

Pharmacological Inhibition of LSD1 for the Treatment of MLL- Rearranged Leukemia

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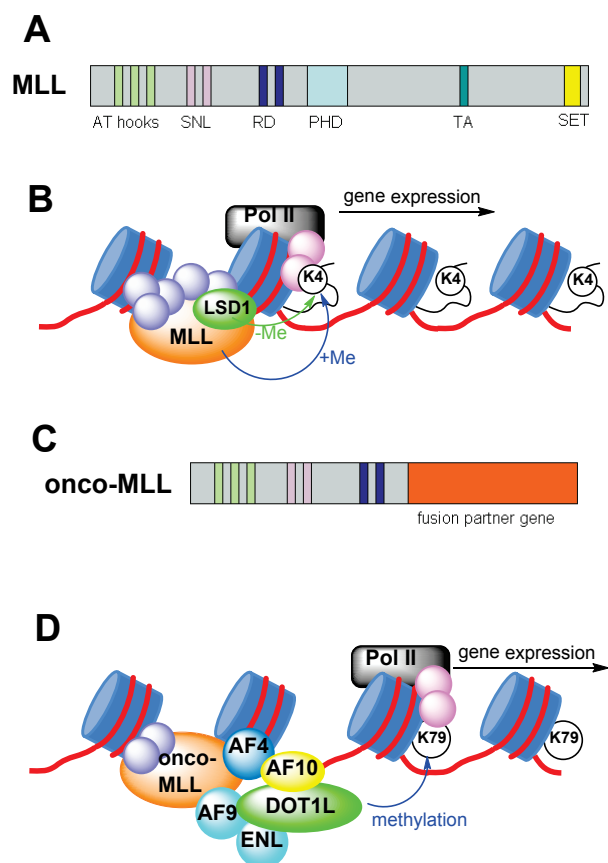


Figure S1. (A) Illustration of the wild-type MLL protein; (B) Functions of wild-type MLL and LSD1. MLL methylates H3K4 and initiates RNA polymerase II (Pol II) mediated gene transcription, while LSD1 removes the methyl group from H3K4-Me1 and 2; (C) Illustration of onco-MLL fusion protein; (D) Function of onco-MLL protein. The MLL protein complex involving AF4, AF9, AF10 or ENL can recruit DOT1L, which methylates H3K79 and causes overexpression of leukemia-relevant genes.

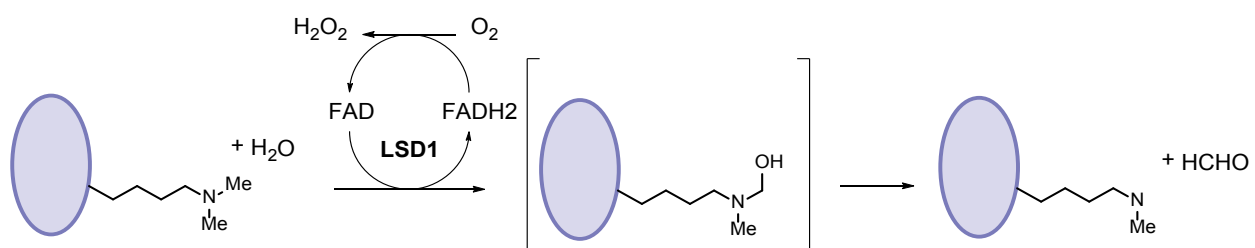


Figure S2. Mechanism of catalysis for LSD1.

