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Article

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Identification of Novel 1-*O*-substituted Aporphine Analogues as Potent 5-HT_{2C} Receptor Agonists

Qi Mao,[a,1] Bingjie Zhang,[b,c,1] Wanwan Li,[a] Sheng Tian,[a] Wenjing Shui,[b,c]** and Na Ye[a,]*

[a]Jiangsu Key Laboratory of Neuropsychiatric Diseases and College of Pharmaceutical Sciences, Soochow University, Suzhou, Jiangsu 215123, China

[b]iHuman Institute, ShanghaiTech University, Shanghai 201210, China

[c]School of Life Science and Technology, ShanghaiTech University, Shanghai, 201210, China

[1]These authors contribute equally to this work.

Corresponding authors:

*Na Ye, PhD
Tel: +86-512-65881161
Email: yena@suda.edu.cn

**Wenjing Shui, PhD
Tel: +86-21-20685595
Email: shuiwq@shanghaitech.edu.cn

Abstract

The 5-HT_{2C} receptor has emerged as a promising target in the treatment of a variety of central nervous system disorders. We have first identified aporphines as a new class of 5-HT_{2C} receptor agonists. SAR results indicate that the aporphine core may be required for 5-HT_{2C} receptor activity, and substitutions on its C1 position are important for 5-HT_{2C} receptor activity. Our efforts to optimize our hit **15781** lead to the identification of the highly potent and selective 5-HT_{2C} agonist **18b** (**MQ02-439**) with an EC_{50} value of 104 nM, and weak antagonism at the 5-HT_{2A} and 5-HT_{2B} receptor. The findings may serve as good starting points for the development of more potent and selective 5-HT_{2C} agonists as valuable pharmacological tools or potential drug candidates.

KEYWORDS*:* Serotonin, 5-HT_{2C} receptor, 5-HT_{2B} receptor, 5-HT_{2A} receptor, agonist, aporphine

INTRODUCTION

The 5-HT_{2C} receptor belongs to 5-HT_2 subfamily of serotonin (5-hydroxytryptamine, 5-HT) receptors.[1] The 5-HT_2 receptors as G protein-coupled receptors (GPCRs) couple preferentially to $G_{q/11}$ to increase the hydrolysis of inositol phosphates and elevate cytosolic calcium concentrations to produce their physiological activity. The 5-HT_{2C} receptor has been explored for over 25 years in the treatment of a variety of central nervous system (CNS) disorders including obesity, depression, anxiety, obsessive-compulsive disorder, addictive disorders, and chronic pain conditions,[2-9] and there is an FDA approved CNS drug (lorcaserin) targeting this receptor. Moreover, 5-HT_{2C} receptor is almost exclusively expressed in the CNS, which provides one of the many advantages of the 5-HT_{2C} receptor as a CNS drug target, and thus compounds selectively activating this receptor should have limited peripheral effects.[10] To date, a number of 5-HT_{2C} receptor agonists have been identified (Fig. 1),[11-14] with the representative drug Lorcaserin as the first-in-class antiobesity drug approved by FDA in 2012.[15] However, it remains a challenge to develop 5-HT_{2C} receptor agonists with high potency and subtype selectivity since the 5-HT_{2C} receptor exhibits 46–50% overall sequence identity with the other two 5-HT_2 subtypes, 5-HT_{2A} and 5-HT_{2B} receptor, which may lead to hallucinogenic and lethal valvulopathic side effect, respectively.[12, 16, 17]

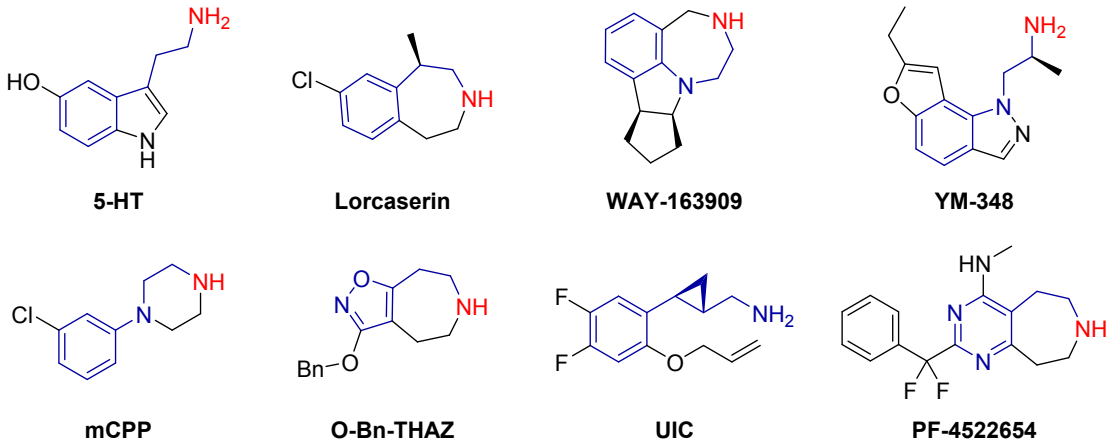
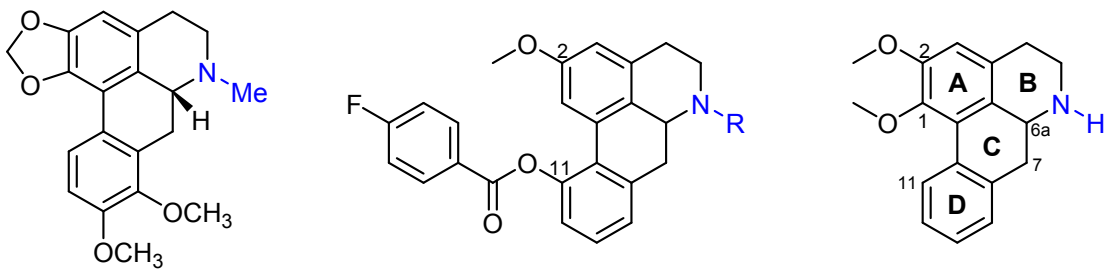


Fig. 1. Structure of 5-HT and representative 5-HT_{2C} selective agonists

Aporphines, a group of tetrahydroisoquinoline alkaloids, exhibit a wide range of CNS pharmacological activities represented by antiparkinsonian drug apomorphine, a dopamine D_2 agonist.[18-26] With regard to serotonin receptors, aporphines have mostly been studied as ligands for 5-HT_{1A} and 5-HT_{2A} receptors.[19, 21, 23, 27-30] However, they are relatively unexplored as 5-HT_{2C} receptor ligands. Recently, our team discovered several aporphine derivatives as 5-HT_{2C} hit ligands by using thermal stability assay to screen a focused alkaloid library of over 300 chemical components isolated from plants, e.g. (–)-crebanine (**1**, Fig. 2) as a 5-HT_{2C} receptor antagonist with no selectivity over 5-HT_{2A} or 5-HT_{2B}.[31] Later, Sromek and coworkers[32] identified a series of fluorinated 11-*O*-aporphine derivatives as potent 5-HT_{2C} receptor ligands but with poor selectivity (e.g. the most potent compounds **2-3**, Fig. 2). All these results indicate that aporphines may serve as a viable source to develop novel ligands for 5-HT_{2C} receptor.



