SUPPLEMENTARY MATERIAL

Synthesis and cytotoxic potential of 3-oxo-19β-trifluoroacetoxy-18α-oleane-28-oic acid

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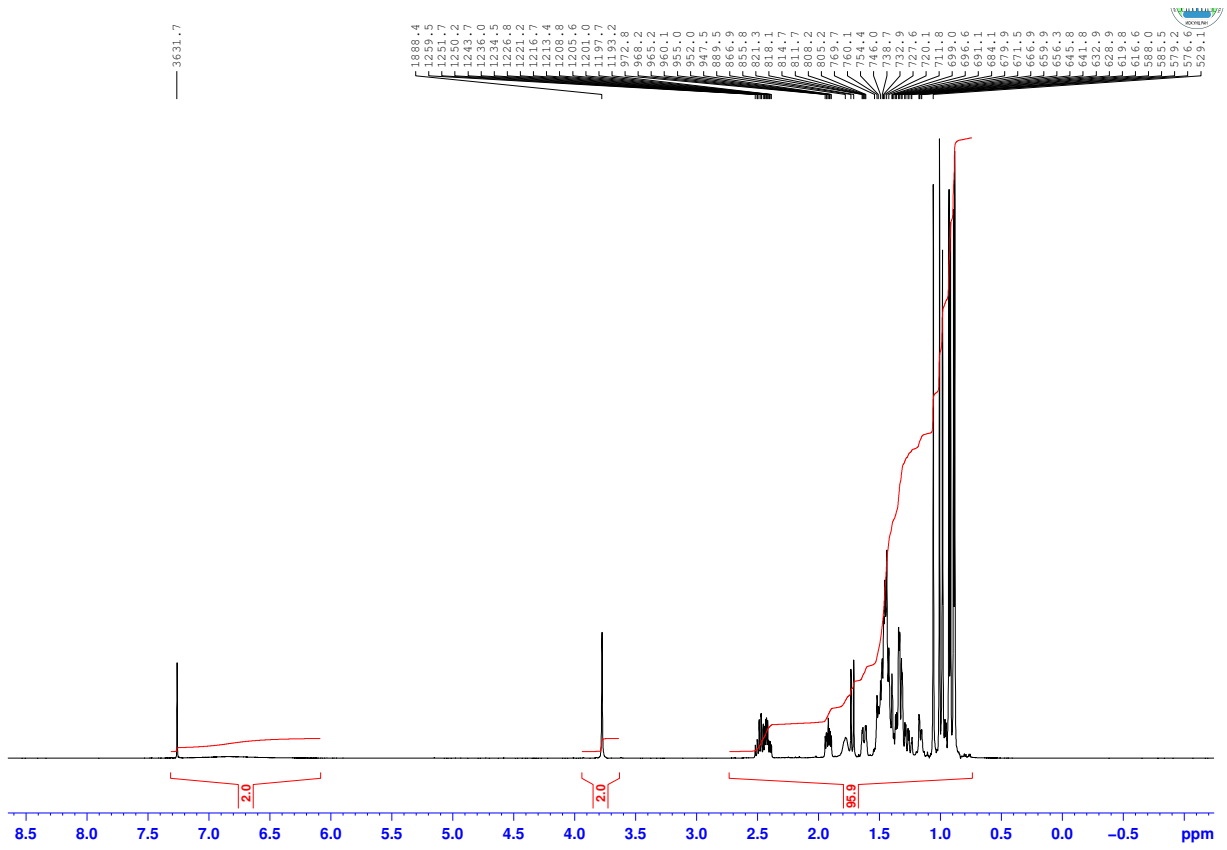


