

Isoquinolinequinone *N*-oxides as Anticancer Agents Effective Against Drug Resistant Cell Lines

Ryan D. Kruschel,^a Alyah Buzid,^a Udaya B. Rao Khandavilli,^a Simon E. Lawrence,^a Jeremy D. Glennon^a
and Florence O. McCarthy^{*a}

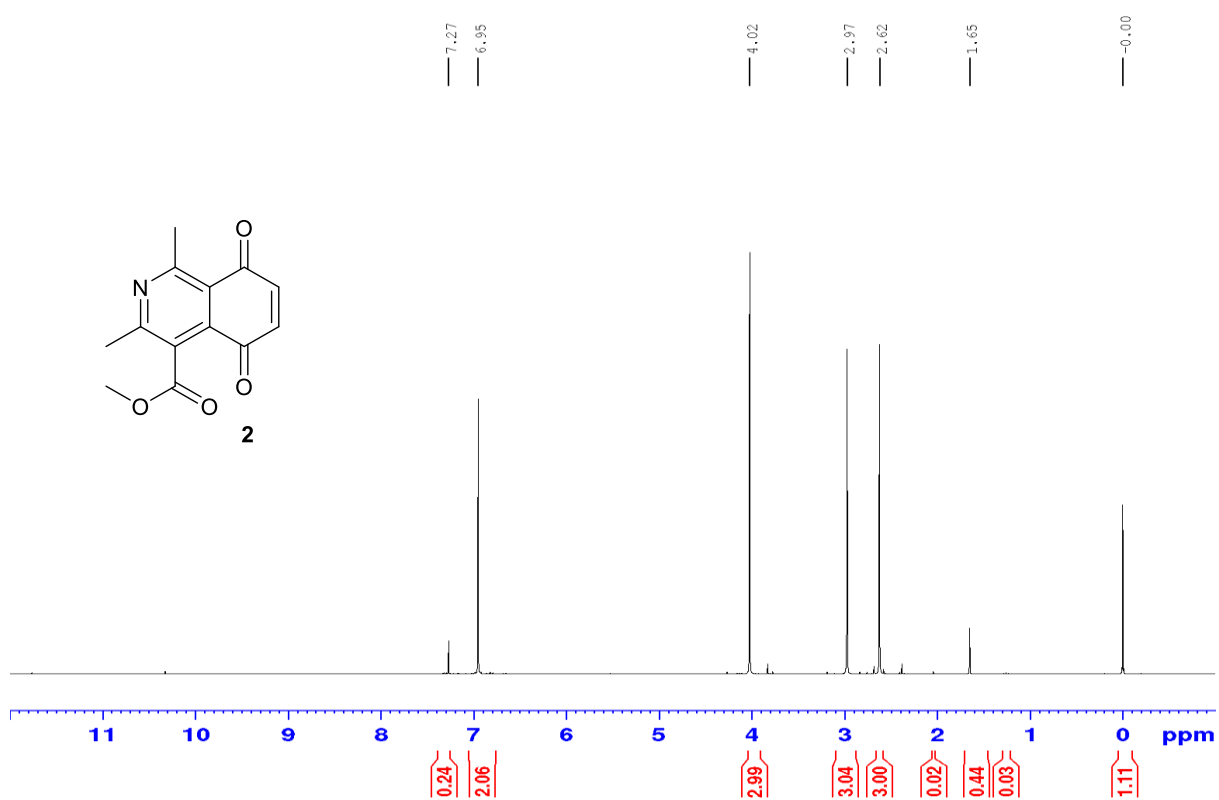
^aSchool of Chemistry, Analytical and Biological Chemistry Research Facility, University College Cork,
Cork T12 K8AF, Ireland; f.mccarthy@ucc.ie, r.kruschel@umail.ucc.ie

*Correspondence: f.mccarthy@ucc.ie; Tel.: 00353214901695

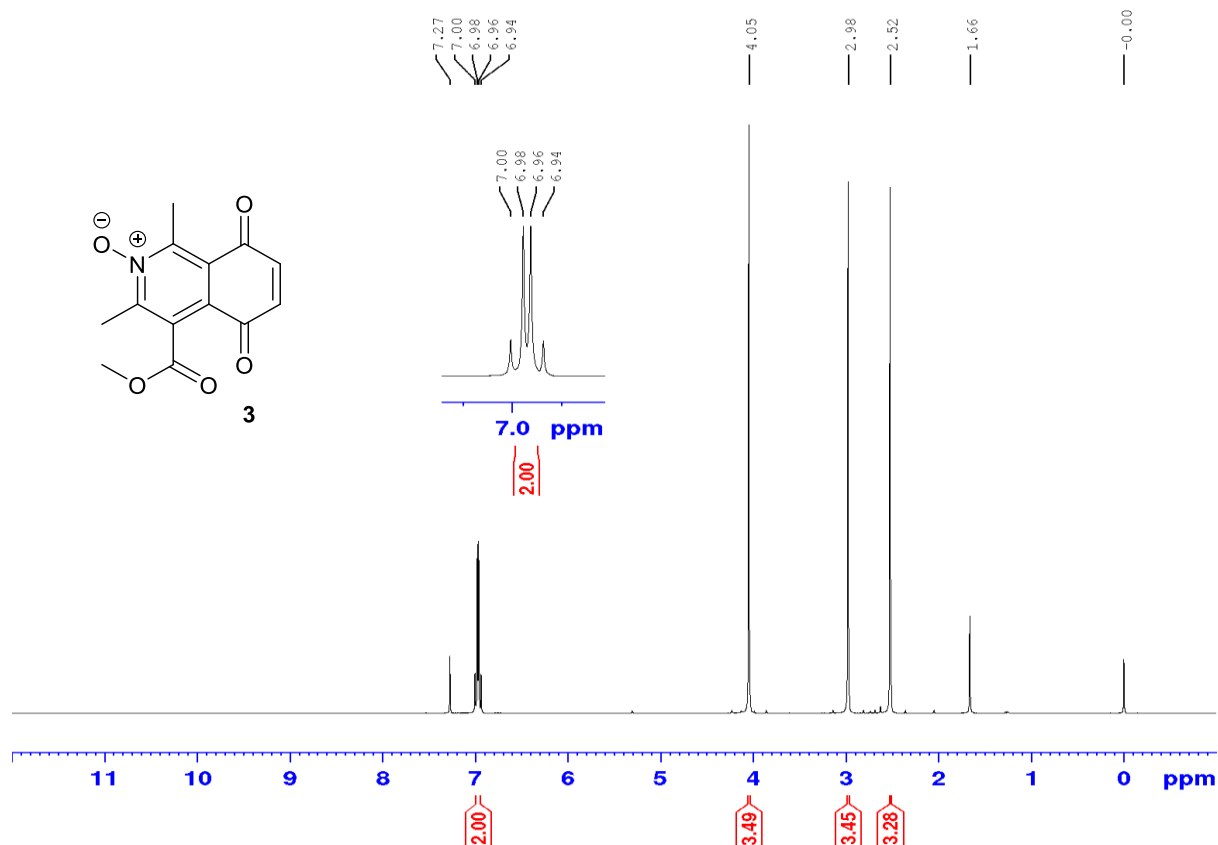
Supplementary Information

¹ H and ¹³ C NMR.....	S2
HMBC analysis of regioisomers 15 and 16	S20
NCI60 One-Dose Graphs	S22
NCI60 Five-Dose Graphs	S36
Calculation of Mean GI ₅₀ and Trends	S48
COMPARE Analysis	S50
Electrochemical Graphs	S60
Adduct Binding Studies	S63

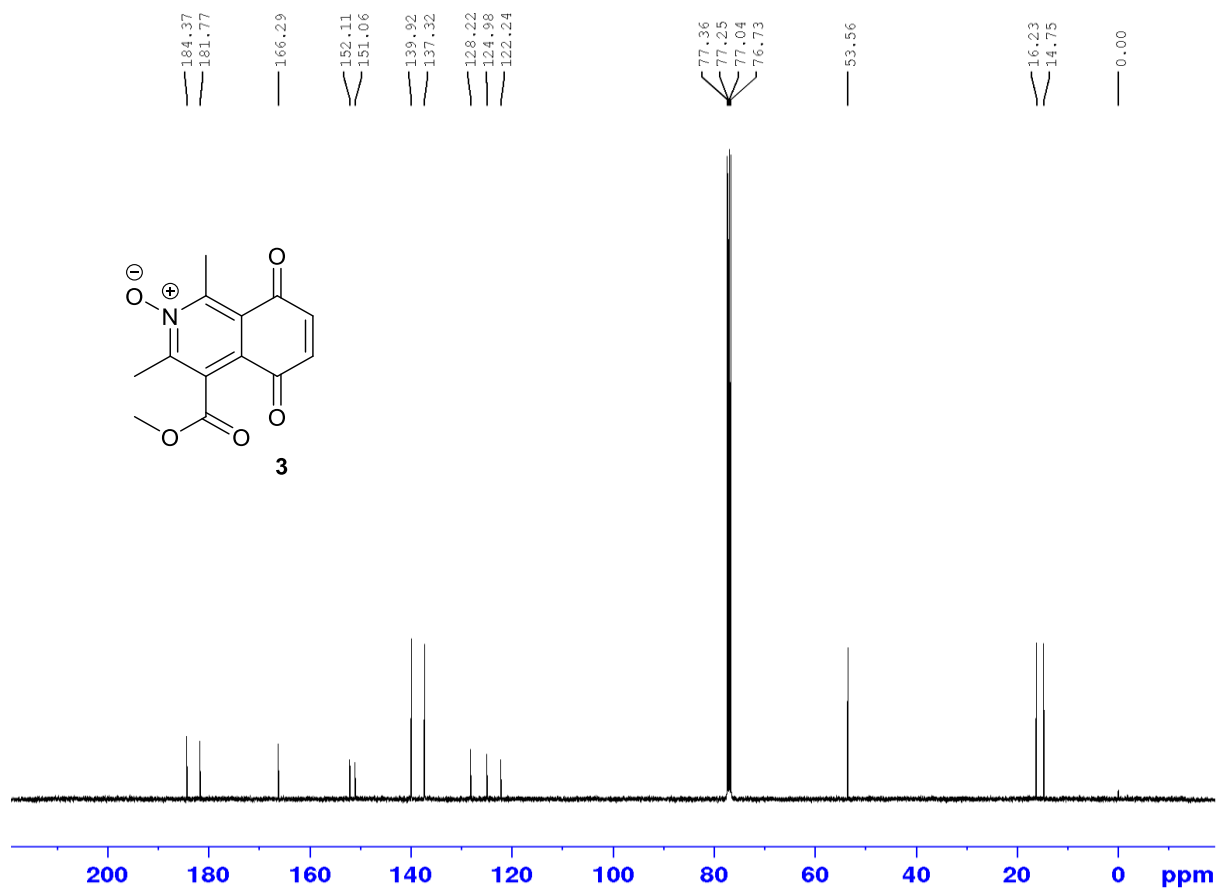
¹H NMR of **2**



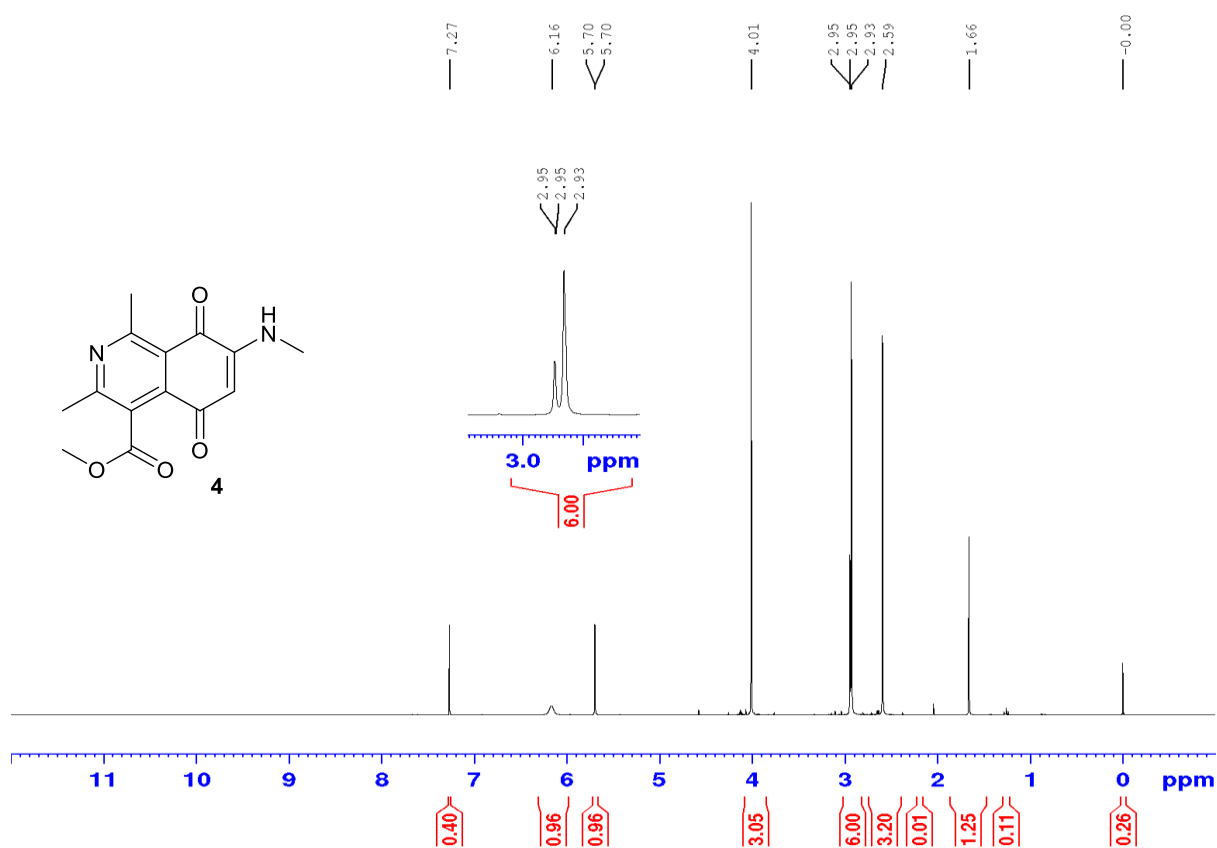
¹H NMR of **3**



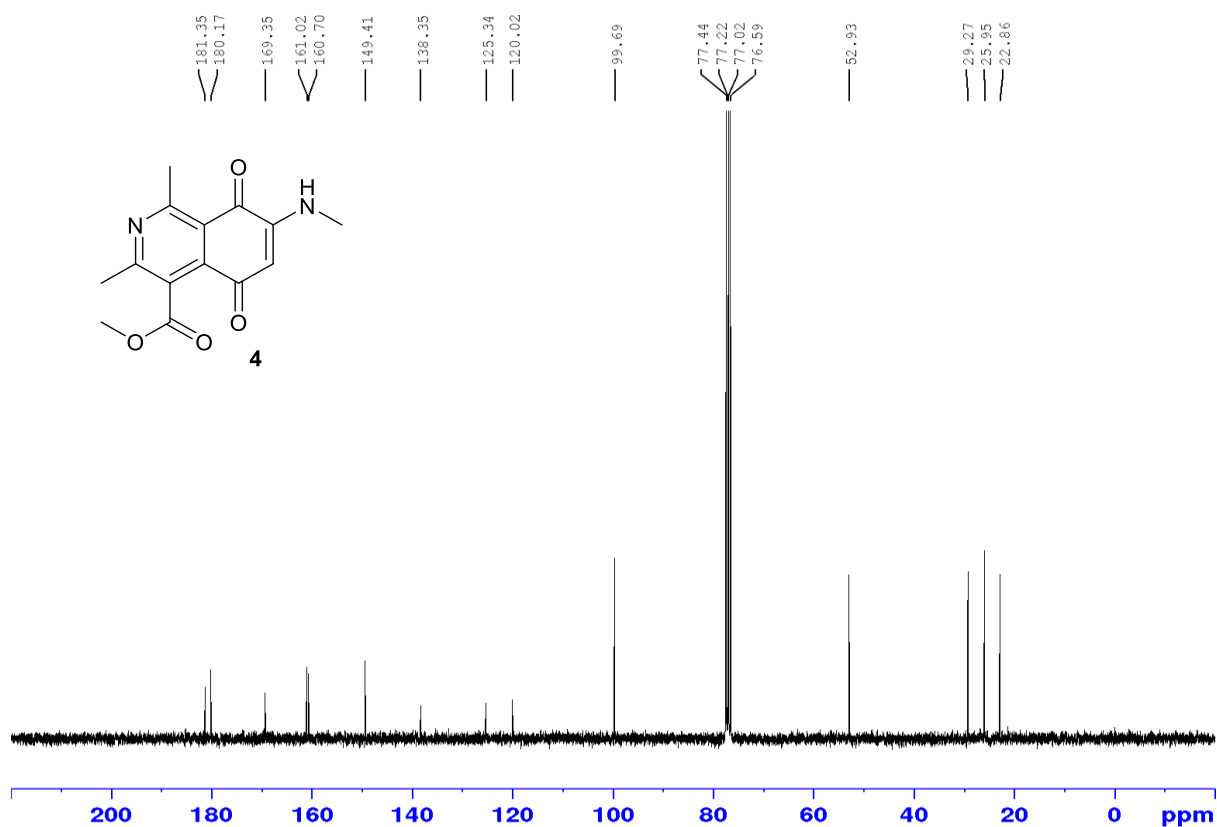
¹³C NMR of **3**



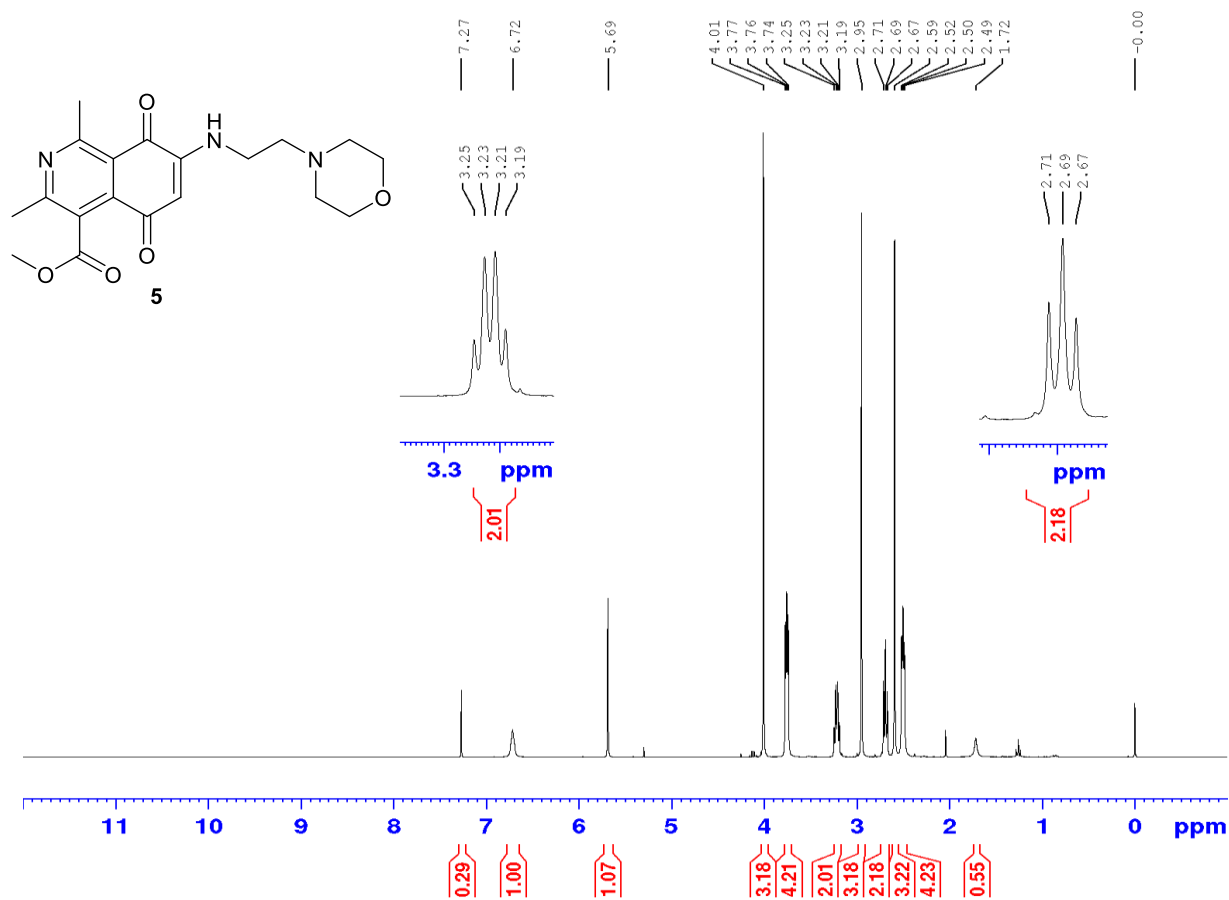
¹H NMR of **4**



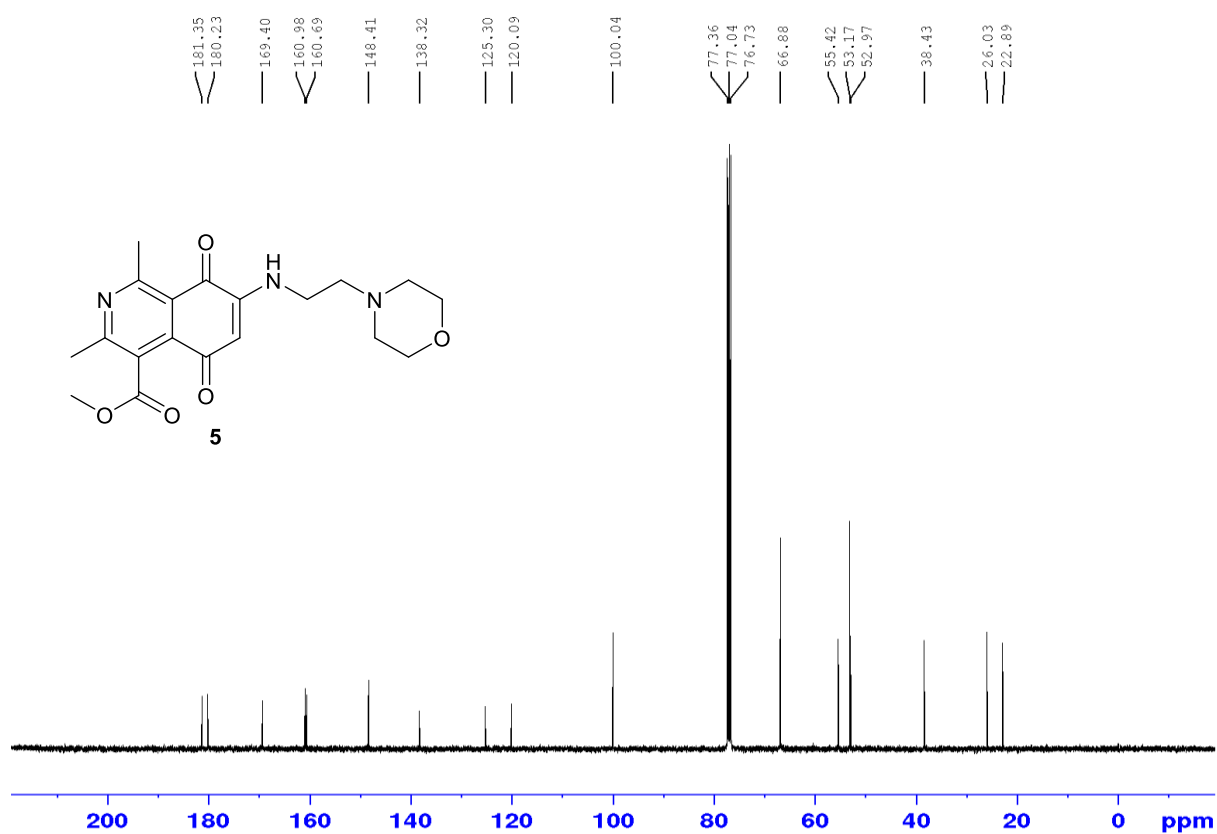
¹³C NMR of **4**



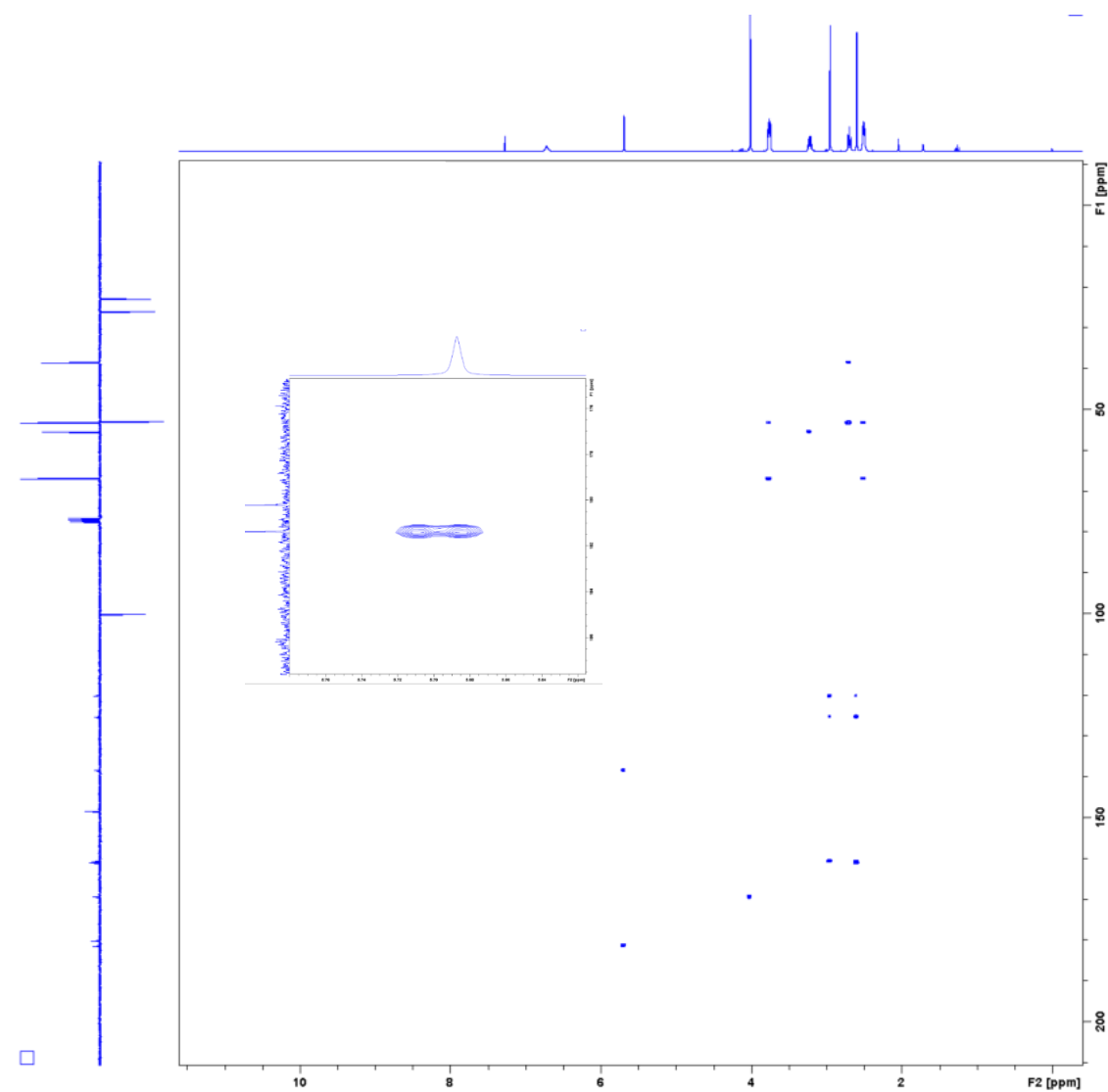
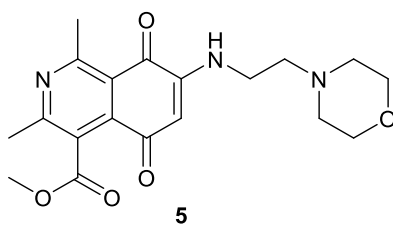
¹H NMR of 5



