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Article

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Design and Discovery of Functionally Selective Serotonin 2C (5-HT_{2C}) Receptor Agonists

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Abstract

Based on the structural similarity of our previous 5-HT_{2C} agonists with the melatonin receptor agonist tasimelteon, and the putative biological cross-talk between serotonergic and melatonergic systems, a series of new (2,3-dihydro)benzofuran-based compounds were designed and synthesized. The compounds were evaluated for their selectivity toward 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors in the calcium flux assay with the ultimate goal to generate selective 5-HT_{2C} agonists. Selected compounds were studied for their functional selectivity by comparing their transduction efficiency at the G protein signaling pathway versus β-arrestin recruitment. The most functionally selective compound (+)–**7e** produced weak β-arrestin recruitment and also demonstrated less receptor desensitization compared to serotonin in both calcium flux and phosphoinositide (PI) hydrolysis assays. We report for the first time that selective 5-HT_{2C} agonists possessing weak β-arrestin recruitment can produce distinct receptor desensitization properties.

Introduction

The serotonin 2C (5-HT_{2C}) receptor has been found to be an invaluable drug target for a variety of central nervous system (CNS) disorders, such as obesity, schizophrenia, and drug addiction.[1-3] The 5-HT_{2C} receptor is activated endogenously by serotonin (5-HT, **1**, Figure 1), which is a major neurotransmitter widely found in both the periphery and the CNS.[4] The 5-HT_{2C} receptor belongs to the family of serotonin receptors comprised of 14 subtypes (5-HT_{1-7}, some of these with further subclassifications).[5] All serotonin receptors belong to the G protein-coupled receptor (GPCR) superfamily except 5-HT_3, which is a ligand-gated ion channel. The 5-HT_{2C} receptor shares high sequence similarity with the other 5-HT_2-family receptors: 5-HT_{2A} and 5-HT_{2B} receptors (55% and 52% homology, respectively).

In the mammalian pineal gland, serotonin can be converted to melatonin (**2**), which is another neurotransmitter that is involved in the control of circadian rhythms linked to certain physiological functions including the timing of sleep, blood pressure regulation, seasonal reproduction, immune function, etc.[6] A close interrelationship between the melatonergic and serotonergic systems has long been suspected, and evidence for their cross-talk has been recently reported. For example, melatonin inhibits the ability of serotonin to phase shift the suprachiasmatic circadian clock.[7] In fact, agomelatine, which is on the market for major depressive disorder, exhibits melatonin receptor agonist activity as well as 5-HT_{2C} antagonism.[8]

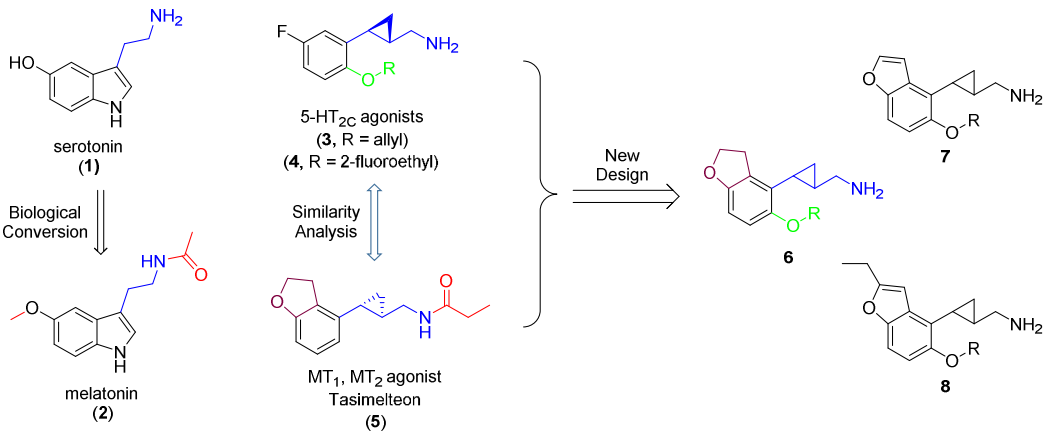


Figure 1. Bio-inspired design of 2,3-dihydrobenzofuran (**6**) and benzofuran (**7** and **8**) compounds as 5-HT_{2C} agonists.

In our previous work, we identified compounds **3** and **4** as highly selective 5-HT_{2C} agonists (Figure 1).[9, 10] Both compounds display excellent selectivity against the 5-HT_{2A} and 5-HT_{2B} receptors, which activation are associated with hallucinogenic effects and cardiac valvulopathy, respectively.[11-13] Interestingly, although compounds **3** and **4** evolved from a hit compound which was identified through a high throughput screening (HTS) campaign,[14] they share the same 2-arylcyclopropylmethylamine backbone with tasimelteon (**5**), a melatonin receptor agonist that was approved recently for the treatment of non-24-hour sleep-wake disorder.[15] The cross-talk between melatonergic and serotonergic signaling pathways, and the structural similarity between compounds **3**, **4**, and tasimelteon, thus inspired us to design compounds possessing the general structure **6** (Figure 1). As a matter of fact, both benzofuran and 2,3-dihydrobenzofuran have been reported as substructures of

5-$HT_{2A/2C}$ receptor ligands previously.[16] We anticipated that compound **6** and its analogs **7** and **8** would function as 5-HT_{2C} agonists.

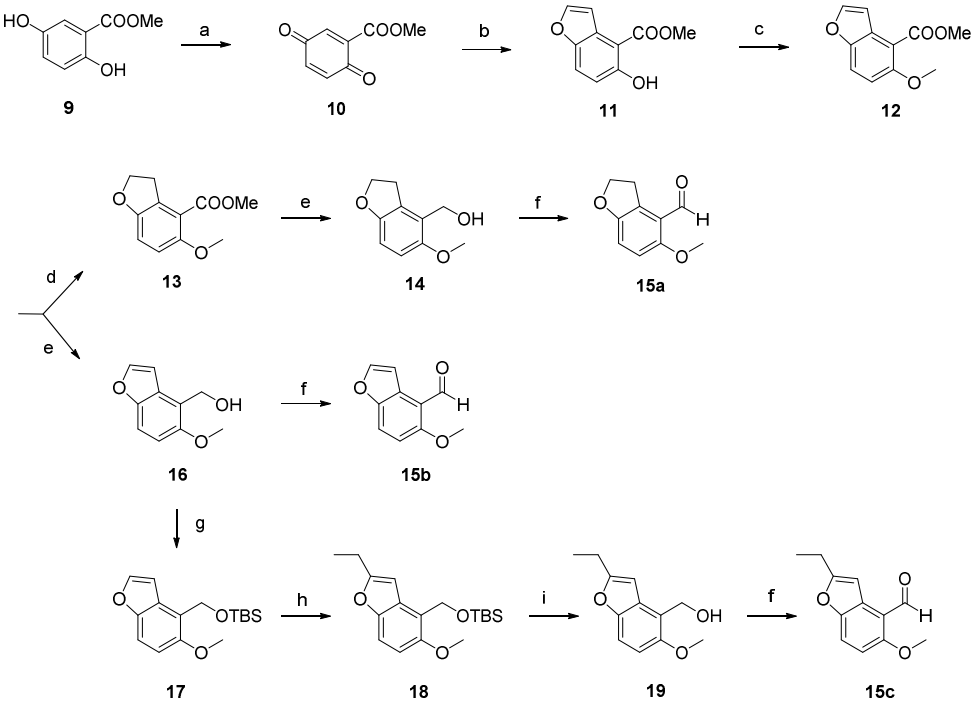
Furthermore, functional selectivity, also known as ligand bias, is a phenomenon whereby a ligand can possess multiple receptor signaling events (i.e G_q–linked calcium flux, β-arrestin recruitment) and is thought to occur through a ligand's ability to stabilize certain receptor conformations.[17] Biased ligands and functional selectivity have emerged as improved therapeutics for several GPCRs including the dopamine D_2 receptor,[18] the kappa opioid receptor,[19] and the mu opioid receptor,[20] and it is likely that the interest in the discovery of novel GPCR ligands targeting specific downstream pathways will continue to grow with time. Although functional selectivity has been actively investigated for several GPCRs, especially for 5-HT receptors,[21] there has been little characterization of biased agonism at the 5-HT_{2C} receptor, especially examining β-arrestin recruitment. The recruitment of β-arrestin to the GPCRs leads to G protein-independent signaling, desensitization and sequestration of the receptor and eventually to GPCR internalization, and it is considered to be an opposing and complementary signaling pathway to G protein signaling.[22] In this article, we report our recent work in synthesizing compounds bearing the general structures **6**–**8**, their pharmacological profiling at 5-HT_{2C}, 5-HT_{2A}, and 5-HT_{2B} receptors, and explore for the first time their G protein versus β-arrestin signaling profiles.

Results and Discussion

Chemistry

We have previously developed a general synthetic approach to various 2-phenylcyclopropylmethylamines,[9, 23-25] which employs appropriate benzaldehydes as the starting materials. Hence, the corresponding aldehydes **15a**–**c** for compounds **6**, **7** and **8** were prepared and the synthetic routes are depicted in Scheme 1. Methyl 2,5-dihydroxybenzoate **9** was used as the starting material, the oxidation of which with Ag_2O provided quinone **10**. The benzofuran intermediate **11** was prepared via a [3 + 2] cycloaddition of quinone **10** with butyl vinyl ether followed by an aromatization reaction under acidic conditions.[26] The phenol **11** was methylated to afford compound **12**, which was then reduced by hydrogenation to provide the 2,3-dihydrobenzofuran intermediate **13**. This carboxylic ester was converted to aldehyde **15a** via a $LiAlH_4$ reduction followed by a Swern oxidation. By omitting the hydrogenation step, direct $LiAlH_4$ reduction and Swern oxidation of **12** provided **15b** in good yields. To incorporate the ethyl substitution at position 2 of the benzofuran scaffold, the benzyl alcohol **16** was protected as the TBS ether **17**, which was treated in turn with *n*-BuLi and EtI to provide intermediate **18**.[27] Desilylation and Swern oxidation of **18** produced aldehyde **15c**.

Scheme 1. Synthesis of Aldehydes **15a**-**c**.[a]

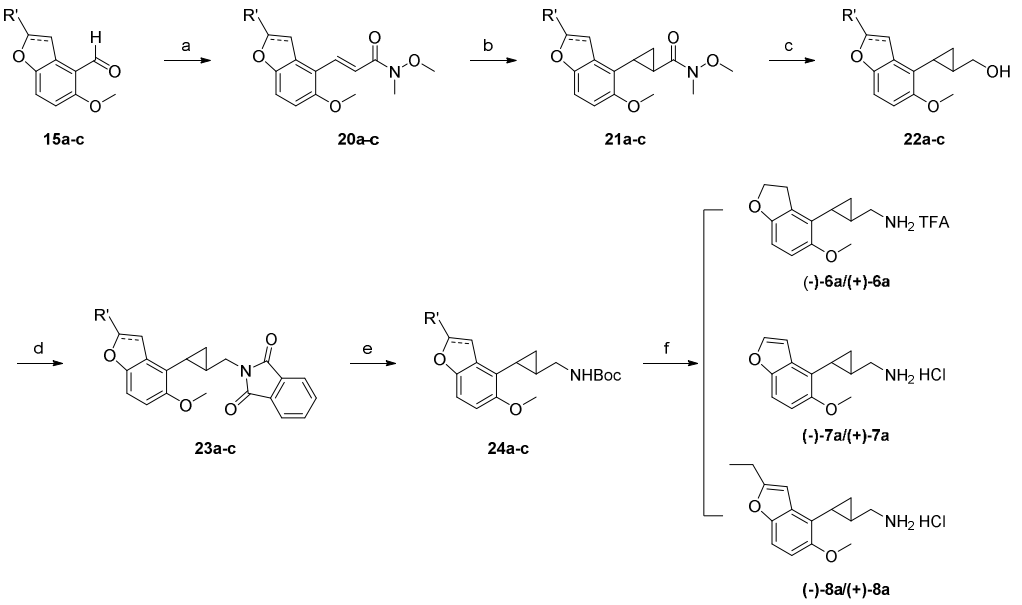


[a]Reagents and conditions: (a) Ag_2O, $MgSO_4$, Et_2O, rt, 3 h; (b) butyl vinyl ether, toluene, 50 ºC, overnight; then TFA, 50 ºC, 3 h, 52% for 2 steps; (c) MeI, K_2CO_3, DMF, rt, 3 h, 96%; (d) H_2 (50 psi), 10% Pd/C, MeOH, overnight, 79%; (e) $LiAlH_4$, THF, 0 ºC to rt, 1 h, 93–95%; (f) DMSO, $(COCl)_2$, DCM, –78 ºC, 45 min; then Et_3N, 75–89%; (g) DMF, TBSCl, imidazole, rt, 2 h; (h) *n*-BuLi, EtI, THF, –78 ºC to rt, 24 h, 94% for 2 steps; (i) TBAF, THF, rt, 1 h, 90%.

With aldehydes **15a**–**c** in hand, the syntheses of the desired target compounds **6a**–**8a** were accomplished following the same approach as we reported previously,[25] as depicted in Scheme 2. Wittig reaction of aldehydes **15a**–**c** with the commercial reagent *N*-methoxy-*N*-methyl(triphenylphosphoranylidene)acetamide provided acrylamides **20a**–**c** as their *E* isomers with complete selectivity. Corey-Chaykovski

cyclopropanation of **20a**–**c** generated the cyclopropanes **21a**–**c** in their *trans* conformations, using the sulfur ylide generated from trimethylsulfoxonium iodide upon treatment with sodium hydride. Next, sequential reduction with DIBAL-H and sodium borohydride provided alcohols **22a**–**c** in good yields, which were then converted by Mitsunobu reaction with phthalimide to afford the Gabriel imides **23a**–**c** in excellent yields. De-protection of the imides with hydrazine hydrate afforded the primary amines, which were then protected as the Boc intermediates **24a**–**c**. Separation of **24a**–**c** using chiral preparative-HPLC followed by the removal of the Boc group provided compounds (–)-**6a**, (+)-**6a**, (–)-**7a**, (+)-**7a**, (–)-**8a**, and (+)-**8a** as the optically pure isomers.

Scheme 2. Synthesis of Compounds **6a**–**8a**.[a]

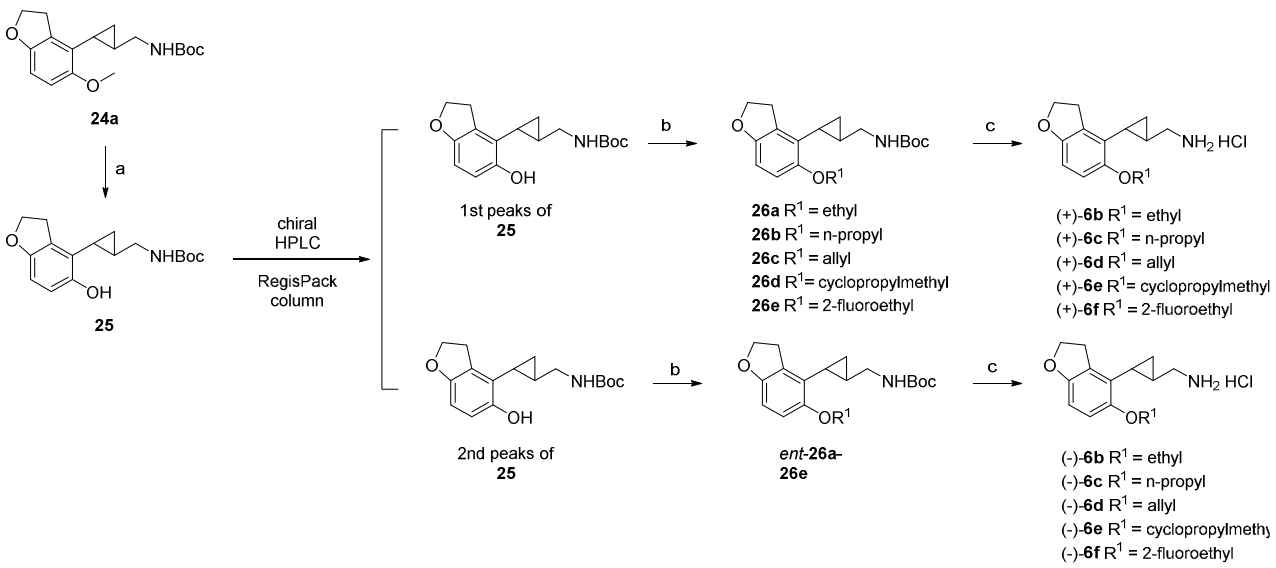


[a]Reagents and conditions: (a) Ph_3P=CHC(O)N(OMe)Me, CH_2Cl_2, rt, overnight, 79–96%; (b) trimethylsulfoxonium iodide, NaH, DMSO, rt, overnight, 83–91%; (c)

DIBAL-H, THF, –78 ºC, 3 h; then MeOH, $NaBH_4$, rt, 0.5 h, 76–90%; (d) phthalimide, Ph_3P, diethyl azodicarboxylate, rt, overnight, 92–97%; (e) N_2H_4-H_2O, EtOH, reflux, 2 h; then DCM, Et_3N, Boc_2O, rt, 30 min, 70–88%; (f) chiral preparative HPLC; then 2M HCl in Et_2O, rt, 24–48h, 67–75% (compounds (–)-**6a** and (+)-**6a** were further purified using preparative HPLC and obtained as TFA salts).

