

# SUPPORTING INFORMATION

## Antiviral Activities of Halogenated Emodin Derivatives against Human Coronavirus NL63

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## 1. GENERAL INFORMATION

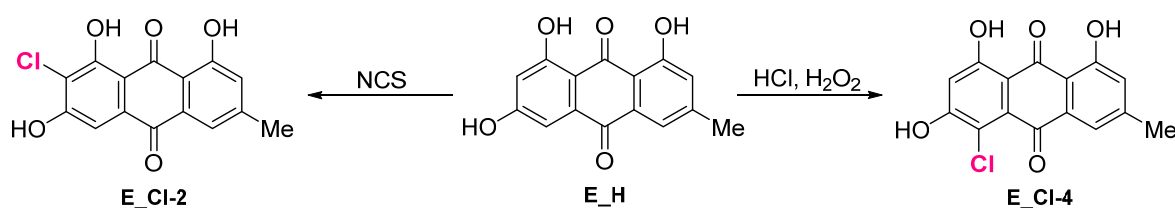
Emodin purchased from Fluorochem was used as received. All other reagents and solvents were of reagent-grade quality and obtained from commercial suppliers (Honeywell, Sigma-Aldrich). TLC was performed on Merck-60-F254 plates using mixtures of EtOAc:EtOH (10:1), CH<sub>2</sub>Cl<sub>2</sub>:EtOH (100:1) or EtOAc:MeOH (20:1). Crude products were purified by column chromatography on silica gel (63-200  $\mu$ m, 70-230 mesh ASTM; Fluka). The products were characterized by <sup>1</sup>H, <sup>13</sup>C NMR spectra, HRMS and IR analysis. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on Bruker Avance III 500 instruments, IR spectra were recorded on Spectrum BX FTIR Perkin-Elmer. HR-MS were recorded on LC MS system Agilent 6224 Accurate Mass TOF LC/MS.

## 2. EXPERIMENTAL SECTION

### Synthesis of emodin derivatives

#### Chlorination

We found out that when the NCS reagent was used, the Cl atom was bonded to side 2 (in HMBC 2D NMR spectra correlation between carbonyl C-10 atom and H-4 and H-5 is indicative as well as methyl C-atom with H-5 and H-7) and when HCl/H<sub>2</sub>O<sub>2</sub> was used, the Cl atom was bonded to side 4 (in HMBC 2D NMR spectra carbonyl C-10 atom is correlated only with H-5, while methyl C-atom is correlated with H-5 and H-7).



**Figure S1.** Chlorination of emodin E\_H with NCS and HCl/H<sub>2</sub>O<sub>2</sub>.

**E\_CI-2:** <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>, 25 °C):  $\delta$  = 7.49 (s, 1H, ArH), 7.35 (s, 1H, ArH), 7.18 (s, 1H, ArH), 2.41 (s, 3H, CH<sub>3</sub>) ppm.

**E\_CI-4:** <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>, 25 °C):  $\delta$  = 7.44 (s, 1H, ArH), 7.13 (s, 1H, ArH), 6.78 (s, 1H, ArH), 2.41 (s, 3H, CH<sub>3</sub>) ppm.

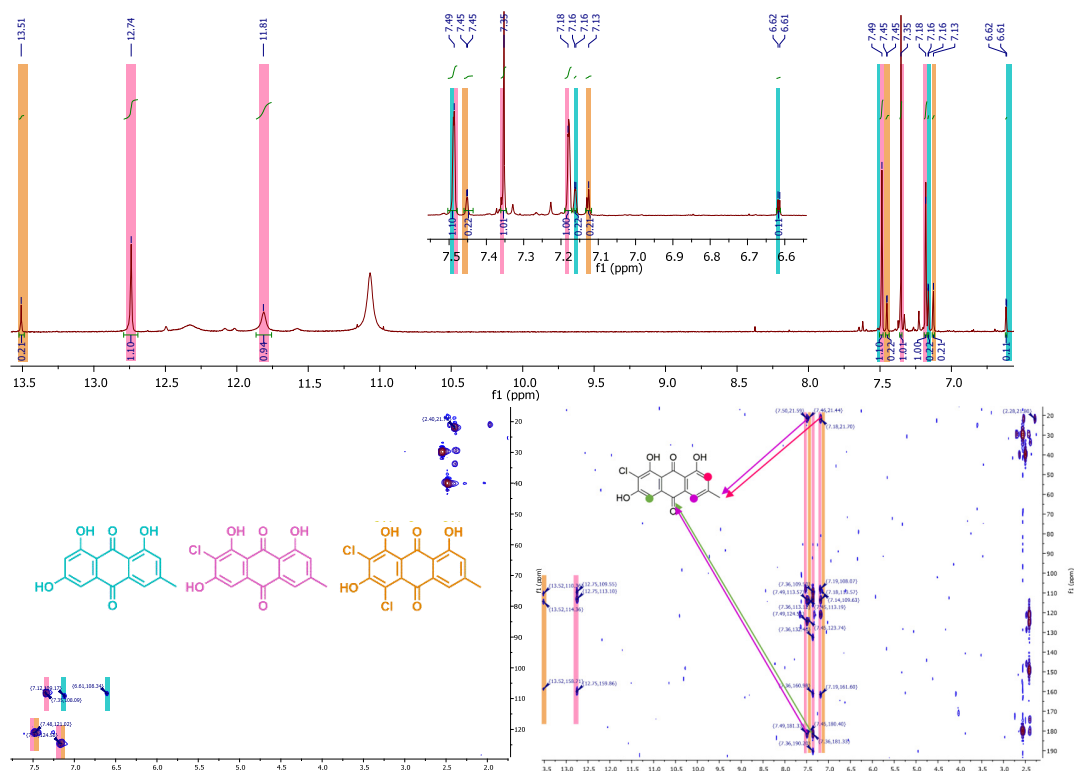


Figure S2. Characterization of  $\text{E\_Cl-2}$  with  $^1\text{H}$  NMR and 2D (HSQC, HMBC) NMR in  $\text{DMSO}$ , 500 MHz.

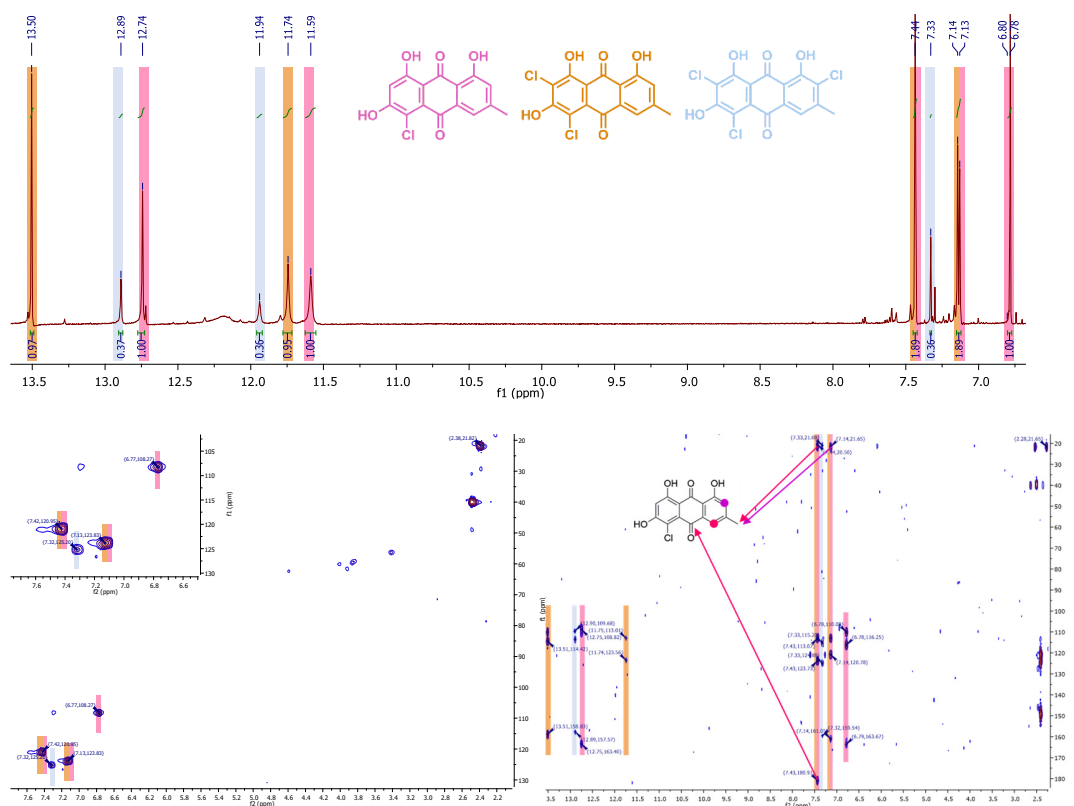
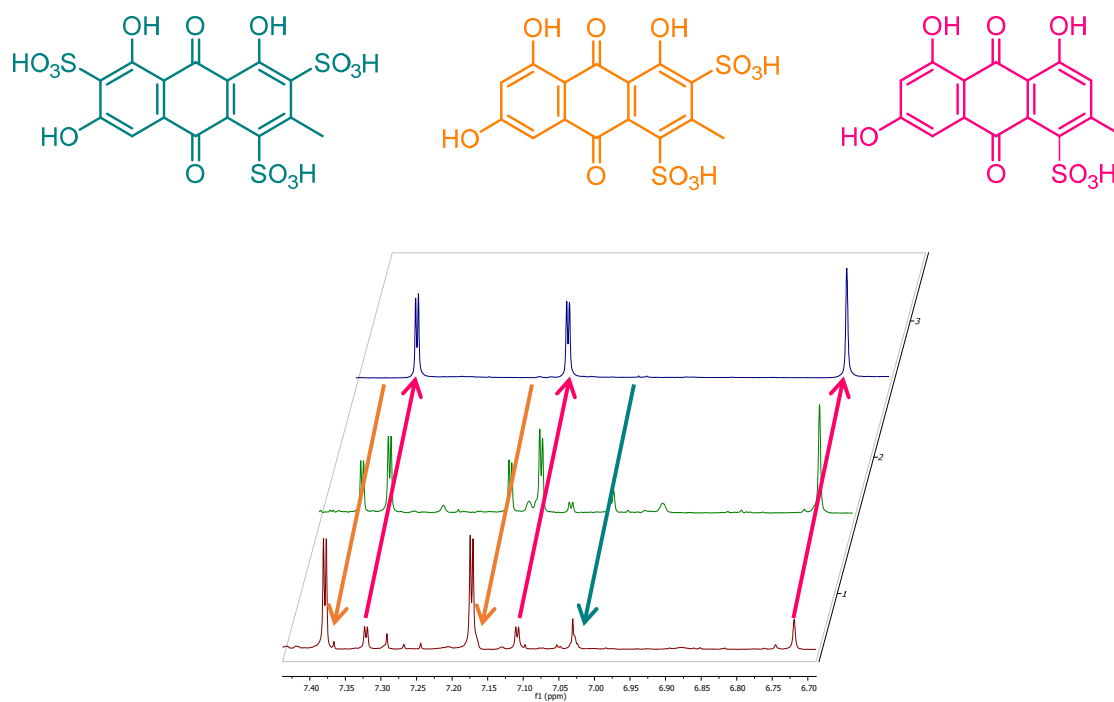


Figure S3. Characterization of  $\text{E\_Cl-4}$  with  $^1\text{H}$  and 2D (HSQC, HMBC) NMR in  $\text{DMSO}$ , 500 MHz.

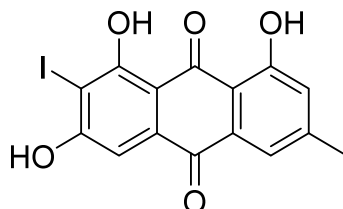
## Sulfonation



**Figure S4.** Following a desulfonation of trisubstituted emodin  $E\_3\text{SO}_3\text{H}$  to monosubstituted  $E\_1\text{SO}_3\text{H}$  in MeCN/hexane at room temperature by  $^1\text{H}$  NMR (bottom spectra: crude reaction mixture, middle spectra: after 1 h, top spectra: after 3 h).

## Synthesis and characterization of products

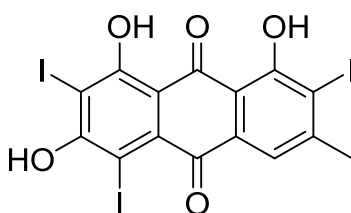
### 1,3,8-trihydroxy-2-iodo-6-methylanthracene-9,10-dione (E\_I).[1]



**Synthesis procedure 1:** Iodine (254 mg, 1.0 mmol) was added at a temperature of 0 °C to a stirred solution of emodin (135 mg, 0.5 mmol) in THF (5 mL) and water (5 mL). Then NaHCO<sub>3</sub> (310 mg, 3.7 mmol) was added in a stepwise manner. The reaction mixture was stirred at room temperature for 1 h. The reaction was monitored by TLC (CH<sub>2</sub>Cl<sub>2</sub>:EtOH=100:1). After the reaction was complete, the mixture was extracted with dichloromethane (3×20 mL). The organic layer was washed with water (3×20 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated under vacuum. The crude reaction product was washed with hexane (5 mL) and acetonitrile (5 mL) to remove soluble impurities. The product was dried in vacuum to provide the product (144 mg, 73%) as an orange solid.

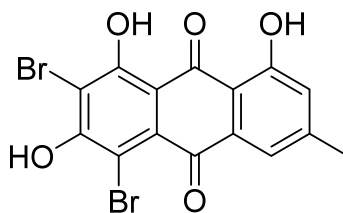
**Synthesis procedure 2:** NIS (146 mg, 0.65 mmol) was added to a stirred solution of emodin (135 mg, 0.5 mmol) in THF (5 mL). The reaction mixture was stirred at room temperature for 3 h. The reaction was monitored by TLC (CH<sub>2</sub>Cl<sub>2</sub>:EtOH=100:1). After the reaction was complete, the mixture was extracted with dichloromethane (3×20 mL). The organic layer was washed with water (3×20 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated under vacuum. The crude reaction product was washed with hexane (5 mL) and acetonitrile (5 mL) to remove soluble impurities. The product was dried in vacuum to provide the product (166 mg, 84%) as an orange solid.

