

Supplementary Information

***In Vitro* and *In Vivo* Evaluation of Novel Chromeno[2,3-*d*] pyrimidinones as Therapeutic Agents for Triple Negative Breast Cancer**

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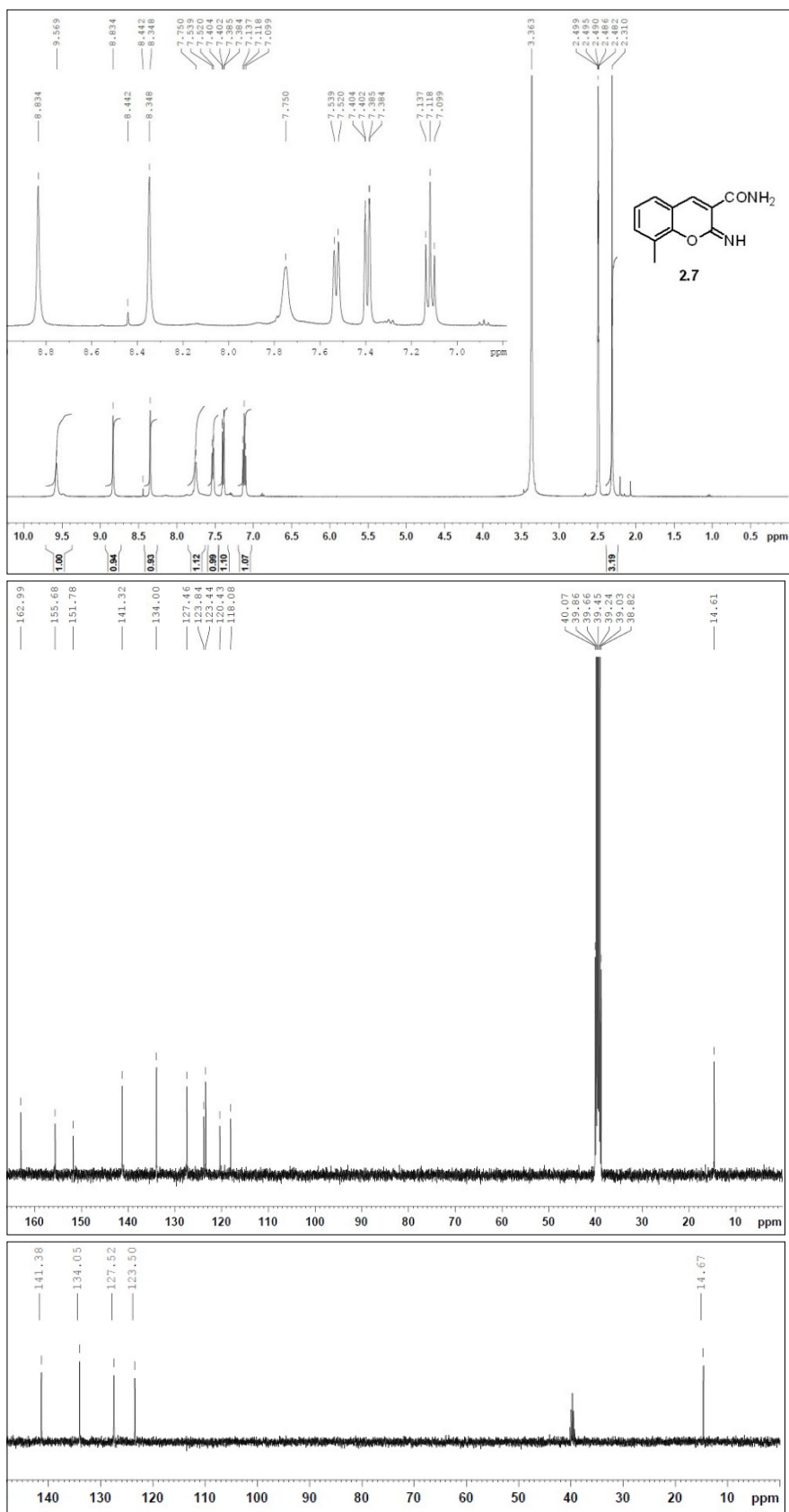
*Corresponding authors.

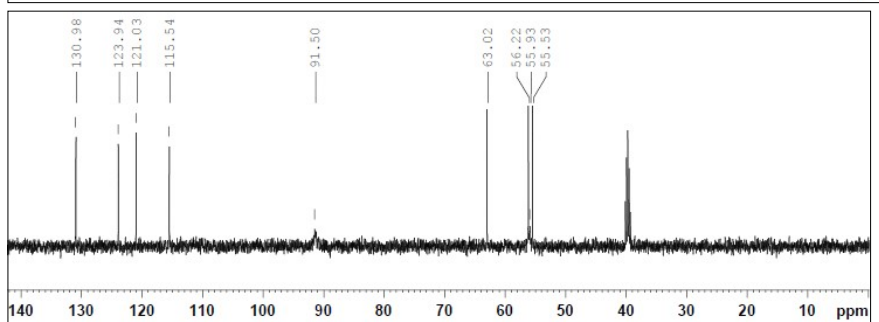
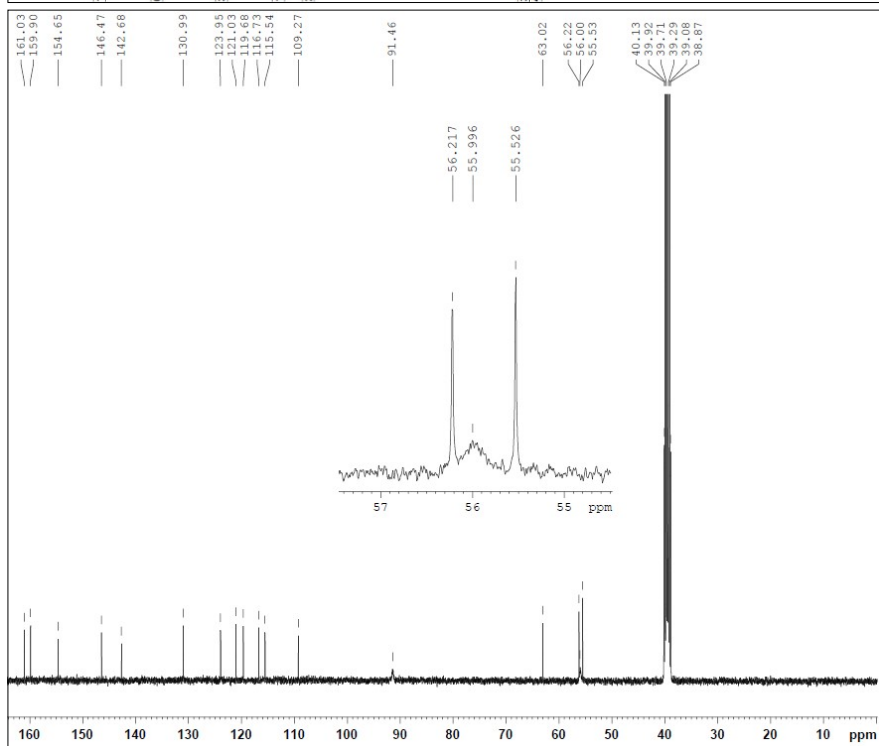
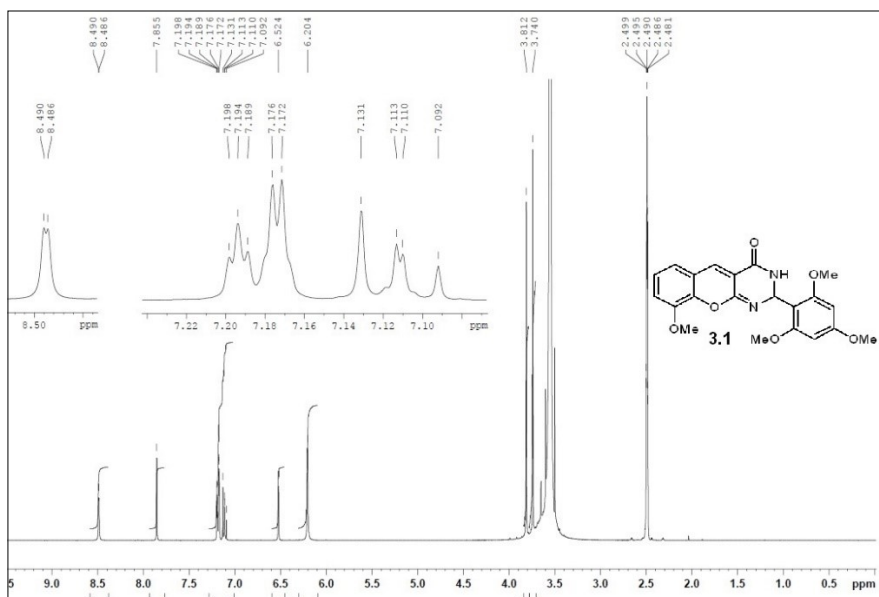
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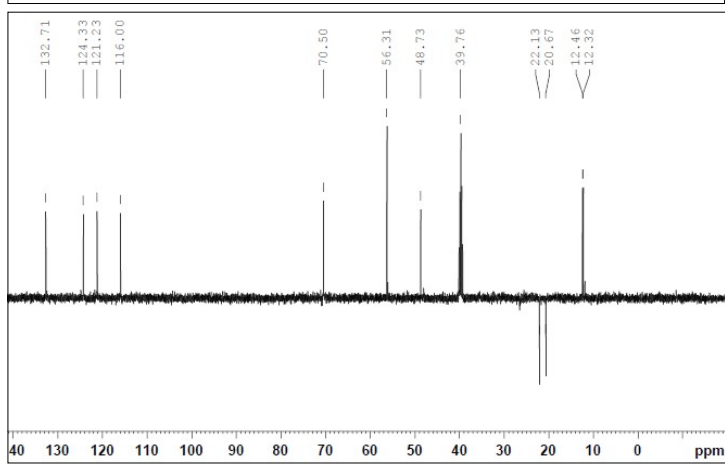
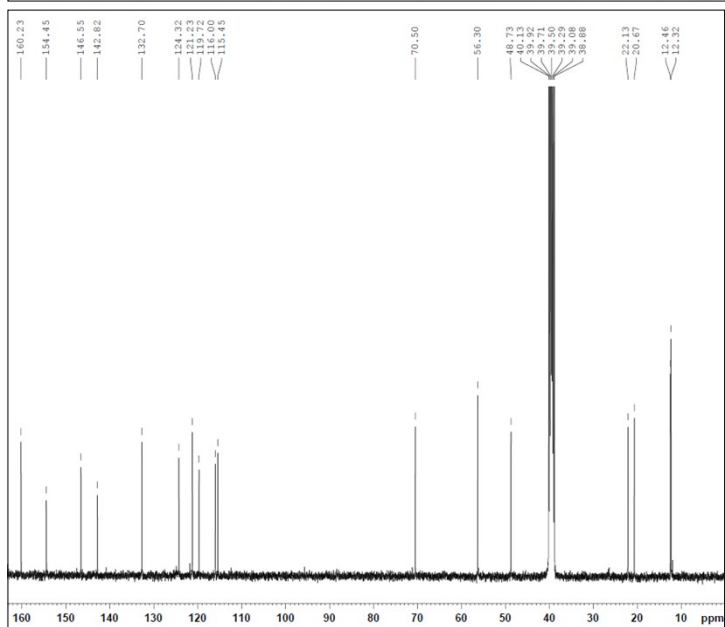
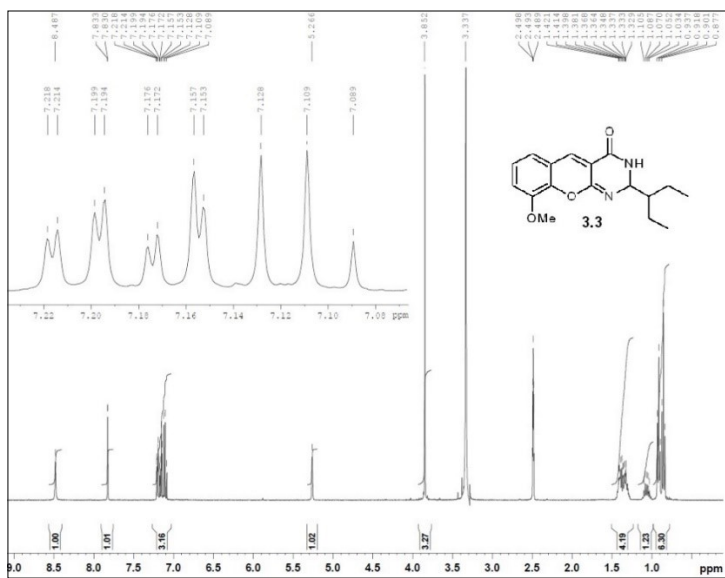
¹These authors contributed equally

