

Supplementary information

Canvass: a crowd-sourced, natural product screening library for exploring biological space

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Additional Figures

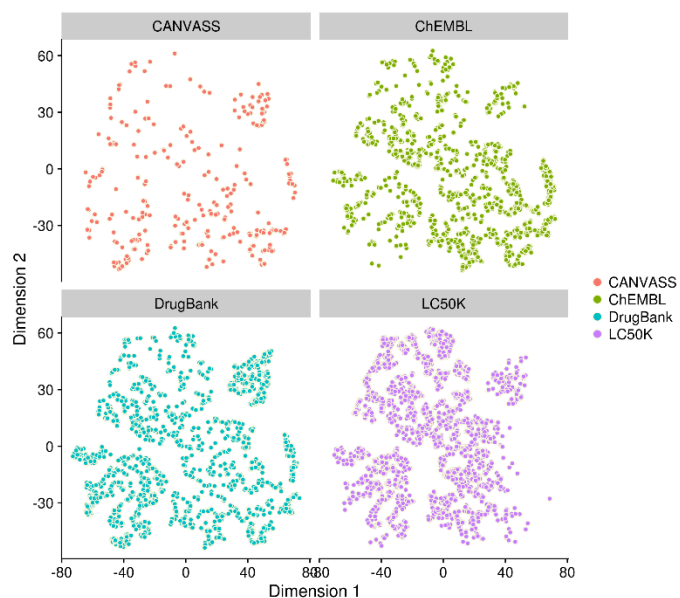


Figure S1. Overlap of the Canvass library with three other libraries in a 7D physicochemical space (MW, HBA, HBD, RotB, PBF, and flexibility), reduced to 2D using tSNE; MW = molecular weight, HBA = H-bond acceptor, HBD = H-bond donor, RotB = number of rotatable bonds, PBF = plane of best fit, tSNE = t-distributed stochastic neighbor embedding.

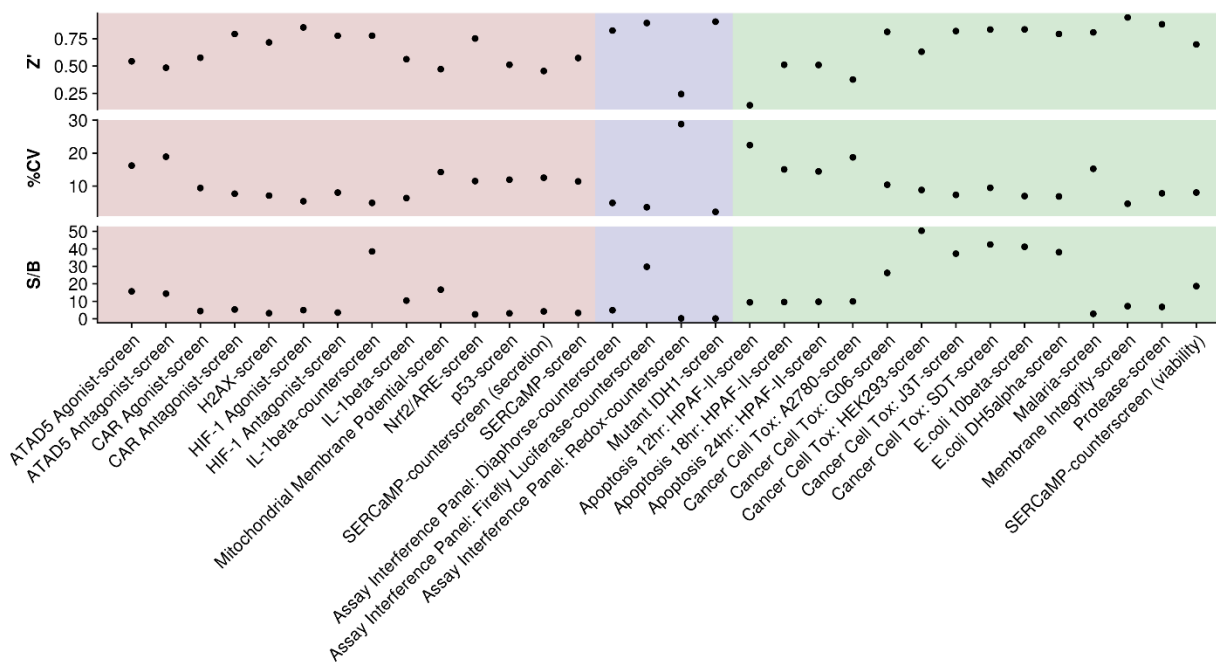


Figure S2. A close-up view of assay QC measure values for assays of $Z' \geq 0$ and of $SB < 60$.

a)



b)

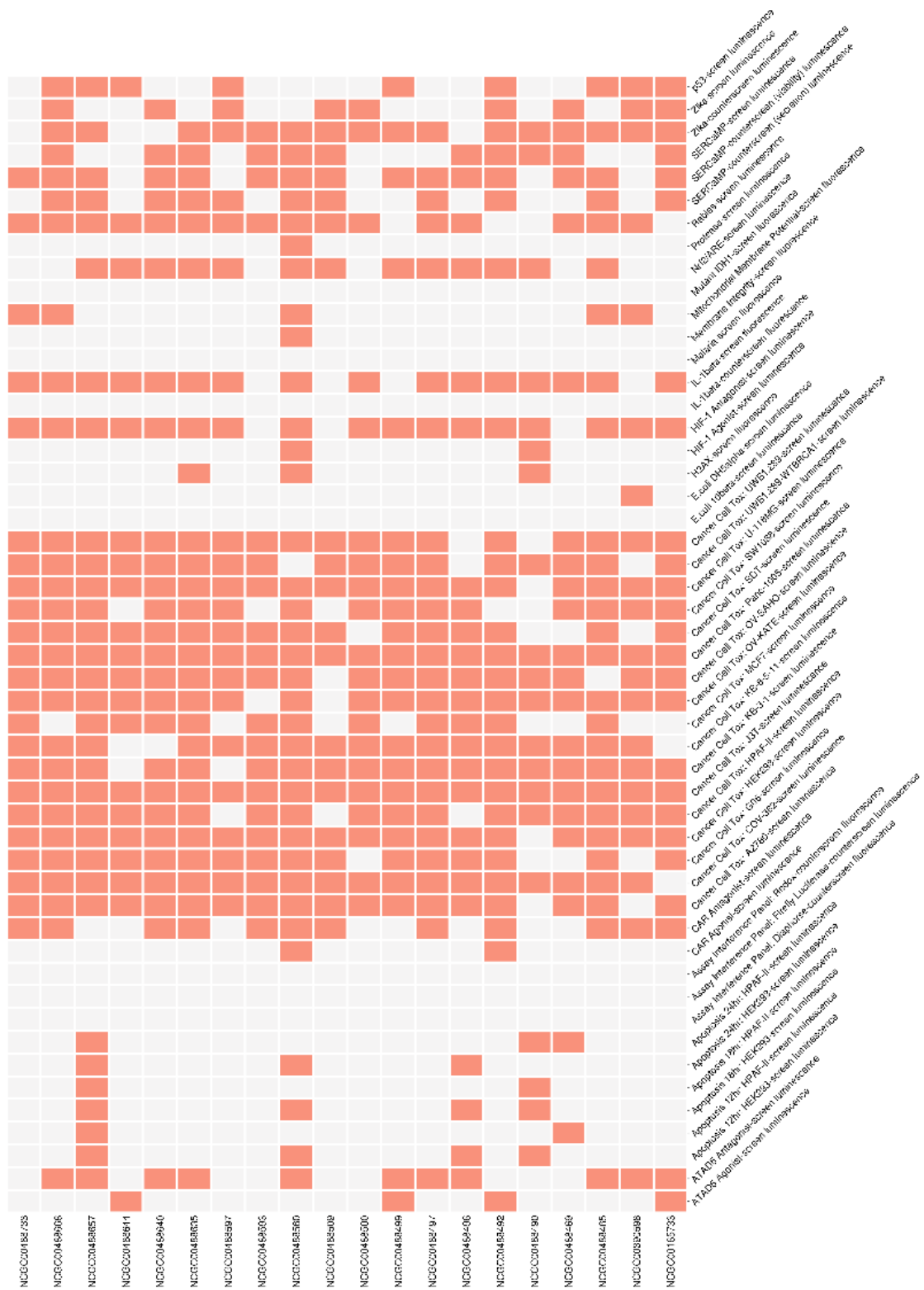


Figure S3. Heat map representations of Canvass compounds identified as promiscuous. Each row corresponds to a compound and a column to an assay. Red cells correspond to compounds of **a**) Abs(nAUC) > 90th percentile, **b**) log AC₅₀ < 10th percentile, **c**) Abs(Efficacy) > 90th percentile for that assay; AC₅₀ = half maximal activity concentration, nAUC = normalized area under the curve, Abs = absolute value.



