Supporting Information for

Oxazinin A, A Pseudodimeric Natural Product of Mixed Biosynthetic Origin from a Filamentous Fungus

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1. Experimental section

1.1 General experimental procedures. UV spectra were obtained using a Perkin-Elmer Lambda2 UV/vis spectrometer. Circular dichroism spectra were obtained on a Jasco J720A spectropolarograph. NMR data were collected using a Varian INOVA 600 (^{1}H 600 MHz, ^{13}C 150 MHz) NMR spectrometer equipped with a 5 mm ^{1}H[^{13}C,^{15}N] triple resonance cold probe with a z-axis gradient, utilizing residual solvent signals for referencing. FT-MS was performed at a LTQ FT Ultra Hybrid Mass Spectrometer (Thermo Scientific) and analyzed both manually using predicted masses from Monoisotopic calculator and using Mascot from Matrix Science. Supelco (St. Louis, MO) Discover HS (4.6 × 150 mm) and semipreparative (10 × 150 mm) C_{18} (5 μm) columns were used for analytical and semipreparative HPLC, respectively, as conducted on a Hitachi (Dallas, TX) Elite Lachrom System equipped with a Diode Array L-2455 detector. Antibacterial and cytotoxicity assay plates were evaluated by measuring A_{580} on a Multiskan FC plate reader (Fisher Scientific, Waltham, MA). Chemicals were sourced from Sigma-Aldrich (St.Louis, MO) or Fisher Scientific (Waltham, MA) with the exception of the following: fetal bovine serum (FBS) was obtained from Atlanta Biologicals (Lawrenceville, GA), ADC supplement from Remel (Lenexa, KS), and antimycotic/antibacterial supplement from MP Biomedicals (Solon, OH).

1.2. Cultivation, extraction, and isolation

Fungal material. Lissoclinum patella was collected in Papua New Guinea (*10.0370785 S 145.767741 E*) with appropriate governmental permission. The sample was immediately processed by aseptically cutting out a small square of tissue. This square was macerated in a sterile tissue grinder containing 10% glycerol in artificial seawater. The resulting ground tissue was serially diluted in the same buffer, and 10 microliters of 1/100 to 1/10,000 of the original sample volume were spread onto plates containing various media. Strain 110162 was purified into an axenic culture, and stored as a glycerol stock at -80 ^{o}C. The strain was subsequently recovered from a glycerol stock and used for further chemical analysis.

Fermentation and extraction. An agar grown culture of fungal strain 110162 was inoculated into 2 × 4L flasks, each containing 2 liters of ISP2 medium[1]. The culture was grown for 25 days at 30 ^{o}C. The broth was centrifuged and the supernatant was extracted with Diaion HP-20 resin for 4 h. The resin was filtered through cheesecloth and washed with H_2O to remove salts. The filtered resin was eluted with MeOH to yield a supernatant extract. The mycelium was extracted with 50% acetone in water, and the acetone extract was combined with the supernatant extract to yield the primary extract. The primary extract was dissolved in water and extracted with 3 × EtOAc to yield the final extract.

Purification. The final extract (452 mg) was dissolved in 2 mL MeOH and then saturated with RP18 silica gel (2g). The RP18 silica gel with extract was dried under vaccum and loaded on a glass column with RP18 silica gel (5g). The column was first flushed with 50 mL H_2O, and followed by 4 more washes using step-gradient elution of MeOH in H_2O (40%, 60%, 70%, 80%, and 100%; 100 mL each), leading to 5 fractions (Fr1-Fr5). Fractions (Fr1-5) were tested in antimicrobial assay, the active fraction Fr5 eluting in 100% MeOH and was further fractionated by C_{18} HPLC using 75% MeCN in H_2O to obtain pure compound **1** (1.5 mg).

Oxazinin A (**1**)**:** pale yellow solid; UV (MeOH) λ_{max} 221, 261, 341 nm; ^{1}H and ^{13}C NMR (see Table 1); HRESIMS *m/z* 947.4460 $[M+H]^{+}$ (calcd for $C_{58}H_{63}N_2O_{10}$, 947.4482).

1.3 Biological activity

Antimicrobial broth dilution assays. The assay was performed as previously described,[2] with the exception that 4 wells per compound were used for each dilution for MIC determination. DMSO served as negative control. Bacterial strains used, along with the positive control antibiotic for each strain, were: *M. tuberculosis* H37Ra (rifampicin MIC = 0.125 ug/mL); *P. aeruginosa* ATCC 27853 (gentamicin MIC = 6.25 ug/mL); *B. subtilis* ATCC 6633 (gentamicin MIC = 1.25 ug/mL); and *B. cepacia ATCC 25608* (chloramphenicol MIC = 12.5 ug/mL).

Cytotoxicity assay. Cytotoxicity testing was performed using CEM-TART lymphoblastoid cells[3] cultured in RPMI 1640 with 20% FBS and antibiotic/antimycotic supplement at 37 ^{0}C with 5% CO_2 in moisture saturated atmosphere in 96 well culture clusters. Test compounds dissolved in 1 µL DMSO, negative controls (DMSO alone) or positive controls (doxorubicin in DMSO) were added in triplicate wells together with 50,000 cells in 200 µL of fresh media and incubated for 72 hours. Each well then received 11 µL of 5 mg/ml MTT (Sigma St. Louis, MO) followed by 2 hours incubation after which the formazan precipitate was pelleted by centrifugation. The spent media was aspirated and the formazan crystals solubilized in DMSO. Absorbance at 580 nm was quantified on a Multiskan FC (Fisher Scientific, Waltham, MA) plate reader and inhibition determined as described in the antimycobacterial assay procedure.

Transient receptor potential channel assay. Fluorometric cell-based Ca^{2+} flux assays were performed using a BMG Labtech NOVOStar fluorescence plate reader equipped with a plate-to-plate reagent delivery system. Human embryonic kidney (HEK-293) and immortalized bronchial epithelial (BEAS-2B) cells that stably overexpress human TRPA1, M8, V1, V2, V3, or V4 have been previously described.[4] TRPV1-overexpressing BEAS-2B cells were grown to confluence in 96-well plates in LHC-9 growth media containing Geneticin (300 µg/mL). TRPA1, M8, V3, and V4 overexpressing HEK-293 cells were grown to confluence in 2% (w/v) gelatin-coated 96-well plates in DMEM:F12 media supplemented with 5% fetal bovine serum and Geneticin (300 µg/mL). Cells were prepared for assay by replacing the growth media with a 1:1 solution of LHC-9 and Fluo 4-Direct (Invitrogen) reagent containing Fluo 4-AM, pluronic F-127, probenecid, and a proprietary quencher dye. BEAS-2B cells were incubated at room temperature (~22 ºC) for 1 h in the dark while HEK-293 cells were incubated at 37ºC for 1h in a cell culture incubator. Cells were subsequently washed by replacing the loading solution with LHC-9 containing 750 μM water-soluble probenecid (Invitrogen), 750 µM Trypan Red (ATT Bioquest), and various concentrations of the test compounds. Both cell types were incubated for an additional 30 min at 37ºC to allow for Fluo 4-AM cleavage and activation as well as equilibration to both the test compounds and Fluo 4. Assays were initiated by addition of TRP channel-specific agonists (3x stock) to the final pre-determined agonist concentration. The agonists were as follows: TRPA1 (2,4-*ditert* buylphenol at 250 µM), TRPM8 (icilin at 50 µM), TRPV1 (nonivamide at 5 µM), TRPV3 (carvacrol at 300 µM), and TRPV4 (GSK1016790A at 30 nM). Changes in cellular fluorescence were monitored for 1 min at 37 ºC. Data were quantified as the maximum rate of change in fluorescence intensity (max ΔF/s), vs. media only treatment (negative control) and were represented as the percentage of response relative to the agonist only (no inhibitor) control. A minimum of three replicates were used for all treatments and IC_{50} values were calculated from dose-response curves of % remaining cell response vs. concentration of test agent using either the

sigmoidal dose response (variable slope) curve fit in GraphPad Prism 5 software (GraphPad Software Inc.).

1.4 Computer modeling

The proposed structure possibilities B and C were initially optimized using Density Functional Theory with the Truhlar functional M06-2X[5] with the 6-31G(d) basis set as implemented in the D.01 revision of the Gaussian package.[6] The resulting structure was parameterized using the General Amber Force Field[7] and was used as the starting structure in a molecular dynamics simulation (MD) using an extensive annealing protocol. After 1000 steps of geometry optimization using steepest descent and conjugate gradients, the structure was heated to 3000K and slowly reducing the temperature every 5000 steps of MD simulation until a target temperature of 300K was reached. After the heating stage, distance restraints shown in Table S2 were applied for 50000 steps. The NOE's considered with a strong signal were restrained with an upper limit of 2.7 Å, medium limit with 3.3 Å and weak with 5.0 Å. The simulation was run using implicit solvation with a time step of 1 femtosecond. The refinement of the structure was made with the AMBER 14 molecular dynamics package.[8]

