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Discovery of Highly Potent Serotonin 5-HT$_2$ Receptor Agonists Inspired by Heteroyohimbine Natural Products

Meghan J. Orr, Andrew B. Cao, Charles Tiancheng Wang, Arsen Gaisin, Adam Csakai, Alec P. Friswold, Herbert Y. Meltzer, John D. McCorvy,* and Karl A. Scheidt*

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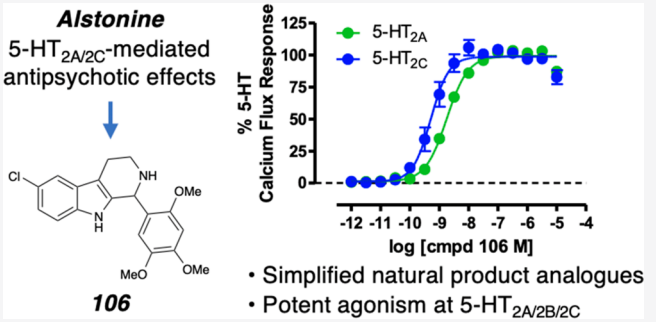
ABSTRACT: The serotonin 5-HT$_2$ receptors are important pharmaceutical targets involved in signaling pathways underlying various neurological, psychiatric, and cardiac functions and dysfunctions. As such, numerous ligands for the investigation of these receptors' activity and downstream effects have been developed synthetically or discovered in nature. For example, the heteroyohimbine natural product alstonine exhibits antispychotic activity mediated by 5-HT$_{2A/2C}$ agonism. In this work, we identified a heteroyohimbine metabolite containing a serotonin pharmacophore and truncated the scaffold, leading to the discovery of potent agonist activity of substituted tetrahydro-β-carbolines across the 5-HT$_2$ receptor family. Extensive SAR development resulted in compound 106 with EC$_{50}$ values of 1.7, 0.58, and 0.50 nM at 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$, respectively. Docking studies suggest a π-stacking interaction between the tetrahydro-β-carboline core and conserved residue Trp$^{6.48}$ as the structural basis for this activity. This work lays a foundation for future investigation of these compounds in neurological and psychiatric disorders.



KEYWORDS: *Serotonin 5-HT$_2$ receptors, tetrahydro-β-carbolines, small molecule agonists, heteroyohimbine metabolism*

From ancient use of the hallucinogen psilocybin to contemporary use of the antihypertensive reserpine, the indole alkaloid class of natural products has long been a source of medicinally relevant bioactive molecules. The degree to which compounds of this family have been studied is highly variable, however, and so there remains untapped potential for the discovery of new and unexpected pharmacological activity among these molecules. One such example is the heteroyohimbine natural product alstonine (**1**, Figure 1). Identified as the active component in a traditional Igbo remedy for psychosis,[1] alstonine was subsequently found to mediate its antipsychotic effects via the serotonin-2A (5-HT$_{2A}$) and 2C (5-HT$_{2C}$) receptors, rather than the dopamine receptor modulation ubiquitous among antipsychotics' pharmacological mechanisms.[2] However, further investigation of alstonine's unique properties was hindered by the limited natural supply of the compound, until our total synthesis of alstonine furnished us with **1**, its diastereomer serpentine (**2**), and their saturated counterparts tetrahydroalstonine (**3**) and ajmalicine (**4**) in sufficient quantities for further evaluation.[3] Though structurally similar, these compounds possess diverse, if underexplored, bioactivity: serpentine has antimalarial[4] and anticancer effects;[5,6] ajmalicine is an alpha-1 adrenergic antagonist;[7] and the pharmacology of tetrahydroalstonine is unknown.

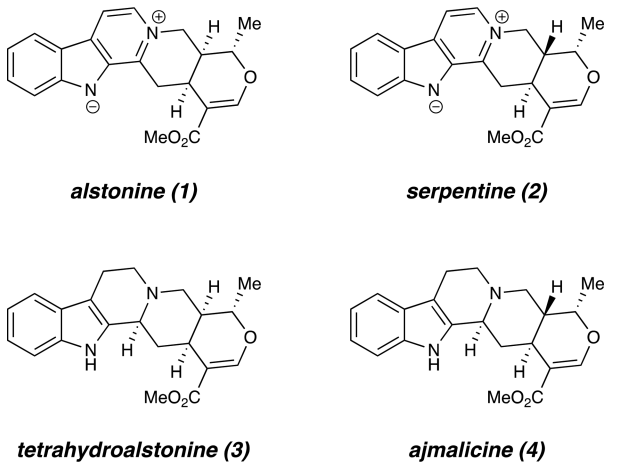


Figure 1. Heteroyohimbine compounds.

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The exposure of ajmalicine to mouse liver microsomes over 1 h produced a metabolite with mass [M + 16], with its concentration surpassing that of the parent compound after 30 min (Figure 2). Spectroscopic characterization of the

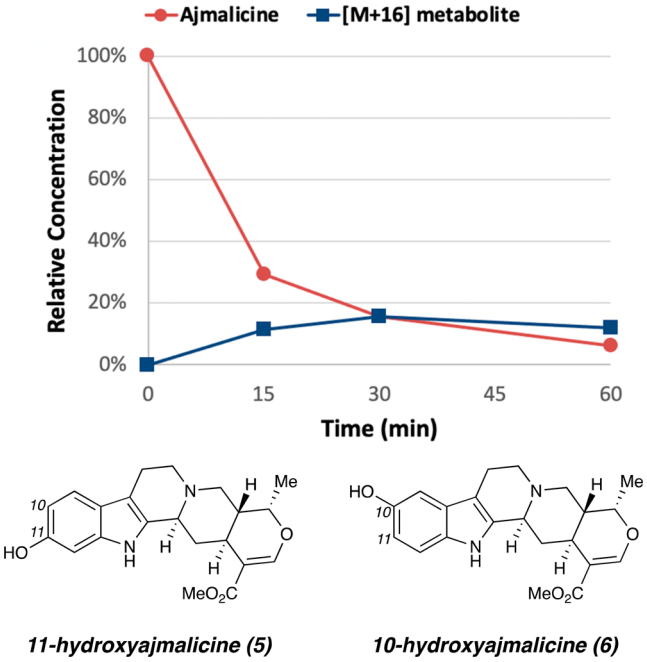
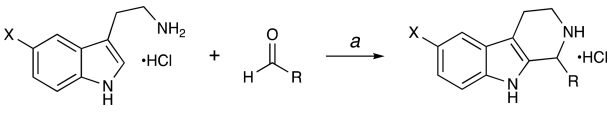


Figure 2. Metabolism of ajmalicine.

metabolite revealed a 3:1 mixture of 11-hydroxyajmalicine (**5**) and 10-hydroxyajmalicine (**6**), consistent with previous reports of indole alkaloid metabolism.[8,9] Primarily interested in profiling the 5-$HT_{2A/2C}$ activity of these compounds, we hypothesized that 10-hydroxyajmalicine was likely the more active metabolite given the serotonin pharmacophore present in its scaffold.