### 1,3,8-trihydroxy-2,4,7-triiodo-6-methylanthracene-9,10-dione (E\_3I).[1]



NIS (450 mg, 2.0 mmol) was added to a stirred solution of emodin (135 mg, 0.5 mmol) in THF (5 mL). The reaction mixture was stirred at 60 °C for 24 h. The reaction was monitored by TLC (CH<sub>2</sub>Cl<sub>2</sub>:EtOH=100:1). After the reaction was complete, the mixture was extracted with dichloromethane (3×20 mL). The organic layer was washed with water (3×20 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated under vacuum. The crude reaction product was washed with hexane (5 mL) and acetonitrile (5 mL) to remove soluble impurities. The product was dried in vacuum to provide the product (256 mg, 79%) as an orange solid.

**2,4-dibromo-1,3,8-trihydroxy-6-methylantracene-9,10-dione (E\_2Br).[1]**



*N*-Bromosuccinimide (196 mg, 1.1 mmol) was added to a solution of emodin (135 mg, 0.5 mmol) in THF (5 mL) and stirred for 30 min at 0 °C. The reaction was monitored by TLC (CH<sub>2</sub>Cl<sub>2</sub>:EtOH = 100:1). After the reaction was complete, the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×30 mL). The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated under vacuum. The crude reaction product was washed with hexane (5 mL) and acetonitrile (3×5 mL) to remove soluble impurities. The product was dried in vacuum to provide the product (188 mg, 88%) as a yellow–orange solid.

**Antiviral activity of emodins**

**Table S1.** IC<sub>50</sub> (μM) values indicating the effect of emodin and emodin analogues on Vero cell viability.

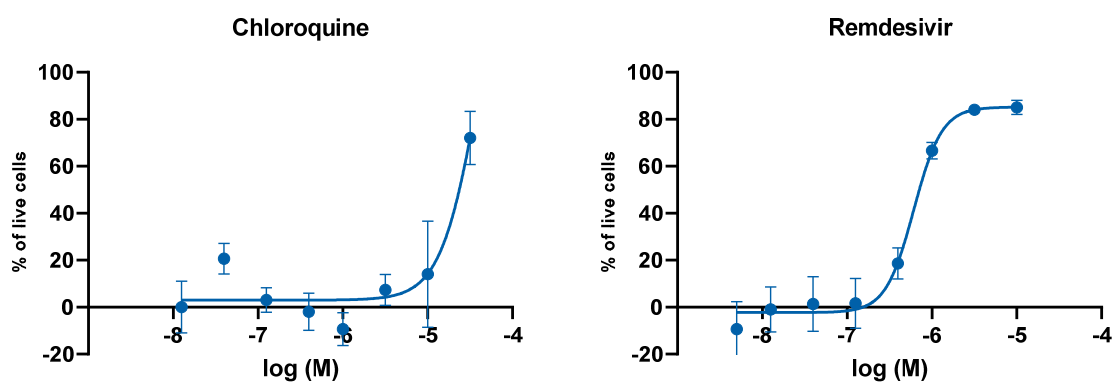
	E_H	E_NO <sub>2</sub>	E_I	E_3I	E_4Br	E_2Br	E_Cl	E_OMe	E_SO <sub>3</sub> H	E_NH <sub>2</sub>	E_Br_OMe
IC <sub>50</sub> (μM)	4.9	6.1	3.6	4.9	7.2	5.4	7.5	8.7	>50	41.8	>50
Bottom	1	1	4	5	4	7	6	= 0.000	= 0.000	= 0.000	n.a.
Top	118	108	92	93	94	99	104	121	111	105	n.a.
HillSlope	-3.8	-3.1	~ -23.63	-5.5	-5.8	-3.3	-4.6	-0.8	-1.8	-2.1	n.a.
R squared	1.00	1.00	1.00	1.00	0.98	0.99	0.97	0.92	0.85	0.94	n.a.

**Table S2.** IC<sub>50</sub> (μM) values for anti HCoV-NL63 effects of emodin and emodin analogs.

	E_H	E_NO <sub>2</sub>	E_I	E_3I	E_4Br	E_2Br	E_Cl	E_OMe	E_SO <sub>3</sub> H	E_NH <sub>2</sub>	E_Br_OMe
IC <sub>50</sub> (μM)	2.5	6.1	1.3	0.5	1.7	1.0	1.1	>50	22.0	6.3	>50
Bottom	= 0.000	= 0.000	= 0.000	= 0.000	= 0.000	= 0.000	= 0.000	n.a.	= 0.000	= 0.000	n.a.
Top	= 100.0	= 100.0	= 100.0	= 100.0	= 100.0	= 100.0	= 100.0	n.a.	= 100.0	= 100.0	n.a.
HillSlope	1.206	2.138	1.308	1.597	1.628	2.434	1.339	n.a.	1.639	1.32	n.a.
R squared	0.89	0.96	0.99	0.97	0.97	0.99	0.97	n.a.	0.95	0.97	n.a.

**Table S3.**  $IC_{50}$  ( $\mu M$ ) values for anti HCoV-NL63 effects of chloroquine and Remdesivir.

	Chloroquine	Remdesivir
$IC_{50}$ ( $\mu M$ )	19.2	0.61
Bottom	= 0.000	= 0.000
Top	= 100.0	= 100.0
HillSlope	1.58	2.16
R squared	0.90	0.99



**Figure S5.**  $IC_{50}$  curves for anti-HCoV-NL63 effects of standard compounds chloroquine and Remdesivir.



## Antiviral activity raw data

### Toxicity to Vero cells

Table S4. Percentage of live cells following exposure to various concentrations of emodin and emodin analogues

Conc. (uM)	E_H	E_NO <sub>2</sub>	E_I	E_3I	E_4Br	E_2Br	E_Cl	E_OMe	E_SO <sub>3</sub> H	E_NH <sub>2</sub>	E_Br_OMe
50	3	3	9	9	3	11	10	38	67	46	108
25	0	2	1	3	7	8	6	34	82	71	102
12.5	2	11	1	2	5	5	11	39	115	106	105
6.25	34	54	3	23	68	44	77	68	115	105	104
3.13	100	96	88	86	82	83	85	85	113	106	102
1.56	113	103	92	89	94	97	113	109	111	108	102
0.78	118	106	90	92	92	93	106	104	108	102	99
0.39	120	113	93	98	108	106	109	105	103	99	106
IC <sub>50</sub> (μM)	4.9	6.1	3.6	4.9	7.2	5.4	7.5	8.7	>50	41.8	>50
Bottom	1	1	4	5	4	7	6	= 0.000	= 0.000	= 0.000	n.a.
Top	118	108	92	93	94	99	104	121	111	105	n.a.
HillSlope	-3.8	-3.1	~ -23.63	-5.5	-5.8	-3.3	-4.6	-0.8	-1.8	-2.1	n.a.
R squared	1.00	1.00	1.00	1.00	0.98	0.99	0.97	0.92	0.85	0.94	n.a.

### Antiviral activity

Table S5. Percentage of live cells following viral infection and exposure to various concentrations of emodin and emodin analogues

Conc. (uM)	E_H	E_NO <sub>2</sub>	E_I	E_3I	E_4Br	E_2Br	E_Cl	E_OMe	E_SO <sub>3</sub> H	E_NH <sub>2</sub>	E_Br_OMe
50	-125	120	15	-17	90	5	-39	1	85	13	-21
25	-130	46	-63	-30	-65	-80	-168	-7	51	77	-17
12.5	-133	84	-143	-122	-45	-72	-118	-23	23	77	-21
6.25	45	52	-13	-118	59	-72	83	-1	26	51	-6
3.13	61	15	74	91	68	54	82	-26	-3	28	-27
1.56	27	13	58	86	52	73	64	-10	-2	13	-17
0.78	27	3	37	74	19	37	39	-6	2	6	-30
0.39	9	10	14	41	5	6	16	-10	-2	-5	-33
IC <sub>50</sub> (μM)	2.5	6.1	1.3	0.5	1.7	1.0	1.1	>50	22.0	6.3	>50
Bottom	= 0.000	= 0.000	= 0.000	= 0.000	= 0.000	= 0.000	= 0.000	n.a.	= 0.000	= 0.000	n.a.

<b>Top</b>	= 100.0	= 100.0	= 100.0	= 100.0	= 100.0	= 100.0	= 100.0	n.a.	= 100.0	= 100.0	n.a.
<b>HillSlope</b>	1.206	2.138	1.308	1.597	1.628	2.434	1.339	n.a.	1.639	1.32	n.a.
<b>R squared</b>	0.89	0.96	0.99	0.97	0.97	0.99	0.97	n.a.	0.95	0.97	n.a.

### Antiviral activity of chloroquine

*Table S6. Percentage of live cells following viral infection and exposure to various concentration of chloroquine*

<b>Conc. (uM)</b>	<b>Chloroquine</b>
30	77
10	14
3.333	7
1.111	-9
0.370	-2
0.123	3
0.041	20
0.014	0
IC50	19.2
Bottom	= 0.000
Top	= 100.0
HillSlope	1.58
R squared	0.90

### Antiviral activity of Remdesivir

*Table S7. Percentage of live cells following viral infection and exposure to various concentration of Remdesivir*

<b>Conc. (uM)</b>	<b>Remdesivir</b>
10	85
3.333	84
1.111	67
0.370	19
0.123	2
0.041	1
0.014	-1
0.005	-9
IC50	0.61
Bottom	-3
Top	86
HillSlope	2.16
R squared	0.99

### 3. COPIES OF NMR SPECTRA

**1,3,8-trihydroxy-2-iodo-6-methylantracene-9,10-dione (E\_I).**

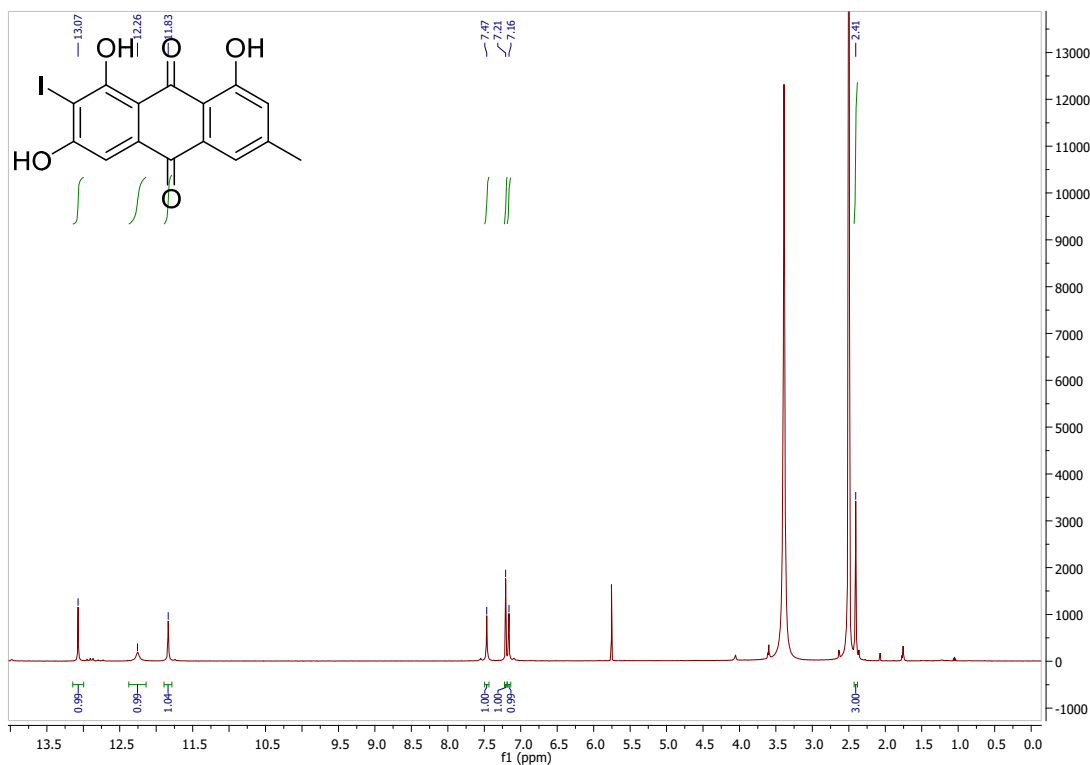


Figure S6. <sup>1</sup>H NMR spectrum of compound E\_I in DMSO, 500 MHz.

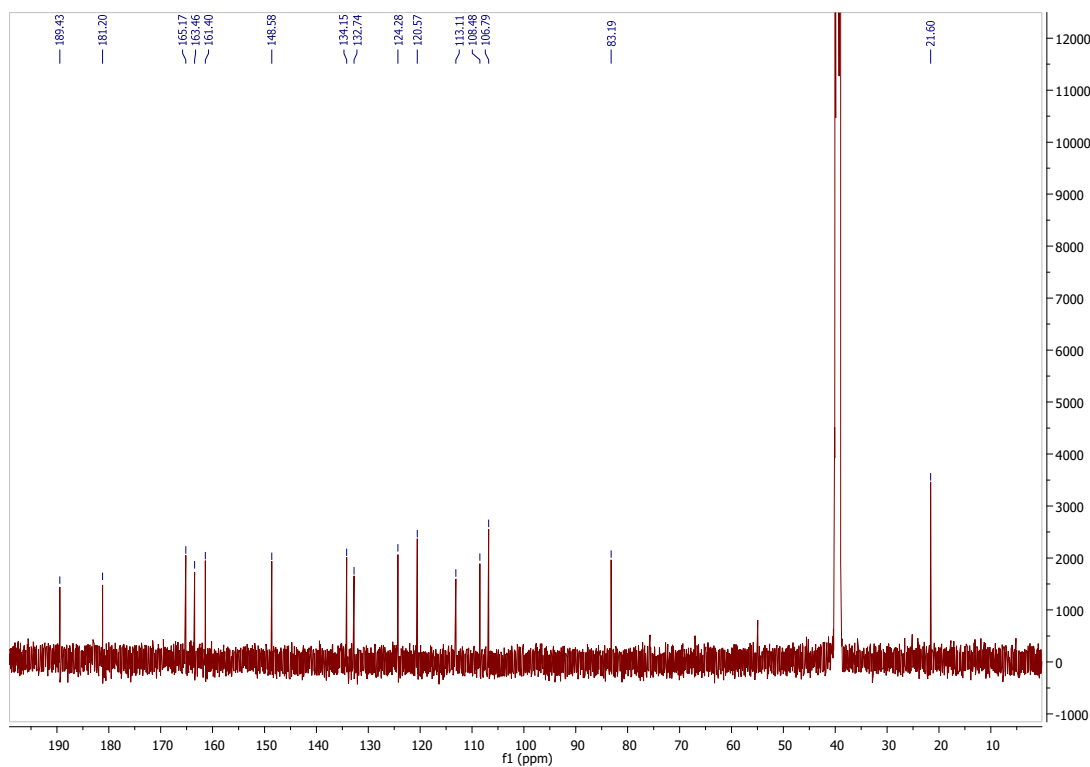


Figure S7. <sup>13</sup>C NMR spectrum of compound E\_I in DMSO, 500 MHz.