2. Table S1. ^{1}H, ^{13}C, ^{15}N NMR data and key ROESY correlations of compound 1.

No.	DMSO-d_6		CD3CN		
	^{1}H NMR (*J* in Hz)	^{13}C NMR	^{1}H NMR (*J* in Hz)	$^{13}C/^{15}N$ NMR	Key ROESY
2		84.6 C		85.0 C	
3	4.22 d (5.0)	76.1 CH	4.48 s	76.9 CH	H-16
3-OH	5.99 d (4.8)				
4		193.7 C		194.1 C	
5		ND		118.9 C	
6	7.18 s	126.2 CH	7.25 s	126.5 CH	H-18, H-19
7		135.2 C		135.7 C	
8		144.3 C		145.6 C	
9		119.8 C		119.8 C	
10		156.1 C		156.6 C	
11	4.47 m	45.5 CH	4.56 brd (10.3)	46.0 CH	H-25', H-18, H-12
12	3.82 brs	58.6 CH	3.85 brs	59.1 CH	H-13, H-14, H-27, H-24'
13	5.19 dd (15.8, 4.3)	128.4 CH	5.22 dd (15.5, 4.5)	128.5 CH	H-24, H-11(weak), H-15
14	5.30 dq (15.8, 5.9)	126.2 CH	5.29 dq (15.5, 6.2)	126.7 CH	H-15, H-12, H-24
15	1.40 d (6.0)	17.6 CH_3	1.42 d (6.2)	16.6 CH_3	
16	1.50 s	26.0 CH_3	1.62 s	26.7 CH_3	H-24 (weak), H-3
17	1.11 s	19.1 CH_3	1.09 s	17.5 CH_3	H-24
18a	3.54 dd (16.3, 4.2)	29.7 CH_2	3.58 dd (15.8, 5.4)	30.3 CH_2	H-16', H-25, H-11
18b	3.27 m		3.35 dd (15.8, 8.2)		H-21, H-6, H-16' (weak), H-25'(weak), H-11 (weak)
19	5.01 t (6.5)	122.3 CH	5.03 t (6.2)	122.0 CH	H-22, H-6
20		133.1 C		134.5 C	
21	1.60 s	18.1 CH_3	1.61 s	17.2 CH_3	
22	1.67 s	25.9 CH_3	1.72 s	25.7 CH_3	
24	6.44 s	80.0 CH	6.51 s	80.8 CH	H-13, H-17
25		-		62.3 N	
26		148.9 C		149.1 C	
27	6.41 d (8.0)	111.9 CH	6.41 d (8.0)	112.4 CH	
28	7.42 dd (7.9, 7.6)	135.0 CH	7.38 dd (7.9, 7.5)	135.1 CH	H-27
29	7.00 dd (7.6, 7.50)	118.8 CH	7.00 dd (7.7, 7.6)	119.1 CH	H-28
30	8.04 d (8.00)	130.1 CH	8.09 d (8.0)	130.4 CH	H-29
31		114.0 C		115.0 C	
32		165.2 C		166.1 C	
2'		84.6 C		84.9 C	
3'	4.46 m	76.2 CH	4.55 s	77.3 CH	H-16'
3'-OH	6.00 d (4.9)				
4'		194.5 C		194.4 C	
5'		119.3 C		119.3 C	
6'	7.35 s	124.1 CH	7.41 s	124.6 CH	H-18', H-19'
7'		132.6 C		134.0 C	
8'		145.5 C		146.9 C	
9'		126.3 C		126.5 C	
10'		156.4 C		157.2 C	
11'	5.29 d (15.6)	124.5 CH	5.40 d (16.0)	125.0 CH	H-18'
12'	5.50 dd (15.6, 10.3)	136.5 CH	5.58 dd (16.0, 10.8)	137.6 CH	
13'	5.80 dd (15.6, 11.0)	130.2 CH	5.90 dd (16.1, 10.8)	130.6 CH	
14'	5.10 dq (14.6, 6.1)	131.3 CH	5.16 dq (16.2, 6.5)	131.8 CH	

15’	1.64 d (6.4)	18.7 CH_3	1.68 d (6.5)	17.6 CH_3	
16’	1.66 s	26.4 CH_3	1.80 s	26.3 CH_3	H-25’(weak), H-18, H-3’
17’	1.44 s	18.5 CH_3	1.46 s	17.2 CH_3	H-25’
18’	2.71 m	31.8 CH_2	2.75 m	31.6 CH_2	H-6’, H-21’, H-11’
19’	4.74 t (7.3)	121.3 CH	4.81t (7.4)	122.0 CH	H-18’, H-22’ H-6’
20’		133.3 C		134.1 C	
21’	1.50 s	19.2 CH_3	1.51 s	17.6 CH_3	
22’	1.57 s	26.2 CH_3	1.66 s	25.5 CH_3	
24’	5.82 dd (10.4, 10.1)	55.7 CH	5.95 dd (10.4, 10.3)	56.4 CH	H-25’, H-12, H-11, H-27’
25’	9.01 d (10.4)		9.02 d (10.6)	73.1 NH	H-16’, H-17’, H-18, H-11, H-24’
26’		150.3 C		150.4 C	
27’	6.22 d (8.0)	111.5 CH	6.25 d (8.1)	112.0 CH	
28’	6.75 dd (8.0, 7.9)	132.8 CH	6.82 dd (8.0, 7.9)	133.4 CH	
29’	6.25 dd (7.6, 7.5)	113.7 CH	6.31 dd (7.7, 7.6)	113.4 CH	H-28’
30’	7.62 d (7.7)	131.1 CH	7.72 d (8.0)	131.5 CH	H-29’
31’		109.5 C		109.0 CH	
32’		171.8 C		170.9 C	
32’-OH	12.53 s	-	10.34 s	-	

Table S2. The distance restraints between the protons that have NOESY correlations for compound 1.

		Possibility C				Possibility B			
First atom	Last atom	curr. Value (Å)	Target (Å)	Deviation (Å)	penalty	curr. Value (Å)	Target (Å)	Deviation (Å)	penalty
H-25’	H-17’	2.255	3.3	0	0	2.2	3.3	0	0
H-25’	H-16’	2.028	3.3	0	0	3.014	3.3	0	0
H-25’	H-18A	2.251	3.3	0	0	3.409	3.3	0.109	0.236
H-25’	H-18B	2.741	3.3	0	0	3.476	3.3	0.176	0.619
H-25’	H-11	2.799	2.7	0.099	0.194	2.24	2.7	0	0
H-24’	H-27’	3.41	3.3	0.11	0.243	3.225	3.3	0	0
H-24’	H-11	3.029	3.3	0	0	2.172	3.3	0	0
H-24’	H-12	3.141	5	0	0	2.919	5	0	0
H-18A	H-17’	2.142	5	0	0	3.697	5	0	0
H-18A	H-11	2.239	3.3	0	0	3.554	3.3	0.254	1.288
H-18B	H-6	2.741	2.7	0.041	0.034	2.742	2.7	0.042	0.035
H-18B	H-21	2.008	2.7	0	0	2.022	2.7	0	0
H-18B	H-11	3.72	5	0	0	4.38	5	0	0
H-18B	H-17’	2.856	5	0	0	2.265	5	0	0
H-19	H-22	2.057	2.7	0	0	2.067	2.7	0	0
H-3’	H-17’	2.329	3.3	0	0	2.3	3.3	0	0
H-13	H-24	2.708	2.7	0.008	0.001	2.204	2.7	0	0
H-24	H-17	3.475	5	0	0	3.379	5	0	0
H-3	H-16	2.248	3.3	0	0	2.576	3.3	0	0
		2.669	2.7	0	0	2.723	2.7	0.023	0.01
Total distance penalty:		0.472				2.188			

2.3. Coordinates of the refined structures of possible stereoisomers B and C.

Table S3. Structure with relative configuration 3*S**,11*R**,12*S**,24*S**,3’*R**,24’*R** (possibility C)

C	-0.68800	-0.26100	0.88400
C	1.48600	0.30400	2.36800
C	-1.55000	0.31100	-0.27600
C	-1.68200	-0.26800	-1.56500
C	-2.35100	0.49200	-2.56800
C	-3.03800	1.66500	-2.22900
C	-3.05300	2.12400	-0.92200
C	-2.28800	1.46200	0.01900
C	1.09800	0.14300	3.71900
C	1.95700	-0.47000	4.63200

C	3.24600	-0.82900	4.25100
C	3.70700	-0.43300	3.00600
C	2.86200	0.20600	2.10400
C	-0.20100	0.69500	4.28500
C	0.00500	1.53600	5.51600
C	-0.44800	1.24200	6.70500
C	-0.14900	2.24100	7.78500
C	-1.20500	-0.02200	7.05200
C	4.17000	-1.55400	5.18400
O	3.78300	-1.92200	6.28600
C	5.61000	-1.81400	4.72300
O	5.02300	-0.66900	2.67500
O	-2.33900	1.92500	1.30200
C	-3.88700	3.29800	-0.48500
O	-4.45600	3.99800	-1.30900
C	-4.02300	3.57100	1.02500
C	3.50800	0.91900	0.92400
C	-2.35500	0.18300	-4.06000
C	-3.63400	-0.49800	-4.51300
C	-3.77600	-1.29000	-5.57800
C	-5.10900	-1.93700	-5.90700
C	-2.67300	-1.59600	-6.57600
C	-2.21700	-1.77700	1.98400
O	4.42800	1.86600	1.49700
C	4.98700	2.79500	0.71800
C	4.28400	3.14700	-0.54800
C	3.03600	2.58300	-0.81200
O	6.02500	3.34200	1.06000
C	4.84400	4.07900	-1.42500
C	4.15700	4.45200	-2.57700
C	2.90600	3.91000	-2.84500
C	2.34200	2.99300	-1.96000
C	5.72200	-1.82000	3.18300
C	7.19500	-1.64100	2.78000
C	5.11900	-3.07900	2.51300
C	-3.59100	2.34300	1.84500
C	-3.25100	2.68100	3.29000
C	-4.61200	1.18100	1.83400
O	6.18800	-3.01000	5.30800
O	-5.35500	4.02900	1.39500
C	0.52400	0.70100	1.21500
N	-1.58900	-0.56200	2.00400
C	1.30600	0.89900	-0.12700
H	0.64000	1.44800	-0.78600

N	2.54800	1.65600	0.08600
C	-0.43200	-2.89000	3.39100
C	-1.75500	-2.88200	2.70300
C	-2.50800	-4.05900	2.72200
C	-3.68200	-4.16200	1.98200
C	-4.09700	-3.09100	1.19900
C	-3.35900	-1.91000	1.19500
O	0.63000	-2.73400	2.82600
C	-1.15700	-1.66100	-1.79500
C	-1.31800	-2.54300	-2.97400
C	-0.64900	-3.94300	-2.89700
C	-0.83800	-4.83500	-3.97100
C	-0.16800	-6.18600	-3.96100
O	-0.48200	-3.16200	4.66000
H	-3.28200	4.33400	1.28000
H	-0.25200	-1.21200	0.62600
H	-3.58700	2.21300	-3.00000
H	1.60400	-0.66300	5.64700
H	-0.69400	1.31300	3.53500
H	-0.86500	-0.12300	4.58700
H	0.53400	2.47600	5.48800
H	0.39700	3.11800	7.43800
H	-1.07300	2.60400	8.18200
H	0.48500	1.77600	8.52000
H	-2.27700	0.14000	6.98800
H	-0.94500	-0.88600	6.44300
H	-0.93500	-0.34800	8.03500
H	6.19100	-0.94900	5.04400
H	4.08000	0.17600	0.33200
H	-2.27500	1.13200	-4.59900
H	-1.45400	-0.37000	-4.33200
H	-4.49100	-0.32200	-3.86300
H	-5.45500	-1.60300	-6.88300
H	-5.01300	-3.02600	-5.94200
H	-5.86800	-1.68300	-5.15100
H	-3.02500	-1.45100	-7.60300
H	-2.36600	-2.63900	-6.49300
H	-1.79200	-0.96400	-6.45000
H	5.81400	4.53300	-1.21300
H	4.60000	5.18600	-3.26100
H	2.36000	4.20100	-3.74300
H	1.36900	2.59500	-2.21800
H	7.76000	-2.55500	2.97500
H	7.65600	-0.83000	3.34400

