Supplementary Materials

New and Bioactive Polyketides from Hawaiian Marine-derived Fungus *Trichoderma* sp. FM652

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Abstract

Two new sorbicillinoid derivatives (**1** and **2**), together with ten other related compounds (**3**−**12**) were isolated from a Hawaiian marine fungal strain *Trichoderma* sp. FM652. The structures of compounds **1** and **2**, including the absolute configuration, were elucidated by extensive analysis of NMR spectroscopy, HRESIMS and electronic circular dichroism (ECD) data. Compounds **6**−**12** exhibited significant anti-proliferative activity against ovarian cancer cell line A2780, with the IC_{50} values ranging from 0.5 to 8.07 μM. Moreover, compounds **1**, **7** and **8** showed significant inhibition against NF-κB with IC_{50} values of 13.83, 24.4 and 14.63 μM, respectively. Compounds **6**, **9** and **12** also demonstrated moderate inhibitory activity against *S. aureus* and methicillin resistant *S. aureus* with the MIC values in the range of 10−40 μg/mL.

Keywords

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1. Experimental

1.1 General experimental procedures

Optical rotations, CD, and FT-IR spectra were measured with a Rudolph Research analytical autoPol automatic polarimeter, JASCO J-815 CD, and Thermo Scientific Nicolet iS10 IR spectrometer, respectively. 1D and 2D NMR spectra were recorded on a Bruker AM-400 spectrometer. The 3.31 and 49.1 ppm resonances for CD_3OD and 2.50 and 39.5 ppm resonances for DMSO-d_6 were used as the internal references for ^1H and ^{13}C NMR spectra, respectively. An Agilent 6530 Accurate-Mass Q-TOF LC-MS spectrometer was used to record high resolution mass spectra. Preparative HPLC was carried out on an Ultimate 3000 chromatographic system with a Phenomenex preparative column (Phenyl-Hexyl, 5 μ, 100 × 21.2 mm) and semipreparative HPLC on an Ultimate 3000 chromatographic system with a Phenomenex semipreparative column (C$_8$, 5 μ, 250 × 10 mm), a Dionex Ultimate 3000 DAD detector, and a Dionex Ultimate 3000 automated fraction collector; all solvents were HPLC grade. Diaion HP-20 was used to run open-column chromatography.

1.2 Strain isolation and fermentation

The strain *Trichoderma* sp. FM652 was isolated from a sea sediment sample collected from offshore sea bed, near Hanauma bay, Hawaii. The rDNA ITS1-4 region sequence of fungus has been submitted to GenBank (Accession number OK626586) and was deposited in an -80 °C freezer at Daniel K. Inouye College of Pharmacy, University of Hawaii at Hilo, HI, USA. After activating on potato dextrose agar (PDA) plates at 28 °C for 5 days, it was cut into small pieces and inoculated into 20 L autoclaved sterilized liquid PDB medium for fermentation at 24 °C for 28 days.

1.3 Extraction and compound isolation

The mycelia of FM652 were filtered and extracted with acetone under ultrasonic (1 L × 3 times), followed by removal of acetone under reduced pressure to afford an aqueous solution. After combining the aqueous mycelia extraction and supernatant solution, it was subjected to HP-20 column eluted with MeOH-H_2O (10, 30, 50, 90 and 100%) to afford five fractions (Fr. 1-5). Fr. 4 (2.4 g) was separated by prep-HPLC (Phenyl-Hexyl, 5μ, 100 ×

21.2 mm; 8 mL/min) eluted with 50-100% MeOH-H$_2$O in 20 min to yield 12 sub-fractions (SFr. 1-20). SFr 4-9 was purified by semi-preparative HPLC (40% isocratic of MeOH-H$_2$O with 0.1% formic acid for 30 min; 3 mL/min) to afford compound **1** (2.1 mg, t_R 26.5 min). Compound **2** (1.36 mg, t_R 12.5 min) was separated from SFr 4-14 by using the same HPLC (65% isocratic of MeOH-H$_2$O with 0.1% formic acid for 18 min).

2,3-Dihydro 2-hydroxy vertinolide (**1**): brown powder; $[\alpha]_D^{25}$ -21.3 (c 0.2, MeOH); UV (MeOH) λmax (log ε) 242 (3.56), 315(3.15) nm; IR (MeOH) v_{max} 3306, 2942, 2834, 1650, 1451, 1403, 1109, 1014 cm^{-1}; ^{1}H NMR (DMSO, 400MHz) δ: 3.83(s; 1H; H-3), 1.86 (m; 2H; H-5), 2.62/2.72 (m; 2H; H-6), 6.09 (d; J = 15; 1H; H-8), 7.21 (d; J = 9,15; 1H; H-9), 6.29(OL; 1H; H-10), 6.32(OL; 1H; H-11), 1.84 (d; J = 6; 1H; H-12), 1.25(s; 3H; H-13), 1.40(s; 3H; H-14) and ^{13}C NMR (DMSO, 100MHz) δ: 177.4(C-1), 76.3(C-2), 80.5(C-3), 86.3(C-4), 29.4(C-5), 34.7(C-6), 199.9(C-7), 127.9(C-8), 143.0(C-9),130.6(C-10), 140.6(C-11), 19.0(C-12), 20.3(C-13), 25.1(C-14); HRESIMS m/z 269.13682 [M + H]$^+$ (calcd for C$_{14}$H$_{21}$O$_5^+$, 269.13890).

(-)-Trichodermatone (**2**): dark yellow powder; $[\alpha]_D^{25}$ -18.7 (*c* 0.002, MeOH); UV (MeOH) λmax (log ε) 291 (3.07), 451 (3.56) nm; IR (MeOH) v_{max} 3327, 2945, 2834, 1652, 1453, 1404, 1114, 1016 cm^{-1}; ^{1}H NMR (CD$_3$OD, 400MHz) δ: 7.65 (s; 1H; H-5), 6.76 (d; J = 15; 1H; H-8), 7.42 (d; J = 11,15; 1H; H-9), 6.45(dd; J=11,15; 1H; H-10), 6.31(m; 1H; H-11), 1.93 (d; J=7; 1H; H-12), 1.95(s; 3H; H-13), 1.49(s; 3H; H-14) and ^{13}C NMR (CD$_3$OD, 100MHz) δ: 204.1(C-1), 81.1(C-2), 199.3(C-3), 124.4(C-4), 136.7(C-5), 105.2(C-6), 172.5(C-7), 117.4(C-8), 143.6(C-9),130.9(C-10), 140.9(C-11), 17.6(C-12), 14.4(C-13), 27.9(C-14); HRESIMS m/z 249.11248 [M + H]$^+$ (calcd for C$_{14}$H$_{17}$O$_4^+$, 249.11268).

1.4 Computational section

All the quantum mechanical calculations were performed using Gaussian 09. Systematic conformational searches were done for each compound in the gas phase using the MMFF force field, implemented in Spartan 08, using an energy cutoff of 5 kcal/mol. The choice for the 5 kcal/mol of cutoff was set as a balance between reducing the overall CPU calculation time and minimizing the possibility of losing further contributing conformers. All conformers were kept for full geometry optimization at the B3LYP/6-31G* level in gas

phase. Frequency calculations were done at the same level to determine the nature of the stationary points found. The magnetic shielding constants (σ) were computed using the gauge including atomic orbitals (GIAO) method, the method of choice to solve the gauge origin problem, at PCM/mPW1PW91/6-31+G** level of theory, the recommended for DP4+ calculations. The unscaled chemical shifts (δ_u) were computed using TMS as reference standard according to $\delta_u = \delta_0 - \delta_x$, where δ_x is the Boltzmann averaged shielding tensor (over all significantly populated conformations) and δ_0 is the shielding tensor of TMS computed at the same level of theory employed for δ_x. The Boltzmann averaging was done according to eq 1:

$$\sigma^x = \frac{\sum_i \sigma_i^x e^{(-E_i/RT)}}{\sum_i e^{(-E_i/RT)}} \qquad (eq.\ 1)$$

where $\delta_i{}^x$ is the shielding constant for nucleus x in conformer i, R is the molar gas constant (8.3145 J K^{-1} mol^{-1}), T is the temperature (298 K), and E_i is the energy of conformer i (relative to the lowest energy conformer), obtained at the SMD/M06-2X/6-31G* level of theory. The scaled chemical shifts (δ_s) were computed as $\delta_s = (\delta_u - b)/m$, where m and b are the slope and intercept, respectively, resulting from a linear regression calculation on a plot of δ_u against δ_{exp}. The DP4+ values were computed using the Excel spread sheet provided in https://sarotti-nmr.weebly.com.

