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Research Article

In-vitro Cytotoxicity Assay of Quinoxalines

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ABSTRACT
Major objective of work is in-vitro cytotoxicity assay of newly synthesized compounds and estimated deaths due to the cancer in human beings in US. Current manuscript also provides the chemotherapy of cancer with highly active and safe anti-cancer synthesized quinoxaline compounds and their *in-vitro* assay at National Cancer Institute (NCI). A series of new quinoxaline derivatives 3 (a-h) has been prepared. The newly synthesized compounds were further evaluated in the National Cancer Institute for their *in-vitro* cytotoxicity assay. Among them compound 3h has been show highest activity against Leukemia RPMI-8226 cell lines (GI_{50}: 1.11 μM) as compared to other tested compounds. It is to be noted that compound 3e has been show significant activity against cancer cell lines. (GI_{50}: 1.11 μM). We conclude that the ongoing studies of targeted agents in conjunction with chemotherapy will show whether there are alternative option for new and safer medicine for cancer in future as well as opens the new doors in era of cancer research.
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KEYWORDS
Quinoxaline, Cytotoxicity, NCI, *In-vitro* assay, DTP.

1. INTRODUCTION

Cancer is a major public health problem in the United States and many other parts of the world. Currently, one in four deaths in the United States is due to the cancer. In given manuscript, we provides the expected numbers of new cancer cases and deaths in 2011, as well as an overview of some new synthetic quinoxaline compounds and its anticancer activity. Table 1 has been show the expected number of deaths from cancer projected for 2011 for men, women, and both sexes combined. It is estimated that about 571,950 Americans will die from cancer, corresponding to more than 1500 deaths per day. Cancers of the lung, bronchus, prostate, colorectum in men and cancers of the lung, bronchus, breast and colorectum in womens continue to be the most common causes of death (Siegel, Ward, Brawley, Jemal, 2006). So it's a moral responsibility of every budding researcher's to go for a development of a new and safer anticancer drugs which can be save the life of maximum in future. On the behalf of a social benefit we provides the expected numbers of new synthesized quinoxaline compounds as well as their glamorous anticancer activity against 60 cell line panel under the Developmental Therapeutic Programme (DTP) at National Cancer Institute (NCI, USA), by keeping in mind that the medicinal importance of quinoxaline moiety and its contribution to this era of research give a ray of hope to the patients suffering from cancer worldwide.

Table1. Estimated New Cancer Cases and Deaths by Sex, United States, 2011

	ESTIMATED NEW CASES			**ESTIMATED DEATHS**		
Sites	BOTH SEXES	MALE	FEMALE	BOTH SEXES	MALE	FEMALE
Leukemia	44600	25320	19280	21780	12740	9040
Acute lymphocytic leukemia	5730	3320	2410	1420	780	640
Chronic lymphocytic leukemia	14570	8520	6050	4380	2660	1720
Acute myeloid leukemia	12950	6830	6120	9050	5440	3610
Chronic myeloid leukemia	5150	3000	2150	270	100	170
Lung & bronchus Cancer	221130	115060	106070	156940	85600	71340
Colon Cancer	101340	48940	52400	49380	25250	24130

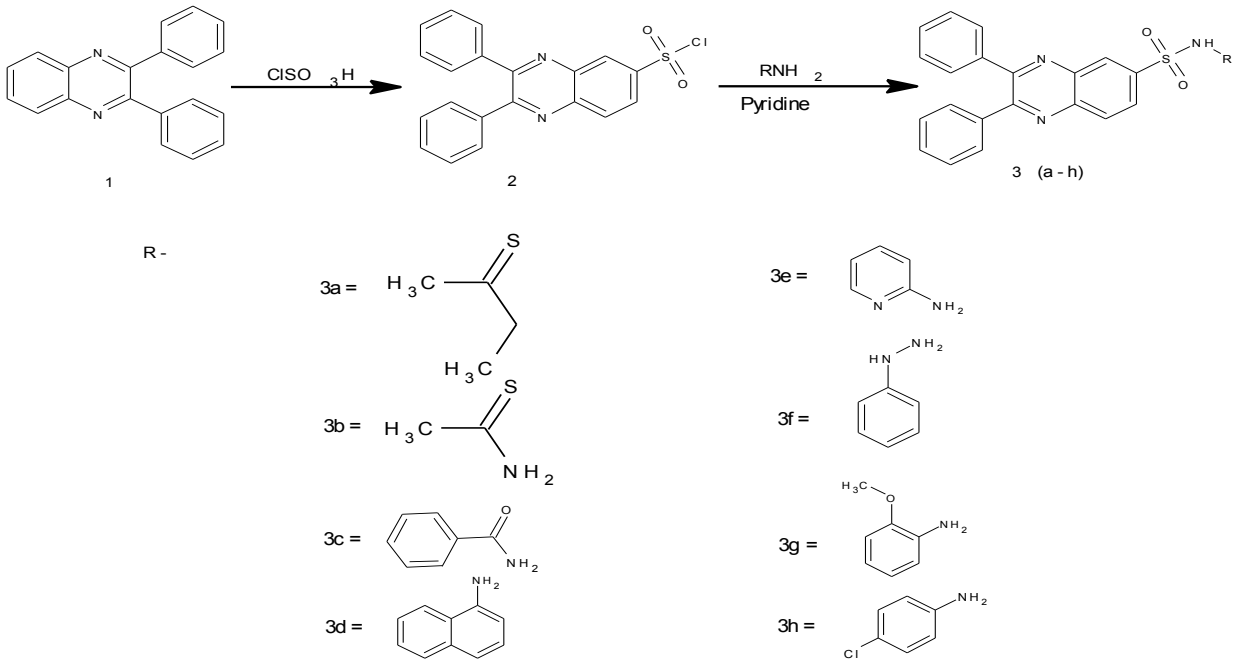
CNS Cancer	22340	12260	10080	13110	7440	5670
Melanoma-skin	70230	40010	30220	8,790	5,750	3,040
Ovarian Cancer	21990	-	21990	15460	-	15460
Kidney & renal pelvis Cancer	60,920	37,120	23,800	13,120	8,270	4,850
Prostate Cancer	240,890	240,890	-	33,720	33,720	-
Breast Cancer	232620	2,140	230,480	39,970	450	39,520

Quinoxalines are attractive chemical candidates in medicinal chemistry due to the their ability to generate biological responses in their interaction with several biological targets. They have been show antiviral (Rong, Chow, Yan, Larson, Hong, Wu, 2007), herbicidal (Li, Wu, Cui, Xiang, Bai, Yang, 2006) and anti-inflammatory action (Burguete, Pontiki, Hadjipavlou-Litina, 2007). Recent investigations reveals the pharmacological potential of quinoxalines as an anticancer agents (Levitzki, 2003).

