Design of fluorinated cyclopropane derivatives of 2-phenylcyclopropylmethylamine leading to identification of a selective serotonin 2C (5-HT_{2C}) receptor agonist without 5-HT_{2B} agonism

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ABSTRACT

A new series of fluorinated 5-HT_{2C} agonists were designed and synthesized on the basis of our previous work on 2-phenylcyclopropylmethylamines as a potential approach for the treatment of central nervous system disorders. Key fluorinated cyclopropane moieties were constructed through transition metal catalyzed [2 + 1]-cycloaddition of aromatic vinyl fluorides, and the absolute stereochemistry of the representative compound (−)-**21a** was established. Functional activity measuring calcium flux at 5-HT_2 receptors reveals high potency for compounds (+)-**21a-d**. In particular, (+)-**21b** had no detectable 5-HT_{2B} agonism and displayed reasonable selectivity against 5-HT_{2A}. Molecular docking studies were further performed to explain the compounds' possible binding poses to the 5-HT_{2C} receptor.

1. Introduction

The 5-HT_2-family of serotonin receptors are members of the G protein-coupled receptor (GPCR) superfamily and consist of three subtypes including 5-HT_{2C}, 5-HT_{2B}, and 5-HT_{2A}, which share high sequence homology [1]. The 5-HT_{2C} receptor represents a promising drug target for obesity and central nervous system disorders, such as schizophrenia and drug addiction [1–4]. Subtype selectivity is essential for the development of potential 5-HT_{2C}-targeted therapeutics [5], as drugs with 5-HT_{2B} agonism may induce potentially life-threatening valvular hypertrophy [6,7], while 5-HT_{2A} agonists are hallucinogenic [8]. However, it is currently a major challenge to develop agonists that are highly selective for 5-HT_{2C} over 5-HT_{2B} and 5-HT_{2A} due to these subtypes' significant homology at structural, genetic, and functional levels. Indeed, the lack of truly selective ligands that are able to fully discriminate among these receptor subtypes, especially between 5-HT_{2C} and 5-HT_{2B}, has hampered the development of therapies based on these compounds. For example, the withdrawal of fenfluramine and pergolide as well as the restriction of sales of cabergoline are related to their off-target agonist activity at the 5-HT_{2B} receptor [7]. Furthermore, concern about the anti-obesity drug lorcaserin has been raised because of its moderate 5-HT_{2B} agonism, which may induce lethal cardiac valvulopathy and pulmonary hypertension

Abbreviations: ADMET, absorption; distribution, metabolism; excretion, and toxicity; 2-PCPMA, 2-phenylcyclopropylmethylamine; RIT, ritanserin; ERG, ergotamine.

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[9,10]. Therefore, high selectivity for 5-HT_{2C} over 5-HT_{2B} has emerged as paramount to develop 5-HT_{2C} agonists as a new generation of therapeutic drugs [1].

Fluorination of bioactive molecules as a drug design strategy has achieved remarkable success as evidenced by a number of marketed drugs, mainly because of the strong electron-withdrawing effects of the fluorine atom and the metabolic stability of the C—F bond [11–13]. In our systematic structural optimization of 2-phenylcyclopropylmethylamines (2-PCPMAs), the introduction of a halogen, especially fluorine, onto the phenyl ring was demonstrated as a robust strategy to develop potent and selective 5-HT_{2C} agonists with improved drug-like properties. For the first generation of 2-PCPMAs, introducing a fluorine substituent at the 3-position of the phenyl ring (compound **3**) generated one of the most selective 5-HT_{2C} agonists reported to date [14]. Subsequent structure-activity relationship (SAR) studies indicated that an additional 2-alkoxy substituent represents a beneficial functional group for maintaining reasonable potency and selectivity as illustrated in compounds **7** and **8** [15]. Introduction of a second halogen substituent resulted in the drug candidate **9**, which displayed exquisite 5-HT_{2C} potency and selectivity over 5-HT_{2A} and 5-HT_{2B}, excellent ADMET profiles, and in vivo antipsychotic drug-like effects [16] (Fig. 1). Further *N*-alkylation of the 2-(5-fluoro-2-alkoxyphenyl)cyclopropylmethylamines led to the identification of the potent *N*-benzyl compound **11** with full selectivity over 5-HT_{2B}, and of the *N*-methyl compound **10**, which is the first fully G_q-biased 5-HT_{2C} agonist reported to date [17].

Based on the promising properties of 2-PCPMA derivatives fluorinated in the phenyl ring reported in our prior work, we further explored the incorporation of fluorine atom(s) into the cyclopropane ring, which could: (1) change the compounds' conformation and thereby possibly lead to higher potency and selectivity; (2) increase lipophilicity for optimal brain penetration; and (3) block potential sites of oxidative metabolism, such as the benzylic position (Fig. 1). Accordingly, a series of fluorinated cyclopropylmethylamine derivatives were designed, synthesized, and evaluated for their activities at 5-HT_2 receptors. Furthermore, modelling studies were performed to understand their possible binding poses at the 5-HT_{2C} receptor.

2. Results and discussion

2.1. Structure-activity relationship studies

Based on our previous findings that the second-generation 2-PCPMAs possessing 2-alkoxy and 5-fluoro substituents exhibited the best 5-HT_{2C} potency and selectivity over 5-HT_{2A} and 5-HT_{2B} receptors [15], an analog of the lead compound, 2-(5-fluoro-2-methoxyphenyl)cyclopropylmethylamine (**6**, cLogP = 1.59, LogBB = −0.04) that is fluorinated at the benzylic position, and which can be readily modified to form different 2-alkoxy derivatives, was synthesized first. Unfortunately, the resulting compound (±)-**12** showed only weak activity at 5-HT_{2C} (EC_{50} = 598 nM). For our third-generation 2-PCPMAs with *N*-substituents, the introduction of an *N*-(2-methoxybenzyl) group led to compounds with high 5-HT_{2C} selectivity over 5-HT_{2B} while maintaining good potency at 5-HT_{2C} [17]. However, the *N*-(2-methoxybenzyl) analog (±)-**13** of compound (±)-**12** exhibited only slightly improved activity at 5-HT_{2C} (EC_{50} = 230 nM, E_{max} = 103%) together with poor selectivity against 5-HT_{2B} and 5-HT_{2A}. Therefore, we concluded that the fluorinated cyclopropane moiety and the 2-alkoxy group could not be accommodated simultaneously, in line with our observation from prior structure-activity relationship studies that suggested strict steric limitations applying to 2-PCPMAs [14–16].

Therefore, we further designed fluorinated cyclopropane derivatives based on the first-generation 2-PCPMAs, which were considered to have less steric hindrance. According to the previous SAR studies on the first-generation 2-PCPMAs (Fig. 1), substitutions at the 3-position of the benzene ring with F (compound **3**), Me (compound **2**), Cl (compound **4**), and even Br and CF_3 groups were well tolerated without significant loss of activity at 5-HT_{2C} despite a gradual increase of the substituent size in this series. Moreover, mono- and disubstituted ligands bearing Br and Cl at the 2-position displayed higher potency than the corresponding analogs substituted with F, Me, or CF_3 groups, while the replacement of the fluorine atom at the 4-position with other substituents led to dramatically reduced 5-HT_{2C} potency and selectivity [14]. On the basis of these previous findings, the fluorinated cyclopropane derivatives **21a-e** of the potent and selective first-generation 5-HT_{2C} agonists **2**–**5** were investigated. The biological results are