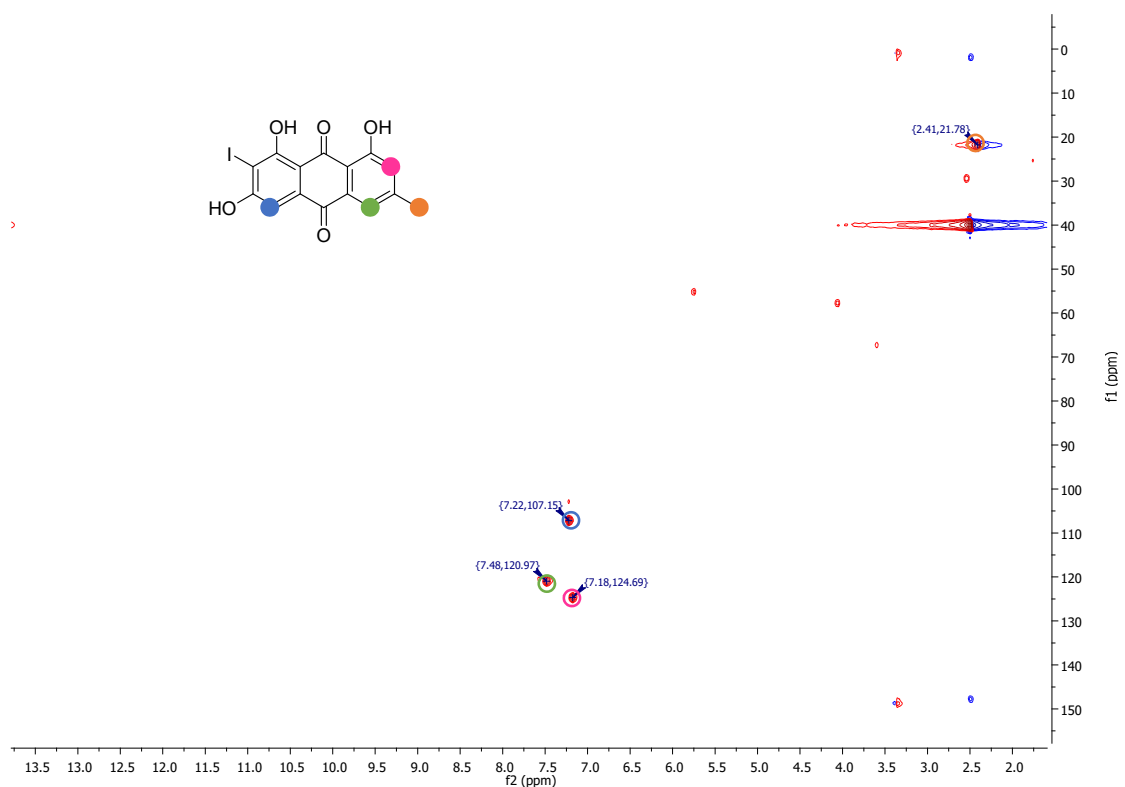


Figure S8. 2D HSQC NMR spectrum of compound E\_I in DMSO, 500 MHz.

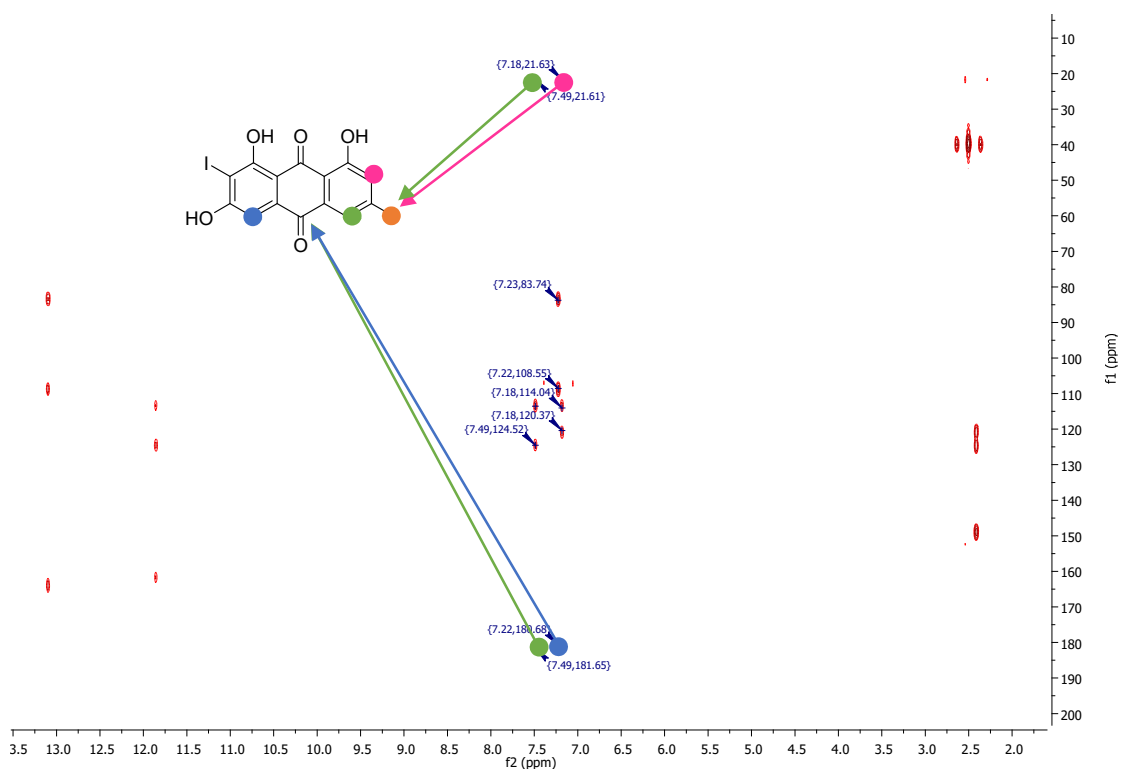
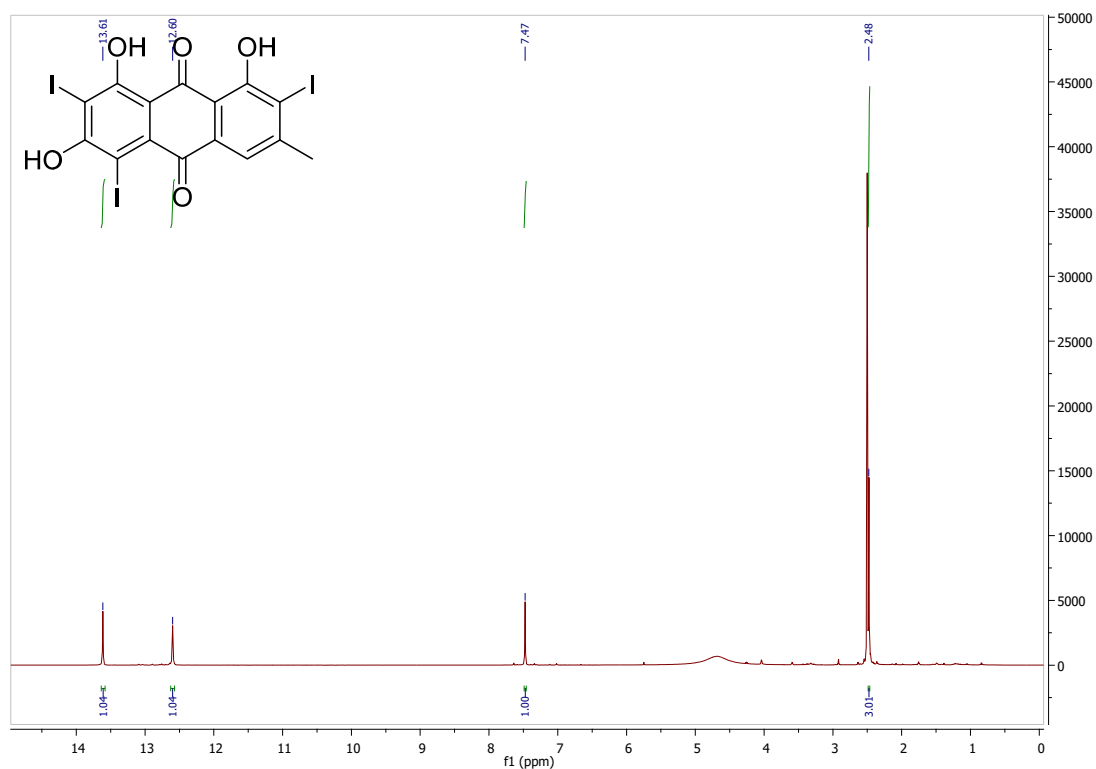
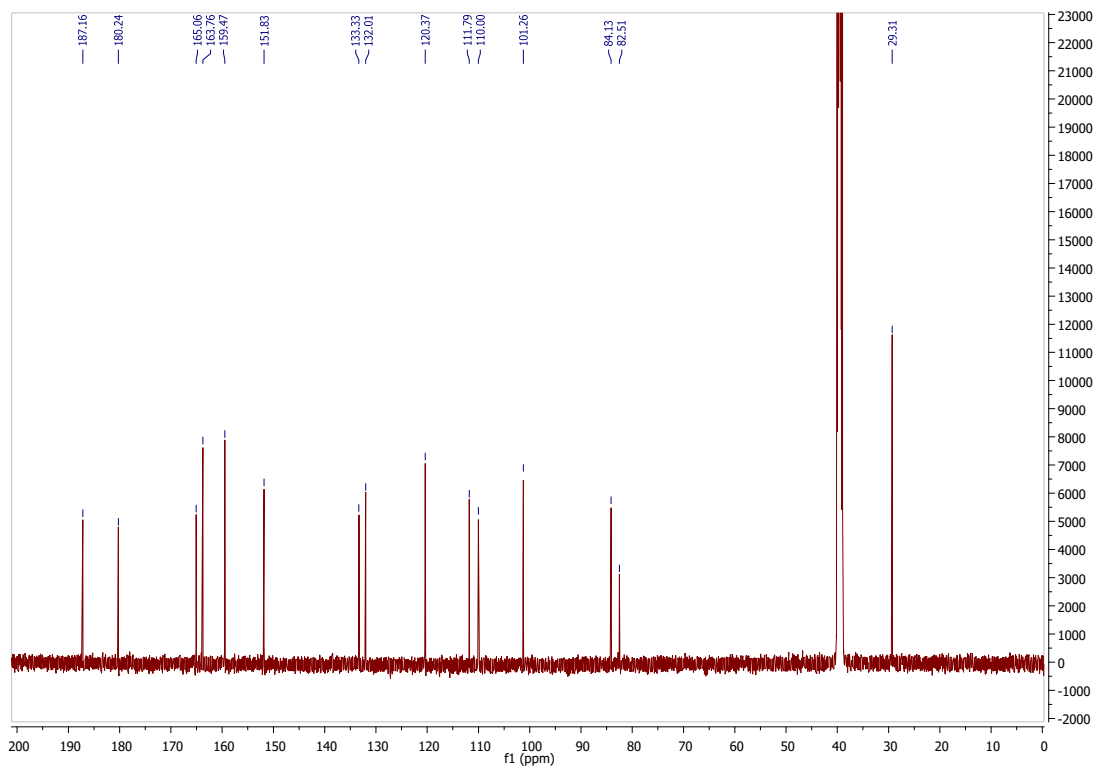


Figure S9. 2D HMBC NMR spectrum of compound E\_I in DMSO, 500 MHz.

**1,3,8-trihydroxy-2,4,7-triiodo-6-methylantracene-9,10-dione (E\_3I).**



**Figure S10.** <sup>1</sup>H NMR spectrum of compound E\_3I in DMSO, 500 MHz.



**Figure S11.** <sup>13</sup>C NMR spectrum of compound E\_3I in DMSO, 500 MHz.

MHE-3I.2.ser

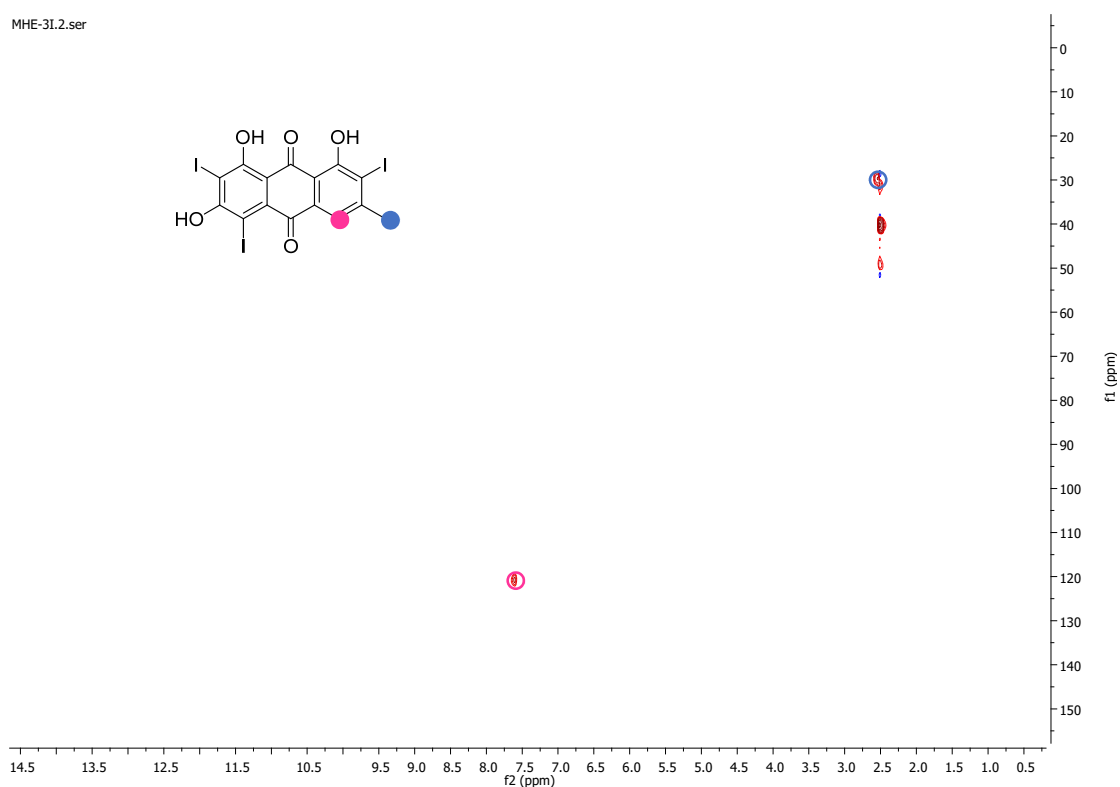


Figure S12. 2D HSQC NMR spectrum of compound E<sub>3</sub>I in DMSO, 500 MHz.

