Comprehensive *in vitro* characterization of the LSD1 small molecule inhibitor class in oncology

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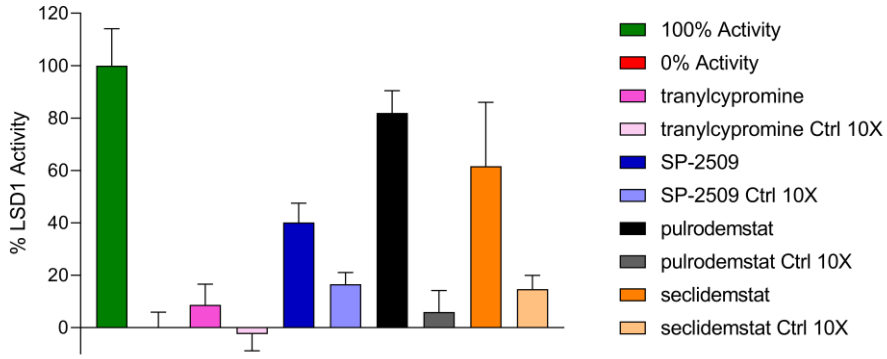

Figure S1. Jump dilution analysis of reversible LSD1 inhibitors. The recovery of LSD1 activity upon jump dilution is reported as a percentage of the activity of LSD1 incubated and diluted in the absence of inhibitor (100% Activity). As a control, the percentage recovery of activity of LSD1 incubated with each compound and diluted with 10-fold the IC_{50} was determined (Ctrl 10X). TCP was used as a control of no recovery upon jump dilution. Data represented as mean ± St. Dev. of N=3, n=1.

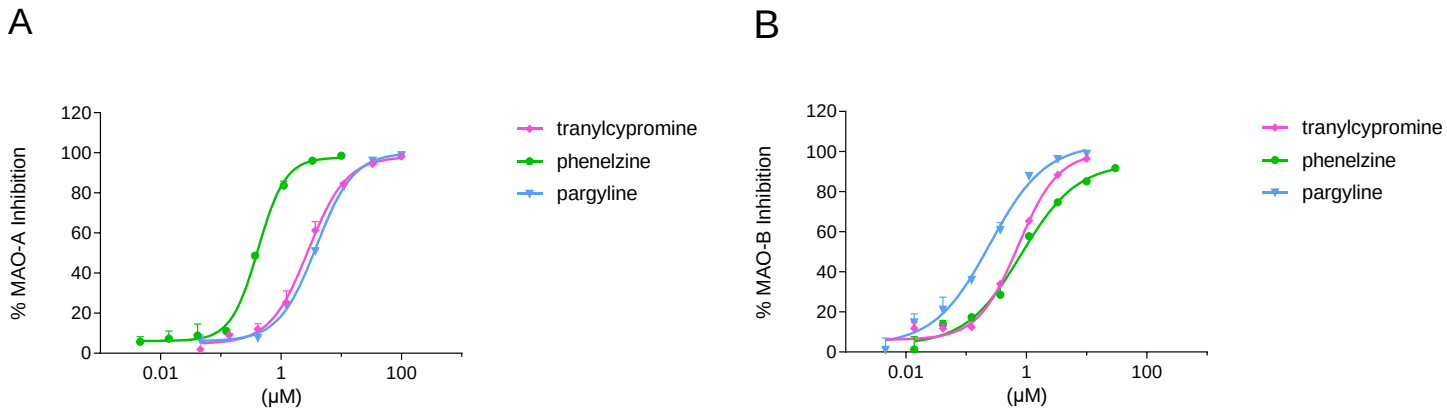

Figure S2. **Dose-response curves of MAO-A (A) and MAO-B (B) inhibition for selected tool compounds.** Data are represented as mean ± St. Dev. of N=2, n=2.

