Supplementary Data

Brain-penetrant LSD1 inhibitors can block memory consolidation

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Materials

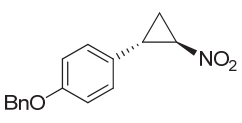
General. Unless otherwise noted, all chemicals were obtained from commercial sources and used without further purification. Analytical thin layer chromatography (TLC) was performed on (SILICYCLE) TLC silica Gel 60-F_{254} plates with visualization by ultraviolet (UV) irradiation at 254 nm. Purifications by flash chromatography were performed using HP silica chromatography column by Teledyne Isco. The eluting system for each purification was determined by TLC analysis. Chromatography solvents were used without distillation. When applicable, reactions were carried out under an argon atmosphere in flame-dried glassware. All organic solvents were removed under reduced pressure using a rotary evaporator.

HPLC and LCMS. HPLC-analysis of organic synthetic reactions was accomplished by using a C-18 column as solid phase. A mixture of MeCN and 0.1 M (aq)-ammonium formate was used as a mobile phase with a flow rate of 2mL/min. All compounds that were tested in the biological assays were analyzed by HPLC and LCMS to confirm the purity.

NMR. ^{1}H and ^{13}C spectra were measured with a Varian 500 MHz spectrometer. ^{1}H NMR chemical shifts are reported as δ in units of parts per million (ppm) relative to chloroform-d (δ 7.27, singlet), methanol-d_4 (δ 3.31, pentet), or dimethylsulfoxide-d_6 (δ 2.50, pentet). Multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), dt (doublet of triplets), or m (multiplet). Coupling constants are reported as a *J* value in Hertz (Hz). ^{13}C NMR chemical shifts are reported as δ in units of parts per million (ppm) relative to chloroform-d (δ 77.1, triplet), methanol-d_4 (δ 49.0, septet), dimethyl sulfoxide-d_6 (δ 39.5 septet), or acetonitrile-d_3 (δ 1.3, singlet; 118.3 septet).

Synthetic procedures and characterization data

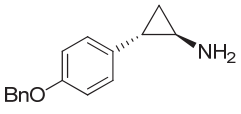
1-(Benzyloxy)-4-((*trans*)-2-nitrocyclopropyl)benzene (6)



Me_3SOI (0.62 g, 2.82 mmol) was added in portions to a solution of *t*-BuOK (0.32 g, 2.82 mmol) in dry DMSO (5 mL). After 10 min, a solution of 4-benzyloxy-*trans*-*β*-nitrostyrene (0.60 g, 2.35 mmol) in DMSO (5 mL) was added slowly and stirred for 6 h. The reaction mixture was poured over water (10 mL) and extracted with Et_2O (3 x 20 mL) and the organic layers were washed with brine (2 x 20 mL), dried (Na_2SO_4) and filtered. After removal of the solvent, the thick liquid was purified by flash column

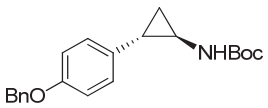
chromatography (5% EtOAc/95% Hexanes) affording white solid (0.16 g, 26% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.61 (m, 1H), 2.19-2.23 (m, 1H), 3.08-3.12 (m, 1H), 4.34-4.36 (m, 1H), 5.07 (s, 2H), 6.94 (d, *J* =9.0 Hz, 2H), 7.05 (d, *J* =9.0 Hz, 2H), 7.35-7.45 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 18.65, 29.09, 61.66, 70.09, 115.21, 127.45, 127.95, 128.08, 128.56, 128.65, 136.75, 158.36.

(*Trans*)-2-(4-(benzyloxy)phenyl)cyclopropanamine (7)



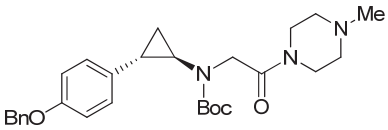
To a stirred solution of 1-(benzyloxy)-4-[(*trans*)-2- nitrocyclopropyl]benzene (0.81 g, 3.0 mmol) in *i*-PrOH (25 mL) and HCl (11 mL, 2.7 N), Zn dust (1.97 g, 30 mmol) was added in 8-10 portions and vigorously stirred for 10 h. The reaction mixture was filtered through celite, washed with MeOH (2 x 10 mL) and solvent was evaporated. To this H_2O (10 mL) was added and extracted with DCM (3 x 20 mL). The organic layers were washed with brine (2 x 20 mL), dried over Na_2SO_4 and filtered. After removal of the solvent, the thick liquid was purified by flash column chromatography (10% MeOH/90% DCM) affording white solid (0.18 g, 27% yield). ^{1}H NMR (500 MHz, CD_3OD): δ 1.23-1.29 (m, 1H), 1.32-1.36 (m, 1H), 2.28-2.32 (m, 1H), 2.73-2.76 (m, 1H), 5.05 (s, 2H), 6.93 (d, *J*=8.0 Hz, 2H), 7.08 (d, *J*=8.5 Hz, 2H), 7.28-7.41 (m, 5H). ^{13}C NMR (125 MHz, CD_3OD): δ 20.51, 27.33, 30.31, 69.59, 114.77, 127.08, 127.20, 127.44, 128.03, 128.06, 137.79, 157.91.

***Tert*-butyl ((*trans*)-2-(4-(benzyloxy)phenyl)cyclopropyl)carbamate (8)**



Boc_2O (0.21 g, 0.97 mmol) was added to a solution of (*trans*)-2-[4-(benzyloxy)phenyl]cyclopropylamine (0.14 g, 0.59 mmol) and Et_3N (0.13 mL, 0.97 mmol) in THF (10 mL) and stirred at rt for 3 h. After removal of the solvent, the crude residue was dissolved in EtOAc and consecutively washed with water and 10% aq. HCl (10 mL) and brine (20 mL). The organic layer was dried over anhydrous Na_2SO_4 and filtered; after removal of the solvent, the residue was purified by column chromatography on silica gel (10-20% EtOAc in Hexanes), affording the target compound (0.14 g, 72% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.08 (br, 2H), 1.45 (s, 9H), 1.98 (br, 1H), 2.64 (br, 1H), 4.86 (br, 1H), 5.02 (s, 2H), 6.87 (d, *J*=7.5 Hz, 2H), 7.07 (d, *J*=7.0 Hz, 2H), 7.31-7.40 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 15.85, 23.10, 28.41, 33.23, 70.06, 80.12, 114.77, 127.48, 127.79, 127.90, 128.56, 128.71, 130.62, 133.04, 137.12, 157.21.

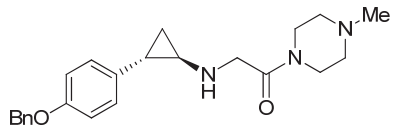
***Tert*-butyl((*trans*)-2-(4-(benzyloxy)phenyl)cyclopropyl)(2-(4-methylpiperazin-1-yl)-2-oxoethyl)carbamate (10)**



To a stirred suspension of NaH (0.01 g, 0.44 mmol) in dry DMF (0.50 mL) at 0 ^{o}C was added a solution of *tert*-butyl (*trans*)-2[4-(benzyloxy)phenyl]cyclopropylcarbamate (0.10 g, 0.29 mmol) in dry DMF (1.0 mL) and stir for 30 min. Then, added a solution of 1-(chloroacetyl)-4-methylpiperazine, **9** (0.10 g, 5.89 mmol) in dry DMF (1.0 mL) at 0 ^{o}C, stirred for 1 h at 0 ^{o}C to rt. The progress of the reaction was monitored by TLC. After completion, reaction mixture was poured into ice water and extracted with EtOAc. The combined extracts were washed with water, brine, dried over anhydrous Na_2SO_4, filtered and evaporated. The crude residue was purified by flash column chromatography on silica gel (20-40% EtOAc in Hexanes), to get *tert*-butyl (*trans*)-2[4-(benzyloxy)phenyl]cyclopropyl(2[4-methylpiperazin-1-yl)2-oxoethyl)carbamate (0.06 g, 45% yield). ^{1}H

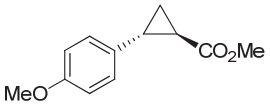
NMR(500 MHz, $CDCl_3$): δ 1.04 (br, 1H), 1.18 (br, 1H), 1.27 (br, 1H), 1.42 (s, 9H), 2.11-2.17 (m, 2H), 2.31 (s, 3H), 2.39 (br, 3H), 3.44 (br, 2H), 3.64 (br, 2H), 3.95-3.97 (m, 1H), 4.16 (br, 1H), 5.03 (s, 2H), 6.88 (d, *J* = 9.0 Hz, 2H), 7.07 (br, 2H), 7.32-7.42 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.36, 28.40, 39.23, 41.91, 44.55, 46.07, 53.47, 54.92, 70.08, 80.22, 114.68, 127.47, 127.91, 128.57, 129.25, 133.44, 137.17, 157.13, 162.29, 167.28.

2-(((*trans*)-2-(4-(Benzyloxy)phenyl)cyclopropyl)amino)-1-(4-methylpiperazin-1-yl)ethanone (11, RN-1)



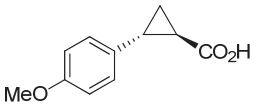
To a solution of *tert*-butyl ((trans)-2-(4-(benzyloxy)phenyl)cyclopropyl)(2-(4-methylpiperazin-1-yl)-2-oxoethyl)carbamate (0.05g, 0.10 mmol) in Et_2O (3.0 mL) at 0 ^oC was added Et_2O-HCl (2.0 mL) drop- wise, stirred for 1 h at 0 ^oC to rt. The progress of the reaction was monitored by TLC. After completion reaction mixture was filtered under inert atmosphere and washed with hexane and EtOAc, and dried under reduced pressure to get compound **11** (RN-1) (0.03 g, 70% yield). ^1H NMR (500 MHz, D_2O): δ 1.22-1.27 (m, 1H), 1.40-1.41 (m, 1H), 2.41-2.44 (m, 1H), 2.81-2.86 (m, 4H), 2.97-3.04 (m, 3H), 3.42-3.49 (m, 3H), 3.85 (d, *J* = 14.5 Hz, 1H), 4.16-4.27 (m, 2H), 4.43 (d, *J* = 13.5 Hz, 1H), 5.00 (s, 2H), 6.88 (d, J = 8.5 Hz, 2H), 7.02 (d, *J* = 8.0 Hz, 2H), 7.26-7.35 (m, 5H). ^{13}C NMR (125 MHz, D_2O): δ 12.04, 20.29, 37.63, 41.60, 42.90, 48.15, 52.37, 70.34, 115.51, 127.71, 128.04, 128.38, 128.77, 130.84, 136.42, 156.72, 164.47. LC-MS calc. $[M+H]^+$ 380.2, found 380.3.

