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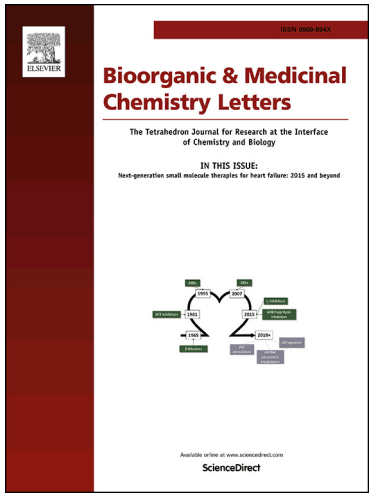

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Identification of Fluorinated (*R*)-(-)-Aporphine Derivatives as Potent and Selective Ligands at Serotonin 5-HT_{2C} Receptor

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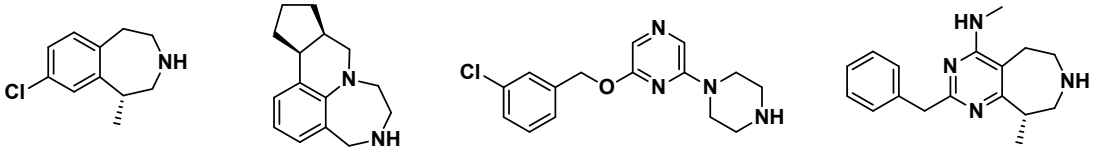
Abstract: A series of novel aporphine derivatives were synthesized for initial screening at the 5-HT_2 receptor subtypes. Among them, Compounds **11a** and **11b** were identified as potent 5-HT_{2C} hit ligands with highly selectivity over other 5-HT_2 receptor subtypes. Molecular docking study revealed that compounds **11a** and **11b** formed two key interactions with the binding site of 5-HT_{2C} receptor, including a salt-bridge to D3.32 and a H-bond interaction with N6.55.

Keywords: Aporphine derivatives; 5-HT_{2C} receptor; Hit ligands; Molecular docking

The serotonin (5-hydroxytryptamine, 5-HT) system is known to play a critical role in the regulation of numerous neurological functions.[1,2] The current classification of the serotonin receptor family includes at least fourteen receptor subtypes classified into seven major classes (5-HT_{1-7}) based upon the conjunction of genetic and

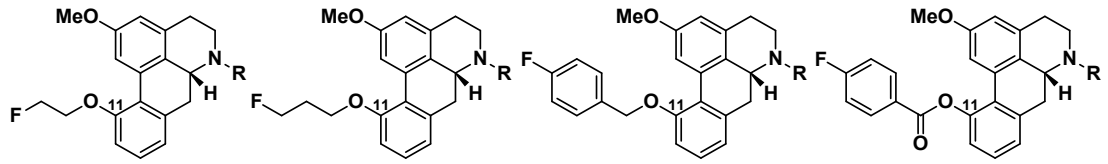
molecular structure, intracellular transduction mechanisms, and pharmacological criteria.[3] All of these serotonin receptor subtypes are G-protein-coupled receptors (GPCR) with the exception of the ligand-gated ion channel 5-HT_3.[4] The 5-HT_2 receptor subtype family is composed of three GPCR members (5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}) which share a high level of amino acid sequence homology within transmembrane regions.[5] In particular, the 5-HT_{2C} receptor is predominantly expressed in the central nervous system (CNS), and has been identified as a promising therapeutic target for the treatment of obesity and other CNS disorders, such as depression, schizophrenia and drug addiction.[6-9] To date, a number of 5-HT_{2C} ligands have been disclosed (Fig. 1). Among them, lorcaserin was approved by the FDA as a first-in-class antiobesity drug in 2012.[10] Its efficacy in treatment of nicotine addiction is currently being evaluated in clinical trials.[11] Despite dramatic advancements in 5-HT_{2C} ligands development, attaining high 5-HT_{2C} receptor selectivity remains an attractive research area, as non-specific interaction with the other two 5-HT_2 receptors, 5-HT_{2A} and 5-HT_{2B} receptors, results in hallucinations and potentially fatal fibrotic cardiac valvulopathy, respectively.[12-14] A major challenge to develop selective 5-HT_{2C} ligands involves the highly conserved ligand recognition transmembrane region of the 5-HT_2 receptor subtypes.[4] Therefore, it is difficult to design a ligand exhibiting high specificity for the 5-HT_{2C} receptor.



Lorcaserin Vabicaserin CP-809101 PF-4479745

Fig. 1. Structures of some representative 5-HT_{2C} receptor ligands.

