Supporting materials

1. Spectroscopic data of target compounds
2. Biological data of target compounds and methods

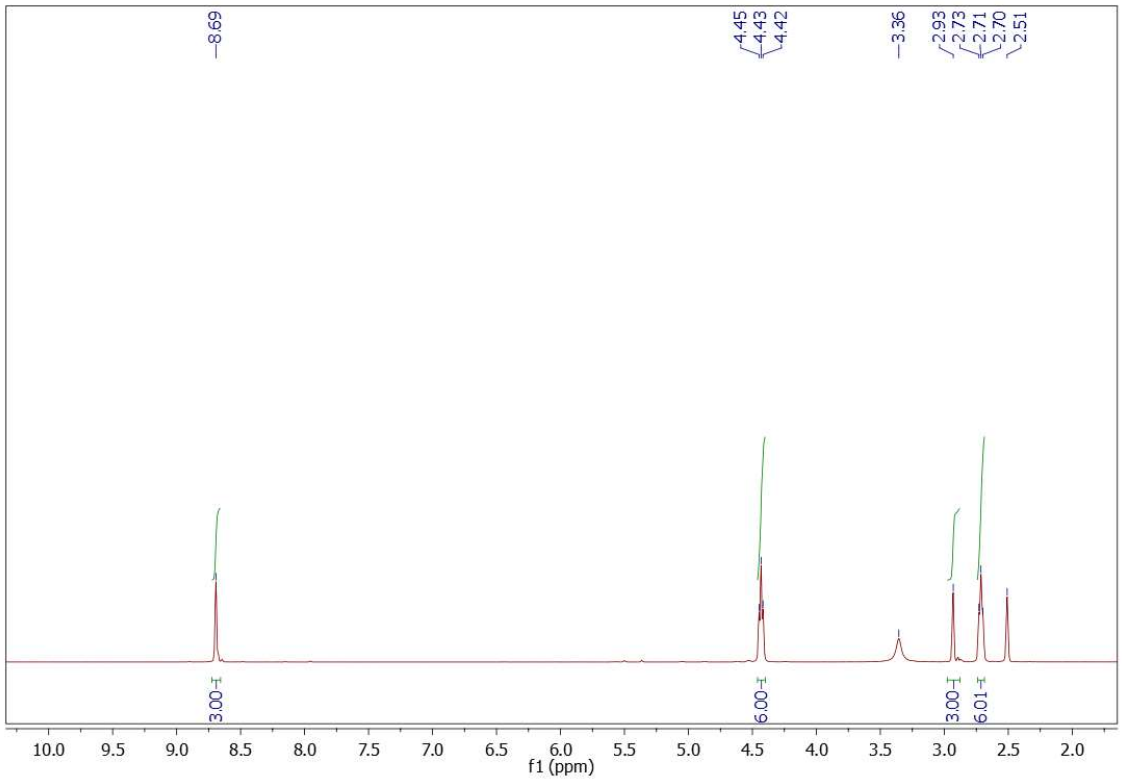


Figure S1: ^{1}H NMR spectrum of compound **2**

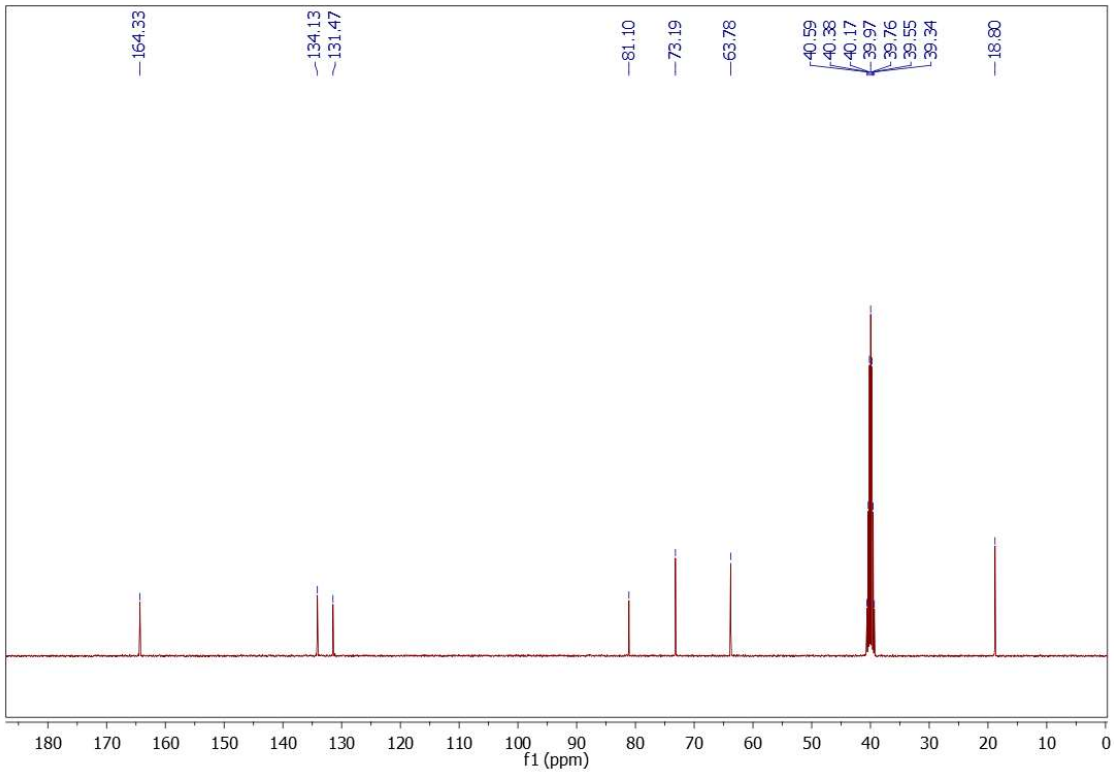


Figure S2: ^{13}C NMR spectrum of compound **2**

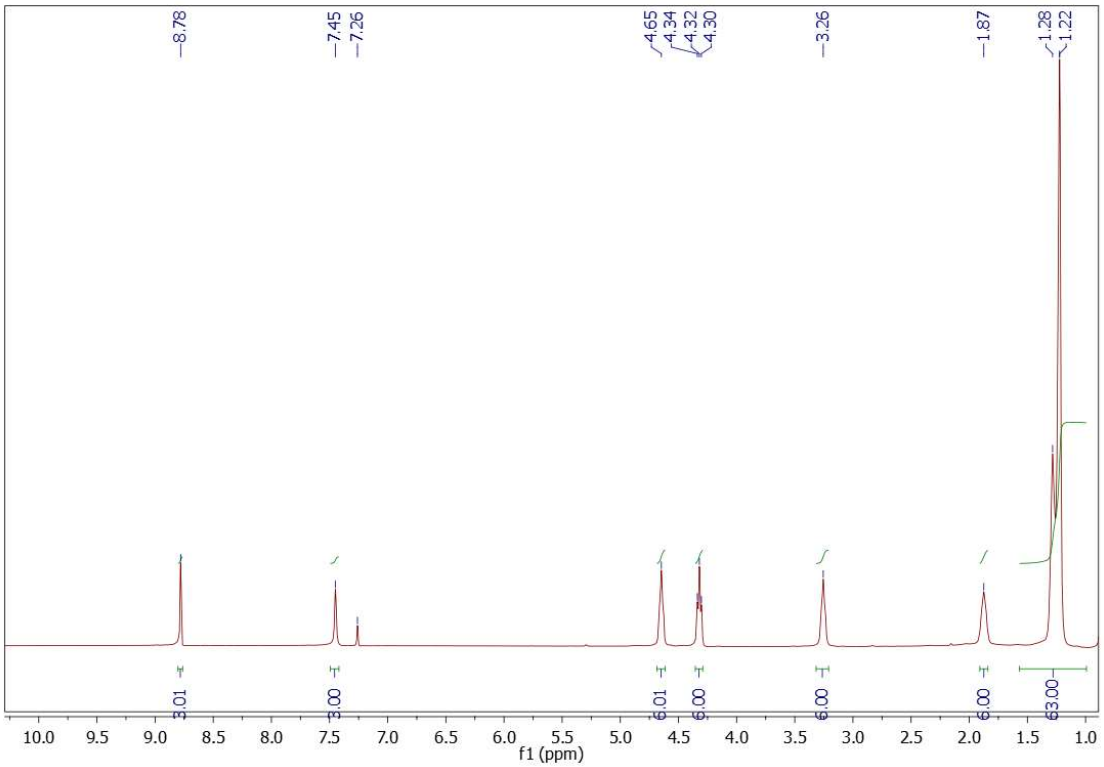


Figure S3: ^{1}H NMR spectrum of compound **4a**

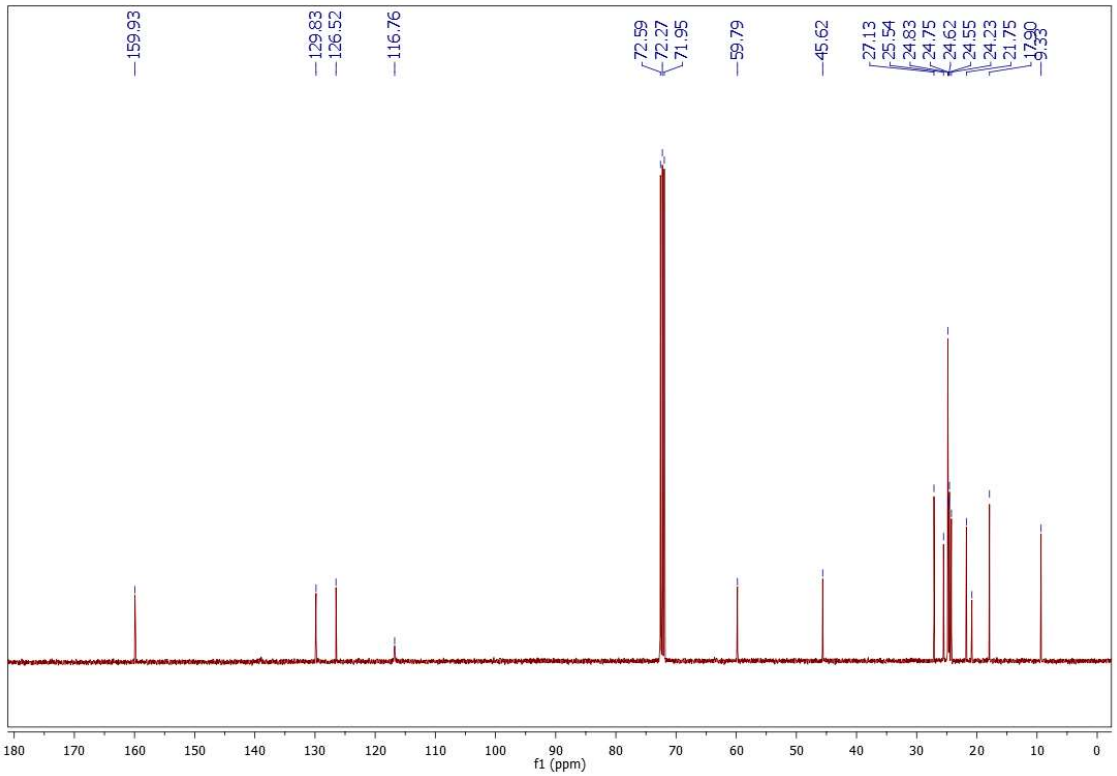


Figure S4: ^{13}C NMR spectrum of compound **4a**

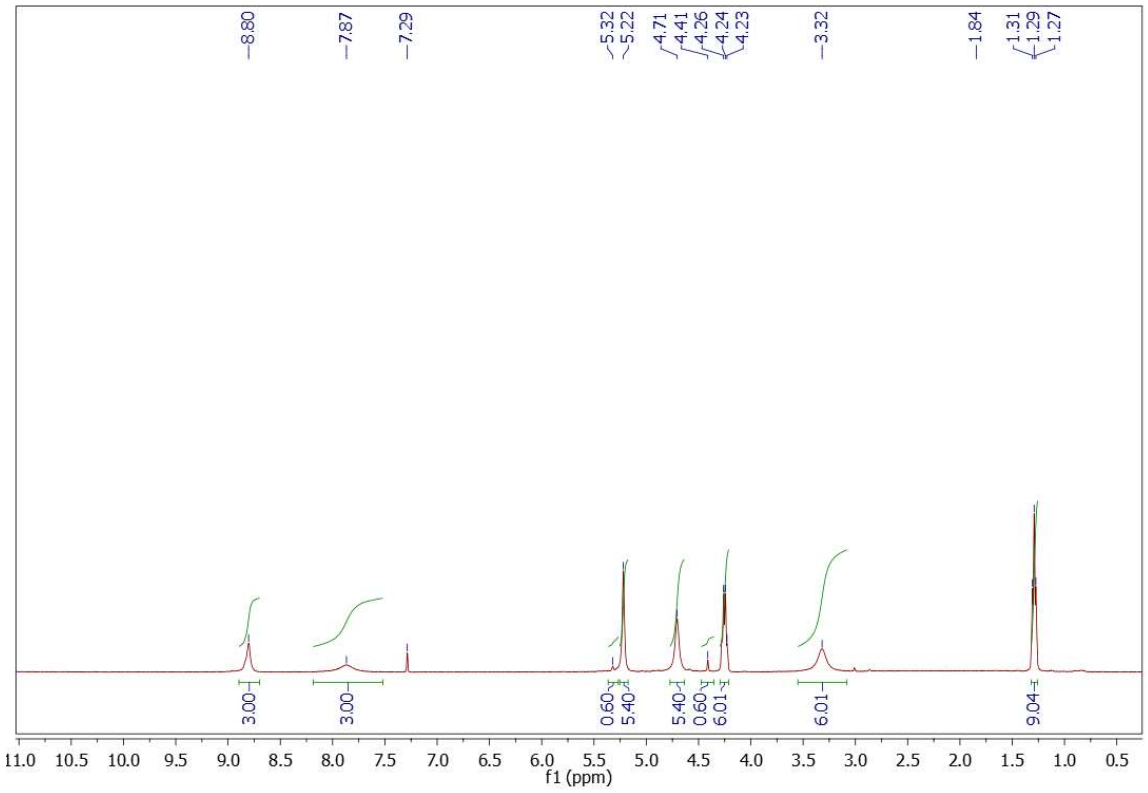


Figure S5: ^{1}H NMR spectrum of compound **4b**

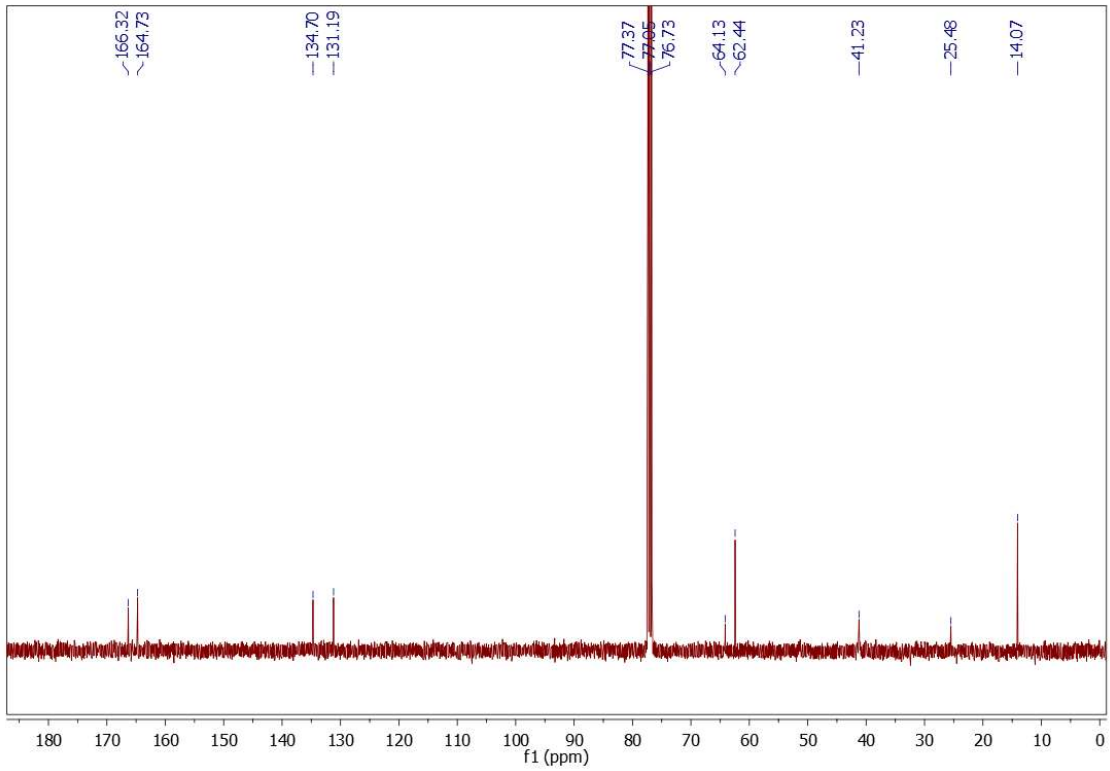


Figure S6: ^{13}C NMR spectrum of compound **4b**

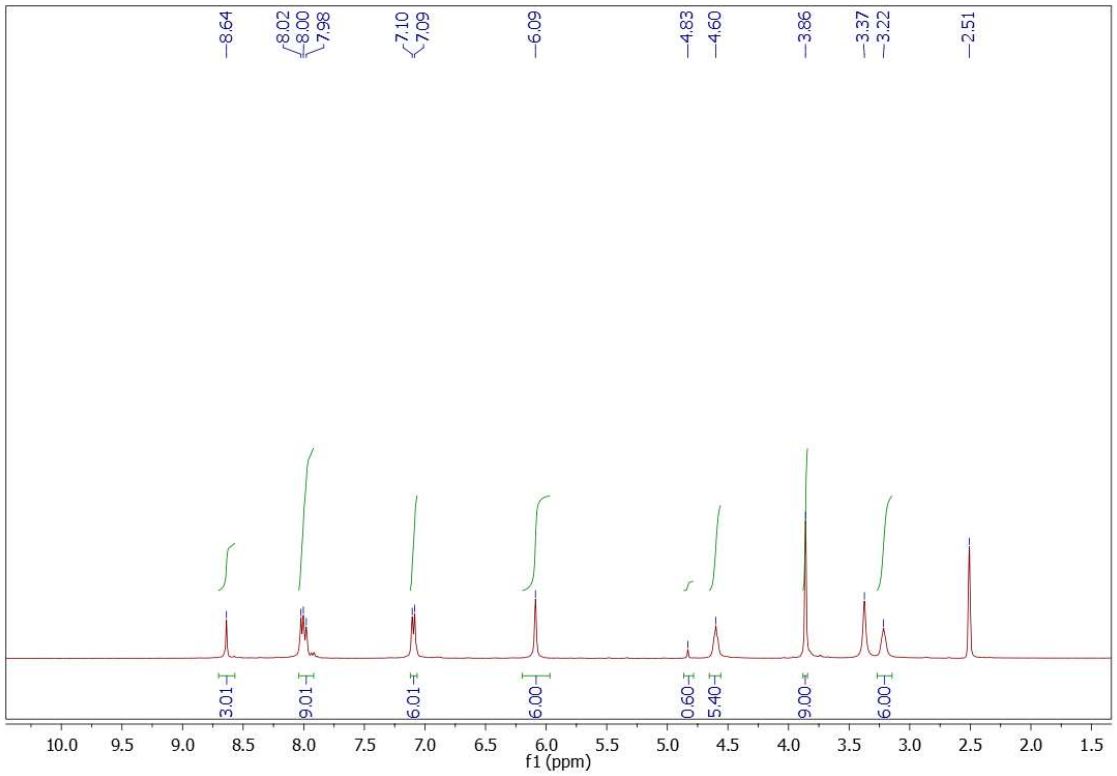


Figure S7: ^{1}H NMR spectrum of compound **4c**

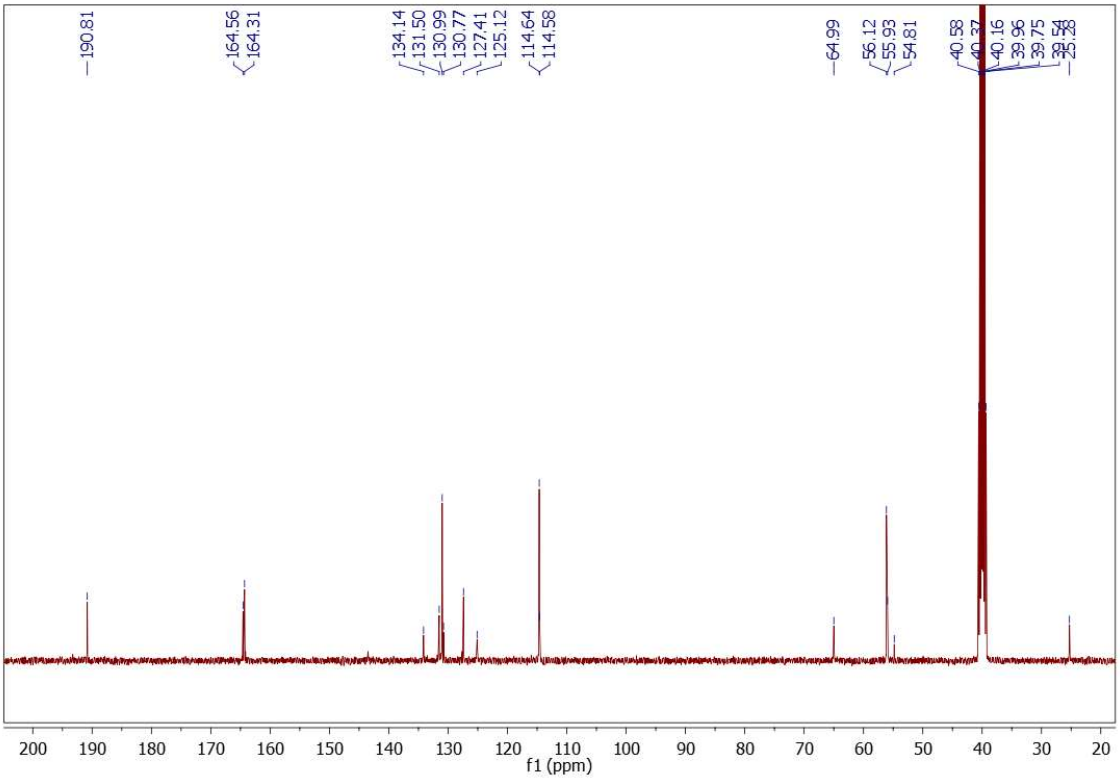


Figure S8: ^{13}C NMR spectrum of compound **4c**

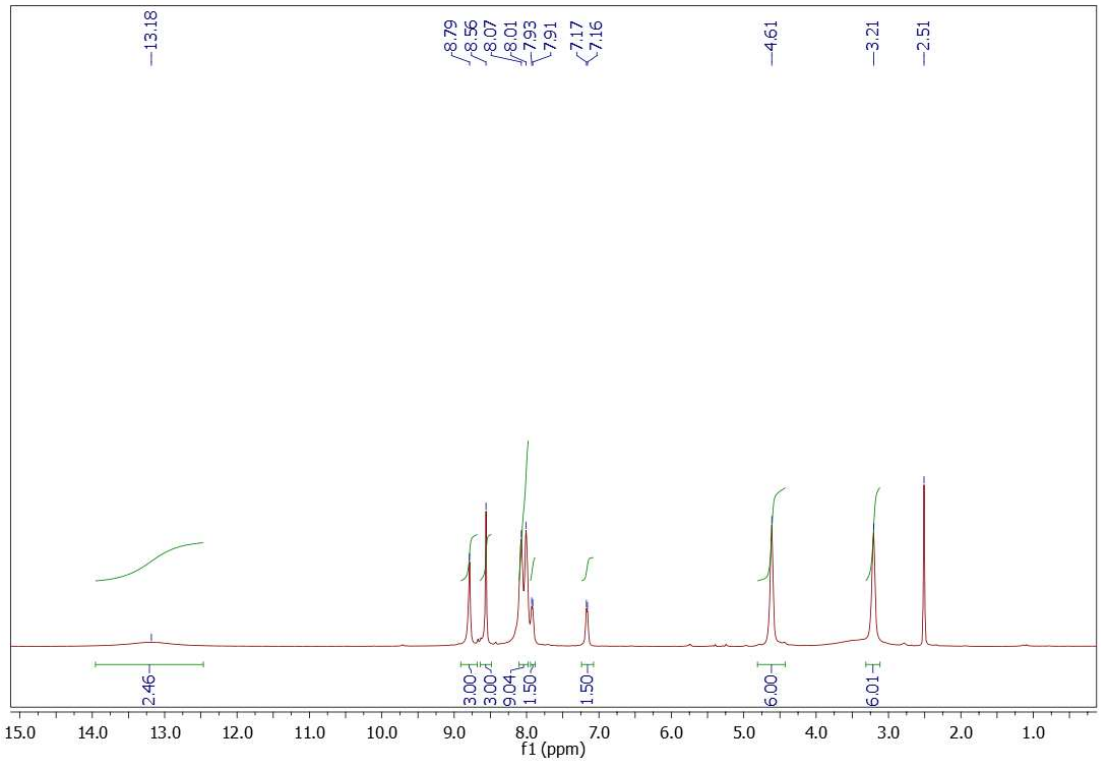


Figure S9: ^{1}H NMR spectrum of compound **6c**

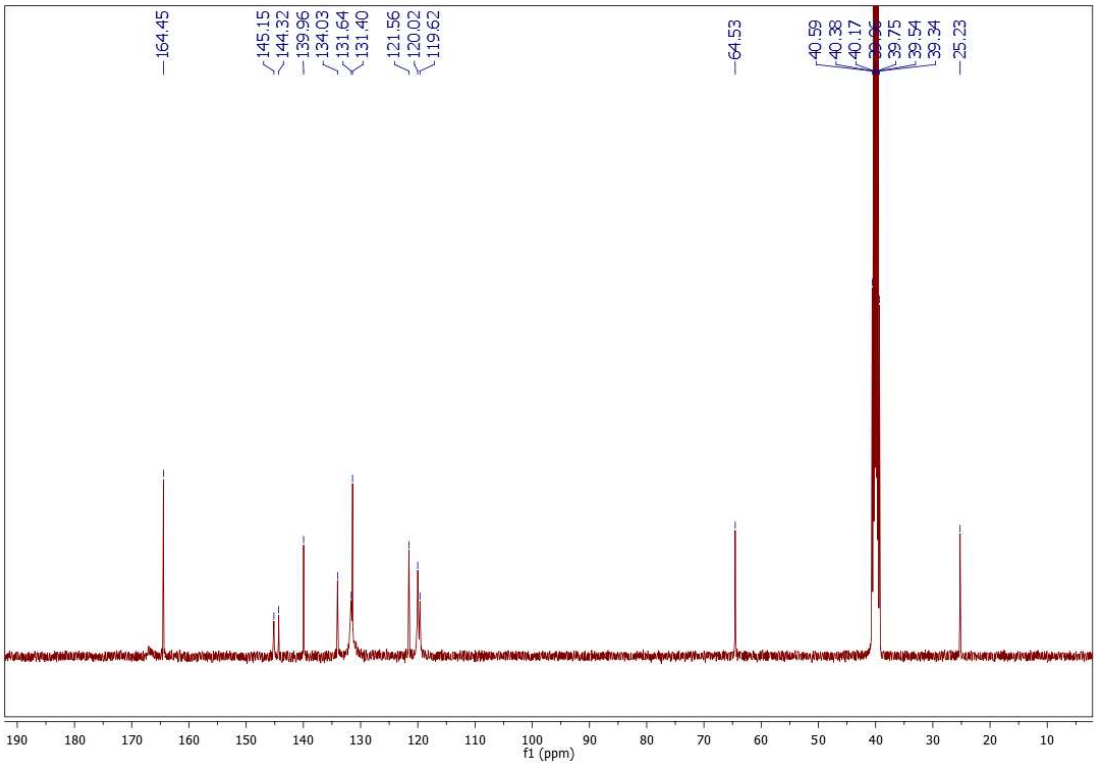


Figure S10: ^{13}C NMR spectrum of compound **6c**

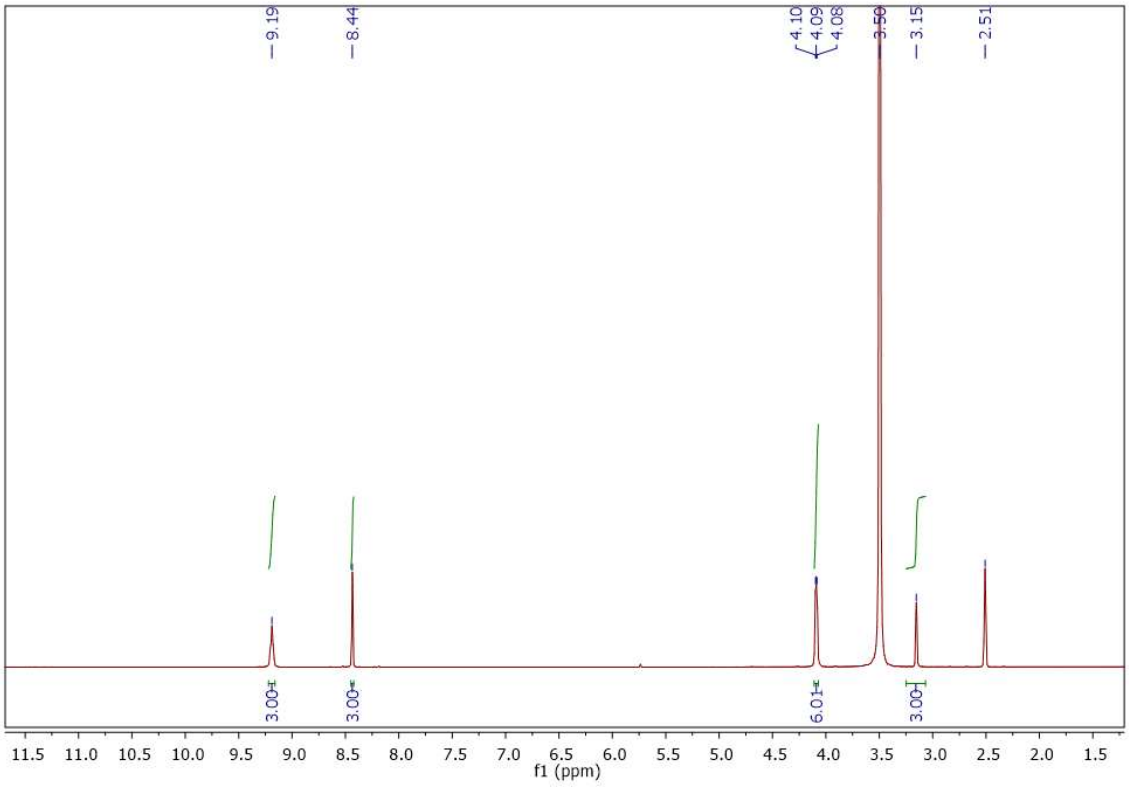


Figure S11: ^{1}H NMR spectrum of compound **7**

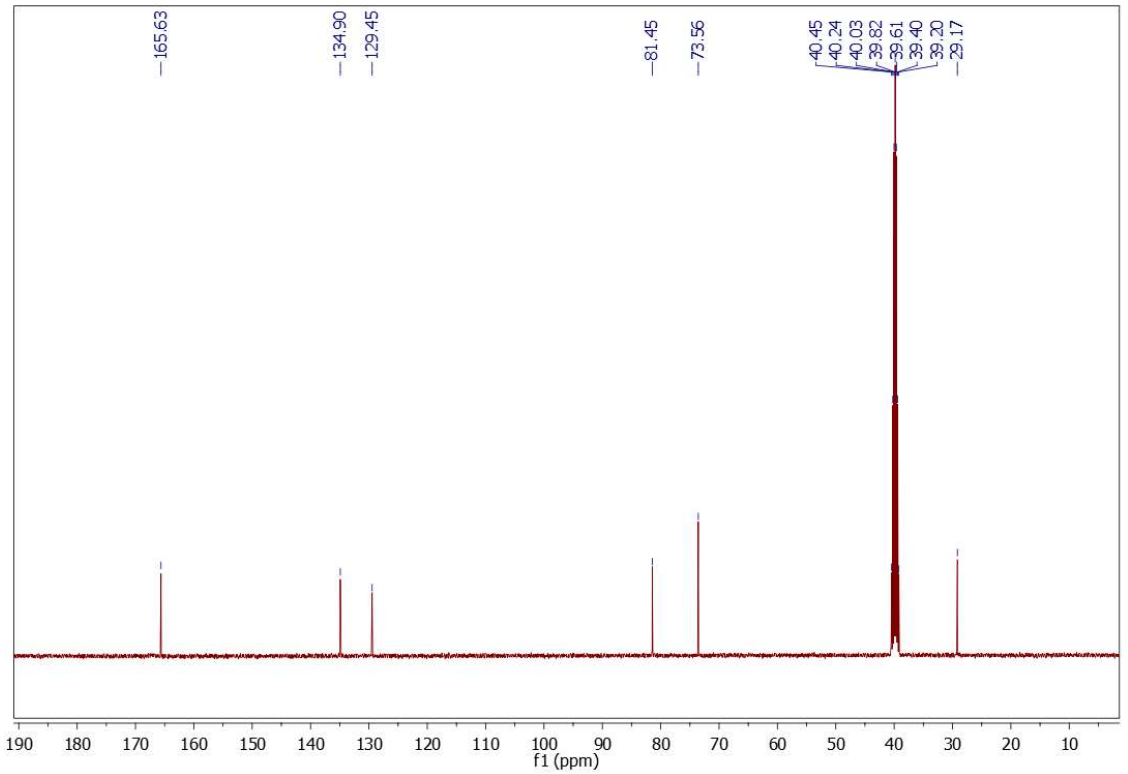


Figure S12: ^{13}C NMR spectrum of compound **7**

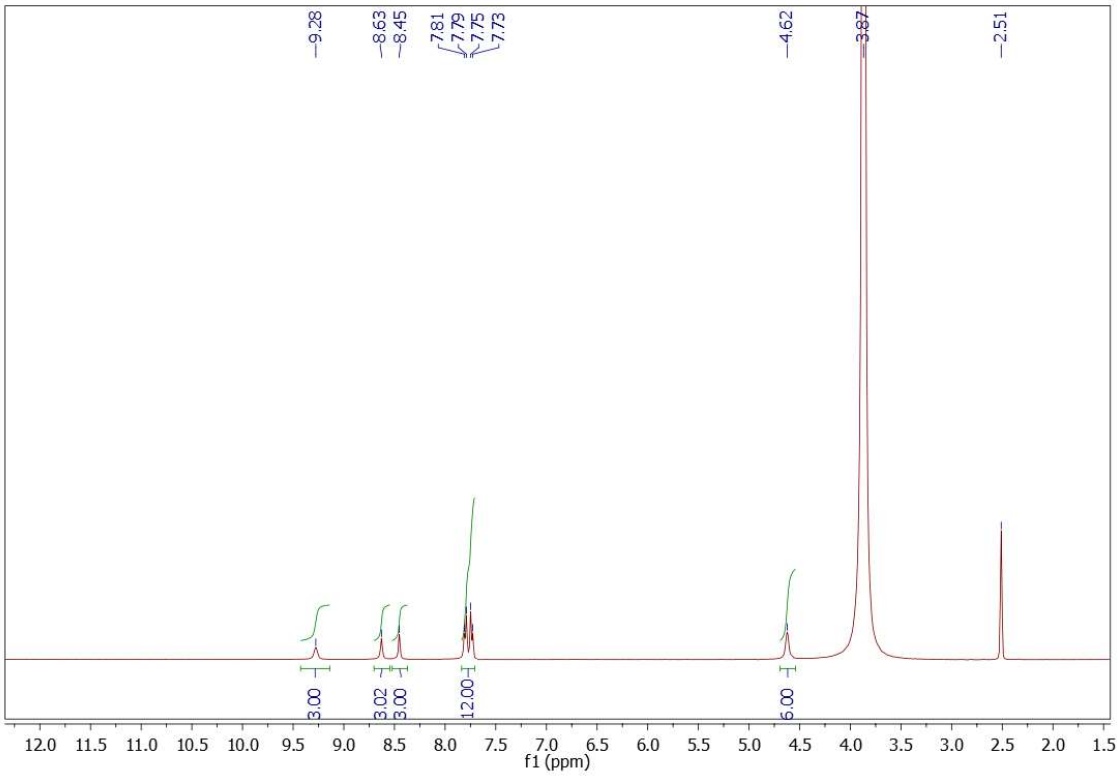


Figure S13: ^{1}H NMR spectrum of compound **8b**

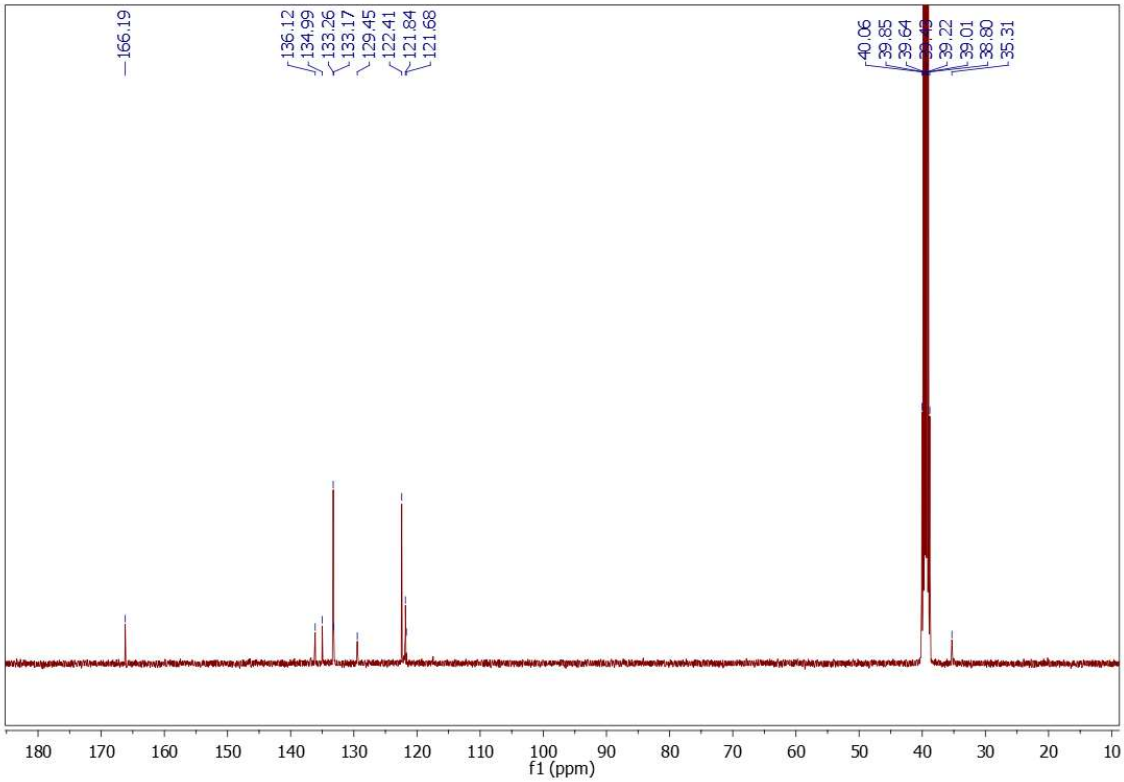


Figure S14: ^{13}C NMR spectrum of compound **8b**

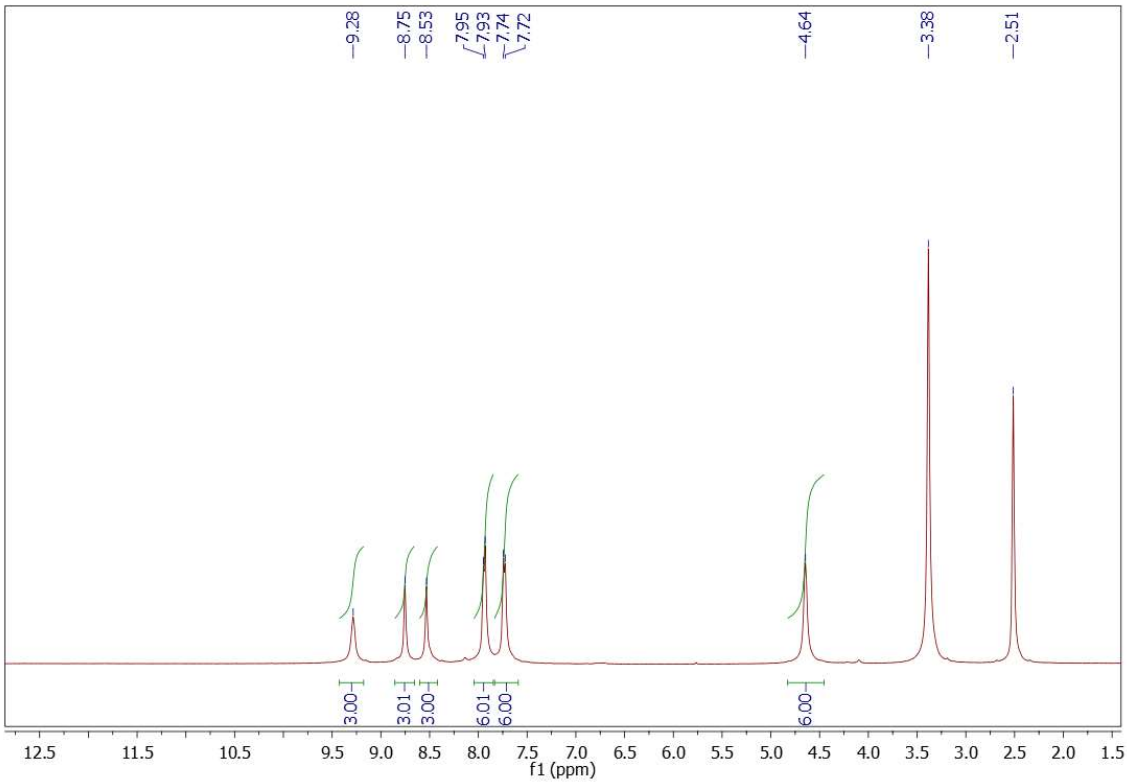


Figure S15: ^{1}H NMR spectrum of compound **8d**

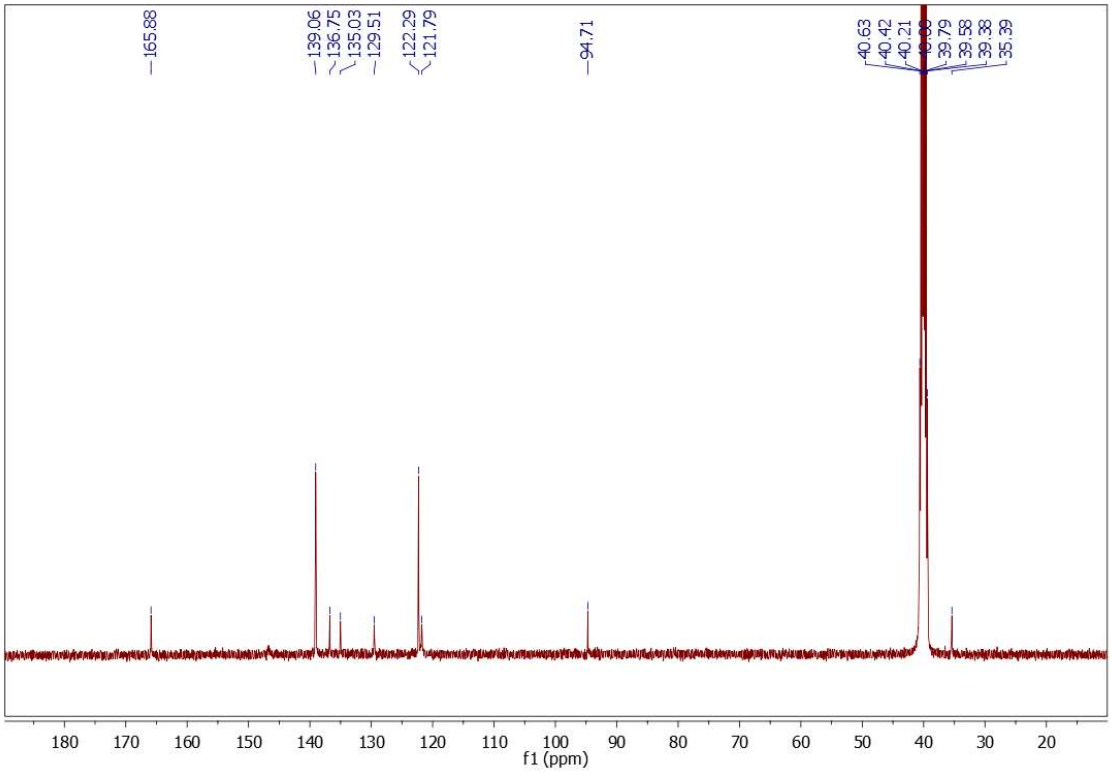


Figure S16: ^{13}C NMR spectrum of compound **8d**

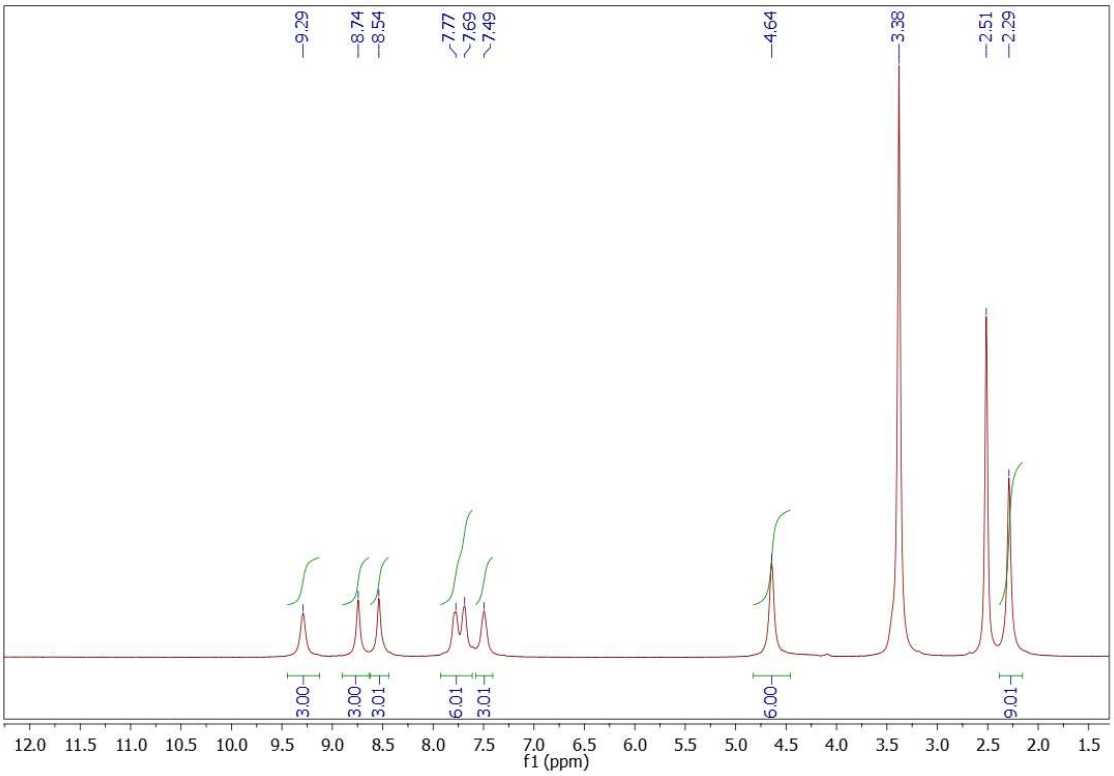


Figure S17: ^{1}H NMR spectrum of compound **8e**

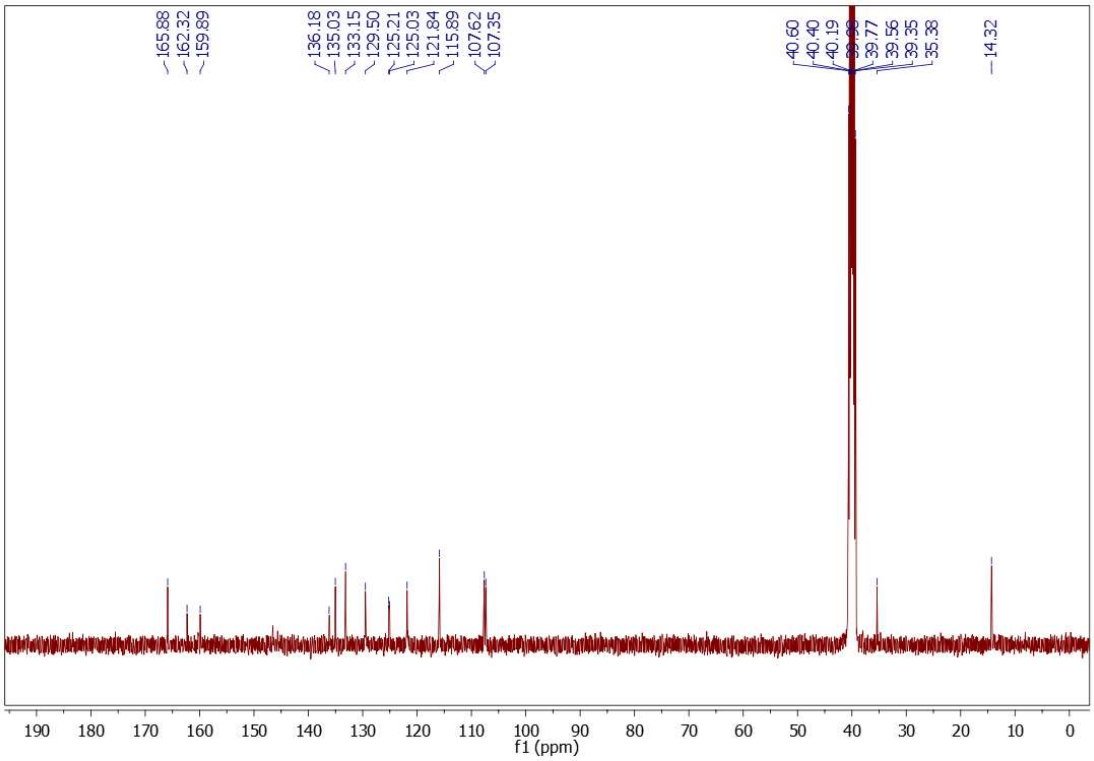


Figure S18: ^{13}C NMR spectrum of compound **8e**

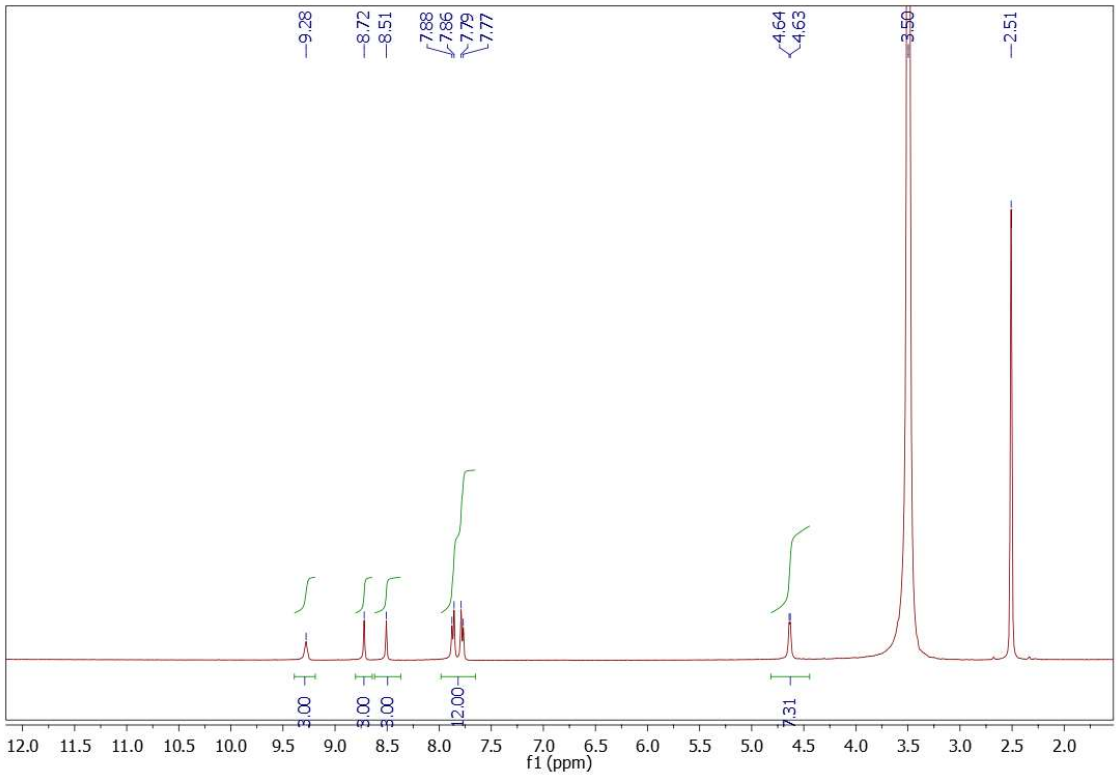


