Supporting Information

Drug Repurposing of Haloperidol: Discovery of New Benzocyclane Derivatives as Potent Antifungal Agents against Cryptococcosis and Candidiasis

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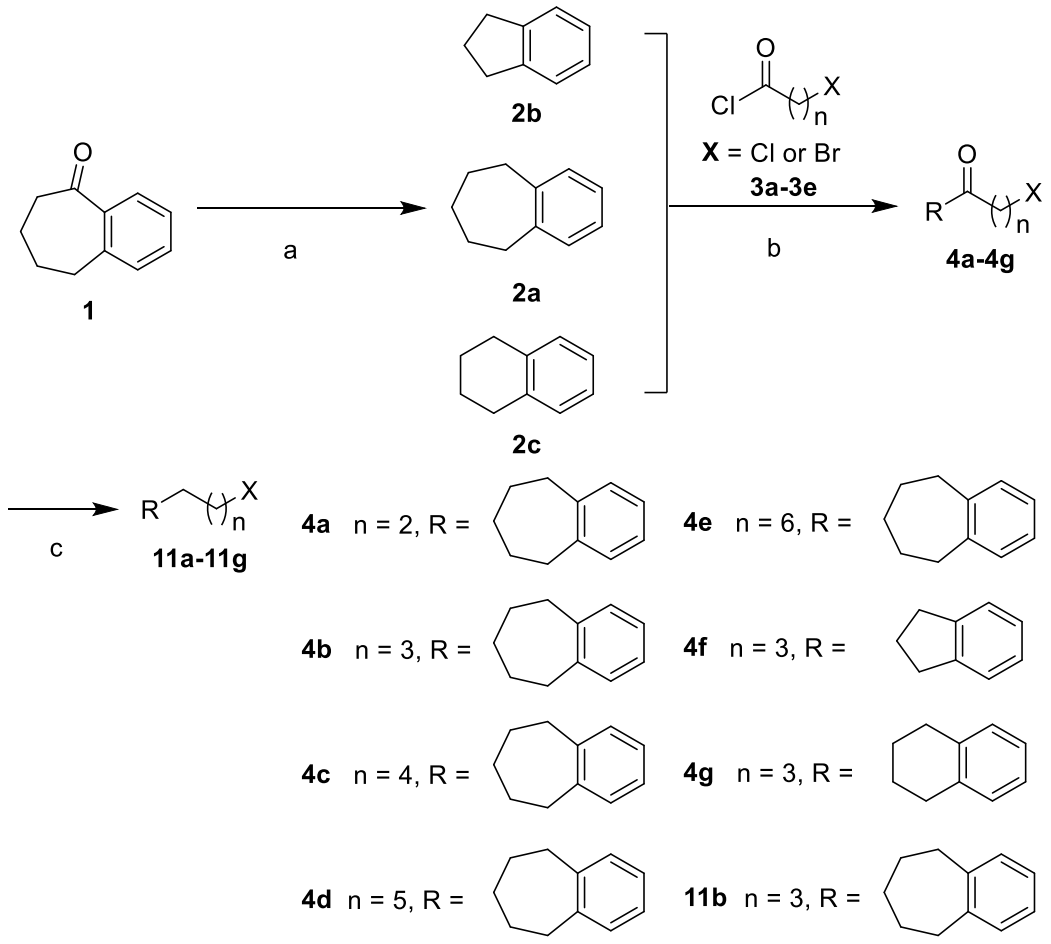
Chemical Synthesis and Structural Characterization of Intermediates 4a-4g, 7, 11b and 15a-15d.

General. ^{1}H NMR spectra were recorded on Bruker AVANCE300 or AVANCE600 spectrometer (Bruker Company, Germany), using TMS as an internal standard and $CDCl_3$ as solvent. Chemical shift is given in ppm (δ). TLC analysis was carried out on silica gel plates GF254 (Qingdao Haiyang Chemical, China). Silica gel column chromatography was performed with Silica gel 60 G (Qingdao Haiyang Chemical, China). Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification.

Scheme S1. Synthesis of Intermediate 4a-4g and 11b[a]



[a]. Reagents and Conditions: a) Et_3SiH, TFA, 60 °C, N_2, 4 h, yield 65.9%; b) $AlCl_3$, CH_2Cl_2, 0 °C, 1.5 h, yield 35.7-86.9%; c) Et_3SiH, TFA, 60 °C, N_2, 4 h, yield56.5-90.5%. **6,7,8,9-tetrahydro-5*H*-benzo[7] annulene (2a).** To a stirred solution of 6,7,8,9- tetrahydro-5*H*-benzo[7]annulen-5-one (200 mg, 1.25 mmol, 1.0

equiv) in 10 mL of TFA was added Et_3SiH (725 mg, 6.25 mmol, 5.0 equiv). The reaction mixture was stirred at 60 °C for 4 h under N_2. Most of TFA was removed by evaporation. Then the reaction mixture was diluted with EtOAc (30 mL) and H_2O (30 mL), separated. The water layer was extracted with EtOAc (30 mL), and the organic layers were combined and washed with saturated $NaHCO_3$ solution (20 mL) and NaCl solution (20 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel, 100% hexane) to afford compound **2a** (122 mg, 65.9% yield) as colorless oil.

4-Bromo-1-(6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-2-yl) butan-1-one (4b). To a stirred solution of compound **2a** (122 mg, 0.83 mmol, 1.0 equiv) and 4-bromopropanoyl chloride (171 mg, 1.00 mmol, 1.2 equiv) in 30 mL of CH_2Cl_2 was added $AlCl_3$ (133 mg, 1.00 mmol, 1.2 equiv) at 0 °C. The reaction mixture was stirred at 0 °C for 1.5 h. Then the reaction mixture was poured into 30 mL of ice water slowly with stirring. When the reaction was quenched, the mixture was poured into separotary funnel and separated. The water layer was extracted with 20 mL of CH_2Cl_2, and the organic layers were combined and washed with saturated $NaHCO_3$ (30 mL) and NaCl (30 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel, hexane/EtOAc = 100:15, v/v) to afford compound **4b** (202 mg, 86.9% yield) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.77-7.72 (m, 2H), 7.22 (d, J = 7.8 Hz, 1H), 3.71 (t, J = 6.6 Hz, 2H), 3.19 (t, J = 7.2 Hz, 2H), 2.93-2.82 (m, 4H), 2.30-2.21 (m, 2H), 1.89 (t, J = 6.0 Hz, 2H), 1.73-1.66 (m, 4H).

The synthesis of compounds **4a and 4c-4g** was similar to that of compound **4b.**

3-Chloro-1-(6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-2-yl)propan-1-one (4a). Light yellow oil (230 mg), yield: 61.7%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.71 (s, 1H), 7.69 (d, J = 7.8 Hz, 1H), 7.20 (d, J = 7.5 Hz, 1H), 3.93 (t, J = 6.9 Hz, 2H), 3.44 (t, J = 6.9 Hz, 2H), 2.91-2.81 (m, 4H), 1.93-1.80 (m, 2H), 1.74-1.60 (m, 4H).

5-Chloro-1-(6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-2-yl)butan-1-one (4c).

White solid (200 mg), yield: 76.4%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.01 (d, J = 7.2 Hz, 1H), 6.91 (s, 1H), 6.88 (d, J = 8.1 Hz), 3.42 (t, J = 6.6 Hz, 2H), 2.79-2.71 (m, 4H), 2.58 (t, J = 7.5 Hz, 2H), 1.94-1.73 (m, 6H), 1.63 (s, 4H).

6-Bromo-1-(6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-2-yl)hexan-1-one (4d). White solid (210 mg), yield: 71.4%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.70 (s, 1H), 7.67 (dd, J = 7.8, 1.8 Hz, 1H), 7.18 (d, J = 7.5 Hz, 1H), 3.43 (t, J = 6.9 Hz, 2H), 2.97 (t, J = 7.2 Hz, 2H), 2.91-2.79 (m, 4H), 1.98-1.89 (m, 2H), 1.89-1.80 (m, 2H), 1.80-1.71 (m, 2H), 1.71-1.60 (m, 4H), 1.60-1.47 (m, 2H).

