

Viability assay

Institute / Researcher: prof.Dr. Modather Farouk

Experiment : **functional assay (MTT)**
(viability/cytotoxicity)

samples number : **6**

experiment design : effect against HepG2, A549 and Mcf7 cells

laboratory comments:

References

- Slater, T. et al. (1963) Biochem. Biophys. Acta 77:383.
van de Loosdrecht, A.A., et al. J. Immunol. Methods 174: 311-320, 1994.
Alley, M.C., et al. Cancer Res. 48: 589-601, 1988.

Viability assay

MTT protocol

Determination of sample cytotoxicity on cells (MTT protocol)

1-the 96 well tissue culture plate was inoculated with 1×10^5 cells / ml (100 ul / well) and incubated at 37°C for 24 hours to develop a complete monolayer sheet.

2- Growth medium was decanted from 96 well micro titer plates after confluent sheet of cells were formed, cell monolayer was washed twice with wash media.

3- two-fold dilutions of tested sample was made in RPMI medium with 2% serum (maintenance medium).

4- 0.1 ml of each dilution was tested in different wells leaving 3 wells as control, receiving only maintenance medium.

5- Plate was incubated at 37°C and examined. Cells were checked for any physical signs of toxicity, e.g. partial or complete loss of the monolayer, rounding, shrinkage, or cell granulation.

6- MTT solution was prepared (5mg/ml in PBS) (BIO BASIC CANADA INC).

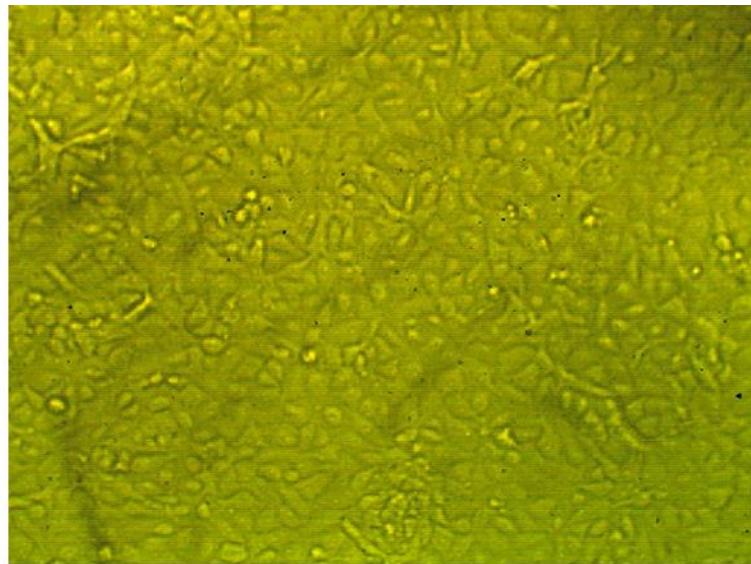
8- 20ul MTT solution were added to each well. Place on a shaking table, 150rpm for 5 minutes, to thoroughly mix the MTT into the media.

9) Incubate (37C, 5% CO₂) for 4 hours to allow the MTT to be metabolized.

10) Dump off the media. (dry plate on paper towels to remove residue if necessary.

- 11) Resuspend formazan (MTT metabolic product) in 200ul DMSO. Place on a shaking table, 150rpm for 5 minutes, to thoroughly mix the formazan into the solvent.
- 12) Read optical density at 560nm and subtract background at 620nm. Optical density should be directly correlated with cell quantity.

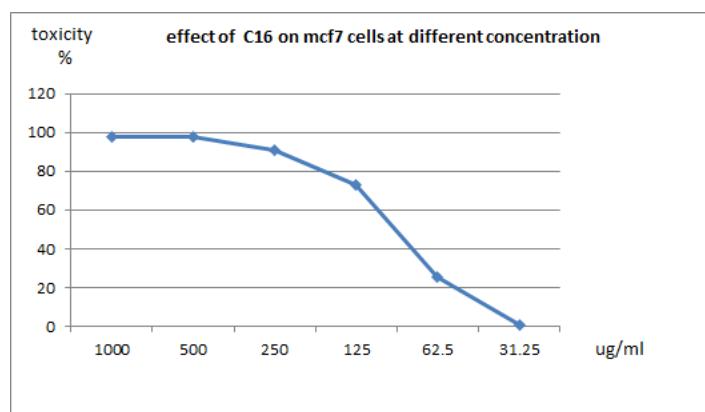
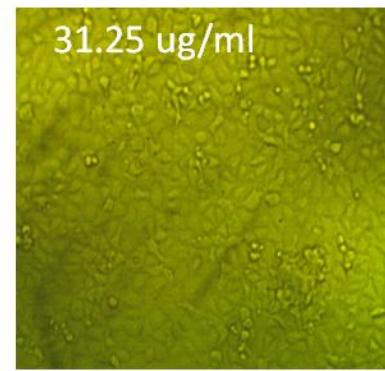
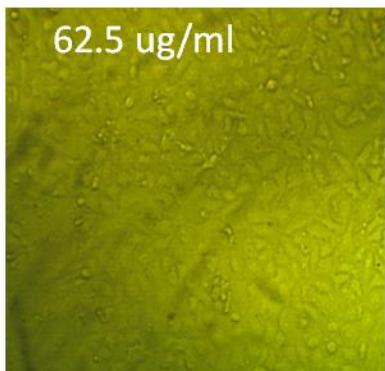
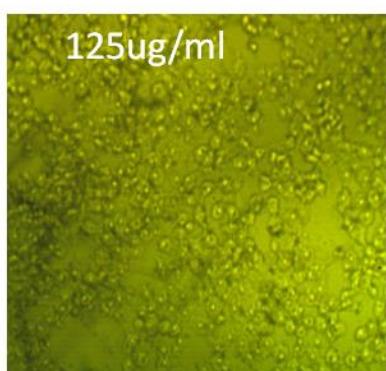
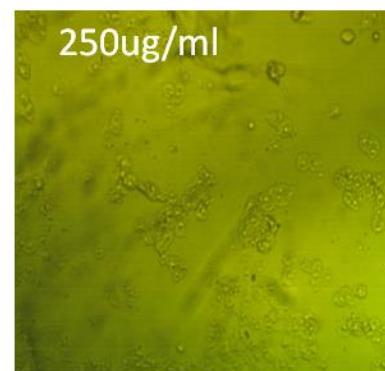
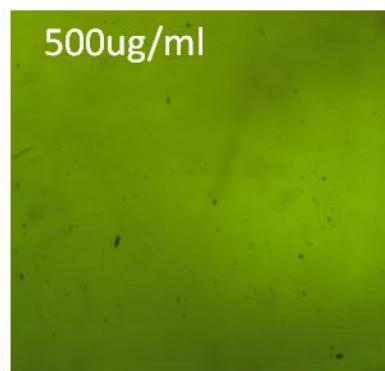
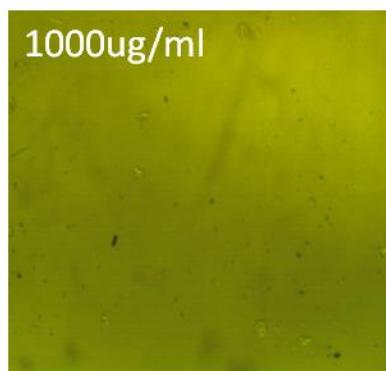
ID	ug/ml	O.D			Mean O.D	±SE	Viability %	Toxicity %	IC50 ± SD
Mcf7	-----	0.782	0.788	0.764	0.778	0.007211	100	0	ug
C16 (2b)	1000	0.016	0.018	0.016	0.016667	0.000667	2.142245073	97.85775493	95 ± 2.01
	500	0.019	0.017	0.018	0.018	0.000577	2.313624679	97.68637532	
	250	0.064	0.068	0.088	0.073333	0.007424	9.42587832	90.57412168	
	125	0.219	0.189	0.224	0.210667	0.010929	27.07797772	72.92202228	
	62.5	0.564	0.588	0.583	0.578333	0.007311	74.33590403	25.66409597	
	31.25	0.774	0.78	0.769	0.774333	0.00318	99.52870608	0.471293916	
C17(2a)	1000	0.033	0.028	0.041	0.034	0.003786	4.370179949	95.62982005	419.33 ± 10.23
	500	0.274	0.234	0.257	0.255	0.01159	32.77634961	67.22365039	
	250	0.758	0.744	0.78	0.760667	0.010477	97.77206512	2.227934876	
	125	0.784	0.764	0.776	0.774667	0.005812	99.57155099	0.428449015	
	62.5	0.772	0.779	0.758	0.769667	0.006173	98.92887746	1.071122536	
	31.25	0.778	0.783	0.756	0.772333	0.008293	99.27163668	0.728363325	
M11(2c)	1000	0.028	0.034	0.031	0.031	0.001732	3.984575835	96.01542416	227.87 ± 3.67
	500	0.056	0.074	0.057	0.062333	0.00584	8.011996572	91.98800343	
	250	0.317	0.324	0.33	0.323667	0.003756	41.60239931	58.39760069	
	125	0.699	0.732	0.718	0.716333	0.009563	92.07369323	7.926306769	
	62.5	0.774	0.769	0.782	0.775	0.003786	99.61439589	0.385604113	
	31.25	0.781	0.774	0.771	0.775333	0.002963	99.65724079	0.342759212	
M12(2d)	1000	0.018	0.02	0.022	0.02	0.001155	2.570694087	97.42930591	307.78 ± 3.3
	500	0.045	0.052	0.044	0.047	0.002517	6.041131105	93.95886889	
	250	0.453	0.472	0.444	0.456333	0.008253	58.65467009	41.34532991	
	125	0.738	0.759	0.766	0.754333	0.008413	96.958012	3.041988003	
	62.5	0.775	0.776	0.778	0.776333	0.000882	99.78577549	0.214224507	
	31.25	0.762	0.775	0.778	0.771667	0.00491	99.18594687	0.814053128	
RM1(2e)	1000	0.023	0.019	0.032	0.024667	0.003844	3.170522708	96.82947729	317.22 ± 4.52
	500	0.087	0.089	0.104	0.093333	0.005364	11.99657241	88.00342759	
	250	0.465	0.488	0.456	0.469667	0.009528	60.36846615	39.63153385	
	125	0.721	0.738	0.743	0.734	0.006658	94.34447301	5.655526992	
	62.5	0.779	0.765	0.781	0.775	0.005033	99.61439589	0.385604113	
	31.25	0.784	0.758	0.775	0.772333	0.007623	99.27163668	0.728363325	
RM22(2f)	1000	0.017	0.015	0.016	0.016	0.000577	2.05655527	97.94344473	87.11 ± 2.08
	500	0.032	0.04	0.02	0.030667	0.005812	3.941730934	96.05826907	
	250	0.089	0.094	0.078	0.087	0.004726	11.18251928	88.81748072	
	125	0.095	0.167	0.143	0.135	0.021166	17.35218509	82.64781491	
	62.5	0.546	0.537	0.55	0.544333	0.003844	69.96572408	30.03427592	
	31.25	0.778	0.772	0.775	0.775	0.001732	99.61439589	0.385604113	