Crebanine
5-HT$_{2C}$: IC$_{50}$ = 148 nM
5-HT$_{2B}$: IC$_{50}$ = 1693 nM
5-HT$_{2A}$: IC$_{50}$ = 564 nM

11a R = Me
5-HT$_{2C}$: K$_i$ = 32 nM
5-HT$_{2B}$: K$_i$ = 102 nM
5-HT$_{2A}$: K$_i$ = 450 nM

11b R = Pr
5-HT$_{2C}$: K$_i$ = 24 nM
5-HT$_{2B}$: K$_i$ = 106 nM
5-HT$_{2A}$: K$_i$ = 414 nM

15781
5-HT$_{2C}$: EC$_{50}$ = 678 nM
5-HT$_{2B, 2A}$: NA

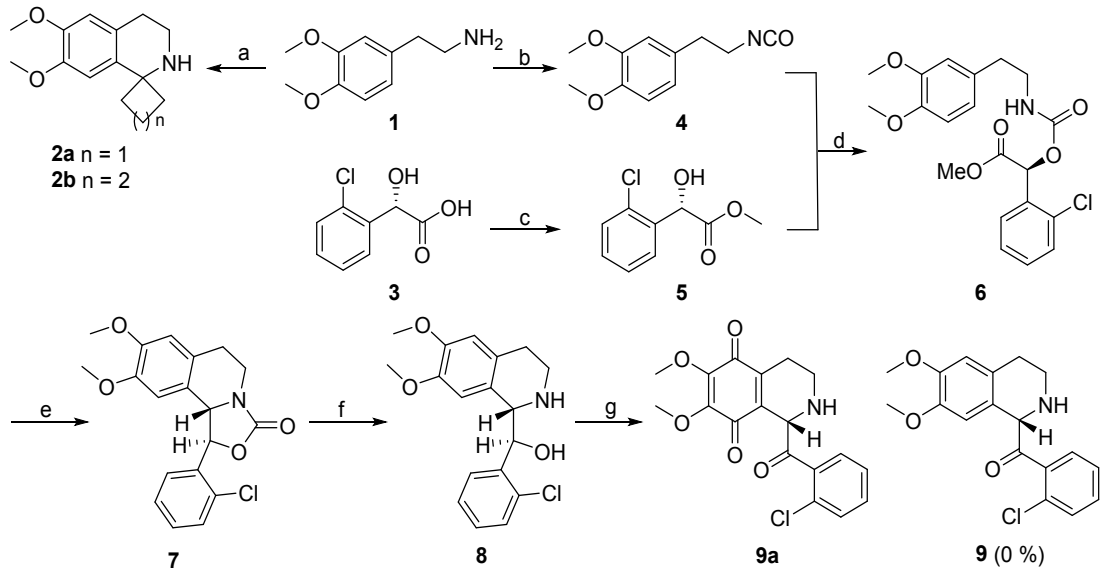
Fig. 2. Structure of aporphine analogs targeting 5-HT$_2$ receptors

We have long been working on the structure-activity relationship (SAR) study of aporphine analogues by structural modifications on the 10,11-dihydroxyl moiety of apomorphine to generate new dopamine D$_2$ receptor agonists with improved metabolic stability.[18-23] Recently, through screening natural herb extracts with the affinity mass spectrometry approach,[33, 34] we identified 1,2-dimethoxy-aporphine **15781** (**3**, Fig. 2) as a novel ligand for 5-HT$_{2C}$ receptor which showed submicromolar agonist activity in the G$_q$-coupled calcium flux assay (EC$_{50}$ = 678 nM). Interestingly, this new agonist (to be disclosed in a separate publication in details) was highly selective on 5-HT$_{2c}$ and had no effects toward 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors (Figure 1). Of note, **15781** lacks the critical structural elements for dopaminergic and adrenergic properties of aporphinoids, the *N*-alkyl substituent and 11-hydroxyphenethylamine component,[24] and 7-hydroxy group,[25] respectively, indicating it may be inactive at these receptors. Inspired by these intriguing results and aiming to develop leads with enhanced activity and selectivity on 5-HT$_{2C}$ receptors, it is imperative to conduct SAR studies on aporphine derivatives by using **15781** as a chemical hit.

RESULTS AND DISCUSSION

Design and Synthesis. As shown in Fig.1, a number of 5-HT_{2C} receptor agonists have been identified with *N* "head" as a mimic of the primary amine in 5-HT, an aromatic ring mimicking its indole core, and a linker between an aromatic ring and the *N* "head".[16, 35] Aporphines share all these key structural features, but have two aromatic rings in the tetracyclic skeleton, phenyl ring A and D. To confirm which phenyl ring mimics the indole core of 5-HT, we initially simplify the compact scaffold of aporphines by removal of phenyl ring C and D as in compounds **2a-b**. To analyze the linker between the desired phenyl ring and the *N* "head", we then investigate the effects of different linkers by opening phenyl ring C as in compounds **8**, **9a** and **13-14b**. And we also explore the necessity of two methoxy groups on the 1,2-position of hit **15781** as in compounds **18a-k**.

Scheme 1[a]