7-Bromo-1-(6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-2-yl)heptan-1-one (4e). **L**ight yellow oil (130 mg), yield: 35.7%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.70 (s, 1H), 7.67 (d, J = 7.8 Hz, 1H), 7.18 (d, J = 7.5 Hz, 1H), 3.42 (t, J = 6.9 Hz, 2H), 2.95 (t, J = 7.2 Hz, 2H), 2.90-2.80 (m, 4H), 1.95-1.80 (m, 4H), 1.80-1.72 (m, 2H), 1.71-1.58 (m, 4H), 1.54-1.37 (m, 4H).

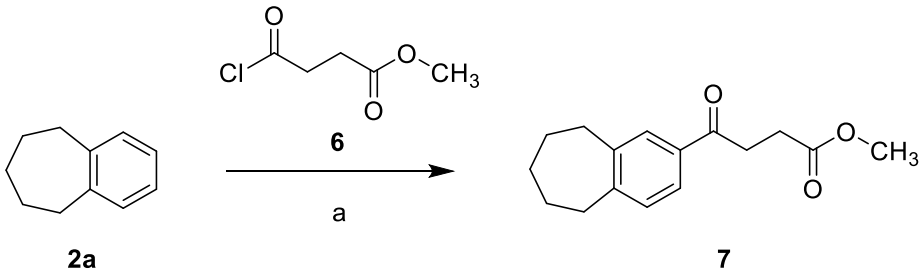
4-Chloro-1-(2,3-dihydro-1*H*-inden-5-yl)butan-1-one (4f). White solid (770 mg), yield: 81.7%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.84 (s, 1H), 7.78 (d, J = 7.8 Hz, 1H), 7.30 (d, J = 7.5 Hz, 1H), 3.68 (t, J = 6.3 Hz, 2H), 3.17 (t, J = 6.9 Hz, 2H), 2.96 (t, J = 7.5 Hz, 4H), 2.23 (p, J = 6.6 Hz, 2H), 2.12 (p, J = 7.5 Hz, 2H).

4-Bromo-1-(5,6,7,8-tetrahydronaphthalen-2-yl)butan-1-one (4g). White solid (850 mg), yield: 80.2%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.72-7.66 (m, 2H), 7.15 (d, J = 8.4 Hz, 1H), 3.55 (t, J = 6.3 Hz, 2H), 3.15 (t, J = 6.9 Hz, 2H), 2.89-2.75 (m, 4H), 2.39-2.26 (m, 2H), 1.93-1.71 (m, 4H).

2-(4-Bromobutyl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulene (11b). To a stirred

solution of compound 4b (202 mg, 0.72 mmol, 1.0 equiv) in 15 mL of TFA was added Et_3SiH (418 mg, 3.60 mmol, 5.0 equiv). The reaction mixture was stirred at 60 °C for 4 h under N_2. Most of TFA was removed by evaporation. Then the reaction mixture was diluted by EtOAc (30 mL) and H_2O (30 mL), separated. The organic layers were combined and washed with saturated $NaHCO_3$ solution (20 mL) and NaCl solution (20 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel, 100% hexane) to afford compound **11b** (174 mg, 86.4% yield) as colorless oil.

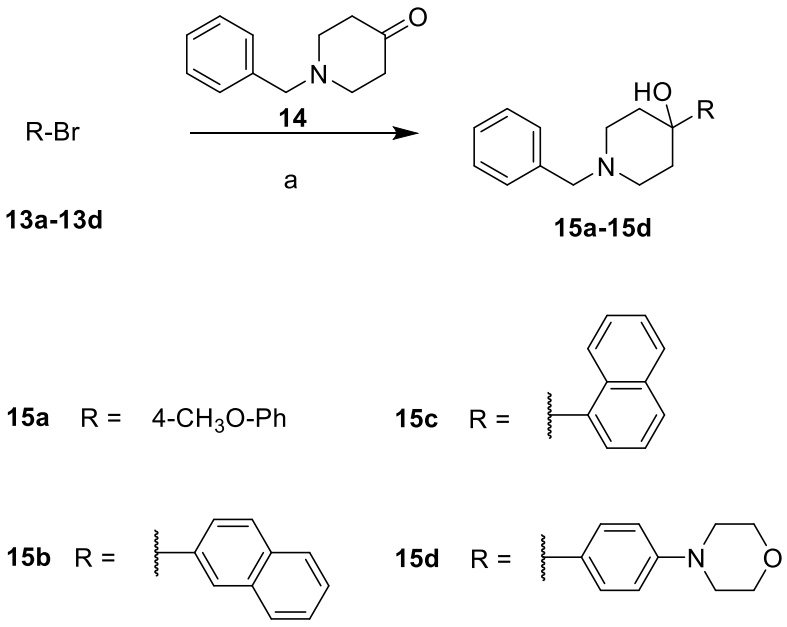
Scheme S2. Synthesis of Intermediate 15a-15d[a]



[a] Reagents and Conditions: a) $AlCl_3$, CH_2Cl_2, 0 °C, 1.5 h, yield 69.1%.

Methyl 4-oxo-4-(6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-2-yl)butanoate (7). To a stirred solution of methyl 4-chloro-4-oxobutanoate (250 mg, 1.66 mmol, 1.0 equiv) and 6,7,8,9-tetrahydro-5*H*-benzo[7]annulene (243 mg, 1.66 mmol, 1.0 equiv) in 30 mL of CH_2Cl_2 was added $AlCl_3$ (265 mg, 1.99 mmol, 1.2 equiv) at 0 °C. The reaction mixture was stirred at 0 °C for 1.5 h. Then the reaction mixture was poured into 30 mL of ice water slowly with stirring. After quenching the reaction, the mixture was poured into separotary funnel and separated. The water layer was extracted with 20 mL of CH_2Cl_2, and the organic layers were combined and washed with saturated $NaHCO_3$ (30 mL) and NaCl (30 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel, hexane/EtOAc = 100:15, v/v) to afford compound **7** (298 mg, 69.1% yield) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.72 (s, 1H), 7.71 (dd, J = 7.8, 1.8 Hz, 1H), 7.18 (d, J = 7.8 Hz, 1H), 3.71 (s, 3H), 3.30 (t, J = 6.6 Hz, 2H), 2.88-2.82 (m, 4H), 2.76 (t, J = 6.6 Hz, 2H), 1.89-1.82 (m, 2H), 1.69-1.62 (m, 4H).

Scheme S3. Synthesis of Intermediate 15a-15d[a]



[a] Reagents and Conditions: a) n-BuLi, THF, -78 °C-rt, N_2, overnight, yield 48.1-55.4%.

1-Benzyl-4-(4-methoxyphenyl)piperidin-4-ol (15a). To a stirred solution of 1-bromo -4-methoxybenzene (374 mg，2.00 mmol, 1.0 equiv) in 20 mL of anhydrous THF was added n-BuLi (0.88 mL, 2.5 M in hexane, 2.1 mmol, 1.05 equiv) dropwith at -78 °C under N_2. The reaction mixture was stirred at -78 °C for 1.0 h, and then 1-benzylpiperidin-4-one (265 mg, 1.4 mmol, 1.2 equiv) was added dropwith. The resulting mixture was stirred for another hour at -78 °C under N_2. The reaction was quenched by the addition of saturated NH_4Cl solution (30 mL). Then the reaction mixture was diluted with EtOAc (30 mL), separated, and the water layer was extracted with EtOAc (20 mL). The organic layers were combined, washed with saturated NaCl solution (30 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH/Et_3N = 100:1:0.5, v/v/v) to afford compound **15a** (295 mg, 49.6% yield) as slight yellow oil. ^1H NMR (300 MHz, $CDCl_3$, TMS) δ: 7.37 (d, J = 8.7 Hz, 2H), 7.33 (s, 2H), 7.32 (s, 2H), 7.28-7.22 (m, 1H), 6.85 (d, J = 9.0 Hz, 2H), 4.68 (s, 1H), 3.72 (s, 3H), 3.49 (s, 2H), 2.63-2.54 (d, J = 10.8 Hz, 2H), 2.41 (t, J = 10.8 Hz, 2H), 1.88 (td, J = 12.6, 4.2 Hz, 2H), 1.58 (s, 1H), 1.53 (s, 1H).