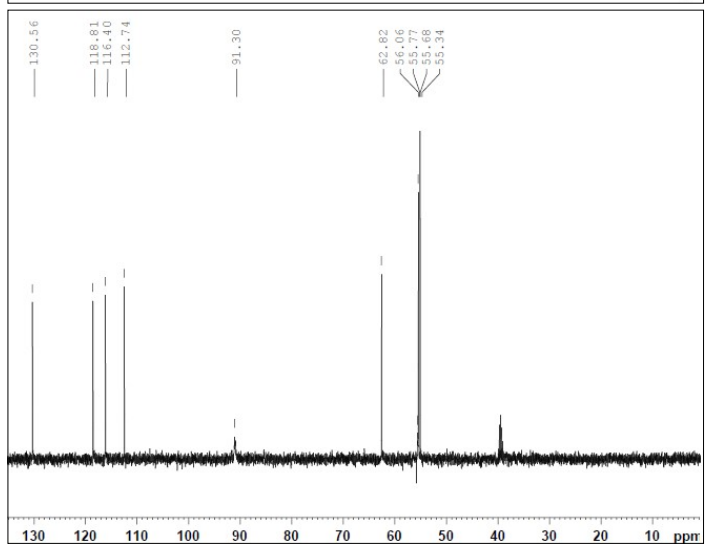
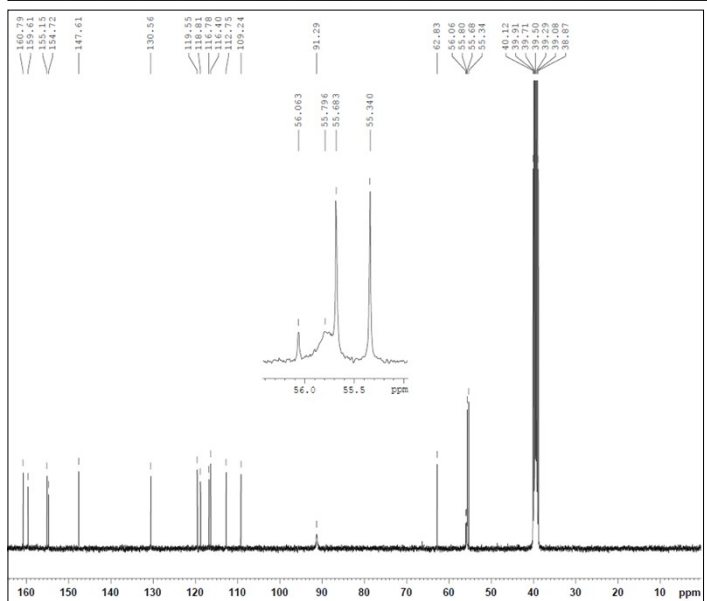
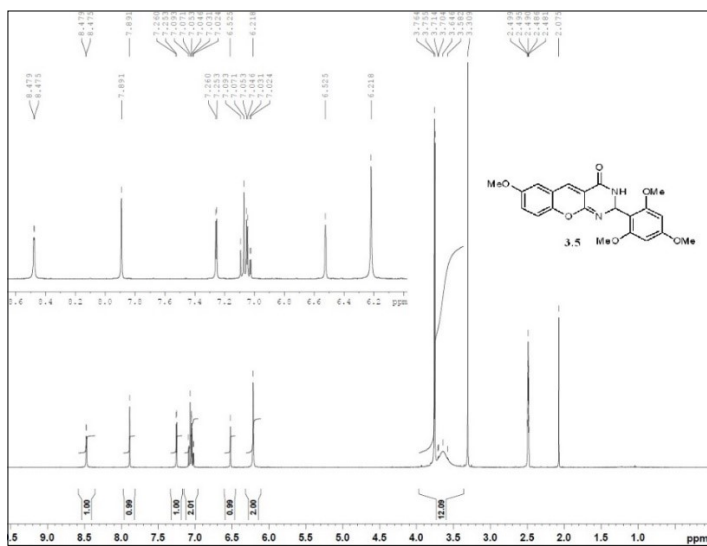
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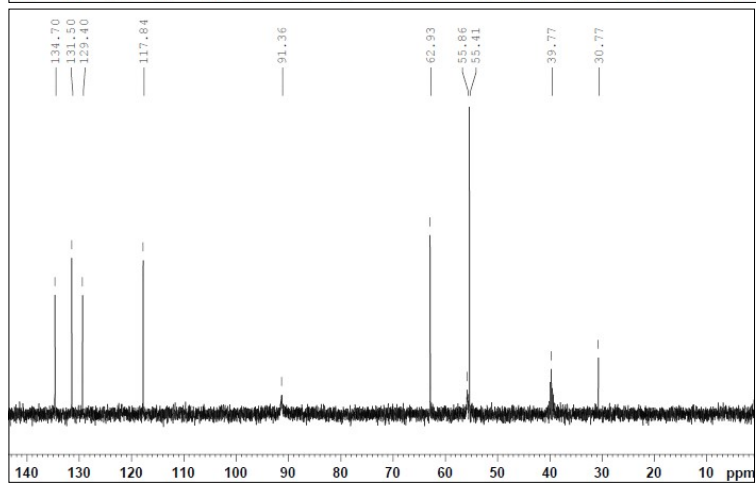
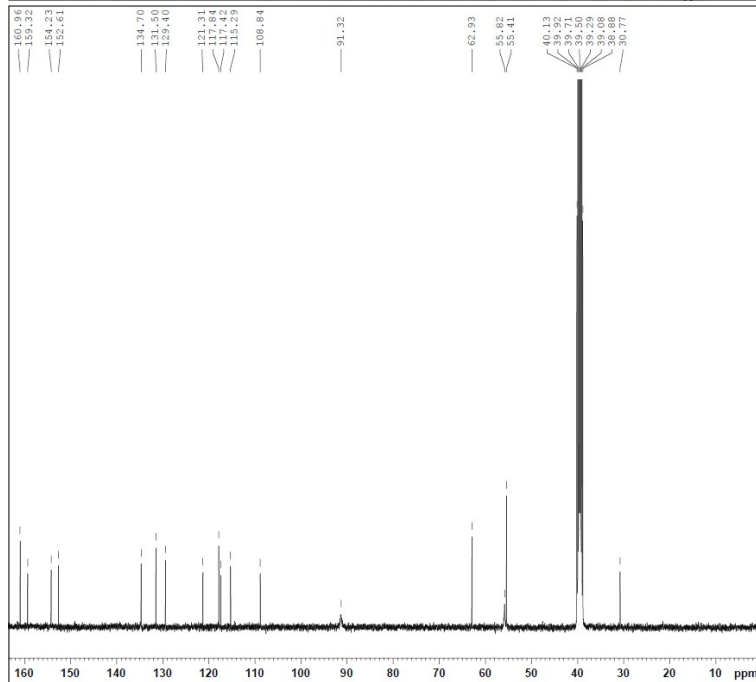
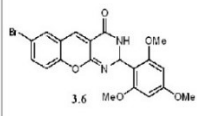
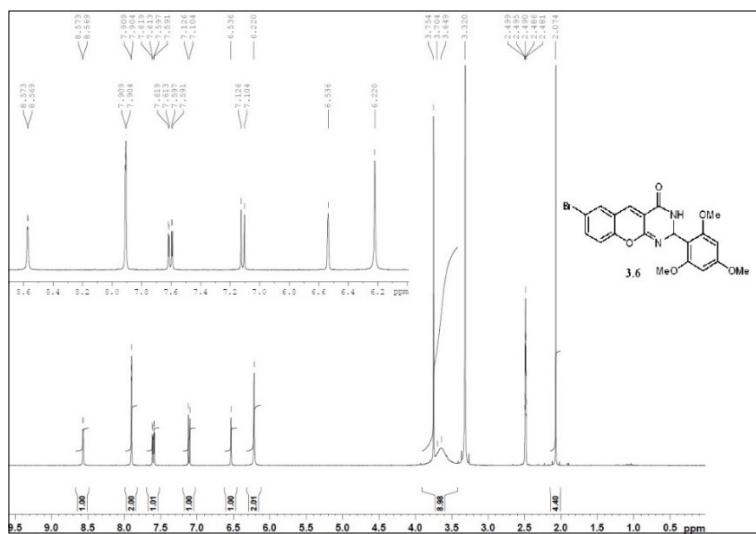
- General compound characterization data, ^1H , ^{13}C and DEPT 135 NMR spectra (400 Mhz, DMSO- d_6 , δ ppm) of compounds **2.6**, **2.7**, **3.1-3.6**, **4.1-4.30**, **5**, **6**, **7** and **8**, page S3-43
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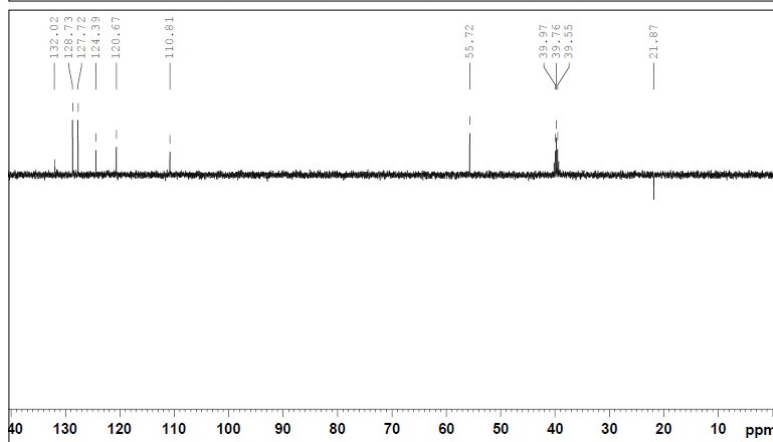
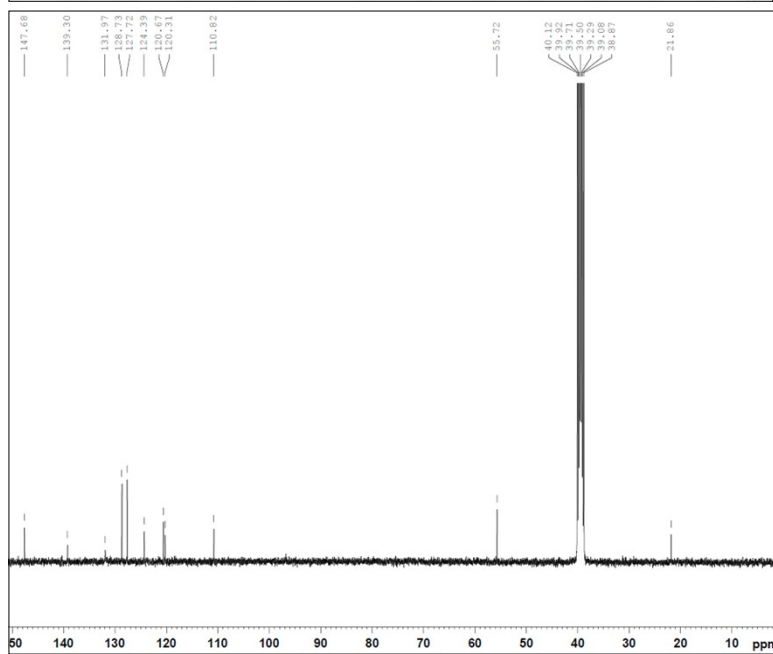
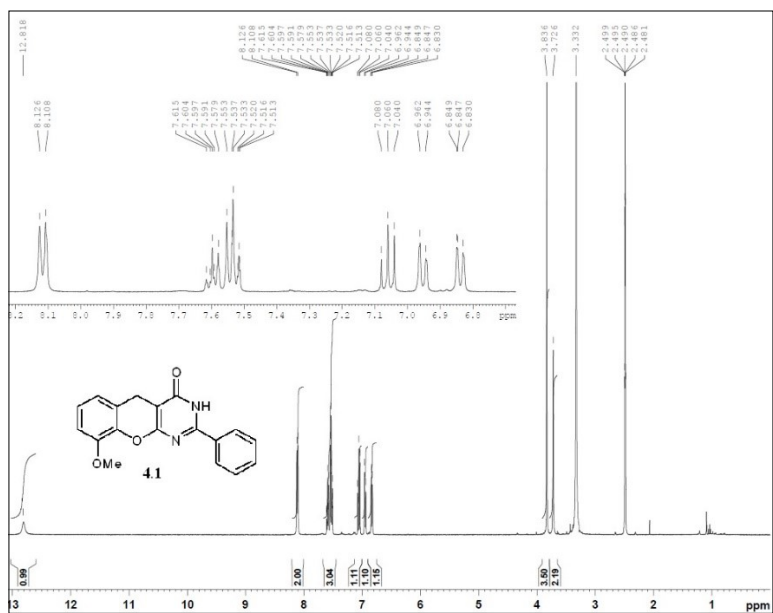


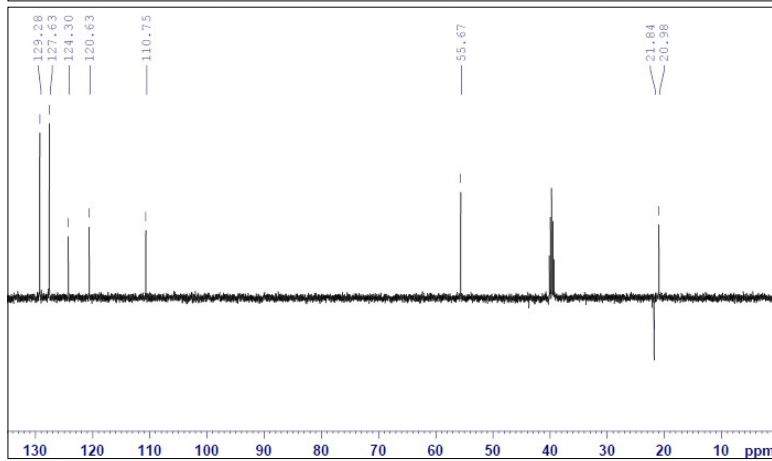
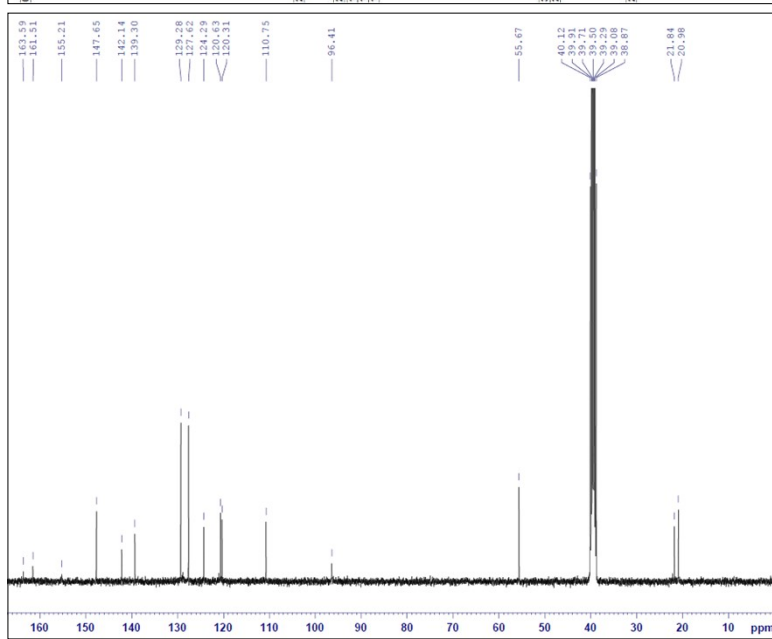
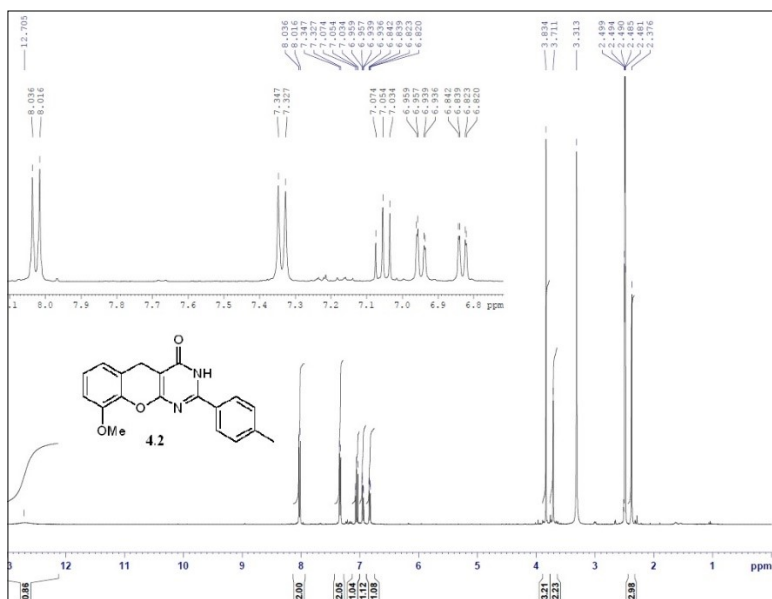


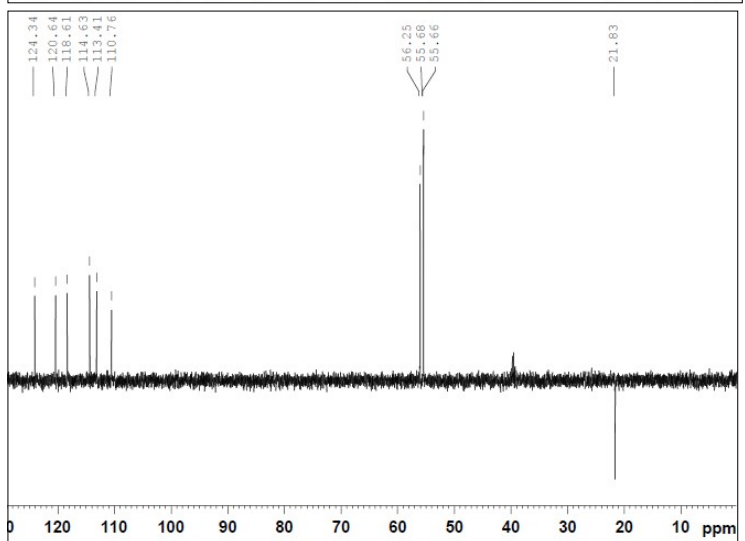
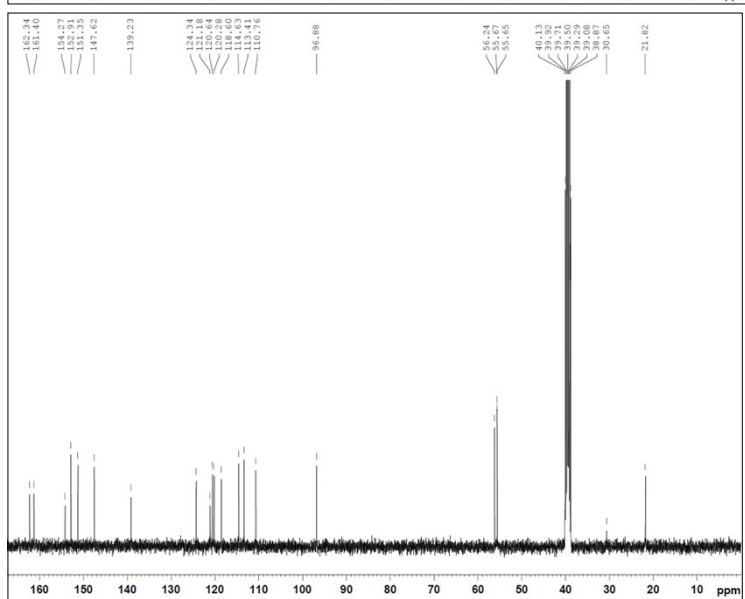
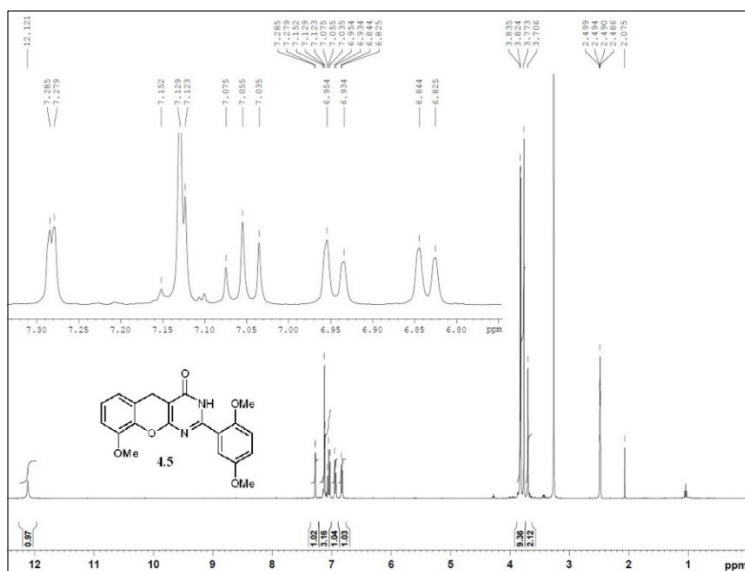


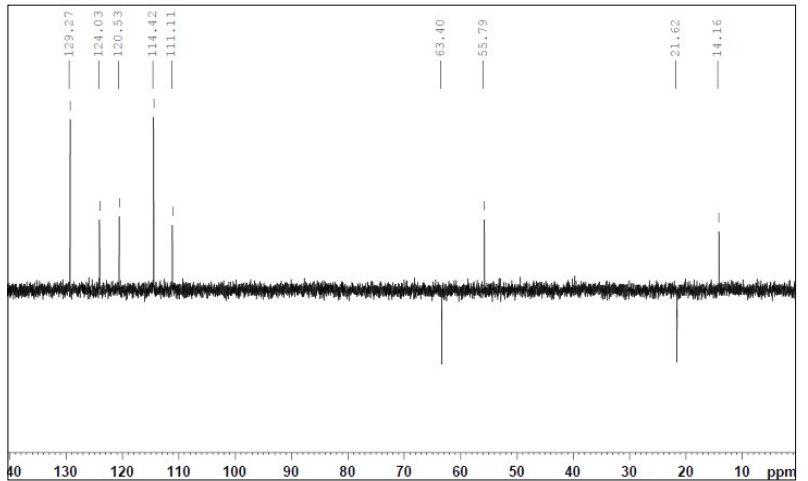
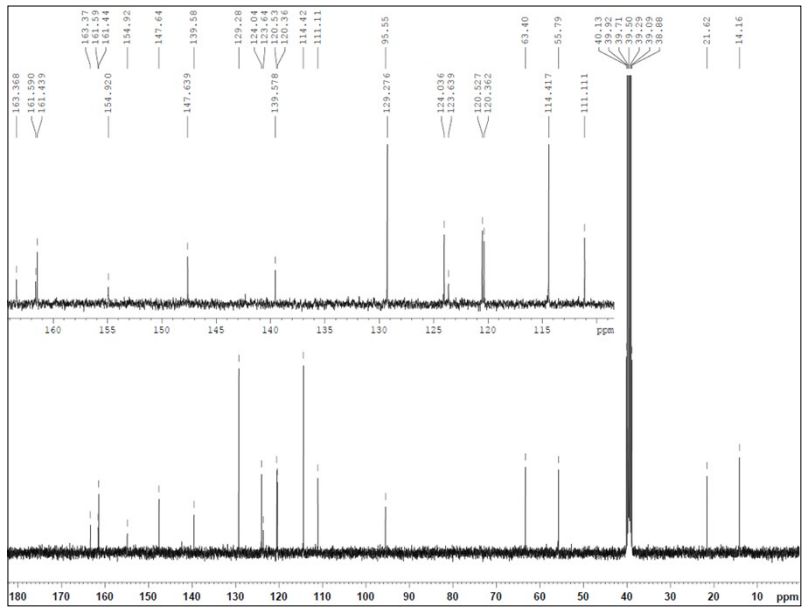
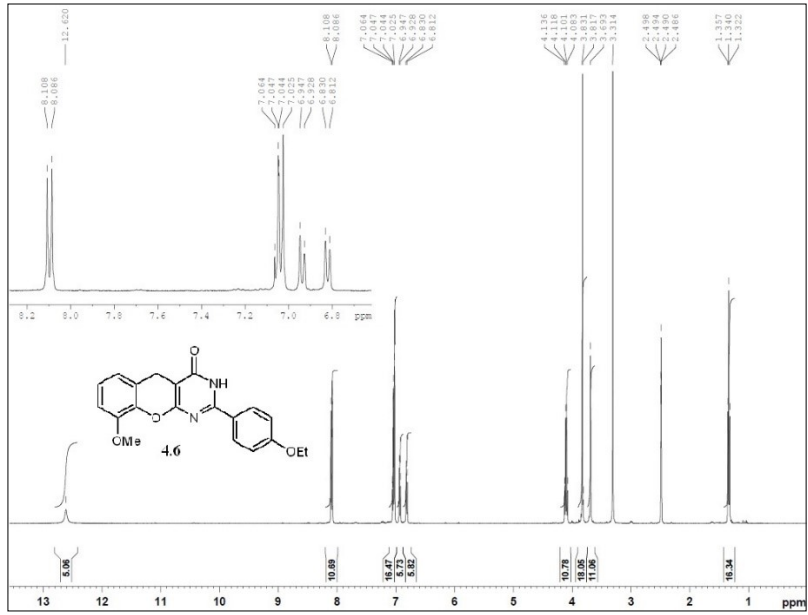