Aporphines are a group of tetrahydroisoquinoline alkaloids which exhibit a wide range of pharmacological activities.[15-20] In the CNS, aporphines have been extensively explored as ligands for serotonin, adrenergic and dopamine receptors.[21-24] Recently, Liu and co-workers identified several aporphine derivatives as 5-HT_{2C} receptor hit ligands by screening a focused target-specific natural compound library, indicating that aporphines can be a viable source of novel ligands for 5-HT_{2C} receptor.[25] Previously reported structure-activity relationship (SAR) studies on aporphines indicated that introducing a more lipophilic group at C11 position leads to the compound possessing high affinity toward the serotonin receptors with a complete loss of affinity for the dopamine receptors.[26] Inspired by these valuable results and aiming to develop potent 5-HT_{2C} ligands with highly selectivity, a series of novel aporphine derivatives with fluorinated alkyl, benzyl or benzoyl groups attached to C11 through an *O*-linkage were synthesized for initial screening at the 5-HT_2 receptor subtypes (Fig. 2). Generally, introduction of a fluorine atom into bioactive molecules is well-established strategy for improving the target affinity.[27,28] Herein, we wish to report the synthetic and pharmacological experiments of these novel aporphine derivatives.



8a: R = Me
8b: R = *n*-Pr

9a: R = Me
9b: R = *n*-Pr

10a: R = Me
10b: R = *n*-Pr

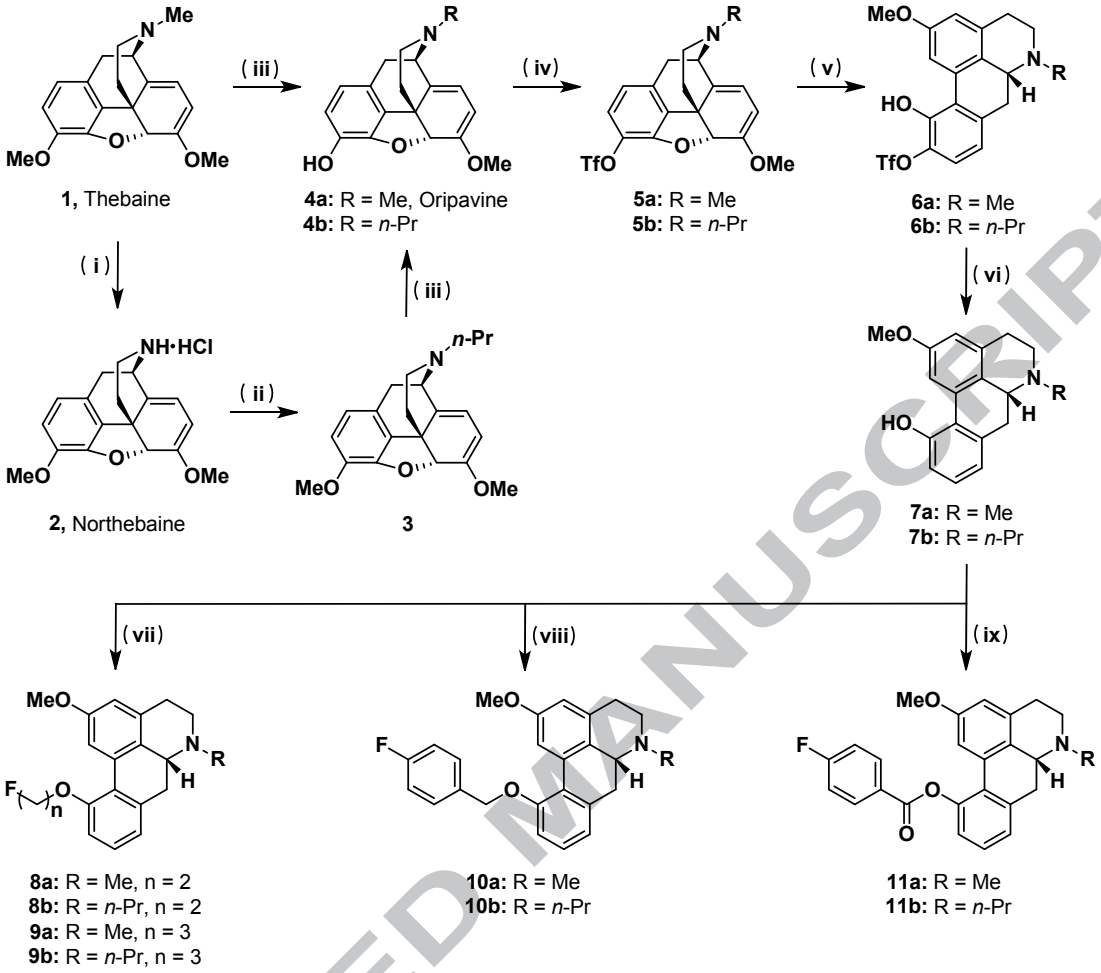
11a: R = Me
11b: R = *n*-Pr

Fig. 2. Fluorinated aporphine derivatives for initial screening at the 5-HT_2 receptor

subtypes.

