

Novel synthesized *N*-ethyl-piperazinyl-amides of C2-substituted oleanonic and ursonic acids exhibit cytotoxic effects through apoptotic cell death regulation

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Abstract

A series of novel hybride chalcone *N*-ethyl-piperazinyl amide derivatives of oleanonic and ursonic acids were synthesized and their antiproliferative potential was *in vitro* evaluated against the NCI-60 cancer cell line panel. Compounds 4 and 6 exhibited the highest overall antiproliferative activity with GI₅₀ values in some cases reaching nanomolar values. Thus, the two compounds were further assessed in detail in order to identify a possible antiproliferative mechanism of action. DAPI staining revealed that both compounds induced nuclei condensation and overall cell morphological changes consistent with apoptotic cell death. rtPCR analysis showed that up-regulation of pro-apoptotic Bak gene combined with the down-regulation of the pro-survival Bcl-XL and Bcl-2 genes caused altered ratios between the proapoptotic and antiapoptotic proteins' levels, leading to overall induced apoptosis. Molecular docking analysis revealed that both compounds exhibited high scores for Bcl-XL inhibition suggesting that compounds may induce apoptotic cell death through targeted anti-apoptotic protein inhibition, as well.

Key words: oleanonic acid, ursonic acid, arylidene, piperazine, anticancer activity, NCI-60, antiproliferative activity, CAM assay, rtPCR, molecular docking

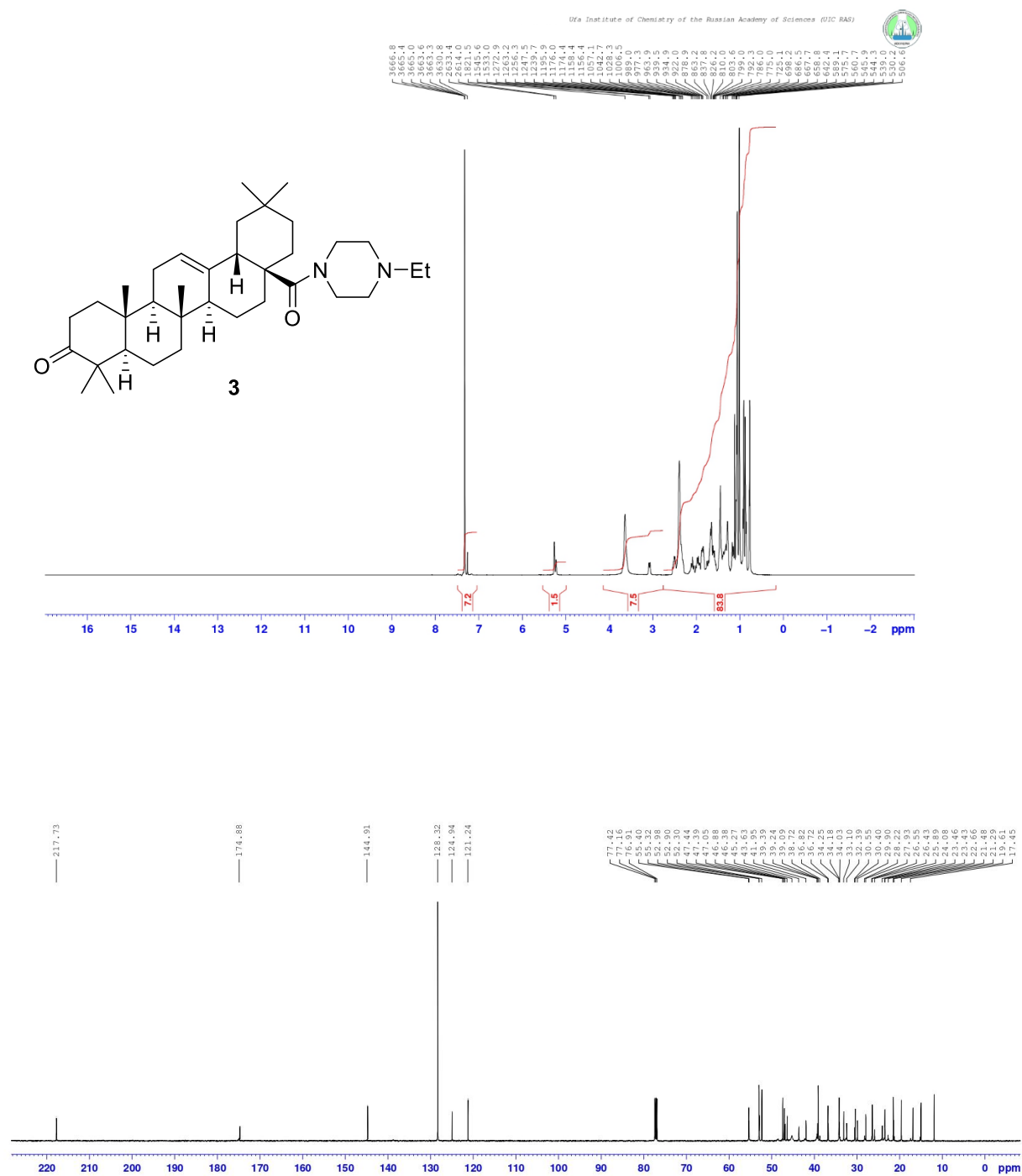
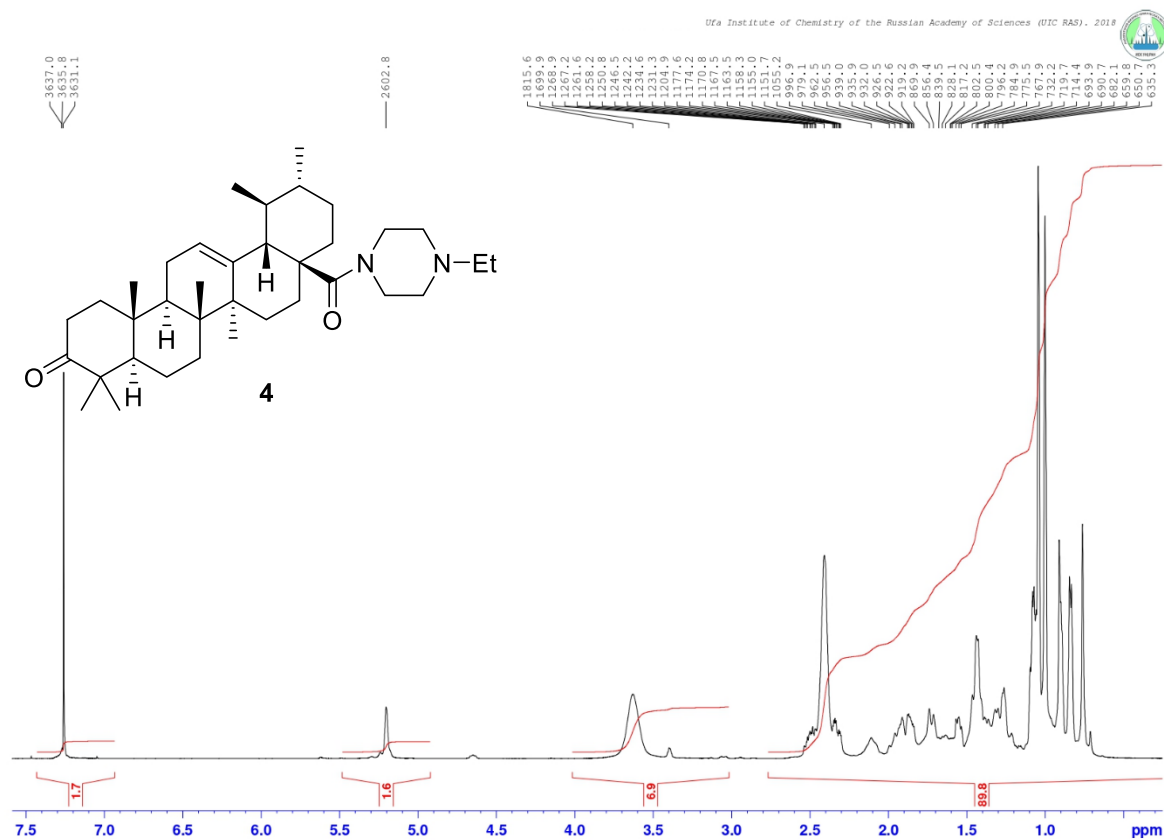


Figure S1. ¹H NMR and ¹³C NMR spectra of compound **3**



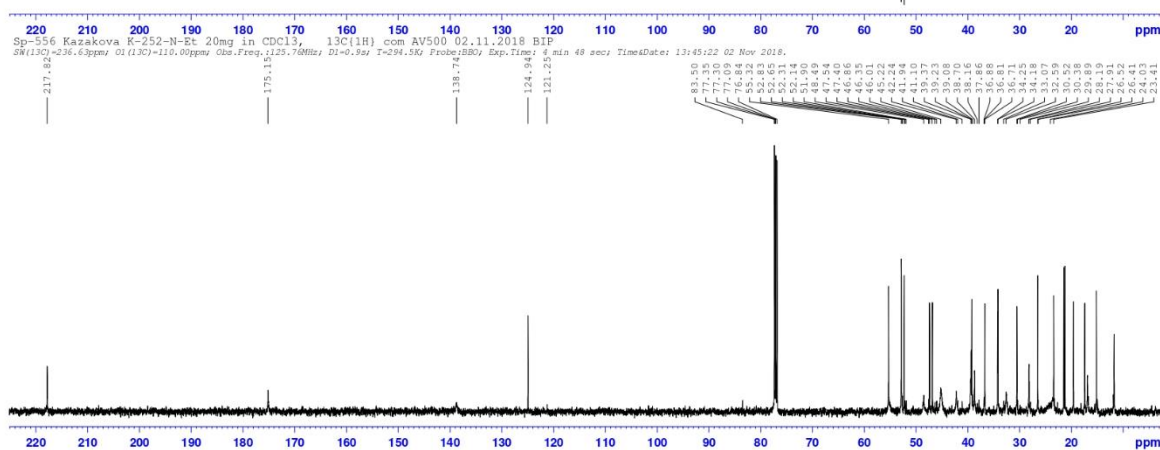


Figure S2. ^1H NMR and ^{13}C NMR spectra of compound **4**.



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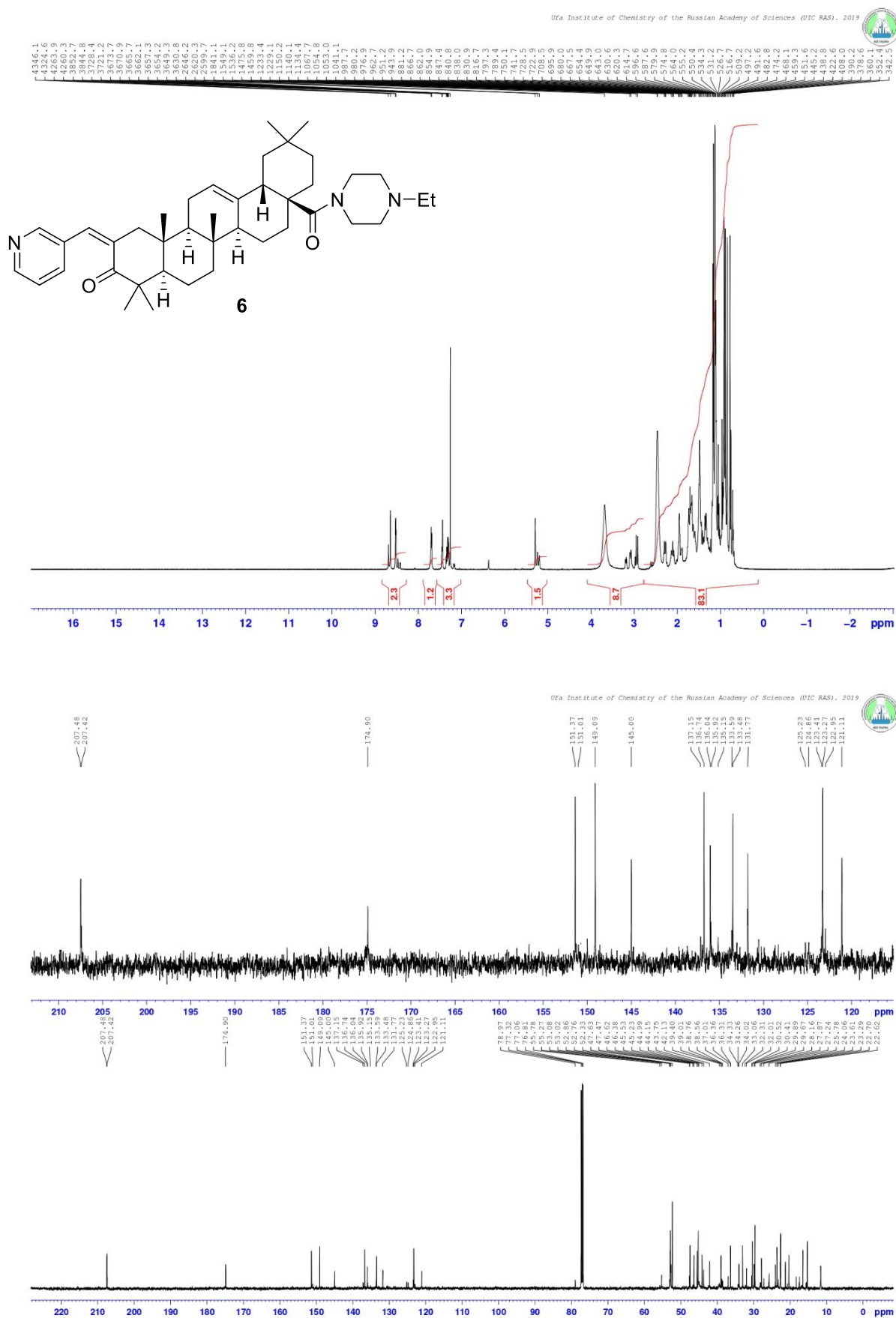
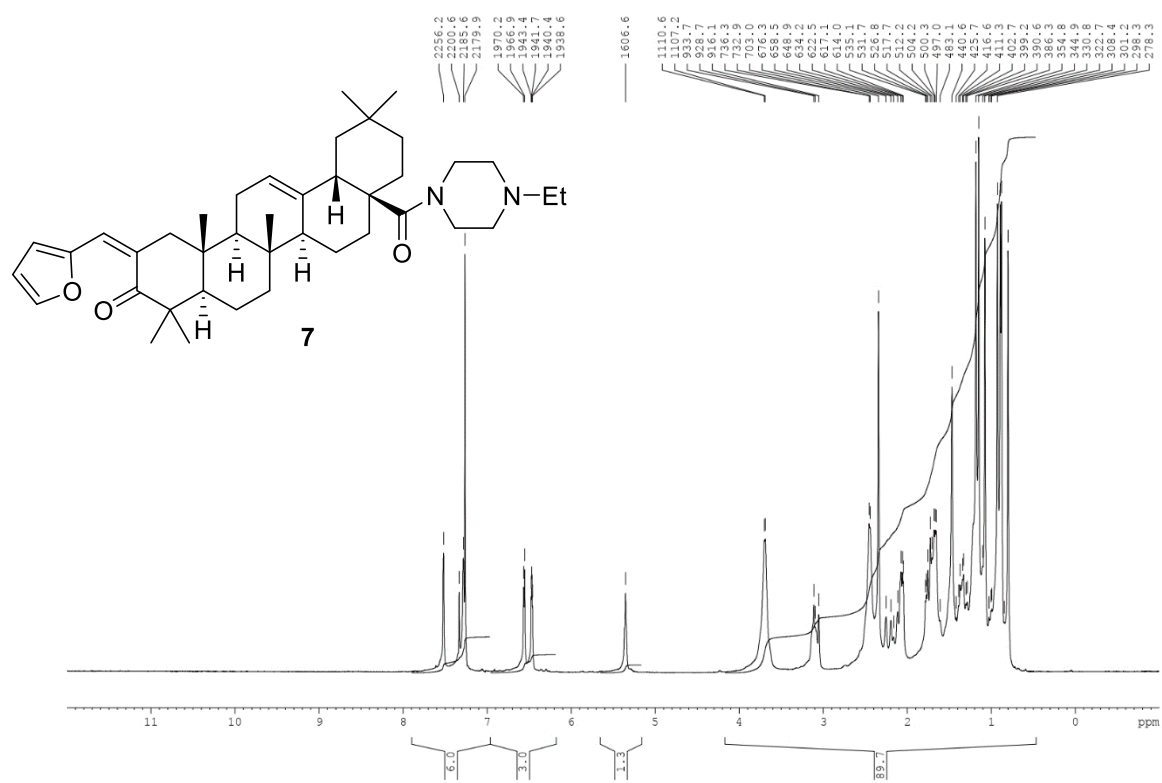


