Supporting Information

Garcinol and Related Polyisoprenylated Benzophenones as Topoisomerase II Inhibitors: Biochemical and Molecular Modeling Studies

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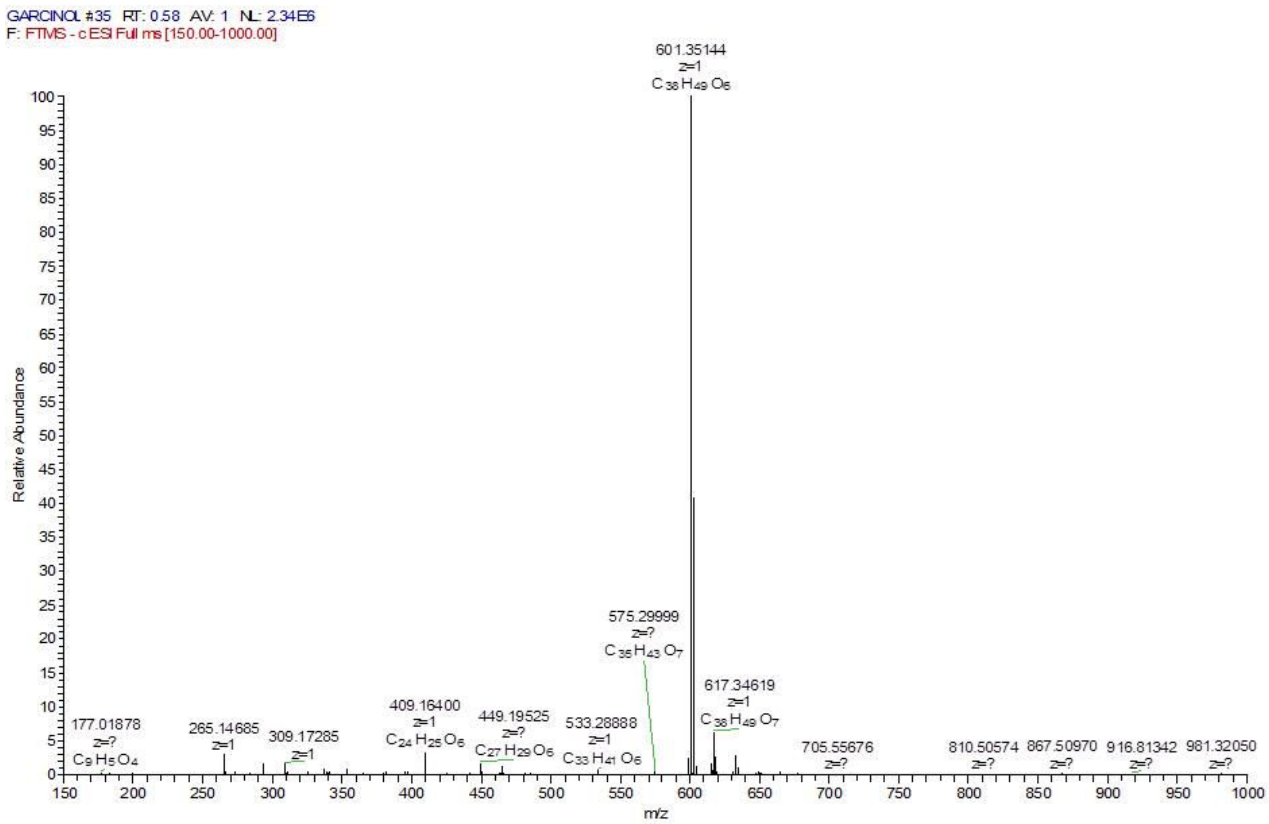

Figure S1. HRMS Spectrum of garcinol (**1**).

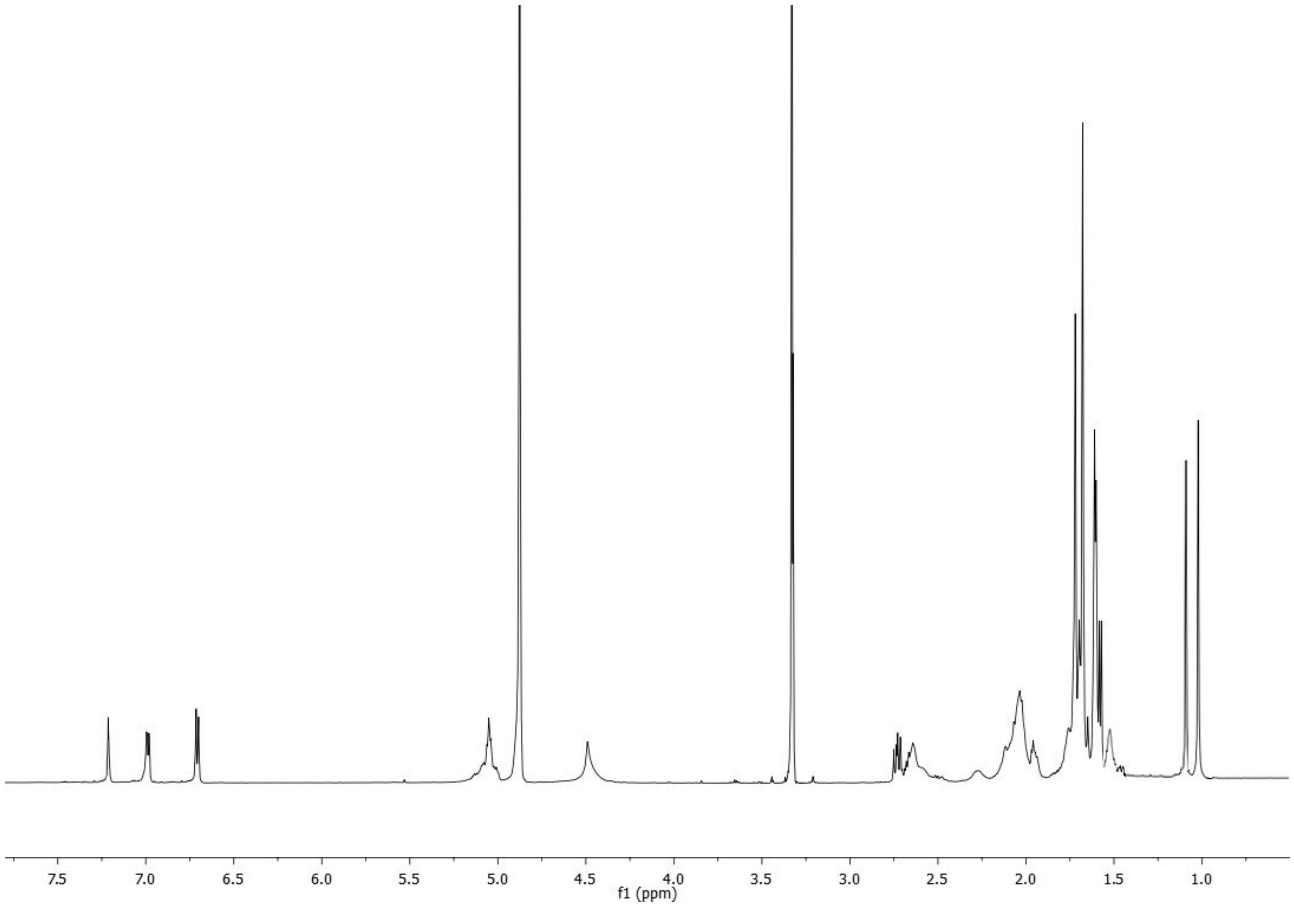

Figure S2. ^{1}H NMR Spectrum (600 MHz, CD_3OD) of garcinol (**1**).

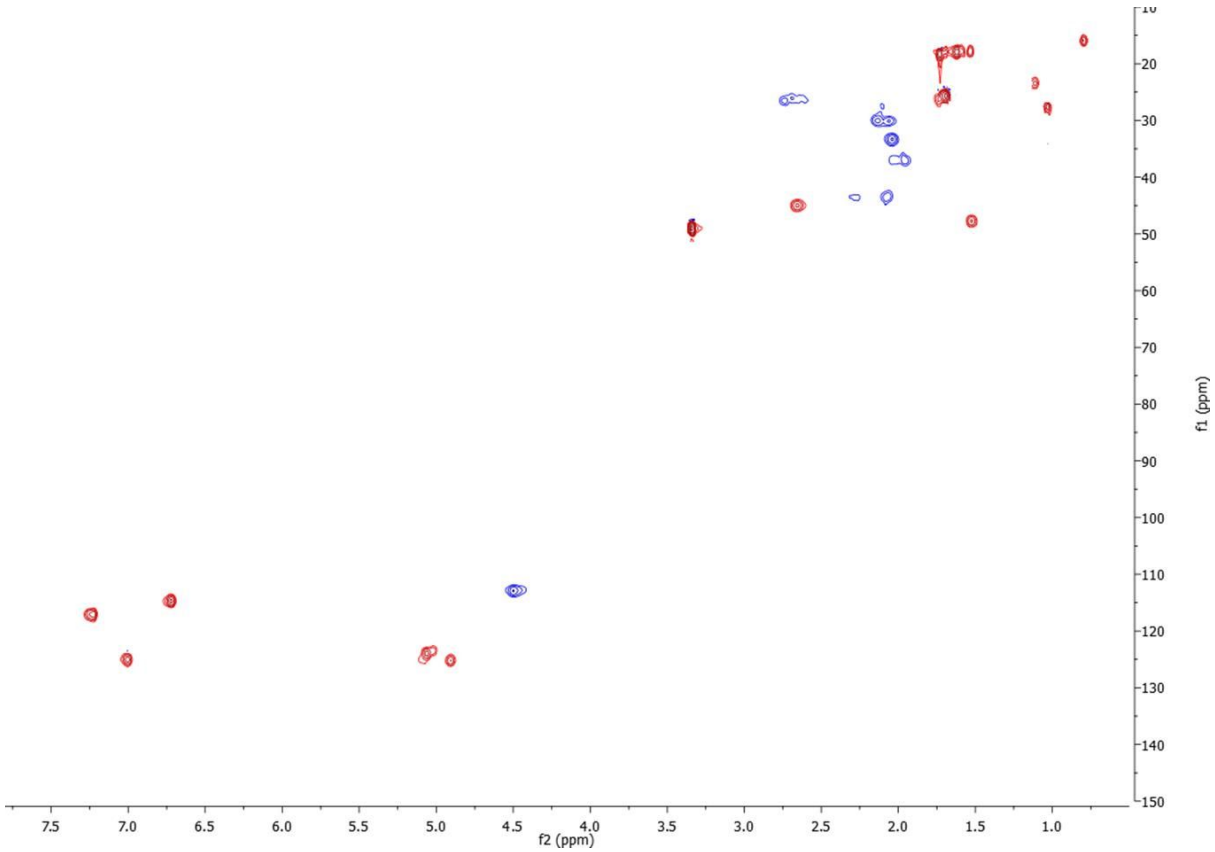

Figure S3. HSQC Spectrum (CD_3OD) of garcinol (**1**).

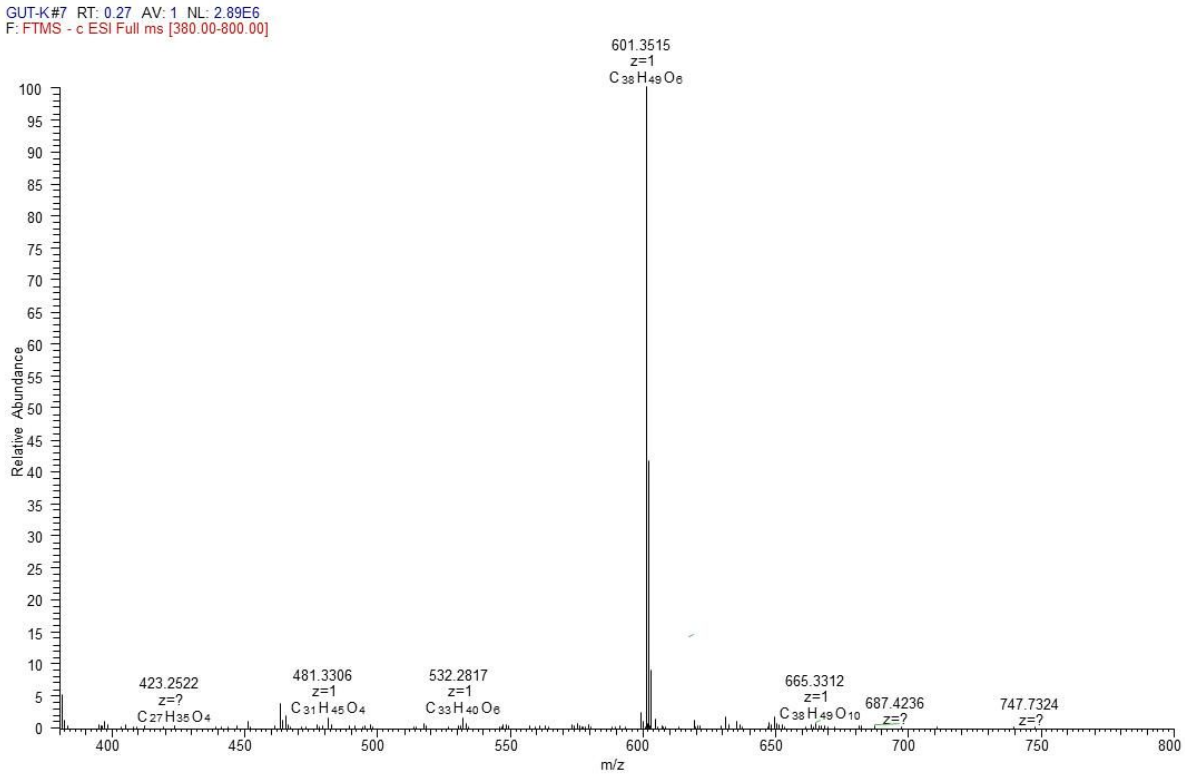

Figure S4. HRMS Spectrum of guttiferone K (**2**).

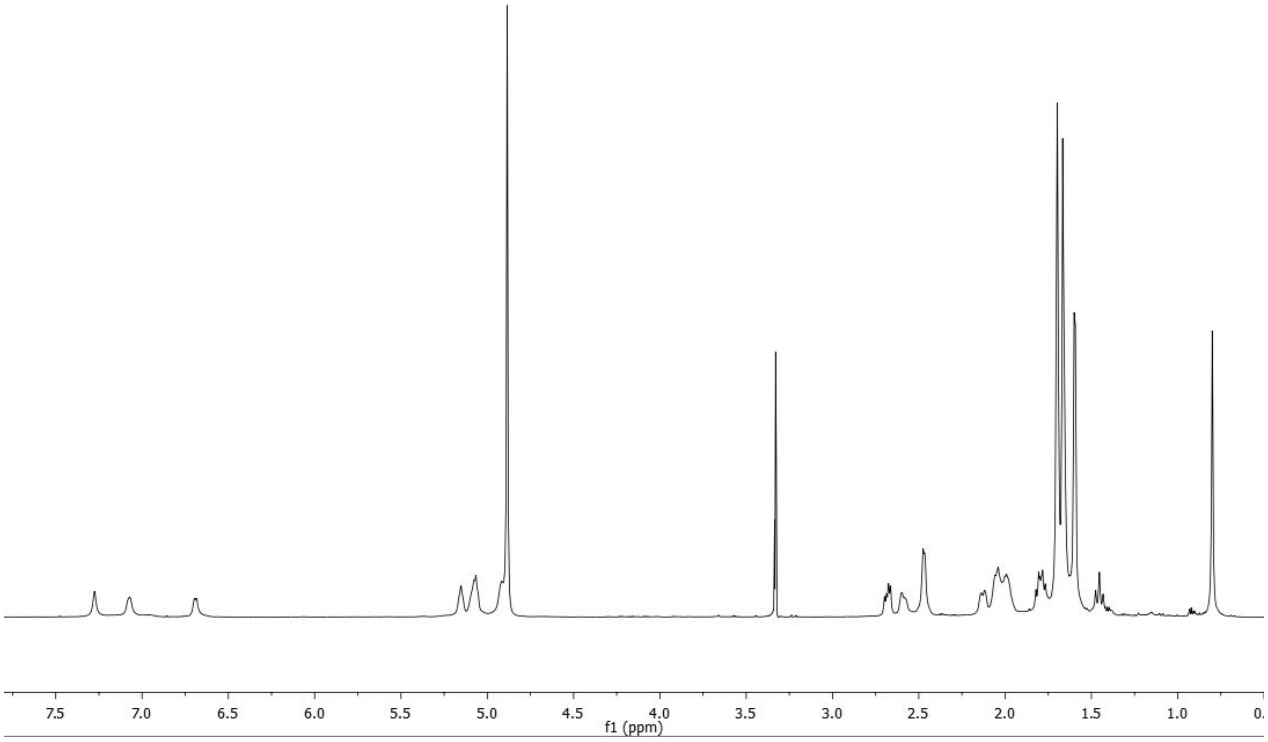

Figure S5. ^{1}H NMR Spectrum (600 MHz, CD_3OD) of guttiferone K (**2**).

