

The Development of Hsp90 β -selective Inhibitors to Overcome Detriments

Associated with *pan*-Hsp90 Inhibition

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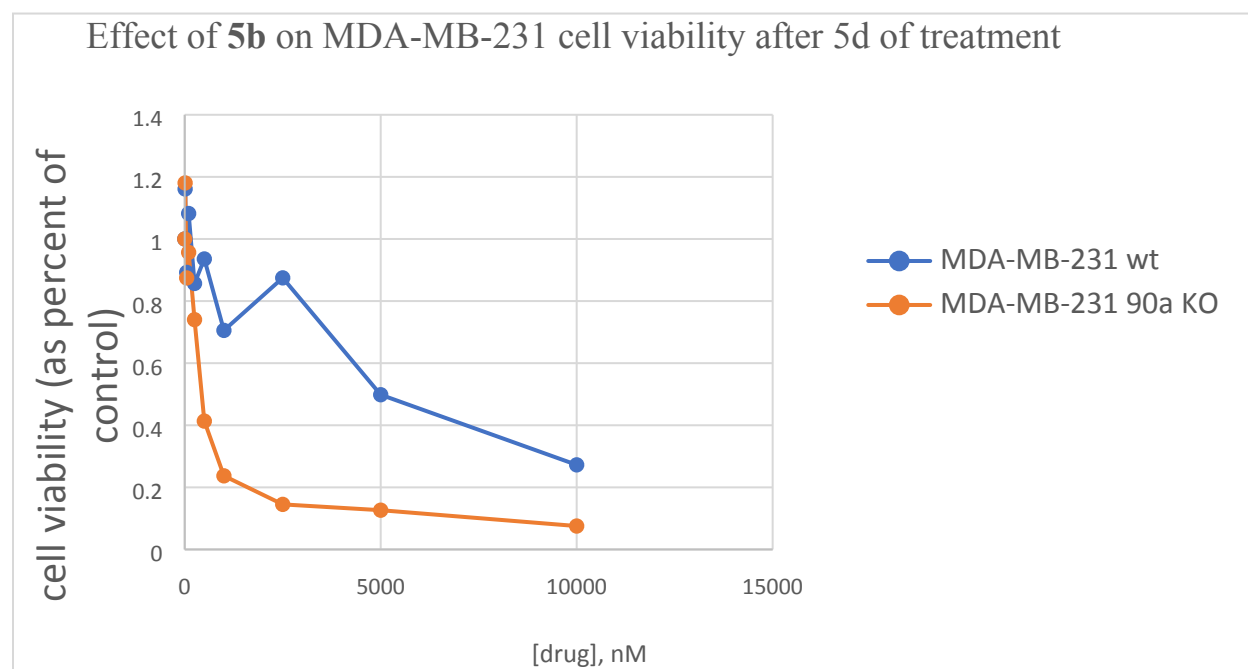
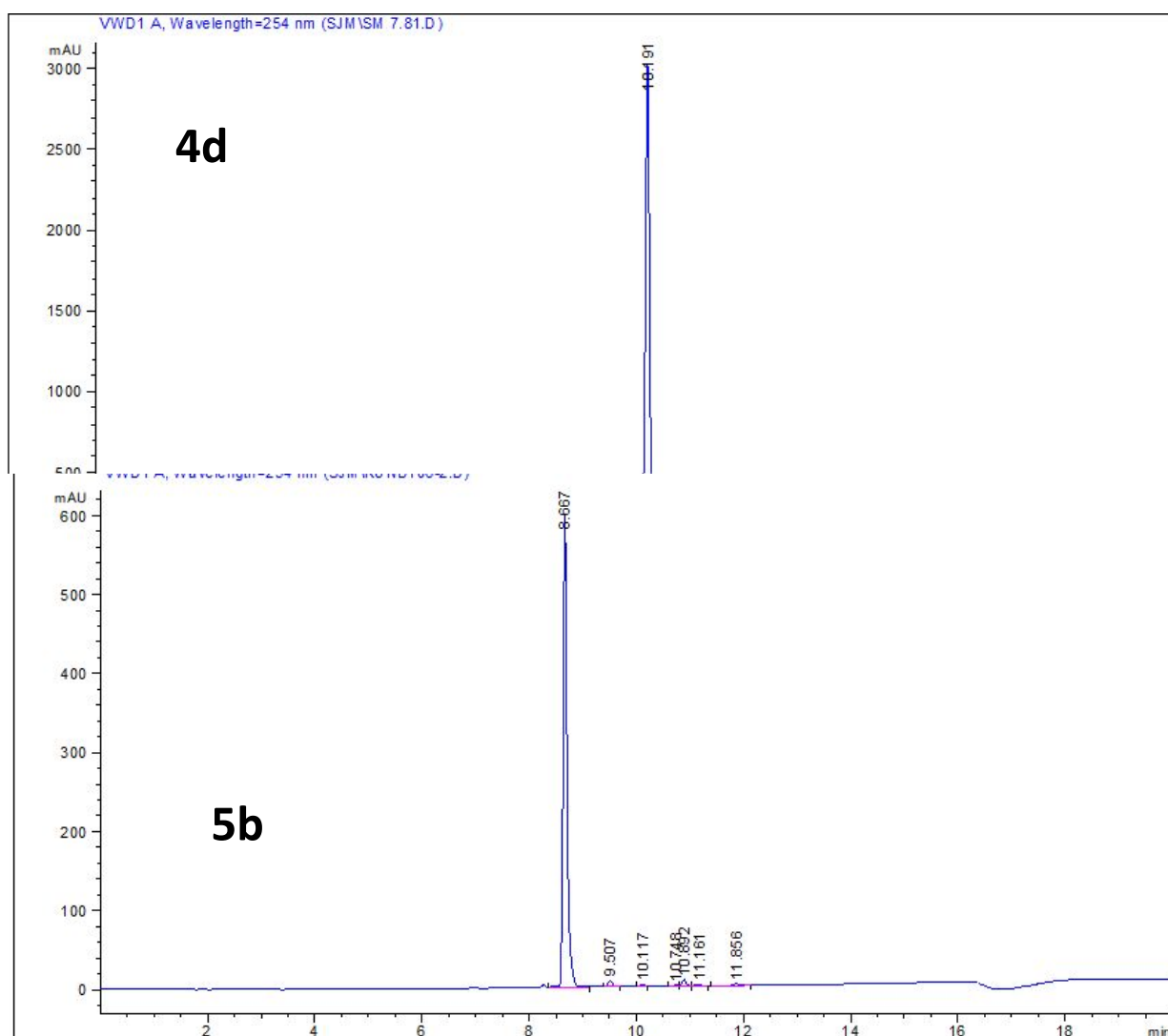
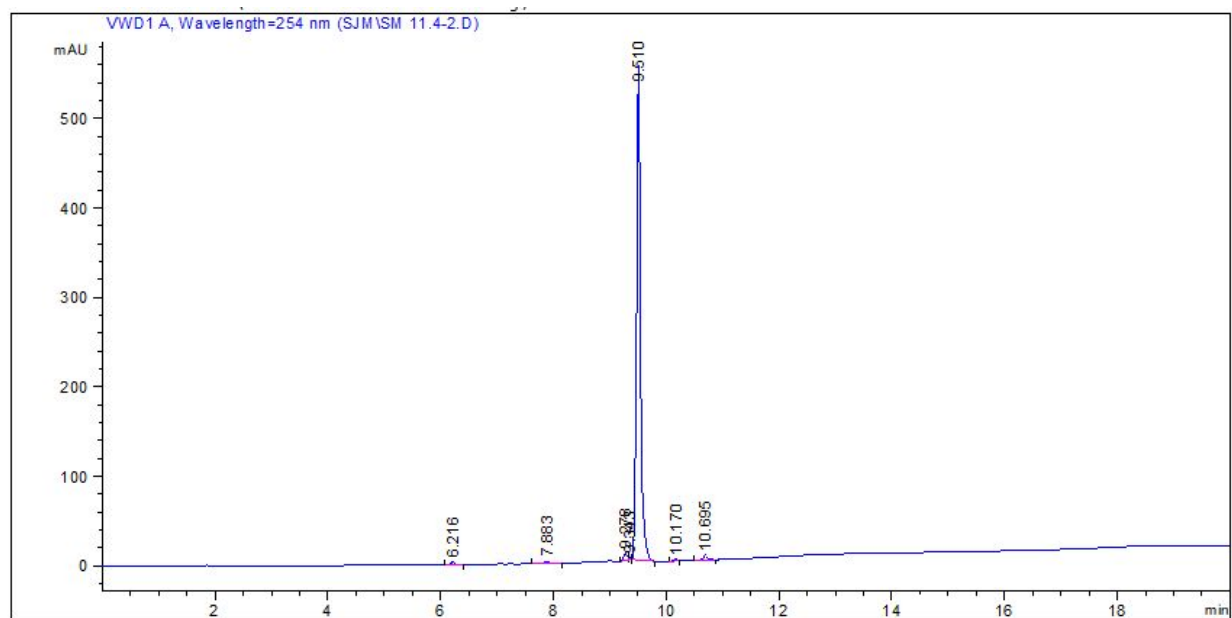