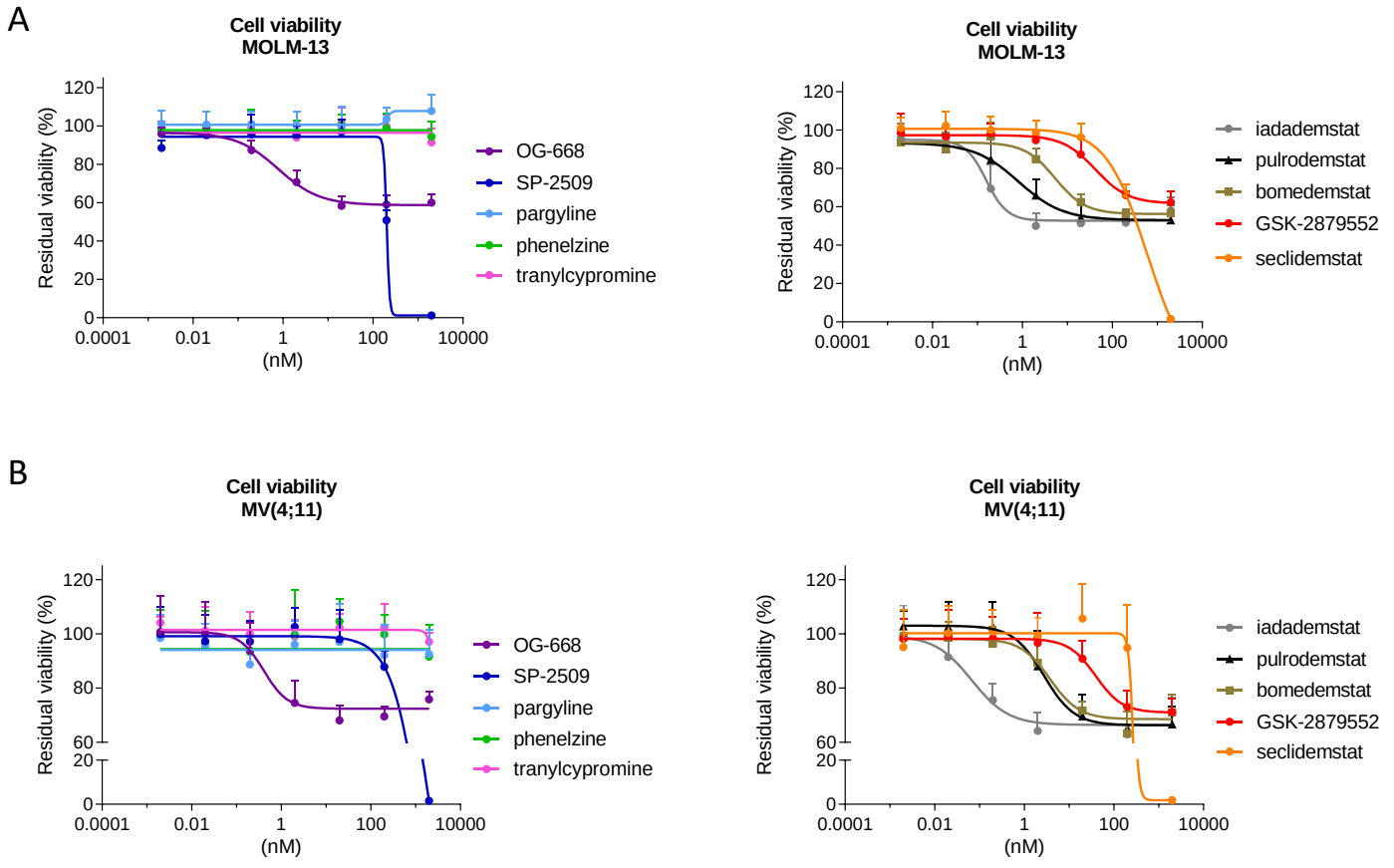

Figure S3. Efficacy in AML. Effect of tool (left panels) and clinical stage (right panels) LSD1 inhibitors on cell viability in the AML cell line MOLM13 (A) and MV(4;11) (B) following 96 hr incubation at concentrations ranging from 0.002 to 2,000 nM. All data are expressed as relative to the vehicle condition and represented as mean ± St. Dev of N=2, n=3.

SUPPLEMENTARY TABLES

Compound	T = 0 days purity (%)[a]	T = 4 days purity (%)[a]	T = 6 days purity (%)[a]
tranylcypromine	96	97	95
phenelzine	94	5	4
pargyline	97	98	95
OG-668	99	96	95
SP-2509	88	74	68
pulrodemstat	98	98	92
GSK-2879552	99	98	96
bomedemstat	100	95	93
seclidemstat	88	74	72
iadademstat	99	94	91

Table S1. Stability of compounds in aqueous buffer at 37ºC. [a] Purity values obtained by LCMS.

