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Asymmetric total synthesis and identification of tetrahydroprotoberberine derivatives as new antipsychotic agents possessing a dopamine D_1, D_2 and serotonin 5-HT_{1A} multi-action profile

Haifeng Sun [a,†], Liyuan Zhu [b,†], Huicui Yang [c], Wangke Qian [a], Lin Guo [b], Shengbin Zhou [a], Bo Gao [c], Zeng Li [a], Yu Zhou [a], Hualiang Jiang [a], Kaixian Chen [a], Xuechu Zhen [b,c,*], Hong Liu [a,*]

[a] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Shanghai 201203, China
[b] Neuropharmacological Laboratory, Shanghai Institute of Materia Medica (SIMM), Chinese Academy of Sciences, Shanghai 201203, China
[c] Department of Pharmacology, Soochow University College of Pharmaceutical Sciences, Suzhou, China

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ABSTRACT

An effective and rapid method for the microwave-assisted preparation of the key intermediate for the total synthesis of tetrahydroprotoberberines (THPBs) including *l*-stepholidine (*l*-SPD) was developed. Thirty-one THPB derivatives with diverse substituents on A and D ring were synthesized, and their binding affinity to dopamine D_1, D_2 and serotonin 5-HT_{1A} and 5-HT_{2A} receptors were determined. Compounds **18k** and **18m** were identified as partial agonists at the D_1 receptor with K_i values of 50 and 6.3 nM, while both compounds act as D_2 receptor antagonists (K_i = 305 and 145 nM, respectively) and 5-HT_{1A} receptor full agonists (K_i = 149 and 908 nM, respectively). These two THPBs compounds exerted antipsychotic actions in animal models. Further electrophysiological studies employing single-unit recording in intact animals demonstrated that **18k**-excited dopaminergic (DA) neurons are associated with its 5-HT_{1A} receptor agonistic activity. These results suggest that these two compounds targeted to multiple neurotransmitter receptors may present novel lead drugs with new pharmacological profiles for the treatment of schizophrenia.

1. Introduction

Schizophrenia, a chronic and devastating psychotic disorder, is clinically characterized by the presentation of positive symptoms such as auditory hallucinations, disorganized thoughts, delusions, and irrational fears, and negative symptoms including social withdrawal, diminished affect, poverty of speech, lack of energy, and the inability to experience pleasure, as well as cognitive impairments.[1–3] It is believed that abnormal neurotransmission, particularly an imbalance of the dopamine system (mesolimbic hyperactivity and mesocortical hypoactivity) is involved in the pathophysiology of schizophrenia.[3,4] Indeed, almost all currently available antipsychotic drugs behave as dopamine D_2 receptor antagonists. However, dopamine D_2 receptor-directed antipsychotic drug therapy is dampened by the lack of efficacies in negative symptoms and cognitive function. Improvements in negative symptoms and cognitive function remain the main challenge in antipsychotic drug development.

Accumulating evidence suggests that dysfunction of dopamine D_1 receptors in the medial prefrontal cortex (mPFC) may be responsible for the negative symptoms and cognitive deficits of schizophrenia.[5,6] Impaired D_1 receptor function has been documented in schizophrenic patients and in experimental animal models.[7,8] Application of a D_1 receptor agonist has been found to improve the negative symptoms and working memory.[9] Unfortunately, currently available antipsychotic drugs do not bear dual D_1 agonistic and D_2 antagonistic functions.

In addition to the dopamine (DA) system, the limbic forebrain structures are also innervated by serotonin (5-HT) neurons. The influence of the 5-HT system on midbrain DA neurons has been demonstrated by a variety of experimental approaches.[10–13] Abnormal serotonin transmission is an important tenet of the serotonergic/dopaminergic hypothesis of schizophrenia which contributes to the pathology of the disease.[14–22] Atypical antipsychotic medications such as clozapine, risperidone solanzapine, ziprasidone, and aripiprazole (Fig. 1), have been shown to produce less severe side-effects whilst effectively relieving positive symptoms.[1,23] The relative advantage of these drugs is thought to be associated with their modulation of 5-HT receptors, particularly the 5-HT_{2A}

* Corresponding authors. Tel.: +86 512 6588 1160; fax: +86 512 6588 0369 (X.Z.); tel./fax: +86 21 5080 7042 (H.L.).
E-mail addresses: zhenxuechu@suda.edu.cn (X. Zhen), hliu@mail.shcnc.ac.cn (H. Liu).
† These two authors contributed equally to this work.

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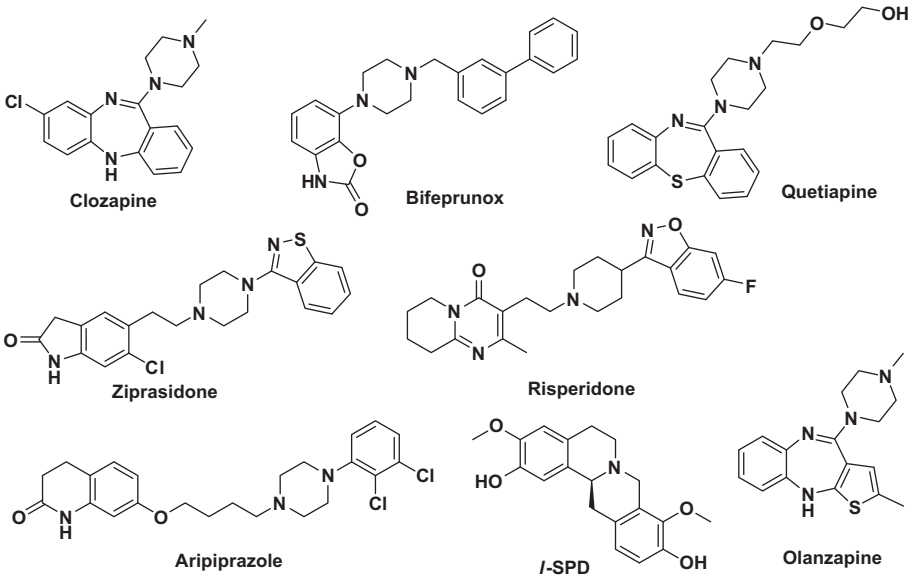


Figure 1. Representative atypical antipsychotic agents.

receptor.[24,25] In addition, the 5-HT_{1A} receptor is believed to control the output of cortical glutamatergic neurons in prefrontal cortex (PFC) and regulate NMDA receptor channels through a microtubule-dependent mechanism.[26–28] Moreover, 5-HT_{1A} stimulation has been found to improve the acute extrapyramidal syndrome (EPS) liability profile,[29] while drugs such as aripiprazole with 5-HT_{1A} receptor agonistic activity have been shown to have a superior effect in the treatment of negative symptoms and cognition impairments in schizophrenic patients.[30–35]

In this regard, development of drugs which are D_1 receptor and 5-HT_{1A} receptor agonists or partial agonists as well as D_2 receptor antagonists may provide a new approach for antipsychotic drug discovery. *l*-Stepholidine (*l*-SPD) (Fig. 1), a tetrahydroberberine (THPB) alkaloid isolated from the Chinese herb *Stephania intermedia*, exhibits a unique pharmacological profile; it elicits dual dopamine receptor action (D_1 agonistic and D_2 antagonistic) while acting as a 5-HT_{1A} receptor partial agonist in vitro and in vivo.[29,36–38] However, its poor physicochemical properties and low oral bioavailability limits the application of *l*-SPD, in addition to its relatively weak agonistic activity on the 5-HT_{1A} receptor. Although it is believed that *l*-SPD present a potential novel category of antipsychotic drug acting as neurotransmitter stabilizer, a detail structure–activity study of *l*-SPD has not been done. We designed and synthesized a series of novel THPBs by introducing various substituents such as methyl, methyoxyl, hydroxymethyl and methylenedioxy groups at different positions on the THPB pharmacophore as initial effort to find better drug candidate. In this paper, we report the synthesis of these novel THPBs compounds and their pharmacological evaluation at dopamine (D_1 and D_2), and serotonin (5-HT_{1A} and 5-HT_{2A}) receptors.

2. Chemistry

2.1. Design of THPBs compounds

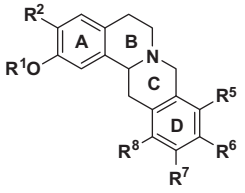
We presumed that the shortcoming of *l*-SPD most likely resulted from the two phenolic hydroxyl groups on the A ring and D ring. Therefore, further structural modification would be necessary to explore natural products with new pharmacological profiles. We designed the THPBs compounds as follows. Firstly, we preserved the hydroxyl group at C2 and introduced diverse substituents the on D ring (**18g**–**18j** and **24a**–**24e**, Table 1); secondly, the hydroxyl group at C10 was preserved and different alkoxy groups were introduced on the A ring (**18k**–**18r**, Table 1); thirdly, C3 or C9 was substituted with a hydroxyl group and C2 or C10 was substituted with an alkoxy groups (**18s–18z** and **17a**–**17d**, Table 1); finally, we investigated compounds devoid of hydroxyl groups and the effect of the C14-configuration.

2.2. Synthesis of target compounds

Derivatives (**17a**–**18z**, Table 1) including *l*-SPD, which required common intermediates **4** and **10**, were synthesized efficiently according to the procedures outlined in Schemes 1 and 2. First, phenylethylamines **4** were prepared from aldehyde **1** using a procedure reported previously.[39] To prepare the intermediates **10**, methyl 2-(4-hydroxyphenyl) acetate was selected as the starting material, and **6** was easily accessible by the bromination of **5** with bromine followed by protection with benzyl bromide or methyl iodide in acetone to give the intermediate **7**. Although the synthesis of phenols from halogen benzene has been previously reported, some methods take a long time to reach completion and produce low yields while others require iodobenzene with electron-withdrawing substituent.[40–42] Therefore, compound **8** was obtained under microwave-assisted condition without ligand. Phenylacetic acid **8** was subjected to hydroxymethylation in the presence of phenylboric acid and *para*-formaldehyde, yielding borate, which may be hydrolyzed without isolation of the lactone **9**.[43,44] Finally, the lactone **9** was methylated with iodomethane to yield the required intermediates **10**.

