Supplementary information for:

Novel Thiazole Derivatives Incorporating Phenyl Sulphonyl Moiety as Potent BRAFV600E Kinase Inhibitors Targeting Melanoma

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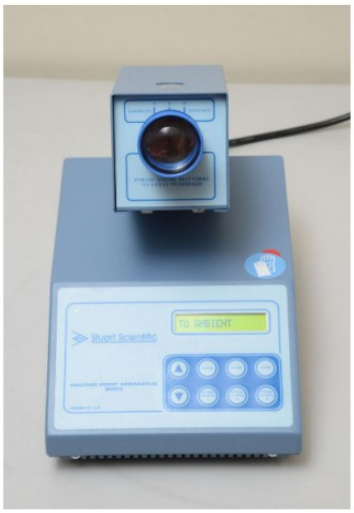
1. Experimental

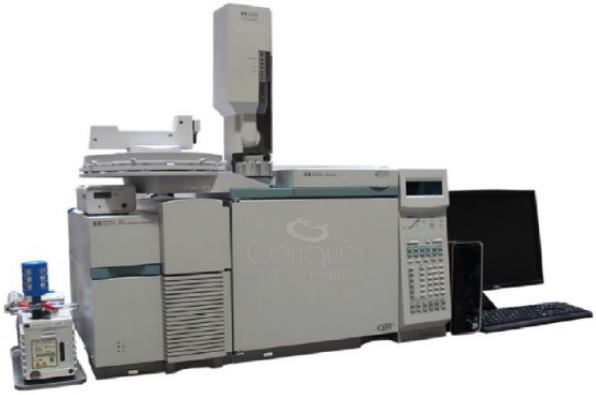
1.1. *Chemistry*

All IR spectra were recorded in KBr discs Shimadzu FT IR 8101 PC IR spectrophotometer.

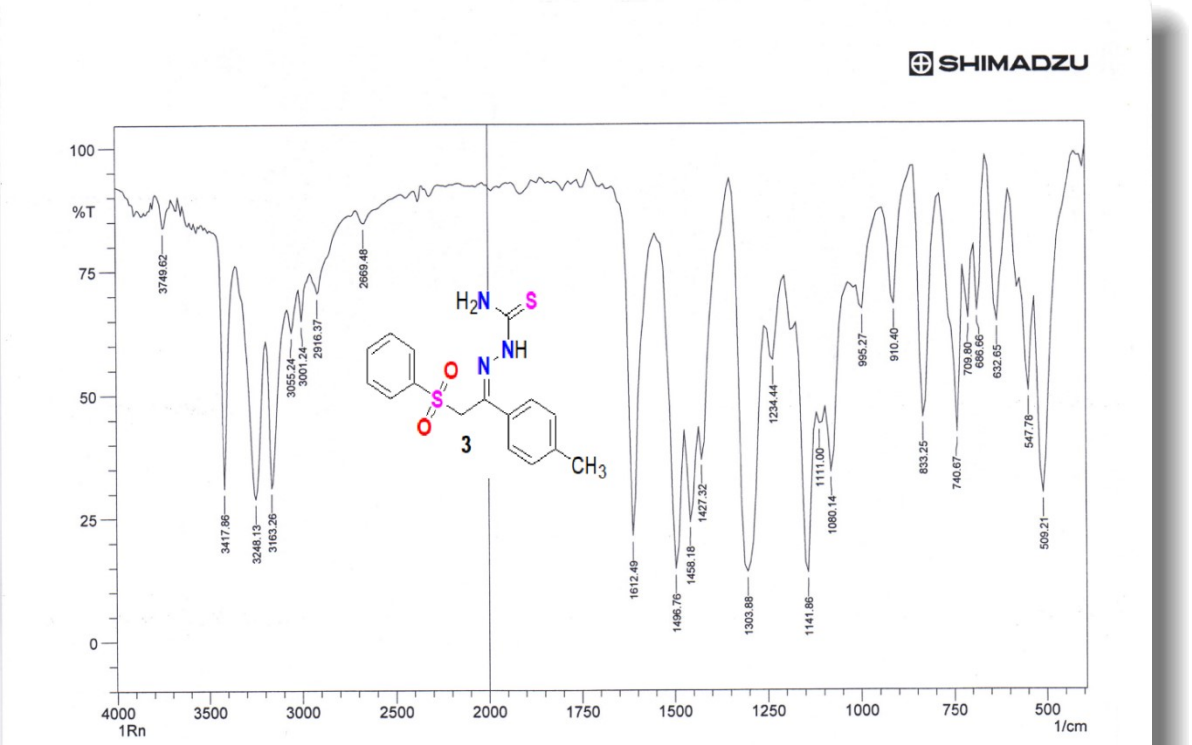
NMR-spectra (Nuclear magnetic resonance spectra) have been determined in deuterated solvent (DMSO-d_6) utilizing a Bruker Avance 300 instrument at 300 MHz for ^{1}H NMR or 75 MHz for ^{13}C NMR in DMSO-d_6 solutions. The measuring units for δ and J are ppm and Hz, respectively.



Melting points were determined by using Stuart in 0.5 mm (o.d.) glass capillaries by a SMP3 melting point apparatus.



Mass spectra were recorded on a GCMS-QP 1000 EX Shimadzu and GCMS 5988-A HP spectrometers



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	509.21	29.706	45.855	532.35	432.05	19.985	11.913
2	547.78	50.223	20.259	570.93	532.35	8.272	2.494
3	632.65	64.322	29.053	655.8	601.79	6.265	4.486
4	686.66	66.403	17.843	694.37	663.51	3.243	1.576
5	709.8	64.883	12.745	725.23	694.37	4.339	0.94
6	740.67	42.021	37.134	786.96	725.23	12.273	6.81
7	833.25	45.174	48.155	856.39	794.67	10.813	8.595
8	910.4	67.859	22.696	941.26	864.11	6.155	3.264
9	995.27	66.817	8.517	1010.7	948.98	6.654	0.803
10	1080.14	34.217	19.192	1095.57	1033.85	16.912	3.595
11	1111	43.839	2.537	1118.71	1095.57	8.07	0.389
12	1141.86	13.739	35.909	1172.72	1126.43	25.233	11.451
13	1234.44	56.646	9.993	1249.87	1211.3	7.65	1.291
14	1303.88	13.882	64.17	1342.46	1257.59	41.602	30.732
15	1427.32	36.759	11.023	1435.04	1350.17	14.138	1.487
16	1458.18	24.124	18.712	1473.62	1435.04	18.66	4.481
17	1496.76	14.535	39.958	1550.77	1473.62	29.554	11.763
18	1612.49	21.268	65.193	1658.78	1558.48	22.609	15.79
19	2669.48	84.555	3.185	2708.06	2468.88	13.546	1.274
20	2916.37	70.405	5.502	2947.23	2746.63	20.367	1.463
21	3001.24	64.849	7.161	3016.67	2954.95	9.325	0.896
22	3055.24	62.589	5.984	3078.39	3024.38	9.745	1.044
23	3163.26	31.205	29.45	3186.4	3086.11	31.553	11.368
24	3248.13	28.889	37.083	3348.42	3194.12	47.716	21.316
25	3417.86	30.996	48.364	3487.3	3356.14	24.756	11.636
26	3749.62	83.738	6.416	3788.19	3703.33	5.007	1.15

Figure S1. IR spectrum of compound 3

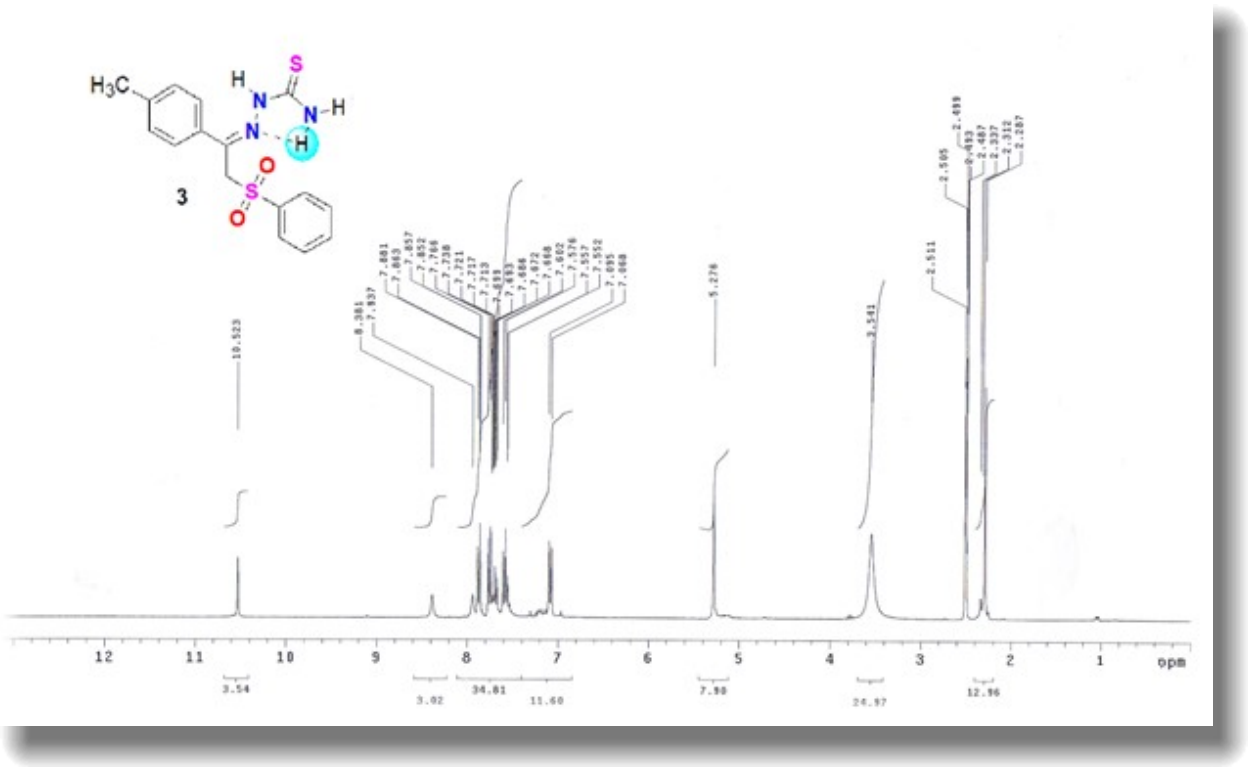


Figure S2. 1H-NMR spectrum of compound 3

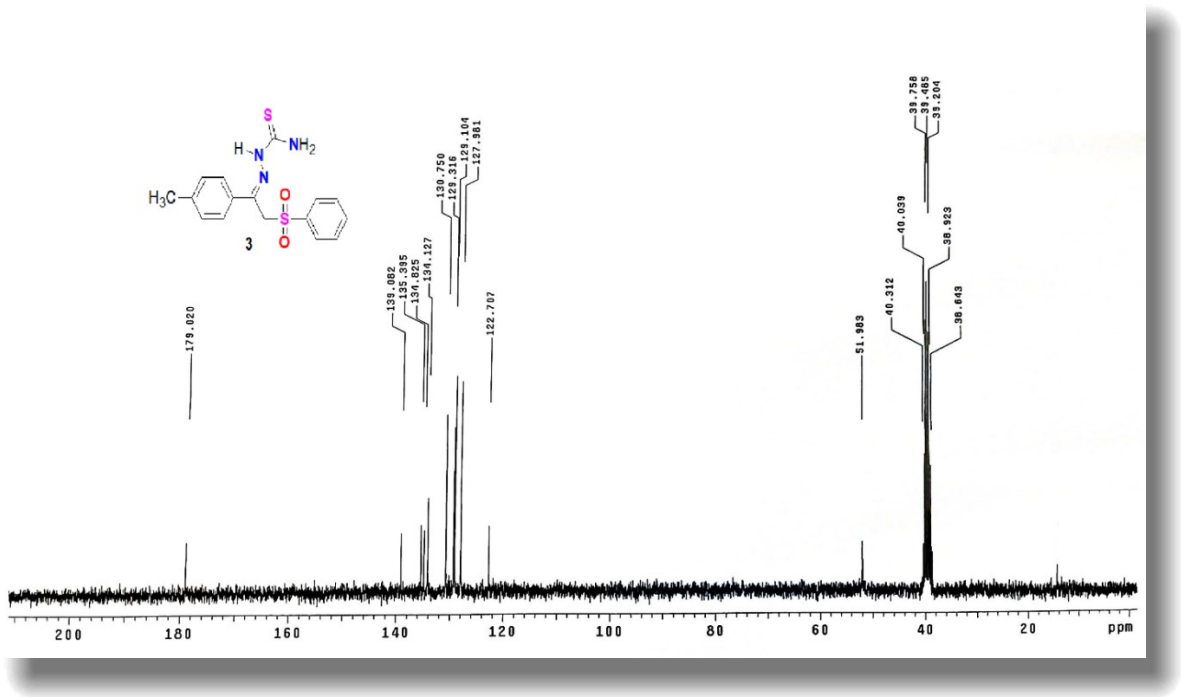


Figure S3. 13C-NMR spectrum of compound 3

Cairo University
Micro Analytical Center

DI Analysis
Shimadzu Qp-2010 Plus

Sample Information

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Analyzed	: 01/01/2007 02:28:15 ص
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Sample ID	:
Customer Name	: Dr. Hoda Kamel - Science - Cairo
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Org Data File	: C:\GCMSsolution\Data\Project1\Rn1.QGD
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Report File	:
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Modified	: 01/01/2007 02:36:47 ص

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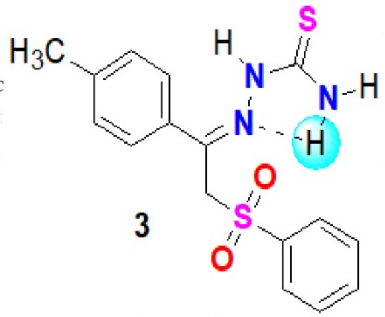
Ionization Mode	: EI



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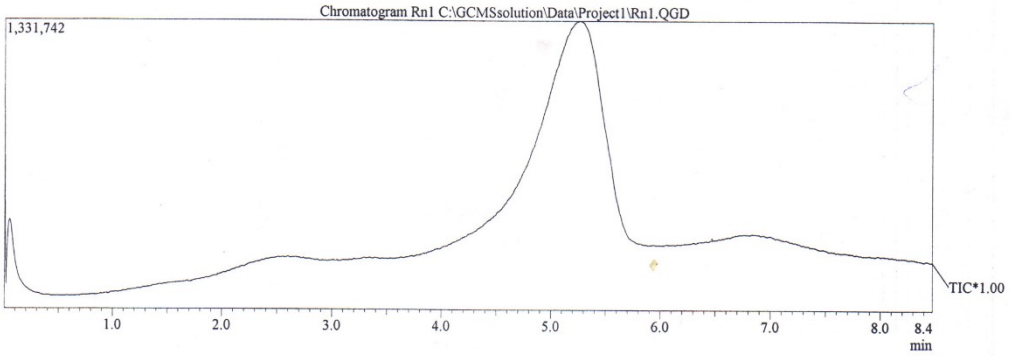

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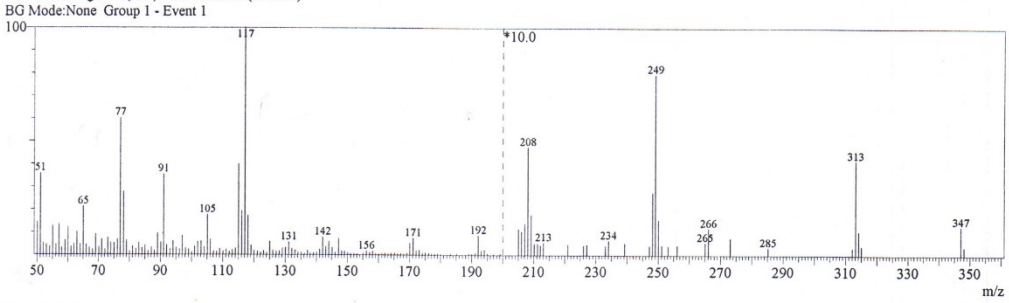

Mass Table
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MassPeaks:173
RawMode:Single 5.2(629) BasePeak:117(163099)
BG Mode:None Group 1 - Event 1

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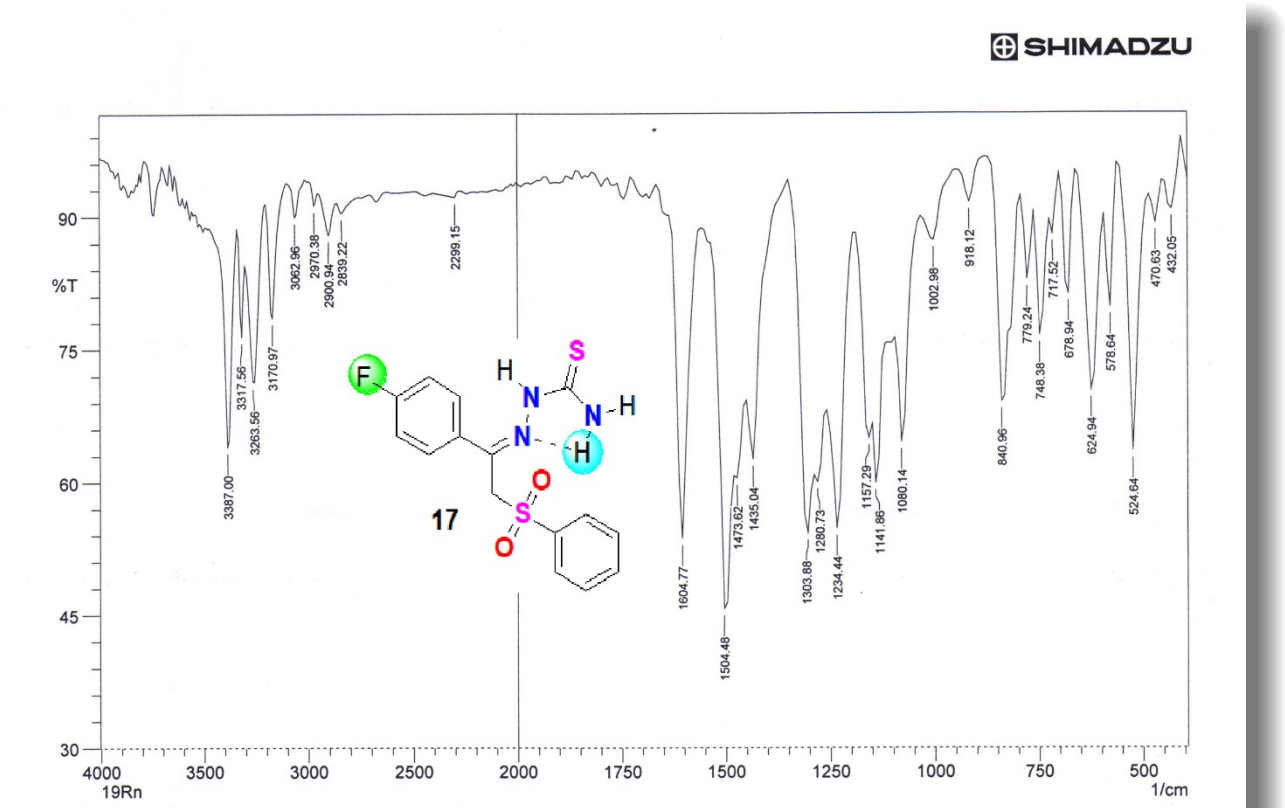
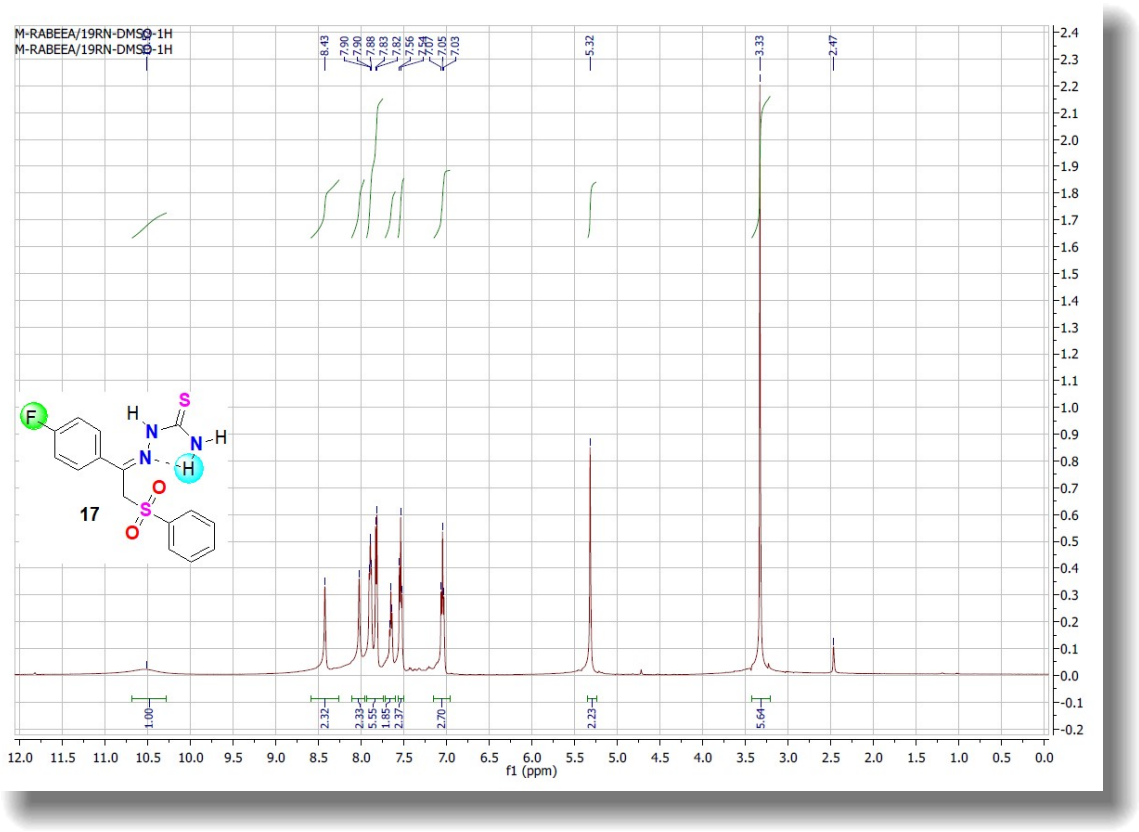
1	50.05	23500	14.41	4	53.05	7138	4.38	7	56.10	7138	4.38
2	51.05	58126	35.64	5	54.10	5623	3.45	8	57.10	21724	13.32
3	52.05	8283	5.08	6	55.05	20664	12.67	9	58.00	4990	3.06

Figure S4. Mass spectrum of compound 3



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	432.05	90.73	5.722	455.2	408.91	1.388	0.655
2	470.63	89.159	3.99	486.06	455.2	1.23	0.278
3	524.64	63.468	30.683	563.21	486.06	6.231	4.205
4	578.64	79.7	13.38	594.08	563.21	1.978	1.01
5	624.94	70.363	20.037	655.8	601.79	5.273	2.888
6	678.94	81.225	13.763	702.09	663.51	2.125	1.258
7	717.52	87.937	3.384	725.23	702.09	0.987	0.165
8	748.38	76.546	13.643	763.81	725.23	3.062	1.313
9	779.24	82.848	8.581	794.67	763.81	1.845	0.644
10	840.96	69.092	24.985	871.82	802.39	6.285	4.233
11	918.12	91.542	4.291	948.98	879.54	1.711	0.453
12	1002.98	87.237	4.589	1033.85	948.98	3.454	0.582
13	1080.14	64.532	15.151	1095.57	1033.85	6.505	1.807
14	1141.86	59.877	8.566	1149.57	1111	6.382	0.652
15	1157.29	65.001	5.91	1188.15	1149.57	5.127	0.661
16	1234.44	54.759	20.827	1257.59	1195.87	9.909	3.484
17	1280.73	60.019	2.604	1288.45	1257.59	6.107	0.199
18	1303.88	54.248	25.346	1350.17	1265.3	14.678	5.8
19	1435.04	62.599	10.667	1450.47	1357.89	8.234	1.077
20	1473.62	60.448	2.416	1481.33	1450.47	5.945	0.141
21	1504.48	45.676	32.149	1558.48	1458.18	17.907	6.978
22	1604.77	53.644	35.656	1635.64	1566.2	9.518	6.039
23	2299.15	92.176	0.672	2399.45	2276	4.201	0.181
24	2839.22	90.405	1.316	2862.36	2762.06	3.932	0.257
25	2900.94	87.932	4.126	2947.23	2862.36	3.785	0.754
26	2970.38	91.256	2.058	2993.52	2947.23	1.553	0.161
27	3062.96	89.954	4.019	3101.54	3016.67	2.869	0.584
28	3170.97	78.588	13.597	3201.83	3101.54	5.831	2.539
29	3263.56	71.356	14.854	3294.42	3209.55	8.392	3.488
30	3317.56	76.431	9.688	3340.71	3302.13	3.294	0.865
31	3387	64.061	24.59	3487.3	3340.71	13.344	5.697

Figure S5. IR spectrum of compound 17



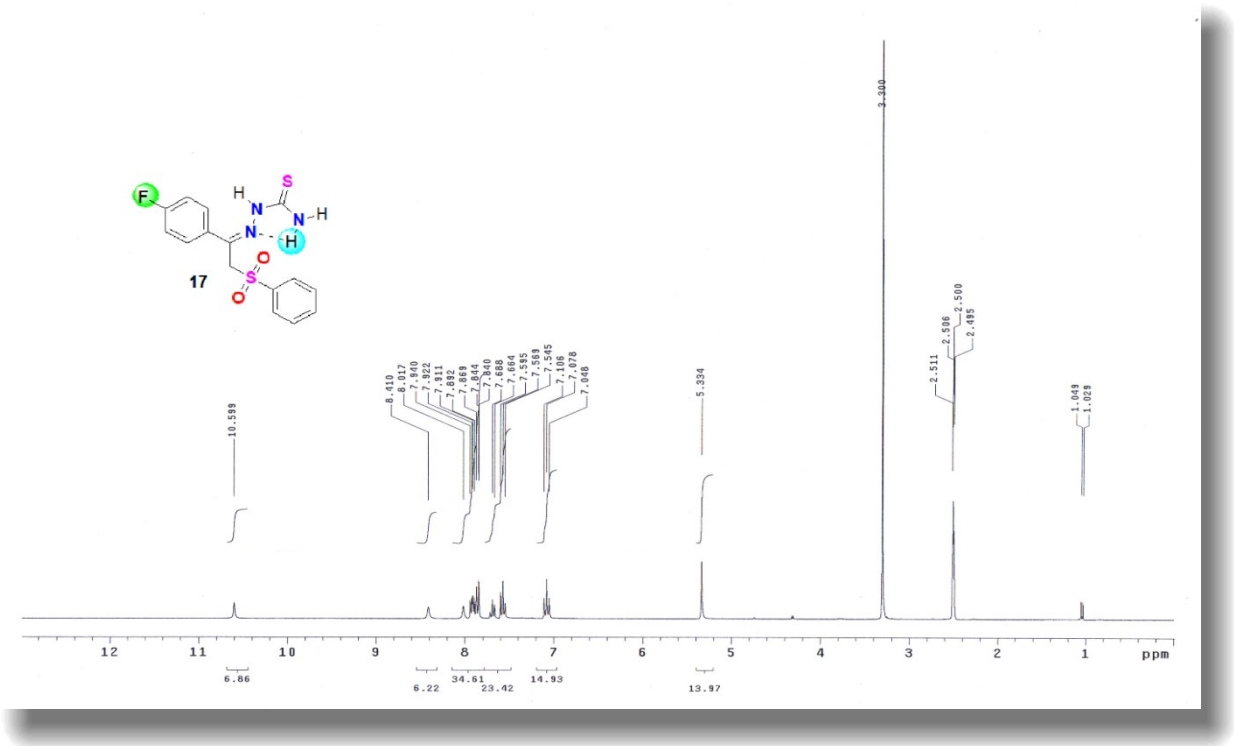


Figure S6. 1H-NMR spectrum of compound 17

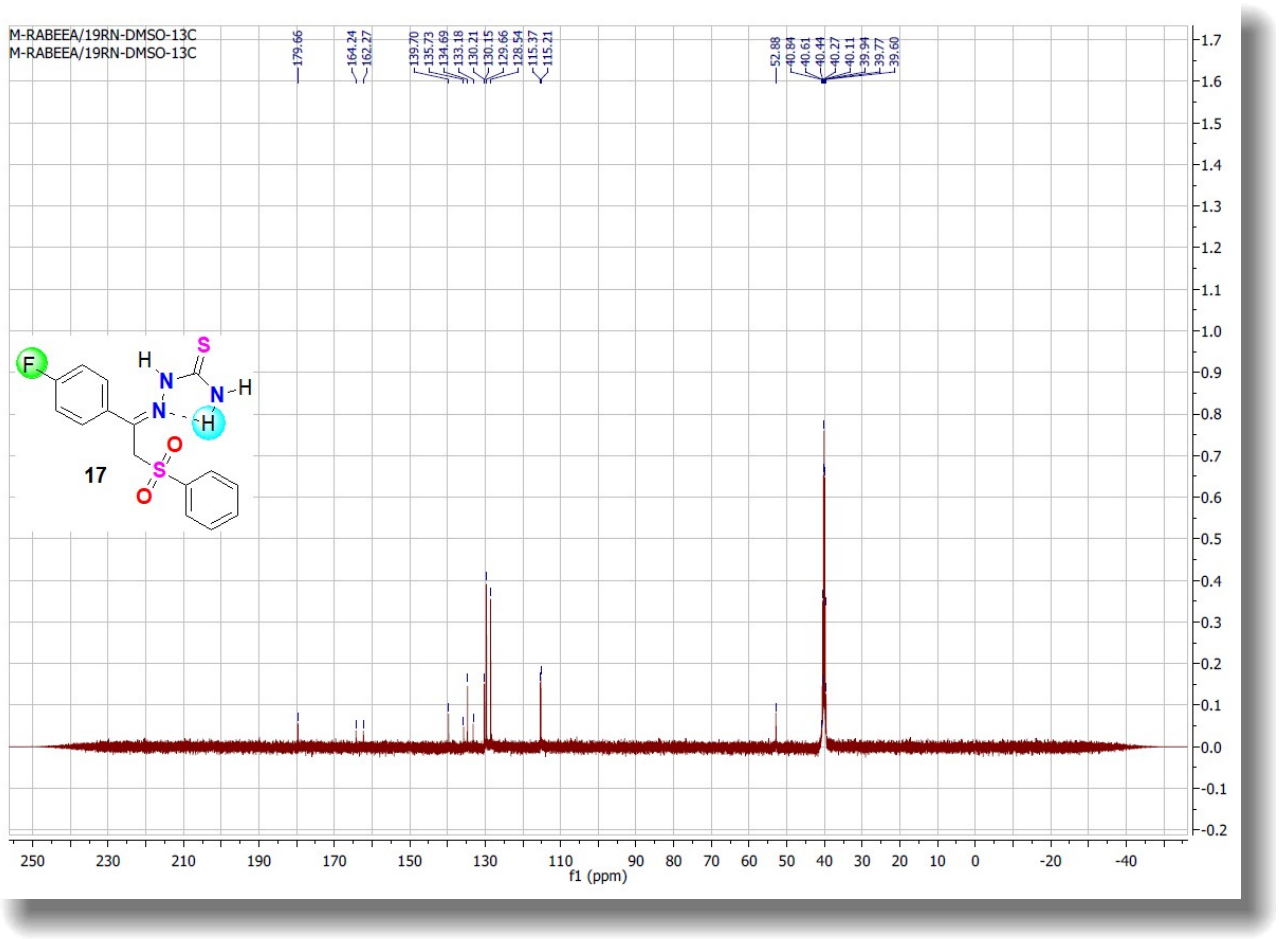


Figure S7. 13C-NMR spectrum of compound 17

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Shimadzu Qp-2010 Plus

Sample Information

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Analyzed	: 03/01/2007 12:11:52 ص
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Sample ID	:
Customer Name	: Dr. Hoda Kamel - Science - Cairo
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Modified	: 03/01/2007 12:15:15 ص