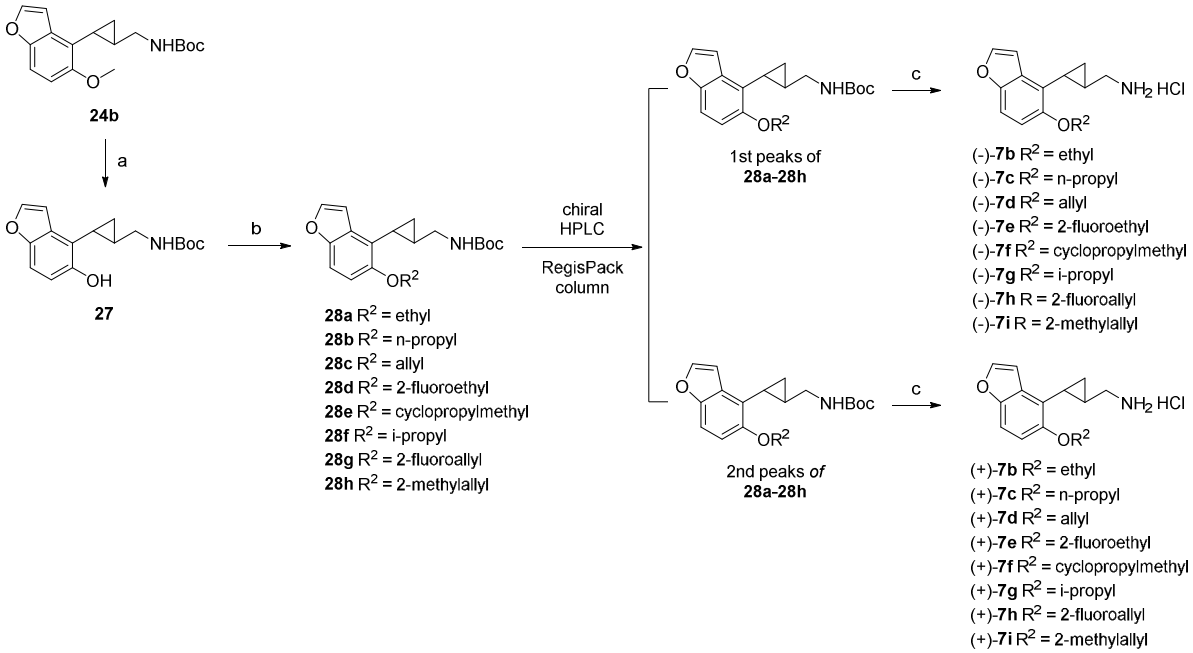
Preparation of the compounds bearing a 5-alkoxy group other than methoxy was accomplished by de-methylation of intermediates **24a**–**c** followed by alkylation with the appropriate alkyl halides. The syntheses of these compounds are outlined in Schemes 3–5 respectively. The demethylation and alkylation conditions are similar to those we had previously reported for the 2-phenylcyclopropylmethylamines.[25] Notably, chiral separation of the 2,3-dihydrobenzofurans was accomplished using the phenol intermediate **25**, while for the benzofuran and 2-ethylbenzofuran compounds the chiral separation was carried out with the ethers **28a**–**h** and **30a**–**e**. These protocols were used due to the fact that chiral separation of intermediates **26a**–**26e** proved very difficult using the chiral columns we had available. Therefore the resolution was performed one step earlier and the phenol intermediate **25** was separated efficiently with a RegisPack column. Similarly, the final compounds were obtained by removal of the Boc protecting group using HCl in diethyl ether.

Scheme 3. Synthesis of compounds **6b**–**6f**. [a]



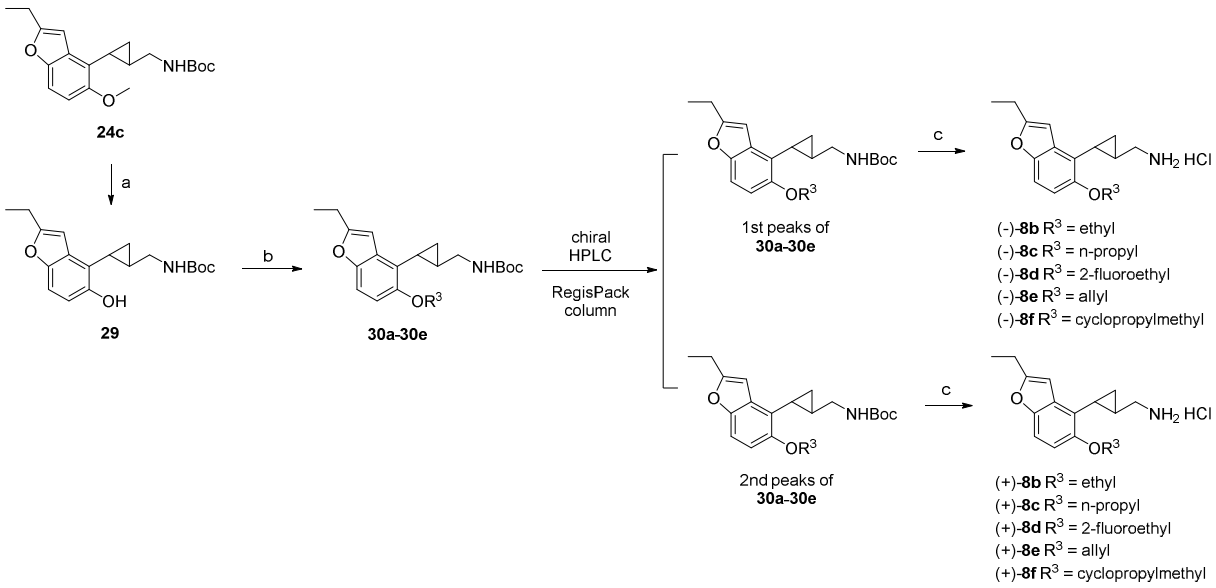
[a]Reagents and conditions: (a) CH_2Cl_2, BBr_3, –78 ºC to rt, 3 h; then Et_3N, Boc_2O, rt, 0.5 h, 90%; (b) R^1X (X = Br or I), Cs_2CO_3, DMF for **26a**–**26d,** 59–94%; 2-fluoroethanol, Ph_3P, diethyl azodicarboxylate, THF for **26e**, 19%; (c) 2M HCl in Et_2O, rt, 24–48h, 62–84%.

Scheme 4. Synthesis of compounds **7b**–**7i**. [a]



[a]Reagents and conditions: (a) CH_2Cl_2, BBr_3, –78 ºC to rt, 3 h; then Et_3N, Boc_2O, rt, 0.5 h, 77%; (b) 2-fluoroethanol, Ph_3P, diethyl azodicarboxylate, THF for **28d**, 99%; R^2X (X = Cl, Br, or I), Cs_2CO_3, DMF for others, 74–95%; (c) 2M HCl in Et_2O, rt, 24–48h, 57–70%.

Scheme 5. Synthesis of compounds **8b**–**8f**. [a]



[a]Reagents and conditions: (a) CH_2Cl_2, BBr_3, –78 ºC to rt, 3 h; then Et_3N, Boc_2O, rt, 0.5 h, 86%; (b) 2-fluoroethanol, Ph_3P, diethyl azodicarboxylate, THF for **30c**, 91%; R^3X (X = Br or I), Cs_2CO_3, DMF for others, 94–100%; (c) 2M HCl in Et_2O, rt, 24–48h, 60–85%.

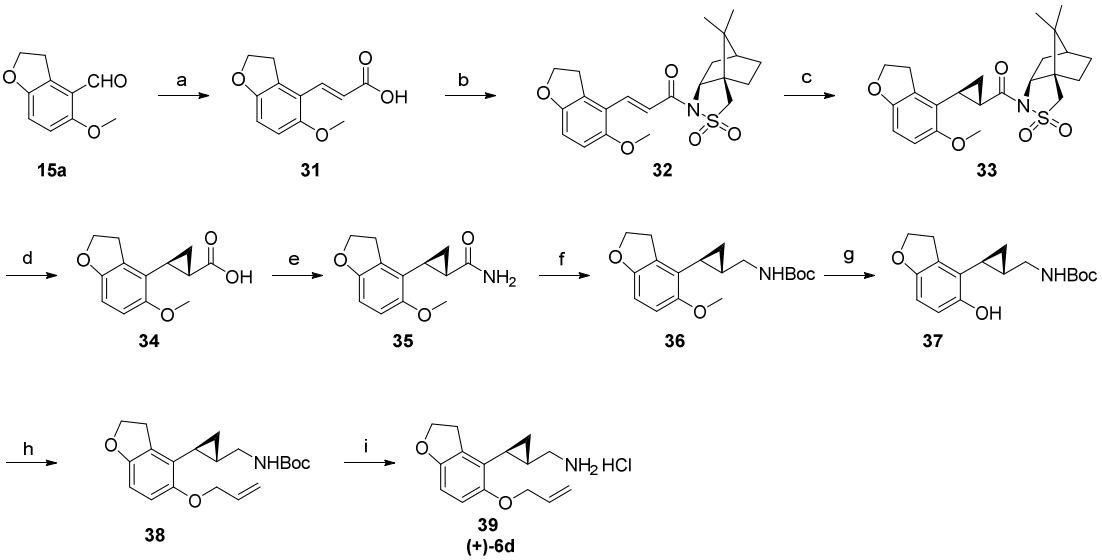
In our previous work, the absolute configurations of both enantiomers of 2-phenylcyclopropylmethylamine were assigned by comparison of the optical rotations of their synthetic intermediates to those of known compounds.[14] Based on that, we subsequently assigned the absolute configurations of other analogs (which possess an extra 2-alkoxy substitution on the benzene ring) based on the very good correlation of compound potency with the direction of their optical rotations (namely, the (+)-enantiomer is always more potent than the (–)-enantiomer).[9, 14, 25] Therefore, we assume that an enantiomer with a negative optical rotation possesses the 1*R*,2*R*-configuration, while a compound showing a positive optical rotation has the

1*S*,2*S*-configuration. An X-ray crystal structure would be an ideal way to test whether this is correct. However, for the new compounds described in this work, we have failed to obtain single crystals for X-ray diffraction. An alternative way to address this problem is to use a well-proven asymmetric synthesis method to prepare a representative compound in the series, and then to compare its optical rotation with that of the equivalent compound obtained from chiral HPLC separation. Since chiral separation in the 2,3-dihydrobenzofuran series was found to be relatively difficult, we chose compound (+)-**6d** as a representative compound to test this approach.

A camphorsultam-directed asymmetric cyclopropanation reaction,[28-30] for which the absolute configuration of the dominant product could be readily predicted, was used as the key step for the asymmetric synthesis of compound (+)-**6d**. As shown in Scheme 6 (see Supporting Information for experimental details), aldehyde **15a** obtained as described in Scheme 1 was converted to the cinnamic acid **31** through Knoevenagel condensation with malonic acid, followed by the coupling with (1*R*)-(+)-2,10-camphorsultam to produce cinnamamide **32**. The cyclopropanation of substrate **32** would favor a desired inward attack on the double bond. Twenty equivalents of diazomethane was used in this reaction to achieve a greater than 95% conversion of the cinnamamide, and the reaction proceeded in a 96.5/3.5 diastereoselectivity based on HPLC analysis of the crude product. Recrystallization of the crude product from ethanol afforded compound **33** with greater than 99% *de* purity and 58% yield. The higher diastereoselectivity of this reaction compared to other reported examples (which are mostly below 10 : 1),[28, 29] is possibly due to the

di-*ortho* substitution pattern of substrate **32**, which would force a higher π-face selectivity. Subsequent hydrolysis of **33** with a solution of lithium hydroxide provided acid **34** in quantitative yield, which was then converted to the corresponding amide **35** in excellent yield. Reduction of **35** was brought about using borane, which was followed by Boc protection of the free amine to provide intermediate **36**. De-methylation of **36** using BBr_3 afforded the phenol **37**, which was subjected to the same allylation and de-protection procedures as described in Scheme 2 to provide compound **39**. The spectral data and optical rotation value of **39** were in agreement with those of (+)-**6d** which was obtained from chiral HPLC separation. This result would thus confirm that the absolute configuration of (+)-enantiomers is (*S*, *S*) and that of (–)-enantiomers is (*R*, *R*). Based on this result, we thus assume that the absolute configurations of compounds (–)-**6a**–**6f**, (–)-**7a**–**7i**, and (–)-**8a**–**8f** are (*R*,*R*) while those of compounds (+)-**6a**–**6f**, (+)-**7a**–**7i**, and (+)-**8a**–**8f** are (*S*,*S*).

Scheme 6. Camphorsultam-Directed Asymmetric Synthesis of Compound (+)-**6d**.[a]



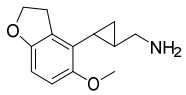
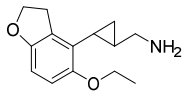
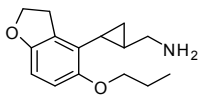
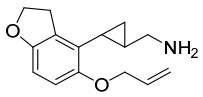
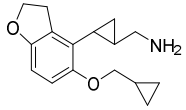
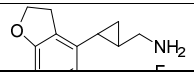
[a]Reagents and conditions: (a) malonic acid, pyrrolidine, pyridine, reflux, 2 h, 94%; (b) $SOCl_2$, rt, 2 h; then (1*R*)-(+)-2,10-camphorsultam, NaH, THF, 0 °C to rt, overnight, 90%; (c) diazomethane, $Pd(OAc)_2$, CH_2Cl_2/Et_2O, 0 °C to rt, overnight, 58%; (d) LiOH, H_2O/THF, 50 °C, 3 h, 100%; (e) $SOCl_2$, toluene, 80 °C, 2 h; then NH_4OH (28%), 1,4-dioxane, 0 °C to rt, 0.5 h, 98%; (f) BH_3-THF (1.0 M in THF), THF, reflux, 4 h; then Boc_2O, Et_3N, CH_2Cl_2, rt, 0.5 h, 74%; (g) BBr_3, CH_2Cl_2, –78 °C to rt, 3 h; then Boc_2O, Et_3N, rt, 0.5 h, 95%; (h) allyl bromide, Cs_2CO_3, DMF, microwave, 80 °C, 0.5 h, 83%; (i) 2M HCl in Et_2O, rt, 48 h, 74%.

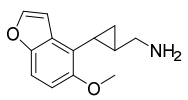
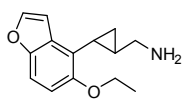
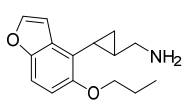
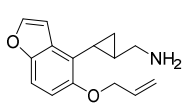
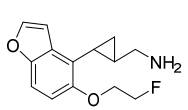
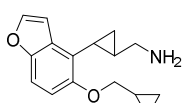
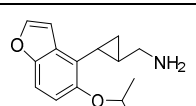
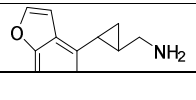
Pharmacology

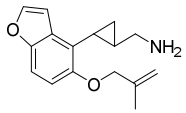
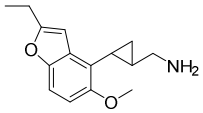
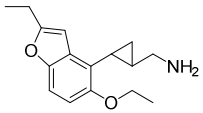
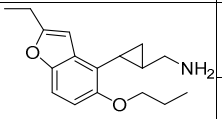
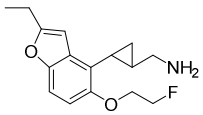
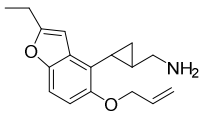
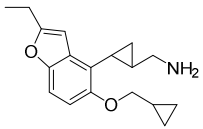
5-HT$_2$ Receptor Screening. All new compounds were screened employing recombinant, stably expressed human 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors in the HEK-293 cell line, using a fluorescence imaging plate reader (FLIPR) assay as we

described previously.[9, 25] Estimates of E_{max}, EC_{50}, and $\log(E_{max}/EC_{50})$, which is a relative activity calculation to account for partial agonist differences, are found in Table 1. Serotonin was used as a positive control and its E_{max} values were normalized to 100% for all receptors. The FDA approved 5-HT_{2C} agonist lorcaserin (EC_{50} = 2.64 nM, E_{max} = 100%, $\log(E_{max}/EC_{50})$ = 8.58) was used as a reference compound for comparison purposes.