Figure S19: ^{1}H NMR spectrum of compound **8f**

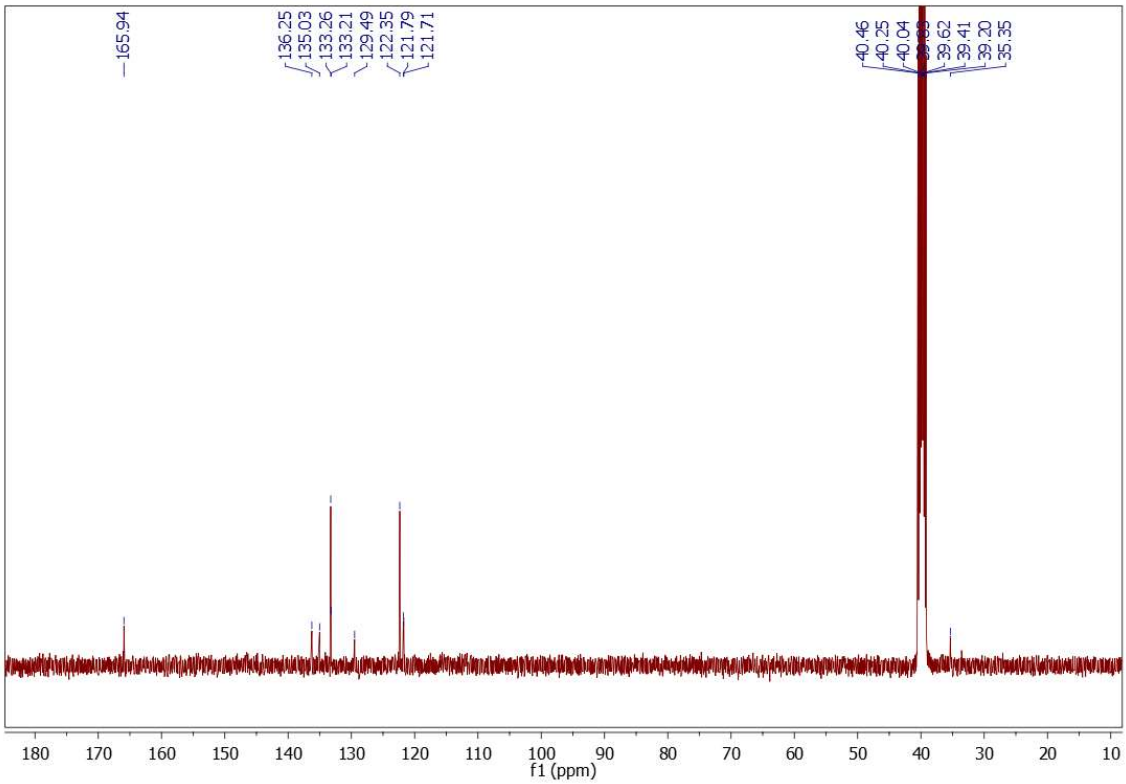


Figure S20: ^{13}C NMR spectrum of compound **8f**

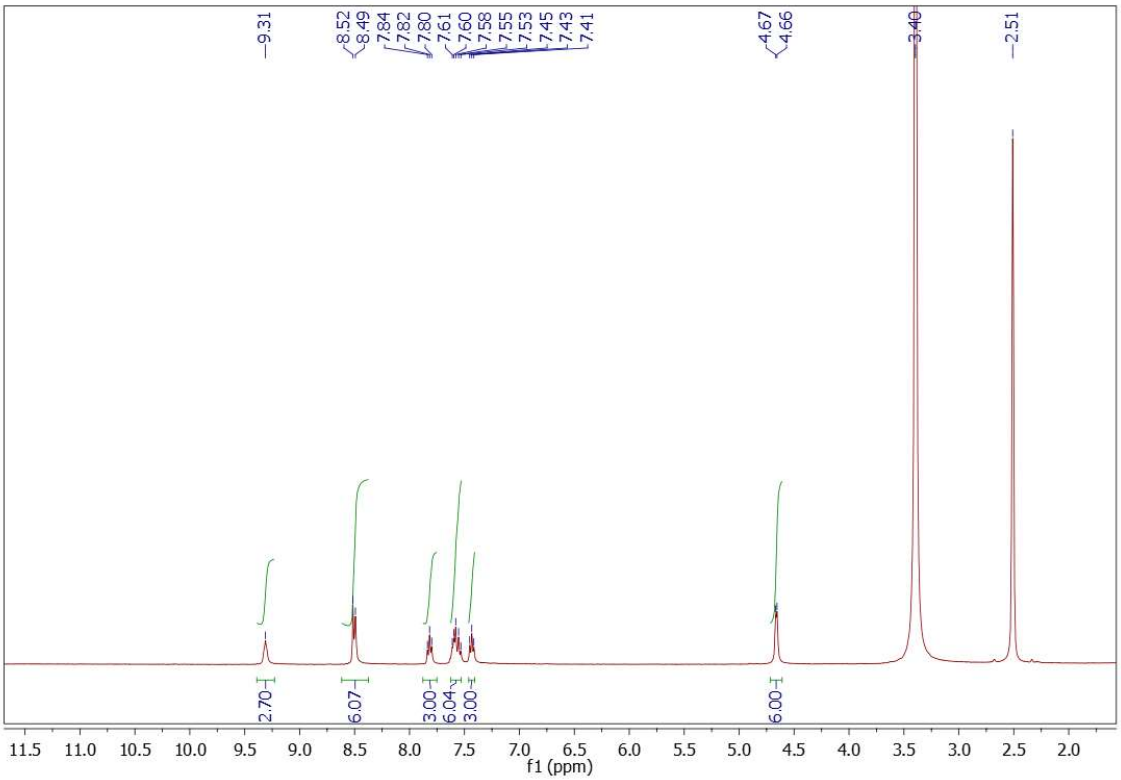


Figure S21: ^{1}H NMR spectrum of compound **8g**

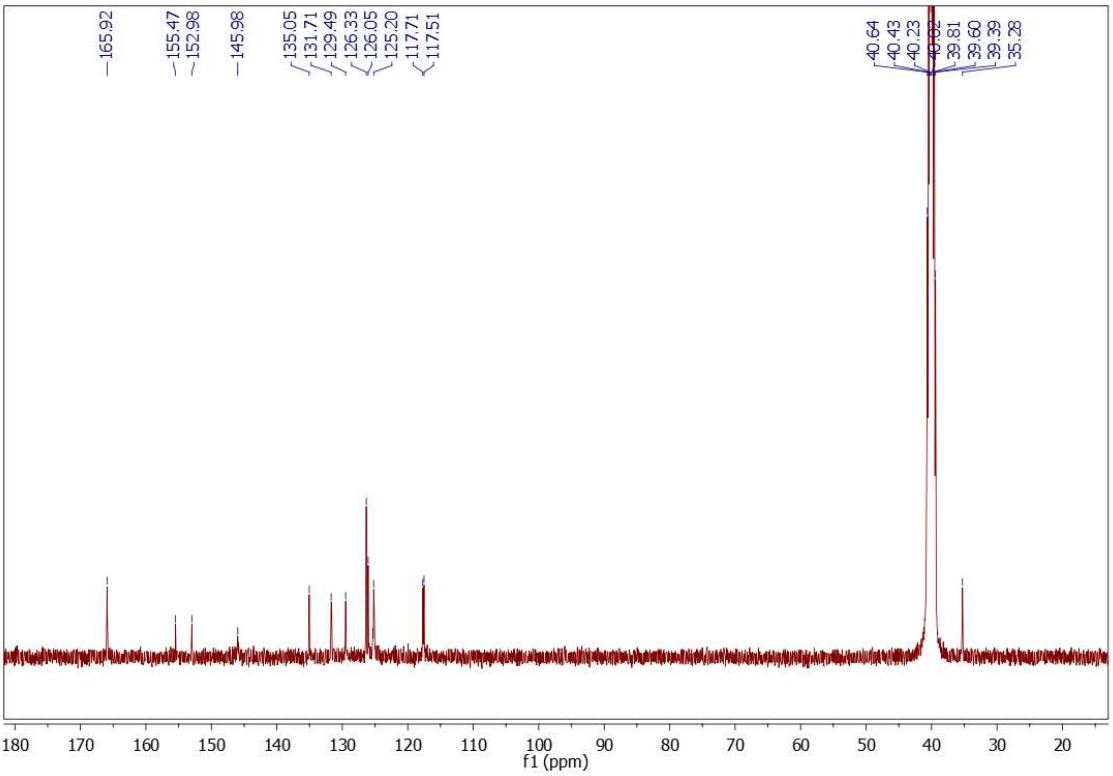


Figure S22: ^{13}C NMR spectrum of compound **8g**

Viability assay

Institute / Researcher: Prof. Dr. Hany

Experiment: **functional assay (MTT)**

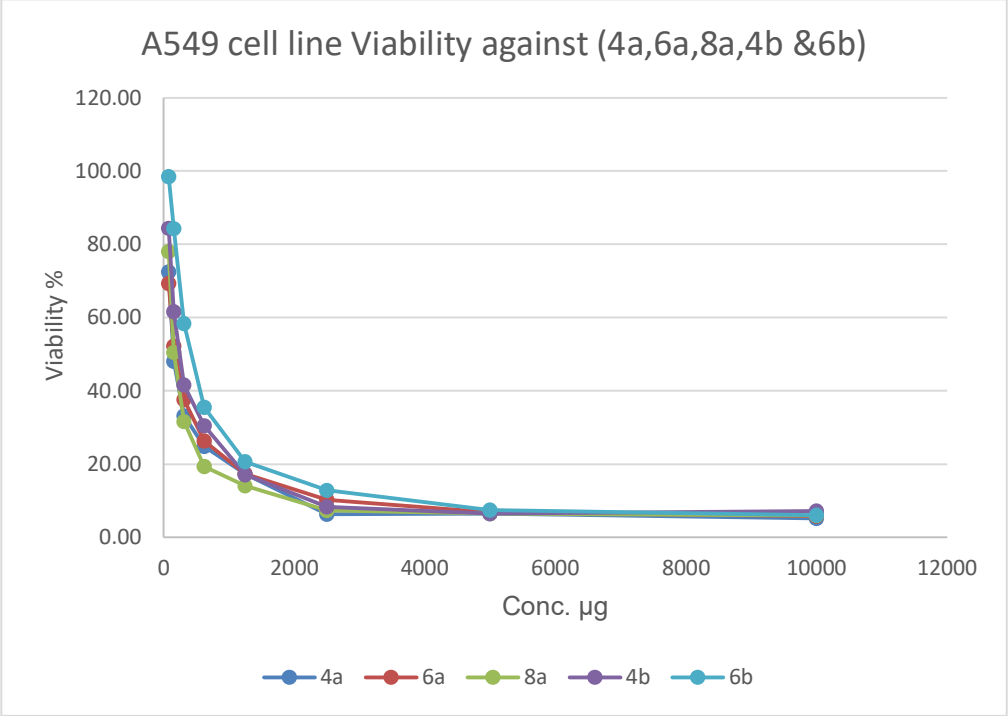
(Viability/cytotoxicity)

Samples number: **16**

Experiment design: viability against A549 cells

Laboratory comments:

ID	Conc. umol	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
A549	dilution	0.621	0.612	0.609	0.614	0.003606	100	0	
4a	25	0.034	0.031	0.030	0.031667	0.001202	5.157437568	94.842562432	**0.1499**
	12.5	0.047	0.040	0.032	0.039667	0.004333	6.460369164	93.539630836	
	6.25	0.050	0.034	0.032	0.038667	0.005696	6.297502714	93.702497286	
	3.125	0.108	0.104	0.107	0.106333	0.001202	17.318132465	82.681867535	
	1.563	0.148	0.152	0.157	0.152333	0.002603	24.809989142	75.190010858	
	0.781	0.198	0.211	0.202	0.203667	0.003844	33.170466884	66.829533116	
	0.391	0.292	0.289	0.304	0.295	0.004583	48.045602606	51.954397394	
	0.195	0.451	0.444	0.440	0.445	0.003215	72.475570033	27.524429967	
6a	25	0.033	0.041	0.032	0.035333	0.002848	5.754614549	94.245385451	**0.209**
	12.5	0.041	0.039	0.046	0.042	0.002082	6.840390879	93.159609121	
	6.25	0.063	0.069	0.057	0.063	0.003464	10.260586319	89.739413681	
	3.125	0.104	0.101	0.114	0.106333	0.00393	17.318132465	82.681867535	
	1.563	0.161	0.164	0.160	0.161667	0.001202	26.330076004	73.669923996	
	0.781	0.225	0.230	0.237	0.230667	0.00348	37.567861021	62.432138979	
	0.391	0.324	0.315	0.322	0.320333	0.002728	52.171552660	47.828447340	
	0.195	0.421	0.427	0.430	0.426	0.002646	69.381107492	30.618892508	
8a	25	0.032	0.037	0.041	0.036667	0.002603	5.971769815	94.028230185	**0.1964**