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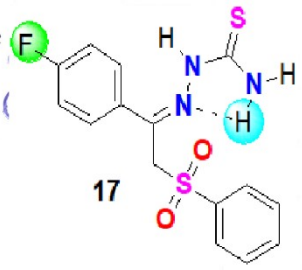

17

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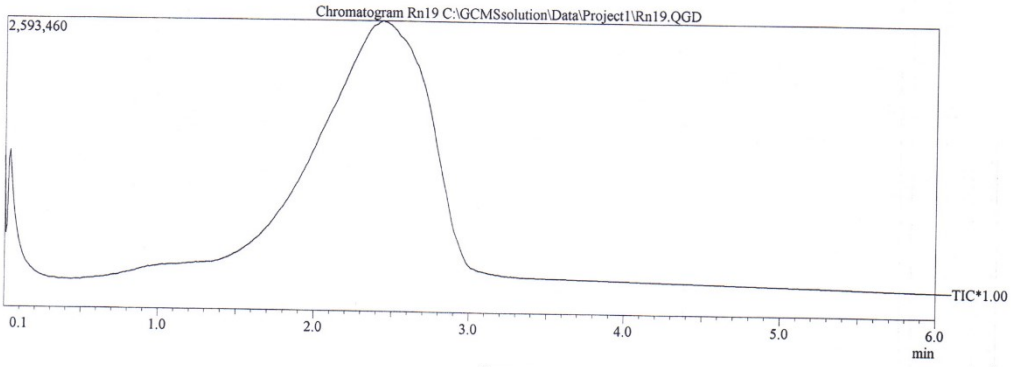

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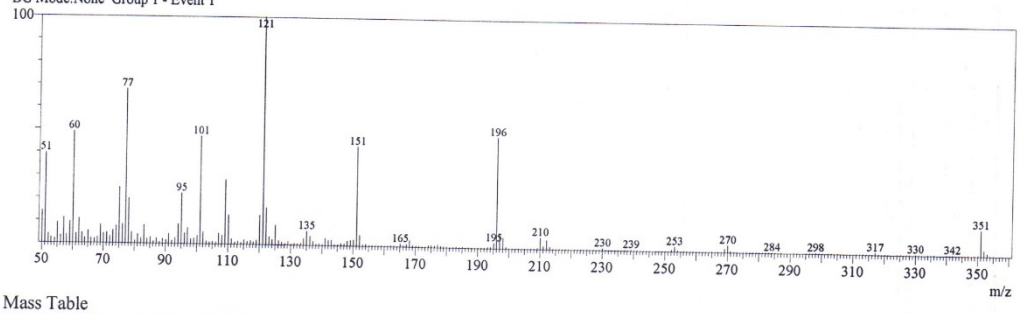

Mass Table
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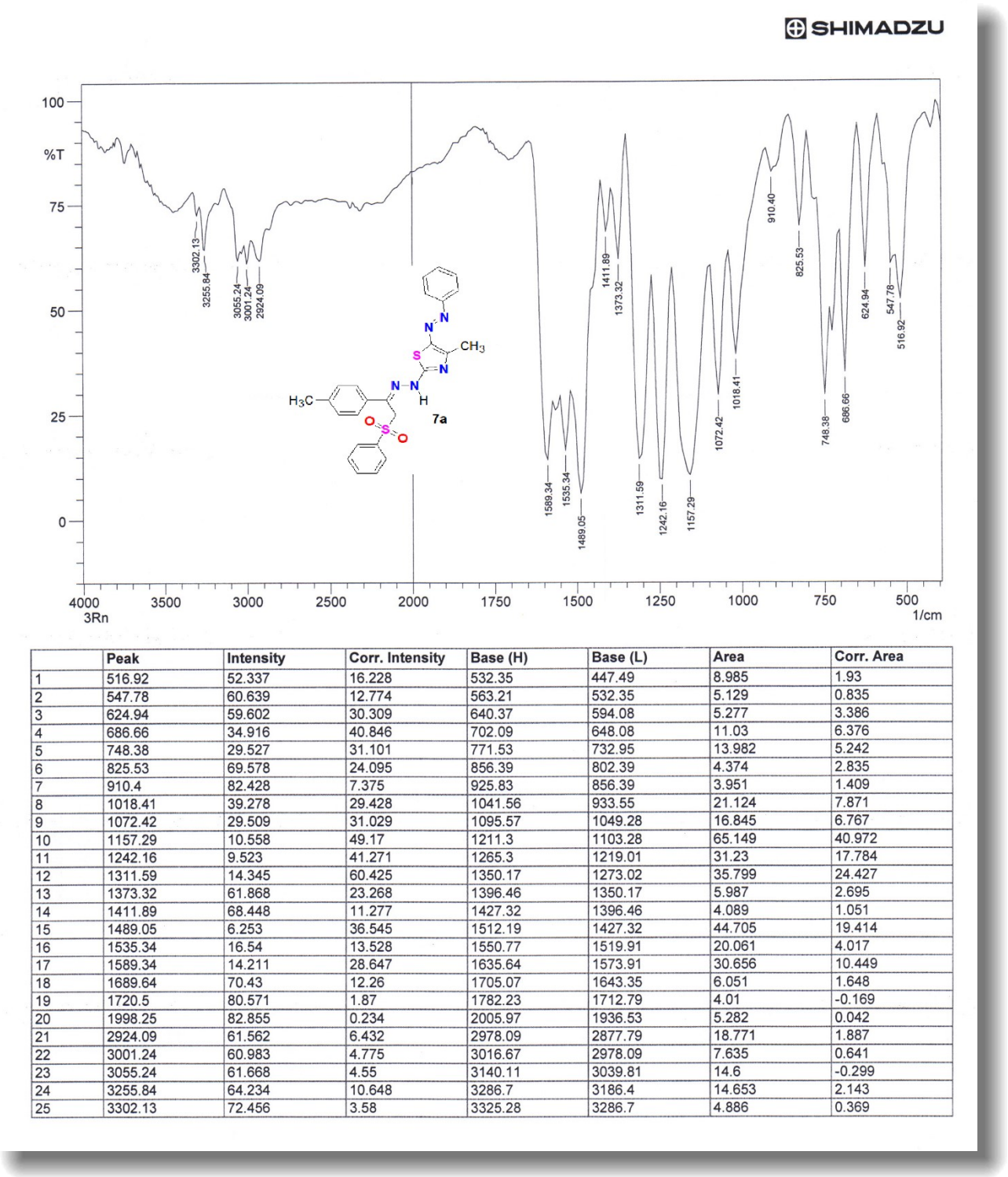
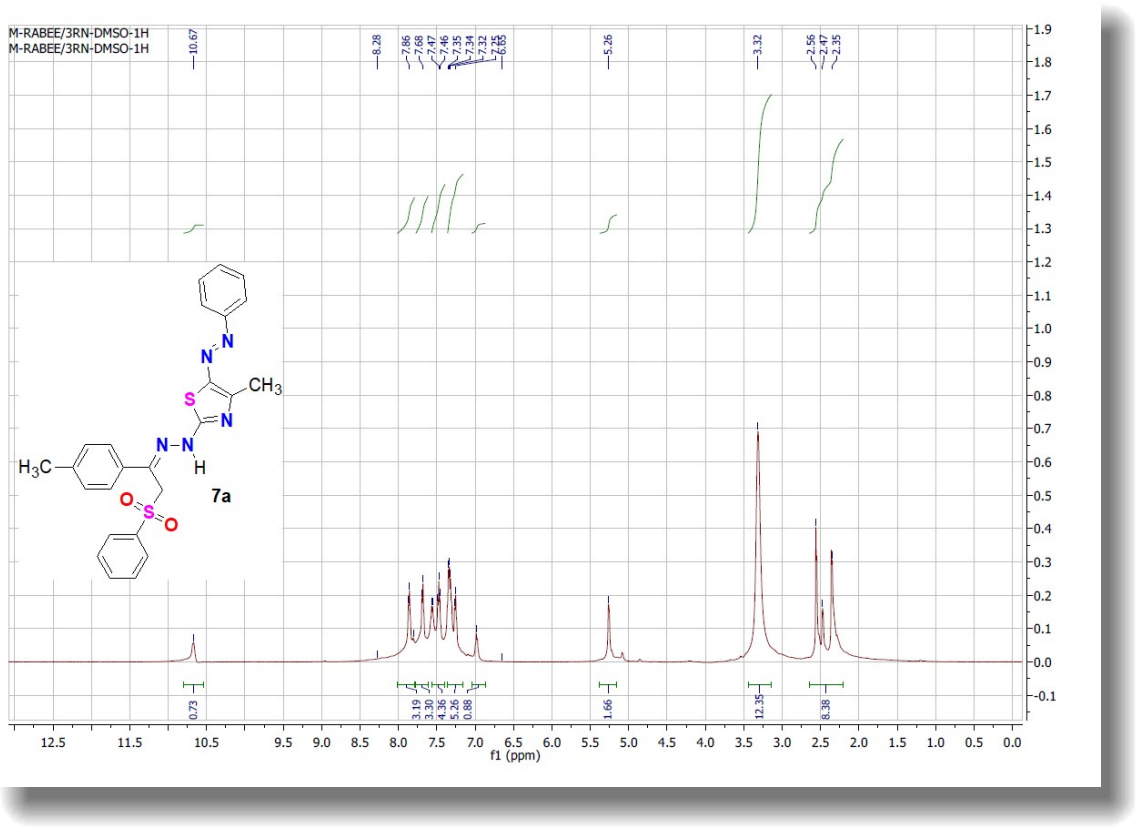
2	51.05	112508	39.36	5	54.15	5139	1.80	8	57.10	31849	11.14
3	52.10	11175	3.91	6	55.10	25764	9.01	9	58.05	10493	3.67

Figure S8. Mass spectrum of compound 17



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	516.92	52.337	16.228	532.35	447.49	8.985	1.93
2	547.78	60.639	12.774	563.21	532.35	5.129	0.835
3	624.94	59.602	30.309	640.37	594.08	5.277	3.386
4	686.66	34.916	40.846	702.09	648.08	11.03	6.376
5	748.38	29.527	31.101	771.53	732.95	13.982	5.242
6	825.53	69.578	24.095	856.39	802.39	4.374	2.835
7	910.4	82.428	7.375	925.83	856.39	3.951	1.409
8	1018.41	39.278	29.428	1041.56	933.55	21.124	7.871
9	1072.42	29.509	31.029	1095.57	1049.28	16.845	6.767
10	1157.29	10.558	49.17	1211.3	1103.28	65.149	40.972
11	1242.16	9.523	41.271	1265.3	1219.01	31.23	17.784
12	1311.59	14.345	60.425	1350.17	1273.02	35.799	24.427
13	1373.32	61.868	23.268	1396.46	1350.17	5.987	2.695
14	1411.89	68.448	11.277	1427.32	1396.46	4.089	1.051
15	1489.05	6.253	36.545	1512.19	1427.32	44.705	19.414
16	1535.34	16.54	13.528	1550.77	1519.91	20.061	4.017
17	1589.34	14.211	28.647	1635.64	1573.91	30.656	10.449
18	1689.64	70.43	12.26	1705.07	1643.35	6.051	1.648
19	1720.5	80.571	1.87	1782.23	1712.79	4.01	-0.169
20	1998.25	82.855	0.234	2005.97	1936.53	5.282	0.042
21	2924.09	61.562	6.432	2978.09	2877.79	18.771	1.887
22	3001.24	60.983	4.775	3016.67	2978.09	7.635	0.641
23	3055.24	61.668	4.55	3140.11	3039.81	14.6	-0.299
24	3255.84	64.234	10.648	3286.7	3186.4	14.653	2.143
25	3302.13	72.456	3.58	3325.28	3286.7	4.886	0.369

Figure S9. IR spectrum of compound 7a



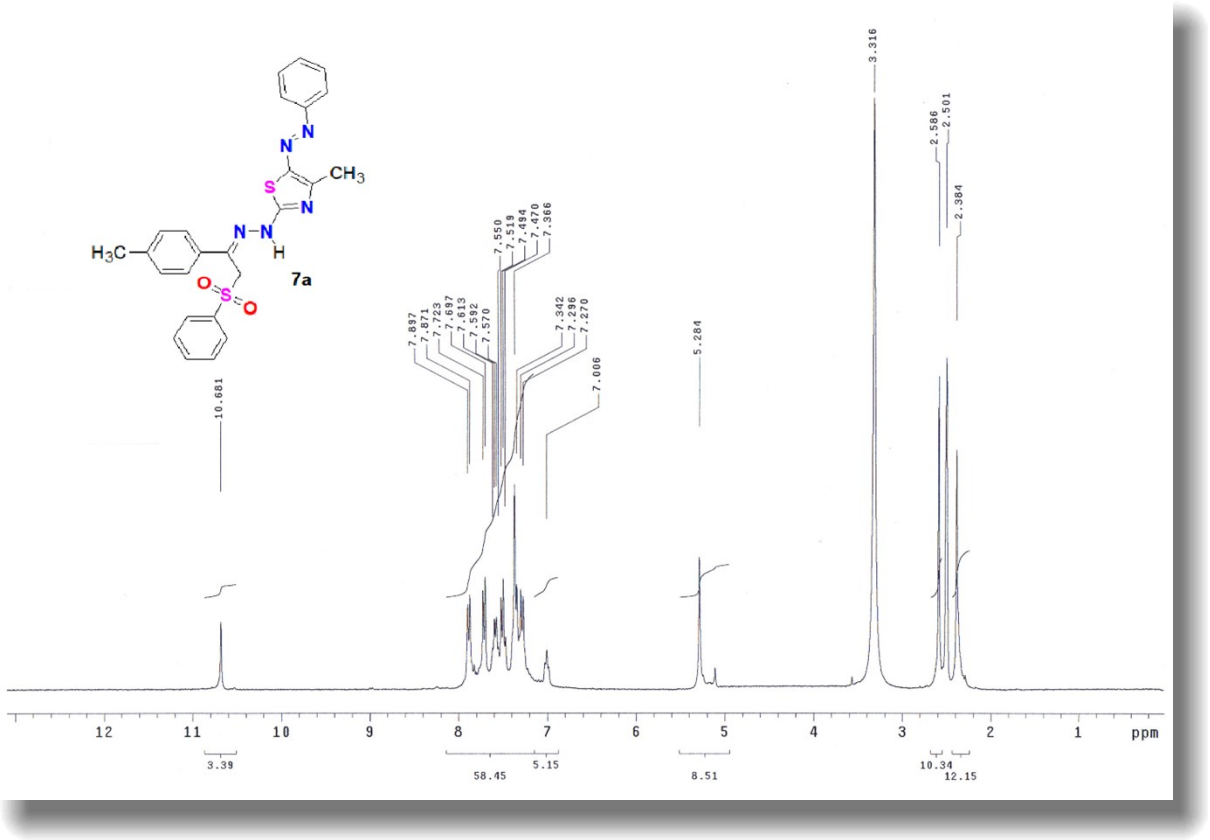


Figure S10. 1H-NMR spectrum of compound 7a

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DI Analysis
Shimadzu Qp-2010 Plus

Sample Information

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Analyzed	: 01/01/2007 02:52:31 ص
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Sample ID	:
Customer Name	: Dr. Hoda Kamel - Science - Cairo
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Modified	: 01/01/2007 02:59:13 ص

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Electron Voltage	: 70 eV
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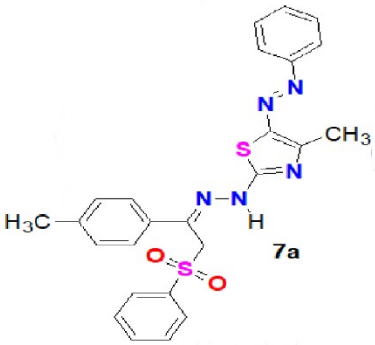

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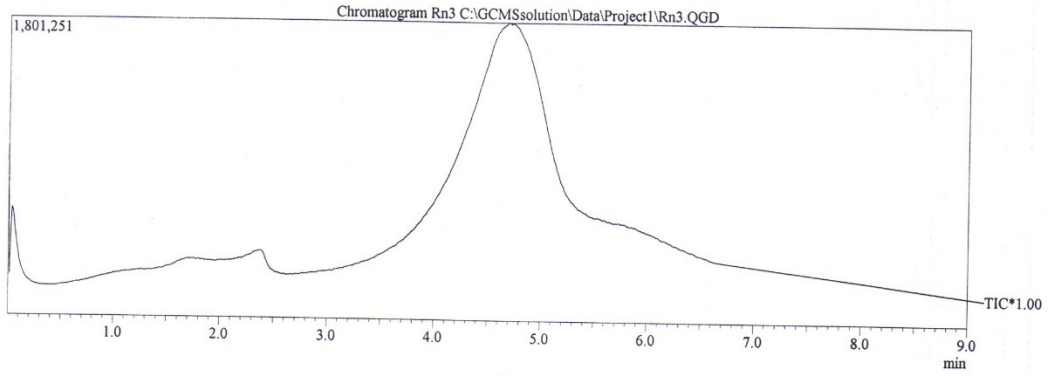

Spectrum

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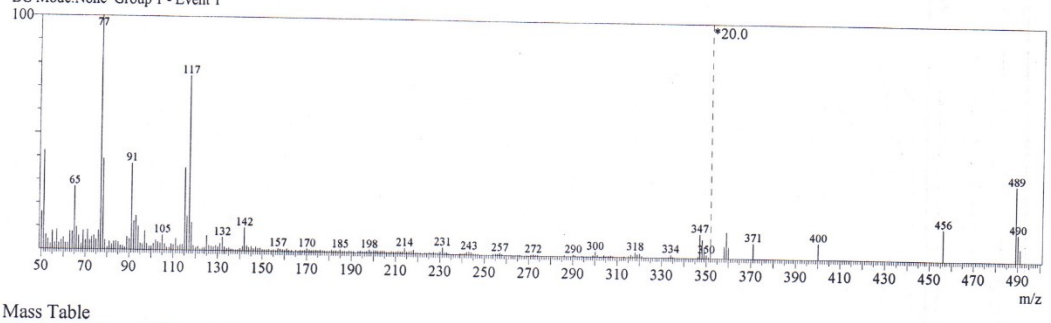

Mass Table

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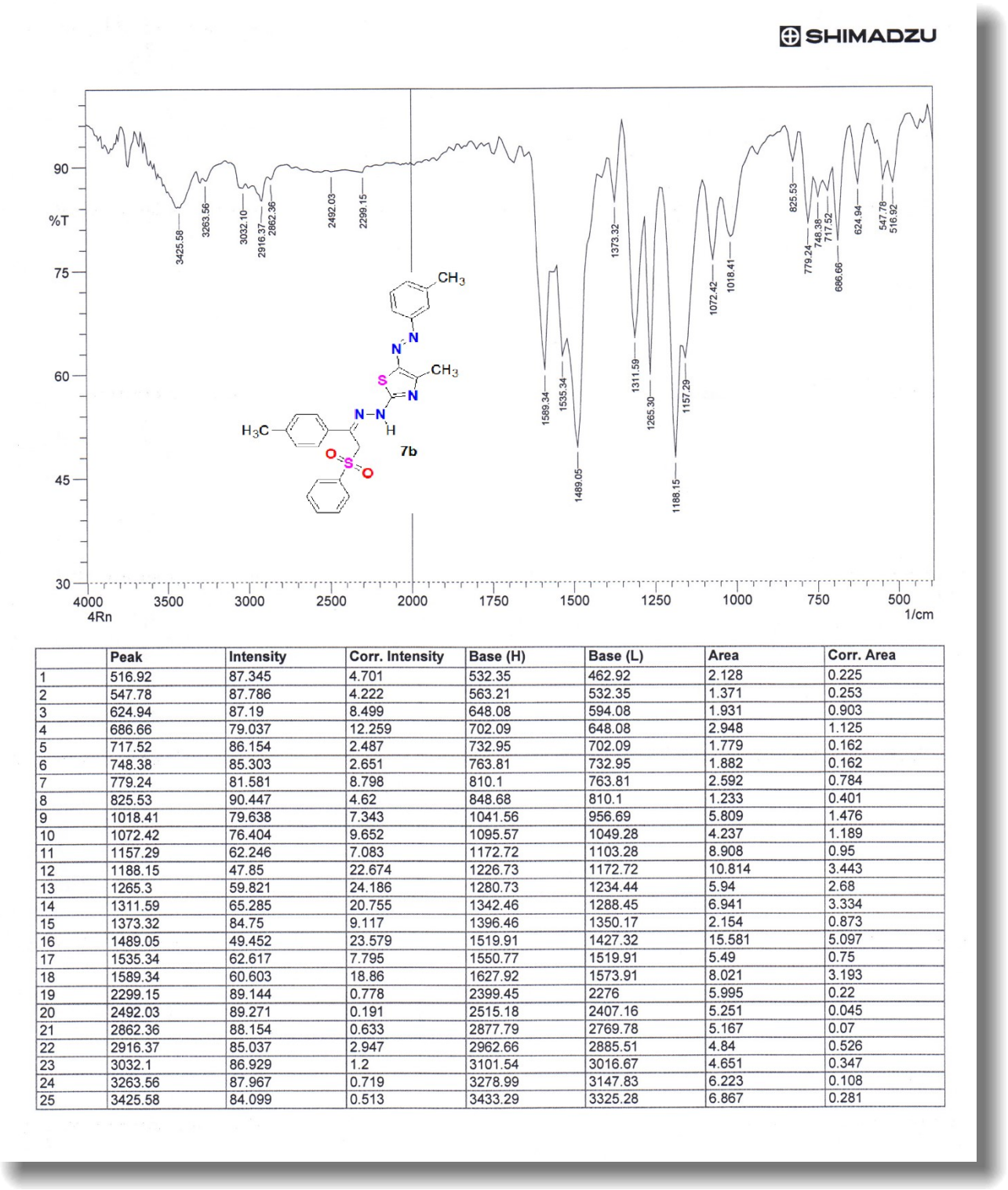
RawMode:Single 4.8(573) BasePeak:77(217825)
BG Mode:None Group 1 - Event 1

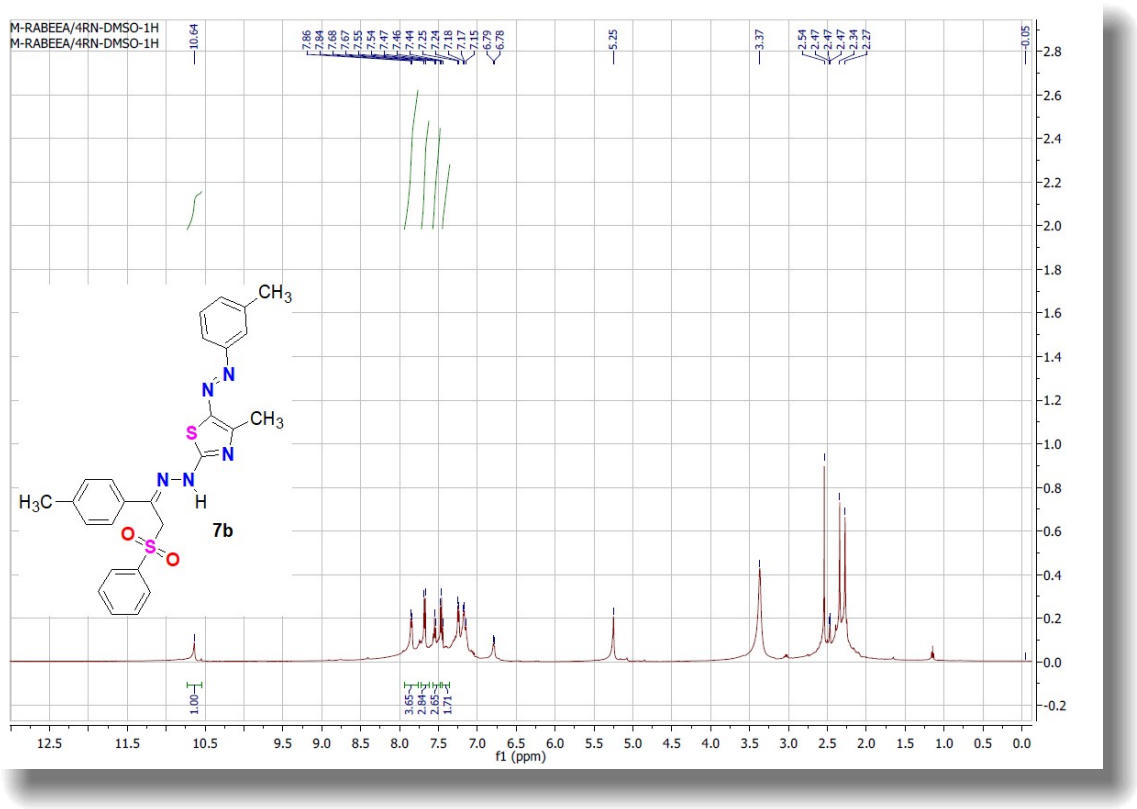
#	m/z	Abs. In	Rel. Int.	#	m/z	Abs. In	Rel. Int.	#	m/z	Abs. In	Rel. Int.
1	50.05	34819	15.98	4	53.05	9152	4.20	7	56.10	5966	2.74
2	51.05	91969	42.22	5	54.10	4850	2.23	8	57.10	18031	8.28
3	52.05	13291	6.10	6	55.05	16773	7.70	9	58.05	5836	2.68