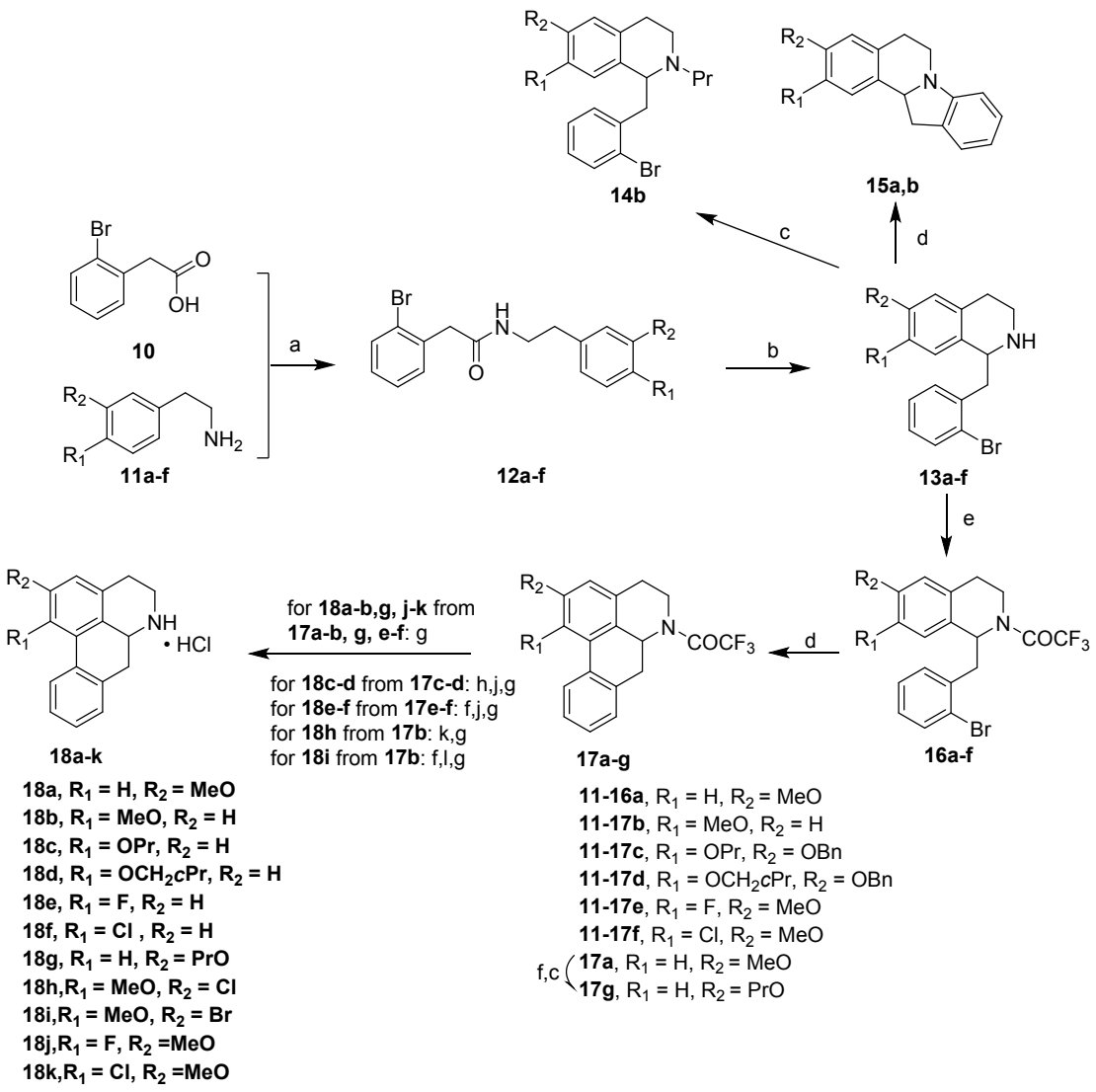


[a]Reagents and conditions: (a) cycloketones, H_3PO_4, 90 °C, 24 - 48 h, 27%-34%. (b) $Cl(OCCl_3)_2$, Et_3N, DCM; 0 °C, 15 min, rt, 1 h, 32%. (c) $SOCl_2$, MeOH, 0 °C, rt, 12 h,

90%. (d) toluene, 110 °C, 12 h, 62%. e) i) DIBAL–H, - 78 °C, 1 h; ii) MeOH, - 20 °C, 10 min; iii) $BF_3 \cdot OEt_2$, rt, 1 h, 40% for three steps. (f) KOH, EtOH, H_2O, THF, 100 °C, 7 h, 90%. (g) i) $(Boc)_2O$, DCM, rt, 2 h, 90%; ii) DMP, DCM, rt, 1 h, 79%; iii) conc. HCl : EtOAc = 1 : 2, rt, 1 h, 46%.

As depicted in Scheme 1, tetrahydroisoquinoline compounds **2a-b** were prepared by the Pictet-Spengler cyclization reaction of 3,4-dimethoxyphenethylamine **1** and the corresponding cycloketone under the treatment of phosphoric acid at 90 °C for 24-48 h in 27-34% yields.[36] On the other hand, coupling of isocyanate **4** and alcohol **5** derived from material amine **1** and acid **3**, respectively, generated the open carbamate **6** at 100 °C in toluene in 62% yield as shown in Scheme 1.[37] Subsequent one-pot reductive cyclization with sequential addition of DIBAL-H, methanol and $BF_3 \cdot OEt_2$ gave enantiomerically pure oxazolidinones **7** in 40% total yield.[38] Hydrolysis of precursor **7** gave 1-hydroxybenzyl substituted analogs **8**. Subsequent oxidation of electron-rich arene **8** with DMP directly generated benzoquinone **9a** in 46% yield, with no detection of desired 1-benzoyl substituted analogs **9**.[39]

Scheme 2[a]



[a]Reagents and conditions: (a) EDCI, HOBt, DCM, rt, 24 h, 60%-90%. (b) i. Method A: PCl_5, DCM, reflux, 20 min, 35%-66%; Method B: $POCl_3$, acetonitrile, reflux, 3 h, 43%-72%; Method C: P_2O_5, toluene, reflux, 3 h; ii. $NaBH_4$, MeOH, 0°C-rt, 1 h, 70%. (c) PrBr, K_2CO_3, KI, acetone, reflux, 4 h, 33%-71%. (d) $Pd(OAc)_2$, PhDavePhos, K_2CO_3, DMA, 130 °C, 4 h, 40%-83%. (e) TEA, TFAA, DCM, 0°C-rt, 1 h, 70%-80%. (f) BBr_3, DCM, -78°C-rt, 1 h, 80%-83%. (g) i. EtOH, $NaBH_4$, N_2, rt, 1.5 h; ii. HCl (1 M in EtOAc), rt, 4-6 h, 53%-72% for two steps. (h) H_2, 10% Pd/C, DCM, MeOH, rt, 4 h, 85%-92%. (j) i. Tf_2O, TEA, DCM, 0°C-rt, 0.5 h; ii. TEA, HCOOH, $Pd(OAc)_2$, DPPF, DMF, 60°C, 15 min, 40-64% for two steps. (k) NCS, ACN, 70°C, 2h, 60%. (l)

i. Br_2, HOAc, rt, 1h; ii. MeI, K_2CO_3, acetone, reflux, 4 h, 50% for two steps.

The synthetic route of 1-benzyltetrahydroisoquinolines **13b** and **14b** is depicted in Scheme 2. Condensation reaction of commercially available 2-bromophenylacetic acid **10** with various phenylethylamine **11a-f**,[40, 41] gave amides **12a-f**, followed by Bischler–Napieralski cyclization with PCl_5 / $POCl_3$ / P_2O_5 and reduction with $NaBH_4$, furnished tetrahydroisoquinolines **13a-f**. Subsequently, **13b** was converted to its *N*-n-propyl analog **14b** by *N*-alkylation reaction with 1-bromopropane under the condition of K_2CO_3 and KI at reflux. To construct the tetracyclic skeleton of aporphines, **17a-b** were initially intended to prepare directly by use unprotected amines **13a-b** via Pd-catalyzed intramolecular C-C coupling reaction. However, only C-N coupling products **15a-b** were yielded. Hence the trifluoroacetyl protected amines **16a-f** were prepared for oxidative cyclization to produce key aporphine immediates **17a-f** in the presence of $Pd(OAc)_2$ and PhDavePhos in DMA at 130 °C. **17a** was then converted to **17g** by *O*-demethylation and subsequent *O*-propylation. Removal of the trifluoroacetyl group of **17a-b, 17g** and **17e-f** using $NaBH_4$ gave the target compounds **18a-b, 18g** and **18j-k** while sequential hydrogenation of **17c-d** or demethylation of **17e-f**, trifluoromethyl sulfonation, palladium-catalyzed hydrogenolysis, and then detrifluoroacetylation yielded **18c-f**. Chlorination of **17b** followed by detrifluoroacetylation obtained **18h**, but **17b** can't be brominated for synthesis of **18i** in the same fashion. Finally, **18i** was prepared sequentially by demethoxylation of **17b**, bromination, methylation, and detrifluoroacetylation.