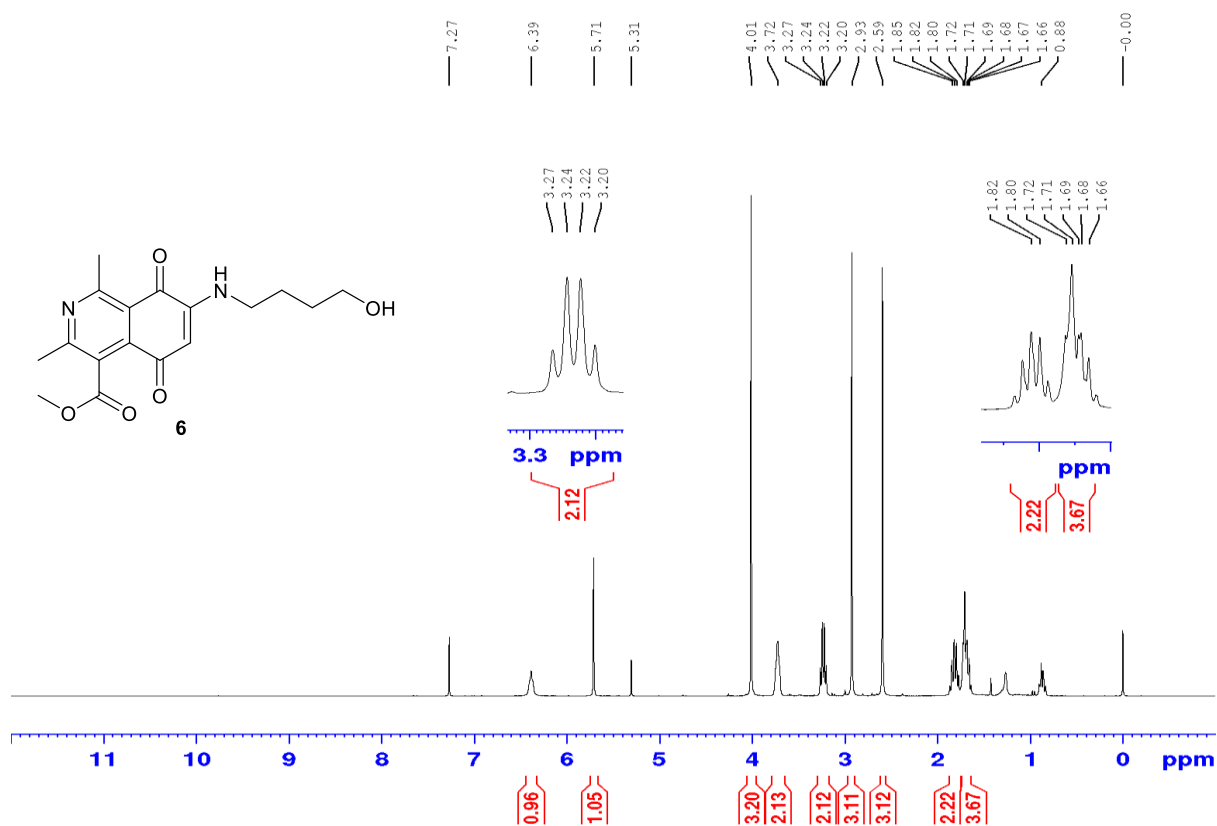
¹³C NMR of 5



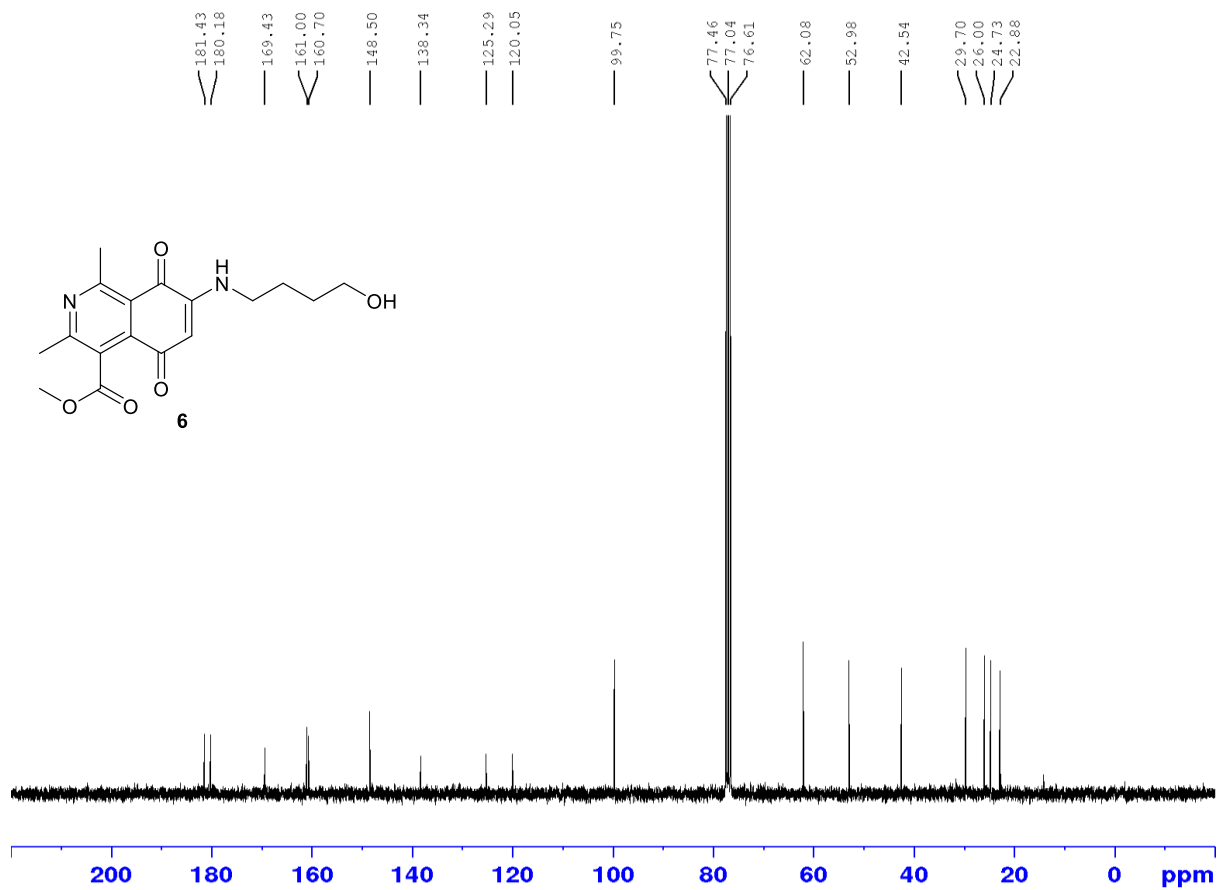
2D NMR- HMBC OF 5



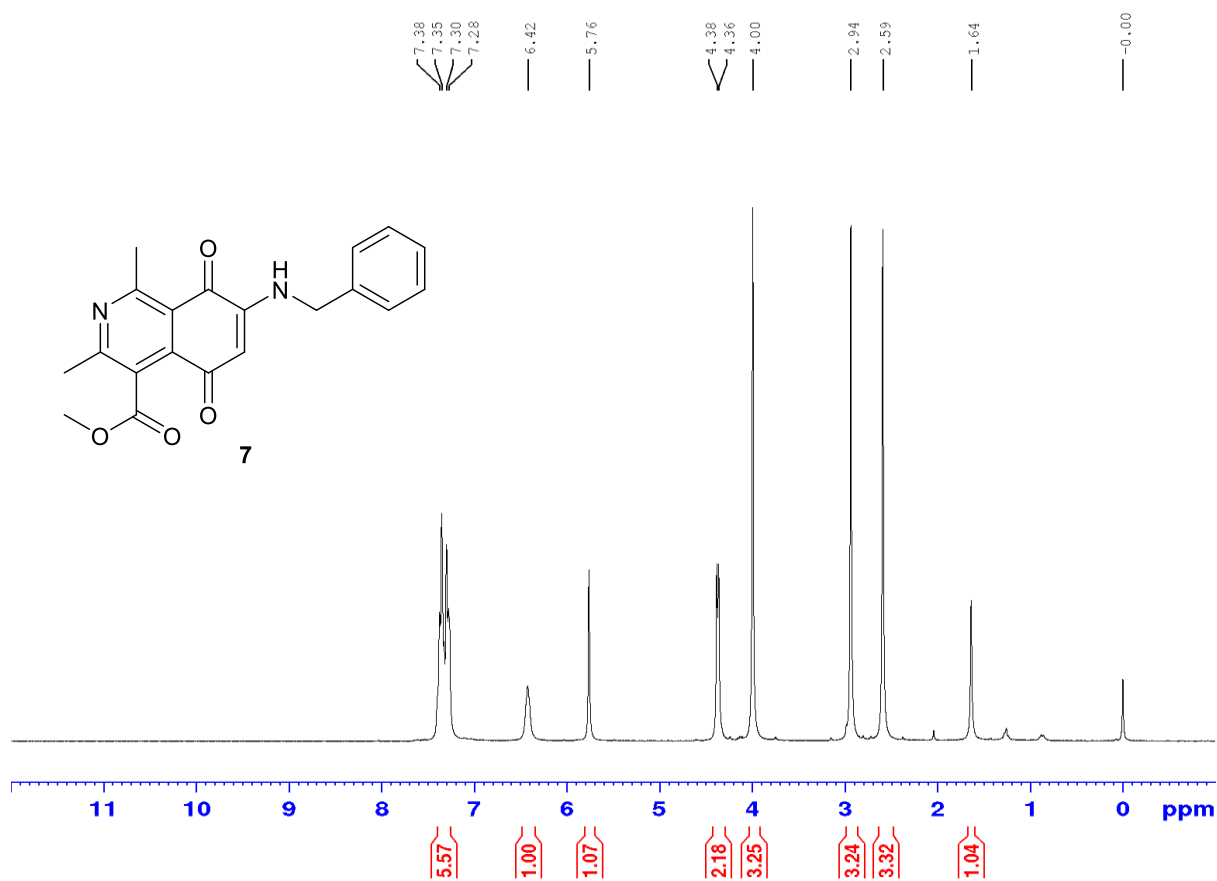
¹H NMR of 6



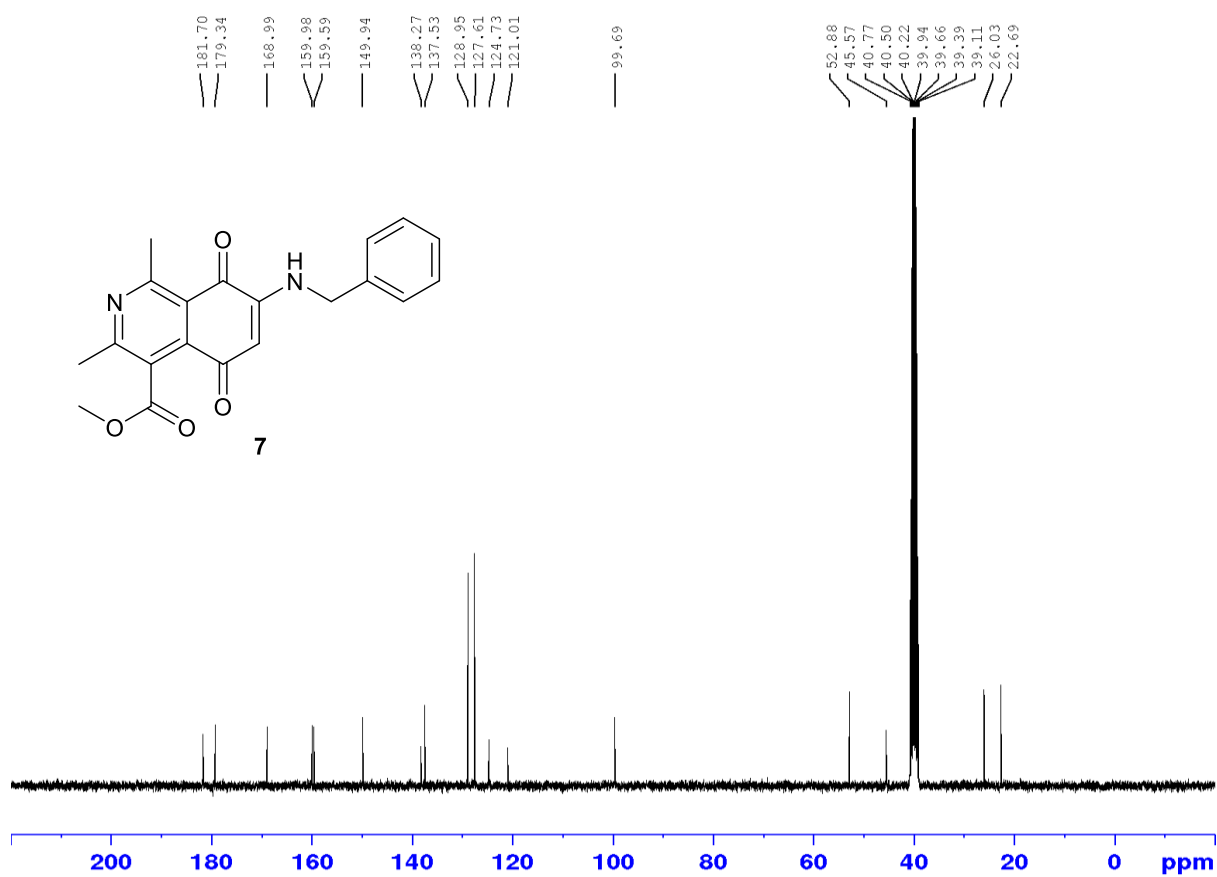
¹³C NMR of 6



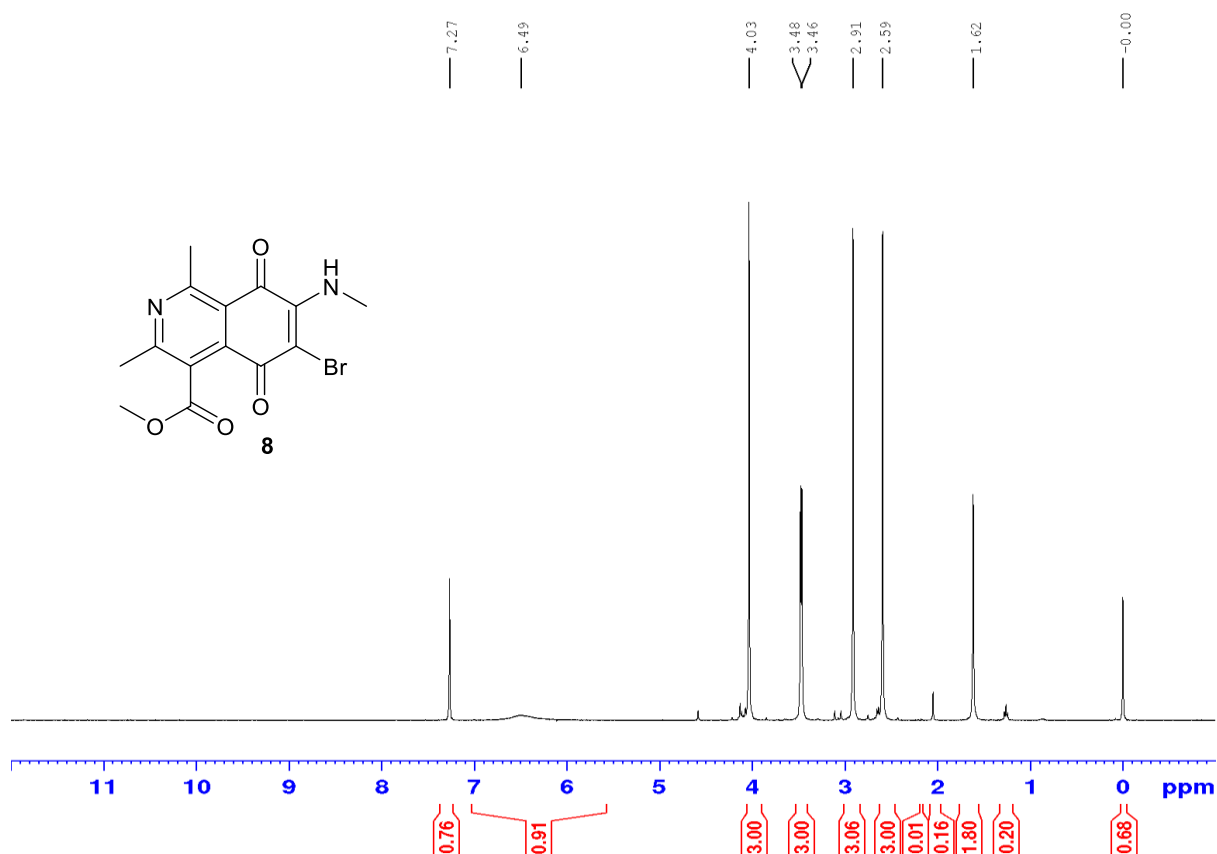
¹H NMR of **7**



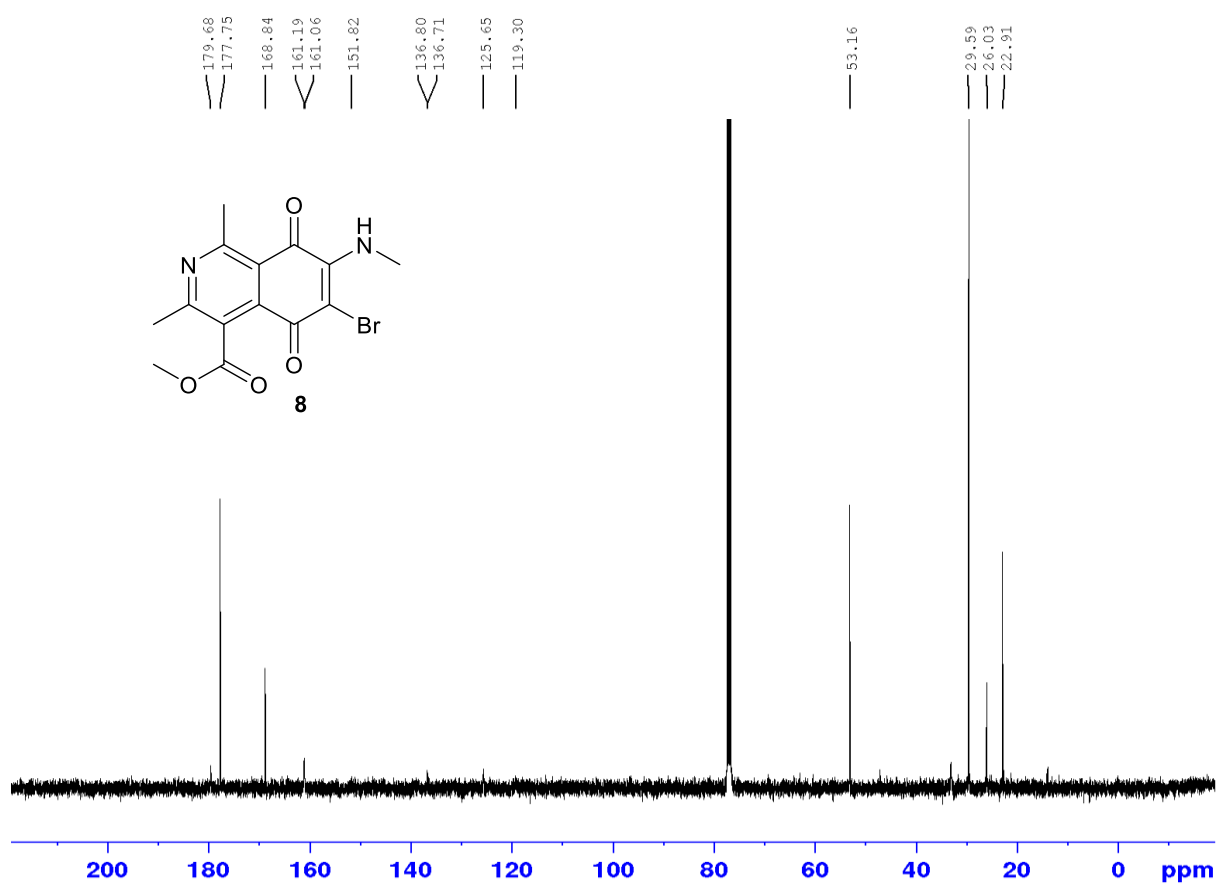
¹³C NMR of **7**



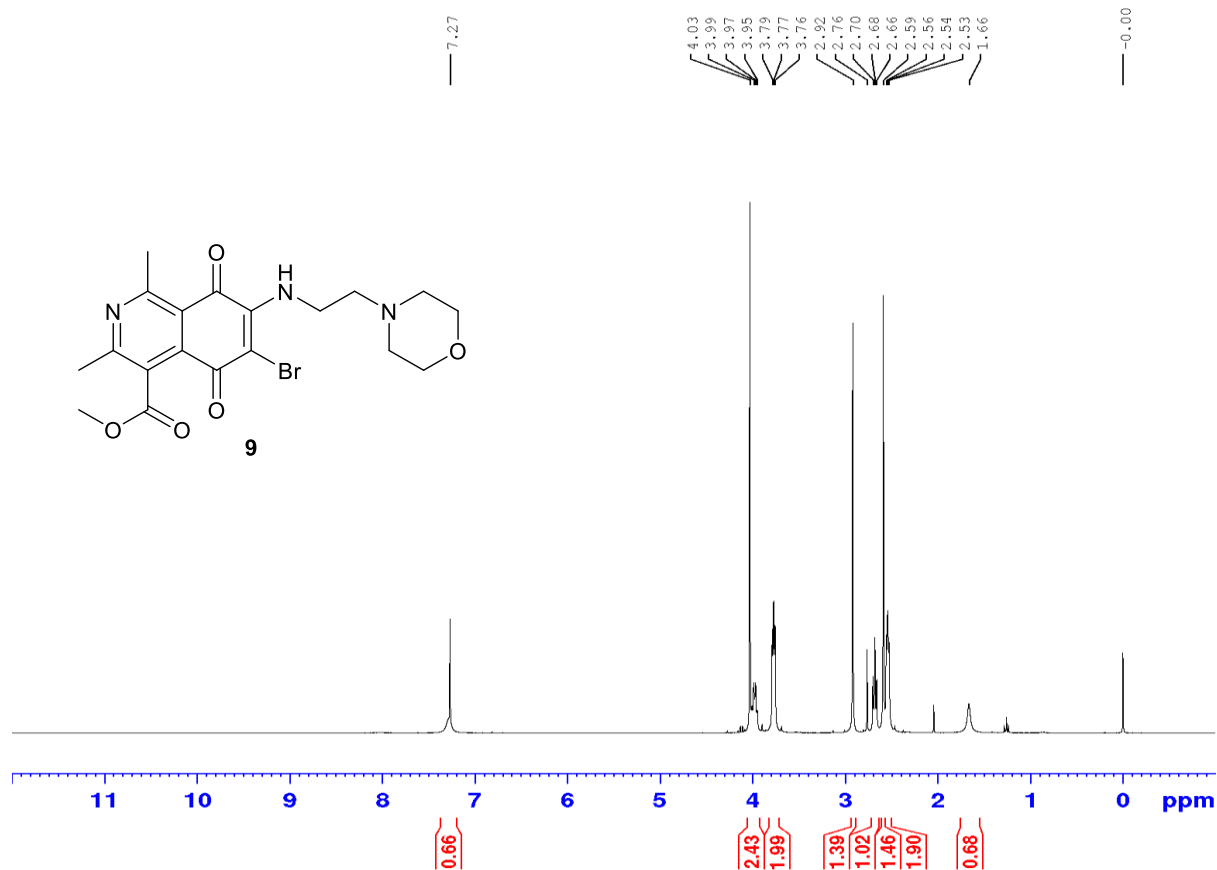
¹H NMR of **8**



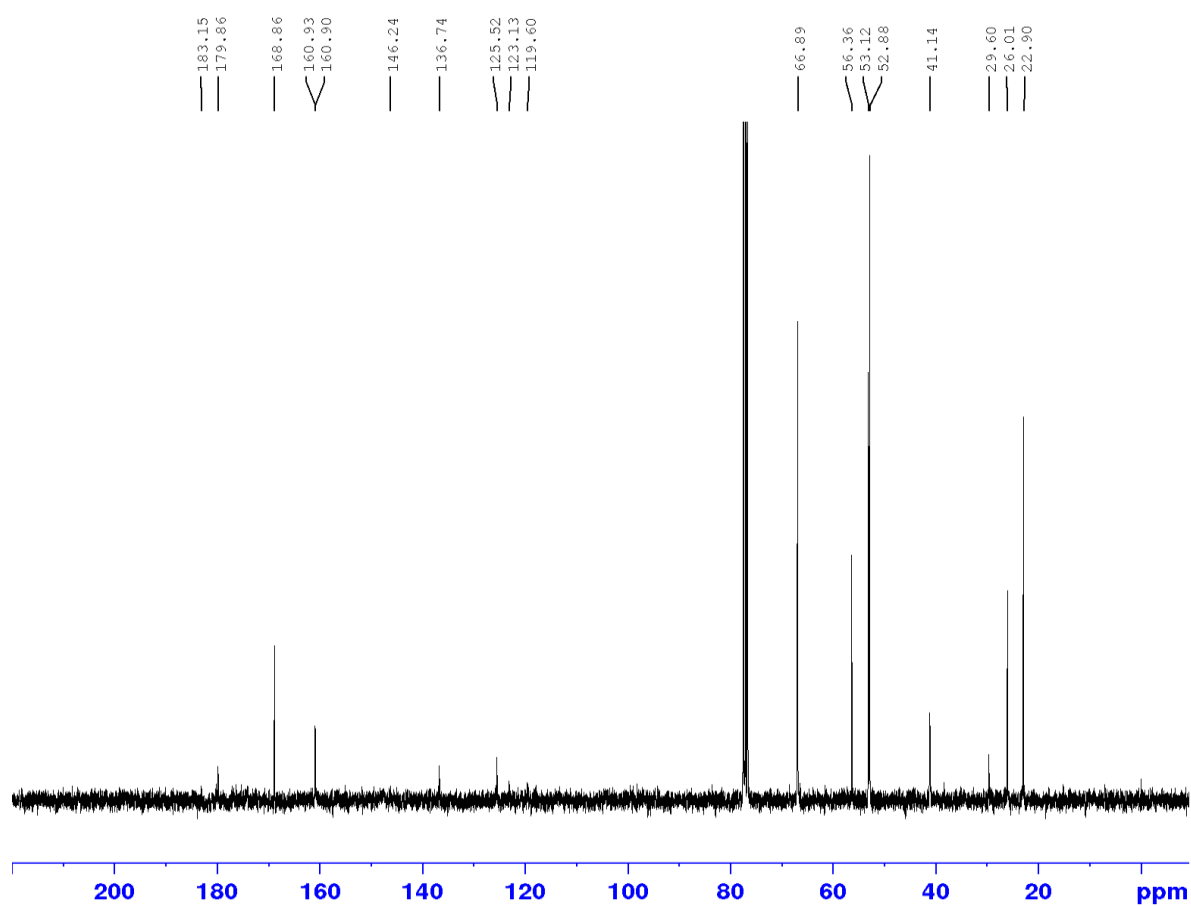
¹³C NMR of **8**



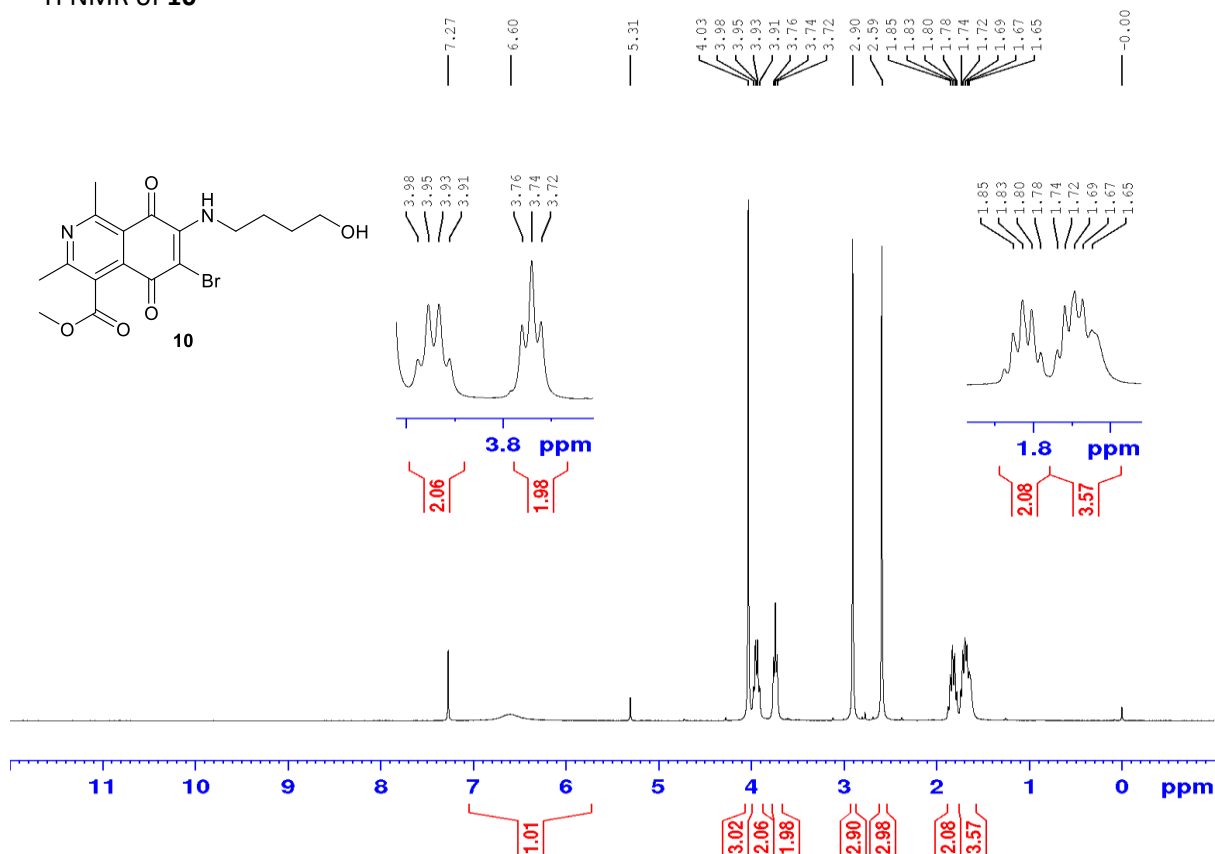
¹H NMR of **9**



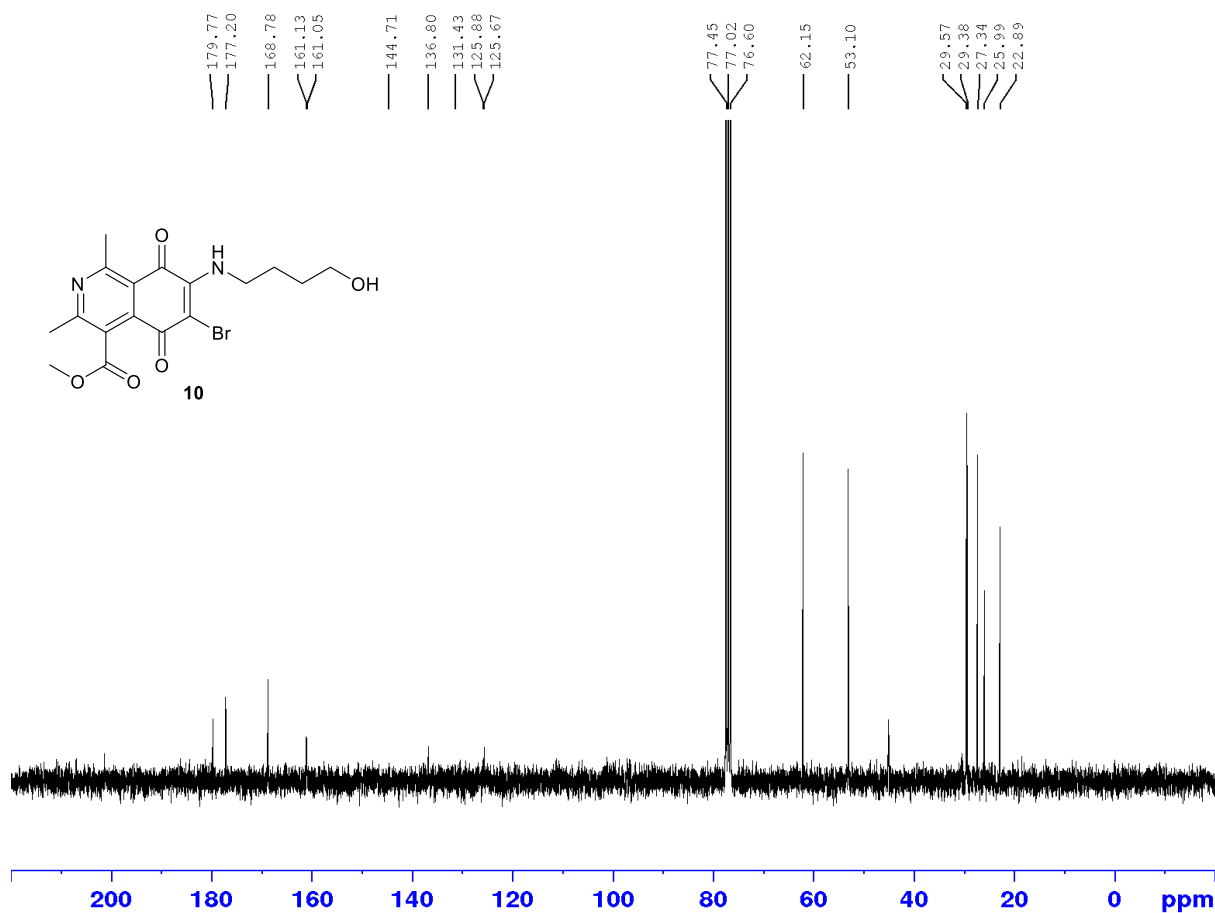
¹³C NMR of **9**



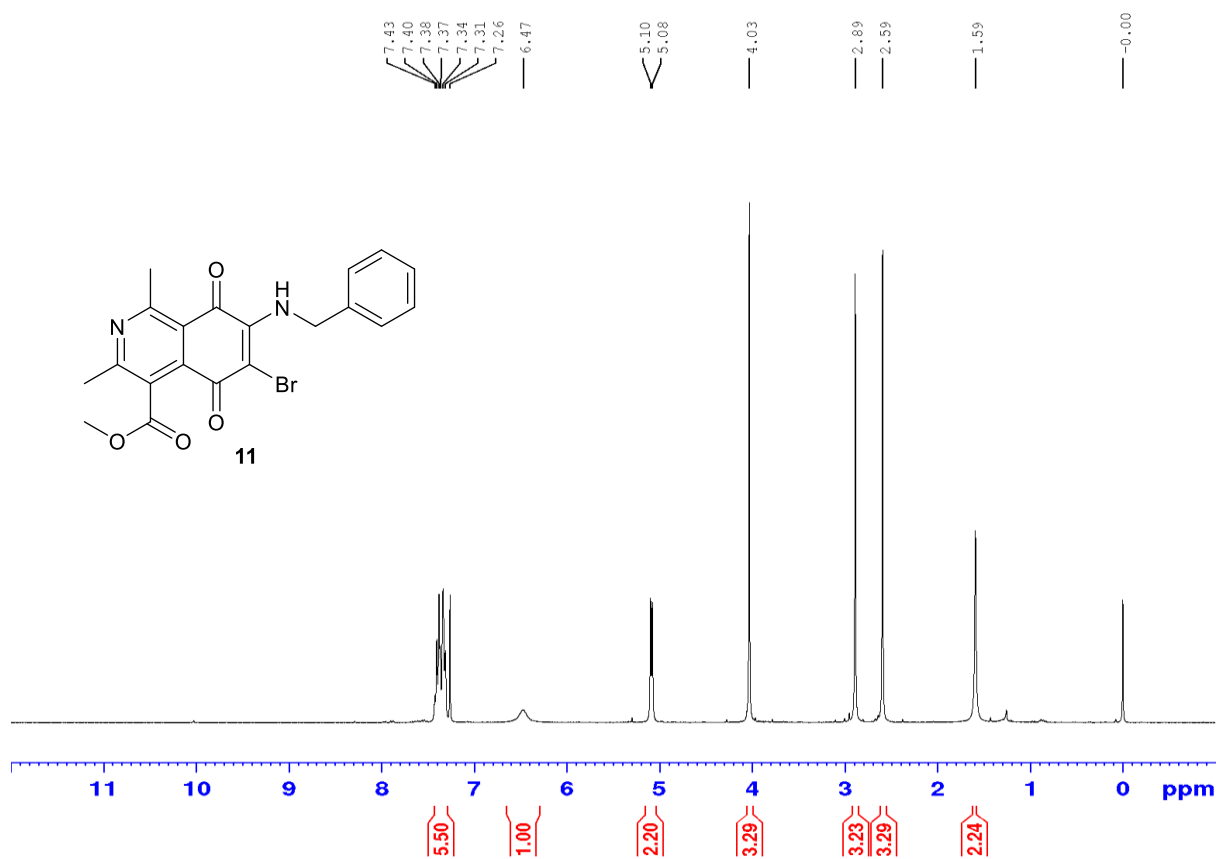
¹H NMR of **10**



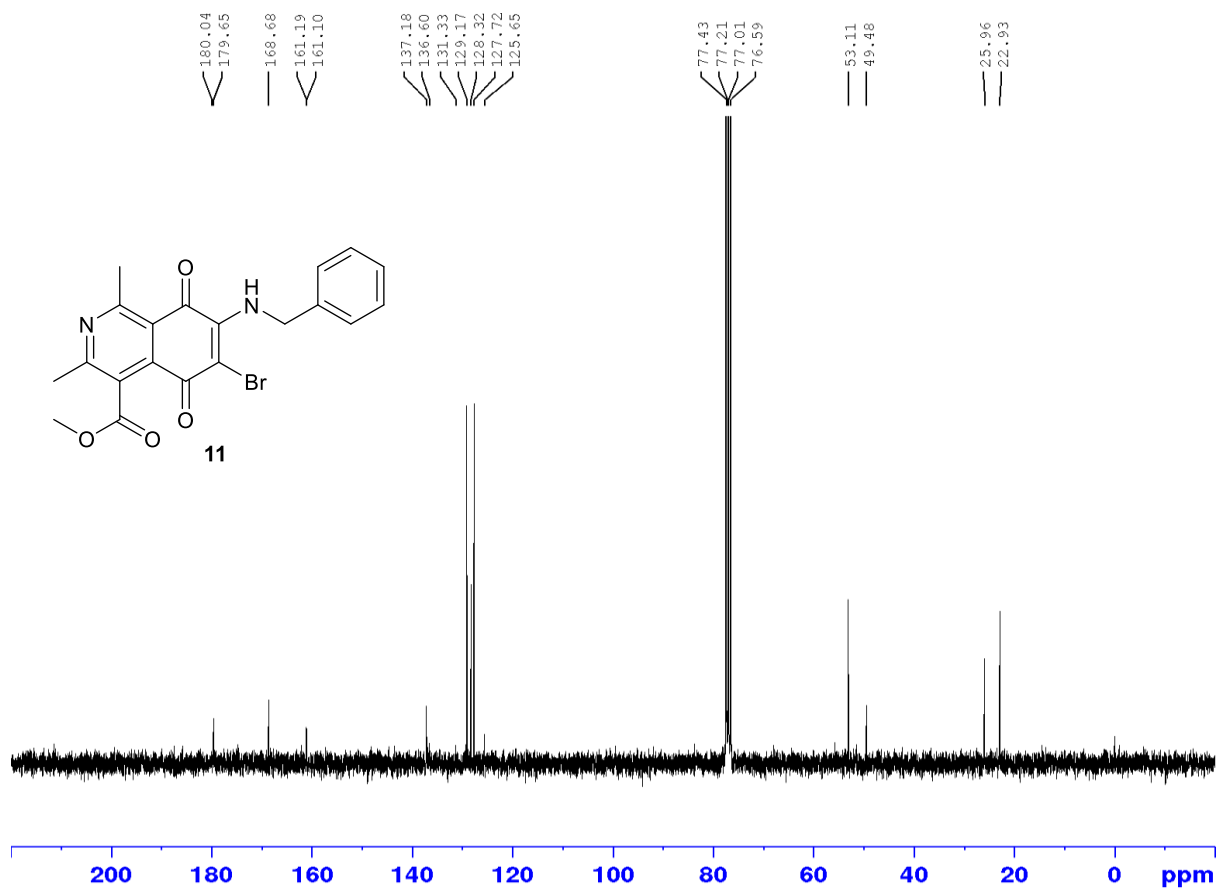
¹³C NMR of **10**



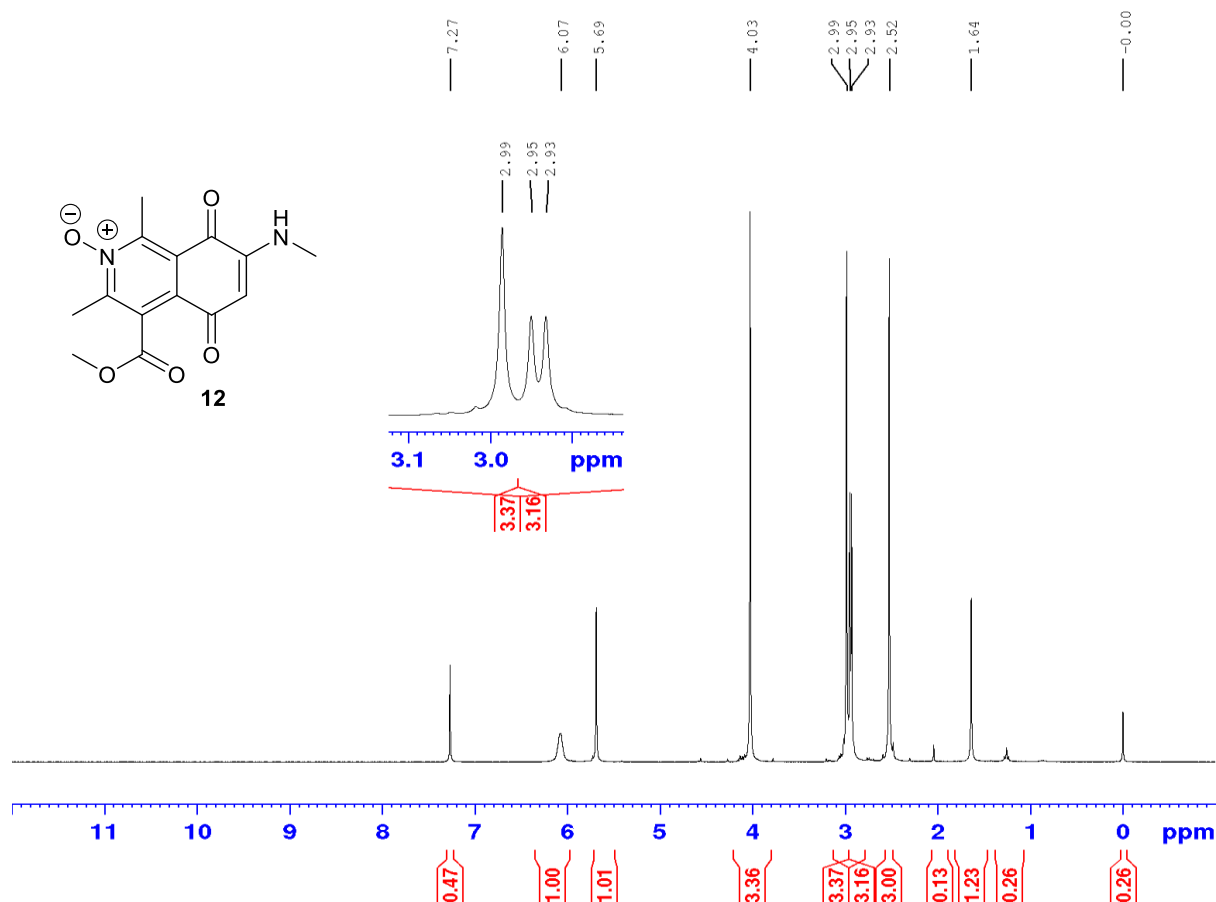
¹H NMR of **11**



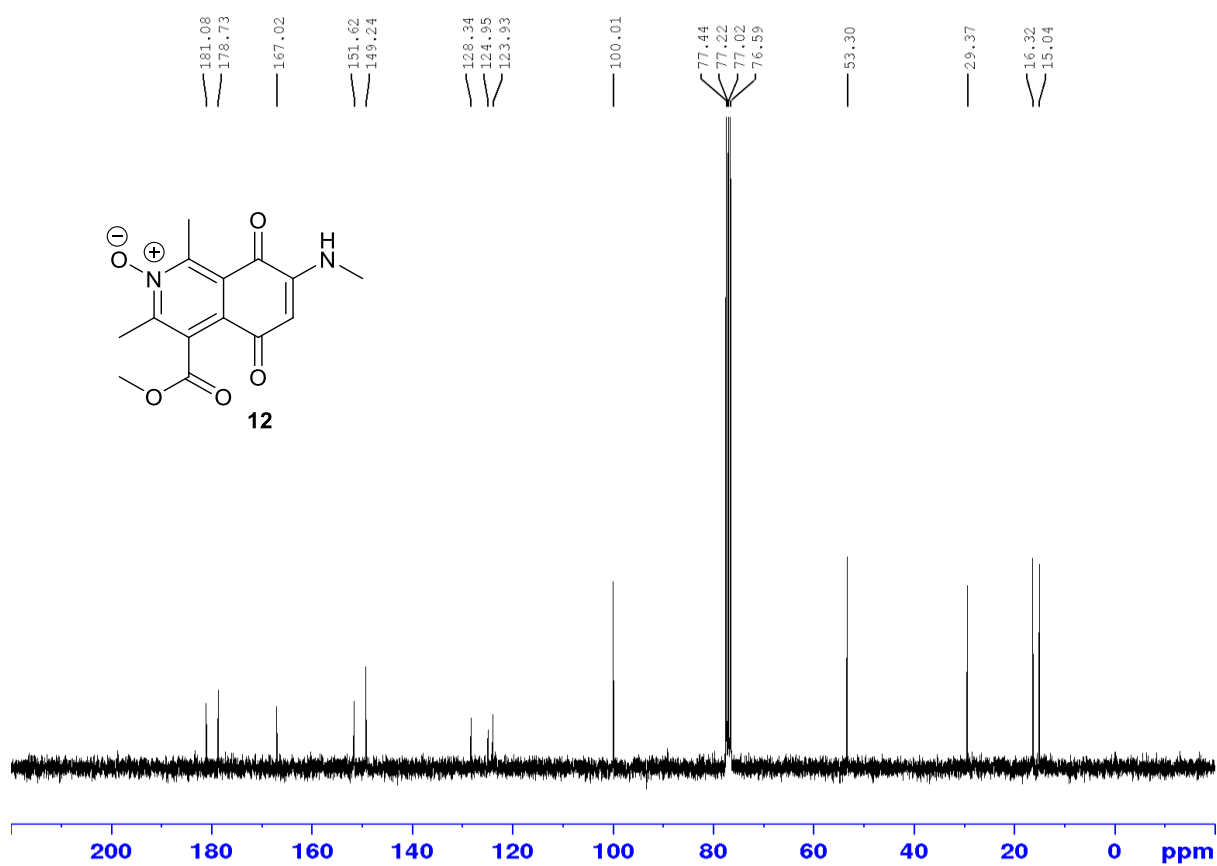
¹³C NMR of **11**



¹H NMR of **12**



¹³C NMR of **12**



Chemical Structure 13: COC1=C(C(=O)N2CCN(CCN2)CC3=CC(=O)C(=C(C1=O)N3[O+])C

¹H NMR Spectrum (DMSO-d₆):

