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Synthesis, *in vitro* evaluation and DNA interaction studies of N-allyl naphthalimide analogues as anticancer agents

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^{1}H and ^{13}C NMR spectra of compounds **4**, **6a-n**

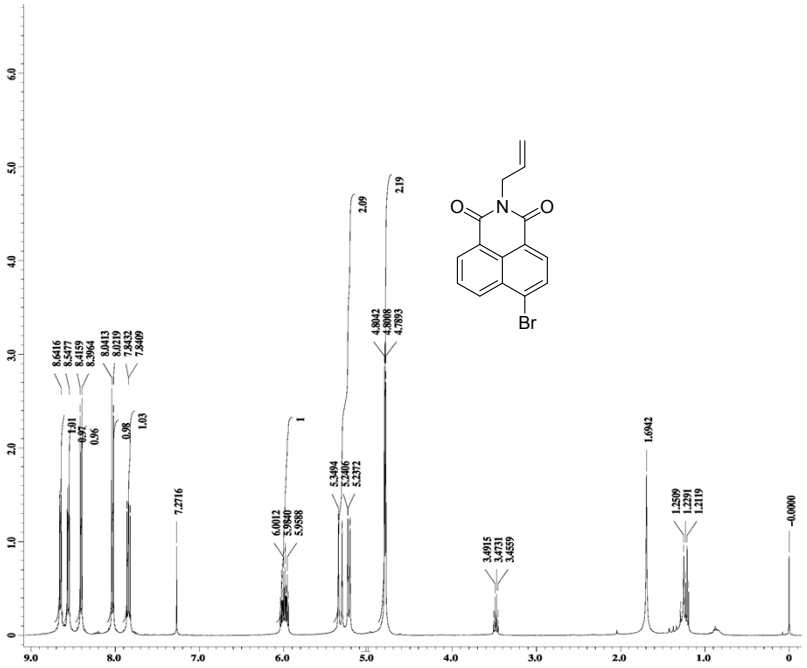

Figure S1: ^{1}H NMR spectrum of 2-allyl-6-bromo-benzo[*de*]isoquinoline-1,3-dione **(4)**

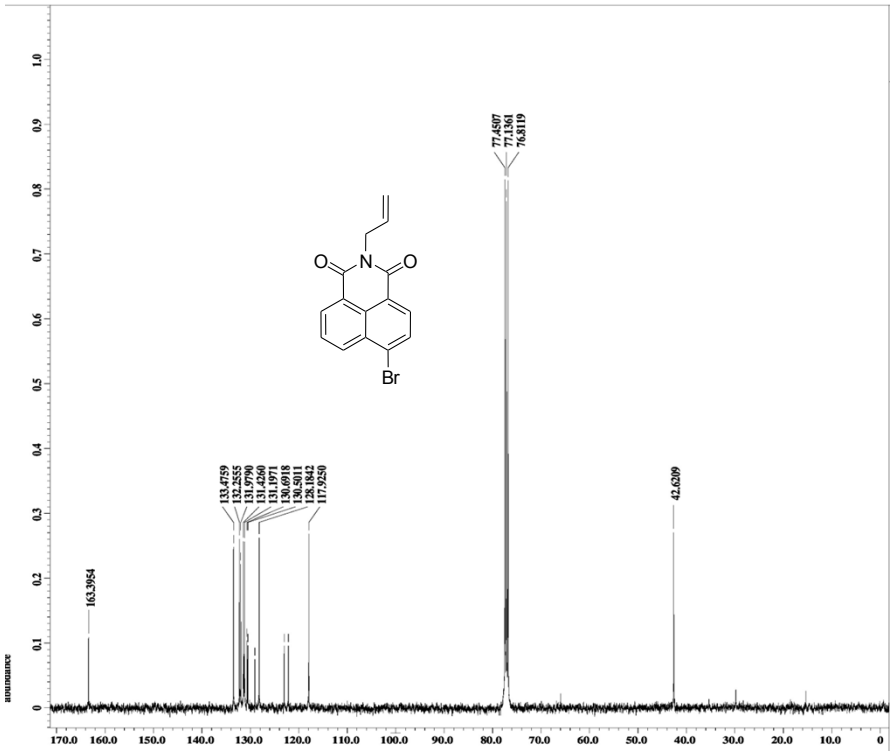

Figure S2: ^{13}C NMR spectrum of 2-allyl-6-bromo-benzo[*de*]isoquinoline-1,3-dione **(4)**

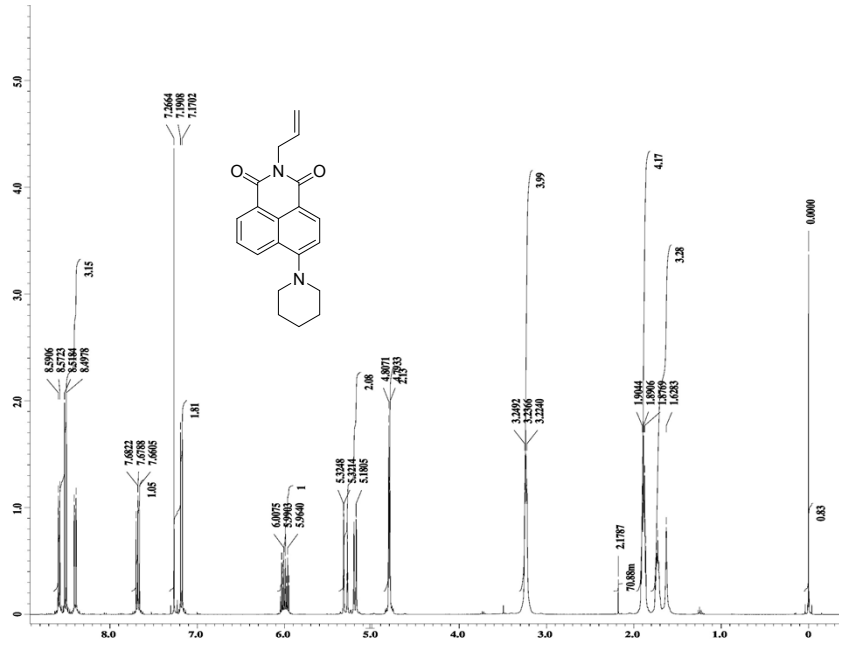

Figure S3: [1]H NMR spectrum of of 2-allyl-6-piperidin-1-yl-benzo[*de*]isoquinoline-1,3-dione **(6a)**

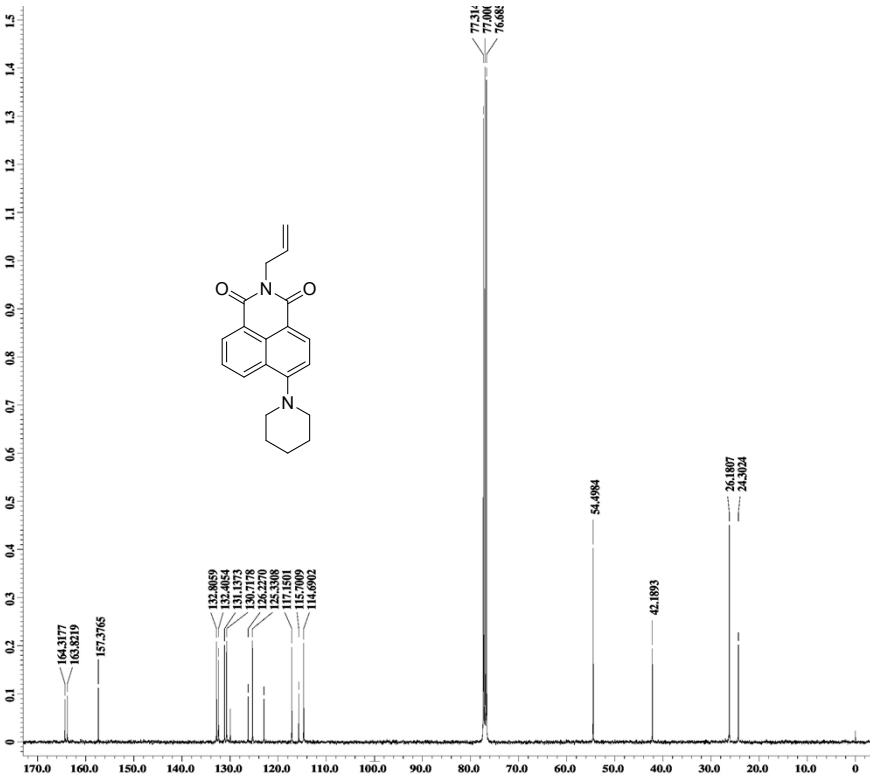

Figure S4: [13]C NMR spectrum of 2-allyl-6-piperidin-1-yl-benzo[*de*]isoquinoline-1,3-dione **(6a)**

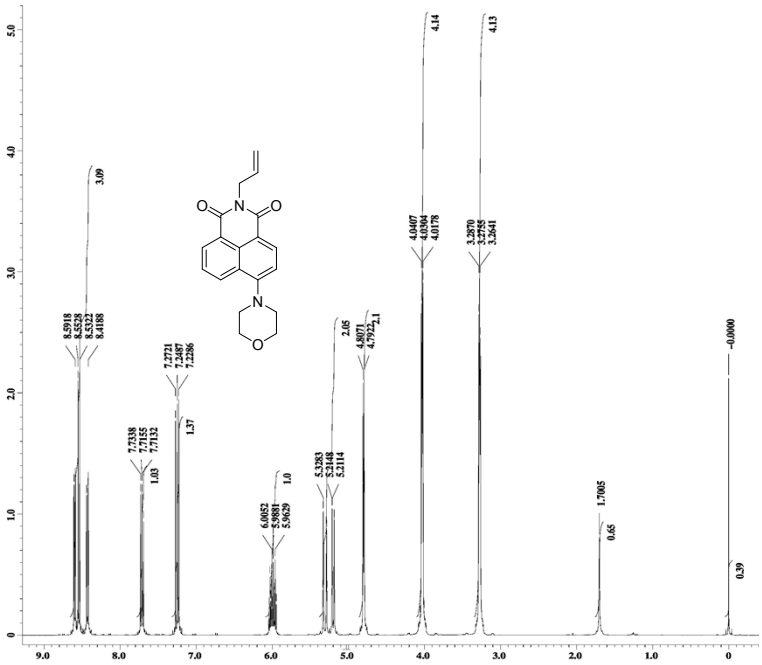

Figure S5: [1]H NMR spectrum of 2-allyl-6-morpholin-4-yl-benzo[*de*]isoquinoline-1,3-dione **(6b)**