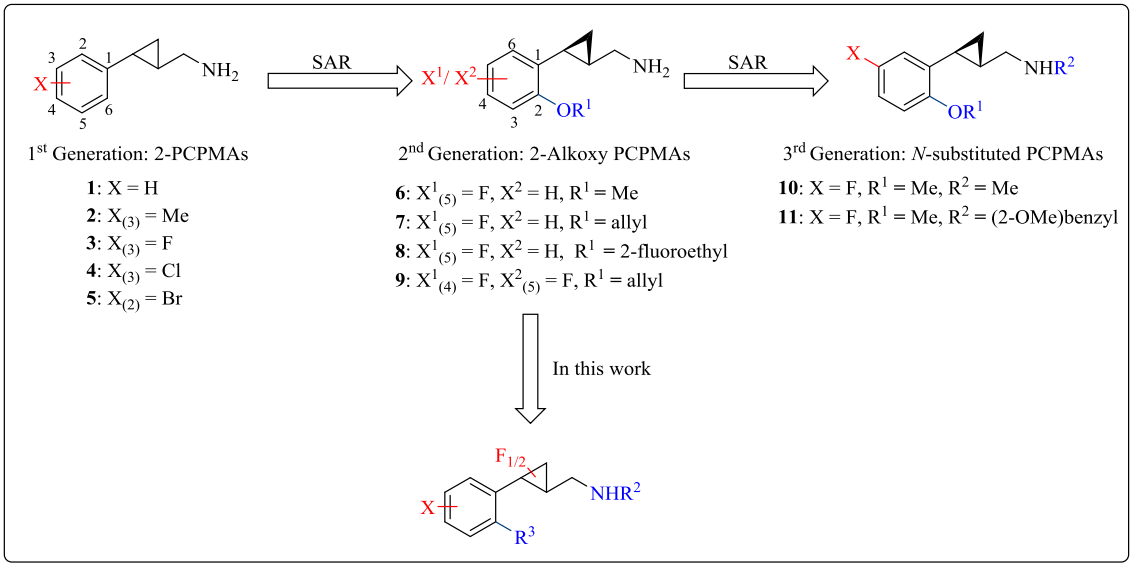


Fig. 1. Selected previously synthesized 5-HT_{2C} agonists **1**–**11** based on the 2-phenylcyclopropylmethylamine scaffold, and new fluorinated cyclopropylmethylamine derivatives.

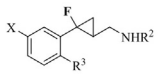
summarized in Table 1. Compared to the parent (2-phenylcyclopropyl)methylamine ((+)-**1**, $EC_{50} = 5.2$ nM, $E_{max} = 108\%$ at 5-HT_{2C}, selectivity (2B/2C) = 7) [14], the fluorinated cyclopropane derivative (+)-**21a** showed comparable 5-HT_{2C} activity ($EC_{50} = 4.7$ nM, $E_{max} = 98\%$) and selectivity (2-fold) over 5-HT_{2B}. Substitution with a methyl group at the 3-position of the phenyl ring of (+)-**21a** led to identification of a potent 5-HT_{2C} agonist ((+)-**21b**, $EC_{50} = 8.0$ nM, $E_{max} = 96\%$) with no detectable agonism at 5-HT_{2B} and about 20-fold selectivity against 5-HT_{2A} ($EC_{50} = 162$ nM, $E_{max} = 71\%$), while intriguingly the corresponding parent (2-(3-methylphenyl)cyclopropyl)methaylamine ((±)-**2**, $EC_{50} = 4.8$ nM, $E_{max} = 95\%$ at 5-HT_{2C}, selectivity (2B/2C) = 14) [14] also had the best selectivity for 5-HT_{2C} over 5-HT_{2B} among the first-generation 2-PCPMAs. The 3-fluoro and 3-chloro analogs ((+)-**21c** and (+)-**21d**, respectively) displayed high potency ($EC_{50} < 15$ nM) at 5-HT_{2C} but poor selectivity against 5-HT_{2B} and 5-HT_{2A} receptors. In contrast, the introduction of a bromo substituent into the 2-position of the phenyl ring (compound (+)-**21e**) resulted in decreased potency ($EC_{50} = 312$ nM, $E_{max} = 71\%$) at 5-HT_{2C}, although the ligand showed no activity at 5-HT_{2B}. *N*-Methylation and *N*-benzylation of (+)-**21a**, which was shown to be a successful optimization strategy in the third-generation *N*-substituted PCPMAs [17], caused loss of potency ($EC_{50} > 250$ nM) at 5-HT_{2C} as found in compounds (+)-**22** and (+)-**23**. Furthermore, the *gem*-difluorinated cyclopropane derivative **24** was almost inactive ($EC_{50} > 4$ μM), which possibly resulted from the bulkier difluorinated cyclopropane moiety.

Compound (+)-**21b** has thus been identified as the best ligand in the series with excellent selectivity for the 5-HT_{2C} receptor against 5-HT_{2B} (no agonism at 10 μM) and a reasonable selectivity against 5-HT_{2A}. Moreover, the introduction of a fluorine atom into the cyclopropane ring is beneficial for improving cLogP and logBB (for example, **21b**: cLogP = 1.92, logBB = 0.38 vs **2**: cLogP = 1.95, LogBB = 0.08), which might contribute to a better brain penetrance of the compound for the treatment of central nervous system (CNS) diseases.

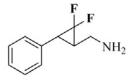
2.2. Chemistry

As shown in Scheme 1, the monofluorinated cyclopropane derivative **12** was synthesized starting from commercially available 5-fluoro-2-methoxybenzaldehyde (**12a**). Wittig reaction of benzaldehyde **12a** with methyltriphenylphosphonium bromide afforded the olefin **12b** in a high yield. Bromofluorination of **12b** with NBS/$Et_3N{\cdot}3HF$ followed by elimination of HBr provided the vinyl fluoride **12d**. Cyclopropanation of **12d** with ethyl diazoacetate in the presence of $Cu(acac)_2$ as catalyst generated the monofluorinated cyclopropanecarboxylate **12e** as an approximate 3:2 racemic mixture of *trans*- and *cis*-isomers. The *trans*-isomer **12e** was obtained by chromatographic separation. Its reduction to the corresponding aldehyde with diisobutylaluminium hydride (DIBAL-H) proceeded in a good yield, but this compound was found to readily decompose under weakly acidic conditions such as in presence of deuterated chloroform. This problem was solved by the direct reduction of **12e** with $LiAlH_4$ to alcohol **12f**, which was transformed to azide **12g** via a Mitsunobu azidation with PPh_3/DEAD/DPPA. Staudinger reduction of **12g** followed by Boc-protection of the primary amine in one pot produced the *N*-Boc amide **12h**, which

Table 1
Functional activity and selectivity of fluorinated cyclopropylmethylamine derivatives 12−13, 21a-e, and 22−24 at 5-HT_2 receptors in the calcium flux assay[a]