	12.5	0.033	0.040	0.047	0.040	0.004041	6.514657980	93.485342020	
	6.25	0.044	0.039	0.052	0.045	0.003786	7.328990228	92.671009772	
	3.125	0.084	0.090	0.085	0.086333	0.001856	14.060803474	85.939196526	
	1.563	0.120	0.115	0.121	0.118667	0.001856	19.326818675	80.673181325	
	0.781	0.195	0.191	0.196	0.194	0.001528	31.596091205	68.403908795	
	0.391	0.305	0.310	0.314	0.309667	0.002603	50.434310532	49.565689468	
	0.195	0.481	0.479	0.478	0.479333	0.000882	78.067318132	21.932681868	
4b	25	0.045	0.048	0.039	0.044	0.002646	7.166123779	92.833876221	**0.3099**
	12.5	0.040	0.038	0.041	0.039667	0.000882	6.460369164	93.539630836	
	6.25	0.047	0.052	0.054	0.051	0.002082	8.306188925	91.693811075	
	3.125	0.103	0.104	0.108	0.105	0.001528	17.100977199	82.899022801	
	1.563	0.183	0.190	0.189	0.187333	0.002186	30.510314875	69.489685125	
	0.781	0.262	0.249	0.255	0.255333	0.003756	41.585233442	58.414766558	
	0.391	0.384	0.379	0.371	0.378	0.003786	61.563517915	38.436482085	
	0.195	0.519	0.514	0.522	0.518333	0.002333	84.419109663	15.580890337	
6b	25	0.039	0.040	0.033	0.037333	0.002186	6.080347448	93.919652552	**0.517**
	12.5	0.043	0.044	0.050	0.045667	0.002186	7.437567861	92.562432139	
	6.25	0.084	0.081	0.072	0.079	0.003606	12.866449511	87.133550489	
	3.125	0.127	0.132	0.122	0.127	0.002887	20.684039088	79.315960912	
	1.563	0.215	0.212	0.227	0.218	0.004583	35.504885993	64.495114007	
	0.781	0.352	0.355	0.369	0.358667	0.005239	58.414766558	41.585233442	
	0.391	0.523	0.512	0.517	0.517333	0.00318	84.256243214	15.743756786	
	0.195	0.601	0.609	0.604	0.604667	0.002333	98.479913138	1.520086862	


A549 cell line Viability against (4a,6a,8a,4b &6b)
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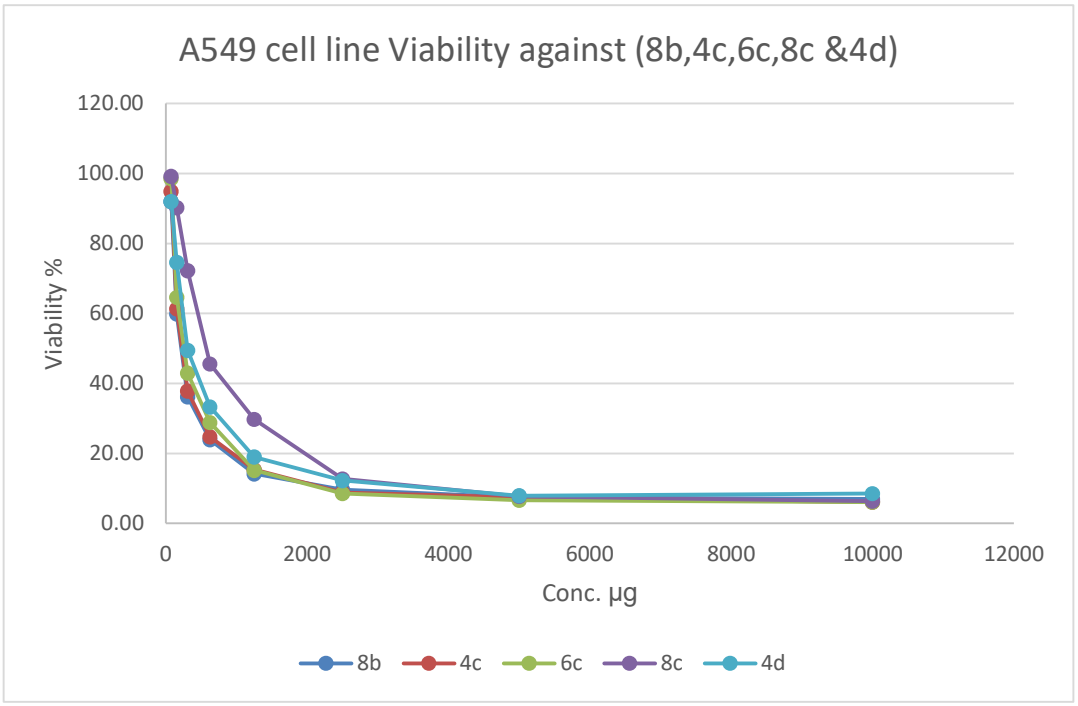
100.00
80.00
60.00
40.00
20.00
0.00
Viability %
0
2000
4000
6000
8000
10000
12000
Conc. µg
4a
6a
8a
4b
6b

