Antibacterial and cytotoxic angucyclines discovered by heterologous expression of a type II polyketide gene cluster

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List of Supporting Information

Figure S1 .[1]H NMR (500 MHz, DMSO) spectrum of compound **1**

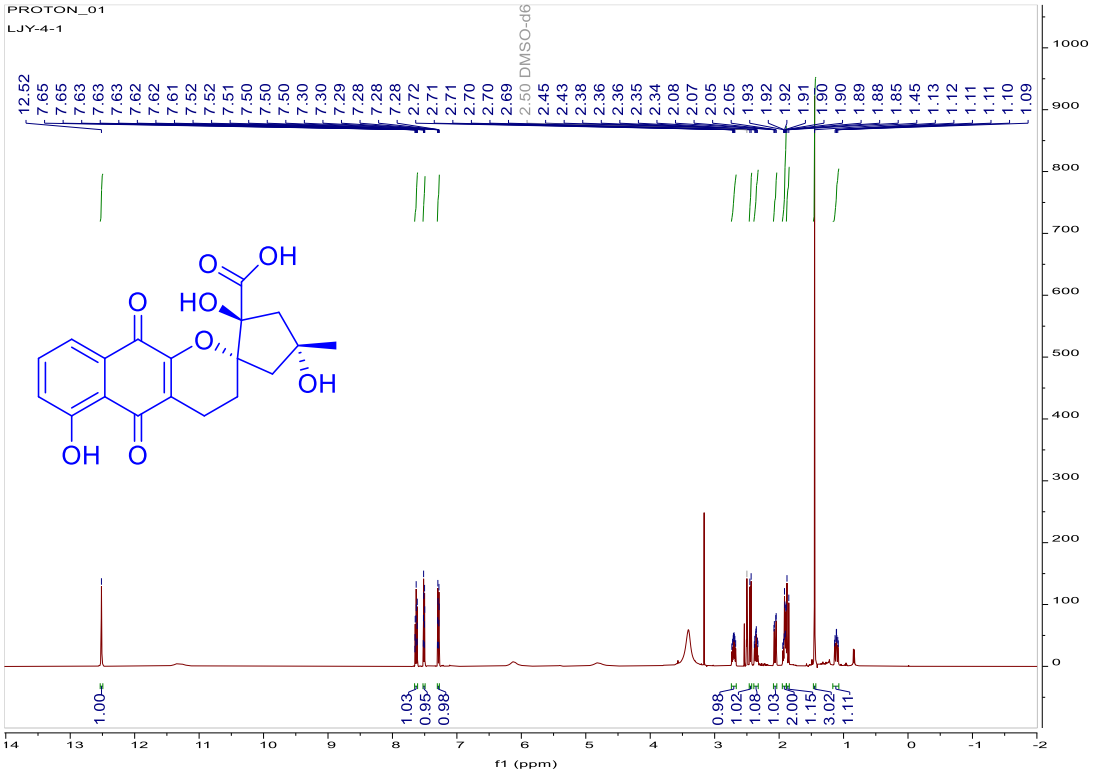

Figure S2 .[13]C NMR (125 MHz, DMSO) spectrum of compound **1**

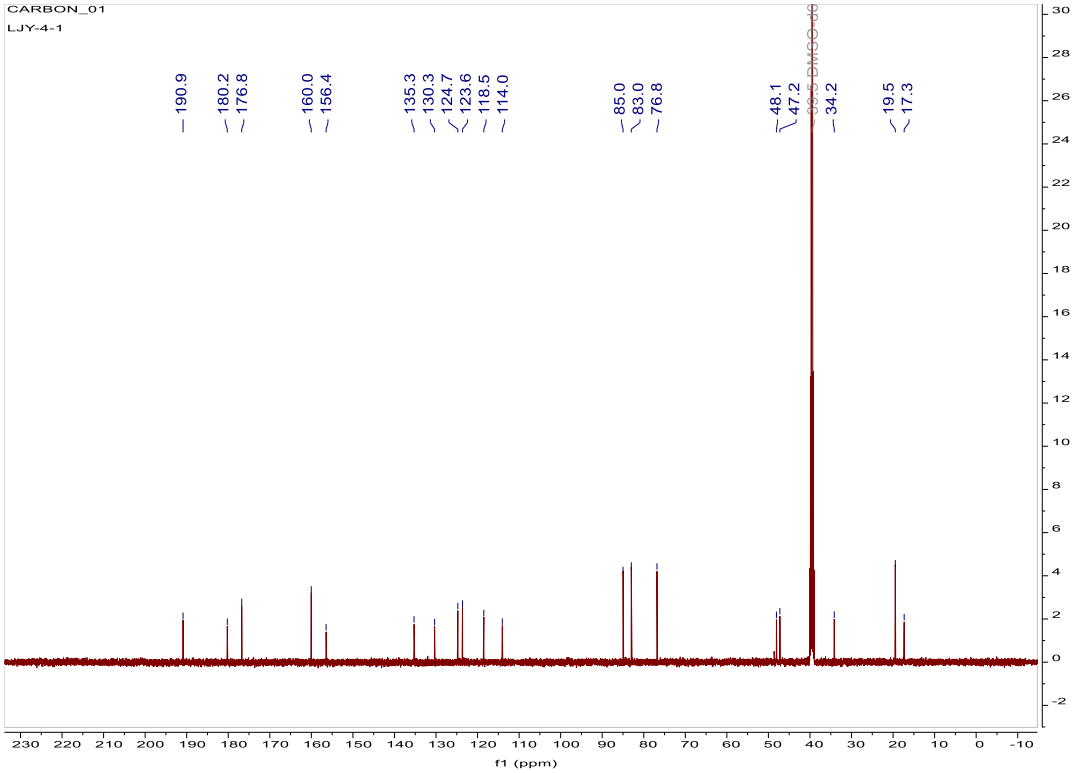