Fig. 1. ^{1}H NMR of compound **2**

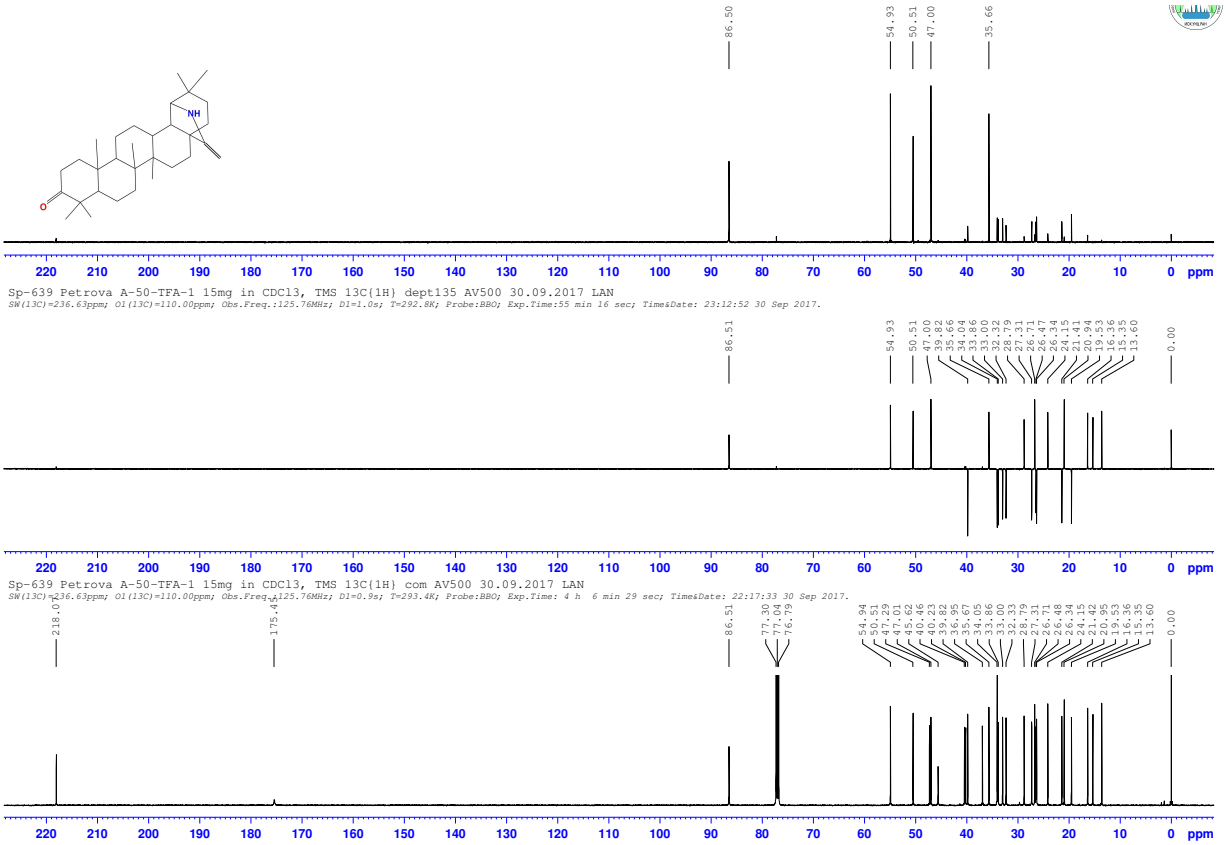


Fig. 2. ^{13}C NMR of compound **2**

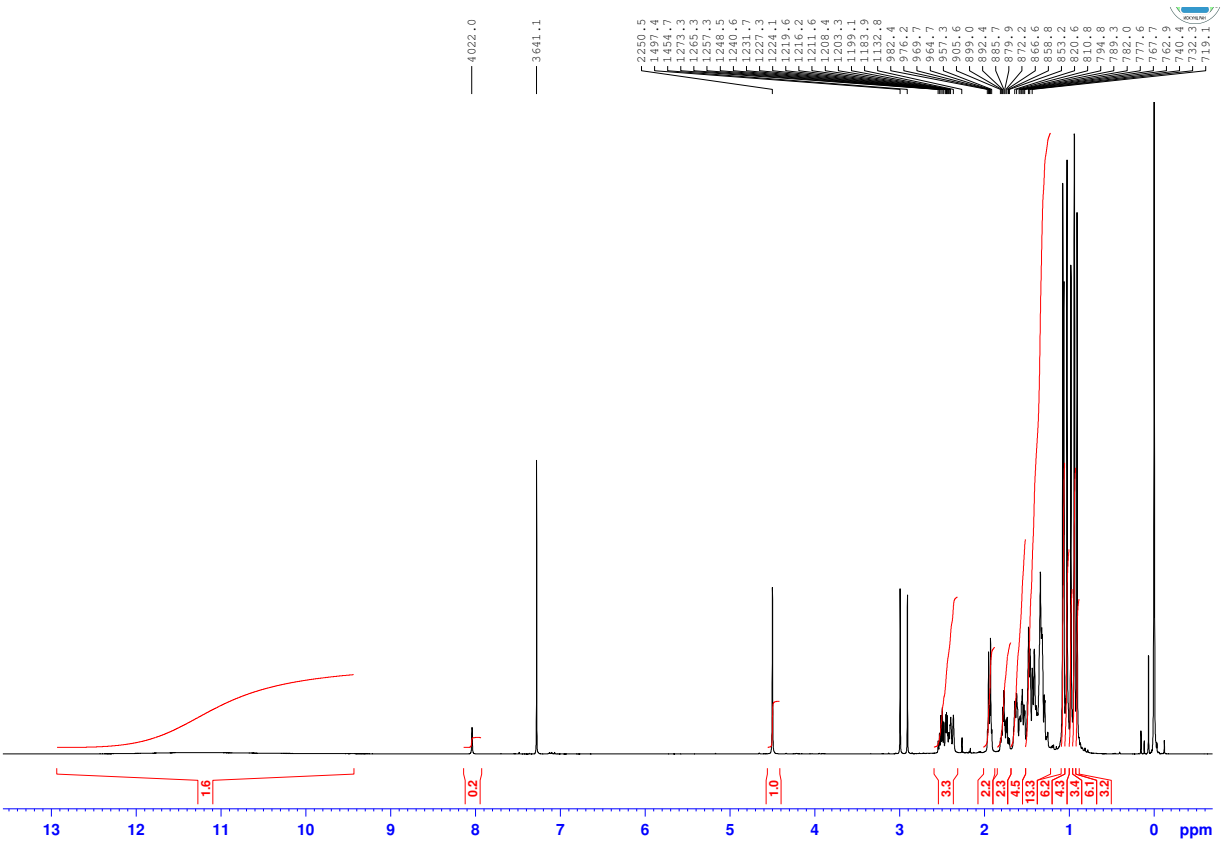


Fig. 3. ^{1}H NMR of compound **3**

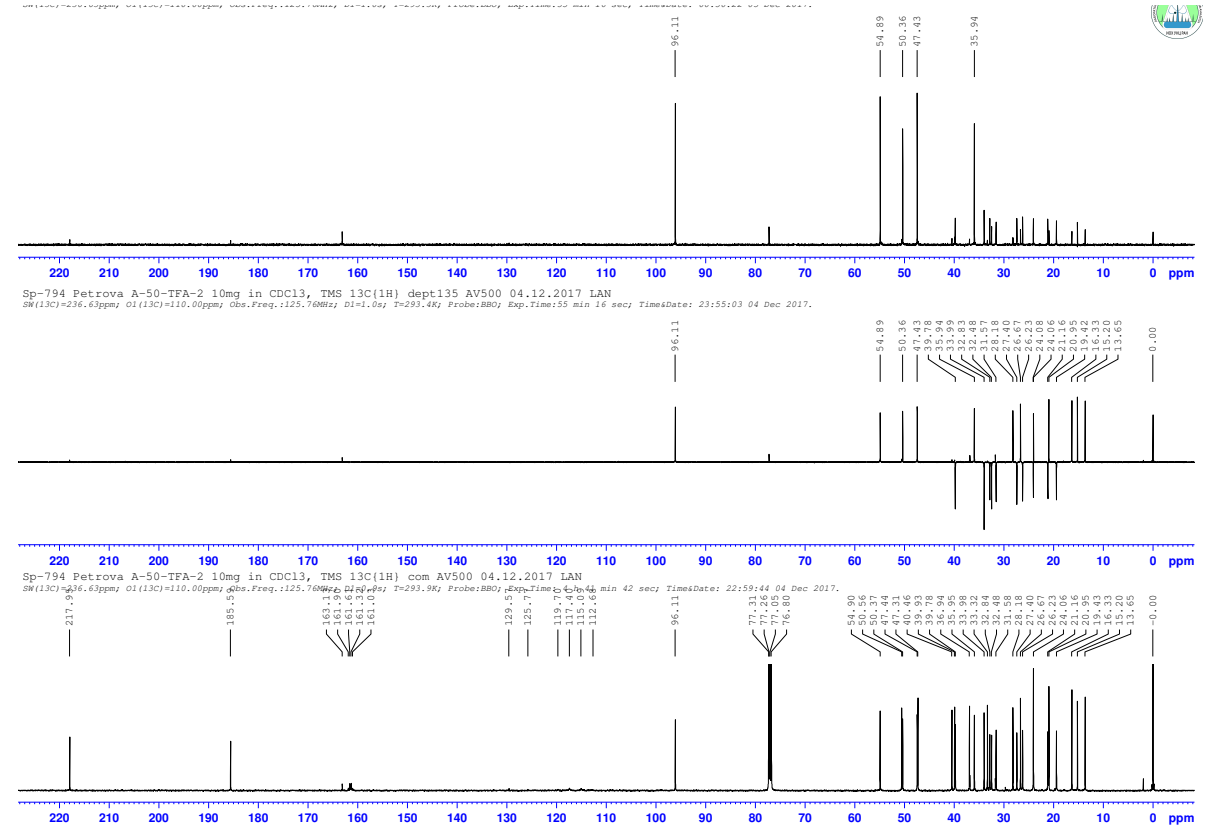