ID	Conc. umol	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
A549	dilution	0.621	0.612	0.609	0.614	0.003606	100	0	
8b	25	0.044	0.031	0.053	0.042667	0.006386	6.948968512	93.051031488	**0.2830**
	12.5	0.058	0.037	0.042	0.045667	0.006333	7.437567861	92.562432139	
	6.25	0.064	0.052	0.061	0.059	0.003606	9.609120521	90.390879479	
	3.125	0.087	0.083	0.091	0.087	0.002309	14.169381107	85.830618893	
	1.563	0.140	0.154	0.146	0.146667	0.004055	23.887079262	76.112920738	
	0.781	0.225	0.222	0.219	0.222	0.001732	36.156351792	63.843648208	
	0.391	0.368	0.361	0.374	0.367667	0.003756	59.880564604	40.119435396	
	0.195	0.561	0.568	0.566	0.565	0.002082	92.019543974	7.980456026	
4c	25	0.033	0.031	0.047	0.037	0.005033	6.026058632	93.973941368	**0.2462**
	12.5	0.041	0.056	0.040	0.045667	0.005175	7.437567861	92.562432139	
	6.25	0.054	0.048	0.060	0.054	0.003464	8.794788274	91.205211726	
	3.125	0.101	0.093	0.089	0.094333	0.003528	15.363735071	84.636264929	
	1.563	0.148	0.150	0.157	0.151667	0.002728	24.701411509	75.298588491	
	0.781	0.232	0.236	0.229	0.232333	0.002028	37.839305103	62.160694897	
	0.391	0.373	0.380	0.376	0.376333	0.002028	61.292073833	38.707926167	
	0.195	0.588	0.579	0.582	0.583	0.002646	94.951140065	5.048859935	
6c	25	0.038	0.030	0.043	0.037	0.003786	6.026058632	93.973941368	**0.305**
	12.5	0.036	0.047	0.039	0.040667	0.003283	6.623235613	93.376764387	
	6.25	0.062	0.045	0.050	0.052333	0.005044	8.523344191	91.476655809	
	3.125	0.090	0.107	0.082	0.093	0.007371	15.146579805	84.853420195	
	1.563	0.178	0.175	0.178	0.177	0.001	28.827361564	71.172638436	
	0.781	0.263	0.262	0.266	0.263667	0.001202	42.942453855	57.057546145	
	0.391	0.397	0.400	0.392	0.396333	0.002333	64.549402823	35.450597177	
	0.195	0.602	0.609	0.604	0.605	0.002082	98.534201954	1.465798046	
8c	25	0.034	0.040	0.042	0.038667	0.002404	6.297502714	93.702497286	**0.7066**
	12.5	0.043	0.042	0.057	0.047333	0.004842	7.709011944	92.290988056	
	6.25	0.073	0.078	0.083	0.078	0.002887	12.703583062	87.296416938	
	3.125	0.179	0.183	0.186	0.182667	0.002028	29.750271444	70.249728556	
	1.563	0.287	0.275	0.277	0.279667	0.003712	45.548317047	54.451682953	
	0.781	0.442	0.447	0.442	0.443667	0.001667	72.258414767	27.741585233	
	0.391	0.543	0.552	0.568	0.554333	0.007311	90.282301846	9.717698154	
	0.195	0.608	0.602	0.618	0.609333	0.004667	99.239956569	0.760043431	
4d	25	0.056	0.050	0.050	0.052	0.002	8.469055375	91.530944625	**0.3243**
	12.5	0.053	0.048	0.044	0.048333	0.002603	7.871878393	92.128121607	
	6.25	0.077	0.064	0.084	0.075	0.005859	12.214983713	87.785016287	
	3.125	0.125	0.113	0.111	0.116333	0.004372	18.946796960	81.053203040	
	1.563	0.202	0.204	0.207	0.204333	0.001453	33.279044517	66.720955483	
	0.781	0.302	0.308	0.301	0.303667	0.002186	49.457111835	50.542888165	
	0.391	0.455	0.462	0.458	0.458333	0.002028	74.647122693	25.352877307	
	0.195	0.564	0.568	0.563	0.565	0.001528	92.019543974	7.980456026	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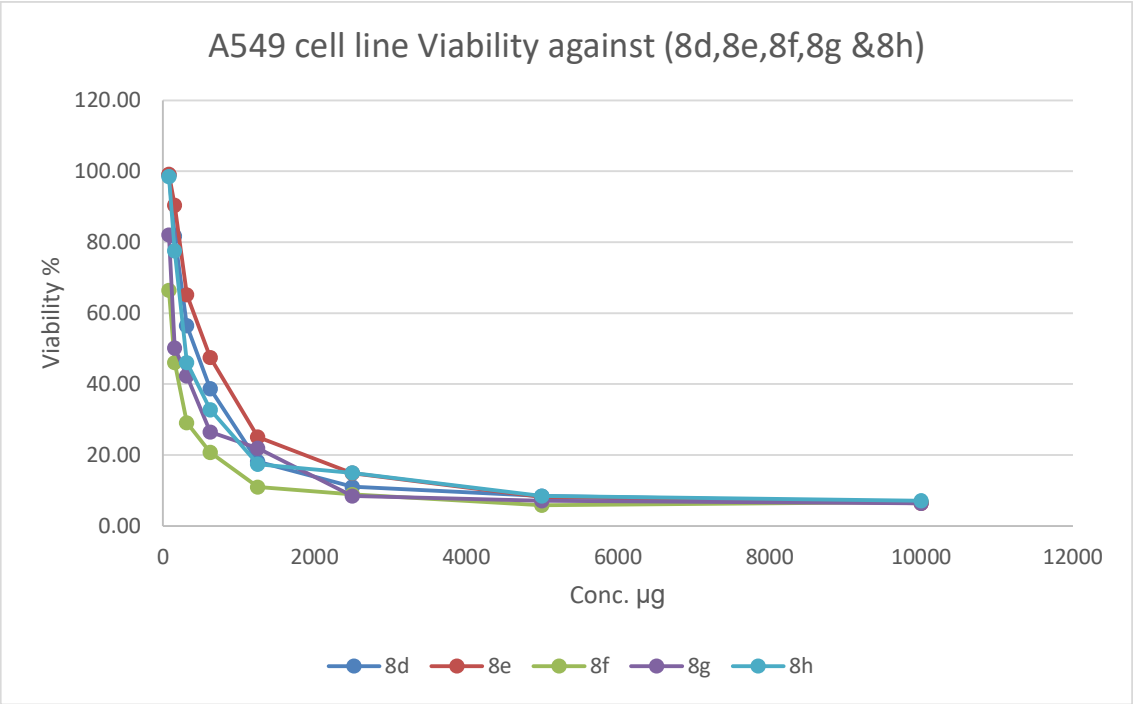
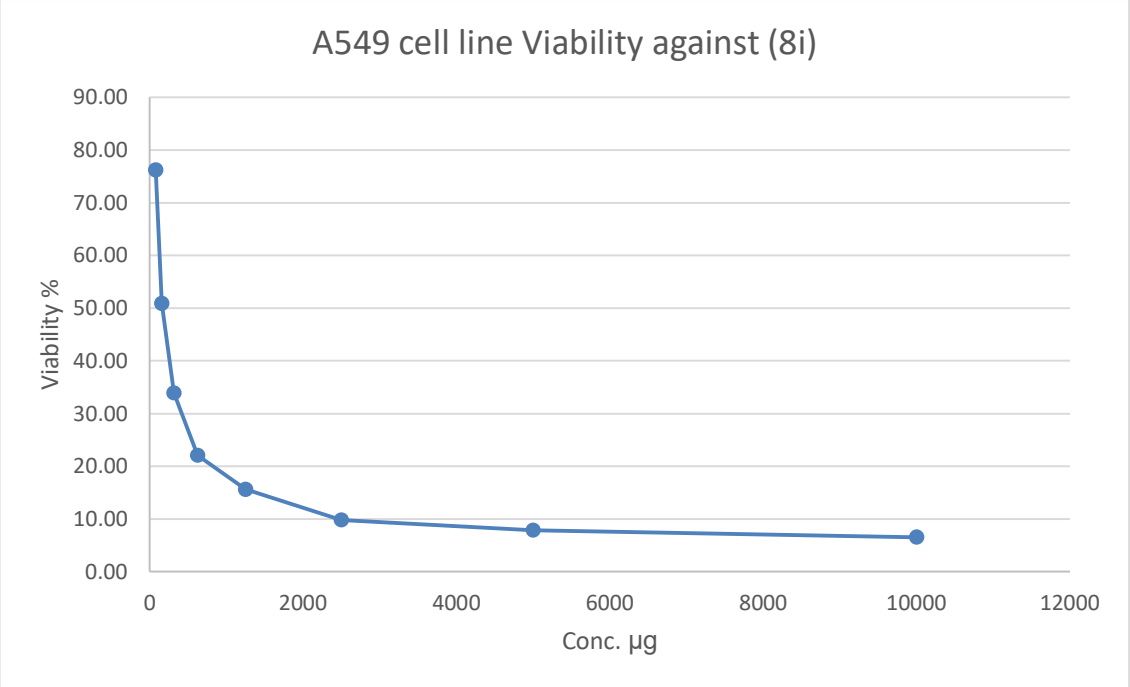
60.00
40.00
20.00
0.00
Viability %
0
2000
4000
6000
8000
10000
12000
Conc. µg
8b
4c
6c
8c
4d

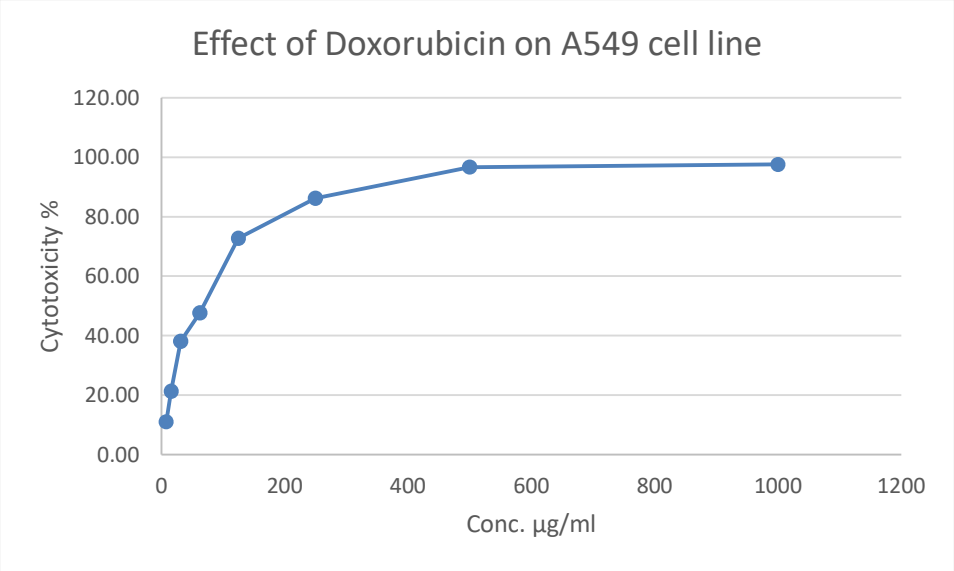
ID	Conc. umol	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
HepG2	dilution	0.621	0.612	0.609	0.614	0.003606	100	0	
8d	25	0.044	0.041	0.037	0.040667	0.002028	6.623235613	93.376764387	**0.4031**
	12.5	0.058	0.048	0.05	0.052	0.003055	8.469055375	91.530944625	
	6.25	0.075	0.06	0.068	0.067667	0.004333	11.020629750	88.979370250	
	3.125	0.112	0.113	0.109	0.111333	0.001202	18.132464712	81.867535288	
	1.563	0.237	0.231	0.244	0.237333	0.003756	38.653637351	61.346362649	
	0.781	0.34	0.343	0.357	0.346667	0.005239	56.460369164	43.539630836	
	0.391	0.501	0.504	0.5	0.501667	0.001202	81.704668838	18.295331162	
	0.195	0.605	0.617	0.601	0.607667	0.004807	98.968512486	1.031487514	
8e	25	0.031	0.045	0.042	0.039333	0.004256	6.406080347	93.593919653	**0.7485**
	12.5	0.054	0.05	0.047	0.050333	0.002028	8.197611292	91.802388708	
	6.25	0.092	0.094	0.088	0.091333	0.001764	14.875135722	85.124864278	
	3.125	0.158	0.154	0.149	0.153667	0.002603	25.027144408	74.972855592	
	1.563	0.295	0.292	0.287	0.291333	0.002333	47.448425624	52.551574376	
	0.781	0.402	0.400	0.398	0.400	0.001155	65.146579805	34.853420195	
	0.391	0.557	0.553	0.555	0.555	0.001155	90.390879479	9.609120521	
	0.195	0.608	0.602	0.615	0.608333	0.003756	99.077090119	0.922909881	
8f	25	0.049	0.04	0.035	0.041333	0.004096	6.731813246	93.268186754	**0.1733**
	12.5	0.033	0.044	0.031	0.036	0.004041	5.863192182	94.136807818	
	6.25	0.06	0.058	0.046	0.054667	0.004372	8.903365907	91.096634093	
	3.125	0.067	0.073	0.061	0.067	0.003464	10.912052117	89.087947883	
	1.563	0.122	0.128	0.132	0.127333	0.002906	20.738327904	79.261672096	
	0.781	0.178	0.181	0.176	0.178333	0.001453	29.044516830	70.955483170	
	0.391	0.284	0.276	0.288	0.282667	0.003528	46.036916395	53.963083605	
	0.195	0.405	0.41	0.408	0.407667	0.001453	66.395222584	33.604777416	
8g	25	0.041	0.037	0.039	0.039	0.001155	6.351791531	93.648208469	**0.2176**
	12.5	0.033	0.054	0.044	0.043667	0.006064	7.111834962	92.888165038	
	6.25	0.048	0.05	0.058	0.052	0.003055	8.469055375	91.530944625	
	3.125	0.134	0.137	0.132	0.134333	0.001453	21.878393051	78.121606949	
	1.563	0.164	0.158	0.166	0.162667	0.002404	26.492942454	73.507057546	
	0.781	0.254	0.264	0.261	0.259667	0.002963	42.290988056	57.709011944	
	0.391	0.314	0.304	0.306	0.308	0.003055	50.162866450	49.837133550	
	0.195	0.503	0.501	0.508	0.504	0.002082	82.084690554	17.915309446	
8h	25	0.037	0.042	0.051	0.043333	0.004096	7.057546145	92.942453855	**0.3201**
	12.5	0.055	0.054	0.046	0.051667	0.002848	8.414766558	91.585233442	
	6.25	0.097	0.097	0.082	0.092	0.005	14.983713355	85.016286645	
	3.125	0.104	0.104	0.112	0.106667	0.002667	17.372421281	82.627578719	
	1.563	0.196	0.2	0.208	0.201333	0.003528	32.790445168	67.209554832	
	0.781	0.284	0.278	0.287	0.283	0.002646	46.091205212	53.908794788	
	0.391	0.48	0.478	0.471	0.476333	0.002728	77.578718784	22.421281216	
	0.195	0.607	0.604	0.603	0.604667	0.001202	98.479913138	1.520086862	

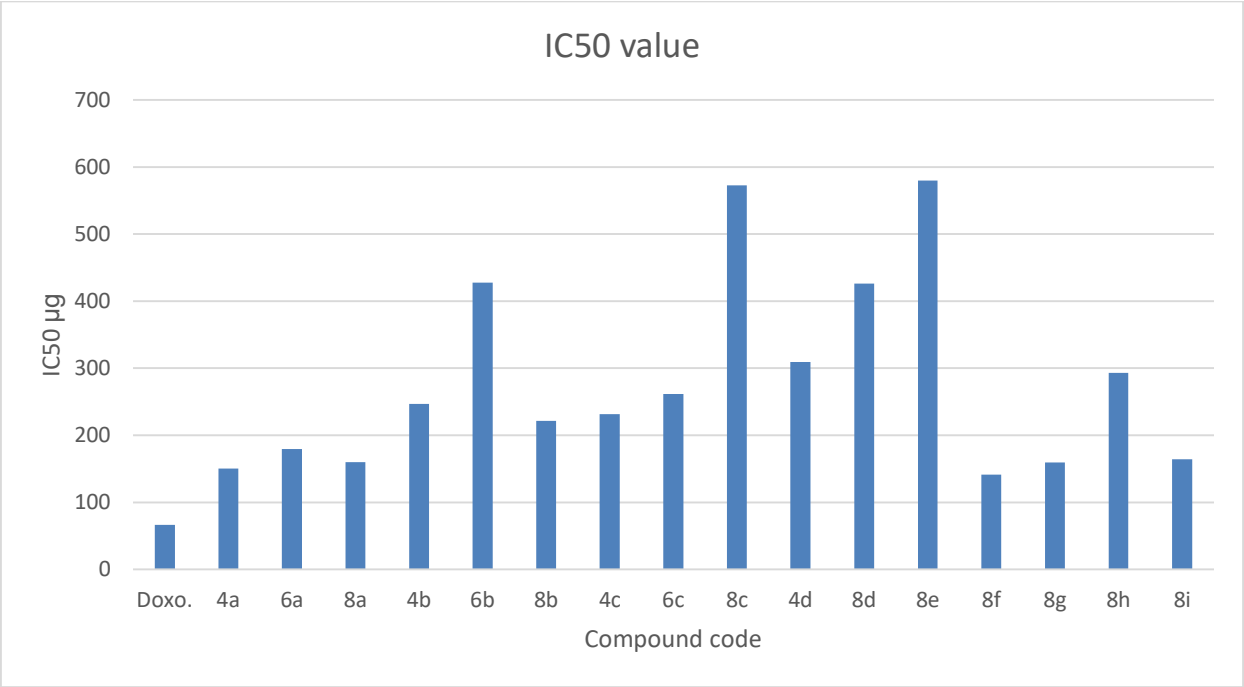

A549 cell line Viability against (8d,8e,8f,8g &8h)
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Viability %
0
2000
4000
6000
8000
10000
12000
Conc. µg
8d
8e
8f
8g
8h

ID	Conc. umol	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
A549	dilution	0.621	0.612	0.609	0.614	0.003606	100	0	
8i	25	0.040	0.042	0.038	0.04	0.001155	6.514657980	93.485342020	**0.1554**
	12.5	0.047	0.053	0.045	0.048333	0.002404	7.871878393	92.128121607	
	6.25	0.064	0.062	0.055	0.060333	0.002728	9.826275787	90.173724213	
	3.125	0.099	0.093	0.095	0.095667	0.001764	15.580890337	84.419109663	
	1.563	0.139	0.135	0.133	0.135667	0.001764	22.095548317	77.904451683	
	0.781	0.211	0.208	0.206	0.208333	0.001453	33.930510315	66.069489685	
	0.391	0.312	0.310	0.315	0.312333	0.001453	50.868621064	49.131378936	
	0.195	0.464	0.477	0.463	0.468	0.004509	76.221498371	23.778501629	



ID	Conc. ug/ml	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
A549	dilution	0.621	0.612	0.609	0.614	0.003606	100	0	
Doxo.	1000	0.018	0.015	0.017	0.016667	0.000882	2.450980392	97.549019608	**0.126**
	500	0.020	0.025	0.023	0.022667	0.001453	3.333333333	96.666666667	
	250	0.097	0.095	0.091	0.094333	0.001764	13.872549020	86.127450980	
	125	0.191	0.179	0.187	0.185667	0.003528	27.303921569	72.696078431	
	62.5	0.359	0.357	0.352	0.356	0.002082	52.352941176	47.647058824	
	31.25	0.417	0.420	0.426	0.421	0.002646	61.911764706	38.088235294	
	15.625	0.532	0.540	0.534	0.535333	0.002404	78.725490196	21.274509804	
	7.812	0.604	0.610	0.602	0.605333	0.002404	89.019607843	10.980392157	




IC50 value
700
600
500
400
300
200
100
0
IC50 µg
Doxo.
4a
6a
8a
4b
6b
8b
4c
6c
8c
4d
8d
8e
8f
8g
8h
8i
Compound code

Viability assay

Institute / Researcher: Prof. Dr. Hany

Experiment: **functional assay (MTT)**

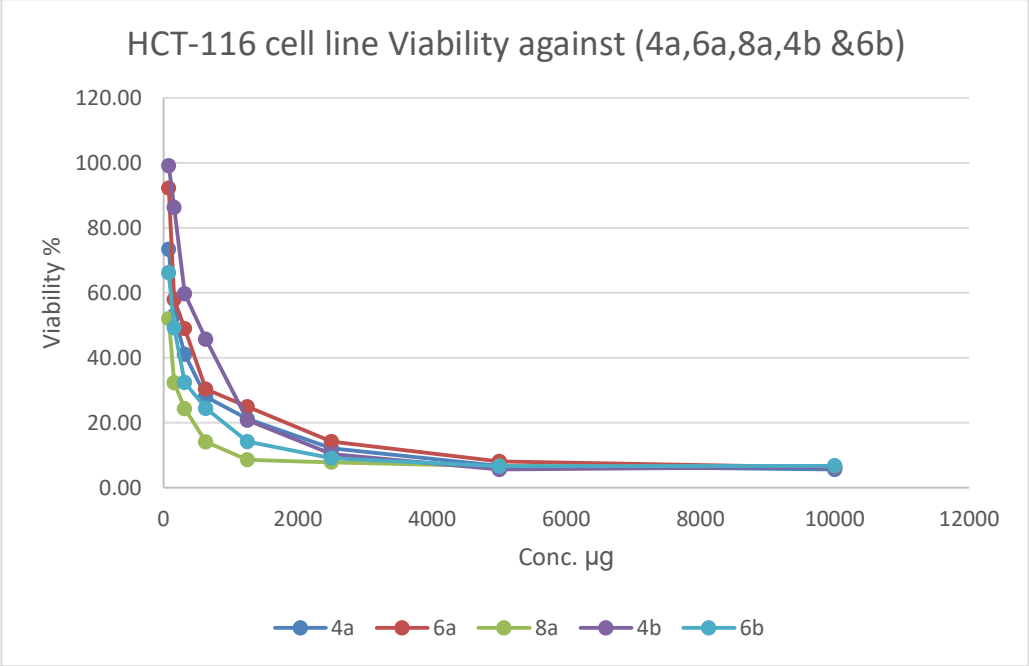
(Viability/cytotoxicity)