Figure S1: Effect of **5b** on Growth Inhibition Wild type and Hsp90 α knockout MDA-MB-231 cells MDA-MB-231 cells (wild-type or Hsp90 α KO) were plated (5000/well) and treated the next day with indicated concentrations of KUNB106. Cell viability was assayed after 5 days of drug

treatment via CellTiter-Glo, which measures ATP via luminescence. Cell viability is represented here as a percent of untreated cells.

HPLC Analysis





6a

Figure S2: NCI-60 Cell screening results with of **4d** (one-dose experiment)

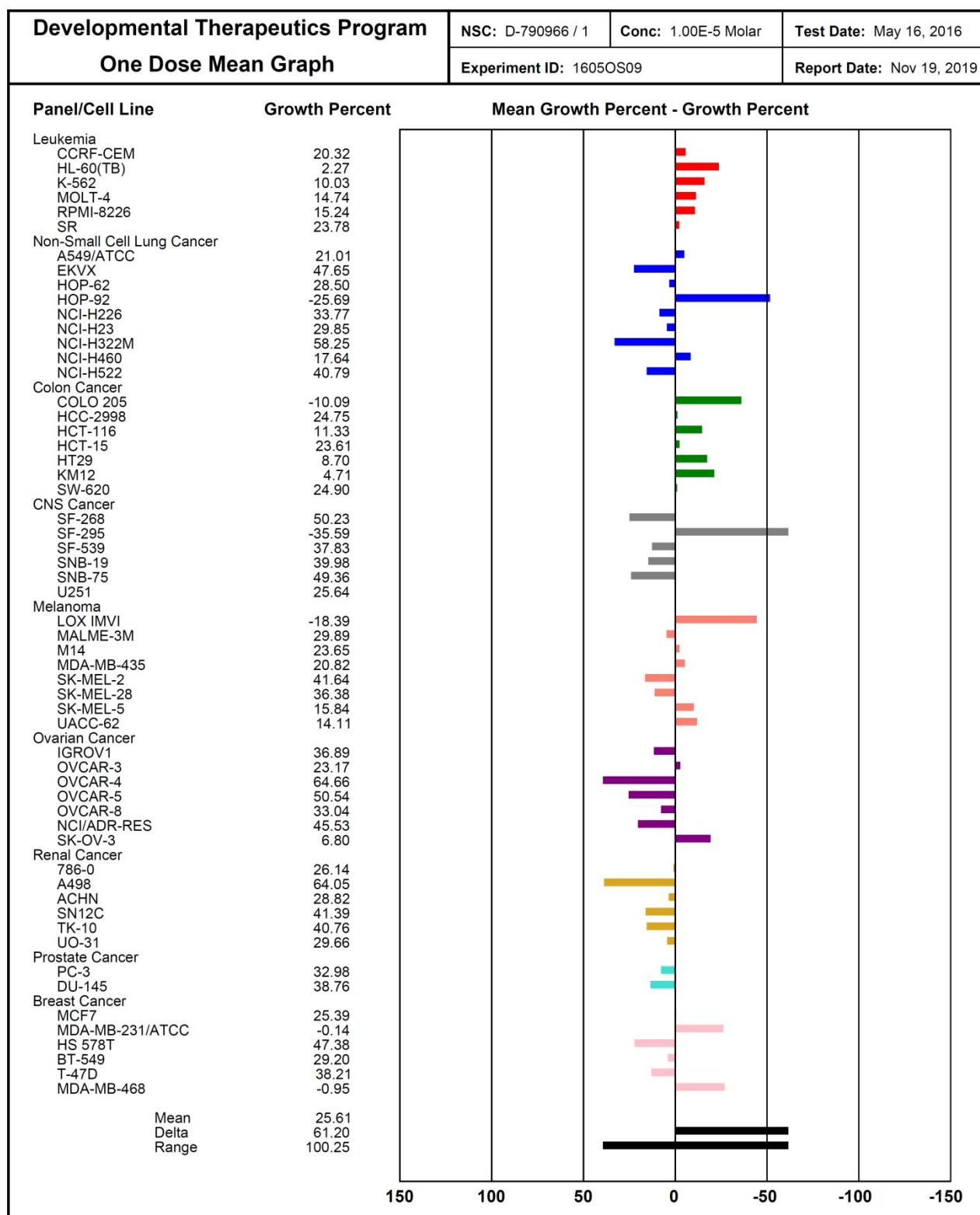


Figure S3: NCI-60 Cell screening results with GI50 values of **4d** (five-dose experiment)

National Cancer Institute Developmental Therapeutics Program In-Vitro Testing Results