Fig. 4. ^{13}C NMR of compound **3**

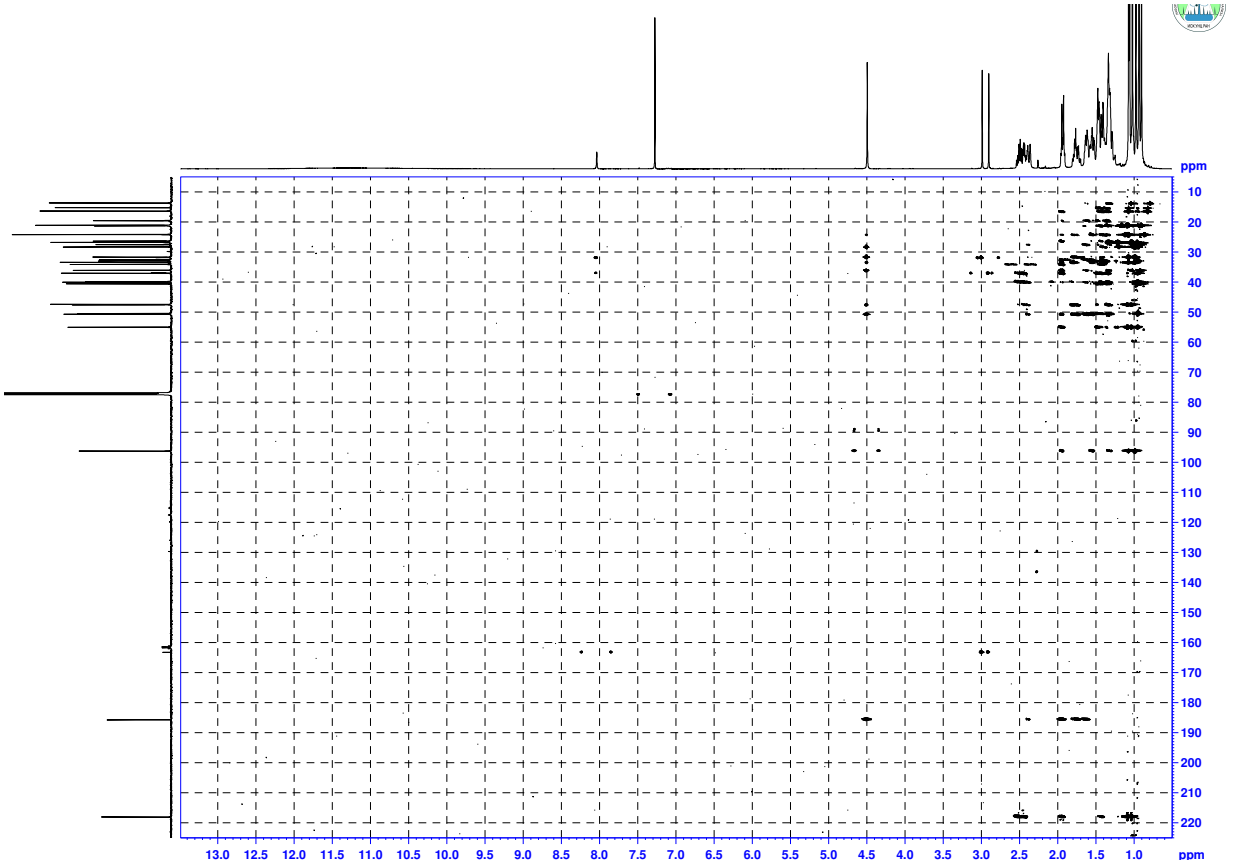


Fig. 5. HMBC spectrum of compound **3**

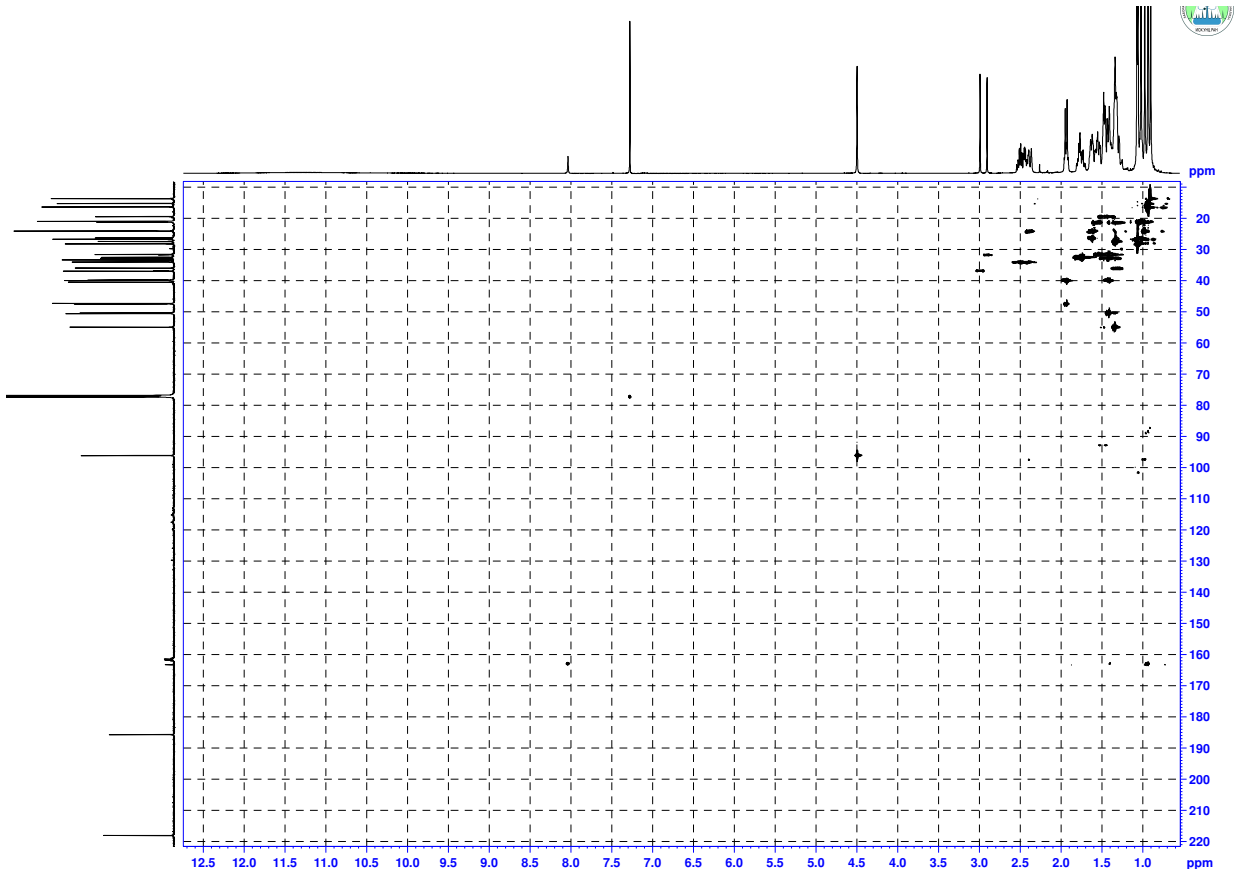


Fig. 6. HSQC spectrum of compound **3**