As the metabolites were obtained in quantities only sufficient for analytical studies, we elected to truncate the scaffold, retaining just the 6-hydroxytetrahydro-β-carboline core for further exploration. These simplified analogues are accessible in a single synthetic step (Scheme 1), rendering

Scheme 1. Pictet–Spengler Reaction of 6-Substituted Tryptamines with Aldehydes to Afford Tetrahydro-β-Carboline Analogues[a]



[a]Conditions (*a*): dimethylformamide, 70 °C, 16–96 h or glacial acetic acid, microwave 110 °C, 5–90 min.

them more amenable to rapid derivatization than the full natural products requiring complex multistep syntheses. The tetrahydro-β-carboline is a privileged scaffold,[10,11] with even its simplest natural derivatives displaying diverse bioactivity.[12] Perhaps the most well-known synthetic tetrahydro-β-carboline is tadalafil, the phosphodiesterase-5 inhibitor marketed as Cialis.[13] Across the 5-HT_2 family, this chemotype is known to display potent yet tunable biased agonist[14] or antagonist activity, as demonstrated in the development of serotonin-2B (5-HT_{2B}) antagonist LY266097.[15]

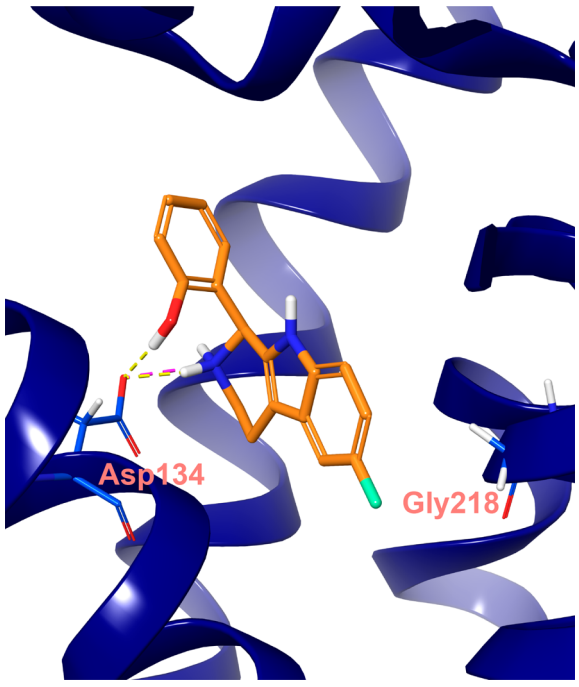


Figure 3. Docked pose of **41** in 5-HT_{2C} (6BQG). Yellow dashed lines indicate hydrogen bonding interactions, and pink dashed line indicates salt bridge formation.

The 5-HT_2 family comprises three G protein-coupled receptors (GPCRs) that share a high degree of sequence homology, and selective targeting of any of the three remains a challenge.[16] 5-HT_{2A} is notable for its mediation of the hallucinogenic effects of psychedelics as well as for its antagonist role in the mechanism of action of second-generation or atypical antipsychotics, such as clozapine.[17] 5-HT_{2C} is also a target of interest in the development of new antipsychotics[18,19] and has been shown to play a role in obesity[20] and substance use disorders.[21−24] Recently, there has been renewed interest in 5-$HT_{2A/2C}$ mixed agonists, such as psilocin,[25] which have shown remarkable long-lasting effects for treatment-resistant depression and other disorders.[26] 5-HT_{2B}, however, is best known as an undesirable off-target whose activation results in valvular heart disease,[27] underscoring the importance of achieving selectivity when targeting 5-HT_{2A} or 5-HT_{2C}. Given the ease of generating several tunable tetrahydro-β-carboline analogues and combined with high-throughput screening technologies, investigation of this scaffold appeared to be a fruitful area for generation of new drug-like compounds for 5-HT_2 receptor subtypes.

We began by constructing a series of 6-hydroxytetrahydro-β-carbolines with variation at the 1 position and profiling their activity at 5-HT_{2A} and 5-HT_{2C} in G_q-mediated calcium flux assays (Table 1). The 1-phenyl analogue **7** was inactive at 5-HT_{2C} and only weakly active at 5-HT_{2A}, but, remarkably, introduction of a fluorine at the 2′ position (**8**) gave full agonism of both receptors with low nanomolar potencies. Switching to 2′-trifluoromethyl (**9**) attenuated potency about 6-fold at both receptors, and chloro (**10**) and methoxy (**11**) substitution at this position again reduced the compounds to

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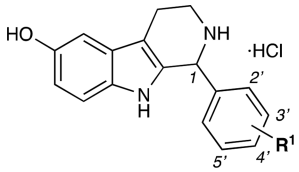
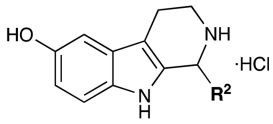
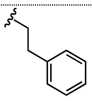
Table 1. Functional Activity of 6-Hydroxytetrahydro-β-carbolines at 5-HT$_{2A}$ and 5-HT$_{2C}$ in the G$_q$-Mediated Calcium Flux Assay[a]