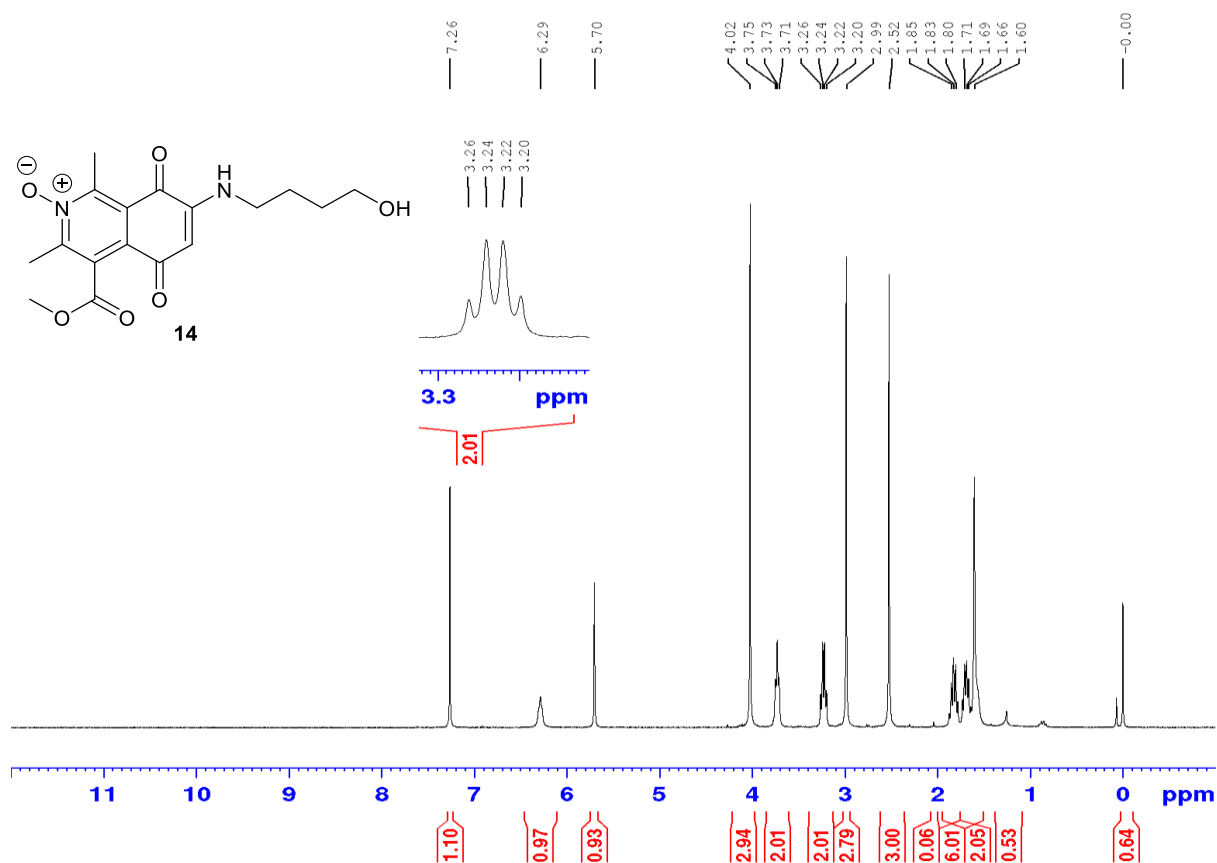
Chemical Shift (ppm)	Integration
7.27	0.56
6.65	1.00
5.68	1.05
3.74	4.20
3.24	2.10
4.02, 3.77, 3.76, 3.74, 3.24, 3.22, 3.20, 3.18, 3.00, 2.72, 2.70, 2.68, 2.52, 2.51, 2.49, 1.67	3.13, 4.20, 2.10, 3.19, 2.19, 7.32
2.72, 2.70, 2.68	2.19
0.00	-

13

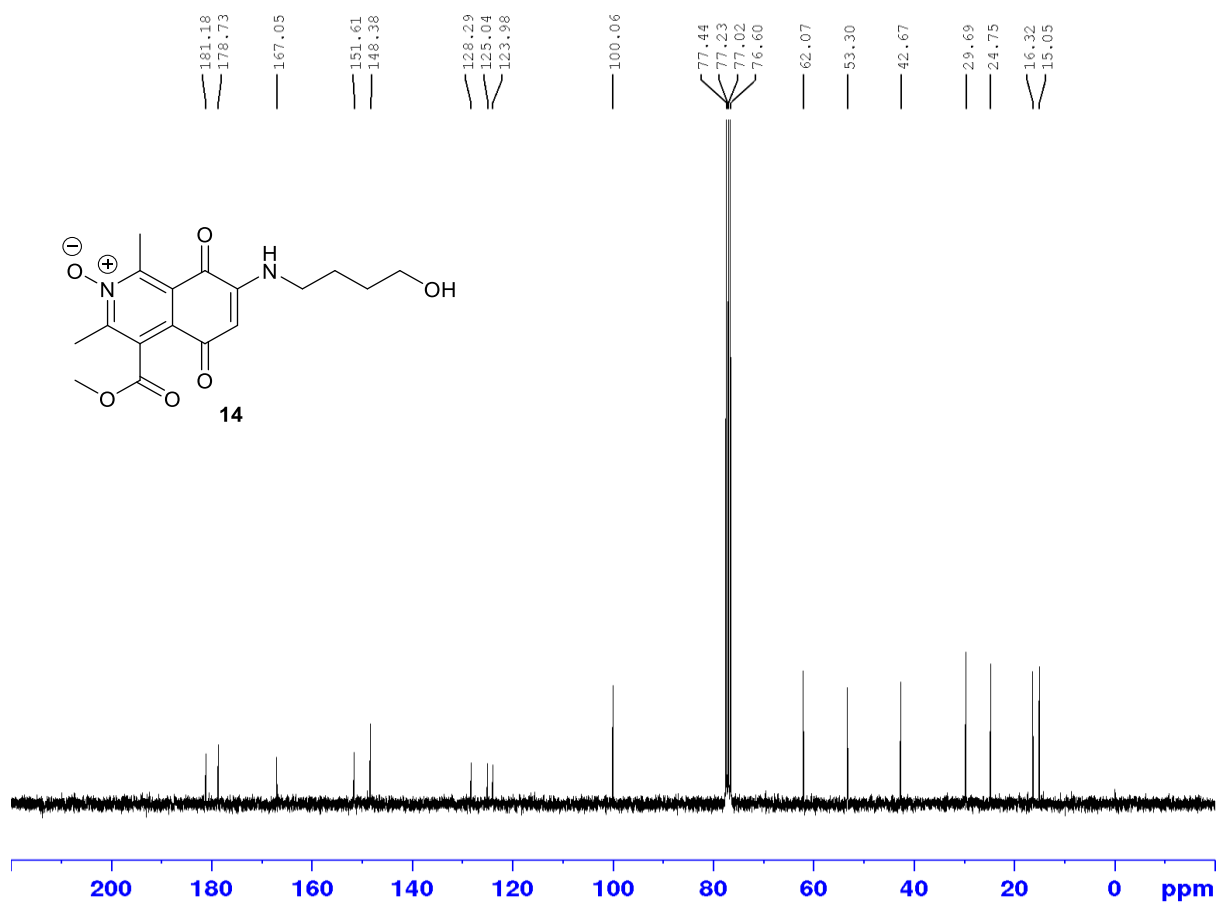
Chemical structure of compound **13** is shown. The structure is a pyridine ring substituted with a methoxy group, a methyl group, and a 2-(2-methoxy-2-oxo-1,2-dihydro-3H-pyridin-3-yl)ethyl group. The spectrum shows peaks corresponding to the structure, with the following chemical shifts (ppm) labeled:

- 181.05
- 178.77
- 167.03
- 151.63
- 151.58
- 148.27
- 128.30
- 124.93
- 124.01
- 100.34
- 77.44
- 77.01
- 76.59
- 66.88
- 55.37
- 53.29
- 53.16
- 38.51
- 16.32
- 15.04

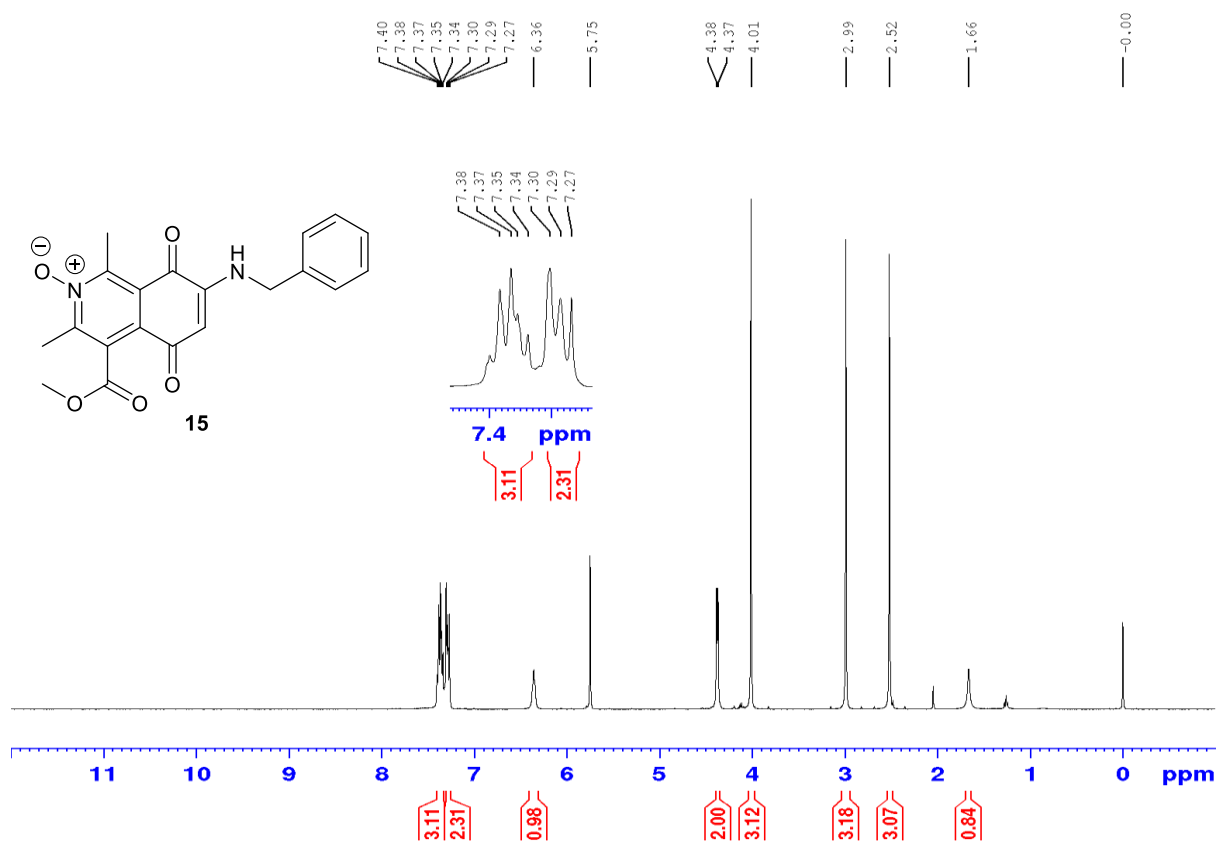
¹H NMR of **14**



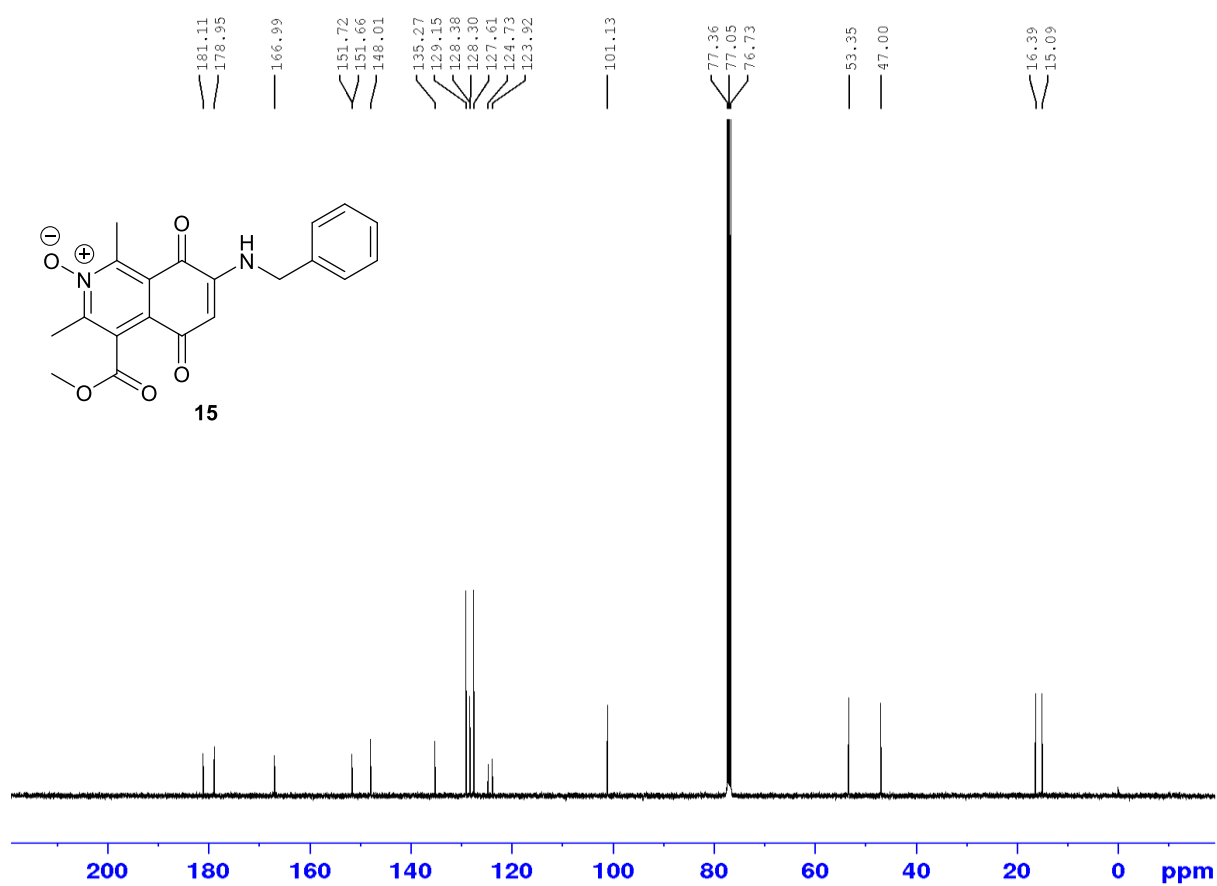
¹³C NMR of **14**



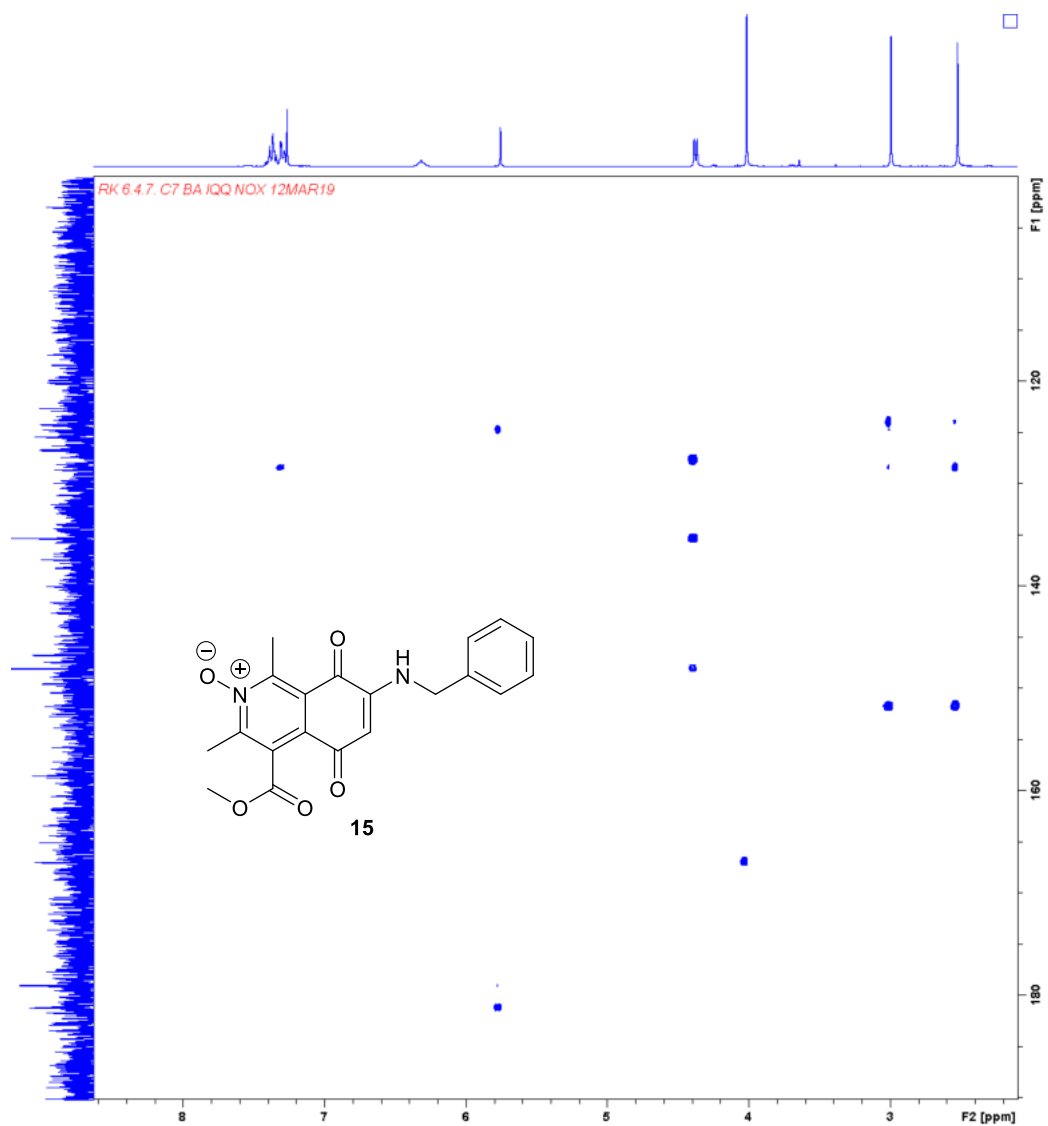
¹H NMR of **15**



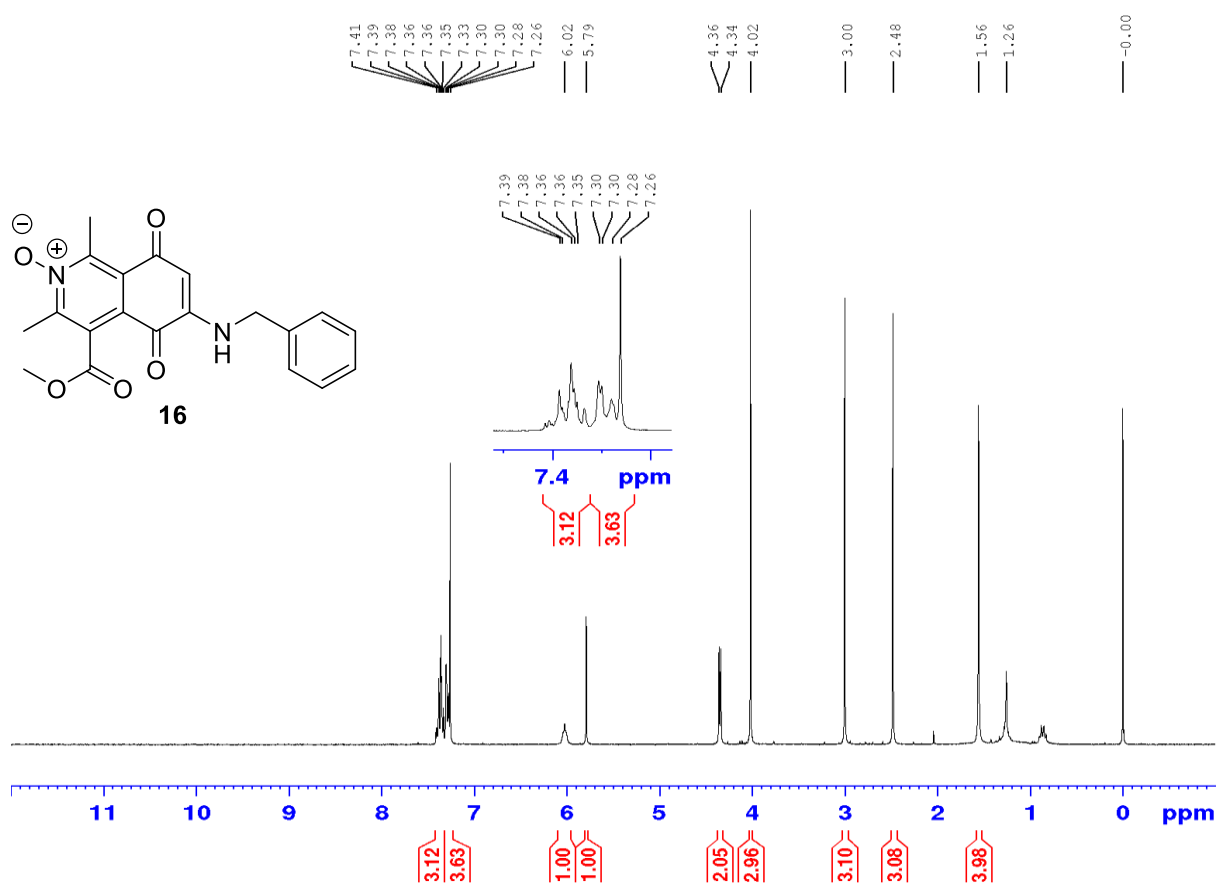
¹³C NMR of **15**



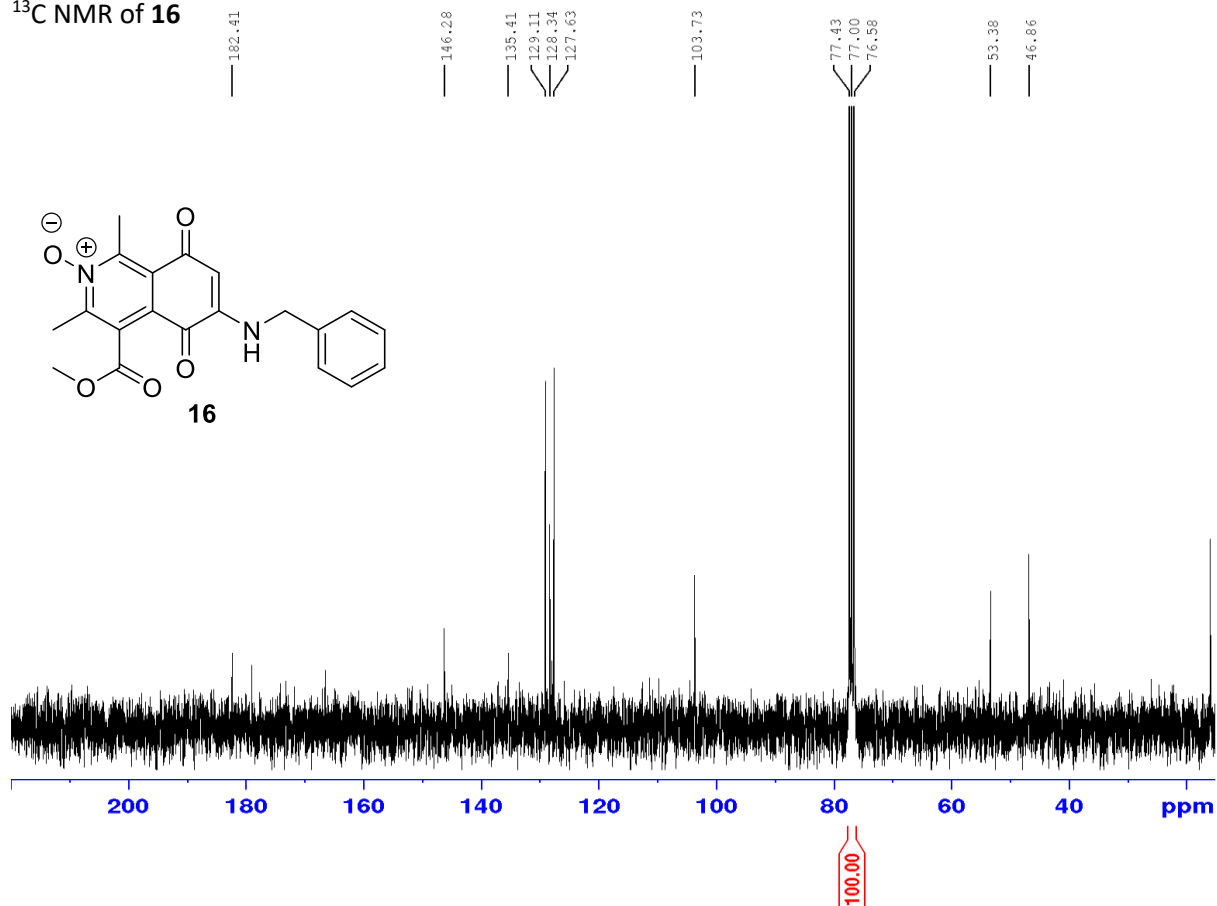
HMBC of **15**



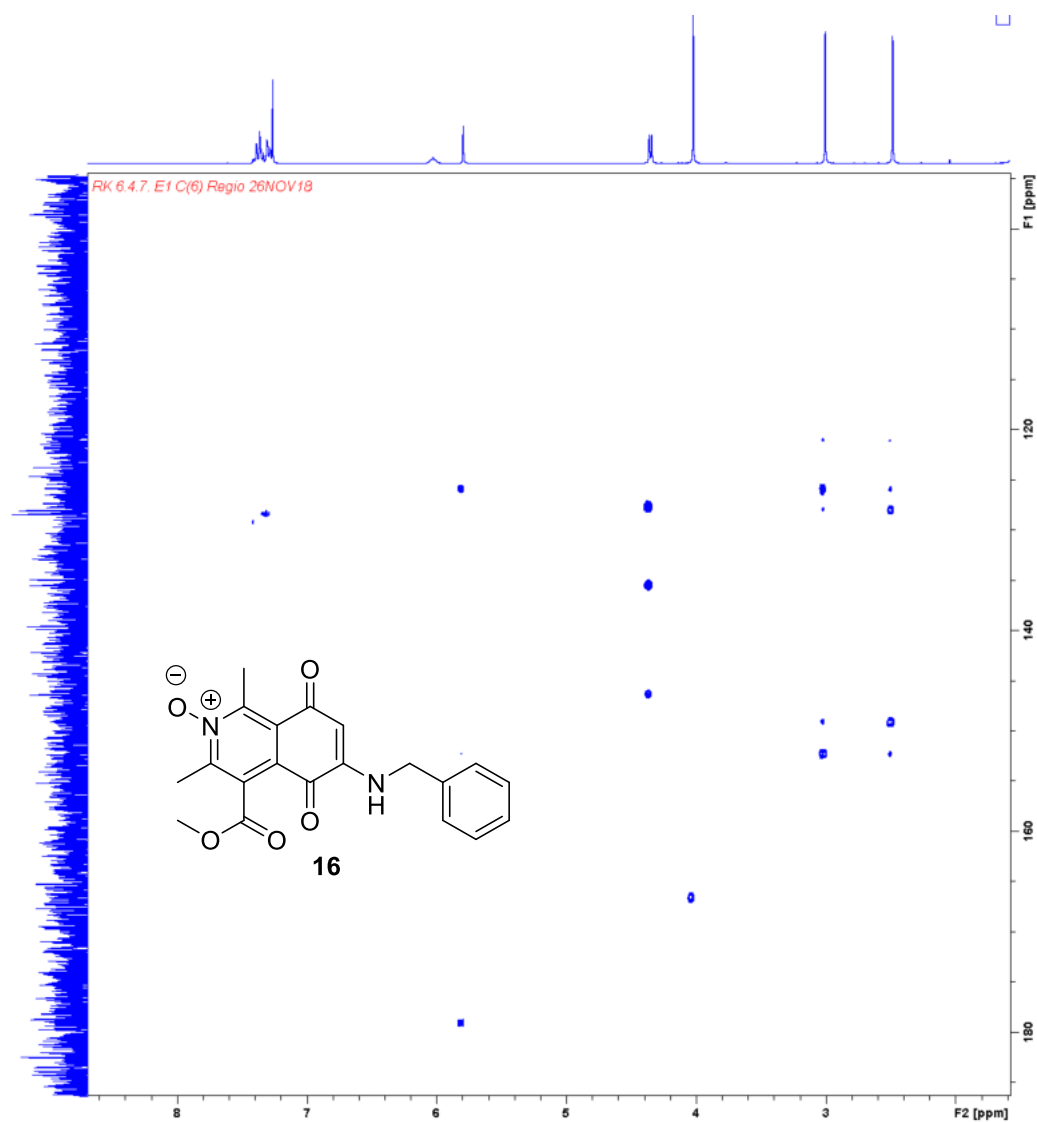
¹H NMR of **16**



¹³C NMR of **16**

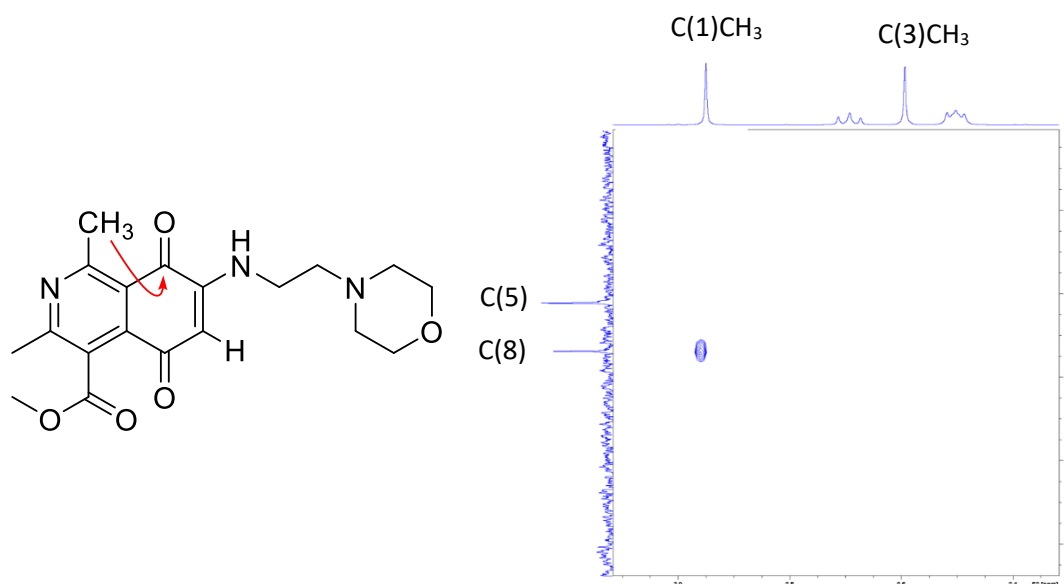


HMBC of **16**

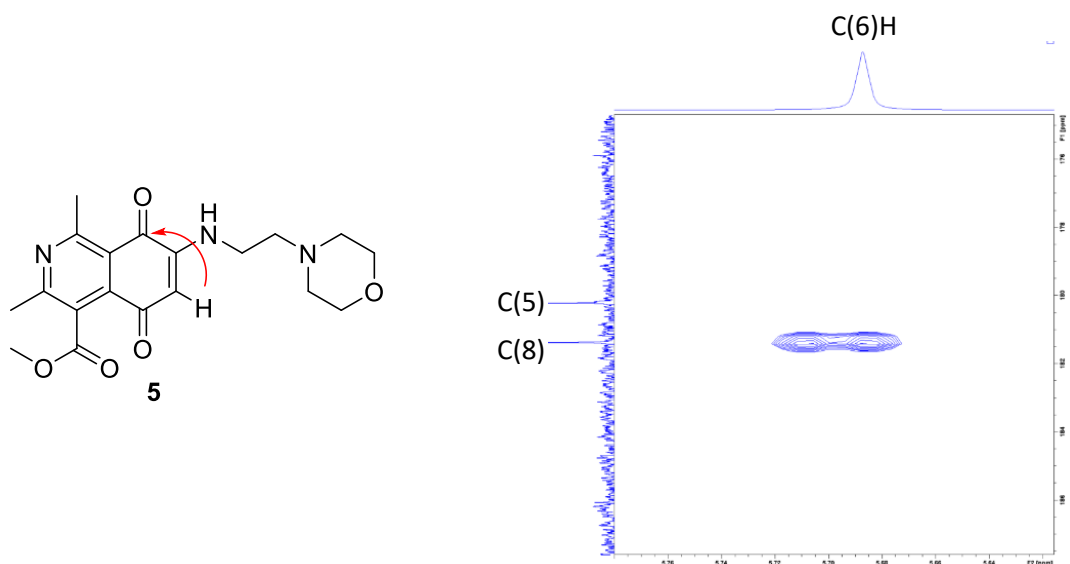


Confirmation of regioisomer assignment of compound 15 and 16

- With respect to morpholino derivative **5** – Crystal structure obtained
- On HMBC analysis, C(1)CH₃ is more downfield and correlated to the C(8) quinone



- C(6)H correlates to C(8) quinone confirmed using X-ray single crystal and HMBC



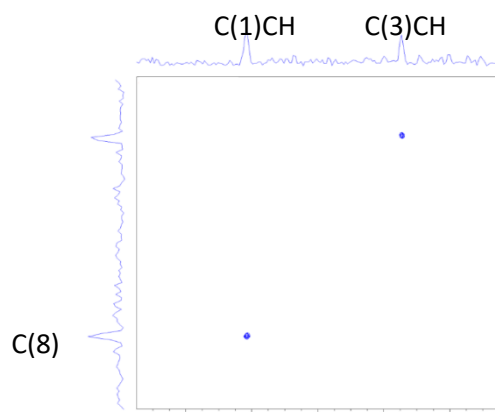
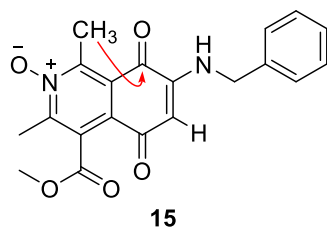
For the benzylamine isoquinolinequinone N-oxides **15** and **16**:

C(7) Regioisomer **15** - Correlation of C(6)H with 181.0 ppm, which is the C(8) carbonyl

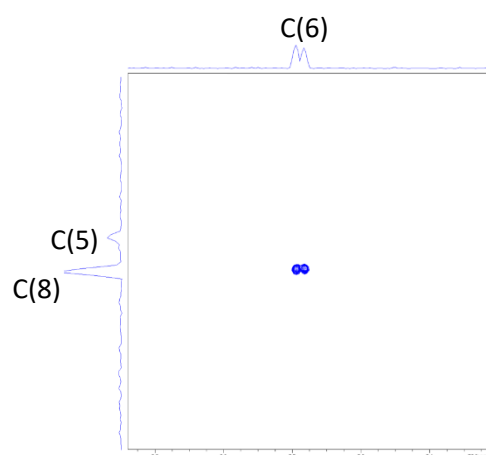
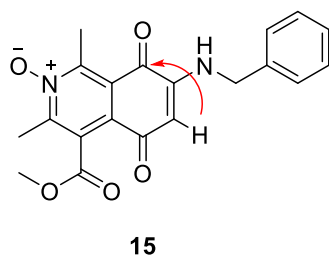
C(6) Regioisomer **16** - Correlation of C(7)H with 179.1 ppm, which is the C(5) carbonyl

C(7) Regioisomer 15

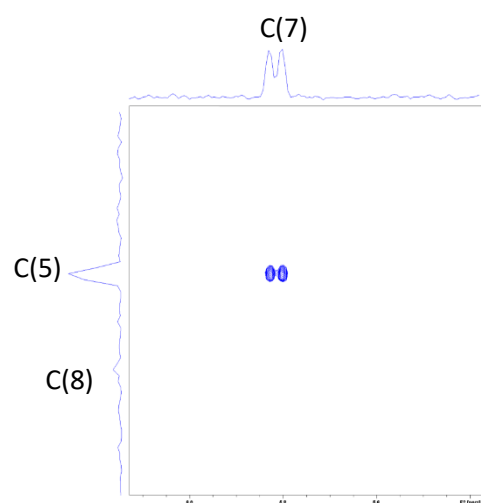
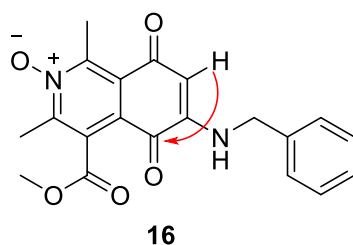
- Like morpholino derivative **5**, C(1)CH₃ correlates to C(8) carbonyl