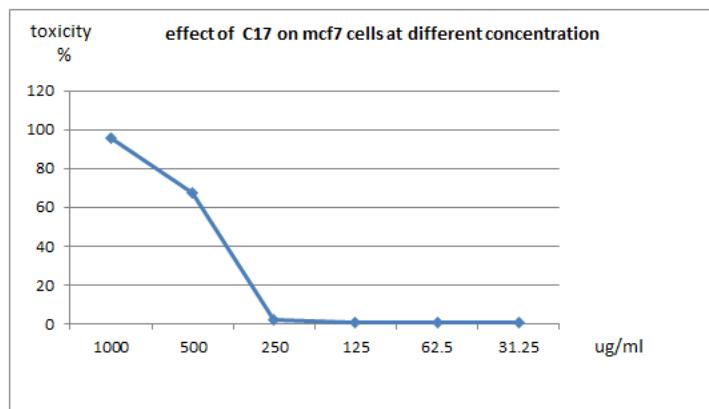
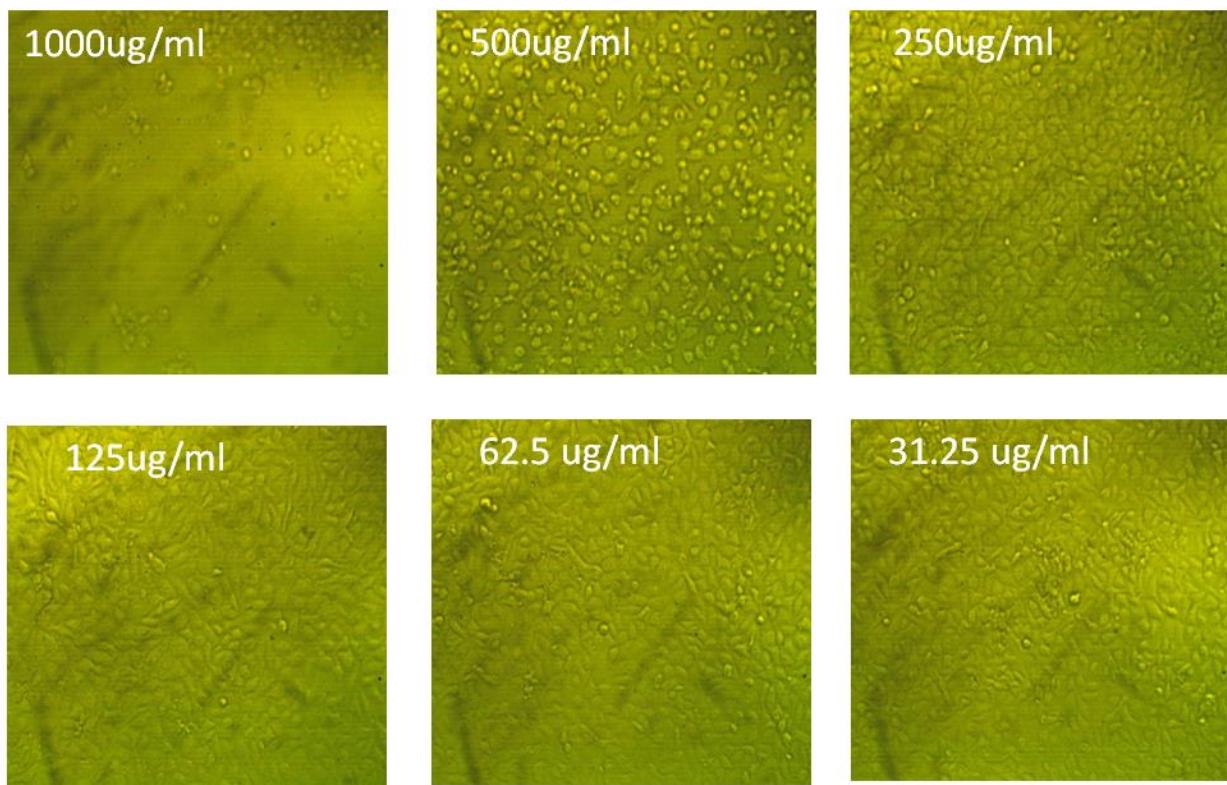
control
Mcf7 cells

Organism : *Homo sapiens*, human
Tissue : mammary gland, breast; derived from metastatic site: pleural effusion
Cell Type : epithelial
Culture Properties : adherent
Disease : adenocarcinoma
ATCC : HTB-22

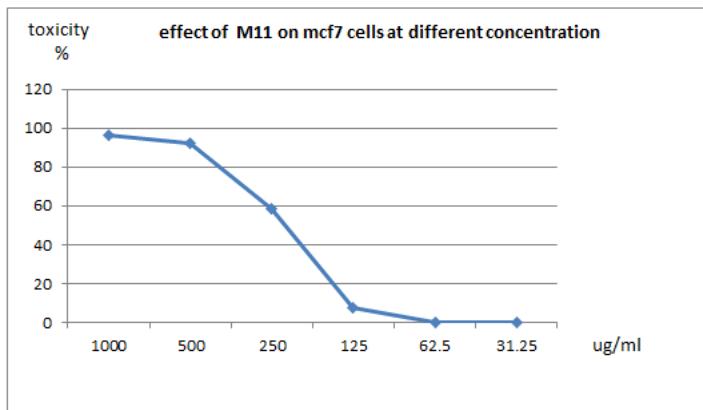
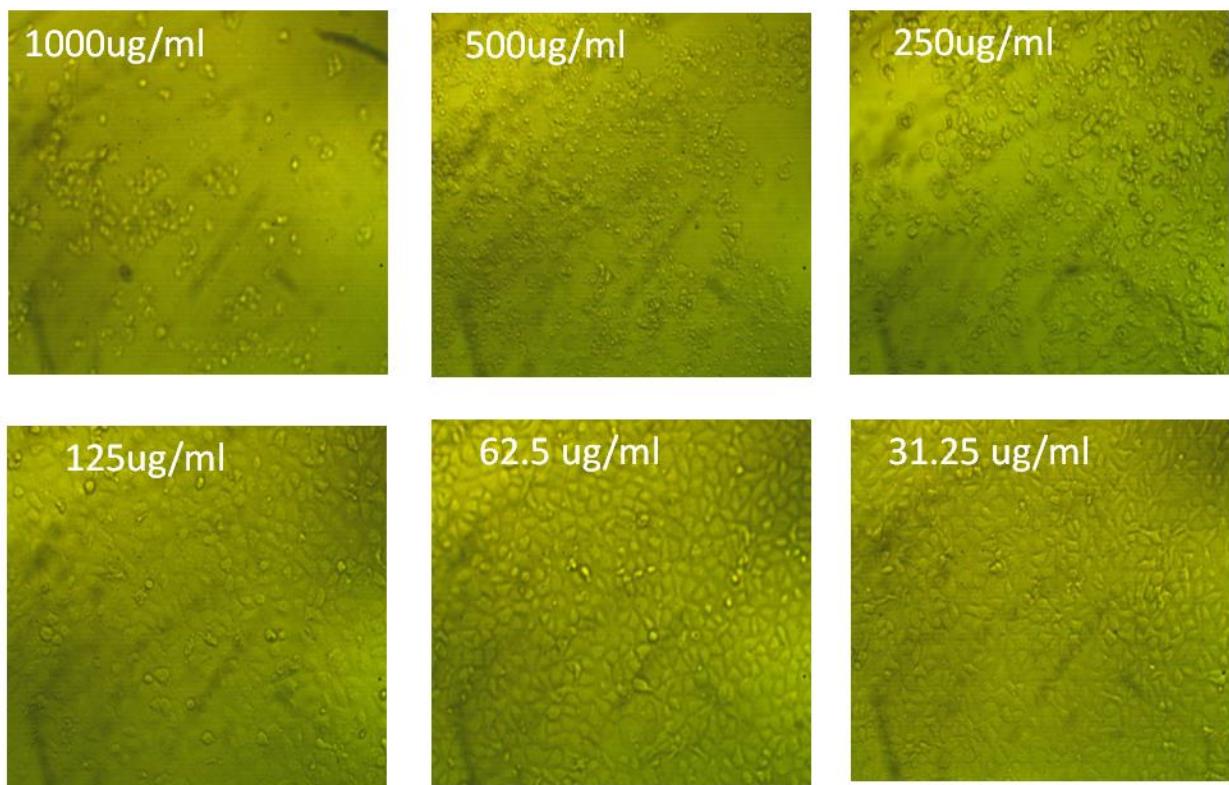
Effect of C16 on Mcf7 cells at different concentration



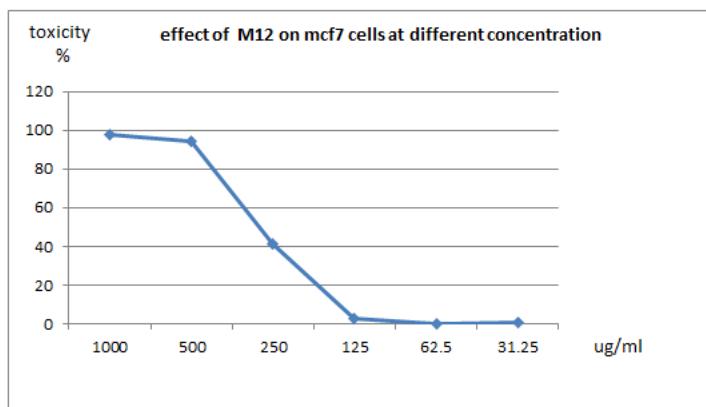
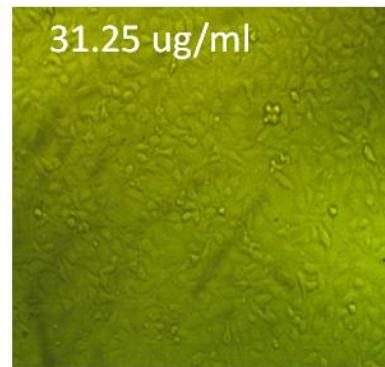
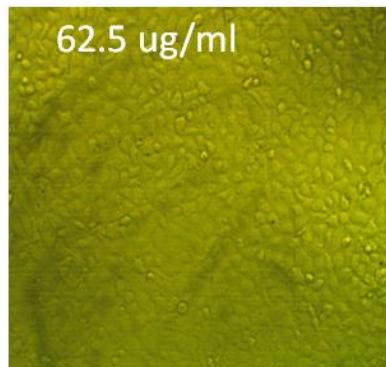
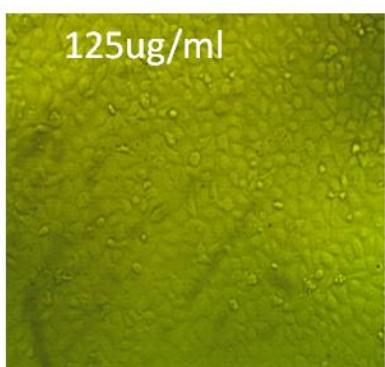
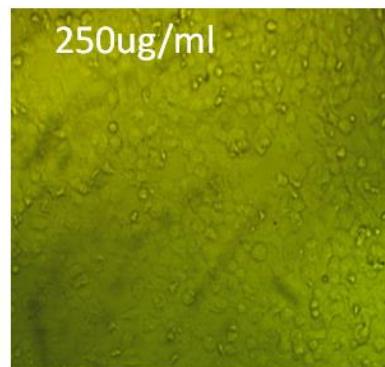
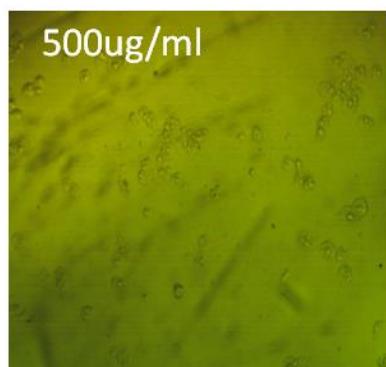
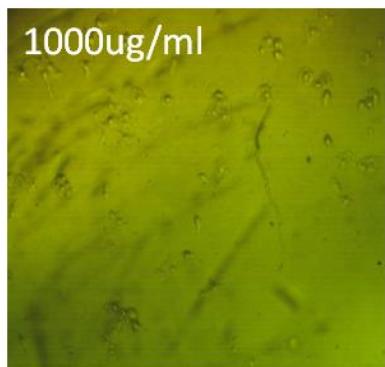
Effect of C17 on Mcf7 cells at different concentration



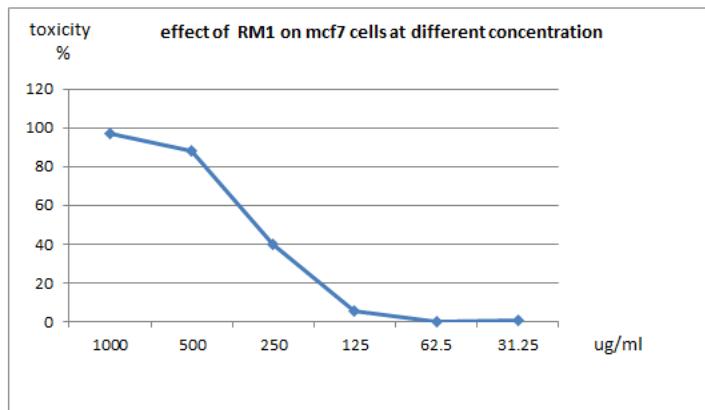
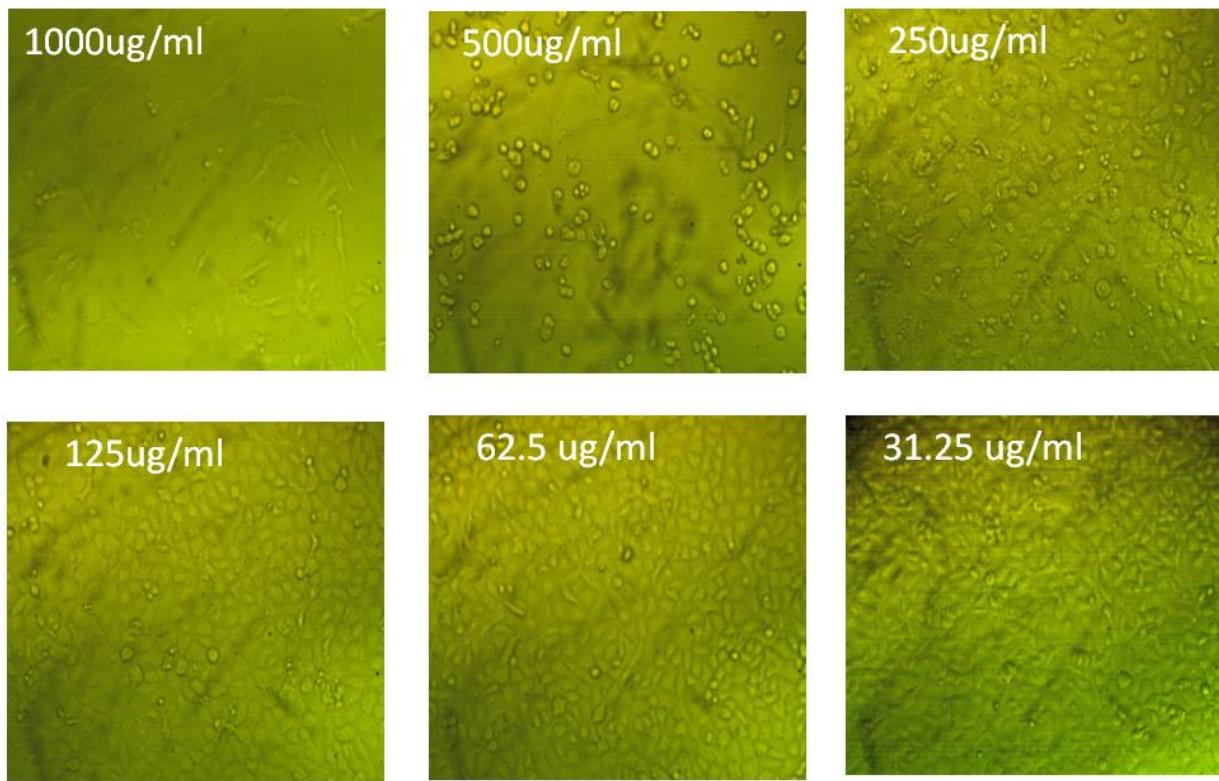
Effect of M11 on Mcf7 cells at different concentration