NSC : D - 790966 / 1			Experiment ID : 1606NS35										Test Type : 08		Units : Molar	
Report Date : November 19, 2019			Test Date : June 27, 2016										QNS :		MC :	
COMI : KUNB105			Stain Reagent : SRB Dual-Pass Related										SSPL : 0XWC			
Panel/Cell Line	Time Zero	Ctrl	Log10 Concentration										GI50	TGI	LC50	
			Mean Optical Densities					Percent Growth								
			-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0				
Leukemia																
CCRF-CEM	0.461	2.251	2.212	1.517	0.775	0.609	0.658	98	59	18	8	11	1.65E-7	> 1.00E-4	> 1.00E-4	
HL-60(TB)	1.083	3.315	3.302	2.009	1.061	0.796	0.661	99	41	-2	-27	-39	7.13E-8	8.98E-7	> 1.00E-4	
K-562	0.291	2.175	2.085	0.669	0.549	0.429	0.446	95	20	14	7	8	4.00E-8	> 1.00E-4	> 1.00E-4	
MOLT-4	0.724	2.800	2.684	1.713	0.950	0.864	0.853	94	48	11	7	6	8.90E-8	> 1.00E-4	> 1.00E-4	
RPMI-8226	0.958	2.876	2.830	2.060	1.591	0.818	0.930	98	57	33	-15	-3	2.02E-7	4.92E-6	> 1.00E-4	
SR	0.525	1.864	1.754	0.966	0.758	0.606	0.610	92	33	17	6	6	5.12E-8	> 1.00E-4	> 1.00E-4	
Non-Small Cell Lung Cancer																
A549/ATCC	0.394	1.589	1.589	1.114	0.672	0.591	0.554	100	60	23	16	13	1.89E-7	> 1.00E-4	> 1.00E-4	
EKVX	0.601	1.832	1.804	1.393	1.217	1.094	1.048	98	64	50	40	36	1.01E-6	> 1.00E-4	> 1.00E-4	
HOP-62	0.716	1.553	1.534	1.089	1.003	0.895	0.780	98	45	34	21	8	7.89E-8	> 1.00E-4	> 1.00E-4	
HOP-92	1.270	1.828	1.841	1.635	1.163	0.947	0.989	102	65	-8	-25	-22	1.62E-7	7.68E-7	> 1.00E-4	
NCI-H226	1.017	1.836	1.773	1.596	1.462	1.271	1.268	92	71	54	31	31	1.52E-6	> 1.00E-4	> 1.00E-4	
NCI-H23	0.524	1.714	1.538	0.887	0.893	0.862	0.846	85	31	31	28	27	4.40E-8	> 1.00E-4	> 1.00E-4	
NCI-H322M	0.885	2.134	1.951	1.538	1.514	1.291	1.429	85	52	50	32	44	1.04E-6	> 1.00E-4	> 1.00E-4	
NCI-H460	0.306	2.723	2.794	1.337	0.688	0.643	0.569	103	43	16	14	11	7.55E-8	> 1.00E-4	> 1.00E-4	
NCI-H522	0.925	1.998	1.940	1.523	1.298	1.148	1.116	95	56	35	21	18	1.87E-7	> 1.00E-4	> 1.00E-4	
Colon Cancer																
COLO 205	0.469	1.611	1.624	0.811	0.457	0.375	0.405	101	30	-3	-20	-14	5.22E-8	8.28E-7	> 1.00E-4	