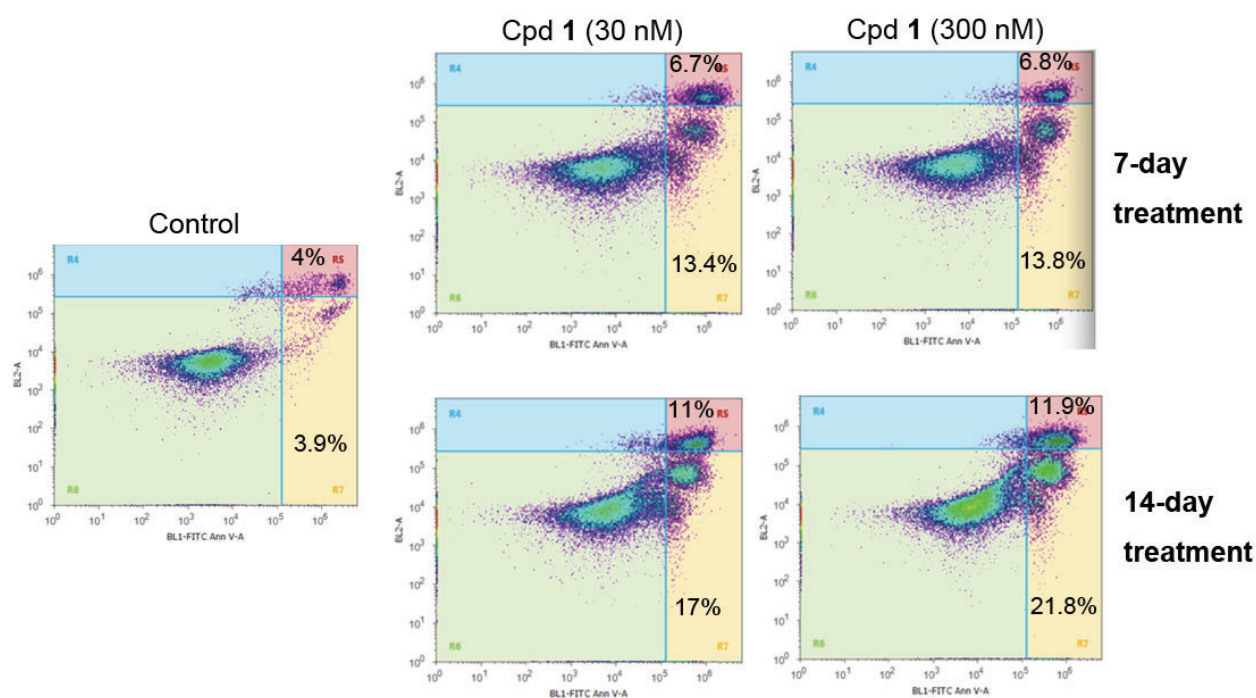


Figure S3. After treatment for 7 or 14 days, compound **1** (30 and 300 nM) induced significant apoptosis of MV4-11 leukemia cells. The upper number in each figure refers to the proportion of propidium iodide-positive, apoptosed cells and the lower number refers to that of annexin-V-positive cells (early apoptosis).

