Genome Mining of *Micromonospora yangpuensis* DSM 45577 as a Producer of an Anthraquinone-fused Enediyne

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Supplementary Information (SI)

Experimental procedures

General materials

The ^{1}H and ^{13}C, and 2D NMR (HSQC, ^{1}H-^{1}H COSY, HMBC, and ROESY) spectra were collected with a Bruker Avance III Ultrashield 700 at 700 MHz for ^{1}H and 175 MHz for ^{13}C nuclei. Optical rotation values were measured using an Autopol IV automatic polarimeter (Rudolph Research Analytical). Circular dichroism spectra were collected with a Jasco J-815 Circular Dichroism Spectropolarimeter. UV spectra were recorded with a NanoDrop 2000C spectrophotometer (Thermo Scientific). IR spectra were collected with a Spectrum One FT-IR spectrometer (PerkinElmer). Column chromatography was conducted on Sephadex LH-20 (GE Healthcare, Glies, UK). Semipreparative HPLC was carried out on a Varian liquid chromatography system with a YMC-pack ODS-A (250 mm × 10 mm, 5 µm) column. HPLC-MS analysis was performed on an Agilent 1260 Infinity LC coupled to a 6230 TOF (HRESI) with an Agilent Poroshell 120 EC-C18 column (2.7 µm, 50 mm × 4.6 mm). MPLC purification was conducted on Biotage Isolera One using a Biotage SNAP Catridge KP-C18-HS column (30 g). Fermentation was carried out in New Brunswick Scientific Innova 44 incubator shakers. All common biochemicals and culture media components were purchased from commercial sources.

Virtual survey of enediyne gene clusters from public genome databases

The five proteins from the C-1027 enediyne PKS cassette (SgcE, SgcE3, SgcE4, SgcE5, and SgcE10) were individually used as queries to search potential enediyne gene clusters in the NCBI and JGI genome databases, using a recently published approach.[S1] The list of strains and gene clusters from the two databases were combined and dereplicated, to afford an initial unique list of potential enediyne gene clusters.

The PKSE sequences from the initial list were aligned with ClustalW and subjected to phylogenetic analysis with MEGA 6.06.[S2] A maximum likelihood phylogenetic tree was generated using the Jones-Taylor-Thornton (JTT) model of amino acid substitution and 1000 bootstrap replications, with the AziB protein as an outgroup.[S3] From the phylogenetic and blastP results, PKSE sequence identities with >90% were regarded as duplicates and their gene clusters were duplicate gene clusters. Duplicate gene clusters were dereplicated to generate unique enediyne biosynthetic gene clusters. Protein sequences from the unique gene clusters were collected and analyzed in an all versus all BlastP using Blast+,[S4] the BLOSUM62 matrix [S5] and an *E* value limit of 10^{-8}. Methods for GNN generation and visualization were the same as previously described.[S1]

Bacterial strains and culture conditions

Micromonospora yangpuensis DSM 45577 was purchased from DSMZ (Genbank accession number GU002071 for 16S rRNA gene, and FMIA00000000 for whole genome shotgun sequences).[S6] The strain was revived on the GPHF plate (1% glucose, 0.5% peptone from Casein, 0.5% yeast extract, 0.5% beef extract, 0.074% $CaCl_2{\cdot}2H_2O$, 1.5% agar, pH 7.2). After growth on the GPHF plate at 28 °C for 21 days, the spores were collected and cultured in 250-mL baffled flasks containing 50 mL of seed medium (1% fish meal, 3% dextrin, 1% lactose, 0.6% $CaSO_4$, 0.5% $CaCO_3$) at 28 °C and 250 rpm for 7 days.

Kocuria rhizophila ATCC 9341 (previously known as *Micrococcus luteus*) was used as the test strain for antibacterial assay of enediyne compounds.[S7] *E. coli* BR513 was used an indicator for DNA-damage activities (biochemical induction assay, BIA), and the assay was performed according to literature procedures.[S8]

Fermentation and isolation of YPM A and its congeners

For large-scale fermentation, forty-five 2.5-L baffled flasks each containing 400 mL of production medium (1% maltose, 1% yeast extract, 1% malt extract, 0.001% $CuSO_4 \cdot 5H_2O$, 0.0005% NaI, 0.2% $CaCO_3$, pH 7.2) were inoculated with 40 mL of the vegetative culture and cultured at 28 °C on a rotary shaker at 250 rpm for 7 days. After the fermentation, 1% (v/v) Amberlite resin XAD-16 and 1% Diaion HP-20 resin were added to the fermentation broth and incubated overnight at 28 °C. The cell pellets and resins were collected by centrifuge, dried on air for 2 days, and then extracted three times with 2 L of MeOH each. The extracts were dried in vacuo and washed three times with 1 L of EtOAC : H_2O (1 : 1). Then the organic phase was dried in vacuo and subjected to LC-MS for analysis or to MPLC for preparation.

To isolate YPM and its congeners, the MPLC fractions with UV absorption at 540 nm were further purified by semipreparative HPLC using a 50-min solvent gradient, from 35% solvent A (water) and 65% solvent B (MeCN) to 0% A and 100% B at a flow rate of 3.2 mL per min and with UV detection at 540 nm. The collected fractions were dried *in vacuo* and subjected to Sephadex LH-20 column chromatography, eluted with MeOH, to afford compounds **1** (0.65 mg), **2** (0.4 mg), **3** (1.3 mg), **4** (1.3 mg), and **5** (1.1 mg), respectively.

Physicochemical properties of YPM A and its congeners

YPM A (**1**): $[\alpha]_D^{25}$ +3000 (C = 0.001, CH_3OH), UV (CH_3OH) λ_{max} nm (logε) 234 (4.48), 254 (4.42), 543 (4.09), 581 (4.02); IR ν_{max} 3413, 2927, 1603, 1558, 1488, 1373, 1294 cm^{-1}

YPM B (**2**): $[\alpha]_D^{25}$ +1500 (C = 0.002, CH_3OH), UV (CH_3OH) λ_{max} nm (logε) 235 (4.60), 570 (4.30), 612 (4.32); IR ν_{max} 3446, 2918, 1592, 1502, 1461, 1376, 1293 cm^{-1}

YPM C (**3**): $[\alpha]_D^{25}$ +1000 (C = 0.001, CH_3OH), UV (CH_3OH) λ_{max} nm (logε) 237 (4.81), 568 (4.53), 613 (4.54); IR ν_{max} 3420, 2921, 1594, 1497, 1459, 1370, 1280 cm^{-1}

YPM D (**4**): $[\alpha]_D^{25}$ +2000 (C = 0.002, CH_3OH), UV (CH_3OH) λ_{max} nm (logε) 235 (4.32), 568 (4.03), 612 (4.05); IR ν_{max} 3415, 2927, 1591, 1501, 1461, 1376, 1294 cm^{-1}

YPM E (**5**): $[\alpha]_D^{25}$ +1000 (C = 0.002, CH_3OH), UV (CH_3OH) λ_{max} nm (logε) 234 (4.84), 568 (4.56), 613 (4.56); IR ν_{max} 3414, 2926, 1594, 1505, 1459, 1372, 1288 cm^{-1}

Cytotoxicity Assay of YPM, UCM, and TNM A. The IC_{50}s of YPM A against selected human cancer cell lines, including melanoma (SK-MEL-5), breast (MDA-MB-231 and SKBR-3), central nervous system (SF-295), and non-small cell lung cancer (NCI-H226), with TNM A and UCM as a control, were determined as follows. Suspended cultures of cells were diluted to a concentration of 5×10^4 cells per mL in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 μg per mL of streptomycin, and 100 U per mL of penicillin. The suspended cultures were dispensed into 96-well plates (100 μL per well), and the plates were incubated for 24 hours at 37 °C in an atmosphere of 5% CO_2, 95% air, and 100% humidity. The original medium was then removed, and 100 μL of fresh medium was added, followed by adding serial dilutions of drugs (1 μL in DMSO with final concentration ranging from 0 to 1000 nM). Plates were incubated under the above conditions for 72 hours. Finally, 20 μL of CellTiter 96® AQueous One Solution Reagent (Promega) was added to the plates and incubation continued at 37 °C in a humidified, 5% CO_2 atmosphere for 30 to 60 min. The absorbance at 490 nm was recorded using an ELISA plate reader. Each point represents the mean ± SD of three replicates, and the IC_{50} was determined by computerized curve fitting using GraphPad Prism (Table 1).

Table S1. Predicted functions of ORFs in the *ypm* gene cluster

Gene[a]	aa[b]	Putative function	Protein homologue	% ID/ %SI
orf(-1)	638	Molecular chaperone HtpG	AWW66_19385 (KXK60353)	90/94
ypmS1	140	Glyoxalase/bleomycin resistance protein/dioxygenase	TnmS1 (AME18020)	44/61
ypmS3	126	Glyoxalase/bleomycin resistance protein/dioxygenase	UcmS3 (AMK92564)	55/69
ypmR4	122	ArsR family transcriptional regulator	AQJ23_20335 (KUN23954)	63/74
ypmT5	327	Transporter ATP-ATPase subunit	DynT5 (ACB47076)	54/72
ypmT8	269	ABC-2 transport permease protein	DynT8 (ACB57075)	53/70
ypmJ1	367	Methyltransferase	TnmJ (AME18009)	52/67
			DynA5 (ACB47069)	49/61
ypmU16	157	Uncharacterized conserved protein YndB, AHSA1/START domain	CalU16 (AAM70339)	32/44
			DynOrf20 (ACB47072)	52/71
ypmM	334	Rieske (2Fe-2S) iron-sulfur protein	UcmM (AMK92573)	56/71
ypmL	365	Cytochrome P450	DynE10 (ACB47071)	56/67
ypmO	163	Putative hydroxylase	TnmO (AME18016)	74/78
ypmI	244	Oxidoreductase	TnmI (AME18007)	55/71
ypmK1	477	Secreted hydrolase	TnmK1 (AME18010)	55/68
ypmK2	485	Secreted hydrolase	UcmK1 (AMK92575)	51/63
ypmN	144	Ester cyclase	TnmN (AME18015)	69/78
ypmJ2	378	Methyltransferase	TnmJ (AME18009)	48/59
ypmR2	436	Putative regulator	UcmR2 (AMK92569)	58/70
ypmP	385	FAD-dependent oxidoreductase	DynE13 (ACB47064)	66/74
ypmR7	259	Transcriptional regulator	DynR7 (ACB47062)	57/69
ypmB	283	Uncharacterized conserved protein YndB, AHSA1/START domain	DynU16 (ACB47061)	60/70
			TnmB (AME17995)	46/56
ypmC	509	Ketone reductase	Dynorf17 (ACB47060)	55/62
ypmD	441	PBS lyase HEAT-like repeat protein	Dynorf16 (ACB	87/93
ypmF	210	Unknown protein	Dynorf15 (ACB47058)	92/97
ypmG	369	Unknown protein	Dynorf14 (ACB47057)	70/78
ypmU20	177	Unknown protein	DynU20 (ACB47056)	49/58
ypmU21	194	Unknown protein	CalU21 (AAM70363)	64/76
ypmR3	453	Unknown protein	DynR3 (ACB47054)	70/79
ypmR5	630	AfsR family transcriptional regulator	DynR2 (ACB47053)	51/61
ypmR1	230	HxlR family transcriptional regulator	DynU8 (ACB47051)	68/80
ypmE10	144	Thioesterase	DynE7 (ACB47049)	74/83
ypmE	1900	Enediyne polyketide synthase	DynE8 (ACB47048)	66/73
ypmE5	335	Unknown protein	DynT3 (ACB47047)	74/81
ypmE4	629	Unknown protein	DynU14 (ACB47046)	72/81
ypmE3	329	Unknown protein	DynU15 (ACB47045)	60/67
ypmR6	502	AfsR family transcriptional regulator	Dynorf13 (ACB47044)	48/59
orf(+1)	94	Hypothetical protein	ADL17_17575 (KUJ44940)	55/60

[a] *orf(-1)* and *orf(+1)* are predicted to represent the upstream and downstream boundaries of the gene cluster.
[b] Number of amino acids.