Samples number: **16**

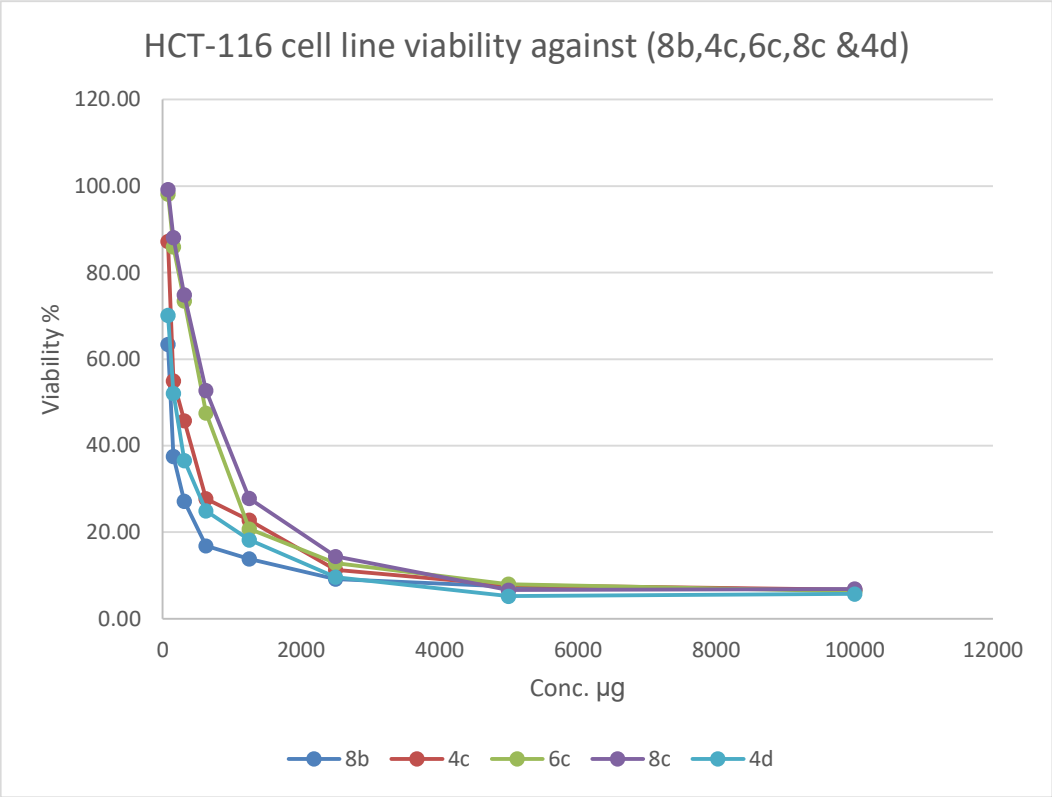
Experiment design: viability against HCT-116 cells

Laboratory comments:

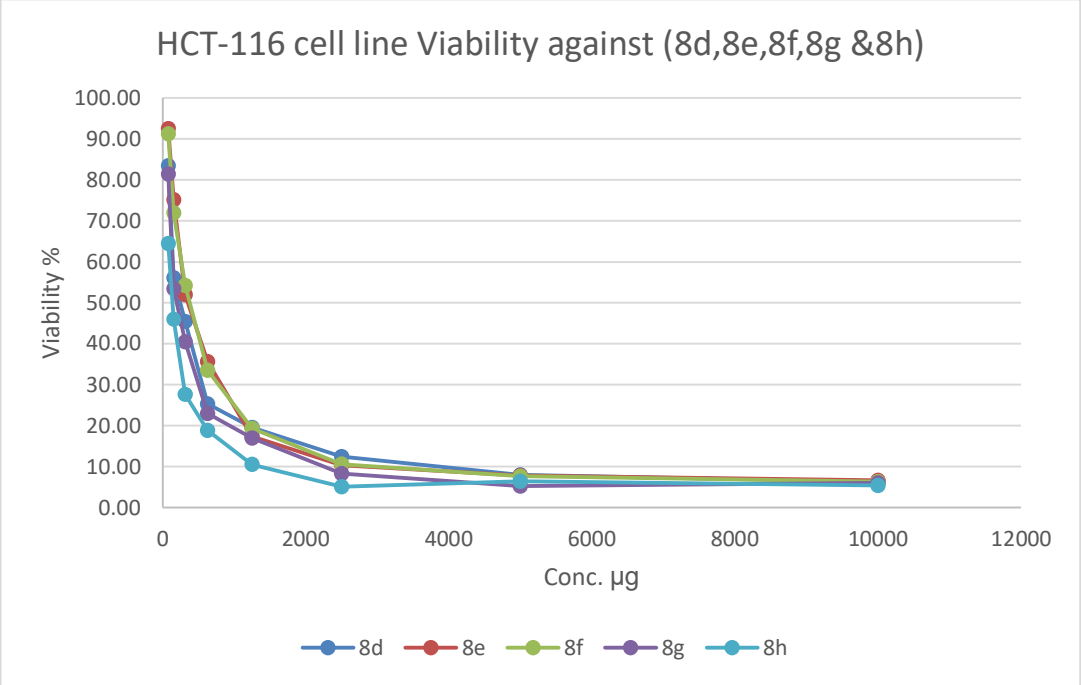
ID	Conc. umol	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
HCT	dilution	0.744	0.758	0.736	0.746	0.006429	100	0	
4a	25	0.045	0.037	0.044	0.042	0.002517	5.630026810	94.369973190	**0.1958**
	12.5	0.060	0.040	0.052	0.050667	0.005812	6.791778374	93.208221626	
	6.25	0.095	0.084	0.093	0.090667	0.003383	12.153708668	87.846291332	
	3.125	0.158	0.162	0.153	0.157667	0.002603	21.134941912	78.865058088	
	1.563	0.204	0.209	0.215	0.209333	0.00318	28.060768543	71.939231457	
	0.781	0.304	0.310	0.305	0.306333	0.001856	41.063449508	58.936550492	
	0.391	0.396	0.394	0.397	0.395667	0.000882	53.038427167	46.961572833	
	0.195	0.555	0.540	0.549	0.548	0.004359	73.458445040	26.541554960	
6a	25	0.044	0.051	0.045	0.046667	0.002186	6.255585344	93.744414656	0.342
	12.5	0.053	0.064	0.064	0.060333	0.003667	8.087578195	91.912421805	
	6.25	0.103	0.109	0.107	0.106333	0.001764	14.253798034	85.746201966	
	3.125	0.185	0.190	0.183	0.186	0.002082	24.932975871	75.067024129	
	1.563	0.221	0.231	0.227	0.226333	0.002906	30.339588919	69.660411081	
	0.781	0.359	0.364	0.373	0.365333	0.004096	48.972296693	51.027703307	
	0.391	0.434	0.425	0.437	0.432	0.003606	57.908847185	42.091152815	
	0.195	0.691	0.688	0.686	0.688333	0.001453	92.269883825	7.730116175	
8a	25	0.046	0.058	0.039	0.047667	0.005548	6.389633601	93.610366399	**0.1060**
	12.5	0.050	0.044	0.052	0.048667	0.002404	6.523681859	93.476318141	
	6.25	0.066	0.048	0.062	0.058667	0.005457	7.864164433	92.135835567	
	3.125	0.057	0.059	0.077	0.064333	0.00636	8.623771224	91.376228776	
	1.563	0.107	0.100	0.108	0.105	0.002517	14.075067024	85.924932976	
	0.781	0.178	0.181	0.185	0.181333	0.002028	24.307417337	75.692582663	
	0.391	0.248	0.233	0.244	0.241667	0.004485	32.394995532	67.605004468	
	0.195	0.392	0.390	0.383	0.388333	0.002728	52.055406613	47.944593387	
4b	25	0.050	0.048	0.044	0.047333	0.001764	6.344950849	93.655049151	**0.6649**
	12.5	0.047	0.040	0.039	0.042	0.002517	5.630026810	94.369973190	
	6.25	0.077	0.072	0.084	0.077667	0.00348	10.411081323	89.588918677	
	3.125	0.160	0.154	0.152	0.155333	0.002404	20.822162645	79.177837355	
	1.563	0.342	0.338	0.343	0.341	0.001528	45.710455764	54.289544236	
	0.781	0.442	0.449	0.445	0.445333	0.002028	59.696157283	40.303842717	
	0.391	0.642	0.647	0.641	0.643333	0.001856	86.237712243	13.762287757	
	0.195	0.739	0.738	0.741	0.739333	0.000882	99.106344951	0.893655049	
6b	25	0.057	0.055	0.040	0.050667	0.005364	6.791778374	93.208221626	0.184
	12.5	0.058	0.043	0.046	0.049	0.004583	6.568364611	93.431635389	
	6.25	0.068	0.071	0.066	0.068333	0.001453	9.159964254	90.840035746	
	3.125	0.109	0.107	0.103	0.106333	0.001764	14.253798034	85.746201966	
	1.563	0.185	0.175	0.187	0.182333	0.003712	24.441465594	75.558534406	
	0.781	0.242	0.239	0.245	0.242	0.001732	32.439678284	67.560321716	
	0.391	0.368	0.364	0.370	0.367333	0.001764	49.240393208	50.759606792	
	0.195	0.493	0.498	0.489	0.493333	0.002603	66.130473637	33.869526363	


HCT-116 cell line Viability against (4a,6a,8a,4b &6b)
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Viability %
0
2000
4000
6000
8000
10000
12000
Conc. µg
4a
6a
8a
4b
6b

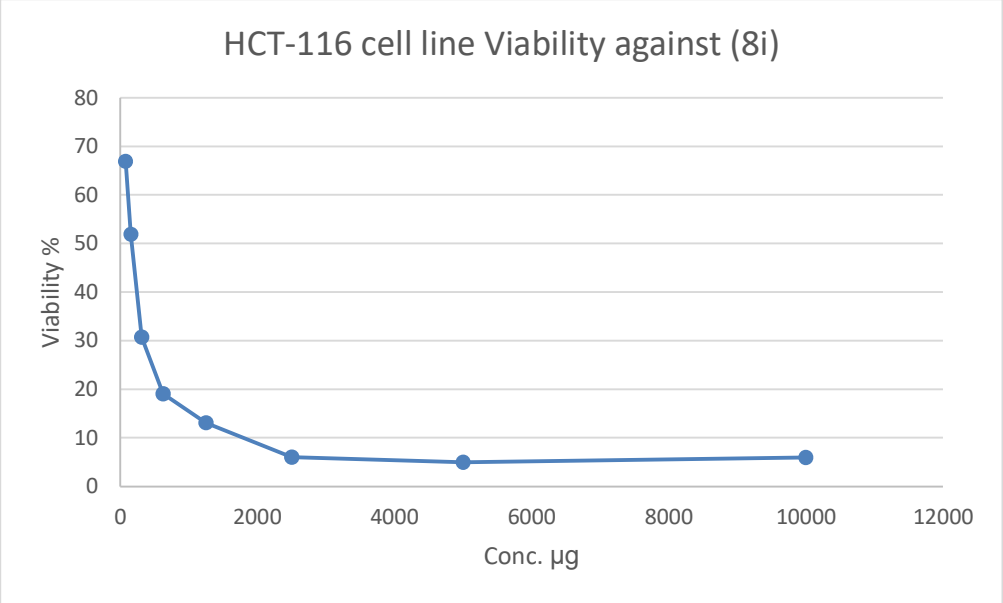
ID	Conc. umol	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
HCT	dilution	0.744	0.758	0.736	0.746	0.006429	100	0	
8b	25	0.050	0.043	0.055	0.049333	0.00348	6.613047364	93.386952636	**0.1515**
	12.5	0.048	0.061	0.058	0.055667	0.00393	7.462019660	92.537980340	
	6.25	0.069	0.073	0.064	0.068667	0.002603	9.204647006	90.795352994	
	3.125	0.103	0.109	0.097	0.103	0.003464	13.806970509	86.193029491	
	1.563	0.119	0.127	0.131	0.125667	0.003528	16.845397676	83.154602324	
	0.781	0.207	0.203	0.197	0.202333	0.002906	27.122430742	72.877569258	
	0.391	0.274	0.286	0.279	0.279667	0.00348	37.488829312	62.511170688	
	0.195	0.477	0.468	0.474	0.473	0.002646	63.404825737	36.595174263	
4c	25	0.050	0.044	0.057	0.050333	0.003756	6.747095621	93.252904379	**0.2558**
	12.5	0.052	0.068	0.055	0.058333	0.00491	7.819481680	92.180518320	
	6.25	0.080	0.083	0.090	0.084333	0.002963	11.304736372	88.695263628	
	3.125	0.176	0.161	0.173	0.170	0.004583	22.788203753	77.211796247	
	1.563	0.205	0.210	0.205	0.206667	0.001667	27.703306524	72.296693476	
	0.781	0.347	0.336	0.341	0.341333	0.00318	45.755138517	54.244861483	
	0.391	0.414	0.405	0.411	0.410	0.002646	54.959785523	45.040214477	
	0.195	0.651	0.646	0.655	0.650667	0.002603	87.220732797	12.779267203	
6c	25	0.054	0.040	0.048	0.047333	0.004055	6.344950849	93.655049151	0.696
	12.5	0.066	0.054	0.060	0.06	0.003464	8.042895442	91.957104558	
	6.25	0.099	0.091	0.098	0.096	0.002517	12.868632708	87.131367292	
	3.125	0.159	0.154	0.151	0.154667	0.002333	20.732797140	79.267202860	
	1.563	0.351	0.353	0.360	0.354667	0.002728	47.542448615	52.457551385	
	0.781	0.548	0.554	0.542	0.548	0.003464	73.458445040	26.541554960	
	0.391	0.648	0.645	0.631	0.641333	0.005239	85.969615728	14.030384272	
	0.195	0.735	0.739	0.724	0.732667	0.004485	98.212689902	1.787310098	
8c	25	0.056	0.046	0.053	0.051667	0.002963	6.925826631	93.074173369	**0.8551**
	12.5	0.055	0.048	0.045	0.049333	0.002963	6.613047364	93.386952636	
	6.25	0.107	0.105	0.110	0.107333	0.001453	14.387846291	85.612153709	
	3.125	0.207	0.204	0.211	0.207333	0.002028	27.792672029	72.207327971	
	1.563	0.388	0.400	0.392	0.393333	0.003528	52.725647900	47.274352100	
	0.781	0.569	0.564	0.542	0.558333	0.008293	74.843610366	25.156389634	
	0.391	0.657	0.650	0.664	0.657	0.004041	88.069705094	11.930294906	
	0.195	0.741	0.747	0.733	0.740333	0.004055	99.240393208	0.759606792	
4d	25	0.046	0.043	0.039	0.042667	0.002028	5.719392315	94.280607685	**0.1855**
	12.5	0.036	0.033	0.048	0.039	0.004583	5.227882038	94.772117962	
	6.25	0.068	0.075	0.072	0.071667	0.002028	9.606791778	90.393208222	
	3.125	0.141	0.132	0.136	0.136333	0.002603	18.275245755	81.724754245	
	1.563	0.183	0.187	0.188	0.186	0.001528	24.932975871	75.067024129	
	0.781	0.280	0.273	0.266	0.273	0.004041	36.595174263	63.404825737	
	0.391	0.396	0.387	0.383	0.388667	0.003844	52.100089366	47.899910634	
	0.195	0.528	0.519	0.523	0.523333	0.002603	70.151921358	29.848078642	


HCT-116 cell line viability against (8b,4c,6c,8c &4d)
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Viability %
0
2000
4000
6000
8000
10000
12000
Conc. µg
8b
4c
6c
8c
4d

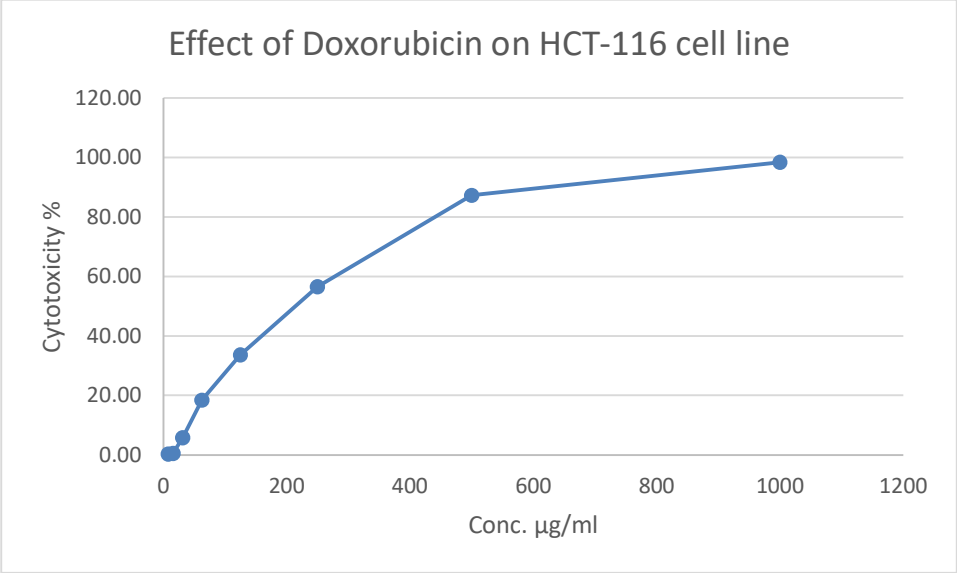
ID	Conc. umol	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
HCT	dilution	0.744	0.758	0.736	0.746	0.006429	100	0	
8d	25	0.039	0.046	0.055	0.046667	0.004631	6.255585344	93.744414656	**0.2324**
	12.5	0.063	0.055	0.060	0.059333	0.002333	7.953529937	92.046470063	
	6.25	0.097	0.092	0.090	0.093	0.002082	12.466487936	87.533512064	
	3.125	0.141	0.152	0.144	0.145667	0.003283	19.526362824	80.473637176	
	1.563	0.185	0.192	0.189	0.188667	0.002028	25.290437891	74.709562109	
	0.781	0.338	0.347	0.332	0.339	0.004359	45.442359249	54.557640751	
	0.391	0.419	0.421	0.415	0.418333	0.001764	56.076854334	43.923145666	
	0.195	0.626	0.623	0.619	0.622667	0.002028	83.467381591	16.532618409	
8e	25	0.051	0.05	0.048	0.049667	0.000882	6.657730116	93.342269884	**0.4508**
	12.5	0.062	0.059	0.053	0.058	0.002646	7.774798928	92.225201072	
	6.25	0.078	0.07	0.084	0.077333	0.004055	10.366398570	89.633601430	
	3.125	0.134	0.131	0.123	0.129333	0.003283	17.336907954	82.663092046	
	1.563	0.263	0.268	0.266	0.265667	0.001453	35.612153709	64.387846291	
	0.781	0.385	0.390	0.387	0.387333	0.001453	51.921358356	48.078641644	
	0.391	0.56	0.564	0.558	0.560667	0.001764	75.156389634	24.843610366	
	0.195	0.697	0.684	0.690	0.690333	0.003756	92.537980340	7.462019660	
8f	25	0.044	0.041	0.057	0.047333	0.00491	6.344950849	93.655049151	**0.4620**
	12.5	0.059	0.06	0.052	0.057	0.002517	7.640750670	92.359249330	
	6.25	0.079	0.083	0.075	0.079	0.002309	10.589812332	89.410187668	
	3.125	0.148	0.146	0.138	0.144	0.003055	19.302949062	80.697050938	
	1.563	0.256	0.250	0.244	0.250	0.003464	33.512064343	66.487935657	
	0.781	0.405	0.407	0.401	0.404333	0.001764	54.200178731	45.799821269	
	0.391	0.540	0.533	0.536	0.536333	0.002028	71.894548704	28.105451296	
	0.195	0.683	0.680	0.678	0.680333	0.001453	91.197497766	8.802502234	
8g	25	0.040	0.043	0.051	0.044667	0.003283	5.987488829	94.012511171	**0.2698**
	12.5	0.039	0.036	0.042	0.039	0.001732	5.227882038	94.772117962	
	6.25	0.067	0.060	0.058	0.061667	0.002728	8.266309205	91.733690795	
	3.125	0.134	0.132	0.114	0.126667	0.00636	16.979445934	83.020554066	
	1.563	0.168	0.169	0.177	0.171333	0.002848	22.966934763	77.033065237	
	0.781	0.305	0.301	0.300	0.302	0.001528	40.482573727	59.517426273	
	0.391	0.397	0.395	0.404	0.398667	0.002728	53.440571939	46.559428061	
	0.195	0.603	0.611	0.608	0.607333	0.002333	81.411974978	18.588025022	
8h	25	0.044	0.042	0.035	0.040333	0.002728	5.406613047	94.593386953	**0.1521**
	12.5	0.048	0.050	0.046	0.048	0.001155	6.434316354	93.565683646	
	6.25	0.038	0.040	0.036	0.038	0.001155	5.093833780	94.906166220	
	3.125	0.073	0.084	0.078	0.078333	0.00318	10.500446828	89.499553172	
	1.563	0.141	0.138	0.143	0.140667	0.001453	18.856121537	81.143878463	
	0.781	0.207	0.209	0.203	0.206333	0.001764	27.658623771	72.341376229	
	0.391	0.336	0.348	0.345	0.343	0.003606	45.978552279	54.021447721	
	0.195	0.480	0.485	0.478	0.481	0.002082	64.477211796	35.522788204	

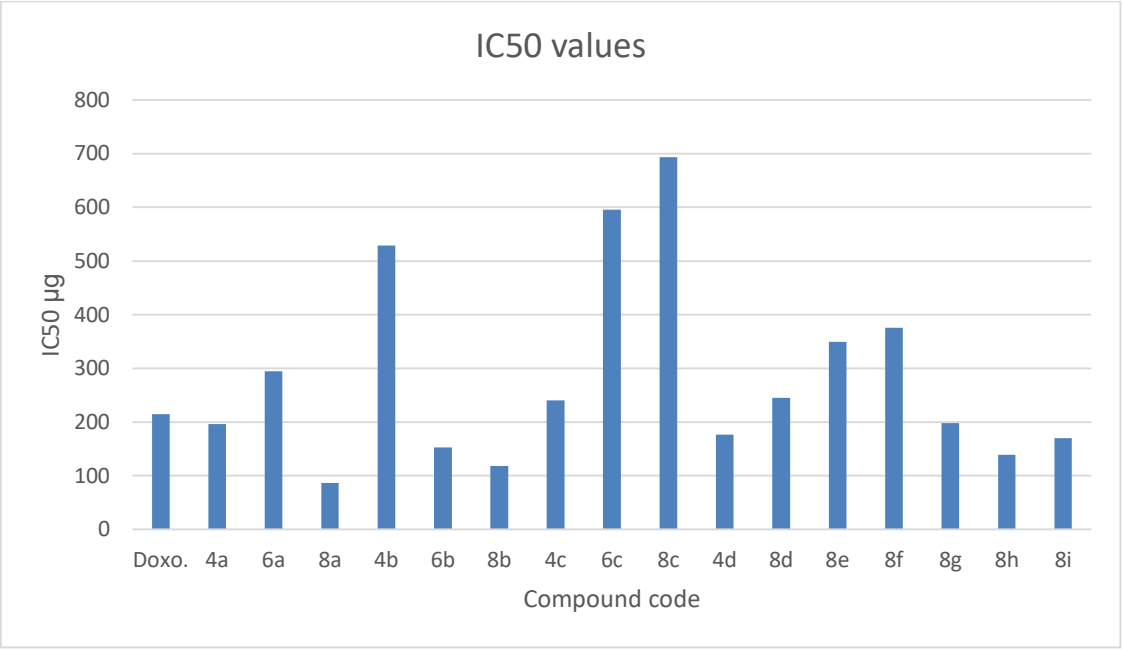

HCT-116 cell line Viability against (8d,8e,8f,8g &8h)
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Viability %
0
2000
4000
6000
8000
10000
12000
Conc. µg
8d
8e
8f
8g
8h