Figure S4. ¹H NMR and ¹³C NMR spectra of compound 6



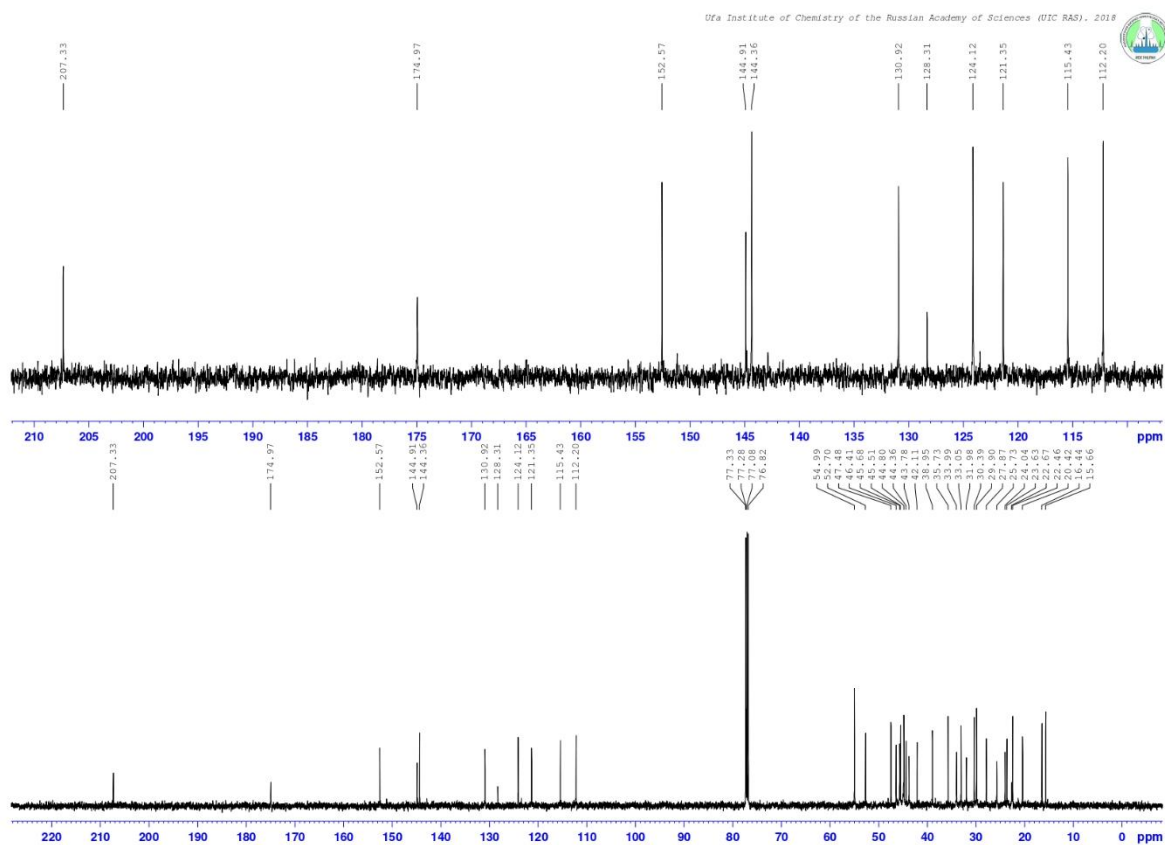
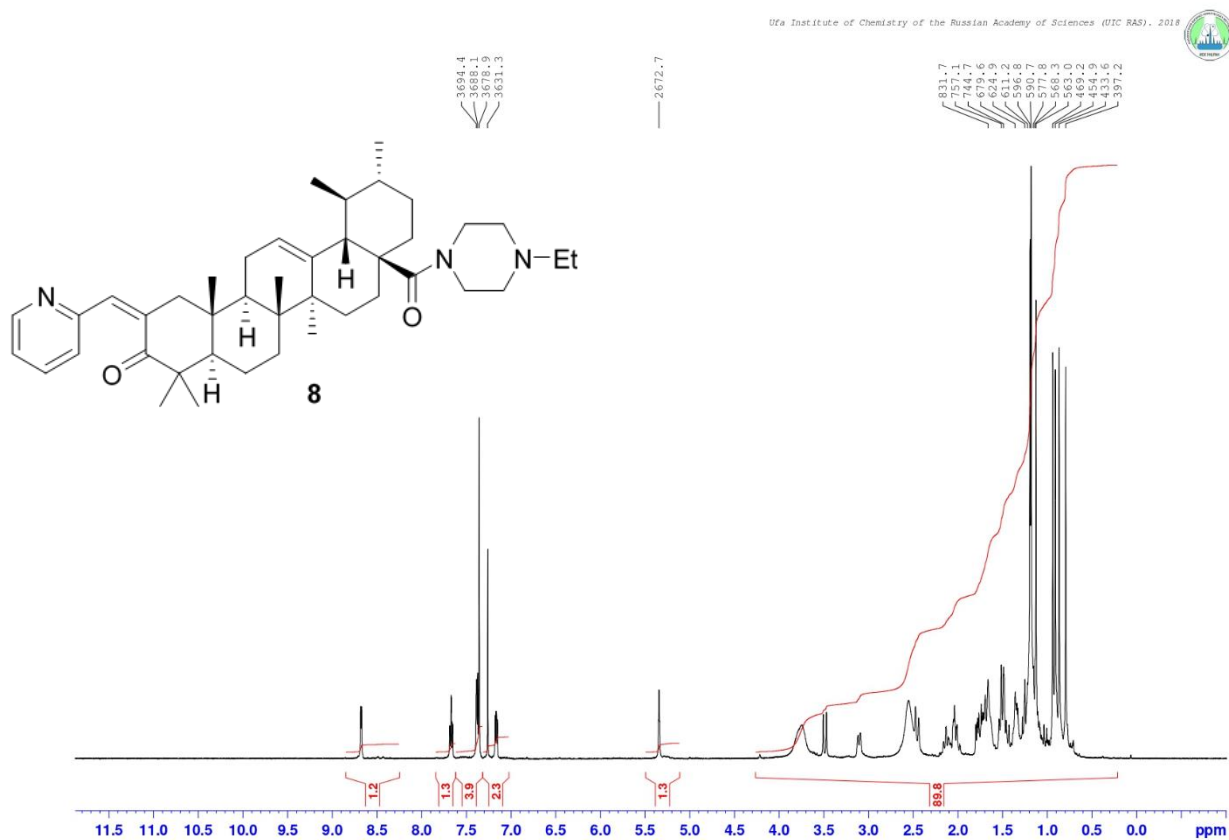


Figure S5. ^1H NMR and ^{13}C NMR spectra of compound **7**



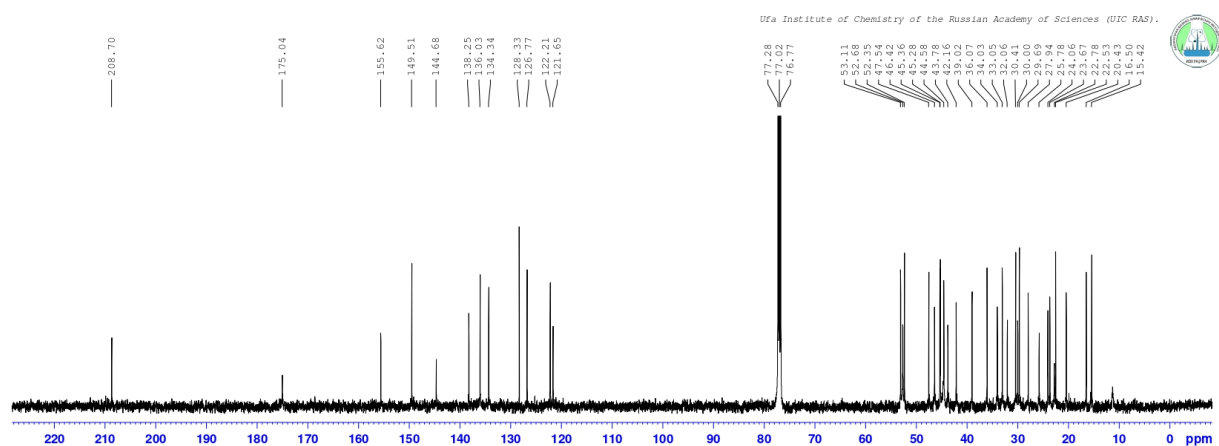
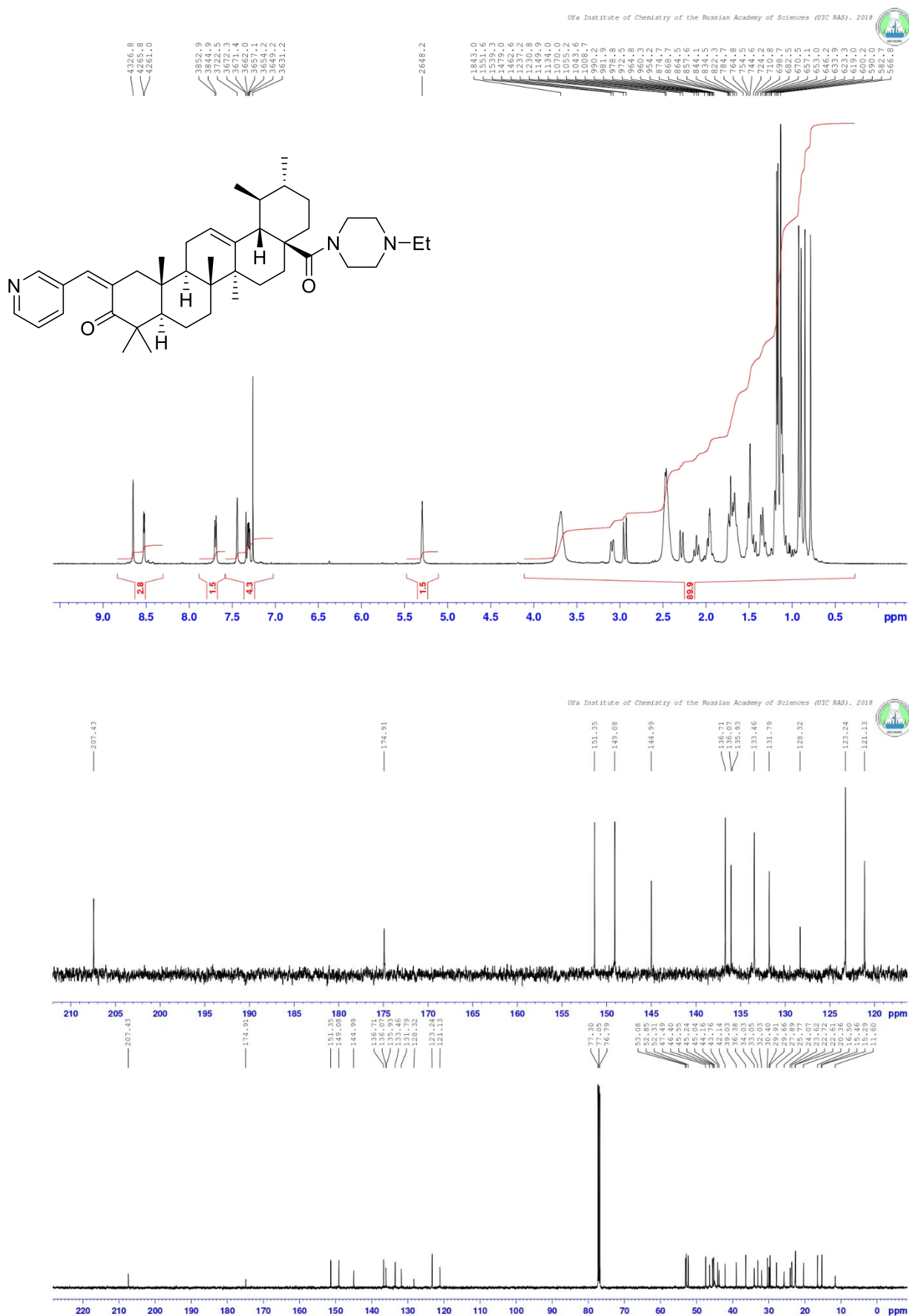