Compound	R$_1$ or R$_2$	5-HT$_{2A}$ EC$_{50}$, nM (pEC$_{50}$)	5-HT$_{2A}$ E$_{max}$ (% 5-HT)	5-HT$_{2C}$ EC$_{50}$, nM (pEC$_{50}$)	5-HT$_{2C}$ E$_{max}$ (% 5-HT)	Selectivity 2C over 2A
5-HT		0.35 (9.46±0.03)	100	0.32 (9.49±0.03)	100	
7	R^1 = H	2514 (5.60±0.06)	26.3±0.6	inactive		
8	R^1 = 2-F	23 (7.63±0.05)	91.4±1.5	13.2 (7.88±0.08)	102.2±2.1	1.97
9	R^1 = 2-CF$_3$	120 (6.92±0.07)	100.0±0.3	76.4 (7.12±0.03)	100±2.3	1.57
10	R^1 = 2-Cl	1422 (5.85±0.10)	14.3±0.8	inactive		
11	R^1 = 2-OMe	269 (6.57±0.07)	21.5±0.7	inactive		
12	R^1 = 2-OH	106 (6.98±0.06)	26.9±0.6	85.9 (7.07±0.07)	103.1±2.5	4.72
13	R^1 = 3-OH	223 (6.65±0.07)	93.4±2.1	148 (6.83±0.08)	95.4±1.5	1.54
14	R^1 = 4-OH	159 (6.80±0.07)	80.0±0.3	152 (6.82±0.12)	99.4±1.4	1.29
15	R^1 = 3-Me	244 (6.61±0.05)	60.9±1.1	191 (6.72±0.03)	99.1±0.9	2.06
16	R^1 = 4-Me	312 (6.50±0.05)	11.2±1.2	1131 (5.95±0.15)	95±2.5	2.42
17	R^1 = 2-OH-4-OMe	0.5 (9.31±0.08)	103.2±2.1	0.8 (9.10±0.06)	114.2±3.6	0.93
18	R^1 = 2-OH-4-Br	364 (6.44±0.50)	67.8±1.3	173 (6.76±0.06)	100.2±1.8	3.10
19	R^1 = 2-OH-5-F	216 (6.66±0.07)	88.4±1.2	77.0 (7.11±0.09)	101.2±0.9	3.22
20	R^1 = 2-OH-5-Cl	1570 (5.80±0.05)	47.6±0.8	754 (6.12±0.03)	97.1±0.9	4.22
21	R^1 = 2-OH-5-Br	455 (6.34±0.05)	85.9±0.5	194 (6.71±0.07)	104.2±2.1	2.85
22	R^1 = 2,4,5-OMe	3.8 (8.40±0.08)	102.3±0.8	7.6 (8.10±0.05)	108.8±1.1	0.52
23	R^2 = 	346 (6.46±0.05)	44.7±1.0	294 (6.53±0.06)	97.0±2.5	2.55
24	R^2 = 	79 (7.10±0.08)	100.0±1.1	97 (7.01±0.03)	87.1±0.5	0.71
25	R^2 = 	800 (6.09±0.03)	89.3±1.4	1800 (5.53±0.03)	83.3±1.7	0.26
26	R^2 = 	281 (6.55±0.05)	74.1±0.8	959 (6.02±0.05)	28.4±0.7	0.11
27	R^2 = 	139 (6.86±0.05)	45.8±0.8	inactive		
28	R^2 = 	288 (6.54±0.08)	59.2±1.3	481 (6.32±0.07)	100.1±2.1	1.01
29	R^2 = 	195 (6.71±0.02)	85.1±0.8	61.0 (7.21±0.05)	97.6±1.8	3.66

[a]All data were generated in stable cell lines expressing 5-HT$_{2A}$ or 5-HT$_{2C}$ receptors and represent at least three independent experiments performed in triplicate. E_{max} is calculated as percent 5-HT response performed for every experiment. Selectivity was calculated by the difference in log(E_{max}/EC$_{50}$) between 5-HT$_{2C}$ and 5-HT$_{2A}$.

inactivity at 5-HT$_{2C}$ and weak partial agonism at 5-HT$_{2A}$. However, 2′-hydroxyl analogue **12** displayed full agonism at 5-HT$_{2C}$ with EC$_{50}$ = 85.9 nM and partial agonism at 5-HT$_{2A}$ with EC$_{50}$ = 106 nM. Shifting the hydroxyl substituent to the 3′ or 4′ position (**13**, **14**) slightly attenuated activity at both receptors. While a 3′-methyl substituent (**15**) maintained similar activity, a 4′-methyl substituent (**16**) had deleterious effects on activity at both receptors.

As **12** was thus far the only fairly potent analogue to exhibit any degree of selectivity between 5-HT$_{2A}$ and 5-HT$_{2C}$, we next synthesized a number of di- and trisubstituted analogues incorporating the 2′-hydroxyl substituent. Introducing a methoxy substituent at the 4′ position (**17**) led to

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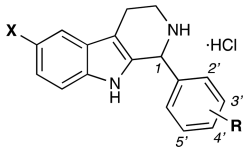
Table 2. Functional Activity of 1-Phenyl-6-halotetrahydro-β-carbolines at 5-HT_{2A} and 5-HT_{2C} in the G_q-Mediated Calcium Flux Assay[a]



			5-HT_{2A}		5-HT_{2C}		
	R	X	EC_{50}, nM (pEC_{50})	E_{max}(% 5-HT)	EC_{50} nM (pEC_{50})	E_{max}(% 5-HT)	selectivity, 2C over 2A
5-HT			0.35 (9.46 ± 0.03)	100	0.32 (9.49 ± 0.03)	100	
30	H	Cl	248 (6.61 ± 0.03)	83.9 ± 0.9	68.9 (7.16 ± 0.06)	98.0 ± 2.0	4.18
31		F	88.5 (7.05 ± 0.03)	86.5 ± 0.9	22.4 (7.65 ± 0.05)	101.6 ± 1.5	4.82
32	2-F	Cl	8004 (5.10 ± 0.12)	23.2 ± 0.8	1164 (5.93 ± 0.08)	13.9 ± 1.2	4.17
33		F	302 (6.52 ± 0.08)	48.9 ± 1.5	1006 (6.00 ± 0.06)	81.0 ± 2.3	0.53
34	2-Cl	Cl	12.8 (7.89 ± 0.07)	95.3 ± 0.8	12.6 (7.90 ± 0.03)	109.1 ± 0.2	1.16
35		F	278 (6.56 ± 0.10)	28.1 ± 0.8	872 (6.06 ± 0.05)	88.1 ± 0.8	1.00
36	2-Br	Cl	403 (6.39 ± 0.05)	91.4 ± 0.5	95.2 (7.02 ± 0.05)	98.4 ± 0.8	4.60
37		F	98 (7.01 ± 0.25)	10.5 ± 1.1	2099 (5.68 ± 0.02)	75.1 ± 1.0	0.33
38	2-OMe	Cl	207 (6.68 ± 0.05)	41.6 ± 0.9	284 (6.55 ± 0.06)	82.9 ± 1.9	1.47
39		F	126 (6.90 ± 0.05)	43.1 ± 0.7	75.4 (7.12 ± 0.07)	94.3 ± 2.2	3.65
40	2-OH	Cl	51.7 (7.29 ± 0.03)	92.7 ± 0.9	15.3 (7.81 ± 0.05)	103.4 ± 1.5	3.85
41		F	26 (7.58 ± 0.07)	96.2 ± 0.8	4.3 (8.36 ± 0.05)	100.3 ± 0.5	6.30
42	3-OH	Cl	3304 (5.48 ± 0.07)	49.1 ± 0.7	688 (6.16 ± 0.03)	67.4 ± 0.8	6.54
43		F	208 (6.68 ± 0.04)	83.2 ± 1.3	130 (6.89 ± 0.04)	122.9 ± 2.1	2.40
44	4-OH	Cl	93.8 (7.03 ± 0.08)	103.1 ± 1.3	105 (6.98 ± 0.07)	99.4 ± 0.9	0.86
45		F	58.2 (7.24 ± 0.05)	99.9 ± 2.1	29.8 (7.52 ± 0.04)	104.3 ± 2.4	2.03
46	3-Me	Cl	481 (6.32 ± 0.08)	63.1 ± 0.5	256 (6.59 ± 0.09)	89.1 ± 0.5	2.67
47		F	160 (6.80 ± 0.07)	39.9 ± 1.2	306 (6.51 ± 0.04)	114.2 ± 2.1	1.55
48	4-Me	Cl	303 (6.52 ± 0.08)	94.1 ± 0.9	62.5 (7.20 ± 0.06)	97.2 ± 0.5	4.98
49		F	208 (6.68 ± 0.07)	39.9 ± 1.2	2058 (5.69 ± 0.04)	85.4 ± 2.0	0.22

