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HTS and Rational Drug Design to Generate a Class of 5-HT_{2C}-Selective Ligands for Possible Use in Schizophrenia.

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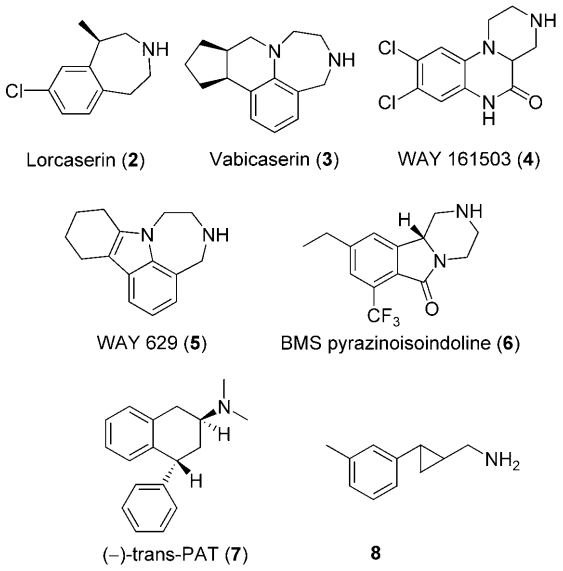
The 5-hydroxytryptamine (**1**, 5-HT) 2C receptor (5-HT_{2C}), a prominent central serotonin receptor subtype, is widely distributed throughout the central nervous system (CNS) and is thought to play a role in regulating a wide variety of behavioral processes, such as mood, appetite, and sexual behavior.[1–4] The 5-HT_{2A} receptor mediates the hallucinogenic activity of drugs, such as lysergic acid diethylamide (LSD), and is a major target for treating schizophrenia, insomnia and other disorders.[5–8] The 5-HT_{2B} receptor mediates the potentially lethal valvulopathic side effects of several compounds that were used as prescription drugs.[9,10]

5-HT_{2C} agonists have demonstrated efficacy in preclinical models of depression, obesity, addiction, and psychosis.[11–13] Thus, targeting the 5-HT_{2C} receptor appears to offer a promising means for developing novel therapeutics for the treatment of CNS-related disorders. However, as this receptor is homologous to the two other family members, 5-HT_{2A} and 5-HT_{2B},[14] it is essential that 5-HT_{2C} agonists being developed for clinical use show little, if any, activity at these subtypes.[15] To date, several 5-HT_{2C} agonists have shown efficacy in preclinical animal models (**2**–**8**),[16–18] and are currently undergoing human trials.[16] In particular, one of the most advanced 5-HT_{2C} ligands is lorcaserin (**2**), which is being developed by Arena Pharmaceuticals and which has been demonstrated in two phase III trials to be an orally active, antiobesity medication.[19]

Based upon the identification of tranylcypromine as the initial hit from an HTS campaign employing a library of FDA-approved drugs, we undertook a structural optimization campaign that led to a potent, but moderately selective, agonist (**8**) with 120- and 14-fold selectivity over 5-HT_{2A} and 5-HT_{2B}, respectively (EC_{50} = 585, 65, and 4.8 nM at the 2A, 2B, and 2C subtypes, respectively). Compound **8** (10–-60 mg kg^{-1}) was also demonstrated to exhibit moderate antidepressant-like effects in a commonly used behavioral assay.[18]

However, because compound **8** fails to exhibit sufficient selectivity over the 5-HT_{2B} receptor, further optimization was required to identify a potential clinical candidate. Currently, the degree of selectivity that is actually needed to avoid side effects is unknown; however, this is a question that can ultimately be addressed only by studies in humans. Thus, we explored additional, selected modifications of these *trans*-2-phenylcyclopropylmethylamine analogues in order to improve the subtype selectivity. Our efforts to modify the 5-HT_{2C} agonist **8** led to the discovery of several new drug candidates with increased subtype selectivity, including dual 5-HT_{2B} antagonism/5-HT_{2C} agonism in the functional assays, and which were thus found suitable for in vivo testing as detailed herein.



