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RESEARCH ARTICLE

WILEY C&B DRUG DESIGN

Functional reversal of (−)-Stepholidine analogues by replacement of benzazepine substructure using the ring-expansion strategy

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(−)-Stepholidine is an active ingredient of the Chinese herb *Stephania* and naturally occurring tetrahydroprotoberberine alkaloid with mixed dopamine receptor D_1 agonistic and dopamine receptor D_2 antagonistic activities. In this work, a series of novel hexahydrobenzo[4,5]azepino [2,1-a]isoquinolines were designed and synthesized as ring-expanded analogues of (−)-Stepholidine. Initial pharmacological assays demonstrated that a benzazepine replacement was associated with significant increase in selectivity and functional reversal at dopamine receptor D_1. Compound-(−)-**15e** ($K_i = 5.32 \pm 0.01$ nM) is more potent than (−)-Stepholidine ($K_i = 13$ nM) and was identified as a selective dopamine receptor D_1 antagonist ($IC_{50} = 0.14$ μM). Moreover, molecular modeling suggested that (−)-**15e** might exert its dopamine receptor D_1 antagonistic activities through interacting with the transmembrane helix 7 of dopamine receptor D_1.

KEYWORDS
dopamine D1 receptor, Receptor and ligands (agonist/antagonist), selective D1R antagonists

As an important monoamine neurotransmitter in the CNS, dopamine plays an important role in reward-motivated behavior,[1,2] mood regulatory functions,[3] learning memory, attention, and executive functions.[4] Dopamine system dysfunction is associated with Parkinson's disease,[5,6] schizophrenia,[7–9] attention-deficit hyperactivity disorder,[10,11] and other CNS diseases. Among the five dopamine receptor subtypes currently identified, the dopamine receptor D1 (D1R) is most abundant. Although the clinical utility of D1R antagonists in the treatment of schizophrenia was limited,[12,13] these ligands were found to be effective in the treatment of other rare CNS conditions, such as pathological gambling[14] and Tourette's syndrome[15] in clinical trials. Therefore, discovery and development of novel D1R antagonists might represent a field of research interests for certain diseases of unmet medical need.

Unlike D_1 agonists with enormous structural diversity, most D_1 antagonists are benzazepine derivatives.[16] SCH 23390[17] (Figure 1) was the first reported selective dopamine D_1 antagonist, with potent binding affinities at both D_1 and D_5 dopamine receptors. This compound is now utilized as a pharmacological tool to investigate the pathological roles of dopamine D_1 receptors in the development of CNS diseases such as seizure, psychosis, and Parkinson's disease.[18,19] Another benzazepine-based D_1 antagonist, ecopipam (SCH 39166)[20] (Figure 1), has ever been developed for the indication of treatment of psychotic disorders. Moreover, it showed efficacy for obesity,[21] although its developability as an antiobesity agent was limited due to the adverse effects such as anxiety and depression.[22] Nevertheless, this agent is now in clinical studies to support the indication of Tourette's syndrome in children (ClinicalTrials.gov Identifier: NCT02102698).[15,23]

(−)-Stepholidine (*l*-SPD) (Figure 1) is a naturally occurring tetrahydroprotoberberine (THPB) alkaloid isolated from the Chinese herb *Stephania intermedia*.[24,25] It was identified as a unique dopaminergic ligand with dual D1R agonistic

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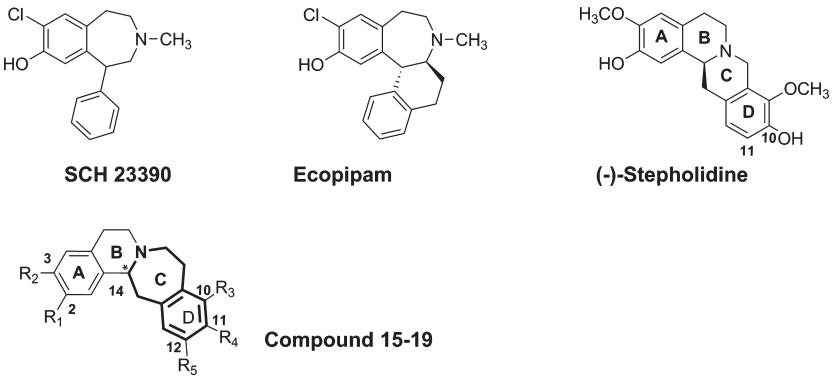


FIGURE 1 Structure of benzazepines, (−)-Stepholidine, and novel hexahydrobenzo[4,5] azepino [2,1-a]isoquinolines as dopamine ligands

and dopamine receptor D2 (D2R) antagonistic activities. It showed antipsychotic activities in animal models *in vivo*,[26] as well as potential efficacy against opiate addiction[27] and sleeping disorders.[28] Our prior efforts have been focused on the molecular mechanism of *l*-SPD to confer multiple pharmacological activities by *in silico* studies,[29,30] and more *l*-SPD derivatives or analogues are expected to be available not only to test the hypotheses we proposed *in silico* studies, but also to provide drug candidates with potential clinical utility.