Figure S6: [13]C NMR spectrum of 2-allyl-6-morpholin-4-yl-benzo[*de*]isoquinoline-1,3-dione **(6b)**

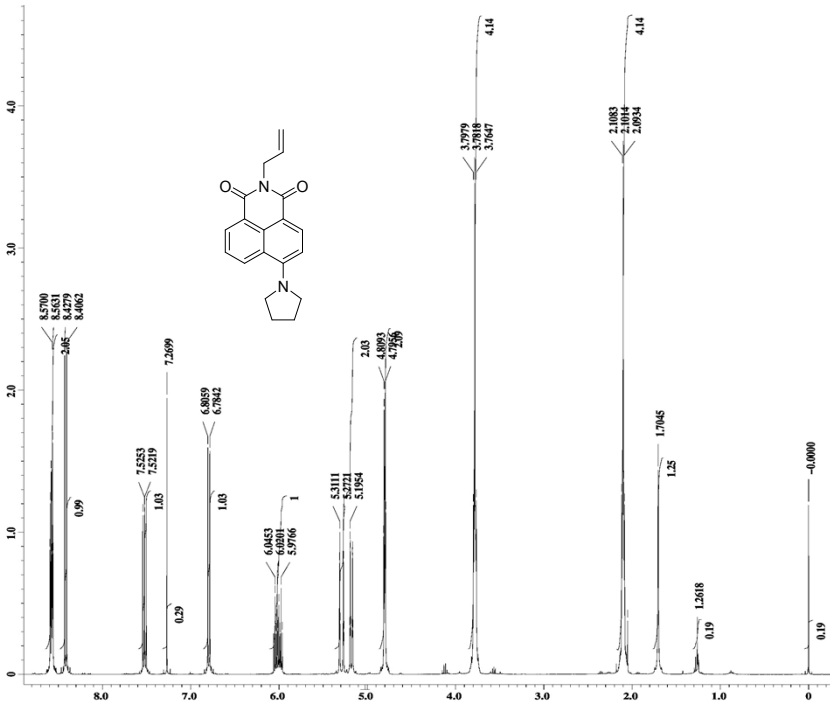

Figure S7: [1]H NMR spectrum of 2-allyl-6-pyrrolidin-1-yl-benzo[*de*]isoquinoline-1,3-dione **(6c)**

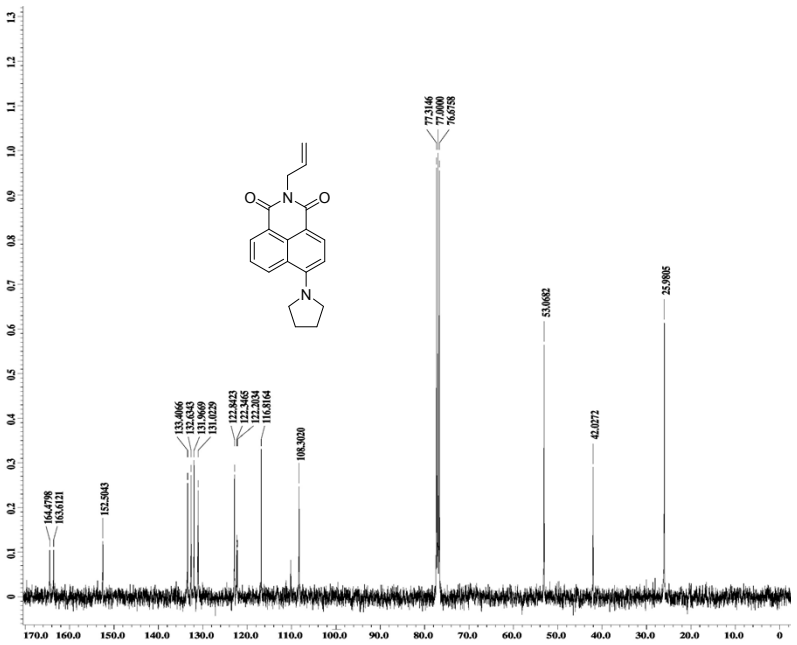

Figure S8: [13]C NMR spectrum of 2-allyl-6-pyrrolidin-1-yl-benzo[*de*]isoquinoline-1,3-dione **(6c)**

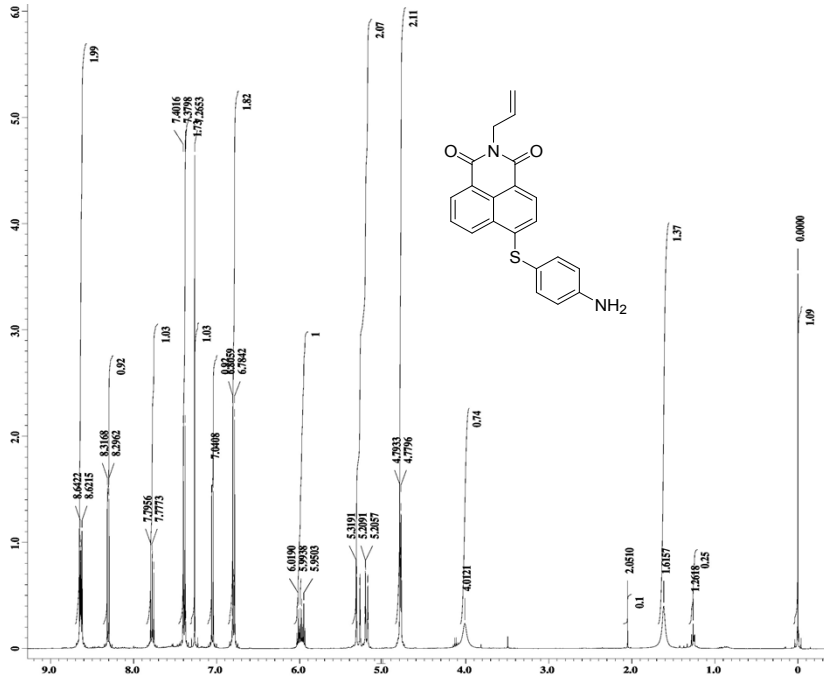

Figure S9: [1]H NMR spectrum of 2-allyl-6-(4-amino-phenylsulfanyl)-benzo[*de*]isoquinoline-1,3-dione **(6d)**

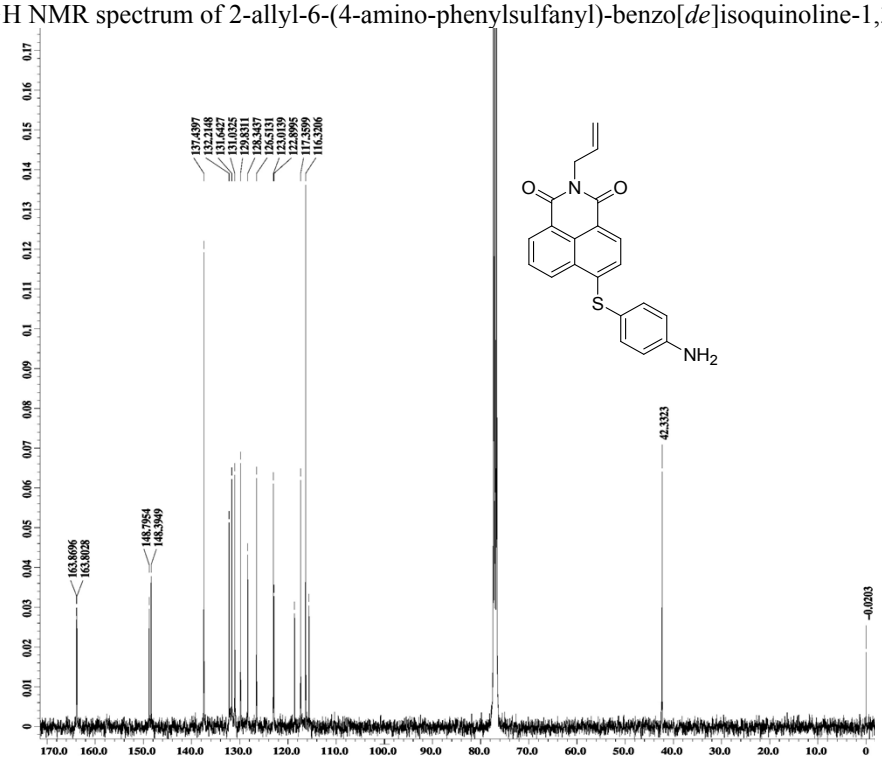

Figure S10: [13]C NMR spectrum of 2-allyl-6-(4-amino-phenylsulfanyl)-benzo[*de*]isoquinoline-1,3-dione **(6d)**

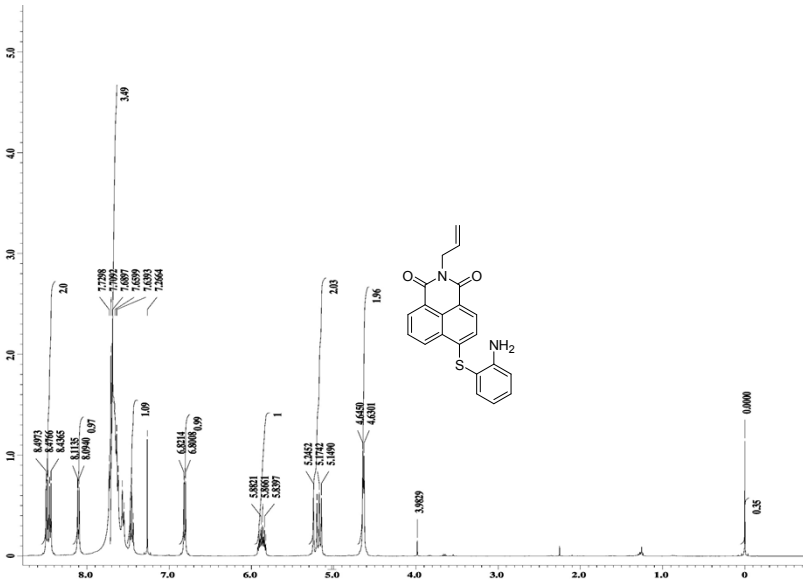

Figure S11: ^{1}H NMR spectrum of 2-allyl-6-(2-amino-phenylsulfanyl)-benzo[*de*]isoquinoline-1,3-dione **(6e)**