Figure S6. ^1H NMR and ^{13}C NMR spectra of compound **8**



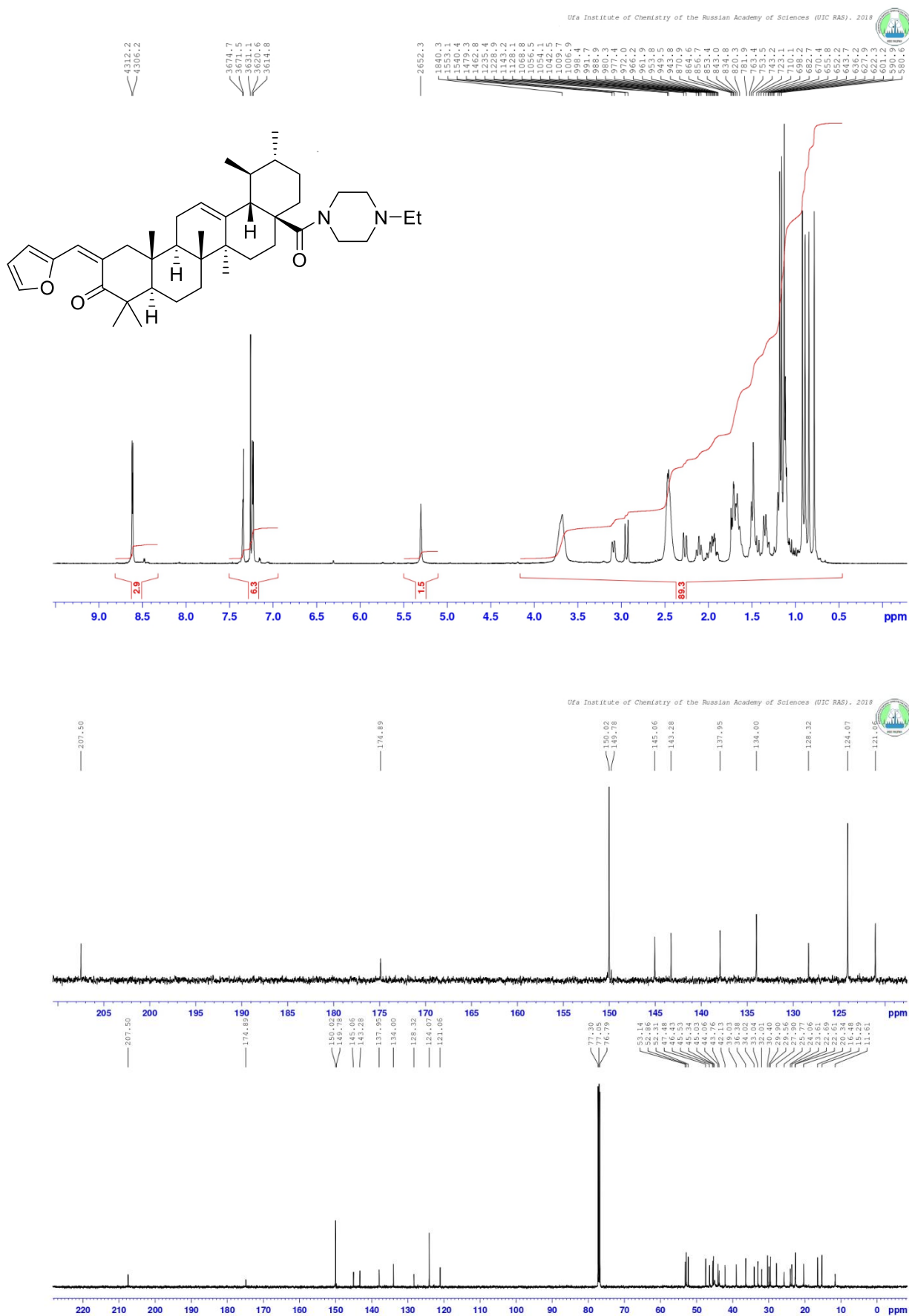


Figure S8. ¹H NMR and ¹³C NMR spectra of compound **10**

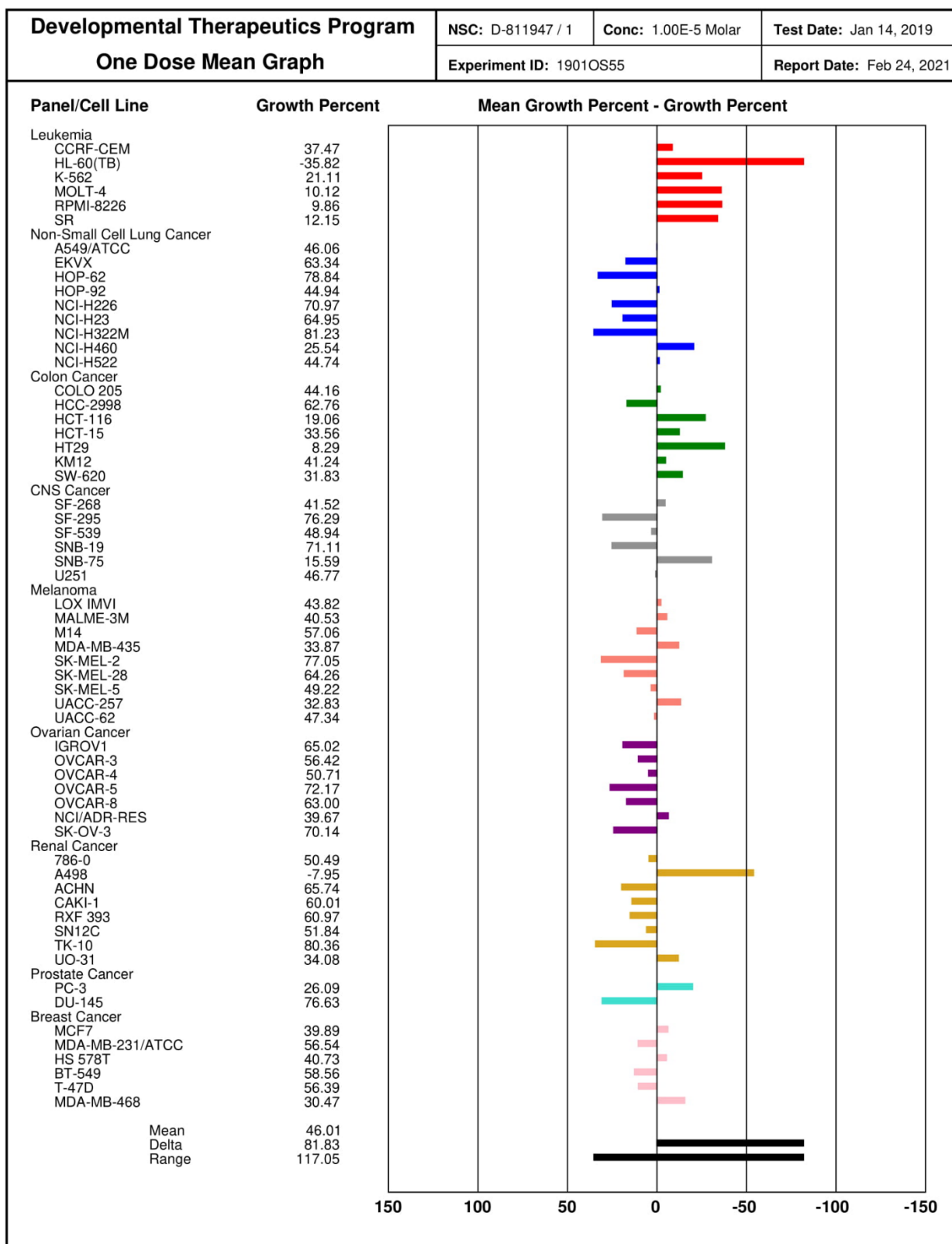


Figure S9. NCI-60 one dose data for compound **3**

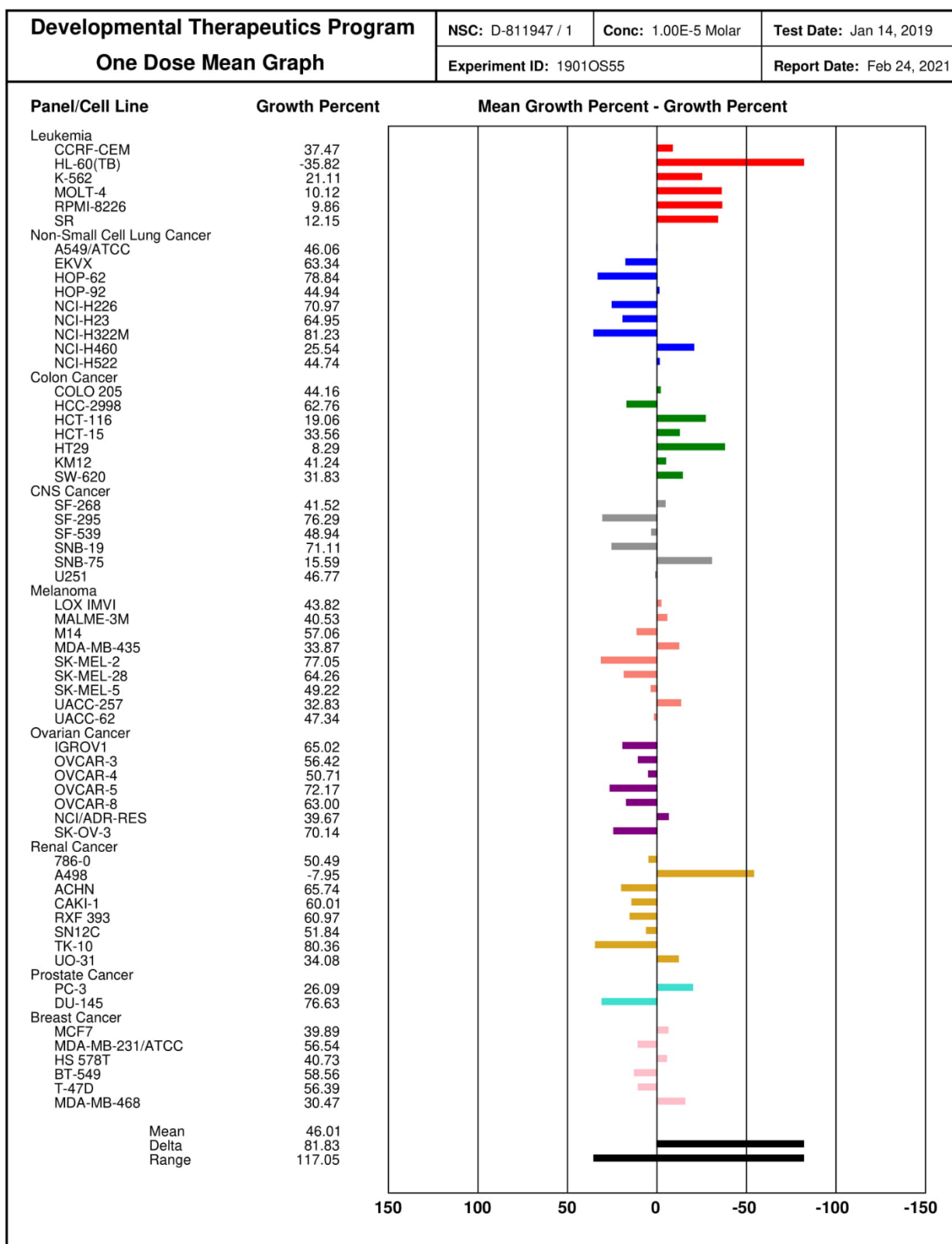


Figure S10. NCI-60 one dose data for compound **4**

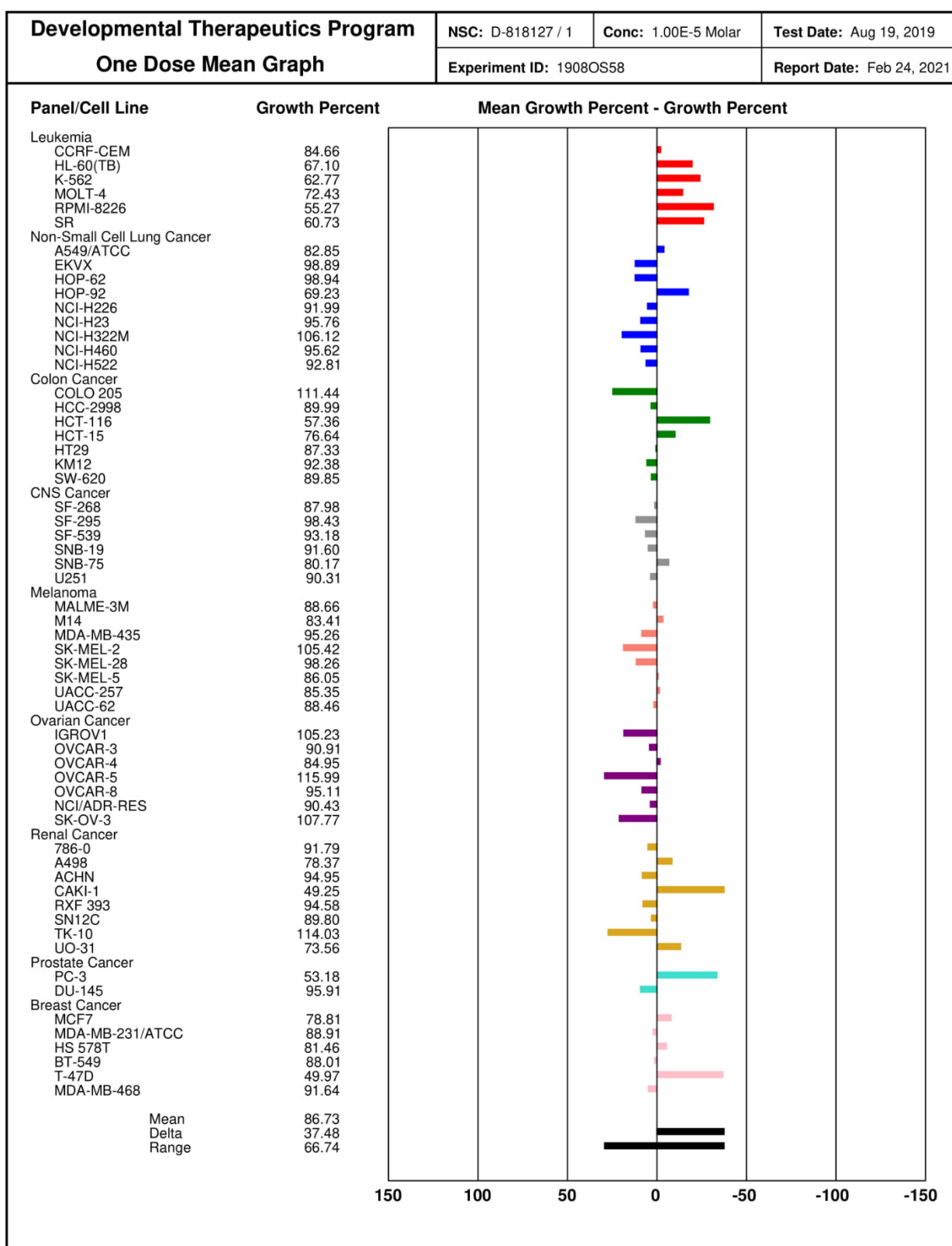


Figure S11. NCI-60 one dose data for compound **5**

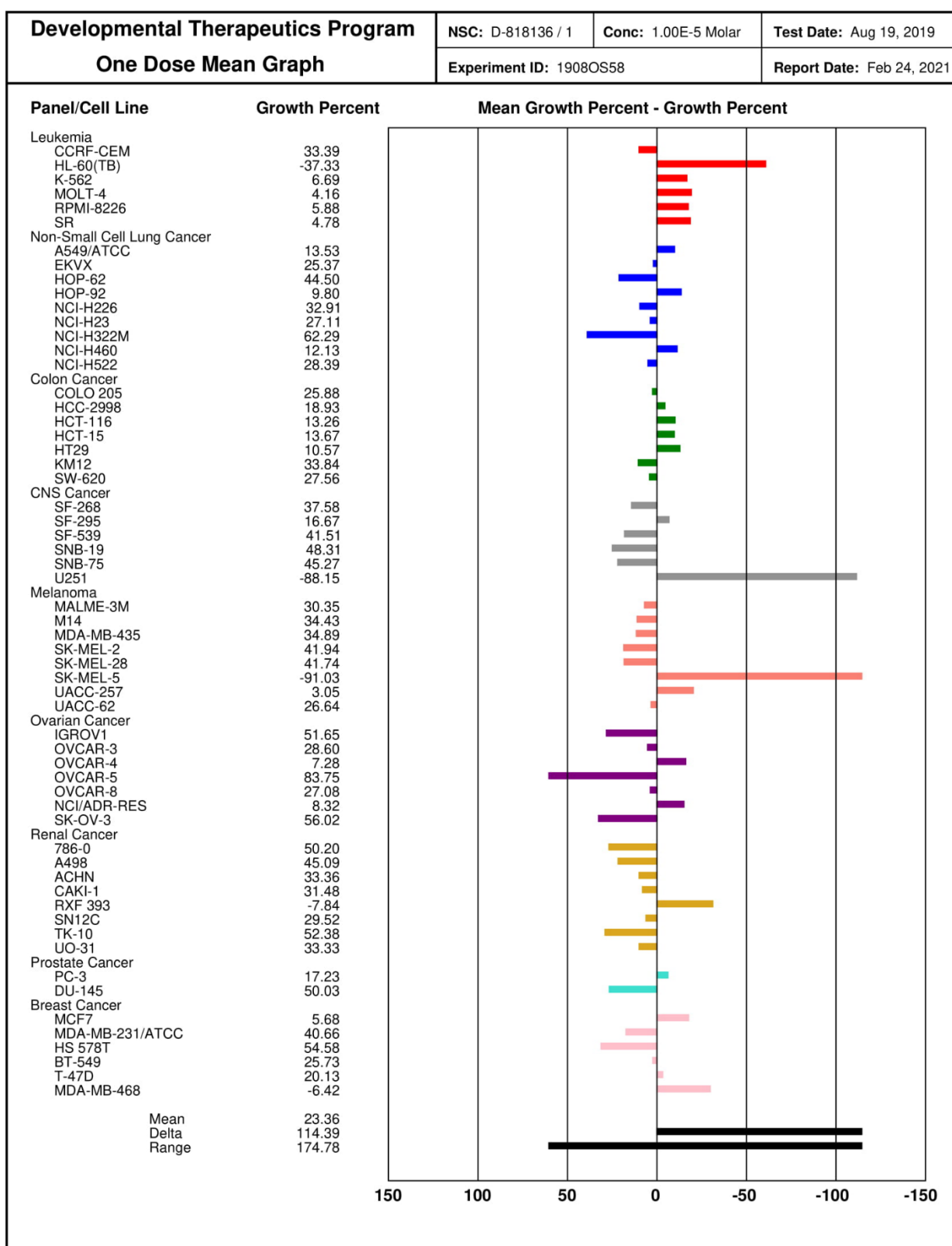


Figure S12. NCI-60 one dose data for compound **6**

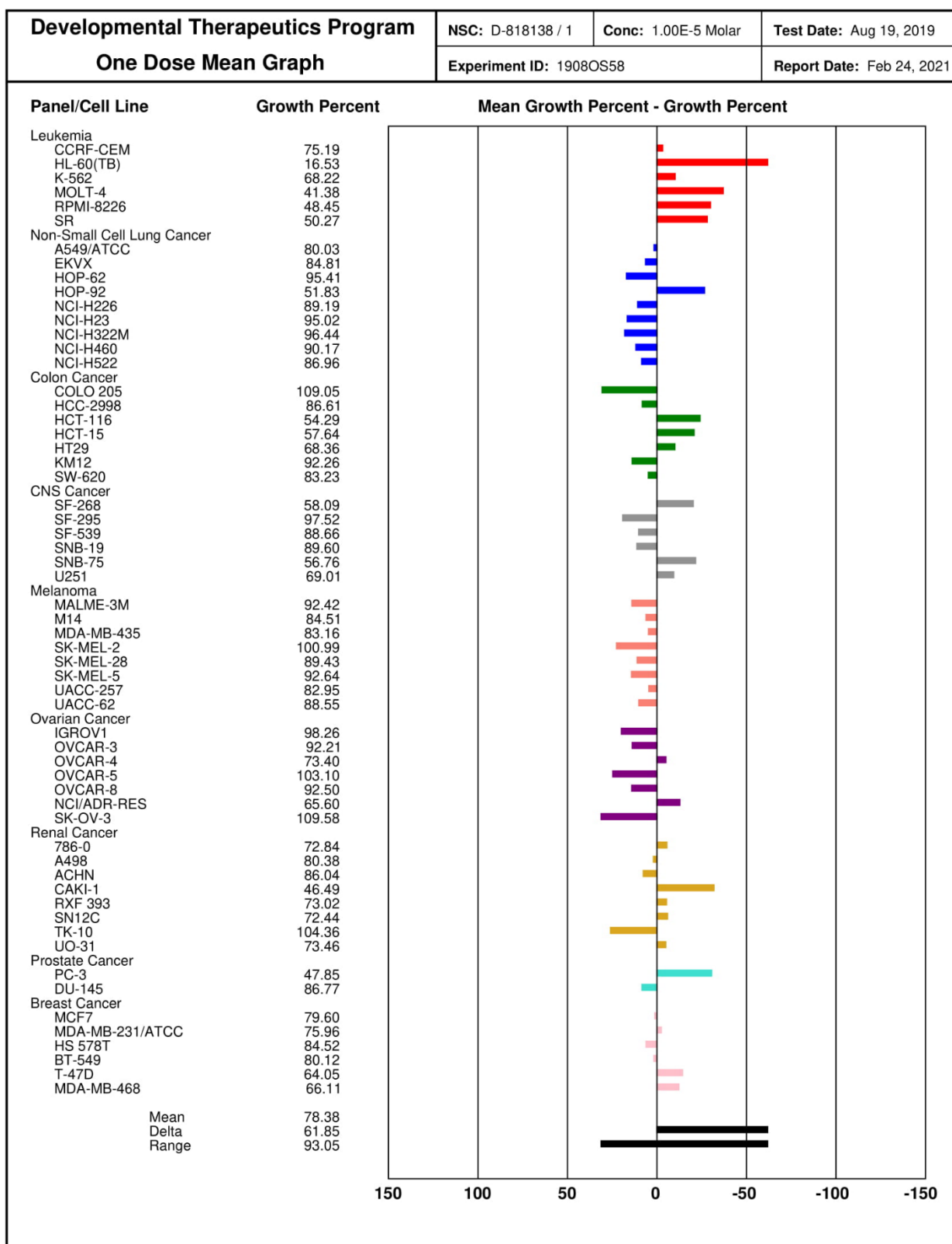


Figure S13. NCI-60 one dose data for compound **7**

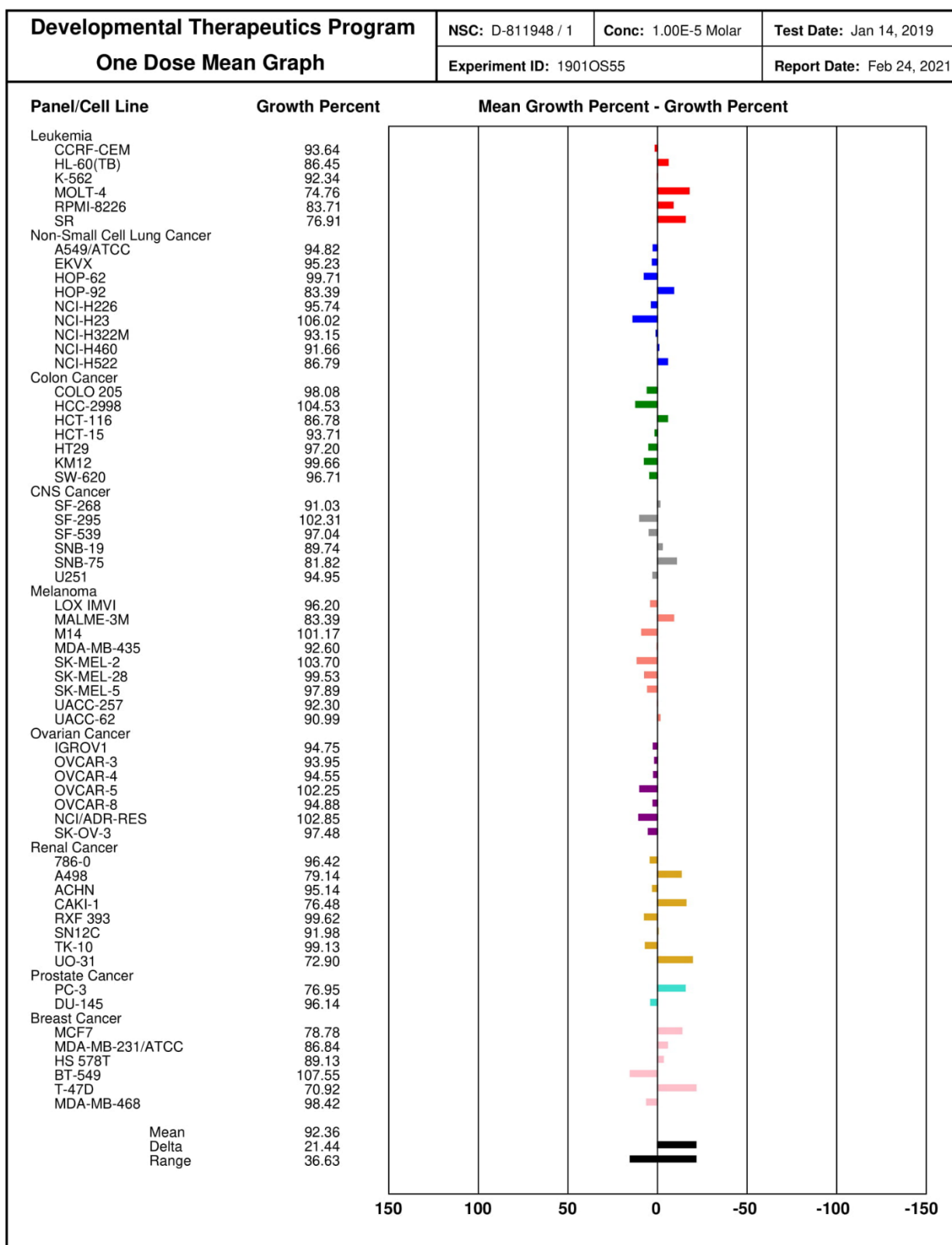


Figure S14. NCI-60 one dose data for compound **8**

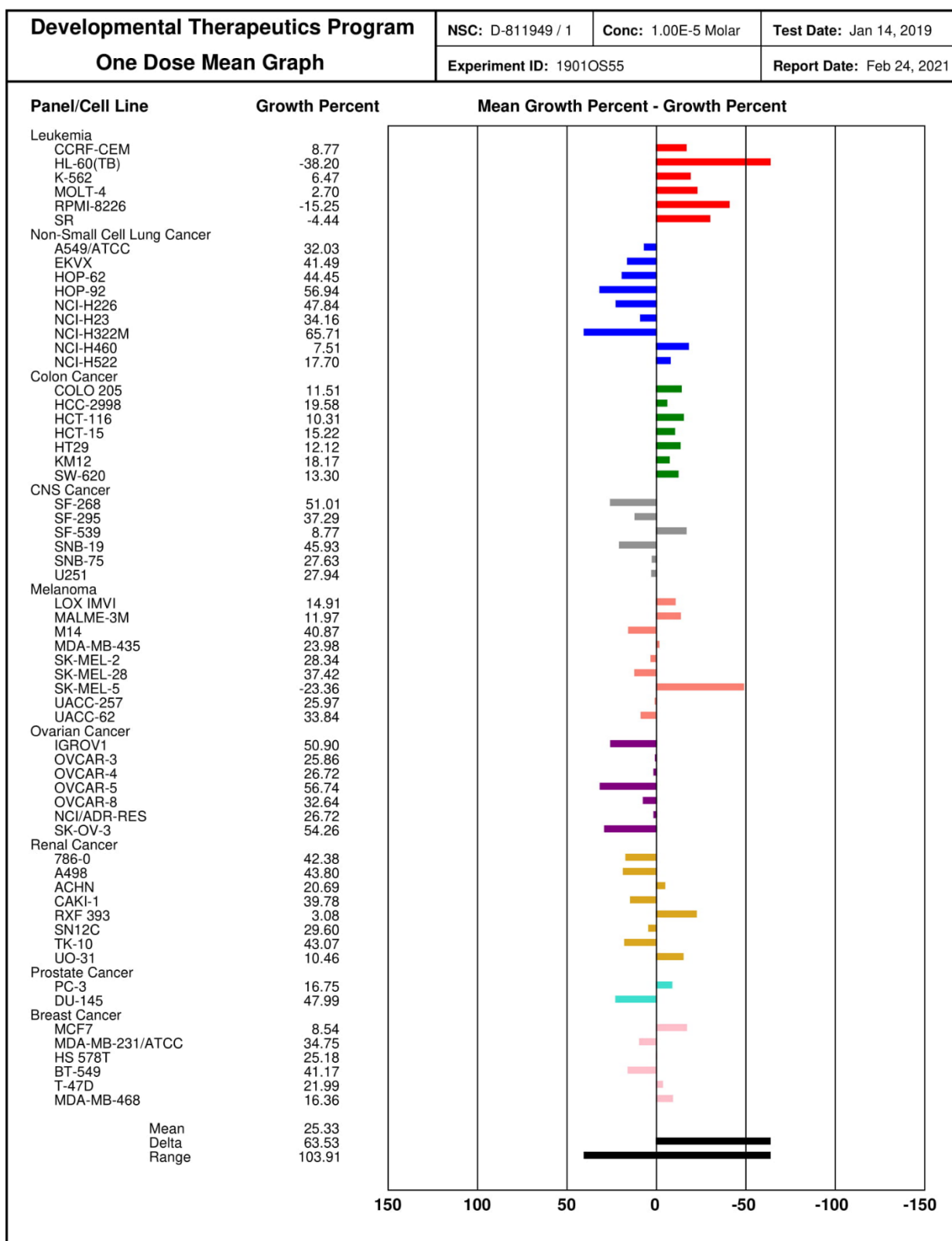


Figure S15. NCI-60 one dose data for compound **9**

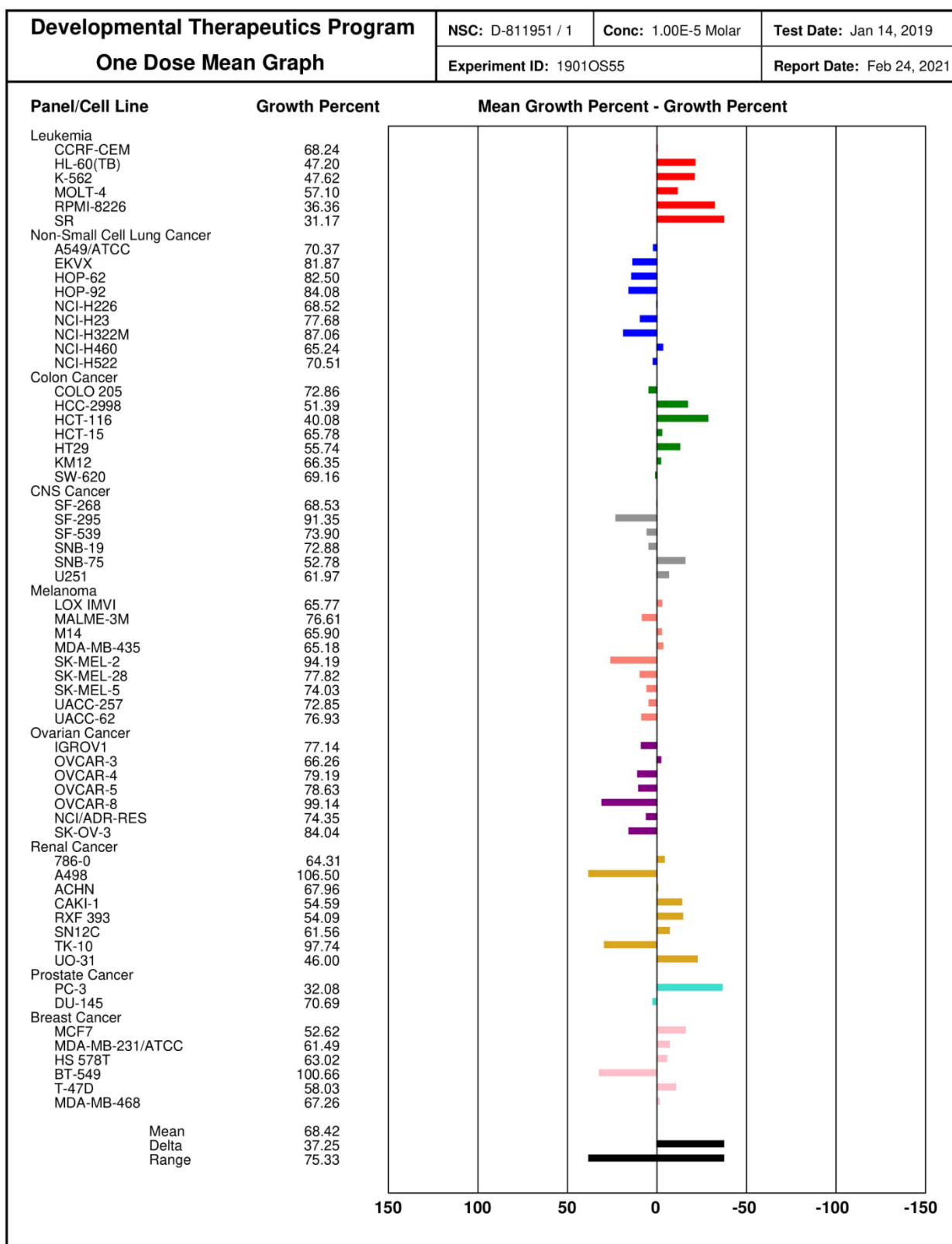
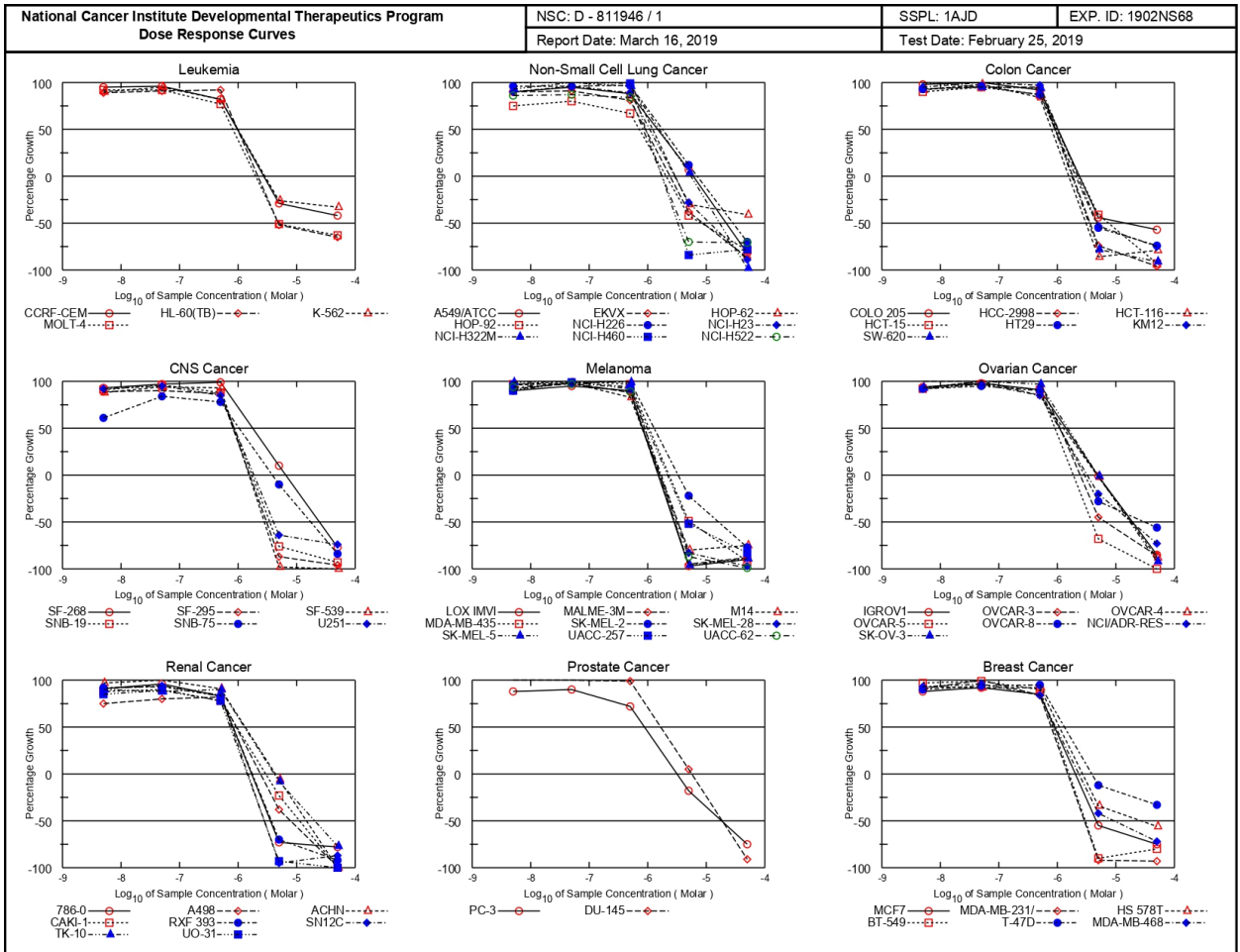
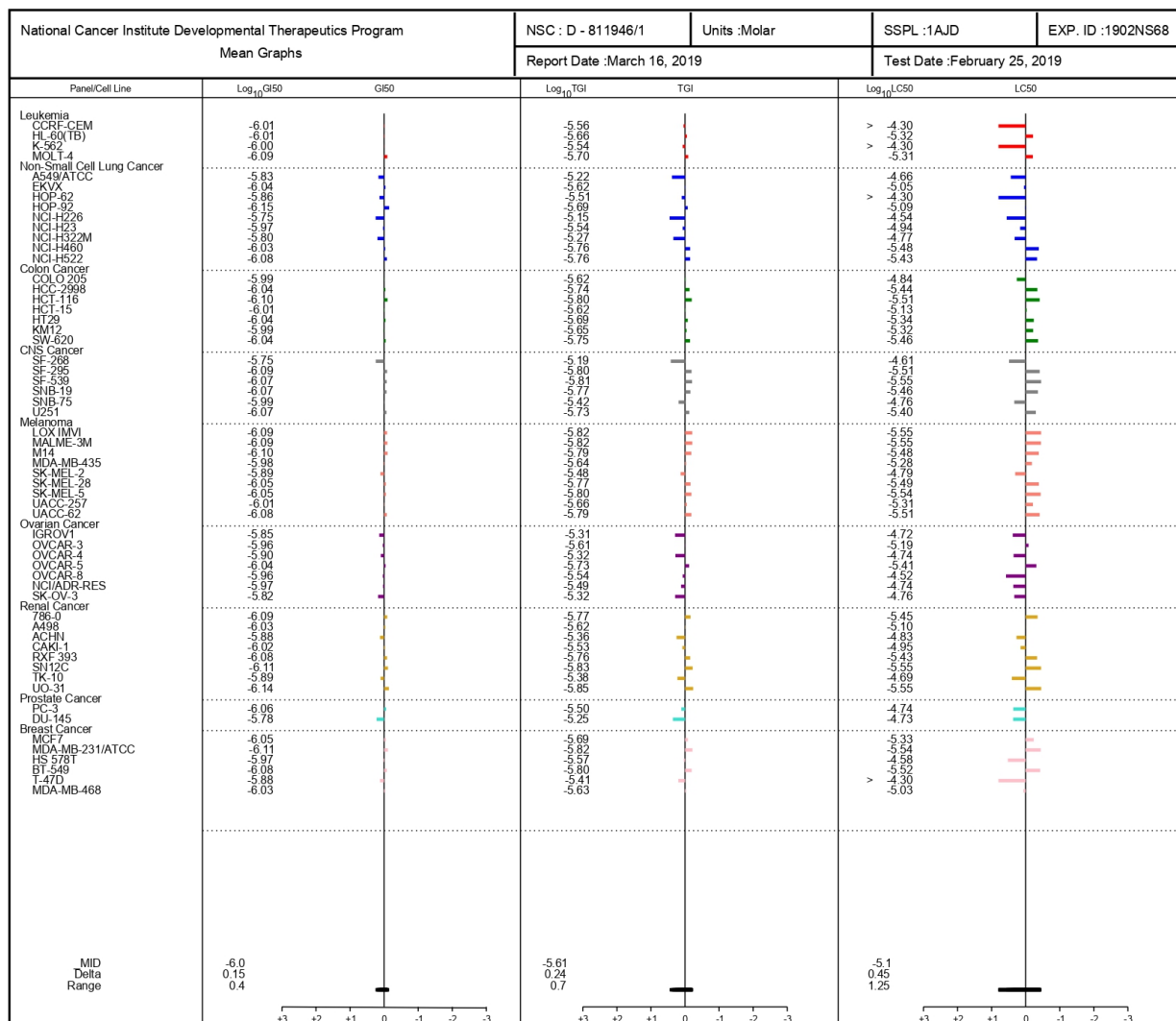


Figure S16. NCI-60 one dose data for compound **10**



**National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results**

NSC : D - 811946 / 1			Experiment ID : 1902NS68										Test Type : 08		Units : Molar	
Report Date : March 16, 2019			Test Date : February 25, 2019										QNS :		MC :	
COMI : PAV-114			Stain Reagent : SRB Dual-Pass Related										SSPL : 1AJD			
Log10 Concentration																
Panel/Cell Line	Time Zero	Ctrl	-8.3	-7.3	-6.3	-5.3	-4.3	-8.3	-7.3	-6.3	-5.3	-4.3	GI50	TGI	LC50	
Leukemia																
CCRF-CEM	0.479	2.636	2.519	2.561	2.257	0.340	0.278	95	96	82	-29	-42	9.76E-7	2.74E-6	> 5.00E-5	
HL-60(TB)	0.854	3.137	2.894	2.941	2.958	0.410	0.300	89	91	92	-52	-65	9.80E-7	2.18E-6	4.84E-6	
K-562	0.266	2.149	1.964	2.060	1.814	0.198	0.179	90	95	82	-26	-33	9.94E-7	2.90E-6	> 5.00E-5	
MOLT-4	0.712	2.871	2.703	2.709	2.368	0.348	0.267	92	92	77	-51	-63	8.09E-7	1.99E-6	4.90E-6	
Non-Small Cell Lung Cancer																
A549/ATCC	0.377	1.875	1.719	1.806	1.698	0.481	0.068	90	95	88	7	-82	1.47E-6	5.98E-6	2.18E-5	
EKVX	0.541	1.478	1.389	1.390	1.302	0.338	0.071	90	91	81	-38	-87	9.16E-7	2.42E-6	8.95E-6	
HOP-62	0.545	1.436	1.432	1.496	1.549	0.383	0.321	100	107	113	-30	-41	1.38E-6	3.09E-6	> 5.00E-5	
HOP-92	1.331	1.918	1.772	1.800	1.723	0.774	0.273	75	80	67	-42	-79	7.13E-7	2.06E-6	8.22E-6	
NCI-H226	1.088	1.968	1.932	1.935	1.941	1.194	0.330	96	96	97	12	-70	1.78E-6	7.01E-6	2.87E-5	