12-13, 21a-e, 22-23



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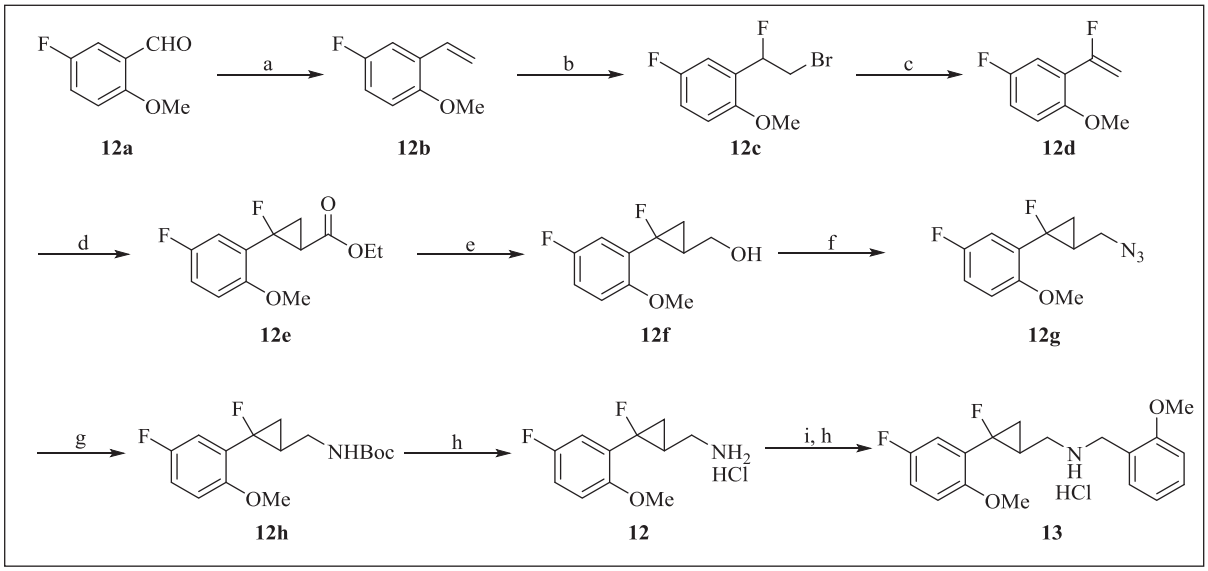
Compd.	X	R^3	R^2	cLogP	LogBB	5-HT_{2C}		5-HT_{2B}		5-HT_{2A}	
						pEC_{50} (EC_{50}, nM)	E_{max} (%)	pEC_{50} (EC_{50}, nM)	E_{max} (%)	pEC_{50} (EC_{50}, nM)	E_{max} (%)
serotonin	-					9.72 ± 0.03 (0.19)	100 ± 0.8	9.07 ± 0.03 (0.86)	100 ± 0.8	9.56 ± 0.02 (0.27)	100 ± 0.7
lorcaserin	-					8.32 ± 0.03 (4.77)	101 ± 1.2	7.10 ± 0.06 (80)	92 ± 2.6	7.49 ± 0.04 (33)	92 ± 1.3
(±)-**12**	F	OMe	H	1.52	0.26	6.22 ± 0.07 (598)	90 ± 3.0	6.94 ± 0.07 (116)	79 ± 2.2	6.45 ± 0.03 (352)	86 ± 1.2
(±)-**13**	F	OMe		3.40	1.02	6.64 ± 0.05 (229)	103 ± 2.4	6.55 ± 0.04 (285)	61 ± 1.2	7.51 ± 0.02 (31)	92 ± 0.6
(+)-**21a**	H	H	H	1.71	0.29	8.33 ± 0.05 (4.7)	98 ± 1.5	8.00 ± 0.08 (10)	104 ± 3.0	8.01 ± 0.05 (10)	106 ± 1.7
(−)-**21a**						5.39 ± 0.05 (4106)	87 ± 3.0	6.04 ± 0.11 (903)	77 ± 4.5	5.74 ± 0.04 (1829)	47 ± 1.1
(+)-**21b**	Me	H	H	1.92	0.38	8.10 ± 0.61 (8.0)	96±1.5	NA	NA	6.79 ± 0.87 (162)	71 ± 1.1
(−)-**21b**						5.28 ± 0.15 (5245)	97 ± 0.8	NA	NA	NA	NA
(+)-**21c**	F	H	H	1.71	0.29	8.56 ± 0.69 (3.0)	107 ± 1.9	8.64 ± 0.77 (2.0)	106 ± 2.0	8.57 ± 0.53 (3.0)	83 ± 1.7
(−)-**21c**						NA	NA	NA	NA	NA	NA
(+)-**21d**	Cl	H	H	2.24	0.42	7.91 ± 0.82 (12)	110 ± 1.6	7.66 ± 0.20 (22)	91 ± 1.8	7.64 ± 0.56 (23)	102 ± 1.3
(−)-**21d**						5.97 ± 0.41 (1064)	92 ± 1.3	NA	NA	NA	NA
(+)-**21e**	H	Br	H	2.29	0.51	6.51 ± 0.30 (312)	71 ± 1.2	NA	NA	6.91 ± 0.19 (122)	38 ± 1.3
(−)-**21e**						NA	NA	NA	NA	NA	NA
(+)-**22**	H	H	Me	2.07	0.46	6.54 ± 0.14 (291)	96 ± 1.1	6.25 ± 0.02 (565)	42 ± 1.3	6.81 ± 0.80 (156)	40 ± 1.1
(+)-**23**	H	H		3.63	1.10	6.10 ± 0.04 (789)	85 ± 1.9	6.44 ± 0.20 (363)	27 ± 2.4	6.50 ± 0.04 (320)	83 ± 1.4
(±)-**24**	-			1.60	0.27	5.35 ± 0.11 (4426)	82 ± 6.4	5.63 ± 0.16 (2347)	62 ± 6.3	5.43 ± 0.12 (3679)	43 ± 3.6

[a] All new compounds were tested as HCl salts. Pharmacological data were acquired with recombinant, stably expressed human 5-HT_2 receptors in the HEK-293 cell line, using a fluorescence imaging plate reader (FLIPR) assay. All data are expressed as average and standard error of the mean and represent three independent experiments performed in triplicate. "NA" indicates no activity up to 10 μM "−" indicates structures of 5-HT and lorcaserin are not shown. cLogP and LogBB values were calculated for the free bases using the ACD Percepta program.



Scheme 1. Synthesis of target compounds 12-13[aa]Reagents and conditions: (a) $Ph_3(CH_3)P^+Br^-$, NaH, THF; (b) $Et_3N{-}3HF$, NBS, CH_2Cl_2; (c) DBU, CH_2Cl_2, reflux; (d) $N_2CHCOOEt$, $Cu(acac)_2$, 1,2-dichloroethane, 60 °C; (e) $LiAlH_4$, THF; (f) DPPA, DEAD, Ph_3P, THF, 0 °C ~ rt; (g) Ph_3P, H_2O/THF; then Boc_2O; (h) 2M HCl/Et_2O; (i) 2-methoxybenzaldehyde, $NaBH_4$, MeOH.

was easily purified by column chromatography. Deprotection of **12h** with HCl solution (2.0M in diethyl ether) afforded the primary amine derivative **12** as its HCl salt. The *N*-benzylated derivate **13** was prepared by reductive alkylation of **12** with 2-methoxybenzaldehyde.

As shown in Scheme 2, the styrenes **14a-e** were converted to the *trans*-isomers of monofluorinated cyclopropanecarboxylates **17a-e** according to the same method as described in Scheme 1. Subsequently, compounds **17a-e** were subjected to a five-step sequence following similar procedures as reported previously by us [16]. Reduction of **17a-e** with $LiAlH_4$ afforded alcohols **18a-e** in good yields, which were then converted into phthalimides **18a-e** via Mitsunobu reactions with phthalimide. Deprotection of the imides with hydrazine hydrate and subsequent Boc-protection produced intermediates **20a-e**. Separation of racemic **20a-e** by chiral preparative HPLC followed by deprotection of the Boc group under acidic conditions (2M HCl/Et_2O) gave optically pure enantiomers (+)-**21a-e** and (−)-**21a-e** as HCl salts. The *N*-methylamine (+)-**22** was prepared by introduction of the *N*-methyl group into the intermediate (−)-**20a** with sodium hydride and iodomethane followed by removal of the Boc group under acidic conditions, while the *N*-benzylamine (+)-**23** was directly synthesized from the enantiomer (+)-**21a** through reductive amination with 2-methoxybenzaldehyde.

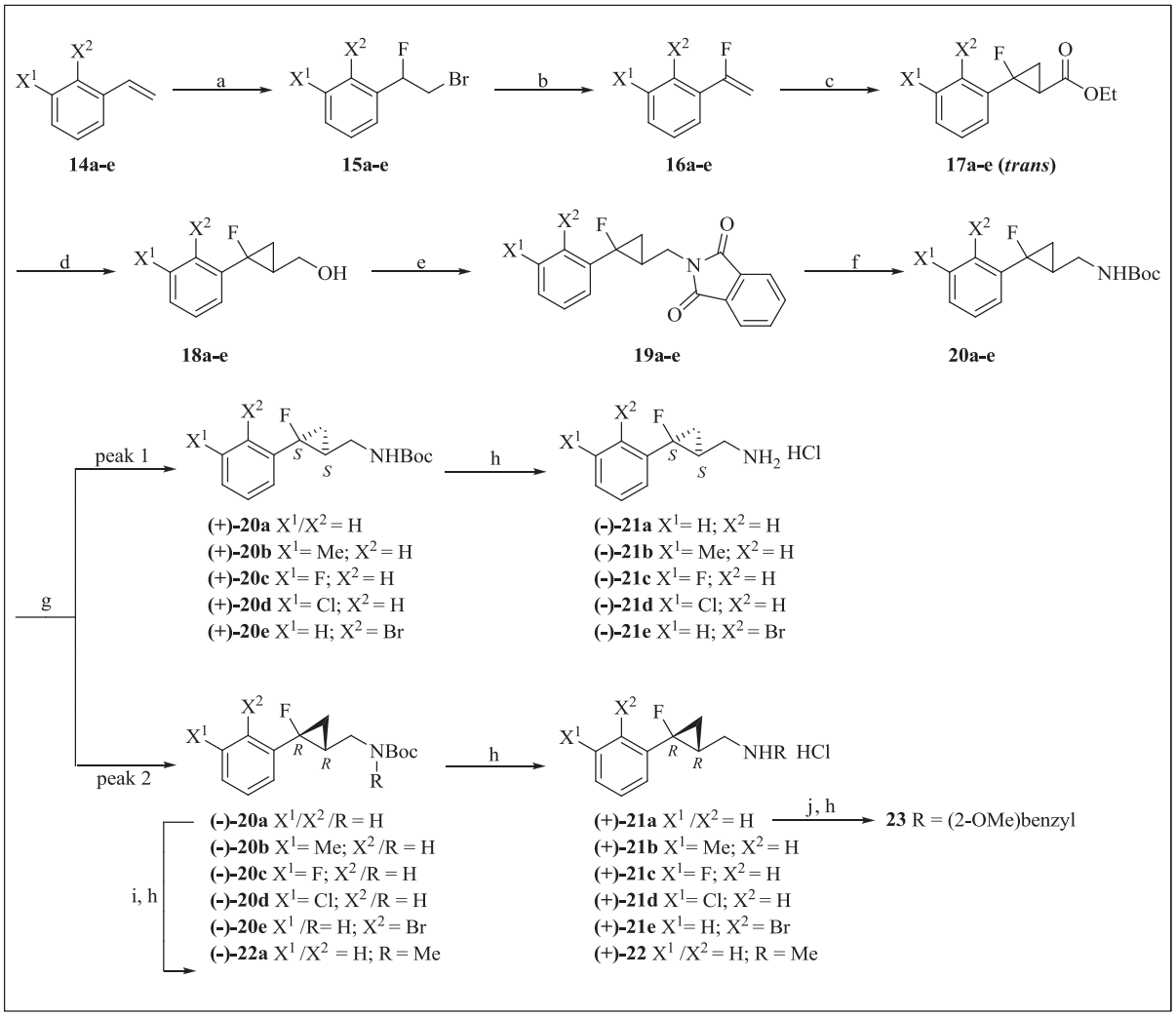
The synthesis of the *gem*-difluorinated cyclopropane **24** was accomplished starting from the precursor **24b** (Scheme 3), obtained through difluorocyclopropanation of cinnamyl acetate (**24a**) with an excesses of sodium chlorodifluoroacetate in diglyme under reflux [18]. Saponified afforded the alcohol **24c** in quantitative yield, which was then subjected to similar synthetic procedures as described above to provide the *gem*-difluorocyclopropane **24**.

