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Synthesis, antidepressant activity and toxicity of the erythro/threo racemates and optical isomers of 2-(4-benzylpiperazin-1-yl)-1-(5-chloro-6- methoxynaphthalen -2-yl)hexan-1-ol

Running title: Synthesis, antidepressant activity and toxicity

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Abstract

The erythro/threo racemates and their four optical isomers of 2-(4-benzylpiperazin-1-yl)-1-(5-chloro-6-methoxynaphthalen-2-yl)hexan-1-ol were synthesized and evaluated for their antidepressant activity, toxicity, and pharmacokinetics as novel triple multiple reuptake inhibitors of monoamine transmitters. The racemates and optical isomers were synthesized respectively through two different routes. Pharmacological data indicate that the erythro racemate (**SIPI5357**) which has better inhibitory activity and lower toxicity than the other racemate and optical isomers is worthy of further evaluation.

Keywords: Erythro/threo racemate; optical isomer; antidepressant; triple reuptake inhibitor

Introduction

Depression is a major health problem estimated to affect 17% of the population during their lifetime. Economically, depression is responsible for tremendous health care costs, with a total of 53 billion dollars per year spent in the US alone (1,2).

Selective serotonin (5-HT) reuptake inhibitors (SSRIs) are extensively used as first-line treatment for major depression, but these drugs are not effective for 30% of depressed patients (3,4). Continuous research for novel antidepressants with improved efficacies and acceptable tolerability prompted discoveries of dual-reuptake inhibitors, such as venlafaxine, which blocks both the 5-HT transporter (SERT) and norepinephrine transporter (NET) (5-7).

Recently, a promising strategy for new antidepressant development targeting monoaminergic circuits

involves triple reuptake inhibitors, which simultaneously inhibit the reuptake of serotonin (5-HT), norepinephrine (NE), and dopamine (DA) by blocking their transporters. Preclinical and clinical studies indicate that a dopaminergic component may improve the speed of onset, efficacy or the side effect profile (8-11), when added to a single- or dual-uptake inhibitor. These triple reuptake inhibitors include DOV-21947, SEP-225289, and NS-2359.

Previously, we described the synthesis of 1-butyl-2-hydroxyl aryl alkanol piperazine derivatives and biological evaluation of their antidepressant activities in our patents (12). We discovered that the erythro (**SIPI5357**) and threo (**SIPI5358**) racemates of 2-(4-benzyl piperazin-1-yl)-1-(5-chloro-6-methoxy- naphthalen-2-yl) hexan-1-ol (**1**) have triple reuptake inhibitory activities for 5-HT, NE and DA. Furthermore, these compounds had potent antidepressant activities in the mouse forced swimming test (FST).

Herein we reported our synthesis of the erythro/threo racemates and their four optical isomers of 2-(4-benzylpiperazin-1-yl)-1-(5-chloro-6-methoxynaphthalen-2-yl)hexan-1-ol, and investigations of their antidepressant activity, toxicity, and pharmacokinetics as novel triple multiple reuptake inhibitors of monoamine transmitters. The results indicate that the erythro racemate (SIPI5357) has better inhibitory activity and lower toxicity than the other racemate and optical isomers is worthy of further evaluation.

Chemistry

Structurally, compound **1** has two chiral carbon centers and therefore contains four optical isomers. We studied the bioactivity and safety of each optical isomer and compared this with the erythro/threo racemates to identify an optimal drug candidate with high efficacy and low toxicity for further preclinical development.

The erythro racemate (**SIPI5357**) and the threo racemate (**SIPI5358**) were synthesized through 5 steps from1-chloro-2-methoxynaphthalene according to the methods described in Scheme **1**. These two racemates (**SIPI5357** and **SIPI5358**) were separated by column chromatography on silica gel (200–300 mesh; dichloromethane/methanol, 20:1 v/v).

Four individual optical isomers shown in Table **1** were prepared through 11 steps from their respective chiral norleucines as chiral educts, according to the methods described in Scheme **2**. Compound **9** was prepared from norleucine according to the procedure described in the literature (13).

Compound **12** was synthesized via Grignand reaction of **9** with **11**, then reduction, hydrolysis and chloro-substitution to obtain the key intermediate **17**, which was subsequently treated with **18** to form the piperazine ring. Compound **19** was a mixture of two epimerides, which had different Rf values by thin-layer chromatography and was separated by column chromatography on silica gel (200–300 mesh; dichloromethane/methanol, 20:1 v/v). Therefore, (1R, 2S)/(1S, 2S) isomers were prepared from L-norleucine and (1S, 2R)/(1R, 2R) isomers were prepared from D-norleucine through the above-mentioned synthetic process.

The yields and the optical rotation values of target compounds are shown in Table 1. All of the isomers were prepared with >99%ee, as determined by chiral HPLC analysis (Daicel 100 OJ-H chiral column, 5 μm, 250×4.6 mm; Mobile phase: N-hexane/isopropanol/diethylamine, 80:20:0.1 v/v/v; Temperature: 30 ℃; Detection: UV, 230 nm).