The ECD calculations were carried out using the B3LYP/6-31G* optimized geometries. The excitation energies (nm) and rotatory strength (R) in dipole velocity (Rvel) of the first forty singlet excitations were calculated using TDDFT implemented in Gaussian 09 at the PBE0/def2-SVP level from all significantly populated conformers, which were averaged using Boltzmann weighting. The Boltzmann amplitudes obtained by refining the Gibbs free energies of all compounds at the SMD/M06-2X/6-31G* level. The calculated rotatory strength were simulated into the ECD curve as the sum of Gaussians with 0.3 eV width at half-heights (σ), which were UV-corrected and scaled.

1.5. Antiproliferative assays

Viability of A2780 human ovarian cancer cells was determined using the CyQuant assay according to the manufacturer's instructions (Life Technologies, CA, USA). Briefly, cells were cultured in 96-well plates at 1000 cells per well for 24 h and subsequently treated with

compounds (20 µg/mL) for 72 h and analyzed. Relative viability of the treated cells was normalized to the DMSO-treated control cells. Cisplatin was used as a positive control, which had an IC$_{50}$ value of 0.36 µM. All experiments were performed in triplicate.

1.6. NF-κB assay

We employed HEK 293 from Panomics for monitoring changes occurring along the NF-κB pathway. Stable constructed cells were seeded into 96-well plates at 20×10^3 cells per well. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen Co.), supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, 100 µg/mL streptomycin, and 2 mML-glutamine. After 48 h of incubation, the medium was replaced and the cells were treated with various concentrations of test substances. TNF-α (human, recombinant, *E. coli*, Calbiochem) was used as an activator at a concentration of 2 ng/mL (0.14 nM). The plate was incubated for 6 h. Spent medium was discarded, and the cells were washed once with PBS. Cells were lysed using 50 µL (for 96-well plate) of reporter lysis buffer from Promega, by incubating for 5 min on a shaker, and stored at −80 °C. The luciferase assay was performed using the Luc assay system from Promega. The gene product, luciferase enzyme, reacts with luciferase substrate, emitting light, which was detected using a luminometer (LUMIstar Galaxy BMG). Data for NF-κB inhibition are expressed as IC$_{50}$ values (i.e., concentration required to inhibit TNF-α-induced NF-κB activity by 50%). As positive controls, two known NF-κB inhibitors were used, TPCK (Nα-tosyl-L-phenylalanine chloromethyl ketone) and BAY-11- 7082 (which selectively and irreversibly inhibits NF-κB activation), yielding IC$_{50}$ values of 5.3 ± 0.9 and 11 ± 1.8 µM, respectively. All experiments were performed in triplicate.

1.7. SRB assay

In order to assess the potential of mediating a cytotoxic response, the cells were treated under the same experimental conditions with each test compound at a concentration of 50 µM, and cell survival was determined by the sulforhodamine B (SRB) assays. After incubation of HEK 293 cells with test compounds, cells were fixed with 10% trichloroacetic acid solution for 30 min and stained with 0.4% SRB in 1% acetic acid solution for 30 min. Protein-bound SRB was dissolved in 10 mM Tris buffer (pH 10.0), and

the absorbance was measured at 515 nm. The effect of compounds on cell survival was demonstrated as percentage survival in comparison with vehicle (DMSO)-treated control cells.

1.8. Antibacterial assay

Antibacterial assay was conducted by using the previously described method (Zaman et al. 2021) with slight modifications. Bacteria were grown on agar plates [Tryptic Soy Agar (TSA) or Brain Heart infusion Agar (BIHA)] for 1 day at 37°C and then added to a liquid medium (TSB for *S. aureus* and methicillin resistant *S. aureus* and BIH for *Bacillus subtilis*). After incubation at 37°C for 20 h, the cultures were diluted with TSB or BIH media to obtain an OD$_{600}$ value of approx. 0.1. One hundred microliter of fresh media with samples at the desired concentration of 160 µg/mL (dissolved in DMSO) was put in the first well and then a two-fold dilution continued to the lowest concentration. The bacterium-containing media (100 µL) were then added to each well of 96-well plates. DMSO (5%) was used as negative controls in these sets of experiments and chloramphenicol, which is active against *S. aureus*, methicillin resistant *S. aureus* and *Bacillus subtilis* at MIC values of 6.25 µg/ml, 6.25 µg/ml and 12.5 µg/ml, respectively, was employed as the a positive control.

Table S1. ^{1}H and ^{13}C NMR data of 1 and 2

no.	1[a]			2[b]	
	δ_H *J (Hz)*[1]	δ_C[2]		δ_H *J (Hz)*[1]	δ_C[2]
1		177.4			204.1
2		76.3			81.1
3	3.83, s	80.5			199.3
4		86.3			124.4
5	1.86, m	29.4		7.65, s	136.7
6	2.62, m	34.7			105.2
	2.72, m				
7		199.9			172.5
8	6.09, d (15)	127.9		6.76, d (15)	117.4
9	7.21, dd (9,15)	143.0		7.42, dd (11,15)	143.6
10	6.29, OL*	130.6		6.45, dd (11,15)	130.9
11	6.32, OL*	140.6		6.31, m	140.9
12	1.84, d (6)	19.0		1.93, d (7)	17.6
13	1.25, s	20.3		1.95, s	14.4
14	1.40, s	25.1		1.49, s	27.9

[a] Solvent→ DMSO, [b]Solvent→CD$_3$OD

[1]Spectra recorded at 400 MHz; [2]Spectra recorded at 100 MHz;

Data based on ^{1}H, ^{13}C, HSQC, and HMBC experiments;

*OL→ overlapped.