ID	Conc. umol	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
HCT	dilution	0.744	0.758	0.736	0.746	0.006429	100	0	
8i	25	0.041	0.053	0.040	0.044667	0.004177	5.987488829	94.012511171	**0.1610**
	12.5	0.043	0.037	0.032	0.037333	0.00318	5.004468275	94.995531725	
	6.25	0.049	0.039	0.047	0.045	0.003055	6.032171582	93.967828418	
	3.125	0.093	0.097	0.103	0.097667	0.002906	13.092046470	86.907953530	
	1.563	0.146	0.139	0.142	0.142333	0.002028	19.079535299	80.920464701	
	0.781	0.224	0.23	0.233	0.229	0.002646	30.697050938	69.302949062	
	0.391	0.395	0.381	0.385	0.387	0.004163	51.876675603	48.123324397	
	0.195	0.494	0.505	0.498	0.499	0.003215	66.890080429	33.109919571	



ID	Conc. ug/ml	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
HCT	dilution	0.744	0.758	0.736	0.746	0.006429	100	0	
Doxo.	1000	0.011	0.014	0.011	0.012	0.001	1.581027668	98.418972332	0.394
	500	0.097	0.099	0.093	0.096333	0.001764	12.692138779	87.307861221	
	250	0.324	0.339	0.327	0.330	0.004583	43.478260870	56.521739130	
	125	0.501	0.507	0.503	0.503667	0.001764	66.359244620	33.640755380	
	62.5	0.618	0.616	0.622	0.618667	0.001764	81.510759772	18.489240228	
	31.25	0.714	0.720	0.711	0.715	0.002646	94.202898551	5.797101449	
	15.625	0.754	0.748	0.761	0.754333	0.003756	99.38515591	0.614844093	
	7.812	0.757	0.758	0.755	0.756667	0.000882	99.69257795	0.307422047	




IC50 values
800
700
600
500
400
300
200
100
0
IC50 µg
Doxo.
4a
6a
8a
4b
6b
8b
4c
6c
8c
4d
8d
8e
8f
8g
8h
8i
Compound code

Viability assay

Institute / Researcher: Prof. Dr. Hany

Experiment: **functional assay (MTT)**

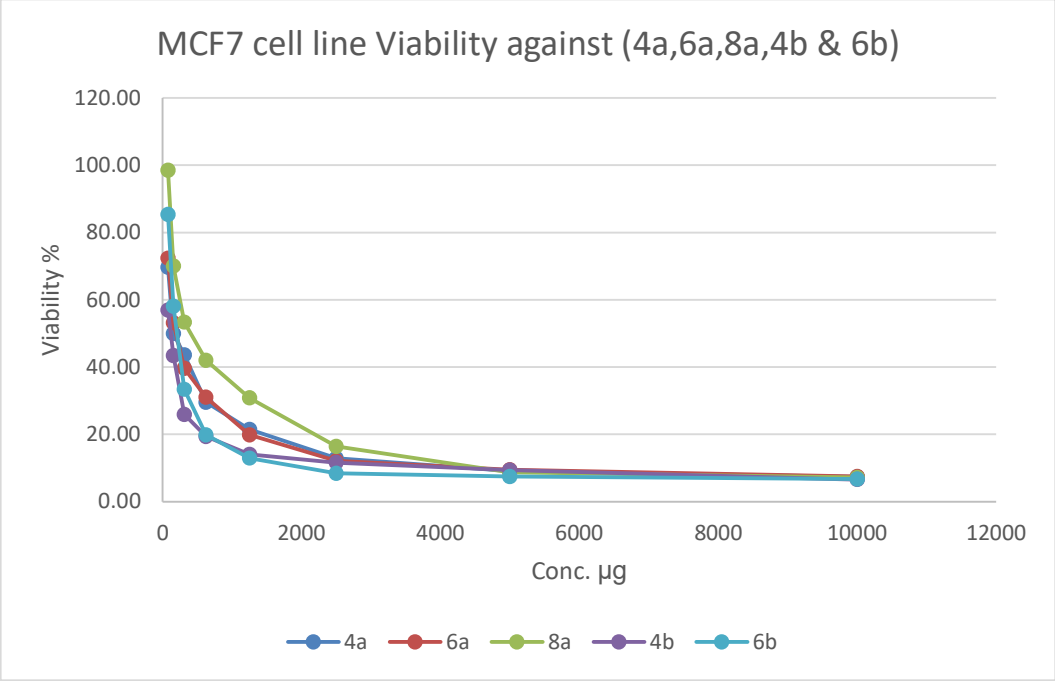
(Viability/cytotoxicity)

Samples number: **16**

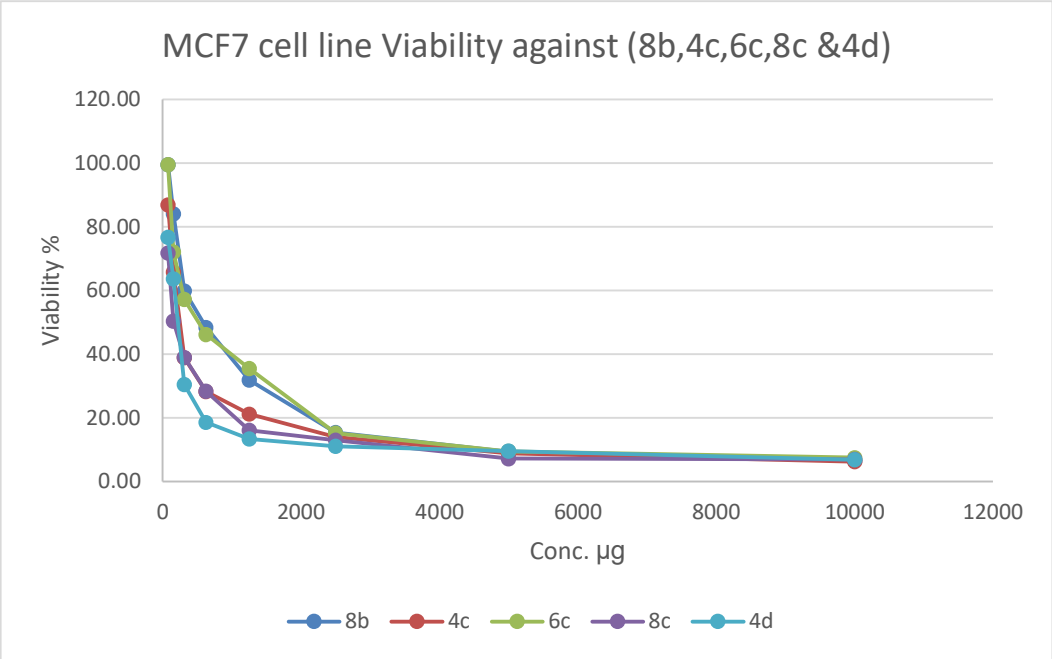
Experiment design: viability against MCF7 cells

Laboratory comments:

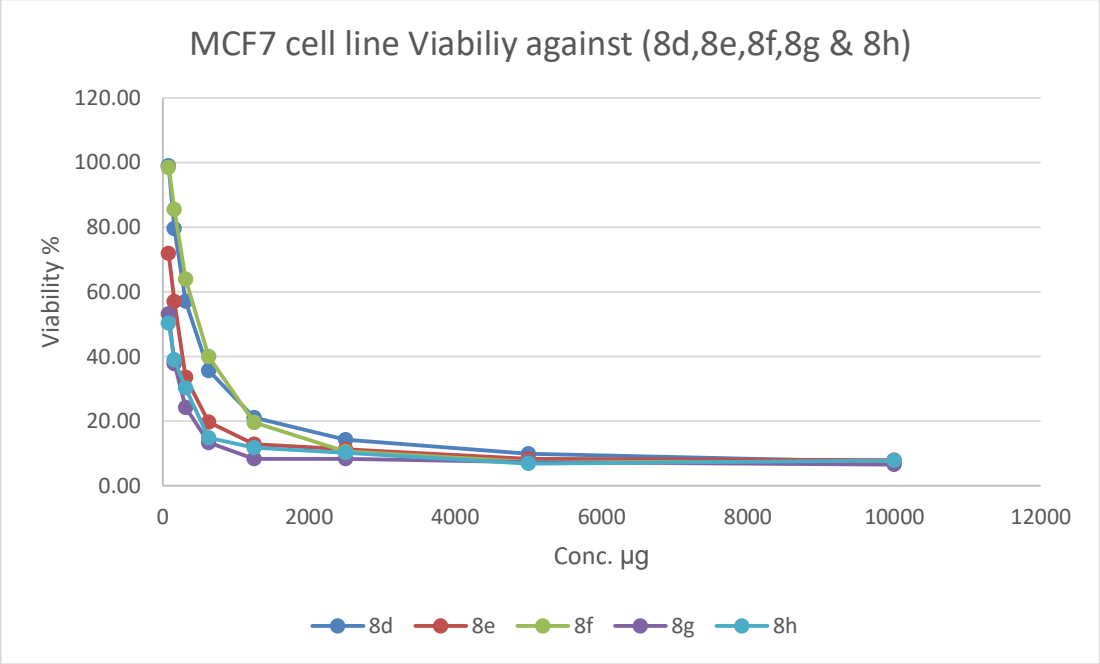
ID	Conc. umol	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
MCF7	dilution	0.802	0.798	0.809	0.803	0.003215	100	0	
4a	25	0.063	0.060	0.055	0.059333	0.002333	7.388958074	92.611041926	**0.1550**
	12.5	0.075	0.075	0.069	0.073	0.002	9.090909091	90.909090909	
	6.25	0.100	0.108	0.104	0.104	0.002309	12.951432130	87.048567870	
	3.125	0.167	0.172	0.178	0.172333	0.00318	21.461187215	78.538812785	
	1.563	0.232	0.236	0.242	0.236667	0.002906	29.472810295	70.527189705	
	0.781	0.349	0.347	0.354	0.35	0.002082	43.586550436	56.413449564	
	0.391	0.394	0.407	0.403	0.401333	0.003844	49.979244500	50.020755500	
	0.195	0.568	0.564	0.545	0.559	0.007095	69.613947696	30.386052304	
6a	25	0.067	0.056	0.057	0.060	0.003512	7.471980075	92.528019925	0.224
	12.5	0.079	0.076	0.073	0.076	0.001732	9.464508095	90.535491905	
	6.25	0.094	0.097	0.103	0.098	0.002646	12.204234122	87.795765878	
	3.125	0.157	0.162	0.159	0.159333	0.001453	19.842258198	80.157741802	
	1.563	0.254	0.250	0.243	0.249	0.003215	31.008717310	68.991282690	
	0.781	0.318	0.320	0.317	0.318333	0.000882	39.643005396	60.356994604	
	0.391	0.435	0.427	0.419	0.427	0.004619	53.175591532	46.824408468	
	0.195	0.585	0.580	0.579	0.581333	0.001856	72.395184724	27.604815276	
8a	25	0.053	0.065	0.055	0.057667	0.003712	7.181403072	92.818596928	**0.4978**
	12.5	0.070	0.066	0.072	0.069333	0.001764	8.634288086	91.365711914	
	6.25	0.131	0.123	0.142	0.132	0.005508	16.438356164	83.561643836	
	3.125	0.246	0.247	0.250	0.247667	0.001202	30.842673308	69.157326692	
	1.563	0.328	0.339	0.344	0.337	0.004726	41.967621420	58.032378580	
	0.781	0.429	0.424	0.433	0.428667	0.002603	53.383146534	46.616853466	
	0.391	0.567	0.553	0.566	0.562	0.004509	69.987546700	30.012453300	
	0.195	0.783	0.797	0.792	0.790667	0.004096	98.464092985	1.535907015	
4b	25	0.059	0.053	0.046	0.052667	0.003756	6.558738066	93.441261934	**0.1486**
	12.5	0.072	0.079	0.075	0.075333	0.002028	9.381486094	90.618513906	
	6.25	0.090	0.098	0.092	0.093333	0.002404	11.623080116	88.376919884	
	3.125	0.113	0.118	0.109	0.113333	0.002603	14.113740141	85.886259859	
	1.563	0.150	0.162	0.155	0.155667	0.00348	19.385637194	80.614362806	
	0.781	0.203	0.208	0.212	0.207667	0.002603	25.861353259	74.138646741	
	0.391	0.340	0.352	0.355	0.349	0.004583	43.462017435	56.537982565	
	0.195	0.456	0.461	0.454	0.457	0.002082	56.911581569	43.088418431	
6b	25	0.055	0.058	0.049	0.054	0.002646	6.724782067	93.275217933	0.251
	12.5	0.053	0.069	0.057	0.059667	0.004807	7.430469074	92.569530926	
	6.25	0.074	0.066	0.062	0.067333	0.003528	8.385222084	91.614777916	
	3.125	0.103	0.109	0.100	0.104	0.002646	12.951432130	87.048567870	
	1.563	0.162	0.164	0.153	0.159667	0.003383	19.883769199	80.116230801	
	0.781	0.272	0.268	0.265	0.268333	0.002028	33.416355334	66.583644666	
	0.391	0.463	0.470	0.467	0.466667	0.002028	58.115400581	41.884599419	
	0.195	0.690	0.681	0.685	0.685333	0.002603	85.346616853	14.653383147	


MCF7 cell line Viability against (4a,6a,8a,4b & 6b)
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Viability %
0
2000
4000
6000
8000
10000
12000
Conc. µg
4a
6a
8a
4b
6b

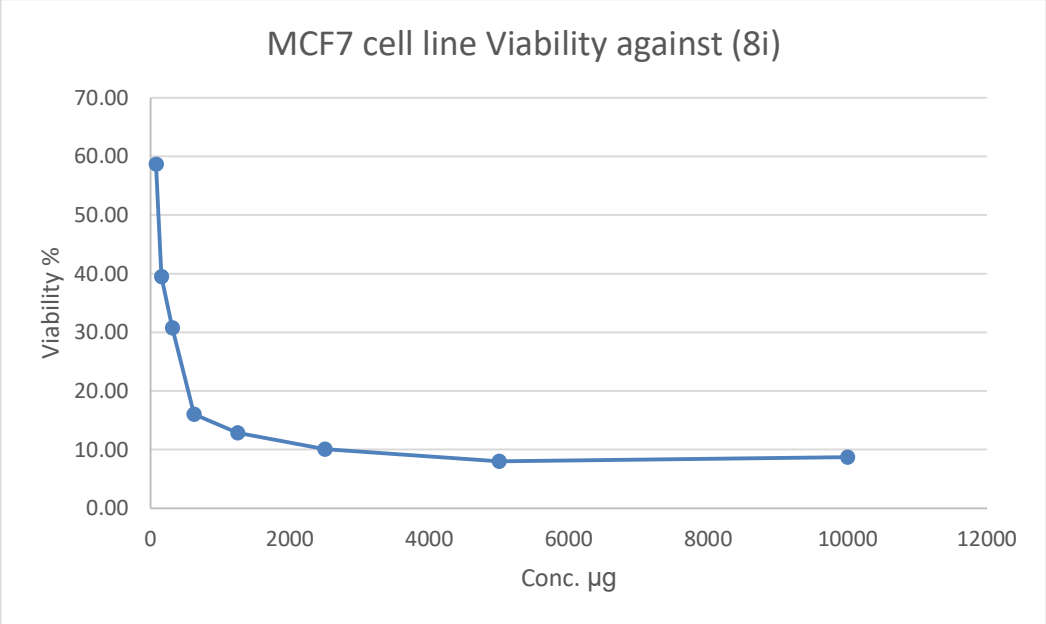
ID	Conc. umol	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
MCF7	dilution	0.802	0.798	0.809	0.803	0.003215	100	0	
8b	25	0.059	0.047	0.050	0.052	0.003606	6.475716065	93.524283935	**0.7438**
	12.5	0.070	0.082	0.074	0.075333	0.003528	9.381486094	90.618513906	
	6.25	0.128	0.124	0.118	0.123333	0.002906	15.359070154	84.640929846	
	3.125	0.255	0.250	0.264	0.256333	0.004096	31.921959319	68.078040681	
	1.563	0.387	0.390	0.389	0.388667	0.000882	48.401826484	51.598173516	
	0.781	0.481	0.486	0.477	0.481333	0.002603	59.941884599	40.058115401	
	0.391	0.680	0.671	0.676	0.675667	0.002603	84.142797841	15.857202159	
	0.195	0.794	0.804	0.801	0.799667	0.002963	99.584889996	0.415110004	
4c	25	0.055	0.053	0.042	0.050	0.004041	6.226650062	93.773349938	**0.2639**
	12.5	0.076	0.060	0.078	0.071333	0.005696	8.883354089	91.116645911	
	6.25	0.108	0.115	0.113	0.112	0.002082	13.947696139	86.052303861	
	3.125	0.176	0.170	0.165	0.170333	0.00318	21.212121212	78.787878788	
	1.563	0.231	0.228	0.222	0.227	0.002646	28.268991283	71.731008717	
	0.781	0.312	0.308	0.317	0.312333	0.002603	38.895807389	61.104192611	
	0.391	0.533	0.523	0.530	0.528667	0.002963	65.836446658	34.163553342	
	0.195	0.696	0.701	0.698	0.698333	0.001453	86.965545870	13.034454130	
6c	25	0.060	0.057	0.063	0.060	0.001732	7.471980075	92.528019925	**0.604**
	12.5	0.083	0.076	0.069	0.076	0.004041	9.464508095	90.535491905	
	6.25	0.121	0.125	0.120	0.122	0.001528	15.193026152	84.806973848	
	3.125	0.283	0.288	0.284	0.285	0.001528	35.491905355	64.508094645	
	1.563	0.370	0.375	0.367	0.370667	0.002333	46.160232462	53.839767538	
	0.781	0.459	0.452	0.468	0.459667	0.004631	57.243669572	42.756330428	
	0.391	0.579	0.583	0.576	0.579333	0.002028	72.146118721	27.853881279	
	0.195	0.798	0.795	0.806	0.799667	0.003283	99.584889996	0.415110004	
8c	25	0.054	0.049	0.064	0.055667	0.00441	6.932337069	93.067662931	**0.2000**
	12.5	0.057	0.056	0.060	0.057667	0.001202	7.181403072	92.818596928	
	6.25	0.116	0.103	0.094	0.104333	0.006386	12.992943130	87.007056870	
	3.125	0.139	0.122	0.125	0.128667	0.005239	16.023246160	83.976753840	
	1.563	0.228	0.230	0.226	0.228	0.001155	28.393524284	71.606475716	
	0.781	0.310	0.316	0.312	0.312667	0.001764	38.937318389	61.062681611	
	0.391	0.405	0.402	0.408	0.405	0.001732	50.435865504	49.564134496	
	0.195	0.570	0.573	0.588	0.577	0.005568	71.855541719	28.144458281	
4d	25	0.058	0.066	0.041	0.055	0.007371	6.849315068	93.150684932	**0.2314**
	12.5	0.082	0.078	0.070	0.076667	0.003528	9.547530095	90.452469905	
	6.25	0.094	0.085	0.088	0.089	0.002646	11.083437111	88.916562889	
	3.125	0.108	0.111	0.104	0.107667	0.002028	13.408053134	86.591946866	
	1.563	0.151	0.140	0.157	0.149333	0.004978	18.596928186	81.403071814	
	0.781	0.242	0.239	0.253	0.244667	0.004256	30.469074305	69.530925695	
	0.391	0.516	0.513	0.505	0.511333	0.003283	63.677874637	36.322125363	
	0.195	0.620	0.618	0.611	0.616333	0.002728	76.753839768	23.246160232	


MCF7 cell line Viability against (8b,4c,6c,8c &4d)
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Viability %
0
2000
4000
6000
8000
10000
12000
Conc. µg
8b
4c
6c
8c
4d

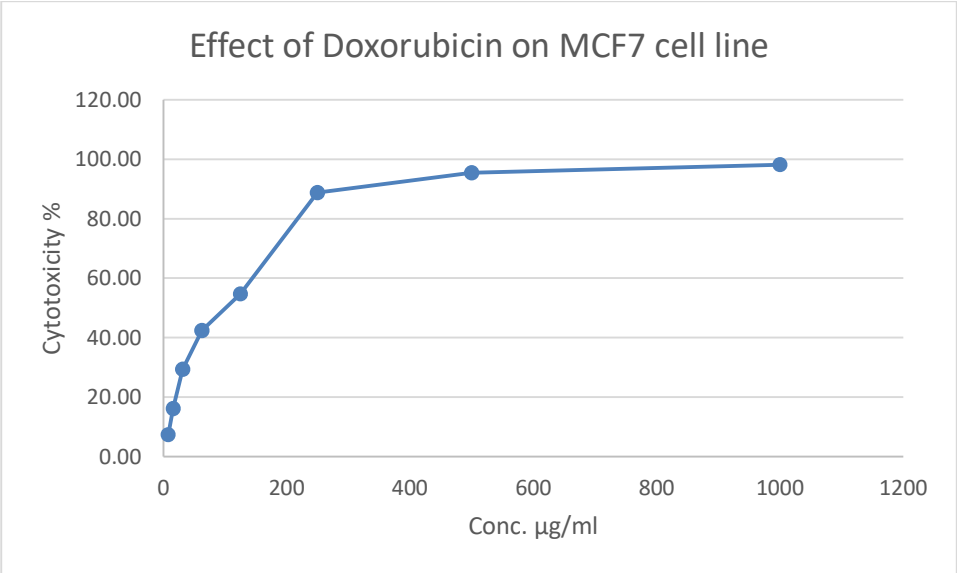
ID	Conc. umol	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
MCF7	dilution	0.802	0.798	0.809	0.803	0.003215	100	0	
8d	25	0.050	0.067	0.061	0.059333	0.004978	7.388958074	92.611041926	**0.3939**
	12.5	0.077	0.089	0.075	0.080333	0.004372	10.004151100	89.995848900	
	6.25	0.120	0.109	0.115	0.114667	0.00318	14.279784143	85.720215857	
	3.125	0.171	0.163	0.175	0.169667	0.003528	21.129099211	78.870900789	
	1.563	0.288	0.290	0.281	0.286333	0.002728	35.657949357	64.342050643	
	0.781	0.462	0.459	0.455	0.458667	0.002028	57.119136571	42.880863429	
	0.391	0.633	0.648	0.637	0.639333	0.004485	79.618098796	20.381901204	
	0.195	0.800	0.793	0.791	0.794667	0.002728	98.962224990	1.037775010	
8e	25	0.068	0.054	0.070	0.064	0.005033	7.970112080	92.029887920	**0.2621**
	12.5	0.070	0.071	0.062	0.067667	0.002848	8.426733084	91.573266916	
	6.25	0.090	0.093	0.089	0.090667	0.001202	11.290992113	88.709007887	
	3.125	0.104	0.100	0.108	0.104	0.002309	12.951432130	87.048567870	
	1.563	0.149	0.157	0.168	0.158	0.005508	19.676214197	80.323785803	
	0.781	0.276	0.273	0.260	0.269667	0.00491	33.582399336	66.417600664	
	0.391	0.458	0.460	0.456	0.458	0.001155	57.036114570	42.963885430	
	0.195	0.580	0.573	0.579	0.577333	0.002186	71.897052719	28.102947281	
8f	25	0.058	0.062	0.055	0.058333	0.002028	7.264425073	92.735574927	**0.6073**
	12.5	0.066	0.050	0.064	0.06	0.005033	7.471980075	92.528019925	
	6.25	0.082	0.079	0.096	0.085667	0.005239	10.668327107	89.331672893	
	3.125	0.153	0.158	0.161	0.157333	0.002333	19.593192196	80.406807804	
	1.563	0.327	0.311	0.325	0.321	0.005033	39.975093400	60.024906600	
	0.781	0.525	0.503	0.512	0.513333	0.006386	63.926940639	36.073059361	
	0.391	0.682	0.690	0.687	0.686333	0.002333	85.471149855	14.528850145	
	0.195	0.798	0.793	0.782	0.791	0.004726	98.505603985	1.494396015	
8g	25	0.051	0.048	0.060	0.053	0.003606	6.600249066	93.399750934	**0.1286**
	12.5	0.069	0.059	0.050	0.059333	0.005487	7.388958074	92.611041926	
	6.25	0.058	0.070	0.075	0.067667	0.005044	8.426733084	91.573266916	
	3.125	0.071	0.064	0.067	0.067333	0.002028	8.385222084	91.614777916	
	1.563	0.102	0.110	0.111	0.107667	0.002848	13.408053134	86.591946866	
	0.781	0.200	0.194	0.189	0.194333	0.00318	24.200913242	75.799086758	
	0.391	0.304	0.307	0.300	0.303667	0.002028	37.816521378	62.183478622	
	0.195	0.424	0.420	0.437	0.427	0.005132	53.175591532	46.824408468	
8h	25	0.071	0.055	0.065	0.063667	0.004667	7.928601079	92.071398921	**0.0879**
	12.5	0.062	0.050	0.054	0.055333	0.003528	6.890826069	93.109173931	
	6.25	0.085	0.089	0.072	0.082	0.005132	10.211706102	89.788293898	
	3.125	0.106	0.092	0.088	0.095333	0.005457	11.872146119	88.127853881	
	1.563	0.126	0.112	0.121	0.119667	0.004096	14.902449149	85.097550851	
	0.781	0.240	0.247	0.242	0.243	0.002082	30.261519303	69.738480697	
	0.391	0.320	0.312	0.307	0.313	0.003786	38.978829390	61.021170610	
	0.195	0.404	0.400	0.409	0.404333	0.002603	50.352843504	49.647156496	