- C(6)H also correlates to the C(8) carbonyl carbon



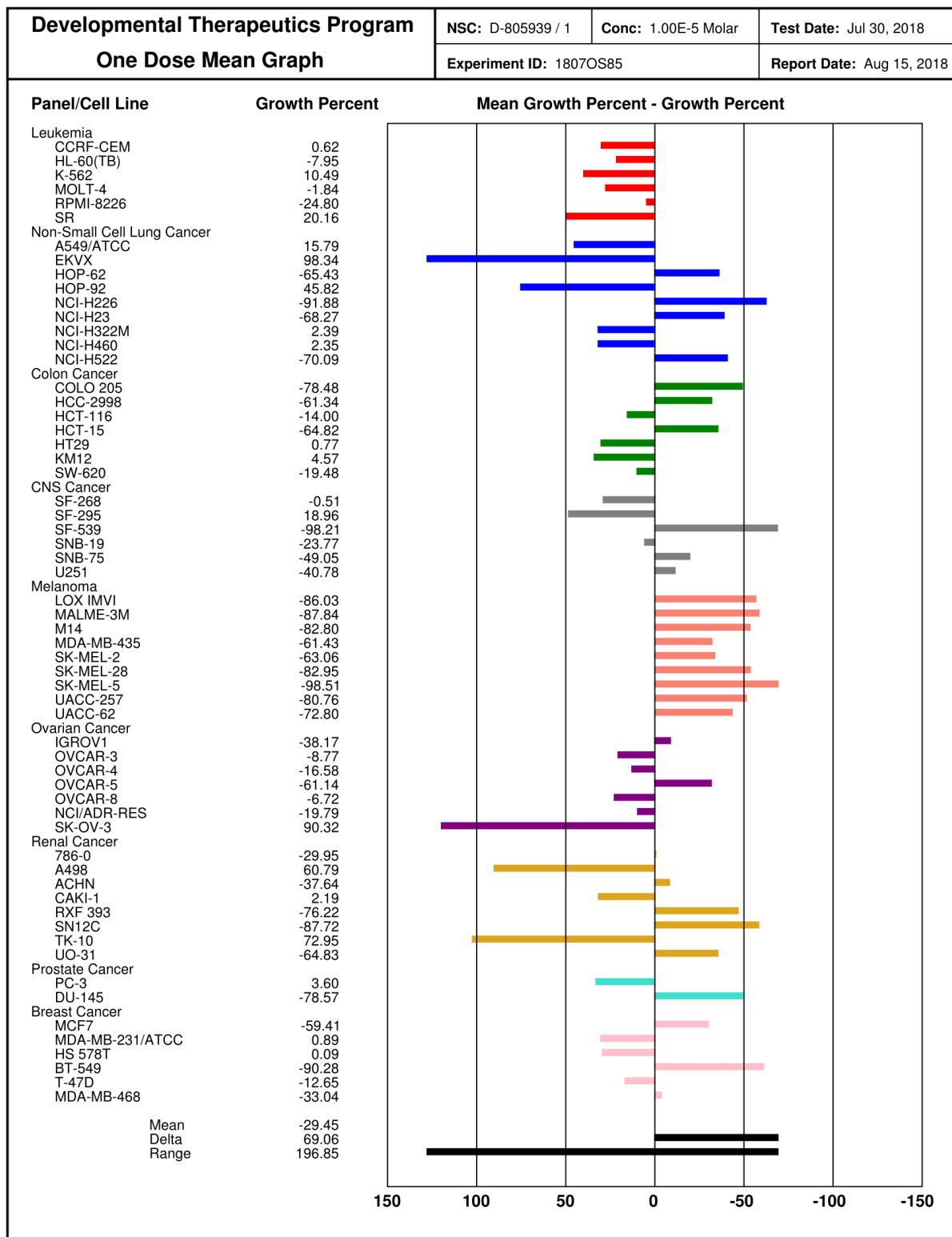
C(6) Regioisomer 16



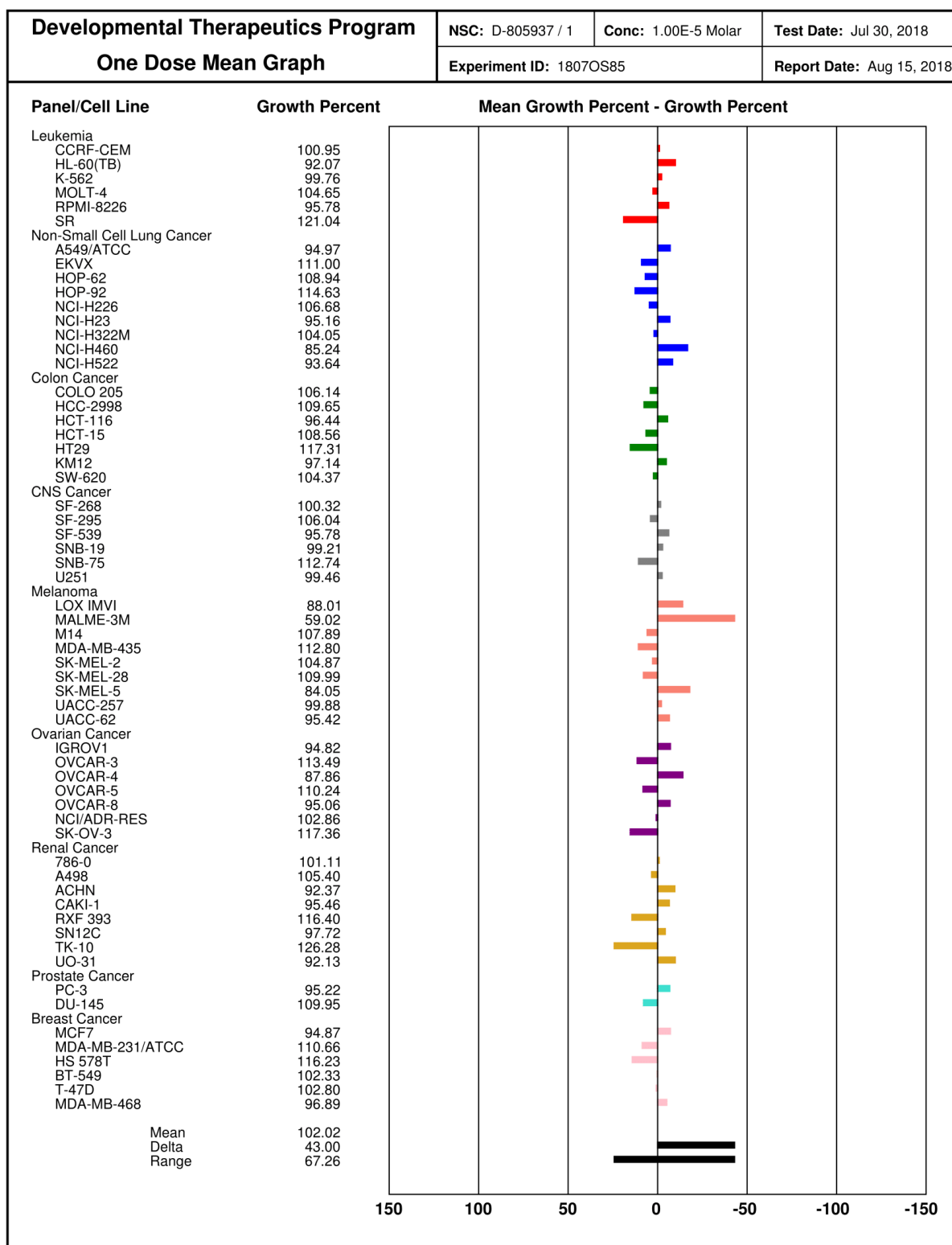
- C(7)H correlates to the C(5) carbonyl carbon