Table S2. Comparison of the *ypm* gene cluster with the *tnm* and *ucm* gene clusters

Gene[a]	aa[b]	Putative function	Protein homolog in *tnm* gene cluster	% ID/ %SI	Protein homolog in *ucm* gene cluster	% ID/ %SI
ypmS1	140	Glyoxalase/bleomycin resistance protein/dioxygenase	TnmS1 (AME18020)	44/61	UcmS1 (AMK92568)	44/61
ypmS3	126	Glyoxalase/bleomycin resistance protein/dioxygenase	TnmS3 (AME18025)	51/68	UcmS3 (AMK92564)	55/69
ypmR4	122	ArsR family transcriptional regulator				
ypmT5	327	Transporter ATP-ATPase subunit				
ypmT8	269	ABC-2 transport permease protein				
ypmJ1	367	Methyltransferase	TnmJ (AME18009)	52/67	UcmJ (AMK92576)	54/66
ypmU16	157	Uncharacterized conserved protein YndB, AHSA1/START domain				
ypmM	334	Rieske (2Fe-2S) iron-sulfur protein	TnmM (AME18014)	57/69	UcmM (AMK92573)	56/71
ypmL	365	Cytochrome P450	TnmL (AME18012)	41/47		
ypmO	163	Putative hydroxylase	TnmO (AME18016)	74/78	UcmO (AMK92571)	76/81
ypmI	244	Oxidoreductase	TnmI (AME18007)	55/71	UcmI (AMK92578)	57/71
ypmK2	477	Secreted hydrolase	TnmK2 (AME18011)	69/77	UcmK2 (AMK92574)	67/77
ypmK1	485	Secreted hydrolase	TnmK1 (AME18010)	51/64	UcmK1 (AMK92575)	51/63
ypmN	144	Ester cyclase	TnmN (AME18015)	69/78	UcmN (AMK92572)	64/75
ypmJ2	378	Methyltransferase	TnmJ (AME18009)	48/59	UcmJ (AMK92576)	47/61
ypmR2	436	Putative regulator	TnmR2 (AME18018)	59/70	UcmR2 (AMK92569)	58/70
ypmP	385	FAD-dependent oxidoreductase	TnmP (AME18017)	52/61	UcmP (AMK92570)	52/62
ypmR7	259	Transcriptional regulator	TnmR7 (AME17994)	41/53	UcmR7 (AMK92581)	41/53
ypmB	283	Uncharacterized conserved protein YndB, AHSA1/START domain	TnmB (AME17995)	46/56	UcmB (AMK92580)	45/54
ypmC	509	Ketone reductase	TnmC (AME17997)	39/47	UcmC (AMK92556)	41/50
ypmD	441	PBS lyase HEAT-like repeat protein	TnmD (AME17998)	82/90	UcmD (AMK92555)	79/88
ypmF	210	Unknown protein	TnmF (AME17999)	80/87	UcmF (AMK92554)	82/88
ypmG	369	Unknown protein	TnmG (AME18000)	56/65	UcmG (AMK92579)	55/65
ypmU20	177	Unknown protein				
ypmU21	194	Unknown protein				
ypmR3	453	Unknown protein	TnmR3 (AME17996)	49/60	UcmR3 (AMK92569)	51/64
ypmR5	630	AfsR family transcriptional regulator				
ypmR1	230	HxlR family transcriptional regulator	TnmR1 (AME18008)	45/61	UcmR1 (AMK92577)	46/61
ypmE10	144	Thioesterase	TnmE10 (AME18002)	63/74	UcmE10 (AMK92559)	46/60
ypmE	1900	Enediyne polyketide synthase	TnmE (AME18003)	51/60	UcmE (AMK92560)	45/54
ypmE5	335	Unknown protein	TnmE5 (AME18004)	61/75	UcmE5 (AMK92561)	56/70
ypmE4	629	Unknown protein	TnmE4 (AME18005)	53/65	UcmE4 (AMK92562)	44/55
ypmE3	329	Unknown protein	TnmE3 (AME18006)	45/54	UcmE3 (AMK92563)	41/50
ypmR6	502	AfsR family transcriptional regulator				

[a] *orf(-1)* and *orf(+1)* are predicted to represent the upstream and downstream boundaries of the gene cluster.
[b] Number of amino acids.

Table S3. ^{1}H NMR Data of Compounds **1–5** (700 MHz, δ in ppm, J in Hz, acetone-d_6)

position	**1**	**2**	**3**	**4**	**5**
1	10.28, br s	11.09, br s	10.99, br s	11.08, br s	11.02, br s
7	7.30, dd (7.7, 1.4)	7.20, d (8.4)	7.20, dd (8.4, 0.7)	7.19, d (8.4)	7.19, d (7.7)
8	7.83, t (7.7)	7.75, t (8.4)	7.75, t (8.4)	7.75, t (7.7)	7.75, t (7.7)
9	7.85, dd (7.7, 1.4)	7.81, d (8.4)	7.80, d (8.4)	7.80, d (7.7)	7.79, d (7.7)
14	8.73, s	7.24, s	7.78, s	7.24, s	7.77, s
16		3.44, dd (6.3, 1.4)		3.65, dd (5.6, 1.4)	
17	5.46, d (4.9)	5.17, dd, (7.7, 6.3)	5.11, d (7.0)	5.36, t (7.0)	5.23, d (6.3)
19		7.61, d (7.0)	7.57, d (7.7)	7.61, d (7.0)	7.57, d (7.7)
20	6.04, dd (9.8, 0.7)	7.26, t (7.0)	7.26, td (7.7, 1.4)	7.26, t (7.0)	7.27, t (7.7)
21	5.96, dt (9.8, 1.4)	7.30, t (7.0)	7.29, t (7.7)	7.30, t (7.0)	7.30, t (7.7)
22		7.55, d (7.7)	7.54, dd (7.7, 1.4)	7.56, d (7.0)	7.56, d (7.7)
24	5.14, dd (4.2, 1.4)	5.02, dd (3.5, 1.4)	5.22, d (5.6)	5.05, dd (4.9, 1.4)	5.25, d (4.9)
26	4.55, qd (6.3, 4.9)	3.74, q (6.3)	3.85, q (6.3)	3.60, m	3.70, m
27	1.46, d (6.3)	1.39, d (6.3)	1.56, d (6.3)	4.05, m	4.19, m
				3.89, m	3.90, m
6-OH	12.19, br s	12.33, br s	12.29, br s	12.32, br s	12.29, br s
13-OH	12.59, br s	12.90, br s	12.81, br s	12.89, br s	12.82, br s

Table S4. ^{13}C NMR Data of Compounds **1**–**5** (175 MHz, δ in ppm, acetone-d_6)

position	**1**	**2**	**3**	**4**	**5**
2	144.6	144.3	144.2	144.3	144.0
3	112.8	106.6	106.5	106.5	106.5
4	191.4	190.5	190.5	190.5	190.6
5	116.1	116.1	116.1	116.1	116.1
6	162.2	162.0	162.0	162.0	162.0
7	122.5	121.3	121.3	121.3	121.3
8	137.1	136.7	136.7	136.6	136.7
9	118.7	118.1	118.1	118.1	118.1
10	135.3	135.9	135.9	135.9	135.9
11	182.2	180.1	180.2	180.1	180.1
12	112.8	111.9	112.1	111.9	112.1
13	156.0	156.6	156.8	156.6	156.7
14	130.6	131.8	130.5	131.8	130.6
15	136.5	137.6	142.1	137.6	141.1
16	64.3	48.7	77.6	48.7	77.7
17	63.9	69.7	74.9	69.9	75.1
18	99.9	136.6	136.2	136.6	136.1
19	90.1	127.7	127.6	127.7	127.7
20	123.1	127.7	127.7	127.8	127.8
21	123.5	128.2	128.3	128.2	128.3
22	87.8	129.1	128.7	129.1	128.6
23	98.4	137.6	137.2	137.6	137.1
24	43.5	55.0	57.3	55.0	57.4
25	76.0	70.7	72.6	70.7	73.2
26	64.9	66.5	67.2	71.0	71.3
27	20.8	16.6	19.7	62.8	63.1

Figure S1. The enediyne GNN consisting of the distinct enediyne gene clusters from the public databases supporting *M. yangpuensis* as a potential producer for anthraquinone-fused enediyne. The GNN was displayed with an *E* value threshold of 10^{-8}.

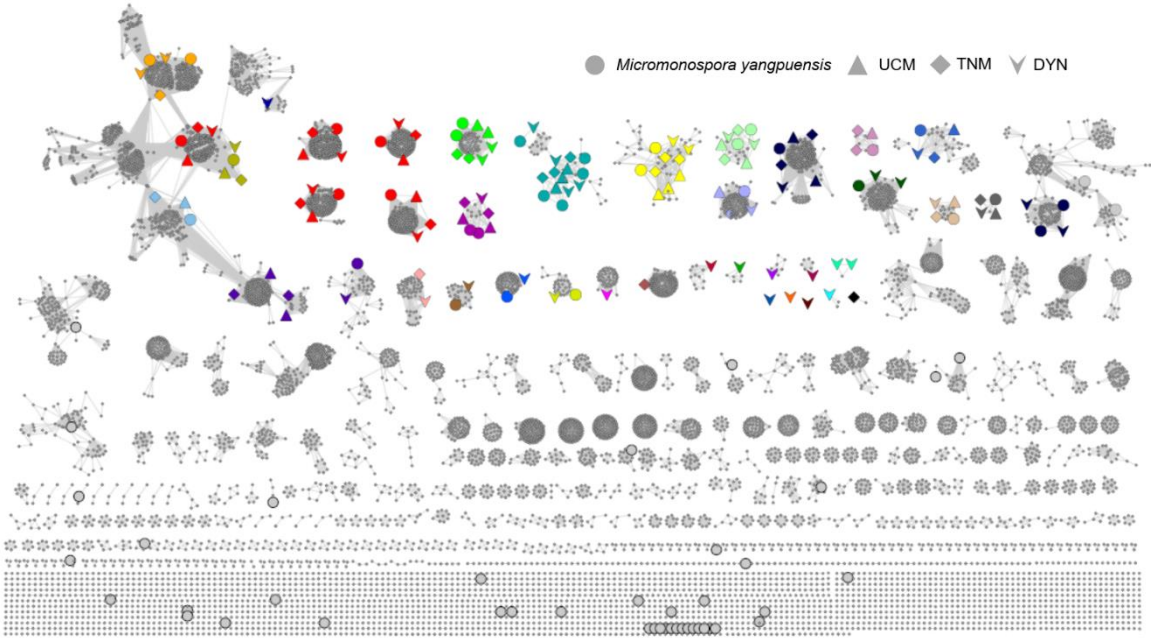


Figure S2. HRESIMS analysis of YPM A–E (**1**–**5**).

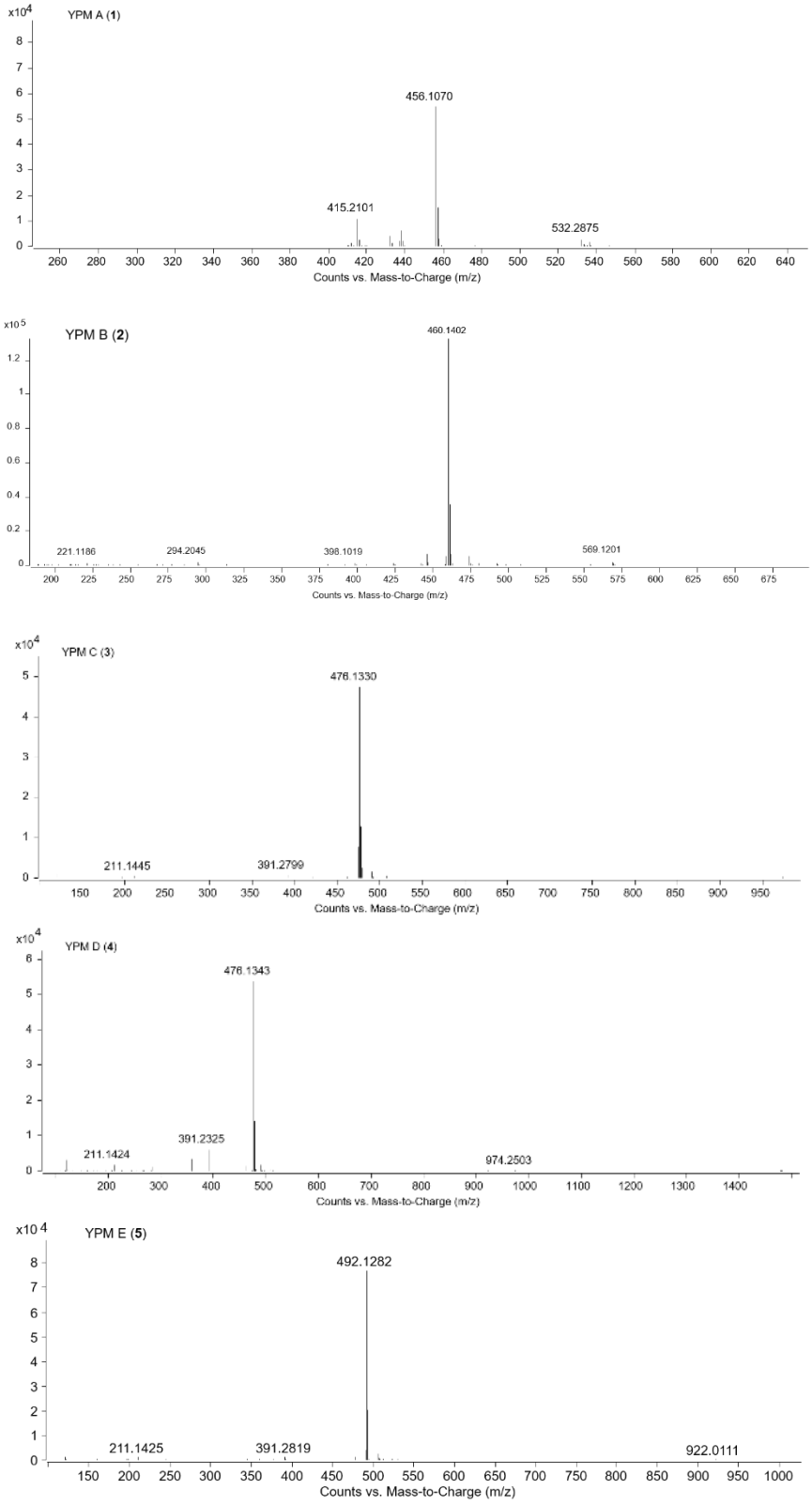


Figure S3. UV spectrum of YPM A (**1**) in comparison with UCM and TNM A

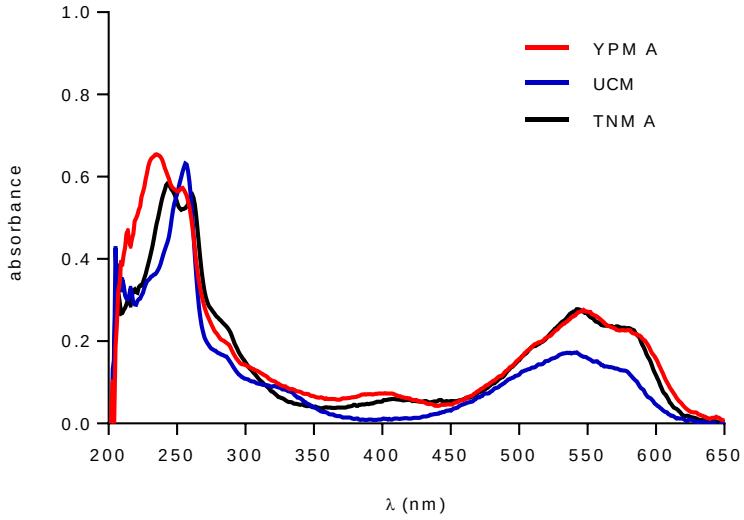


Figure S4. ^{1}H NMR spectrum of YPM A (**1**) (700 MHz, acetone-d_6)