In this work, the six-membered piperidine substructure (Ring-C) of *l*-SPD (Figure 1) was expanded to seven-membered benzazepine to yield hexahydrobenzo[4,5]azepino [2,1-a]isoquinolines (Figure 1, **15**–**19**), which hops over the THPB scaffold of *l*-SPD. The effect of this scaffold hopping on the pharmacological profiles of this series of derivatives was examined at the molecular level. As a consequence, unlike *l*-SPD that functions as a D1R agonist and D2R antagonist, some of hexahydrobenzo[4,5]azepino [2,1-a]isoquinolines were identified as potent and selective D1R antagonists, suggesting the probable existence of functional activity cliff between these structure categories.

1 | MATERIALS AND METHODS

1.1 | Chemistry

Commercially available chemicals were used directly without further purification. Moisture-sensitive reactions were implemented under an atmosphere of dry argon, and relevant solvents were dried according to standard procedures. Reactions were monitored using thin-layer chromatography on silica-coated plastic sheets (silica gel $60F_{254}$; Rushan Taiyang Desiccant Co., Ltd., Rushan, China) with the indicated eluant. Nuclear magnetic resonance spectra were recorded on a Brucker-DPX 400 MHz spectrometer (Bruker, Scientific Technology Co., Ltd., Beijing, China), and mass spectral data were collected on a HP5973N analytical mass spectrometer. Separation works of chiral compounds were implemented by DAICEL Daicel Chiral Technologies (Shanghai, China) using preparative chiral HPLC (Column: CHIRALCEL OJHs, Size: 0.46 cm I.D. × 15 cm L). The optical rotation of chiral compounds was determined on a Jasco (Easton, MD, USA) P-1020 Polarimeter.

1.2 | Synthesis procedures

The procedure for all intermediates and characterization was described in the supporting information supplemented.

1.3 | 2,3,11,12-tetramethoxy-5,6,8,9,14,14a-hexahydrobenzo[4,5]azepino[2,1-a]isoquinoline (15a)

This compound was prepared in the manner described above for compound **15e** using **14a** to afford the title compound in 70.1% yield as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.75 (s, 1H), 6.71 (s, 1H), 6.66 (s, 1H), 6.58 (s, 1H), 3.91–3.86 (m, 13H), 3.54 (dd, J = 15.7, 9.8 Hz, 1H), 3.31–3.59 (m, 3H), 3.01–2.89 (m, 2H), 2.81–2.74 (m, 3H), 2.58 (dd, J = 15.3, 5.9 Hz, 1H). ESI-MS m/z 370.3 $[M + H]^+$.

1.4 | 3,11,12-trimethoxy-5,6,8,9,14,14a-hexahydrobenzo[4,5]azepino[2,1-a]isoquinolin-2-ol (15b)

This compound was prepared in the method described above for compound **15e** using **14a** to afford the title compound in 52.6% yield as a light yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.84 (s, 1H), 6.77 (s, 1H), 6.64 (s, 1H), 6.55 (s, 1H), 3.91 (s, 3H), 3.87 (s, 3H),3.86 (s, 3H), 3.78 (d, J = 9.3 Hz, 1H), 3.47 (dd, J = 15.3, 9.5 Hz, 1H), 3.33–3.19 (m, 3H), 3.00–2.70 (m, 5H), 2.59 (dd, J = 14.5, 5.4 Hz, 1H).^{13}C NMR (100 MHz, $CDCl_3$) δ 147.0, 147.0, 145.1, 143.8, 134.1, 133.3, 131.9, 126.3, 113.4, 113.1, 113.0, 110.5, 63.3, 57.5, 56.1, 56.1, 55.9, 49.2, 42.9, 34.5, 29.3. ESI-MS m/z 356.2 $[M + H]^+$.

1.5 | 2,3,10,11-tetramethoxy-5,6,8,9,14,14a-hexahydrobenzo[4,5]azepino[2,1-a]isoquinoline (15c)

This compound was prepared in the manner described above for compound **15e** using **14c** to afford the title compound in 81.3% yield as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.95 (d, J = 8.2 Hz, 1H), 6.70 (d, J = 8.0 Hz, 2H), 6.57 (s, 1H), 3.89 (s, 3H), 3.86 (d, J = 2.5 Hz, 7H), 3.81 (d, J = 9.5 Hz, 1H), 3.76 (s, 3H), 3.51 (dd, J = 15.1, 9.5 Hz, 1H), 3.32–3.18 (m, 3H), 3.02–2.72 (m, 6H).^{13}C NMR (100 MHz, $CDCl_3$) δ 25.0, 29.1, 42.6, 48.2, 55.7, 55.8, 56.0, 56.8, 61.0, 63.0, 109.1, 110.2, 111.0, 124.3, 126.9, 131.1, 134.7, 136.1, 146.3, 147.1, 147.4, 151.2. ESI-MS m/z 370.1 $[M + H]^+$.