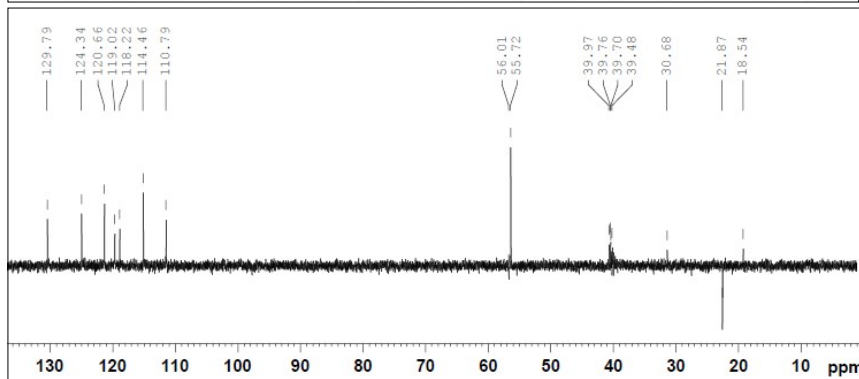
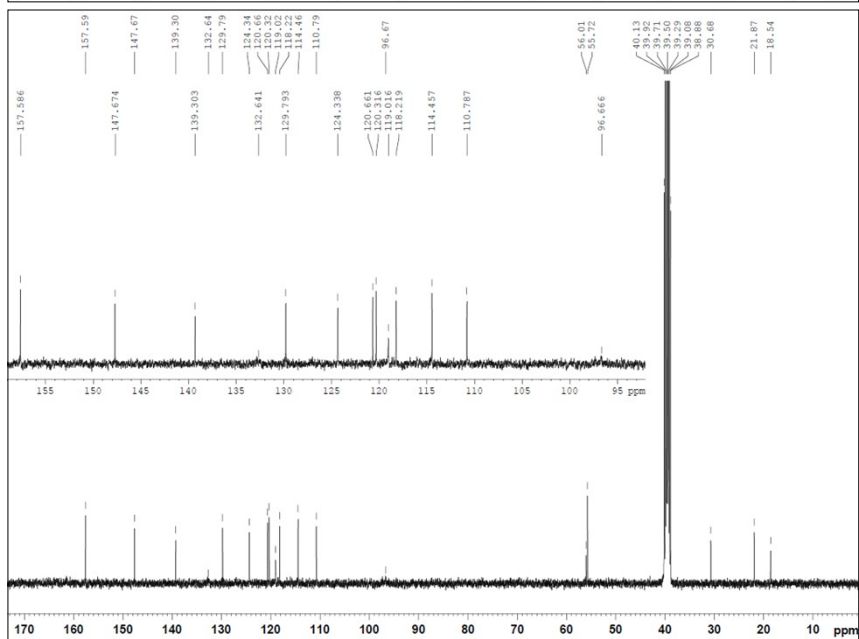
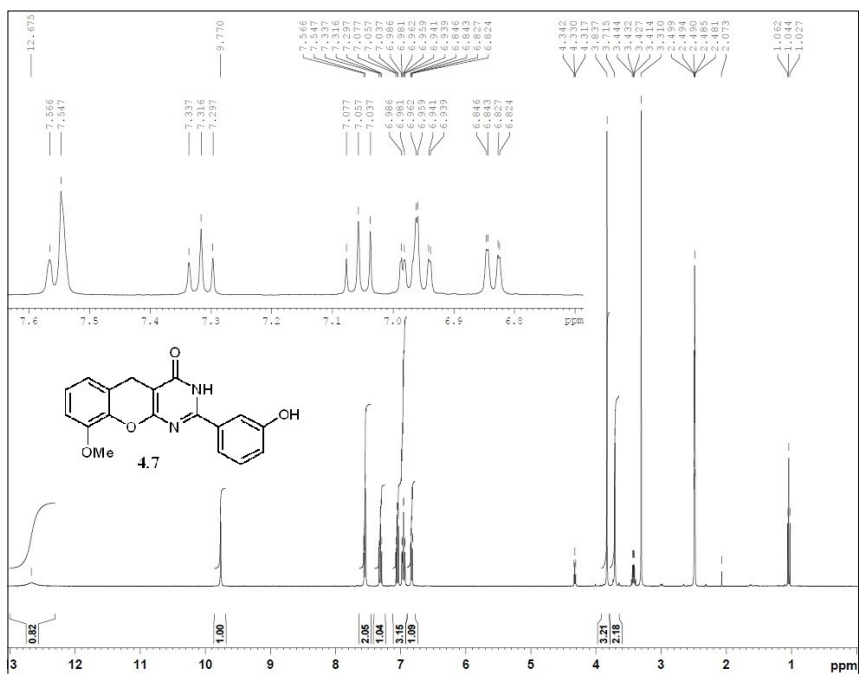


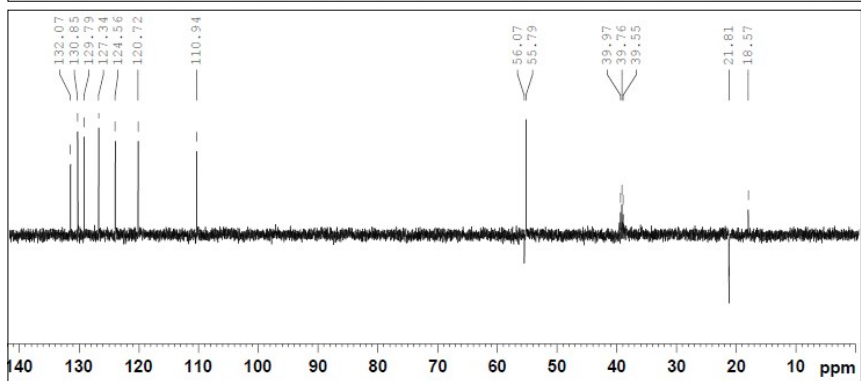
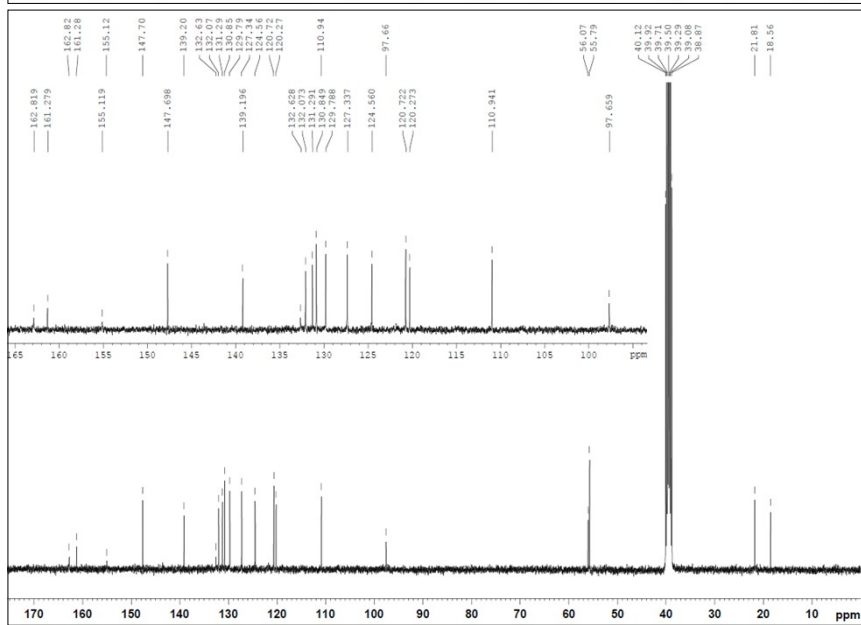
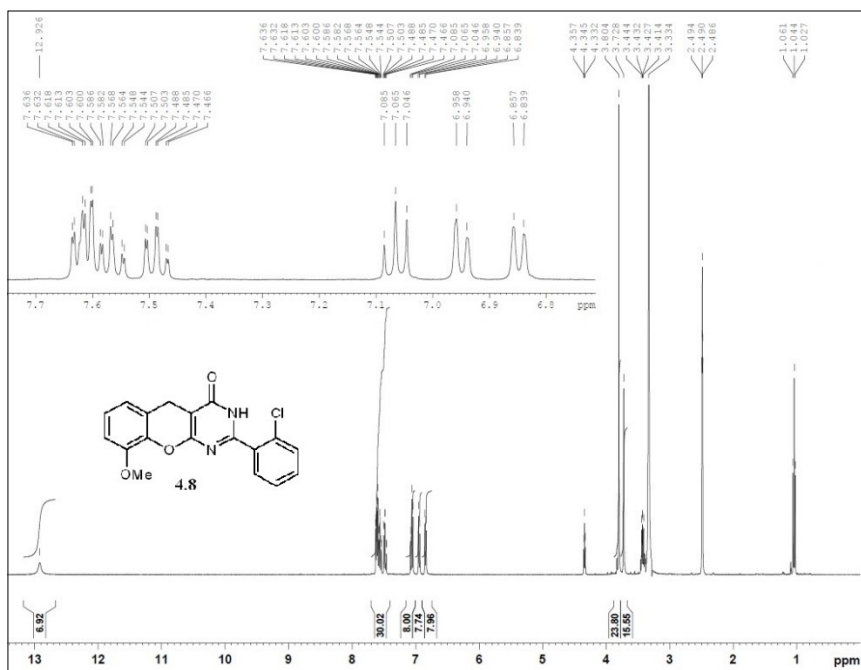


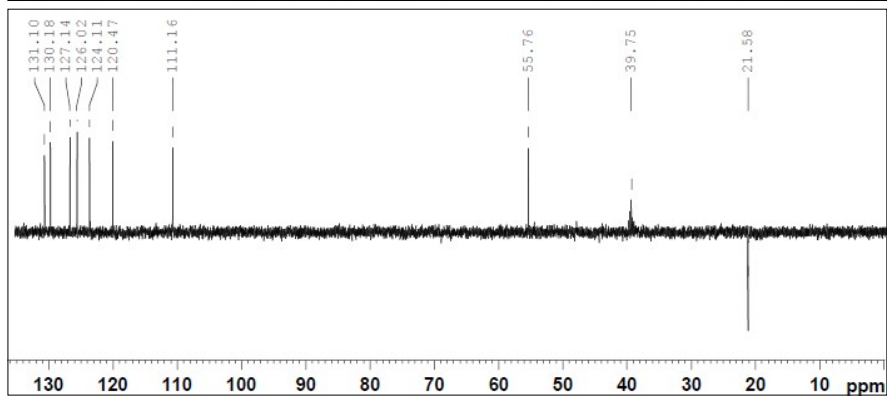
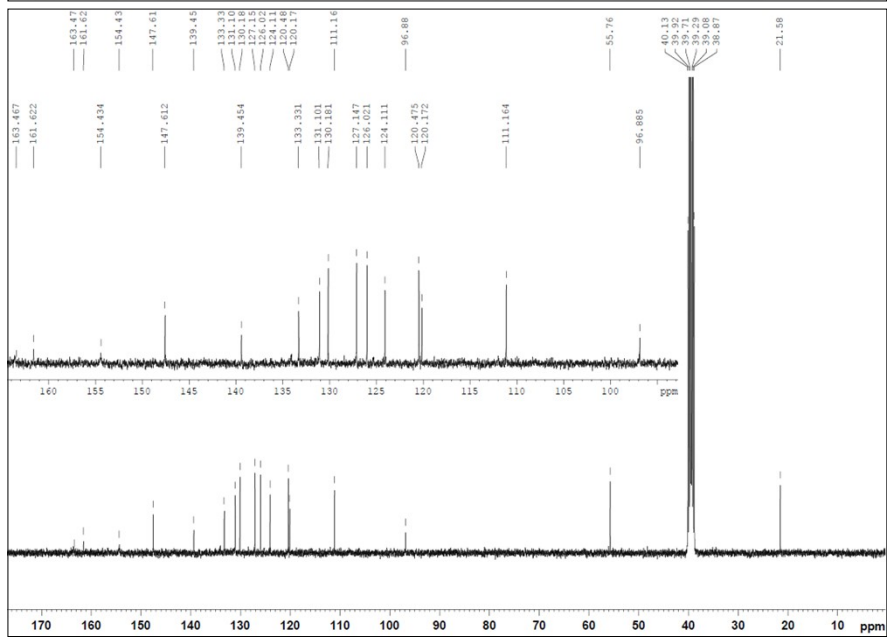
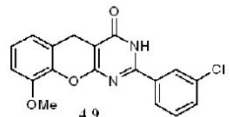
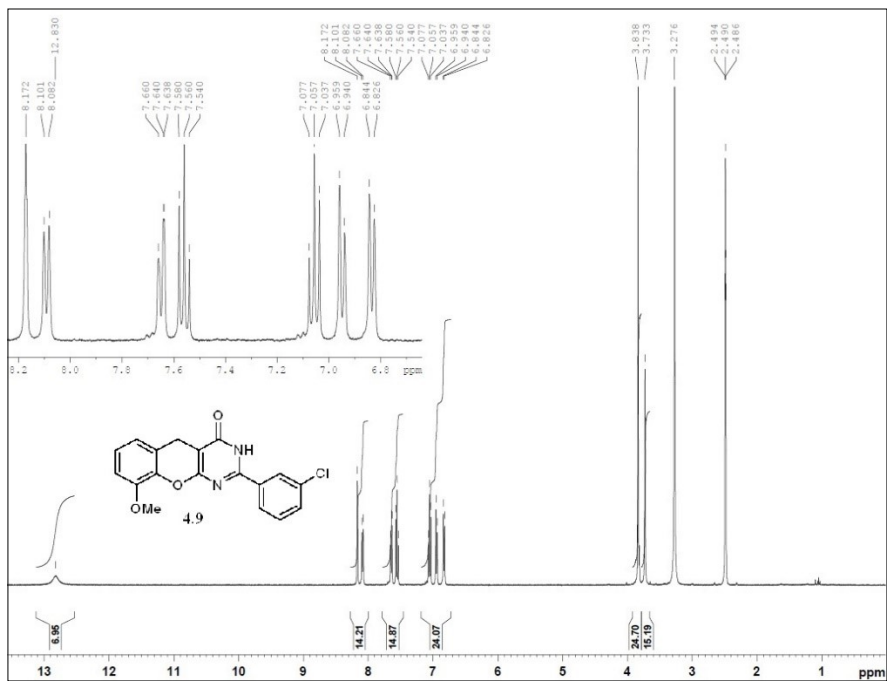


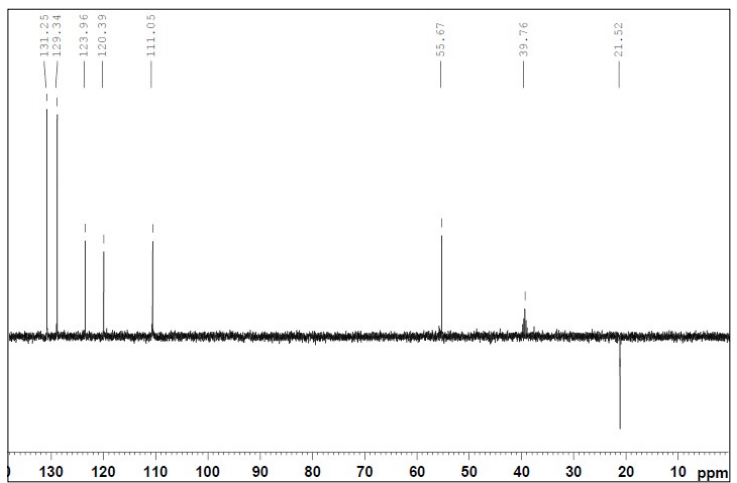
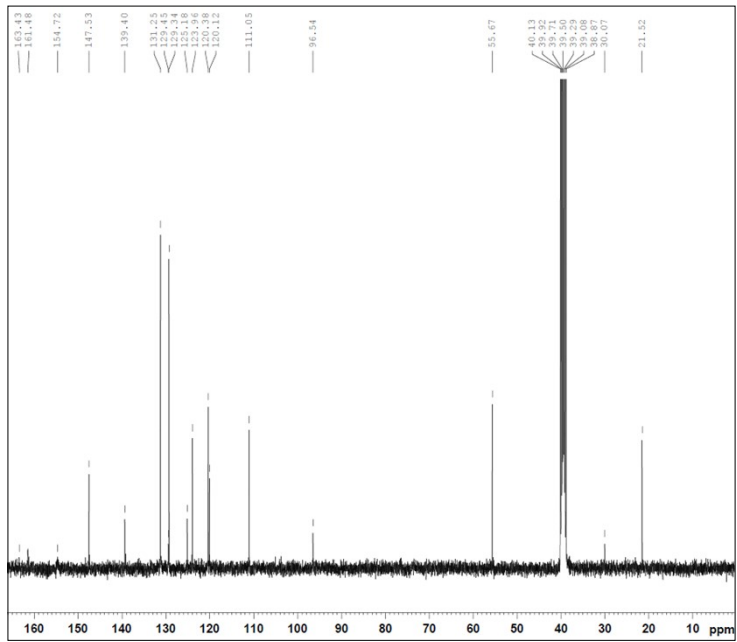
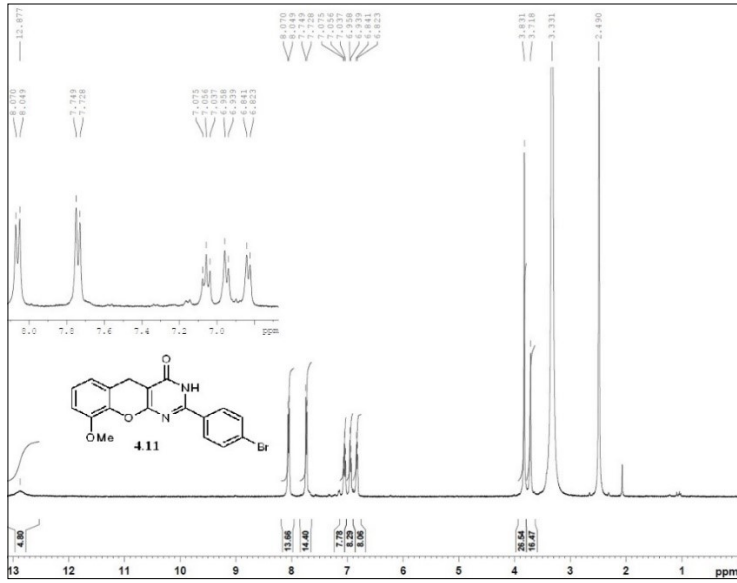