Figure S1: HRESIMS of compound 1
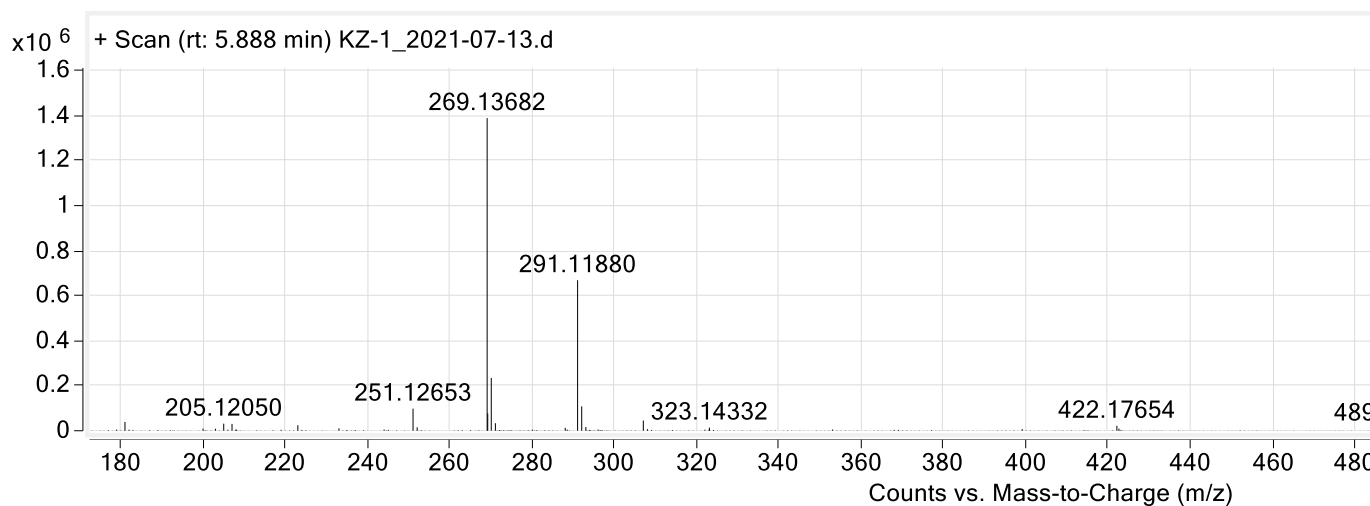
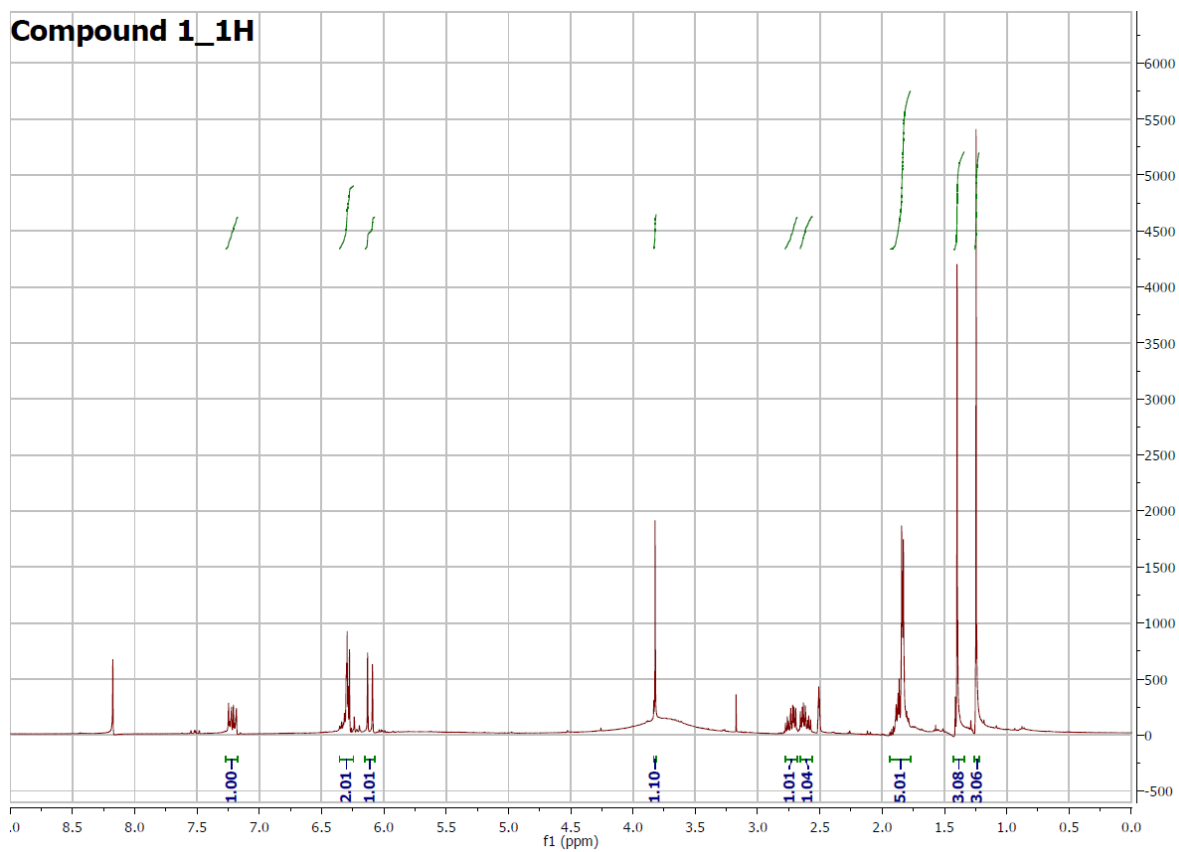**Figure S2: ^{1}H NMR spectrum of compound 1 in DMSO-d₆ (400MHz)**

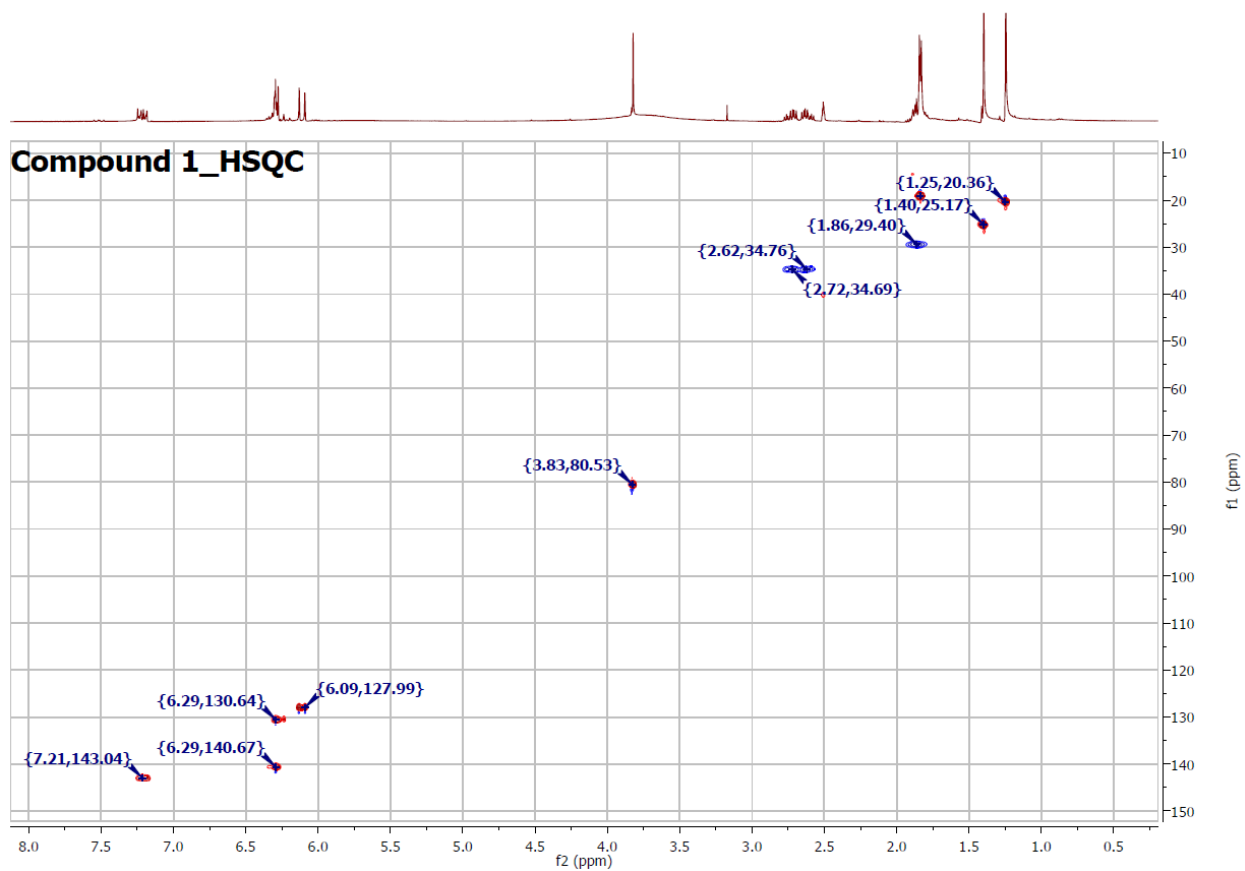**Figure S3: HSQC spectrum of compound 1 in DMSO-d₆ (400MHz)**