H	7.26300	-1.38600	1.72700
H	5.70600	-3.96400	2.74000
H	4.09300	-3.26500	2.84100
H	5.10000	-2.96100	1.42400
H	-2.56400	3.52200	3.34100
H	-4.14500	2.85500	3.86000
H	-2.76200	1.83500	3.73600
H	-4.93600	0.92100	0.82300
H	-4.21500	0.30000	2.33200
H	-5.49100	1.45900	2.40500
H	6.03400	-2.98400	6.30100
H	-5.59700	4.81700	0.82200
H	0.11300	1.67100	1.47800
H	-2.19000	0.26500	2.22000
C	1.69800	-0.40100	-0.82000
H	-2.16300	-4.91300	3.30400
H	-4.25200	-5.09100	1.99500
H	-4.99300	-3.17400	0.58000
H	-3.65900	-1.09200	0.55200
H	-0.64000	-2.15900	-0.98500
H	-2.05300	-2.35000	-3.75500
H	-0.00900	-4.22100	-2.04400
H	-1.45900	-4.58700	-4.82800
H	0.89400	-6.09000	-3.72300
H	-0.64200	-6.85200	-3.23200
H	-0.24500	-6.65200	-4.94800
H	0.47900	-3.19700	4.90700
H	1.76800	0.33400	-2.79100
C	1.89200	-0.52100	-2.12700
H	1.84200	-1.26200	-0.17300
C	2.29700	-1.81100	-2.79600
H	1.55400	-2.09700	-3.54300
H	2.40300	-2.62800	-2.07000
H	3.25200	-1.68200	-3.31700

Table S4. Structure with relative configuration 3*S**,11*S**,12*S**,24*S**,3'*R**,24'*R** (possibility B)

C	-0.77300	-0.68100	-0.81900
C	2.00100	-0.70300	-0.21300
C	-1.40000	0.33800	0.08200
C	-2.76800	0.71000	-0.19500
C	-3.49200	1.56600	0.74000

C	-2.93800	1.82800	2.07700
C	-1.61900	1.37700	2.27300
C	-0.91100	0.54700	1.33700
C	2.58200	-1.83600	0.49400
C	3.95500	-1.90500	0.74900
C	4.81800	-0.99400	0.33100
C	4.20600	0.19900	-0.05300
C	2.83200	0.46200	-0.12800
C	1.86900	-3.19100	0.54800
C	2.38200	-4.36000	1.29300
C	3.01700	-5.43300	0.81800
C	3.47300	-6.52400	1.80400
C	3.24100	-5.62000	-0.71700
C	6.33500	-1.18600	0.07700
O	7.04300	-1.86000	0.87800
C	6.92600	-0.51000	-1.20200
O	5.06700	1.06000	-0.73800
O	0.23500	-0.15800	1.83200
C	-0.80500	1.96100	3.33100
O	-1.29700	2.82200	3.98800
C	0.58600	1.39900	3.57300
C	2.33800	1.82500	-0.58700
C	-4.85200	2.44000	0.38500
C	-6.21500	2.08000	1.23000
C	-7.20900	2.82700	1.82200
C	-8.45800	2.16100	2.42300
C	-7.19100	4.38200	1.90600
C	-1.96600	-2.79900	-0.77900
O	2.66400	2.87000	0.37700
C	2.08300	4.14100	0.11800
C	0.71300	4.20300	-0.39100
C	0.23700	3.06800	-1.00000

O	2.72300	5.09300	0.29900
C	-0.06500	5.43500	-0.35800
C	-1.29800	5.54600	-0.99300
C	-1.76300	4.36500	-1.53700
C	-1.06900	3.17400	-1.48600
C	5.96400	0.54700	-1.78700
C	6.79000	1.72300	-2.26900
C	5.05700	-0.02800	-2.87400
C	0.69000	-0.12600	3.23800
C	2.21100	-0.47000	3.47400
C	-0.25100	-0.91700	4.18800
O	7.31600	-1.49400	-2.14700
O	0.89600	1.51200	4.89200
C	0.77300	-0.49500	-1.16200
N	-0.78600	-2.03200	-0.34800
C	0.71100	0.83000	-1.98100
H	-0.30700	0.89000	-2.34900
N	0.95100	1.90100	-0.99100
C	-2.82700	-2.96900	1.61200
C	-2.89300	-3.13300	0.14300
C	-3.95000	-4.00000	-0.24900
C	-4.02500	-4.64800	-1.43000
C	-2.99300	-4.32200	-2.31100
C	-1.98700	-3.44100	-2.01900
O	-3.04500	-3.78400	2.51300
C	-3.43000	0.20200	-1.44400
C	-4.70900	-0.35900	-1.48600
C	-5.18800	-0.96000	-2.90600
C	-6.72300	-0.96800	-3.08600
C	-7.21400	-1.95400	-4.19600
O	-2.28800	-1.89500	2.08300
H	1.09700	2.01500	2.80100

H	-1.23900	-0.63400	-1.84600
H	-3.51800	2.45300	2.76600
H	4.36300	-2.91200	0.90600
H	0.99500	-2.86600	1.12100
H	1.62100	-3.45300	-0.51000
H	2.20700	-4.32100	2.38300
H	3.16700	-7.51900	1.47200
H	4.55500	-6.53400	1.82500
H	3.16200	-6.36600	2.81200
H	3.16300	-6.65500	-0.98800
H	2.55800	-5.04600	-1.30500
H	4.20600	-5.20700	-0.98800
H	7.79700	-0.00500	-0.88700
H	1.07000	-1.23700	-1.92100
H	3.02400	2.07800	-1.39400
H	-5.17000	2.13600	-0.62200
H	-4.65800	3.51000	0.20500
H	-6.33300	1.00000	1.36900
H	-8.40600	2.13600	3.50900
H	-8.55200	1.15000	2.08400
H	-9.38300	2.69700	2.17300
H	-7.11500	4.58000	2.95400
H	-8.20400	4.69800	1.68600
H	-6.46700	4.90600	1.29300
H	0.48200	6.21100	0.11400
H	-1.85200	6.50400	-1.06600
H	-2.74700	4.38700	-1.99600
H	-1.59000	2.26000	-1.82600
H	6.18600	2.60000	-2.49600
H	7.39300	1.43700	-3.13300
H	7.44300	2.04800	-1.47900
H	4.30700	-0.72500	-2.51500

H	4.54700	0.81100	-3.31100
H	5.65500	-0.47100	-3.67100
H	2.96200	-0.00400	2.82900
H	2.43700	-0.18600	4.50000
H	2.43700	-1.51600	3.27400
H	-1.29100	-0.61900	4.09600
H	0.04300	-0.75100	5.21700
H	-0.21300	-1.97000	3.88700
H	8.28000	-1.32300	-2.62200
H	1.95500	1.50600	5.10400
H	-0.50800	-2.00800	0.58900
C	1.66800	0.93700	-3.09300
H	-4.63000	-4.20600	0.62400
H	-4.81300	-5.33700	-1.59300
H	-2.99500	-4.90300	-3.25900
H	-1.10700	-3.31100	-2.77300
H	-3.02900	0.19000	-2.43400
H	-5.60500	-0.13900	-0.55300
H	-4.59300	-1.25900	-3.79800
H	-7.65200	-0.81700	-2.35600
H	-7.36800	-1.46300	-5.23500
H	-6.51700	-2.86500	-4.20800
H	-8.21000	-2.28800	-3.91200
H	-2.33700	-1.99600	3.12400
H	0.30600	1.66100	-4.36600
C	1.33200	1.27200	-4.30300
H	2.56400	0.38900	-2.94400
C	2.05600	1.20900	-5.67500
H	1.50800	0.51500	-6.31500
H	3.08700	0.89800	-5.66400
H	1.94300	2.16500	-6.19100

3. NMR and ESI-FT-ICR-MS spectra of 1.

Figure S1. ^{1}H NMR spectrum of compound **1** in DMSO-d_6.

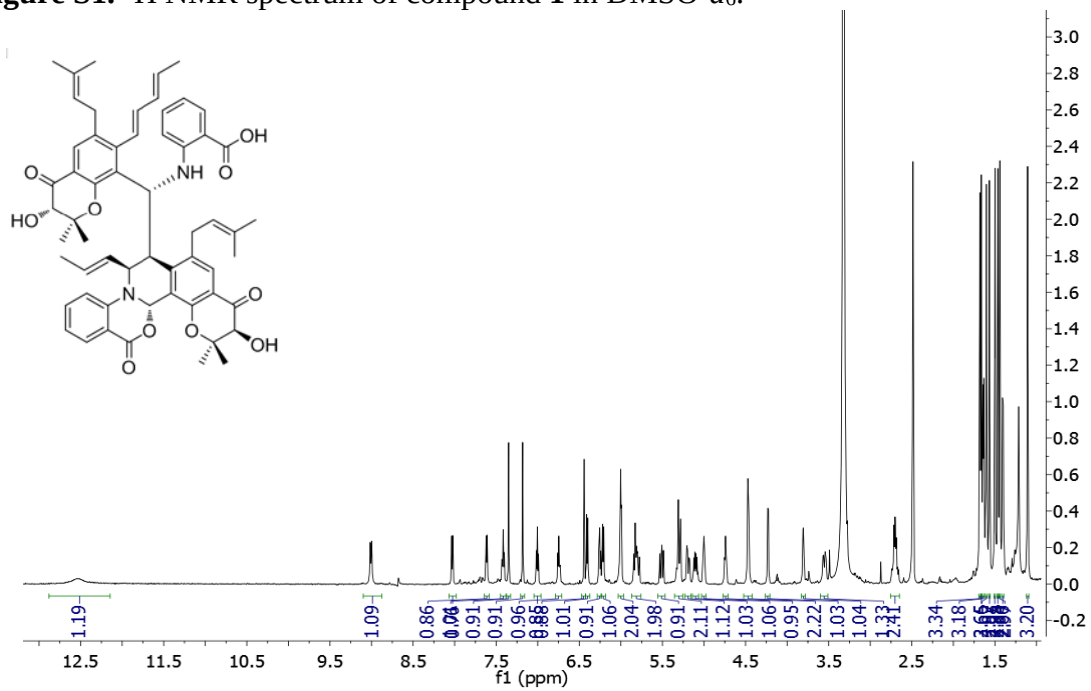


Figure S2. HSQC spectrum of compound **1** in DMSO-d_6.

