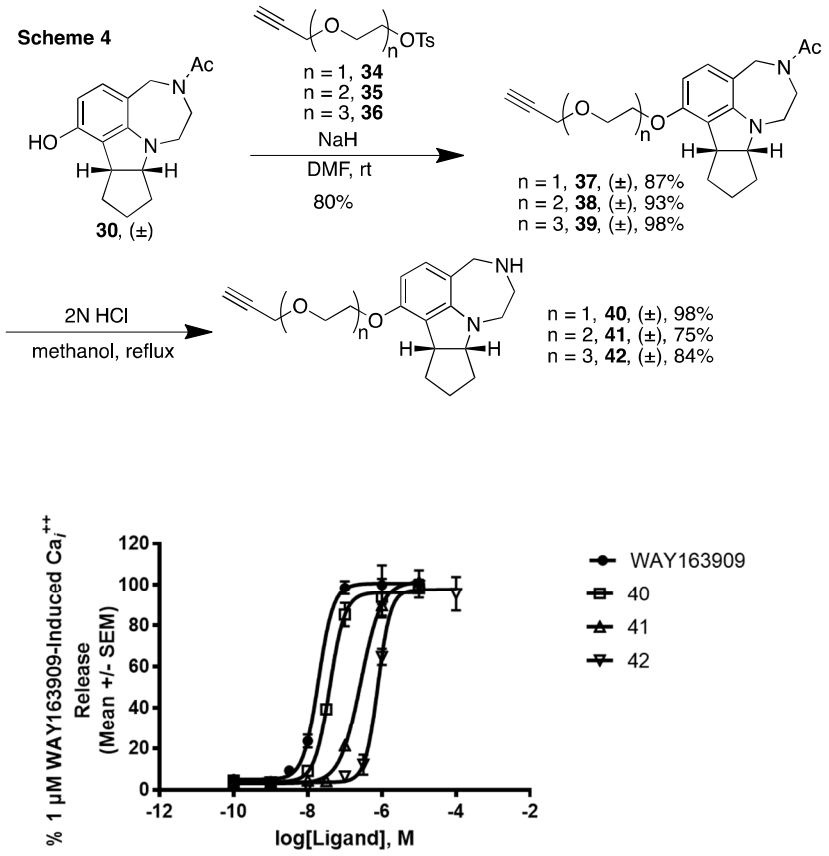


Figure 4. Representative intracellular calcium release response of WAY163909 derivatives at human 5-HT_{2C}R compared with WAY163909. The E_{max} and EC_{50} of these compounds are listed in Table 1.

Through the synthesis of a number of analogs of WAY163909, we have identified a site on the scaffold of WAY163909 for the attachment of a tether to create tool compounds to bind to other molecules. Derivatives where site **d** (**2**, **Figure 1**) has a methoxyl group or PEG chain attached retain nanomolar potency with little effect on efficacy. The identification that site **d** can be modified without significant loss of activity will allow for the synthesis of molecules that bind to the 5-HT_{2C}R and retain activity as full agonists. Versions where the PEG chain is terminated with an alkyne will be valuable as tools to link WAY163909 derivatives to other biologically active molecules, fluorescent tags, or affinity probes through the alkyne/azide click reaction. The pharmacological characterization of these molecules are an active research focus of our group and future biological studies will be reported as they are completed.

Materials and Methods

Cell lines and cell culture. The PathHunter® U2OS *HTR2C* β-Arrestin cell line (5-HT_{2C}R-U2OS; DiscoveRx) stably express the non-edited (INI) human 5-HT_{2C}R isoform (h5-HT_{2C}R). The 5-$HT_{2C\text{-}INI}$R-U2OS cells were grown in Assay Complete™ U2OS Medium 31 (DiscoveRx) at 37°C, 5% CO_2 and 85% relative humidity per manufacturer's recommendations utilizing AssayComplete™ Cell Detachment Reagent (DiscoveRx). Cells were passaged at 70-80% confluence and all experiments were conducted using cells in log phase growth.