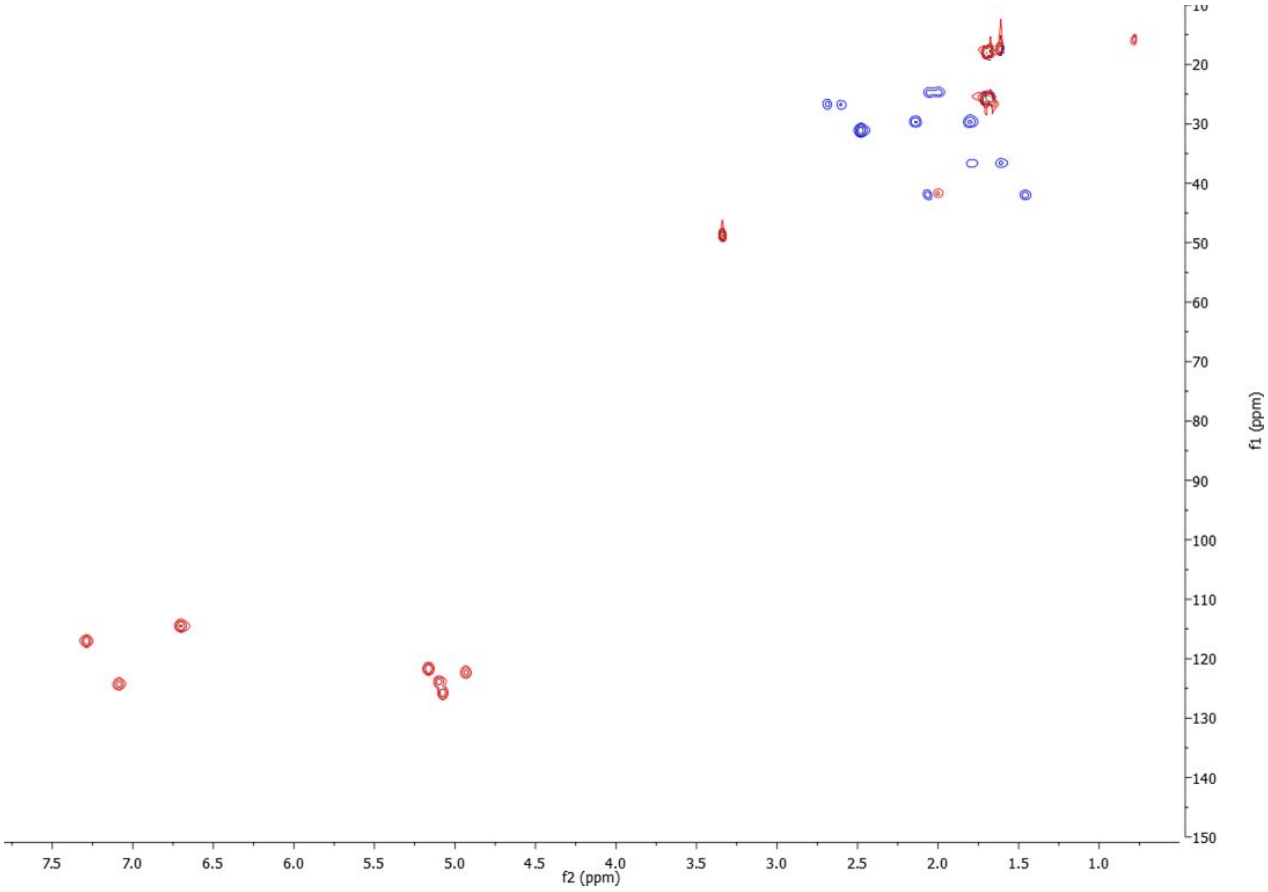

Figure S6. HSQC Spectrum (CD_3OD) of guttiferone K (**2**).

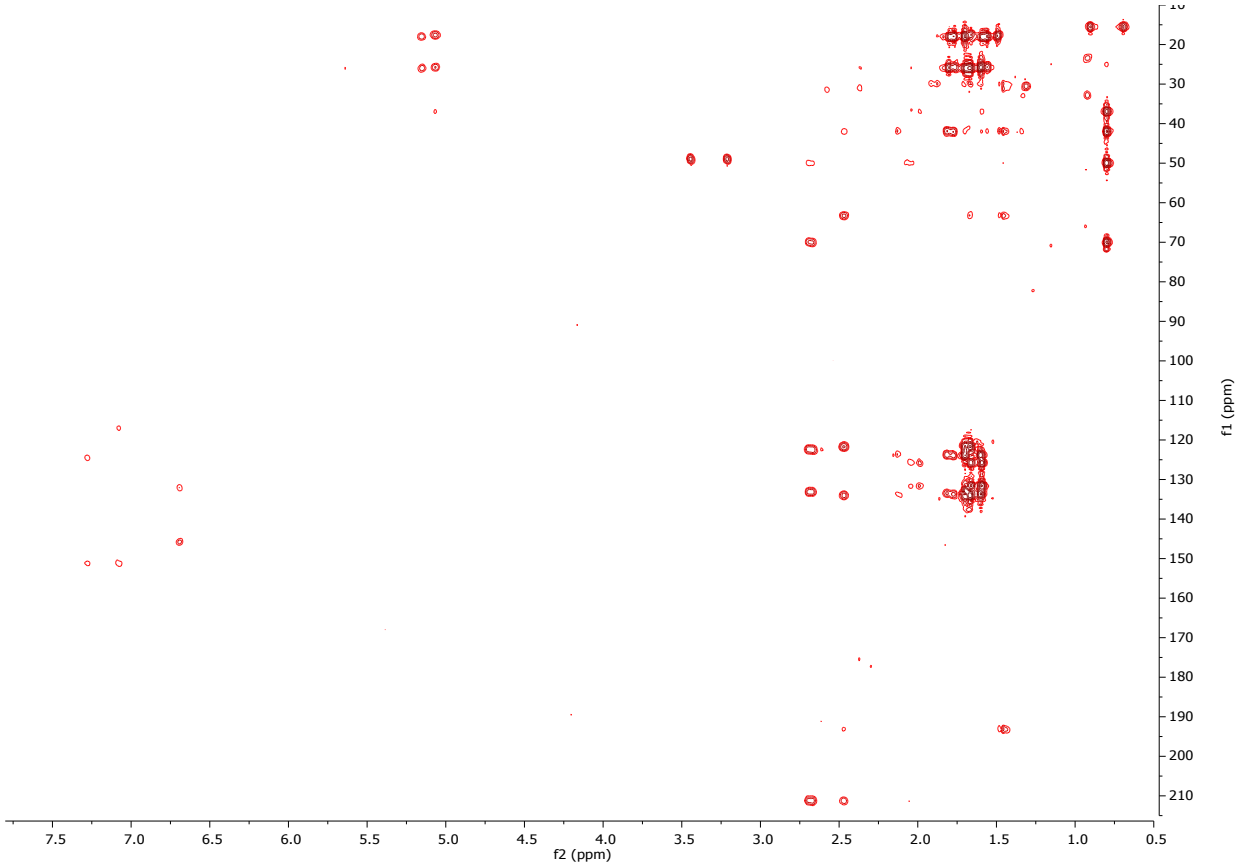

Figure S7. HMBC Spectrum (CD_3OD) of guttiferone K (**2**).

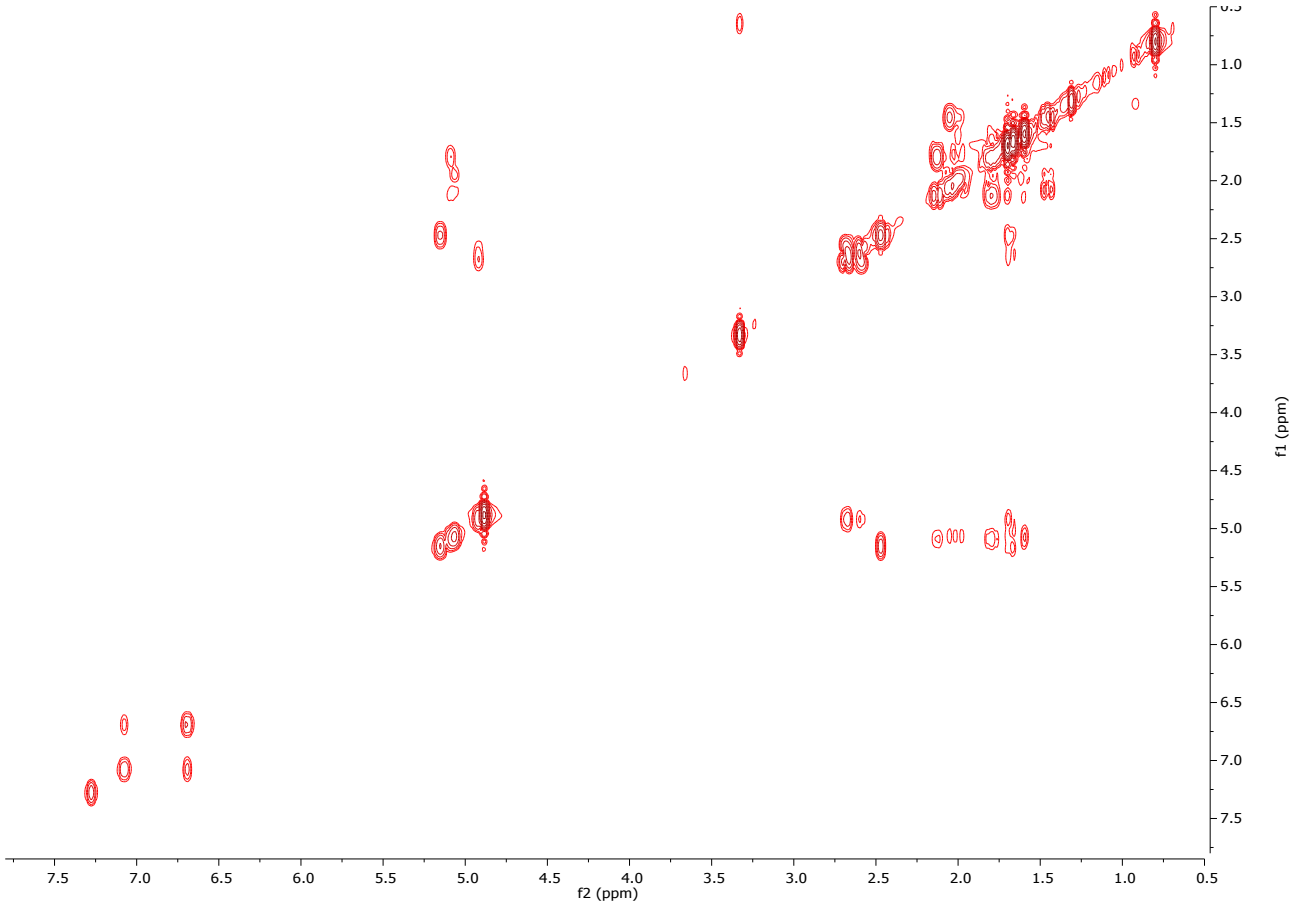

Figure S8. COSY Spectrum (CD_3OD) of guttiferone K (**2**).

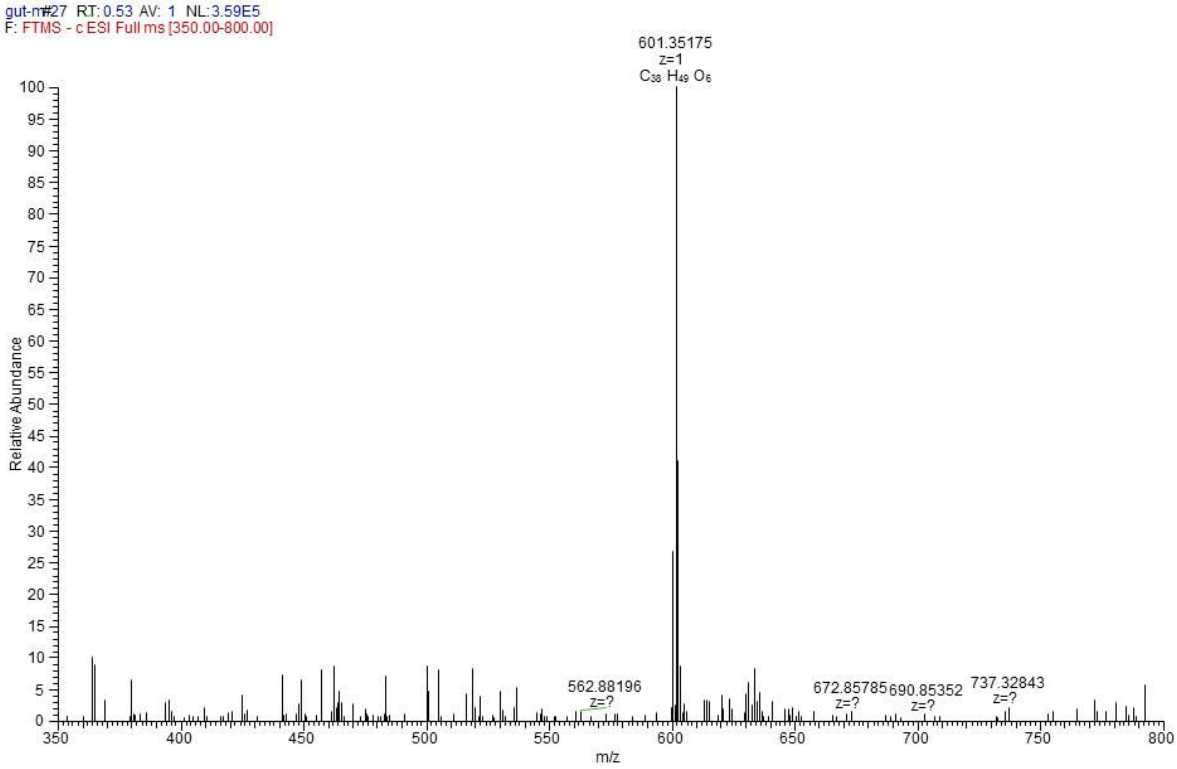

Figure S9. HRMS Spectrum of guttiferone M (**3**).

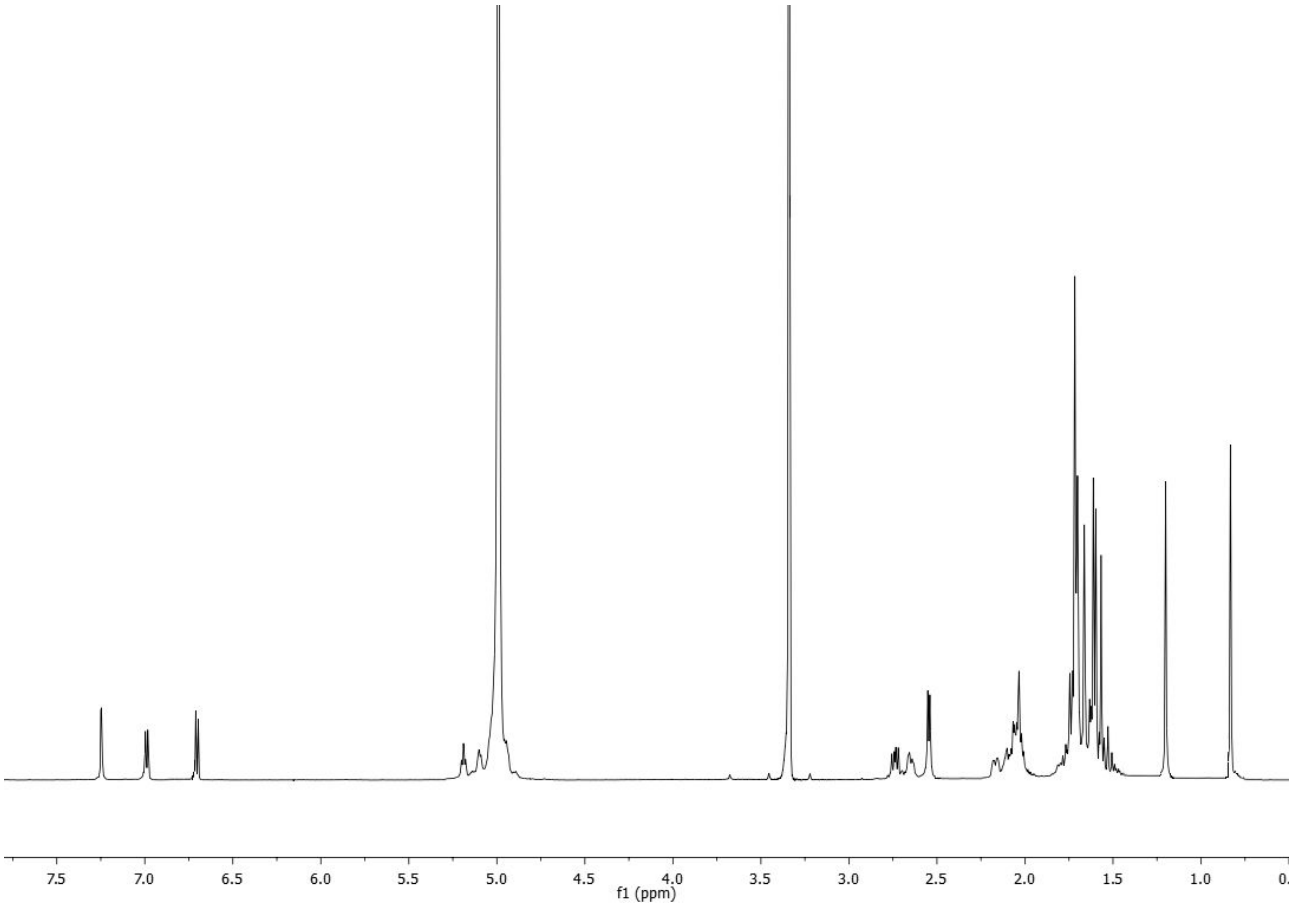

Figure S10. ^{1}H NMR Spectrum (600 MHz, CD_3OD) of guttiferone M (**3**).