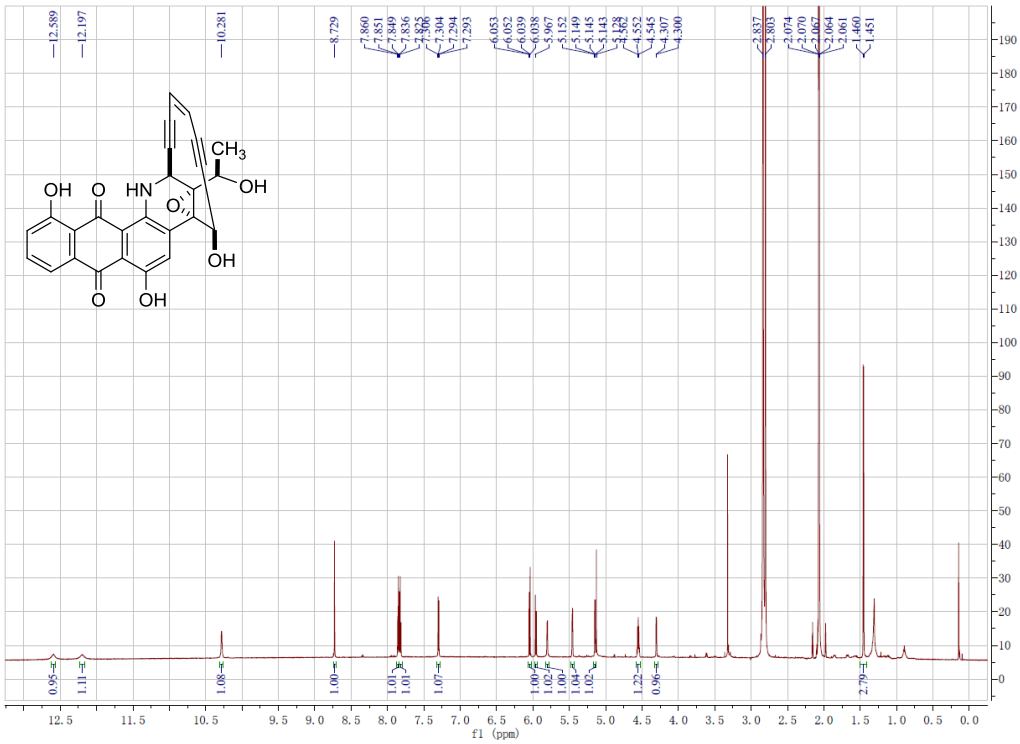


Figure S5. ^{13}C NMR spectrum of YPM A (**1**) (175 MHz, acetone-d_6)

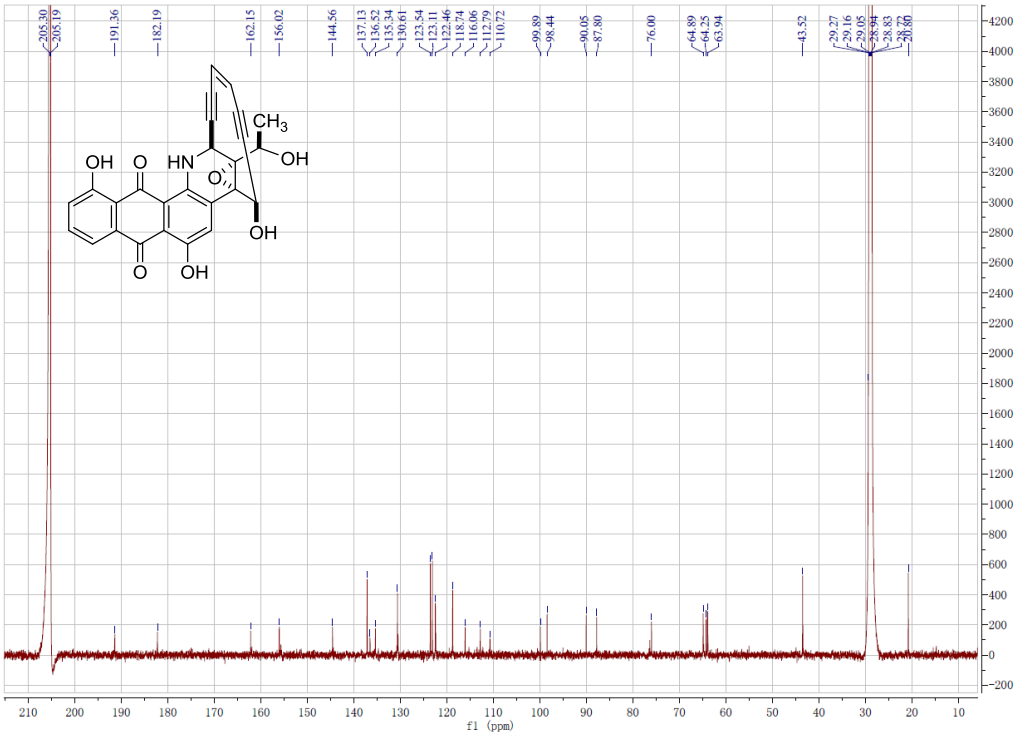


Figure S6. ^{1}H-^{1}H COSY spectrum of YPM A (**1**) (acetone-d_6)

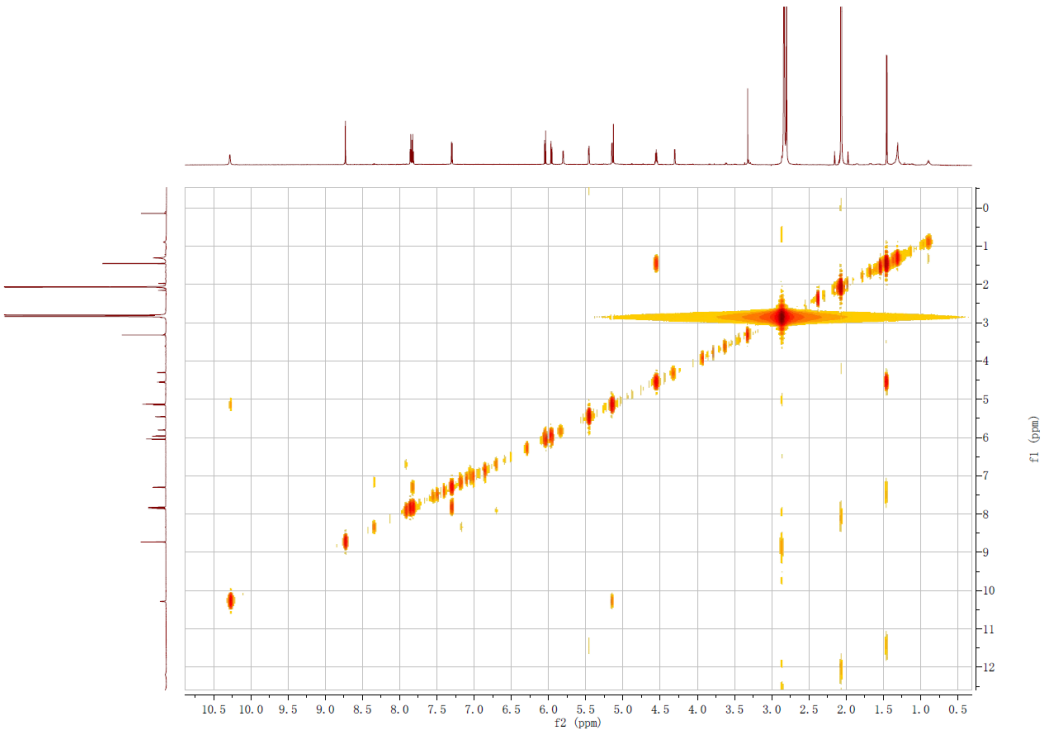


Figure S7. HSQC spectrum of YPM A (**1**) (acetone-d_6)

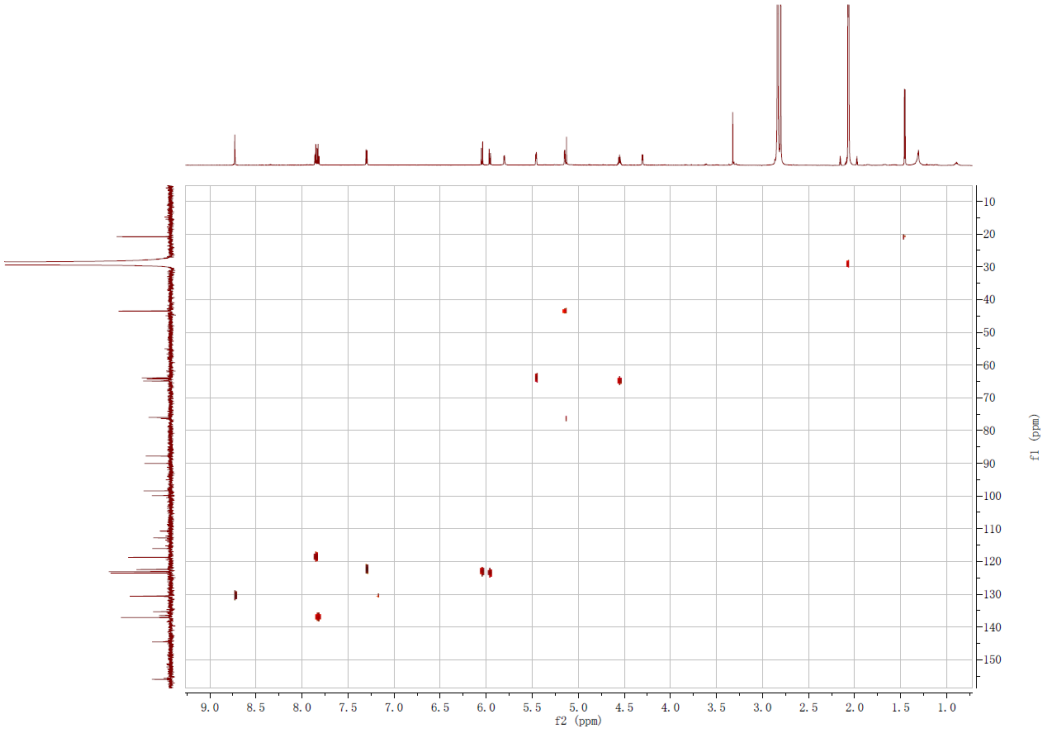


Figure S8. HMBC spectrum of YPM A (**1**) (acetone-d_6)

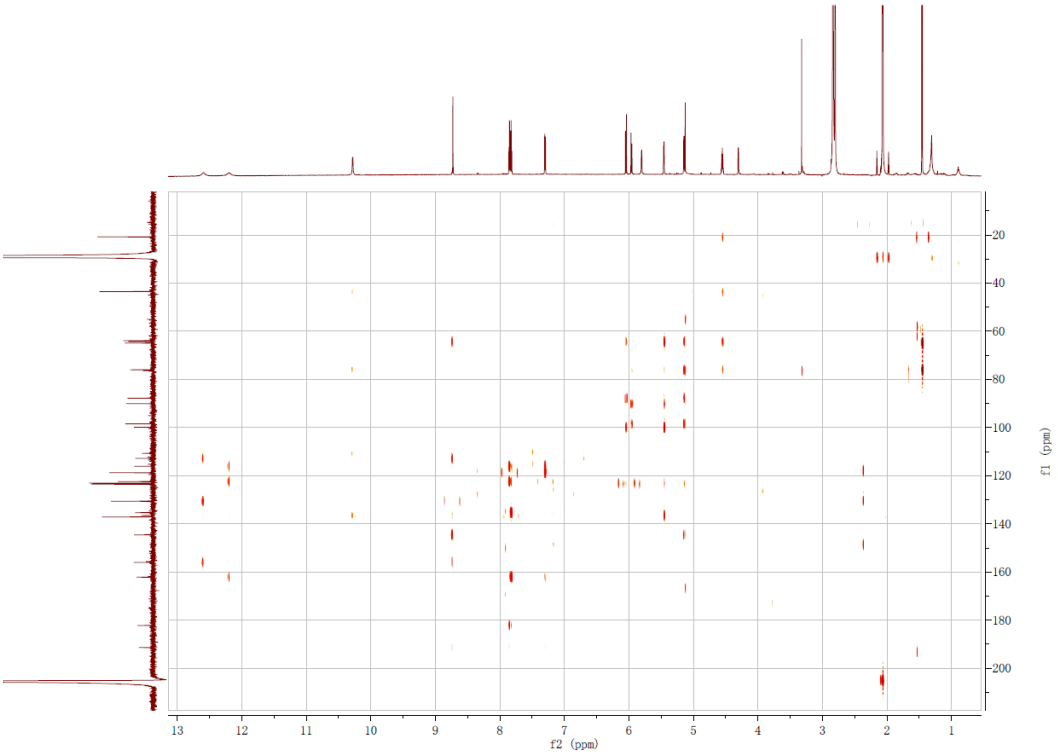


Figure S9. ROESY spectrum of YPM A (**1**) (acetone-d_6)