[a]All data were generated in stable cell lines expressing 5-HT_{2A} or 5-HT_{2C} receptors and represent at least three independent experiments performed in triplicate. E_{max} is calculated as percent 5-HT response performed for every experiment. Selectivity was calculated by the difference in log(E_{max}/EC_{50}) between 5-HT_{2C} and 5-HT_{2A}.

complete loss of selectivity but demonstrated full agonism with subnanomolar potency at both receptors. This activity was not maintained with a switch to 4′-bromo (**18**), nor was it matched by any 2′-hydroxyl-5′-halo analogues (**19**–**21**). However, 2′,4′,5′-trimethoxy analogue **22** was a full agonist with EC_{50} values of 3.8 and 7.6 nM at 5-HT_{2A} and 5-HT_{2C}, respectively.

We then evaluated the effects of heterocyclic substituents at the 1 position. Unlike phenyl compound **7**, the bioisosteric thiophene compound **23** was active at both 5-HT_{2A} and 5-HT_{2C}. Pyrimidine analogue **25** was a weaker agonist at both, but *N*-methyl imidazole compound **24** proved to be fairly potent with EC_{50} = 79 nM for 5-HT_{2A} and 97 nM for 5-HT_{2C}. Aliphatic substitution at the 1 position gave rise to active compounds as well (**26**–**29**), though only 1-ethyl compound **29** had full agonist activity at both receptors.

We next turned our attention to the 6 position of the tetrahydro-β-carboline scaffold to examine the feasibility of tuning the selectivity of our compounds. As halogenation at the analogous position on tranylcypromine derivatives previously demonstrated substantial impact on 5-HT_{2A} versus 5-HT_{2C} selectivity,[28] we synthesized and evaluated parallel series of 6-chloro- and 6-fluorotetrahydro-β-carbolines (Table 2). In contrast to **7**, 1-phenyl compounds **30** and **31** displayed robust activity at both 5-HT_{2A} and 5-HT_{2C}. Introduction of a 2′-fluoro substituent (**32**, **33**) dramatically attenuated potency, perhaps rendering these analogues too electron-deficient. Other 2′- halo compounds (**34**–**37**) fared generally better, as did the 2′-methoxy analogues in both series (**38**, **39**). As observed in the 6-hydroxyl series, the most favorable substituent 2′ substituent proved to be a hydroxyl group, affording potent agonists **40** and **41** with EC_{50} = 51.7 and 26 nM at 5-HT_{2A} and EC_{50} = 15.3 and 4.3 nM at 5-HT_{2C}. Shifting the hydroxyl to the 3′ or 4′ position reduced potency at both receptors (**42**–**45**), and the 3′- and 4′-methyl analogues were similarly less active (**46**–**49**).

Fluoropyridine derivatives **50**–**52** were partial agonists at 5-HT_{2A} and 5-HT_{2C}, and they were 2–14-fold selective for the former over the latter (Table 3). Though pyrimidine compound **53** was a partial agonist at 5-HT_{2A} and 5-HT_{2C}, its 6-fluoro counterpart **54** was a full agonist at both receptors with EC_{50} = 54.9 and 10.1 nM at 5-HT_{2A} and 5-HT_{2C}, respectively. As for five-membered heterocycles, isoxazole analogues **55** and **56** were fairly potent at both receptors, while thiazole compound **57** was a micromolar partial agonist at 5-HT_{2C} and inactive at 5-HT_{2A}. Thiophene compounds **58**–**62** demonstrated markedly greater efficacy at 5-HT_{2C} than 5-HT_{2A}, a trend likewise observed in benzthiophene analogues **63** and **64**. Other compounds with larger substituents at the 1 position generally had diminished activity (**65**–**68**), with the exception of naphthalene derivative **65**, a full agonist at 5-HT_{2A} and 5-HT_{2C} with EC_{50} = 15.1 and 24.5 nM, respectively.

As in the 6-hydroxyl series, aliphatic substituents at the 1 position gave rise to some active compounds as well (Table 4). Hydrocinnamyl compound **75** was particularly potent at

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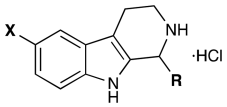
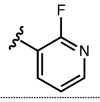
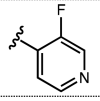
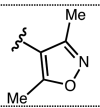
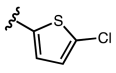
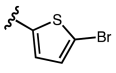
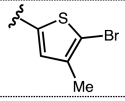
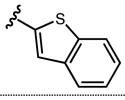
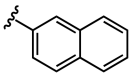
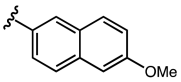
Table 3. Functional Activity of 6-Halotetrahydro-β-carbolines with Heteroaromatic and Bicyclic 1-Substituents at 5-HT_{2A} and 5-HT_{2C} in the G_q-Mediated Calcium Flux assay[a]