Figure S11. Mass spectrum of compound 7a



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	516.92	87.345	4.701	532.35	462.92	2.128	0.225
2	547.78	87.786	4.222	563.21	532.35	1.371	0.253
3	624.94	87.19	8.499	648.08	594.08	1.931	0.903
4	686.66	79.037	12.259	702.09	648.08	2.948	1.125
5	717.52	86.154	2.487	732.95	702.09	1.779	0.162
6	748.38	85.303	2.651	763.81	732.95	1.882	0.162
7	779.24	81.581	8.798	810.1	763.81	2.592	0.784
8	825.53	90.447	4.62	848.68	810.1	1.233	0.401
9	1018.41	79.638	7.343	1041.56	956.69	5.809	1.476
10	1072.42	76.404	9.652	1095.57	1049.28	4.237	1.189
11	1157.29	62.246	7.083	1172.72	1103.28	8.908	0.95
12	1188.15	47.85	22.674	1226.73	1172.72	10.814	3.443
13	1265.3	59.821	24.186	1280.73	1234.44	5.94	2.68
14	1311.59	65.285	20.755	1342.46	1288.45	6.941	3.334
15	1373.32	84.75	9.117	1396.46	1350.17	2.154	0.873
16	1489.05	49.452	23.579	1519.91	1427.32	15.581	5.097
17	1535.34	62.617	7.795	1550.77	1519.91	5.49	0.75
18	1589.34	60.603	18.86	1627.92	1573.91	8.021	3.193
19	2299.15	89.144	0.778	2399.45	2276	5.995	0.22
20	2492.03	89.271	0.191	2515.18	2407.16	5.251	0.045
21	2862.36	88.154	0.633	2877.79	2769.78	5.167	0.07
22	2916.37	85.037	2.947	2962.66	2885.51	4.84	0.526
23	3032.1	86.929	1.2	3101.54	3016.67	4.651	0.347
24	3263.56	87.967	0.719	3278.99	3147.83	6.223	0.108
25	3425.58	84.099	0.513	3433.29	3325.28	6.867	0.281

Figure S12. IR spectrum of compound 7b



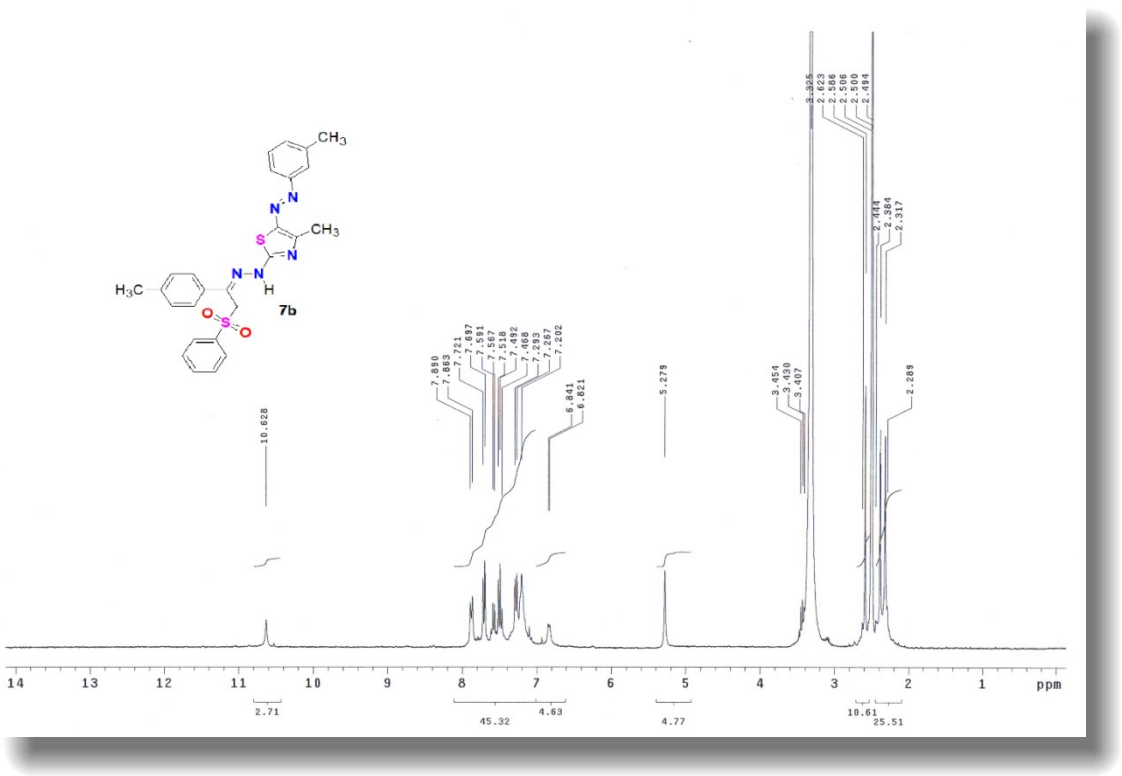


Figure S13. 1H-NMR spectrum of compound 7b

Cairo University
Micro Analytical Center

DI Analysis
Shimadzu Qp-2010 Plus

Sample Information

Analyzed by	: Dr. Mai Younis
Analyzed	: 01/01/2007 03:21:49 ص
Sample Name	: Rn4.
Sample ID	:
Customer Name	: Dr. Hoda Kamel - Science - Cairo
Data File	: C:\GCMSsolution\Data\Project1\Rn4..QGD
Org Data File	: C:\GCMSsolution\Data\Project1\Rn4..QGD
Method File	: C:\GCMSsolution\Data\Project1\High Temperature Op
Org Method File	: C:\GCMSsolution\Data\Project1\High Temperature Op
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1_default.qgt
$EndIf$Modified by	: Dr. Mai Younis
Modified	: 01/01/2007 03:31:49 ص

Method

===== Analytical Line 1 =====	
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[MS Table]	
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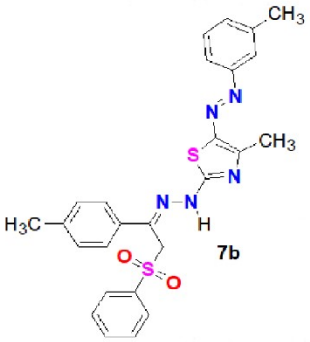
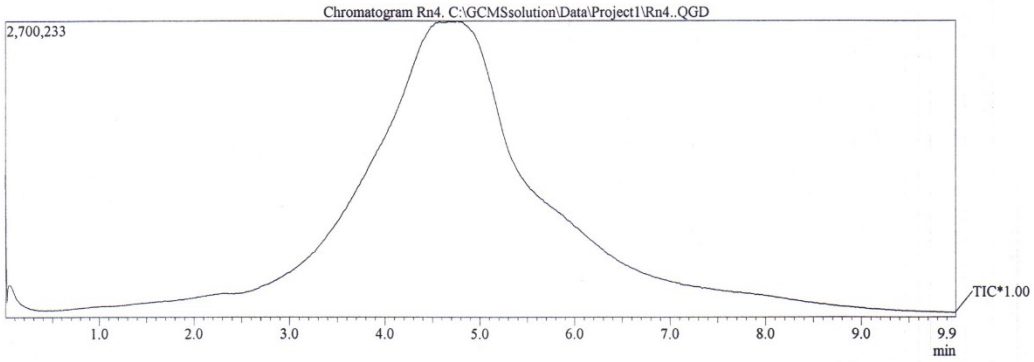
End Time	:10.00min
ACQ Mode	:Scan
Event Time	:0.50sec
Scan Speed	:1250
Start m/z	:50.00
End m/z	:600.00
Electron Voltage	: 70 eV
Ionization Mode	: EI



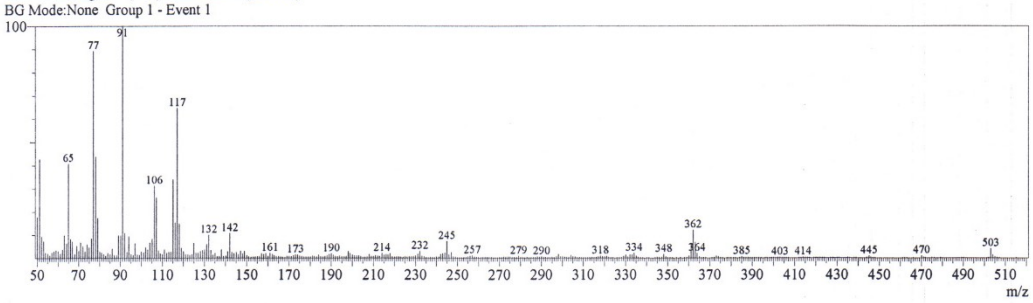
7b

C:\GCMSsolution\Data\Project1\Rn4..QGD



Spectrum

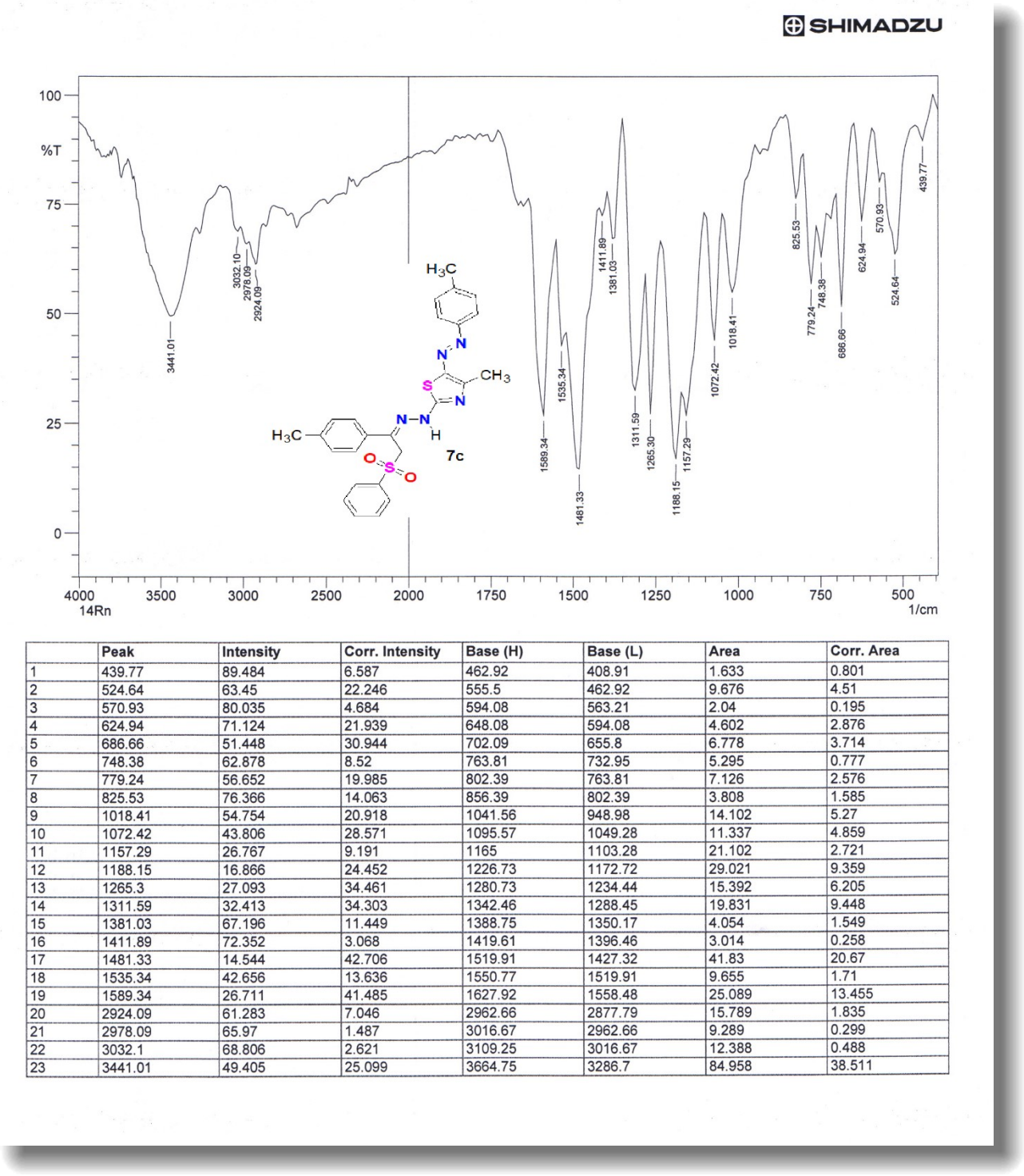
Line#:1 R.Time:4.7(Scan#:565)
MassPeaks:390
RawMode:Single 4.7(565) BasePeak:91(259532)
BG Mode:None Group 1 - Event 1



Mass Table
Line#:1 R.Time:4.7(Scan#:565)
MassPeaks:390
RawMode:Single 4.7(565) BasePeak:91(259532)
BG Mode:None Group 1 - Event 1

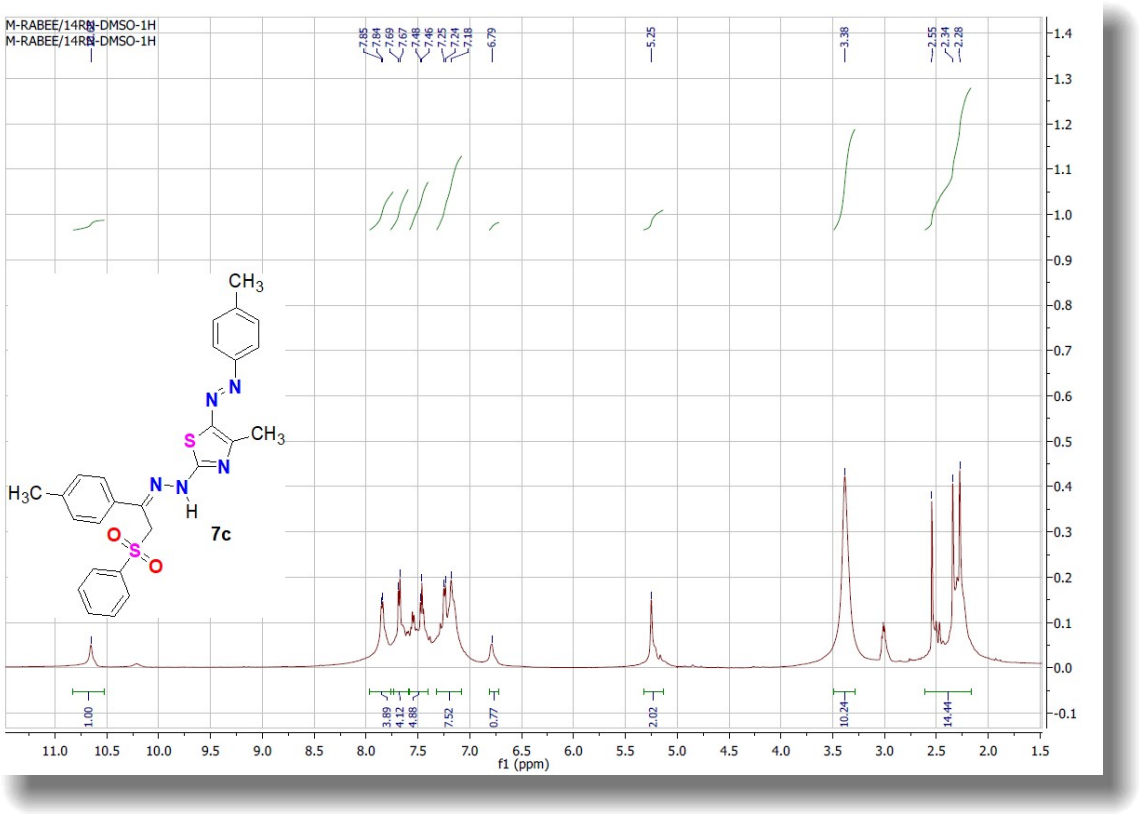
#	m/z	Abs. In	Rel. Int.	#	m/z	Abs. In	Rel. Int.	#	m/z	Abs. In	Rel. Int.
1	50.05	45778	17.64	4	53.05	18517	7.13	7	56.05	2585	1.00
2	51.05	110597	42.61	5	54.00	5710	2.20	8	57.05	5786	2.23
3	52.05	23795	9.17	6	55.05	4677	1.80	9	58.05	7324	2.82

Figure S14. Mass spectrum of compound 7b



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	439.77	89.484	6.587	462.92	408.91	1.633	0.801
2	524.64	63.45	22.246	555.5	462.92	9.676	4.51
3	570.93	80.035	4.684	594.08	563.21	2.04	0.195
4	624.94	71.124	21.939	648.08	594.08	4.602	2.876
5	686.66	51.448	30.944	702.09	655.8	6.778	3.714
6	748.38	62.878	8.52	763.81	732.95	5.295	0.777
7	779.24	56.652	19.985	802.39	763.81	7.126	2.576
8	825.53	76.366	14.063	856.39	802.39	3.808	1.585
9	1018.41	54.754	20.918	1041.56	948.98	14.102	5.27
10	1072.42	43.806	28.571	1095.57	1049.28	11.337	4.859
11	1157.29	26.767	9.191	1165	1103.28	21.102	2.721
12	1188.15	16.866	24.452	1226.73	1172.72	29.021	9.359
13	1265.3	27.093	34.461	1280.73	1234.44	15.392	6.205
14	1311.59	32.413	34.303	1342.46	1288.45	19.831	9.448
15	1381.03	67.196	11.449	1388.75	1350.17	4.054	1.549
16	1411.89	72.352	3.068	1419.61	1396.46	3.014	0.258
17	1481.33	14.544	42.706	1519.91	1427.32	41.83	20.67
18	1535.34	42.656	13.636	1550.77	1519.91	9.655	1.71
19	1589.34	26.711	41.485	1627.92	1558.48	25.089	13.455
20	2924.09	61.283	7.046	2962.66	2877.79	15.789	1.835
21	2978.09	65.97	1.487	3016.67	2962.66	9.289	0.299
22	3032.1	68.806	2.621	3109.25	3016.67	12.388	0.488
23	3441.01	49.405	25.099	3664.75	3286.7	84.958	38.511

Figure S15. IR spectrum of compound 7b



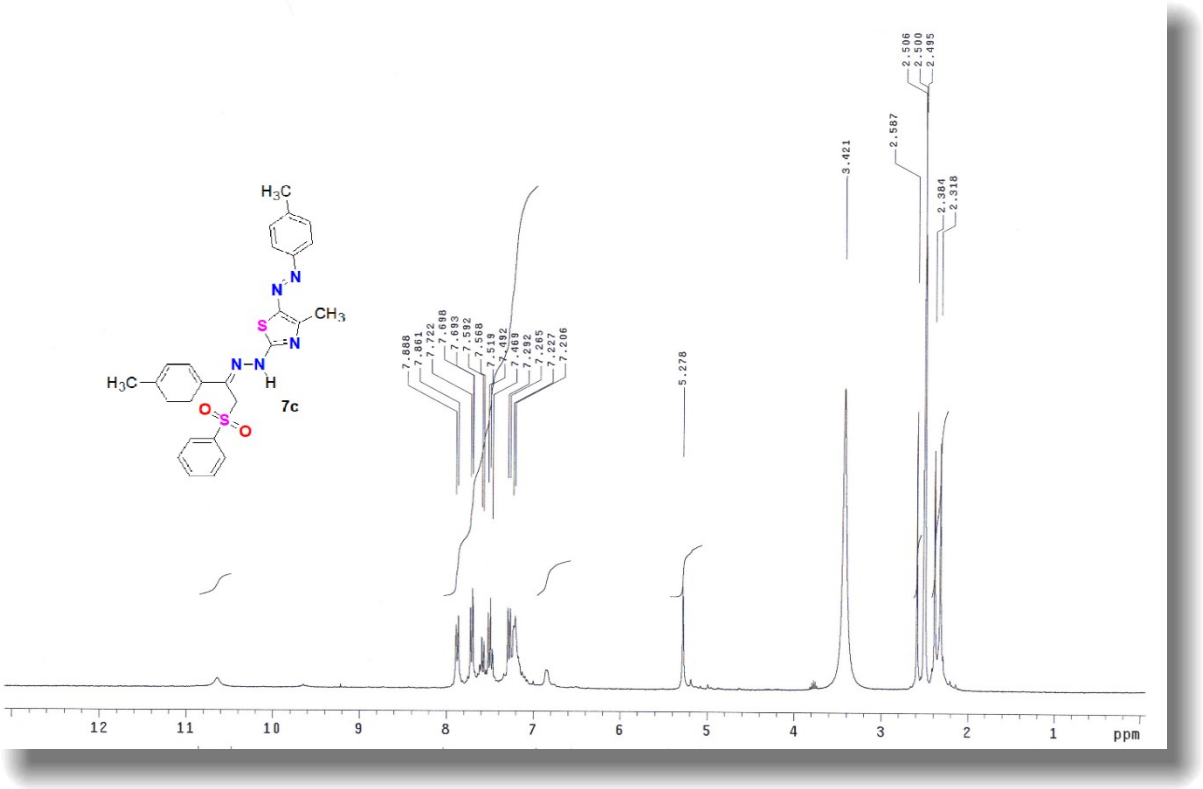


Figure S16. 1H-NMR spectrum of compound 7c

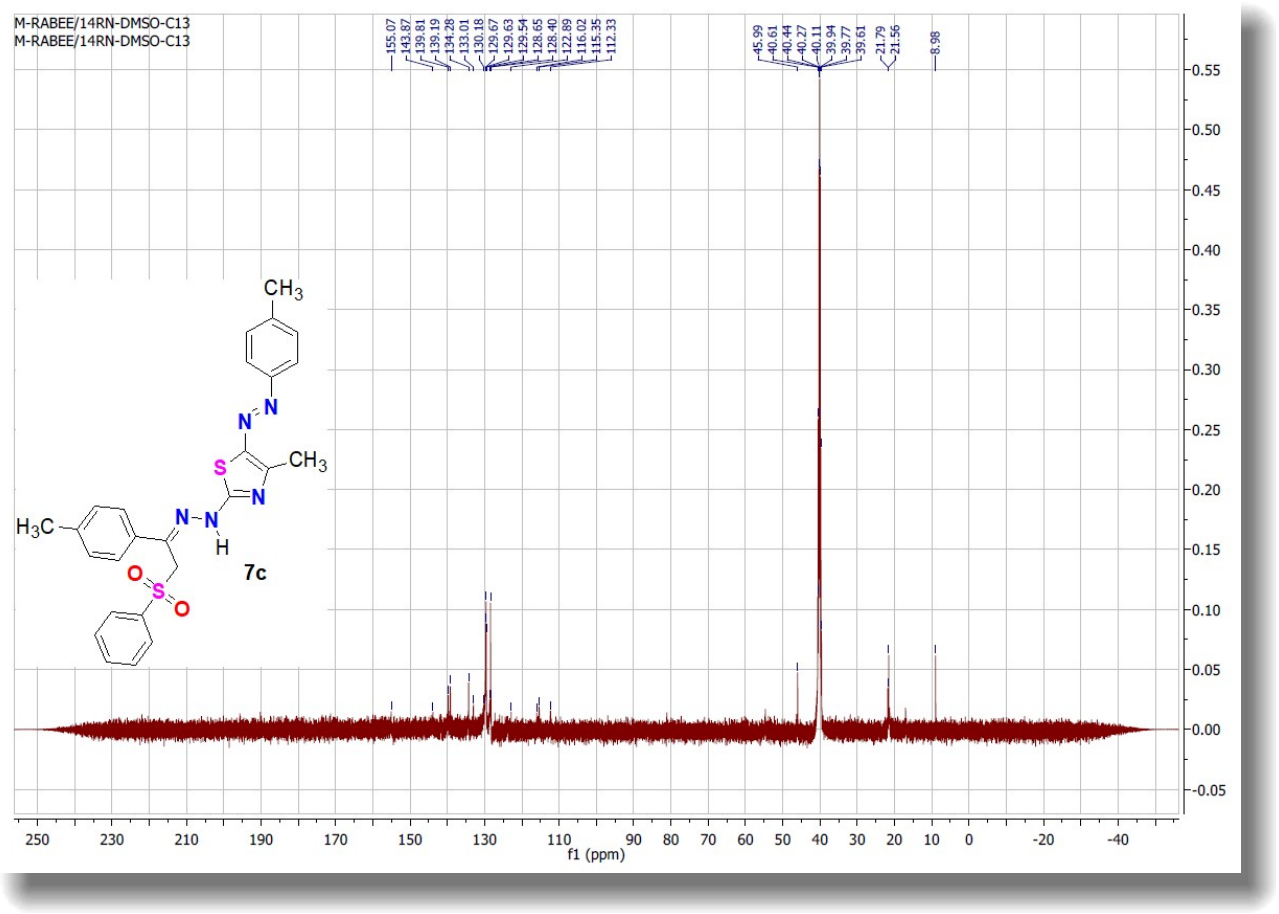


Figure S17. 13C-NMR spectrum of compound 7c

Cairo University
Micro Analytical Center

DI Analysis
Shimadzu Qp-2010 Plus

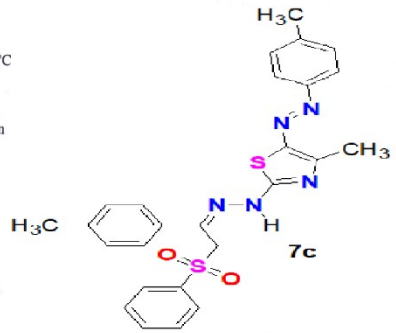
Sample Information

Analyzed by	: Dr. Mai Younis
Analyzed	: 02/01/2007 11:23:59
Sample Name	: Rn14
Sample ID	:
Customer Name	: Dr. Hoda Kamel - Science - Cairo
Data File	: C:\GCMSsolution\Data\Project1\Rn14.QGD
Org Data File	: C:\GCMSsolution\Data\Project1\Rn14.QGD
Method File	: C:\GCMSsolution\Data\Project1\High Temperature Op
Org Method File	: C:\GCMSsolution\Data\Project1\High Temperature Op
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1_default.qgt
$EndIf$Modified by	: Dr. Mai Younis
Modified	: 02/01/2007 11:28:36

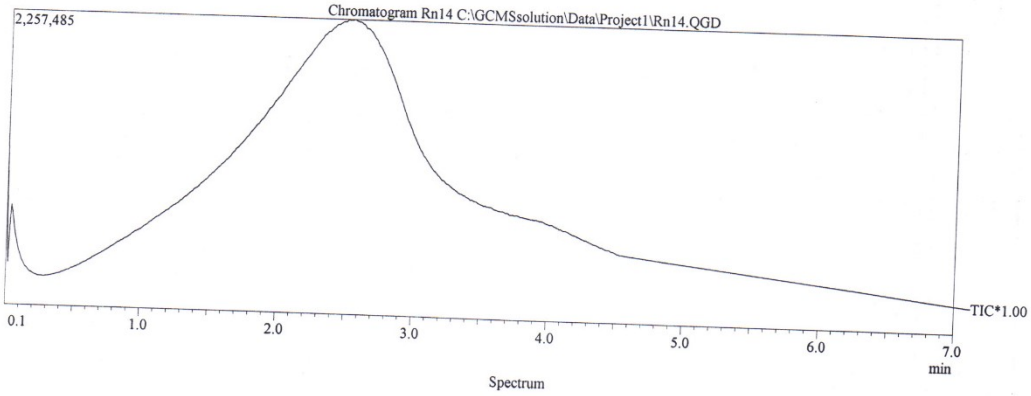
Method

====== Analytical Line 1 ======

IonSourceTemp	:250.00 °C
[MS Table]	
--Group 1 - Event 1--	
Start Time	:0.00min
End Time	:10.00min
ACQ Mode	:Scan
Event Time	:0.50sec
Scan Speed	:1250
Start m/z	:50.00
End m/z	:600.00
Electron Voltage	: 70 eV
Ionization Mode	: EI



C:\GCMSsolution\Data\Project1\Rn14.QGD



Spectrum

Line#:1 R.Time:2.5(Scan#:304)
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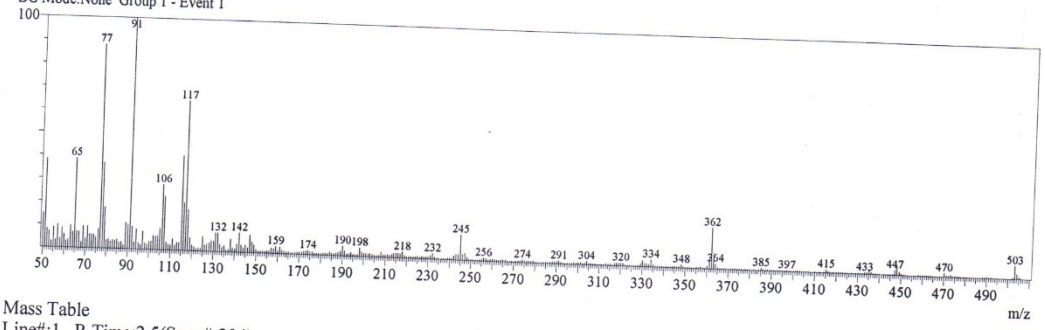
RawMode:Single 2.5(304) BasePeak:91(190426)
BG Mode:None Group 1 - Event 1