1.6 | 3,10,11-trimethoxy-5,6,8,9,14,14a-hexahydrobenzo[4,5]azepino[2,1-a]isoquinolin-2-ol (15d)

This compound was prepared in the manner described above for compound **15e** using **14d** to afford the title compound in 85.3% yield as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.95 (d, J = 8.2 Hz, 1H), 6.81 (s, 1H), 6.71 (d, J = 8.0 Hz,1H), 6.55 (s, 1H), 3.86 (s, 3H), 3.85 (s, 3H),3.76 (s, 3H), 3.72 (t, J = 5.1 Hz, 1H), 3.45 (dd, J = 15.3, 9.4 Hz, 1H), 3.29–3.21 (m, 3H), 3.00–2.68 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.3, 146.4, 145.1, 143.8, 136.0, 134.9, 132.0, 126.3, 124.5, 113.0, 110.5, 110.0, 63.4, 61.0, 57.2, 56.0, 57.8, 49.2, 42.9, 29.3, 25.4. ESI-MS m/z 356.2 $[M + H]^+$.

1.7 | 3,11-dimethoxy-5,6,8,9,14,14a-hexahydrobenzo[4,5]azepino[2,1-a] isoquinoline-2,10-diol (15e)

To a solution of $LiAlH_4$ (0.2 g, 5.27 mmol) suspended in dry THF (30 mL) was added **14e** (0.4 g, 1.13 mmol) in THF (10 mL) at 80 °C. The mixture was stirred for 3 h, and then, H_2O (1.9 mL) and 15% aqueous HCl (1.9 mL) were added successively. The reaction mixture was concentrated, and water (50 mL) was added and extracted with ethyl acetate (3 × 50 mL).The organic extract was washed three times with brine, dried over Na_2SO_4, filtered, and the solvent was removed. The resulting residue was purified by column chromatography, eluting with CH_2Cl_2: MeOH=20:1 to yield 0.11 g (28.6%) of **15e** as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.81 (s, 1H), 6.74 (d, J = 8.1 Hz, 1H), 6.66 (d, J = 8.1 Hz, 1H), 6.54 (s, 1H), 3.87–3.85 (m, 7H), 3.48 (dd, J = 15.3, 9.2 Hz, 1H), 3.36–3.19 (m, 3H), 3.05–2.89 (m, 4H), 2.85–2.72 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.3, 145.1, 143.9, 142.7, 127.1, 125.6, 120.0, 113.0, 110.4, 107.9, 63.0, 56.1, 55.9, 53.4, 49.2, 31.9, 29.7, 29.4, 24.2, 22.7, 14.1. ESI-MS m/z 342.1 $[M + H]^+$.

1.8 | 3,11-dimethoxy-5,6,8,9,14,14a-hexahydrobenzo[4,5]azepino[2,1-a] isoquinoline-2,10-diol (−)-15e

This compound was separated by preparative chiral HPLC (Column: CHIRALCEL OJHs, Size: 0.46 cm I.D. × 15 cm L MeOH/CH_3CN/DEA = 90/10/0.1 (v/v/v), Flow rate:1.0 mL/min, Wave length: UV 254 nm) from **15e** as an white solid: RT = 5.057 min, e.e. > 99%, $[\alpha]_D^{24} = -3.377°$ (c = 1.14, DMSO); ^{1}H NMR (400 MHz, DMSO) δ 8.69 (s, 1H), 8.35 (s, 1H), 6.67–6.62 (m, 3H), 6.55 (s, 1H), 3.74 (s, 3H), 3.69 (s, 3H), 3.43–3.36 (m, 2H), 3.27–3.21 (m, 1H), 3.14–3.07 (m, 3H), 2.76–2.67 (m, 3H), 2.57–2.46 (m, 2H). ESI-MS m/z 342.0 $[M + H]^+$.

1.9 | 3,11-dimethoxy-5,6,8,9,14,14a-hexahydrobenzo[4,5]azepino[2,1-a] isoquinoline-2,10-diol (+)-15e

This compound was separated by preparative chiral HPLC (Column: CHIRALCEL OJHs, Size: 0.46 cm I.D. × 15 cm L MeOH/CH_3CN/DEA = 90/10/0.1 (v/v/v), Flow rate: 1.0 mL/min, Wave length: UV 254 nm) from **15e** as an white solid: RT = 3.452 min, e.e. > 99%, ^{1}H NMR (400 MHz, DMSO) δ 8.69 (s, 1H), 8.35 (s, 1H), 6.67–6.62 (m, 3H), 6.55 (s, 1H), 3.74 (s, 3H), 3.69 (s, 3H), 3.43–3.36 (m, 2H), 3.27–3.21 (m, 1H), 3.14–3.07 (m, 3H), 2.76–2.67 (m, 3H), 2.57–2.46 (m, 2H). ESI-MS m/z 342.0 $[M + H]^+$.