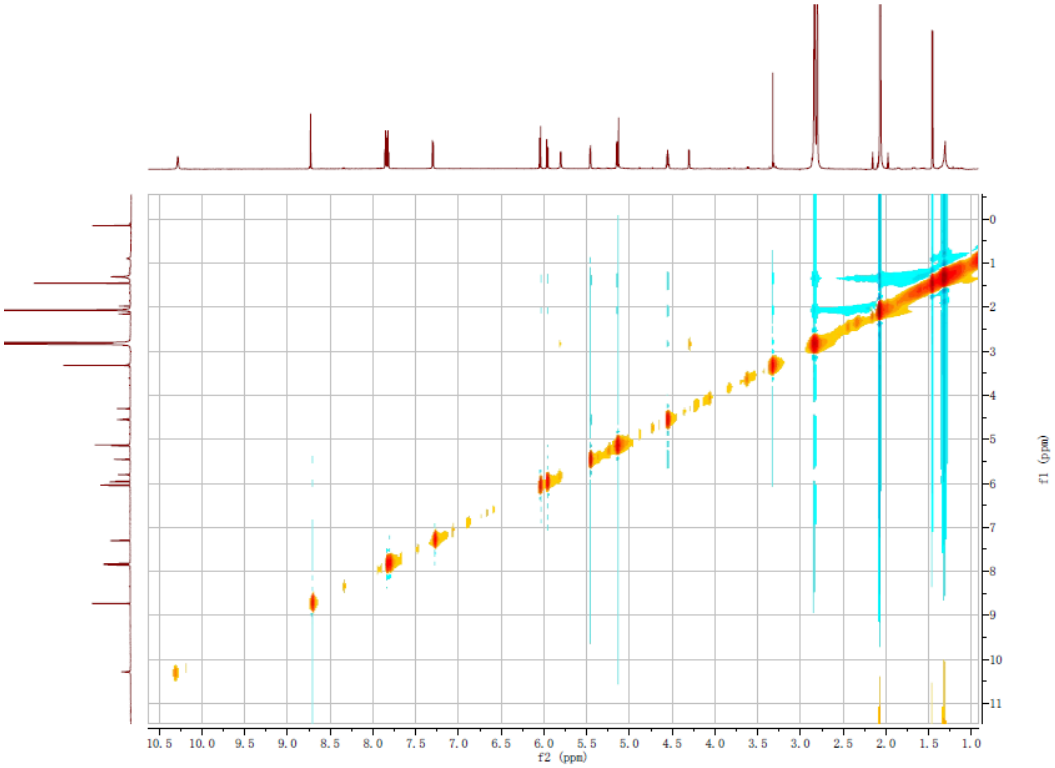


Figure S10. ^{1}H NMR spectrum of YPM-A (**1**) (700 MHz, DMSO-d_6)

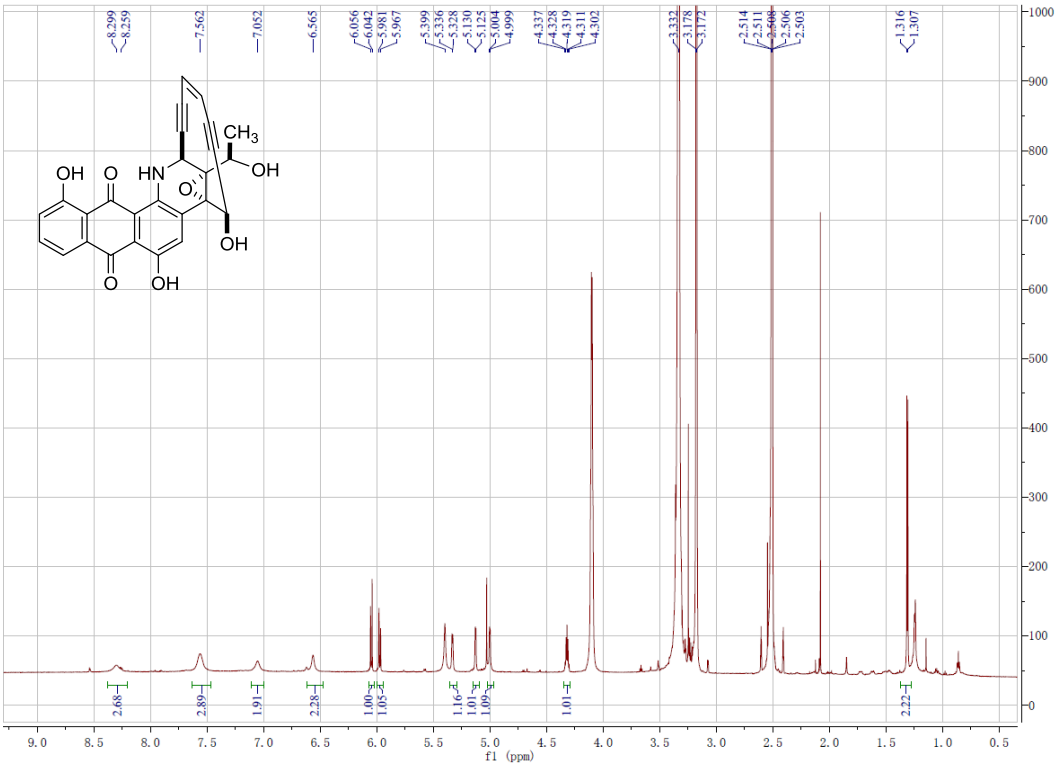


Figure S11. ^{1}H NMR spectrum of YPM B (**2**) (700 MHz, acetone-d_6)

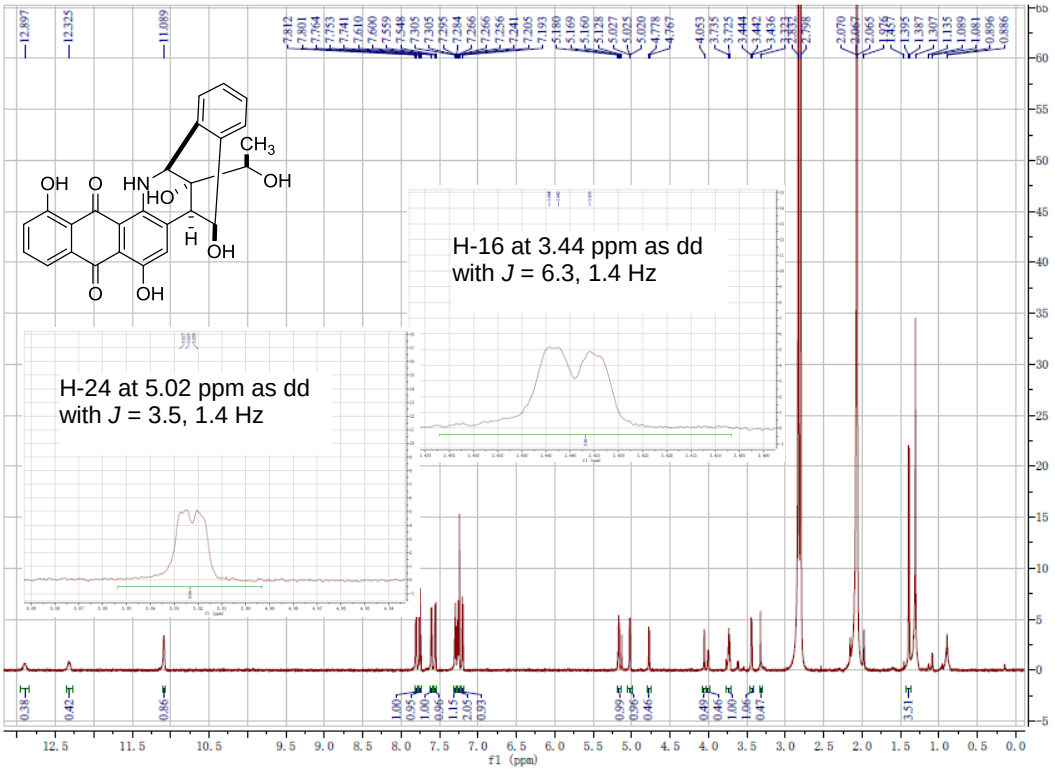


Figure S12. ^{13}C NMR spectrum of YPM B (**2**) (175 MHz, acetone-d_6)

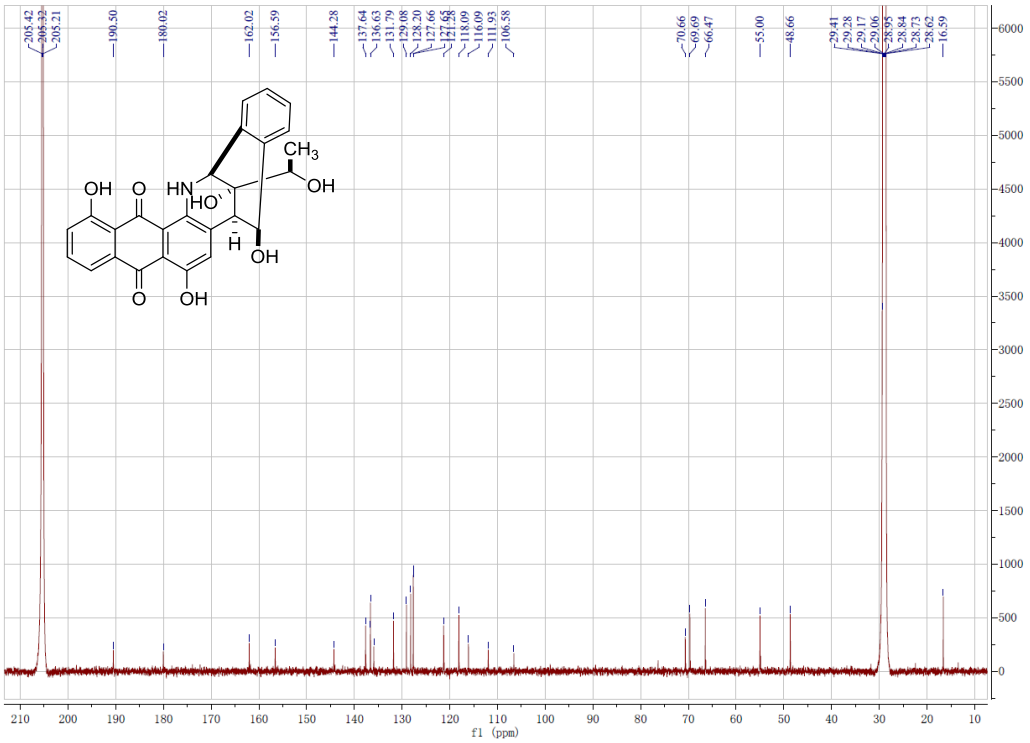


Figure S13. Proposed biogenesis of 2 and 3 from 1 by the Bergman cycloaromatization

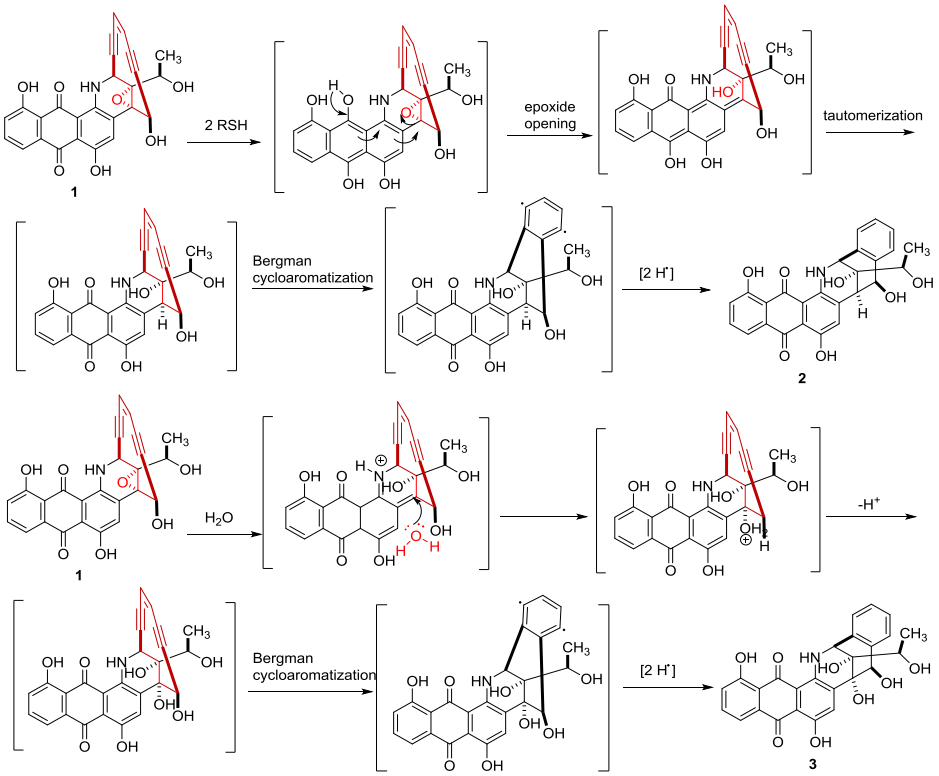


Figure S14. ROESY spectrum of YPM B (**2**) (acetone-d_6)