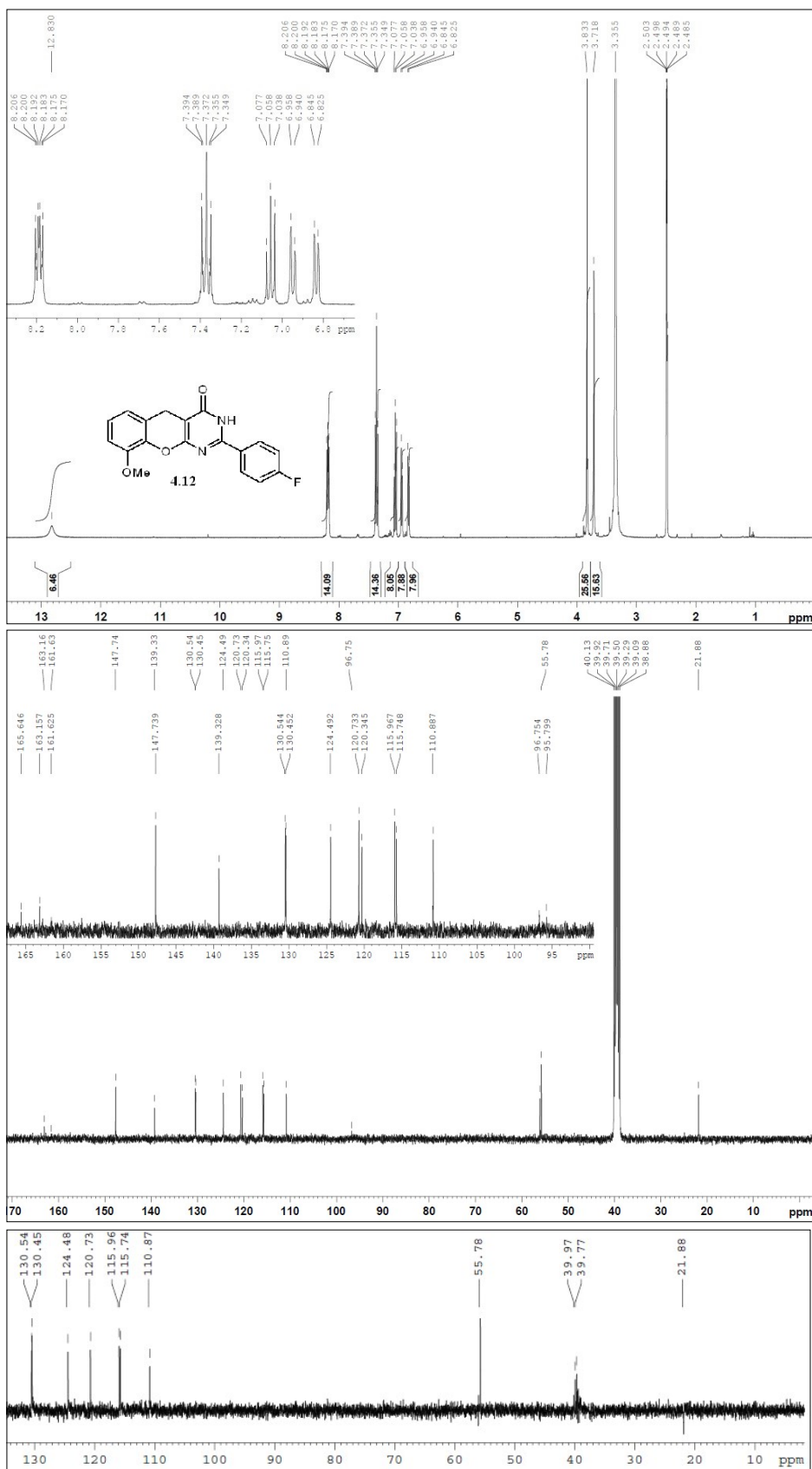


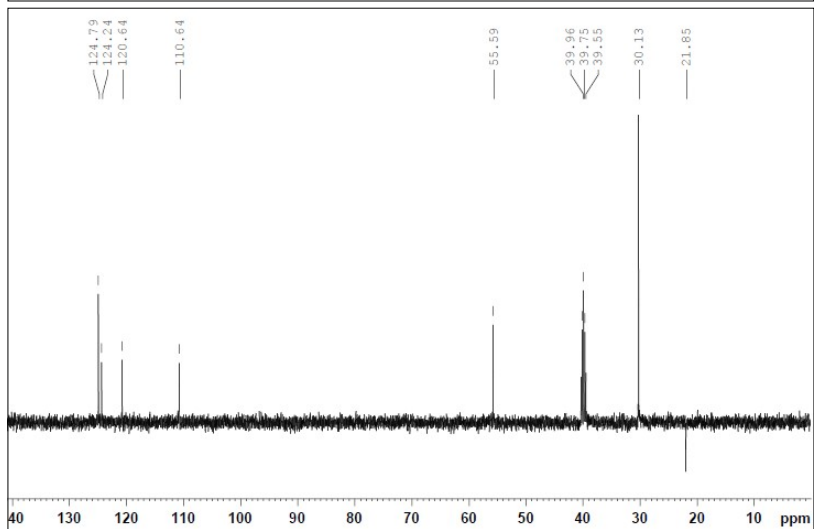
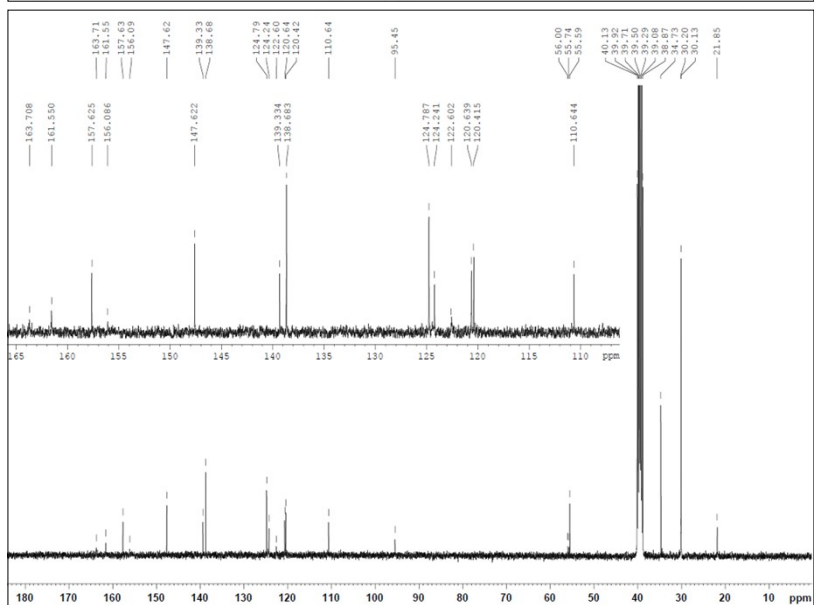
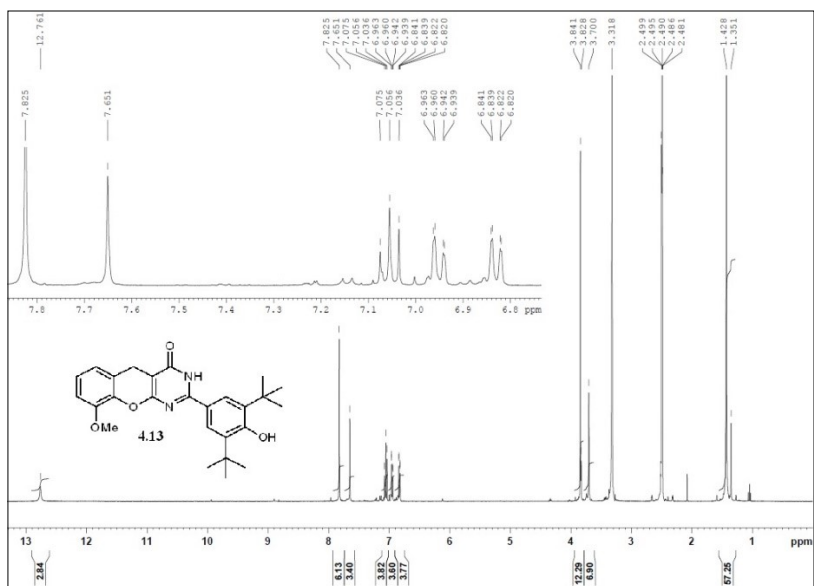


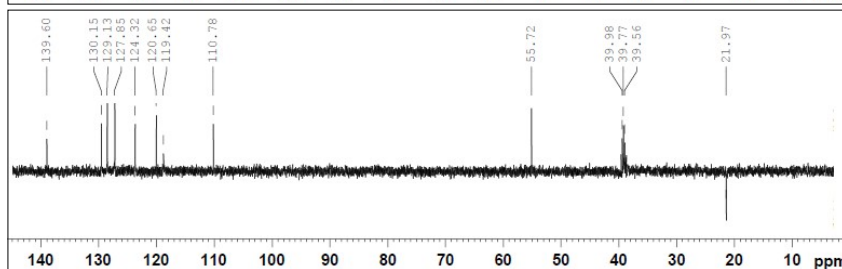
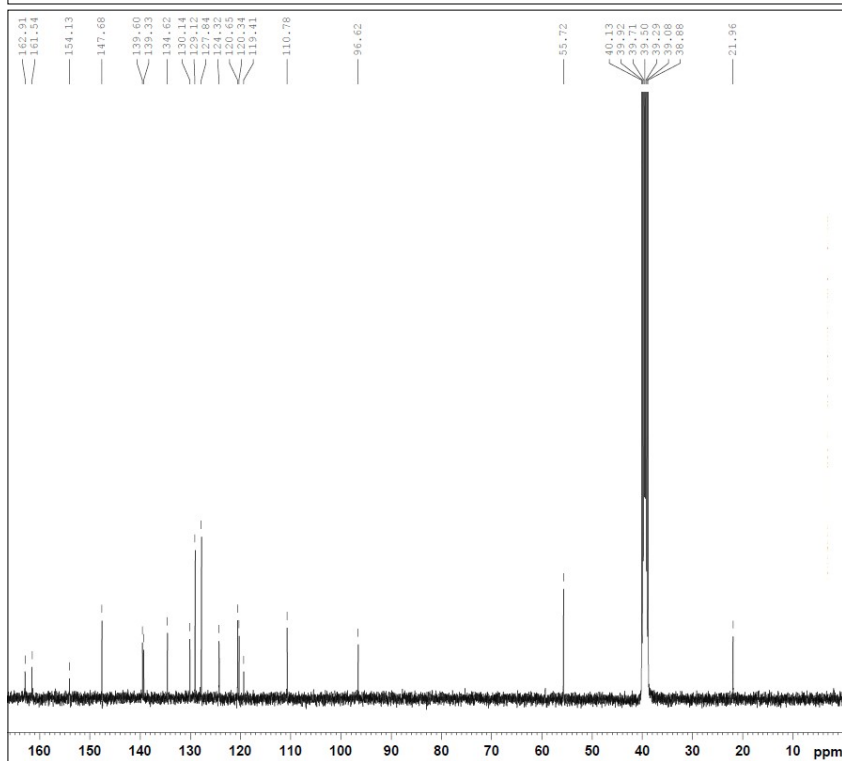
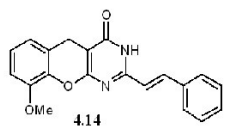
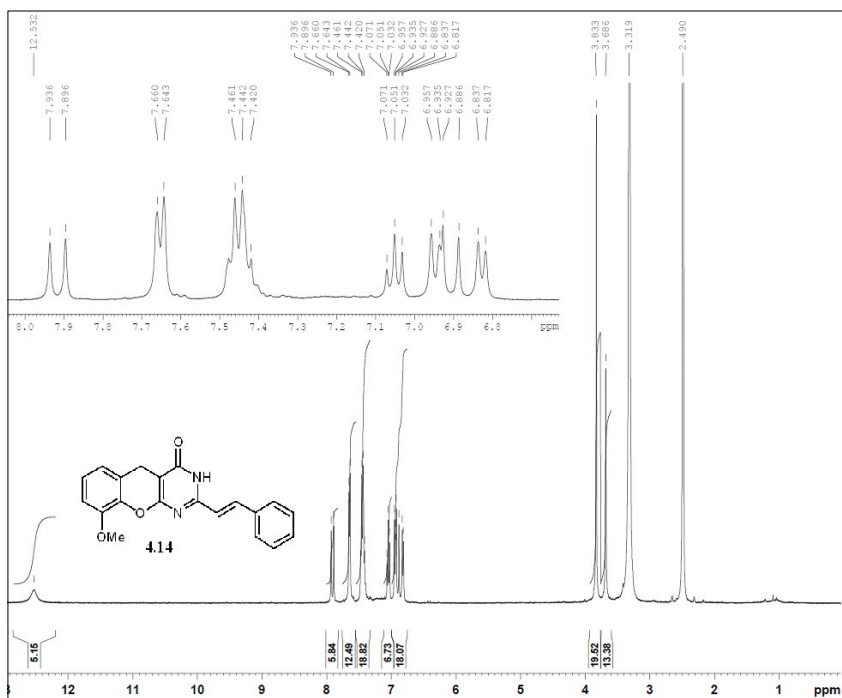


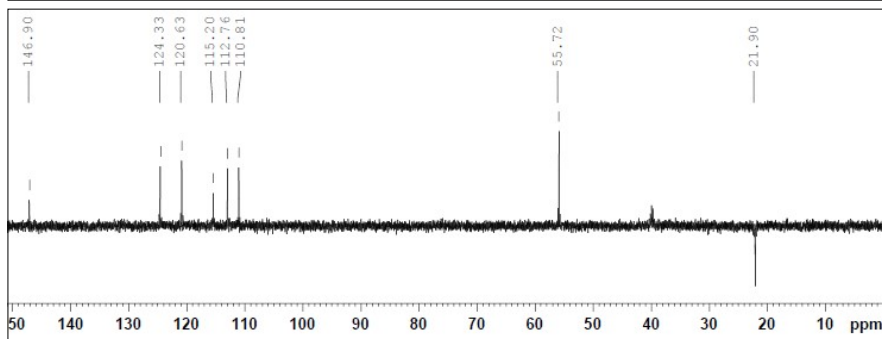
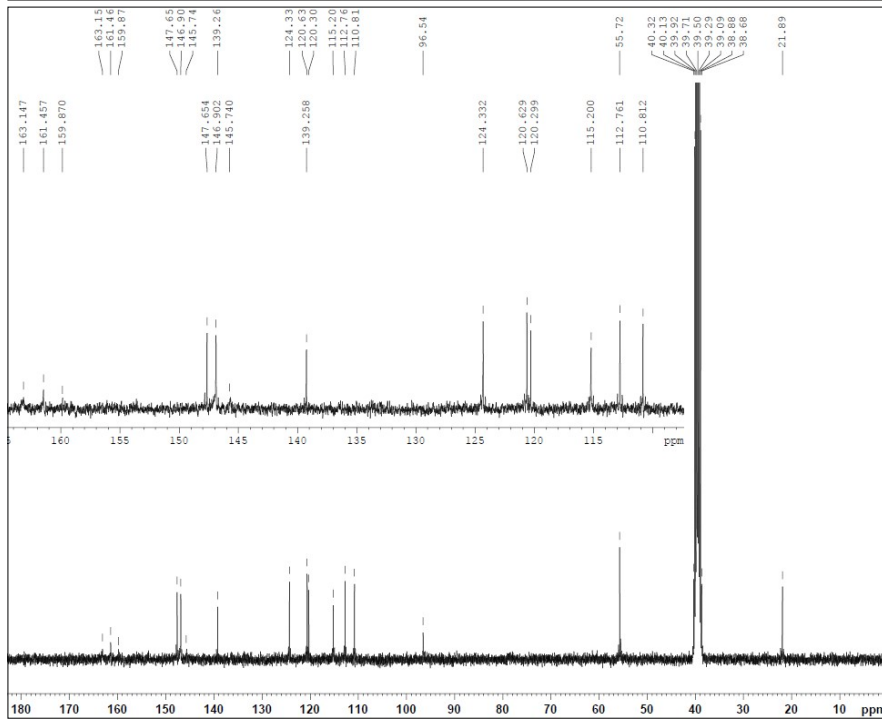
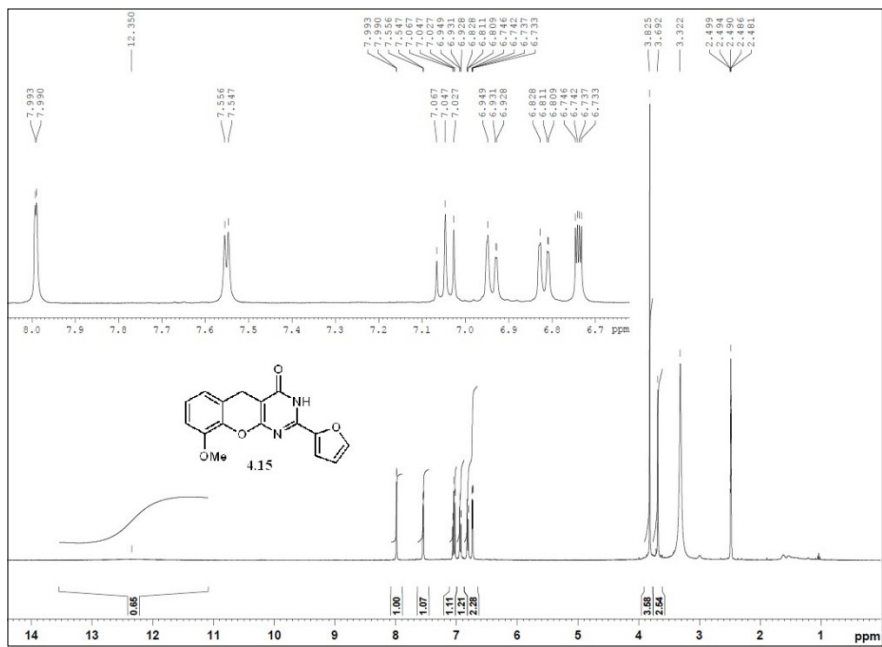