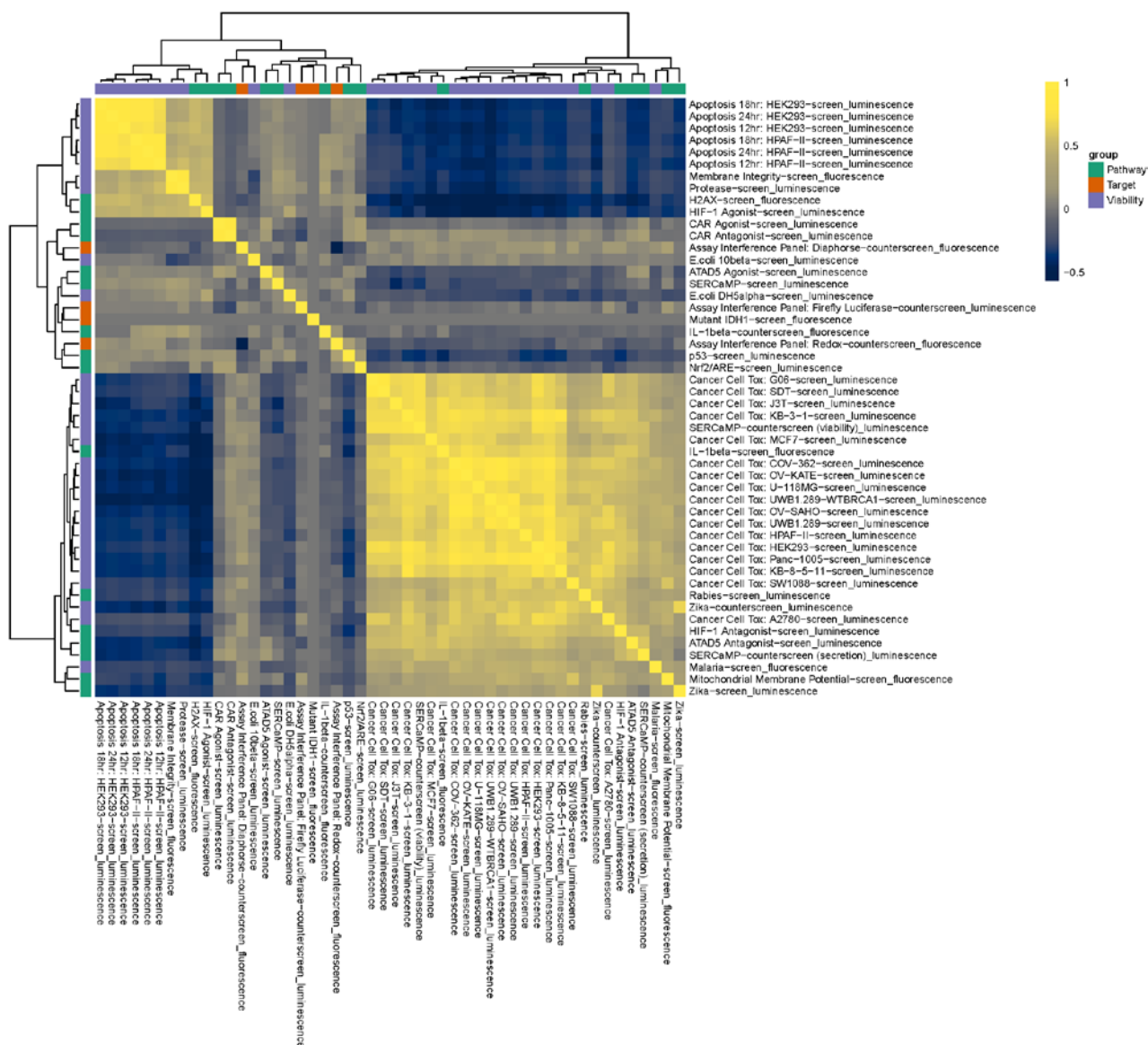


Figure S6. Assay clustering based on efficacy.

Novelty of Canvass Compounds

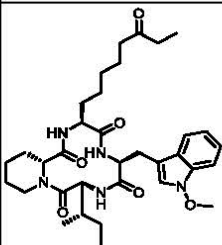
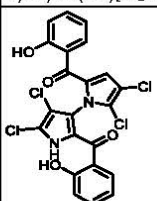
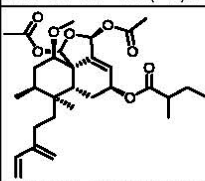
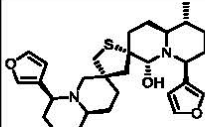
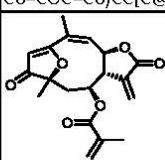
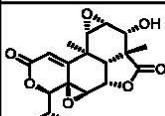
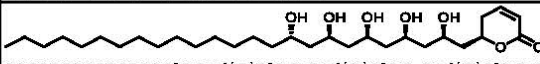
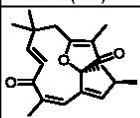
The novelty of the Canvass compounds was assessed as described in the Experimental Section. The number of unique BMSs for the Canvass, DrugBank, ChEMBL Natural Products, and the entire LC50K compound collections were found to be 250, 868, 1,129 and 2,775, respectively. According to Table S1 the majority BMSs in the Canvass collection were not present in the comparator libraries. This further supports our observation that the Canvass compounds represent unique chemical space.

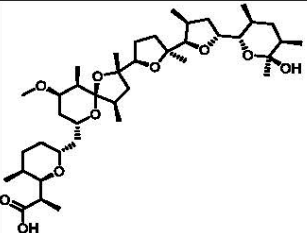
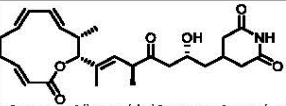
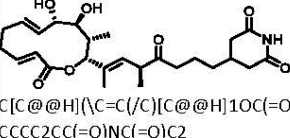
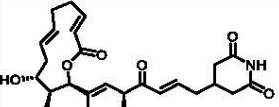
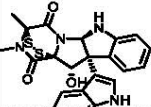

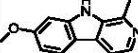
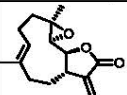
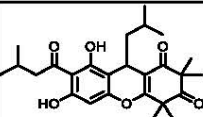
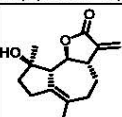
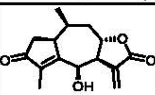
Table S1. Overlap of unique Bemis-Murcko Scaffolds between pairs of molecular libraries. The table depicts the fraction of overlap between unique BMSs of libraries relative to the number of unique BMSs of the library listed in the first column. On a green-white-red heat scale, the green and red extremes indicate no or complete overlap, respectively.

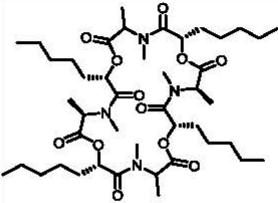
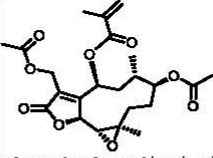
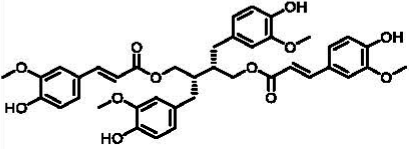
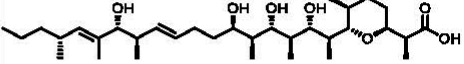
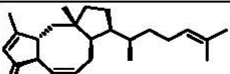
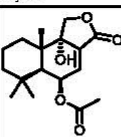
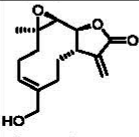
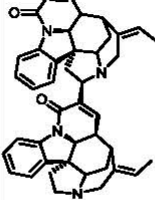
	CANVASS	ChEMBL	DrugBank	LC50K (entire 50K)
CANVASS	1.00	0.04	0.06	0.02
ChEMBL	0.01	1.00	0.28	0.01
DrugBank	0.01	0.22	1.00	0.03
LC50K (entire 50K)	0.00	0.00	0.01	1.00

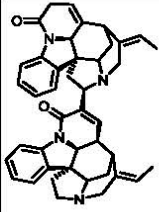
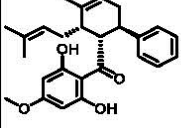
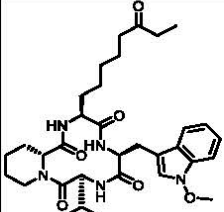
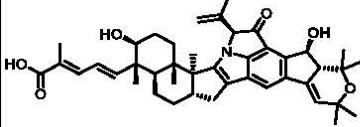
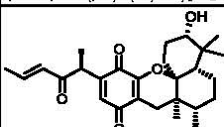
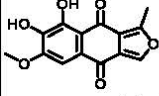
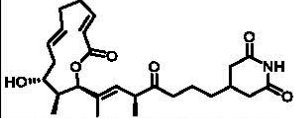
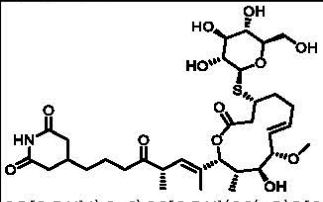
Additional information for promiscuous compounds in Canvass library

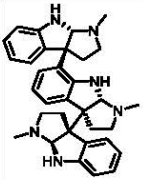
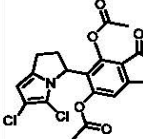
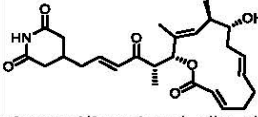
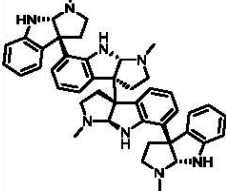
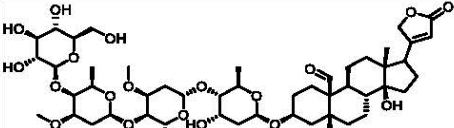
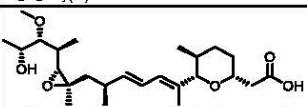
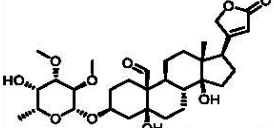
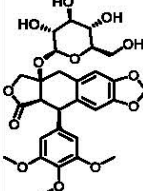
Table S2. Promiscuous Compounds

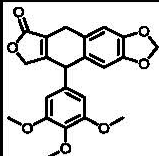
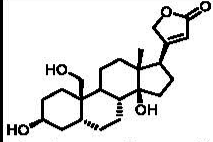
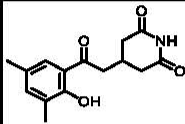
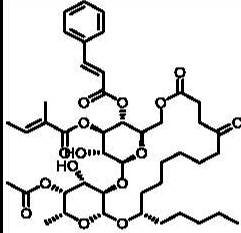
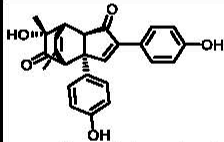
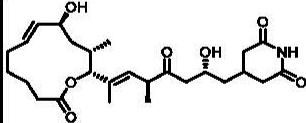
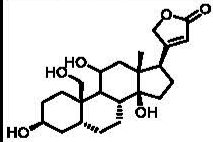
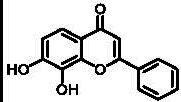
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NCGC00390598-03	 <chem>OC1=CC=CC=C1C(=O)C2=CC(Cl)=C(Cl)N2C3=C(NC(Cl)=C3Cl)C(=O)C4=CC=CC=C4O</chem>	1	1	1
NCGC00488469-01	 <chem>CCC(C)C(=O)O[C@H]1C[C@H]2[C@]3{[C@@H](OC(C)=O)O[C@@H](OC(C)=O)C3=C1}[C@@H](C[C@H](C)[C@]2(C)CCC(=C)C=C)OC</chem>	1	1	1
NCGC00488490-01	 <chem>C[C@@H]1CC[C@H](N2C[C@]3{CS[C@]4(C3)CC[C@H]5[C@H](C)CC[C@H](N5[C@@H]4O)C6=COC=C6)CC[C@H]12)C7=COC=C7</chem>	1	1	1
NCGC00488496-01	 <chem>CC(=C)C(=O)O[C@H]1C[C@@]2(C)OC(=CC2=O)C(C)=C[C@H]3OC(=O)C(=C)[C@H]13</chem>	1	1	1
NCGC00488499-01	 <chem>C[C@H](O)[C@H]1OC(=O)C=C2[C@@]13O[C@@H]3[C@H]4OC(=O)[C@]5(C)[C@H]4[C@@]2(C)[C@@H]6O[C@@H]6[C@@H]5O</chem>	1	1	1
NCGC00488500-01	 <chem>CCCCCCCCCCCCCCCC[C@H](O)C[C@@H](O)C[C@H](O)C[C@H](O)C[C@H](O)C[C@H](O)C[C@H]1CC=CC(=O)O1</chem>	1	1	1
NCGC00488560-01	 <chem>C[C@@H]1C[C@@]23OC(CC(C)(C)\C=C\C(=O)C(C)=CC2=C1)=C(C)C3=O</chem>	1	1	1