By stepwise structural modifications of the *trans*-(2-arylcyclopropyl)methylamine aromatic moiety, we found that potent, 5-HT_{2C}-selective compounds could be produced using 5-hydroxy- or 5-fluoro-substituted systems with a medium sized 2-alkyloxy group. The 2-cyclopropylmethyloxy-5-fluoro-substituted derivative **12** was synthesized according to the steps shown in Scheme 1. Thus, the starting compound **9** was prepared by employing a standard sequence of reactions as previously reported.[18] The amino group of the phenolic derivative **9** was protected using di-*tert*-butyl dicarbonate (Boc_2O). The *N*-Boc-protected derivative **10** was then alkylated with cyclopropylmethyl bromide followed by subsequent deprotection to provide the racemic compound **12**.

To synthesize the 2-cyclopropylmethyloxy-5-hydroxy substituted derivative **18**, the 2-cyclopropylmethyloxy intermediate **16** was prepared in a straightforward manner through a sequence of selective protection and alkylation steps.[20] Next, the pivaloyl and Boc protecting groups were removed sequentially to afford the final product **18** (Scheme 2).

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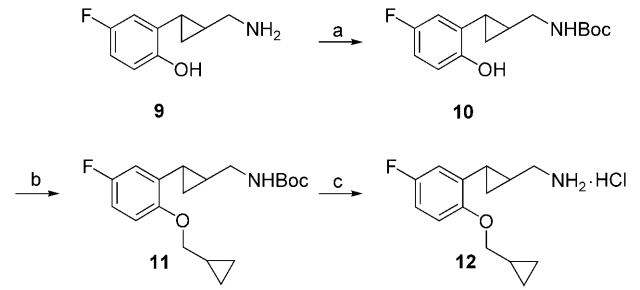
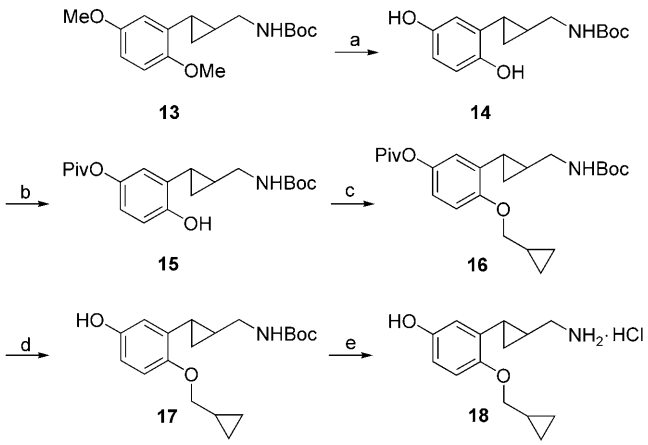


Scheme 1. *Reagents and conditions*: a) Boc_2O, Et_3N, CH_2Cl_2, 0 °C→RT, 5 h; b) (bromomethyl)cyclopropane, K_2CO_3, DMF, 60 °C, 20 h; c) 2 M HCl, RT, 48 h.

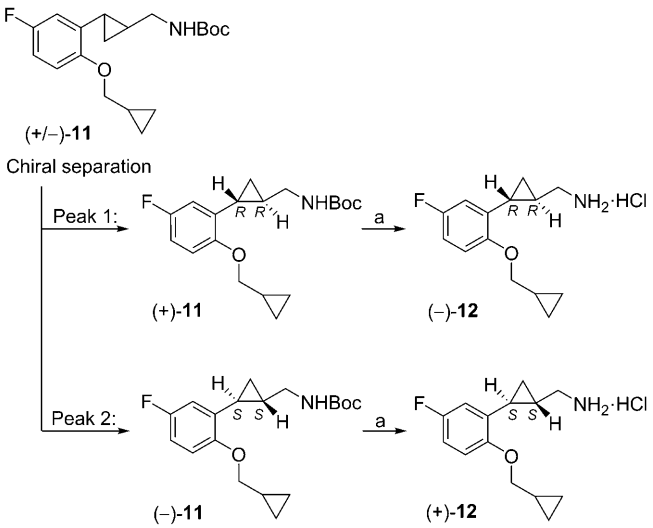


Scheme 2. *Reagents and conditions*: a) 1. BBr_3, CH_2Cl_2, −78 °C→RT, 6 h; 2. Boc_2O, Et_3N, CH_2Cl_2, 0 °C→RT, 1 h; b) Piv-Cl, CH_2Cl_2, Et_3N, 0 °C→RT, 6 h; c) (bromomethyl)cyclopropane, K_2CO_3, DMF, 60 °C, 20 h; d) NaO*t*Bu, MeOH, RT, 1 h; e) 2 M HCl, RT, 48 h.