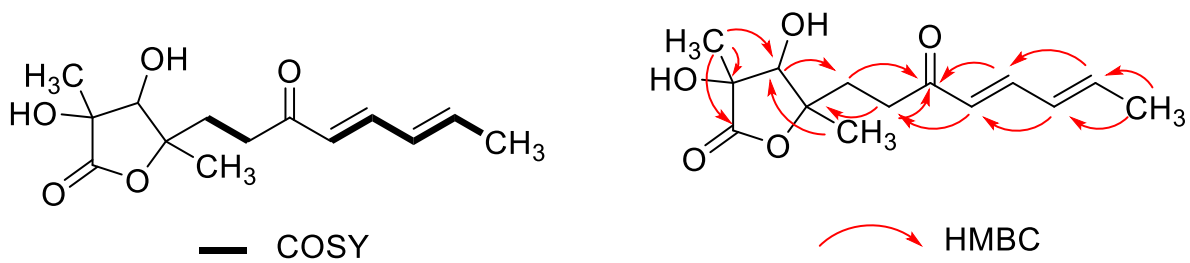**Figure S4. Key COSY (Bold) and HMBC (Single headed) correlations of 1**

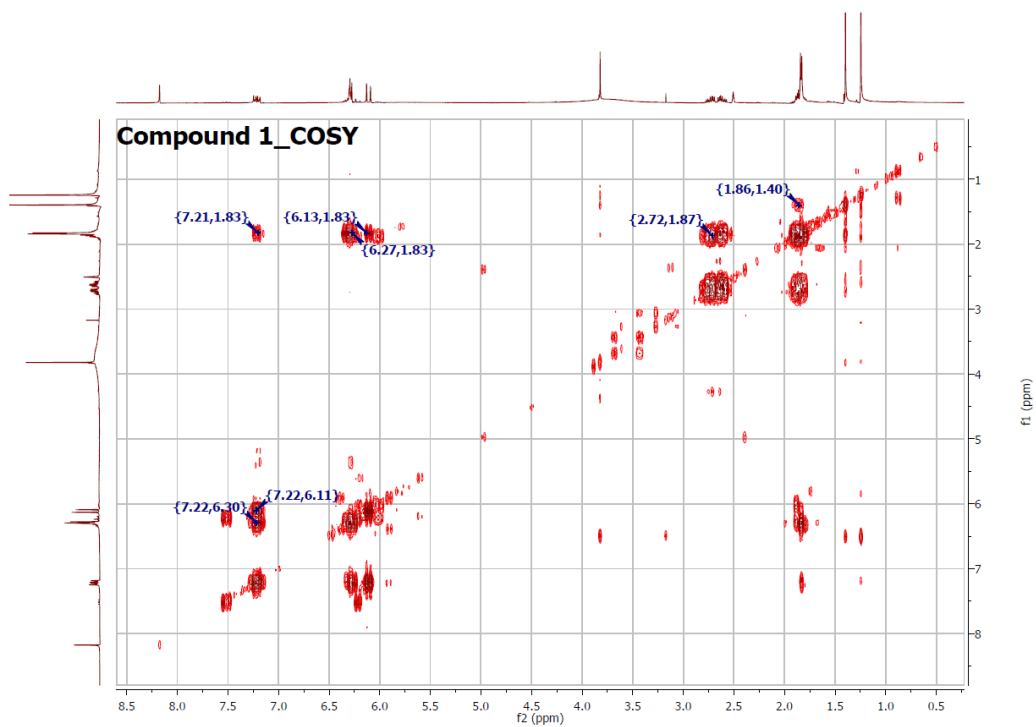**Figure S5: COSY spectrum of compound 1 in DMSO-d₆ (400MHz)**

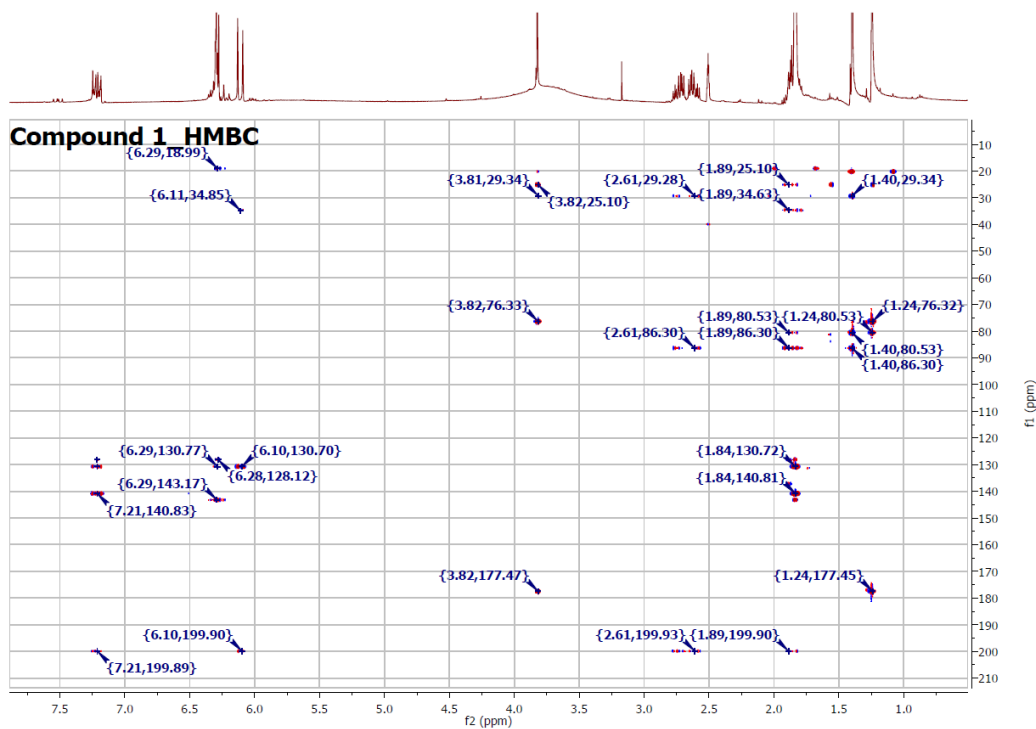**Figure S6: HMBC spectrum of compound 1 in DMSO-d₆ (400MHz)**

Figure S7: ROESY spectrum of compound 1 in DMSO-d6 (400MHz)

Figure S8: 1D NOE spectrum of compound 1 in DMSO-d6 (400MHz)
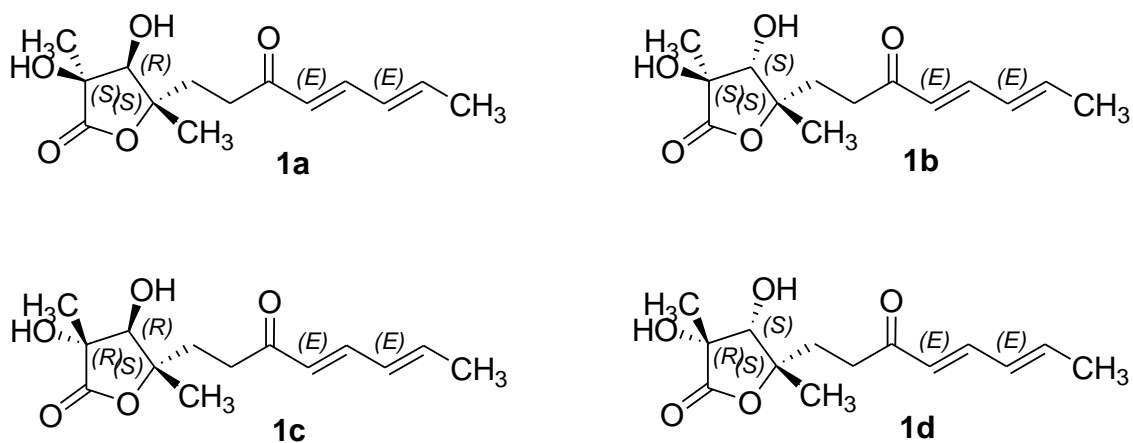
Figure S9: Structures of compounds 1a-d

Figure S10: DP4+ values computed for 1a-d

	1a	1b	1c	1d
sDP4+ (H data)	20.20%	65.46%	9.47%	4.87%
sDP4+ (C data)	0.03%	94.19%	0.09%	5.69%
sDP4+ (all data)	0.01%	99.53%	0.01%	0.45%
uDP4+ (H data)	39.43%	4.48%	0.98%	55.11%
uDP4+ (C data)	0.00%	99.75%	0.09%	0.16%
uDP4+ (all data)	0.02%	98.07%	0.02%	1.89%
DP4+ (H data)	58.24%	21.45%	0.68%	19.63%
DP4+ (C data)	0.00%	99.99%	0.00%	0.01%
DP4+ (all data)	0.00%	99.99%	0.00%	0.01%