Aporphine derivatives **8a**-**11a** and **8b**-**11b** were synthesized from natural alkaloid thebaine (**1**) as depicted in Scheme 1, and the detailed protocols are found in Supporting Information. (*R*)-(-)-2-Methoxy-11-hydroxyaporphine (**7a**) and (*R*)-(-)-*N*-propyl-2-methoxy-11-hydroxynoraporphine (**7b**) were used as key intermediates, and were obtained as we have detailed in previous publication.[29] Deprotonation of intermediate **7a** or **7b** with potassium carbonate in dry DMF followed by alkylation with 1-fluoro-2-iodoethane, 1-fluoro-3-iodopropane or 4-fluorobenzyl bromide provided aporphine derivatives **8a**-**10a** or **8b**-**10b**. In addition, esters **11a** and **11b** were prepared by facile coupling 4-fluorobenzoyl chloride with intermediates **7a** and **7b** in the presence of 4-(Dimethylamino)pyridine, respectively. Spectral data (^{1}H and ^{13}C NMR) and elemental analysis for these target compounds were consistent with their proposed structures.

Scheme 1. Synthesis of novel aporphine derivatives **8a**-**11a** and **8b**-**11b**[a]



[a] Reagents and conditions: (i): 1. DIAD, Benzene, reflux; 2. Pyridine hydrochloride, MeOH, rt, yield 90%. (ii): 1-Iodopropane, K_2CO_3, EtOH, reflux, yield 65%. (iii): L-Selectride, THF, reflux, yields 22-35%. (iv): *N*-Phenyl-bis(trifluoromethanesulfonimide), Et_3N, CH_2Cl_2, rt, yields 71-72%. (v): $MeSO_3H$, 90 °C, yields 55-59%. (vi): Pd/C, Mg, NH_4OAc, MeOH, rt, yields 62-87%. (vii): 1-Fluoro-2-iodoethane or 1-fluoro-3-iodopropane, K_2CO_3, DMF, 80 °C, yields 56-89%. (viii): 4-Fluorobenzyl bromide, K_2CO_3, DMF, 80 °C, yields 74-76%. (ix): 4-Fluorobenzoyl chloride, 4-(Dimethylamino)pyridine, Et_3N, THF, rt, yields 77-86%.

The binding affinities of aporphine derivatives **8a-11a** and **8b-11b** for the 5-HT_2

receptor subtypes were examined by the National Institute of Mental Health (NIMH) Psychoactive Drug Screening Program (PDSP), using competitive binding assays with membrane preparations obtained from transiently transfected HEK293 T cells (for 5-HT_{2A}) and stably transfected HEK293 (for 5-HT_{2B}), HEK293 T cells (for 5-HT_{2C}).[30] Data from these evaluations are presented in Table 1.

Among these newly synthesized aporphine derivatives, compounds **8a**, **9a**, **11a** and **11b** were identified as potential hits for 5-HT_{2C} receptor. Compounds **11a** and **11b** showed more significant binding property to 5-HT_{2C} receptor, with inhibitory constant (K_i) value of 32 nM and 24 nM, respectively. The other two aporphine derivatives, **8a** and **9a**, exhibited moderate binding affinity (K_i = 343 and 338 nM, respectively). These results indicated that the carbonyl moiety of C11 fluorinated benzoyl group in compounds **11a** and **11b** might form a strong interaction with the binding site of 5-HT_{2C} receptor, while *N*- substituent might not play a large role here. To our delight, compounds **11a** and **11b** also possessed good 5-HT_2 receptor subtype selectivity. Compound **11a** showed 14-fold selectivity over the 5-HT_{2A} receptor (K_i = 450 nM) and 3.2-fold selectivity versus the 5-HT_{2B} receptor (K_i = 102 nM). Compound **11b** showed 17-fold selectivity over the 5-HT_{2A} receptor (K_i = 414 nM) and 4.4-fold selectivity versus the 5-HT_{2B} receptor (K_i = 106 nM). Notably, in comparison with the reference drug lorcaserin, compounds **11a** and **11b** exhibited slightly lower affinity at the 5-HT_{2C} receptor [**11a** (K_i = 32 nM) and **11b** (K_i = 24 nM) vs. **Lorcaserin** (K_i = 15 nM)] and lower selectivity over the 5-HT_{2B} receptor, but

improved selectivity over the 5-HT_{2A} receptor.