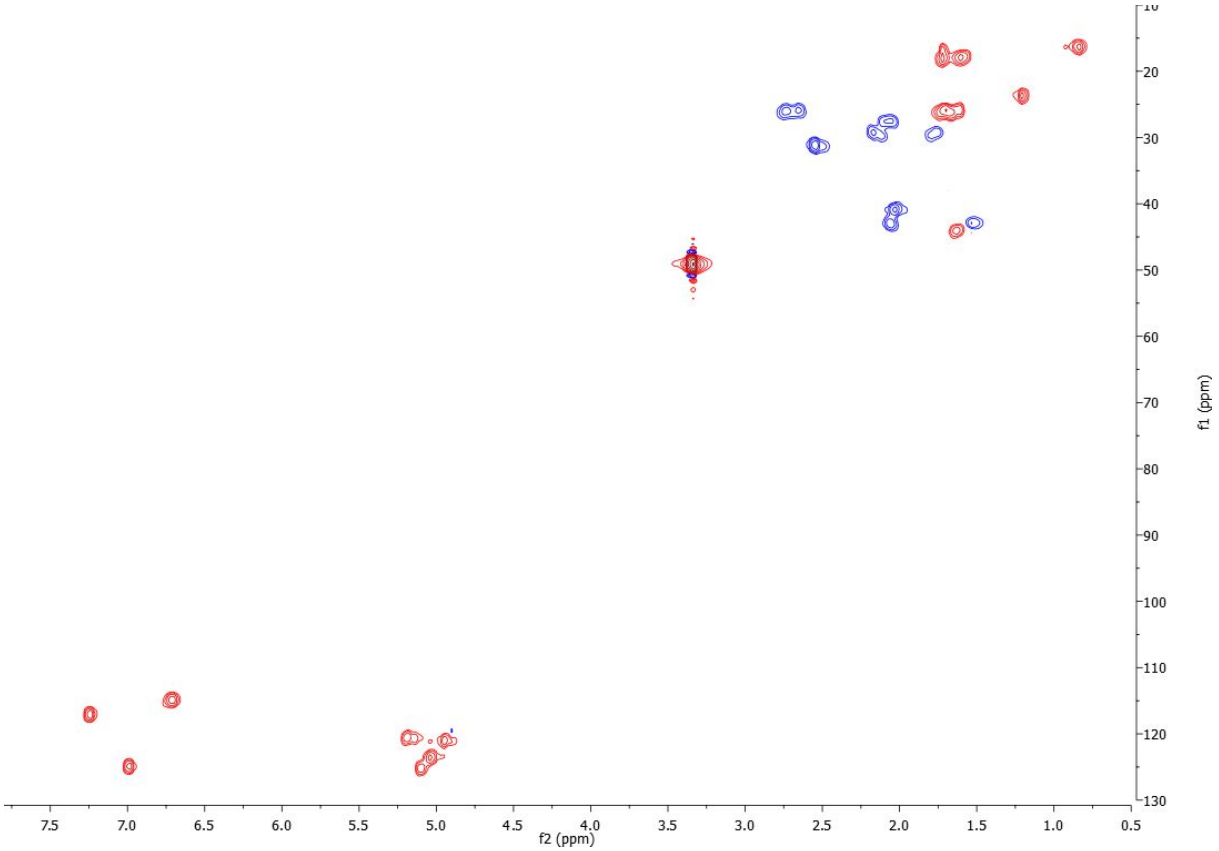

Figure S11. HSQC Spectrum (CD_3OD) of guttiferone M (**3**).

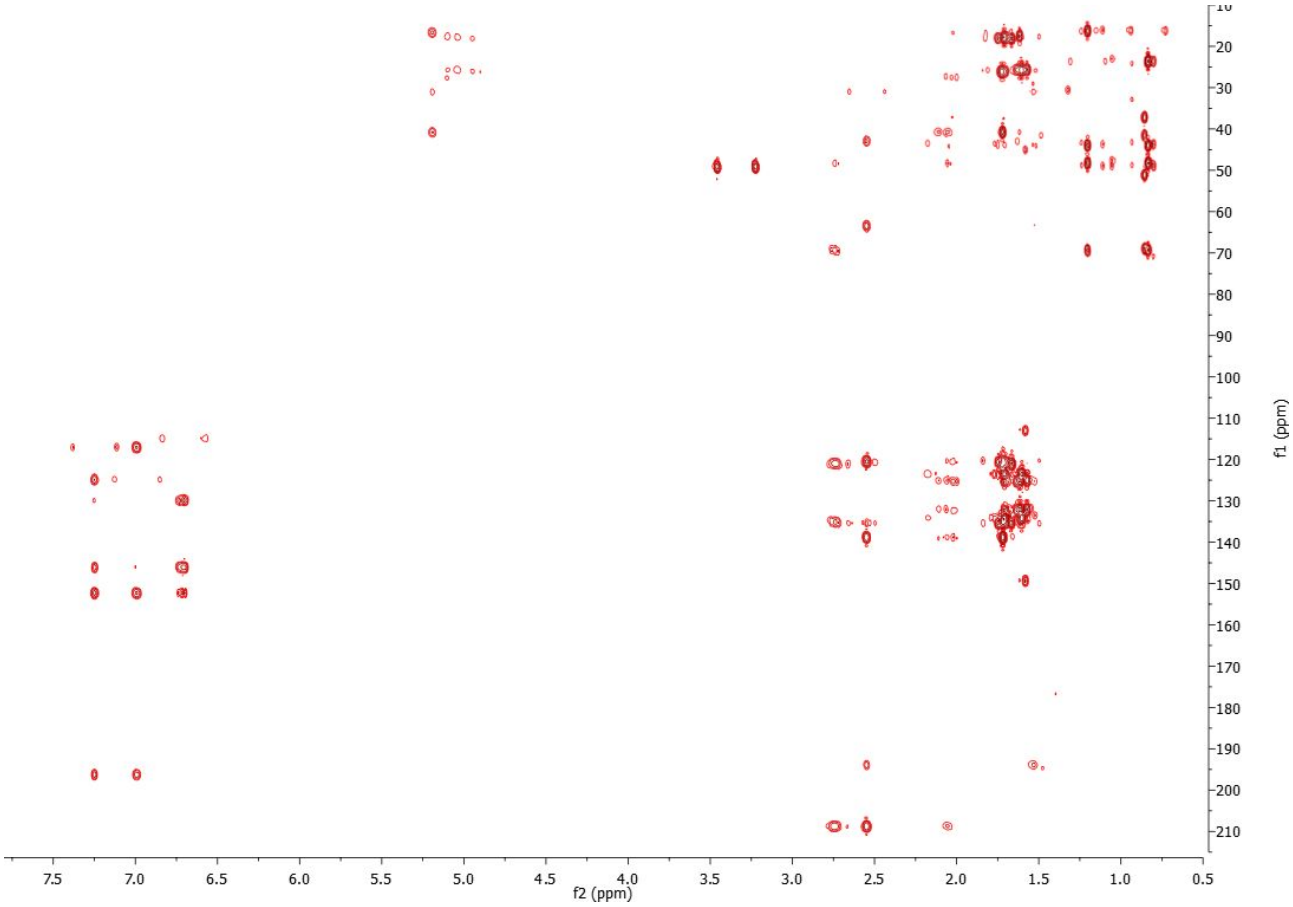

Figure S12. HMBC Spectrum (CD_3OD) of guttiferone M (**3**).

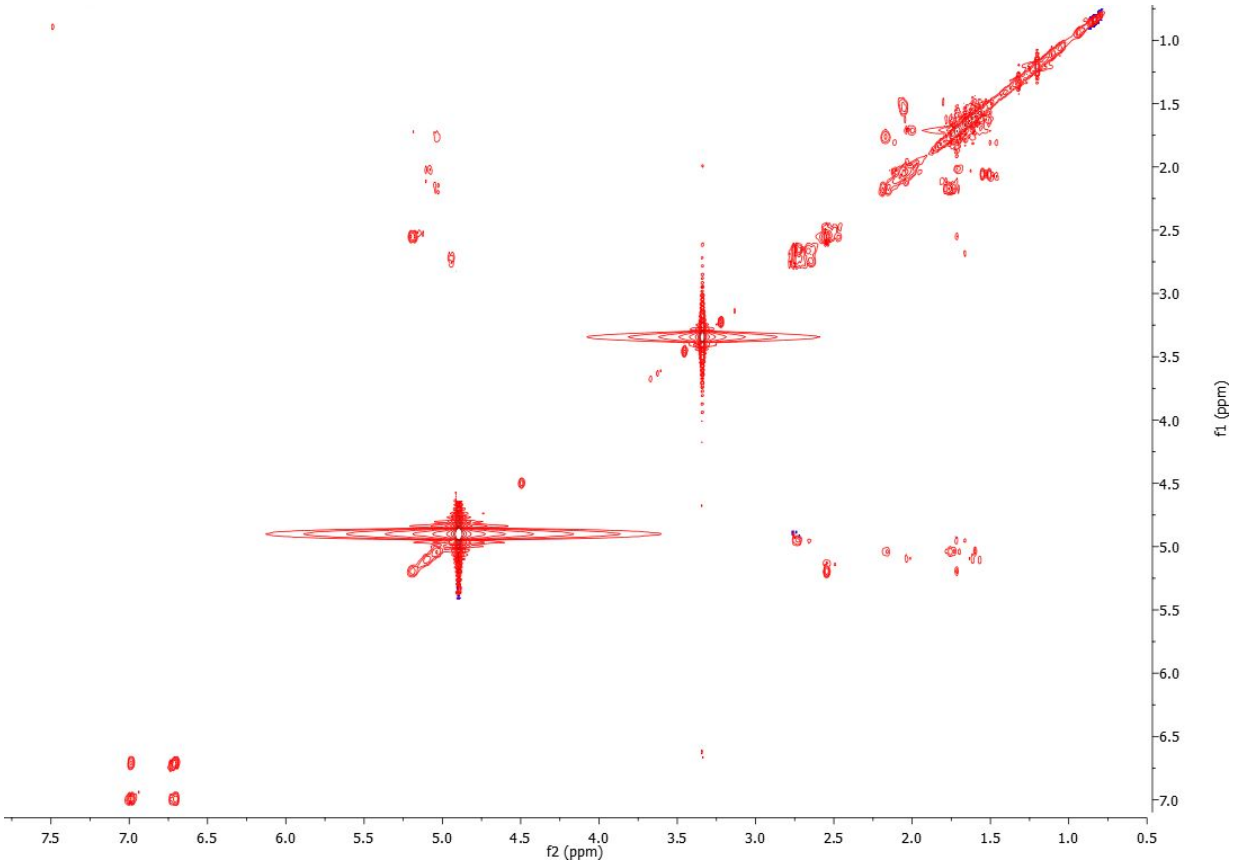

Figure S13. COSY Spectrum (CD_3OD) of guttiferone M (**3**).

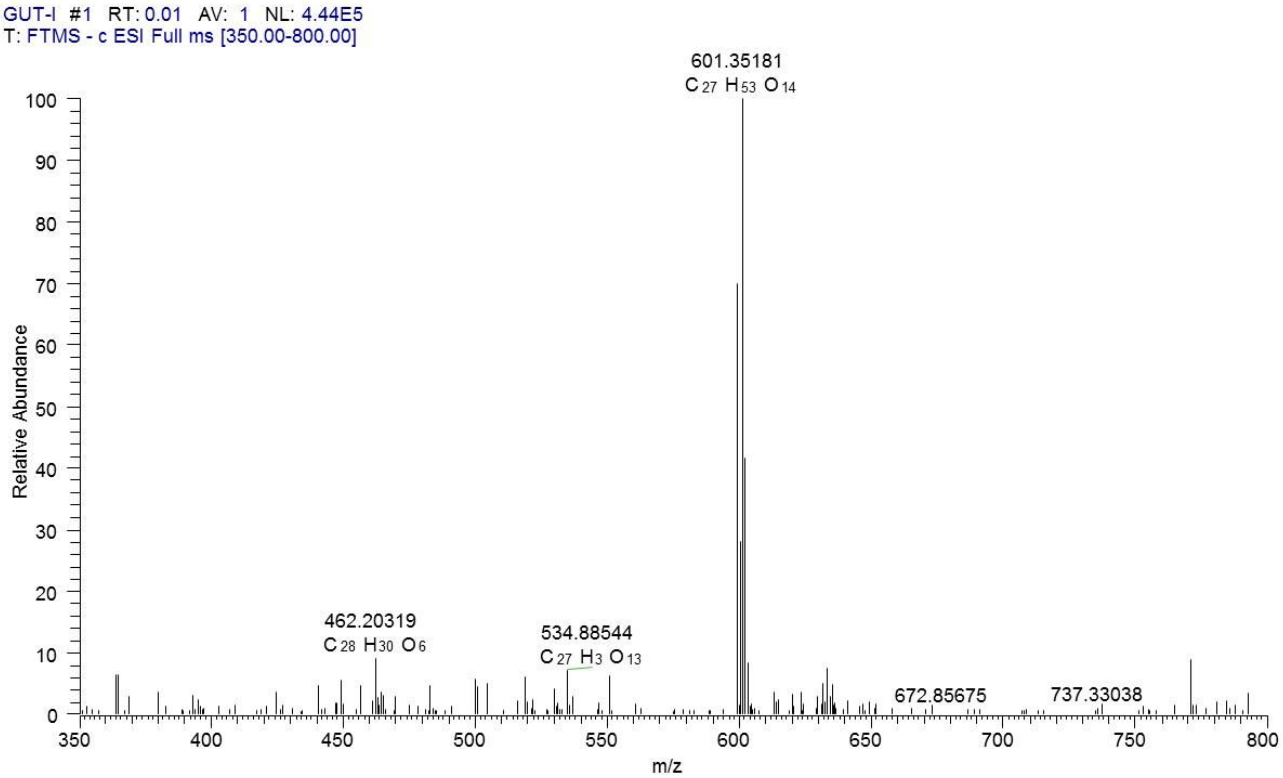

Figure S14. HRMS Spectrum of guttiferone I (**4**).

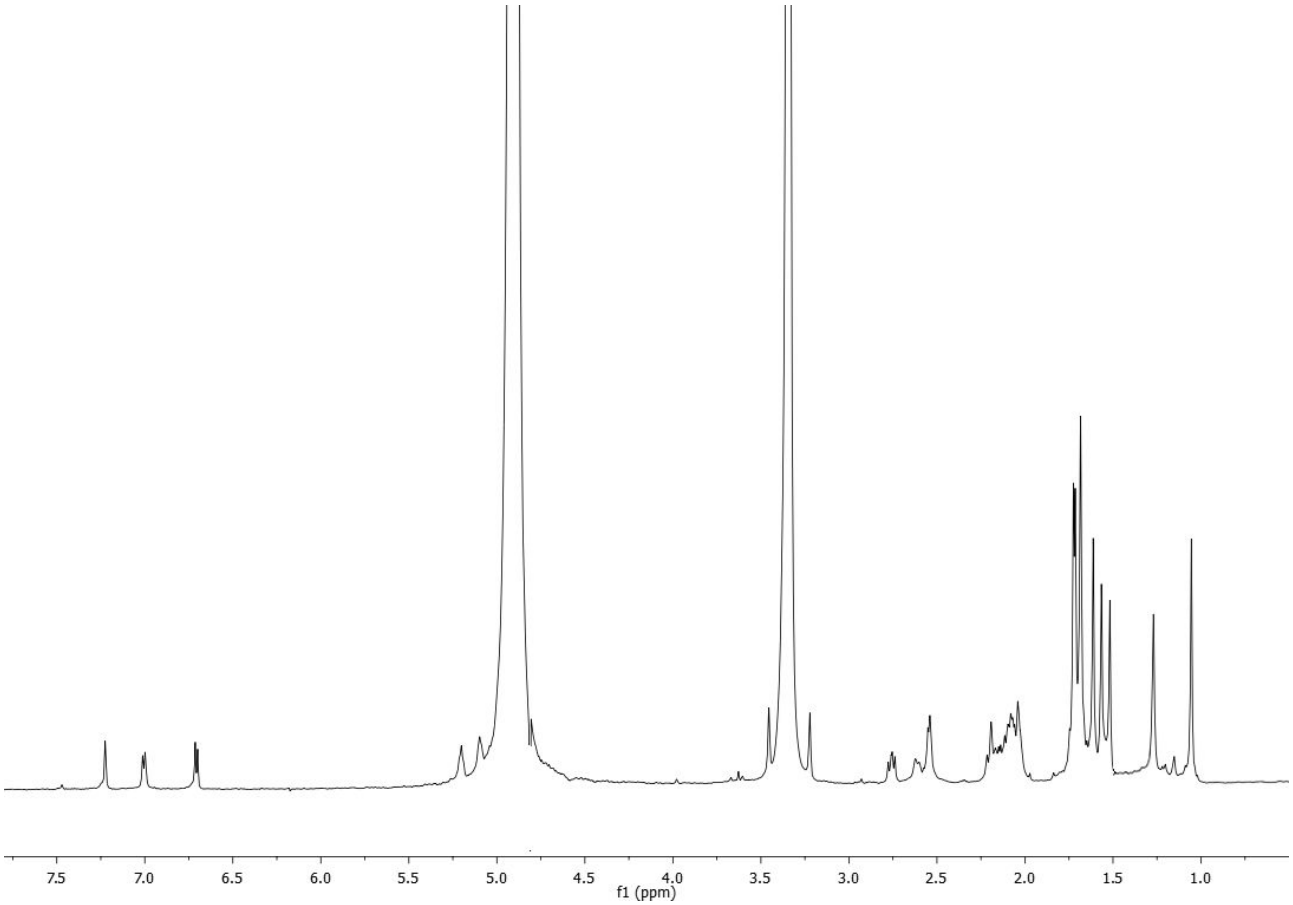

Figure S15. ^{1}H NMR Spectrum (600 MHz, CD_3OD) of guttiferone I (**4**).

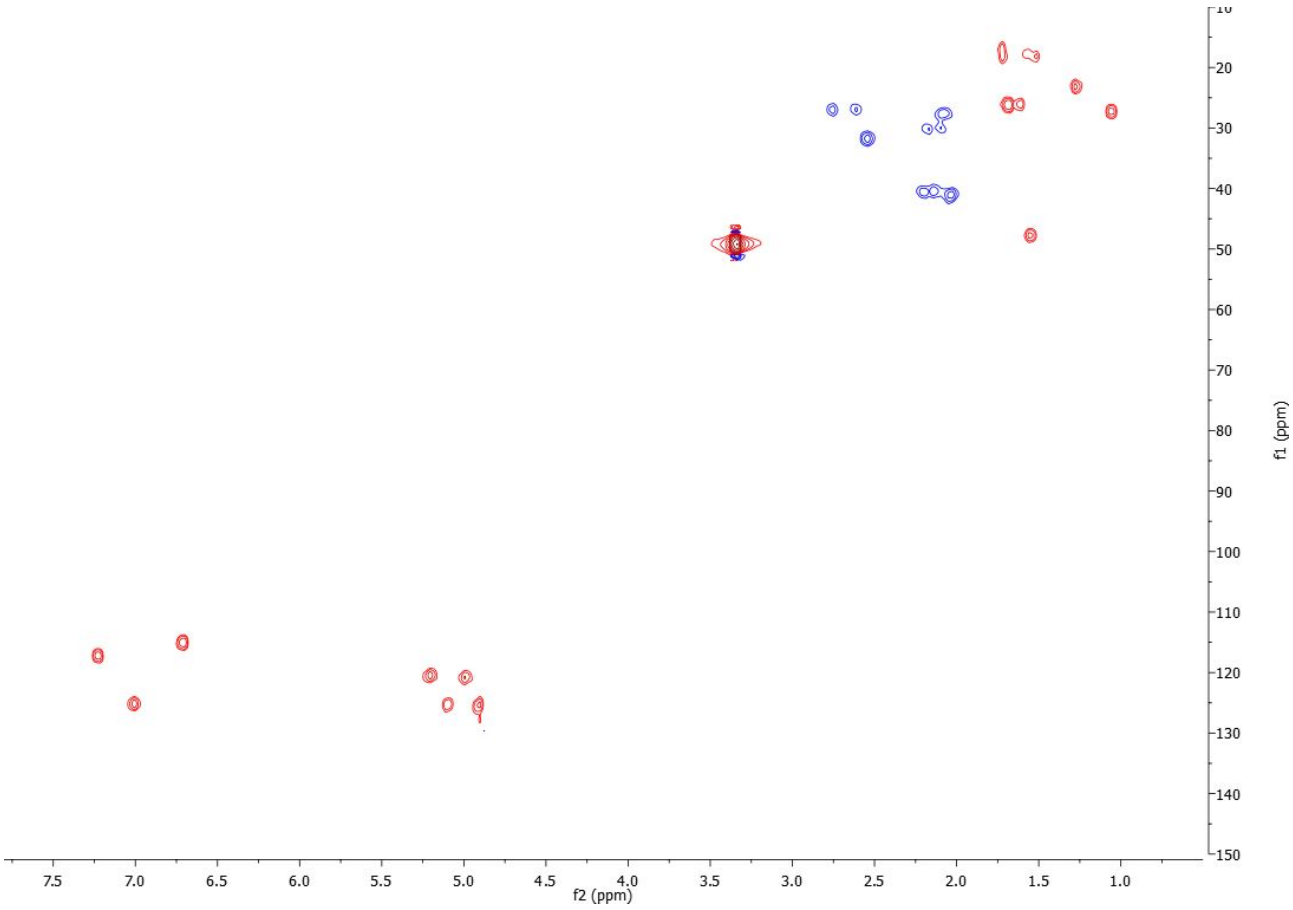

Figure S16. HSQC Spectrum (CD_3OD) of guttiferone I (**4**).