Table 1. Pharmacological Profiling of Compounds (–)-/(+)-**6a**–**6f**, (–)-/(+)-**7a**–**7i**, and (–)-/(+)-**8a**–**8f** at 5-HT_2 Receptors in Calcium Flux Assay.[a]

STRUCTURE	ID	h5-HT_{2C}				h5-HT_{2B}				h5-HT_{2A}			
		pEC_{50}	EC_{50} (nM)	E_{max}	Log(E_{max} / EC_{50})	pEC_{50}	EC_{50} (nM)	E_{max}	Log(E_{max} / EC_{50})	pEC_{50}	EC_{50} (nM)	E_{max}	Log(E_{max} / EC_{50})
-	serotonin	9.78 ± 0.02	0.17	100 ± 0.5	9.77	8.84 ± 0.03	1.46	100 ± 1.1	8.84	8.63 ± 0.02	2.35	100 ± 0.7	8.63
-	lorcaserin	8.58 ± 0.01	2.64	100 ± 0.7	8.58	6.36 ± 0.03	433	80 ± 1.6	6.27	6.61 ± 0.01	248	68 ± 0.5	6.44
	(–)-**6a**	7.71 ± 0.02	19	102 ± 1.1	7.73	6.56 ± 0.02	279	45 ± 0.6	6.21	6.53 ± 0.01	295	66 ± 0.7	6.35
	(+)-**6a**	8.05 ± 0.02	8.9	101 ± 0.8	8.05	7.12 ± 0.01	77	70 ± 0.5	6.96	6.93 ± 0.02	117	90 ± 0.9	6.89
	(–)-**6b**	7.39 ± 0.01	41	105 ± 0.6	7.41	6.11 ± 0.03	781	30 ± 0.8	5.58	6.01 ± 0.02	976	35 ± 0.6	5.55
	(+)-**6b**	8.57 ± 0.01	2.7	106 ± 0.7	8.59	7.23 ± 0.02	54	58 ± 0.6	7.03	6.95 ± 0.02	113	81 ± 1.0	6.86
	(–)-**6c**	7.02 ± 0.01	96	94 ± 0.7	6.99	NA	NA	NA	NA	NA	NA	NA	NA
	(+)-**6c**	7.69 ± 0.01	20	104 ± 0.6	7.72	6.59 ± 0.03	258	31 ± 0.5	6.08	6.13 ± 0.01	749	31 ± 0.3	5.62
	(–)-**6d**	7.03 ± 0.01	94	90 ± 0.8	6.98	NA	NA	NA	NA	NA	NA	NA	NA
	(+)-**6d**	7.96 ± 0.01	11	101 ± 0.7	7.96	6.59 ± 0.03	258	35 ± 0.7	6.13	6.20 ± 0.06	618	49 ± 0.7	5.90
	(–)-**6e**	6.55 ± 0.02	283	65 ± 1.0	6.36	NA	NA	NA	NA	NA	NA	NA	NA
	(+)-**6e**	7.58 ± 0.01	26	95 ± 0.7	7.56	6.57 ± 0.05	269	33 ± 1.0	6.09	6.12 ± 0.04	754	17 ± 0.5	5.35
	(–)-**6f**	6.52±0.04	302	79±3.7	6.42	NA	NA	NA	NA	5.71±0.08	1960	55±4.3	5.45

	(+)-**6f**	7.13±0.03	73	76±3.4	7.02	NA	NA	NA	NA	5.63±0.09	2320	63±4.5	5.43
	(–)-**7a**	8.17 ± 0.02	6.6	96 ± 0.9	8.16	6.14 ± 0.18	725	55 ± 8.8	5.88	6.96 ± 0.02	109	66 ± 0.6	6.78
	(+)-**7a**	8.79 ± 0.03	1.6	93 ± 0.9	8.76	6.96 ± 0.12	111	64 ± 5.12	6.76	7.43 ± 0.02	37	86 ± 1.0	7.37
	(–)-**7b**	7.59 ± 0.02	26	107 ± 1.2	7.61	NA	NA	NA	NA	6.23 ± 0.02	585	15 ± 0.2	5.41
	(+)-**7b**	8.59 ± 0.01	2.6	106 ± 0.7	8.61	7.07 ± 0.03	84	50 ± 0.8	6.77	6.96 ± 0.02	111	74 ± 1.0	6.82
	(–)-**7c**	7.06 ± 0.02	87	88 ± 1.1	7.00	NA	NA	NA	NA	NA	NA	NA	NA
	(+)-**7c**	7.99 ± 0.02	10	98 ± 1.1	7.99	NA	NA	NA	NA	6.41 ± 0.02	392	21 ± 0.3	5.73
	(–)-**7d**	7.25 ± 0.01	56	101 ± 0.9	7.26	NA	NA	NA	NA	NA	NA	NA	NA
	(+)-**7d**	8.27 ± 0.03	5.3	103 ± 1.6	8.29	6.46 ± 0.06	348	31 ± 1.1	5.95	6.67 ± 0.02	213	46 ± 0.8	6.33
	(–)-**7e**	7.50 ± 0.02	33	98 ± 1.1	7.47	6.35 ± 0.04	448	34 ± 0.9	5.88	6.41 ± 0.02	393	22 ± 0.4	5.75
	(+)-**7e**	9.22 ± 0.05	0.59	97 ± 1.5	9.22	7.34 ± 0.06	46	103 ± 2.8	7.35	7.65 ± 0.05	22	98 ± 1.8	7.65
	(–)-**7f**	6.95 ± 0.01	113	87 ± 0.8	6.89	6.29 ± 0.06	524	27 ± 1.2	5.71	NA	NA	NA	NA
	(+)-**7f**	7.91 ± 0.01	12	99 ± 0.8	7.92	6.47 ± 0.05	180	27 ± 0.8	6.18	6.34 ± 0.03	456	25 ± 0.6	5.74
	(–)-**7g**	7.67 ± 0.02	21	97 ± 1.0	7.66	6.34 ± 0.24	453	25 ± 4.2	5.74	6.03 ± 0.11	938	38 ± 3.8	5.61
	(+)-**7g**	8.21 ± 0.01	6.2	99 ± 0.6	8.20	6.76 ± 0.04	174	39 ± 1.0	6.35	6.53 ± 0.03	298	79 ± 1.4	6.42
	(–)-**7h**	7.00 ± 0.01	99	93 ± 0.8	6.97	6.23 ± 0.05	585	29 ± 1.2	5.70	NA	NA	NA	NA

	(+)-**7h**	8.03 ± 0.01	9.2	103 ± 0.8	8.05	6.89 ± 0.03	128	38 ± 0.6	6.47	6.45 ± 0.02	358	49 ± 0.7	6.14
	(–)-**7i**	6.58 ± 0.03	261	70 ± 0.9	6.43	6.14 ± 0.07	733	33 ± 1.7	5.65	NA	NA	NA	NA
	(+)-**7i**	7.60 ± 0.02	25	99 ± 0.9	7.60	6.82 ± 0.03	150	40 ± 0.6	6.43	6.59 ± 0.02	260	19 ± 0.3	5.86
	(–)-**8a**	6.99 ± 0.01	101	88 ± 0.7	6.94	NA	NA	NA	NA	NA	NA	NA	NA
	(+)-**8a**	8.57 ± 0.02	2.7	101 ± 1.0	8.57	7.22 ± 0.03	60	55 ± 0.7	6.96	6.03 ± 0.02	939	72 ± 1.6	5.88
	(–)-**8b**	6.94 ± 0.01	115	76 ± 0.8	6.82	NA	NA	NA	NA	NA	NA	NA	NA
	(+)-**8b**	7.84 ± 0.02	15	100 ± 0.8	7.82	6.51 ± 0.04	309	36 ± 0.9	6.07	5.58 ± 0.01	2598	35 ± 0.5	5.13
	(–)-**8c**	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	(+)-**8c**	7.09 ± 0.01	81	96 ± 0.6	7.07	NA	NA	NA	NA	NA	NA	NA	NA
	(–)-**8d**	7.09 ± 0.01	81	84 ± 1.7	7.02	NA	NA	NA	NA	NA	NA	NA	NA
	(+)-**8d**	7.96 ± 0.01	11	106 ± 0.9	7.98	6.26 ± 0.19	550	27 ± 3.7	5.69	5.66 ± 0.04	2210	44 ± 2.0	5.30
	(–)-**8e**	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	(+)-**8e**	7.65 ± 0.02	23	102 ± 0.9	7.65	6.36 ± 0.15	433	19 ± 2.1	5.64	NA	NA	NA	NA
	(–)-**8f**	6.35 ± 0.02	446	69 ± 1.0	6.19	NA	NA	NA	NA	NA	NA	NA	NA
	(+)-**8f**	6.86 ± 0.01	138	101 ± 0.6	6.86	NA	NA	NA	NA	NA	NA	NA	NA

[a]All new compounds were tested as HCl salts except compounds (–)-**6a** and (+)-**6a** which are TFA salts; pharmacological data were acquired with recombinant, stably expressed human 5-HT_{2C}, 5-HT_{2A}, and 5-HT_{2B} receptors in the HEK-293 cell line, using a fluorescence imaging plate reader (FLIPR) assay; pEC_{50} and E_{max} values are shown as mean ± SEM ($n \geqq 2$); EC_{50} values were calculated from averaged pEC_{50}s; "NA", no activity up to 10 μM; "-", structures of serotonin and lorcaserin are not shown.

As can be seen from Table 1, most of the new compounds show potent agonism of the 5-HT_{2C} receptor and display selectivity against the 5-HT_{2A} and 5-HT_{2B} receptors. Overall the (+)-isomers are more potent than their (–)-isomers within each pair of enantiomers, and increasing the size of the alkoxyl group at position 5 of the 2,3-dihydrobenzofuran or benzofuran scaffold decreases potency in most cases, while enhancing compound selectivity for the 5-HT_{2C} receptor. These observations are consistent with our previous findings. [9, 25] For compounds **6a**–**f** which bear the 2,3-dihydrobenzofuran scaffold **6**, ethoxy substitution provides the best potency (compound (+)-**6b**: EC_{50} = 2.7 nM, E_{max} = 106%, log(E_{max}/EC_{50}) = 8.59), whereas the elongation of carbon chain leads to a significant loss of potency as exemplified by compound (+)-**6c** (EC_{50} = 20 nM). Maintaining the four-atom length of the alkoxy chain, allyloxy substitution in compound (+)-**6d** (EC_{50} = 11 nM) increases compound potency while cyclopropylmethyoxy slightly decreases the activity (compound (+)-**6e**, EC_{50} = 26 nM). The presence of a 2-fluoroethoxy substituent as in compound (+)-**6f** results in a reduction in potency at the 5-HT_{2C} receptors (EC_{50} = 73 nM), but a gain of selectivity against the 5-HT_{2B} receptors was achieved as (+)-**6f** shows no 5-HT_{2B} activation at concentrations up to 10 μM.

Changing from the 2,3-dihydrobenzofuran backbone to benzofuran leads to an overall enhancement of 5-HT_{2C} potency, as observed for compound **7a**–**7i**. Compared to compound (+)-**6a**, (+)-**7a** shows improved potency at 5-HT_{2C} (EC_{50} = 1.6 nM, E_{max} = 93%) as well as better selectivity against 5-HT_{2B} (111/1.6 = 69-fold) and 5-HT_{2A} (37/1.6 = 23-fold). For the propyl compound **7c**, the (+)-enantiomer shows 10 nM potency at 5-HT_{2C} receptors, no activity at 5-HT_{2B}, and good selectivity against 5-HT_{2A} (EC_{50} = 392 nM). Compared to lorcaserin, (+)-**7c** represents one of the best ligands in this series of compounds in terms of both potency and selectivity. Our efforts in introducing other alkoxy substitutions as shown for compounds **7d**–**7i** did not lead to any significant improvements in selectivity as compared

to compound (+)-**7c**, but the majority of these compounds showed high potency and moderate selectivity. Compound (+)-**7e** which bears the 2-fluoroethoxy substitution, displays the best potency among all compounds (EC_{50} = 0.59 nM).

The medium sized ethyl group was introduced to position 2 of the benzofuran backbone to further explore the ligand chemical space at this position. As can be seen from compounds **8a**–**8f**, the additional ethyl group slightly decreases the 5-HT_{2C} potency of these compounds compared to compounds **7a**–**7i**, but provides much better selectivity against the 5-HT_{2B} and 5-HT_{2A} receptors. Among them, compound (+)-**8d** showed an EC_{50} value of 11 nM at 5-HT_{2C} receptors, 50-fold selectivity against 5-HT_{2B} (EC_{50} = 550 nM and over 200-fold selectivity against 5-HT_{2A} (EC_{50} = 2210 nM). Given that log(E_{max}/EC_{50}) values of (+)-**8d** at both the 5-HT_{2B} and 5-HT_{2A} receptors are much lower (5.69 and 5.30, respectively), we would consider this ligand to be more selective than lorcaserin (5-HT_{2B} log(E_{max}/EC_{50}) = 6.27; 5-HT_{2A} log(E_{max}/EC_{50}) = 6.44). Compound (+)-**8e** (EC_{50} = 23 nM) is 2-fold weaker than (+)-**8d** at 5-HT_{2C}, but shows similar weak 5-HT_{2B} activity (EC_{50} = 433 nM, E_{max} = 19%) and no 5-HT_{2A} activation. Compounds (+)-**8c** and (+)-**8f** are weaker in terms of their activation of 5-HT_{2C} receptors (EC_{50} = 81 and 138 nM respectively), but both of them show excellent selectivity against the other two receptors as no agonist activity was observed for either 5-HT_{2B} or 5-HT_{2A}.

Taken together, the majority of the compounds bearing the new scaffolds **6**–**8** are selective 5-HT_{2C} agonists showing moderate to high selectivity against both the 5-HT_{2A} and 5-HT_{2B} receptors. Given the fact that the E_{max} ranges of most of these compounds at 5-HT_{2A} and 5-HT_{2B} receptors are much lower than those at 5-HT_{2C} receptors, a number of compounds such as (+)-**6d**, (+)-**7c**, (+)-**8d**, and (+)-**8e** represent good candidates for further studies. It is important to note that some of these compounds bind to the 5-HT_{2A} and 5-HT_{2B} receptors at moderate to weak affinities (see Supporting Information, Table S1), but their low intrinsic

efficacy exclude them from mediating side effects through activation of those receptors. Although these compounds possess close structural similarity with the melatonin agonist tasimelteon, no activity at the melatonin receptors was anticipated, as the melatonin receptor agonists bear an amide functional group rather than a primary amine.[31] We did in fact test some of these compounds at melatonin MT_1 and MT_2 receptors examining $G_{i/o}$ function, and observed either no activity or very weak agonist activity (EC_{50} > 1 μM) at both receptors (see Supporting Information, Figures S1 and S2).

