Supplementary Information
for
Antimicrobial bianthrones from the crinoid *Heterometra* sp.

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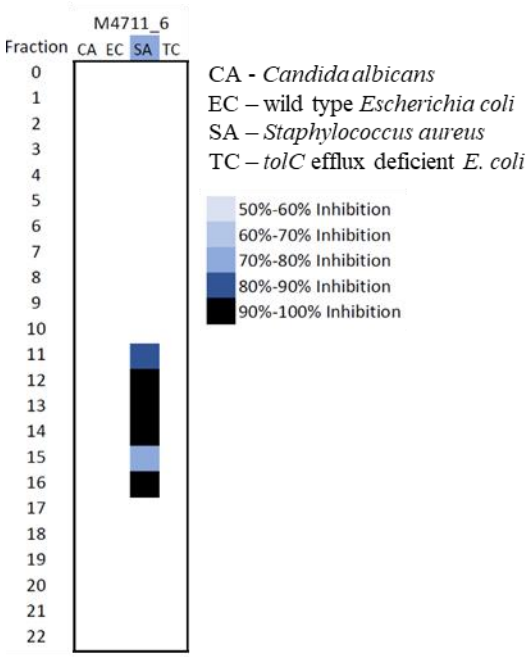

Figure S1. Screening of subfractions generatedfor the an antimicrobial campaing between NIAID and NCI.[1]

1. L. Martinez-Fructuoso, S. J. R. Arends, V. F. Freire, J. R. Evans, S. DeVries, B. D. Peyser, R. K. Akee, C. C. Thornburg, R. Kumar, S. Ensel, G. M. Morgan, G. D. McConachie, N. Veeder, L. R. Duncan, T. Grkovic and B. R. O'Keefe, *ACS Infect. Dis.*, 2023, **9**, 1245-1256.

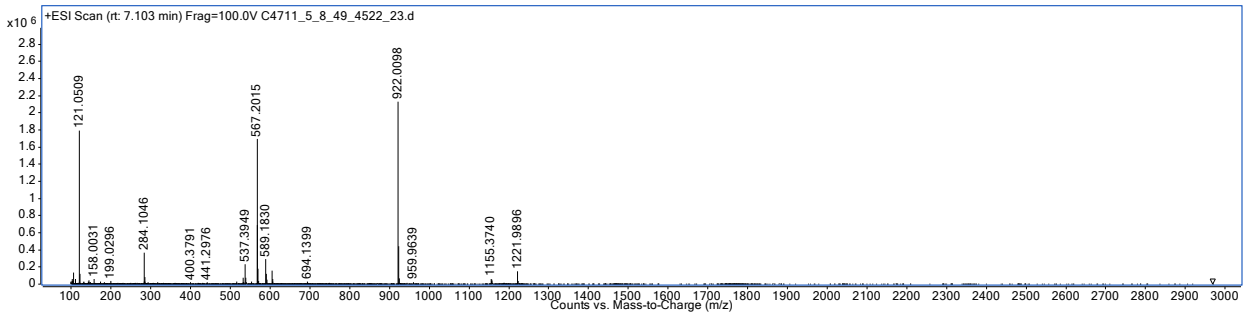

Figure S2. HRESIMS spectrum of crinemodin bianthrone (**1**).

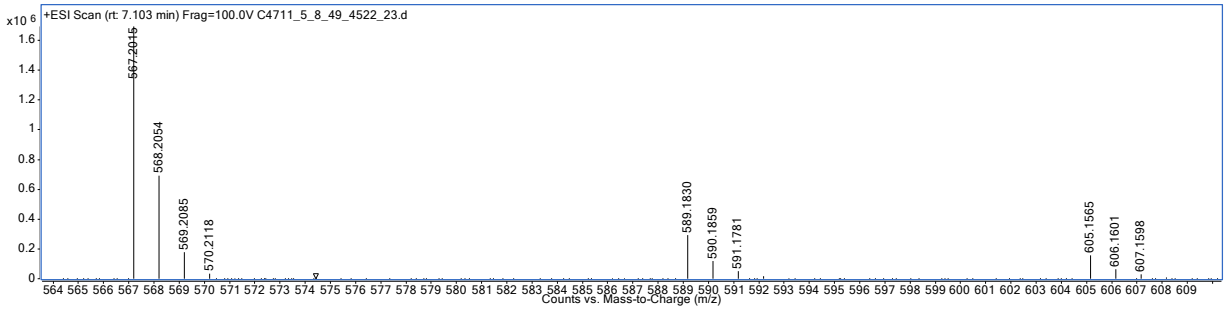

Figure S3. Expansion of HRESIMS spectrum of crinemodin bianthrone (**1**).

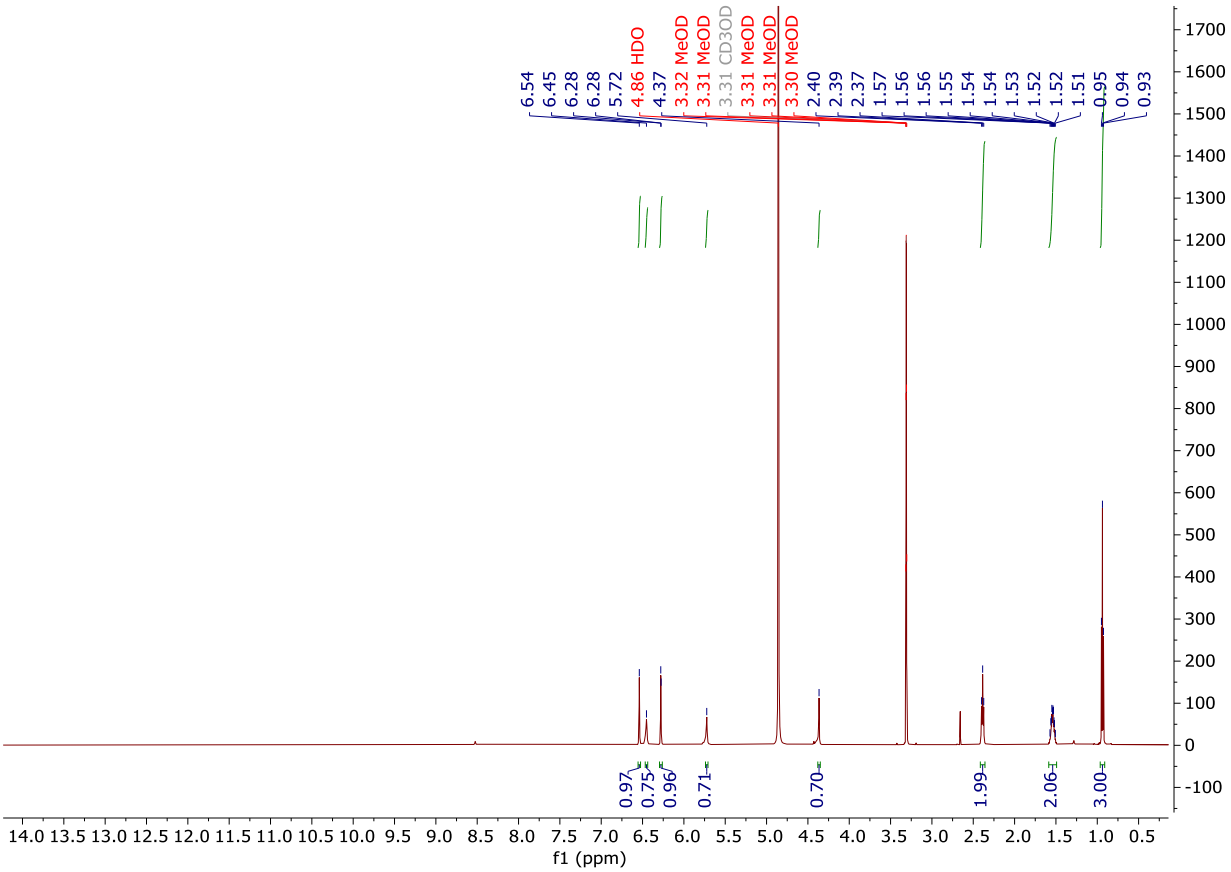

Figure S4. 600 MHz ^{1}H NMR spectrum of crinemodin bianthrone (**1**) in MeOH-d_4. The spectrum was acquired over 4 scans using the standard zg30 Bruker pulse sequence.

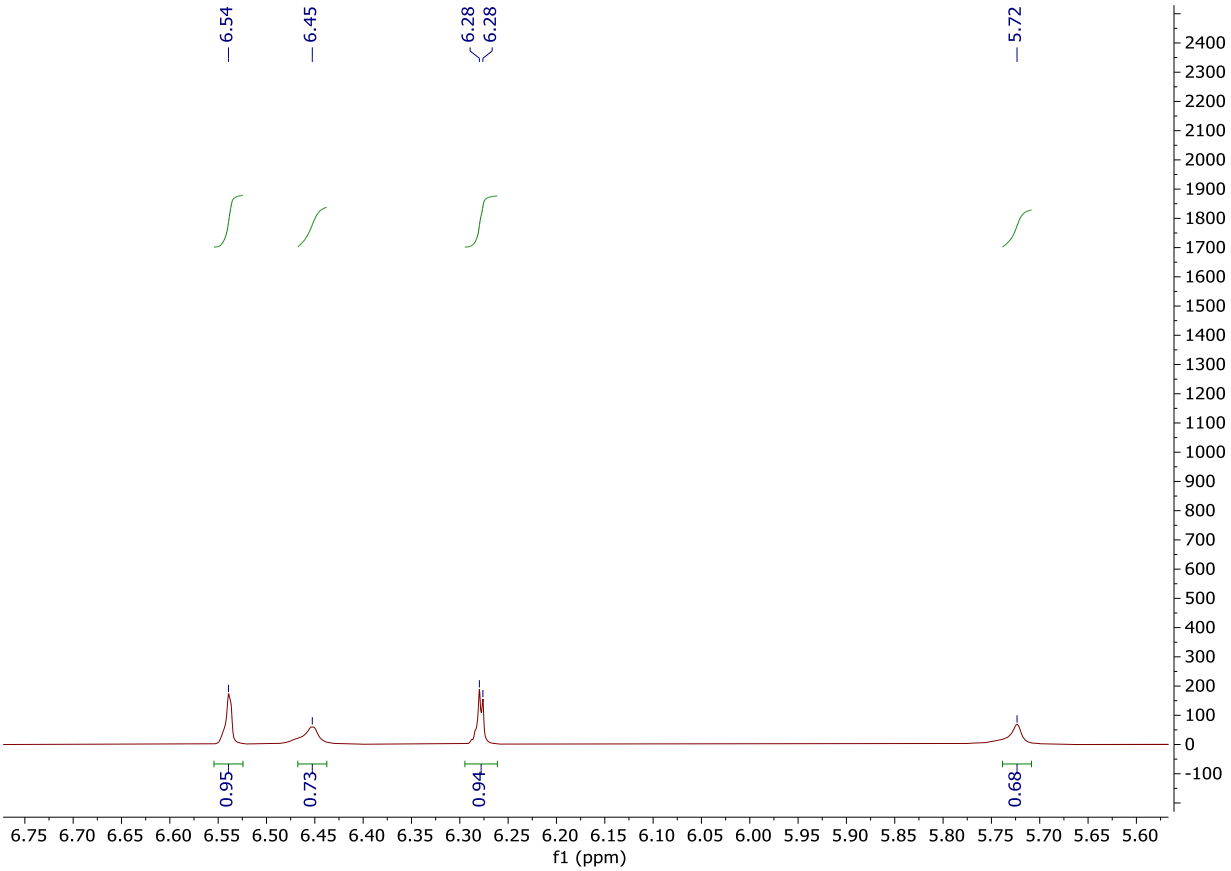

Figure S5. Expansion of 600 MHz ^{1}H NMR spectrum of crinemodin bianthrone (**1**) in MeOH-d_4. The spectrum was acquired over 4 scans using the standard zg30 Bruker pulse sequence.

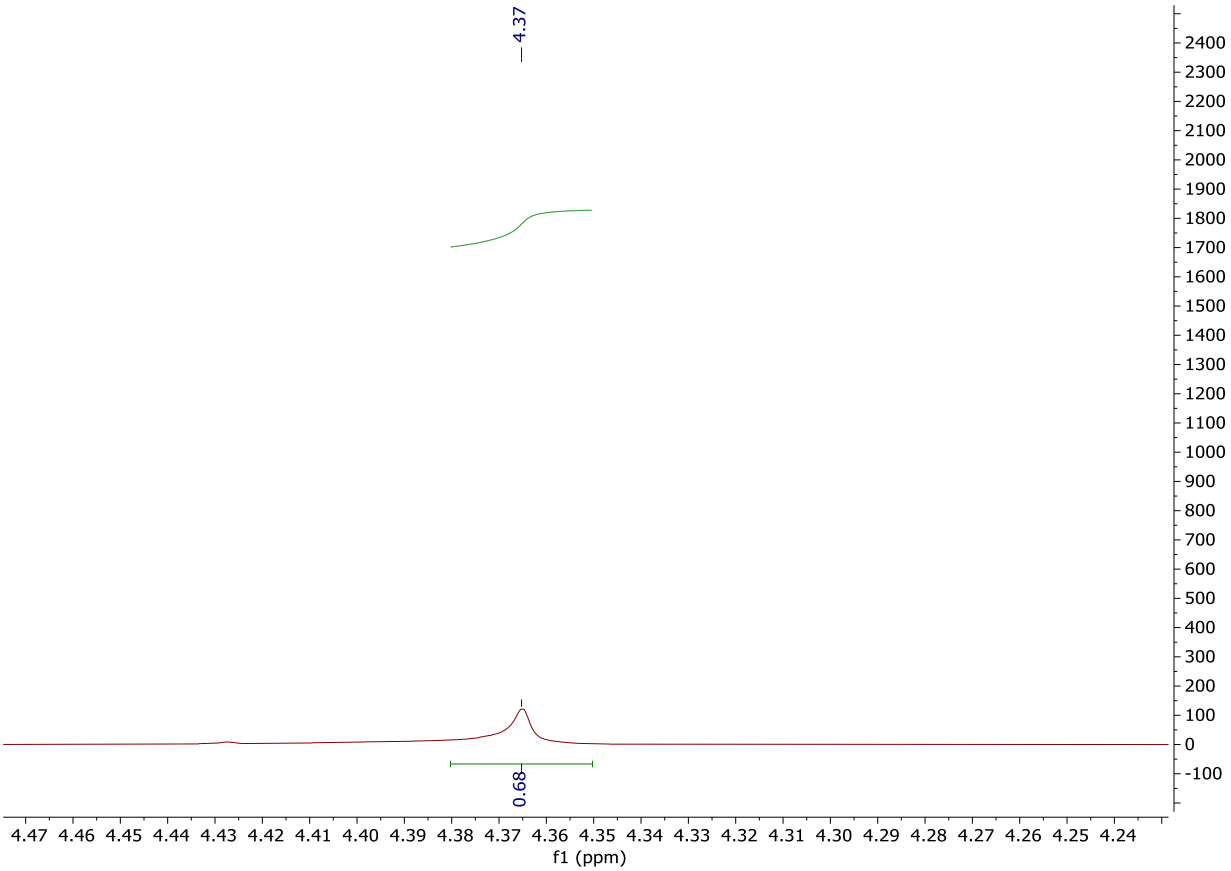

Figure S6. Expansion of 600 MHz ^{1}H NMR spectrum of crinemodin bianthrone (**1**) in MeOH-d_4. The spectrum was acquired over 4 scans using the standard zg30 Bruker pulse sequence.