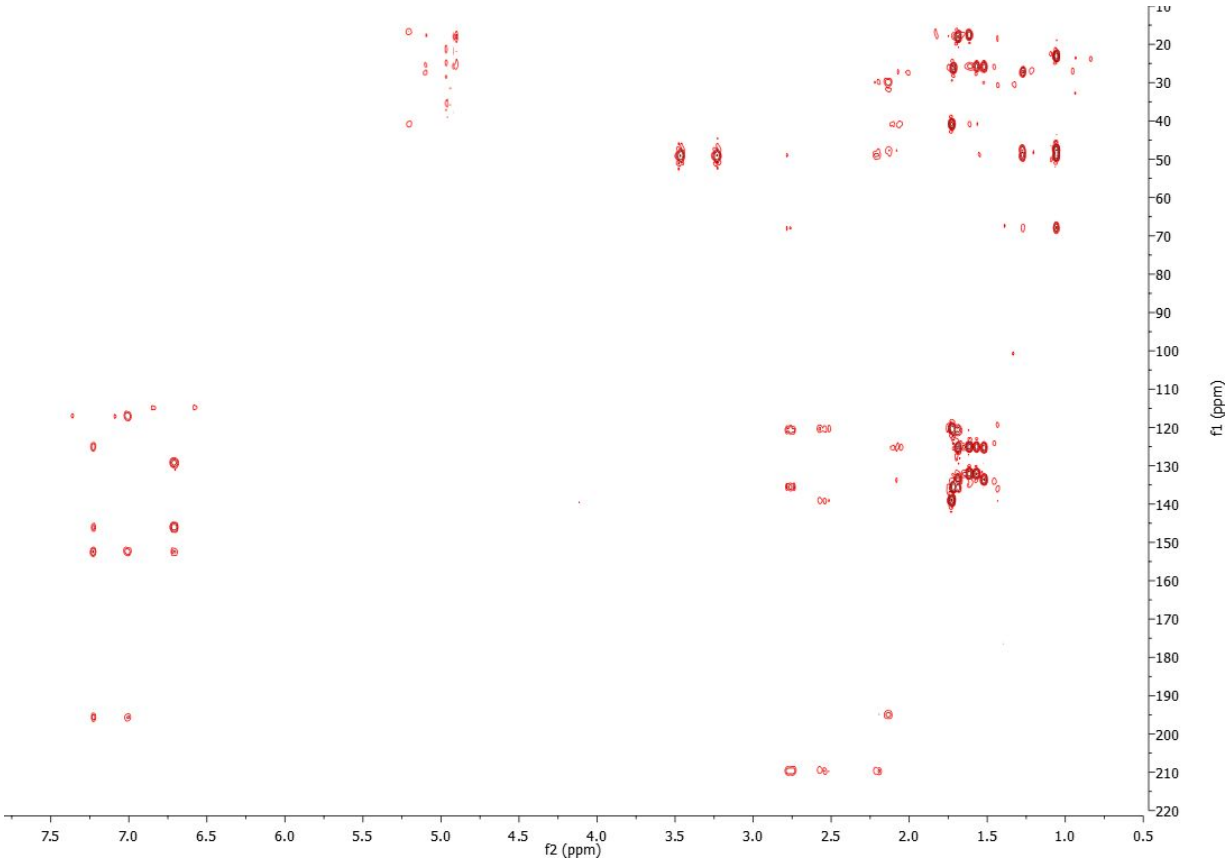

Figure S17. HMBC Spectrum (CD_3OD) of guttiferone I (**4**).

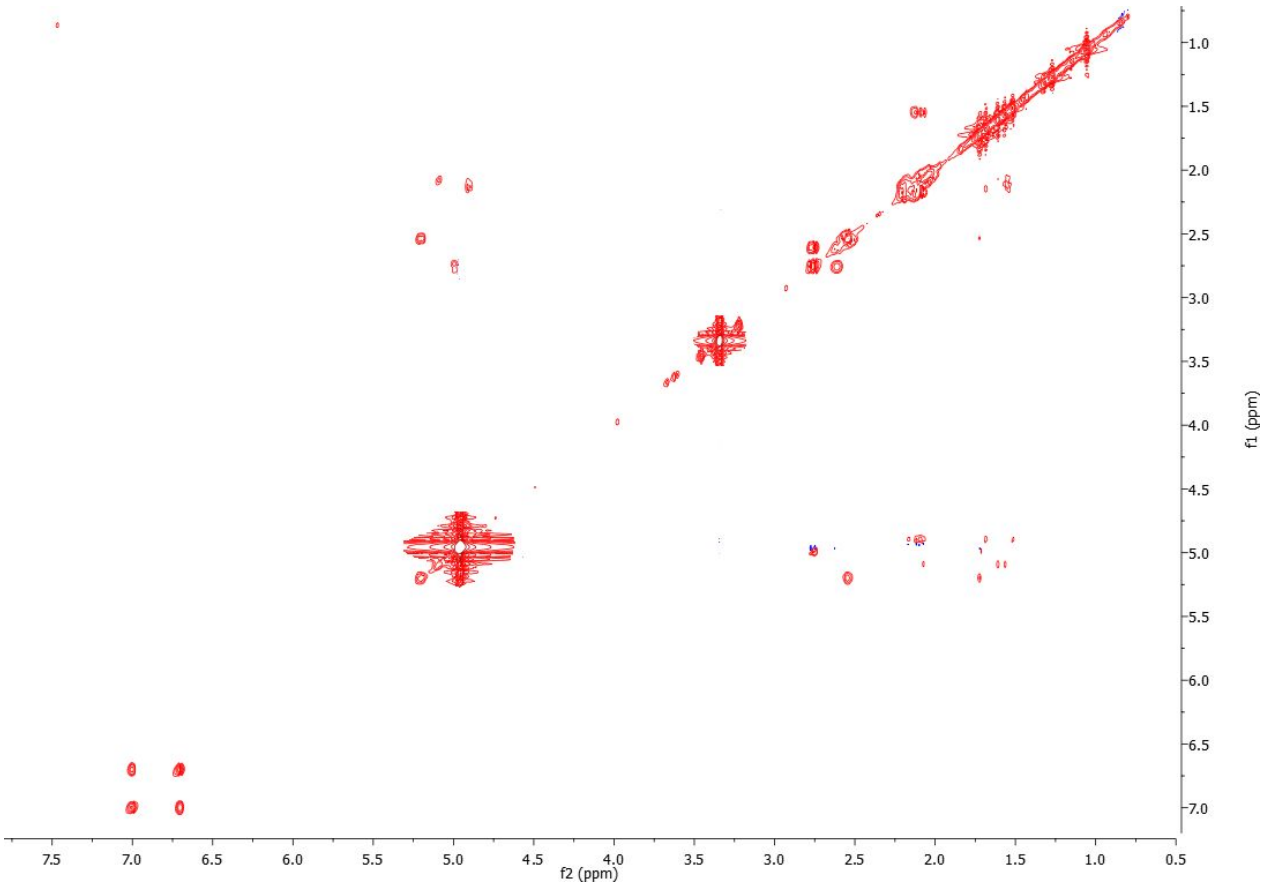

Figure S18. COSY Spectrum (CD_3OD) of guttiferone I (**4**).

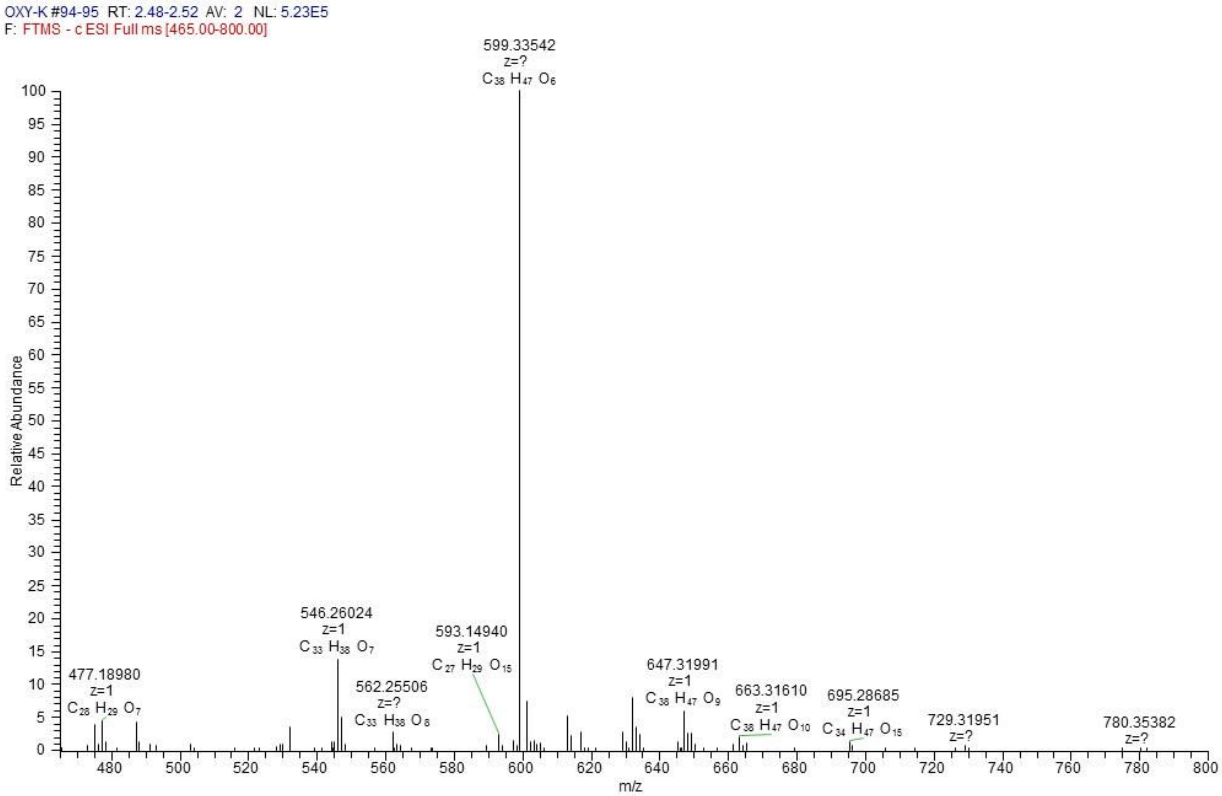

Figure S19. HRMS Spectrum of oxyguttiferone K (**5**).

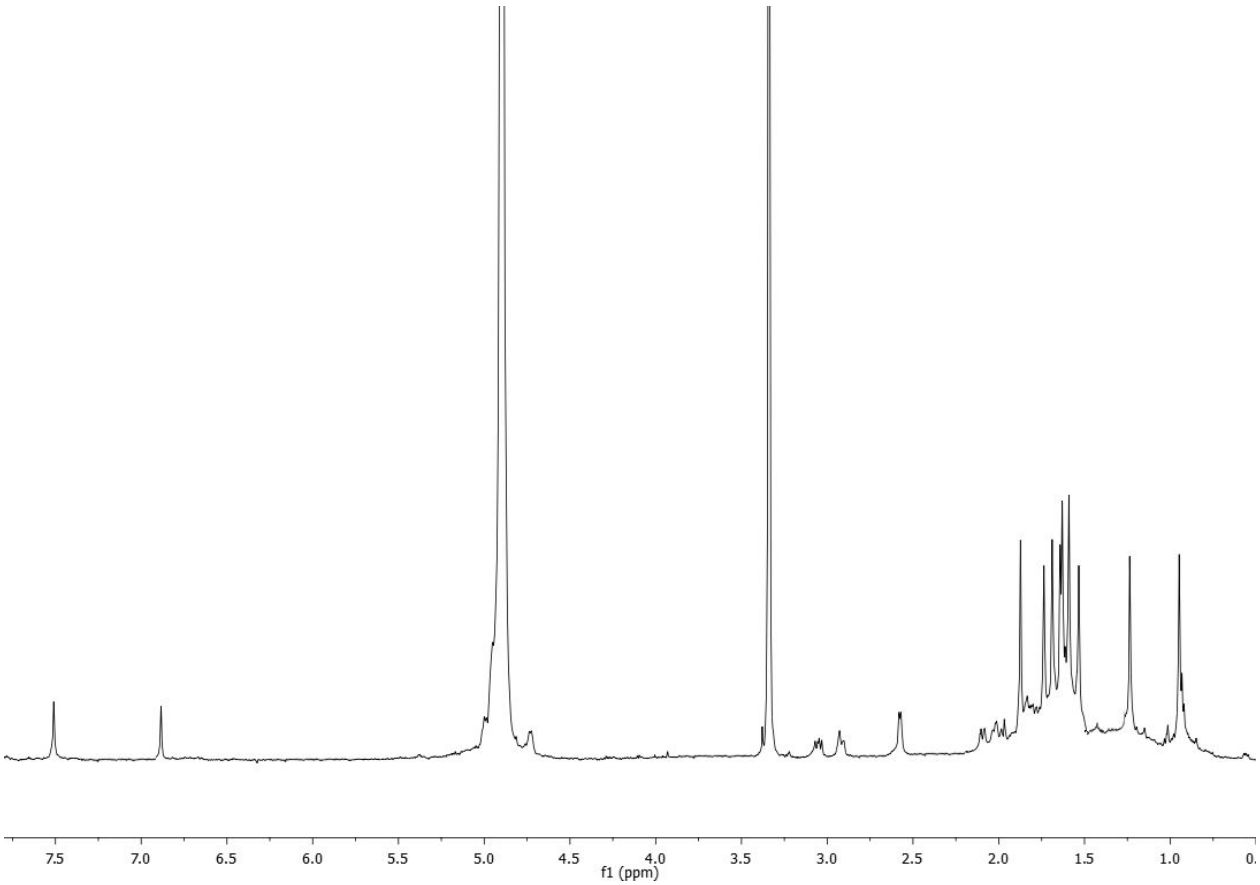

Figure S20. ^1H NMR Spectrum (600 MHz, CD_3OD) of oxyguttiferone K (**5**).

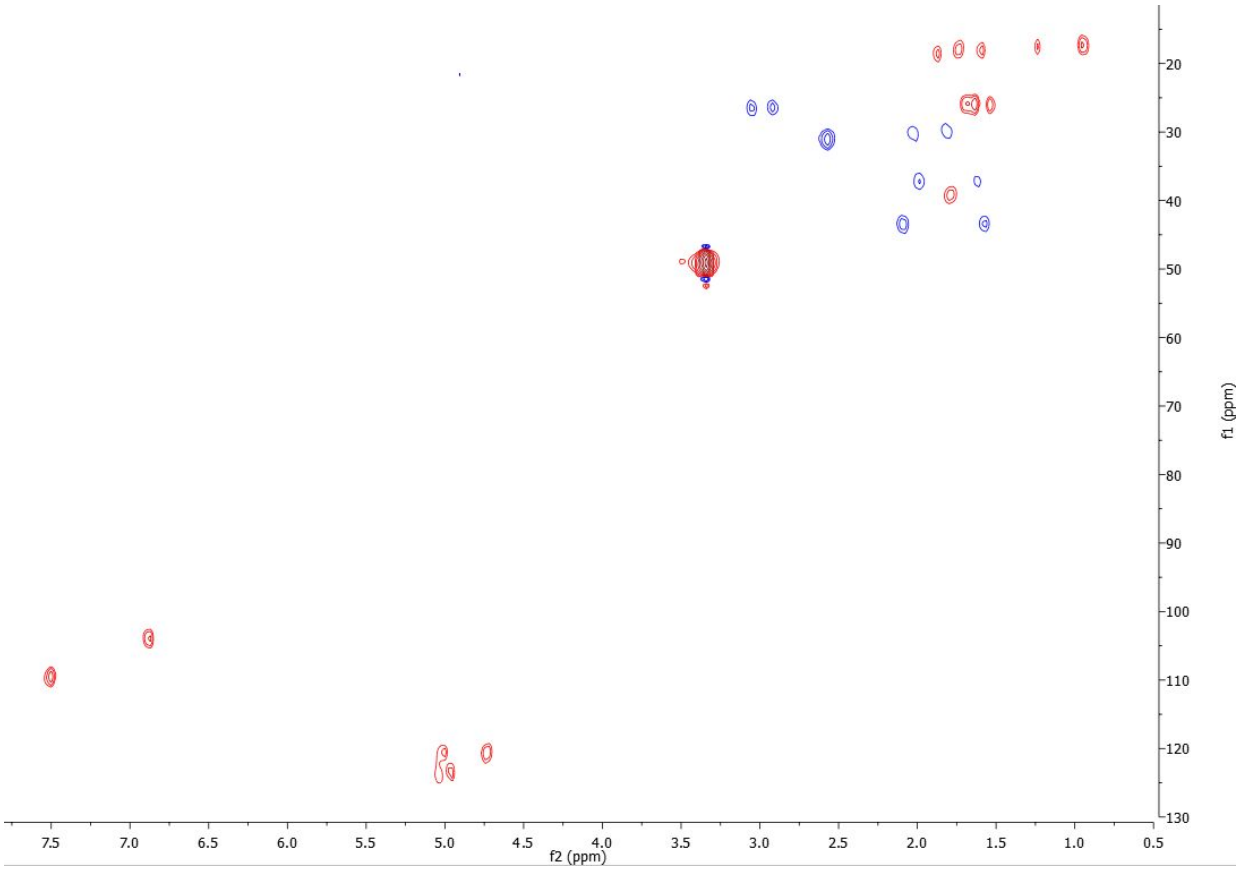

Figure S21. HSQC Spectrum of oxyguttiferone K (**5**).