2. RESULTS AND DISCUSSION

A series of new quinoxaline derivatives 3 (a-h) has been prepared. The newly synthesized compounds 3a, 3b, 3c, 3d, 3e, 3f, 3g and 3h were further evaluated in the National Cancer Institute for *in-vitro* cytotoxicity assay. Among them compound 3e has been show highest activity against Leukemia RPMI-8226 cell lines (GI_{50}: 1.11 μM) as compared to other tested compounds. It is to be noted that compound 3h has been show significant activity against cancer cell lines. (GI_{50}: 1.11 μM)

The synthesis of compounds 3 (a-h) is given in Scheme-1. The derivatives were characterized by spectral studies and confirmed to the structures.



Scheme1. Reaction scheme for the synthesis of target compounds 3(a-h).

The *in vitro* anticancer screening at NCI is a two-stage process, beginning with the evaluation of all compounds against the 60 cell lines at a single dose of 10 µM. The output from the single dose screen is reported as a mean graph and is available for analysis by the COMPARE programme. Compounds which exhibit significant growth inhibition are evaluated against the 60 cell panel at five concentration levels as shown in table 2. The human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5 % fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well microtiter plates in 100 µL at plating densities ranging from 5,000 to 40,000 cells/well depending on

the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37 °C, 5% CO_2, 95 % air and 100% relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, two plates of each cell line are 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 are solubilized in dimethyl sulfoxide 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 is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 µg/ml gentamycin. Additional four, 10-fold or ½ log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 µl of these different drug dilutions are added to the appropriate microtiter wells already containing 100 µl of medium, resulting in the required final drug concentrations.

Following the drug addition, the plates are incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed *in situ* by the gentle addition of 50 µl of cold 50% (w/v) TCA (final

concentration, 10% TCA) and incubated for 60 minutes at 4 °C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µl) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 minutes at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 µl of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

[(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz

[(Ti-Tz)/Tz] x 100 for concentrations for which Ti<Tz.

Three dose response parameters are calculated for each experimental agent. Growth inhibition of 50% (GI_{50}) is calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) is calculated from Ti = Tz. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50. Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested. (Alley, Scudierom, Monks, Hursey, Czerwinski, Fine, Abbott, Mayo, Shoemaker, Boyd, 1998), (Grever, Schepartz, Chabner, 1992), (Boyd and Paull, 1995)

Table 2: Percentage growth inhibition (GI %) of in vitro subpanel tumor cell lines at 10_5 mM (Single Dose Assay).

Compound Code → / **Cancer Cell Line ↓**	**NSC:763437**	**NSC:763438**	**NSC:763442**	**NSC:763441**	**NSC:763440**	**NSC:763439**	**NSC:763435**	**NSC :763 436**
Leukemia								
CCRF-CEM	41.34	58.32	**61.94**	49.84	32.64	**70.73**	35.83	24.82
HL-60(TB)	49.43	70.98	**66.73**	60.73	39.92	**80.72**	40.63	35.26
K-562	45.75	49.56	**55.63**	45.52	34.92	**64.42**	34.82	26.83
MOLT-4	44.86	47.78	**60.82**	56.93	35.52	**86.83**	28.12	20.54
RPMI-8226	58.54	44.34	**86.04**	65.72	47.78	**89.93**	46.37	39.62
SR	71.12	81.12	**88.12**	81.92	53.78	**-3.25**	49.98	40.95
Non-Small Cell Lung								

Cancer								
A549/ATCC	21.12	15.34	**41.93**	36.45	17.57	**54.67**	34.72	7.74
EKVX	26.54	71.23	**57.83**	48.82	30.57	**80.82**	23.72	8.53
HOP-62	2.87	2.24	**12.83**	2.12	2.52	**22.76**	38.73	2.84
NCI-H226	61.97	43.97	**-2.45**	69.93	59.84	**-1.38**	52.93	53.64
NCI-H23	19.56	21.34	**26.64**	23.03	16.56	**46.52**	11.62	11.67
NCI-H322M	20.87	22.23	**32.82**	23.23	10.77	**59.42**	2.92	17.83
NCI-H460	31.97	23.56	**47.12**	45.54	26.63	**63.42**	15.63	8.63
NCI-H522	23.54	47.82	**50.83**	28.82	18.67	**65.63**	13.74	3.73
Colon Cancer								
COLO 205	19.54	27.87	**26.64**	28.94	12.67	**54.23**	4.83	4.64
HCC-2998	15.32	4.56	**37.92**	19.92	1.83	**60.63**	8.92	6.23
HCT-116	36.67	49.87	**59.62**	55.72	36.56	**72.12**	17.83	14.53
HCT-15	44.94	34.34	**44.72**	44.72	32.53	**62.43**	29.92	26.21
HT 29	48.43	82.45	**70.43**	63.92	39.61	**81.72**	30.82	21.46
KM 12	20.98	29.92	**38.74**	8.62	6.93	**61.52**	15.92	16.75
SW-620	17.43	26.78	**30.32**	21.54	13.56	**47.92**	3.72	3.84
CNS Cancer								
SF-268	6.89	2.78	**14.12**	23.82	1.63	**27.63**	2.92	1.86
SF-295	20.98	3.88	**40.73**	15.92	14.52	**76.83**	8.92	5.83
SF-539	3.89	5.57	**25.93**	24.72	7.76	**61.92**	21.72	3.29
SNB-19	21.98	9.78	**31.93**	41.92	16.52	**43.63**	14.82	2.12
SNB-75	34.67	39.88	**45.12**	31.93	29.64	**61.82**	25.63	26.85
U251	21.56	7.23	**39.45**	ND	15.62	**56.53**	26.82	10.32
Melanoma								
LOX IMVI	20.54	32.87	**38.42**	23.34	15.34	**63.32**	11.23	12.23
MALME-3M	7.67	9.98	**6.64**	1.87	2.94	**32.85**	2.94	2.85
M14	2.45	2.23	**30.96**	25.98	4.84	**62.84**	4.23	2.85
MDA-MB-435	12.89	20.45	**35.63**	22.53	7.72	**54.24**	11.13	4.73
SK-MEL-2	15.84	14.87	**23.93**	24.97	2.94	**39.29**	7.94	8.53
SK-MEL-28	7.63	5.98	**14.64**	12.67	4.26	**30.29**	6.93	1.73
SK-MEL-5	48.93	48.56	**73.95**	61.42	42.28	**-6.25**	39.13	28.93
UACC-257	15.24	21.66	**17.85**	18.87	1.94	**42.45**	8.83	2.23
UACC-62	19.65	28.86	**30.56**	23.42	30.23	**41.13**	24.34	20.72
Ovarian Cancer								
IGROV1	16.54	1.78	**20.83**	11.78	1.84	**31.82**	8.83	14.84
OVCAR-3	19.25	30.88	**28.84**	13.46	2.86	**48.92**	1.84	1.94
OVCAR-4	16.53	31.23	**30.28**	29.98	17.25	**40.12**	16.54	13.43
OVCAR-5	8.43	2.98	**18.28**	12.73	5.94	**24.34**	4.85	2.84
OVCAR-8	8.02	20.56	**34.84**	24.12	4.27	**52.75**	5.83	3.93
NCI/ADR-RES	27.53	22.62	**55.21**	40.98	13.26	**73.20**	11.88	8.63
SK-OV-3	12.83	5.86	**18.48**	18.63	2.37	**44.93**	5.98	4.85
Renal Cancer								
786-0	16.54	4.34	**66.93**	41.54	25.74	**99.23**	2.34	2.83
A-498	42.22	31.85	**44.29**	42.83	33.38	**70.23**	26.75	22.73
ACHN	11.84	21.89	**23.93**	13.12	7.63	**34.34**	01.83	16.62
CAKI-1	7.22	3.34	**33.93**	23.87	3.23	**54.12**	2.94	5.83
RXF-393	39.12	48.64	**98.83**	70.66	30.27	**-40.38**	29.24	14.53
SN 12C	17.43	8.87	**26.93**	32.65	10.45	**38.49**	6.98	2.83
TK-10	2.54	6.34	**2.84**	1.26	1.25	**30.23**	2.74	6.93
UO-31	15.23	17.86	**29.25**	22.84	2.23	**45.23**	13.73	10.63
Prostate Cancer								
PC-3	41.85	41.76	**60.03**	52.82	34.43	**72.19**	34.64	33.93
DU-145	6.85	10.87	**24.38**	18.85	3.74	**37.43**	2.73	2.63