1-Benzyl-4-(naphthalen-2-yl)piperidin-4-ol (15b). White solid (273 mg), yield: 48.1%. ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ: 7.96 (s, 1H), 7.89-7.78 (m, 3H), 7.65 (d, J = 8.7 Hz, 1H), 7.51-7.44 (m, 2H), 7.44-7.28 (m, 5H), 3.65 (s, 2H), 2.96-2.79 (m, 2H), 2.67-2.51 (m, 2H), 2.42-2.25 (m, 2H), 1.85 (s, 1H) , 1.81 (s, 1H).

1-Benzyl-4-(naphthalen-1-yl)piperidin-4-ol (15c). White solid (242 mg), yield: 55.4%. ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ: 8.90 (d, J = 9.0 Hz, 1H), 7.91-7.82 (m, 1H), 7.77 (d, J = 8.1 Hz, 1H), 7.55 (d, J = 7.2 Hz, 1H), 7.51-7.44 (m, 2H), 7.44-7.26 (m, 7H), 3.65 (s, 2H), 2.87 (d, J = 10.8 Hz, 2H), 2.69 (t, J = 11.7 Hz, 2H), 2.46-2.31 (m, 2H), 2.26 (s, 2H) , 2.22 (s, 2H).

4-(4-Morpholinophenyl)piperidin-4-ol (15d). White solid (241 mg), yield: 54.1%. ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ: 7.37-7.28 (m, 6H), 7.28-7.21 (m, 1H), 6.87 (d, J = 9.0 Hz, 2H), 4.61 (s, 1H), 3.80-3.68 (m, 4H), 3.49 (s, 2H), 3.12-3.00 (m, 4H), 2.58 (d, J = 10.8 Hz, 2H), 2.41 (t, J = 10.8 Hz, 2H), 1.87 (td, J = 12.6, 3.6 Hz, 2H), 1.55 (d, J = 12.6 Hz, 2H).

***In vitro* Blood-Brain Barrier (BBB) Permeation Assay.** Brain penetration of compounds was performed using PAMPA, according to reported protocol.[1, 2] The acceptor 96-well microplate was filled with 300 μL of PBS solution (70% PBS in EtOH), and the filter membrane was soaked with 4 μL of porcine brain lipid (PBL) solution (20 mg/mL in dodecane). 200μL of compound solution (100 μg/mL in PBS solution) was added to the donor wells (PVDF membrane, pore size 0.45 mm). The acceptor filter plate was placed on the donor plate, which was allowed to stand for 12 h at 25°C. After incubation, the donor plate was carefully removed and the concentration of compounds in the acceptor wells was determined using a UV plate reader (SpectraMax i3). Three replicates were performed for each group.

Table S1 Permeability ($P_e \times 10^{-6}$ cm/s) of compound **B10** in the PAMPA-BBB assay.

	P_e[a] (10^{-6} cm/s)	prediction[b]
Clonidine	3.71 ± 0.33	CNS±
B10	1.18 ± 0.22	CNS±

[a] Results were represented as the mean ± standard deviation for three independent experiments .

[b] Compounds with $Pe > 3.08 \times 10^{-6}$ cm/s was considered able to cross the BBB by passive diffusion (CNS+). Compounds with $Pe < 1.13 \times 10^{-6}$ cm/s was considered doesn't able to cross the BBB (CNS−), and compounds with 1.13×10^{-6} cm/s < Pe < 3.08×10^{-6} cm/s showed uncertain BBB permeation (CNS±).[2]

Table S2. Primers for real-time RT-PCR in this study (5' to 3')

Name	Sequence
ERG11-F	ACTCATGGGGTTGCCAATGT
ERG11-R	GAGCAGCATCACGTCTCCAA
MDR1-F	CCACTGGTGGTGCAAGTGTT
MDR1-R	TCGTTACCGGTGATGGCTCT

HTRF assay. G protein-coupled receptors (including D2R) assays are often carried on by detecting the production of intracellular secondary messengers such as cyclic AMP (cAMP), Ca^{2+} and inositol triphosphate.[3] Herein, the binding activity of compound **B10** with D2R was determined by the HTRF cAMP dynamic kit according to the manufacturer's protocol with a few modifications. In brief, Chinese hamster ovary (CHO) cells were grown to 80% confluence, then the culture medium was removed and the cells were washed with 5 mL PBS. PBS was removed and 1.5 mL versene was added , then the cells were incubated at 37 ℃ for 2-5 min. Stimulation Buffer (5 mM HEPES, 0.5 mM IBMX and 0.1% BSA in HBSS) was used to adjust the cell suspension to 2.5×10^5 cells/ml. 10ul of cell solution was transfered to a white 384-well ProxiPlate with 3 μM of forskolin, 30 nM of dopamine and different concentrations of compounds, incubated 60 minutes at room temperature. Subsequently, 5 μL 4X Eu-cAMP tracer solution and 5 μL 4X ULight™-anti-cAMP solution was added to the assay plate, and further incubated 60 minutes at room temperature before reading with an EnVision™ plate reader (PerkinElmer).

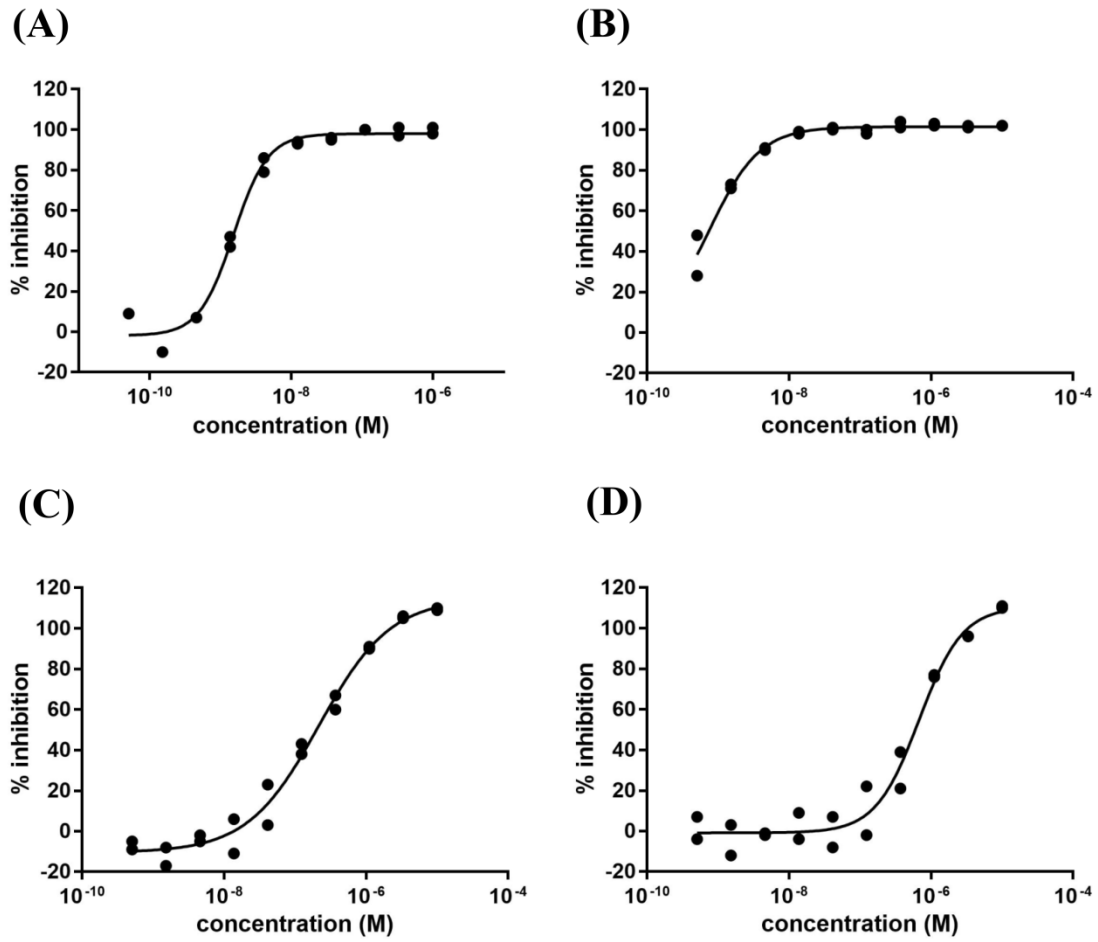


Figure S1. The dose response curve of Risperidone (A), Haloperidol (**B**), compound **A1** (**C**), compound **B10** (**D**) by HTRF cAMP assay.