1.10 | 5,6,8,9,14,14a-hexahydrobenzo[4,5] azepino[2,1-a]isoquinoline-2,3,10,11-tetraol (18)

A solution of **15a** (50 mg, 0.14 mmol) in 48%HBr was stirred at 90 °C for 7 h. The mixture was then quenched with saturated $NaHCO_3$ and extracted with EtOAc (3 × 20 mL). The organic layer was dried over anhydrous Na_2SO_4 filtered and purified by chromatography (DCM:MeOH = 20:1) to get the desired product as a light yellow solid (23 mg, 41.8%):^{1}H NMR (400 MHz, DMSO) δ 9.05 (s, 1H), 8.67 (s, 1H), 8.59 (s, 1H), 8.03 (s, 1H), 6.57 (s, 1H), 6.48 (d, J = 3.4 Hz, 2H), 6.37 (s, 1H), 4.01 (d, J = 7.2 Hz, 1H), 3.72 (d, J = 17.8 Hz, 1H), 3.19 (dd, J = 14.3, 9.1 Hz, 2H), 3.22–3.05 (m, 3H), 2.71–2.62 (m, 4H).ESI-MS m/z 314.2 $[M + H]^+$.

1.11 | 3,10,11-trimethoxy-5,6,8,9,14,14a-hexahydrobenzo[4,5]azepino[2,1-a]isoquinolin-2-yl acetate (19)

To a solution of **15d** (40 mg, 0.11 mmol), TEA (24 μL, 0.17 mmol) in DCM (10 mL) was added acetylchloride (12 μL, 0.11 mmol) and DMAP (13 mg, 0.11 mmol) at 0 °C, then stirred at room temperature for 4 h. The reaction mixture was poured into water (20 mL) and extracted with DCM (3 × 15 mL). The organic layer was washed with water (3 × 15 mL), dried over anhydrous Na_2SO_4, and filtered, and the solvent was removed under vacuum. Chromatography (DCM:MeOH = 50:1) afforded 35 mg (80.1%) of the desired product as a light yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.94 (d, J = 8.2 Hz, 1H), 6.92 (s, 1H), 6.70 (d, J = 8.2 Hz, 1H), 6.66 (s, 1H), 3.86 (s, 3H), 3.81 (s, 3H), 3.76 (s, 3H), 3.70 (d, J = 9.3 Hz, 1H), 3.43 (dd, J = 15.1, 9.5 Hz, 1H), 3.28–3.20 (m, 3H), 3.03–2.69 (m, 6H), 2.33 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 169.2, 151.3, 149.1, 146.4, 138.0, 136.0, 134.7, 133.6, 131.8, 124.5, 121.2, 112.2, 109.5, 63.3, 61.0, 57.4, 55.9, 55.8, 49.2, 43.2, 29.8, 25.6, 20.7. ESI-MS m/z 398.2 $[M + H]^+$.

1.12 | Biological evaluations

1.12.1 | Binding assays

All target molecules were profiled by the competitive binding assays for D1R, D2R, and D3R, respectively, using a membrane preparation harvested from stably transfected HEK293 cells. The transfection of plasmid and membrane preparations was conducted as described in our previous report, with 0.7 nM [^{3}H] SCH23390 (D1R) or [^{3}H]Spiperone (D2R and D3R) as the standard radioligands. 1 nM–10 μM *l*-SPD and *l*-SLR were used as the control for the assay. For the preliminary screening, the potential of each compound at a concentration of 10 μM to inhibit the binding of a tritiated-labeled ligand to its receptor was examined. For compounds with the percent inhibition greater than 90%, K_i/IC_{50} was determined. K_i was calculated based on the following formula: $K_i = IC_{50}/(1 + C/K_d)$.

1.12.2 | Functional assays

The membrane protein of HEK cells stably transfected with D1 receptor was used.[31] The [^{35}S]GTPγS binding assay was performed at 30 °C for 30 min containing 10 μg of membrane protein in a final volume of 100 μL with various concentration of the compound. The antagonism effects of the compounds were tested in the existence of 10 μM haloperidol for the D1R. The binding buffer contains 50 mM Tris, pH 7.5, 5 mM $MgCl_2$, 1 mM ethylenediamine tetraacetic acid (EDTA), 100 mM NaCl, 1 mM DL-dithiothreitol, and 40 μM guanosine triphosphate. The reaction was initiated by the addition of [^{35}S]GTPγS (final concentration of 0.1 nM). Non-specific binding was measured in the presence of 100 μM 50-guanylimidodiphosphate (Gpp(NH)p). The reaction was terminated by the addition of 1 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 washed three times. Radioactivity was determined by liquid scintillation counting. Again, *l*-SPD was used as the control of D1R agonist.[31,32]