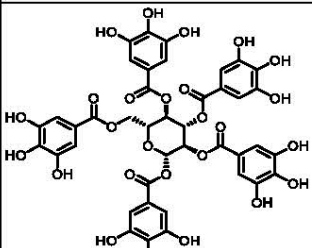
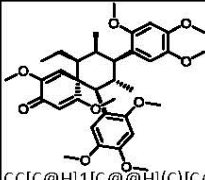
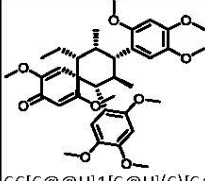
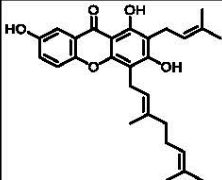
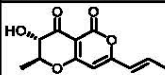
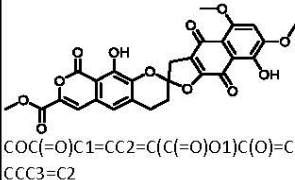
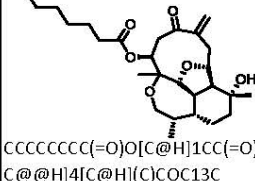
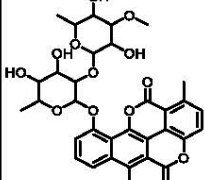
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NCGC00488635-01	 <chem>C[C@@H](\C=C(/C)[C@@H]1OC(=O)\C=C\CC\C=C\C=C\C[C@@H]1C)C(=O)C[C@H](O)CC2C(=O)NC(=O)C2</chem>	1	1	1
NCGC00488640-01	 <chem>C[C@@H](\C=C(/C)[C@@H]1OC(=O)\C=C\CC\C=C\C[C@H](O)[C@@H](O)[C@@H]1C)C(=O)CCCC2C(=O)NC(=O)C2</chem>	1	1	1
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NCGC00488657-01	 <chem>CN1C(=O)N2[C@H](C(=O)N2[C@H]4NC5=CC=CC=C5[C@]4([C@@H]3O)C6=CNC7=CC=CC=C67)C1</chem>	1	1	1
NCGC00488668-01	 <chem>CO\C=C\C(=O)C1=NC2=C3C(C=NC3=C(N)C=C2NC(=O)C(C)C)=C1</chem>	1	1	1
NCGC00016435-20	 <chem>COC1=CC2=C(C=C1)C3=CC=NC(C)=C3N2</chem>	1	1	0
NCGC00024683-06	 <chem>C\C1=C/CC[C@@]2(C)O[C@H]2[C@H]3OC(=O)C(=C)[C@@H]3CC1</chem>	1	1	0
NCGC00347528-03	 <chem>CC(C)CC1C2=C(O)C(C(=O)CC(C)C)=C(O)C=C2OC3=C1C(=O)C(C)(C)C(=O)C3(C)C</chem>	1	1	0
NCGC00390768-03	 <chem>CC1=C2CC[C@@](C)(O)[C@@H]2[C@H]3OC(=O)C(=C)[C@@H]3CC1</chem>	1	1	0
NCGC00488457-01	 <chem>C[C@H]1C[C@@H]2OC(=O)C(=C)[C@H]2[C@@H](O)C3=C(C)C(=O)C[C@@H]13</chem>	1	1	0

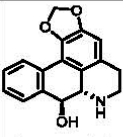
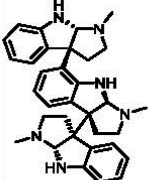
ID	Structure + SMILES	nAUC	Efficacy	LogAC50
NCGC00488466-01	 <chem>CCCC[C@H]1OC(=O)[C@@H](C)N(C)C(=O)[C@H](CCCC)OC(=O)C(C)N(C)C(=O)[C@H](CCCC)OC(=O)[C@@H](C)N(C)C(=O)[C@H](CCCC)OC(=O)C(C)N(C)C1=O</chem>	1	1	0
NCGC00488474-01	 <chem>C[C@H]1C[C@H](OC(=O)C(C)=C)C2=C(COC(C)=O)C(=O)O[C@@H]2[C@H]3O[C@]3(CC[C@H]1OC(C)=O</chem>	1	1	0
NCGC00488498-01	 <chem>COC1=CC[C@H](COC(=O)\C=C\2=CC=C(O)C(OC)=C2)[C@@H](COC(=O)\C=C\3=CC(OC)=C(O)C=C3)CC4=CC(OC)=C(O)C=C4)=CC=C1O</chem>	1	1	0
NCGC00488501-01	 <chem>CCC[C@H](C)\C=C(/C)[C@H](O)[C@H](C)\C=C\CC[C@H](O)[C@H](C)[C@H](O)[C@H](C)[C@H](O)[C@H](C)[C@H]1O[C@@H](CC[C@H]1C)[C@H](C)C(=O)O</chem>	1	1	0
NCGC00488503-01	 <chem>C[C@H](CCC=C(C)C)[C@@H]1CC[C@@]2(C)C[C@@H]3[C@H](C(=O)C=C3C)C(=O)=CC[C@H]12</chem>	1	1	0
NCGC00488506-01	 <chem>CC(=O)O[C@@H]1C=C2C(=O)OC[C@]2(O)[C@@]3(C)CCCC(C)(C)[C@H]13</chem>	1	1	0
NCGC00488522-01	 <chem>C[C@@]12CCC=C(CO)CC[C@@H]3[C@H](OC(=O)C3=C)[C@@H]1O2</chem>	1	1	0
NCGC00488533-01	 <chem>CC=C1CN2CC[C@@]34C2CC1[C@@H]5C=C([C@H]6C[C@@]78C9CC(C%10C=CC(=O)N([C@H]7%10)C%11=CC=CC=C8%11)C(CN69)=CC(=O)N([C@H]35)C%12=CC=CC=C4%12</chem>	1	1	0

ID	Structure + SMILES	nAUC	Efficacy	LogAC50
NCGC00488534-01	 <chem>CC=C1CN2CC[C@@]34C2CC1[C@@H]5C=C([C@H]6C[C@@]78C9CC(C(CN69)=C)C%10=C</chem> <chem>CC(=O)N([C@H]7%10)C%11=CC=CC=C8%11)C(=O)N([C@H]35)C%12=CC=CC=C4%12</chem>	1	1	0
NCGC00488548-01	 <chem>COC1=CC(O)=C(C(=O)[C@@H]2[C@H](CC=C(C)C)C(C)=CC[C@H]2C3=CC=CC=C3)C(O)=C1</chem>	1	1	0
NCGC00488594-01	 <chem>CCC(=O)CCCC[C@@H]1NC(=O)[C@H]2CCCCN2C(=O)[C@@H](NC(=O)[C@H](CC3=CN(OC)</chem> <chem>C4=C3C=CC=C4)NC1=O)C(C)C</chem>	1	1	0
NCGC00488600-01	 <chem>CC(=C)[C@@H]1N2C3=C(C1=O)C4=C(C=C3C5=C2[C@@]6(C)[C@H](C5)CC[C@H]7[C@](C){</chem> <chem>\C=C\=C(/C)C(O)=O)[C@@H](O)CC[C@]67C)C8=CC(C)(C)OC(C)(C)[C@H]8[C@@H]4O</chem>	1	1	0
NCGC00488601-01	 <chem>C\C=C\C(=O)[C@@H](C)C1=CC(=O)C2=C(O[C@@]34CC[C@H](O)C(C)(C)[C@@H]3CC[C@H</chem> <chem>]C)[C@@]4(C)C2)C1=O</chem>	1	1	0
NCGC00488626-01	 <chem>COC1=CC2=C(C(=O)C3=C(C)OC=C3C2=O)C(O)=C1O</chem>	1	1	0
NCGC00488643-01	 <chem>C[C@@H](\C=C(/C)[C@@H]1OC(=O)\C=C\CC\C=C\C[C@@H](O)[C@@H]1C)C(=O)CCCC2C</chem> <chem>C(=O)NC(=O)C2</chem>	1	1	0
NCGC00488645-01	 <chem>CO[C@H]1\C=C\CC[C@H](CC(=O)O[C@H]([C@@H](C)[C@@H]1O)C(\C)=C\C[C@H](C)C(=O)</chem> <chem>CCCC2CC(=O)NC(=O)C2)S[C@@H]3O[C@H](CO)[C@@H](O)[C@H](O)[C@H]3O</chem>	1	1	0

ID	Structure + SMILES	nAUC	Efficacy	LogAC50
NCGC00488665-01	 <chem>CN1CC[C@]2([C@@H]1NC3=CC=CC=C23)C4=C5N[C@H]6N(C)CC[C@@]6(C5=CC=C4)[C@@]78CCN(C)[C@H]7NC9=CC=CC=C89</chem>	0	1	0
NCGC00488667-01	 <chem>CC(=O)OC1=CC2=C(C(=O)N3C2=CC(Cl)=C3Cl)C(OC(C)=O)=C1C4CCC5=CC(Cl)=C(Cl)N45</chem>	1	1	0
NCGC00488744-01	 <chem>C[C@@H]([C@H]1OC(=O)\C=C\CC\C=C\C[C@H](O)[C@H](C)C=C1C)C(=O)\C=C\CC2CC(=O)NC(=O)C2</chem>	1	1	0
NCGC00488751-01	 <chem>CN1CC[C@]2([C@@H]1NC3=C2C=CC=C3)C4=CC=CC5=C4N[C@H]6N(C)CC[C@@]56[C@]78CCN(C)[C@H]7NC9=C8C=CC=C9[C@H]10%10%11CCN(C)[C@H]10NC%12=C%11C=CC=C%12</chem>	1	1	0
NCGC00488465-01	 <chem>CO[C@H]1C[C@H](O)[C@H]2[C@@H](O)C[C@H](O)[C@H]3CC[C@]4(C=O)[C@H]5CC[C@]6(C)C(CC[C@]6(O)[C@@H]5CC[C@]4(O)C3)C7=CC(=O)OC7)O[C@@H]2C)O[C@H](C)[C@H]1O[C@H]8C[C@H](OC)[C@H](O)[C@H]9O[C@H](CO)[C@H](O)[C@H](O)[C@H]9O)[C@H](C)O8</chem>	1	0	1
NCGC00488492-01	 <chem>CO[C@H]([C@@H](C)O)[C@H](C)[C@H]1O[C@]1(C)C[C@H](C)\C=C\C=C/[C@H]2O[C@H](CC(O)=O)CC[C@H]2C</chem>	1	0	1
NCGC00488497-01	 <chem>CO[C@H]1[C@@H](O)[C@H](C)O[C@@H](O)[C@H]2CC[C@]3(C=O)[C@H]4CC[C@]5(C)[C@H](CC[C@]5(O)[C@H]4CC[C@]3(O)C2)C6=CC(=O)OC6)[C@@H]1OC</chem>	1	0	1
NCGC00488509-01	 <chem>COC1=CC(=CC(OC)=C1OC)[C@H]2[C@H]3C(=O)OC[C@@]3(CC4=CC5=C(OCO5)C=C24)O[C@@H]6O[C@H](CO)[C@@H](O)[C@H](O)[C@H]6O</chem>	1	0	1