Figure S3. HSQC spectrum of compound **1**

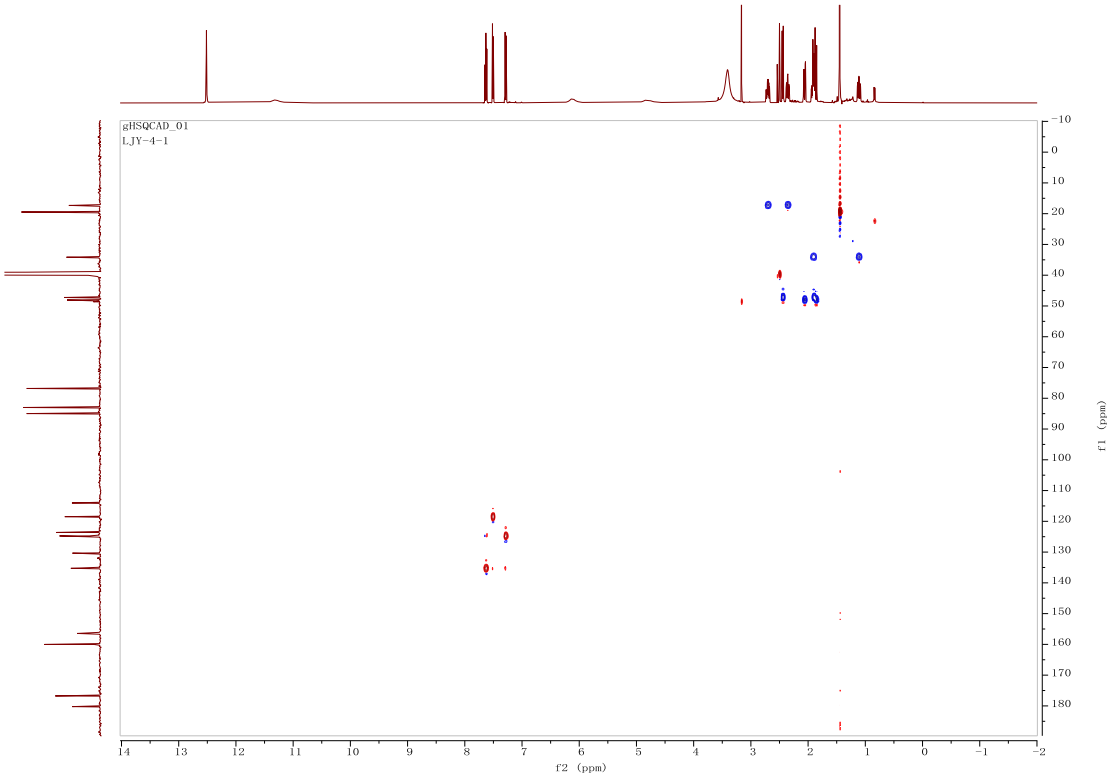

Figure S4. ^{1}H-^{1}H COSY spectrum of compound **1**

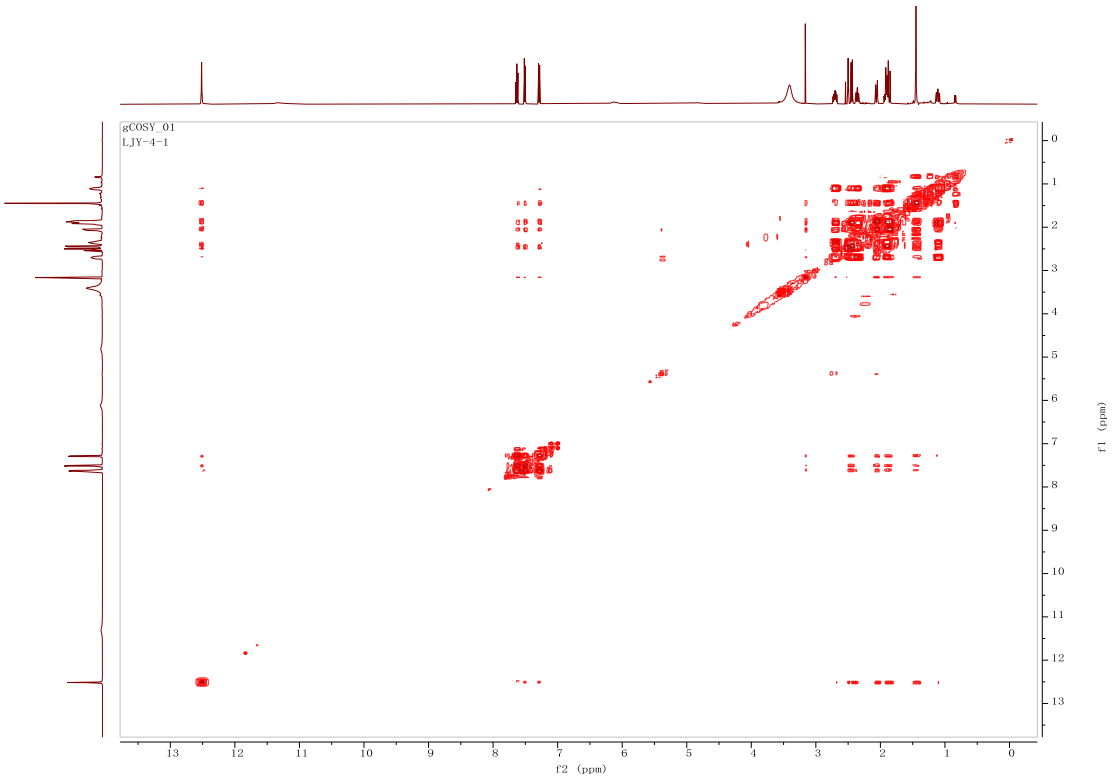

Figure S5. ^{1}H-^{13}C HMBC spectrum of compound **1**

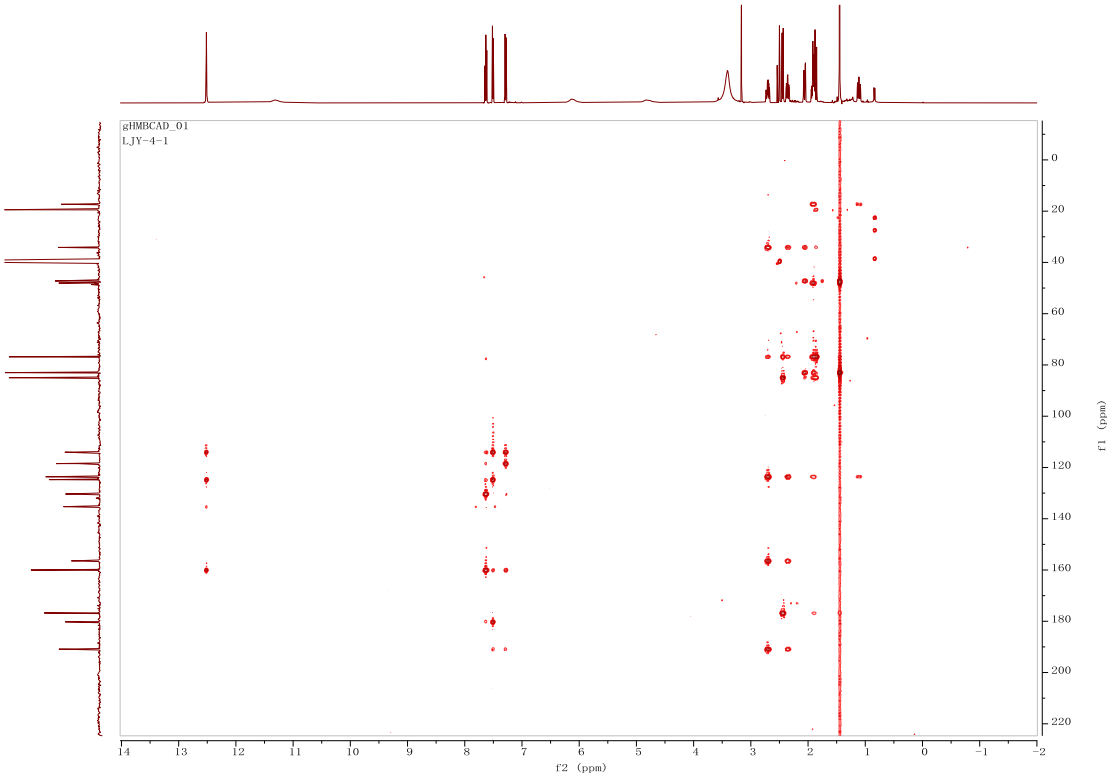

Figure S6. HRESIMS spectrum of compound **1**

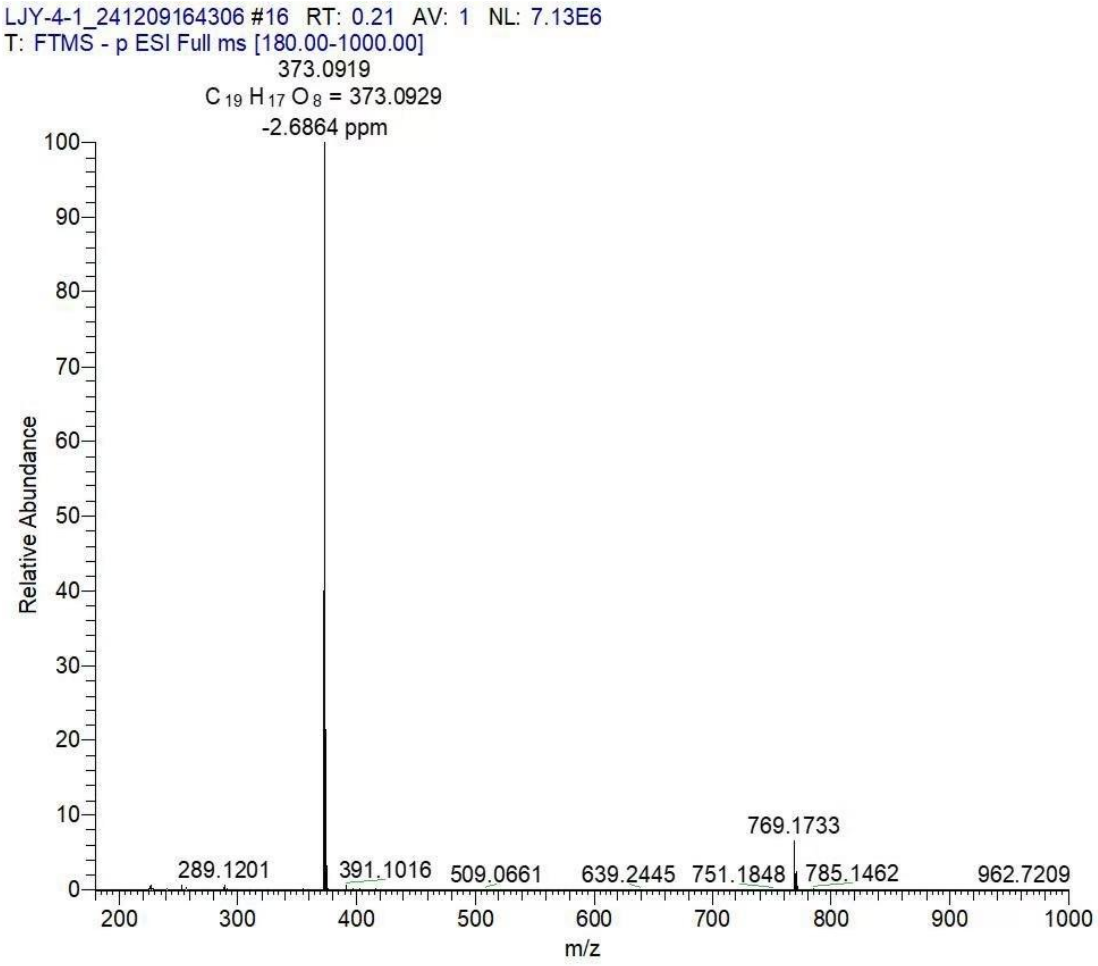

Figure S7. IR spectrum of compound **1**