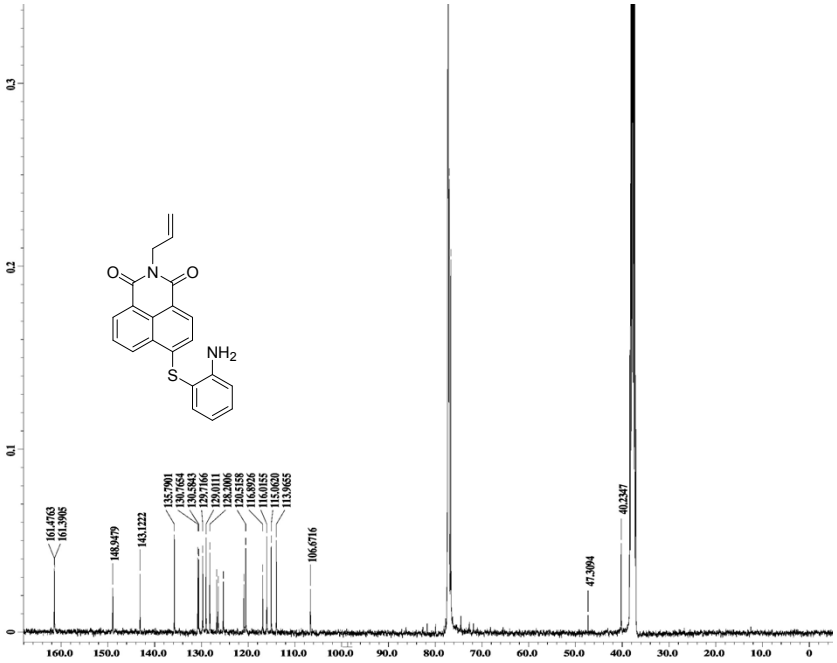

Figure S12: ^{13}C NMR spectrum of 2-allyl-6-(2-amino-phenylsulfanyl)-benzo[*de*]isoquinoline-1,3-dione **(6e)**

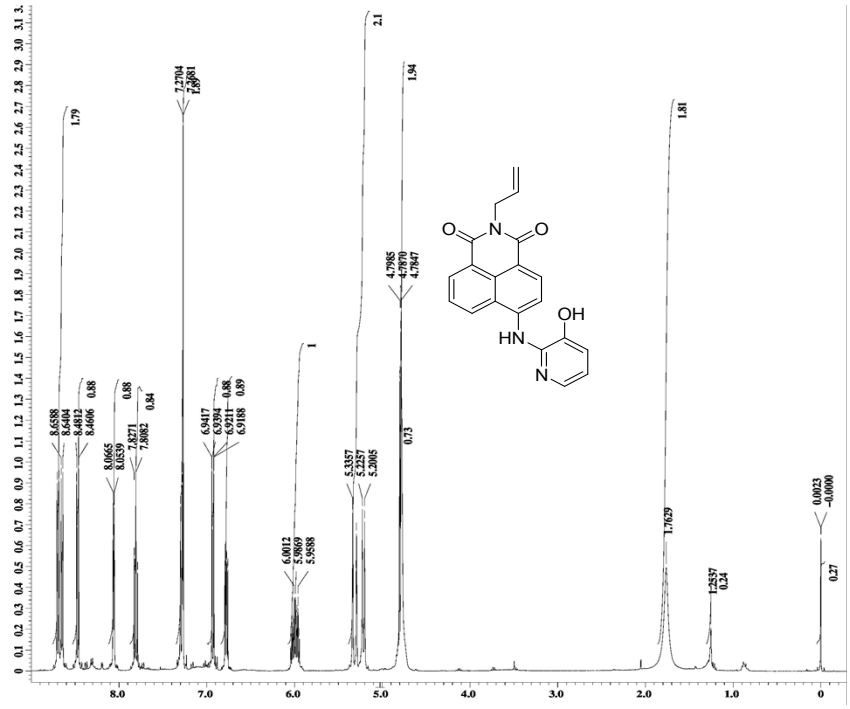

Figure S13: ^{1}H NMR spectrum of 2-allyl-6-(3-hydroxy-pyridin-2-ylamino)-benzo[*de*]isoquinoline-1,3-dione **(6f)**

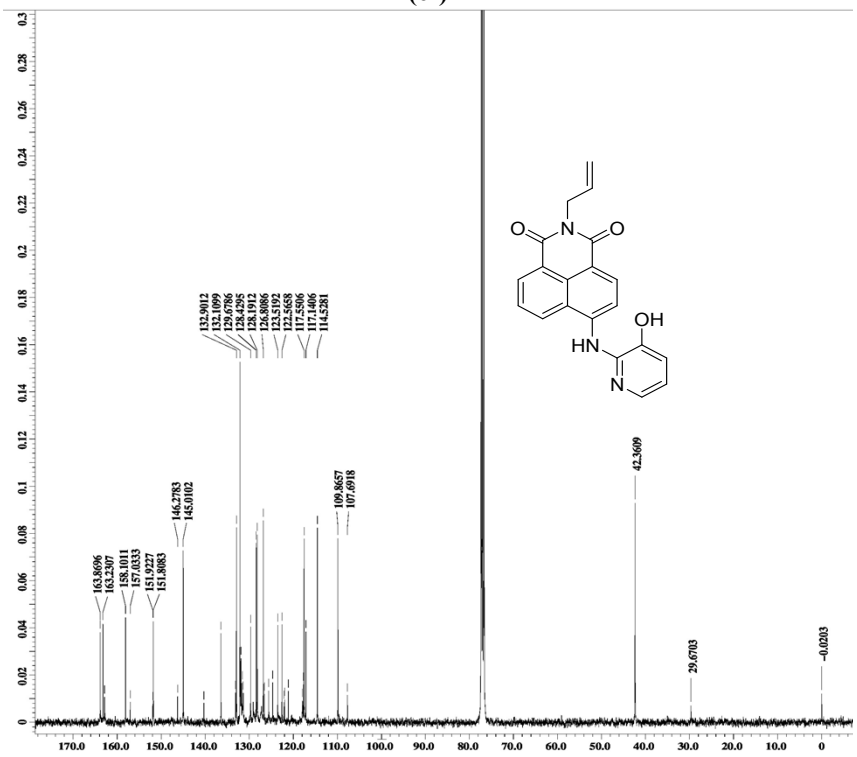

Figure S14: ^{13}C NMR of spectrum 2-allyl-6-(3-hydroxy-pyridin-2-ylamino)-benzo[*de*]isoquinoline-1,3-dione **(6f)**

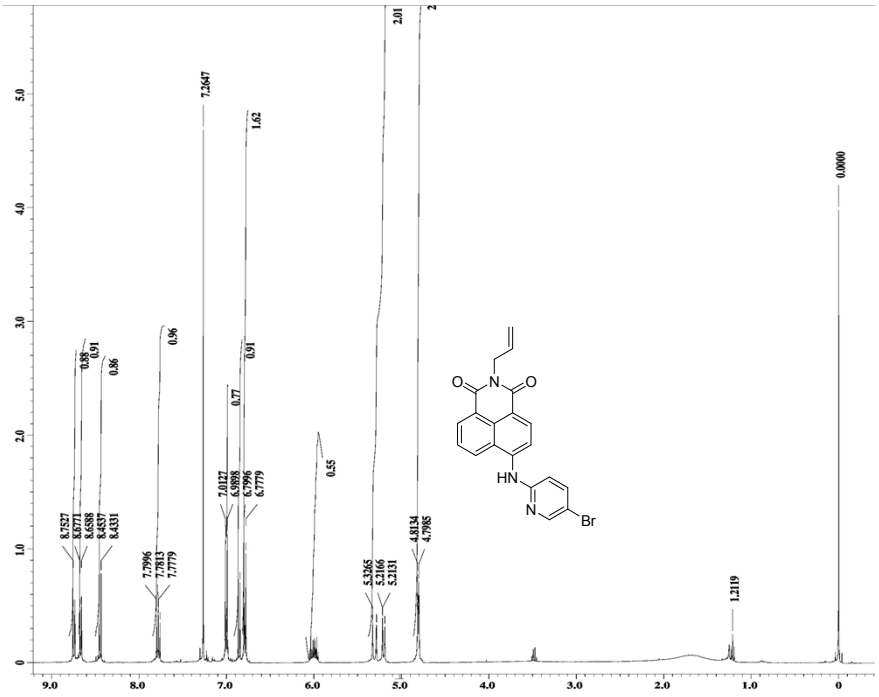

Figure S15: ^{1}H NMR spectrum of 2-allyl-6-(5-bromo-pyridin-2-ylamino)-benzo[*de*]isoquinoline-1,3-dione **(6g)**