Compound **12** was reduced with $NaBH_4$ to yield **13** as a mixture of two isomers. The erythro-isomer was obtained as major product with formation of 5% of threo-isomer when $NaBH_4$ or AIP (aluminum isopropoxide) was used as the reductant. Meanwhile, compound **13** was obtained as a 1:1 isomer mixture using Pd/C catalyzed hydrogenation.

The conformation inherited from its chiral norleucine starting material was confirmed by comparing the coupling constants from their collective NMR spectra. The definitive configuration of **SIPI5357** was established by X-ray diffraction (Fig. 1). Crystallographic data (including structure factors) for the structure in this paper have been submitted with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 837280. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223-336033 or e-mail: deposit@ccdc.cam.ac. uk].

Pharmacology

According to the method described in the literature (14,15), rat synaptosomes were prepared from cerebral cortices and used with ^{3}H-labeled 5-HT, NE and DA to test reuptake inhibition of **SIPI5357**, **SIPI5358** and their four isomers *in vitro*. **SIPI5357** was also tested in a commercial (CEREP, www.CEREP.com) panel of 53 human targets (G-protein coupled receptors, ion channels and transporters).

Two depression-related behavioral models—the mouse forced tail suspension test (TST) and the rat forced swim test (FST) (16, 17) were used to measure the antidepressant activity of the six compounds. Acute and chronic (two-week) toxicities of these compounds were studied in the rat. Pharmacokinetics of the six compounds was also tested in the rat.

Results and discussion

The synthesized compounds described above were test for their inhibition of functional uptake of 5-HT, NE, and DA. The detailed results were summarized in **Table 1**.

Studies on the erythro and threo compounds revealed that the erythro conformation was relatively better for 5-HT and DA reuptake inhibitions (**SIPI5357** and **SIPI5358**, **Table 1**). The erythro compound **SIPI5357** showed good selectivity for 5-HT and DA reuptake inhibitions (5-HT, IC_{50}=0.87 μM; NE, IC_{50}=1.40 μM; DA, IC_{50}=0.96 μM), whereas the threo isomer **SIPI5358** was less potent and equal for all three transporters (5-HT, IC_{50}=1.59 μM; NE, IC_{50}=1.50 μM; DA, IC_{50}=1.30 μM).

For their four optical isomers, the D-conformation compounds **D-SIPI5357**, and **D-SIPI5358** exhibited higher potency for all three monoamine transporters than their L-conformation compounds.

On the basis of in vitro studies, the six target compounds were tested in the mouse TST and rat FST, which have predictive value for antidepressant-like activity. For comparative purpose, venlafaxine was also included in the experiment. The results of single administration and chronic treatment were displayed in **Table 2** and **Table 3**.

As show in **Table 2** (the single administration), the six target compounds have antidepressant efficacy in the mouse TST and rat FST. Compounds **SIPI5357**, **L-SIPI5357**, **SIPI5358**, and **D-SIPI5358** are dose dependently reduced the immobility time in the TST, which are statistically significant compared to the vehicle at 20 and 40 mg/kg dose. In the FST model, compounds **SIPI5357**, and **L-SIPI5358** are dose dependently reduced the immobility time.

All six target compounds reduced the immobility time in the TST and FST model after the chronic treatment (10 days, **Table 3**). Compounds **D-SIPI5357**, and **SIPI5358** are dose dependently reduced the immobility time in the TST, meanwhile, **SIPI5358** is dose dependently reduced the immobility time in the FST.

To explore the safety profile, the acute and chronic (two-week) toxicity of these six compounds were measured in SD rat. In the acute toxicity test, compound **SIPI5358** and its two threo-isomers

(**L-SIPI5358** and **D-SIPI5358**) took more safety risks than **SIPI5358** and its two erythro-isomers (**L-SIPI5357** and **D-SIPI5357**) at the dose of 400, 800, and 1600 mg/kg. In chronic toxicity tests, all of the six compounds were observed to induce apparent toxic reactions and animal death except **SIPI5357**. The minimum effective dosage of **SIPI5357** in the chronic toxicity test exceeded 400 mg/kg in rat (Table 4). These data suggest that compound **SIPI5357** have higher safety factor than the other five compounds.

Compound **SIPI5357** was further characterized in several CNS receptor binding assays to assess the selectivity and specific interactions with monoamine transporters. Compound **SIPI5357** was performed against 53 receptors at Cerep, and the default concentration for primary binding experiments was 10 μM. Results indicating inhibition greater than 50% were considered to represent significant effects test compound effects. Binding tests revealed that **SIPI5357** was not only a triple reuptake inhibitor but could inhibit 100% of 5-HT_{1A} at 10 μM, the further studies shown compound **SIPI5357** exhibit high affinity for 5-HT_{1A} receptor (IC_{50} = 21 nM, K_i = 13 nM). Thus, these results suggested that compound **SIPI5357** might be regarded as multi-target agent.

