Supporting Information

Design and synthesis of styrenylcyclopropylamine LSD1 inhibitors.

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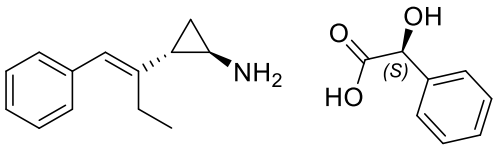
Methods and Materials

All commercial reagents and anhydrous solvents were purchased and used without purification, unless specified. Column chromatography was performed using a Biotage chromatography system on Biotage or Silicycle normal phase silica gel columns. NMR spectra were recorded on a Varian Unity Inova (400 MHz) or an Oxford (Varian, 300 MHz) instrument. LCMS were recorded on an Agilent 1200 series LC connected to an Agilent 6120 MS or Agilent 1100 series LC connected to an Agilent 1956B MS or a Shimadzu LC-MS-2020 system. Preparatory HPLC were performed using a Gilson GX-281 or P230 Gradient System (Elite). Chiral preparatory HPLC were performed using Elite P230 Preparative Gradient System, Thar Prep-80 and Thar SFC X-5 systems.

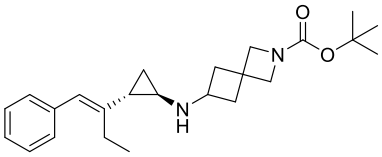
Experimental Procedures



(1R,2S)-2-((E)-1-phenylbut-1-en-2-yl)cyclopropan-1-amine (S)-2-hydroxy-2-phenylacetate

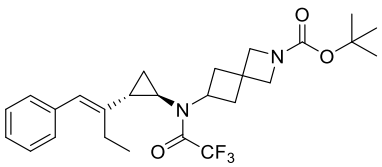
The synthesis has been reported previously.[1]

Synthesis of CPI-0670242: 2-(6-(((1R,2S)-2-((E)-1-phenylbut-1-en-2-yl)cyclopropyl)amino)-2-azaspiro[3.3]heptan-2-yl)ethanol bis-(2,2,2-trifluoroacetic acid) salt



***tert*-butyl 6-((((*(*1R,2S*)*)-2-((E)-1-phenylbut-1-en-2-yl)cyclopropyl)amino)-2-azaspiro[3.3]heptane-2-carboxylate**

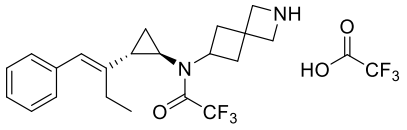
To ((1R,2S or 1S,2R))-2-((E)-1-phenylbut-1-en-2-yl)cyclopropanamine (S)-2-hydroxy-2-phenylacetate (6.82 g, 20.1 mmol) and *tert*-butyl 6-oxo-2-azaspiro[3.3]heptane-2-carboxylate (4.25 g, 20.1 mmol) in 1,2-DCE (100 mL) was added sodium triacetoxyborohydride (8.98 g, 42.4 mmol). After 30 min., the reaction was quenched with K_2CO_3 (aq.) and extracted with DCM (2X150 mL). The organic phase was concentrated and the crude residue was purified via column chromatography (40g column, 5% to 100% EtOAc:hexanes)to afford *tert*-butyl 6-((((1R,2S or 1S,2R))-2-((E)-1-phenylbut-1-en-2-yl)cyclopropyl)amino)-2-azaspiro[3.3]heptane-2-carboxylate (single unknown stereoisomer) (1.35 g, 3.52 mmol). LCMS $[M+H]^+$ *m/Z:* calc'd 383.26 *found* 383.7.



[1] Albrecht, B. K.; Audia, J. E.; Cote, A.; Duplessis, M.; Gehling, V. S.; Harmange, J.-C.; Vaswani, R. G. Preparation of 2-styrylcyclopropan-1-amine derivatives as LSD1 inhibitors and uses thereof. WO2016172496.