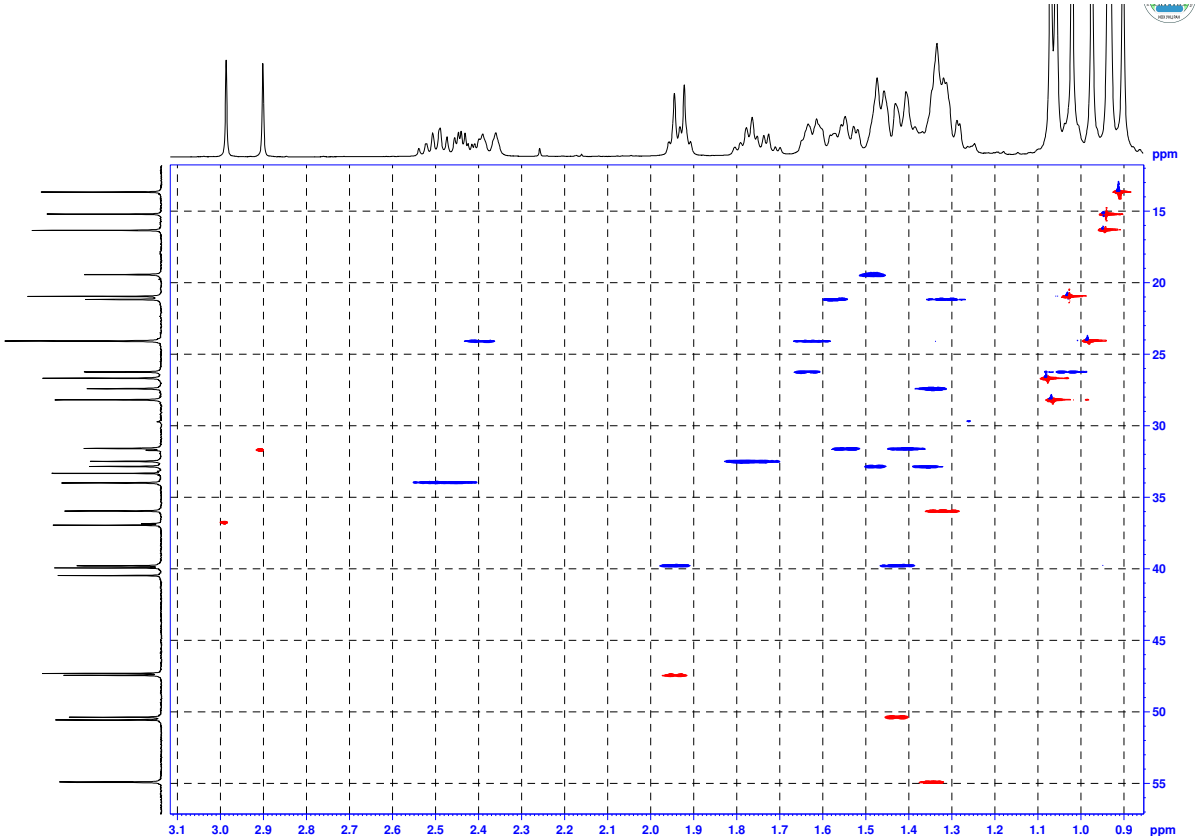


Fig. 7. HSQCED spectrum of compound **3**

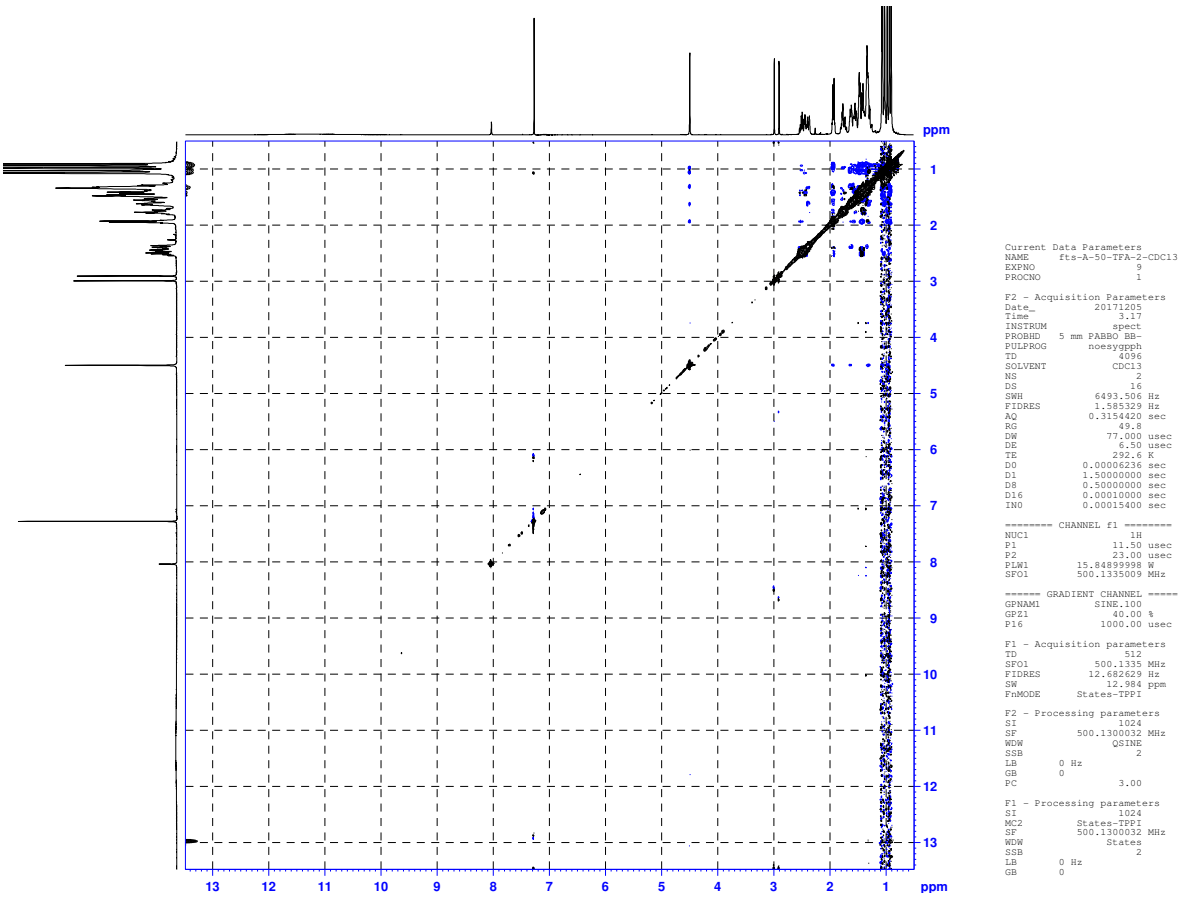


Fig. 8. NOESY spectrum of compound **3**

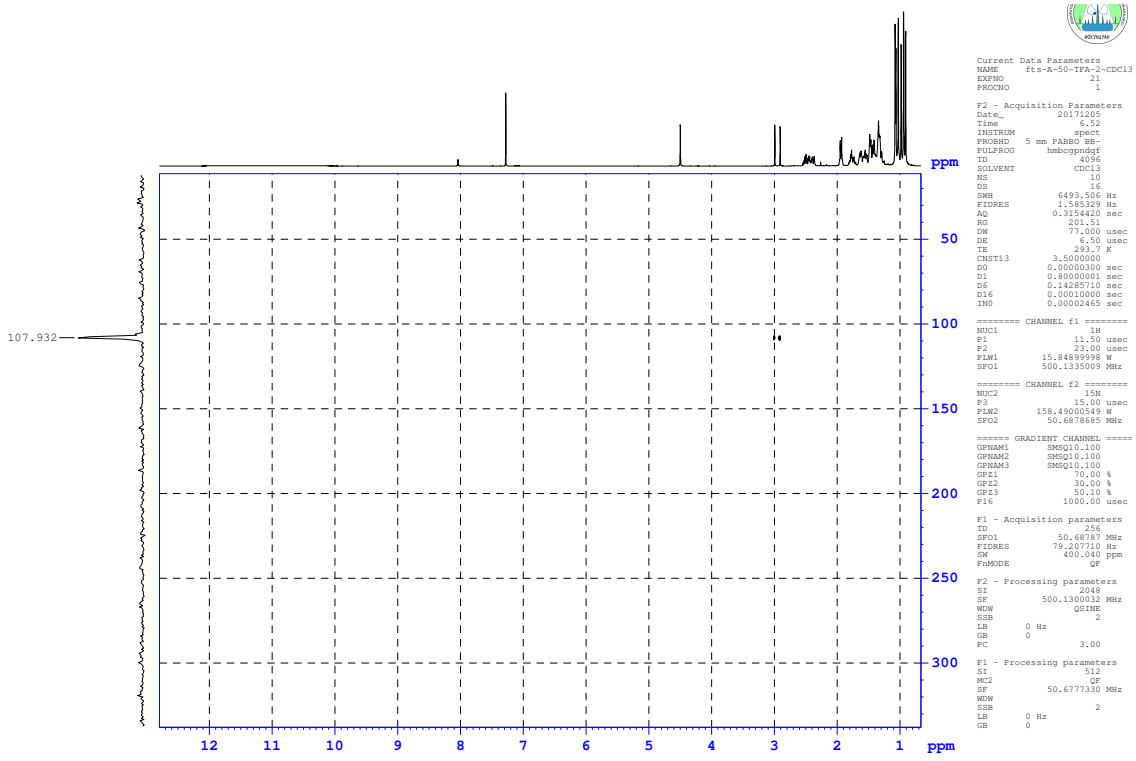