To prepare the optically pure enantiomers of **12** (Scheme 3), we first carried out a chiral separation of the *N*-Boc-protected fluoro derivative **11** using a Chiralpack AD column. Under isocratic conditions (4% isopropanol in hexane), the individual enantiomers could be conveniently separated in pure form (>99%). Due to the high resolution, stacked injections could be employed in order to increase throughput. The choice of intermediate **11** for the chiral resolution was based on the ease of the separation and the ready cleavage of the resulting enantiomers under acidic conditions.

The resulting enantiomers (+)- and (−)-**11** were then converted individually to (−)- and (+)-*trans*-[2-(2-cyclopropylmethyloxy-5-fluorophenyl)cyclopropyl]methylamine hydrochlorides [(−)-**12** and (+)-**12**], respectively, using the same method as described above for the racemate. For preparation of the pure enantiomers of compound **18**, intermediate **17** was used for the chiral separation.

The functional activity of these two sets of compounds was determined by measuring $G_{\alpha q}$-mediated intracellular calcium mobilization in HEK-293 cells stably expressing the human 5-HT_{2A}, human 5-HT_{2B}, and human 5-HT_{2C} (INI) receptors.[21] The results are summarized in Table 1. In the first round of functional assays, racemic **12** was found not to activate either the 5-HT_{2A} or 5-HT_{2B} receptors and to have an EC_{50} value of 19 nM



Scheme 3. *Reagents and conditions*: a) 2 M HCl, RT, 48 h. Chiral separation was carried out on a Chiralpak AD column (30×250 mm, DAICEL); mobile phase: hexane/*i*PrOH, 4% isocratic. Optical purity: (+)-**11**: D = +47.2°; Chiral HPLC purity: 13.2 min, >99%; (−)-**11**: D = −45.9°; Chiral HPLC purity: 16.8 min, >99%.

at the 5-HT_{2C} receptor. The more active enantiomer (+)-**12** also showed the expected selectivity profile in these functional assays. In contrast, the less active isomer (−)-**12** had an EC_{50} value of 918 nM in the 5-HT_{2C} assay. For comparison, we also tested the 5-HT_{2C} ligand **2**, which is in phase III clinical trials for obesity. In our hands, this compound has a low nanomolar potency at the 5-HT_{2C} receptor, however, it is also fairly active at the 5-HT_{2B} subtype, with an EC_{50} value of 85 nM and an E_{max} value of 93%. As such, there may be some risk for possible valvulopathic side effects induced by this drug. Compound (+)-**12** has an even lower selectivity margin towards the $5HT_{2B}$-subtype, but due to its low maximal activity (E_{max} = 21%), we believe this will be a very minor concern, especially in view of the 2B isoform antagonist activity described below. Three other known 5-HT_{2C} agonists, vabicaserin (**3**) and the Way compounds **4** and **5**, along with 5-HT (**1**) are also included in Table 1 for reference purposes.

In order to further characterize the pharmacology of the lead compounds, both (+)-**12** and (+)-**18**, which show minimal 5-HT_{2B} activation, were tested for their ability to function as 5-HT_{2B} antagonists. Compounds (+)-**12** and (+)-**18** were found to shift the concentration curve of 5-HT in calcium flux assays to the right, without depressing the maximal 5-HT response, indicating fast binding kinetics (Figure 1). Schild analyses yielded pA2 (±SEM) values of 5.50±0.06, 5.79±0.07, and 5.92±0.02, respectively (n = 3). These results show that the compounds tested act as moderately potent, full antagonists at the 5-HT_{2B} receptor.

On the basis of their promising in vitro pharmacology, the active enantiomers (+)-**12** and (+)-**18** were selected for further in vivo studies. Specifically, we examined the ability of these compounds to normalize disrupted prepulse inhibition (PPI) in animals treated with phencyclidine (PCP; an NMDA receptor antagonist).[22] The ability to normalize the effects of PCP-

Table 1. Functional activity and selectivity of racemic **12** and **18**, their enantiomers, and comparison compounds at the Human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors in calcium flux assays using stably transfected HEK-293 cells.