***tert*-butyl 6-(2,2,2-trifluoro-N-(((1R,2S))-2-((E)-1-phenylbut-1-en-2-yl)cyclopropyl)acetamido)-2-azaspiro[3.3]heptane-2-carboxylate**

To *tert*-butyl 6-((((1R,2S or 1S,2R))-2-((E)-1-phenylbut-1-en-2-yl)cyclopropyl)amino)-2-azaspiro[3.3]heptane-2-carboxylate (1.35 g, 3.52 mmol) dissolved in DCM (9 mL) was added diisopropylethylamine (826 µL, 4.75 mmol). The solution was cooled to 0 °C before addition of trifluoroacetic anhydride (561 µL, 4.04 mmol). The reaction mixture was stirred for 24 h while warming to room temperature. The volatiles were evaporated under reduced pressure and the crude residue purified by column chromatography on silica gel (0% to 40% EtOAc in hexanes, 40g) to afford *tert*-butyl 6-(2,2,2-trifluoro-N-(((1R,2S or 1S,2R))-2-((E)-1-phenylbut-1-en-2-yl)cyclopropyl)acetamido)-2-azaspiro[3.3]heptane-2-carboxylate (1.34 g, 2.80 mmol). LCMS $[M+Na]^+$ *m/Z:* calc'd 501.23 *found* 501.1

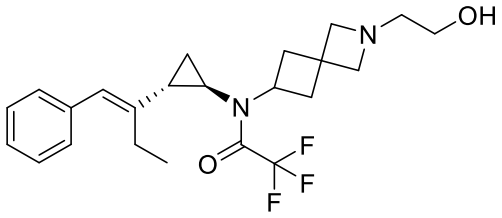


2,2,2-trifluoro-N-(((1R,2S))-2-((E)-1-phenylbut-1-en-2-yl)cyclopropyl)-N-(2-azaspiro[3.3]heptan-6-yl)acetamide 2,2,2-trifluoroacetate

To *tert*-butyl 6-(2,2,2-trifluoro-N-(((1R,2S or 1S,2R))-2-((E)-1-phenylbut-1-en-2-yl)cyclopropyl)acetamido)-2-azaspiro[3.3]heptane-2-carboxylate (1.36 g, 2.84 mmol) dissolved in DCM (10 mL) was added trifluoroacetic acid (2.16 mL, 28.4 mmol) and the solution stirred for 5 h. The reaction was concentrated to afford 2,2,2-trifluoro-N-(((1R,2S or 1S,2R))-2-((E)-1-phenylbut-1-en-2-yl)cyclopropyl)-N-(2-azaspiro[3.3]heptan-6-yl)acetamide 2,2,2-trifluoroacetate (1.39 g, 2.82 mmol). LCMS $[M+H]^+$ *m/Z:* calc'd 379.20 *found* 379.3.

Example 1: 2-(6-(((1R,2S)-2-((E)-1-phenylbut-1-en-2-yl)cyclopropyl)amino)-2-azaspiro[3.3]heptan-2-yl)ethanol bis-(2,2,2-trifluoroacetic acid) salt

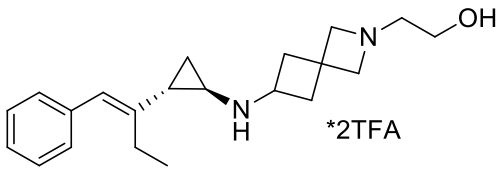
Step 1:



2,2,2-trifluoro-*N*-(2-(2-hydroxyethyl)-2-azaspiro[3.3]heptan-6-yl)-*N*-((1R,2S)-2-((E)-1-phenylbut-1-en-2-yl)cyclopropyl)acetamide

To a solution of 2,2,2-trifluoro-N-((1R,2S or 1S,2R)-2-((E)-1-phenylbut-1-en-2-yl)cyclopropyl)-N-(2-azaspiro[3.3]heptan-6-yl)acetamide 2,2,2-trifluoroacetate (400 mg, 812 µmol) and glycoaldehyde dimer (89.2 mg, 1.36 mmol) in methanol was added 5 drops of acetic acid and sodium cyanoborohydride (46.6 mg, 743 µmol) . The reaction was heated to 40°C and monitored by LCMS. After 1 hour, the reaction was quenched with 10% aqueous potassium carbonate to a pH of 8. Volatiles were then removed under vacuum. The crude mixture was extracted twice with 2-methyltetrahydrofuran. The organic layer was then dried with sodium sulfate, filtered and volatiles were removed under vacuum to afford 2,2,2-trifluoro-*N*-(2-(2-hydroxyethyl)-2-azaspiro[3.3]heptan-6-yl)-N-((1R,2S or 1S,2R)-2-((E)-1-phenylbut-1-en-2-yl)cyclopropyl)acetamide which was used without further purification. LCMS $[M+H]^+$ *m/Z:* calc'd 423.23 *found* 423.1.