ID	Structure + SMILES	nAUC	Efficacy	LogAC50
NCGC00488593-01	 <chem>COC1=CC(=CC(OC)=C1OC)C2C3=C(C(C4=CC5=C(OCO5)C=C24)C(=O)OC3</chem>	1	0	1
NCGC00488733-01	 <chem>C[C@]12CCC3[C@@H](CC[C@@H]4C[C@@H](O)CC[C@]34CO)[C@@]1(O)CC[C@@H]2C5=CC(=O)OC5</chem>	1	0	1
NCGC00180403-02	 <chem>CC1=CC(C(=O)CC2CC(=O)NC(=O)C2)=C(O)C(C)=C1</chem>	1	0	0
NCGC00488452-01	 <chem>CCCCC[C@H]1CCCCCCC(=O)CCC(=O)OC[C@H]2O[C@@H](O[C@H]3[C@@H](O)[C@@H](OC(C)=O)[C@@H](C)O[C@H]3O1)[C@H](O)[C@@H](OC(=O)C(\C)=C\C)[C@@H]2OC(=O)C=CC4=CC=CC=C4</chem>	1	0	0
NCGC00488556-01	 <chem>CC1=C[C@H]2[C@H]3C(=O)C(=C[C@]3([C@@H]1C(=O)[C@]2(C)O)C4=CC=C(O)C=C4)C5=C(C=C(O)C=C5</chem>	1	0	0
NCGC00488638-01	 <chem>C[C@@H](\C=C/C)[C@@H]1OC(=O)CCCC\C=C\[C@@H](O)C[C@@H]1C(=O)C[C@H](O)CC2CC(=O)NC(=O)C2</chem>	1	0	0
NCGC00488734-01	 <chem>C[C@]12CC(O)C3[C@@H](CC[C@@H]4C[C@@H](O)CC[C@]34CO)[C@@]1(O)CC[C@@H]2C5=CC(=O)OC5</chem>	1	0	0
NCGC00095217-09	 <chem>OC1=CC=C2C(=O)C=C(OC2=C1O)C3=CC=CC=C3</chem>	0	1	0

ID	Structure + SMILES	nAUC	Efficacy	LogAC50
NCGC00180839-03	 <chem>OC1=CC(=CC(=O)C1O)C(=O)OC[C@H]2O[C@@H](OC(=O)C3=CC(=O)C(=O)C(=O)C3)[C@H](OC(=O)C4=CC(=O)C(=O)C(=O)C4)[C@@H](OC(=O)C5=CC(=O)C(=O)C(=O)C5)[C@@H]2OC(=O)C6=CC(=O)C(=O)C(=O)C6</chem>	0	1	0
NCGC00488478-01	 <chem>CC[C@H]1[C@@H](C)[C@H]([C@H](C)[C@@H](C2=C(OC)C=C(OC)C(OC)=C2)[C@@]13C=C(OC)C(=O)C=C3OC)C4=CC(OC)=C(OC)C=C4OC</chem>	0	1	0
NCGC00488479-01	 <chem>CC[C@H]1[C@@H](C)[C@H]([C@H](C)[C@@H](C2=C(OC)C=C(OC)C(OC)=C2)[C@@]13C=C(OC)C(=O)C=C3OC)C4=CC(OC)=C(OC)C=C4OC</chem>	0	1	0
NCGC00488495-01	 <chem>CC(C)=CCC\C(C)=C\CC1=C2OC3=C(C=C(O)C=C3)C(=O)C2=C(O)C(CC=C(C)C)=C1O</chem>	0	1	0
NCGC00488693-01	 <chem>C\C=C\C1=CC2=C(C(=O)O1)C(=O)[C@@H](O)[C@H](C)O2</chem>	0	1	0
NCGC00488745-01	 <chem>COC(=O)C1=CC2=C(C(=O)O1)C(=O)C3OC4(CC5=C(O4)C(=O)C6=C(C5=O)C(OC)=CC(OC)=C6O)CCC3=C2</chem>	0	1	0
NCGC00488748-01	 <chem>CCCCCCCC(=O)O[C@H]1CC(=O)C(=C)C[C@H]2O[C@@H]3[C@H]4[C@@H]2[C@@](C)(O)CC[C@@H]4[C@H](C)COC13C</chem>	0	1	0
NCGC00488808-01	 <chem>COC1C(O)C(C)OC(OC2C(OC3=C4C(=CC=C3)C(=O)=C5C(=O)OC6=C7C(=O)OC4=C57)=C(C)C=C6)OC(C)C(O)C2O)C1O</chem>	0	1	0

ID	Structure + SMILES	nAUC	Efficacy	LogAC50
NCGC00488809-01	 <chem>O[C@@H]1[C@H]2NCCC3=CC4=C(C(=C1C=CC=C5)C23</chem>	0	1	0
NCGC00489874-01	 <chem>CN1CC[C@]2{[C@@H]1NC3=CC=CC=C23)C4=C5N[C@H]6N(C)CC[C@]6(C5=CC=C4)[C@]78</chem> <chem>CCN(C)[C@H]7NC9=CC=CC=C89</chem>	0	1	0

Assay protocol tables for the Canvass screen

Table S3. Assay protocol tables for the Canvass screen.

Cancer Cell Apoptosis Assay: HEK293 and HPAF-II protocol			
Step	Parameter	Value	Description
1	Plate Cells	5 μ L	[1x]: 500 cells/well with 10% FBS DMEM culture medium
2a 2b	Library & Control Compounds	23 nL 23 nL	Compounds in DMSO solution; qHTS format Doxorubicin [0.03 μ M – 1000 μ M]
3	Incubation	12, 18, 24 hours	Incubation at 37°C/5% CO ₂ /95% RH
4	Reagent	3 μ L	Caspase-Glo 3/7 Assay reagent
5	Incubation	15 minutes	Incubation at room temperature, covered from light
6	Detection	Clear Filter	ViewLux Luminescence Read

Step	Notes
1	White solid bottom tissue culture treated plates. Multidrop Combi dispenser dispense 5 μ L of [1x]: 500 cells/well into columns 2 to 48, rows 1 to 32 in DMEM + 10% FBS + 1% P/S. Media into column 1, rows 1-32.
2a	Compound plates. Single pin-transfer columns 5 to 48.
2b	Control compounds. Doxorubicin (1mM; 1:2 dilution; 16 points; n=2; column 3, rows 1 to 32), DMSO (column 1, 2, and 4, rows 1 to 32) Single pin-transfer.
3	Incubation at 37°C/5% CO ₂ /95% RH
4	Caspase-Glo 3/7 Reagent (Promega), dispense 3 μ L into columns 1 to 48, rows 1 to 32
5	Incubation at room temperature, covered from light
6	Online ViewLux: Clear filter, 20 second exposure, 2X binning

Assay Interference Profile-counterscreen 1 protocol			
Step	Parameter	Value	Description
1	Reagent	3 μ L	[1x]: 0.4 mg/mL Diaphorase in 1X PBS-T ([final] = 0.03 mg/mL)
2a	Compound	23 nL	Compound (qHTS format) Plates PTC124 [25 μ M]
2b	Control	23 nL	
3	Time	~15 minutes	Incubation at room temperature, covered
4	Reagent	1 μ L	[4x]: 40 μ M Resazurin + 40 μ M NADH in PBS-T ([final] = 10 μ M NADH, 10 μ M Resazurin)
5	Time	5 minutes	Incubation at room temperature, covered
6	Detector	525/598	ViewLux fluorescence read

Step	Notes
1	Black solid bottom medium binding plates (corning 7494). Bioraptr dispenser, dispense 3 μ L of [1x]: Diaphorase from Clostridium kluyveri in PBS + 0.01% Tween-20 into columns 2 to 48, rows 1 to 32, PBS-T solution in column 1, rows 1 to 32.
2a	Compound Plates. Single Pin-transferred.
2b	PTC (25 μ M; n = 32; column 2, rows 1 – 32) single pin-transferred for a [final] of 114.5 nM
3	Incubation at room temperature, covered from light
4	Bioraptr, dispense 1 μ L of [4x]: 40 μ M Resazurin + 40 μ M NADH in PBS-T into column 1 to 48, rows 1-32
6	Incubation at room temperature, covered from light
9	Offline ViewLux #1: Protocol (Dori_525/598_1536-well); Excitation filter 525/20, Emission filter 598/25; 1000 light energy, 2 second exposure; 2X Binning.

Assay Interference Profile-counterscreen 2 protocol			
Step	Parameter	Value	Description
1	Reagent	3 μ L	[1x]: 50 mM Tris Acetate substrate solution containing 13.3 mM Mg-Acetate, 0.013 mM D-Luciferin, 0.013 mM ATP, 0.013% Tween-20, 0.067% BSA ([final] = 10 mM Mg-Acetate, 10 mM D-Luciferin, 10 mM ATP, 0.01% Tween-20, 0.05% BSA)
2a	Compound	23 nL	Compound (qHTS format) Plates PTC124 [25 μ M]
2b	Control	23 nL	
3	Time	~15 minutes	Incubation at room temperature, covered
4	Reagent	1 μ L	[4x]: 0.04 μ g/mL P. pyralis Luciferase in 50 mM Tris-Acetate ([final] = 0.01 μ g/mL Luciferase)
5	Time	5 minutes	Incubation at room temperature, covered
6	Detector	clear	ViewLux luminescence read

Step	Notes
1	Black solid bottom medium binding plates (corning 7494). Bioraptr dispenser, dispense 3 μ L of [1x]: 50 mM Tris Acetate substrate solution containing 13.3 mM Mg-Acetate, 0.013 mM D-Luciferin, 0.013 mM ATP, 0.013% Tween-20, 0.067% BSA into columns 1 to 48, rows 1 to 32.
2a	Compound Plates. Single Pin-transferred.
2b	PTC124 (25 μ M; n = 32; column 2, rows 1 to 32) single pin-transferred for a [final] of 142.9 nM
3	Incubation at room temperature, covered from light
4	Bioraptr, dispense 1 μ L of [4x]: 0.04 μ g/mL P. pyralis Luciferase in 50 mM Tris-Acetate into column 2 to 48, rows 1 to 32, 50 mM Tris-Acetate into column 1, rows 1 to 32
6	Incubation at room temperature, covered from light
9	Offline ViewLux #1: Protocol 1382 (lum 1536 dc); 2 second exposure; 2X Binning, Excitation filter: none, emission filter: clear

