

# Supplementary Materials

## 1. Figures

### 1.1. The IR spectra

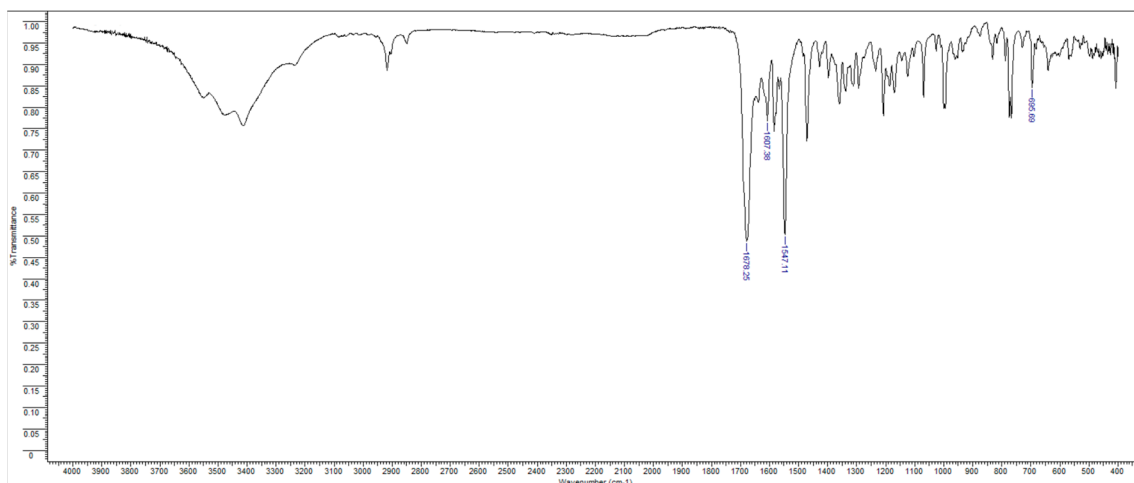


Figure S1. The IR spectrum for the compound 1a.

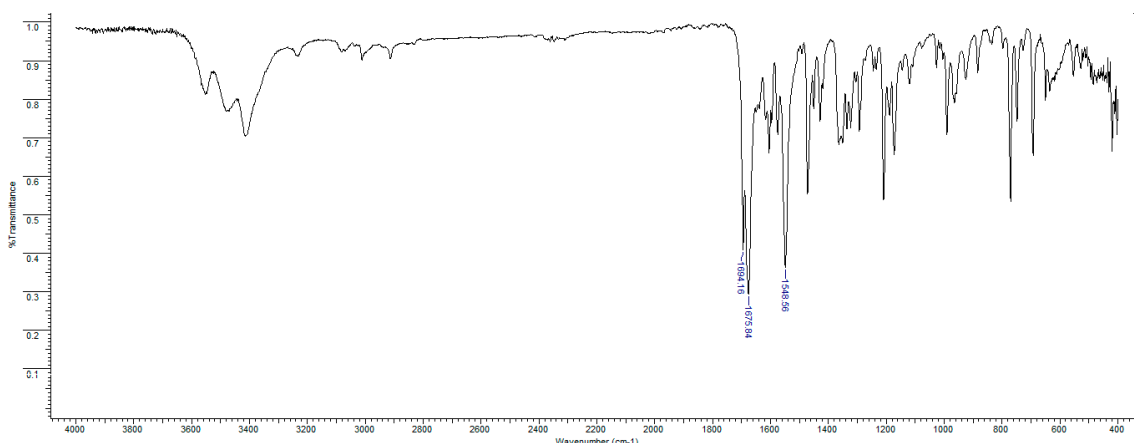


Figure S2. The IR spectrum for the compound 2a.

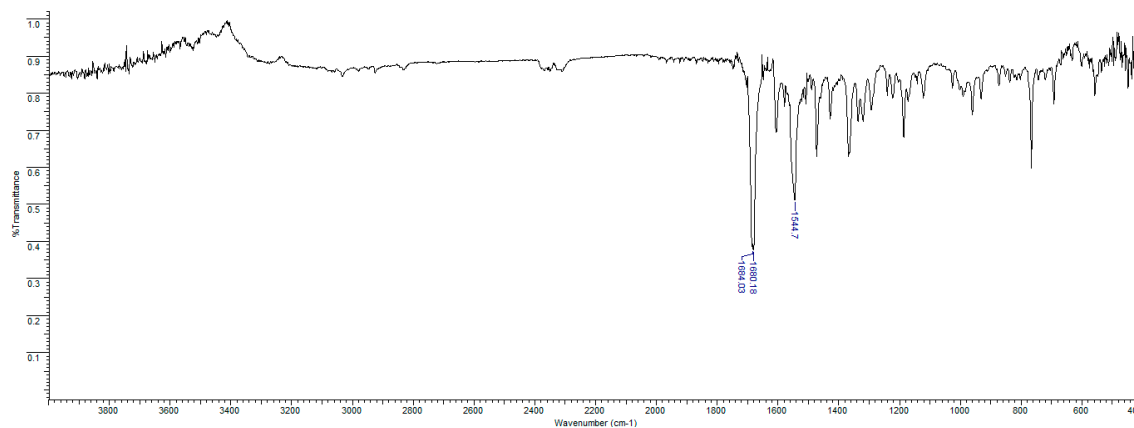


Figure S3. The IR spectrum for the compound 3a.

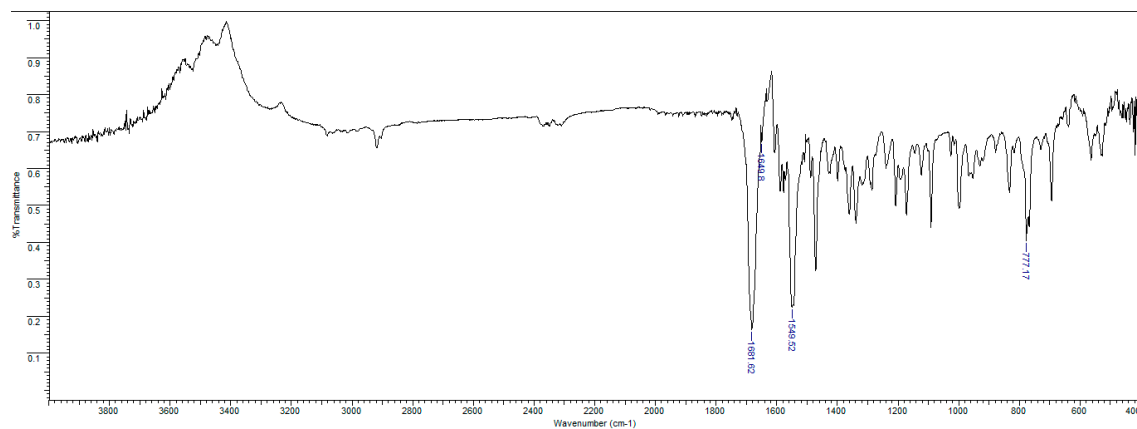


Figure S4. The IR spectrum for the compound 4a.

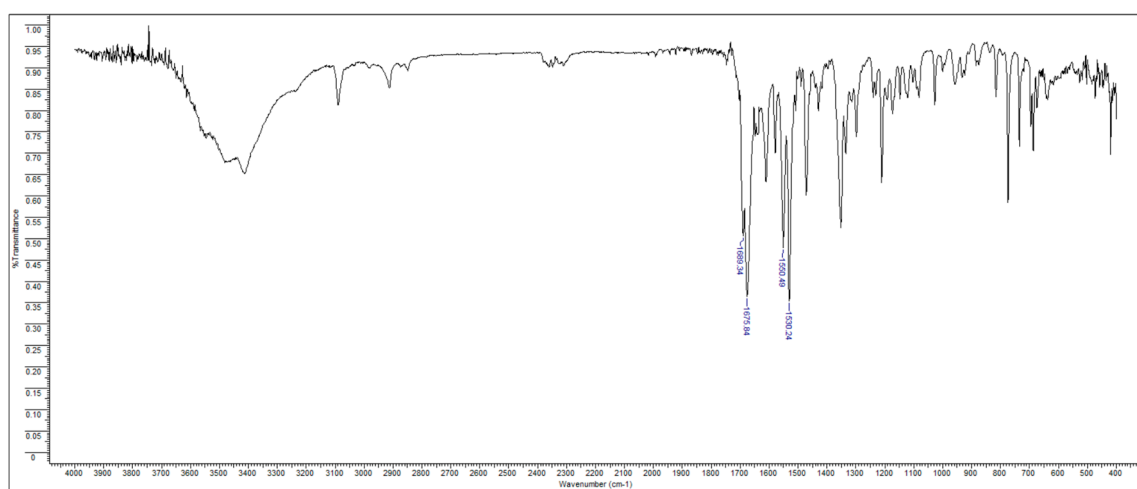


Figure S5. The IR spectrum for the compound 5a.

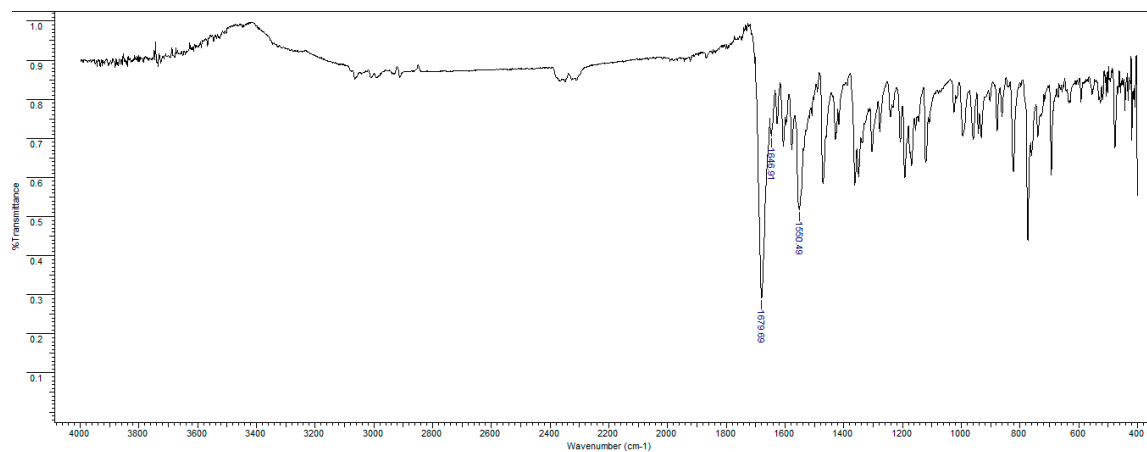


Figure S6. The IR spectrum for the compound 6a.

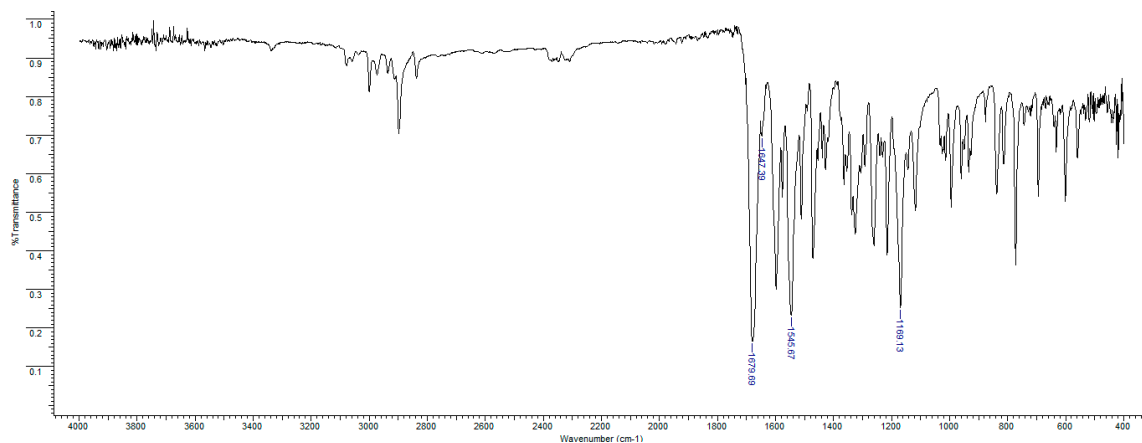


Figure S7. The IR spectrum for the compound 7a.

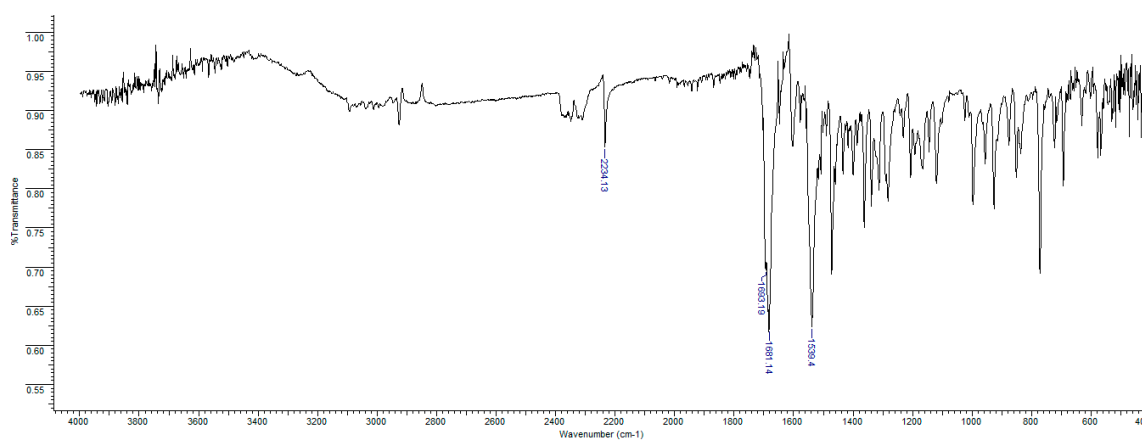


Figure S8. The IR spectrum for the compound 8a.

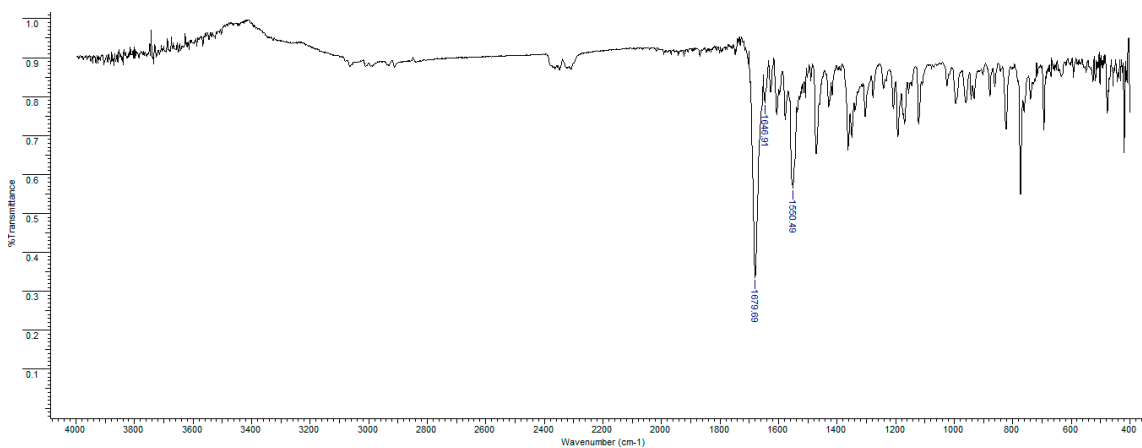
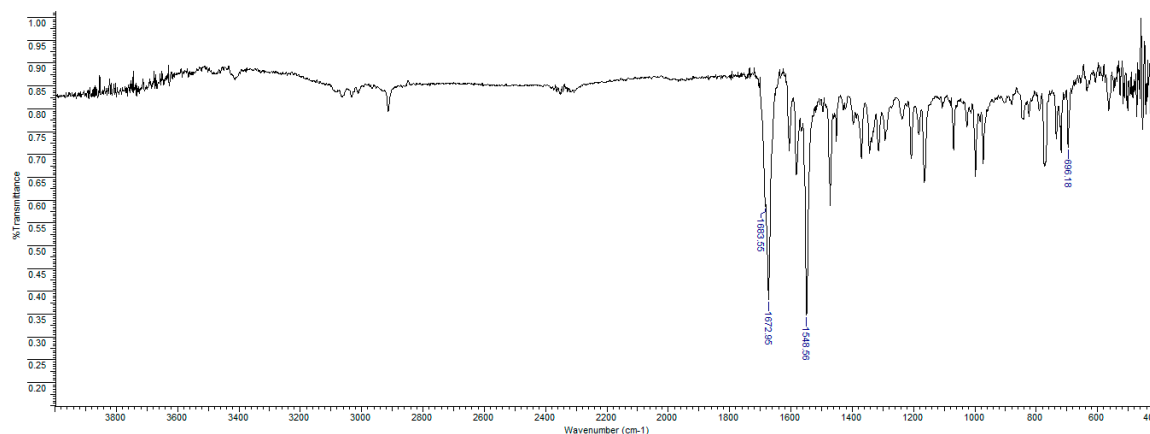
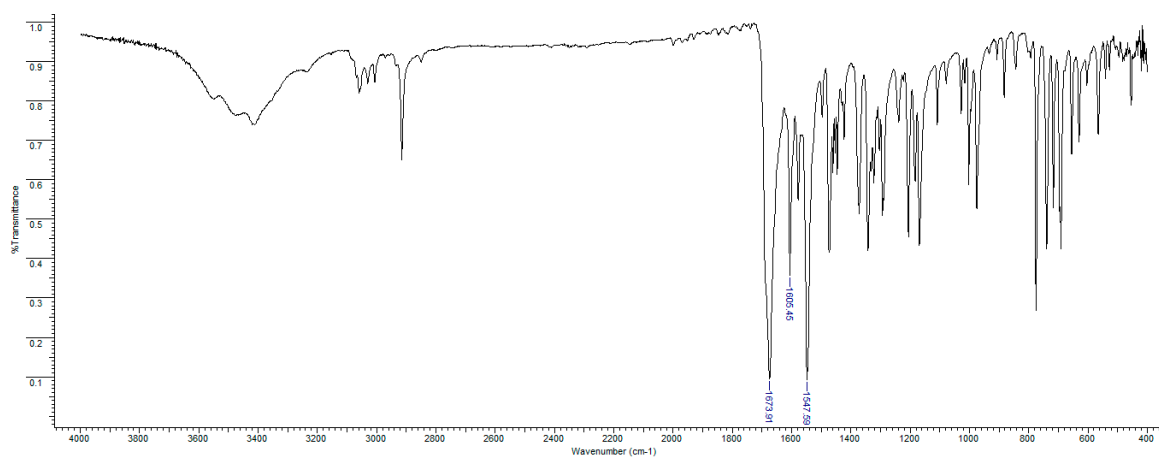


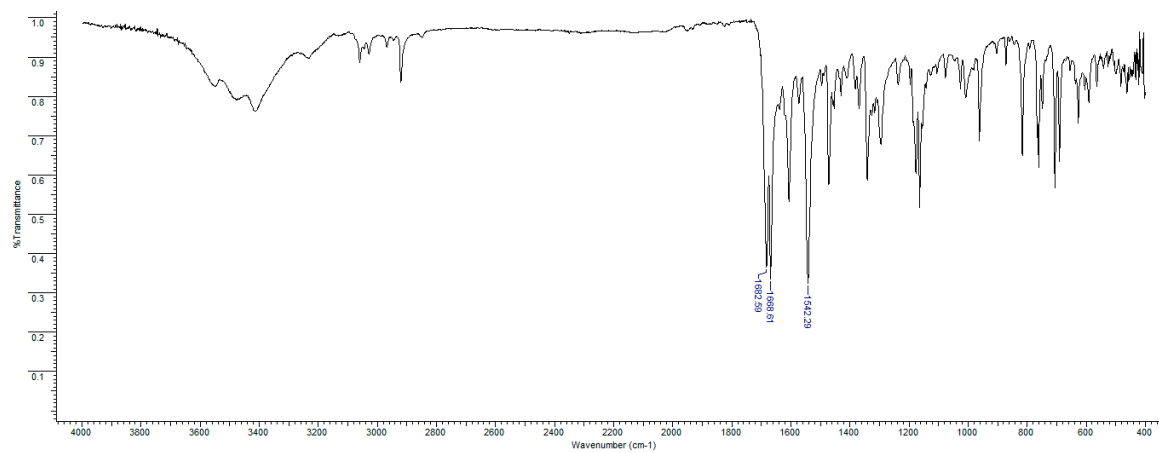
Figure S9. The IR spectrum for the compound 9a.