Fig. 9. ^{1}H and ^{15}N NMR HMBC spectrum of compound **3**

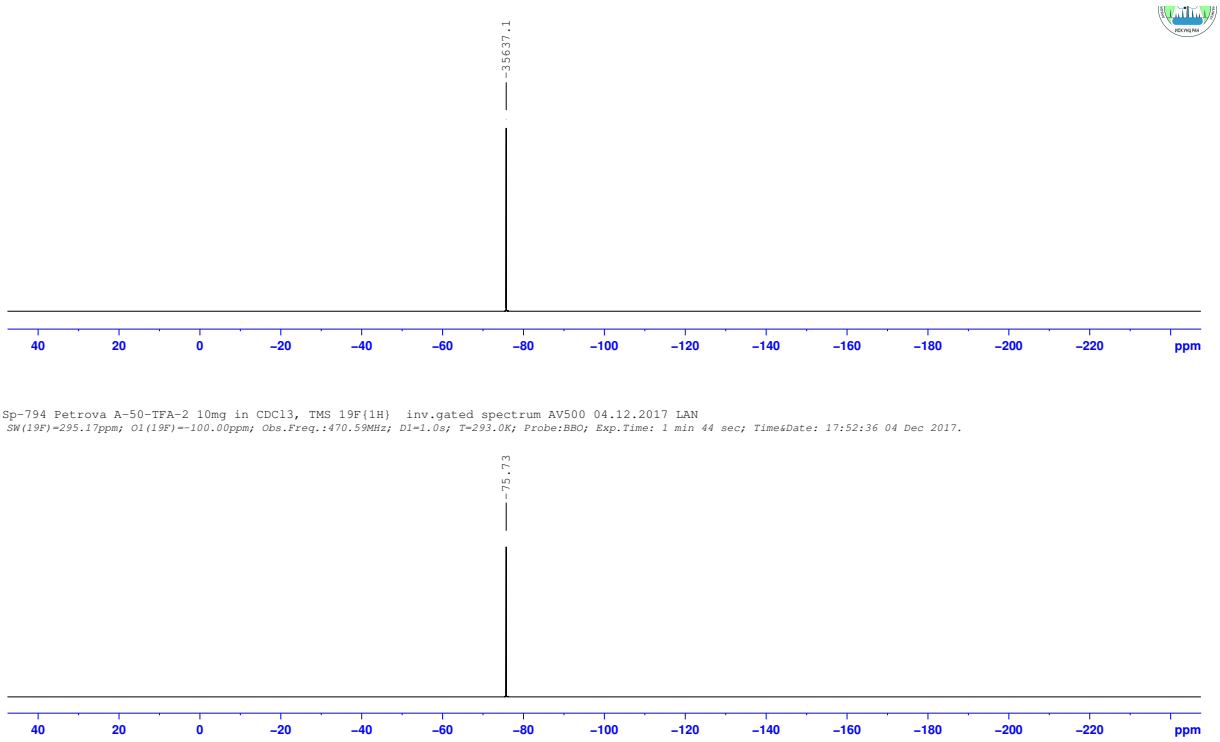


Fig. 10. ^{19}F NMR spectrum of compound **3**

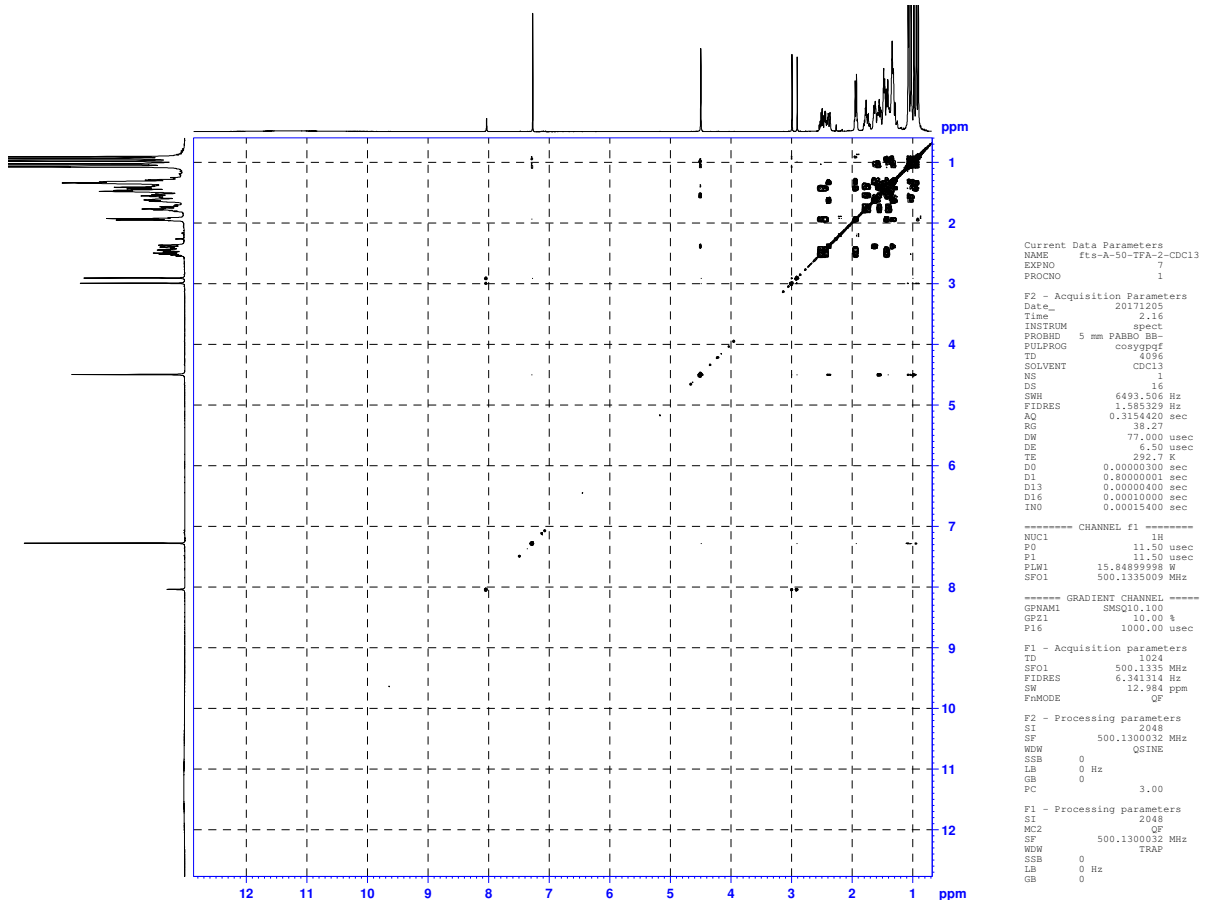
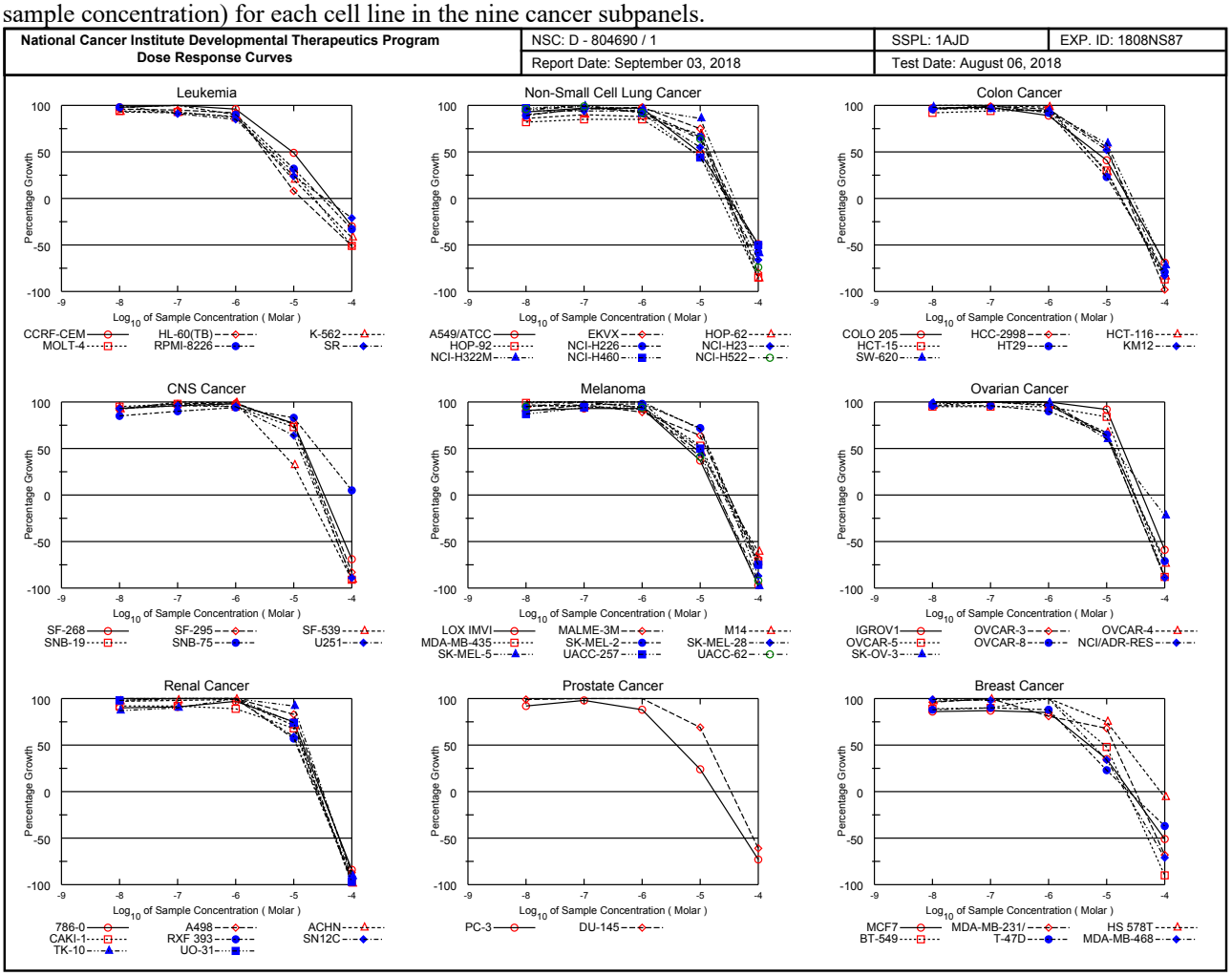