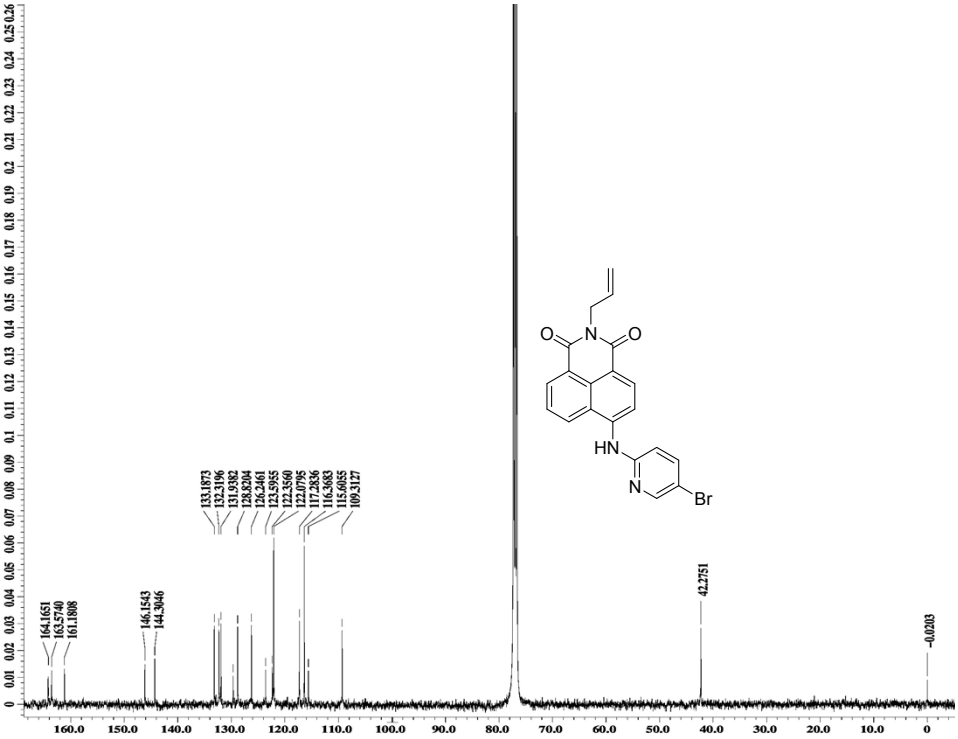

Figure S16: ^{13}C NMR spectrum of 2-allyl-6-(5-bromo-pyridin-2-ylamino)-benzo[*de*]isoquinoline-1,3-dione **(6g)**

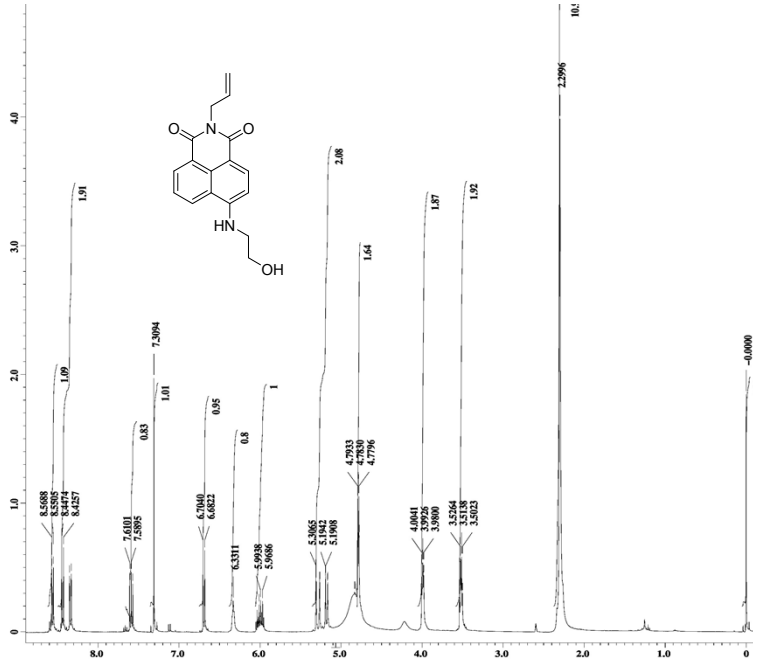

Figure S17: [1]H NMR spectrum of 2-allyl-6-(2-hydroxy-ethylamino)-benzo[*de*]isoquinoline-1,3-dione **(6h)**

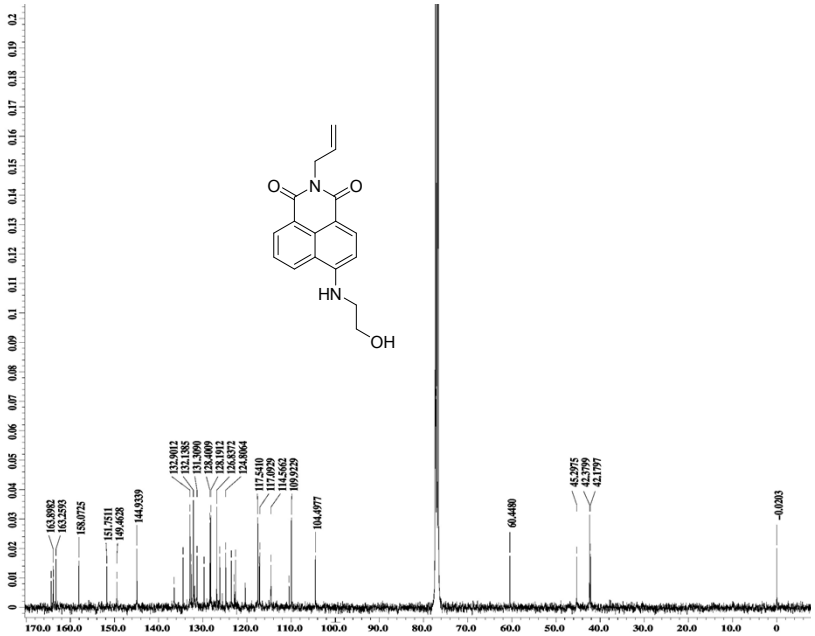

Figure S18: [13]C NMR spectrum of 2-allyl-6-(2-hydroxy-ethylamino)-benzo[*de*]isoquinoline-1,3-dione **(6h)**

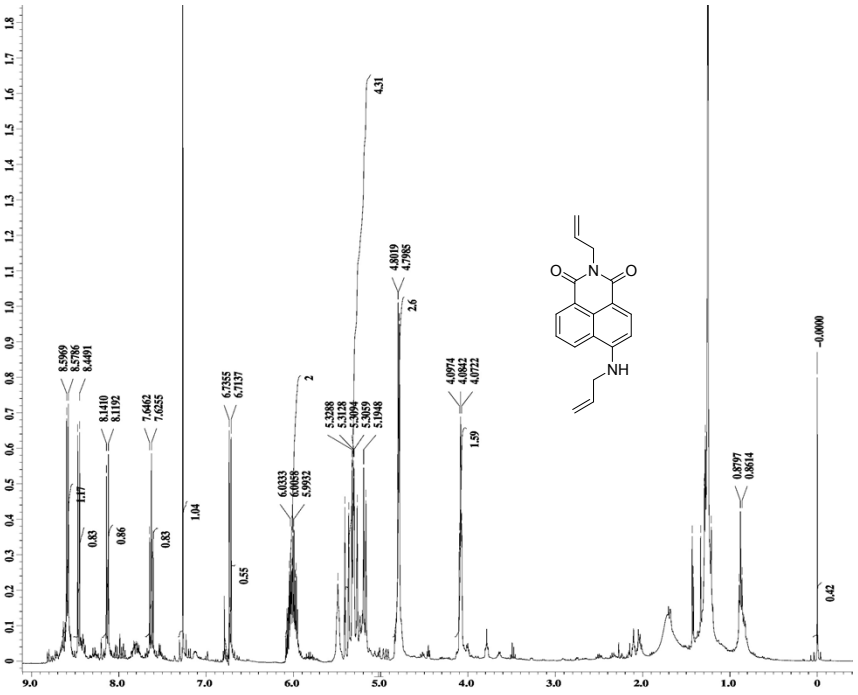

Figure S19: ^{1}H NMR spectrum of 2-allyl-6-allylamino-benzo[*de*]isoquinoline-1,3-dione **(6i)**

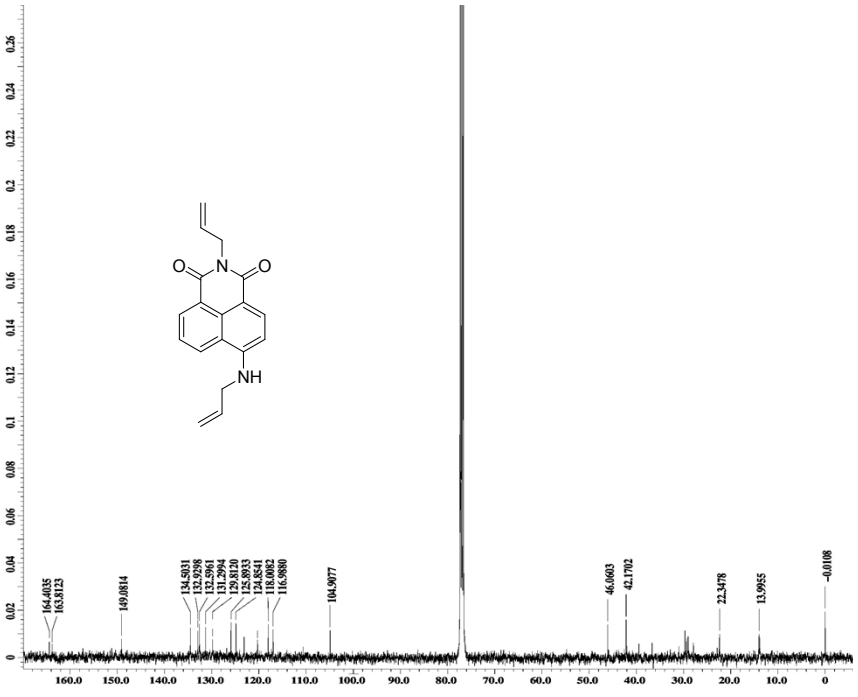

Figure S20: ^{13}C NMR spectrum of 2-allyl-6-allylamino-benzo[*de*]isoquinoline-1,3-dione **(6i)**

