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Optimization of 2-Phenylcyclopropylmethylamines as Selective Serotonin 2C Receptor Agonists and Their Evaluation as Potential Antipsychotic Agents

Jianjun Cheng,[†] Patrick M. Giguère,[‡] Oluseye K. Onajole,^{†,#} Wei Lv,[†] Arsen Gaisin,^{†,▽} Hendra Gunosewoyo,^{†,○} Claire M. Schmerberg,[§] Vladimir M. Pogorelov,[§] Ramona M. Rodriguez,[§] Giulio Vistoli,^{||} William C. Wetsel,[⊥] Bryan L. Roth,[‡] and Alan P. Kozikowski*,[†]

[†]Drug Discovery Program, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, Illinois 60612, United States

[‡]National Institute of Mental Health Psychoactive Drug Screening Program, Department of Pharmacology and Division of Chemical Biology and Medicinal Chemistry, University of North Carolina Chapel Hill Medical School, Chapel Hill, North Carolina 27599, United States

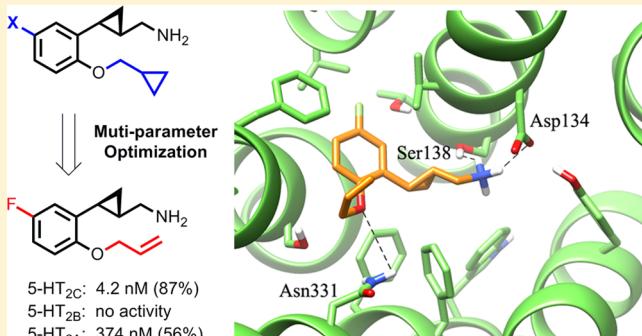
[§]Department of Psychiatry and Behavioral Sciences, Mouse Behavioral and Neuroendocrine Analysis Core Facility, Duke University Medical Center, Durham, North Carolina 27710, United States

^{||}Dipartimento di Scienze Farmaceutiche, Università degli Studi di Milano, Via Mangiagalli 25, 20133 Milano, Italy

[⊥]Departments of Psychiatry and Behavioral Sciences, Cell Biology, and Neurobiology, Mouse Behavioral and Neuroendocrine Analysis Core Facility, Duke University Medical Center, Durham, North Carolina 27710, United States

S Supporting Information

ABSTRACT: The discovery of a new series of compounds that are potent, selective 5-HT_{2C} receptor agonists is described herein as we continue our efforts to optimize the 2-phenylcyclopropylmethylamine scaffold. Modifications focused on the alkoxy substituent present on the aromatic ring led to the identification of improved ligands with better potency at the 5-HT_{2C} receptor and excellent selectivity against the 5-HT_{2A} and 5-HT_{2B} receptors. ADMET studies coupled with a behavioral test using the amphetamine-induced hyperactivity model identified four compounds possessing drug-like profiles and having antipsychotic properties. Compound (+)-16b, which displayed an EC₅₀ of 4.2 nM at 5-HT_{2C}, no activity at 5-HT_{2B}, and an 89-fold selectivity against 5-HT_{2A}, is one of the most potent and selective 5-HT_{2C} agonists reported to date. The likely binding mode of this series of compounds to the 5-HT_{2C} receptor was also investigated in a modeling study, using optimized models incorporating the structures of β₂-adrenergic receptor and 5-HT_{2B} receptor.



INTRODUCTION

Serotonin or 5-hydroxytryptamine (5-HT) is a major neurotransmitter that is primarily found in the gastrointestinal tract, platelets, and the central nervous system (CNS). It is believed to be involved in the regulation of a variety of physiological functions such as intestinal movements, mood, cognition, and appetite.¹ These functions are mediated through serotonin receptors, which belong to the G-protein coupled receptor (GPCR) superfamily and are composed of seven subfamilies (5-HT_{1–7}) and 14 isoforms.²

Recently, the serotonin 2C (5-HT_{2C}) receptor has been shown to be a promising drug target for the treatment of a variety of CNS disorders, including obesity and mental disorders such as schizophrenia, depression, and anxiety.^{3–7} One of the many advantages of the 5-HT_{2C} receptor as a CNS

drug target stems from the fact that it is found almost exclusively in the CNS,^{8,9} and thus compounds that selectively activate this receptor should have limited impact on peripheral tissues. However, the activation of two other closely related 5-HT₂ subtypes, namely the 5-HT_{2A} and 5-HT_{2B} receptors, has been reported to be associated with hallucinations and cardiac valvulopathy, respectively.¹⁰ Therefore, the identification of ligands possessing exquisite selectivity against the 5-HT_{2A} and 5-HT_{2B} receptors is a key criterion for the therapeutic advancement of 5-HT_{2C} agonists. The achievement of this goal has been challenging due to the high conservation of

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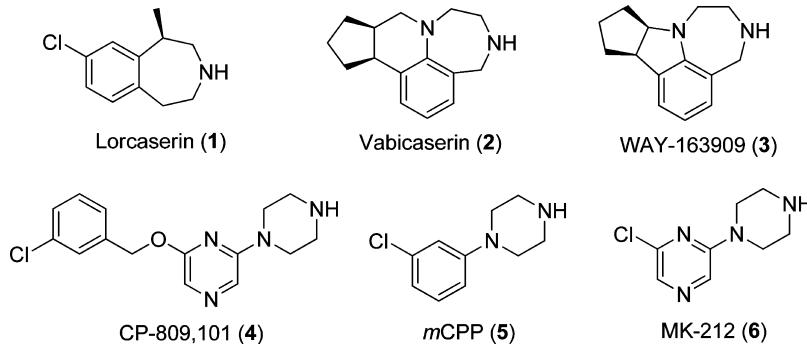


Figure 1. Representative 5-HT_{2C} agonists.

molecular determinants involved in ligand recognition within this subfamily of receptors.¹¹

Currently, there are a number of 5-HT_{2C} agonists (Figure 1, 1–6) generated for potential therapeutic uses or as chemical tools for the study of the biological roles of 5-HT_{2C} receptor. Among them, lorcaserin (Belviq, 1) was approved by the FDA in 2012 for the treatment of obesity. Although it was reported to have 100-fold selectivity for 5-HT_{2C} relative to the 5-HT_{2B} subtype, lorcaserin possesses full agonist activity at 5-HT_{2B} ($EC_{50} = 943 \pm 90$ nM, $E_{max} = 100\%$).¹² Hence, it is not surprising that lorcaserin was found to cause a higher incidence of cardiac valve disorders in clinical trials compared to the placebo group.¹³

Vabicaserin (SCA-136, 2), by targeting 5-HT_{2C} receptors ($EC_{50} = 8$ nM, $E_{max} = 100\%$), was tested in clinical trials for the treatment of schizophrenia;¹⁴ however, it displayed moderate efficacy on 5-HT_{2B} receptors ($E_{max} = 50\%$) and good potency ($EC_{50} = 12$ or 102 nM depending on receptor densities).¹⁵ WAY-163909 (3), an analogue of vabicaserin, was shown to have selectivity toward 5-HT_{2C} ($EC_{50} = 8$ nM; $E_{max} = 90\%$) while possessing no agonist activity at 5-HT_{2A} and weak efficacy at 5-HT_{2B} receptors ($E_{max} = 40\%$); it has proven to have good preclinical antipsychotic-like activity in several animal models.¹⁶ Compound CP-809,101 (4) is one of the most selective and potent 5-HT_{2C} ($EC_{50} = 0.11$ nM, $E_{max} = 93\%$) ligands developed, with about 600-fold 5-HT_{2C} selectivity against 5-HT_{2B}. However, it is still relatively potent at 5-HT_{2B} ($EC_{50} = 65.3$ nM, $E_{max} = 57\%$).¹⁷ Because of the genotoxicity of this compound, it could not be advanced to clinical evaluation.¹⁸ Nonetheless, CP-809,101 has structural similarities to mCPP (5) and MK-212 (6), two compounds discovered decades ago and used as tools for the pharmacological study of 5-HT_{2C} receptors.^{19,20}

In our own research, we focused on compounds that possessed the 2-phenylcyclopropylmethylamine scaffold (7) to develop selective 5-HT_{2C} agonists. This particular scaffold was derived from an initial high throughput screening (HTS) screening campaign in which tranylcypromine was identified as a hit as described in an earlier publication.²¹ Rational drug design principles coupled with the evolution of a body of structure–activity relationships (SARs) led to the identification of compounds possessing a 2-cyclopropylmethoxy group at position 2, as illustrated by compounds 8, 9, and 10 (Figure 2). Compound 8, which possesses a fluorine substitution at position 5 of the benzene ring, showed good potency on the 5-HT_{2C} receptor ($EC_{50} = 21$ nM), with only moderate selectivity for 5-HT_{2B} ($EC_{50} = 289$ nM).²² The replacement of the fluorine atom with a hydroxyl group, as in compound 9,