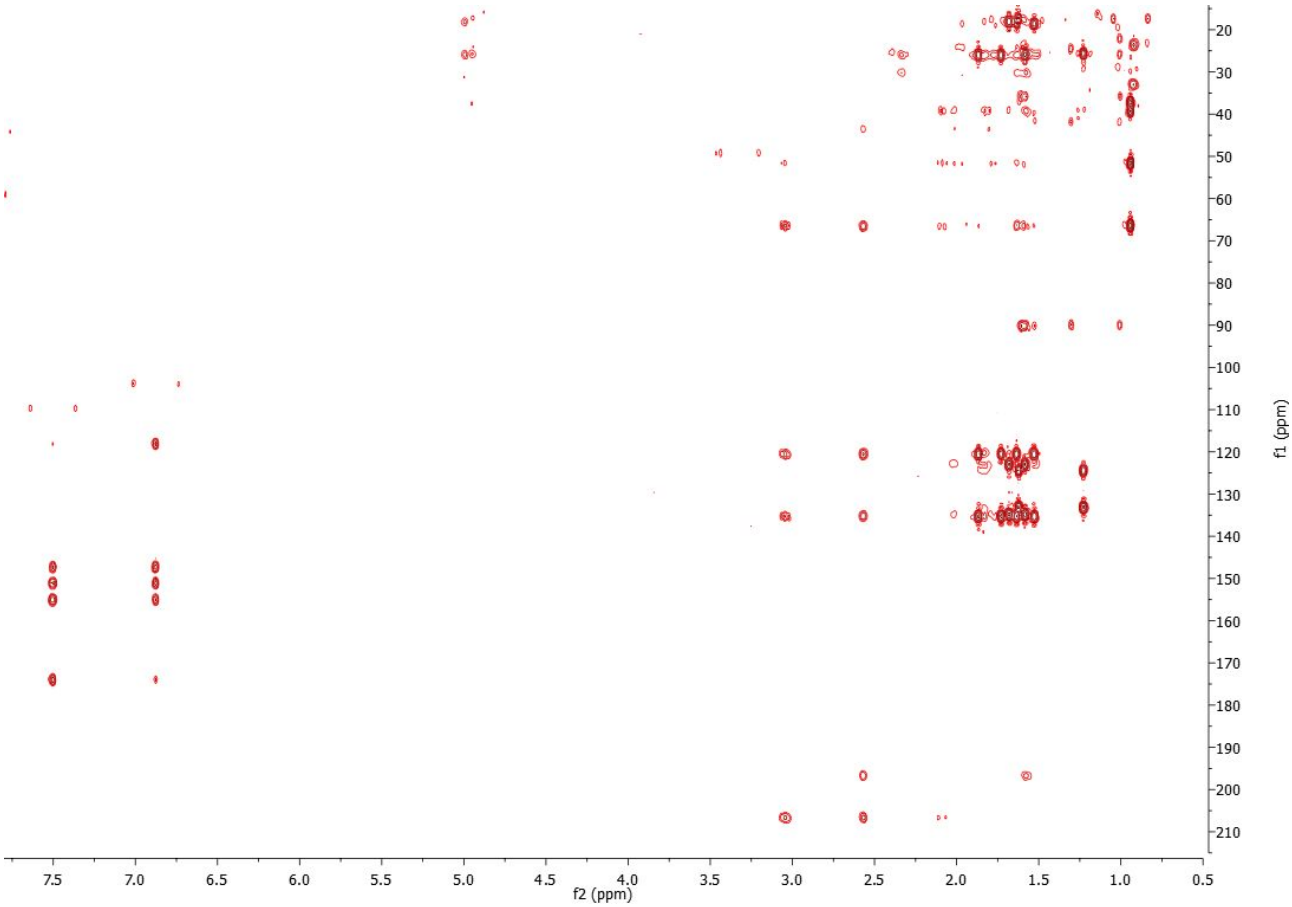

Figure S22. HMBC Spectrum of oxyguttiferone K (**5**).

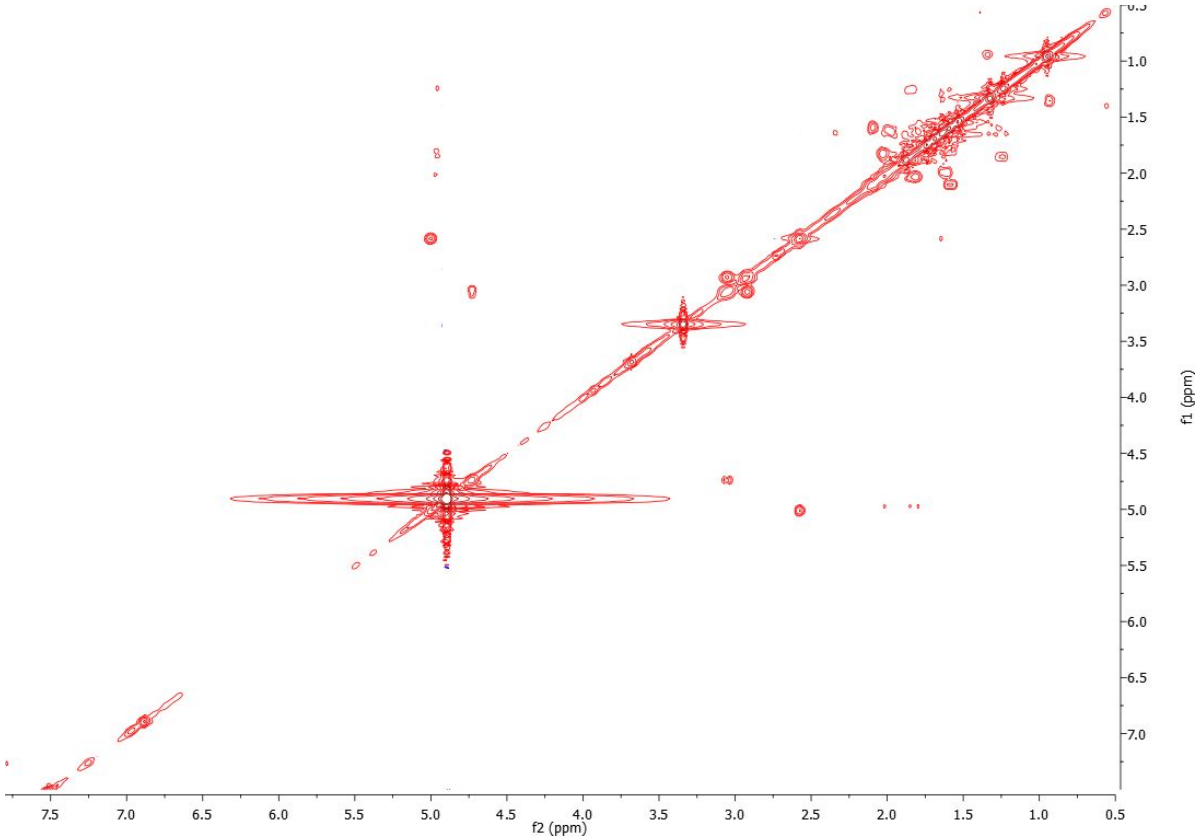

Figure S23. COSY Spectrum of oxyguttiferone K (**5**).

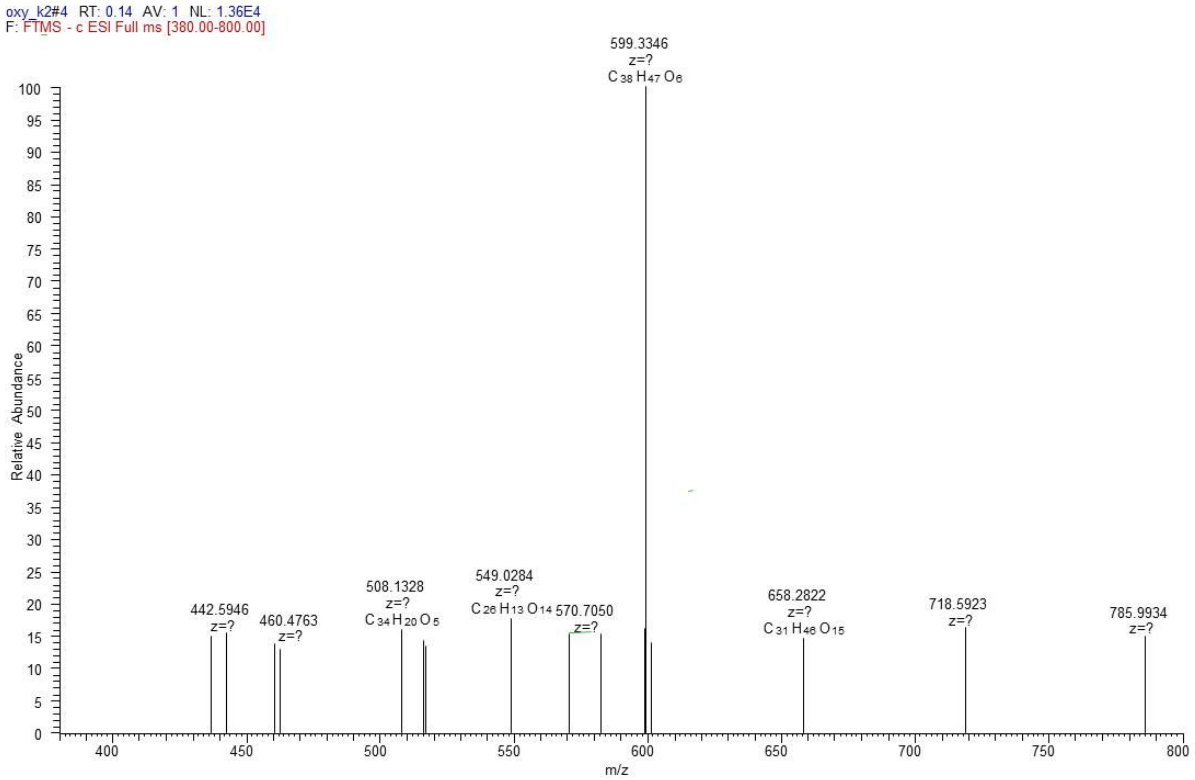

Figure S24. HRMS Spectrum of oxyguttiferone K2 (**6**).

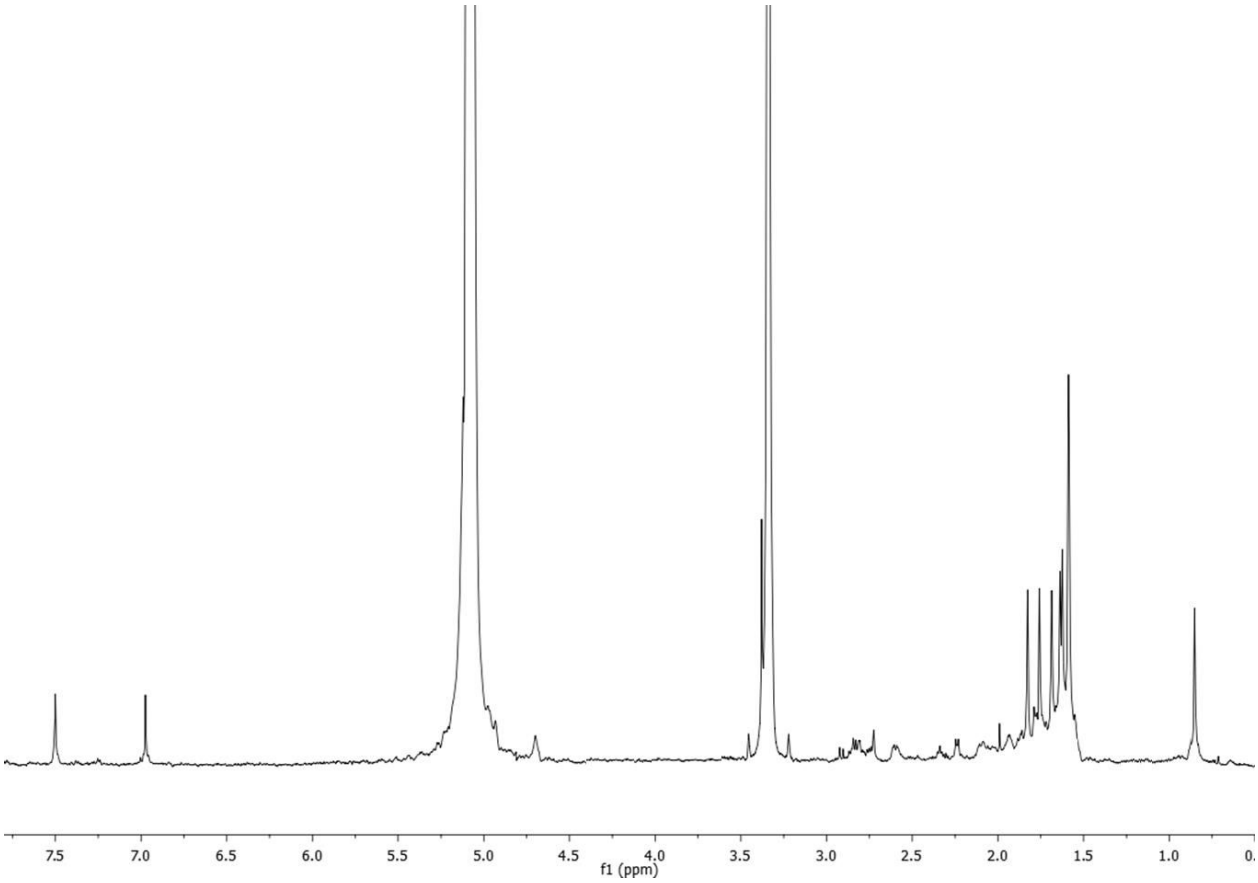

Figure S25. ^{1}H NMR Spectrum (600 MHz, CD_3OD) of oxyguttiferone K2 (**6**).

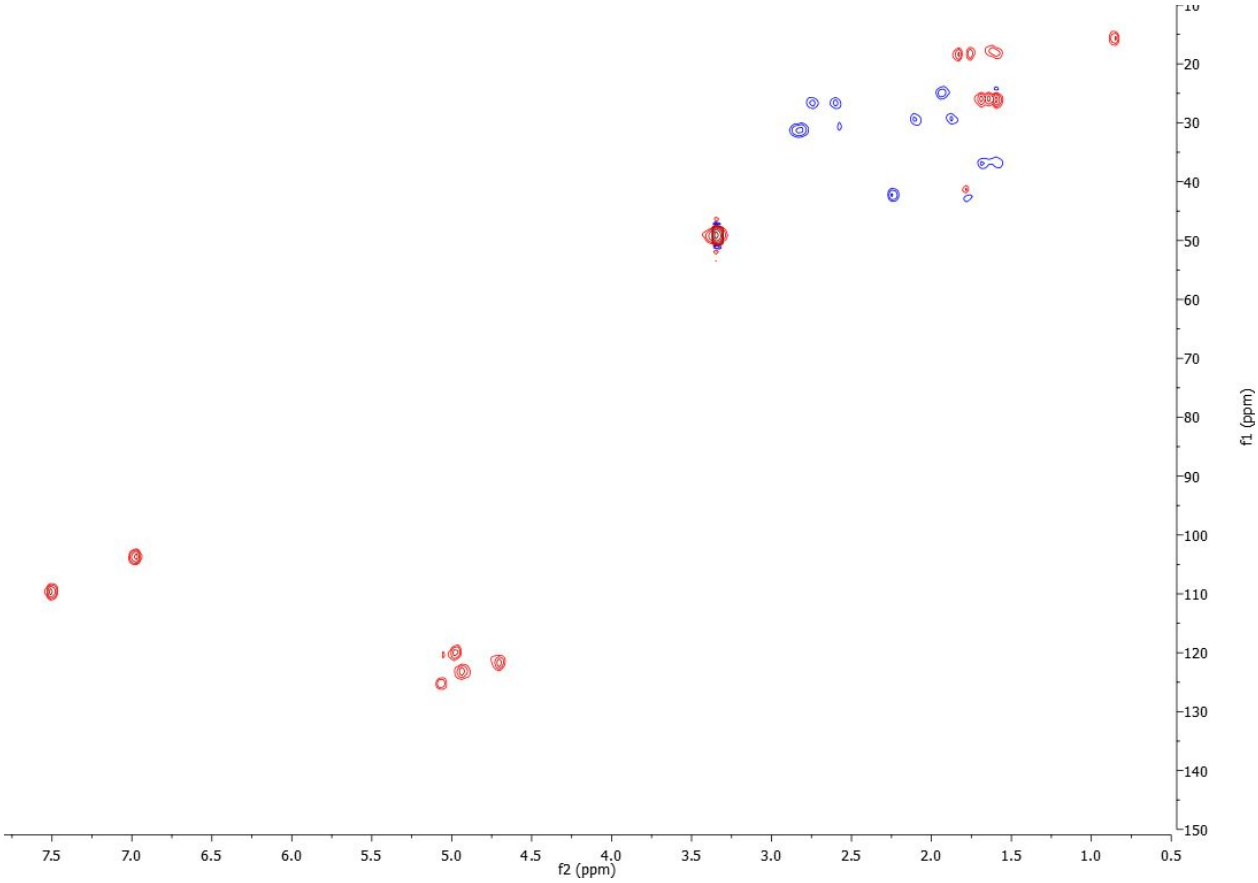

Figure S26. HSQC Spectrum (CD_3OD) of oxyguttiferone K2 (**6**).