MHE-3I.3.ser

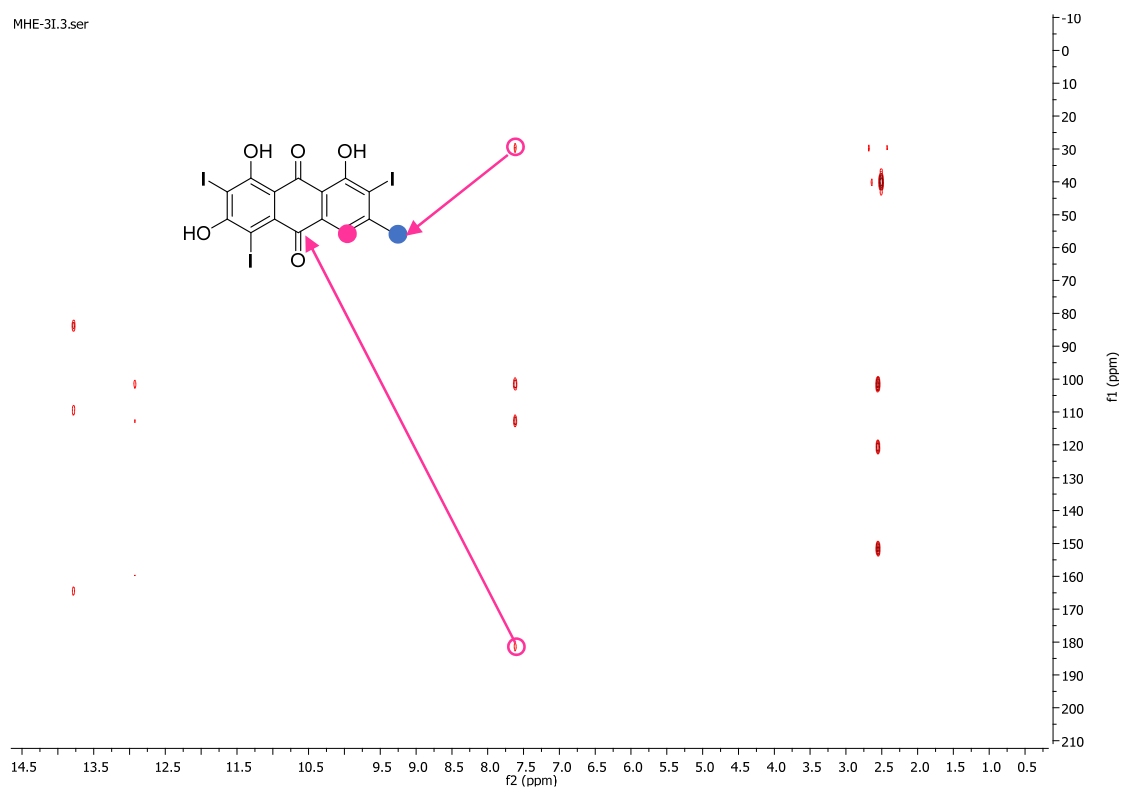


Figure S13. 2D HMBC NMR spectrum of compound E<sub>3</sub>I in DMSO, 500 MHz.

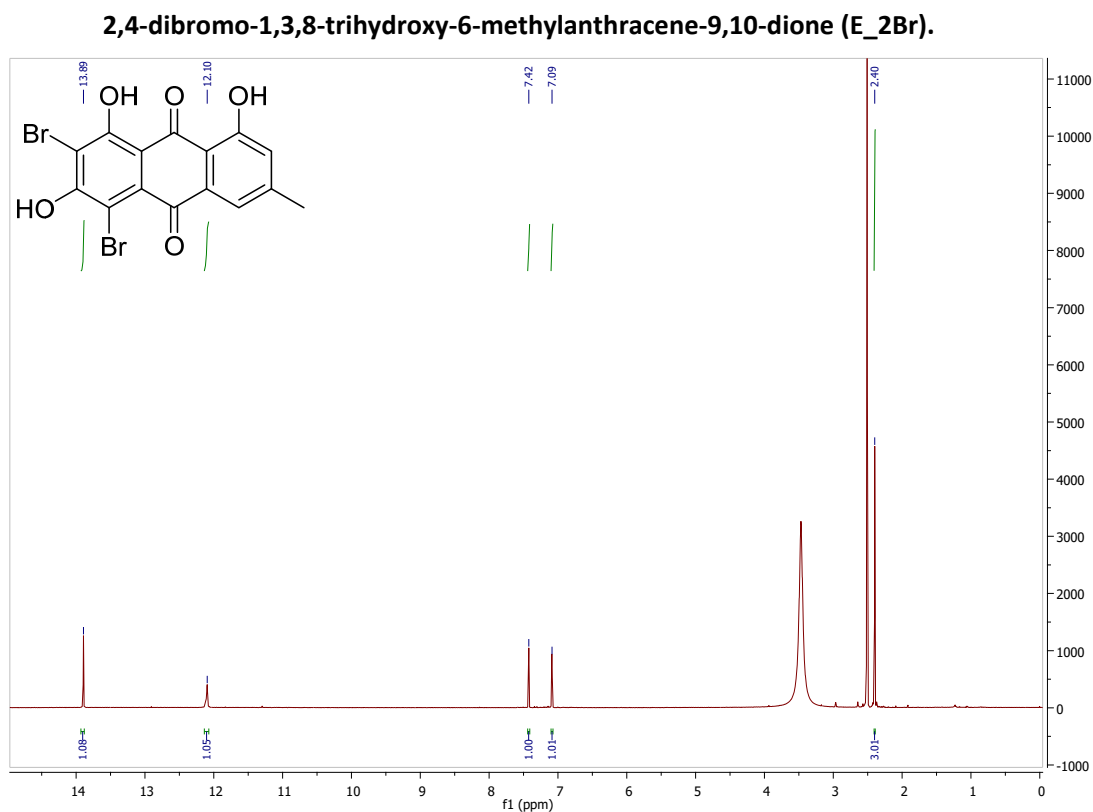


Figure S14.  $^1\text{H}$  NMR spectrum of compound E\_2Br in DMSO, 500 MHz.

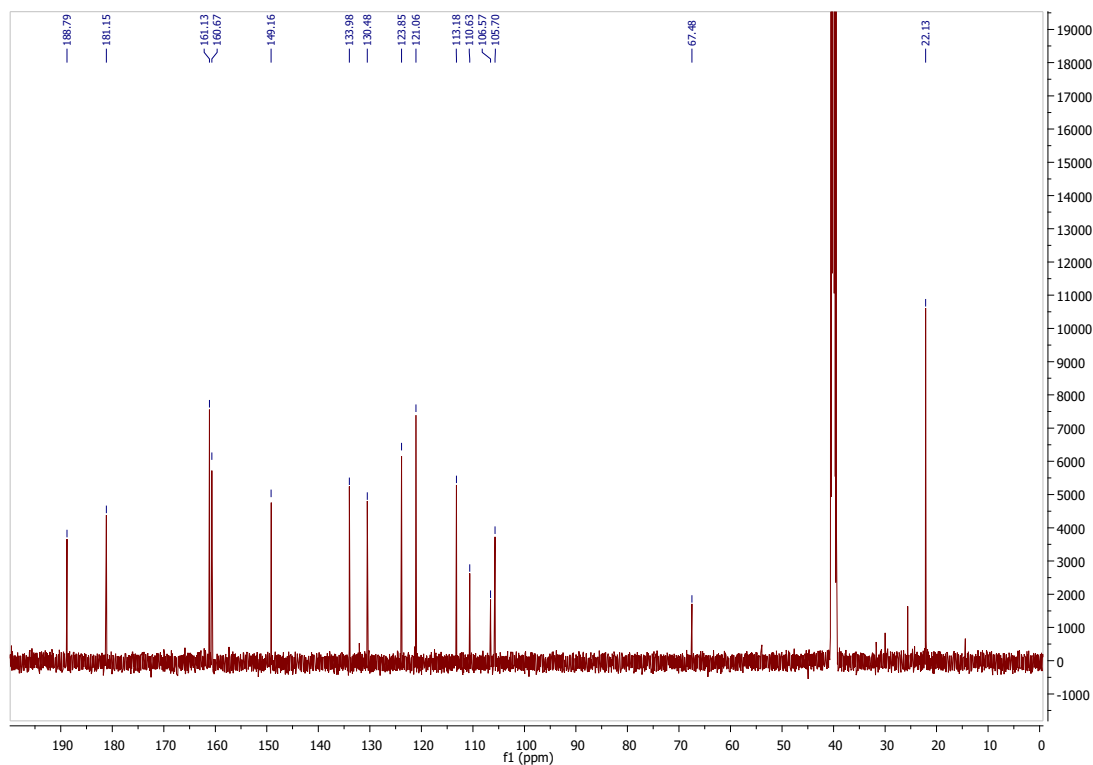


Figure S15.  $^{13}\text{C}$  NMR spectrum of compound E\_2Br in DMSO, 500 MHz.

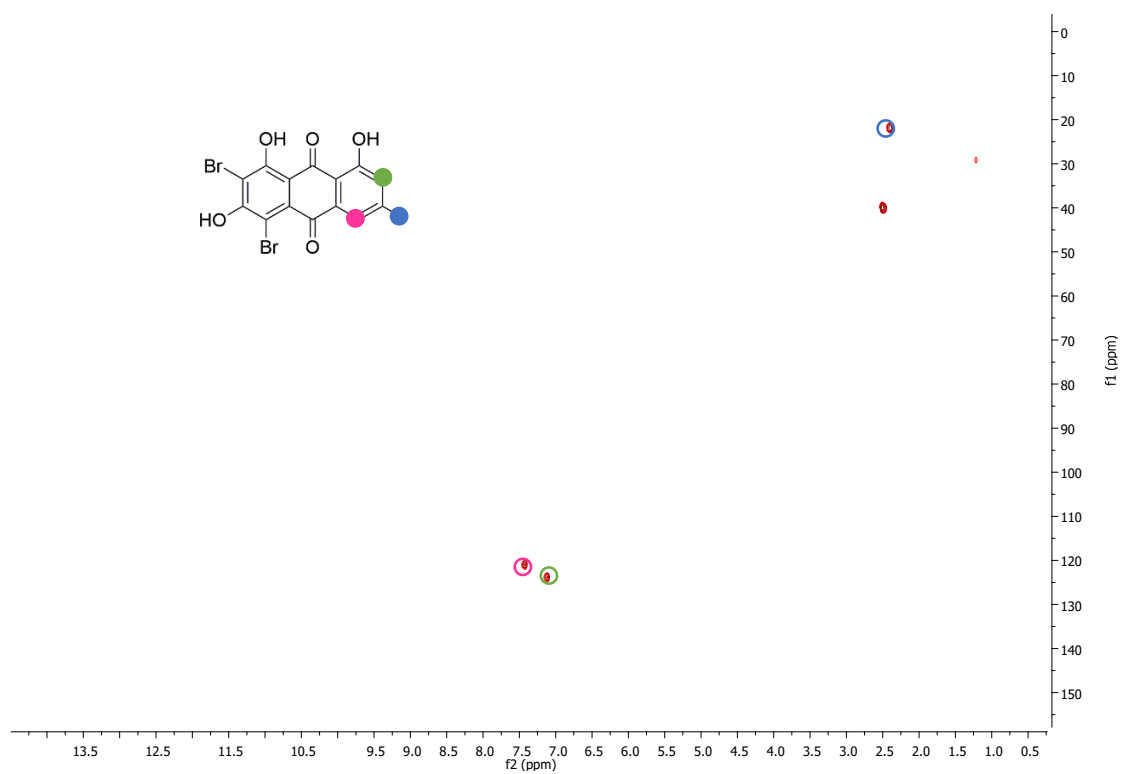


Figure S16. 2D HSQC NMR spectrum of compound *E*<sub>2</sub>Br in DMSO, 500 MHz.