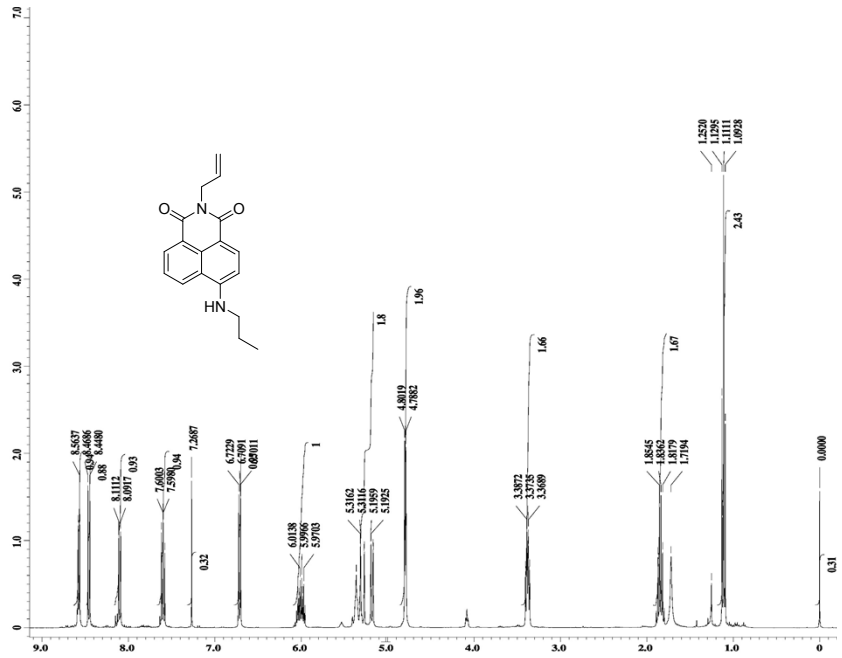

Figure S21: ^{1}H NMR spectrum of 2-allyl-6-propylamino-benzo[*de*]isoquinoline-1,3-dione **(6j)**

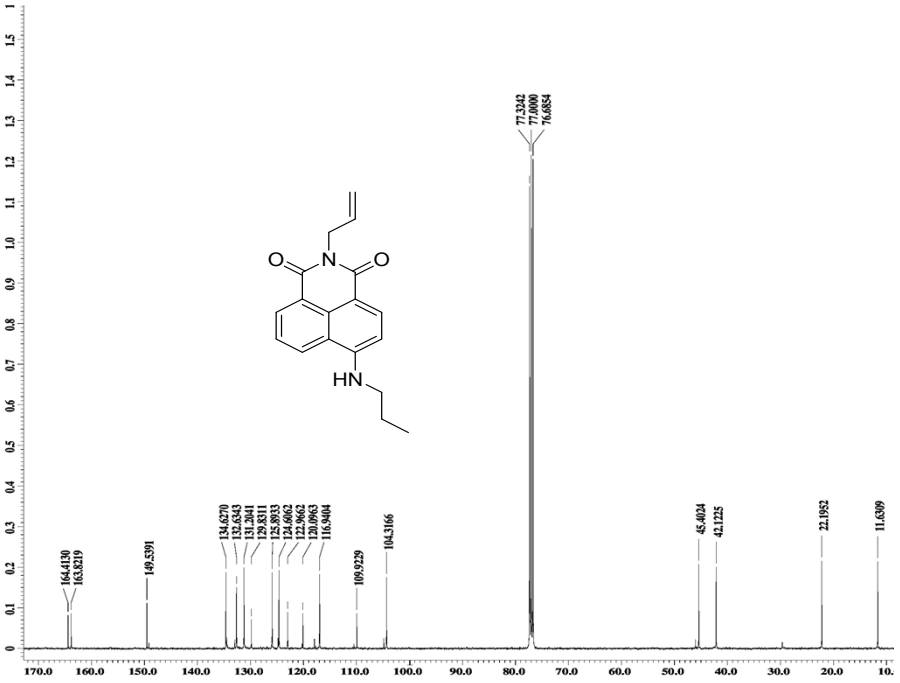

Figure S22: ^{13}C NMR spectrum of 2-allyl-6-propylamino-benzo[*de*]isoquinoline-1,3-dione **(6j)**

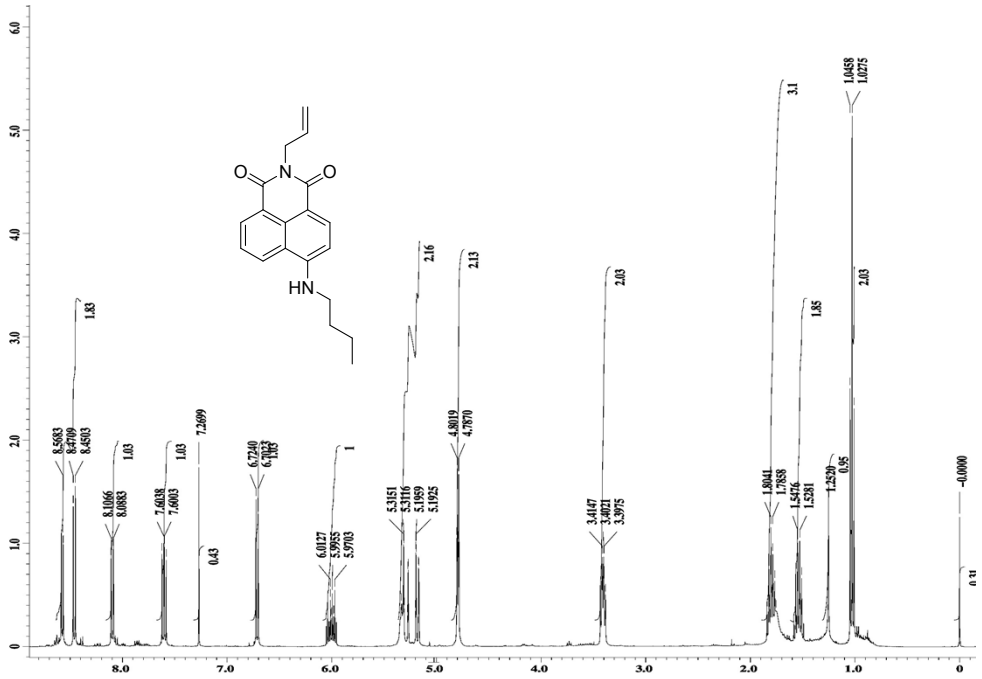

Figure S23: [1]H NMR spectrum of 2-allyl-6-butylamino-benzo[*de*]isoquinoline-1,3-dione **(6k)**

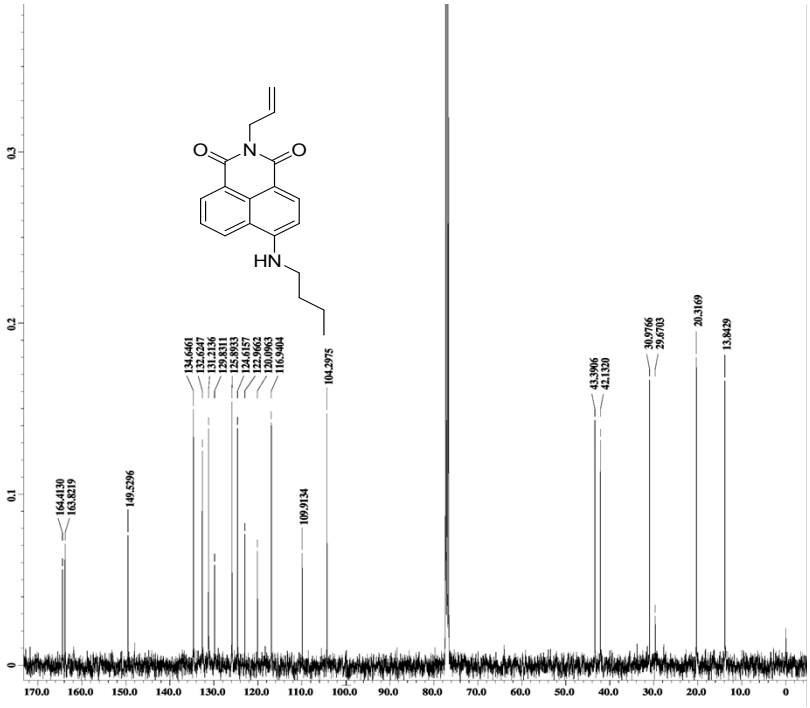

Figure S24: [13]C NMR spectrum of 2-allyl-6-butylamino-benzo[*de*]isoquinoline-1,3-dione **(6k)**

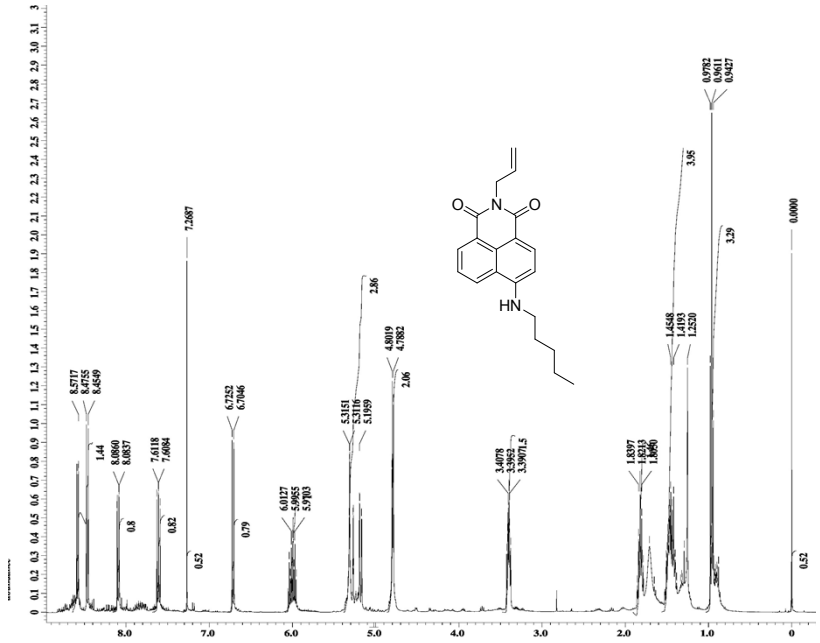

Figure S25: ^{1}H NMR spectrum of 2-allyl-6-pentylamino-benzo[*de*]isoquinoline-1,3-dione **(6l)**