HCC-2998	0.837	2.902	2.629	1.785	1.144	0.997	1.141	87	46	15	8	15	7.94E-8	> 1.00E-4	> 1.00E-4	
HCT-116	0.561	3.003	2.854	1.426	1.009	0.708	0.958	94	35	18	6	16	5.63E-8	> 1.00E-4	> 1.00E-4	
HCT-15	0.384	2.295	2.185	1.396	0.803	0.674	0.655	94	53	22	15	14	1.24E-7	> 1.00E-4	> 1.00E-4	
HT29	0.313	1.678	1.388	0.441	0.372	0.356	0.329	79	9	4	3	1	2.60E-8	> 1.00E-4	> 1.00E-4	
KM12	0.500	2.756	2.584	1.354	0.640	0.616	0.594	92	38	6	5	4	5.99E-8	> 1.00E-4	> 1.00E-4	
SW-620	0.291	1.818	1.811	0.806	0.613	0.593	0.526	100	34	21	20	15	5.66E-8	> 1.00E-4	> 1.00E-4	
CNS Cancer																
SF-268	0.578	1.959	1.927	1.614	1.222	1.040	0.875	98	75	47	33	21	7.61E-7	> 1.00E-4	> 1.00E-4	
SF-295	0.996	2.748	2.682	2.485	1.317	0.600	0.738	96	85	18	-40	-26	3.35E-7	2.06E-6	> 1.00E-4	
SF-539	1.027	2.919	2.944	2.603	1.817	1.425	1.489	101	83	42	21	24	6.34E-7	> 1.00E-4	> 1.00E-4	
SNB-19	0.423	1.698	1.711	1.342	0.973	0.753	0.766	101	72	43	26	27	5.77E-7	> 1.00E-4	> 1.00E-4	
SNB-75	0.868	1.716	1.681	1.693	1.387	1.005	0.801	96	97	61	16	-8	1.77E-6	4.74E-5	> 1.00E-4	
U251	0.434	1.818	2.071	1.635	0.944	0.668	0.613	118	87	37	17	13	5.45E-7	> 1.00E-4	> 1.00E-4	
Melanoma																
LOX IMVI	0.380	2.440	2.343	1.359	0.397	0.299	0.330	95	48	1	-21	-13	8.88E-8	1.09E-6	> 1.00E-4	
MALME-3M	0.623	1.099	1.161	0.871	0.628	0.593	0.597	113	52	1	-5	-4	1.10E-7	1.45E-6	> 1.00E-4	
M14	0.574	2.167	2.084	1.111	0.993	0.554	0.763	95	34	26	-4	12	5.41E-8	.	> 1.00E-4	
MDA-MB-435	0.465	2.334	2.355	1.232	0.910	0.678	0.674	101	41	24	11	11	7.09E-8	> 1.00E-4	> 1.00E-4	
SK-MEL-2	0.978	1.975	1.969	1.635	1.326	1.228	1.179	99	66	35	25	20	3.26E-7	> 1.00E-4	> 1.00E-4	
SK-MEL-28	0.742	2.170	2.138	1.577	1.158	0.963	1.083	98	58	29	15	24	1.95E-7	> 1.00E-4	> 1.00E-4	
SK-MEL-5	0.770	2.790	2.729	2.104	1.241	0.508	0.943	97	66	23	-34	9	2.37E-7	.	> 1.00E-4	
UACC-257	1.062	2.047	2.080	1.886	1.274	1.289	1.298	103	84	22	23	24	3.48E-7	> 1.00E-4	> 1.00E-4	
UACC-62	0.761	2.872	2.726	1.822	1.006	0.726	0.881	93	50	12	-5	6	1.01E-7	.	> 1.00E-4	
Ovarian Cancer																
IGROV1	0.585	1.949	1.940	1.247	0.845	0.854	0.785	99	49	19	20	15	9.35E-8	> 1.00E-4	> 1.00E-4	