Condensation of lactone **10** with several phenylethylamines proceeded in ethanol afforded **11** in high yields. The benzyl alcohol group was converted to its acetate **12** using acetyl chloride and pyridine. The imine **13** was obtained in excellent yields by promotion with phosphoryl trichloride in acetonitrile according to the Bischler–Napieralski reaction. Asymmetric transfer hydrogenation of imines with formic acid/triethylamine catalyzed by a suitably designed chiral Ru complex developed by Noyori and co-workers[45] generated the amines **14** and their enantiomers asymmetrically in dimethylformamide (DMF) at room temperature; the corresponding amines were hydrolyzed to benzyl alcohol with sodium hydroxide without purification. Closure of the C ring was

Table 1
Binding affinity of THPBs for D_1, D_2, 5-HT_{1A} and 5-HT_{2A} receptors from HEK293 or CHO cells[a]



No.	R^1	R^2	R^5	R^6	R^7	R^8	Config	D_1		D_2		5-HT_{1A}		5-HT_{2A}	
							C-14[b]	IR[c] (%)	K_i (nM)	IR[c] (%)	K_i (nM)	IR[c] (%)	K_i (nM)	IR[c] (%)	K_i (nM)
17a	CH_3	CH_3O	CH_3O	CH_3O	H	H	S	96.9	231 ± 41	85.4	>5000	84.9	>5000	33.0	—
17b	CH_3	CH_3O	CH_3O	CH_3O	H	H	R	39.8	—	20.5	—	30.4	—	29.7	—
17c	CH_2O		CH_3O	CH_3O	H	H	S	98.3	66 ± 18	88.7	119 ± 2	84.4	>5000	46.6	—
17d	CH_2O		CH_3O	CH_3O	H	H	R	75.5	>5000	26.6	—	34.5	—	18.3	—
18e[d]	H	CH_3O	CH_3O	OH	H	H	S	99.8	3.4 ± 0.7	97.5	11 ± 1	89.3	>5000	45.6	—
18f	H	CH_3O	CH_3O	OH	H	H	R	94.4	135 ± 15	53.7	—	53.6	—	16.4	—
18g	H	CH_3O	CH_3O	CH_3O	H	H	S	99.2	4.5 ± 0.4	90.9	91 ± 9	71.1	—	40.5	—
18h	H	CH_3O	CH_3O	CH_3O	H	H	R	99.5	6.6 ± 0.8	90.5	105 ± 3	64.2	—	40.6	—
18i	H	CH_3O	OH	CH_3O	H	H	S	99.1	22 ± 4	93.4	214 ± 2	14.2	—	20.9	—
18j	H	CH_3O	OH	CH_3O	H	H	R	84.5	529 ± 64	31.8	—	2.0	—	26.3	—
24a	H	CH_3O	H	H	CH_3O	H	S	97.5	35.8 ± 2.6	90.0	347 ± 3	65.6	—	68.7	—
24b	H	CH_3O	CH_3O	H	CH_3O	H	S	99.8	2.5 ± 0.2	83.8	—	56.9	—	32.4	—
24c	H	CH_3O	CH_3	H	CH_3	H	S	99.0	28.9 ± 2.7	90.1	161 ± 2	74.6	—	27.1	—
24d	H	CH_3O	CH_3O	H	CH_3O	CH_2OH	S	104	17.3 ± 0.5	94.8	147 ± 1	80.9	>5000	18.4	—
24e	H	CH_3O	H	OCH_2O		H	S	99.6	4.2 ± 0.1	100	32 ± 3	61.0	—	49.9	—
18k	CH_3	CH_3O	CH_3O	OH	H	H	S	98.8	50 ± 10	88.8	305 ± 4	97.4	149 ± 4	68.8	—
18l	CH_3	CH_3O	CH_3O	OH	H	H	R	54.4	—	3.4	—	65.5	—	27.9	—
18m	CH_2O		CH_3O	OH	H	H	S	99.7	6.3 ± 0.4	92.8	145 ± 5	95.7	908 ± 10	73.6	—
18n	CH_2O		CH_3O	OH	H	H	R	95.8	565 ± 12	40.5	—	63.6	—	25.3	—
18o	CH_2CH_2O		CH_3O	OH	H	H	S	22.6	—	73.1	—	90.8	208 ± 45	97.3	363 ± 4
18p	CH_2CH_2		CH_3O	OH	H	H	S	99.3	7.5 ± 1.0	63.9	—	79.5	>5000	91.7	480 ± 6
18q	CH_3	OH	CH_3O	OH	H	H	S	98.3	200 ± 25	94.2	113 ± 2	76.7	>5000	84.0	>5000
18r	CH_3	OH	CH_3O	OH	H	H	R	67.1	—	27.7	—	13.2	—	13.9	—
18s	CH_3	CH_3O	OH	CH_3O	H	H	S	87.7	443 ± 42	22.7	—	30.5	—	20.0	—
18t	CH_3	CH_3O	OH	CH_3O	H	H	R	35.9	—	1.5	—	6.2	—	19.3	—
18u	CH_2O		OH	CH_3O	H	H	S	98.7	60 ± 8.4	78.0	>5000	82.8	>5000	49.1	
18v	CH_2O		OH	CH_3O	H	H	R	78.9	>5000	20.4	—	25.5	—	10.9	
18w	CH_3	OH	CH_3O	CH_3O	H	H	S	84.1	310 ± 61	63.2%	—	50.5	—	38.0	—
18x	CH_3	OH	CH_3O	CH_3O	H	H	R	88.8	1189 ± 23	71.5	—	55.4	—	40.4	—
18y	CH_3	OH	OH	CH_3O	H	H	S	95.2%	530 ± 74	79.8	>5000	60.5	—	59.1	—
18z	CH_3	OH	OH	CH_3O	H	H	R	49.4	—	8.16	—	31.5	—	36.0	—
SCH-23390								100	1.2 ± 0.4	—	—	—	—	—	—
Spiperone								—	—	100	1.2 ± 0.3	—	—	100	2.9 ± 0.1
5-HT								—	—	—	—	100	0.9 ± 0.1	—	—

[a] The initial screening was carried out at a concentration of 10 μM for each compound and K_is were measured for compounds that inhibited binding by more than 80% for DA receptors or 90% for the serotonin receptors.
[b] Config C-14 represents the configuration of C-14.
[c] IR represents inhibition ratio.
[d] Compound **18e** is *l*-SPD; dash line denote that no experiment was conducted.

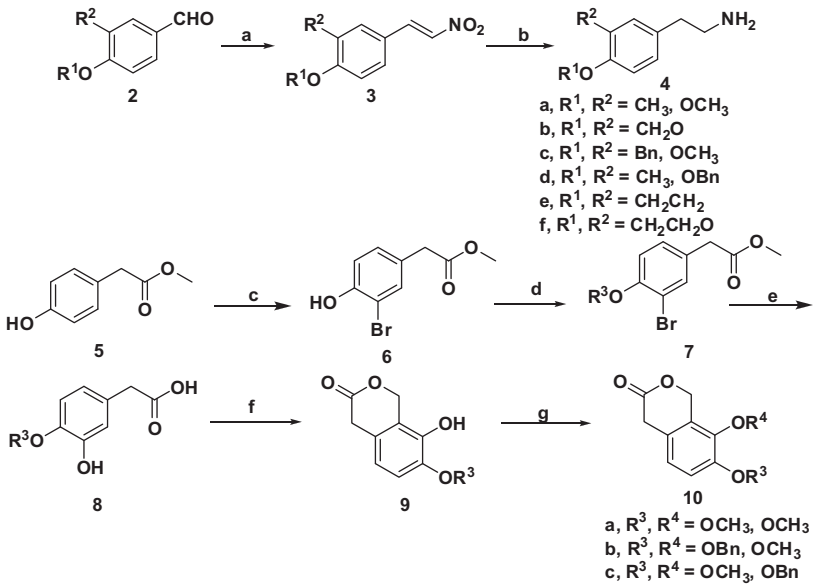
accomplished in one pot in the presence of thionyl chloride and aqueous $NaHCO_3$ in good yields. The deprotection was carried out by refluxing **17** in concentrated hydrochloric acid and ethanol, when **18** was collected in moderate isolated yield and good enantiomeric excess (ee). All compounds (**17a**–**18z**) prepared by these general methods are listed in Table 1.

Compounds **24a**–**24e** (Table 1) were synthesized from **3c** and **19a–19c and 19e** in five steps (Scheme 3). Condensation of intermediates **3c** with commercially available 2-phenylacetic acids **19** generated amides **20** in good yields. Using the Bischler–Napieralski reaction, treatment of the amides **20** with phosphoryl trichloride ($POCl_3$) gave access to imines **21** in excellent yields. Asymmetric hydrogenation of **21** catalyzed by a chiral Ru-(II) complex (Noyori's catalyst) afforded chiral amines **22**. Cyclization of amines **22** via the Pictect–Spengler reaction provided products **23a–c** and **23e** in good isolated yields and excellent enantiomeric excess. Products **24a–e** were obtained by deprotection of compounds **23**.