To further evaluate this series of compounds, the pharmacokinetics of the six compounds were studied in the rat. After intravenous injection 5mg/kg or intragastric administration 25mg/kg of the six compounds to Wister rats, there were no significant differences in pharmacokinetic behavior between the racemate and it's two optical isomers (**Table 5**). The absolute bioavailability of SIPI5358 was slightly higher than that of **SIPI5357**. After a single oral dose (25 mg/kg) of **SIPI5357** to rats, a Cmax = 291.1 g/L was obtained at 1.88 h. The elimination half-life of **SIPI5357** following oral administration was 6.77 h in rats. This compound also have a moderately orally bioavailable (F = 21.1%) in rats (**Table 5**).

Conclusion

The erythro/threo racemates and four optical isomers of 2-(4-benzylpiperazin-1-yl)-1-(5-chloro-6-methoxynaphthalen-2-yl) hexan-1-ol were synthesized and evaluated. Their *in vitro* and *in vivo* properties were studied and compared. Data indicate that **SIPI5357** is worthy of further investigation due to its significant antidepressant activity and lower toxicity compared with the other five compounds.

Experimental protocols

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian INOVA-400 spectrometer with TMS as an internal standard and DMSO-d_6 as solvent. Chemical shifts (d values) and coupling constants (*J* values) are given in ppm and Hz, respectively. ESI mass spectra were performed on an Agilent 6210 MS spectrometer. TLC analysis was carried out on silica gel plates GF254 (Qindao Haiyang Chemical, China). Silica gel column chromatography was performed with Silica gel 200~300 m (Sinopharm Chemical Reagent, China). Commercial solvents were used without purification. Analyses indicated by the symbols of the elements or functions were within ± 0.4 % of the theoretical values.

Synthesis of 1-(5-chloro-6-methoxynaphthalen-2-yl) hexan-1-one (**3**)

$AlCl_3$ (4.11 g, 0.031 mol, 1.3 equiv) was added to a solution of 1-chloro-2-methoxynaphthalene (5.45 g, 0.028 mol, 1.2 equiv) in dichloromethane 30 mL. This reactant was stirred for 1 hour at room temperature. Subsequently, hexanoyl chloride (3.19 g, 0.024 mol, 1 equiv) was added dropwise to the mixture, with the temperature controlled below 10℃. Then the reactant was warmed naturally to room temperature and stirred for another 1 h. The reaction solution was poured into a mixture of hydrochloric acid 20 mL and crashed ice 50 g under stirring. The organic phase was separated, washed with water till the aqueous phase being neutral, dried with Na_2SO_4 and concentrated under reduced pressure to give **3** as yellow oil (5.55 g, 80.5% yield). The product was used in the next step without further purification.

Synthesis of 2-bromo-1-(5-chloro-6-methoxynaphthalen-2-yl)hexan- 1-one (**4**)

$CuBr_2$ (8.93 g, 0.040 mol, 2 equiv) was added to a solution of compound **3** (5.82 g, 0.020 mol, 1 equiv) in ethyl acetate 50 mL and chloroform 50 mL. The reaction mixture was refluxed for 3 h and then filtered. The filtrate was washed with 100 mL water , dried with Na_2SO_4 and concentrated under reduced pressure. Light yellow crystalline solid **4** was obtained by recrystallized with ethanol (6.28 g, 85.0% yield). MS (ESI) m/z: 370 (M+1).

Synthesis of 2-(4-benzylpiperazin-1-yl)-1-(5-chloro-6-methoxynaphthalen-2-yl)hexan-1-one (**5**)

Potassium iodide (0.17 g, 0.001 mol, 0.1 equiv) and K_2CO_3 (4.84 g, 0.035 mol, 3.5 equiv) were added to the solution of 1-Benzylpiperazine (1.76 g, 0.01 mol, 1 equiv) and compound **4** (4.44 g, 0.012 mol, 1.2 equiv) in acetone 50 mL. The reaction was refluxed for 8 h. After filtered, the solvent was evaporated under reduced pressure. The residue was diluted with water 50 mL and extracted with ethyl

acetate (100 mL × 3). The organic phase was separated, dried with Na_2SO_4 and concentrated under reduced pressure. Then the concentrate was dissolved in 30 mL ethanol, and adjusted to pH 2 with HCl/ethanol (5N). The precipitated solid was filtered and recrystallized in methanol to obtain compound **5** as white solid (3.80 g, 82.0% yield). MS (ESI) m/z: 466 (M+1)

Synthesis of 2-(4-benzylpiperazin-1-yl)-1-(5-chloro-6- methoxynaphthalen-2-yl)hexan-1-ol (**1**)

$AlCl_3$ (4.67 g, 0.035 mol, 1 equiv) was added to a solution of aluminium isopropoxide (7.15 g, 0.035 mol, 1 equiv) in isopropanol 80 mL. The suspension was stirred for 30 min until it turned to clear at 45-50 ℃. A solution of compound **5** (16.28 g, 0.035 mol, 1 equiv) in isopropanol 160 mL was added to it. The reaction mixture was heated to 60-65 ℃ and stirred for 10h. Then the reactant was adjusted pH to 7 with 15% of NaOH aqueous solution (w/w) and extracted with dichloromethane (100 mL × 3). The organic phase was separated, dried with Na_2SO_4 and concentrated under reduced pressure to give **1** as yellow oil (14.25 g, 87.2% yield).