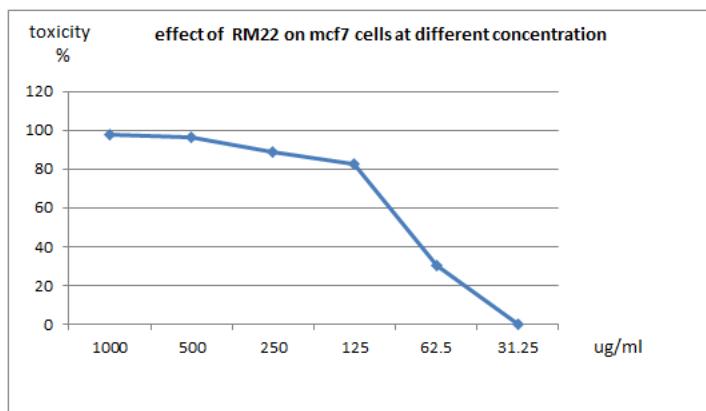
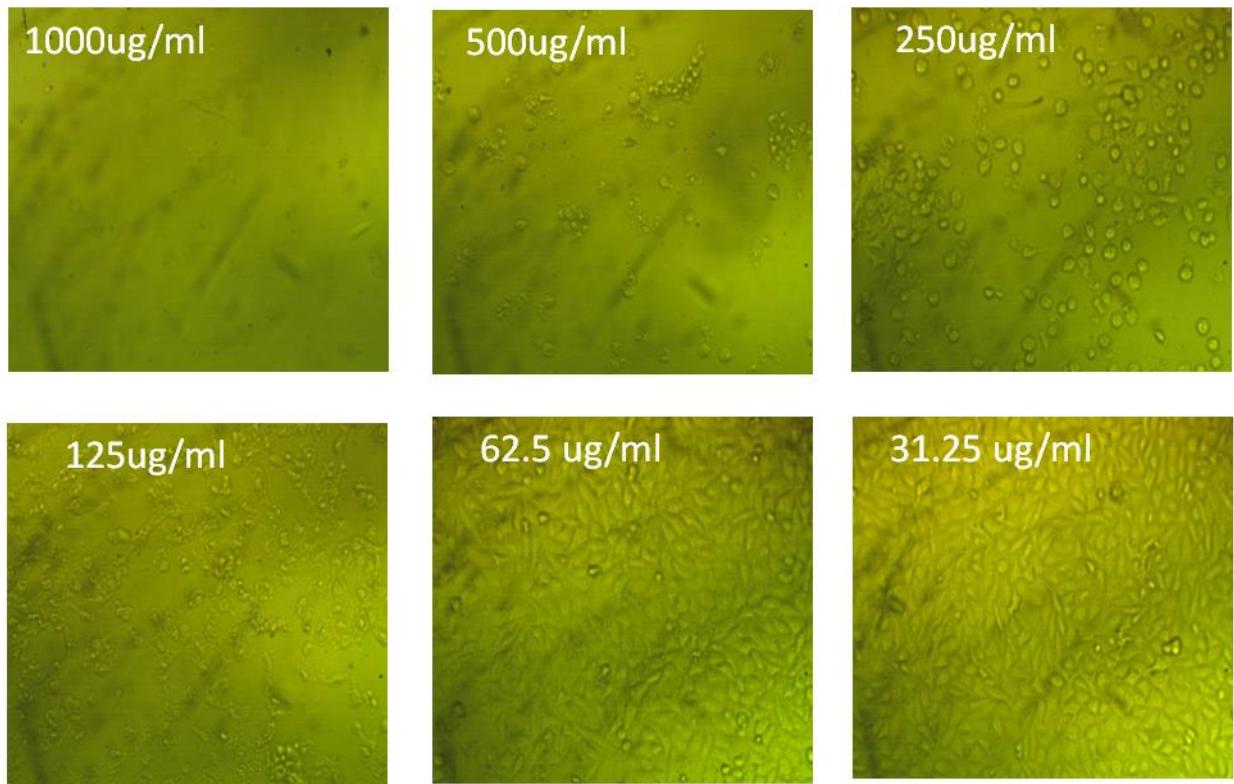
Effect of M12 on Mcf7 cells at different concentration



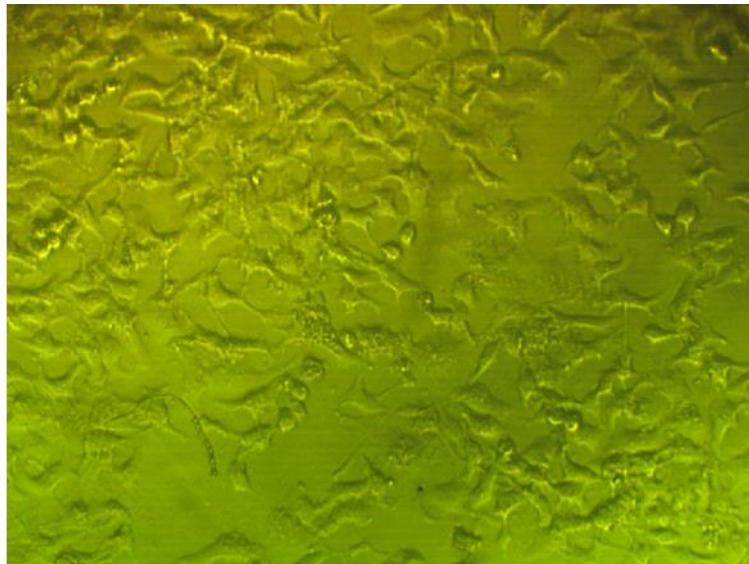
Effect of RM1 on Mcf7 cells at different concentration



Effect of RM22 on Mcf7 cells at different concentration



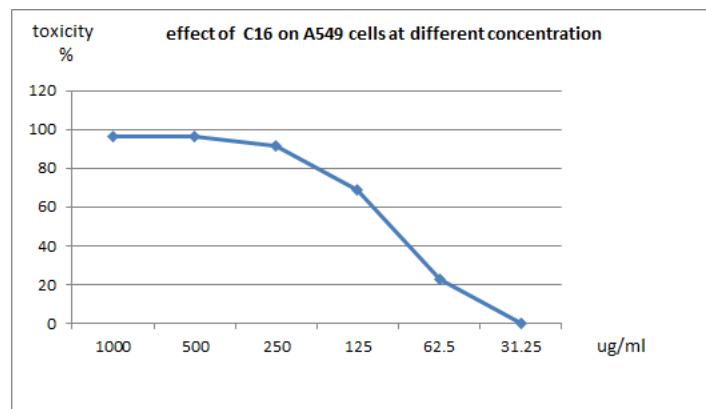
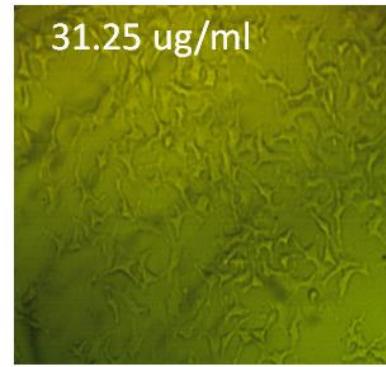
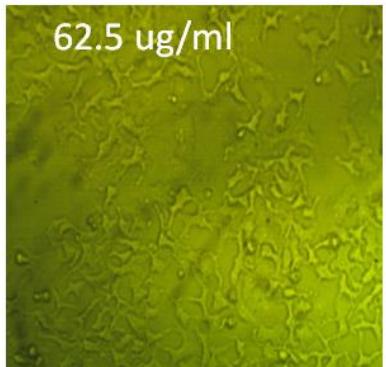
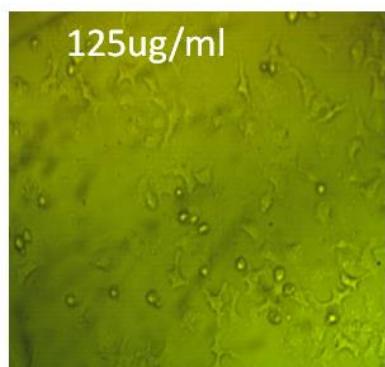
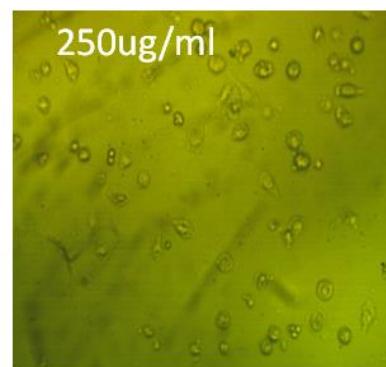
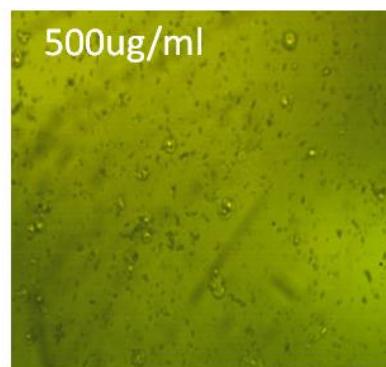
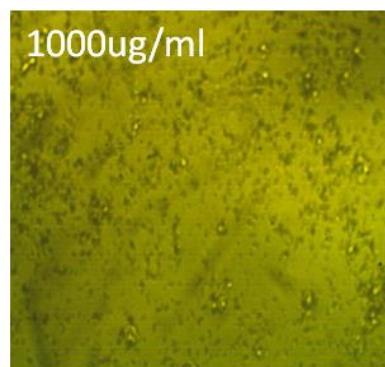
ID	ug/ml	O.D			Mean O.D	±SE	Viability %	Toxicity %	IC50 ± SD
A549	-----	0.478	0.484	0.499	0.487	0.006245	100	0	ug 99.57 ± 5.21
C16(2b)	1000	0.019	0.017	0.018	0.018	0.000577	3.696098563	96.30390144	
	500	0.019	0.018	0.019	0.018667	0.000333	3.832991102	96.1670089	
	250	0.028	0.043	0.056	0.042333	0.00809	8.692676249	91.30732375	
	125	0.174	0.139	0.144	0.152333	0.010929	31.27994524	68.72005476	
	62.5	0.395	0.337	0.401	0.377667	0.020407	77.54962355	22.45037645	
	31.25	0.498	0.475	0.488	0.487	0.006658	100	0	
C17(2a)	1000	0.018	0.017	0.019	0.018	0.000577	3.696098563	96.30390144	164.46 ± 0.99
	500	0.019	0.031	0.027	0.025667	0.003528	5.270362765	94.72963723	
	250	0.075	0.068	0.08	0.074333	0.00348	15.26351814	84.73648186	
	125	0.318	0.348	0.352	0.339333	0.010729	69.67830253	30.32169747	
	62.5	0.467	0.427	0.441	0.445	0.011719	91.37577002	8.624229979	
	31.25	0.473	0.49	0.465	0.476	0.007371	97.7412731	2.258726899	
M11(2c)	1000	0.016	0.018	0.016	0.016667	0.000667	3.422313484	96.57768652	129.93 ± 3.14
	500	0.019	0.015	0.017	0.017	0.001155	3.490759754	96.50924025	
	250	0.022	0.028	0.023	0.024333	0.001856	4.996577687	95.00342231	
	125	0.218	0.237	0.204	0.219667	0.009563	45.10609172	54.89390828	
	62.5	0.385	0.369	0.392	0.382	0.006807	78.43942505	21.56057495	
	31.25	0.428	0.465	0.452	0.448333	0.010837	92.06023272	7.939767283	
M12(2d)	1000	0.014	0.018	0.017	0.016333	0.001202	3.353867214	96.64613279	134.01 ± 2.79
	500	0.017	0.016	0.017	0.016667	0.000333	3.422313484	96.57768652	
	250	0.02	0.018	0.034	0.024	0.005033	4.928131417	95.07186858	
	125	0.216	0.246	0.223	0.228333	0.009062	46.88569473	53.11430527	
	62.5	0.411	0.385	0.358	0.384667	0.015301	78.98699521	21.01300479	
	31.25	0.475	0.468	0.489	0.477333	0.006173	98.01505818	1.984941821	
RM1(2e)	1000	0.017	0.019	0.017	0.017667	0.000667	3.627652293	96.37234771	222.27 ± 9.43
	500	0.018	0.019	0.022	0.019667	0.001202	4.038329911	95.96167009	
	250	0.189	0.216	0.204	0.203	0.00781	41.68377823	58.31622177	
	125	0.423	0.395	0.355	0.391	0.019732	80.28747433	19.71252567	
	62.5	0.478	0.453	0.496	0.475667	0.012468	97.67282683	2.327173169	
	31.25	0.489	0.476	0.473	0.479333	0.00491	98.4257358	1.574264203	
RM22(2f)	1000	0.032	0.046	0.054	0.044	0.006429	9.034907598	90.9650924	64.88 ± 0.53
	500	0.039	0.047	0.057	0.047667	0.005207	9.787816564	90.21218344	
	250	0.074	0.058	0.069	0.067	0.004726	13.75770021	86.24229979	
	125	0.084	0.079	0.094	0.085667	0.00441	17.59069131	82.40930869	
	62.5	0.147	0.138	0.188	0.157667	0.015388	32.37508556	67.62491444	
	31.25	0.389	0.402	0.391	0.394	0.004041	80.90349076	19.09650924	