NCI60 One dose Screen

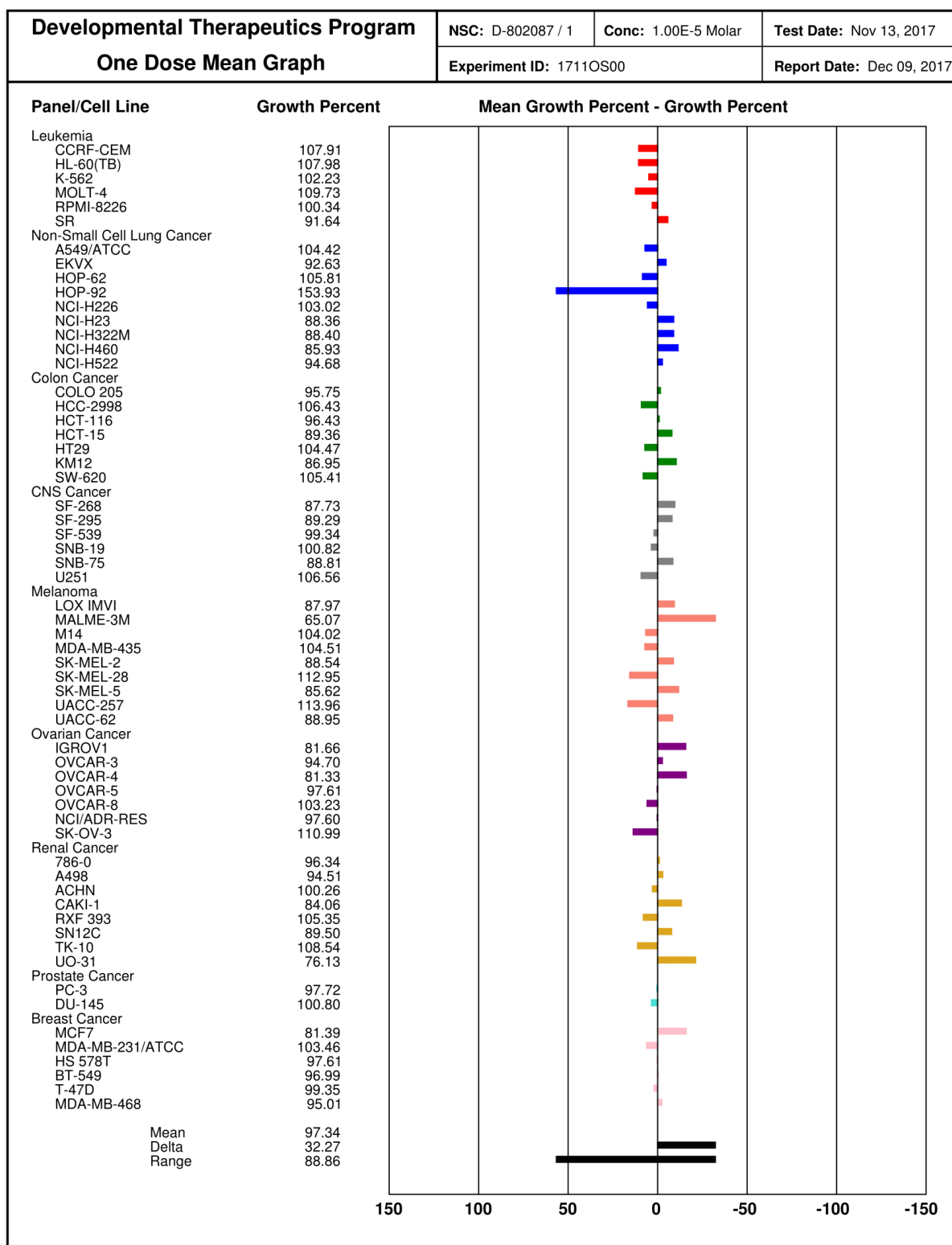
Compound 3



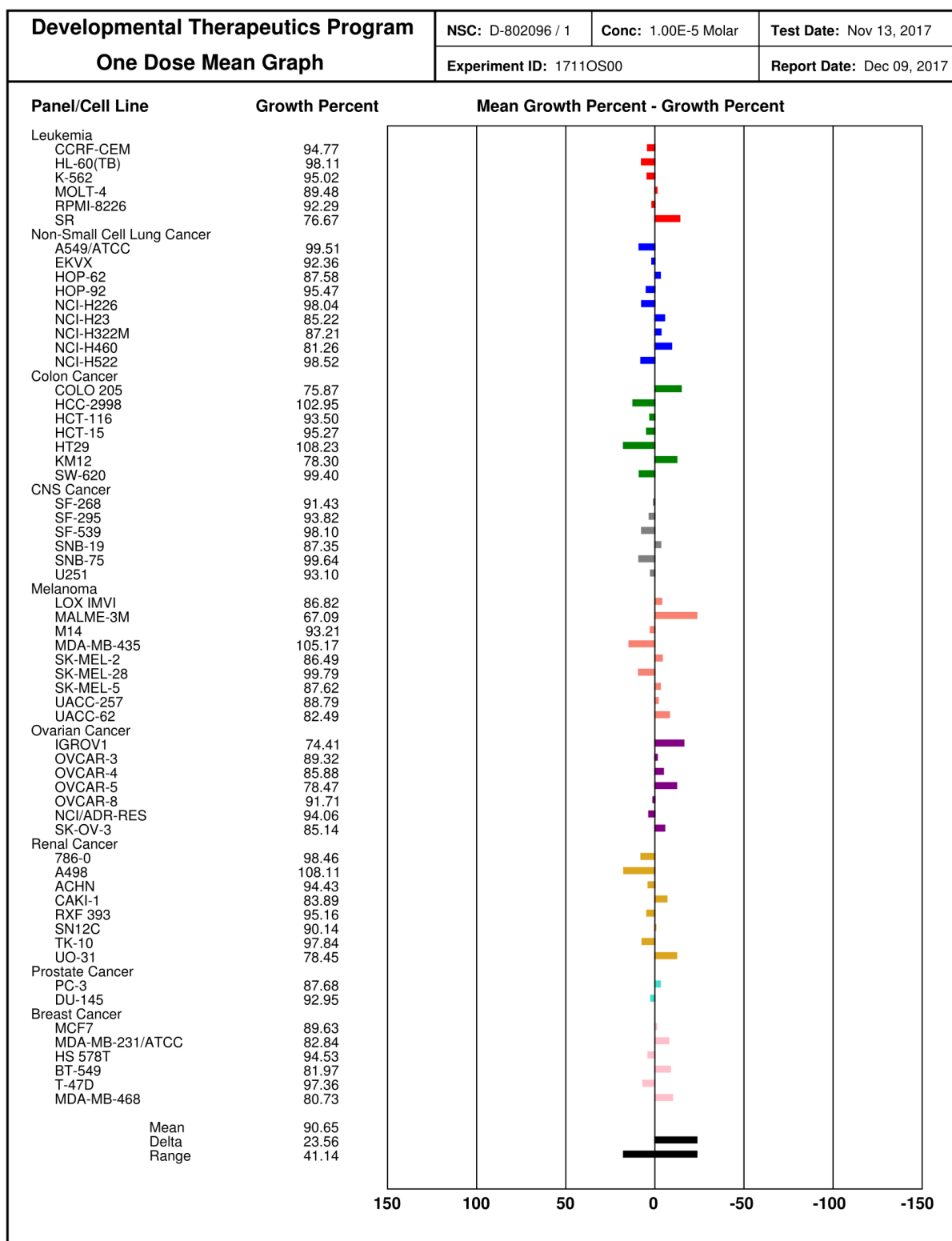
Compound 4



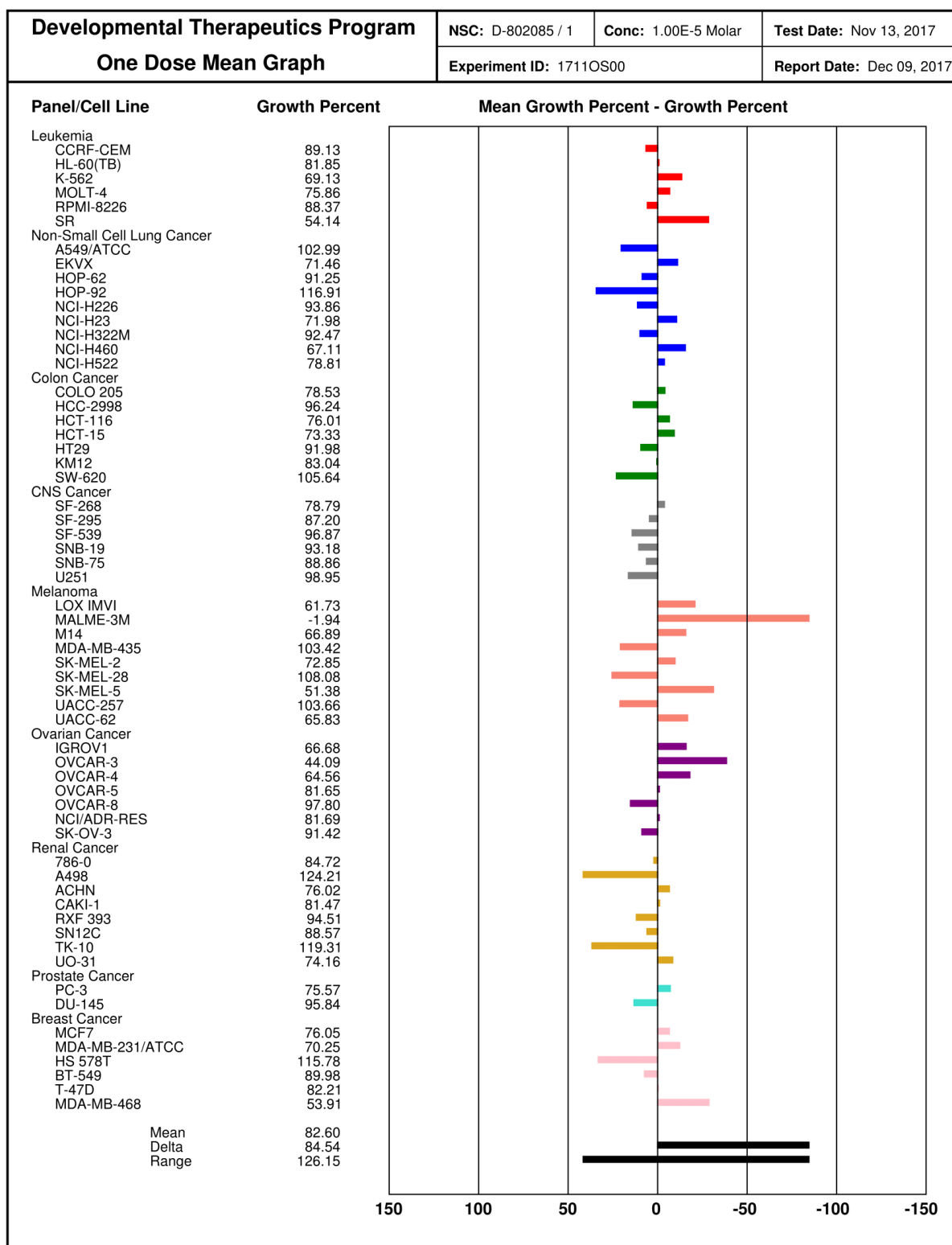
Compound 5



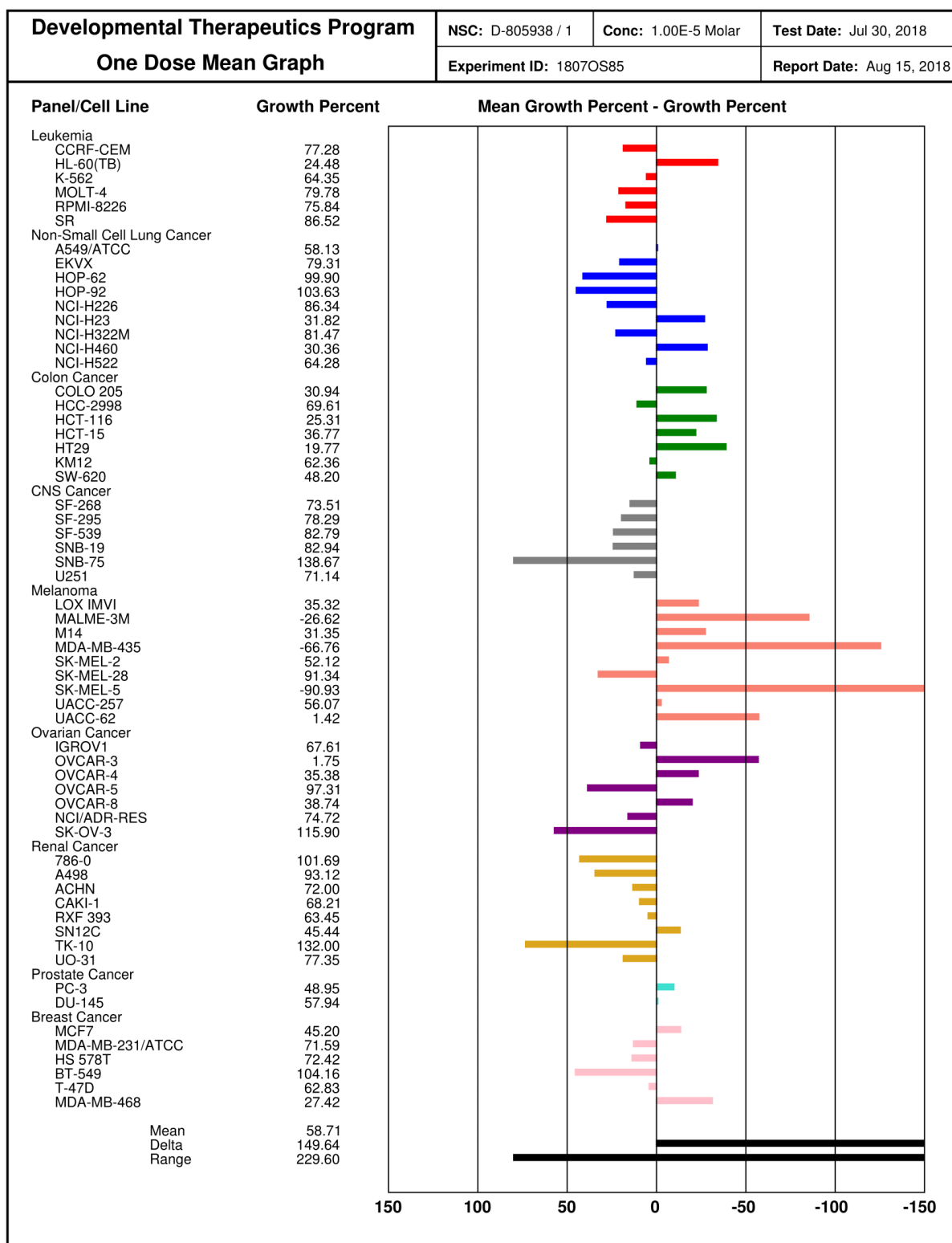
Compound 6



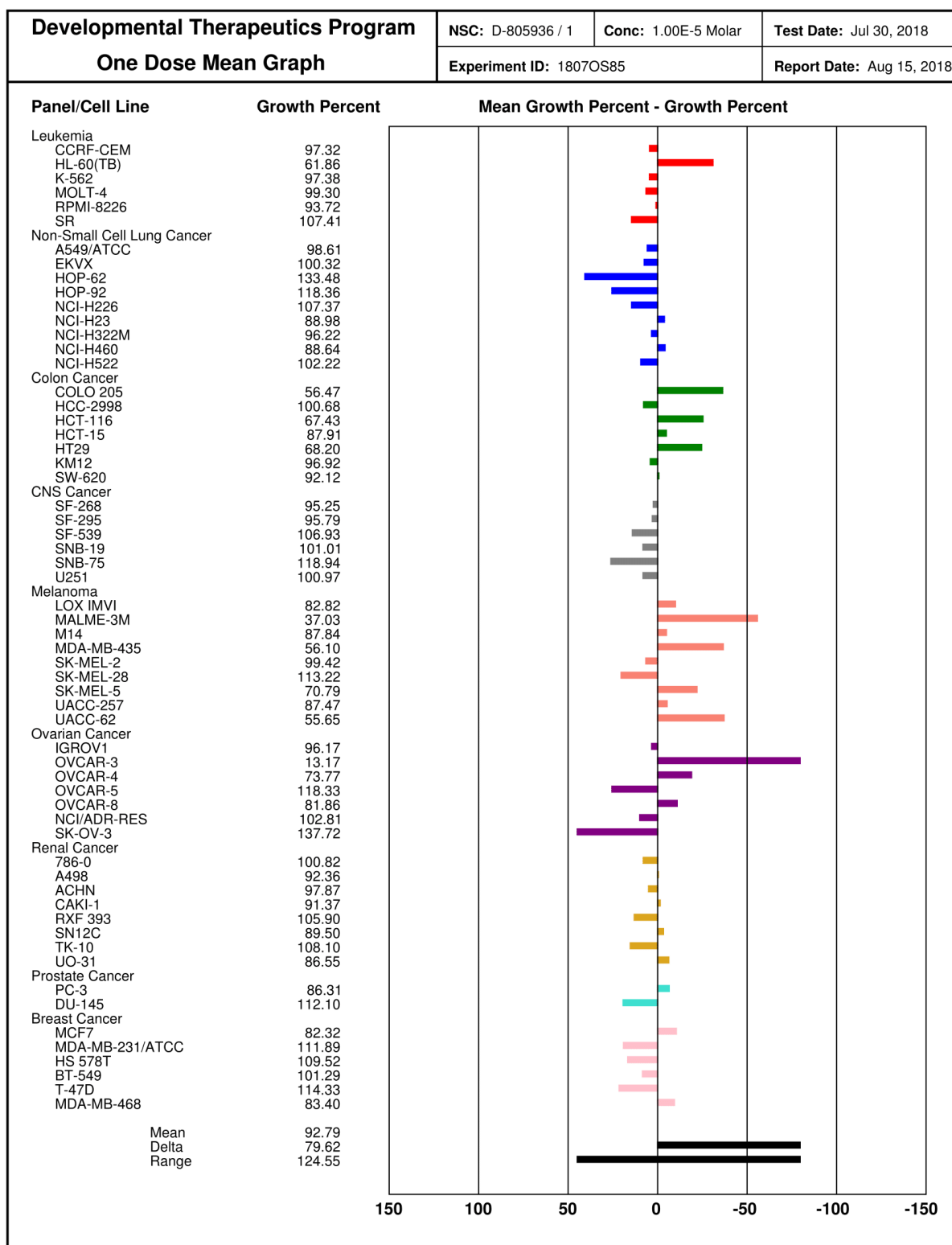
Compound 7



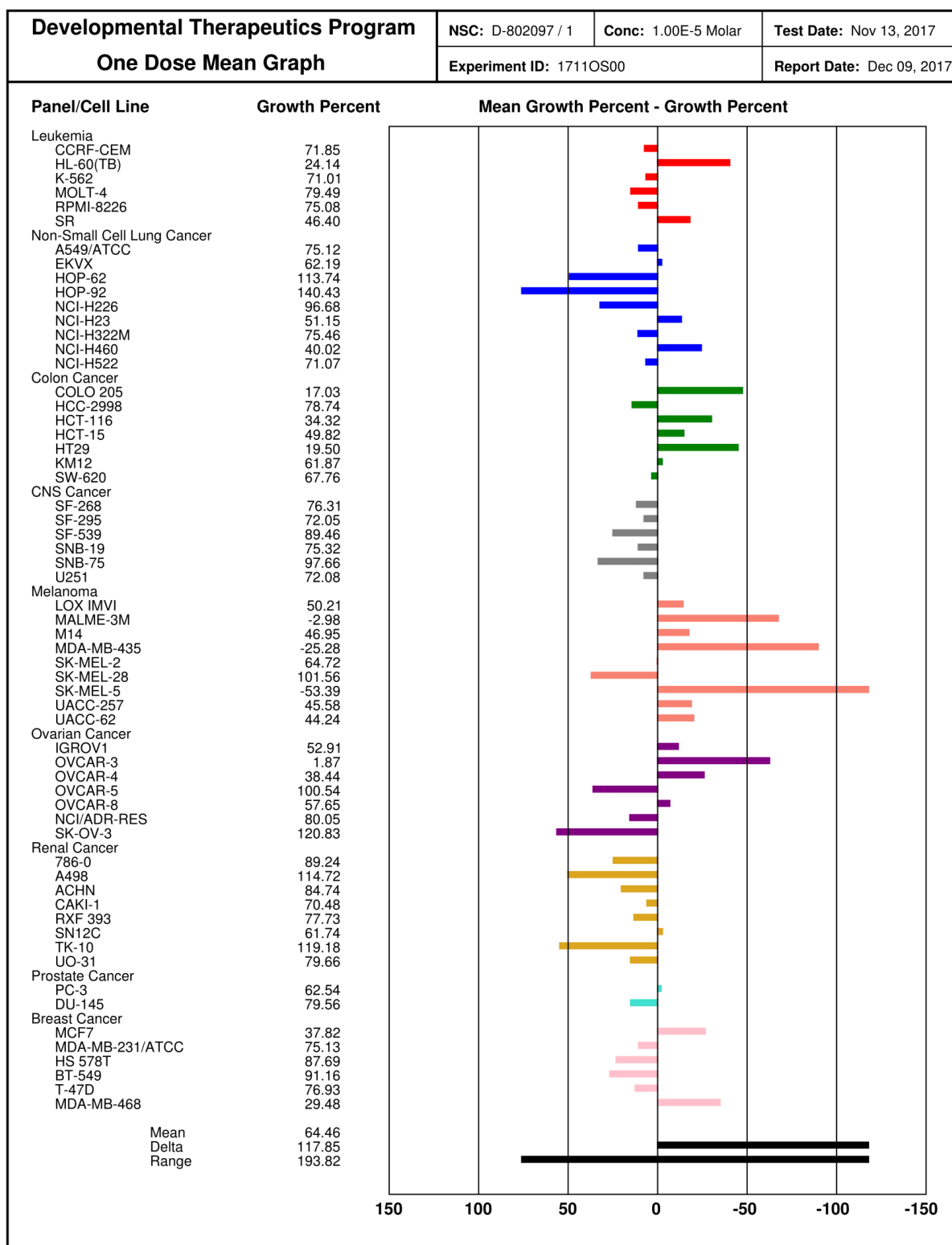
Compound 8



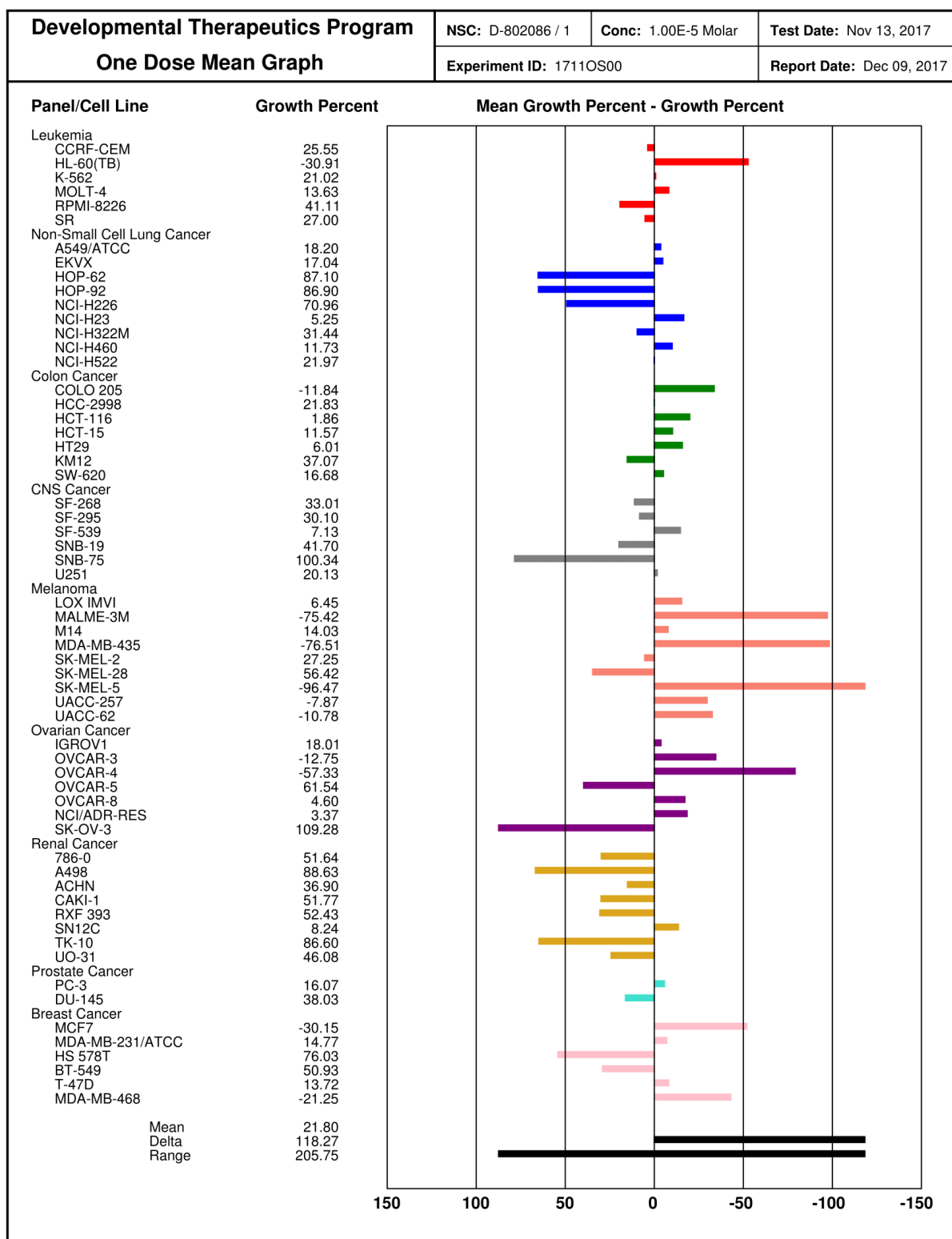
Compound 9



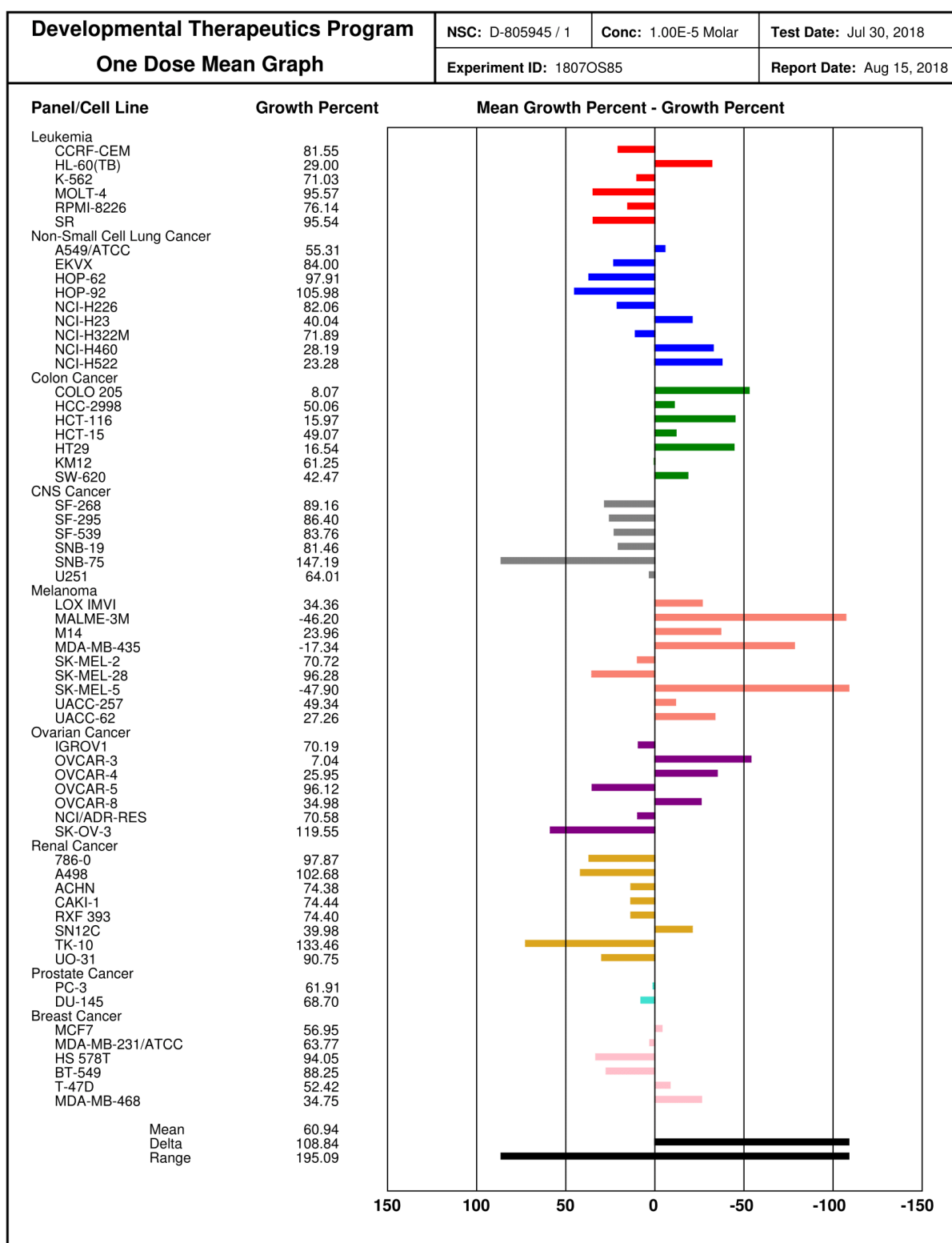
Compound 10



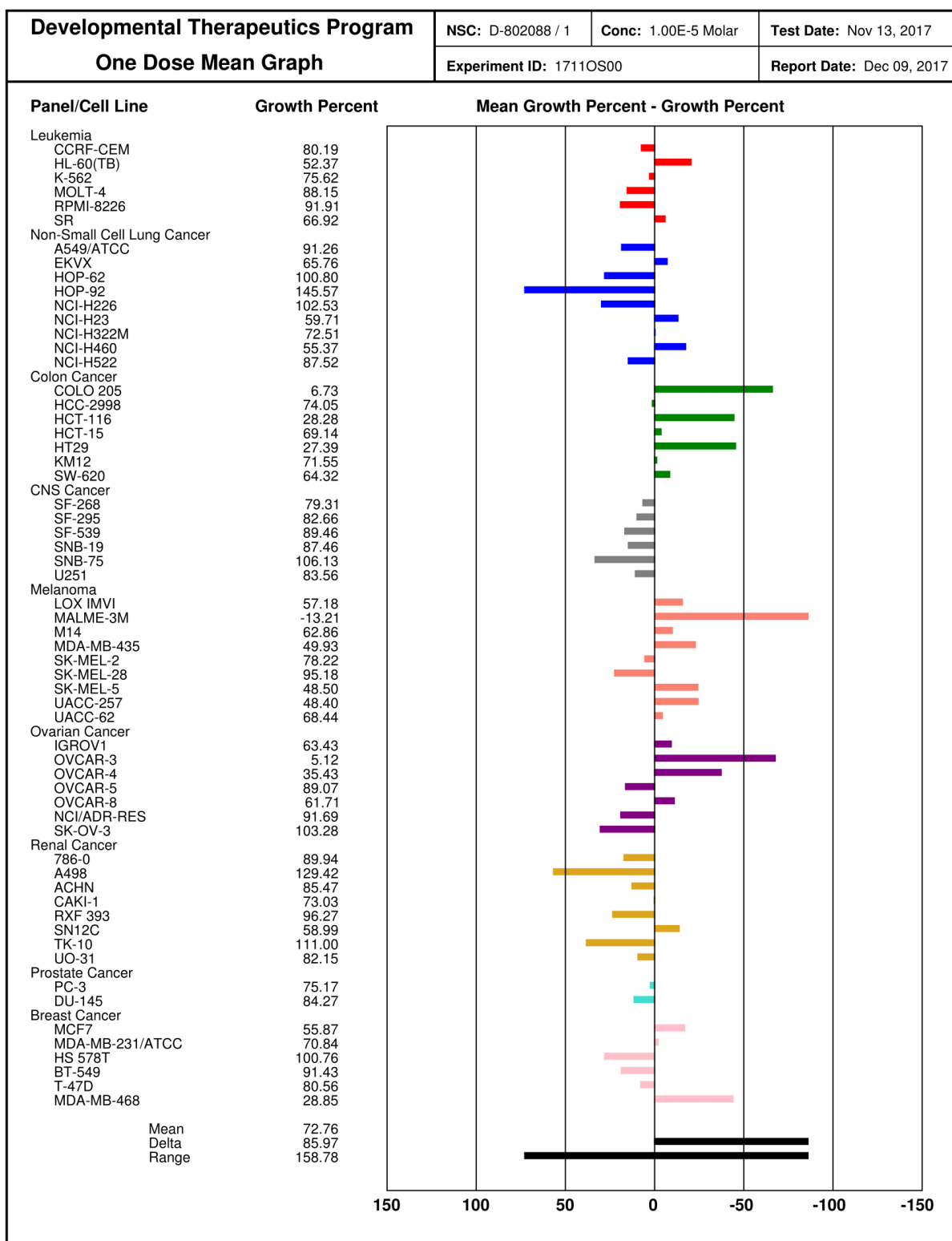
Compound 11



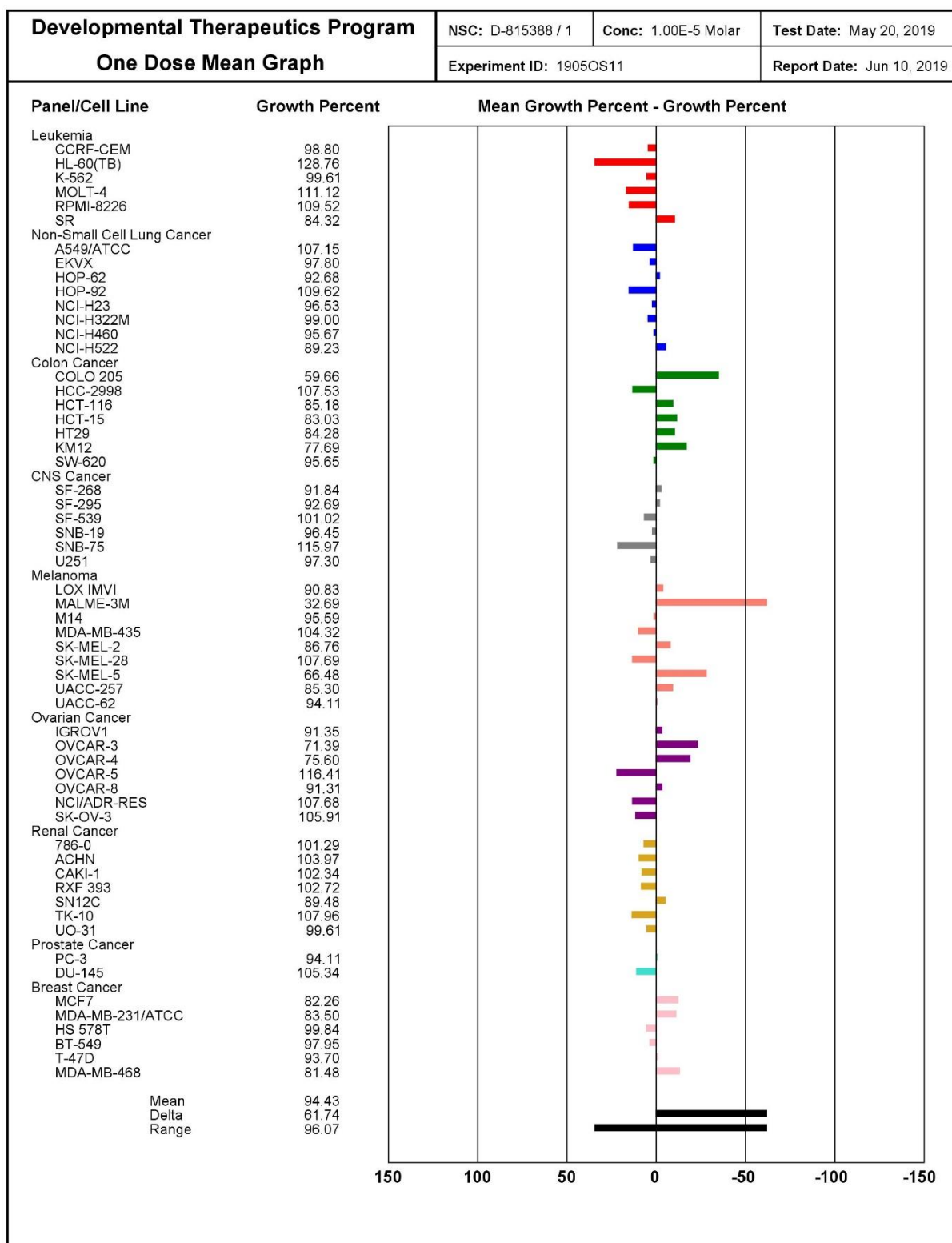
Compound 12



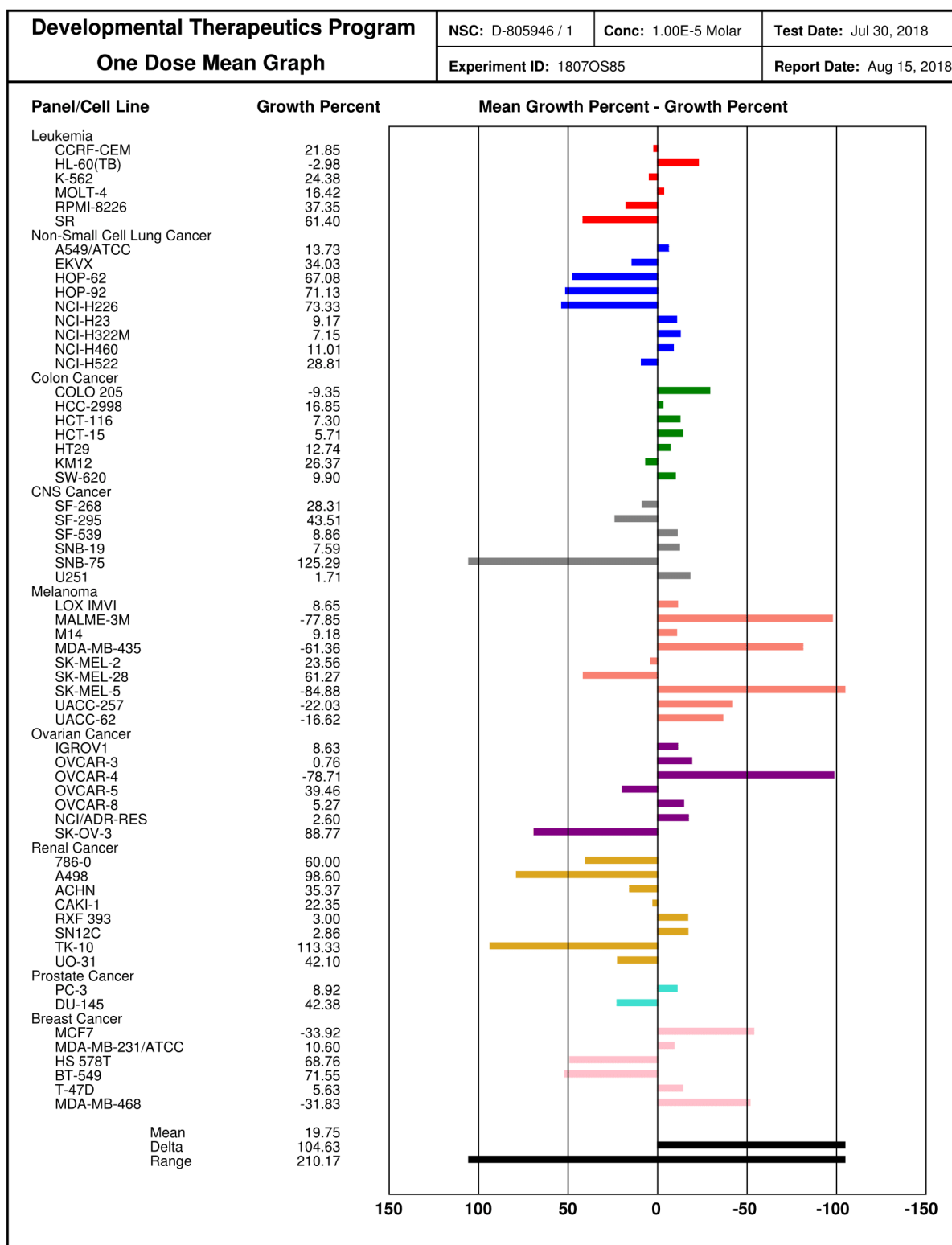
Compound 13



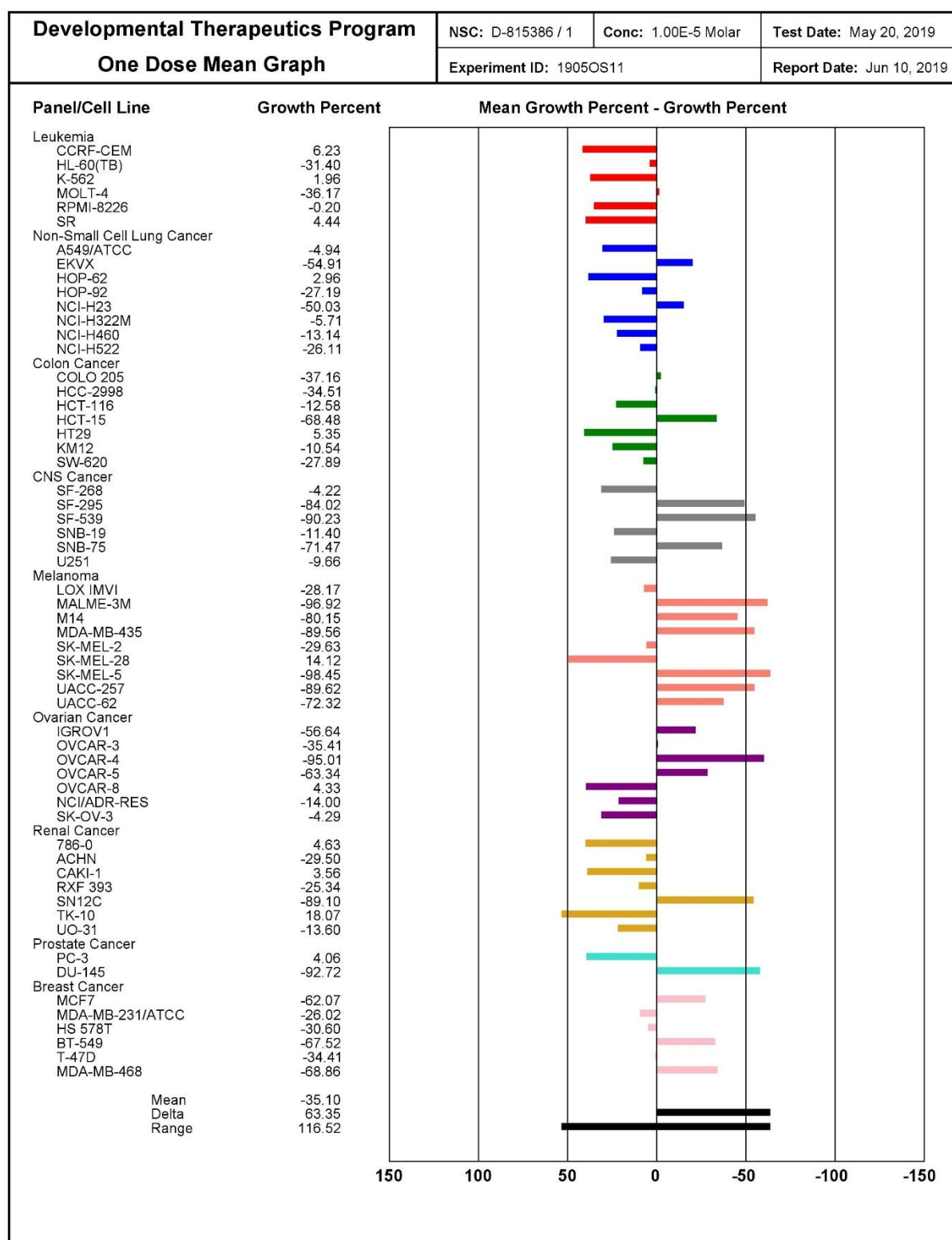
Compound 14



Compound 15

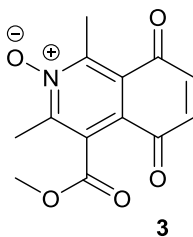
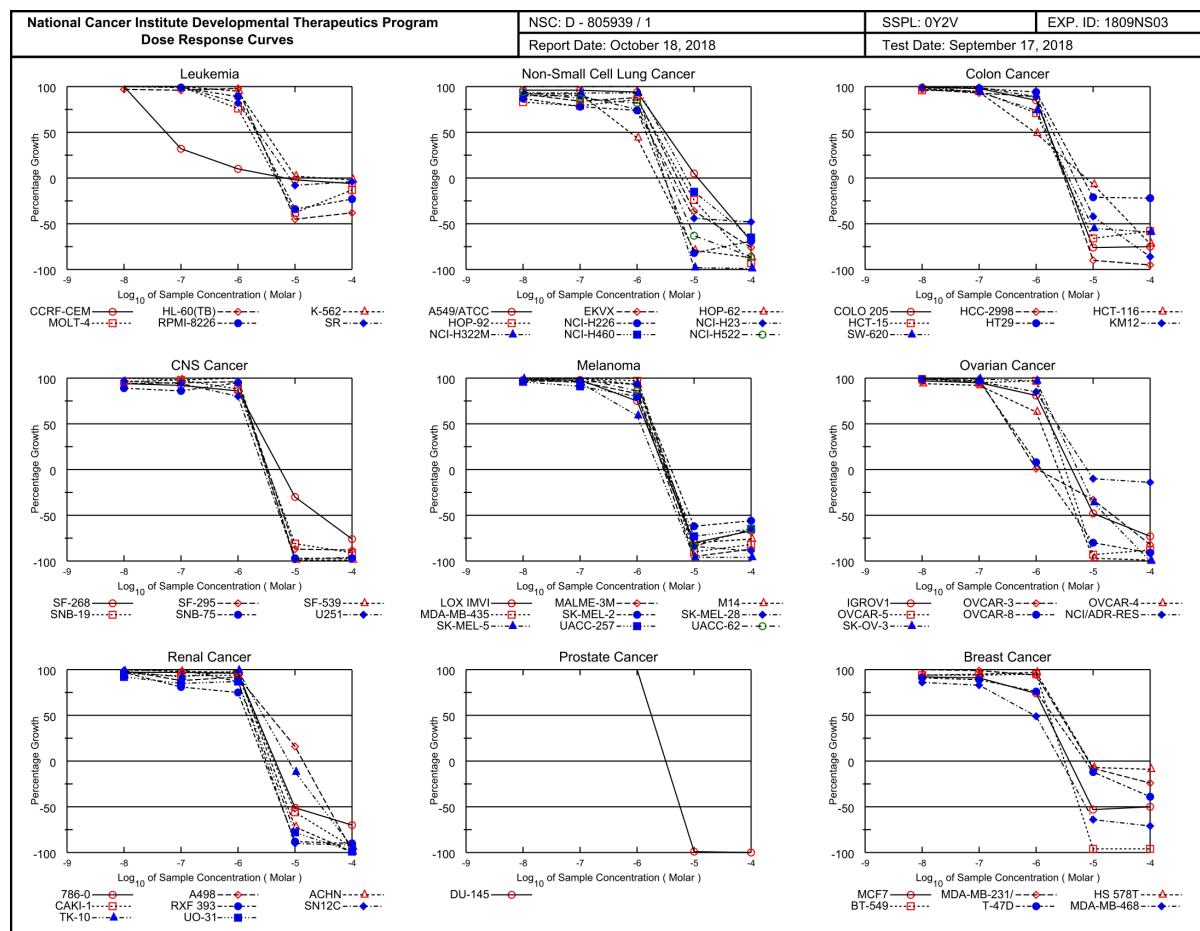


Compound 16



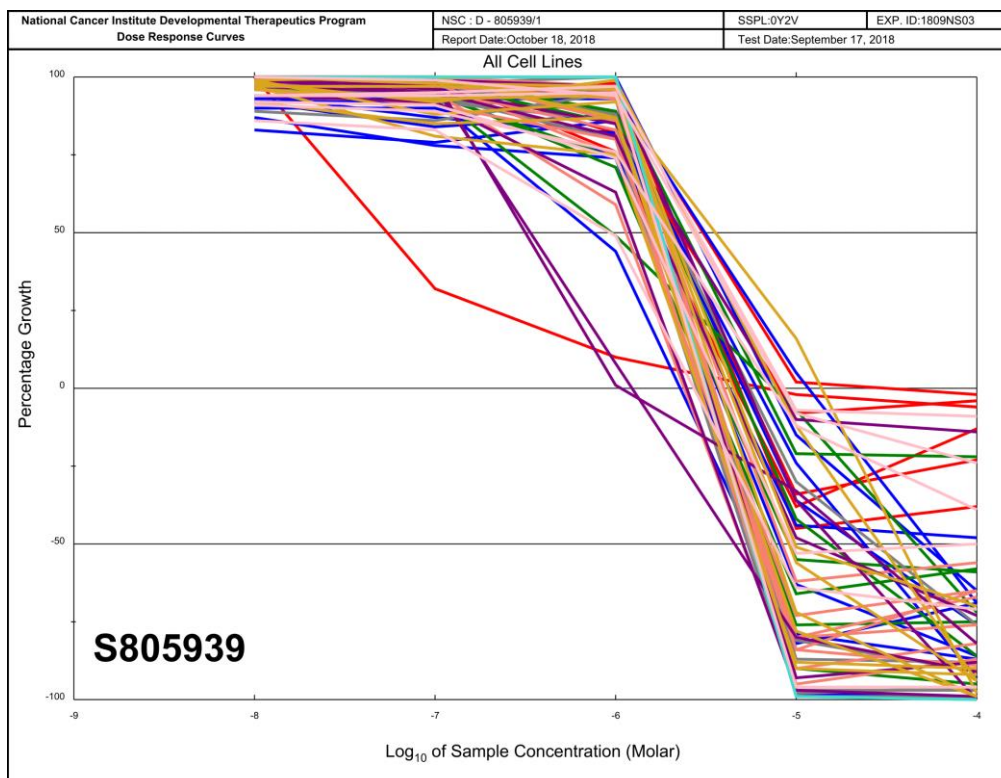
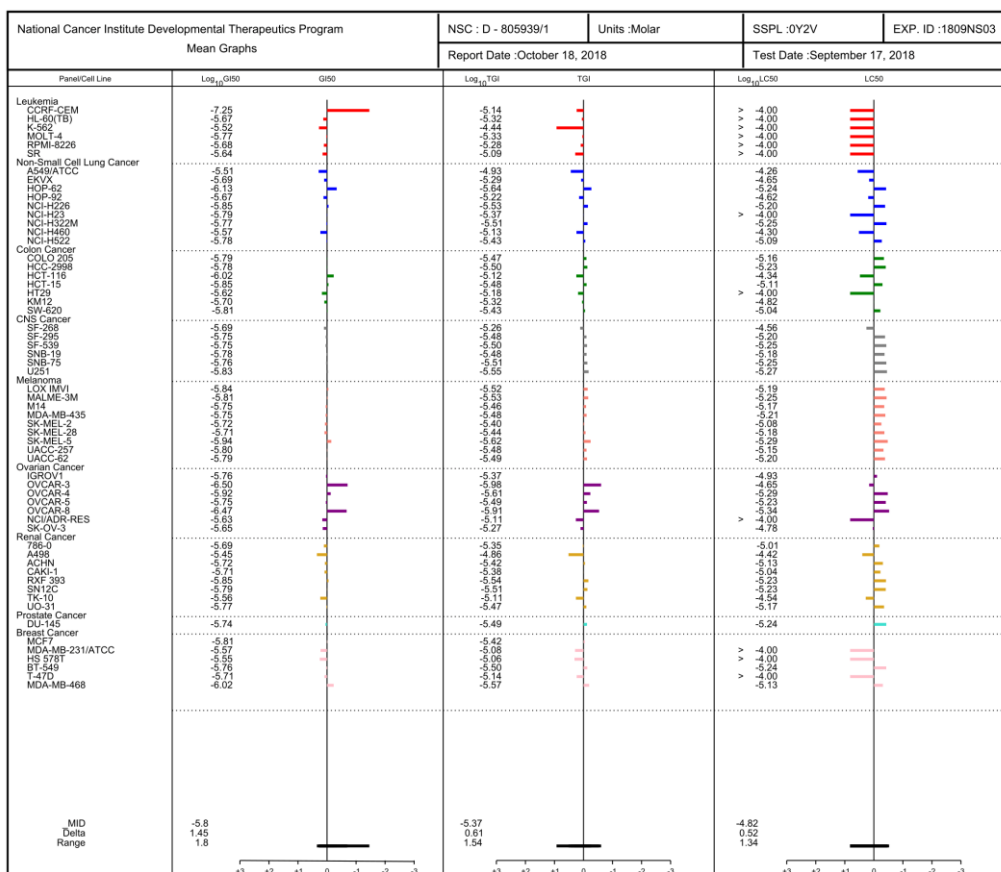
NCI60 – Five Dose Data

Compound 3



National Cancer Institute Developmental Therapeutics Program In-Vitro Testing Results

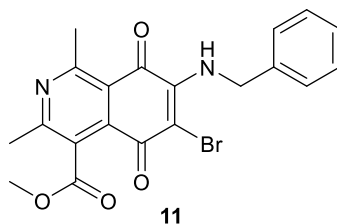
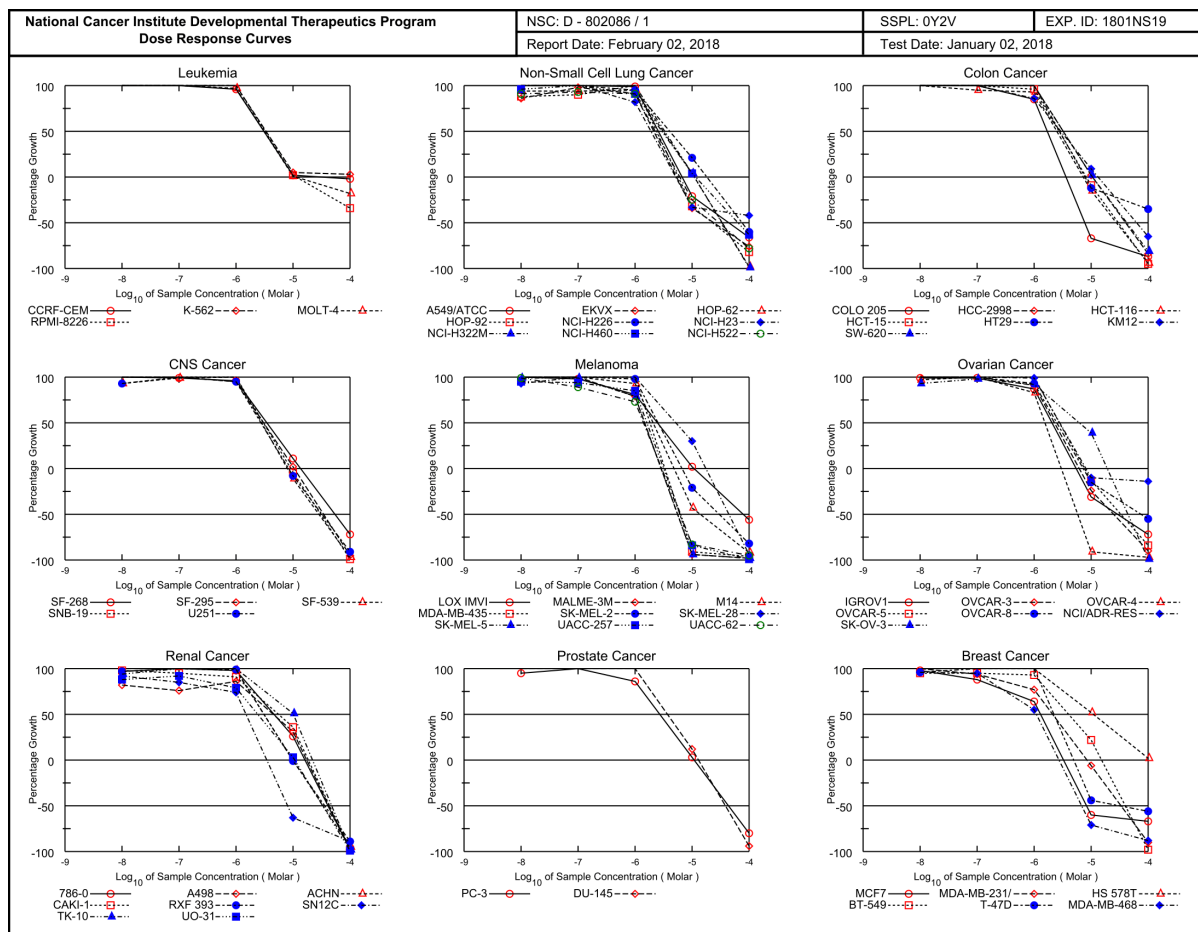
NSC : D - 805939 / 1			Experiment ID : 1809NS03							Test Type : 08			Units : Molar		
Report Date : October 18, 2018			Test Date : September 17, 2018							QNS :			MC :		
COMI : RK CH 0.0.2			Stain Reagent : SRB Dual-Pass Related							SSPL : 0Y2V					
Log10 Concentration															
Panel/Cell Line	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	0.555	2.485	2.576	1.164	0.753	0.546	0.521	105	32	10	-2	-6	5.59E-8	7.30E-6	> 1.00E-4
HL-60(TB)	0.925	3.282	3.219	3.192	3.233	0.508	0.577	97	96	98	-45	-38	2.16E-6	4.84E-6	> 1.00E-4
K-562	0.278	2.640	2.781	2.715	2.516	0.326	0.274	106	103	95	2	-2	3.04E-6	3.60E-5	> 1.00E-4
MOLT-4	0.645	2.975	3.012	2.966	2.415	0.401	0.561	102	100	76	-38	-13	1.69E-6	4.65E-6	> 1.00E-4
RPMI-8226	0.838	2.851	2.887	2.838	2.622	0.554	0.644	102	99	89	-34	-23	2.07E-6	5.29E-6	> 1.00E-4
SR	0.345	1.836	1.871	1.878	1.571	0.318	0.330	102	103	82	-8	-4	2.28E-6	8.19E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.431	2.311	2.240	2.229	2.199	0.523	0.134	96	96	94	5	-69	3.12E-6	1.16E-5	5.53E-5
EKVX	0.872	2.152	2.054	1.953	1.999	0.558	0.207	92	84	88	-36	-76	2.02E-6	5.12E-6	2.22E-5
HOP-62	0.326	1.375	1.291	1.296	0.785	0.068	0.043	92	92	44	-79	-87	7.44E-7	2.27E-6	5.79E-6
HOP-92	0.922	1.477	1.383	1.360	1.400	0.699	0.069	83	79	86	-24	-93	2.12E-6	6.03E-6	2.38E-5
NCI-H226	1.257	2.301	2.163	2.069	2.026	0.232	0.385	87	78	74	-82	-69	1.42E-6	2.98E-6	6.26E-6
NCI-H23	0.664	2.229	2.066	2.074	1.843	0.371	0.345	90	90	75	-44	-48	1.63E-6	4.27E-6	> 1.00E-4
NCI-H322M	0.681	2.241	2.137	2.137	2.136	0.015	0.005	93	93	93	-98	-99	1.68E-6	3.08E-6	5.62E-6
NCI-H460	0.353	3.149	3.196	3.184	3.149	0.300	0.123	102	101	100	-15	-65	2.72E-6	7.40E-6	4.99E-5
NCI-H522	0.827	2.555	2.456	2.334	2.247	0.304	0.117	94	87	82	-63	-86	1.66E-6	3.67E-6	8.11E-6
Colon Cancer															
COLO 205	0.375	2.012	1.989	1.976	1.759	0.089	0.095	99	98	85	-76	-75	1.64E-6	3.35E-6	6.85E-6
HCC-2998	1.137	3.308	3.252	3.161	3.062	0.109	0.056	97	93	89	-90	-95	1.64E-6	3.13E-6	5.95E-6
HCT-116	0.201	2.397	2.353	2.262	1.275	0.187	0.057	98	94	49	-7	-72	9.45E-7	7.50E-6	4.60E-5
HCT-15	0.362	2.375	2.285	2.266	1.793	0.125	0.151	96	95	71	-66	-58	1.43E-6	3.31E-6	7.69E-6
HT29	0.256	1.988	2.047	1.951	1.884	0.202	0.200	103	98	94	-21	-22	2.41E-6	6.54E-6	> 1.00E-4
KM12	0.576	2.845	2.820	2.818	2.602	0.333	0.084	99	99	89	-42	-86	1.99E-6	4.77E-6	1.51E-5
SW-620	0.290	1.997	2.057	1.901	1.557	0.130	0.119	104	94	74	-55	-59	1.54E-6	3.74E-6	9.09E-6
CNS Cancer															
SF-268	0.781	2.278	2.195	2.153	2.070	0.548	0.191	94	92	86	-30	-76	2.05E-6	5.52E-6	2.76E-5
SF-295	1.102	3.203	3.083	3.096	3.110	0.146	0.132	94	95	96	-87	-88	1.78E-6	3.34E-6	6.28E-6
SF-539	1.016	2.836	2.769	2.792	2.855	0.008	0.011	96	98	101	-99	-99	1.80E-6	3.20E-6	5.68E-6
SNB-19	0.588	2.250	2.244	2.215	2.048	0.112	0.055	100	98	88	-81	-91	1.68E-6	3.31E-6	6.55E-6
SNB-75	0.854	1.672	1.581	1.555	1.634	0.022	0.028	89	86	95	-97	-97	1.72E-6	3.12E-6	5.67E-6
U251	0.520	2.449	2.390	2.336	2.070	0.013	0.022	97	94	80	-98	-96	1.48E-6	2.83E-6	5.40E-6
Melanoma															
LOX IMVI	0.758	3.337	3.279	3.275	2.681	0.154	0.251	98	98	75	-80	-67	1.44E-6	3.04E-6	6.42E-6
MALME-3M	0.842	1.822	1.797	1.786	1.658	0.040	0.114	97	96	83	-95	-87	1.53E-6	2.92E-6	5.57E-6
M14	0.458	2.172	2.140	2.179	2.058	0.090	0.110	98	100	93	-80	-76	1.78E-6	3.45E-6	6.69E-6
MDA-MB-435	0.666	2.990	2.981	3.016	2.920	0.069	0.119	100	101	97	-90	-82	1.79E-6	3.31E-6	6.13E-6
SK-MEL-2	1.247	2.886	2.888	2.832	2.770	0.479	0.553	100	97	93	-62	-56	1.89E-6	3.99E-6	8.41E-6
SK-MEL-28	0.839	2.540	2.611	2.609	2.630	0.135	0.095	104	104	105	-84	-89	1.96E-6	3.60E-6	6.62E-6
SK-MEL-5	1.028	3.204	3.187	3.095	2.313	0.046	0.037	99	95	59	-96	-96	1.14E-6	2.41E-6	5.07E-6
UACC-257	0.764	2.132	2.082	2.009	1.856	0.210	0.270	96	91	80	-73	-65	1.57E-6	3.34E-6	7.11E-6
UACC-62	0.978	2.861	2.961	2.913	2.602	0.155	0.344	105	103	86	-84	-65	1.63E-6	3.21E-6	6.30E-6
Ovarian Cancer															
IGROV1	0.412	1.984	1.937	1.900	1.690	0.214	0.112	97	95	81	-48	-73	1.74E-6	4.24E-6	1.18E-5
OVCAR-3	0.511	1.863	1.895	1.846	0.521	0.341	0.094	102	99	1	-33	-82	3.14E-7	1.05E-6	2.22E-5
OVCAR-4	0.631	1.631	1.572	1.550	1.257	0.019	0.009	94	92	63	-97	-99	1.20E-6	2.47E-6	5.08E-6
OVCAR-5	0.576	1.648	1.636	1.594	1.615	0.038	0.069	99	95	97	-93	-88	1.76E-6	3.23E-6	5.91E-6
OVCAR-8	0.342	1.768	1.757	1.730	0.458	0.068	0.030	99	97	8	-80	-91	3.39E-7	1.24E-6	4.54E-6
NCI/ADR-RES	0.682	2.396	2.392	2.321	2.142	0.611	0.586	100	96	85	-10	-14	2.33E-6	7.78E-6	> 1.00E-4
SK-OV-3	0.558	1.690	1.694	1.681	1.656	0.359	0.002	100	99	97	-36	-100	2.26E-6	5.38E-6	1.67E-5
Renal Cancer															
786-O	0.670	2.699	2.631	2.641	2.627	0.328	0.201	97	97	96	-51	-70	2.07E-6	4.51E-6	9.84E-6
A498	1.579	2.461	2.454	2.354	2.388	1.724	0.034	99	88	92	16	-98	3.58E-6	1.39E-5	3.81E-5
ACHN	0.355	1.655	1.670	1.634	1.619	0.101	0.003	101	98	97	-72	-99	1.90E-6	3.77E-6	7.45E-6
CAKI-1	0.878	2.444	2.376	2.337	2.344	0.382	0.051	96	93	94	-56	-94	1.95E-6	4.20E-6	9.05E-6
RXF 393	1.198	1.965	1.945	1.816	1.772	0.143	0.115	97	81	75	-88	-90	1.42E-6	2.88E-6	5.84E-6
SN12C	0.754	2.932	2.901	2.970	2.657	0.074	0.062	99	102	87	-90	-92	1.62E-6	3.10E-6	5.93E-6
TK-10	0.792	2.121	2.089	2.013	2.104	0.694	0.050	98	92	99	-12	-94	2.74E-6	7.74E-6	2.90E-5
UO-31	0.771	2.208	2.097	1.989	2.029	0.172	0.006	92	85	87	-78	-99	1.69E-6	3.39E-6	6.80E-6
Prostate Cancer															
DU-145	0.477	1.878	1.929	1.941	1.912	0.003	0.001	104	105	102	-99	-100	1.82E-6	3.22E-6	5.69E-6
Breast Cancer															
MCF7	0.631	3.142	2.937	2.922	2.493	0.295	0.317	92	91	74	-53	-50	1.55E-6	3.82E-6	.
MDA-MB-231/ATCC	0.725	1.663	1.711	1.653	1.604	0.665	0.549	105	99	94	-8	-24	2.68E-6	8.28E-6	> 1.00E-4
HS 578T	1.104	2.260	2.190	2.199	2.225	1.032	1.009	94	95	97	-7	-9	2.84E-6	8.64E-6	> 1.00E-4
BT-549	1.199	2.726	2.638	2.636	2.650	0.045	0.046	94	94	95	-96	-96	1.72E-6	3.14E-6	5.73E-6
T-47D	0.489	1.196	1.132	1.116	1.026	0.429	0.299	91	89	76	-12	-39	1.97E-6	7.26E-6	> 1.00E-4
MDA-MB-468	0.945	1.631	1.539	1.517	1.283	0.337	0.276	86	83	49	-64	-71	9.46E-7	2.71E-6	7.48E-6