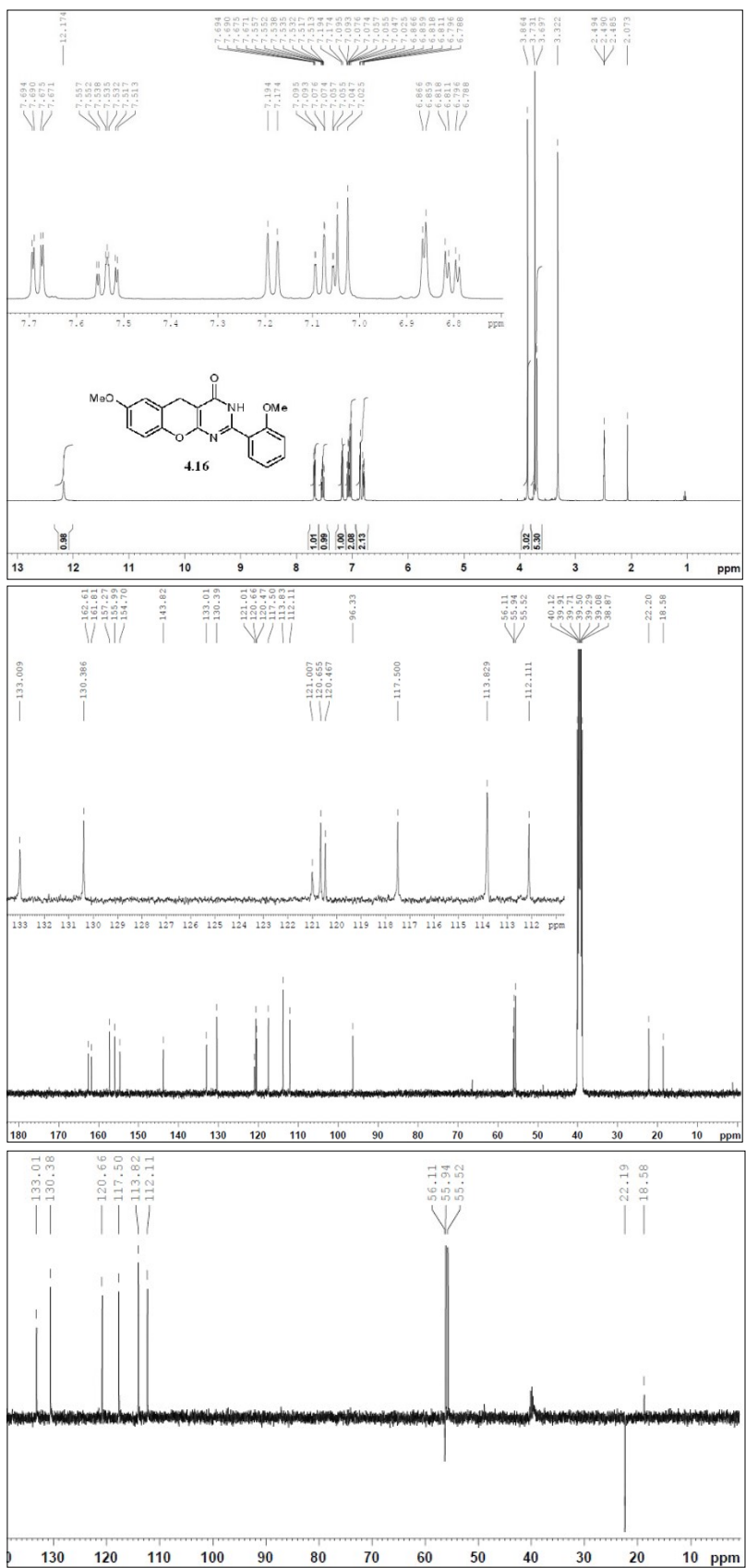


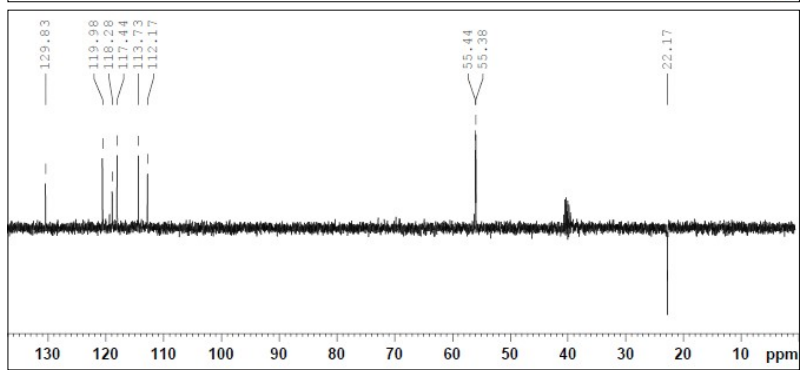
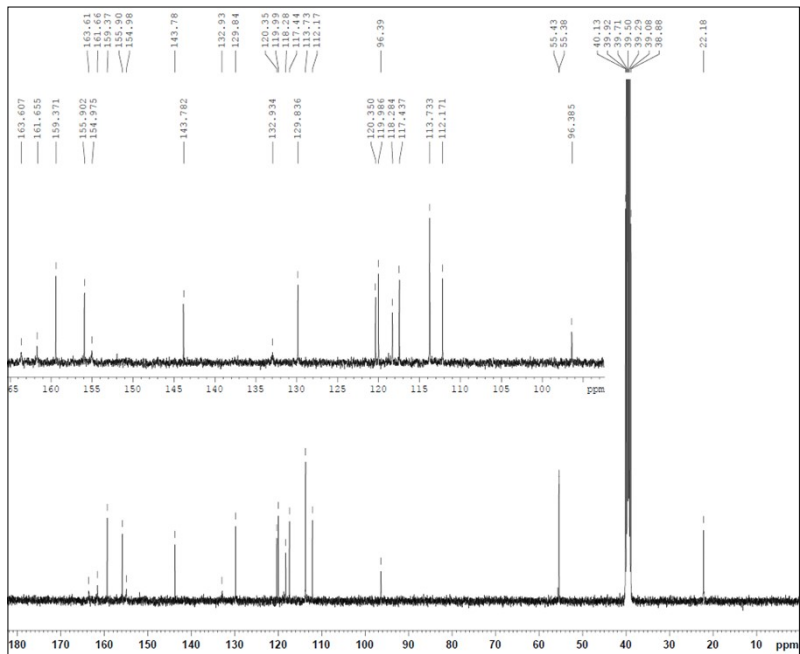
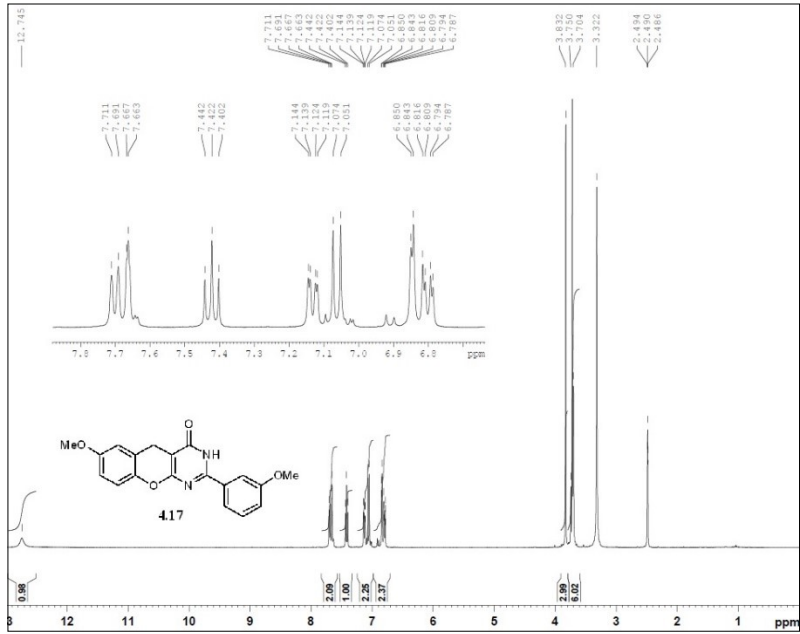


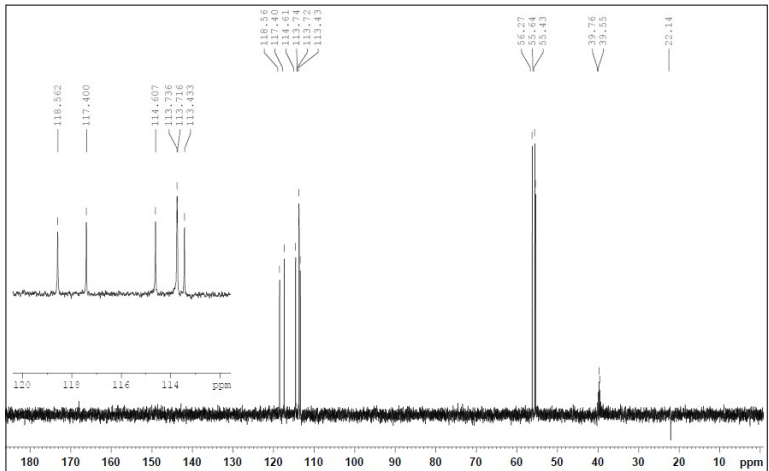
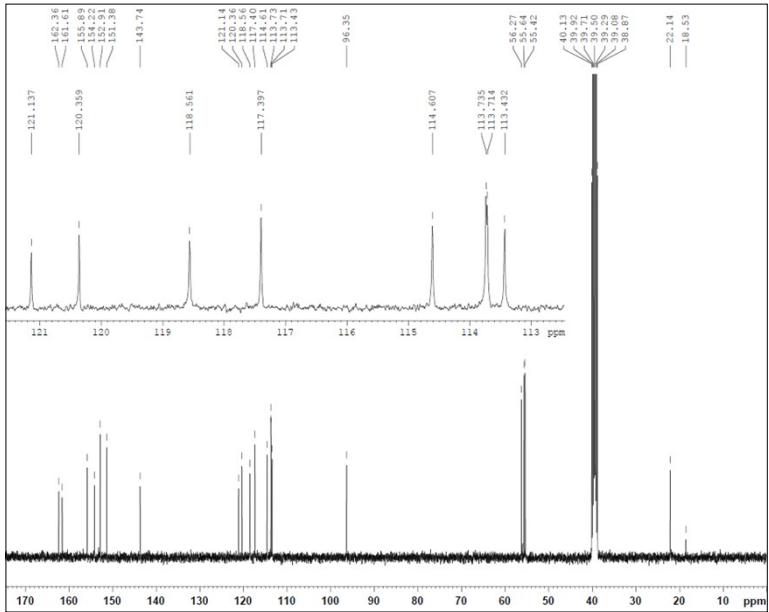
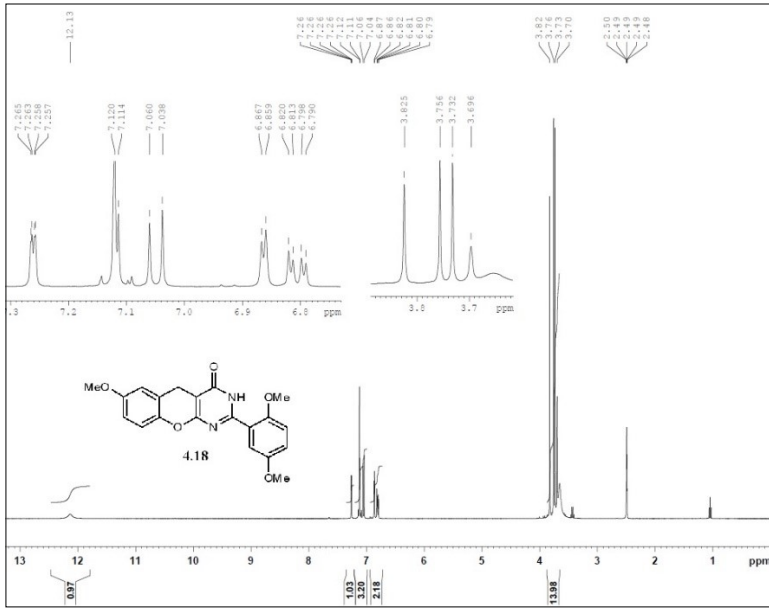


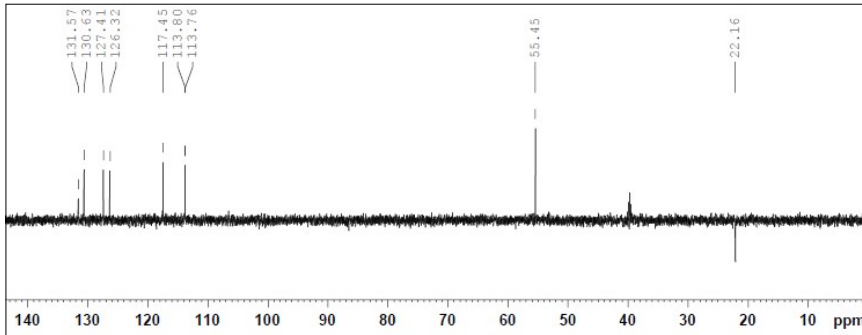
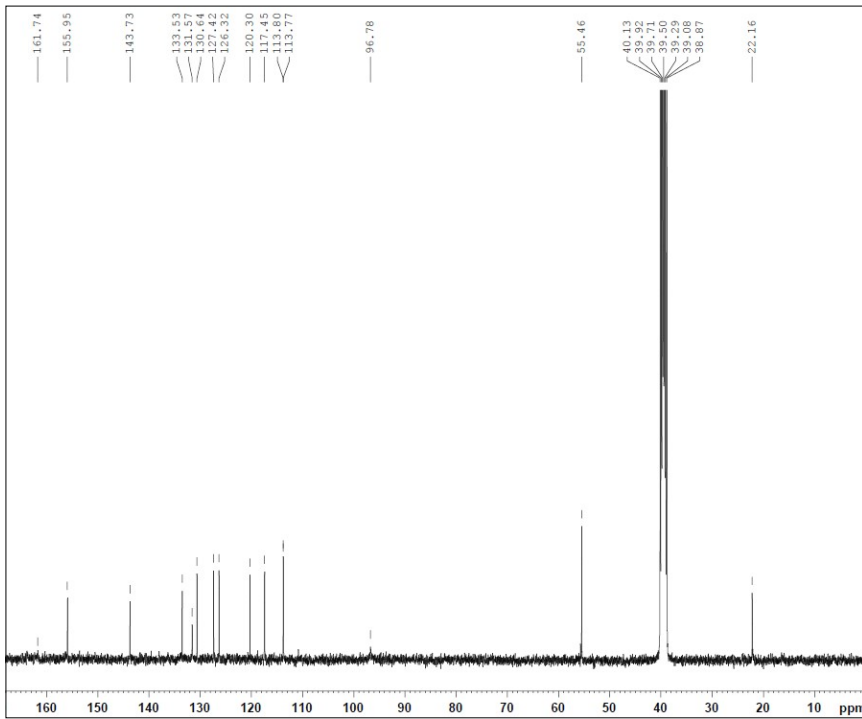
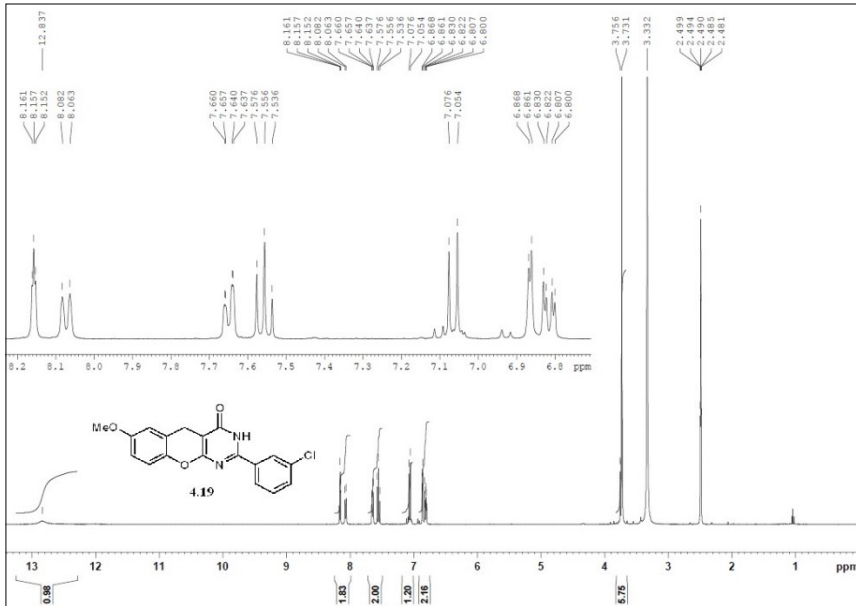


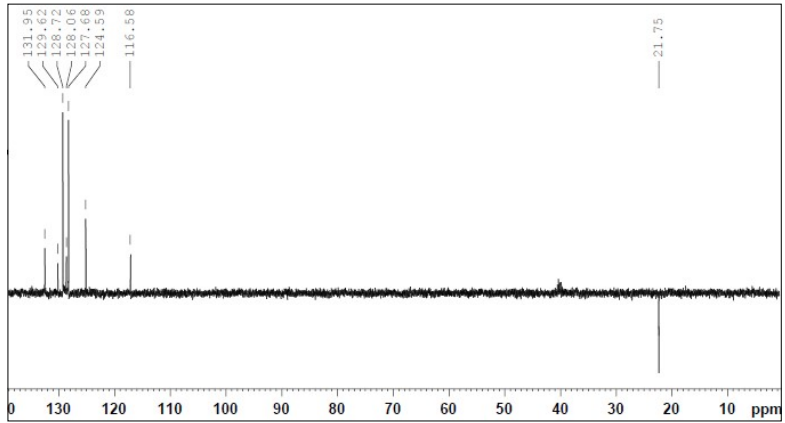
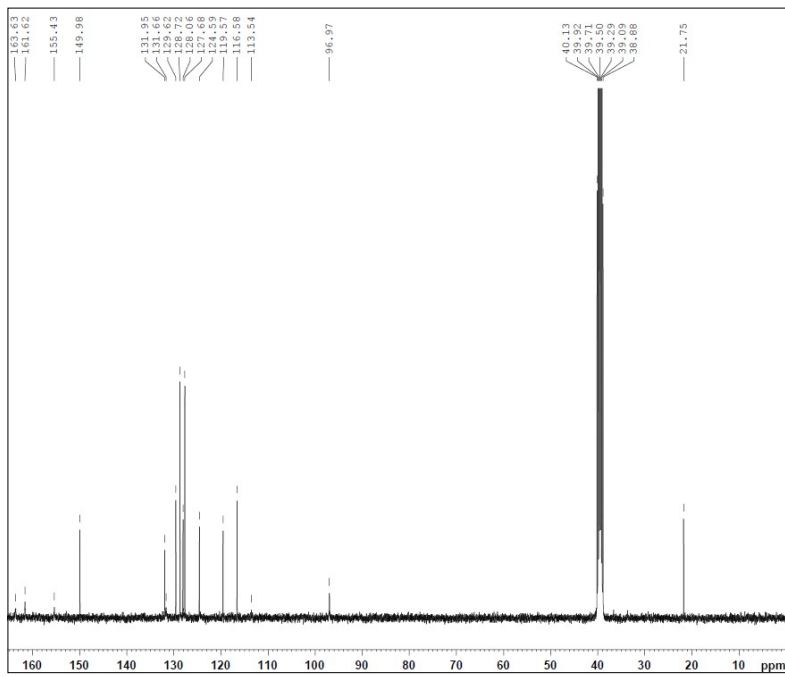
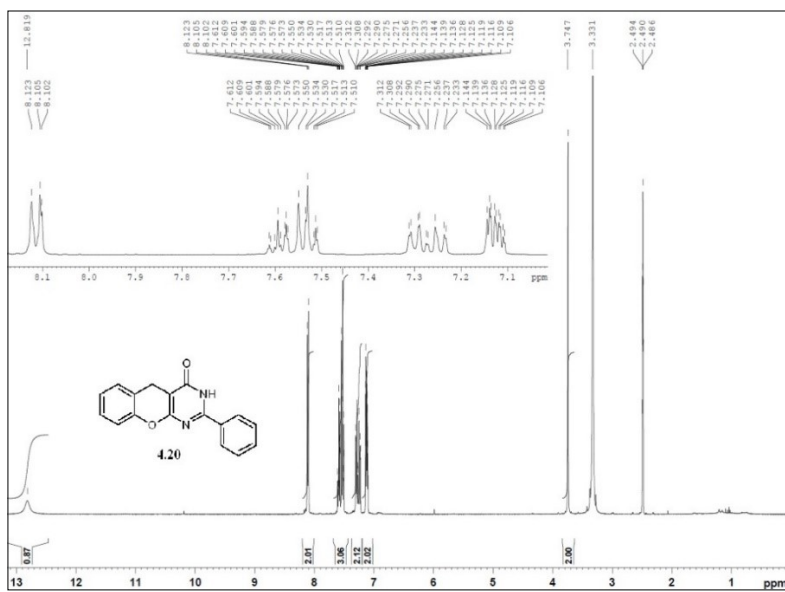