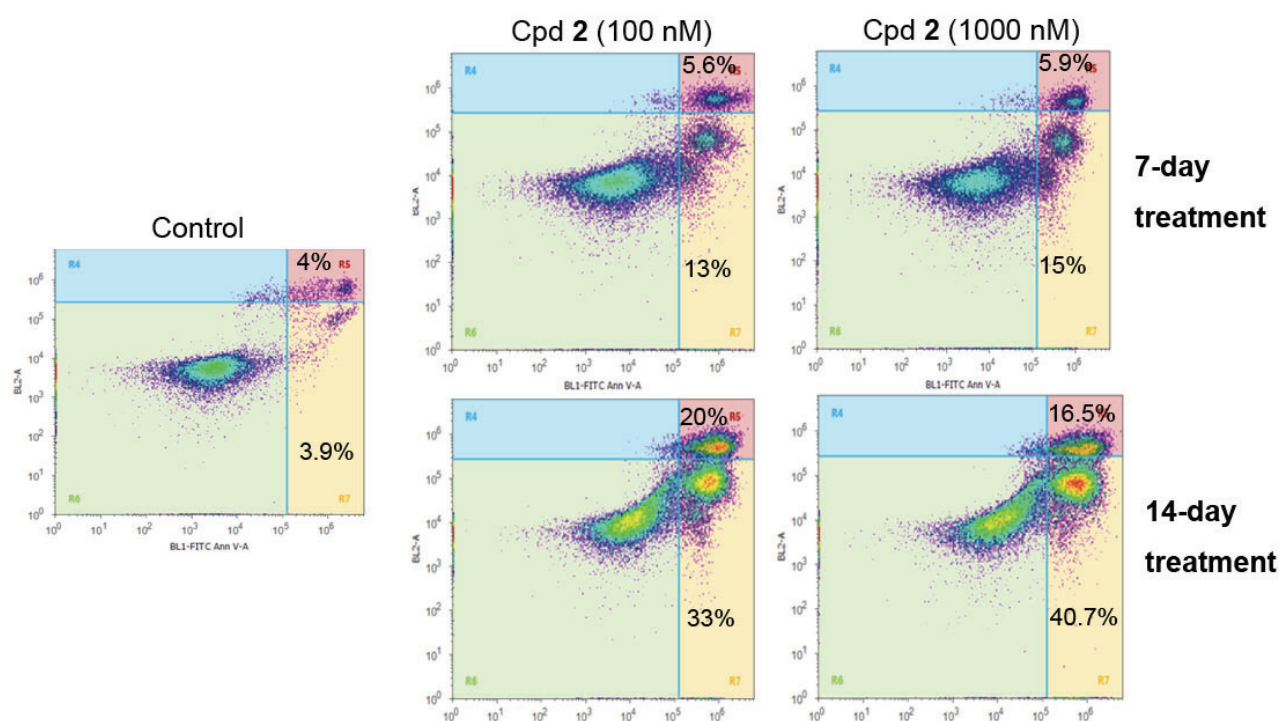


Figure S4. After treatment for 7 or 14 days, compound **2** (100 and 1000 nM) induced significant apoptosis of MV4-11 leukemia cells. The upper number in each figure refers to the proportion of propidium iodide-positive, apoptosed cells and the lower number refers to that of annexin-V-positive cells (early apoptosis).

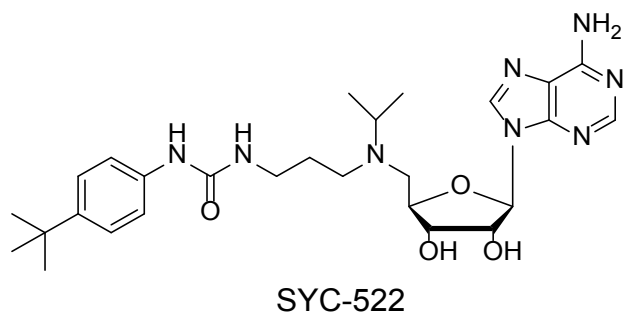


Figure S5. Chemical structure of DOT1L inhibitor SYC-522.

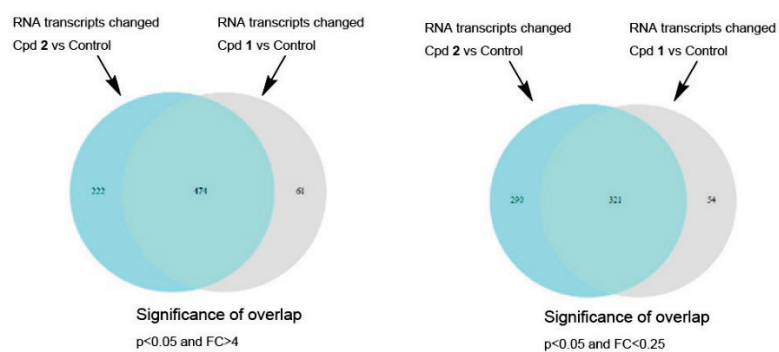


Figure S6. Microarray results of LSD1 inhibition in MV4-11 cells show that treatment with compounds **1** and **2** caused highly similar changes in gene expression pattern.