Step 2:



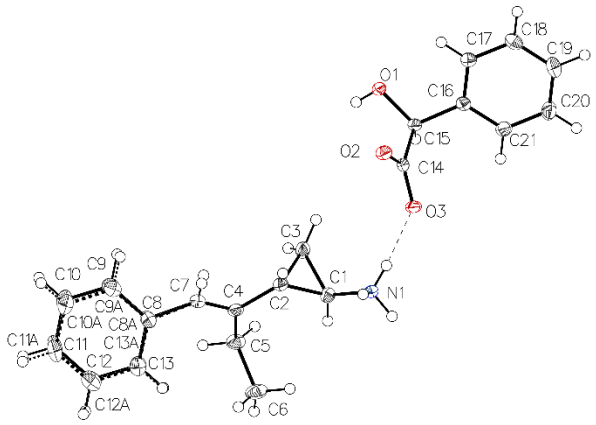
2-(6-(((1R,2S)-2-((E)-1-phenylbut-1-en-2-yl)cyclopropyl)amino)-2-azaspiro[3.3]heptan-2-yl)ethanol bis-(2,2,2-trifluoroacetic acid) salt

To a round bottom flask charged with 2,2,2-trifluoro-*N*-(2-(2-hydroxyethyl)-2-azaspiro[3.3]heptan-6-yl)-*N*-((1R,2S or 1S,2R)-2-((E)-1-phenylbut-1-en-2-yl)cyclopropyl)acetamide (343mg, 812 µmol) was added methanol (4.1 mL) and the solution cooled to 0°C before addition of 1.0 mL of 10% aqueous sodium hydroxide. The reaction was stirred under nitrogen atmosphere for 30 min and then was quenched with 2,2,2-trifluoroacetic acid to a pH of 2.0. Volatiles were then removed under vacuum. The desired product was purified by reverse phase column chromatography using 10 - 30 % CH_3CN / 0.1% aqueous 2,2,2-trifluoroacetic acid. Pure fractions were combined, frozen and lyophilized over 4 days to afford 2-(6-(((1R,2S or 1S,2R)-2-((E)-1-phenylbut-1-en-2-yl)cyclopropyl)amino)-2-azaspiro[3.3]heptan-2-yl)ethanol bis-(2,2,2-trifluoroacetic acid) salt (115 mg, 207 µmol). LCMS $[M+H]^+$ *m/Z:* calc'd 327.24 *found* 327.3.^{1}H NMR (400 MHz, DMSO-d_6) δ 9.87 (br s, 1H), 9.21 (br s, 2H), 7.39 – 7.31 (m, 2H), 7.27 – 7.17 (m, 3H), 6.21 (s, 1H), 4.25 – 4.11 (m, 2H), 4.11 – 4.06 (m, 1H), 3.98 (m, 1H), 3.85 – 3.69 (m, 1H), 3.55 (s, 2H), 3.25 – 3.11

(m, 2H), 2.80 – 2.72 (m, 1H), 2.72 – 2.65 (m, 1H), 2.65 – 2.53 (m, 1H), 2.48 – 2.35 (m, 1H), 2.30 – 2.18 (m, 2H), 2.02 – 1.90 (m, 1H), 1.22 – 1.14 (m, 2H), 1.12 (t, *J* = 7.6 Hz, 3H).

Small molecule X-ray crystallography

Confirmation of 1*R*,2*S*-stereochemistry



Parameters:

Bond precision: C-C = 0.0031 A Wavelength=1.54178

Cell: a=6.3649(1) b=7.6194(1) c=18.2235(3)
alpha=90 beta=93.7903(11) gamma=90 Temperature: 100 K

	Calculated	Reported
Volume	881.85(2)	881.85(2)
Space group	P 21	P 1 21 1
Hall group	P 2yb	P 2yb
Moiety formula	C13 H18 N, C8 H7 O3	C13 H18 N, C8 H7 O3
Sum formula	C21 H25 N O3	C21 H25 N O3
Mr	339.42	339.42
Dx,g cm-3	1.278	1.278 Z 2 2
Mu (mm-1)	0.678	0.678
F000	364.0	364.0
F000'	365.07	
h,k,lmax	7,9,22	7,9,22
Nref	3470[1873]	3359
Tmin,Tmax	0.914,0.973	0.693,0.754
Tmin'	0.873	

Correction method= # Reported T Limits: Tmin=0.693 Tmax=0.754
AbsCorr = MULTI-SCAN
Data completeness= 1.79/0.97 Theta(max)= 72.027
R(reflections)= 0.0360(3272) wR2(reflections)= 0.0965(3359)
S = 1.024 Npar= 238