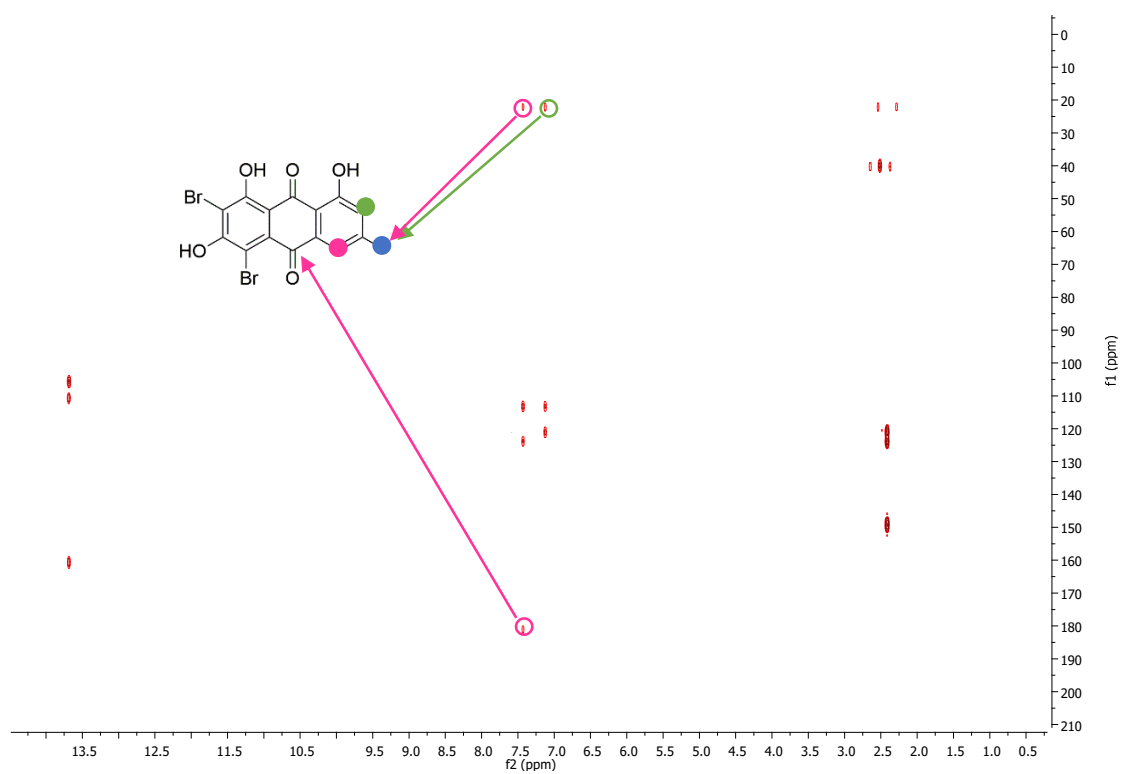
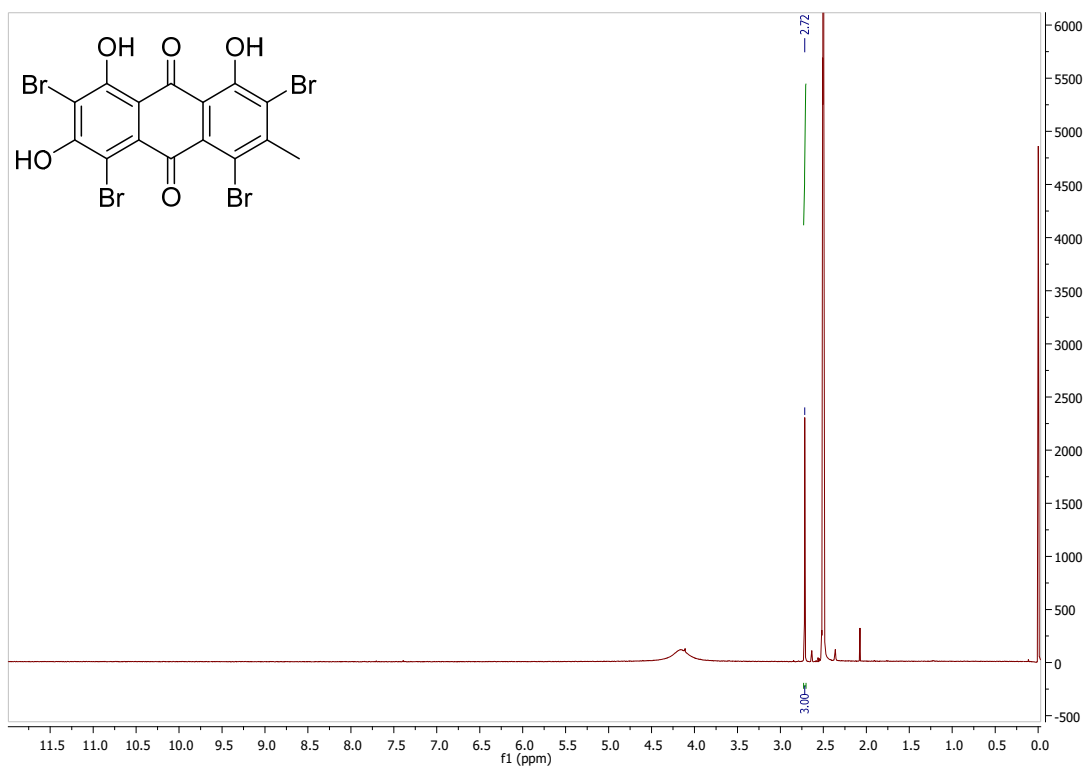


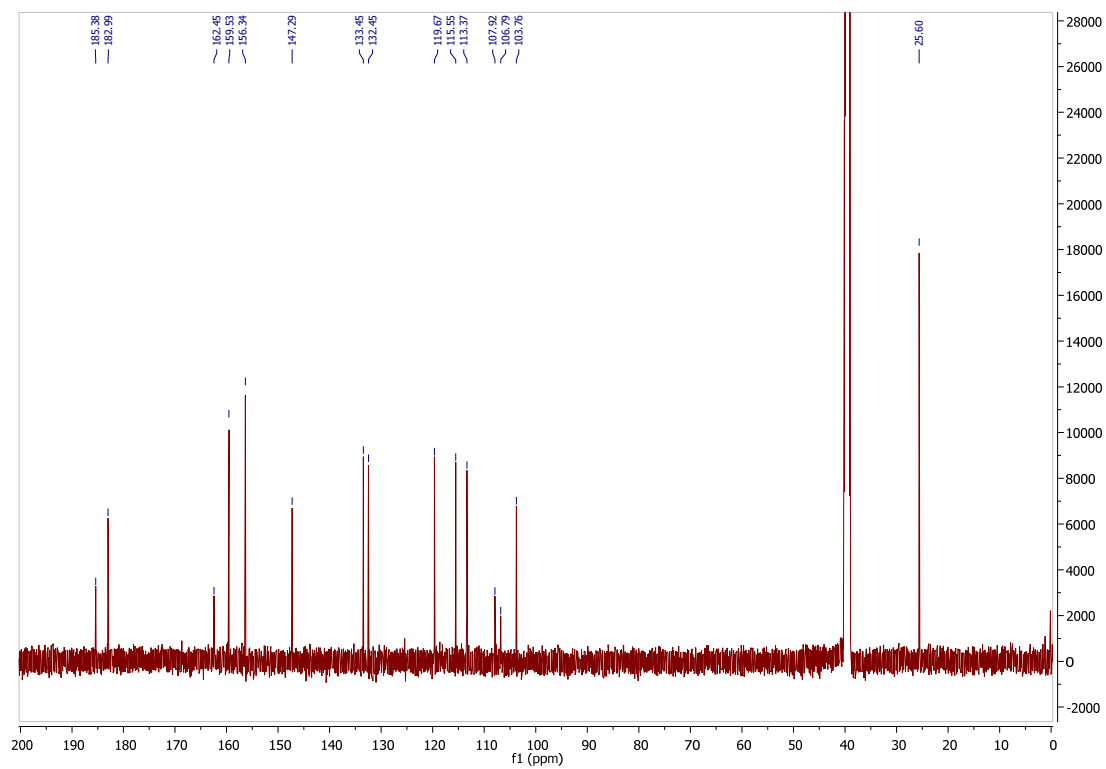
Figure S17. 2D HMBC NMR spectrum of compound *E*<sub>2</sub>Br in DMSO, 500 MHz.



**2,4,5,7-tetrabromo-1,3,8-trihydroxy-6-methylantracene-9,10-dione (E\_4Br).**



**Figure S18.**  $^1\text{H}$  NMR spectrum of compound E\_4Br in DMSO, 500 MHz.



**Figure S19.**  $^{13}\text{C}$  NMR spectrum of compound E\_4Br in DMSO, 500 MHz.

**2,4-dichloro-1,3,8-trihydroxy-6-methylantracene-9,10-dione (E\_2Cl).**

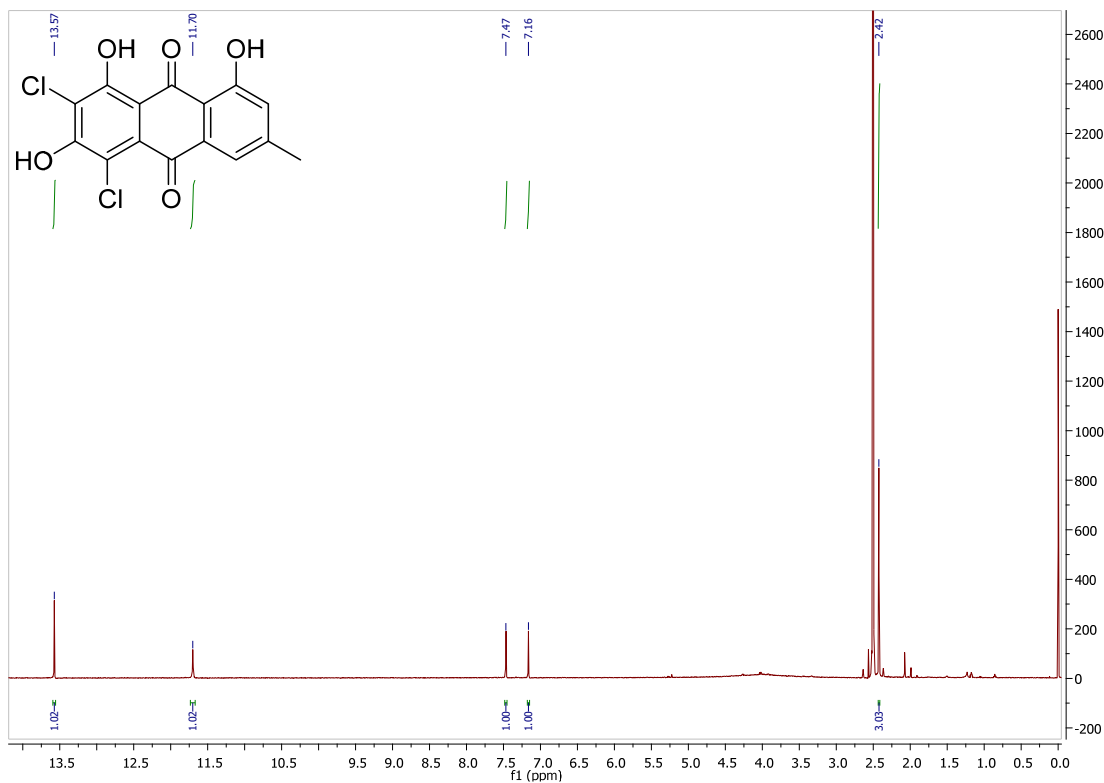


Figure S20. <sup>1</sup>H NMR spectrum of compound E\_2Cl in DMSO, 500 MHz.

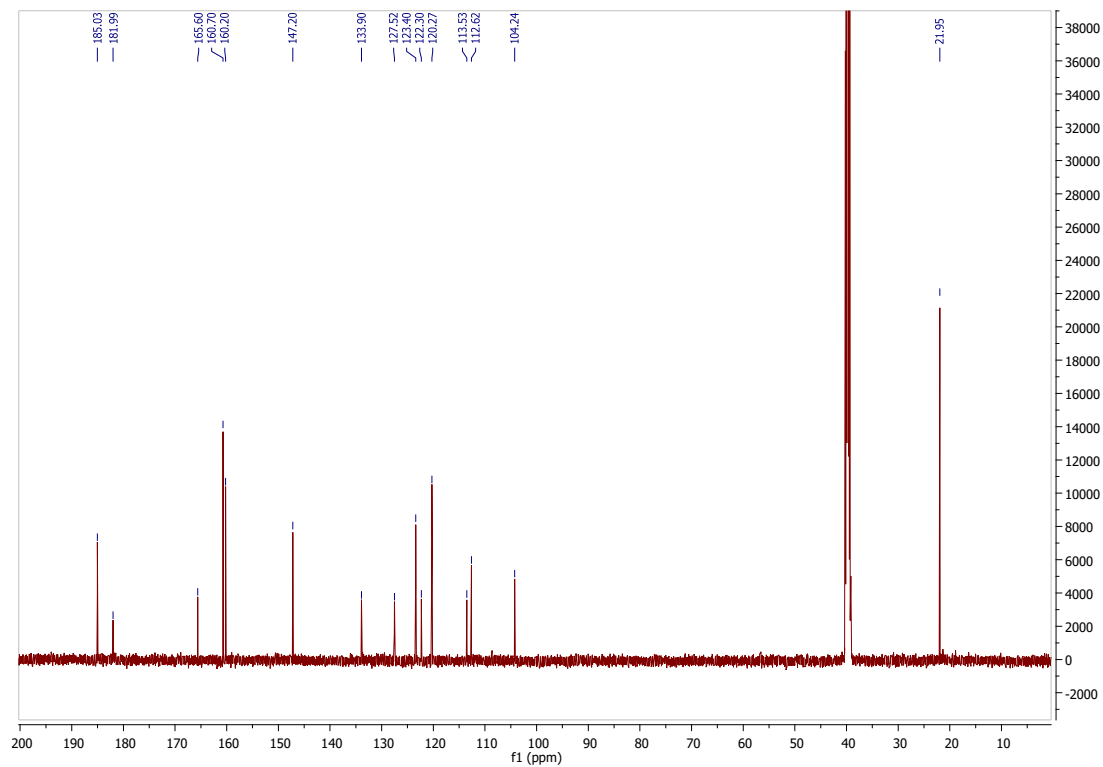


Figure S21. <sup>13</sup>C NMR spectrum of compound E\_2Cl in DMSO, 500 MHz.

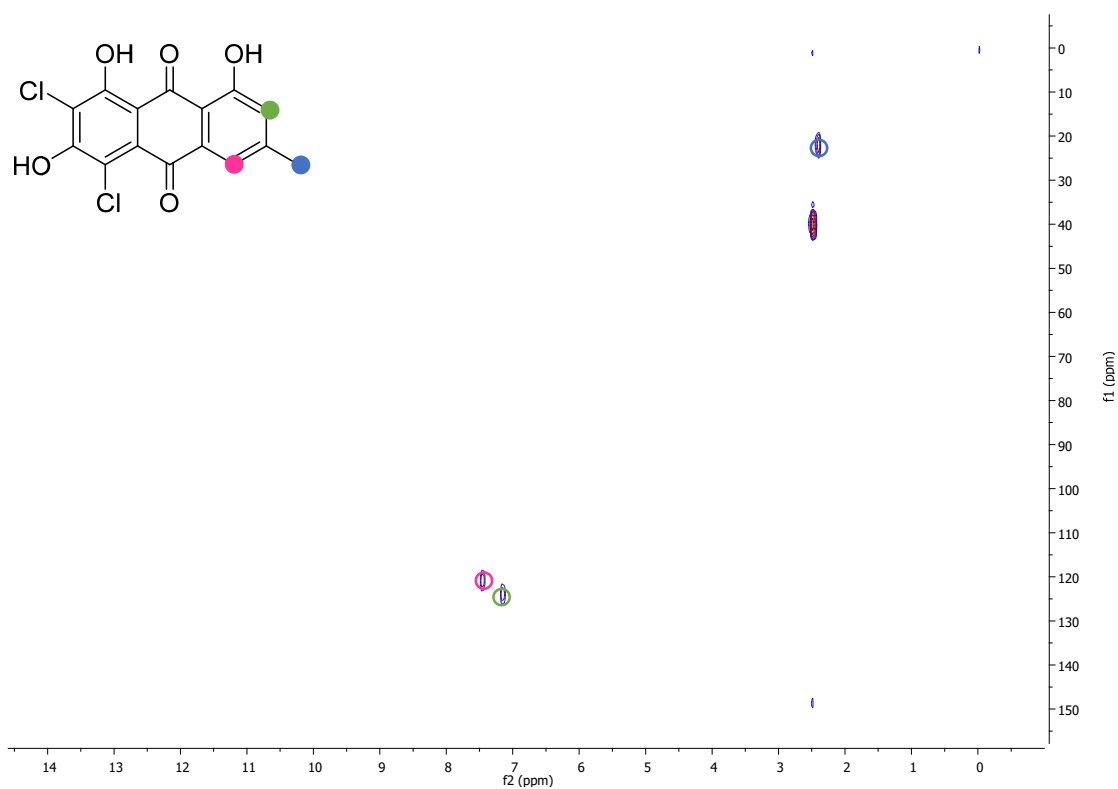


Figure S22. 2D HSQC NMR spectrum of compound E<sub>2</sub>Cl in DMSO, 500 MHz.