Synthesis of **SIPI5357** and **SIPI5358**

Compound **1** (14.25 g, 0.03 mol) was separated to the erythro and threo form by silica gel column chromatography (dichloromethane/methanol, 20:1, v/v). The erythro form was dissolved in 30 mL ethanol, and adjusted to pH 2 with HCl/ethanol (5 N). The precipitated solid was filtered and recrystallized in methanol to obtain **SIPI5357** as white solid (6.92g, 42.0% yield). Mp: 225.0-225.8 ℃. ^{1}H NMR δ (ppm): 0.72-1.60 (m, 9H, $CH_2CH_2CH_2CH_3$), 2.27-2.70 (m, 9H, CH & piperazine-H), 3.40 (s, 2H, CH_2Ph), 3.98 (s, 3H, OCH_3), 5.000-5.011 (d, 1H, CH, *J* = 4.4 Hz), 5.10 (s, 1H, OH), 7.20-8.02 (m, 10H, Ar-H); MS (ESI) m/z: 468 (M+1); Anal. Calcd for $C_{28}H_{35}ClN_2O_2$.2HCl: C, 62.28; H, 6.91; N, 5.19. Found: C, 62.20; H, 6.78; N, 5.30.

SIPI5358 was prepared from the threo form through the above synthetic process as white solid (6.87g, 41.7% yield). Mp: 223.1-224.3 ℃. ^{1}H NMR δ (ppm): 0.66-1.50 (m, 9H, $CH_2CH_2CH_2CH_3$), 2.42-2.84 (m, 9H, CH & piperazine-H), 3.49 (s, 2H, CH_2Ph), 4.02 (s, 3H, OCH_3), 4.501-4.523 (d, 1H, CH, *J* = 8.8 Hz), 5.11(s, 1H, OH), 7.26-8.08 (m, 10H, Ar-H); MS (ESI) m/z: 468 (M+1); Anal. Calcd for $C_{28}H_{35}ClN_2O_2$.2HCl: C, 62.28; H, 6.91; N, 5.19. Found: C, 62.41; H, 6.94; N, 5.09.

Synthesis of methyl 1-(6-methoxynaphthalen-2-yl)- 1-oxohexan-2-ylcarbamate (**12)**

Compound **9** and **11** was prepared according to the procedure described in the literature (13). Compound **9** (23.2 g, 0.1 mol, 1 equiv) prepared from L-norleucine was dissolved in anhydrous tetrahydrofuran 400 mL and the solution was stirred under N_2 atmosphere at -20 ℃. Then a solution of Compound **11** (52.28 g, 0.2 mol, 2 equiv) in tetrahydrofuran 350 mL was added dropwise to it. After stirring at that temperature for 2 hours, the mixture was elevated gradually to room temperature. The reactant was stirred for 10 h and concentrated under reduced pressure. Dichloromethane 500 mL and 1M phosphoric acid aqueous solution 200 mL was added to the residue and stirred for 30 min. The aqueous phase was separated and extracted with dichloromethane (200 mL × 1). The combined extract was dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum /ethyl acetate, 20:1, v/v) to obtain **12** as white solid (28.8 g, 87.4% yield). MS (ESI) m/z: 330 (M+1).

Synthesis of methyl 1-hydroxy-1-(6-methoxynaphthalen- 2-yl)hexan-2-ylcarbamate (**13)**

10% Pd/C 0.7 g (10% w/w) was added to the solution of compound **12** (7 g, 0.021 mol, 1 equiv) in methanol 70 mL. The mixture was stirred under H_2 (2.0~2.5 MPa) atmosphere at 45 ℃ for 20 h. Pd/C was filtered off and the filtrate was concentrated under reduced pressure to give **13** as white solid (7 g, 99.4% yield). MS (ESI) m/z: 332 (M+1).

Synthesis of 2-amino-1-(6-methoxynaphthalen-2-yl)hexan-1-ol (**14**)

30% of KOH aqueous solution 60 mL (w/w) was added to the solution of compound **13** (6 g, 0.018 mol, 1 equiv) in methanol 300 mL. The mixture was refluxed for 8 h and concentrated under reduced pressure. The residue was diluted with water 100 mL and extracted with dichloromethane (50 mL × 3). The extract was dried with Na_2SO_4 and concentrated under reduced pressure to obtain **14** as yellow oil (5.3 g, 97.7% yield). The product was used in the next step without further purification.