ATAD5-agonist-screen protocol			
Step	Parameter	Value	Description
1	Plate cells	2000 cells/5uL	ATAD5 Cells in white solid-bottom 1536 well plates, using Multidrop Combi dispenser (8-tips)
2	Incubation time	5hr	Incubate at 37° C, 5% CO ₂ for the cells to acclimate and attach
3	Library & Control Compounds	23nL	Compound transfer to columns 5-48 and positive control to columns 1-4 using Pintool station
4	Incubation time	16hr	Incubate at 37° C, 5% CO ₂
5	Reagent	1uL (add after 15hr incubation)	Add CellTiter-Fluor Reagent using BioRAPTR dispenser (single tip)
6	Incubation time	1hr	Incubate at 37°C, 5% CO ₂
7	Assay Readout	ViewLux	Measure fluorescence signal using ViewLux plate reader [Settings; exposure time: 1sec]
8	Reagent	4uL	Add Amplite Reagent using BioRAPTR dispenser (single tip)
9	Incubation time	30min	Incubate at room temperature to develop and stabilize Luminescence signal
10	Assay Readout	ViewLux	Measure luminescence signal using ViewLux plate reader [Settings; exposure time: 10sec]

ATAD5-antagonist-screen protocol			
Step	Parameter	Value	Description
1	Plate cells	2000 cells/4uL	ATAD5 Cells in white solid-bottom 1536 well plates, using Multidrop Combi dispenser (8-tips)
2	Incubation time	5hr	Incubate at 37° C, 5% CO ₂ for the cells to acclimate and attach
3	Library & Control Compounds	23nL	Compound transfer to columns 5-48 and positive control to columns 1-4 using Pintool station
4	Reagent	1uL	Add 10uM of 5-Fluorouridine and assay medium using BioRAPTR dispenser (two separate tips)
5	Incubation time	16hr	Incubate at 37° C, 5% CO ₂
6	Reagent	1uL (add after 15hr incubation)	Add CellTiter-Fluor Reagent using BioRAPTR dispenser (single tip)
7	Incubation time	1hr	Incubate at 37°C, 5% CO ₂
8	Assay Readout	ViewLux	Measure fluorescence signal using ViewLux plate reader [Settings; exposure time: 1sec]
9	Reagent	4uL	Add Amplite Reagent using BioRAPTR dispenser (single tip)
10	Incubation time	30min	Incubate at room temperature to develop and stabilize Luminescence signal
11	Assay Readout	ViewLux	Measure luminescence signal using ViewLux plate reader [Settings; exposure time: 10sec]

Cancer Cell Tox: A2780-screen protocol			
Step	Parameter	Value	Description
1	OVA cells*	5 uL/well	Ovarian cancer cells (750 cells/well) *cisplatin sensitive cells and cisplatin resistant cells were screened, respectively.
2	Compound	0.023 uL/well	Compound in DMSO solution
3	Incubation	72 hr	37°C, 5% CO ₂
4	Detection reagent	4 uL/well	ATPLite reagent
5	Incubation	10 min	Room temperature
7	Plate reading	Luminescence mode	ViewLux plate reader

Cancer Cell Tox: COV-362, OV-KATE, OV-SAHO, UWB1.289, UWB1.289-WTBRCA1, HEK-293, HPAF-II, Panc-1005, SW1088, and U-118MG-screen protocol			
Step	Parameter	Value	Description
1	Reagent	6 µL	[1x]: 1,000 cells/well (1.7x10 ⁵ cells/mL); with 5% FBS culture medium
2	Time	~4 hours	Incubation at 37°C/5% CO ₂ /95% RH
3a	Compound	23 nL	Compound (qHTS format) Plates Bortezomib [0.0305 µM – 1000µM] & Top dose 500µM
3b	Control	23 nL	
4	Time	72 hours	Incubation at 37°C/5% CO ₂ /95% RH
5	Reagent	3µL	Cell titer Glo detect reagent
6	Time	15min	Incubation at 37°C/5% CO ₂ /95% RH
9	Detector	Clear Filter	ViewLux Luminescence Read

Step	Notes
1	White solid bottom tissue culture plates (corning 7494). Multidrop Combi dispenser, dispense 5 µL of [1x]: 1,000 cells/well (1.7 x 10 ⁵ cells/mL) into columns 1 – 48, rows 1 to 32. Plate using multidrop. Cell lines:
2	Incubation at 37°C/5% CO ₂ /95% R _h
3a	Compound Plates. Single Pin-transferred.
3b	Bortezomib (1mM; 1:2 dilution; 16-points; n = 2; column 3, rows 1 – 32) single pin-transferred for a [final] range of 0.0001 µM – 3.8 µM, Bortezomib (0.5mM column 4, rows 1 – 32) single pin-transferred for a [final] range of 1.9 µM
4	Incubation at 37°C/5% CO ₂ /95% R _h
5	Cell titer Glo detect reagent, dispense 3µL into column 2-48, rows 1 to 32.
6	Incubation at room temperature
9	Offline ViewLux #1: Protocol (Yaqin_Luminescence_1536-well); Excitation filter wheel A (None), Emission filter wheel A (Clear filter); 2 second exposure; 2X Binning.

Cancer Cell Tox: G06, J3T and SDT-screen protocol			
Step	Parameter	Value	Description
1	Reagent	5 μ L	500 cells/well
2a	Compound	23 nL	Compound (qHTS format) Plates Bortezomib [0.001 μ M – 46 μ M]
2b	Control	23 nL	
3	Time	72 hours	Incubation at 37°C/5% CO ₂ /95% RH
4	Reagent	3 μ L	CellTiterGlo reagent
5	Time	15 sec, 1000 rpm	Centrifuge
6	Detector	Clear Filter	ViewLux Luminescence Read

Step	Notes
1	White solid bottom tissue culture plates (corning 7494). Multidrop Combi dispenser, dispense 5 μ L: 500 cells/well (1 x 10 ⁵ cells/mL) into columns 2, 3, 5 – 48. Plate using multidrop.
2a	Compound Plates. Single Pin-transferred.
2b	Bortezomib (1 mM; 1:2 dilution; 16-points; n = 2; column 2) single pin-transferred for a [final] range of 0.0001 μ M – 4.6 μ M
3	Incubation at 37°C/5% CO ₂ /95% R _h
4	Multidrop dispense
5	Centrifugation step to remove bubbles.
6	Online ViewLux #1: Protocol 1262 (YASGAR_Luminescence_120210_1536-well); Excitation filter wheel A (None), Emission filter wheel A (Clear filter); 2 second exposure; 1X Binning.

Cancer Cell Tox: MCF7-screen protocol			
Step	Parameter	Value	Description
1	Reagent	5 μ L	[1x]: 500 cells/well (1 x 10 ⁵ cells/mL); MCF-7 (HTB-22, ATCC)
2a	Compound	23 nL	Compound (qHTS format) Plates [Bortezomib [0.03 μ M – 1,000 μ M]
2b	Control	23 nL	
3	Time	~72 hours	Incubation at 37°C/5% CO ₂ /95% RH
4	Reagent	3 μ L	CellTiterGlo reagent
5	Time	15 sec, 1000 rpm	Centrifuge
6	Detector	Clear Filter	ViewLux Luminescence Read

Step	Notes
1	White solid bottom tissue culture plates (corning 7494). Multidrop Combi dispenser, dispense 5 μ L of [1x]: 500 cells/well (1 x 10 ⁵ cells/mL) into columns 2 to 48, rows 1 to 32. Plate using multidrop. DMEM + 10% FBS + 1% P/S dispensed into column 1, rows 1 to 32 using multidrop.
2a	Compound Plates. Single Pin-transferred.
2b	Bortezomib (2 mM; 1:2 dilution; 16-points; n = 2; column 2, rows 1 – 32) single pin-transferred for a [final] range of 0.0003 μ M – 9.2 μ M
3	Incubation at 37°C/5% CO ₂ /95% R _h
4	Multidrop dispense into columns 1 to 48, rows 1 to 32
5	Centrifugation step to remove bubbles.
6	Online ViewLux #1: Protocol 1262 (YASGAR_Luminescence_120210_1536-well); Excitation filter wheel A (None), Emission filter wheel A (Clear filter); 2 second exposure; 1X Binning.

Cancer Cell Tox: KB-3-1 and KB-8-5-11 screen protocol			
Step	Parameter	Value	Description
1	Reagent	5 μ L	500 cells/well (1×10^5 cells/mL); HeLa derived KB 3-1 or KB 8-5-11
2a	Compound	23 nL	Compound (qHTS format) Plates Bortezomib [0.061 μ M – 2000 μ M]
2b	Control	23 nL	
3	Time	~72 hours	Incubation at 37°C/5% CO ₂ /85% RH
4	Reagent	2.5 μ L	CellTiter-Glo Reagent
5	Time	10 minutes	Incubation at room temperature (22°C)
6	Detector	Clear Filter	ViewLux Luminescence Read

Step	Notes
1	Multidrop Combi dispenser, dispense 5 μ L of 500 cells/well (1×10^5 cells/mL) columns 3 – 48, rows 1 to 32 of white solid bottom tissue culture plate (Corning 7464). Columns 1 – 2, rows 1 to 32 received medium only: DMEM (Life Technologies), 10% FBS (Hyclone), 100 U/ml penicillin and 100 μ g/ml streptomycin. For follow-up confirmation, KB 8-5-11 were also plated in medium supplemented with 1 μ M tariquidar.
2a	Compound plates were single pin-transferred to cell plates.
2b	Bortezomib (2 mM; 1:2 dilution; 16-points; n = 2; column 3, rows 1 – 32) single pin-transferred to cell plates for a [final] range of 0.2806 nM – 9.2 μ M
4	Incubation at 37°C/5% CO ₂ /85% RH
7	Multidrop Combi dispenser, dispense 2.5 μ L of CellTiter-Glo Reagent into columns 1 – 48, rows 1 to 32.
8	Incubation at room temperature (22°C)
9	ViewLux (Perkin Elmer) luminescence read; 1 second exposure; 1X Binning.