	5-HT_{2A}			5-HT_{2B}			5-HT_{2C}			Selectivity	
Compound[a]	EC_{50} [nM]	E_{max}[b] [%]	n[c]	EC_{50} [nM]	E_{max}[b] [%]	n[c]	EC_{50} [nM]	E_{max}[b] [%]	n[c]	2A/2C	2B/2C
First round											
1 (5-HT)	6.9±0.42	100	15	0.7±0.02	100	16	0.1±0.01	100	16	63	6.7
(+/-)-**12**	NA	7±1.8	3	NA	6±0.6	3	19±3.5	68±5.2	3	–	–
(+/-)-**18**	761±160	11±1.9	3	NA	2±0.9	3	9.9±1.7	68±4.5	3	77	–
2 (lorcaserin)	264±31	24±0.9	6	85±7.0	93±1.2	6	2.1±0.29	99±1.0	7	123	40
3 (vabicaserin)	NA	2±0.1	6	NA	5±1.0	6	6.0±1.0	95±0.8	7	–	–
4 (WAY-161503)	76±9.1	80±1.0	3	15±1.6	92±1.3	3	1.1±0.16	97±1.6	4	70	14
5 (WAY 629)	NA	1±0.2	6	NA	6±1.3	6	286±40	80±1.1	7	–	–
Second round											
1 (5-HT)	6.1±0.38	100	9	0.8±0.08	100	9	0.1±0.0	100	9	62	7.7
(+)-**12**	894±91	29±1.6	3	289±34	21±4.9	3	21±2.2	71±4.4	3	42	14
(−)-**12**	NA	0±0.0	3	NA	4±2.7	3	918±83	35±2.4	3	–	–
(+)-**18**	372±131	18±3.0	3	NA	6±2.8	3	9.3±0.05	70±5.4	3	40	–
(−)-**18**	NA	3±0.6	3	NA	0±0.5	3	361±21	51±2.4	3	–	–
2 (lorcaserin)	136±24	31±0.5	3	50±10	86±2.3	3	1.7±0.09	94±0.8	3	79	29
3 (vabicaserin)	NA	7±0.1	3	47±6.4	15±3.3	3	8.0±1.6	88±1.8	3	–	5.8
4 (WAY-161503)	61±8.3	79±4.5	3	16±3.2	86±4.7	3	1.5±0.18	94±3.6	3	40	11
5 (WAY 629)	NA	7±2.0	3	> 5 μM	17±2.7	3	451±52	82±3.5	3	–	–

[a] Tested in two independent screening campaigns using different cell lines/passages; direct comparisons of the potencies and efficacies are only valid within the bounds of each particular table section. [b] Percent of maximal activation by 5-HT (E_{max}); activation at 10 μM for compounds without EC_{50} value; values are given ±SEM (standard error of the mean). [c] n: number of concentration curves from ≥2 (typically ≥3) independent experiments; values are given ±SEM. NA: not applicable (E_{max} ≤ 12%). In contrast to binding affinities, the potencies in functional assays can vary strongly depending on cell type and receptor-expression level.