**Figure S10.** The IR spectrum for the compound **1b**.

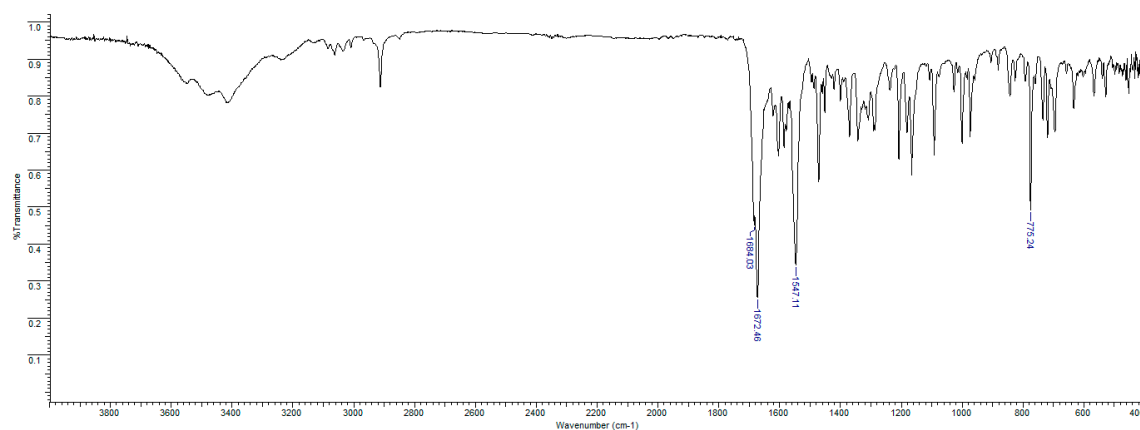


**Figure S11.** The IR spectrum for the compound **2b**.

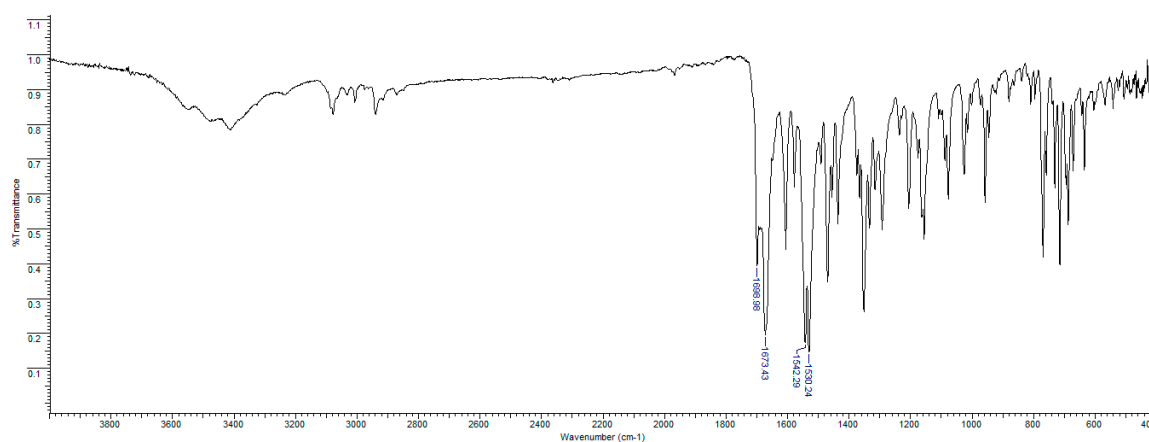


**Figure S12.** The IR spectrum for the compound **3b**.

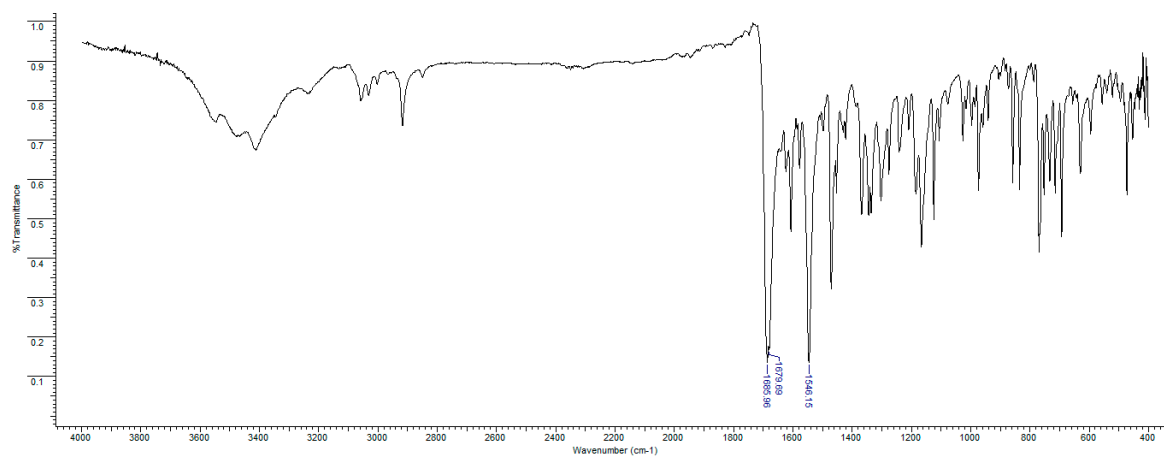




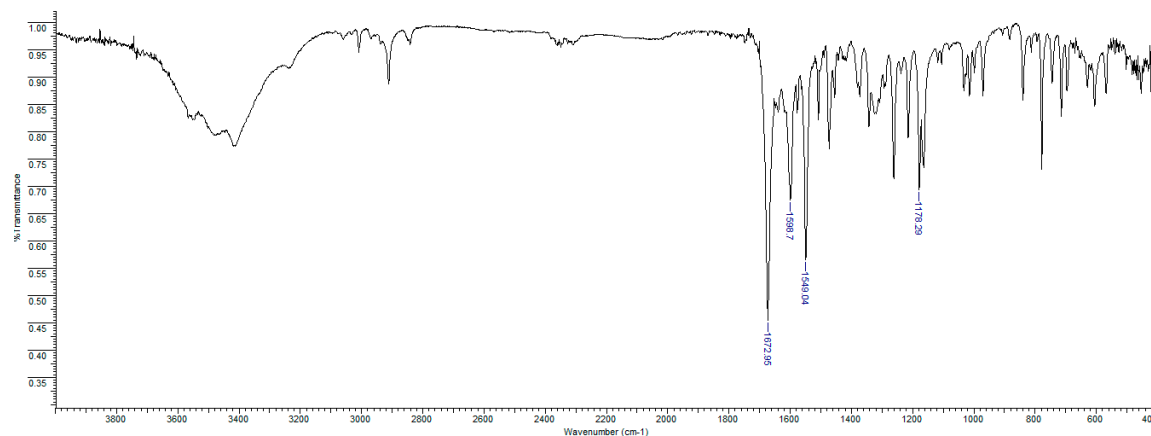
**Figure S13.** The IR spectrum for the compound **4b**.



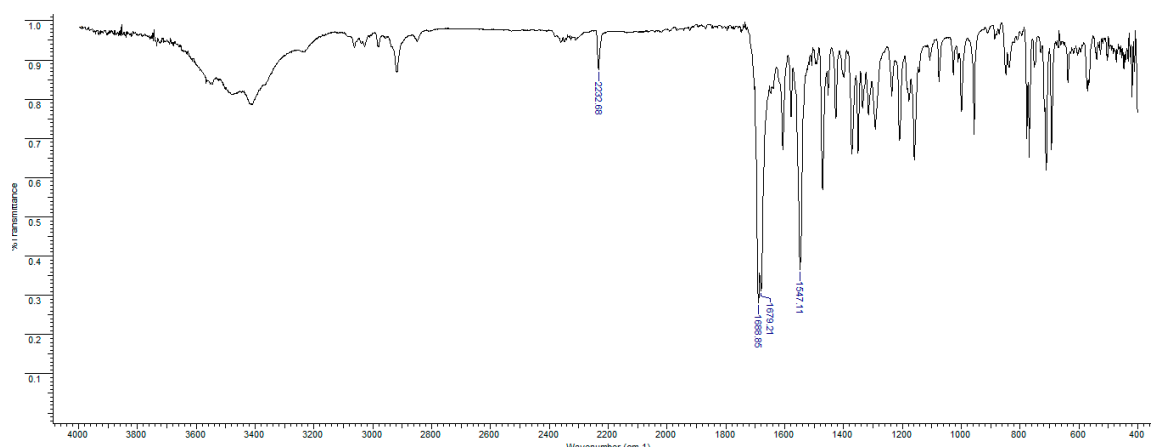
**Figure S14.** The IR spectrum for the compound **5b**.



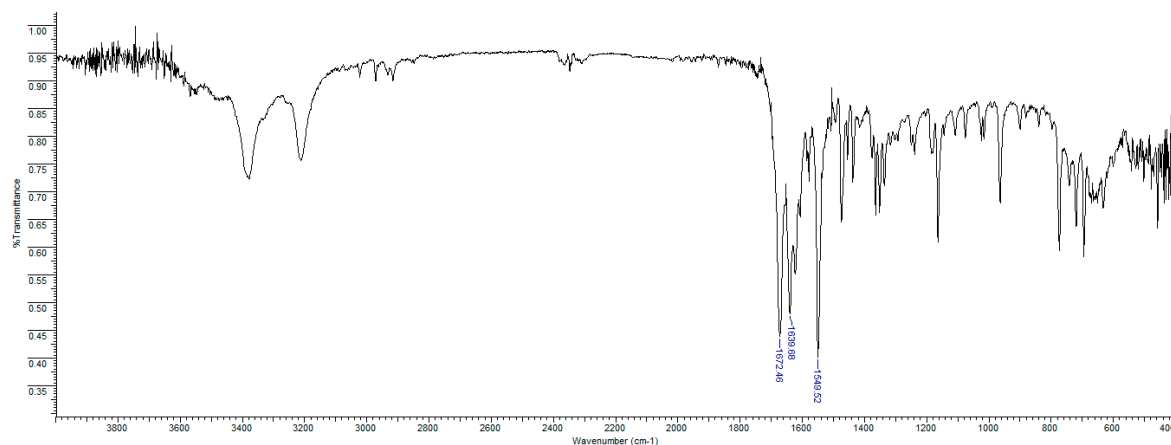
**Figure S15.** The IR spectrum for the compound **6b**.



**Figure S16.** The IR spectrum for the compound **7b**.

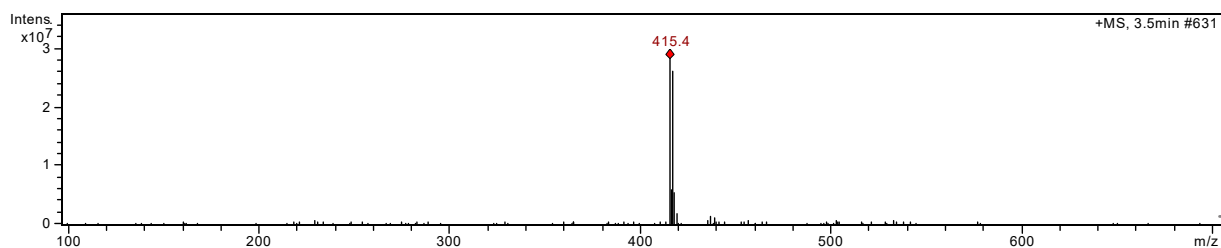


**Figure S17.** The IR spectrum for the compound **8b**.



**Figure S18.** The IR spectrum for the compound **9b**.

### 1.1. The MS spectra



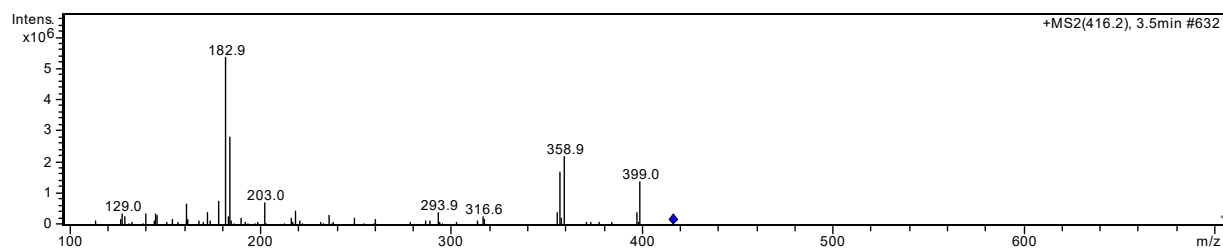


Figure S19. The MS spectrum for the compound 1a.

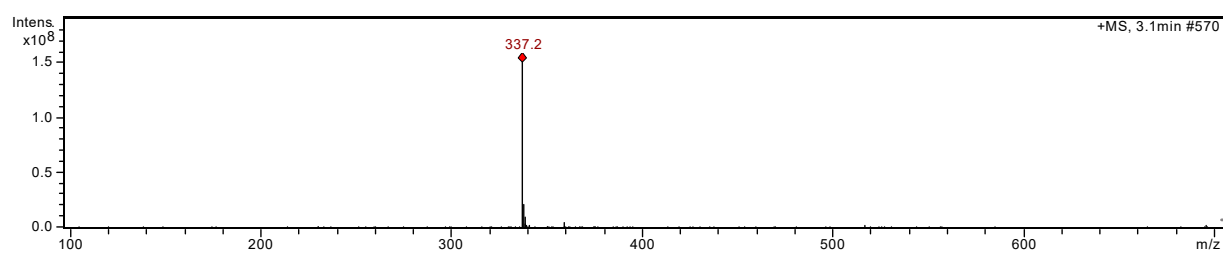


Figure S20. The MS spectrum for the compound 2a.

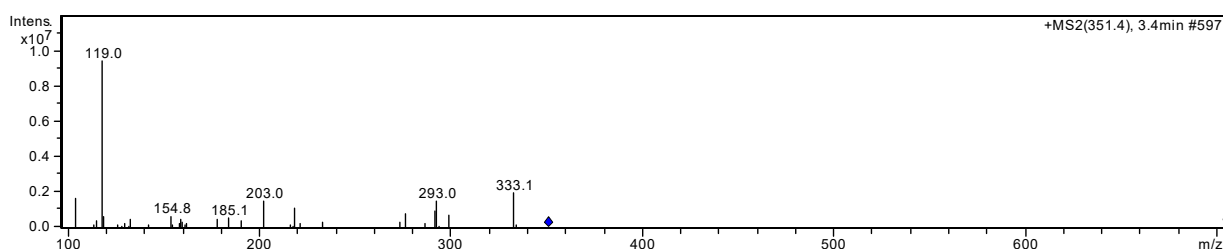
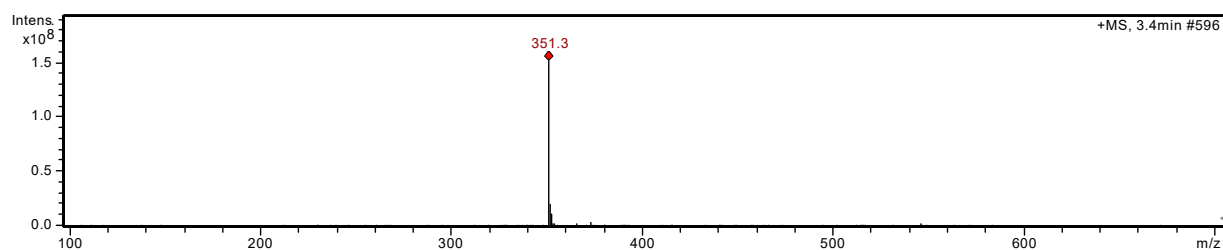


Figure S21. The MS spectrum for the compound 3a.

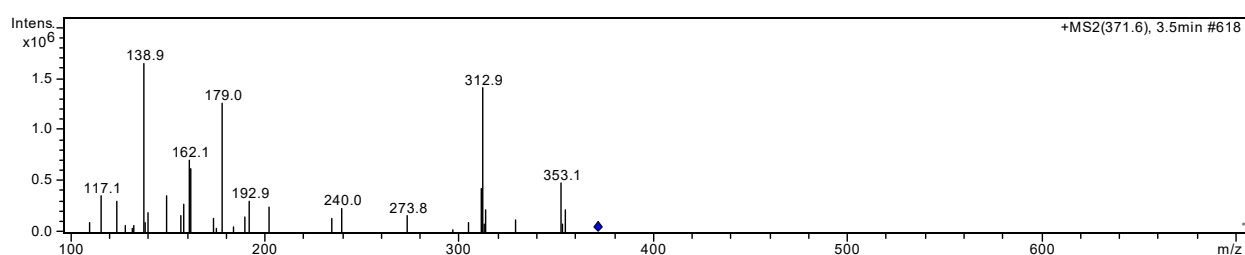
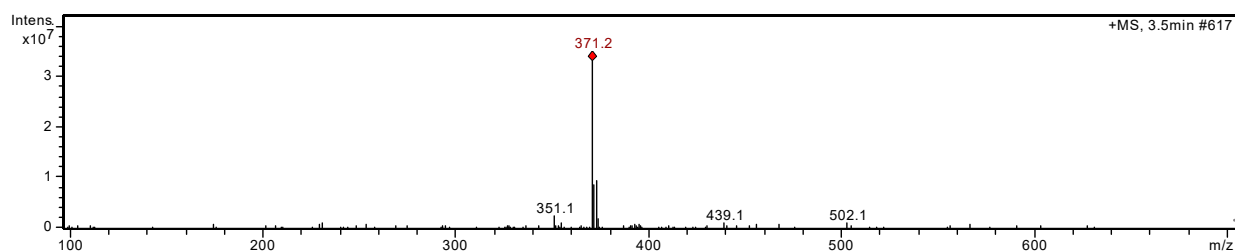


Figure S22. The MS spectrum for the compound 4a.

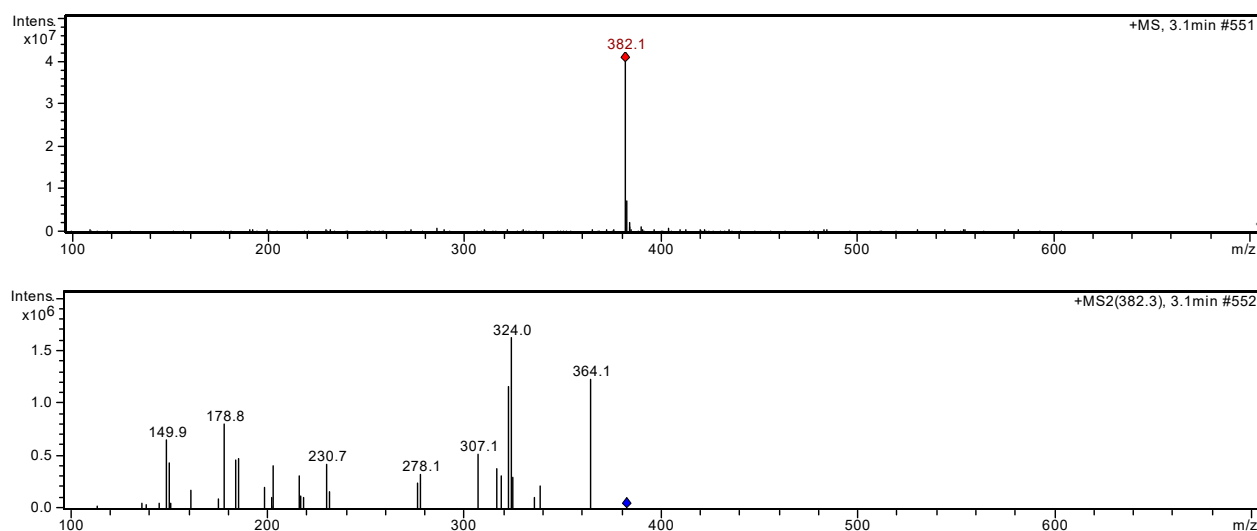


Figure S21. The MS spectrum for the compound 5a.

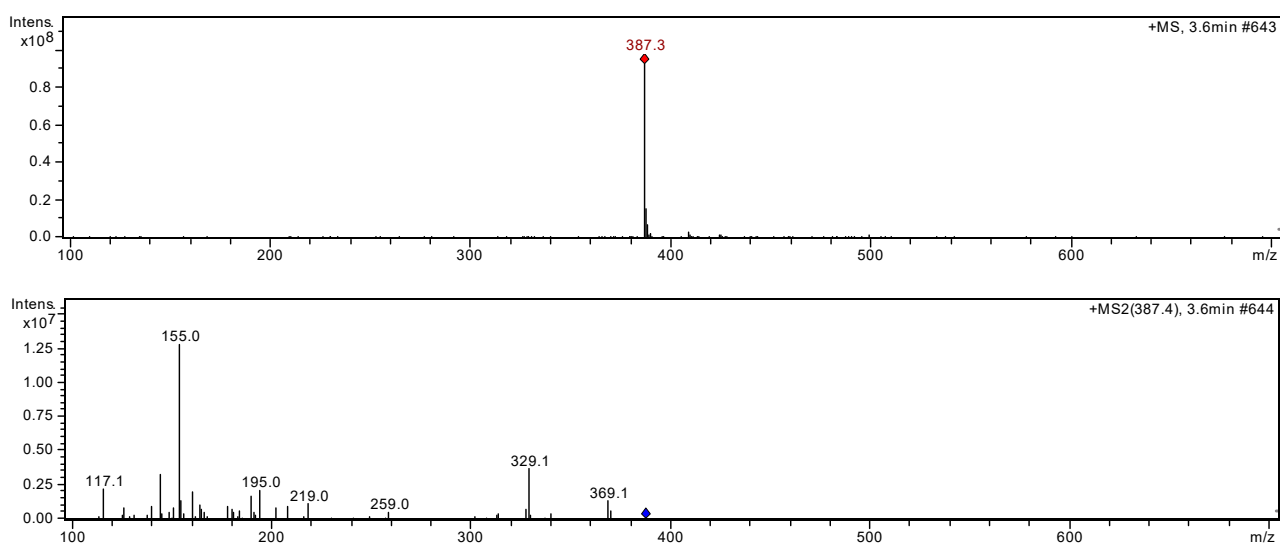


Figure S22. The MS spectrum for the compound 6a.