(*Trans*)-methyl 2-(4-methoxyphenyl)cyclopropanecarboxylate (13a)



Compound **13a** prepared from **12a** using the procedure described for **6** (yield 22%). ^1H NMR (500 MHz, $CDCl_3$): δ 1.26-1.29 (m, 1H), 1.55-1.58 (m, 1H), 1.81-1.84 (m, 1H), 2.47-2.50 (m, 1H), 3.71 (s, 3H), 3.78 (s, 3H), 6.81 (d, J=8.5 Hz, 2H), 7.02 (d, J=8.0 Hz, 2H).

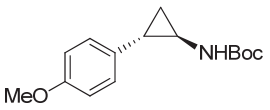
(*Trans*)-2-(4-methoxyphenyl)cyclopropanecarboxylic acid (14a)



To a solution of of an ester (0.64 g, 3.11 mmol) in MeOH (10 mL) was added an aqeous solution of K_2CO_3 (1.30 g, 9.42 mmol) in water (10 mL) and the reaction mixture was refluxed for 3 h. The solvent was evaporated under reduced pressure and the residue was suspended in water and extracted with CH_2Cl_2. The organic layer was discarded and the aqueous phase was acidified with 2 N aqueous HCl to pH 1 and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4. Filtration and concentration in vacuo gave 0.50 g (83%) of an acid, **14a** as an off-white solid. ^1H NMR (500 MHz, $CDCl_3$): δ 1.35-1.38 (m, 1H), 1.60-1.64 (m, 1H), 1.81-1.84 (m, 1H), 2.55-2.59 (m, 1H), 3.79 (s, 1H), 3.78 (s, 1H), 6.82 (d, *J*=9.0 Hz, 2H), 7.04 (d, *J*=9.0 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.21, 23.65, 26.61, 55.52, 113.95, 127.48, 131.47, 158.46, 179.54.

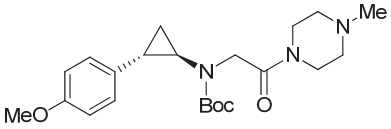
***Tert*-butyl ((*trans*)-2-(4-methoxyphenyl)cyclopropyl)carbamate (15a)**

To a solution of an acid, **14a** (0.50 g, 2.60 mmol) obtained above in dry toluene (10 mL) was added diphenyl phosphoryl azide (0.68 mL, 3.12 mmol) and Et_3N (0.73 mL, 5.23 mmol) at rt for 30 min under an argon atmosphere. The reaction mixture was refluxed for 1.5 h, dry *t*-BuOH (3.0 mL) was added, and the resulting solution was refluxed for overnight. The reaction mixture was cooled to room temperature, and the solvent was removed by evaporation. The residue was purified by silica gel flash column chromatography (EtOAc/Hexanes = 2/8) to give 0.47 g (70 %) of carbamate, **15a** as a white



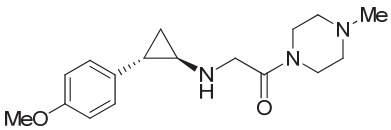
solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.13 (m, 2H), 1.46 (s, 9H), 2.10 (m, 1H), 2.68 (br, 1H), 3.77 (s, 3H), 4.85 (br, 1H, NH), 6.81 (d, *J*=8.5 Hz, 2H), 7.07 (d, *J*=8.0 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 15.80, 26.32, 27.97, 28.08, 28.40, 55.27, 113.75, 127.76, 130.58, 132.73, 157.99.

Tert-butyl((_trans_)-2-(4-methoxyphenyl)cyclopropyl)(2-(4-methylpiperazin-1-yl)-2-oxoethyl) carbamate (16a)



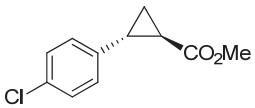
Compound **16a** prepared from **15a** using the procedure described for **10** (yield 67%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.00 (br, 1H), 1.16 (br, 2H), 1.39 (s, 9H), 2.12 (br, 1H), 2.28 (s, 3H), 2.37 (br, 4H), 3.42 (br, 2H), 3.60 (br, 2H), 3.74 (s, 3H), 3.93-3.96 (m, 1H), 4.14 (br, 1H), 6.77 (d, J=8.5 Hz, 2H), 7.00 (br, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.27, 26.31, 28.43, 29.66, 39.08, 41.86, 44.49, 46.02, 54.59, 80.15, 113.59, 113.74, 127.29, 133.06, 157.87, 167.21.

2-(((_trans_)-2-(4-Methoxyphenyl)cyclopropyl)amino)-1-(4-methylpiperazin-1-yl)ethanone (17a, RN-22)



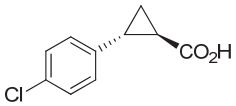
Compound **17a** prepared from **16a** using the procedure described for **11** (yield 20%). ^{1}H NMR (500 MHz, CD_3OD): δ 1.30 (q, *J*=7.5 Hz, 2H), 1.48-1.52 (m, 1H), 2.51 (br, 1H), 2.96 (s, 3H), 3.31 (br, 8H), 3.57 (br, 3H), 3.76 (s, 3H), 6.86 (d, *J*=8.5 Hz, 2H), 7.11 (d, *J*=8.0 Hz, 2H). ^{13}C NMR (500 MHz, CD_3OD): δ 11.79, 14.02, 20.53, 37.58, 41.47, 42.29, 52.52, 65.48, 113.69, 127.33, 129.69, 158.86, 163.84. LC-MS calc. $[M+H]^+$ 304.19, found 304.2.

(_Trans_)-methyl 2-(4-chlorophenyl)cyclopropanecarboxylate (13b)



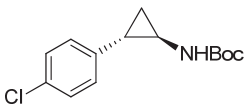
Compound **13b** prepared from **12b** using the procedure described for **6** (yield 23%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.19-1.26 (m, 1H), 1.54-1.58 (m, 1H), 1.81-1.84 (m, 1H), 2.43-2.49 (m, 1H), 3.67 (s, 3H), 6.98 (d, *J*=7.5 Hz, 2H), 7.19 (d, *J*=7.5 Hz, 2H).

(_Trans_)-2-(4-chlorophenyl)cyclopropanecarboxylic acid (14b)



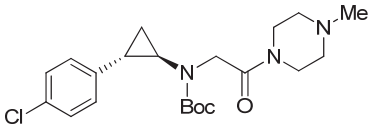
Compound **14b** prepared from **13b** using the procedure described for **14a** (yield 85%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.35-1.39 (m, 1H), 1.65-1.70 (m, 1H), 1.84-1.88 (m, 1H), 2.57-2.60 (m, 1H), 7.05 (d, *J*=8.0 Hz, 2H), 7.30 (d, *J*=8.0 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.45, 23.89, 26.42, 127.66, 128.66, 132.46, 137.97, 179.21.

Tert-butyl ((_trans_)-2-(4-chlorophenyl)cyclopropyl)carbamate (15b)



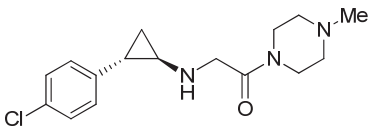
Compound **15b** prepared from **14b** using the procedure described for **15a** (yield 60%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.10-1.16 (m, 2H), 1.45 (s, 9H), 2.02 (br, 1H), 2.67 (br, 1H), 4.85 (br, 1H, NH), 7.06 (d, *J*=8.0 Hz, 2H), 7.22 (d, *J*=9.0 Hz, 2H).

Tert-butyl ((_trans_)-2-(4-chlorophenyl)cyclopropyl)(2-(4-methylpiperazin-1-yl)-2-oxoethyl)carbamate (16b)



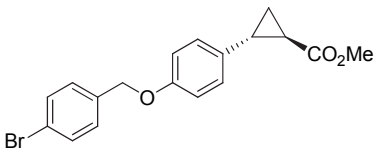
Compound **16b** prepared from **15b** using the procedure described for **10** (yield 74%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.02-1.06 (m, 1H), 1.21-1.47 (m, 2H), 1.34 (s, 9H), 2.12 (br, 1H), 2.31 (s, 3H), 2.40 (br, 4H), 2.94 (br, 1H), 3.46 (br, 2H), 3.62 (br, 2H), 4.16 (br, 1H), 7.02 (br, 2H), 7.32 (d, *J* = 8.5 Hz, 2H).

2-(((*trans*)-2-(4-Chlorophenyl)cyclopropyl)amino)-1-(4-methylpiperazin-1-yl)ethanone (17b, RN-23)



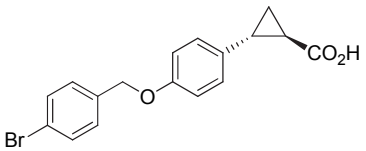
Compound **17b** prepared from **16b** using the procedure described for **11** (yield 28%). ^{1}H NMR (500 MHz, CD_3OD): δ 1.24-1.27 (m, 1H), 1.44 (br, 1H), 2.43-2.47 (m, 2H), 2.97-3.05 (m, 3H), 3.45-3.48 (m, 3H), 3.84-3.86 (m, 2H), 4.17-4.26 (m, 4H), 4.45-4.48 (m, 2H), 7.03 (d, *J*=8.5 Hz, 2H), 7.22 (d, *J*=8.5 Hz, 2H). LC-MS calc. $[M+H]^+$ 308.15, found 308.2.

(*Trans*)-methyl 2-(4-((4-bromobenzyl)oxy)phenyl)cyclopropanecarboxylate (13c)



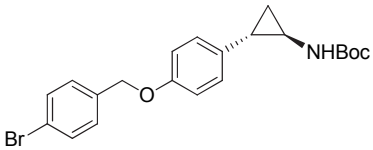
Compound **13c** prepared from **12c** using the procedure described for **6** (yield 19%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.24-1.28 (m, 1H), 1.54-1.58 (m, 1H), 1.81-1.84 (m, 1H), 2.47-2.51 (m, 1H), 4.99 (s, 2H), 6.85 (d, *J*=8.5 Hz, 2H), 7.02 (d, *J*=8.5 Hz, 2H), 7.29 (d, *J*=8.5 Hz, 2H), 7.50 (d, *J*=9.0 Hz, 2H).

(*Trans*)-2-(4-((4-bromobenzyl)oxy)phenyl)cyclopropanecarboxylic acid (14c)



Compound **14c** prepared from **13c** using the procedure described for **14a** (yield 80%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.33-1.37 (m, 1H), 1.60-1.64 (m, 1H), 1.81-1.84 (m, 1H), 2.54-2.58 (m, 1H), 4.99 (s, 2H), 6.87 (d, *J* = 8.0 Hz, 2H), 7.04 (d, *J* = 9.0 Hz, 2H), 7.29 (d, *J* = 8.0 Hz, 2H), 7.50 (d, *J* = 8.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 16.56, 23.53, 25.63, 69.22, 114.77, 121.77, 127.32, 128.93, 131.52, 132.52, 136.19, 157.07, 176.14.