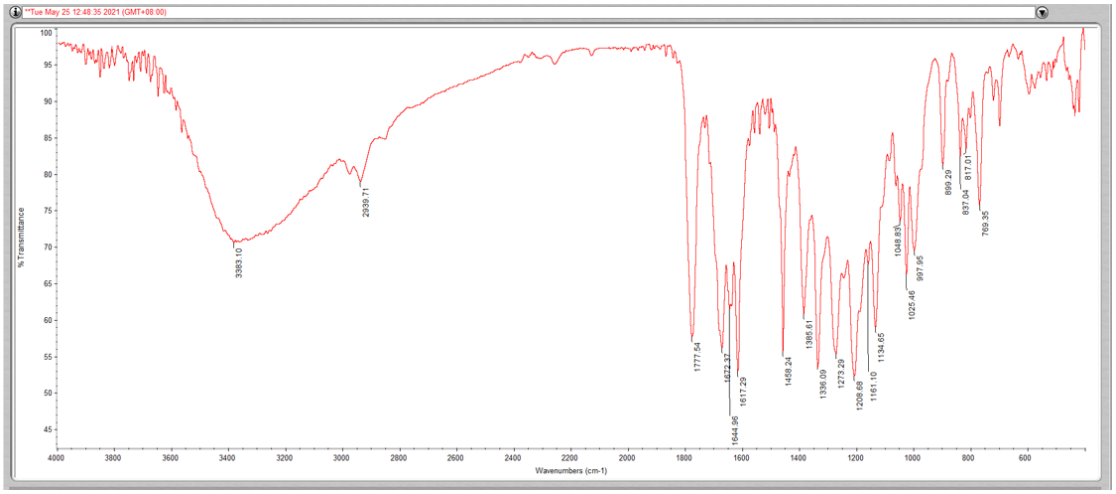

Figure S8. ^{1}H NMR (600 MHz, DMSO) spectrum of compound **2**

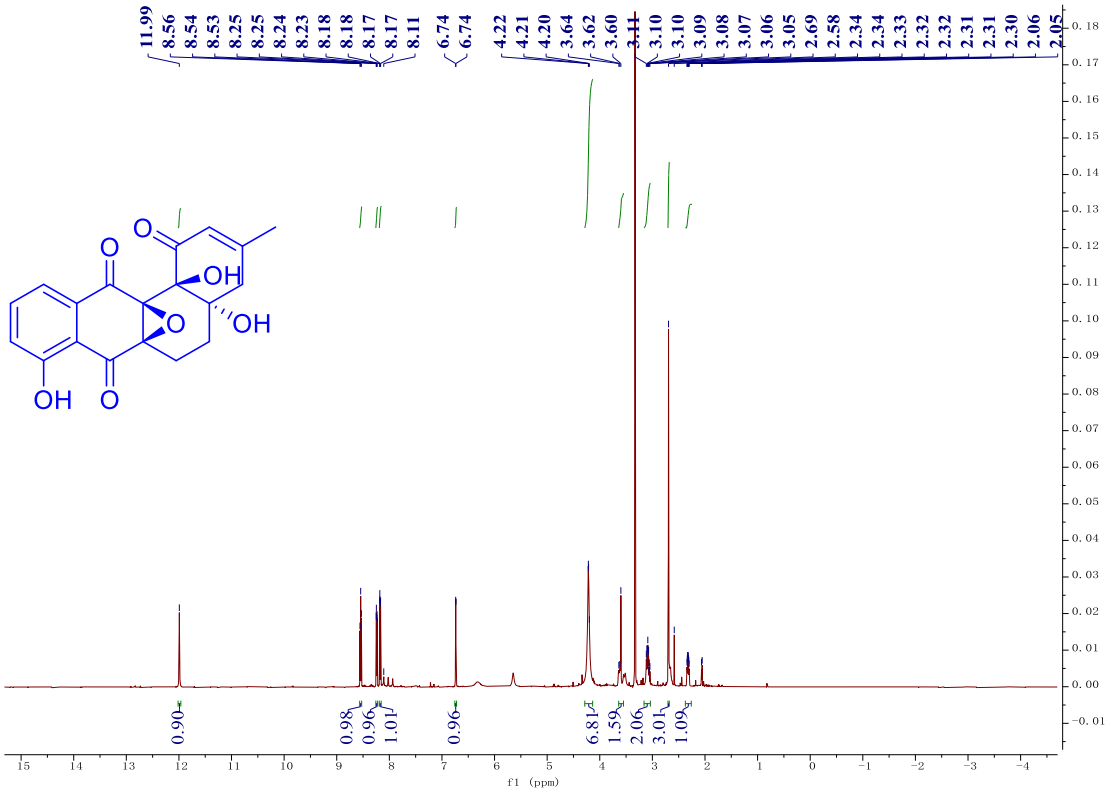

Figure S9. ^{13}C NMR (150 MHz, DMSO) spectrum of compound **2**

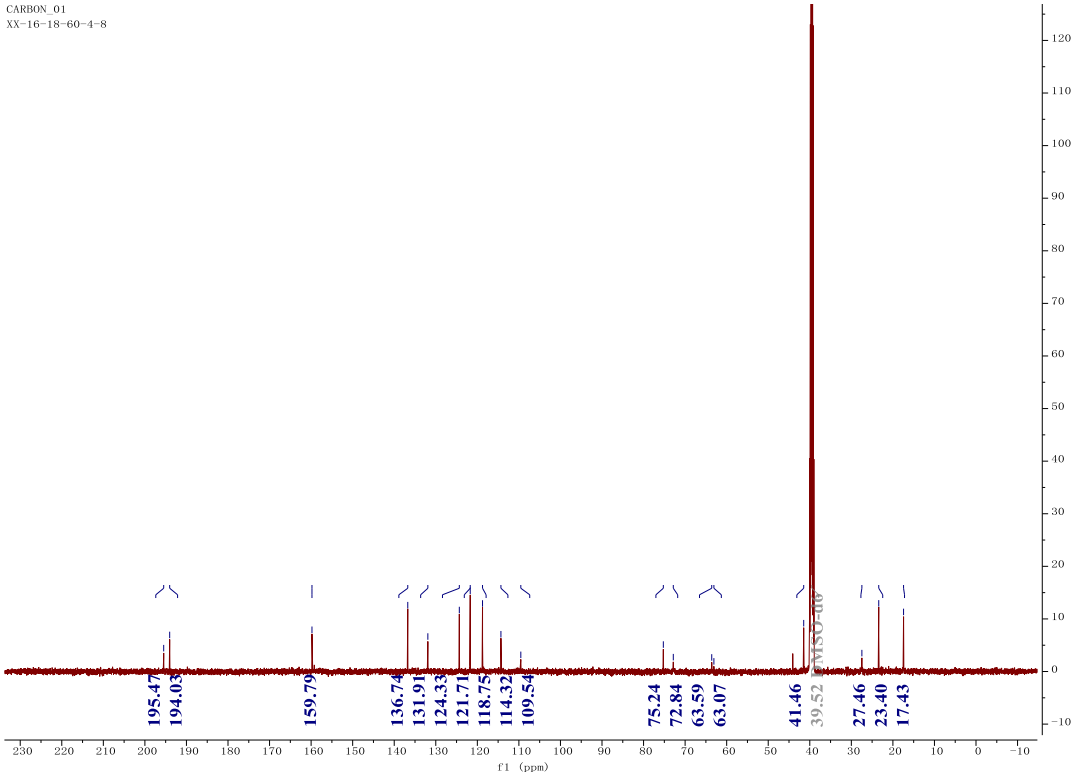

Figure S10. HSQC spectrum of compound **2**

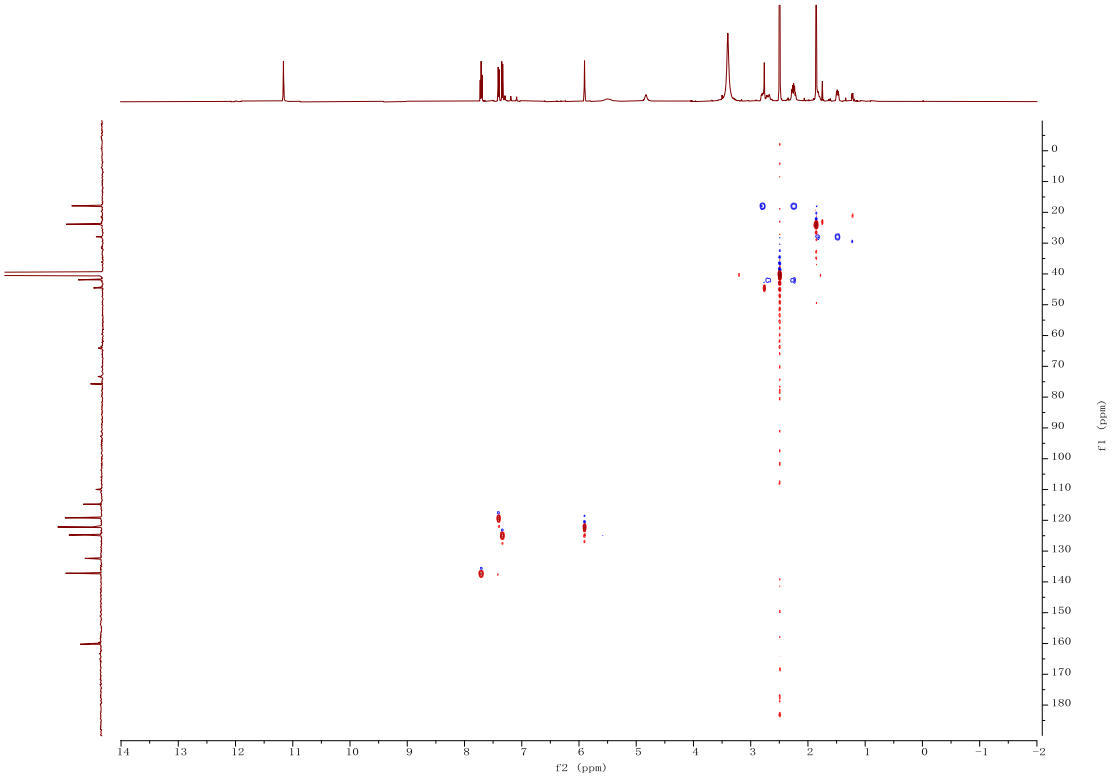

Figure S11. ^{1}H-^{1}H COSY spectrum of compound **2**

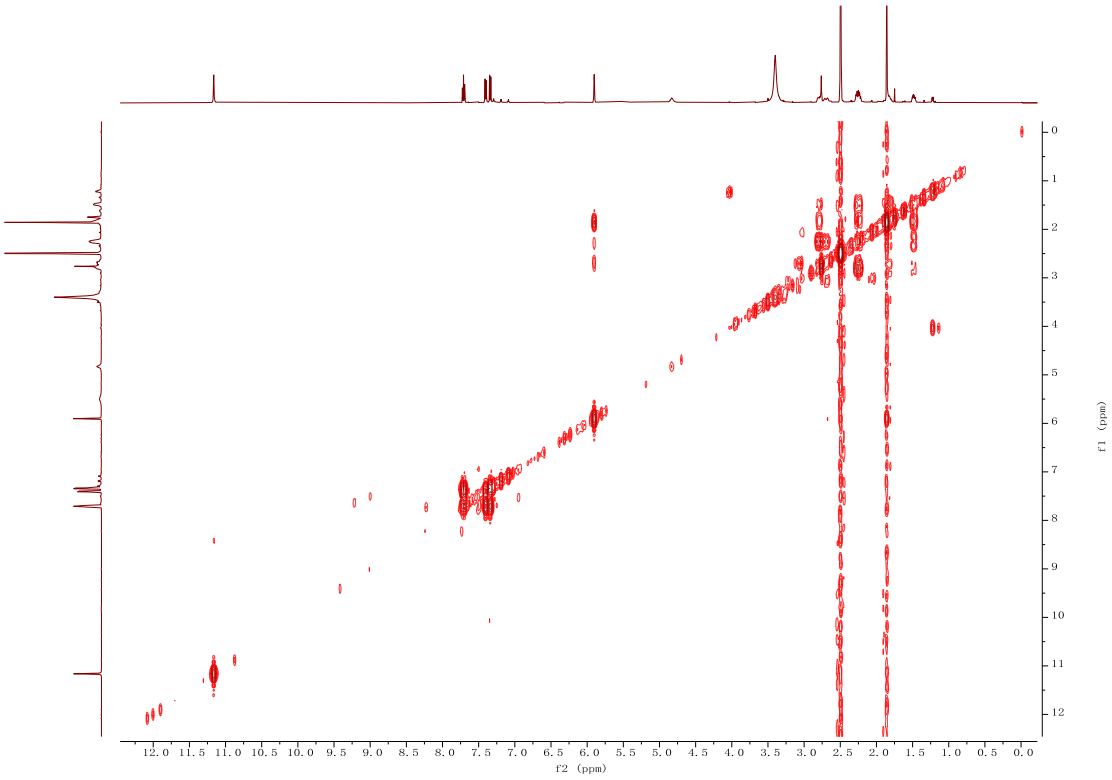

