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Rational Drug Design Leading to the Identification of a Potent 5-HT$_{2C}$ Agonist Lacking 5-HT$_{2B}$ Activity

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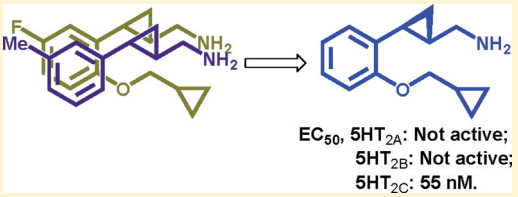
Supporting Information

ABSTRACT: The 5-HT$_{2C}$ receptor is an attractive drug target in the quest for new therapeutics to treat a variety of human disorders. We have previously undertaken a structural optimization campaign that has led to some potent and moderately selective 5-HT$_{2C}$ receptor agonists. After expanding our structure−function library, we were able to combine our data sets so as to allow the design of compounds of improved selectivity and potency. We disclose herein the structural optimization of our previously reported 5-HT$_{2B}$/5-HT$_{2C}$ agonists, which has led to the identification of a highly selective 5-HT$_{2C}$ agonist, (+)-*trans*-[2-(2-cyclopropylmethoxyphenyl)cyclopropyl]methylamine hydrochloride, with an EC$_{50}$ of 55 nM and no detectable agonism at the 5-HT$_{2B}$ receptor.



KEYWORDS: *Serotonin, 5-HT$_{2C}$ receptor, 5-HT$_{2B}$ receptor, agonist, hydrophobic interactions*

Serotonin, or 5-hydroxytryptamine (5-HT), is a major neurotransmitter that is believed to be involved in a wide variety of behaviors, including cognition, emotion, attention, and appetite.[1,2] These physiological effects of serotonin are mediated by the activation of 14 receptor subtypes, which have been classified into seven major families (5-HT$_{1-7}$) on the basis of sequence similarity, signal transduction coupling, and pharmacological characteristics.[3,4] The 5-HT$_2$ subtype family consists of three members: 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$, all of which are G protein-coupled receptors (GPCR) sharing a high level of amino acid sequence similarity, especially within the transmembrane regions.[5] The 5-HT$_{2A}$ receptor seems to be the key site for the hallucinogenic action of many drugs, such as lysergic acid diethylamide (LSD), and this receptor is a major target for treating disorders such as schizophrenia and insomnia.[6] Activation of the 5-HT$_{2B}$ receptor, on the other hand, has been associated with severe side effects, such as heart valvulopathy and pulmonary hypertension, and was responsible for the removal of several prescription drugs from the marketplace.[7] The 5-HT$_{2C}$ receptor, unlike the majority of 5-HT receptors, appears to be exclusively localized in the central nervous system (CNS) and is attractive as a promising drug target in the treatment of a number of conditions, including depression, anxiety, obesity, schizophrenia, and erectile dysfunction.[8−14]

To date, a number of 5-HT$_{2C}$ agonists (**1**−**8**, Figure 1) have been generated as possible treatments for several diseases, including obesity, schizophrenia, and diabetes.[15−17] Some of these synthetic ligands have undergone clinical trials, and their further development is currently pending.[15,17] However, due to the complexity of the 5-HT$_{2C}$ receptor structure and its high sequence similarity to the other two subfamily members, the advancement of 5-HT$_{2C}$ ligands to clinical trials, let alone to the marketplace, has proven challenging.

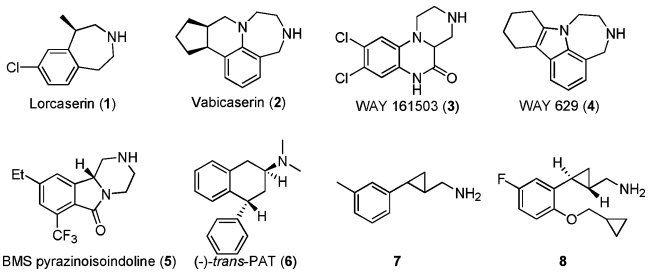


Figure 1. Selected 5-HT$_{2C}$ agonists showing efficacy in preclinical models.

