Discovery of novel indene-based hybrids as breast cancer inhibitors targeting Hsp90: Synthesis, bio-evaluation and molecular docking study

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Viability assay

Test code: T-2-022-33

Institute / Researcher: **prof.Dr. rehab sabour**

Experiment : **functional assay (MTT)**

(viability/cytotoxicity)

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samples number : **13**

experiment design : effect against Mcf7 and MDA cells

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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 X 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.

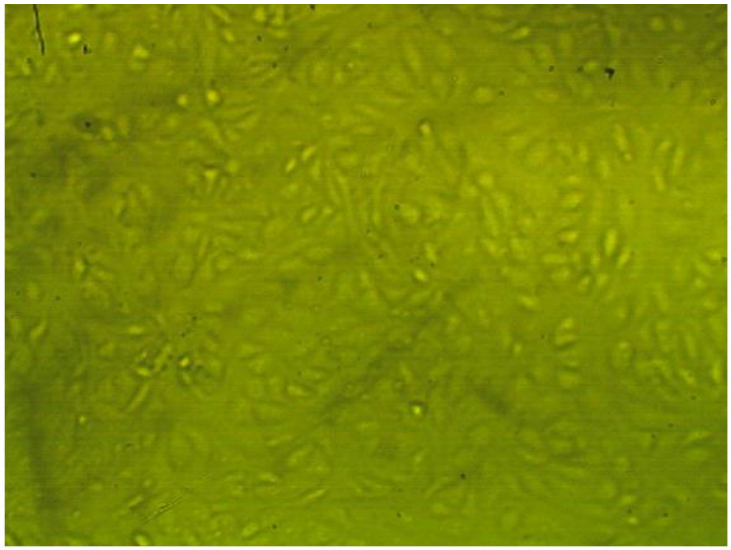
12) Read optical density at 560nm and subtract background at 620nm. Optical density should be directly correlated with cell quantity.

ID	uM	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC_{50}
Mcf7	--------	0.688	0.658	0.664	0.67	0.009165	100	0	uM
3	1000	0.013	0.015	0.014	0.014	0.000577	2.089552239	97.91044776	172.48 ± 5.39
	500	0.019	0.017	0.018	0.018	0.000577	2.686567164	97.31343284	
	250	0.097	0.086	0.115	0.099333	0.008452	14.82587065	85.17412935	
	125	0.458	0.511	0.488	0.485667	0.015344	72.48756219	27.51243781	
	62.5	0.643	0.689	0.656	0.662667	0.013691	98.90547264	1.094527363	
	31.25	0.667	0.675	0.659	0.667	0.004619	99.55223881	0.447761194	
20	1000	0.015	0.016	0.016	0.015667	0.000333	2.338308458	97.66169154	61.62 ± 2.34
	500	0.032	0.016	0.02	0.022667	0.004807	3.383084577	96.61691542	
	250	0.096	0.11	0.089	0.098333	0.006173	14.67661692	85.32338308	
	125	0.177	0.134	0.184	0.165	0.015631	24.62686567	75.37313433	
	62.5	0.297	0.316	0.276	0.296333	0.011552	44.22885572	55.77114428	
	31.25	0.478	0.421	0.428	0.442333	0.017947	66.0199005	33.9800995	
21	1000	0.015	0.017	0.016	0.016	0.000577	2.388059701	97.6119403	76.31 ± 2.61
	500	0.016	0.018	0.018	0.017333	0.000667	2.587064677	97.41293532	
	250	0.019	0.018	0.021	0.019333	0.000882	2.885572139	97.11442786	
	125	0.112	0.143	0.128	0.127667	0.00895	19.05472637	80.94527363	
	62.5	0.288	0.267	0.323	0.292667	0.016333	43.68159204	56.31840796	
	31.25	0.632	0.648	0.659	0.646333	0.007839	96.46766169	3.532338308	
8a	1000	0.017	0.018	0.016	0.017	0.000577	2.537313433	97.46268657	26.44 ± 4.47
	500	0.016	0.018	0.017	0.017	0.000577	2.537313433	97.46268657	
	250	0.018	0.02	0.019	0.019	0.000577	2.835820896	97.1641791	
	125	0.022	0.019	0.024	0.021667	0.001453	3.233830846	96.76616915	
	62.5	0.087	0.095	0.058	0.08	0.01124	11.94029851	88.05970149	
	31.25	0.277	0.324	0.31	0.303667	0.013932	45.32338308	54.67661692	
25	1000	0.017	0.016	0.017	0.016667	0.000333	2.487562189	97.51243781	32.85 ± 3.97
	500	0.089	0.066	0.105	0.086667	0.011319	12.93532338	87.06467662	
	250	0.436	0.411	0.396	0.414333	0.011667	61.84079602	38.15920398	
	125	0.649	0.643	0.668	0.653333	0.007535	97.51243781	2.487562189	
	62.5	0.678	0.674	0.649	0.667	0.009074	99.55223881	0.447761194	
	31.25	0.682	0.657	0.669	0.669333	0.007219	99.90049751	0.099502488	
26	1000	0.021	0.024	0.018	0.021	0.001732	3.134328358	96.86567164	236.95 ± 8.29
	500	0.034	0.032	0.023	0.029667	0.003383	4.427860697	95.5721393	
	250	0.286	0.317	0.301	0.301333	0.00895	44.97512438	55.02487562	
	125	0.629	0.618	0.642	0.629667	0.006936	93.9800995	6.019900498	
	62.5	0.669	0.68	0.652	0.667	0.008145	99.55223881	0.447761194	
	31.25	0.658	0.678	0.671	0.669	0.005859	99.85074627	0.149253731	

ID	uM	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
Mcf7	--------	0.688	0.658	0.664	0.67	0.009165	100	0	uM
27	1000	0.016	0.014	0.017	0.015667	0.000882	2.338308458	97.66169154	114.46 ± 4
	500	0.018	0.016	0.019	0.017667	0.000882	2.63681592	97.36318408	
	250	0.019	0.024	0.02	0.021	0.001528	3.134328358	96.86567164	
	125	0.267	0.3	0.278	0.281667	0.009701	42.039801	57.960199	
	62.5	0.611	0.589	0.638	0.612667	0.01417	91.44278607	8.55721393	
	31.25	0.658	0.682	0.667	0.669	0.007	99.85074627	0.149253731	
17b	1000	0.015	0.018	0.014	0.015667	0.001202	2.338308458	97.66169154	184.96 ± 4.48
	500	0.022	0.034	0.021	0.025667	0.004177	3.830845771	96.16915423	
	250	0.116	0.102	0.145	0.121	0.012662	18.05970149	81.94029851	
	125	0.567	0.61	0.592	0.589667	0.012468	88.00995025	11.99004975	
	62.5	0.678	0.645	0.67	0.664333	0.009939	99.15422886	0.845771144	
	31.25	0.664	0.682	0.659	0.668333	0.006984	99.75124378	0.248756219	
17a	1000	0.018	0.019	0.019	0.018667	0.000333	2.786069652	97.21393035	38.41 ± 4.3
	500	0.018	0.022	0.019	0.019667	0.001202	2.935323383	97.06467662	
	250	0.043	0.067	0.08	0.063333	0.010837	9.452736318	90.54726368	
	125	0.297	0.316	0.321	0.311333	0.007311	46.46766169	53.53233831	
	62.5	0.572	0.614	0.587	0.591	0.012288	88.20895522	11.79104478	
	31.25	0.644	0.689	0.656	0.663	0.013454	98.95522388	1.044776119	
8d	1000	0.015	0.019	0.017	0.017	0.001155	2.537313433	97.46268657	182.18 ± 3.78
	500	0.026	0.034	0.031	0.030333	0.002333	4.527363184	95.47263682	
	250	0.1	0.089	0.113	0.100667	0.006936	15.02487562	84.97512438	
	125	0.588	0.609	0.613	0.603333	0.007753	90.04975124	9.950248756	
	62.5	0.647	0.679	0.669	0.665	0.009452	99.25373134	0.746268657	
	31.25	0.681	0.66	0.668	0.669667	0.006119	99.95024876	0.049751244	
8b	1000	0.018	0.02	0.021	0.019667	0.000882	2.935323383	97.06467662	37.01 ± 7.54
	500	0.078	0.092	0.099	0.089667	0.006173	13.38308458	86.61691542	
	250	0.258	0.242	0.289	0.263	0.013796	39.25373134	60.74626866	
	125	0.631	0.629	0.657	0.639	0.009018	95.37313433	4.626865672	
	62.5	0.652	0.659	0.667	0.659333	0.004333	98.4079602	1.592039801	
	31.25	0.663	0.674	0.67	0.669	0.003215	99.85074627	0.149253731	

ID	uM	O.D	Mean O.D	ST.E	Viability %	Toxicity %	IC50

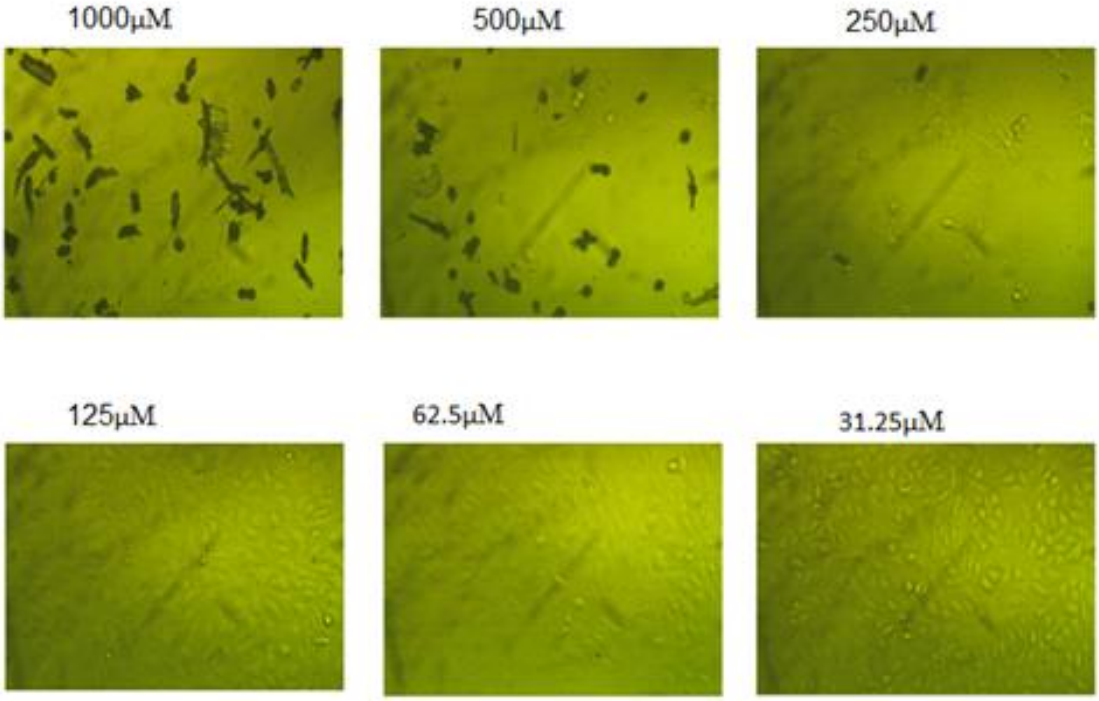
Mcf7	--------	0.688	0.658	0.664	0.67	0.009165	100	0	uM
8c	1000	0.065	0.078	0.043	0.062	0.010214	9.253731343	90.74626866	39.62 ± 10.21
	500	0.175	0.139	0.193	0.169	0.015875	25.2238806	74.7761194	
	250	0.593	0.634	0.621	0.616	0.012097	91.94029851	8.059701493	
	125	0.643	0.657	0.659	0.653	0.005033	97.46268657	2.537313433	
	62.5	0.664	0.672	0.673	0.669667	0.002848	99.95024876	0.049751244	
	31.25	0.679	0.654	0.666	0.666333	0.007219	99.45273632	0.547263682	
doxo	1000	0.016	0.015	0.017	0.016	0.000577	2.388059701	97.6119403	31.16 ± 3.43
	500	0.018	0.018	0.019	0.018333	0.000333	2.736318408	97.26368159	
	250	0.034	0.023	0.022	0.026333	0.003844	3.930348259	96.06965174	
	125	0.167	0.132	0.185	0.161333	0.01556	24.07960199	75.92039801	
	62.5	0.246	0.281	0.234	0.253667	0.014099	37.86069652	62.13930348	
	31.25	0.351	0.332	0.34	0.341	0.005508	50.89552239	49.10447761	

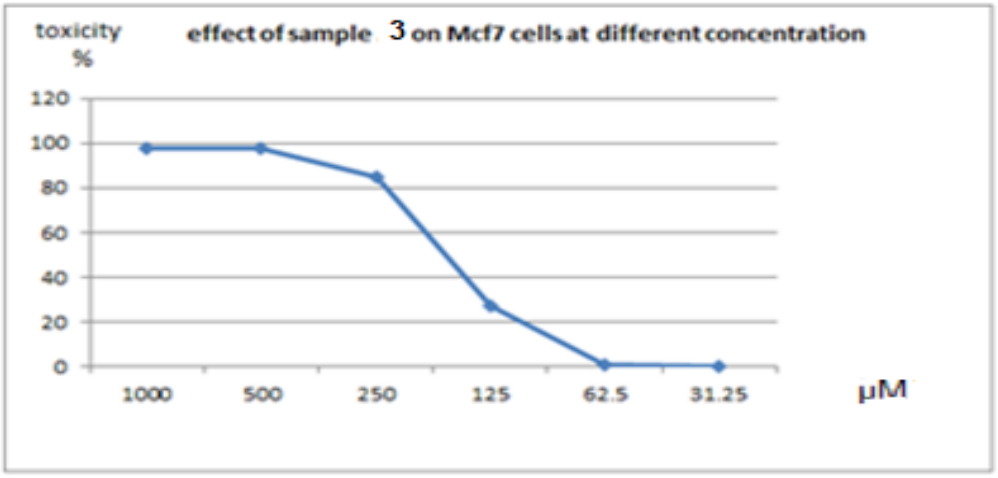


control
Mcf7 cells

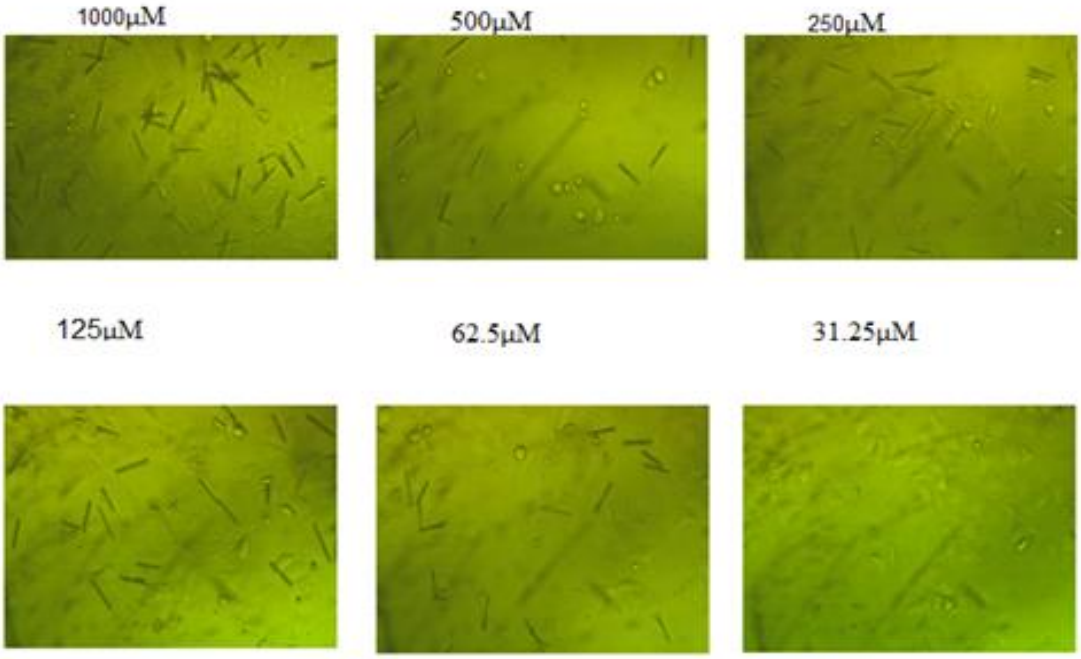
Organism : *Homo sapiens*, human
Tissue : mammary gland, breast; derived from metastatic site: pleural effusion
Cell Type : epithelial
Culture Properties : adherent
Disease : adenocarcinoma
ATCC : HTB-22

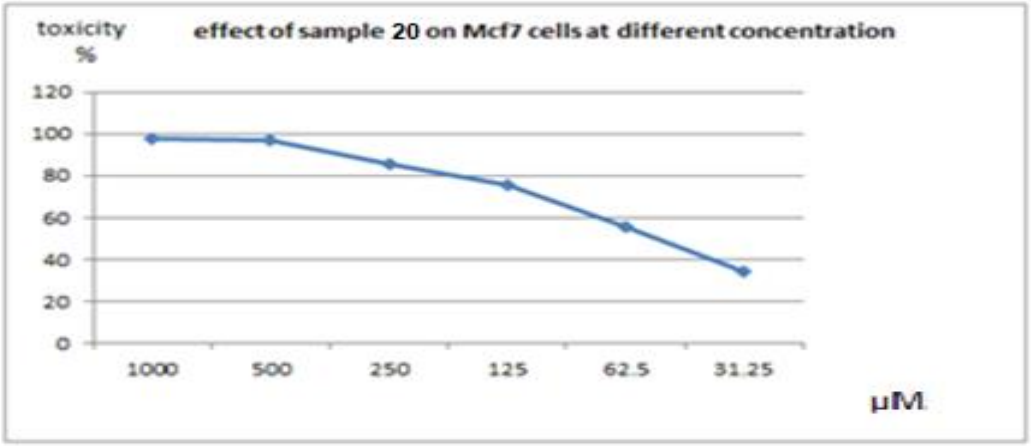
Effect of sample 3 on Mcf7 cells at different concentration



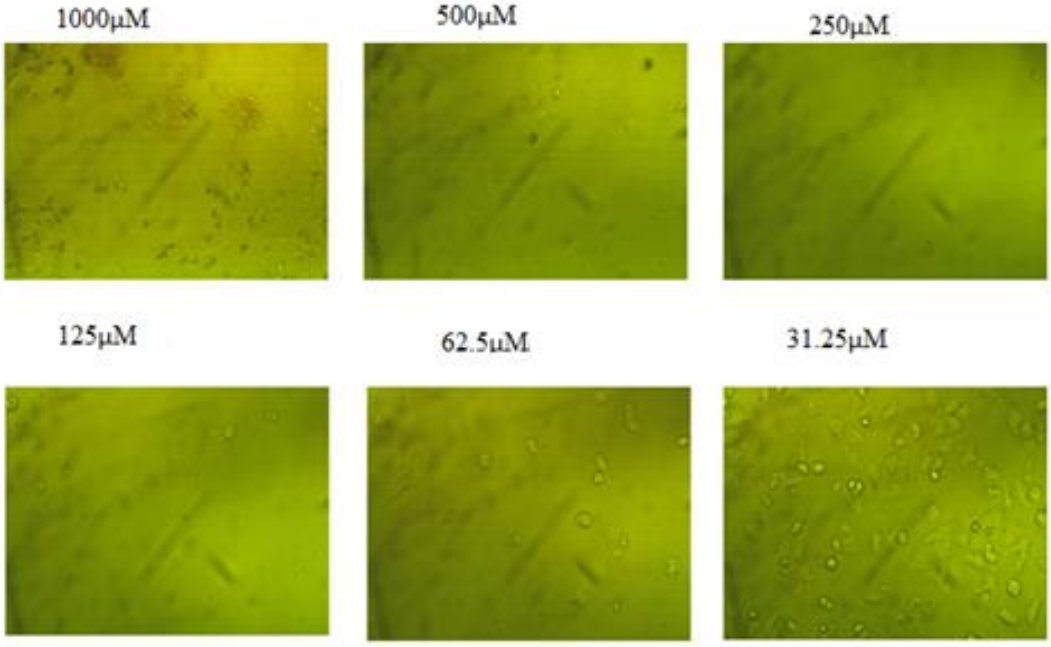


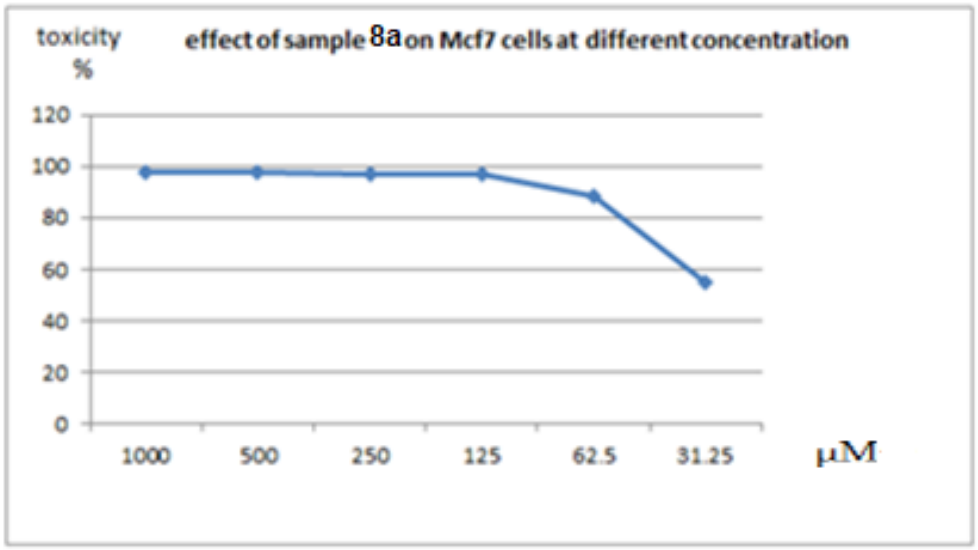
Effect of sample 20 on Mcf7 cells at different concentrationa



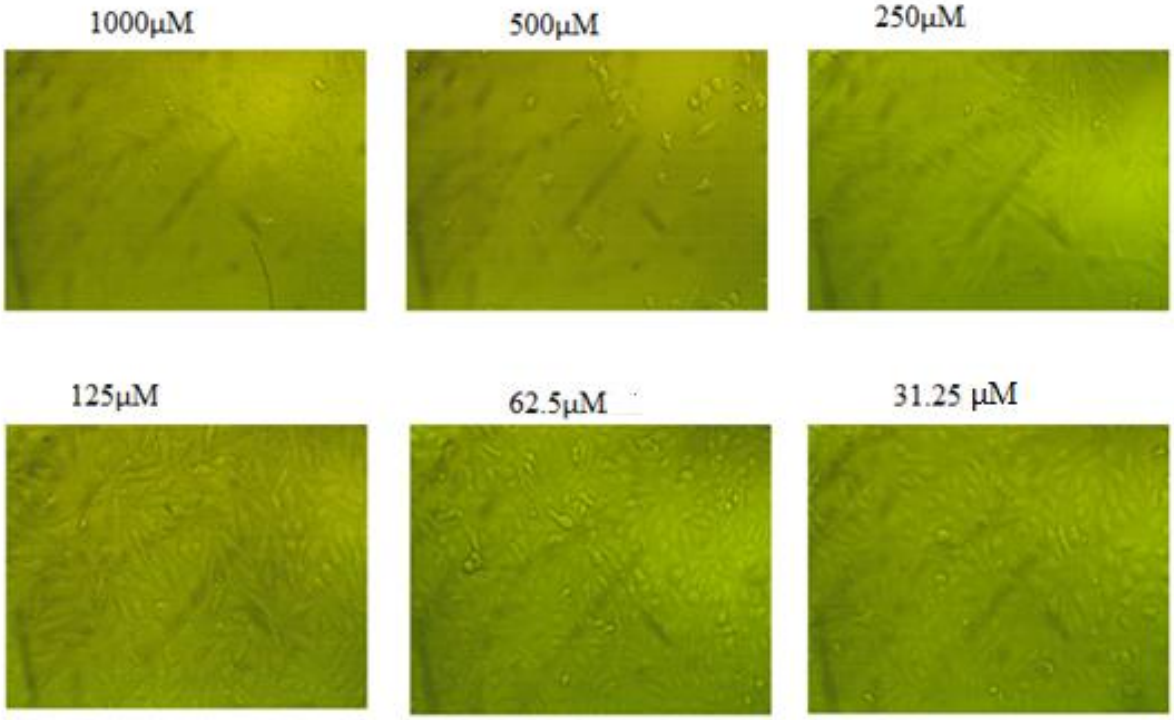


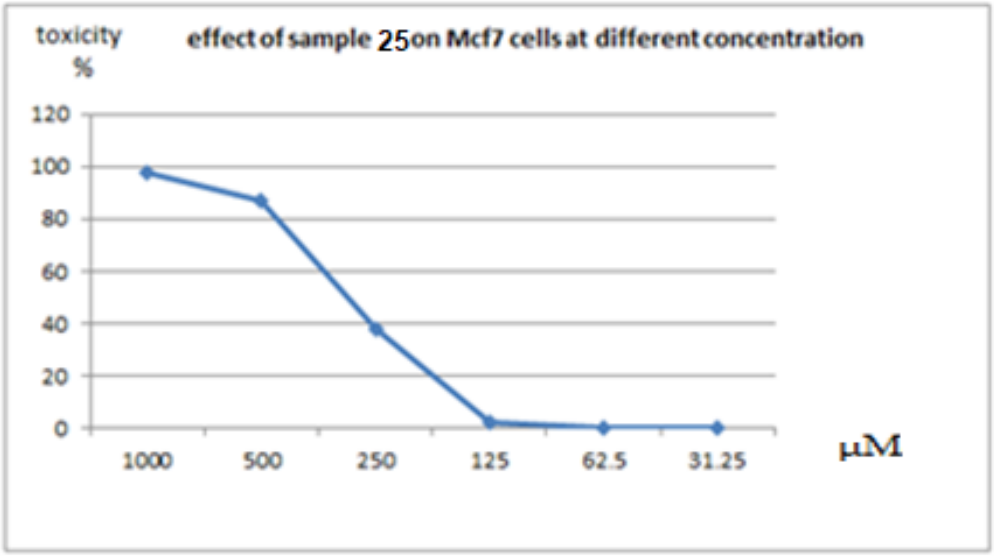
Effect of sample 8a on Mcf7 cells at different concentration



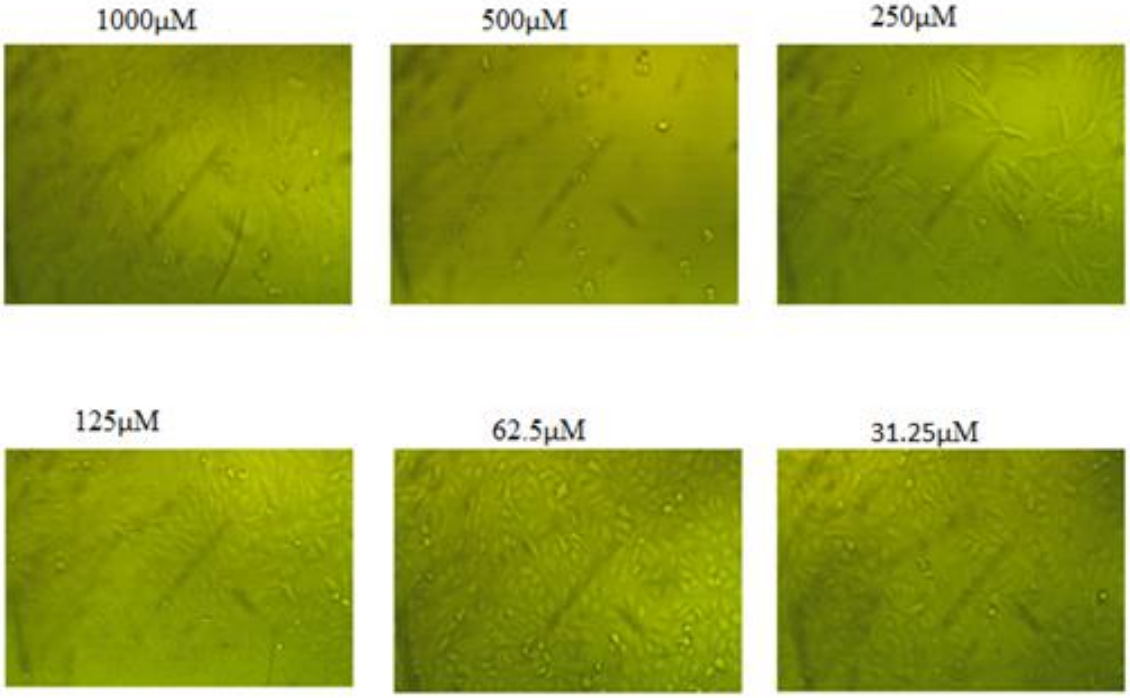


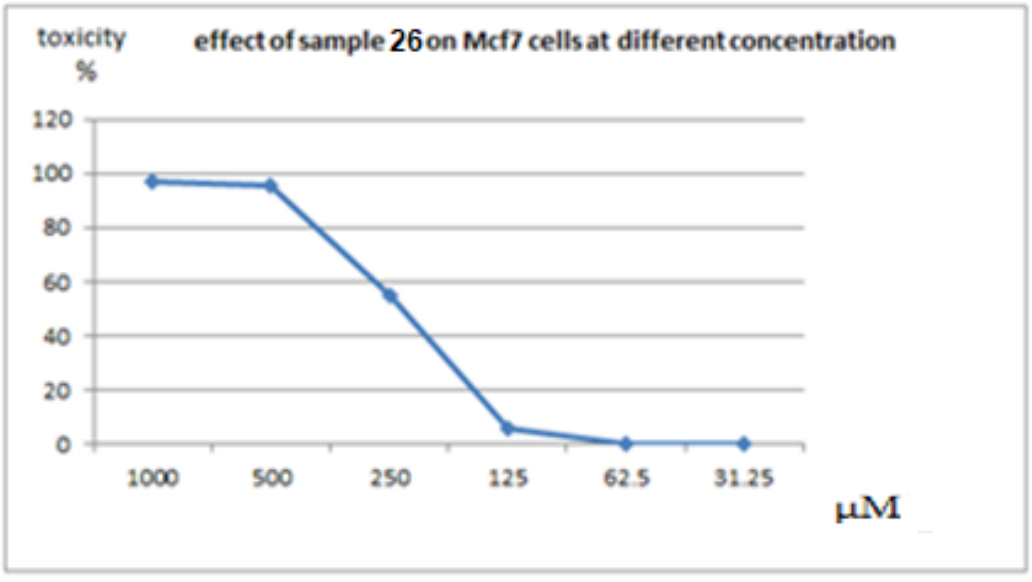
Effect of sample 25 on Mcf7 cells at different concentration