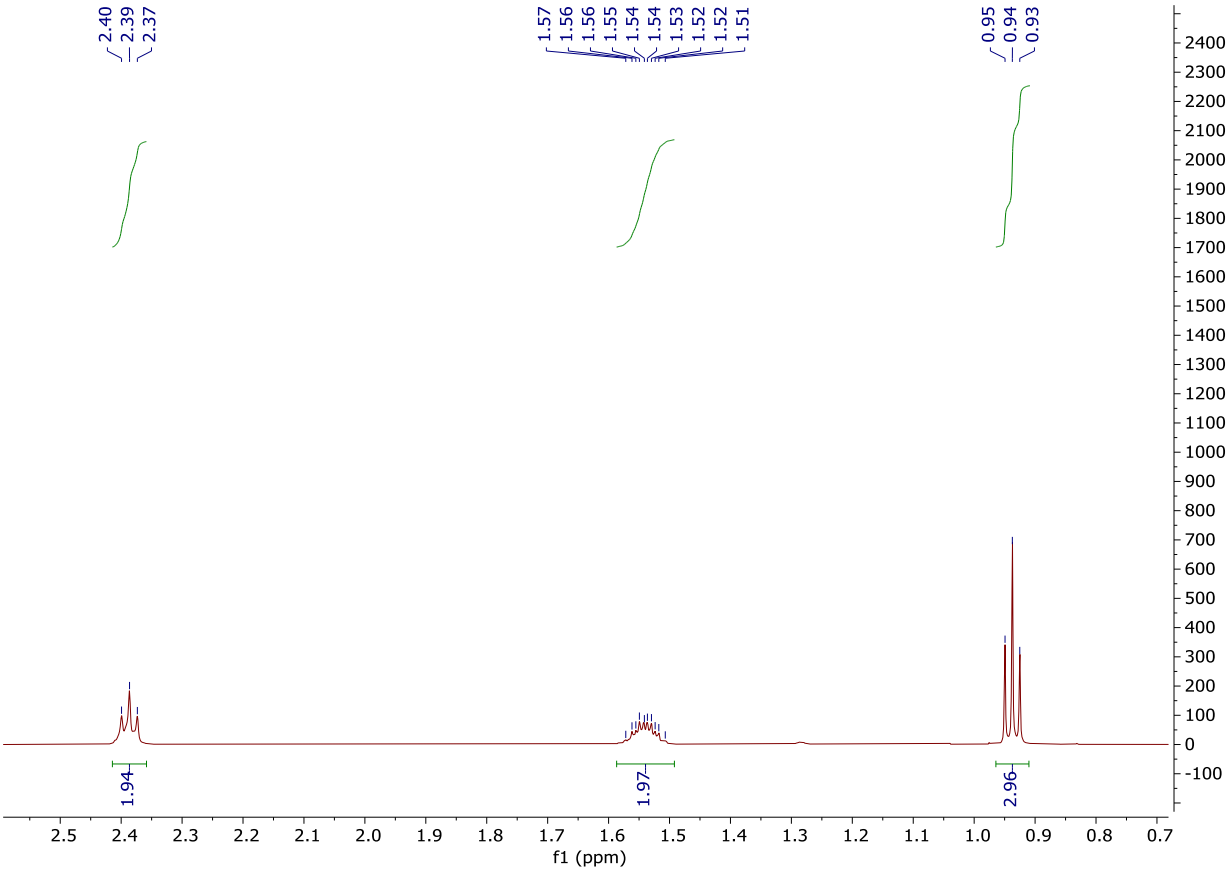

Figure S7. Expansion of 600 MHz ^{1}H NMR spectrum of crinemodin bianthrone (**1**) in MeOH-d_4. The spectrum was acquired over 4 scans using the standard zg30 Bruker pulse sequence.

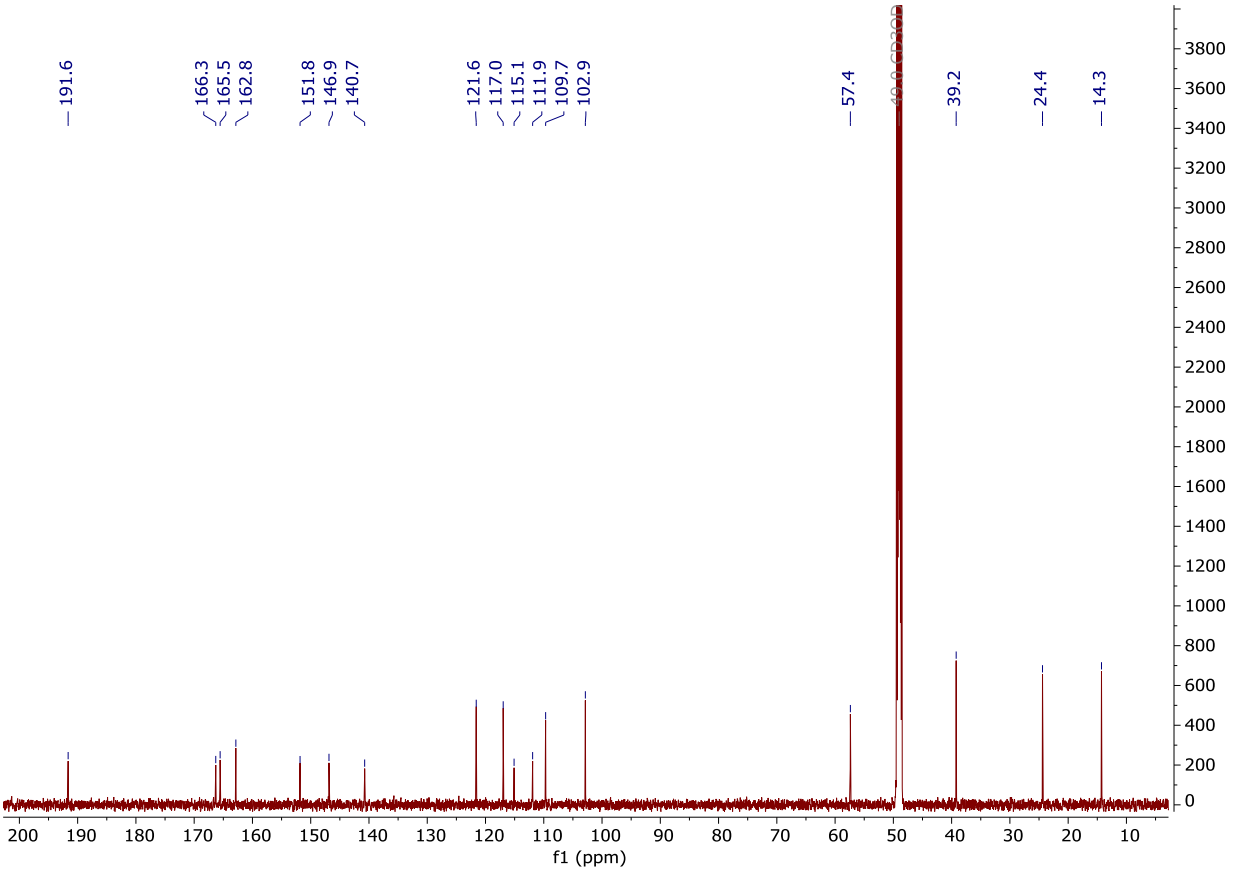

Figure S8. 151 MHz ^{13}C NMR spectrum of crinemodin bianthrone (**1**) in MeOH-*d*$_4$. The spectrum was acquired over 10000 scans using the standard zgpg30 Bruker pulse sequence.

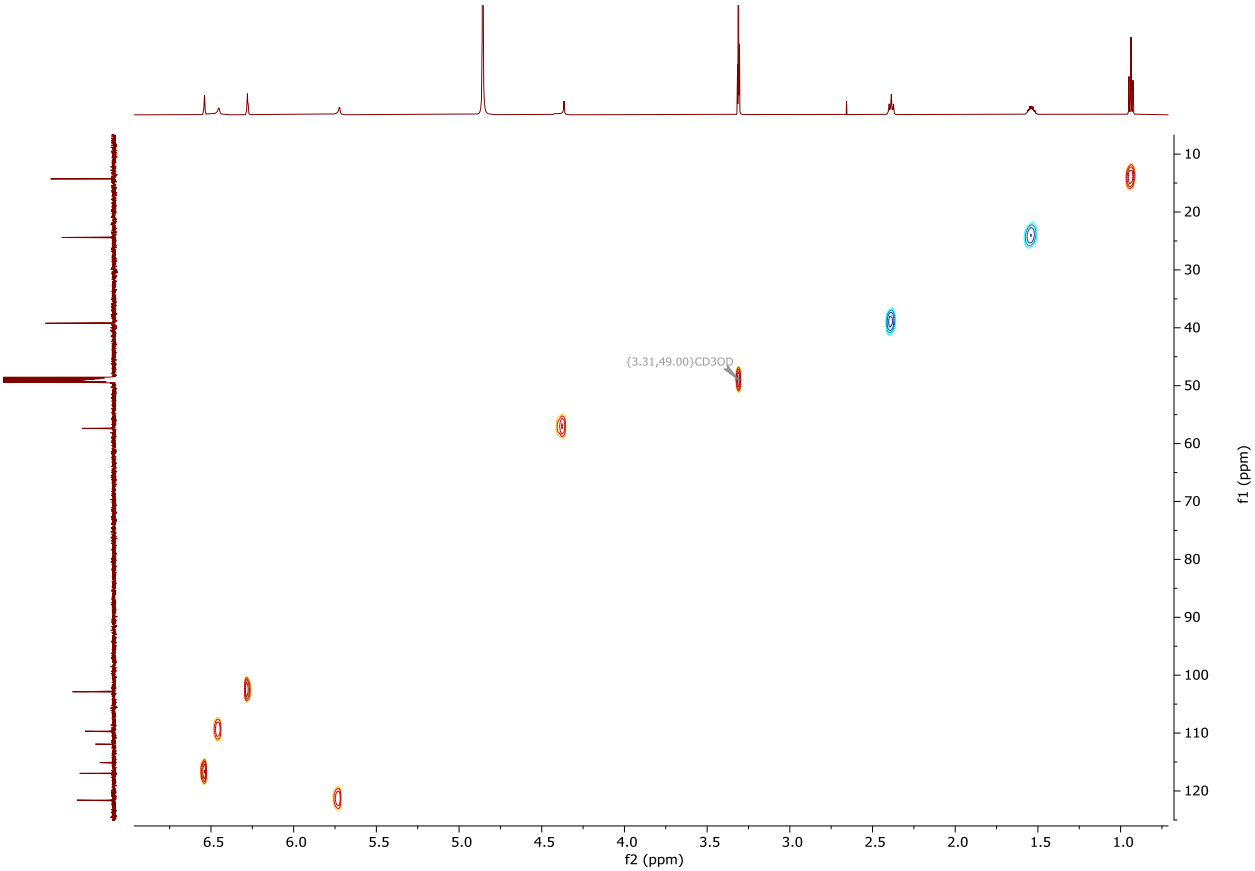

Figure S9. ^{1}H–^{13}C HSQC NMR spectrum of crinemodin bianthrone (**1**) in MeOH-d_4. The spectrum was acquired over 80 scans at 1024 t1 increments using the standard hsqcedetgpsisp2.3 Bruker pulse sequence.

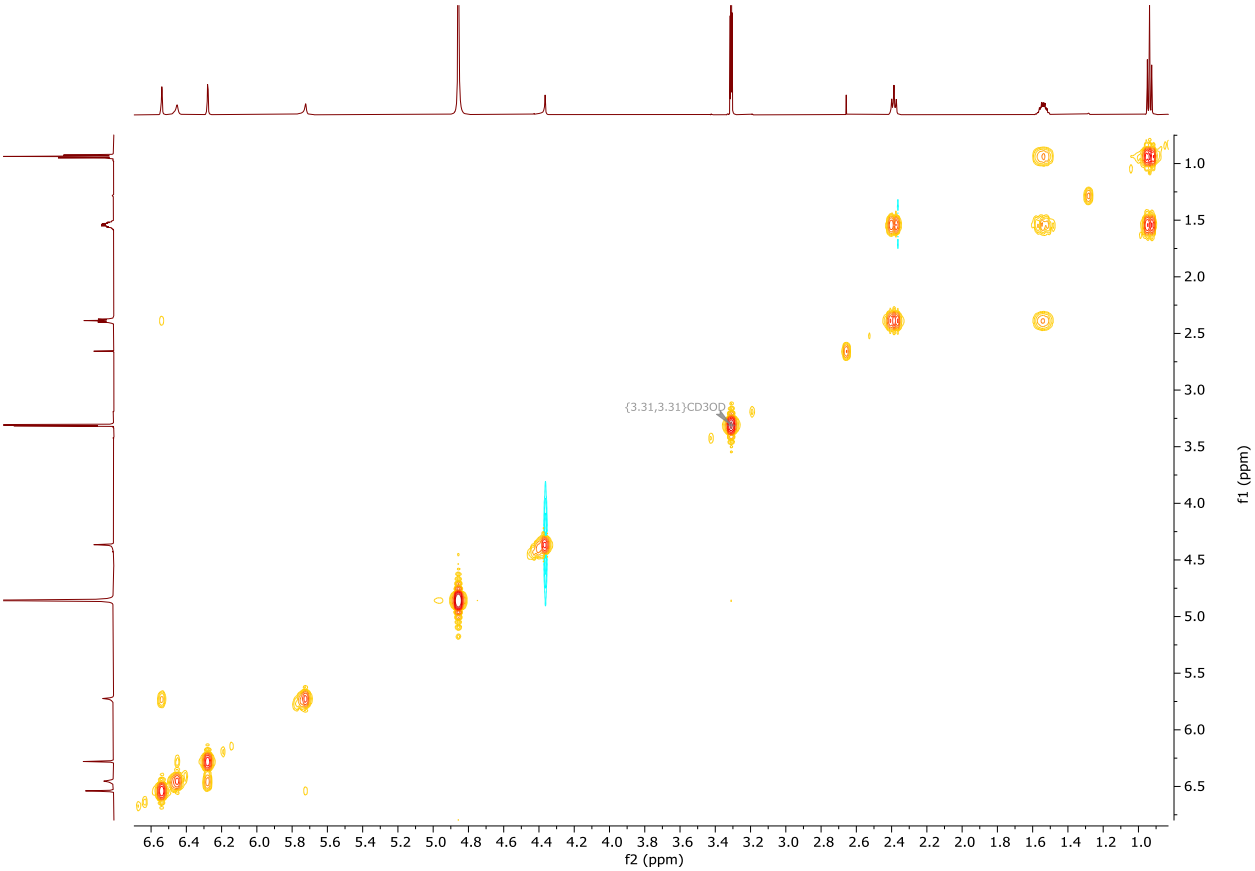

Figure S10. ^{1}H–^{1}H COSY NMR spectrum of crinemodin bianthrone (**1**) in MeOH-d_4. The spectrum was acquired over 8 scans at 1024 t1 increments using the standard cosygpppqf Bruker pulse sequence.