We have previously undertaken a preliminary structural optimization campaign that led to some potent and moderately selective 5-HT$_{2C}$ receptor agonists (**7** and **8**).[18,19] However, for clinical use, the compounds must have good agonist activity at the 5-HT$_{2C}$ receptor while showing no activity at the 5-HT$_{2B}$ receptor. Unfortunately, neither compound **7** nor **8** is adequate (EC$_{50}$ of **7**: 5-HT$_{2A}$, 585 nM; 5-HT$_{2B}$, 65 nM; 5-HT$_{2C}$, 4.8 nM;

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EC_{50} of **8**: 5-HT_{2A}, 894 nM; 5-HT_{2B}, 289 nM; 5-HT_{2C}, 21 nM). After enlarging our structure–function library, we were able to combine our data sets so as to allow the design of compounds of possibly improved selectivity and potency. As shown in Figure 2a, the overlay of our best first- and second-generation

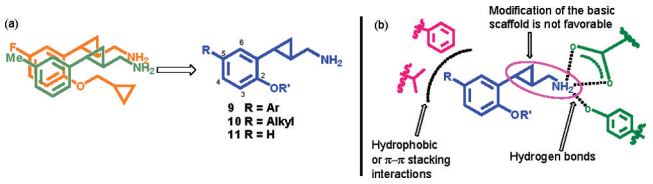


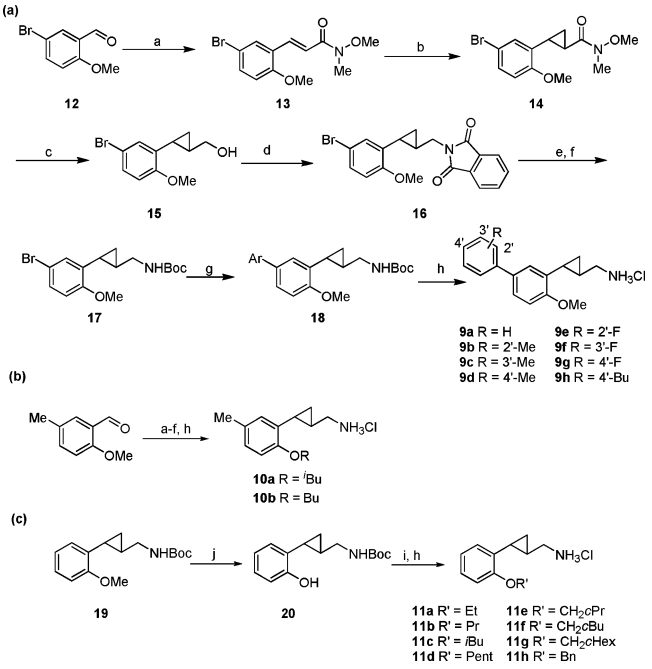
Figure 2. (a) Modification of the C-2 and C-5 substituents is suggested by the overlay of compounds **7** and **8**. (b) Schematic representation of postulated interactions of our ligands with the 5-HT_{2C} receptor.

compounds, **7** and **8**, suggested that it might be rewarding to focus on the substituents at the C-2 and C-5 positions. Moreover, on the basis of the homology modeling studies[20] reported by Jiang's group, we assume that our ligands interact with the 5-HT_{2C} receptor by hydrogen bonding to the amino group, whereas hydrophobic and/or $\pi-\pi$ stacking interactions predominate at the aromatic moiety (Figure 2b). We disclose herein a new series of compounds (**9–11**) bearing nonpolar groups at the C-5 position and different alkoxyl groups at the C-2 position, some of which show good agonist activity at the 5-HT_{2C} receptor with no agonist activity at the 5-HT_{2B} receptor.

The target molecules **9–11** were prepared through a concise route employing the commercially available aldehyde **12** as the starting material (Scheme 1). By taking advantage of the Weinreb amide **13**, compound **14** was obtained exclusively as its *trans* isomer.[21] This intermediate was then reduced to alcohol **15** and condensed with phthalimide under Mitsunobu reaction conditions. Hydrazinolysis[22] and protection with Boc_2O provided the corresponding urethane **17**. Standard Suzuki coupling of **17** with arylboronic acids provided the biaryls **18**, which upon deprotection with hydrogen chloride in diethyl ether provided the target molecules **9a–h** (Scheme 1a). Similar procedures were adopted for the synthesis of compounds **10** (Scheme 1b). Demethylation of **19** with BBr_3 and reprotection of the amine with Boc_2O provided phenol **20**, which afforded in turn **11a–h** by alkylation with the requisite alkyl halide and *N*-deprotection with hydrogen chloride in diethyl ether (Scheme 1c).