Intracellular calcium assay. Intracellular calcium (Ca_i^{++}) release was monitored using the FLIPR Calcium 4 Assay Kit (Molecular Devices) according to previously published protocols with minor modifications.[13, 29-31] Cells were plated at 5,000-7,000 cells/well in Assay Complete™ Cell Plating Reagent 16 (DiscoveRx) in black-sided, clear bottomed 96-well tissue culture plates and allowed to adhere overnight. Medium was removed and replaced with 40 µl Hank's balanced salt solution without calcium, magnesium and phenol red (HBSS; Corning) plus 40 µl Calcium 4 dye solution in Buffer B supplemented with 2.5 mM probenecid (Sigma-Aldrich) to inhibit extracellular dye transport. Plates were incubated for 60 min at 37 °C followed by 30 min at room temperature in the dark. Fluorescence (λ_{ex} = 485 nm, λ_{em} = 525 nm) was measured using a FlexStation3 (Molecular Devices). Baseline was established for 17 sec before addition of 20 µl vehicle (HBSS without calcium or magnesium) or 5x concentrated compound. Addition of 5-HT, WAY163909, or compound occurred at the 17-sec timepoint and fluorescence was recorded every 1.7 sec for 120 sec. Maximum peak height was determined using FlexStation software (SoftMax Pro 5.4). After the final readings, cells were fixed in 2% paraformaldehyde overnight.

Data analysis. Peak responses from each well were normalized to total cell mass as determined with crystal violet staining.[30] The E_{max} is defined as the maximum possible Ca_i^{++} response and data are expressed as a percent of the Ca_i^{++} release (mean ± SEM) obtained with 1 µM of WAY163909. Potency of the compounds was determined using the EC_{50} (concentration of compound required to achieve half-maximal response). The EC_{50} values were determined using 4-parameter nonlinear regression analysis (GraphPad Prism Version 7.02) and calculated from at least three independent experiments, each conducted in triplicate, and are presented as the mean and the 95% confidence interval.[32-33] An EC_{50} or E_{max} value was not calculated for ligands that failed to reach a plateau [reported as not determined (ND) in Table 1].

CEREP binding assays. Compounds 32, 33 and 41 were submitted to CEREP to determine receptor selectivity to displace binding to the 5-HT_{2A}R, 5-HT_{2B}R and 5-HT_{2C}R per their standard assay protocols. (http://www.cerep.fr/cerep/users/pages/Downloads/Documents/Marketing/Pharmacology%20&%20ADME/Assay%20lists/Binding%20assays_2013.pdf). Compounds were tested at a single concentration of 10 µM in duplicate to determine their ability to displace [125I] (+/-) DOI. Data are presented as mean % inhibition of control specific binding for compound tested at each receptor subtype. Significant inhibition is considered > 50%; 25-50% inhibition is indicative of weak to moderate effects.

Chemistry. Details for the synthesis of the individual compounds are presented in the Supplementary Information. The characterization data for the five most active compounds are presented below.

7-methoxy-2,3,4,7b,8,9,10,10a-octahydro-1*H*-cyclopenta[*b*][1,4]diazepino[6,7,1-*hi*]indole (racemate of 32/33) ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.80 (d, *J* = 8.1 Hz, 1H), 6.22 (d, *J* = 8.1 Hz, 1H), 3.98-3.90 (m, 2H), 3.84-3.76 (m, 4H), 3.68 (d, *J* = 15.2 Hz, 1H), 3.28-3.22 (m, 1H), 3.19-3.15 (m, 1H), 2.85 (p, *J* = 11.3 Hz, 2H), 1.95-1.80 (m, 2H), 1.76-1.53 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.7, 153.9, 127.7, 121.5, 119.4, 101.0, 73.4, 56.9, 55.1, 54.3, 51.2, 43.7, 34.2, 33.4, 24.6. HRMS (ESI-TOF) Calcd. for $C_{15}H_{20}N_2O$ $[M+H]^+$: 245.1648; found: 245.1650.