Table S2: NMR data calculated for 1a-d

	Boltzmann averaged isotropic shielding values				Scaled chemical shifts			
	1a	1b	1c	1d	1a	1b	1c	1d
C1	23.7889	20.6948	22.4956	24.2647	174.6	175.4	174.4	173.3
C2	121.1896	116.0669	117.8501	122.1840	74.3	78.4	77.4	73.2
C3	119.1845	114.5515	113.4366	116.0933	76.3	79.9	81.9	79.4
C4	107.6427	107.1418	110.3009	110.3339	88.2	87.5	85.0	85.3
C5	160.4109	165.0703	161.6255	166.0748	33.8	28.5	32.8	28.3
C6	156.0973	155.8928	156.8311	157.7129	38.3	37.9	37.7	36.8
C7	-1.4244	-4.1647	-2.8783	-3.3249	200.6	200.7	200.2	201.6
C8	74.0797	74.0988	74.3532	74.4614	122.8	121.1	121.6	122.0
C9	52.1167	50.7280	50.1903	50.0103	145.4	144.9	146.2	147.0
C10	68.7027	69.0271	68.7374	68.7494	128.3	126.3	127.3	127.8
C11	48.9115	47.1945	47.8236	47.5465	148.7	148.5	148.6	149.5
C12	174.3011	174.2810	174.1244	174.1923	19.5	19.2	20.1	20.0
C13	173.5078	175.3946	176.2801	173.2498	20.3	18.0	17.9	20.9
C14	174.2169	168.7780	174.3264	168.5106	19.6	24.8	19.9	25.8
H3	27.3791	27.7226	27.4165	27.8494	3.93	3.58	3.89	3.54
H5	29.4946	29.3680	29.3942	29.5968	1.96	2.03	2.07	1.93
H6a	28.8735	28.7033	28.6767	28.6586	2.54	2.66	2.73	2.79
H6b	28.9852	28.7160	29.0647	29.0192	2.44	2.65	2.37	2.46
H8	25.1741	25.1799	25.1668	25.2102	5.98	5.98	5.96	5.97
H9	23.8128	23.8043	23.7927	23.7929	7.25	7.27	7.23	7.27
H10	24.9100	24.8975	24.9130	24.8976	6.23	6.24	6.20	6.26
H11	24.6705	24.6322	24.6055	24.6185	6.45	6.49	6.48	6.51
H12	29.5344	29.5174	29.5198	29.5105	1.93	1.89	1.95	2.01

Figure 11: Experimental and calculated ECD spectra of **1**
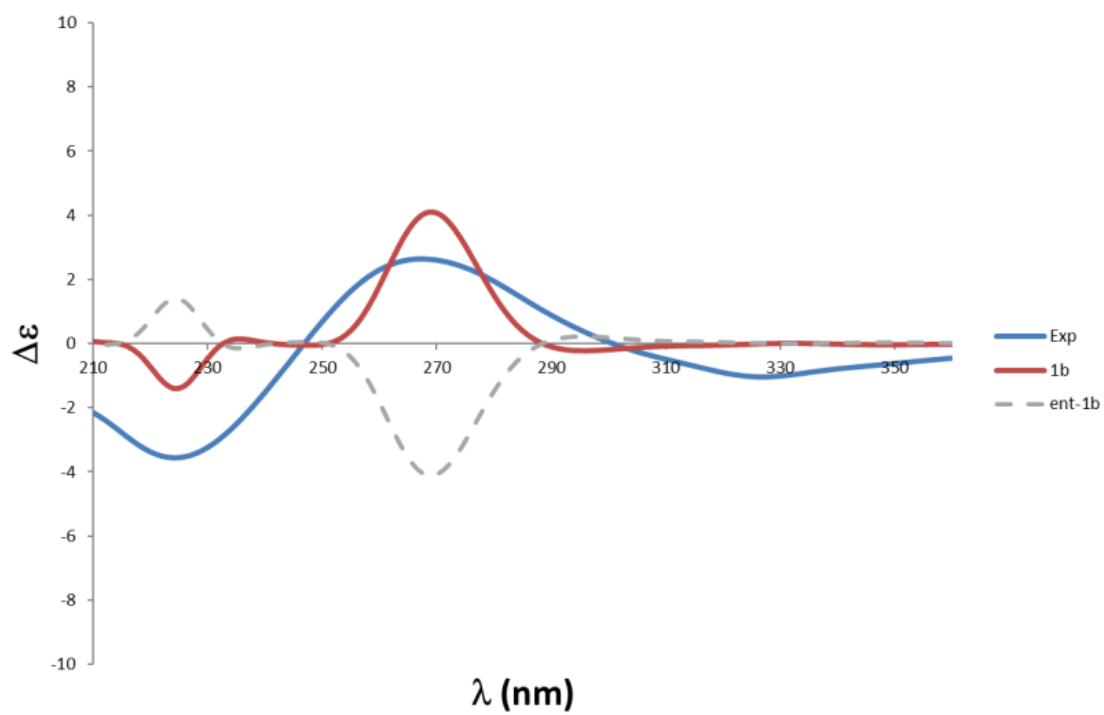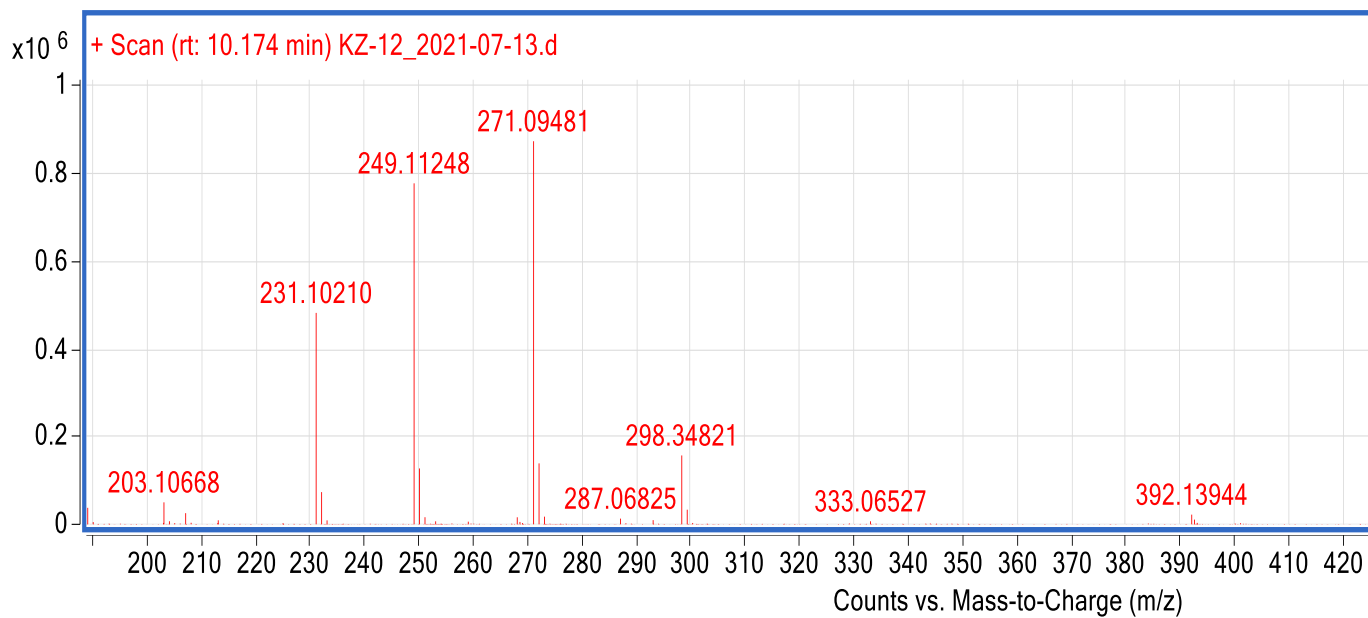