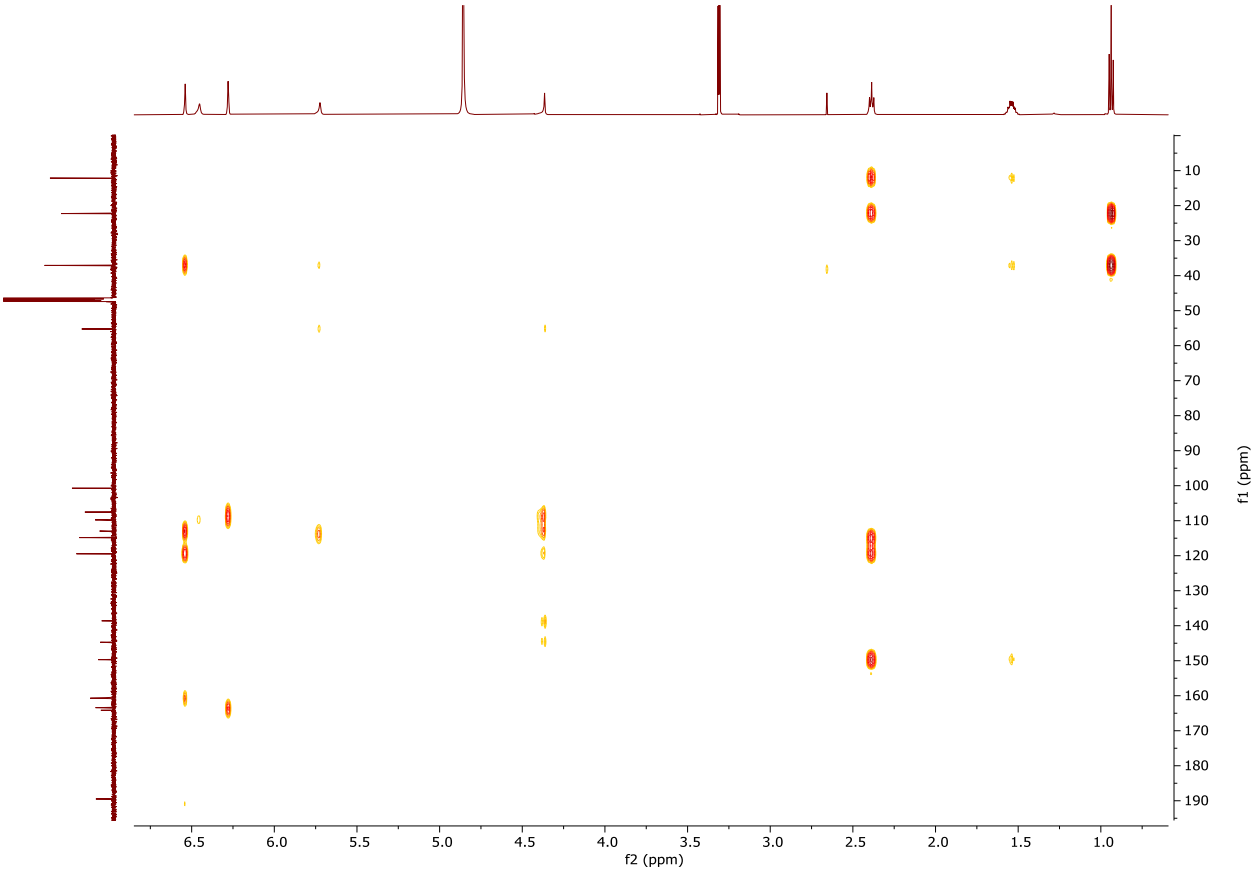

Figure S11. $^{1}H–^{13}C$ HMBC NMR spectrum of crinemodin bianthrone (**1**) in MeOH-d_4. The spectrum was acquired over 296 scans at 2048 t1 increments using the standard hmbcetgpl3nd Bruker pulse sequence.

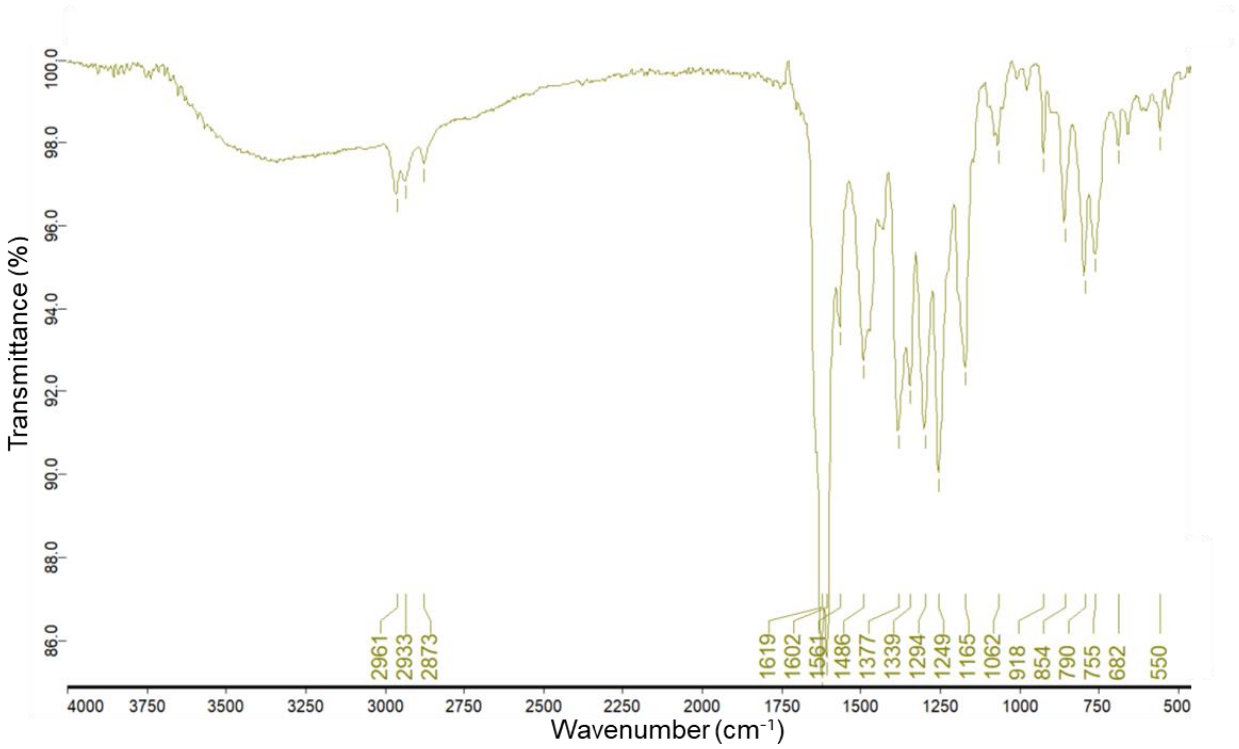

Figure S12. FTIR spectrum of crinemodin bianthrone (**1**).

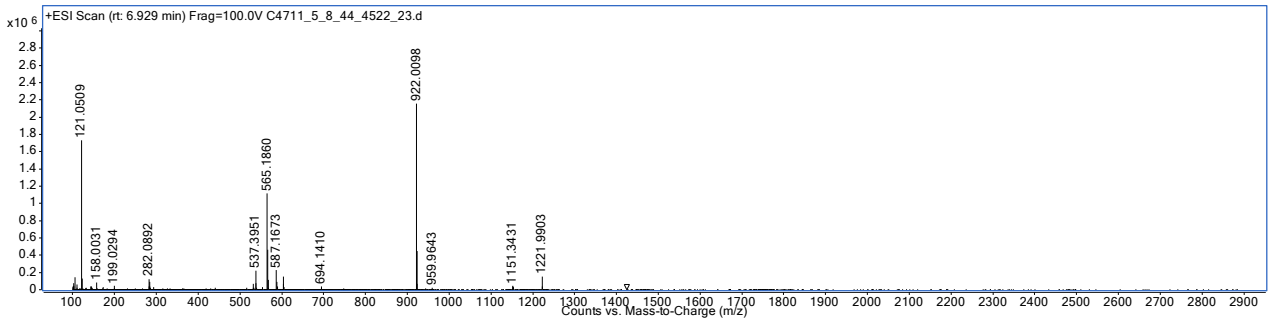

Figure S13. HRESIMS spectrum of 1"–dehydrocrinemodin bianthrone (**2**).

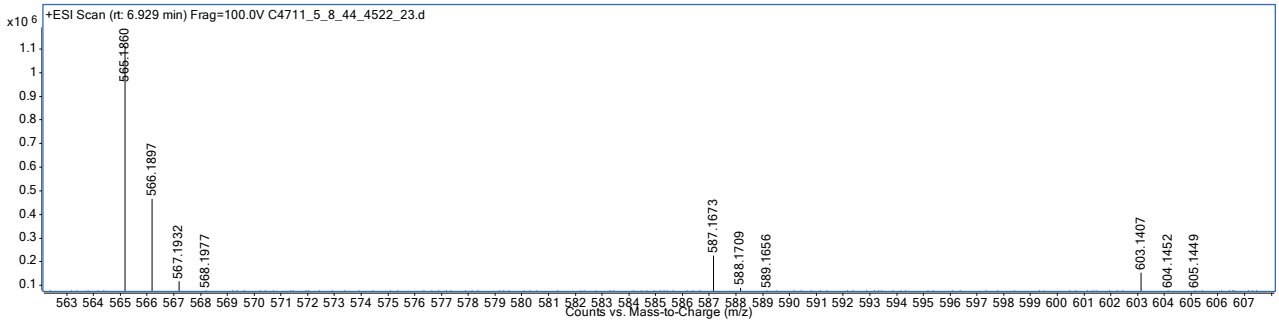

Figure S14. Expansion of HRESIMS spectrum of 1"–dehydrocrinemodin bianthrone (**2**).

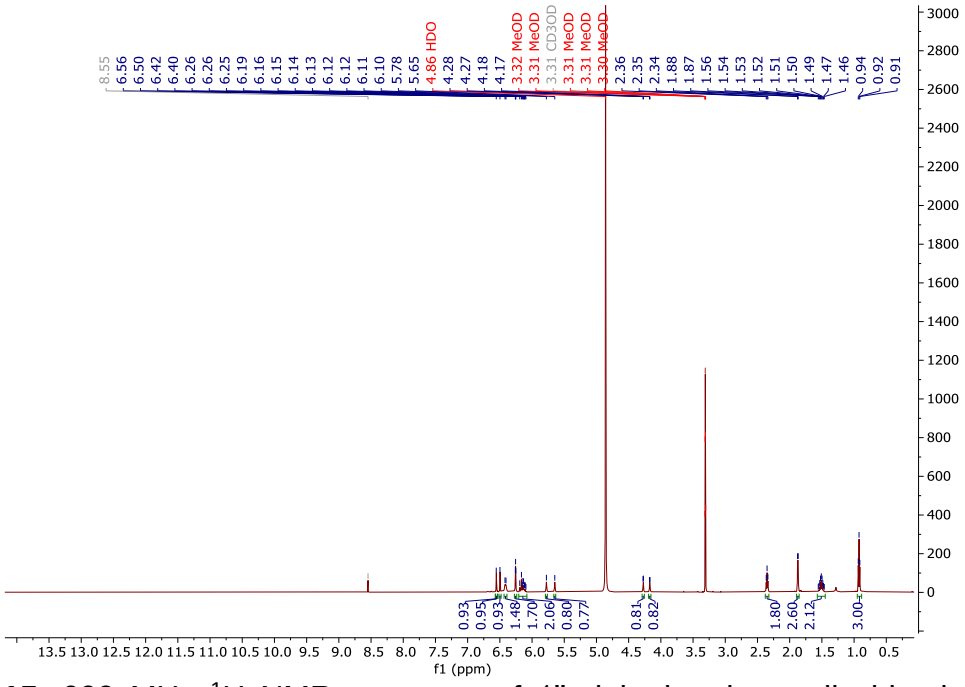

Figure S15. 600 MHz ^{1}H NMR spectrum of 1"–dehydrocrinemodin bianthrone (**2**) in MeOH-d_4. The spectrum was acquired over 4 scans using the standard zg30 Bruker pulse sequence.

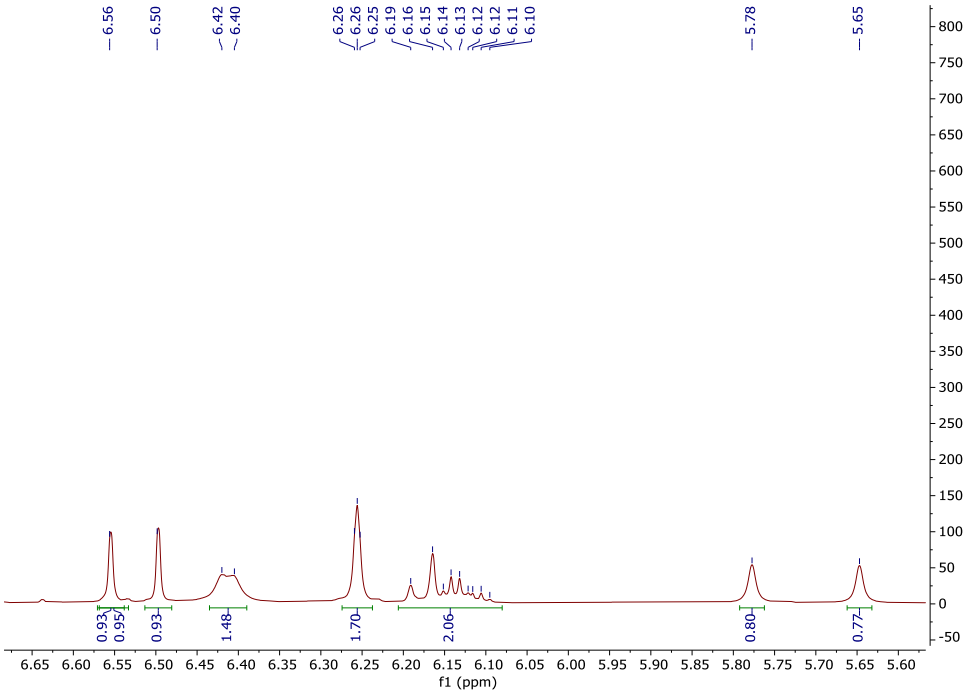

Figure S16. Expansion of 600 MHz ^{1}H NMR spectrum of 1"–dehydrocrinemodin bianthrone (**2**) in MeOH-*d*$_4$. The spectrum was acquired over 4 scans using the standard zg30 Bruker pulse sequence.

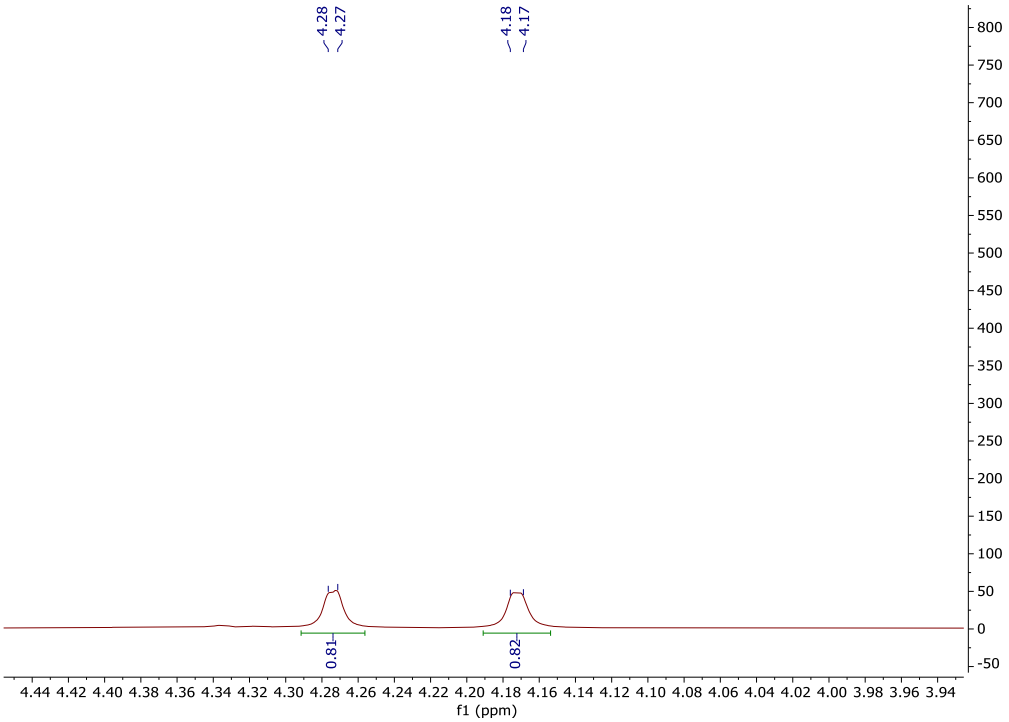

Figure S17. Expansion of 600 MHz ^{1}H NMR spectrum of 1"–dehydrocrinemodin bianthrone (**2**) in MeOH-*d*$_4$. The spectrum was acquired over 4 scans using the standard zg30 Bruker pulse sequence.