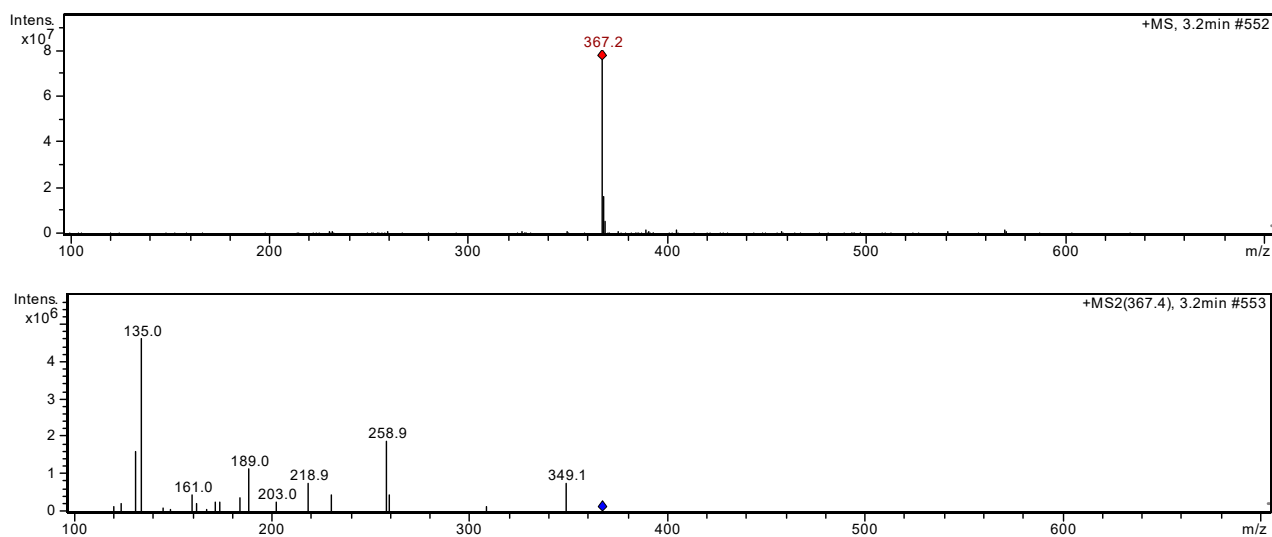


Figure S23. The MS spectrum for the compound 7a.

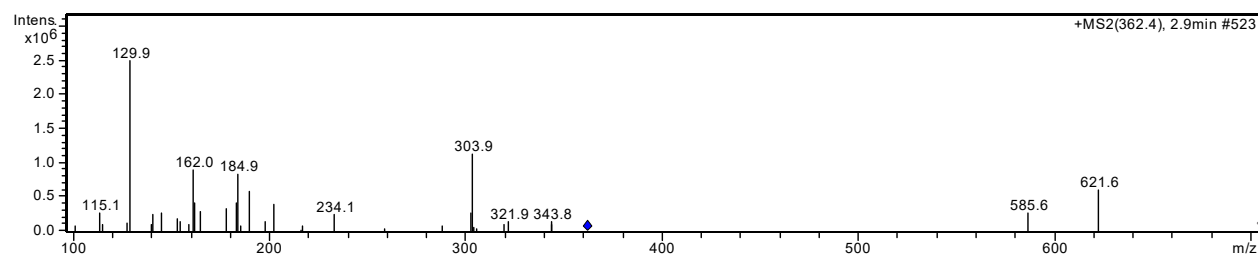
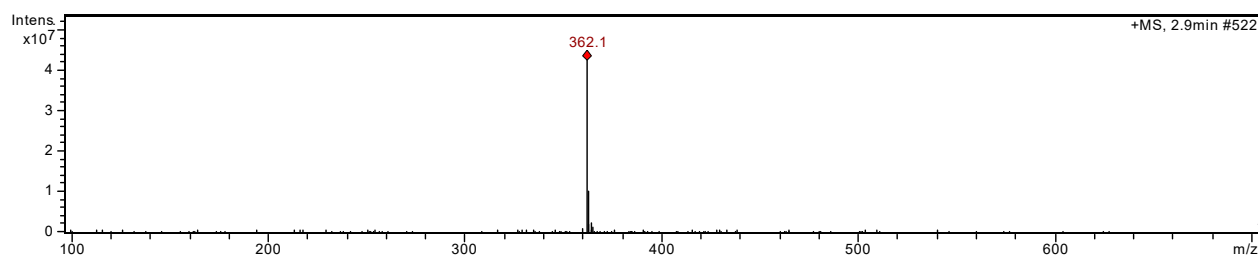


Figure S24. The MS spectrum for the compound 8a.

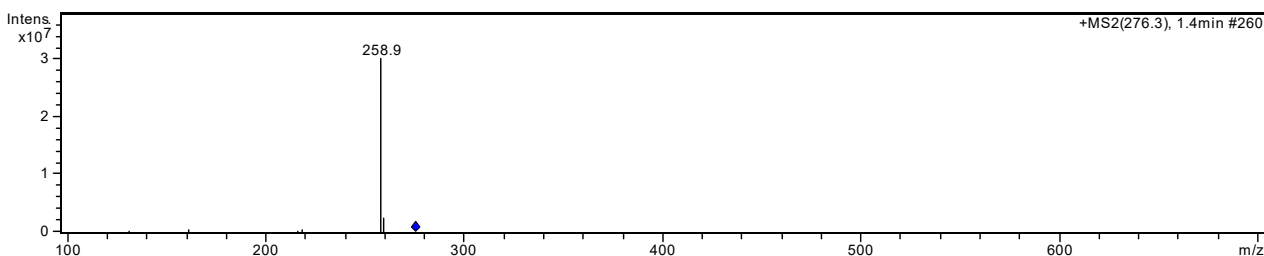
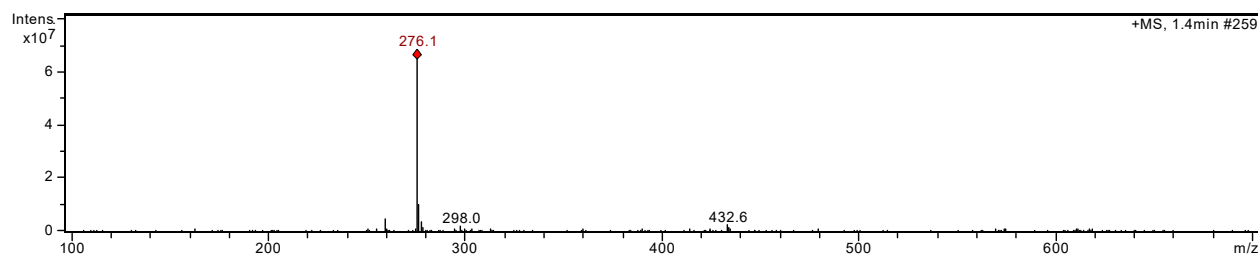


Figure S25. The MS spectrum for the compound 9a.

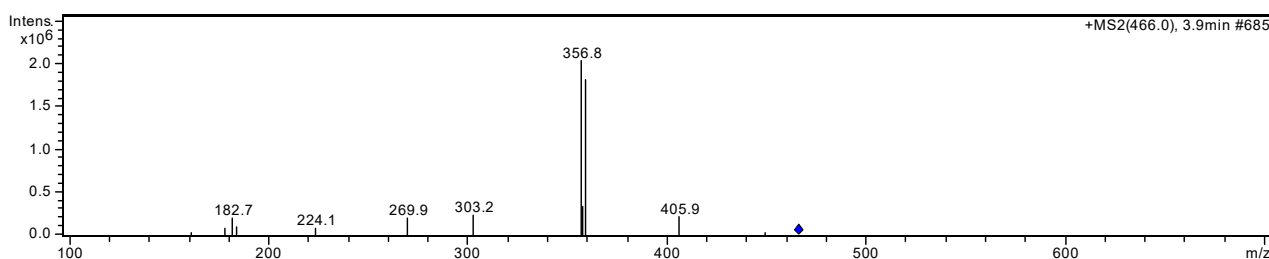
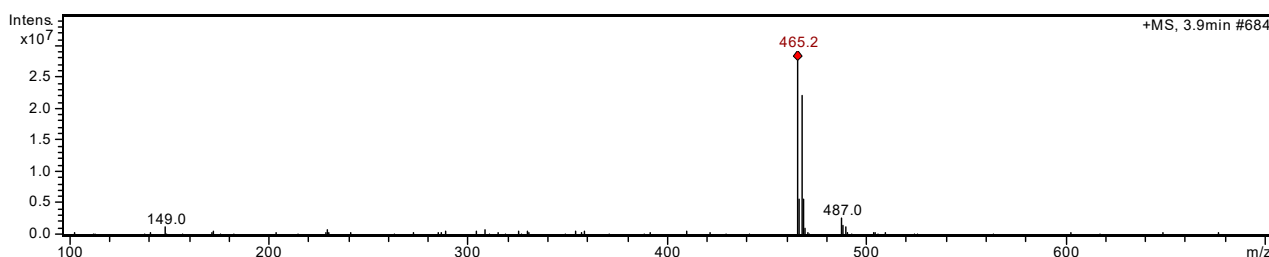


Figure S26. The MS spectrum for the compound 1b.

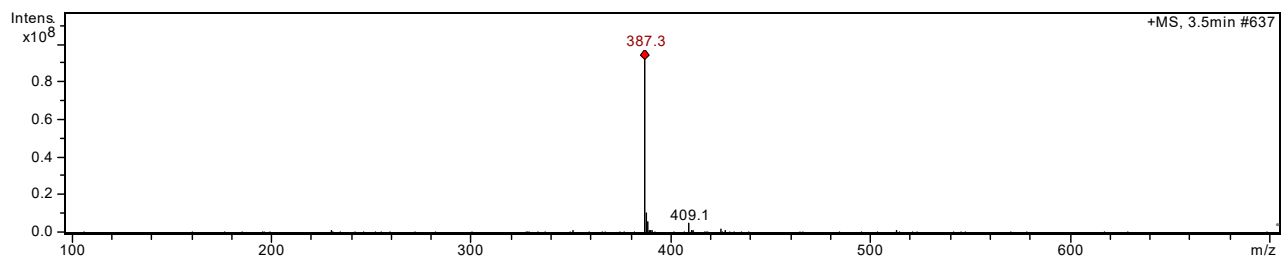


Figure S27. The MS spectrum for the compound 2b.

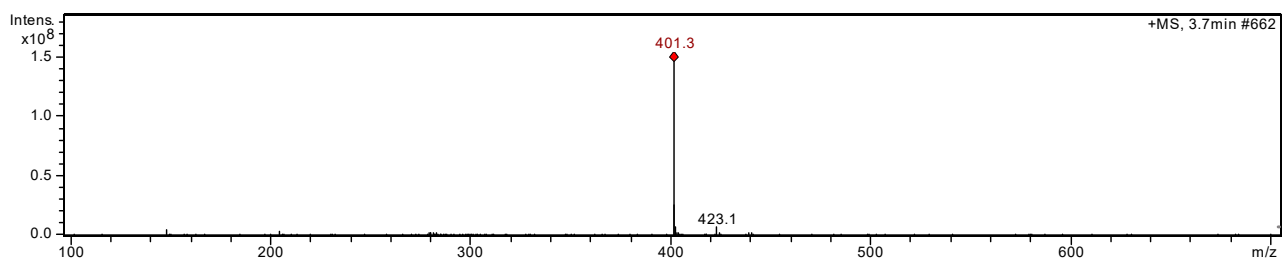
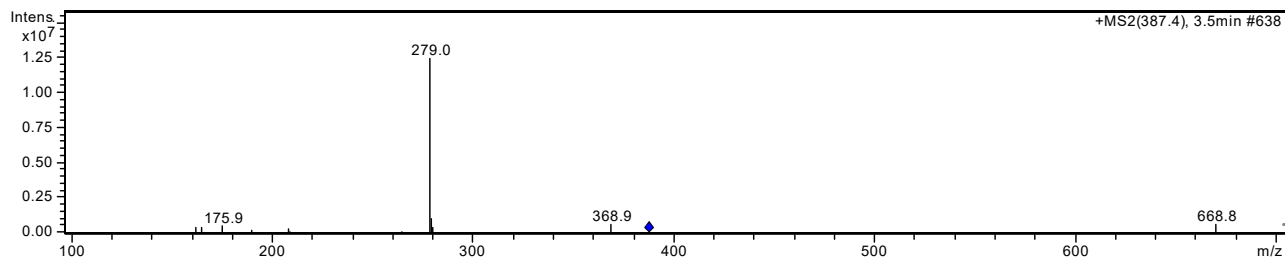


Figure S28. The MS spectrum for the compound 3b.

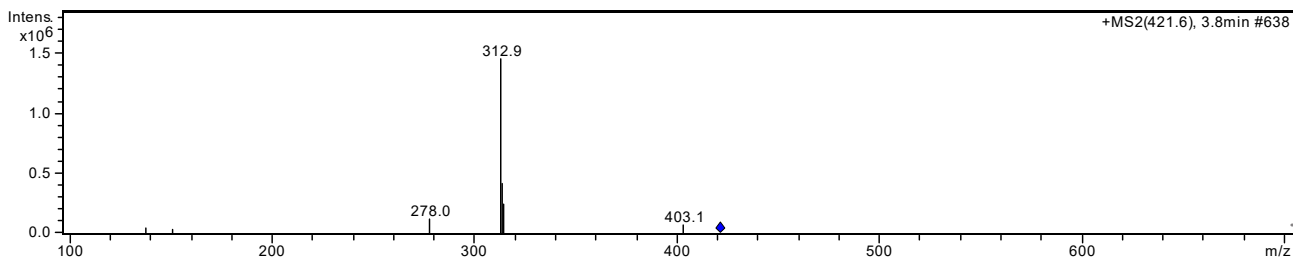
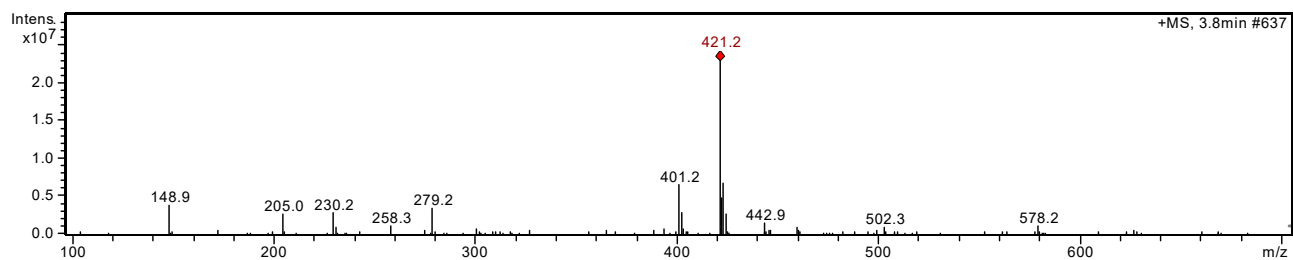
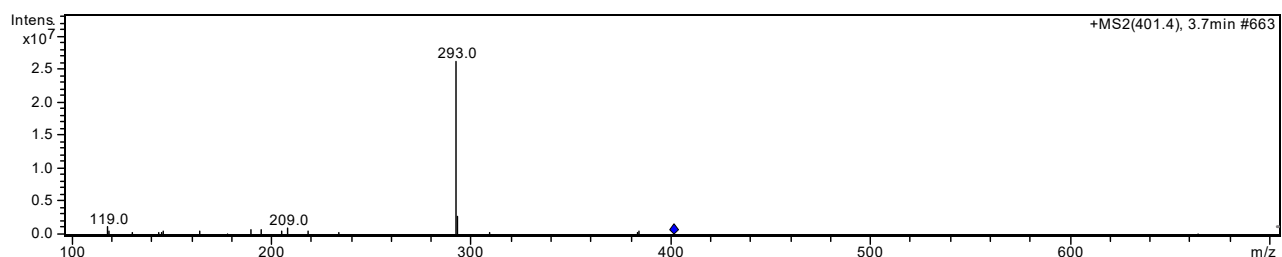


Figure S29. The MS spectrum for the compound 4b.

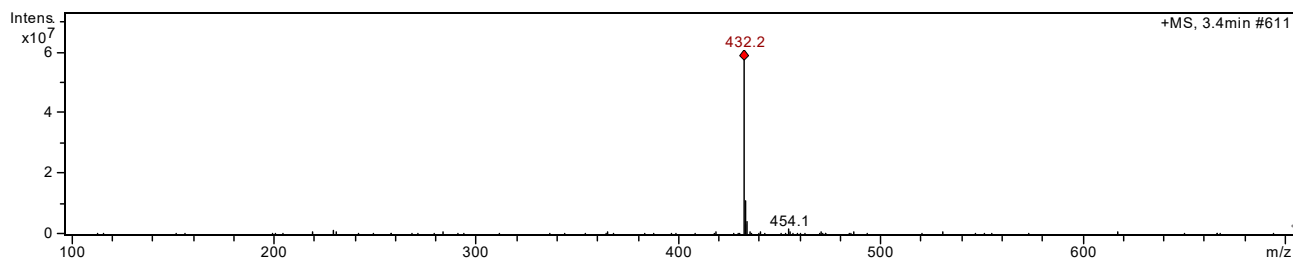


Figure S30. The MS spectrum for the compound 5b.

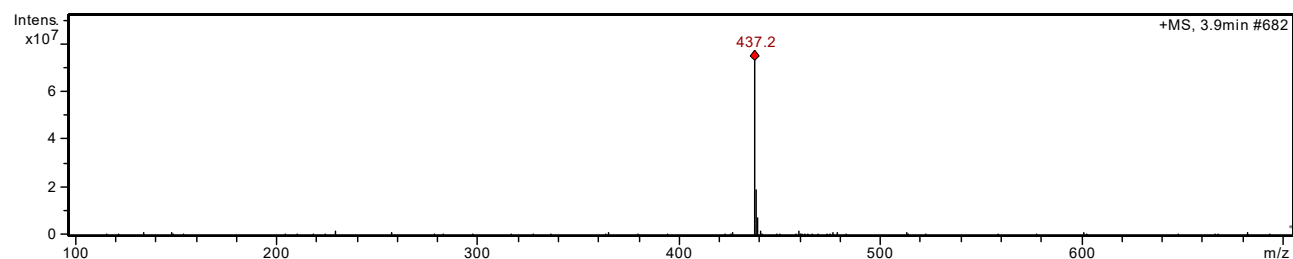
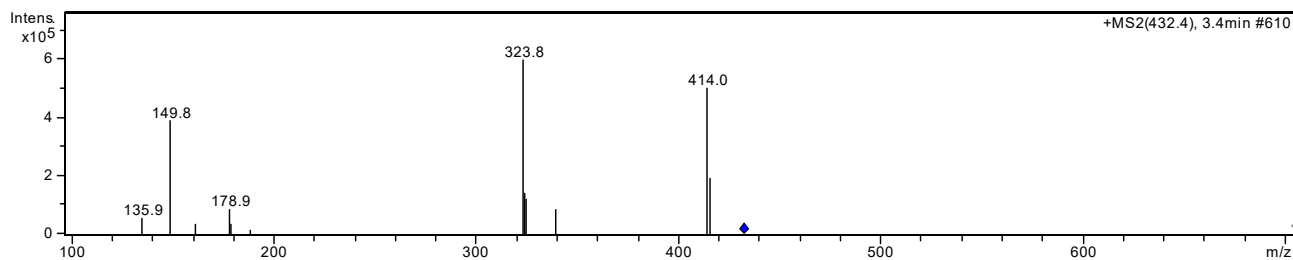


Figure S31. The MS spectrum for the compound 6b.

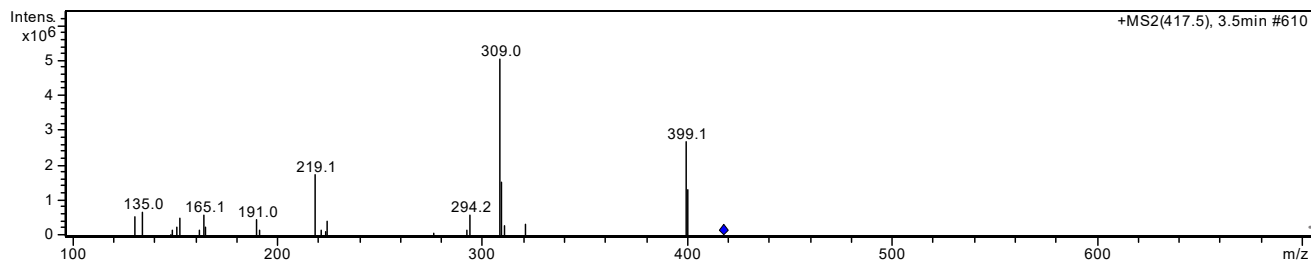
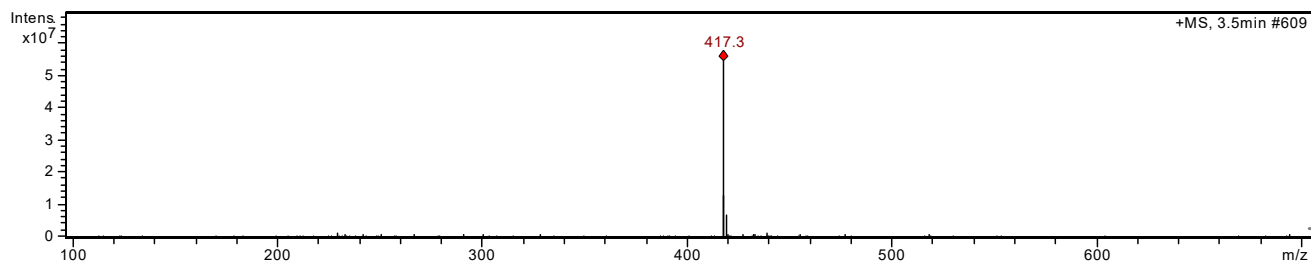
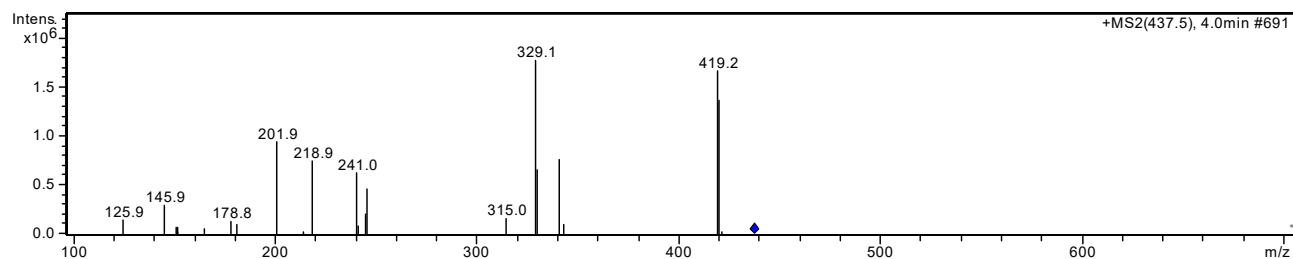


Figure S32. The MS spectrum for the compound 7b.