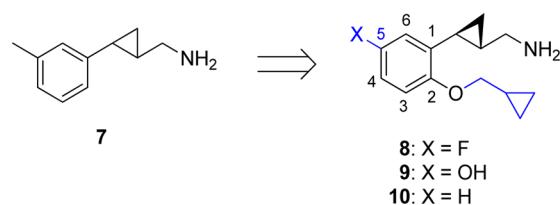


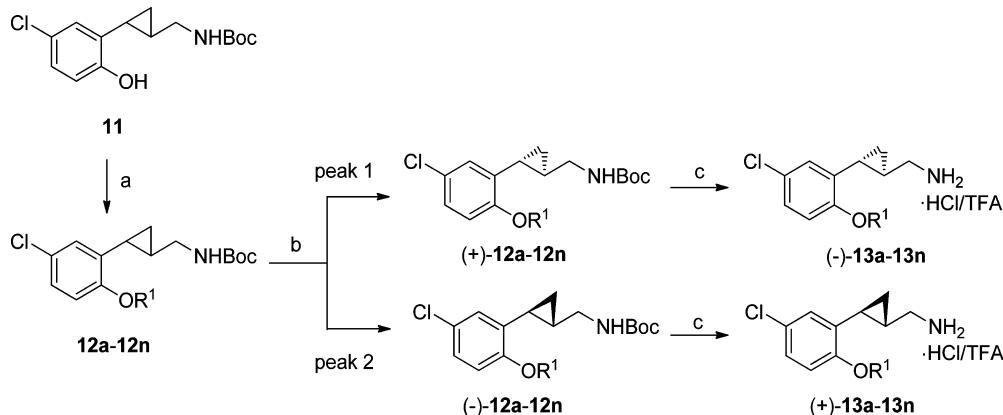
Figure 2. Selective 5-HT_{2C} agonists based on 2-phenylcyclopropylmethylamine scaffold.

led to the enhancement of both potency and selectivity, but the bioavailability of compound 9 was found to be too low in a later study ($F = 3.2\%$ in mice, unpublished data). Compound 10, with no substitution at the same position, showed good potency as a partial agonist ($EC_{50} = 55$ nM, $E_{max} = 61\%$), with great selectivity against both 5-HT_{2A} and 5-HT_{2B}.²³ Considering the needs of more selective and potent 5-HT_{2C} agonists for the validation of their therapeutic potential in the treatment of various illnesses associated with 5-HT_{2C}, we conducted a new round of lead optimization on this promising scaffold. The chemical synthesis and SAR results obtained for the new compounds are reported herein, together with a rodent behavioral test and a homology modeling study. These new analogues provide improvements compared to previous compounds.

RESULTS

Design and Synthesis of 5-Chlorine Substituted Compounds. Prior SAR studies showed that compounds with an alkoxy group at position 2 provided the most favorable 5-HT_{2C} agonism and selectivity toward 5-HT_{2A} and 5-HT_{2B}, and thus this substitution pattern was therefore retained in further rounds of structural modifications. Through a careful structural analysis of known 5-HT_{2C} agonists as well as other CNS drugs, it is apparent that a chlorine atom represents one of the most frequently used substituents for aromatic rings. This observation is exemplified by mCPP, MK-212, and lorcaserin, all of which contain a chlorine substituent located at a position *meta* to the nitrogen-containing group. Thus, we introduced the chlorine atom as the substituent at position 5, and further optimization of the alkyl ether group was conducted to improve 5-HT_{2C} potency as well as selectivity over both 5-HT_{2A} and 5-HT_{2B}.

As depicted in Scheme 1, the synthesis of the 5-chlorine containing analogues started from the corresponding phenol 11, which was prepared using methods similar to those reported previously by us for the 5-bromo analogue.²³ The cyclopropane ring of 11 was prepared in its *trans* configuration,

Scheme 1. Synthesis of 5-Chloro Compounds^a

^aReagents and conditions: (a) Williamson ether synthesis with R^1X or Mitsunobu reaction with appropriate alcohols; (b) chiral prep-HPLC separation; (c) $13c$, $13d$, $13f$, $13h$, and $13i$, TFA/CH_2Cl_2 , rt, 1h; $13a$, $13b$, $13e$, $13g$, $13j$ – $13n$, 2 M HCl in diethyl ether, rt, 24–48 h.

as this stereochemistry has proven to be favored in our previous SAR studies. The ether derivatives **12a**–**12n** were prepared either by a standard Williamson ether synthesis with an alkyl halide or by use of the Mitsunobu reaction with the appropriate alcohol (for details of the synthesis of **12a**–**12n**, see Supporting Information, Table S1). Chiral separations of **12a**–**12n** were achieved with preparative chiral HPLC and the corresponding (+)- and (−)-enantiomers were then subjected to Boc deprotection reactions to provide both (−)- and (+)-enantiomers of compounds **13a**–**13n** as their HCl or TFA salts. The absolute configurations of the enantiomers were assigned from the measured optical rotations coupled with the stereochemical correlations reported previously by us.²¹

On the basis of the SAR data from our previous work, we hypothesized that the alkoxy substituents at position 2 on the benzene ring might be accommodated within a hydrophobic cavity, which could vary in size among the 5-HT_{2C}, 5-HT_{2A}, and 5-HT_{2B} receptors. This cavity might be slightly larger for the 5-HT_{2C} receptors compared to the 5-HT_{2A} and 5-HT_{2B} receptors based on the observation that (1) small groups attached to position 2 displayed good activity at all three subtypes (less selectivity) and (2) increasing the size of the substituent resulted in a decrease in activity at 5-HT_{2A} and 5-HT_{2B} while retaining good activity at 5-HT_{2C}. Accordingly, we believed that by achieving a proper balance of size and lipophilicity, we should be able to identify more potent 5-HT_{2C} agonists with improved selectivity against the 5-HT_{2A} and 5-HT_{2B} subtypes.

To explore this assumption, compounds with varied alkoxy groups were designed and synthesized. Functional activities at the 5-HT_{2C}, 5-HT_{2A}, and 5-HT_{2B} receptors were determined, and physiochemical properties related to possible CNS penetration such as the CLogP and LogBB values were calculated using the ACD Percepta program. These values are listed in Table 1. Generally, the (+)-isomers of all new compounds were found to be more potent than the (−)-isomers, which is consistent with our previously published SAR data.²³ As evidenced by compounds **13a**, **13b**, and **13c**, potency at the 5-HT_{2C} receptor decreases with elongation of the alkyl chain, while selectivity over 5-HT_{2B} and 5-HT_{2A} improves. *n*-Propyl substitution as in compound (+)-**13b** was favored with respect to both activity and selectivity. The change from an *n*-propyl group to an isopropyl group gave compound (+)-**13d** that has similar 5-HT_{2C} activity but decreased selectivity against 5-HT_{2B} (33-fold selectivity for (+)-**13b** vs

11-fold selectivity for (+)-**13d**). The cyclopropylmethoxy group was the preferred ether substituent at position 2 of the benzene ring based on our previous findings;^{22,23} however, the use of this group as found in compound (+)-**13e** afforded only a moderate potency at 5-HT_{2C} receptors ($EC_{50} = 251$ nM, $E_{max} = 75\%$). Incorporation of an additional heteroatom such as oxygen or sulfur into the ether appendage, as exemplified by compounds **13f** and **13g**, led to a large reduction in 5-HT_{2C} activity as well as a predicted decrease in blood–brain barrier (BBB) penetration based upon the calculated LogBB values.

Unsaturated alkyl groups, such as allyl and propargyl, were preferred both for their potency and selectivity, as revealed by compounds (+)-**13h** and (+)-**13i**. (+)-**13h** showed a moderately good pharmacological profile, with 60 nM potency at 5-HT_{2C}, 37-fold selectivity against 5-HT_{2B} (2211 nM), and no activity at 5-HT_{2A}. Furthermore, good physiochemical properties were predicted for this compound. Similar potency and selectivity was observed for (+)-**13i** as well, however, it was predicted to have low brain penetration, as indicated by its LogBB value of −0.43. Allyl ethers bearing small modifications were also explored, as exemplified by compounds **13j** and **13k**. The presence of a fluorine substituent as in compound (+)-**13j** resulted in a 2-fold reduction in 5-HT_{2C} activity compared to (+)-**13h** (133 vs 60 nM) while maintaining the same general selectivity profile against 5-HT_{2B} and 5-HT_{2A}. The presence of the fluorine atom might provide some advantage in terms of pharmacokinetics due to the possibility of reducing oxidative metabolism of the double bond. By adding an additional methyl group to the position 2 of the allyl appendage as in compound (+)-**13k**, the potency was reduced to 212 nM at the 5-HT_{2C} receptors while its selectivity also decreased in comparison to compounds **13h** and **13j**.

The 2-fluoroethyl ether analogue (+)-**13l** displayed 32 nM potency at 5-HT_{2C} and good selectivity against both 5-HT_{2A} and 5-HT_{2B}. Upon introducing one additional fluorine atom into the molecule by way of a 2,2-difluoroethyl appendage as in analogue **13m**, a 3-fold decrease in activity at 5-HT_{2C} ($EC_{50} = 91$ nM vs 32 nM) was observed. This drop in activity may result from either increased steric hindrance or electronic effects. Additionally, the incorporation of a 3-fluoropropyl ether appendage as in (+)-**13n** also led to a drop in potency ($EC_{50} = 206$ nM) compared to the simple unsubstituted propyl derivative (+)-**13b**, although (+)-**13n** possessed a reasonably good selectivity profile.