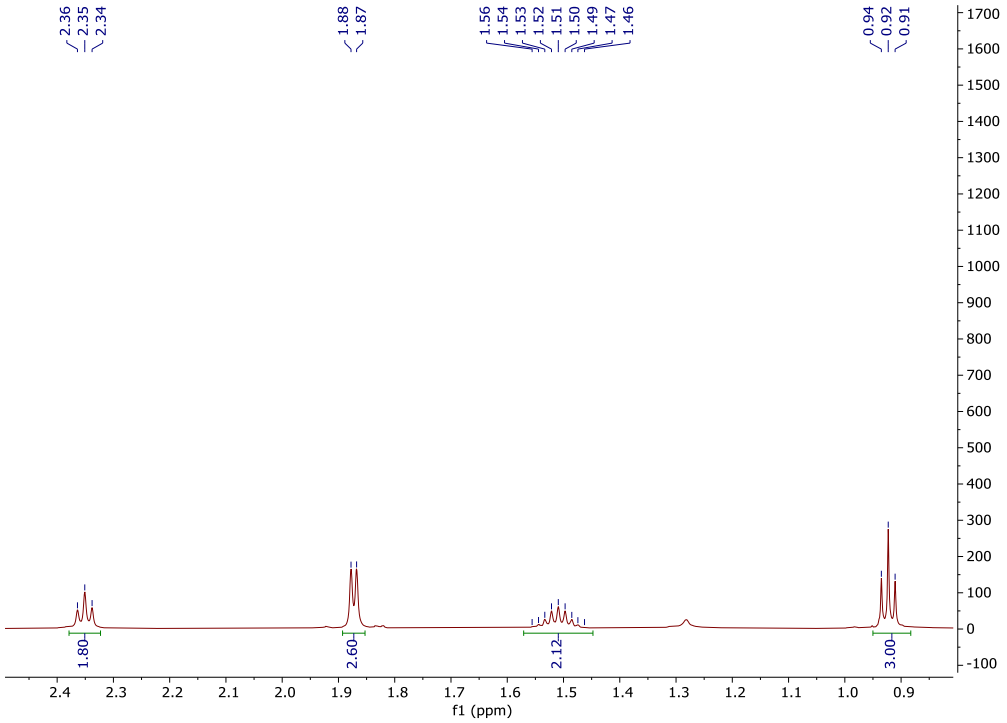

Figure S18. Expansion of 600 MHz ^{1}H NMR spectrum of 1"–dehydrocrinemodin bianthrone (**2**) in MeOH-d_4. The spectrum was acquired over 4 scans using the standard zg30 Bruker pulse sequence.

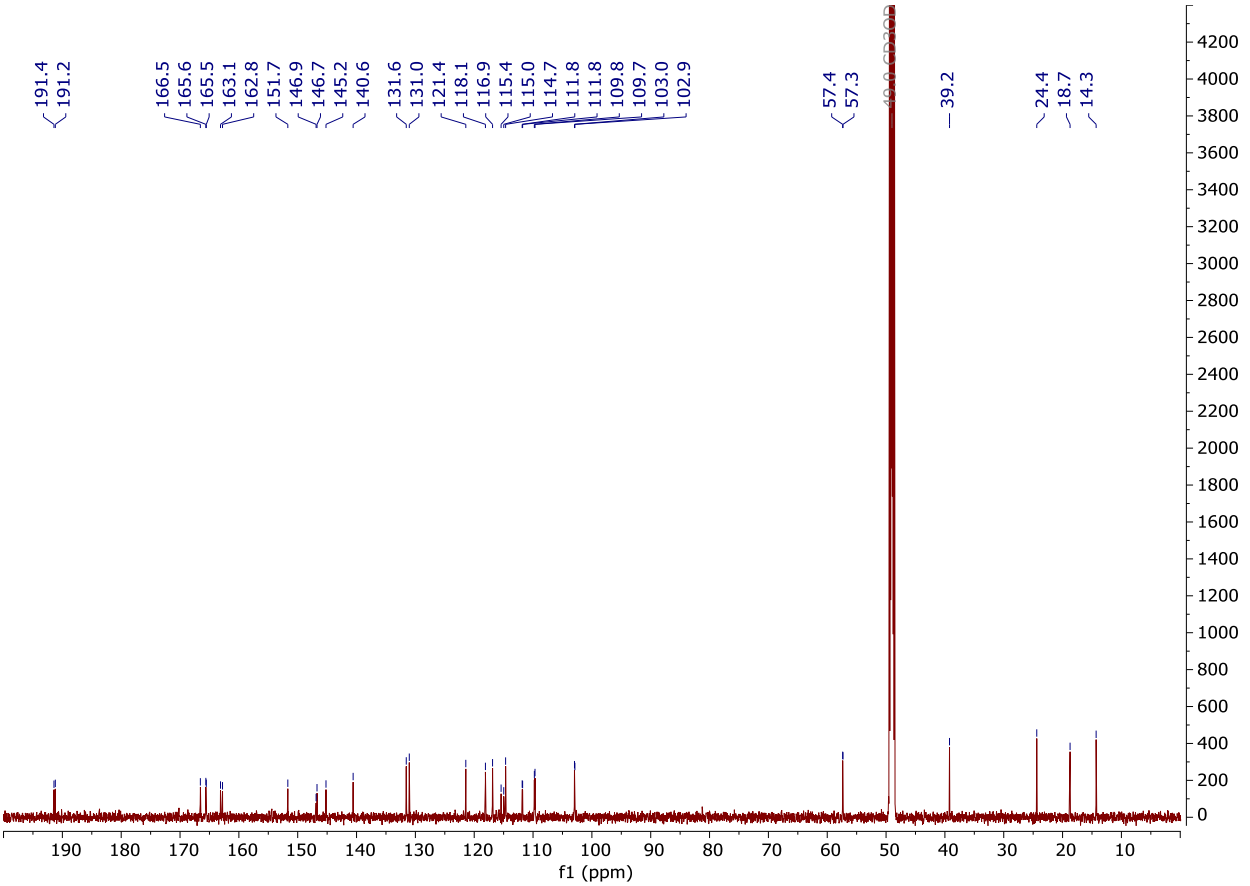

Figure S19. 151 MHz ^{13}C NMR spectrum of 1"–dehydrocrinemodin bianthrone (**2**) in MeOH-d_4. The spectrum was acquired over 10000 scans using the standard zgpg30 Bruker pulse sequence.

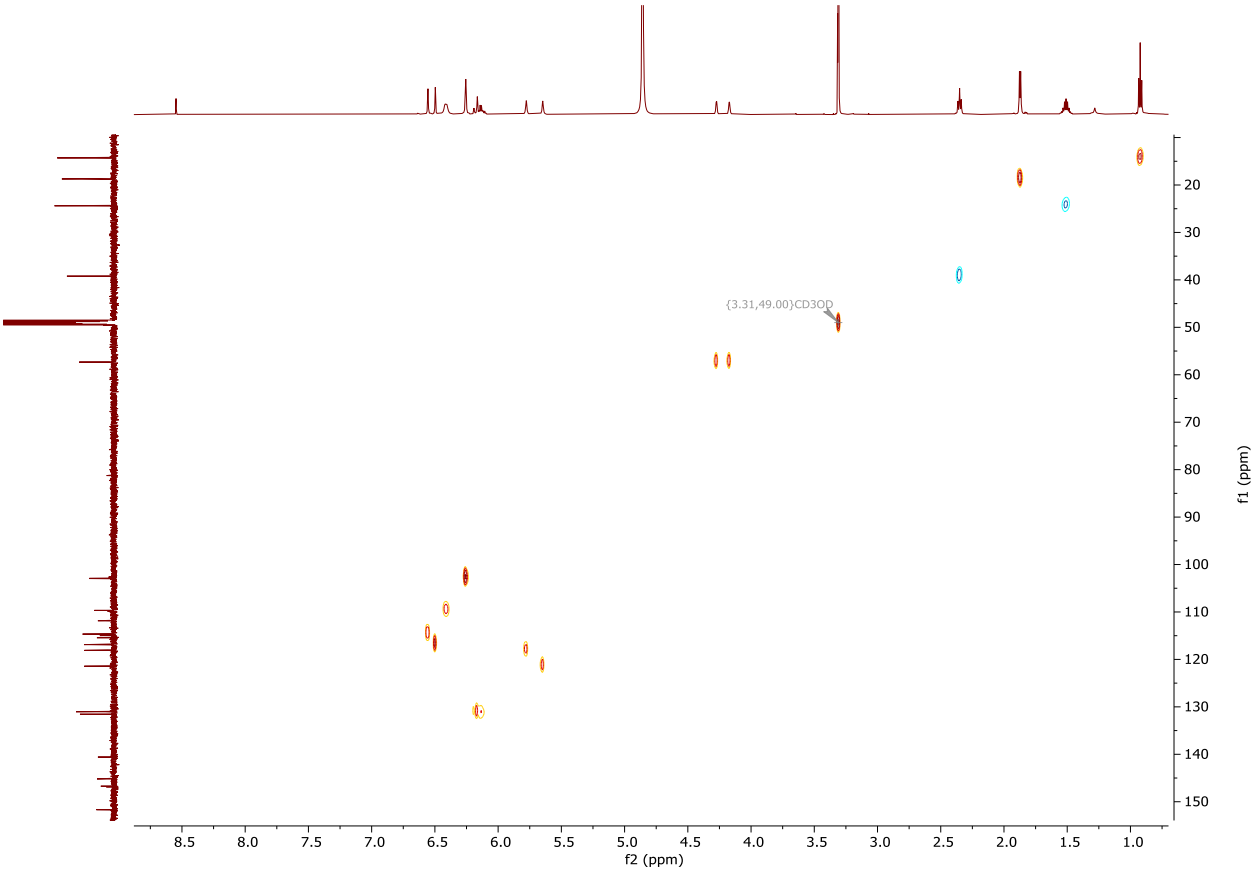

Figure S20. $^{1}H–^{13}C$ HSQC NMR spectrum of 1''–dehydrocrinemodin bianthrone (**2**) in MeOH-d_4. The spectrum was acquired over 64 scans at 1024 t1 increments using the standard hsqcedetgpsisp2.3 Bruker pulse sequence.

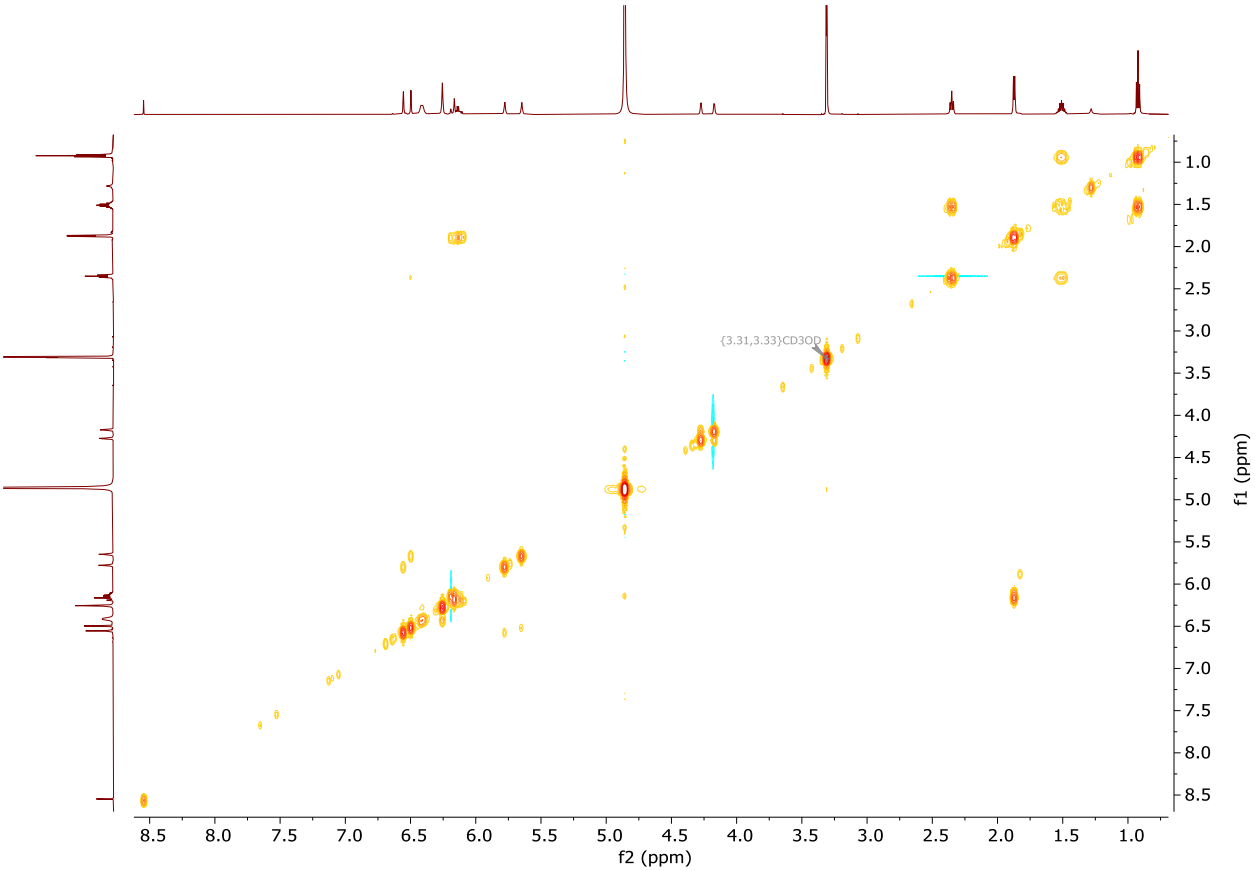

Figure S21. ^{1}H–^{1}H COSY NMR spectrum of 1"–dehydrocrinemodin bianthrone (**2**) in MeOH-d_4. The spectrum was acquired over 4 scans at 1024 t1 increments using the standard cosygpppqf Bruker pulse sequence.