In vitro **Metabolic Stability Assay.** The i*n vitro* metabolic stability was determined according to reported protocol[4] with a few modifications. Potassium phosphate buffer (0.1 mM, pH 7.4) was preheated at first. Dispense 30 μL of 1.5 μM spiking solution of test and reference compounds (Ketanserin) containing 0.75 mg/mL microsomes solution to the assay plates designated for different time points (0, 5,15,30,45 min). For 0 min, add 150 μL of ACN containing the internal standard (200 ng/mL Tolbutamide) to the wells of 0 min plate and then add 15 μL of NADPH stock solution (6 mM). Pre-incubate all other plate at 37 °C for 5 minutes, and then add 15

μL of NADPH stock solution to the plates to start the reaction and timing. At 5-min, 15-min，30-min and 45-min add 150 μL of ACN containing Tolbutamide to the wells of corresponding plates to stop the reaction, respectively. After quenching, shake the plates at the vibrator for 10 min (600 rpm/min) and then centrifuge at 6000rmp for 15 min. Transfer 80 μL of the supernatant from each well into a 96-well sample plate containing 120 μL of water for LC/MS analysis.

The Evaluation of the Production of Capsular Polysaccharide. The evaluation of the production of capsular polysaccharide was performed according to the reported protocol with a few modifications.[5] A colony of *C. neoformans* was inoculated in 100 mL of Yeast Nitrogen Base (YNB) medium, and cultured for 4 days at 30 °C with constant shaking (200 r.p.m.). The *C. neoformans* was spinned down (9000 r.p.m, 10 min) and weighed, as the supernatant was collected. Then 3.0 volumes of EtOH were added slowly to the supernatant as a white precipitate appeared. The suspension was stayed still overnight at 4 °C, then the precipitate was collected by centrifugation and air dried to remove EtOH. The precipitate was dissolved with 3 mL of dH_2O, and that was the capsular polysaccharides (GXM and GalXM). The polysaccharide concentration by the phenol sulfuric method.[6]

Sterol Composition Analysis. Sterol composition analysis was performed according to the reported protocol with a few modifications.[7] *C. albicans* cells during the exponential growth period were harvested and re-suspended to 1×10^6 CFU/mL in 30 mL of YPD medium untreated or treated with 16 μg/mL of FLC and/or compound B10. The cells were collected and weighed after being cultured at 35 ºC for 24 h, and

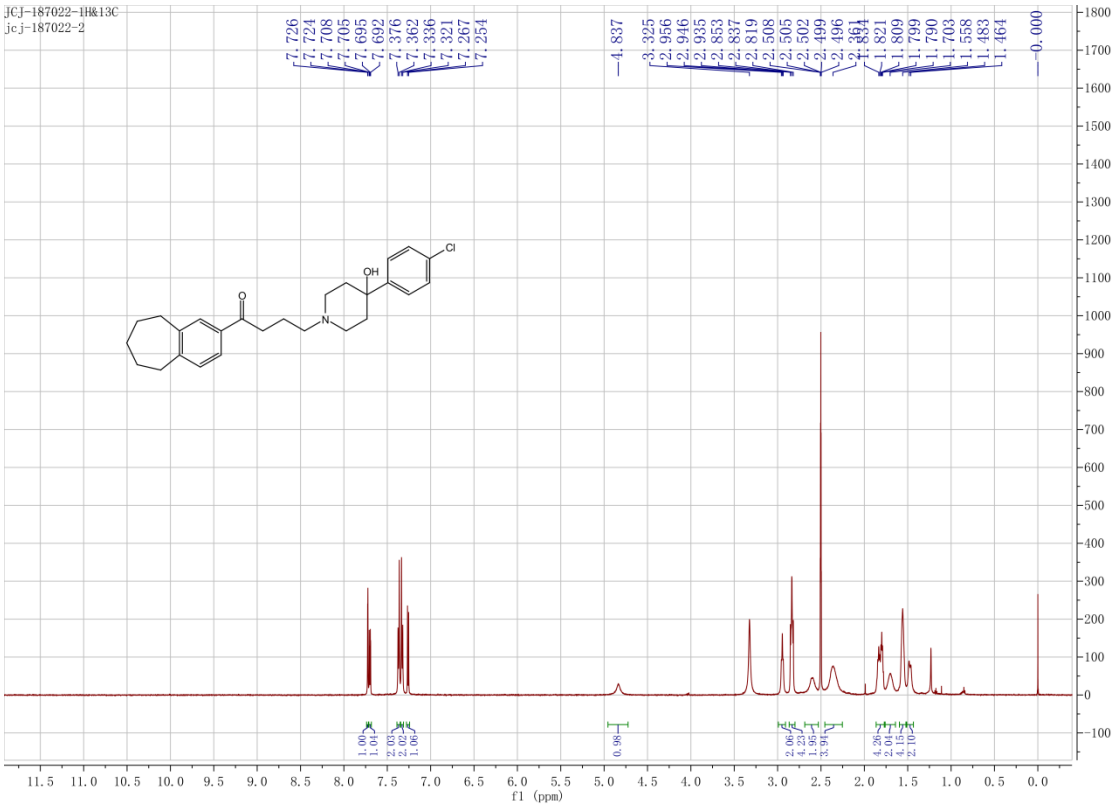
then washed twice with PBS solution, saponified at 80 ºC for 1 h with a mixture of 1 mL of 15% NaOH and 9 mL of EtOH. The suspensions were then extracted three times with 5 mL of heptane. The extracts were evaporated *in vacuum* and dissolved in 400 μL of cyclohexane. GC-MS analyses were performed on an Agilent 7890A gas chromatograph with splitless injection, coupled to an Agilent 5970C inert XL mass spectrometer with a triple-axis detector and an Agilent 19091S-433 capillary column (30 m × 250 μm)，and 100 μg/mL of cholesterol was added as internal standard. The oven temperature was programmed to hold at 70 °C for 2 min and then ramped to 270 °C at a rate of 20 °C/min. Helium (10 psi) was used as the carrier gas, the electron ionization energy was 70 eV, and the inlet temperature 250 °C. Identification of sterols was achieved using the NIST (the National Institute of Standards and Technology) reference database.