Compound	R	X	5-HT_{2A} EC_{50}, nM (pEC_{50})	5-HT_{2A} E_{max} (% 5-HT)	5-HT_{2C} EC_{50}, nM (pEC_{50})	5-HT_{2C} E_{max} (% 5-HT)	Selectivity 2C over 2A
5-HT			0.35 (9.46±0.03)	100	0.32 (9.49±0.03)	100	
50		Cl	346 (6.46±0.05)	56.1±1.6	1060 (5.97±0.07)	75.2±0.8	0.44
51		F	390 (6.41±0.03)	40.9±0.8	4027 (5.40±0.04)	30.2±0.5	0.07
52		Cl	849 (6.07±0.04)	57.1±0.8	2806 (5.55±0.05)	27.3±0.8	0.14
53		Cl	898 (6.05±0.10)	32.1±0.8	420 (6.38±0.07)	38.7±0.4	2.60
54		F	54.9 (7.26±0.03)	95.1±1.0	10.1 (8.00±0.06)	100.8±1.7	6.30
55		Cl	513 (6.29±0.02)	84.5±1.5	191 (6.72±0.03)	103.6±2.0	3.31
56		F	239 (6.62±0.02)	90.7±1.2	50.1 (7.30±0.03)	110.6±1.9	5.92
57		F	inactive		1259 (5.90±0.22)	39.4±4.9	
58		Cl	724 (6.14±0.08)	23.7±0.9	602 (6.22±0.09)	48.6±2.6	2.47
59		F	409 (6.39±0.08)	36.4±1.3	147 (6.83)	102.0±2.1	7.75
60		Cl	177 (6.75±0.03)	100.4±1.5	44 (7.36±0.08)	117±4.5	4.84
61		Cl	912 (6.04±0.04)	62.4±1.2	447 (6.35±0.08)	104±3.8	3.42
62		Cl	776 (6.11±0.04)	97.1±3.1	173 (6.76±0.08)	118±5.5	5.46
63		Cl	151 (6.82±0.02)	88±1.5	39 (7.41±0.07)	105±3.9	4.62
64		F	1548 (5.81±0.05)	40.0±1.2	912 (6.04±0.08)	110.2±4.4	4.68
65		Cl	15.1 (7.82±0.04)	96.5±1.5	24.5 (7.61±0.02)	102.2±0.9	0.64
66		F	195 (6.71±0.04)	79.1±1.5	186 (6.73±0.03)	103.4±1.1	1.37
67		Cl	1318 (5.88±0.05)	66.8±2.0	1063 (5.97±0.04)	93.7±2.0	1.73
68		F	2818 (5.55±0.14)	30.5±2.7	3388 (5.47±0.06)	76.3±3.1	2.08

[a]All data were generated in stable cell lines expressing 5-HT_{2A} or 5-HT_{2C} receptors and represent at least three independent experiments performed in triplicate. E_{max} is calculated as percent 5-HT response performed for every experiment. Selectivity was calculated by the difference in log(E_{max}/EC_{50}) between 5-HT_{2C} and 5-HT_{2A}.

both receptors with EC_{50} = 64.0 nM at 5-HT_{2A} and 15.8 nM at 5-HT_{2C}. Notably, deletion of a methylene unit gave compound **77**, which was inactive at 5-HT_{2C} and a weak partial agonist at 5-HT_{2A}, as was its 6-fluoro counterpart **78**. This finding is perhaps unsurprising given the structural similarity of these compounds to 5-HT_{2B} antagonist LY266097.

Docking studies performed with some of our most potent compounds and structures of both 5-HT_{2A}[29] and 5-HT_{2C}[30] indicate that in both receptors the tetrahydro-β-carboline core occupies the orthosteric site with its protonated amine forming a salt bridge with $Asp^{3.32}$, the 6-substituent lying in the vicinity of transmembrane domain 5, and the indole N–H bond oriented upward toward the extracellular end of the

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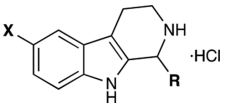
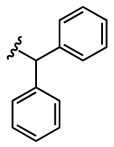
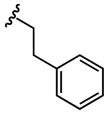
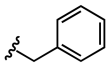
Table 4. Functional Activity of 6-Halotetrahydro-β-carbolines with Aliphatic 1-Substituents at 5-HT_{2A} and 5-HT_{2C} in the G_q-Mediated Calcium Flux Assay[a]



Compound	R	X	5-HT_{2A} EC_{50}, nM (pEC_{50})	5-HT_{2A} E_{max} (% 5-HT)	5-HT_{2C} EC_{50}, nM (pEC_{50})	5-HT_{2C} E_{max} (% 5-HT)	Selectivity 2C over 2A
5-HT			0.35 (9.46±0.03)	100	0.32 (9.49±0.03)	100	
69		Cl	1348 (5.87±0.05)	88.1±2.7	3235 (5.49±0.02)	106.2±1.6	0.50
70		F	1905 (5.72±0.03)	92.5±0.1	954 (6.02±0.02)	103.4±1.2	2.23
71		Cl	295 (6.53±0.03)	59.9±0.6	95.5 (7.02±0.06)	98.5±2.2	5.11
72		F	91.2 (7.04±0.02)	87.3±0.8	20.4 (7.69±0.06)	102.9±2.1	5.45
73		Cl	371 (6.43±0.11)	42.8±2.2	295 (6.53±0.05)	63.8±1.6	1.88
74		F	354 (6.45±0.10)	34.1±1.6	891 (6.05±0.05)	42.3±1.0	0.49
75		Cl	64.0 (7.19±0.07)	101.1±2.0	15.8 (7.80±0.07)	98.5±2.1	4.15
76		F	812 (6.09±0.08)	31.7±1.1	467 (6.33±0.05)	91.3±2.0	5.01
77		Cl	2622 (5.58±0.04)	56.1±1.1	inactive		
78		F	1329 (5.88±0.06)	44.4±0.9	inactive		
79		Cl	398 (6.40±0.04)	74.3±1.4	7943 (5.10±0.02)	80.8±1.6	0.05
80		F	597 (6.22±0.04)	75.4±0.8	200 (6.70±0.03)	96.2±0.7	3.84
81		Cl	646 (6.19±0.09)	50.1±2.3	>10000		
82		F	>10000		>10000		

[a]All data were generated in stable cell lines expressing 5-HT_{2A} or 5-HT_{2C} receptors and represent at least three independent experiments performed in triplicate. E_{max} is calculated as percent 5-HT response performed for every experiment. Selectivity was calculated by the difference in log(E_{max}/EC_{50}) between 5-HT_{2C} and 5-HT_{2A}.

receptor (representative pose with 5-HT_{2C} shown in Figure 3; 5-HT_{2A} not shown). This pose is in accordance with that observed in the crystal structure of LY266097 in complex with 5-HT_{2B}.[14] The favorable effects of incorporating a 2′-hydroxyl substituent, as demonstrated by analogue **41**, are likely reflective of the additional hydrogen bond which can be formed with $Asp^{3.32}$.