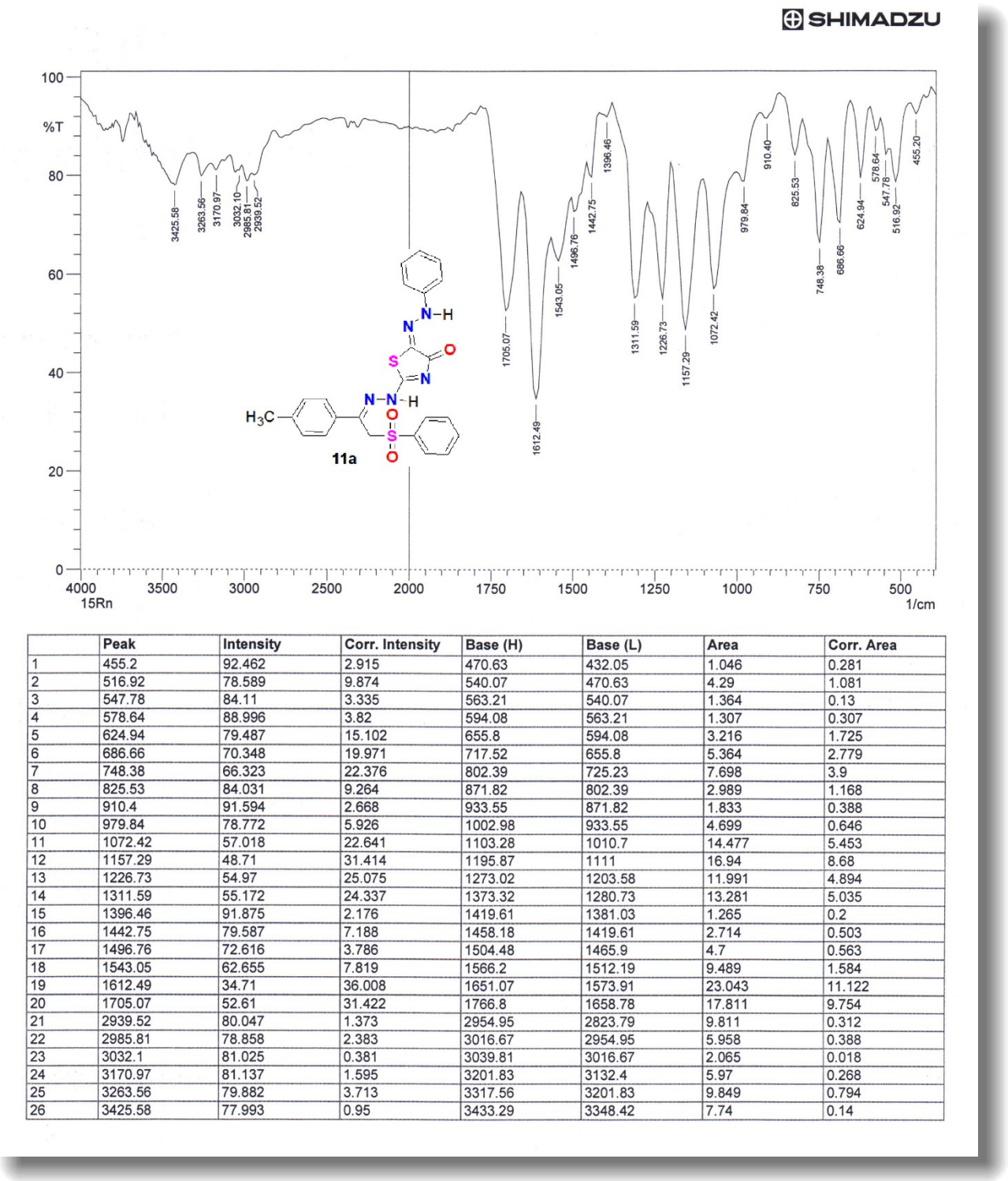


Mass Table

Line#:1 R.Time:2.5(Scan#:304)
MassPeaks:418
RawMode:Single 2.5(304) BasePeak:91(190426)
BG Mode:None Group 1 - Event 1

#	m/z	Abs. In	Rel. Int.	#	m/z	Abs. In	Rel. Int.	#	m/z	Abs. In	Rel. Int.
1	50.05	28087	14.75	4	53.05	13490	7.08	7	56.10	6159	3.23
2	51.05	72364	38.00	5	54.10	5162	2.71	8	57.05	18703	9.82
3	52.05	14858	7.80	6	55.05	16437	8.63	9	58.05	7291	3.83

Figure S18. Mass spectrum of compound 7c



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	455.2	92.462	2.915	470.63	432.05	1.046	0.281
2	516.92	78.589	9.874	540.07	470.63	4.29	1.081
3	547.78	84.11	3.335	563.21	540.07	1.364	0.13
4	578.64	88.996	3.82	594.08	563.21	1.307	0.307
5	624.94	79.487	15.102	655.8	594.08	3.216	1.725
6	686.66	70.348	19.971	717.52	655.8	5.364	2.779
7	748.38	66.323	22.376	802.39	725.23	7.698	3.9
8	825.53	84.031	9.264	871.82	802.39	2.989	1.168
9	910.4	91.594	2.668	933.55	871.82	1.833	0.388
10	979.84	78.772	5.926	1002.98	933.55	4.699	0.646
11	1072.42	57.018	22.641	1103.28	1010.7	14.477	5.453
12	1157.29	48.71	31.414	1195.87	1111	16.94	8.68
13	1226.73	54.97	25.075	1273.02	1203.58	11.991	4.894
14	1311.59	55.172	24.337	1373.32	1280.73	13.281	5.035
15	1396.46	91.875	2.176	1419.61	1381.03	1.265	0.2
16	1442.75	79.587	7.188	1458.18	1419.61	2.714	0.503
17	1496.76	72.616	3.786	1504.48	1465.9	4.7	0.563
18	1543.05	62.655	7.819	1566.2	1512.19	9.489	1.584
19	1612.49	34.71	36.008	1651.07	1573.91	23.043	11.122
20	1705.07	52.61	31.422	1766.8	1658.78	17.811	9.754
21	2939.52	80.047	1.373	2954.95	2823.79	9.811	0.312
22	2985.81	78.858	2.383	3016.67	2954.95	5.958	0.388
23	3032.1	81.025	0.381	3039.81	3016.67	2.065	0.018
24	3170.97	81.137	1.595	3201.83	3132.4	5.97	0.268
25	3263.56	79.882	3.713	3317.56	3201.83	9.849	0.794
26	3425.58	77.993	0.95	3433.29	3348.42	7.74	0.14

Figure S19. IR spectrum of compound 11a

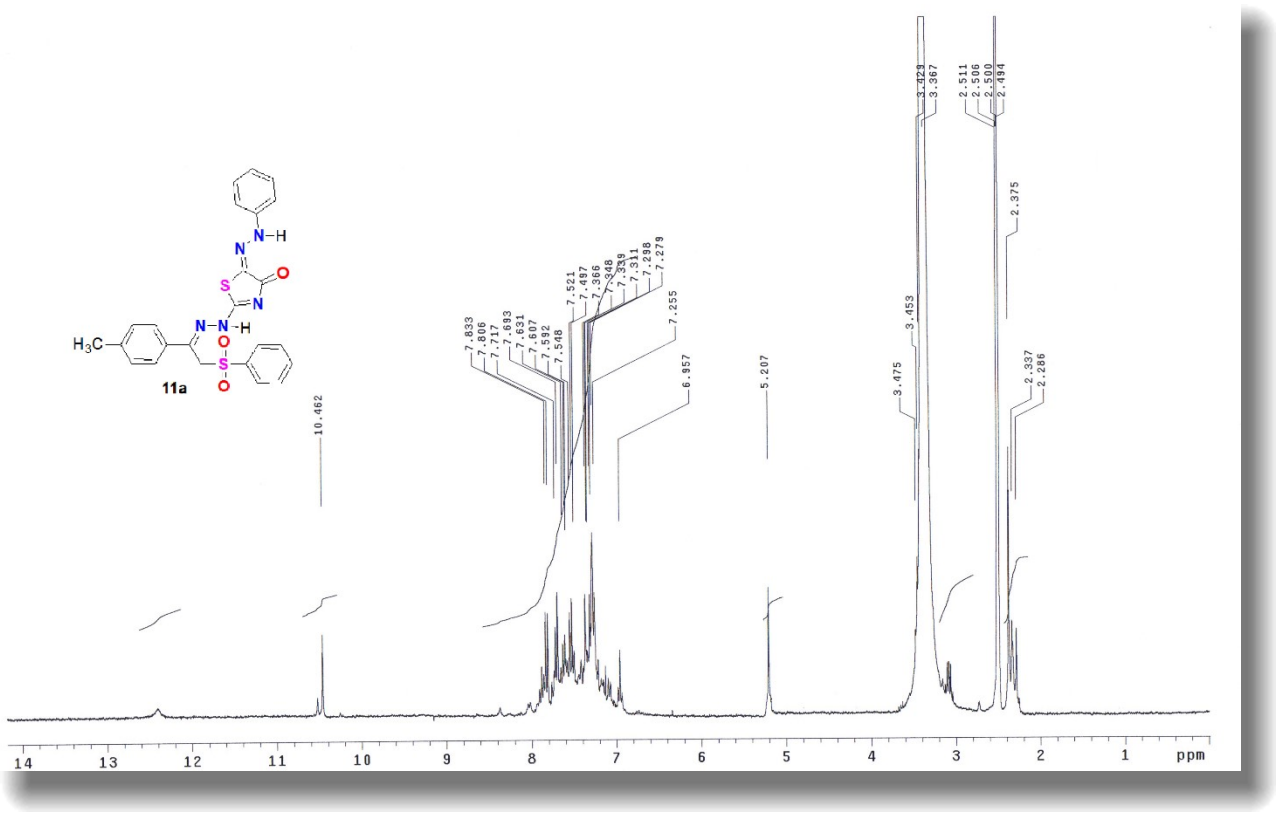


Figure S20. 1H-NMR spectrum of compound 11a

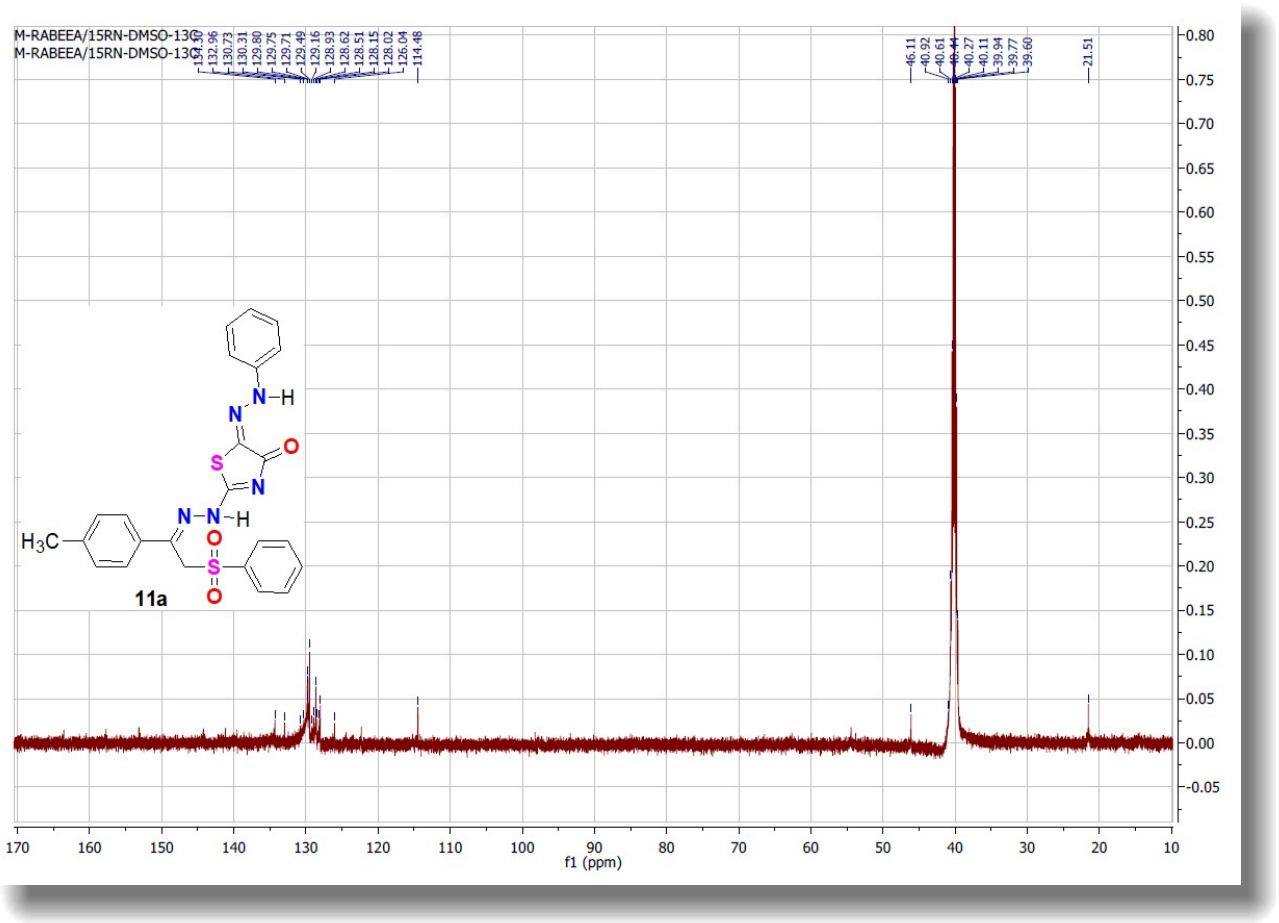
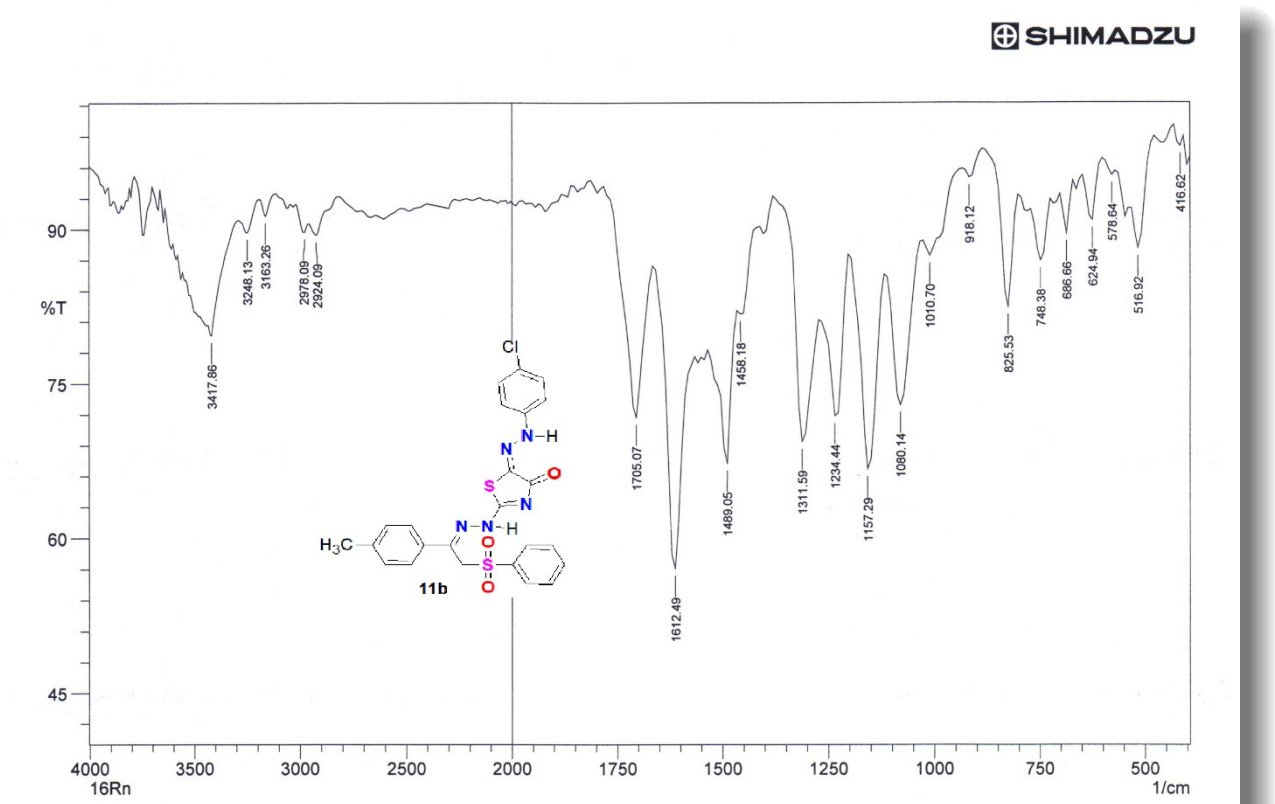


Figure S21. 13C-NMR spectrum of compound 11a



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	416.62	98.04	1.337	432.05	408.91	0.135	0.09
2	516.92	88.151	6.505	540.07	478.35	1.991	0.746
3	578.64	95.239	0.722	601.79	570.93	0.551	0.039
4	624.94	90.862	5.184	648.08	601.79	1.319	0.507
5	686.66	89.562	4.544	702.09	671.23	1.125	0.31
6	748.38	86.946	5.512	771.53	725.23	2.254	0.678
7	825.53	82.446	12.133	887.26	802.39	3.169	1.494
8	918.12	95.012	1.518	933.55	887.26	0.802	0.156
9	1010.7	87.427	2.657	1026.13	933.55	3.665	0.462
10	1080.14	72.941	13.773	1111	1033.85	7.551	2.967
11	1157.29	66.694	19.692	1195.87	1118.71	9.09	4.156
12	1234.44	71.866	12.428	1265.3	1203.58	6.623	2.154
13	1311.59	69.336	16.382	1373.32	1273.02	9.241	3.052
14	1458.18	81.754	1.516	1465.9	1411.89	3.549	0.036
15	1489.05	67.261	13.047	1527.62	1465.9	7.941	1.951
16	1612.49	57.039	24.8	1658.78	1566.2	13.963	5.678
17	1705.07	71.724	17.339	1782.23	1666.5	9.617	4.33
18	2924.09	89.454	1.841	2962.66	2823.79	5.562	0.453
19	2978.09	89.753	1.341	3016.67	2962.66	2.309	0.224
20	3163.26	91.299	1.89	3194.12	3116.97	2.61	0.265
21	3248.13	89.726	2.127	3294.42	3194.12	4.111	0.445
22	3417.86	79.669	7.202	3518.16	3294.42	16.701	3.361

Figure S22. IR spectrum of compound 11b

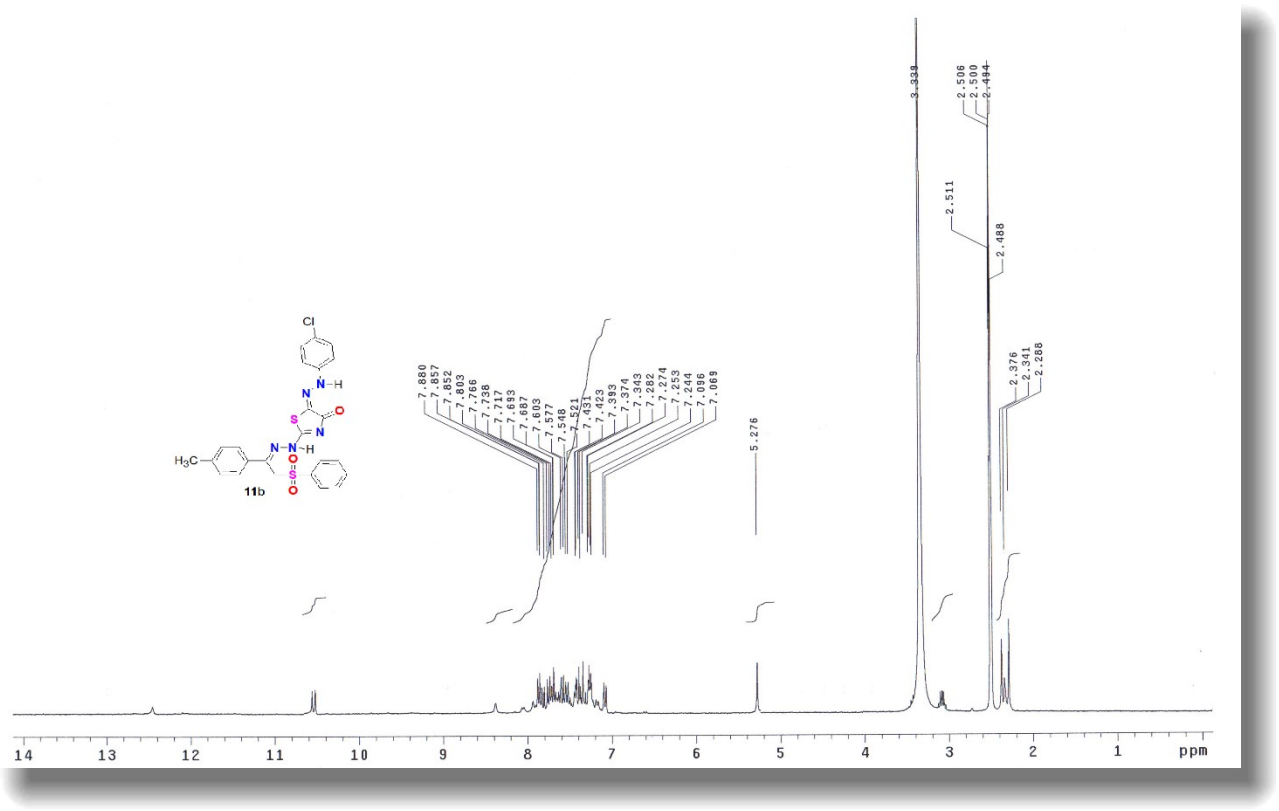
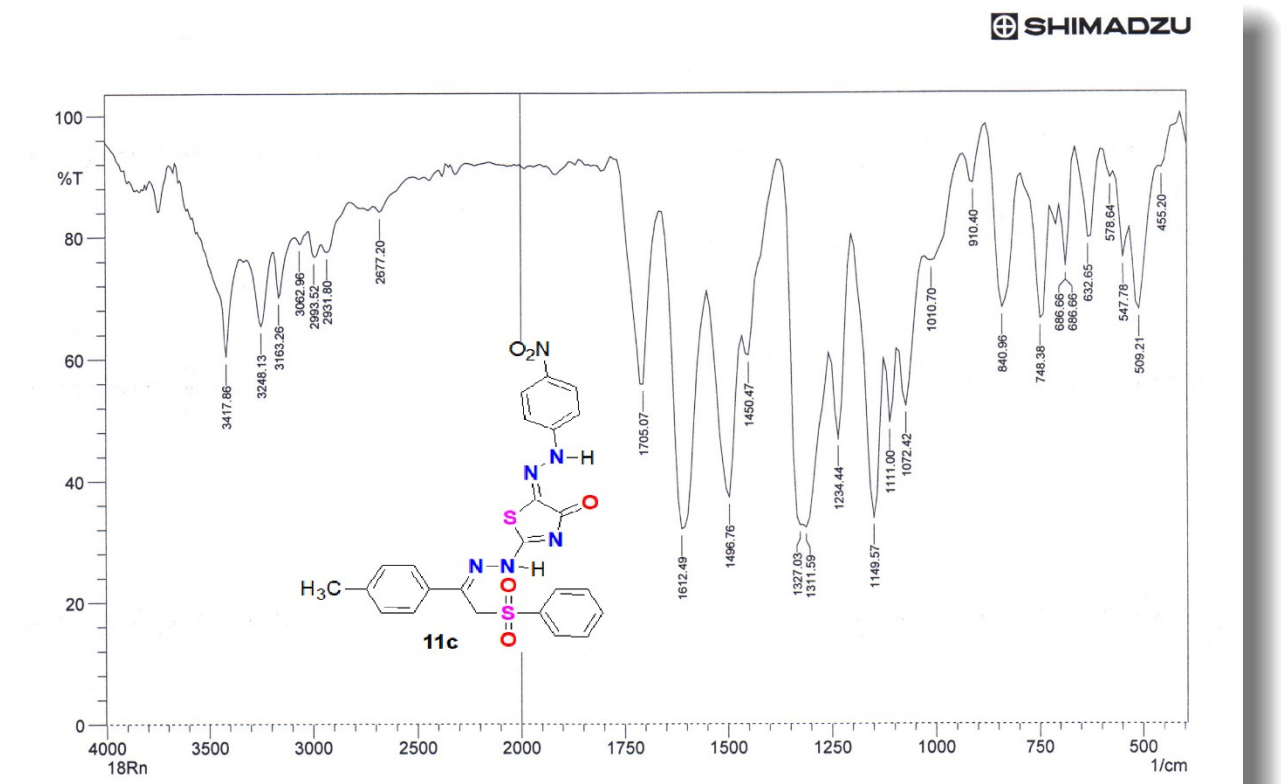
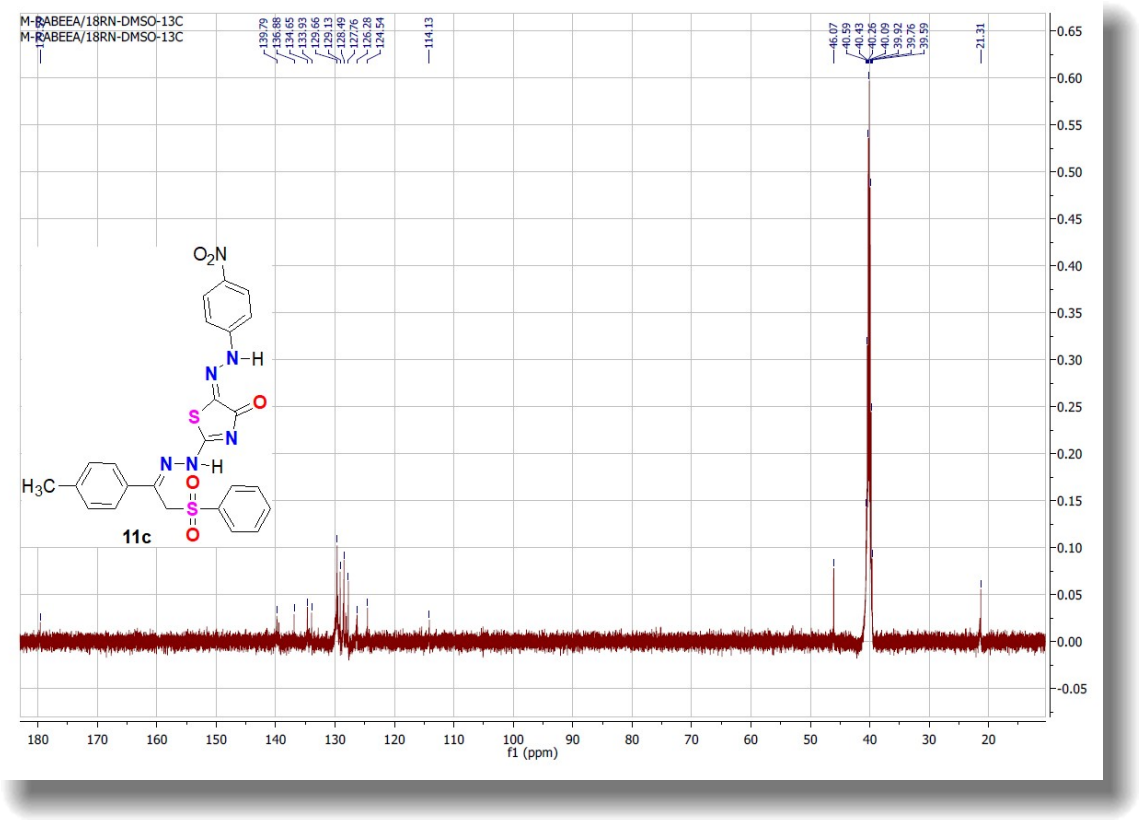