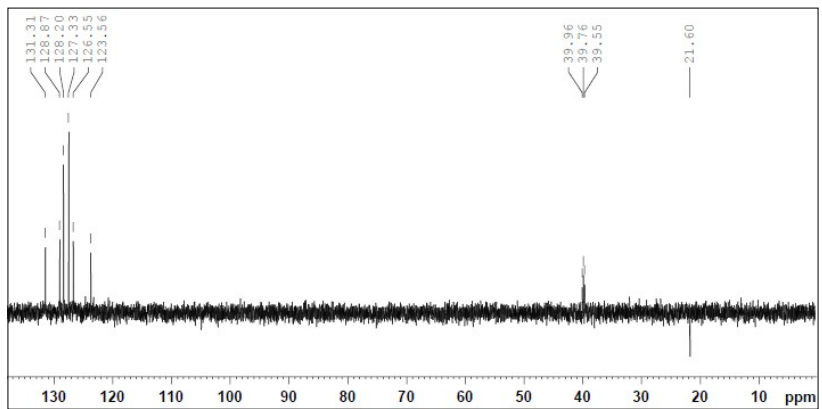
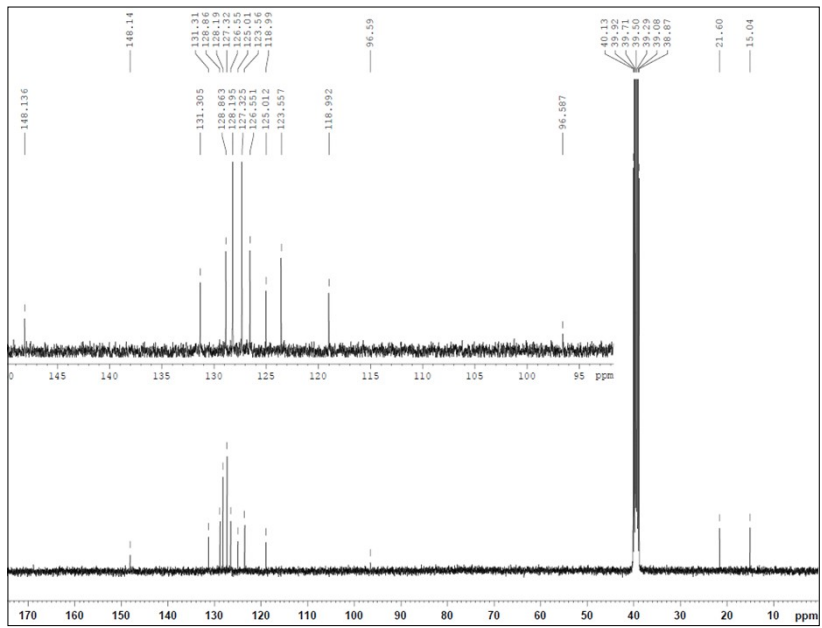
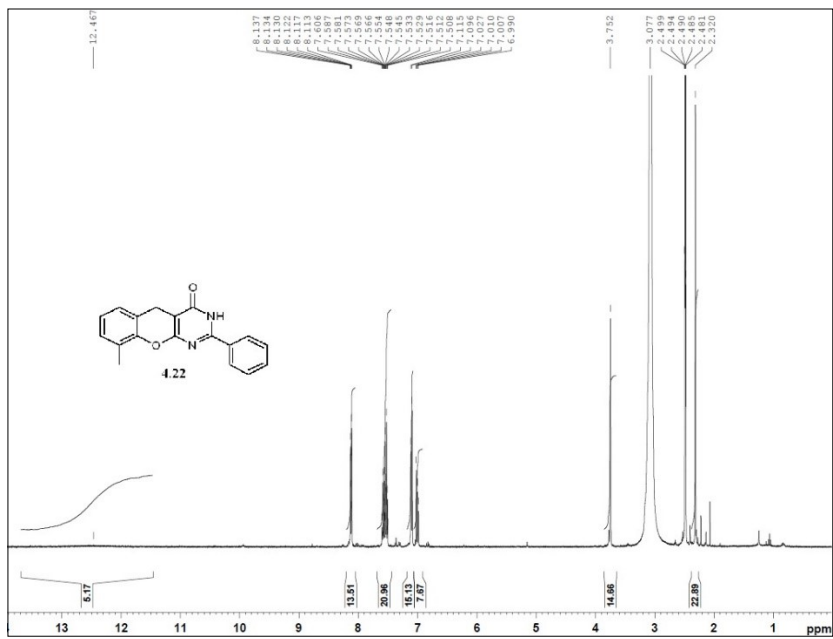


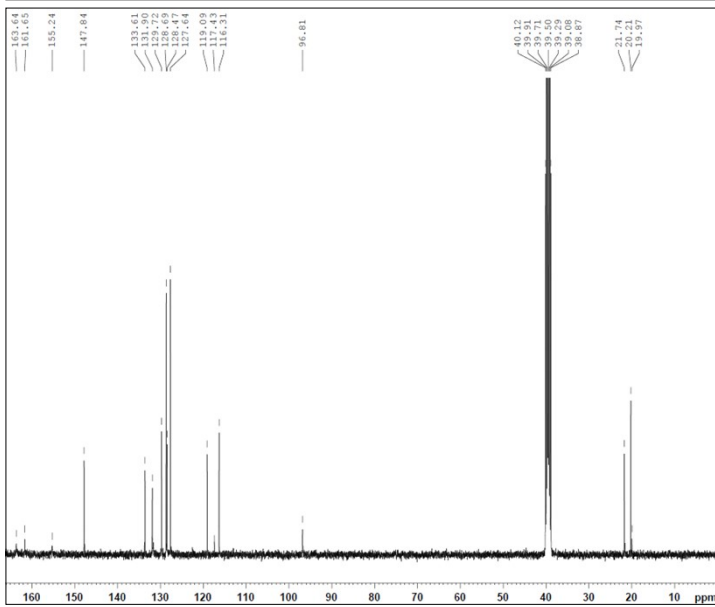
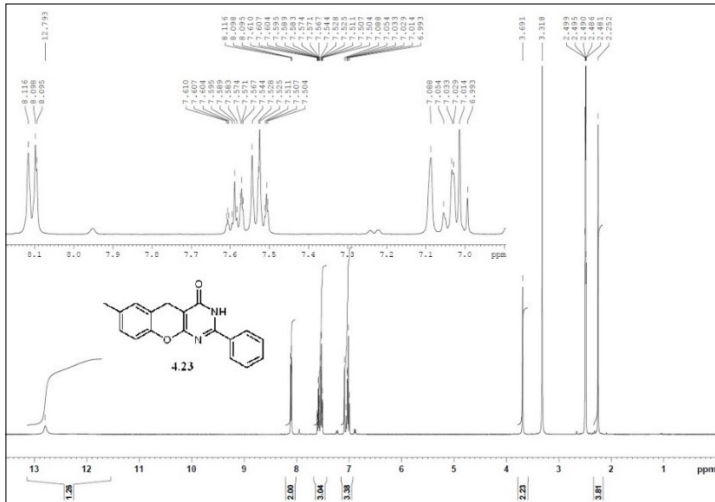


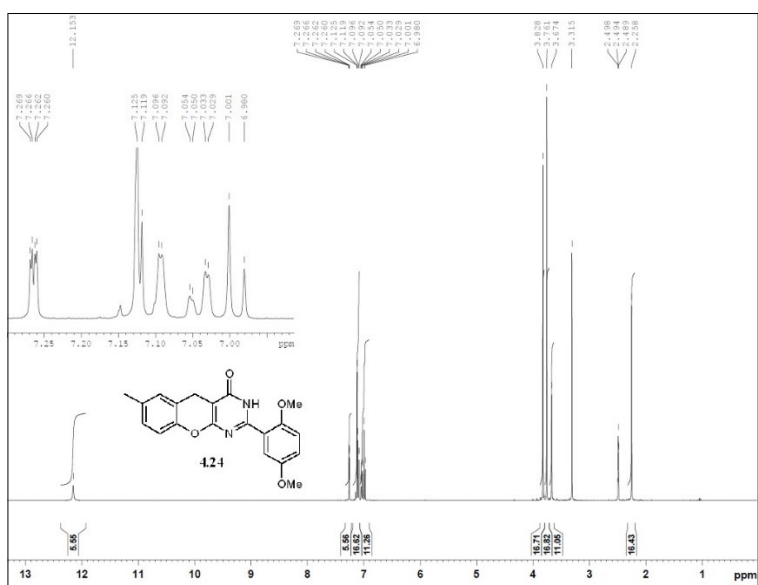
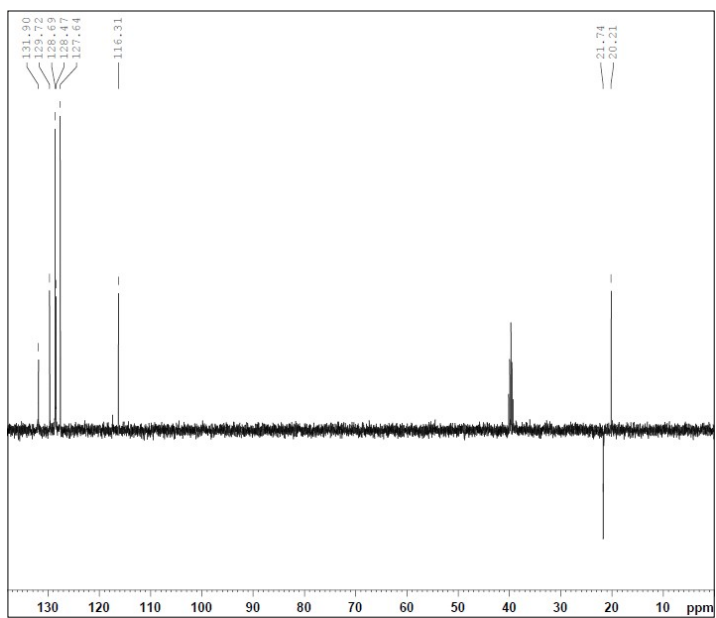


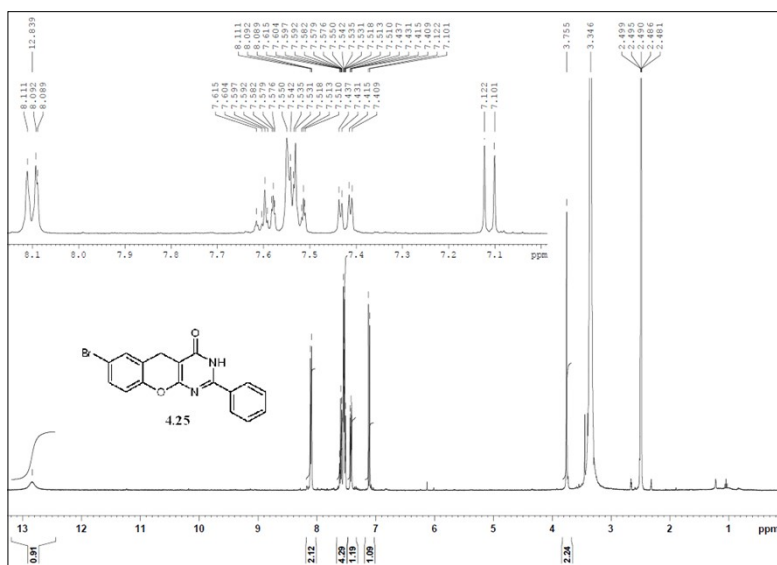
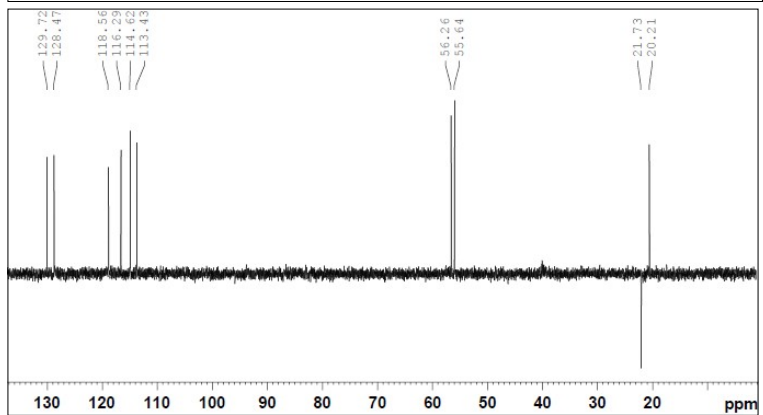
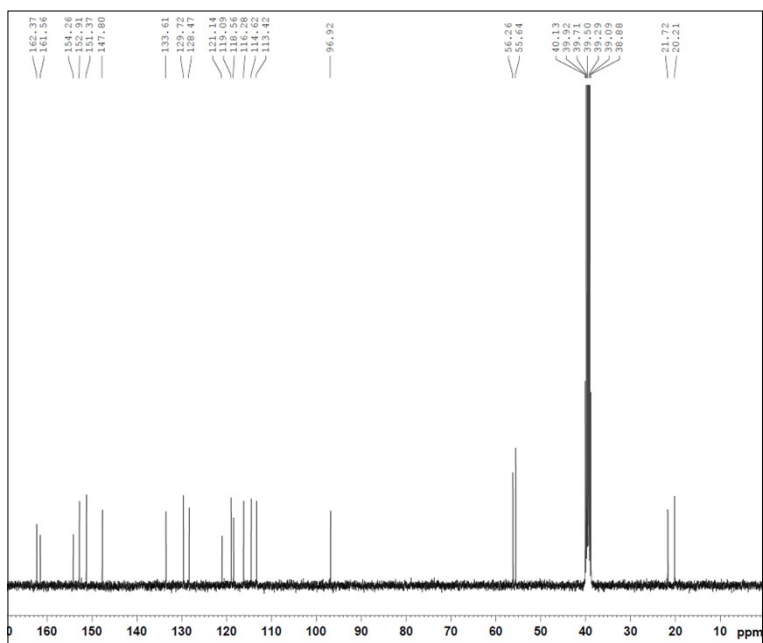