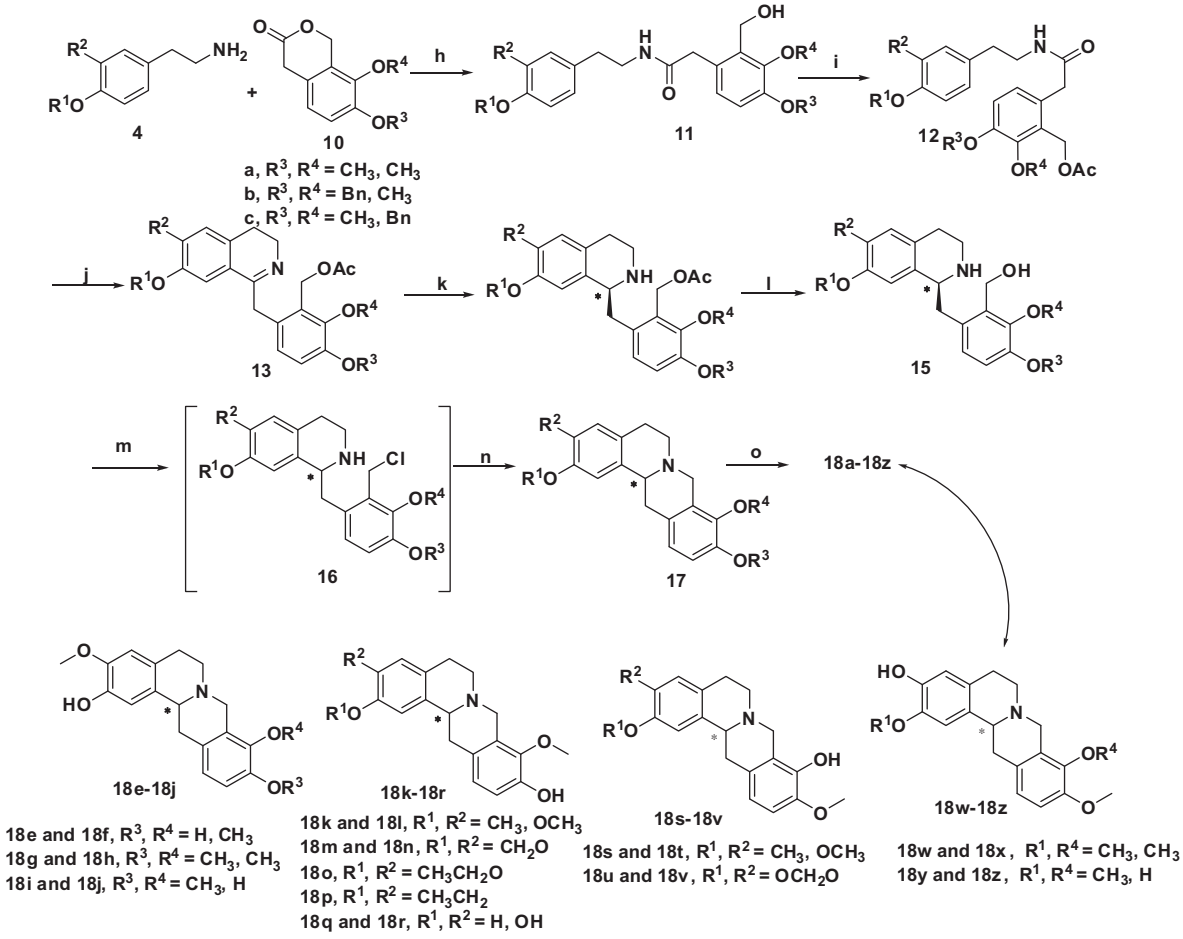
3. Results and discussion

3.1. Chemistry

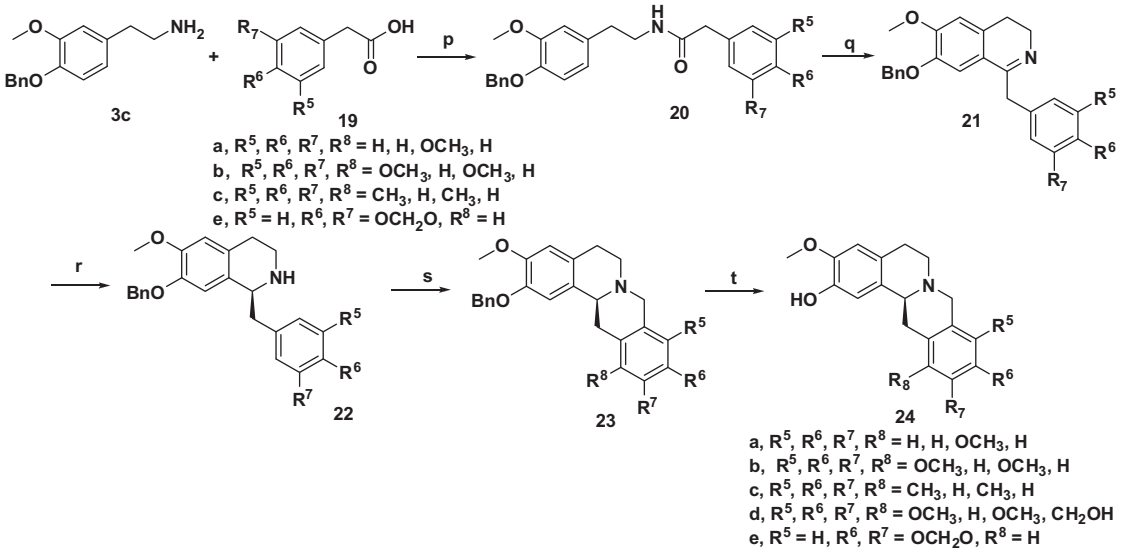
Although several racemic syntheses of tetrahydroberberines (THPBs) with substituents at C9 and C10 have been reported along with a few asymmetric syntheses, there remains a lack of general methods to prepare THPBs in enantiopure form with the desired ring substitution patterns.[46–51] Although the racemic synthesis of (±)-SPD was reported by Chiang and Brochmann-Hassen in 1977,[52] the total synthesis of *l*-SPD with good yield and high enantiomeric excess was only recently reported.[53] We developed an effective and rapid microwave-assisted method to prepare the key intermediate of THPBs, which was then applied in the total synthesis of THPBs including *l*-SPD. The chemical structures of 31 designed compounds (**17a–18z** and **24a–24e**) are shown in Table 1. These compounds were synthesized through the routes outlined in



Scheme 1. Preparation of the amine and isochroman-3-one. Reagents and conditions: (a) CH_3NO_2, CH_3COOH, CH_3COONH_4, 100 °C, 70–85%; (b) THF, $LiAlH_4$, reflux, 80–87%; (c) Br_2, CH_3COOH, 90%; (d) BnBr, acetone, K_2CO_3, reflux, 95–99%; (e) μw, 140 °C, 60 min, KOH, H_2O, CuO/Cu, 56%; (f) $C_6H_5B(OH)_2$, toluene, 110 °C, 1 h; $(HCHO)_n$, 4 Å molecular sieve, 46 h, then H_2O, reflux, 2 h, 82–85%; (g) CH_3I or BnBr, acetone, K_2CO_3, reflux, 92–95%.



Scheme 2. Synthesis of (−)-THPBs and (+)-THPBs from amines and isochroman-3-ones. Reagents and conditions: (h) C_2H_5OH, reflux, 84–92%; (i) CH_3COCl, pyridine, CH_2Cl_2, 89–95%; (j) $POCl_3$, CH_3CN, reflux; (k) (Ru[(*R*,*R*)-Tsdpen](η^6-*p*-cymene) or (Ru[(*S*,*S*)-Tsdpen](η^6-*p*-cymene), DMF, HCOOH/TEA, rt, 89–95%; (l) NaOH, EtOH/H_2O, 90–98%; (m) $SOCl_2$, CH_2Cl_2; (n) saturated $NaHCO_3$, 84–92%; (o) concentrated HCl, C_2H_5OH, reflux, 72–85%.



Scheme 3. Synthesis of compounds **24a**–**24e**. Reagents and conditions: (p) EDCI, HOBt, DCM, TEA, 87–93%; (q) $POCl_3$, CH_3CN, reflux; (r) (Ru[(*R*,*R*)-Tsdpen](η^6-*p*-cymene), DMF, HCOOH/TEA, rt, 87–96%; (s) HCOOH, 40% HCHO, 25–90 °C, 2 h, 79–91%; (t) concentrated HCl, C_2H_5OH, reflux, 83–90%.

Schemes 1–3, and the details of the synthetic procedures and structural characterizations are described in the Section 5.

3.2. Binding assays

All THPBs compounds were subjected to the competitive binding assays for DA (D_1 and D_2) and serotonin (5-HT_{1A} and 5-HT_{2A}) receptors using a membrane preparation obtained from HEK293T cells stably transfected with the respective receptors. The initial screening was carried out at a concentration of 10 μM for each compound to test its ability to inhibit the binding of a tritiated radioligand to the corresponding receptor. K_is were measured for compounds that inhibited binding by more than 80% for DA receptors or 90% for the serotonin receptors. The procedures used were similar to those reported previously by us.[13,21,29] [3*H*]-8-Chloro-3-methyl-5-phenyl-2,3,4,5-tetrahydro-1*H*-benzo-[*d*]azepin-7-ol (SCH23390), [3*H*] spiperone, [3*H*]-8-OH-DPAT, and [3*H*] Ketanserin were used as standard radioligands for DA D_1 and D_2 and serotonin 5-HT_{1A} and 5-HT_{2A} receptors, respectively.

The inhibition ratio and K_i values of the newly synthesized THPBs (**17a–18z** and **24a–24e**) are summarized in Table 1. Similar to the natural product **18e** (*l*-SPD), compared to the high binding affinity of C14-S-configured THPBs, the R-configured compounds showed remarkably weakened binding affinity to all the receptors (D_1, D_2, 5-HT_{1A} and 5-HT_{2A} receptors) except compound **18h**, indicating that the C14-S configuration in THPBs is an important determinant of receptor binding affinity.

Unlike **18e** (*l*-SPD, K_i = 3.4 nM for D_1 receptor), **18g** bearing only a hydroxyl group at C2 showed a similar high binding affinity for the D_1 receptor with a K_i value of 4.5 nM. However, it was nine times less potent than **18e** (*l*-SPD, K_i = 11 nM for D_2 receptor) at the D_2 receptor with a K_i value of 91 nM. Unlike other enantiomers, a similar binding affinity was also observed for the enantiomer of **18g** and **18h** with K_i values of 6.6 and 105 nM at the D_1 and D_2 receptors respectively. **18i**, produced by introducing a hydroxyl group at C9 in **18g**, displayed a somewhat reduced binding affinity compared to **18g** at the D_1 and D_2 receptors. These results suggest that the hydroxyl group at C2 may be essential to maintain potency at the D_1 and D_2 receptors. Based on the early results achieved from compounds **18g** to **18j**, further SAR was explored retaining hydroxyl group at C2. Compounds **24a** and **24b** with methoxyl group at C11 exhibited good binding affinities at the D_1 receptor (35.8 and 2.5 nM), especially **24a** were more potent than **18e** (*l*-SPD) at the D_1 receptor. Compared to compound **24b**, compound **24c** with methyl group at C9 and C11 was found to be more potent than **24b** at the D_2 receptor and lower binding affinity at the D_1 receptor (28.9 nM for D_1 and 161 nM for D_2). Compound **24d**, originated from **24b** by introducing hydroxymethyl at the C12, also displayed higher binding affinity than **24a–c** at the D2 receptor (147 nM) and good binding affinity at the D1 receptor (17.3 nM). In addition, **24e** which formed a ring between C10 and C11 with methylenedioxy showed similar high binding affinity at the D_1 and D_2 receptors (4.2 nM for D_1 receptor and 32.1 nM for D_2 receptor) with **18e**. Therefore, compounds with good potency at the D_1 and D_2 receptors can be explored through retaining hydroxyl group at the C2 and diverse substituents on the D ring.

Compounds **18k**, **18m**, **18o** and **18p** retained hydroxyl groups at C10 but with an alkoxyl group at C2 showed an improved binding affinity to 5-HT_{1A} and 5-HT_{2A} receptors except for **18p**, and compounds **18k** and **18o** displayed K_i values of 149 and 208 nM at the 5-HT_{1A} receptor (inhibition ratio 97.4% and 95.7% for 5-HT_{1A} receptor, respectively), which indicated a moderate improvement compared to **18e** (*l*-SPD, inhibition ratio 89.3% for 5-HT_{1A} receptor). In addition, **18o** and **18p** were found to be more potent than **18e** (*l*-SPD) at the 5-HT_{2A} receptor (363 and 468 nM, respectively). Meanwhile, **18m** and **18p** showed a similar high binding affinity to the D_1 receptor with K_i values of 6.3 and 7.5 nM, respectively. However, only moderate affinity was observed for **18k** and **18m** at the D_2 receptor (305 and 145 nM, respectively), while **18o** and **18p** exhibited remarkably weakened binding to the D_2 receptor. Introducing a hydroxyl group to **18k** at C3 generated compound **18q,** which exhibited improved binding potency compared to **18k** at the D_2 receptor and a somewhat reduced affinity for D_1 and 5-HT_{1A} receptors. Compared to**18e** (*l*-SPD), a hydroxyl group at C10 and alkyloxyl group at C2 improved binding affinity at the 5-HT_{1A} receptor. In addition, the cyclization of C2 and C3 also boosted binding potency at the 5-HT_{2A} receptor. The reduced affinity for D_2 receptors following substitution with an alkyloxyl group at C2 further confirmed that the alkyloxyl group at C2 hampered the interaction of the ligand with D_2 receptor, and the hydroxyl group at C10 can improve the binding affinity to the D_1 and 5-HT_{1A} receptors. As enantiomers **18f** and **18e**, compounds **18l**, **18n**

and **18r** exhibited reduced potency at D_1, D_2 and 5-HT_{1A} receptors compared to their corresponding enantiomers. Another important difference between **18e** (*l*-SPD) and compounds such as **18k**, **18m**, **18o** and **18p** was that the latter were found to be more soluble in organic solvents.

Compounds **18s** and **18u** containing only a hydroxyl group at C9 showed reduced binding affinity for the D_1 receptor with K_i values of 334 and 60 nM, with weak binding affinity for 5-HT_{1A}, 5-HT_{2A} and D_2 receptors. Thus, a hydroxyl group at C9 can have a relatively strong influence on the interaction between compounds and receptors. Compounds **18w** and **18y** represented a subseries of C3 hydroxyl group THPBs. Compound **18w** bearing a hydroxyl group at C3 retained a moderate affinity for the D_1 receptor, with a K_i value of 310 nM. Not unexpectedly, **18y** containing two hydroxyl groups at C3 and C9 with a K_i value of 530 nM were less potent than **18w** at the D_1 receptor. Compound **18q** with K_i values of 200 and 113 nM at D_1 and D_2 receptors respectively, introducing hydroxyl group to **18w** at C10, was more potent than **18w** at all tested receptors, which indicated that hydroxyl group at C10 was beneficial to improve binding affinity to dopamine (D_1 and D_2) and serotonin (5-HT_{1A} and 5-HT_{2A}) receptors. Just as other enantiomers, **18t**, **18v**, **18x** and **18z** also showed lower potency than their corresponding enantiomer, which once again demonstrated that the R-configured compounds showed remarkably weakened binding affinity to all the receptors.

A direct comparison between **18e** (l-SPD) and **17a** and **17c** indicated that the hydrogen bonding donor (OH) at C2 and C10 is a determining factor to D1 and D2 receptor binding. To our surprise, non-hydroxyl THPBs retained a similar binding affinity to the 5-HT1A and 5-HT2A receptors.