7-(2-(prop-2-ynyloxy)ethoxy)-2,3,4,7b,8,9,10,10a-octahydro-1*H*-cyclopenta[*b*][1,4]diazepino[6,7,1-*hi*]indole (40): ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.76 (d, *J* = 8.2 Hz, 1H), 6.19 (d, *J* = 8.2 Hz, 1H), 4.19 (d, *J* = 2.3 Hz, 2H), 4.11 (td, *J* = 4.8, 2.6 Hz, 2H), 3.94-3.89 (m, 2H), 3.83 (t, *J* = 5.0 Hz, 2H), 3.80 (dd, *J* = 9.0, 3.1 Hz, 1H), 3.74 (dd, *J* = 5.8, 3.6 Hz, 2H), 3.71-3.65 (m, 7H), 3.30-3.23 (m, 1H), 3.20-3.14 (m, 1H), 2.95 (br, 1H), 2.90-2.82 (m,

2H), 2.42 (t, *J* = 2.3 Hz, 1H), 1.93-1.79 (m, 2H), 1.76-1.50 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.4, 153.9, 128.6, 122.1, 114.7, 102.7, 79.5, 74.5, 73.4, 70.8, 70.6, 70.4, 69.7, 69.0, 67.4, 58.3, 53.9, 52.4, 49.9, 43.8, 34.4, 33.3, 24.5. HRMS (ESI-TOF) Calcd. for $C_{23}H_{32}N_2O_4$ $[M+H]^+$: 401.2435; found: 401.2440.

7-(2-(2-(prop-2-ynyloxy)ethoxy)ethoxy)-2,3,4,7b,8,9,10,10a-octahydro-1*H*-cyclopenta[*b*][1,4]diazepino[6,7,1-*hi*]indole (41): ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.80 (d, *J* = 8.3 Hz, 1H), 6.22 (d, *J* = 8.2 Hz, 1H), 4.96 (br, NH), 4.21 (d, *J* = 2.3 Hz, 2H), 4.16-4.09 (m, 2H), 4.04 (d, *J* = 15.1 Hz, 1H), 3.92 (dd, *J* = 8.8, 5.1 Hz, 1H), 3.84 (t, *J* = 4.9 Hz, 2H), 3.81 (dd, *J* = 8.8, 3.2 Hz, 1H), 3.77-3.74 (m, 2H), 3.74-3.69 (m, 3H), 3.39 (dd, *J* = 13.2, 3.1 Hz, 1H), 3.20 (dd, *J* = 12.6, 2.5 Hz, 1H), 3.03-2.88 (m, 2H), 2.43 (t, *J* = 2.3 Hz, 1H), 1.93-1.80 (m, 2H), 1.77-1.60 (m, 3H), 1.60-1.49 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.3, 153.9, 128.4, 122.1, 115.7, 102.6, 79.6, 74.5, 73.5, 70.6, 69.8, 69.1, 67.5, 58.4, 54.5, 52.7, 50.0, 43.8, 34.4, 33.3, 24.5. HRMS (ESI-TOF) Calcd. for $C_{21}H_{28}N_2O_3$ $[M+H]^+$: 357.2173; found: 357.2178.