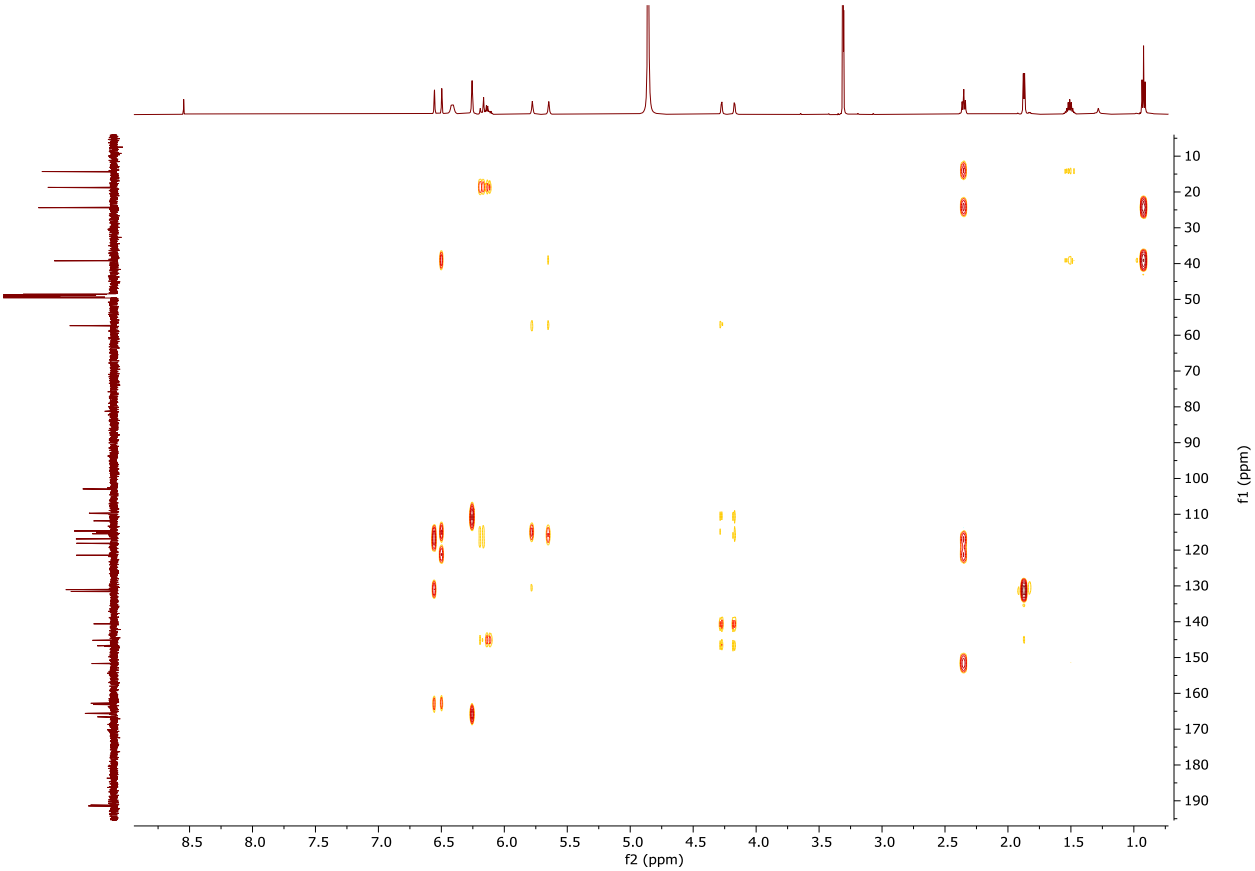

Figure S22. $^{1}H–^{13}C$ HMBC NMR spectrum of 1"–dehydrocrinemodin bianthrone (**2**) in MeOH-d_4. The spectrum was acquired over 296 scans at 2048 t1 increments using the standard hmbcetgpl3nd Bruker pulse sequence.

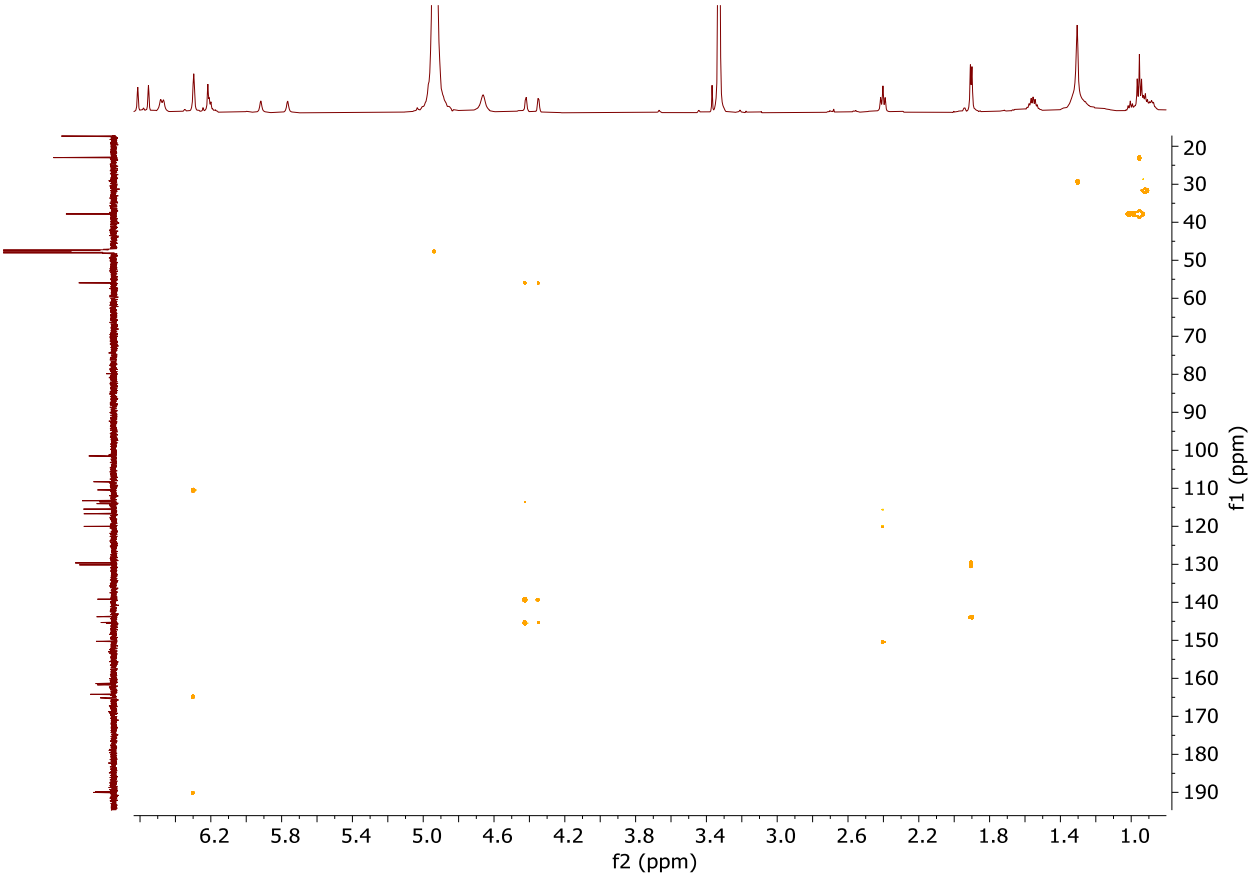

Figure S23. $^{1}H–^{13}C$ LRHSQMBC NMR spectrum of 1"–dehydrocrinemodin bianthrone (**2**) in MeOH-d_4. The spectrum was acquired over 300 scans at 2048 t1 increments using the js_lrhsqmbc_final pulse sequence.

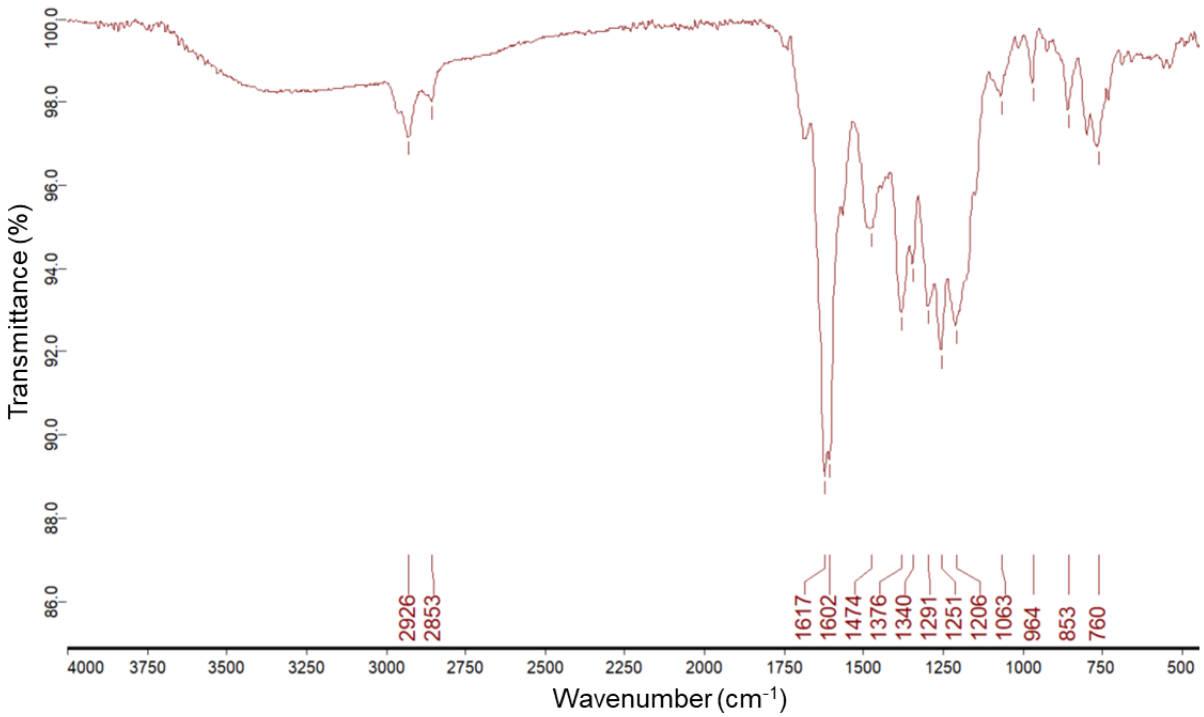

Figure S24. FTIR spectrum for 1"–dehydrocrinemodin bianthrone (**2**).

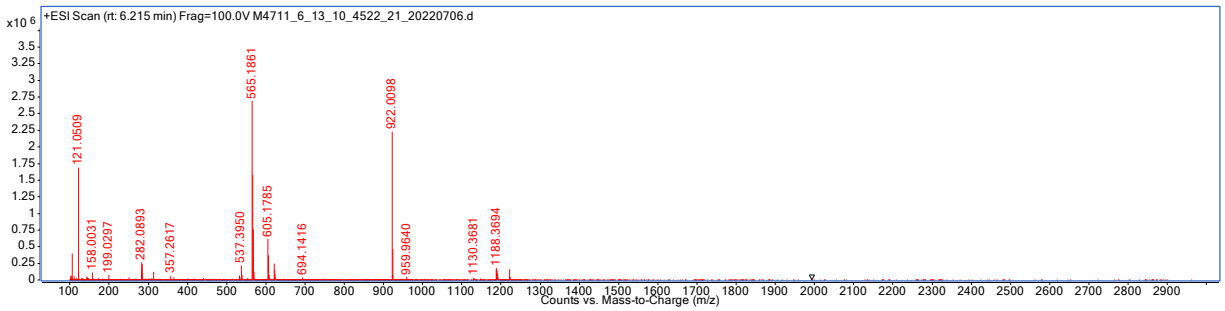

Figure S25. HRESIMS spectrum of 1"–hydroxycrinemodin bianthrone (**3**).

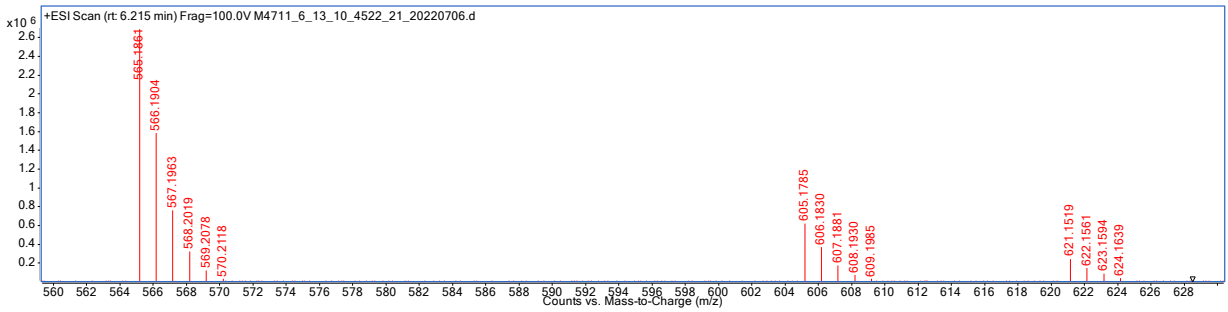

Figure S26. Expansion of HRESIMS spectrum of 1"–hydroxycrinemodin bianthrone (**3**).

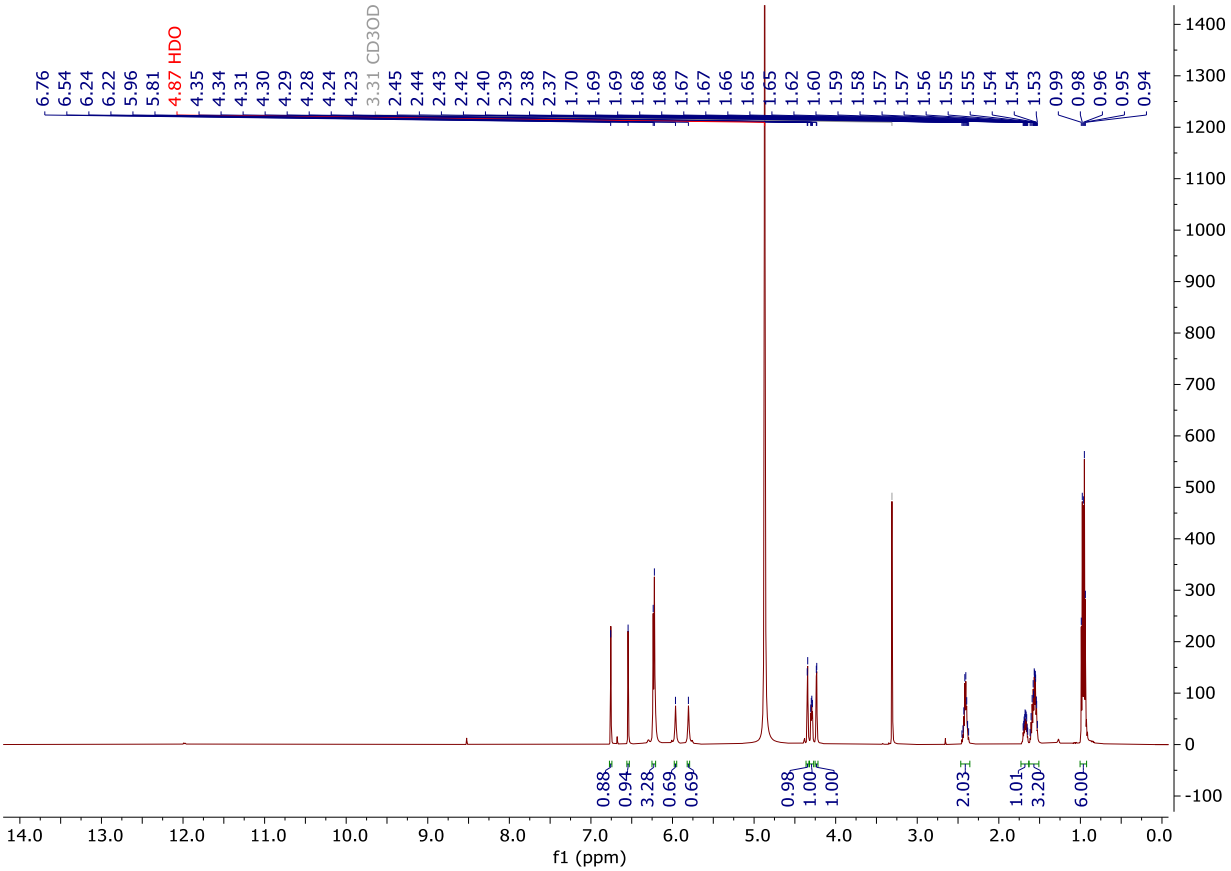

Figure S27. 600 MHz ^{1}H NMR spectrum of 1"–hydroxycrinemodin bianthrone (**3**) in MeOH-d_4. The spectrum was acquired over 4 scans using the standard zg30 Bruker pulse sequence.