Figure S12. ^{1}H-^{13}C HMBC spectrum of compound **2**

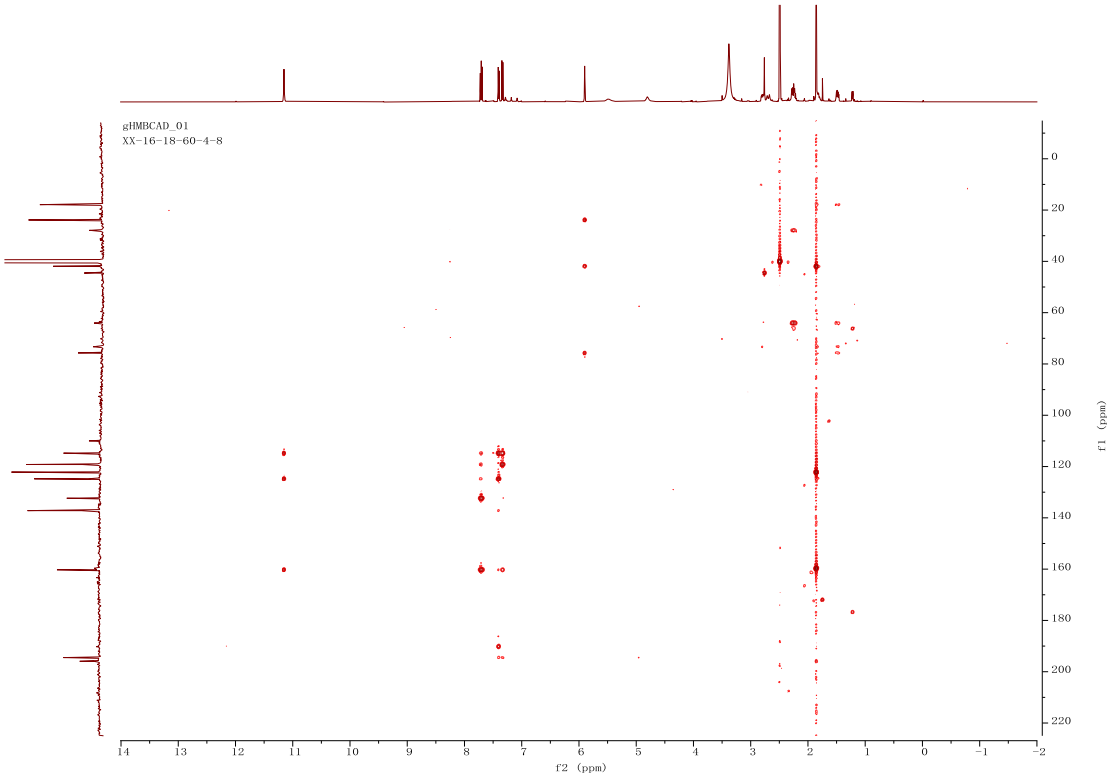

Figure S13. HRESIMS spectrum of compound **2**

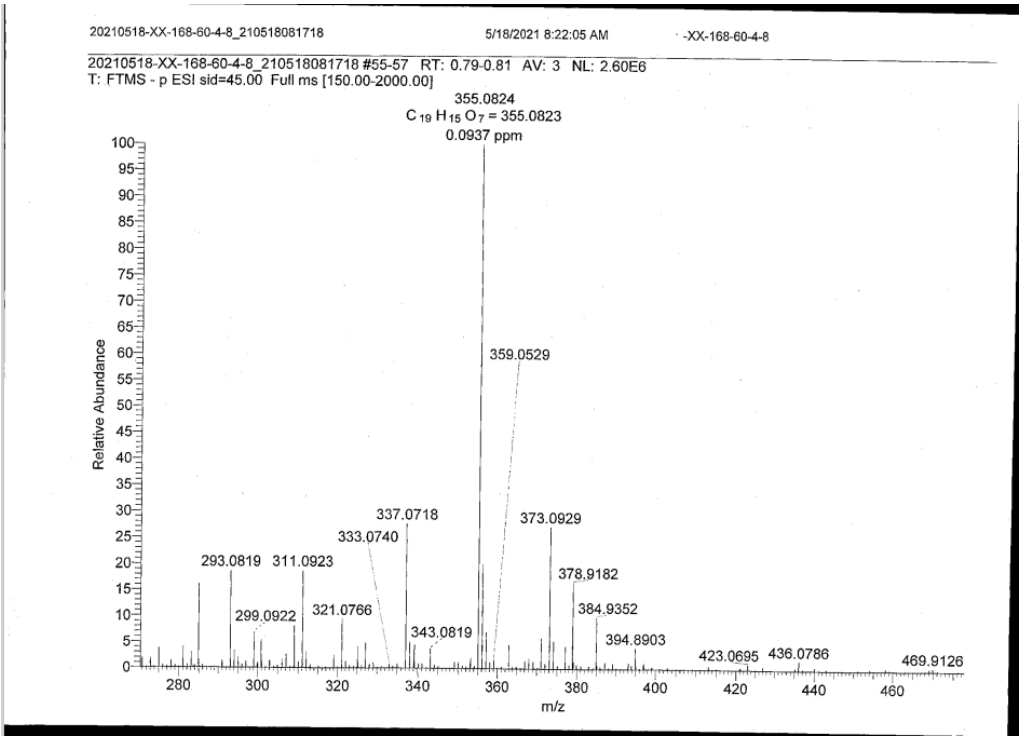

Figure S14. IR spectrum of compound **2**

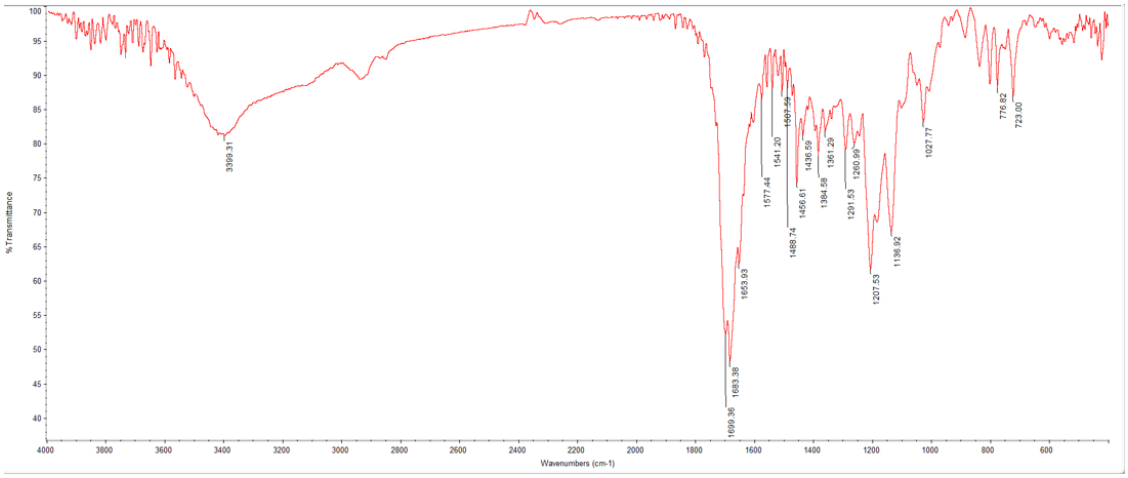

Figure S15. ^{1}H NMR (500 MHz, DMSO) spectrum of compound **3**

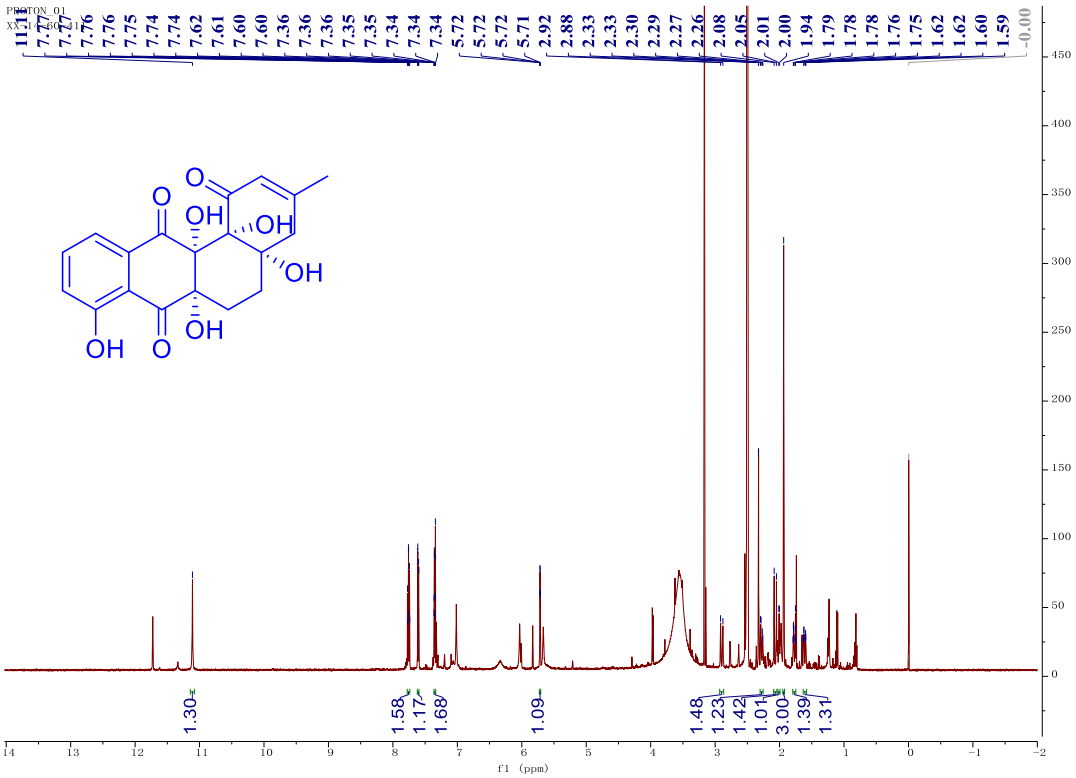

Figure S16. ^{13}C NMR (125 MHz, DMSO) spectrum of compound **3**