Figure S12: IR spectrum of compound 1
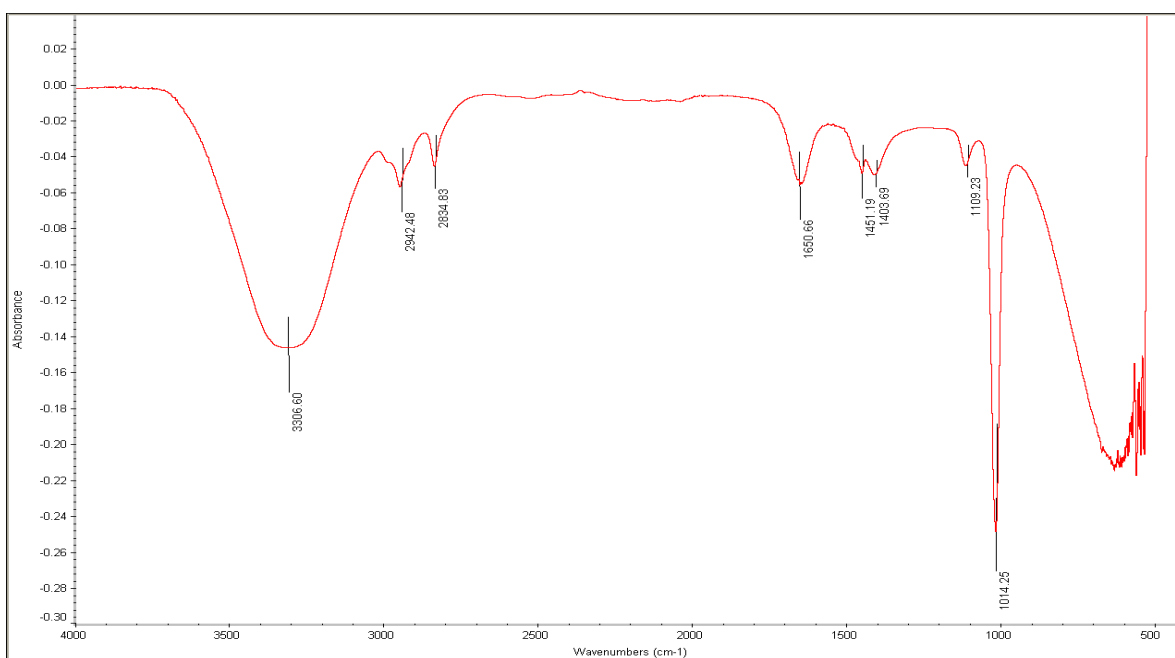
Figure S13: HRESIMS of compound 2

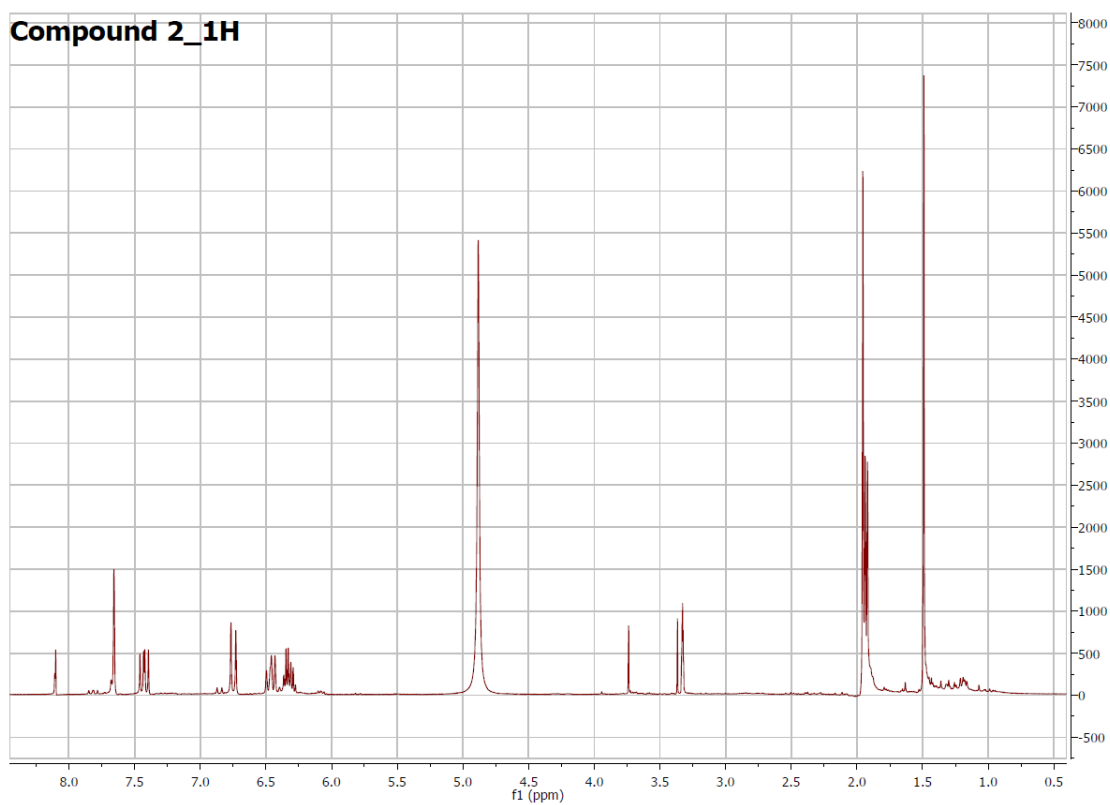**Figure S14: ¹H NMR spectrum of compound 2 in CD₃OD (400MHz)**
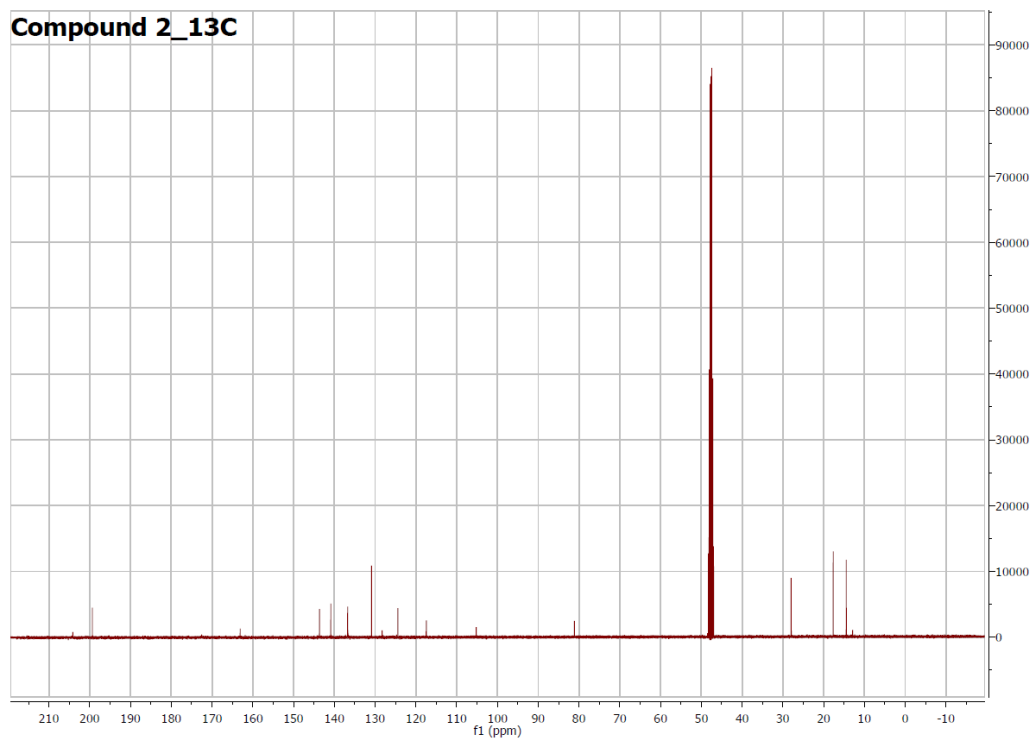
Figure S15: ^{13}C spectrum of compound 2 in CD₃OD (100MHz)