OVCAR-3	0.533	1.878	1.915	1.647	0.908	0.675	0.675	103	83	28	11	11	3.96E-7	> 1.00E-4	> 1.00E-4	
OVCAR-4	0.776	1.596	1.617	1.535	1.345	1.246	1.202	103	93	69	57	52	> 1.00E-4	> 1.00E-4	> 1.00E-4	
OVCAR-5	0.742	1.941	1.808	1.688	1.276	1.129	1.104	89	79	44	32	30	6.91E-7	> 1.00E-4	> 1.00E-4	
OVCAR-8	0.509	2.011	2.081	1.862	1.029	0.866	0.825	105	90	35	24	21	5.27E-7	> 1.00E-4	> 1.00E-4	
NCI/ADR-RES	0.502	1.786	1.748	1.729	1.031	0.745	0.754	97	96	41	19	20	6.89E-7	> 1.00E-4	> 1.00E-4	
SK-OV-3	0.912	2.098	2.023	1.556	1.006	1.008	0.957	94	54	8	8	4	1.24E-7	> 1.00E-4	> 1.00E-4	
Renal Cancer																
786-0	0.656	2.224	2.151	1.716	1.338	0.772	0.876	95	68	44	7	14	5.38E-7	> 1.00E-4	> 1.00E-4	
A498	1.368	2.011	2.083	1.846	1.586	1.451	1.484	111	74	34	13	18	3.98E-7	> 1.00E-4	> 1.00E-4	
ACHN	0.446	2.030	2.005	1.105	0.848	0.702	0.750	98	42	25	16	19	7.12E-8	> 1.00E-4	> 1.00E-4	
RXF 393	0.773	1.581	1.571	1.157	1.027	0.466	0.556	99	47	31	-40	-28	8.92E-8	2.76E-6	> 1.00E-4	
SN12C	0.516	2.191	1.976	1.233	1.107	1.035	0.992	87	43	35	31	28	6.89E-8	> 1.00E-4	> 1.00E-4	
TK-10	1.079	2.167	2.126	1.617	1.376	1.384	1.309	96	49	27	28	21	9.71E-8	> 1.00E-4	> 1.00E-4	
UO-31	0.629	1.667	1.508	1.140	0.858	0.715	0.777	85	49	22	8	14	9.51E-8	> 1.00E-4	> 1.00E-4	
Prostate Cancer																
PC-3	0.617	1.674	1.545	1.217	1.116	0.931	0.884	88	57	47	30	25	5.09E-7	> 1.00E-4	> 1.00E-4	
DU-145	0.388	1.648	1.558	0.757	0.811	0.473	0.449	93	29	34	7	5	4.72E-8	> 1.00E-4	> 1.00E-4	
Breast Cancer																
MCF7	0.444	2.102	1.873	1.005	0.898	0.766	0.671	86	34	27	19	14	4.91E-8	> 1.00E-4	> 1.00E-4	
MDA-MB-231/ATCC	0.547	1.393	1.473	1.063	0.732	0.500	0.531	109	61	22	-9	-3	1.90E-7	5.22E-6	> 1.00E-4	
HS 578T	1.100	2.097	2.181	1.892	1.610	1.345	1.381	108	79	51	25	28	1.11E-6	> 1.00E-4	> 1.00E-4	
BT-549	1.182	2.414	2.399	1.746	1.775	0.999	1.399	99	46	48	-15	18	8.32E-8	.	> 1.00E-4	
T-47D	0.873	1.590	1.514	1.190	1.023	1.005	1.024	89	44	21	18	21	7.45E-8	> 1.00E-4	> 1.00E-4	
MDA-MB-468	0.706	1.406	1.372	0.895	0.697	0.719	0.693	95	27	-1	2	-2	4.59E-8	.	> 1.00E-4	