Table 1. 5-HT_{2C} Activity and Selectivity Profiles of Compounds 13a–13n and Their Predicted Physiochemical Properties^{a,b}

R ¹	Compound	EC ₅₀ , nM (E _{max})			CLogP	LogBB
		5-HT _{2C}	5-HT _{2B}	5-HT _{2A}		
-	5-HT	0.21 (100%)	0.92 (100%)	1.88 (100%)	-	-
-	lorcaserin	3.6 (99%)	478 (92%)	302 (68%)	-	-
	(-)13a	646 (72%)	2307 (26%)	NA	2.50	-0.02
	(+)-13a	13 (91%)	86 (45%)	1215 (49%)		
	(-)13b	1228 (31%)	3004 (21%)	NA	2.94	0.23
	(+)-13b	103 (72%)	3436 (25%)	NA		
	(-)13c	683 (50%)	NA	NA	3.36	0.42
	(+)-13c	288 (33%)	NA	NA		
	(-)13d	2481 (37%)	5660 (19%)	NA	2.84	0.15
	(+)-13d	91 (86%)	997 (37%)	4002 (19%)		
	(-)13e	1082 (51%)	3034 (32%)	NA	2.87	0.23
	(+)-13e	251 (75%)	5012 (20%)	NA		
	(-)13f	667 (45%)	5300 (32%)	NA	2.11	-0.22
	(+)-13f	183 (8%)	NA	NA		
	(-)13g	1762 (11%)	NA	NA	2.87	-0.06
	(+)-13g	244 (24%)	NA	NA		
	(-)13h	737 (42%)	4295 (28%)	NA	2.69	0.07
	(+)-13h	60 (80%)	2211 (32%)	NA		
	(-)13i	365 (71%)	4411 (30%)	NA	2.17	-0.43
	(+)-13i	63 (65%)	4602 (29%)	1005 (21%)		
	(-)13j	NA	6877 (33%)	NA	2.98	0.16
	(+)-13j	133 (81%)	1688 (27%)	NA		
	(-)13k	NA	6094 (39%)	NA	3.23	0.37
	(+)-13k	212 (90%)	422 (32%)	903 (26%)		
	(-)13l	1127 (59%)	4521 (31%)	NA	2.23	0.01
	(+)-13l	32 (86%)	632 (31%)	1476 (35%)		
	(-)13m	914 (64%)	1485 (30%)	1305 (43%)	2.43	0.11
	(+)-13m	91 (81%)	839 (27%)	1811 (31%)		
	(-)13n	1258 (24%)	6166 (22%)	NA	2.48	0.07
	(+)-13n	206 (60%)	4911 (22%)	NA		

^aFunctional data were acquired with recombinant, stably expressed human serotonin receptors in the HEK-293 cell line, using a fluorescence imaging plate reader (FLIPR) assay; "NA", no activity at 10 μ M. ^bCLogP and LogBB values were calculated for the free bases using the ACD Percepta program.

On the basis of the data from Table 1, compounds (+)-13h and (+)-13l represent the best ligands among this series of compounds, but they are about 10-fold less potent than lorcaserin, which shows an EC₅₀ value of 3.6 nM on 5-HT_{2C} in the functional calcium-based assay. Although lorcaserin displays around 100-fold selectivity over both 5-HT_{2B} and 5-HT_{2A}, it acts as a full or partial agonist at both receptors. As such, lorcaserin has potential side effect issues as discussed previously. Compounds (+)-13h and (+)-13l, along with (+)-13j, which might provide better PK properties due to the presence of the fluorine substitution, were advanced to in vitro ADMET studies.

ADMET Studies. Compounds (+)-13h, (+)-13j, and (+)-13l were evaluated for selected in vitro ADME properties and toxicity profiles (Table 2). In Caco-2 permeability studies,

Table 2. In Vitro ADMET Data for Compounds (+)-13h, (+)-13j, and (+)-13l^a

compd	(+)-13h	(+)-13j	(+)-13l
Caco-2 permeability (pH 7.4, $\times 10^{-6}$ cm/s)	A-B	35.6	38.3
	B-A	9.8	10.8
microsomal stability (%)	human	52	80
	mouse	60	95
human PPB at 10 μ M (%)		75	68
CYP inhibition at 10 μ M (%)	3A	27	22
	2D6	91	80
	2C9	56	51
	2C19	32	33
	2B6	86	58
hERG IC ₅₀ (μ M)		0.27	3.98
Ames fluctuation test	negative	negative	negative

^aThis study was conducted at Cerep, Inc. (Redmond, USA); microsomal stability is presented as % compound remaining after 1 h incubation with human/mouse liver microsomes; the CYP inhibition assays were performed using human liver microsomes; midazolam, dextromethorphan, diclofenac, omeprazole, and bupropion were used as test substrates for the 3A, 2D6, 2C9, 2C19, and 2B6 isoforms, respectively; hERG inhibition was tested on CHO cells using the automated patch-clamp method; the Ames fluctuation test was performed with the TA98, TA100, TA1535, and TA1537 strains, with/without S9, at concentrations of 10, 50, and 100 μ M.

compounds (+)-13h, (+)-13j, and (+)-13l displayed greater than 35×10^{-6} cm/s A-B permeability in pH 7.4 buffer solution, and A-B/B-A ratios of 3.6, 3.5, and 2.0, respectively. When incubated with human and CD-1 mouse liver microsomes, all three compounds retained reasonably good stability, with more than 50% compound remaining after 1 h. Interestingly, although compound (+)-13h and (+)-13j displayed similar stability in the mouse liver microsomes test (60% vs 62%), (+)-13j had much better stability in the human microsomes study compared to (+)-13h (80% vs 52%), which could be due to the presence of the fluorine substitution as expected. Relatively low human plasma protein binding (57–75%) was measured for compounds (+)-13h, (+)-13j, and (+)-13l, which was expected based on their good CLogP values

(between 2 and 3 as in Table 1), as Log P is an important indicator that determines protein binding properties.²⁴

Human cytochrome P450 (CYP) enzyme inhibition studies were conducted using the five most important isoforms, 3A, 2D6, 2C9, 2C19, and 2B6, at compound concentrations of 10 μ M. Similar profiles were observed for all three compounds, with low inhibition of CYP3A and CYP2C19 and relatively higher inhibition of the other three isoforms (>50%). Compound (+)-13j shows a slightly better profile compared to (+)-13h, which indicates another benefit of the fluorine substituent. In the hERG inhibition test with the automated patch-clamp assay, moderate inhibition was observed with compounds (+)-13j and (+)-13l, while (+)-13h was found to have an IC₅₀ of 0.27 μ M. The Ames test was also conducted to preclude any mutagenic potential of these compounds; all three compounds showed negative results. Because of the high hERG activity of (+)-13h, this compound was not further evaluated. Thus, only compounds (+)-13j and (+)-13l were submitted to the in vivo brain penetration studies.

Brain penetration studies of (+)-13j and (+)-13l were conducted with CD1 mice, and the results are listed in Table 3.

Table 3. Brain Penetration Study of Compounds (+)-13j and (+)-13l in CD1 Mice^a

compd	time-point (h)	brain concentration (ng/g)	plasma concentration (ng/mL)	ratio (brain/plasma) ^b
(+)-13j	0.5	17460	1445	12.1
	2	14670	903	16.2
(+)-13l	0.5	12795	1200	10.7
	2	13350	874	15.3

^aCD1 mice were administrated a single dose of both compounds (10 mg/kg, ip, formulation in pure water). ^bDensity of mouse brain tissue was calculated as 1 g/mL.

Excellent brain penetration was observed for both compounds, with brain concentrations of greater than 50 μ M achieved at a dose of 10 mg/kg (ip) and with brain/plasma ratios over 10 at both time-points (0.5 and 2 h). The excellent brain penetration for these two compounds coupled with low plasma protein binding should ensure optimal CNS distribution of both compounds. The lower plasma concentrations would also help

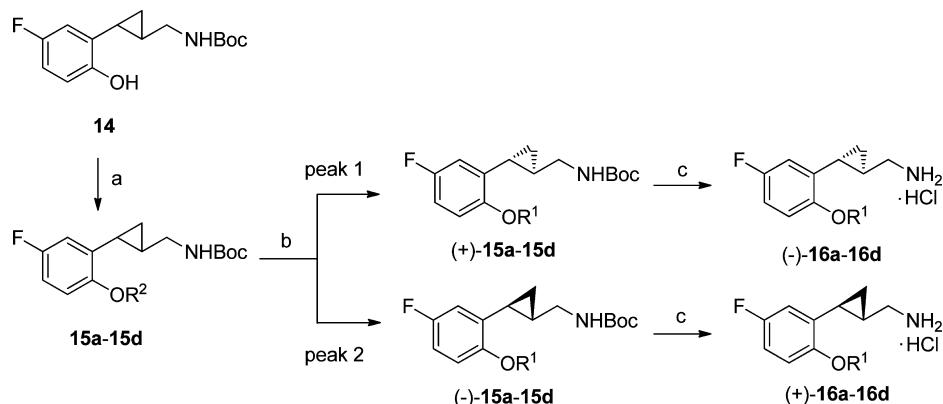
to mitigate possible cardiac toxicity related to hERG inhibition observed in the previous ADMET studies.