MCF7 cell line Viabiliy against (8d,8e,8f,8g & 8h)
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Viability %
0
2000
4000
6000
8000
10000
12000
Conc. µg
8d
8e
8f
8g
8h

ID	Conc. umol	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
MCF7	dilution	0.802	0.798	0.809	0.803	0.003215	100	0	
8i	25	0.071	0.079	0.060	0.07	0.005508	8.717310087	91.282689913	**0.1073**
	12.5	0.073	0.062	0.058	0.064333	0.004485	8.011623080	91.988376920	
	6.25	0.068	0.084	0.091	0.081	0.006807	10.087173101	89.912826899	
	3.125	0.107	0.103	0.100	0.103333	0.002028	12.868410129	87.131589871	
	1.563	0.120	0.137	0.129	0.128667	0.00491	16.023246160	83.976753840	
	0.781	0.241	0.246	0.254	0.247	0.003786	30.759651308	69.240348692	
	0.391	0.312	0.318	0.321	0.317	0.002646	39.476961395	60.523038605	
	0.195	0.472	0.473	0.468	0.471	0.001528	58.655043587	41.344956413	



ID	Conc. ug/ml	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
MCF7	dilution	0.802	0.798	0.809	0.803	0.005568	100	0	
Doxo.	1000	0.017	0.018	0.014	0.016333	0.001203	1.851851852	98.148148148	**0.186**
	500	0.048	0.041	0.034	0.041000	0.004045	4.648526077	95.351473923	
	250	0.104	0.093	0.100	0.099000	0.003217	11.224489796	88.775510204	
	125	0.397	0.400	0.402	0.399667	0.001454	45.313681028	54.686318972	
	62.5	0.513	0.511	0.500	0.508000	0.004045	57.596371882	42.403628118	
	31.25	0.621	0.627	0.622	0.623333	0.001858	70.672713530	29.327286470	
	15.625	0.740	0.734	0.744	0.739333	0.002909	83.824640967	16.175359033	
	7.812	0.793	0.785	0.804	0.817333	0.003386	92.668178382	7.331821618	



Researcher: Prof. Dr. Hany

Experiment: (**MAPK-p38**) activity in (HCT-116) cell line.

Number of Sample: 8 + **Flagecidin as reference drug**

Method: Enzyme Assay (Activity)

Principles:

Sandwich ELISA

MAPK-p38 in (HCT-116 cell line):

Sample code	Concentration (µmol/L)			O.D			Activity %	IC50 (µmol/L)
HCT-116	-----			**0.1**	**0.1**	**0.1**	----	
Flag.	**100**	**50**	**25**	**5.5**	**3.0**	**2.3**	**100**	
4a	25			0.9814			**32.621**	**33.96**
	12.5			0.6189			**20.572**	
	6.25			0.5931			**19.714**	
	3.125			0.3648			**12.126**	
	1.563			0.3180			**10.570**	
	0.781			0.1869			**6.212**	
	0.391			0.1766			**5.870**	
	0.195			0.1634			**5.431**	

Sample code	Concentration (µmol)			O.D			Activity %	IC50 (µmol/L)
HCT-116	-----			**0.1**	**0.1**	**0.1**		
Flag.	**100**	**50**	**25**	**5.5**	**3.0**	**2.3**	**100**	
8a	25			1.0223			**33.980**	**33.01**
	12.5			0.8582			**28.526**	
	6.25			0.8331			**27.692**	
	3.125			0.6155			**20.459**	
	1.563			0.5523			**18.358**	
	0.781			0.3516			**11.687**	
	0.391			0.2298			**7.638**	
	0.195			0.1834			**6.096**	

Sample code	Concentration (µmol)			O.D			Activity %	IC50 (µmol/L)
HCT-116	-----			**0.1**	**0.1**	**0.1**		
Flag.	**100**	**50**	**25**	**5.5**	**3.0**	**2.3**	**100**	
4b	25			1.2042			**40.027**	**29.97**
	12.5			1.1300			**37.560**	
	6.25			1.0942			**36.370**	
	3.125			0.8270			**27.489**	
	1.563			0.5421			**18.019**	
	0.781			0.4873			**16.197**	
	0.391			0.3166			**10.524**	
	0.195			0.1877			**6.239**	

Sample code	Concentration (µmol)			O.D			Activity %	IC50 (µmol/L)
HCT-116	**-----**			**0.1**	**0.1**	**0.1**		
Flag.	**100**	**50**	**25**	**5.5**	**3.0**	**2.3**	**100**	
8b	25			1.6173			**53.758**	**18.51**
	12.5			1.3997			**46.525**	
	6.25			1.2441			**41.353**	
	3.125			1.0199			**33.901**	
	1.563			0.8710			**28.951**	
	0.781			0.6960			**23.134**	
	0.391			0.5209			**17.314**	
	0.195			0.2168			**7.206**	

Sample code	Concentration (µmol)			O.D			Activity %	IC50 (µmol/L)
HCT-116	**-----**			**0.1**	**0.1**	**0.1**		
Flag.	**100**	**50**	**25**	**5.5**	**3.0**	**2.3**	**100**	
8f	25			0.8583			**28.529**	**35.74**
	12.5			0.7336			**24.384**	
	6.25			0.7566			**25.149**	
	3.125			0.5646			**18.767**	
	1.563			0.2926			**9.726**	
	0.781			0.2227			**7.402**	
	0.391			0.1728			**5.744**	
	0.195			0.1767			**5.873**	

Sample code	Concentration (µmol)			O.D			Activity %	IC50 (µmol/L)
HCT-116	**-----**			**0.1**	**0.1**	**0.1**		
Flag.	**100**	**50**	**25**	**5.5**	**3.0**	**2.3**	**100**	
8g	25			2.0866			**69.357**	**5.75**
	12.5			1.5733			**52.295**	
	6.25			1.5404			**51.202**	
	3.125			1.3133			**43.653**	
	1.563			0.8713			**28.961**	
	0.781			0.7476			**24.850**	
	0.391			0.7359			**24.461**	
	0.195			0.2039			**6.777**	

Sample code	Concentration (µmol)			O.D			Activity %	IC50 (µmol/L)
HCT-116	**-----**			**0.1**	**0.1**	**0.1**		**20.16**
Flag.	**100**	**50**	**25**	**5.5**	**3.0**	**2.3**	**100**	
8h	25			1.7982			**59.771**	
	12.5			1.0390			**34.535**	
	6.25			1.0924			**36.310**	
	3.125			1.1079			**36.826**	
	1.563			0.6280			**20.874**	
	0.781			0.3590			**11.933**	
	0.391			0.2061			**6.851**	
	0.195			0.1906			**6.335**	

Sample code	Concentration (µmol)			O.D			Activity %	IC50 (µmol/L)
HCT-116	**-----**			**0.1**	**0.1**	**0.1**		**19.05**
Flag.	**100**	**50**	**25**	**5.5**	**3.0**	**2.3**	**100**	
8i	25			1.5160			**50.391**	
	12.5			1.4913			**49.570**	
	6.25			1.1112			**36.935**	
	3.125			1.2558			**41.742**	
	1.563			0.5988			**19.904**	
	0.781			0.6216			**20.661**	
	0.391			0.3122			**10.377**	
	0.195			0.1654			**5.498**	

Researcher: Prof. Dr. Hany

Experiment: (**MAPK-p38**) activity in (MCF7) cell line.

Number of Sample: 8 + **Flagecidin as reference drug**

Method: Enzyme Assay (Activity)

Principles:

Sandwich ELISA

MAPK-p38 in (MCF7 cell line):

Sample code	Concentration (µmol)			O.D			Activity %	IC50 (µmo/L)
MCF7	-----			**0.1**	**0.0**	**0.09**	----	
Flag.	**100**	**50**	**25**	**4.4**	**3.2**	**1.98**	**100**	
4a	25			1.0700			**31.950**	34.025
	12.5			0.7252			**21.654**	
	6.25			0.5013			**14.969**	
	3.125			0.3072			**9.173**	
	1.563			0.3212			**9.591**	
	0.781			0.1395			**4.165**	
	0.391			0.1366			**4.079**	
	0.195			0.1057			**3.156**	

Sample code	Concentration (µmol)			O.D			Activity %	IC50
MCF7	-----			**0.1**	**0.0**	**0.0**		
Flag.	**100**	**50**	**25**	**4.4**	**3.2**	**1.9**	**100**	
8a	25			1.2579			**37.560**	
	12.5			1.0781			**32.192**	31.22
	6.25			0.8599			**25.676**	
	3.125			0.8062			**24.073**	
	1.563			0.7063			**21.090**	
	0.781			0.4438			**13.252**	
	0.391			0.2511			**7.498**	
	0.195			0.1685			**5.031**	

Sample code	Concentration (µmol)			O.D			Activity %	IC50
MCF7	-----			**0.1**	**0.0**	**0.0**		
Flag.	**100**	**50**	**25**	**4.4**	**3.2**	**1.9**	**100**	
4b	25			1.5103			**45.097**	
	12.5			0.5709			**17.047**	27.45
	6.25			0.5405			**16.139**	
	3.125			0.5429			**16.211**	
	1.563			0.5427			**16.205**	
	0.781			0.3904			**11.657**	
	0.391			0.2904			**8.671**	
	0.195			0.1801			**5.378**	

Sample code	Concentration (µmol)			O.D			Activity %	IC50
MCF7	-----			0.1	0.0	0.0		
Flag.	100	50	25	4.4	3.2	1.9	100	
8b	25			1.5769			**47.086**	**26.46**
	12.5			0.5145			**15.363**	
	6.25			0.4843			**14.461**	
	3.125			0.4010			**11.974**	
	1.563			0.2718			**8.116**	
	0.781			0.2555			**7.629**	
	0.391			0.1999			**5.969**	
	0.195			0.2348			**7.011**	

Sample code	Concentration (µmol)			O.D			Activity %	IC50
MCF7	-----			0.1	0.0	0.0		
Flag.	100	50	25	4.4	3.2	1.9	100	
8f	25			0.8189			**24.452**	**37.77**
	12.5			0.8861			**26.459**	
	6.25			0.7735			**23.096**	
	3.125			0.6371			**19.024**	
	1.563			0.4478			**13.371**	
	0.781			0.3659			**10.926**	
	0.391			0.2174			**6.491**	
	0.195			0.1710			**5.106**	

Sample code	Concentration (µmol)			O.D			Activity %	IC50
MCF7	-----			0.1	0.0	0.0		
Flag.	100	50	25	4.4	3.2	1.9	100	
8g	25			1.6076			**48.002**	**25.99**
	12.5			0.9731			**29.056**	
	6.25			0.8762			**26.163**	
	3.125			0.7591			**22.666**	
	1.563			0.6314			**18.853**	
	0.781			0.7261			**21.681**	
	0.391			0.4676			**13.962**	
	0.195			0.2082			**6.217**	

Sample code	Concentration (µmol)			O.D			Activity %	IC50
MCF7	**-----**			**0.1**	**0.0**	**0.0**		
Flag.	**100**	**50**	**25**	**4.4**	**3.1**	**1.9**	**100**	
8h	25			1.7047			**50.902**	**24.15**
	12.5			1.2601			**37.626**	
	6.25			0.8051			**24.040**	
	3.125			0.8919			**26.632**	
	1.563			0.5862			**17.504**	
	0.781			0.5056			**15.097**	
	0.391			0.5399			**16.121**	
	0.195			0.2172			**6.486**	

Sample code	Concentration (µmol)			O.D			Activity %	IC50
MCF7	**-----**			**0.1**	**0.0**	**0.0**		
Flag.	**100**	**50**	**25**	**4.4**	**3.1**	**1.9**	**100**	
8i	25			1.2472			**37.241**	**31.38**
	12.5			0.6148			**18.358**	
	6.25			0.6271			**18.725**	
	3.125			0.3777			**11.278**	
	1.563			0.2679			**7.999**	
	0.781			0.1634			**4.879**	
	0.391			0.1440			**4.300**	
	0.195			0.1450			**4.330**	

Researcher: Prof. Dr. Hany

Experiment: (**MAPK-p38**) activity in (MCF7) cell line.

Number of Sample: 8 + **Flagecidin as reference drug**

Method: Enzyme Assay (Activity)

Principles:

Sandwich ELISA

MAPK-p38 in (MCF7 cell line):

Sample code	Concentration (µg/ml)			O.D			Activity %	IC50
MCF7	-----			**0.1**	**0.0**	**0.09**	----	
Flag.	**100**	**50**	**25**	**4.4**	**3.2**	**1.98**	**100**	
4a	10000			1.0700			**31.950**	
	5000			0.7252			**21.654**	
	2500			0.5013			**14.969**	
	1250			0.3072			**9.173**	
	625			0.3212			**9.591**	
	312.5			0.1395			**4.165**	
	156.25			0.1366			**4.079**	
	78.12			0.1057			**3.156**	

Sample code	Concentration (µg/ml)			O.D			Activity %	IC50
MCF7	-----			**0.1**	**0.0**	**0.0**		
Flag.	**100**	**50**	**25**	**4.4**	**3.2**	**1.9**	**100**	
8a	10000			1.2579			**37.560**	
	5000			1.0781			**32.192**	
	2500			0.8599			**25.676**	
	1250			0.8062			**24.073**	
	625			0.7063			**21.090**	
	312.5			0.4438			**13.252**	
	156.25			0.2511			**7.498**	
	78.12			0.1685			**5.031**	

Sample code	Concentration (µg/ml)			O.D			Activity %	IC50
MCF7	-----			**0.1**	**0.0**	**0.0**		
Flag.	**100**	**50**	**25**	**4.4**	**3.2**	**1.9**	**100**	
4b	10000			1.5103			**45.097**	
	5000			0.5709			**17.047**	
	2500			0.5405			**16.139**	
	1250			0.5429			**16.211**	
	625			0.5427			**16.205**	
	312.5			0.3904			**11.657**	
	156.25			0.2904			**8.671**	
	78.12			0.1801			**5.378**	

Sample code	Concentration (µg/ml)			O.D			Activity %	IC50
MCF7	-----			0.1	0.0	0.0		
Flag.	100	50	25	4.4	3.2	1.9	100	
8b	10000			1.5769			47.086	
	5000			0.5145			15.363	
	2500			0.4843			14.461	
	1250			0.4010			11.974	
	625			0.2718			8.116	
	312.5			0.2555			7.629	
	156.25			0.1999			5.969	
	78.12			0.2348			7.011	

Sample code	Concentration (µg/ml)			O.D			Activity %	IC50
MCF7	-----			0.1	0.0	0.0		
Flag.	100	50	25	4.4	3.2	1.9	100	
8f	10000			0.8189			24.452	
	5000			0.8861			26.459	
	2500			0.7735			23.096	
	1250			0.6371			19.024	
	625			0.4478			13.371	
	312.5			0.3659			10.926	
	156.25			0.2174			6.491	
	78.12			0.1710			5.106	

Sample code	Concentration (µg/ml)			O.D			Activity %	IC50
MCF7	-----			0.1	0.0	0.0		
Flag.	100	50	25	4.4	3.2	1.9	100	
8g	10000			1.6076			48.002	
	5000			0.9731			29.056	
	2500			0.8762			26.163	
	1250			0.7591			22.666	
	625			0.6314			18.853	
	312.5			0.7261			21.681	
	156.25			0.4676			13.962	
	78.12			0.2082			6.217	

Sample code	Concentration (µg/ml)			O.D			Activity %	IC50
MCF7	**-----**			**0.1**	**0.0**	**0.0**		
Flag.	**100**	**50**	**25**	**4.4**	**3.1**	**1.9**	**100**	
8h	**10000**			1.7047			**50.902**	
	5000			1.2601			**37.626**	
	2500			0.8051			**24.040**	
	1250			0.8919			**26.632**	
	625			0.5862			**17.504**	
	312.5			0.5056			**15.097**	
	156.25			0.5399			**16.121**	
	78.12			0.2172			**6.486**	

Sample code	Concentration (µg/ml)			O.D			Activity %	IC50
MCF7	**-----**			**0.1**	**0.0**	**0.0**		
Flag.	**100**	**50**	**25**	**4.4**	**3.1**	**1.9**	**100**	
8i	**10000**			1.2472			**37.241**	
	5000			0.6148			**18.358**	
	2500			0.6271			**18.725**	
	1250			0.3777			**11.278**	
	625			0.2679			**7.999**	
	312.5			0.1634			**4.879**	
	156.25			0.1440			**4.300**	
	78.12			0.1450			**4.330**	

Researcher: Prof. Dr. Hany

Experiment: (**VEGFR-2 & EGFR**) level in (MCF7 and HCT-116) cell line.

Number of Sample: 8 + **Vandetanib as reference drug**

Method: Enzyme-Linked Immunosorbent Assay (ELISA)

Principles:

Sandwich ELISA

EGFR Level (ng/ml) in (MCF7 cell line):

Sample code	Conc. ng/ml			Mean conc.	St. D.
4a	5.835	4.903	5.948	**5.562**	0.573501
8a	6.584	6.890	6.988	**6.821**	0.210735
4b	2.665	2.722	3.098	**2.828**	0.235271
8b	10.54	11.21	10.98	**10.910**	0.340441
8f	4.577	4.752	4.420	**4.583**	0.166081
8g	15.53	8.816	9.335	**11.227**	3.735532
8h	2.631	2.218	2.265	**2.371**	0.226102
8i	3.219	4.529	4.405	**4.051**	0.723196
Vandetanib	2.512	1.870	2.511	**2.298**	0.370371
MCF7	12.564	12.308	12.636	**12.503**	0.172387

EGFR Level (ng/ml) in (HCT-116 cell line):

Sample code	Conc. ng/ml			Mean conc.	St. D.
4a	10.36	7.831	8.871	**9.021**	1.271126
8a	5.085	5.607	4.698	**5.130**	0.456168
4b	9.200	15.26	9.663	**11.374**	3.37304
8b	11.38	12.09	19.50	**14.323**	4.497158
8f	9.872	8.801	8.934	**9.202**	0.583749
8g	15.06	10.82	10.08	**11.987**	2.687179
8h	3.621	2.574	4.525	**3.573**	0.976373
8i	6.913	5.584	3.978	**5.492**	1.469677
Vandetanib	3.056	2.563	2.432	**2.684**	0.329035
HCT-116	10.776	10.803	11.089	**10.889**	0.173443

VEGFR-2 Level (ng/ml) in (MCF7 cell line):

Sample code	Conc. ng/ml			Mean conc.	St. D.
4a	3.553	3.190	3.604	**3.449**	0.225745
8a	2.942	2.812	2.993	**2.916**	0.093329
4b	2.312	2.529	2.477	**2.439**	0.113298
8b	2.660	2.659	2.683	**2.667**	0.013577
8f	1.875	2.150	2.198	**2.074**	0.174288
8g	3.842	3.729	4.017	**3.863**	0.145108
8h	1.463	1.382	1.469	**1.438**	0.04859
8i	1.811	1.824	1.739	**1.791**	0.045786
Vandetanib	1.321	1.231	1.240	**1.264**	0.049568
MCF7	8.996	8.228	9.011	**8.745**	0.447798

VEGFR-2 Level (ng/ml) in (HCT-116 cell line):

Sample code	Conc. ng/ml			Mean conc.	St. D.
4a	2.229	2.214	2.275	**2.239**	0.031786
8a	2.209	2.852	2.544	**2.535**	0.321594
4b	3.932	4.059	4.122	**4.038**	0.09678
8b	1.657	1.593	1.494	**1.581**	0.082124
8f	2.748	2.989	2.712	**2.816**	0.150613
8g	3.354	3.547	3.273	**3.391**	0.140763
8h	1.229	0.9645	1.454	**1.216**	0.245015
8i	1.396	1.456	1.571	**1.474**	0.088929
Vandetanib	0.981	1.076	0.979	**1.012**	0.055524
HCT-116	7.108	7.452	7.242	**7.267**	0.173394

Prof. Dr. Hany

Enzyme assay (EGFR kinase):

A- MCF7

code	O.D.			Mean	Activity %	Inhibition %
MCF7	584.20	515.50	559.00	552.900	100.0	0.0
Doxo.	392.20	362.30	382.80	379.100	68.566	31.434
4a	92.69	159.70	242.00	164.797	29.806	70.194
4b	141.40	236.00	127.00	168.133	30.409	69.591
8a	110.50	124.70	215.00	150.067	27.142	72.858
8b	219.20	109.20	214.70	181.033	32.743	67.257
8f	127.40	174.00	164.00	155.133	28.058	71.942
8g	208.10	157.80	195.30	187.067	33.834	66.166
8h	235.40	175.70	149.20	186.767	33.779	66.221
8i	135.80	195.40	110.20	147.133	26.611	73.389

B- HCT-116

code	O.D.			Mean	Activity %	Inhibition %
HCT	451.0	483.9	454.1	463.000	100.0	0.0
Doxo.	240.6	262.0	278.4	260.333	56.228	43.772
4a	123.7	155.2	172.4	150.433	32.491	67.509
4b	224.8	156.4	190.6	190.600	41.166	58.834
8a	317.5	177.6	197.2	230.767	49.842	50.158
8b	178.2	168.2	213.3	186.567	40.295	59.705
8f	144.4	111.1	172.5	142.667	30.814	69.186
8g	233.1	134.8	143.5	170.467	36.818	63.182
8h	238.5	209.4	141.0	196.300	42.397	57.603
8i	184.9	202.4	142.6	176.633	38.150	61.850

Institute / Researcher: **Dr. Hany**

Experiment: **Relative Quantification of Gene expression**

(Fold change)

Samples number: **3 group**

Experiment design: **Gene Expression of (Bax, Pcl2 and HSP-70 genes) in different cell line.**

Laboratory comments:

Extraction of the total RNA is according to Qiagen Kit

Purification of total RNA from testis tissue using the RNeasy® Mini Kit

- At the end of purification procedure the extracted RNA will be evaluated to insure that the extracted RNA well purified and free of contamination, this will be achieved by measuring the extract by U.V spectrophotometer at wavelength 260/280 nm (**we use Denovix Spectrophotometer AGBL USA**).