Figure S16: HSQC spectrum of compound 2 in CD₃OD (400MHz)
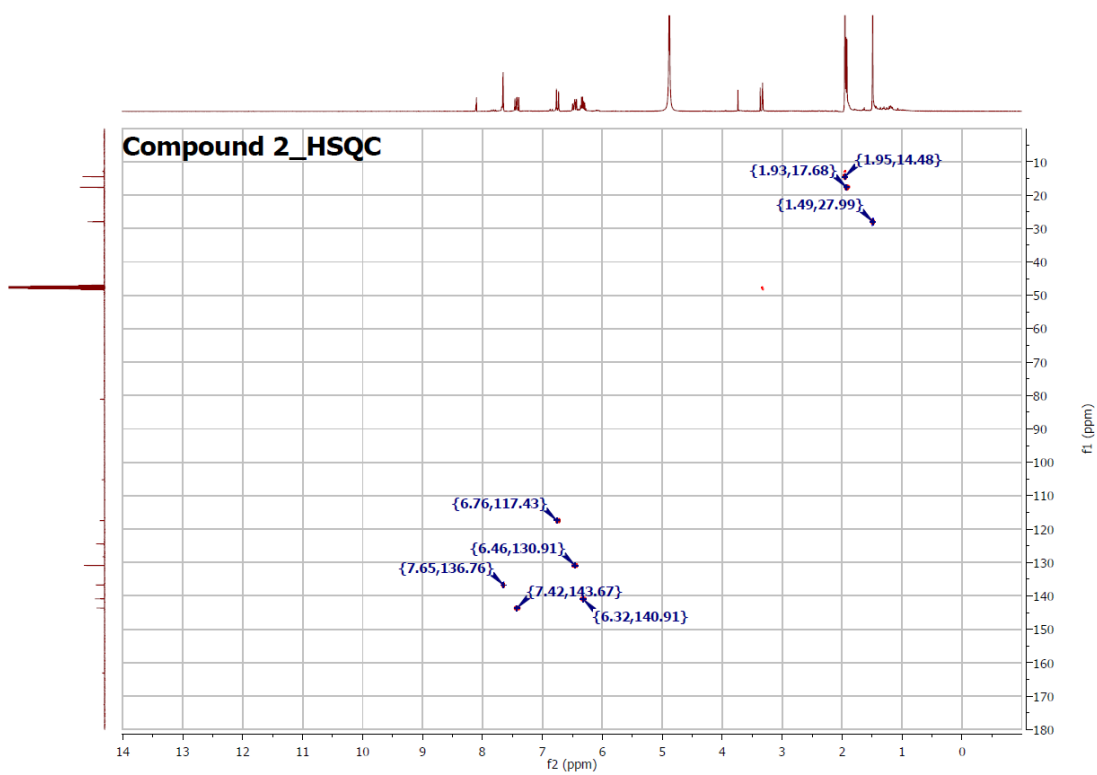
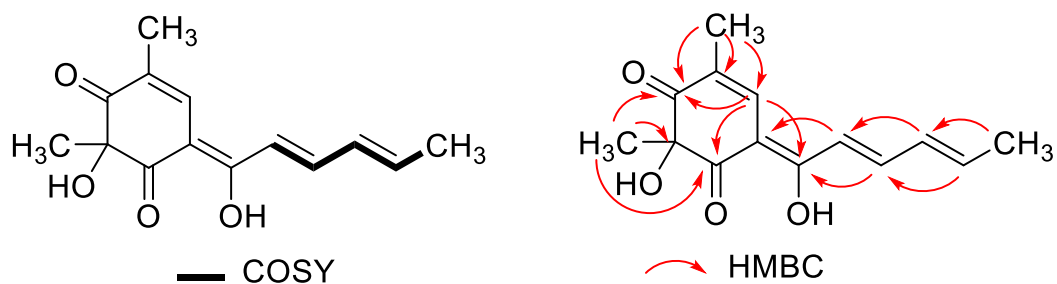**Figure S17: Key COSY (Bold) and HMBC (Single headed) correlations of 2.**
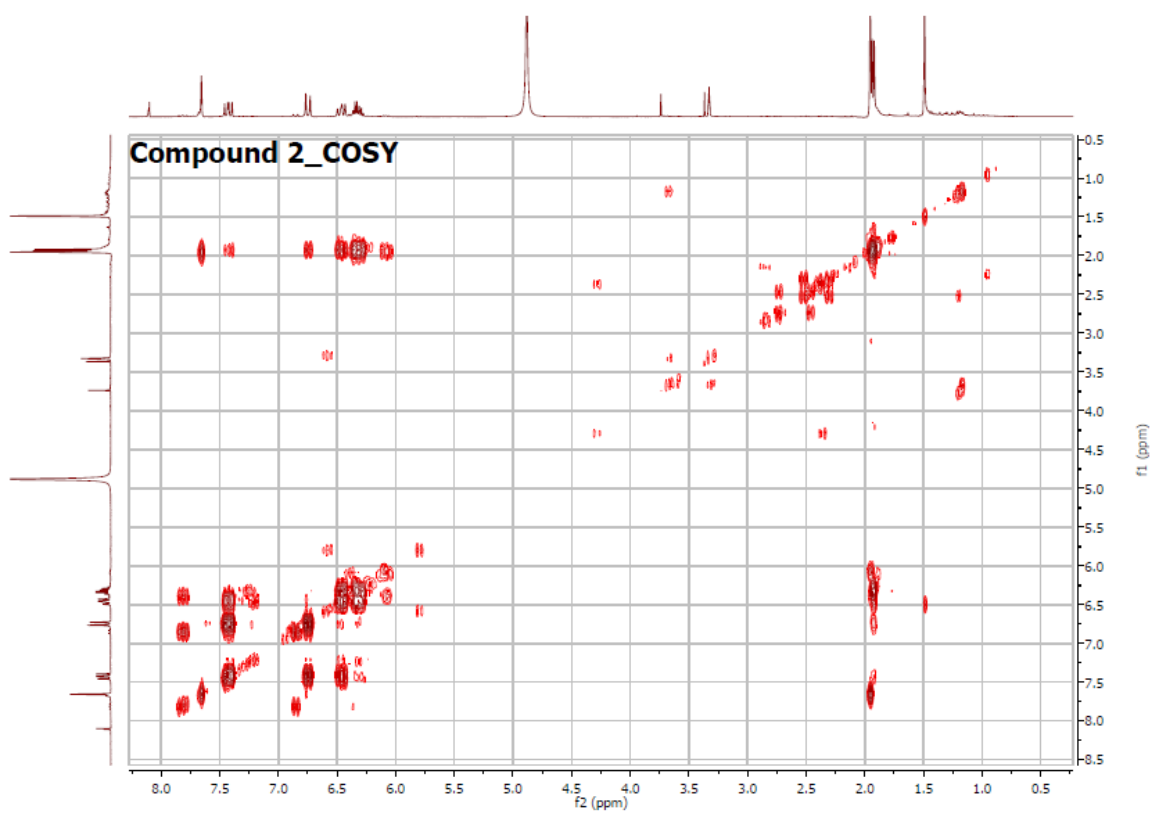

COSY

HMBC

Figure S18: COSY spectrum of compound 2 in CD₃OD (400MHz)