Compound	Turbidimetric Solubility (μM)[a]	LSD1 IC_{50} (μM) HRP	LSD1 IC_{50} (μM) HTRF	LSD2 IC_{50} (μM) HRP	LSD2 IC_{50} (μM) HTRF	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)
tranylcypromine	500	69.86 ± 8.95	5.63 ± 0.47	>> 100	> 100	2.84 ± 0.45	0.73 ± 0.04
phenelzine	200,000	ND[b]	1,049.34 ± 150.10	ND[b]	832.33 ± 99.01	0.42 ± 0.02	0.83 ± 0.005
pargyline	200,000	9,316.87 ± 97.97	7,102.93 ± 2,940.99	>> 100	3,009.68 ± 143.33	3.84 ± 0.14	0.24 ± 0.02
OG-668	100	$4.72 \times 10^{-2} \pm 1.38 \times 10^{-2}$	$7.60 \times 10^{-3} \pm 1.31 \times 10^{-3}$	> 100	10 < IC_{50} < 100	> 100	> 100
SP-2509	33	2.56 ± 0.39	2.48 ± 1.21	> 10[c]	> 10[c]	> 10[c]	> 10[c]
pulrodemstat	100	$1.68 \times 10^{-2} \pm 1.97 \times 10^{-3}$	$6.55 \times 10^{-4} \pm 1.10 \times 10^{-4}$	> 100	≥ 100	≥ 100	> 100
GSK-2879552	100	1.10 ± 0.06	0.16 ± 0.002	>> 100	≥ 100	> 100	> 100
bomedemstat	100	0.25 ± 0.03	$5.68 \times 10^{-2} \pm 9.58 \times 10^{-3}$	> 100	10 < IC_{50} < 100	> 100	> 100
seclidemstat	33	2.44 ± 0.66	1.33 ± 0.004	> 10[c]	> 10[c]	> 10[c]	> 10[c]
iadademstat	100	$8.57 \times 10^{-3} \pm 6.61 \times 10^{-4}$	$3.26 \times 10^{-4} \pm 1.42 \times 10^{-4}$	> 100	10 < IC_{50} < 100	> 100	> 100

Table S2. Kinetic solubility, inhibition of LSD1 and other FAD dependent enzymes. [a]Highest soluble concentration. ND[b] not determined: the compound is fluorescent at HRP assay wavelength. [c]Compound not soluble at 100 μM. Data are represented as mean ± St. Dev. of N=2 or 6, n=2 or 3.

Compound	LSD1 K_I (μM)	LSD1 k_{inact} (sec^{-1})	LSD1 k_{inact}/K_I (M^{-1} sec^{-1})
tranylcypromine	360.13 ± 244.54	0.011 ± 0.004	33.67 ± 11.56
phenelzine	ND[a]	ND[a]	ND[a]
pargyline	ND[b]	ND[b]	ND[b]
OG-668	0.626 ± 0.186	0.016 ± 0.005	$2.57 \times 10^4 \pm 1.04 \times 10^3$
GSK-2879552	2.137 ± 1.267	0.013 ± 0.003	$6.73 \times 10^3 \pm 2.43 \times 10^3$
bomedemstat	1.189 ± 0.435	0.018 ± 0.001	$1.63 \times 10^4 \pm 6.68 \times 10^3$
iadademstat	0.017 ± 0.001	0.020 ± 0.003	$1.19 \times 10^6 \pm 2.39 \times 10^5$

Table S3. Kinetic parameters of LSD1 irreversible inhibition. ND[a] not determined: the compound is fluorescent at HRP assay wavelength. ND[b] not determined: no time-dependent inhibition was observed at the tested concentrations. Data are represented as mean ± St. Dev. of N=2, n=1.

	EC_{50} (nM)			
	TF1a	MOLM-13	MV(4;11)	NCI-H510A
Compound	Mean ± St. Dev.			
tranylcypromine	>2,000	>2,000	>2,000	>2,000
phenelzine	>2,000	>2,000	>2,000	>2,000
pargyline	>2,000	>2,000	>2,000	>2,000
OG-668	1.55 ± 0.57	0.91 ± 0.61	0.48 ± 0.15	1.17 ± 0.84
SP-2509	1,624.50 ± 260.92	203.35 ± 6.72	422.00 ± 42.99	56.24 ± 1.75
pulrodemstat	7.87 ± 3.65	0.96 ± 0.51	2.90 ± 1.98	1.77 ± 0.67
GSK-2879552	76.43 ± 20.71	40.19 ± 0.47	39.59 ± 3.21	109.48 ± 30.72
bomedemstat	31.06 ± 9.24	5.38 ± 0.42	4.20 ± 0.78	10.46 ± 0.73
seclidemstat	1,261.50 ± 96.87	284.65 ± 18.03	885.65 ± 692.05	184.35 ± 0.92
iadademstat	0.09 ± 0.04	0.21 ± 0.17	0.10 ± 0.06	0.16 ± 0.03

Table S4. EC_{50} viability values for AML and SCLC cell lines. EC_{50} values were calculated using the GraphPad Prism 9 software. Data are represented as mean ± St. Dev. of N=2, n=3

Relative EC_{50} (nM)	
Compound	THP-1 cells
tranylcypromine	1,402.00
phenelzine	>2,000
pargyline	>2,000
OG-668	0.62
SP-2509	>2,000
pulrodemstat	2.34
GSK-2879552	31.23
bomedemstat	4.15
seclidemstat	>2,000
iadademstat	0.04

Table S5. EC_{50} differentiation values for the AML cell line THP1. EC_{50} values were calculated using the GraphPad 9 Prism software. N=1