In our studies, we found that the configuration of the C14 position in the THPB skeleton is a critical factor determining receptor binding affinity. Moreover, the hydrogen bonding donor (OH) at C2 and C10 has a strong impact on both D_1 and D_2 receptor binding, and also with much effect on binding to 5-HT_{1A} and 5-HT_{2A}. We also demonstrated that the hydroxyl group at C2 is a key factor in maintaining activity at D_1 and D_2 receptors, evidenced from the data on **18g** (D_1, 4.5 nM; D_2, 91 nM) and **24e** (D_1, 4.2 nM; D_2, 32 nM). Compounds **18k**, **18m**, **18o** with a hydroxyl group at C10 exhibited higher potency than *l*-SPD at 5-HT_{1A} and 5-HT_{2A} receptors, indicating that the C10 hydroxyl group is crucial for improving 5-HT_{1A} and 5-HT_{2A} receptors binding. Most of the corresponding THPB compounds with C10 hydroxyl group showed appreciable binding to the D_1 receptor and reduced potency at the D_2 receptor. Given that **18k** and **18m** bore the optimal receptor binding profiles, and these were selected as the lead compounds for further study.

3.3. [^{35}S]GTPγS binding assays for compounds 18k and 18m

To determine the agonistic or antagonistic activities of compounds **18k** and **18m**, [^{34}S]GTPγS binding assays were employed. Stably transfected respective D_1, D_2 or 5-HT_{1A} cell membrane fractions were prepared, and the [^{35}S]GTPγS binding assays were performed as previously described.[9,54] Compounds **18k** and **18m** were diluted to various concentrations and added to the reaction tubes. The D_1 receptor agonist SKF38393 (1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine-7,8-diol) and antagonist SCH23390 (7-chloro-3-methyl-1-phenyl-1,2,4,5-tetrahydro-3-benzazepin-8-ol), D_2 receptor agonist quinpirole [(4a*R*,8a*R*)-5-propyl-4,4a,5,6,7,8,8a,9-octahydro-1*H*-pyrazolo[3,4-*g*]quinoline] and 5-HT_{1A} receptor agonist 5-HT (5-hydroxytryptamine) were used for reference. The results are summarized in Table 2, where **18k** and **18m** showed partial agonistic activity at the D_1 receptor. Both **18k** and **18m** exhibited full agonistic activity at the 5-HT_{1A} receptor with E_{max} values of 156.4% and 80.7%, respectively. Compound **18k** (EC_{50}, 0.19 μM) had a 70-fold higher potency in activating the 5-HT_{1A} receptor than that of compound **18m** (EC_{50}, 13.5 μM). In agreement with *l*-SPD, both **18k** and **18m** displayed antagonistic activity at the D_2 receptor, where **18m** was 20 times more potent in antagonizing the D_2 receptor than that of **18k**. Thus, **18k** and **18m** appear to act as partial D_1 receptor agonists and full 5-HT_{1A} agonists while exerting antagonistic activity at the D_2 receptor.

We are searching for compounds that act as neurotransmitter stabilizer that can target to multiple related neurotransmitter receptors, presumably has advantage than that of single receptor-targeted drug such as classical D_2 receptor antipsychotic drug. Since the pharmacological profile of **18k** and**18m** meet such criteria, they therefore were selected for efficacy study in vivo to prove the concept.

3.4. Efficacy studies of compound 18k and 18m in vivo

Phencyclidine (PCP)-induced hyperlocomotion rat model is a widely used in schizophrenia drug research. We therefore employed this model to study the potential antipsychotic efficacy of compounds **18k** and **18m**.

3.4.1. Compounds 18k and 18m attenuated phencyclidine (PCP)-induced hyperlocomotion

Rats received intraperitoneal injections of saline or the respective compounds 10 min prior to the administration of PCP. Animal activity was monitored 5 min after PCP treatment for a period of 30 min. As shown in Figure 2, acute 5 mg/kg PCP treatment caused hyperlocomotion in rats. Pretreatment with **18k** or **18m** significantly attenuated PCP-induced hyperlocomotive activity in a dose-dependent manner. A significant decrease in total travel distance was already observed with 2.5 mg/kg **18k** and 10 mg/kg **18m**.

3.4.2. The effect of 18k and 18m on phencyclidine-induced PPI disruption

Impairment of sensorimotor gating is a common feature in schizophrenia. Rats were pretreated with either saline or the respective compounds 10 min prior to the administration of PCP. Prepulse inhibition (PPI) was measured 5 min after PCP treatment. As shown in Figure 3, pretreatment with **18k** (5 mg/kg, ip) or **18m** (5 mg/kg and 10 mg/kg, ip) or atypical antipsychotic risperidone (0.5 mg/kg, ip) significantly attenuated PPI disruption induced by PCP(5 mg/kg, ip), at least at one prepulse level. There was no significant effect of drug treatment (**18k**, **18m**, risperidone and/or PCP) on absolute acoustic startle responses produced by a 120 dB noise stimulus (Fig. 3D–F), indicating that the drug treatment, at the dosage used in the present study, had no effect on baseline startle reactivity. In addition, there was no significant effect on PPI when **18k** was administered alone (data not shown).

3.5. Compound 18k excited tegmental area (VTA) DA neurons in vivo via its 5-HT_{1A} receptor agonistic effect

To further determine the agonistic properties of **18k** at the 5-HT_{1A} receptor, we studied the effect of this compound on the ventral tegmental area (VTA) dopaminergic (DA) neuron firing using single-unit recording system as reported previously.[55–62] Because the effect of 5-HT_{1A} agonists on VTA DA neurons are known to be masked by D_2 receptor activation, the 5-HT_{1A}-excited VTA DA neurons can only be observed while the D_2 action is blocked by a D_2 receptor selective antagonist.[62] Therefore, rats were administrated with raclopride, a D_2-like receptor antagonist, before treatment with **18k**. Consistent with literature reports,[58,62] raclopride (0.1 mg/kg) alone produced only a small effect on DA neuron activities (n = 5, data not shown). Subsequent injection of **18k** (1 mg/kg)

Table 2
[^{35}S]GTPγS binding assays of compounds **18k** and **18m** for D_1, D_2 and 5-HT_{1A} receptors

Compounds	Agonist D_1 Receptor EC_{50} (μM)	E_{max} %	Antagonist IC_{50} (μM)	I_{max} %	Antagonist D_2 Receptor IC_{50} (μM)	I_{max} %	Agonist 5-HT_{1A} Receptor EC_{50} (μM)	E_{max} %
18k	1.35 ± 0.09	31.3 ± 4.5	21.27 ± 1.30	60.1 ± 4.2	18.73 ± 2.72	82.3 ± 2.4	0.19 ± 0.02	156.4 ± 44
18m	15.97 ± 4.75	35.6 ± 9.1	17.37 ± 0.58	78.2 ± 4.7	1.00 ± 0.06	58.9 ± 6.0	13.5 ± 1.3	80.7 ± 2.9
SKF38393	0.13 ± 0.01	100	—	—	—	—	—	—
SCH23390	—	—	0.60 ± 0.01	83.5 ± 0.3	—	—	—	—
Haloperidol	—	—	—	—	0.55 ± 0.01	94.6 ± 0.6	—	—
5-HT	—	—	—	—	—	—	3.75 ± 0.50($\times10^{-3}$)	100

Note, the dash line denote that no experiment was conducted.

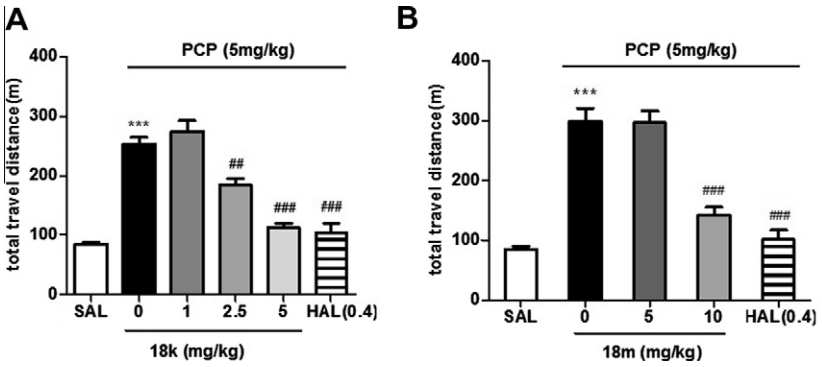


Figure 2. Compound **18k** and **18m** dose-dependently inhibit acute PCP-induced hyperlocomotion in rats. The locomotor activity was measured for a 30 min duration after PCP administration and the total traveled distance was expressed as mean ± SEM. Statistical analysis showed 2.5 mg/kg and 5 mg/kg **18k** (A, one-way ANOVA followed by Tamhane's post hoc test: $F(5,51) = 48.883$, $n = 7–12$), as well as 10 mg/kg **18m** (B, one-way ANOVA followed by Tamhane's post hoc test: $F(4,33) = 42.928$, $n = 6–10$) significantly attenuated hyperlocomotor activity induced by PCP ($^{***}P$ <0.001 vs saline, $^{\#\#}P$ <0.01 $^{\#\#\#}P$ <0.001 vs PCP). SAL: saline; PCP: phencyclidine; HAL: haloperidol.

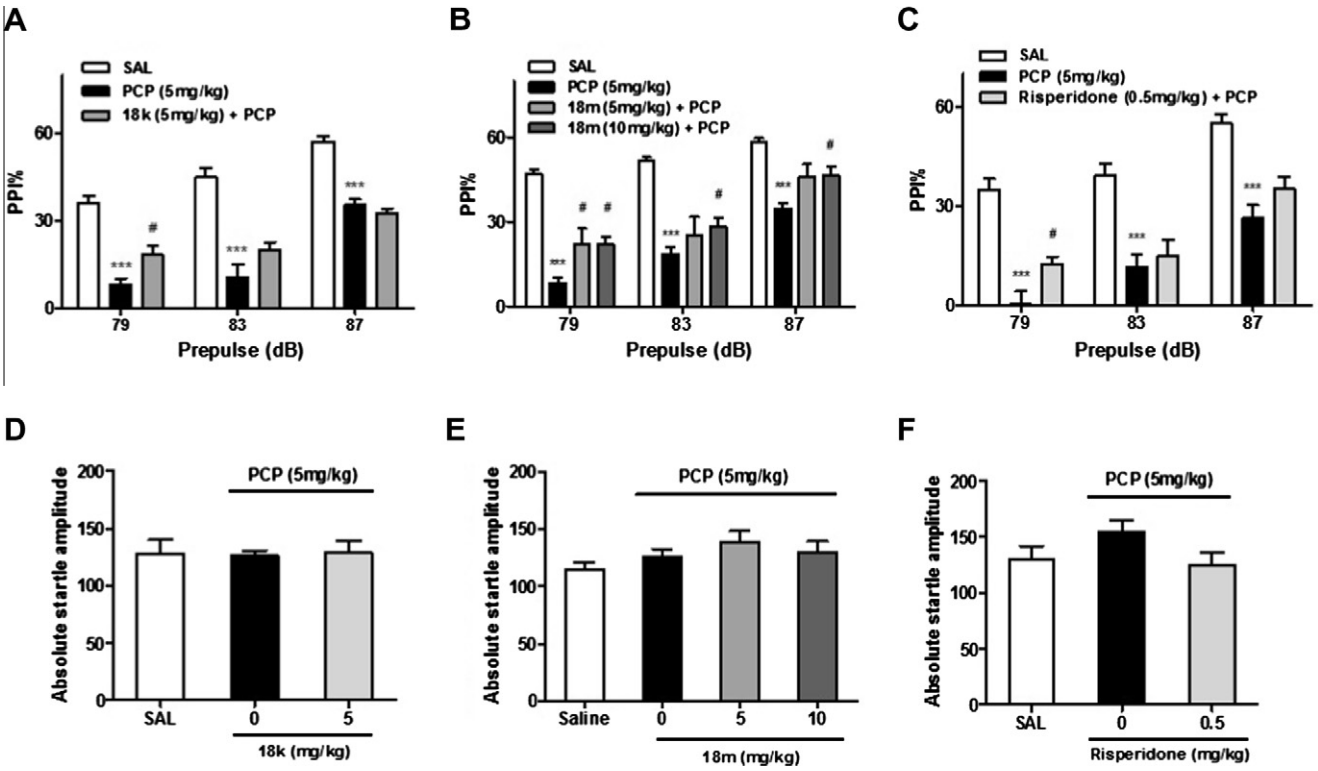


Figure 3. Compounds **18k** and **18m** alleviated PCP-induced PPI deficit without affecting the baseline startle reactivity and normal PPI function. (A, B, C) **18k** (5 mg/kg, ip) or **18m** (5 and 10 mg/kg, ip) or atypical antipsychotic risperidone (0.5 mg/kg, ip) significantly attenuated PPI disruption induced by PCP (5 mg/kg, ip) at least at one prepulse level. (Two-way ANOVA followed by Tamhane's post hoc test: $^{***}P$ <0.001 vs saline, $^{\#}P$ <0.05 vs PCP, $n = 9–15$.); (D, E, F) There's no significant effect of drug treatment on the absolute startle amplitude (one way ANOVA, $n = 9–15$). SAL: saline; PCP: phencyclidine.