Fig. 11. COSY spectrum of compound **3**

Fig. 12. Cytostatic and cytotoxic effects of compound **3** (NSC 804690) on the NCI-60 panel of cancer cell lines at different doses, ranging from 10 nM to 100 μM. Results are displayed as dose-response curves (% growth versus sample concentration) for each cell line in the nine cancer subpanels.

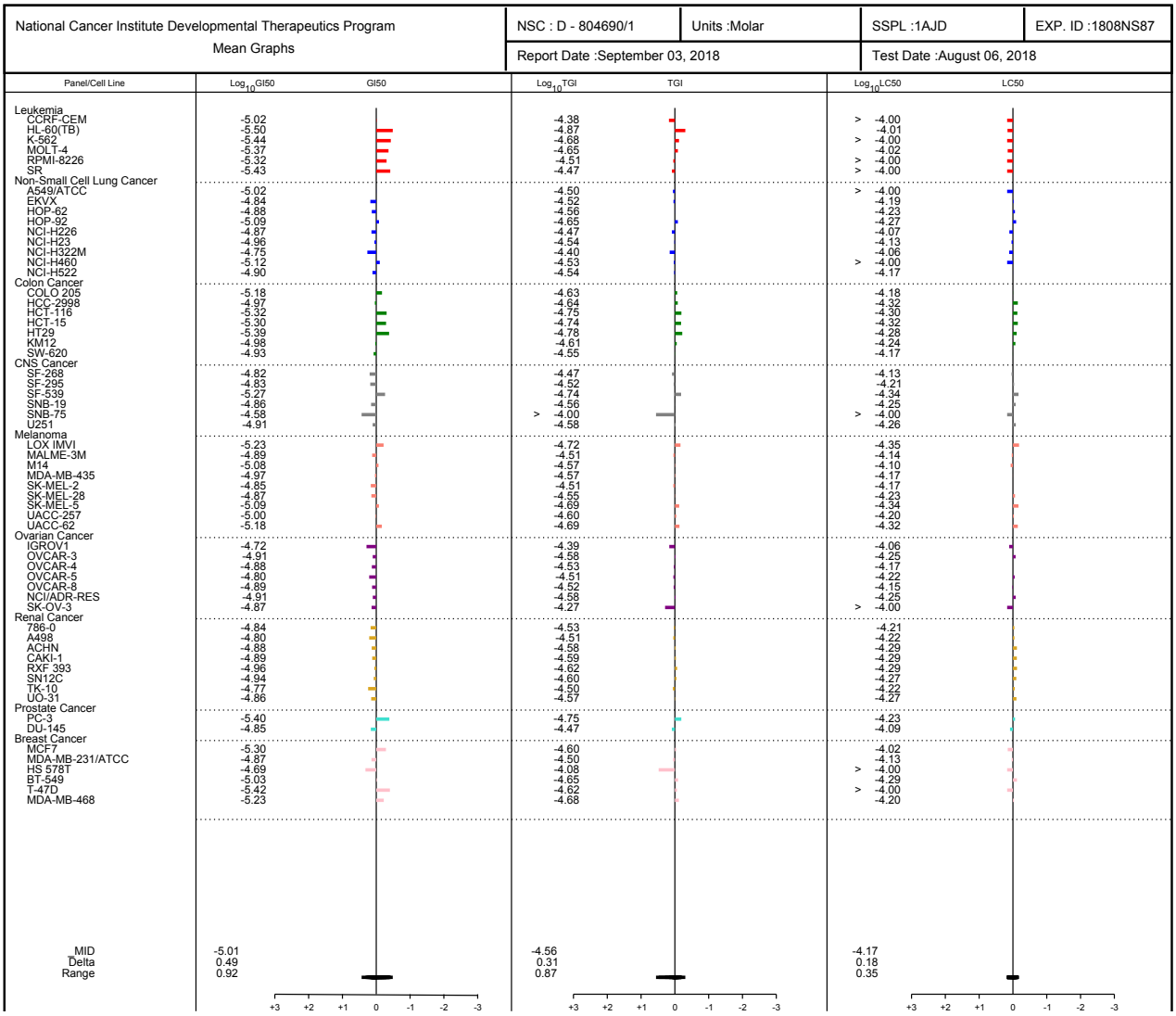


National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

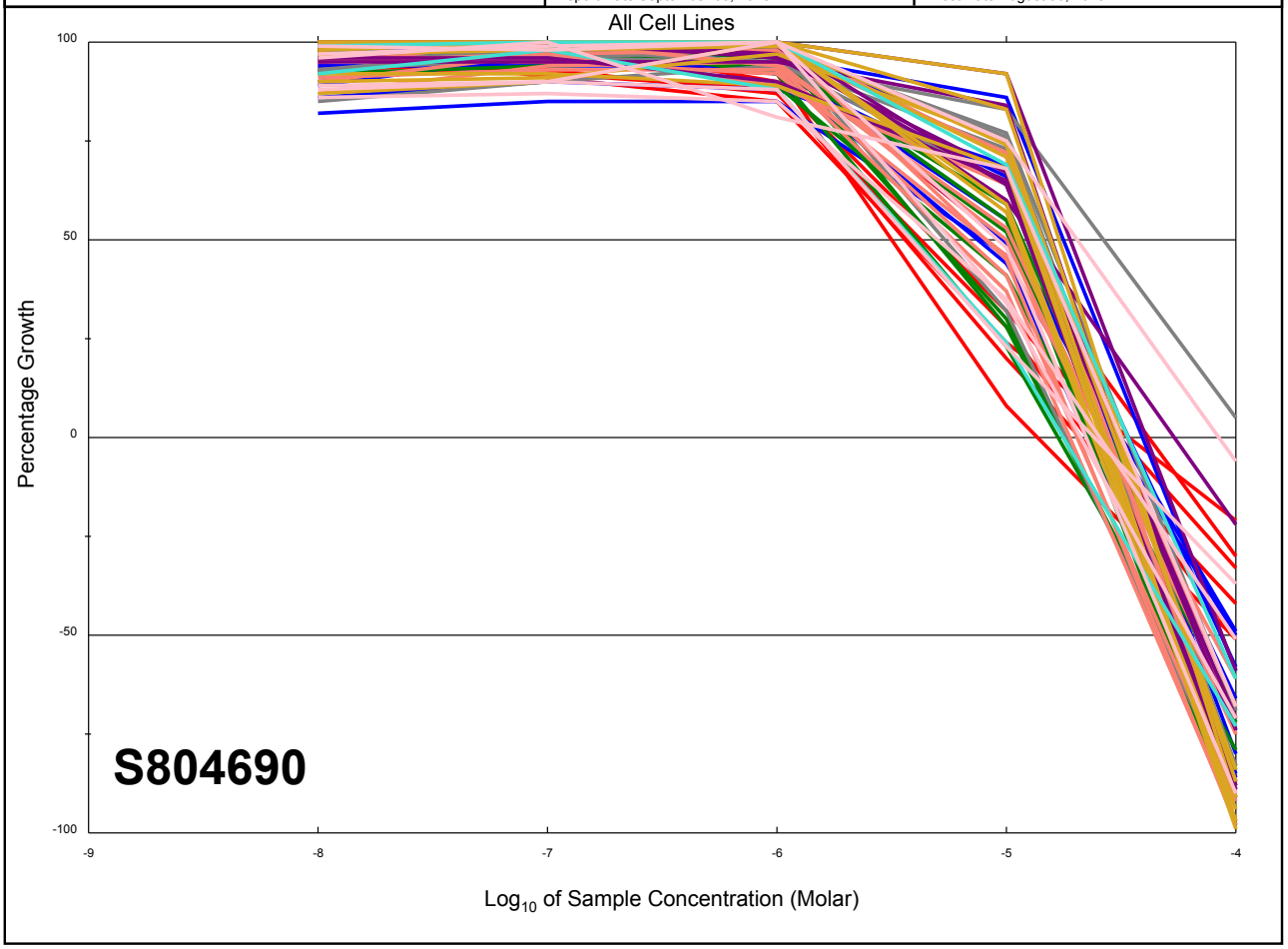
NSC : D - 804690 / 1	Experiment ID : 1808NS87	Test Type : 08	Units : Molar
Report Date : September 03, 2018	Test Date : August 06, 2018	QNS :	MC :
COMI : A-50-TFA-2	Stain Reagent : SRB Dual-Pass Related	SSPL : 1AJD	