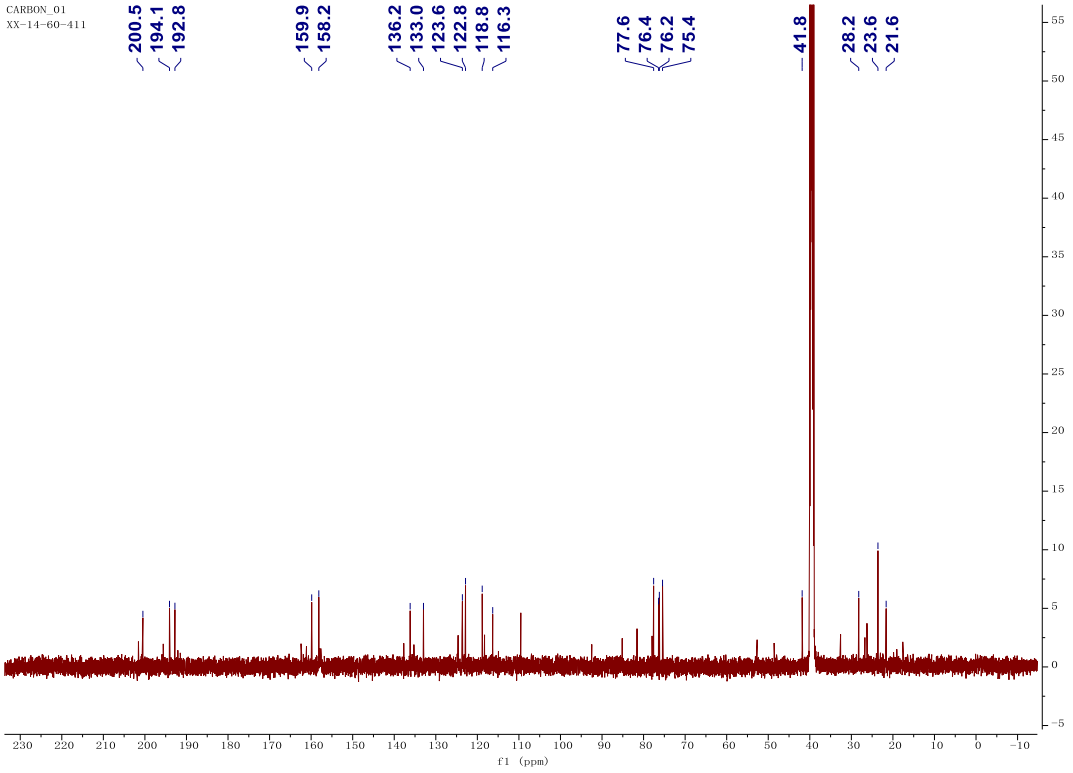

Figure S17. HSQC spectrum of compound **3**

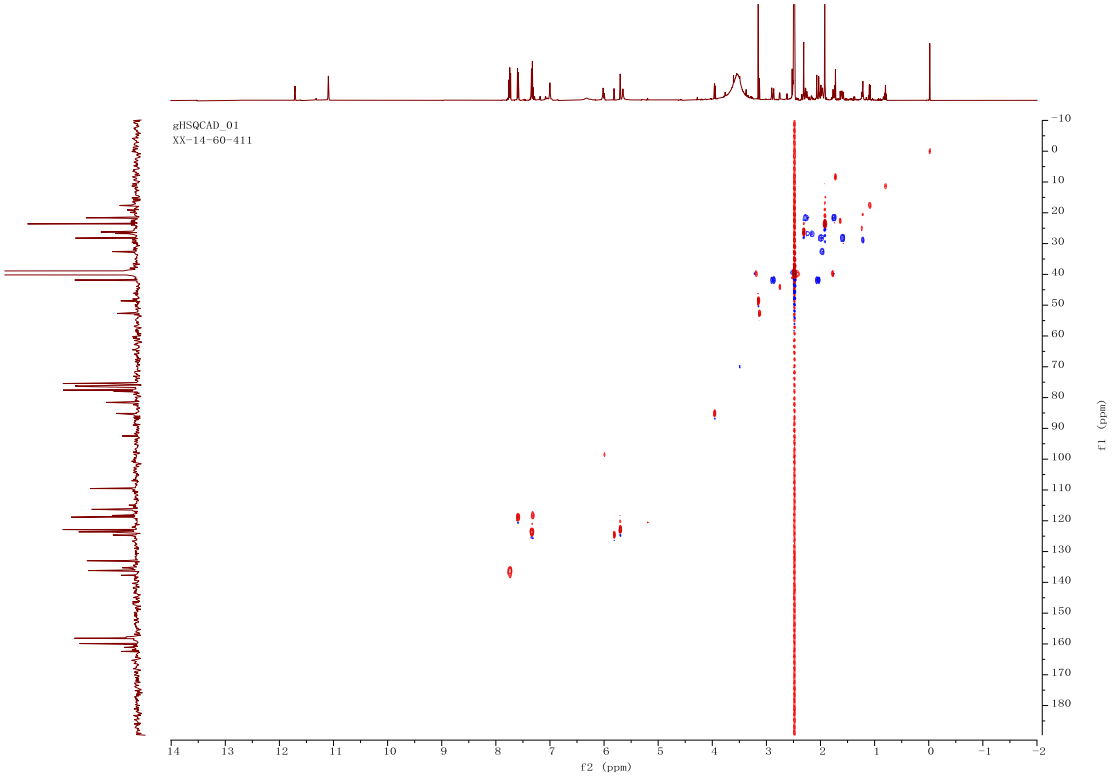

Figure S18. ^{1}H-^{1}H COSY spectrum of compound **3**

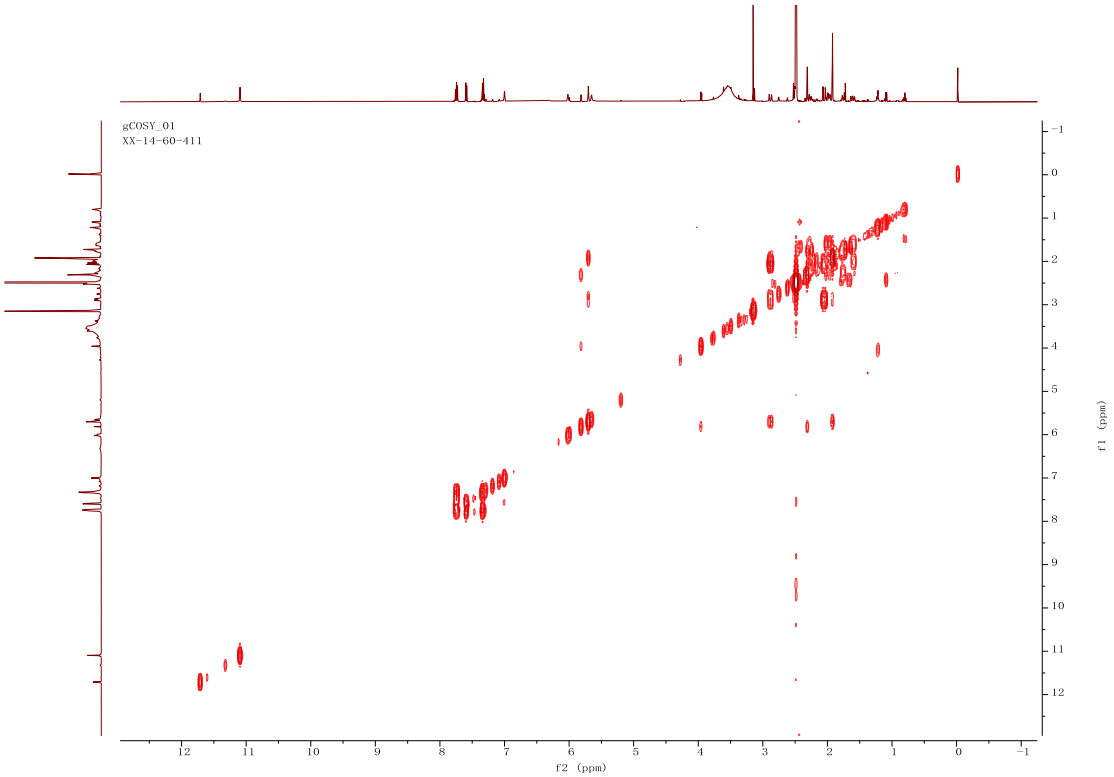

Figure S19. ^{1}H-^{13}C HMBC spectrum of compound **3**

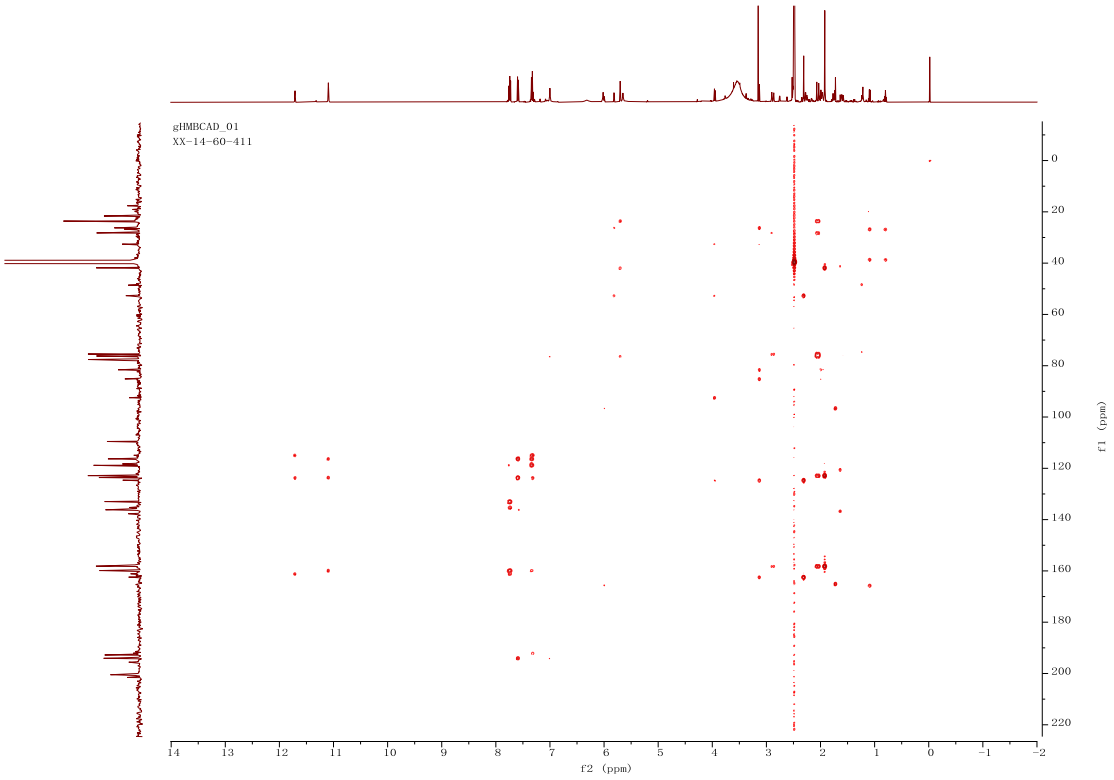

Figure S20. HRESIMS spectrum of compound **3**