While both compounds (+)-13j and (+)-13l showed good pharmacological profiles, acceptable ADMET properties, and excellent brain penetration, their moderate hERG inhibition could be a concern for further development. A similarity analysis of their structures with known hERG inhibitors revealed that the scaffold embodied by this series of compounds matched one of the common structural templates known to cause hERG inhibition, which is comprised of a three-point pharmacophore containing an aromatic ring, an adjacent hydrogen-bond donor, and an ionizable N atom.²⁵ However, the 5-fluoro containing compound 8 displayed no hERG inhibition in our previous studies.²² Thus, replacement of the chlorine atom by the less lipophilic fluorine atom at position 5 might lead to a decrease in hERG inhibition. Moreover, from the SAR data gleaned from the study of the 5-chloro bearing compounds, we concluded that some of the alkoxy substitutions at position 2 on the benzene ring may provide advantages over the cyclopropylmethoxy group found in compounds 13e and 8 in terms of potency and selectivity. Therefore, the introduction of these substituents into the 5-fluoro containing scaffold might produce compounds with better potency and, more importantly, reduced hERG inhibitory activity. Four different substituents from Table 1 were chosen for this purpose, and the synthesis and evaluation of these compounds are described below.

Routes to the 5-fluoro compounds are depicted in Scheme 2. 5-Fluorophenol 14 was prepared and used as the starting material, the synthesis of which has been reported previously.²² Alkylation of 14 with 1-iodopropane, allyl bromide, or 3-chloro-2-fluoropropene and a Mitsunobu reaction with 2-fluoroethanol provided the corresponding ethers 15a–15d in excellent yields (for details of the synthesis of 15a–15d, see Supporting Information, Table S2). These intermediates were separated using chiral preparative HPLC and then deprotected with HCl in diethyl ether to give both (−)- and (+)-enantiomers of compound 16a–16d. Similarly, absolute configurations of the enantiomers were assigned based on the measured optical rotations as described above.

Pharmacological profiles of the 5-fluoro compounds are summarized in Table 4. All (+)-enantiomers had better efficacy than their (−)-enantiomers. However, a significant increase in

Scheme 2. Synthesis of 5-Fluoro Compounds^a



^aReagents and conditions: (a) Williamson ether synthesis with R¹X or Mitsunobu reaction with appropriate alcohols; (b) chiral prep-HPLC separation; (c) 2 M HCl in diethyl ether, rt, 24 h, 64–80%.

Table 4. Efficacy and Selectivity Data of Compound 16a–16d^{a,b}

R ²	Compound	EC ₅₀ , nM (E _{max})			CLogP	LogBB
		5-HT _{2C}	5-HT _{2B}	5-HT _{2A}		
-	5-HT	0.21 (100%)	0.92 (100%)	1.88 (100%)	-	-
-	lorcaserin	3.6 (99%)	478 (92%)	302 (68%)	-	-
	(-)-16a	296 (80%)	735 (11%)	NA	2.35	0.24
	(+)-16a	11 (88%)	1994 (33%)	1025 (15%)		
	(-)-16b	157 (87%)	NA	697 (56%)	2.50	-0.02
	(+)-16b	4.2 (87%)	NA	374 (56%)		
	(-)-16c	985 (62%)	NA	NA	2.48	0.23
	(+)-16c	22 (91%)	NA	1666 (17%)		
	(-)-16d	514 (77%)	NA	2994 (20%)	2.94	0.23
	(+)-16d	3.4 (89%)	NA	359 (76%)		

^aFunctional data were acquired with recombinant, stably expressed human 5-HT receptors in the HEK-293 cell line, using a fluorescence imaging plate reader (FLIPR) assay; “NA”, no activity at 10 μ M. ^bCLogP and LogBB values were calculated for the free bases using the ACD Percepta program.

potency at the 5-HT_{2C} receptor was observed for these compounds compared to their 5-chloro analogues, while excellent selectivity was maintained. Compound (+)-16a, bearing an *n*-propyl group, displayed an EC₅₀ value of 11 nM at 5-HT_{2C} and was 9-fold more potent than its 5-chloro analogue (+)-13b (EC₅₀ = 103 nM). It showed a high degree of selectivity over 5-HT_{2B} (EC₅₀ = 1994 nM) and 5-HT_{2A} (no activity). The allyl ether bearing derivative (+)-16b (EC₅₀ = 4.2 nM) has a potency equal to that of lorcaserin. It showed much better selectivity over 5-HT_{2B} (no activity observed), with an 89-fold selectivity against 5-HT_{2A} (EC₅₀ = 374 nM). The attachment of a fluorine atom to the allyl group, with the intent to enhance metabolic stability, led to (+)-16c that was slightly less potent at 5-HT_{2C} (22 nM) but had an improved selectivity over 5-HT_{2A}. The 2-fluoroethoxy bearing compound (+)-16d showed enhanced potency at 5-HT_{2C} (EC₅₀ = 3.4 nM), along with excellent functional selectivity over both 5-HT_{2B} (no activity observed) and 5-HT_{2A} (>100-fold selectivity).

To further investigate their drug-like character, compounds (+)-16b and (+)-16d were tested for both CYP and hERG inhibition. As shown in Table 5, improved CYP inhibition profiles were measured for (+)-16b and (+)-16d relative to compounds (+)-13h, (+)-13j, and (+)-13l. Less than 50% inhibition was observed at CYPs 3A4, 2D6, 2C9, and 2C19 for both compounds, while higher inhibition was observed at 2B6. As the 2B6 isoform is present in relatively low abundance in comparison to the other CYPs,²⁶ these results are encouraging. In the hERG inhibition assay, although concentration-dependent inhibition was observed for (+)-16b and (+)-16d, both compounds afforded less than 50% inhibition at the highest concentration tested, which was 30 μ M. These results suggest that the compounds may present a reasonable safety margin, as both compounds are active at the 5-HT_{2C} receptor in the low nanomolar range.

Further pharmacological profiling was conducted for compounds (+)-16b and (+)-16d to investigate potential off-target activity. Binding studies were performed at 45 targets,

Table 5. CYP and hERG Inhibition Data for Compounds (+)-16b and (+)-16d

assay	(+)-16b	(+)-16d	
CYP inhibition at 10 μ M ^a (%)	3A4	15	10
	2D6	43	24
	2C9	35	16
	2C19	22	26
	2B6	80	6
hERG inhibition ^b (%)	0.37 μ M	6.4	6.0
	1.1 μ M	8.7	11
	3.3 μ M	18	15
	10 μ M	29	25
	30 μ M	45	35

^aCYP inhibition test was performed using human liver microsomes; midazolam, dextromethorphan, tolbutamide, (*S*)-mephentyoin, and bupropion were used as test substrates for the 3A4, 2D6, 2C9, 2C19, and 2B6 isoforms, respectively. ^bhERG inhibition was tested on CHO cells using the automated patch-clamp method.

including GPCRs, ion channels, neurotransmitter transporters, and sigma receptors, by the Psychoactive Drug Screening Program (PDSP). (+)-16b and (+)-16d were found to display high affinity for the 5-HT_{2B} and 5-HT_{2C} receptors, with K_i values in the range of 30–50 nM. However, as already described, both compounds have been shown to be “functionally” selective for the 5-HT_{2C} receptor. (+)-16b showed <1 μ M K_i values at the 5-HT_{1A}, 5-HT₆, 5-HT₇, α _{2A}, α _{2B}, α _{2C}, and dopamine D₃ receptor, while (+)-16d showed binding at this same level at only α _{2A}. None of the other targets analyzed were found to show any significant off-target affinity for these two compounds (Supporting Information, Table S3). Compared to the other known 5-HT_{2C} agonists reported to date, compounds (+)-16b and (+)-16d thus represent attractive molecules in terms of both their 5-HT_{2C} potency and selectivity.