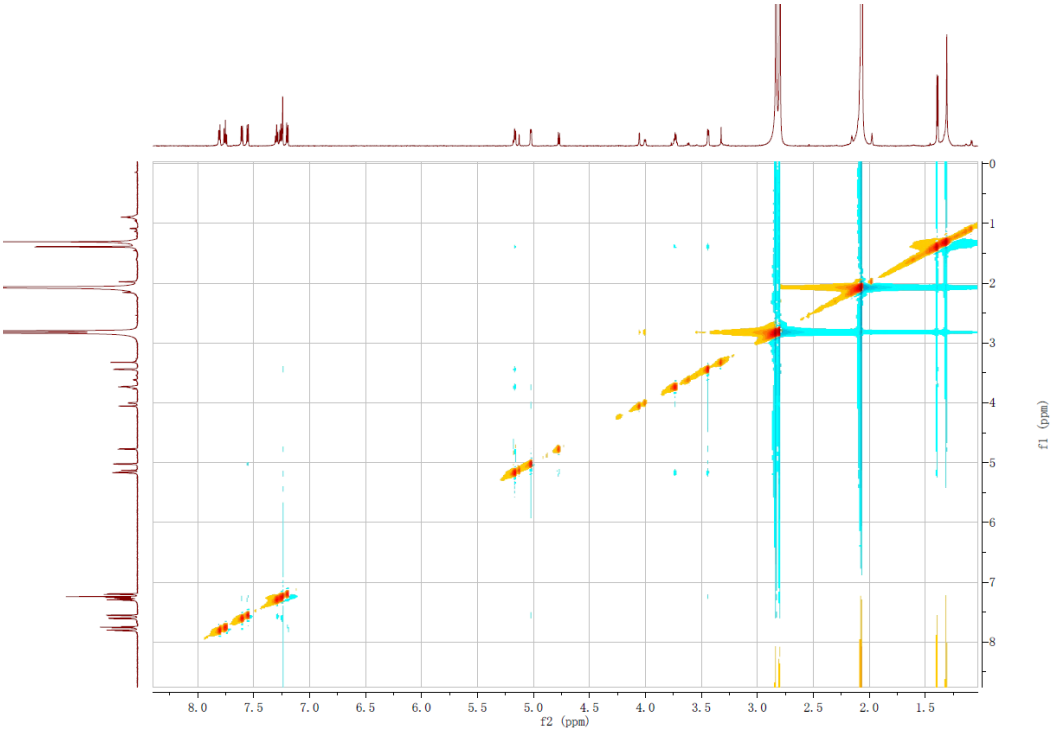


Figure S15. ^{1}H-^{1}H COSY spectrum of YPM B (**2**) (acetone-d_6)

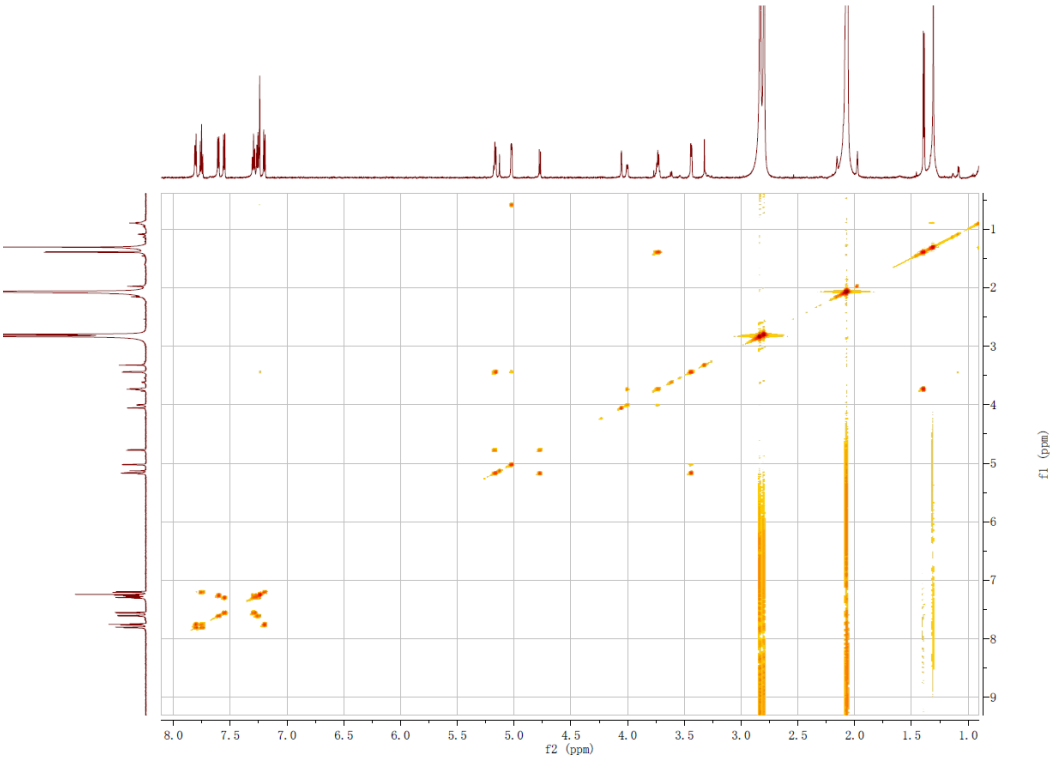


Figure S16. HSQC spectrum of YPM B (**2**) (acetone-d_6)

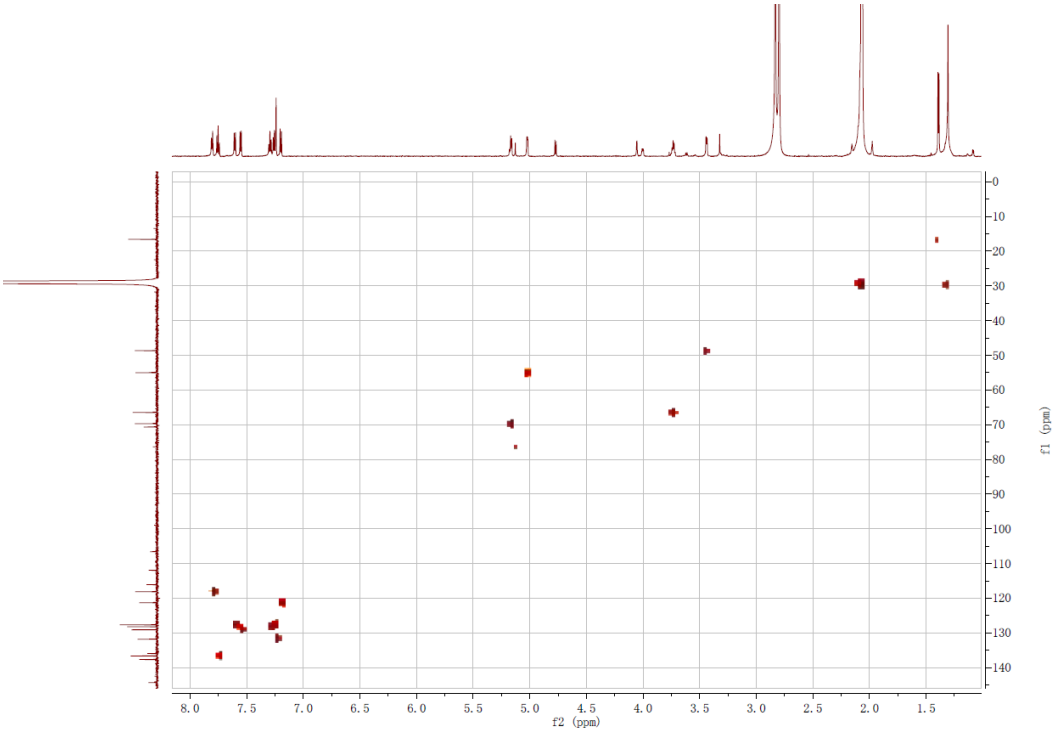


Figure S17. HMBC spectrum of YPM B (**2**) (acetone-d_6)

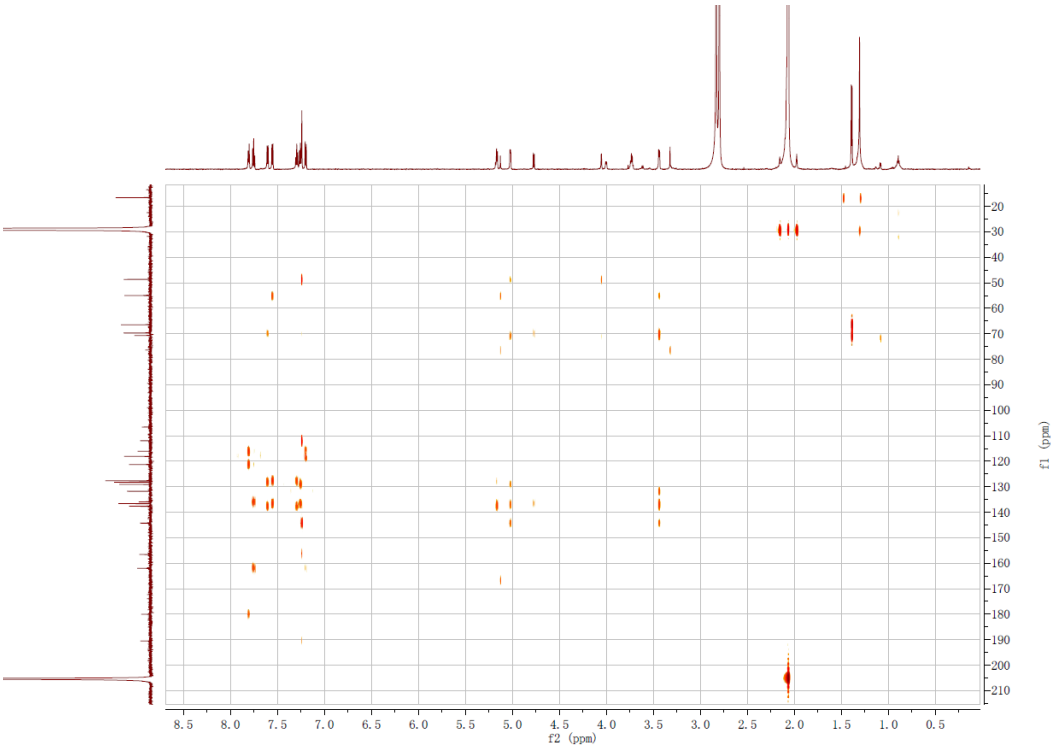


Figure S18. ^{1}H NMR spectrum of YPM C (**3**) (700 MHz, acetone-d_6)

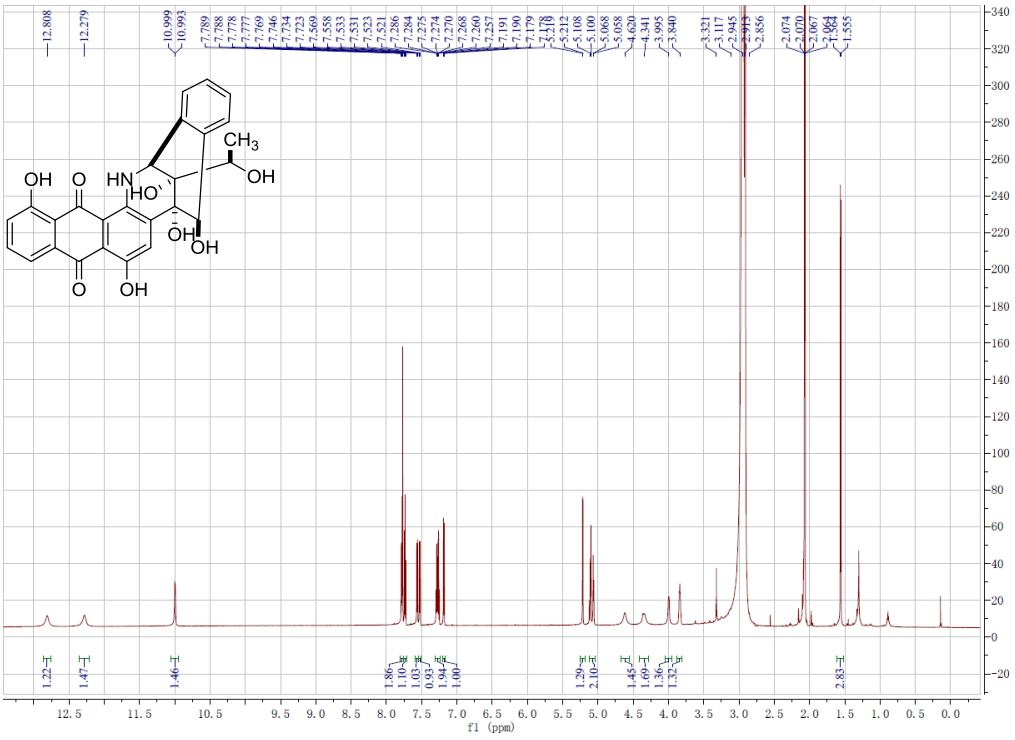


Figure S19. ^{13}C NMR spectrum of YPM C (**3**) (175 MHz, acetone-d_6)

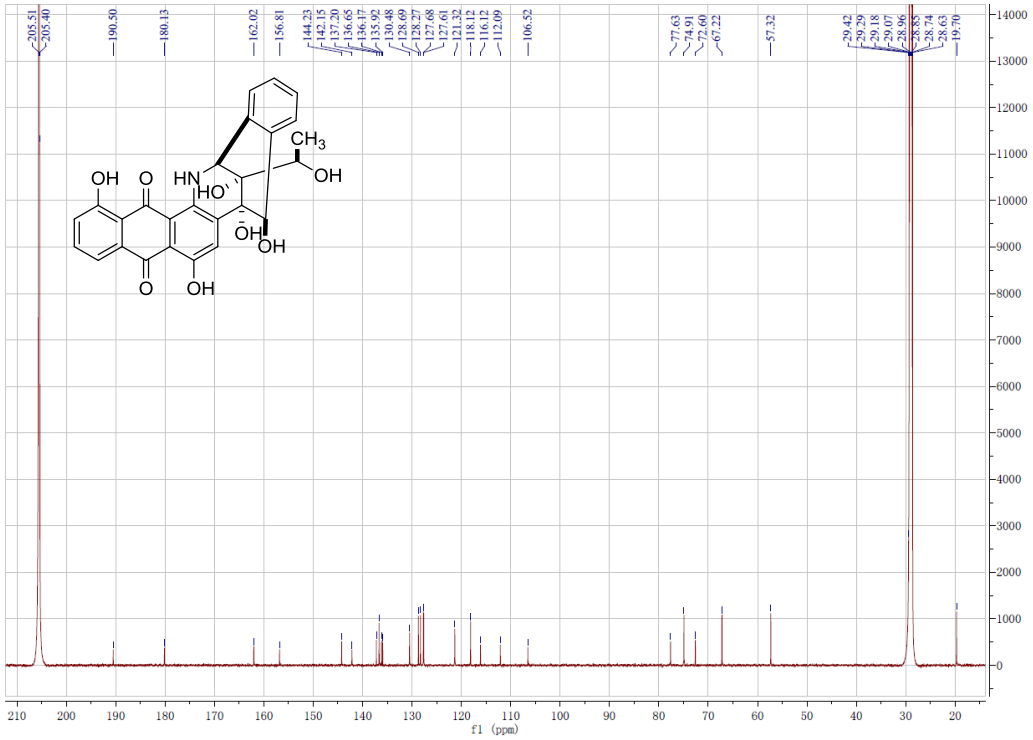


Figure S20. ^{1}H-^{1}H COSY spectrum of YPM C (**3**) (acetone-d_6)