7-(2-(2-(2-(prop-2-ynyloxy)ethoxy)ethoxy)ethoxy)-2,3,4,7b,8,9,10,10a-octahydro-1*H*-cyclopenta[*b*][1,4]diazepino[6,7,1-*hi*]indole (42): ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.76 (d, *J* = 8.2 Hz, 1H), 6.19 (d, *J* = 8.2 Hz, 1H), 4.19 (d, *J* = 2.3 Hz, 2H), 4.11 (td, *J* = 4.8, 2.6 Hz, 2H), 3.94-3.89 (m, 2H), 3.83 (t, *J* = 5.0 Hz, 2H), 3.80 (dd, *J* = 9.0, 3.1 Hz, 1H), 3.74 (dd, *J* = 5.8, 3.6 Hz, 2H), 3.71-3.65 (m, 7H), 3.30-3.23 (m, 1H), 3.20-3.14 (m, 1H), 2.95 (br, 1H), 2.90-2.82 (m, 2H), 2.42 (t, *J* = 2.3 Hz, 1H), 1.93-1.79 (m, 2H), 1.76-1.50 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.4, 153.9, 128.6, 122.1, 114.7, 102.7, 79.5, 74.5, 73.4, 70.8, 70.6, 70.4, 69.7, 69.0, 67.4, 58.3, 53.9, 52.4, 49.9, 43.8, 34.4, 33.3, 24.5. HRMS (ESI-TOF) Calcd. for $C_{23}H_{32}N_2O_4$ $[M+H]^+$: 401.2435; found: 401.2440.

Author Contributions

Y-C.C. performed the chemical syntheses and analyses and drafted the manuscript; R.M.H. performed the *in vitro* biological assays and analyses and drafted the manuscript; N.C.A. conducted pharmacological analyses; N.C.A., K.A.C. and S.R.G. conceptualized the project, oversaw experimental design/interpretation/analyses, and wrote/edited the manuscript.

Corresponding Author: E-mail srgilbe2@central.uh.edu. Voice 832-842-8821

Funding Sources

This work was supported by NIDA grant P50 DA033935.

Conflicts of Interest

The remaining authors declare no competing financial interests.

References

1. 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-HT_{2A} Receptor (5-HT_{2A}R) Antagonist and 5-HT_{2C}R Agonist Suggests New Pharmacotherapeutics for Cocaine Addiction. *ACS Chem. Neurosci.* **2013,** *4* (1), 110-121.
2. Cunningham, K. A.; Anastasio, N. C., Serotonin at the nexus of impulsivity and cue reactivity in cocaine addiction. *Neuropharmacology* **2014,** *76* (Part B), 460-478.
3. Kamal, M.; Gbahou, F.; Guillaume, J. L.; Daulat, A. M.; Benleulmi-Chaachoua, A.; Luka, M. C., P.; Kalbasi Anaraki, D.; Baroncini, M.; Mannoury la Cour, C.; Millan, M. J.; Prevot, V.;