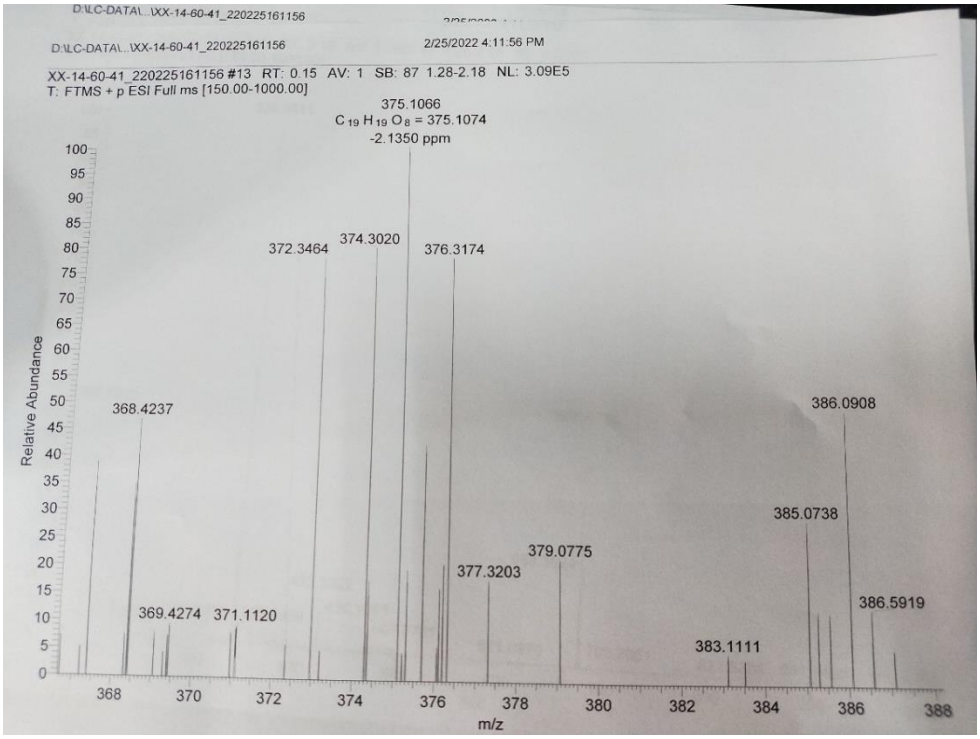

Figure S21. IR spectrum of compound **3**

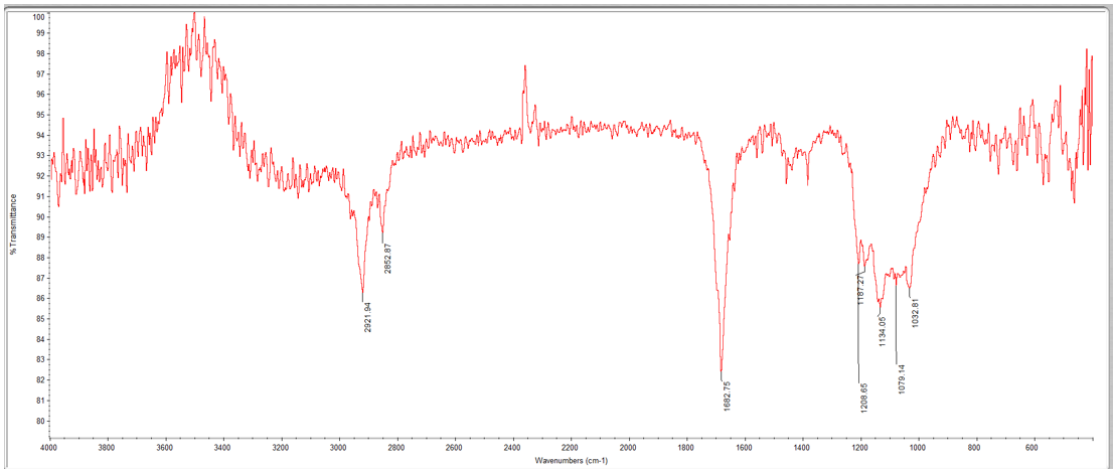

Figure S22. ^{13}C NMR calculations of compound **1** with stereoisomers (8a*S**,10*S**,12*R**)-isomer 1, (8a*S**,10*S**,12*S**)- isomer 2, (8a*R**,10*S**,12*R**)- isomer 3, and (8a*S**,10*R**,12*R**)-isomer 4 using the means of gauge-independent atomic orbital (GIAO) at the pcSseg-1/ revtpssrevtpss leve.