In Vivo Test in a Schizophrenia-like Animal Model. The pharmacological profiles and ADMET properties of the best compounds from both the 5-chloro and 5-fluoro series, (+)-13j, (+)-13l, (+)-16b, and (+)-16d, prompted their assessment in an animal model to explore their potential antipsychotic effects. One of the advantages of developing 5-HT_{2C} agonists as antipsychotics is that they decrease levels of dopamine in limbic brain regions,²⁷ which have been proven to mediate the antipsychotic effects of current antischizophrenia drugs. Meanwhile, agonism of the 5-HT_{2C} receptor does not affect dopamine levels in the striatum, the region associated with extrapyramidal side effect of the “typical” antipsychotics.²⁸ Moreover, the antiobesity effect of 5-HT_{2C} agonists is well-known, exemplified by the approval of lorcaserin as an antiobesity drug. Thus, these agonists do not have the potential side effect of weight gain, which is observed with many antipsychotics which have properties of 5-HT_{2C} antagonists or inverse agonists.²⁹

Amphetamine-induced hyperactivity is a well-recognized rodent test used to assess the antipsychotic potential of a compound. To examine this possibility, compounds (+)-13j, (+)-13l, (+)-16b, and (+)-16d were tested in this model (details of the behavioral test are provided in the Experimental Section). Adult male C57BL/6J mice were given the vehicle (Veh) or one of the test compounds and were placed into the open field for 15 min. They were removed and administered the Veh or D-amphetamine (AMPH) and returned immediately

to the open field for 105 min. As shown in Figure 3, d-amphetamine significantly increased locomotor activity from 16

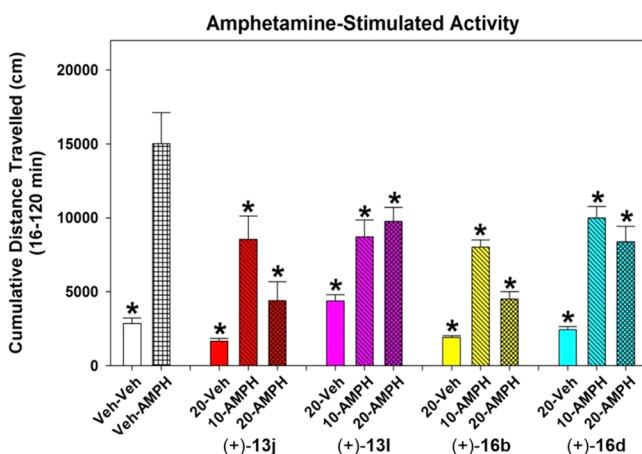


Figure 3. Cumulative locomotor activity following injection of the vehicle (Veh) or 3 mg/kg d-amphetamine (AMPH). AMPH-stimulated locomotor activity in the Veh–AMPH group was higher than that in all other groups tested. Locomotion in the Veh–Veh group was similar to that in the (+)-13j–Veh, (+)-13l–Veh, (+)-16b–Veh, and (+)-16d–Veh groups (i.e., 20-Veh groups). Both 10 and 20 mg/kg of each of the four compounds decreased the AMPH-stimulated hyperlocomotion, with 20 mg/kg (+)-13j and (+)-16b being the most efficacious. $N = 8\text{--}16$ mice/group; *, $p < 0.035$, from the Veh–AMPH group.

to 120 min in the Veh–AMPH compared to the Veh–Veh group. Administration of each of the four compounds at the 10 or 20 mg/kg doses significantly reduced the AMPH-stimulated hyperlocomotion relative to the Veh–AMPH group (p -values <0.035). Although responses to 10 and 20 mg/kg (+)-13l and (+)-16d were not differentiated by dose, compounds (+)-13j and (+)-16b dose dependently suppressed the hyperactivity such that locomotion at the 20 mg/kg doses was similar to that of the Veh–Veh group. Importantly, the 20 mg/kg dose of each the four compounds alone (20-Veh groups) had little influence on the spontaneous activity of the injected animals. The reduction in AMPH-stimulated hyperlocomotion, especially by compounds (+)-13j and (+)-16b, indicate their potential as antipsychotic agents and encourage further studies of other behaviors.

Homology Modeling to Elucidate the Binding Mode of Key Compounds to the 5-HT_{2C} Receptor. Following our SAR studies, we wanted to obtain some idea as to how (+)-16b might interact with the 5-HT_{2C} receptor. Thus, the 5-HT_{2C} primary sequence was retrieved from UniProt (P28335, SHT_{2C_HUMAN}), and two 5-HT_{2C} homology models were generated. The first model (5-HT_{2C_inact}) was obtained using the β_2 -adrenergic receptor (β_2 -AR) as a template in its inactive state (PDB: 2RH1), while the second model (5-HT_{2C_act}) was generated by combining two templates, namely the resolved structure of 5-HT_{2B} in a complex with ergotamine (PDB: 4IB4) and the β_2 -AR in its fully active state (PDB: 3SN6). This 5-HT_{2B}–ergotamine model was selected due to the fact that ergotamine has functional selectivity for 5-HT_{2B}, and thus the resolved structure does not represent a fully active state.³⁰ By combining the two templates, the generated active model should benefit from the high homology in the orthosteric site between 5-HT_{2B} and 5-HT_{2C} while conserving features responsible for the fully activated state as taken from the β_2 -

AR. The homology models were built by using Modeller9.12,³¹ and the best structures were selected for docking studies. Docking simulations were performed using PLANTS, which finds plausible ligand poses through ant colony optimization algorithms (ACO).³² (For details of the modeling study, see Supporting Information.)

The macroscopic view of compound (+)-16b binding to both the inactive and active conformations of the 5-HT_{2C} and the magnified views of the binding sites are shown in Figure 4. As exemplified in Figure 4a, a significant difference was observed between the binding poses of compound (+)-16b with 5-HT_{2C_inact} and 5-HT_{2C_act} as the compound is inserted much deeper into a tighter niche when docking to 5-HT_{2C_act}. As shown with the magnified view in Figure 4b, the computed complexes for the 5-HT_{2C_inact} model are vastly stabilized by the ion pair between the ligand ammonium head and Asp134, a key contact reinforced by clear H-bonds with Ser138 and Tyr358 plus a charge transfer interaction with Trp130. By contrast, the remaining part of the ligand is seen to elicit only weak apolar contacts: (a) the cyclopropane ring approaches Ile131, (b) the phenyl ring is engaged in π – π stacking with Phe327, Trp355, and Tyr358, with the halogen atom approaching the indole nitrogen atom of Trp355, and (c) the alkenyl chain is stabilized by a possible π – π stacking interaction with Phe327.

A significantly different interaction pattern is observed when analyzing the computed complexes for the 5-HT_{2C_act} model. As shown in Figure 4c, the interactions involving the ligand ammonium group are almost superimposable on those seen using the inactive model and involve Asp134, Ser138, and Tyr358, while the cyclopropane ring elicits apolar contacts with Val135 and Val185. The largest differences between the two 5-HT_{2C} models are observed for the contacts that stabilize the remaining portion of the ligands because the phenyl ring is inserted into a tight niche lined by aromatic residues (i.e., Phe214, Trp324, Phe327, Phe328, and Tyr358) with which it can interact via extended π – π stacking interactions, while the ether bridge engages in two possible H-bonds with Ser219 and Asn331. Finally, the alkenyl tail also engages in a possible π – π stacking interaction with Phe214.

Some differences were also observed when the binding pose of (+)-16b was compared to that found for (+)-13h, as is apparent from the overlay shown in Figure 4d. The interactions displayed by the ammonium group of (+)-13h are superimposable to those of (+)-16b, but the positions of the benzene ring and the direction of the ether side chain are significantly different. The increase of steric hindrance upon changing the fluorine atom to a chlorine atom probably prevents the benzene ring from going deeper into the pocket, thus the ether side chain moves inside while the benzene ring is outside. The π – π stacking interactions within the tight niche lined by aromatic residues would therefore be anticipated to be weaker than those experienced by (+)-16b. This property might explain the fact that the potency of (+)-16b is about 15-fold higher than that of (+)-13h (4.2 vs 60 nM).

When the binding mode of compound (+)-16b is compared to that of lorcaserin, as shown in Figure 5, (+)-16b shares with lorcaserin the same key interactions that stabilize the ammonium group as well as the extended set of hydrophobic and stacking contacts, with the cyclopropane ring suitably replacing the carbon skeleton of the benzazepine ring. The major differences in the 5-HT_{2C_act} model is that the polar contact, namely the likely H-bond with Asn331, is seen only