NCI-H23	0.618	1.929	1.798	1.881	1.788	0.447	0.069	90	96	89	-28	-89	1.08E-6	2.90E-6	1.16E-5	
NCI-H322M	0.722	1.960	1.880	1.956	1.911	0.767	0.013	93	100	96	4	-98	1.57E-6	5.43E-6	1.68E-5	
NCI-H460	0.281	2.379	2.396	2.508	2.366	0.046	0.061	101	106	99	-84	-78	9.31E-7	1.75E-6	3.27E-6	
NCI-H522	0.575	1.516	1.382	1.395	1.366	0.170	0.165	86	87	84	-70	-71	8.31E-7	1.75E-6	3.69E-6	
Colon Cancer																
COLO 205	0.565	2.235	2.197	2.325	2.098	0.317	0.243	98	105	92	-44	-57	1.02E-6	2.37E-6	1.45E-5	
HCC-2998	0.399	1.355	1.291	1.309	1.307	0.104	0.017	93	95	95	-74	-96	9.22E-7	1.82E-6	3.60E-6	
HCT-116	0.281	2.368	2.203	2.317	2.045	0.041	0.060	92	98	84	-86	-79	7.98E-7	1.57E-6	3.09E-6	
HCT-15	0.330	2.258	2.072	2.166	2.016	0.194	0.024	90	95	87	-41	-93	9.77E-7	2.39E-6	7.41E-6	
HT29	0.343	1.887	1.784	1.823	1.690	0.154	0.088	93	96	87	-55	-74	9.13E-7	2.05E-6	4.59E-6	
KM12	0.403	1.907	1.834	1.931	1.863	0.191	0.103	95	102	97	-53	-75	1.03E-6	2.22E-6	4.79E-6	
SW-620	0.296	1.795	1.793	1.726	1.707	0.065	0.027	100	95	94	-78	-91	9.02E-7	1.76E-6	3.44E-6	
CNS Cancer																
SF-268	0.554	1.914	1.817	1.873	1.901	0.687	0.129	93	97	99	10	-77	1.77E-6	6.49E-6	2.46E-5	
SF-295	0.604	2.350	2.152	2.176	2.129	0.081	0.023	89	90	87	-87	-96	8.20E-7	1.59E-6	3.08E-6	
SF-539	0.892	2.637	2.433	2.541	2.523	0.016	-0.007	88	94	93	-98	-100	8.43E-7	1.54E-6	2.80E-6	
SNB-19	0.500	2.090	1.946	2.021	1.894	0.121	0.035	91	96	88	-76	-93	8.49E-7	1.72E-6	3.47E-6	
SNB-75	1.185	2.038	1.708	1.900	1.849	1.063	0.187	61	84	78	-10	-84	1.03E-6	3.82E-6	1.72E-5	
U251	0.333	1.723	1.606	1.657	1.509	0.119	0.086	92	95	85	-64	-74	8.54E-7	1.85E-6	4.00E-6	
Melanoma																
LOX IMVI	0.260	1.870	1.708	1.786	1.715	0.008	0.026	90	95	90	-97	-90	8.21E-7	1.52E-6	2.80E-6	
MALME-3M	0.711	1.775	1.695	1.758	1.648	0.035	0.082	92	98	88	-95	-88	8.07E-7	1.51E-6	2.84E-6	
M14	0.415	1.670	1.615	1.648	1.452	0.085	0.103	96	98	83	-80	-75	7.94E-7	1.61E-6	3.28E-6	
MDA-MB-435	0.560	2.344	2.295	2.369	2.289	0.285	0.047	97	101	97	-49	-92	1.05E-6	2.30E-6	5.22E-6	
SK-MEL-2	1.442	2.923	2.874	2.893	2.929	1.131	0.329	97	98	100	-22	-77	1.29E-6	3.33E-6	1.62E-5	
SK-MEL-28	0.632	1.856	1.752	1.849	1.786	0.109	0.016	91	99	94	-83	-97	8.89E-7	1.70E-6	3.27E-6	
SK-MEL-5	0.998	3.271	3.241	3.275	3.241	0.036	0.108	99	100	99	-96	-89	8.88E-7	1.60E-6	2.89E-6	
UACC-257	0.984	2.353	2.214	2.338	2.249	0.477	0.169	90	99	92	-52	-83	9.85E-7	2.19E-6	4.88E-6	