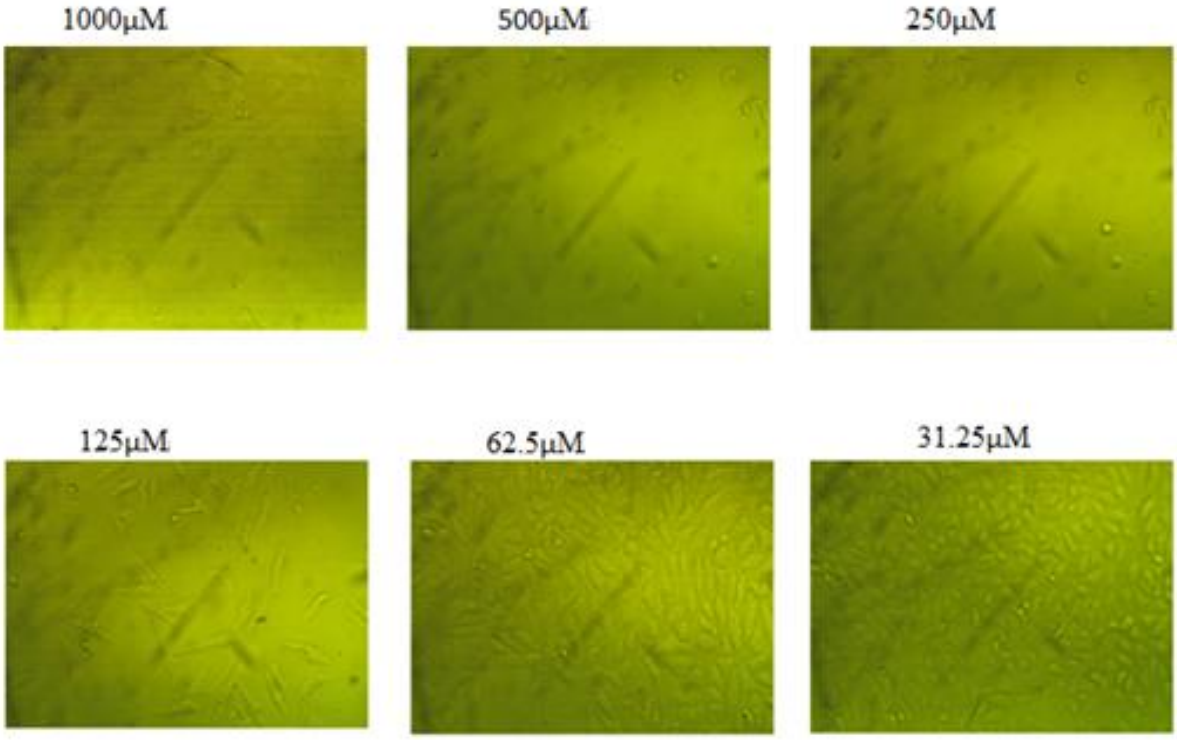


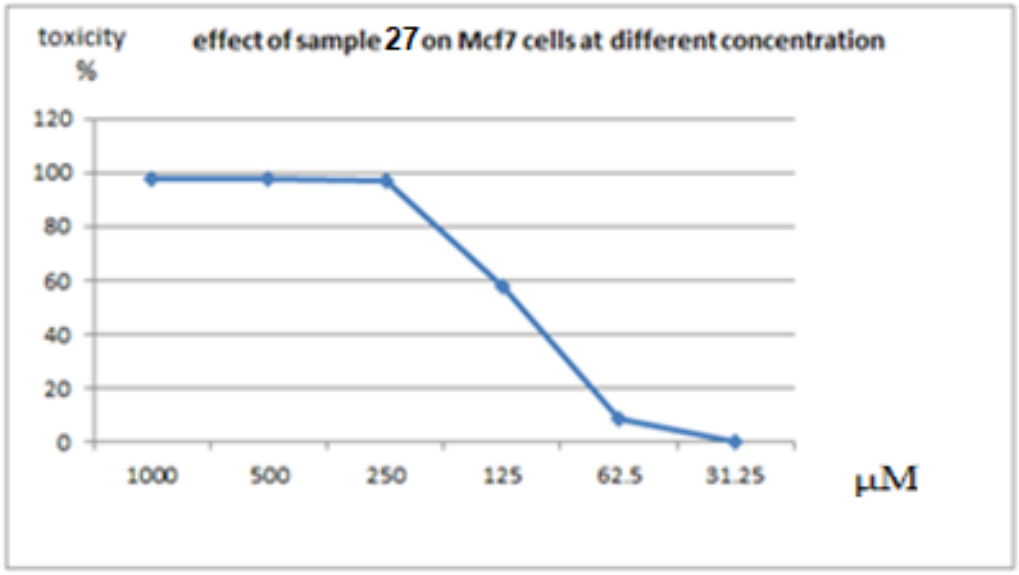
Effect of sample 26 on Mcf7 cells at different concentration



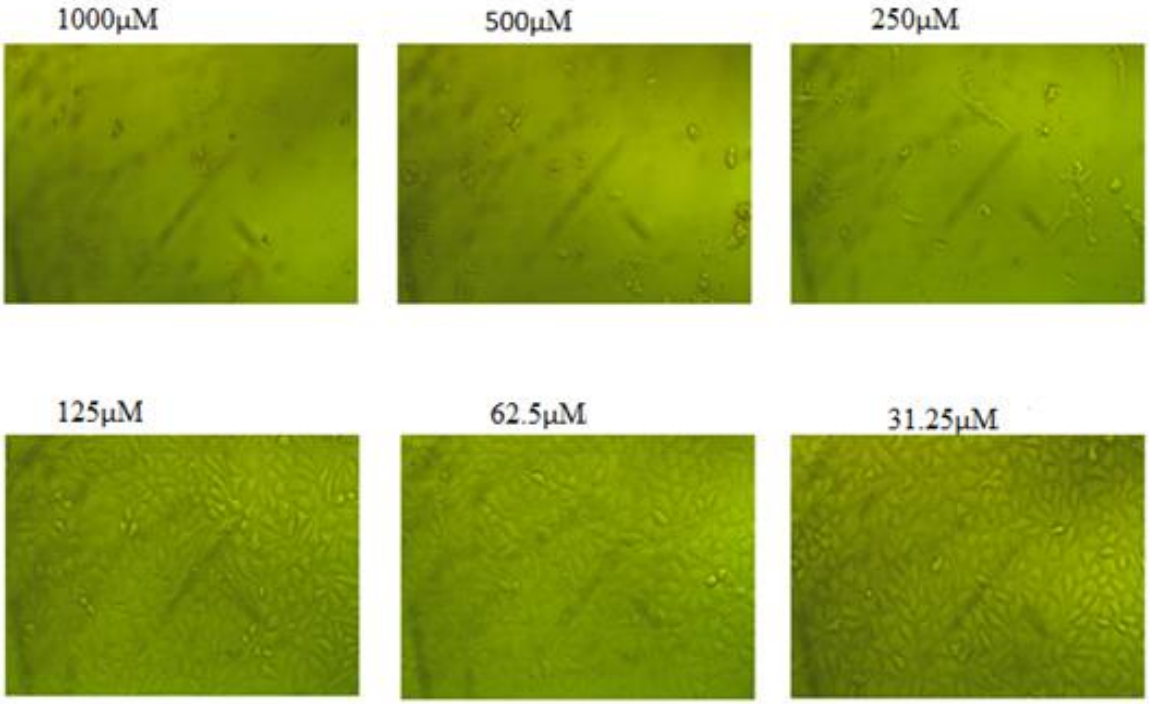


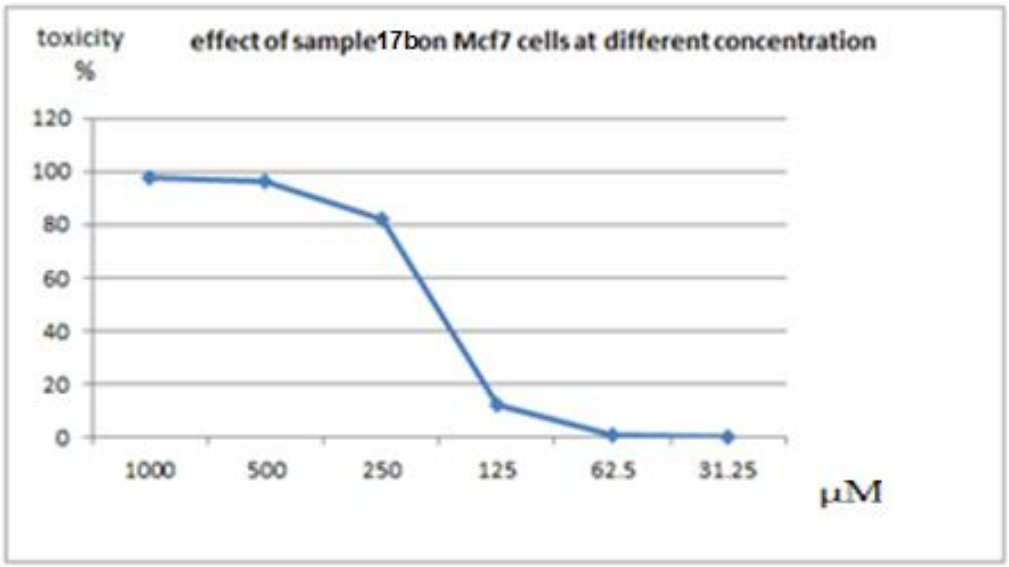
Effect of sample 27 on Mcf7 cells at different concentration



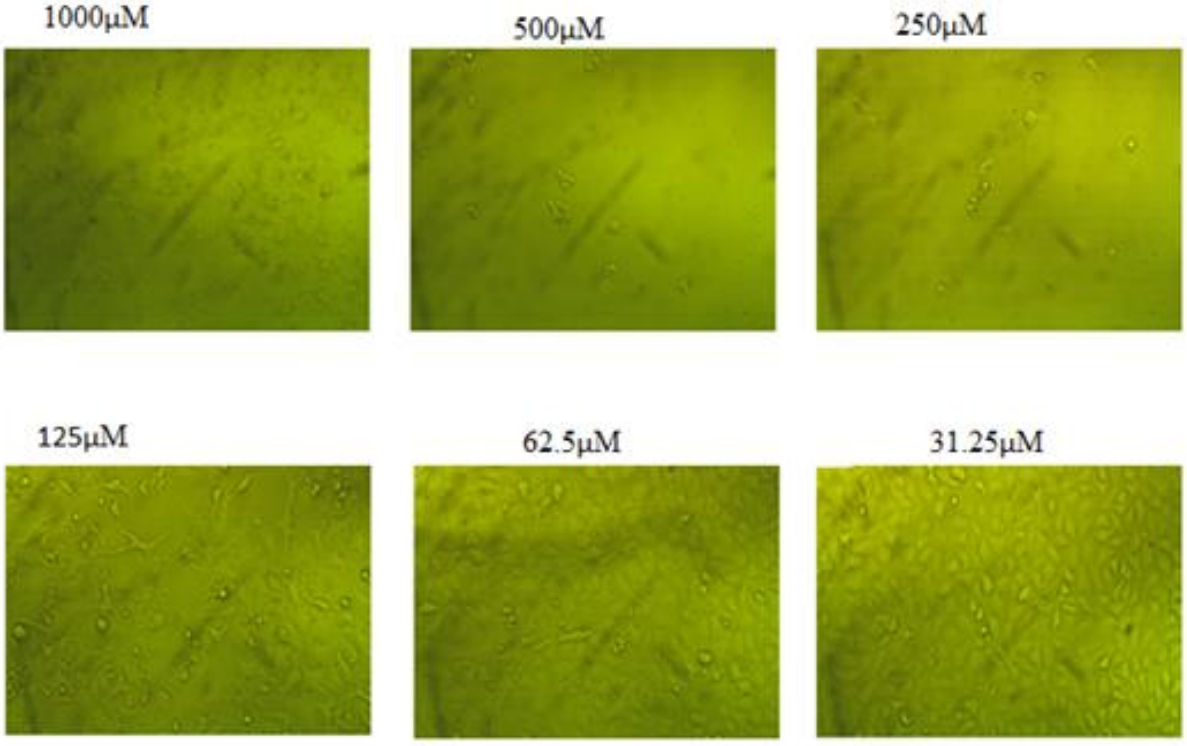


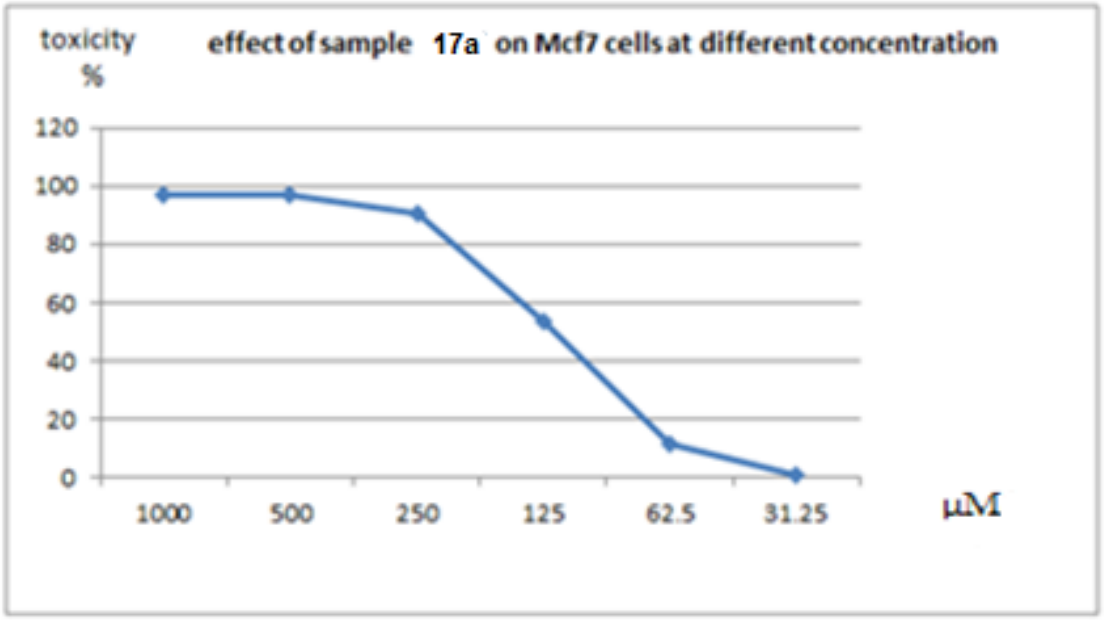
Effect of sample17bon Mcf7 cells at different concentration



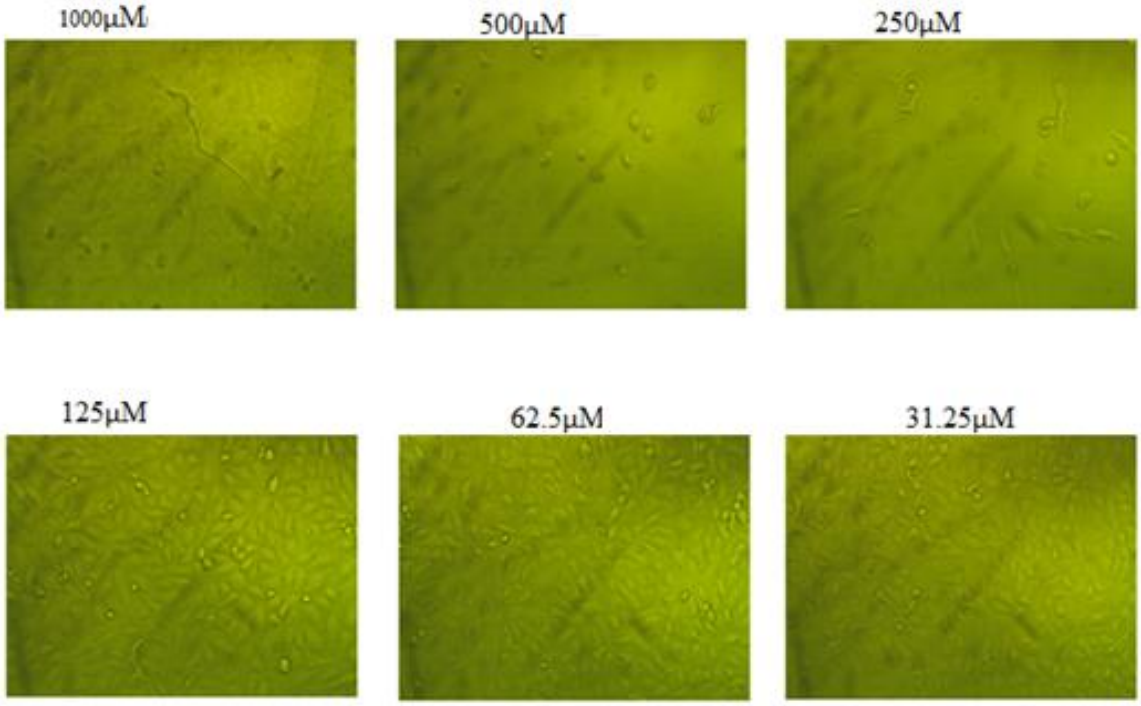


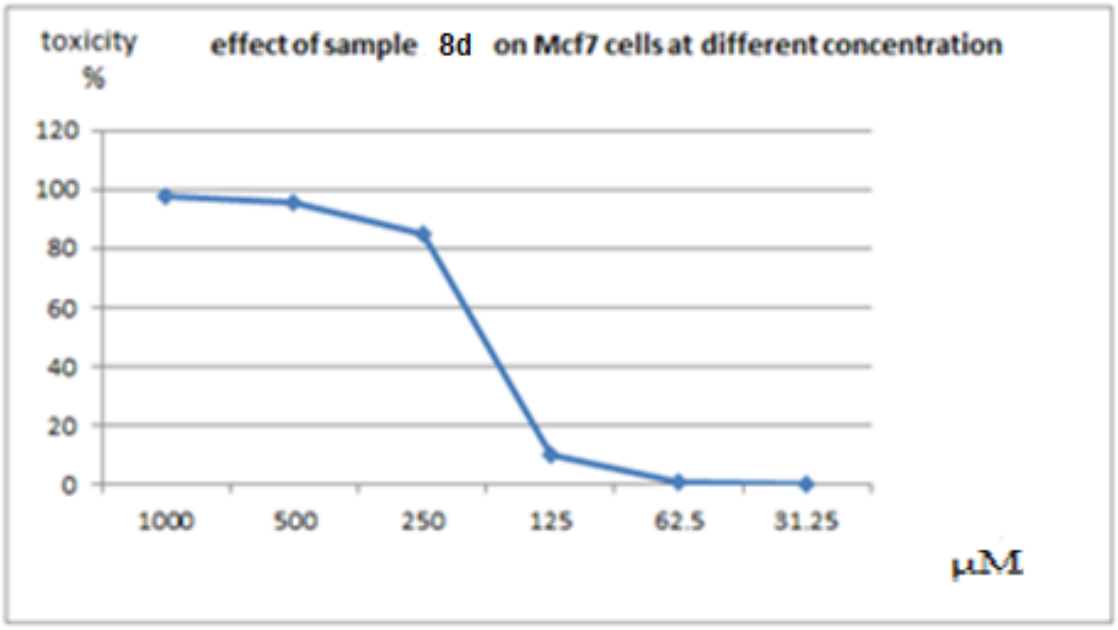
Effect of sample17a on Mcf7 cells at different concentration



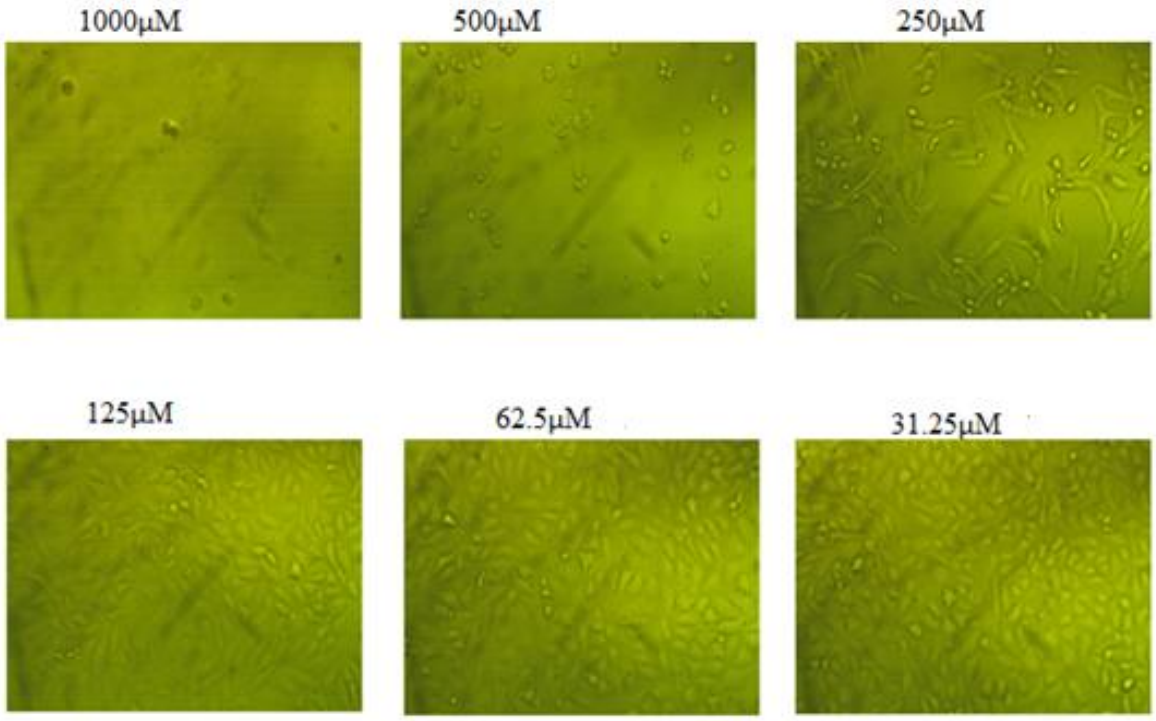


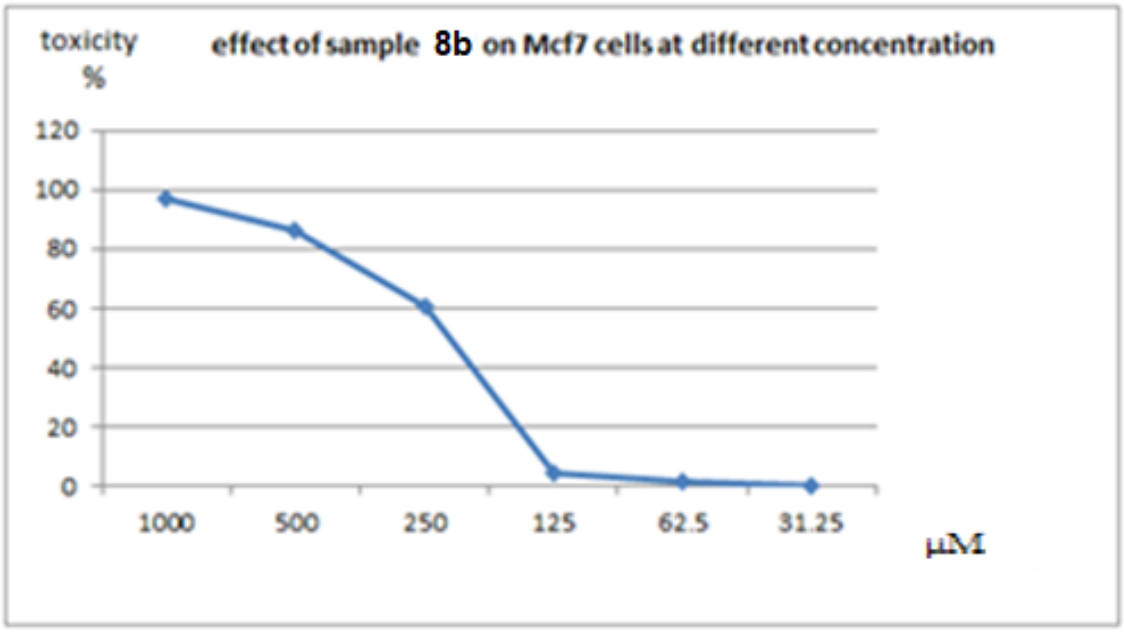
Effect of sample 8d on Mcf7 cells at different concentration



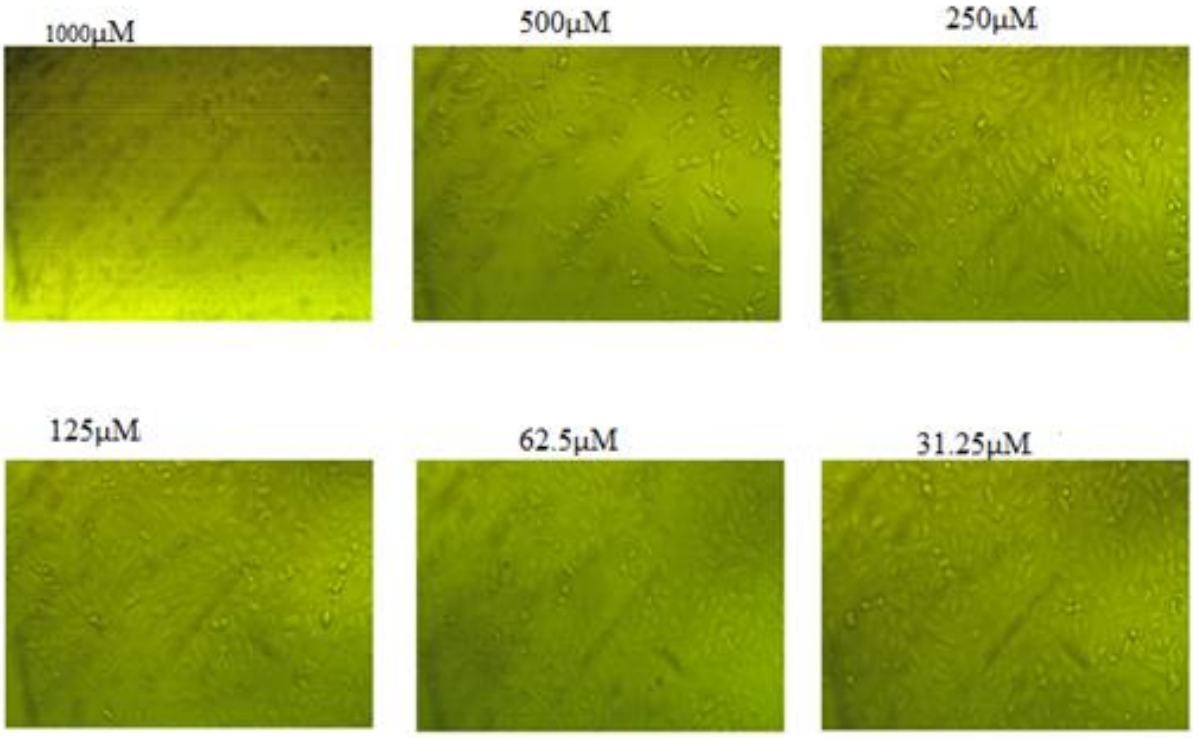


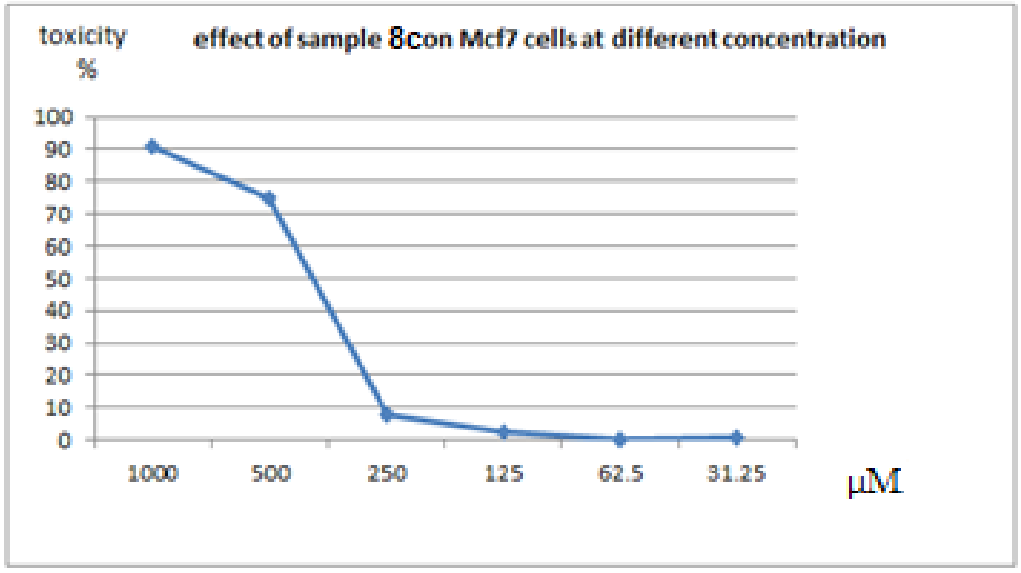
Effect of sample 8b on Mcf7 cells at different concentration

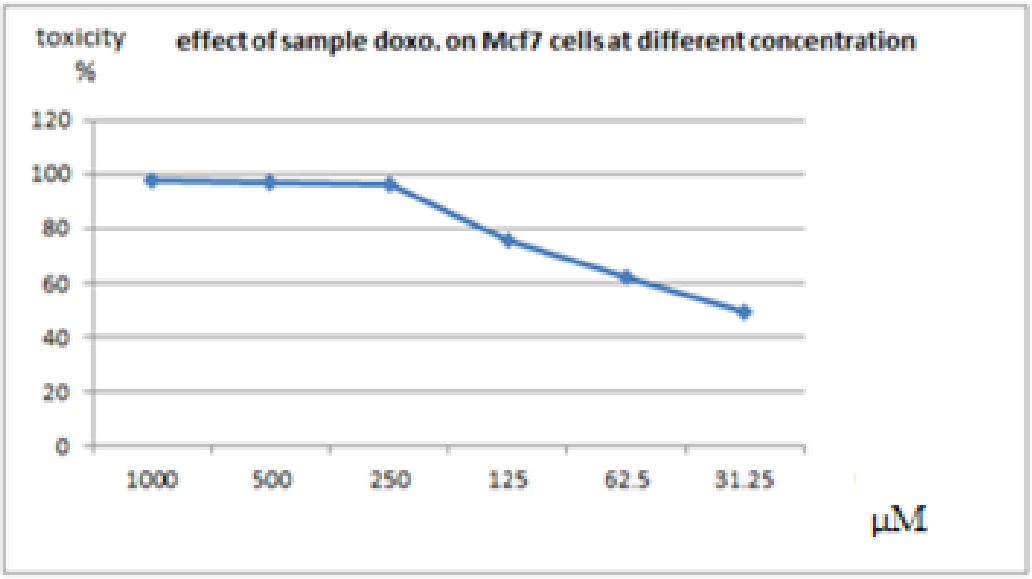




Effect of sample 8c on Mcf7 cells at different concentration







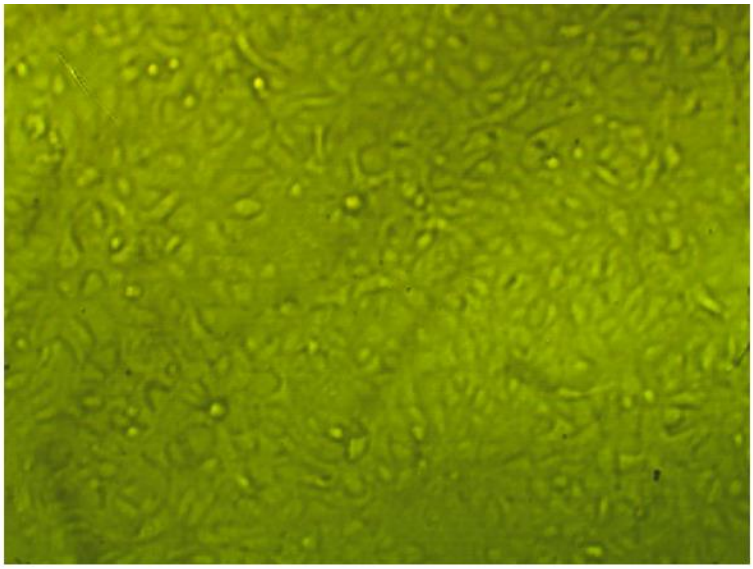
ID	uM	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
MDA	--------	0.749	0.722	0.731	0.734	0.007937	100	0	uM
3	1000	0.016	0.018	0.019	0.017667	0.000882	2.406902816	97.59309718	222.29 ± 9.01
	500	0.034	0.052	0.089	0.058333	0.01619	7.947320618	92.05267938	
	250	0.248	0.316	0.295	0.286333	0.020103	39.00999092	60.99000908	
	125	0.719	0.723	0.741	0.727667	0.006766	99.13714805	0.862851953	
	62.5	0.746	0.711	0.739	0.732	0.010693	99.72752044	0.272479564	
	31.25	0.733	0.729	0.73	0.730667	0.001202	99.54586739	0.454132607	
20	1000	0.024	0.031	0.035	0.03	0.003215	4.08719346	95.91280654	93.58 ± 1.39
	500	0.056	0.042	0.047	0.048333	0.004096	6.584922797	93.4150772	
	250	0.089	0.092	0.079	0.086667	0.00393	11.80744777	88.19255223	
	125	0.288	0.269	0.305	0.287333	0.010398	39.1462307	60.8537693	
	62.5	0.431	0.444	0.386	0.420333	0.017572	57.26612171	42.73387829	
	31.25	0.658	0.631	0.649	0.646	0.007937	88.01089918	11.98910082	
21	1000	0.015	0.018	0.014	0.015667	0.001202	2.134423252	97.86557675	88.85 ± 1.55
	500	0.016	0.018	0.018	0.017333	0.000667	2.361489555	97.63851045	
	250	0.044	0.023	0.037	0.034667	0.006173	4.72297911	95.27702089	
	125	0.067	0.084	0.092	0.081	0.007371	11.03542234	88.96457766	
	62.5	0.645	0.679	0.638	0.654	0.012662	89.10081744	10.89918256	
	31.25	0.733	0.729	0.731	0.731	0.001155	99.59128065	0.408719346	
8a	1000	0.017	0.019	0.018	0.018	0.000577	2.452316076	97.54768392	69.95 ± 2.45
	500	0.019	0.018	0.018	0.018333	0.000333	2.497729337	97.50227066	
	250	0.035	0.022	0.036	0.031	0.004509	4.223433243	95.77656676	
	125	0.056	0.06	0.072	0.062667	0.004807	8.537693006	91.46230699	
	62.5	0.245	0.271	0.238	0.251333	0.010039	34.24159855	65.75840145	