Breast Cancer								
MCF7	22.94	22.97	**47.74**	31.84	18.75	**65.94**	18.93	11.84
MDA-MB-231/ATCC	28.54	38.66	**54.28**	48.28	30.54	**80.12**	17.63	14.78
HS 578T	16.97	23.97	**37.54**	33.23	27.23	**76.34**	6.63	20.84
BT -549	7.67	32.56	**77.75**	55.13	35.65	**80.25**	1.83	2.74
T-47D	57.45	49.43	**72.13**	63.94	50.93	**81.12**	42.62	40.64
MDA-MB-468	40.23	54.87	**76.75**	56.83	35.13	**-4.67**	30.83	23.66

2.1. In-vitro 5 dose full NCI 60 cell panel assay

All the cell lines (about 60), representing nine tumor subpanels, were incubated at five different concentrations (0.01, 0.1, 1, 10 & 100 μM). The outcomes were used to create log concentration Vs % growth inhibition curves and three response parameters (GI_{50}, TGI and LC_{50}) were calculated for each cell line. The GI_{50} value (growth inhibitory activity) corresponds to the concentration of the compound causing 50% decrease in net cell growth, the TGI value (cytostatic activity) is the concentration of the compound resulting in total growth inhibition and LC_{50} value (cytotoxic activity) is the concentration of the compound causing net 50% loss of initial cells at the end of the incubation period of 48 h.

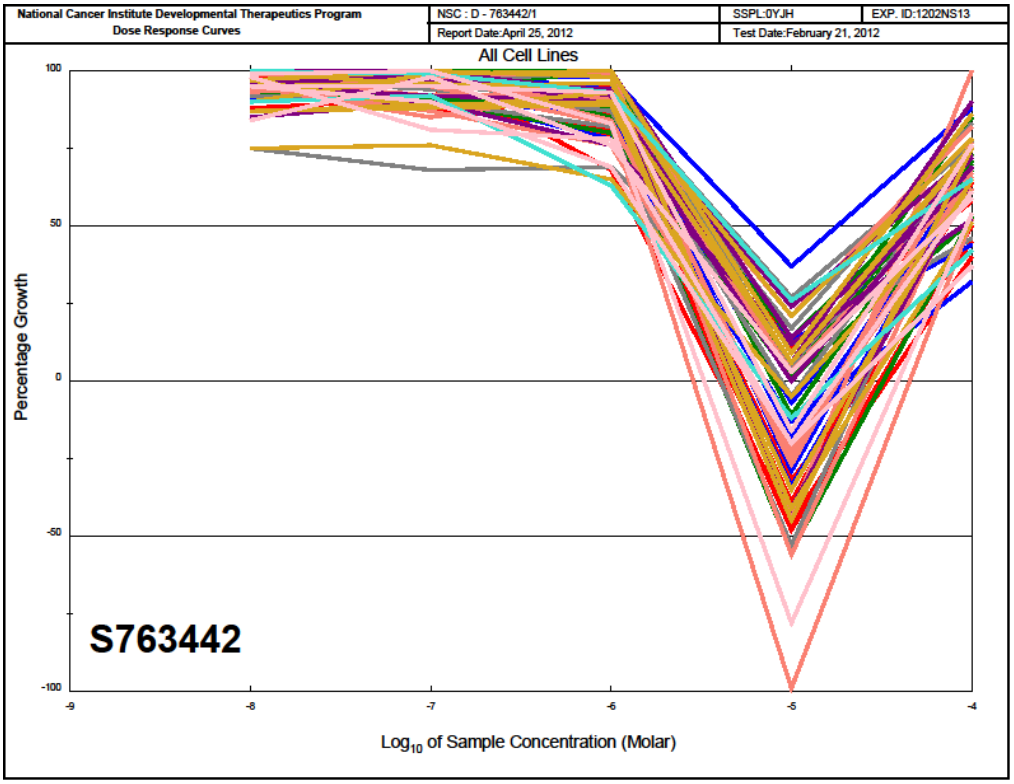
Compound **3h** (NSC: 763442**)** exhibited high activity against Leukemia HL-60 (GI_{50}: 2.09 μM) and RPMI-8226 cell lines (GI_{50}: 1.43 μM); Non Small Cell Lung Cancer HOP-62 (GI_{50}: 3.95 μM) and HOP-92 cell line (GI_{50}: 2.03 μM); CNS Cancer SNB-75 cell line (GI_{50}: 2.12 μM); Prostate Cancer PC-3 cell line (GI_{50}: 1.47 μM) and Breast T-47D Cancer cell line (GI_{50}: 1.62 μM) as shown in Fig. 1, 2 and 3. Similarly compound under investigation **3e** (NSC: 763439**)** exhibited significant anticancer activity against most of the tested cell lines representing nine different subpanels with GI50 values between 1.11 – 4.54 μM and found to be potential candidate of the series as shown in Fig. 4, 5 and 6. With regards to the sensitivity against some individual cell lines the compound **3h** shown highest activity against Leukemia RPMI-8226 cell lines (GI_{50}: 1.11 μM) and least against Non Small Cell Lung Cancer HOP-62 cell line (GI_{50}: 4.54 μM). It is to be noted that compound **3h** shows significant activity (GI_{50}: 1.11 μM) as compared to the High Throughput Screening (HTS) hit identified by Porter and Collaborator with IC_{50} = 1.3 *M*m [23]. Toxicity is measured in terms of lethality; both compounds are not lethal and safe in nature as it is obvious by examining the LC_{50} value as shown in Fig. 1 and 4.