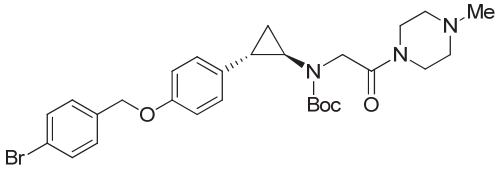
***Tert*-butyl ((*trans*)-2-(4-((4-bromobenzyl)oxy)phenyl)cyclopropyl)carbamate (15c)**



Compound **15c** prepared from **14c** using the procedure described for **15a** (yield 60%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.07-1.11 (m, 1H), 1.46 (s, 9H), 1.97-2.01 (m, 1H), 2.65 (s, 1H), 4.83 (s, 1H, NH), 4.98 (s, 2H), 6.84-6.86 (m, 2H), 7.07 (d, *J*=8.5 Hz, 2H), 7.26-7.29 (m, 2H), 7.48-7.50 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 15.81, 24.55, 28.39, 32.05, 69.30, 79.98, 114.76, 121.77, 127.83, 129.01, 130.62, 131.66, 133.29, 136.17, 156.91.

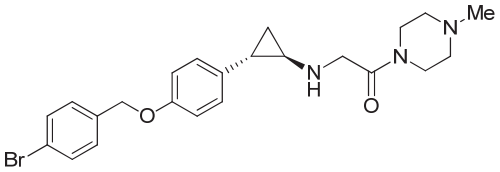
***Tert*-butyl ((*trans*)-2-(4-((4-bromobenzyl)oxy)phenyl)cyclopropyl)(2-(4-methylpiperazin-1-yl)-2-oxoethyl)carbamate (16c)**

Compound **16c** prepared from **15c** using the procedure described for **10** (yield 56%). ^{1}H NMR(500 MHz, $CDCl_3$): δ 1.04 (br, 1H), 1.21 (br, 1H), 1.26 (br, 1H), 1.39 (s, 9H), 2.11-2.15 (m, 2H), 2.28 (s, 3H), 2.41 (br, 3H), 3.44 (br,



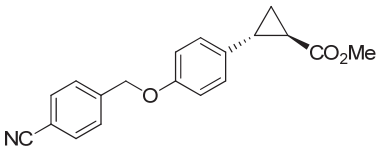
2H), 3.64 (br, 2H), 3.96-3.99 (m, 1H), 4.09 (br, 1H), 5.04 (s, 2H),), 6.81 (d, *J*=8.5 Hz, 2H), 6.92-6.98 (m, 4H), 7.46 (d, *J*=8.0 Hz, 2H).

2-(((*trans*)-2-(4-((4-Bromobenzyl)oxy)phenyl)cyclopropyl)amino)-1-(4-methylpiperazin-1-yl)ethanone (17c, RN-21)



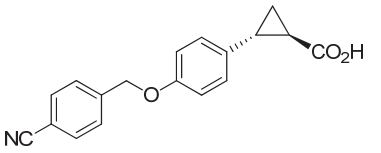
Compound **17c** prepared from **16c** using the procedure described for **11** (yield 28%). ^{1}H NMR (500 MHz, CD_3OD): δ 1.24-1.28 (m, 1H), 1.54 (br, 1H), 2.54 (br, 1H), 3.13 (br, 1H), 3.15 (br, 1H), 3.30 (s, 3H), 3.31 (s, 2H), 3.48 (br, 1H), 3.63 (br, 1H), 3.98 (d, *J* = 8.5 Hz, 1H), 4.11-4.28 (m, 2H), 4.45 (t, *J* = 4.0 Hz, 1H), 4.63 (br, 1H), 5.00 (s, 2H), 6.89 (d, J=9.0 Hz, 2H), 7.08 (d, *J*=8.5 Hz, 2H), 7.29 (d, *J*=8.0 Hz, 2H), 7.47 (d, *J*=8.5 Hz, 2H). LC-MS calc. $[M+H]^+$ 458.14, found 458.10 and 460.10.

(*Trans*)-methyl 2-(4-((4-cyanobenzyl)oxy)phenyl)cyclopropanecarboxylate (13d)



Compound **13d** prepared from **12d** using the procedure described for **6** (yield 24%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.24-1.28 (m, 1H), 1.55-1.58 (m, 1H), 1.81-1.84 (m, 1H), 2.46-2.51 (m,1H), 3.71 (s, 3H), 5.09 (s, 2H), 6.86 (d, *J*=8.5 Hz, 2H), 7.03 (d, *J*=9.0 Hz, 2H), 7.52 (d, *J*=8.0 Hz, 2H), 7.66 (d, *J*=7.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 16.75, 23.72, 25.67, 51.92, 68.96, 111.67, 114.83, 118.69, 126.12, 127.51, 132.37, 132.39, 132.87, 142.52, 156.90, 173.96.

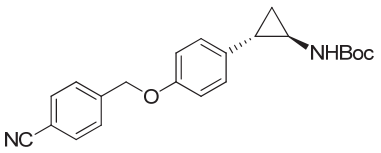
(*Trans*)-2-(4-((4-cyanobenzyl)oxy)phenyl)cyclopropanecarboxylic acid (14d)



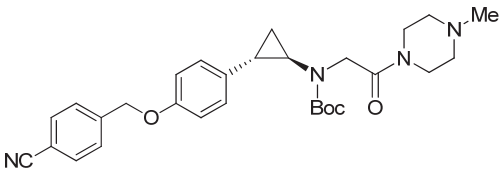
Compound **14d** prepared from **13d** using the procedure described for **14a** (yield 82%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.28 (br, 2H), 1.47 (br, 1H), 1.73 (br, 1H), 2.41 (br, 1H), 5.14 (s, 2H), 6.91 (d, *J* = 8.0 Hz, 2H), 7.06 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.72 (d, *J* = 7.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 16.99, 24.68, 26.51, 69.83, 112.38, 115.92, 119.59, 128.34, 128.82, 133.31, 134.31, 144.82, 156.64, 177.26.

***Tert*-butyl ((*trans*)-2-(4-((4-cyanobenzyl)oxy)phenyl)cyclopropyl)carbamate (15d)**

Compound **15d** prepared from **14d** using the procedure described for **15a** (yield 65%). ^{1}H NMR (500 MHz, CDCl3): δ 1.07-1.11 (m, 1H), 1.46 (s, 9H), 1.97-2.01 (m, 1H), 2.65 (s, 1H), 4.83 (s, 1H), 4.98 (s, 2H), 6.84-6.86 (m, 2H), 7.07 (d, *J*=8.5 Hz, 2H), 7.26-7.29 (m, 2H), 7.48-7.50 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 15.77, 24.32, 28.39, 32.29, 69.07, 79.80, 111.59, 114.71, 118.71, 120.09, 127.51, 127.91, 132.36, 133.68, 142.68, 156.57.



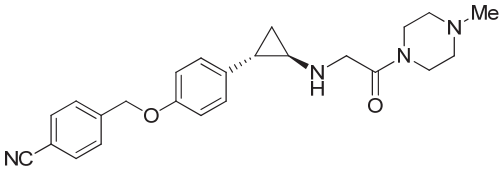
***Tert*-butyl ((*trans*)-2-(4-((4-cyanobenzyl)oxy)phenyl)cyclopropyl)(2-(4-methylpiperazin-1-yl)-2-oxoethyl)carbamate (16d)**



Compound **16d** prepared from **15d** using the procedure described for **10** (yield 63%). ^{1}H NMR(500 MHz, $CDCl_3$): δ 1.19

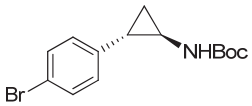
(br, 1H), 1.25 (br, 1H), 1.26 (br, 1H), 1.40 (s, 9H), 2.15 (br, 2H), 2.29 (s, 3H), 2.38 (br, 3H), 3.43 (br, 2H), 3.63 (br, 2H), 3.93-3.96 (m, 1H), 4.17 (br, 1H), 5.08 (s, 2H), 6.83 (d, *J*=8.0 Hz, 2H), 7.03 (br, 2H), 7.52 (d, *J*=7.5 Hz, 2H), 7.65 (d, *J*=7.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.52, 28.43, 29.65, 41.67, 44.67, 46.02, 54.60, 54.87, 68.97, 80.42, 111.96, 114.57, 115.23, 118.86, 127.52, 129.98, 132.34, 134.18, 142.90, 156.71, 167.50.

Tert-butyl ((_trans_)-2-(4-((4-cyanobenzyl)oxy)phenyl)cyclopropyl)(2-(4-methylpiperazin-1-yl)-2-oxoethyl)carbamate (17d, RN-27)



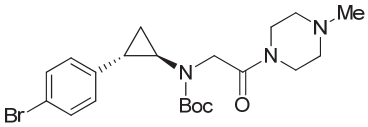
Compound **17d** prepared from **16d** using the procedure described for **11** (yield 20%). ^{1}H NMR (500 MHz, CD_3OD): δ 1.16-1.19 (m, 2H), 1.24-1.33 (m, 1H), 1.52 (br, 1H), 2.52 (br, 1H), 2.95 (s, 3H), 3.16-3.22 (m, 4H), 3.56 (br, 2H), 4.01 (br, 1H), 4.31 (br, 1H), 4.43 (br, 1H), 4.64 (br, 1H), 5.17 (s, 2H), 6.94 (d, *J*=8.0 Hz, 2H), 7.13 (d, *J*=8.5 Hz, 2H), 7.60 (d, *J*=8.0 Hz, 2H), 7.72 (d, *J*=8.0 Hz, 2H). ^{13}C NMR (125 MHz, CD_3OD): δ 13.72, 15.81, 22.39, 40.25, 43.09, 43.92, 54.17, 70.11, 112.68, 113.28, 116.42, 116.87, 129.09, 129.15, 133.63, 144.97, 159.32, 165.60. LC-MS calc. $[M+H]^+$ 405.22, found 405.3.

Tert-butyl ((_trans_)-2-(4-bromophenyl)cyclopropyl)carbamate (15e)



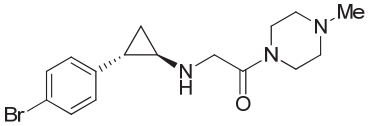
Compound **15e** was prepared from 4-bromoparnate (purchased from UORSY/Ukrorgsyntez Ltd) using the procedure described for **8** (yield 75%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.13 (m, 2H), 1.45 (s, 9H), 2.78 (s, 1H), 4.91 (br, 1H), 7.03 (d, *J* = 8.0 Hz, 2H), 7.38 (d, *J* = 8.0 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 15.58, 24.63, 28.69, 32.75, 79.57, 119.52, 128.89, 131.70, 140.13, 156.36.