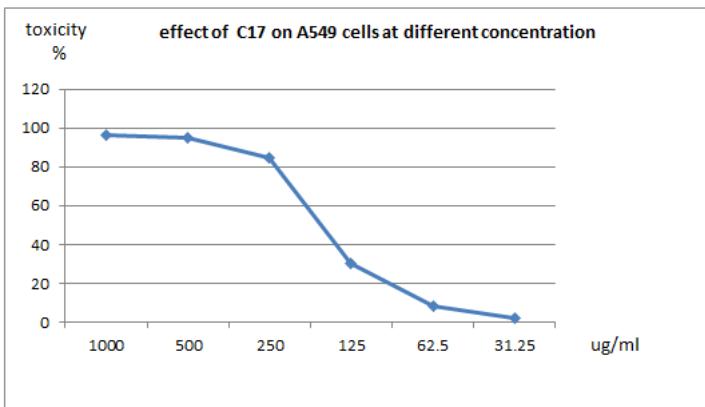
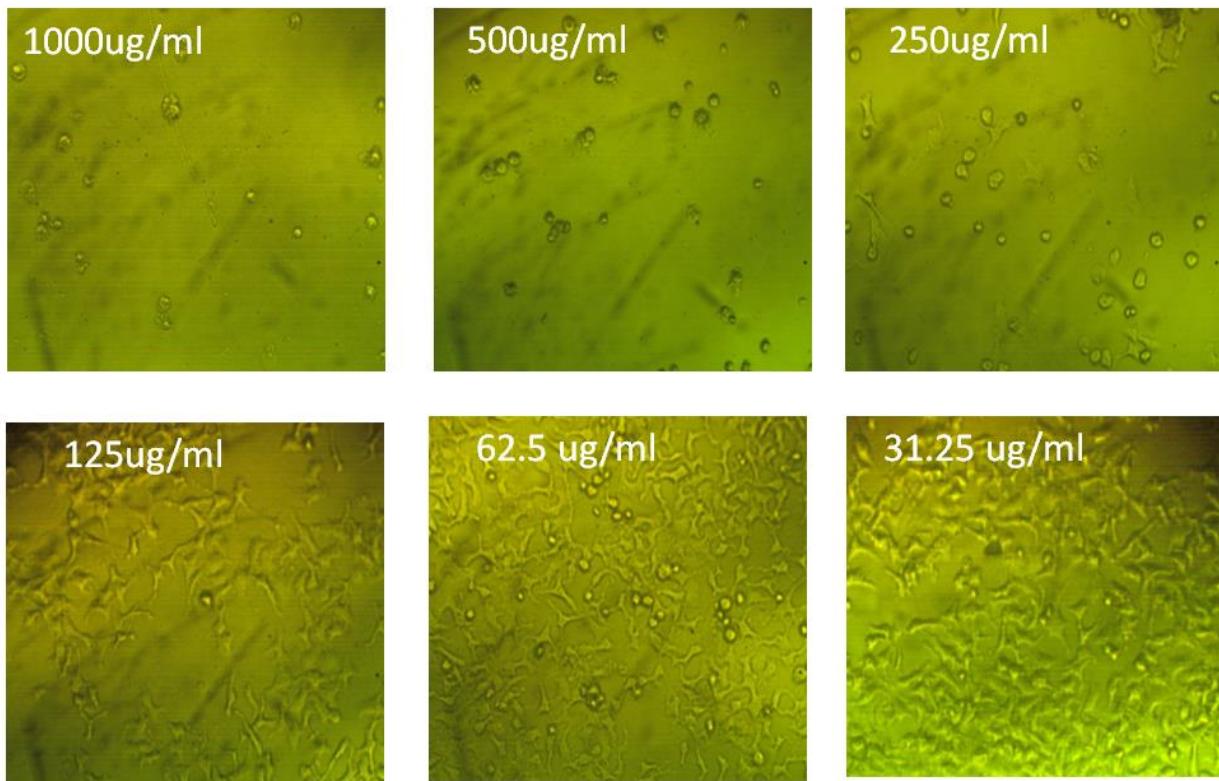
control
A549 cells

Organism : *Homo sapiens*, human
Tissue : lung
Cell Type : epithelial
Culture Properties : adherent
Disease : Carcinoma

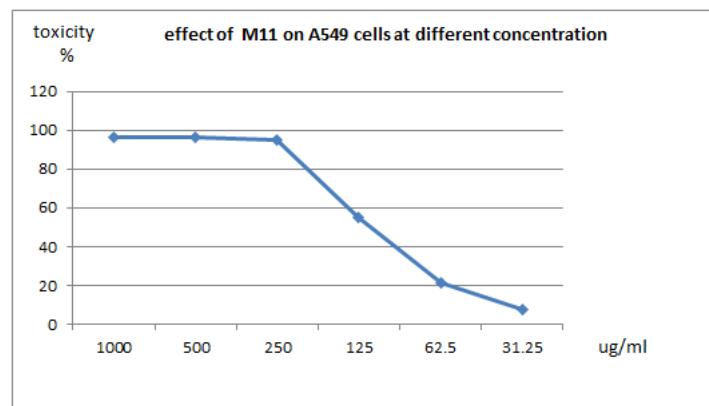
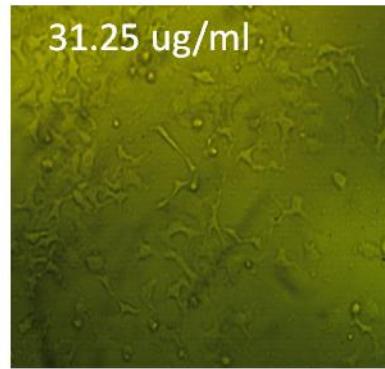
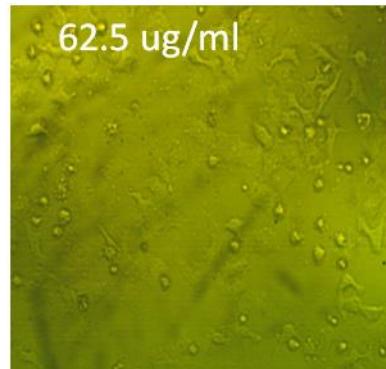
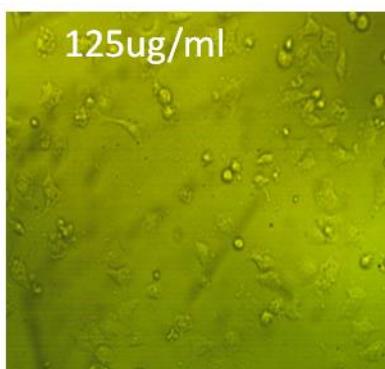
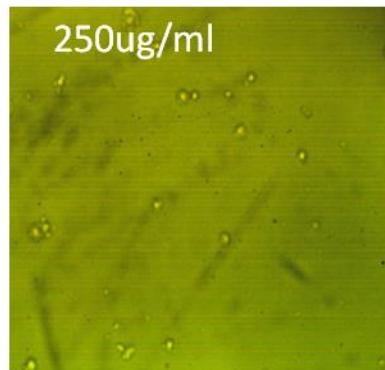
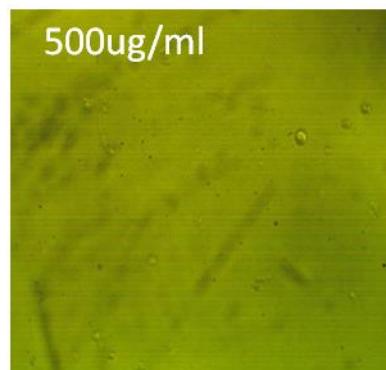
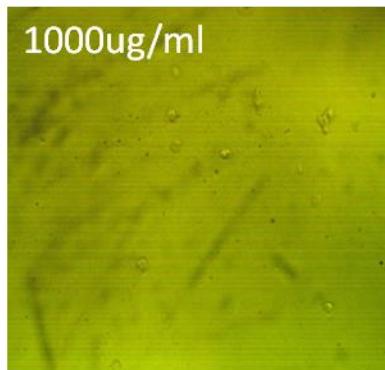
Effect of C16 on A549 cells at different concentration



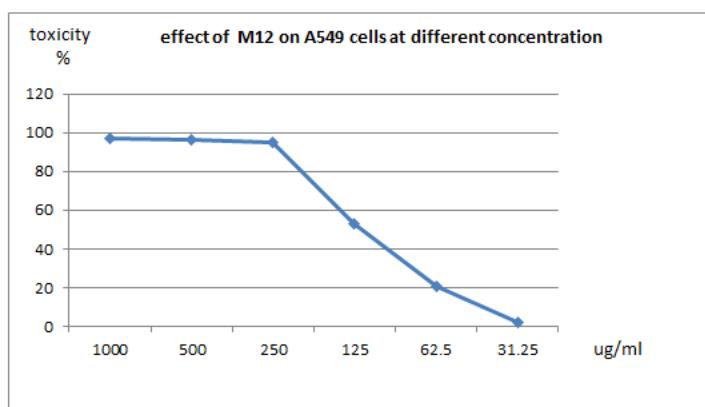
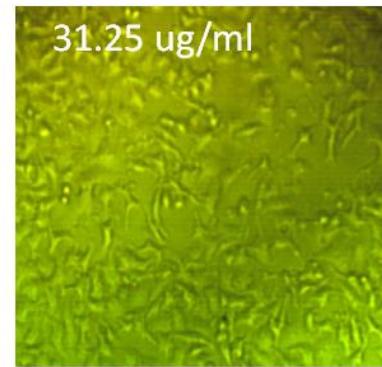
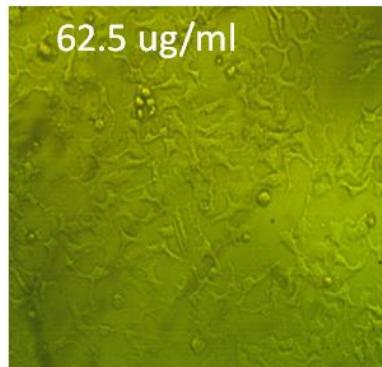
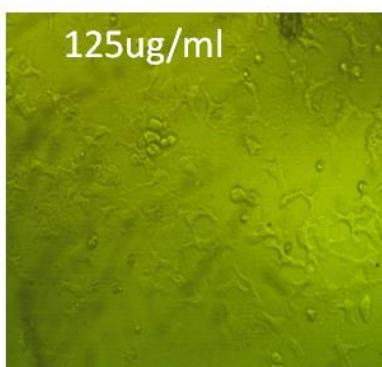
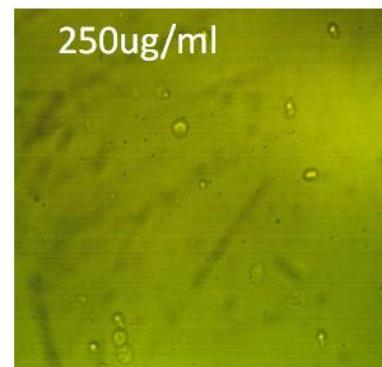
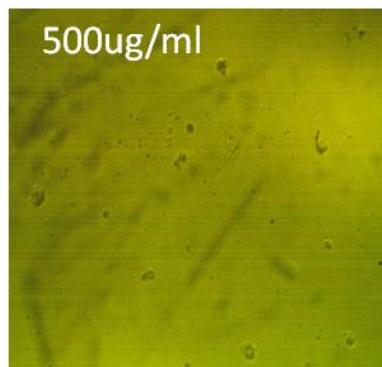
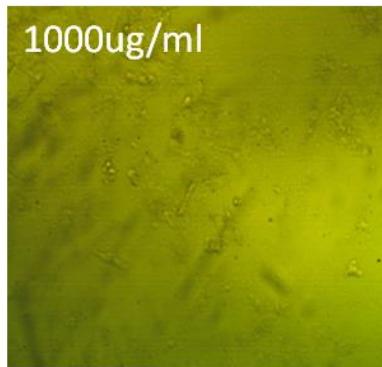
Effect of C17 on A549 cells at different concentration