Concentration and purity of Extracted RNA:

Sample	Conc. ng/µl			A 260			A mean (260/280)	Notes
MCF10A	66.25	65.86	66.48	1.6563	1.6464	1.6621	1.66	
MCF7	57.90	57.87	57.73	1.4476	1.4467	1.4433	1.61	
MCF7-8h	63.19	63.63	63.49	1.5797	1.5908	1.5873	1.58	
CCD841	59.95	60.18	59.77	1.4987	1.5045	1.4942	1.67	
HCT116	68.62	67.54	68.10	1.7154	1.6886	1.7024	1.73	
HCT-8h	67.16	66.62	66.90	1.6789	1.6655	1.6724	1.60	
HLF	63.20	63.44	63.69	1.5799	1.5861	1.5922	1.71	
A549	72.25	71.83	71.19	1.8062	1.7958	1.7798	1.93	
A549-8h	62.04	61.95	61.30	1.5509	1.5487	1.5324	1.64	

Converting extracted RNA into Double Stranded DNA (ds DNA) for PCR reaction

The conversion achieved by reverse transcriptase according to Qiagen QuantiTect RT kit.

For quantitative, real-time PCR:

Optimized kit for quantitative, real-time PCR, which includes Taq polymerase; quantitative, real-time PCR buffer; primers; SYBR® Green I dye; and nucleotides

Primer sequence:

Gene	Sequence	Tm	Product size (bp)	Accession Number
BAX	**F:** 5′-GATTACAGACCCCAGGCAGG-3′ **R:** 5′-TGGCTCAAGTAGGACGGGTA-3′	54	130	**(NM_001291428)**
Bcl-2	**F:** 5′-GCAATGGGCACGAGTTTGTT-3′ **R:** 5′- AGTGTGTTCACCAGGCCAAA-3′	60	170	**(NM_000633)**
HSP-70	**F:** : 5′-GGACATCAGCCAGAACAAGC-3′ **R:** 5-CCCTCGAACAGAGTCGAT-3′	56	230	**(NM_005345)**
GAPDH	**F:** 5′-CCATCAACGACCCCTTCATT-3′ **R:** 5′-CACGACATACTCAGCACCAGC-3′	**58**	193	**(NM_001256799)**

- qPCR data analyzed by double delta Ct analysis

The double delta Ct analysis assumes that:

- There is equal primer efficiency between primer sets (i.e. within 5%);
- There is near 100% amplification efficacy of the reference and the target genes;
- The internal control genes are constantly expressed and aren't affected by the treatment.

The method generally caters to experiments with a large number of DNA samples and a low number of genes to be tested.

1. Take the average of the Ct values for the housekeeping gene and the gene being tested in the experimental and control conditions, returning 4 values. The 4 values are Gene being Tested

Experimental (TE), Gene being Tested Control (TC), Housekeeping Gene Experimental (HE), and Housekeeping Gene Control (HC).

2. Calculate the differences between experimental values (TE – HE) and the control values (TC – HC). These are your \DeltaΔCt values for the experimental (\DeltaΔCTE) and control (\DeltaΔCTC) conditions, respectively.

3. Then, calculate the difference between the \DeltaΔCT values for the experimental and the control conditions (\DeltaΔCTE – \DeltaΔCTC) to arrive at the double delta Ct value (ddCt).

4. Since all calculations are in logarithm base 2, every time there is twice as much DNA, your Ct values decrease by 1 and will not halve. You need to calculate the value of 2^{-2\Delta\Delta C_{t}}−2ΔΔ*Ct* to get the expression fold change. **(Kenneth J. Livak and Thomas D. Schmittgen 2001)**.

PCR condition:

Start Activation Temperature: 95 C for 3 min.

Denaturation Temperature: 95 C for 30 sec.

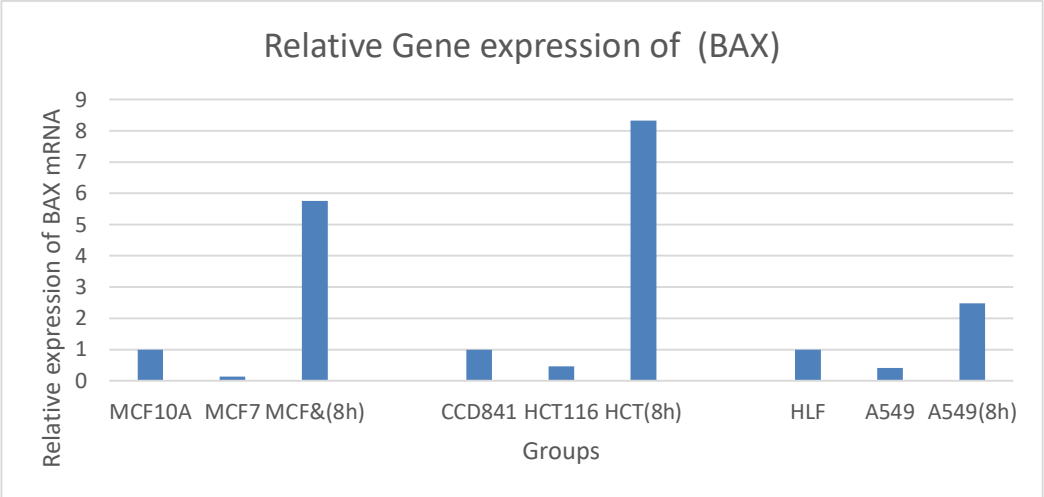
Annealing Temperature: (54 C for 40 sec.)

Extension Temperature: 72 C for 45 Sec.

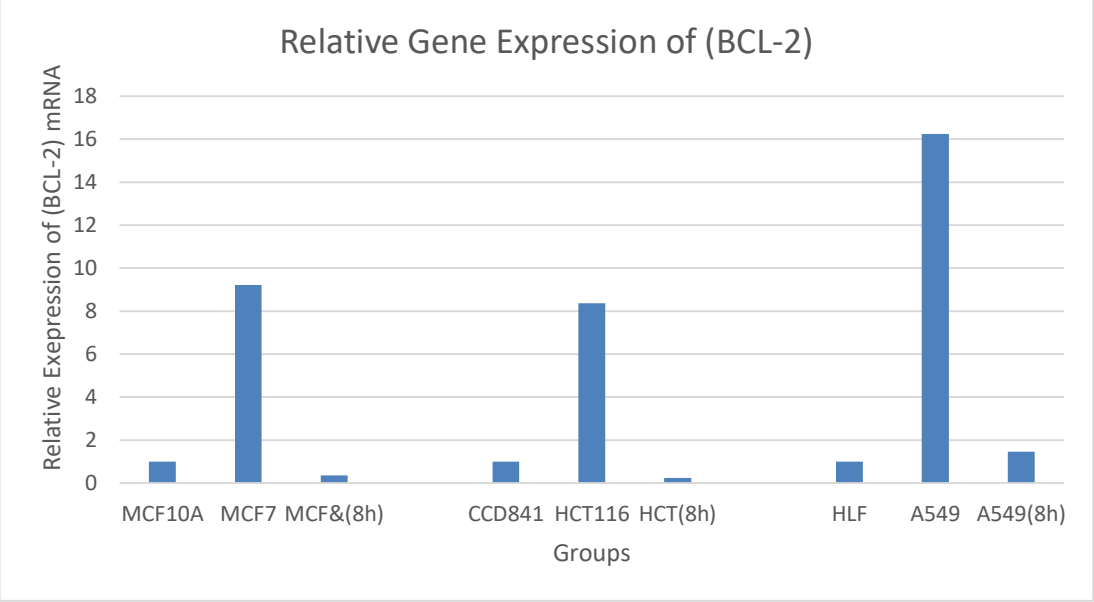
- There are 40 cycles for the complete run.
- Reaction Volume **10 µL**: Master mix 10 µL, 0.5 µL for each Primer (F-R), 2 µL of DNA template and 7 µL DD – RNAse – DNAse- free Water

Results:

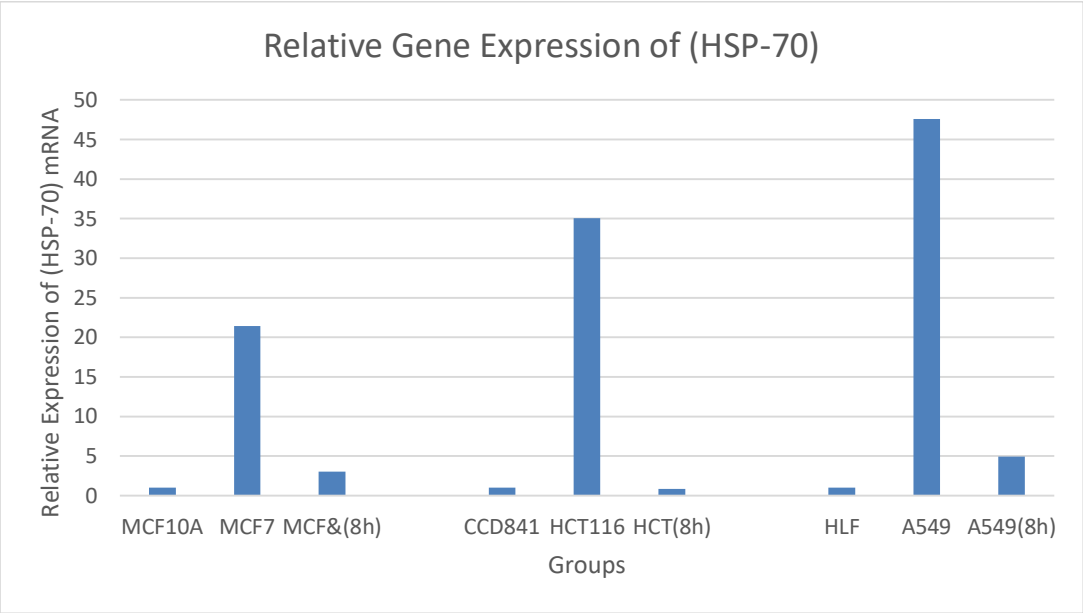
Group type	BAX: Ct			Mean Bax-Ct	GAPDH (H.K.G): Ct			Mean HKG-Ct	ΔCt	ΔΔCt	$2^{-\Delta\Delta Ct}$ Fold Change
MCF-10A	26.97	27.28	27.15	**27.13**	18.32	21.45	21.29	**20.35**	**6.78**	**0.0556**	**0.9622**
MCF-10A	27.87	26.69	27.12	**27.23**	20.53	20.61	19.88	**20.34**	**6.89**	**-0.0511**	**1.0361**
MCF-10A	27.18	26.22	26.51	**26.64**	20.18	20.09	19.12	**19.80**	**6.84**	**-0.0044**	**1.0031**
MCF-7	29.18	28.63	30.77	**29.53**	20.77	18.68	19.71	**19.72**	**9.81**	**2.9711**	**0.1275**
MCF-7(8h)	25.91	26.12	26.73	**26.25**	22.03	21.87	21.93	**21.94**	**4.31**	**-2.5256**	**5.7580**
CCD841-CoN	28.58	28.39	28.92	**28.63**	20.42	19.05	20.11	**19.86**	**8.77**	**0.0711**	**0.9519**
CCD841-CoN	29.17	28.12	29.03	**28.77**	18.88	20.43	20.32	**19.88**	**8.90**	**-0.0556**	**1.0393**
CCD841-CoN	28.88	28.42	27.2	**28.17**	19.66	19.32	18.95	**19.31**	**8.86**	**-0.0156**	**1.0108**
HCT-116	30.08	30.87	31.32	30.76	21.77	20.68	20.04	20.83	9.93	**1.0856**	**0.4712**
HCT-116(8h)	27.92	28.03	27.23	27.73	21.83	21.07	22.93	21.94	5.78	**-3.0578**	**8.3269**
HLF	27.18	27.06	27.12	**27.12**	22.12	23.28	23.73	**23.04**	**4.08**	**-0.0144**	**1.0101**
HLF	27.44	27.32	27.97	**27.58**	22.98	23.52	24.11	**23.54**	**4.04**	**0.0222**	**0.9847**
HLF	27.58	27.72	26.02	**27.11**	23.06	22.92	23.13	**23.04**	**4.07**	**-0.0078**	**1.0054**
A549	28.15	29.11	28.99	28.75	22.96	23.18	24.12	23.42	5.33	**1.2678**	**0.4153**
A549(8h)	25.42	24.98	24.23	24.88	22.13	23.71	20.54	22.13	2.75	**-1.3122**	**2.4832**



Group type	BCL2: Ct			Mean Bcl2-Ct	GAPDH (H.K.G): Ct			Mean HKG-Ct	ΔCt	ΔΔCt	$2^{-\Delta\Delta Ct}$ Fold Change
MCF-10A	31.96	28.48	29.71	**30.05**	18.32	21.45	21.29	**20.35**	**9.70**	**0.0144**	**0.9900**
MCF-10A	29.94	29.88	30.21	**30.01**	20.53	20.61	19.88	**20.34**	**9.67**	**0.0411**	**0.9719**
MCF-10A	30.15	28.62	29.92	**29.56**	20.18	20.09	19.12	**19.80**	**9.77**	**-0.0556**	**1.0393**
MCF-7	25.03	27.21	26.44	**26.23**	20.77	18.68	19.71	**19.72**	**6.51**	**-3.2044**	**9.2179**
MCF-7(8h)	32.88	34.42	32.12	**33.14**	22.03	21.87	21.93	**21.94**	**11.20**	**1.4856**	**0.3571**
CCD841-CoN	30.93	31.98	33.52	**32.14**	20.42	19.05	20.11	**19.86**	**12.28**	**0.0489**	**0.9667**
CCD841-CoN	32.64	32.44	31.51	**32.20**	18.88	20.43	20.32	**19.88**	**12.32**	**0.0122**	**0.9916**
CCD841-CoN	32.75	31.49	30.87	**31.70**	19.66	19.32	18.95	**19.31**	**12.39**	**-0.0611**	**1.0433**
HCT-116	30.73	29.83	29.73	**30.10**	21.77	20.68	20.04	**20.83**	**9.27**	**-3.0656**	**8.3719**
HCT-116(8h)	34.02	38.12	36.80	**36.31**	21.83	21.07	22.93	**21.94**	**14.37**	**2.0378**	**0.2435**
HLF	34.64	34.59	34.17	**34.47**	22.12	23.28	23.73	**23.04**	**11.42**	**-0.0178**	**1.0124**
HLF	34.55	34.73	35.41	**34.90**	22.98	23.52	24.11	**23.54**	**11.36**	**0.0456**	**0.9689**
HLF	35.35	34.84	33.22	**34.47**	23.06	22.92	23.13	**23.04**	**11.43**	**-0.0278**	**1.0194**
A549	29.89	30.97	31.55	**30.80**	22.96	23.18	24.12	**23.42**	**7.38**	**-4.0222**	**16.2484**
A549(8h)	31.99	34.38	32.54	**32.97**	22.13	23.71	20.54	**22.13**	**10.84**	**-0.5622**	**1.4765**



Group type	HSP70: Ct			Mean HSP-Ct	GAPDH (H.K.G): Ct			Mean HKG-Ct	ΔCt	ΔΔCt	$2^{-\Delta\Delta Ct}$ Fold Change
MCF-10A	36.73	37.15	36.10	**36.66**	18.32	21.45	21.29	**20.35**	16.31	**-0.1356**	**1.0985**
MCF-10A	36.22	36.37	36.52	**36.37**	20.53	20.61	19.88	**20.34**	16.03	**0.1411**	**0.9068**
MCF-10A	35.87	37.04	35.01	**35.97**	20.18	20.09	19.12	**19.80**	16.18	**-0.0056**	**1.0039**
MCF-7	32.24	32.12	30.05	**31.47**	20.77	18.68	19.71	**19.72**	11.75	**-4.4211**	**21.4233**
MCF-7(8h)	37.59	36.92	35.02	**36.51**	22.03	21.87	21.93	**21.94**	14.57	**-1.6044**	**3.0408**
CCD841-CoN	36.24	36.62	34.53	**35.80**	20.42	19.05	20.11	**19.86**	**15.94**	**0.0044**	**0.9969**
CCD841-CoN	34.91	36.73	36.12	**35.92**	18.88	20.43	20.32	**19.88**	**16.04**	**-0.1022**	**1.0734**
CCD841-CoN	35.47	35.66	34.33	**35.15**	19.66	19.32	18.95	**19.31**	**15.84**	**0.0978**	**0.9345**
HCT-116	31.88	29.92	33.12	**31.64**	21.77	20.68	20.04	**20.83**	**10.81**	**-5.1311**	**35.0444**
HCT-116(8h)	38.19	37.77	38.45	**38.14**	21.83	21.07	22.93	**21.94**	**16.19**	**0.2522**	**0.8396**
HLF	38.25	37.65	34.99	**36.96**	22.12	23.28	23.73	**23.04**	**13.92**	**0.1256**	**0.9167**
HLF	38.23	37.28	37.26	**37.59**	22.98	23.52	24.11	**23.54**	**14.05**	**-0.0078**	**1.0054**
HLF	38.18	37.31	36.11	**37.20**	23.06	22.92	23.13	**23.04**	**14.16**	**-0.1178**	**1.0851**
A549	30.28	31.87	33.53	**31.89**	22.96	23.18	24.12	23.42	**8.47**	**-5.5722**	**47.5780**
A549(8h)	33.24	34.17	34.21	**33.87**	22.13	23.71	20.54	22.13	**11.75**	**-2.2989**	**4.9208**



Institute / Researcher: **Prof. Dr. Hany**

Experiment: **Western Blotting**

(HSP70, Bax & Bcl2)

Samples number: **1 (8h) sample**

Experiment design: first step protein separation by (SDS-PAGE), then second step which consists of applying specific Antibody for the protein.

Laboratory comments:

Solutions and reagents:

lysis buffers:-

These buffers may be stored at 4°C for several weeks or aliquoted and stored at -20°C for up to a year.

NP-40 buffer

- 150 mM NaCl
- 1.0% NP-40 (possible to substitute with 0.1% Triton X-100)
- 50 mM Tris-HCl, pH 8.0
- Protease inhibitors

RIPA buffer (radioimmunoprecipitation assay buffer)

- 150 mM NaCl
- 1% IGEPAL CA-630
- 0.5% sodium deoxycholate
- 0.1% SDS (sodium dodecyl sulphate)
- 50 mM Tris-HCl, pH 8.0
- Protease inhibitors

Tris-HCl

- 20 mM Tris-HCl
- Protease inhibitors

Solutions and reagents: running, transfer, and blocking buffers:-

Laemmli 2X buffer/loading buffer

- 4% SDS
- 10% 2-mercaptoethanol
- 20% glycerol
- 0.004% bromophenol blue
- 0.125 M Tris-HCl

  Check the pH and adjust to 6.8

Running buffer (Tris-Glycine/SDS)

- 25 mM Tris base
- 190 mM glycine
- 0.1% SDS

  Check the pH and adjust to 8.3

Transfer buffer (wet)

- 25 mM Tris base
- 190 mM glycine
- 20% methanol
- Check the pH and adjust to 8.3

For proteins larger than 80 kDa, we recommend that SDS is included at a final concentration of 0.1%.

Transfer buffer (semi-dry)

- 48 mM Tris
- 39 mM glycine
- 20% methanol
- 0.04% SDS

Blocking buffer

3–5% milk or BSA (bovine serum albumin)
Add to TBST buffer. Mix well and filter. Failure to filter can lead to spotting, where tiny dark grains will contaminate the blot during color development.

Sample lysis

Preparation of lysate from tissues

1. Dissect the tissue of interest with clean tools, on ice preferably, and as quickly as possible to prevent degradation by proteases.
2. Place the tissue in round-bottom microcentrifuge tubes or Eppendorf tubes and immerse in liquid nitrogen to snap freeze. Store samples at -80°C for later use or keep on ice for immediate homogenization. For a ~5 mg piece of tissue, add ~300 µL of ice-cold lysis buffer rapidly to the tube, homogenize with an electric homogenizer, rinse the blade twice with another 2 x 200 µL lysis buffer, then maintain constant agitation for 2 h at 4°C (eg place on an orbital shaker in the fridge). Volumes of lysis buffer must be determined in relation to the amount of tissue present; protein extract should not be too dilute to avoid loss of protein and large volumes of samples to be loaded onto gels. The minimum concentration is 0.1 mg/mL, optimal concentration is 1–5 mg/mL.
3. Centrifuge for 20 min at 12,000 rpm at 4°C in a microcentrifuge. Gently remove the tubes from the centrifuge and place on ice, aspirate the supernatant, and place in a fresh tube kept on ice; discard the pellet.

Sample preparation

1. Remove a small volume of lysate to perform a protein quantification assay. Determine the protein concentration for each cell lysate.
2. Determine how much protein to load and add an equal volume 2X Laemmli sample buffer.

To reduce and denature your samples, boil each tissue homogenate in sample buffer at 100°C for 5 min. Lysates can be aliquoted and stored at -20°C for future use.

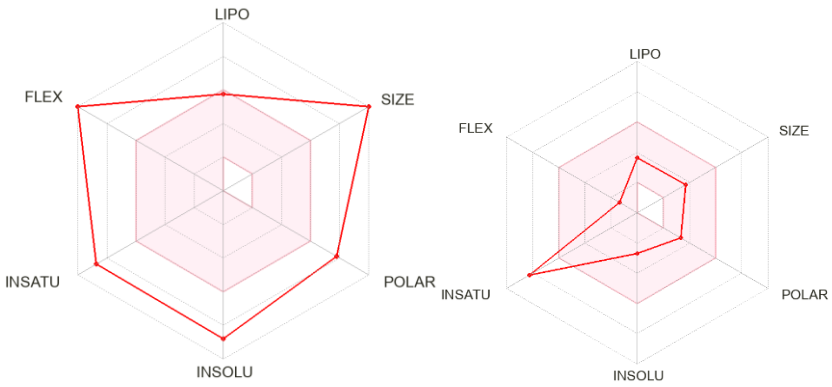


Fig. S4. ADME radar plots of (a) 8i from the SwissADME server.