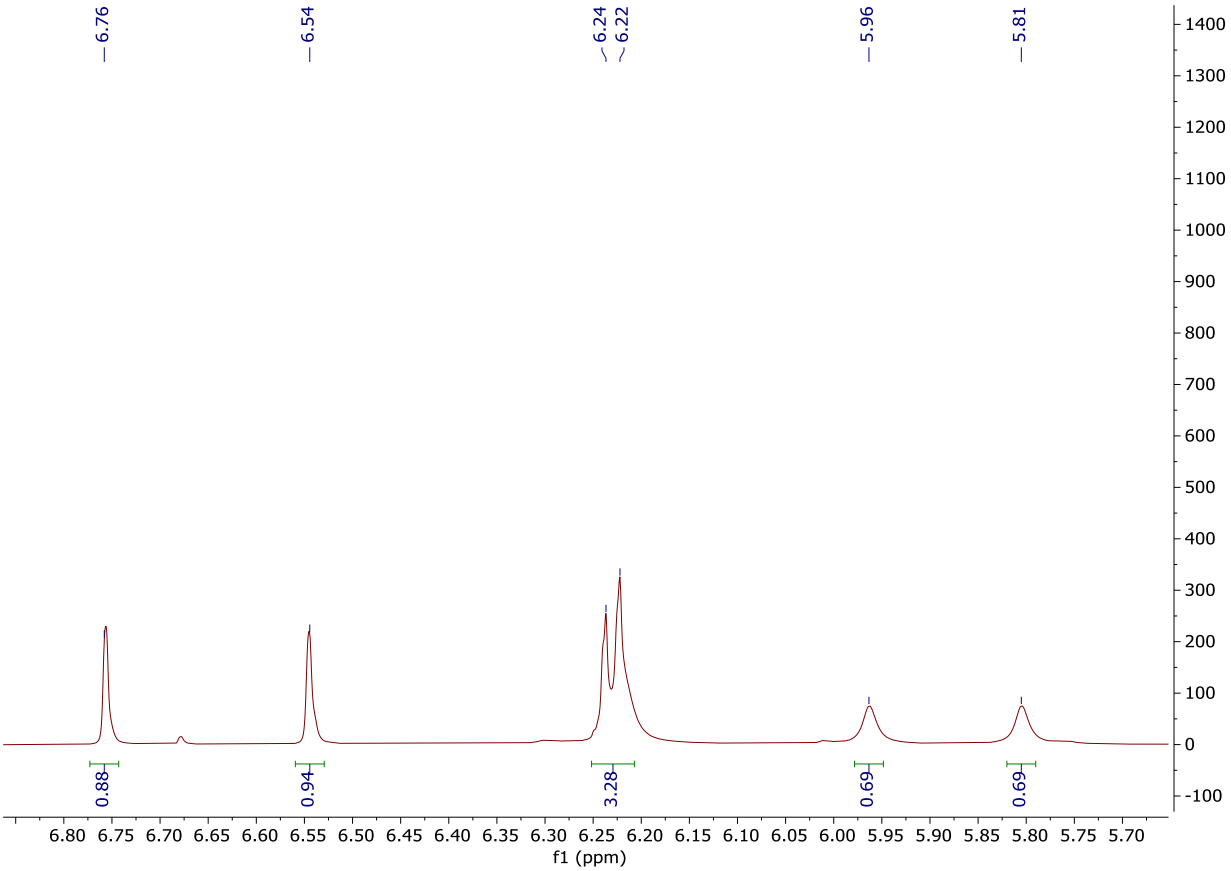

Figure S28. Expansion of 600 MHz ^{1}H NMR spectrum of 1"–hydroxycrinemodin bianthrone (**3**) in MeOH-d_4. The spectrum was acquired over 4 scans using the standard zg30 Bruker pulse sequence.

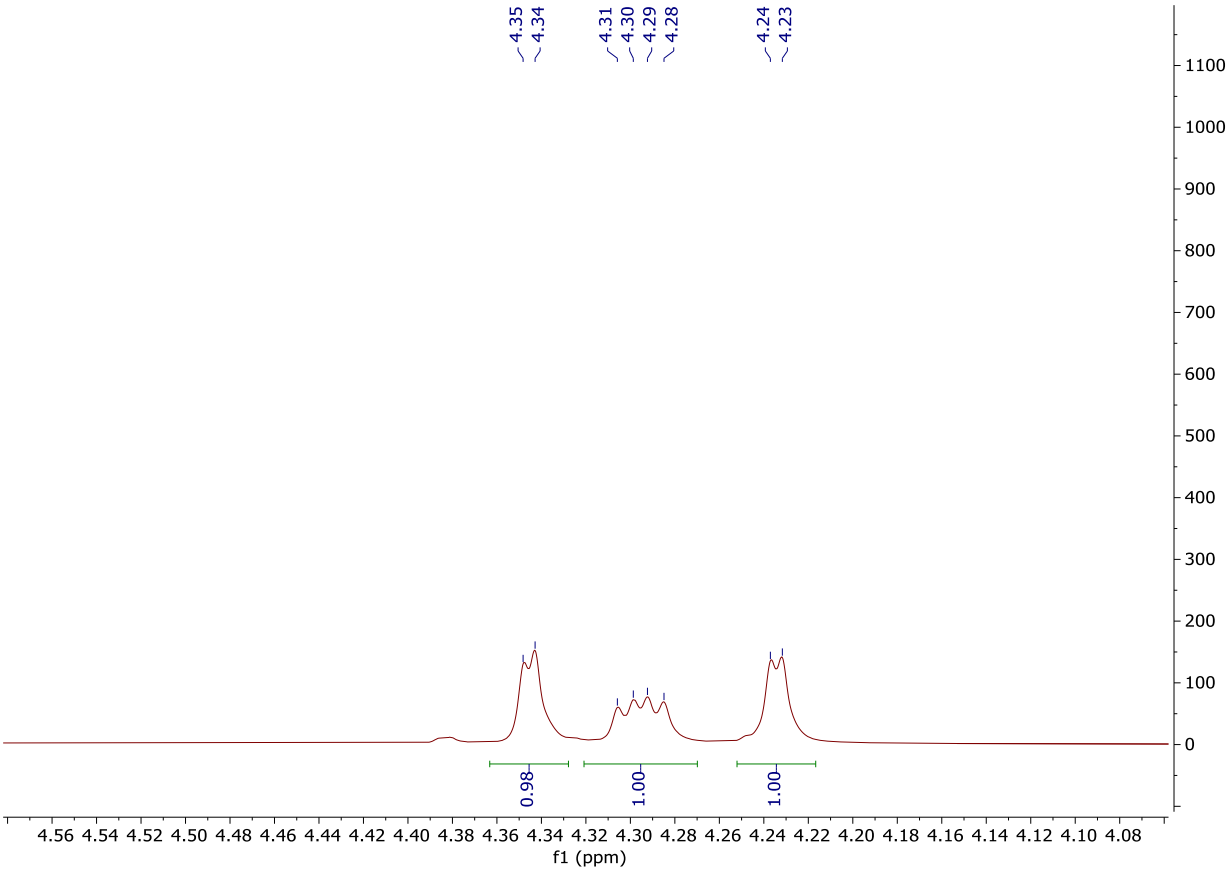

Figure S29. Expansion of 600 MHz ^{1}H NMR spectrum of 1"–hydroxycrinemodin bianthrone (**3**) in MeOH-d_4. The spectrum was acquired over 4 scans using the standard zg30 Bruker pulse sequence.

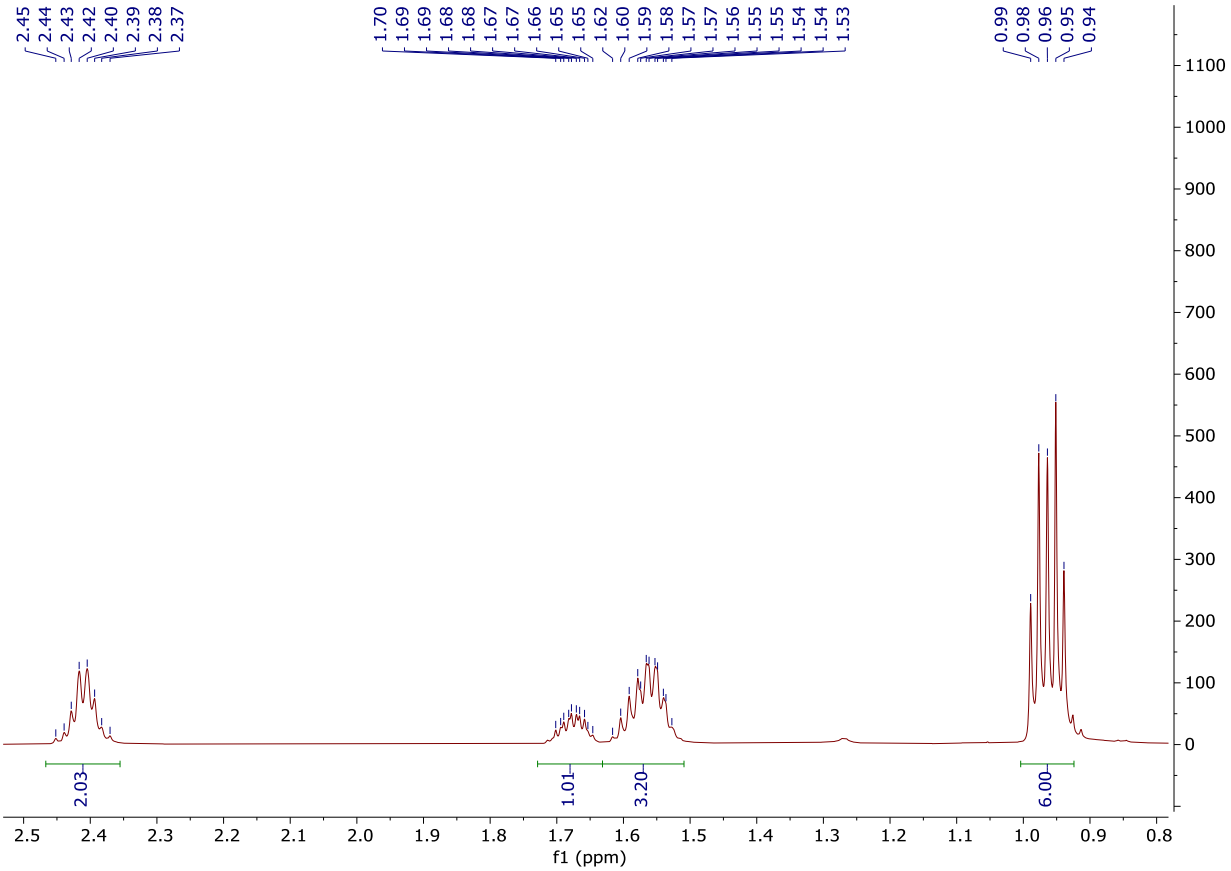

Figure S30. Expansion of 600 MHz ^{1}H NMR spectrum of 1"–hydroxycrinemodin bianthrone (**3**) in MeOH-d_4. The spectrum was acquired over 4 scans using the standard zg30 Bruker pulse sequence.

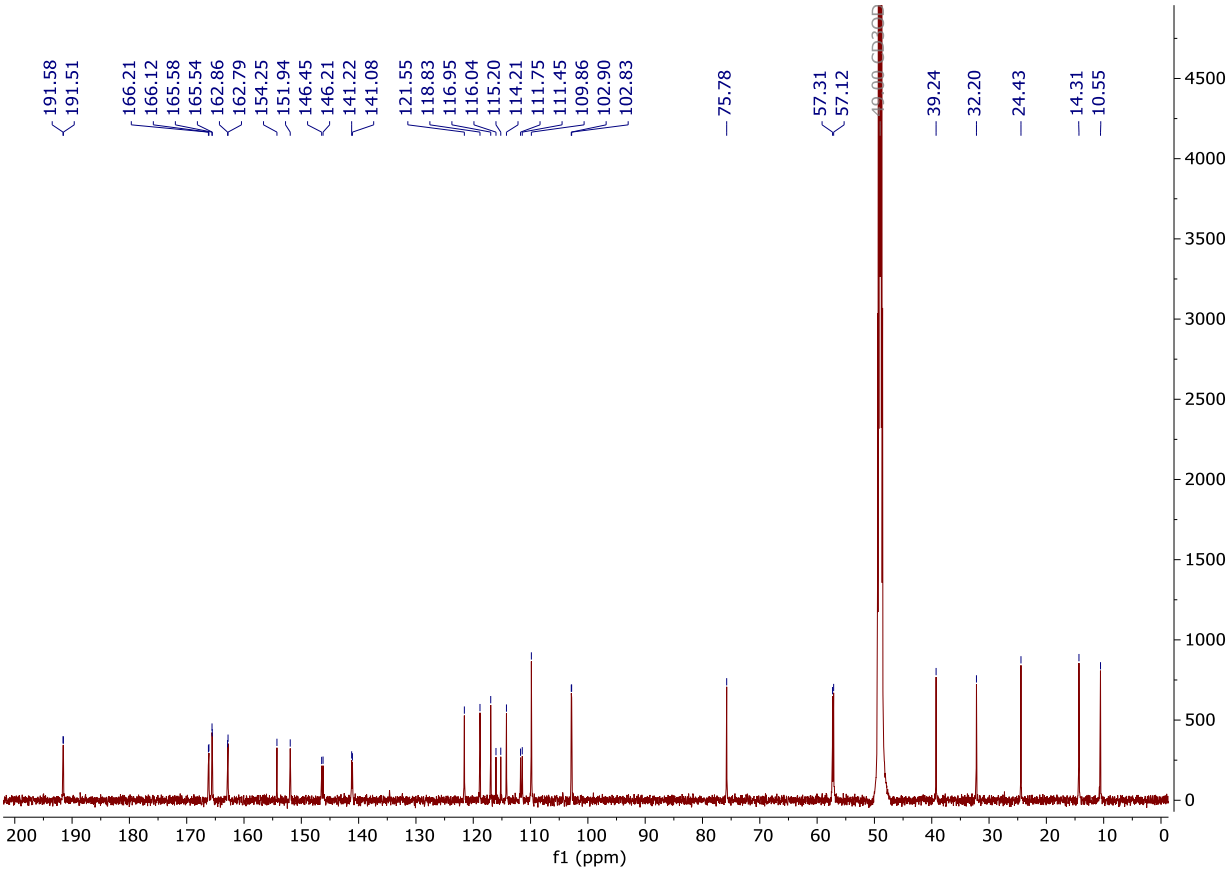

Figure S31. 151 MHz ^{13}C NMR spectrum of 1"–hydroxycrinemodin bianthrone (**3**) in MeOH-d_4. The spectrum was acquired over 12000 scans using the standard zgpg30 Bruker pulse sequence.