5-HT_{2C} Functional Selectivity

Agomelatine has been investigated for its antidepressant potential and shown to be a neutral antagonist blocking both G_q activity and β-arrestin recruitment at 5-HT_{2C} receptors.[8] Despite this study, few 5-HT_{2C} ligands have been thoroughly and systematically investigated for β-arrestin recruitment and ligand bias. Therefore, we sought to investigate β-arrestin recruitment efficacy with the above 5-HT_{2C}-selective benzofuran ligands and to compare their functional selectivity profile to lorcaserin and 5-HT. We measured β-arrestin recruitment activity using a modified reporter assay,[32] and compared activities to G_q-mediated calcium flux for our newly synthesized 5-HT_{2C}-selective benzofuran ligands, which was performed in parallel using the same drug dilutions and conditions. Our results indicate that lorcaserin recruited β-arrestin with almost full efficacy compared to 5-HT (Table 2, Figure 2). By contrast, all tested benzofuran compounds exhibited diminished β-arrestin recruitment efficacy (range 22–49%) compared to lorcaserin (89%) and 5-HT (100%), indicating that the 5-HT_{2C}-selective benzofuran ligands exhibit functional selectivity with preference for Gq-mediated calcium flux (Figure 2). To quantify the extent of preference for Gq signaling, we calculated bias factors[17] using relative activities (log E_{max}/EC_{50}) for each respective pathway. The relative activity (log E_{max}/EC_{50}) of compounds can be used as a surrogate for the Black and Leff operational model estimation of log (τ/K_A) provided that the

hill slope does not substantially deviate from 1,[33] which in the case of these compounds it did not. Using lorcaserin as the reference ligand to distinguish our newly synthesized 5-HT_{2C}-selective benzofuran ligands from it, we found that compound (+)-**7e** showed the best preference for Gq signaling (bias factor = 3.8, Table 2). This Gq preference of (+)-**7e** is mainly driven by its extremely weak arrestin recruitment efficacy (E_{max} = 28%), thus demonstrating functionally selective effects compared to lorcaserin and 5-HT.

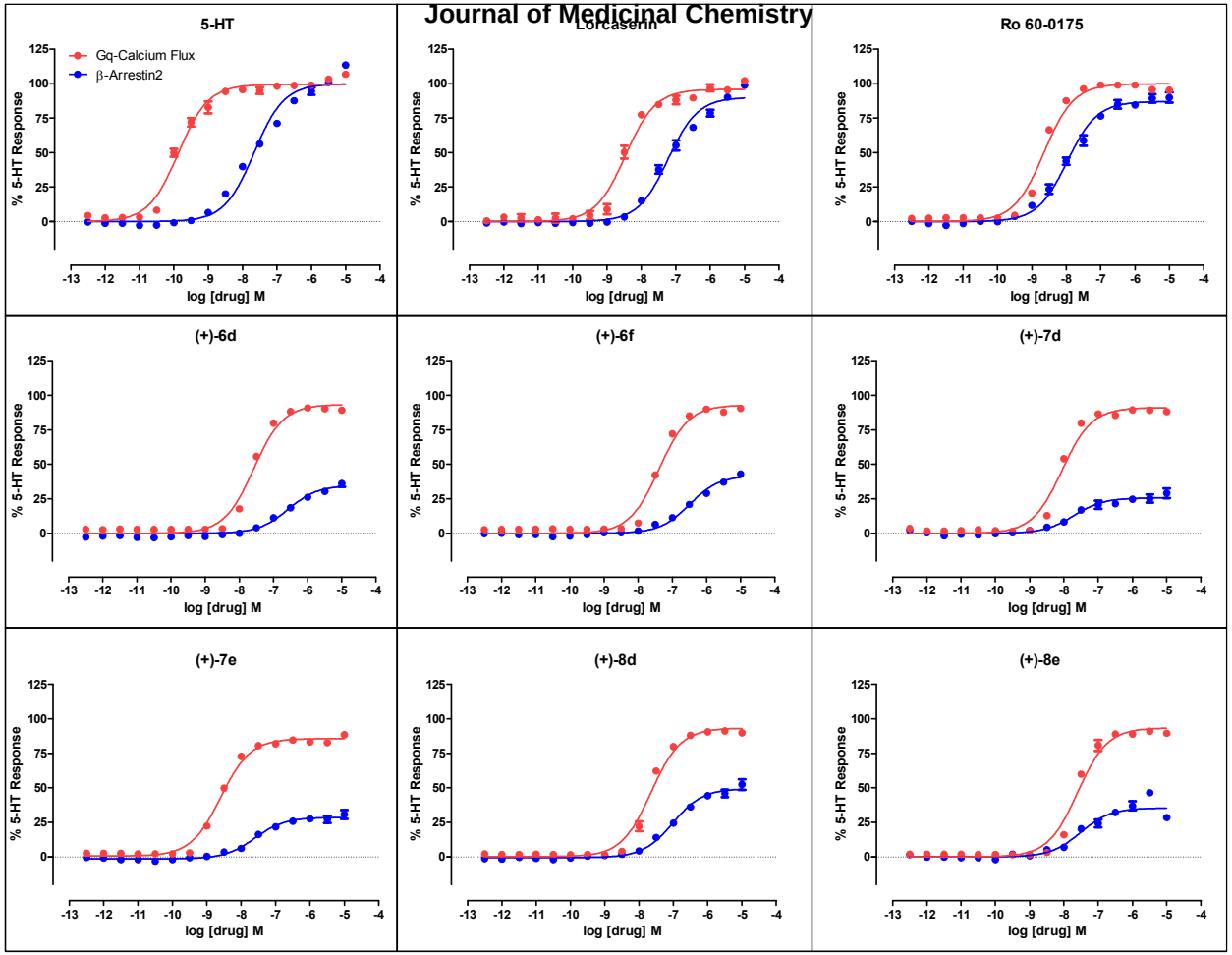


Figure 2. Parallel 5-HT_{2C} screening for functional selectivity of 5-HT_{2C}-selective compounds examining Gq-mediated calcium flux (FLIPR, red) and β-arrestin-2 recruitment (Tango, blue). Data were acquired with the human 5-HT_{2C} INI receptor isoform measuring Gq-calcium flux (FLIPR) or β-arrestin-2 recruitment (Tango) performed in triplicate. Assays were conducted in parallel with the same drug dilutions

Table 2. Assessment of functional selectivity for 5-HT_{2C}-selective compounds.[a]

Compound	Gq-Calcium Flux				β-arrestin-2				BIAS Factor (Gq)	BIAS Factor (β-Arr)
	pEC_{50}	EC_{50} (nM)	E_{max}	$Log(E_{max} / EC_{50})$	pEC_{50}	EC_{50} (nM)	E_{max}	$Log(E_{max} / EC_{50})$		
lorcaserin (reference)	8.46 ± 0.03	3.4	96 ± 1.1	8.45	7.23 ± 0.03	59	91 ± 1.2	7.19	1.0	1.0
5-HT	9.87 ± 0.03	0.14	100 ± 0.8	9.85	7.66 ± 0.02	21	100 ± 1.0	7.68	8.2	0.12
Ro 60-0175	8.65 ± 0.04	2.2	99 ± 1.2	8.65	7.97 ± 0.04	10	87 ± 1.2	7.94	0.3	3.3
(+)-**6d**	7.58 ± 0.03	26	93 ± 1.2	7.55	6.57 ± 0.06	267	35 ± 1.0	6.12	1.5	0.7
(+)-**6f**	7.39 ± 0.03	41	93 ± 1.3	7.36	6.49 ± 0.04	318	42 ± 0.8	6.12	0.9	1.1
(+)-**7d**	8.06 ± 0.04	8.7	92 ± 1.3	8.02	7.71 ± 0.01	20	25 ± 0.9	7.10	0.5	2.0
(+)-**7e**	8.92 ± 0.03	1.2	94 ± 0.8	8.89	7.60 ± 0.06	25	28 ± 0.7	7.05	3.8	0.3
(+)-**8d**	7.65 ± 0.04	22	93 ± 1.3	7.63	7.02 ± 0.04	96	50 ± 1.0	6.72	0.4	2.5
(+)-**8e**	7.60 ± 0.04	25	94 ± 0.8	7.58	7.52 ± 0.08	30	35 ± 1.1	7.07	0.2	5.0

[a]Data were acquired with the human 5-HT_{2C} INI receptor isoform measuring Gq-calcium flux (FLIPR) and β-arrestin-2 recruitment (Tango); pEC_{50} and E_{max} values are shown as mean ± SEM (n = 3) performed in triplicate and assays were conducted in parallel with the same drug

dilutions. Bias factors toward Gq or β-arrestin-2 were calculated using change in respective relative activity, log (E_{max}/EC_{50}), as described previously.[17]

5-HT$_{2C}$ Desensitization. Considering that arrestin recruitment is reported to cause desensitization at numerous GPCRs[22] and that compound (+)-**7e** exhibited weak arrestin recruitment efficacy, we sought to examine the 5-HT$_{2C}$ desensitization properties comparing full arrestin agonist 5-HT to weak partial arrestin agonist (+)-**7e**. To measure Gq-mediated 5-HT$_{2C}$ desensitization, we first pre-treated 5-HT$_{2C}$-expressing HEK cells with increasing concentrations of either 5-HT or (+)-**7e** for 30 min and then challenged cells after washout to release calcium upon further drug stimulation. Pre-treatment with 5-HT concentrations as low as 32 nM depressed the absolute E_{max} without affecting 5-HT's potency, and a 10 μM 5-HT pre-treatment concentration completely abolished any additional 5-HT-stimulated calcium flux (Figure 3a). By contrast, (+)-**7e** did not show E_{max} depression at pre-treatment concentrations below 1 μM (Figure 3b), suggesting that (+)-**7e** was not as efficient to desensitize 5-HT$_{2C}$ Gq-mediated calcium flux compared to similar 5-HT concentrations.

Although calcium flux is commonly investigated as a functional readout of G_q-coupled receptor desensitization, calcium flux does not allow full receptor occupancy given its short time duration. In addition, calcium flux is downstream from PLC-mediated phosphoinositide (PI) hydrolysis, which has been investigated as functional readout of 5-HT$_{2C}$ desensitization by measuring ^{3}H-inositol phosphates (IP) accumulation over time.[34] Therefore, we compared full arrestin agonist 5-HT to partial arrestin agonist (+)-**7e** expecting to observe a loss of IP accumulation over time indicative of 5-HT$_{2C}$ desensitization. First, 5-HT$_{2C}$-expressing cells produced expected elevated basal levels of ^{3}H-inositol phosphates (Figure 3e, f), consistent with a high level of constitutive activity at this receptor isoform.[35, 36] Despite the elevated level of ^{3}H-phosphoinositides, both 5-HT and (+)-**7e** at maximum receptor occupancy (1 μM, Figure S3 in Supporting Information) produced a rapid and robust increase in ^{3}H-phosphoinositides after

only 5 min drug incubation peaking around 60 min (Figure 3c). In fact, IP accumulation for both 5-HT and (+)-**7e** was sustained up to 5 hours (300 min) with no depression in absolute E_{max} (Figure 3e, f). 5-HT incubation at longer time points, however, showed decreasing IP accumulation at 600 min (10 h) with a gradual reduction back to basal levels at 40 hours (Figure 3d). Although the 5-HT E_{max} was completely abolished by at least 30 hours of incubation (Figure 3e), (+)-**7e** produced a sustained IP accumulation and only began to show a significant decrease in E_{max} after 20 hours of drug incubation (Figure 3d). Comparison of the desensitization rates of 5-HT and (+)-**7e** revealed that (+)-**7e** at 1 μM produced slower reduction in IP accumulation over time (Figure S4). In fact, (+)-**7e** still retained a dose-response at 30 h (Figure 3f), whereas 5-HT did not. Given that (+)-**7e** was previously found to produce weaker arrestin recruitment compared to 5-HT, these results support the finding that weaker desensitization is a unique contributor to (+)-**7e**'s functional selectivity profile.

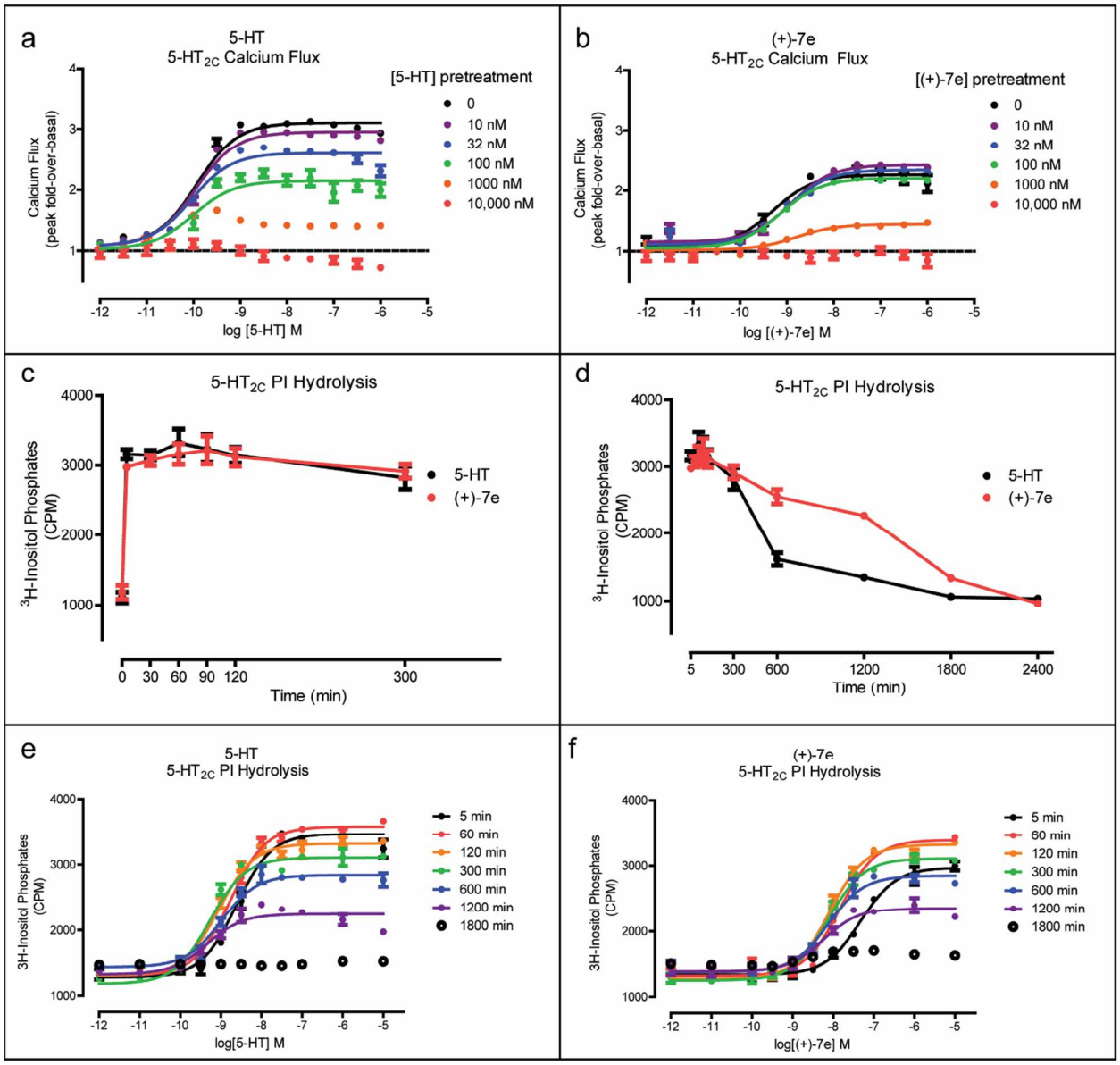


Figure 3. Functionally selective (+)-**7e** produces less 5-HT$_{2C}$ G$_q$-mediated desensitization compared to 5-HT as measured by calcium flux (**a**, **b**) and by IP accumulation (**c**–**f**). 5-HT$_{2C}$ G$_q$-mediated calcium flux comparing desensitization with different pre-treatment concentrations of either 5-HT (**a**) or (+)-**7e** (**b**) on subsequent stimulated calcium release. Time course for 5-HT$_{2C}$ IP accumulation comparing 1 μM 5-HT or (+)-**7e** at early time points (5–300 min, **c**) and at later time points (300–2400 min, **d**). Effect of IP accumulation over time on dose-response curves of 5-HT (**e**) and (+)-**7e** (**f**).

Conclusions

Inspired by the cross-talk between serotonergic and melatonergic biological functions and the structural similarity of our previous 2-phenylcyclopropylmethylamine scaffold with the melatonin receptor agonist tasimelteon, we designed and synthesized a series of compounds bearing either a 2,3-dihydrobenzofuran or a benzofuran scaffold. Pharmacological profiling of these new compounds at 5-HT_{2C}, 5-HT_{2B}, and 5-HT_{2A} receptors identified a number of compounds showing high potency and good selectivity as 5-HT_{2C} agonists. Parallel testing of selected compounds in both Gq-mediated calcium flux and β-arrestin recruitment assays revealed that these compounds show functional selectivity with weaker arrestin recruitment and less desensitization properties compared to 5-HT. These findings open the possibility of discovering novel functionally selective compounds for the 5-HT_{2C} receptor, which will serve as tools to identify therapeutically-relevant 5-HT_{2C} signaling pathways.