Figure S4: NCI-60 Cell screening results with GI50 values of a pan-inhibitor of Hsp90, **17-AAG** (five-dose experiment) **Source:** NCI DTP

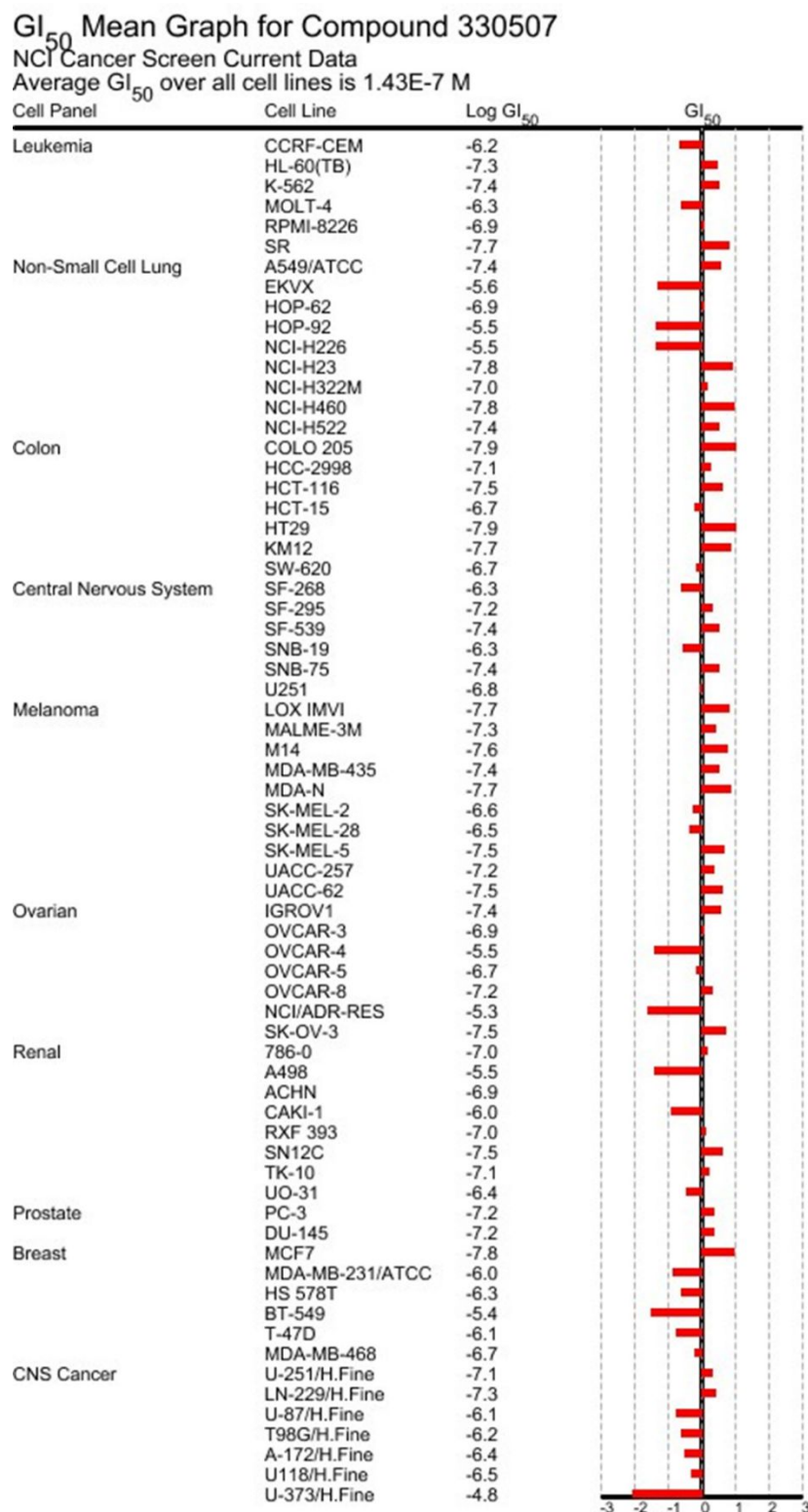


Figure S5: NCI-60 Cell screening results with **6a** (10 μ M)