Tert-butyl((_trans_)-2-(4-bromophenyl)cyclopropyl)(2-(4-methylpiperazin-1-yl)-2-oxoethyl)carbamate (16e)



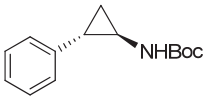
Compound **16e** prepared from **15e** using the procedure described for **10** (yield 67%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.06 (br, 1H), 1.24-1.27 (m, 2H), 1.39 (s, 9H), 2.16 (br, 1H), 2.34 (s, 3H), 2.45 (br, 4H), 2.98 (br, 1H), 3.48 (br,2H), 3.66 (br, 2H), 4.16 (br, 1H), 6.96 (br, 2H), 7.36 (d, *J* = 9.0 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.47, 28.28, 39.46, 41.72, 44.35, 45.86, 54.41, 54.69, 79.99, 119.26, 127.71, 130.99, 140.06, 156.92, 167.03.

2-(((_trans_)-2-(4-Bromophenyl)cyclopropyl)amino)-1-(4-methylpiperazin-1-yl)ethanone (17e, RN-24)



Compound **17e** prepared from **16e** using the procedure described for **11** (yield 32%). ^{1}H NMR (500 MHz, CD_3OD): δ 1.37-1.39 (m, 1H), 1.59 (br, 1H), 2.56 (br, 1H), 2.95 (s, 3H), 3.03-3.27 (m, 5H), 3.57 (d, *J*=9.5 Hz, 3H), 4.03 (d, *J*=13.5 Hz, 1H), 4.29-4.33 (m, 1H), 4.43-4.49 (m, 1H), 4.64 (d, *J*=14.0 Hz, 1H), 7.13 (d, *J*=8.0 Hz, 2H), 7.46 (d, *J*=7.5 Hz, 2H). ^{13}C NMR (125 MHz, CD_3OD): δ 12.23, 14.01, 20.67, 38.62, 42.28, 52.51, 65.47, 120.23, 128.15, 131.35, 137.32, 163.79. LC-MS calc. $[M+H]^+$ 352.09, found 352.20.

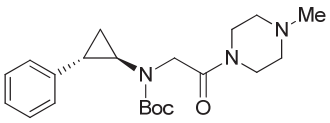
***Tert*-butyl ((*trans*)-2-phenylcyclopropyl)carbamate (15f)**



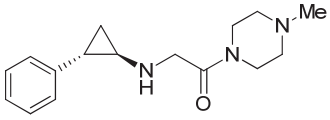
Compound **15f** was prepared from parnate (purchased from Aldrich) using the procedure described for **8** (yield 62%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.13-1.18 (m, 2H), 1.46 (s, 9H), 1.58-2.05 (m, 1H), 2.73 (br, 1H), 4.91 (br, 1H, NH), 7.09-7.18 (m, 3H), 7.24-7.27 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 16.35, 27.83, 32.44, 67.14, 79.55, 125.60, 126.45, 128.18, 140.74, 156.41.

***tert*-butyl (2-(4-Methylpiperazin-1-yl)-2-oxoethyl)((*trans*)-2-phenylcyclopropyl)carbamate (16f)**

Compound **16f** was prepared from **15f** using the procedure described for **10** (yield 60%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.08 (br, 1H), 1.22 (br, 1H), 1.37 (s, 9H), 2.14-2.18 (m, 1H), 2.27 (s, 3H), 2.37 (br, 4H), 2.98 (br, 1H), 3.41 (br, 2H), 3.60 (br, 2H), 3.93-3.96 (m, 1H), 4.14 (br, 1H), 7.06 (br, 2H), 7.10-7.13 (m, 1H), 7.19-7.22 (m, 2H). ^{13}C NMR (125 MHz, CD_3OD): δ 17.71, 28.39, 36.44, 39.56, 41.87, 44.51, 46.03, 54.59, 80.18, 125.81, 126.10, 128.38, 141.05, 162.77, 167.19.

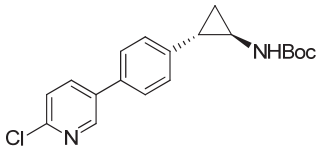


1-(4-Methylpiperazin-1-yl)-2-(((*trans*)-2-phenylcyclopropyl)amino)ethanone (17f, RN-11)



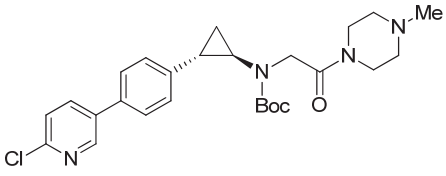
Compound **17f** prepared from **16f** using the procedure described for **11** (yield 23%). ^{1}H NMR (500 MHz, CD_3OD): δ 1.18-1.22 (m, 1H), 1.38-1.42 (m, 1H), 2.39-2.44 (m, 1H), 2.75-2.78 (m, 4H), 2.83-2.97 (m, 3H), 3.40-3.47 (m, 3H), 3.78-3.85 (m, 4H), 7.17-7.24 (m, 5H). LC-MS calc. $[M+H]^+$ 274.2, found 274.2.

***Tert*-butyl ((*trans*)-2-(4-(6-chloropyridin-3-yl)phenyl)cyclopropyl)carbamate (18a)**



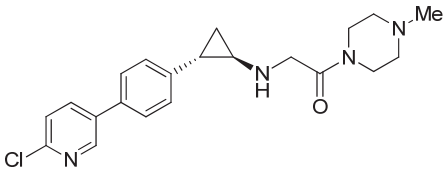
Compound **18a** prepared from **15e** using the procedure described for **18b** (yield 33%). ^{1}H NMR(500 MHz, $CDCl_3$): δ 1.20 (t, J = 7.5 Hz, 2H), 1.45 (s, 9H), 2.07-2.11 (m, 1H), 2.75 (s, 1H), 4.92 (bs, 1H, NH), 7.22 (d, *J* = 7.0 Hz, 2H), 7.36 (d, *J*= 8.5 Hz, 1H), 7.43 (d, *J*= 8.0 Hz, 2H), 7.78 (dd, *J* = 8.0 and 2.5 Hz, 1H), 8.56 (d, *J* = 2.5 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 16.21, 24.64, 28.26, 32.77, 80.00, 124.17, 126.93, 127.27, 134.13, 135.41, 136.94, 141.44, 147.76, 150.05, 156.52.

***Tert*-butyl ((*trans*)-2-(4-(6-chloropyridin-3-yl)phenyl)cyclopropyl)(2-(4-methylpiperazin-1-yl)-2-oxoethyl)carbamate (19a)**



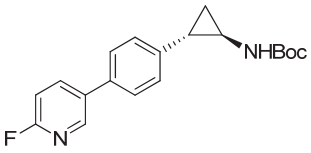
Compound **19a** prepared from **18a** using the procedure described for **10** (yield 48%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.15-1.29 (m, 2H), 1.39 (s, 9H), 2.24 (br, 1H), 2.30 (s, 3H), 2.42 (br, 4H), 3.15 (br, 1H), 3.42 (br, 2H),3.63 (br, 2H), 4.20 (br, 1H), 7.18 (br, 2H), 7.35 (d, *J*=8.0 Hz, 1H), 7.42 (d, *J*=8.5 Hz, 2H), 7.78-7.80 (m, 1H), 8.55 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 18.08, 27.44, 28.22, 39.61, 41.75, 44.41, 48.98, 54.76, 80.19, 124.15, 124.83, 126.74, 126.73, 126.93, 133.92, 135.44, 136.91, 137.63, 141.80, 147.73, 149.99, 156.98, 167.28.

2-(((*trans*)-2-(4-(6-Chloropyridin-3-yl)phenyl)cyclopropyl)amino)-1-(4-methylpiperazin-1-yl)ethanone (20a, RN-5)



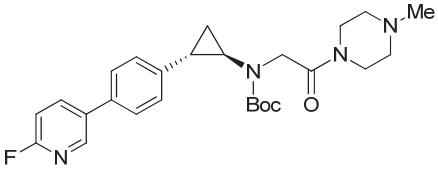
Compound **20a** prepared from **19a** using the procedure described for **11** (yield 52%). ^{1}H NMR(500 MHz, CD_3OD): δ 1.45 (br, 1H), 1.51 (br, 1H), 2.35 (br, 1H), 2.85 (s, 3H), 3.0 (s, 2H), 3.31 (br, 3H), 3.58 (br, 3H), 4.08 (br, 1H), 4.35 (br, 1H), 4.41 (br, 1H), 4.65 (m, 1H), 7.34 (d, *J* = 8.5 Hz, 1H), 7.53 (d, *J* = 7.5 Hz, 2H), 7.63 (d, *J* = 8.0 Hz, 2H), 8.05 (m, 1H), 8.59 (s, 1H). LC-MS calc. $[M+H]^+$ 385.17, found 385.1.

***Tert*-butyl ((*trans*)-2-(4-(6-fluoropyridin-3-yl)phenyl)cyclopropyl)carbamate (18b)**



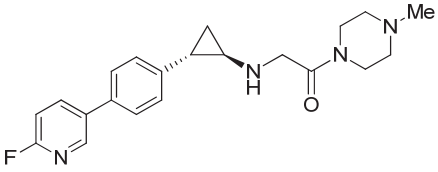
A solution of tert-butyl (trans)-2-(4-bromophenyl)cyclopropylcarbamate, **15e** (0.20 g, 0.64 mmol), 2-flouropyridine-5-boronic acid (0.11 g, 0.77 mmol), K_2CO_3 (0.27 g, 1.93 mmol) in CH_3CN and H_2O (4:1) was degassed for 30 min with argon gas, added $Pd(PPh_3)_4$ (0.007 g, 0.0064 mmol) heated the reaction mixture at reflux temp for 4 h. The progress of the reaction was monitored by TLC, after completion, poured the reaction mixture into water, extracted with EtOAc (2 x 20 mL). The combined extracts were washed with water (10 mL), brine (10 mL), dried over anhydrous Na_2SO_4, filtered and evaporated. The crude compound was purified by flash column chromatography (20-40% EtOAc in Hexanes) to give tert-butyl ((trans)-2-(4-(6-fluoropyridin-3-yl)phenyl)cyclopropyl)carbamate as a white solid (0.07 g, 35% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.19 (t, J = 6.0 Hz, 2H), 1.44 (s, 9H), 2.04-2.11 (m, 1H), 2.76 (s, 1H), 4.90 (br, 1H, NH), 6.99 (dd, *J* = 8.5 and 3.0 Hz, 1H), 7.24 (d, *J*= 8.0 Hz, 2H), 7.44 (d, *J*= 8.5 Hz, 2H), 7.93 (ddd, *J* = 8.0, 3.0 and 2.5 Hz, 1H), 8.37 (d, *J* = 2.5 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 16.40, 28.85, 28.39, 32.74, 79.80, 109.54, 126.94, 128.68, 132.09, 134.37, 139.49, 141.05, 145.62, 162.01, 163.92.