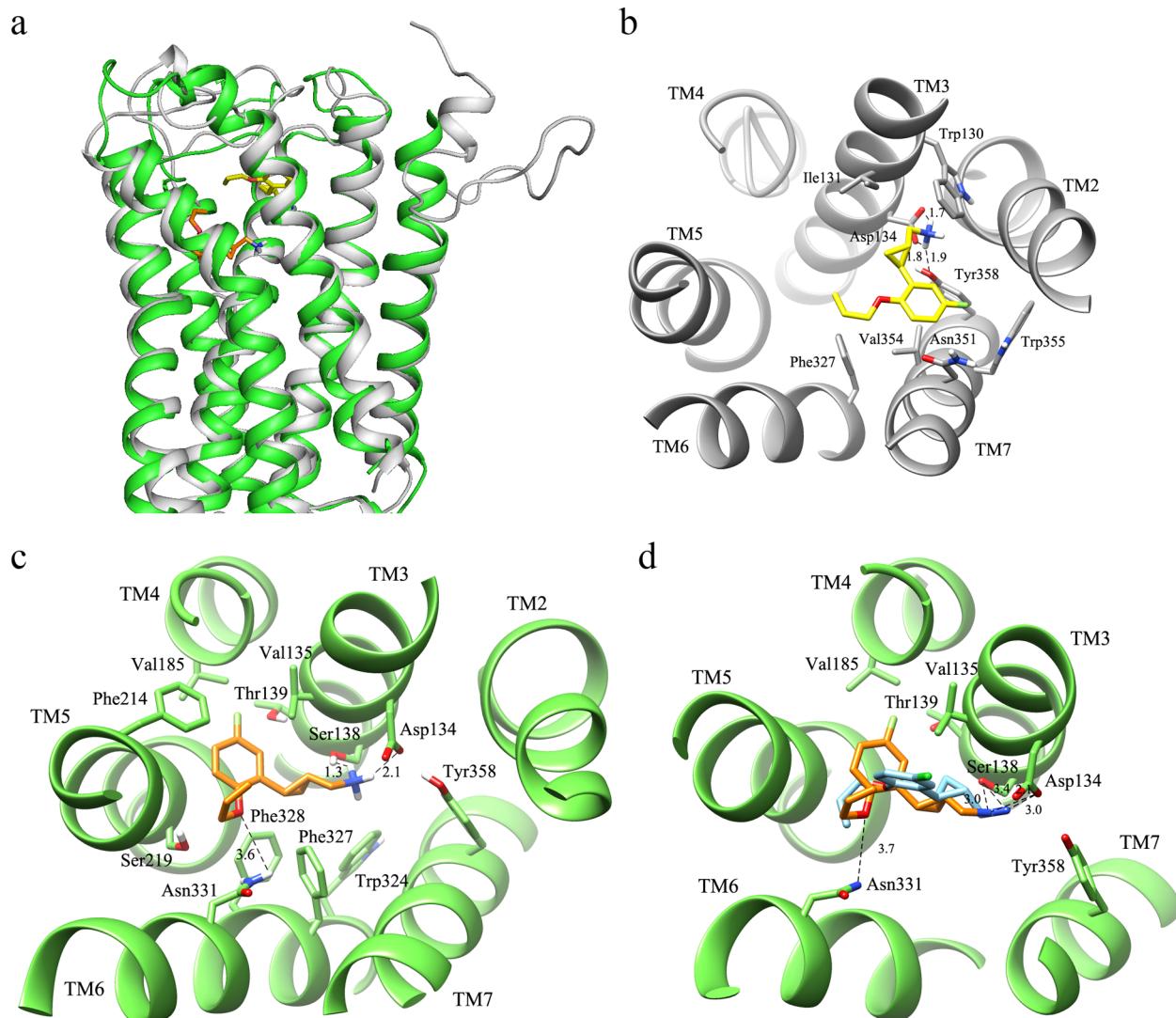


Figure 4. Predicted binding poses of compound (+)-16b in 5-HT_{2C}. (a) Docking poses of compound (+)-16b in the 7-transmembrane domain (7TMD) of the 5-HT_{2C}_inactive (compound shown in light yellow, protein shown in gray) and 5-HT_{2C}_active (compound shown in orange, protein shown in green) conformations. (b) Magnified view of binding sites of compound (+)-16b in 5-HT_{2C}_inactive. (c) Magnified view of binding sites of compound (+)-16b in 5-HT_{2C}_active. (d) Overlay of compound (+)-16b (orange) with (+)-13h (sky blue) in the binding pocket.

with compound (+)-16b because of its ether oxygen atom, while the chlorine atom of lorcaserin engages Asn331 in possible hydrophobic contacts.

CONCLUSION

As our continuing efforts to optimize the 2-phenylcyclopropylmethylamine scaffold, new compounds bearing chlorine or fluorine substitutions at position 5 on the benzene ring were synthesized, and these modifications together with optimization of the ether substituent at position 2 led to compounds with good potency at the 5-HT_{2C} receptor as well as improved selectivity against the 5-HT_{2B} and 5-HT_{2A} receptors. In turn, ADMET studies coupled with behavioral analysis in the amphetamine-stimulated hyperlocomotion model identified four compounds with good profiles as antipsychotic agents. Compound (+)-16b displayed an EC₅₀ value of 4.2 nM at 5-HT_{2C} was 89-fold selective against 5-HT_{2A}, and had no significant activity at 5-HT_{2B}. It is one of the most potent and selective 5-HT_{2C} agonists reported to date. At a dose of 20 mg/kg, (+)-16b completely reversed amphetamine-induced hyper-

activity in mice while showing no significant influence on the spontaneous activity of the tested animals. Molecular modeling studies were also performed to elucidate the possible binding poses of compound (+)-16b to the 5-HT_{2C} receptor, using a homology model incorporating both the well-known β₂-adrenergic receptor template and recently reported 5-HT_{2B} receptor.

The high potency and excellent selectivity profile of compound (+)-16b, together with its significant effects in an animal behavior study, strongly support the further evaluation of this compound as a chemical tool that can be used to elucidate the importance of the 5-HT_{2C} receptors in normal human behaviors as well as in pathophysiological states. Further animal studies are being conducted to explore the use of this compound in the possible treatment of schizophrenia-like behaviors, and these studies will be reported separately.

EXPERIMENTAL SECTION

General. All chemicals and solvents were purchased from Sigma-Aldrich or Fisher Scientific and used without further purification.

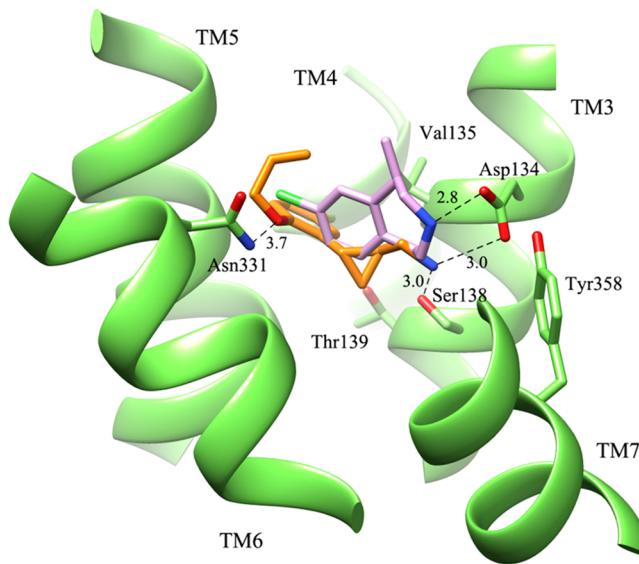


Figure 5. Overlay of compound (+)-16b (orange) with lorcaserin (pink) in the binding pocket of 5-HT_{2C}_active.

Microwave reactions were run in Biotage Initiator microwave synthesizer. Synthetic intermediates were purified by CombiFlash flash chromatography on 230–400 mesh silica gel. ¹H and ¹³C NMR spectra were recorded on Bruker DPX-400 or AVANCE-400 spectrometer at 400 and 100 MHz, respectively. NMR chemical shifts were reported in δ (ppm) using residual solvent peaks as standard (CDCl_3 , 7.26 ppm (¹H), 77.23 ppm (¹³C); CD_3OD , 3.31 ppm (¹H), 49.15 ppm (¹³C); $\text{DMSO}-d_6$, 2.50 ppm (¹H), 39.52 ppm (¹³C)). Mass spectra were measured in the ESI mode at an ionization potential of 70 eV with an LC-MS MSD (Hewlett-Packard). Purity of all final compounds (greater than 95%) was determined by analytical HPLC (ACE 3AQ C₁₈ column (150 mm × 4.6 mm, particle size 3 μm), 0.05% TFA in $\text{H}_2\text{O}/0.05\%$ TFA in MeOH gradient eluting system). Optical rotation values were recorded on Autopol IV automatic polarimeter.

General Method A: Preparation of N-Boc-amines 12a–12n and 15a–15d. Intermediate 11 and 14 was treated with the Williamson ether synthesis or Mitsunobu reaction conditions as described in Scheme 1 and Scheme 2 (for detailed conditions, see Supporting Information, Tables S1 and S2). The reactions were monitored with TLC, and the workup was done with ethyl acetate and water. Crude product was purified with flash chromatography.

General Method B: Chiral Separation of N-Boc-amines 12a–12n and 15a–15d. The racemic intermediates were separated by chiral HPLC. Analytical conditions: RegisCell chiral column (25 cm × 4.6 mm, 10 μM), 1.5–15% EtOH in *n*-hexane as the fluent phase. Preparative conditions: RegisPack chiral column (25 cm × 21.1 mm, 10 μM), 3–7.5% EtOH in *n*-hexane as the eluting system (isocratic eluent, stacked injections, flow rate = 18 mL/min, λ = 254 and 280 nm). (+)-12a–12n and (+)-15a–15d were isolated as the first-eluting peaks, with (−)-12a–12n and (−)-15a–15d as the second-eluting peaks, both after evaporation appeared as colorless oil or white solids. Optical purity of both enantiomers was determined on analysis HPLC after the separation, and a second separation was done when necessary to guarantee >90% ee optical purity.

General Method C: Deprotection of N-Boc-Amines to Afford HCl Salts (13a, 13b, 13e–13g, 13i–13n, and 16a–16d). N-Boc-Amines was dissolved in 2 M HCl (g) in diethyl ether (10 mL/mmol substrate) and stirred at room temperature for 24–48 h. The white solids formed were collected by filtration, washed with diethyl ether, and dried over vacuum to give the HCl salts as white solids.