1.12.3 | Molecular modeling

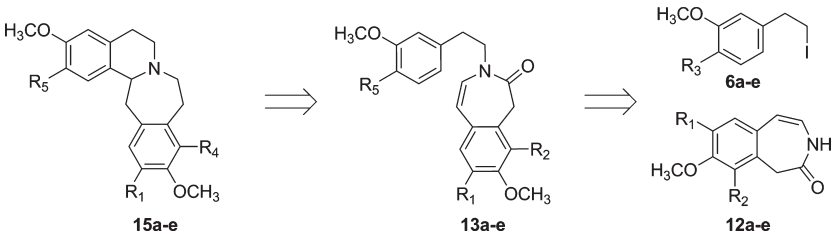
The active and inactive structures of D1R were built by homology modeling module encoded in Discovery Studio 3.5[33] by taking the crystal structures of active-state and inactive-state β_2AR as templates (PDB codes: 3SN6 and 2RIH). The sequence alignment of D1R and β_2AR is shown in Figure S1 (Supporting information), and the sequence identity and similarity is 38.5% and 63.8% between D1R and β_2AR, respectively. Fifty models for both active and inactive structures of D1R were generated after loop refinement and the one with the lowest discrete optimized protein energy score was submitted to the stepwise energy minimization to get the structure of D1R.

Molecular docking of *l*-SLR and (−)-**15e** (constructed and energy minimized in vacuum using SYBYL 6.9 with the MMFF94s force field) in the binding site of D1R was performed using GOLDSUITE 5.0 (The Cambridge Crystallographic Data Centre (CCDC), Cambridge, UK). The binding pocket was defined as amino acids within 15 Å of the Cγ carbon atom of D3.32. Ten conformations were produced for each ligand. Other parameters were set as default. Thirty docking orientations were obtained. The final docking pose was chosen based on the GOLD score and the predicted binding energy; in the meantime, the reported binding mode between *l*-SPD and D1 receptor and the results of mutation experiments on these receptors were also considered.

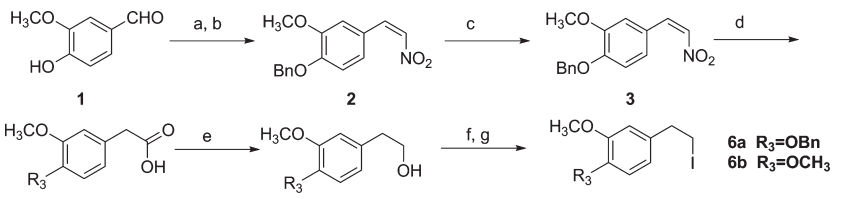
2 | RESULTS

2.1 | Chemistry

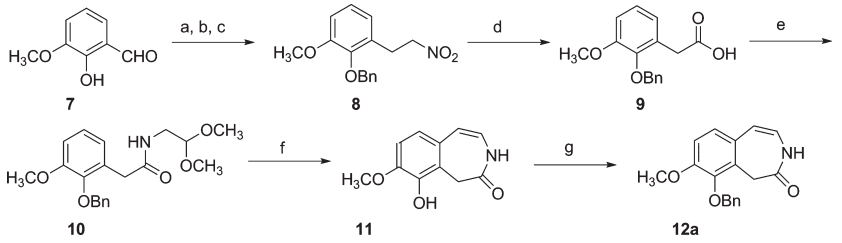
As depicted in retro-synthetic analysis (Scheme 1), the target molecule **15a–e** could be disconnected into the key intermediate **13a–e**, which could be further decomposed into two critical fragment **6a–e** and **12a–e**. With commercially available vanillin and nitromethane as the starting material, the critical fragment **6a–b** was prepared



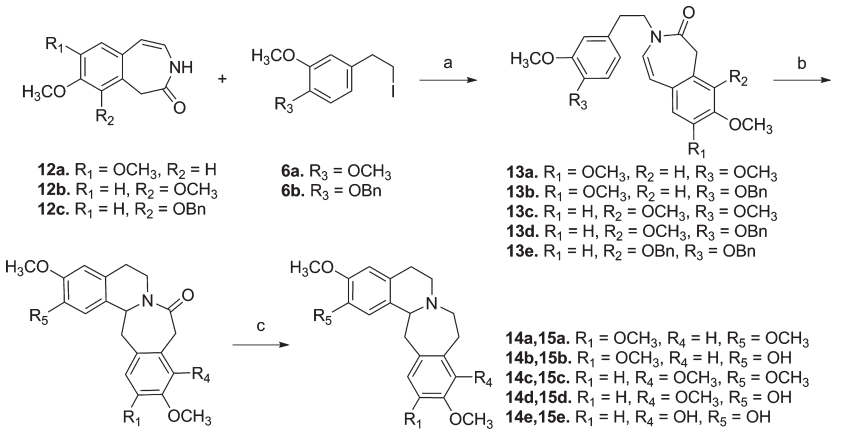
SCHEME 1 Retrosynthetic analysis of compounds **15–19**