UACC-62	0.847	2.792	2.678	2.731	2.581	0.113	0.006	94	97	89	-87	-99	8.35E-7	1.61E-6	3.09E-6	
Ovarian Cancer																
IGROV1	0.413	1.809	1.728	1.778	1.687	0.408	0.062	94	98	91	-1	-85	1.40E-6	4.85E-6	1.91E-5	
OVCAR-3	0.274	0.830	0.838	0.866	0.828	0.150	0.038	101	106	100	-45	-86	1.10E-6	2.44E-6	6.52E-6	
OVCAR-4	0.583	1.202	1.157	1.250	1.108	0.572	0.069	93	108	85	-2	-88	1.26E-6	4.74E-6	1.80E-5	
OVCAR-5	0.585	1.647	1.557	1.611	1.552	0.189	-0.007	92	97	91	-68	-100	9.07E-7	1.87E-6	3.87E-6	
OVCAR-8	0.412	1.911	1.787	1.840	1.768	0.296	0.181	92	95	90	-28	-56	1.10E-6	2.89E-6	3.01E-5	
NCI/ADR-RES	0.644	2.341	2.228	2.289	2.091	0.516	0.172	93	97	85	-20	-73	1.08E-6	3.24E-6	1.83E-5	
SK-OV-3	0.956	2.102	2.026	2.125	2.073	0.942	0.077	93	102	97	-1	-92	1.51E-6	4.83E-6	1.72E-5	
Renal Cancer																
786-0	0.557	2.467	2.302	2.381	2.144	0.150	0.123	91	96	83	-73	-78	8.14E-7	1.70E-6	3.56E-6	
A498	1.673	2.468	2.270	2.311	2.326	1.043	0.010	75	80	82	-38	-99	9.27E-7	2.42E-6	7.92E-6	
ACHN	0.436	1.870	1.823	1.903	1.740	0.412	-0.027	97	102	91	-6	-100	1.33E-6	4.38E-6	1.48E-5	
CAKI-1	0.499	2.233	2.034	2.056	1.851	0.384	0.002	88	90	78	-23	-100	9.46E-7	2.96E-6	1.12E-5	
RXF 393	0.772	1.582	1.513	1.529	1.446	0.229	0.065	92	93	83	-70	-92	8.22E-7	1.74E-6	3.69E-6	
SN12C	0.477	1.876	1.746	1.795	1.654	0.025	0.064	91	94	84	-95	-87	7.76E-7	1.48E-6	2.81E-6	
TK-10	0.963	2.433	2.282	2.254	2.283	0.886	0.222	90	88	90	-8	-77	1.27E-6	4.14E-6	2.03E-5	
UO-31	0.484	1.685	1.504	1.551	1.418	0.033	-0.028	85	89	78	-93	-100	7.26E-7	1.42E-6	2.79E-6	
Prostate Cancer																
PC-3	0.655	1.917	1.766	1.785	1.560	0.540	0.164	88	90	72	-18	-75	8.75E-7	3.17E-6	1.83E-5	
DU-145	0.348	1.407	1.405	1.496	1.402	0.400	0.031	100	108	99	5	-91	1.67E-6	5.62E-6	1.86E-5	
Breast Cancer																
MCF7	0.309	1.762	1.589	1.651	1.546	0.140	0.077	88	92	85	-55	-75	8.92E-7	2.03E-6	4.63E-6	
MDA-MB-231/ATCC	0.556	1.485	1.403	1.502	1.335	0.044	0.038	91	102	84	-92	-93	7.79E-7	1.50E-6	2.88E-6	
HS 578T	0.942	1.807	1.744	1.735	1.735	0.621	0.414	93	92	92	-34	-56	1.07E-6	2.68E-6	2.65E-5	
BT-549	1.116	2.293	2.256	2.286	2.174	0.112	0.222	97	99	90	-90	-80	8.33E-7	1.58E-6	3.00E-6	
T-47D	0.853	2.013	1.900	1.946	1.960	0.753	0.574	90	94	95	-12	-33	1.33E-6	3.89E-6	> 5.00E-5	
MDA-MB-468	0.913	1.700	1.645	1.673	1.575	0.530	0.257	93	96	84	-42	-72	9.31E-7	2.32E-6	9.27E-6	