Unless otherwise noted, the fluorinated cyclopropylmethylamine derivatives were tested as racemic mixtures. The absolute stereochemistry of compound (−)-**21a** was established by comparison of the optical rotation of its *N*-Boc precursor (+)-**20a** obtained from chiral HPLC separation to that of a sample synthesized asymmetrically. The enantioselective synthesis of the key intermediate *trans*-(−)-(1*S*,2*S*)-**17a** for preparation of (+)-(1*S*,2*S*)-**20a** was achieved by asymmetric cyclopropanation of α-fluorostyrene (**16a**) in the presence of an enantiopure copper catalytic system consisting of the chiral bis(oxazoline) ligand **25** and copper(I) triflate [19,20] (Scheme 4). Following the same synthetic route as shown in Scheme 2, *trans*-(−)-(1*S*,2*S*)-**17a** was transformed into (+)-(1*S*,2*S*)-**20a**, which was identical to the enantiomer (+)-**20a** obtained from chiral HPLC separation in terms of optical rotation and spectral data. Based on this result, the absolute configuration of (−)-**21a** obtained by removal of the *N*-Boc group from (+)-**20a** was assigned as (1*S*,2*S*) and that of the other enantiomer, (+)-**20a**, as (1*R*,2*R*). Furthermore, our finding that the (+)-enantiomers of all new fluorinated cyclopropylmethylamine derivatives **21a-e** are more potent than their (−)-enantiomers is in agreement with that previously observed for the 2-phenylcyclopropylmethylamine scaffold [14]. Taken together, the absolute configuration of compounds (+)-**21a-e** is generally assumed as (1*R*,2*R*) and that of (−)-**21a-e** as (1*S*,2*S*).

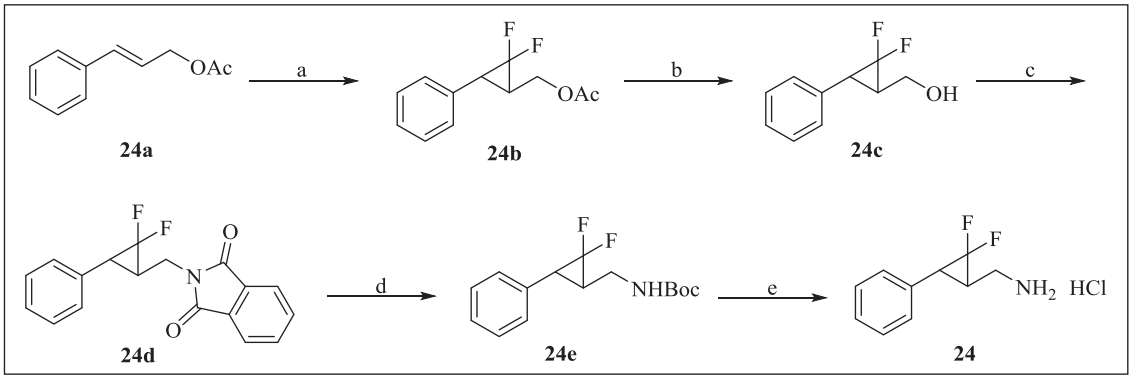
2.3. Molecular docking studies

To clarify the potential binding poses of the new fluorinated cyclopropane compounds compared to those of our previous compounds, we conducted molecular docking studies using the recently published crystal structures of the 5-HT_{2C} receptor [21].

The transition from 5-HT_{2C} inactive to active states is thought to be triggered by helix movements accompanied by rotamer switches in the conserved P326$^{5.50}$-I142$^{3.40}$-F320$^{6.44}$ (P—I—F) motif and a shift of the W324$^{6.48}$ in helix VI, a hallmark of representative active-state-like structures at biogenic amine and other GPCRs [22,23]. The recently reported inactive and active structures of the 5-HT_{2C} receptor in complex with ritanserin (RIT) and ergotamine (ERG), respectively, demonstrate that, by comparing the conformational changes between inverse agonist- and agonist-bound 5-HT_{2C}, hydrogen bonds between the highly conserved D134$^{3.32}$ and Y358$^{7.43}$ residues are in place in both structures, while one of RIT's 4-fluorophenyl rings forms a strong, tight interaction with W324$^{6.48}$ and I142$^{3.40}$, as well as F320$^{6.44}$ side chains, thus apparently preventing the conformational changes in these key activation microswitch structures [21].

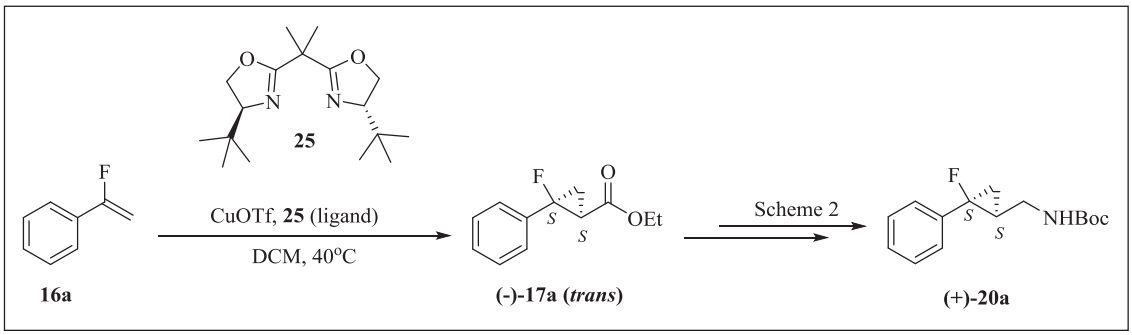


Scheme 2. Synthesis of Target Compounds 21a-e, 22, and 23[aa]Reagents and conditions: (a) $Et_3N{-}3HF$, NBS, CH_2Cl_2; (b) DBU, CH_2Cl_2, reflux; (c) $N_2CHCOOEt$, $Cu(acac)_2$, CH_2Cl_2, 40 °C; (d) $LiAlH_4$, THF; (e) phthalimide, PPh_3, DEAD, THF, 0 °C ~ rt; (f) 1. $N_2H_4{-}H_2O$, EtOH, reflux; 2. Boc_2O, Et_3N, DCM; (g) chiral preparative HPLC separation; (h) 2M HCl/Et_2O; (i) NaH, MeI, THF; (j) 2-methoxybenzaldehyde, $NaBH_4$, MeOH.



Scheme 3. Synthesis of target compounds 24[aa]Reagents and conditions: (a) $ClCF_2COONa$, diglyme, 162 °C; (b) 2N NaOH, MeOH; (c) phthalimide, PPh_3, DEAD, THF, 0 °C ~ rt; (d) 1. $N_2H_4{-}H_2O$, EtOH, reflux; 2. Boc_2O, Et_3N, DCM; (e) 2M HCl/Et_2O.