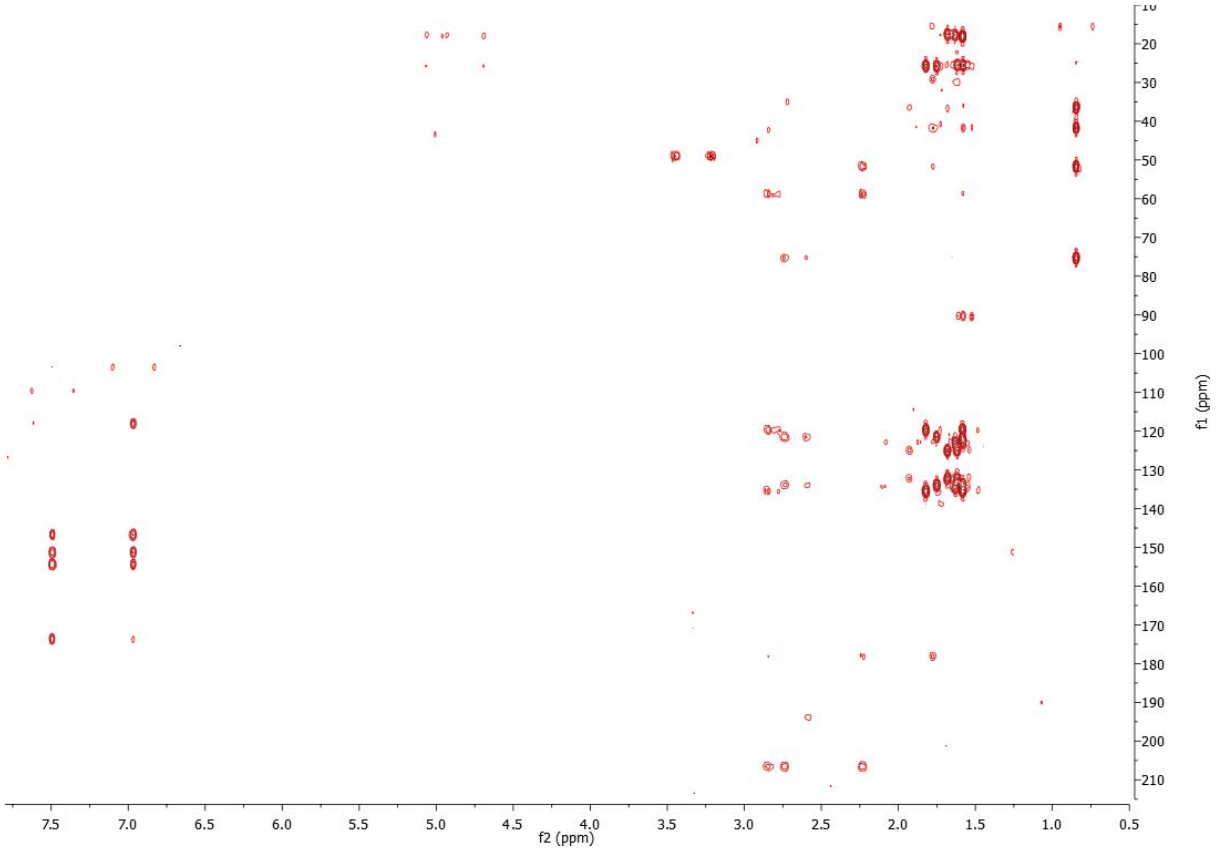

Figure S27. HMBC Spectrum (CD_3OD) of oxyguttiferone K2 (**6**).

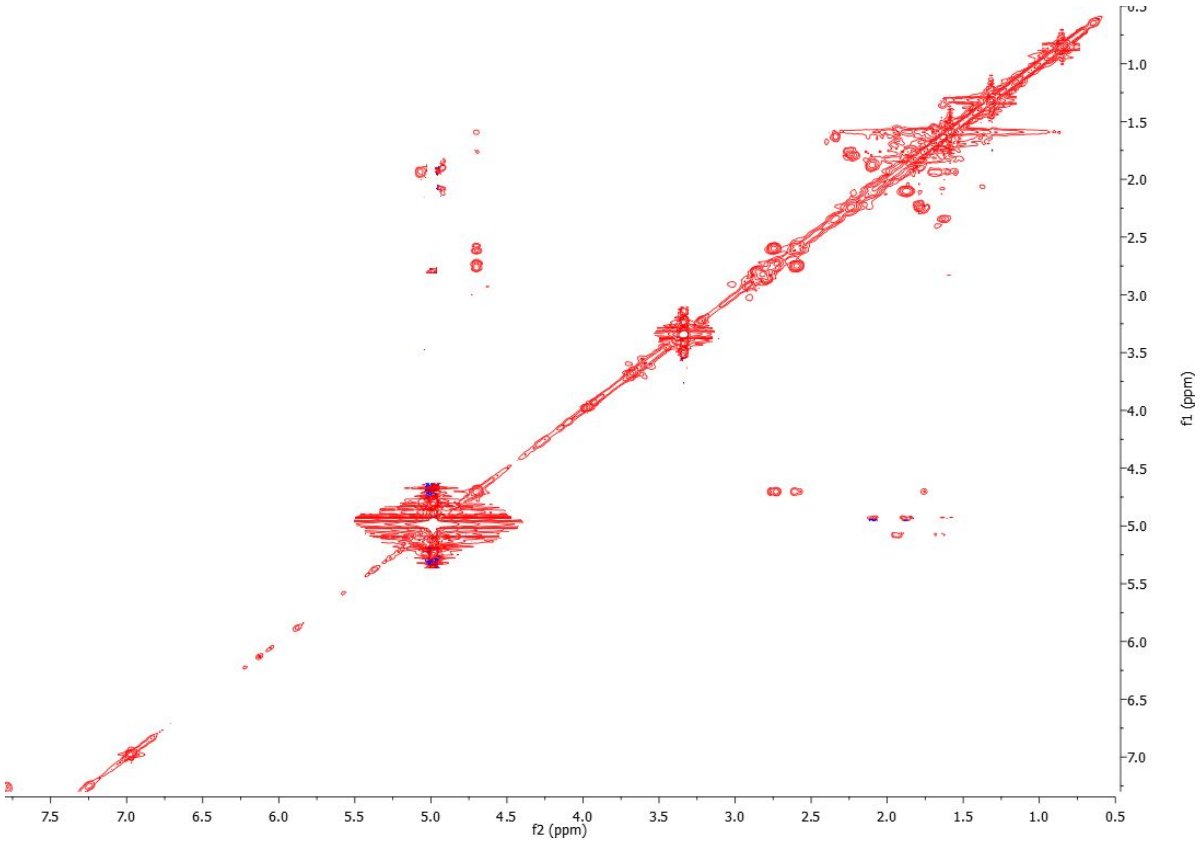

Figure S28. COSY Spectrum (CD_3OD) of oxyguttiferone K2 (**6**).

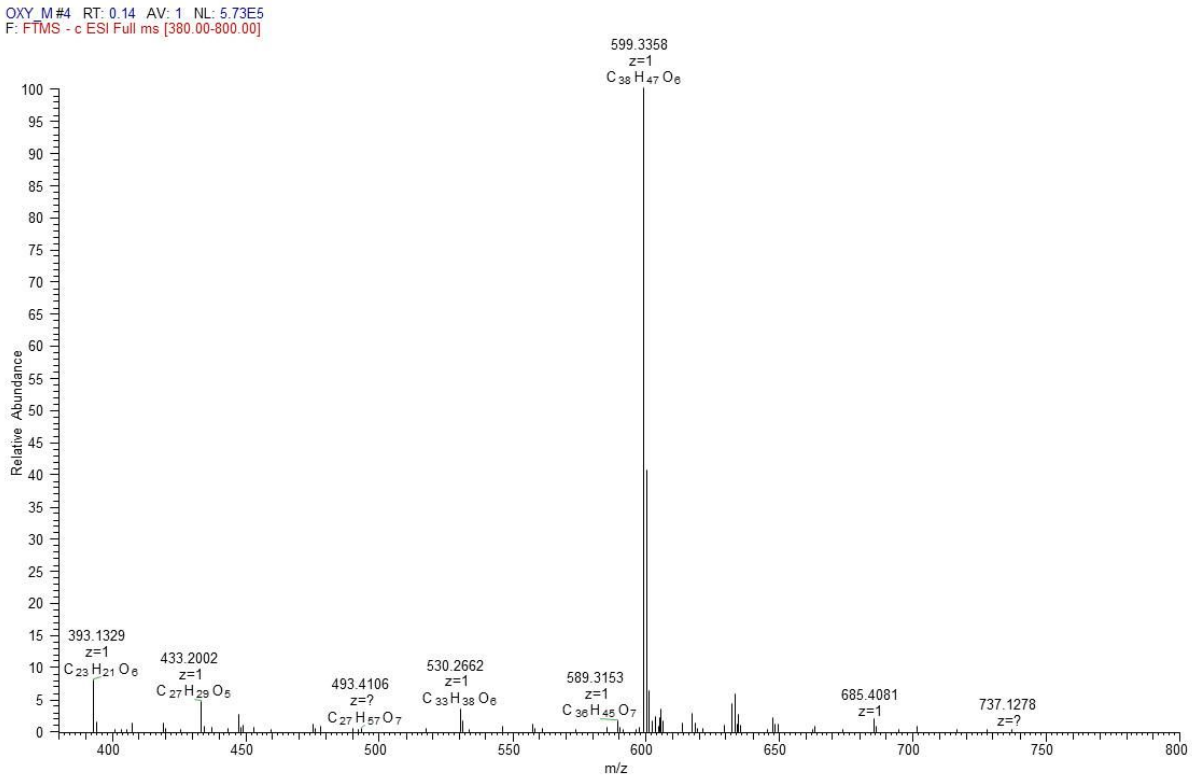

Figure S29. HRMS Spectrum of oxyguttiferone M (**7**).

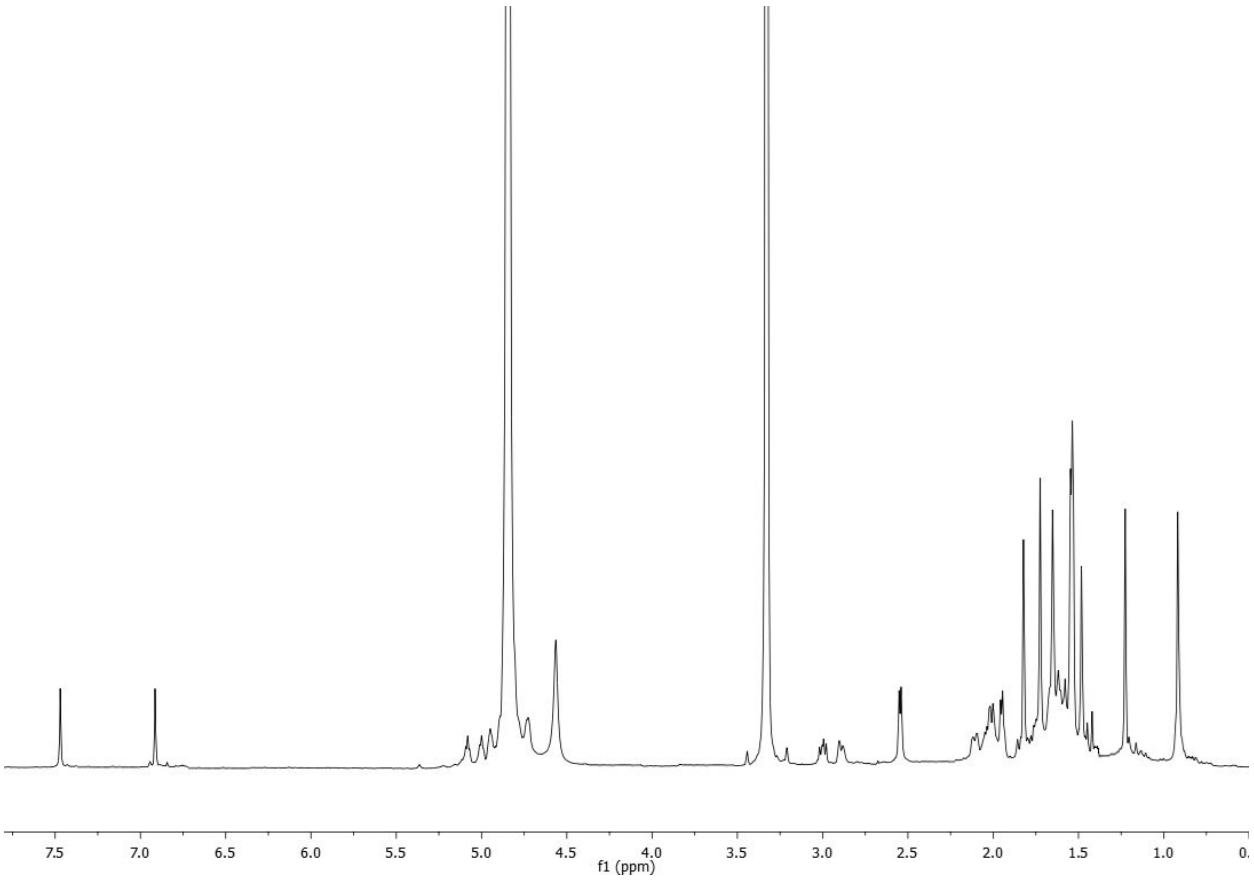

Figure S30. ^{1}H NMR Spectrum (600 MHz, CD_3OD) of oxyguttiferone M (**7**).

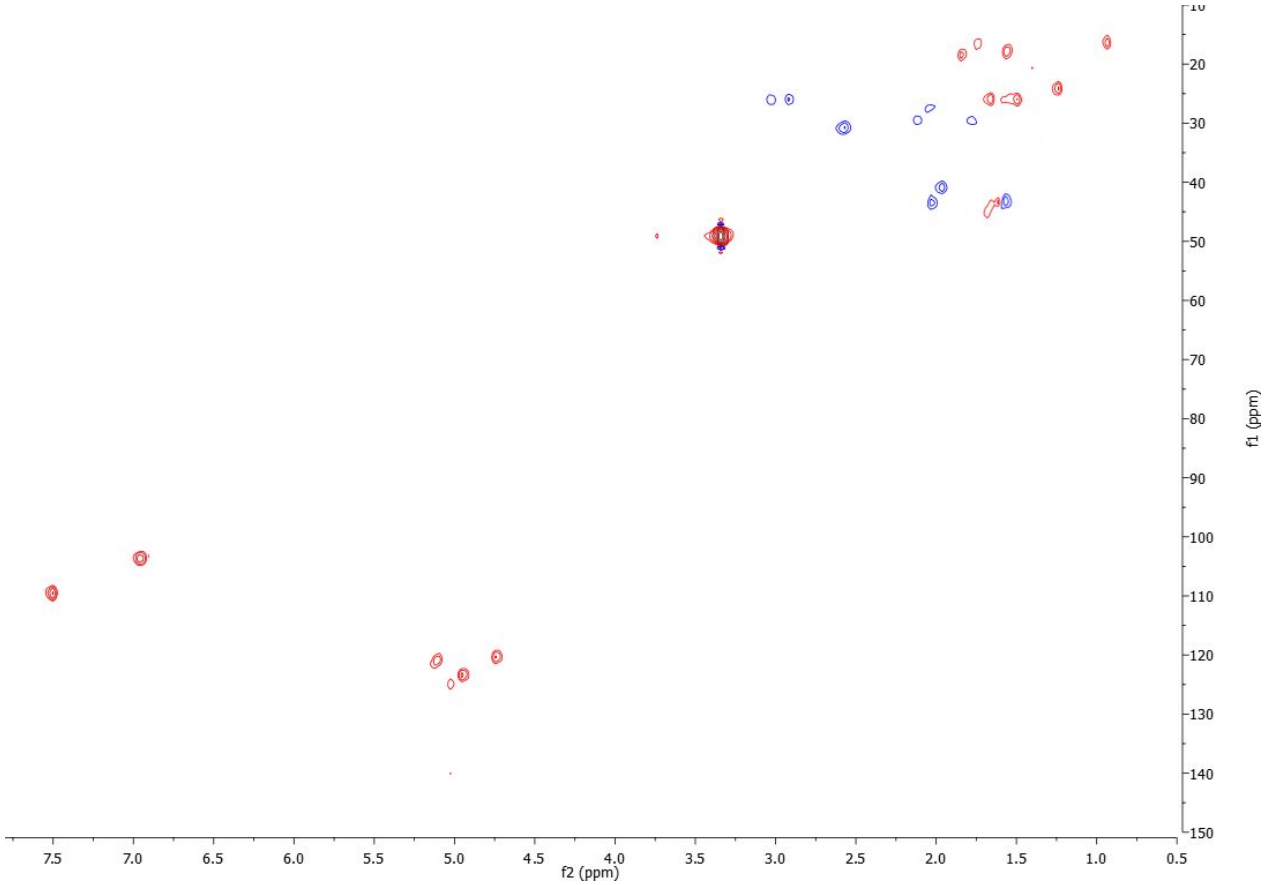

Figure S31. HSQC Spectrum of oxyguttiferone M (**7**).