Tert-butyl ((*trans*)-2-(4-(6-fluoropyridin-3-yl)phenyl)cyclopropyl)(2-(4methyl piperazin-1-yl)-2-oxoethyl)carbamate (19b)



Compound **19b** prepared from **18b** using the procedure described for **10** (yield 52%). ^{1}H NMR(500 MHz, $CDCl_3$): δ 1.12 (br, 1H), 1.28 (br, 1H), 1.39 (s, 9H), 1.99 (br, 2H), 2.24-2.29 (m, 3H), 2.39 (br, 4H), 3.04 (br, 1H), 3.43 (br, 2H), 3.62 (br, 2H), 4.17 (br, 1H), 6.97 (d, *J* = 7.0 Hz, 1H), 7.18 (br, 2H), 7.40 (d, *J* = 8.0 Hz, 2H), 7.92 (t, *J* = 7.0 Hz, 1H), 8.36 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.94, 26.95, 28.42, 39.71, 44.52, 46.02, 49.18, 54.59, 80.32, 109.21, 126.75, 126.89, 134.17, 139.44, 139.51, 141.42, 145.47, 161.98, 163.88, 167.22.

2-(((*trans*)-2-(4-(6-Fluoropyridin-3-yl)phenyl)cyclopropyl)amino)-1-(4-methylpiperazin-1-yl)ethanone (20b, RN-7)



Compound **20b** prepared from **19b** using the procedure described for **11** (yield 50%). ^{1}H NMR(500 MHz, CD_3OD): δ 1.44 (br, 1H), 1.63 (br, 1H), 2.64 (br, 1H), 2.95 (s, 3H), 3.09 (br, 2H), 3.30 (br, 3H), 3.57 (br, 3H), 4.03 (br, 1H), 4.35 (br, 1H), 4.47 (br, 1H), 4.65 (d, *J* =

8.5 Hz, 1H), 7.14 (d, *J* = 8.5 Hz, 1H), 7.33 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 7.5 Hz, 2H), 8.16 (t, *J* = 8.0 Hz, 1H), 8.41 (s, 1H). ^{13}C NMR (125 MHz, CD_3OD): δ 12.53, 20.64, 38.06, 38.66, 41.37, 42.57, 52.48, 109.25, 109.55, 127.28, 135.39, 138.39, 139.89, 144.70, 161.82, 163.62. LC-MS calc. $[M+H]^+$ 369.20, found 369.1.

Biochemical Methods:

LSD1 inhibition. Three independent biochemical assay formats were employed for determination and confirmation of test compound potency against LSD1. All the assays were carried out in a 384 well format at 25 °C. The assay quality (Z') was evaluated by inclusion of 2 columns (32 wells) each for MAX (no inhibitor, DMSO) and MIN (10 μM LSD1 inhibitor, RN-1) control samples on the left and right side of the assay plate. Percentage inhibition of LSD1 activity was calculated according to the following equation: % inhibition = 100* (MAX avearge – test compound)/(average of MAX–average of MIN). For IC_{50} determination, dose response curves were fitted using Graphpad Prism 5 (GraphPad Software Inc., La Jolla, CA) with the equation for log[inhibitor] vs. response- variable slope (four parameters).

HRP-Coupled assay: Recombinant LSD1 (GenBank Accession No. NM_015013, amino acids 158-end with N-terminal GST tag) was expressed in *E. coli* and purified using glutathione agarose (BPS Biosciences) based upon the published studies of Forneris et al. 2007 [1]. The 21-mer peptide corresponding to the H3 histone tail (NH2-ARTK(me2)--QTARKSTGGKAPRKQKA-COOH) was synthesized by the Massachusetts Institute of Technology Biopolymers Laboratory (http://web.mit.edu/biopolymers), and the purity was confirmed by HPLC and MALDI mass spectrometry (expected mass= 2297.71, observed ion= 2298.30). 100 ng of LSD1 in a reaction volume of 30 μL (32 nM) was determined to afford satisfactory signal over background. The substrate K_m for 32 nM LSD1 was determined to be 10 μM, and this concentration was used for all HRP-coupled and mass spectrometry assays. The assay was determined to be linear over at least 30 minutes. Reactions were run in 50 mM sodium phosphate buffer (Boston BioProducts #BB-185, pH 7.4) with 0.01% BRIJ35 detergent (Calbiochem #203728) in 383-well black non-sterile plates (Corning #3573). To 100 ng of LSD1 in 20 μL of buffer was added inhibitor stock solution in DMSO. Typical volumes of DMSO were 50-500 nL. After pre-incubation of the enzyme and inhibitor for 10 minutes, 10 μL of substrate in buffer was added to a final concentration of 10 μM. The reaction ran for 20 minutes at room temperature, during which time the detection reagent was prepared. HRP (Sigma, #P2088, 5KU) and Amplite (ABD Bioquest #11000, in DMSO) were diluted in buffer, then 30 μL was added to each well for a final concentration of 0.06 U HRP and 40 μM Amplite. The plates were immediately read on a PerkinElmer Wallac Envision 2103 Multilabel plate reader (excitation filter: FITC 485; emission filter: photometric

595; gain: 75; excitation light: 10%). Background subtraction of no-enzyme controls and IC_{50} determination was performed in GraphPad Prism v5.03.

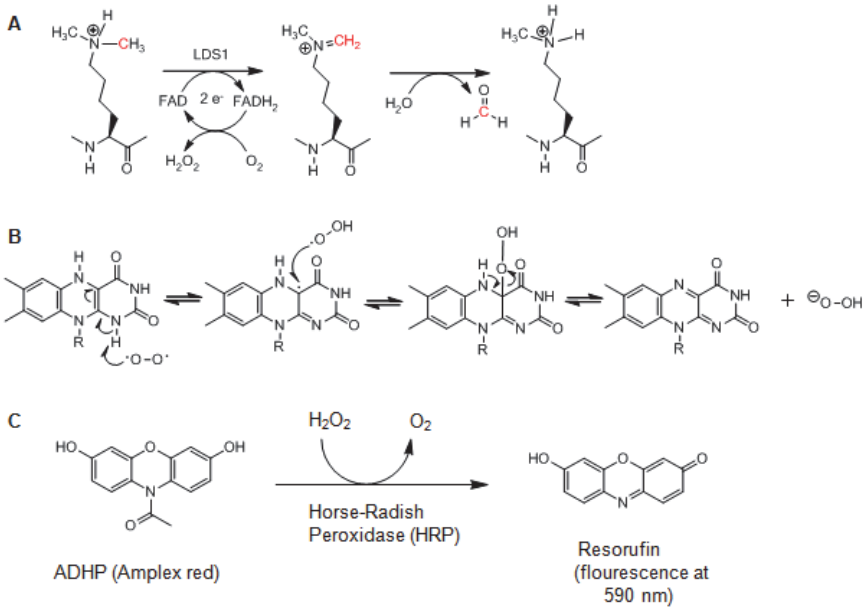


Figure SI-1. **HRP coupled assay scheme**. (**A**) LSD1 catalyzes the demethylation of Lys4 of histone H3 through a flavin-dependent oxidative reaction. LSD1 can act both on mono- and dimethylated H3K4. First, the histone substrate is bound and its methylated Lys4 side chain is oxidized (i) by the FAD prosthetic group with resultant reduction of oxygen to hydrogen peroxide. The resulting imine intermediate is hydrolyzed (ii) to generate the demethylated H3 tail and formaldehyde. (**B**) Regeneration of FAD oxidizing agent releases peroxide. **(C)** Peroxide-based coupled assay mechanism: Conversion of Amplex Red into Resorufin: Horseradish peroxidase uses ADHP (Amplex red) as an electron donor during the reduction of hydrogen peroxide to oxygen. The resulting product, resorufin, is a highly colored and fluorescent compound.

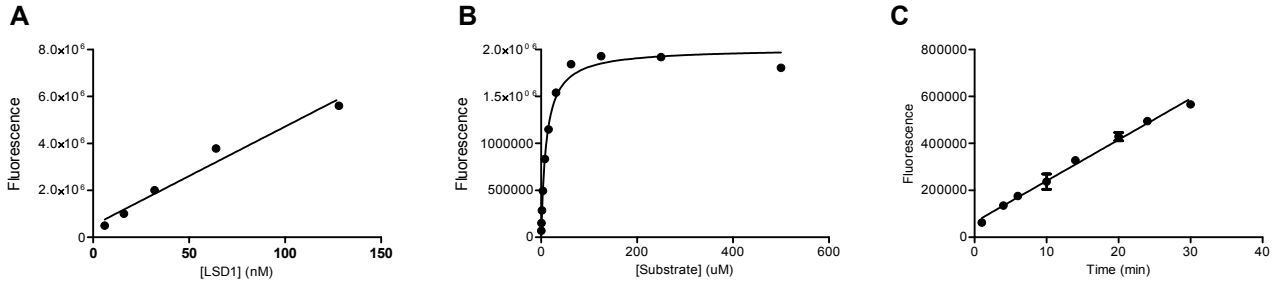


Figure SI-2. HRP-coupled LSD1 assay: (**A**) LSD1 reaction progress as a function of enzyme concentration indicates a direct relationship between enzyme concentration and activity in this assay. (**B**) K_m determination of substrate H3K4Me$_2$ peptide. The substrate K_m for 32 nM LSD1 was determined to be 10 μM. (**C**) LSD1 reaction progress as a function of time indicates linear response with substrate reaction time. Duration of 20 minutes was chosen for LSD1 demethylation reaction in the HRP-coupled assay based on robust signal to background value.

Mass-Spectrometry Assay: The experimental procedure is same as the HRP-coupled assay except the addition of the detection reagent. Reactions were terminated after 20 min incubation time by addition of 0.2N formic acid (30 µL). The plates were stored at -80 ^{o}C prior to submission for RapidFire High-throughput Mass Spectrometric analysis (Agilent Technologies, Wakefield, MA). The LSD1 demethylation reaction was monitored by detecting the levels of unmethyl-, monomethyl-, and dimethyl-H3K4 analytes using a selective reaction monitoring protocol. For determination of IC_{50} percentage substrate conversion [%Substrate Conversion=100*[Product/ (Product+Substrate)] H3K4Me_1 was plotted against test compound concentration using GraphPad Prism.