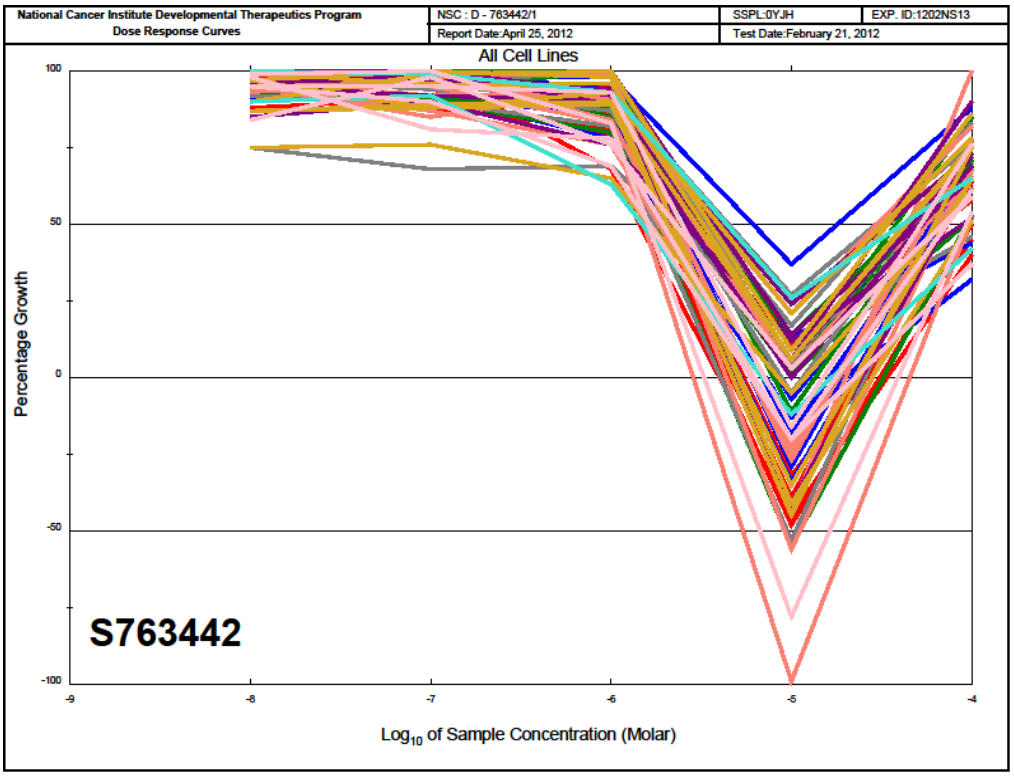
National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 763442 / 1	Experiment ID : 1202NS13	Test Type : 08	Units : Molar
Report Date : August 08, 2012	Test Date : February 21, 2012	QNS :	MC :
COMI : 114502	Stain Reagent : SRB Dual-Pass Related	SSPL : 0YJH	