LSD1 Biochemical Assay

Compound potency was assessed through demethylation of a biotinylated H3K4Me1 peptide substrate in a TR-FRET assay. Specifically, 5 μL of biotinylated substrate (ART-K(Me1)-QTARKSTGGKAPRKQLA-GGK(Biotin))(Anaspec) in reaction buffer (50 mM Hepes (pH 7.4), 100 mM NaCl, 1 mM DTT, 0.1 mg/mL BSA, and 0.01% (v/v) Tween-20) was added to a 384 plate (Greiner) which already contained compound (80 nL) at varying concentrations as a 10 point dose-response in DMSO. To this was added 5 μL of N-terminally His tagged full length LSD1 (Expressed in *E. coli* and purified in house) and FAD in reaction buffer. Final concentrations were 10 nM LSD1, 40 nM FAD, and 4 μM substrate in reaction buffer with 0.8% DMSO (v/v). After 50 minutes at room temperature reactions were quenched by the addition of 90 μL of quench buffer (same as reaction buffer except 500 mM NaCl). After quenching 10 μL was transferred to a black 384 well Proxiplate Plus (Perkin-Elmer). To this was added 10 μL of detection mix (SA-APC and Eu-α-H3K4) (both Perkin-Elmer) in quench buffer. Final concentrations in the detection reaction were 200 nM biotinylated peptide, 200 nM SA-APC (biotin binding sites) and 0.3 nM Eu antibody in a volume of 20 μL. After a 60 minute incubation at room temperature plates were read in a laser sourced Envision (Perkin-Elmer) measuring fluorescence at 615 and 665 nm. IC_{50}s were calculated by comparing the 665/615 nm ratio relative to a no enzyme (100% inhibition) and no inhibitor (0% inhibition) controls as using non-linear least square four parameter fits and either Activity Base (IDBS) or GraphPad Prism (GraphPad Software, Inc.).

LSD1 kinact/Ki studies

Inactivation parameters for LSD1 inhibitors were determined from the time-dependence of the compound IC_{50} values. Specifically, 1 μL of 10X compound or DMSO solutions in assay buffer (50 mM HEPES, pH 8.0, 20 mM NaCl, 1 mM DTT, 0.1 mg/mL BSA, 0.01% (v/v) Tween-20 and 40 nM FAD) were plated into a 384-well plate (Greiner). A 1.11X solution of enzyme (f.c. 1 nM) and substrate (ART-K(Me1)-QTARKSTGGKAPRKQLA-GGK(Biotin), f.c. 4 μM) were incubated for 5 minutes. Nine microliters of 1.11X enzyme/substrate solutions were added to compound/DMSO-containing wells. Reactions were quenched at indicated time pointes using 90 μL of quench buffer (50 mM HEPES, pH 7.4, 500 mM NaCl, 1 mM DTT, 0.1 mg/mL BSA and 0.01% (v/v) Tween-20). Ten microliters of quenched reaction mixtures were transferred to a black, 384-well ProxiPlate Plus (Perkin Elmer) containing 10 μL of Detection mix. IC_{50} values were calculated by comparing the 665/615 nm ratio relative to a no enzyme (100% inhibition) and no inhibitor (0% inhibition) controls as using non-linear least square four parameter fits in GraphPad

Prism (GraphPad Software, Inc.). Determination of K_I and k_{inact} were determined by fitting IC_{50} values at the indicated time points to the following equation, described previously[12]:

$$IC_{50}(t) = K_I\left(1 + \frac{[S]}{K_m}\right)\left(\frac{2 - 2e^{-\eta_{IC_{50}} * k_{inact} * t}}{\eta_{IC_{50}*} k_{ianct} * t} - 1\right)$$

$$Where:$$

$$\eta_{IC_{50}} = \frac{IC_{50}(t)}{K_I\left(1 + \frac{[S]}{K_m}\right) + IC_{50}(t)}$$

*Substrate K_m was experimentally determined to be 5.4 μM under these conditions

LSD1 LY96 Quantigene assay

MV4-11 cells were cultured at a density of 4 × 104 cells per well in a 96-well plates and treated with various doses inhibitor starting from 10 μM up to 0.0005 μM for 16h. The LY-96 mRNA induction was quantified using the Quantigene 2.0 system (Affymetrix). The cells were lysed with Lysis Mixture containing Proteinase K. The working reagent for capturing the RNA was prepared according to the steps detailed in "Capturing Target RNA from Cultured Cell or Blood Lysates" in the Quantigene handbook. The subsequent hybridization with LY-96 probe, signal amplification and detection steps were performed as described in the manual. The chemiluminescence was read using Envision (PerkinElmer) and Abase (IDBS software) was used to plot the dose response curves and calculate IC50.