Figure S21. HSQC spectrum of YPM C (**3**) (acetone-d_6)

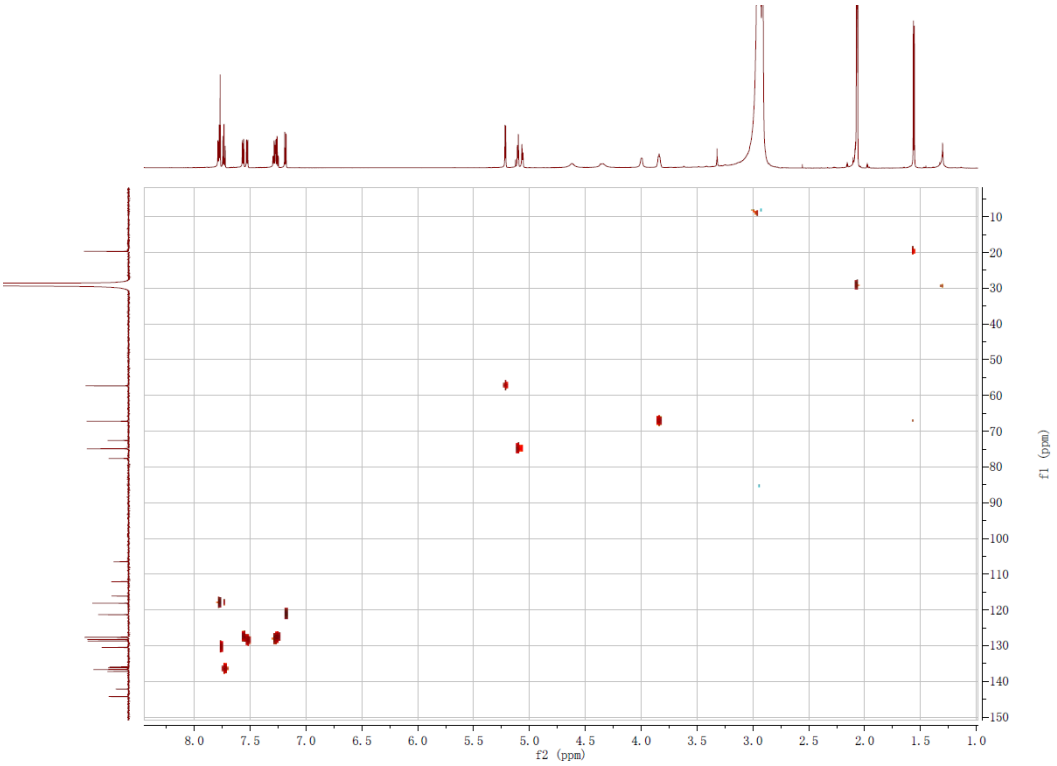


Figure S22. HMBC spectrum of YPM C (**3**) (acetone-d_6)

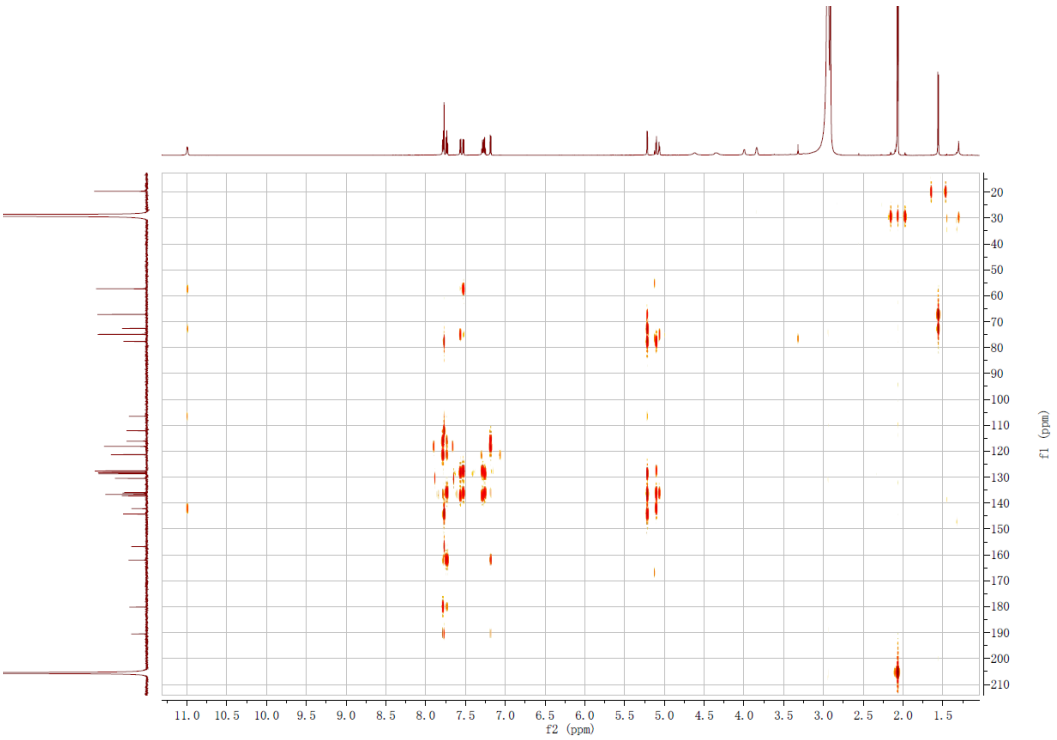


Figure S23. ROESY spectrum of YPM C (**3**) (acetone-d_6)

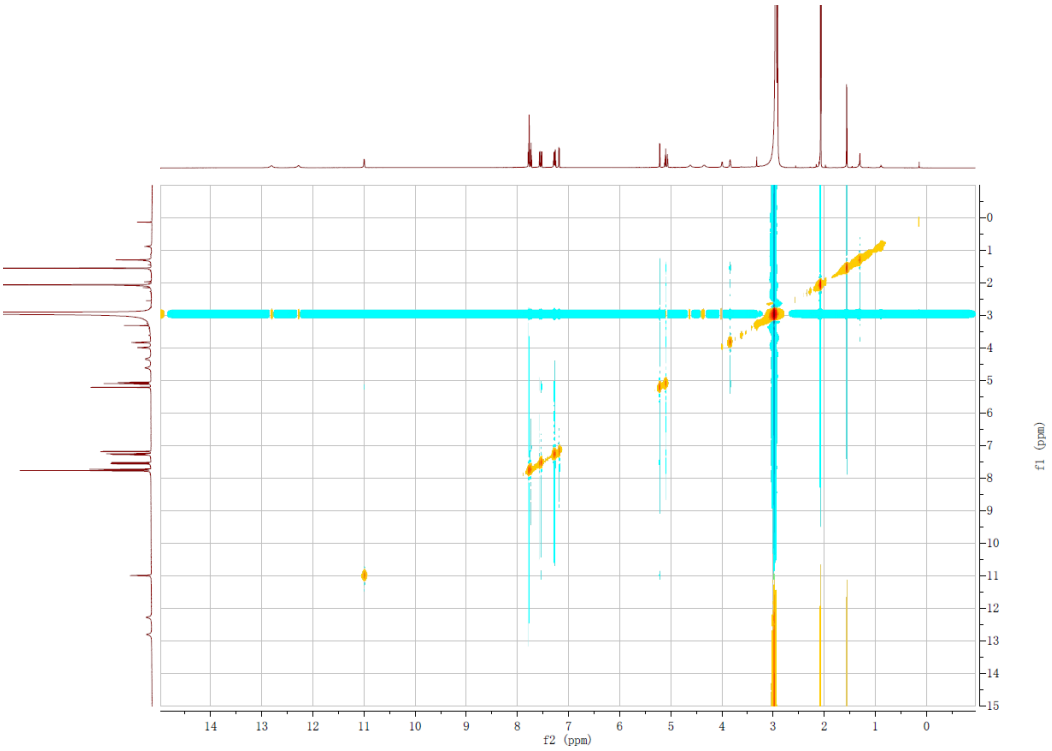


Figure S24. ^{1}H NMR spectrum of YPM D (**4**) (700 MHz, acetone-d_6)

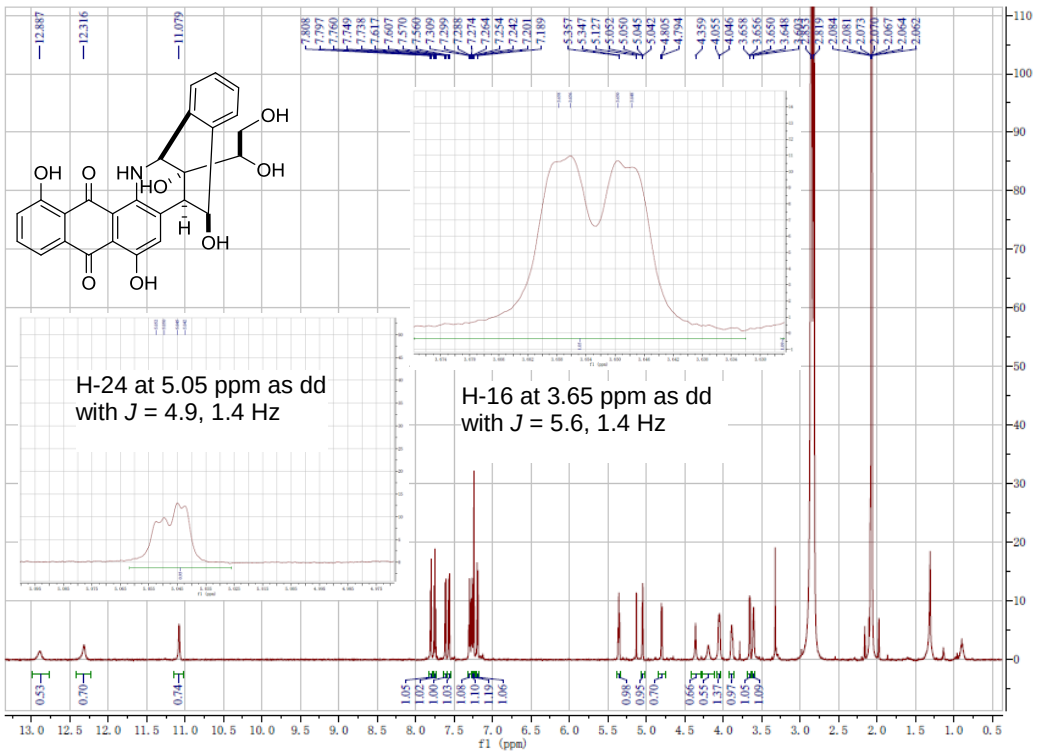


Figure S25. ^{13}C NMR spectrum of YPM D (**4**) (175 MHz, acetone-d_6)

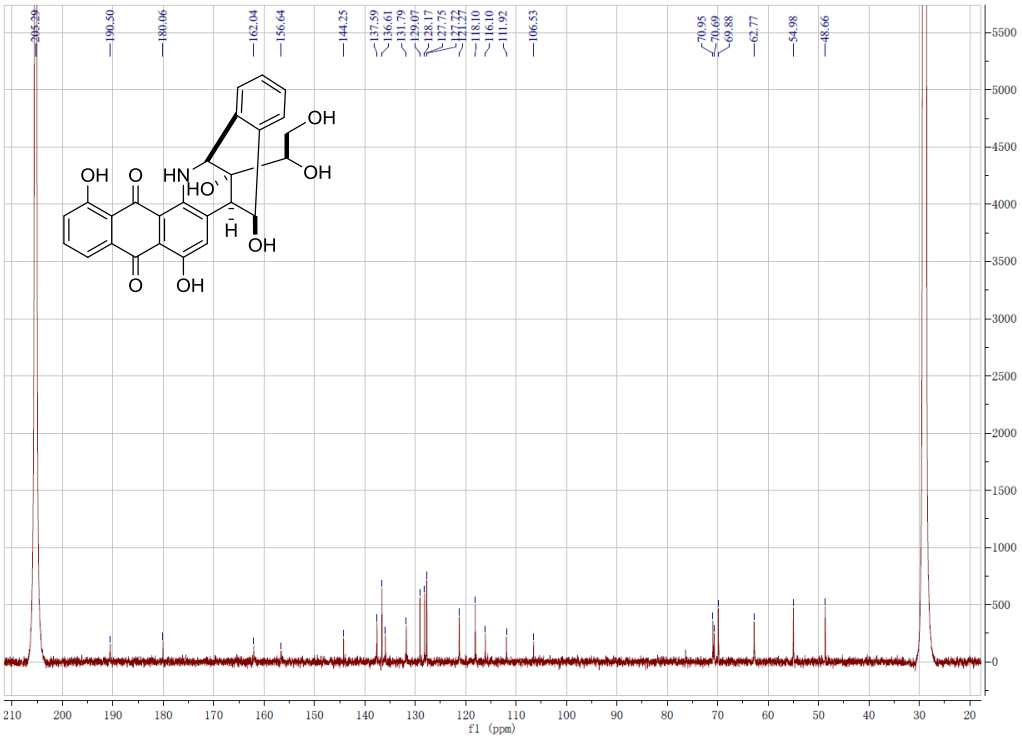


Figure S26. ^{1}H-^{1}H COSY spectrum of YPM D (**4**) (acetone-d_6)