		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	0.592	2.069	1.887	1.940	1.766	0.354	1.539	88	91	79	-40	64	.	.	> 1.00E-4
HL-60(TB)	1.003	2.945	2.877	2.918	2.723	0.681	1.869	97	99	89	-32	45	2.09E-6	.	> 1.00E-4
K-562	0.410	2.217	2.188	2.185	2.125	0.603	1.453	98	98	95	11	58	.	> 1.00E-4	> 1.00E-4
MOLT-4	0.649	2.268	2.217	2.177	2.046	0.399	1.748	97	94	86	-39	68	.	.	> 1.00E-4
RPMI-8226	0.985	2.313	2.356	2.331	1.882	0.541	1.512	103	101	68	-45	40	1.43E-6	.	> 1.00E-4
SR	0.663	1.796	1.711	1.656	1.580	0.347	1.232	92	88	81	-48	50	.	.	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.366	1.782	1.698	1.746	1.600	0.434	1.403	94	97	87	5	73	.	> 1.00E-4	> 1.00E-4
EKVX	0.800	1.826	1.797	1.825	1.738	0.745	1.467	97	100	91	-7	65	.	.	> 1.00E-4
HOP-62	0.286	0.847	0.825	0.845	0.873	0.360	0.532	96	100	105	13	44	3.95E-6	> 1.00E-4	> 1.00E-4
HOP-92	0.534	0.945	0.930	0.913	0.855	0.462	0.665	96	92	78	-13	32	2.03E-6	.	> 1.00E-4
NCI-H226	0.736	1.516	1.445	1.509	1.527	0.524	1.302	91	99	101	-29	73	.	.	> 1.00E-4
NCI-H23	0.533	1.641	1.605	1.553	1.472	0.576	1.333	97	92	85	4	72	.	> 1.00E-4	> 1.00E-4
NCI-H322M	0.775	1.490	1.438	1.475	1.473	1.043	1.408	93	98	98	37	88	.	> 1.00E-4	> 1.00E-4
NCI-H460	0.274	2.358	2.374	2.452	2.239	0.225	1.695	101	105	94	-18	68	.	.	> 1.00E-4
NCI-H522	0.702	1.888	1.804	1.767	1.744	0.465	1.501	93	90	88	-34	67	.	.	> 1.00E-4
Colon Cancer															
COLO 205	0.502	2.054	2.062	2.073	2.099	0.446	1.631	101	101	103	-11	73	.	.	> 1.00E-4
HCC-2998	0.739	2.616	2.605	2.590	2.658	0.778	2.337	99	99	102	2	85	.	> 1.00E-4	> 1.00E-4
HCT-116	0.221	1.602	1.550	1.459	1.324	0.236	0.945	96	90	80	1	52	.	> 1.00E-4	> 1.00E-4
HCT-15	0.468	2.268	2.274	2.241	2.032	0.513	1.695	100	98	87	3	68	.	> 1.00E-4	> 1.00E-4
HT29	0.187	0.978	0.981	0.975	0.862	0.084	0.610	100	100	85	-55	53	.	.	.
KM12	0.455	2.036	2.085	1.944	2.068	0.529	1.572	103	94	102	5	71	.	> 1.00E-4	> 1.00E-4
SW-620	0.241	1.488	1.405	1.381	1.337	0.412	1.113	93	91	88	14	70	.	> 1.00E-4	> 1.00E-4
CNS Cancer															
SF-268	0.565	1.759	1.688	1.734	1.676	0.884	1.468	94	98	93	27	76	.	> 1.00E-4	> 1.00E-4
SF-295	0.996	2.111	2.017	2.004	1.905	0.472	1.857	92	90	82	-53	77	.	.	.
SF-539	0.661	1.927	1.918	1.878	1.940	0.721	1.590	99	96	101	5	73	.	> 1.00E-4	> 1.00E-4
SNB-19	0.569	1.747	1.749	1.698	1.616	0.765	1.549	100	96	89	17	83	.	> 1.00E-4	> 1.00E-4
SNB-75	0.784	1.408	1.255	1.206	1.213	0.856	1.069	75	68	69	12	46	2.12E-6	> 1.00E-4	> 1.00E-4
U251	0.360	1.566	1.525	1.500	1.415	0.344	1.271	97	94	87	-5	75	.	.	> 1.00E-4
Melanoma															
LOX IMVI	0.195	1.784	1.764	1.761	1.602	0.235	1.252	99	99	89	3	66	.	> 1.00E-4	> 1.00E-4
MALME-3M	0.531	0.927	0.950	0.924	0.938	0.304	0.958	106	99	103	-43	108	.	.	> 1.00E-4
M14	0.367	1.309	1.263	1.168	1.253	0.267	1.092	95	85	94	-27	77	.	.	> 1.00E-4
MDA-MB-435	0.382	1.641	1.599	1.544	1.543	0.288	1.336	97	92	92	-25	76	.	.	> 1.00E-4
SK-MEL-2	0.548	0.942	0.914	0.939	0.936	0.241	0.822	93	99	99	-56	69	.	.	.
SK-MEL-28	0.592	1.647	1.617	1.610	1.555	0.811	1.456	97	96	91	21	82	.	> 1.00E-4	> 1.00E-4
SK-MEL-5	0.657	2.027	1.963	1.961	1.790	0.009	1.371	95	95	83	-99	52	.	.	.
UACC-257	0.603	1.340	1.295	1.334	1.221	0.461	1.096	94	99	84	-24	67	.	.	> 1.00E-4
UACC-62	0.606	2.289	2.204	2.073	1.883	0.473	1.657	95	87	76	-22	62	.	.	> 1.00E-4
Ovarian Cancer															
IGROV1	0.653	1.952	1.972	1.959	1.861	0.968	1.597	102	101	93	24	73	.	> 1.00E-4	> 1.00E-4
OVCAR-3	0.508	1.575	1.579	1.545	1.515	0.302	1.207	100	97	94	-41	65	.	.	> 1.00E-4
OVCAR-4	0.434	0.837	0.775	0.795	0.739	0.481	0.642	85	90	76	12	52	.	> 1.00E-4	> 1.00E-4
OVCAR-5	0.594	1.357	1.321	1.299	1.277	0.636	1.285	95	92	90	6	90	.	> 1.00E-4	> 1.00E-4
OVCAR-8	0.356	1.449	1.405	1.457	1.360	0.419	1.142	96	101	92	6	72	.	> 1.00E-4	> 1.00E-4
NCI/ADR-RES	0.408	1.537	1.530	1.560	1.409	0.412	1.185	99	102	89	.	69	.	> 1.00E-4	> 1.00E-4
SK-OV-3	0.497	1.153	1.121	1.148	1.184	0.586	0.925	95	99	105	14	65	.	> 1.00E-4	> 1.00E-4
Renal Cancer															
786-0	0.576	2.028	1.980	1.851	1.920	0.315	1.669	97	88	93	-45	75	.	.	> 1.00E-4
A498	1.116	1.746	1.590	1.596	1.527	1.058	1.497	75	76	65	-5	61	.	.	> 1.00E-4
ACHN	0.541	2.018	2.042	1.942	1.954	0.626	1.658	102	95	96	6	76	.	> 1.00E-4	> 1.00E-4
CAKI-1	0.748	1.816	1.679	1.689	1.700	0.486	1.439	87	88	89	-35	65	.	.	> 1.00E-4
RXF 393	0.646	1.087	1.072	1.101	1.077	0.383	0.871	97	103	98	-41	51	.	.	> 1.00E-4
SN12C	0.508	2.155	2.066	2.095	2.046	0.861	1.796	95	96	93	21	78	.	> 1.00E-4	> 1.00E-4
TK-10	0.594	1.183	1.126	1.177	1.232	0.649	1.100	90	99	108	9	86	.	> 1.00E-4	> 1.00E-4
UO-31	0.621	1.610	1.485	1.501	1.508	0.679	1.362	87	89	90	6	75	.	> 1.00E-4	> 1.00E-4
Prostate Cancer															
PC-3	0.434	1.492	1.389	1.408	1.096	0.380	0.874	90	92	63	-12	42	1.47E-6	.	> 1.00E-4
DU-145	0.357	1.413	1.460	1.397	1.340	0.634	1.046	105	99	93	26	65	.	> 1.00E-4	> 1.00E-4
Breast Cancer															
MCF7	0.448	2.179	2.098	2.101	2.066	0.376	1.757	95	95	93	-16	76	.	.	> 1.00E-4
MDA-MB-231/ATCC	0.411	1.114	1.107	1.124	0.993	0.090	0.789	99	101	83	-78	54	.	.	.
HS 578T	0.926	1.701	1.580	1.685	1.512	0.953	1.385	84	98	76	3	59	.	> 1.00E-4	> 1.00E-4
BT-549	0.785	1.681	1.659	1.513	1.486	0.627	1.331	98	81	78	-20	61	.	.	> 1.00E-4
T-47D	0.766	1.450	1.413	1.384	1.235	0.612	1.021	95	90	69	-20	37	1.62E-6	.	> 1.00E-4

Figure 1. Five dose assay of compound **3f** (NSC: 763442).


National Cancer Institute Developmental Therapeutics Program
Dose Response Curves
NSC : D - 763442/1
Report Date:April 25, 2012
SSPL:0YJH
Test Date:February 21, 2012
EXP. ID:1202NS13
All Cell Lines
Percentage Growth
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
S763442
Log10 of Sample Concentration (Molar)


National Cancer Institute Developmental Therapeutics Program
Dose Response Curves
NSC : D - 763442/1
Report Date:April 25, 2012
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EXP. ID:1202NS13
All Cell Lines
Percentage Growth
100
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0
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-7
-6
-5
-4
S763442
Log10 of Sample Concentration (Molar)