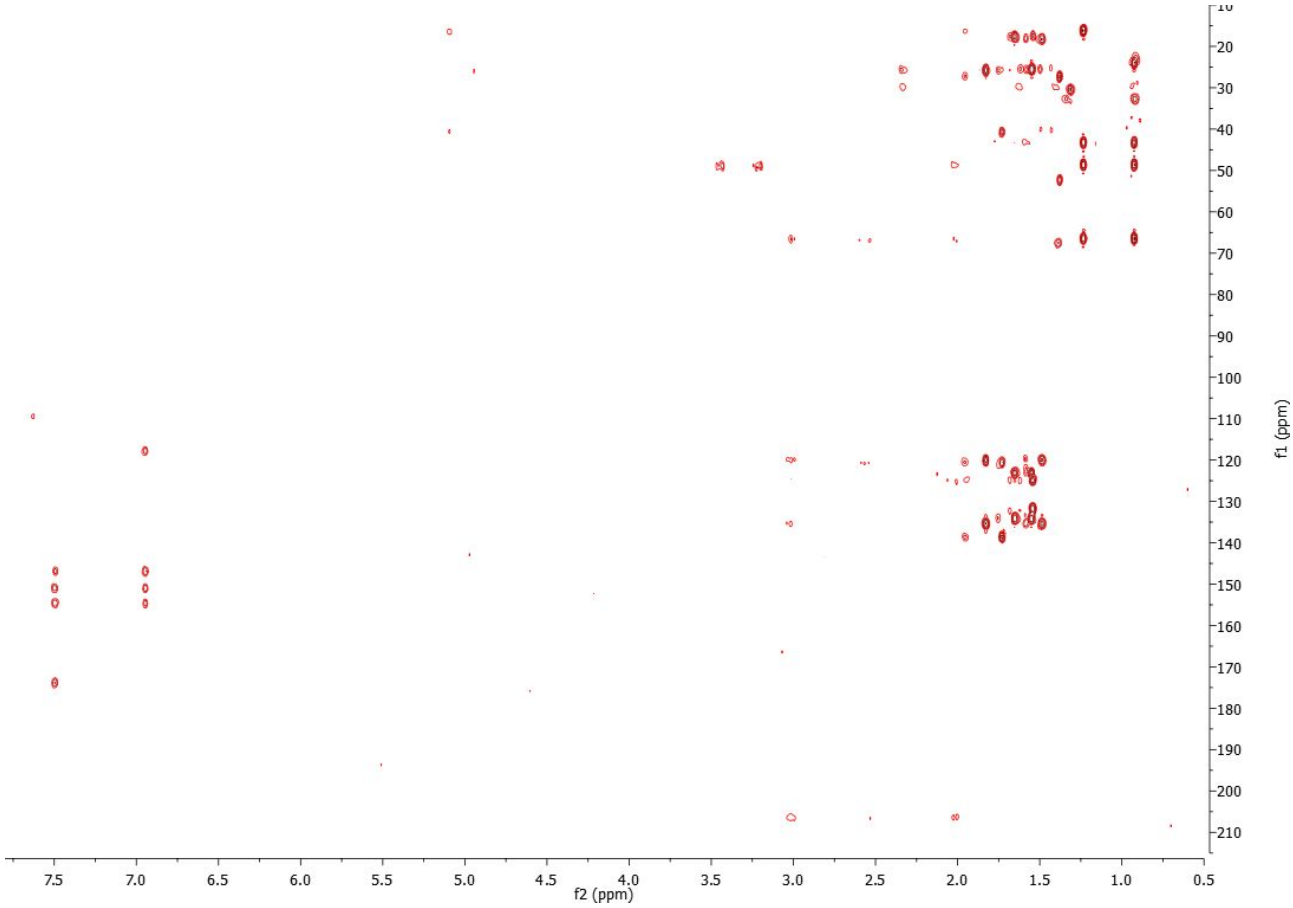

Figure S32. HMBC Spectrum of oxyguttiferone M (**7**).

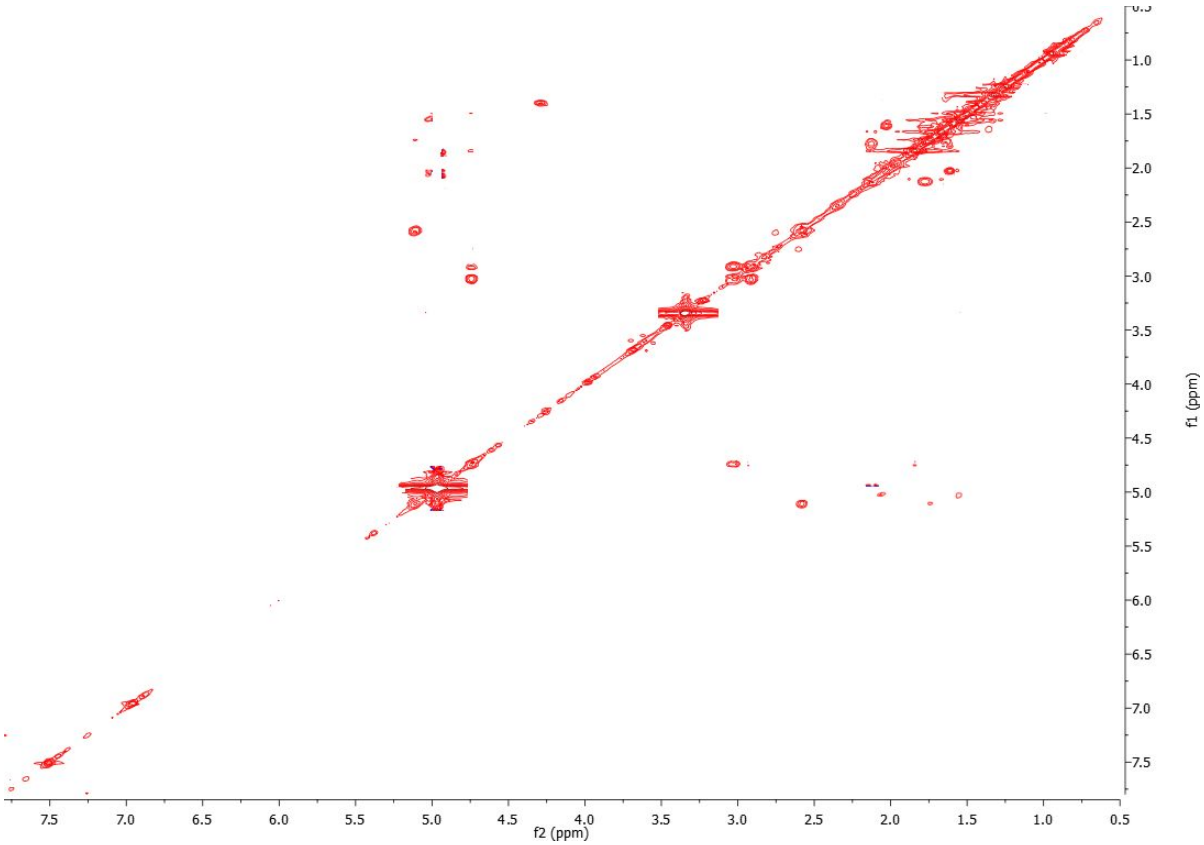

Figure S33. COSY Spectrum of oxyguttiferone M (**7**).

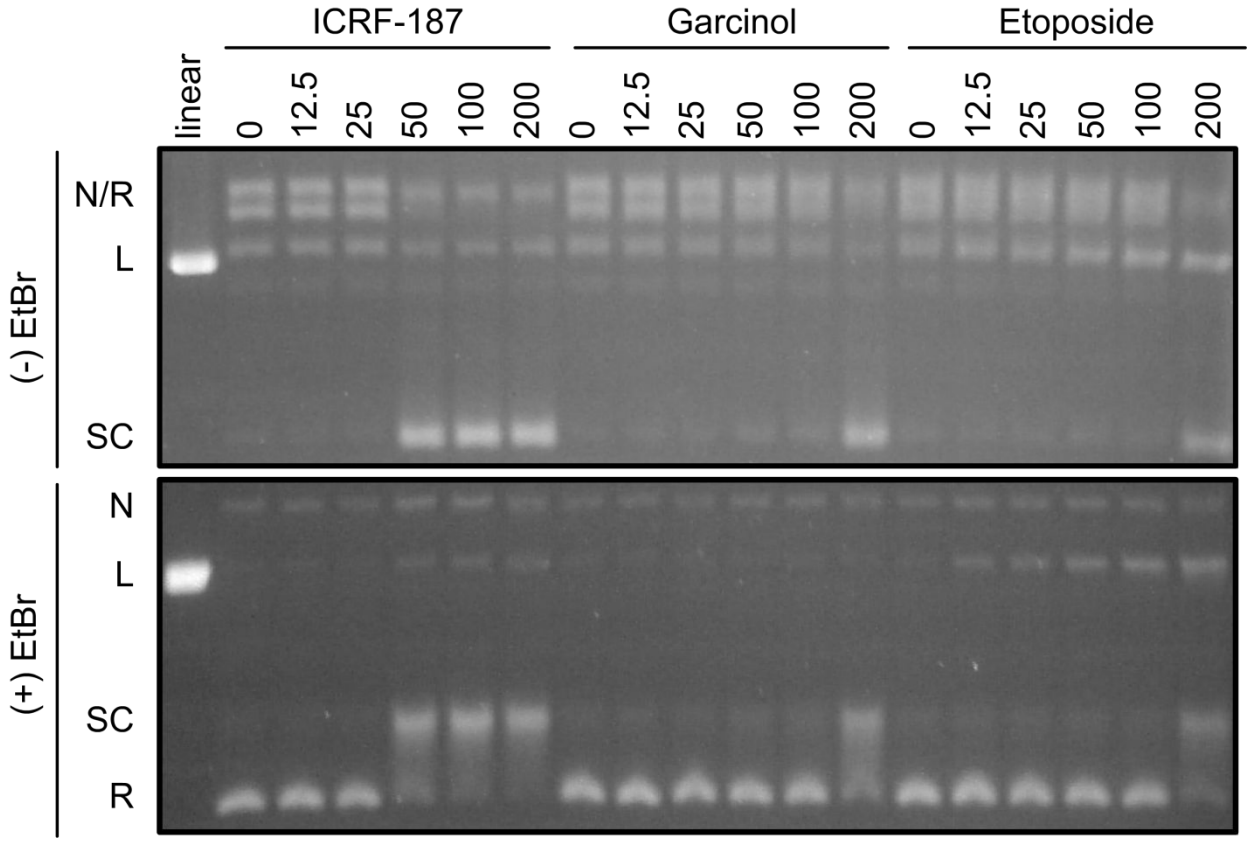

Figure S34. Garcinol inhibits DNA supercoil relaxation by topo IIα roughly four-fold more weakly than ICRF-187 (reference compound), and does not appear to stimulate linearization of DNA by topoIIα**.** Samples were run on native agarose gels with and without ethidium bromide to aid in the visualization of nicked and linear products (bottom).

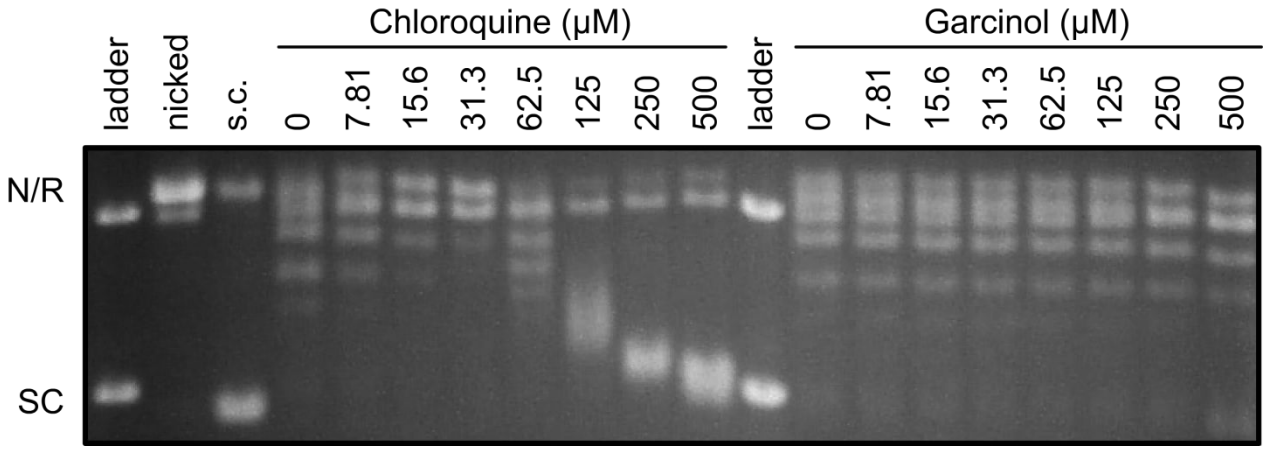

Figure S35. Garcinol does not act as a DNA intercalator. Samples of nicked plasmid DNA were treated with garcinol and chloroquine (known intercalator), then ligated overnight with T4 ligase to trap supercoiled topoisomers. Increasing concentrations of chloroquine promoted supercoiling, while no such effect was seen for Garcinol.

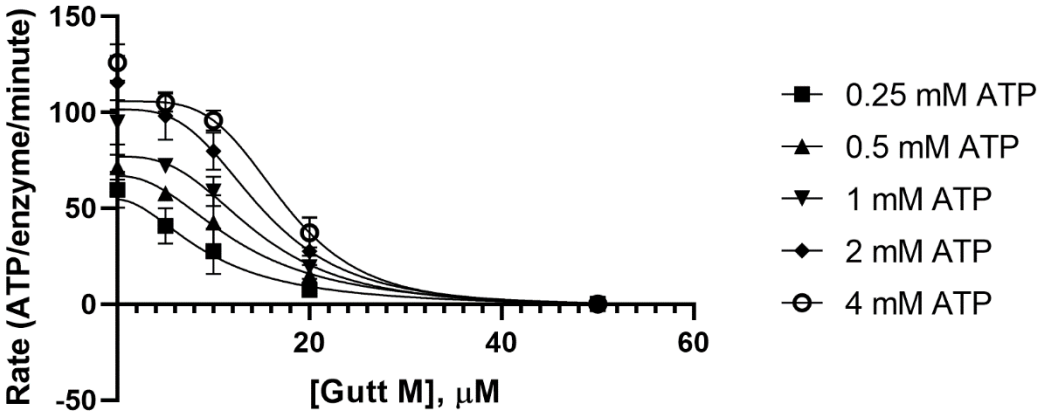

Figure S36. Inhibition of DNA-stimulated ATP hydrolysis activity of topo IIα by guttiferone M. Reaction rates plotted against drug concentrations were used to compute IC_{50} values at various concentrations of ATP (IC_{50} values are shown in Table S2).

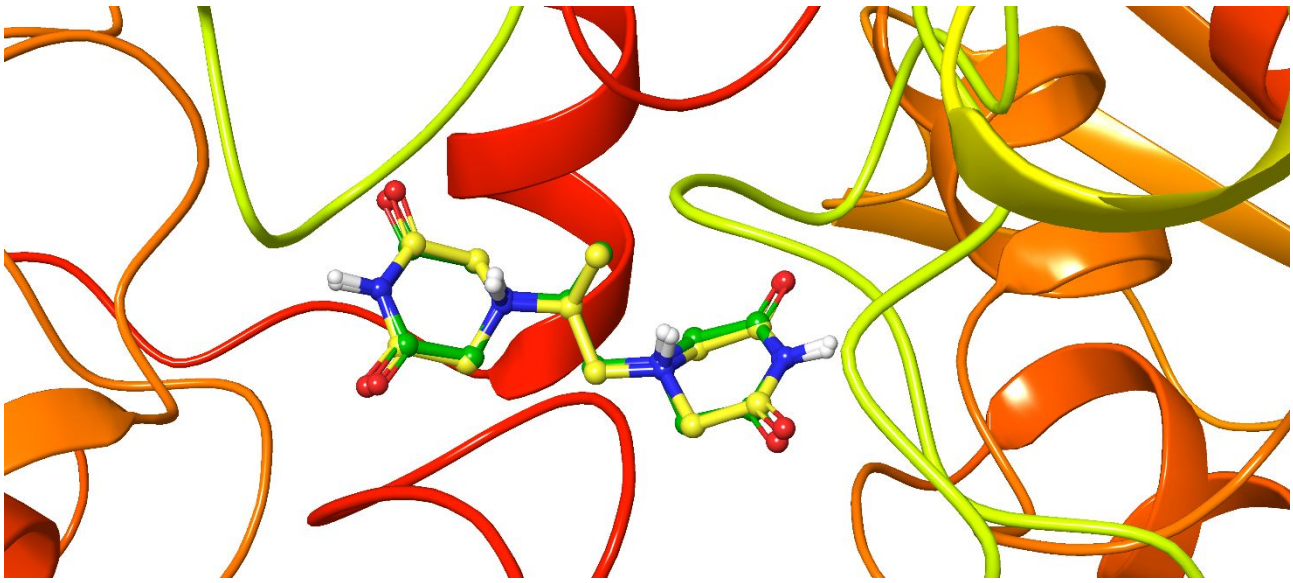

Figure S37. Superimposition (0.241 Å) of co-crystallized (green, PDB ID: 1QZR) and docked pose (yellow) structures of ICRF-187. The protein is depicted by ribbons. The ligand is represented by stick-and-ball (colored: C, as for the stick; polar H, white; N, dark-blue; O, red).

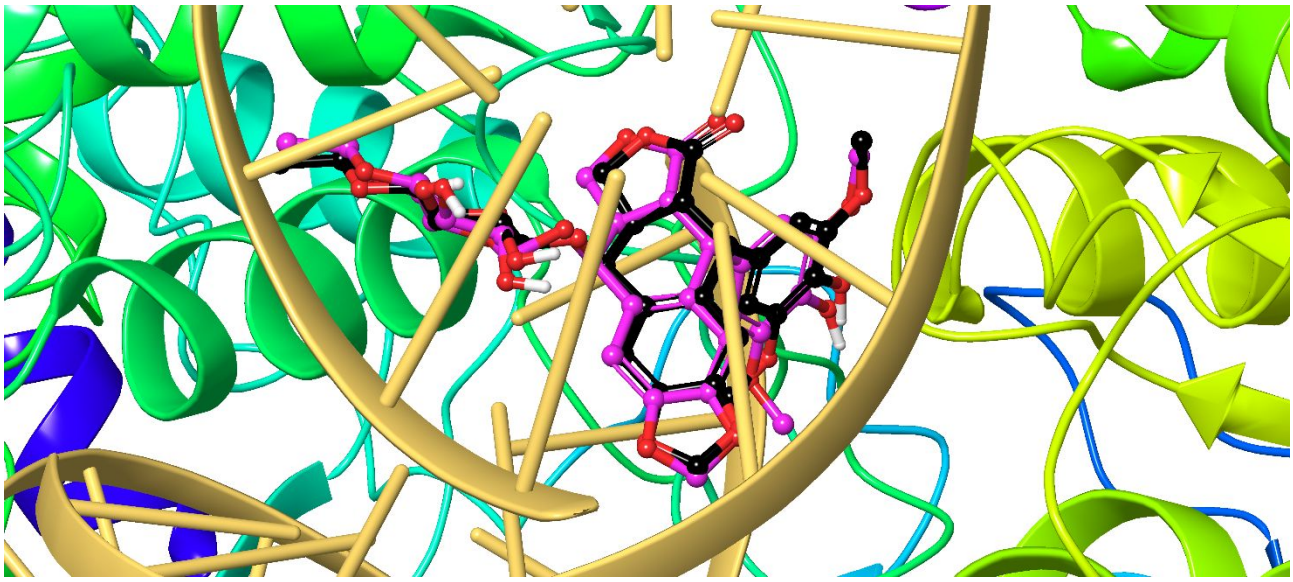

Figure S38. Superimposition (0.557 Å) of co-crystallized (pink, PDB ID: 5GWK) and docked pose (black) structures of etoposide. The protein and DNA are depicted by ribbons. The ligand is represented by stick-and-ball (colored: C, as for the stick-and-ball; polar H, white; O, red).