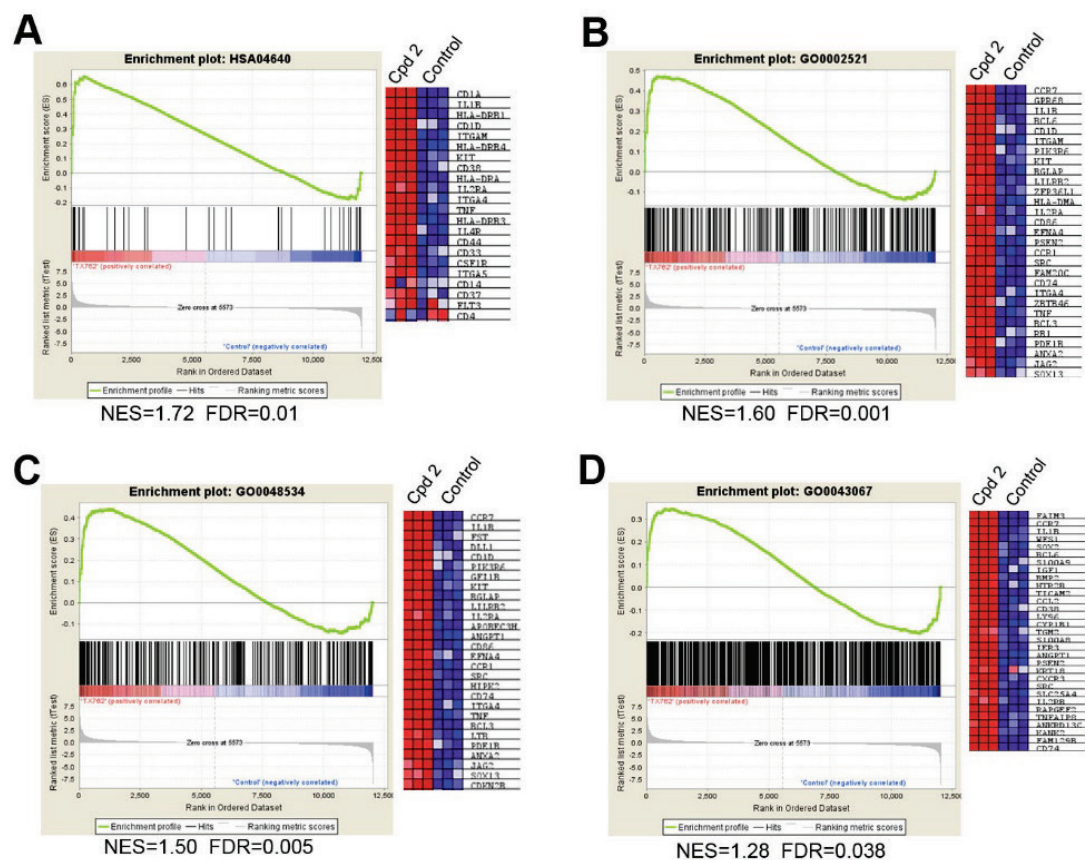
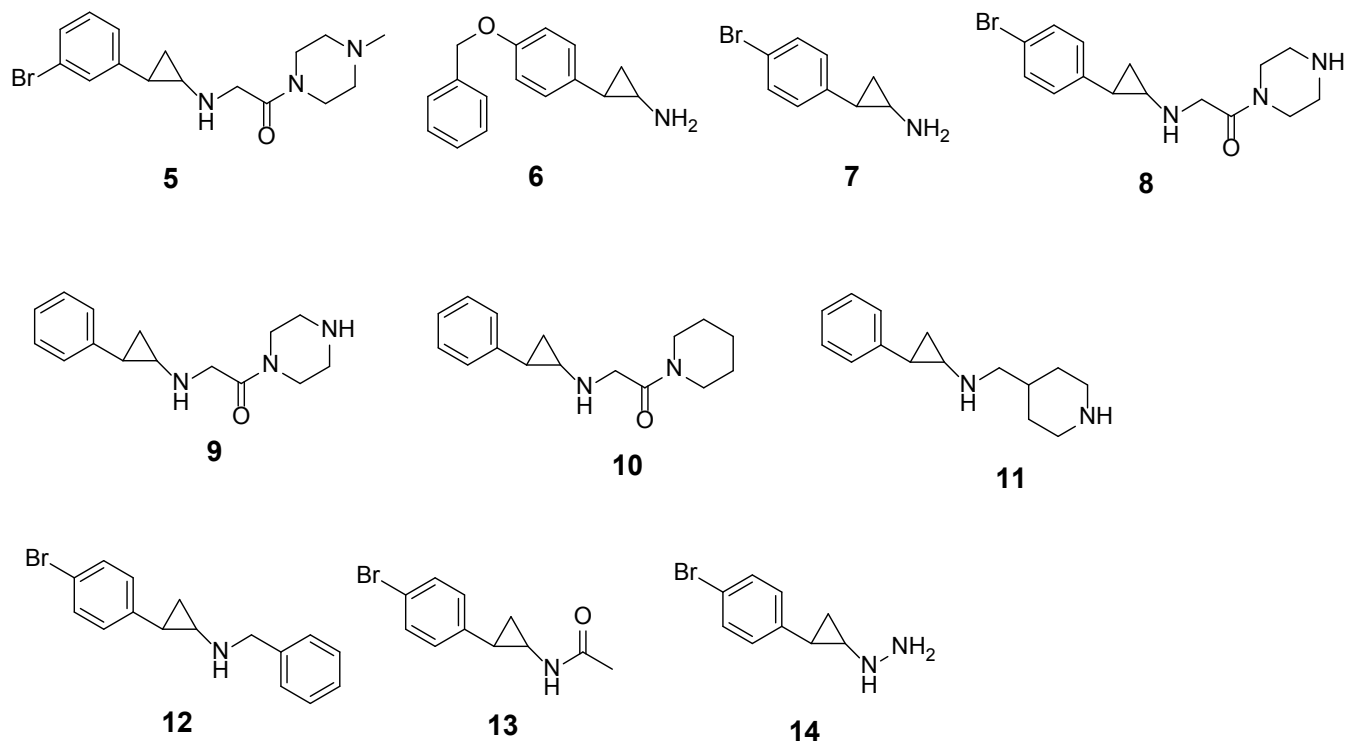


Fig. S7. Microarray results of LSD1 inhibition in MV4-11 cells. Upon treatment with compound **2** (300 nM), GSEA plots showed significant upregulation of the gene sets of (A) hematopoietic cell lineage (HSA04640), (B) leukocyte differentiation (GO:0002521) and (C) hematopoietic or lymphoid organ development (GO:0048534), as well as (D) the pro-apoptotic gene set of regulation of programmed cell death (GO:0043067). The right panels in (A-D) are heat maps showing expression levels of selected genes in the leading edges of the GSEA plots.