	31.25	0.684	0.743	0.732	0.719667	0.018114	98.04722979	1.952770209	
25	1000	0.018	0.023	0.019	0.02	0.001528	2.72479564	97.27520436	170.46 ± 2.04
	500	0.018	0.02	0.019	0.019	0.000577	2.588555858	97.41144414	
	250	0.218	0.178	0.194	0.196667	0.011624	26.7938238	73.2061762	
	125	0.437	0.399	0.409	0.415	0.011372	56.53950954	43.46049046	
	62.5	0.658	0.708	0.693	0.686333	0.014814	93.50590372	6.494096276	
	31.25	0.722	0.738	0.733	0.731	0.004726	99.59128065	0.408719346	
26	1000	0.019	0.023	0.027	0.023	0.002309	3.133514986	96.86648501	237.72 ± 3.06
	500	0.056	0.062	0.07	0.062667	0.004055	8.537693006	91.46230699	
	250	0.34	0.318	0.339	0.332333	0.007172	45.27702089	54.72297911	
	125	0.632	0.619	0.658	0.636333	0.011465	86.69391462	13.30608538	
	62.5	0.729	0.715	0.73	0.724667	0.004842	98.7284287	1.271571299	
	31.25	0.731	0.738	0.721	0.73	0.004933	99.45504087	0.544959128	

ID	**uM**	**O.D**			**Mean O.D**	**ST.E**	**Viability %**	**Toxicity %**	**IC_{50}**
MDA	--------	0.749	0.722	0.731	0.734	0.007937	100	0	uM
27	1000	0.018	0.016	0.018	0.017333	0.000667	2.361489555	97.63851045	78.52 ± 2.24
	500	0.018	0.015	0.019	0.017333	0.001202	2.361489555	97.63851045	
	250	0.018	0.02	0.019	0.019	0.000577	2.588555858	97.41144414	
	125	0.14	0.139	0.174	0.151	0.011504	20.57220708	79.42779292	
	62.5	0.349	0.326	0.368	0.347667	0.012143	47.36603088	52.63396912	
	31.25	0.688	0.732	0.705	0.708333	0.012811	96.50317893	3.496821072	
17b	1000	0.014	0.018	0.016	0.016	0.001155	2.179836512	97.82016349	114.11 ± 1.8
	500	0.017	0.018	0.014	0.016333	0.001202	2.225249773	97.77475023	
	250	0.02	0.024	0.018	0.020667	0.001764	2.815622162	97.18437784	
	125	0.32	0.285	0.316	0.307	0.01106	41.82561308	58.17438692	
	62.5	0.711	0.679	0.72	0.703333	0.012441	95.82198002	4.178019982	
	31.25	0.738	0.722	0.724	0.728	0.005033	99.18256131	0.817438692	
17a	1000	0.014	0.015	0.014	0.014333	0.000333	1.952770209	98.04722979	103.59 ± 0.54
	500	0.017	0.015	0.017	0.016333	0.000667	2.225249773	97.77475023	
	250	0.019	0.018	0.019	0.018667	0.000333	2.543142598	97.4568574	
	125	0.24	0.218	0.237	0.231667	0.006888	31.56221617	68.43778383	
	62.5	0.701	0.684	0.722	0.702333	0.01099	95.68574024	4.314259764	
	31.25	0.738	0.734	0.72	0.730667	0.005457	99.54586739	0.454132607	
8d	1000	0.018	0.016	0.018	0.017333	0.000667	2.361489555	97.63851045	109.26 ± 1.73
	500	0.02	0.017	0.018	0.018333	0.000882	2.497729337	97.50227066	
	250	0.045	0.063	0.058	0.055333	0.005364	7.538601272	92.46139873	
	125	0.268	0.283	0.277	0.276	0.004359	37.60217984	62.39782016	
	62.5	0.729	0.711	0.717	0.719	0.005292	97.95640327	2.04359673	
	31.25	0.73	0.721	0.725	0.725333	0.002603	98.81925522	1.180744777	

8b	1000	0.017	0.019	0.018	0.018	0.000577	2.452316076	97.54768392	197.83 ± 4.51
	500	0.023	0.025	0.032	0.026667	0.002728	3.633060854	96.36693915	
	250	0.177	0.209	0.198	0.194667	0.009387	26.52134423	73.47865577	
	125	0.683	0.725	0.689	0.699	0.013115	95.23160763	4.768392371	
	62.5	0.727	0.719	0.699	0.715	0.008327	97.41144414	2.588555858	
	31.25	0.733	0.721	0.73	0.728	0.003606	99.18256131	0.817438692	

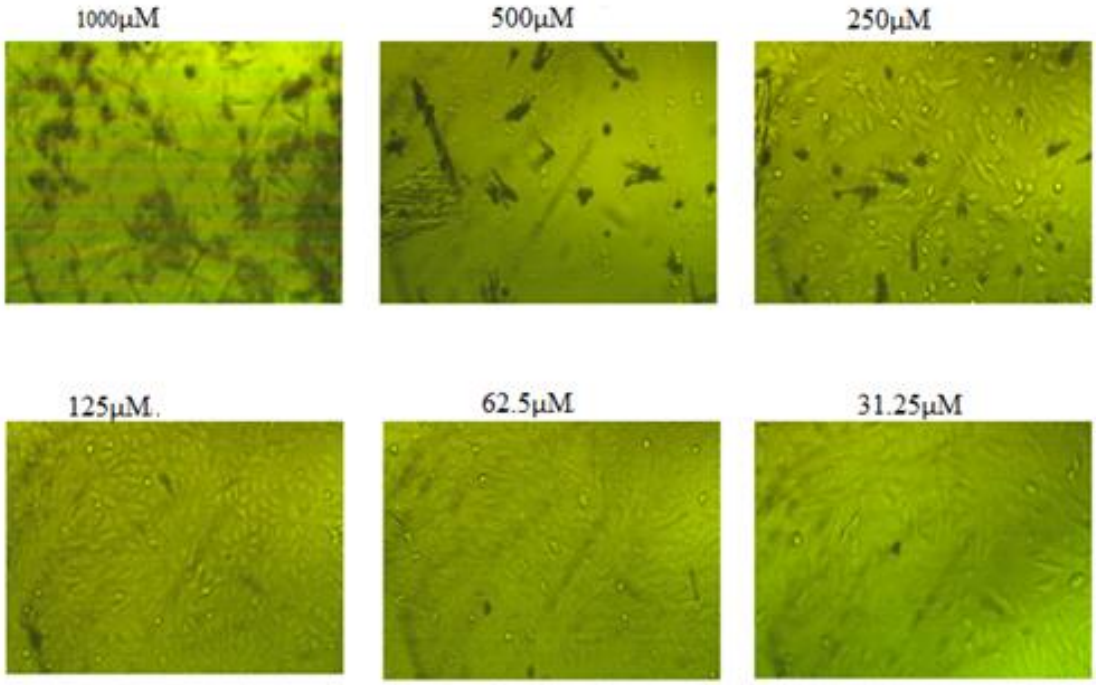
ID	uM	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
MDA	--------	0.749	0.722	0.731	0.734	0.007937	100	0	uM
8c	1000	0.067	0.065	0.07	0.067333	0.001453	9.173478656	90.82652134	335.63 ± 7.58
	500	0.089	0.099	0.134	0.107333	0.013642	14.62306994	85.37693006	
	250	0.589	0.549	0.528	0.555333	0.017892	75.65849228	24.34150772	
	125	0.658	0.694	0.677	0.676333	0.010398	92.1435059	7.856494096	
	62.5	0.722	0.722	0.745	0.729667	0.007667	99.40962761	0.590372389	
	31.25	0.698	0.742	0.726	0.722	0.012858	98.36512262	1.634877384	
doxo	1000	0.016	0.014	0.017	0.015667	0.000882	2.134423252	97.86557675	74.32 ± 1.1
	500	0.018	0.019	0.017	0.018	0.000577	2.452316076	97.54768392	
	250	0.045	0.068	0.073	0.062	0.008622	8.446866485	91.55313351	
	125	0.148	0.189	0.165	0.167333	0.011893	22.79745686	77.20254314	
	62.5	0.348	0.341	0.328	0.339	0.005859	46.1852861	53.8147139	
	31.25	0.64	0.593	0.611	0.614667	0.013691	83.74205268	16.25794732	

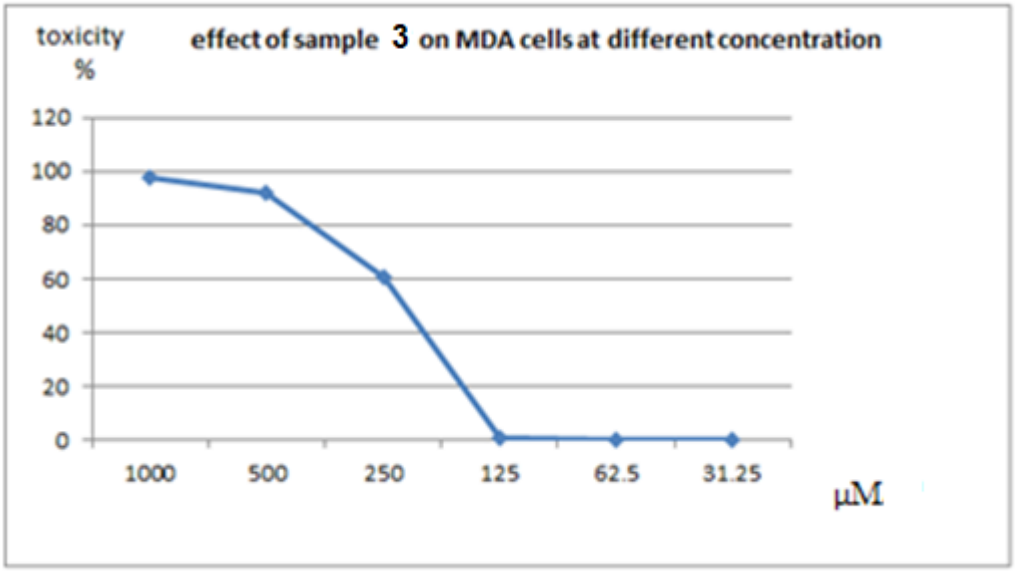


control
MDA-MB cells

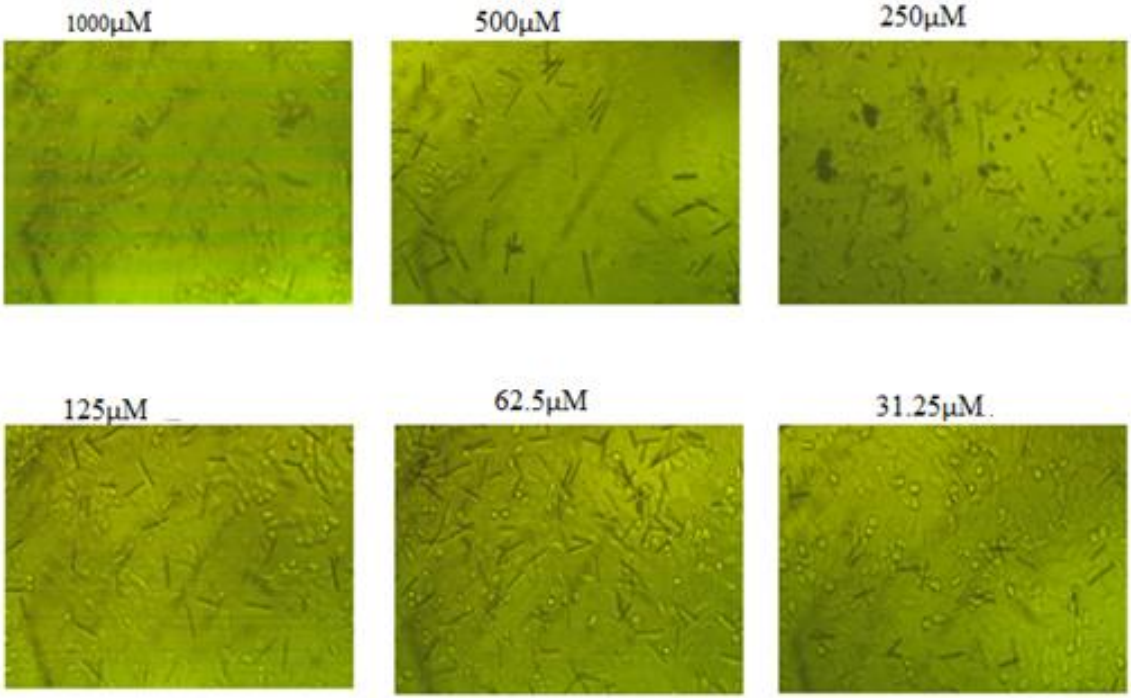
Organism :	*Homo sapiens*, human
Tissue :	mammary gland, breast; derived from metastatic site: pleural effusion
Cell Type :	epithelial
Culture Properties :	adherent
Disease :	adenocarcinoma

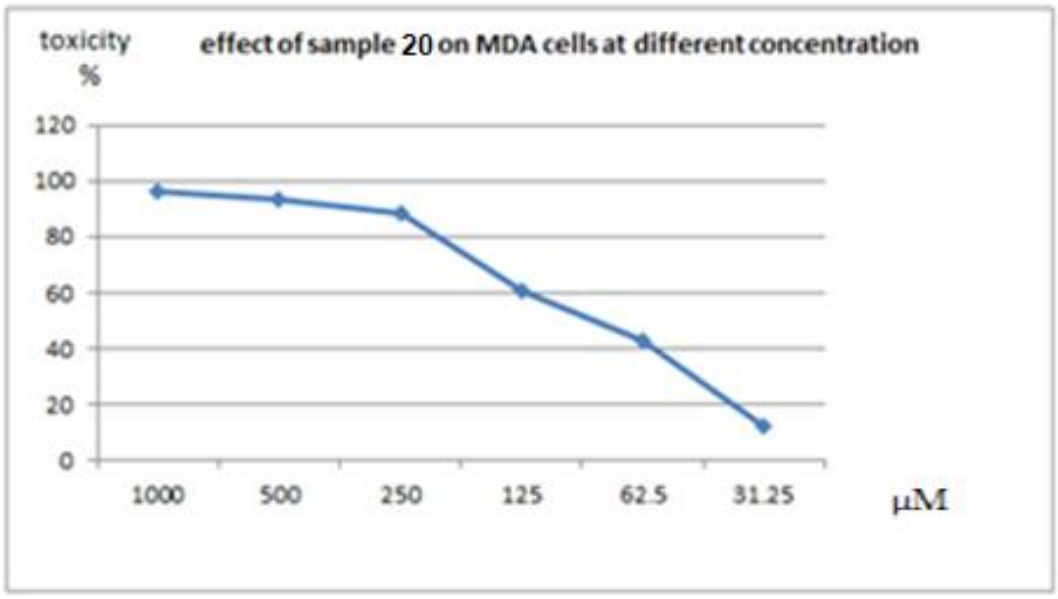
Effect of sample 3 on MDA cells at different concentration



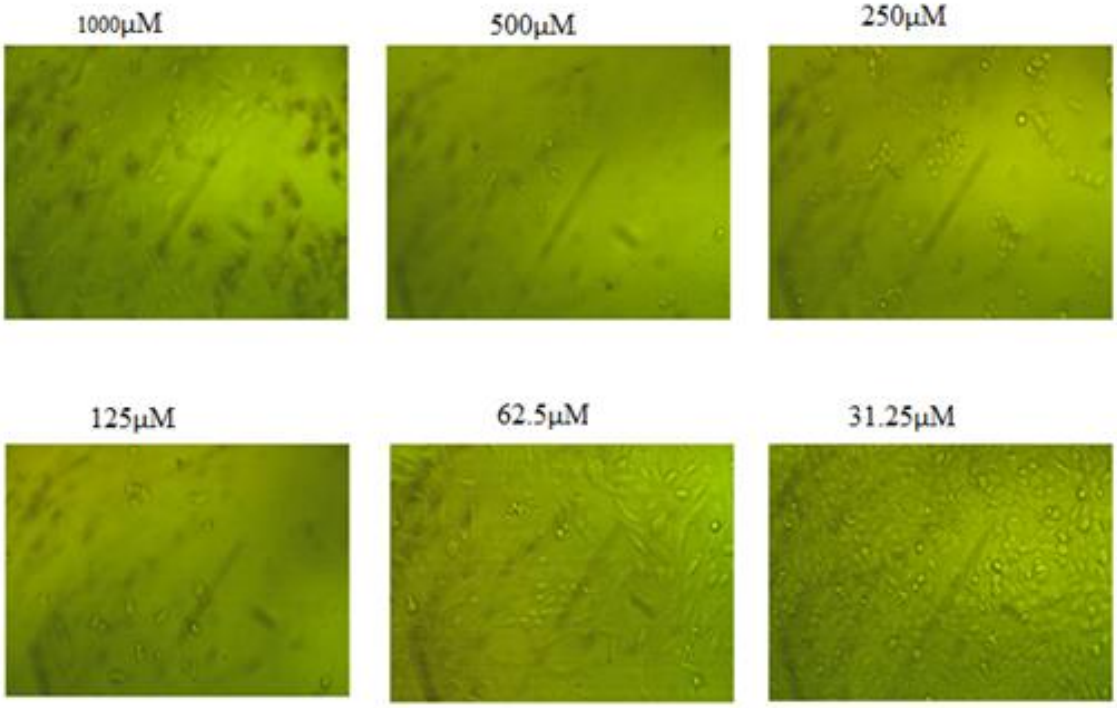


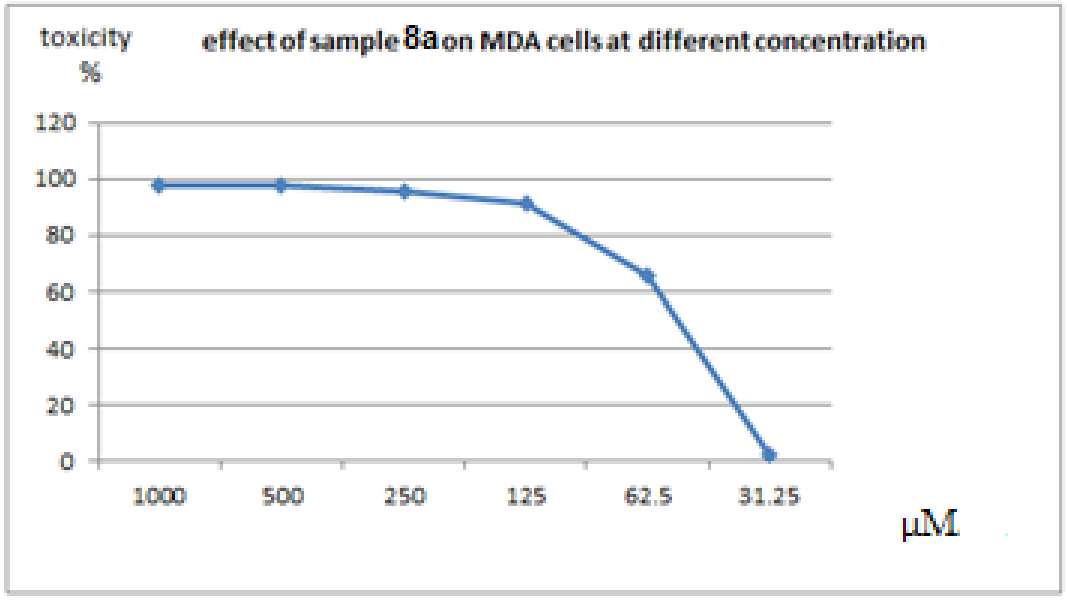
Effect of sample 20 on MDA cells at different concentration



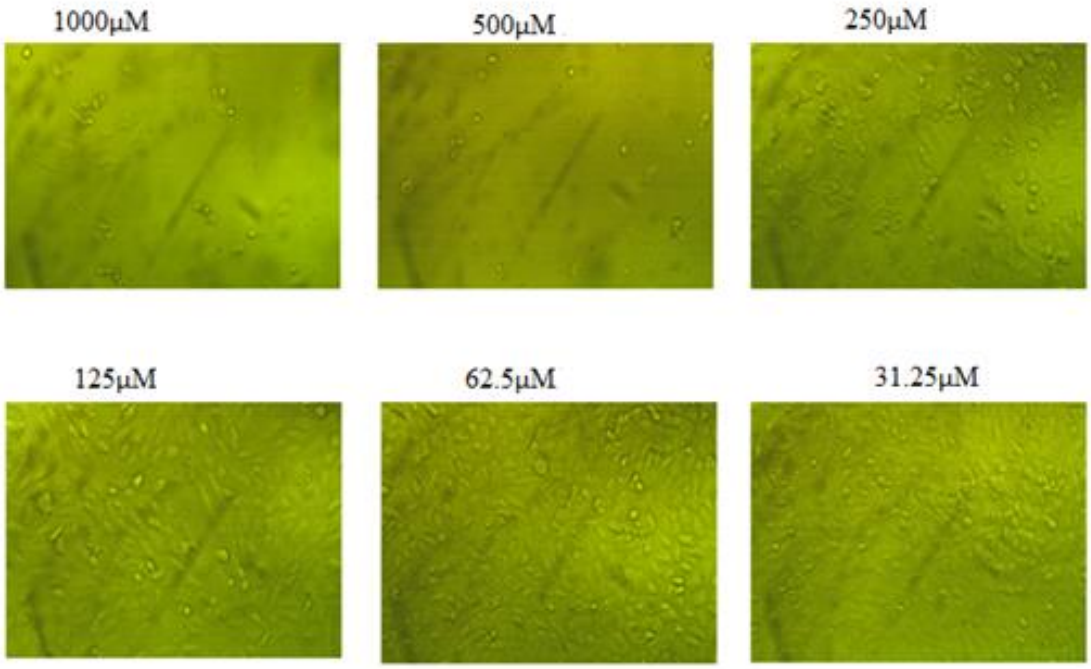


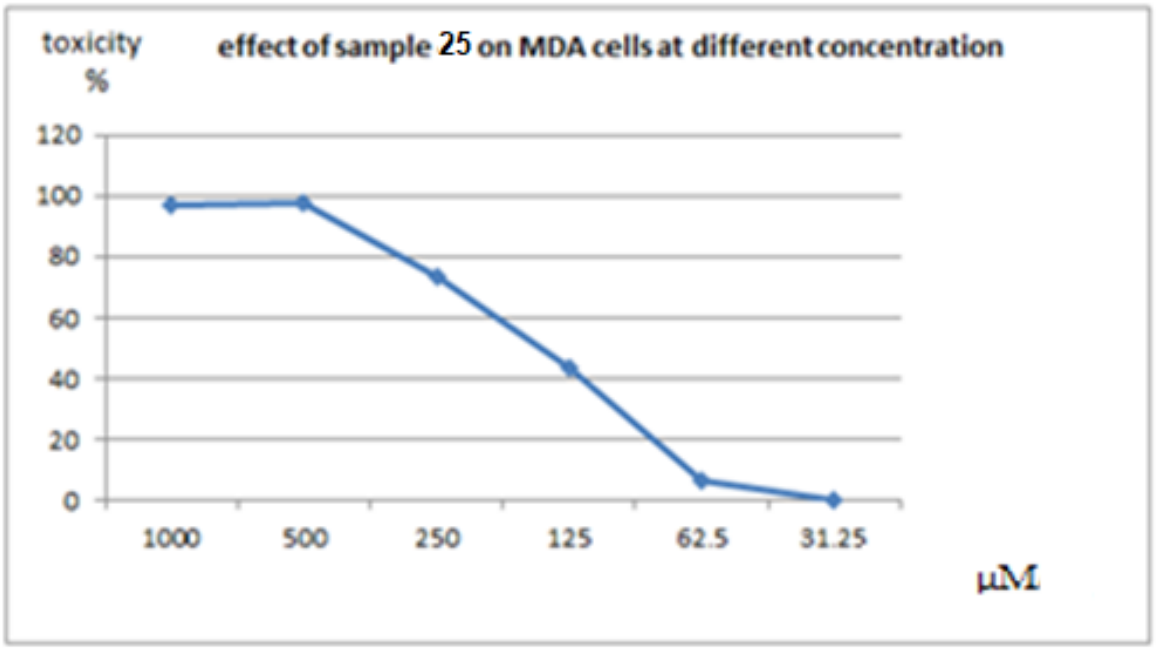
Effect of sample 8a on MDA cells at different concentration