Pharmacological Assays and Structure−Activity Relationship (SAR). All new

compounds were screened employing recombinant, stably expressed human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors in the Flp-In T-rex293 cell line, using a fluorescence imaging plate reader (FLIPR) assay. Estimates of E_{max} and EC_{50} are found in Table 1. Serotonin was used as a positive control, and its E_{max} values were normalized to 100% for all receptors.

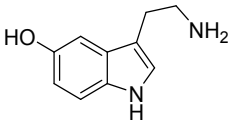
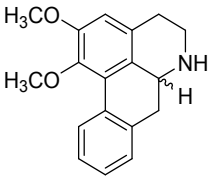
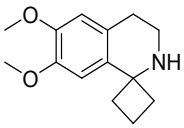
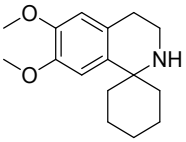
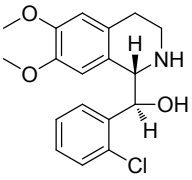
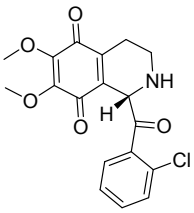
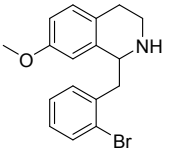
As shown in Table 1, removal of ring C and D in hit **15781** leads to the spiro-tetrahydroisoquinolines **2a-b** with significant loss of activity on 5-HT_{2C} receptors, suggesting that it may be phenyl ring D mimicking the indole core of 5-HT rather than ring A. And opening ring C also results in C-1 substituted tetrahydroisoquinolines **8**, **9a** and **13-14b** with no activity on 5-HT_{2C} receptors even if these tetrahydroisoquinolines keep pivotal structural characteristics of 5-HT_{2C} receptor agonists. It is noted that the linker of **8**, **9a** and **13-14b** is flexible while the linker in hit **15781** is restricted. However, C-N coupling byproducts **15a-b** are also inactive, suggesting that it is of great importance to keep the linker between the phenyl ring D and the *N* "head" in an appropriate configuration. As we expected, cyclization of **13b** to aporphines **18b** lead to regain 5-HT_{2C} receptor activity. All these above results suggest that the aporphine core may be required for 5-HT_{2C} receptor activity.

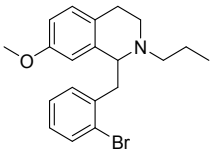
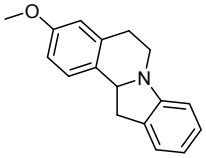
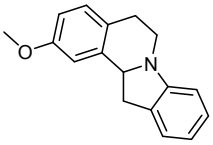
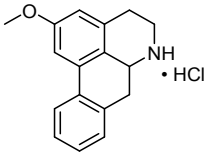
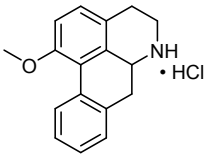
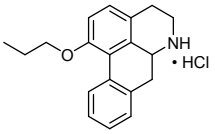
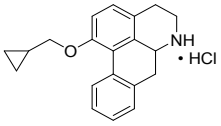
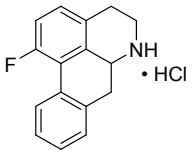
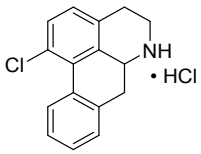
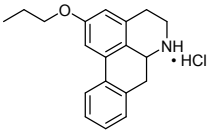
We later focused on investigating the effect of the monosubstituent on the 1- or 2-position of our hit **15781**. Although ring A is not the desired aromatic ring mimicking the indole core of 5-HT, its substitutions such as two methoxy groups on the 1,2-position of hit **15781** play an important role in 5-HT_{2C} effects. All the tested

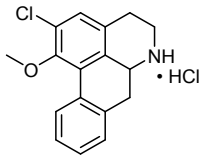
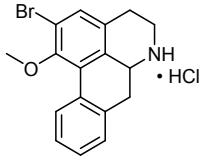
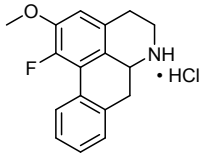
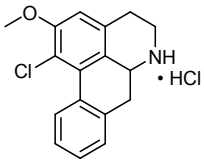
new compounds **18b-f** with various substitutions on C1 position show potent agonism of the 5-HT_{2C} receptor, while compounds **18a** and **18g** with alkyloxy groups on C2 position completely lost activity (Fig. 3). The most potent compound **18b** (**MQ02-439**) with C1 methoxy group is 6-fold more potent than hit **15781** on 5-HT_{2C} activity (EC_{50} 103 *vs* 678 nM), and also shows great selectivity with no effect on the 5-HT_{2A} and 5-HT_{2B} receptors (Fig. 4). Increasing the size of the C1-methoxy group of **18b** as in compounds **18c-d** results in decreased potency on 5-HT_{2C} receptors, and poor selectivity against 5-HT_{2A} and 5-HT_{2B} receptors. Replacement of the C1-methoxy group of **18b** with halogen as in compounds **18e-f** also decreases potency on 5-HT_{2C} receptors, and selectivity against the 5-HT_{2B} receptors. And compound **18f** with a chloro group is almost 3-fold potent than **18e** with a fluoro group, and displays higher efficacy on 5-HT_{2C} (EC_{50} 205 *vs* 568 nM; E_{max} 98% *vs* 51%) and 5-HT_{2B} receptors (EC_{50} 222 *vs* 598 nM; E_{max} 72% *vs* 48%). Taken together, C1 position of aporphine core may prefer small lipophilic and electro-donating groups for the 5-HT_{2C} receptor activity. And it is noted that **18e-f** are inactive at the 5-HT_{2A} receptors while **18c-d** display moderate potency, indicating C1 position may be tolerant of bulky lipophilic and electro-donating groups for 5-HT_{2A} receptor activity. Further halogenated **18b** at 2-position as in compounds **18h-i**, dramatically decreased activities. However, fluorinated **18a** (as in **18j**) at 1-position was still no active while chlorinated **18a** (as in **18k**) gained weak 5-HT_{2C} agonist activity. All the SAR studies indicates that C1 position on the aporphine core may be a key position to modify for increasing agonist activity for 5-HT_{2C} receptors, and appropriate groups on C2

position may modulate the agonist activity.