Experimental Section

General. All chemicals and solvents were purchased from Sigma-Aldrich or Fisher Scientific, and were used as obtained without further purification. Microwave reactions were run in a Biotage Initiator microwave reactor. Synthetic intermediates were purified on 230−400 mesh silica gel using a Teledyne CombiFlash R_f flash chromatography. ^{1}H and ^{13}C NMR spectra were recorded on Bruker DPX-400 or AVANCE-400 spectrometers at 400 MHz and 100 MHz respectively. NMR chemical shifts were reported in δ (ppm) using residual solvent peaks as standards ($CDCl_3$, 7.26 (H), 77.16 (C); CD_3OD, 3.31 (H), 49.00 (C); DMSO-d_6, 2.50 (H), 39.52 (C)). Mass spectra were measured using an LCMS-IT-TOF (Shimadzu) mass spectrometer in ESI mode. Purity of all final compounds (greater than 95% in all cases) was determined by analytical HPLC (ACE 3AQ C_{18} column (150 × 4.6 mm, particle size 3 μm); 0.05% TFA in

H_2O/0.05% TFA in MeOH gradient eluting system; flow rate = 1.0 mL/min). Chiral separation of synthetic intermediates was conducted using RegisPack (25 cm × 21.1 mm, 10 μm) or ChromegaChiral CCJ (25 cm × 20 mm, 10 μm) chiral columns, and *n*-hexane/ethanol as the eluent. Optical rotations were recorded on a Rudolph Research Autopol IV automatic polarimeter.

The synthetic procedures, chiral separation methods, and characterization data of all intermediates can be found in Supporting Information. All intermediates subjected to chiral preparative-HPLC separation were prepared with an optical purity of > 90% *ee* (determined with analytical HPLC using a RegisPack (25 cm × 4.6 mm, 10 μm) or ChromegaChiral CCJ (25 cm × 4.6 mm, 10 μm) chiral column, and *n*-hexane/ethanol as the eluent). Compounds (–)-**6a–6f**, (+)-**6a–6f**, (–)-**7a–7i**, (+)-**7a–7i**, (–)-**8a–8f**, and (+)-**8a–8f** were all obtained as white solids.

(–)-(2-(5-Methoxy-2,3-dihydrobenzofuran-4-yl)cyclopropyl)methanamine Trifluoroacetate ((–)-6a). The HCl salt obtained using the general methods as described was further purified using preparative HPLC (Shimadzu HPLC system; ACE 5AQ C18 column (150 × 21.2 mm, particle size 5 μm); 0.05% TFA in H_2O/0.05% TFA in MeOH gradient eluting system; flow rate = 17.0 mL/min), and the title compound was obtained as a white solid. ^{1}H NMR (CD_3OD) δ 6.66 (d, J = 8.8 Hz, 1H), 6.54 (d, J = 8.8 Hz, 1H), 4.53 – 4.42 (m, 2H), 3.78 (s, 3H), 3.25 – 3.16 (m, 2H), 3.11 (dd, J = 12.8, 6.8 Hz, 1H), 2.89 (dd, J = 12.8, 8.4 Hz, 1H), 1.78 – 1.75 (m, 1H), 1.39 – 1.37 (m, 1H), 1.15 – 1.09 (m, 4H), 0.98 – 0.93 (m, 1H); ^{13}C NMR (CD_3OD) δ 162.8 (q, J_{CF} = 34.9 Hz), 155.5, 154.4, 129.6, 126.3, 118.2 (q, J_{CF} = 290.7 Hz), 111.1, 107.9, 72.4, 56.6, 45.6, 30.5, 18.4, 17.8, 12.6; HRMS calcd for $C_{13}H_{18}NO_2^+$ ($[M + H]^+$): 220.1332, found: 220.1333; $[\alpha]_D^{20}$ – 39.0 (*c* 0.1, MeOH).

(+)-(2-(5-Methoxy-2,3-dihydrobenzofuran-4-yl)cyclopropyl)methanamine Trifluoroacetate ((+)-6a). The HCl salt obtained using the general methods as described was further purified using preparative HPLC as described for compound (–)-**6a**, and the title compound was obtained as a white solid. ^{1}H NMR (CD_3OD) δ 6.66 (d, J = 8.8 Hz, 1H), 6.54 (d, J = 8.8 Hz, 1H), 4.53 – 4.42 (m, 2H), 3.78 (s, 3H), 3.25 – 3.16 (m, 2H), 3.11 (dd, J = 12.8, 6.8 Hz, 1H), 2.89 (dd, J = 12.8, 8.4 Hz, 1H), 1.78 – 1.75 (m, 1H), 1.39 – 1.37 (m, 1H), 1.15 – 1.09 (m, 4H), 0.98 – 0.93 (m, 1H); ^{13}C NMR (CD_3OD) δ 162.8 (q, J_{CF} = 34.8 Hz), 155.5, 154.4, 129.6, 126.3, 118.2 (q, J_{CF} = 290.7 Hz), 111.1, 107.9, 72.4, 56.6, 45.6, 30.5, 18.4, 17.8, 12.6; HRMS calcd for $C_{13}H_{18}NO_2^+$ ($[M + H]^+$): 220.1332, found: 220.1332; $[\alpha]_D^{20}$ +34.7 (*c* 0.5, MeOH).

(–)-(2-(5-Ethoxy-2,3-dihydrobenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((–)-6b). ^{1}H NMR (CD_3OD) δ 6.65 (d, J = 8.4 Hz, 1H), 6.51 (d, J = 8.4 Hz, 1H), 4.59 – 4.44 (m, 2H), 4.02 – 3.95 (m, 2H), 3.25 – 3.18 (m, 2H), 3.14 (dd, J = 13.2, 6.8 Hz, 1H), 2.89 (dd, J = 13.2, 8.0 Hz, 1H), 1.81 – 1.77 (m, 1H), 1.51 – 1.46 (m, 1H), 1.41 (t, J = 6.8 Hz, 3H), 1.21 – 1.16 (m, 1H), 0.99 – 0.94 (m, 1H); HRMS calcd for $C_{14}H_{20}NO_2^+$ ($[M + H]^+$): 234.1489, found: 234.1486; $[\alpha]_D^{20}$ –42.0 (*c* 0.1, MeOH).

(+)-(2-(5-Ethoxy-2,3-dihydrobenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((+)-6b). ^{1}H NMR (CD_3OD) δ 6.65 (d, J = 8.4 Hz, 1H), 6.51 (d, J = 8.4 Hz, 1H), 4.59 – 4.44 (m, 2H), 4.01 – 3.95 (m, 2H), 3.25 – 3.18 (m, 2H), 3.14 (dd, J = 13.2, 6.8 Hz, 1H), 2.89 (dd, J = 13.2, 8.0 Hz, 1H), 1.80 – 1.77 (m, 1H), 1.51 – 1.48 (m, 1H), 1.41 (t, J = 7.2 Hz, 3H), 1.21 – 1.17 (m, 1H), 0.99 – 0.94 (m, 1H); ^{13}C NMR (CD_3OD) δ 155.4, 153.6, 129.4, 126.6, 112.6, 107.9, 72.4, 65.8, 45.6, 30.6, 18.4, 18.2, 15.5, 12.8; HRMS calcd for $C_{14}H_{20}NO_2^+$ ($[M + H]^+$): 234.1489, found: 234.1482; $[\alpha]_D^{20}$ +37.0 (*c* 0.1, MeOH).

(–)-(2-(5-Propoxy-2,3-dihydrobenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((–)-6c). ^{1}H NMR (CD_3OD) δ 6.64 (d, J = 8.4 Hz, 1H), 6.51 (d, J = 8.4 Hz, 1H), 4.51 – 4.45 (m, 2H), 3.91 – 3.83 (m, 2H), 3.25 – 3.20 (m, 3H), 2.81 (dd, J = 13.2, 8.8 Hz, 1H), 1.86 – 1.77 (m, 3H), 1.55 – 1.52 (m, 1H), 1.23 – 1.20 (m, 1H), 1.07 (t, J = 7.2 Hz, 3H), 0.99 – 0.94 (m, 1H); HRMS calcd for $C_{15}H_{22}NO_2^+$ ([M + H]$^+$): 248.1645, found: 248.1648; $[\alpha]_D^{20}$ –47.2 (*c* 0.1, MeOH).

(+)-(2-(5-Propoxy-2,3-dihydrobenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((+)-6c). ^{1}H NMR (CD_3OD) δ 6.64 (d, J = 8.4 Hz, 1H), 6.51 (d, J = 8.4 Hz, 1H), 4.53 – 4.45 (m, 2H), 3.91 – 3.83 (m, 2H), 3.25 – 3.20 (m, 3H), 2.81 (dd, J = 13.2, 8.8 Hz, 1H), 1.86 – 1.77 (m, 3H), 1.57 – 1.52 (m, 1H), 1.23 – 1.19 (m, 1H), 1.07 (t, J = 7.2 Hz, 3H), 0.99 – 0.94 (m, 1H); ^{13}C NMR (CD_3OD) δ 155.4, 153.7, 129.4, 126.7, 112.5, 107.9, 72.3, 71.9, 45.6, 30.6, 24.0, 18.4, 18.2, 12.9, 11.2; HRMS calcd for $C_{15}H_{22}NO_2^+$ ([M + H]$^+$): 248.1645, found: 248.1638; $[\alpha]_D^{20}$ +44.7 (*c* 0.15, MeOH).

(–)-(2-(5-(Allyloxy)-2,3-dihydrobenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((–)-6d). ^{1}H NMR (CD_3OD) δ 6.67 (d, J = 8.4 Hz, 1H), 6.51 (d, J = 8.4 Hz, 1H), 6.15 – 6.08 (m, 1H), 5.42 (dd, J = 17.2, 1.6 Hz, 1H), 5.28 (dd, J = 10.4, 1.2 Hz, 1H), 4.53 – 4.45 (m, 4H), 3.27 – 3.19 (m, 2H), 3.11 (dd, J = 12.8, 7.2 Hz, 1H), 2.92 (dd, J = 12.8, 8.0 Hz, 1H), 1.83 – 1.80 (m, 1H), 1.49 – 1.47 (m, 1H), 1.22 – 1.16 (m, 1H), 1.00 – 0.95 (m, 1H); HRMS calcd for $C_{15}H_{20}NO_2^+$ ([M + H]$^+$): 246.1489, found: 246.1483; $[\alpha]_D^{20}$ –62.7 (*c* 0.1, MeOH).

(+)-(2-(5-(Allyloxy)-2,3-dihydrobenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((–)-6d). ^{1}H NMR (CD_3OD) δ 6.67 (d, J = 8.4 Hz, 1H), 6.51 (d, J = 8.4 Hz, 1H), 6.15 – 6.08 (m, 1H), 5.42 (dd, J = 17.2, 1.6 Hz, 1H), 5.28 (dd, J = 10.4, 1.2 Hz, 1H), 4.51 – 4.45 (m, 4H), 3.26 –

3.19 (m, 2H), 3.11 (dd, *J* = 12.8, 7.2 Hz, 1H), 2.92 (dd, *J* = 12.8, 8.0 Hz, 1H), 1.83 – 1.80 (m, 1H), 1.49 – 1.47 (m, 1H), 1.22 – 1.16 (m, 1H), 1.00 – 0.95 (m, 1H); ^{13}C NMR (CD_3OD) δ 155.7, 153.3, 135.5, 129.5, 126.9, 118.2, 113.1, 107.9, 72.4, 71.4, 45.6, 30.6, 18.4, 18.2, 12.8; HRMS calcd for $C_{15}H_{20}NO_2^+$ ($[M + H]^+$): 246.1489, found: 246.1485; $[\alpha]_D^{20}$ +56.3 (*c* 0.15, MeOH).

(–)-(2-(5-(Cyclopropylmethoxy)-2,3-dihydrobenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((–)-6e). ^{1}H NMR (CD_3OD) δ 6.63 (d, *J* = 8.8 Hz, 1H), 6.51 (d, *J* = 8.4 Hz, 1H), 4.53 – 4.44 (m, 2H), 3.77 (d, *J* = 6.8 Hz, 2H), 3.24 – 3.18 (m, 2H), 3.13 (dd, *J* = 12.8, 7.2 Hz, 1H), 2.98 (dd, *J* = 12.8, 8.0 Hz, 1H), 1.83 – 1.80 (m, 1H), 1.48 – 1.45 (m, 1H), 1.30 – 1.22 (m, 2H), 1.01 – 0.98 (m, 1H), 0.65 – 0.62 (m, 2H), 0.39 – 0.37 (m, 2H); HRMS calcd for $C_{16}H_{22}NO_2^+$ ($[M + H]^+$): 260.1645, found: 260.1641; $[\alpha]_D^{20}$ –46.7 (*c* 0.05, MeOH).

(+)-(2-(5-(Cyclopropylmethoxy)-2,3-dihydrobenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((+)-6e). ^{1}H NMR (CD_3OD) δ 6.62 (d, *J* = 8.8 Hz, 1H), 6.50 (d, *J* = 8.4 Hz, 1H), 4.53 – 4.42 (m, 2H), 3.77 (d, *J* = 7.2 Hz, 2H), 3.24 – 3.18 (m, 2H), 3.13 (dd, *J* = 12.8, 7.2 Hz, 1H), 2.99 (dd, *J* = 12.8, 8.0 Hz, 1H), 1.83 – 1.80 (m, 1H), 1.48 – 1.45 (m, 1H), 1.30 – 1.22 (m, 2H), 1.01 – 0.98 (m, 1H), 0.65 – 0.62 (m, 2H), 0.40 – 0.37 (m, 2H); ^{13}C NMR (CD_3OD) δ 155.5, 153.8, 129.4, 126.7, 112.7, 107.9, 75.3, 72.4, 45.7, 30.6, 18.6, 18.2, 12.7, 11.5, 4.0, 3.7; HRMS calcd for $C_{16}H_{22}NO_2^+$ ($[M + H]^+$): 260.1645, found: 260.1635; $[\alpha]_D^{20}$ +34.7 (*c* 0.2, MeOH).

(–)-(2-(5-(2-Fluoroethoxy)-2,3-dihydrobenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((–)-6f). ^{1}H NMR (CD_3OD) δ 6.69 (d, *J* = 8.4 Hz, 1H), 6.54 (d, *J* = 8.4 Hz, 1H), 4.84 – 4.82 (m, 1H), 4.73 – 4.71 (m, 1H), 4.53 – 4.45 (m, 2H), 4.23 – 4.12 (m, 2H), 3.25 – 3.15 (m, 2H), 3.13 (dd, *J* = 13.2, 7.2 Hz, 1H), 2.93 (dd, *J* = 12.8, 8.0 Hz, 1H), 1.82 – 1.78 (m, 1H), 1.47 – 1.44 (m, 1H), 1.22 – 1.19 (m, 1H), 1.00 – 0.95 (m, 1H); ^{13}C NMR (CD_3OD) δ 155.9,

153.2, 129.7, 127.0, 112.8, 108.0, 83.8 (d, J_{CF} = 166.5 Hz), 72.5, 69.8 (d, J_{CF} = 18.8 Hz), 45.5, 30.6, 18.6, 18.0, 12.6; HRMS calcd for $C_{14}H_{19}FNO_2^+$ ($[M + H]^+$): 252.1394, found: 252.1388; $[\alpha]_D^{20}$ –56.0 (*c* 0.1, MeOH).

(+)-(2-(5-(2-Fluoroethoxy)-2,3-dihydrobenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((+)-6f). ^{1}H NMR (CD_3OD) δ 6.69 (d, J = 8.4 Hz, 1H), 6.54 (d, J = 8.8 Hz, 1H), 4.84 – 4.82 (m, 1H), 4.73 – 4.70 (m, 1H), 4.53 – 4.45 (m, 2H), 4.23 – 4.12 (m, 2H), 3.26 – 3.19 (m, 2H), 3.13 (dd, J = 13.2, 7.2 Hz, 1H), 2.93 (dd, J = 12.8, 8.0 Hz, 1H), 1.83 – 1.79 (m, 1H), 1.47 – 1.45 (m, 1H), 1.23 – 1.18 (m, 1H), 1.00 – 0.95 (m, 1H); ^{13}C NMR (CD_3OD) δ 156.0, 153.2, 129.7, 127.1, 112.9, 108.0, 83.8 (d, J_{CF} = 166.3 Hz), 72.5, 69.8 (d, J_{CF} = 18.7 Hz), 45.5, 30.6, 18.6, 18.0, 12.6; HRMS calcd for $C_{14}H_{19}FNO_2^+$ ($[M + H]^+$): 252.1394, found: 252.1397; $[\alpha]_D^{20}$ +68.0 (*c* 0.1, MeOH).