We next expanded our library of compounds to include di- and trisubstituted phenyl substituents at the 1 position, holding the 2′-hydroxyl constant to retain that favorable hydrogen bonding interaction (Table 5). Though addition of halogens at the 3′ and 4′ positions did not improve activity (**83**−**90**), incorporating a second hydroxyl substituent at the 4′ position afforded compound **91**, a full agonist at both receptors with EC_{50} = 1.5 and 1.9 nM at 5-HT_{2A} and 5-HT_{2C}, respectively. By contrast, 2′,4′-dimethoxy analogue **92** was around 100-fold less potent. However, 2′- hydroxy-4′-methoxy analogues **93** and **94** exhibited activity more akin to that of **91**, possibly underscoring the impact of hydrogen bond donation from the 2′ position. As expected, dimethyl analogues **95** and **96** were far less active at both receptors.

Compounds with 2,5-substitution patterns (**97**−**105**) generally displayed decreased activity as compared to **40** and **41**. Though 2′,5′-dimethoxy analogue **104** was a weak partial agonist at both receptors, incorporation of a third methoxy substituent at the 4′ position gave compound **106** with EC_{50} = 1.7 and 0.50 nM at 5-HT_{2A} and 5-HT_{2C}, one of the most potent compounds across all our series. Removal of the 2′-methoxy substituent resulted in 80- and 200-fold loss of potency at 5-HT_{2A} and 5-HT_{2C}, respectively (**108**).

The subnanomolar activity of **106** was particularly intriguing. The SAR thus far indicated that a hydrogen bond donor was preferred at the 2′ position and that activity at both receptors was diminished with replacement by a 2′-methoxy group, observations supported by the compounds' docked poses. Additionally, the much weaker activity profiles of 2′,4′-dimethoxy analogue **92** and 2′,5′-dimethoxy analogues **104** and **105** did not suggest that a combination of their characteristics would lead to dramatic improvements in potency. However, the 2,4,5-trisubstituted phenyl motif has a well-documented history of giving rise to powerful psychedelics in the phenethylamine class.[31,32] Docking studies

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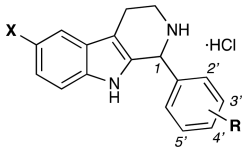
Table 5. Functional Activity of 6-Halotetrahydro-β-carbolines at 5-HT$_{2A}$, 5-HT$_{2C}$, and 5-HT$_{2B}$ in the G$_q$-Mediated Calcium Flux Assay[a]



			5-HT$_{2A}$		5-HT$_{2C}$			5-HT$_{2B}$	
	R	X	EC$_{50}$ nM (pEC$_{50}$)	E_{max} (% 5-HT)	EC$_{50}$ nM (pEC$_{50}$)	E_{max} (% 5-HT)	sel	EC$_{50}$ nM (pEC$_{50}$)	E_{max} (% 5-HT)
5-HT			0.35 (9.46 ± 0.03)	100	0.32 (9.49 ± 0.03)	100		0.36 (9.44 ± 0.05)	100
83	2-OH-3-Cl	Cl	336 (6.47 ± 0.03)	70.1 ± 2.3	176 (6.75 ± 0.04)	94.0 ± 2.7	2.56	106 (6.97 ± 0.03)	95.9 ± 1.2
84		F	273 (6.56 ± 0.04)	65.3 ± 2.8	78 (7.11 ± 0.04)	99.2 ± 2.3	5.33	88 (7.06 ± 0.02)	96.2 ± 1.6
85	2-OH-3-Br	Cl	74.5 (7.13 ± 0.04)	97.9 ± 0.8	68.6 (7.16 ± 0.08)	107.2 ± 2.1	1.18	nt	
86		F	116 (6.94 ± 0.08)	93.1 ± 0.7	66.5 (7.18 ± 0.02)	109.4 ± 3.1	2.05	nt	
87	2-OH-4-Cl	Cl	52 (7.29 ± 0.02)	98.5 ± 0.6	47 (7.33 ± 0.02)	102.4 ± 0.8	1.14	25 (7.61 ± 0.03)	108.9 ± 1.2
88		F	229 (6.64 ± 0.03)	73.2 ± 0.8	209 (6.68 ± 0.03)	106.6 ± 1.5	1.60	128 (6.90 ± 0.02)	105.2 ± 0.9
89	2-OH-4-Br	Cl	537 (6.27 ± 0.02)	94.5 ± 1.0	794 (6.10 ± 0.02)	106.8 ± 1.4	0.76	204 (6.69 ± 0.02)	106.2 ± 0.9
90		F	147 (6.83 ± 0.02)	98.0 ± 0.7	95 (7.02 ± 0.02)	110.8 ± 0.8	1.76	59 (7.23 ± 0.02)	112.1 ± 0.7
91	2,4-OH	Cl	1.5 (8.82 ± 0.02)	91.2 ± 0.2	1.9 (8.72 ± 0.04)	101.7 ± 1.1	0.89	nt	
92	2,4-OMe	F	167 (6.78 ± 0.04)	45.6 ± 0.7	159 (6.80 ± 0.03)	100.5 ± 1.1	2.31	98 (7.01 ± 0.01)	91.3 ± 0.5
93	2-OH-4-OMe	Cl	8.3 (8.08 ± 0.02)	100.0 ± 0.8	8.9 (8.05 ± 0.02)	97.2 ± 0.8	0.90	3.1 (8.50 ± 0.02)	99.4 ± 0.7
94		F	3.2 (8.50 ± 0.02)	102.3 ± 0.8	1.02 (8.99 ± 0.03)	105.2 ± 1.0	3.23	1.3 (8.88 ± 0.07)	101.9 ± 2.1
95	2,4-Me	Cl	562 (6.25 ± 0.03)	69.8 ± 1.1	407 (6.39 ± 0.05)	99.2 ± 2.2	1.96	977 (6.01 ± 0.03)	89.4 ± 1.5
96		F	363 (6.44 ± 0.05)	47.8 ± 1.2	159 (6.80 ± 0.04)	104.3 ± 1.3	5.01	537 (6.27 ± 0.02)	107.4 ± 1.3
97	2-OH-5-Cl	Cl	>10 000		3560 (5.45 ± 0.05)	58.9 ± 1.9		1995 (5.70 ± 0.02)	110.9 ± 1.1
98	2-OH-5-Br	Cl	812 (6.09 ± 0.06)	79.7 ± 2.4	1318 (5.88 ± 0.04)	95.2 ± 2.3	0.74	457 (6.34 ± 0.02)	110.7 ± 0.9
99		F	1112 (5.95 ± 0.02)	80.8 ± 0.9	376 (6.42 ± 0.02)	101.3 ± 0.9	3.71	338 (6.47 ± 0.03)	111.4 ± 1.4
100	2-OH-5-Me	Cl	inactive		inactive			4265 (5.37 ± 0.04)	106.2 ± 2.8
101		F	1230 (5.91 ± 0.07)	36.8 ± 1.5	446 (6.35 ± 0.03)	99.2 ± 1.2	7.43	316 (6.50 ± 0.02)	115.2 ± 1.1
102	2,5-OH	Cl	inactive		inactive			inactive	
103		F	72 (7.14 ± 0.02)	95.2 ± 0.9	42 (7.38 ± 0.03)	119.6 ± 1.1	2.02	25 (7.60 ± 0.01)	102.3 ± 0.4
104	2,5-OMe	Cl	1820 (5.74 ± 0.15)	14.4 ± 1.3	2691 (5.57 ± 0.04)	42.4 ± 1.2	1.94	416 (6.38 ± 0.05)	32.0 ± 0.7
105		F	>10000		1604 (5.80 ± 0.04)	55.3 ± 1.2		588 (6.23 ± 0.04)	52.1 ± 1.1
106	2,4,5-OMe	Cl	1.7 (8.77 ± 0.04)	98.4 ± 1.1	0.50 (9.33 ± 0.07)	97.5 ± 1.9	3.58	0.58 (9.24 ± 0.06)	96.7 ± 1.7
107		F	39 (7.40 ± 0.01)	97.1 ± 0.5	20 (7.69 ± 0.02)	110.6 ± 1.1	2.19	10 (7.96 ± 0.02)	105.9 ± 0.6
108	3,4-OMe	Cl	81 (7.09 ± 0.03)	96.0 ± 1.0	123 (6.91 ± 0.02)	99.3 ± 1.0	0.68	34 (7.47 ± 0.02)	101.6 ± 0.6
109		F	254 (6.60 ± 0.02)	83.2 ± 0.7	152 (6.82 ± 0.03)	102.7 ± 1.3	2.05	102 (6.99 ± 0.03)	89.8 ± 1.0