Mean of effective endpoints across 59 cell lines for compound 3 as Log₁₀ Concentration (SD)

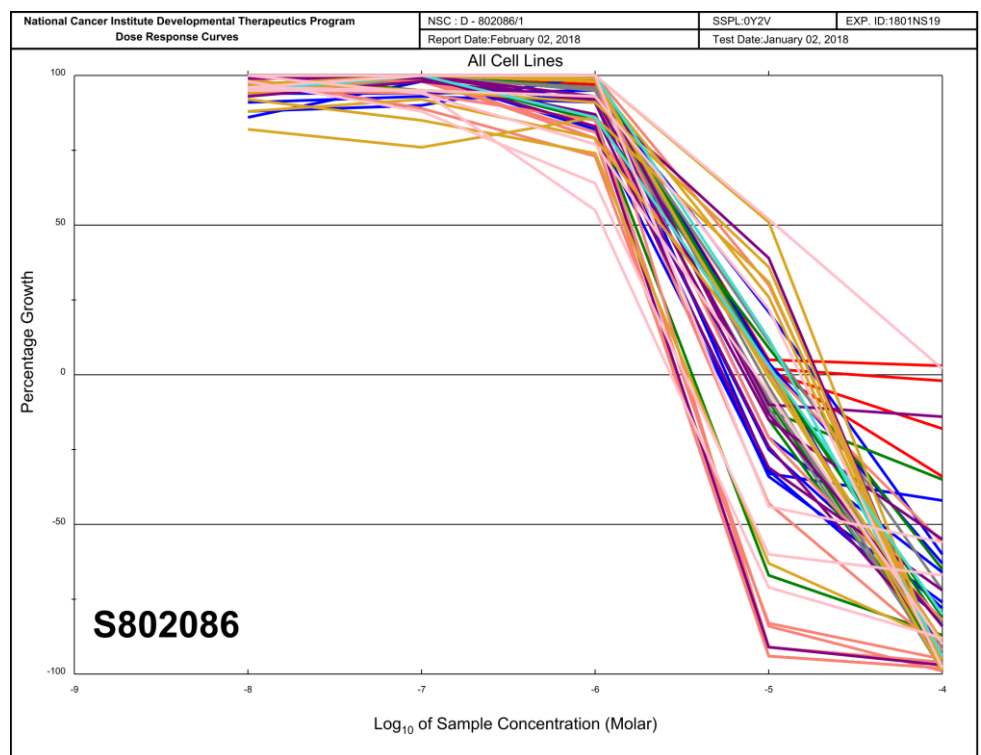
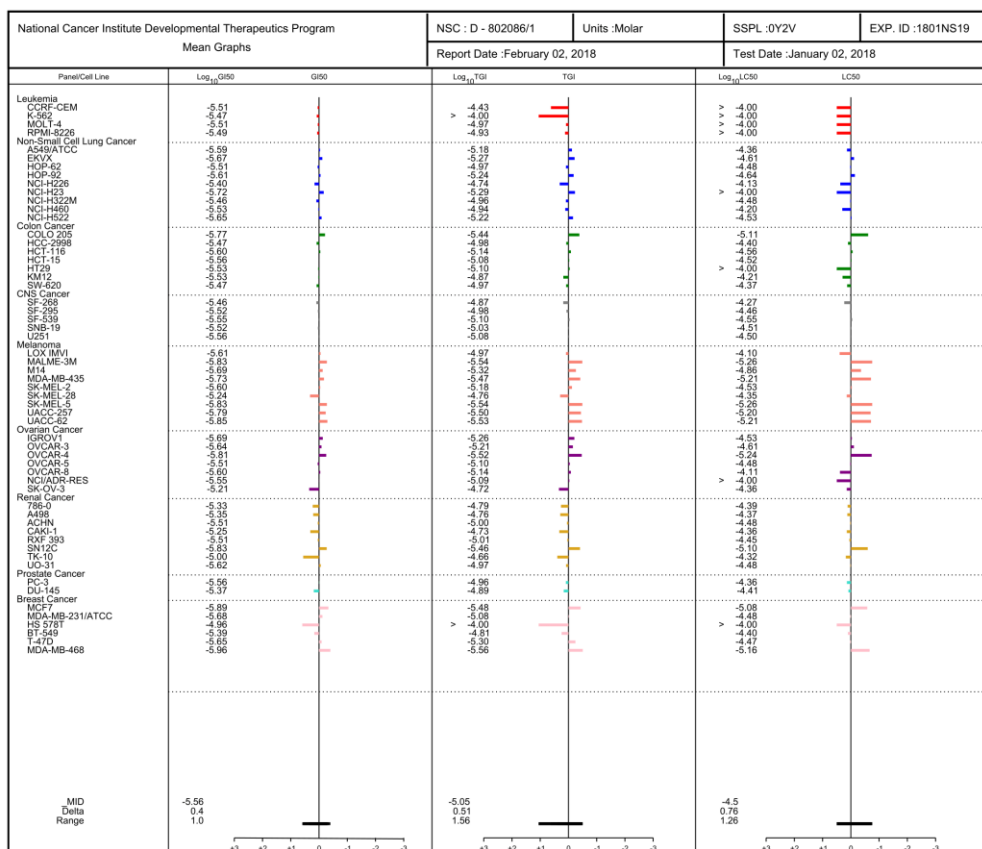
GI₅₀ -5.8 (±0.26); TGI -5.37 (±0.24); LC₅₀ -4.82 (±0.41)

Compound 11



National Cancer Institute Developmental Therapeutics Program In-Vitro Testing Results

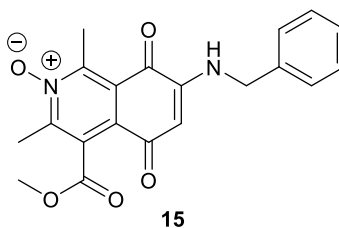
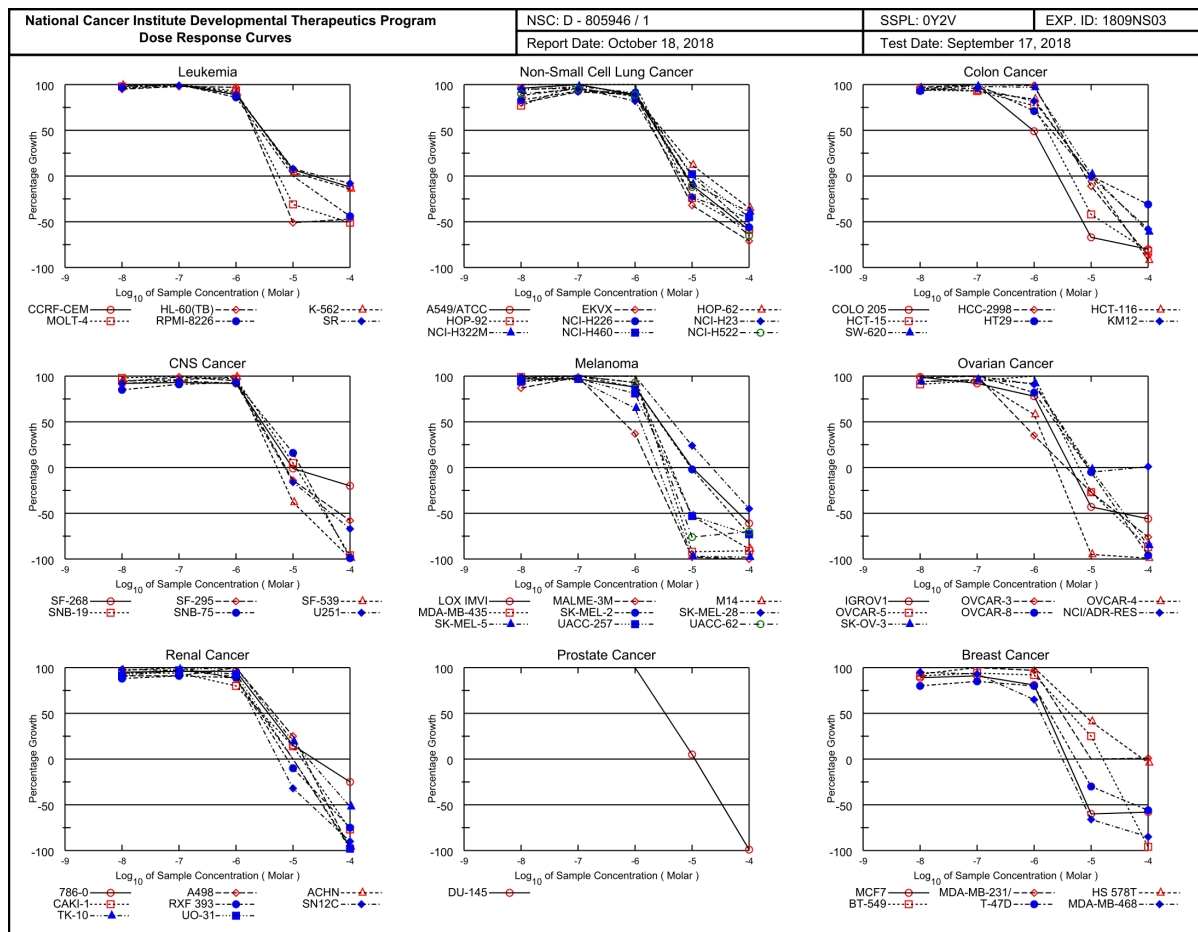
NSC : D - 802086 / 1			Experiment ID : 1801NS19										Test Type : 08		Units : Molar	
Report Date : February 02, 2018			Test Date : January 02, 2018										QNS :		MC :	
COMI : AGS 1.1.7.1			Stain Reagent : SRB Dual-Pass Related										SSPL : 0Y2V			
Log10 Concentration																
Panel/Cell 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	0.289	1.705	1.732	1.758	1.654	0.321	0.284	102	104	96	2	-2	3.11E-6	3.68E-5	> 1.00E-4	
K-562	0.074	0.882	0.919	0.907	0.880	0.116	0.102	104	103	100	5	3	3.36E-6	> 1.00E-4	> 1.00E-4	
MOLT-4	0.476	2.189	2.264	2.254	2.144	0.487	0.391	104	104	97	1	-18	3.09E-6	1.08E-5	> 1.00E-4	
RPMI-8226	0.592	2.130	2.134	2.224	2.125	0.630	0.392	100	106	100	2	-34	3.24E-6	1.17E-5	> 1.00E-4	
Non-Small Cell Lung Cancer																
A549/ATCC	0.319	1.621	1.616	1.626	1.612	0.251	0.108	100	100	99	-21	-66	2.56E-6	6.64E-6	4.35E-5	
EKVX	0.408	1.173	1.063	1.157	1.101	0.270	0.100	86	98	91	-34	-76	2.12E-6	5.35E-6	2.44E-5	
HOP-62	0.856	1.965	1.896	1.901	1.914	0.895	0.013	94	94	95	4	-98	3.12E-6	1.08E-5	3.35E-5	
HOP-92	1.068	1.524	1.471	1.479	1.537	0.726	0.188	88	90	103	-32	-82	2.46E-6	5.78E-6	2.27E-5	
NCI-H226	0.617	1.238	1.254	1.244	1.205	0.745	0.246	103	101	95	21	-60	4.01E-6	1.80E-5	7.49E-5	
NCI-H23	0.479	1.855	1.883	1.916	1.603	0.323	0.280	102	104	82	-33	-42	1.89E-6	5.19E-6	> 1.00E-4	
NCI-H322M	0.544	1.409	1.452	1.468	1.444	0.576	0.006	105	107	104	4	-99	3.45E-6	1.09E-5	3.34E-5	
NCI-H460	0.160	1.514	1.465	1.658	1.387	0.214	0.059	96	111	91	4	-63	2.94E-6	1.15E-5	6.37E-5	
NCI-H522	0.913	2.105	1.995	2.017	1.996	0.685	0.204	91	93	91	-25	-78	2.25E-6	6.09E-6	2.98E-5	
Colon Cancer																
COLO 205	0.426	1.092	1.122	1.108	0.994	0.141	0.054	104	102	85	-67	-87	1.70E-6	3.63E-6	7.73E-6	
HCC-2998	0.576	2.115	2.133	2.136	2.182	0.607	0.091	101	101	104	2	-84	3.40E-6	1.05E-5	4.00E-5	
HCT-116	0.234	1.855	1.873	1.772	1.747	0.198	0.014	101	95	93	-15	-94	2.50E-6	7.22E-6	2.75E-5	
HCT-15	0.247	1.404	1.403	1.463	1.362	0.226	0.013	100	105	96	-9	-95	2.76E-6	8.26E-6	3.01E-5	
HT29	0.221	1.239	1.326	1.315	1.291	0.196	0.143	109	107	105	-12	-35	2.97E-6	7.96E-6	> 1.00E-4	
KM12	0.408	1.831	1.963	1.936	1.626	0.543	0.141	109	107	86	9	-65	2.93E-6	1.34E-5	6.22E-5	
SW-620	0.273	1.897	2.004	1.968	1.959	0.312	0.053	107	104	104	2	-81	3.39E-6	1.07E-5	4.28E-5	
CNS Cancer																
SF-268	0.510	1.729	1.731	1.715	1.677	0.643	0.142	100	99	96	11	-72	3.46E-6	1.35E-5	5.41E-5	
SF-295	0.542	2.092	2.140	2.201	2.020	0.566	0.029	103	107	95	2	-95	3.04E-6	1.04E-5	3.43E-5	
SF-539	0.664	2.045	1.951	2.031	2.053	0.593	0.017	93	99	101	-11	-97	2.85E-6	8.02E-6	2.84E-5	
SNB-19	0.431	1.469	1.526	1.502	1.469	0.416	0.004	105	103	100	-4	-99	3.04E-6	9.23E-6	3.06E-5	
U251	0.289	1.344	1.269	1.352	1.294	0.266	0.025	93	101	95	-8	-91	2.74E-6	8.34E-6	3.19E-5	
Melanoma																
LOX IMVI	0.315	2.204	2.211	2.166	1.839	0.352	0.139	100	98	81	2	-56	2.45E-6	1.08E-5	7.91E-5	
MALME-3M	0.432	0.902	0.922	0.938	0.802	0.025	0.008	104	108	79	-94	-98	1.47E-6	2.85E-6	5.55E-6	
M14	0.362	1.264	1.268	1.298	1.197	0.206	0.029	100	104	93	-43	-92	2.06E-6	4.81E-6	1.38E-5	
MDA-MB-435	0.513	2.161	2.162	2.229	2.190	0.048	0.019	100	104	102	-91	-96	1.86E-6	3.38E-6	6.15E-6	
SK-MEL-2	1.130	2.075	2.088	2.086	2.054	0.890	0.199	101	101	98	-21	-82	2.52E-6	6.63E-6	2.95E-5	
SK-MEL-28	0.633	1.768	1.694	1.921	1.931	0.973	0.039	93	113	114	30	-94	5.79E-6	1.74E-5	4.42E-5	
SK-MEL-5	0.716	2.965	2.952	2.940	2.488	0.041	0.014	99	99	79	-94	-98	1.47E-6	2.85E-6	5.54E-6	
UACC-257	1.049	2.162	2.101	2.092	1.998	0.168	0.009	95	94	85	-84	-99	1.62E-6	3.19E-6	6.30E-6	
UACC-62	1.056	2.718	2.696	2.540	2.271	0.176	0.048	99	89	73	-83	-95	1.41E-6	2.93E-6	6.12E-6	
Ovarian Cancer																
IGROV1	0.426	1.584	1.567	1.574	1.428	0.294	0.120	99	99	87	-31	-72	2.05E-6	5.45E-6	2.92E-5	
OVCAR-3	0.434	1.580	1.718	1.701	1.478	0.329	0.044	112	110	91	-24	-90	2.27E-6	6.16E-6	2.46E-5	
OVCAR-4	0.753	1.696	1.663	1.728	1.536	0.066	0.021	97	103	83	-91	-97	1.55E-6	2.99E-6	5.80E-6	
OVCAR-5	0.588	1.394	1.411	1.422	1.480	0.514	0.094	102	104	111	-13	-84	3.11E-6	7.91E-6	3.34E-5	
OVCAR-8	0.444	1.735	1.765	1.739	1.650	0.377	0.202	102	100	93	-15	-55	2.51E-6	7.24E-6	7.68E-5	
NCI/ADR-RES	0.407	1.409	1.416	1.443	1.400	0.368	0.350	101	103	99	-10	-14	2.83E-6	8.16E-6	> 1.00E-4	
SK-OV-3	0.819	1.515	1.467	1.504	1.459	1.091	0.008	93	98	92	39	-99	6.20E-6	1.92E-5	4.41E-5	
Renal Cancer																
786-0	0.554	2.000	1.951	2.014	1.970	0.932	0.012	97	101	98	26	-98	4.65E-6	1.63E-5	4.11E-5	
A498	1.381	2.098	1.971	1.927	2.000	1.600	0.024	82	76	86	31	-98	4.48E-6	1.73E-5	4.22E-5	
ACHN	0.419	1.401	1.531	1.506	1.380	0.419	0.020	113	111	98	.	-95	3.08E-6	9.97E-6	3.35E-5	
CAKI-1	0.532	1.741	1.714	1.681	1.636	0.973	0.011	98	95	91	36	-98	5.66E-6	1.87E-5	4.40E-5	
RXF 393	1.040	1.684	1.663	1.756	1.677	1.026	0.110	97	111	99	-1	-89	3.08E-6	9.69E-6	3.57E-5	
SN12C	0.569	1.838	1.738	1.654	1.502	0.209	0.060	92	85	74	-63	-89	1.49E-6	3.45E-6	8.00E-6	
TK-10	0.742	1.485	1.440	1.510	1.687	1.118	0.019	94	103	127	51	-98	1.01E-5	2.19E-5	4.78E-5	
UO-31	0.737	2.138	1.969	2.021	1.844	0.781	0.004	88	92	79	3	-99	2.41E-6	1.07E-5	3.30E-5	
Prostate Cancer																
PC-3	0.442	1.815	1.748	1.819	1.624	0.487	0.089	95	100	86	3	-80	2.73E-6	1.09E-5	4.37E-5	
DU-145	0.292	1.087	1.159	1.322	1.202	0.388	0.018	109	130	114	12	-94	4.26E-6	1.30E-5	3.85E-5	
Breast Cancer																
MCF7	0.278	1.615	1.585	1.458	1.136	0.112	0.093	98	88	64	-60	-67	1.30E-6	3.29E-6	8.32E-6	
MDA-MB-231/ATCC	0.485	1.227	1.235	1.181	1.056	0.455	0.049	101	94	77	-6	-90	2.11E-6	8.40E-6	3.33E-5	
HS 578T	1.070	2.082	2.137	2.116	2.150	1.594	1.093	105	103	107	52	2	1.08E-5	> 1.00E-4	> 1.00E-4	
BT-549	1.061	2.154	2.103	2.096	2.080	1.307	0.025	95	95	93	22	-98	4.08E-6	1.54E-5	4.01E-5	
T-47D	0.737	1.337	1.315	1.361	1.346	0.416	0.327	96	104	101	-44	-56	2.26E-6	5.00E-6	3.37E-5	
MDA-MB-468	0.726	1.339	1.318	1.309	1.066	0.214	0.086	96	95	55	-71	-88	1.10E-6	2.75E-6	6.87E-6	



Mean of effective endpoints across 57 cell lines for compound 11 as Log₁₀ Concentration (SD)

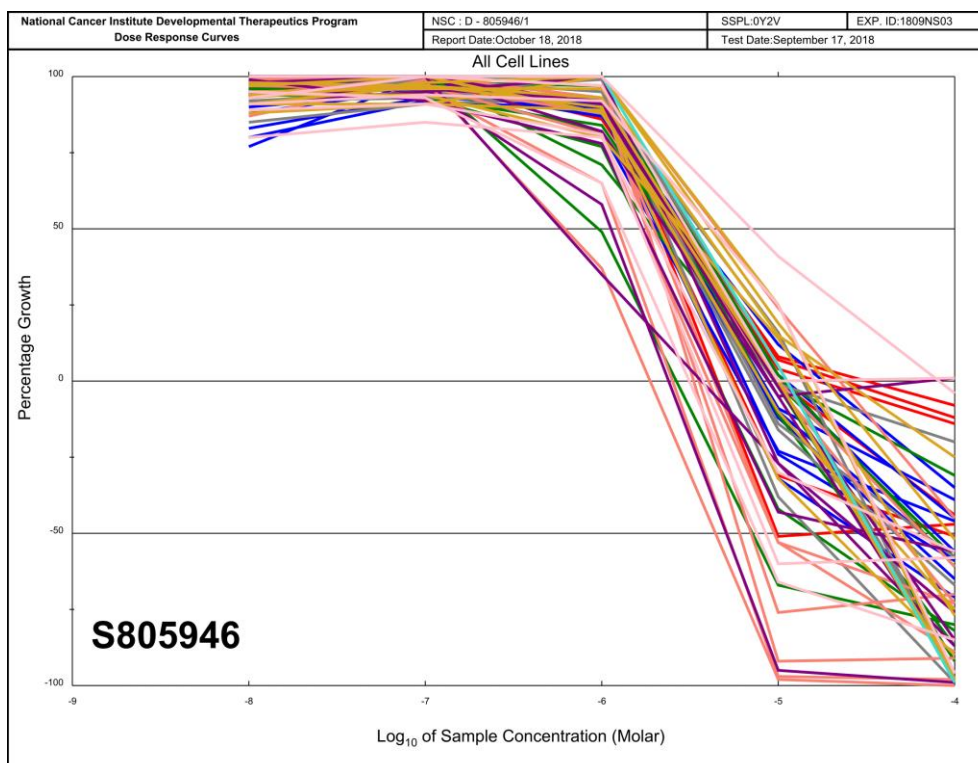
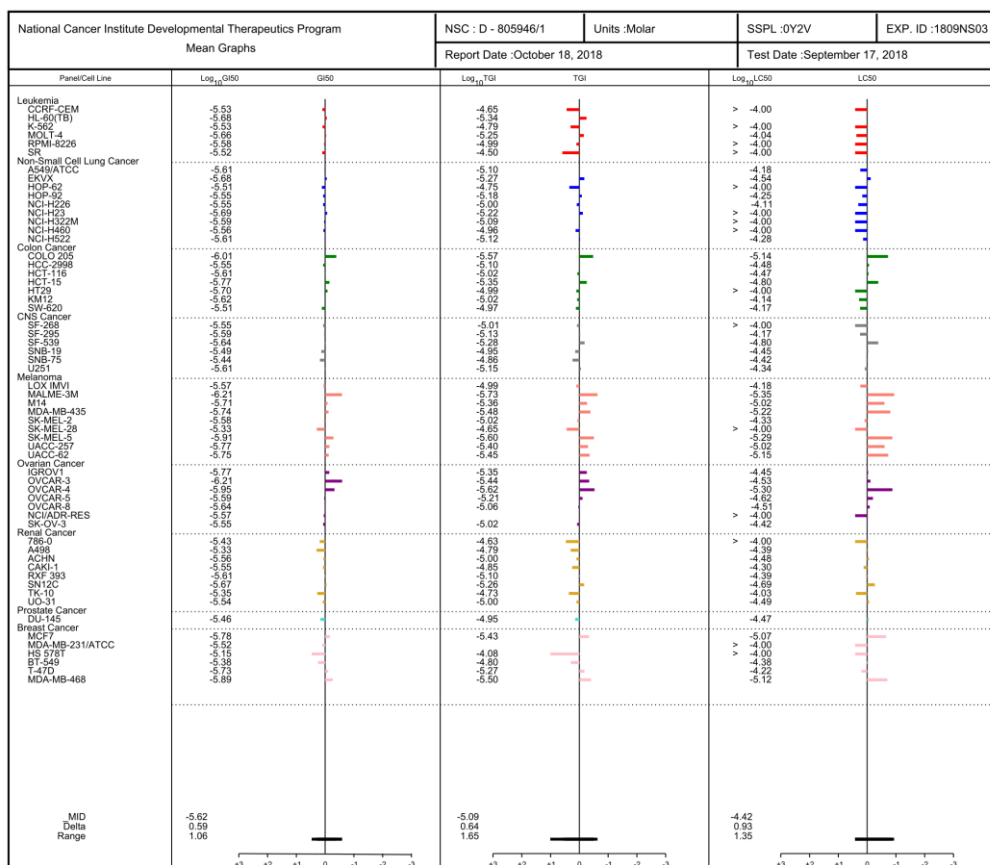
GI₅₀ -5.56 (±0.19); TGI -5.05 (±0.32); LC₅₀ -4.5 (±0.36)

Compound 15



National Cancer Institute Developmental Therapeutics Program In-Vitro Testing Results

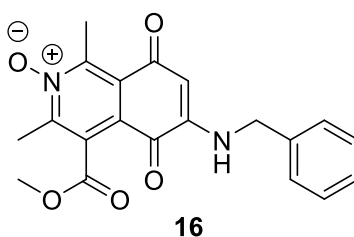
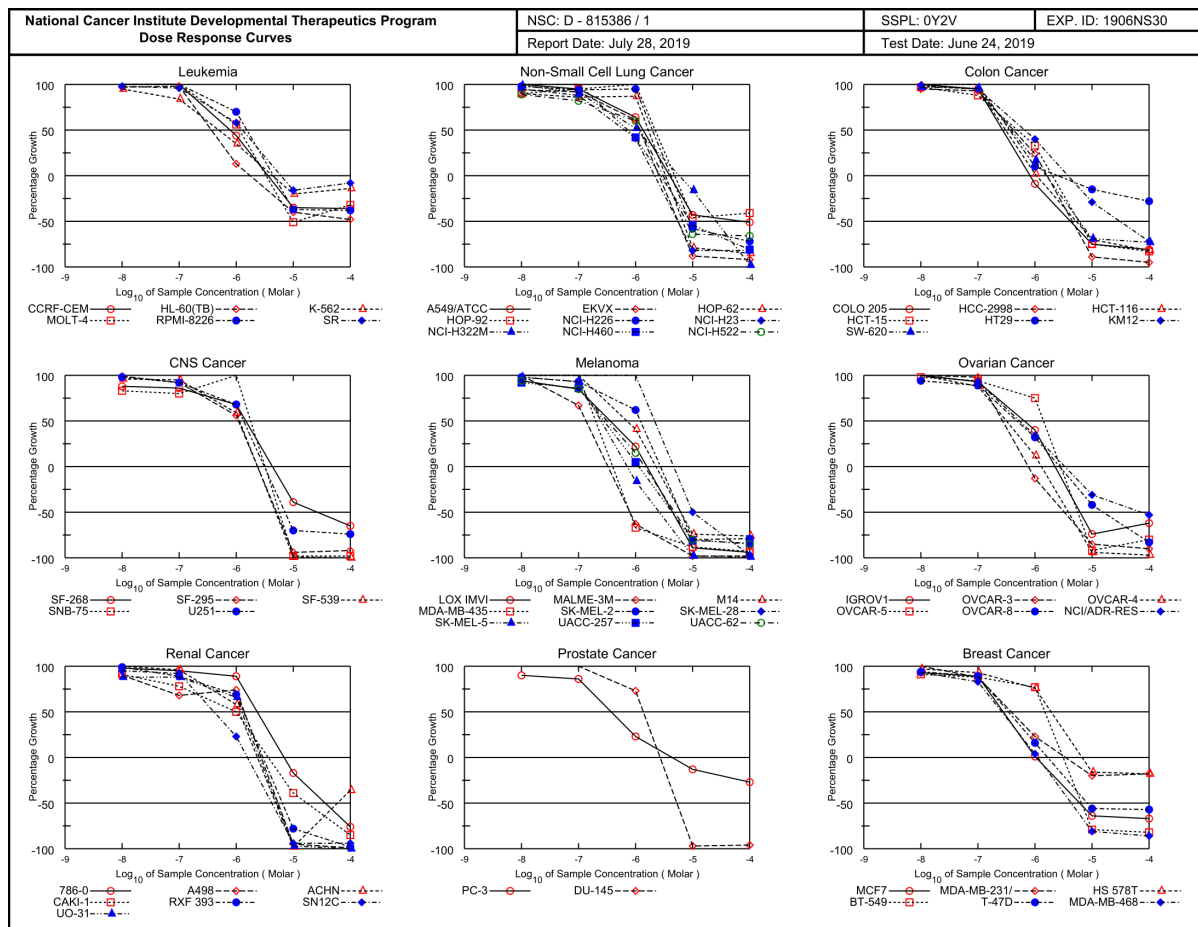
NSC : D - 805946 / 1			Experiment ID : 1809NS03							Test Type : 08			Units : Molar		
Report Date : October 18, 2018			Test Date : September 17, 2018							QNS :			MC :		
COMI : RK LEI 1-007			Stain Reagent : SRB Dual-Pass Related							SSPL : 0Y2V					
Log10 Concentration															
Panel/Cell 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	0.555	2.485	2.507	2.558	2.277	0.683	0.487	101	104	89	7	-12	2.98E-6	2.23E-5	> 1.00E-4
HL-60(TB)	0.925	3.282	3.170	3.242	3.216	0.455	0.493	95	98	97	-51	-47	2.08E-6	4.54E-6	.
K-562	0.278	2.640	2.623	2.709	2.421	0.367	0.239	99	103	91	4	-14	2.94E-6	1.63E-5	> 1.00E-4
MOLT-4	0.645	2.975	2.932	3.041	2.803	0.444	0.317	98	103	93	-31	-51	2.21E-6	5.60E-6	9.05E-5
RPMI-8226	0.838	2.851	2.782	2.866	2.561	0.843	0.469	97	101	86	.	-44	2.61E-6	1.01E-5	> 1.00E-4
SR	0.345	1.836	1.772	1.822	1.667	0.461	0.318	96	99	89	8	-8	3.00E-6	3.14E-5	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.431	2.311	2.236	2.318	2.101	0.389	0.178	96	100	89	-10	-59	2.47E-6	7.94E-6	6.61E-5
EKVX	0.872	2.152	1.902	2.044	2.011	0.590	0.254	80	92	89	-32	-71	2.09E-6	5.41E-6	2.87E-5
HOP-62	0.326	1.375	1.249	1.336	1.236	0.452	0.211	88	96	87	12	-35	3.10E-6	1.79E-5	> 1.00E-4
HOP-92	0.922	1.477	1.349	1.486	1.533	0.699	0.383	77	102	110	-24	-59	2.80E-6	6.61E-6	5.65E-5
NCI-H226	1.257	2.301	2.119	2.226	2.207	1.252	0.552	83	93	91	.	-56	2.81E-6	9.90E-6	7.78E-5
NCI-H23	0.664	2.229	2.157	2.169	1.952	0.514	0.357	95	96	82	-23	-46	2.03E-6	6.09E-6	> 1.00E-4
NCI-H322M	0.681	2.241	2.153	2.206	2.106	0.621	0.414	94	98	91	-9	-39	2.59E-6	8.15E-6	> 1.00E-4
NCI-H460	0.353	3.149	3.156	3.200	2.805	0.400	0.195	100	102	88	2	-45	2.74E-6	1.09E-5	> 1.00E-4
NCI-H522	0.827	2.555	2.380	2.474	2.371	0.728	0.294	90	95	89	-12	-65	2.45E-6	7.62E-6	5.29E-5
Colon Cancer															
COLO 205	0.375	2.012	2.005	2.025	1.185	0.125	0.075	100	101	49	-67	-80	9.77E-7	2.67E-6	7.19E-6
HCC-2998	1.137	3.308	3.244	3.312	3.290	1.015	0.151	97	100	99	-11	-87	2.80E-6	7.98E-6	3.28E-5
HCT-116	0.201	2.397	2.310	2.249	2.038	0.197	0.017	96	93	84	-2	-92	2.47E-6	9.48E-6	3.42E-5
HCT-15	0.362	2.375	2.261	2.238	1.921	0.210	0.065	94	93	77	-42	-82	1.70E-6	4.44E-6	1.57E-5
HT29	0.256	1.988	1.866	2.083	1.482	0.261	0.178	93	106	71	.	-31	1.97E-6	1.02E-5	> 1.00E-4
KM12	0.576	2.845	2.708	2.759	2.430	0.568	0.244	94	96	82	-1	-58	2.40E-6	9.60E-6	7.31E-5
SW-620	0.290	1.997	1.937	1.958	1.953	0.317	0.114	96	98	97	2	-61	3.12E-6	1.06E-5	6.70E-5
CNS Cancer															
SF-268	0.781	2.278	2.165	2.180	2.154	0.771	0.624	92	93	92	-1	-20	2.81E-6	9.69E-6	> 1.00E-4
SF-295	1.102	3.203	3.080	3.181	3.099	0.952	0.468	94	99	95	-14	-58	2.60E-6	7.49E-6	6.74E-5
SF-539	1.016	2.836	2.742	2.767	2.823	0.632	0.006	95	96	99	-38	-99	2.29E-6	5.30E-6	1.58E-5
SNB-19	0.588	2.250	2.217	2.360	2.200	0.673	0.025	98	107	97	5	-96	3.25E-6	1.12E-5	3.52E-5
SNB-75	0.854	1.672	1.552	1.603	1.612	0.988	0.013	85	91	93	16	-99	3.62E-6	1.39E-5	3.78E-5
U251	0.520	2.449	2.288	2.354	2.302	0.437	0.170	92	95	92	-16	-67	2.46E-6	7.11E-6	4.60E-5
Melanoma															
LOX IMVI	0.758	3.337	3.255	3.272	3.031	0.770	0.296	97	97	88	.	-61	2.72E-6	1.02E-5	6.62E-5
MALME-3M	0.842	1.822	1.692	1.815	1.204	0.020	.	87	99	37	-98	-100	6.17E-7	1.88E-6	4.43E-6
M14	0.458	2.172	2.061	2.167	2.051	0.217	0.049	94	100	93	-53	-89	1.97E-6	4.35E-6	9.58E-6
MDA-MB-435	0.666	2.990	2.966	3.062	2.982	0.050	0.058	99	103	100	-92	-91	1.81E-6	3.30E-6	6.01E-6
SK-MEL-2	1.247	2.886	2.853	2.933	2.685	1.221	0.331	98	103	88	-2	-73	2.63E-6	9.48E-6	4.69E-5
SK-MEL-28	0.839	2.540	2.543	2.718	2.582	1.241	0.465	100	110	102	24	-45	4.63E-6	2.22E-5	> 1.00E-4
SK-MEL-5	1.028	3.204	3.140	3.118	2.432	0.028	0.019	97	96	65	-97	-98	1.23E-6	2.50E-6	5.10E-6
UACC-257	0.764	2.132	2.047	2.095	1.869	0.359	0.204	94	97	81	-53	-73	1.70E-6	4.02E-6	9.49E-6
UACC-62	0.978	2.861	2.918	3.044	2.727	0.239	0.292	103	110	93	-76	-70	1.80E-6	3.56E-6	7.05E-6
Ovarian Cancer															
IGROV1	0.412	1.984	1.970	1.855	1.640	0.236	0.182	99	92	78	-43	-56	1.71E-6	4.42E-6	3.52E-5
OVCAR-3	0.511	1.863	1.838	1.936	0.985	0.373	0.121	98	105	35	-27	-76	6.13E-7	3.67E-6	2.93E-5
OVCAR-4	0.631	1.631	1.573	1.595	1.214	0.030	0.006	94	96	58	-95	-99	1.13E-6	2.40E-6	5.07E-6
OVCAR-5	0.576	1.648	1.550	1.590	1.689	0.421	0.073	91	95	104	-27	-87	2.58E-6	6.22E-6	2.41E-5
OVCAR-8	0.342	1.768	1.774	1.774	1.506	0.325	0.013	100	100	82	-5	-96	2.32E-6	8.73E-6	3.10E-5
NCI/ADR-RES	0.682	2.396	2.388	2.451	2.241	0.648	0.693	100	103	91	-5	1	2.67E-6	.	> 1.00E-4
SK-OV-3	0.558	1.690	1.624	1.648	1.599	0.550	0.081	94	96	92	-2	-85	2.81E-6	9.63E-6	3.78E-5
Renal Cancer															
786-O	0.670	2.699	2.596	2.610	2.615	0.972	0.504	95	96	96	15	-25	3.68E-6	2.37E-5	> 1.00E-4
A498	1.579	2.461	2.380	2.380	2.471	1.803	0.036	91	91	101	25	-98	4.72E-6	1.61E-5	4.09E-5
ACHN	0.355	1.655	1.618	1.662	1.504	0.360	0.013	97	101	88	.	-96	2.73E-6	1.01E-5	3.32E-5
CAKI-1	0.878	2.444	2.343	2.350	2.126	1.100	0.201	94	94	80	14	-77	2.84E-6	1.43E-5	5.04E-5
RXF 393	1.198	1.965	1.876	1.899	1.882	1.075	0.301	88	91	89	-10	-75	2.47E-6	7.88E-6	4.12E-5
SN12C	0.754	2.932	2.882	2.865	2.711	0.515	0.077	98	97	90	-32	-90	2.13E-6	5.48E-6	2.06E-5
TK-10	0.792	2.121	2.033	2.096	2.215	1.050	0.380	93	98	107	19	-52	4.48E-6	1.87E-5	9.35E-5
UO-31	0.771	2.208	2.081	2.164	2.102	0.777	0.019	91	97	93	.	-98	2.90E-6	1.01E-5	3.27E-5
Prostate Cancer															
DU-145	0.477	1.878	1.894	1.988	1.926	0.550	0.005	101	108	103	5	-99	3.50E-6	1.12E-5	3.39E-5
Breast Cancer															
MCF7	0.631	3.142	2.862	2.928	2.653	0.250	0.265	89	91	81	-60	-58	1.65E-6	3.73E-6	8.44E-6
MDA-MB-231/ATCC	0.725	1.663	1.713	1.719	1.636	0.721	0.734	105	106	97	.	1	3.03E-6	.	> 1.00E-4
HS 578T	1.104	2.260	2.180	2.272	2.280	1.573	1.063	93	101	102	41	-4	7.00E-6	8.24E-5	> 1.00E-4
BT-549	1.199	2.726	2.589	2.635	2.600	1.576	0.046	91	94	92	25	-96	4.19E-6	1.60E-5	4.15E-5
T-47D	0.489	1.196	1.051	1.090	1.056	0.343	0.217	80	85	80	-30	-56	1.88E-6	5.35E-6	5.99E-5
MDA-MB-468	0.945	1.631	1.598	1.585	1.391	0.326	0.141	95	93	65	-66	-85	1.30E-6	3.15E-6	7.61E-6