LSD1 TR-FRET assay for detection of H3K4Me_1 product: LSD1 enzymatic reactions for compound testing were performed in duplicate in a 10 µl reaction volume using an assay buffer of 50 mM Tris, pH 7.5, 0.01% *Brij*-35 in Proxiplate 384 Plus white plates (Perkin Elmer). Appropriate concentration of test compounds were pre-incubated for 15 minutes with 25 nM LSD1 (BPS Biosciences # 50100) and the enzymatic reactions were initiated by the addition of 0.0625 µM biotinylated H3K4Me_2 substrate peptide (Anaspec # 64356-1). Reactions were terminated after eight min by addition of 10 µl of reaction termination and detection reagent consisting of 50 µM RN-1 inhibitor in 1X LANCE detection buffer, 2 nM Mono-Methyl-Histone H3 (lys4) D1A9 Rabbit mAb (Cell Signaling Technology), 2 nM LANCE Eu-W1024 anti-rabbit IgG (Perkin Elmer) and 50 nM streptavidin-Ulight in (Perkin Elmer). Following incubation for an h at room temperature, the samples were read using a multi-label plate reader (Envision, Perkin Elmer). H3K4monomethyl product formation results in an increase in acceptor specific fluorescence at 665 nm indicating demethylation reaction progression. **Fig SI-3A:** The assay is designed to detect H3K4Me_1 product formed as results of LSD1 enzymatic reaction. Europium labeled to anti-rabbit IgG acts as the donor label for TR-FRET and the acceptor dye (Ulight) labeled streptavidin captures the biotinylated H3K4Me_1 product. A successful binding reaction of the primary H3K4me1 antibody to the biotinylated H3K4me1 product captured by Ulight-SA facilitates a time resolved fluorescence resonance energy transfer from the donor Europium label upon excitation to the acceptor Ulight, if the above mentioned assay components are in close proximity (~10 nm). The acceptor specific fluorescence is directly proportional to the amount of H3k4Me_1 product formed.

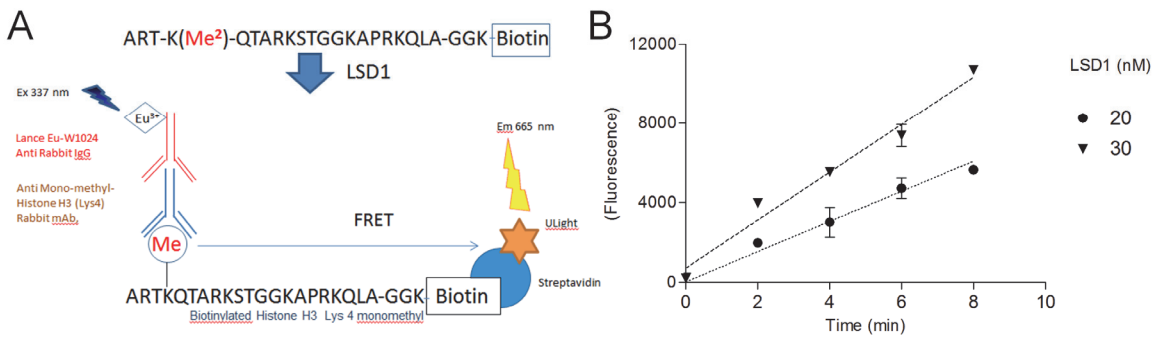


Figure SI-3. (A) **Schematic of a homogeneous TR-FRET assay for measuring mono-methylated H3K4:** The unmodified peptide is captured by the Eu-labeled antibody (Eu-Ab) and ULight-Streptavidin (ULight-SA), which brings the Eu donor and ULight acceptor dye molecules into close proximity. Upon irradiation at 320 -340 nm, the energy from the Eu donor is transferred to the ULight acceptor dye which, in turn, generates light at 665 nm. The intensity of the light emission is proportional to the level of biotinylated reaction product. (B) LSD1 Reaction progression as a function of enzyme concentration and time in a TR-FRET assay designed for detection of Monomethyl-H3K4. LSD1 enzymatic reaction was performed in duplicate with 0.0625 μM Biotinylated H3K4me2 substrate peptide in a 10 µL reaction volume. Reactions were terminated at specific time intervals by addition of 5 µl 40 μM RN-1 inhibitor in 1X LANCE detection buffer. For experiments designed to monitor reaction progression, detection reagent (2 nM Mono-Methyl-Histone H3 (lys4) D1A9 Rabbit mAb , 2 nM LANCE Eu-W1024 anti-rabbit IgG and 50 nM streptavidin-Ulight in 1X LANCE detection buffer) were added simultaneously to all the wells. Following incubation for an hour at room temperature, the samples were read using a multi-label plate reader (Envision, Perkin Elmer).

Assay for MAO A and MAO B inhibitory activity: The MAO activity assays were performed using a MAO-Glo™ assay kit (Promega), according to the manufacturer's protocol in 384 well Proxiplate 384 Plus white plates (Perkin Elmer) with miniaturization of final assay volume to 20 µL. Reactions were stopped after 60 min by adding reconstituted Luciferin Detection Reagent (50 µL/well). Then, 20 min after addition of this reagent, the fluorescence at 570 nm of the wells was measured with a fluorescence reader. The value of % inhibition was calculated from the fluorescence readings of inhibited wells relative to those of control wells. The compound concentration resulting in 50% inhibition was determined by plotting log[Inhibitor] versus the Relative Luminescence Units (RLU). IC_{50} values were determined by means of regression analysis of the concentration/inhibition data.

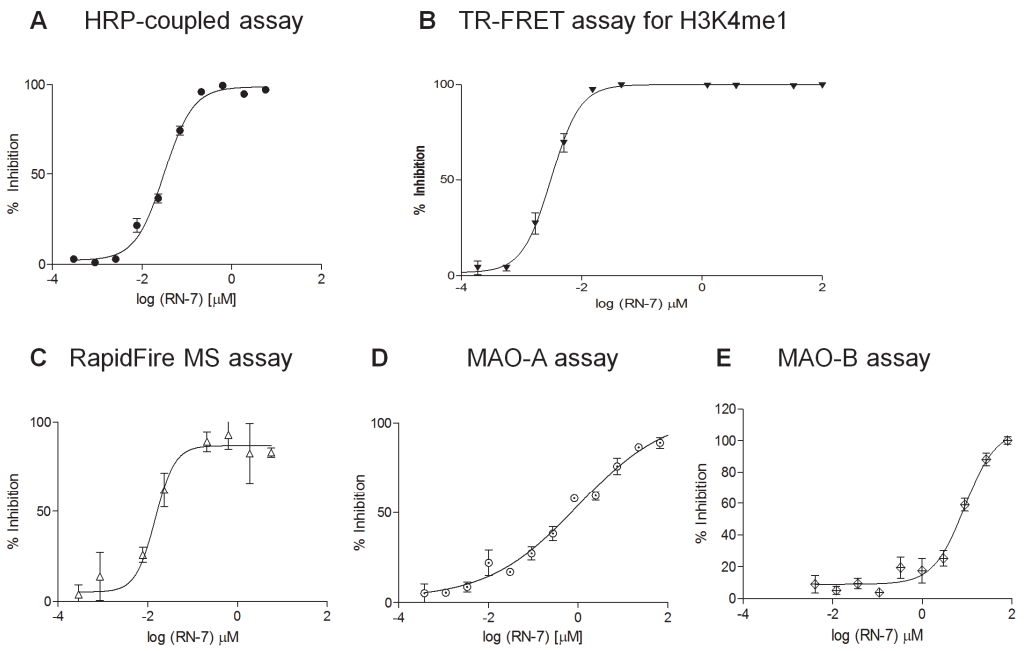


Figure SI-4. **Representative Inhibitor Assay Data (provided here for RN-7)** (**A**) Dose-dependent LSD1 inhibition using the HRP-coupled assay (**B**) Dose-dependent LSD1 inhibition using the TR-FRET assay and titration of RN-7 compound. (**C**) Dose-dependent LSD1 inhibition using the Rapidfire mass spectrometry assay (Biocius). (**D**) Dose-dependent MAO A inhibition using a MAO-Glo^{TM} assay kit (Promega) (**E**) Dose-dependent MAO B inhibition using a MAO-Glo^{TM} assay kit (Promega)

LSD1 inhibition kinetics: LSD1 (3.2 μM) was incubated with RN-1 (7.0 μM) in reaction buffer. An equal volume of DMSO was added to a separate stock of LSD1 (3.2 μM) as a control. At one minute intervals, 0.3 μL of each pre-incubated enzyme solution were diluted to 30 μL of substrate (10 μM) solution in buffer. The final concentration of reagents after this 100-fold dilution is the same as typically used in the coupled-assay; however, significant dissociation is expected if the inhibitor is non-covalent. Twenty minutes after dilution, the HRP and Amplite detection reagent was added and the plate was read immediately.

Behavior Assay Methods

Novel Object Recognition. Training and testing for novel object recognition (NOR) was carried out as previously described [2-5]. Briefly, before training, mice were handled 1-2 min for 4 days and then habituated to the experimental apparatus (white rectangular open field, 30 x 23 x 21.5 cm) 5 min a day for 6 consecutive days in the absence of objects. During training, mice were placed into the experimental apparatus with two identical objects (A1 and A2; either 100 ml beakers, 2.5 cm diameter, 4 cm height; or large blue Lego blocks, 2.5 x 2.5 x 5 cm) and were allowed to explore for 10 min [2].

Immediately following training, mice were systemically administered either 30 mg/kg RN-1, dissolved in a vehicle of 3% DMSO in ddH_2O, or vehicle alone (10 ml/kg volume; intraperitoneal). During the retention test, (90 min for short-term memory or 24 h for long-term memory, tested in different sets of animals), mice explored the experimental apparatus for 5 min. For the novel object recognition task, one familiar object (A3) and one novel object (B1) were placed in the same location as during training. All combinations and locations of objects were used in a balanced manner to reduce potential biases due to preference for particular locations or objects. All training and testing trials were videotaped and analyzed by individuals blind to the treatment condition. A mouse was scored as exploring an object when its head was oriented toward the object within a distance of 1 cm or when the nose was touching the object. The relative exploration time was recorded and expressed by a discrimination index [DI = $(t_{novel} - t_{familiar})/(t_{novel} + t_{familiar})$ X 100]. *Statistics.* Datasets were analyzed using Student's *t* tests with α levels held at 0.05.