Functional		Solvent?	Basis Set		Type of Data	
mPW1PW91		PCM	6-311+G(d,p)		Shielding Tensors	
		DP4+	0.00%	100.00%	0.00%	0.00%	-
Nuclei	sp2?	xperimenta	Isomer 1	Isomer 2	Isomer 3	Isomer 4	Isomer 5
C	x	130	51.8	51.6	51.7	51.7	
C	x	114	69.3	69.0	69.5	69.1	
C	x	190.9	-10.2	-10.5	-10.5	-10.4	
C	x	123.6	60.5	58.9	60.0	59.9	
C	x	156.4	26.1	26.5	25.4	26.7	
C	x	180.2	-0.4	-0.4	-0.4	-0.5	
C	x	118.5	61.6	61.7	61.7	61.7	
C	x	160	19.4	19.5	19.6	19.4	
C	x	124.7	54.5	55.1	54.9	54.8	
C	x	135.3	44.6	44.7	44.6	44.6	
C		17.7	168.6	168.4	168.5	168.3	
C		34.2	160.6	158.1	161.8	160.7	
C		76.8	89.6	88.2	90.7	93.5	
C		47.2	133.2	132.5	137.0	137.2	
C		83	104.8	101.8	107.4	106.1	
C		48.1	129.0	135.5	134.6	134.6	
C		85	95.7	95.7	94.7	94.8	
C	x	176.8	3.2	2.5	4.1	5.9	
C		19.5	159.6	161.1	157.2	155.5	

Figure S23. ^{13}C NMR calculations of compound **2** stereoisomers (*6aR**, *8aR**, *12aS**, *12bS**)- isomer 1, (*6aR**, *8aR**, *12aR**, *12bS**)- isomer 2, (*6aR**, *8aS**, *12aR**, *12bS**)- isomer 3, and (*6aR**, *8aS**, *12aS**, *12bS**)- isomer 4 using the means of gauge-independent atomic orbital (GIAO) at the pcSseg-1/ revtpssrevtpss leve.

A	B	C	D	E	F	G	H
Functional		Solvent?		Basis Set		Type of Data	
mPW1PW91		PCM		6-311+G(d,p)		Shielding Tensors	
		DP4+	0.00%	0.13%	0.00%	99.87%	-
Nuclei	sp2?	Experimenta	Isomer 1	Isomer 2	Isomer 3	Isomer 4	Isomer 5
C	x	132.4	51.2	48.4	49.5	50.0	
C	x	114.8	69.1	68.8	68.7	68.5	
C	x	194.5	-19.3	-17.8	-20.0	-19.6	
C		71.6	118.1	115.7	115.6	116.6	
C		64.1	117.8	117.4	122.8	117.2	
C	x	192.1	-15.7	-20.9	-21.2	-12.8	
C	x	119.2	60.7	61.2	60.3	61.2	
C	x	160.3	17.1	18.4	18.4	17.1	
C	x	125.2	54.4	55.8	54.9	55.1	
C	x	137.2	41.7	41.6	42.1	41.7	
C		17.9	166.5	167.7	166.8	165.4	
C		27.9	157.4	157.0	160.6	158.7	
C		63.5	107.5	106.4	111.5	107.7	
C		75.7	103.7	108.3	105.9	105.3	
C	x	196	-20.7	-18.8	-17.3	-20.6	
C	x	122.2	59.5	58.6	58.6	57.9	
C		41.9	140.3	143.1	141.8	142.5	
C	x	156.9	6.7	11.4	7.7	13.2	
C		23.9	160.5	160.6	160.0	161.0	

A	B	C	D	E	F	G	H
Functional		Solvent?		Basis Set		Type of Data	
mPW1PW91		PCM		6-311+G(d,p)		Shielding Tensors	
		Isomer 1	Isomer 2	Isomer 3	Isomer 4	Isomer 5	Isomer 6
sDP4+ (H data)		-	-	-	-	-	-
sDP4+ (C data)		0.64%	1.94%	0.04%	97.38%	-	-
sDP4+ (all data)		0.64%	1.94%	0.04%	97.38%	-	-
uDP4+ (H data)		-	-	-	-	-	-
uDP4+ (C data)		0.00%	6.01%	0.03%	93.95%	-	-
uDP4+ (all data)		0.00%	6.01%	0.03%	93.95%	-	-
DP4+ (H data)		-	-	-	-	-	-
DP4+ (C data)		0.00%	0.13%	0.00%	99.87%	-	-
DP4+ (all data)		0.00%	0.13%	0.00%	99.87%	-	-

Table S1. Optimized Z-matrixes of compound **1**

C	5.05382	1.98345	0.46197	O	-2.92268	1.0231	1.95864
C	5.59504	0.70937	0.36867	O	0.85939	2.42916	0.08243
C	4.75677	-0.40095	0.14619	O	2.93668	-2.521	-0.32223
C	3.36156	-0.21508	0.01887	O	5.33249	-1.61663	0.06224
C	2.83309	1.09483	0.11932	H	5.70833	2.83297	0.63346
C	3.66959	2.18512	0.33799	H	6.66351	0.54106	0.4629
C	2.47207	-1.36133	-0.22562	H	3.24343	3.17954	0.41196
C	1.02812	-1.1479	-0.35847	H	0.60892	-2.96273	-1.40207
C	0.51075	0.10469	-0.22725	H	0.01943	-2.94276	0.24486
C	1.36712	1.31517	-0.00097	H	-1.12861	-1.46621	-2.19399
C	0.13328	-2.32026	-0.65368	H	-1.94803	-2.65592	-1.18884
C	-1.22769	-1.83511	-1.16733	H	-2.25941	-2.20744	1.20328
C	-1.76421	-0.70949	-0.28441	H	-1.16165	-0.97613	1.82315
O	-0.8007	0.39645	-0.28826	H	-4.89441	0.48996	0.4367
C	-2.03187	-1.13546	1.17791	H	-4.63668	-1.24571	0.23518
C	-3.27342	-0.36199	1.65292	H	-2.85125	1.93682	0.58148
C	-4.12731	-0.2885	0.38317	H	-3.45761	-0.27603	-2.71078
C	-3.15091	-0.10604	-0.80633	H	-4.86185	-0.38496	3.12159
C	-2.99576	1.37526	-1.25877	H	-3.30788	-0.99861	3.72652
O	-2.90465	2.32376	-0.34167	H	-4.29299	-2.00081	2.64371
O	-3.00507	1.64387	-2.45131	H	-2.17064	1.05261	2.57458
O	-3.65416	-0.81788	-1.91984	H	4.60028	-2.27083	-0.09983
C	-3.97723	-0.97436	2.8585				

Table S2. Optimized Z-matrixes of compound **2**

C	4.39304	-2.12147	-0.23631	O	-1.06953	-1.97995	-1.21628
C	4.93789	-0.89082	-0.57095	C	-5.73016	-0.71842	-0.71258
C	4.15011	0.27323	-0.50777	O	0.3645	0.7796	1.75256
C	2.79007	0.19276	-0.10613	O	-2.00183	1.20099	-1.46269
C	2.25784	-1.08706	0.22918	O	-2.09611	-0.62765	1.71275
C	3.05373	-2.22531	0.17033	H	5.00956	-3.01372	-0.29227
C	1.98376	1.4144	-0.03921	H	5.97201	-0.79776	-0.88777
C	0.54583	1.3367	0.42933	H	2.62537	-3.18663	0.43134
C	-0.04971	-0.01226	0.60591	H	-0.27646	2.82988	-0.86523
C	0.84769	-1.23218	0.66919	H	0.0878	3.38603	0.76556
C	-0.32801	2.55193	0.19006	H	-2.40673	3.14163	0.29707
C	-1.78684	2.29307	0.60655	H	-1.8715	2.1979	1.69326
C	-2.31236	1.02489	-0.06301	H	-4.08001	0.79813	1.18091
C	-1.55401	-0.24726	0.44458	H	-4.37045	1.59505	-0.36027
C	-3.81653	0.77598	0.11627	H	-3.67154	-2.42502	-1.29889
C	-4.25858	-0.5477	-0.47615	H	4.05385	2.15025	-0.76484
C	-3.36	-1.50692	-0.80604	H	-6.10065	0.04314	-1.41153
C	-1.91373	-1.33568	-0.60031	H	-5.96938	-1.7084	-1.11061
O	4.73844	1.43591	-0.84091	H	-6.28192	-0.57431	0.22624
O	0.4194	-2.24355	1.21574	H	-2.57828	0.63699	-2.00471
O	2.4379	2.5217	-0.37119	H	-1.58388	-1.39706	2.02428