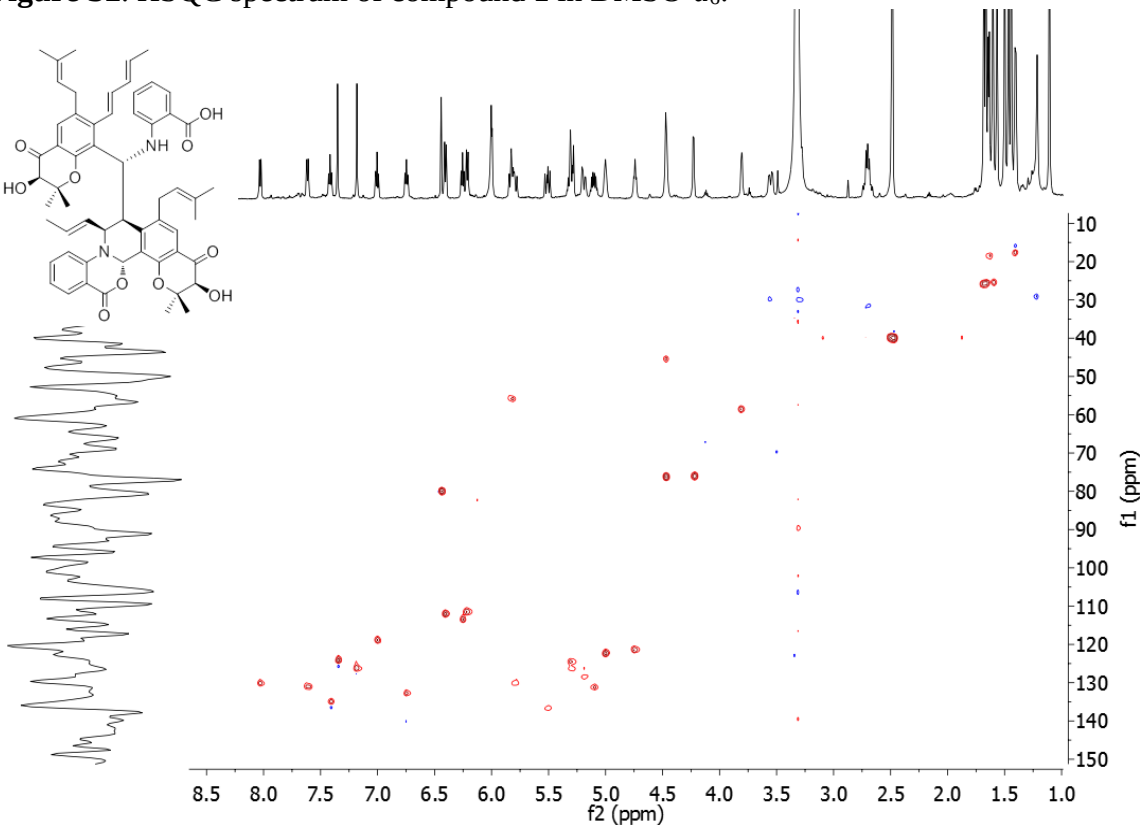


Figure S3. HMBC spectrum of compound **1** in DMSO-d_6.

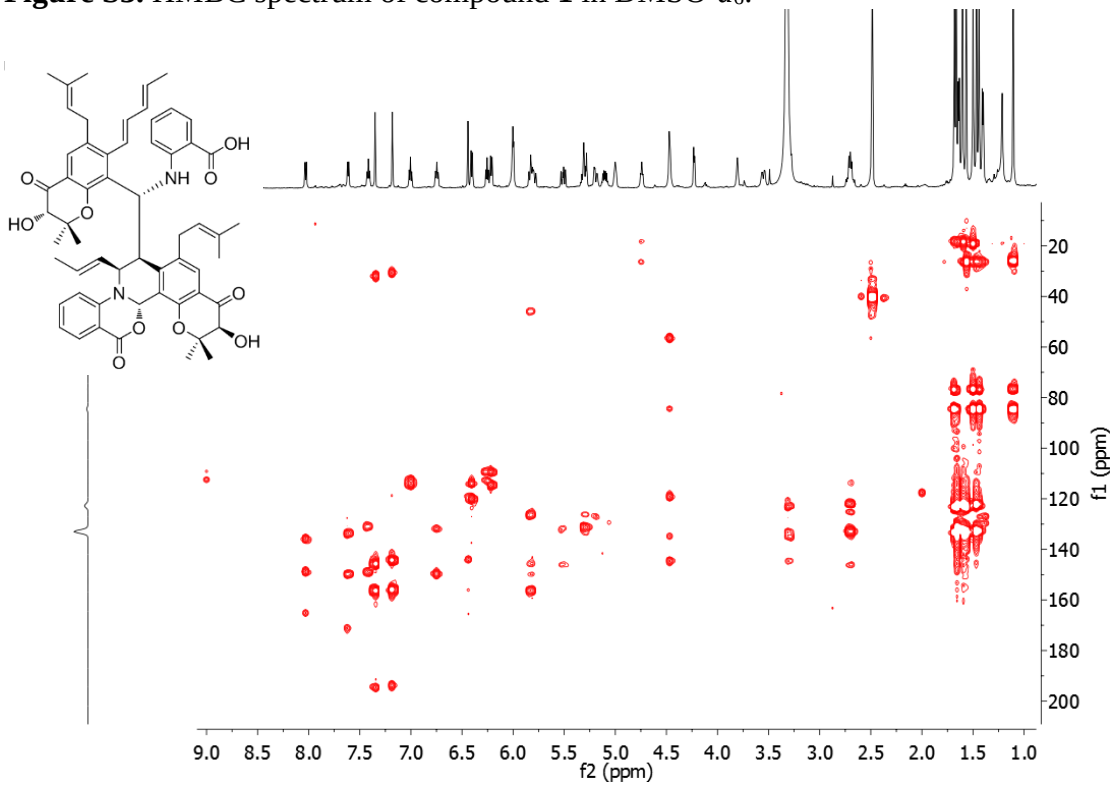


Figure S4. ^{1}H-^{1}H COSY spectrum of compound **1** in DMSO-d_6.

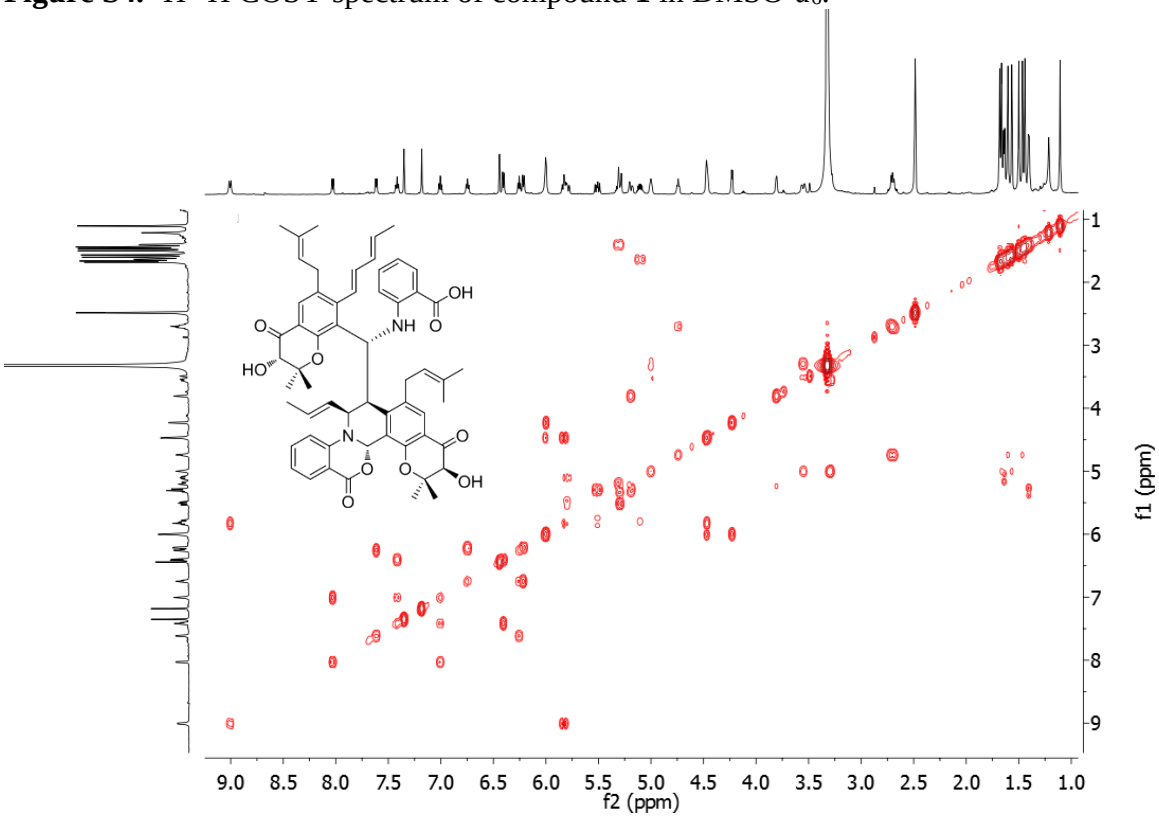


Figure S5. NOESY spectrum of compound **1** in DMSO-d_6.