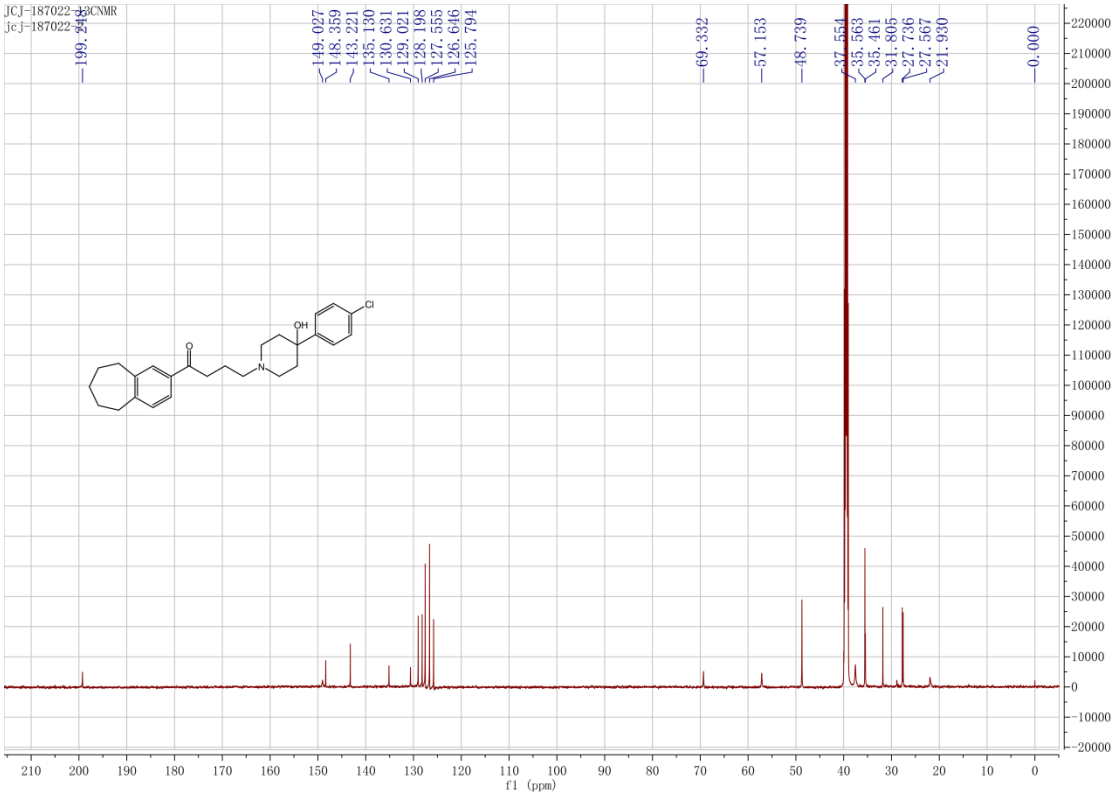
The Evaluation of the Efflux of Rhodamine 6G . The evaluation of the Efflux of Rhodamine 6G by *C. albicans* was performed according to the reported protocol with a few modifications.[7] *C. albicans* cells during the exponential growth period were harvested and re-suspended to 1×10^6 CFU/mL in 30 mL of YPD medium untreated or treated with 16 μg/mL of FLC and/or compound B10. The cells were collected after being cultured at 35 ºC for 24 h, and then washed twice with PBS solution, re-suspended to 5×10^7 CFU/mL in 10 mL of YPD. The PBS suspension was cultured at 35 °C for 1 h (200 rpm), until the energy and glucose were fully depleted. Then 1% 10 mm rhodamine 6G was added to obtain a final concentration of 10 μM, the resulting miture was cultured at 35 °C for 30 min. The rhodamine-saturated cell

solution was labeled, centrifuged (3000 pm, 5 min), washed 3 times with PBS, and the supernatant was discarded. The cell suspension was divided into two equal parts. Glucose was added to the centrifuge tube of the glucose group to a final concentration of 2 mM, and the other group was left untreated. After being cultured for 60 min, the suspension was centrifuged, and the fluorescence intensity of the supernatant was measured at an excitation wavelength of 515 nm and an emission wavelength of 555 nm, and three replicate wells were made at each time point. At the same time, the rhodamine 6G standard curve was used, and 10 μM of Rhodamine 6G was used as the highest concentration, and the gain value was adjusted based on it, and the ratio was diluted by 2 times with PBS. The efflux amount of fluorescent material rhodamine 6G was calculated.

Spectral data

Compound **A1**





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Comment			

Sample Group **Info.**

User Spectra

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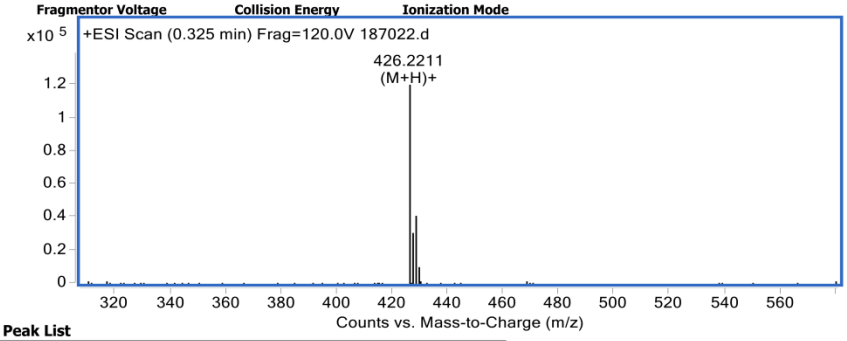

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Formula Calculator Results

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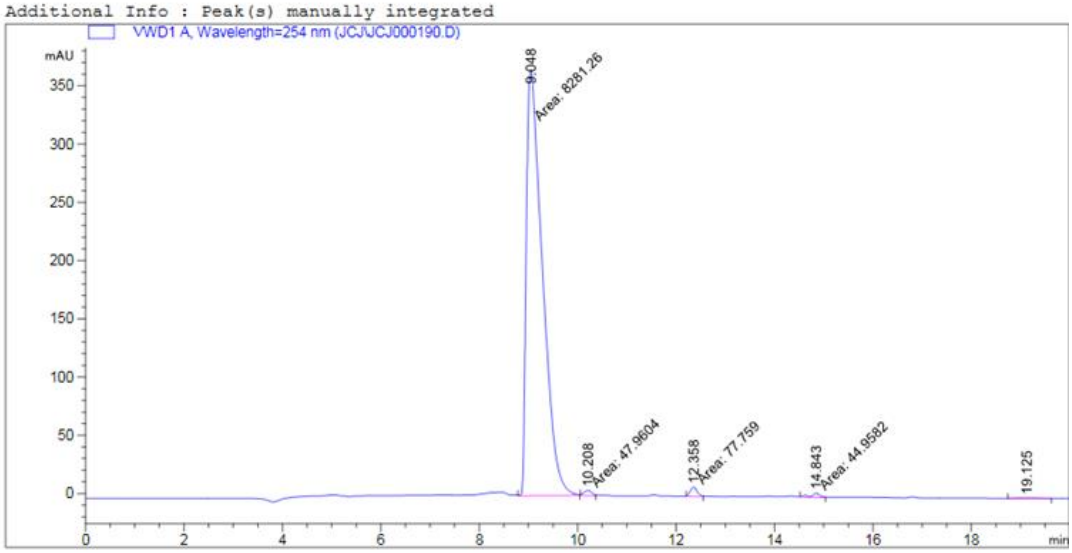


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                        Area Percent Report
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Use Multiplier & Dilution Factor with ISTDs