[a]All data were generated in stable cell lines expressing 5-HT$_{2A}$, 5-HT$_{2C}$, or 5-HT$_{2B}$ receptors and represent at least three independent experiments performed in triplicate. E_{max} is calculated as percent 5-HT response performed for every experiment. "sel" = selectivity for 5-HT$_{2C}$ over 5-HT$_{2A}$ and was calculated by the difference in log(E_{max}/EC$_{50}$) between 5-HT$_{2C}$ and 5-HT$_{2A}$. "nt" = not tested.

with **106** and the structure of 5-HT$_{2A}$[29] (Figure 4) gave insight into its activity profile: the 2,4,5-substituted phenyl ring occupies the orthosteric site within the binding pocket, and the tetrahydro-β-carboline unit extends downward toward transmembrane domain 6, engaging in π interactions with Phe340$^{6.52}$. Finally, an edge-to-face π-stacking interaction with Trp336$^{6.48}$, the canonical "toggle switch" residue, may account for this compound's robust agonist activity.

We next evaluated a subset of our compounds at 5-HT$_{2B}$. These results for our final series of compounds are summarized in Table 5 (see Table S1 for 5-HT$_{2B}$ data for other compounds). In general, the activity of analogues at 5-HT$_{2B}$ was similar to their activity at 5-HT$_{2A}$ and 5-HT$_{2C}$ with no distinct pattern of selectivity for any one receptor over the others (Figure 5). Another selection of compounds was submitted to the Psychoactive Drug Screening Program (PDSP) for screening across a wide array of GPCRs and other targets (Table 6).[33] Outside of the 5-HT family, these analogues demonstrated little to weak affinity for off-target receptors. (See Table S2 for PDSP data for additional compounds.)

Inspired by a newly identified ajmalicine metabolite and the motivation to prepare simplified analogues of neuroactive heteroyohimbine natural products, we synthesized three series of tetrahydro-β-carbolines and evaluated their activity at 5-HT$_{2A}$ and 5-HT$_{2C}$. The 2′,4′,5′-trimethoxy compound **106** was found to be a highly potent agonist at both receptors, with EC$_{50}$ = 1.7 and 0.50 nM for 5-HT$_{2A}$ and 5-HT$_{2C}$, respectively. Docking studies suggest that a π-stacking interaction between the tetrahydro-β-carboline core and conserved residue Trp$^{6.48}$ is the structural basis for this activity. Our work reveals determinants of 5-HT$_2$ subtype activation and highlights 6-hydroxyl and 6-halo tetrahydro-β-carbolines as highly potent agonists of the 5-HT$_2$ receptor family. Considering the importance of these receptors in various neurological and psychiatric diseases, this investigation lays a foundation for further development of this compound scaffold toward these targets.

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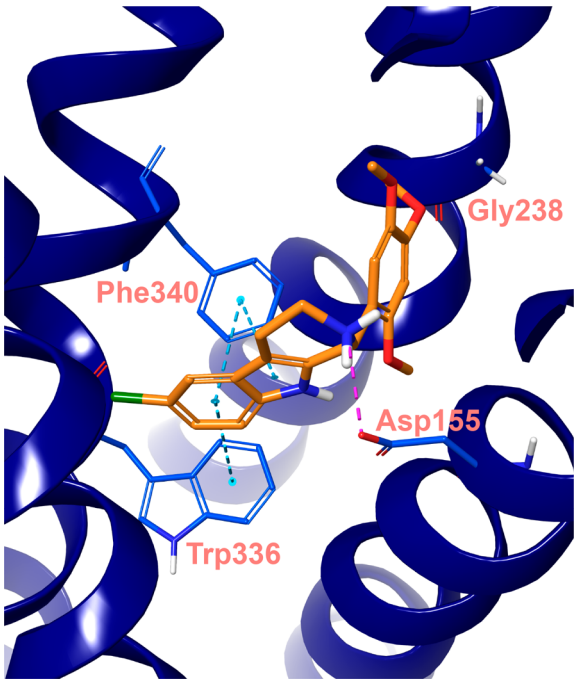

Figure 4. Docked pose of **106** in 5-HT$_{2A}$ (6WHA). Pink dashed line indicates salt bridge formation, and blue dashed lines indicate π interactions.