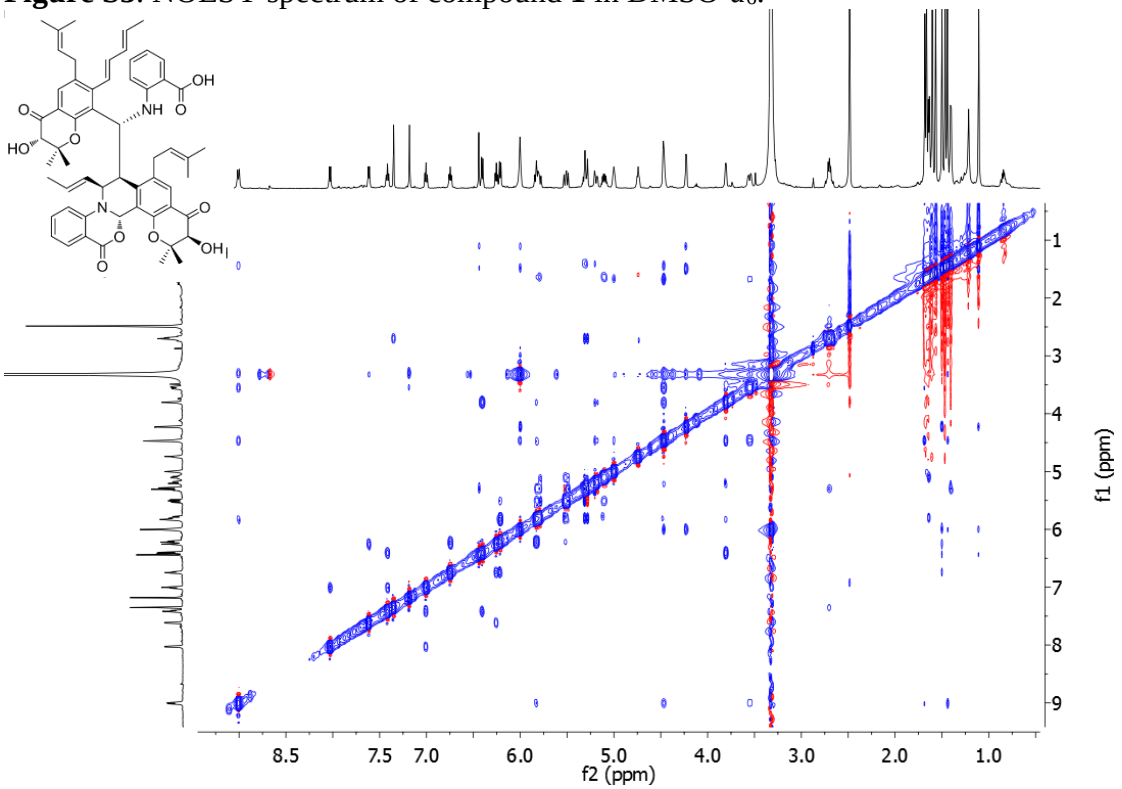


Figure S6. ^{1}H NMR spectrum of compound **1** in CD_3CN.

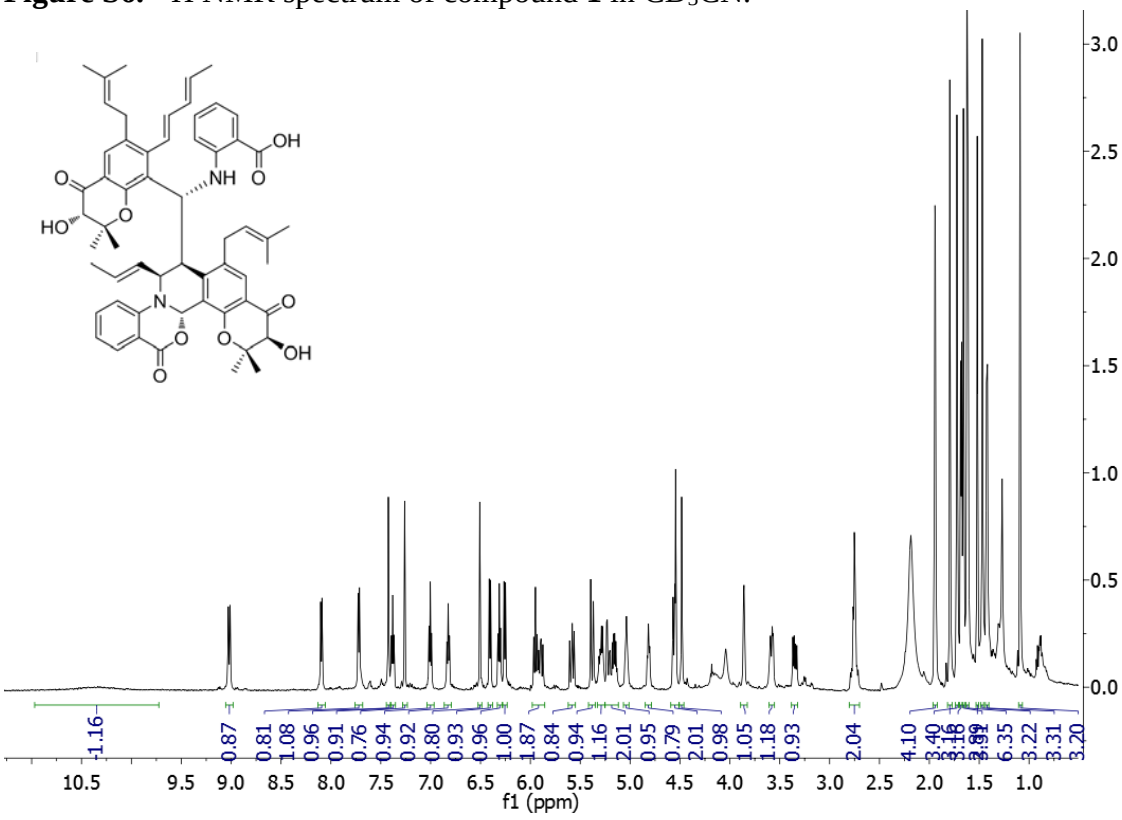


Figure S7. HSQC spectrum of compound **1** in CD_3CN.

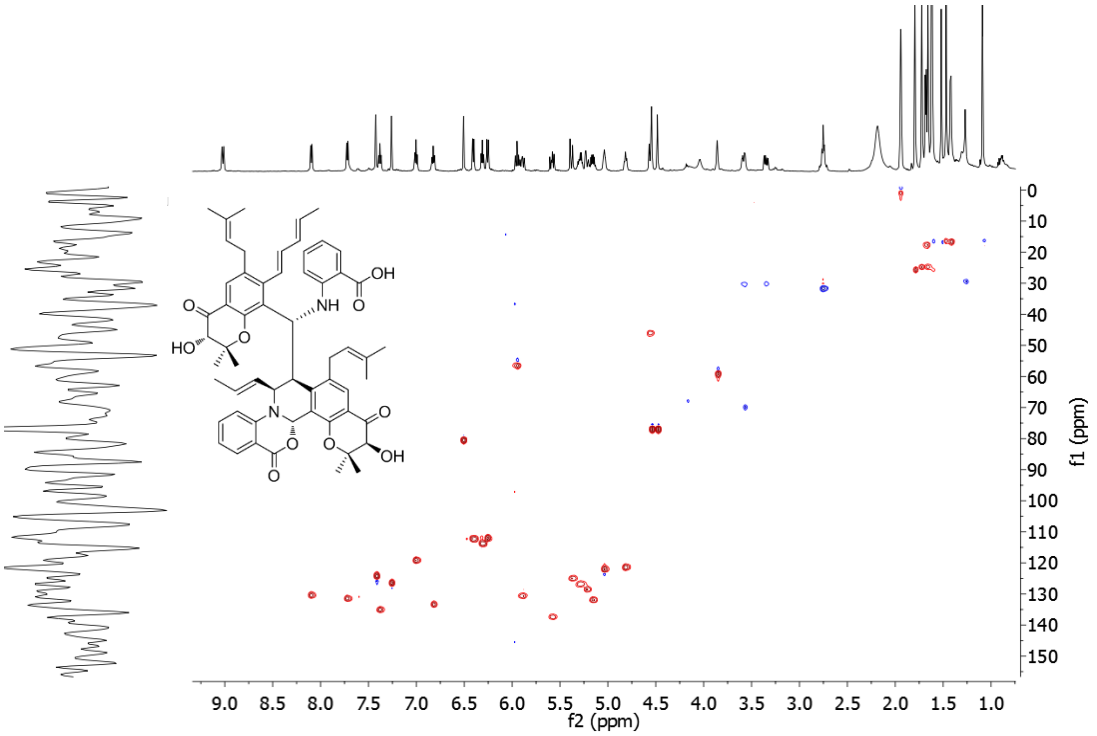


Figure S8. HMBC spectrum (the long-range C-H coupling constant, Jnxh = 4 Hz) of compound **1** in CD_3CN.

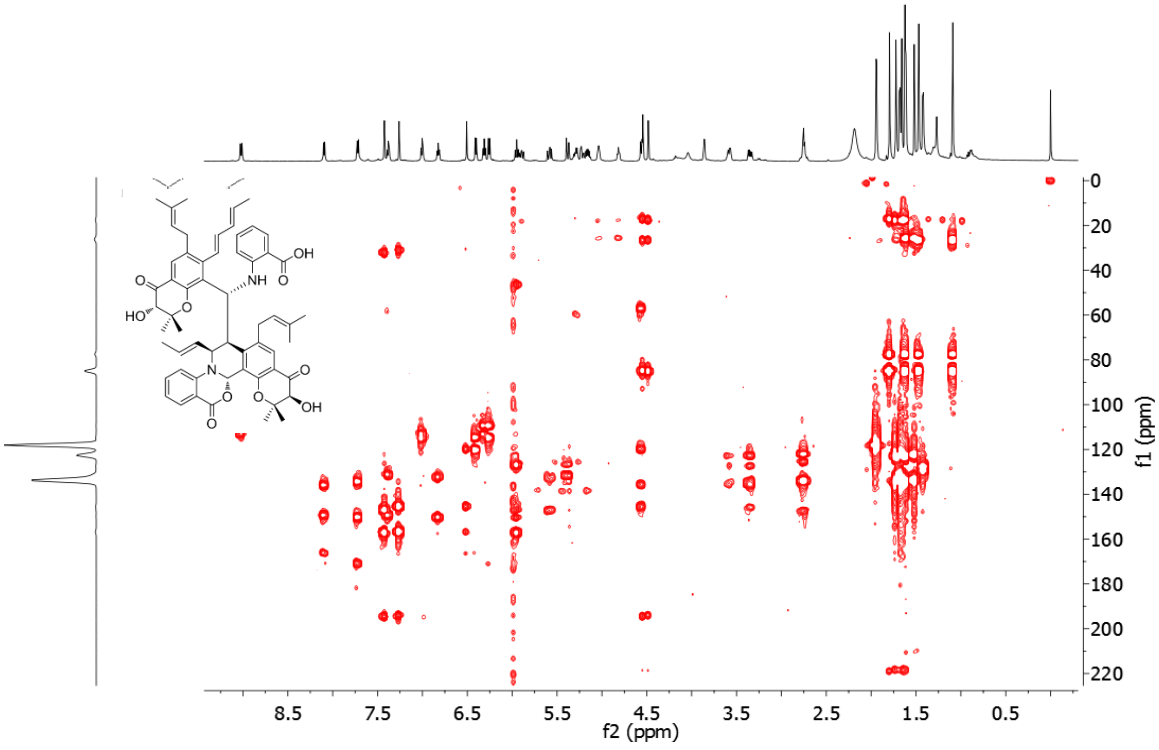


Figure S9. HMBC spectrum (Jnxh = 4 Hz) of compound **1** in CD_3CN.