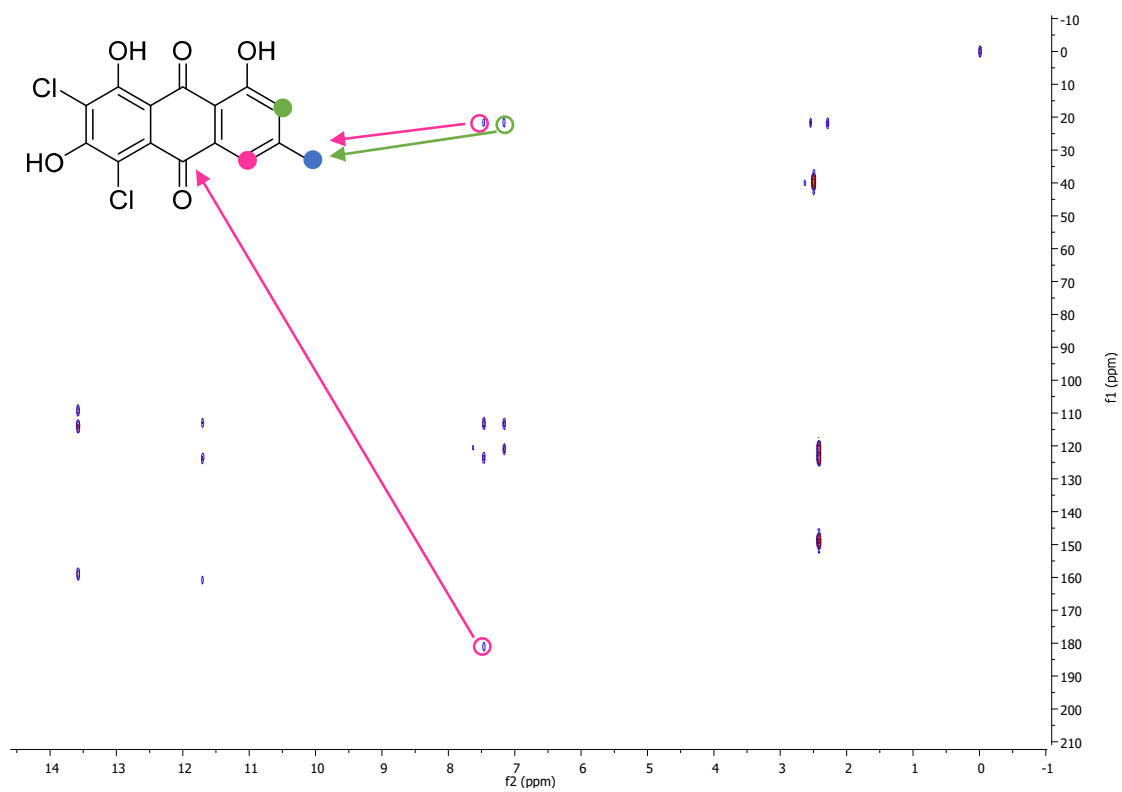
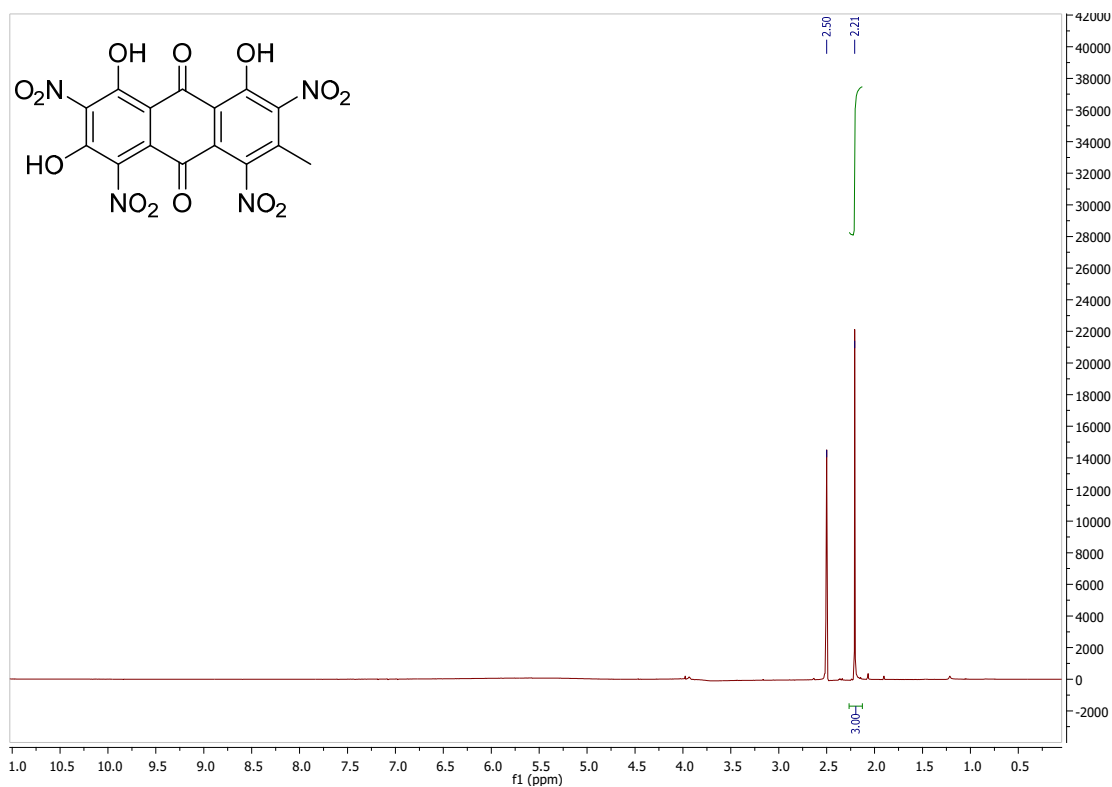
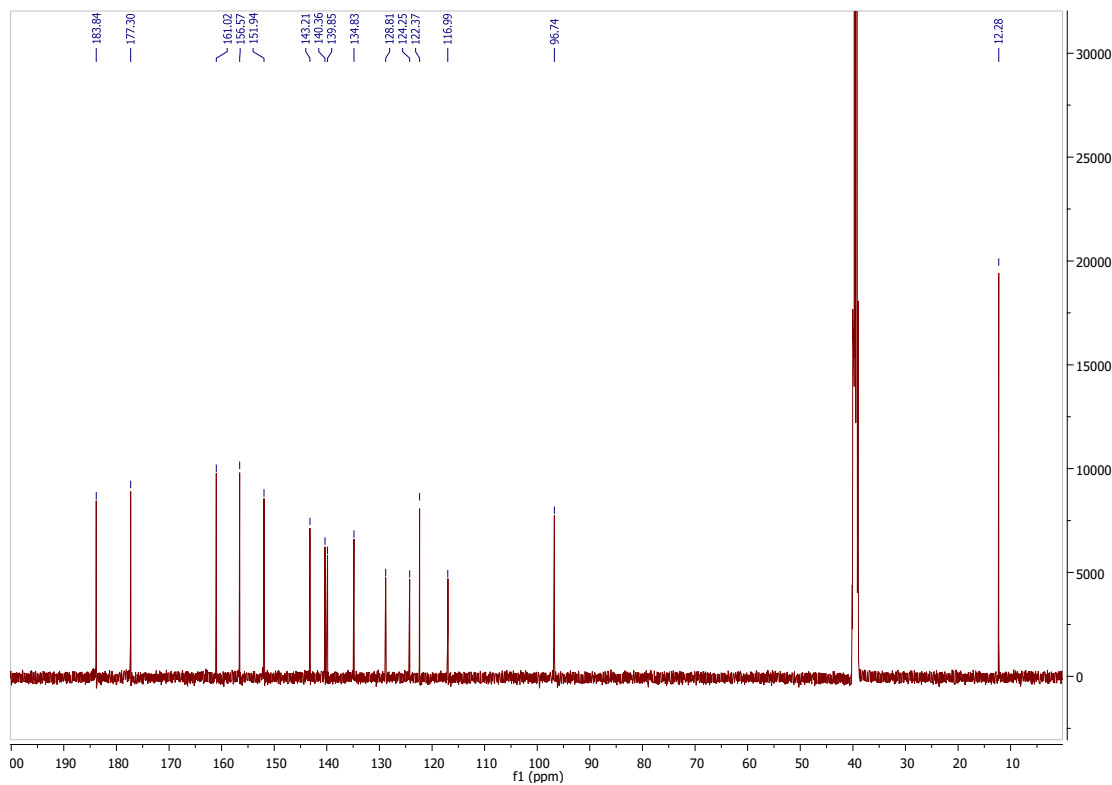


Figure S23. 2D HMBC NMR spectrum of compound E<sub>2</sub>Cl in DMSO, 500 MHz.

**1,3,8-trihydroxy-6-methyl-2,4,5,7-tetranitroanthracene-9,10-dione (E\_4NO<sub>2</sub>).**



**Figure S24.** <sup>1</sup>H NMR spectrum of compound E\_4NO<sub>2</sub> in DMSO, 500 MHz.



**Figure S25.** <sup>13</sup>C NMR spectrum of compound E\_4NO<sub>2</sub> in DMSO, 500 MHz.

**4-((3-aminopropyl)amino)-1,3,8-trihydroxy-6-methylantracene-9,10-dione (E\_NH<sub>2</sub>).**

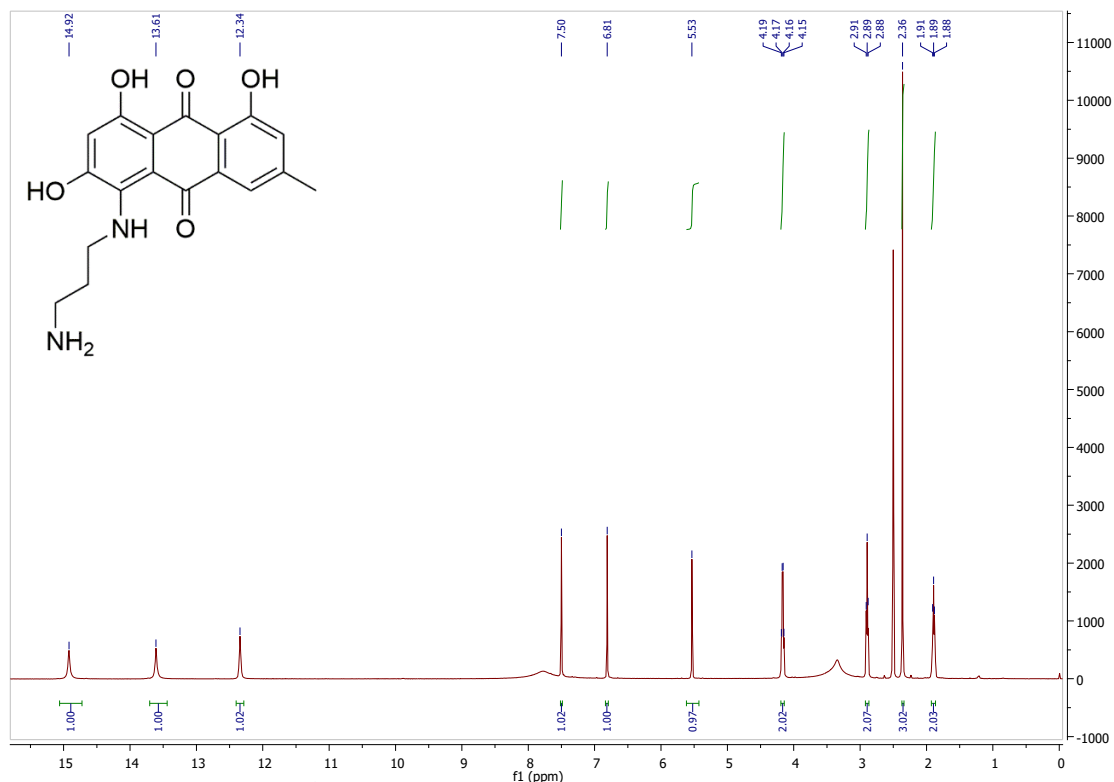


Figure S26. <sup>1</sup>H NMR spectrum of compound E\_NH<sub>2</sub> in DMSO, 500 MHz.