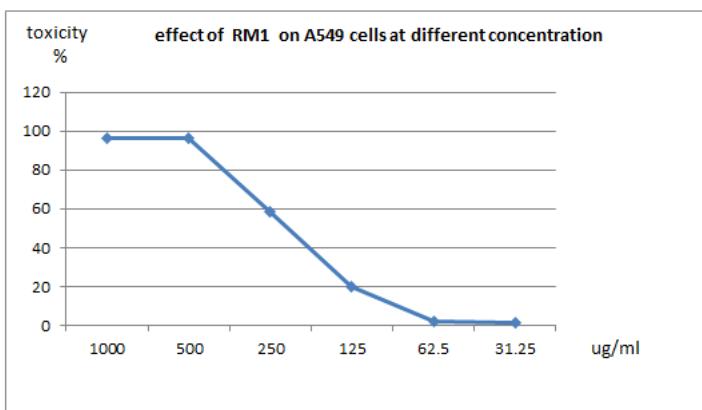
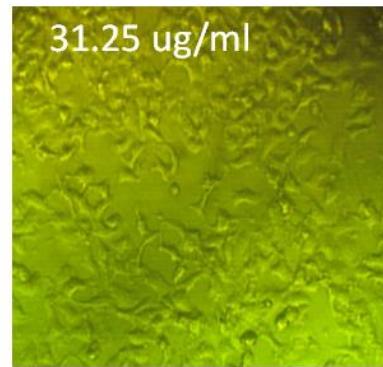
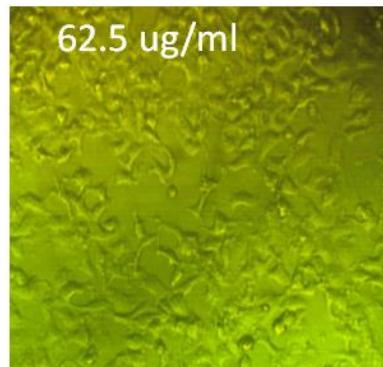
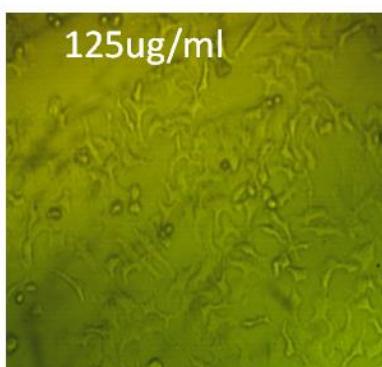
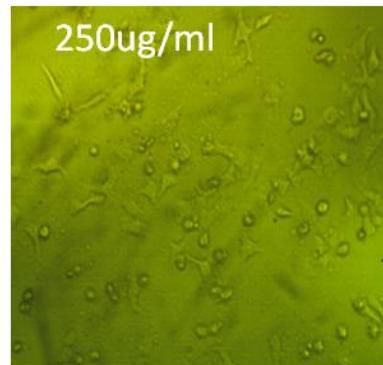
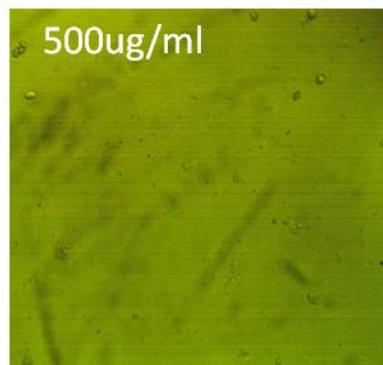
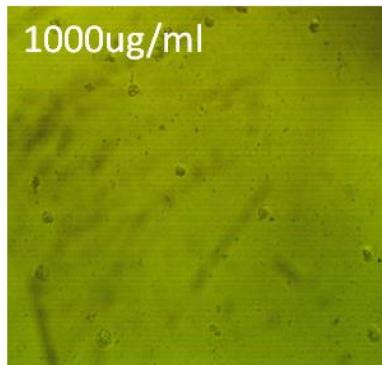
Effect of M11 on A549 cells at different concentration



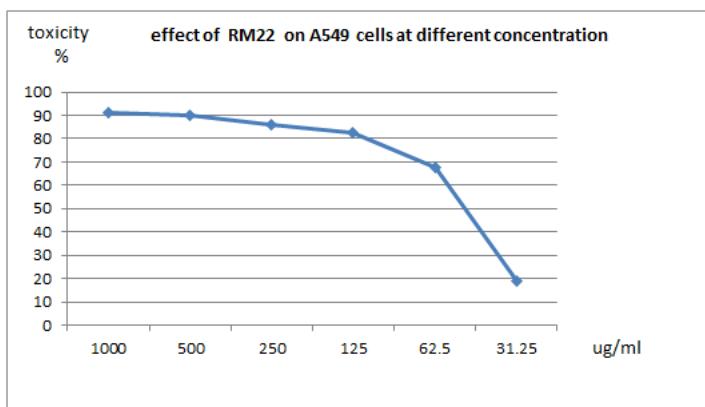
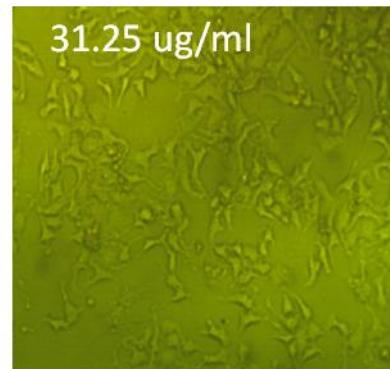
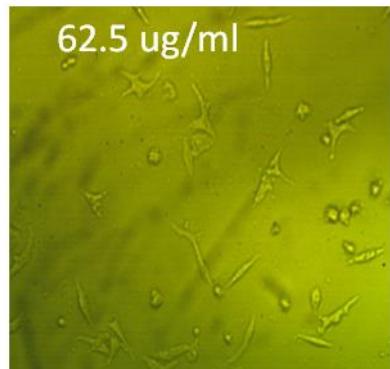
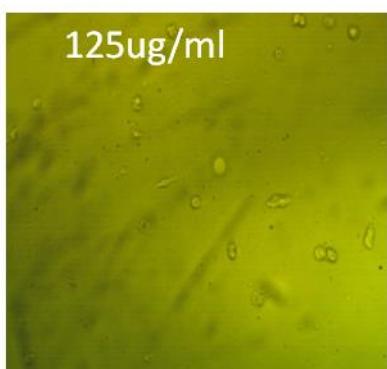
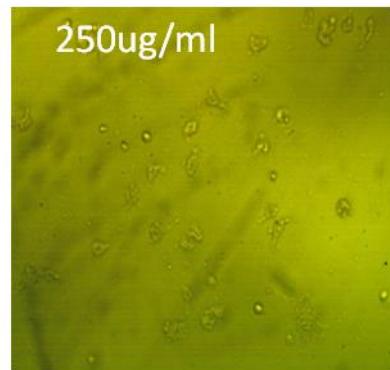
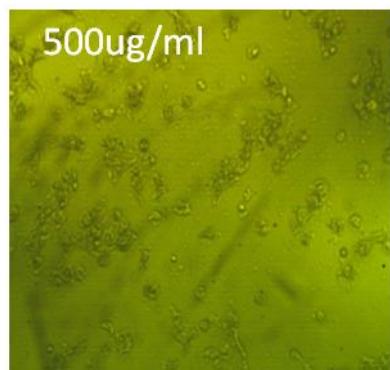
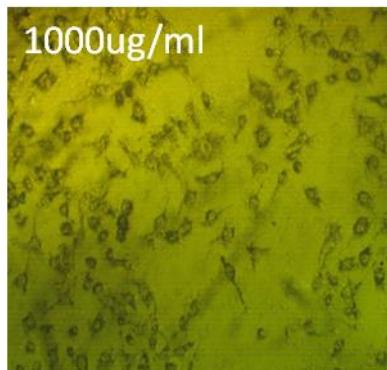
Effect of M12 on A549 cells at different concentration



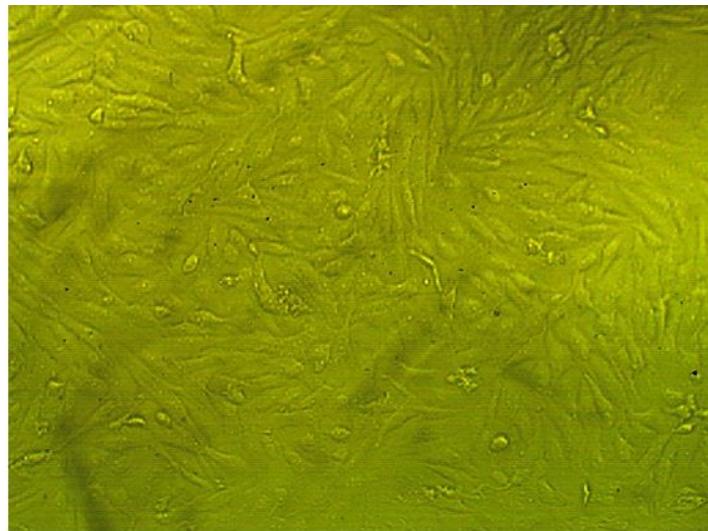
Effect of RM1 on A549 cells at different concentration