CAR-agonist-screen protocol			
Step	Parameter	Value	Description
1	Plate cells	2.5k cells/ 4 μ L	Plate Cells in white-flat bottom 1536 well plates, using 8 tip dispense (Multidrop)
2	Incubation time	4 h	Incubate at 37° C, 5% CO ₂
3	Compound Addition	23 nL	Pintool transfer of control (1-4 columns) and compound library (5-48 columns).
4	PK11195 Addition	1 μ L	Add 1.5 μ M PK11195 using Bioraptr (solution in media)
5	Incubation time	24 h	Incubate at 37° C, 5% CO ₂
6	Reagent	1 μ L	Addition of cell-titer fluor using a single tip dispense (Bioraptr)
7	Incubation time	1 h	Incubate at 37° C, 5% CO ₂
8	Readout	ViewLux	Light energy – 65 1 s Excitation – 405/10 (A01) Emission – 540/25 (FITC) (A06) Mirror 3 (FITC dichroic) Low, low, 2x
9	Reagent	4 μ L	Addition of ONEglo using a Multidrop
10	Incubation	0.5 h	Incubate at RT.
11	Readout	ViewLux	Luminescent, 10°, low, high, 2x

CAR-antagonist-screen protocol			
Step	Parameter	Value	Description
1	Plate cells	2.5k cells/ 4 μ L	Plate Cells in white-flat bottom 1536 well plates, using 8 tip dispense (Multidrop)
2	Incubation time	4 h	Incubate at 37° C, 5% CO ₂
3	Compound Addition	23 nL	Pintool transfer of control (1-4 columns) and compound library (5-48 columns).
4	CITCO Addition	1 μ L	Add 50 nM CITCO using Bioraptr (solution in media)
5	Incubation time	24 h	Incubate at 37° C, 5% CO ₂
6	Reagent	1 μ L	Addition of cell-titer fluor using a single tip dispense (Bioraptr)
7	Incubation time	1 h	Incubate at 37° C, 5% CO ₂
8	Readout	ViewLux	Light energy – 65 1 s Excitation – 405/10 (A01) Emission – 540/25 (FITC) (A06) Mirror 3 (FITC dichroic) Low, low, 2x
9	Reagent	4 μ L	Addition of ONEglo using a Multidrop
10	Incubation	0.5 h	Incubate at RT.
11	Readout	ViewLux	Luminescent, 10°, low, high, 2x

<i>E.coli</i> 10beta and DH5alpha-screen protocol			
Step	Parameter	Value	Description
1	Reagent	5 µL	Dilute overnight bacterial cell culture (0.9 OD)
2a	Compound	23 nL	Compound (qHTS format) Plates
2b	Control	23 nL	Daunomycin [0.06–6000µM]
3	Time	Overnight culture	Incubation at 37°C/5% CO ₂ /95% RH
4	Reagent	2.5 µL	BacTiterGlo Solution
5	Time	20 min	Incubation in RT
6	Detector	Clear Filter	ViewLux Luminescence Read

Step	Notes
1	White solid clear bottom tissue culture plates (Greiner). Multidrop Combi dispenser, dispense 5 µL (0.9 OD culture diluted 00.2OD) into columns 1, 2 and 5– 48, medium columns 3 and 4
2a	Compound Plates. Single Pin-transferred.
2b	Daunomycin (1 mM; 1:2 dilution; 16-points; n = 2; column 2, rows) single pin-transferred for a [final] range of 0.0002 µM – 9.2 µM
3	Incubation at 37°C
4	Multidrop dispensing of 2.5 uL of BacTiterGlo
5	Incubation at RT
6	Offline ViewLux #1: Protocol (George_Luminescence_1536-well); Excitation filter wheel A (None), Emission filter wheel A (Clear filterBinning)

H2AX-screen protocol			
Step	Parameter	Value	Description
1	Plate cells	1000 cells/3uL	CHO-K1 Cells in white solid-bottom 1536 well plates, using Multidrop Combi dispenser (8-tips)
2	Incubation time	Overnight (18-20hr)	Incubate at 37° C, 5% CO ₂ for the cells to acclimate and attach
3	Library & Control Compounds	23nL	Compound transfer to columns 5-48 and positive control to columns 1- 4 using Pintool station
4	Incubation time	3hr	Incubate at 37° C, 5% CO ₂
5	Reagent	1uL	Add blocking reagent made in lysis buffer (100-fold diluted in lysis buffer) using BioRAPTR dispenser (single tip). The assay plate centrifuged for 10sec at 1000rpm.
6	Incubation time	30min	Incubate at room temperature
7	Reagent	1uL	Add antibody solution made in detection buffer (Anti H2AX-d2 and Anti-pH2AX (S139)-K mixed in 1:1 ratio and diluted to 20-fold in detection buffer) using BioRAPTR dispenser (single tip).
8	Incubation time	24hr	Incubate at room temperature
9	Assay Readout	Envision	Measure fluorescence signal using Envision plate reader [excitation 320nm and emissions at 665nm and 620nm]

HIF-1-agonist-screen protocol			
Step	Parameter	Value	Description
1	Plate cells	2000 cells/6uL	HRE-bla ME180 Cells in black clear-bottom 1536 well plates, using Multidrop Combi dispenser (8-tips)
2	Incubation time	5hr	Incubate at 37° C, 5% CO ₂ for the cells to acclimate and attach
3	Library & Control Compounds	23nL	Compound transfer to columns 5-48 and positive control to columns 1-4 using Pintool station
4	Incubation time	17hr	Incubate at 37° C, 5% CO ₂
5	Reagent	1uL	Add CCF4 dye using BioRAPTR dispenser (single tip)
6	Incubation time	2hr	Incubate at room temperature
7	Assay Readout	Envision	Measure fluorescence signal using Envision plate reader [Excitation at 405nm and emissions at 460 & 530nm]
8	Reagent	4uL	Add CellTiter-Glo Reagent using BioRAPTR dispenser (single tip)
9	Incubation time	30min	Incubate at room temperature to develop and stabilize Luminescence signal
10	Assay Readout	ViewLux	Measure luminescence signal using ViewLux plate reader [Settings; exposure time: 15sec]

HIF-1-antagonist-screen protocol			
Step	Parameter	Value	Description
1	Plate cells	2000 cells/5uL	HRE-bla ME180 Cells in black clear-bottom 1536 well plates, using Multidrop Combi dispenser (8-tips)
2	Incubation time	5hr	Incubate at 37° C, 5% CO ₂ for the cells to acclimate and attach
3	Library & Control Compounds	23nL	Compound transfer to columns 5-48 and positive control to columns 1-4 using Pintool station
4	Reagent	1uL	Add 60uM CoCl ₂ and assay medium using BioRAPTR dispenser (two separate tips)
5	Incubation time	17hr	Incubate at 37° C, 5% CO ₂
6	Reagent	1uL	Add CCF4 dye using BioRAPTR dispenser (single tip)
7	Incubation time	2hr	Incubate at room temperature
8	Assay Readout	Envision	Measure fluorescence signal using Envision plate reader [Excitation at 405nm and emissions at 460 & 530nm]
9	Reagent	4uL	Add CellTiter-Glo Reagent using BioRAPTR dispenser (single tip)
10	Incubation time	30min	Incubate at room temperature to develop and stabilize Luminescence signal
11	Assay Readout	ViewLux	Measure luminescence signal using ViewLux plate reader [Settings; exposure time: 15sec]

IL-1 β -screen protocol			PubChem AID: 743279
Step	Parameter	Value	Description
1	Reagent	3.0 μ L	THP1 cells
2	Test and control compounds	23 nL	DMSO solutions
3	Time	1 hour	Incubation (37°C, 5% CO ₂)
4	Reagent	1.0 μ L	1 μ g / mL LPS and 5 mM ATP
5	Time	16 hours	Incubation (37°C, 5% CO ₂)
6	Reagent	1.5 μ L	AlphaLISA acceptor beads and biotinylated antibody
7	Time	1 hour	Ambient temperature
8	Reagent	1.5 μ L	AlphaLISA donor beads
9	Time	30 min	Ambient temperature, dark
10	Read	EnVision	AlphaScreen 1536-well plate protocol

Step	Notes
1	Dispense 3000 THP1 cells per well into solid, white, tissue culture-treated 1536-well plates
2	Canvass compounds or control (glyburide) are introduced by Kalypsis pin-tool
4	1 μ g/mL LPS and 5 mM ATP in culture medium are dispensed by BioRapTR
6	Anti-IL-1 β AlphaLISA acceptor beads (6 μ g/mL, final) and anti-IL-1 β biotinylated antibody (0.6 nM, final)
8	AlphaLISA SA-Donor beads (24 μ g/mL, final)
10	EnVision AlphaLISA protocol: Excitation 680 nm / Emission 615 nm

IL-1 β -counterscreen protocol			
Step	Parameter	Value	Description
1	Reagent	4.0 μ L	Cell culture media (low control, columns 3-4) with 4 pg purified IL-1 β (high control; columns 1-2; 5-48)
2	Test and control compounds	23 nL	DMSO solutions
3	Reagent	1.5 μ L	AlphaLISA acceptor beads and biotinylated antibody
4	Time	1 hour	Ambient temperature
5	Reagent	1.5 μ L	AlphaLISA donor beads
6	Time	30 min	Ambient temperature, dark
7	Read	EnVision	AlphaScreen 1536-well plate protocol

Step	Notes
1	Dispensed by BioRapTR into solid, white, tissue culture-treated 1536-well plates
2	Canvass compounds or vehicle control (DMSO) are introduced by Kalypsis pin-tool
3	Anti-IL-1 β AlphaLISA acceptor beads (6 μ g/mL, final) and anti-IL-1 β biotinylated antibody (0.6 nM, final)
5	AlphaLISA SA-Donor beads (24 μ g/mL, final)
7	EnVision AlphaLISA protocol: Excitation 680 nm / Emission 615 nm

Malaria-screen protocol			
Step	Parameter	Value	Description
1	Reagent	3 μ L	Complete Malaria Growth medium
2a 2b	Library Compounds Control Compounds	23 nL 23 nL	Compound (qHTS format) Plates Dihydroartemisinin 29 μ M; 1.84 μ M to 56 pM titration series
3	Reagent	5 μ L	Malaria-infected RBCs
4	Time	72 hr	37°C incubation (5% O ₂ , 5% CO ₂ , 90% N ₂ ; 95% R _h)
5	Reagent	2 μ L	Lysis buffer + SYBRGreen
7	Time	Overnight	37°C incubation (5% O ₂ , 5% CO ₂ , 90% N ₂ ; 95% R _h)
8	Detector	Fluorescence	EnVision