Radiolabeling by automation with GN

Computer-controlled GN automated system was used for the preparation of ^{18}F[RN-7]. An aqueous solution of $[^{18}F]F^-$, produced by cyclotron using ^{18}O (*p*,*n*) ^{18}F reaction, was passed through a Sep-Pak Light QMA cartridge. The cartridge was dried with argon, and the ^{18}F activity was eluted with 1.0 ml of K_{222}/K_2CO_3 solution (6.8 mg K_{222}/mL and 2.8 mg K_2CO_3/mL). The solvent was then evaporated at 110 °C under an argon stream. Furthermore, the residue was azeotropically dried twice with 1 mL anhydrous CH_3CN at 110 °C under an argon stream. A solution of chloride precursor **9a** (1.5 mg) in 0.2 ml DMSO was added to the dried $[^{18}F]$ KF/K_{222} complex and heated at 140 °C for 10 min. The mixture was cooled down for 1 min, then diluted with 4 mL HPLC solvent, loaded into the reverse phase column chromatography and using 50% ACN/50% (0.1 M-aqueous ammonium formate) solvent system, the $[^{18}F]$ labeled product came out at 8 min and removal of tert-butoxycarbonyl group by TFA at 90 ^oC for 10 min gave the product **12**. The reaction mixture was diluted with deionized water (20 mL), passed through the Sep-Pak® plus cartridge and washed with water (10 mL). The yield of formation of ^{18}F[RN-7] is in the range of 15-20 mCi (5% yield, based on EOB. The product fraction was reformulated with EtOH (0.3 mL) and saline (3 mL). The average time required for the $[^{18}F]$-labeling, purification, deprotection, and reformulation was 70 min from the end-of-bombardment (EOB). Aliquots of the formulated solution was analyzed by analytical HPLC column using UV and gamma detectors (Agilent; Gemini C18 [250×4.6 mm]; 254 nm; mobile phase: flow rate 2 mL/min; 0.1 M AF /CH_3CN=65%/35%) to determine the specific activity and chemical and radiochemical purity. The radiochemical identity was confirmed by coinjection with "cold" fluorinated compounds that gave the same retention time of **20b (RN-7)** and **(^{18}F[RN-7])** are 3.50 and 3.68 min, respectively (35% ACN/65% 0.1M AF). Subsequently,

purity was verified using TLC (30% MeOH/70% CH_2Cl_2) by co-spotting the labeled product with a standard.

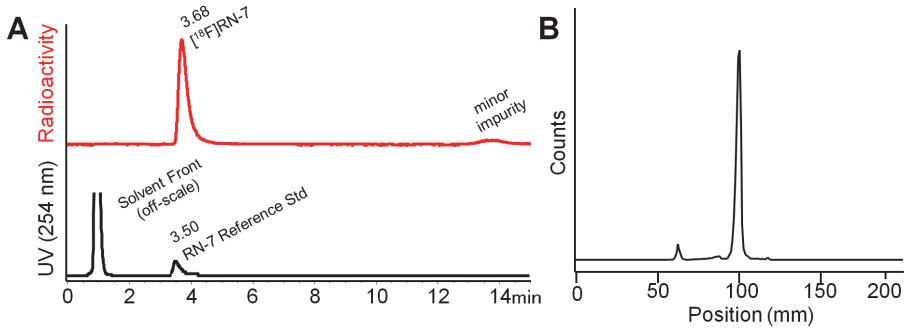


Figure SI-5. **Analysis of [^{18}F]RN-7** (**A**) Aliquots of the formulated solution was analyzed by analytical HPLC column using UV and gamma detectors (Agilent; Gemini C18 [250×4.6 mm]; 254 nm; mobile phase: flow rate 2 ml/min; 0.1 M AF /CH_3CN=6.5/3.5, retention time: 3.50 and 3.68 min for UV and Rad spectra respectively. (**B**) The RCY was determined by radio TLC method, with a mobile phase-MeOH:DCM-30:70). The retention factors (R_f) for [^{18}F]F^- and **of** [^{18}F]RN-7 were 0 and 0.6 respectively.

Bio-distribution studies

Saline (1.0 mL) containing [^{18}F]RN-7 (~100 µCi) was injected into the tail vein of two Sprague-Dawley rats. The rats were sacrificed at 10 and 40 min post-injection. The organs of interest (brain, heart, liver, spleen, lung, kidney, muscle, bone, and urine) were removed and weighed, and the corresponding radioactivity was assayed with an automatic gamma counter (Perkin Elmer Wizard). Data were normalized to blood.

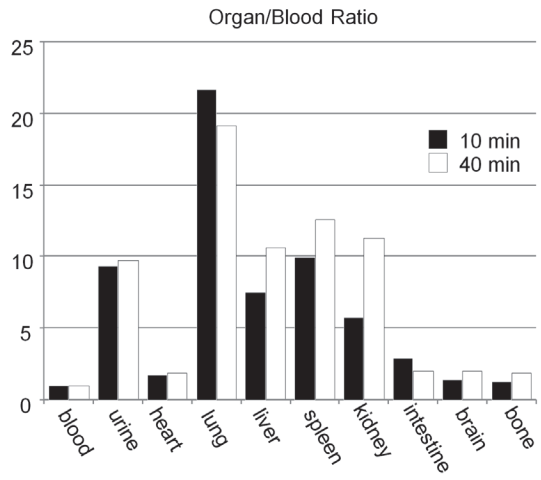


Figure SI-6. **Preliminary biodistribution experiments.** [^{18}F]RN-7 was administered intravenously to two Sprague-Dawley rats. One animal was killed at 10 min post injection and the other at 40 min. Organs and fluids were harvested and the amount of radioactivity per mass was determined. The ratio of organ:blood was used to compare regional uptake. The preliminary experiment indicated normal excretion with some uptake in the lungs and a minimal amount of defluorination as observed by uptake in bone.

Pharmacokinetics and Biodistribution studies of RN-1

The plasma and concentration-time data (ng/mL) for RN-1 was obtained from bioanalytical group of Sai Advantium Pharma Ltd, Pune, India. The brain concentrations were converted to ng/g by considering total homogenate volume and brain weight {conc. (ng/g) = conc. (ng/mL) * total homogenate volume (mL)/ weight of tissue (g)}. The plasma and brain concentration-time data was then used for the pharmacokinetic analysis. Non-Compartmental-Analysis module in WinNonlin® (Version 5.2) was used to assess the pharmacokinetic parameters. Peak plasma concentrations (C_{max}) and time required to achieve the peak plasma concentration (T_{max}) was the back observed value in plasma concentration time data. The areas under the concentration time curve (AUC) were calculated by linear trapezoidal rule.

Twenty seven male mice were dosed with freshly prepared RN-1 solution formulation at 10 mg/kg, intraperitoneally. The concentration of formulation was 1 mg/mL for intraperitoneal route and solution formulation was prepared by dissolving RN-1 in 0.9% *w/v* normal saline (pH-3 adjusted with HCl). All

animals were provided laboratory rodent diet (Vetcare India Pvt Ltd, Bengaluru) *ad libitum,* except for 2 h before and 2 h after RN-1 administration.

Blood samples were collected at 0.08, 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h from set of three mice at each time point. The blood samples (~120 µL) were collected from the retro-orbital plexus of each mouse into labeled micro-tubes, containing 20% *w/v* K_2EDTA solution, as an anticoagulant. Plasma was harvested from the blood by centrifugation at 4000 rpm for 10 min at 4 ± 2 ºC and was stored below -70 ºC until bioanalysis. After collecting blood samples, mice were sacrificed and brain was collected at 0.08, 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h. Following collection, the brain samples were washed in ice-cold buffer, weighed and placed in polypropylene tubes. Further brain samples were homogenized using ice cold phosphate buffer pH 7.4 and the total homogenate volume was thrice the brain weight. The brain samples were stored at -70 ºC until bioanalysis. Concentrations of RN-1 in mouse plasma and brain samples were determined by developed LC-MS/MS method. The developed bioanalytical method was found linear from 1.07 to 5338.54 ng/ml in both the matrices using a linear fit, with $1/X^2$ (where X is concentration) weighting factor. The calibration curves of RN-1 are depicted in **Figure SI-7**.