Table S3. Optimized Z-matrixes of compound **3**

C	3.94768	2.57755	-0.26123	O	0.50309	-0.18745	2.01752
C	4.73187	1.4249	-0.32259	O	0.33221	-2.53927	0.73135
C	4.13039	0.16725	-0.29238	O	-1.95876	-0.93768	2.01781
C	2.73971	0.04789	-0.18979	O	-2.38603	-2.39352	-0.04744
C	1.96629	1.20521	-0.11444	H	4.4194	3.55741	-0.29079
C	2.56082	2.47114	-0.16337	H	5.81395	1.50522	-0.40068
C	2.08324	-1.27898	-0.20034	H	1.94941	3.37089	-0.11826
C	0.55295	-1.36574	-0.08038	H	1.8987	-2.20285	0.96797
C	-0.04165	-0.16251	0.67294	H	0.67046	-1.4499	1.9656
C	0.50118	1.08696	-0.03046	H	2.82242	-0.15015	1.41079
C	-0.05315	-1.56983	-1.48087	H	1.29999	0.71706	1.40924
C	-1.43935	-0.93256	-1.66429	H	3.84397	1.50278	0.05831
C	-2.28353	-0.99156	-0.38392	H	3.49572	1.68195	-1.63885
C	-1.60392	-0.21952	0.78644	H	0.48052	4.00988	-0.68045
C	-3.73028	-0.53465	-0.64871	H	-2.4717	-4.28566	1.13868
C	-3.89657	0.95549	-0.69749	H	3.9589	4.24423	-0.31137
C	-3.16984	1.75903	0.08957	H	2.47702	4.9157	0.3841
C	-2.18974	1.18298	1.03519	H	3.3816	3.68338	1.27402
O	-0.18658	1.9258	-0.60118	H	-0.67967	-1.43248	-2.40932
O	2.77734	-2.29105	-0.32837	H	0.90566	-3.34585	-0.76341
O	4.97188	-0.9084	-0.37817	H	0.24418	0.38137	-3.24329
O	-1.88876	1.83111	2.04079	H	2.57618	-0.77411	-2.09456
C	-4.95253	1.48898	-1.61928				

Figure S24. The purification procedures of compounds in the strain *S. coelicolor* A3(2)/*spi1*

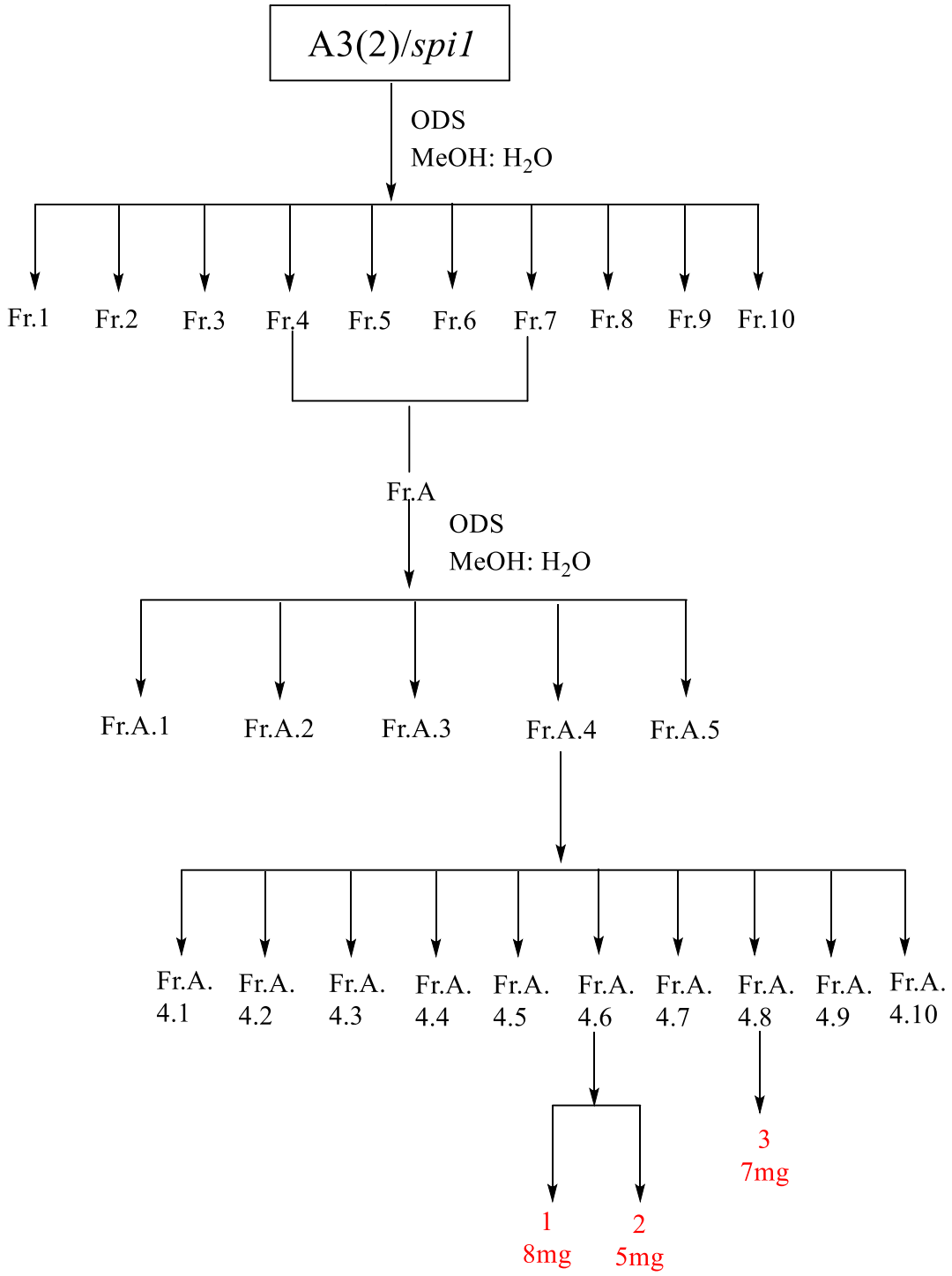

Table S4. The predicted gene functions in *spi1* BGC in comparison with homologous proteins in other type II PKS biosynthesis systems

Gene	AA[a]	Putative function	Homologous gene (Origin, Accession No.)	I/S [b] (%)
Orf1	82	HP		
*spi*H1	238	Antibiotic biosynthesis monooxygenase	*BexI*, *Amycolatopsis orientalis* subsp. *vinearia*, ADI71452	46/60
*spi*L	438	putative glycosyltransferase	*Lct36, S. rishiriensis,* ABX71119	45/62
*spi*M	257	putative MT	*BexP, A. orientalis* subsp. *vinearia,* ADI71455	57/70
*spi*N	377	glycosyl transferase	*SaqGT5, Micromonospora* sp. Tu 6368, ACP19370	46/63
*spi*O	518	drug resistance transporter EmrB/ QacA	*May1, Streptomyces* sp.120454, AVO00800	48/62
*spi*P	246	N, N-dimethyltransferase	*NbmE, S. venezuelae,* AAM88354	53/67
*spi*Q	385	NDP-hexose aminotransferase	*PgaC1, Streptomyces* sp. PGA64, AHW57776	72/82
*spi*R	196	NDP-hexose 3, 5-epimerase	*PgaZ1, Streptomyces* sp. PGA64, AHW57777	67/77
*spi*S	355	glucose-1-phosphate thymidylyltransferase	*PgaG, Streptomyces* sp. PGA64, AHW57786	71/82
*spi*T	338	dTDP-glucose 4, 6-dehydratase	*PgaH1, Streptomyces* sp. PGA64, AHW57787	76/86
*spi*U	434	NDP-hexose 3, 4-dehydratase	*PgaQ, Streptomyces* sp. PGA64, AHW57788	84/91
*spi*V	460	NDP-hexose 2, 3-dehydratase	*PgaS, Streptomyces* sp. PGA64, AHW57789	69/76

*spi*W	319	dNDP-hexose 3-ketoreductase	*SaqT, M.* sp. Tü6368, ACP19378	63/73
*spi*I	407	putative methyltransferase	*ssfQ, Streptomyces* sp. SF2575, ADE34497	88/92
*spi*J	327	putative carbohydrate kinase	*ssfX2, Streptomyces* sp. SF2575, ADE34496	63/72
*spi*K	289	Methylenetetrahydrofolate reductase	*Lct19, S. rishiriensis,* ABX71102	79/86
*spi*G	298	Thioesterase	*May4, Streptomyces* sp. 120454, AVO00803	58/69
*spiH*2	775	oxygenase-reductase	*PgaM, Streptomyces* sp. PGA64, AAK57530	73/80
*spi*E	311	aromatase/cyclase	*PgaL, Streptomyces* sp. PGA64, AAK57529	77/84
*spi*F	260	polyketide ketoreductase	*May13, Streptomyces* sp. 120454, AVO00812	84/91
*spi*A	101	ACP	*PgaC, Streptomyces* sp. PGA64, AAK57527	66/78
*spi*B	405	ketosynthase β subunit	*PgaB, Streptomyces* sp. PGA64, AAK57526	77/84
*spi*C	422	ketosynthase α subunit	*PgaA, Streptomyces* sp. PGA64, AAK57525	91/94
*spi*D	109	polyketide cyclase	*PgaY, Streptomyces* sp. PGA64, AAK57524	83/88
*Spi*H3	477	FAD-binding protein	*PgaE, Streptomyces* sp. PGA64, AAK57522	82/88

[a] Amino acid. [b] Identity /similarity.