Mean of effective endpoints across 59 cell lines for compound 15 as Log₁₀ Concentration (SD)

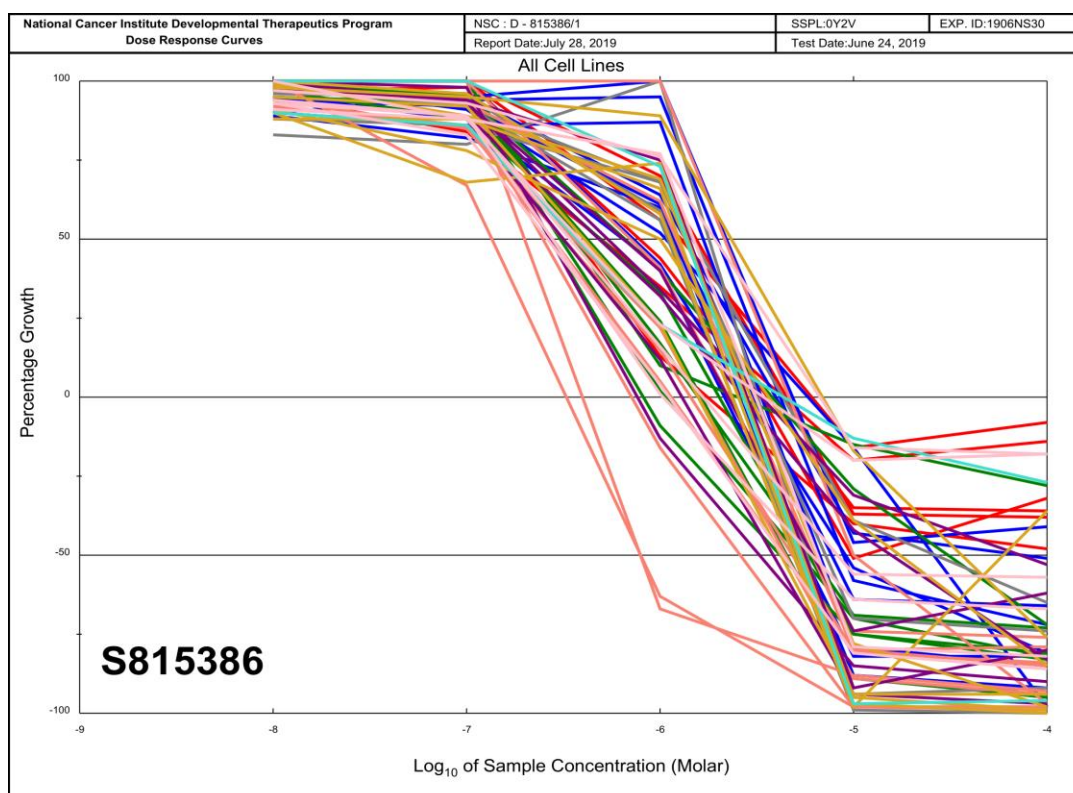
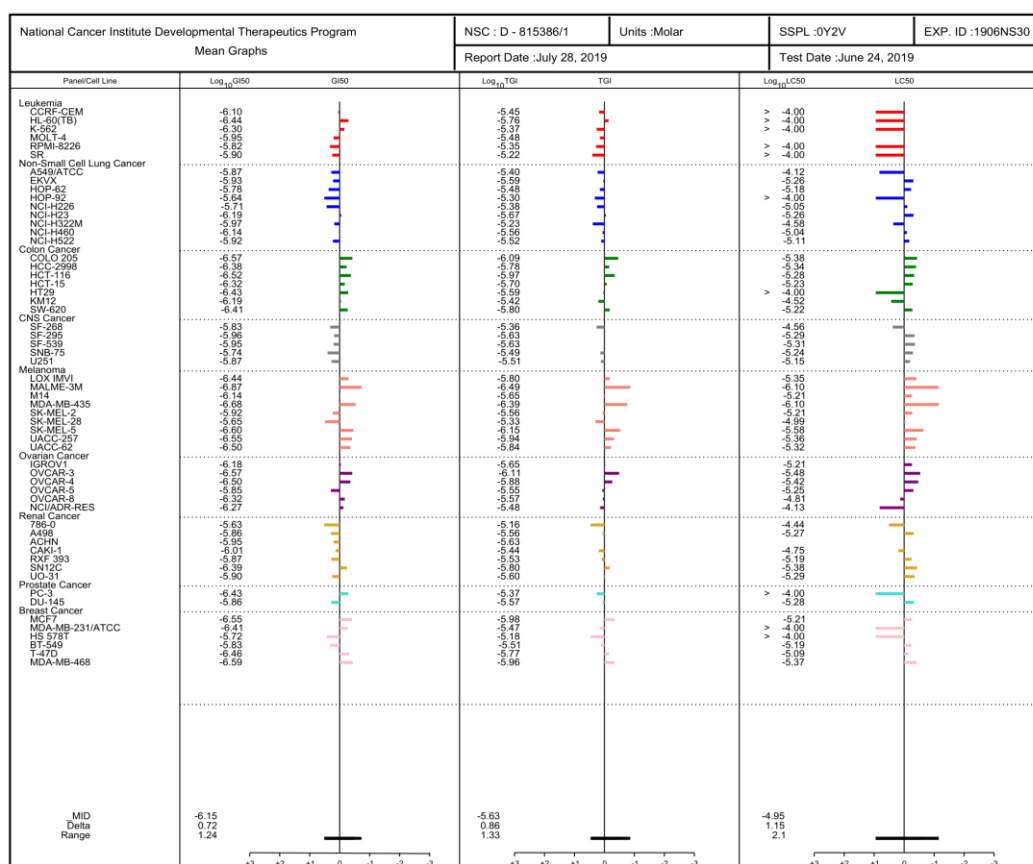
GI₅₀ -5.62 (±0.19); TGI -5.09 (±0.3); LC₅₀ -4.42 (±0.41)

Compound 16



National Cancer Institute Developmental Therapeutics Program In-Vitro Testing Results

NSC : D - 815386 / 1			Experiment ID : 1906NS30							Test Type : 08			Units : Molar		
Report Date : July 28, 2019			Test Date : June 24, 2019							QNS :			MC :		
COMI : RK 6.4.7			Stain Reagent : SRB Dual-Pass Related							SSPL : 0Y2V					
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	0.555	2.338	2.392	2.432	1.336	0.359	0.357	103	105	44	-35	-36	7.93E-7	3.58E-6	> 1.00E-4
HL-60(TB)	0.970	3.133	3.065	3.081	1.247	0.578	0.503	97	98	13	-40	-48	3.64E-7	1.74E-6	> 1.00E-4
K-562	0.175	1.369	1.308	1.181	0.594	0.140	0.150	95	84	35	-20	-14	4.97E-7	4.30E-6	> 1.00E-4
MOLT-4	0.690	2.474	2.574	2.525	1.687	0.341	0.470	106	103	56	-51	-32	1.14E-6	3.35E-6	
RPMI-8226	0.896	2.775	2.781	2.774	2.203	0.563	0.555	100	100	70	-37	-38	1.52E-6	4.48E-6	> 1.00E-4
SR	0.401	1.135	1.120	1.109	0.824	0.336	0.367	98	96	58	-16	-8	1.27E-6	6.01E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549(ATCC	0.290	1.919	1.913	1.834	1.336	0.167	0.142	100	95	64	-43	-51	1.36E-6	3.99E-6	7.54E-5
EKVX	0.554	1.867	1.788	1.756	1.352	0.067	0.047	94	92	61	-88	-92	1.18E-6	2.56E-6	5.55E-6
HOP-62	0.711	1.813	1.706	1.662	1.668	0.148	0.110	90	86	87	-79	-85	1.67E-6	3.34E-6	6.67E-6
HOP-92	1.036	1.609	1.553	1.582	1.639	0.559	0.614	90	95	105	-46	-41	2.31E-6	4.96E-6	> 1.00E-4
NCI-H226	1.126	2.385	2.357	2.309	2.316	0.474	0.316	98	94	95	-58	-72	1.96E-6	4.17E-6	8.87E-6
NCI-H23	0.613	1.868	1.800	1.717	1.126	0.113	0.110	95	88	41	-82	-82	6.40E-7	2.16E-6	5.52E-6
NCI-H322M	0.743	1.960	1.952	1.849	1.380	0.625	0.015	99	91	52	-16	-98	1.08E-6	5.84E-6	2.60E-5
NCI-H460	0.287	2.764	2.847	2.801	1.325	0.133	0.055	103	101	42	-54	-81	7.31E-7	2.74E-6	9.16E-6
NCI-H522	0.820	2.272	2.117	2.005	1.690	0.294	0.281	89	82	60	-64	-66	1.20E-6	3.04E-6	7.68E-6
Colon Cancer															
COLO 205	0.585	2.203	2.229	2.115	0.533	0.146	0.110	102	95	-9	-75	-81	2.70E-7	8.21E-7	4.17E-6
HCC-2998	0.932	2.543	2.462	2.425	1.325	0.107	0.045	95	93	24	-89	-95	4.21E-7	1.64E-6	4.55E-6
HCT-116	0.244	2.059	2.024	1.968	0.283	0.072	0.044	98	95	2	-70	-82	3.05E-7	1.07E-6	5.22E-6
HCT-15	0.278	2.113	2.060	1.885	0.877	0.069	0.047	97	88	33	-75	-83	4.83E-7	2.01E-6	5.84E-6
HT29	0.208	1.712	1.717	1.761	0.364	0.178	0.150	100	103	10	-15	-28	3.74E-7	2.60E-6	> 1.00E-4
KM12	0.579	2.965	2.950	2.834	1.530	0.410	0.162	99	95	40	-29	-72	6.53E-7	3.77E-6	3.05E-5
SW-620	0.258	1.891	1.836	1.829	0.539	0.080	0.070	97	96	17	-69	-73	3.85E-7	1.58E-6	6.00E-6
CNS Cancer															
SF-268	0.838	2.441	2.244	2.221	1.930	0.515	0.295	88	86	68	-39	-65	1.48E-6	4.35E-6	2.72E-5
SF-295	0.781	2.926	2.904	2.764	1.980	0.049	0.060	99	92	56	-94	-92	1.09E-6	2.36E-6	5.10E-6
SF-539	1.054	2.819	2.745	2.732	2.089	0.013	0.004	96	95	59	-99	-100	1.13E-6	2.36E-6	4.90E-6
SNB-75	1.027	1.811	1.679	1.656	1.820	0.019	0.025	83	80	101	-98	-98	1.81E-6	3.22E-6	5.73E-6
U251	0.272	1.582	1.550	1.475	1.157	0.082	0.071	98	92	68	-70	-74	1.34E-6	3.10E-6	7.15E-6
Melanoma															
LOX IMVI	0.496	2.775	2.644	2.429	1.002	0.054	0.031	94	85	22	-89	-94	3.60E-7	1.58E-6	4.45E-6
MALME-3M	0.763	1.675	1.672	1.371	0.280	0.013	0.015	100	67	-63	-98	-98	1.34E-7	3.26E-7	7.89E-7
M14	0.430	1.807	1.840	1.940	0.988	0.113	0.104	102	110	41	-74	-76	7.29E-7	2.26E-6	6.19E-6
MDA-MB-435	0.512	2.664	2.692	2.765	0.170	0.061	0.036	101	105	-67	-88	-93	2.08E-7	4.08E-7	7.98E-7
SK-MEL-2	1.127	2.562	2.536	2.466	2.016	0.221	0.233	98	93	62	-80	-79	1.21E-6	2.72E-6	6.12E-6
SK-MEL-28	0.772	1.914	1.979	2.042	1.942	0.390	0.027	106	111	102	-50	-97	2.21E-6	4.72E-6	1.02E-5
SK-MEL-5	0.784	3.159	3.121	3.004	0.657	0.020	0.007	98	93	-16	-98	-99	2.49E-7	7.12E-7	2.60E-6
UACC-257	0.739	1.905	1.816	1.746	0.796	0.139	0.115	92	86	5	-81	-84	2.79E-7	1.14E-6	4.33E-6
UACC-62	0.975	2.652	2.555	2.401	1.227	0.193	0.143	94	85	15	-80	-85	3.16E-7	1.44E-6	4.82E-6
Ovarian Cancer															
IGROV1	0.547	2.134	2.177	2.020	1.188	0.144	0.206	103	93	40	-74	-62	6.56E-7	2.26E-6	6.19E-6
OVCAR-3	0.469	1.531	1.602	1.511	0.410	0.070	0.048	107	98	-13	-85	-90	2.72E-7	7.70E-7	3.28E-6
OVCAR-4	0.560	1.500	1.496	1.384	0.676	0.031	0.018	100	88	12	-94	-97	3.16E-7	1.30E-6	3.83E-6
OVCAR-5	0.600	1.584	1.569	1.522	1.334	0.046	0.118	98	94	75	-92	-80	1.40E-6	2.80E-6	5.58E-6
OVCAR-8	0.444	2.368	2.252	2.151	1.063	0.256	0.075	94	89	32	-42	-83	4.84E-7	2.70E-6	1.53E-5
NCI/ADR-RES	0.563	2.074	2.091	1.970	1.076	0.387	0.266	101	93	34	-31	-53	5.35E-7	3.31E-6	7.38E-5
Renal Cancer															
786-0	0.921	2.757	2.719	2.670	2.556	0.762	0.222	98	95	89	-17	-76	2.33E-6	6.88E-6	3.61E-5
A498	1.553	2.098	2.041	1.921	1.957	0.073	0.010	90	68	74	-95	-99	1.39E-6	2.74E-6	5.40E-6
ACHN	0.354	1.569	1.597	1.521	1.065	0.008	0.228	102	96	58	-98	-36	1.13E-6	2.37E-6	
CAKI-1	0.772	2.673	2.506	2.258	1.716	0.475	0.117	91	78	50	-39	-85	9.72E-7	3.66E-6	1.77E-5
RXF 393	0.883	1.677	1.668	1.589	1.429	0.193	0.018	99	89	69	-78	-98	1.34E-6	2.94E-6	6.43E-6
SN12C	0.558	1.964	1.894	1.858	0.881	0.034	0.033	95	92	23	-94	-94	4.08E-7	1.57E-6	4.21E-6
UO-31	0.542	1.595	1.465	1.465	1.239	0.015	-0.001	88	88	66	-97	-100	1.26E-6	2.54E-6	5.13E-6
Prostate Cancer															
PC-3	0.573	1.664	1.551	1.511	0.819	0.497	0.420	90	86	23	-13	-27	3.69E-7	4.26E-6	> 1.00E-4
DU-145	0.410	1.710	1.774	1.764	1.359	0.014	0.017	105	104	73	-97	-96	1.37E-6	2.69E-6	5.31E-6
Breast Cancer															
MCF7	0.467	2.487	2.350	2.268	0.497	0.170	0.154	93	89	1	-64	-67	2.80E-7	1.05E-6	6.18E-6
MDA-MB-231/ATCC	0.775	1.855	1.871	1.727	1.024	0.617	0.636	102	88	23	-20	-18	3.85E-7	3.68E-6	> 1.00E-4
HS 578T	0.869	1.941	1.911	1.861	1.679	0.730	0.709	97	93	76	-16	-18	1.90E-6	6.38E-6	> 1.00E-4
BT-549	1.031	2.013	1.927	1.897	1.790	0.213	0.184	91	88	77	-79	-82	1.49E-6	3.12E-6	6.50E-6
T-47D	0.723	1.736	1.680	1.629	0.889	0.317	0.308	94	89	16	-56	-57	3.46E-7	1.68E-6	8.21E-6
MDA-MB-468	0.732	1.442	1.390	1.319	0.758	0.137	0.106	93	83	4	-81	-86	2.59E-7	1.10E-6	4.28E-6



Mean of effective endpoints across 59 cell lines for compound 16 as Log₁₀ Concentration (SD)

GI₅₀ -6.15 (±0.34); TGI -5.63 (±0.31); LC₅₀ -4.95 (±0.6)

Calculation of Mean GI₅₀ and Trends

Mean GI₅₀ was calculated from 53 human cancer cell lines that were common across the four compounds **3**, **11**, **15** and **18**. Conditional formatting was applied to the GI₅₀ values per compound row with the colour green representing more un-responsive cell lines and red representing more-responsive cell lines.

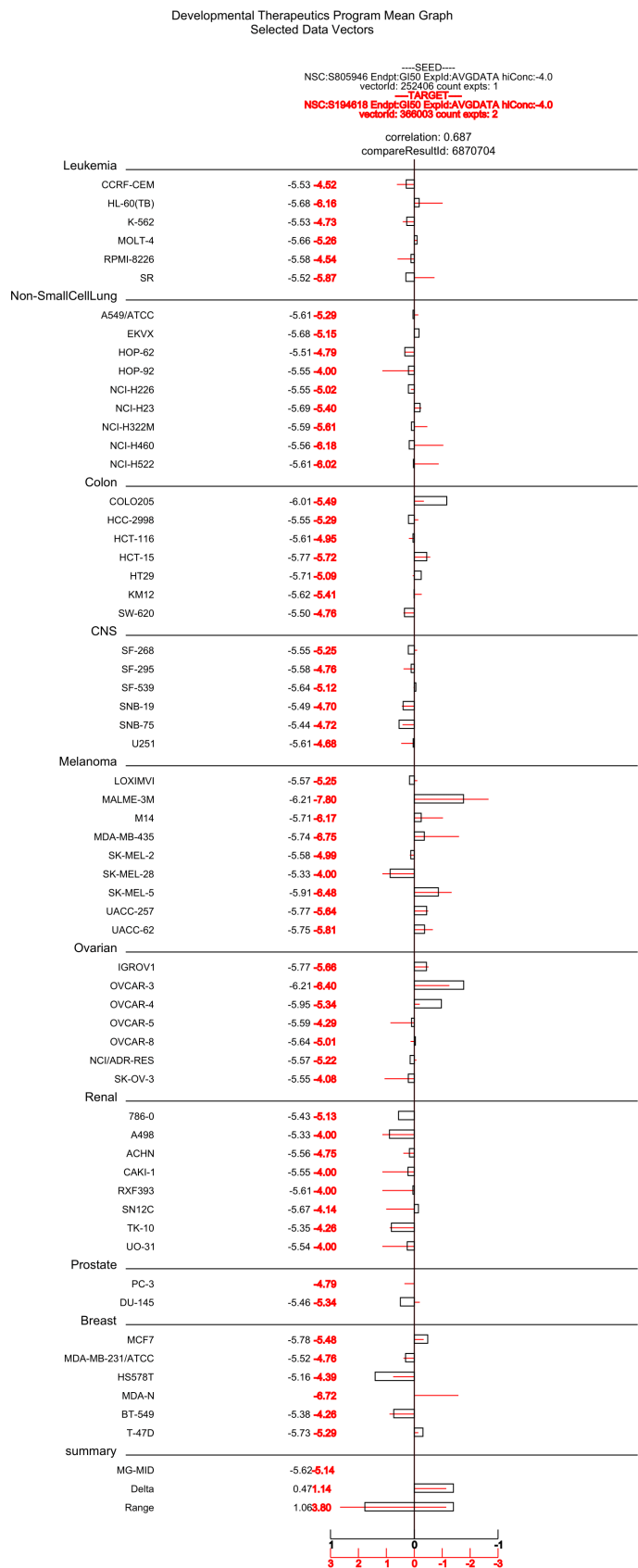
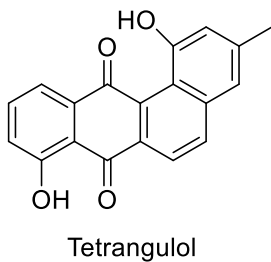
	3	11	15	18
CCRF-CEM	0.0559	3.11	2.98	0.793
K-562	3.04	3.36	2.94	0.497
MOLT-4	1.69	3.09	2.21	1.14
RPMI-8226	2.07	3.24	2.61	1.52
AS49/ATCC	3.12	2.56	2.47	1.36
EKVX	2.02	2.12	2.09	1.18
HOP-62	0.744	3.12	3.1	1.67
HOP-92	2.12	2.46	2.8	2.31
NCI-H226	1.42	4.01	2.81	1.96
NCI-H23	1.63	1.89	2.03	0.64
NCI-H322M	1.68	3.45	2.59	1.08
NCI-H460	2.72	2.94	2.74	0.731
NCI-H522	1.66	2.25	2.45	1.2
COLO-205	1.64	1.7	0.977	0.27
HCC-2998	1.64	3.4	2.8	0.421
HCT-116	0.945	2.5	2.47	0.305
HCT-15	1.43	2.76	1.7	0.483
HT-29	2.41	2.97	1.97	0.374
KM12	1.99	2.93	2.4	0.653
SW-620	1.54	3.39	3.12	0.385
SF-268	2.05	3.46	2.81	1.48
SF-295	1.78	3.04	2.6	1.09
SF-539	1.8	2.85	2.29	1.13
U251	1.48	2.74	2.46	1.34
LOX IMVI	1.44	2.45	2.72	0.36
MALME-3M	1.53	1.47	0.617	0.134
M14	1.78	2.06	1.97	0.729
MDA-MB-435	1.79	1.86	1.81	0.208
SK-MEL-2	1.89	2.52	2.63	1.21
SK-MEL-28	1.96	5.79	4.63	2.21
SK-MEL-5	1.14	1.47	1.23	0.249
UACC-257	1.57	1.62	1.7	0.279
UACC-62	1.63	1.41	1.8	0.316
IGROV1	1.74	2.05	1.71	0.656
OVCAR-3	0.314	2.27	0.613	0.272
OVCAR-4	1.2	1.55	1.13	0.316
OVCAR-5	1.76	3.11	2.58	1.4
OVCAR-8	0.339	2.51	2.32	0.484
NCI-ADR/RES	2.33	2.83	2.67	0.535
786-0	2.07	4.65	3.68	2.33
A498	3.58	4.48	4.72	1.39
ACHN	1.9	3.08	2.73	1.13
CAKI-1	1.95	5.66	2.84	0.972
RXF 393	1.42	3.08	2.47	1.34
SN12C	1.62	1.49	2.13	0.408
UO-31	1.69	2.41	2.9	1.26
DU-145	1.82	4.26	3.5	1.37
MCF7	1.55	1.3	1.65	0.29
MDA-MB-231/ATCC	2.68	2.11	3.03	0.385
HS 578T	2.84	10.8	7	1.9
BT-549	1.72	4.08	4.19	1.49
T-47D	1.97	2.26	1.88	0.346
MDA-MB-468	0.946	1.1	1.3	0.259
Mean GI ₅₀	1.75	2.93	2.52	0.91

Mean LC₅₀ was calculated from 50 human cancer cell lines that were common across the four compounds **3**, **11**, **15** and **16**. Conditional formatting was applied to the GI₅₀ values per compound row with the colour green representing more un-responsive cell lines and red representing more-responsive cell lines.

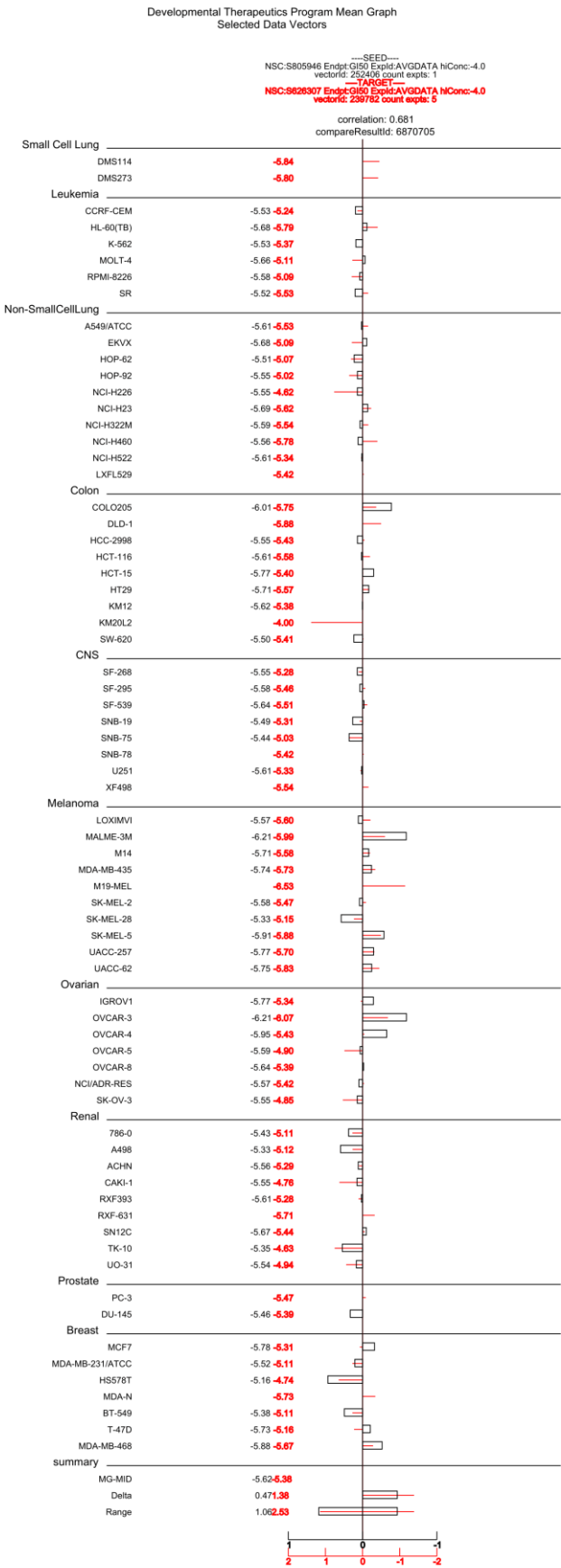
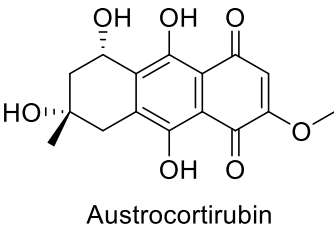
	3	11	15	16
CCRF-CEM	100	100	100	100
K-562	100	100	100	100
RPMI-8226	100	100	100	100
A549/ATCC	55.3	43.5	66.1	75.4
EKVX	22.2	24.4	28.7	5.55
HOP-62	5.79	33.5	100	6.67
HOP-92	23.8	22.7	56.5	100
NCI-H226	6.26	74.9	77.8	8.87
NCI-H23	100	100	100	5.52
NCI-H322M	5.62	33.4	100	26
NCI-H460	49.9	63.7	100	9.16
NCI-H522	8.11	29.8	52.9	7.68
COLO-205	6.85	7.73	7.19	4.17
HCC-2998	5.95	40	32.8	4.55
HCT-116	46	27.5	34.2	5.22
HCT-15	7.69	30.1	15.7	5.84
HT-29	100	100	100	100
KM12	15.1	62.2	73.1	30.5
SW-620	9.09	42.8	67	6
SF-268	27.6	54.1	100	27.2
SF-295	6.28	34.3	67.4	5.1
SF-539	5.68	28.4	15.8	4.9
U251	5.4	31.9	46	7.15
LOX IMVI	6.42	79.1	66.2	4.45
MALME-3M	5.57	5.55	4.43	0.789
M14	6.69	13.8	9.58	6.19
MDA-MB-435	6.13	6.15	6.01	0.798
SK-MEL-2	8.41	29.5	46.9	6.12
SK-MEL-28	6.62	44.2	100	10.2
SK-MEL-5	5.07	5.54	5.1	2.6
UACC-257	7.11	6.3	9.49	4.33
UACC-62	6.3	6.12	7.05	4.82
IGROV1	11.8	29.2	35.2	6.19
OVCAR-3	22.2	24.6	29.3	3.28
OVCAR-4	5.08	5.8	5.07	3.83
OVCAR-5	5.91	33.4	24.1	5.58
OVCAR-8	4.54	76.8	31	15.3
NCI-ADR/RES	100	100	100	73.8
786-0	9.84	41.1	100	36.1
A498	38.1	42.2	40.9	5.4
CAKI-1	9.05	44	50.4	17.7
RXF 393	5.84	35.7	41.2	6.43
SN12C	5.93	8	20.6	4.21
UO-31	6.8	33	32.7	5.13
DU-145	5.69	38.5	33.9	5.31
MDA-MB-231/ATCC	100	33.3	100	100
HS 578T	100	100	100	100
BT-549	5.73	40.1	41.5	6.5
T-47D	100	33.7	59.9	8.21
MDA-MB-468	7.48	6.87	7.61	4.28
Mean LC ₅₀	28.3	42.14	52.99	23.86

COMPARE Analysis

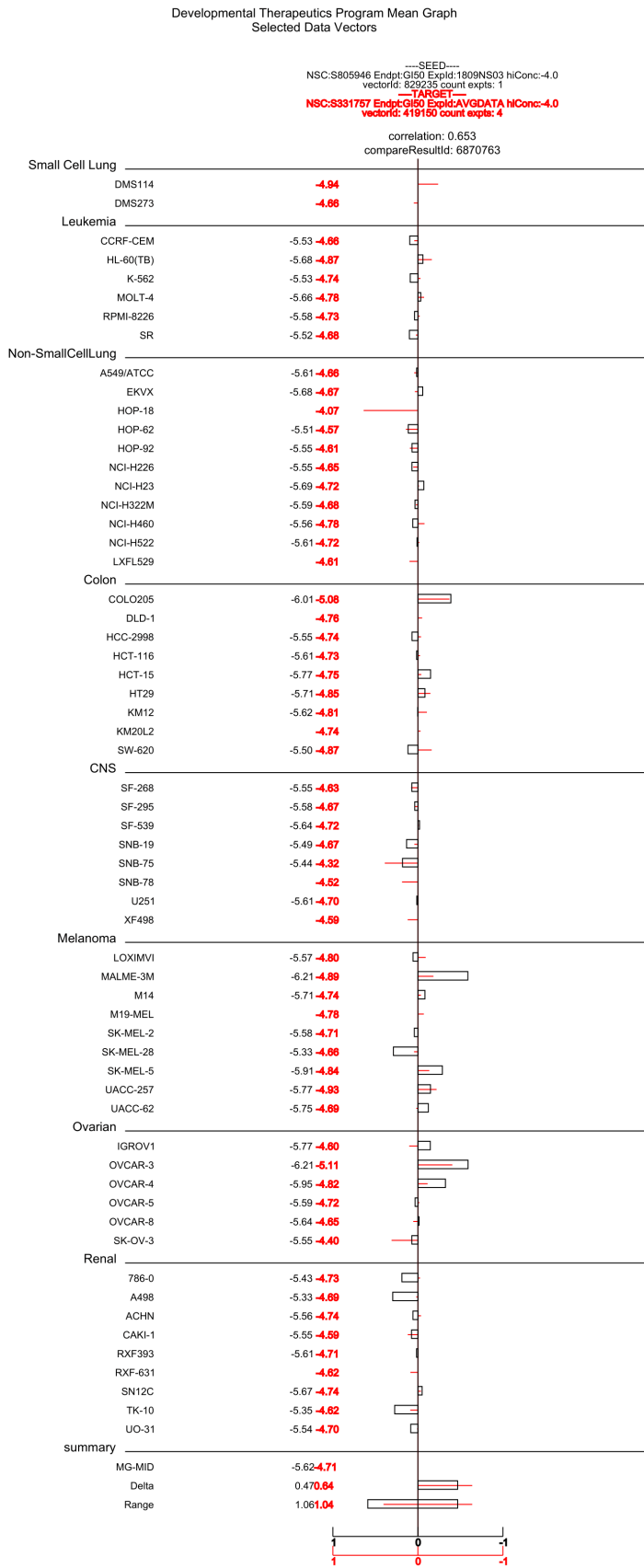
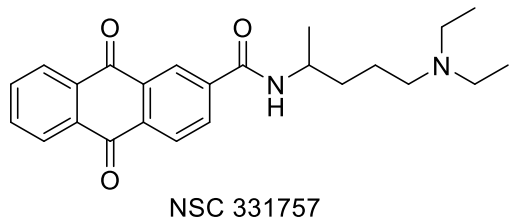
Correlation of 15 - Tetrangulol



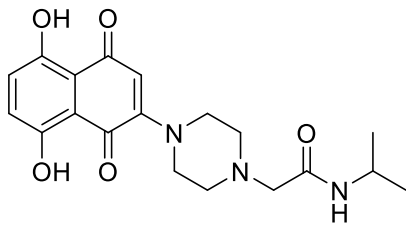
Correlation of **15** - (1S,3S)-austrocortirubin



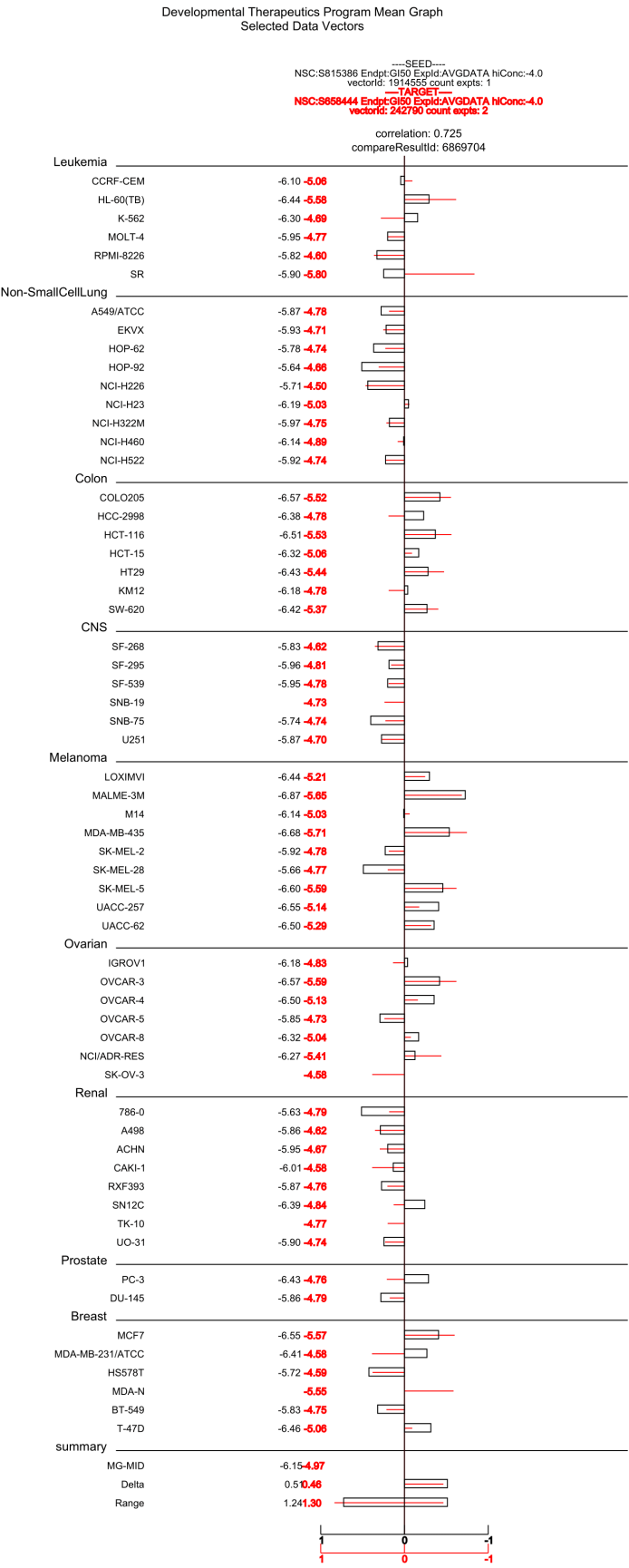
Correlation of 15 – NSC 331757



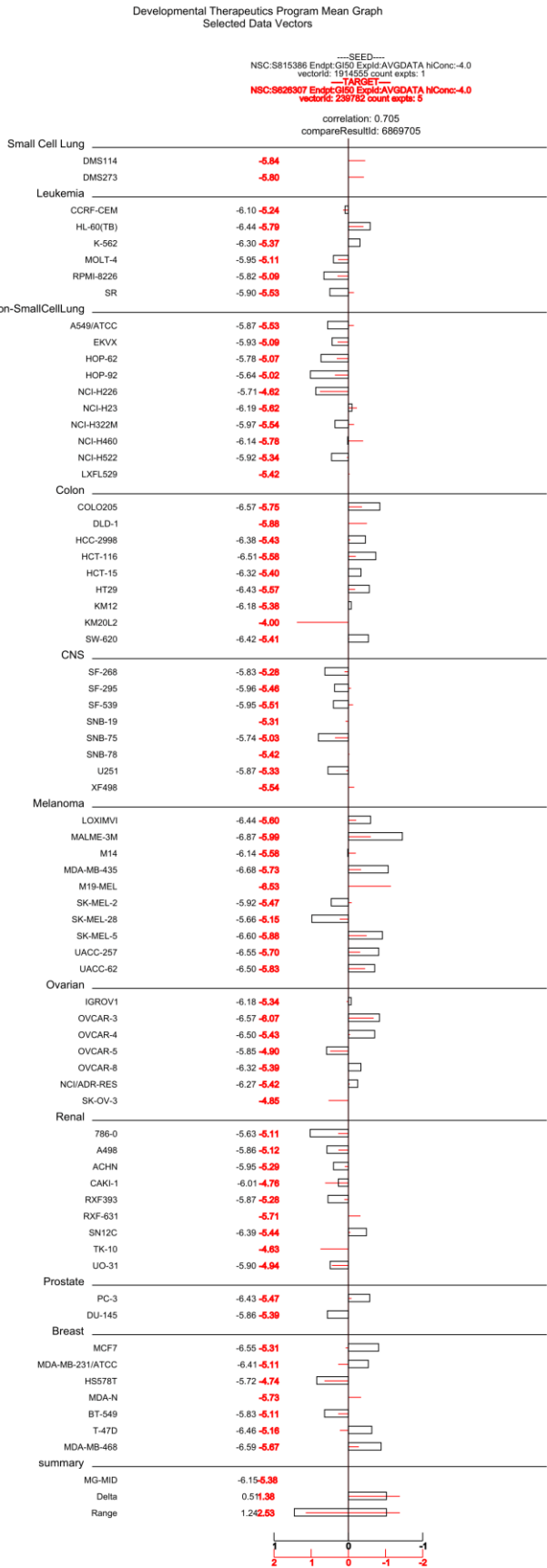
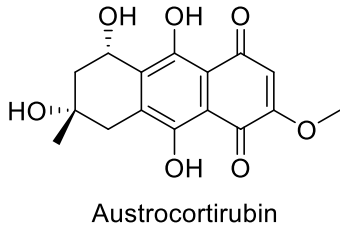
Correlation of 16 – NSC658444