Figure S27. HSQC spectrum of YPM D (**4**) (acetone-d_6)

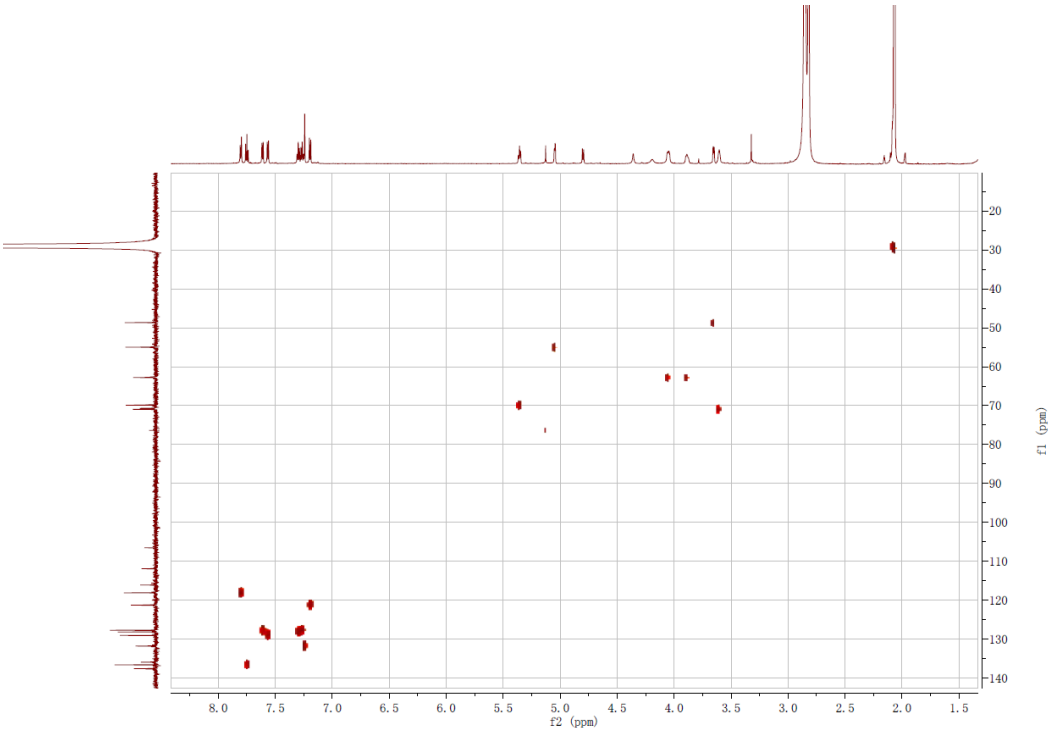


Figure S28. HMBC spectrum of YPM D (**4**) (acetone-d_6)

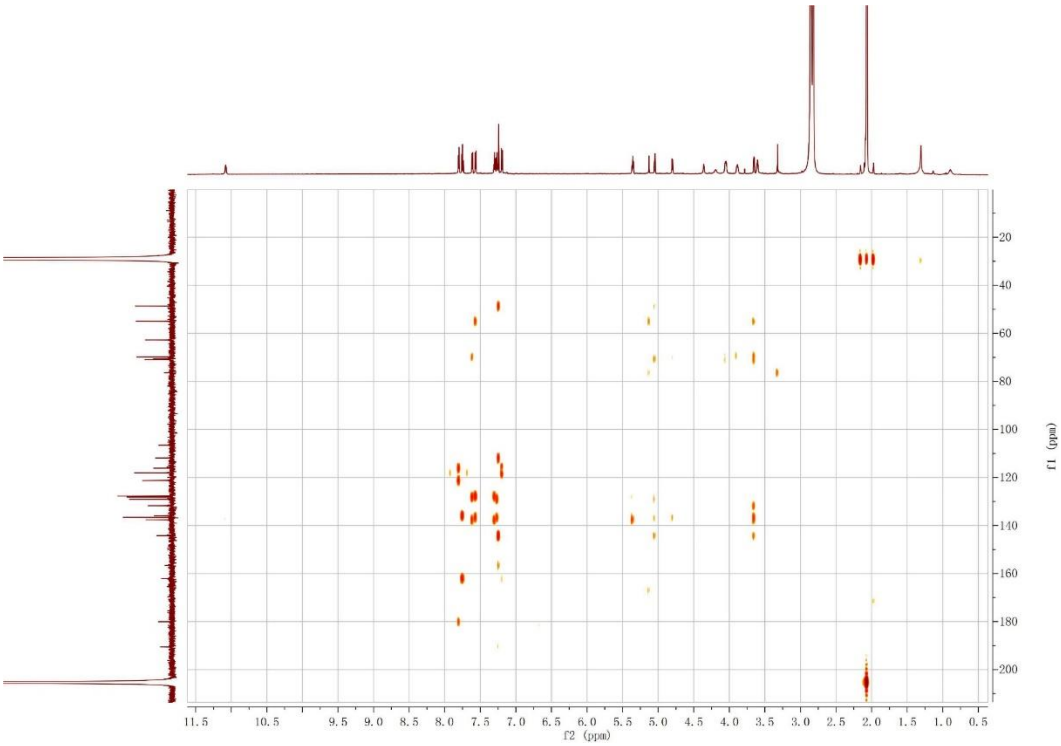


Figure S29. ROESY spectrum of YPM D (**4**) (acetone-d_6)

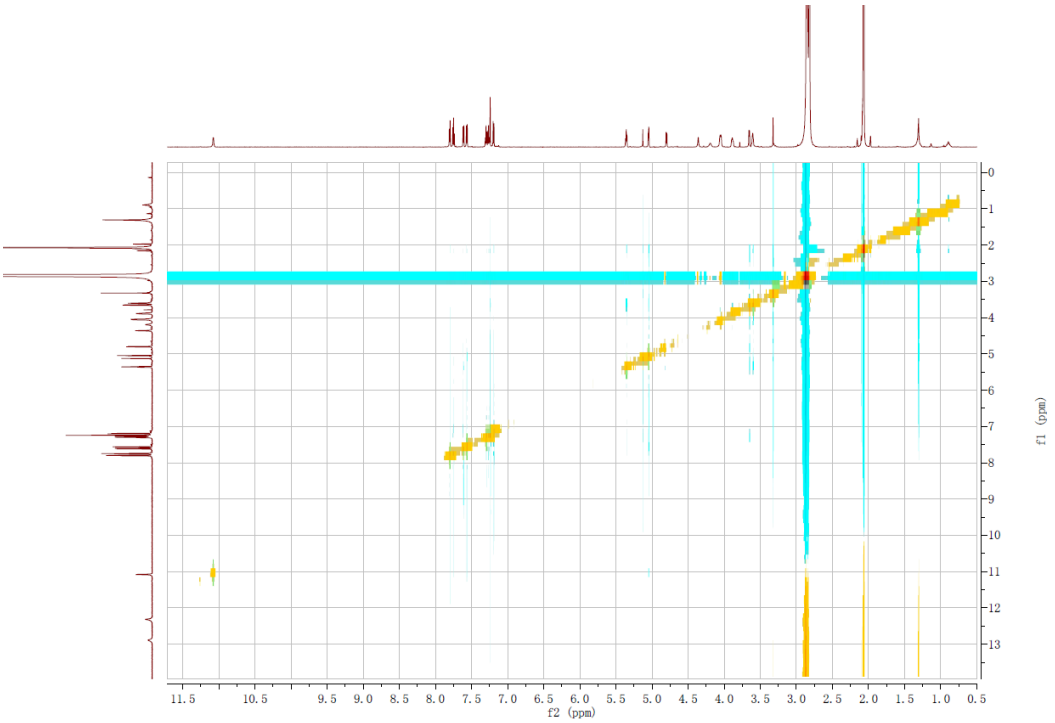


Figure S30. ^{1}H NMR spectrum of YPM E (**5**) (700 MHz, acetone-d_6)

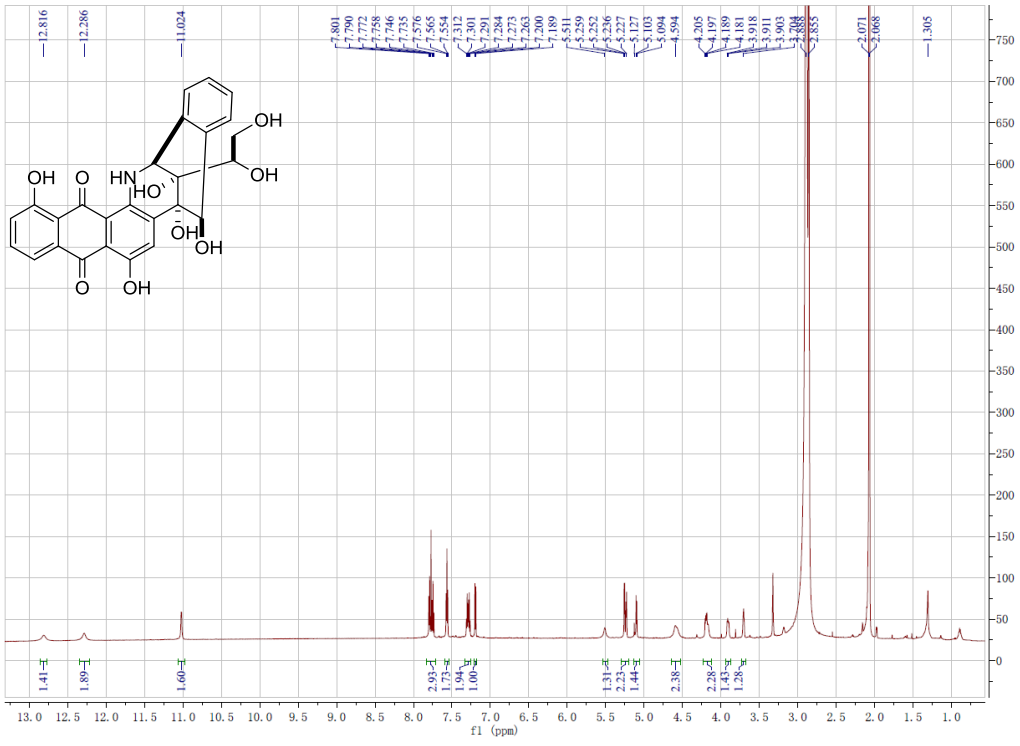


Figure S31. ^{13}C NMR spectrum of YPM E (**5**) (175 MHz, acetone-d_6)

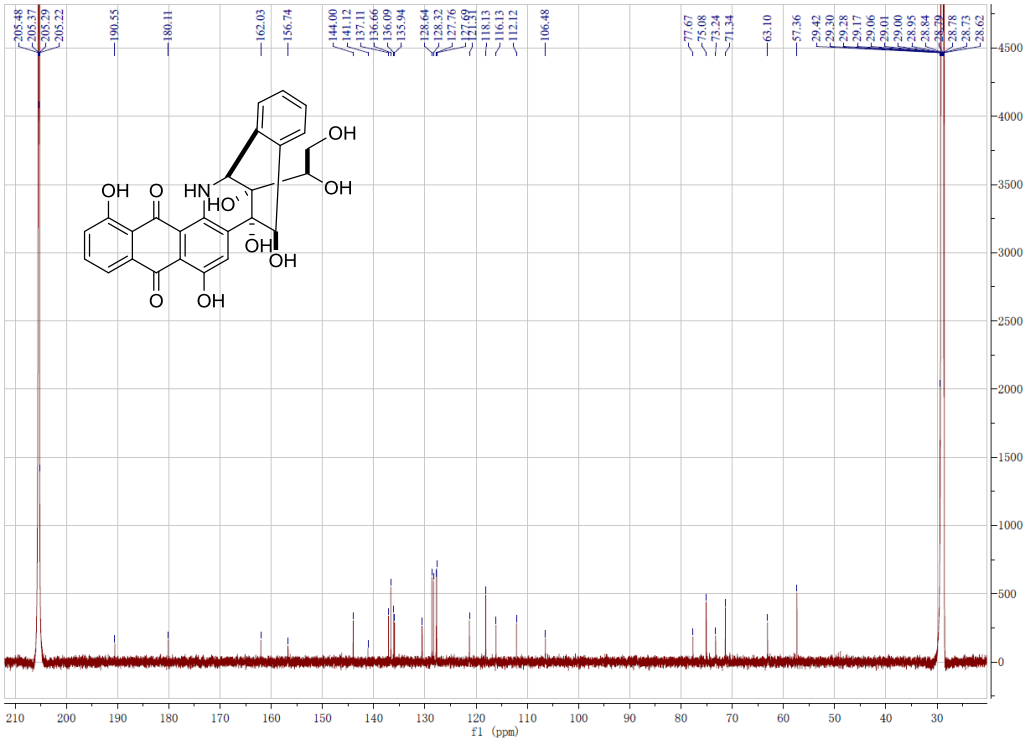


Figure S32. ^{1}H-^{1}H COSY spectrum of YPM E (**5**) (acetone-d_6)