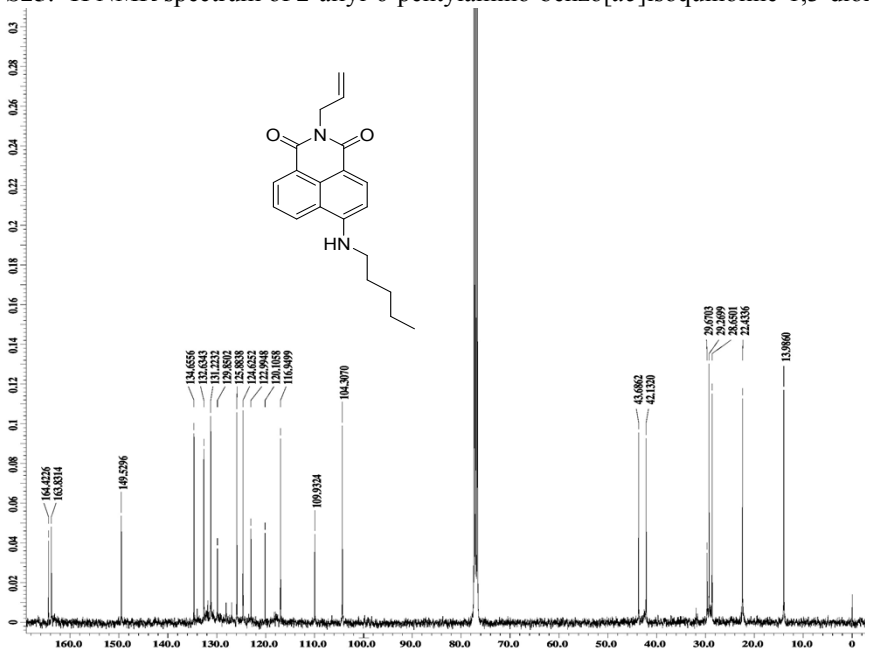

Figure S26: ^{13}C NMR spectrum of 2-allyl-6-pentylamino-benzo[*de*]isoquinoline-1,3-dione **(6l)**

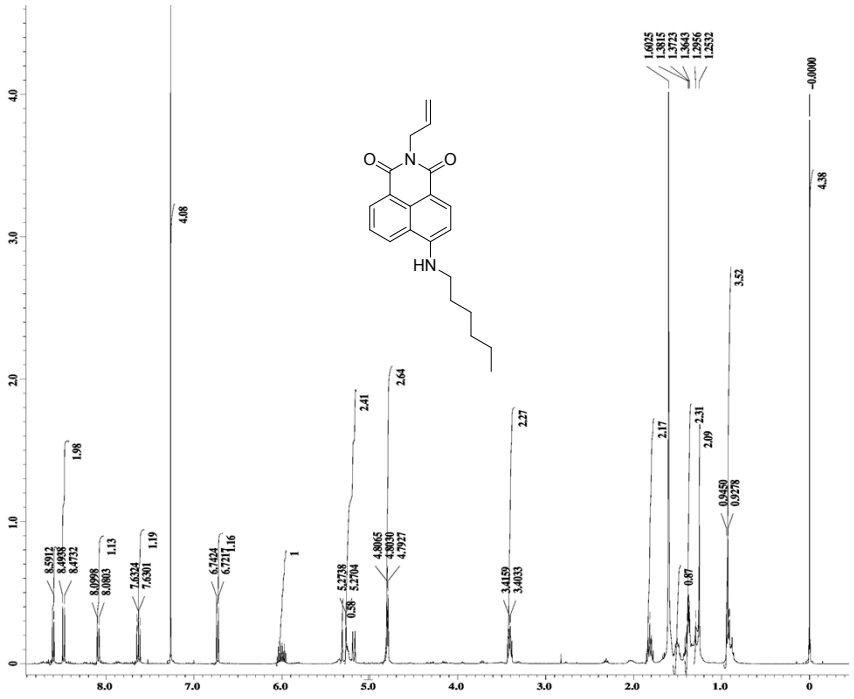

Figure S27: ^{1}H NMR spectrum of 2-allyl-6-hexylamino-benzo[*de*]isoquinoline-1,3-dione **(6m)**

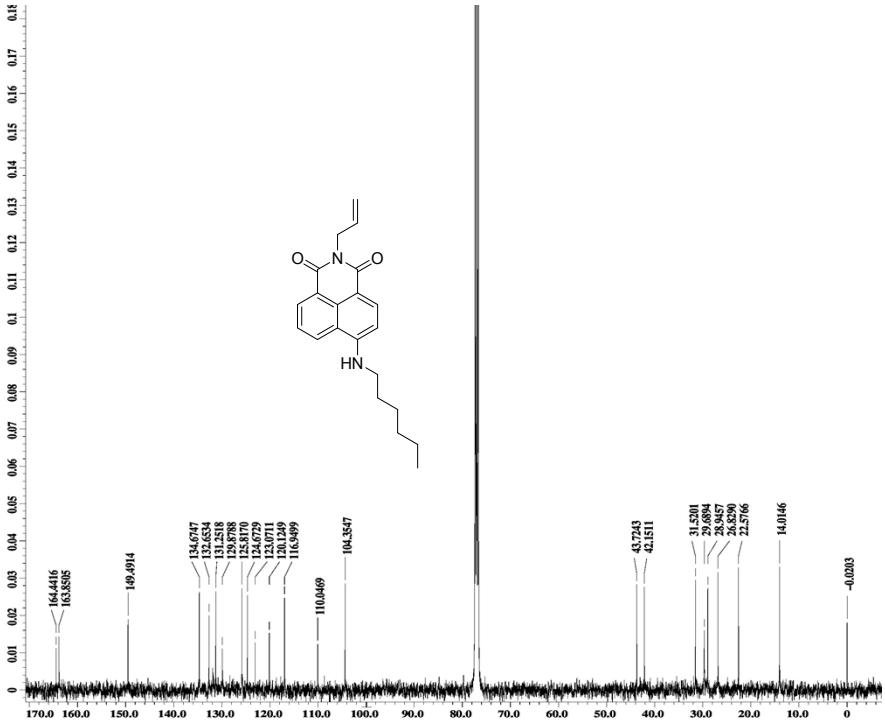

Figure S28: ^{13}C NMR spectrum of 2-allyl-6-hexylamino-benzo[*de*]isoquinoline-1,3-dione **(6m)**

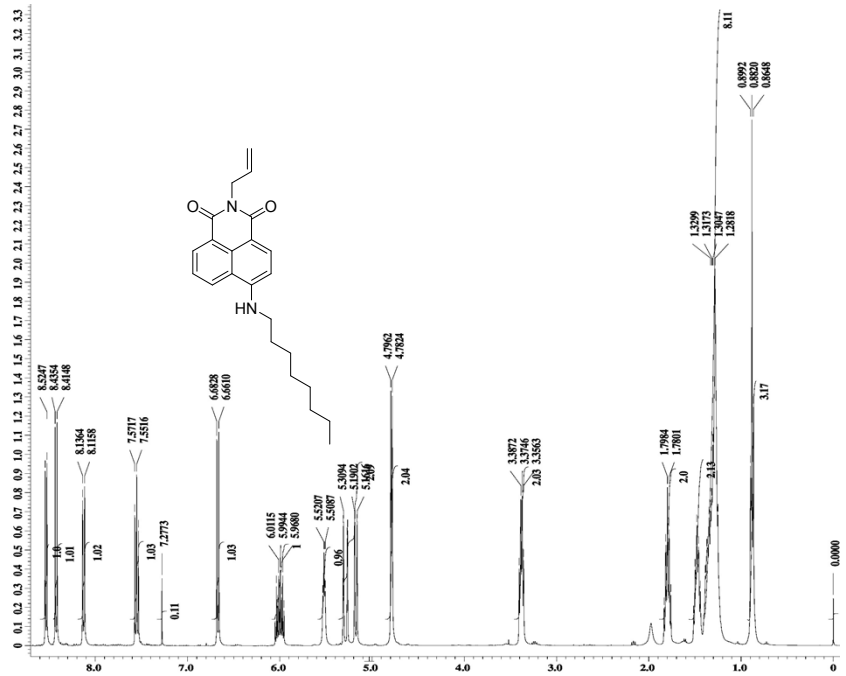

Figure S29: ^{1}H NMR spectrum of 2-allyl-6-octylamino-benzo[*de*]isoquinoline-1,3-dione **(6n)**

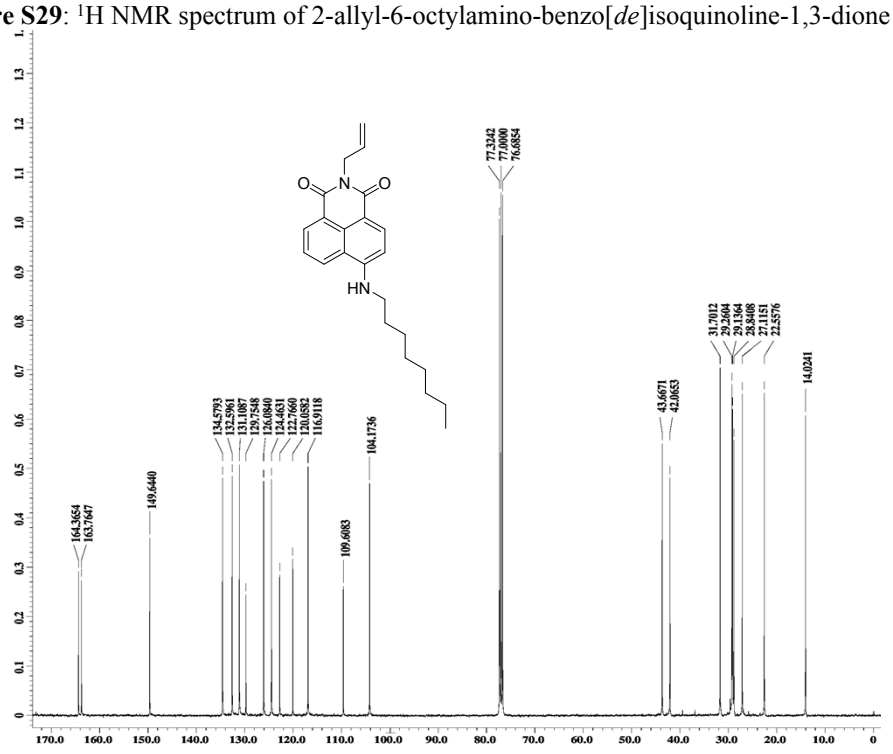

Figure S30: ^{13}C NMR spectrum of 2-allyl-6-octylamino-benzo[*de*]isoquinoline-1,3-dione **(6n)**