General Method D: Deprotection of N-Boc-Amines to Afford TFA Salts (13c, 13d, and 13h). To a solution of the N-Boc protected precursor (1 mmol) in CH_2Cl_2 (10 mL) was added to TFA (1 mL) at 0 °C under an argon atmosphere. The mixture was stirred at

room temperature for 1 h. The reaction mixture was concentrated and the residue was dissolved in water and methanol (ratio 4:1). The solution was filtered and then purified by Shidmadzu preparative LC using the following conditions: ACE 5AQ column (150 mm × 21.2 mm, particle size 5 μm). Method: 8–100% 0.05% TFA in MeOH/0.05% TFA in H_2O , 30 min. Flow rate = 17 mL/min with monitoring at 254 and 280 nm wavelengths. After the solvent was evaporated, the residue was dissolved in distilled water (2–3 mL) and lyophilized to obtain the TFA salt.

Detailed synthetic procedures were described for compounds (−)-16b, (+)-16b, (−)-16d, and (+)-16d. Other compounds were prepared similarly with the general methods described above and the characterization data are provided in the Supporting Information.

tert-Butyl ((2-(Allyloxy)-5-fluorophenyl)cyclopropyl)methyl)carbamate (15b). To a solution of compound 14 (282 mg, 1.0 mmol) in anhydrous DMF (2 mL) was added Cs_2CO_3 (489 mg, 1.5 mmol) and allyl bromide (242 mg, 2.0 mmol), and the mixture was heated in a microwave at 80 °C for 30 min. Water was added, and the mixture was extracted with ethyl acetate, the combined extracts were dried over Na_2SO_4 , concentrated, and purified with flash chromatography (0–30% ethyl acetate in hexanes) to render the title compound as a colorless oil (250 mg, 78%). ¹H NMR (400 MHz, CDCl_3) δ 6.80 (dt, J = 8.8, 3.2 Hz, 1H), 6.75 (dd, J = 8.8, 4.8 Hz, 1H), 6.61 (dd, J = 9.2, 2.8 Hz), 6.18–6.08 (m, 1H), 5.42 (dd, J = 17.2, 1.6 Hz, 1H), 5.31 (dd, J = 10.4, 1.2 Hz, 1H), 5.08 (br, 1H), 4.61–4.57 (m, 2H), 3.54–3.51 (m, 1H), 2.79–2.76 (m, 1H), 1.93–1.88 (m, 1H), 1.46 (s, 9H), 1.07–1.98 (m, 2H), 0.88–0.82 (m, 1H). ¹³C NMR (100 MHz, CDCl_3) δ 157.3 (d, J_{CF} = 236.9 Hz), 155.9, 153.5, 133.3, 132.6 (d, J_{CF} = 7.4 Hz), 118.1, 113.4 (d, J_{CF} = 23.3 Hz), 112.5 (d, J_{CF} = 22.5 Hz), 112.4 (d, J_{CF} = 8.4 Hz), 79.0, 69.8, 45.2, 28.5, 21.8, 17.0, 11.4. The racemate was separated with RegisPack chiral column (25 cm × 21.1 mm, 10 μM), 3.25% EtOH in *n*-hexane as the eluting system (isocratic eluent, stacked injections, flow rate = 18 mL/min, λ = 254 and 280 nm). (+)-15b (92 mg, 97% ee) was separated as the first peaks and (−)-15b (78 mg, 96% ee) as the second peaks. (+)-15b, $[\alpha]_D^{20} +26.4$ (c 0.5, CHCl_3); (−)-15b, $[\alpha]_D^{20} -26.8$ (c 0.5, CHCl_3).

(−)-((1*R*,2*R*)-2-(Allyloxy)-5-fluorophenyl)cyclopropyl)methanamine Hydrochloride ((−)-16b). (+)-15b (80 mg, 0.25 mmol) was dissolved in 2 M HCl in diethyl ether (10 mL) and stirred at room temperature for 24 h. The precipitation was collected by filtration, washed with diethyl ether, and dried on vacuum to render the title compound as a white solid (48 mg, 75%). ¹H NMR (400 MHz, CD_3OD) δ 6.93–6.86 (m, 2H), 6.73 (dd, J = 9.4, 3.2 Hz, 1H), 6.17–6.10 (m, 1H), 5.45 (dd, J = 17.2, 1.6 Hz, 1H), 5.30 (dd, J = 10.4, 1.6 Hz, 1H), 4.60 (d, J = 5.6 Hz, 2H), 3.06 (dd, J = 13.2, 7.6 Hz, 1H), 2.98 (dd, J = 13.2, 4.2 Hz, 1H), 2.21–2.15 (m, 1H), 1.34–1.30 (m, 1H), 1.14–1.02 (m, 2H). ¹³C NMR (100 MHz, CD_3OD) δ 158.8 (d, J_{CF} = 225.7 Hz), 154.9, 135.0, 133.0 (d, J_{CF} = 7.4 Hz), 118.2, 114.3 (d, J_{CF} = 17.8 Hz), 114.1, 114.0 (d, J_{CF} = 37.3 Hz), 71.0, 45.1, 19.7, 18.3, 13.7. HRMS (ESI) calculated for $\text{C}_{13}\text{H}_{17}\text{FNO}$ ([M + H]⁺), 222.1294; found, 222.1273. $[\alpha]_D^{20} -16.2$ (c 0.4, MeOH).

(+)-((1*S*,2*S*)-2-(Allyloxy)-5-fluorophenyl)cyclopropyl)methanamine Hydrochloride ((+)-16b). (−)-15b (60 mg, 0.19 mmol) was dissolved in 2 M HCl in diethyl ether (8 mL) and stirred at room temperature for 24 h. The precipitation was collected by filtration, washed with diethyl ether, and dried on vacuum to render the title compound as a white solid (32 mg, 66%). ¹H NMR (400 MHz, CD_3OD) δ 6.93–6.86 (m, 2H), 6.73 (dd, J = 9.6, 3.2 Hz, 1H), 6.17–6.10 (m, 1H), 5.45 (dd, J = 17.2, 1.6 Hz, 1H), 5.30 (dd, J = 10.4, 1.6 Hz, 1H), 4.60 (d, J = 4.2 Hz, 2H), 3.07 (dd, J = 12.8, 7.2 Hz, 1H), 2.98 (dd, J = 13.2, 5.6 Hz, 1H), 2.21–2.17 (m, 1H), 1.35–1.31 (m, 1H), 1.14–1.04 (m, 2H). ¹³C NMR (100 MHz, CD_3OD) δ 158.8 (d, J_{CF} = 235.7 Hz), 155.0, 135.0, 133.0 (d, J_{CF} = 7.4 Hz), 118.2, 114.3 (d, J_{CF} = 17.0 Hz), 114.1, 114.0 (d, J_{CF} = 36.1 Hz), 71.0, 45.1, 19.7, 18.3, 13.7. HRMS (ESI) calculated for $\text{C}_{13}\text{H}_{17}\text{FNO}$ ([M + H]⁺), 222.1294; found, 222.1273. $[\alpha]_D^{20} +17.5$ (c 0.2, MeOH).

tert-Butyl ((2-(5-Fluoro-2-fluoroethoxy)phenyl)cyclopropyl)methyl)carbamate (15d). To a solution of compound 14 (282 mg, 1.0 mmol), triphenylphosphine (787 mg, 3.0 mmol) and 2-fluoroethanol (192 mg, 3.0 mmol) in anhydrous THF (3 mL) was

cooled at 0 °C and was added diethylazodicarboxylate (522 mg, 3.0 mmol) dropwise. The solution was then heated in microwave reactor at 60 °C for 40 min. The mixture was purified with flash chromatography (0–30% ethyl acetate in hexanes) after concentration to render **15d** as a colorless oil (320 mg, 98%). ¹H NMR (400 MHz, CDCl₃) δ 6.82 (dt, *J* = 8.8, 2.8 Hz, 1H), 6.77 (dd, *J* = 8.0, 4.8 Hz, 1H), 6.62 (dd, *J* = 9.2, 2.8 Hz, 1H), 5.02 (br, 1H), 4.88–4.73 (m, 2H), 4.31–4.20 (m, 2H), 3.53–3.48 (m, 1H), 2.84–2.78 (m, 1H), 1.96–1.92 (m, 1H), 1.46 (s, 9H), 1.10–1.05 (m, 1H), 1.02–0.98 (m, 1H), 0.88–0.82 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 157.7 (d, *J*_{CF} = 237.5 Hz), 155.9, 153.4, 133.2 (d, *J*_{CF} = 7.4 Hz), 113.5 (d, *J*_{CF} = 23.4 Hz), 112.9 (d, *J*_{CF} = 8.5 Hz), 112.6 (d, *J*_{CF} = 22.7 Hz), 81.9 (d, *J*_{CF} = 169.7 Hz), 79.1, 68.5 (d, *J*_{CF} = 20.2 Hz), 45.1, 28.5, 20.0, 16.8, 11.5. The racemate was separated with RegisPack chiral column (25 cm × 21.1 mm, 10 μM), 7.5% EtOH in *n*-hexane as the eluting system (isocratic eluent, stacked injections, flow rate = 18 mL/min, *λ* = 254 and 280 nm). (+)-**15d** (120 mg, > 99% ee) was separated as the first peaks and (−)-**15d** (110 mg, > 99% ee) as the second peaks. (+)-**15d**, [α]_D²⁰ +20.2 (c 0.3, CHCl₃); (−)-**15d**, [α]_D²⁰ −18.7 (c 0.3, CHCl₃).