Synthesis of 4-butyl-5-(6-methoxynaphthalen-2-yl)oxazolidin-2-one (**15**)

CDI (6.3 g, 0.039 mol, 2 equiv) and triethylamine（5.9 ml, 0.058 mol, 3 equiv） was added to the solution of compound **14** (5.33 g, 0.0195 mol, 1 equiv) in dichloromethane 100 mL. The mixture was stirred for 24 h at room temperature. Then water (25 mL) was added and the mixture was stirred for 30 min. The organic phase was separated and washed with water again, dried with Na_2SO_4 and concentrated under reduced pressure to obtain **15** as yellow oil (5.75 g, 98.5% yield). The product was used in the next step without further purification.

Synthesis of 4-butyl-5-(5-chloro-6-methoxynaphthalen-2-yl) oxazolidin-2-one (**16)**

NaCl (2.3 g, 0.04 mol, 1.2 equiv) and 40% H_2SO_4 aqueous solution (10 ml, w/w) was added to the solution of compound **15** (10 g, 0.033 mol, 1.0 equiv) in dichloromethane (100 ml). 11% NaClO aqueous solution (50 ml, w/w, 0.074 mol, 2.2 equiv) was added dropwise to the mixture. After the addition, the reactant was stirred for 15 min at room temperature. Then saturated NaCl aqueous solution（100 mL）was added and the solution was extracted with dichloromethane (50 mL × 3). The extract was dried with Na_2SO_4 and concentrated under reduced pressure to give yellow oil. The oil was dissolved in the solution of hexane (50 mL) and ethyl acetate (25 mL) when heated to 60 ℃. The residue was cooled to room temperature. The precipitation was filtrated to give **16** as yellow solid (6 g , 54.5% yield). MS (ESI) m/z: 334 (M+1).

Synthesis of 2-amino-1-(5-chloro-6-methoxynaphthalen -2-yl)hexan-1-ol（**17**）

Compound **16** (5 g, 0.015 mol, 1 equiv) was added to the solution of NaOH aqueous solution (1 N, 27 mL) and methanol 50 mL. The mixture was refluxed for 20 h and concentrated under reduced pressure to remove methanol. The precipitation from the solution was filtrated to give **17** as yellow solid (4.2g, 91.3% yield). MS (ESI) m/z: 308 (M+1).

Synthesis of 2-(4-benzylpiperazin-1-yl)-1- (5-chloro-6-methoxynaphthalen-2-yl)hexan-1-ol (**19**)

Compound **18** was prepared according to the procedure described in the literature (18). Compound **17** (3.5 g, 0.011 mol, 1 equiv) and Compound **18** (5.3 g, 0.022 mol, 2 equiv） was added to the solution of triethylamine (5.7 g, 0.055 mol, 5 equiv) and CH_3CN (50 mL). The reactant was refluxed for 20 h and concentrated under reduced pressure. The residue was diluted with water (100 mL) and extracted with dichloromethane (50 mL × 3). The extract was dried with Na_2SO_4 and concentrated under reduced pressure to give **19** as yellow oil (5g, 95.6% yield).

Synthesis of **L-SIPI5357** and **L-SIPI5358**

Compound **19** was separated to the erythro and threo form by silica gel column chromatography (dichloromethane/methanol, 20:1 v/v). The erythro form was dissolved in 30 mL ethanol, and

adjusted to pH 2 with HCl/ ethanol (5N). The precipitated solid was filtered and recrystallized in methanol to obtain **L-SIPI5357** as white solid (2.34g, 40.5% yield). Mp: 225.1-225.8 ℃. ^{1}H NMR δ (ppm): 0.36-1.86 (m, 9H, $CH_2CH_2CH_2CH_3$), 3.54-3.80 (m, 9H, CH, piperazine-H), 3.96 (s, 2H, CH_2Ph), 4.42 (s, 3H, OCH_3), 5.519-5.530 (d, 1H, CH, *J* = 4.4 Hz), 5.77 (s, 1H, OH), 7.43-8.14 (m, 10H, Ar-H); EI-MS m/z: 468 (M+1); Anal. Calcd for $C_{28}H_{35}ClN_2O_2$.2HCl: C, 62.28; H, 6.91; N, 5.19. Found: C, 62.30; H, 6.94; N, 5.17.

L-SIPI5358 was prepared from the threo form through the above synthetic process (2.2g, 38.2% yield). Mp: 225.0-226.1 ℃. ^{1}H NMR δ (ppm): 0.33-1.84 (m, 9H, $CH_2CH_2CH_2CH_3$), 3.57-3.82 (m, 9H, CH, piperazine-H), 4.00 (s, 2H, CH_2Ph), 4.42 (s, 3H, OCH_3), 5.520-5.542 (d, 1H, CH, *J* = 8.8 Hz), 5.82 (s, 1H, OH), 7.44-8.18 (m, 10H, Ar-H); MS(ESI) m/z: 468 (M+1); Anal. Calcd for $C_{28}H_{35}ClN_2O_2$.2HCl: C, 62.28; H, 6.91; N, 5.19. Found: C, 62.50; H, 7.01; N, 5.02.