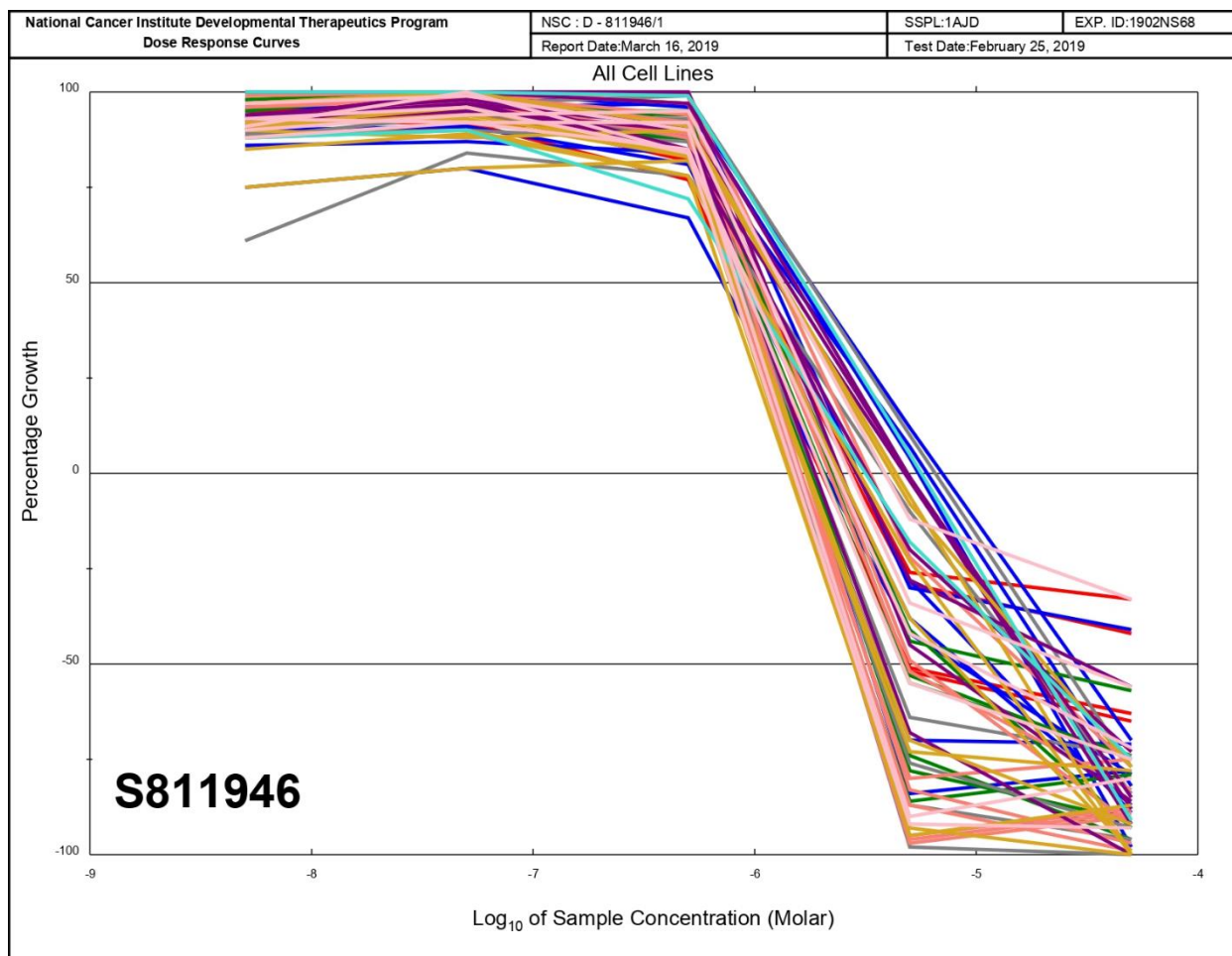
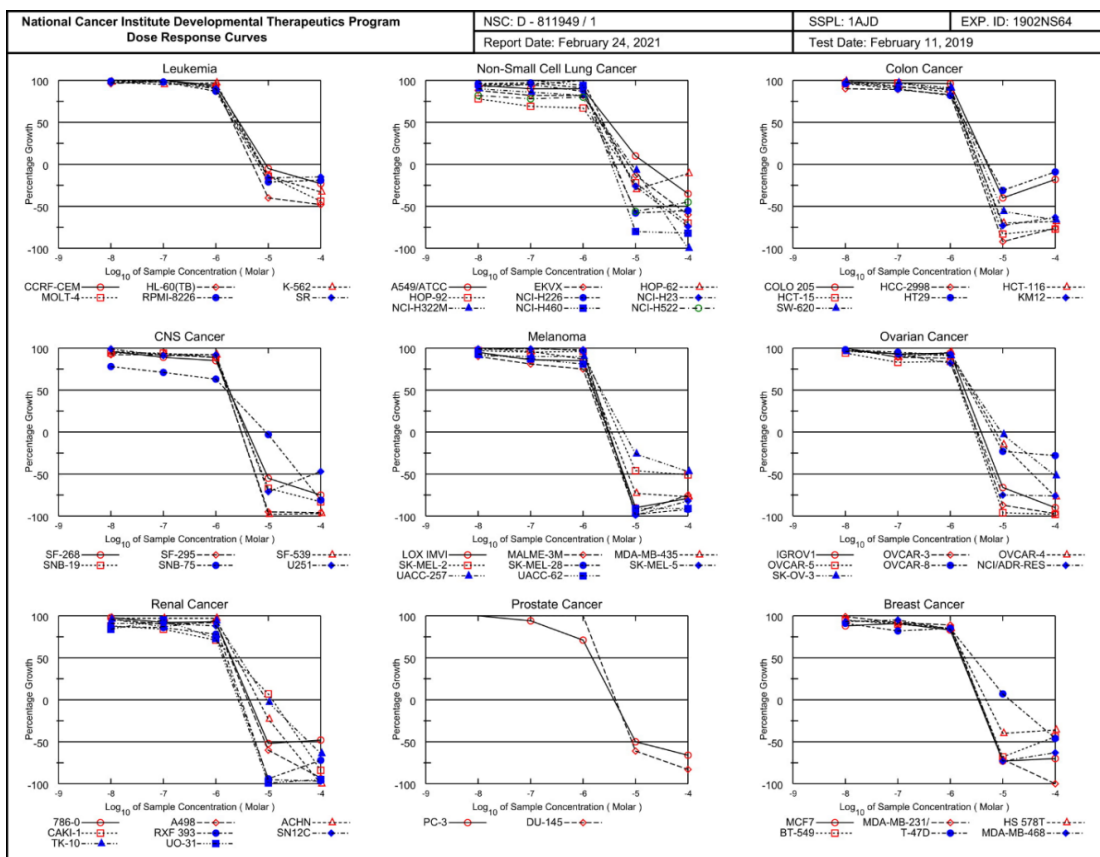
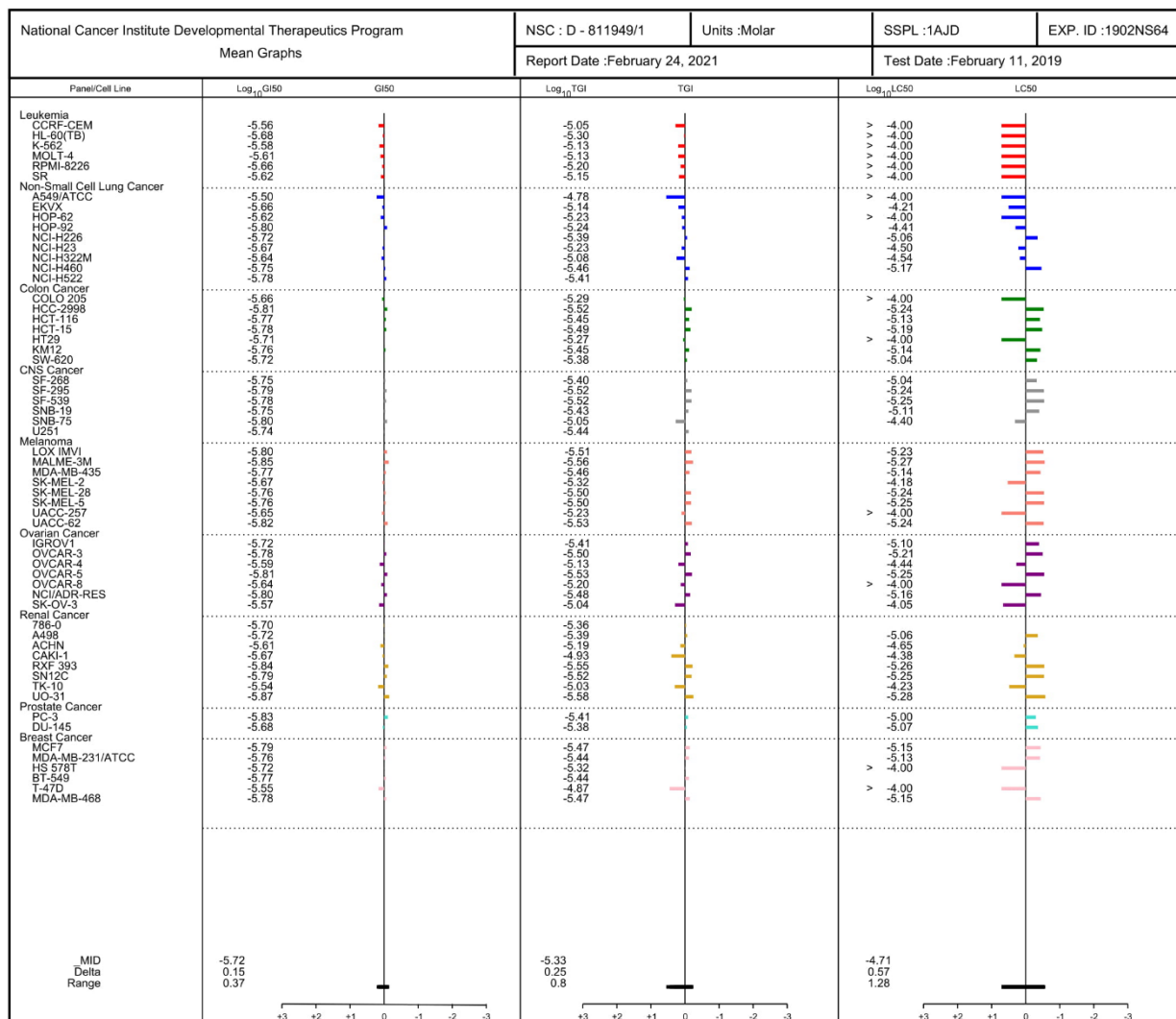


Figure S17. The five-dose assay data for compound **4**



National Cancer Institute Developmental Therapeutics Program In-Vitro Testing Results