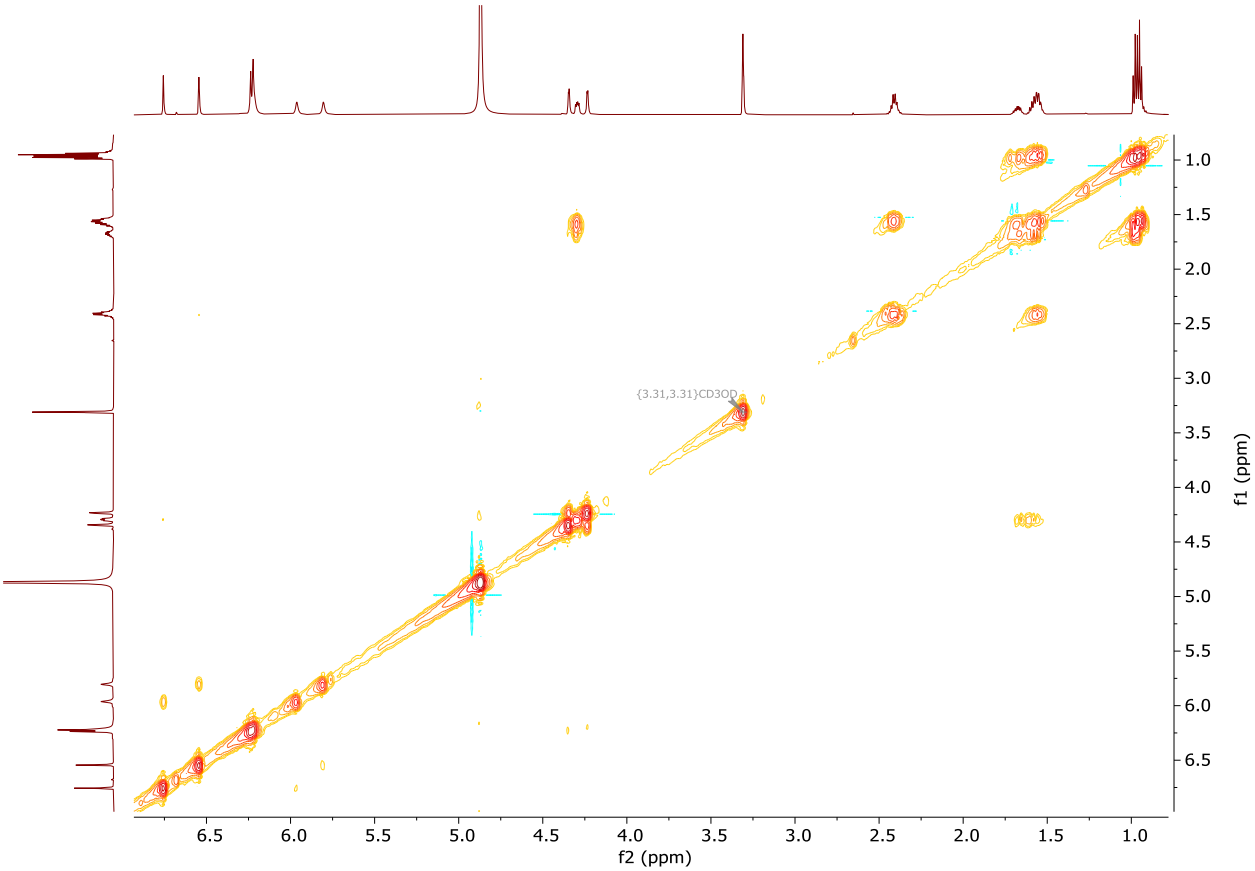

Figure S32. ^{1}H–^{1}H COSY NMR spectrum of 1''–hydroxycrinemodin bianthrone (**3**) in MeOH-d_4. The spectrum was acquired over 4 scans at 1024 t1 increments using the standard cosygpppqf Bruker pulse sequence.

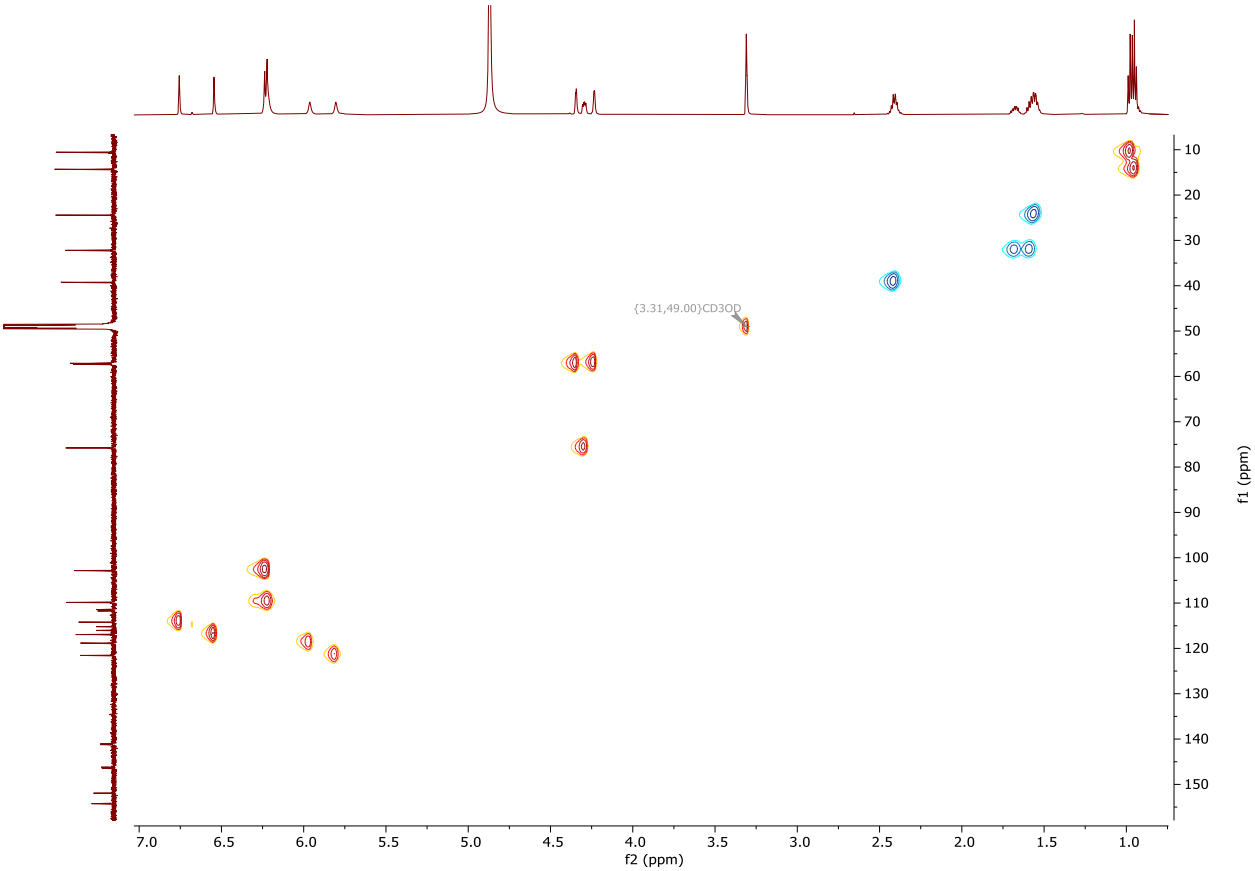

Figure S33. $^{1}H–^{13}C$ HSQC NMR spectrum of 1"–hydroxycrinemodin bianthrone (**3**) in MeOH-d_4. The spectrum was acquired over 64 scans at 1024 t1 increments using the standard hsqcedetgpsisp2.3 Bruker pulse sequence.

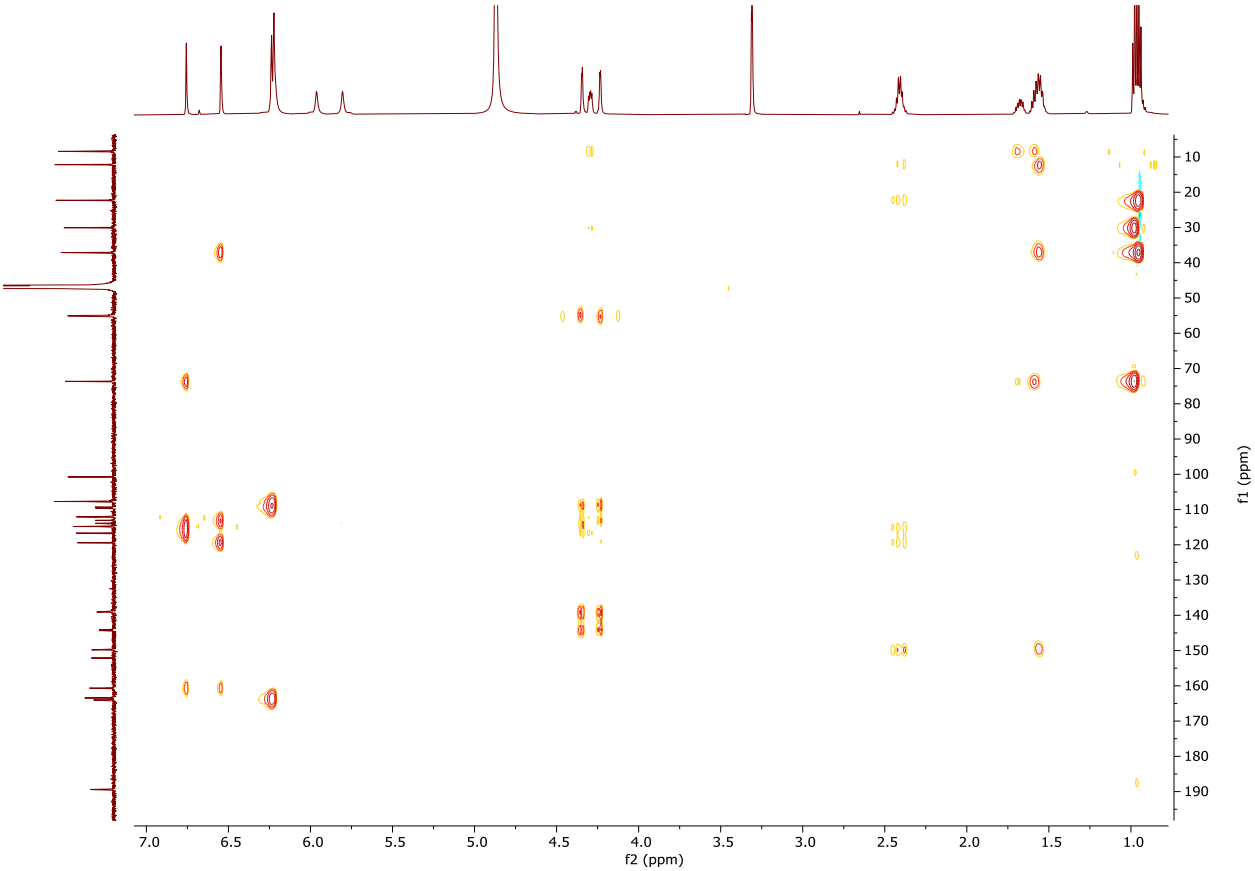

Figure S34. $^{1}H–^{13}C$ HMBC NMR spectrum of 1"–hydroxycrinemodin bianthrone (**3**) in MeOH-d_4. The spectrum was acquired over 240 scans at 2048 t1 increments using the standard hmbcetgpl3nd Bruker pulse sequence.

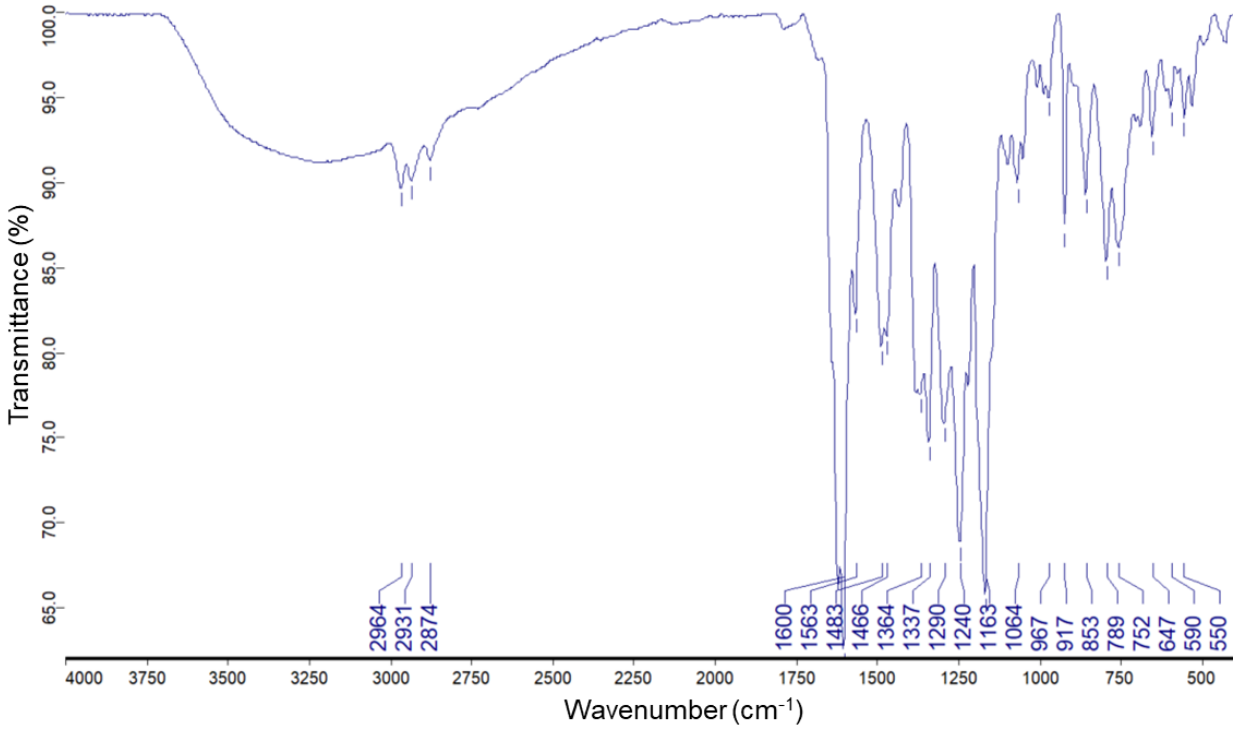

Figure S35. FTIR spectrum of 1"–hydroxycrinemodin bianthrone (**3**).

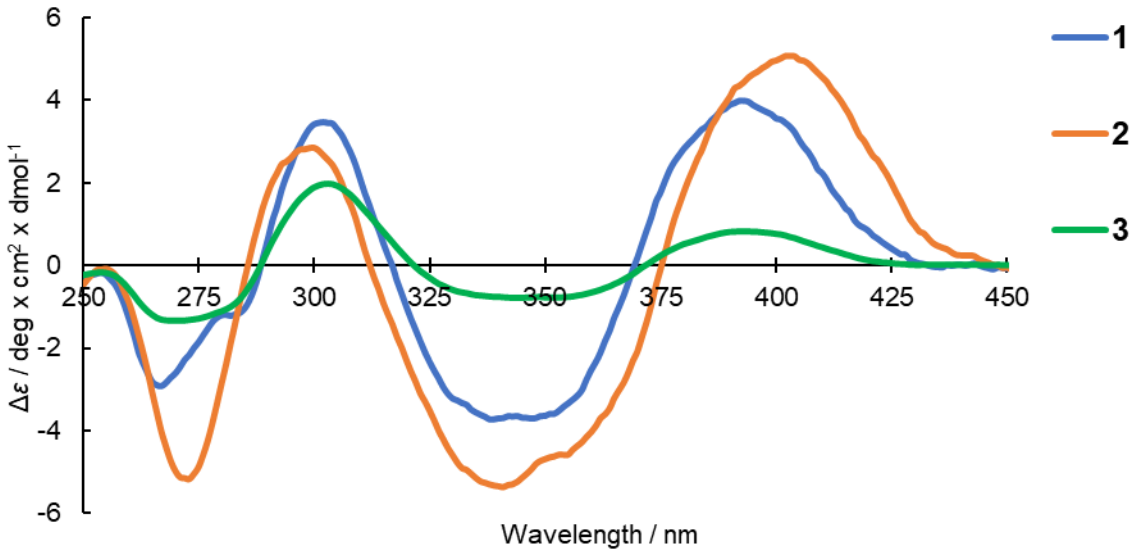

Figure S36. ECD spectra overlap of compounds **1**–**3** in acetonitrile.