Effect of sample 25 on MDA cells at different concentration



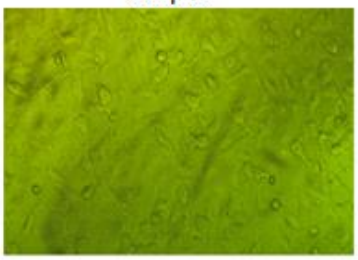


Effect of sample 26 on MDA cells at different concentration

1000µM

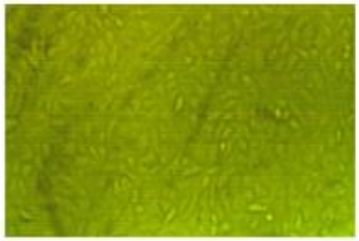
500µM

250µM

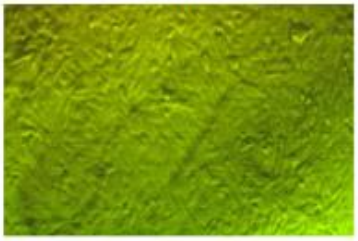


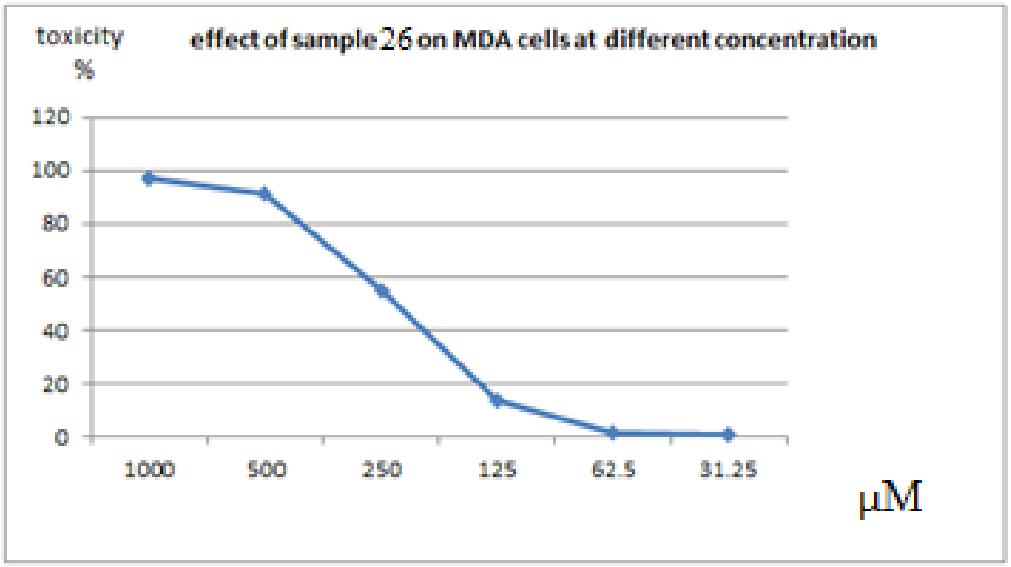
125µM

62.5µM



31.25µM





Effect of sample 27on Mcf7 cells at different concentration

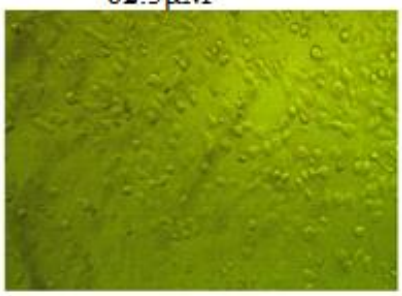
1000 µM

500µM

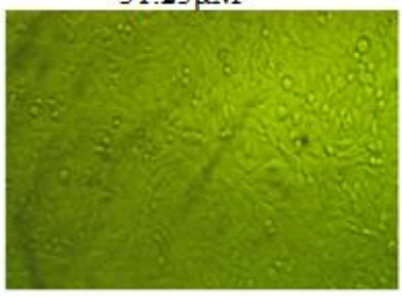
250µM

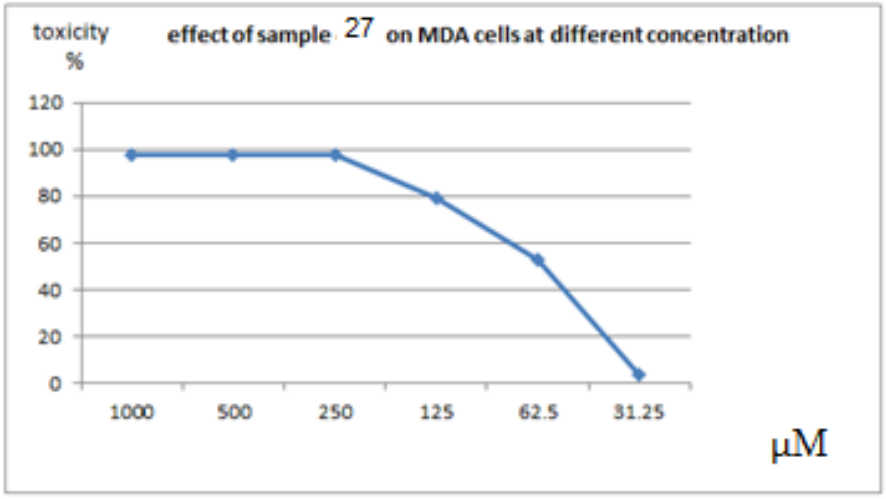
125µM

62.5µM

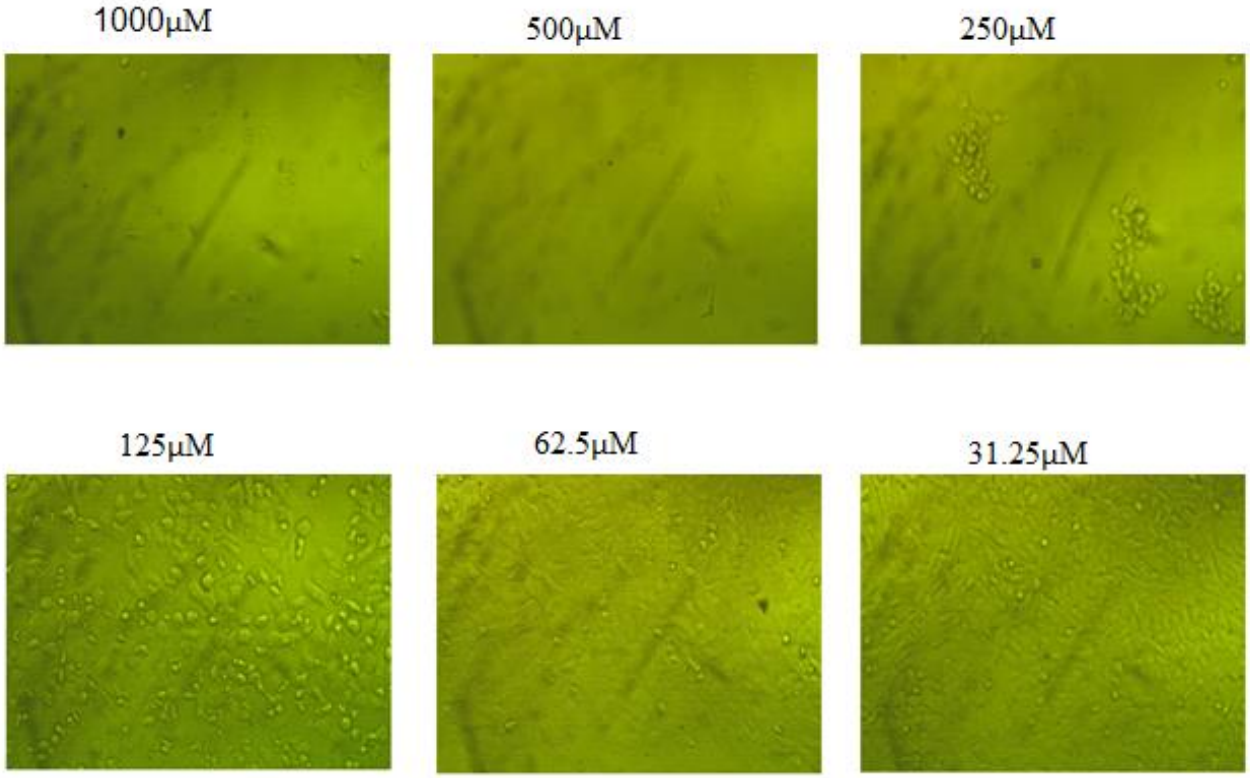


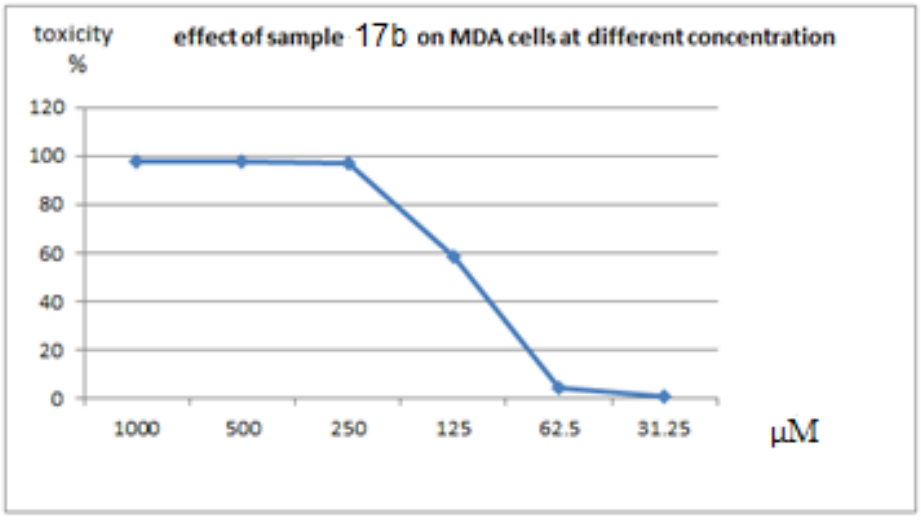
31.25µM



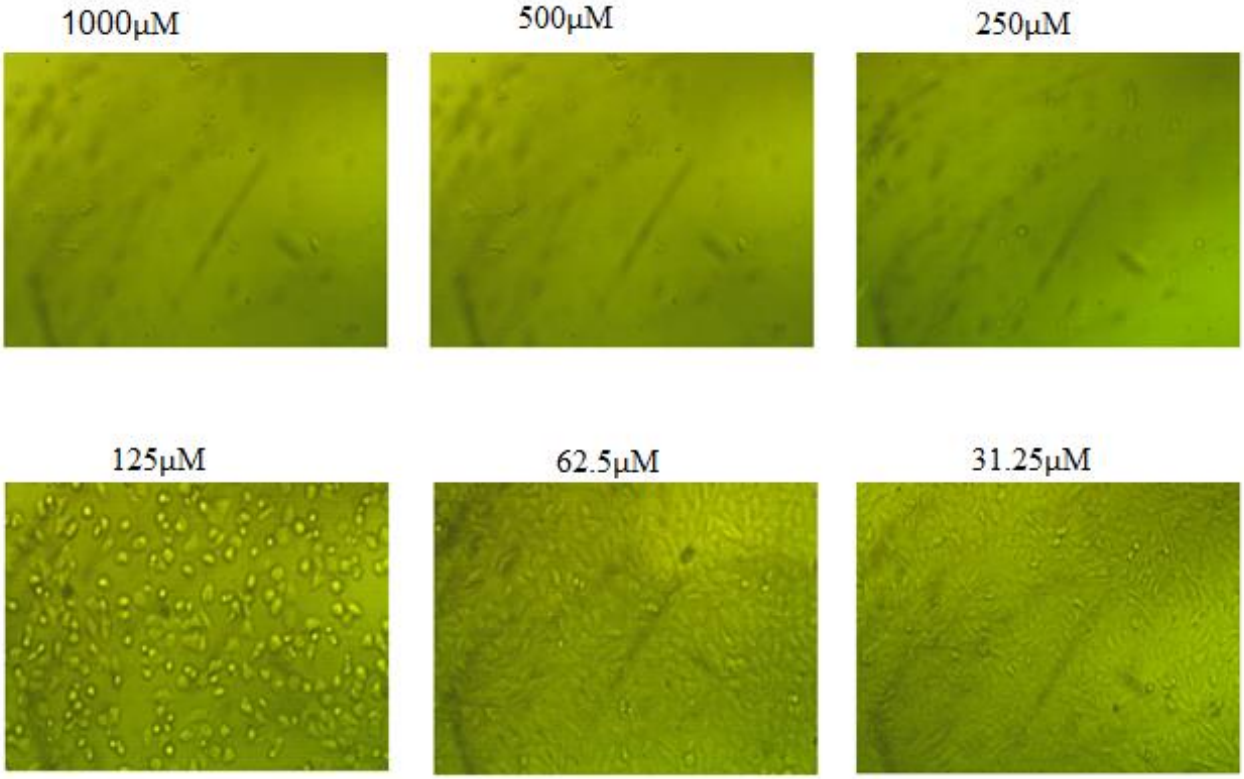


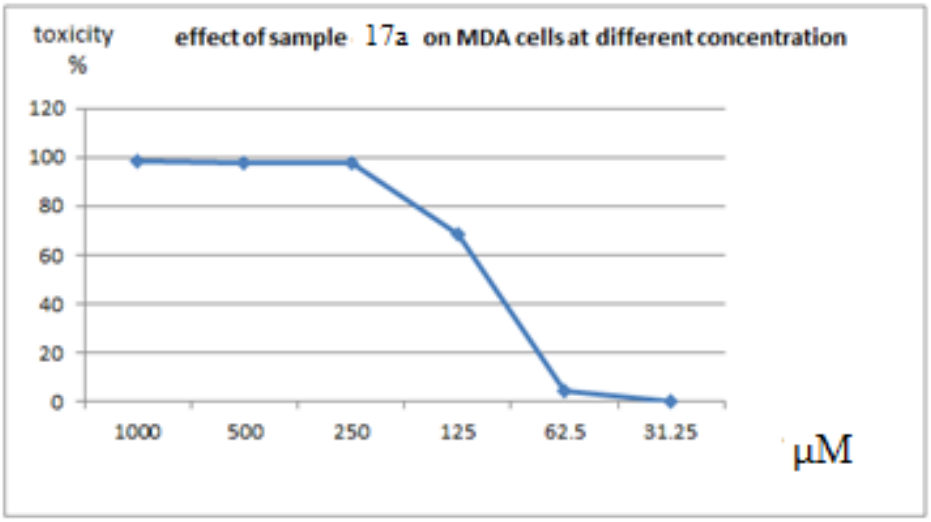
Effect of sample 17b on MDA cells at different concentration



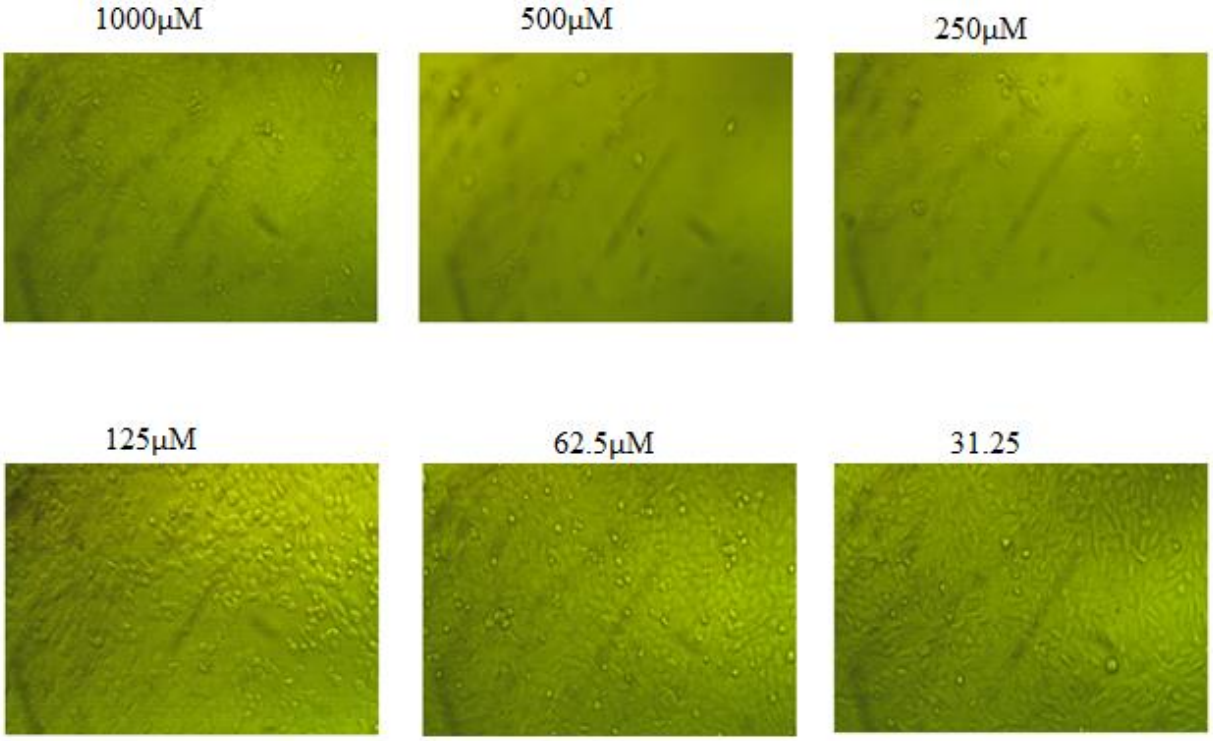


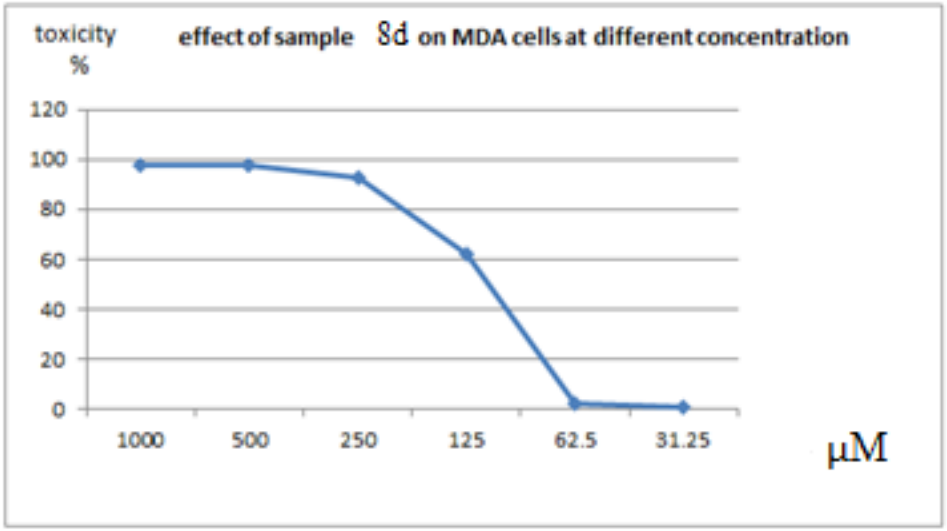
Effect of sample 17a on MDA cells at different concentration



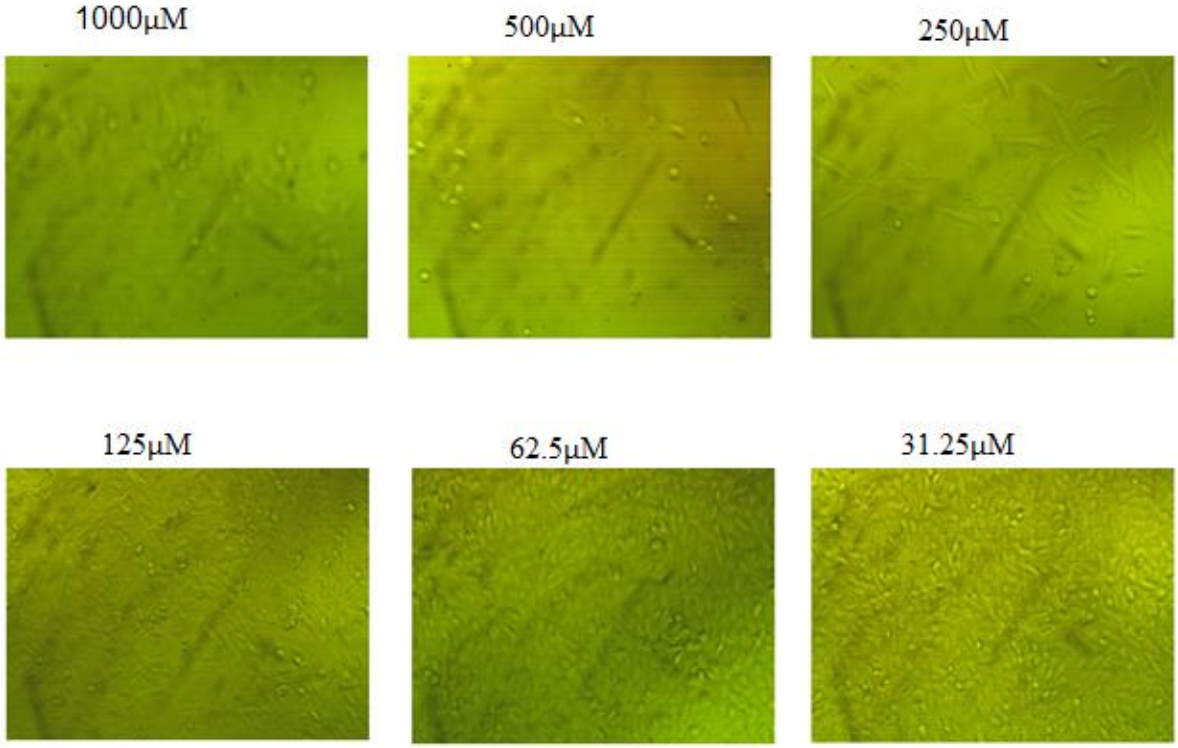


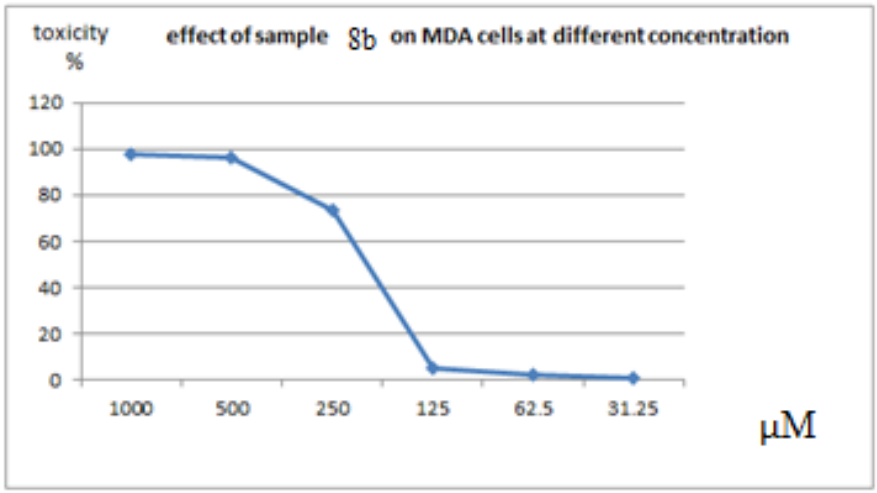
Effect of sample 8d on MDA cells at different concentration



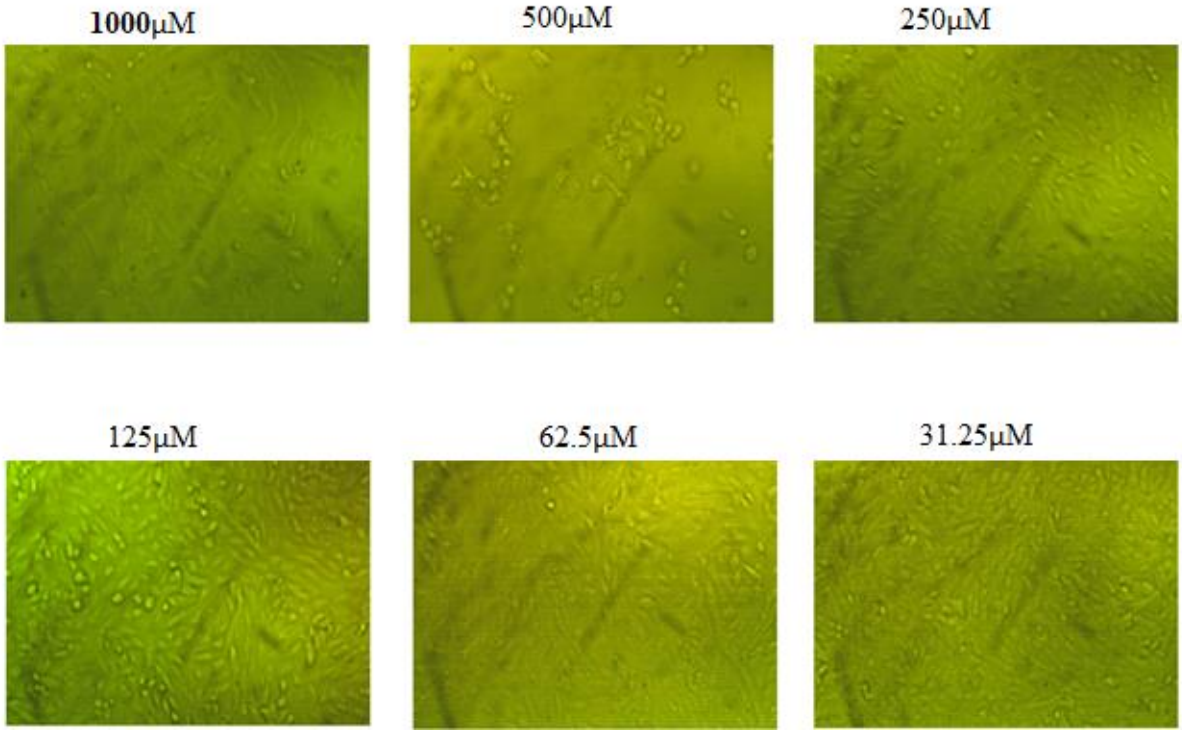


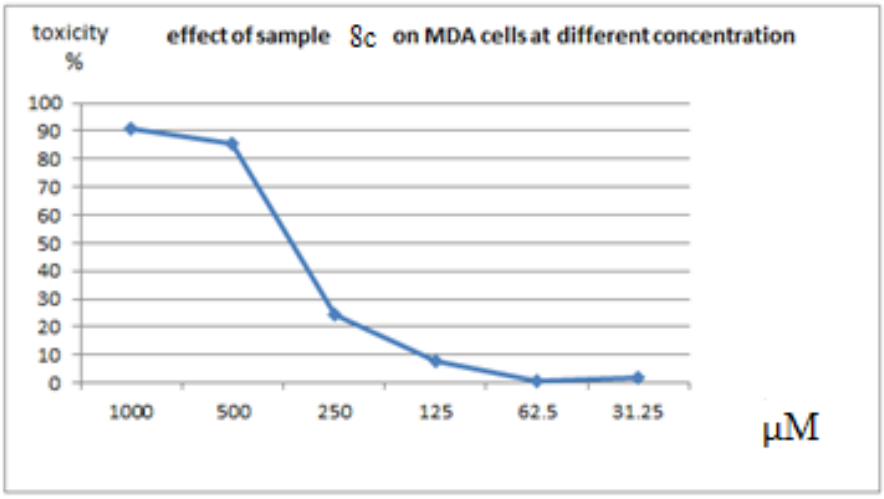
Effect of sample 8b on MDA cells at different concentration

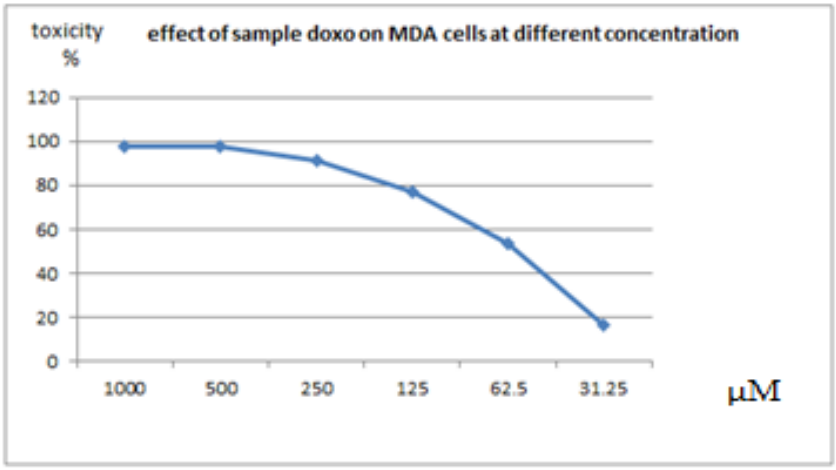




Effect of sample 8c on MDA cells at different concentration







Science Way
Science Way
For Scientific Researches

Viability assay

Test code: E-4-022-2

Institute / Researcher: Dr.Rehab Sabour

Experiment :

Human hormone shock protein 90 ELISA kit

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samples number : 2 samples with 4 conc./each.

experiment design : ELISA technique

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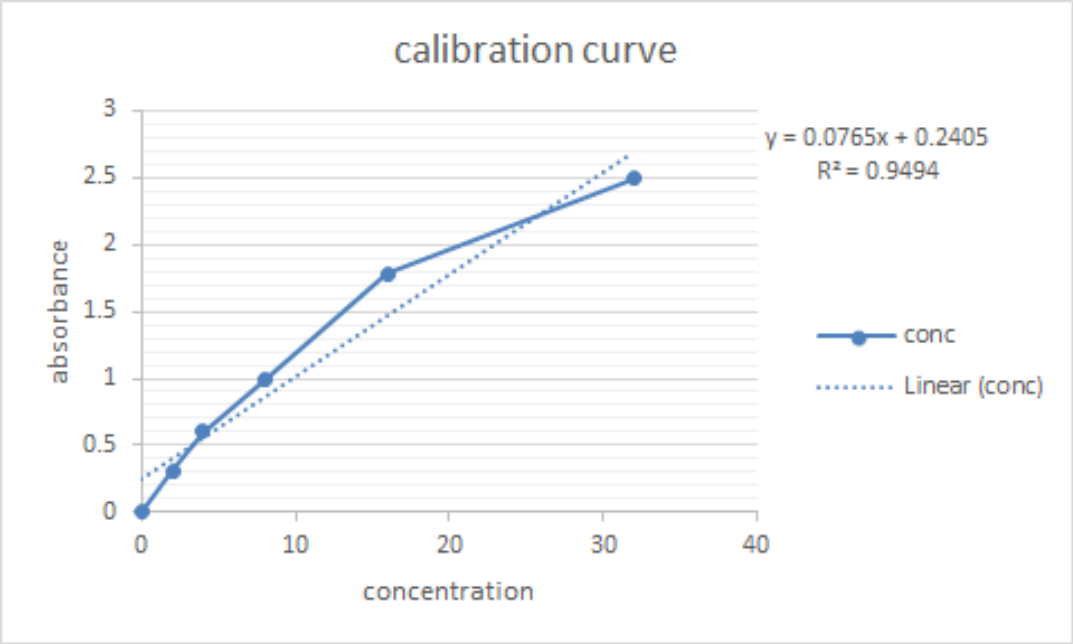
laboratory comments:

cell based tequ. On Mcf7

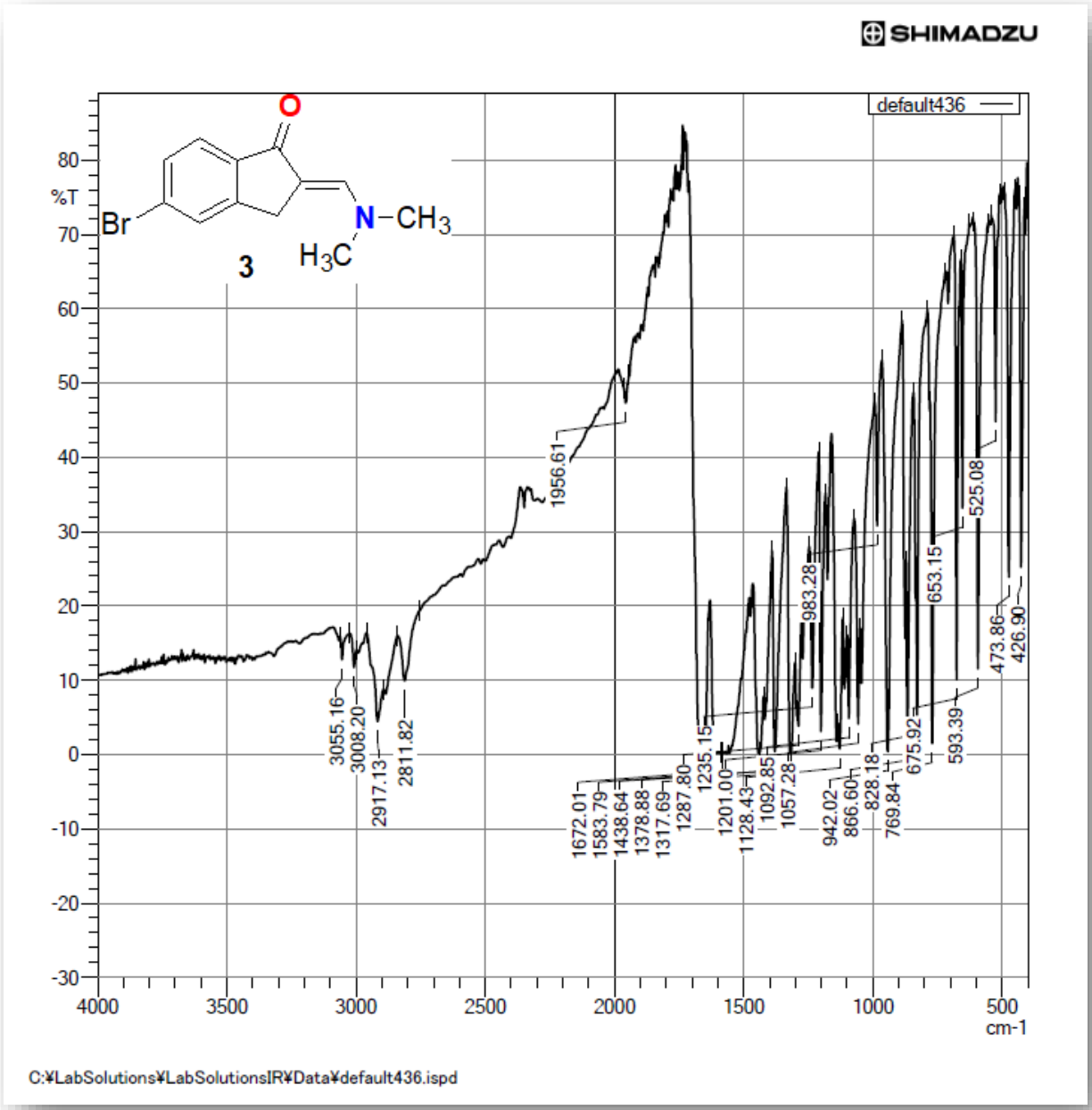
Calibration curve

Standard	Absorbance	Concentration nmol/ml
S1	0.004	0
S2	0.314	2
S3	0.600	4
S4	0.991	8
S5	1.785	16
S6	2.492	32



Results

Sample	Conc. nM	ng/ml	IC_{50}% nM
8a	100	1.388	18.79 ± 0.68
	50	1.518	
	25	1.977	
	12.5	2.02	
doxo	100	1.642	21.45 ± 1.88
	50	1.839	
	25	2.022	
	12.5	2.467	


