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

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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*.

Graphical Abstract

Keywords

Serotonin; 5-HT_{2C} receptor agonist; WAY163909 derivatives

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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.

Conflicts of Interest

The authors declare no competing financial interests.

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-HT₂R 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.

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.

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.

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 (EC₅₀ >10 μM) in the Ca_i⁺⁺ assay (data not shown).

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.

The product from 2-amino-5-methoxy-benzoic acid, (**14**) proved to be less potent (EC₅₀ ~1.5 μM) relative to WAY163909 (EC₅₀~18.4 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 (EC₅₀ ~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.

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 μ M to determine their ability to displace [¹²⁵I]-(±)-2,5-dimethoxy-4-iodoamphetamine ([¹²⁵I]-(±)-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 [¹²⁵I]-(±)-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).

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 μ M) displaced 100% of [¹²⁵I]-(±)-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 (**33**) will be used.

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}-INI R-U2OS cells were grown in Assay Complete™ U2OS Medium 31 (DiscoveRx) at 37°C, 5% CO₂ 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 ($\lambda_{\text{ex}} = 485 \text{ nm}$, $\lambda_{\text{em}} = 525 \text{ nm}$) 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 5× 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 EC₅₀ (concentration of compound required to achieve half-maximal response). The EC₅₀ 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 EC₅₀ 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 μ M 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-1H-cyclopenta[b][1,4]diazepino[6,7,1-h]indole (racemate of 32/33)—¹H NMR (500 MHz, CDCl₃) 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). ¹³C NMR (126 MHz, CDCl₃) 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 C₁₅H₂₀N₂O [M+H]⁺: 245.1648; found: 245.1650.

7-(2-(prop-2-ynyloxy)ethoxy)-2,3,4,7b,8,9,10,10a-octahydro-1H-cyclopenta[b][1,4]diazepino[6,7,1-h]indole (40)—¹H NMR (500 MHz, CDCl₃) 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). ¹³C NMR (126 MHz, CDCl₃) 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 C₂₃H₃₂N₂O₄ [M+H]⁺: 401.2435; found: 401.2440.

7-(2-(2-(prop-2-ynyloxy)ethoxy)ethoxy)-2,3,4,7b,8,9,10,10a-octahydro-1H-cyclopenta[b][1,4]diazepino[6,7,1-h]indole (41)—¹H NMR (500 MHz, CDCl₃) 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). ¹³C NMR (126 MHz, CDCl₃) 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 C₂₁H₂₈N₂O₃ [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-1H-cyclopenta[b][1,4]diazepino[6,7,1-h]indole (42)—¹H NMR (500 MHz, CDCl₃) 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). ¹³C NMR (126 MHz, CDCl₃) 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 C₂₃H₃₂N₂O₄ [M+H]⁺: 401.2435; found: 401.2440.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Figure 2.

Figure 3. Representative intracellular calcium release response of WAY163909 derivatives at human 5-HT_{2C}R compared with WAY163909. The E_{max} and EC₅₀ of these compounds are listed in Table 1.

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.

Scheme 1.

Reagents and conditions: (i) a. 6N NaOH, 100 °C, 12 h, b. glycine, 6N NaOH, H₂O, reflux, 12 h, then L-tartaric acid, 100 °C, 2 h, 86%; (ii) a. LAH, THF, 24 h, 93%; b. CbzCl, Et₃N, CH₂Cl₂, rt, 18 h, 88%; (iii) a. NaNO₂, 1,4-dioxane, b. 1N HCl, rt, 24 h, 98%; c. TiCl₄, Mg, CH₂Cl₂, Et₂O, rt, 0.75 h, 97% crude product; (iv) ketone **8**, acetic acid, reflux, 18 h, 64%; (v) a. NaH, CH₃I, DMF, 0 °C-rt 16h; b. NaBH₄, TFA, 18 h, rt, 78%; c. 10% Pd/C, H₂, EtOH, rt, 48 h, 88%; (vi) 4-(methoxy)cyclohexanone, acetic acid, reflux, 16 h, 45%; (vii) a. NaBH₄, TFA, 18 h, rt, 70%; b. 10% Pd/C, H₂, EtOH, rt, 48 h 42%.

Scheme 2.

Reagents and conditions: (i) a. triphosgene, THF, rt, 18 h, 97%; b. glycine, 6N NaOH, H₂O, reflux, 12 h, then L-tartaric acid, reflux, 2 h, 77%; (ii) a. LAH, THF, reflux, 48 h, 98%; b. benzyl chloroformate, Et₃N, CH₂Cl₂, rt, 18 h, 77%; (iii) NaNO₂, 1N HCl, H₂O, 1,4-dioxane, rt, 15 min, 99%; (iv) TiCl₄, Mg, CH₂Cl₂, diethyl ether, rt, 15 min, 99% crude product; (v) cyclopentanone, *p*-TSA, toluene, reflux, 3 h, 48% over steps iv and v; (vi) a. NaBH₄, TFA, rt, 10 min, 99%; b. 10% Pd/C, H₂, MeOH, rt, 3 h, 99%. Steps for **14** are the same as **15**. Yields and details are reported in the supporting information.

Scheme 3.

Scheme 4.

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 _{7c}	Cpd #	Structure	EC ₅₀ (nM) ^a (95% C.I.)	E _{max} b _{7c}
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	103 ± 3.8	41 (±)		236.7	96.8 ± 5.2

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}
24 (±)		(1.3–3.1)	ND	42 (±)		(37–437)	94 ± 2.5
25 (±)		>10 μM	ND			647.3 (271–1024)	

^aEC₅₀ is presented as the mean; 95% confidence interval (CI) is presented as the range

^bE_{max} is presented as mean ± SEM; % Ca²⁺ release of compounds (except 5-HT) normalized to 1 μM of WAY163909 (1)

^cND = not determined

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 AlCl₃ were premixed for 1 hour at 0 °C

Table 3Selectivity profile of WAY163909 derivatives at 5-HT₂R

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-HT₂ Compounds were tested at a single concentration of 10 μ M 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.