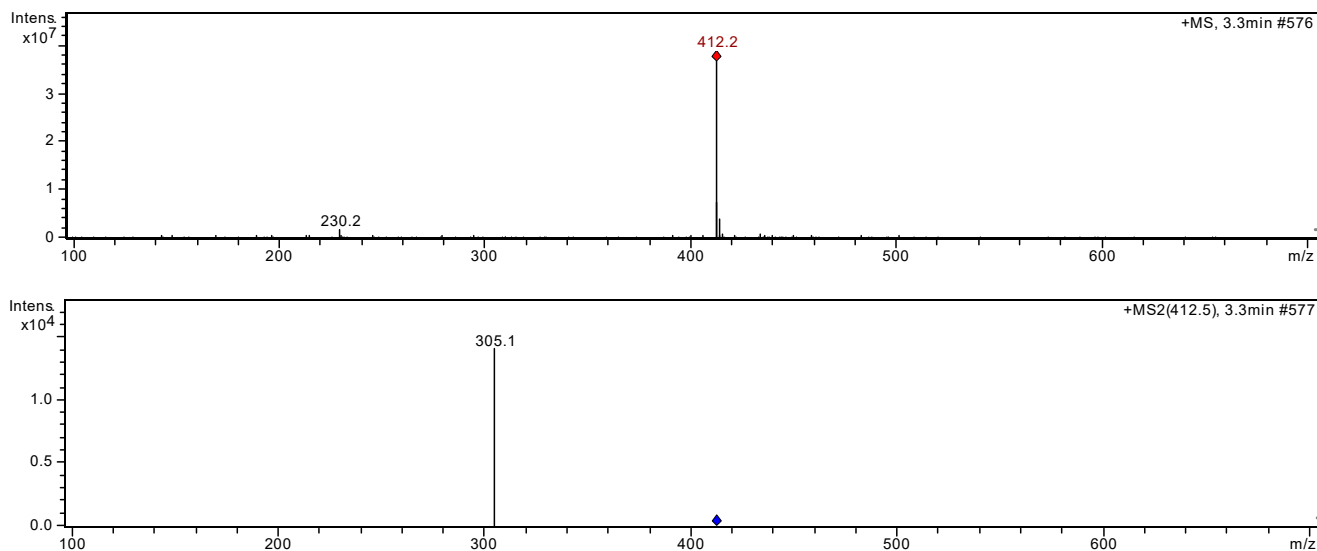


Figure S33. The MS spectrum for the compound **8b**.

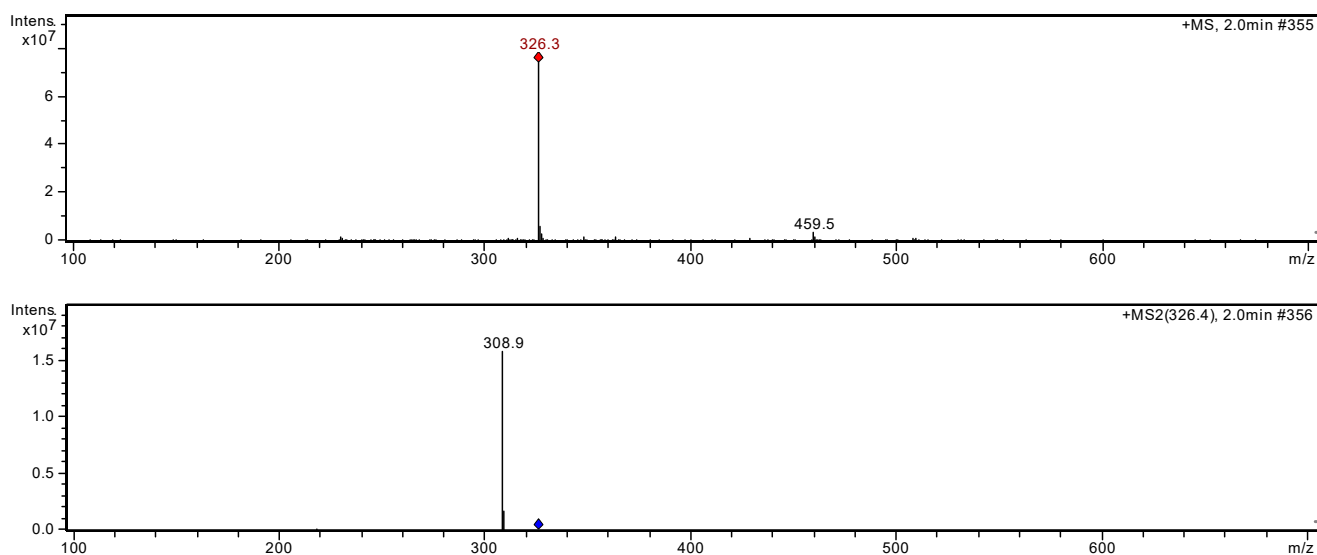


Figure S34. The MS spectrum for the compound **9b**.

### 1.2. The $^1\text{H}$ -NMR spectrum

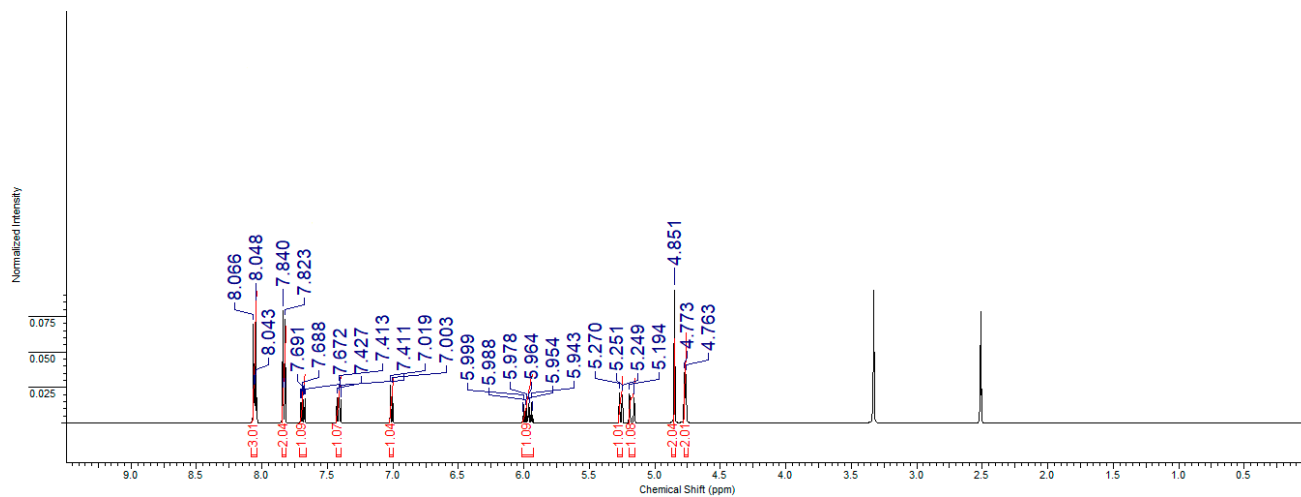
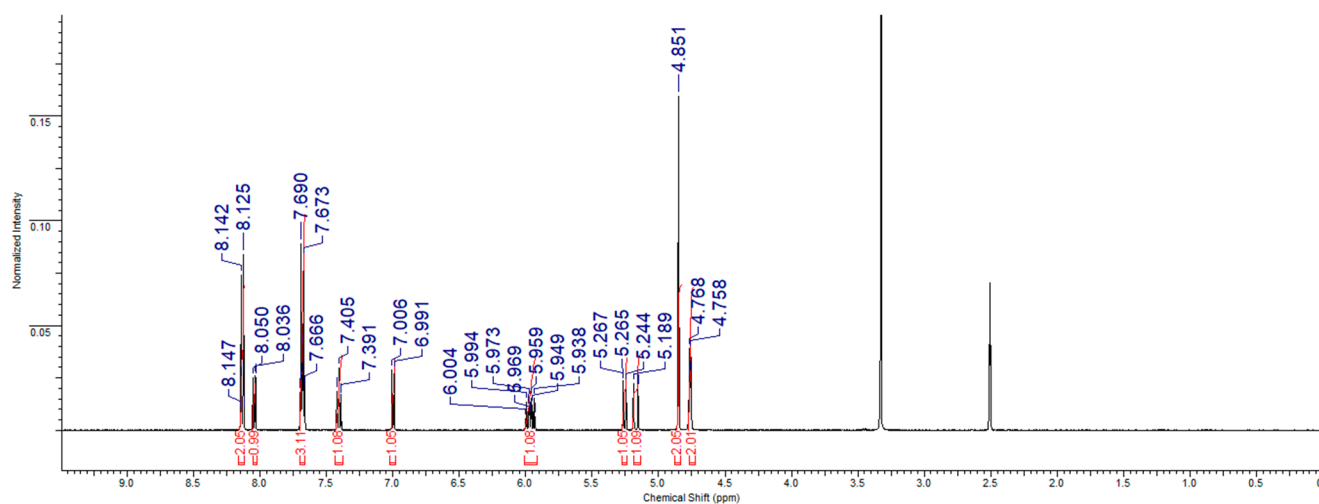
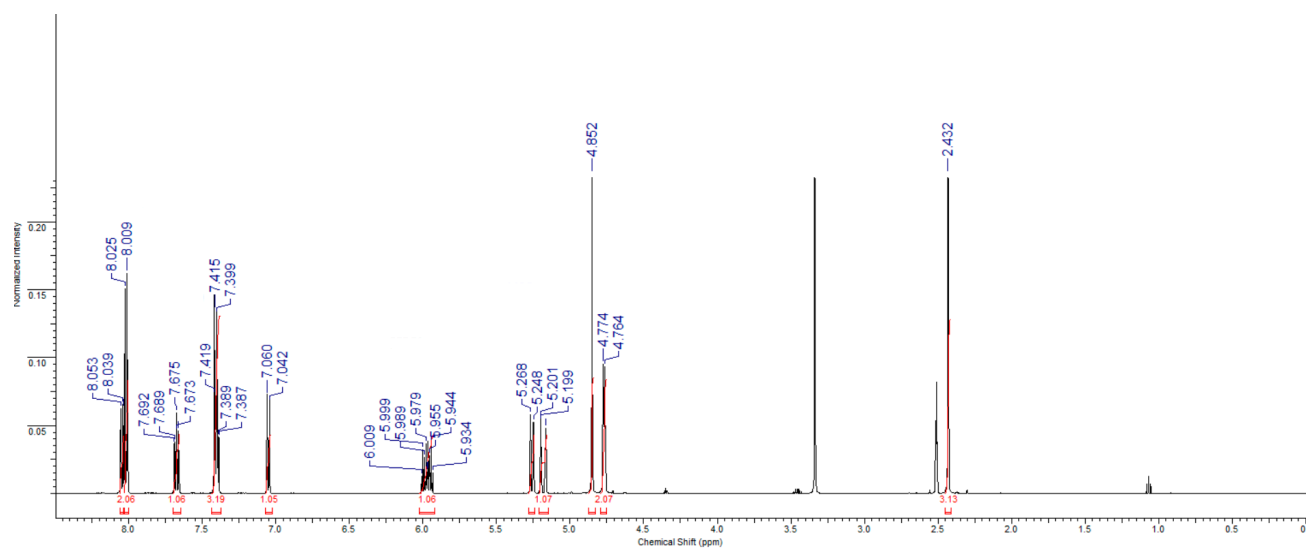
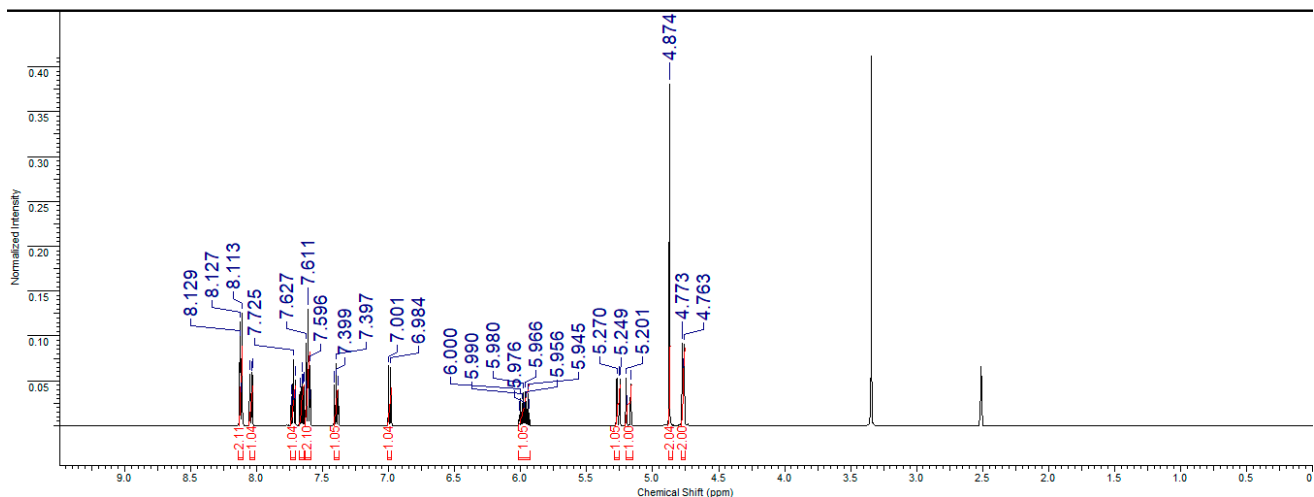


Figure S35. The  $^1\text{H}$ -NMR spectrum for the compound **1a**.





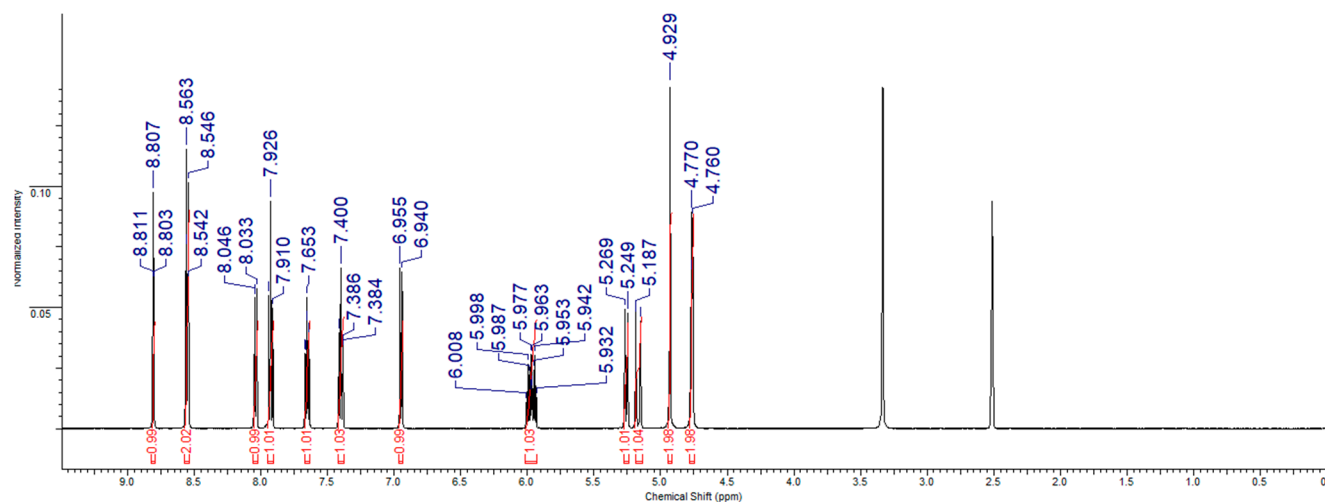


Figure S39. The  $^1\text{H}$ -NMR spectrum for the compound **5a**.

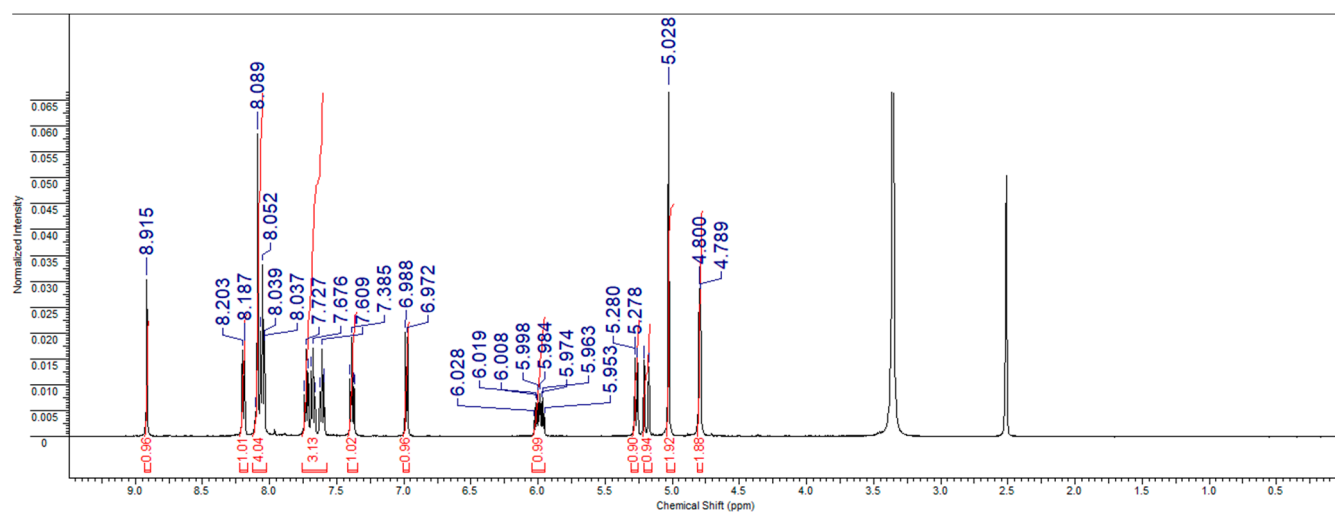


Figure S40. The  $^1\text{H}$ -NMR spectrum for the compound **6a**.

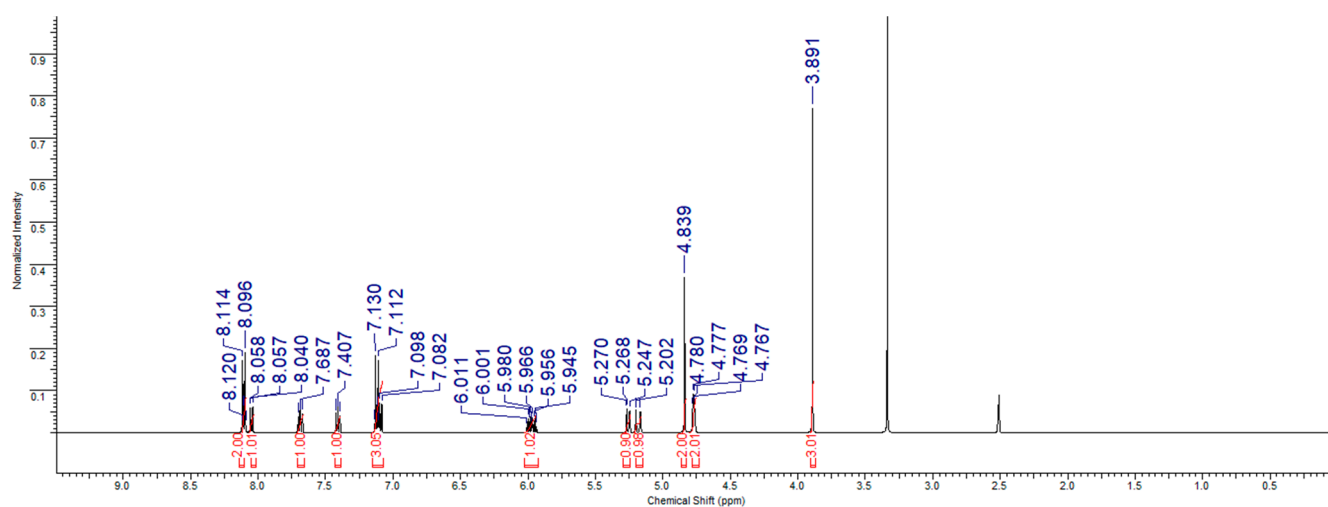


Figure S41. The  $^1\text{H}$ -NMR spectrum for the compound **7a**.