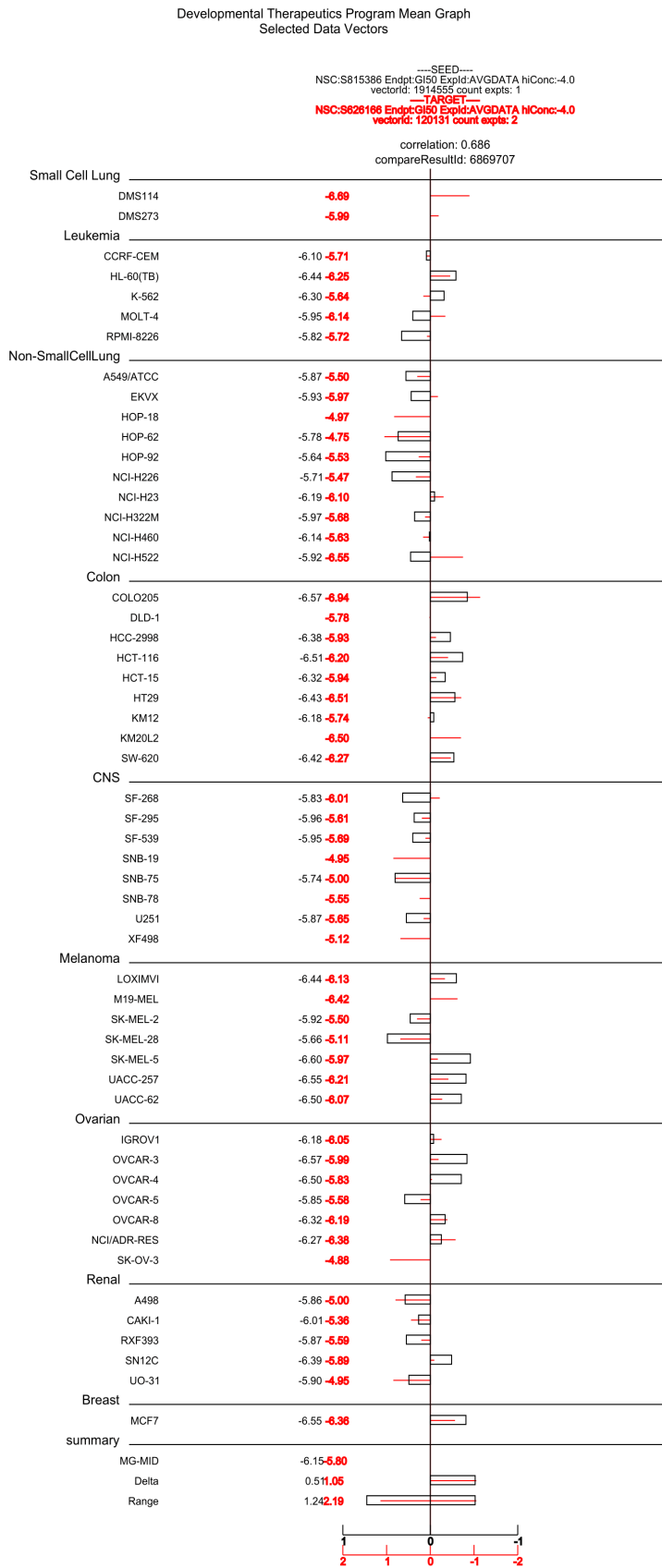
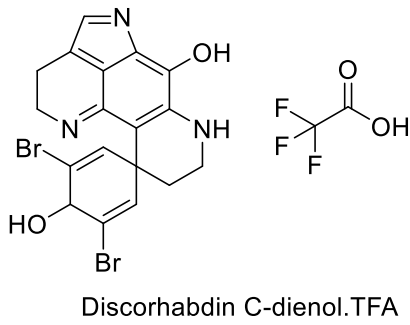
NSC 658444



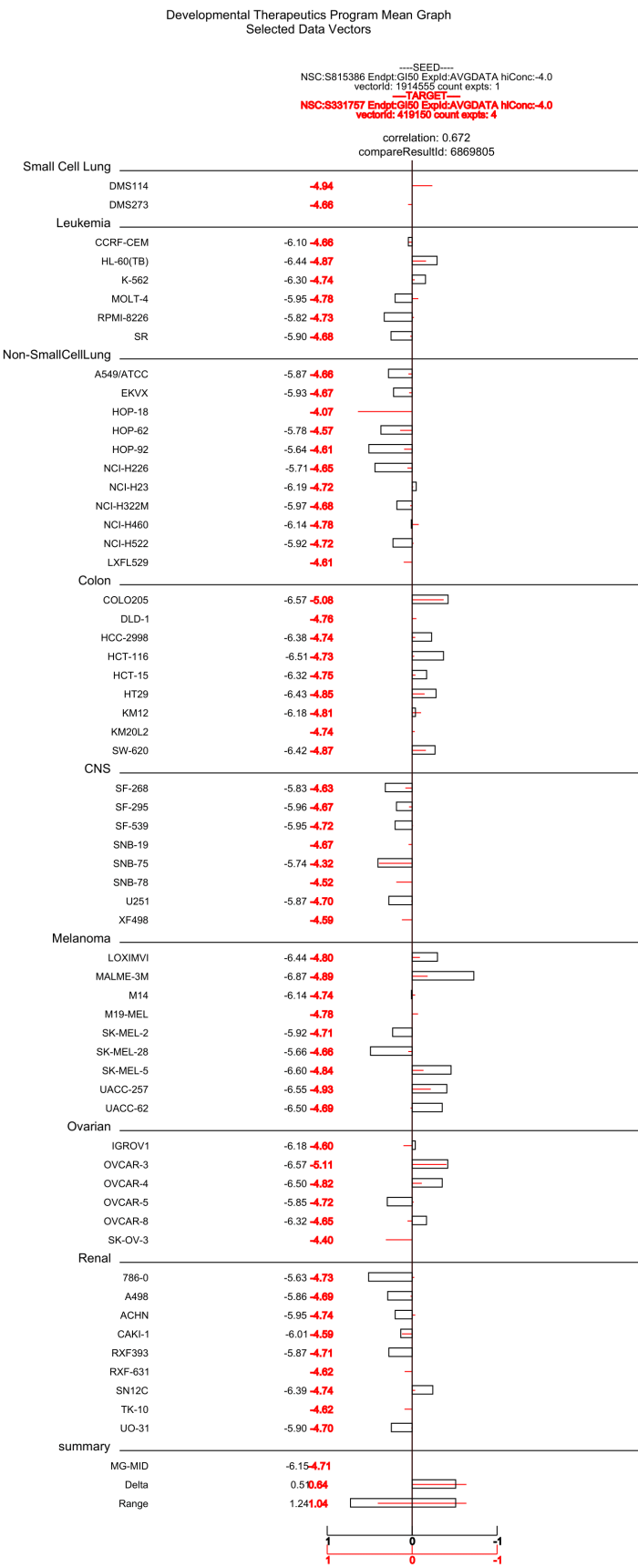
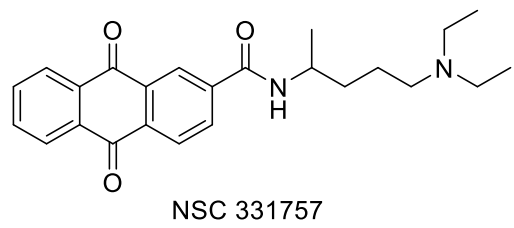
Correlation of **16** - (1S,3S)-austrocrotirubin



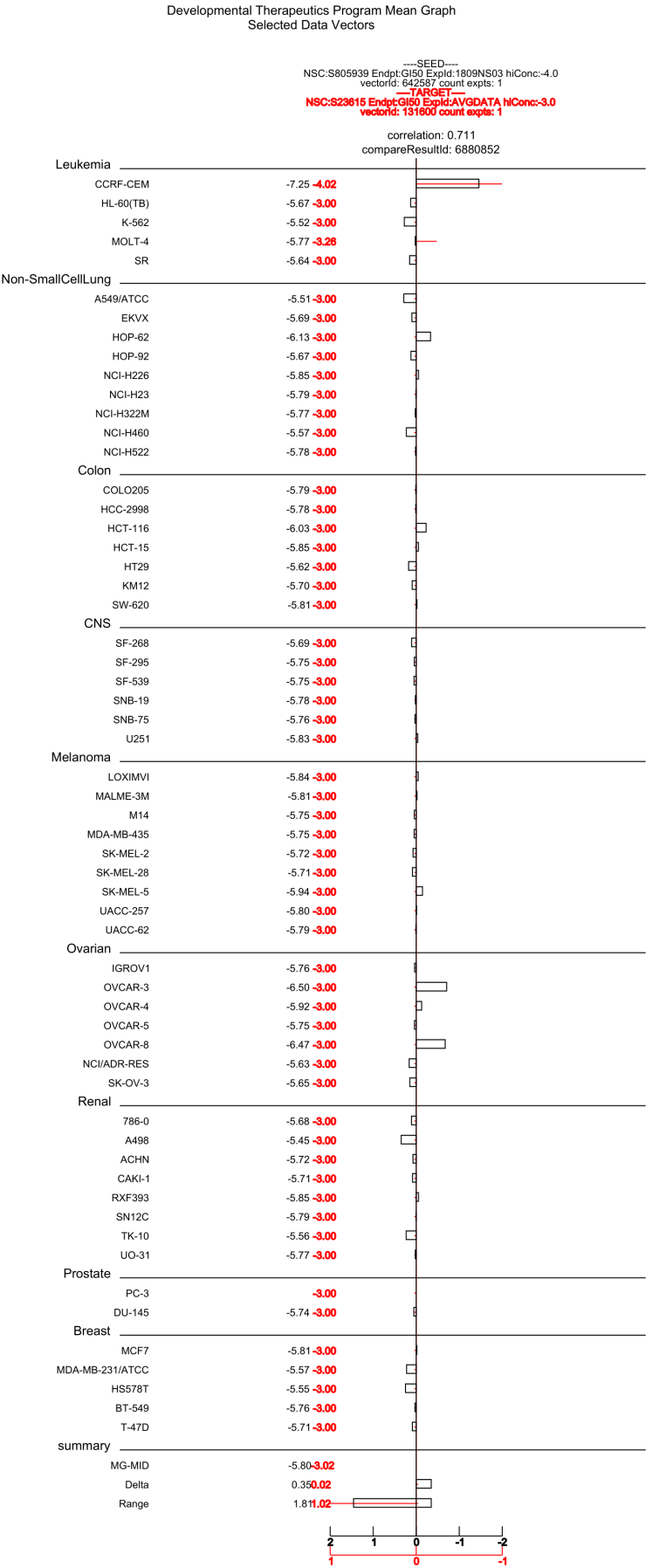
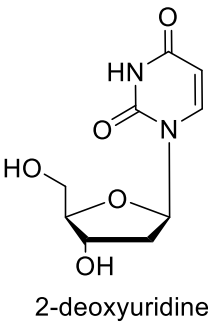
Correlation of **16** – Discorhabdin C-dienol.TFA



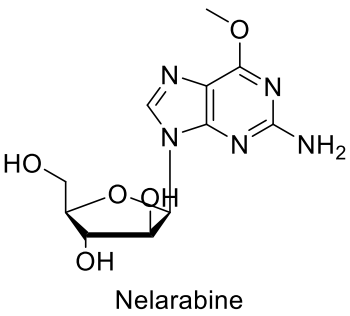
Correlation of 16 – NSC331757



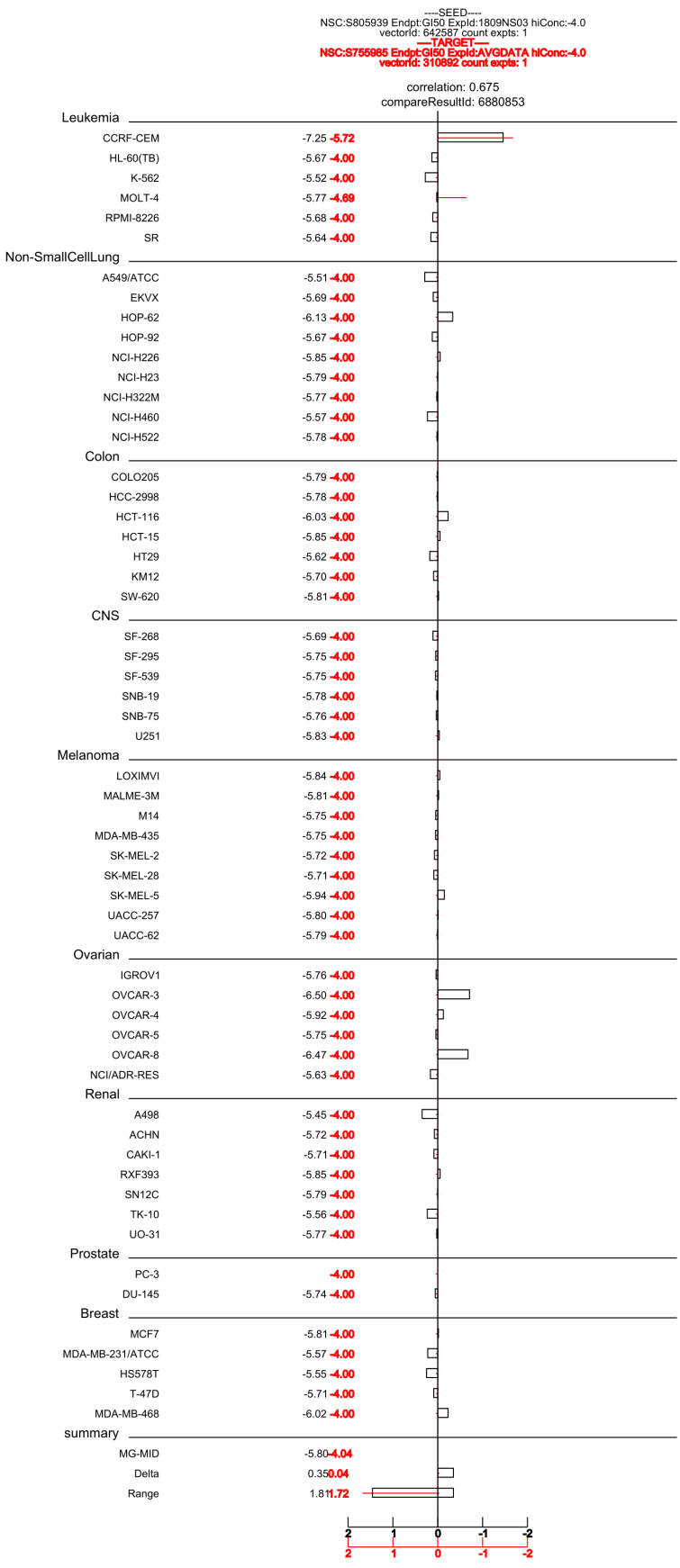
Correlation of 3 – 2-Deoxyuridine



Correlation of 3- Nelarabine



Developmental Therapeutics Program Mean Graph
Selected Data Vectors



Matrix COMPARE of 15 (NSC 805946) and 16 (NSC 815386)

Row Label	829235 NSC:S805946 Endpt:GI50 Expld:1809NS03 hiConc:-4.0 null	1914555 NSC:S815386 Endpt:GI50 Expld:AVGDATA hiConc:-4.0 null
829235 NSC:S805946 Endpt:GI50 Expld:1809NS03 hiConc:-4.0 null	correlation: 1.0 count cell lines: 59 seed stdDev: 0.185 target stdDev: 0.185 ☐ Select this result	correlation: 0.725 count cell lines: 56 seed stdDev: 0.185 target stdDev: 0.319 ☐ Select this result
1914555 NSC:S815386 Endpt:GI50 Expld:AVGDATA hiConc:-4.0 null	correlation: 0.725 count cell lines: 56 seed stdDev: 0.319 target stdDev: 0.185 ☐ Select this result	correlation: 1.0 count cell lines: 57 seed stdDev: 0.319 target stdDev: 0.319 ☐ Select this result

Matrix COMPARE of 15 (NSC 805946) and 3 (NSC 805939)

Row Label	252406 NSC:S805946 Endpt:GI50 Expld:AVGDATA hiConc:-4.0 null	642587 NSC:S805939 Endpt:GI50 Expld:1809NS03 hiConc:-4.0 null
252406 NSC:S805946 Endpt:GI50 Expld:AVGDATA hiConc:-4.0 null	correlation: 1.0 count cell lines: 59 seed stdDev: 0.185 target stdDev: 0.185 ☐ Select this result	correlation: 0.291 count cell lines: 59 seed stdDev: 0.185 target stdDev: 0.263 ☐ Select this result
642587 NSC:S805939 Endpt:GI50 Expld:1809NS03 hiConc:-4.0 null	correlation: 0.291 count cell lines: 59 seed stdDev: 0.263 target stdDev: 0.185 ☐ Select this result	correlation: 1.0 count cell lines: 59 seed stdDev: 0.263 target stdDev: 0.263 ☐ Select this result

Graph selected matrix COMPARE results Select/Deselect All

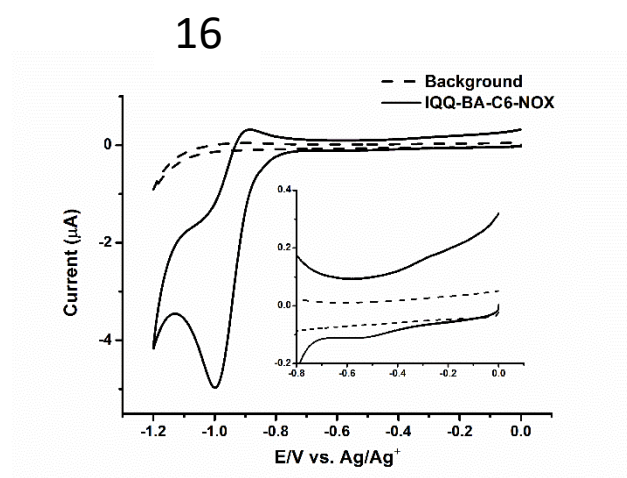
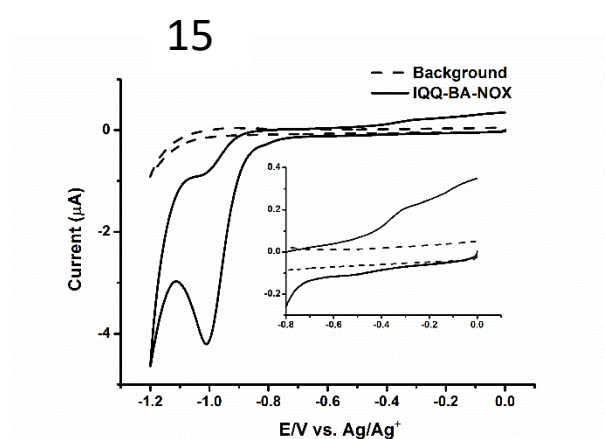
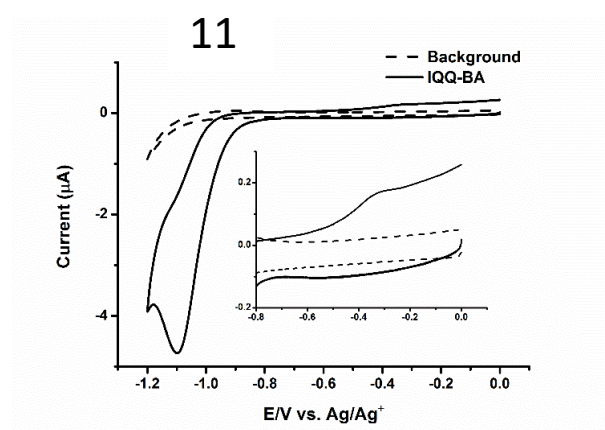
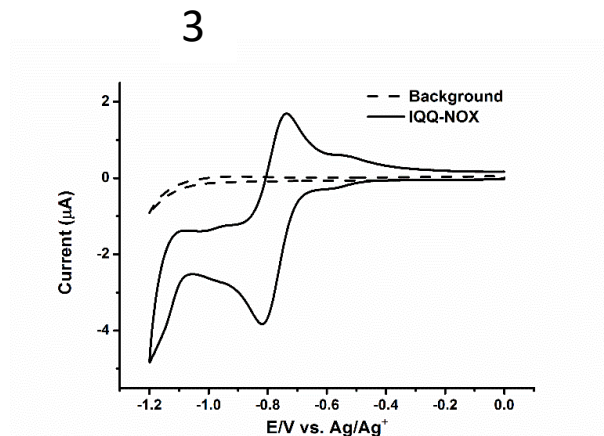
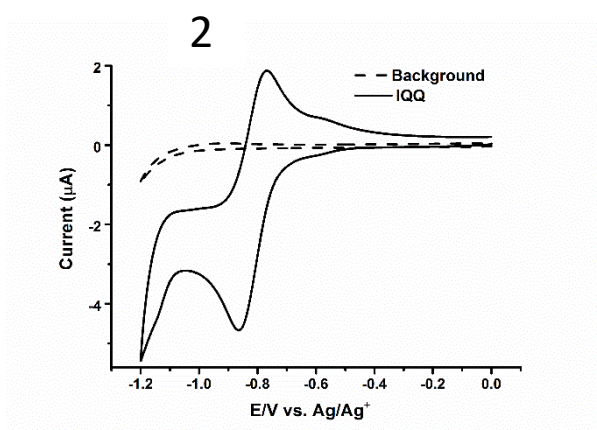
Matrix COMPARE of 16 (NSC 815386) and 3 (NSC 805939)

Row Label	642587 NSC:S805939 Endpt:GI50 Expld:1809NS03 hiConc:-4.0 null	1914555 NSC:S815386 Endpt:GI50 Expld:AVGDATA hiConc:-4.0 null
642587 NSC:S805939 Endpt:GI50 Expld:1809NS03 hiConc:-4.0 null	correlation: 1.0 count cell lines: 59 seed stdDev: 0.263 target stdDev: 0.263 ☐ Select this result	correlation: 0.2 count cell lines: 56 seed stdDev: 0.268 target stdDev: 0.319 ☐ Select this result
1914555 NSC:S815386 Endpt:GI50 Expld:AVGDATA hiConc:-4.0 null	correlation: 0.2 count cell lines: 56 seed stdDev: 0.319 target stdDev: 0.268 ☐ Select this result	correlation: 1.0 count cell lines: 57 seed stdDev: 0.319 target stdDev: 0.319 ☐ Select this result

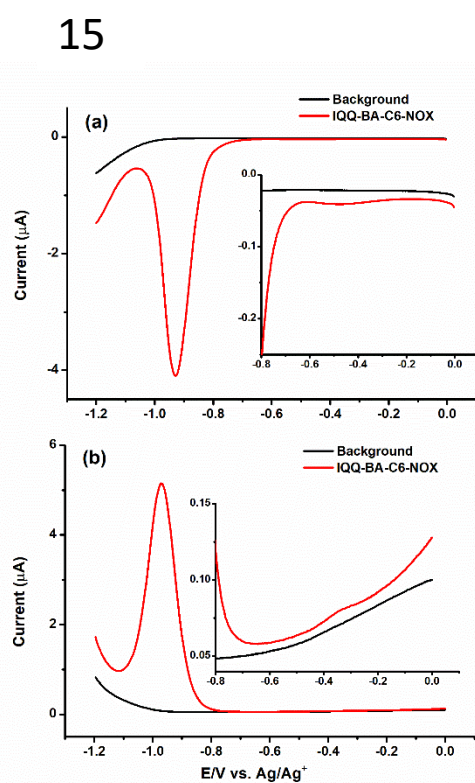
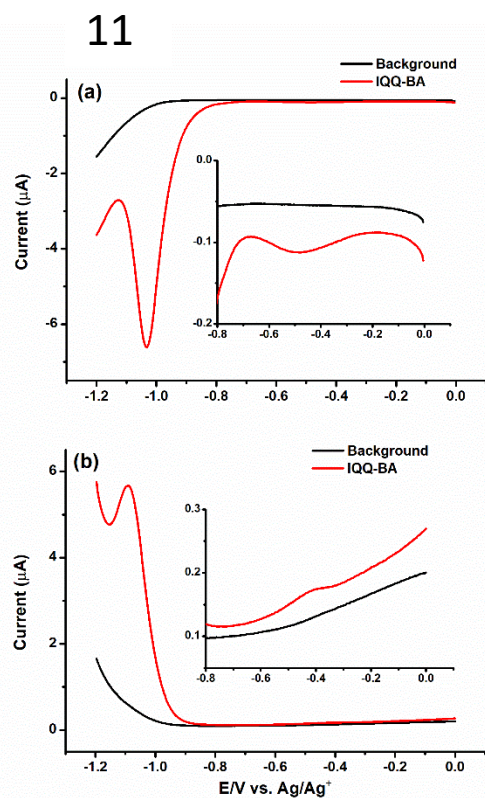
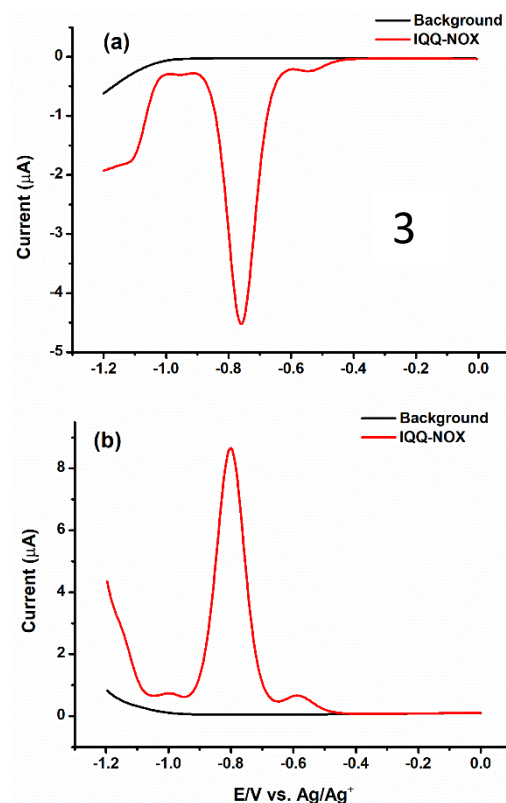
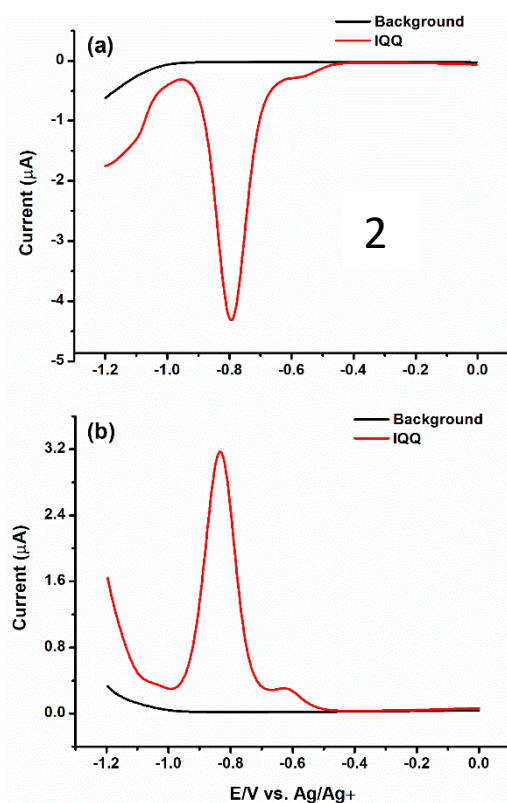
Graph selected matrix COMPARE results Select/Deselect All

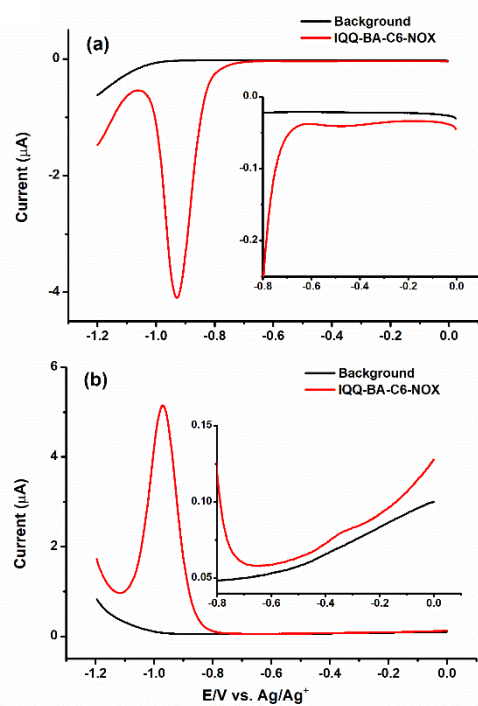
Electrochemical Graphs

CV of 2 mM **2**, 2 mM **3**, 2 mM **11**, 2 mM **15**, and 3 mM **16** obtained by a Pt electrode vs. Ag/Ag⁺ using 0.1 M of tetrabutylammonium tetrafluoroborate in ACN, a scan rate of 100 mVs⁻¹.



Squarewave voltammetry (SWV) of 3 mM **2**, 2 mM **3**, 2 mM **11**, 3 mM **15**, and 3 mM **16** obtained by a Pt electrode vs. Ag/Ag⁺ using 0.1 M of tetrabutylammonium tetrafluoroborate in ACN.

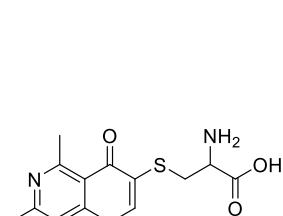




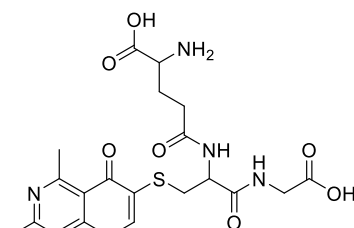
Adduct Binding Studies

Predicted adducts

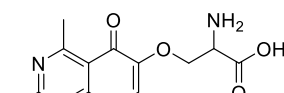
Compounds **2**, **3**, **7** and **15** proposed adducts are shown. Compound **11** adducts are identical to compound **7** adducts due to loss of bromine. Compound **16** is opposite isomer to compound **15**, thus mass of adducts formed would be identical.



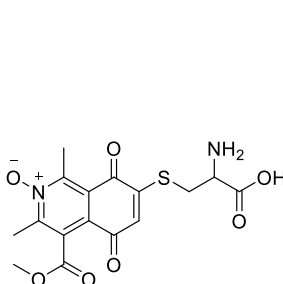
Molecular Weight: 364.37
2-cys



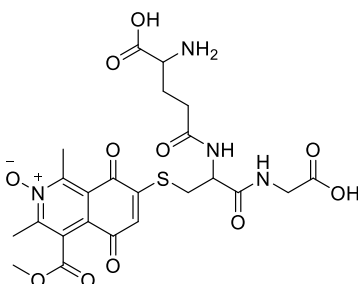
Molecular Weight: 550.54
2-glu



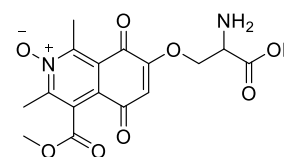
Molecular Weight: 348.31
2-ser



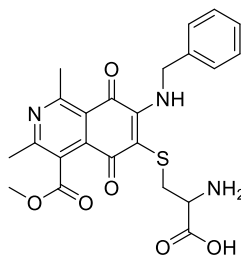
Molecular Weight: 380.37
3-cys



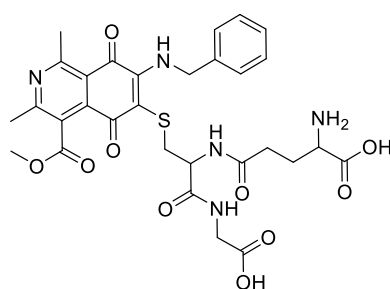
Molecular Weight: 566.54
3-glu



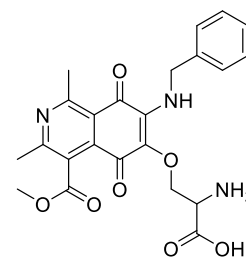
Molecular Weight: 364.31
3-ser



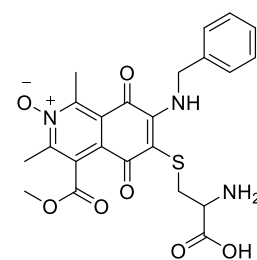
Molecular Weight: 469.51
7-cys



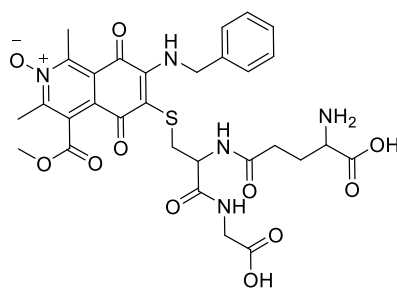
Molecular Weight: 655.68
7-glu



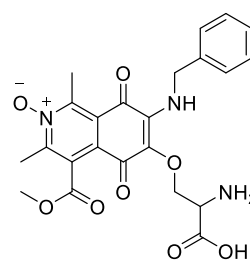
Molecular Weight: 453.45
7-ser



Molecular Weight: 485.51
15-cys



Molecular Weight: 671.68
15-glu



Molecular Weight: 469.45
15-ser

LC/MS procedure and information

LC/MS : Acquity BSM, Acquity Sample Manager, Vion IMS QTof (SAA055K)

Column: Acquity UPLC-BEH-C18

Column Dimension : 2.1 mm x 50 mm

Column Particle Size : 1.7 μ m

Mode: ESI⁺

Mobile Phase: See Below

Gradient: See Below

Flow Rate: 0.600 mL/min

Sample Run Time: 5 minutes

Injection Volume: 1 μ L with a 2 μ L dilution

Mobile Phases and Gradients

Solvent A = Water + 0.1% Formic Acid

Solvent B = Acetonitrile + 0.1 % Formic Acid

Time (min)	Composition A (%)	Composition B (%)	Curve
0.00	98.0	2.0	Initial
0.50	98.0	2.0	6
2.00	50.0	50.0	6
4.00	5.0	95.0	6
4.10	98.0	2.0	6
5.00	98.0	2.0	6

Vion IMS QTof Source Parameters

Desolvation temperature:	450 °C	Cone gas:	50 L/hr
Desolvation gas:	800 L/hr	Capillary Voltage:	1.00 kV
Source Temperature:	120 °C	Scan Time:	0.200 s

LC/MS Results

Entry	Biological Nucleophile	Retention Time	Observed mass [m/z, (M+H ⁺)]	Detector Response
2	Cysteine	1.3	365.0798	20,237
	Glutathione	1.18	551.1445	10,072
	Serine	1.6	349.1026	20,342
3	Cysteine	1.39	381.0750	11,416
	Glutathione	1.14	567.1396	4154
	Serine	1.41	365.0981	1,017
7	Cysteine	1.88	470.1385	516
	Glutathione	1.83	656.2014	737
	Serine	N/A	N/A	N/A
11	Cysteine	1.88	470.1376	1,742
	Glutathione	1.83	656.2030	3,856
	Serine	N/A	N/A	N/A
15	Cysteine	1.72	486.1328	3,893
	Glutathione	1.68	672.1991	4,324
	Serine	N/A	N/A	N/A
16	Cysteine	1.78	486.1348	208
	Glutathione	1.76	672.1984	4,713
	Serine	N/A	N/A	N/A