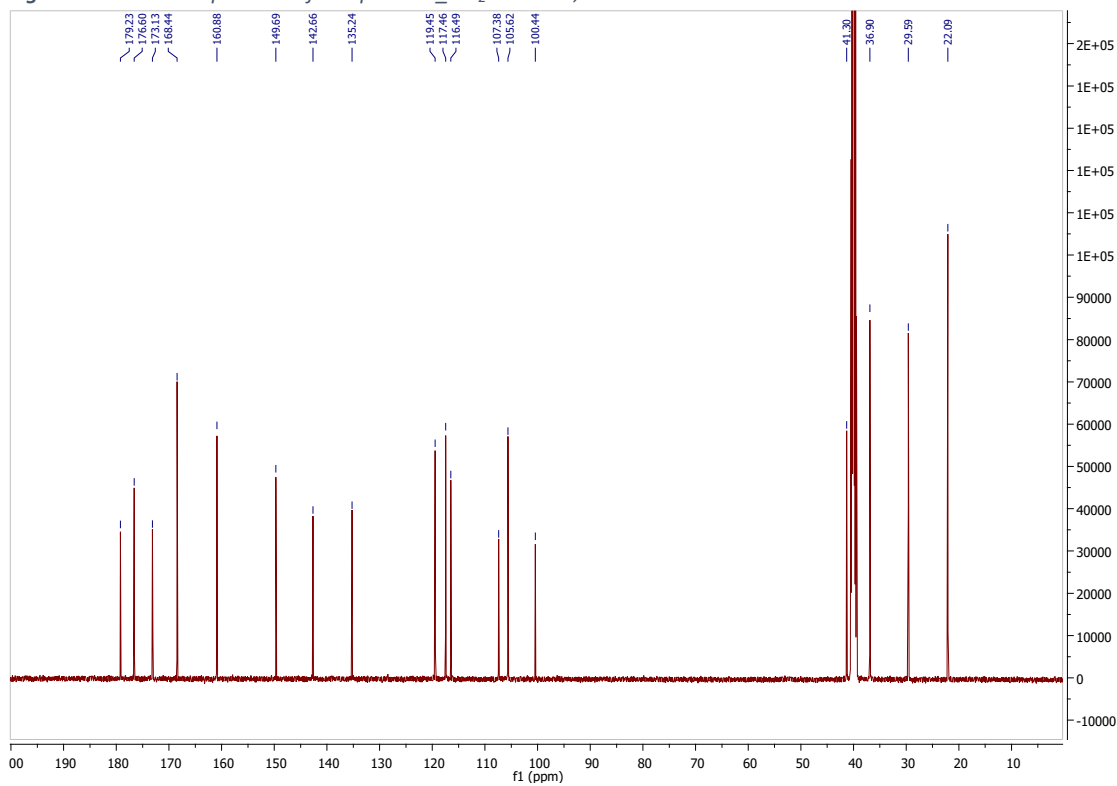
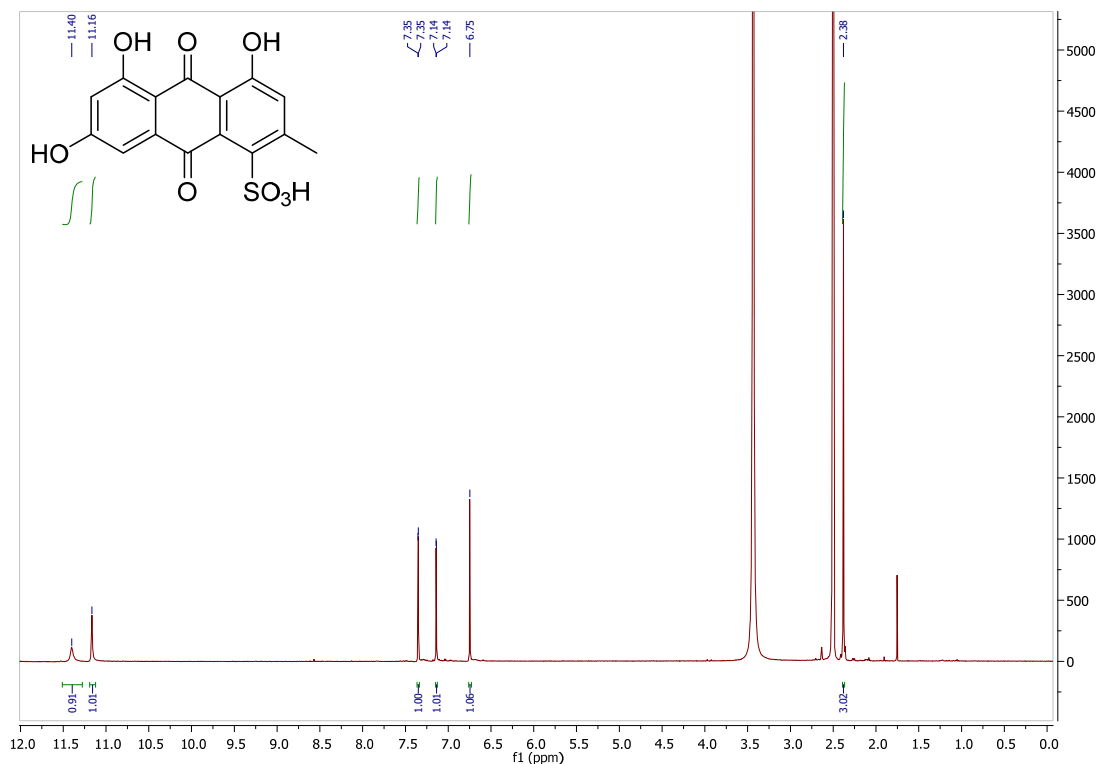
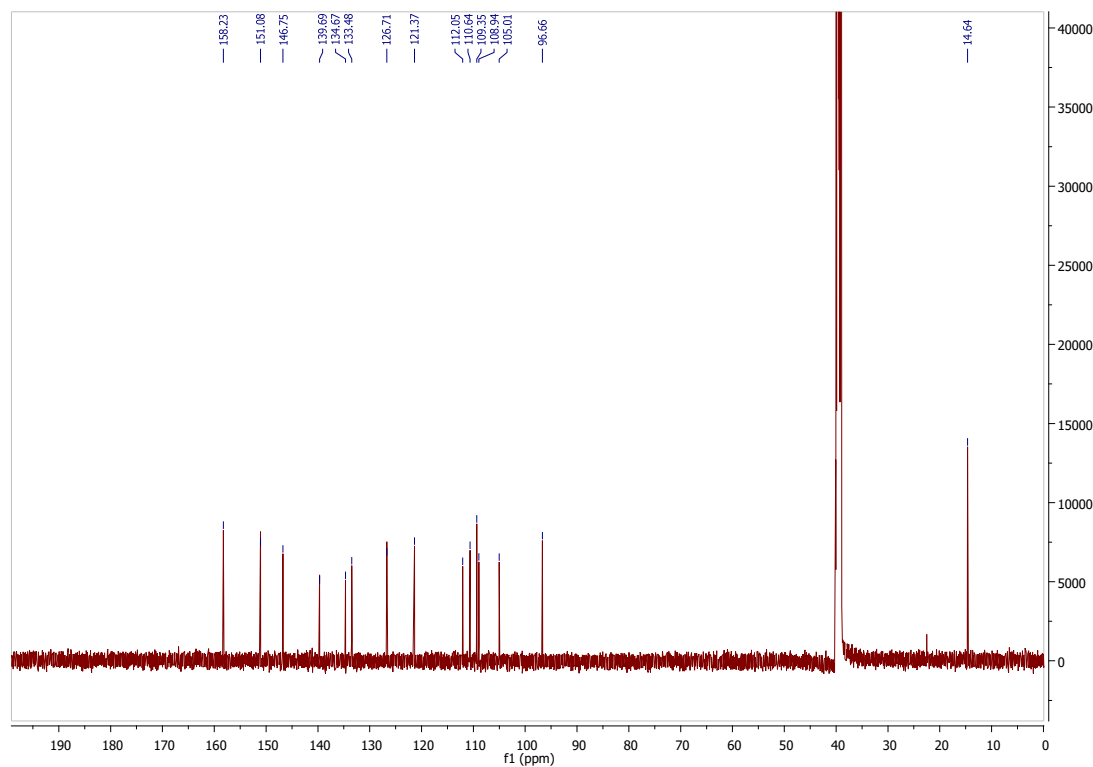


Figure S27. <sup>13</sup>C NMR spectrum of compound E\_NH<sub>2</sub> in DMSO, 500 MHz.

**1,3,8-trihydroxy-6-methyl-9,10-dioxo-9,10-dihydroanthracene-5-sulfonic acid (E\_SO<sub>3</sub>H).**



**Figure S28.** <sup>1</sup>H NMR spectrum of compound E\_SO<sub>3</sub>H in DMSO, 500 MHz.



**Figure S29.** <sup>13</sup>C NMR spectrum of compound E\_SO<sub>3</sub>H in DMSO, 500 MHz.

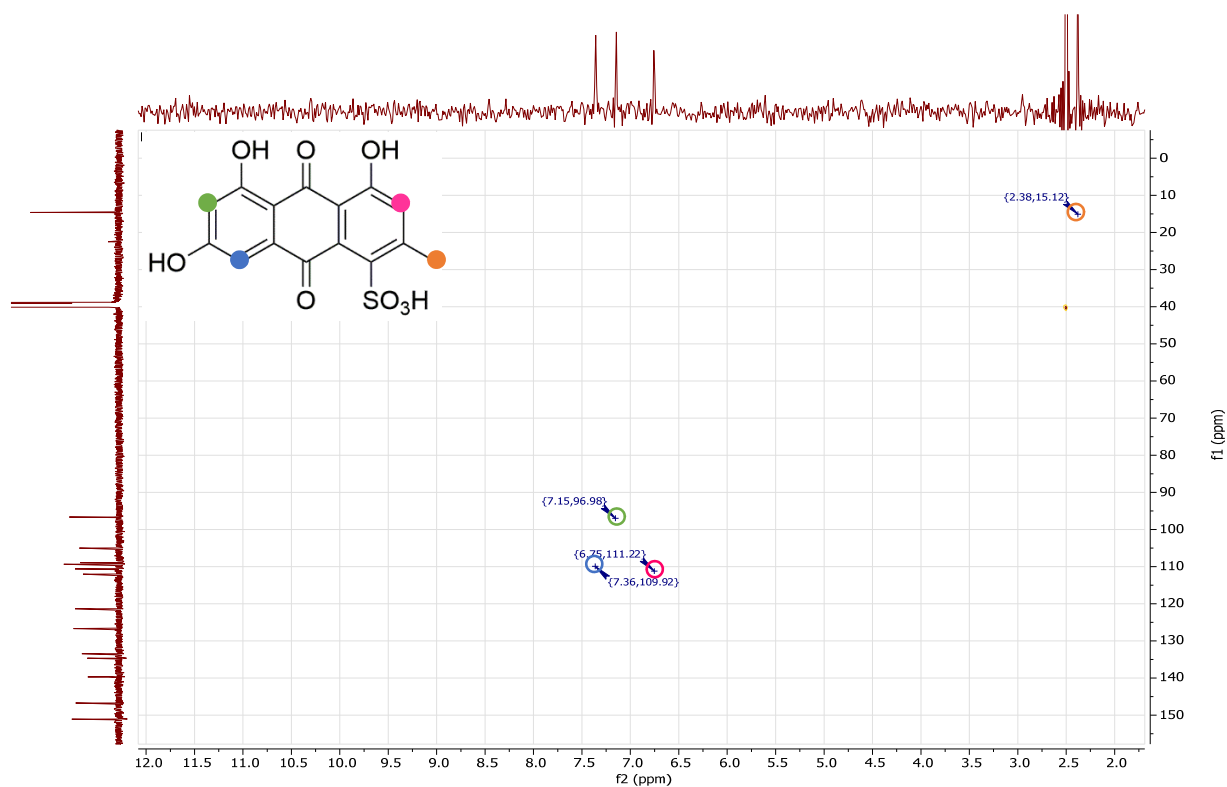


Figure S30. 2D HSQC NMR spectrum of compound  $E\_SO_3H$  in DMSO, 500 MHz.