SHIMADZU
default436
%T
cm-1
Br
N−CH3
H3C
3
3055.16
3008.20
2917.13
2811.82
1956.61
1672.01
1583.79
1438.64
1378.88
1317.69
1287.80
1235.15
1201.00
1128.43
1092.85
1057.28
983.28
942.02
866.60
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426.90
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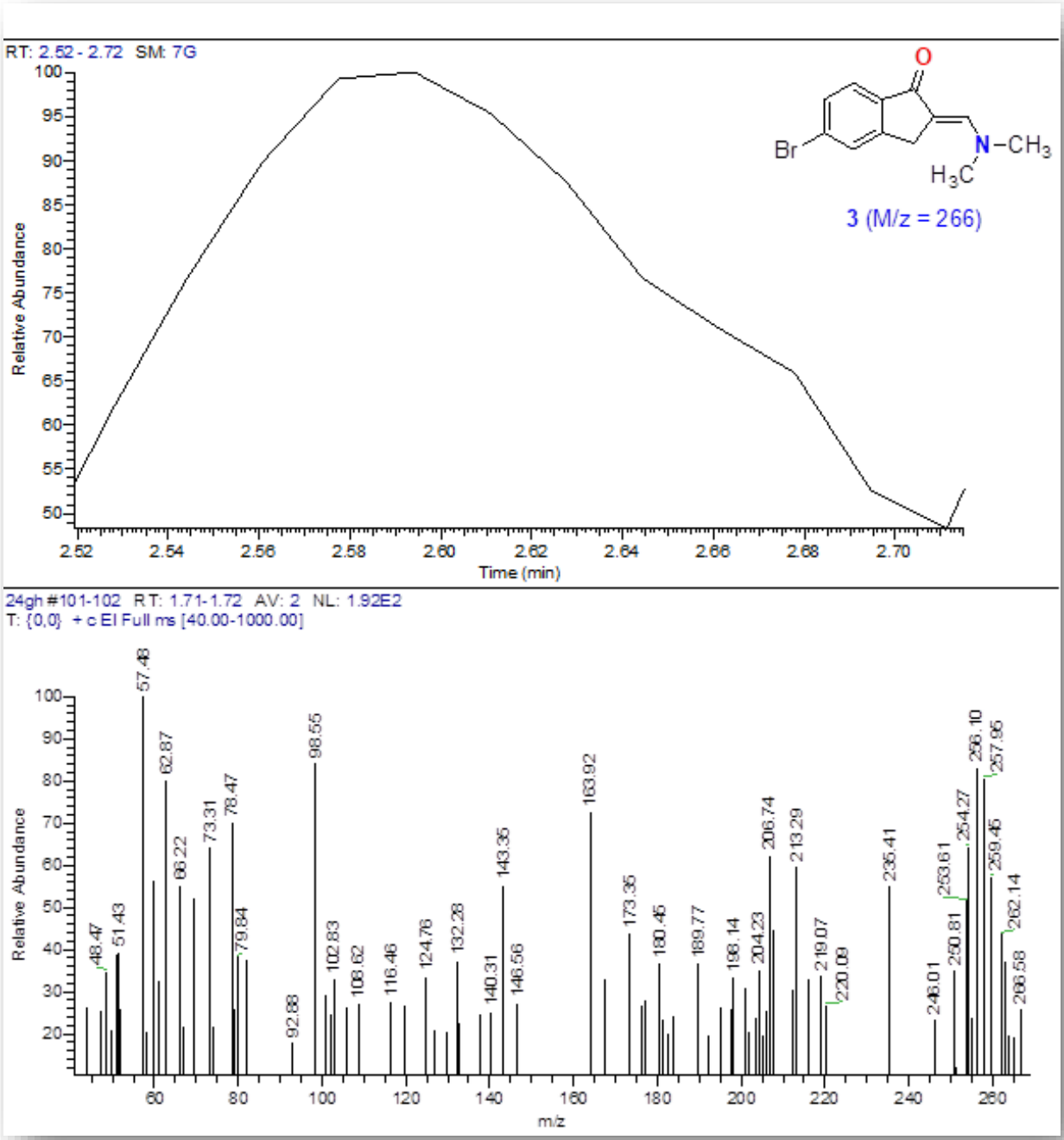
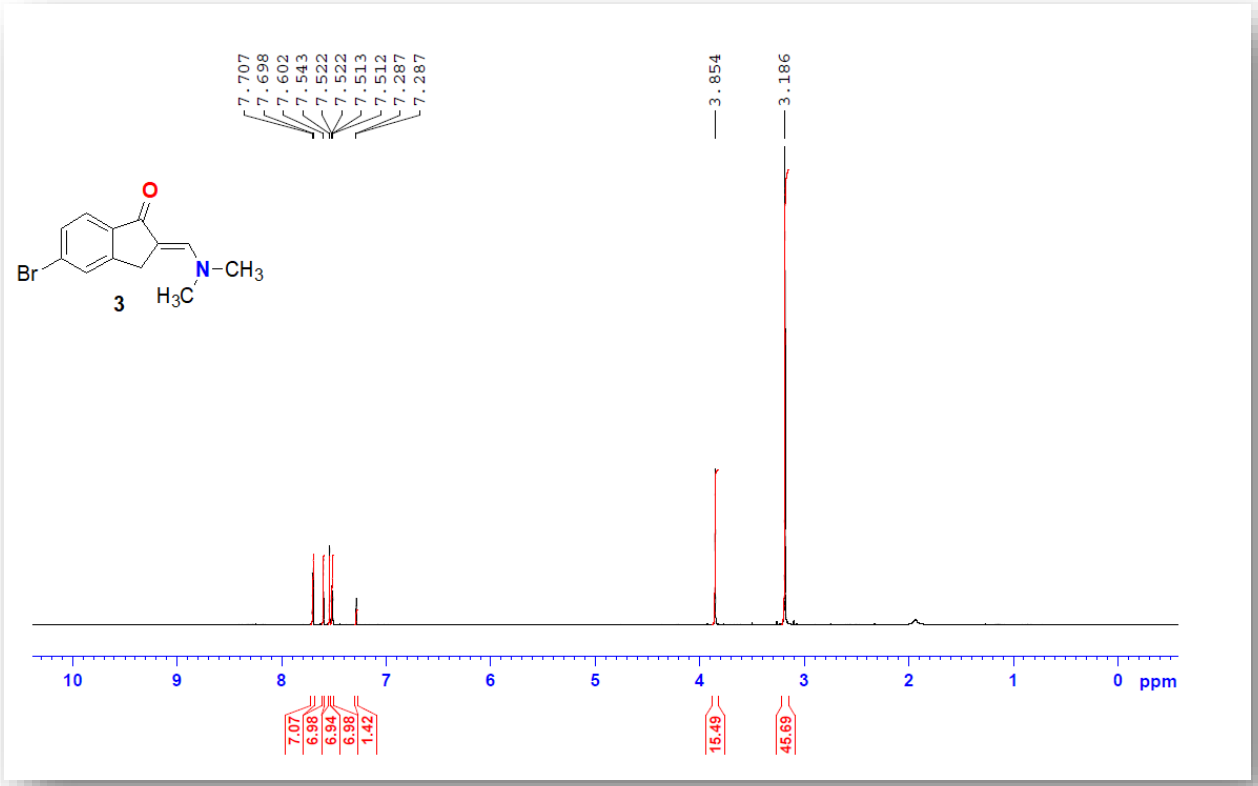
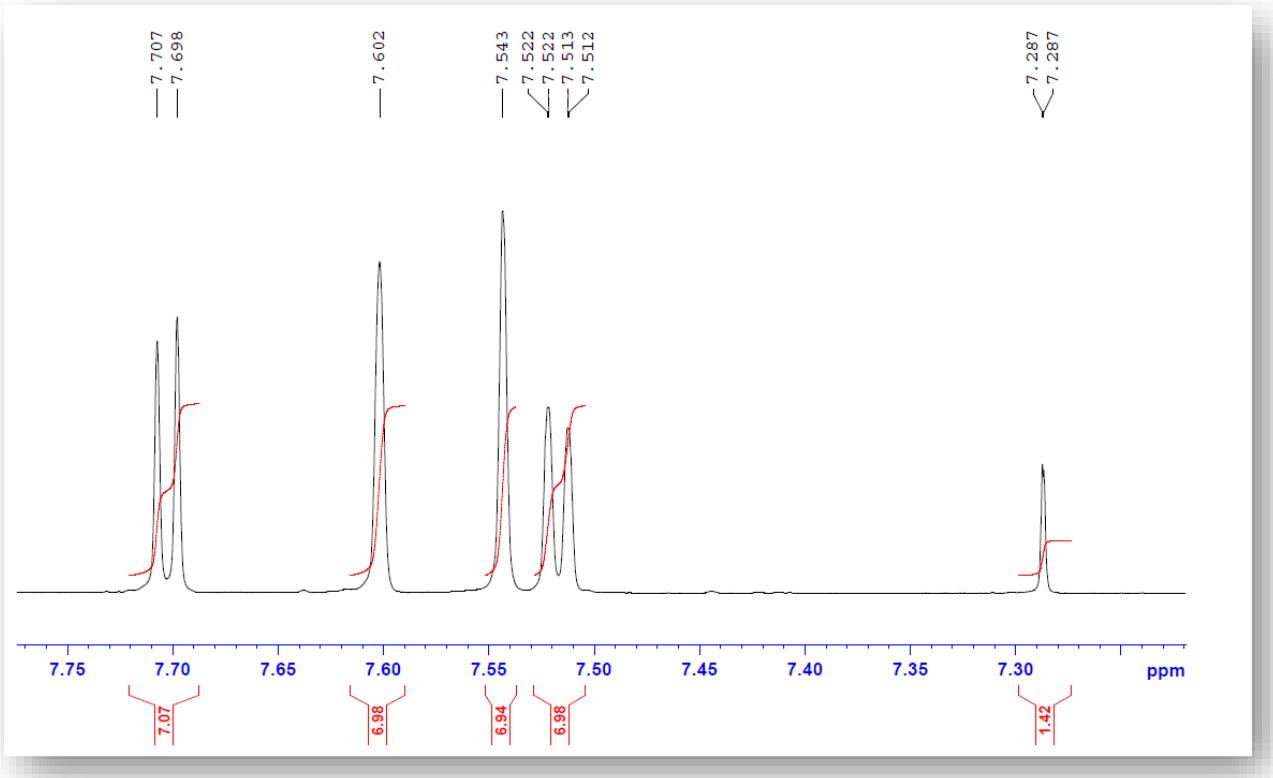
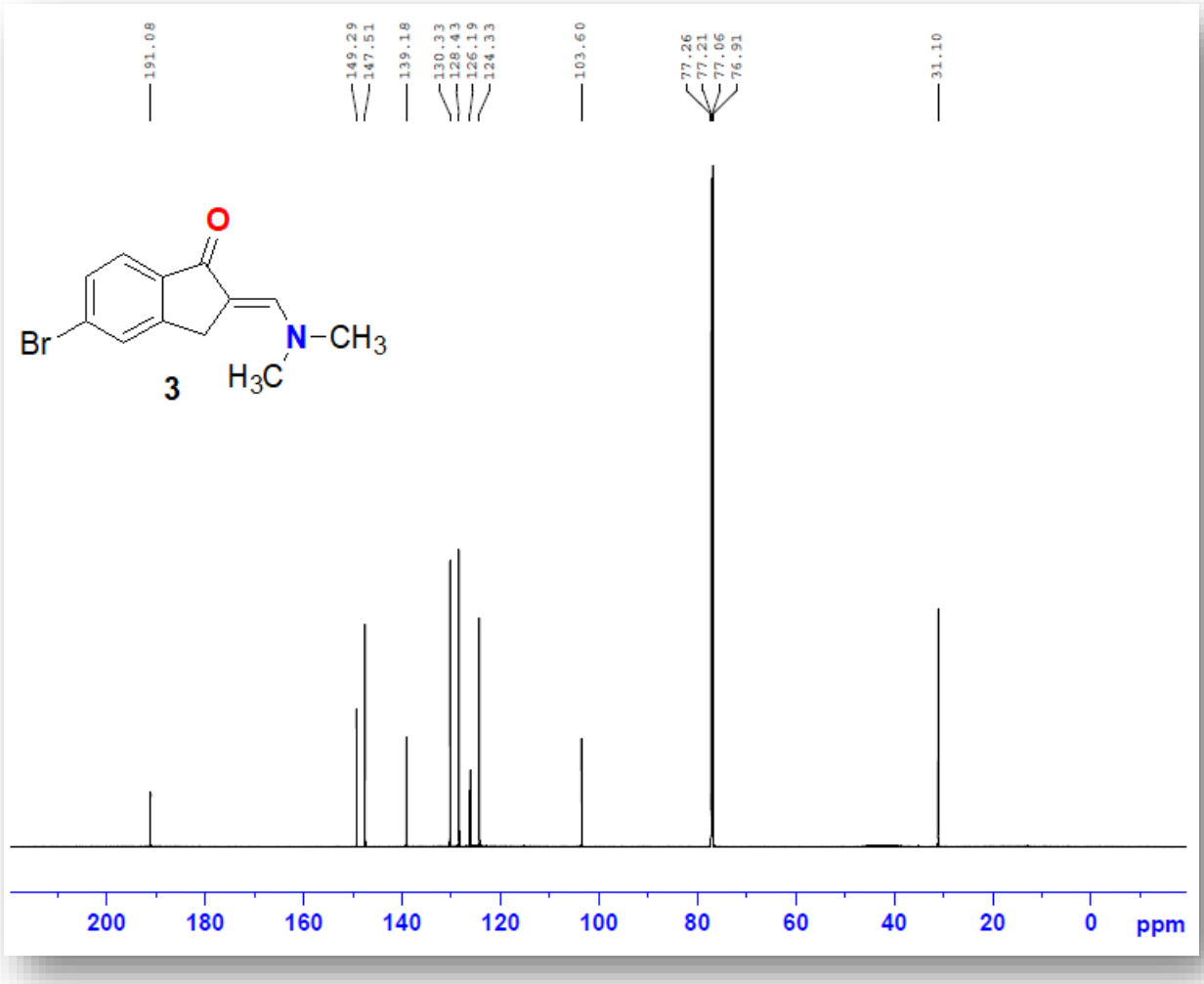
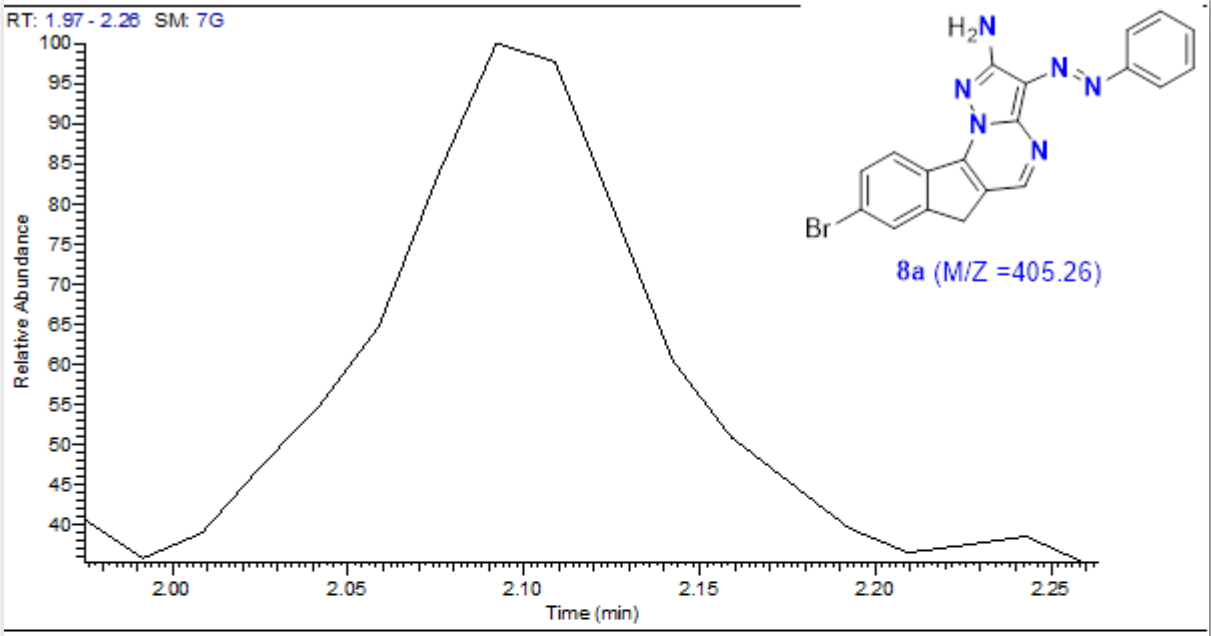
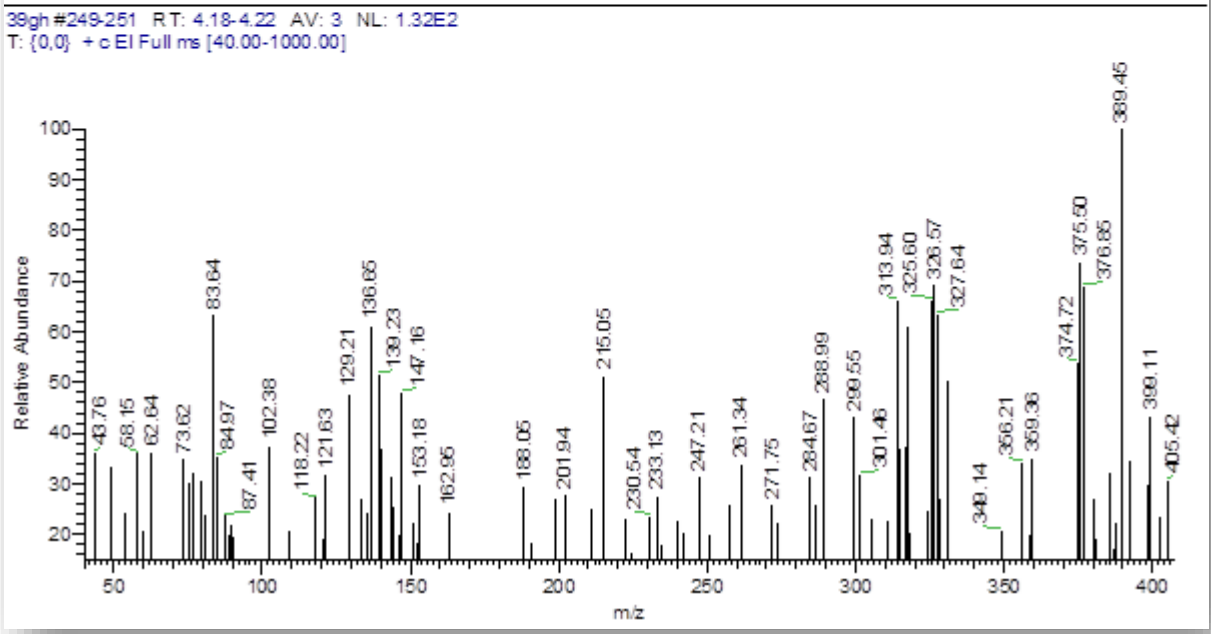

RT: 2.52 - 2.72 SM: 7G
Relative Abundance
Time (min)
O
Br
N–CH3
H3C
3 (M/z = 266)
24gh #101-102 RT: 1.71-1.72 AV: 2 NL: 1.92E2
T: {0,0} + c EI Full ms [40.00-1000.00]
Relative Abundance
m/z
57.48
62.87
66.22
73.31
78.47
79.84
92.88
98.55
102.83
108.62
116.46
124.76
132.28
140.31
143.35
146.56
163.92
173.35
180.45
189.77
196.14
204.23
206.74
213.29
219.07
220.09
235.41
246.01
250.81
253.61
254.27
256.10
257.95
259.45
262.14
266.58

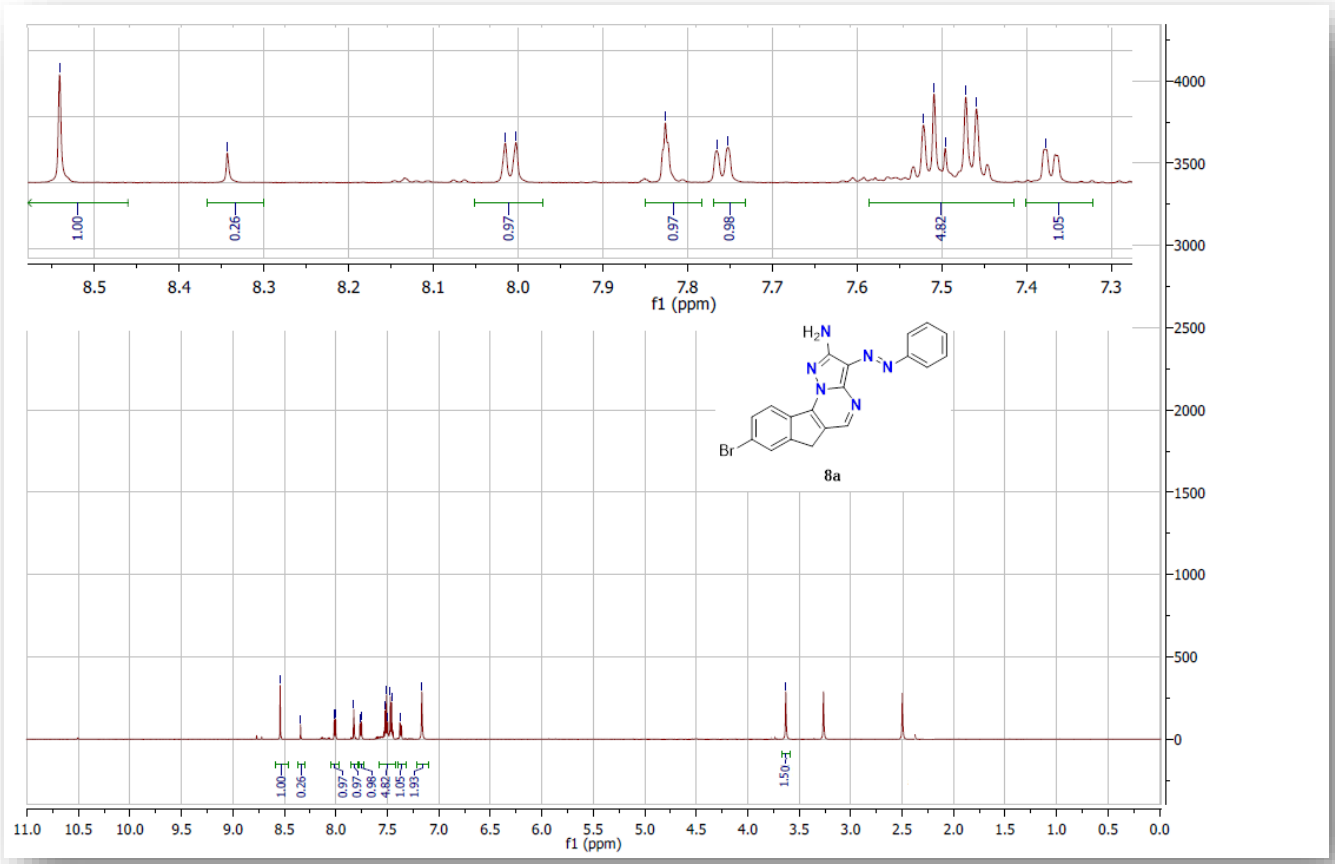

7.707
7.698
7.602
7.543
7.522
7.522
7.513
7.512
7.287
7.287
3.854
3.186
O
Br
N–CH3
H3C
3
10
9
8
7
6
5
4
3
2
1
0
ppm
7.07
6.98
6.94
6.98
1.42
15.49
45.69

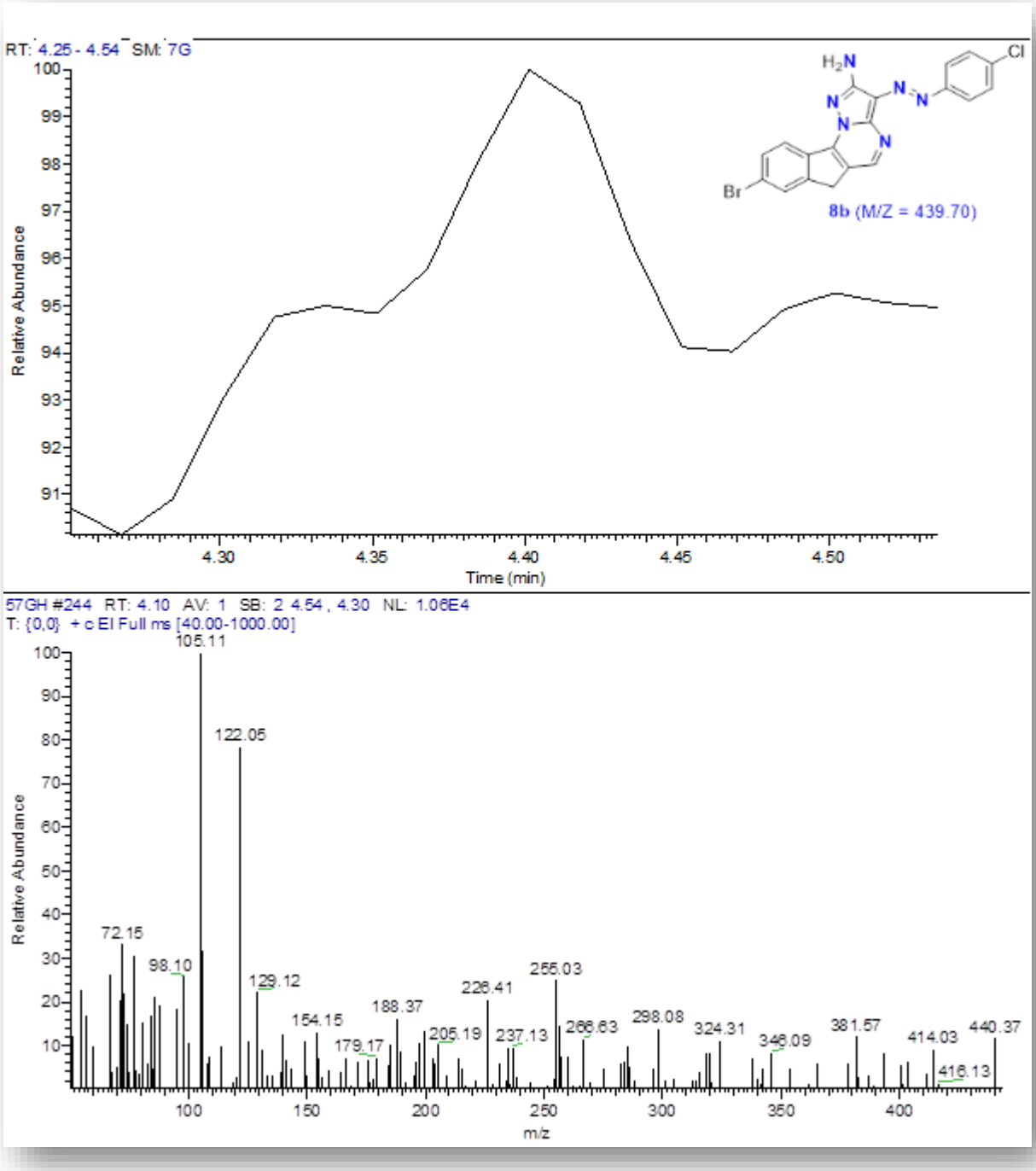

7.707
7.698
7.602
7.543
7.522
7.522
7.513
7.512
7.287
7.287
7.75
7.70
7.65
7.60
7.55
7.50
7.45
7.40
7.35
7.30
ppm
7.07
6.98
6.94
6.98
1.42

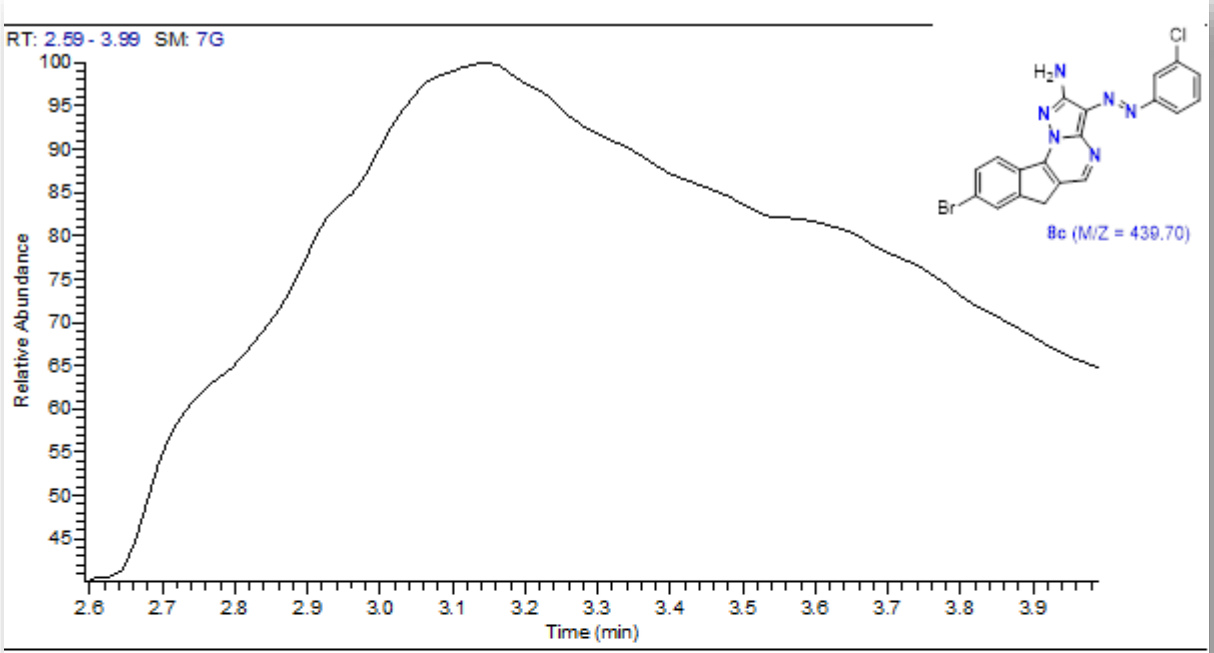

191.08
149.29
147.51
139.18
130.33
128.43
126.19
124.33
103.60
77.26
77.21
77.06
76.91
31.10
O
Br
N–CH3
H3C
3
200
180
160
140
120
100
80
60
40
20
0
ppm

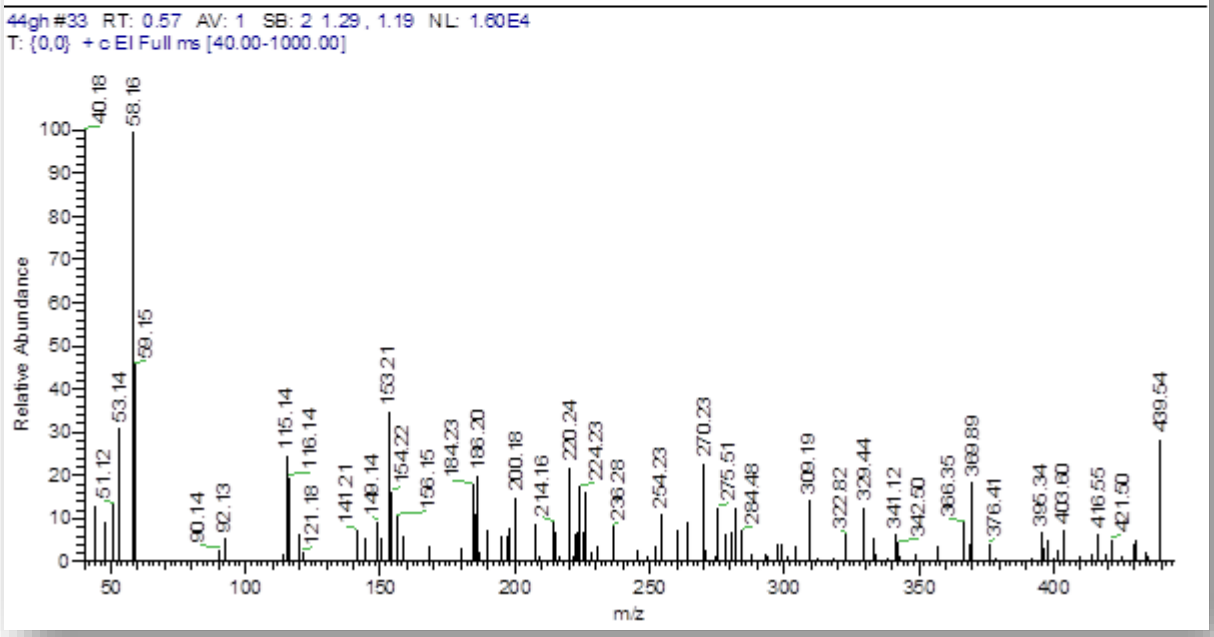

RT: 1.97 - 2.26 SM: 7G
Relative Abundance
Time (min)
H2N
N
N
N
N
N
Br
8a (M/Z =405.26)

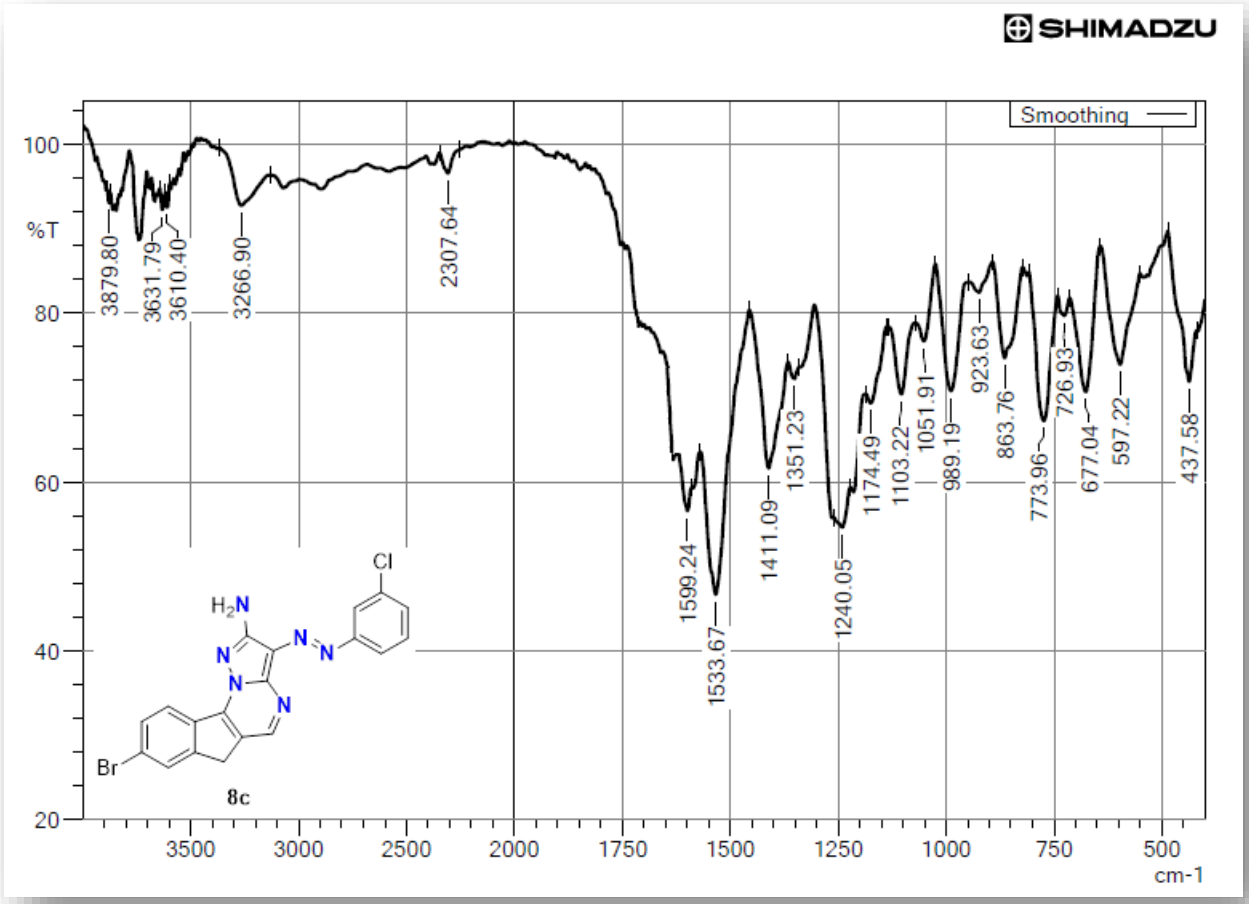

39gh #249-251 RT: 4.18-4.22 AV: 3 NL: 1.32E2
T: {0,0} + c EI Full ms [40.00-1000.00]
Relative Abundance
m/z
43.76
58.15
62.64
73.62
83.64
84.97
87.41
102.38
118.22
121.63
129.21
136.85
139.23
147.16
153.18
162.95
188.05
201.94
215.05
230.54
233.13
247.21
261.34
271.75
284.67
288.99
299.55
301.46
313.94
325.60
326.57
327.64
349.14
356.21
359.36
374.72
375.50
376.85
389.45
399.11
405.42