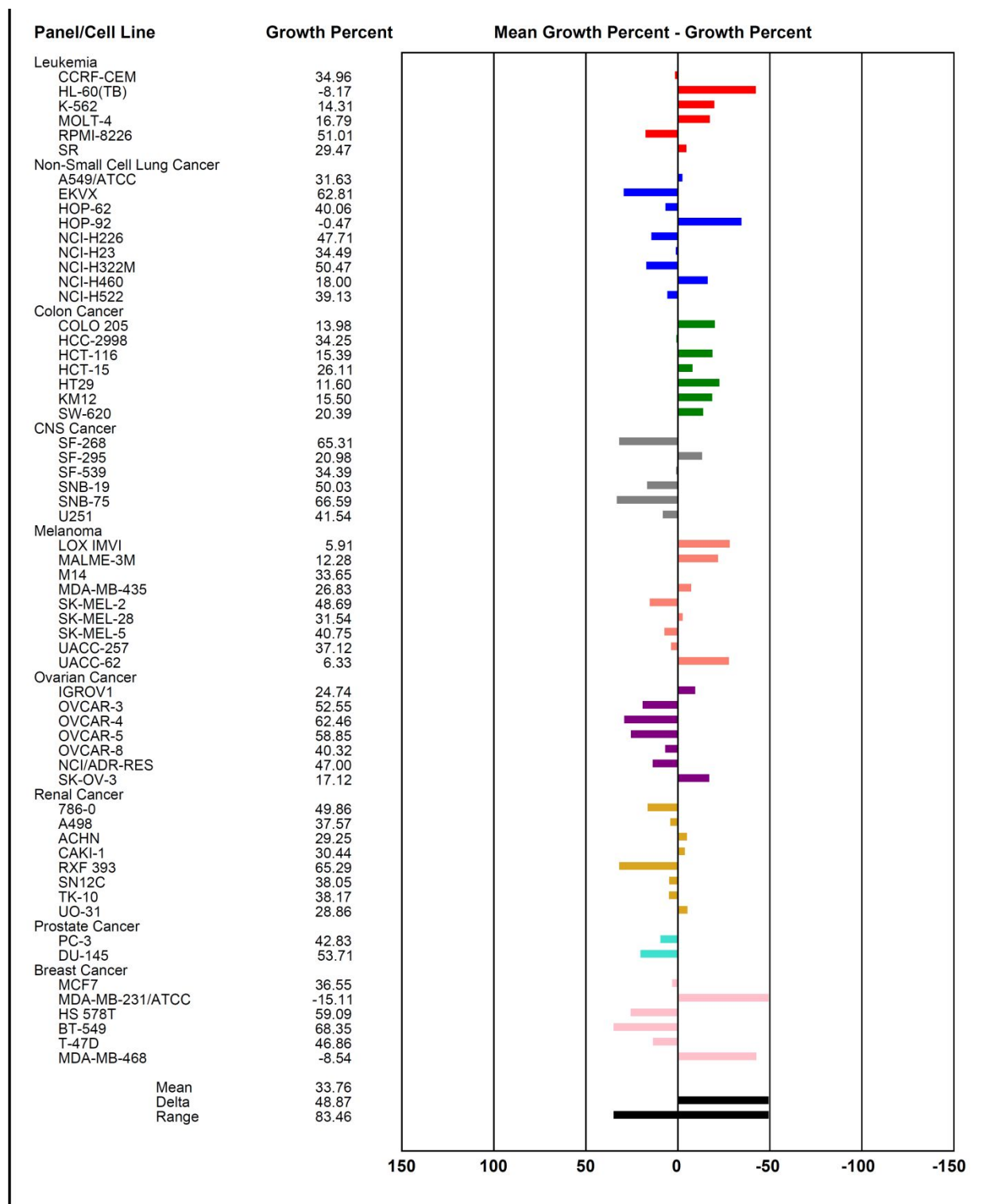


Table S1: IC₅₀ of **4d**, **5b** and **6a** for Grp94 and Trap1.

Compound	Grp94 IC50 (μM)	Trap1 IC50 (μM)
4d	>50	>50
5b	>50	50
6a	>50	>50

Table S2: GI50 values against Hct116 and Hek293 cells

Compound	Hct116 GI50 (μM)	Hek293 GI50 (μM)
4d	0.056	0.587± 0.02
5b	>5	2.338± 0.33
6a	0.427	0.943± 0.07
Geldanamycin	0.0158	0.0246