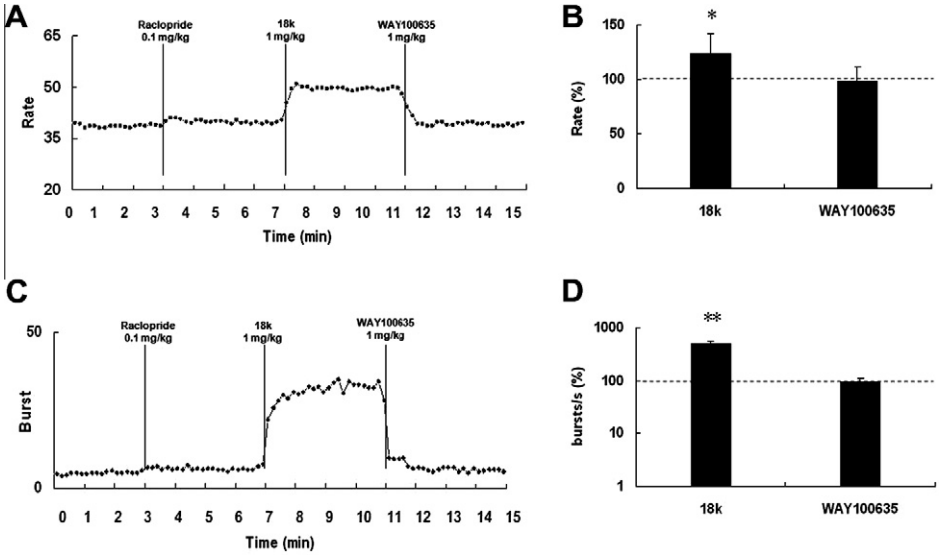


Figure 4. Compound **18k** excited VTA DA neurons in vivo. (A) Typical rate scatter diagram showing the excitatory effect by **18k** (1.0 mg/kg), suggesting that **18k** can increase the firing rate, which can be reversed by 5-HT_{1A} receptor antagonist WAY100635. (B) Summary bar graph showing that firing rates were all increased after **18k** injection compared to the baseline. (C) Typical burst scatter diagram showing the excitatory effect by **18k** (1.0 mg/kg), suggesting that **18k** can increase the bursts, which also can be reversed by WAY100635. (D) Summary bar graph showing that bursting level was increased after **18k** injection compared to the baseline. All values were expressed as percent of baseline (the firing rates or bursting levels after raclopride administration, respectively) in B and D ($^{*}P$ <0.05, $^{**}P$ <0.01 compared to baseline).

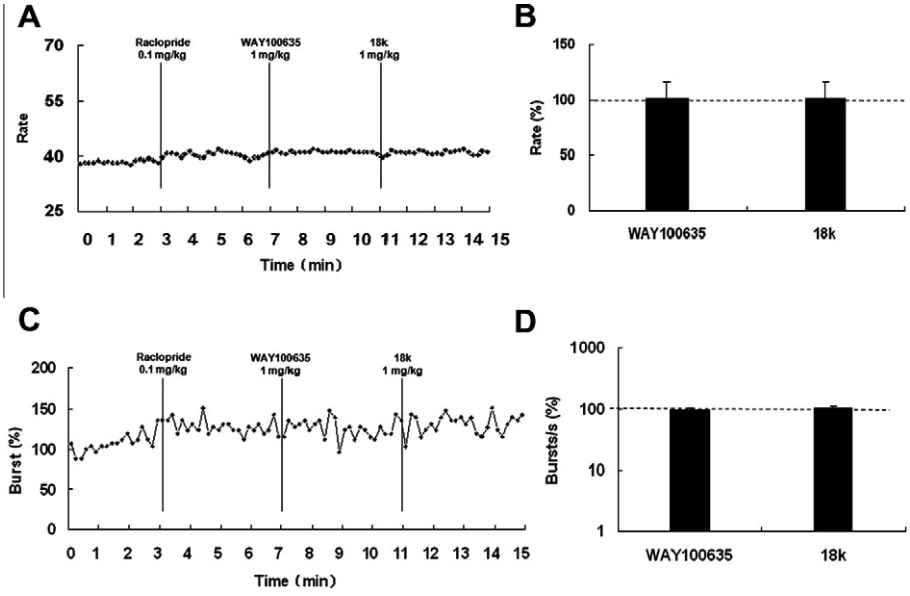


Figure 5. Compound **18k** fails to excite VTA DA neurons in antagonist WAY100635-pretreated rats. (A) Typical rate scatter diagram showing that 5-HT_{1A} antagonist WAY100635 completely blocks the firing rate increase induced by **18k** injection. (B) Summary bar graph showing that firing rate had no significantly change after **18k** injection compared to the baseline. (C) Typical rate scatter diagram showing that 5-HT_{1A} antagonist WAY100635 also completely blocks the bursting increase induced by **18k** injection. (D) Summary bar graph showing that bursting level had no significantly change after **18k** injection compared to the baseline. All values are expressed as percent of baseline (the firing rates or bursting levels after raclopride administration, respectively) in B and D.

significantly increased the firing rate of DA neurons in all cells tested (n = 5, Fig. 4). Compared to the results after raclopride administration (paired t-test, p <0.05), the firing rate of DA neurons significantly increased to 128.3 ± 9.89% after treatment with **18k**. Compound **18k** also increased the bursts/s (bursts per second) of DA neurons compared to the basal control (paired t-test, p <0.01). To confirm the role of 5-HT_{1A} receptor activation in **18k**-enhanced firing of VTA DA neurons, compound WAY100635 (1.0 mg/kg), a selective 5-HT_{1A} antagonist, was administered following injection with **18k**. Compound **18k**-enhanced DA neuron firing rate and bursting were blunted, suggesting that 5-HT_{1A} agonistic activity plays an important role in **18k** excitement of DA neurons.

To further confirm excitation by **18k** via the 5-HT_{1A} receptor, rats were injected with 5-HT_{1A} antagonist WAY100635 prior to administration of **18k**. WAY100635 alone had little effect on DA neuron activities. However, pretreatment with WAY100635 abolished the **18k**-enhanced firing and bursting of DA neurons (n = 5, Fig. 5). Taken together, these results demonstrate that 5-HT_{1A} receptor agonistic activity contributes to the excitation of VTA DA neurons by **18k** in vivo.

As can be seen from the above results, compounds **18k** and **18m** displayed full agonistic response at the 5-HT_{1A} receptor, partial agonistic activity at the D_1 receptor and antagonistic activity at the D_2 receptor. In the PCP-induced rat models of schizophrenia,

both **18k** and **18m** dose-dependently reduced PCP-induced spontaneous activity, indicating that **18k** and **18m** may effectively relieve the positive symptoms of schizophrenia. Although **18k** was 20 times less potent than **18m** in antagonizing the D_2 receptor in the [^{34}S]GTPγS assays in vitro (Table 2), **18k** was more efficacious than **18m** in the locomotion test in vivo (Fig. 2). We further found that **18k** and **18m** significantly improved PCP-impaired PPI. Improvement of PPI is a common feature of antipsychotic drugs.[63–65] These results demonstrated that compounds with agonistic activity at the 5-HT_{1A} receptor could be more effective in vivo. This is further supported by our data from the in vivo electrophysiological studies (Fig. 5), in which we showed that, like other antipsychotic drugs that excite DA neurons,[66,67] **18k**-excited DA neurons and this excitation effects are found to associate with its 5-HT_{1A} receptor agonistic activity. It is noted that **18k** with the weaker affinity for D_1 and D_2 shows a higher potency in attenuating PCP-induced locomotor activity than compound **18m** with the higher binding affinity to D_1 and D_2. The discrepancy may result from the distinct in PK/PD property, penetration of brain blood-barrier and receptor occupancy in vivo.

4. Conclusions

This study reported the development, characterization, and antischizophrenic effects of a series of THPB compounds. The rationale is based on the recent consensus that an ideal antipsychotic drug should behave as a neurotransmitter stabilizer with multiple targets to correct imbalances of neurotransmission in schizophrenia.[68] In addition to D_2 receptor antagonistic activity, the beneficial effects of D_1 and 5-HT_{1A} receptor agonistic activities have been proven in human and experimental animals studies of schizophrenia, by improving cognitive function or negative symptoms which are the major challenge of schizophrenia drug therapy.[69,70]

In conclusion, we have developed a series of derivatives of THPB, among them, pharmacological profile assays indicate that **18k** and **18m** act as partial D_1 receptor agonists and full 5-HT_{1A} agonists while exerting antagonistic activity at the D_2 receptor. Further animal and electrophysiological studies in vivo indicate that **18k** and **18m** are potential antipsychotic agents with new pharmacological profiles.

5. Experimental section

The reagents (chemicals) were purchased from Lancaster, Sigma, Acros and Shanghai Chemical Reagent Company, and used without further purification. Analytical thin-layer chromatography (TLC) was performed on HSGF 254 (150–200 μm thickness, Yantai Huiyou Company, China). Yields were not optimized. Column chromatography was performed with Combi*Flash*® Companion system (Teledyne Isco, Inc.). Nuclear magnetic resonance (NMR) spectra were performed on a Brucker AMX-400 and AMX-300 NMR (TMS as IS). Chemical shifts were reported in parts per million (ppm, δ) downfield from tetramethylsilane. Proton coupling patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). Low- and high-resolution mass spectra (LRMS and HRMS) were given with electric, electrospray and matrix-assisted laser desorption ionization (EI, ESI and MALDI) produced by Finnigan MAT-95, LCQ-DECA spectrometer and IonSpec 4.7 Tesla. Optical rotations of compounds were obtained on PE-431 polarimeter and the melting points were performed on SGW X-4 (SHANGHAI PRECISION & SCIENTIFIC INSTRUMENT CO., LTD). LC–MS analysis were conducted on Agilent 1100 Series HPLC with an Agilent Extend-C18 (4.6 × 50 mm, 5 μm) reversed phase column. Compounds **17a–17d**, **18e–18z** and **24a–24e** were confirmed ⩾95% purity (Supplementary data, Table S1) and the compounds enantiomeric excess (ee) were also indentified by HPLC (Supplementary data, Table S2). The details for purity analyses of compounds **17a–17d**, **18e–18z** and **24a–24e** are described in the Supplementary data.

5.1. General procedure for the synthesis of THPBs

5.1.1. 4-(Benzyloxy)-3-methoxybenzaldehyde (2c)

To a solution of 4-hydroxy-3-methoxybenzaldehyde (**1c**, 7.6 g, 50 mmol) in acetone (150 mL) was added K_2CO_3 (10.4 g, 75 mmol) and BnBr (9.41 g, 55 mmol), and the mixture was heated at reflux for 4 h before it was cooled to room temperature. The solid was filtered off, and the filtrate was concentrated under reduced pressure to get yellow oil, which was purified by flash chromatography (ethyl acetate/petroleum ether = 1:6) to yield 4-(benzyloxy)-3-methoxybenzaldehyde (**2c**, 11.5 g, 95%) as white solid.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.85 (s, 1H), 7.44–7.34 (m, 7H), 6.99 (d, J = 8.1 Hz, 1H), 5.26 (s, 2H), 3.96 (s, 3H).