60 human cancer cell line results of compound 6b at five dose concentrations

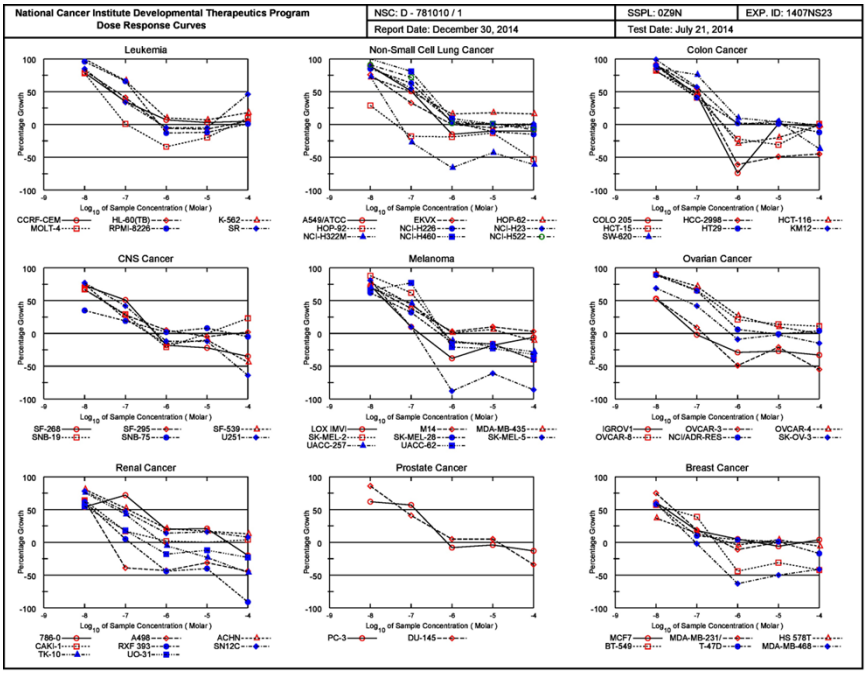

Figure S31 National Cancer Institute developmental therapeutics program *in-vitro* testing results of compound **6b** at five dose level in μM

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 781010 / 1	Experiment ID : 1407NS23	Test Type : 08	Units : Molar
Report Date : December 30, 2014	Test Date : July 21, 2014	QNS :	MC :
COMI : Meen-376	Stain Reagent : SRB Dual-Pass Related	SSPL : 0Z9N	

		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	0.621	2.635	2.203	1.325	0.756	0.675	0.730	79	35	7	3	5	4.52E-8	> 1.00E-4	> 1.00E-4
HL-60(TB)	1.063	3.285	2.894	1.981	1.003	0.988	1.171	82	41	-6	-7	5	6.14E-8	.	> 1.00E-4
K-562	0.344	2.397	2.469	1.729	0.556	0.488	0.720	104	67	10	7	18	2.02E-7	> 1.00E-4	> 1.00E-4
MOLT-4	0.679	2.824	2.343	0.708	0.447	0.541	0.902	78	1	-34	-20	10	2.30E-8	.	> 1.00E-4
RPMI-8226	1.053	2.754	2.691	2.179	0.920	0.928	1.078	96	66	-13	-12	1	1.61E-7	.	> 1.00E-4
SR	0.229	0.873	0.778	0.447	0.219	0.217	0.526	85	34	-5	-5	46	4.85E-8	.	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.335	2.046	1.866	1.202	0.286	0.301	0.301	89	51	-15	-10	-10	1.02E-7	5.95E-7	> 1.00E-4
EKVX	0.661	1.722	1.464	1.016	0.659	0.627	0.658	76	33	.	-5	.	4.05E-8	9.80E-7	> 1.00E-4
HOP-62	0.538	1.506	1.231	1.024	0.690	0.709	0.689	72	50	16	18	16	1.01E-7	> 1.00E-4	> 1.00E-4
HOP-92	1.253	1.576	1.346	1.032	1.010	1.096	0.589	29	-18	-19	-13	-53	< 1.00E-8	4.16E-8	8.41E-5
NCI-H226	0.671	1.642	1.499	1.286	0.705	0.598	0.571	85	63	4	-11	-15	1.67E-7	1.75E-6	> 1.00E-4
NCI-H23	0.657	2.289	2.113	1.554	0.734	0.659	0.676	89	55	5	.	1	1.26E-7	> 1.00E-4	> 1.00E-4
NCI-H322M	0.772	1.909	1.605	0.564	0.263	0.442	0.304	73	-27	-66	-43	-61	1.71E-8	5.38E-8	.
NCI-H460	0.287	2.054	2.112	1.722	0.453	0.298	0.273	103	81	9	1	-5	2.72E-7	1.27E-5	> 1.00E-4
NCI-H522	0.671	2.179	2.041	1.758	0.697	0.682	0.621	91	72	2	1	-8	2.06E-7	1.23E-5	> 1.00E-4
Colon Cancer															
COLO 205	0.463	1.550	1.448	0.957	0.119	0.469	0.449	91	45	-74	1	-3	7.93E-8	.	.
HCC-2998	1.104	3.223	3.042	2.268	0.435	0.567	0.607	91	55	-61	-49	-45	1.10E-7	2.99E-7	.
HCT-116	0.210	1.753	1.463	0.973	0.150	0.167	0.208	81	49	-29	-20	-1	9.61E-8	4.28E-7	> 1.00E-4
HCT-15	0.231	1.666	1.409	0.820	0.180	0.160	0.239	82	41	-22	-31	1	6.05E-8	.	> 1.00E-4
HT29	0.170	1.108	1.014	0.553	0.187	0.176	0.150	90	41	2	1	-12	6.50E-8	1.11E-5	> 1.00E-4
KM12	0.370	1.975	1.965	1.277	0.375	0.457	0.385	99	57	.	5	-1	1.31E-7	6.09E-5	> 1.00E-4
SW-620	0.325	1.388	1.237	1.137	0.428	0.368	0.204	86	76	10	4	-37	2.49E-7	1.25E-5	> 1.00E-4
CNS Cancer															
SF-268	0.423	1.384	1.129	0.914	0.347	0.329	0.274	73	51	-18	-22	-35	1.04E-7	5.48E-7	> 1.00E-4
SF-295	0.629	2.289	1.746	1.100	0.708	0.601	0.663	67	28	5	-5	2	2.78E-8	.	> 1.00E-4
SF-539	0.787	2.512	2.079	1.160	0.662	0.711	0.444	75	22	-16	-10	-44	2.93E-8	3.76E-7	> 1.00E-4
SNB-19	0.523	1.251	1.012	0.736	0.416	0.527	0.692	67	29	-21	.	23	2.84E-8	.	> 1.00E-4
SNB-75	0.831	1.524	1.076	0.963	0.845	0.886	0.789	35	19	2	8	-5	< 1.00E-8	4.06E-5	> 1.00E-4
U251	0.376	1.940	1.576	1.035	0.332	0.331	0.137	77	42	-12	-12	-64	5.92E-8	6.06E-7	5.45E-5
Melanoma															
LOX IMVI	0.449	2.717	2.103	0.668	0.279	0.370	0.423	73	10	-38	-18	-6	2.30E-8	1.59E-7	> 1.00E-4
M14	0.552	2.023	1.598	1.108	0.596	0.704	0.594	71	38	3	10	3	4.29E-8	> 1.00E-4	> 1.00E-4
MDA-MB-435	0.446	1.813	1.514	1.045	0.465	0.523	0.399	78	44	1	6	-11	6.59E-8	2.22E-5	> 1.00E-4
SK-MEL-2	0.832	2.306	2.137	1.750	0.726	0.697	0.502	88	62	-13	-16	-40	1.46E-7	6.75E-7	> 1.00E-4
SK-MEL-28	0.830	2.185	1.672	1.267	0.711	0.695	0.502	62	32	-14	-16	-40	2.54E-8	4.92E-7	> 1.00E-4
SK-MEL-5	0.664	2.733	2.341	0.876	0.082	0.260	0.094	81	10	-88	-61	-86	2.74E-8	1.27E-7	4.12E-7
UACC-257	1.031	2.442	2.014	1.682	0.918	0.822	0.747	70	46	-11	-20	-28	6.85E-8	6.42E-7	> 1.00E-4
UACC-62	0.885	2.648	2.053	2.238	0.702	0.679	0.600	66	77	-21	-23	-32	1.88E-7	6.13E-7	> 1.00E-4
Ovarian Cancer															
IGROV1	0.586	1.867	1.267	0.574	0.416	0.428	0.390	53	-2	-29	-27	-33	1.14E-8	9.15E-8	> 1.00E-4
OVCAR-3	0.650	1.898	1.314	0.762	0.329	0.511	0.293	53	9	-49	-21	-55	1.18E-8	1.42E-7	7.10E-5
OVCAR-4	0.702	1.397	1.327	1.201	0.893	0.771	0.705	90	72	27	10	.	3.10E-7	> 1.00E-4	> 1.00E-4
OVCAR-8	0.422	1.979	1.824	1.453	0.753	0.640	0.598	90	66	21	14	11	2.29E-7	> 1.00E-4	> 1.00E-4
NCI/ADR-RES	0.567	1.871	1.731	1.416	0.645	0.564	0.619	89	65	6	-1	4	1.80E-7	.	> 1.00E-4
SK-OV-3	0.786	1.584	1.334	1.106	0.698	0.748	0.650	69	42	-9	-2	-15	4.98E-8	6.67E-7	> 1.00E-4
Renal Cancer															
786-0	0.964	2.820	1.970	2.294	1.336	1.351	0.774	54	72	20	21	-20	2.63E-7	3.27E-5	> 1.00E-4
A498	1.357	1.742	1.605	0.831	0.774	0.932	0.758	64	-39	-43	-31	-44	1.38E-8	4.21E-8	> 1.00E-4
ACHN	0.528	2.155	1.840	1.367	0.875	0.798	0.740	81	52	21	17	13	1.13E-7	> 1.00E-4	> 1.00E-4
CAKI-1	0.473	2.045	1.474	0.735	0.503	0.471	0.534	64	17	2	.	4	1.95E-8	.	> 1.00E-4
RXF 393	0.594	1.182	0.958	0.626	0.333	0.359	0.054	62	5	-44	-40	-91	1.62E-8	1.28E-7	1.59E-5
SN12C	0.702	2.406	2.031	1.502	0.937	0.976	0.836	78	47	14	16	8	7.96E-8	> 1.00E-4	> 1.00E-4
TK-10	0.922	2.195	1.896	1.472	0.877	0.710	0.501	76	43	-5	-23	-46	6.23E-8	7.91E-7	> 1.00E-4
UO-31	0.641	1.896	1.326	0.873	0.527	0.562	0.494	55	18	-18	-12	-23	1.34E-8	3.22E-7	> 1.00E-4
Prostate Cancer															
PC-3	0.718	1.967	1.494	1.431	0.659	0.690	0.623	62	57	-8	-4	-13	1.28E-7	7.48E-7	> 1.00E-4
DU-145	0.444	1.501	1.354	0.880	0.502	0.499	0.293	86	41	5	5	-34	6.38E-8	1.36E-5	> 1.00E-4
Breast Cancer															
MCF7	0.310	1.731	1.177	0.565	0.374	0.292	0.383	61	18	5	-6	4	1.80E-8	.	> 1.00E-4
MDA-MB-231/ATCC	0.653	1.437	1.241	0.803	0.583	0.655	0.657	75	19	-11	.	.	2.80E-8	.	> 1.00E-4
HS 578T	1.127	1.924	1.419	1.238	1.088	1.157	1.082	37	14	-4	4	-6	< 1.00E-8	.	> 1.00E-4
BT-549	0.977	2.183	1.659	1.445	0.548	0.671	0.570	57	39	-44	-31	-42	2.34E-8	2.94E-7	> 1.00E-4
T-47D	0.533	1.021	0.817	0.584	0.552	0.539	0.441	58	10	4	1	-17	1.48E-8	1.15E-5	> 1.00E-4
MDA-MB-468	0.768	1.665	1.294	0.751	0.281	0.387	0.455	59	-2	-63	-50	-41	1.39E-8	9.20E-8	.