Table 1. Pharmacological Profiling of Aporphine Derivatives at 5-HT_2 Receptors in Calcium Flux Assay

NO	Structure	$5HT_{2C}$		$5HT_{2B}$		$5HT_{2A}$	
		EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)
5-HT		0.12 ± 0.01	100 ± 0.6	1.19 ± 0.09	100 ± 0.9	0.23 ± 0.04	100 ± 0.2
15781		653 ± 0.51	65.61 ± 3.1	NA	-	NA	-
2a		NA	NA	-	-	-	-
2b		NA	NA	-	-	-	-
8		NA	NA	-	-	-	-
9a		6755 ± 0.125	67.28 ± 1.45	NA	NA	6412 ± 0.83	46.58 ± 5.01
13b		NA	NA				

14b		NA	NA	-	-	-	-
15a		NA	NA	-	-	-	-
15b		NA	NA				
18a		NA	NA	-	-	-	-
18b		103.1 ± 0.04	95.91 ± 1.06	NA	NA	NA	NA
18c		461 ± 0.50	96 ± 2.64	211.2 ± 0.85	71.85 ± 3.53	372.0 ± 0.69	85.26 ± 3.73
18d		855.7 ± 0.66	89.53 ± 2.82	1920 ± 0.92	85 ± 5.83	3566 ± 0.09	73.20 ± 0.47
18e		568.4 ± 0.54	51.44 ± 1.62	598.6 ± 1.27	48.47 ± 13.44	NA	NA
18f		205.2 ± 0.23	98.50 ± 3.20	222 ± 0..79	72 ± 1.12	NA	NA
18g		NA	NA	-	-	-	-

18h		1643 ± 0.08	82.81 ± 1.31	14600	121.48	NA	NA
18i		1680 ± 0.14	82.27 ± 2.46	3935 ± 0.25	85.28 ± 6.13	NA	NA
18j		NA	NA	-	-	-	-
18k		1163 ± 0.23	53.28 ± 4.62	-	-	-	-

[a]Pharmacological data were acquired with recombinant, stably expressed human 5-HT_{2C}, 5-HT_{2B}, and 5-HT_{2A} receptors in the Flp-In T-rex293 cell line, using a fluorescence imaging plate reader (FLIPR) assay. EC_{50} and E_{max} values are shown as the mean ± SEM ($n \geq 2$). "NA" indicates no activity up to 10 μM. "–" indicates the activity are not tested. Data were obtained from two independent experiments performed in triplicate.

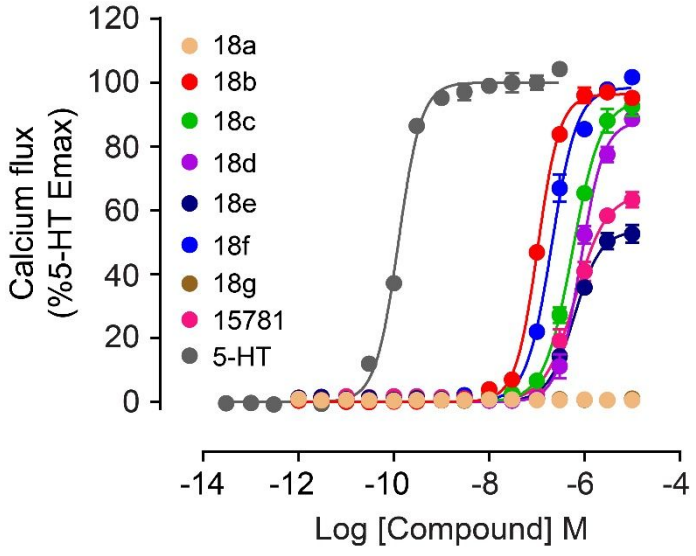


Fig. 3. Representative intracellular calcium release response of Aporphine derivatives at human 5-HT_{2C} receptors compared with **15781** and 5-HT. E_{max} and EC_{50} values of these compounds are listed in Table 1.

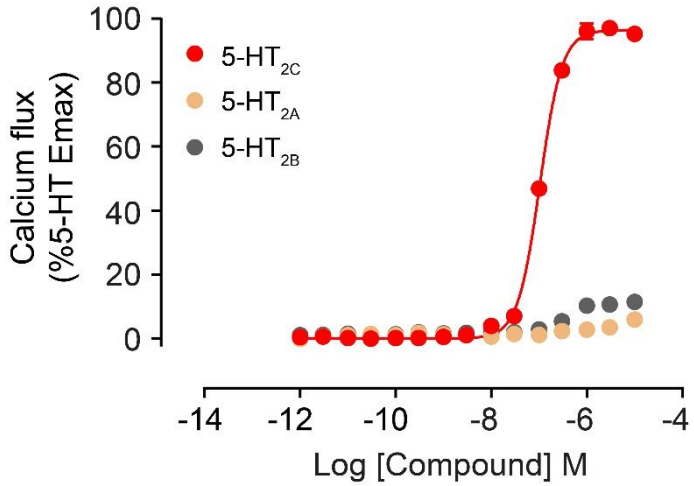
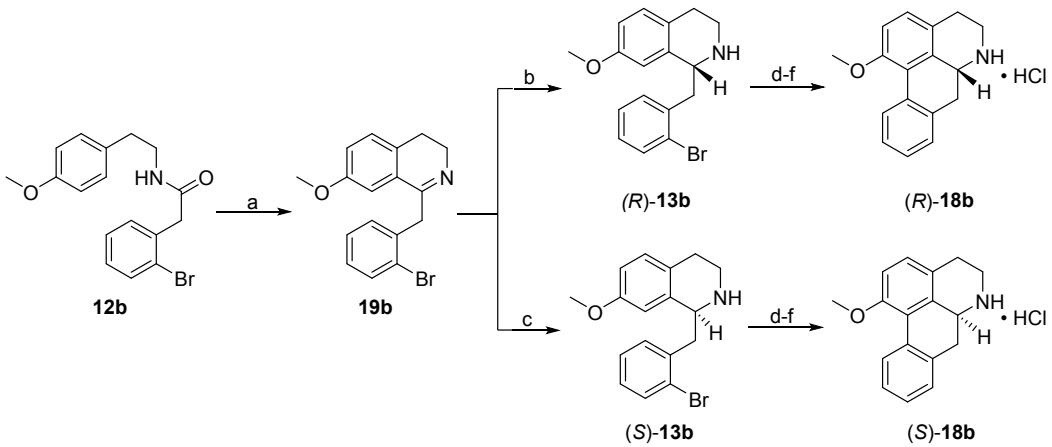


Fig. 4. Representative intracellular calcium release responses of **18b** at human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors. E_{max} and EC_{50} values of these compounds are listed in Table 1.