On the basis of the available 5-$HT_{2C\text{-active}}$ crystal structure (PDB code: 6BQG, Fig. 2A), a docking simulation was carried out on the potent and highly selective agonist **8** and its corresponding fluorinated cyclopropane derivative **21c** to interpret their possible binding modes (Fig. 2B and C). Expectedly, both ligands engage in a strong salt bridge with $D134^{3.32}$, which is critical for charged aminergic ligand recognition. Furthermore, the salt bridge is enhanced by an interaction between $D134^{3.32}$ and the fluorine-substituted



Scheme 4. Asymmetric synthesis of intermediate (+)-20a.

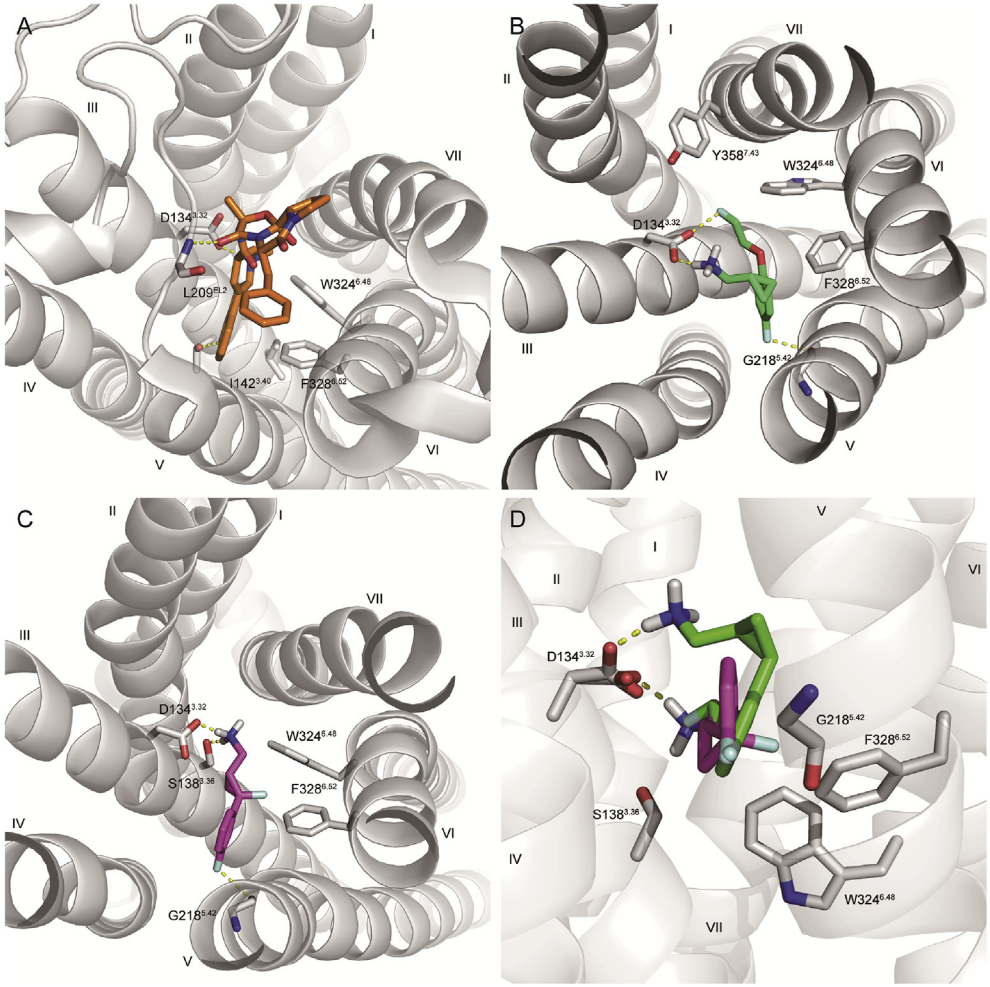


Fig. 2. Docking simulations of 8 and 21c with 5-HT$_{2C\text{-active}}$. (A) Empirical conformation of ERG (orange) in 5-HT$_{2C\text{-active}}$ (PDB: 6BQG); (B) docking pose of **8** (green) in the binding site of 5-HT$_{2C\text{-active}}$; (C) docking pose of **21c** (magenta) in the binding site of 5-HT$_{2C\text{-active}}$; (D) superimposed structures of **8** (green) and **21c** (magenta) in the binding site of 5-HT$_{2C\text{-active}}$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

alkoxy tail in the case of **8**, while an additional interaction between the amino group and S138$^{3.36}$ is observed in the complex of **21c**. Both the alkyl side chain in **8** and the cyclopropyl ring in **21c** also participate in additional π-alkyl interactions with W324$^{6.48}$ that may reinforce interactions with helix VI toward an active conformation. In addition, the fluorophenyl rings in both compounds engage in an interaction with G218$^{5.42}$ and an edge-to-face π-π stacking with F328$^{6.52}$, suggesting that interhelical interactions are involved to stabilize helices III, V, and VI in 5-HT$_{2C\text{-active}}$. The superimposed structures of **8** and **21c** in Fig. 2D further demonstrate that both fluorophenyl rings occupy relatively similar binding poses (Fig. 2D). However, the cyclopropane moiety of **8** forms a salt bridge from the top of D134$^{3.32}$, while the fluorinated cyclopropane moiety of **21c** binds to D134$^{3.32}$ from the downside of the residue and locates at a similar position as the 2-fluoroethoxy group of **8**.

3. Conclusions

Based on the expected favorable drug-like properties imparted by fluorine substituent(s) on the phenyl ring of the 2-PCPMA scaffold, new fluorinated cyclopropylmethylamine derivatives were designed and synthesized by construction of the cyclopropane ring via transition metal catalyzed [2 + 1]-cycloaddition of a diazo compound to an aromatic vinyl fluoride. The absolute stereochemistry of the enantiomers (+)-**21a-e** and (−)-**21a-e** was indirectly determined by comparison of the *N*-Boc protected precursor of the representative compound (−)-**21a** obtained by chiral HPLC separation to a sample synthesized asymmetrically by a well-proven methodology. Further pharmacological profiling of these compounds led to identification of a potent and highly selective 5-HT_{2C} agonist, (+)-**21b**, without 5-HT_{2B} agonism. Molecular docking studies using recently disclosed crystal structures of the 5-HT_{2C} receptor indicate that the new fluorinated cyclopropane compounds such as **21c** have similar interaction patterns with 5-HT_{2C} compared to the best ligand **8** reported previously by us. Introduction of the fluorine atom at the benzylic position of 2-PCPMA may contribute to improved drug-like properties with regard to metabolic stability and brain penetration crucial for CNS drugs.

4. Experimental section

4.1. General

All chemicals and solvents were purchased from Sigma-Aldrich or Fisher Scientific and were used as obtained without further purification. Synthetic intermediates were purified on 230−400 mesh silica gel using a Teledyne CombiFlash R_f flash chromatograph. ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded on Bruker DPX-400 or AVANCE-400 spectrometers at 400 MHz, 100 MHz, and 376 MHz, respectively. NMR chemical shifts are 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)). High resolution mass spectra (HRMS) were acquired using an LCMS-IT-TOF (Shimadzu) mass spectrometer in ESI mode. Preparative HPLC purification of synthetic intermediates was performed on a Shimadzu LC-8A instrument with an ACE 5AQ column (150 × 21.2 mm, particle size 5 μm; eluent: 8−100% MeOH (0.05% TFA)/H_2O (0.05% TFA) gradient, 30 min; flow rate: 17 mL/min; UV detection at 220 and 254 nm). The purity of all final compounds (greater than 95% in all cases) was determined by analytical HPLC on an ACE 3AQ C_{18} column (150 × 4.6 mm, particle size 3 μm; eluent: MeOH (0.05% TFA)/H_2O (0.05% TFA) gradient, 25 min; flow rate: 1.0 mL/min).

The synthetic procedures and characterization data of all intermediates can be found in the Supporting Information.