Synthesis of **D-SIPI5357** and **D-SIPI5358:**

D-SIPI5357 was prepared from D-norleucine through the above synthetic process (2.05 g, 35.5% yield). Mp: 223.1-224.3 ℃. ^{1}H NMR δ (ppm): 0.33-1.58 (m, 9H, $CH_2CH_2CH_2CH_3$), 3.62-3.79 (m, 9H, CH, piperazine-H), 3.95 (s, 2H, CH_2Ph), 4.42 (s, 3H, OCH_3), 4.913-4.924 (d, 1H, CH, *J* = 4.4 Hz), 5.07 (s, 1H, OH), 7.43-8.17 (m, 10H, Ar-H); MS(ESI) m/z: 468 (M+1); Anal. Calcd for $C_{28}H_{35}ClN_2O_2$.2HCl: C, 62.28; H, 6.91; N, 5.19. Found: C, 62.34; H, 6.88; N, 5.04.

D-SIPI5358: White solid (1.92 g, 33.2% yield). Mp: 223.0-224.2 ℃. ^{1}H NMR δ (ppm): 0.37-1.58 (m, 9H, $CH_2CH_2CH_2CH_3$), 3.60-3.74 (m, 9H, CH, piperazine-H), 3.97 (s, 2H, CH_2Ph), 4.39 (s, 3H, OCH_3), 4.910-4.932 (d, 1H, CH, *J* = 8.8 Hz), 5.22 (s, 1H, OH), 7.43-8.19 (m, 10H, Ar-H); MS(ESI) m/z: 468 (M+1); Anal. Calcd for $C_{28}H_{35}ClN_2O_2$.2HCl: C, 62.28; H, 6.91; N, 5.19. Found: C, 62.37; H, 6.88; N, 5.22.

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Figure, Table and Scheme Legends

Figure 1 SIPI5357 is mixture of (1S,2R) and (1R,2S) isomer.

Table 1 *In vitro* reuptake inhibition of target compounds for 5-HT, NE, DA receptors.

Table 2 *In vivo* antidepressant activity of target compounds after a single administration (n=12, PO).

Table 3 *In vivo* antidepressant activity of target compounds after chronic treatment (10 days, n=12, PO).

Table 4 Acute toxicity (n=10, female: male =1:1, PO) and chronic toxicity (two weeks, n=6, female: male =1:1, PO) of target compounds.

Table 5 Pharmacokinetic characterizations of target compounds（n=4）

Scheme 1. Reagents and conditions: (a) Hexanoyl chloride, $AlCl_3$, CH_2Cl_2, 0 ºC; (b) $CuBr_2$, ethyl acetate, $CHCl_3$, reflux; (c) 1-Benzylpiperazine, K_2CO_3, KI, acetone, reflux; (d) Aluminum isopropoxide, isopropyl alcohol, $AlCl_3$, 45–50 ºC; (e) silica gel (200–300 mesh) column chromatography, dichloromethane: methanol, 20:1 v/v.

Scheme 2. Reagents and conditions: (f) methyl chloroformate, 1N NaOH, rt; (g) $SOCl_2$, CH_2Cl_2, -10ºC; (h) N,O-dimethyl hydroxylamine hydrochloride, CH_2Cl_2, rt; (i)Mg, THF, 70ºC; (j) THF, -78 ºC; (k) H_2, 10%Pd/C, 2-2.5atm, CH_3OH, 45ºC; (i) 30% KOH/H_2O, CH_3OH, reflux; (m) CDI, Et_3N, CH_2Cl_2, rt;(n) NaClO/NaCl/40% H_2SO_4, CH_2Cl_2, rt; (o) 1N NaOH, MeOH, reflux; (p) TEA, CH_3CN, reflux; (q) silica gel (200–300 mesh) column chromatography, dichloromethane: methanol, 20:1 v/v.

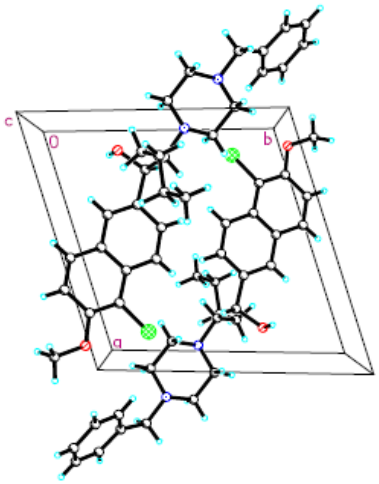
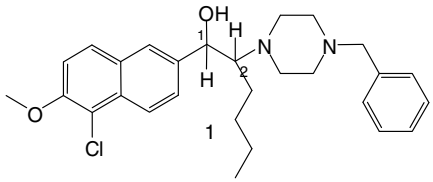


Figure 1. SIPI5357 is mixture of (1S,2R) and (1R,2S) isomer

Table 1 *In vitro* reuptake inhibition of target compounds for 5-HT, NE, DA receptors