Table S1. Structures and biological activity of compounds **5** - **14**.



Cpd #	LSD1 IC ₅₀ (μM)	MV4-11 EC ₅₀ (μM)
5	>100	25
6	0.91	6.0
7	25	>50
8	0.064	0.10
9	0.19	0.28
10	1.3	2.5
11	0.062	0.13
12	5.0	25
13	15.4	40
14	5.8	42

Compound synthesis and characterization. All reagents were purchased from Alfa Aesar (Ward Hill, MA) or Aldrich (Milwaukee, WI). Compounds were characterized by ^1H NMR on a Varian (Palo Alto, CA) 400-MR spectrometer. The purities of all compounds were determined by a Shimadzu Prominence HPLC with a Zorbax C18 or C8 column (4.6 x 250 mm) monitored by UV absorbance at 254 nm, or ^1H (at 400 MHz) absolute spin-count quantitative NMR analysis using imidazole as an internal standard. The purities of all compounds were found to be >95%.

Compound 1. It was prepared from 4-benzyloxy-benzaldehyde and 1-chloroacetyl-4-methylpiperazine, following a published method, as a dihydrochloric acid salt (white powder). ^1H NMR (400 MHz, DMSO- d_6): δ 11.81-11.42 (m, 1H), 9.88-9.54 (m, 1H), 7.49-7.30 (m, 5H), 7.11-6.86 (m, 4H), 5.06 (d, J = 10.4 Hz, 2H), 4.48-3.81 (m, 4H), 3.48-3.35 (m, 2H), 3.34-2.86 (m, 4H), 2.74 (s, 3H), 2.59-2.52 (m, 1H), 2.13-2.00 (m, 1H), 1.59-1.50 (m, 1H), 1.21-1.08 (m, 1H).

Compound 2. It was prepared from 4-(6-fluoropyridin-3-yl)-benzaldehyde and 1-chloroacetyl-4-methylpiperazine, following a published method, as a dihydrochloric acid salt (white powder). ^1H NMR (400 MHz, DMSO- d_6): δ 8.27 (s, 1H), 8.07 (t, J = 8.0 Hz, 1H), 7.53 (d, J = 7.2 Hz, 2H), 7.22 (d, J = 7.2 Hz, 2H), 7.07 (d, J = 8.0 Hz, 1H), 4.63 (d, J = 8.4 Hz, 1H), 4.51-4.41 (m, 1H), 4.33-4.22 (m, 1H), 3.93-3.82 (m, 1H), 3.65-3.57 (m, 4H), 3.11-2.98 (m, 4H), 2.83 (s, 3H), 1.55-1.50 (m, 1H), 1.41-1.32 (m, 1H).

Compound 3. It was prepared from 4-bromobenzaldehyde and 1-chloroacetyl-4-methylpiperazine, following a published method, as a dihydrochloric acid salt (white powder). ^1H NMR (400 MHz, DMSO- d_6): δ 11.61-11.50 (bs, 1H), 9.88-9.59 (bs, 1H), 7.49 (d, J = 8.4 Hz, 2H), 7.16 (d, J = 8.4 Hz, 2H), 4.38-4.20 (m, 2H), 3.95-3.81 (m, 1H), 3.66-3.03 (m, 8H), 2.76 (s, 3H), 2.59-2.52 (m, 1H), 1.62-1.57 (m, 1H), 1.29-1.24 (m, 1H).

Compound 4. It was prepared from 4-(6-chloropyridin-3-yl)benzaldehyde and 1-chloroacetyl-4-methylpiperazine, following a published method, as a dihydrochloric acid salt (yellow solid). ^1H NMR (400 MHz, D_2O): δ 8.63 (s, 1H), 8.12 (d, J = 7.6 Hz, 1H), 7.70 (d, J = 7.2 Hz, 2H), 7.61 (d, J = 7.2 Hz,

2H), 7.38 (d, $J = 7.6$ Hz, 1H), 4.71-4.66 (m, 1H), 4.52-4.38 (m, 1H), 4.36-4.18 (m, 1H), 4.07-3.81 (m, 1H), 3.59-3.38 (m, 4H), 3.17-2.93 (m, 4H), 2.84 (s, 3H), 1.55-1.37 (m, 2H).