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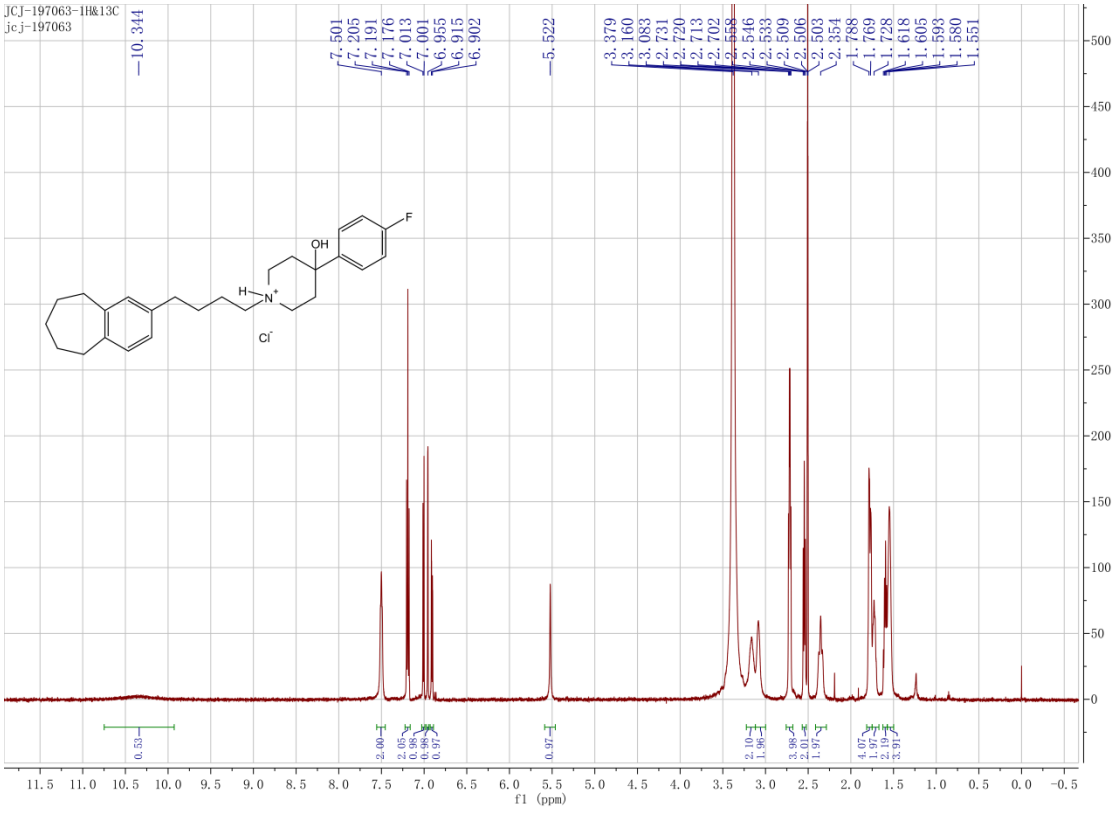
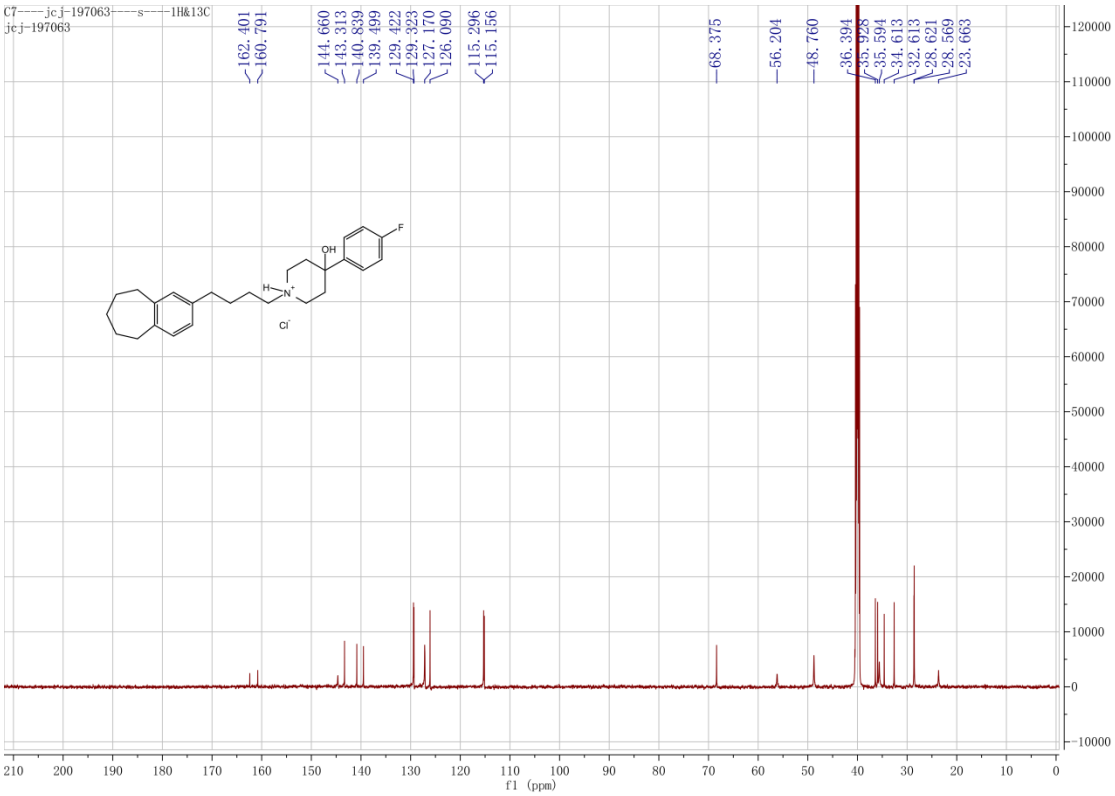
   2  10.208 MM    0.1832   47.96038    4.36232   0.5672
   3  12.358 MM    0.1620   77.75903    8.00089   0.9196
   4  14.843 MM    0.2075   44.95820    3.61060   0.5317
   5  19.125 BB    0.3292    4.21771 1.94131e-1   0.0499

Totals :                  8456.15119  382.07977

==========================================================================
                        *** End of Report ***
```

Compound **B10**





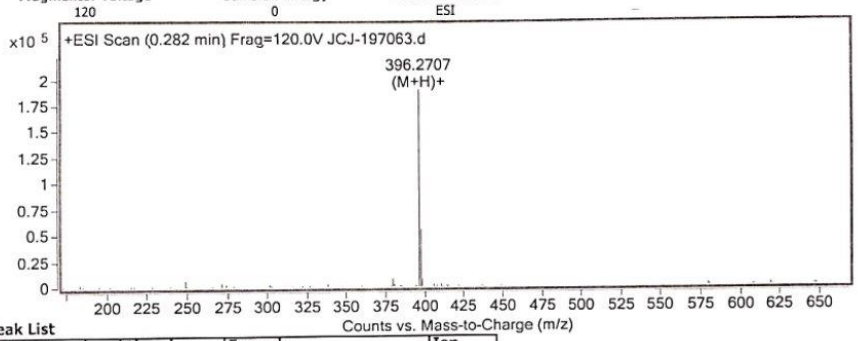
Qualitative Analysis Report

Data Filename	JCJ-197063.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-D2
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	7/20/2018 11:06:10 AM
IRM Calibration Status	Success	**DA Method**	MZ-1.0%_TEST.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
120	0	ESI



Peak List

m/z	z	Abund	Formula	Ion
396.2707	1	191660.8	C26 H35 F N O	(M+H)+
397.273	1	56001.9	C26 H35 F N O	(M+H)+

Formula Calculator Element Limits

Element	Min	Max
C	0	60
H	0	60
O	1	30
N	1	10
F	0	1

Formula Calculator Results

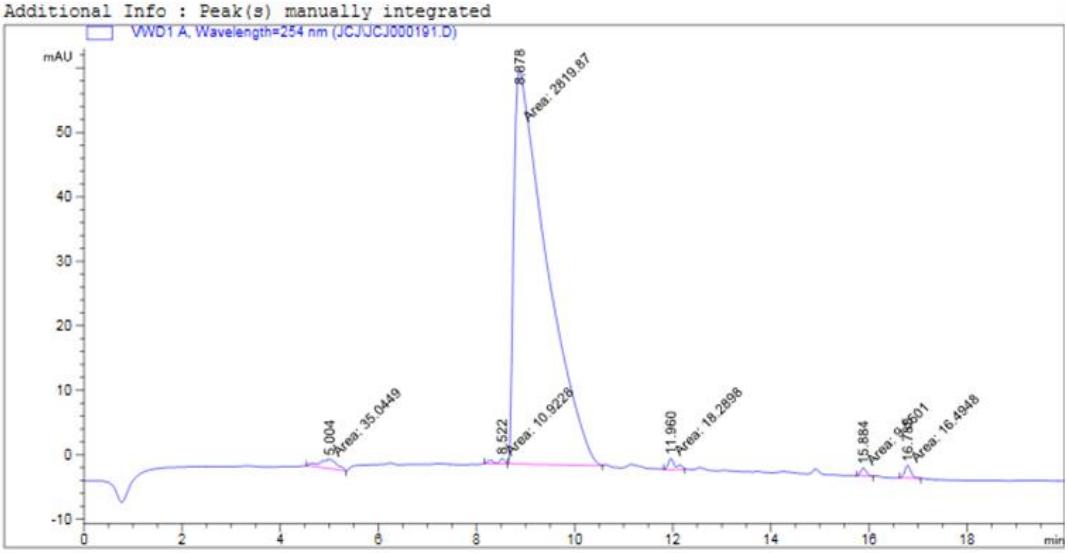
Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C26 H34 F N O	TRUE	395.2634	395.2624	-2.5	C26 H35 F N O	96.08

--- End Of Report ---

Data File D:\CHEM32\1\DATA\JCJ\JCJ000191.D
Sample Name: JCJ-197063