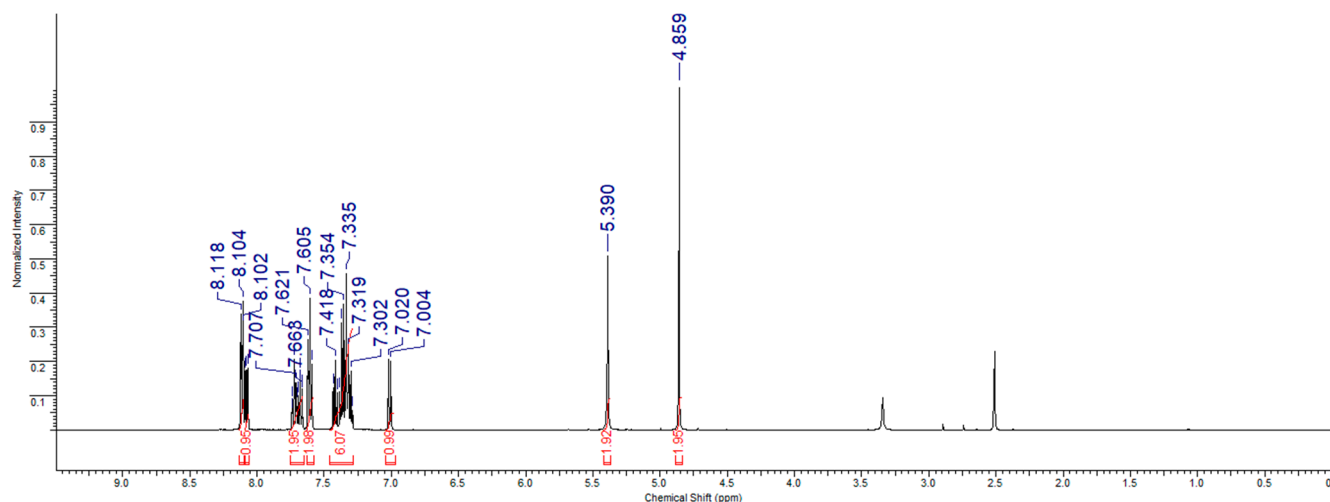


Figure S45. The <sup>1</sup>H-NMR spectrum for the compound 2b.

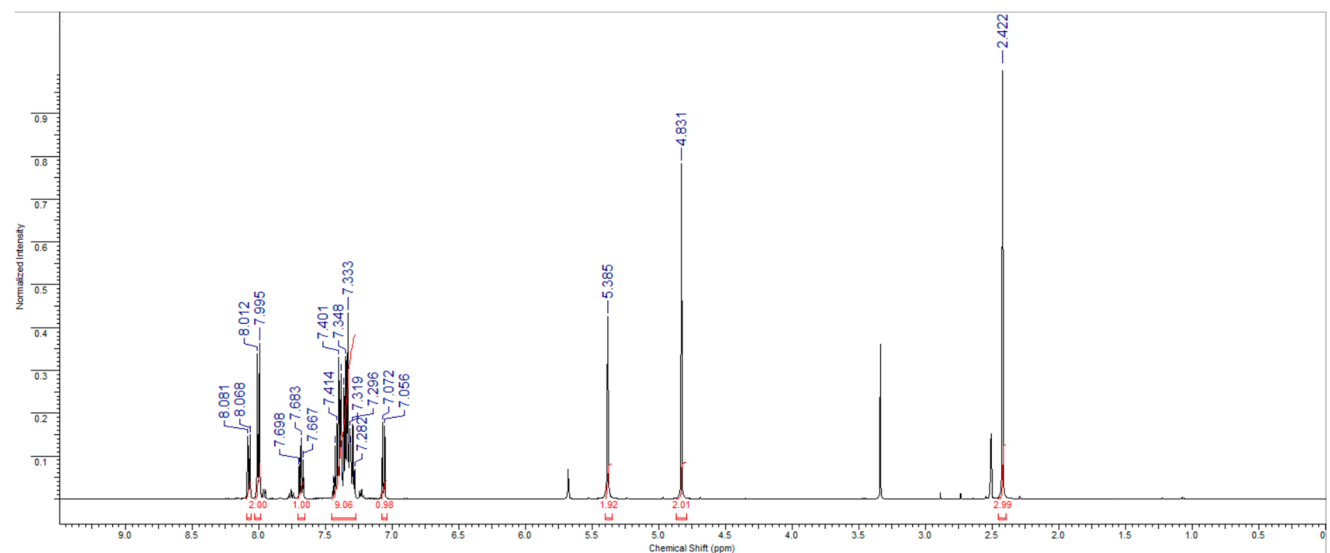


Figure S46. The <sup>1</sup>H-NMR spectrum for the compound 3b.

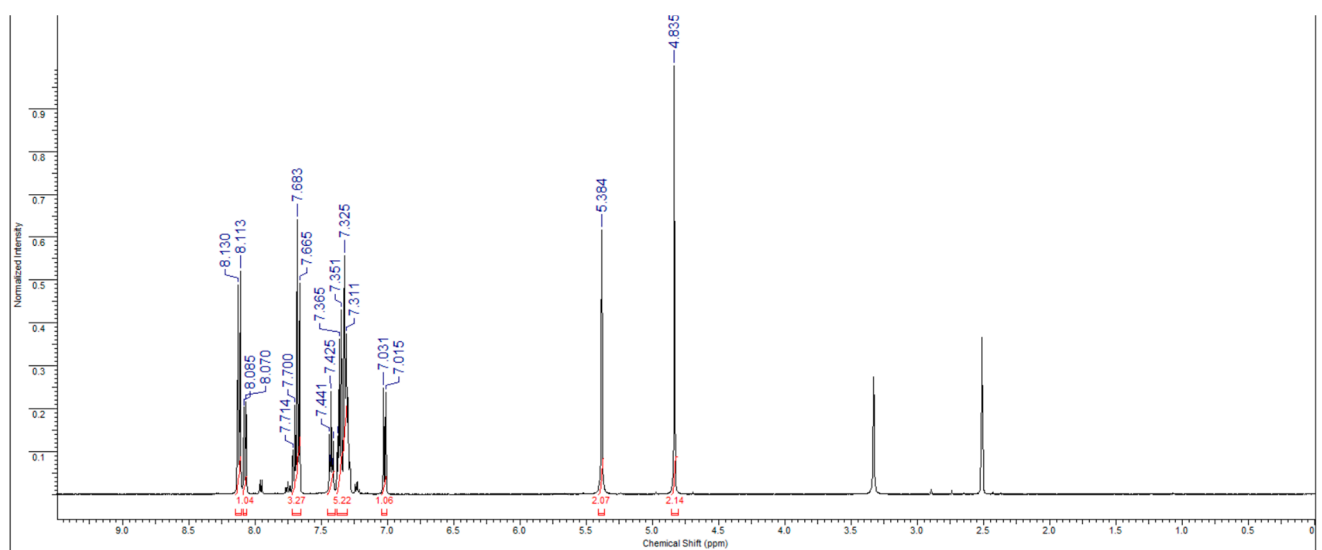


Figure S47. The <sup>1</sup>H-NMR spectrum for the compound 4b.

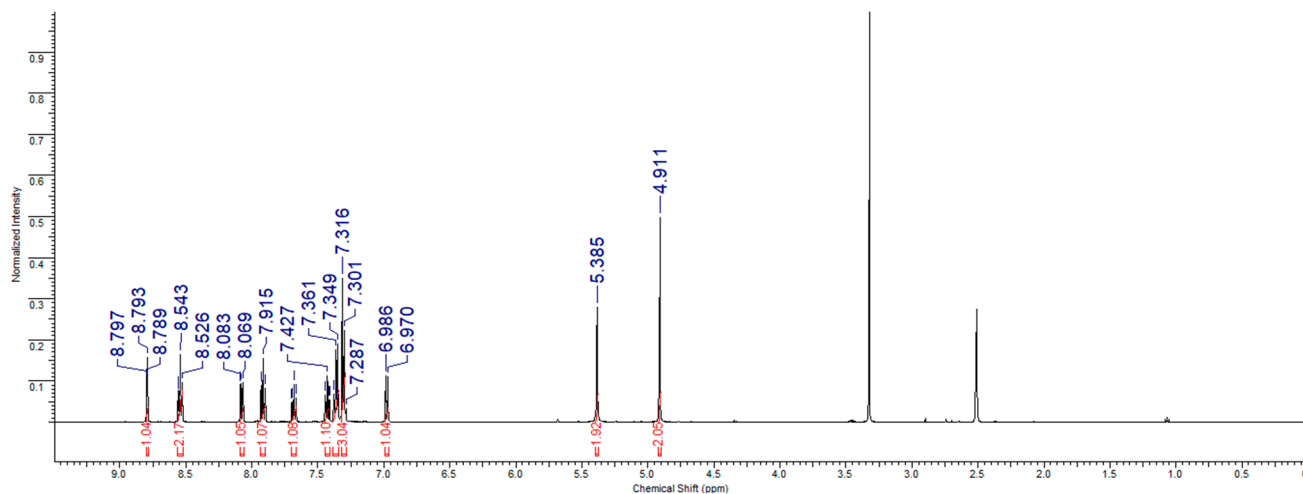


Figure S48. The  $^1\text{H}$ -NMR spectrum for the compound **5b**.

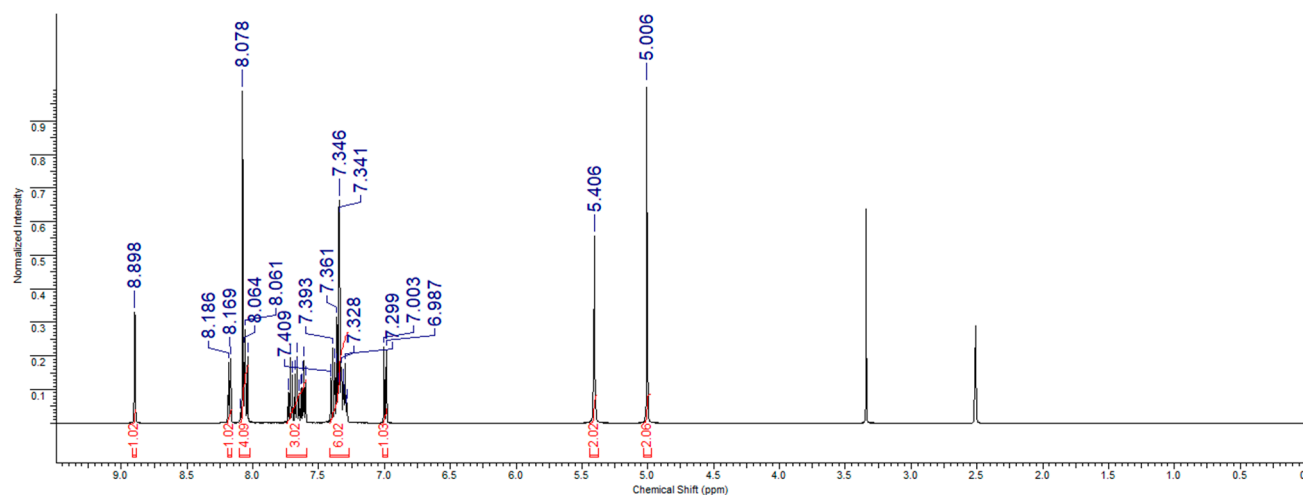


Figure S49. The  $^1\text{H}$ -NMR spectrum for the compound **6b**.

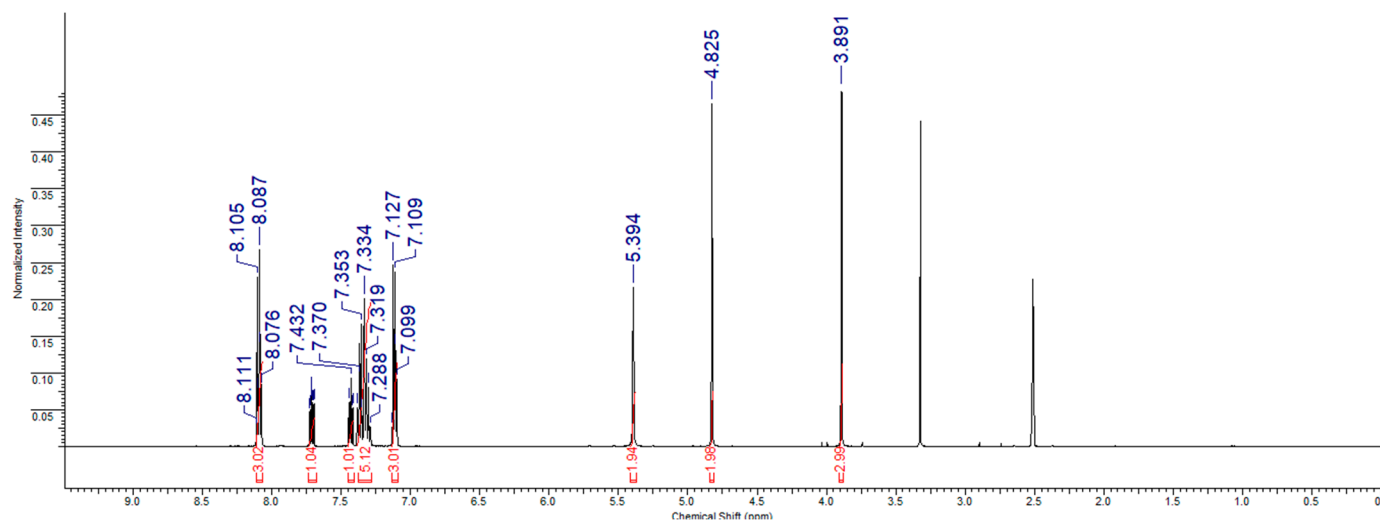


Figure S50. The  $^1\text{H}$ -NMR spectrum for the compound **7b**.

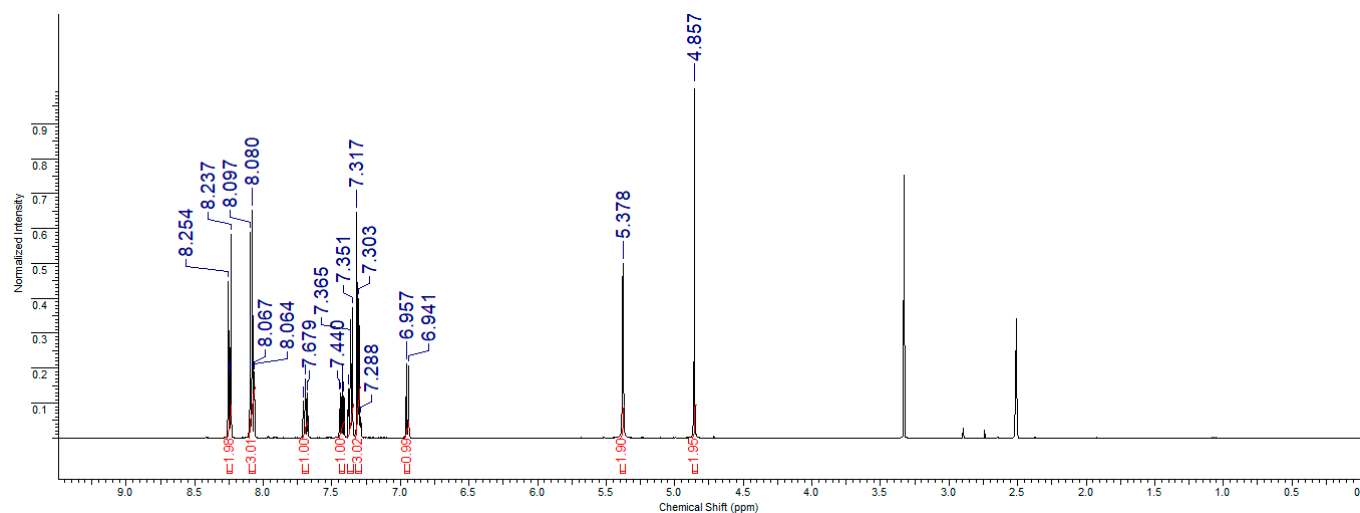


Figure S51. The <sup>1</sup>H-NMR spectrum for the compound 8b.

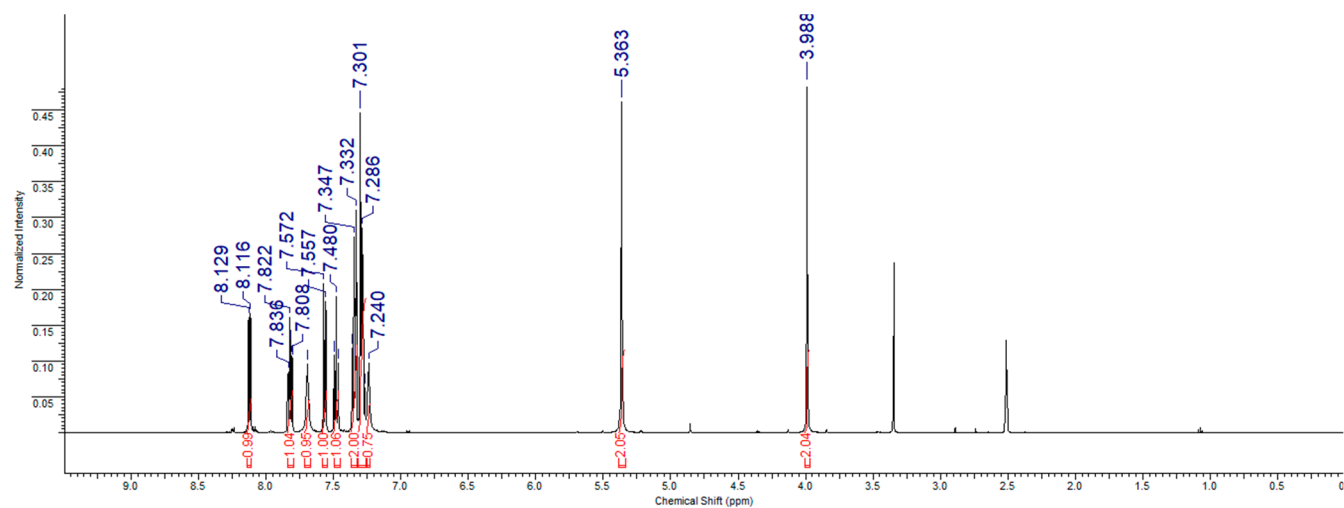


Figure S52. The <sup>1</sup>H-NMR spectrum for the compound 9b.

### 1.3. The <sup>13</sup>C-NMR spectrum

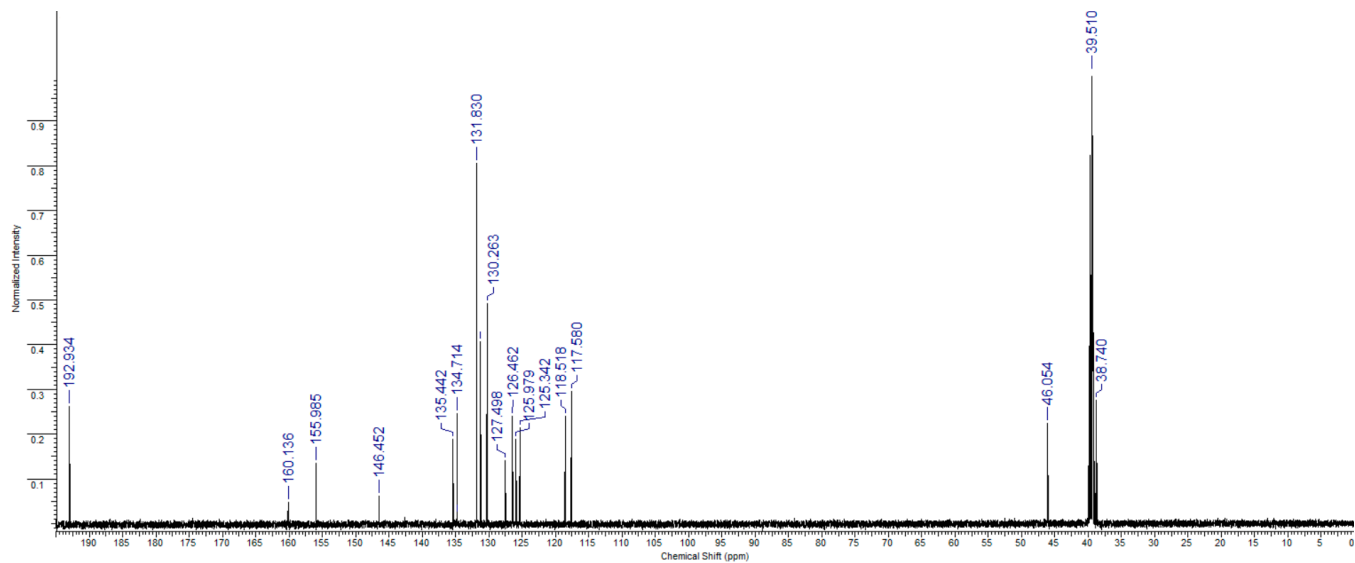


Figure S53. The  $^{13}\text{C}$ -NMR spectrum for the compound 1a.