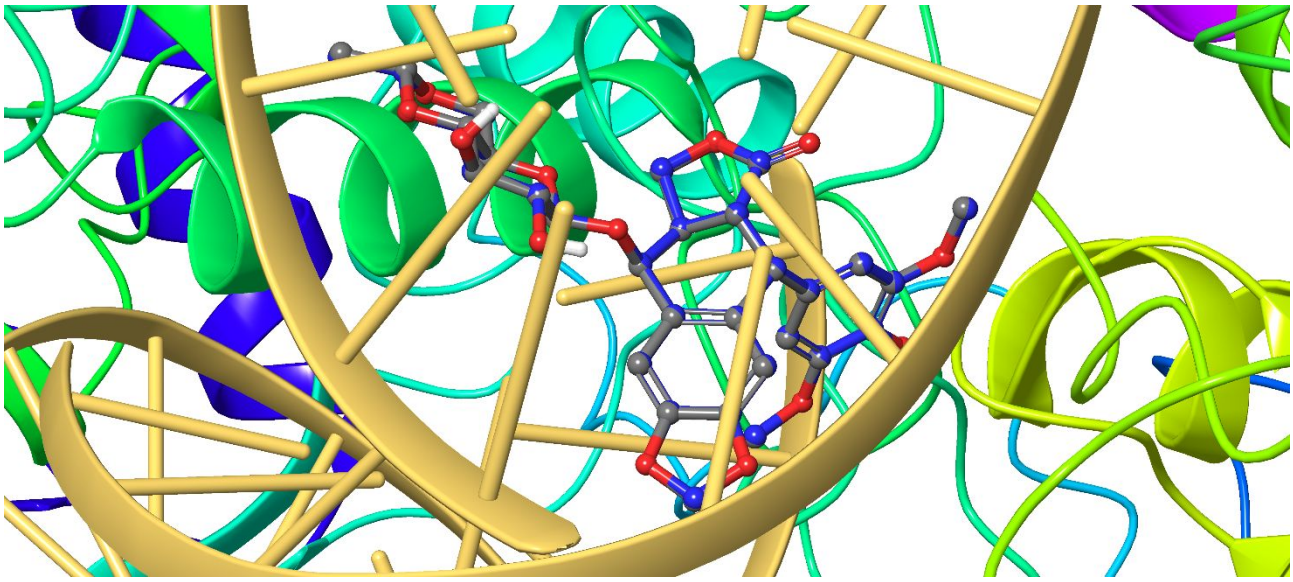

Figure S39. Superimposition (0.038 Å) of co-crystallized (blue, PDB ID: 3QX3) and docked pose (grey) structures of etoposide. The protein and DNA are depicted by ribbons. The ligand is represented by stick-and-ball (colored: C, as for the stick-and-ball; polar H, white; O, red).

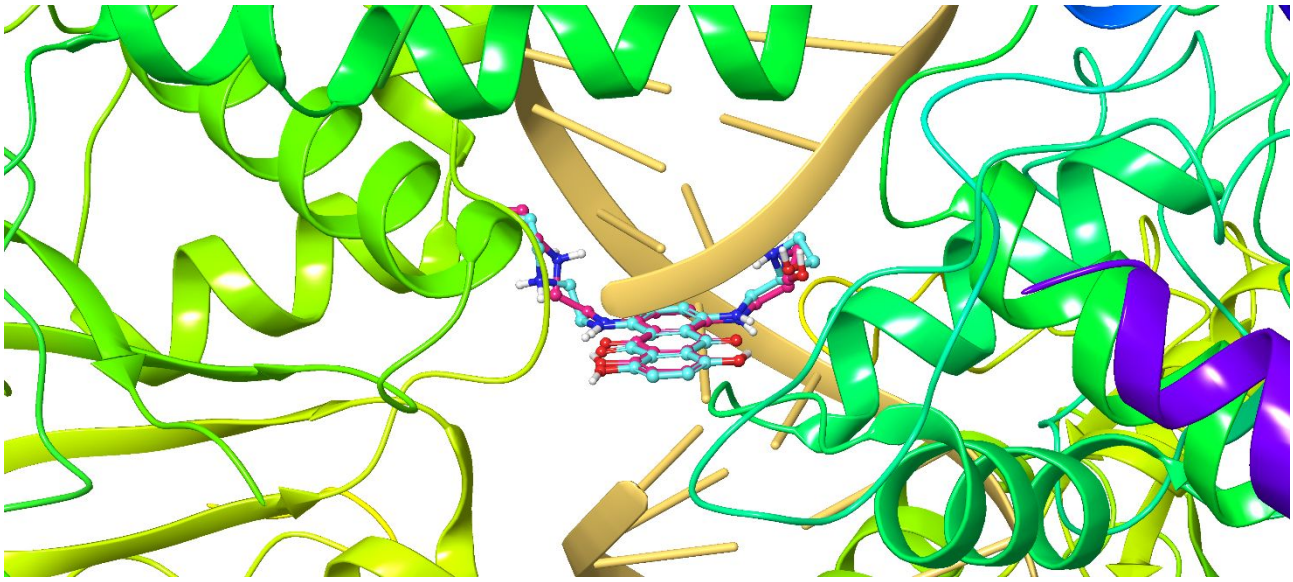

Figure S40. Superimposition (0.515 Å) of co-crystallized (pink salmon, PDB ID: 4G0V) and docked pose (cyan) structures of mitoxantrone. The protein and DNA are depicted by ribbons. The ligand is represented by stick-and-ball (colored: C, as for the stick-and-ball ; polar H, white; N, dark-blue; O, red).

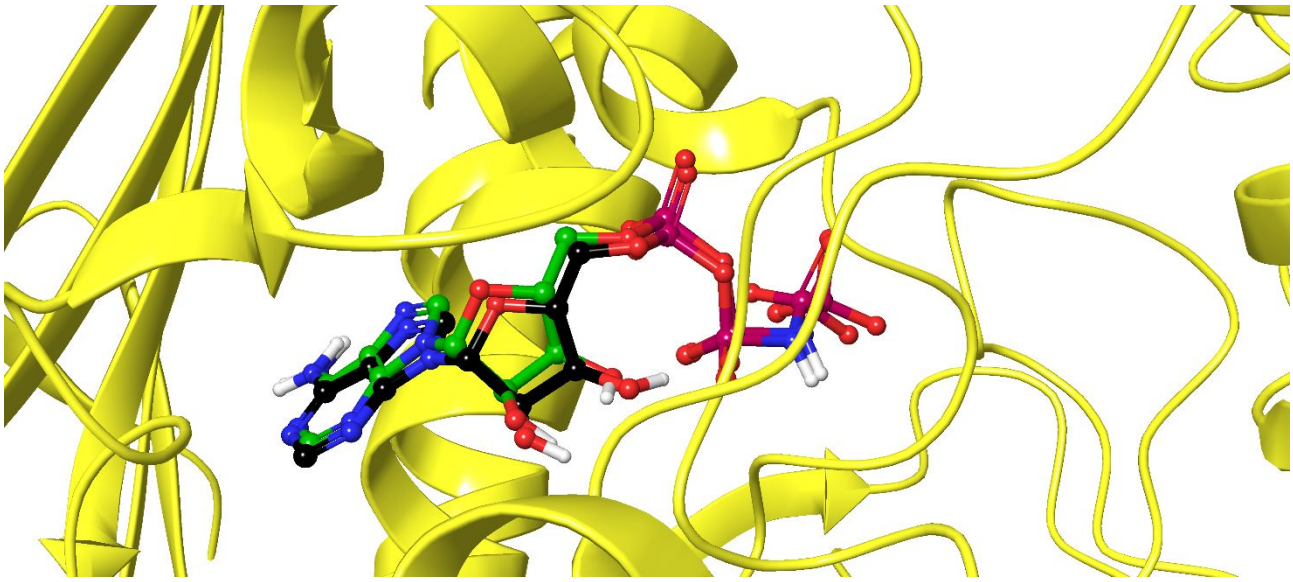

Figure S41. Superimposition (0.485 Å) of co-crystallized (black, PDB ID: 1ZXM) and docked pose (green) structures of AMPPNP. The protein is depicted by ribbons. The ligand is represented by stick-and-ball (colored: C, as for the stick-and-ball; polar H, white; N, dark-blue; O, red; P, dark-purple).

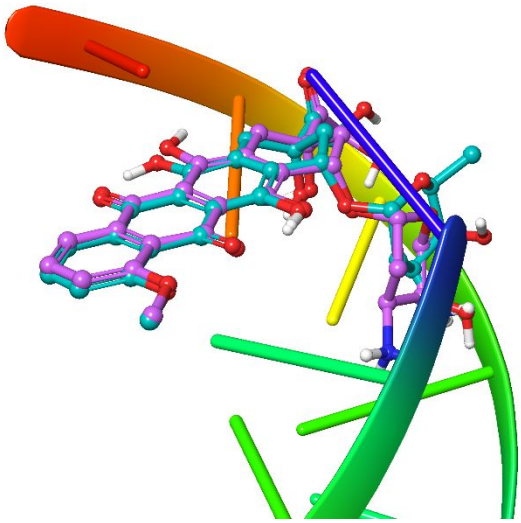

Figure S42. Superimposition (0.972 Å) of co-crystallized (aquamarine carbon, PDB ID: 1DA9) and docked pose (violet) structures of doxorubicin. The DNA is depicted by ribbons. The ligand is represented by stick-and-ball (colored: C, as for the stick-and-ball; polar H, white; O, red).

Table S1. ^{1}H and ^{13}C NMR data of garcinol (**1**) and guttiferones K (**2**), M (**3**) and I (**4**) (CD_3OD).

	Garcinol (1)		**Guttiferone K (2)**		**Guttiferone M (3)**		**Guttiferone I (4)**	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	194.2	-	193.2	-	194.0	-	195.1	-
2	116.8	-	119.6	-	119.5	-	118.4	-
3	194.9	-	192.6	-	191.2	-	195.4	-
4	70.7	-	70.2	-	69.3	-	67.9	-
5	50.4	-	50.7	-	48.0	-	49.1	-
6	47.7	1.52 m	41.7	2.00 m	44.0	1.63 m	47.6	1.54, m
7	43.3	2.30, m 2.08, m	42.0	eq. 2.06, m ax. 1.48, t (13.1)	43.1	2.06, m 1.53, t (13.1)	40.4	2.20, m 2.14, m
8	58.8	-	63.7	-	63.4	-	62.8	-
9	210.4	-	211.0	-	208.8	-	209.8	-
10	194.9	-	197.8	-	196.5	-	196.0	-
11	128.9	-	131.0	-	129.6	-	129.2	-
12	117.0	7.22, d (2.0)	116.9	7.29, d (2.0)	116.9	7.25, d (2.0)	116.9	7.24, d (2.0)
13	146.1	-	145.8	-	145.9	-	146.4	-
14	152.2	-	151.2	-	152.2	-	152.5	-
15	114.6	6.72, d (8.3)	114.6	6.70, d (8.3)	114.7	6.70, d (8.3)	114.8	6.70, d (8.3)
16	125.0	7.00, dd (8.3, 2.0)	124.5	7.09, dd (8.3, 2.0)	124.8	6.99, dd (8.3, 2.0)	125.1	7.00, dd (8.3, 2.0)
17	26.5	2.73, dd (13.3, 8.2) 2.60, m	26.8	2.68, dd (13.3, 8.2) 2.59, m	25.6	2.73, dd (13.3, 8.2) 2.65, m	26.7	2.76, dd (13.7, 8.8) 2.61, m
18	125.1	4.91, m	120.9	4.93, m	120.8	4.94, m	120.6	4.99, m
19	135.7	-	135.4	-	135.4	-	135.9	-
20	26.4	1.73, s	25.7	1.68. s	26.1	1.66, s	26.0	1.68, s
21	17.9	1.62, s	18.2	1.73, s	18.0	1.72, s	18.0	1.72, s
22	23.0	1.12, s	15.7	0.82, s	16.2	0.83, s	27.1	1.05, s
23	26.9	1.02, s	37.0	1.78, m 1.61, m	23.6	1.20, s	23.0	1.27, s
24	30.1	2.13, m 2.06, m	24.8	2.04, m 2.00, m	28.9	2.17, m 1.76, m	29.9	2.16, m 2.08, m
25	123.4	5.06, m	125.3	5.09, t (6.8)	123.4	5.04, m	125.3	4.91, m
26	134.0	-	134.0	-	134.0	-	133.7	-
27	25.9	1.69, s	26.0	1.70, s	25.7	1.71, s	26.0	1.68, s
28	18.4	1.73, s	17.4	1.62, s	17.7	1.60, s	17.9	1.51, s
29	36.9	2.02, 1.96, m	29.6	2.14 (2H), d (7.3)	30.8	2.55 (2H), d (7.3)	31.5	2.55 (2H), d (6.8)
30	44.9	2.65, m	123.7	5.04, t (6.8)	120.3	5.20, t (7.3)	120.3	5.20, t (6.8)
31	149.4	-	135.0	-	138.8	-	139.3	-
32	113.0	4.50 (2H), s	25.9	1.67, s	40.8	2.03 (2H), m	40.8	2.02 (2H), m
33	18.0	1.58, brd	17.8	1.68, s	27.3	2.09.m 2.03, m	27.4	2.08 (2H), m
34	33.4	2.04 (2H), m	31.2	2.46, m 2.52, m	125.1	5.10, m	125.1	5.10, m
35	124.0	5.06, t (6.8)	121.6	5.16, t (6.8)	132.0	-	132.1	-
36	133.0		133.7	-	25.7	1.60, s	25.9	1.61, s
37	25.9	1.69, s	25.9	1.71, s	17.5	1.57, s	17.5	1.57, s
38	17.9	1.61, s	18.0	1.69, s	16.5	1.72, s	16.5	1.73, s

Table S2. ^{1}H and ^{13}C NMR data of oxyguttiferones K (**5**), K2 (**6**) and M (**7**) (CD_3OD).

	Oxyguttiferone k (5)		**Oxyguttiferone k (6)**		**Oxyguttiferone M (7)**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	196.5	-	178.5	-	194.7	-
2	119.8	-	114.6	-	119.5	-
3	177.6	-	194.0	-	176.0	-
4	66.2	-	75.6	-	66.5	-
5	51.4	-	51.7	-	48.7	-
6	39.2	1.79, m	41.7	1.78, m	43.2	1.62 m
7	43.5	2.09, dd (13.5, 3.8) 1.57, m	42.2	2.24, m 1.78, m	43.5	2.02, m 1.52, m
8	66.2	-	58.8	-	67.1	-
9	206.4	-	207.0	-	206.5	-
10	173.9	-	174.1	-	174.1	-
11	118.0	-	118.1	-	117.7	-
12	109.5	7.50, s	109.6	7.50, s	109.5	7.51, s
13	150.9	-	151.3	-	150.9	-
14	154.8	-	154.8	-	154.6	-
15	103.9	6.88, s	103.5	6.98, s	103.6	6.96, s
16	147.0	-	146.9	-	147.0	-
17	26.6	3.04, dd (14.1, 8.8) 2.91, dd (14.1, 3.6)	26.6	2.74, dd (13.7, 8.8) 2.57, m	25.8	3.02, dd (13.9, 9.0) 2.91, dd (13.9, 4.6)
18	120.6	4.73, dd (8.8, 3.6)	121.6	4.70, m	120.2	4.74, m
19	135.2	-	134.0	-	135.3	-
20	26.0	1.53, s	25.9	1.58, s	25.8	1.50, s
21	18.5	1.87, s	18.1	1.75, s	18.3	1.84, s
22	17.2	0.95, s	15.4	0.85, s	16.2	0.93, s
23	37.2	1.99, m 1.62, m	36.6	1.68 (2H), m	23.9	1.24, s
24	30.2	2.03, m 1.81, m	24.8	1.93 (2H), m	29.4	2.12, m 1.77, m
25	122.6	4.96, t (6.8)	125.2	5.07, m	123.2	4.95, m
26	134.7	-	132.4	-	134.2	-
27	25.7	1.69, s	25.9	1.69, s	25.8	1.66, s
28	18.0	1.59, s	17.6	1.62, s	17.6	1.56, s
29	31.1	2.57 (2H), d (7.3)	29.3	2.10, m 1.88, m	30.5	2.57 (2H), d (6.8)
30	120.4	5.01, t (6.8)	123.1	4.93, m	120.7	5.10, m
31	135.1	-	134.8	-	138.6	-
32	25.9	1.64, s	25.8	1.64, s	40.8	1.96 (2H), m
33	17.9	1.73, s	17.9	1.58, s	27.4	2.04. (2H), m
34	29.8	1.81, m 1.39, m	31.1	2.82 (2H), d (6.8)	124.9	5.02, m
35	123.7	4.96, t (6.8)	119.8	4.97, m	131.8	-
36	133.7	-	135.5	-	25.6	1.55, s
37	25.9	1.63, s	25.9	1.58, s	17.6	1.63, s
38	17.5	1.24, s	18.2	1.83, s	16.2	1.74, s

Table S3. IC_{50} values for inhibition of supercoil relaxation activity by compounds **1-7** (measured from Figure 2, fits performed with PRISM - Graphpad).

compound	IC_{50} (µM)
garcinol (1)	101.2
guttiferone K (2)	107.5
guttiferone M (3)	73.0
guttiferone I (4)	not detectable
oxyguttiferone K (5)	109.0
oxyguttiferone K2 (6)	241.2
oxyguttiferone M (7)	105.5

Table S4. IC_{50} values for inhibition of ATP hydrolysis activity at different concentrations of guttiferone M (fits performed with PRISM - Graphpad).

[ATP], mM	IC_{50}, mM	Error, mM
0.25	9.6	± 2.6
0.5	12.7	± 2.7
1	14.6	± 2.2
2	15.1	± 3.8
4	17.4	± 1.6

Error values are representative of triplicate experiments.