					Log10 Concentration										
	Time				Mean Optical Densities					Percent Growth					
Panel/Cell Line	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	0.546	2.855	2.816	2.848	2.769	1.677	0.381	98	100	96	49	-30	9.51E-6	4.15E-5	> 1.00E-4
HL-60(TB)	0.909	3.212	3.114	3.105	3.032	1.088	0.449	96	95	92	8	-51	3.16E-6	1.36E-5	9.76E-5
K-562	0.184	2.112	1.979	1.949	1.882	0.566	0.107	93	92	88	20	-42	3.61E-6	2.09E-5	> 1.00E-4
MOLT-4	0.571	2.569	2.444	2.430	2.305	1.130	0.279	94	93	87	28	-51	4.22E-6	2.25E-5	9.65E-5
RPMI-8226	1.200	3.150	3.116	3.145	2.946	1.817	0.804	98	100	90	32	-33	4.82E-6	3.09E-5	> 1.00E-4
SR	0.279	1.331	1.333	1.232	1.177	0.529	0.220	100	91	85	24	-21	3.75E-6	3.38E-5	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.550	2.943	2.775	2.864	2.876	1.729	0.279	93	97	97	49	-49	9.66E-6	3.16E-5	> 1.00E-4
EKVX	0.967	2.742	2.559	2.677	2.707	2.302	0.194	90	96	98	75	-80	1.45E-5	3.05E-5	6.41E-5
HOP-62	0.589	2.080	1.876	1.927	1.901	1.611	0.085	86	90	88	69	-86	1.32E-5	2.78E-5	5.88E-5
HOP-92	1.052	1.845	1.706	1.725	1.723	1.421	0.159	82	85	85	46	-85	8.08E-6	2.26E-5	5.42E-5
NCI-H226	0.715	2.160	2.001	2.124	2.067	1.670	0.300	89	97	94	66	-58	1.35E-5	3.40E-5	8.60E-5
NCI-H23	0.567	2.100	2.013	2.006	2.014	1.412	0.195	94	94	94	55	-66	1.10E-5	2.86E-5	7.43E-5
NCI-H322M	0.770	2.306	2.233	2.291	2.245	2.099	0.318	95	99	96	86	-59	1.78E-5	3.94E-5	8.71E-5
NCI-H460	0.103	2.156	2.096	2.174	1.991	1.017	0.052	97	101	92	44	-50	7.65E-6	2.97E-5	> 1.00E-4
NCI-H522	0.984	2.944	2.862	2.918	2.789	2.234	0.258	96	99	92	64	-74	1.26E-5	2.91E-5	6.71E-5
Colon Cancer															
COLO 205	0.485	2.228	2.171	2.187	2.040	1.205	0.148	97	98	89	41	-69	6.58E-6	2.36E-5	6.67E-5
HCC-2998	0.509	1.839	1.786	1.826	1.792	1.235	0.009	96	99	96	55	-98	1.07E-5	2.28E-5	4.83E-5
HCT-116	0.172	2.055	2.060	2.102	2.008	0.696	0.028	100	103	98	28	-84	4.80E-6	1.78E-5	4.98E-5
HCT-15	0.270	2.118	1.966	2.012	2.032	0.831	0.035	92	94	95	30	-87	4.99E-6	1.81E-5	4.84E-5
HT29	0.150	1.519	1.466	1.526	1.415	0.461	0.032	96	101	92	23	-79	4.06E-6	1.67E-5	5.21E-5
KM12	0.419	2.336	2.261	2.283	2.212	1.425	0.071	96	97	94	52	-83	1.04E-5	2.44E-5	5.70E-5
SW-620	0.173	2.314	2.264	2.233	2.186	1.428	0.049	98	96	94	59	-72	1.16E-5	2.81E-5	6.79E-5
CNS Cancer															
SF-268	0.672	2.180	2.077	2.113	2.149	1.829	0.208	93	96	98	77	-69	1.53E-5	3.36E-5	7.40E-5
SF-295	0.914	3.076	2.935	3.026	3.045	2.564	0.156	93	98	99	76	-83	1.46E-5	3.01E-5	6.21E-5
SF-539	0.940	2.620	2.480	2.614	2.598	1.474	0.081	92	100	99	32	-91	5.34E-6	1.81E-5	4.61E-5
SNB-19	0.727	2.508	2.413	2.475	2.428	2.030	0.064	95	98	95	73	-91	1.38E-5	2.79E-5	5.61E-5
SNB-75	0.453	1.584	1.416	1.474	1.521	1.388	0.510	85	90	94	83	5	2.64E-5	> 1.00E-4	> 1.00E-4
U251	0.560	2.493	2.355	2.425	2.401	1.807	0.060	93	96	95	64	-89	1.24E-5	2.62E-5	5.55E-5
Melanoma															
LOX IMVI	0.303	2.347	2.161	2.208	2.213	1.062	0.009	91	93	93	37	-97	5.91E-6	1.89E-5	4.45E-5
MALME-3M	0.390	1.096	1.024	1.077	1.020	0.845	0.125	90	97	89	64	-68	1.29E-5	3.07E-5	7.31E-5
M14	0.418	2.142	2.094	2.057	1.997	1.219	0.163	97	95	92	46	-61	8.34E-6	2.70E-5	7.88E-5
MDA-MB-435	0.360	2.665	2.643	2.689	2.517	1.590	0.105	99	101	94	53	-71	1.06E-5	2.69E-5	6.78E-5
SK-MEL-2	0.861	2.316	2.240	2.274	2.286	1.910	0.214	95	97	98	72	-75	1.41E-5	3.09E-5	6.74E-5
SK-MEL-28	0.798	2.371	2.491	2.582	2.529	1.914	0.106	108	113	110	71	-87	1.36E-5	2.82E-5	5.85E-5
SK-MEL-5	1.043	3.235	3.233	3.276	3.272	2.026	0.023	100	102	102	45	-98	8.11E-6	2.06E-5	4.62E-5
UACC-257	1.108	2.673	2.463	2.585	2.590	1.897	0.275	87	94	95	50	-75	1.01E-5	2.52E-5	6.30E-5
UACC-62	0.891	2.957	2.856	2.976	2.832	1.730	0.073	95	101	94	41	-92	6.67E-6	2.03E-5	4.83E-5
Ovarian Cancer															
IGROV1	0.550	2.328	2.339	2.382	2.558	2.181	0.225	101	103	113	92	-59	1.89E-5	4.05E-5	8.69E-5
OVCAR-3	0.435	1.525	1.528	1.562	1.547	1.134	0.052	100	103	102	64	-88	1.24E-5	2.64E-5	5.61E-5
OVCAR-4	0.196	0.853	0.828	0.855	0.827	0.634	0.051	96	100	96	67	-74	1.31E-5	2.98E-5	6.75E-5
OVCAR-5	0.553	1.426	1.382	1.385	1.386	1.285	0.065	95	95	95	84	-88	1.57E-5	3.07E-5	5.99E-5
OVCAR-8	0.559	2.622	2.538	2.534	2.423	1.903	0.163	96	96	90	65	-71	1.29E-5	3.01E-5	7.03E-5
NCI/ADR-RES	0.631	2.227	2.212	2.248	2.173	1.650	0.072	99	101	97	64	-89	1.23E-5	2.62E-5	5.58E-5
SK-OV-3	0.941	2.062	2.043	2.086	2.055	1.618	0.733	98	102	99	60	-22	1.34E-5	5.40E-5	> 1.00E-4
Renal Cancer															
786-0	0.509	2.467	2.280	2.296	2.413	1.970	0.082	90	91	97	75	-84	1.43E-5	2.95E-5	6.10E-5
A498	0.817	1.854	1.867	1.907	1.875	1.681	0.108	101	105	102	83	-87	1.57E-5	3.09E-5	6.08E-5
ACHN	0.375	1.763	1.724	1.736	1.752	1.358	0.003	97	98	99	71	-99	1.33E-5	2.61E-5	5.14E-5
CAKI-1	0.173	1.569	1.458	1.453	1.411	1.121	0.005	92	92	89	68	-97	1.28E-5	2.57E-5	5.17E-5
RXF 393	1.038	2.047	2.025	2.128	2.050	1.610	0.063	98	108	100	57	-94	1.11E-5	2.38E-5	5.11E-5
SN12C	0.613	2.584	2.577	2.667	2.603	1.782	0.057	100	104	101	59	-91	1.15E-5	2.49E-5	5.35E-5
TK-10	0.579	1.717	1.571	1.598	1.750	1.622	0.054	87	90	103	92	-91	1.69E-5	3.18E-5	5.98E-5
UO-31	0.729	2.021	1.995	2.039	2.111	1.680	0.023	98	101	107	74	-97	1.37E-5	2.70E-5	5.31E-5
Prostate Cancer															
PC-3	0.514	2.173	2.044	2.135	1.980	0.920	0.141	92	98	88	24	-73	3.98E-6	1.79E-5	5.84E-5
DU-145	0.292	1.388	1.375	1.466	1.417	1.054	0.114	99	107	103	69	-61	1.41E-5	3.40E-5	8.22E-5
Breast Cancer															
MCF7	0.304	2.203	1.931	1.957	1.924	0.967	0.148	86	87	85	35	-51	5.02E-6	2.54E-5	9.61E-5
MDA-MB-231/ATCC	0.601	1.563	1.525	1.627	1.384	1.251	0.191	96	107	81	68	-68	1.35E-5	3.14E-5	7.33E-5
HS 578T	0.587	1.985	1.937	1.969	1.990	1.637	0.550	97	99	100	75	-6	2.03E-5	8.35E-5	> 1.00E-4
BT-549	0.991	2.220	2.088	2.097	2.236	1.585	0.098	89	90	101	48	-90	9.30E-6	2.23E-5	5.13E-5
T-47D	0.641	1.515	1.406	1.430	1.412	0.841	0.402	88	90	88	23	-37	3.84E-6	2.39E-5	> 1.00E-4
MDA-MB-468	0.807	2.141	2.133	2.113	2.167	1.262	0.233	99	98	102	34	-71	5.83E-6	2.11E-5	6.30E-5