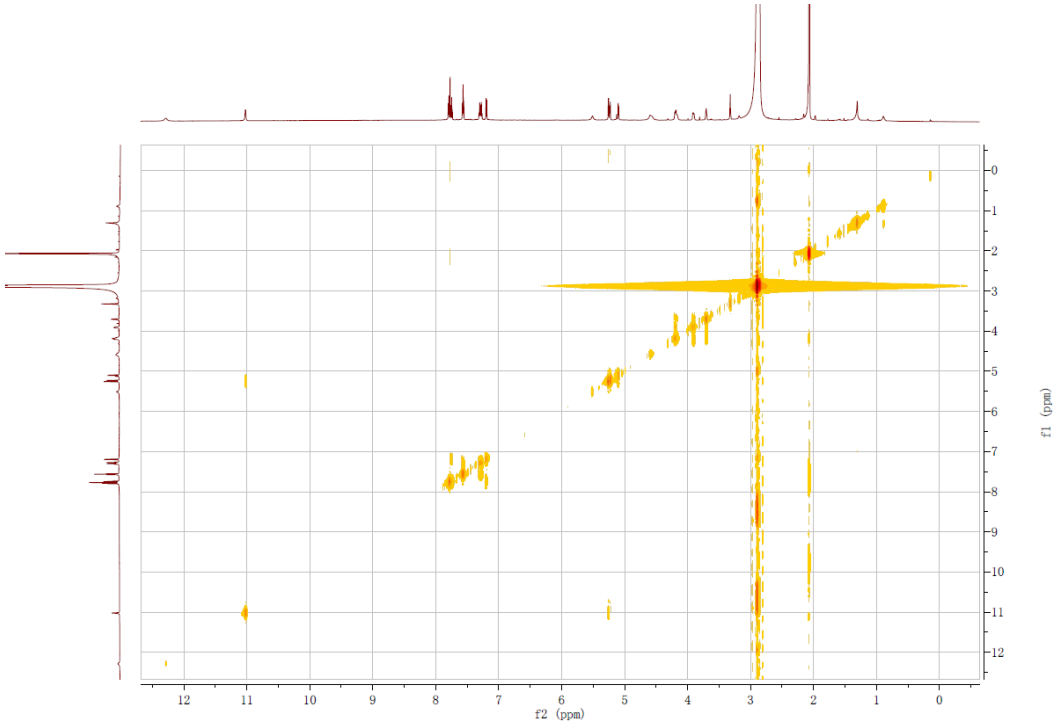


Figure S33. HSQC spectrum of YPM E (**5**) (acetone-d_6)

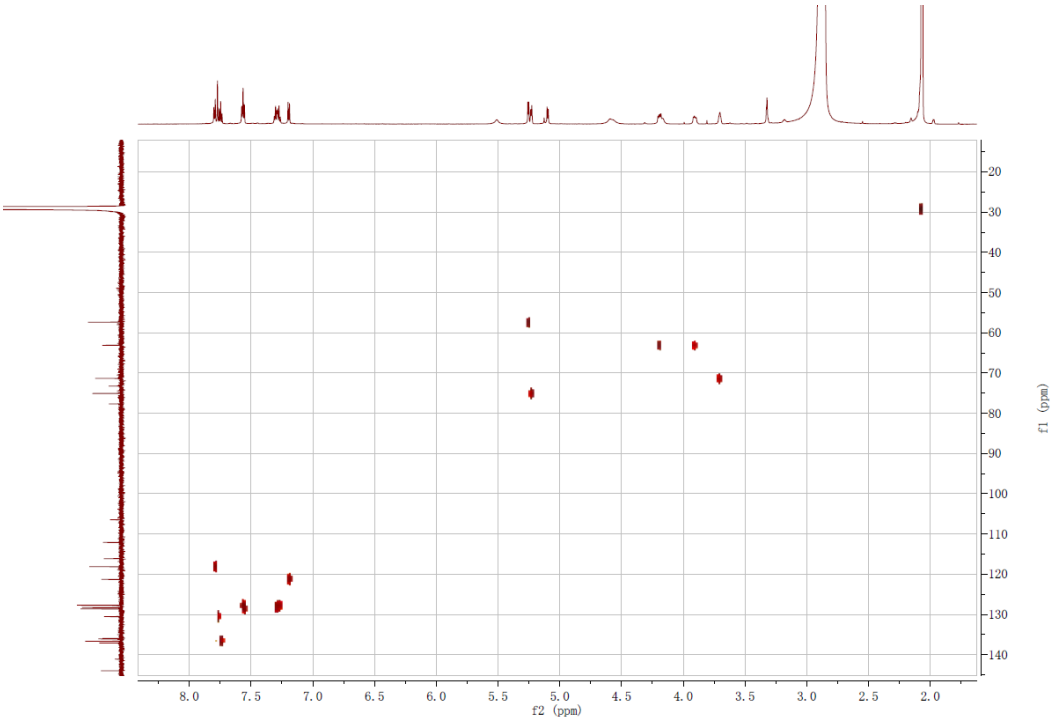


Figure S34. HMBC NMR spectra of YPM E (**5**) (acetone-d_6)

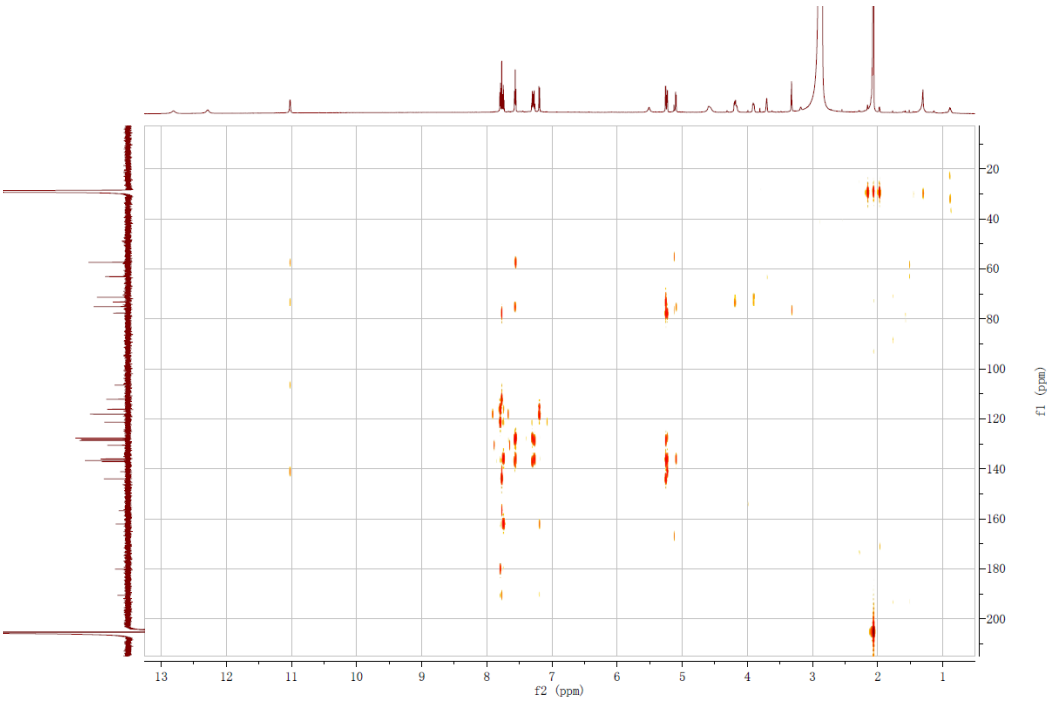


Figure S35. ROESY NMR spectra of YPM E (**5**) (acetone-d_6)

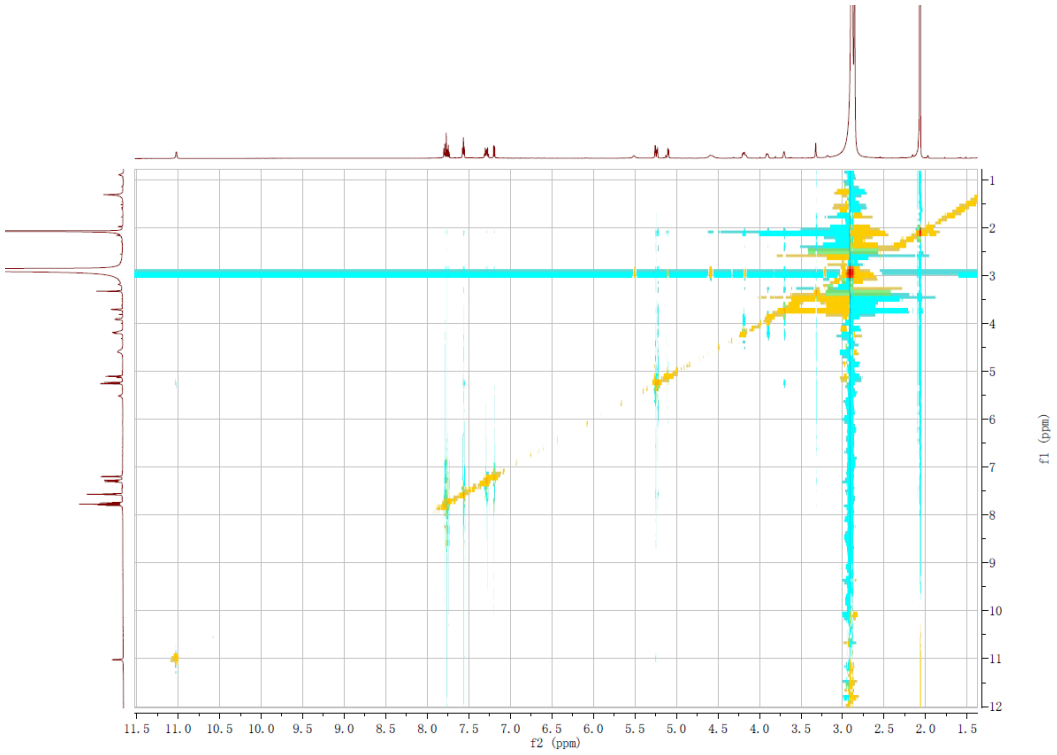
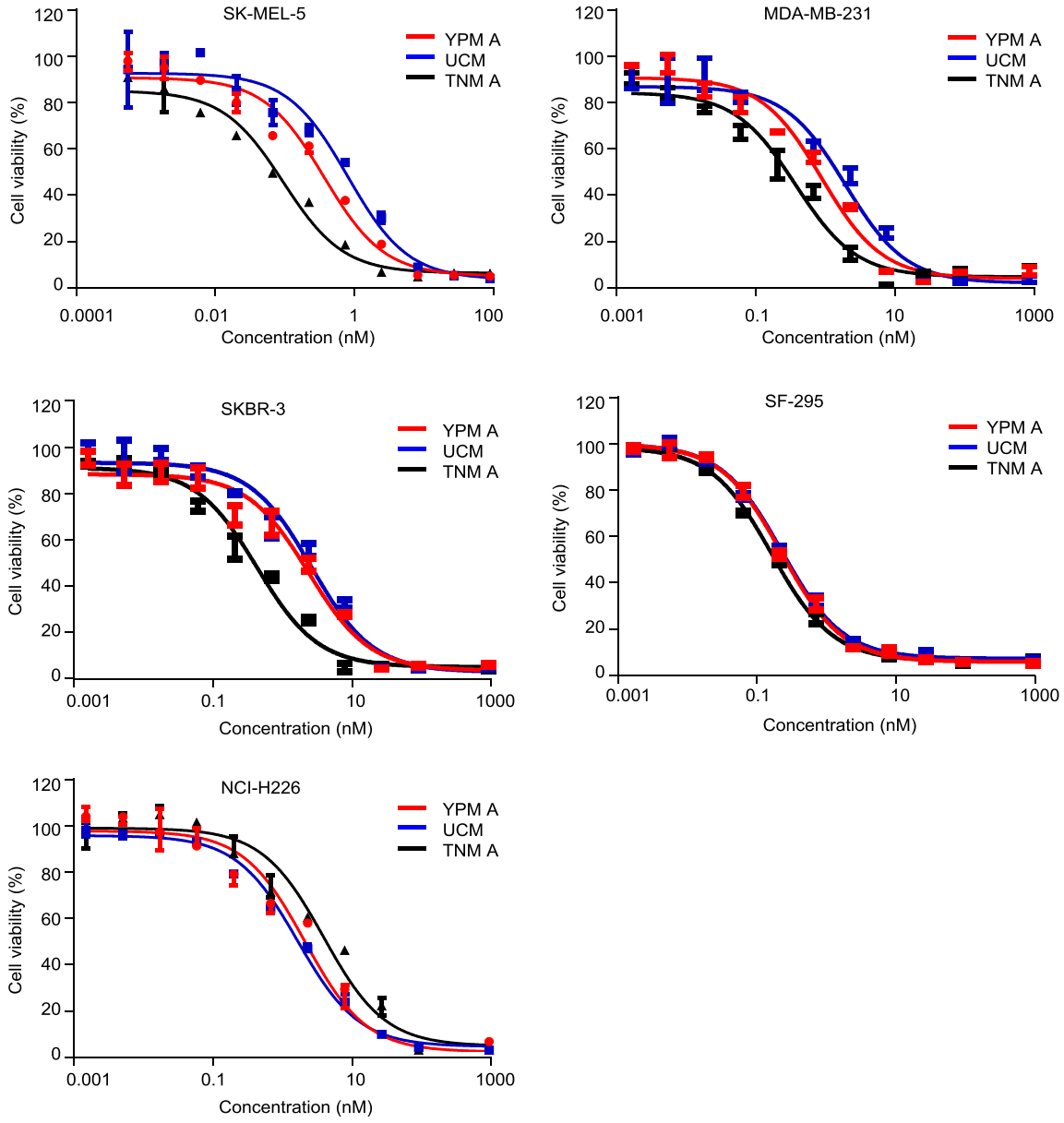


Figure S36. Cytotoxicity assay of YPM A in comparison with UCM and TNM A.



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