Step	Notes
1	Assay adopted from Plouffe <i>et al.</i> 2008 PNAS 105: 9059. Reagents were dispensed into 1536-well black clear Cyclo-Olefin Polymer plate (Aurora Microplates) using a Multidrop Combi (Thermo Fisher Scientific Inc.) contained in a biosafety cabinet.
1a	Complete Malaria Growth Media: 1X RPMI-1640 w/L-glutamine, 5.5g/L Albumax II, 367 μ M Hypoxanthine, 25 mM HEPES, 0.25% Sodium Bicarbonate, 35 μ M Gentamicin sulfate
2	Compound Plates. Single Pin-transferred.
3	Hematocrit 4% (final 2.5%), 0.3% parasitemia
5	Lysis buffer: 20 mM Tris-HCl, 10 mM EDTA, 0.16% Saponin, 1.6% Triton-X, 10X SYBR Green. 25 sec shake immediately following dispense
7	Approximately 18 hours
8	EnVision (PerkinElmer) bottom read at 485/14nm excitation and 535/25 nm emission

NOTE: To gain confidence in the primary screening hits, an additional replicate utilizing SYBRGreen against wild-type *P. falciparum* Dd2 parasites was conducted. In addition, an orthogonal assay using a Nanoluc (Nluc) recombinant *P. falciparum* NF54 parasite was also performed. Analysis of compounds that produced higher quality dose-response curves demonstrated variance between the two replicates of the *P. falciparum* Dd2 assay ($r^2 = 0.344$). Higher correlation was obtained between the replicate *P. falciparum* Dd2 SYBRGreen and orthogonal *P. falciparum* NF54 Nluc assay performed in parallel ($r^2 = 0.595$). Divergence between compound activity in the Dd2 SYBRGreen replicate assay and NF54 Nluc assay is likely partially explained by the altered drug susceptibility of the Dd2 parasite (multidrug resistance transporter SNPs and CNV; chloroquine resistance transporter SNPs). The exact basis underlying the divergence between the primary screen and secondary screens remains unclear; however, the higher correlation between the assay run in parallel may suggest a reagent dispense or compound transfer issue in the primary screen.

Membrane Integrity-screen protocol			
Step	Parameter	Value	Description
1	Plate cells	2000 cells/5uL	HepG2 Cells in black solid-bottom 1536 well plates, using Multidrop Combi dispenser (8-tips)
2	Incubation time	Overnight (18-20hr)	Incubate at 37° C, 5% CO ₂ for the cells to acclimate and attach
3	Library & Control Compounds	23nL	Compound transfer to columns 5-48 and positive control to columns 1-4 using Pintool station
4	Incubation time	5hr	Compound treatment and incubation at 37° C, 5% CO ₂
5	Reagent	5uL	Addition of CytoTox-ONE Membrane Integrity assay Reagent using BioRAPTR dispenser (single tip)
6	Incubation time	10min	Incubation at room temperature
7	Assay Readout	Envision	Measurement of fluorescence signal using Envision plate reader [Excitation at 560nm and Emission at 590nm]

Mutant IDH1-screen protocol			
Step	Parameter	Value	Description
1	Reagent	3 µL	[2x]: 0.0001 mg/mL IDH1 R132C in 50 mM K ₂ PO ₄ , pH 6.5 buffer containing 5 mM MgCl ₂ , 0.03% protease-free BSA, 10% glycerol ([final] = 0.00005 mg/mL IDH1 R132C)
2a	Compound	23 nL	Compound (qHTS format) Plates AGX-7205 [0.3—10000 µM]
2b	Control	23 nL	
3	Time	30 minutes	Incubation at room temperature, covered
4	Reagent	3 µL	[2x]: 0.012 mM NADPH, 0.6 mM α-KG substrate solution in 50 mM K ₂ PO ₄ , pH 6.5 buffer containing 5 mM MgCl ₂ , 0.03% protease-free BSA, 10% glycerol ([final] = 0.006 mM NADPH, 0.3 mM α-KG)
5	Time	50 minutes	Incubation at room temperature, covered
6	Reagent	3 µL	[1x]: 0.03 mg/mL Diaphorase, 0.03 mM Resazurin detection solution in 50 mM K ₂ PO ₄ , pH 6.5 buffer containing 5 mM MgCl ₂ , 0.03% protease-free BSA, 10% glycerol
7	Time	5 minutes	Incubation at room temperature, covered
8	Detector	540/590	Envision fluorescence read

Step	Notes
1	Black solid bottom medium binding culture plates (corning 7494). Bioraptr dispenser, dispense 3 µL of [2x]: 0.0001 mg/mL IDH1 R132C in 50 mM K ₂ PO ₄ , pH 6.5 buffer containing 5 mM MgCl ₂ , 0.03% protease-free BSA, 10% glycerol into columns 2 – 48, rows 1 to 32, 50 mM K ₂ PO ₄ , pH 6.5 buffer solution in column 1, rows 1 to 32
2a	Compound Plates. Single Pin-transferred.
2b	AGX-7205 (10 mM; 1:2 dilution; 16-points; n = 2; column 2, rows 1 – 32) single pin-transferred for a [final] range of 0.0012 µM – 38.2 µM
3	Incubation at room temperature, covered
4	Bioraptr dispense, dispense 3 µL of 0.012 mM NADPH, 0.6 mM α-KG substrate solution in 50 mM K ₂ PO ₄ , pH 6.5 buffer containing 5 mM MgCl ₂ , 0.03% protease-free BSA, 10% glycerol into columns 1 – 48, rows 1 to 32
5	Incubation at room temperature, covered
5	Bioraptr, dispense 3 µL of 0.03 mg/mL Diaphorase, 0.03 mM Resazurin detection solution in 50 mM K ₂ PO ₄ , pH 6.5 buffer containing 5 mM MgCl ₂ , 0.03% protease-free BSA, 10% glycerol into columns 1 – 48, rows 1 to 32.
6	Incubation at room temperature, covered
7	Envision #1 (Mindy resorufurin_topread); Excitation 540 (#311), Emission 590 (#217), mirror D555 (#405), 4 flashes

Nrf2/ARE-screen protocol			
Step	Parameter	Value	Description
1	Plate cells	2000 cells/well/5uL	Plate cells in 1536-well black-clear bottom plate using a Multidrop
2	Incubation	5 hrs	Incubate at 37° C, 5% CO ₂ .
3	Compound Addition	23nL	Pintool transfer of control (1-4 columns) and compound library (5-48 columns).
4	Incubation	16 hrs	Incubate at 37° C, 5% CO ₂ .
5	Reagent	1uL	Addition of CCF4 dye using a BioRAPTR
6	Incubation	2 hrs	Incubate at RT
7	Readout	Envision	Excitation : 405nm Emission: 460nm and 530nm. Data express: ratio of 460nm/530nm
8	Reagent	4uL	Addition of CellTiter Glo using a BioRAPTR
9	Incubation	0.5 hr	Incubate at RT.
10	Readaout	ViewLux	Measure luminescence signal

p53-screen protocol			
Step	Parameter	Value	Description
1	Plate cells	4000 cells/well/5uL	Plate cells in 1536-well black-clear bottom plate using a Multidrop
2	Incubation	5 hrs	Incubate at 37° C, 5% CO ₂ .
3	Compound Addition	23nL	Pintool transfer of control (1-4 columns) and compound library (5-48 columns).
4	Incubation	16 hrs	Incubate at 37° C, 5% CO ₂ .
5	Reagent	1uL	Addition of CCF4 dye using a BioRAPTR
6	Incubation	2 hrs	Incubate at RT
7	Readout	Envision	Excitation : 405nm Emission: 460nm and 530nm. Data express: ratio of 460nm/530nm
8	Reagent	4uL	Addition of CellTiter Glo using a BioRAPTR
9	Incubation	0.5 hr	Incubate at RT.
10	Readaout	ViewLux	Measure luminescence signal

Protease-screen protocol			
Step	Parameter	Value	Description
1	Plate cells	2000 cells/5uL	HepG2 Cells in white solid-bottom 1536 well plates, using Multidrop Combi dispenser (8-tips)
2	Incubation time	Overnight (18-20hr)	Incubate at 37° C, 5% CO ₂ for the cells to acclimate and attach
3	Library & Control Compounds	23nL	Compound transfer to columns 5-48 and positive control to columns 1-4 using Pintool station
4	Incubation time	5hr	Compound treatment and incubation at 37° C, 5% CO ₂
5	Reagent	5uL	Addition of CytoTox-Glo Cytotoxicity assay Reagent using BioRAPTR dispenser (single tip)
6	Incubation time	15min	Incubation at room temperature
7	Assay Readout	ViewLux	Measurement of luminescence signal using ViewLux plate reader [Settings; exposure time: 1sec]

Rabies-screen protocol			
Step	Parameter	Value	Description
1	Cell dispensing	3 μ L	~1,000 cells/well (3x10 ⁴ cells/mL); Vero 766 cells derived (African Green Monkey)
2	Time	overnight	Incubation at 37°C/5% CO ₂ /95% RH
3	Compound	23 nL	Compound (qHTS format) Plates
4a 4b	Virus dispensing	2 μ L 2 μ L	Calculate the volume with desired MOI of Rabies virus Media + vehicle
5	Time	18 hours	Incubation at 37°C/5% CO ₂ /95% RH
6	Reagent	2 μ L	OneGlo luciferase
7	Detector	Clear Filter	ViewLux Luminescence Read

Step	Notes
1	White solid bottom tissue culture plates (corning 7494). Multidrop Combi dispenser, dispense 3 μ L of [1x]: 1,000 cells/well (3 x 10 ⁴ cells/mL) into columns 1 – 48, rows 1 to 32. Plate using multidrop.
2	Incubation at 37°C/5% CO ₂ /95% R _h
3	Compound Plates. Single Pin-transferred.
3	10 mM; 1:2 dilution; 16-points; n = 2; column 2, rows 1 – 32) single pin-transferred for a [final] range of 0.001 μ M – 38.2 μ M
4	Incubation at 37°C/5% CO ₂ /95% R _h
4a	Multidrop Combi dispense 2 μ L of diluted Rabies virus [2.5x]: into columns 1,2 and - 48
4b	Multidrop Combi dispense 2 μ L of Media into columns 3 and 4
5	Incubation for 18 hrs at 37°C/5% CO ₂ /95% R _h
7	OneGlo luciferase solution by Multidrop dispensing
8	Offline ViewLux #1: Protocol (George_Luminescence_1536-well); Excitation filter wheel A (None), Emission filter wheel A (Clear filter)

Redox Profiling-screen protocol			
Step	Parameter	Value	Description
1	Reagent	4 μ L	[1x]: 5 μ M Resazurin and 200 μ M DTT
2a 2b	Compound Control	23 nL 23 nL	qHTS or cherry pick format Chloranil
3	Time	15 sec, 1000 rpm	Centrifuge
4	Detector	(E _x /E _m) = 525 nm /598 nm	ViewLux Fluorescence (Read 1)
5	Time	15 min	RT Incubation
6	Detector	(E _x /E _m) = 525 nm /598 nm	ViewLux Fluorescence (Read 2)

Step	Notes
1	Black solid bottom medium binding plates (Greiner 789176). BioRAPTR dispenser, dispense 4 μ L of [1x]: 5 μ M Resazurin and 200 μ M DTT (prepare DTT fresh; Assay Buffer = 50 mM HEPES pH 7.3 with 50 mM NaCl) into columns 1 – 48, rows 1 to 32.
2a	qHTS format (10 mM, 1:5 dilution, 7-points; DMSO). Cherry-pick (10 mM, 1:3, 11-points; DMSO). Single Pin-transfer.
2b	Chloranil (NCGC00091254-06) Intraplate Titration (10 mM; 1:2 dilution; 16-points; n = 2; column 2, rows 1 – 32) single pin-transferred for a [final] range of 0.00174 μ M to 57.2 μ M. Chloranil positive control titration (350 μ M; column 3 and 4, rows 1 – 32) single pin-transferred for a [final] range of 2 μ M.
3	Centrifugation step to remove bubbles.
4	ViewLux Optics: Excitation filter wheel (525(20)nm), Emission filter wheel (598(25)nm).
5	Incubation at room temperature. Cover, protected from light.
6	ViewLux Optics: Excitation filter wheel (525(20)nm), Emission filter wheel (598(25)nm).