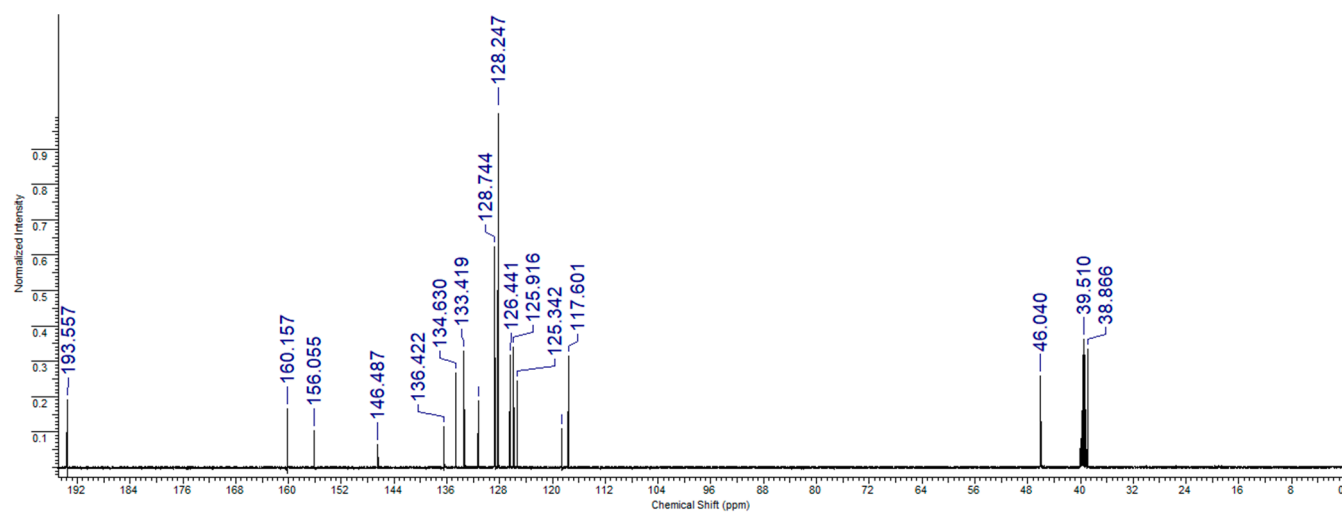


Figure S54. The  $^{13}\text{C}$ -NMR spectrum for the compound 2a.

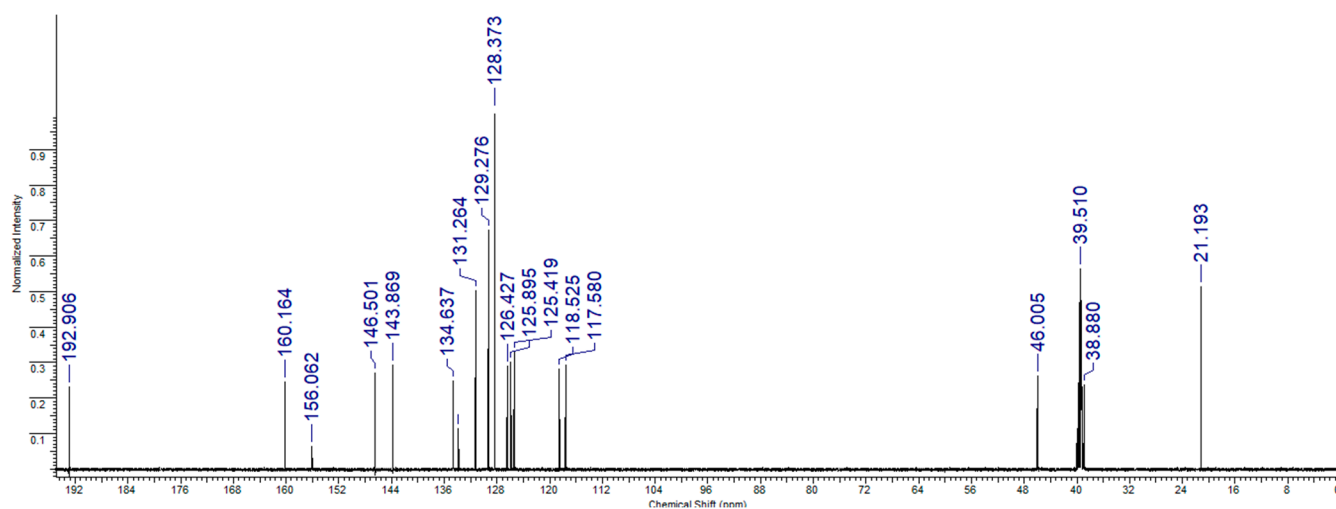


Figure S55. The  $^{13}\text{C}$ -NMR spectrum for the compound 3a.

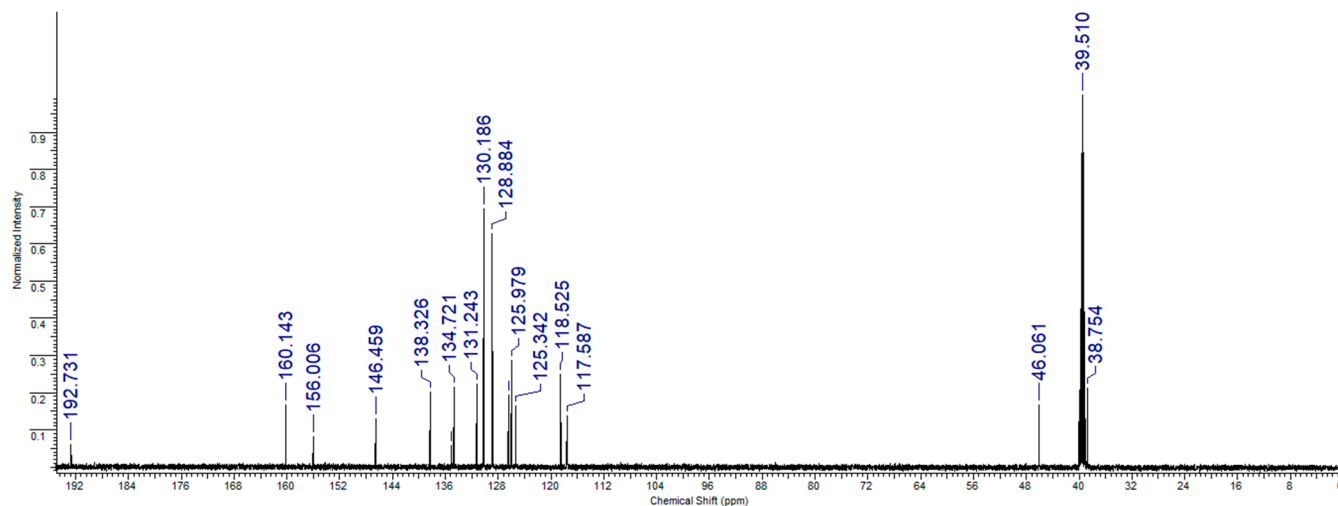


Figure S56. The <sup>13</sup>C-NMR spectrum for the compound 4a.

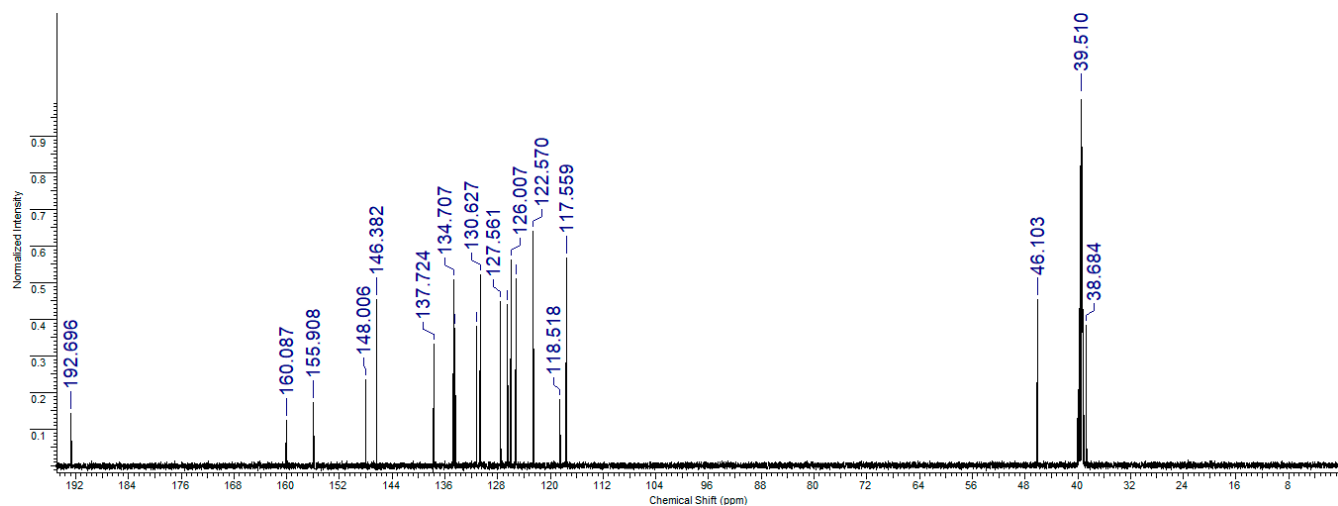


Figure S57. The <sup>13</sup>C-NMR spectrum for the compound 5a.

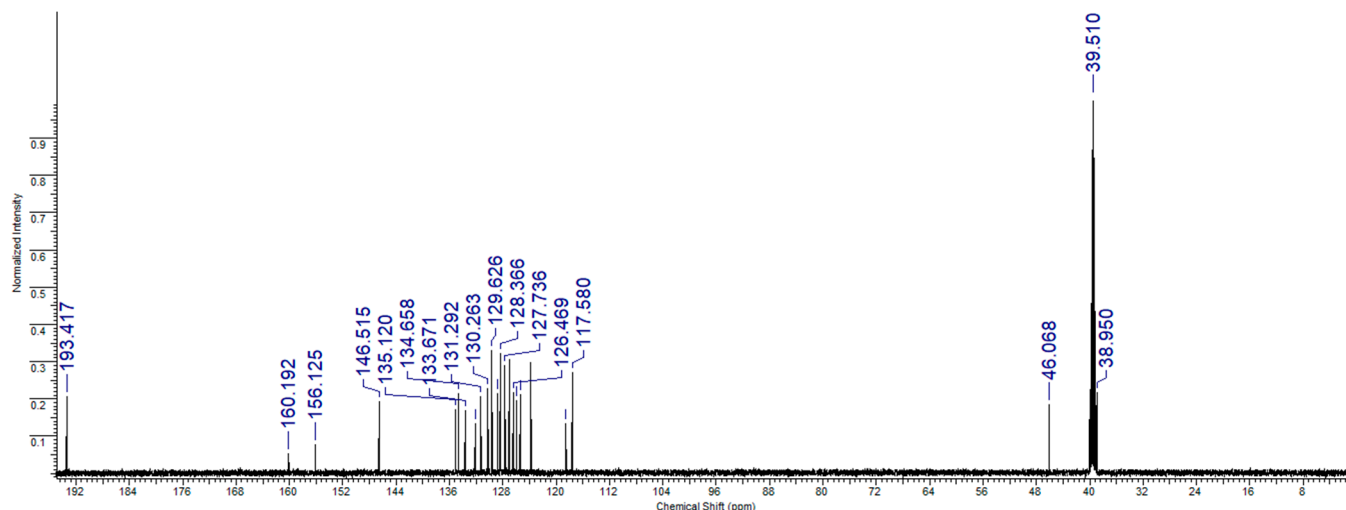


Figure S58. The <sup>13</sup>C-NMR spectrum for the compound 6a.



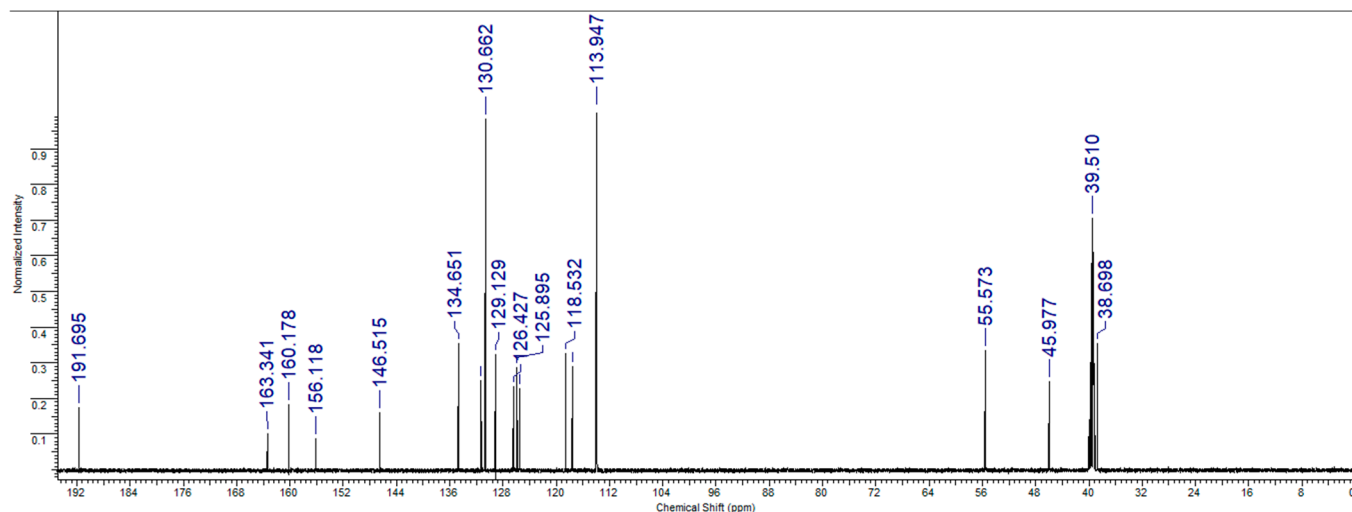


Figure S59. The  $^{13}\text{C}$ -NMR spectrum for the compound 7a.

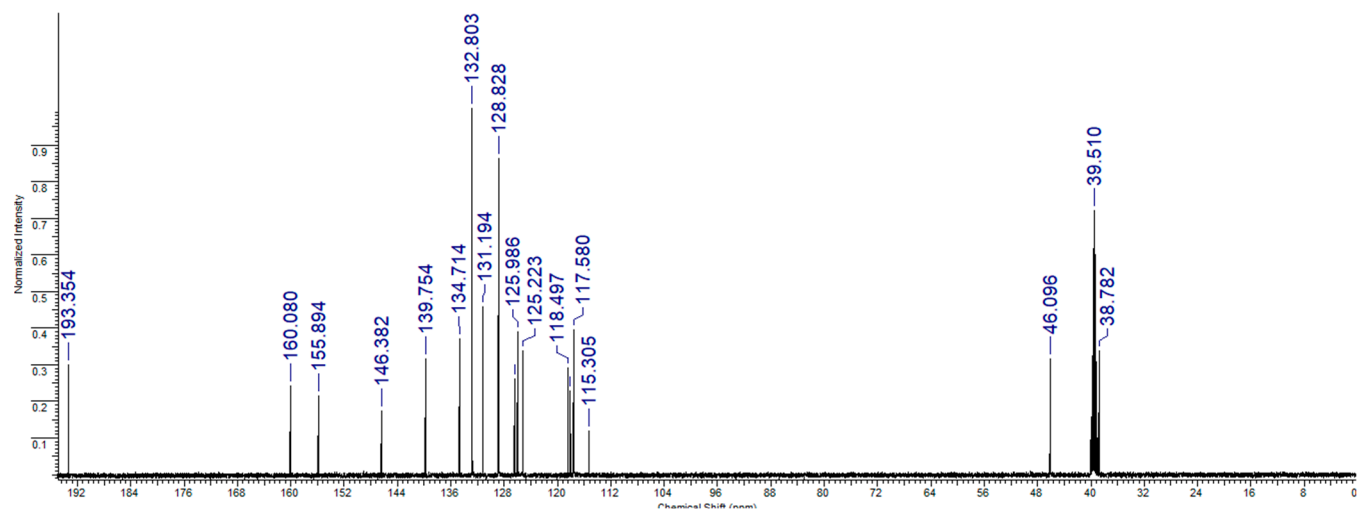


Figure S60. The  $^{13}\text{C}$ -NMR spectrum for the compound 8a.

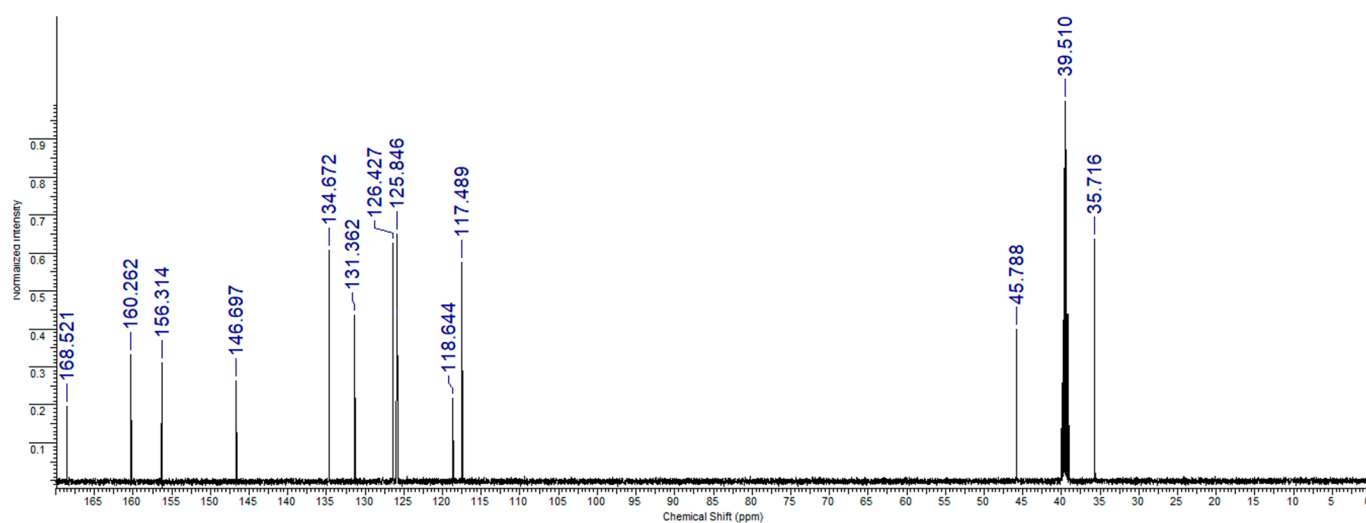


Figure S61. The  $^{13}\text{C}$ -NMR spectrum for the compound 9a.

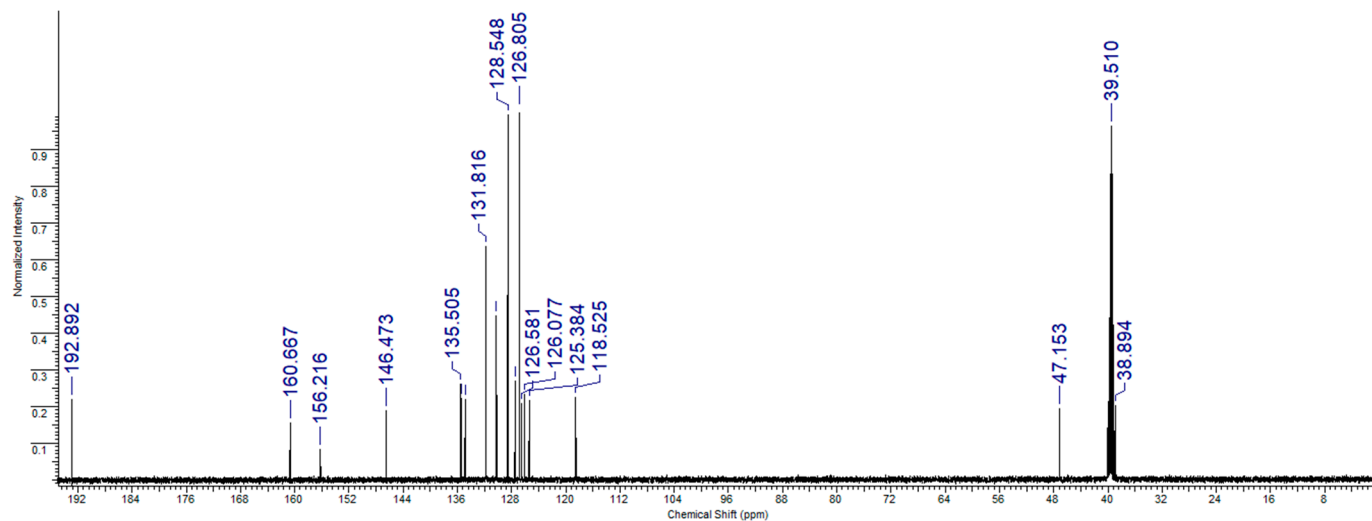


Figure S62. The <sup>13</sup>C-NMR spectrum for the compound 1b.