The functional activity of the three sets of compounds was determined by measuring Gαq-mediated intracellular calcium mobilization in HEK-293 cells stably expressing the human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} (INI) receptors.[23] In over-expressing cell lines such as those utilized in the current screening, it is common to observe EC_{50} potency concentrations much lower than the K_i binding constant, particularly when antagonist radioligands are used for competition binding studies.[24] The results are summarized in Table 1, in which serotonin (5-HT) is also included for reference purposes. Compounds **10a** and **10b** were designed on the basis of compound **8** in an effort to probe the effect of added hydrophobic interactions at C-5. Both ligands were much less potent at the 5-HT_{2C} receptor than compound **8**, which indicates that the envisaged hydrophobic interactions do not contribute significantly to binding at this site while, in contrast,

Scheme 1. Synthesis of Compounds 9, 10, and 11[a]



[a](a) $Ph_3P{=}CHC(O)N(OMe)Me$, CH_2Cl_2, rt. (b) $Me_3S^+(O)I^-$, NaH, DMSO. (c) DIBAL-H, THF, −78 °C; then $NaBH_4$, MeOH, 0 °C to rt. (d) Phthalimide, PPh_3, DEAD, THF. (e) $N_2H_4-H_2O$, EtOH, reflux. (f) Boc_2O, Et_3N, CH_2Cl_2. (g) $ArB(OH)_2$, $Pd(PPh_3)_4$ (5 mol %), DMF, microwave heating, 120 °C, 2 h. (h) HCl (2 M in Et_2O). (i) RI, K_2CO_3, DMF. (j) BBr_3, CH_2Cl_2, −78 °C to rt; then Boc_2O, Et_3N, CH_2Cl_2.

the methyl group's bulk might account for the decreased potency. To probe the possibility of engaging a 5-substituent in $\pi-\pi$ stacking interactions, the aryl analogues **9a–9h** were synthesized. Unfortunately, most of these compounds also proved to be inactive at the 5-HT_{2C} receptor or had potencies above 1 μM at all tested receptor subtypes. The 2-methylphenyl substituted compound **9b** showed very similar potency (3800 nM vs 3470 nM) and efficacy (64% vs 67%) at the 5-HT_{2C} receptor as the phenyl substituted compound **9a**. Altering the position of the methyl group from C-2′ (**9b**) to C-3′ (**9c**) and then to C-4′ (**9d**) led to a progressive drop in 5-HT_{2C} potency. However, when the methyl group was replaced by a smaller fluorine atom as in compounds **9e–9g**, a less obvious trend was observed. The low potency of this series of methyl- and fluoro-substituted compounds may be a consequence of steric factors that render them less able to fit into the binding pocket of the 5-HT_{2C} receptor. Because of the smaller size of the fluorine atom, its position of attachment has a smaller impact on functional activity in comparison to a methyl group. This hypothesis is further supported by the failure of compound **9h**, which bears a bulky butyl group at C-4′, to exhibit agonism at any 5-HT_2 subtype.

If the loss in potency of compounds **9a–9h** is indeed due to the large volume occupied by the C-5 substituent, we hypothesized that its removal may conversely increase 5-HT_{2C} receptor potency. As the variable substituent, the C-2 alkoxy group was chosen. For the initially synthesized ligands **11a** and **11b**, their potencies and efficacies at the 5-HT_{2C} receptor were found to be higher than those measured at the 5-HT_{2B} subtype. For both compounds, efficacies decreased with increasing bulk of R′, and this decrease was more pronounced

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Table 1. 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ Agonist Activity of Compounds 9a–h, 10a–b, and 11a–h[a]

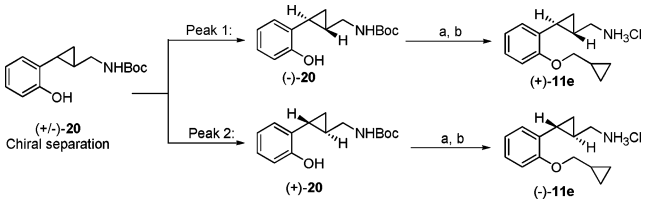
	5-HT$_{2A}$		5-HT$_{2B}$		5-HT$_{2C}$	
compd	EC$_{50}$ (nM)	E_{max} (%)[c]	EC$_{50}$ (nM)	E_{max} (%)[c]	EC$_{50}$ (nM)	E_{max} (%)[c]
5-HT	7.6	100	0.86	100	0.09	100
9a	NA[b]	18	NA	2	3800	64
9b	NA	23	NA	2	3470	67
9c	NA	16	NA	1	8710	69
9d	NA	20	NA	1	NA	19
9e	NA	21	NA	1	3310	69
9f	NA	18	NA	2	7240	59
9g	NA	20	NA	8	2190	83
9h	NA	15	NA	1	NA	22
10a	NA	3	NA	2	NA	24
10b	NA	NA	NA	NA	NA	NA
11a	3020	64	331	48	18	99
11b	NA	1	304	16	24	70
11c	NA	1	NA	0	254	84
11d	NA	7	NA	3	NA	6
11e	NA	0	NA	2	120	60
11f	NA	1	NA	3	1210	31
11g	NA	0	NA	1	NA	1
11h	NA	0	1080	20	304	74
(-)-**11e**	NA	0	NA	4	978	51
(+)-**11e**	NA	1	NA	1	55	61