(–)-(2-(5-Methoxybenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((–)-7a). ^{1}H NMR (CD_3OD) δ 7.71 (d, J = 2.4 Hz, 1H), 7.33 (d, J = 9.2 Hz, 1H), 6.99 (d, J = 8.8 Hz, 1H), 6.91 (d, J = 2.4 Hz, 1H), 3.91 (s, 3H), 3.17 (dd, J = 12.8, 6.8 Hz, 1H), 2.97 (dd, J = 12.8, 8.4 Hz, 1H), 2.07 – 2.02 (m, 1H), 1.46 – 1.41 (m, 1H), 1.28 – 1.23 (m, 1H), 1.14 – 1.09 (m, 1H); ^{13}C NMR (CD_3OD) δ 155.8, 151.6, 147.4, 129.5, 121.0, 110.7, 109.8, 106.4, 57.0, 45.6, 18.6, 17.2, 12.9; HRMS calcd for $C_{13}H_{16}NO_2^+$ ($[M + H]^+$): 218.1176, found: 252.1181; $[\alpha]_D^{20}$ –56.0 (*c* 0.2, MeOH).

(+)-(2-(5-Methoxybenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((+)-7a). ^{1}H NMR (CD_3OD) δ 7.71 (d, J = 2.0 Hz, 1H), 7.33 (d, J = 8.8 Hz, 1H), 6.99 (d, J = 9.2 Hz, 1H), 6.91 (d, J = 1.6 Hz, 1H), 3.91 (s, 3H), 3.17 (dd, J = 13.2, 6.8 Hz, 1H), 2.98 (dd, J = 12.8, 8.0 Hz, 1H), 2.08 – 2.03 (m, 1H), 1.47 – 1.43 (m, 1H), 1.29 – 1.24 (m, 1H), 1.15 – 1.10 (m, 1H); ^{13}C

NMR (CD_3OD) δ 155.8, 151.6, 147.4, 129.5, 121.0, 110.7, 109.9, 106.3, 57.1, 45.6, 18.6, 17.3, 13.0; HRMS calcd for $C_{13}H_{16}NO_2^+$ ($[M + H]^+$): 218.1176, found: 252.1165; $[\alpha]_D^{20}$ +60.3 (*c* 0.3, MeOH).

(–)-(2-(5-Ethoxybenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((–)-7b). ^1H NMR (CD_3OD) δ 7.70 (d, *J* = 2.0 Hz, 1H), 7.29 (d, *J* = 8.8 Hz, 1H), 6.95 (d, *J* = 8.8 Hz, 1H), 6.90 (d, *J* = 2.0 Hz, 1H), 4.15 – 4.07 (m, 2H), 3.22 (dd, *J* = 13.2, 6.8 Hz, 1H), 2.96 (dd, *J* = 13.2, 8.0 Hz, 1H), 2.11 – 2.05 (m, 1H), 1.58 – 1.54 (m, 1H), 1.46 (t, *J* = 7.2 Hz, 3H), 1.32 – 1.28 (m, 1H), 1.14 – 1.09 (m, 1H); ^{13}C NMR (CD_3OD) δ 155.0, 151.6, 147.2, 129.3, 121.5, 111.4, 110.5, 106.4, 66.3, 45.6, 18.6, 17.6, 15.6, 13.2; HRMS calcd for $C_{14}H_{18}NO_2^+$ ($[M + H]^+$): 232.1332, found: 232.1329; $[\alpha]_D^{20}$ –53.4 (*c* 0.5, MeOH).

(+)-(2-(5-Ethoxybenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((+)-7b). ^1H NMR (CD_3OD) δ 7.70 (d, *J* = 2.4 Hz, 1H), 7.29 (d, *J* = 8.8 Hz, 1H), 6.96 (d, *J* = 9.2 Hz, 1H), 6.90 (d, *J* = 2.4 Hz, 1H), 4.15 – 4.08 (m, 2H), 3.22 (dd, *J* = 13.2, 6.8 Hz, 1H), 2.96 (dd, *J* = 13.2, 8.0 Hz, 1H), 2.10 – 2.07 (m, 1H), 1.57 – 1.55 (m, 1H), 1.46 (t, *J* = 7.2 Hz, 3H), 1.33 – 1.28 (m, 1H), 1.14 – 1.10 (m, 1H); ^{13}C NMR (CD_3OD) δ 155.0, 151.6, 147.3, 129.3, 121.5, 111.4, 110.6, 106.4, 66.3, 45.6, 18.6, 17.6, 15.6, 13.2; HRMS calcd for $C_{14}H_{18}NO_2^+$ ($[M + H]^+$): 232.1332, found: 232.1332; $[\alpha]_D^{20}$ +50.7 (*c* 0.3, MeOH).

(–)-(2-(5-Propoxybenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((–)-7c). ^1H NMR (CD_3OD) δ 7.71 (d, *J* = 2.0 Hz, 1H), 7.29 (d, *J* = 8.8 Hz, 1H), 6.96 (d, *J* = 8.8 Hz, 1H), 6.91 (d, *J* = 1.6 Hz, 1H), 4.04 – 3.97 (m, 2H), 3.32 (dd, *J* = 13.2, 6.8 Hz, 1H), 2.88 (dd, *J* = 13.2, 8.8 Hz, 1H), 2.14 – 2.08 (m, 1H), 1.91 – 1.85 (m, 2H), 1.62 – 1.59 (m, 1H), 1.35 – 1.29 (m, 1H), 1.14 – 1.09 (m, 4H); ^{13}C NMR (CD_3OD) δ 155.1, 151.6, 147.3, 129.2, 121.5, 111.4, 110.5,

106.4, 72.5, 45.5, 24.2, 18.5, 17.8, 13.3, 11.2; HRMS calcd for $C_{15}H_{20}NO_2^+$ ([M + H]$^+$): 246.1489, found: 246.1484; $[\alpha]_D^{20}$ –53.7 (*c* 0.3, MeOH).

(+)-(2-(5-Propoxybenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((+)-7c). ^{1}H NMR (CD_3OD) δ 7.71 (d, J = 2.0 Hz, 1H), 7.30 (d, J = 8.8 Hz, 1H), 6.97 (d, J = 8.8 Hz, 1H), 6.91 (d, J = 1.2 Hz, 1H), 4.05 – 3.98 (m, 2H), 3.32 (dd, J = 12.8, 6.4 Hz, 1H), 2.88 (dd, J = 12.8, 8.8 Hz, 1H), 2.14 – 2.09 (m, 1H), 1.91 – 1.85 (m, 2H), 1.64 – 1.60 (m, 1H), 1.35 – 1.30 (m, 1H), 1.15 – 1.09 (m, 4H); ^{13}C NMR (CD_3OD) δ 155.1, 151.6, 147.2, 129.2, 121.5, 111.5, 110.5, 106.4, 72.5, 45.6, 24.2, 18.6, 17.8, 13.3, 11.2; HRMS calcd for $C_{15}H_{20}NO_2^+$ ([M + H]$^+$): 246.1489, found: 246.1494; $[\alpha]_D^{20}$ +50.5 (*c* 0.2, MeOH).

(–)-(2-(5-(Allyloxy)benzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((–)-7d). ^{1}H NMR (CD_3OD) δ 7.72 (d, J = 2.0 Hz, 1H), 7.31 (d, J = 9.2 Hz, 1H), 6.99 (d, J = 8.8 Hz, 1H), 6.92 (d, J = 2.0 Hz, 1H), 6.23 – 6.12 (m, 1H), 5.47 (dd, J = 17.6, 1.6 Hz, 1H), 5.32 (d, J = 9.6 Hz, 1H), 4.63 (d, J = 5.2 Hz, 2H), 3.20 (dd, J = 12.8, 7.2 Hz, 1H), 2.98 (dd, J = 12.8, 8.0 Hz, 1H), 2.14 – 2.08 (m, 1H), 1.57 – 1.52 (m, 1H), 1.34 – 1.29 (m, 1H), 1.15 – 1.10 (m, 1H); ^{13}C NMR (CD_3OD) δ 154.8, 151.7, 147.4, 135.5, 129.3, 121.7, 118.3, 111.8, 110.6, 106.4, 71.8, 45.5, 18.7, 17.6, 13.1; HRMS calcd for $C_{15}H_{18}NO_2^+$ ([M + H]$^+$): 244.1332, found: 244.1328; $[\alpha]_D^{20}$ –52.5 (*c* 0.2, MeOH).

(+)-(2-(5-(Allyloxy)benzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((+)-7d). ^{1}H NMR (CD_3OD) δ 7.72 (d, J = 2.4 Hz, 1H), 7.31 (d, J = 9.2 Hz, 1H), 6.99 (d, J = 8.8 Hz, 1H), 6.92 (d, J = 2.4 Hz, 1H), 6.22 – 6.14 (m, 1H), 5.48 (dd, J = 17.2, 1.6 Hz, 1H), 5.32 (dd, J = 10.4, 1.2 Hz, 1H), 4.64 (d, J = 5.6 Hz, 2H), 3.21 (dd, J = 12.8, 6.8 Hz, 1H), 2.98 (dd, J = 13.2, 8.0 Hz, 1H), 2.15 – 2.09 (m, 1H), 1.58 – 1.54 (m, 1H), 1.35 – 1.30 (m, 1H), 1.16 – 1.11 (m, 1H); ^{13}C

NMR (CD_3OD) δ 154.8, 151.8, 147.4, 135.5, 129.3, 121.8, 118.3, 111.9, 110.6, 106.4, 71.9, 45.6, 18.7, 17.6, 13.2; HRMS calcd for $C_{15}H_{18}NO_2^+$ ($[M + H]^+$): 244.1332, found: 244.1324; $[\alpha]_D^{20}$ +57.0 (*c* 0.2, MeOH).

(–)-(2-(5-(2-Fluoroethoxy)benzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((–)-7e). ^1H NMR (CD_3OD) δ 7.72 (d, J = 2.4 Hz, 1H), 7.33 (d, J = 8.8 Hz, 1H), 6.99 (d, J = 8.8 Hz, 1H), 6.92 (d, J = 2.0 Hz, 1H), 4.90 – 4.88 (m, 1H), 4.78 – 4.75 (m, 1H), 4.36 – 4.26 (m, 2H), 3.21 (dd, J = 13.2, 7.2 Hz, 1H), 2.98 (dd, J = 13.2, 8.0 Hz, 1H), 2.12 – 2.09 (m, 1H), 1.53 – 1.50 (m, 1H), 1.35 – 1.30 (m, 1H), 1.15 – 1.11 (m, 1H); ^{13}C NMR (CD_3OD) δ 154.7, 151.9, 147.5, 129.5, 122.1, 111.7, 110.8, 106.5, 83.9 (d, J_{CF} = 166.5 Hz), 70.4 (d, J_{CF} = 18.7 Hz), 45.5, 18.8, 17.5, 13.0; HRMS calcd for $C_{14}H_{17}FNO_2^+$ ($[M + H]^+$): 250.1238, found: 250.1231; $[\alpha]_D^{20}$ –93.0 (*c* 0.2, MeOH).

(+)-(2-(5-(2-Fluoroethoxy)benzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((+)-7e). ^1H NMR (CD_3OD) δ 7.72 (d, J = 2.0 Hz, 1H), 7.33 (d, J = 8.8 Hz, 1H), 6.99 (d, J = 8.8 Hz, 1H), 6.93 (d, J = 2.0 Hz, 1H), 4.90 – 4.88 (m, 1H), 4.77 – 4.75 (m, 1H), 4.36 – 4.25 (m, 2H), 3.20 (dd, J = 12.8, 7.2 Hz, 1H), 2.98 (dd, J = 12.8, 8.0 Hz, 1H), 2.13 – 2.08 (m, 1H), 1.53 – 1.49 (m, 1H), 1.35 – 1.30 (m, 1H), 1.15 – 1.10 (m, 1H); HRMS calcd for $C_{14}H_{17}FNO_2^+$ ($[M + H]^+$): 250.1238, found: 250.1231; $[\alpha]_D^{20}$ +88.9 (*c* 0.1, MeOH).

(–)-(2-(5-(Cyclopropylmethoxy)benzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((–)-7f). ^1H NMR (CD_3OD) δ 7.70 (d, J = 2.0 Hz, 1H), 7.29 (d, J = 8.8 Hz, 1H), 6.94 (d, J = 8.8 Hz, 1H), 6.91 (d, J = 2.0 Hz, 1H), 3.90 (d, J = 7.2 Hz, 2H), 3.21 (dd, J = 12.8, 7.2 Hz, 1H), 3.03 (dd, J = 12.8, 8.0 Hz, 1H), 2.12 – 2.09 (m, 1H), 1.54 – 1.51 (m, 1H), 1.37 – 1.32 (m, 2H), 1.17 – 1.13 (m, 1H), 0.68 – 0.63 (m, 2H), 0.44 – 0.40 (m, 2H); ^{13}C NMR (CD_3OD) δ 155.2, 151.6,

147.3, 129.2, 121.6, 111.7, 110.6, 106.4, 75.9, 45.6, 18.8, 17.6, 13.1, 11.6, 4.0, 3.7; HRMS calcd for $C_{16}H_{20}NO_2^+$ ([M + H]$^+$): 258.1489, found: 258.1501; $[\alpha]_D^{20}$ –76.0 (*c* 0.2, MeOH).

(+)-(2-(5-(Cyclopropylmethoxy)benzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((+)-7f). ^{1}H NMR (CD_3OD) δ 7.70 (d, *J* = 2.0 Hz, 1H), 7.29 (d, *J* = 8.8 Hz, 1H), 6.94 (d, *J* = 8.8 Hz, 1H), 6.91 (d, *J* = 2.0 Hz, 1H), 3.90 (d, J = 6.8 Hz, 2H), 3.21 (dd, *J* = 12.8, 7.2 Hz, 1H), 3.03 (dd, *J* = 12.8, 8.0 Hz, 1H), 2.13 – 2.08 (m, 1H), 1.54 – 1.51 (m, 1H), 1.38 – 1.32 (m, 2H), 1.16 – 1.11 (m, 1H), 0.68 – 0.63 (m, 2H), 0.43 – 0.39 (m, 2H); HRMS calcd for $C_{16}H_{20}NO_2^+$ ([M + H]$^+$): 258.1489, found: 258.1477; $[\alpha]_D^{20}$ +73.3 (*c* 0.3, MeOH).

(–)-(2-(5-Isopropoxybenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((–)-7g). ^{1}H NMR (CD_3OD) δ 7.70 (d, *J* = 2.0 Hz, 1H), 7.28 (d, *J* = 8.8 Hz, 1H), 6.97 (d, *J* = 8.8 Hz, 1H), 6.88 (d, *J* = 2.0 Hz, 1H), 4.65 – 4.57 (m, 1H), 3.37 (dd, *J* = 12.8, 6.0 Hz, 1H), 2.83 (dd, *J* = 12.8, 8.8 Hz, 1H), 2.14 – 2.08 (m, 1H), 1.62 – 1.56 (m, 1H), 1.38 (d, *J* = 6.0 Hz, 3H), 1.34 (d, *J* = 6.0 Hz, 3H), 1.32 – 1.29 (m, 1H), 1.13 – 1.08 (m, 1H); ^{13}C NMR (CD_3OD) δ 153.6, 151.7, 147.2, 129.0, 123.1, 114.0, 110.6, 106.4, 73.3, 45.6, 22.9, 22.7, 18.7, 18.0, 13.3; HRMS calcd for $C_{15}H_{20}NO_2^+$ ([M + H]$^+$): 246.1489, found: 246.1486; $[\alpha]_D^{20}$ –48.5 (*c* 0.2, MeOH).

(+)-(2-(5-Isopropoxybenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((+)-7g). ^{1}H NMR (CD_3OD) δ 7.70 (d, *J* = 2.4 Hz, 1H), 7.29 (d, *J* = 8.8 Hz, 1H), 6.97 (d, *J* = 8.8 Hz, 1H), 6.87 (d, *J* = 1.2 Hz, 1H), 4.65 – 4.57 (m, 1H), 3.36 (dd, *J* = 13.2, 6.0 Hz, 1H), 2.83 (dd, *J* = 12.8, 8.8 Hz, 1H), 2.14 – 2.08 (m, 1H), 1.62 – 1.56 (m, 1H), 1.38 (d, *J* = 6.0 Hz, 3H), 1.34 (d, *J* = 6.0 Hz, 3H), 1.32 – 1.29 (m, 1H), 1.12 – 1.07 (m, 1H); HRMS calcd for $C_{15}H_{20}NO_2^+$ ([M + H]$^+$): 246.1489, found: 246.1487; $[\alpha]_D^{20}$ +46.0 (*c* 0.2, MeOH).