5.1.2. 1-(Benzyloxy)-2-methoxy-4-(2-nitrovinyl) benzene (3c)

To the solution of **2c** (11.5 g, 49.5 mmol) in acetic acid (50 mL) was added ammonium acetate (3.82 g, 49.5 mmol) and nitro methane (9.2 g, 150 mmol), and the mixture was reflux overnight, then the reaction mixture was cooled to room temperature and the product was obtained by filtration (9.88 g; yellow solid, 70%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.95 (d, J = 13.2 Hz, 1H), 7.51 (d, J = 13.2 Hz, 1H), 7.42–7.32 (m, 5H), 7.10 (dd, J = 8.4 Hz, J = 1.8 Hz, 1H), 7.02 (d, J = 1.8 Hz, 1H), 6.92 (d, J = 8.4 Hz, 1H), 5.22 (s, 2H), 3.92 (s, 3H).

5.1.3. 2-(4-(Benzyloxy)-3-methoxyphenyl)ethanamine (4c)

To a slurry of $LiAlH_4$ (2.28 g, 60 mmol) in THF (40 mL) at room temperature was added **3c** (5.7 g, 20 mmol) as a solution in THF (20 mL), and the reaction mixture was heated to reflux for 2 h. The reaction was cooled to room temperature, and certain amount of water was added slowly to the reaction mixture. Then, the solid was filtered off, and the filtrate was dried with Na_2SO_4, concentrated to give yellow oil.

Yellow oil, yield 85%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.20 (br, 2H), 7.46–7.33 (m, 5H), 6.99 (d, J = 8.4 Hz, 1H), 6.92 (d, J = 1.5 Hz, 1H), 6.75 (dd, J = 8.4 Hz, J = 1.5 Hz, 1H), 5.06 (s, 2H), 3.79 (s, 3H), 3.01 (t, J = 6.6 Hz, 2H), 2.85 (m, 2H).

5.1.4. Methyl 2-(3-bromo-4-hydroxyphenyl) acetate (6)

To a solution of methyl 4-hydroxyphenyl acetate (16.6 g, 100 mmol) in 200 mL acetic acid was added liquid bromine (16.8 g, 105 mmol) in 10 mL acetic acid slowly. The mixture was stirred for 2 h at room temperature, then saturation $NaHSO_3$ was added to quench the reaction. The solvent was evaporated and the residue was purified by flash chromatography (ethyl acetate/petroleum ether = 1:4) to give compound **6**.

Yield 90%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.39 (d, J = 1.5 Hz, 1H), 7.11 (dd, J = 8.4 Hz, J = 1.5 Hz, 1H), 6.93 (d, J = 8.4 Hz, 1H), 3.70 (s, 3H), 3.54 (s, 2H).

5.1.5. Methyl 2-(4-(benzyloxy)-3-bromophenyl) acetate (7b)

To methyl 2-(4-(hydroxyl)-3-bromophenyl) (22.9 g) was added potassium carbonate and acetone, benzyl bromide was added to the stirring solution and the mixture was heated at reflux. The mixture cooled after 3 h, and the solution was filtered. The acetone was evaporated to give an oil crude product, which was purified by flash chromatography (ethyl acetate/petroleum ether = 1:6) to give **7b** (31.0 g).

Yield 99%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.51–7.33 (m, 6H), 7.15 (dd, J = 8.4 Hz, J = 2.4 Hz, 1H), 6.89 (d, J = 8.4 Hz, 1H), 5.15 (s, 2H), 3.70 (s, 3H), 3.55 (s, 2H).

5.1.6. 2-(4-(Benzyloxy)-3-hydroxyphenyl)acetic acid (8b)

To a solution of **7b** (2.0 g) and KOH (2.0 g) in water (8 mL) was added 200 mg copper power and 200 mg copper oxide, and the mixture was stirred 10 min at room temperature. The sealed tube was filled with Ar gas, and the mixture was irradiated on microwave under 140 °C for 60 min. The solution was filtered and adjusted to acid with hydrochloric acid, and then the precipitation was collected and purified by column chromatography (MeOH/CH_2Cl_2 = 1:100) to give **8b** (white solid, yield 56%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.43–7.38 (m, 5H), 6.90 (s, 1H), 6.89 (dd, J = 8.1 Hz, J = 2.1 Hz, 1H), 6.74 (d, J = 8.1 Hz, 1H), 5.10 (s, 2H), 3.56 (s, 2H).

5.1.7. 7-Methoxy-8-hydroxyisochroman-3-one (9a)

To homoisovanillic acid (3.0 g, 16.5 mmol) was added phenylboric acid (4.0 g, 33.0 mmol) and dry toluene (70 mL). The mixture was heated at reflux for 1 h, and H_2O was collected in a Dean–Stark trap. The hot solution was poured over molecular sieves (4 Å, 2.07 g) in a sealed tube. Para formaldehyde (2.72 g) was added along with enough toluene (10 mL). The tube was heated at 100 °C for 46 h. The sealed tube was opened and the hot solution filtered. The toluene was evaporated, and 70 mL water was added to the residue. After heating at reflux for 2 h, the mixture was cooled to room temperature and extracted with CH_2Cl_2 (150 mL). The solution was dried ($MgSO_4$) and the solvent evaporated. The residue was stirred in Et_2O (60 mL) for 3 h and the solid lactone **9a** (white solid; 2.2 g, 85%) was filtered.

White solid, yield 85%; ^{1}H NMR ($CDCl_3$, 300 MHz): 6.82 (d, J = 8.1 Hz, 1H), 6.69 (d, J = 8.1 Hz, 1H), 5.82 (s, 1H), 5.41 (s, 1H), 3.89 (s, 3H), 3.63 (s, 2H).

5.1.8. 7-Benzyloxy-8-hydroxyisochroman-3-one (9c)

White solid, yield 82%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.45–7.34 (m, 5H), 6.93 (d, J = 8.4 Hz, 1H), 6.86 (d, J = 8.4 Hz, 1H), 5.40 (s, 1H), 5.12 (s, 1H), 3.91 (s, 3H), 3.62 (s, 2H).

5.1.9. 7,8-Dimethoxyisochroman-3-one (10a)

To a solution of **9a** (1.94 g, 10 mmol) in acetone (50 mL) was added K2CO3 (2.07 g, 15 mmol) and CH_3I (0.77 mL, 15 mmol), and the mixture was heated at reflux for 4 h before it was cooled to room temperature. The solid was filtered off, and the filtrate was concentrated under reduced pressure to yield a yellow oil, which was purified by flash chromatography (ethyl acetate/petroleum ether = 1:4) to yield **10a** (1.98 g, 92%) as white solid.

^{1}H NMR ($CDCl_3$, 300 MHz): 6.83 (d, J = 8.1 Hz, 1H), 6.69 (d, J = 8.1 Hz, 1H), 5.41 (s, 1H), 3.92 (s, 3H), 3.89 (s, 3H), 3.62 (s, 2H).

5.1.10. 7-(Benzyloxy)-8-methoxyisochromen-3-one (10b)

White solid, yield 95%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.45–7.34 (m, 5H), 6.92 (d, J = 8.1 Hz, 1H), 6.85 (d, J = 8.1 Hz, 1H), 5.40 (s, 2H), 5.12 (s, 2H), 3.91 (s, 3H), 3.62 (s, 2H).

5.1.11. *N*-[2-(3,4-Dimethoxyphenyl)ethyl]-2-(hydroxymethyl)-3,4-dimethoxybenzeneacetamide (11a)

3,4-Dimethoxyphenylethanamine (0.54 g, 3.6 mmol) was added to a stirred solution of compound **10a** (0.8 g, 3.0 mmol) in C_2H_5OH (5 mL). The mixture was reflux for 14 h. Evaporation of the solvent left a little yellow powder, which was purified by flash chromatography (MeOH/CH_2Cl_2 = 1:80) to yield a white solid **11a** (1.0, 89%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.88 (d, J = 8.4 Hz, 1H), 6.82 (d, J = 8.4 Hz, 1H), 6.74 (d, J = 8.0 Hz, 1H), 6.64 (d, J = 2.0 Hz, 1H), 6.57 (dd, J = 8.0 Hz, J = 2.0 Hz, 1H), 6.03 (m, 1H), 4.68 (d, J = 4.5 Hz, 2H), 3.89 (s, 3H), 3.86 (s, 3H), 3.85 (s, 3H), 3.83 (s, 3H), 3.51 (s, 2H), 3.45 (m, 2H), 2.70 (t, 2H).

5.1.12. *N*-(2-(Benzo[*d*][1,3]dioxol-5-yl)ethyl)-2-(2-(hydroxymethyl)-3,4-dimethoxyphenyl)acetamide (11c)

White solid, yield 88%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.92 (d, J = 8.4 Hz, 1H), 6.82 (d, J = 8.4 Hz, 1H), 6.68 (d, J = 7.8 Hz, 1H), 6.57 (d, J = 1.8 Hz, 1H), 6.48 (dd, J = 7.8 Hz, J = 1.8 Hz, 1H), 5.44 (m, 1H), 4.69 (s, 2H), 3.88 (s, 3H), 3.86 (s, 3H), 3.52 (s, 2H), 3.40 (m, 2H), 2.60 (m, 2H).

5.1.13. 2-(4-(Benzyloxy)-2-(hydroxymethyl)-3-methoxyphenyl)-*N*-(4-(benzyloxy)-3-methoxyphenethyl)acetamide (11e)

White solid, yield 84%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.42–7.25 (m, 10H), 6.82 (m, 2H), 6.75 (d, J = 8.4 Hz, 1H), 6.64 (d, J = 1.9 Hz, 1H), 6.47 (dd, J = 8.4, 1.9 Hz, 1H), 6.32 (m, 1H), 5.08 (s, 2H), 5.06 (s, 2H), 4.65 (s, 2H), 3.91 (s, 3H), 3.79 (s, 3H), 3.47 (s, 2H), 3.39 (q, J = 6.8 Hz, 2H), 2.65 (t, J = 6.8 Hz, 2H).

5.1.14. 6-(2-(3,4-Dimethoxyphenethylamino)-2-oxoethyl)-2,3-dimethoxyphenyl acetate (12a)

To a solution of compound **11a** (1.0 g, 2.57 mmol) in CH_2Cl_2 (15 mL) was added pyridine (3.8 mL, 3.86 mmol). The mixture was cooled to 0 °C, and acetyl chloride (1.6 mL, 3.86 mmol) was added dropwise. After being stirred 1.5 h at room temperature, the reaction mixture was diluted with CH_2Cl_2 (10 mL), washed with 1 N HCl and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography (MeOH/CH_2Cl_2 = 1:100) to afford a white solid **12a** (1.09 g, 90%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.90 (d, J = 8.4, 1H), 6.86 (d, J = 8.4 Hz, 1H), 6.72 (d, J = 8.1 Hz, 1H), 6.62 (d, J = 1.8 Hz, 1H), 6.55 (dd, J = 8.1 Hz, J = 1.8 Hz, 1H), 5.45 (m, 1H), 5.14 (s, 2H), 3.87 (s, 3H), 3.85 (s, 3H), 3.83 (s, 3H), 3.83 (s, 3H), 3.54 (s, 2H), 3.43 (m, 2H), 2.68 (t, J = 6.9 Hz, 2H), 2.00 (s, 3H).