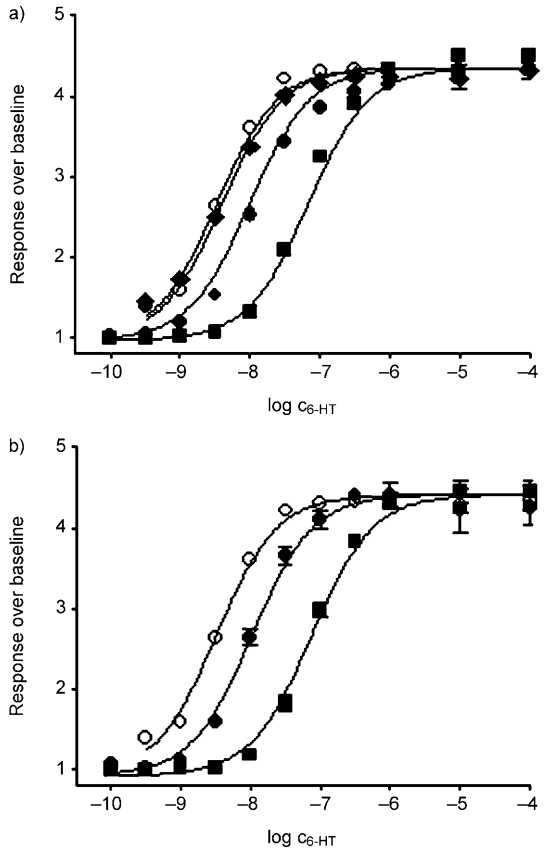


Figure 1. 5-HT_{2B} antagonism (Schild plots) of compounds a) (+)-**18** (○, no test compound; ◆, 316 nM; ●, 3.16 μM; ■, 31.6 μM) and b) (+)-**12** (○, no test compound; ●, 3.16 μM; ■, 31.6 μM). pA2 (±SEM): 5.79±0.07 and 5.92±0.02; n=3.

induced disruption of PPI in mice is a well accepted model for measuring atypical antipsychotic activity,[23,24] and it has been used previously to characterize the antipsychotic activity of 5-HT_{2C} agonists.[25] Previously, we successfully used an identical PPI testing paradigm to demonstrate clozapine, a classic atypical antipsychotic, robustly normalizes PCP-disrupted PPI.[26] We tested the ability of compounds (+)-**12** and (+)-**18**, as well as the reference compound **3**, to normalize PCP effects on PPI (Figure 2). As is apparent from the accompanying figures, both (+)-**12** and (+)-**18** are able to normalize PCP-disrupted PPI at doses of 10 mg kg^{-1} (and are comparable in activity to **3**), while at the 5 mg kg^{-1} dose level, (+)-**12** and **3** are still effective.

Because of its robust activity at normalizing PCP-disrupted PPI, we carried out cytochrome P450 screening, metabolic stability studies, and hERG assays on (+)-**12** in order to qualify it as a possible candidate for further development. In the recombinant CYP inhibition test, (+)-**12** showed relatively low inhibition against CYP2C9 (19.6%), CYP2D6 (27.0%), and CYP3 A4 (21.7% and 25.1%) using midazolam and testosterone as the substrates, respectively, at 10 μM (Table 2). Furthermore, compound (+)-**12** had an acceptable microsomal stability in a 1 h assay (90.5% left in human microsomes and 66% in rat, respectively) compared with the control drug, verapamil (39.6% using human microsomes). Additionally, no hERG inhibition was detected for (+)-**12**, (−)-**12**, or **18**, nor for the comparison compounds **2**, **3**, and **5**. However, compound **4** caused very modest inhibition (3.6 mM, 97%), and the standard cisapride gave full inhibition (35 nM, 100%).