(–)-(2-(5-((2-Fluoroallyl)oxy)benzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((–)-7h). ^{1}H NMR (DMSO-d_6) δ 8.00 (br, 3H), 7.95 (d, J = 2.0 Hz, 1H), 7.39 (d, J = 8.8 Hz, 1H), 7.04 (d, J = 8.8 Hz, 1H), 7.00 (d, J = 2.0 Hz, 1H), 4.94 (dd, J = 8.8, 3.2 Hz, 1H), 4.86 (dd, J = 25.2, 2.8 Hz, 1H), 4.68 (d, J = 14.8 Hz, 2H), 3.16 – 3.14 (m, 1H), 2.76 – 2.71 (m, 1H), 2.13 – 2.08 (m, 1H), 1.65 – 1.59 (m, 1H), 1.17 – 1.13 (m, 1H), 1.09 – 1.05 (m, 1H); ^{13}C NMR (CD_3OD) δ 163.4 (d, J_{CF} = 256.4 Hz), 154.3, 152.1, 147.5, 129.4, 122.6, 112.4, 110.8, 106.5, 94.8 (d, J_{CF} = 16.9 Hz), 68.8 (d, J_{CF} = 31.7 Hz), 45.4, 18.7, 17.5, 13.2; HRMS calcd for $C_{15}H_{17}FNO_2^+$ ([M + H]$^+$): 262.1238, found: 262.1234; $[\alpha]_D^{20}$ –67.0 (*c* 0.2, MeOH).

(+)-(2-(5-((2-Fluoroallyl)oxy)benzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((+)-7h). ^{1}H NMR (DMSO-d_6) δ 8.09 (br, 3H), 7.95 (d, J = 2.0 Hz, 1H), 7.38 (d, J = 8.8 Hz, 1H), 7.04 (d, J = 8.8 Hz, 1H), 7.01 (d, J = 2.0 Hz, 1H), 4.93 (dd, J = 10.2, 3.2 Hz, 1H), 4.85 (dd, J = 23.2, 3.2 Hz, 1H), 4.68 (d, J = 14.8 Hz, 2H), 3.14 (dd, J = 12.8, 6.0 Hz, 1H), 2.72 (dd, J = 12.8, 8.8 Hz, 1H), 2.13 – 2.08 (m, 1H), 1.64 – 1.60 (m, 1H), 1.17 – 1.12 (m, 1H), 1.09 – 1.04 (m, 1H); HRMS calcd for $C_{15}H_{17}FNO_2^+$ ([M + H]$^+$): 262.1238, found: 262.1227; $[\alpha]_D^{20}$ +57.2 (*c* 0.1, MeOH).

(–)-(2-(5-((2-Methylallyl)oxy)benzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((–)-7i). ^{1}H NMR (CD_3OD) δ 7.71 (d, J = 2.0 Hz, 1H), 7.28 (d, J = 8.8 Hz, 1H), 6.96 (d, J = 8.8 Hz, 1H), 6.91 (d, J = 2.0 Hz, 1H), 5.14 (s, 1H), 5.01 (s, 1H), 4.53 (s, 2H), 3.32 (dd, J = 12.8, 6.0 Hz, 1H), 2.88 (dd, J = 12.8, 8.8 Hz, 1H), 2.17 – 2.11 (m, 1H), 1.88 (s, 3H), 1.62 – 1.58 (m, 1H), 1.34 – 1.29 (m, 1H), 1.15 – 1.10 (m, 1H); ^{13}C NMR (CD_3OD) δ 154.9, 151.7, 147.3, 143.2, 129.2, 121.8, 113.1, 112.0, 110.6, 106.4, 74.6, 45.5, 19.9, 18.6, 17.8, 13.3; HRMS calcd for $C_{16}H_{20}NO_2^+$ ([M + H]$^+$): 258.1489, found: 258.1477; $[\alpha]_D^{20}$ –82.5 (*c* 0.15, MeOH).

(+)-(2-(5-((2-Methylallyl)oxy)benzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((+)-7i). ^{1}H NMR (CD_3OD) δ 7.71 (d, J = 2.4 Hz, 1H), 7.29 (d, J = 8.8 Hz, 1H), 6.96 (d, J = 8.8 Hz, 1H), 6.91 (d, J = 2.4 Hz, 1H), 5.14 (s, 1H), 5.01 (s, 1H), 4.53 (s, 2H), 3.32 (dd, J = 12.8, 6.0 Hz, 1H), 2.88 (dd, J = 12.8, 8.8 Hz, 1H), 2.17 – 2.11 (m, 1H), 1.88 (s, 3H), 1.62 – 1.57 (m, 1H), 1.34 – 1.29 (m, 1H), 1.15 – 1.09 (m, 1H); HRMS calcd for $C_{16}H_{20}NO_2^+$ ($[M + H]^+$): 258.1489, found: 258.1477; $[\alpha]_D^{20}$ +63.4 (*c* 0.15, MeOH).

(–)-(2-(2-Ethyl-5-methoxybenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((–)-8a). ^{1}H NMR (CD_3OD) δ 7.20 (d, J = 9.2 Hz, 1H), 6.86 (d, J = 9.2 Hz, 1H), 6.50 (s, 1H), 3.88 (s, 3H), 3.15 (dd, J = 13.2, 7.2 Hz, 1H), 2.95 (dd, J = 12.8, 8.4 Hz, 1H), 2.76 (q, J = 7.6 Hz, 2H), 2.01 – 1.98 (m, 1H), 1.43 – 1.41 (m, 1H), 1.32 (t, J = 7.6 Hz, 3H), 1.25 – 1.21 (m, 1H), 1.10 – 1.07 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 163.2, 155.7, 151.3, 130.9, 120.3, 109.9, 108.5, 100.9, 57.0, 45.7, 22.9, 18.6, 17.3, 12.9, 12.5; HRMS calcd for $C_{15}H_{20}NO_2^+$ ($[M + H]^+$): 246.1489, found: 246.1477; $[\alpha]_D^{20}$ –71.2 (*c* 0.5, MeOH).

(+)-(2-(2-Ethyl-5-methoxybenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((+)-8a). ^{1}H NMR (CD_3OD) δ 7.21 (d, J = 9.2 Hz, 1H), 6.87 (d, J = 9.2 Hz, 1H), 6.50 (s, 1H), 3.88 (s, 3H), 3.15 (dd, J = 12.8, 6.8 Hz, 1H), 2.95 (dd, J = 12.8, 8.0 Hz, 1H), 2.77 (q, J = 7.6 Hz, 2H), 2.01 – 1.98 (m, 1H), 1.42 – 1.40 (m, 1H), 1.32 (t, J = 7.6 Hz, 3H), 1.26 – 1.21 (m, 1H), 1.10 – 1.06 (m, 1H); HRMS calcd for $C_{15}H_{20}NO_2^+$ ($[M + H]^+$): 246.1489, found: 246.1481; $[\alpha]_D^{20}$ +78.7 (*c* 0.15, MeOH).

(–)-(2-(5-Ethoxy-2-ethylbenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((–)-8b). ^{1}H NMR (CD_3OD) δ 7.18 (d, J = 8.8 Hz, 1H), 6.86 (d, J = 8.8 Hz, 1H), 6.50 (s, 1H), 4.13 – 4.05 (m, 2H), 3.21 (dd, J = 13.2, 7.6 Hz, 1H), 2.95 (dd, J = 12.8, 8.0 Hz, 1H), 2.77 (q, J = 7.6 Hz,

2H), 2.05 – 2.03 (m, 1H), 1.53 – 1.51 (m, 1H), 1.45 (t, J = 6.8 Hz, 3H), 1.32 (t, J = 7.6 Hz, 3H), 1.29 – 1.26 (m, 1H), 1.11 – 1.07 (m, 1H); ^{13}C NMR (CD_3OD) δ 163.1, 154.8, 151.3, 130.7, 120.8, 110.1, 109.9, 100.9, 66.3, 45.6, 22.9, 18.6, 17.6, 15.6, 13.1, 12.5; HRMS calcd for $C_{16}H_{22}NO_2^+$ ($[M + H]^+$): 260.1645, found: 260.1639; $[\alpha]_D^{20}$ –57.0 (*c* 0.2, MeOH).

(+)-(2-(5-Ethoxy-2-ethylbenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((+)-8b). ^{1}H NMR (CD_3OD) δ 7.18 (d, J = 8.8 Hz, 1H), 6.85 (d, J = 8.8 Hz, 1H), 6.50 (s, 1H), 4.14 – 4.05 (m, 2H), 3.20 (dd, J = 13.2, 7.6 Hz, 1H), 2.95 (dd, J = 12.8, 8.0 Hz, 1H), 2.77 (q, J = 7.6 Hz, 2H), 2.05 – 2.02 (m, 1H), 1.53 – 1.51 (m, 1H), 1.45 (t, J = 6.8 Hz, 3H), 1.32 (t, J = 7.6 Hz, 3H), 1.29 – 1.25 (m, 1H), 1.11 – 1.06 (m, 1H); HRMS calcd for $C_{16}H_{22}NO_2^+$ ($[M + H]^+$): 260.1645, found: 260.1643; $[\alpha]_D^{20}$ +66.0 (*c* 0.1, MeOH).

(–)-(2-(2-Ethyl-5-propoxybenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((–)-8c). ^{1}H NMR (CD_3OD) δ 7.17 (d, J = 8.8 Hz, 1H), 6.84 (d, J = 8.8 Hz, 1H), 6.50 (s, 1H), 4.01 – 3.94 (m, 2H), 3.30 (dd, J = 12.8, 7.6 Hz, 1H), 2.86 (dd, J = 12.8, 8.8 Hz, 1H), 2.77 (q, J = 7.6 Hz, 2H), 2.07 – 2.05 (m, 1H), 1.89 – 1.83 (m, 2H), 1.59 – 1.54 (m, 1H), 1.34 – 1.27 (m, 4H), 1.11 – 1.06 (m, 4H); ^{13}C NMR (CD_3OD) δ 163.1, 155.0, 151.3, 130.6, 120.8, 110.2, 109.8, 100.9, 72.4, 45.6, 24.2, 22.9, 18.5, 17.8, 13.2, 12.5, 11.2; HRMS calcd for $C_{17}H_{24}NO_2^+$ ($[M + H]^+$): 274.1802, found: 274.1789; $[\alpha]_D^{20}$ –66.5 (*c* 0.1, MeOH).

(+)-(2-(2-Ethyl-5-propoxybenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((+)-8c). ^{1}H NMR (CD_3OD) δ 7.17 (d, J = 8.8 Hz, 1H), 6.84 (d, J = 8.8 Hz, 1H), 6.50 (s, 1H), 4.01 – 3.94 (m, 2H), 3.30 (dd, J = 12.8, 7.6 Hz, 1H), 2.86 (dd, J = 12.8, 8.8 Hz, 1H), 2.77 (q, J = 7.6 Hz, 2H), 2.07 – 2.04 (m, 1H), 1.89 – 1.83 (m, 2H), 1.59 – 1.54 (m, 1H), 1.34 – 1.26 (m, 4H),

1.11 – 1.06 (m, 4H); HRMS calcd for $C_{17}H_{24}NO_2^+$ ([M + H]$^+$): 274.1802, found: 274.1796; $[\alpha]_D^{20}$ +68.7 (*c* 0.1, MeOH).

(–)-(2-(2-Ethyl-5-(2-fluoroethoxy)benzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((–)-8d). ^{1}H NMR (CD_3OD) δ 7.21 (d, J = 8.8 Hz, 1H), 6.88 (d, J = 8.8 Hz, 1H), 6.53 (s, 1H), 4.89 – 4.87 (m, 1H), 4.77 – 4.74 (m, 1H), 4.34 – 4.23 (m, 2H), 3.18 (dd, J = 12.8, 7.2 Hz, 1H), 2.98 (dd, J = 12.8, 8.0 Hz, 1H), 2.78 (q, J = 7.6 Hz, 2H), 2.07 – 2.04 (m, 1H), 1.49 – 1.46 (m, 1H), 1.35 – 1.28 (m, 4H), 1.12 – 1.09 (m, 1H); HRMS calcd for $C_{16}H_{21}FNO_2^+$ ([M + H]$^+$): 278.1551, found: 278.1563; $[\alpha]_D^{20}$ –118.0 (*c* 0.1, MeOH).

(+)-(2-(2-Ethyl-5-(2-fluoroethoxy)benzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((+)-8d). ^{1}H NMR (CD_3OD) δ 7.21 (d, J = 8.8 Hz, 1H), 6.88 (d, J = 8.8 Hz, 1H), 6.53 (s, 1H), 4.89 – 4.87 (m, 1H), 4.77 – 4.74 (m, 1H), 4.34 – 4.23 (m, 2H), 3.18 (dd, J = 12.8, 7.2 Hz, 1H), 2.98 (dd, J = 12.8, 8.0 Hz, 1H), 2.78 (q, J = 7.6 Hz, 2H), 2.07 – 2.04 (m, 1H), 1.49 – 1.46 (m, 1H), 1.35 – 1.28 (m, 4H), 1.12 – 1.09 (m, 1H); ^{13}C NMR (CD_3OD) δ 163.4, 154.6, 151.6, 131.0, 121.3, 110.3, 110.1, 101.0, 83.9 (d, J_{CF} = 166.4 Hz), 70.3 (d, J_{CF} = 18.7 Hz), 45.5, 22.9, 18.8, 17.4, 12.9, 12.5; HRMS calcd for $C_{16}H_{21}FNO_2^+$ ([M + H]$^+$): 278.1551, found: 278.1549; $[\alpha]_D^{20}$ +113.5 (*c* 0.1, MeOH).

(–)-(2-(5-(Allyloxy)-2-ethylbenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((–)-8e). ^{1}H NMR (CD_3OD) δ 7.18 (d, J = 8.8 Hz, 1H), 6.86 (d, J = 8.8 Hz, 1H), 6.51 (s, 1H), 6.21 – 6.11 (m, 1H), 5.46 (dd, J = 15.6, 1.6 Hz, 1H), 5.30 (dd, J = 10.4, 1.6 Hz, 1H), 4.60 (d, J = 5.6 Hz, 2H), 3.18 (dd, J = 13.2, 6.8 Hz, 1H), 2.96 (dd, J = 13.2, 8.0 Hz, 1H), 2.77 (q, J = 7.6 Hz, 2H), 2.09 – 2.05 (m, 1H), 1.53 – 1.50 (m, 1H), 1.32 (t, J = 7.6 Hz, 3H), 1.29 – 1.27 (m, 1H), 1.12 – 1.08 (m, 1H); ^{13}C NMR (CD_3OD) δ 163.2, 154.6, 151.5, 135.6, 130.7, 121.0, 118.3, 110.6,

109.9, 101.0, 71.8, 45.6, 22.9, 18.6, 17.6, 13.1, 12.5; HRMS calcd for $C_{17}H_{22}NO_2^+$ ([M + H]$^+$): 272.1645, found: 272.1635; $[\alpha]_D^{20}$ –92.5 (*c* 0.2, MeOH).

(+)-(2-(5-(Allyloxy)-2-ethylbenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((+)-8e). ^{1}H NMR (CD_3OD) δ 7.18 (d, *J* = 8.8 Hz, 1H), 6.86 (d, *J* = 8.8 Hz, 1H), 6.51 (s, 1H), 6.21 – 6.11 (m, 1H), 5.46 (dd, *J* = 15.6, 1.6 Hz, 1H), 5.30 (dd, *J* = 10.4, 1.6 Hz, 1H), 4.60 (d, *J* = 5.6 Hz, 2H), 3.19 (dd, *J* = 13.2, 6.8 Hz, 1H), 2.98 (dd, *J* = 13.2, 8.0 Hz, 1H), 2.77 (q, *J* = 7.6 Hz, 2H), 2.07 – 2.03 (m, 1H), 1.53 – 1.50 (m, 1H), 1.32 (t, *J* = 7.6 Hz, 3H), 1.29 – 1.26 (m, 1H), 1.12 – 1.07 (m, 1H); HRMS calcd for $C_{17}H_{22}NO_2^+$ ([M + H]$^+$): 272.1645, found: 272.1640; $[\alpha]_D^{20}$ +107.6 (*c* 0.05, MeOH).