5.1.15. 6-(2-(2-(Benzo[*d*][1,3]dioxol-5-yl)ethylamino)-2-oxoethyl)-2,3-dimethoxybenzyl acetate (12c)

White solid, yield 93%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.92 (d, J = 8.4 Hz, 1H), 6.87 (d, J = 8.4 Hz, 1H), 8.64 (d, J = 8.1 Hz, 1H), 8.52 (d, J = 2.1 Hz, 1H), 8.43 (dd, J = 8.1 Hz, J = 2.1 Hz, 1H), 5.91 (s, 2H), 5.13 (s, 2H), 3.87, (s, 3H), 3.86 (s, 3H), 3.53 (s, 2H), 3.38 (m, 2H), 2.82 (m, 2H), 1.99 (s, 3H).

5.1.16. 6-((4-(Benzyloxy-3-methoxyphenethyl)carbamoyl)methyl)-3-(benzyloxy)-2-methoxybenzyl acetate (12e)

White solid, yield 92%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.46–7.28 (m, 10H), 6.90 (d, J = 8.4 Hz, 1H), 6.84 (d, J = 8.4 Hz, 1H), 6.73 (d, J = 8.1 Hz, 1H), 6.64 (d, J = 1.9 Hz, 1H), 6.46 (dd, J = 8.1 Hz, 1.9 Hz, 1H), 5.46 (m, 1H), 5.15 (s, 2H), 5.12 (s, 2H), 5.10 (s, 2H), 3.88 (s, 3H), 3.83 (s, 3H), 3.53 (s, 2H), 3.42 (q, J = 7.0 Hz, 2H), 2.65 (t, J = 7.0 Hz, 2H), 2.00 (s, 3H).

5.1.17. 6-((-6,7-Dimethoxy-3,4-dihydroisoquinolin-1-yl)methyl)-2,3-dimethyloxybenzyl acetate (13a)

To a solution of the **12a** (1.09 g, 2.52 mmol) in dry acetonitrile (25 mL) was added $POCl_3$ (1.38 mL, 15.1 mmol) then the mixture was refluxed for 40 min under nitrogen. The reaction mixture was cooled to room temperature and concentrated under vacuum. The residue was dissolved in CH_2Cl_2, washed with saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated to yield a yellow solid (1.0 g) without further purification.

Yellow solid, yield 99%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.90 (s, 1H), 6.88 (d, J = 8.7 Hz, 1H), 6.82 (d, J = 8.7 Hz, 1H), 6.69 (s, 1H), 5.28 (s, 2H), 5.28 (s, 2H), 4.09 (s, 2H), 3.91 (s, 3H), 3.85 (s, 3H), 3.83 (s, 3H), 3.74 (s, 3H), 3.70 (m, 2H), 2.68 (m, 2H), 2.04 (s, 2H).

5.1.18. 3-(Benzyloxy)-6-((7-(benzyloxy)-3,4-dihydro-6-methoxyisoquinolin-1-yl)methyl)-2-methoxybenzyl acetate (13e)

Yellow solid, yield 98%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.44–7.28 (m, 10H), 6.96 (s, 1H), 6.83 (d, J = 8.4 Hz, 1H), 6.72 (d, J = 8.4 Hz, 1H), 6.70 (s, 1H), 5.24 (s, 2H), 5.08 (s, 2H), 5.00 (s, 2H), 3.98 (s, 2H), 3.92 (s, 3H), 3.90 (s, 3H), 3.69 (t, J = 7.5 Hz, 2H), 2.66 (t, J = 7.5 Hz, 2H), 2.03 (s, 3H).

5.1.19. (*S*)-6-((6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl)-2,3-dimethoxylbenzyl acetate (14a)

The imine **13a** (0.42 g, 1.0 mmol) was dissolved in anhydrous DMF (3 mL), and RuCl[(R,R)-TsDPEN(*P*-cymene)] (7.0 mg, 1 mol %) was added followed by formic acid/triethylamine (v/v = 5/2, 300 μL), and the reaction mixture was stirred at room temperature overnight. The reaction mixture was adjusted pH to 8 with saturated $NaHCO_3$ and extracted with ethyl acetate. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was without further purified and directly employed in the next step.

Green solid, yield 93%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.01 (d, J = 8.4 Hz, 1H), 6.88 (d, J = 8.4 Hz, 1H), 6.75 (s, 1H), 6.60 (s, 1H), 5.29 (s, 2H), 4.84 (d, J = 7.6 Hz, 1H), 4.48 (d, J = 7.6 Hz, 1H), 4.11–4.08 (m, 1H), 3.91 (s, 3H), 3.85 (s, 3H), 3.83 (s, 3H), 3.74 (s, 3H), 3.24–3.18 (m, 2H), 2.76–2.67 (m, 2H), 2.61 (s, 3H).

5.1.20. (*S*)-2,3-Dimethoxy-6-((5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-*g*]isoquinolin-5-yl)methyl)benzyl acetate (14c)

Yellow solid, yield 91%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.01 (d, J = 8.4 Hz, 1H), 6.92 (d, J = 8.4 Hz, 1H), 6.66 (s, 1H), 6.57 (s, 1H), 5.90 (s, 2H), 5.28 (s, 2H), 4.02–3.98 (m, 1H), 3.90 (s, 3H), 3.89 (s, 3H), 3.22–3.16 (m, 2H), 2.90–2.84 (m, 3H), 2.74–2.72 (m, 3H), 2.06 (s, 2H).

5.1.21. (*S*)-3-(Benzyloxy)-6-((7-(benzyloxy)-6-methoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl)-2-methoxybenzyl acetate (14e)

Green solid, yield 92%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.48–7.30 (m, 10H), 6.96 (d, J = 8.4 Hz, 1H), 6.90 (d, J = 8.4 Hz, 1H), 6.79 (s, 1H), 6.66 (s, 1H), 5.35 (d, J = 11.1 Hz, 1H), 5.20 (d, J = 11.1 Hz, 1H), 5.12 (s, 2H), 5.08 (s, 2H), 3.97–3.93 (m, 1H), 3.95 (s, 3H), 3.89 (s, 3H), 3.24–3.16 (m, 1H), 3.12–2.87 (m, 3H), 2.82–2.71 (m, 2H), 2.06 (s, 3H).

5.1.22. (*S*)-(6-((6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl)-2,3-dimethoxyphenyl)methanol (15a)

To the solution of **14a** (370 mg) in 3 mL ethanol was added 1 mL H_2O and 120 mg NaOH, the mixture was stirred for 3 h at room temperature. 10 mL water was added to the solution and the solid was filtered and dried in vacuum. Slightly green solid was obtained (300 mg; yield 80%, two steps).

A slightly green solid, yield 80%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.01 (d, J = 8.4 Hz, 1H), 6.88 (d, J = 8.4 Hz, 1H), 6.76 (s, 1H), 6.62 (s, 1H), 5.29 (m, 2H), 4.84 (d, J = 11.7 Hz, 1H), 4.49 (d, J = 11.7 Hz, 1H), 4.11–3.90 (m, 1H), 3.91 (s, 3H), 3.89 (s, 3H), 3.85 (s, 3H), 3.82 (s,3H), 3.24–3.18 (m, 1H), 3.01–2.85 (m, 3H), 2.76–267 (m, 2H).

5.1.23. (*S*)-(2,3-Dimethoxy-6-((5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-*g*]isoquinolin-5-yl)methyl)phenyl)methanol (15c)

A slightly green solid, yield 83%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.01 (d, J = 8.4 Hz, 1H), 6.92 (d, J = 8.4 Hz, 1H), 6.66 (s, 1H), 6.59 (s, 1H), 5.90 (m, 2H), 5.33 (d, J = 11.2 Hz, 1H), 5.22 (d, J = 11.2 Hz, 1H), 4.02–3.90 (m, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.22–3.16 (m, 2H), 2.90–2.83 (m, 2H), 2.74–2.71 (m, 2H).

5.1.24. (3-(Benzyloxy)-6-(((*S*)-7-(benzyloxy)-1,2,3,4-tetrahydro-6-methoxyisoquinolin-1-yl)-methyl)-2-methoxyphenyl)methanol (15e)

A slightly green solid, yield 75%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.56–7.25 (m, 10H), 7.04 (d, J = 8.4 Hz, 1H), 6.91 (d, J = 8.4 Hz, 1H), 6.83 (s, 1H), 6.64 (s, 1H), 5.17 (s, 2H), 5.13 (s, 2H), 4.89 (d, J = 11.1 Hz, 1H), 4.47 (d, J = 11.1 Hz, 1H), 4.12–4.09 (m, 1H), 3.92 (s, 3H), 3.89 (s, 3H), 3.26–3.20 (m, 2H), 3.07–2.89 (m, 3H), 2.74–2.72 (m, 1H), 2.56–2.54 (m, 1H).

5.1.25. (−)-Tetrahydropalmatine (17a)

A solution of **15a** (300 mg, 0.8 mmol) in CH_2Cl_2 (10 mL) was cooled to 0 °C, and thionyl chloride (350 μL, 4.8 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 1.5 h before saturated $NaHCO_3$ (20 mL) was added slowly. After being stirred for another 1.5 h, the organic layer was washed with CH_2Cl_2 (20 mL) and brine, dried over Na_2SO_4, filtered, and concentrated to yield a yellow solid, which was purified by flash chromatography (ethyl acetate/petrol ether = 1:3) to give white solid **17a** (247 mg, 87%).

Mp 140–141 °C; $[\alpha]_D^{24}$ +238.7 (c 0.45, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.87 (d, J = 8 Hz, 1H), 6.77 (d, J = 8 Hz, 1H), 6.72 (s, 1H), 6.61 (s, 1H), 4.25–4.21 (m, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.84 (s, 6H), 3.55–3.51 (m, 2H), 3.28–3.11 (m, 3H), 2.79–2.85 (m, 1H), 2.68–2.61 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): 150.2, 147.4, 147.3, 144.9, 129.6, 128.6, 127.6, 126.7, 123.8, 111.2, 110.8, 108.5, 60.1, 59.2, 56.0, 55.8, 53.9, 51.4, 36.2, 29.0. MS (ESI) m/z: 355(M^+). HRMS (ESI): Calcd for 356.1682, found 356.1684. The optical purity of **17a** was determined to be 99.9% by HPLC analyses (Chiralcel AD-H (0.46 cm × 25 cm; hexanes/*i*-PrOH = 70/30 with flow rate = 1.0 mL/min and a UV detector at 214 nm; retain time = 17.35 min).

5.1.26. (+)-Tetrahydropalmatine (17b)

White solid, yield, 87% (for two steps); Mp 138–139 °C; $[\alpha]_D^{24}$ −244.3 (c 0.42, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.87 (d, J = 8.0 Hz, 1H), 6.78 (d, J = 8.0 Hz, 1H), 6.72 (s, 1H), 6.61 (s, 1H), 4.25–4.22 (m, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.84 (s, 6H), 3.55–3.51 (m, 2H), 3.29–3.14 (m, 3H), 2.86–2.79 (m, 1H), 2.69–2.61 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): 150.2, 147.4, 147.3, 144.9, 129.6, 128.6, 127.6, 123.8, 111.2, 110.8, 108.5, 60.1, 59.2, 56.0, 55.8, 53.9, 51.4, 36.2, 29.0. MS (ESI) m/z: 355(M^+). HRMS (ESI): Calcd for 356.1682, found 356.1853. The optical purity of **17a** was determined to be 99.5% by HPLC analyses (Chiralcel AD-H (0.46 cm × 25 cm; hexanes/*i*-PrOH = 70/30 with flow rate = 1.0 mL/min and a UV detector at 214 nm; retain time = 9.10 min).