SCHEME 2 Synthesis of the key fragment **6a–b**. Reagents and conditions: (a) BnBr, K_2CO_3, MeOH; (b) CH_3NO_2, NH_4OAc, AcOH; (c) KBH_4, THF; (d) $NaNO_2$, AcOH, DMSO; (e) $LiAlH_4$, THF; (f) TsCl, TEA, DCM; (g) NaI, acetone



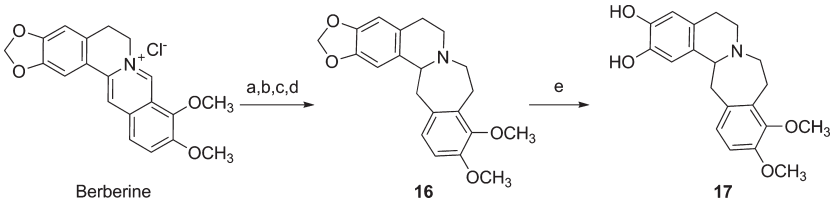
SCHEME 3 Synthesis of the key fragment **12a**. Reagents and conditions: (a) BnBr,K_2CO_3; (b) CH_3NO_2, NH_4OAc, AcOH; (c) KBH_4; (d) $NaNO_2$, AcOH, DMSO; (e) aminoacetaldehyde dimethyl acetal, EDC, HOBt, TEA; (f) HCl, HOAc; (g) BnBr, K_2CO_3



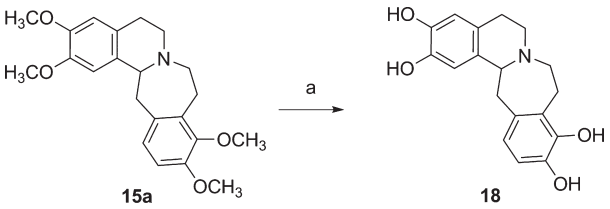
SCHEME 4 Synthesis of **15a–15e**. Reagents and conditions: (a) ^tBuOK, DMF; (b) HCl/EtOH; (c) $LiAlH_4$, THF

subsequently via Henry condensation, selective olefinic reduction, nitro group oxidation into carboxylic acids, reduction of carboxylate, and iodide substitution, as depicted in Scheme 2. Meanwhile, with *o*-vanillin as the starting material, the key intermediate **12a** was synthesized via alkylation, aldol-like condensation with nitromethane, selective olefinic reduction, nitro group oxidation into carboxylic acids, amidation, cyclization, and alkylation again[34] (Scheme 3). Furthermore, the other key fragments **12b** and **12c** (Scheme 4) were prepared via similar conditions, with 2-(3,4-dimethoxyphenyl) acetic acid and 2-(2,3-dimethoxyphenyl) acetic acid as the corresponding starting material. Intermediate **6a–b** and **12a–c** were condensed to afford the key intermediate **13a–e**, which was subsequently cyclized under acidic conditions to produce the expected molecule **15a–e** (Scheme 4).

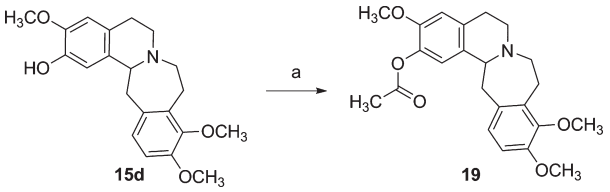
In addition, compound **16**, a hexahydrobenzo[4,5]azepino [2,1-a]isoquinoline, as well as its demethylated derivative compound **17** was prepared according to the procedure described in the literature[35] (Scheme 5). Compound **15c** was demethylated to produce compound **18** (Scheme 6). Compound **15d** was treated with acetyl chloride to afford the acylate compound **19** (Scheme 7). As to compound **15e**, the racemic mixture was resolved using preparative chiral HPLC to afford its optical isomers, for example, (+)-**15e** and (−)-**15e**.



SCHEME 5 Synthesis of **16** and **17**. Reagents and conditions: (a) $NaBH_4$, MeOH; (b) $BrCH_2CN$; (c) NaH, THF; (d) $NaBH_4$, EtOH; (e) BCl_3, DCM



SCHEME 6 Synthesis of compound **18**. Reagents and conditions: (a) 48% HBr, reflux



SCHEME 7 Synthesis of compound **19**. Reagents and conditions: (a) CH_3COCl, TEA, DCM

2.2 | Biological evaluation

Results of percent inhibition or K_i values and functional assays are summarized in Table 1 and Table 2, respectively.