Figure SI-7A: Calibration curve for LC-MS/MS analysis of RN-1 in plasma

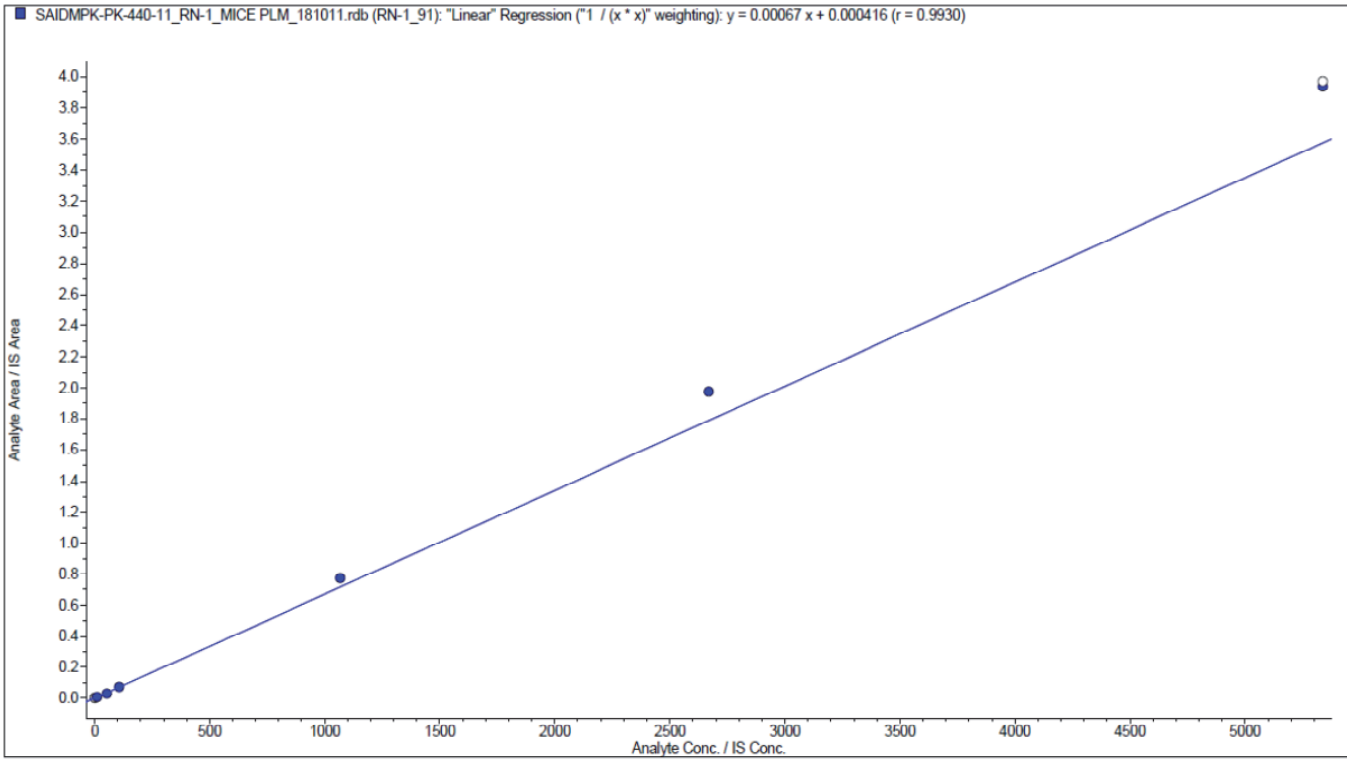
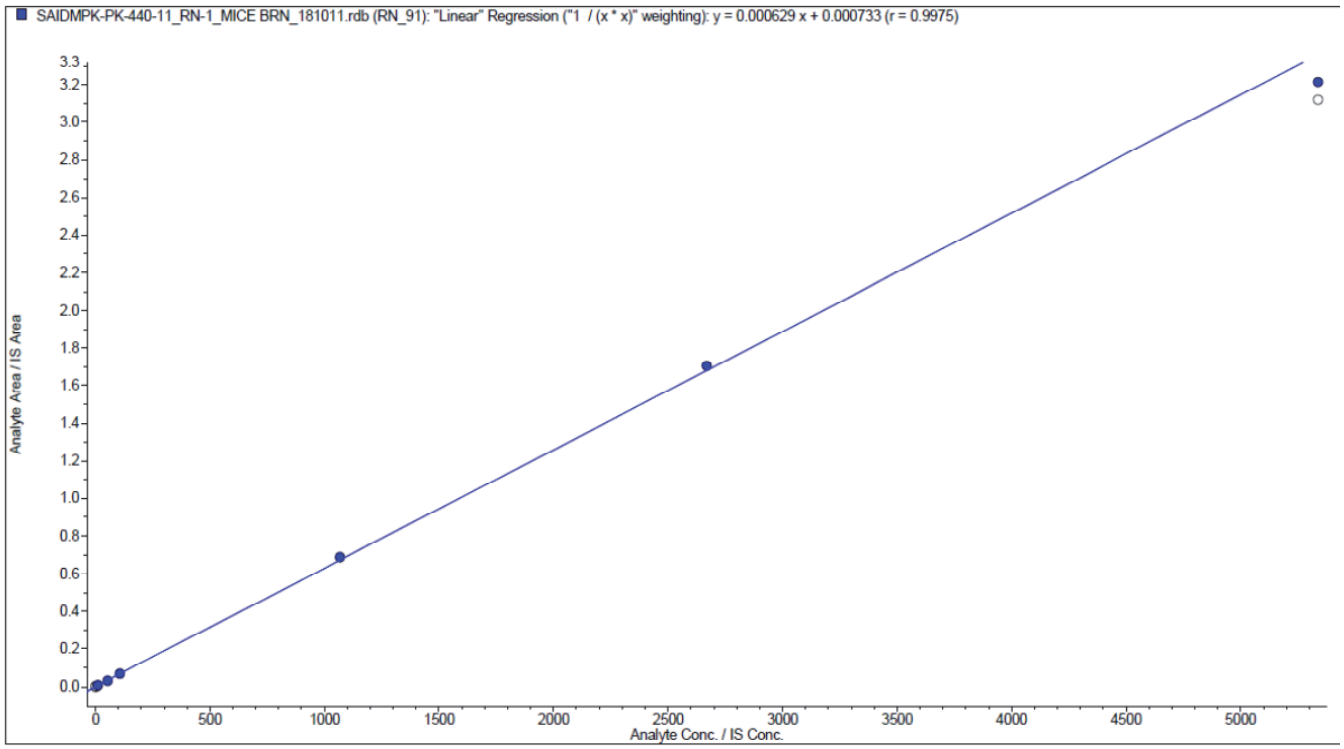


Figure SI-7B: Calibration curve for LC-MS/MS analysis of RN-1 in brain homogenate



Sample Processing and extraction procedure

Plasma/brain was spiked with analyte (RN-1) by taking 10 µL of analyte into 190 µL of plasma or brain. Samples were vortexed for two minutes. 25 µL of above spiked sample were taken in centrifuge tubes and 100 µL of IS (Albendazole, 125 ng/mL) in acetonitrile was added into it; except in blank sample where only acetonitrile was added, and then vortexed for 5 min. After vortexing, centrifuged at 15000 rpm 4 ^{o}C for 10 min. 100 µL of supernatant was collected in insert vials and analyzed using LC-MS/MS.

Recovery of RN-1 in mice plasma & brain homogenate was evaluated at three concentrations 12.01, 106.77, 2669.27 ng/mL. Average recoveries of RN-1 in plasma was 100.89 ± 19.2. Average recoveries of RN-1 in brain homogenate was 106.01 ± 10.0. Recovery of Internal standard (Albendazole) was 100.43 ± 2.7 from plasma and 102.53 ± 0.9 from brain homogenate

Table SI1: Plasma concentration-time data of RN-1 following a single intraperitoneal administration in male C57BL/6 mice (Dose: 10 mg/kg)

Animal ID	Plasma Conc. (ng/mL)								
	Time (hr)								
	0.08	**0.25**	**0.50**	**1.00**	**2.00**	**4.00**	**6.00**	**8.00**	**24.00**
2601	508.5								
2602	624.0								
2603	492.5								
2604		325.9							
2605		407.3							
2606		436.5							
2607			336.8						
2608			315.3						
2609			279.2						
2610				259.6					
2611				223.1					
2612				253.3					
2613					208.9				
2614					175.2				
2615					222.0				
2616						82.1			
2617						86.7			
2618						109.9			
2619							55.8		
2620							77.6		
2621							92.5		
2622								45.7	
2623								61.3	
2624								63.0	
2625									7.3
2626									8.4
2627									7.4
Mean	**541.7**	**389.9**	**310.4**	**245.3**	**202.0**	**92.9**	**75.3**	**56.7**	**7.7**
SD	71.7	57.3	29.1	19.5	24.2	14.9	18.5	9.6	0.6
CV%	13.2	14.7	9.4	7.9	12.0	16.0	24.5	16.9	8.0

LLOQ –1.07 ng/mL,

Table SI2: Brain concentration-time data (ng/mL) of RN-1 following a single intraperitoneal administration in male C57BL/6 mice (Dose: 10 mg/kg)

Animal ID	Brain Conc. (ng/mL)								
	Time (hr)								
	0.08	**0.25**	**0.50**	**1.00**	**2.00**	**4.00**	**6.00**	**8.00**	**24.00**
2601	1872.2								
2602	2671.1								
2603	1678.9								
2604		3003.3							
2605		2971.2							
2606		2466.2							
2607			3429.7						
2608			2261.0						
2609			2520.9						
2610				3493.4					
2611				3715.6					
2612				2662.8					
2613					3784.9				
2614					3306.8				
2615					4298.8				
2616						3251.6			
2617						3177.0			
2618						3577.3			
2619							2424.9		
2620							4111.2		
2621							3489.8		
2622								1878.2	
2623								2829.2	
2624								2858.0	
2625									384.0
2626									433.6
2627									358.7
Mean	**2074.1**	**2813.6**	**2737.2**	**3290.6**	**3796.8**	**3335.3**	**3342.0**	**2521.8**	**392.1**
SD	526.0	301.2	613.6	555.0	496.1	212.9	852.8	557.5	38.1
CV%	25.4	10.7	22.4	16.9	13.1	6.4	25.5	22.1	9.7

LLOQ –1.07 ng/mL,

Table SI3: Brain concentration-time data (ng/g) of RN-1 following single intraperitoneal administration in male C57BL/6 mice (Dose: 10 mg/kg)

Animal ID	Brain Conc. (ng/g)								
	Time (hr)								
	0.08	**0.25**	**0.50**	**1.00**	**2.00**	**4.00**	**6.00**	**8.00**	**24.00**
2601	5616.5								
2602	8013.3								
2603	5036.8								
2604		9009.9							
2605		8913.6							
2606		7398.6							
2607			10289.2						
2608			6783.1						
2609			7562.7						
2610				10480.2					
2611				11146.9					
2612				7988.3					
2613					11354.7				
2614					9920.5				
2615					12896.4				
2616						9754.7			
2617						9531.1			
2618						10731.9			
2619							7274.8		
2620							12333.6		
2621							10469.5		
2622								5634.7	
2623								8487.5	
2624								8574.0	
2625									1152.0
2626									1300.7
2627									1076.2
Mean	**6222.2**	**8440.7**	**8211.7**	**9871.8**	**11390.5**	**10005.9**	**10026.0**	**7565.4**	**1176.3**
SD	1578.0	903.7	1840.9	1664.9	1488.3	638.6	2558.4	1672.6	114.2
CV%	25.4	10.7	22.4	16.9	13.1	6.4	25.5	22.1	9.7

LLOQ –1.07 ng/mL,

Determination of mice brain tissue binding of compound RN-1 using rapid equilibrium dialysis method:

Brain tissue homogenate samples were prepared by diluting one part of whole brain tissue with three volumes of dialysis buffer (phosphate buffered saline pH 7.4 - 0.1 M sodium phosphate and 0.15 M sodium chloride) to yield 4 times diluted homogenate. RN-1 at a concentration of 5 µM was added to mice brain homogenate. This was immediately followed by dialyzing against buffer until equilibrium was achieved. Concentrations of RN-1 in brain homogenate and buffer were determined to calculate unbound and bound percentages of compound to the brain tissue.

Assay Procedure: Rapid equilibrium dialysis was performed with a rapid equilibrium dialysis (RED) device containing dialysis membrane with a molecular weight cut-off of 8,000 Daltons. Each dialysis insert contains two chambers. The red chamber was used for brain homogenate while the white chamber for buffer. A 200 µL aliquot of RN-1 in brain homogenate (triplicates) was added to the red chamber of dialysis inserts. A 350 µL aliquot of dialysis buffer was added to the buffer chamber of the inserts. Carbamazepine was used as positive control for brain tissue binding. After sealing the RED device with an adhesive film, dialysis was done at 37 ^{o}C with shaking at 100 rpm for 4 h as per manufacturer's recommendation.

Recovery and stability: A 50 µL aliquot of test compounds and positive controls was separately added to 0.5 mL micro fuse tubes. Two aliquots were frozen immediately (0 minute sample). Two other aliquots were incubated at 37 ^{o}C for 4 h along with the RED device. Following dialysis, an aliquot of 50 µL was removed from each well (brain homogenate and buffer) and diluted with equal volume of opposite matrix to nullify the matrix effect. Similarly, buffer was added to recovery and stability samples. An aliquot of 100 µL was submitted for LC-MS/MS analysis.

Sample preparation and Bio-analysis: A 25 µL aliquot of the carbamazepine, RN-1 was crashed with 100 µL of acetonitrile containing internal standards (Antipyrine 250 ng/mL in acetonitrile) and vortexed for 5 min. The samples were centrifuged at 15000 rpm at 4 ^{o}C for 10 min and 100 µL of supernatant was submitted LC-MS/MS analysis. Samples were monitored for parent compound in MRM mode using LC-MS/MS.

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