SERCAMP- screen protocol			
Step	Parameter	Value	Description
1	Reagent	5 µL	[1x]: 1,000 cells/well (2x10 ⁵ cells/mL); SH-SY5Y-GLuc SERCaMP MJH297 derived (MP_G)
2	Time	~4 hours	Incubation at 37°C/5% CO ₂ /95% RH
3a 3b	Compound Control	23 nL 23 nL	Compound (qHTS format) Plates Bromocriptine [0.305 µM – 10,000 µM]
4	Time	~16 hours	Incubation at 37°C/5% CO ₂ /95% RH
5a 5b	Reagent	1 µL 1 µL	[6x]: 600 nM Thapsigargin (final conc = 100 nM) [1x]: Media + vehicle
6	Time	4 hours	Incubation at 37°C/5% CO ₂ /95% RH
7	Reagent	1 µL	[7x]: Coelenterazine (Substrate) diluted 1:200 in Pierce Glow Assay Buffer
8	Time	15 sec, 1000 rpm	Centrifuge
9	Detector	Clear Filter	ViewLux Luminescence Read

Step	Notes
1	White solid bottom tissue culture plates (corning 7494). Multidrop Combi dispenser, dispense 5 µL of [1x]: 1,000 cells/well (2 x 10 ⁵ cells/mL) into columns 1 – 48, rows 1 to 32. Plate using multidrop.
2	Incubation at 37°C/5% CO ₂ /95% R _h
3a	Compound Plates. Single Pin-transferred.
3b	Bromocriptine (10 mM; 1:2 dilution; 16-points; n = 2; column 2, rows 1 – 32) single pin-transferred for a [final] range of 0.001 µM – 38.2 µM
4	Incubation at 37°C/5% CO ₂ /95% R _h
5a	Bioraptr, dispense 1 µL of [6x]: 600 nM Thapsigargin into column 1 rows 17-32; column 2 rows 1 to 32; columns 4- 48, rows 1 to 32.
5b	Bioraptr, dispense 1 µL of [1x]: Media into column 1 rows 1-16; column 3, rows 1 to 32.
6	Incubation at 37°C/5% CO ₂ /95% R _h
7	Bioraptr, dispense 1 µL of [7x]: Coelenterazine (substrate) into columns 1 – 48, rows 1 to 32.
8	Centrifugation step to remove bubbles.
9	Online ViewLux #1: Excitation filter wheel A (None), Emission filter wheel A (Clear filter); 5 second exposure; 1X Binning.

SERCAMP-counterscreen 1 protocol			
Step	Parameter	Value	Description
1	Reagent	5 µL	[1x]: 1,000 cells/well (2x10 ⁵ cells/mL); SH-SY5Y-GLuc-STOP
2	Time	~4 hours	Incubation at 37°C/5% CO ₂ /95% RH
3a 3b	Compound Control	23 nL 23 nL	Compound (qHTS format) Plates Brefeldin A [0.03--1000µM]
4	Time	~20 hours	Incubation at 37°C/5% CO ₂ /95% RH
5	Reagent	1 µL	[7x]: Coelenterazine (Substrate) diluted 1:200 in Pierce Glow Assay Buffer
6	Time	15 sec, 1000 rpm	Centrifuge
7	Detector	Clear Filter	ViewLux Luminescence Read

Step	Notes
1	White solid bottom tissue culture plates (corning 7494). Multidrop Combi dispenser, dispense 5 µL of [1x]: 1,000 cells/well (2 x 10 ⁵ cells/mL) into columns 1 – 48, rows 1 to 32. Plate using multitidrop.
2	Incubation at 37°C/5% CO ₂ /95% R _h
3a	Compound Plates. Single Pin-transferred.
3b	Brefeldin A (1 mM; 1:2 dilution; 16-points; n = 2; column 2, rows 1 – 32) single pin-transferred for a [final] range of 0.0001 µM – 4.6 µM
4	Incubation at 37°C/5% CO ₂ /95% R _h
5	Bioraptr, dispense 1 µL of [7x]: Coelenterazine (substrate) into columns 1 – 48, rows 1 to 32.
6	Centrifugation step to remove bubbles.
7	Online ViewLux #1: Protocol 1262 (YASGAR_Luminescence_120210_1536-well); Excitation filter wheel A (None), Emission filter wheel A (Clear filter); 5 second exposure; 1X Binning.

SERCAMP-counterscreen 2			
Step	Parameter	Value	Description
1	Reagent	5 μ L	[1x]: 1,000 cells/well (2x10 ⁵ cells/mL); SH-SY5Y-GLuc SERCaMP MJH297 derived (MP_G)
2	Time	~4 hours	Incubation at 37°C/5% CO ₂ /95% RH
3a 3b	Compound Control	23 nL 23 nL	Compound (qHTS format) Plates [Bortezomib [0.03 μ M – 1,000 μ M]
4	Time	~48 hours	Incubation at 37°C/5% CO ₂ /95% RH
5	Reagent	3 μ L	CellTiterGlo reagent
6	Time	15 sec, 1000 rpm	Centrifuge
7	Detector	Clear Filter	ViewLux Luminescence Read

Step	Notes
1	White solid bottom tissue culture plates (corning 7494). Multidrop Combi dispenser, dispense 5 μ L of [1x]: 1,000 cells/well (2 x 10 ⁵ cells/mL) into columns 1 – 48, rows 1 to 32. Plate using multidrop.
2	Incubation at 37°C/5% CO ₂ /95% R _h
3a	Compound Plates. Single Pin-transferred.
3b	Bortezomib (1 mM; 1:2 dilution; 16-points; n = 2; column 2, rows 1 – 32) single pin-transferred for a [final] range of 0.0001 μ M – 4.6 μ M
4	Incubation at 37°C/5% CO ₂ /95% R _h
5	Multidrop dispense
6	Centrifugation step to remove bubbles.
7	Online ViewLux #1: Protocol 1262 (YASGAR_Luminescence_120210_1536-well); Excitation filter wheel A (None), Emission filter wheel A (Clear filter); 2 second exposure; 1X Binning.

Zika-screen protocol			
Step	Parameter	Value	Description
1	Cell dispensing	3 μ L	~1,000 cells/well (3x10 ⁴ cells/mL); Vero 766 cells derived (African Green Monkey)
2	Time	overnight	Incubation at 37°C/5% CO ₂ /95% RH
3	Compound	23 nL	Compound (qHTS format) Plates
4a 4b	Virus dispensing	2 μ L 2 μ L	Calculate the volume with desired MOI Zika virus Media + vehicle
5	Time	72 hours	Incubation at 37°C/5% CO ₂ /95% RH
6	Time	Peeling off plate films	Film peeler
7	Reagent	2.5 μ L	Cell TiterGlo assay
8	Detector	Clear Filter	ViewLux Luminescence Read

Step	Notes
1	White solid bottom tissue culture plates (corning 7494). Multidrop Combi dispenser, dispense 3 μ L of [1x]: 1,000 cells/well (3 x 10 ⁴ cells/mL) into columns 1 – 48, rows 1 to 32.
2	Incubation at 37°C/5% CO ₂ /95% R _h
3	Compound Plates. Single Pin-transferred.
3	10 mM; 1:2 dilution; 16-points; n = 2; column 2, rows 1 – 32) single pin-transferred for a [final] range of 0.001 μ M – 38.2 μ M
4	Incubation at 37°C/5% CO ₂ /95% R _h
4a	Multidrop Combi dispense 2 μ L of [2.5x]: into columns 1,2 and 5- 48
4b	Multidrop Combi dispense 2 μ L of Media into columns 3 and 4
5	After dispensing virus seal with breathable film Incubation at 37°C/5% CO ₂ /95% R _h
6	Peeling off breathable films by Xpeal
7	Adding CellTiter Glo reagent
8	Offline ViewLux #1: Protocol (George_Luminescence_1536-well); Excitation filter wheel A (None), Emission filter wheel A (Clear filter)

Zika-counterscreen protocol			
Step	Parameter	Value	Description
1	Cell dispensing	3 μ L	~1,000 cells/well (3x10 ⁴ cells/mL); Vero 766 cells derived (African Green Monkey)
2	Time	overnight	Incubation at 37°C/5% CO ₂ /95% RH
3	Compound	23 nL	Compound (qHTS format) Plates
4	reagent	2 μ L	Media + vehicle
5	Time	72 hours	Incubation at 37°C/5% CO ₂ /95% RH
6	Time	Peeling off plate films	Film peeler
7	Reagent	2.5 μ L	Cell TiterGlo assay
8	Detector	Clear Filter	ViewLux Luminescence Read

Step	Notes
1	White solid bottom tissue culture plates (corning 7494). Multidrop Combi dispenser, dispense 3 μ L of [1x]: 1,000 cells/well (3 x 10 ⁴ cells/mL) into columns 1,2 and 5 – 48, medium. cols 3 and 4.
2	Incubation at 37°C/5% CO ₂ /95% R _h
3	Compound Plates. Single Pin-transferred.
3	10 mM; 1:2 dilution; 16-points; n = 2; column 2, rows 1 – 32) single pin-transferred for a [final] range of 0.001 μ M – 38.2 μ M
4	Incubation at 37°C/5% CO ₂ /95% R _h
4	Multidrop Combi dispense 2 μ L to all wells
5	After dispensing virus seal with breathable film Incubation at 37°C/5% CO ₂ /95% R _h
6	Peeling off breathable films by Xpeal
7	Adding CellTiter Glo reagent
8	Offline ViewLux #1: Protocol (George_Luminescence_1536-well); Excitation filter wheel A (None), Emission filter wheel A (Clear filter)