Synthesis and Pharmacological Effects of (+)-Enantiomers and (-)-Enantiomers of Compound 18b in Calcium Flux Assay. From the results above, aporlogue **18b** stood out as the most potent 5-HT_{2C} agonist identified in this report. Since the compound was recemate, **(+)-18b** and **(-)-18b** were prepared using the synthetic route depicted in Scheme 3 based on the asymmetric hydrogenation of 1-benzyl-3,4-dihydroisoquinoline intermediates **19b**. As shown in Scheme 3, Bischler–Napieralski cyclisation of **12b** using phosphorus pentoxide in refluxing toluene, followed by catalytic asymmetric hydrogenation of the resulting imines **19b**,

gave 1-benzyltetrahydroisoquinoline enantiomers (*R*)-**13b** with an e.e. value of 90% (Table 2, entry 1) by using previously reported Noyori's ruthenium-based catalyst, RuCl[*S*,*S*)-TsDPEN](*p*-cymene).[42] After optimization of the reaction conditions (Table 2), the e.e. value was improved to 95.5% (entry 5; see Supporting Information, Fig. S2). Subsequently, (*S*)-**13b** was also synthesized under the optimal reaction condition using RuCl[*R*,*R*)-TsDPEN](*p*-cymene) as the catalyst with identical e.e. value (95.4%) as (*R*)-**13b** (Fig. S3). Finally, the desired products (*R*)-**18b** and (*S*)-**18b** were accomplished from (*R*)-**13b** and (*S*)-**13b** respectively with remaining steps in the same fashion as those described for the synthesis of **18b**. (*R*)-**18b** and (*S*)-**18b** were determined by chiral IC column with e.e. value of 96% and 94%, respectively with *n*-hexane/ethyl alcohol/diethylamine as the mobile phase (Fig. S5-S6).

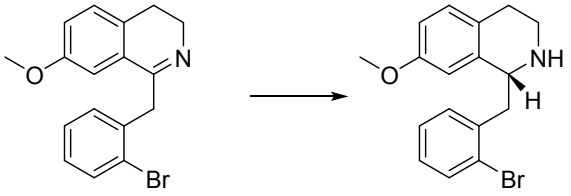
Scheme 3[a]



[a]Reagents and conditions: (a) P_2O_5, toluene, reflux, 3 h, 70%. (b) TEA, HCOOH, RuCl[*S*,*S*)-TsDPEN](*p*-cymene), DMF, 0°C-rt, 12 h, 57%. (c) TEA, HCOOH, RuCl[*R*,*R*)-TsDPEN](*p*-cymene), DMF, 0°C-rt, 12 h, 57%. (d) TEA, TFAA, DCM, 0°C - rt, 1 h, 80%-85%; (e) $Pd(OAc)_2$, PhDavePhos, K_2CO_3, DMA, 130 °C, 4 h,

60%-70%. (f) i) EtOH, $NaBH_4$, N_2, rt, 1.5 h; ii) HCl (1 M in EtOAc), rt, 4-6 h, 60% for two steps.

Table 2. Optimization conditions for enantiomeric asymmetric synthesis[a]



19b (*R*)-**13b**

Entry	S/C[b]	F/T[c]	Time	e.e.[d]
1[e]	50	0.5 mL	12h	90%
2	50	0.5 mL	12h	90.7%
3[e]	50	1.0 mL	12h	92%
4	50	1.0 mL	12h	91%
5	**30**	**1.0** mL	**12h**	**95.4**%
6	25	1.0 mL	12h	92.5%
7	30	0.5 mL	12h	91.5%
8	30	1.0 mL	4h	94%

[a]Reaction conditions: **19b** (1.0 mmol), RuCl[*S*,*S*)-TsDPEN](*p*-cymene), HCOOH, TEA, DMF (2 mL); 0℃-rt. [b]S/C = Substrate/Catalyst (molar ratio). [c]F/T = HCOOH / TEA (molar ratio = 5:2). [d]Determined by HPLC equipped with a chiral IC column. [e]under N_2 atmosphere.

From the results in Table 3, racemate **18b** and its enantiomers are all selective 5-HT_{2C} receptor agonist with no effect on 5-HT_{2A} and 5-HT_{2B} receptor, whereas both of the enantiomers show weaker potency at the 5-HT_{2C} receptor than its racemate (Fig. 5 and 6). Although (*S*)**-18b** (**MQ02-592**) showed slightly less potency than racemate **18b** (139.3 *vs* 103.1 nM)**,** it exhibited 12-fold more potency than (*R*)**-18b** at the

5-HT_{2C} receptor (139.3 *vs* 1658 nM). The significant effect of C*6a*-stereochemistry in aporphine skeleton on the 5-HT_{2C} receptor functionality assay was first observed in our studies. It is of note that *R*-(-)-enantiomer aporphines typically are more potent DA agonists while *S*-(+)-antipode aporphines usually have DA antagonist effects in various assays,[43, 44] e.g. *R*-(-)-apomorphine as a DA agonist and *S*-(+)-apomorphine as weak agonist, partial-agonist, or antagonist effects at DA receptors.[24]

Table 3. Effects of (+)- and (-)-enantiomers of the most potent compound **18b** in Calcium Flux Assay

No	e.e.	$[\alpha]_D^{25}$	$5HT_{2C}$		$5HT_{2B}$		$5HT_{2A}$	
			EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)
18b	0	-	103.1 ± 0.04	96.0 ± 1.06	NA	NA	NA	NA
(*S*)-18b	96.1	+92.9	139.3 ± 0.15	81.8 ± 2.27	NA	NA	NA	NA
(*R*)-18b	94.0	-92.0	1658 ± 0.10	70.3 ± 1.93	NA	NA	NA	NA

EC_{50} and E_{max} values are shown as the mean ± SEM ($n \geq 2$). "NA" indicates no activity up to 10 μM. Data were obtained from two independent experiments performed in triplicate.

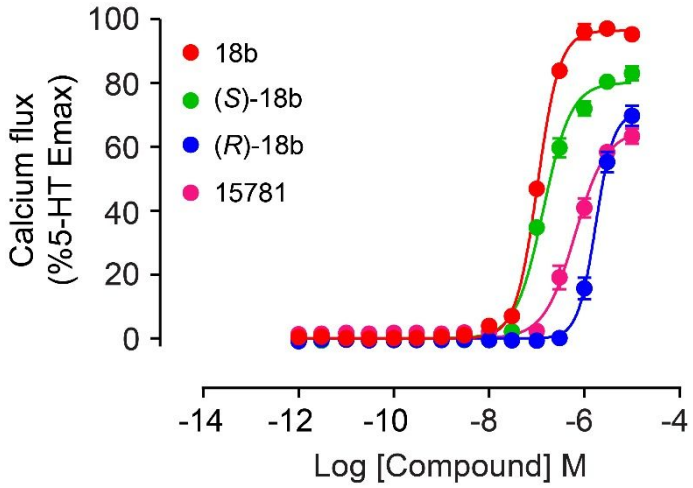


Fig. 5. Representative intracellular calcium release responses of **18b** and its isomers (*S*)-**18b** and (*R*)-**18b** at human 5-HT_{2C} receptors compared with **15781**. E_{max} and EC_{50} values of these compounds are listed in Table 2.