Delagrange, P.; Jockers, R., Convergence of melatonin and serotonin (5-HT) signaling at MT2/5-HT_{2C} receptor heteromers. *J. Biol. Chem.* **2015,** *290* (18), 11537-11546.
4. Bigford, G. E.; Chaudhry, N. S.; Keane, R. W.; Holohean, A. M., 5-Hydroxytryptamine $5HT_{2C}$ receptors form a protein complex with N-methyl-D-aspartate $GluN_{2A}$ subunits and activate phosphorylation of Src protein to modulate motoneuronal depolarization. *J. Biol. Chem.* **2012,** *287* (14), 11049-11059.
5. Iglesias, A.; Cimadevila, M.; Cadavid, M. I.; Loza, M. I.; Brea, J., Serotonin-2A homodimers are needed for signalling via both phospholipase A_2 and phospholipase C in transfected CHO cells. *Eur. J. Pharmacol.* **2017,** *800*, 63-69.
6. Mancia, F.; Assur, Z.; Herman, A. G.; Siegel, R.; Hendrickson, W. A., Ligand sensitivity in dimeric associations of the serotonin $5HT_{2C}$ receptor. *EMBO reports* **2008,** *9* (4), 363-369.
7. Teitler, M.; Klein, M. T., A new approach for studying GPCR dimers: drug-induced inactivation and reactivation to reveal GPCR dimer function in vitro, in primary culture, and in vivo. *Pharmacol. Ther.* **2012,** *133* (2), 205-217.
8. Pellissier, L. P.; Barthet, G.; Gaven, F.; Cassier, E.; Trinquet, E.; Pin, J. P.; Marin, P.; Dumuis, A.; Bockaert, J.; Baneres, J. L.; Claeysen, S., G Protein Activation by Serotonin Type 4 Receptor Dimers: Evidence That Turning on Two Progomers is More Efficient. *J Biol Chem* **2011,** *286* (12), 9985-9997.
9. Herrick-Davis, K., Inhibition of Serotonin 5-Hydroxytryptamine2C Receptor Function through Heterodimerization: Recptor Dimers Bind Two Molecules of Ligand and One G-Protein. *J. Biol. Chem.* **2005,** *280* (48), 40144-40151.
10. Herrick-Davis, K., Functional significance of serotonin receptor dimerization. *Exp. Brain Res.* **2013,** *230* (4), 375-386.
11. Herrick-Davis, K.; Weaver, B. A.; Grinde, E.; Mazurkiewicz, J. E., Serotonin 5-HT_{2C} receptor homodimer biogenesis in the endoplasmic reticulum: real-time visualization with confocal fluorescence resonance energy transfer. *J. Biol. Chem.* **2006,** *281* (37), 27109-27116.
12. Herrick-Davis, K.; Grinde, E.; Mazurkiewicz, J. E., Biochemical and Biophysical Characterization of Serotonin 5-HT_{2C} Receptor Homodimers on the Plasma Membrane of Living Cells. *Biochemistry* **2004,** *43* (44), 13963-13971.
13. Shashack, M. J.; Cunningham, K. A.; Seitz, P. K.; McGinnis, A.; Smith, T. D.; Watson, C. S.; Gilbertson, S. R., Synthesis and Evaluation of Dimeric Derivatives of 5-HT_{2A} Receptor (5-HT2AR) Antagonist M-100907. *ACS Chem. Neurosci.* **2011,** *2* (11), 640-644.
14. 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_{2C} receptor system aligns with vulnerability to cocaine cue reactivity. *Translational Psychiatry* **2014,** *4* (3), e369.
15. Neelamegam, R.; Hellenbrand, T.; Schroeder, F. A.; Wang, C.; Hooker, J. M., Imaging evaluation of $5HT_{2C}$ agonists, [(11)C]WAY-163909 and [(11)C]vabicaserin, formed by Pictet-Spengler cyclization. *Journal of Medicinal Chemistry* **2014,** *57* (4), 1488-1494.
16. Navailles, S.; Lagière, M.; Roumegous, A.; Polito, M.; Boujema, M. B.; Cador, M.; Dunlop, J.; Chesselet, M.-F.; Millan, M. J.; De Deurwaerdère, P., Serotonin2C ligands exhibiting full negative and positive intrinsic activity elicit purposeless oral movements in rats: distinct effects of agonists and inverse agonists in a rat model of Parkinson's disease. *The International Journal of Neuropsychopharmacology* **2012,** *16* (03), 593-606.
17. 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* (3), 513-523.