Figure S23. 1H-NMR spectrum of compound 11b



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	455.2	91.118	1.379	462.92	408.91	1.097	0.035
2	509.21	67.833	16.968	532.35	470.63	6.768	2.852
3	547.78	76.36	8.577	570.93	532.35	3.233	0.652
4	578.64	89.441	1.833	601.79	570.93	1.17	0.08
5	632.65	79.641	14.61	663.51	601.79	3.709	2.122
6	686.66	74.897	13.808	702.09	663.51	2.999	1.146
7	686.66	74.897	13.808	702.09	663.51	2.999	1.146
8	748.38	66.407	20.086	794.67	725.23	7.418	3.236
9	840.96	68.198	25.748	879.54	802.39	7.752	5.467
10	910.4	88.691	6.808	933.55	879.54	1.695	0.694
11	1010.7	75.867	3.663	1026.13	941.26	7.104	1.201
12	1072.42	52.256	13.054	1087.85	1033.85	10.941	2.331
13	1111	49.436	11.318	1126.43	1095.57	7.993	1.313
14	1149.57	33.885	32.148	1195.87	1126.43	20.731	8.861
15	1234.44	46.818	19.302	1249.87	1203.58	10.111	3.12
16	1311.59	32.273	43.335	1373.32	1257.59	37.763	22.655
17	1327.03	32.682	7.516	1381.03	1319.31	14.666	-1.342
18	1450.47	60.514	8.398	1465.9	1381.03	9.98	1.001
19	1496.76	37.242	26.647	1543.05	1473.62	21.865	8.657
20	1612.49	32.058	46.373	1658.78	1550.77	31.521	19.122
21	1705.07	55.87	31.341	1774.51	1666.5	14.446	8.484
22	2677.2	84.052	1.732	2708.06	2515.18	11.57	0.276
23	2931.8	77.441	2.497	2954.95	2823.79	11.8	0.578
24	2993.52	76.707	3.219	3024.38	2954.95	7.39	0.584
25	3062.96	78.824	1.457	3086.11	3024.38	6.082	0.235
26	3163.26	70.065	7.979	3186.4	3086.11	12.095	1.655
27	3248.13	65.395	11.527	3317.56	3186.4	18.782	3.809
28	3417.86	60.322	17.575	3556.74	3348.42	28.901	7.131

Figure S24. IR spectrum of compound 11c



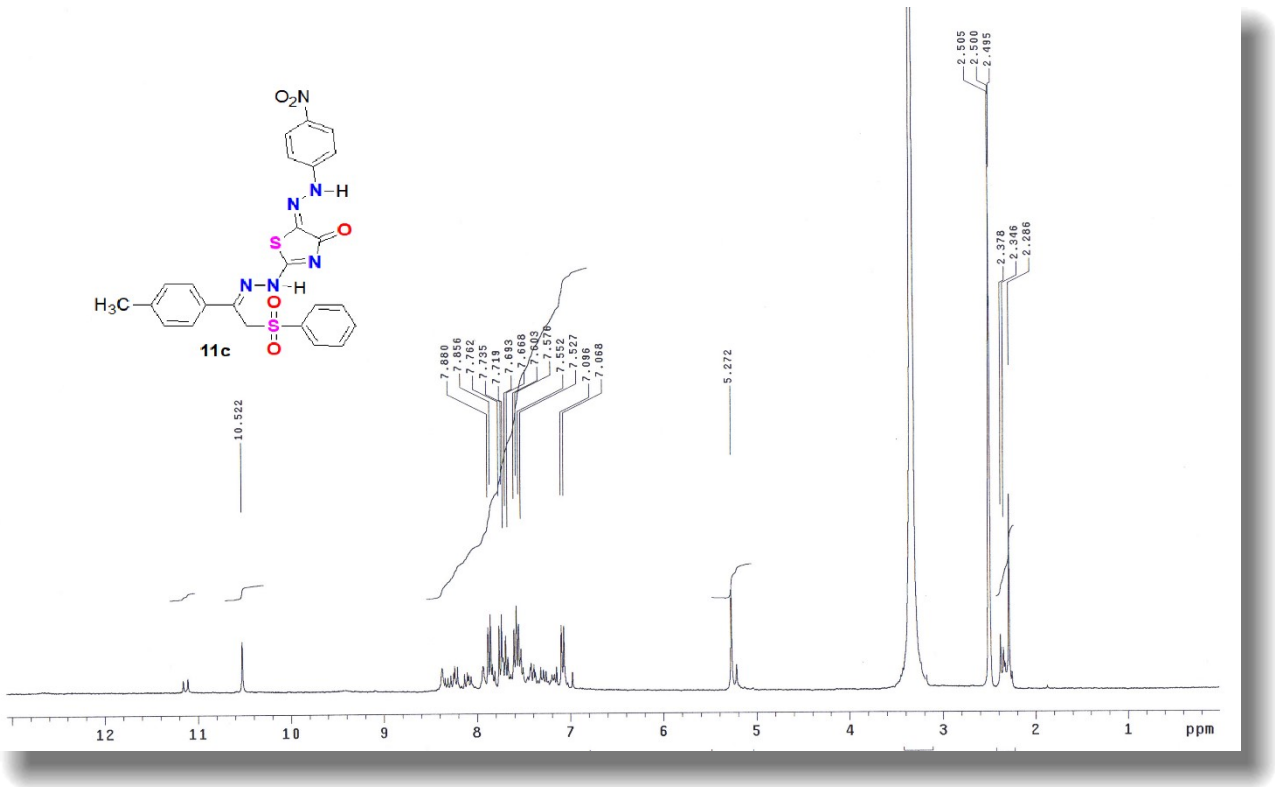


Figure S25. 1H-NMR spectrum of compound 11c

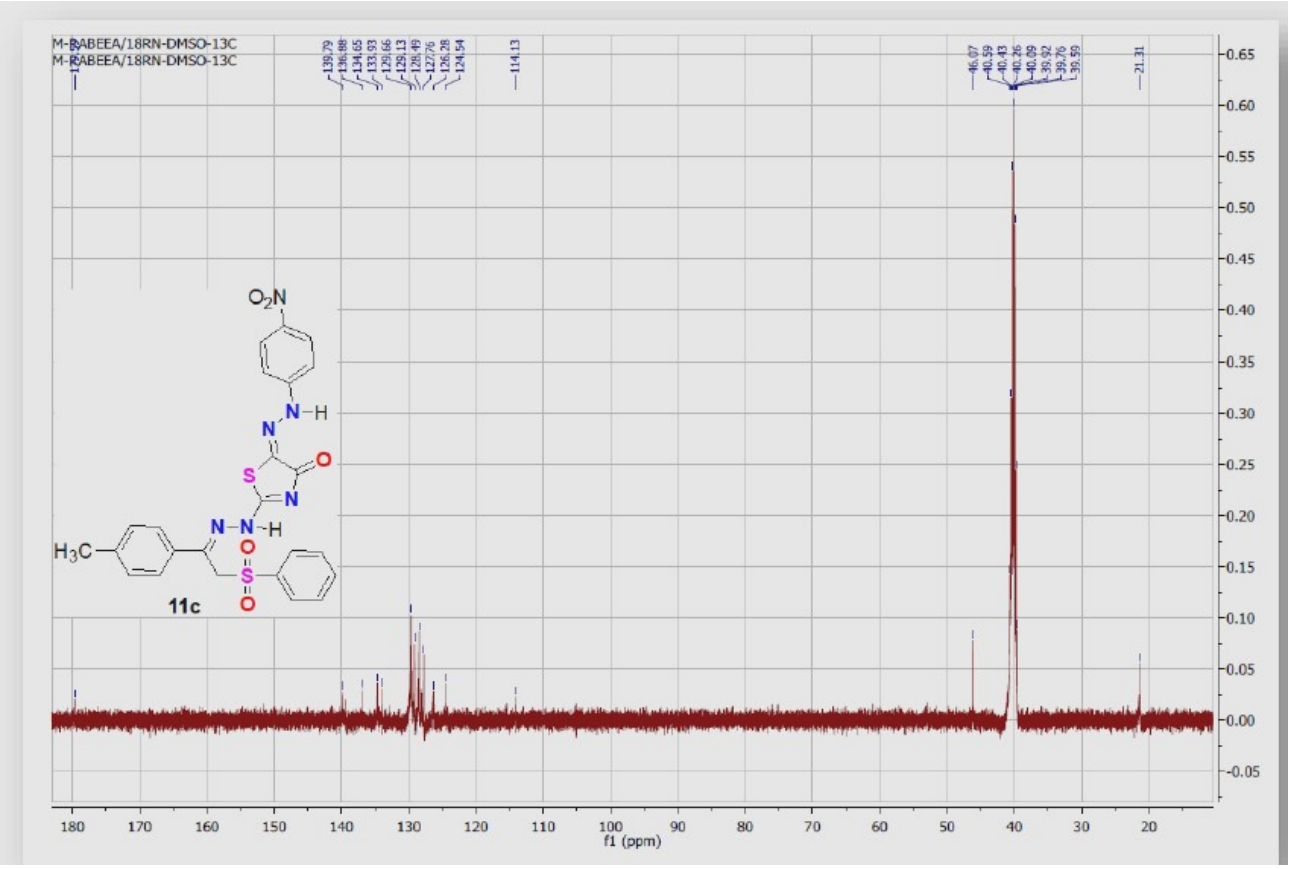
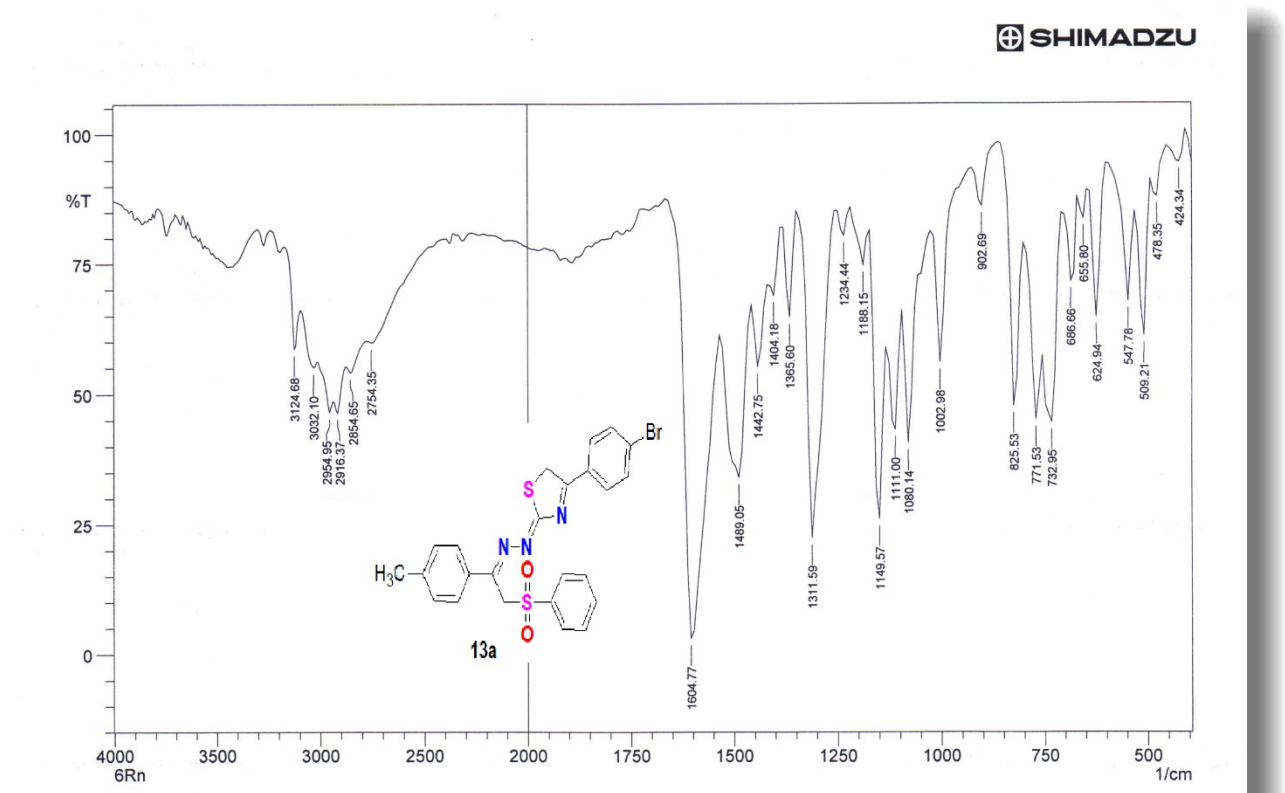


Figure S26. 13C-NMR spectrum of compound 11c



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	424.34	94.453	5.306	455.2	408.91	0.754	0.591
2	478.35	87.841	5.842	493.78	455.2	1.397	0.408
3	509.21	61.043	27.607	532.35	493.78	4.878	2.732
4	547.78	67.693	19.556	594.08	532.35	5.124	1.955
5	624.94	64.651	26.244	640.37	601.79	3.998	2.593
6	655.8	83.658	5.08	671.23	648.08	1.539	0.313
7	686.66	71.512	15.096	709.8	671.23	3.944	1.473
8	732.95	44.565	26.434	756.1	709.8	11.36	4.747
9	771.53	45.083	19.396	802.39	756.1	10.321	2.337
10	825.53	47.678	39.452	856.39	802.39	8.336	4.972
11	902.69	86.018	9.137	925.83	864.11	1.991	0.83
12	1002.98	55.961	26.538	1018.41	933.55	8.017	2.957
13	1080.14	40.671	28.753	1095.57	1026.13	13.215	4.184
14	1111	43.158	19.963	1134.14	1095.57	10.958	3.026
15	1149.57	26.054	41.768	1172.72	1134.14	14.342	7.618
16	1188.15	74.669	8.258	1219.01	1172.72	4.535	0.942
17	1234.44	80.405	5.13	1249.87	1219.01	2.506	0.412
18	1311.59	22.467	62.693	1350.17	1249.87	25.28	18.285
19	1365.6	64.622	18.96	1381.03	1350.17	4.108	1.702
20	1404.18	68.855	6.471	1419.61	1381.03	4.983	0.438
21	1442.75	55.208	13.337	1458.18	1419.61	7.98	1.741
22	1489.05	34.106	30.576	1535.34	1458.18	26.013	11.079
23	1604.77	3.095	72.066	1666.5	1535.34	63.676	45.912
24	2754.35	59.775	1.735	2777.5	2468.88	49.957	-0.45
25	2854.65	54.09	2.302	2877.79	2777.5	24.615	0.626
26	2916.37	46.459	4.651	2939.52	2877.79	18.635	1.02
27	2954.95	46.552	3.572	3016.67	2939.52	22.218	0.468
28	3032.1	55.067	3.077	3093.82	3016.67	17.66	1.055
29	3124.68	58.666	12.338	3170.97	3093.82	12.854	1.832