Compd.	Formula	Config.	Yield (%)	$[\alpha]_D^{25}$(C1, CH_3OH)	IC_{50}(μM)		
					5-HT	NE	DA
SIPI5357	$C_{28}H_{35}ClN_2O_2.2HCl$	(1S,2R)/ (1R,2S)	42.0%	0	0.87	1.40	0.96
L-SIPI5357	$C_{28}H_{35}ClN_2O_2.2HCl$	(1R,2S)	37.3%	-2.2	1.89	2.10	1.01
D-SIPI5357	$C_{28}H_{35}ClN_2O_2.2HCl$	(1S,2R)	35.5%	2.9	0.83	1.40	0.67
SIPI5358	$C_{28}H_{35}ClN_2O_2.2HCl$	(1S,2S)/ (1R,2R)	41.7%	0	1.59	1.50	1.30
L-SIPI5358	$C_{28}H_{35}ClN_2O_2.2HCl$	(1S,2S)	38.2%	32.9	3.10	1.40	1.11
D-SIPI5358	$C_{28}H_{35}ClN_2O_2.2HCl$	(1R,2R)	33.2%	-32.7	1.19	1.21	1.10

Table 2 *In vivo* antidepressant activity of target compounds after a single administration (n=12, PO)

Compd.	Immobility (s) in mouse TST			Immobility (s) in rat FST		
	10mg/kg	20mg/kg	40mg/kg	5mg/kg	10mg/kg	20mg/kg
Blank		155.8±42.8			139.2±8.0	
Venlafaxine	NT	72.8±58.2[a]	NT	NT	123.4±8.3	NT
SIPI5357	127.8±27.9[a]	90.0±49.9[a]	63.2±38.7[a]	124.9±10.1	123.2±12.3	106.0±14.1[a]
L-SIPI5357	93.0±46.1[a]	75.5±58.3[a]	71.0±34.2[a]	105.5±12.6[a]	112.3±12.7	97.6±10.4[b]
D-SIPI5357	66.8±47.4[a]	62.3±57.5[a]	73.8±37.5[a]	110.2±11.4[a]	111.5±12.2	98.6±11.0[b]
SIPI5358	125.9±47.0	82.0±35.7[a]	55.7±33.2[a]	106.3±11.5[a]	107.7±11.2[a]	100.6±13.4[b]
L-SIPI5358	105.9±53.8	113.7±62.1	75.9±50.2	124.5±10.3	122.4±12.3	96.6±13.2[b]
D-SIPI5358	101.42±64.0	78.83±63.1	66.42±57.3	117.4±12.5	93.2±8.1[c]	104.5±11.5[a]

a: p<0.05; b: p<0.01; c: p<0.001; NT: no test

Table 3 *In vivo* antidepressant activity of target compounds after chronic treatment (10 days, n=12, PO)

Compd.	Immobility (s) in mouse TST			Immobility (s) in rat FST		
	10mg/kg	20mg/kg	40mg/kg	5mg/kg	10mg/kg	20mg/kg
Blank		124.7±65.9			154.2±11.4	
Venlafaxine	NT	82.1±48.7[a]	NT	NT	124.5±7.3[a]	NT
SIPI5357	80.2±51.9[a]	94.6±61.4	81.2±52.5[a]	107.2±10.4[b]	126.5±9.1[a]	121.3±12.2[a]
L-SIPI5357	57.2±47.7[a]	65.8±56.4[a]	61.5±37.5[a]	113.6±11.4[a]	148.5±7.9	127.3±9.4
D-SIPI5357	102.7±53.9	87.1±60.8	85.6±75.2	143.5±14.6	112.5±10.2[b]	140.4±12.5
SIPI5358	71.3±57.1[a]	63.3±36.9[a]	48.3±55.9[a]	130.5±10.2	119.4±10.5[a]	117.5±11.5[a]
L-SIPI5358	65.3±65.7[a]	84.7±56.7[a]	81.2±44.2[a]	125.4±13.5	131.6±9.1	131.9±11.2
D-SIPI5358	67.8±59.2[a]	107.0±67.8	64.8±51.1[a]	139.3±7.6	131.3±11.4	137.2±9.4

a: p<0.05; b: p<0.01; NT: no test

Table 4 Acute toxicity (n=10, female: male =1:1, PO) and chronic toxicity (two weeks, n=6, female: male =1:1, PO) of target compounds

Compd.	Acute toxicity/ mortality (%)				Chronic toxicity/ mortality (%)			
	0mg/kg	400mg/kg	800mg/kg	1600mg/kg	0mg/kg	100mg/kg	200mg/kg	400mg/kg
SIPI5357	0	0	0	0	0	0	0	0
L-SIPI5357	0	0	0	NT	0	0	0	0[a]
D-SIPI5357	0	0	0	NT	0	0	0[a]	0[a]
SIPI5358	0	0	60	100	0	0[a]	100[a]	100[a]
L-SIPI5358	0	0	0	0	0	0[a]	0[a]	50[a]
D-SIPI5358	0	0	30	80	0	16.7[a]	83.3[a]	100[a]