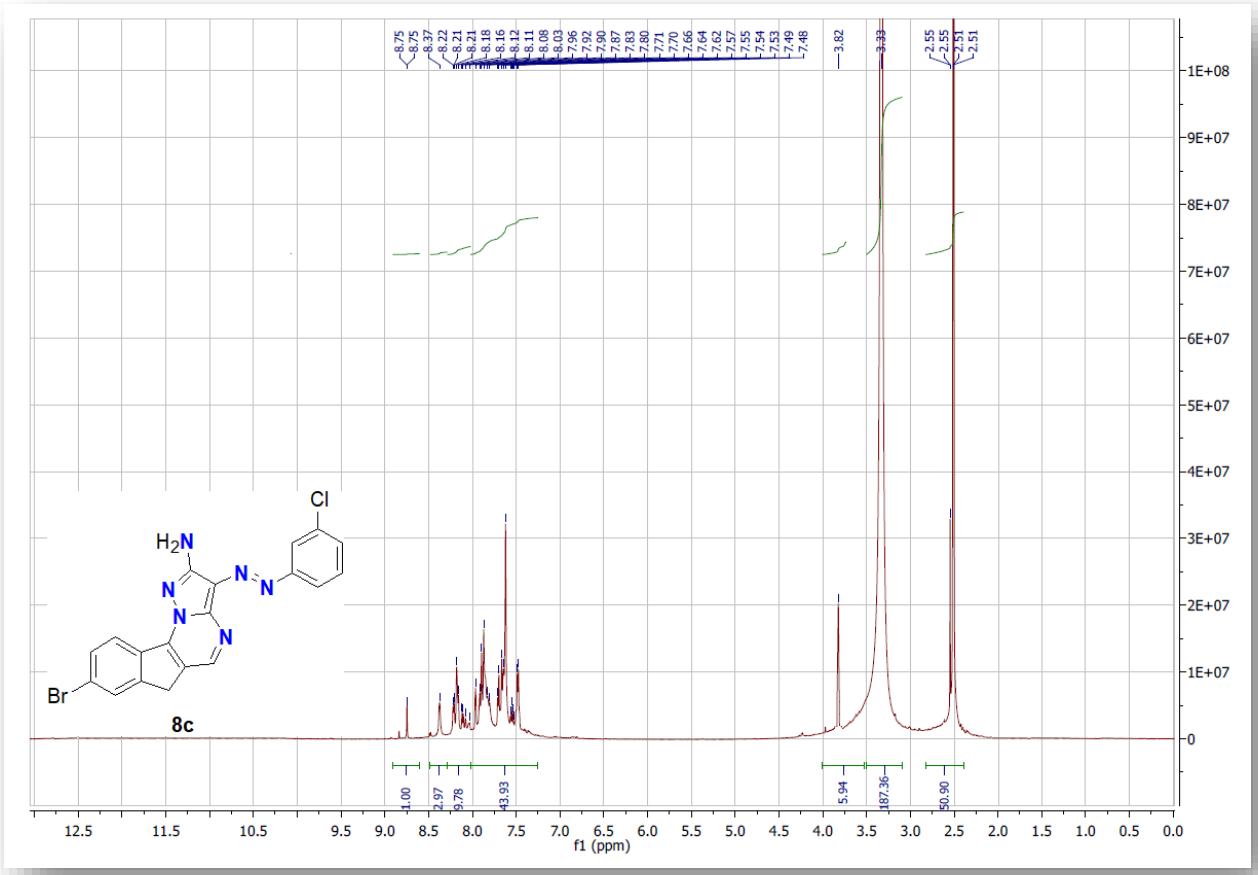

1.00
0.26
0.97
0.97
0.98
4.82
1.05
8.5
8.4
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
f1 (ppm)
4000
3500
3000
2500
2000
1500
1000
500
0
H_2N
N
N
N
N
Br
8a
1.00
0.26
0.97
0.97
0.98
4.82
1.05
1.93
1.50
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

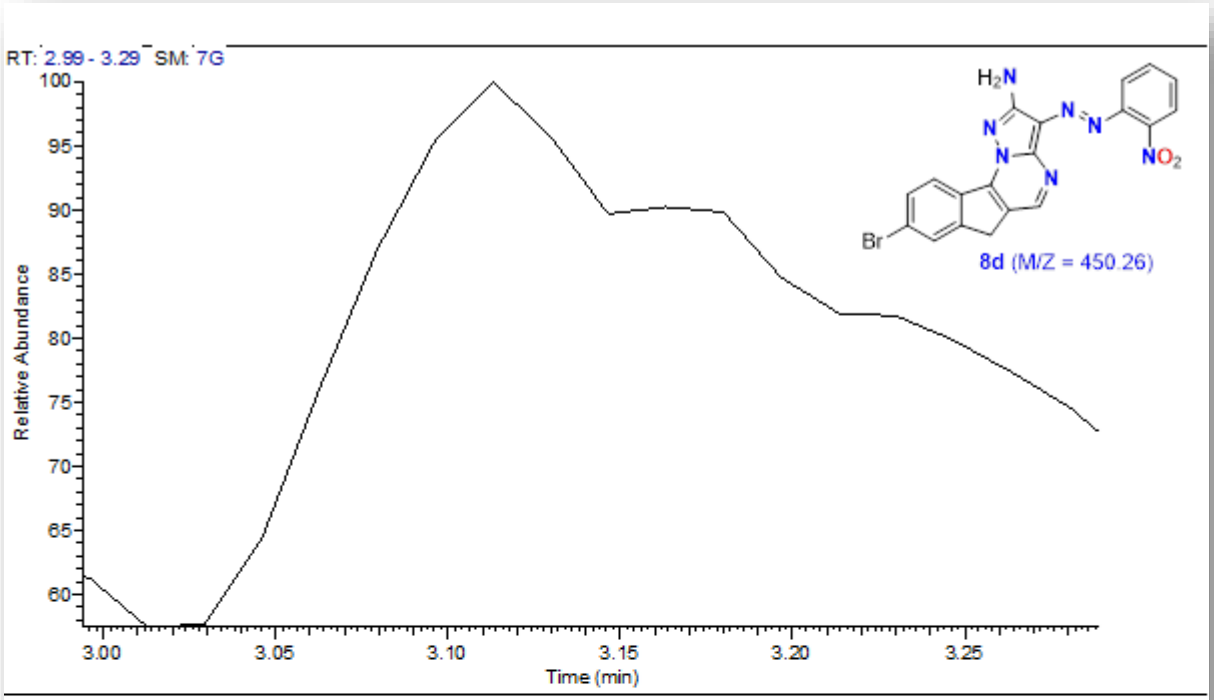

RT: 4.25 - 4.54 SM: 7G
Relative Abundance
Time (min)
8b (M/Z = 439.70)
57GH #244 RT: 4.10 AV: 1 SB: 2 4.54 , 4.30 NL: 1.06E4
T: {0,0} + c EI Full ms [40.00-1000.00]
105.11
122.05
72.15
98.10
129.12
154.15
188.37
179.17
205.19
226.41
237.13
255.03
266.63
298.08
324.31
346.09
381.57
414.03
416.13
440.37
m/z

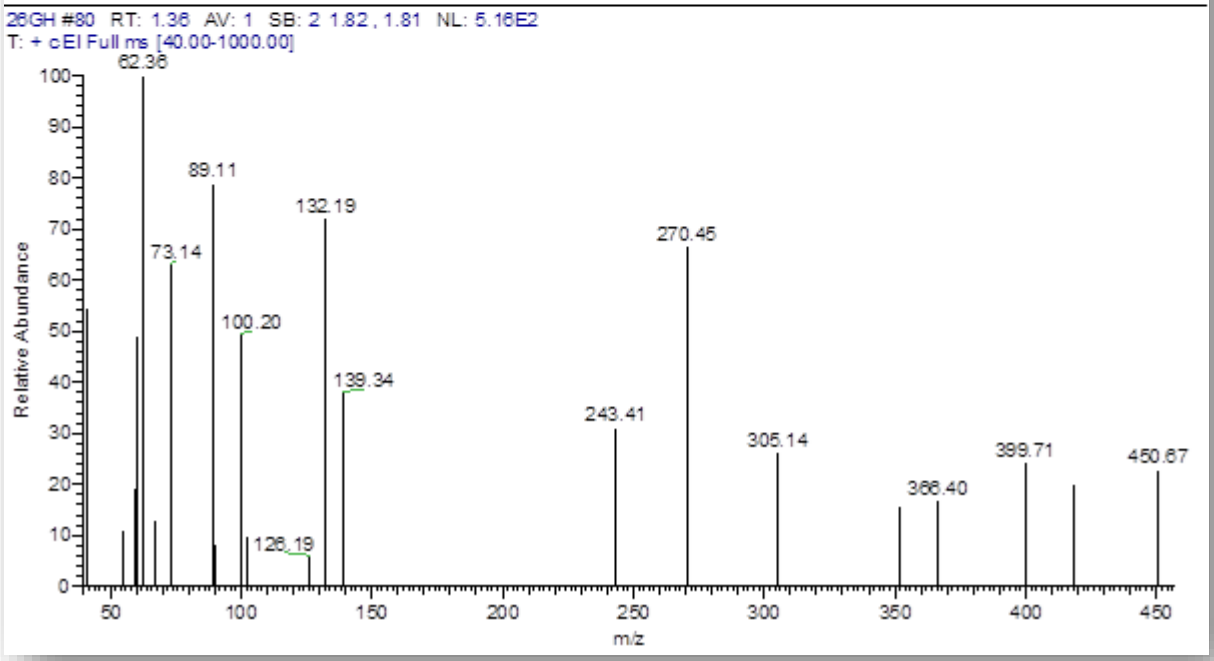

RT: 2.59 - 3.99 SM: 7G
Relative Abundance
Time (min)
8c (M/Z = 439.70)

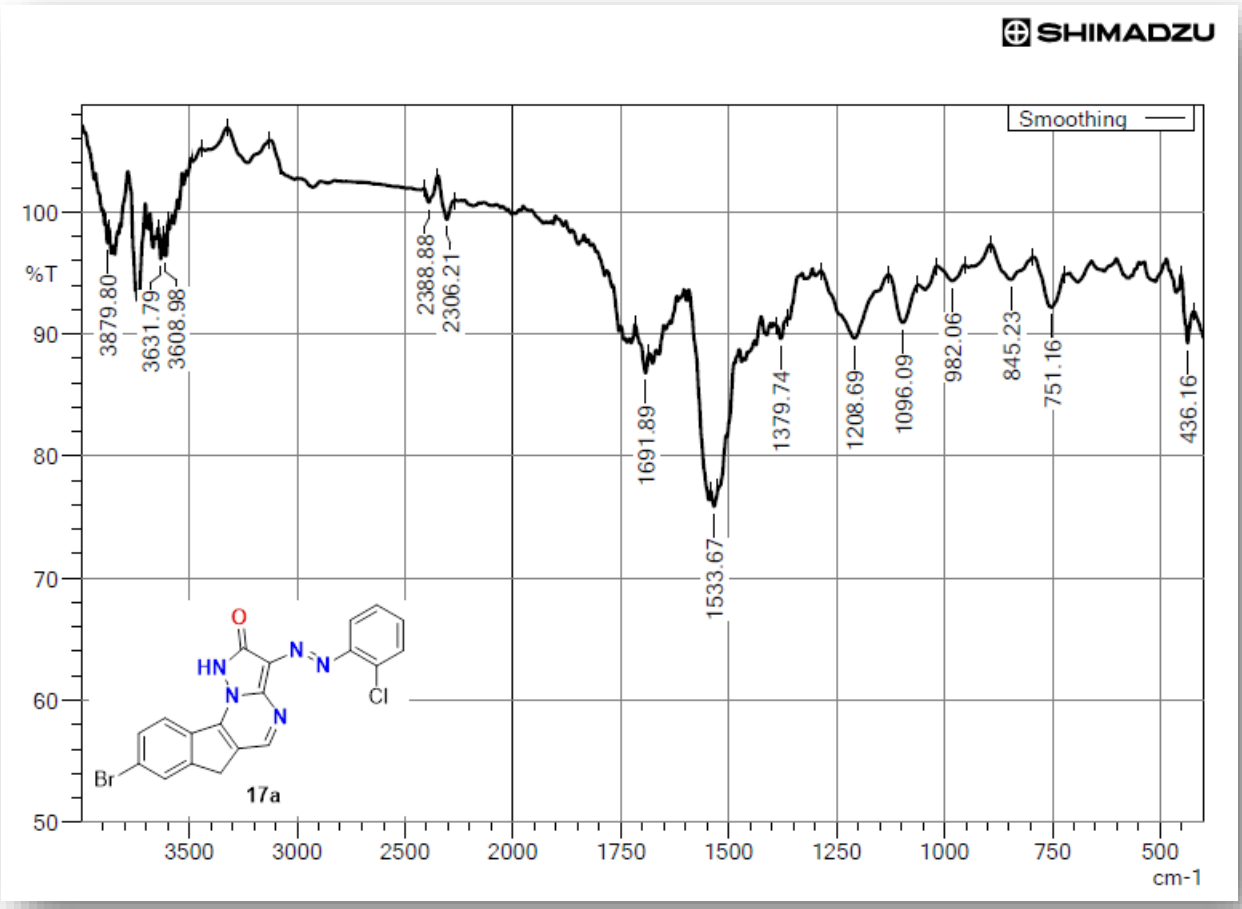

44gh #33 RT: 0.57 AV: 1 SB: 2 1.29 , 1.19 NL: 1.60E4
T: {0,0} + c EI Full ms [40.00-1000.00]
Relative Abundance
m/z

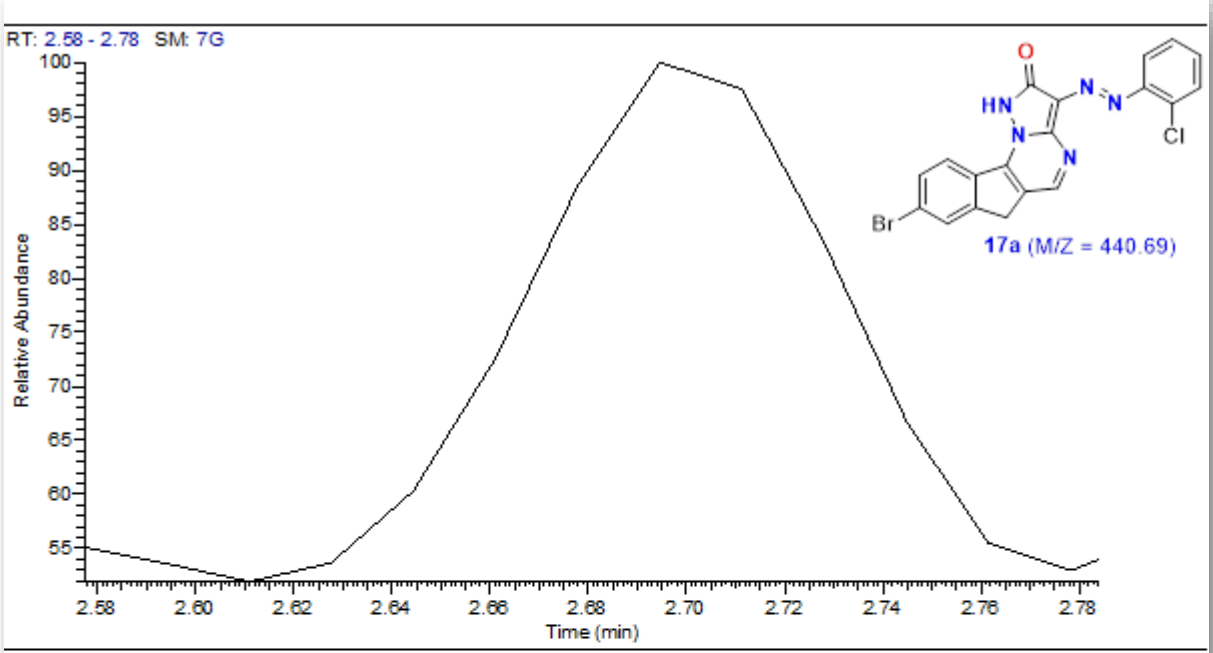

SHIMADZU
Smoothing
%T
100
80
60
40
20
3879.80
3631.79
3610.40
3266.90
2307.64
1599.24
1533.67
1411.09
1351.23
1240.05
1174.49
1103.22
1051.91
989.19
923.63
863.76
773.96
726.93
677.04
597.22
437.58
3500
3000
2500
2000
1750
1500
1250
1000
750
500
cm-1
Cl
H2N
N
N
N
N
Br
8c

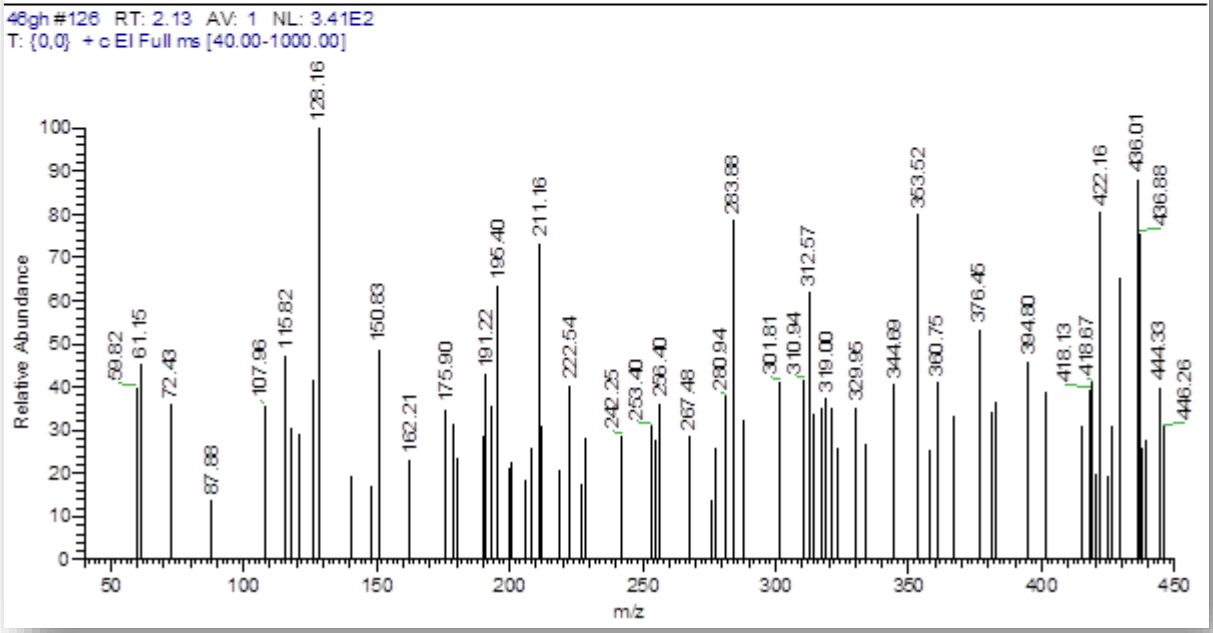

8.75
8.75
8.37
8.22
8.21
8.21
8.18
8.16
8.12
8.11
8.08
8.03
7.96
7.92
7.90
7.87
7.83
7.80
7.71
7.70
7.66
7.64
7.62
7.57
7.55
7.54
7.53
7.49
7.48
3.82
3.33
2.55
2.55
2.51
2.51
1E+08
9E+07
8E+07
7E+07
6E+07
5E+07
4E+07
3E+07
2E+07
1E+07
0
Cl
H2N
N
N
N
N
N
Br
8c
1.00
2.97
9.78
43.93
5.94
187.36
50.90
12.5
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

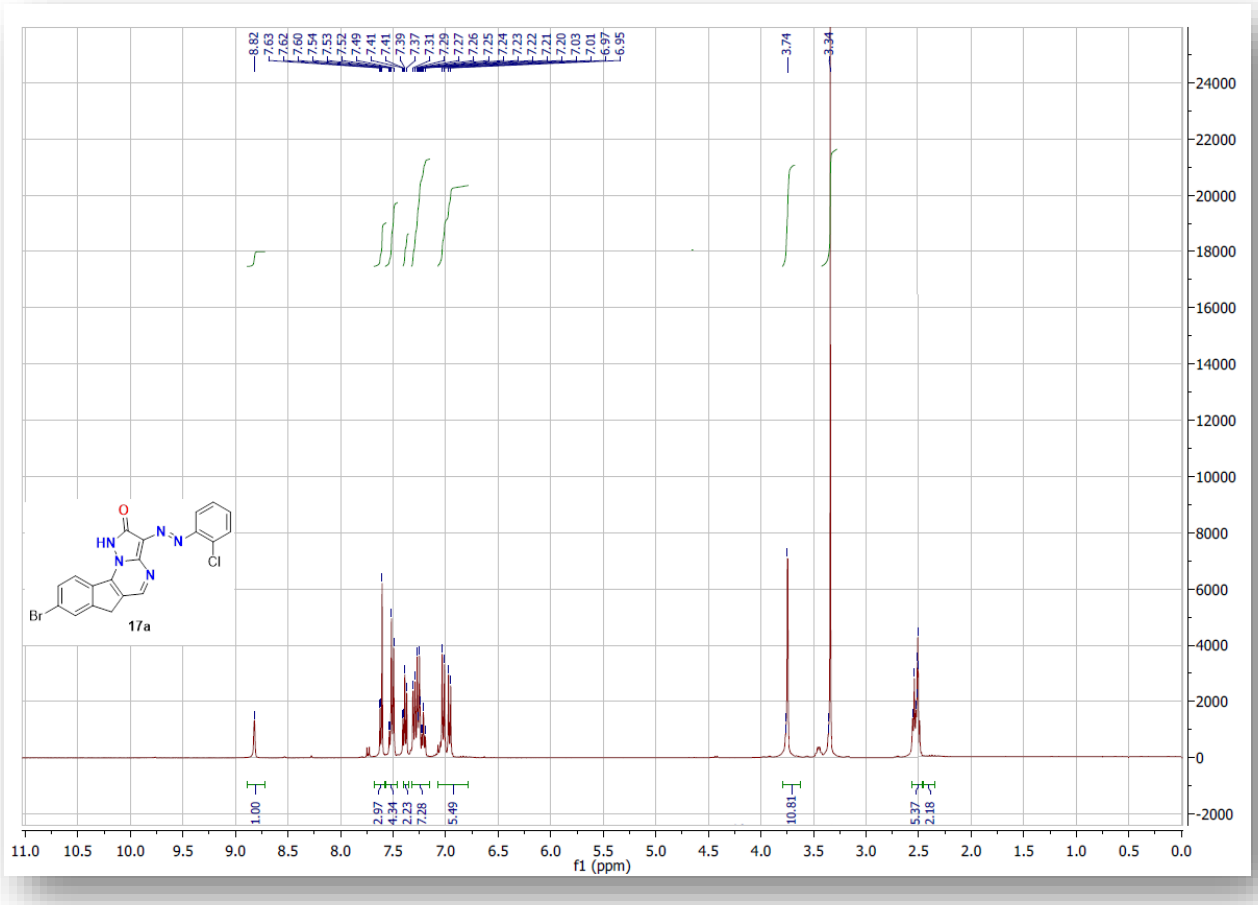

RT: 2.99 - 3.29 SM: 7G
Relative Abundance
Time (min)
8d (M/Z = 450.26)

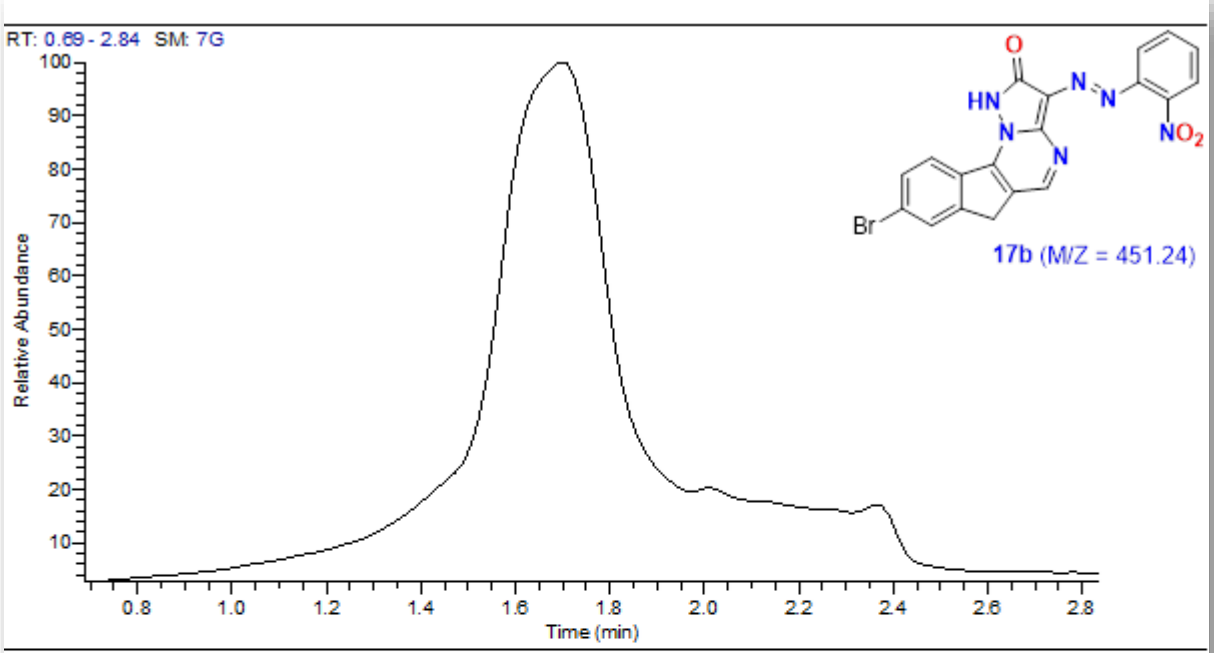

26GH #80 RT: 1.36 AV: 1 SB: 2 1.82 , 1.81 NL: 5.16E2
T: + cEI Full ms [40.00-1000.00]
Relative Abundance
m/z
62.36
89.11
73.14
132.19
270.45
100.20
139.34
243.41
305.14
399.71
450.67
366.40
126.19

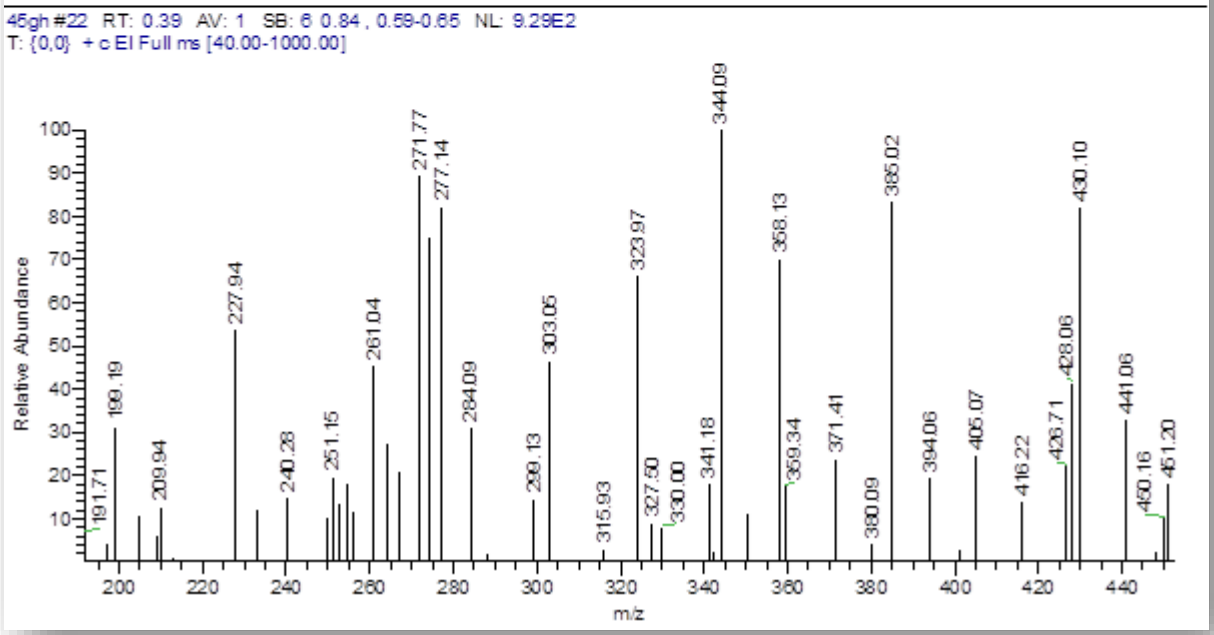

SHIMADZU
Smoothing
%T
100
90
80
70
60
50
3879.80
3631.79
3608.98
2388.88
2306.21
1691.89
1533.67
1379.74
1208.69
1096.09
982.06
845.23
751.16
436.16
3500
3000
2500
2000
1750
1500
1250
1000
750
500
cm-1
O
HN
N
N
N
N
Cl
Br
17a