3 | DISCUSSION

3.1 | Binding assays

Compared with the prototype compound *l*-SPD with THPBs scaffold, the ring-expanded analogues generally demonstrated loss in D2R binding affinities. However, the D1R binding affinities were maintained or minimally decreased for analogues with 2-hydroxy substitution, resulting in significant increase in D1R selectivity. In fact, the structure motif of *m*-hydroxy phenethylamine motif (as for *l*-SPD and its analogues, 2-hydroxy substitution) was considered to be the conserved substructure for ligands with dopamine agonistic activities, which was also supported by the significant loss of binding affinities in D1R for the analogues with 2-methoxy (compound-**15a**, compound-**15c**) or 2,3-dioxole (compound-**16**) without such substitution. In contrast, 3-hydroxy substitution also contributed to D1R binding affinities, although there were no substantial differences in D1R binding affinities between compound-**17** (220.27 ± 23.71 nM) with 3-hydroxy substitution versus compound-**15d** (30.28 ± 2.13 nM) with 3-methoxy substitution. In addition, compound-**15e** (8.80 ± 0.44 nM) with 10-hydroxy substitution demonstrated relatively higher D1R affinities than that of compound-**15d** (30.28 ± 2.13 nM) with 10-methoxy substitution, suggesting 10-hydroxy substitution

TABLE 1 Binding affinities of novel hexahydrobenzo[4,5]azepino [2,1-a]isoquinolines for dopamine receptors D1R, D2R, and D3R

	Inhibition % or K_i (±SEM, nM)		
Compound	**D1R**	**D2R**	**D3R**
15a	33.94	26.53	13.32
15b	69.09 ± 8.04	6.96	39.22
15c	57.27	29.82	21.97
15d	30.28 ± 2.13	83.95	284.34 ± 7.45
15e	8.80 ± 0.44	36.59	88.63
16	60.32	4.26	27.28
17	220.27 ± 23.71	39.24	61.20
18	33.74 ± 2.36	72.15	470.86 ± 15.58
19	474.10 ± 19.30	51.97	73.67
(+)-**15e**	72.76	34.40	19.85
(−)-**15e**	5.32 ± 0.01	58.26	1185.20 ± 43.31
l-SPD[a]	13	85	–
l-SLR[a]	42	180	–
SCH23390	1.69 ± 0.12	ND	ND
Spiperone	ND	1.08 ± 0.09	0.48 ± 0.07

ND, not determined.
[a]The binding affinities of *l*-SPD and *l*-SLR were cited from Xu's paper.[37]

TABLE 2 The [^{35}S]GTPγS binding assays of compound-**15d**, (−)-**15e**, and **18** for D1R

	D1R	
Compound	**EC_{50} (μM)**	**IC_{50} (μM)**
15d	–[a]	33.45
(−)**15e**	–	0.14
18	–	114.50
l-SPD	41.10[b]	–
SCH23390	–	0.52
SKF-38393	0.14	

[a][^{35}S] GTPγS binding activity could not be detected.
[b]EC_{50} value of *l*-SPD was cited from Dong et al.'s paper.[32]

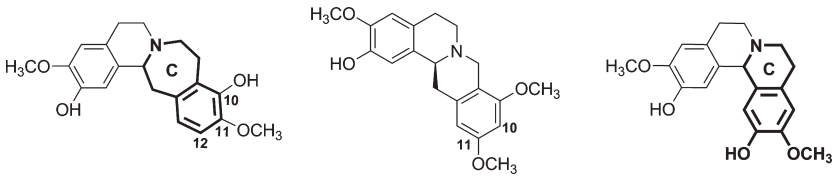


FIGURE 2 The chemical structures of related *l*-SPD analogues

might also contribute to D1R affinities. As to the optimal compound-**15e**, its (−)-isomer [(−)-**15e**] (5.32 ± 0.01 nM) showed much more potent binding affinities at D1R compared with its (+)-isomer [(+)-**15e**] (72.76% @ 10 μM), suggesting certain stereochemical requirement for these analogues to maintain favorable D1R binding affinities.

As for D3R binding affinities, only compound-**15**, compound-**18**, and compound-**15e** with 2-hydroxy substitution demonstrated mild-to-moderate D3R binding affinities, suggesting a role of this substituent conferring D3R binding activities.

3.2 | Functional assays

The optimal compounds identified in binding assays were further examined by functional assays to evaluate their biological functions (Table 2). In contrast to *l*-SPD with no intrinsic antagonistic activities on D1R, **15d**, (−)-**15e,** and **18** with the benzazepine replacement generally demonstrated pure D1R antagonistic activities. Moreover, in comparison with SCH23390 (IC_{50} = 0.52 μM), (−)-**15e** (IC_{50} = 0.14 μM) showed comparable or even higher antagonistic activities for D1R. According to the work depicted by Qian et al.[36] removal of C10 hydroxy group and introduction of a methoxy group at C11 of the pharmacophore of *l*-SPD can reverse the function of THPB s at the D1 receptor (Figure 2), resulting in the antagonistic activities against D1R. As the benzazepine replacement changed, the spatial orientation of the D-ring and all compounds with the benzazepine replacement assayed showed antagonistic activities against D1R, suggesting the disparity in the mechanism of functional reversal at D1R for the ring-expanded analogues of *l*-SPD might be different. Moreover, the substituents similar to those described by Qian et al.[36] could only regulate the extent of antagonistic potency other than the nature of biological function, indicating the correlation between the benzazepine replacement and the D1R antagonistic nature for *l*-SPD analogues.