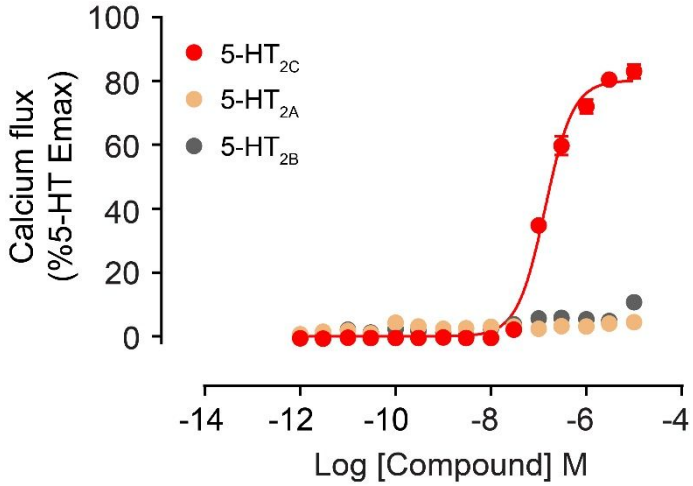


Fig. 6. Representative intracellular calcium release responses of (*S*)-**18b** (**MQ02-592**) at human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors. E_{max} and EC_{50} values of these compounds are listed in Table 2.

Functional profiles of Compound 18b and its Enantiomers at 5-HT_{2A} and 5-HT_{2B} receptors. To further determine the functional profiles of **18b** and its enantiomers, (*S*)-**18b** and (*R*)-**18b,** at 5-HT_{2A} and 5-HT_{2B} receptors, these three highly potent 5-HT_{2C} agonists were evaluated in an antagonist mode. As shown in Fig. 7, all

of them acted as weak antagonists, and they were more potent in inhibiting the calcium flux activated by 5-HT at 5-HT_{2B} receptors than 5-HT_{2A} receptors. Moreover, (*S*)-**18b** was similarly more potent than (*R*)-**18b** in inhibiting the calcium flux at both 5-HT_{2A} (IC_{50}: 4.5 *vs* 111.5 μM) and 5-HT_{2B} receptors (IC_{50}: 1.4 *vs* 90.5 μM). Thus, **18b** and its enantiomers are potent 5-HT_{2C} receptor agonists, and weak 5-HT_{2A} and 5-HT_{2B} antagonists. Compared with nonselective 5-HT_2 antagonist (–)-crebanine (**1**, Fig. 2), it indicates the substitutions on aporphine scaffolds may result in their function difference at 5-HT_2 subfamily members.

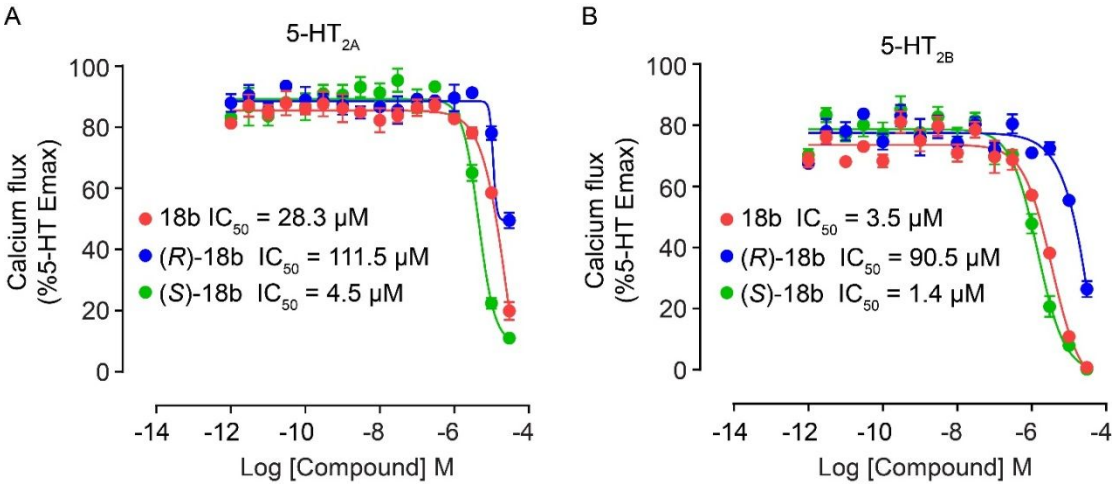


Fig. 7. Functional profiles of **18b**, (*S*)-**18b**, and (*R*)-**18b** at human 5-HT_{2A} and 5-HT_{2B} receptors.

Molecular docking studies. In order to examine the interaction pattern between the most potent and selective agonist, compound (*S*)-**18b** and 5-HT_{2C}, the compound (*S*)-**18b** was selected and docked into the binding pocket of ergotamine-5-HT_{2C} crystal complex (PDB ID: 6BQG)[45] using extra precision (XP) scoring function of *Glide* docking. The binding pose and interaction pattern between compound (*S*)-**18b** and 5-HT_{2c} were shown in Fig. 8. As can be seen in Fig. 8, the molecular docking

prediction results demonstrated that the most important key residue for compound (*S*)-**18b** binding was V135. The arene-H interaction can be explicitly observed between compound (*S*)-**18b** and V135 in the binding site of 6BQG. As described in the previous study,[46] the most important favorable residue for ergotamine binding with 5-HT_{2C} was D134. A strong salt bridge interaction can be found between ergotamine and D134. Considering the inherent nature of GPCR targets, different chemical scaffolds or even minor modifications for same chemical scaffold may generate different interaction patterns in the binding site of 5-HT_{2c}. This finding may provide some clues to develop more promising 5-HT_{2c} agonists with novel scaffold architectures by employing rational-drug-design approaches.

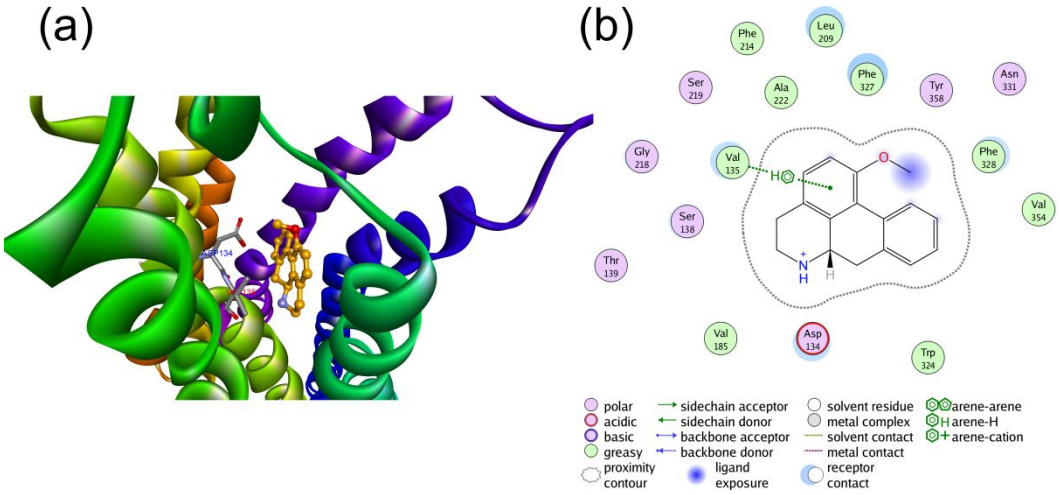


Fig. 8. The predicted binding pose (a) and interaction pattern (b) between compound (*S*)-**18b** and 5-HT_{2c} (PDB ID: 6BQG).