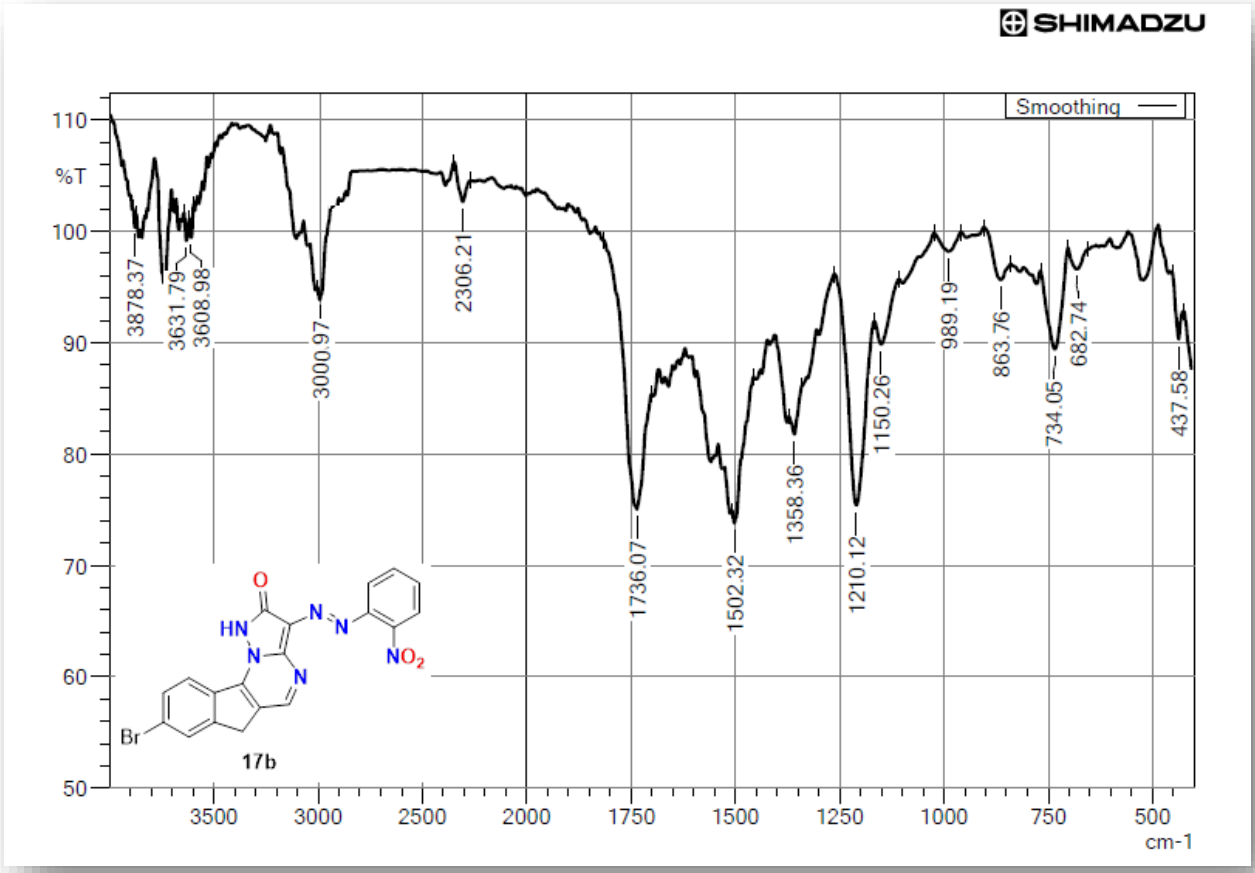

RT: 2.58 - 2.78 SM: 7G
Relative Abundance
Time (min)
17a (M/Z = 440.69)

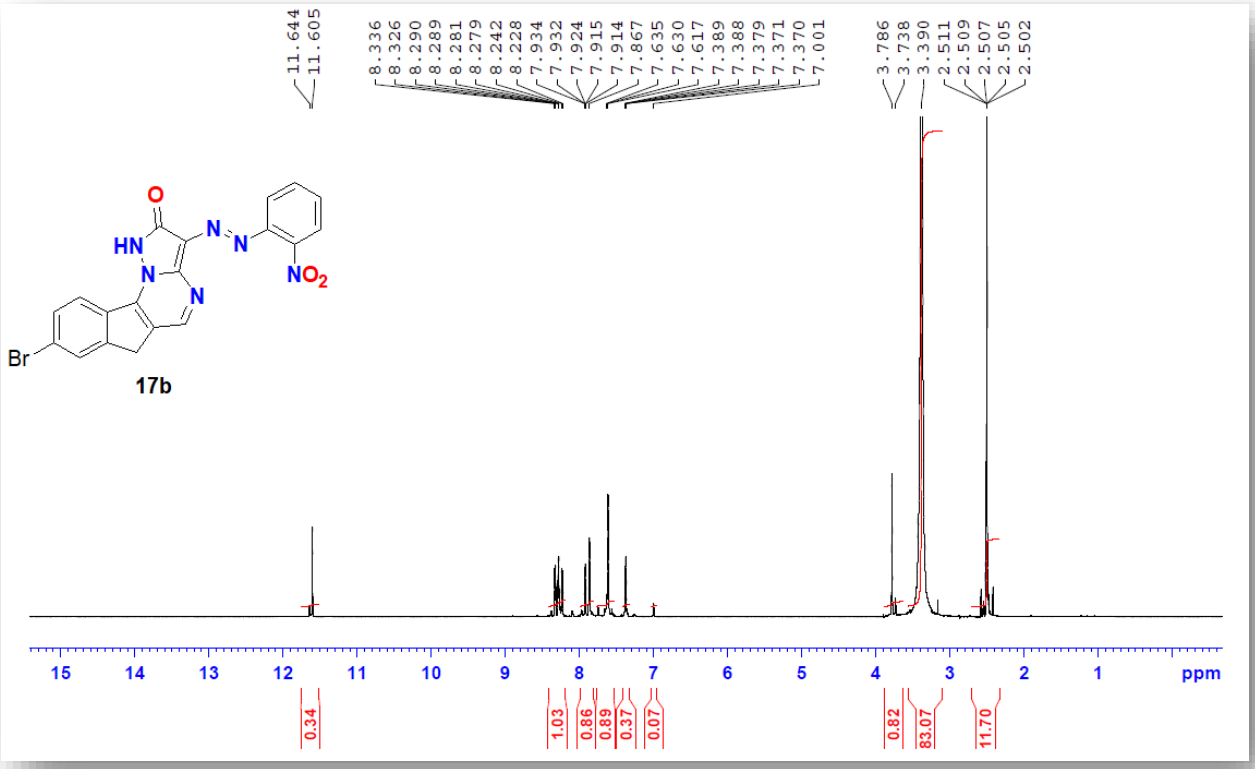

46gh #126 RT: 2.13 AV: 1 NL: 3.41E2
T: {0,0} + c EI Full ms [40.00-1000.00]
Relative Abundance
m/z

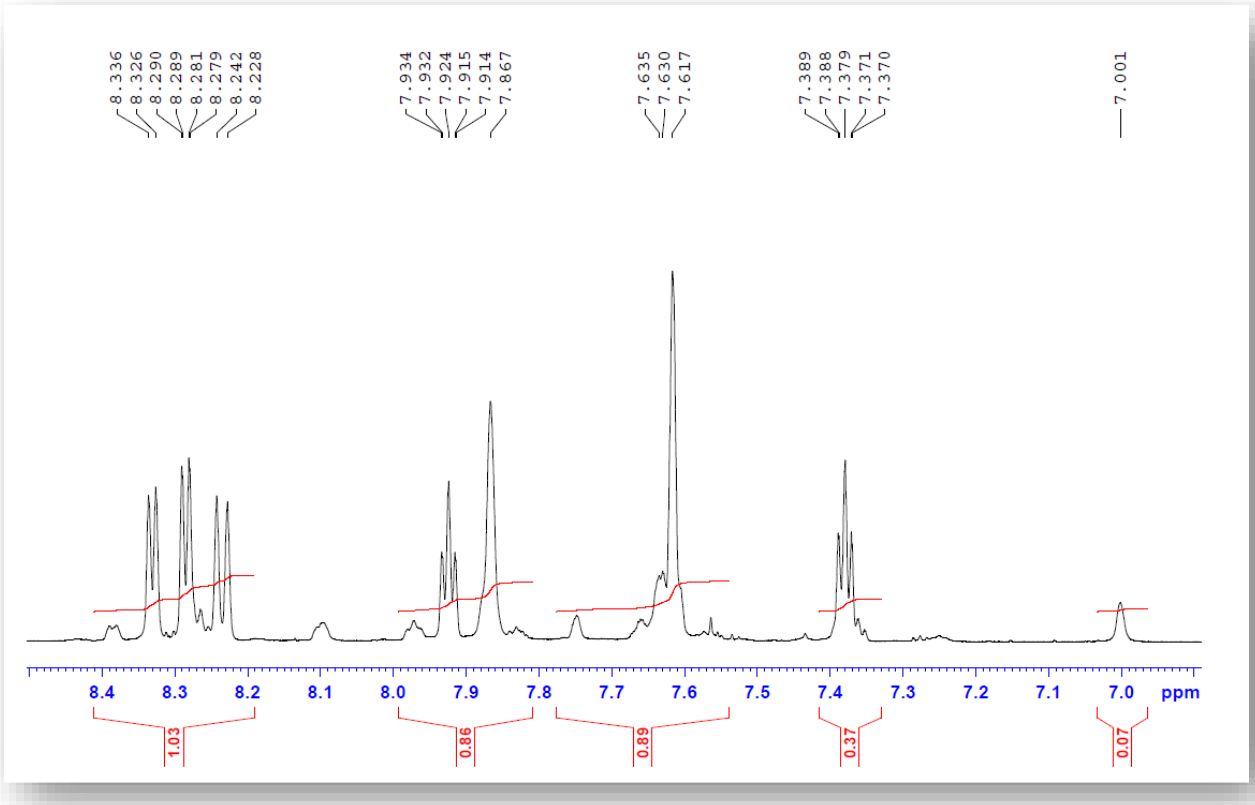

8.82
7.63
7.62
7.60
7.54
7.53
7.52
7.49
7.41
7.41
7.39
7.37
7.31
7.29
7.27
7.26
7.25
7.24
7.23
7.22
7.21
7.20
7.03
7.01
6.97
6.95
3.74
3.34
1.00
2.97
4.34
2.23
7.28
5.49
10.81
5.37
2.18
f1 (ppm)
17a

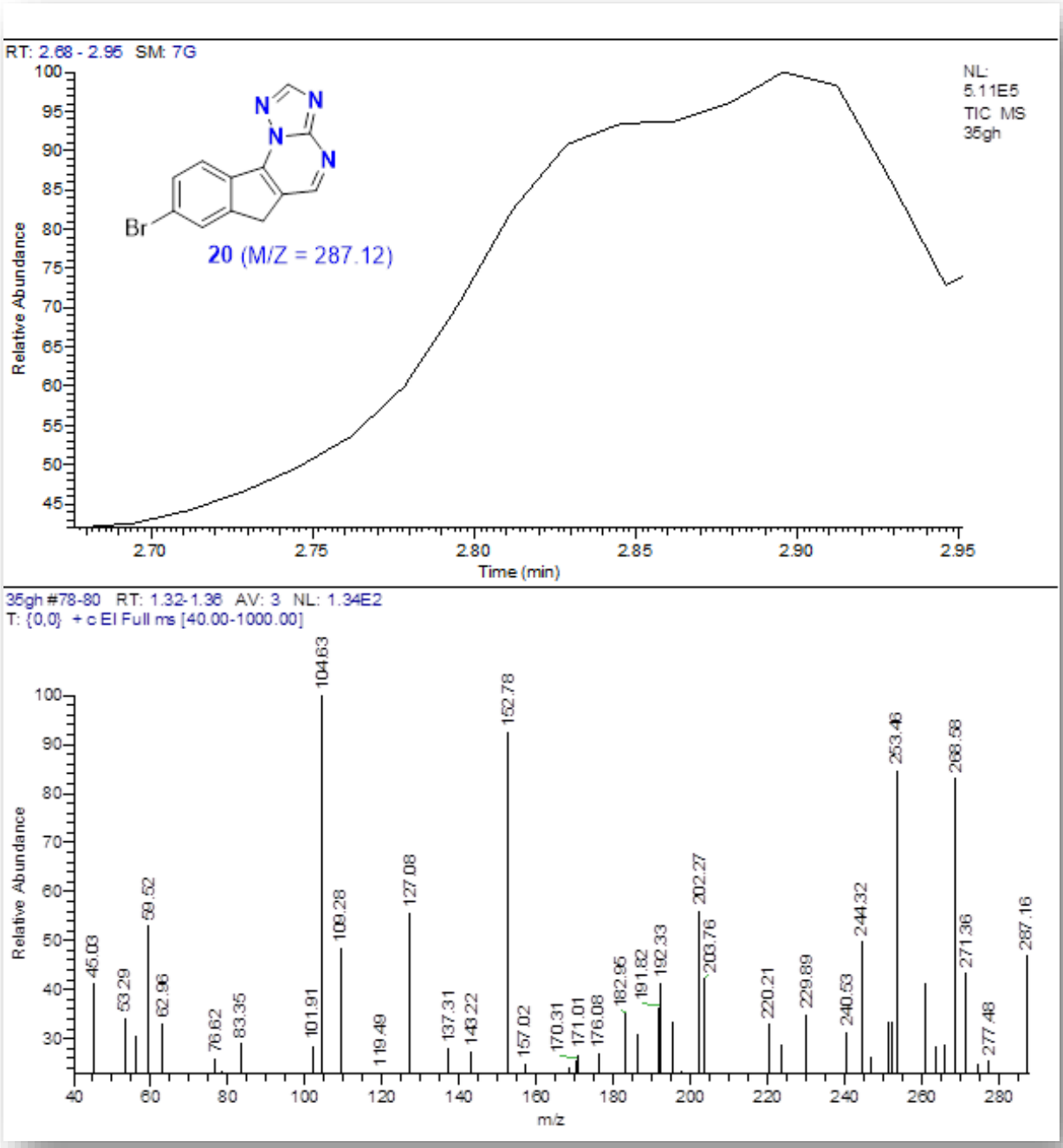

RT: 0.69 - 2.84 SM: 7G
Relative Abundance
Time (min)
O
HN
N
N
N
N
NO2
Br
17b (M/Z = 451.24)

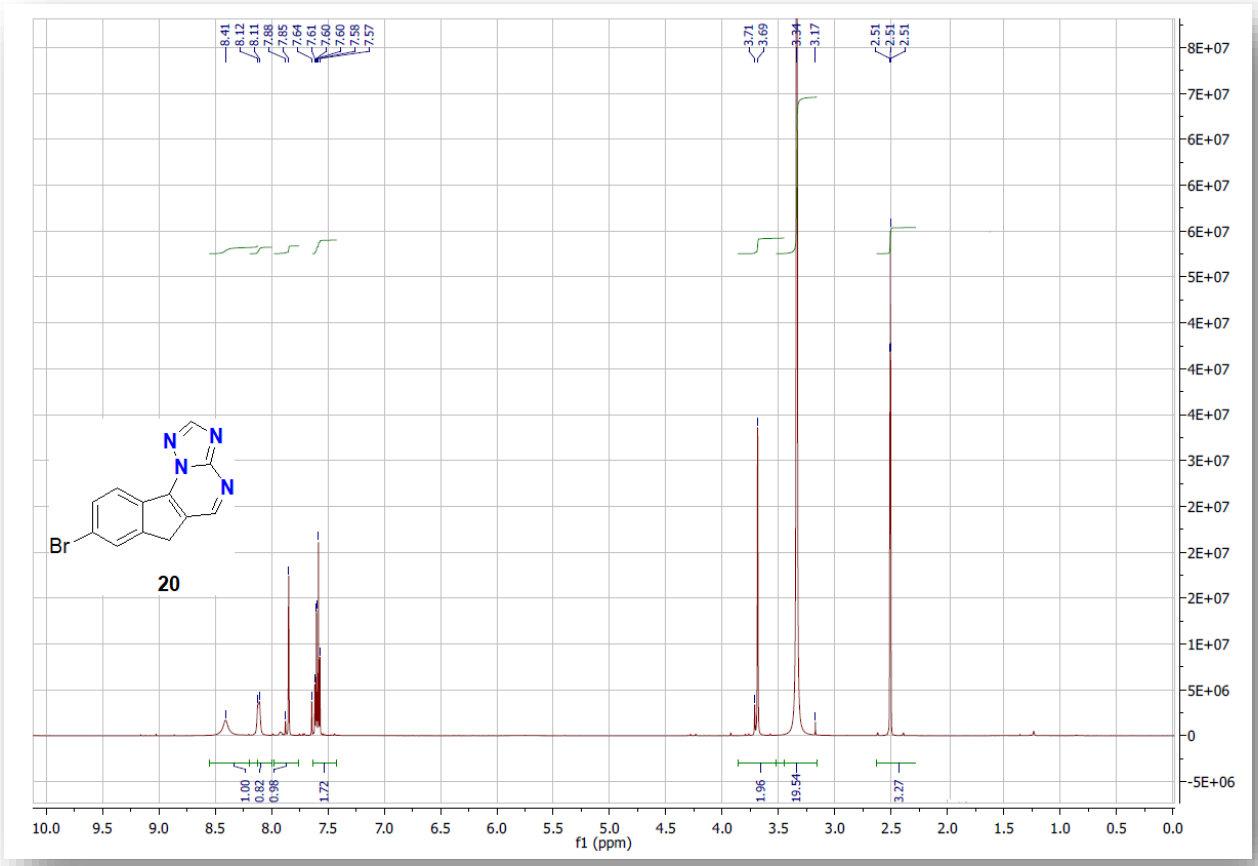

45gh #22 RT: 0.39 AV: 1 SB: 6 0.84 , 0.69-0.85 NL: 9.29E2
T: {0,0} + c EI Full ms [40.00-1000.00]
Relative Abundance
m/z
191.71
199.19
209.94
227.94
240.28
251.15
261.04
271.77
277.14
284.09
299.13
303.05
315.93
323.97
327.50
330.00
341.18
344.09
358.13
359.34
371.41
380.09
385.02
394.06
405.07
416.22
426.71
428.06
430.10
441.06
450.16
451.20

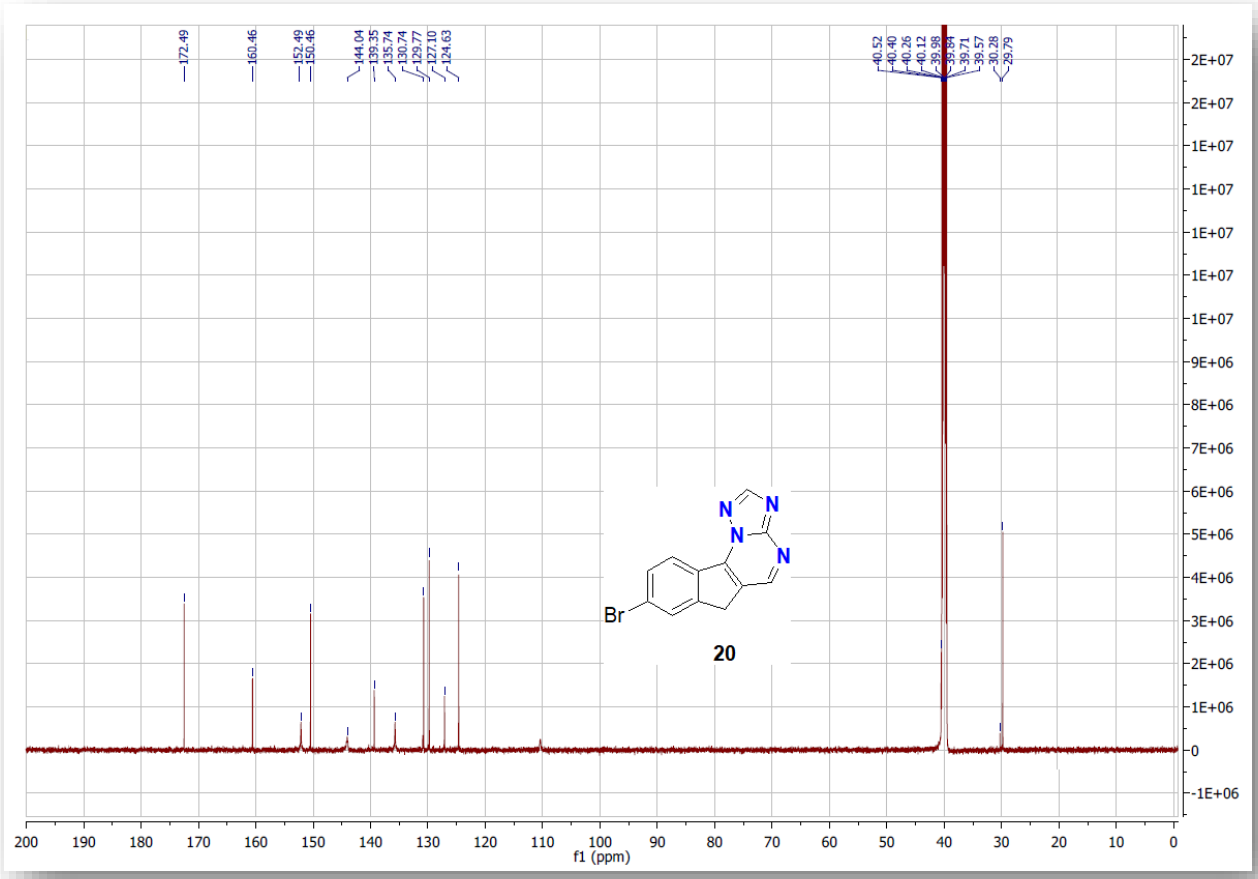

SHIMADZU
Smoothing
%T
110
100
90
80
70
60
50
3878.37
3631.79
3608.98
3000.97
2306.21
1736.07
1502.32
1358.36
1210.12
1150.26
989.19
863.76
734.05
682.74
437.58
3500
3000
2500
2000
1750
1500
1250
1000
750
500
cm-1
O
HN
N
N
N
N
NO2
Br
17b

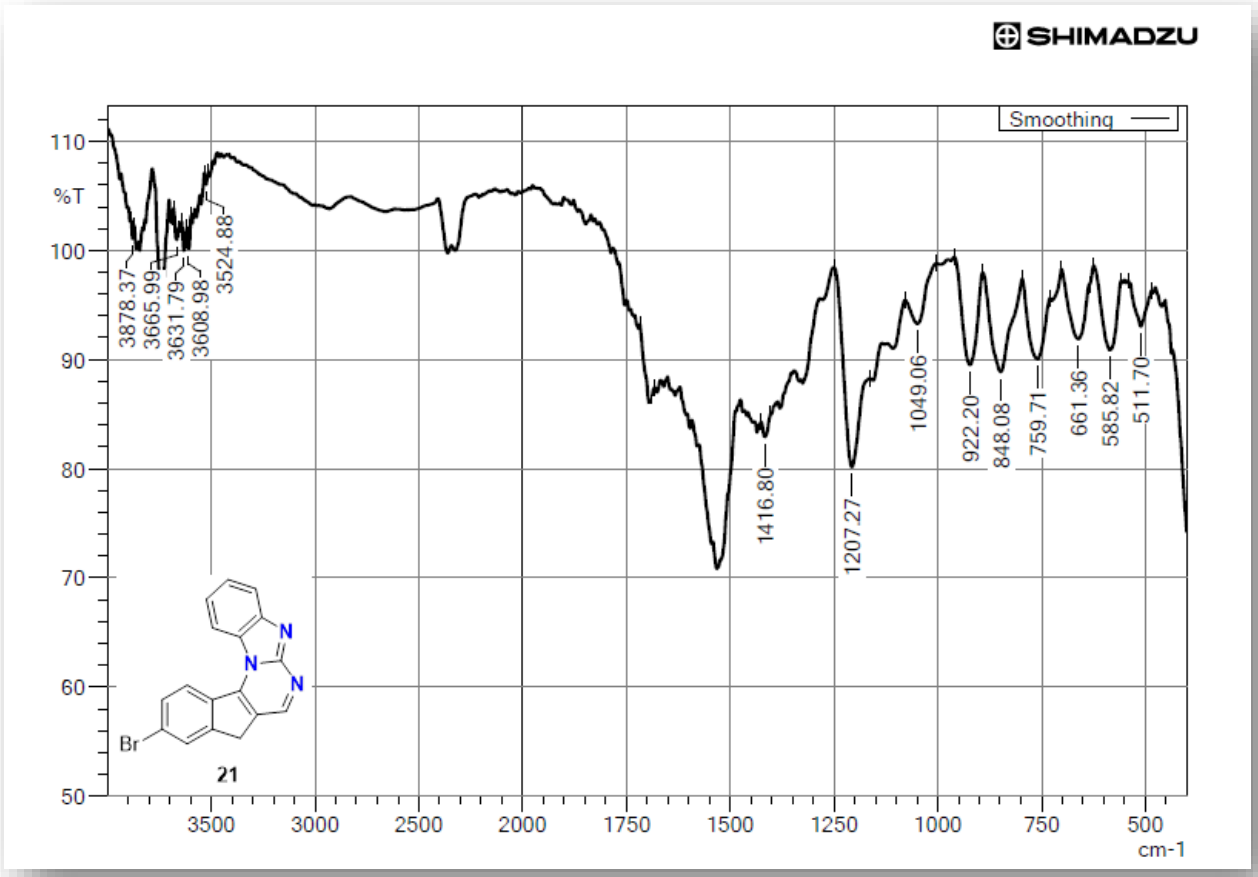

11.644
11.605
8.336
8.326
8.290
8.289
8.281
8.279
8.242
8.228
7.934
7.932
7.924
7.915
7.914
7.867
7.635
7.630
7.617
7.389
7.388
7.379
7.371
7.370
7.001
3.786
3.738
3.390
2.511
2.509
2.507
2.505
2.502
O
HN
N
N
N
N
NO2
Br
17b
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.34
1.03
0.86
0.89
0.37
0.07
0.82
83.07
11.70

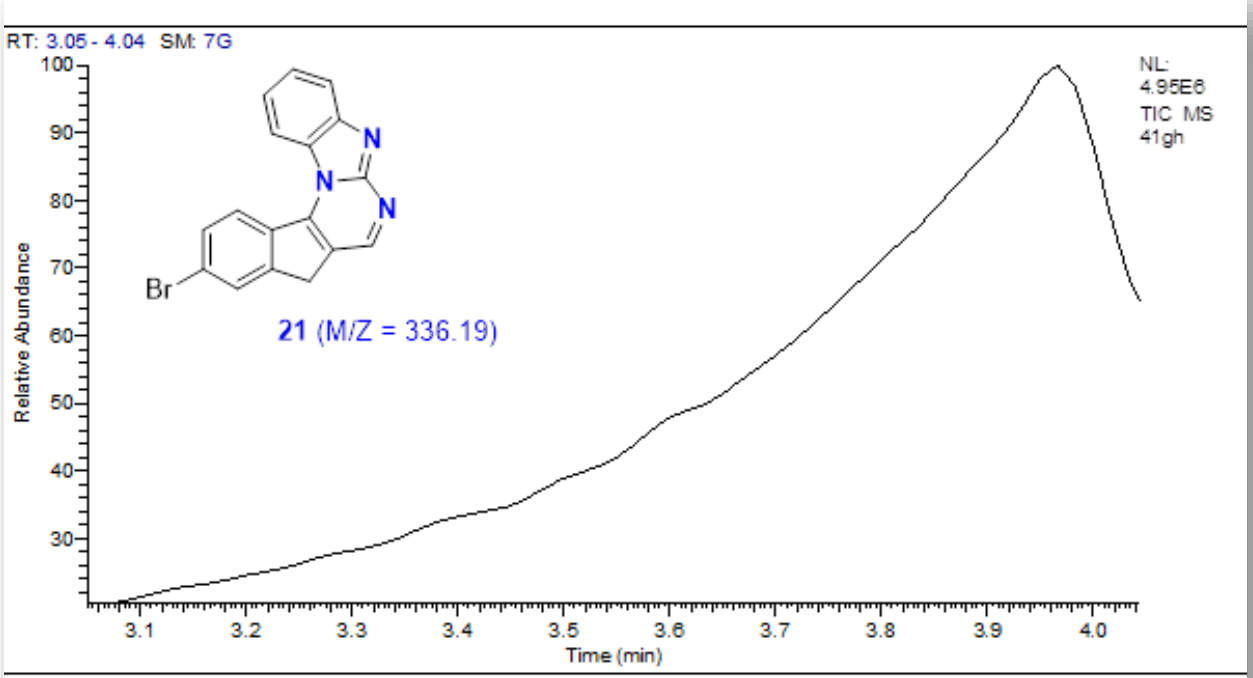

8.336
8.326
8.290
8.289
8.281
8.279
8.242
8.228
7.934
7.932
7.924
7.915
7.914
7.867
7.635
7.630
7.617
7.389
7.388
7.379
7.371
7.370
7.001
8.4
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
ppm
1.03
0.86
0.89
0.37
0.07

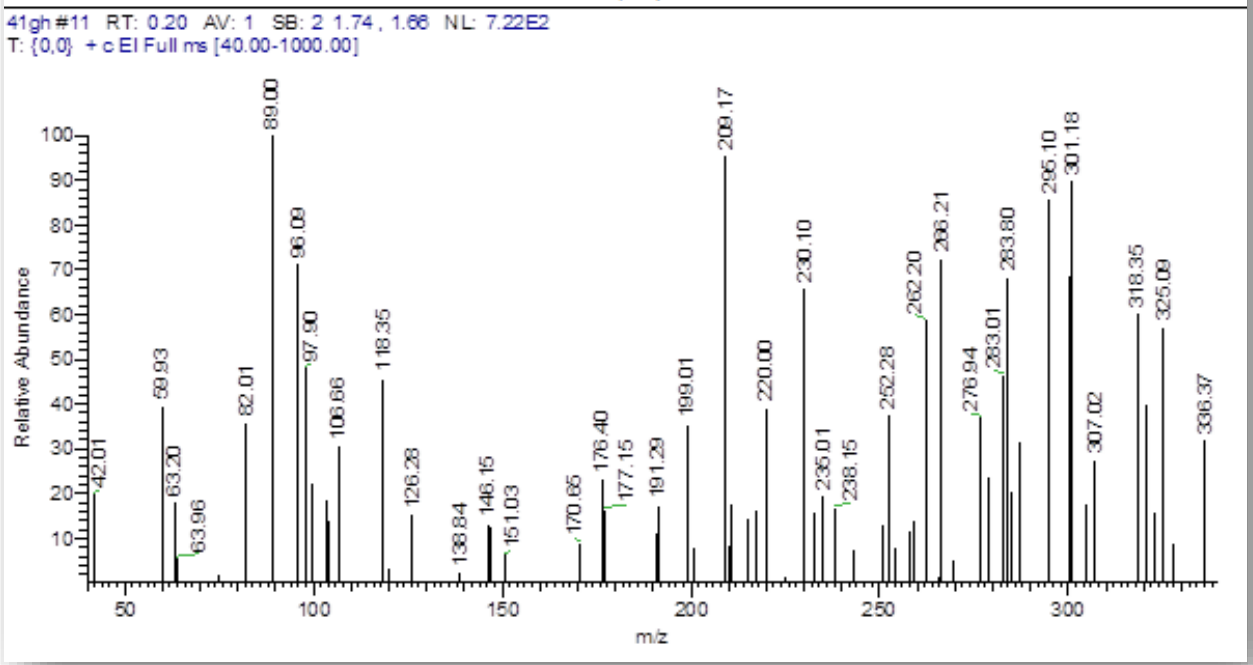

RT: 2.68 - 2.95 SM: 7G
NL:
5.11E5
TIC MS
35gh
Relative Abundance
Time (min)
Br
20 (M/Z = 287.12)
35gh #78-80 RT: 1.32-1.36 AV: 3 NL: 1.34E2
T: {0,0} + c EI Full ms [40.00-1000.00]
m/z
45.03
53.29
59.52
62.96
76.62
83.35
101.91
104.63
109.28
119.49
127.08
137.31
143.22
152.78
157.02
170.31
171.01
176.08
182.95
191.82
192.33
202.27
203.76
220.21
229.89
240.53
244.32
253.46
268.58
271.36
277.48
287.16