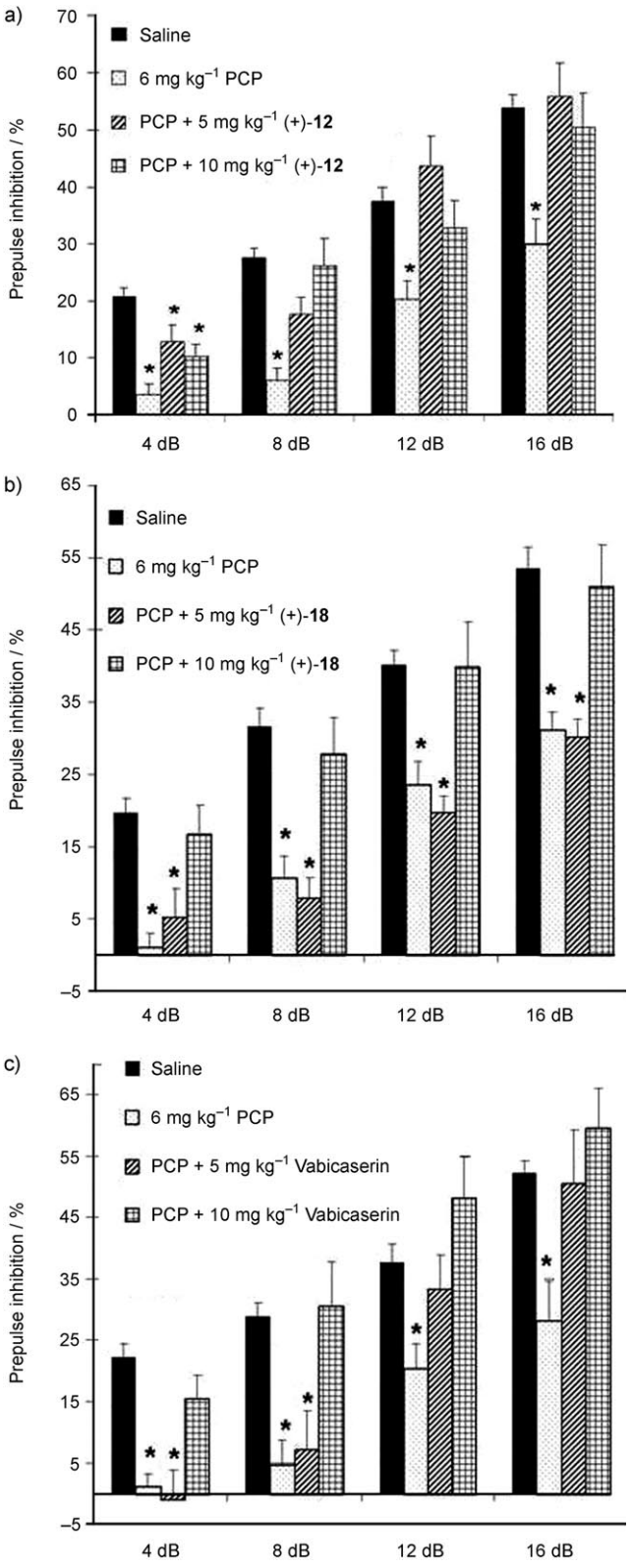


Figure 2. Compounds (+)-**12** and (+)-**18** normalize PCP-disrupted PPI in mice. Mice were administered (ip) with saline or 6 mg kg^{-1} PCP and immediately tested for PPI of the acoustic startle response. The pretreatments of mice (30 min) with a) (+)-**12** or b) (+)-**18** effectively normalize PCP-disruption of PPI. c) Compound **3**, a positive reference control compound, also normalizes PCP-disrupted PPI. *, $p < 0.05$ versus saline treated animals. Data are the mean ± SEM. $n = 9$, 10 or 7 animals in each testing group for (+)-**12**, (+)-**18** or **3**, respectively.

Table 2. Cytochrome P450 inhibition profile of compound (+)-**12**.

CYP[a]	Substrate	Test inhibitors	Inhibition[b] [%]
2C9	tolbutamide	sulfaphenazole	94.7
		(+)-**12**	19.6
2D6	dextrometorphan	quinidine	98.7
		(+)-**12**	27.0
3A4	midazolam	ketokonazole	99.8
		(+)-**12**	21.7
	testosterone	ketokonazole	99.7
		(+)-**12**	25.1

[a] Cytochrome P450 enzyme. [b] Percent inhibition was measured at 10 μM.

In conclusion, our optimization efforts led to the discovery of the highly selective 5-HT$_{2C}$ agonists (±)-**12** and (±)-**18**, and to the identification of (+)-**12** and (+)-**18** as the more active enantiomers. Compounds (+)-**12** and (+)-**18** were as effective as the known reference compound **3** in the PCP-PPI animal model of antipsychotic drug activity. Compound **3** was recently in phase II clinical trials for the treatment of acute exacerbations of schizophrenia. Compound (+)-**12** has an acceptable DMPK profile, while showing no adverse side effects in animals. Compound (+)-**12** represents a structurally simple 5-HT$_{2C}$ partial agonist of excellent potency and high selectivity. The structural relationship of **12** with the marketed monamine oxidase inhibitor tranylcypromine is also noteworthy, and when combined with the information now in hand, bodes well for the further development of this class of molecules. The study of (+)-**12** in other animal disease models, including cocaine addiction, is underway.

Experimental Section

Full experimental protocols can be found in the Supporting Information.

All experiments were conducted in accordance with National Institutes of Health (USA) guidelines and under an approved protocol from the Institutional Animal Care and Use Committee at the University of North Carolina (USA).

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Keywords: 5-HT$_{2C}$ · CNS · drug discovery · medicinal chemistry · schizophrenia

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