18. Steed, E.; Jones, C. A.; Mccreary, A. C., Serotonergic involvement in methamphetamine-induced locomotor activity: A detailed pharmacological study. *Behav. Brain. Res.* **2011,** *220* (1), 9-19.
19. Farrell, M.; Rosenzweig-Lipson, S.; Walker, E., Discriminative stimulus effects of serotonin agonists, neutral antagonists, and inverse agonists in pigeons: perspectives on intrinsic efficacy measurements in vivo. *Psychopharmacology* **2010,** *211* (2), 149-159.
20. Zaniewska, M.; Mccreary, A. C.; Wydra, K.; Filip, M., Effects of serotonin (5-HT)2 receptor ligands on depression-like behavior during nicotine withdrawal. *Neuropharmacology* **2010,** *58* (7), 1140-1146.
21. Navarra, R.; Comery, T. A.; Graf, R.; Rosenzweig-Lipson, S.; Day, M., The 5-HT(2C) receptor agonist WAY-163909 decreases impulsivity in the 5-choice serial reaction time test. *Behav. Brain. Res.* **2008,** *188* (2), 412-415.
22. Rosenzweig-Lipson, S.; Sabb, A.; Stack, G.; Mitchell, P.; Lucki, I.; Malberg, J. E.; Grauer, S.; Brennan, J.; Cryan, J. F.; Sukoff Rizzo, S. J.; Dunlop, J.; Barrett, J. E.; Marquis, K. L., Antidepressant-like effects of the novel, selective, 5-HT2C receptor agonist WAY-163909 in rodents. *Psychopharmacology* **2007,** *192* (2), 159-170.
23. Dunlop, J.; Marquis, K.; Lim, H.; Leung, L., Pharmacological Profile of the 5‐HT2C Receptor Agonist WAY‐163909; Therapeutic Potential in Multiple Indications. *CNS Drug Reviews* **2006,** *12* (3-4), 167-177.
24. Marquis, K. L.; Sabb, A. L.; Logue, S. F.; Brennan, J. A.; Piesla, M. J.; Comery, T. A.; Grauer, S. M.; Ashby, C. R.; Nguyen, H. Q.; Dawson, L. A.; Barrett, J. E.; Stack, G.; Meltzer, H. Y.; Harrison, B. L.; Rosenzweig-Lipson, S., WAY-163909 [(7bR,10aR)-1,2,3,4,8,9,10,10a-octahydro-7bH-cyclopenta-[b][1,4]diazepino[6,7,1hi]indole]: A novel 5-hydroxytryptamine 2C receptor-selective agonist with preclinical antipsychotic-like activity. *The Journal of pharmacology and experimental therapeutics* **2007,** *320* (1), 486-496.
25. Dunlop, J., Sabb, A. L.., Mazandarani, H., Zhang, J., Kalgaonker, S., Shukhina, E., Sukoff, S., Vogel, R., Stack, G., Schechter, L., Harrison, B. L., Rosenzweig-Lipson, S., WAY-163909 [(7bR,10aR)-1,2,3,4,8,9,10,10a-Octahydro-7bHcyclopenta-[b][1,4]diazepino[6,7,1hi]indole], a Novel 5-Hydroxytryptamine 2C Receptor-Selective Agonist with Anorectic Activity. *J. Pharmacol. Exp. Ther.* **2005,** *313*, 862-869.
26. Welmaker, G. S.; Sabalski, J. E., A process for the preparation of 1,2,3,4,8,9,10,10a-octahydro-7bH-cyclopenta[b][1,4]diazepino[6,7,1-hi]indole. *Tetrahedron Lett* **2004,** *45* (25), 4851-4854.
27. Sabb, A. L.; Vogel, R. L.; Nelson, J. A.; Rosenzweig-Lipson, S. J.; Welmaker, G. S.; Sabalski, J. E.; Smith, M. D.; Chan, A. W.-Y.; Antane, M. M.; Raveendranath, P.; Megati, S. Preparation of cyclopenta[b][1,4]diazepino[6,7,1-hi]indoles as selective 5-HT2c receptor agonists. May 30, 2002.
28. Sabb, A. L.; Vogel, R. L.; Welmaker, G. S.; Sabalski, J. E.; Coupet, J.; Dunlop, J.; Rosenzweig-Lipson, S.; Harrison, B., Cycloalkyl[b][1,4]benzodiazepinoindoles are agonists at the human 5-HT2C receptor. *Bioorg. Med. Chem. Lett.* **2004,** *14* (10), 2603-2607.
29. Seitz, P. K.; Bremer, N. M.; McGinnis, A. G.; 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.
30. 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* (7), 538-545.
31. 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.; 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* (4), 1615-1630.
32. Ratkowsky, D. A.; Reedy, T. J., Choosing near-linear parameters in the four-parameter logistic model for radioligand and related assays. *Biometrics* **1986,** *42*, 575-582
.
33. Tallarida, R. J., and Murray, R.B, *Manual of Pharmacologic Calculations with Computer Programs*. 2nd ed.; Springer-Verlag: New York, 1986.