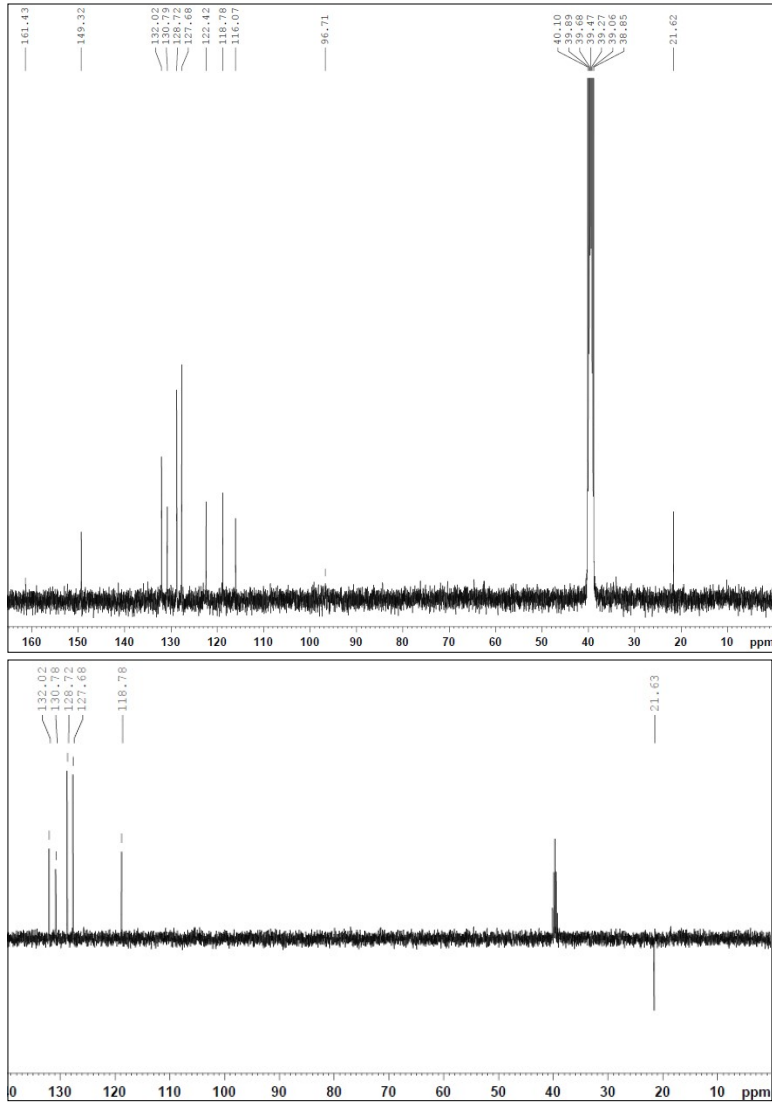


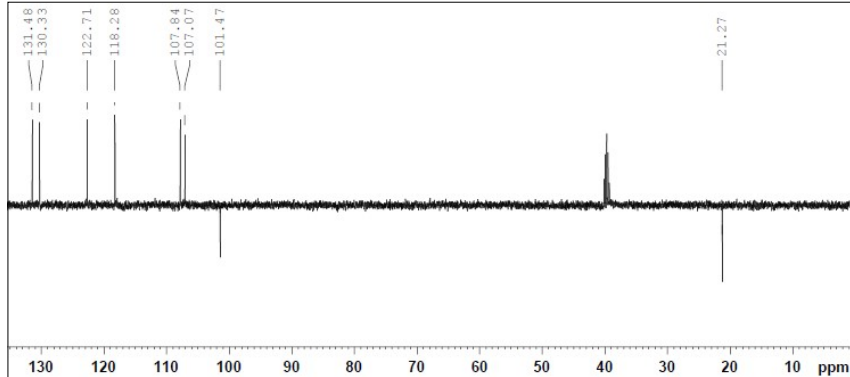
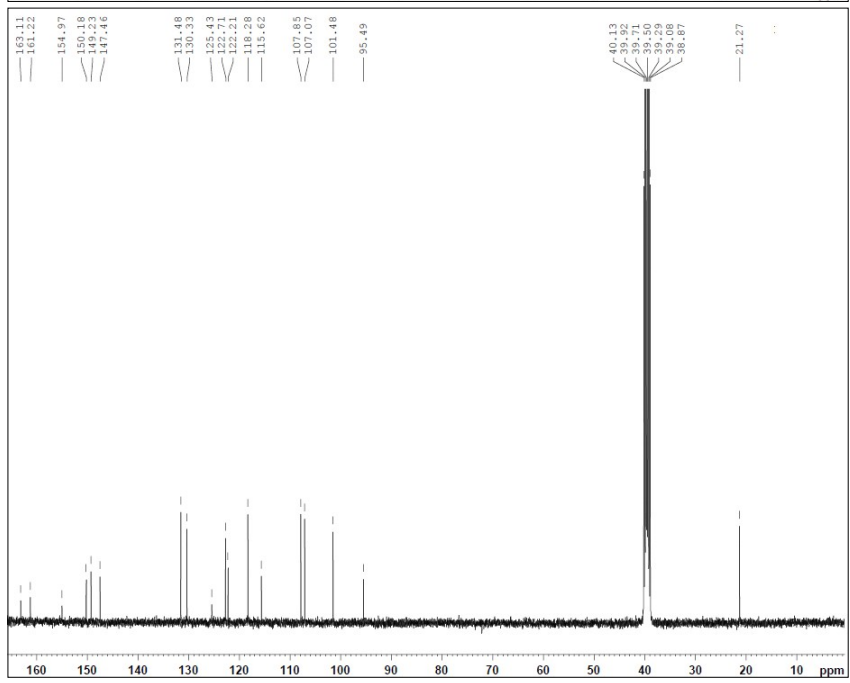
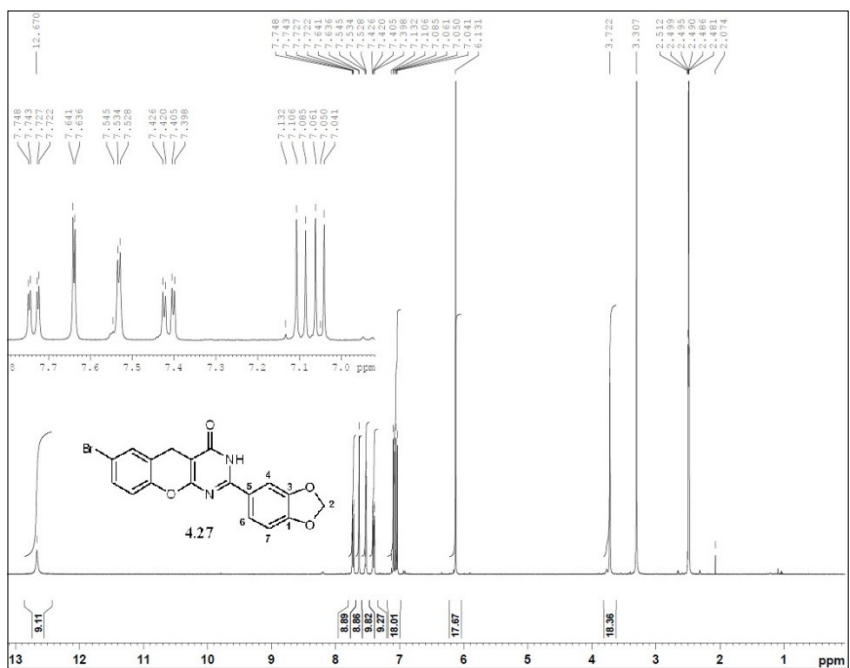


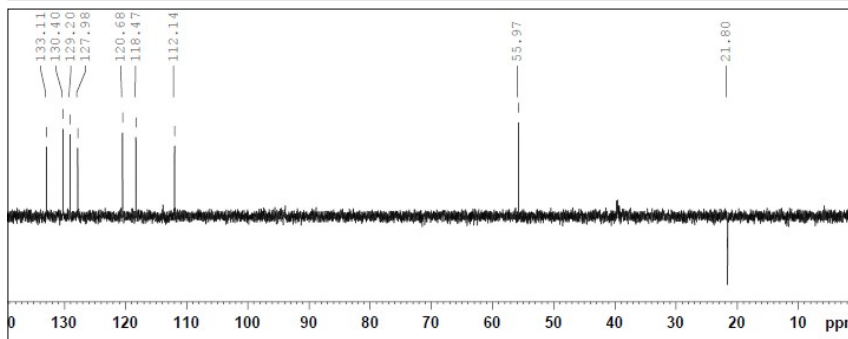
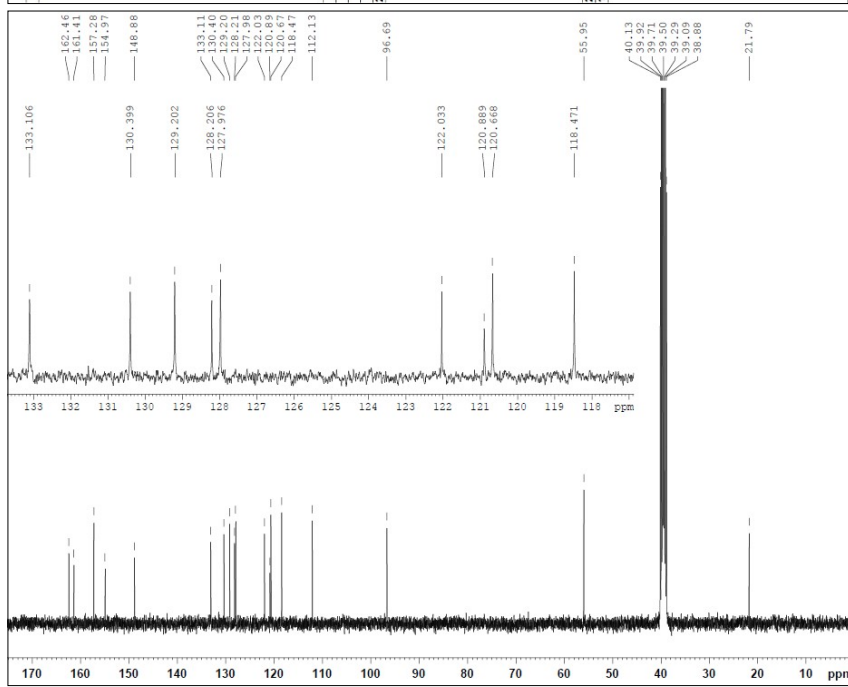
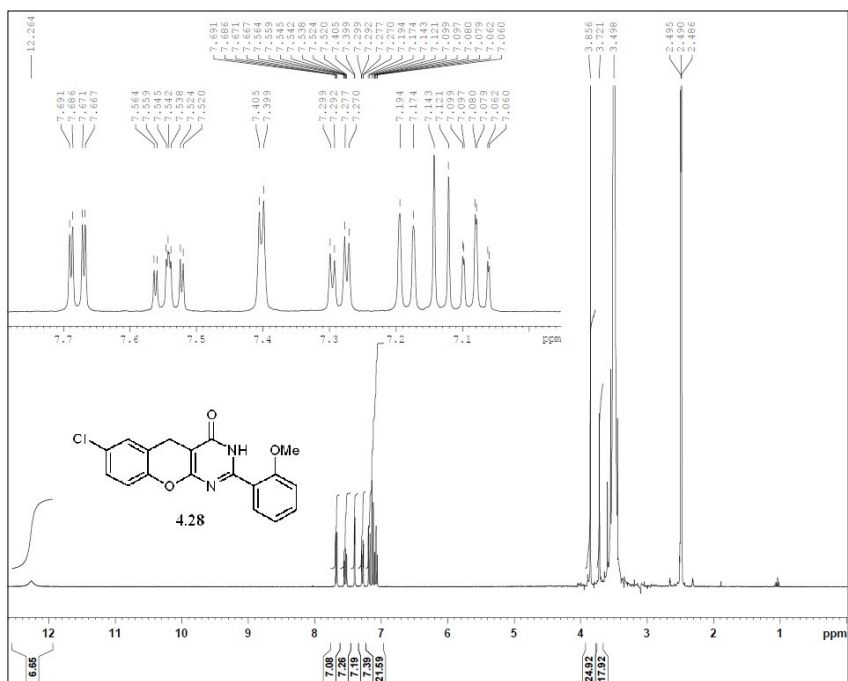


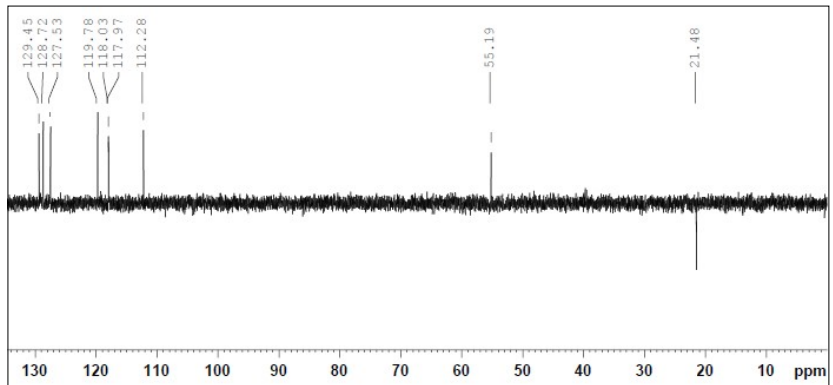
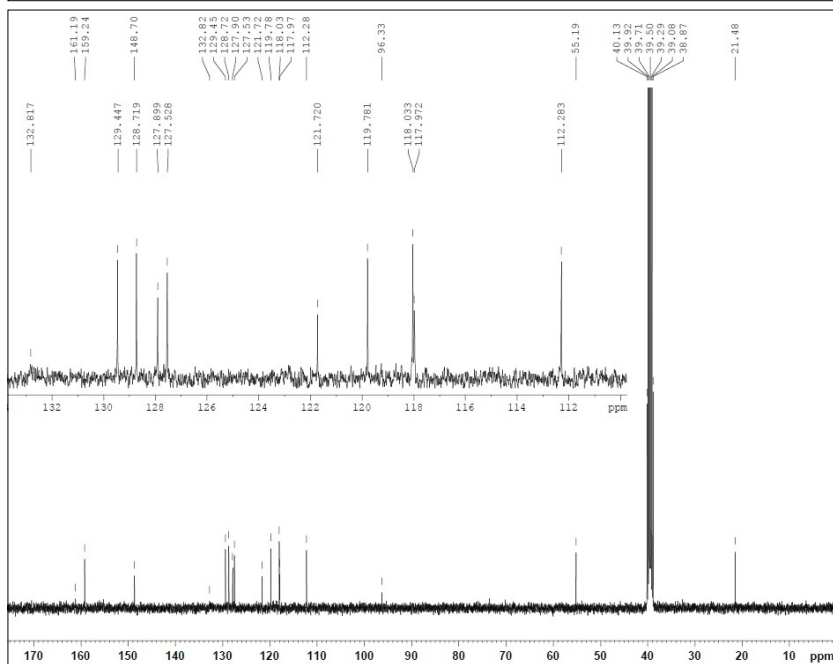
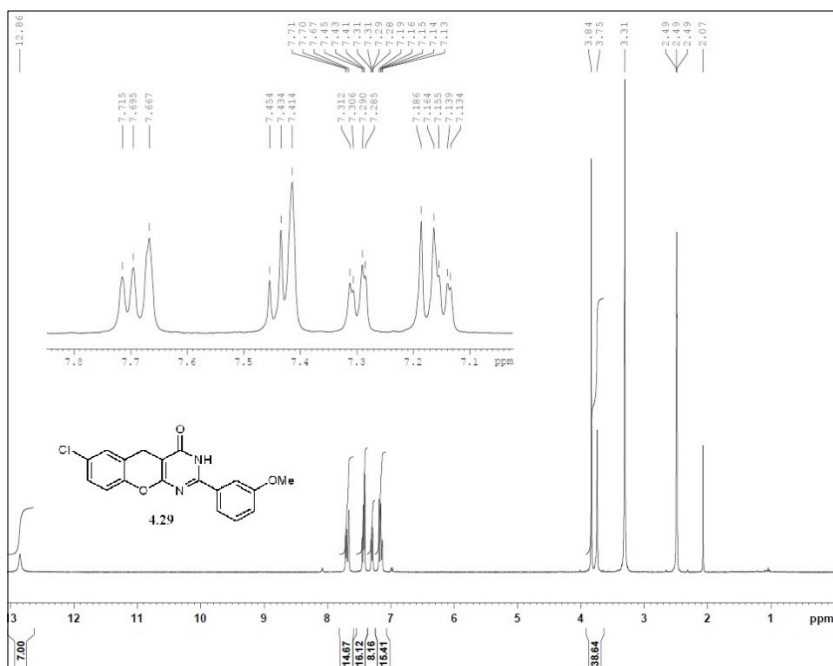


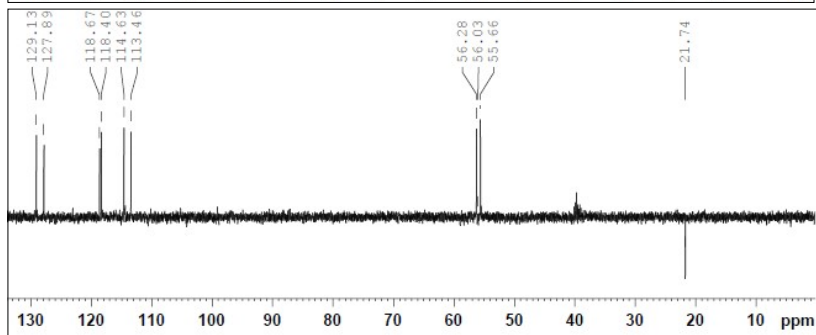
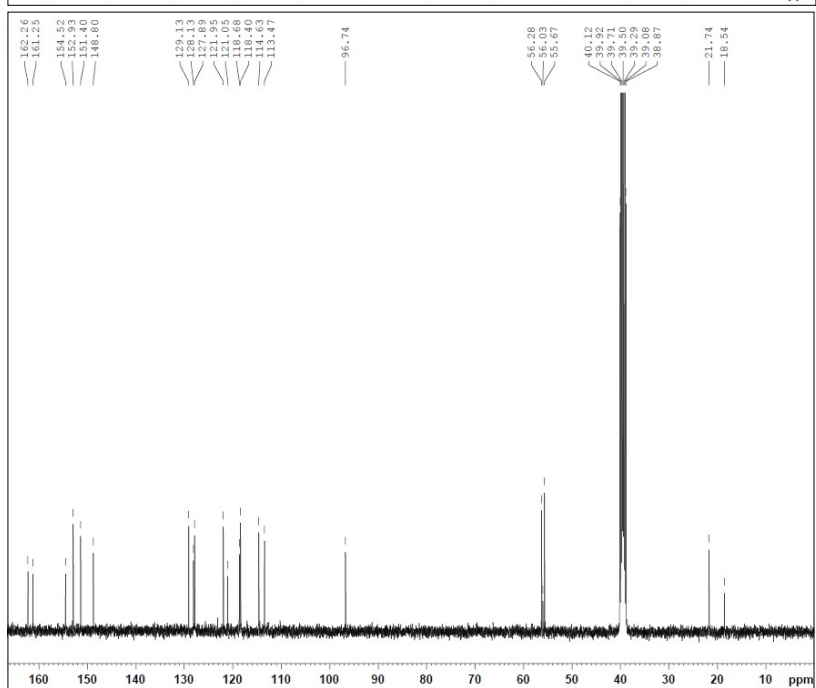
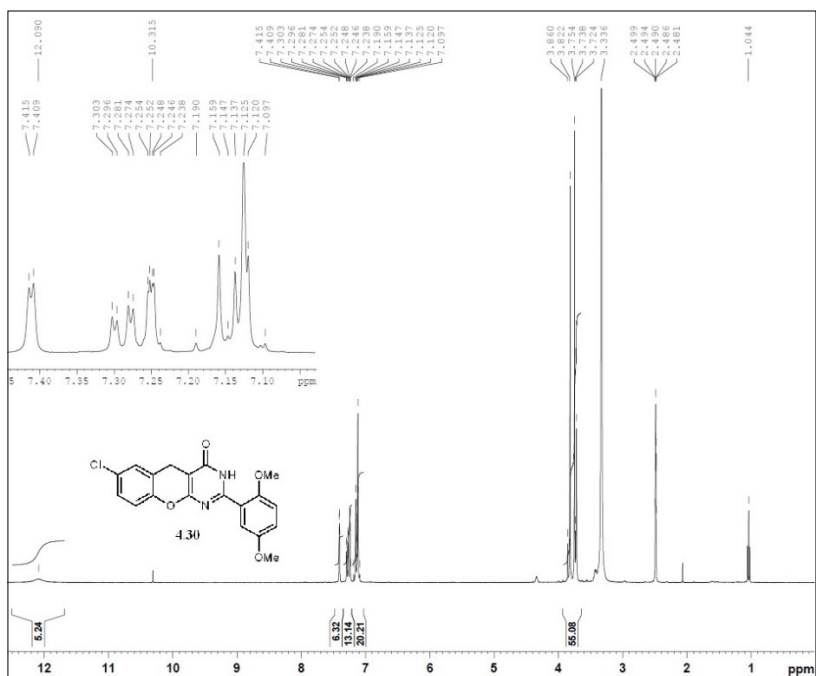