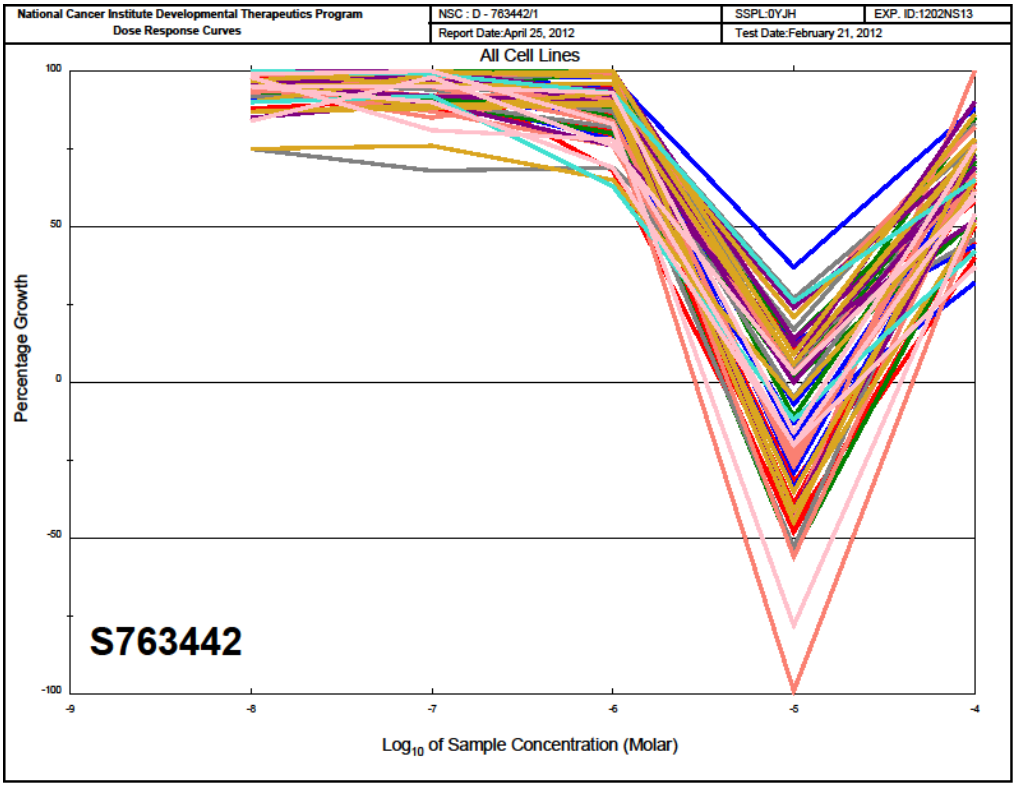

Figure 2. Dose response curves of compound **3f** (NSC: 763442) against all cancer cell lines at five dose assay level.

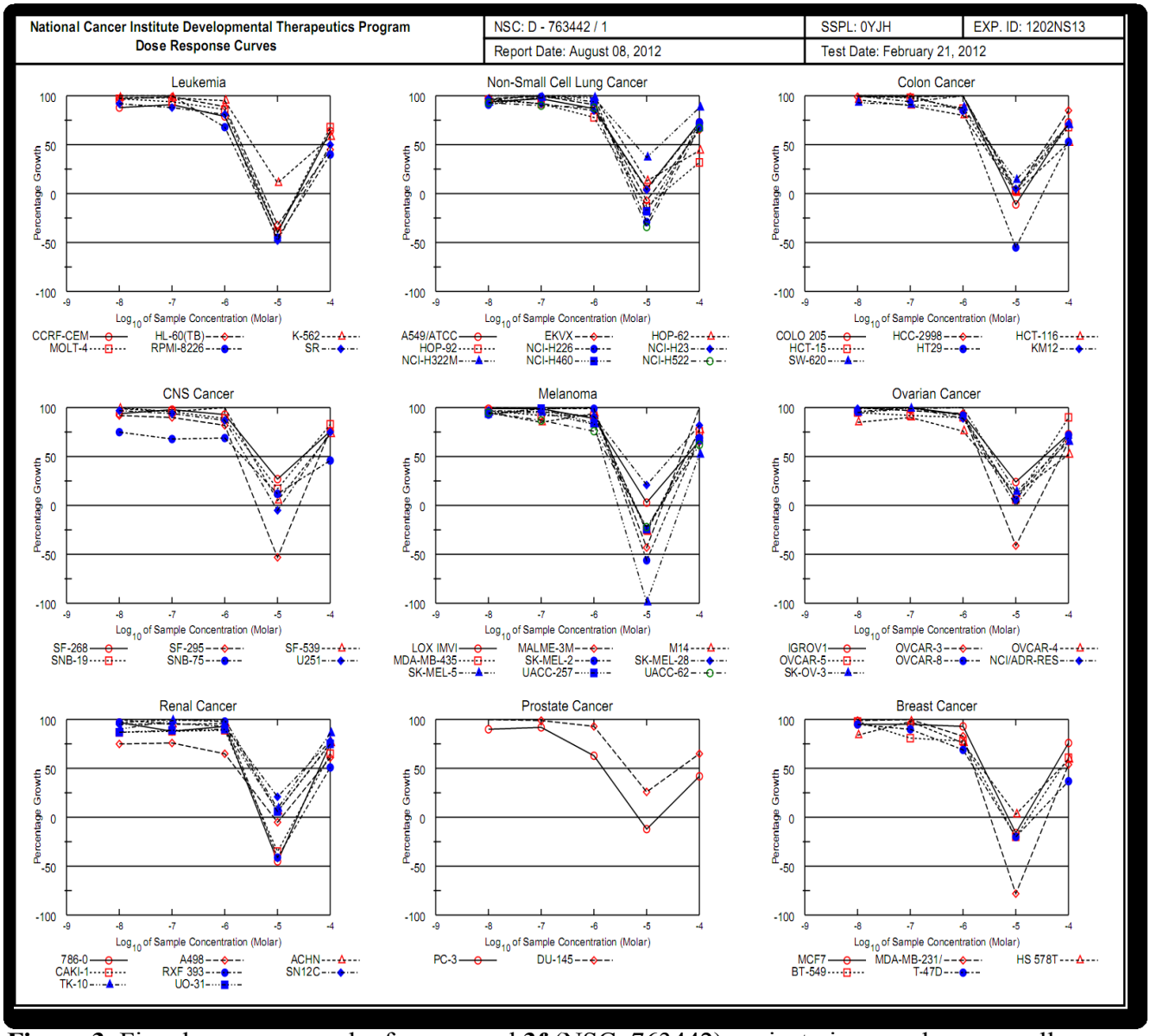

Figure 3. Five dose assay graph of compound **3f** (NSC: 763442) against nine panel cancer cell

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 763439 / 1	Experiment ID : 1202NS13	Test Type : 08	Units : Molar
Report Date : August 08, 2012	Test Date : February 21, 2012	QNS :	MC :
COMI : 114496	Stain Reagent : SRB Dual-Pass Related	SSPL : 0YJH	