Figure S27. IR spectrum of compound 13a

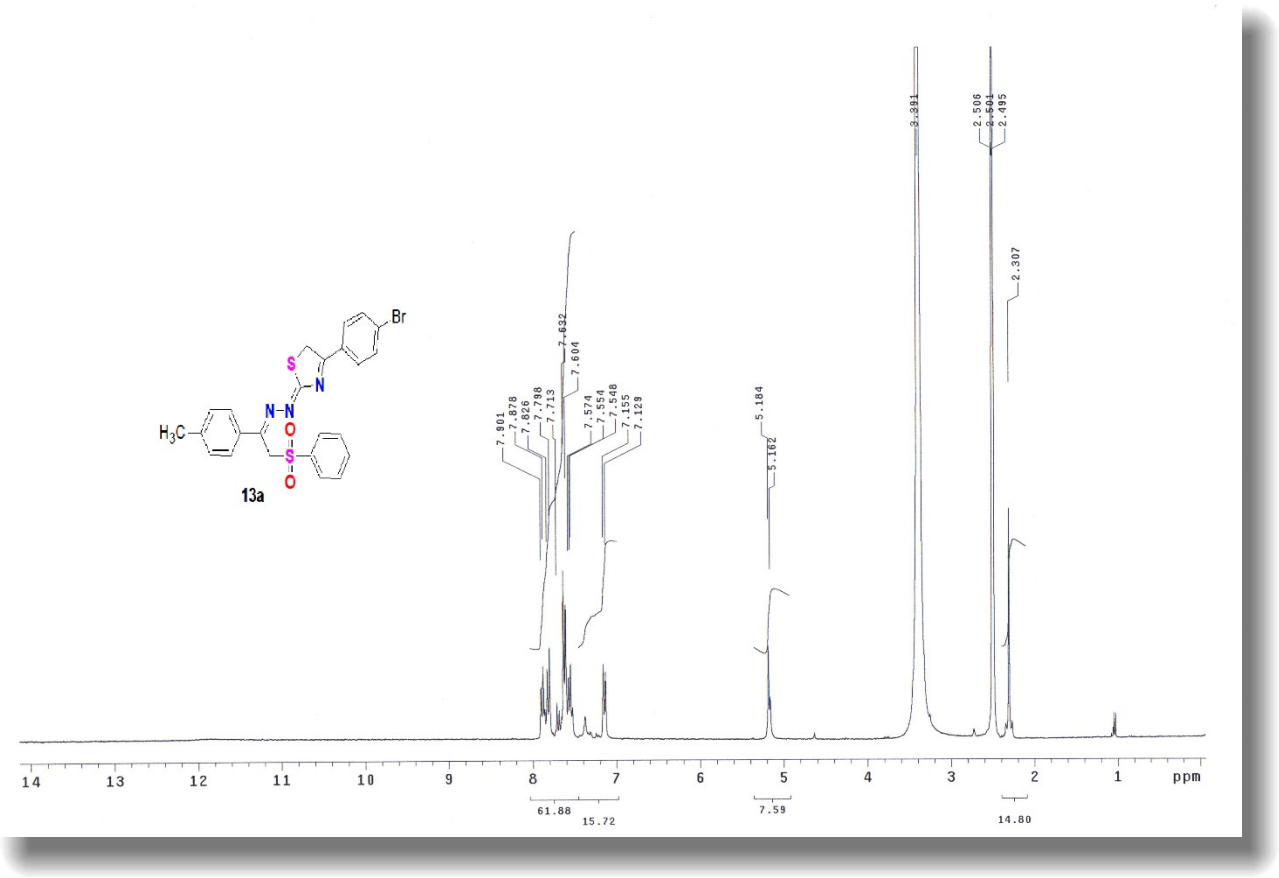


Figure S28. 1H-NMR spectrum of compound 13a

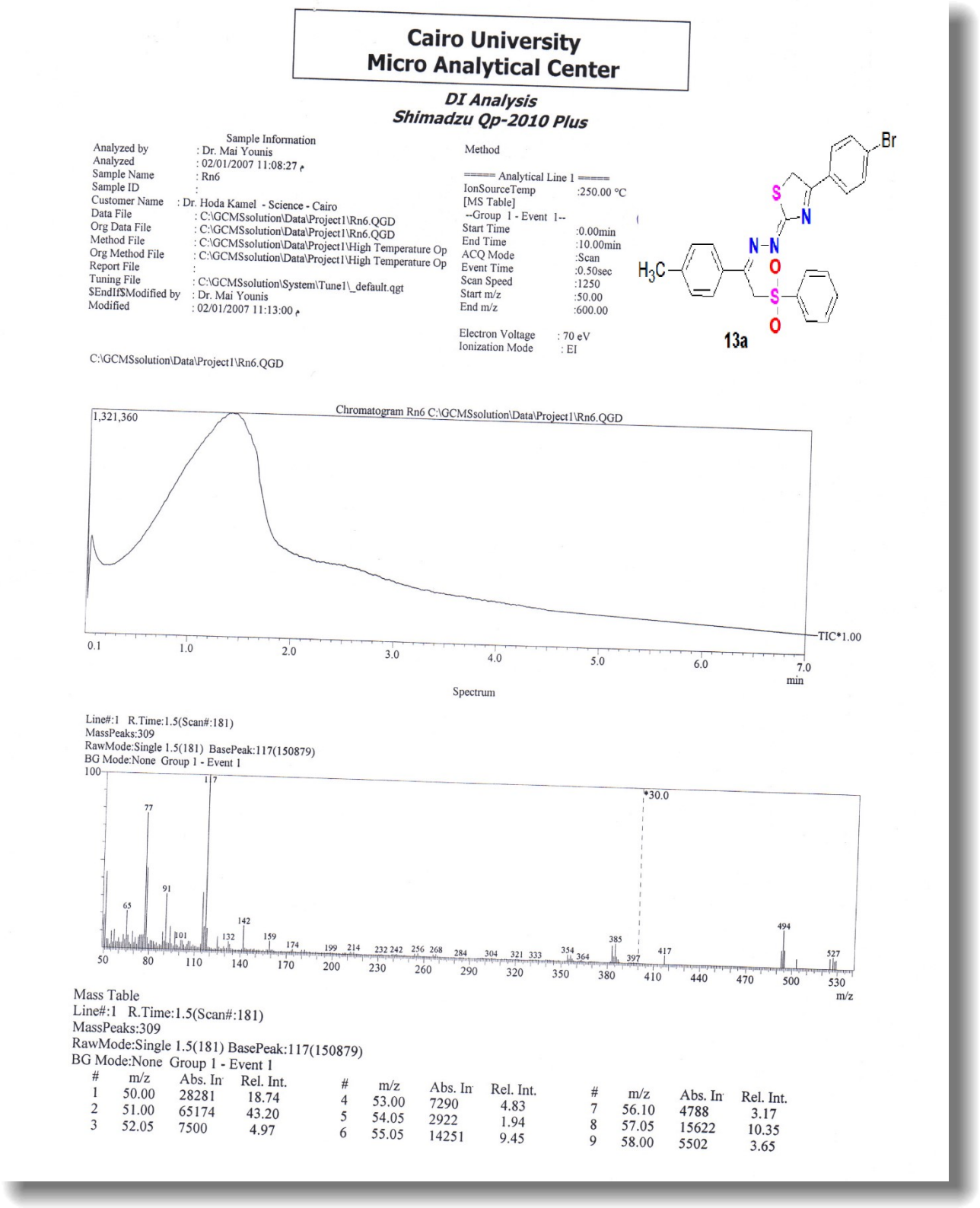


Figure S29. Mass spectrum of compound 13a

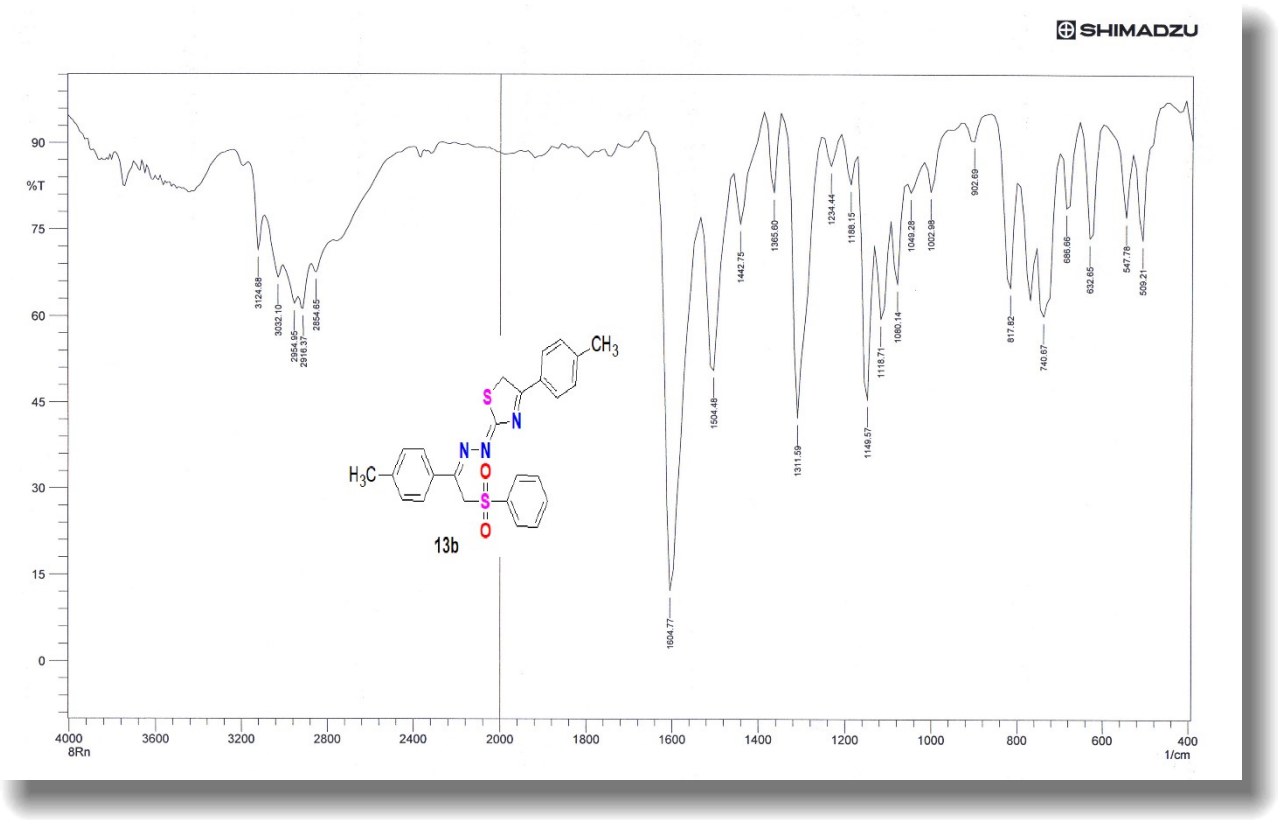


Figure S30. IR spectrum of compound 13b

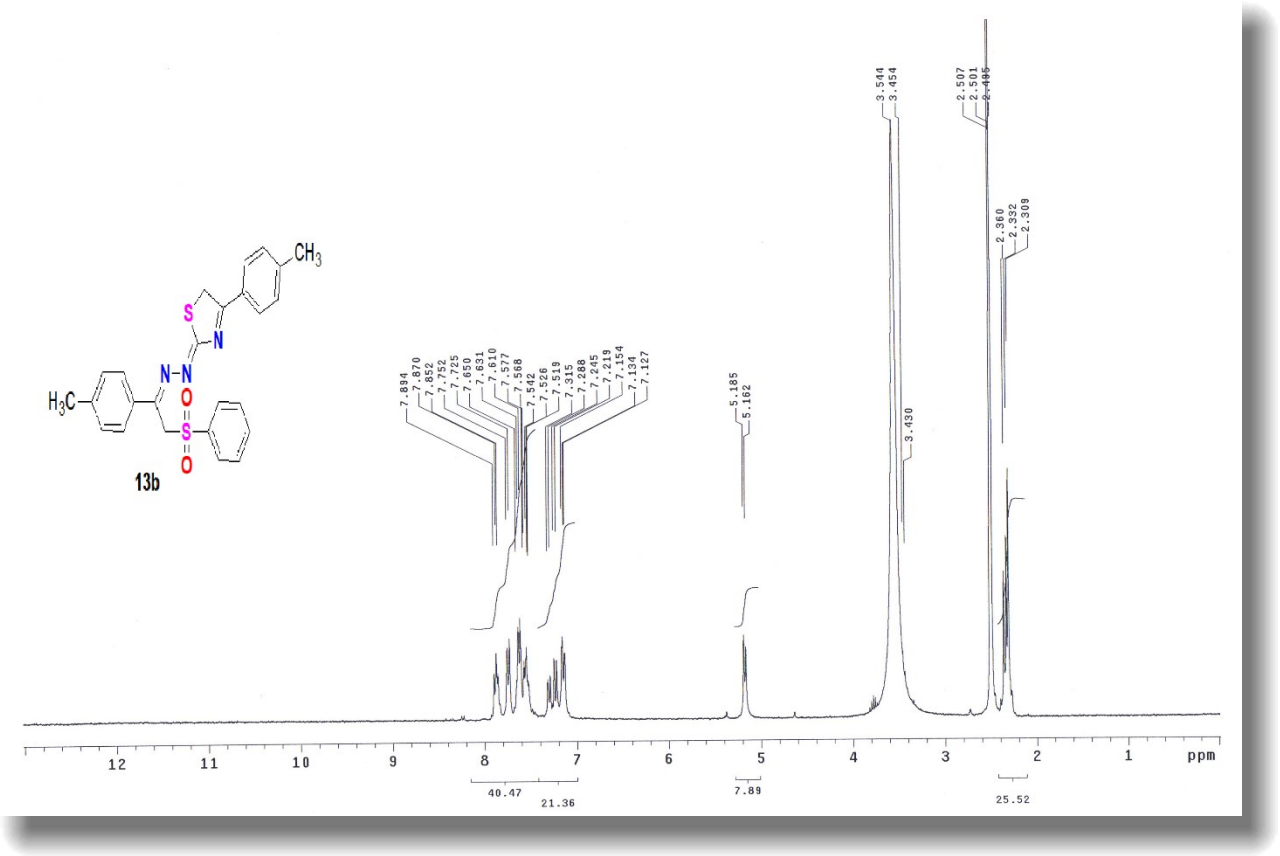


Figure S31. 1H-NMR spectrum of compound 13b

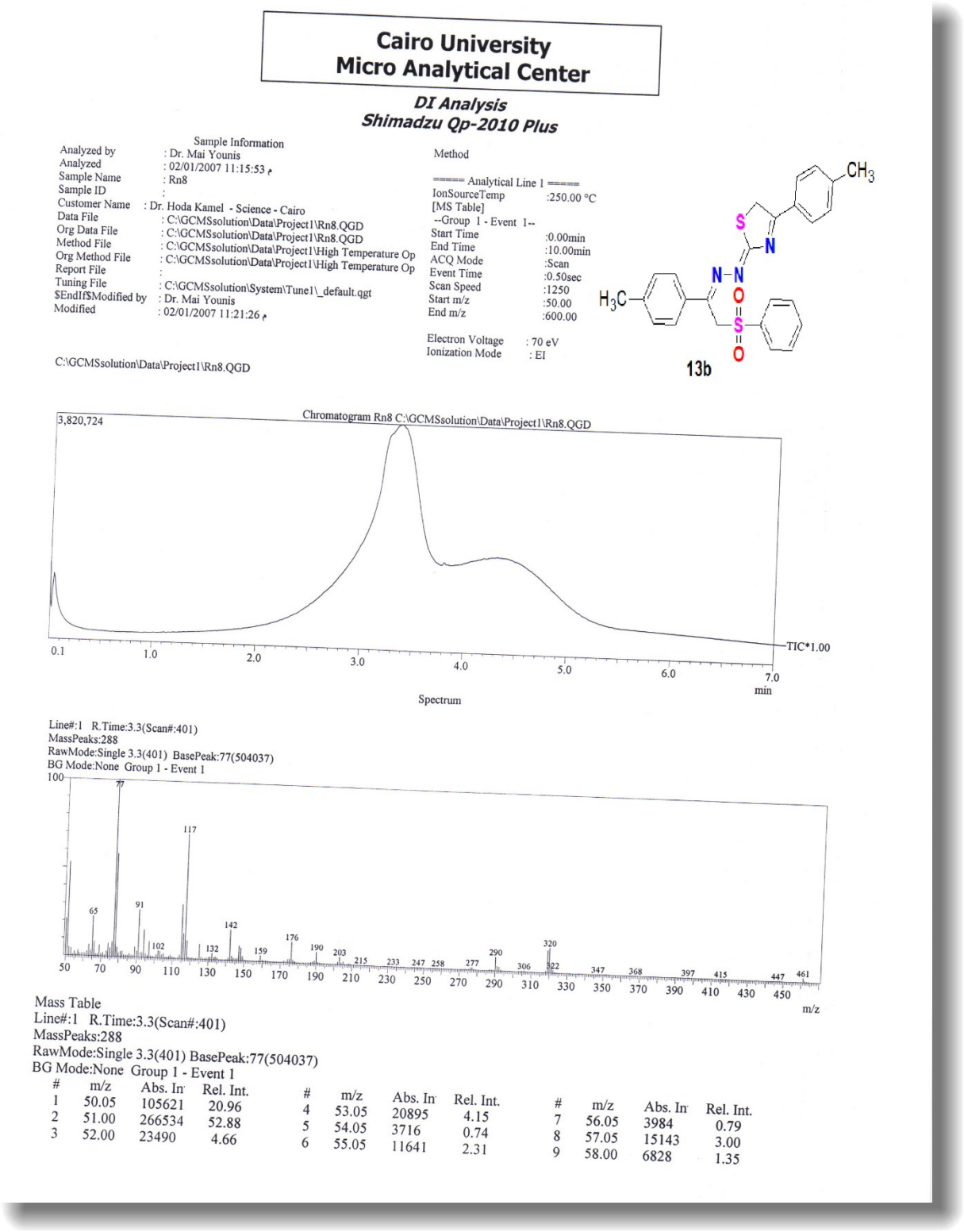


Figure S32. Mass spectrum of compound 13b

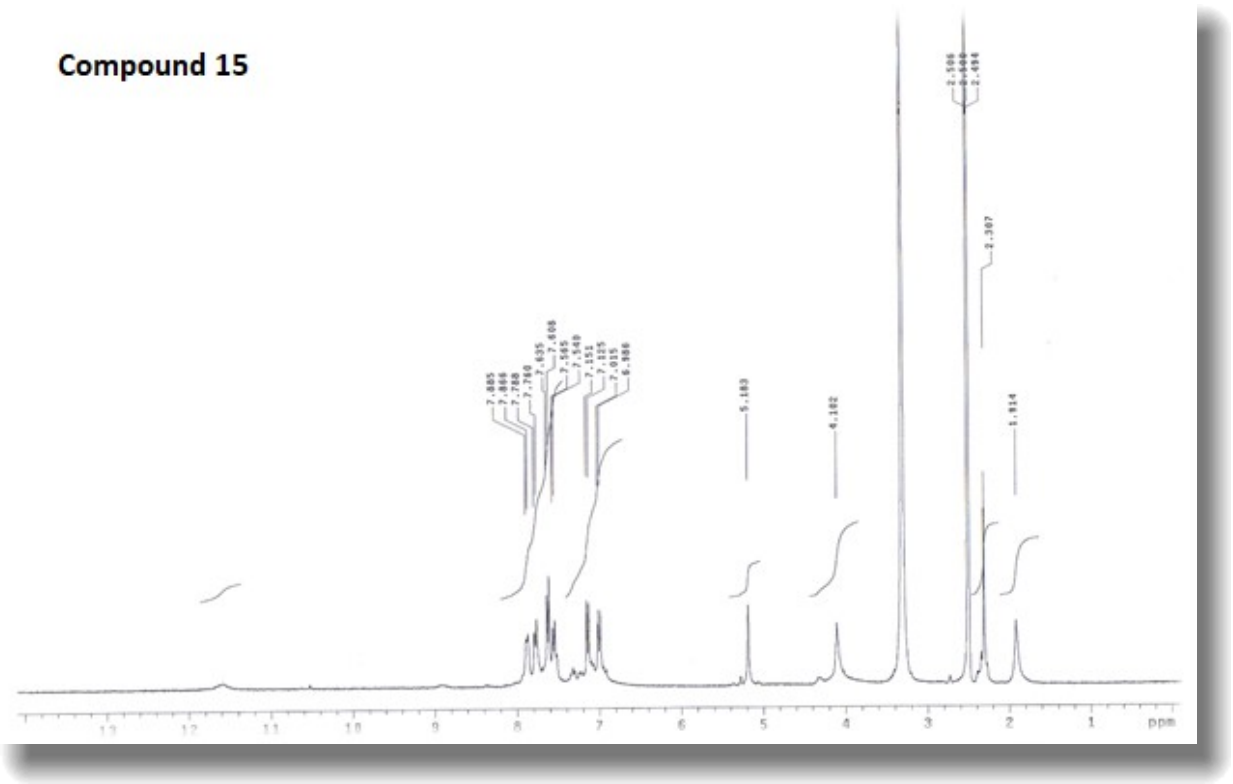


Figure S33. 1H-NMR spectrum of compound 15

Cairo University
Micro Analytical Center

DI Analysis
Shimadzu Qp-2010 Plus

Compound 15

Sample Information

Analyzed by	: Dr. Mai Younis
Analyzed	: 02/01/2007 11:47:42 م
Sample Name	: Rn17
Sample ID	:
Customer Name	: Dr. Hoda Kamel - Science - Cairo
Data File	: C:\GCMSsolution\Data\Project1\Rn17.QGD
Org Data File	: C:\GCMSsolution\Data\Project1\Rn17.QGD
Method File	: C:\GCMSsolution\Data\Project1\High Temperature Op
Org Method File	: C:\GCMSsolution\Data\Project1\High Temperature Op
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1_default.qgt
$End If$Modified by	: Dr. Mai Younis
Modified	: 02/01/2007 11:54:06 م

Method

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End Time	:10.00min
ACQ Mode	:Scan
Event Time	:0.50sec
Scan Speed	:2000
Start m/z	:50.00
End m/z	:1000.00
Electron Voltage	: 70 eV
Ionization Mode	: EI

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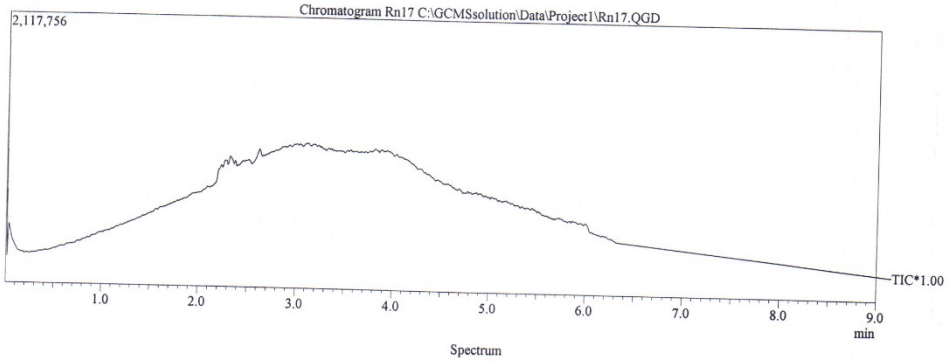

Spectrum

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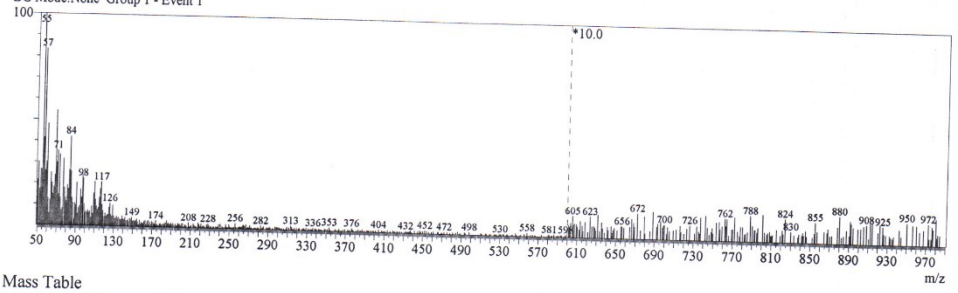
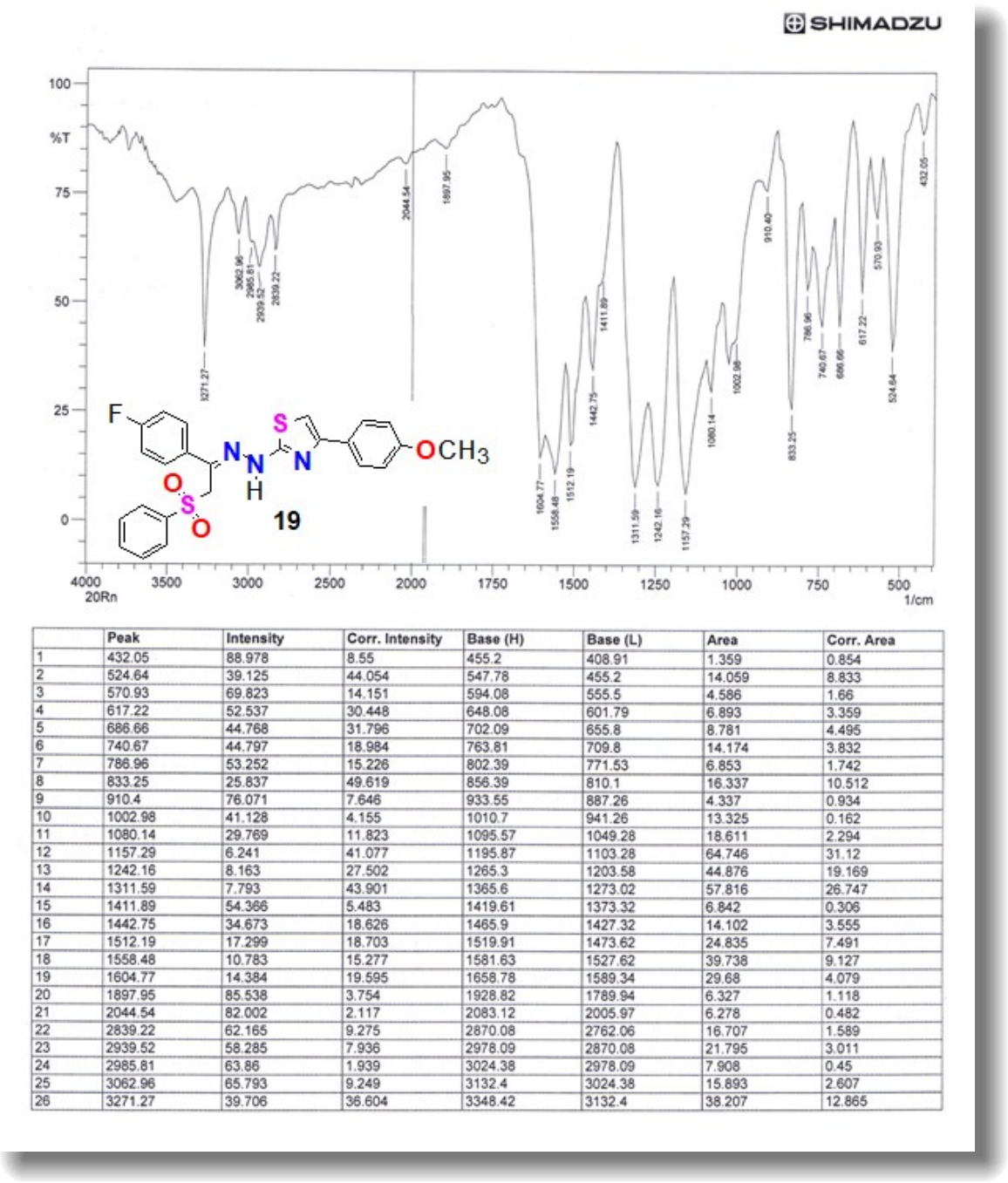
RawMode:Single 4.9(588) BasePeak:55(41954)
BG Mode:None Group 1 - Event 1



Mass Table
Line#:1 R.Time:4.9(Scan#:588)
MassPeaks:558
RawMode:Single 4.9(588) BasePeak:55(41954)
BG Mode:None Group 1 - Event 1

#	m/z	Abs. In	Rel. Int.	#	m/z	Abs. In	Rel. Int.	#	m/z	Abs. In	Rel. Int.
1	50.10	9002	21.46	4	53.10	11094	26.44	7	56.15	17440	41.57
2	51.10	12556	29.93	5	54.15	11064	26.37	8	57.10	34962	83.33
3	52.10	7092	16.90	6	55.10	41954	100.00	9	58.10	10921	26.03

Figure S34. Mass spectrum of compound 15



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	432.05	88.978	8.55	455.2	408.91	1.359	0.854
2	524.64	39.125	44.054	547.78	455.2	14.059	8.833
3	570.93	69.823	14.151	594.08	555.5	4.586	1.66
4	617.22	52.537	30.448	648.08	601.79	6.893	3.359
5	686.66	44.768	31.796	702.09	655.8	8.781	4.495
6	740.67	44.797	18.984	763.81	709.8	14.174	3.832
7	786.96	53.252	15.226	802.39	771.53	6.853	1.742
8	833.25	25.837	49.619	856.39	810.1	16.337	10.512
9	910.4	76.071	7.646	933.55	887.26	4.337	0.934
10	1002.98	41.128	4.155	1010.7	941.26	13.325	0.162
11	1080.14	29.769	11.823	1095.57	1049.28	18.611	2.294
12	1157.29	6.241	41.077	1195.87	1103.28	64.746	31.12
13	1242.16	8.163	27.502	1265.3	1203.58	44.876	19.169
14	1311.59	7.793	43.901	1365.6	1273.02	57.816	26.747
15	1411.89	54.366	5.483	1419.61	1373.32	6.842	0.306
16	1442.75	34.673	18.626	1465.9	1427.32	14.102	3.555
17	1512.19	17.299	18.703	1519.91	1473.62	24.835	7.491
18	1558.48	10.783	15.277	1581.63	1527.62	39.738	9.127
19	1604.77	14.384	19.595	1658.78	1589.34	29.68	4.079
20	1897.95	85.538	3.754	1928.82	1789.94	6.327	1.118
21	2044.54	82.002	2.117	2083.12	2005.97	6.278	0.482
22	2839.22	62.165	9.275	2870.08	2762.06	16.707	1.589
23	2939.52	58.285	7.936	2978.09	2870.08	21.795	3.011
24	2985.81	63.86	1.939	3024.38	2978.09	7.908	0.45
25	3062.96	65.793	9.249	3132.4	3024.38	15.893	2.607
26	3271.27	39.706	36.604	3348.42	3132.4	38.207	12.865

Figure S35. IR spectrum of compound 19

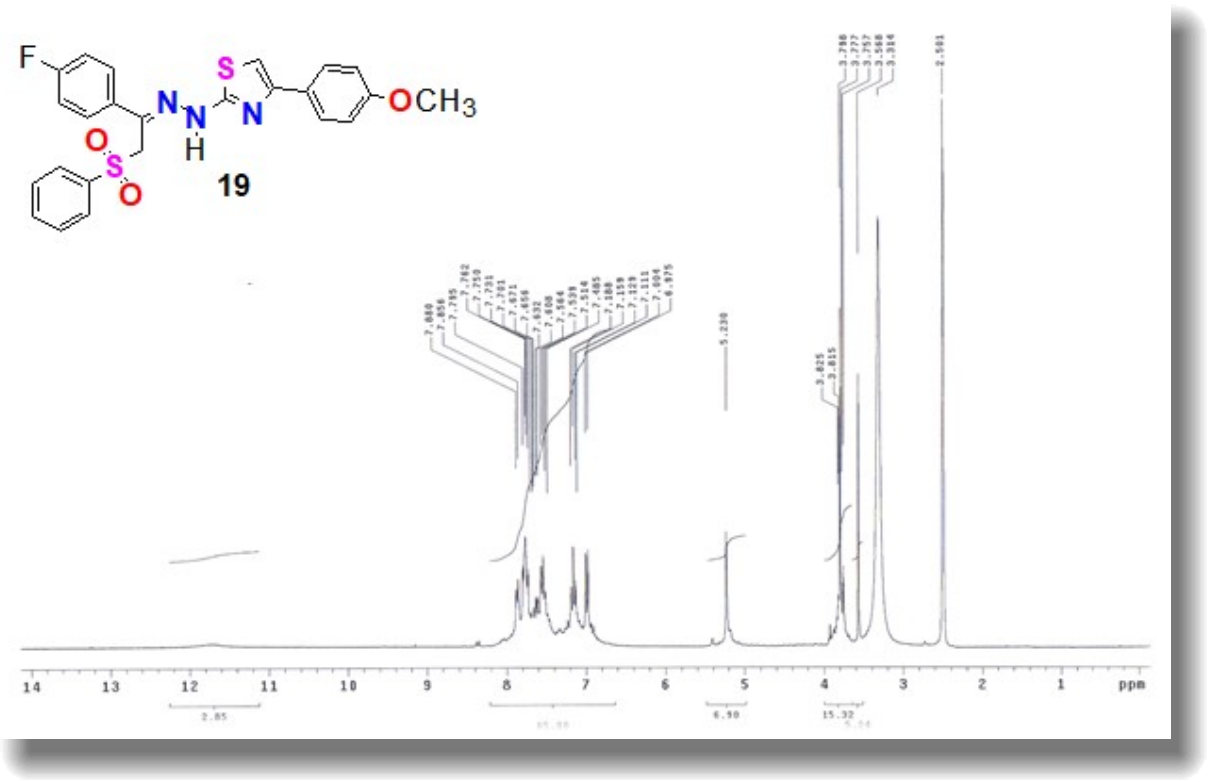
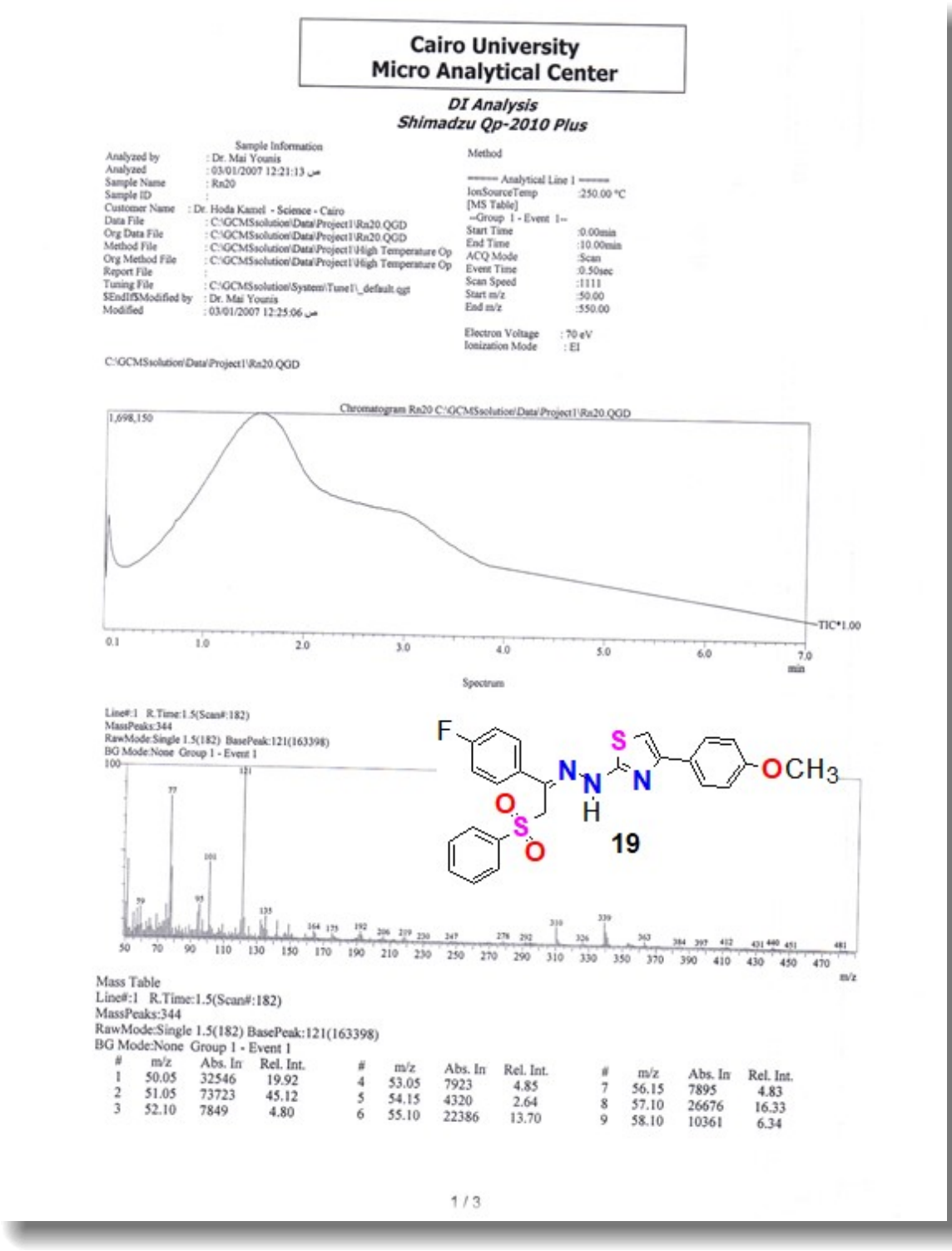


Figure S35. 1H-NMR spectrum of compound 19



Cairo University
Micro Analytical Center

DI Analysis
Shimadzu Qp-2010 Plus

Sample Information

Analyzed by	: Dr. Mai Younis
Analyzed	: 03/01/2007 12:21:13 ص
Sample Name	: Rn20
Sample ID	:
Customer Name	: Dr. Hoda Kamel - Science - Cairo
Data File	: C:\GCMSsolution\Data\Project1\Rn20.QGD
Org Data File	: C:\GCMSsolution\Data\Project1\Rn20.QGD
Method File	: C:\GCMSsolution\Data\Project1\High Temperature Op
Org Method File	: C:\GCMSsolution\Data\Project1\High Temperature Op
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1_default.qgt
$EndIf$Modified by	: Dr. Mai Younis
Modified	: 03/01/2007 12:25:06 ص

Method

====== Analytical Line 1 ======	
IonSourceTemp	:250.00 °C
[MS Table]	
--Group 1 - Event 1--	
Start Time	:0.00min
End Time	:10.00min
ACQ Mode	:Scan
Event Time	:0.50sec
Scan Speed	:1111
Start m/z	:50.00
End m/z	:550.00
Electron Voltage	: 70 eV
Ionization Mode	: EI

C:\GCMSsolution\Data\Project1\Rn20.QGD

Chromatogram Rn20 C:\GCMSsolution\Data\Project1\Rn20.QGD

1,698,150

TIC*1.00

0.1 1.0 2.0 3.0 4.0 5.0 6.0 7.0 min

Spectrum

Line#:1 R.Time:1.5(Scan#:182)
MassPeaks:344
RawMode:Single 1.5(182) BasePeak:121(163398)
BG Mode:None Group 1 - Event 1

100

50 70 90 110 130 150 170 190 210 230 250 270 290 310 330 350 370 390 410 430 450 470 m/z

19

Mass Table
Line#:1 R.Time:1.5(Scan#:182)
MassPeaks:344
RawMode:Single 1.5(182) BasePeak:121(163398)
BG Mode:None Group 1 - Event 1

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1	50.05	32546	19.92	4	53.05	7923	4.85	7	56.15	7895	4.83
2	51.05	73723	45.12	5	54.15	4320	2.64	8	57.10	26676	16.33
3	52.10	7849	4.80	6	55.10	22386	13.70	9	58.10	10361	6.34

1 / 3

Figure S36. Mass spectrum of compound 19

1.2. Biological activity

1.2.1. B-RAFV600E kinase assay

Researcher : Dr.Hanan Gaber email: hanangaber1980@hotmail.com mob. 01000788565

Date : 20-10-2020

Assay : B-Raf(V600E) Kinase Assay
Samples :.