(−)-((1*R*,2*R*)-2-(5-Fluoro-2-(2-fluoroethoxy)phenyl)cyclopropyl)methanamine Hydrochloride ((−)-**16d**). Compound (+)-**15d** (110 mg, 0.34 mmol) was dissolved in 2 M HCl in diethyl ether (8 mL) and stirred at room temperature for 24 h. The precipitation was collected by filtration, washed with diethyl ether, and dried on vacuum to render the title compound as a white solid (61 mg, 69%). ¹H NMR (400 MHz, CD₃OD) δ 6.97–6.86 (m, 2H), 6.76 (dd, *J* = 9.2, 2.8 Hz, 1H), 4.87–4.75 (m, 2H), 4.32–4.20 (m, 2H), 3.04–3.00 (m, 2H), 2.19–2.15 (m, 1H), 1.28–1.17 (m, 2H), 1.06–1.02 (m, 1H). ¹³C NMR (100 MHz, CD₃OD) δ 159.0 (d, *J*_{CF} = 236.0 Hz), 155.0, 133.0 (d, *J*_{CF} = 7.4 Hz), 114.6 (d, *J*_{CF} = 24.0 Hz), 114.2 (d, *J*_{CF} = 22.8 Hz), 114.0 (d, *J*_{CF} = 8.1 Hz), 83.6 (d, *J*_{CF} = 166.6 Hz), 69.7 (d, *J*_{CF} = 18.9 Hz), 45.0, 20.0, 18.3, 13.0. HRMS (ESI) calculated for C₁₂H₁₆F₂NO ([M + H]⁺), 228.1200; found, 228.1179. [α]_D²⁰ −3.3 (c 0.3, MeOH).

(+)-((1*S*,2*S*)-2-(5-Fluoro-2-(2-fluoroethoxy)phenyl)cyclopropyl)methanamine Hydrochloride ((+)-**16d**). This compound was prepared from (−)-**15d** as described for (−)-**16d** as a white solid. ¹H NMR (400 MHz, CD₃OD) δ 6.97–6.88 (m, 2H), 6.76 (dd, *J* = 9.6, 3.2 Hz, 1H), 4.87–4.74 (m, 2H), 4.32–4.22 (m, 2H), 3.03 (d, *J* = 7.6 Hz, 2H), 2.20–2.15 (m, 1H), 1.28–1.25 (m, 1H), 1.22–1.17 (m, 1H), 1.07–1.02 (m, 1H). ¹³C NMR (100 MHz, CD₃OD) δ 159.0 (d, *J*_{CF} = 236.2 Hz), 155.0, 133.1 (d, *J*_{CF} = 7.3 Hz), 114.6 (d, *J*_{CF} = 24.0 Hz), 114.2 (d, *J*_{CF} = 17.5 Hz), 114.1 (d, *J*_{CF} = 3.1 Hz), 83.6 (d, *J*_{CF} = 166.6 Hz), 69.7 (d, *J*_{CF} = 19.8 Hz), 45.0, 20.0, 18.3, 13.1. HRMS (ESI) calculated for C₁₂H₁₆F₂NO ([M + H]⁺), 228.1200; found, 228.1173. [α]_D²⁰ +3.6 (c 0.3, MeOH).

Calcium Flux Assay. Flp-In-293 cells stably expressing the human 5-HT_{2A}, 5-HT_{2B}, or 5-HT_{2C-IN1} were grown for 24–48 h in DMEM containing 10% dialyzed FBS before seeding. Cells were plated into Poly-L-Lys-coated 384-well black clear bottom cell culture plates in DMEM with 1% dialyzed FBS at a density of 12000 cells per 50 μL per well for 24 h. Preceding the experiment, culture medium was removed and 20 μL of assay buffer (20 mM Hepes, pH 7.40, Hanks' balanced salt solution, 2.5 mM probenecid, 1× FLIPR calcium dye) was added and cells were incubated at 37 °C for 1 h. Serial dilutions of each tested drug were prepared at 3× final concentration and transferred to 384-well plates. Each drug plate contained 5-HT and lorcaserin in serial dilutions for internal reference. Cell and drug plates were placed in a FLIPR^{TETRA} fluorescence imaging plate reader (Molecular Dynamics). The FLIPR^{TETRA} was programmed to read baseline for 10 s (1 read/s) and then add 10 μL of drug/well and read for an additional 120 s. Fluorescence was normalized to the average of the baseline (first 10 reads), and the maximum fold increase peak was determined for each drug and controls (5-HT and lorcaserin). Data were plotted as a function of drug concentration and were normalized compared to the internal 5-HT reference for each plate recorded. Normalized data were regressed using a sigmoidal dose–response function. Data of two independent experiments (*n* = 2) conducted in quadruplicate are presented. Analyses were performed using the software from GraphPad Prism 6.0. 5-HT_{2C} EC₅₀ confidence intervals

(5-HT, 0.16–0.26 nM; lorcaserin, 3.1–3.9 nM), E_{max} Std Error (5-HT, 100% ± 1.04; lorcaserin, 99% ± 0.75); 5-HT_{2B} EC₅₀ confidence intervals (5-HT, 0.66–1.17 nM; lorcaserin, 429–527 nM), E_{max} Std Error (5-HT, 100% ± 1.69; lorcaserin, 92% ± 3.01); 5-HT_{2A} EC₅₀ confidence intervals (5-HT, 1.60–2.16 nM; lorcaserin, 275–329 nM), E_{max} Std Error (5-HT, 100% ± 1.03; lorcaserin, 68% ± 0.83).

Open Field Activity. Adult male CS7BL/6J mice (Jackson Laboratories, Bar Harbor, ME) were injected (ip) with the vehicle (Veh, 0.9% saline from Butler Schein Animal Health, Dublin, Ohio) or different doses of compounds (+)-**13j**, (+)-**13l**, (+)-**16b**, or (+)-**16d** (compounds were dissolved in 0.9% saline and injected intraperitoneally at 5 mL/kg) and placed into the open field for 15 min. The mice were removed and administered (ip) the Veh or 3 mg/kg amphetamine (AMPH; Sigma-Aldrich, St. Louis, MO) and returned to the open field for 105 min. Locomotor activity was monitored as distance traveled in an automated Omnitech Digiscan apparatus using VersaMax software (AccuScan Instruments, Columbus, OH). The results are presented as means and standard errors of the mean using SPSS software (IBM, Armonk, NY). The data from the 0–15 min interval were analyzed by three-way ANOVA for condition (vehicle and AMPH), treatment (compounds (+)-**13j**, (+)-**13l**, (+)-**16b**, and (+)-**16d**), dose (10 or 20 mg/kg), and dose nested in treatment (to reflect the different doses tested per compound). Because activities among the groups at the 0–15 min interval were significantly different, a RMANOVA was run with a sequential sum of squares (to control for the group differences at 0–15 min) for test interval (0–15 and 16–120 min), condition, treatment, dose, and dose (treatment) to control for these group differences. All posthoc analyses were by Bonferroni corrected pairwise comparisons where a *p* < 0.05 was considered significant.

ASSOCIATED CONTENT

Supporting Information

Reaction conditions for the preparation of **12a–12n** and **15a–15d** intermediates, characterization data for all other compounds, pharmacological profiling data of compounds (+)-**16b** and (+)-**16d**, and details of the modeling study. This material is available free of charge via the Internet at <http://pubs.acs.org>.

AUTHOR INFORMATION

Corresponding Author

*Phone: +1 312 996-7577. E-mail: kozikowa@uic.edu.

Present Addresses

[†]For O.K.O.: Department of Biological, Chemical and Physical Sciences, College of Arts and Sciences, Roosevelt University, Chicago, Illinois, 60605, United States.

[‡]For A.G.: Center for Molecular Innovation & Drug Discovery, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208–3113, United States.

[○]For H.G.: School of Pharmacy, Faculty of Health Sciences, Curtin University, Building 306, Kent Street, Bentley, Perth, Western Australia 6102, Australia.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

S-HT, serotonin; β₂-AR, β₂-adrenergic receptor; ADMET, absorption, distribution, metabolism, excretion and toxicity; AMPH, D-amphetamine; Boc, *tert*-butyloxycarbonyl; BBB, blood–brain barrier; CHO, Chinese hamster ovary cells;

CNS, central nervous system; CYP, cytochrome P450; DMF, dimethylformamide; FDA, US Food and Drug Administration; FLIPR, fluorescence imaging plate reader; GPCR, G-protein coupled receptor; HEK-293, human embryonic kidney 293 cells; hERG, human ether-a-go-go-related gene; HPLC, high-performance liquid chromatography; PDB, Protein Data Bank; PPB, plasma protein binding; SAR, structure–activity relationship; TFA, trifluoroacetic acid; TM, transmembrane; TMD, transmembrane domain; Veh, vehicle

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