Figure 13. GI_{50} (50% Growth Inhibition), TGI (Total Growth Inhibition) and LC_{50} (50% Lethal Concentration) mean graphs obtained for compound **3** (NSC 804690) tested at five concentrations (0.01, 0.1, 1, 10, 100 μM) against the NCI-60 human cancer cell lines.



National Cancer Institute Developmental Therapeutics Program Dose Response Curves	NSC : D - 804690/1	SSPL:1AJD	EXP. ID:1808NS87
	Report Date:September 03, 2018	Test Date:August 06, 2018	



Anticancer activity assays

In vitro cytotoxic activity (NCI, USA)

Evaluation of compounds against the 60 cell lines started at a single dose of10^{-5}M. The human tumor cell lines of the cancer-screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM *L*-glutamine. For a typical screening experiment, cells were inoculated into 96 well microtiter plates in 100 mL at plating densities ranging from 5000 to 40.000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the micro titer plates were incubated at 37 ºC, 5% CO, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After24 h, two plates of each cell line were fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs were solubilized in dimethylsulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was dissolved and diluted totwice the desired final maximum test concentration with complete medium containing 50 mg/mL gentamicin. Additional four, 10-foldor ½ log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 mL of these different drug dilutions were added to the appropriate micro titer wells already containing 100 mL of medium, resulting in the required final drug concentrations. Following drug addition, the plates were incubated for an additional 48 h at 37 ºC, 5% CO, 95% air, and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 mL of cold 50% TCA (final concentration, 10% TCA) and incubated for 60 min at 4 ºC. The supernatant is discarded, and the plates werewashed five times with tap water and air dried.Sulforhodamine B (SRB) solution (100 mL) at 0.4% in 1% acetic acidis added to each well, and plates wereincubated for 10 min at roomtemperature. After staining, the unbound dye wasremoved by washingfive times with 1% acetic acid and the plates wereair dried. The boundstain wassubsequently solubilized with 10 N Mtrizma base, and theabsorbance wasread on an automated plate reader at a wavelengthof 515 nM. For suspension cells, the methodology wasthe sameexcept that the assay wasterminated by fixing settled cells at thebottom of the wells by gently adding 50 mLof 80% TCA (final concentration,16% TCA). Using the seven absorbance measurements[time zero (Tz), control growth (C), and test growth in the presenceof drug at the five concentration levels (Ti)], the percentage growthwascalculated at each of the drug concentrations levels. Percentagegrowth inhibition wascalculated as:

[(Ti_Tz)/(C_Tz)]_100 for concentrations for which Ti_Tz

[(Ti_Tz)/Tz]_100 for concentrations for which Ti < Tz

Antimicrobial Activity assays

Procedure

All bacteria were cultured in Cation-adjusted Mueller Hinton broth (CAMHB) at 37ºC overnight. A sample of each culture was then diluted 40-fold in fresh broth and incubated at 37ºC for 1.5-3 h. The resultant mid-log phase cultures were diluted (CFU/ ml measured by OD_{600}), then added to each well of the compound containing plates, giving a cell density of 5×10^5 CFU/ml and a total volume of 50 µl. All the plates were covered and incubated at 37ºC for 18 h without shaking.

Analysis

Inhibition of bacterial growth was determined measuring absorbance at 600 nm (OD_{600}), using a Tecan M1000 Pro monochromator plate reader. The percentage of growth inhibition was calculated

for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate as references. The significance of the inhibition values was determined by modified Z-scores, calculated using the median and MAD of the samples (no controls) on the same plate. Samples with inhibition value above 80% and Z-Score above 2.5 for either replicate were classed as actives. Samples with inhibition values between 50 and 80% and Z-Score above 2.5 for either replicate were classed as partial actives.

Hit-Confirmation.

The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate. The MIC was determined as the lowest concentration at which the growth was fully inhibited, defined by an inhibition ≥ 80%. In addition, the maximal percentage of growth inhibition is reported as DMax , indicating any compounds with partial activity. Hits were classified by MIC ≤ 16 μg/ml in either replicate.