Summary. Aporphines were first identified as a new class of 5-HT_{2C} receptor agonists. SAR results indicate that the aporphine core may be required for 5-HT_{2C} receptor activity, and C1 position may be a key position to modify in order to improve

agonist activity for 5-HT_{2C} receptors. Our efforts to optimize our hit **15781** led to the identification of the highly potent and selective 5-HT_{2C} agonist **18b** (**MQ02-439**) possessing an EC_{50} of 104 nM, and weak antagonism at the 5-HT_{2A} and 5-HT_{2B} receptor. Further profiling of the biological effects in animal models of behavior and systematic optimizations based upon identified lead **18b** toward high potency and selectivity are under way, and the findings will be reported in due course.

MATERIALS AND METHODS

Cell Lines and Cell Culture. The calcium flux assays were performed with Flp-In T-rex 293 cells stably expressed 5-$HT_{2A/2B/2C}$ receptors.[47] The stable cell lines were cultured in DMEM containing 10% fetal bovine serum (FBS), 0.5% Penicillin/Streptomycin and contained selection antibotics, 10 μg/mL Blasticidin and 100 μg/mL Hygromycin B in a humidified atmosphere at 37°C and 5% CO_2.

Intracellular Calcium Flux Assay. Intracellular calcium (Ca_i^{+2}) release was monitored using the Fluo-4 Direct Calcium Assay Kit (Invitrogen) according to previously published protocols with minor modifications.[31] Cells were plated at 15000 cells/well in black-sided, clear bottomed 384-well tissue culture plates and allowed to adhere overnight. Medium was removed and replaced with Fluo-4 Direct dye reconstituted in FLIPR buffer (1× HBSS, 2.5 mM probenecid and 20 mM HEPES, pH 7.4) Plates were incubated for 60 min at 37 °C followed by 30 min at room temperature in the dark. Drug dilutions were prepared at 3× final concentration in FLIPR buffer and aliquotted into 384-well source plate. The cell plate and the

source plate were placed to the FLIPR^{TETRA}. The fluidics module and plate reader of the FLIPR^{TETRA} were programmed to read baseline fluorescence prior to reading drug dilutions (1 read/s per well). For the antagonist mode, 5-HT (3 nM) was used to activate the receptor.

Data Analysis. The response in each well was normalized to the average of the baseline fluorescence. Then, the fold was determined by the maximum response over the baseline fluorescence. The folds were plotted as a function of drug concentration, normalized to the percent of 5-HT with 100% as the 5-HT E_{max} and 0% as the baseline, and the EC_{50} was determined using log (agonist) vs. response (4-parameter) or log (antagonist) vs response in GraphPad Prism 7.0. Data represent means ± SEM of two independent experiments performed in triplicate.

Molecular Docking Procedure. First of all, the crystal structure of 5-HT_{2C} interacting with agonist, ergotamine was retrieved from the RCSB Protein Data Bank (PDB ID; 6BQG).[45] The *Receptor Grid Generation* mode in *Glide* of Schrödinger 9.0[48] was used to generate binding pocket for molecular docking. The binding site with the size of 10 Å × 10 Å × 10 Å and centered on the centroid of the ergotamine of 6BQG. Then, the most potent 5-HT_{2C} agonist, compound (*S*)-**18b** was preprocessed using the *LigPrep* module in *Glide*. The ionized states and tautomers were generated at pH = 7.0 ± 2.0 and the different combinations of chiralities were also generated by setting the maximum number of stereoisomers to 32 by using *Epik*. Finally, the all tautomers of compound (*S*)-**18b** were docked into the binding site of 6BQG and scored by applying *Glide* docking.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschemneuro.xxxx.xx.xxx.

Procedure for synthesis of new compounds; optimization of asymmetric hydrogenation reaction conditions; ^{1}H and ^{13}C NMR and HPLC files of synthetic intermediates and final products.

AUTHOR INFORMATION

Corresponding Author

Tel: +86-512-65881161; E-mail, yena@suda.edu.cn. (N. Ye).

Tel:+86-21-20685595; E-mail, shuiwq@shanghaitech.edu.cn. (W. Shui).

Author Contributions

[1]Q.M. and B.Z. contributed equally to this work. N.Y. conceived the project, and oversaw and designed the chemistry. Q.M. and W.L. performed synthetic chemistry work. B.Z. performed the pharmacology experiments, and W.S. interpreted the data. S. T. performed molecular docking. N. Y., W.S., Q.M., B.Z., S.T. and W.L. wrote the paper.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS

CNS, central nervous system; SAR, structure-activity relationship; HPLC, high-performance liquid chromatography; DCM, dichloromethane; EtOAc, ethyl acetate; e.e., enantionmeric excesses; CDI, *N*,*N*'-Carbonyldiimidazole; DMSO, dimethyl sulfoxide; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt, 1-hydroxybenzotriazole; DMA, dimethylacetamide; TEA, triethylamine; TFAA, trifluoroacetic anhydride; Tf_2O, trifluoromethanesulfonic anhydride; PhDavePhos, 2-diphenylphosphino-2'-(*N*,*N*-dimethylamino)biphenyl; THF, tetrahydrofuran; DMF, *N*,*N*-dimethylformamide; DPPF, 1,1'-bis(diphenylphosphino)ferrocene; LAH, lithium aluminum hydride; Et_2O, diethyl ether; RuCl[*R*,*R*)-TsDPEN](*p*-cymene), Chloro{[(1*R*,2*R*)-(-)-2-amino-1,2-diphenylethyl](4-toluenesulfonyl)amido}(p-cymene

)ruthenium(II); $(Boc)_2O$, *di*-tert-butyl dicarbonate; RuCl[*S*,*S*)-TsDPEN](*p*-cymene), Chloro{[(1*S*,2*S*)-(-)-2-amino-1,2-diphenylethyl](4-toluenesulfonyl)amido}(p-cymene) ruthenium(II); $BF_3 \cdot OEt_2$, boron trifluoride etherate; DIBAL–H, diisobutylaluminium hydride; DMP, Dess-Martin Periodinane. e.e., enantionmeric excesses; NCS, N-chlorosuccinimide; ACN, acetonitrile.

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Identification of Novel 1-*O*-substituted Aporphine Analogues as Potent 5-HT_{2C} Receptor Agonists

Qi Mao,[a,l] Bingjie Zhang,[b,c,l] Wanwan Li,[a] Sheng Tian,[a] Wenjing Shui,[b,c]** and Na Ye[a,]*