Effect of RM22 on A549 cells at different concentration



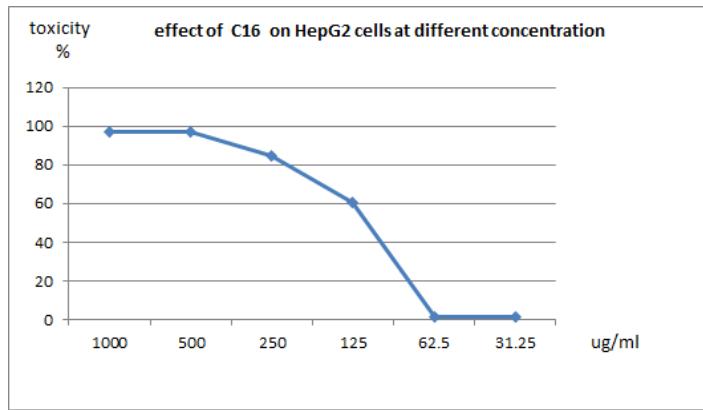
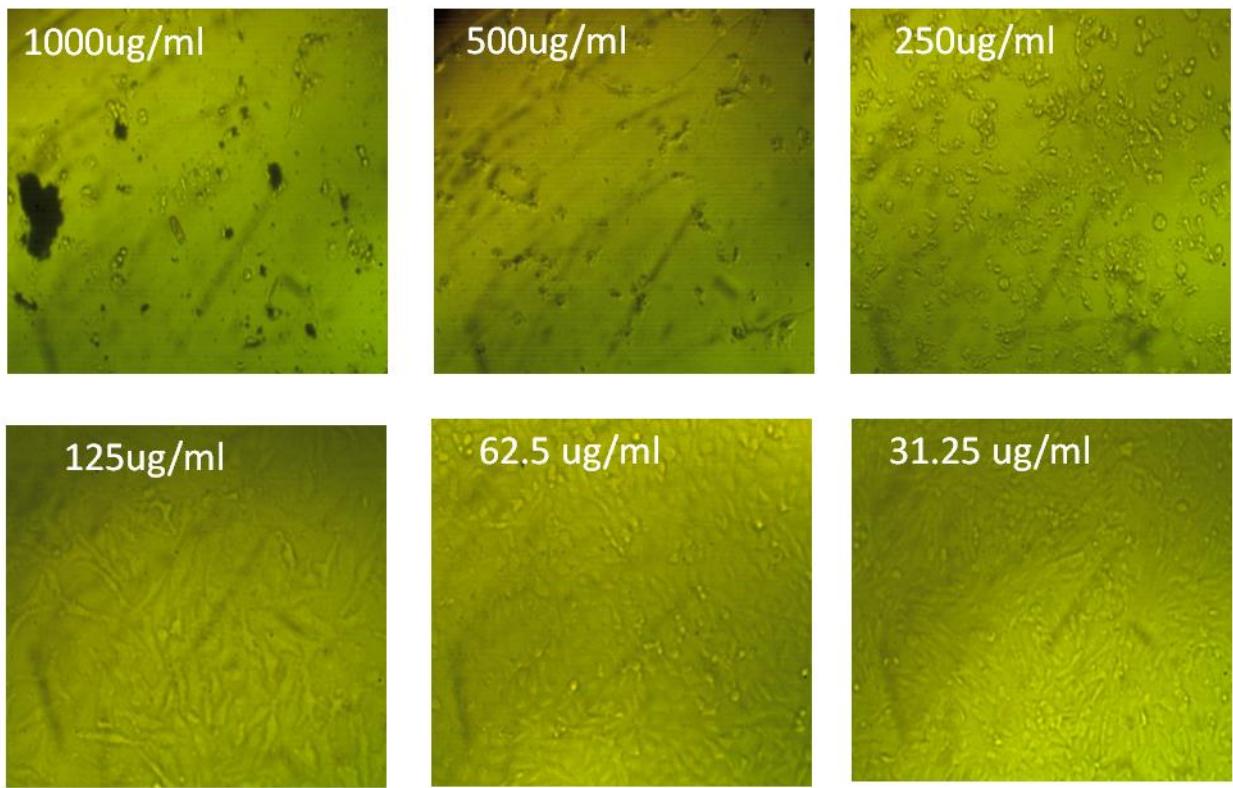
ID	ug/ml	O.D			Mean O.D	±SE	Viability %	Toxicity %	IC50 ± SD
HepG2	-----	0.683	0.647	0.677	0.669	0.011136	100	0	ug
C16(2b)	1000	0.018	0.023	0.015	0.018667	0.002333	2.79023418	97.20976582	110.65 ± 1.51
	500	0.018	0.019	0.021	0.019333	0.000882	2.889885401	97.1101146	
	250	0.111	0.094	0.109	0.104667	0.005364	15.64524165	84.35475835	
	125	0.274	0.248	0.266	0.262667	0.007688	39.26258097	60.73741903	
	62.5	0.637	0.657	0.689	0.661	0.015144	98.80418535	1.195814649	
	31.25	0.667	0.684	0.633	0.661333	0.014993	98.85401096	1.145989038	
C17(2a)	1000	0.033	0.042	0.056	0.043667	0.006692	6.527154958	93.47284504	217.82 ± 4.61
	500	0.104	0.119	0.147	0.123333	0.012601	18.43547583	81.56452417	
	250	0.274	0.226	0.248	0.249333	0.013872	37.26955655	62.73044345	
	125	0.657	0.667	0.662	0.662	0.002887	98.95366218	1.046337818	
	62.5	0.679	0.658	0.648	0.661667	0.009135	98.90383657	1.096163428	
	31.25	0.662	0.674	0.664	0.666667	0.003712	99.65122073	0.348779273	
M11(2c)	1000	0.017	0.019	0.018	0.018	0.000577	2.69058296	97.30941704	165.92 ± 3.91
	500	0.037	0.047	0.053	0.045667	0.004667	6.82610862	93.17389138	
	250	0.189	0.163	0.142	0.164667	0.013593	24.61385152	75.38614848	
	125	0.327	0.348	0.362	0.345667	0.010171	51.66915795	48.33084205	
	62.5	0.647	0.689	0.66	0.665333	0.012414	99.45191829	0.548081714	
	31.25	0.663	0.668	0.659	0.663333	0.002603	99.15296462	0.847035376	
M12(2d)	1000	0.017	0.014	0.017	0.016	0.001	2.391629297	97.6083707	154.83 ± 4.6
	500	0.016	0.017	0.016	0.016333	0.000333	2.441454908	97.55854509	
	250	0.094	0.103	0.115	0.104	0.006083	15.54559043	84.45440957	
	125	0.295	0.346	0.321	0.320667	0.014723	47.93223717	52.06776283	
	62.5	0.669	0.653	0.661	0.661	0.004619	98.80418535	1.195814649	
	31.25	0.664	0.666	0.67	0.666667	0.001764	99.65122073	0.348779273	
RM1(2e)	1000	0.064	0.058	0.064	0.062	0.002	9.267563528	90.73243647	211.36 ± 13.5
	500	0.068	0.084	0.069	0.073667	0.005175	11.01145989	88.98854011	
	250	0.194	0.275	0.214	0.227667	0.024361	34.03089188	65.96910812	
	125	0.584	0.628	0.611	0.607667	0.012811	90.83208769	9.167912307	
	62.5	0.678	0.645	0.66	0.661	0.009539	98.80418535	1.195814649	
	31.25	0.663	0.674	0.662	0.666333	0.003844	99.60139512	0.398604883	
RM22(2f)	1000	0.014	0.017	0.016	0.015667	0.000882	2.341803687	97.65819631	39.18 ± 1.95
	500	0.017	0.016	0.018	0.017	0.000577	2.541106129	97.45889387	
	250	0.018	0.017	0.017	0.017333	0.000333	2.590931739	97.40906826	
	125	0.019	0.016	0.109	0.048	0.030512	7.174887892	92.82511211	
	62.5	0.085	0.099	0.093	0.092333	0.004055	13.80169407	86.19830593	
	31.25	0.396	0.433	0.424	0.417667	0.011141	62.43148979	37.56851021	



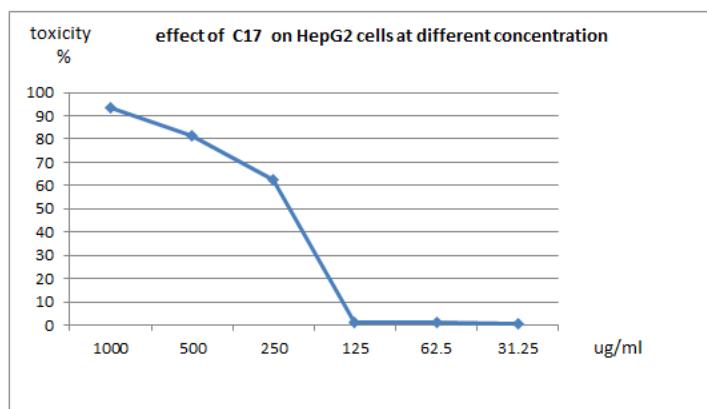
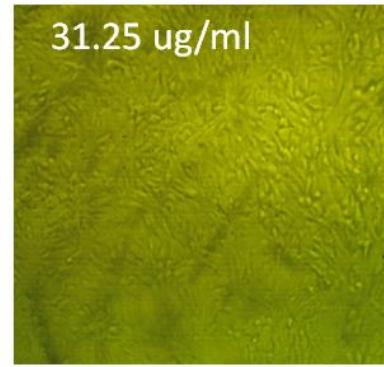
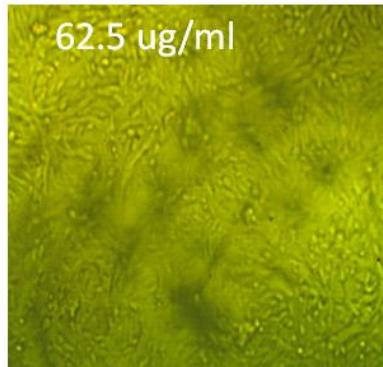
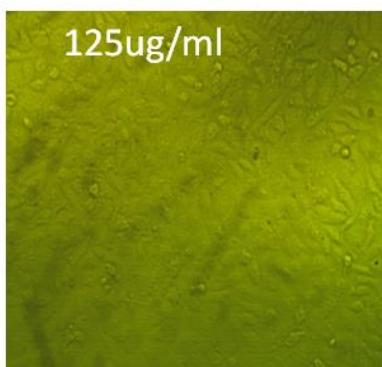
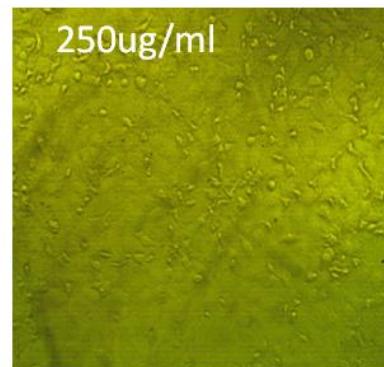
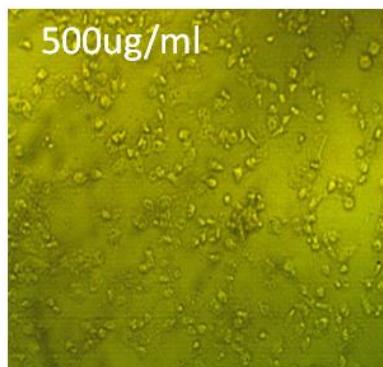
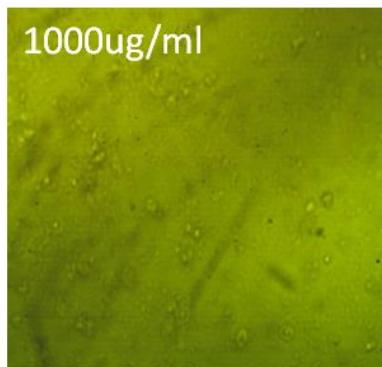
**control
HepG2 cells**

Organism : *Homo sapiens*, human
Tissue : liver
Cell Type : epithelial
Culture Properties : adherent
Disease : hepatocellular carcinoma

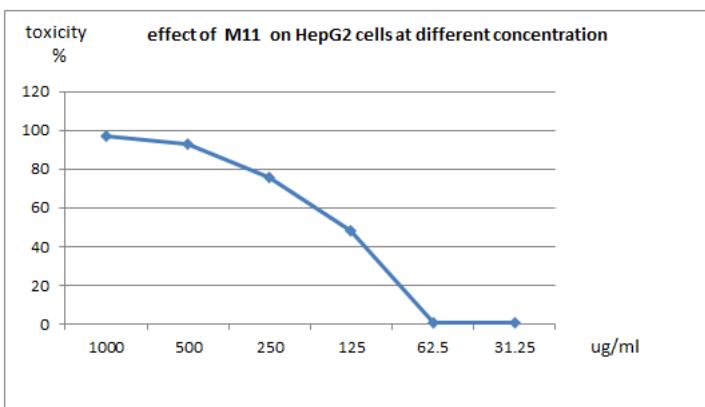
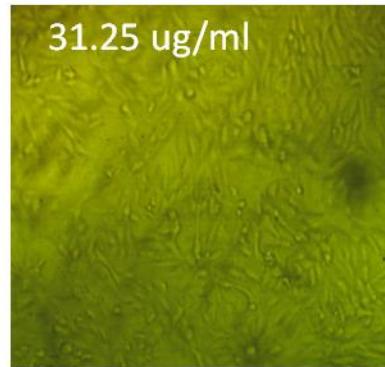
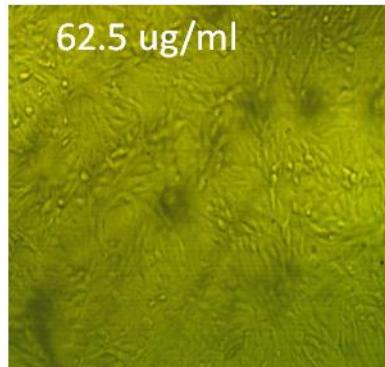
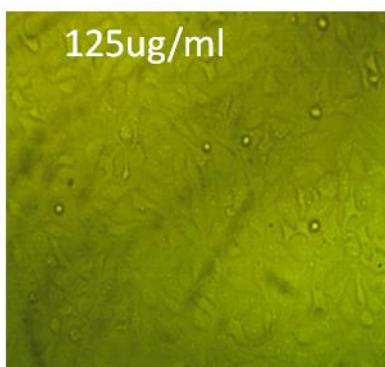
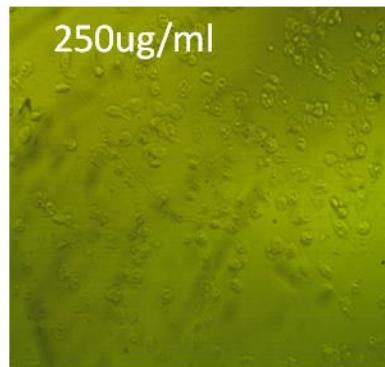
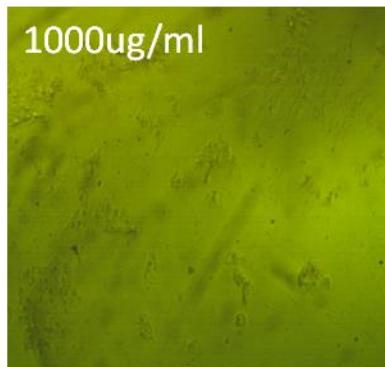
Effect of C16 on HepG2 cells at different concentration