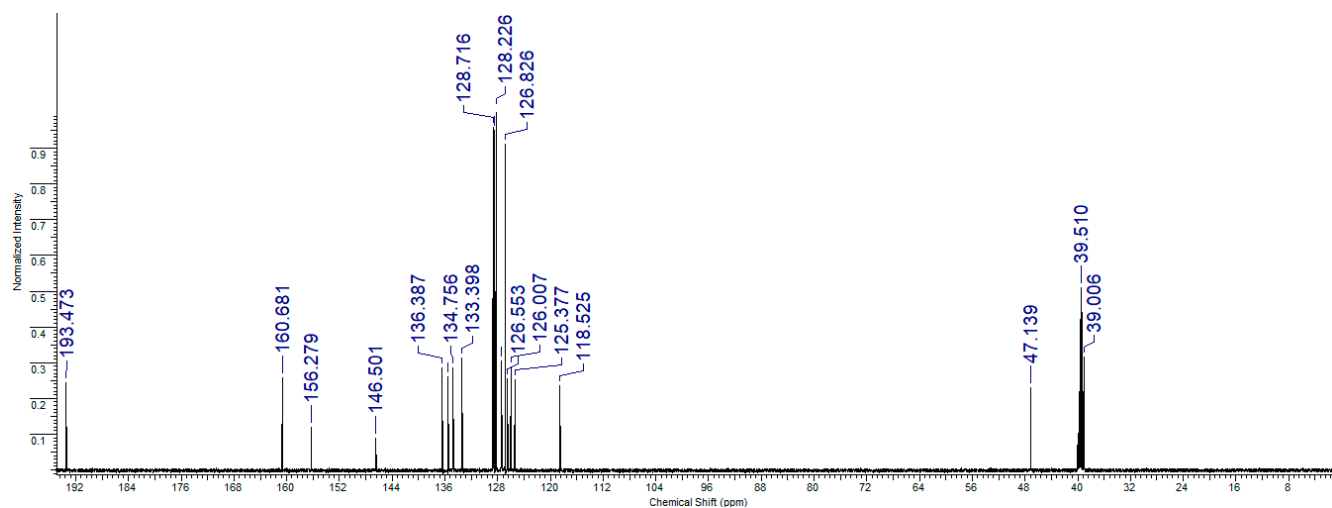


Figure S63. The <sup>13</sup>C-NMR spectrum for the compound 2b.

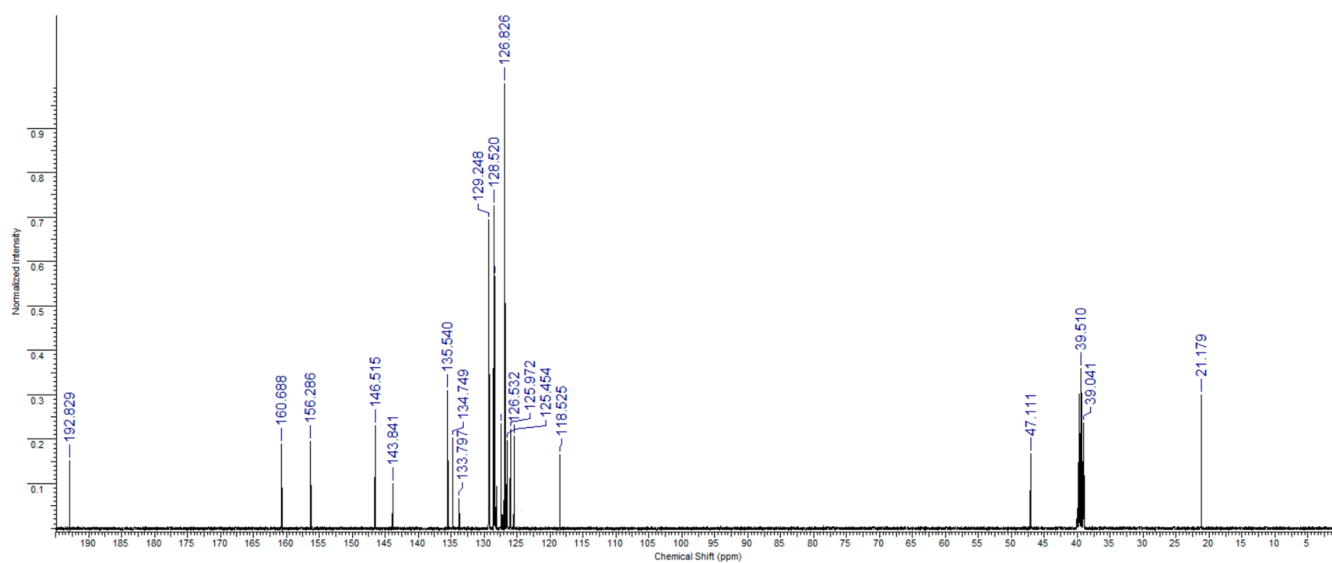


Figure S64. The <sup>13</sup>C-NMR spectrum for the compound 3b.

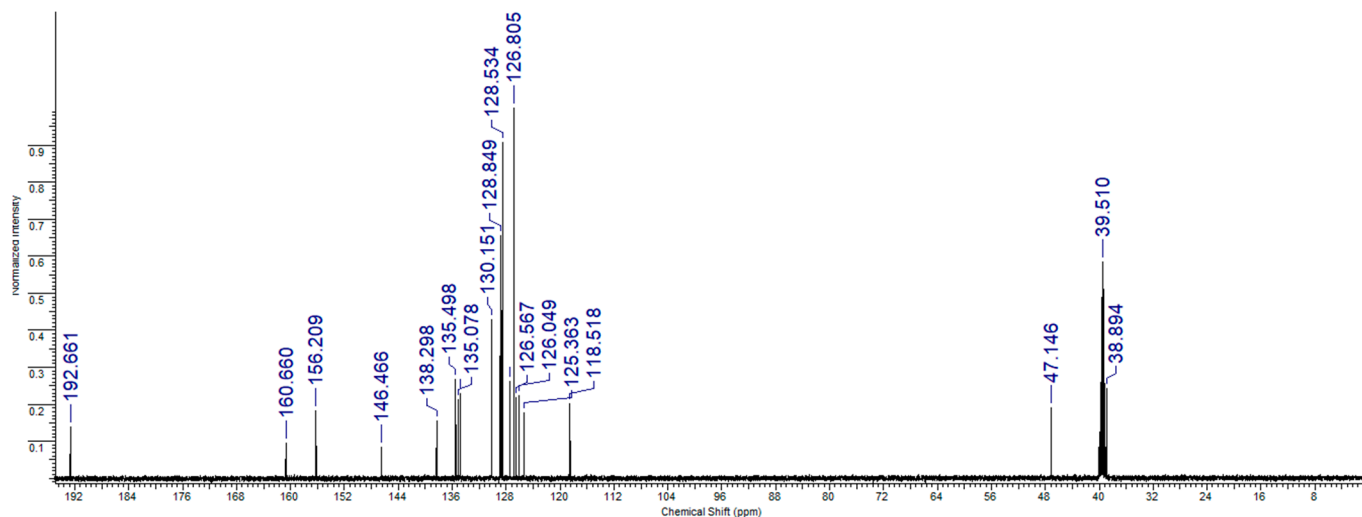


Figure S65. The <sup>13</sup>C-NMR spectrum for the compound 4b.

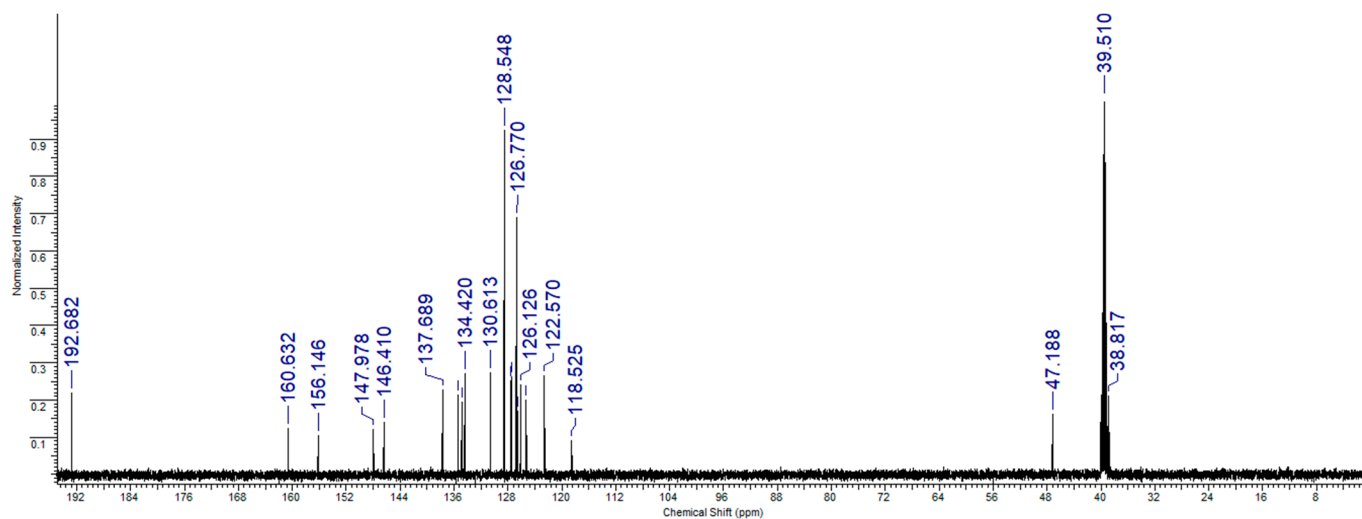


Figure S66. The <sup>13</sup>C-NMR spectrum for the compound 5b.

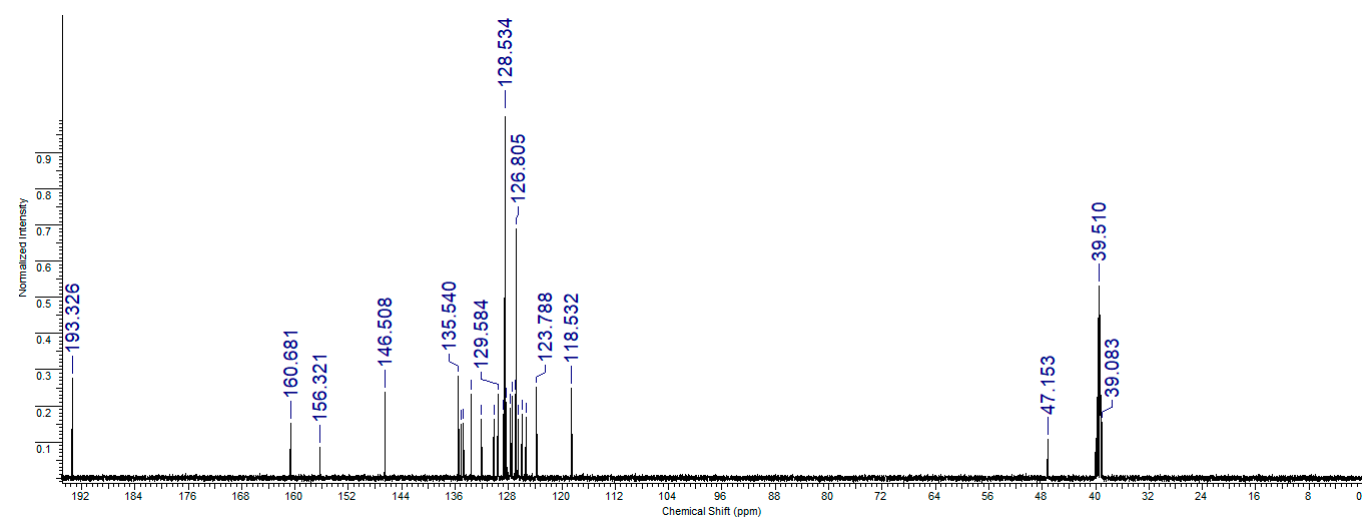


Figure S67. The <sup>13</sup>C-NMR spectrum for the compound 6b.

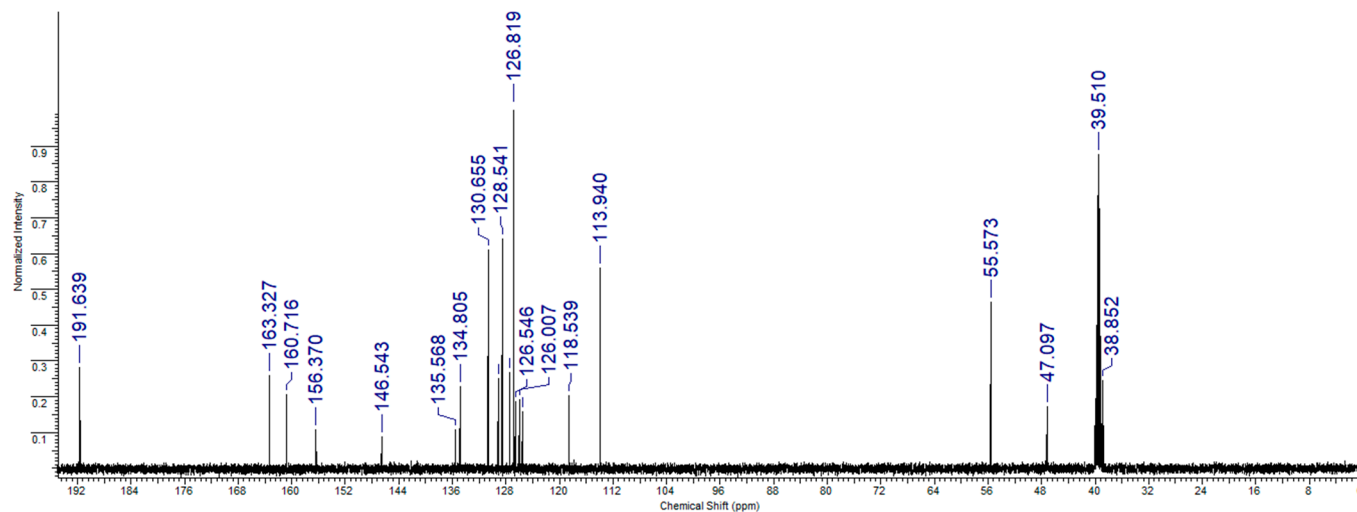


Figure S68. The  $^{13}\text{C}$ -NMR spectrum for the compound **7b**.

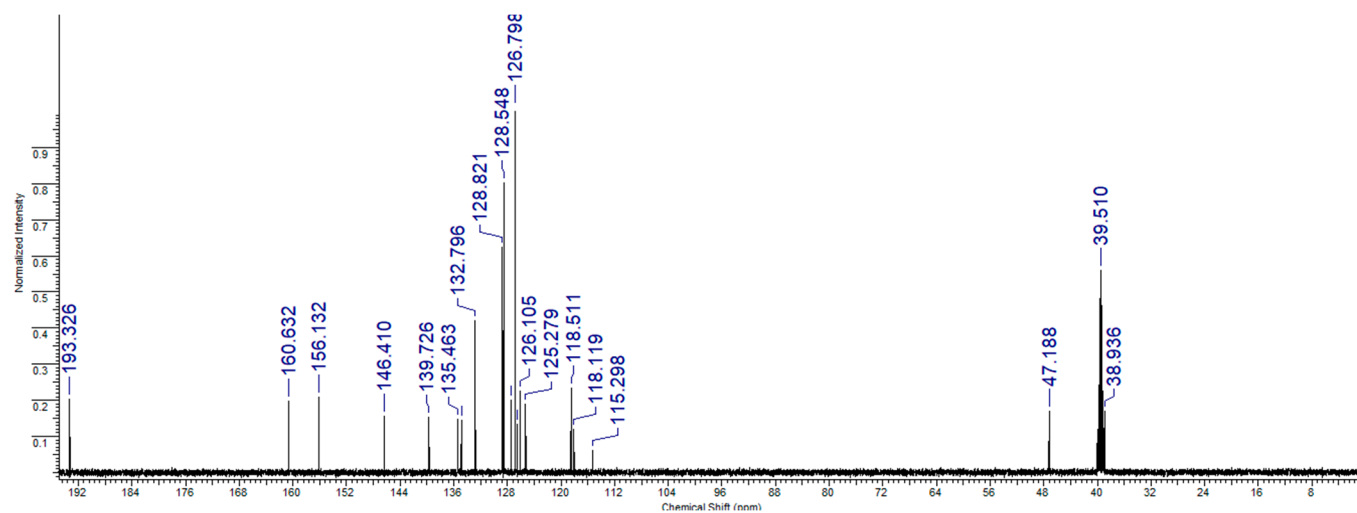


Figure S69. The  $^{13}\text{C}$ -NMR spectrum for the compound **8b**.

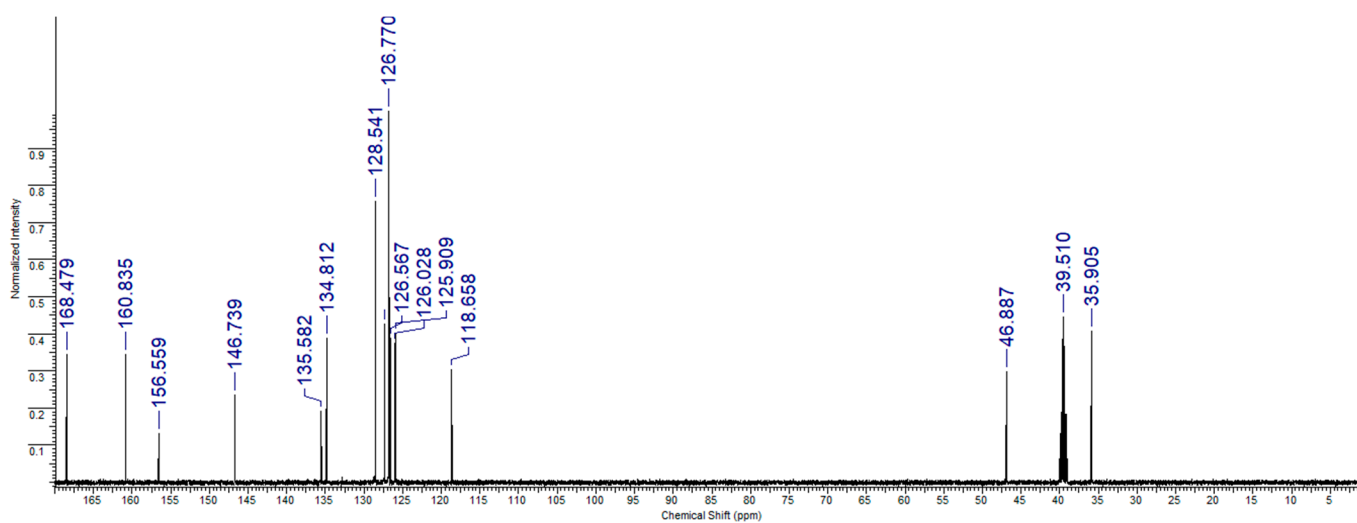
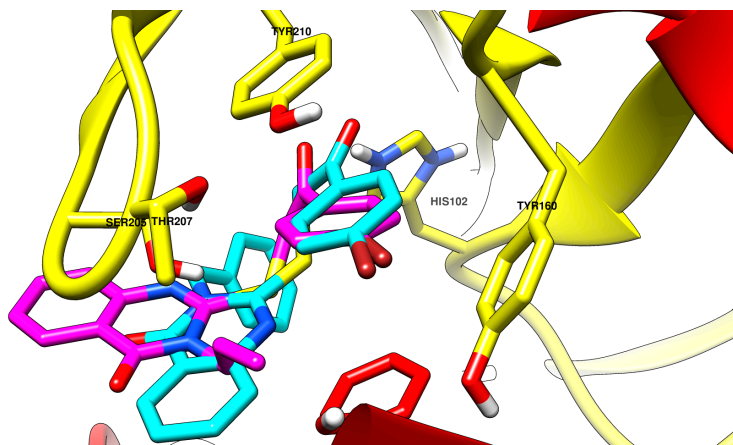
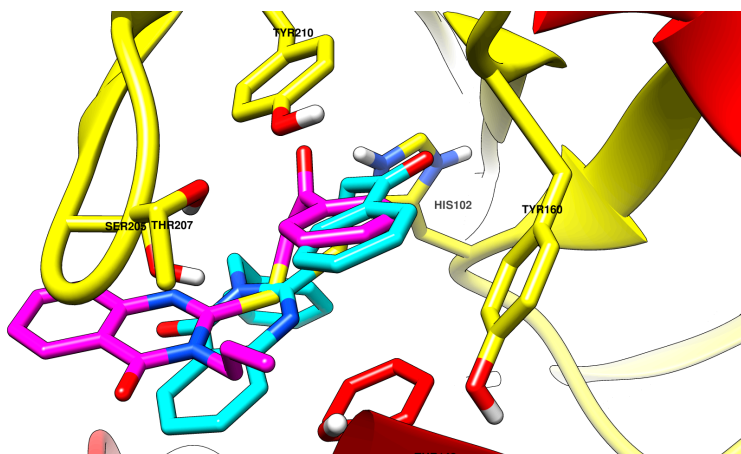


Figure S70. The  $^{13}\text{C}$ -NMR spectrum for the compound **9b**.

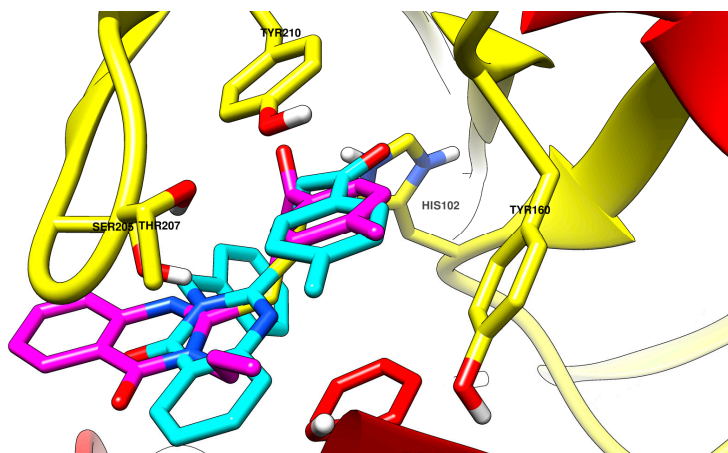
#### 1.4. Molecular docking



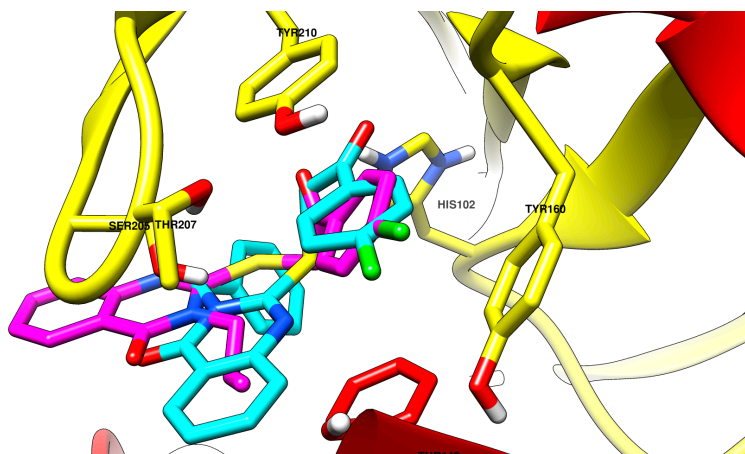
**Figure S71.** The best binding pose for the compounds **1a** and **1b** (carbon atoms in magenta) in the benzodiazepine binding site of GABA<sub>A</sub> receptor. The  $\gamma$ 2 subunit is depicted in red, while  $\alpha$ 1 subunit is depicted in yellow.