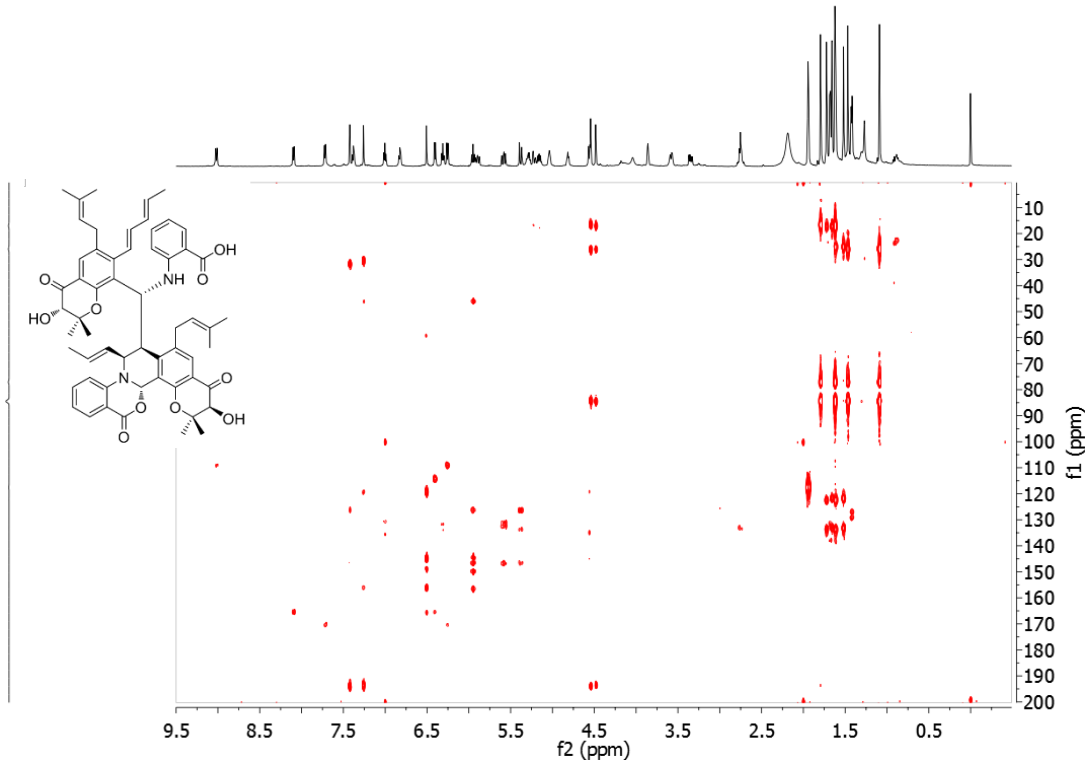


Figure S10. ^{1}H-^{1}H COSY spectrum of compound **1** in CD_3CN.

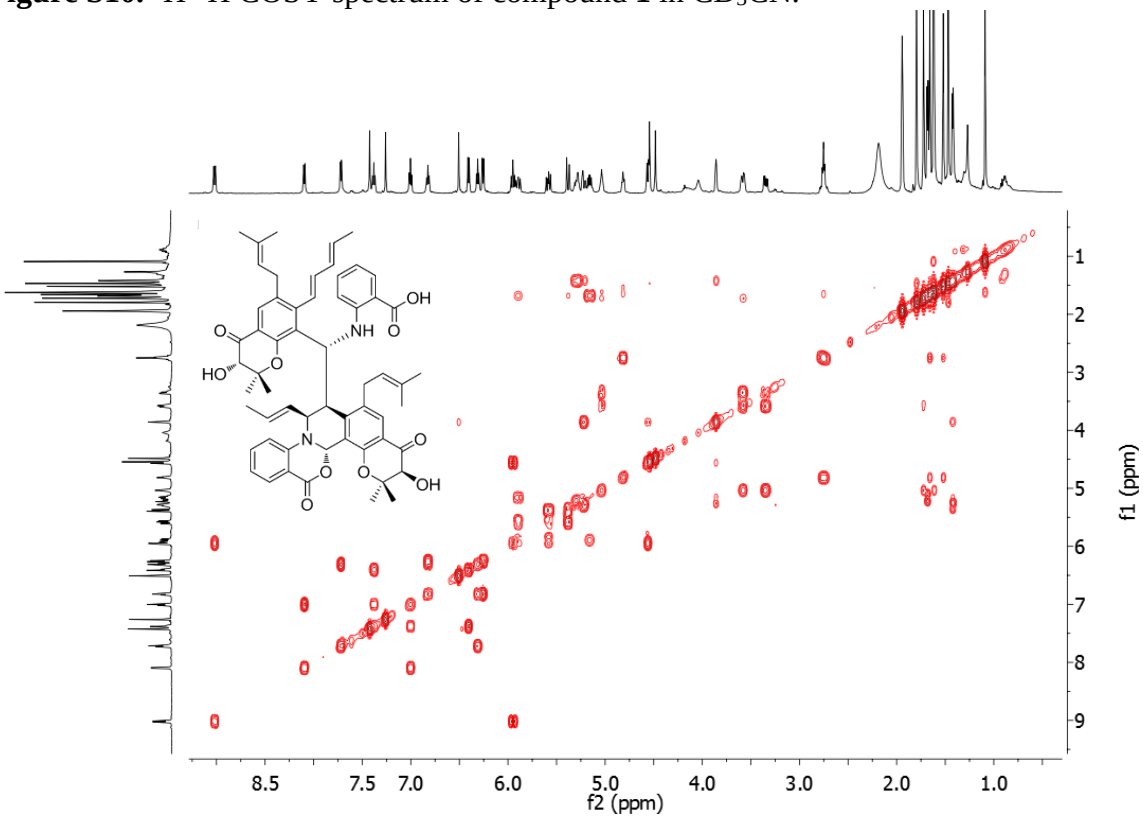
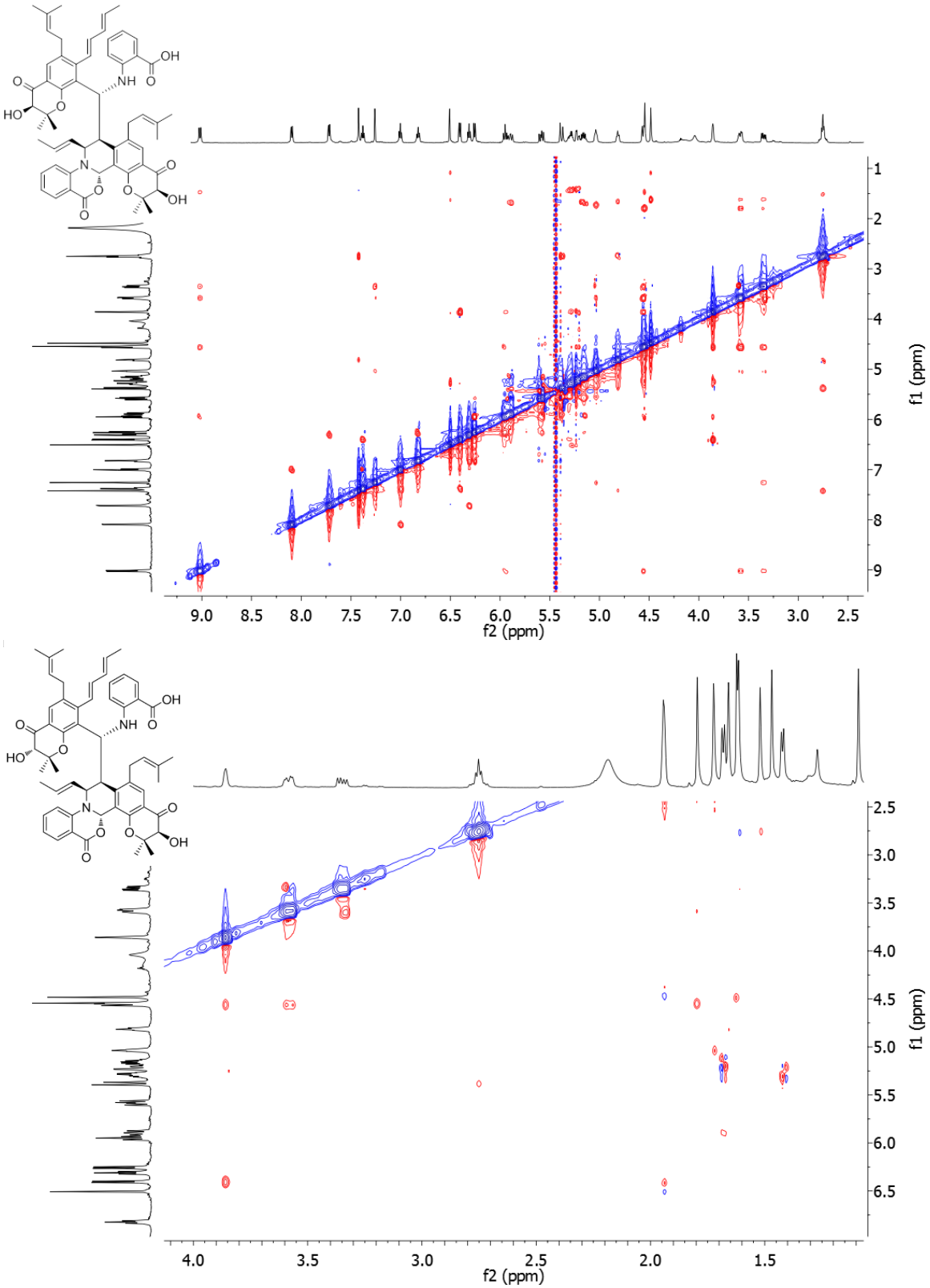


Figure S11. ROESY spectrum of compound **1** in CD_3CN.