Reference : ---

Cell lines : ---

Lab Report

ser	compound			IC_{50} nM	
		M.W	formula	**B-Raf(V600E)**	
1	1Rn (3)	347.45		115.3±6	
2	3Rn (7a)	489.61		198.6±10	
3	4Rn (7b)	503.64		36.3±1.9	
4	14Rn (7c)	503.64		108.4±5.6	
5	15Rn (11a)	491.58		84.9±4.4	
6	16Rn (11b)	526.03		185.2±9.6	
7	18Rn (11c)	536.58		95.4±5	
8	6Rn (13a)	526.47		23.1±1.2	
9	8Rn (13b)	461.60		205.6±11	
10	17Rn (15)	981.23		51.9±2.7	
11	19Rn (17)	351.41		68.2±3.5	
12	20Rn (19)	481.56		116.3±8	
**	Dabrafenib	519.56		47.2±2.5	

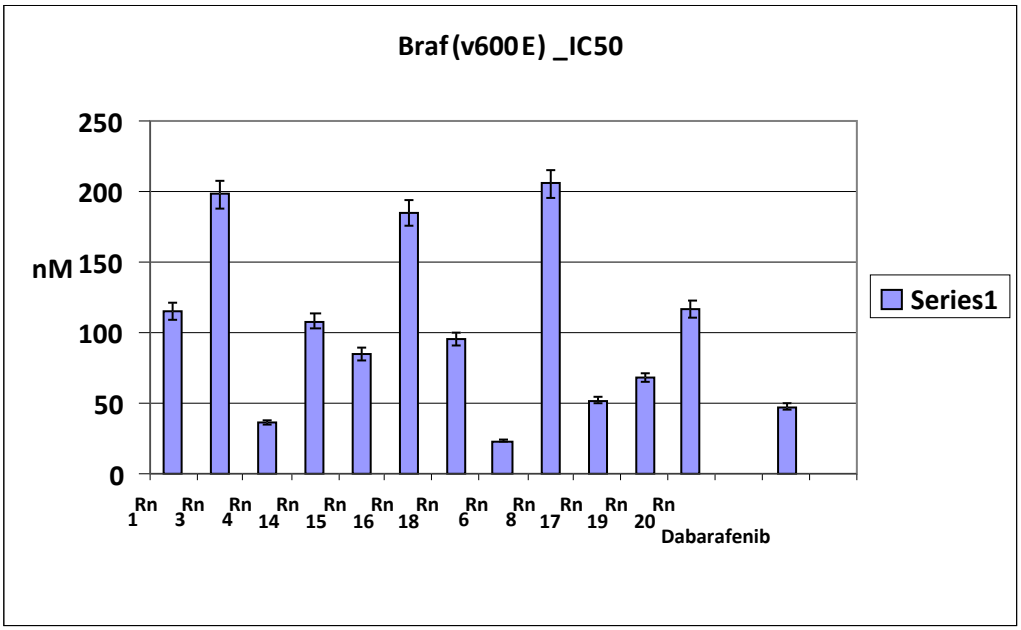
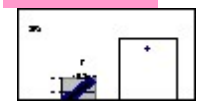


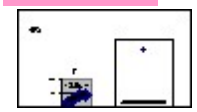
Figure S37 B-RAFV600E assay

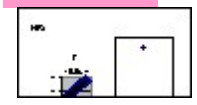
Detailed results:

B-Raf(V600E)

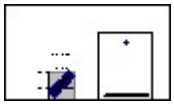
code	IC50	conc.	log	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
1Rn		10	4	88	30	0	30	12	0	12	3.3333333	14.4	120
		1	3	69	30	0	30	31	0	31	3.3333333	37.2	120
		0.1	2	46	30	0	30	54	0	54	3.3333333	64.8	120
		0.01	1	31	30	0	30	69	0	69	3.3333333	82.8	120
EC				0	30	0	30	100	0	100	3.3333333	120	120

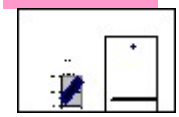
code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
3Rn		10	4	85	30	0	30	15	0	15	3.3333333	18	120
		1	3	63	30	0	30	37	0	37	3.3333333	44.4	120
		0.1	2	42	30	0	30	58	0	58	3.3333333	69.6	120
		0.01	1	26	30	0	30	74	0	74	3.3333333	88.8	120
EC				0	30	0	30	100	0	100	3.3333333	120	120

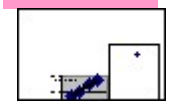
code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
4Rn		10	4	92	30	0	30	8	0	8	3.3333333	9.6	120
		1	3	81	30	0	30	19	0	19	3.3333333	22.8	120
		0.1	2	63	30	0	30	37	0	37	3.3333333	44.4	120
		0.01	1	35	30	0	30	65	0	65	3.3333333	78	120
EC				0	30	0	30	100	0	100	3.3333333	120	120

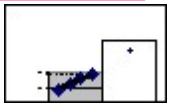
code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
14Rn		10	4	87	30	0	30	13	0	13	3.3333333	15.6	120
		1	3	73	30	0	30	27	0	27	3.3333333	32.4	120

	0.1	2	48	30	0	30	52	0	52	3.3333333	62.4	120
	0.01	1	29	30	0	30	71	0	71	3.3333333	85.2	120
EC			0	30	0	30	100	0	100	3.3333333	120	120

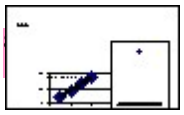
code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
		10	4	89	30	0	30	11	0	11	3.3333333	13.2	120
		1	3	67	30	0	30	33	0	33	3.3333333	39.6	120
		0.1	2	54	30	0	30	46	0	46	3.3333333	55.2	120
		0.01	1	32	30	0	30	68	0	68	3.3333333	81.6	120
EC				0	30	0	30	100	0	100	3.3333333	120	120

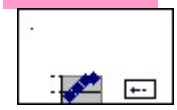
code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
16Rn		10	4	83	30	0	30	17	0	17	3.3333333	20.4	120
		1	3	61	30	0	30	39	0	39	3.3333333	46.8	120
		0.1	2	46	30	0	30	54	0	54	3.3333333	64.8	120
		0.01	1	27	30	0	30	73	0	73	3.3333333	87.6	120
EC				0	30	0	30	100	0	100	3.3333333	120	120

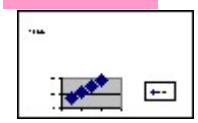
code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
18Rn		10	4	89	30	0	30	11	0	11	3.3333333	13.2	120
		1	3	72	30	0	30	28	0	28	3.3333333	33.6	120
		0.1	2	49	30	0	30	51	0	51	3.3333333	61.2	120
		0.01	1	31	30	0	30	69	0	69	3.3333333	82.8	120
EC				0	30	0	30	100	0	100	3.3333333	120	120

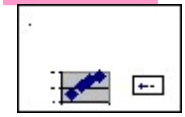
code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
6Rn		10	4	92	30	0	30	8	0	8	3.3333333	9.6	120
		1	3	79	30	0	30	21	0	21	3.3333333	25.2	120
		0.1	2	61	30	0	30	39	0	39	3.3333333	46.8	120
		0.01	1	43	30	0	30	57	0	57	3.3333333	68.4	120

EC				0	30	0	30	100	0	100	3.3333333	120	120

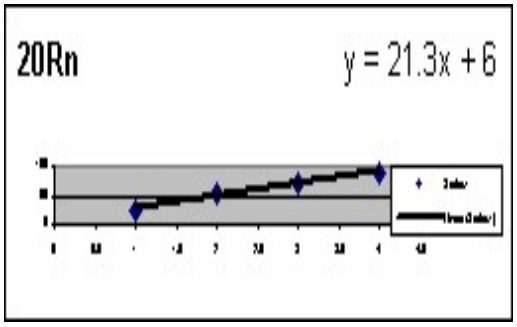
code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
		10	4	86	30	0	30	14	0	14	3.3333333	16.8	120
		1	3	65	30	0	30	35	0	35	3.3333333	42	120
		0.1	2	43	30	0	30	57	0	57	3.3333333	68.4	120
		0.01	1	22	30	0	30	78	0	78	3.3333333	93.6	120
EC				0	30	0	30	100	0	100	3.3333333	120	120

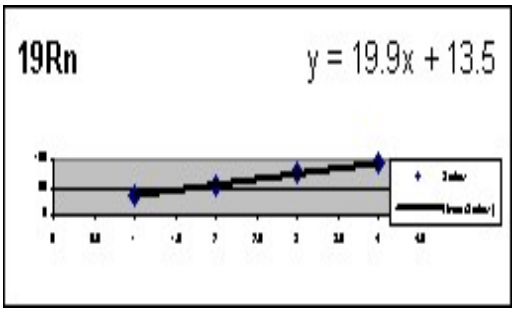
code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
17Rn		10	4	91	30	0	30	9	0	9	3.3333333	10.8	120
		1	3	84	30	0	30	16	0	16	3.3333333	19.2	120
		0.1	2	57	30	0	30	43	0	43	3.3333333	51.6	120
		0.01	1	32	30	0	30	68	0	68	3.3333333	81.6	120
EC				0	30	0	30	100	0	100	3.3333333	120	120

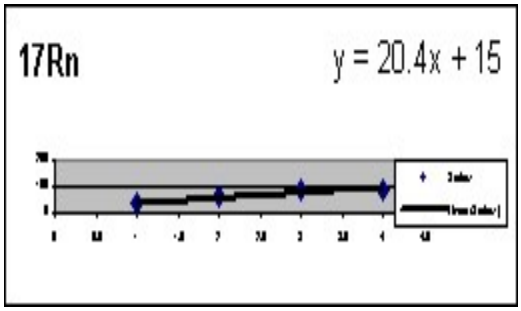
code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
19Rn		10	4	92	30	0	30	8	0	8	3.3333333	9.6	120
		1	3	76	30	0	30	24	0	24	3.3333333	28.8	120
		0.1	2	51	30	0	30	49	0	49	3.3333333	58.8	120
		0.01	1	34	30	0	30	66	0	66	3.3333333	79.2	120
EC				0	30	0	30	100	0	100	3.3333333	120	120

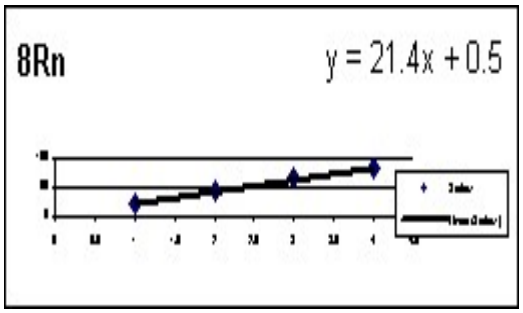
code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
20Rn		10	4	89	30	0	30	11	0	11	3.3333333	13.2	120
		1	3	72	30	0	30	28	0	28	3.3333333	33.6	120
		0.1	2	51	30	0	30	49	0	49	3.3333333	58.8	120
		0.01	1	25	30	0	30	75	0	75	3.3333333	90	120
EC				0	30	0	30	100	0	100	3.3333333	120	120

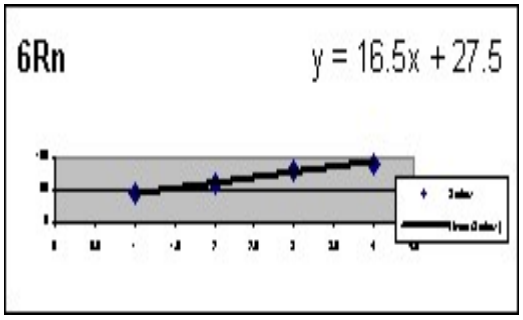
code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
Dabarafenib		10	4	95	30	0	30	5	0	5	3.3333333	6	120
		1	3	81	30	0	30	19	0	19	3.3333333	22.8	120
		0.1	2	58	30	0	30	42	0	42	3.3333333	50.4	120
		0.01	1	34	30	0	30	66	0	66	3.3333333	79.2	120
EC				0	30	0	30	100	0	100	3.3333333	120	120

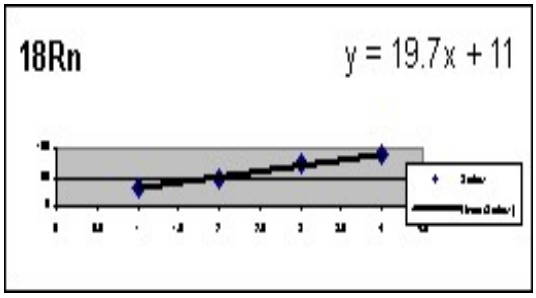


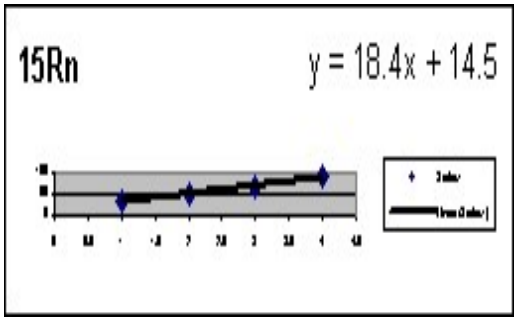






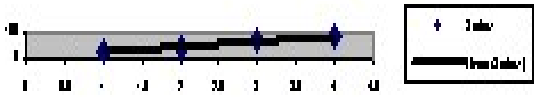






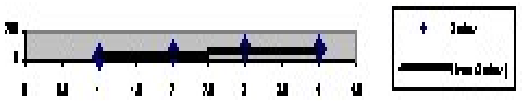
14Rn

$y = 19.9x + 9.5$



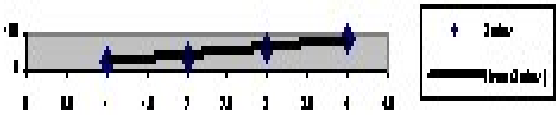
4Rn

$y = 18.9x + 20.5$



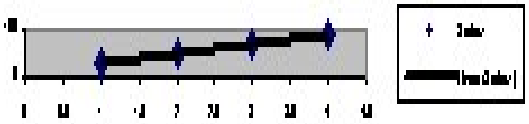
3Rn

$y = 19.8x + 4.5$



1Rn

$y = 19.4x + 10$



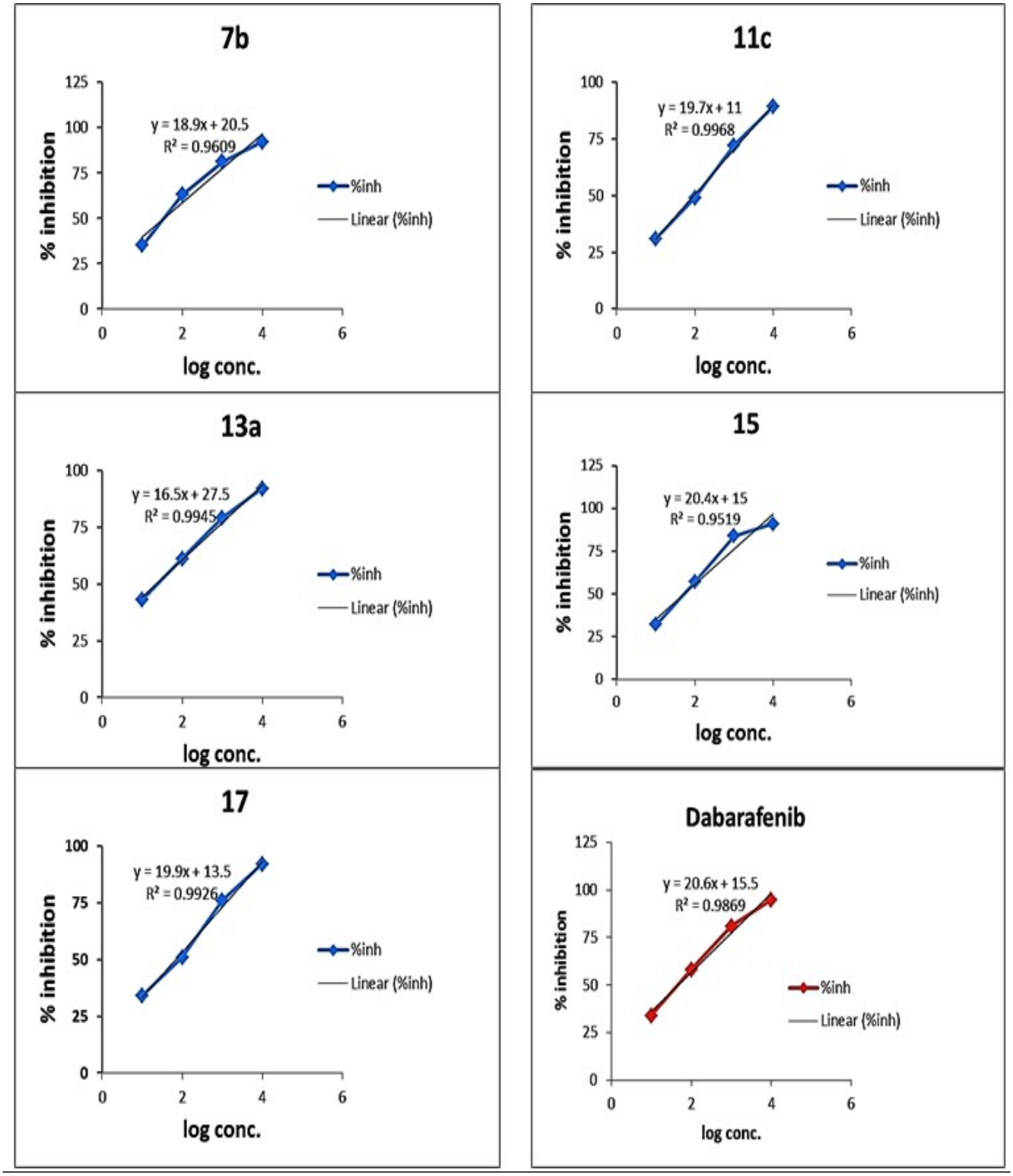


Figure S38 In vitro B-RAFV600E assay curves of compounds 7b, 11c, 13a, 15, 17 and dabrafenib

1.2.2. B-RAF wild-type kinase assay

Researcher	: Dr.Hanan Gaber email : Hanangaber1980@hotmail.com mob. 01000788565
Date	: 14-11-2020
Assay	: B-Raf(WT) Kinase Assay
Samples	:
Reference	: ---
Cell lines	: ---
Kit used	: ---

Lab Report

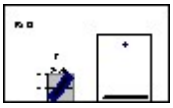
ser	compound			B-Raf (WT) IC50 nM	
		M.W	conc		
1	**Rn4 (7b)**	**503.64**		123.5±6.6	
2	**Rn15 (11a)**	**491.58**		361.1±19.4	
3	**Rn18 (11c)**	**436.58**		229±12.3	
4	**Rn6 (13a)**	**516.47**		97.9±5.2	
5	**Rn17 (15)**	**981.23**		251.2±13.5	
6	**Rn19 (17)**	**351.41**		153±8.2	
8	**Dabrafenib**	**519.56**		231.2±12.4	

Detailed results:

B-raf (WT)

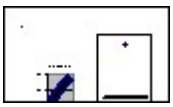
code	IC50	conc	log	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
Rn4		10	4	84	30	0	30	16	0	16	3.3333333	19.2	120
													
		1	3	71	30	0	30	29	0	29	3.3333333	34.8	120
		0.1	2	52	30	0	30	48	0	48	3.3333333	57.6	120
		0.01	1	25	30	0	30	75	0	75	3.3333333	90	120
EC				0	30	0	30	100	0	100	3.3333333	120	120

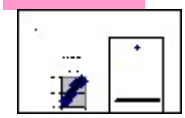
code	IC50	conc	log	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
Rn15		10	4	79	30	0	30	21	0	21	3.3333333	25.2	120
													
		1	3	64	30	0	30	36	0	36	3.3333333	43.2	120
		0.1	2	36	30	0	30	64	0	64	3.3333333	76.8	120
		0.01	1	16	30	0	30	84	0	84	3.3333333	100.8	120
EC				0	30	0	30	100	0	100	3.3333333	120	120

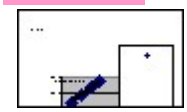
code	IC50	conc	log	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
Rn18		10	4	85	30	0	30	15	0	15	3.3333333	18	120
													
		1	3	66	30	0	30	34	0	34	3.3333333	40.8	120
		0.1	2	38	30	0	30	62	0	62	3.3333333	74.4	120
		0.01	1	23	30	0	30	77	0	77	3.3333333	92.4	120
EC				0	30	0	30	100	0	100	3.3333333	120	120

code	IC50	conc	log	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
Rn6		10	4	91	30	0	30	9	0	9	3.3333333	10.8	120
													
		1	3	79	30	0	30	21	0	21	3.3333333	25.2	120
		0.1	2	47	30	0	30	53	0	53	3.3333333	63.6	120

		0.01	1	28	30	0	30	72	0	72	3.3333333	86.4	120
EC				0	30	0	30	100	0	100	3.3333333	120	120

code	IC50	conc	log	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
		10	4	81	30	0	30	19	0	19	3.3333333	22.8	120
		1	3	64	30	0	30	36	0	36	3.3333333	43.2	120
		0.1	2	41	30	0	30	59	0	59	3.3333333	70.8	120
		0.01	1	22	30	0	30	78	0	78	3.3333333	93.6	120
EC				0	30	0	30	100	0	100	3.3333333	120	120

code	IC50	conc	log	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
Rn19		10	4	87	30	0	30	13	0	13	3.3333333	15.6	120
		1	3	72	30	0	30	28	0	28	3.3333333	33.6	120
		0.1	2	48	30	0	30	52	0	52	3.3333333	62.4	120
		0.01	1	21	30	0	30	79	0	79	3.3333333	94.8	120
EC				0	30	0	30	100	0	100	3.3333333	120	120

code	IC50	conc	log	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
Dabrafenib		10	4	86	30	0	30	14	0	14	3.3333333	16.8	120
		1	3	69	30	0	30	31	0	31	3.3333333	37.2	120
		0.1	2	43	30	0	30	57	0	57	3.3333333	68.4	120
		0.01	1	15	30	0	30	85	0	85	3.3333333	102	120
EC				0	30	0	30	100	0	100	3.3333333	120	120

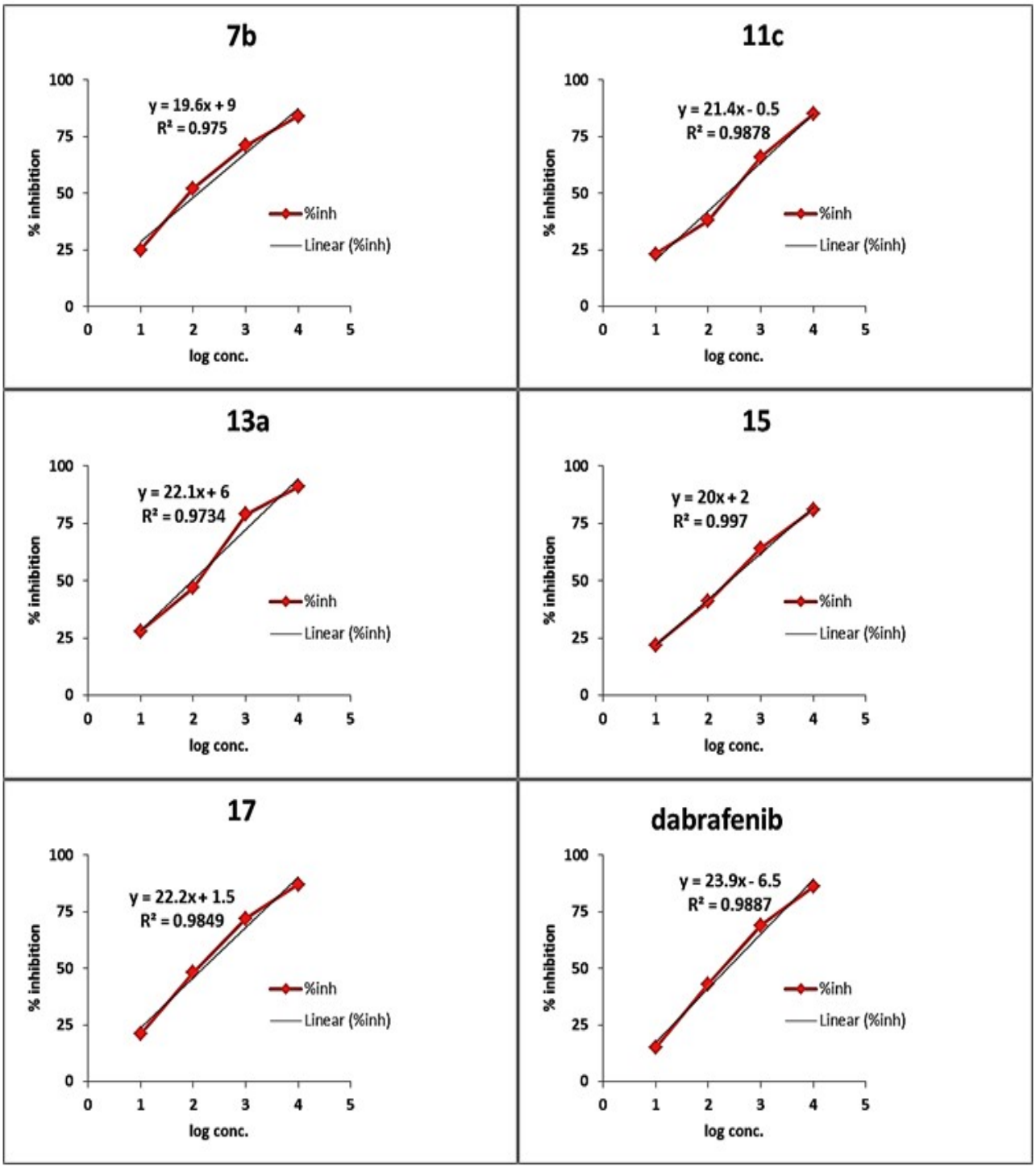
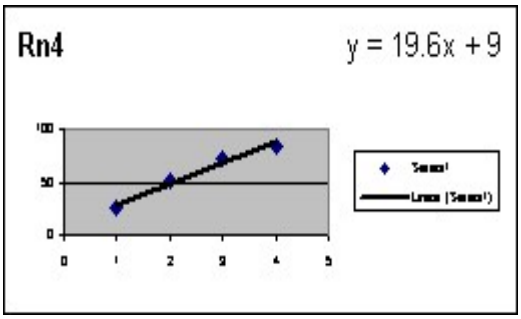
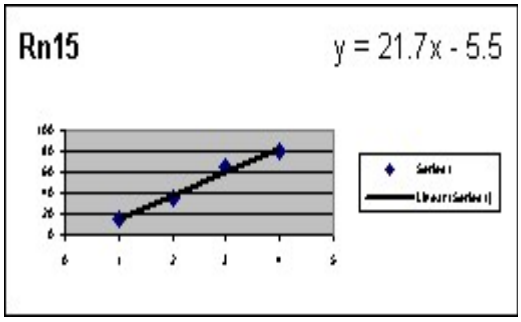
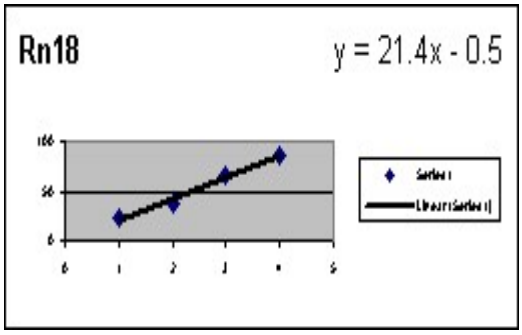
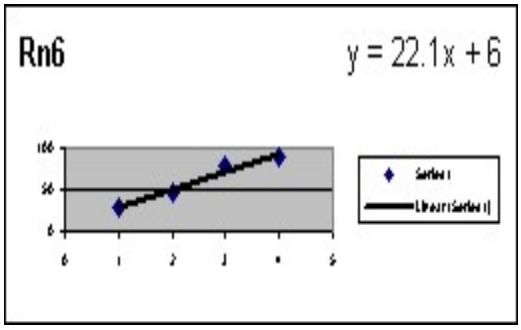


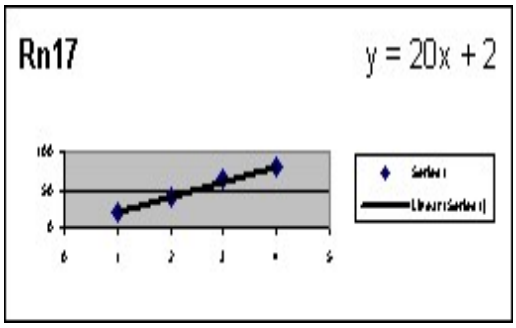
Figure S39 In vitro B-RAF Wild Type assay curves of compounds 7b, 11c, 13a, 15, 17 and dabrafenib

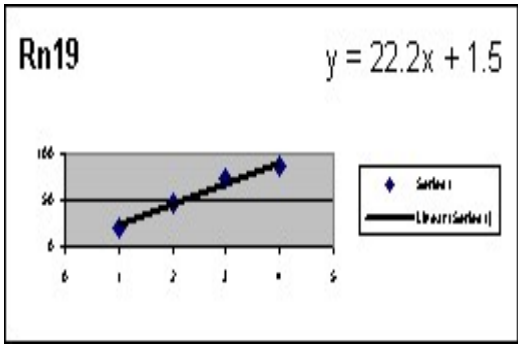

Rn4
y = 19.6x + 9

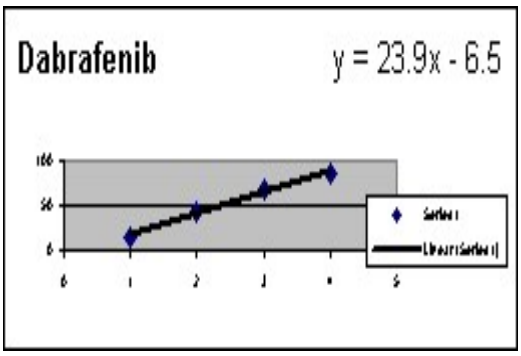

Rn15
y = 21.7x - 5.5


Rn18
y = 21.4x - 0.5


Rn6
y = 22.1x + 6


Rn17
y = 20x + 2


Rn19
y = 22.2x + 1.5


Dabrafenib
y = 23.9x - 6.5

1.2.3. Cytotoxicity against B-RAFV600E-mutated melanoma cells:

Researcher	**:** Dr.Hanan Gaber email : Hanangaber1980@hotmail.com mob. 01000788565
Assay	: MTT cytotoxicity assay
Samples	:
Cell lines	: ---
Ref.	: ---
Date	: 14-11-2020

Lab REPORT

* Cytotoxicity results

Ser	**Sample**		Cytotoxicity **IC50** **uM**	
	code	**MW**	WM266.4	
1	**Rn4 (7b)**	**503.64**	3.18±0.17	
2	**Rn15 (11a)**	**491.58**	6.18±0.34	
3	**Rn18 (11c)**	**436.58**	1.53±0.08	
4	**Rn6 (13a)**	**516.47**	4.52±0.25	
5	**Rn17 (15)**	**981.23**	17.1±0.94	
6	**Rn19 (17)**	**351.41**	1.24±0.07	
8	**Dabrafenib**	**519.56**	16.5±0.91	

Assay Protocol

Cell Line cells were obtained from American Type Culture Collection , cells were cultured using DMEM (Invitrogen/Life Technologies) supplemented with 10% FBS (Hyclone,), 10 ug/ml of insulin (Sigma), and 1% penicillin-streptomycin. All of the other chemicals and reagents were from Sigma, or Invitrogen.

Plate cells (cells density 1.2 – 1.8 × 10,000 cells/well) in a volume of 100µl complete growth medium + 100 ul of the tested compound per well in a 96-well plate for 24 hours before the MTT assay .

Cell cuture protocol

1. Remove culture medium to a centrifuge tube.

2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin 0.53 mM EDTA solution to remove all traces of serum which contains Trypsin inhibitor.

3. Add 2.0 to 3.0 ml of Trypsin EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).

Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.

5. Transfer the cell suspension to the centrifuge tube with the medium and cells from step 1, and centrifuge at approximately 125 xg for 5 to 10 minutes. Discard the supernatant.

6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels.

7. Incubate cultures at 37°C for 24 hrs.

8-After treatment of cells with the serial concentrations of the compound to be tested incubation is carried out for 48 h at 37°C ,then the plates are to be examined under the inverted microscope and proceed for the MTT assay

MTT – Cytotoxicity assay protocol

The MTT method of monitoring in vitro cytotoxicity is

well suited for use with multiwell plates. For best results, cells in the log phase of growth should be employed and final cell number should not exceed 106 cells/cm2. Each test should include a blank containing complete medium without cells.