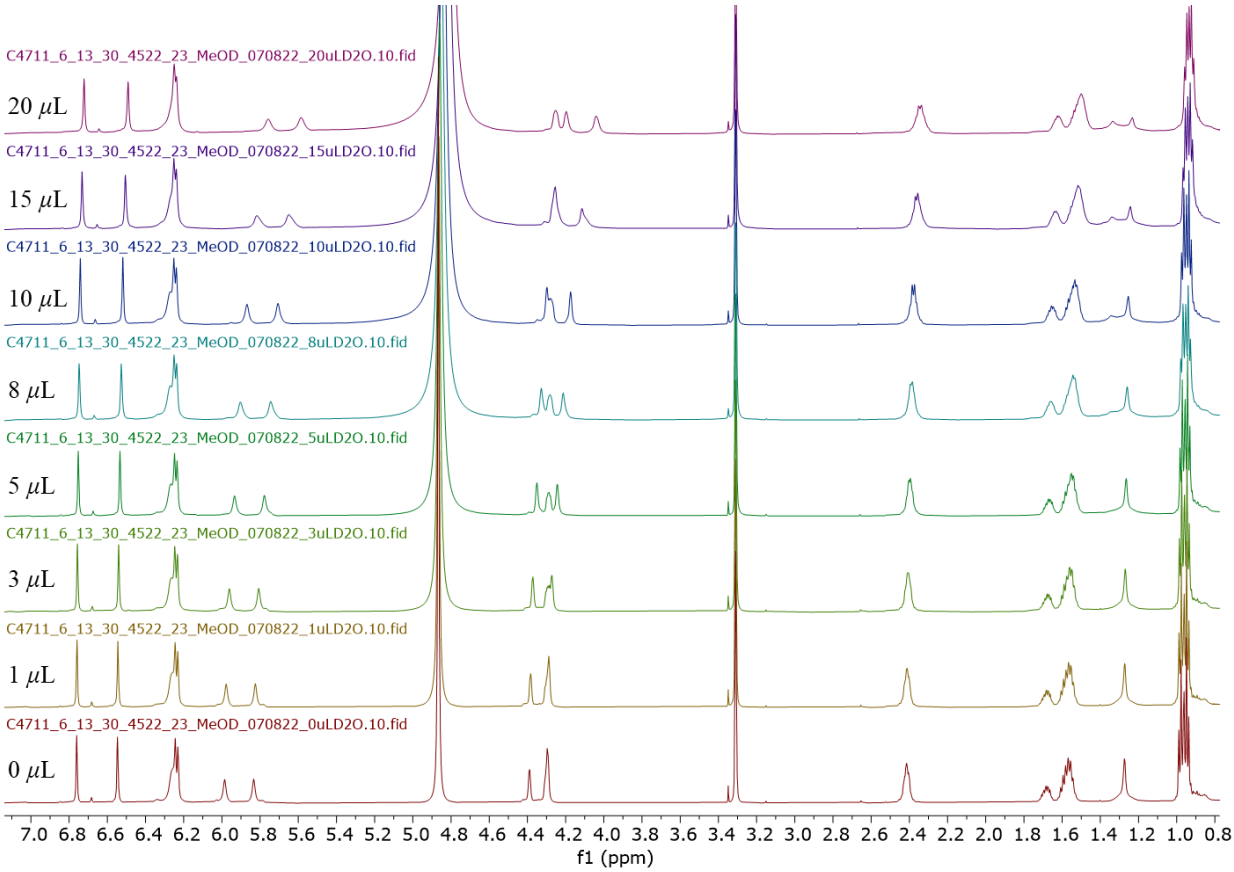

Figure S37. ^{1}H NMR spectra of **3** (in MeOH-d_4) titration with D_2O, from 0 μL (bottom) to 20 μL (top) of D_2O.

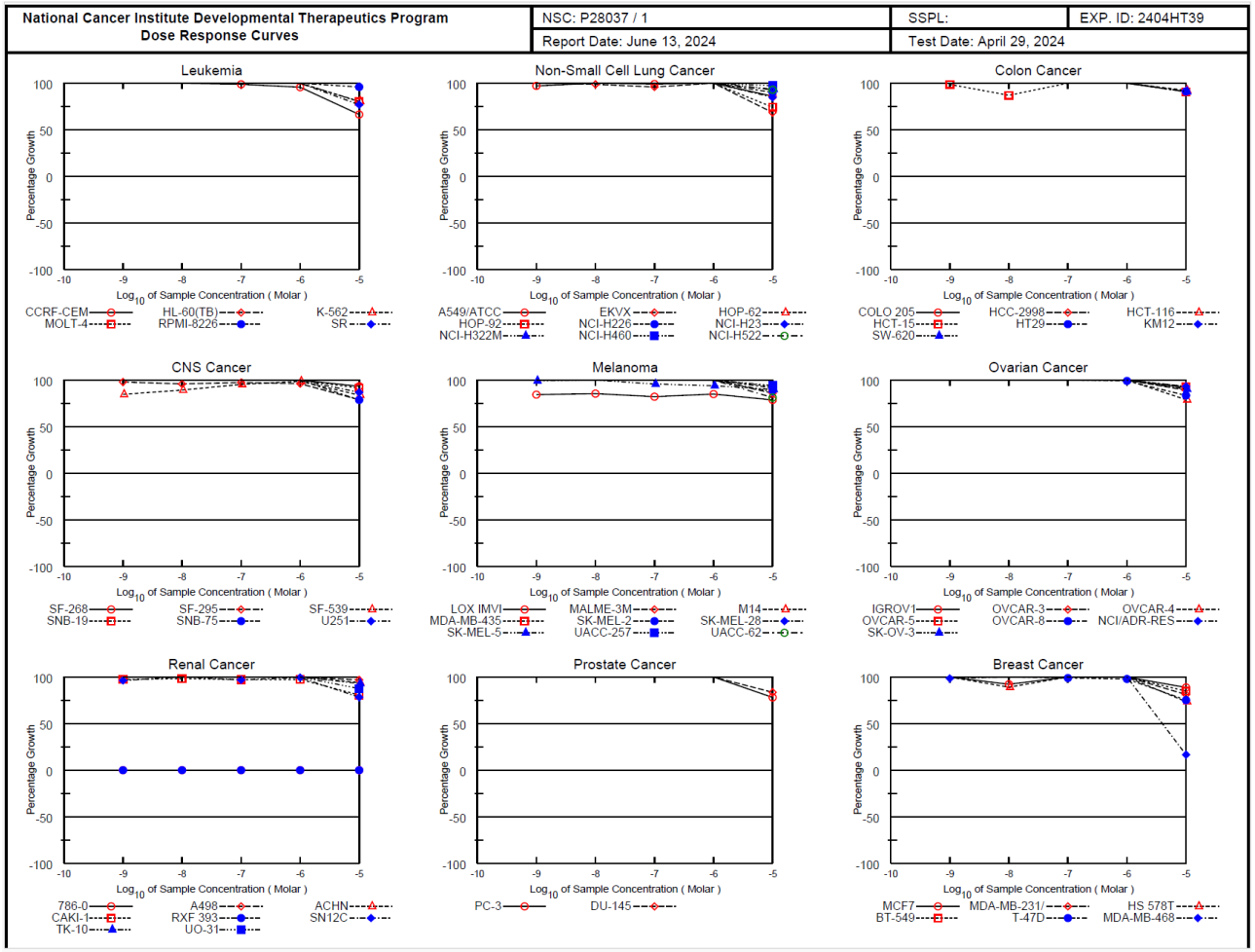

Figure S38. HTS384 NCI60 screen data for crinemodin bianthrone (**1**).

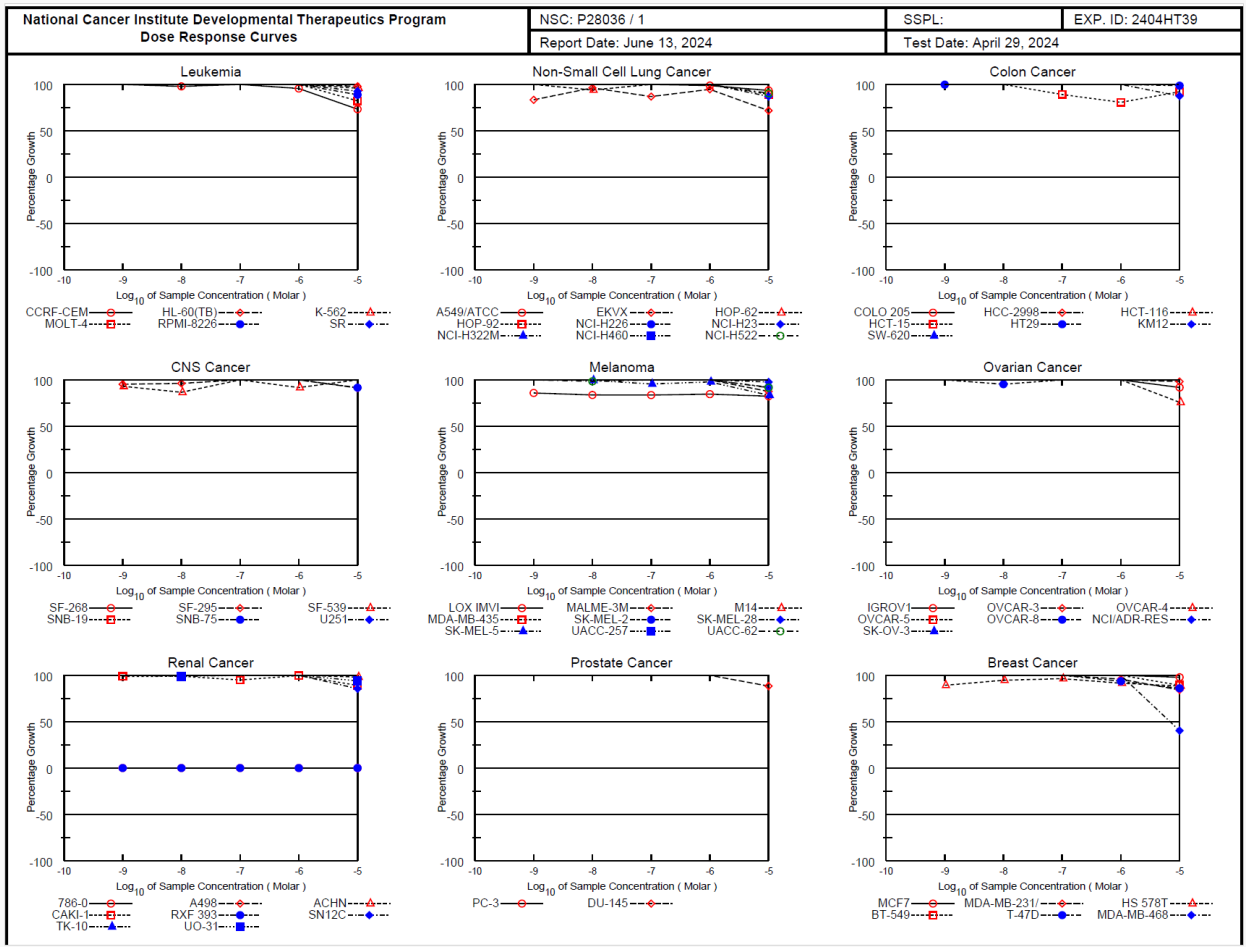

Figure S39. HTS384 NCI60 screen data for 1"–dehydrocrinemodin bianthrone (**2**).

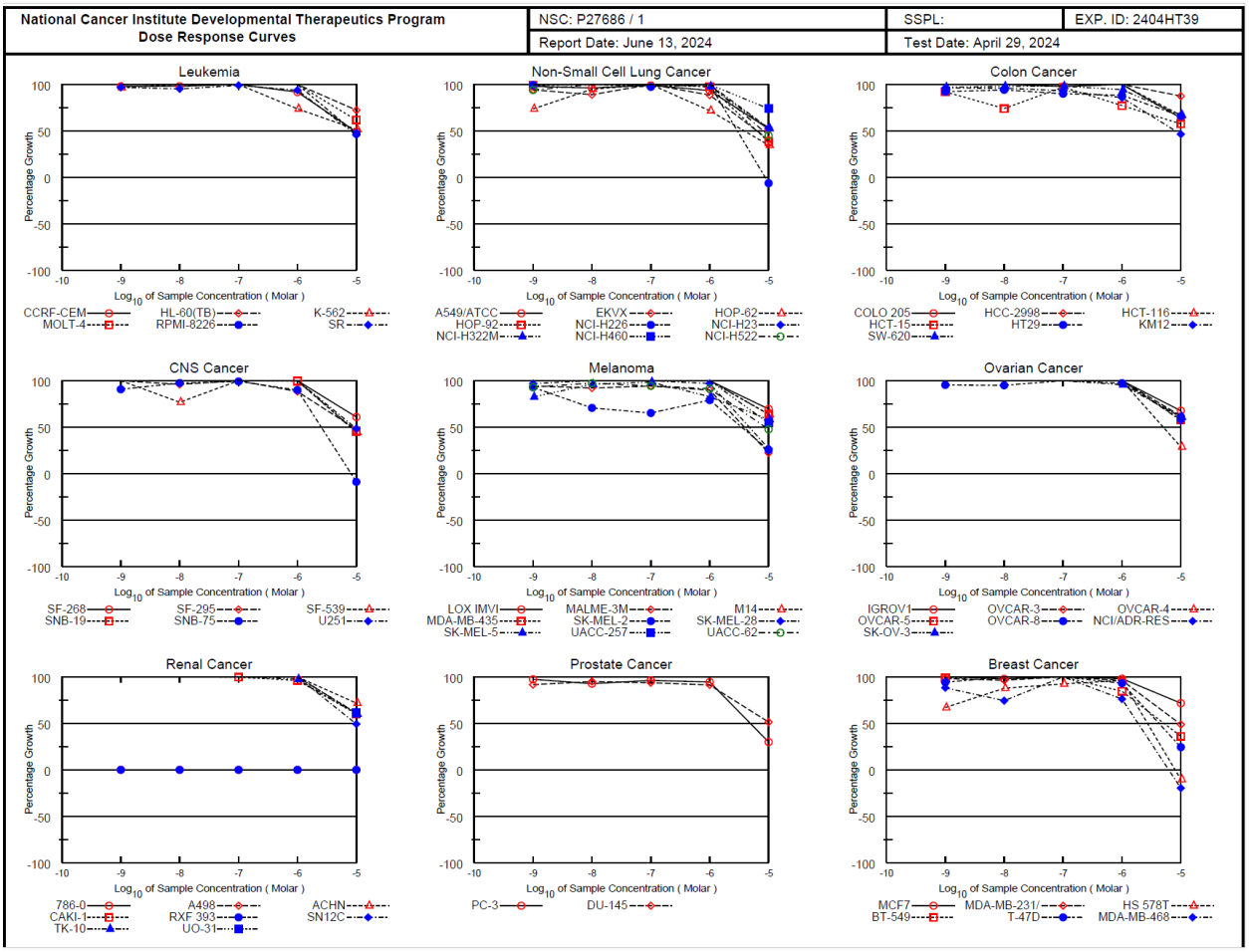

Figure S40. HTS384 NCI60 screen data for 1"–hydroxycrinemodin bianthrone (**3**).

Table S1. Reaction rates of **3** reacted with propionic anhydride using *R*- or *S*-HBTM catalysts. The reaction rate was achieved by measuring the peak areas for the fully acylated derivative, using the extracted ion chromatogram (EIC) for the sodiated molecule $[M + Na]^+$ at *m/z* 997.3617 (± 20 ppm).

Time (min)	***R*-HBTM** (peak area)	***S*-HBTM** (peak area)
5	5.16E+06	3.63E+06
10	1.26E+07	8.05E+06
15	1.98E+07	1.12E+07
20	2.43E+07	1.53E+07
25	3.10E+07	1.89E+07
30	3.40E+07	2.13E+07
35	3.82E+07	2.40E+07
40	4.20E+07	2.49E+07
45	4.42E+07	2.81E+07
50	4.80E+07	2.97E+07
55	5.09E+07	3.26E+07
60	5.33E+07	3.47E+07