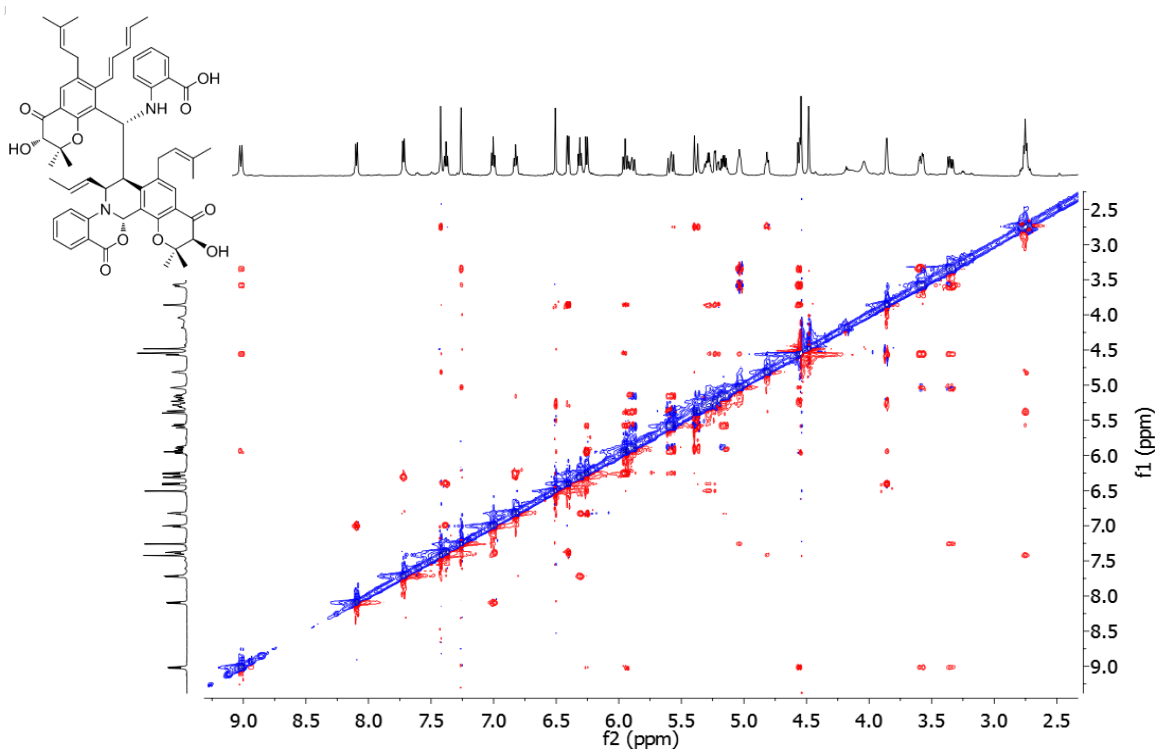


Figure S12. 13C NMR spectrum of compound **1** in CD_3CN.

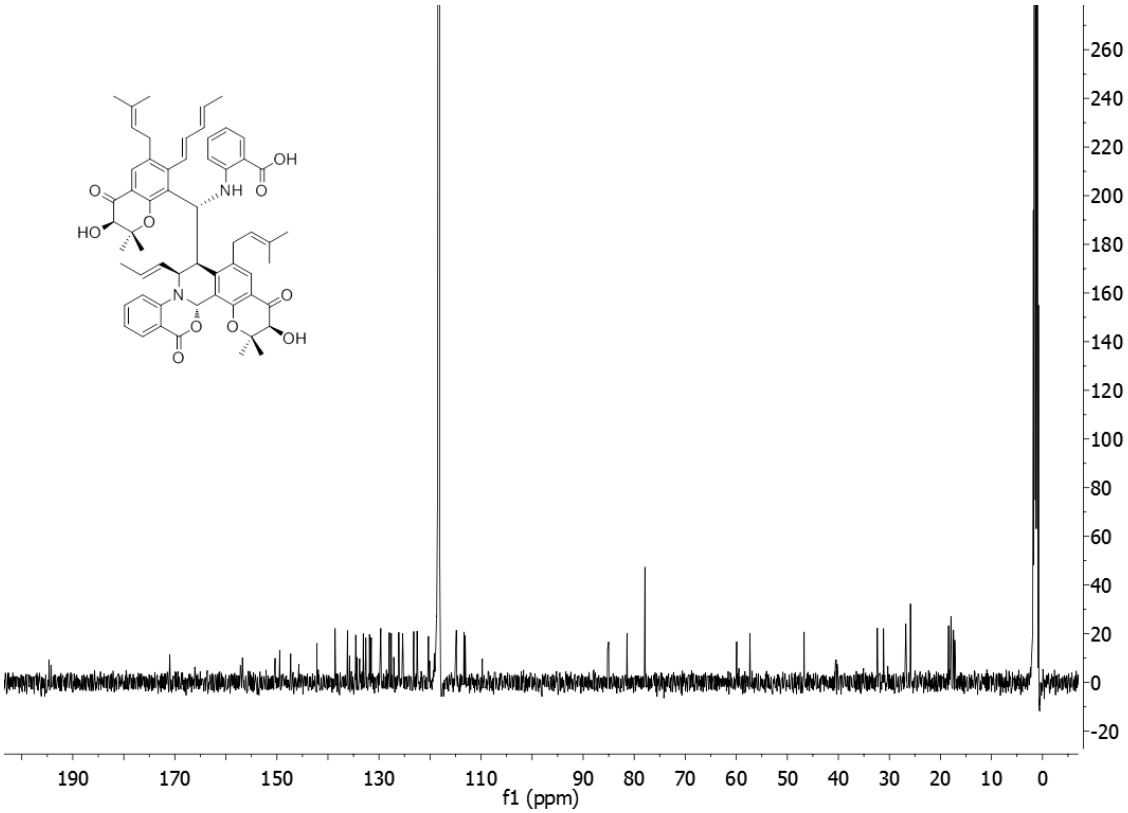


Figure S13. ^{1}H-^{15}N HMBC spectrum of compound **1** in CD_3CN.

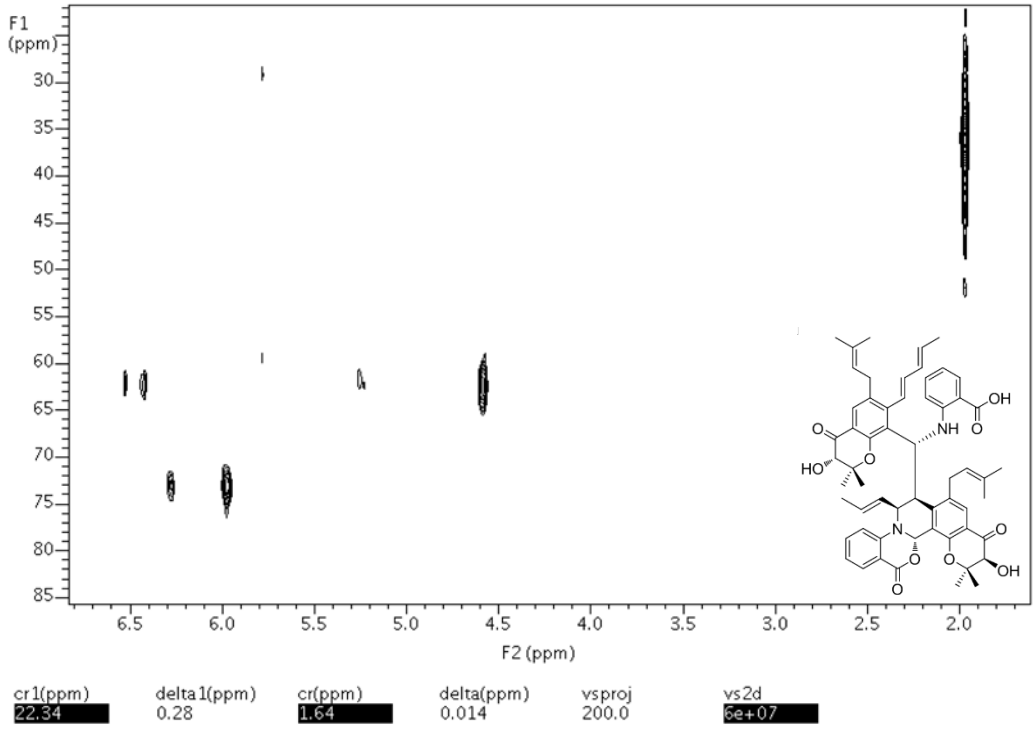
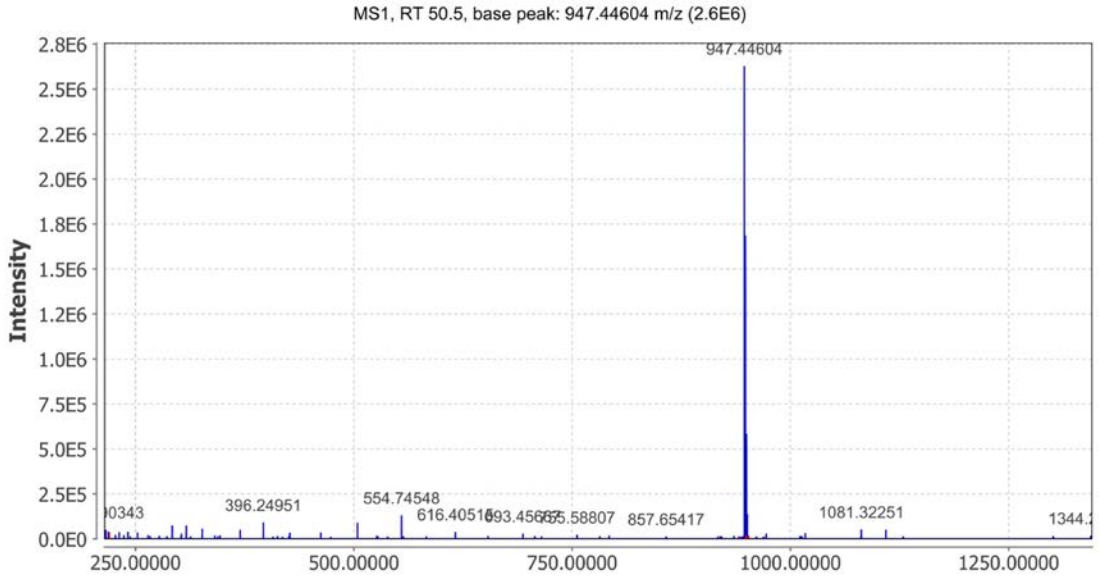


Figure S14. ESI-FT-ICR MS data of compound **1**.