4.2. General Method A

Chiral Separation of *N*-Boc-amines 20a-e. The racemic *N*-Boc-amines **20a-e** were separated by preparative HPLC using a RegisPack chiral column (25 cm × 21.1 mm, particle size 10 μM) and 2−10% ethanol in *n*-hexane as the eluent (flow rate = 18 mL/min, λ = 220 and 254 nm; isocratic elution, stacked injections). The first- and second-eluting peaks were collected and concentrated, and separations were repeated when necessary to ensure obtention of both enantiomers with optical purities >90% *ee* (determined by analytical HPLC using a RegisPack (25 cm × 4.6 mm, particle size 10 μm) chiral column and ethanol/*n*-hexane as the eluent). Specific rotations were recorded on a Rudolph Research Autopol IV automatic polarimeter. Compounds (+)-**20a-e** were isolated as the first-eluting peaks, and (−)-**20a-e** as the second-eluting peaks.

4.3. General Method B

Preparation of HCl Salts 12, (+)/(−)-**21a-e, 22, and 24.** The *N*-Boc-amines **12h,** (+)/(−)-**20a-e,** (+)-**22a,** and **24e** were dissolved in 2M HCl (g) in diethyl ether (5 equiv.) and stirred at room temperature for 24−48 h. The resulting white solids were filtered off, washed with diethyl ether, and dried under vacuum to give the HCl salts as white solids in high yields (75−90%).

4.4. [2-Fluoro-2-(5-fluoro-2-methoxyphenyl)cyclopropyl] methanamine hydrochloride (12)

Obtained from the intermediate **12h** employing General Method B as a white solid. ^{1}H NMR (CD_3OD) δ 7.24 (ddd, J = 8.6, 3.0, 1.9 Hz, 1H), 7.17 (tdd, J = 8.1, 3.1, 1.7 Hz, 1H), 7.08 (dd, J = 9.1, 4.3 Hz, 1H), 3.91 (s, 3H), 3.42 (ddd, J = 13.3, 6.3, 1.2 Hz, 1H), 3.22−3.14 (m, 1H), 1.66−1.56 (m, 1H), 1.52 (td, J = 10.2, 7.1 Hz, 1H), 1.35 (dt, J = 20.0, 7.0 Hz, 1H). ^{13}C NMR (CD_3OD) δ 157.86 (d, J = 238.0 Hz), 156.15 (d, J = 8.5 Hz), 126.64 (dd, J = 19.8, 7.3 Hz), 118.16 (dd, J = 22.7, 2.2 Hz), 118.02 (dd, J = 24.0, 4.2 Hz), 113.53 (d, J = 8.0 Hz), 79.24 (d, J = 216.1 Hz), 56.58 (s), 40.16 (d, J = 9.3 Hz), 21.38 (d, J = 12.1 Hz), 16.28 (d, J = 13.4 Hz). ^{19}F NMR (CD_3OD) δ −125.90 (s), −184.10 (s); HRMS (ESI) calculated for $C_{11}H_{14}F_2NO$ ($[M+H]^+$): *m*/*z* 214.1038; found: 214.1021.

4.5. 1-[2-Fluoro-2-(5-fluoro-2-methoxyphenyl)cyclopropyl]-N-(2-methoxybenzyl)methanamine hydrochloride (13)

To a solution of the HCl salt **12** (50 mg, 0.30 mmol) and 2-methoxybenzaldehyde (40 mg, 0.30 mmol) in methanol (5 mL) was added triethylamine (90 mg, 0.90 mmol). The reaction mixture was stirred at rt for 6 h, and then $NaBH_4$ (35 mg, 0.90 mmol) was added in portions. The resulting mixture was further stirred for 30 min, quenched by the addition of water and extracted with DCM. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The resulting residue was purified by silica gel chromatography to give the free base as a colorless oil (72 mg). The above free base was dissolved in 2 M HCl in diethyl ether (3 mL) and stirred at room temperature for 2 h. The precipitate was collected by filtration, washed with diethyl ether, and dried under vacuum to obtain the desired salt as a white solid (76 mg, 78%). ^{1}H NMR ($CDCl_3$) δ 12.19 (s, 2H), 7.50−7.36 (m, 2H), 7.18−7.00 (m, 3H), 6.95 (d, J = 8.3 Hz, 1H), 6.91 (dd, J = 9.0, 4.1 Hz, 1H), 4.55 (d, J = 12.7 Hz, 1H), 4.02 (d, J = 11.9 Hz, 1H), 3.91 (s, 3H), 3.86 (s, 3H), 3.83−3.70 (m, 1H), 2.77−2.62 (m, 1H), 2.04−1.84 (m, 1H), 1.46−1.36 (m, 1H), 1.24−1.12 (m, 1H); ^{19}F NMR ($CDCl_3$) δ −123.00 (s), −184.03 (s). HRMS (ESI) calculated for $C_{11}H_{14}F_2NO$ ($[M+H]^+$): *m*/*z* 334.1613; found: 334.1602.

4.6. (+)-[(1R,2R)-2-fluoro-2-phenylcyclopropyl]methanamine hydrochloride ((+)-21a)

Obtained from the intermediate **(-)-20a** employing General Method B as a white solid. ^{1}H NMR (CD_3OD) δ 7.52−7.30 (m, 5H), 3.40−3.21 (m, 2H), 1.81−1.68 (m, 1H), 1.60−1.44 (m, 2H); ^{13}C NMR (CD_3OD) δ 139.36 (d, J = 20.8 Hz), 129.64 (s, 2C), 129.15 (s), 125.67 (d, J = 6.4 Hz, 2C), 82.24 (d, J = 217.7 Hz), 39.65 (d, J = 9.8 Hz), 23.68 (d, J = 11.2 Hz), 19.17 (d, J = 12.3 Hz); HRMS (ESI) calculated for $C_{10}H_{13}FN$ ($[M+H]^+$): *m*/*z* 166.1027; found: 166.1008. $[\alpha]_D^{20}$ +68.3 (*c* 0.3, MeOH).

4.7. (-)-[(1S,2S)-2-fluoro-2-phenylcyclopropyl]methanamine hydrochloride ((-)-21a)

Obtained from the intermediate **(+)-20a** employing General

Method B as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.53–7.30 (m, 5H), 3.40–3.20 (m, 2H), 1.81–1.68 (m, 1H), 1.60–1.44 (m, 2H); ^{13}C NMR (CD_3OD) δ 139.36 (d, J = 20.8 Hz), 129.64 (s, 2C), 129.15 (s), 125.67 (d, J = 6.4 Hz, 2C), 82.24 (d, J = 217.6 Hz), 39.65 (d, J = 9.8 Hz), 23.68 (d, J = 11.2 Hz), 19.17 (d, J = 12.3 Hz); HRMS (ESI) calculated for $C_{10}H_{13}FN$ ([M+H]$^+$): m/z 166.1027; found: 166.1008. $[\alpha]_D^{20}$ **-**67.4 (c 0.2, MeOH).

4.8. (+)-[(1R,2R)-2-fluoro-2-(m-tolyl)cyclopropyl]methanamine hydrochloride ((+)-21b)

Obtained from the intermediate **(-)-20b** employing General Method B as a white solid. ^{1}H NMR (CD_3OD) δ 7.30 (t, J = 7.6 Hz, 1H), 7.24–7.13 (m, 3H), 3.37–3.30 (m, 1H), 3.24 (dd, J = 13.3, 8.2 Hz, 1H), 2.38 (s, 3H), 1.73–1.61 (m, 1H), 1.60–1.41 (m, 2H); ^{13}C NMR (CD_3OD) δ 139.56 (s), 139.25 (d, J = 20.3 Hz), 129.87 (s), 129.53 (d, J = 10.7 Hz), 126.28 (d, J = 6.4 Hz), 122.77 (d, J = 6.5 Hz), 82.22 (d, J = 217.7 Hz), 39.68 (d, J = 9.9 Hz), 23.63 (d, J = 11.2 Hz), 21.43 (s), 19.02 (d, J = 12.5 Hz); ^{19}F NMR (CD_3OD) δ −192.4 (s); HRMS (ESI) calculated for $C_{11}H_{15}FN$ ([M+H]$^+$): m/z 180.1183; found: 180.1176. $[\alpha]_D^{20}$ +53.6 (c 0.5, MeOH).