NSC : D - 811949 / 1				Experiment ID : 1902NS64								Test Type : 08			Units : Molar	
Report Date : February 24, 2021				Test Date : February 11, 2019								QNS :			MC :	
COMI : PAV-114				Stain Reagent : SRB Dual-Pass Related								SSPL : 1AJD				
Log10 Concentration																
Panel/Cell Line	Time Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50	
Leukemia																
CCRF-CEM	0.622	2.800	2.814	2.826	2.662	0.593	0.476	101	101	94	-5	-23	2.78E-6	8.97E-6	> 1.00E-4	
HL-60(TB)	1.030	3.033	2.963	3.105	2.865	0.623	0.538	96	104	92	-40	-48	2.08E-6	5.00E-6	> 1.00E-4	
K-562	0.277	1.732	1.710	1.662	1.683	0.238	0.185	98	95	97	-14	-33	2.63E-6	7.44E-6	> 1.00E-4	
MOLT-4	0.799	2.830	3.012	2.967	2.643	0.689	0.449	109	107	91	-14	-44	2.45E-6	7.38E-6	> 1.00E-4	
RPMI-8226	0.747	2.030	2.021	2.000	1.858	0.588	0.606	99	98	87	-21	-19	2.18E-6	6.35E-6	> 1.00E-4	
SR	0.381	1.717	1.677	1.687	1.578	0.321	0.325	97	98	90	-16	-15	2.38E-6	7.09E-6	> 1.00E-4	
Non-Small Cell Lung Cancer																
A549/ATCC	0.489	2.069	1.979	1.907	1.906	0.649	0.318	94	90	90	10	-35	3.15E-6	1.68E-5	> 1.00E-4	
EKVX	0.764	2.386	2.184	2.101	2.090	0.666	0.307	88	82	82	-13	-60	2.17E-6	7.32E-6	6.17E-5	
HOP-62	0.392	1.140	1.105	1.104	1.143	0.273	0.348	95	95	100	-30	-11	2.43E-6	5.86E-6	> 1.00E-4	
HOP-92	1.558	2.226	2.078	2.016	2.009	1.223	0.469	78	69	67	-22	-70	1.57E-6	5.73E-6	3.88E-5	
NCI-H226	1.598	3.110	3.047	3.061	2.979	0.664	0.712	96	97	91	-58	-55	1.89E-6	4.07E-6	8.78E-6	
NCI-H23	0.658	2.000	1.893	1.928	1.825	0.489	0.171	92	95	87	-26	-74	2.13E-6	5.91E-6	3.18E-5	
NCI-H322M	0.616	1.511	1.425	1.382	1.349	0.571	-0.023	90	86	82	-7	-100	2.28E-6	8.27E-6	2.88E-5	
NCI-H460	0.242	1.934	1.982	1.928	1.828	0.048	0.044	103	100	94	-80	-82	1.78E-6	3.45E-6	6.69E-6	
NCI-H522	0.957	2.450	2.175	2.125	2.147	0.420	0.528	82	78	80	-56	-45	1.66E-6	3.86E-6	.	
Colon Cancer																
COLO 205	0.455	1.711	1.683	1.673	1.665	0.275	0.372	98	97	96	-40	-18	2.19E-6	5.12E-6	> 1.00E-4	
HCC-2998	0.647	2.114	1.962	1.953	1.870	0.055	0.155	90	89	83	-92	-76	1.55E-6	3.00E-6	5.79E-6	
HCT-116	0.117	1.290	1.273	1.207	1.122	0.035	0.038	99	93	86	-70	-68	1.69E-6	3.55E-6	7.43E-6	
HCT-15	0.376	2.767	2.677	2.693	2.468	0.064	0.087	96	97	88	-83	-77	1.66E-6	3.26E-6	6.41E-6	
HT29	0.234	1.556	1.507	1.426	1.321	0.163	0.213	96	90	82	-31	-9	1.93E-6	5.36E-6	> 1.00E-4	
KM12	0.477	2.173	2.126	2.045	1.999	0.129	0.175	97	92	90	-73	-63	1.75E-6	3.56E-6	7.23E-6	
SW-620	0.298	1.690	1.658	1.653	1.564	0.130	0.101	98	97	91	-56	-66	1.90E-6	4.14E-6	9.05E-6	
CNS Cancer																
SF-268	0.687	2.020	1.967	1.877	1.815	0.307	0.171	96	89	85	-55	-75	1.77E-6	4.02E-6	9.15E-6	
SF-295	0.929	3.113	2.946	2.942	2.878	0.046	0.034	92	92	89	-95	-96	1.63E-6	3.05E-6	5.70E-6	
SF-539	0.669	1.958	1.907	1.871	1.855	0.013	0.017	96	93	92	-98	-97	1.66E-6	3.05E-6	5.58E-6	
SNB-19	0.555	1.816	1.739	1.738	1.681	0.185	0.092	94	94	89	-67	-83	1.79E-6	3.74E-6	7.82E-6	
SNB-75	1.008	1.667	1.522	1.478	1.424	0.975	0.192	78	71	63	-3	-81	1.57E-6	8.91E-6	3.99E-5	
U251	0.453	1.936	1.918	1.803	1.823	0.131	0.240	99	91	92	-71	-47	1.82E-6	3.67E-6	.	
Melanoma																
LOX IMVI	0.316	1.861	1.786	1.644	1.628	0.032	0.066	95	86	85	-90	-79	1.58E-6	3.06E-6	5.91E-6	
MALME-3M	0.731	1.590	1.508	1.425	1.377	0.030	0.180	90	81	75	-96	-75	1.40E-6	2.75E-6	5.39E-6	
MDA-MB-435	0.486	1.909	1.929	1.846	1.718	0.132	0.113	101	96	87	-73	-77	1.70E-6	3.49E-6	7.18E-6	
SK-MEL-2	1.650	3.143	3.103	3.065	3.090	0.894	0.810	97	95	96	-46	-51	2.12E-6	4.76E-6	6.55E-5	
SK-MEL-28	0.726	1.799	1.779	1.827	1.768	0.017	0.060	98	103	97	-98	-92	1.74E-6	3.15E-6	5.69E-6	
SK-MEL-5	1.339	3.342	3.326	3.322	3.296	0.011	0.245	99	99	98	-99	-82	1.75E-6	3.14E-6	5.63E-6	
UACC-257	1.139	2.248	2.145	2.137	2.139	0.841	0.601	91	90	90	-26	-47	2.21E-6	5.95E-6	> 1.00E-4	
UACC-62	0.932	2.583	2.461	2.373	2.262	0.089	0.080	93	87	81	-91	-91	1.51E-6	2.96E-6	5.80E-6	
Ovarian Cancer																
IGROV1	0.463	1.727	1.688	1.638	1.657	0.157	0.046	97	93	94	-66	-90	1.89E-6	3.87E-6	7.94E-6	
OVCAR-3	0.421	1.138	1.160	1.062	1.052	0.057	0.012	103	89	88	-87	-97	1.65E-6	3.19E-6	6.17E-6	
OVCAR-4	0.554	1.149	1.171	1.085	1.120	0.473	0.126	104	89	95	-15	-77	2.57E-6	7.36E-6	3.66E-5	
OVCAR-5	0.547	1.463	1.413	1.312	1.315	0.025	0.012	94	83	84	-96	-98	1.54E-6	2.93E-6	5.57E-6	
OVCAR-8	0.470	1.975	1.941	1.905	1.856	0.361	0.338	98	95	92	-23	-28	2.32E-6	6.29E-6	> 1.00E-4	
NCI/ADR-RES	0.562	2.168	2.141	2.049	1.871	0.140	0.136	98	93	82	-75	-76	1.59E-6	3.31E-6	6.91E-6	
SK-OV-3	0.834	1.944	1.898	1.865	1.839	0.806	0.399	96	93	91	-3	-52	2.70E-6	9.20E-6	9.01E-5	
Renal Cancer																
786-O	0.599	2.825	2.792	2.649	2.677	0.286	0.312	98	92	93	-52	-48	1.98E-6	4.37E-6	.	
A498	1.687	2.877	2.830	2.757	2.783	0.682	0.119	96	90	92	-60	-93	1.90E-6	4.05E-6	8.65E-6	
ACHN	0.282	1.246	1.213	1.221	1.213	0.218	-0.012	97	97	97	-23	-100	2.46E-6	6.45E-6	2.26E-5	
CAKI-1	0.658	2.557	2.330	2.250	2.007	0.784	0.103	88	84	71	7	-84	2.12E-6	1.18E-5	4.19E-5	
RXF 393	1.105	1.582	1.521	1.516	1.475	0.064	0.312	87	86	78	-94	-72	1.45E-6	2.83E-6	5.53E-6	
SN12C	0.507	1.712	1.601	1.609	1.563	0.024	0.015	91	91	88	-95	-97	1.61E-6	3.01E-6	5.66E-6	
TK-10	0.916	2.333	2.278	2.148	2.253	0.892	0.331	96	87	94	-3	-64	2.87E-6	9.40E-6	5.93E-5	
UO-31	0.496	1.442	1.290	1.391	1.184	0.006	0.026	84	95	73	-99	-95	1.36E-6	2.65E-6	5.19E-6	
Prostate Cancer																
PC-3	0.567	2.121	2.119	2.023	1.673	0.281	0.195	100	94	71	-50	-66	1.49E-6	3.85E-6	9.92E-6	
DU-145	0.314	1.148	1.200	1.151	1.164	0.122	0.054	106	100	102	-61	-83	2.08E-6	4.21E-6	8.53E-6	
Breast Cancer																
MCF7	0.481	2.565	2.322	2.380	2.220	0.131	0.142	88	91	83	-73	-70	1.64E-6	3.42E-6	7.14E-6	
MDA-MB-231/ATCC	0.636	1.419	1.412	1.360	1.330	0.185	-0.023	99	92	89	-71	-100	1.75E-6	3.59E-6	7.39E-6	
HS 578T	0.816	1.791	1.736	1.712	1.647	0.487	0.519	94	92	85	-40	-36	1.91E-6	4.77E-6	> 1.00E-4	
BT-549	1.028	2.554	2.476	2.408	2.323	0.329	0.571	95	90	85	-68	-44	1.69E-6	3.59E-6	.	
T-47D	0.830	1.875	1.782	1.691	1.717	0.899	0.452	91	82	85	7	-46	2.79E-6	1.34E-5	> 1.00E-4	
MDA-MB-468	0.895	1.528	1.483	1.496	1.430	0.238	0.330	93	95	84	-73	-63	1.65E-6	3.43E-6	7.10E-6	



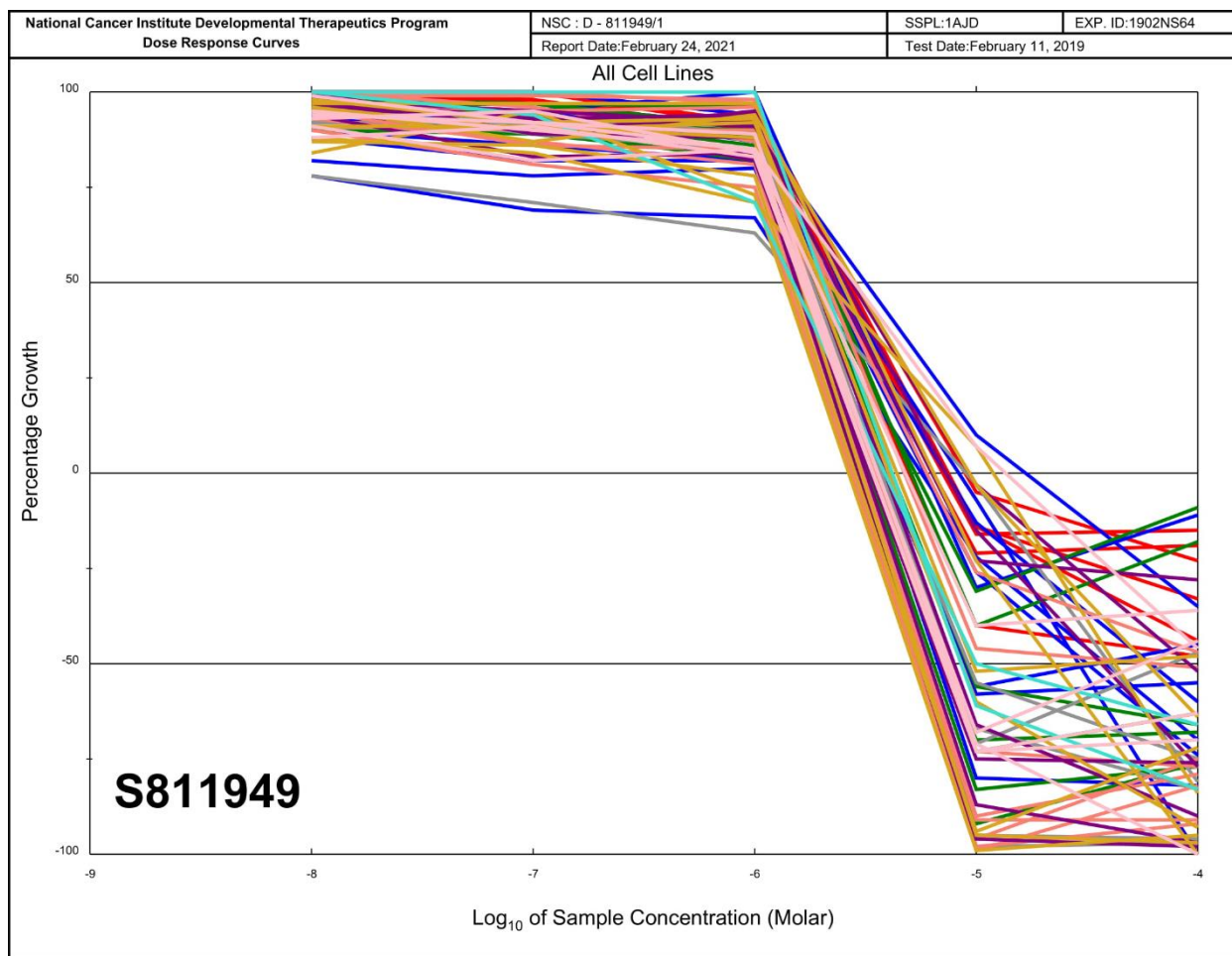
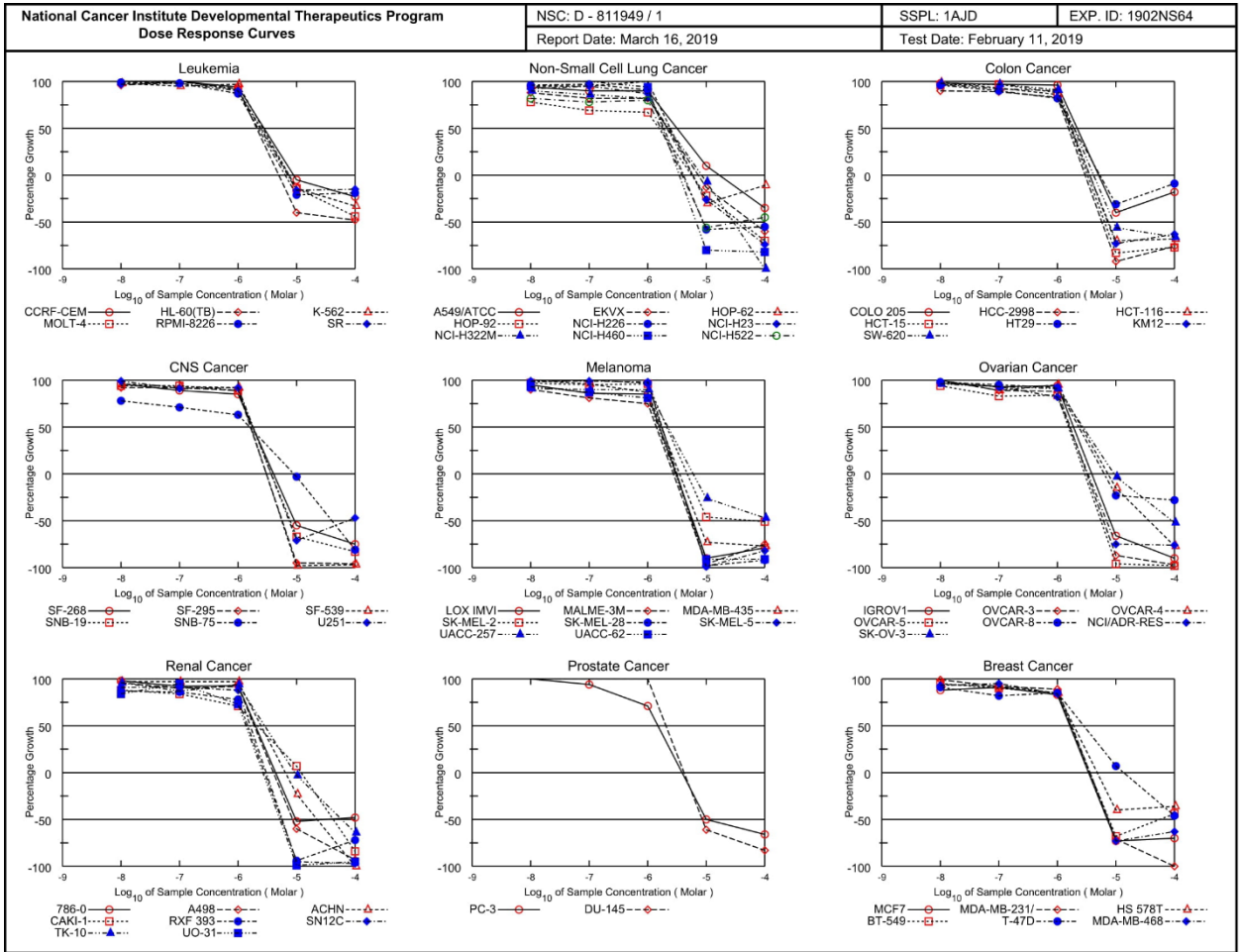
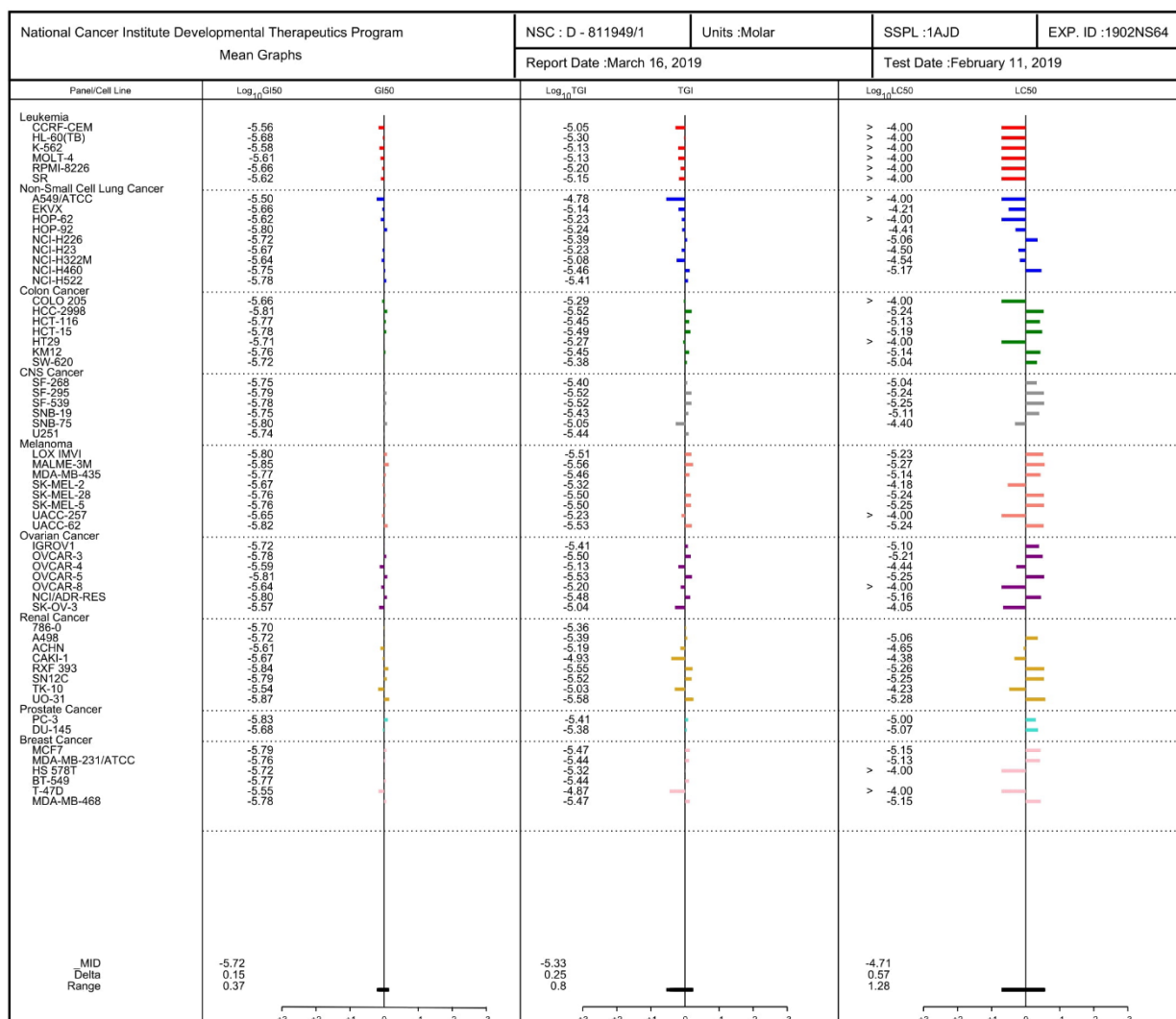