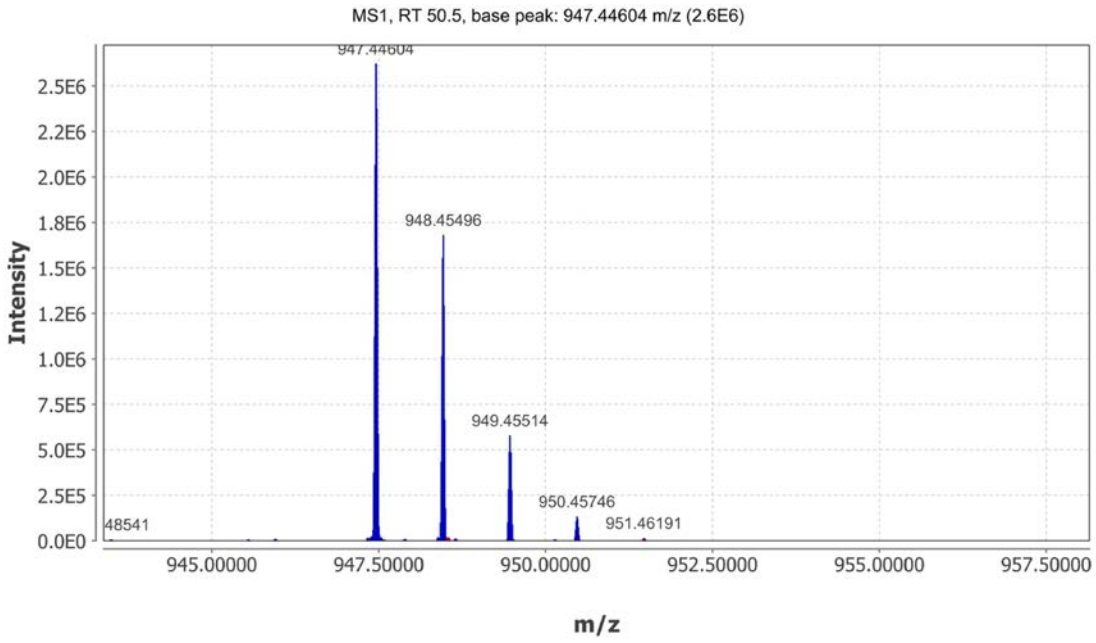
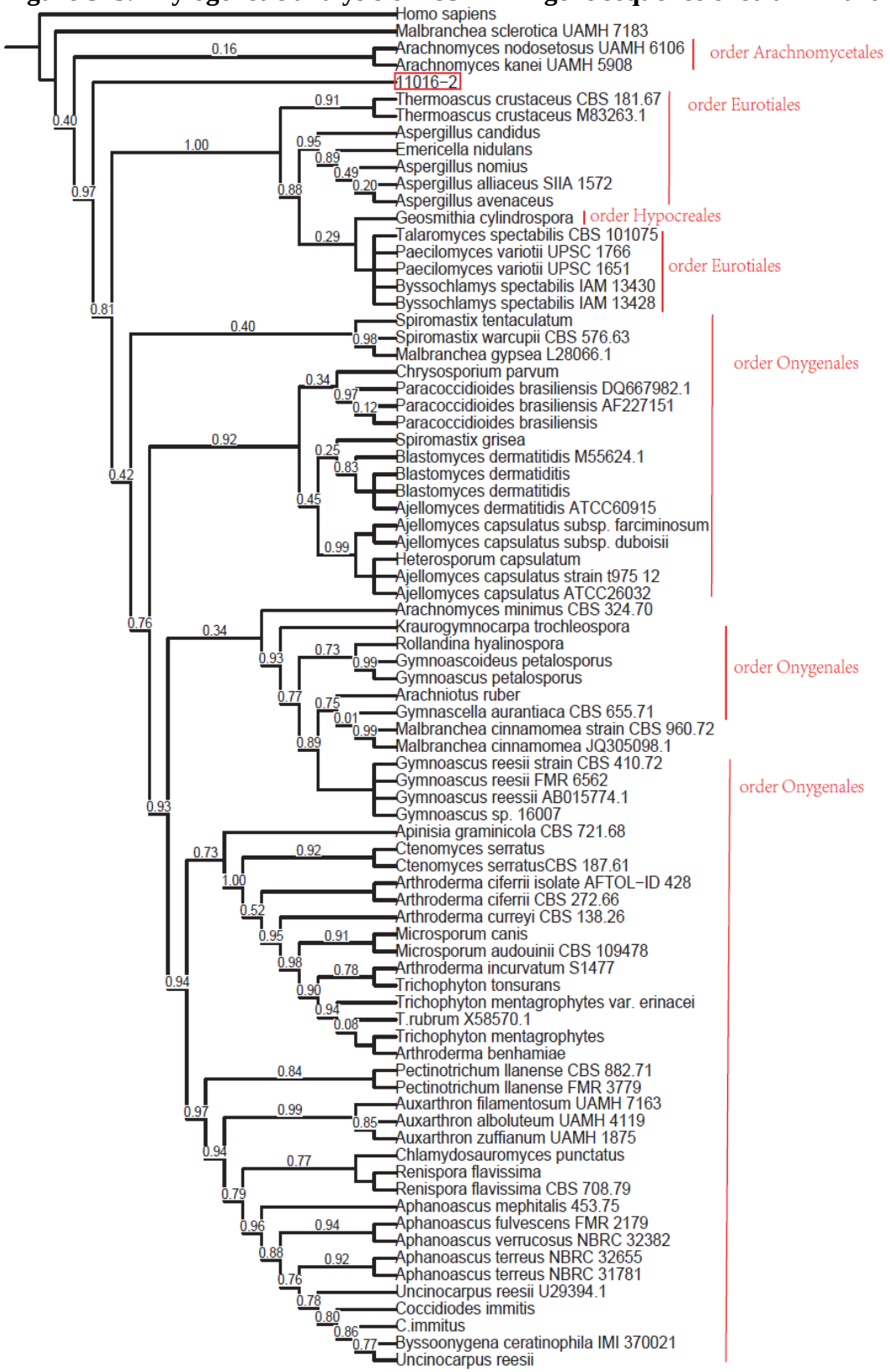
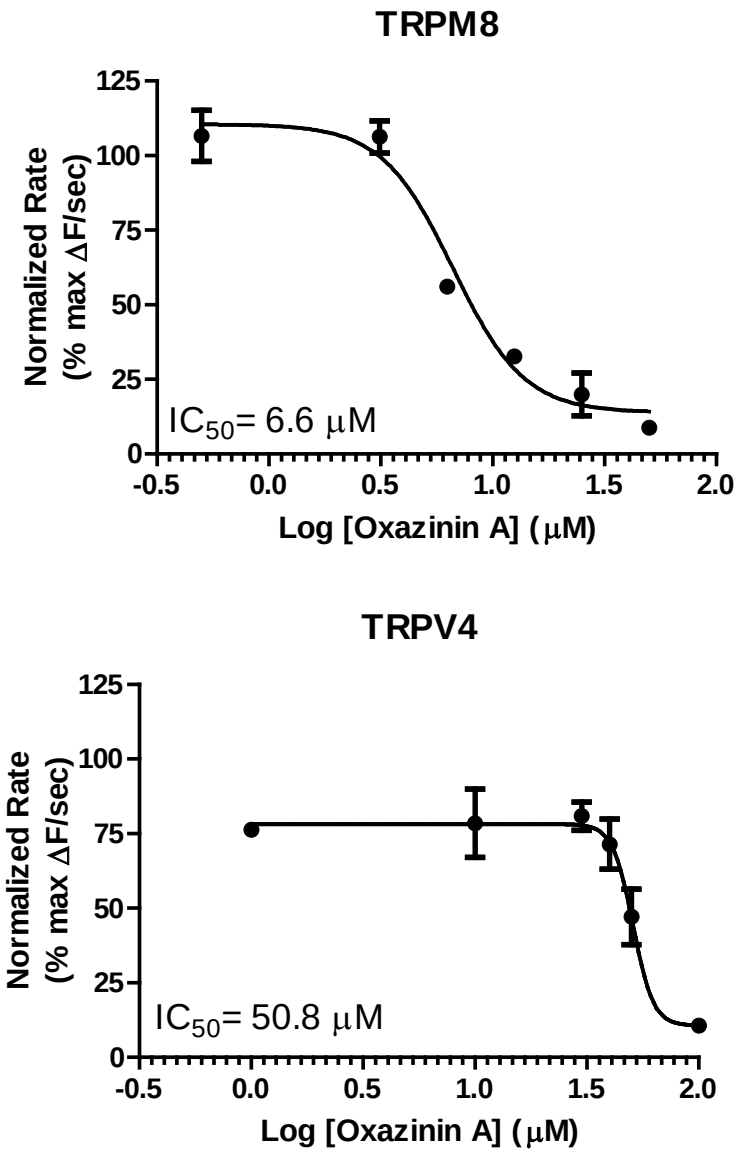

MS1, RT 50.5, base peak: 947.44604 m/z (2.6E6)
947.44604
948.45496
949.45514
950.45746
951.46191
48541
Intensity
2.5E6
2.2E6
2.0E6
1.8E6
1.5E6
1.2E6
1.0E6
7.5E5
5.0E5
2.5E5
0.0E0
945.00000
947.50000
950.00000
952.50000
955.00000
957.50000
m/z

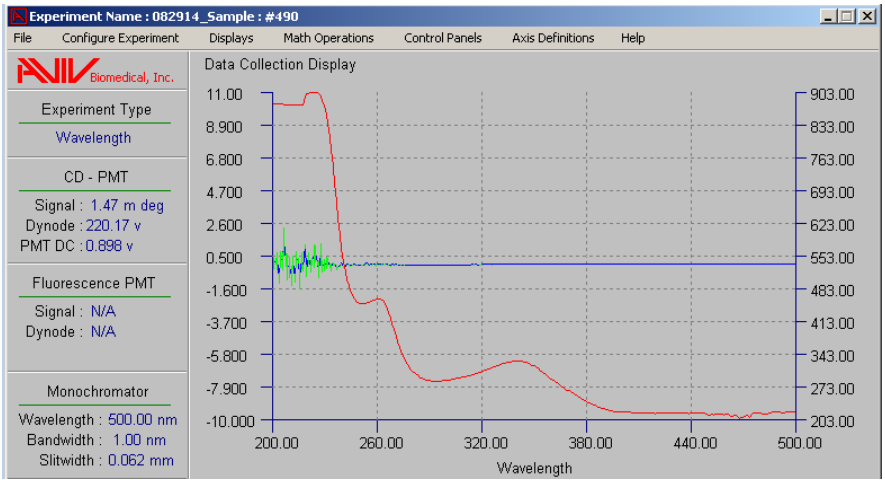
Figure S15. Phylogenetic analysis of 18S rRNA gene sequence of strain 110162.



5. Figure S16. The antagonist activity of compound 1 against transient receptor poten-tial (TRP) channels.



6. Figure S17. Circular dichroism spectrum of compound 1. Red line is the dynode voltage curve and the blue line is CD signal (m deg)**.**



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Ferrer, R.; Seabra, G.; Simmerling, C.L.; Smith, W.; Swails, J.; Walker, R.C.; Wang, J.; Wolf, R.M.; Wu X.; Kollman, P.A. **2014**, *AMBER 14*, University of California, San Francisco.