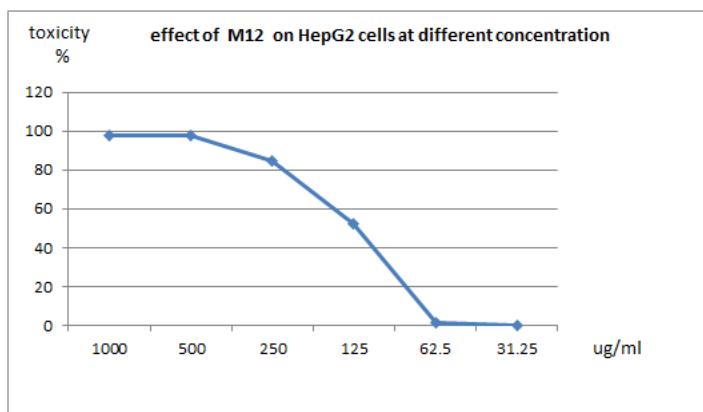
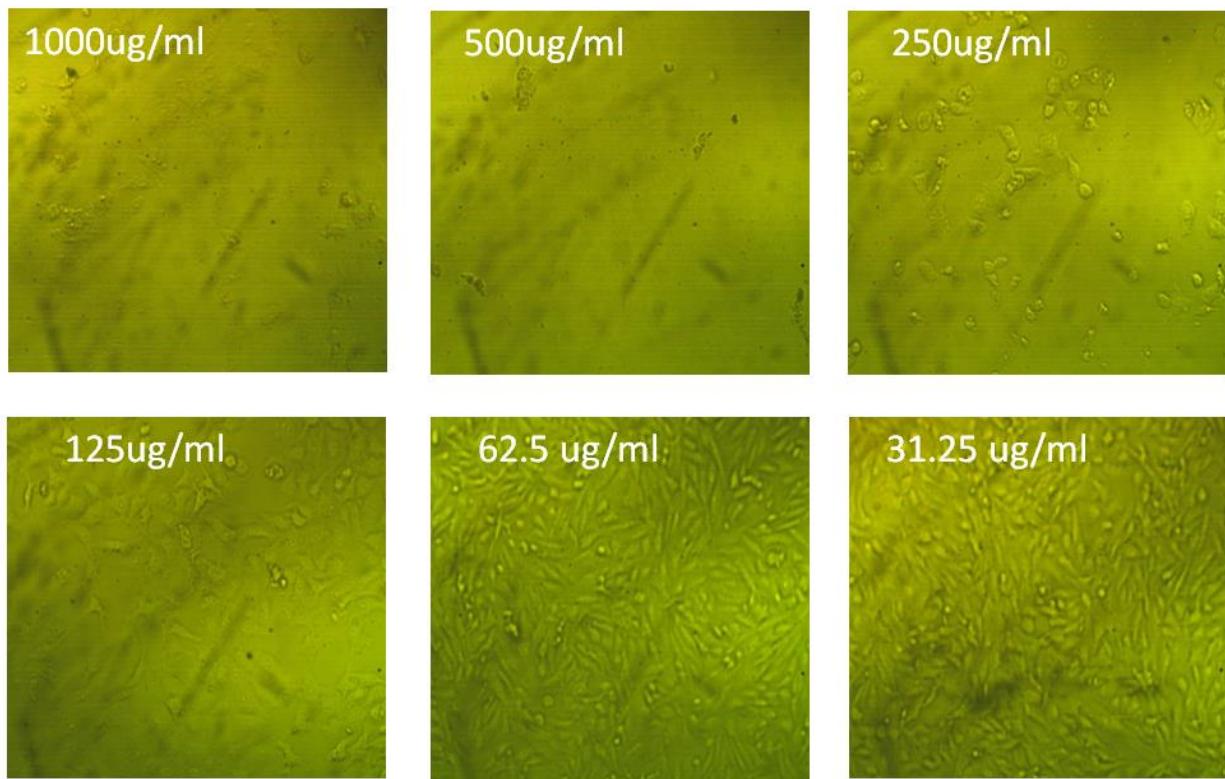
Effect of C17 on HepG2 cells at different concentration



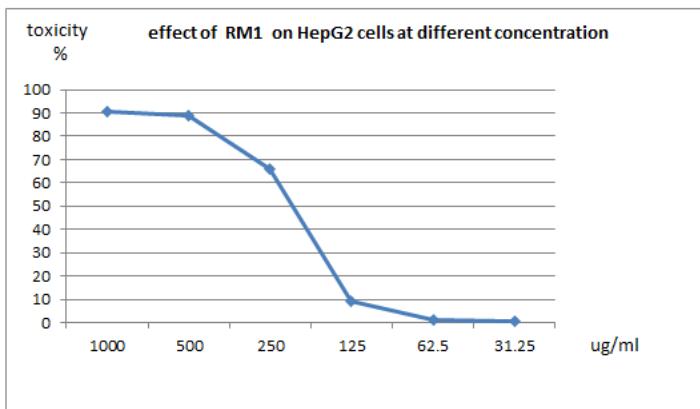
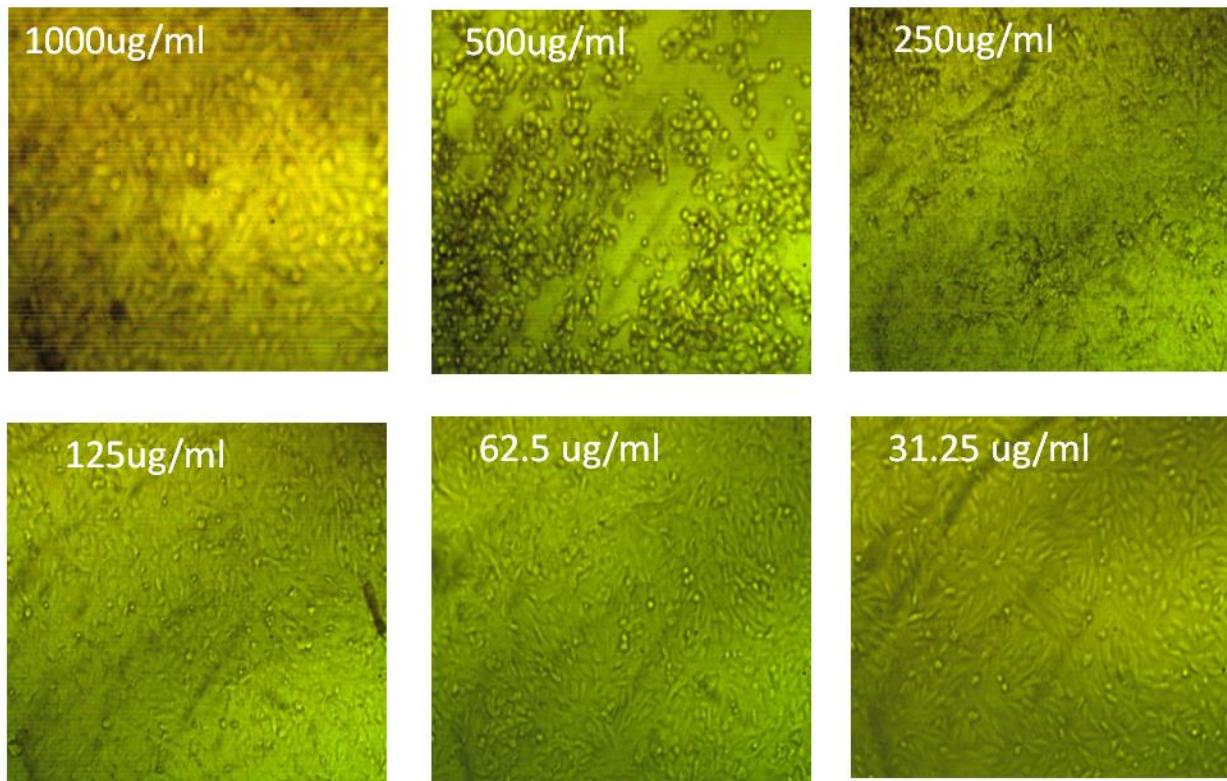
Effect of M11 on HepG2 cells at different concentration



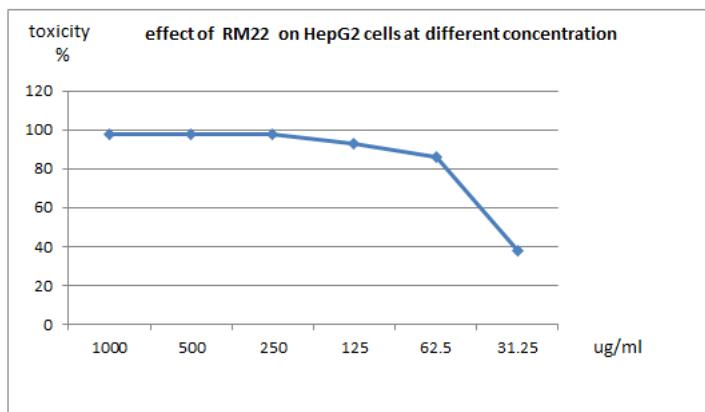
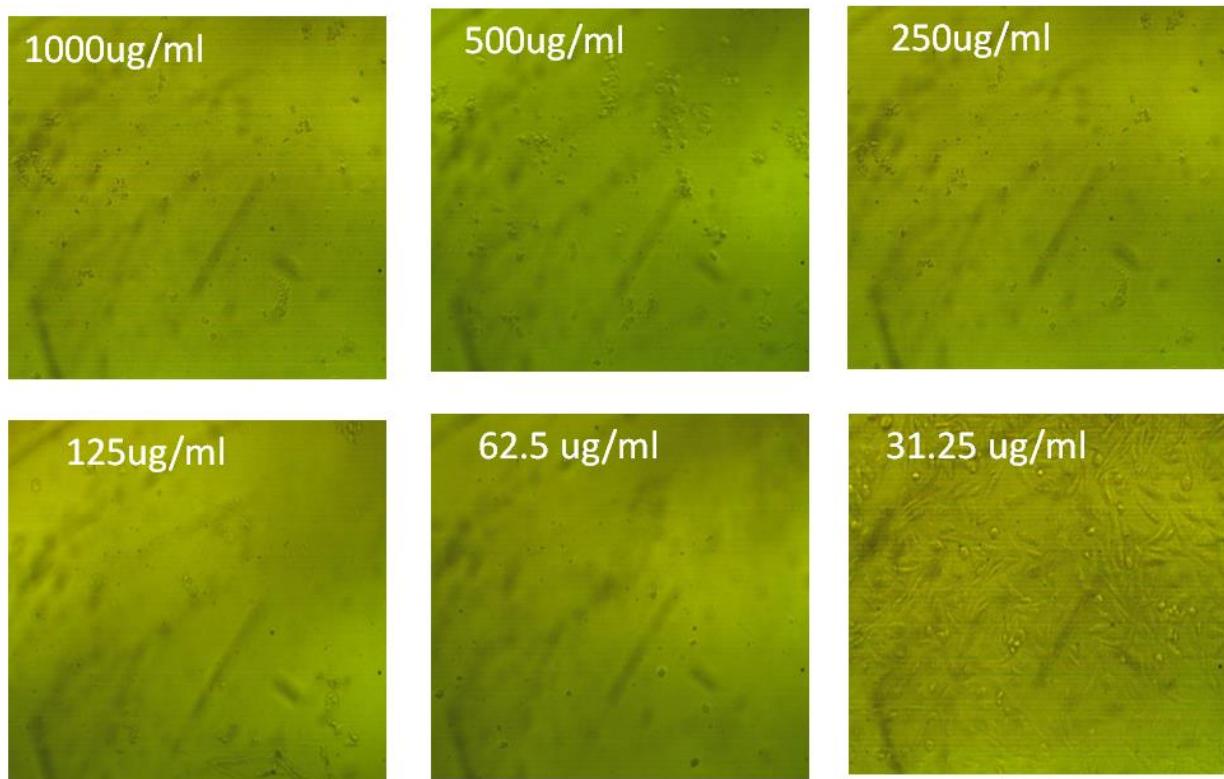
Effect of M12 on HepG2 cells at different concentration



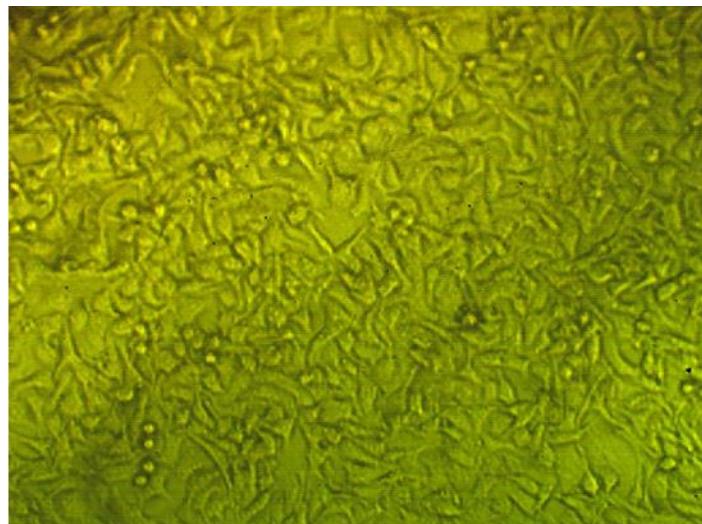
Effect of RM1 on HepG2 cells at different concentration