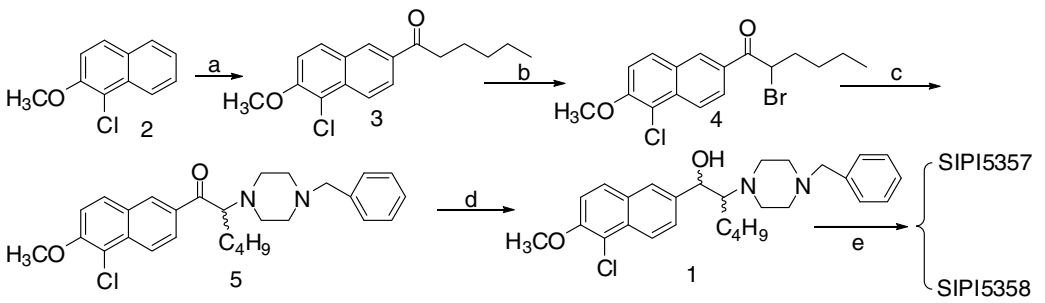
a: toxic reactions were observed, including: ALT, AST, ALP ↑;TP, ALB ↓;weight loss; drooling; adrenal glands tumefaction, et al. ; NT: no test

Table 5 Pharmacokinetic characterizations of target compounds （n=4）

Compd.	Dose (mg/kg)	*C*max	AUC (0-t)	AUC (0-∞)	MRT (0-t)	t1/2	Tmax	CLz	Vz	*F* (%)
		ug/L	ug/L*h	ug/L*h	h	h	h	L/h/kg	L/kg	
SIPI5357(iv)	5	910.5	2530.0	2724.9	5.55	6.47	0.13	7.46	69.5	21.1
SIPI5357(po)	25	291.1	2665.1	2909.0	7.37	6.77	1.88	6.89	67.5	
L-SIPI5357(iv)	5	853.3	3194.0	3853.1	7.47	9.29	NT	5.92	70.5	33.1
L-SIPI5357(po)	25	408.3	4328.3	5285.1	8.23	9.96	2.75	4.17	60.1	
D-SIPI5357(iv)	5	970.5	2822.3	2934.1	7.53	5.71	NT	5.83	84.9	23.1
D-SIPI5357(po)	25	399.5	2998.0	3172.4	6.41	5.46	2.00	6.31	49.5	
SIPI5358(iv)	5	887.1	1547.2	1574.1	3.93	4.08	NT	12.9	80.8	44.8

SIPI5358(po)	25	379.3	3467.1	3612.0	6.53	5.33	2.13	5.68	43.9	
L-SIPI5358(iv)	5	1137.2	2220.1	2253.0	4.32	3.51	NT	9.09	44.9	50.2
L-SIPI5358(po)	25	847.3	5577.0	5632.9	5.90	3.44	1.75	3.91	20.3	
D-SIPI5358(iv)	5	738.5	1638.1	1823.0	5.51	8.44	NT	11.0	134.1	32.7
D-SIPI5358(po)	25	318.0	2674.0	3264.1	7.95	9.14	1.00	7.94	99.7	

F: absolute bioavailability; NT: no test



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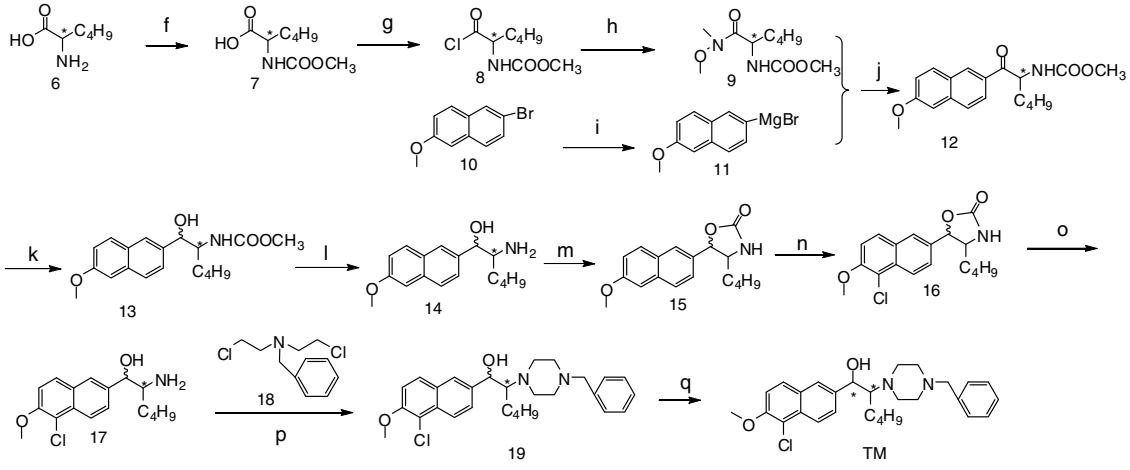

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