Figure S32 National Cancer Institute developmental therapeutics program *in-vitro* testing results of compound **6b** at five dose level in μM.

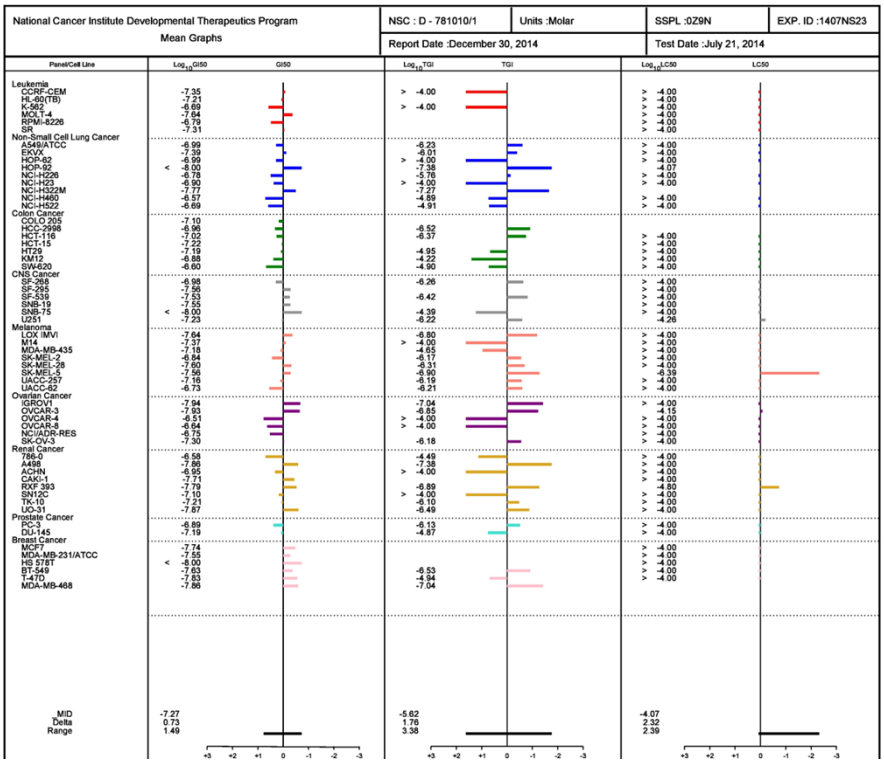

Figure S33 National Cancer Institute developmental therapeutics program *in-vitro* testing results of compound **6b** at five dose level in μM.

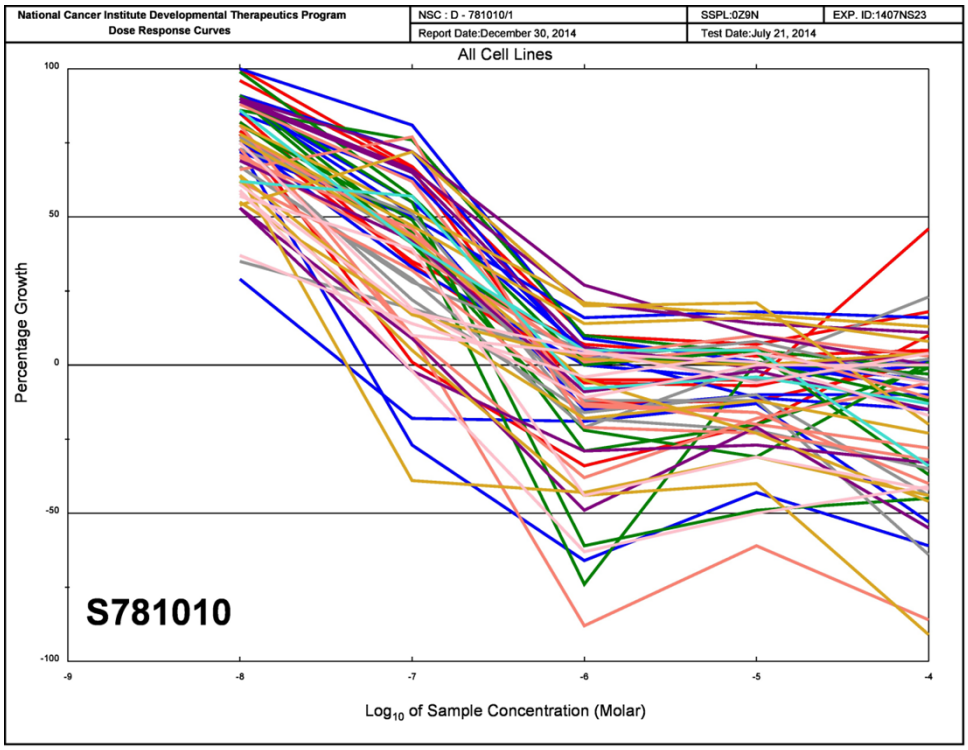

Figure S34 Five dose assay graph of compound **6b** against nine panel cancer cell line at NCI

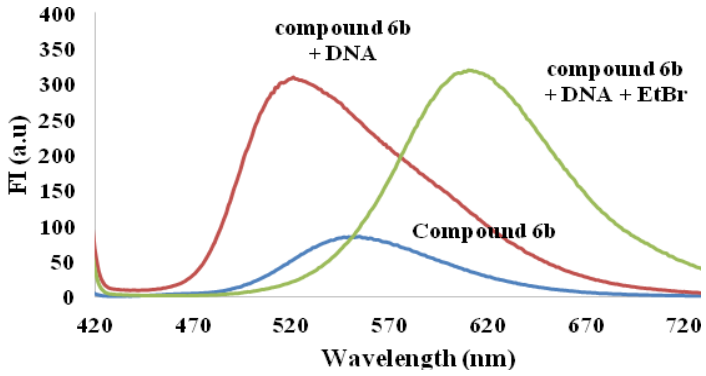

Figure S35 EtBr indicator displacement assay to show reversibility of compound **6b**:DNA binding

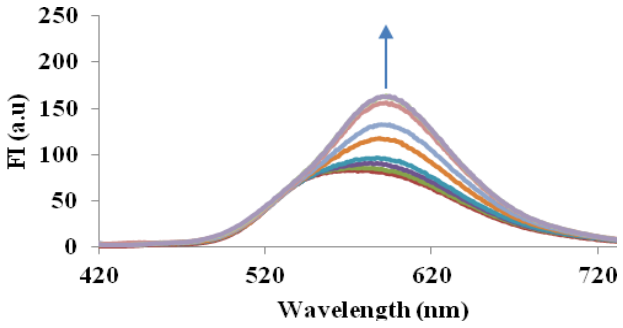

Figure S36 Effect of incremental addition of compound **6b** on EtBr:DNA complex

Molecular Modelling (Docking): Coordinates from the X-ray crystal structure of DNA (pdb ID 1BNA) were taken from the RCSB Protein Data Bank. Compounds were constructed with the builder toolkit of the software package ArgusLab 4.0.1 (www.arguslab.com) and energy minimized using the semiempirical quantum mechanical method PM3. The DNA structure was chosen, and the active site was defined around the ligand. The molecule to be docked in the DNA was inserted into the work space carrying the structure of the enzyme. The docking program implements an efficient grid-based docking algorithm, which approximates an exhaustive search within the free volume of the binding site cavity. The conformational space was surveyed by the geometry optimization of the flexible ligand (rings are treated as rigid) in combination with the incremental construction of the ligand torsions. Thus, docking occurred between the flexible ligand parts of the compound and DNA. The ligand orientation was determined by a shape scoring function based on Ascore and the final positions were ranked by lowest interaction energy values. Van der Waal's and hydrophobic interactions between the compound and DNA were explored.