[a]Functional activity and selectivity at human 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors in calcium flux assays using stably transfected HEK-293 cells. Compounds were tested as racemic mixtures of the *trans*-(*R*,*R*) and *trans*-(*S*,*S*) isomers unless mentioned otherwise. [b]NA: no activity (E_{max} < 12%). [c]Percentage of maximal activation by 5-HT, or activation at 10 μM for NA.

at 5-HT$_{2B}$ (**11a**, 5-HT$_{2B}$/5-HT$_{2C}$ 62% vs 96%; **11b**, 16% vs 70%). These data suggest that judicious choice of R′ might result in analogues that are partial 5-HT$_{2C}$ agonists with negligible efficacies at 5-HT$_{2B}$. We therefore synthesized a series of additional C-2 alkoxy substituted compounds (**11c–h**). In this series, particularly notable are compounds **11c** and **11e**, which exhibited fair 5-HT$_{2C}$ potency and efficacy while being inactive at the 5-HT$_{2B}$ receptor. Excessive bulk of the R′ groups as in compounds **11d** and **11g** abolished agonist activity at all 5-HT$_2$ subtypes. However, in the case where R′ = benzyl, this relationship does not hold, possibly as a result of the ability of this group to engage in additional noncovalent interactions with the receptor that may modify its mode of binding.

Because of the subtype selectivity shown by racemic **11e**, we sought to prepare this compound in optically pure form. The pure enantiomers of **11e** were obtained by carrying out a chiral HPLC separation of the *N*-Boc-protected derivative (±)-**20** using procedures similar to those reported earlier.[19] The resulting enantiomers (−)- and (+)-**20** were then converted individually to (+)- and (−)-*trans*-[2-(2-cyclopropylmethoxyphenyl)cyclopropyl]methylamine hydrochloride [(+)-**11e** and (−)-**11e**], respectively, using the same method as described above for the racemate (Scheme 2). To our delight, the more active enantiomer (+)-**11e** was found to activate neither the 5-HT$_{2A}$ nor the 5-HT$_{2B}$ receptor while having a lower EC$_{50}$ value of 55 nM at the 5-HT$_{2C}$ receptor in comparison to the racemate. In contrast, the less active isomer (−)-**11e** had an EC$_{50}$ value of 978 nM at the 5-HT$_{2C}$ receptor.

Scheme 2. Creation of (+)-11e and (−)-11e[a]



[a]Reagents and conditions: (a) BrCH$_2$cPr, K$_2$CO$_3$, DMF, microwave heating, 100 °C, 1 h. (b) 2 M HCl, rt, 48 h. Chiral separation was carried out on a Chiralpak AD column (20 mm × 250 mm, DAICEL); mobile phase: 7.5% *i*PrOH in hexane, isocratic. (+)-**11e**: Specific rotation: $[\alpha]_D$ = +4.3 (*c* 1.0, MeOH); chiral HPLC: t_R 11.6 min, purity 99.3%. (−)-**11e**: Specific rotation: $[\alpha]_D$ = −4.5 (*c* 1.0, MeOH); chiral HPLC: t_R 11.7 min, purity 96.4%.

In summary, our efforts to further optimize the previously reported 5-HT$_{2B}$/5-HT$_{2C}$ agonists **7** and **8** led to the identification of the highly selective 5-HT$_{2C}$ agonist (+)-**11e**, possessing an EC$_{50}$ of 55 nM and no detectable agonism at the 5-HT$_{2B}$ receptor. Further profiling of the biological effects of these compounds in animal models of behavior will be reported separately.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic procedures, characterization of final products, and biological assay protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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