5.1.27. (−)-Tetrahydroberberine (17c)

White solid, yield, 91% (for two steps); Mp 130–131 °C; $[\alpha]_D^{24}$ +250.0 (c 0.40, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.85 (d, J = 8.0 Hz, 1H), 6.77 (d, J = 8.0 Hz, 1H), 6.72 (s, 1H), 6.58 (s, 1H), 5.91 (s, 2H), 4.25–4.21 (m, 1H), 3.84 (s, 6H), 3.55–3.51 (m, 2H), 3.24–3.11 (m, 3H), 2.84–2.77 (m, 1H), 2.67–2.59 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): 150.2, 146.1, 145.9, 145.0, 130.8, 128.6, 127.8, 127.6, 123.9, 110.9, 108.4, 105.5, 100.7, 60.2, 59.6, 55.8, 53.9, 51.4, 36.4, 29.5. MS (ESI) m/z: 339(M^+). HRMS (ESI): Calcd for 340.1549, found 340.1542. The optical purity of **17a** was determined to be 97.8% by HPLC analyses (Chiralcel OD-H (0.46 cm × 25 cm; hexanes/*i*-PrOH = 70/30 with flow rate = 1.0 mL/min and a UV detector at 214 nm; retain time = 44 min).

5.1.28. (+)-Tetrahydroberberine (17d)

White solid, yield, 87% (for two steps); Mp 133–134 °C; $[\alpha]_D^{24}$ −184.3 (c 0.46, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.85 (d,

J = 8 Hz, 1H), 6.77 (d, J = 8 Hz, 1H), 6.72 (s, 1H), 6.58 (s, 1H), 5.91 (s, 2H), 4.25–4.21 (m, 1H), 3.84 (s, 6H), 3.55–3.51 (m, 2H), 3.24–3.11 (m, 3H), 2.84–2.77 (m, 1H), 2.67–2.59 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): 150.2, 146.1, 145.9, 145.0, 130.8, 128.6, 127.8, 127.6, 123.9, 110.9, 108.4, 105.5, 100.7, 60.2, 59.6, 55.8, 53.9, 51.4, 36.4, 29.5. MS (ESI) m/z: 339(M^+). HRMS (ESI): Calcd for 340.1549, found 340.1541. The optical purity of **17a** was determined to be 99.9% by HPLC analyses (Chiralcel OD-H (0.46 cm × 25 cm; hexanes/*i*-PrOH = 70/30 with flow rate = 1.0 mL/min and a UV detector at 214 nm; retain time = 21.84 min).

5.1.29. (−)-Stepholidine (18e)

Compound **17e** (254 mg, 0.5 mmol) was refluxed in a mixture of concentrated hydrochloric acid (13 mL) and EtOH (50 mL) for 2 h. The reaction mixture was cooled to 0 °C and basified with concentrated ammonia solution. The mixture was extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated to give the crude product, which was purified by column chromatograph ($MeOH/CH_2Cl_2$ = 1:60) to give **18e**.

White solid, yield, 75%; Mp 127–128 °C; $[\alpha]_D^{24}$ −197.3 (c 0.52, $CHCl_3$). ^{1}H NMR (CD_3OD, 300 MHz): δ 6.78–6.69 (m, 3H), 6.63 (d, 1H), 4.15 (d, J = 15.6 Hz, 1H), 3.79 (s, 3H), 3.78 (s, 3H), 3.49–3.41 (m, 2H), 3.30–3.06 (m, 3H), 2.73–2.57 (m, 3H). ^{13}C NMR (CD_3OD, 100 MHz): 149.2, 148.3, 146.4, 145.4, 131.2, 129.2, 127.7, 126.7, 125.9, 116.8, 113.6, 112.9, 61.1, 60.9, 56.8, 55.3, 53.3, 37.0, 29.7. MS (ESI) m/z: 327(M^+). HRMS (ESI): Calcd for 328.1549, found 328.1565. The optical purity of 17a was determined to be 99.9% by HPLC analyses (Chiralcel AD-H (0.46 cm × 25 cm; hexanes/*i*-PrOH = 70/30 with flow rate = 1.0 mL/min and a UV detector at 214 nm; retain time = 14.68 min).

5.1.30. (+)-Stepholidine (18f)

White solid, yield, 80%; Mp 128–129 °C; $[\alpha]_D^{24}$ −195.7 (c 0.53, $CHCl_3$). ^{1}H NMR (CD_3OD, 300 MHz): δ 6.79–6.70 (m, 3H), 6.65 (d, 1H), 4.16 (d, J = 15.6 Hz, 1H), 3.80 (s, 3H), 3.79 (s, 3H), 3.50–3.45 (m, 2H), 3.26–3.02 (m, 3H), 2.74–2.57 (m, 3H). ^{13}C NMR (CD_3OD, 100 MHz): 149.3, 148.3, 146.5, 145.5, 131.2, 129.3, 127.7, 126.8, 125.9, 116.8, 113.6, 113.0, 61.2, 60.9, 56.8, 55.4, 53.4, 37.0, 29.8. MS (ESI) m/z: 327(M^+). HRMS (ESI): Calcd for 328.1549, found 328.1530. The optical purity of **17a** was determined to be 99.9% by HPLC analyses (Chiralcel AD-H (0.46 cm × 25 cm; hexanes/*i*-PrOH = 70/30 with flow rate = 1.0 mL/min and a UV detector at 214 nm; retain time = 8.74 min).

5.2. Biology

5.2.1. Radioligand binding assays

The affinity of compounds to D_1 and D_2 dopamine receptors, 5-HT_{1A} and 5-HT_{2A} receptors were determined by competition binding assays. Membrane homogenates of HEK293T cells stably transfected with D_1, D_2, 5-HT_{1A} or 5-HT_{2A} receptors were prepared as described previously.[14,22] Duplicated tubes were incubated at 30 °C for 50 min(for D_1, D_2 and 5-HT_{1A}) or 37 °C for 15 min (for 5-HT_{2A}) with increasing concentrations of respective compound and with [^{3}H]SCH23390 (for D_1 dopamine receptors), [^{3}H]Spiperone (for dopamine D_2 receptor), [^{3}H]8-OH-DPAT (for 5-HT_{1A} receptor), or [^{3}H] Ketanserin (for 5-HT_{2A} receptor) in a final volume of 200 μL binding buffer containing 50 mM Tris, 4 mM $MgCl_2$, pH 7.4. Nonspecific binding was determined by parallel incubations with either 10 μM SCH23390 for D_1, Spiperone for D_2, WAY100635 for 5-HT_{1A}, or spiperone for 5-HT_{2A} receptors respectively. The reaction was started by addition of membranes (15 ng/tube) and stopped by rapid filtration through Whatman GF/B glass fiber filter and subsequent washing with cold buffer (50 mM Tris, 5 mM EDTA, pH 7.4) using a Brandel 24-well cell harvester. Scintillation cocktail was added and the radioactivity was determined in a MicroBeta liquid scintillation counter. The IC_{50} and K_i values were calculated by nonlinear regression (PRISM, Graphpad, San Diego, CA) using a sigmoidal function.

5.2.2. [^{35}S]GTPγS binding assays

For detection of the agonism action of the compounds, the [^{35}S]GTPγS binding assay was performed at 30 °C for 40 min (for D_1 and D_2) or 20 min (for 5-HT_{1A}) in reaction buffer containing 50 mM Tris, pH 7.5, 5 mM $MgCl_2$, 1 mM EDTA, 100 mM NaCl, and 1 mM DL-dithiothreitol (DTT). The assay mixture (200 μL) contained 20 μg (for D_2) or 30 μg (for D_1 and 5-HT_{1A}) of membrane protein, 0.1 nM [^{35}S]GTPγS, and 40 μM guanosine triphosphate (GDP) with various concentration of the compound. The antagonism effects of the compounds were tested in the existence of 100 μM SKF38393 for D_1 or quinpirole for D_2 receptor. Nonspecific binding was measured in the presence of 100 μM 50-guanylimidodiphosphate (Gpp(NH)p). The reaction was terminated by addition of 3 mL of ice-cold washing buffer (50 mM Tris, pH 7.5, 5 mM $MgCl_2$, 1 mM EDTA, and 100 mM NaCl) and was rapidly filtered with GF/C glass fiber filters (Whatman) and rinsed three times. Filters were dried and radioactivity was determined by liquid scintillation counting.

5.3. Animals

Male Sprague–Dawley rats (230–280 g), were purchased from Shanghai Slac Laboratory Animal Co. Ltd. (Shanghai, China). All animals were maintained under standard laboratory conditions and kept in temperature and humidity controlled rooms (22 ± 1 °C, 55 ± 5%) on a 12:12 h of light:dark cycle. Food and filtered water were available ad libitum. All procedures were in compliance with the Guidelines for the Care and Use of Laboratory Animals (National Research Council, People's Republic of China, 1996). The animal protocols were approved by the Institutional Animal Care and Use Committee of Shanghai Institute of Materia Medica (SIMM).

5.3.1. Locomotion test

Rats were placed into a Plexiglas open field arena (40 × 40 × 45 cm, Jiliang Co. Ltd., Shanghai, China) with a video camera connected to a video recorder. Automated activity was recorded for 30 min. The total distance and the distance traveled per 5 min were calculated by Jiliang Vision software (Jiliang Co. Ltd., Shanghai, China).

5.3.2. Prepulse inhibition test

PPI of the acoustic startle response was measured as described previously[47,48] in three startle chambers from Coulbourn Instruments (Whitehall, PA, USA). Briefly, following a 3-min acclimation period, animals were exposed to four different types of trial: a PULSEALONE trial in which a 40 ms, 120 dB white noise burst was presented; three PREPULSE (79 dB, 83 dB, 87 dB) + PULSE trials in which 20 ms, 3 kHz sounds were presented for 100 ms, respectively, before the onset of a 120 dB pulse. All trial types were presented 12 times in random order. In addition, 3 PULSE ALONE trials, which were not used in data analyses, were presented at the beginning of the test session to achieve a relatively stable level of startle reactivity for the remainder of the session. The inter-trial interval was ranged from 15 to 25 s. The total duration of the session was approximately 20 min. A ventilating fan built into the chamber provided a background noise of 74 dB throughout the test.

PPI values were calculated as percentages using the following formulation: %PPI = [1 − (startle response for PREPULSE + PULSE trial)/(startle response for PULSE ALONE trial)] × 100%. The acoustic startle magnitude was calculated as the average response of the PULSE ALONE trials (excluding the first 3 PULSE ALONE trials).

5.3.3. 5-HT_{1A} agonism-involved DA neuron firing

Rats were anesthetized with chloral hydrate (400 mg/kg, ip with supplemental doses administered via a lateral tail vein) and mounted in a stereotaxic instrument. Their body temperatures were maintained at 37 °C by means of a heating pad throughout the experiment. The skull was exposed, and the wound margins were infiltrated with a 0.3% solution of mepivacaine hydrochloride. A glass microelectrode (5–10 MΩ) filled with 2 M NaCl solution containing 2% pontamine skyblue dye was lowered through a small burr hole drilled above the VTA (3.0 mm anterior to the lambda, 0.5–0.9 mm lateral to the midline, 6.5–8.5 mm ventral to the cortical surface). DA neurons were identified and recorded as described:[55–58] positive/negative action potentials of long duration (2–5 ms), firing rate (1–10 Hz) with slow irregular or burst firing pattern, low pitch sound produced on audio amplifier, duration of 1.1 ms from the start of the action potential to the negative trough.[59] Interspike intervals (ISI) and firing rates were collected online to a personal computer.

All data analyses were performed using programs written in Visual Basic for Applications in Microsoft Excel.[60] Bursting was identified according to the '80/160 ms' definition proposed by Grace and Bunney.[55,56] Thus, the onset of a burst was identified as the concurrence of two spikes with an interspike interval less than 80 ms and the termination of a burst was defined as an interspike interval greater than 160 ms.[61] Data were analyzed off-line with a software package from BSPS Mathlib (Fudan University, Shanghai, China). The firing rate and bursting were analyzed every 10 s.[62] Effects of drugs were determined by comparing measures before and after drug injection using paired *t* test. All numerical data were expressed as mean (±SEM).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.12.016.

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