Table 6. Binding Affinity Data for Selected Compounds[a]

	K_i (nM)			
	93	**94**	**106**	**107**
5-HT$_{1A}$	79.9	94.4	22.4	1367
5-HT$_{1B}$	164	70.8	52.1	nd
5-HT$_{1D}$	31.5	110	38.8	660
5-HT$_{1E}$	1750	1409	666	nd
5-HT$_{2A}$	225	250	113	2445
5-HT$_{2B}$	4.53	4.42	2.93	55.7
5-HT$_{2C}$	35.9	49.8	25.2	671
5-HT$_3$	2790	nd	2785	nd
5-HT$_{5A}$	177	229	69.5	2839
5-HT$_6$	47.7	68.8	29.0	530
5-HT$_7$	7.98	25.4	2.50	249
α_{2C}	nd	nd	nd	964
β_1	nd	nd	1475	961
D3	1246	nd	nd	nd
D4	609	362	nd	nd
SERT	nd	1841	nd	nd
σ_1	1032	nd	1493	760
σ_2	nd	nd	nd	2328

[a]"nd" indicates K_i not determined as binding inhibition was <50%. Additionally, all four compounds were found to have binding inhibition < 50% at α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B}, BZP, β_2, β_3, D1, D2, D5, H1, H2, H3, H4, M1, M2, M3, M4, M5, DOR, KOR, MOR, DAT, NET, BZP, PBR, and GABA-A.

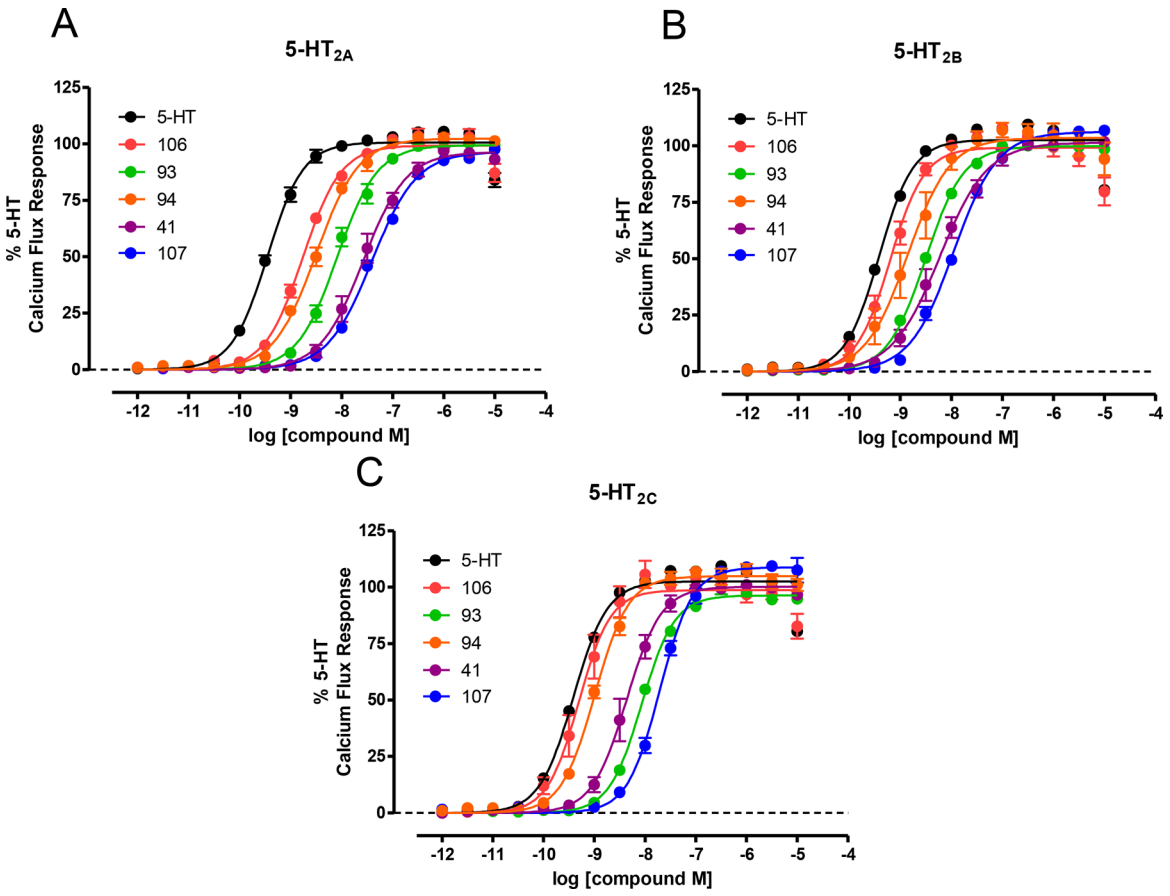

Figure 5. 5-HT$_2$ G$_q$-mediated calcium flux activity of highly potent tetrahydro-β-carbolines. All compounds were assayed in stable cell lines measuring G$_q$-mediated calcium flux at (A) 5-HT$_{2A}$, (B) 5-HT$_{2B}$, and (C) 5-HT$_{2C}$ receptors. Data represent mean and standard error of the mean from at least three independent experiments performed in triplicate. All data were normalized to percent 5-HT response.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.1c00694.

Additional 5-HT_{2B} and PDSP assay results, experimental details for functional assays and metabolism study, modeling protocol, compound synthesis and characterization (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

John D. McCorvy − *Department of Cell Biology, Neurobiology, and Anatomy, Medical College of Wisconsin, Milwaukee, Wisconsin 53226, United States*; Email: jmccorvy@mcw.edu

Karl A. Scheidt − *Department of Chemistry, Northwestern University, Evanston, Illinois 60208, United States; Department of Pharmacology, Northwestern University, Chicago, Illinois 60208, United States*; orcid.org/0000-0003-4856-3569; Email: scheidt@northwestern.edu

Authors

Meghan J. Orr − *Department of Chemistry, Northwestern University, Evanston, Illinois 60208, United States*

Andrew B. Cao − *Department of Cell Biology, Neurobiology, and Anatomy, Medical College of Wisconsin, Milwaukee, Wisconsin 53226, United States*

Charles Tiancheng Wang − *Department of Chemistry, Northwestern University, Evanston, Illinois 60208, United States*

Arsen Gaisin − *Department of Chemistry, Northwestern University, Evanston, Illinois 60208, United States*

Adam Csakai − *Department of Chemistry, Northwestern University, Evanston, Illinois 60208, United States*

Alec P. Friswold − *Department of Chemistry, Northwestern University, Evanston, Illinois 60208, United States*

Herbert Y. Meltzer − *Department of Pharmacology and Department of Psychiatry and Behavioral Sciences, Northwestern University, Chicago, Illinois 60208, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsmedchemlett.1c00694

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

GPCR, G protein-coupled receptor; 5-HT_{2C}, serotonin-2C receptor; 5-HT_{2A}, serotonin-2A receptor; 5-HT_{2B}, serotonin-2B receptor; PDSP, Psychoactive Drug Screening Program

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