```
======================================================================
Acq. Operator   : JCJ
Acq. Instrument : HPLC1260                      Location :   -
Injection Date  : 4/24/2019 9:42:05 PM
                                                Inj Volume : No inj
Acq. Method     : D:\CHEM32\1\METHODS\CL2019.M
Last changed    : 4/24/2019 9:38:09 PM by JCJ
                  (modified after loading)
Analysis Method : D:\CHEM32\1\METHODS\CL2019.M
Last changed    : 4/25/2019 10:06:07 PM by MHJ
                  (modified after loading)
Sample Info     : 70%-95% MEOH, 0.1% TFA;0.5ml/min

Additional Info : Peak(s) manually integrated
```




```
======================================================================
                        Area Percent Report
======================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs

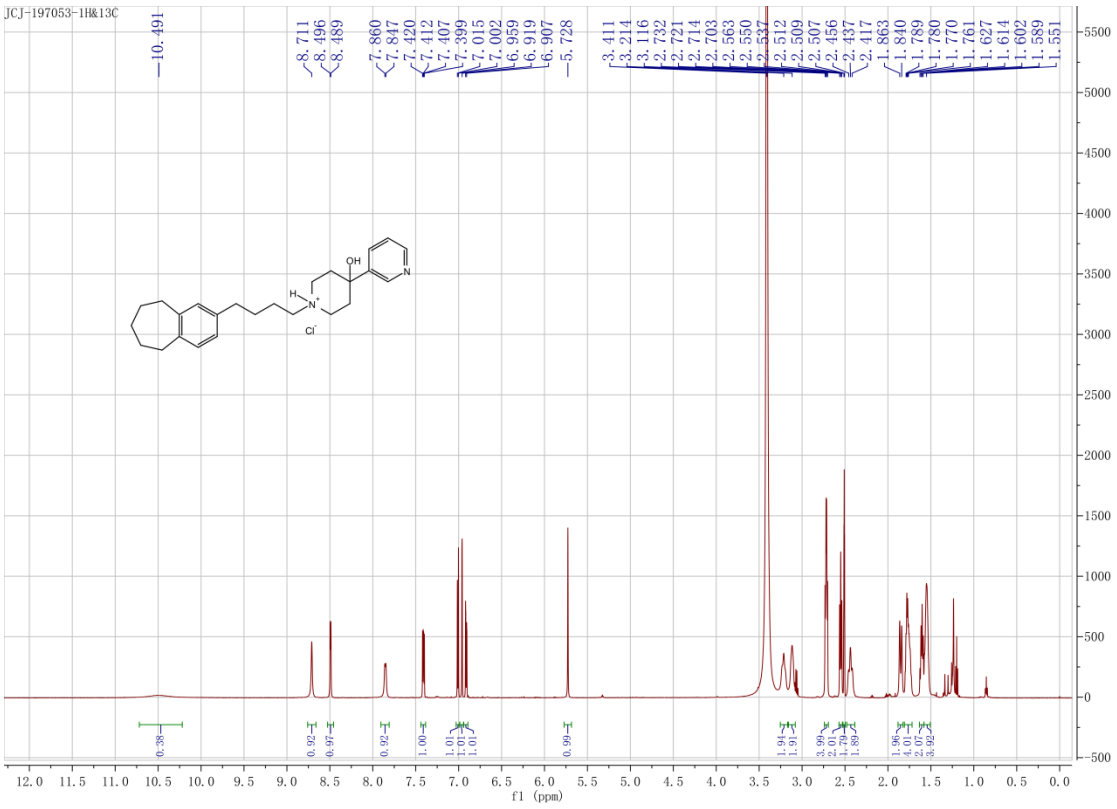

Signal 1: VWD1 A, Wavelength=254 nm

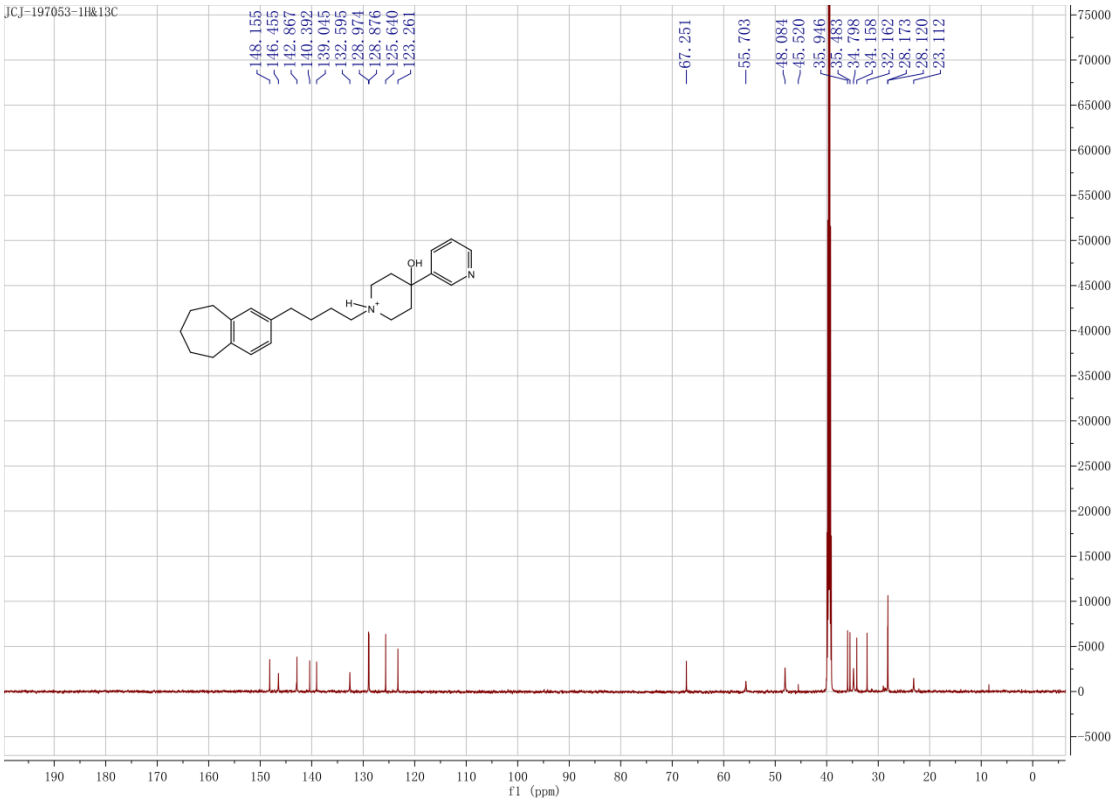
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   5.004 MM    0.4096   35.04487    1.42582   1.2042
   2   8.522 MM    0.2220   10.92281 8.20092e-1   0.3753
   3   8.878 MM    0.7690 2819.87231   61.11896  96.8970
   4  11.960 MM    0.1754   18.28978    1.73749   0.6285
   5  15.884 MM    0.1334    9.55010    1.19283   0.3282
   6  16.785 MM    0.1407   16.49477    1.95385   0.5668