		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															
HL-60(TB)	1.003	2.880	2.841	2.819	2.398	0.705	1.539	98	97	74	-30	29	1.71E-6	.	> 1.00E-4
K-562	0.410	2.170	2.260	2.148	1.848	0.562	1.253	105	99	82	9	48	2.72E-6	> 1.00E-4	> 1.00E-4
MOLT-4	0.649	2.153	2.093	2.081	1.852	0.463	1.374	96	95	80	-29	48	1.89E-6	.	> 1.00E-4
RPMI-8226	0.985	2.012	2.096	2.057	1.548	0.509	1.258	108	104	55	-48	27	1.11E-6	.	> 1.00E-4
SR	0.663	1.726	1.652	1.609	1.364	0.383	0.857	93	89	66	-42	18	1.40E-6	.	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.366	1.639	1.572	1.613	1.591	0.485	1.012	95	98	96	9	51	.	> 1.00E-4	> 1.00E-4
EKVX	0.800	1.806	1.804	1.793	1.699	0.782	1.303	100	99	89	-2	50	2.69E-6	.	> 1.00E-4
HOP-62	0.286	0.775	0.757	0.779	0.849	0.365	0.475	96	101	115	16	39	4.54E-6	> 1.00E-4	> 1.00E-4
HOP-92	0.534	0.839	0.820	0.814	0.770	0.439	0.537	94	92	77	-18	1	1.94E-6	.	> 1.00E-4
NCI-H226	0.736	1.406	1.403	1.369	1.377	0.475	1.203	100	95	96	-35	70	.	.	> 1.00E-4
NCI-H23	0.533	1.568	1.511	1.503	1.352	0.579	0.972	95	94	79	4	42	2.45E-6	> 1.00E-4	> 1.00E-4
NCI-H322M	0.775	1.412	1.387	1.422	1.462	0.996	1.188	96	102	108	35	65	.	> 1.00E-4	> 1.00E-4
NCI-H460	0.274	2.286	2.365	2.405	2.159	0.298	1.580	104	106	94	1	65	.	> 1.00E-4	> 1.00E-4
NCI-H522	0.702	1.873	1.748	1.731	1.563	0.409	1.060	89	88	74	-42	31	1.60E-6	.	> 1.00E-4
Colon Cancer															
COLO 205	0.502	1.899	1.878	1.870	1.814	0.135	1.304	98	98	94	-73	57	.	.	.
HCC-2998	0.739	2.522	2.504	2.522	2.513	0.639	1.876	99	100	100	-14	64	.	.	> 1.00E-4
HCT-116	0.221	1.438	1.396	1.282	1.129	0.239	0.697	97	87	75	1	39	2.17E-6	> 1.00E-4	> 1.00E-4
HCT-15	0.468	2.206	2.209	2.164	1.816	0.527	1.558	100	98	78	3	63	.	> 1.00E-4	> 1.00E-4
HT29	0.187	0.861	0.877	0.840	0.719	0.119	0.351	102	97	79	-37	24	1.78E-6	.	> 1.00E-4
KM12	0.455	1.898	1.771	1.886	1.887	0.546	1.218	91	99	99	6	53	.	> 1.00E-4	> 1.00E-4
SW-620	0.241	1.400	1.408	1.428	1.255	0.399	0.929	101	102	87	14	59	.	> 1.00E-4	> 1.00E-4
CNS Cancer															
SF-268	0.565	1.646	1.569	1.548	1.637	0.857	1.256	93	91	99	27	64	.	> 1.00E-4	> 1.00E-4
SF-295	0.996	2.058	1.911	1.959	1.947	0.442	1.416	86	91	90	-56	40	1.87E-6	.	.
SF-539	0.661	1.821	1.769	1.748	1.776	0.742	1.282	96	94	96	7	54	.	> 1.00E-4	> 1.00E-4
SNB-19	0.569	1.687	1.687	1.608	1.541	0.754	1.266	100	93	87	17	62	.	> 1.00E-4	> 1.00E-4
SNB-75	0.784	1.260	1.187	1.215	1.142	0.799	0.879	85	90	75	3	20	2.23E-6	> 1.00E-4	> 1.00E-4
U251	0.360	1.323	1.312	1.290	1.169	0.381	0.816	99	97	84	2	47	2.61E-6	> 1.00E-4	> 1.00E-4
Melanoma															
LOX IMVI	0.195	1.445	1.404	1.411	1.299	0.014	0.910	97	97	88	-93	57	.	.	.
MALME-3M	0.531	0.848	0.838	0.871	0.894	0.318	0.840	97	107	115	-40	97	.	.	> 1.00E-4
M14	0.367	1.248	1.211	1.153	1.230	0.214	0.979	96	89	98	-42	69	.	.	> 1.00E-4
MDA-MB-435	0.382	1.455	1.442	1.477	1.354	0.286	1.199	99	102	91	-25	76	.	.	> 1.00E-4
SK-MEL-28	0.592	1.459	1.469	1.435	1.423	0.753	1.300	101	97	96	19	82	.	> 1.00E-4	> 1.00E-4
SK-MEL-5	0.657	2.012	1.951	1.901	1.713	0.047	1.215	95	92	78	-93	41	1.46E-6	.	.
UACC-257	0.603	1.169	1.145	1.186	1.060	0.451	0.902	96	103	81	-25	53	.	.	> 1.00E-4
UACC-62	0.606	2.063	1.953	1.948	1.696	0.525	1.541	92	92	75	-13	64	.	.	> 1.00E-4
Ovarian Cancer															
IGROV1	0.653	1.912	1.951	1.967	1.937	1.031	1.441	103	104	102	30	63	.	> 1.00E-4	> 1.00E-4
OVCAR-3	0.508	1.499	1.421	1.419	1.417	0.345	0.953	92	92	92	-32	45	2.17E-6	.	> 1.00E-4
OVCAR-4	0.434	0.741	0.741	0.747	0.685	0.466	0.497	100	102	82	10	21	2.79E-6	> 1.00E-4	> 1.00E-4
OVCAR-5	0.594	1.256	1.210	1.213	1.285	0.649	1.250	93	93	104	8	99	.	> 1.00E-4	> 1.00E-4
OVCAR-8	0.356	1.251	1.255	1.223	1.121	0.358	0.765	100	97	85	.	46	2.60E-6	> 1.00E-4	> 1.00E-4
NCI/ADR-RES	0.408	1.394	1.406	1.409	1.234	0.404	0.979	101	102	84	-1	58	.	.	> 1.00E-4
SK-OV-3	0.497	1.115	1.094	1.102	1.103	0.567	0.726	97	98	98	11	37	3.58E-6	> 1.00E-4	> 1.00E-4
Renal Cancer															
786-0	0.576	1.904	1.906	1.796	1.854	0.405	1.247	100	92	96	-30	50	.	.	> 1.00E-4
A498	1.116	1.736	1.664	1.655	1.528	1.030	1.419	88	87	66	-8	49	1.67E-6	.	> 1.00E-4
ACHN	0.541	1.912	1.902	1.888	1.828	0.618	1.387	99	98	94	6	62	.	> 1.00E-4	> 1.00E-4
CAKI-1	0.748	1.673	1.592	1.610	1.689	0.666	1.422	91	93	102	-11	73	.	.	> 1.00E-4
RXF 393	0.646	1.005	1.023	1.037	0.992	0.362	0.782	105	109	96	-44	38	2.14E-6	.	> 1.00E-4
SN12C	0.508	1.955	1.880	1.848	1.827	0.887	1.430	95	93	91	26	64	.	> 1.00E-4	> 1.00E-4
TK-10	0.594	1.051	1.015	1.048	1.115	0.613	0.840	92	99	114	4	54	.	> 1.00E-4	> 1.00E-4
UO-31	0.621	1.545	1.432	1.434	1.409	0.587	1.163	88	88	85	-5	59	.	.	> 1.00E-4
Prostate Cancer															
PC-3	0.434	1.358	1.330	1.289	0.993	0.382	0.651	97	93	60	-12	23	1.39E-6	.	> 1.00E-4
DU-145	0.357	1.349	1.368	1.336	1.303	0.664	0.938	102	99	95	31	59	.	> 1.00E-4	> 1.00E-4
Breast Cancer															
MCF7	0.448	1.912	1.834	1.855	1.721	0.348	1.040	95	96	87	-22	40	2.18E-6	.	> 1.00E-4
MDA-MB-231/ATCC	0.411	1.015	1.073	1.060	0.947	0.163	0.661	110	107	89	-60	41	1.82E-6	.	.
HS 578T	0.926	1.674	1.618	1.629	1.410	0.822	1.267	93	94	65	-11	46	1.56E-6	.	> 1.00E-4
BT-549	0.785	1.614	1.602	1.455	1.372	0.520	1.194	99	81	71	-34	49	1.58E-6	.	> 1.00E-4
T-47D	0.766	1.271	1.245	1.197	1.038	0.599	0.759	95	85	54	-22	-1	1.12E-6	5.14E-6	> 1.00E-4