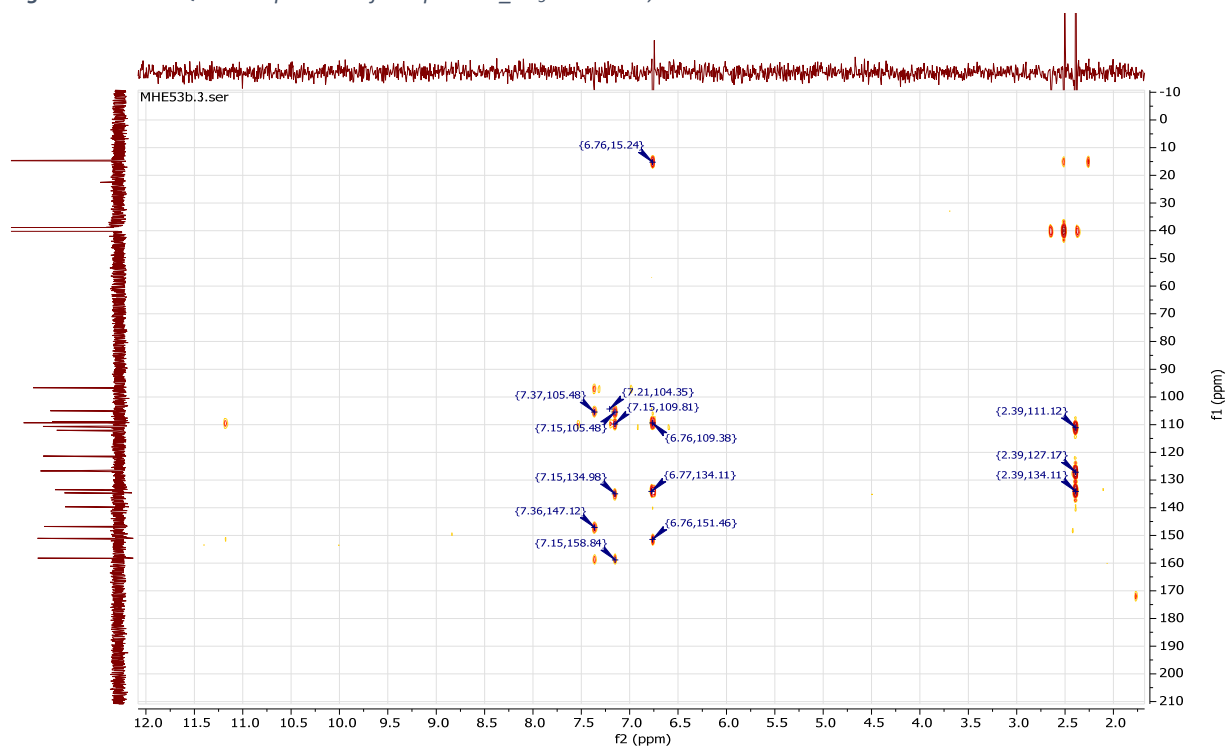
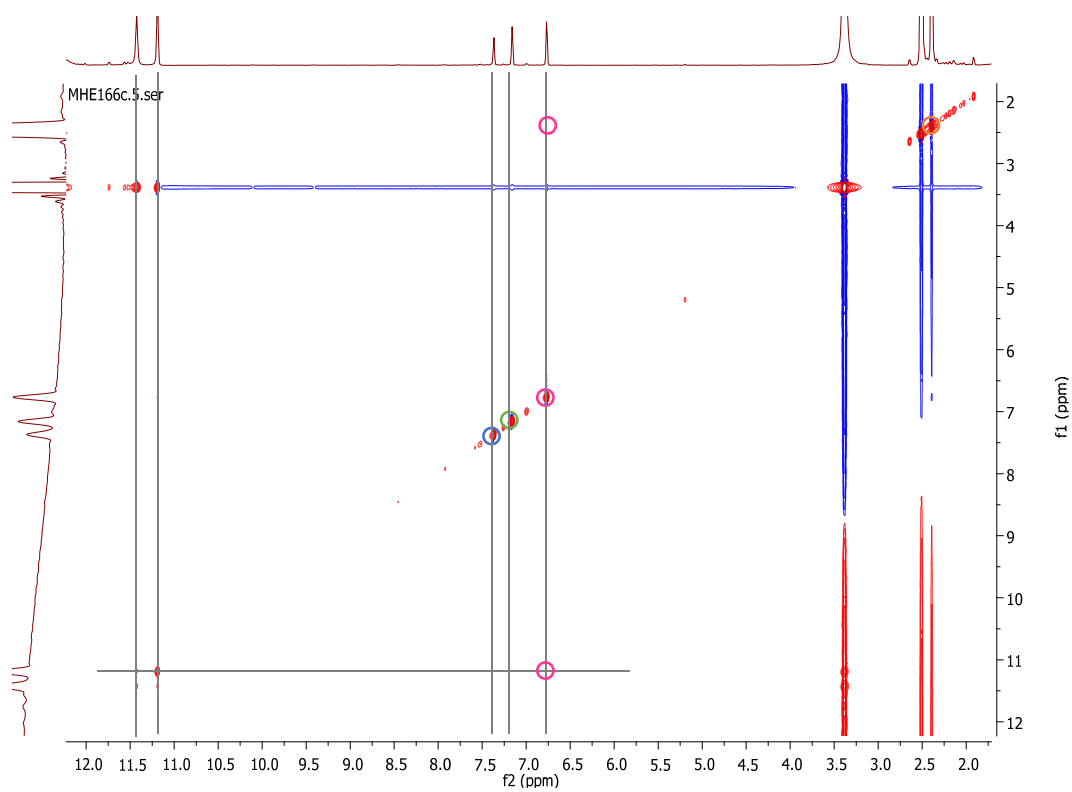
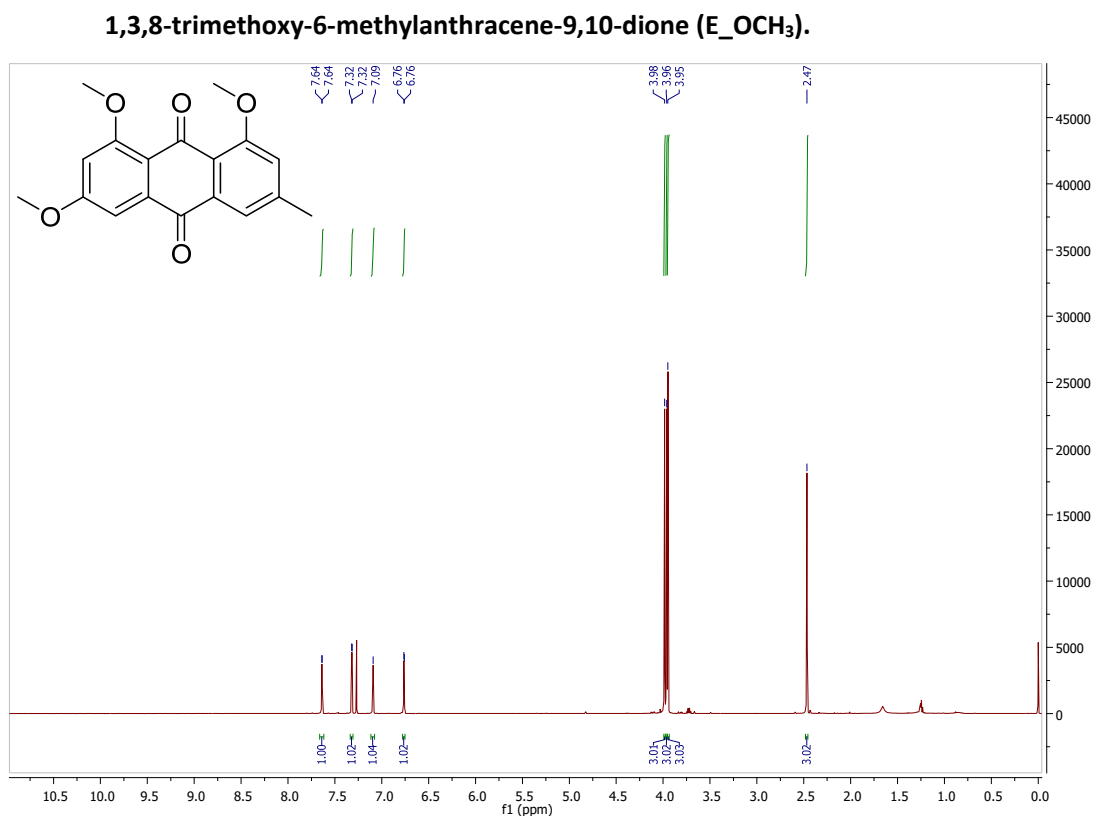


Figure S31. 2D HMBC NMR spectrum of compound  $E\_SO_3H$  in DMSO, 500 MHz.

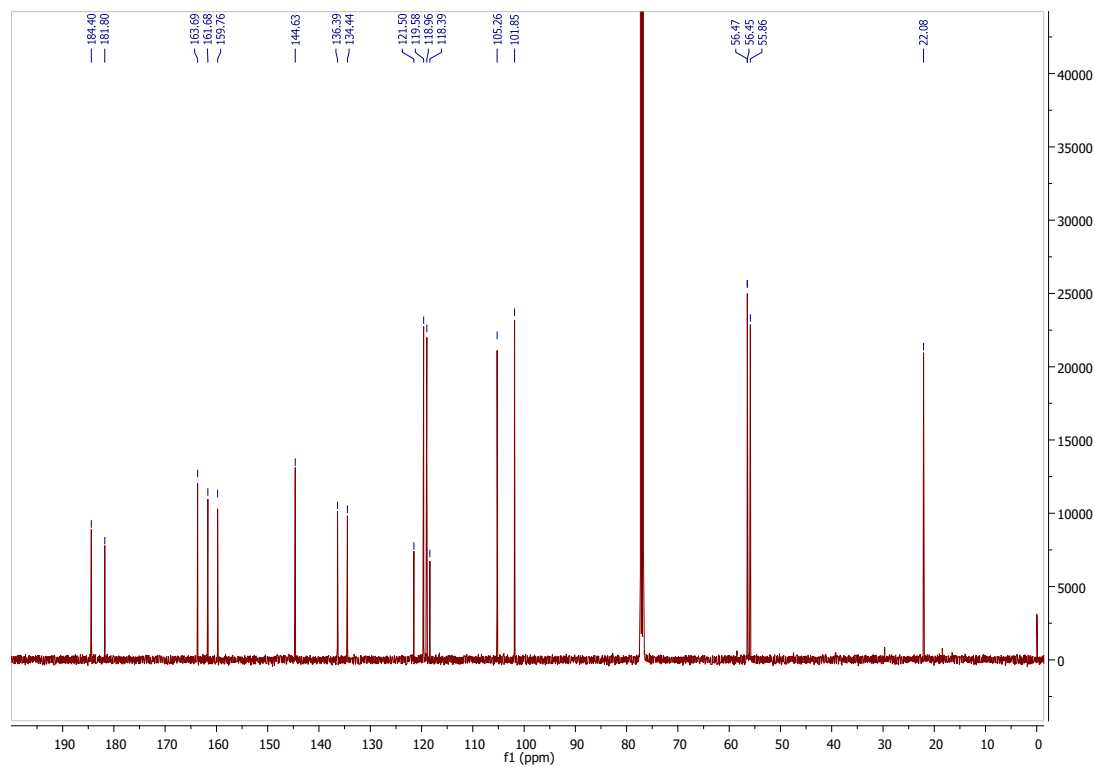


**Figure S32.** 2D NOESY NMR spectrum of compound  $E_{SO_3H}$  in DMSO, 500 MHz.





**Figure S33.** <sup>1</sup>H NMR spectrum of compound E\_OCH<sub>3</sub> in CDCl<sub>3</sub>, 500 MHz.



**Figure S34.** <sup>13</sup>C NMR spectrum of compound E\_OCH<sub>3</sub> in CDCl<sub>3</sub>, 500 MHz.

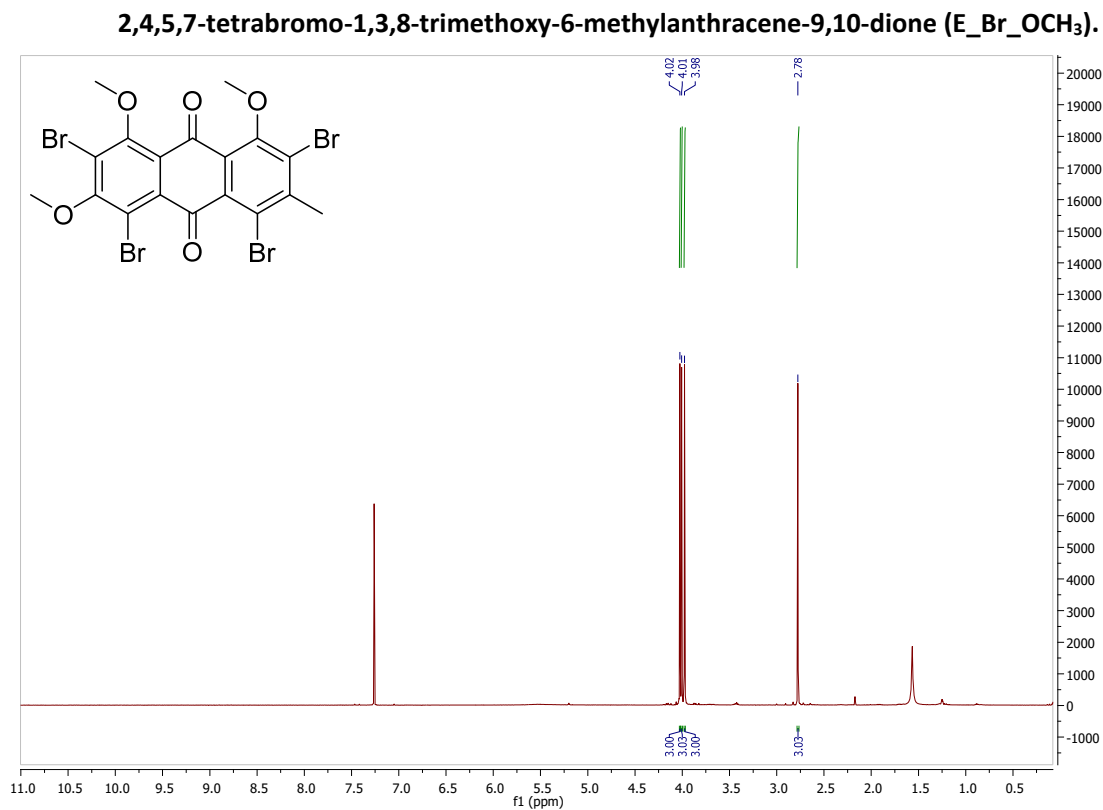


Figure S35. <sup>1</sup>H NMR spectrum of compound E\_Br\_OCH<sub>3</sub> in CDCl<sub>3</sub>, 500 MHz.

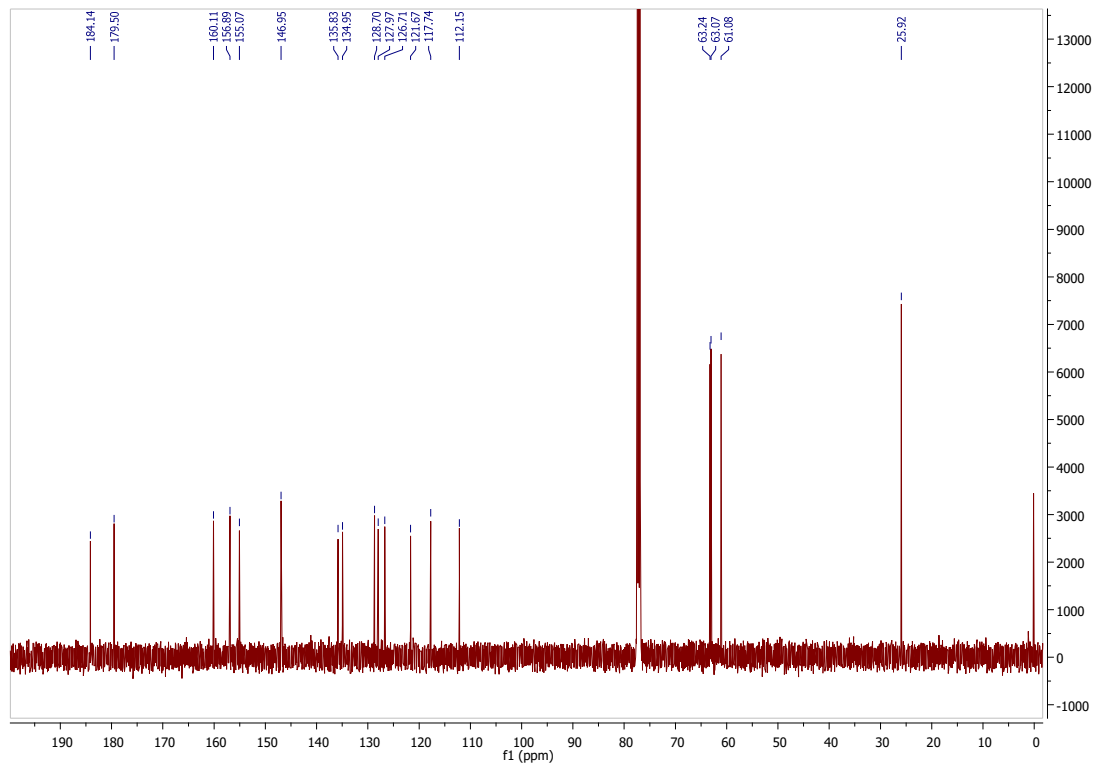


Figure S36. <sup>13</sup>C NMR spectrum of compound E\_Br\_OCH<sub>3</sub> in CDCl<sub>3</sub>, 500 MHz.

#### 4. LITERATURE

[1] F. Duan, X. Li, S. Cai, G. Xin, Y. Wang, D. Du, S. He, B. Huang, X. Guo, H. Zhao, R. Zhang, L. Ma, Y. Liu, Q. Du, Z. Wei, Z. Xing, Y. Liang, X. Wu, C. Fan, C. Ji, D. Zeng, Q. Chen, Y. He, X. Liu, W. Huang, Haloemodin as Novel Antibacterial Agent Inhibiting DNA Gyrase and Bacterial Topoisomerase I, *Journal of Medicinal Chemistry*, 57 (2014) 3707-3714.