(–)-(2-(5-(Cyclopropylmethoxy)-2-ethylbenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((–)-8f). ^{1}H NMR (CD_3OD) δ 7.17 (d, *J* = 8.8 Hz, 1H), 6.82 (d, *J* = 8.8 Hz, 1H), 6.51 (s, 1H), 3.87 (d, *J* = 7.2 Hz, 2H), 3.19 (dd, *J* = 12.8, 7.2 Hz, 1H), 3.01 (dd, *J* = 12.8, 7.6 Hz, 1H), 2.77 (q, *J* = 7.6 Hz, 2H), 2.07 – 2.05 (m, 1H), 1.51 – 1.48 (m, 1H), 1.36 – 1.29 (m, 5H), 1.13 – 1.10 (m, 1H), 0.68 – 0.64 (m, 2H), 0.43 – 0.40 (m, 2H); ^{13}C NMR (CD_3OD) δ 163.2, 155.0, 151.4, 130.7, 120.9, 110.3, 109.9, 100.9, 75.8, 45.7, 22.9, 18.8, 17.6, 13.0, 12.5, 11.6, 4.0, 3.7; HRMS calcd for $C_{18}H_{24}NO_2^+$ ([M + H]$^+$): 286.1802, found: 286.1788; $[\alpha]_D^{20}$ –92.5 (*c* 0.2, MeOH).

(+)-(2-(5-(Cyclopropylmethoxy)-2-ethylbenzofuran-4-yl)cyclopropyl)methanamine Hydrochloride ((+)-8f). ^{1}H NMR (CD_3OD) δ 7.17 (d, *J* = 8.8 Hz, 1H), 6.83 (d, *J* = 8.8 Hz, 1H), 6.51 (s, 1H), 3.88 (d, *J* = 7.2 Hz, 2H), 3.19 (dd, *J* = 13.2, 7.2 Hz, 1H), 3.02 (dd, *J* = 12.8, 8.0 Hz, 1H), 2.77 (q, *J* = 7.6 Hz, 2H), 2.08 – 2.03 (m, 1H), 1.51 – 1.47 (m, 1H), 1.34 – 1.30 (m, 5H),

1.13 – 1.08 (m, 1H), 0.67 – 0.63 (m, 2H), 0.42 – 0.39 (m, 2H); HRMS calcd for $C_{18}H_{24}NO_2^+$ ([M + H]$^+$): 286.1802, found: 286.1789; $[\alpha]_D^{20}$ +98.8 (*c* 0.1, MeOH).

Calcium Flux Assay.

Calcium flux assay were performed with Flp-In-293 cells stably expressing the human 5-HT_{2A}, 5-HT_{2B}, or 5-$HT_{2C\text{-}INI}$ using a FLIPR^{TETRA} fluorescence imaging plate reader (Molecular Dynamics) as previously described.[9] Briefly, cells were seeded in 384-well poly-L-lysine plates at a density of 10,000 cells/well, and next day, cells were loaded with Fluo-4 Direct dye (Invitrogen, (20 μL/well) for 1 h at 37 °C in drug buffer (1× HBSS, 2.5 mM probenecid, and 20 mM HEPES, pH 7.4). Drug dilutions were prepared at 3X final concentration in drug buffer (1× HBSS, 20 mM HEPES, 0.1% BSA, 0.01% ascorbic acid, pH 7.4) and 10 μL per well of drug was added and calcium flux was measured (1 read/s) for 300 seconds. For experiments to determine bias, drug solutions used for FLIPR assay were exactly the same as used for the Tango assay. Compounds were routinely tested at concentrations from 1 pM up to 10 μM, but in the cases of weak potency, compounds were re-tested up to at least 100 μM. Fluorescence in each well was normalized to the average of the first 10 reads (i.e., baseline fluorescence), then the maximum-fold increase was determined and fold over baseline was plotted as a function of drug concentration. Data were normalized to % 5-HT stimulation and analyzed using log(agonist) vs. response in Graphpad Prism 5.0.

Tango Arrestin Recruitment Assay.

The Tango assay measuring β-arrestin-2 recruitment utilizes an HEK cell line expressing TEV fused-β-Arrestin2 (HTLA cells, kindly provided by Dr. Richard Axel) and a tetracycline transactivator (tTA)-driven luciferase. HTLA cells are transfected with the 5-HT_{2C} INI receptor

fused to tTA containing a TEV cleavage site. Cells were plated exactly like for the FLIPR assay in a 40 μL volume except into white 384-well plates, and stimulated with the exact same drugs used for FLIPR (3X, 20 μL per well in HBSS, 20 mM HEPES, 0.1% BSA, 0.01% ascorbic acid, pH 7.4). After 20 hours incubation at 37° C and 5% CO_2, media containing drugs was decanted and 20 μL of Bright-Glo reagent was added per well (Promega). The plate was incubated for 20 min at room temperature for complete cell lysis before being counted using a Wallac MicroBeta Trilux luminescence counter (Perkin Elmer). Results (relative luminescence units) were plotted as a function of drug concentration, normalized to % 5-HT, and subjected to non-linear least-squares regression analysis using the sigmoidal dose-response function in GraphPad Prism 5.0.

Desensitization Assays.

For calcium flux assays measuring desensitization, Flp-In-293 cells expressing the 5-HT_{2C} INI receptor were plated exactly as described for the FLIPR assay. First, addition of drug was initiated by decanting media and adding 20 μL per well of drug buffer (HBSS, 20 mM HEPES, 0.1% BSA, 0.01% ascorbic acid, pH 7.4). Cells were then pretreated with 10 μL per well of varying concentrations of drugs (3X) and allowed to incubate for 30 min at 37° C. Afterwards drugs were decanted and cells were washed three times with 20 μL per well of drug buffer. Dye was applied as previously described and cells were incubated for an additional 30 min. Cells were then challenged with varying concentrations of agonist to measure calcium flux as previously described.[9, 25]

For PI hydrolysis assays measuring time-dependent desensitization, Flp-In-293 5-HT_{2C} INI cells were plated into 96-well poly-L-lysine coated plates at 25,000 cells per well in inositol-free

DMEM containing 2 μCi/well of [^{3}H]-myo-inositol. After labeling with [^{3}H]-myo-inositol for 16-18 hours, media was decanted and cells were washed twice with inositol-free DMEM and 200 μL of inositol-free DMEM was added per well. Drugs (5X) at varying concentrations were diluted in drug buffer (HBSS, 20 mM HEPES, 0.1% BSA, 0.01% ascorbic acid, pH 7.4) and 50 μL was added per well and incubated for indicated time points. Exactly 2 hours before lysing, 10 μL of LiCl (15 mM final concentration) was added to each well. After indicated time points, media was decanted and 50 μL of cold 50 mM formic acid was added to lyse cells. Plates were incubated at 4° C overnight and next day, 10 μL of lysate was added to 75 μL of 0.2 mg/mL RNA binding yttrium silicate beads (Perkin Elmer). Plates containing lysate and beads were incubated for one hour on a shaker, centrifuged at 300xg for 1 min, and counted using a Wallac MicroBeta Trilux plate reader (Perkin Elmer). Data were plotted (CPM) and analyzed in Graphpad Prism 5.0.

Associated Content

Supporting Information

Supporting Information is available free of charge on the ACS Publications website.

Synthetic procedures, chiral separation methods, and characterization data of all intermediates; asymmetric synthesis of compound **39**/(+)-**6d**; methods and results of selected compounds in the binding and MT_1, MT_2 assays (PDF).

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Abbreviations

5-HT, serotonin; CNS, central nervous system; FDA, US Food and Drug Administration; FLIPR, fluorescence imaging plate reader; GPCR, G protein-coupled receptor; HEK-293, Human Embryonic Kidney-293 cell; HPLC, high-performance liquid chromatography; HTS, high throughput screening; IP, inositol phosphate; MT, melatonin; PI, phosphoinositide.

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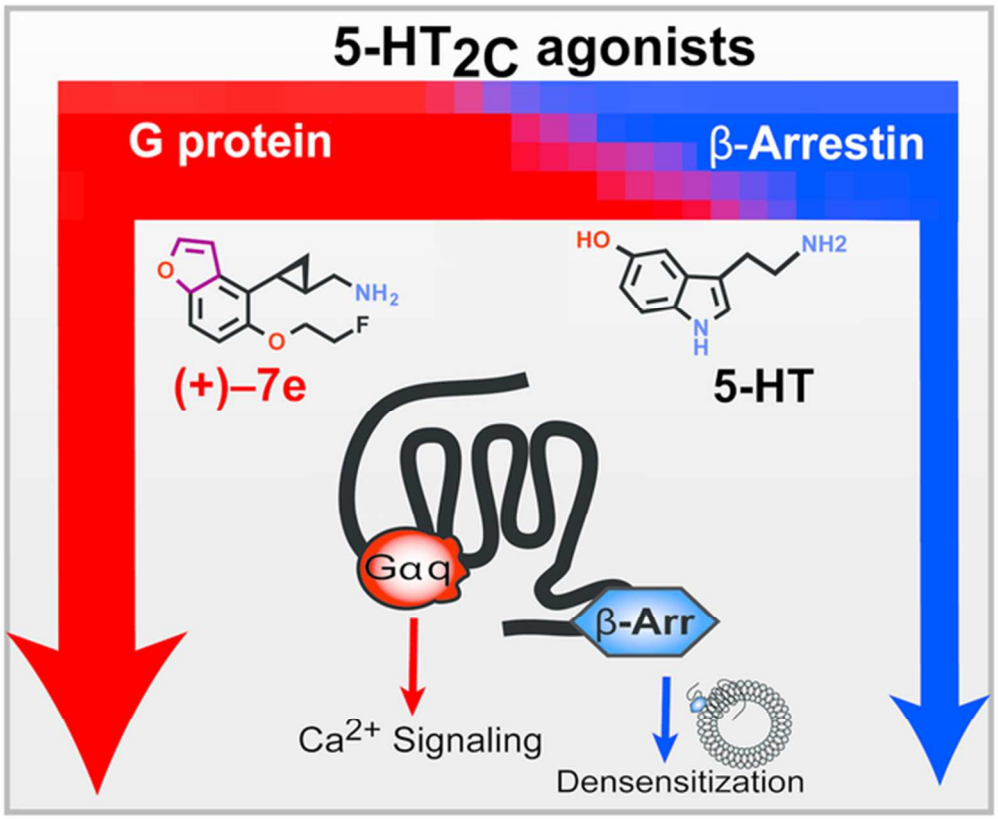

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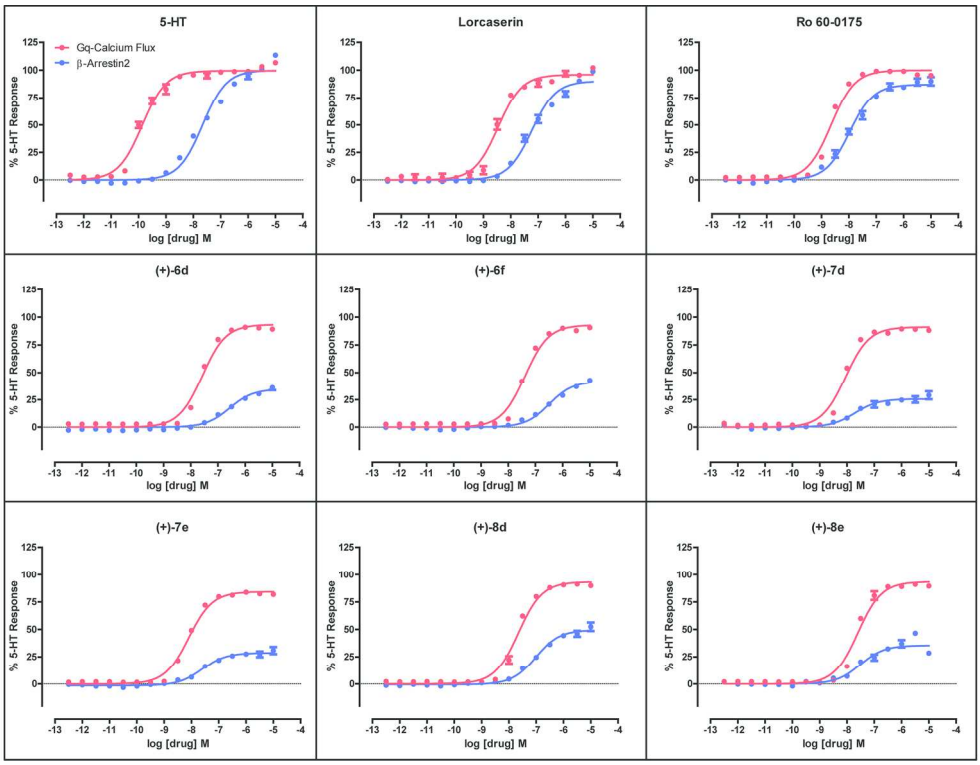

Figure 2. Parallel 5-HT2C screening for functional selectivity of 5-HT2C-selective compounds examining Gq-mediated calcium flux (FLIPR, red) and β-arrestin-2 recruitment (Tango, blue).

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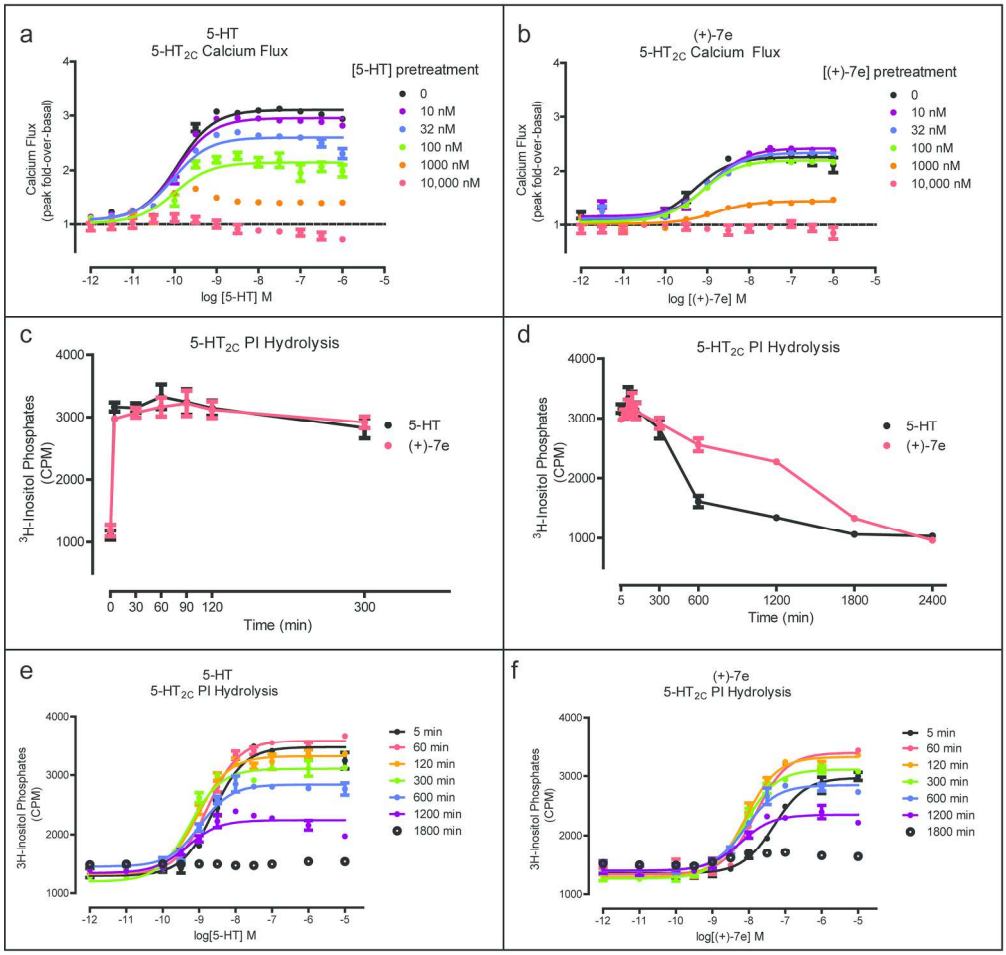

Figure 3. Functionally selective (+)-7e produces less 5-HT2C Gq-mediated desensitization compared to 5-HT as measured by calcium flux (a, b) and by IP accumulation (c-f).

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