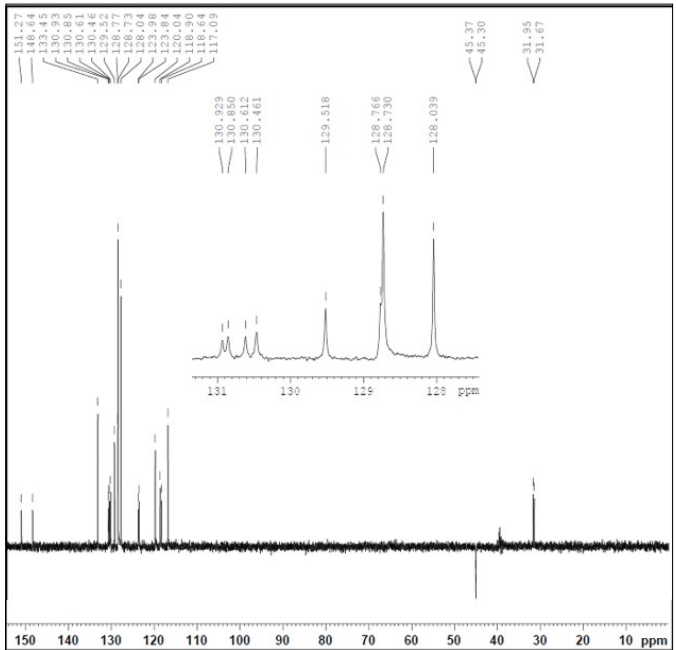
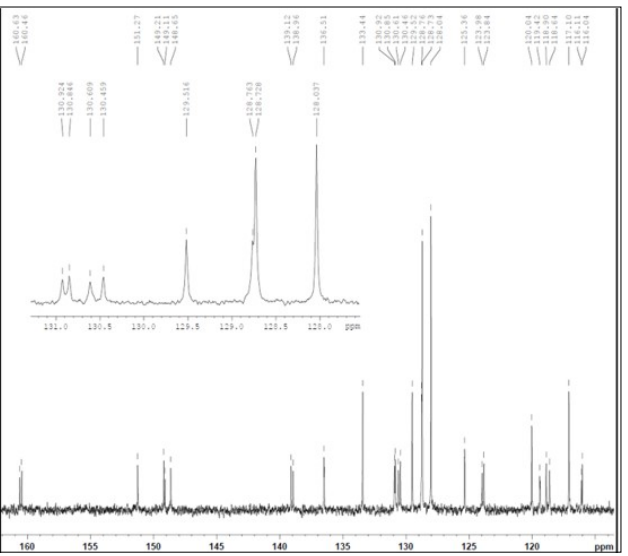
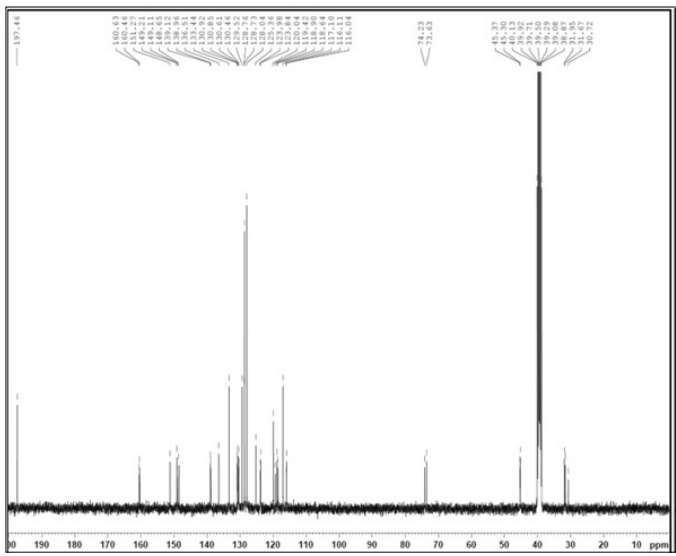
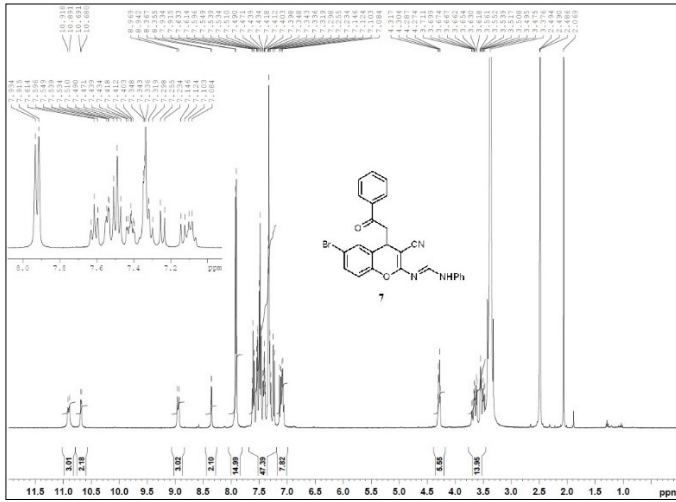


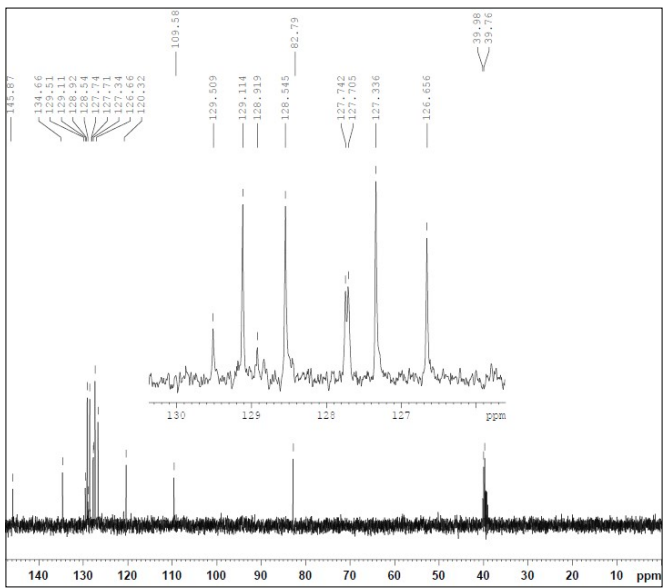
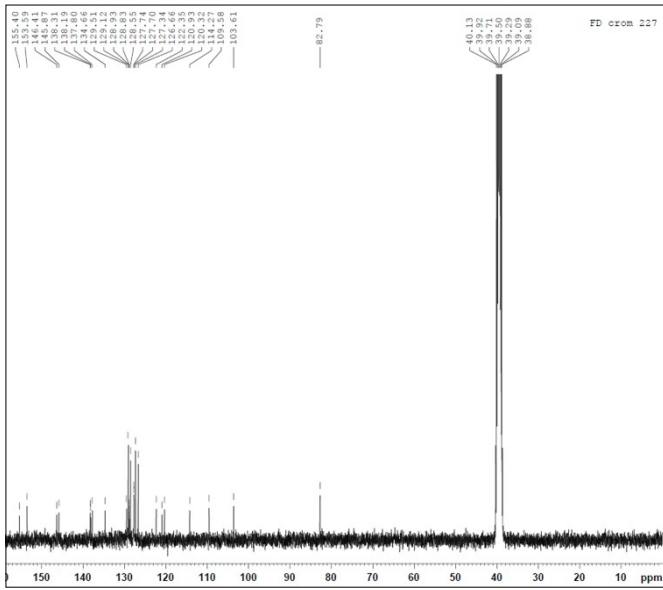
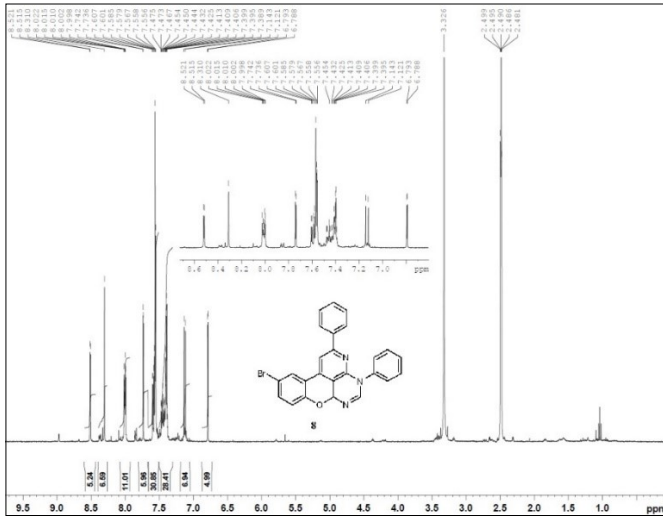












Biology

Table S1 Cell viability results of chromene-based compounds **3** and **4** in Hs578t, MDA-MB-231 and MCF-7 breast cancer cell lines, after 72 h of treatment with two concentrations (10 and 30 μ M).

Compounds	Cell viability (%)					
	Hs578t		MDA-MB-231		MCF-7	
	10 μ M	30 μ M	10 μ M	30 μ M	10 μ M	30 μ M
3.1	94.8	86.0	83.6	77.4	99.9	91.8
3.2	100.3	96.5	88.2	62.2	84.9	82.1
3.3	98.4	83.0	85.8	64.1	85.9	84.8
3.5	97.0	65.5	86.3	78.5	100.7	100.0
3.6	95.2	68.6	80.6	64.4	90.6	76.2
4.1	91.1	75.8	91.8	89.2	91.2	88.5
4.2	86.8	63.3	99.2	91.1	78.5	77.2
4.3	86.0	80.2	82.9	63.6	85.5	54.8
4.4	99.3	85.3	70.5	51.6	93.6	71.3
4.5	72.7	74.0	87.1	88.4	77.7	76.6
4.7	75.9	67.3	101.6	101.8	38.0	25.4
4.8	81.7	72.5	92.5	90.1	74.7	58.2
4.9	72.0	49.9	80.8	76.9	69.8	40.4
4.10	66.8	40.6	92.0	80.8	78.4	52.4
4.11	97.7	84.4	83.7	80.4	83.8	75.3
4.12	97.5	84.4	105.2	97.8	88.8	82.9
4.13	30.0	18.7	68.1	58.6	59.8	43.5
4.14	99.8	97.9	99.2	92.5	84.7	56.7

4.15	99.8	90.7	92.8	85.8	98.9	100.0
4.16	87.5	65.6	85.0	65.9	90.6	70.4
4.17	88.0	77.0	69.3	41.2	73.1	49.8
4.18	95.9	96.5	60.9	49.7	63.7	57.8
4.19	76.0	74.7	80.7	44.2	79.3	45.8
4.20	76.3	65.4	69.3	53.2	88.5	79.2
4.21	80.2	81.0	95.1	95.2	97.7	94.7
4.22	81.3	62.1	32.6	15.9	34.7	23.8
4.23	96.0	73.3	90.1	65.6	85.8	73.1
4.25	40.2	21.1	62.4	52.1	39.2	36.7
4.27	86.1	78.5	85.3	77.8	83.4	83.1
4.28	78.4	38.7	88.2	65.0	80.0	57.3
4.29	54.7	33.9	85.1	18.4	81.5	35.4
4.30	91.7	92.0	91.0	78.0	69.2	62.6
7	96.1	89.9	98.0	96.0	74.6	37.9
8	50.4	32.6	49.9	40.5	58.0	25.9
Doxorubicin	8.65	7.61	6.72	5.85	24.5	28.8

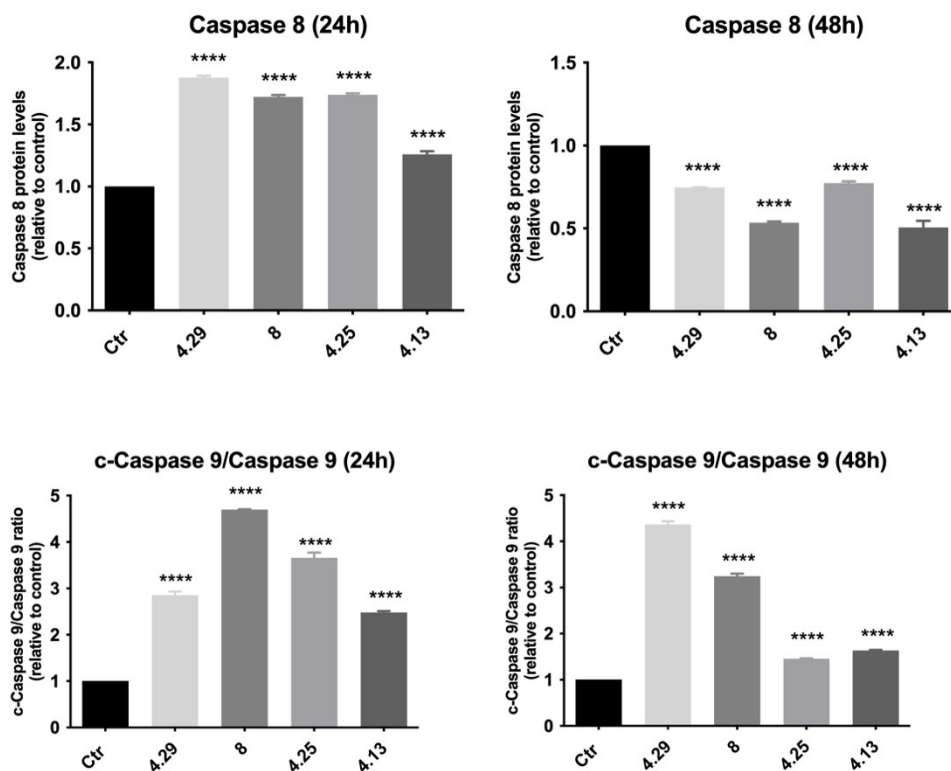


Figure S1. Quantification of the expression levels of Caspase 8 levels and ratio c-Caspase 9/Caspase 9, after 24 and 48 h of incubation of Hs578t cells with compounds **4.13**, **4.25**, **4.29** and **8**. Values are mean \pm SEM of 3 independent experiments. **** p<0.001, ***p<0.005, **p<0.01, *p<0.05 when compared with control by one-way ANOVA.

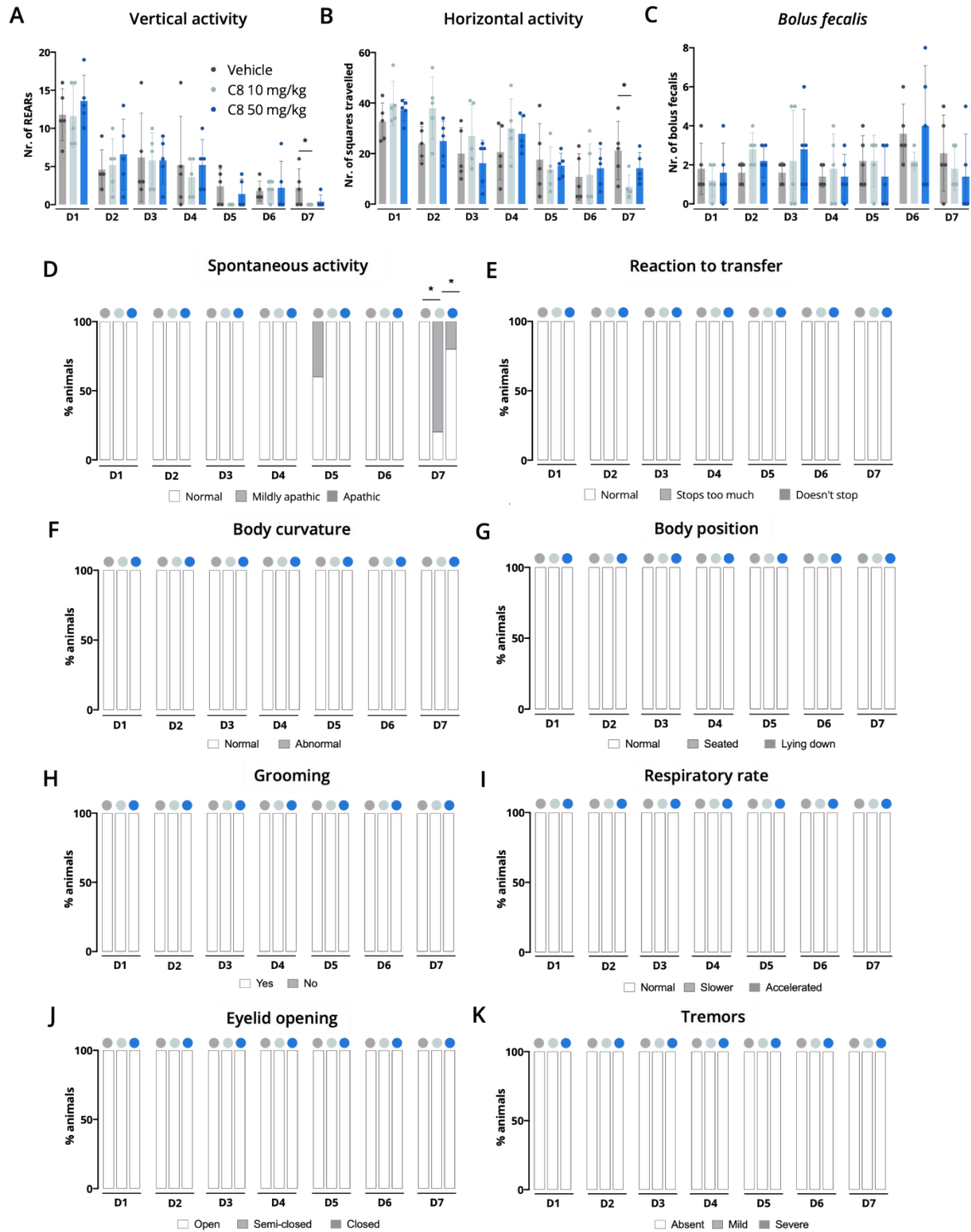


Figure S2. Mouse welfare evaluation tests. Overall, no differences were found between vehicle- and chromene **8** 10 mg/kg- and 50 mg/kg-treated animals in any of the tests. Only on day 7, animals treated with 10 mg/kg of chromene **8** were mildly apathic, as seen in spontaneous activity (**D**), which was further

supported by the decreased number of rears and number of squares travelled in vertical (A) and horizontal (B) exploration, respectively. One-way ANOVA with Tukey's or Dunnett T3 Post-Hoc analysis (n=5/group). All data are expressed as group mean \pm SD (* p<0.05).

Table S2. Primary and secondary antibodies used in the Western blot assays.

Antibody designation	Label	Reference	Dilution
Rabbit anti-PARP	Cell signaling	#9542	1:500 5% BSA
Mouse anti-Caspase 9	Cell signaling	#9508	1:1000 5% BSA
Mouse anti-Caspase 8	Santa Cruz Biotechnology	sc-81656	1:1000 5%BSA
Rabbit anti-Caspase 3	Cell signaling	#14220	1:1000 5% BSA
Rabbit anti-Bad	Cell signaling	#9239	1:1000 5%BSA
Rabbit anti-Bid	Cell signaling	#2002	1:500 5% BSA
Rabbit anti-BIM	Cell signaling	#2933	1:500 5% BSA
Rabbit anti-β-tubulin	Abcam	ab6046	1:10000 5% BSA
Goat-anti-rabbit IgG-HRP	Cell signaling	#7074	1:1000 5% BSA
Horse-anti-mouse IgG-HRP	Cell signaling	#7076	1:1000 5% milk