Figure S18. The five-dose assay data for compound **6**



National Cancer Institute Developmental Therapeutics Program In-Vitro Testing Results

NSC : D - 811949 / 1				Experiment ID : 1902NS64								Test Type : 08			Units : Molar	
Report Date : March 16, 2019				Test Date : February 11, 2019								QNS :			MC :	
COMI : PAV-114				Stain Reagent : SRB Dual-Pass Related								SSPL : 1AJD				
Log10 Concentration																
Panel/Cell Line	Time Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50	
Leukemia																
CCRF-CEM	0.622	2.800	2.814	2.826	2.662	0.593	0.476	101	101	94	-5	-23	2.78E-6	8.97E-6	> 1.00E-4	
HL-60(TB)	1.030	3.033	2.963	3.105	2.865	0.623	0.538	96	104	92	-40	-48	2.08E-6	5.00E-6	> 1.00E-4	
K-562	0.277	1.732	1.710	1.662	1.683	0.238	0.185	98	95	97	-14	-33	2.63E-6	7.44E-6	> 1.00E-4	
MOLT-4	0.799	2.830	3.012	2.967	2.643	0.689	0.449	109	107	91	-14	-44	2.45E-6	7.38E-6	> 1.00E-4	
RPMT-8226	0.747	2.030	2.021	2.000	1.858	0.588	0.606	99	98	87	-21	-19	2.18E-6	6.35E-6	> 1.00E-4	
SR	0.381	1.717	1.677	1.687	1.578	0.321	0.325	97	98	90	-16	-15	2.38E-6	7.09E-6	> 1.00E-4	
Non-Small Cell Lung Cancer																
A549/ATCC	0.489	2.069	1.979	1.907	1.906	0.649	0.318	94	90	90	10	-35	3.15E-6	1.68E-5	> 1.00E-4	
EKVX	0.764	2.386	2.184	2.101	2.090	0.666	0.307	88	82	82	-13	-60	2.17E-6	7.32E-6	6.17E-5	
HOP-62	0.392	1.140	1.105	1.104	1.143	0.273	0.348	95	95	100	-30	-11	2.43E-6	5.86E-6	> 1.00E-4	
HOP-92	1.558	2.226	2.078	2.016	2.009	1.223	0.469	78	69	67	-22	-70	1.57E-6	5.73E-6	3.88E-5	
NCI-H226	1.598	3.110	3.047	3.061	2.979	0.664	0.712	96	97	91	-58	-55	1.89E-6	4.07E-6	8.78E-6	
NCI-H23	0.658	2.000	1.893	1.928	1.825	0.489	0.171	92	95	87	-26	-74	2.13E-6	5.91E-6	3.18E-5	
NCI-H322M	0.616	1.511	1.425	1.382	1.349	0.571	-0.023	90	86	82	-7	-100	2.28E-6	8.27E-6	2.88E-5	
NCI-H460	0.242	1.934	1.982	1.928	1.828	0.048	0.044	103	100	94	-80	-82	1.78E-6	3.45E-6	6.69E-6	
NCI-H522	0.957	2.450	2.175	2.125	2.147	0.420	0.528	82	78	80	-56	-45	1.66E-6	3.86E-6	.	
Colon Cancer																
COLO 205	0.455	1.711	1.683	1.673	1.665	0.275	0.372	98	97	96	-40	-18	2.19E-6	5.12E-6	> 1.00E-4	
HCC-2998	0.647	2.114	1.962	1.953	1.870	0.055	0.155	90	89	83	-92	-76	1.55E-6	3.00E-6	5.79E-6	
HCT-116	0.117	1.290	1.273	1.207	1.122	0.035	0.038	99	93	86	-70	-68	1.69E-6	3.55E-6	7.43E-6	
HCT-15	0.376	2.767	2.677	2.693	2.468	0.064	0.087	96	97	88	-83	-77	1.66E-6	3.26E-6	6.41E-6	
HT29	0.234	1.556	1.507	1.426	1.321	0.163	0.213	96	90	82	-31	-9	1.93E-6	5.36E-6	> 1.00E-4	
KM12	0.477	2.173	2.126	2.045	1.999	0.129	0.175	97	92	90	-73	-63	1.75E-6	3.56E-6	7.23E-6	
SW-620	0.298	1.690	1.658	1.653	1.564	0.130	0.101	98	97	91	-56	-66	1.90E-6	4.14E-6	9.05E-6	
CNS Cancer																
SF-268	0.687	2.020	1.967	1.877	1.815	0.307	0.171	96	89	85	-55	-75	1.77E-6	4.02E-6	9.15E-6	
SF-295	0.929	3.113	2.946	2.942	2.878	0.046	0.034	92	92	89	-95	-96	1.63E-6	3.05E-6	5.70E-6	
SF-539	0.669	1.958	1.907	1.871	1.855	0.013	0.017	96	93	92	-98	-97	1.66E-6	3.05E-6	5.58E-6	
SNB-19	0.555	1.816	1.739	1.738	1.681	0.185	0.092	94	94	89	-67	-83	1.79E-6	3.74E-6	7.82E-6	
SNB-75	1.008	1.667	1.522	1.478	1.424	0.975	0.192	78	71	63	-3	-81	1.57E-6	8.91E-6	3.99E-5	
U251	0.453	1.936	1.918	1.803	1.823	0.131	0.240	99	91	92	-71	-47	1.82E-6	3.67E-6	.	
Melanoma																
LOX IMVI	0.316	1.861	1.786	1.644	1.628	0.032	0.066	95	86	85	-90	-79	1.58E-6	3.06E-6	5.91E-6	
MALME-3M	0.731	1.590	1.508	1.425	1.377	0.030	0.180	90	81	75	-96	-75	1.40E-6	2.75E-6	5.39E-6	
MDA-MB-435	0.486	1.909	1.929	1.846	1.718	0.132	0.113	101	96	87	-73	-77	1.70E-6	3.49E-6	7.18E-6	
SK-MEL-2	1.650	3.143	3.103	3.065	3.090	0.894	0.810	97	95	96	-46	-51	2.12E-6	4.76E-6	6.55E-5	
SK-MEL-28	0.726	1.799	1.779	1.827	1.768	0.017	0.060	98	103	97	-98	-92	1.74E-6	3.15E-6	5.69E-6	
SK-MEL-5	1.339	3.342	3.326	3.322	3.296	0.011	0.245	99	99	98	-99	-82	1.75E-6	3.14E-6	5.63E-6	
UACC-257	1.139	2.248	2.145	2.137	2.139	0.841	0.601	91	90	90	-26	-47	2.21E-6	5.95E-6	> 1.00E-4	
UACC-62	0.932	2.583	2.461	2.373	2.262	0.089	0.080	93	87	81	-91	-91	1.51E-6	2.96E-6	5.80E-6	
Ovarian Cancer																
IGROV1	0.463	1.727	1.688	1.638	1.657	0.157	0.046	97	93	94	-66	-90	1.89E-6	3.87E-6	7.94E-6	
OVCAR-3	0.421	1.138	1.160	1.062	1.052	0.057	0.012	103	89	88	-87	-97	1.65E-6	3.19E-6	6.17E-6	
OVCAR-4	0.554	1.149	1.171	1.085	1.120	0.473	0.126	104	89	95	-15	-77	2.57E-6	7.36E-6	3.66E-5	
OVCAR-5	0.547	1.463	1.413	1.312	1.315	0.025	0.012	94	83	84	-96	-98	1.54E-6	2.93E-6	5.57E-6	
OVCAR-8	0.470	1.975	1.941	1.905	1.856	0.361	0.338	98	95	92	-23	-28	2.32E-6	6.29E-6	> 1.00E-4	
NCI/ADR-RES	0.562	2.168	2.141	2.049	1.871	0.140	0.136	98	93	82	-75	-76	1.59E-6	3.31E-6	6.91E-6	
SK-OV-3	0.834	1.944	1.898	1.865	1.839	0.806	0.399	96	93	91	-3	-52	2.70E-6	9.20E-6	9.01E-5	
Renal Cancer																
786-O	0.599	2.825	2.792	2.649	2.677	0.286	0.312	98	92	93	-52	-48	1.98E-6	4.37E-6	.	
A498	1.687	2.877	2.830	2.757	2.783	0.682	0.119	96	90	92	-60	-93	1.90E-6	4.05E-6	8.65E-6	
ACHN	0.282	1.246	1.213	1.221	1.213	0.218	-0.012	97	97	97	-23	-100	2.46E-6	6.45E-6	2.26E-5	
CAKI-1	0.658	2.557	2.330	2.250	2.007	0.784	0.103	88	84	71	7	-84	2.12E-6	1.18E-5	4.19E-5	
RXF 393	1.105	1.582	1.521	1.516	1.475	0.064	0.312	87	86	78	-94	-72	1.45E-6	2.83E-6	5.53E-6	
SN12C	0.507	1.712	1.601	1.609	1.563	0.024	0.015	91	91	88	-95	-97	1.61E-6	3.01E-6	5.66E-6	
TK-10	0.916	2.333	2.278	2.148	2.253	0.892	0.331	96	87	94	-3	-64	2.87E-6	9.40E-6	5.93E-5	
UO-31	0.496	1.442	1.290	1.391	1.184	0.006	0.026	84	95	73	-99	-95	1.36E-6	2.65E-6	5.19E-6	
Prostate Cancer																
PC-3	0.567	2.121	2.119	2.023	1.673	0.281	0.195	100	94	71	-50	-66	1.49E-6	3.85E-6	9.92E-6	
DU-145	0.314	1.148	1.200	1.151	1.164	0.122	0.054	106	100	102	-61	-83	2.08E-6	4.21E-6	8.53E-6	
Breast Cancer																
MCF7	0.481	2.565	2.322	2.380	2.220	0.131	0.142	88	91	83	-73	-70	1.64E-6	3.42E-6	7.14E-6	
MDA-MB-231/ATCC	0.636	1.419	1.412	1.360	1.330	0.185	-0.023	99	92	89	-71	-100	1.75E-6	3.59E-6	7.39E-6	
HS 578T	0.816	1.791	1.736	1.712	1.647	0.487	0.519	94	92	85	-40	-36	1.91E-6	4.77E-6	> 1.00E-4	
BT-549	1.028	2.554	2.476	2.408	2.323	0.329	0.571	95	90	85	-68	-44	1.69E-6	3.59E-6	.	
T-47D	0.830	1.875	1.782	1.691	1.717	0.899	0.452	91	82	85	7	-46	2.79E-6	1.34E-5	> 1.00E-4	
MDA-MB-468	0.895	1.528	1.483	1.496	1.430	0.238	0.330	93	95	84	-73	-63	1.65E-6	3.43E-6	7.10E-6	



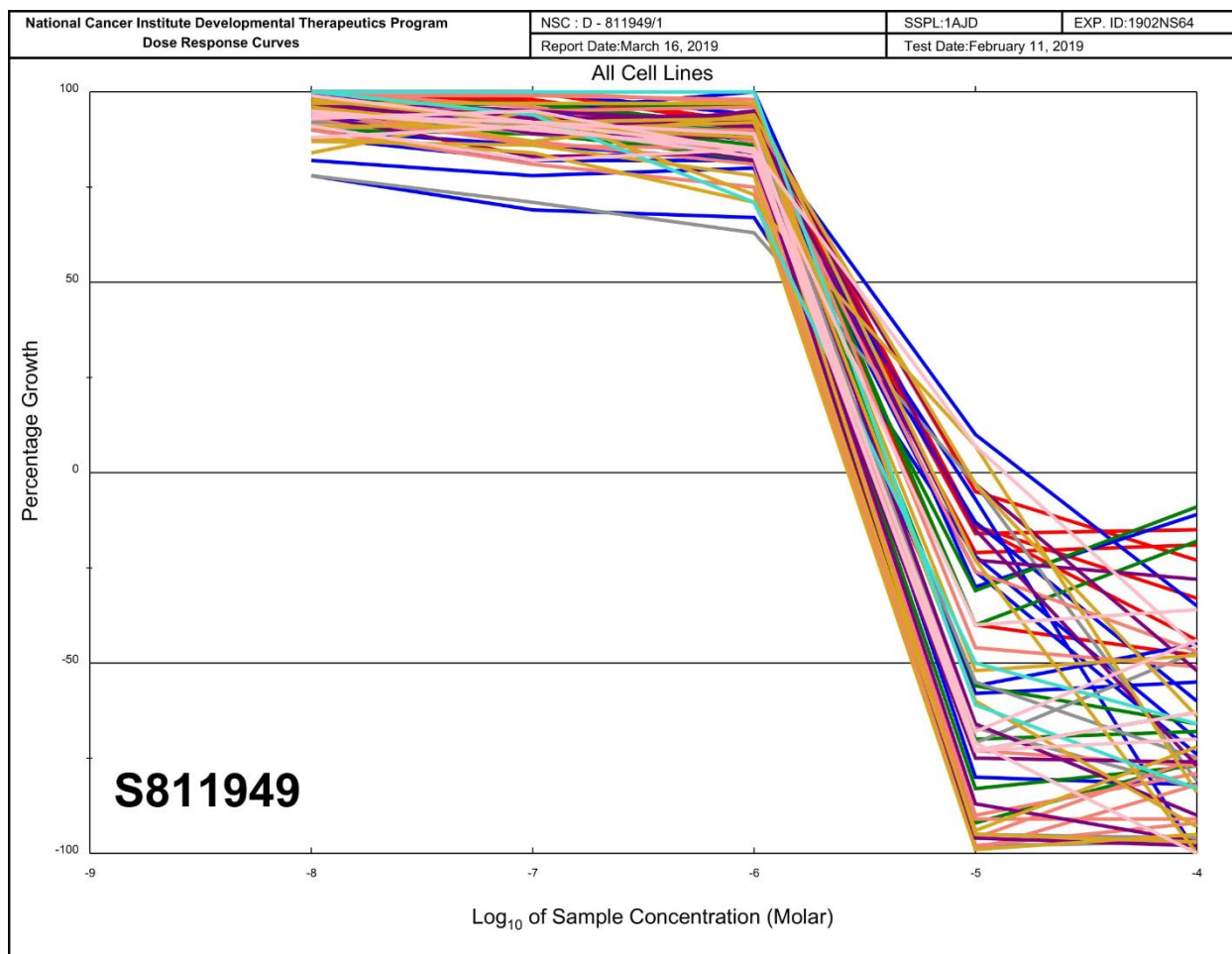


Figure S19. The five-dose assay data for compound **9**