Figure 4. Five dose assay of compound **3l** (NSC: 763439).

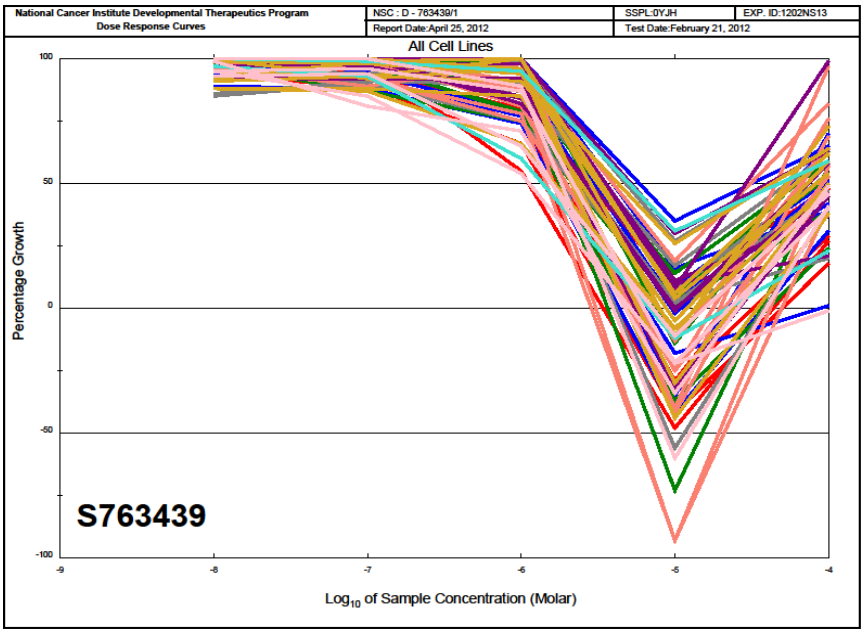

Figure 5. Dose response curves of compound **3l** (NSC: 763439) against all cancer cell lines at five dose assay level

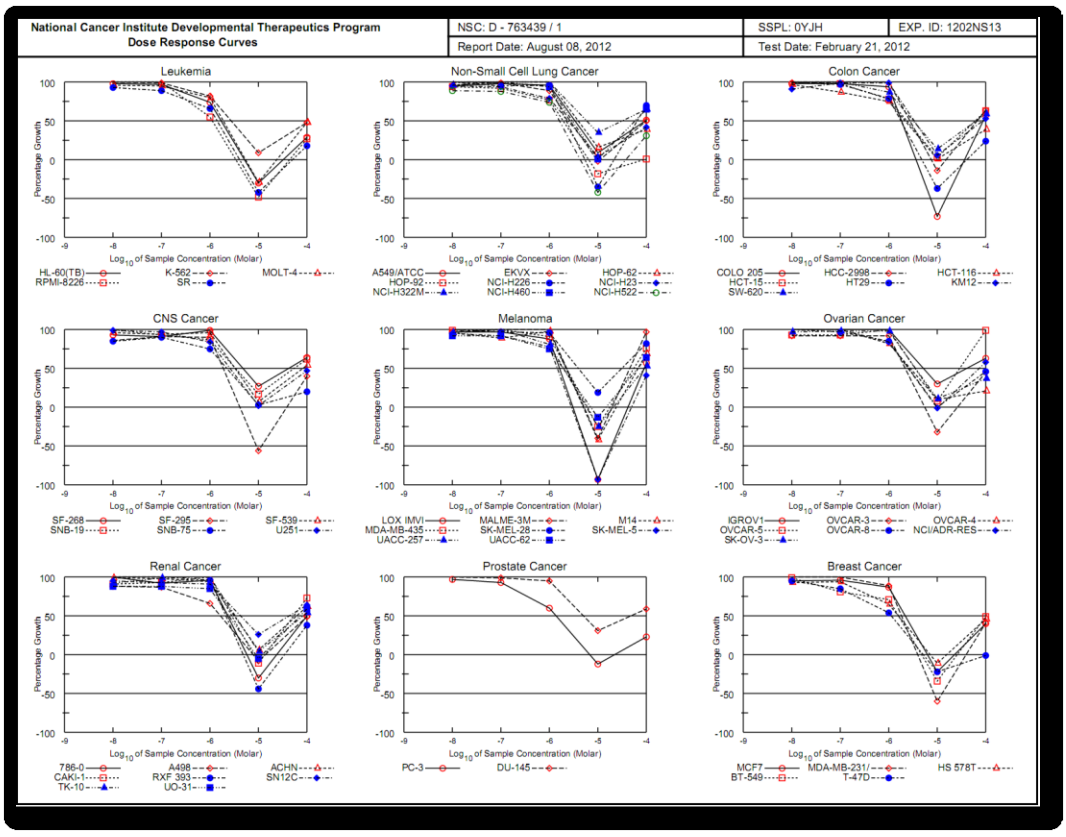

Figure 6. Five dose assay graph of compound **3l** (NSC: 763439) against nine panel cancer cell line at NCI.

3. CONCLUSION

A new series of sulphonamido-quinoxalines **3** (**a-h**) were synthesized. All of these derivatives, compounds **3a** (NSC:763435) , **3b**(NSC:763436) , **3c** (NSC: 763437), **3d** (NSC:763438), **3e** (NSC:763439), **3f** (NSC:763440), **3g** (NSC:763441) and **3h** (NSC:763442) were tested at a single dose of 10^{-5} M concentration at the NCI over 60 cell line panel, and compounds **3e** and **3h** were subsequently tested in 5-dose testing mode. These encouraging results of biological screening of the tested compounds could offer an excellent framework in this field that may lead to discovery of potent antitumor agent.

We conclude that the ongoing studies of targeted agents in conjunction with chemotherapy will show whether there are alternative option for new and safer medicine for cancer in future which may decline the ongoing incidence of deaths due to the cancer.

4. ACKNOWLEDGEMENTS

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