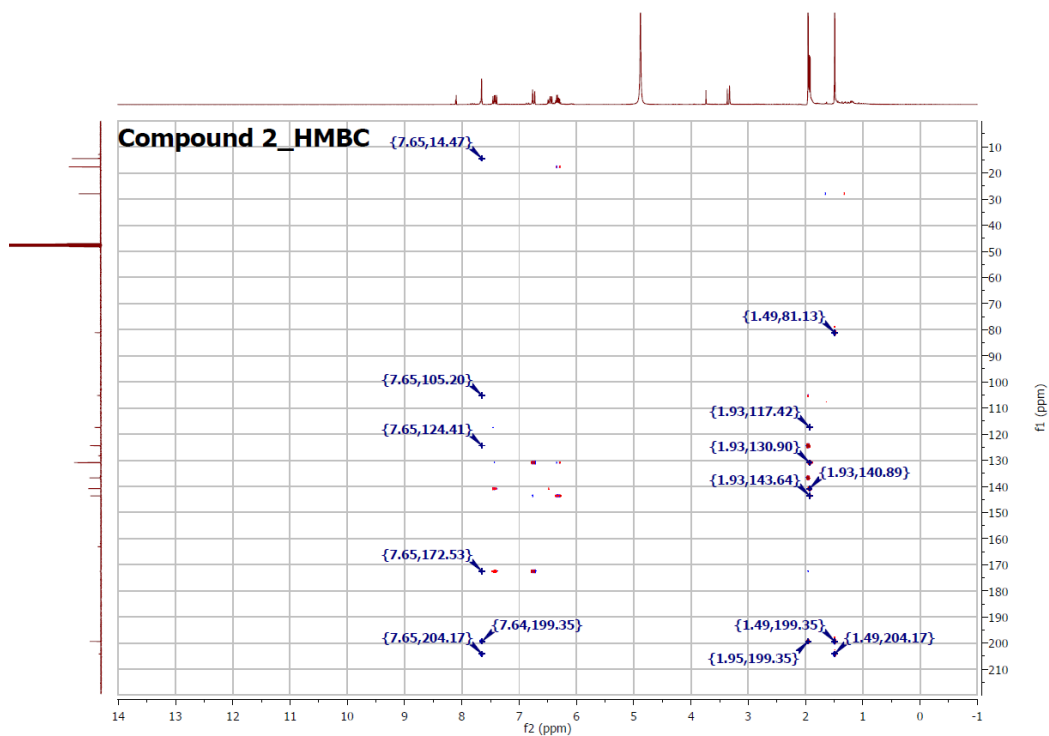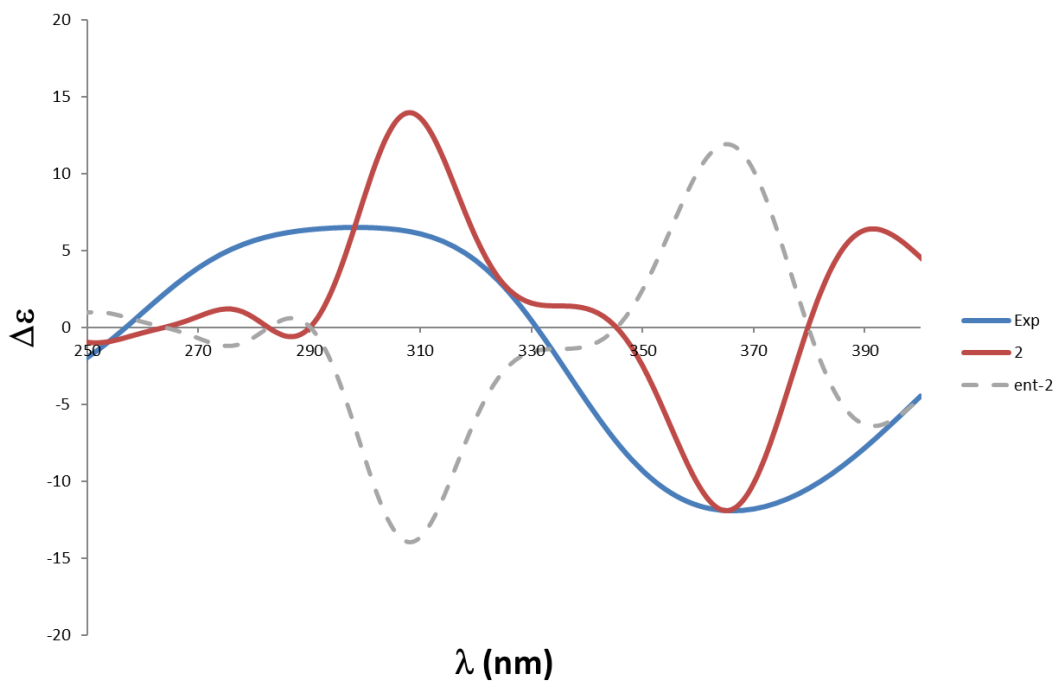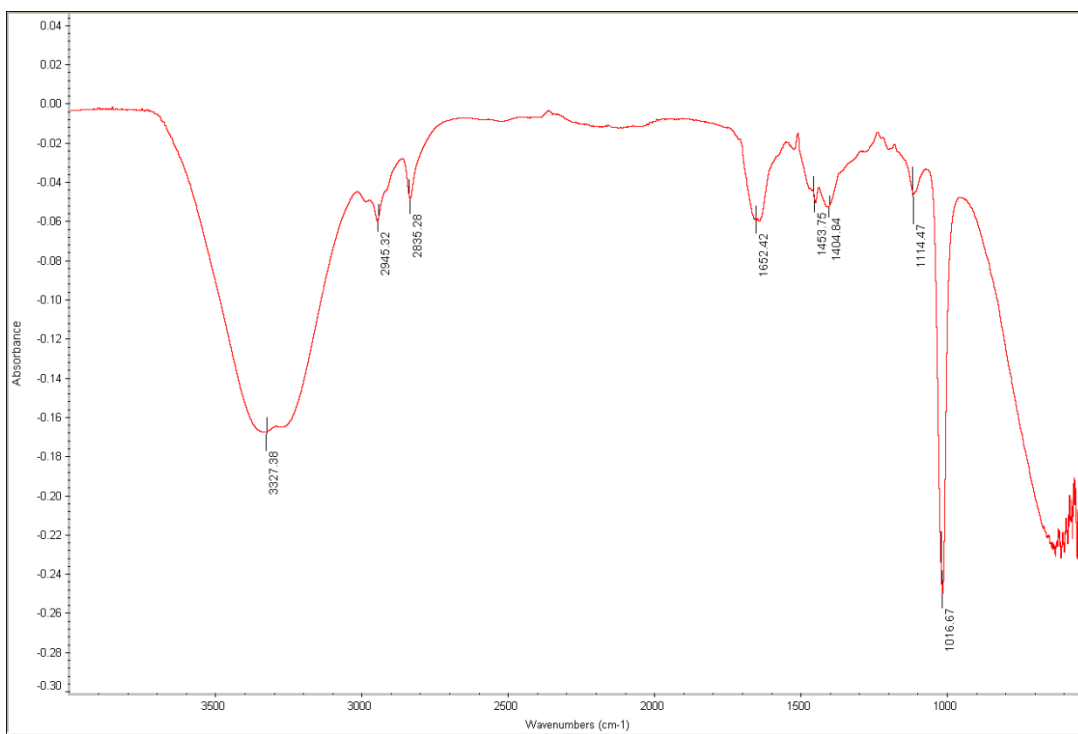**Figure S19: HMBC spectrum of compound 2 in CD₃OD (400MHz)**

Figure 20. Experimental and calculated ECD spectra of 2

Figure S21: IR spectrum of compound 2

Table S3. Activities of compounds **1**–**12** against human ovarian cancer cell line A2780 and

TNF-α-induced NF-κB

Compounds	IC$_{50}$ [µM]	
	A2780	**TNF-α-induced NF-κB**
1	13.60	13.83
2	16.50	-
3	36.20	-
4	47.40	-
5	11.03	-
6	4.60	-
7	0.50	24.40
8	3.90	14.63
9	4.53	-
10	5.80	-
11	8.07	-
12	3.76	-

Table S4. Antibacterial activities of compounds **6, 9, 10 and 12** against *S. aureus* (ATCC® 12600™), methicillin resistant *S. aureus* (ATCC®43300™), *B. subtilis* (ATCC®6633™)

Compounds	MIC [µg/mL]		
	S. aureus	**Methicillin resistant** *S. aureus*	*Bacillus subtilis*
6	40	40	-
9	20	20	-
10	-	-	80
12	10	10	20
Chloramphenicol	6.25	6.25	12.5