4.9. (-)-[(1S,2S)-2-fluoro-2-(m-tolyl)cyclopropyl]methanamine hydrochloride ((-)-21b)

Obtained from the intermediate **(+)-20b** employing General Method B as a white solid. ^{1}H NMR (CD_3OD) δ 7.30 (t, J = 7.6 Hz, 1H), 7.25–7.13 (m, 3H), 3.37–3.30 (m, 1H), 3.24 (dd, J = 13.3, 8.0 Hz, 1H), 2.38 (s, 3H), 1.73–1.62 (m, 1H), 1.60–1.41 (m, 2H); ^{13}C NMR (CD_3OD) δ 139.56 (s), 139.25 (d, J = 20.3 Hz), 129.87 (s), 129.53 (d, J = 10.7 Hz), 126.28 (d, J = 6.4 Hz), 122.77 (d, J = 6.5 Hz), 82.22 (d, J = 217.6 Hz), 39.68 (d, J = 9.9 Hz), 23.63 (d, J = 11.2 Hz), 21.43 (s), 19.02 (d, J = 12.5 Hz); ^{19}F NMR (CD_3OD) δ −192.4 (s); HRMS (ESI) calculated for $C_{11}H_{15}FN$ ([M+H]$^+$): m/z 180.1183; found: 180.1175. $[\alpha]_D^{20}$ **-**49.8 (c 0.1, MeOH).

4.10. (+)-[(1R,2R)-2-fluoro-2-(3-fluorophenyl)cyclopropyl] methanamine hydrochloride ((+)-21c)

*4.10.1. Obtained from the intermediate **(-)-20c** employing General Method B as a white solid*

^{1}H NMR (CD_3OD) δ 7.48–7.39 (m, 1H), 7.19–7.13 (m, 2H), 7.13–7.05 (m, 1H), 3.39–3.34 (m, 1H), 3.23 (dd, J = 13.4, 8.1 Hz, 1H), 1.78–1.68 (m, 1H), 1.65–1.48 (m, 2H); ^{13}C NMR (CD_3OD) δ 164.40 (d, J = 245.1 Hz), 142.39 (dd, J = 21.6, 7.5 Hz), 131.65 (d, J = 8.4 Hz), 120.89 (d, J = 4.3 Hz), 115.72 (d, J = 21.4 Hz), 112.45 (dd, J = 23.9, 7.8 Hz), 81.69 (d, J = 218.7 Hz), 39.50 (d, J = 9.9 Hz), 24.34 (d, J = 11.0 Hz), 19.56 (d, J = 11.9 Hz); ^{19}F NMR (CD_3OD) δ −114.77 (s), −194.54 (s); HRMS (ESI) calculated for $C_{10}H_{12}F_2N$ ([M+H]$^+$): m/z 184.0932; found: 184.0948. $[\alpha]_D^{20}$ +40.8 (c 0.2, MeOH).

4.11. (-)-[(1S,2S)-2-fluoro-2-(3-fluorophenyl)cyclopropyl] methanamine hydrochloride ((-)-21c)

Obtained from the intermediate **(+)-20c** employing General Method B as a white solid. ^{1}H NMR (CD_3OD) δ 7.48–7.39 (m, 1H), 7.19–7.13 (m, 2H), 7.13–7.05 (m, 1H), 3.39–3.34 (m, 1H), 3.23 (dd, J = 13.4, 8.0 Hz, 1H), 1.78–1.68 (m, 1H), 1.65–1.48 (m, 2H); ^{13}C NMR (CD_3OD) δ 164.40 (d, J = 245.2 Hz), 142.39 (dd, J = 21.6, 7.5 Hz), 131.65 (d, J = 8.4 Hz), 120.89 (d, J = 4.3 Hz), 115.72 (d, J = 21.4 Hz), 112.45 (dd, J = 23.9, 7.8 Hz), 81.69 (d, J = 218.7 Hz), 39.50 (d, J = 9.9 Hz), 24.34 (d, J = 11.0 Hz), 19.56 (d, J = 11.9 Hz); ^{19}F NMR (CD_3OD) δ −114.77 (s), −194.54 (s); HRMS (ESI) calculated for $C_{10}H_{12}F_2N$ ([M+H]$^+$): m/z 184.0932; found: 184.0935. $[\alpha]_D^{20}$ **-**42.6 (c 0.1, MeOH).

4.12. (+)-[(1R,2R)-2-(3-chlorophenyl)-2-fluorocyclopropyl] methanamine hydrochloride ((+)-21d)

Obtained from the intermediate **(-)-20d** employing General Method B as a white solid. ^{1}H NMR (CD_3OD) δ 7.46–7.34 (m, 3H), 7.28 (d, J = 7.5 Hz, 1H), 3.39–3.33 (m, 1H), 3.24 (dd, J = 13.4, 8.1 Hz, 1H), 1.81–1.68 (m, 1H), 1.66–1.48 (m, 2H); ^{13}C NMR (CD_3OD) δ 141.84 (d, J = 21.3 Hz), 135.69 (s), 131.31 (s), 129.08 (d, J = 9.7 Hz), 125.64 (d, J = 7.6 Hz), 123.66 (d, J = 6.8 Hz), 81.66 (d, J = 218.5 Hz), 39.48 (d, J = 9.8 Hz), 24.19 (d, J = 10.9 Hz), 19.42 (d, J = 12.1 Hz); ^{19}F NMR (CD_3OD) δ −194.37 (s); HRMS (ESI) calculated for $C_{10}H_{12}ClFN$ ([M+H]$^+$): m/z 200.0637; found: 200.0626. $[\alpha]_D^{20}$ +38.2 (c 0.2, MeOH).

4.13. (-)-[(1R,2R)-2-(3-chlorophenyl)-2-fluorocyclopropyl] methanamine hydrochloride ((-)-21d)

Obtained from the intermediate **(+)-20d** employing General Method B as a white solid. ^{1}H NMR (CD_3OD) δ 7.46–7.34 (m, 3H), 7.28 (d, J = 7.5 Hz, 1H), 3.39–3.33 (m, 1H), 3.24 (dd, J = 13.4, 8.0 Hz, 1H), 1.81–1.68 (m, 1H), 1.66–1.48 (m, 2H); ^{13}C NMR (CD_3OD) δ 141.84 (d, J = 21.3 Hz), 135.69 (s), 131.31 (s), 129.08 (d, J = 9.7 Hz), 125.64 (d, J = 7.6 Hz), 123.66 (d, J = 6.8 Hz), 81.66 (d, J = 218.5 Hz), 39.48 (d, J = 9.8 Hz), 24.19 (d, J = 10.9 Hz), 19.42 (d, J = 12.0 Hz); ^{19}F NMR (CD_3OD) δ −194.37 (s); HRMS (ESI) calculated for $C_{10}H_{12}ClFN$ ([M+H]$^+$): m/z 200.0637; found: 200.0626. $[\alpha]_D^{20}$ **-**37.8 (c 0.2, MeOH).

4.14. (+)-[(1R,2R)-2-(2-bromophenyl)-2-fluorocyclopropyl] methanamine hydrochloride ((+)-21e)

*4.14.1. Obtained from the intermediate **(-)-20e** employing General Method B as a white solid*

^{1}H NMR (CD_3OD) δ 7.72 (d, J = 7.9 Hz, 1H), 7.60 (dt, J = 7.6, 1.9 Hz, 1H), 7.45 (t, J = 7.5 Hz, 1H), 7.38 (m, 1H), 3.52 (dd, J = 13.4, 6.2 Hz, 1H), 3.19 (ddd, J = 13.4, 8.7, 1.2 Hz, 1H), 1.72–1.61 (m, 1H), 1.60–1.45 (m, 2H); ^{13}C NMR (CD_3OD) δ 136.51 (d, J = 19.0 Hz), 134.54 (s), 133.15 (d, J = 2.3 Hz), 132.76 (d, J = 2.4 Hz), 128.93 (s), 126.94 (s), 82.88 (d, J = 218.9 Hz), 39.94 (d, J = 8.4 Hz), 21.70 (d, J = 11.8 Hz), 17.40 (d, J = 13.3 Hz); ^{19}F NMR (CD_3OD) δ −181.76 (s); HRMS (ESI) calculated for $C_{10}H_{12}BrFN$ ([M+H]$^+$): m/z 244.0132; found: 244.0105. $[\alpha]_D^{20}$ +80.3 (c 0.2, MeOH).