Table 1. Binding affinities of aporphine derivatives **8a-11a** and **8b-11b** and the reference for the 5-HT_2 receptor subtypes[a]

Compound	K_i ± SEM (nM)[b]			Selectivity	
	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}	5-HT_{2A}/5-HT_{2C}	5-HT_{2B}/5-HT_{2C}
8a	1138 ± 205	552 ± 95	343 ± 41	3.3	1.6
9a	434 ± 62	422 ± 67	338 ± 36	1.3	1.2
10a	2313 ± 389	2681 ± 451	>10000	-	-
11a	450 ± 61	102 ± 13	32 ± 3	14	3.2
8b	>10000	3044 ± 589	>10000	-	-
9b	>10000	>10000	>10000	-	-
10b	>10000	>10000	>10000	-	-
11b	414 ± 51	106 ± 13	24 ± 3	17	4.4
Lorcaserin	112[c]	174[c]	15[c]	7.5	12

[a] All compounds were tested as their HCl salts. The following tritiated radioligands were used: [^{3}H]Ketanserin (5-HT_{2A}), [^{3}H]LSD (5-HT_{2B}), [^{3}H]Mesulergine (5-HT_{2C}). [b] The K_i values are means ± standard errors of 2-3 experiments. [c] Data from ref. 31.

To explain the higher binding affinity of compounds **11a** and **11b** at the 5-HT_{2C} receptor and explore a potential binding mode, molecular docking study on compound **11b** using the GoldSuite was performed based on 5-HT_{2C} receptor model which was disclosed very recently (PDB 6BQG).[32] As shown in Fig. 3, compound **11b** is

properly stabilized into the 5-HT_{2C} receptor binding site through a key salt-bridge between the positively charged nitrogen atom of compound **11b** and the carboxylate of D3.32, which is fully conserved in 5-HT and other monoamine receptors.[33] Compound **11b** also forms a H-bond interaction with N6.55 through the carbonyl oxygen atom of C11 fluorinated benzoyl group. This observation provided a potential explanation as to why compounds **11a** and **11b** possessed higher binding affinity than other newly synthesized aporphine derivatives, and supported our previous prediction. *N*-*n*-Propyl substituent of compound **11b** orients toward the vacant binding site of 5-HT_{2C} receptor, thereby explained the comparable binding affinity between compounds **11a** and **11b** at the 5-HT_{2C} receptor. In addition, the diphenyl moiety of aporphine structure may elicit π-alkyl interactions with some residues on TMH3, TMH6 and TMH7 (transmembrane helix) to further stabilize the ligand binding.

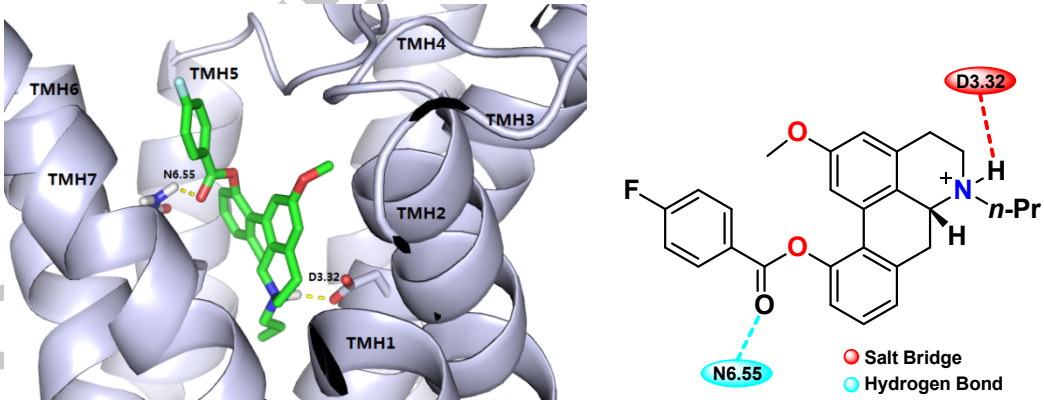


Fig. 3. Binding mode of compound **11b** in the binding site of 5-HT_{2C} receptor. **Left** is 3D view of the receptor-ligand interactions. Amino acid residues engaged in ligand binding are shown as sticks. Dotted yellow lines represent H-bonds with polar residues. **Right** is 2D view of the receptor-ligand interactions.

In summary, a series of novel aporphine derivatives were synthesized for initial screening at the 5-HT_2 receptor subtypes. Compounds **11a** and **11b** were identified as potent 5-HT_{2C} hit ligands with highly selectivity over other 5-HT_2 receptor subtypes. Molecular docking study revealed that compounds **11a** and **11b** formed two key interactions with the binding site of 5-HT_{2C} receptor, including a salt-bridge to D3.32 and a H-bond interaction with N6.55, which result in higher binding affinity than other newly synthesized aporphine derivatives. Compounds **11a** and **11b** are useful starting points for further SAR exploration and optimization studies. We are continuing in this vein and will report our findings in due course.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online

version.

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