3.3 | Molecular modeling

The effect of benzazepine replacement on the binding and function at D1R was further investigated for compound (−)-**15e**. Due to the similarities in the spatial orientation of ring-C and relevant substituents, *l*-Scoulerine (*l*-SLR, Figure 2), another naturally occurring product with similar binding profiles at dopamine receptors[37] was chosen as the reference compound for molecular modeling. *l*-SLR and (−)-**15e** were docked to the D1R by using GOLDSUITE 5.0[38] (Figure 3). The active and inactive structures of D1R were built by homology modeling, taking

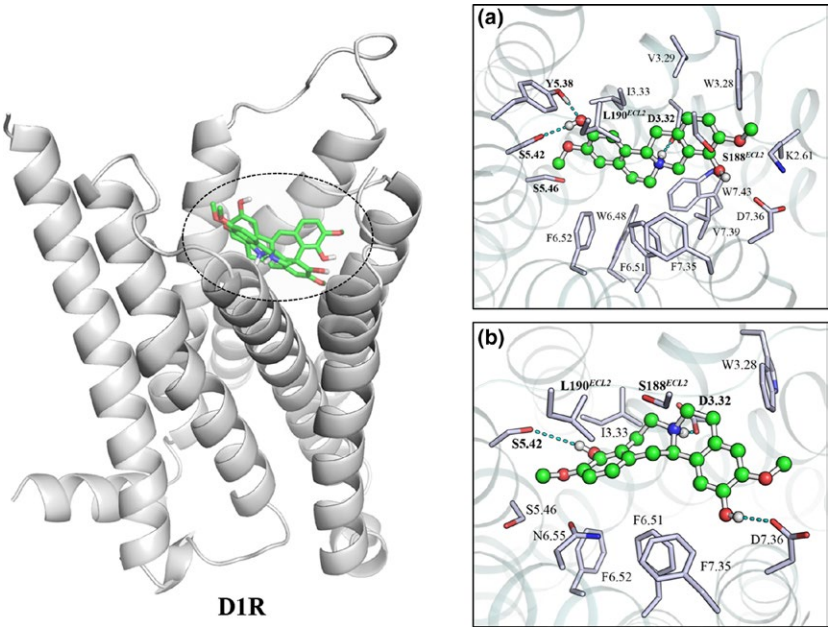


FIGURE 3 Predicted Binding mode of *l*-SLR (a) and (−)-**15e** (b) with D1R

the crystal structures of active-state and inactive-state β_2AR as templates (PDB ID: 3SN6[39] and 2RH1[40]). Based on the results of molecular docking, (−)-**15e** took a reverse docking pose in the active site of (−)-**15e**-D1R complex compared with *l*-SLR. In both models, the salt bridge between the protonated nitrogen atom of ligand and D3.32 was maintained, consistent with the fact that mutation of D3.32 is associated with the binding affinities of both antagonist and agonist among catecholamine receptors.[41,42] In the *l*-SLR-D1R complex, the 2-hydroxy group of *l*-SLR formed a hydrogen bond with S5.42. But in (−)-**15e**-D1R complex, the residue S5.42 interacted with the 10-hydroxy group of ligand via a hydrogen bonding interaction. Furthermore, in comparison with the rigid and flat THPB substructure of *l*-SLR, compound (−)-**15e** could take more flexible and twisted conformations as the consequence of benzazepine replacement. In addition, according to the docked model of (−)-**15e**-D1R complex, ring-A of ligand was extended to the transmembrane VII and the 10-hydroxy group formed another hydrogen bond with D7.36, indicating the possible role of TM7 in the antagonistic binding of compound-(−)-**15e**.

4 | CONCLUSIONS

In conclusion, as the ring-expanded analogues of *l*-SPD, a series of novel hexahydrobenzo[4,5]azepino [2,1-a]isoquinolines were designed, synthesized, and assayed. As demonstrated by preliminary SAR investigations, the benzazepine replacement was associated with significant increase in selectivity of D1R and its functional reversal. Compound (−)-**15e** was identified as a potent and selective D1R antagonist. This was in comparison with *l*-SPD with dual D1R agonistic and D2R antagonistic activities. Moreover, molecular modeling suggested (−)-**15e** might exert its D1R antagonistic activities through interacting with the TM7 of D1R.

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CONFLICT OF INTEREST

All authors declared no conflicts of interest.

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