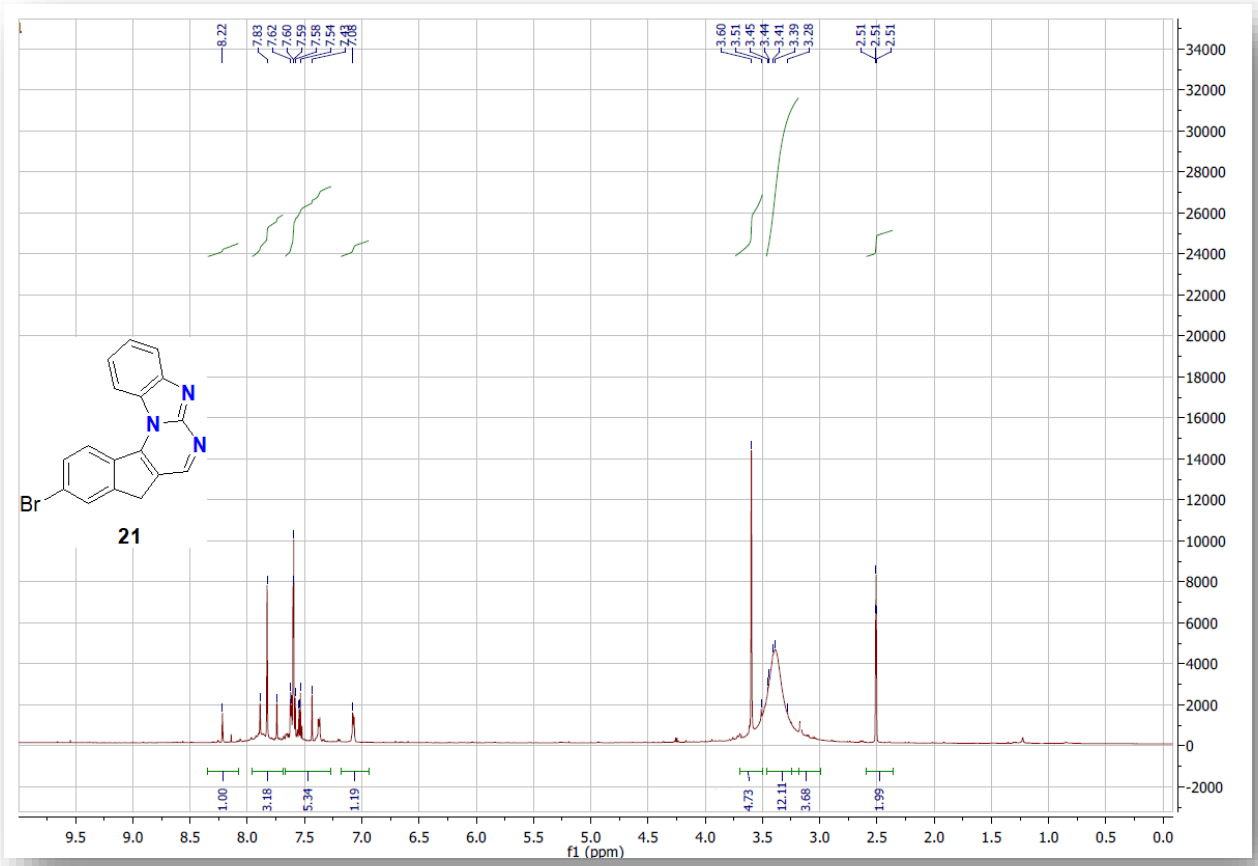

8.41
8.12
8.11
7.88
7.85
7.64
7.61
7.60
7.60
7.58
7.57
3.71
3.69
3.34
3.17
2.51
2.51
2.51
Br
20
1.00
0.82
0.98
1.72
1.96
19.54
3.27
f1 (ppm)

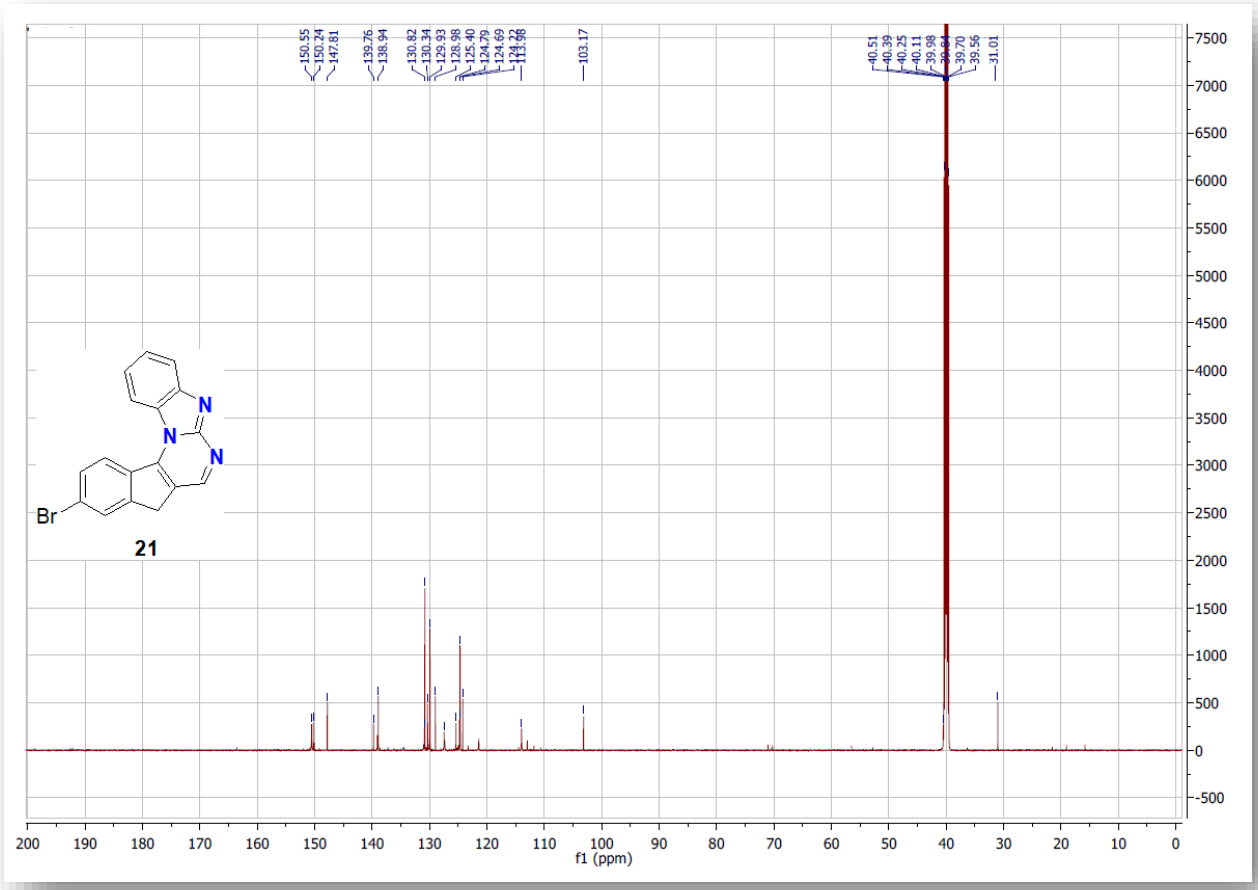

172.49
160.46
152.49
150.46
144.04
139.35
135.74
130.74
129.77
127.10
124.63
40.52
40.40
40.26
40.12
39.98
39.84
39.71
39.57
30.28
29.79
2E+07
2E+07
1E+07
1E+07
1E+07
1E+07
1E+07
9E+06
8E+06
7E+06
6E+06
5E+06
4E+06
3E+06
2E+06
1E+06
0
-1E+06
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)
N
N
N
N
Br
20

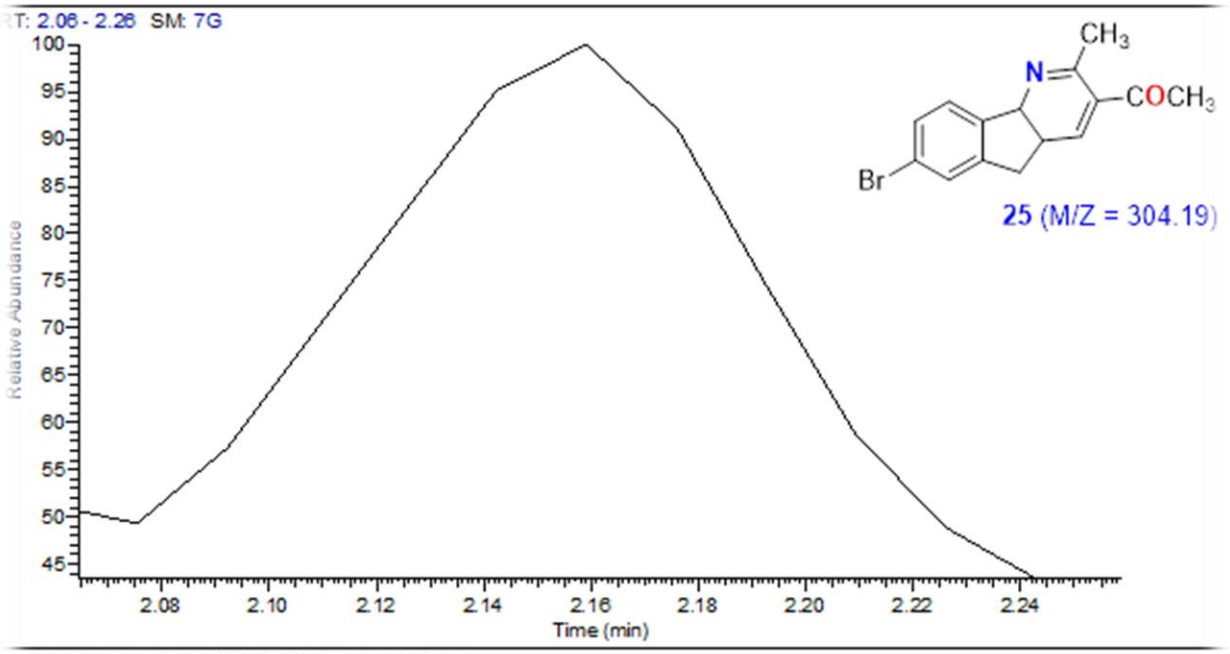

SHIMADZU
Smoothing
%T
110
100
90
80
70
60
50
3878.37
3665.99
3631.79
3608.98
3524.88
1416.80
1207.27
1049.06
922.20
848.08
759.71
661.36
585.82
511.70
3500
3000
2500
2000
1750
1500
1250
1000
750
500
cm-1
N
N
N
Br
21

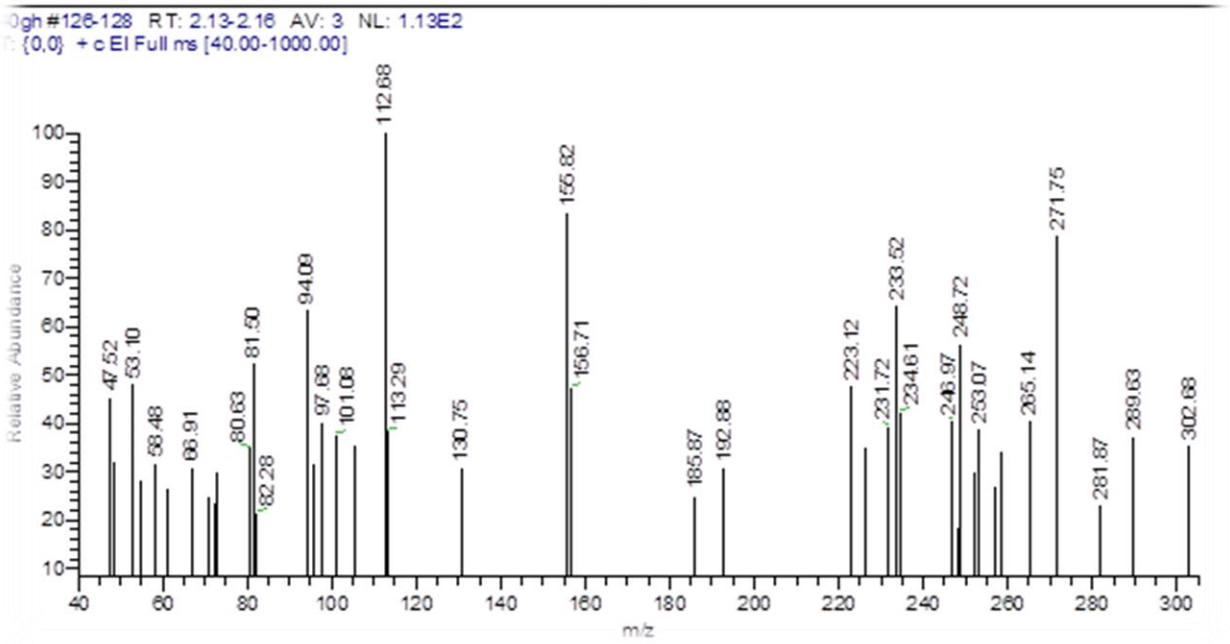

RT: 3.05 - 4.04 SM: 7G
NL:
4.95E6
TIC MS
41gh
Relative Abundance
Time (min)
Br
21 (M/Z = 336.19)

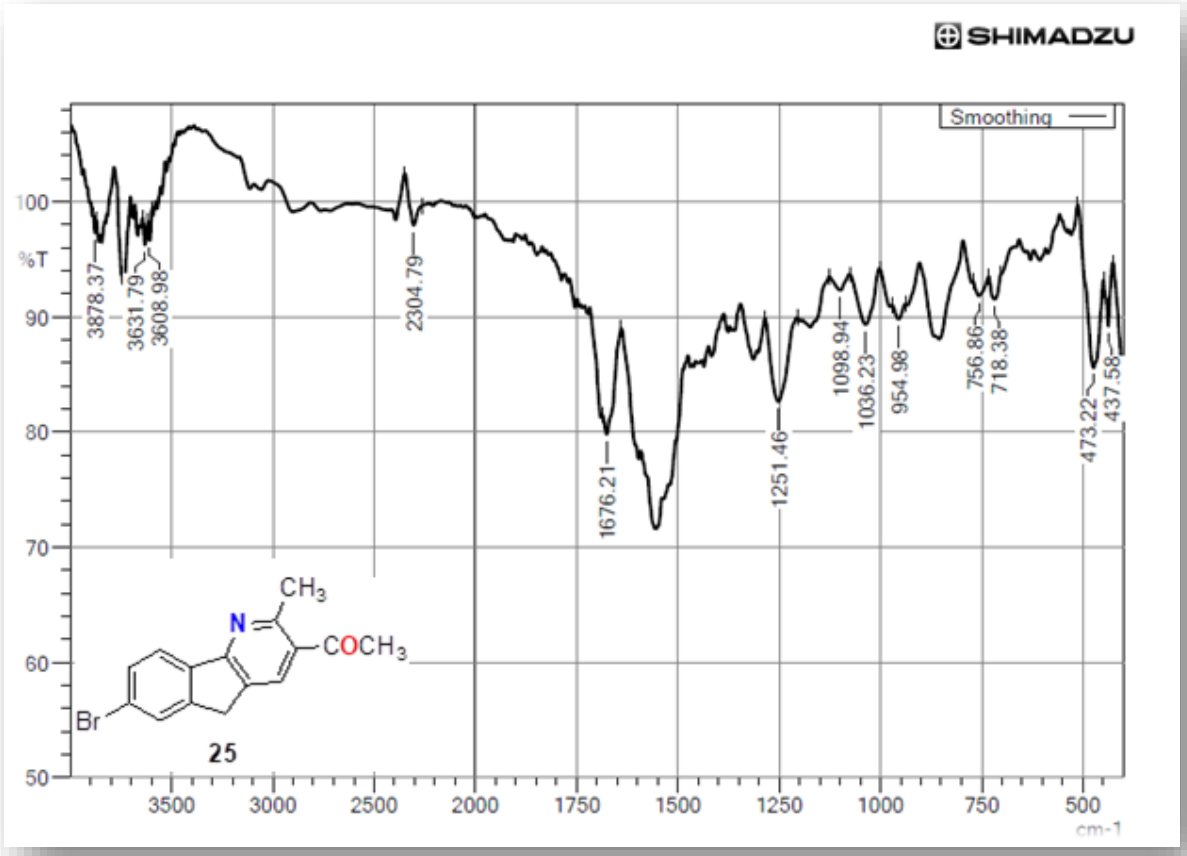

41gh #11 RT: 0.20 AV: 1 SB: 2 1.74 , 1.66 NL: 7.22E2
T: {0,0} + c EI Full ms [40.00-1000.00]
Relative Abundance
m/z
89.00
209.17
96.09
301.18
295.10
230.10
266.21
262.20
283.80
318.35
325.09
97.90
118.35
283.01
220.00
252.28
276.94
336.37
106.66
199.01
307.02
59.93
82.01
126.28
235.01
238.15
63.20
146.15
176.40
177.15
191.29
42.01
63.96
138.84
151.03
170.65

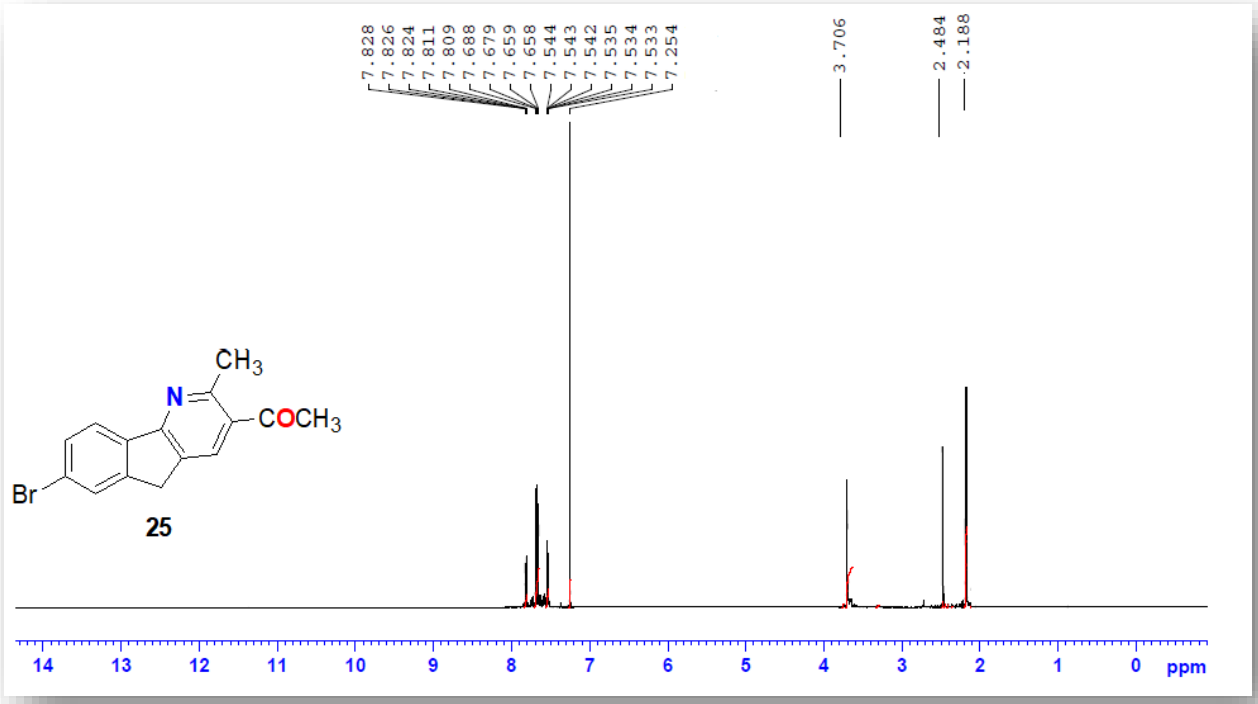

8.22
7.83
7.62
7.60
7.59
7.58
7.54
7.48
7.08
3.60
3.51
3.45
3.44
3.41
3.39
3.28
2.51
2.51
2.51
N
N
N
Br
21
1.00
3.18
5.34
1.19
4.73
12.11
3.68
1.99
f1 (ppm)

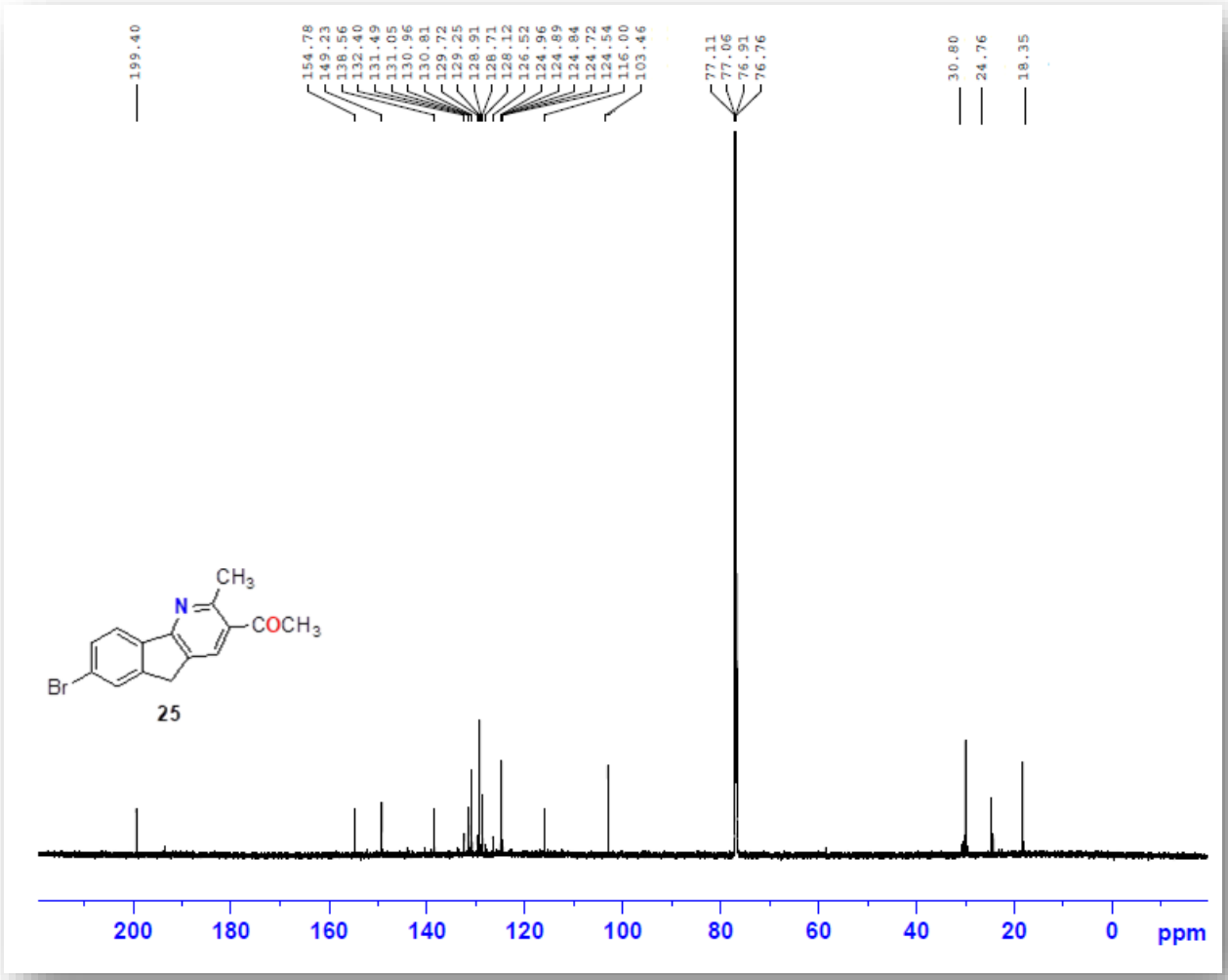

150.55
150.24
147.81
139.76
138.94
130.82
130.34
129.93
128.98
125.40
124.79
124.69
103.17
40.51
40.39
40.25
40.11
39.98
39.84
39.70
39.56
31.01
N
N
N
Br
21
f1 (ppm)

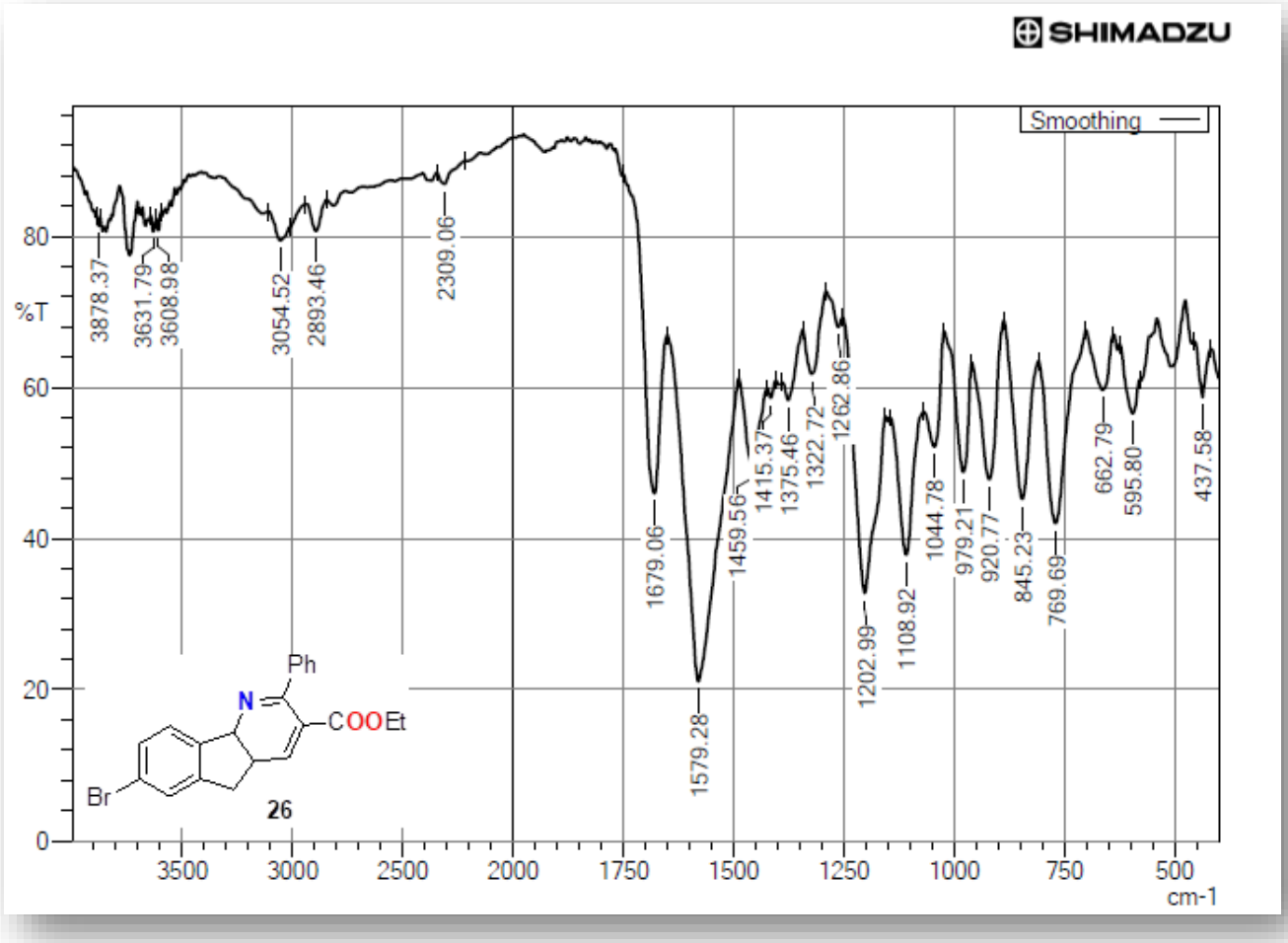

RT: 2.06 - 2.26 SM: 7G
Relative Abundance
Time (min)
CH3
N
COCH3
Br
25 (M/Z = 304.19)

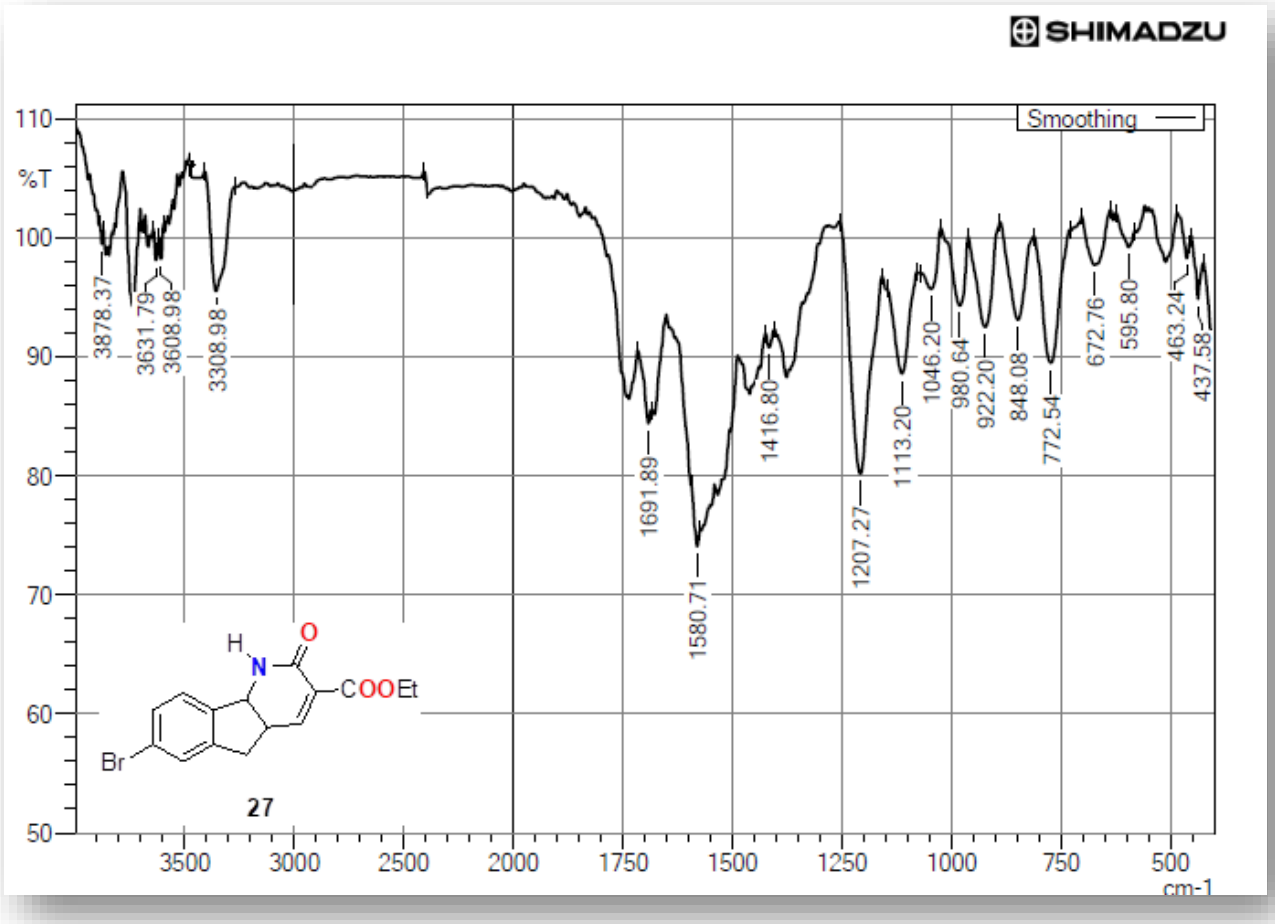

gh #126-128 RT: 2.13-2.16 AV: 3 NL: 1.13E2
{0,0} + c EI Full ms [40.00-1000.00]
Relative Abundance
m/z
47.52
53.10
58.48
66.91
80.63
81.50
82.28
94.09
97.68
101.08
112.68
113.29
130.75
155.82
156.71
185.67
192.88
223.12
231.72
233.52
234.61
246.97
248.72
253.07
265.14
271.75
281.87
289.63
302.68


SHIMADZU
Smoothing
100
%T
90
80
70
60
50
3878.37
3631.79
3608.98
2304.79
1676.21
1251.46
1098.94
1036.23
954.98
756.86
718.38
473.22
437.58
3500
3000
2500
2000
1750
1500
1250
1000
750
500
cm-1
CH3
N
COCH3
Br
25


7.828
7.826
7.824
7.811
7.809
7.688
7.679
7.659
7.658
7.544
7.543
7.542
7.535
7.534
7.533
7.254
3.706
2.484
2.188
CH3
N
COCH3
Br
25
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm


199.40
154.78
149.23
138.56
132.40
131.49
131.05
130.96
130.81
129.72
129.25
128.91
128.71
128.12
126.52
124.96
124.89
124.84
124.72
124.54
116.00
103.46
77.11
77.06
76.91
76.76
30.80
24.76
18.35
CH3
N
COCH3
Br
25
200
180
160
140
120
100
80
60
40
20
0
ppm


SHIMADZU
Smoothing
%T
80
60
40
20
0
3878.37
3631.79
3608.98
3054.52
2893.46
2309.06
1679.06
1579.28
1459.56
1415.37
1375.46
1322.72
1262.86
1202.99
1108.92
1044.78
979.21
920.77
845.23
769.69
662.79
595.80
437.58
3500
3000
2500
2000
1750
1500
1250
1000
750
500
cm-1
Ph
N
COOEt
Br
26


SHIMADZU
Smoothing
%T
110
100
90
80
70
60
50
3878.37
3631.79
3608.98
3308.98
1691.89
1580.71
1416.80
1207.27
1113.20
1046.20
980.64
922.20
848.08
772.54
672.76
595.80
463.24
437.58
3500
3000
2500
2000
1750
1500
1250
1000
750
500
cm-1
H
N
O
COOEt
Br
27