1. Remove cultures from incubator into laminar

flow hood or other sterile work area.

2. Reconstitute each vial of MTT [M-5655] to be used with 3 ml of medium or balanced salt solution without phenol red and serum. Add reconstituted MTT in an amount equal to 10% of the culture medium volume.

3. Return cultures to incubator for 2-4 hours depending on cell type and maximum cell density. (An incubation period of 2 hours is generally adequate but may be lengthened for low cell densities or cells with lower metabolic activity.) Incubation times should beconsistent when making comparisons.

4. After the incubation period, remove cultures from incubator and dissolve the resulting formazan crystals by adding an amount of MTT Solubilization Solution [M-8910] equal to the original culture medium volume.

5. Gentle mixing in a gyratory shaker will enhance dissolution. Occasionally, especially in dense cultures, pipetting up and down [trituration] may be required to completely dissolve the MTT formazan crystals.

6. Spectrophotometrically measure absorbance

at a wavelength of 450 nm. Measure the background absorbance of multiwell plates at 690 nm and subtract from the 450 nm measurement. Tests performed in multiwell plates can be read using the appropriate type of plate

reader or the contents of individual wells may be transferred to appropriate size cuvets for spectrophotometric measurement.

researcher	assay	Date	cells	
Dr.Hanan Gaber	MTT	14-Nov	WM266.4	

	Blank	CC	Sample No. Rn4/WM266.4					Sample No. Rn15/WM266.4				
	1	2	3	4	5	6	7	8	9	10	11	12
A	B	C	100uM	25uM	6.3uM	1.6ug	0.4uM	100uM	25uM	6.3uM	1.6ug	0.4uM
B	B	C	100uM	25uM	6.3uM	1.6ug	0.4uM	100uM	25uM	6.3uM	1.6ug	0.4uM
C	B	C	100uM	25uM	6.3uM	1.6ug	0.4uM	100uM	25uM	6.3uM	1.6ug	0.4uM

ROBONIK P2000 eia reader

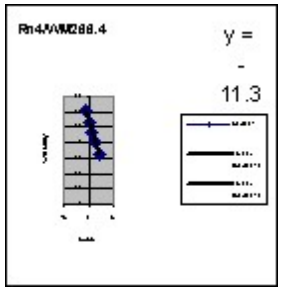
Wave length: 450 nm

Reference: 630 nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001	0.592	0.187	0.232	0.267	0.302	0.359	0.205	0.245	0.284	0.336	0.385
B	0.001	0.585	0.191	0.239	0.274	0.311	0.345	0.214	0.239	0.283	0.342	0.379
C	0.001	0.573	0.196	0.235	0.277	0.308	0.361	0.206	0.237	0.277	0.356	0.382
mean	0.0004	0.5833	0.1913	0.2353	0.2727	0.307	0.355	0.2083	0.2403	0.2813	0.3447	0.382
%		`	32.8	40.343	46.743	52.629	60.857	35.714	41.2	48.229	59.086	65.486

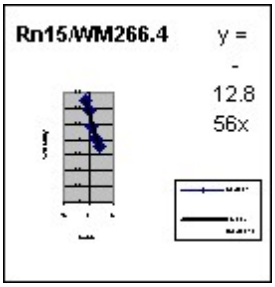
Rn4/WM266.4

log conc.	% viability
2	32.8
1.3979	40.343
0.7959	46.743
0.1931	52.629
-0.409	60.857



Rn15/WM266.4

log conc.	% viability
2	35.714
1.3979	41.2
0.7959	48.229
0.1931	59.086
-0.4089	65.486



IC50=

IC50=

	Blank	CC	Sample No. Rn18/WM266.4					Sample No. Rn6/WM266.4				
	1	2	3	4	5	6	7	8	9	10	11	12
A	B	C	100uM	25uM	6.3uM	1.6ug	0.4uM	100uM	25uM	6.3uM	1.6ug	0.4uM
B	B	C	100uM	25uM	6.3uM	1.6ug	0.4uM	100uM	25uM	6.3uM	1.6ug	0.4uM
C	B	C	100uM	25uM	6.3uM	1.6ug	0.4uM	100uM	25uM	6.3uM	1.6ug	0.4uM

ROBONIK P2000 eia reader

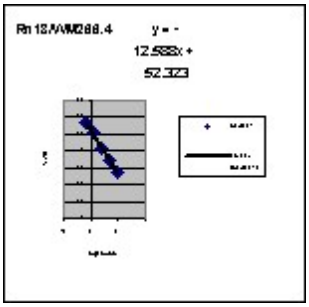
Wave length: 450 nm

Reference: 630 nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001	0.555	0.153	0.188	0.231	0.276	0.312	0.176	0.227	0.264	0.297	0.344
B	0.001	0.525	0.147	0.185	0.226	0.272	0.306	0.169	0.231	0.269	0.295	0.359
C	0.001	0.549	0.146	0.192	0.221	0.274	0.317	0.171	0.228	0.254	0.305	0.337
mean	0.001	0.543	0.1487	0.1883	0.226	0.274	0.3117	0.172	0.2287	0.2623	0.299	0.3467
% viability			27.379	34.684	41.621	50.46	57.397	31.676	42.112	48.312	55.064	63.843

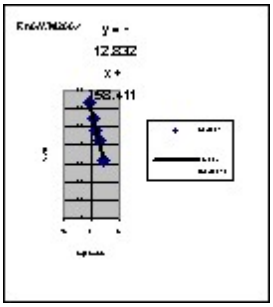
Rn18/WM266.4

log conc.	% viability
2	27.379
1.3979	34.684
0.7959	41.621
0.1931	50.46
-0.409	57.397



Rn6/WM266.4

log conc.	% viability
2	31.676
1.3979	42.112
0.7959	48.312
0.1931	55.064
-0.4089	63.843



IC50=

IC50=

	Blank	CC	Sample No. Rn17/WM266.4					Sample No. Rn19/WM266.4				
	1	2	3	4	5	6	7	8	9	10	11	12
A	B	C	100uM	25uM	6.3uM	1.6ug	0.4uM	100uM	25uM	6.3uM	1.6ug	0.4uM
B	B	C	100uM	25uM	6.3uM	1.6ug	0.4uM	100uM	25uM	6.3uM	1.6ug	0.4uM
C	B	C	100uM	25uM	6.3uM	1.6ug	0.4uM	100uM	25uM	6.3uM	1.6ug	0.4uM

ROBONIK P2000 eia reader

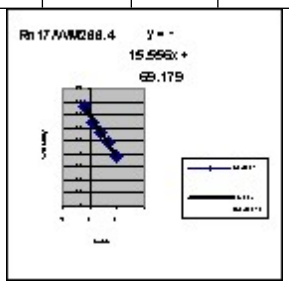
Wave length: 450 nm

Reference: 630 nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001	0.511	0.195	0.239	0.287	0.323	0.376	0.112	0.154	0.197	0.235	0.284
B	0.003	0.492	0.182	0.246	0.276	0.319	0.391	0.132	0.159	0.185	0.245	0.286
C	0.001	0.486	0.187	0.242	0.271	0.316	0.379	0.119	0.153	0.191	0.243	0.294
mean	0.0017	0.4963	0.188	0.2423	0.278	0.3193	0.382	0.121	0.1553	0.191	0.241	0.288
% viability			37.878	48.825	56.011	64.338	76.964	24.379	31.296	38.482	48.556	58.026

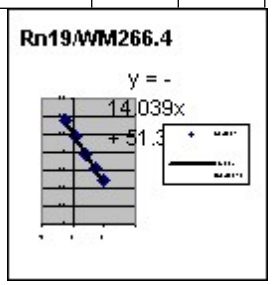
Rn17/WM266.4

2	37.878
1.3979	48.825
0.7959	56.011
0.1931	64.338
-0.409	76.964



Rn19/WM266.4

2	24.379
1.3979	31.296
0.7959	38.482
0.1931	48.556
-0.4089	58.026



IC50=

IC50=

	Blank	CC	Sample No. sorafenib/WM266.4					Sample No. Dabrafenib/WM266.4				
	1	2	3	4	5	6	7	8	9	10	11	12
A	B	C	100u	25uM	6.3uM	1.6ug	0.4uM	100uM	25uM	6.3uM	1.6ug	0.4uM

			M									
B	B	C	100uM	25uM	6.3uM	1.6ug	0.4uM	100uM	25uM	6.3uM	1.6ug	0.4uM
C	B	C	100uM	25uM	6.3uM	1.6ug	0.4uM	100uM	25uM	6.3uM	1.6ug	0.4uM

ROBONIK P2000 eia reader

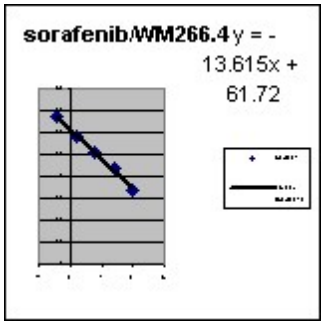
Wave length: 450 nm

Reference: 630 nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001	0.539	0.179	0.223	0.276	0.299	0.356	0.205	0.247	0.292	0.339	0.383
B	0.001	0.525	0.188	0.241	0.264	0.311	0.359	0.211	0.255	0.303	0.342	0.392
C	0.001	0.536	0.176	0.236	0.271	0.326	0.366	0.193	0.261	0.316	0.353	0.399
mean	0.001	0.5333	0.181	0.2333	0.2703	0.312	0.3603	0.203	0.2543	0.3037	0.3447	0.3913
% viability			33.938	43.75	50.688	58.5	67.563	38.063	47.688	56.938	64.625	73.375

sorafenib/WM266.4

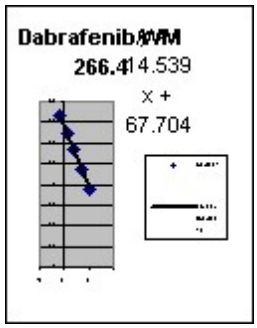
log conc.	% viability
2	33.938
1.3979	43.75
0.7959	50.688
0.1931	58.5
-0.409	67.563



IC50=

Dabrafenib/WM266.4

log conc.	% viability
2	38.063
1.3979	47.688
0.7959	56.938
0.1931	64.625
-0.4089	73.375



IC50=

1.2.4. Cytotoxicity against B-RAF wild type melanoma cells:

Researcher	: Dr.hanan gaber email: hanangaber1980@hotmail.com mob. 01000788565
Assay	: MTT cytotoxicity assay
Samples	: 06 compounds.
Cell lines	: ---
Ref.	: ---

Lab REPORT

* Cytotoxicity results

	Ser	Sample		Cytotoxicity IC50 uM	SD ±
		code	MW	sk-mel-23	
7b	1	**Rn4**	**503.64**	**7.87**	0.4
11a	2	**Rn15**	**491.58**	**0.43**	0.02
11c	3	**Rn18**	**436.58**	**22.3**	1.14

13a	4	**Rn6**	**516.47**	**50.2**	2.56
15	5	**Rn17**	**981.23**	**17.6**	0.89
17	6	**Rn19**	**351.41**	**12.4**	0.63
	7	**Dabrafenib**	**519.56**	**9.03**	0.46

Assay Protocol

Cell Line cells were obtained from American Type Culture Collection , cells were cultured using DMEM (Invitrogen/Life Technologies) supplemented with 10% FBS (Hyclone,), 10 ug/ml of insulin (Sigma), and 1% penicillin-streptomycin. All of the other chemicals and reagents were from Sigma, or Invitrogen.

Plate cells (cells density 1.2 – 1.8 × 10,000 cells/well) in a volume of 100µl complete growth medium + 100 ul of the tested compound per well in a 96-well plate for 24 hours before the MTT assay .

Cell cuture protocol

1. Remove culture medium to a centrifuge tube.

2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin 0.53 mM EDTA solution to remove all traces of serum which contains Trypsin inhibitor.

3. Add 2.0 to 3.0 ml of Trypsin EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).

Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.

5. Transfer the cell suspension to the centrifuge tube with the medium and cells from step 1, and centrifuge at approximately 125 xg for 5 to 10 minutes. Discard the supernatant.

6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels.

7. Incubate cultures at 37°C for 24 hrs.

8-After treatment of cells with the serial concentrations of the compound to be tested incubation is carried out for 48 h at 37°C ,then the plates are to be examined under the inverted microscope and proceed for the MTT assay

MTT – Cytotoxicity assay protocol

The MTT method of monitoring in vitro cytotoxicity is

well suited for use with multiwell plates. For best results, cells in the log phase of growth should be employed and final cell number should not

exceed 106 cells/cm2. Each test should include a blank containing complete medium without cells.

1. Remove cultures from incubator into laminar

flow hood or other sterile work area.

2. Reconstitute each vial of MTT [M-5655] to be used with 3 ml of medium or balanced salt solution without phenol red and serum. Add reconstituted MTT in an amount equal to 10% of the culture medium volume.

3. Return cultures to incubator for 2-4 hours depending on cell type and maximum cell density. (An incubation period of 2 hours is generally adequate but may be lengthened for low cell densities or cells with lower metabolic activity.) Incubation times should beconsistent when making comparisons.

4. After the incubation period, remove cultures from incubator and dissolve the resulting formazan crystals by adding an amount of MTT Solubilization Solution [M-8910] equal to the original culture medium volume.

5. Gentle mixing in a gyratory shaker will enhance dissolution. Occasionally, especially in dense cultures, pipetting up and down [trituration] may be required to completely dissolve the MTT formazan crystals.

6. Spectrophotometrically measure absorbance

at a wavelength of 450 nm. Measure the background absorbance of multiwell plates at 690 nm and subtract from the 450 nm measurement. Tests performed in multiwell plates can be read using the appropriate type of plate

reader or the contents of individual wells may be transferred to appropriate size cuvets for spectrophotometric measurement.

researcher	assay	Date	cells
Dr.Hanan Gaber	MTT	01-Aug	sk-mel-23

	Blank	CC	Sample No.			Rn4/sk-mel-23		Sample No.		Rn15/sk-mel-23		
	1	2	3	4	5	6	7	8	9	10	11	12
A	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM
B	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM
C	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM

ROBONIK P2000 eia reader

Wave length: 450 nm

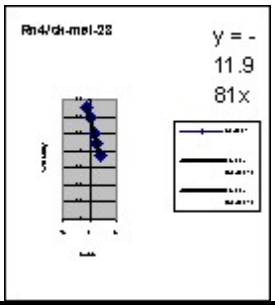
Reference: 630 nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001	0.594	0.227	0.265	0.311	0.355	0.388	0.129	0.175	0.229	0.264	0.311
B	0.001	0.622	0.231	0.269	0.303	0.364	0.412	0.141	0.184	0.213	0.259	0.319
C	0.001	0.612	0.219	0.271	0.301	0.369	0.395	0.117	0.163	0.222	0.262	0.293
mean	0.001	0.609	0.2257	0.2683	0.305	0.3627	0.398	0.129	0.174	0.221	0.2617	0.308
%		`	37.035	44.037	50.05	59.519	65.37	21.17	28.56	36.32	42.943	50.49

Rn4/sk-mel-23

log conc.	% viability
2	37.04
1.398	44.04
0.796	50.05
0.193	59.52
-0.41	65.37

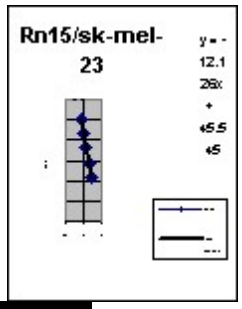
IC50=



Rn15/sk-mel-23

log conc.	% viability
2	21.17
1.398	28.56
0.796	36.32
0.193	42.94
-0.409	50.49

IC50=



	Blank	CC	Sample No.			Rn18/sk-mel-23		Sample No.		Rn6/sk-mel-23		
	1	2	3	4	5	6	7	8	9	10	11	12
A	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM

B	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM
C	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM

ROBONIK P2000 eia reader

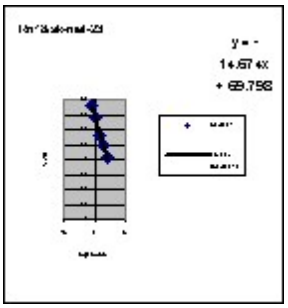
Wave length: 450 nm

Reference: 630 nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001	0.541	0.212	0.253	0.287	0.344	0.408	0.242	0.289	0.327	0.379	0.436
B	0.001	0.526	0.208	0.254	0.296	0.361	0.393	0.239	0.284	0.334	0.376	0.444
C	0.001	0.499	0.221	0.266	0.303	0.358	0.387	0.234	0.287	0.336	0.374	0.429
mean	0.001	0.522	0.2137	0.2577	0.295	0.3543	0.396	0.238	0.287	0.332	0.3763	0.436
% viability			40.932	49.361	56.58	67.88	75.86	45.66	54.92	63.67	72.095	83.59

Rn18/sk-mel-23

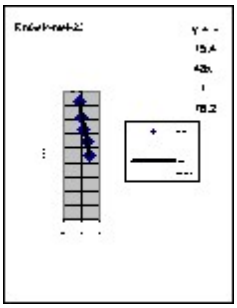
log conc.	% viability
2	40.93
1.398	49.36
0.796	56.58
0.193	67.88
-0.41	75.86



IC50=

Rn6/sk-mel-23

log conc.	% viability
2	45.66
1.398	54.92
0.796	63.67
0.193	72.09
-0.409	83.59



IC50=

[redacted]

	Blank	CC	Sample No. Rn17/sk-mel-23					Sample No. Rn19/sk-mel-23				
	1	2	3	4	5	6	7	8	9	10	11	12
A	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM
B	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM
C	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM

ROBONIK P2000 eia reader

Wave length: 450 nm

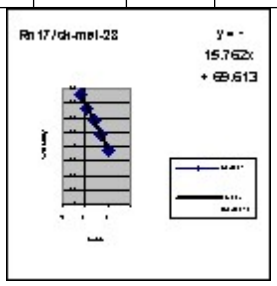
Reference: 630 nm

	1	2	3	4	5	6	7	8	9	10	11	12

A	0.001	0.531	0.203	0.254	0.292	0.343	0.386	0.177	0.231	0.282	0.361	0.405
B	0.003	0.509	0.186	0.238	0.304	0.352	0.401	0.165	0.248	0.277	0.354	0.387
C	0.001	0.518	0.191	0.256	0.317	0.328	0.395	0.167	0.229	0.279	0.357	0.412
mean	0.002	0.519	0.1933	0.2493	0.304	0.341	0.394	0.17	0.236	0.279	0.3573	0.401
% viability			37.227	48.01	58.6	65.661	75.87	32.67	45.44	53.79	68.806	77.28

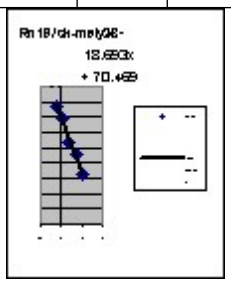
Rn17/sk-mel-23

2	37.23
1.398	48.01
0.796	58.6
0.193	65.66
-0.41	75.87



Rn19/sk-mel-23

2	32.67
1.398	45.44
0.796	53.79
0.193	68.81
-0.409	77.28



IC50=

IC50=

[redacted]

	Blank	CC	Sample No.		Dabrafenib/sk-mel-23			Sample No.				
	1	2	3	4	5	6	7	8	9	10	11	12
A	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM					
B	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM					
C	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM					

ROBONIK P2000 eia reader

Wave length: 450 nm

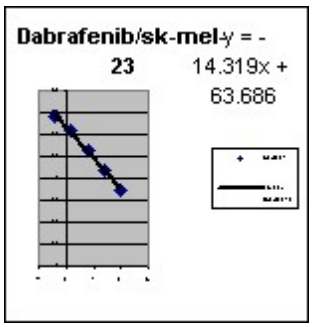
Reference: 630 nm

	1	2	3	4	5	6	7	8	9	10	11	12

A	0.001	0.566	0.194	0.243	0.295	0.346	0.395					
B	0.001	0.549	0.178	0.231	0.282	0.355	0.374					
C	0.001	0.564	0.205	0.264	0.309	0.337	0.382					
mean	0.001	0.56	0.1923	0.246	0.295	0.346	0.384	0	0	0	0	0
% viability			34.366	43.955	52.77	61.823	68.55	0	0	0	0	0

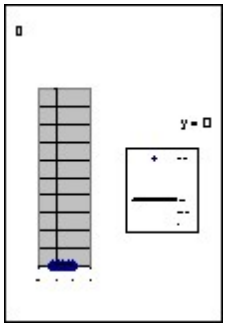
Dabrafenib/sk-mel-23

log conc.	% viability
2	34.37
1.398	43.95
0.796	52.77
0.193	61.82
-0.41	68.55



IC50=

log conc.	% viability
2	0
1.398	0
0.796	0
0.193	0
-0.409	0



IC50=

[redacted]

1.2.5. Cell-based B-RAFV600E kinase assay

The blocking effect of title compounds, represented by compound 7b, on the phosphorylation of downstream ERK in B-RAFV600E-mutated WM266.4 melanoma cells was measured using ELISA kit (Abcam's ERK1/2 (pT202/Y204) and ERK1/2 (Total) in vitro Simple Step ELISA™ Kit). This kit employs an affinity tag-labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. Briefly, cells in culture medium were treated with compound 7b and dabrafenib, dissolved in DMSO. The expression of phosphorylated ERK (pERK) in samples was measured (ng/ml) as duplicate determinations and the data were compared with dabrafenib as standard B-RAFV600E kinase inhibitors.

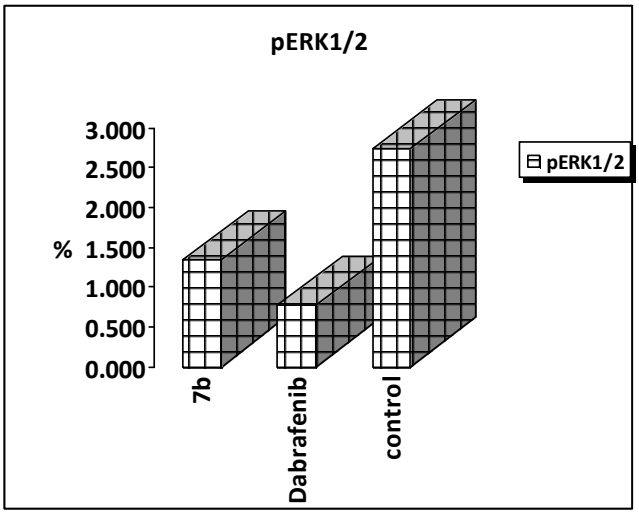
Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- To assay phospho and total ERK1/2, please use separate wells.
- Prepare all reagents, working standards, and samples as directed in the previous sections.
- Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
- Add 50 µL of all samples and standards to appropriate wells.
- Add 50 µL of the corresponding Antibody Cocktail (phospho or total ERK1/2) to each well.
- Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.
- Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.
- Add 100 µL of TMB Substrate to each well and incubate for 15 minutes in the dark on a plate shaker set to 400 rpm.
- Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

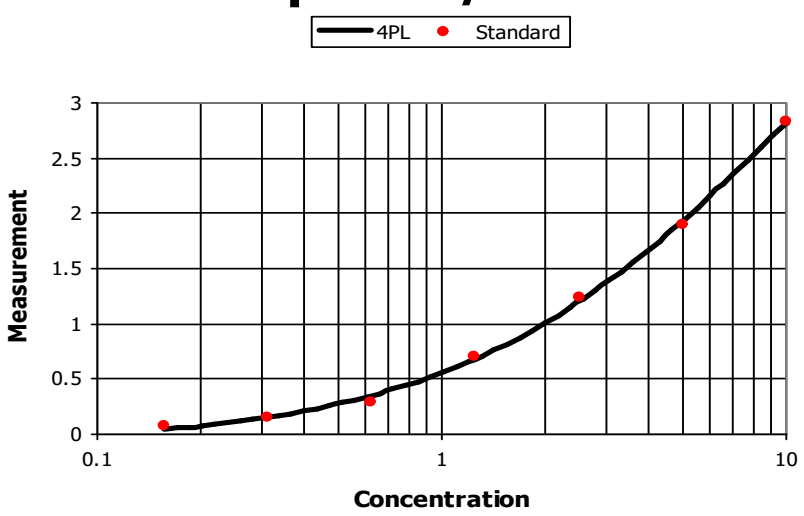
Researcher	: Dr.hanan gaber email: hanangaber1980@hotmail.com mob. 01000788565
Assay	: **pERK 1/2**(**ERK1/2 (pT202/Y204)** enzyme assay
Samples	: 01 sample
Reference	: ---
Cell line	: **WM266.4**
Reader	: ROBONIK P2000 ELISA READER WL 450 nm

Lab. report

s	sample		ERK 1/2(ERK1/2 (pT202/Y204)	fld
	code	cells	%	
1	**7b**	**WM266.4**	**1.339±0.035**	**0.490**
2	**Dabrafenib**	**WM266.4**	**0.769±0.018**	**0.281**
3	**control**	**WM266.4**	**2.732±0.045**	**1**



Results:



ST.	CONC.%
St.1	10
St.2	5
St.3	2.5
St.4	1.25
St.5	0.625
St.6	0.313
St.7	0.157

Detailed Results:

Plate map

	1	2	3	4	5	6	7	8	9	10	11	12
A	ST1	**7b**	--	--	--	--	--	--	--	--	--	
B	ST2	**7b**	--	--	--	--	--	--	--	--	--	
C	ST3	**Dabrafenib**	--	--	--	--	--	--	--	--	--	
D	ST4	**Dabrafenib**	--	--	--	--	--	--	--	--	--	--
E	ST5	**control**	--	--	--	--	--	--	--	--	--	--
F	ST6	**control**	--	--	--	--	--	--	--	--	--	--
G	ST7	---	--	--	--	--	--	--	--	--	--	--
H	B	---	--	--	--	--	--	--	--	--	--	--

Sample ODs

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.851	**0.755**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
B	1.911	**0.732**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
C	1.257	**0.447**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
D	0.729	**0.461**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
E	0.311	**1.289**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
F	0.171	**1.311**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

G	0.088	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
H	0.019	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Calibrator	**Wells**	**Conc.**	**Raw (Corrected)**	**Backfit**	**Recovery %**
Standard1	A1	10	2.83	10.07	100.7
Standard2	B1	5	1.89	4.847	96.93
Standard3	C1	2.5	1.24	2.609	104.3
Standard4	D1	1.25	0.71	1.308	104.6
Standard5	E1	0.625	0.292	0.5236	83.77
Standard6	F1	0.3125	0.152	0.3068	98.18
Standard7	G1	0.1563	0.069	0.1905	121.9

Sample	**Wells**	**Raw**	**Background Corrected**	**Conc.**	**Conc. (Average)**	**%CV**	**SD**	**SEM**
7b	A2	0.755	0.724	1.363	1.339	2.6	0.0348	0.0246
	B2	0.732		1.314				
Dabrafenib	C2	0.447	0.435	0.7565	0.7691	2.32	0.0178	0.0126
	D2	0.461		0.7817				
control	E2	1.29	1.28	2.7	2.732	1.65	0.0452	0.032
	F2	1.31		2.764				
Blank	H1	0.019	0	0.1021	0.1021	-	-	0

1.3. Docking study

A docking study of the most promising compounds in this work was performed using MOE (2014.0901). A high resolution B-RAFV600E kinase enzyme co-crystallized with dabrafenib was retrieved form protein data bank (PDB code: 4XV2) to be used in docking study.

Table S1. The docking results of selected compounds into the active site of B-RAF V600E kinase

Compound No.	Docking score (kcal/mol)	Residues	Interaction	Distance (A°)
7b	-8.99	LYS 483 CYS 532 LEU 514	H-acceptor H-acceptor pi-H	3.29 3.54 4.25
11a	-8.74	ASP 594 PHE 595 PHE 583 LYS 483 LEU 505 LEU 514 CYS 532	H-acceptor H-pi H-pi pi-H pi-H pi-H pi-H	3.03 3.68 3.88 3.67 4.45, 3.65 4.54, 4.29 4.95
11c	-8.80	CYS532 ASP 594 LYS 483 PHE 583 PHE 583	H-donor H-acceptor pi-H pi-H pi-pi	4.32, 3.99 2.97 4.23 4.37 4.00
13a	-7.88	CYS 532 THR 508 ASP 594 CYS 532 VAL 471 LEU 514	H-donor H-donor H-donor H-acceptor pi-H pi-H	3.98 2.83 4.21 3.39 4.54 4.54
15	-8.68	CYS 532 GLU533 GLU 533 LYS 483	H-donor H-donor H-donor H-acceptor	4.04 3.09 3.35 3.21
17	-5.01	CYS 532 CYS 532 VAL 471	H-acceptor H-acceptor pi-H	4.26 4.26 3.96
Co-crystallized ligand (dabrafenib)	-9.95	GLN 530 CYS 532 LYS 483 PHE 595 LEU 505 LEU 514	H-donor H-acceptor H-acceptor H-acceptor pi-H pi-H	2.77 3.16 2.97 3.08 3.72 4.23