Totals :                  2910.17464   68.24905

======================================================================
                        *** End of Report ***
```

Compound **B12**




JCJ-197053-1H&13C
148.155
146.455
142.867
140.392
139.045
132.595
128.974
128.876
125.640
123.261
67.251
55.703
48.084
45.520
35.946
35.483
34.798
34.158
32.162
28.173
28.120
23.112
OH
N
H
N+
f1 (ppm)

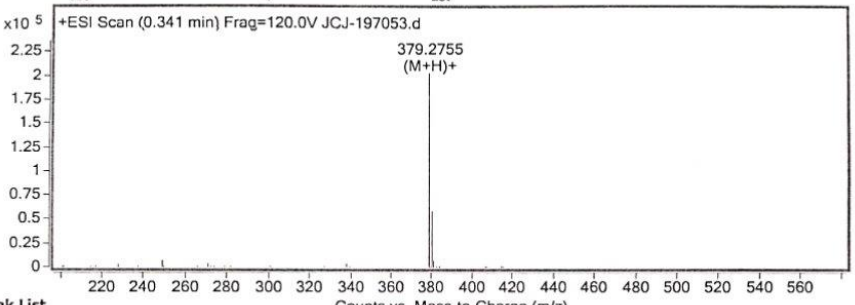
Qualitative Analysis Report

Data Filename	JCJ-197053.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-D1
Instrument Name	Instrument 1	**User Name**	
Acq Method	TEST-POS-WL.m	**Acquired Time**	7/20/2018 11:04:33 AM
IRM Calibration Status	Success	**DA Method**	MZ-1.0%_TEST.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
120	0	ESI



Peak List

m/z	z	Abund	Formula	Ion
121.0509		44069.6		
379.2755	1	204327.4	C22 H36 F N2 O2	(M+H)+
380.2778	1	59253.5	C22 H36 F N2 O2	(M+H)+
922.0098		51012.2		

Formula Calculator Element Limits

Element	Min	Max
C	0	60
H	0	60
O	1	30
N	1	10
F	0	1

Formula Calculator Results

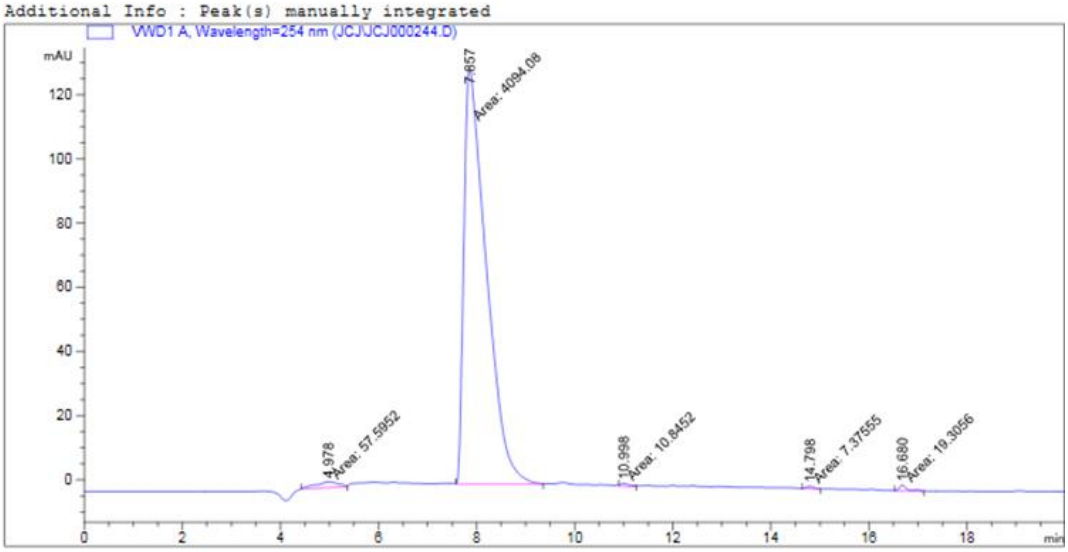
Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C25 H34 N2 O	TRUE	378.2682	378.2671	-2.77	C25 H35 N2 O	95.81

--- End Of Report ---

Data File D:\CHEM32\1\DATA\JCJ\JCJ000244.D
Sample Name: JCJ-197053

```
==========================================================================
Acq. Operator    : JCJ
Acq. Instrument : HPLC1260                        Location :    -
Injection Date  : 5/6/2019 11:04:13 AM
                                                  Inj Volume : No inj
Acq. Method      : D:\CHEM32\1\METHODS\CL2019.M
Last changed     : 5/6/2019 11:03:39 AM by JCJ
                   (modified after loading)
Analysis Method : D:\CHEM32\1\METHODS\CL2019.M
Last changed     : 5/6/2019 11:25:14 AM by JCJ
                   (modified after loading)
Sample Info      : 70%-95% MeOH, 0.1% TFA, 0.5 mL/min

Additional Info : Peak(s) manually integrated
```




```
==========================================================================
                        Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm
```

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.978	MM	0.5405	57.59525	1.77592	1.3748
2	7.857	MM	0.5274	4094.08374	129.37256	97.7294
3	10.998	MM	0.2180	10.84521	8.29247e-1	0.2589
4	14.798	MM	0.1559	7.37555	7.88627e-1	0.1761
5	16.680	MM	0.1817	19.30561	1.77071	0.4608

```
Totals :                  4189.20536  134.53706

==========================================================================
                        *** End of Report ***
```

References

1. Di, L.; Kerns, E. H.; Fan, K.; Mcconnell, O. J.; Carter, G. T. (2003) High throughput artificial membrane permeability assay for blood-brain barrier. *Eur. J. Med. Chem.* 38, 223-232. DOI: 10.1016/s0223-5234(03)00012-6.

2. Xu, Y.-X.; Wang, H.; Li, X.-k.; Dong, S.-n.; Liu, W.-w.; Gong, Q.; Wang, T.-d.-y.; Tang, Y.; Zhu, J.; Li, J.; Zhang, H.-y.; Mao, F. (2018) Discovery of novel propargylamine-modified 4-aminoalkyl imidazole substituted pyrimidinylthiourea derivatives as multifunctional agents for the treatment of Alzheimer's disease. *Eur. J. Med. Chem.* 143, 33-47. DOI: 10.1016/j.ejmech.2017.08.025.

3. Eglen, R. M. (2005) An overview of high throughput screening at G protein coupled receptors. *Front. Drug Des. Discovery***.** 1, 97-111. DOI: 10.2174/ 1574088054583381.

4. Tu, J.; Li, Z.; Jiang, Y.; Ji, C.; Han, G.; Wang, Y.; Liu, N.; Sheng, C. (2019) Discovery of Carboline Derivatives as Potent Antifungal Agents for the Treatment of Cryptococcal Meningitis. *J. Med. Chem.* 62, 2376-2389. DOI: 10.1021/acs.jmedchem.8b01598.

5. Wozniak, K. L., Levitz, S. M. (2009) Isolation and Purification of Antigenic Components of Cryptococcus. Host-Pathogen Interactions, 7nd ed., pp 71–83, Humana Press, Totowa, NJ.

6. Dubois, M.; Gilles, K.; Hamilton, J. K.; Rebers, P. A.; Smith, F. (1951) A Colorimetric Method for the Determination of Sugars. *Nature*, 168, 167-167. DOI: 10.1038/168167a0.

7. Thamban, C. N.; Shrestha, S. K.; Ngo, H. X.; Tsodikov, O. V.; Howard, K. C.; Garneau-Tsodikova, S. (2017) Alkylated Piperazines and Piperazine-Azole Hybrids as Antifungal Agents. *J. Med. Chem.* 61, 158-173. DOI: 10.1021/acs.jmedchem.7b01138.