Cell Proliferation Assays

Cells were plated in 96-well tissue culture dishes at various cell line-dependent densities, such that cell growth would remain exponential over a 4-day growth period. CPI-670242 and other LSD1 inhibitors were arrayed in a 10-point dose curve at 4-fold dilutions (10 μM maximum concentration). Cells were split every fourth day and an aliquot was removed for viable cell counts and the remainder was replated at a fixed ratio specific for each individual cell line, such that the cell number input re-established the original cell plating density in the DMSO-treated control wells. Viable cell numbers were assessed at days 4, 8, and 12 using the using the Cell Titer-Glo® luminescent cell viability assay (Promega, Madison, WI USA) and read on an EnVision® Multilabel Plate Reader (Perkin Elmer, Waltham, MA USA). GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA USA) was used for curve fitting and determination of GI_{50} values.

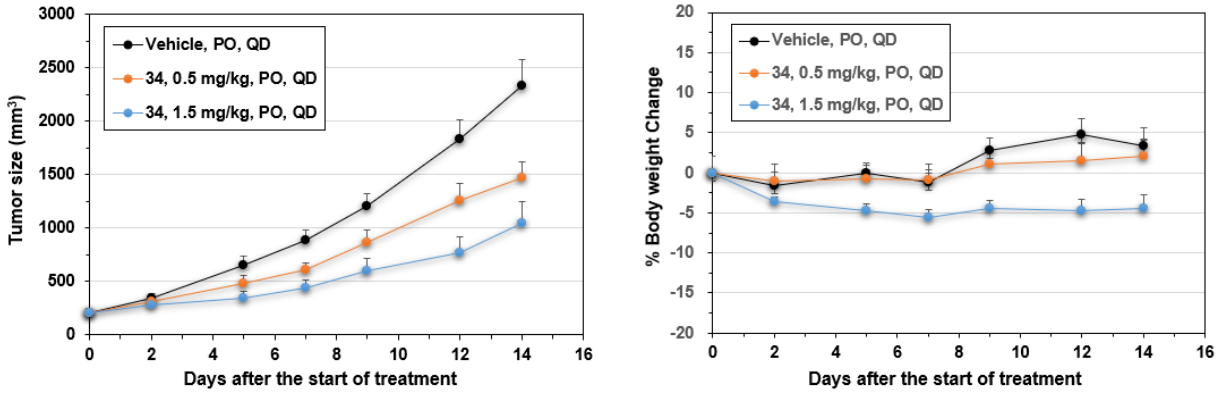
[2] Kripendorff, B.F.; Neuhaus, R.; Lienau, P.; Reichel A.; Huisinga, W. Mechanism-based inhibition: deriving K(I) and k(inact) directly from time-dependent IC(50) values. *J Biomol Screen* 2009*,* 14*,* 913-923.

Animal Use Care Statement

All the procedures were performed according to the guidelines approved by the Institutional Animal Care and Use Committee (IACUC) of WuXi AppTec, Shanghai, China, following the guidance of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

In vivo Kasumi-1 Xenograft Experiment

Figure S1



CB-17 SCID mice were inoculated subcutaneously at the right flank with exponentially growing Kasumi-1 cells (1 x 10^7) in 0.2 mL of a PBS:Matrigel (1:1) mixture. Tumor growth was assessed daily, until the average tumor volume reached approximately 120 mm^3. At this point, tumor-bearing mice were randomly assigned to the treatment groups. Each group consisted of 10 tumor bearing mice. Mice were treated with either vehicle (0.5% methylcellulose + 0.2% Tween 80) or CPI-0670242 at 1.5 mg/kg QD, formulated in the vehicle. Tumor size was measured three times a week using a caliper, and the tumor volume (V) was expressed in mm^3 using the formula: $V = 0.5a \times b^2$ where "a" and "b" were the long and short diameters of the tumor, respectively. The mice were weighed every day to monitor for changes in body weight. TGI% was calculated according to the following equation: $TGI\ (\%) = [1-(T_1-T_0) / (V_1-V_0)] \times 100$, where V_1 is the mean tumor volume of control mice at the specific time point, t; T_1 is the mean tumor volume of treated mice at the same time point, t; V_0 is the mean tumor volume of control mice at the start of treatment, time 0; and T_0 is the mean tumor volume of treated mice at the treatment start, time 0. Treatment continued until the tumor volume in the vehicle-treated cohort reached an average size approaching 2000 mm^3, as per IACUC guidelines. At study termination, plasma and tumor samples were collected for pharmacokinetic analysis and pharmacodynamic gene expression studies.