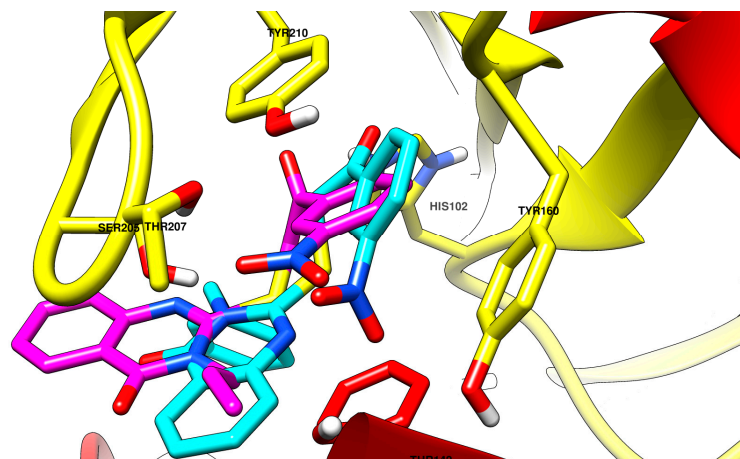
**Figure S72.** The best binding pose for the compounds **2a** and **2b** (carbon atoms in magenta) in the benzodiazepine binding site of GABA<sub>A</sub> receptor. The  $\gamma$ 2 subunit is depicted in red, while  $\alpha$ 1 subunit is depicted in yellow.



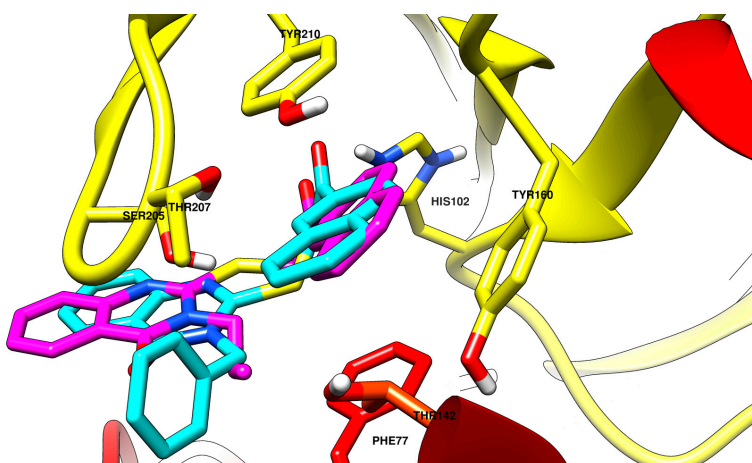
**Figure S73.** The best binding pose for the compounds **3a** and **3b** (carbon atoms in magenta) in the benzodiazepine binding site of GABA<sub>A</sub> receptor. The  $\gamma$ 2 subunit is depicted in red, while  $\alpha$ 1 subunit is depicted in yellow.



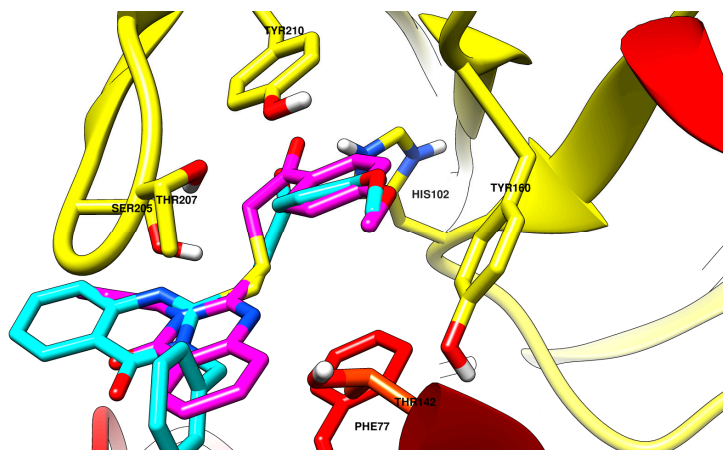
**Figure S74.** The best binding pose for the compounds **4a** and **4b** (carbon atoms in magenta) in the benzodiazepine binding site of GABA<sub>A</sub> receptor. The  $\gamma$ 2 subunit is depicted in red, while  $\alpha$ 1 subunit is depicted in yellow.



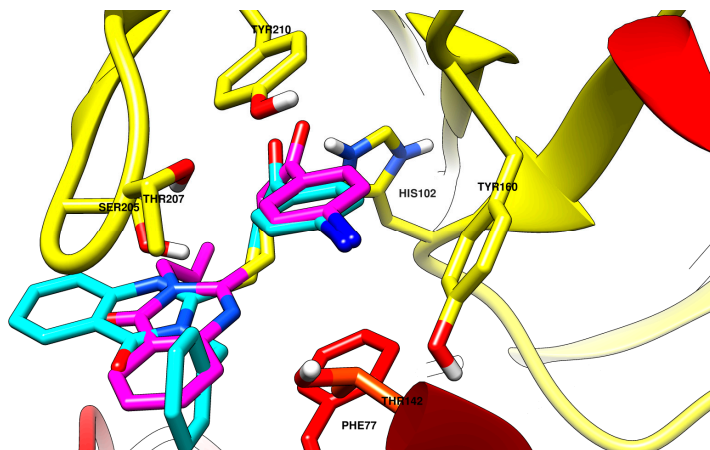
**Figure S75.** The best binding pose for the compounds **5a** and **5b** (carbon atoms in magenta) in the benzodiazepine binding site of GABA<sub>A</sub> receptor. The  $\gamma$ 2 subunit is depicted in red, while  $\alpha$ 1 subunit is depicted in yellow.



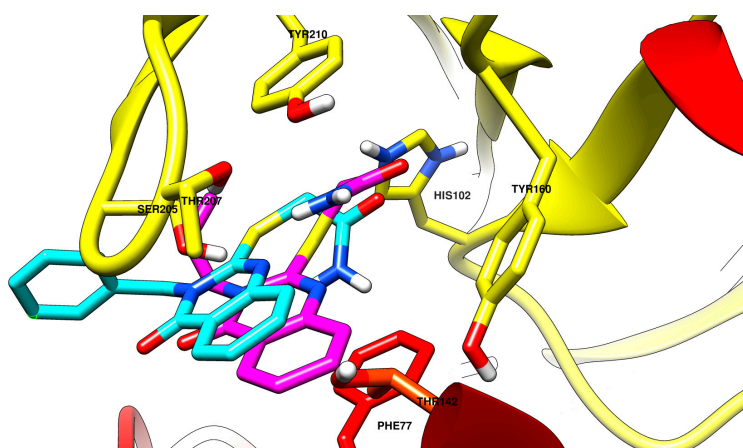
**Figure S76.** The best binding pose for the compounds **6a** and **6b** (carbon atoms in magenta) in the benzodiazepine binding site of GABA<sub>A</sub> receptor. The  $\gamma$ 2 subunit is depicted in red, while  $\alpha$ 1 subunit is depicted in yellow.



**Figure S77.** The best binding pose for the compounds **7a** and **7b** (carbon atoms in magenta) in the benzodiazepine binding site of GABA<sub>A</sub> receptor. The  $\gamma$ 2 subunit is depicted in red, while  $\alpha$ 1 subunit is depicted in yellow.



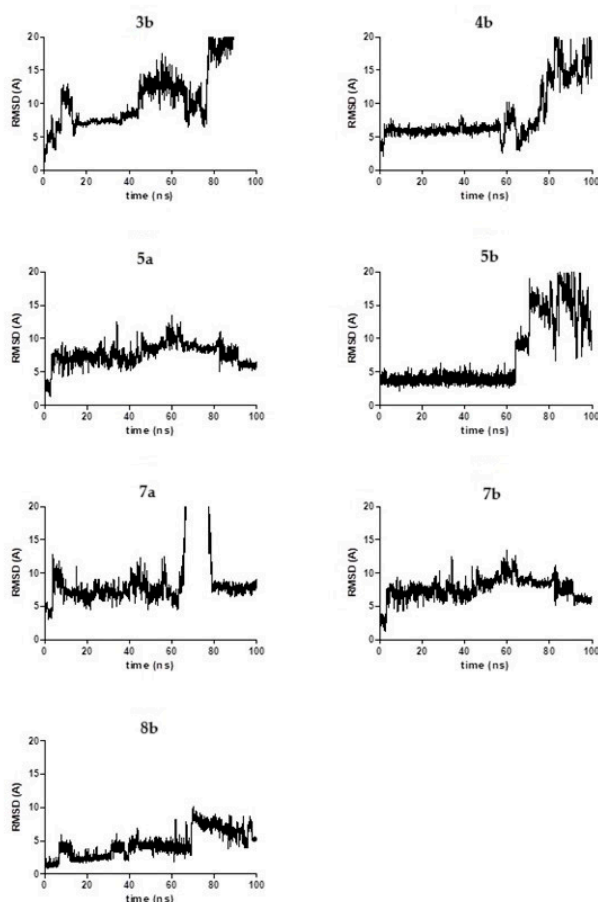
**Figure S78.** The best binding pose for the compounds **8a** and **8b** (carbon atoms in magenta) in the benzodiazepine binding site of GABA<sub>A</sub> receptor. The  $\gamma$ 2 subunit is depicted in red, while  $\alpha$ 1 subunit is depicted in yellow.



**Figure S79.** The best binding pose for the compounds **9a** and **9b** (carbon atoms in magenta) in the benzodiazepine binding site of GABA<sub>A</sub> receptor. The  $\gamma$ 2 subunit is depicted in red, while  $\alpha$ 1 subunit is depicted in yellow.

### 1.5. Molecular dynamics simulation





**Figure S80.** RMSD (Å) of the ligands during the 100 ns simulation in complex with carbonic anhydrase II for the compounds **3b**, **4b**, **5a**, **5b**, **7a**, **7b**, **8a** and **8b**.

## 2. Tables

### 2.1. Pentylentetrazole (PTZ)-induced seizures

**Table S1.** Protection against PTZ-induced seizure model, time until the first seizure, mean number of seizures and LogP, for the compounds of the "a" series, in the first 30 minutes after PTZ administration.

Comp.	Doses (mg/kg)	PTZ P/T <sup>a</sup> (% protection)	T <sub>c</sub> (s)	No. seizures <sup>b</sup>	Log P
NC	-	0/6 (0%)	111.67 ± 27.38	9.33 ± 1.65	-
<b>1a</b>	D <sub>1</sub>	0/6 (0%)	398.33 ± 40.37	7.83 ± 2.09	3.56
	D <sub>2</sub>	1/6 (16.67%)	660.00 ± 248.35	2.00 ± 0.68	
	D <sub>3</sub>	3/6 (50%)	1600.00*** ± 143.39	2.00 ± 1.18	
<b>2a</b>	D <sub>1</sub>	0/6 (0%)	630.00 ± 45.83	13.17 ± 3.75	3.16
	D <sub>2</sub>	0/6 (0%)	1210.00*** ± 118.41	6.00 ± 1.46	
	D <sub>3</sub>	3/6 (50%)	1130.00*** ± 307.66	4.83 ± 2.48	
<b>3a</b>	D <sub>1</sub>	1/6 (16.67%)	590.00 ± 242.69	8.33 ± 2.26	3.21
	D <sub>2</sub>	1/6 (16.67%)	600.00 ± 253.61	5.17 ± 1.05	
	D <sub>3</sub>	0/6 (0%)	590.00 ± 170.70	9.67 ± 3.06	
<b>4a</b>	D <sub>1</sub>	0/6 (0%)	671.67 ± 156.77	4.00 ± 0.52	3.46
	D <sub>2</sub>	0/6 (0%)	153.33 ± 21.08	4.00 ± 1.06	
	D <sub>3</sub>	0/6 (0%)	690.00 ± 33.76	7.83 ± 1.11	
<b>5a</b>	D <sub>1</sub>	0/6 (0%)	1080.00** ± 63.87	9.83 ± 1.74	2.65



Comp.	Doses (mg/kg)	PTZ P/T <sup>a</sup> (% protection)	T <sub>c</sub> (s)	No. seizures <sup>b</sup>	Log P
	D <sub>2</sub>	0/6 (0%)	1030.00** ± 78.10	15.67 ± 3.52	
	D <sub>3</sub>	3/6 (50%)	1800.00*** ± 0.00	0.83* ± 0.40	
6a	D <sub>1</sub>	1/6 (16.67%)	540.00 ± 254.09	7.83 ± 2.33	3.60
	D <sub>2</sub>	3/6 (50%)	1700.00*** ± 55.14	2.17 ± 1.05	
	D <sub>3</sub>	1/6 (16.67%)	960.00** ± 174.59	4.83 ± 1.19	
7a	D <sub>1</sub>	0/6 (0%)	710.00 ± 74.97	8.67 ± 2.56	3.16
	D <sub>2</sub>	1/6 (16.67%)	740.00 ± 253.46	5.00 ± 1.39	
	D <sub>3</sub>	6/6 (100%)	1800.00*** ± 0.00	0.00** ± 0.00	
8a	D <sub>1</sub>	1/6 (16.67%)	830.00* ± 230.52	2.00 ± 0.58	3.00
	D <sub>2</sub>	2/6 (33.33%)	853.33* ± 308.96	2.17 ± 0.87	
	D <sub>3</sub>	3/6 (50%)	1220.00*** ± 259.54	8.33 ± 3.96	
9a	D <sub>1</sub>	1/6 (16.67%)	940.00** ± 277.42	3.67 ± 1.76	1.91
	D <sub>2</sub>	0/6 (0%)	510.00 ± 207.12	6.67 ± 1.15	
	D <sub>3</sub>	0/6 (0%)	1180.00*** ± 131.15	12.17 ± 3.09	
D	D = 2 mg/kg	6/6 (100%)	1800.00*** ± 0.00	0.00** ± 0.00	2.68
Phe	D = 15 mg/kg	6/6 (100%)	1800.00*** ± 0.00	0.00** ± 0.00	1.06

Comp. – compound ("a" series); NC – negative control (solvent).; D – diazepam.;

Phe – phenobarbital;

D<sub>1</sub> = 50 mg/kg; D<sub>2</sub> = 100 mg/kg; D<sub>3</sub> = 150 mg/kg;

<sup>a</sup> – PTZ – pentylenetetrazole; (P) - Number of mice protected (no seizures)/(T) - number of mice tested;

T<sub>c</sub> – Time (seconds) until the first seizure ± SEM (Standard error of mean);

<sup>b</sup> – mean number of seizures in the first 30 minutes/animal lot ± SEM (Standard error of mean);

\*\*\*p<0.001; \*\*p<0.01; \*p<0.05; Statistically significant compared to negative control group.

**Table S2.** Protection against PTZ-induced seizure model, time until the first seizure, mean number of seizures and LogP, for the compounds of the "b" series, in the first 30 minutes after PTZ administration.

Comp.	Doses (mg/kg)	PTZ P/T <sup>a</sup> (% protection)	T <sub>c</sub> (s)	No. seizures <sup>b</sup>	LogP
NC	-	0/6 (0%)	111.67 ± 27.38	9.33 ± 1.65	-
1b	D <sub>1</sub>	1/6 (16.67%)	1450.00*** ± 124.34	2.33*** ± 0.71	3.28
	D <sub>2</sub>	2/6 (33.33%)	1190.00*** ± 198.04	1.17*** ± 0.47	
	D <sub>3</sub>	1/6 (16.67%)	850.00** ± 205.77	2.33*** ± 0.71	
2b	D <sub>1</sub>	0/6 (0%)	1100.00*** ± 150.73	5.67 ± 0.71	2.99
	D <sub>2</sub>	0/6 (0%)	820.00** ± 97.57	5.17 ± 0.83	
	D <sub>3</sub>	0/6 (0%)	580.00 ± 115.58	3.83* ± 1.35	
3b	D <sub>1</sub>	0/6 (0%)	990.00*** ± 73.89	4.83 ± 0.79	3.73
	D <sub>2</sub>	1/6 (16.67%)	950.00*** ± 174.87	3.33** ± 1.05	
	D <sub>3</sub>	4/6 (66.66%)	1390.00*** ± 259.42	1.17*** ± 0.83	
4b	D <sub>1</sub>	0/6 (0%)	900.00** ± 26.83	8.17 ± 2.32	3.13
	D <sub>2</sub>	1/6 (16.67%)	590.00 ± 242.69	4.67 ± 1.72	
	D <sub>3</sub>	4/6 (66.66%)	1500.00*** ± 189.73	0.67*** ± 0.49	
5b	D <sub>1</sub>	0/6 (0%)	1030.00*** ± 47.53	6.50 ± 1.38	2.74
	D <sub>2</sub>	1/6 (16.67%)	1020.00*** ± 168.29	3.83* ± 1.07	
	D <sub>3</sub>	3/6 (50%)	1650.00*** ± 150.00	0.17*** ± 0.16	
6b	D <sub>1</sub>	0/6 (0%)	780.00* ± 63.87	5.33 ± 0.84	3.65
	D <sub>2</sub>	0/6 (0%)	510.00 ± 59.49	3.00** ± 0.96	
	D <sub>3</sub>	1/6 (16.67%)	620.00 ± 241.83	2.50** ± 0.76	
7b	D <sub>1</sub>	0/6 (0%)	460.00 ± 85.79	7.50 ± 2.07	3.74

Comp.	Doses (mg/kg)	PTZ P/T <sup>a</sup> (% protection)	T <sub>c</sub> (s)	No. seizures <sup>b</sup>	LogP
	D <sub>2</sub>	0/6 (0%)	1110.00*** ± 150.80	3.00** ± 1.15	
	D <sub>3</sub>	3/6 (50%)	1340.00*** ± 212.79	2.33*** ± 1.30	
8b	D <sub>1</sub>	1/6 (16.67%)	1330.00*** ± 167.87	3.83* ± 1.81	3.29
	D <sub>2</sub>	3/6 (50%)	1480.00*** ± 176.41	4.33* ± 2.43	
	D <sub>3</sub>	6/6 (100%)	1800.00*** ± 0.00	0.00*** ± 0.00	
9b	D <sub>1</sub>	0/6 (0%)	470.00 ± 76.55	6.00 ± 0.89	2.15
	D <sub>2</sub>	0/6 (0%)	350.00 ± 36.05	5.33 ± 0.88	
	D <sub>3</sub>	0/6 (0%)	300.00 ± 40.98	7.83 ± 1.22	
D	D = 2 mg/kg	6/6 (100%)	1800.00*** ± 0.00	0.00*** ± 0.00	2.68
Phe	D = 15 mg/kg	6/6 (100%)	1800.00*** ± 0.00	0.00*** ± 0.00	1.06

**Comp.** – compound ("b" series); NC – negative control (solvent).; **D** – diazepam.;

**Phe** – phenobarbital;

**D**<sub>1</sub> = 50 mg/kg; **D**<sub>2</sub> = 100 mg/kg; **D**<sub>3</sub> = 150 mg/kg;

<sup>a</sup> – **PTZ** – pentylenetetrazole; (**P**) - Number of mice protected (no seizures)/(**T**) - number of mice tested;

**T<sub>c</sub>** – Time (seconds) until the first seizure ± **SEM** (Standard error of mean);

<sup>b</sup> – mean number of seizures in the first 30 minutes/animal lot ± **SEM** (Standard error of mean);

\*\*\*p<0.001; \*\*p<0.01; \*p<0.05; Statistically significant compared to negative control group.

## 2.2. Flumazenil antagonism assay

**Table S3.** Results obtained in flumazenil antagonism assay for the compounds 7a and 8b.

Comp.	Dose (mg/kg)	PTZ P/T <sup>a</sup> (% protection)	T <sub>c</sub> (s)	No. seizures <sup>b</sup>
7a	D <sub>3</sub>	0/6 (0%)	580.00 ± 59.32	5.17 ± 0.60
8b	D <sub>3</sub>	0/6 (0%)	500.00 ± 59.32	4.00 ± 0.77
D	D = 2 mg/kg	0/6 (0%)	591.00 ± 243.69	4.70 ± 1.75

**Comp.** – compound; **D** – diazepam; **D**<sub>3</sub> = 150 mg/kg;

**T<sub>c</sub>** – Time (seconds) until the first seizure (mean) ± **SEM** (Standard error of mean);

<sup>a</sup> – **PTZ** – pentylenetetrazole; (**P**) - Number of mice protected (no seizures)/(**T**) - number of mice tested;

<sup>b</sup> – mean number of seizures in the first 30 minutes/animal lot ± **SEM** (Standard error of mean);