Effect of RM22 on HepG2 cells at different concentration



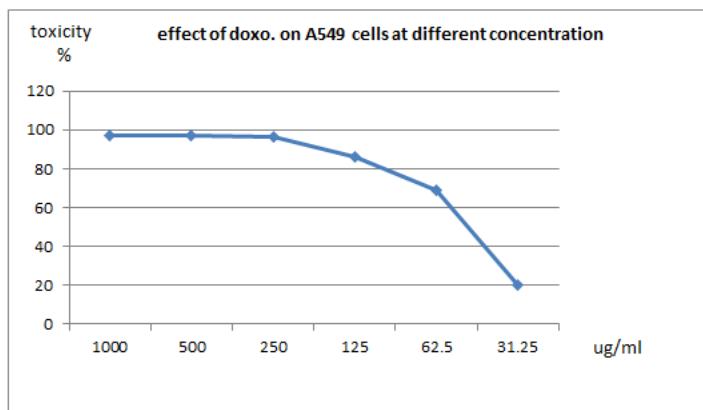
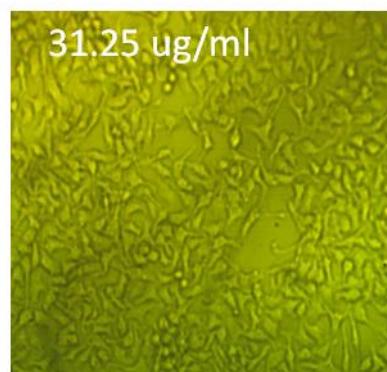
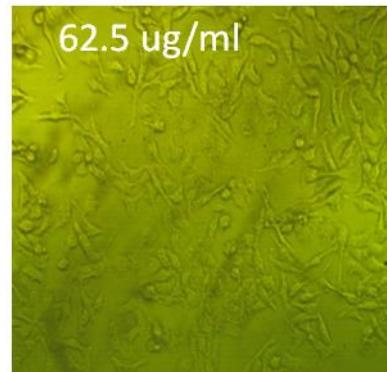
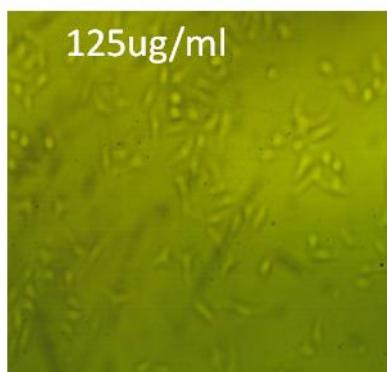
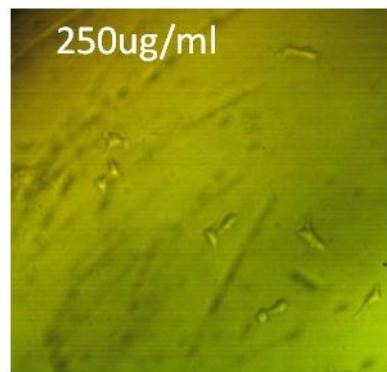
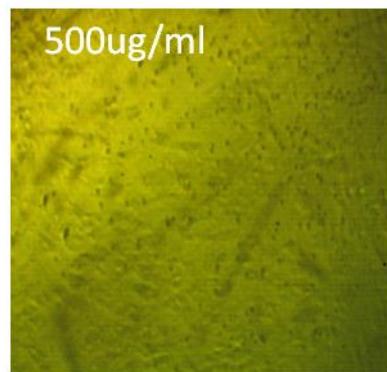
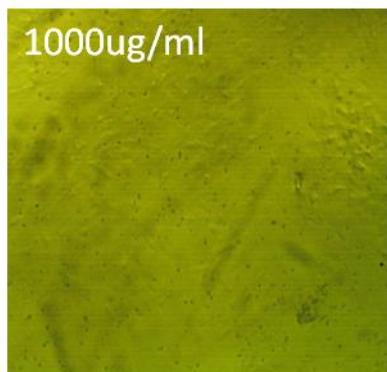
ID	ug/ml	O.D			Mean O.D	\pm SE	Viability %	Toxicity %	IC50 \pm SD
A549	-----	0.683	0.689	0.695	0.689	0.003464	100	0	ug
doxo	1000	0.018	0.02	0.018	0.018667	0.000667	2.709240445	97.29075955	50.47 \pm 1.32
	500	0.019	0.017	0.019	0.018333	0.000667	2.660861151	97.33913885	
	250	0.02	0.034	0.025	0.026333	0.004096	3.821964199	96.1780358	
	125	0.089	0.11	0.094	0.097667	0.006333	14.17513304	85.82486696	
	62.5	0.219	0.235	0.194	0.216	0.01193	31.34978229	68.65021771	
	31.25	0.546	0.558	0.542	0.548667	0.004807	79.63231737	20.36768263	



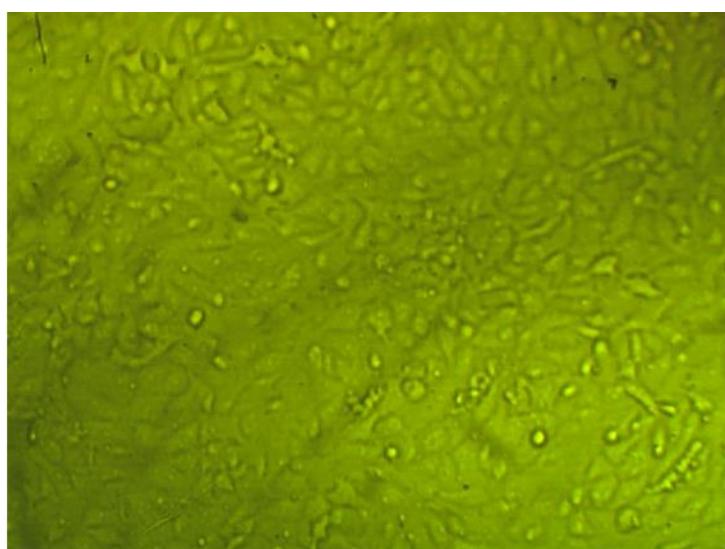
**control
A549 cells**

Organism : *Homo sapiens*, human
 Tissue : lung
 Cell Type : epithelial
 Culture Properties : adherent
 Disease : Carcinoma
 ATCC : CCL-185

Effect of doxo on A549 cells at different concentration



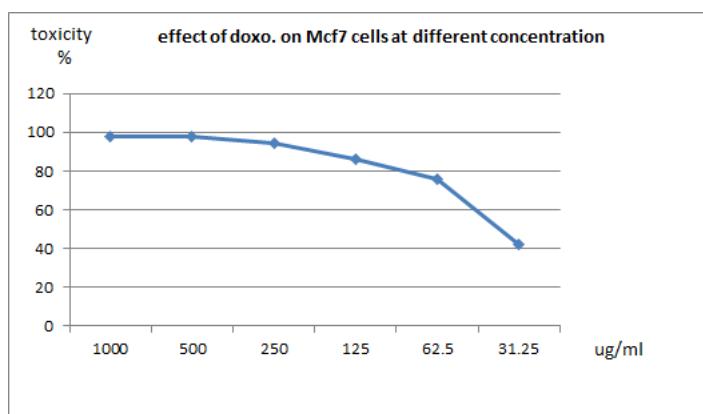
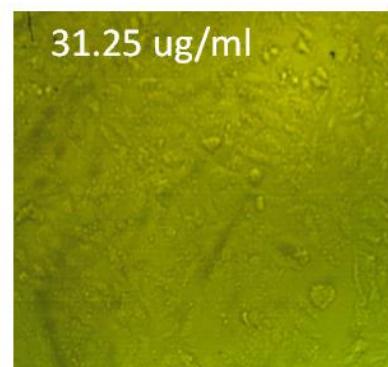
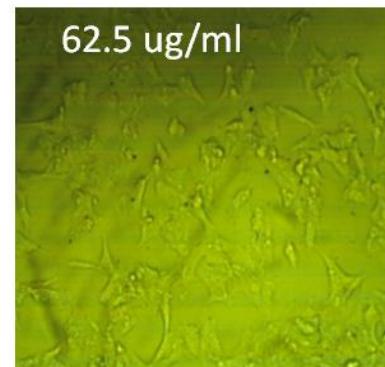
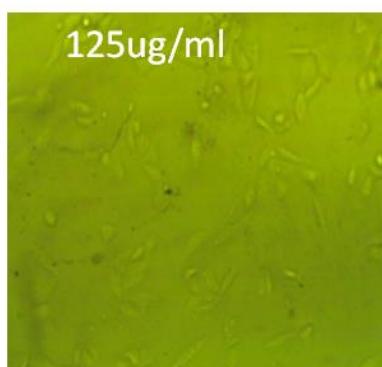
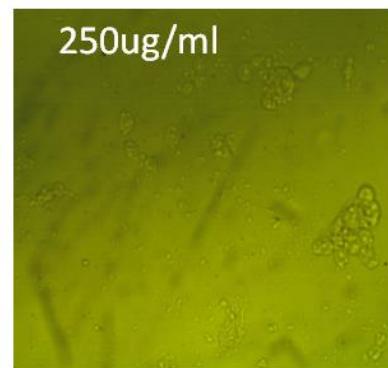
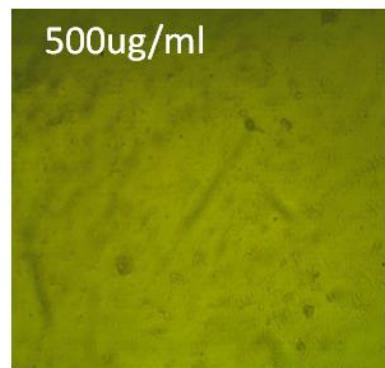
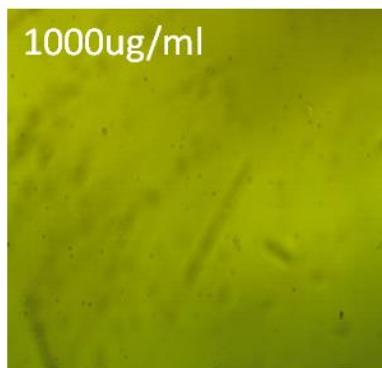
ID	ug/ml	O.D			Mean O.D	±SE	Viability %	Toxicity %	IC50 ± SD
Mcf7	-----	0.784	0.762	0.758	0.768	0.008083	100	0	ug
doxo	1000	0.018	0.015	0.017	0.016667	0.000882	2.170138889	97.82986111	38.53 ± 0.49
	500	0.018	0.017	0.019	0.018	0.000577	2.34375	97.65625	
	250	0.036	0.053	0.048	0.045667	0.005044	5.946180556	94.05381944	
	125	0.089	0.119	0.107	0.105	0.008718	13.671875	86.328125	
	62.5	0.174	0.189	0.188	0.183667	0.004842	23.91493056	76.08506944	
	31.25	0.452	0.438	0.444	0.444667	0.004055	57.89930556	42.10069444	



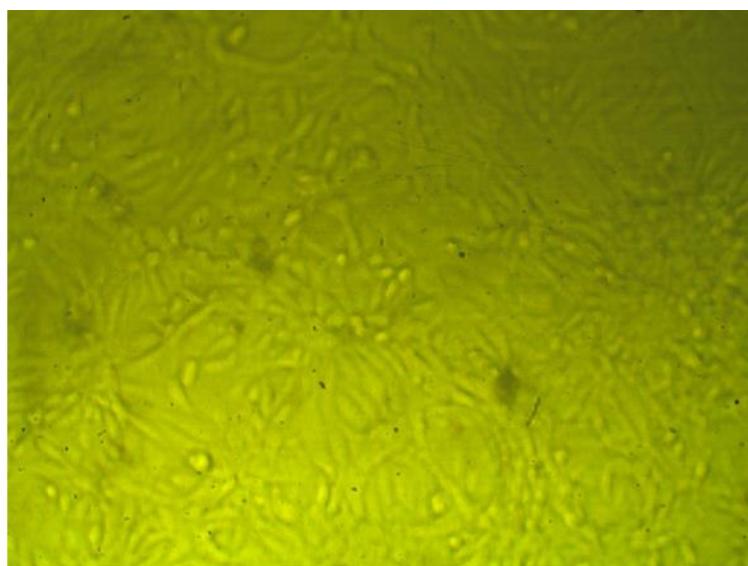
**control
Mcf7 cells**

Organism : *Homo sapiens*, human
 Tissue : mammary gland, breast; derived from metastatic site: pleural effusion
 Cell Type : epithelial
 Culture Properties : adherent
 Disease : adenocarcinoma
 ATCC : HTB-22

Effect of doxo on Mcf7 cells at different concentration



ID	ug/ml	O.D			Mean O.D	±SE	Viability %	Toxicity %	IC50 ± SD
HepG2	-----	0.822	0.813	0.819	0.818	0.002646	100	0	ug
doxo	1000	0.018	0.017	0.019	0.018	0.000577	2.200488998	97.799511	47.59 ± 0.88
	500	0.016	0.018	0.018	0.017333	0.000667	2.118989405	97.88101059	
	250	0.034	0.021	0.04	0.031667	0.005608	3.871230644	96.12876936	
	125	0.094	0.089	0.11	0.097667	0.006333	11.9396903	88.0603097	
	62.5	0.164	0.189	0.174	0.175667	0.007265	21.47514262	78.52485738	
	31.25	0.645	0.678	0.672	0.665	0.010149	81.29584352	18.70415648	



**control
HepG2 cells**

Organism : *Homo sapiens*, human
 Tissue : liver
 Cell Type : epithelial
 Culture Properties : adherent
 Disease : hepatocellular carcinoma
 ATCC : HB-8065

Effect of doxo on HepG2 cells at different concentration