4.15. (-)-[(1R,2R)-2-(2-bromophenyl)-2-fluorocyclopropyl] methanamine hydrochloride ((-)-21e)

Obtained from the intermediate **(+)-20e** employing General Method B as a white solid. ^{1}H NMR (CD_3OD) δ 7.72 (d, J = 8.0 Hz, 1H), 7.60 (dt, J = 7.6, 1.9 Hz, 1H), 7.45 (t, J = 7.5 Hz, 1H), 7.38 (m, 1H), 3.52 (dd, J = 13.4, 6.2 Hz, 1H), 3.19 (ddd, J = 13.4, 8.7, 1.2 Hz, 1H), 1.72–1.62 (m, 1H), 1.60–1.45 (m, 2H); ^{13}C NMR (CD_3OD) δ 136.51 (d, J = 19.0 Hz), 134.54 (s), 133.15 (d, J = 2.3 Hz), 132.76 (d, J = 2.4 Hz), 128.93 (s), 126.94 (s), 82.88 (d, J = 218.9 Hz), 39.94 (d, J = 8.4 Hz), 21.70 (d, J = 11.8 Hz), 17.40 (d, J = 13.3 Hz); ^{19}F NMR (CD_3OD) δ −181.76 (s); HRMS (ESI) calculated for $C_{10}H_{12}BrFN$ ([M+H]$^+$): m/z 244.0132; found: 244.0110. $[\alpha]_D^{20}$ **-**85.6 (c 0.1, MeOH).

4.16. (+)-1-[(1R,2R)-2-fluoro-2-phenylcyclopropyl]-N-methylmethanaminehydrochloride ((+)-22)

To a solution of **(-)-21a** (23 mg, 0.09 mmol) in THF (12 mL) was added NaH (60% dispersion in mineral oil, 6 mg, 0.13 mmol). The mixture was stirred at room temperature for 30 min, and then methyl iodide (20 mg, 0.13 mmol) was added. The reaction mixture was stirred overnight at room temperature, quenched with water,

and extracted with EtOAc. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The crude product was purified by flash chromatography to give the intermediate *N*-Boc-*N*-methylmethanamine **22a** as a colorless oil (20 mg). Subsequent *N*-Boc deprotection afforded the desired HCl salt according to General Method B as a white solid (12 mg, 64% yield). ^{1}H NMR ($CDCl_3$) δ 9.40 (s, 2H), 7.42–7.35 (m, 2H), 7.35–7.29 (m, 3H), 3.64–3.44 (m, 1H), 3.27–3.09 (m, 1H), 2.73 (s, 3H), 2.00–1.81 (m, 1H), 1.58–1.40 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 137.48 (d, J = 20.8 Hz), 128.83 (s, 2C), 128.32 (s), 124.54 (d, J = 6.7 Hz, 2C), 80.81 (d, J = 219.2 Hz), 47.24 (d, J = 8.6 Hz), 32.40 (s), 21.07 (d, J = 10.9 Hz), 18.81 (d, J = 12.5 Hz); ^{19}F NMR ($CDCl_3$) δ −188.7 (s); HRMS (ESI) calculated for $C_{11}H_{15}FN$ ($[M+H]^+$): m/z 180.1183; found: 180.1175. $[\alpha]_D^{20}$ +40.5 (c 0.1, MeOH).

4.17. (+)-1-[(1R,2R)-2-fluoro-2-phenylcyclopropyl]-N-(2-methoxybenzyl)methanamine hydrochloride ((+)-23)

To a solution of the HCl salt **(+)-21a** (50 mg, 0.30 mmol) and 2-methoxybenzaldehyde (40 mg, 0.30 mmol) in methanol (5 mL) was added Et_3N (90 mg, 0.90 mmol). The reaction mixture was stirred at rt for 6 h, and then $NaBH_4$ (35 mg, 0.90 mmol) was added in portions. The resulting mixture was further stirred for 30 min, quenched by the addition of water, and extracted with DCM. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The resulting residue was purified by silica gel chromatography to give the free base as a colorless oil (72 mg). The above free base was dissolved in 2 M HCl in diethyl ether (3 mL) and stirred at room temperature for 2 h. The precipitate was collected by filtration, washed with diethyl ether, and dried under vacuum to obtain the desired salt as a white solid (76 mg, 78%). ^{1}H NMR ($CDCl_3$) δ 9.98 (br s, 2H), 7.48 (dd, J = 7.4, 1.2 Hz, 1H), 7.39–7.35 (m, 2H), 7.32–7.28 (m, 4H), 6.92 (t, J = 7.4 Hz, 1H), 6.85 (d, J = 8.2 Hz, 1H), 4.18–4.06 (m, 2H), 3.82 (s, 3H), 3.35–3.28 (m, 1H), 3.06–3.01 (m, 1H), 1.97–1.93 (m, 1H), 1.46–1.28 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 157.97 (s), 137.77 (d, J = 20.7 Hz), 132.14 (s), 131.22 (s), 128.67 (s, 2C), 128.15 (s), 124.65 (d, J = 6.6 Hz, 2C), 120.95 (s), 118.88 (s), 110.60 (s), 81.11 (d, J = 218.6 Hz), 55.54 (s), 45.55 (s), 44.77 (d, J = 8.7 Hz), 21.23 (d, J = 10.8 Hz), 19.13 (d, J = 12.5 Hz); HRMS (ESI) calculated for $C_{18}H_{21}FNO$ ($[M+H]^+$): m/z 286.1602; found: 286.1589. $[\alpha]_D^{20}$ +53.6 (c 0.1, MeOH).

4.18. (2,2-Difluoro-3-phenylcyclopropyl)methanamine hydrochloride (24)

Obtained from the intermediate **24e** employing General Method B as a white solid. ^{1}H NMR (CD_3OD) δ 7.42–7.27 (m, 5H), 3.40–3.23 (m, 2H), 2.99 (dd, J = 14.8, 7.5 Hz, 1H), 2.46–2.33 (m, 1H); ^{13}C NMR (CD_3OD) δ 131.99 (s), 128.26 (s, 2C), 127.78 (s, 2C), 127.28 (s), 112.80 (t, J = 288.0 Hz), 36.69 (d, J = 5.9 Hz), 32.00 (t, J = 11.0 Hz), 25.63 (t, J = 10.7 Hz); HRMS (ESI) calculated for $C_{10}H_{12}F_2N$ ($[M+H]^+$): m/z 184.0932; found: 184.0914.

4.19. 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-INI} using a FLIPR^{TETRA} fluorescence imaging plate reader (Molecular Dynamics) as previously described [17,24]. Briefly, cells were seeded in 384-well poly-L-lysine plates at a density of 10,000 cells/well in 1% dialyzed FBS DMEM containing 1 μg/mL tetracycline for inducible expression. Next day, the medium was decanted, and 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 5 × final concentration in drug buffer (1 × HBSS, 20 mM HEPES, 0.1% BSA, 0.01% ascorbic acid, pH 7.4). Plates were loaded into the FLIPR, a 10 s baseline was acquired, 5 μL per well of drug was added, and calcium flux was measured (1 read/s) for 300 s. Fluorescence in each well was normalized to the average of the first 10 reads (i.e., baseline fluorescence), then the maximum-fold increase and fold-over-baseline were acquired and 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.

4.20. Docking simulation

The three-dimensional (3D) structures of **8** and **21c** were built up in their protonated forms and energy-minimized by molecular mechanics using ChemBio3D 12.0 (MM2). The crystallographic structure of 5-$HT_{2C\text{-active}}$ was obtained from the Protein Data Bank (PDB) with the access code 6BQG (resolution of 3 Å). All docking simulations were conducted in the binding pocket of 5-$HT_{2C\text{-active}}$ by using GOLD 5.4 software (CCDC Software Limited). GOLD 5.4 has four fitness functions available: GoldScore, ChemScore, ASP, and ChemPLP. All fitness functions were evaluated by re-docking of the co-crystallized ligands of each crystallographic complex to identify the most suitable fitness function for the docking into 5-$HT_{2C\text{-active}}$. Crystallographic waters were removed during docking. Hydrogen atoms were added to the protein according to the data inferred by the program on the ionization and tautomeric states. The set of amino acid residues selected as the binding site was determined within a 6 Å radius from each co-crystallized ligand. After re-docking, the root-mean-square deviation (RMSD) between the best result for each fitness function, and the experimental conformation of ERG were calculated. The fitness function with the lower value of RMSD (no more than 2.0 Å), and the best performance in the re-docking was used for the docking of **8** and **21c**. The GoldScore was the best fitness function found for both structures (higher RMSD obtained equal to 1.15 Å). The program optimizes hydrogen-bond geometries by rotating hydroxyl and amino groups of the amino acid side chains. The score of each pose identified is calculated as the negative of the sum of a series of energy terms involved in the protein-ligand interaction process, so higher positive score values means better interactions. The figures of the best docking poses for each compound were generated using PyMOL (Schrödinger, LLC).

Notes

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2019.111626.

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