Supporting Information

Tandem Prenyltransferases Catalyze Isoprenoid Elongation and Complexity Generation in Biosynthesis of Quinolone Alkaloids

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Experimental Procedures

1. Strain and Culture Condition

P. thymicola IBT 5891was obtained from the IBT culture collection (Kgs. Lyngby, Denmark) and maintained on PDA (potato dextrose agar, BD) at 24°C, 6 days for sporation or on SDA (sabouraud agar, BD) at 24°C, 7 days for the production of penigequinolone A/B.

2. General DNA Manipulation Techniques

E. coli TOPO10 and *E. coli* XL-1 were used for cloning, following standard recombinant DNA techniques. DNA restriction enzymes were used as recommended by the manufacturer (New England Biolabs, NEB). PCR was performed using Phusion® High-Fidelity DNA Polymerase (NEB). PCR products were confirmed by DNA sequencing. *E. coli* BL21(DE3) (Novagen) was used for protein expression. *Saccharomyces cerevisiae* strain BJ5464-NpgA (*MATα ura3-52 his3-Δ200 leu2-Δ1 trp1 pep4::HIS3 prb1 Δ1.6R can1 GAL*) was used as the yeast expression host.

3. Gene Knock-out in *P. thymicola*

Target genes of *pen* gene cluster were deleted in *P. thymicola* based on the hygromycin split-marker apporach.[1] Hygromycin resistance-gene *hph* upstream and downstream fragments were amplified from plasmid pAN-7 (Addgene) with primers hph-up F, hph-up R and hph-dn F, hph-dn R, respectively, and digested with *Not*I/*Sac*II and *Sac*I/*Not*I to insert into the self-ligated T-vector pTA2 (Toyobo) to create plasmid phph-up and phph-dn, respectively. The upstream and downstream homologous fragments of each gene (*penG-I, penG, penH* and *penE*) were ligated to phph-up and phph-up, respectively. All these deletion cassettes were amplified with universal primers M13-F and M13-R from the above plasmids and were precipitated with ethanol and dissolved in STC buffer (1.2 M sorbitol, 10 mM $CaC1_2$, 10 mM Tris-HCI, pH 7.5).

Polyethylene glycol-mediated protoplast transformation of *P. thymicola* was performed essentially as described previously for *P. aethiopicum*.[2] Briefly, germinated cells were collected, washed twice with osmotic medium (1.2 M $MgCl_2$, 10 mM sodium phosphate, pH 5.8) and resuspended in enzyme cocktail solution (3 mg/ml Lysing Enzymes, 3 mg/ml Yatalase in osmotic medium) at 28°C for 8 hours. After wash twice with STC buffer, protoplasts were gently mixed with DNA and incubated for 1 hour on ice. 500 μl of PEG 4000 solution (60% PEG 4000, 50 mM $CaC1_2$, 50 mM Tris-HCI, pH 7.5) was added for 100 μl protoplast mixture, incubated at room

temperature for 20 min and plated on regeneration selection medium (PSA, PDA agar supplemented with 1.2 M sorbitol, 100 μg/ml hygromycin B). After incubation at room temperature for about 4 days, the transformants were transfered into fresh 2 ml PDB medium at 24°C, 200 rpm, 2 days. Mycelia were collected, lyophilized and grounded to disrupt cells. Cell lysate was solubilized in LETS buffer (10 mM Tris-HCI, pH 8.0, 20 mM EDTA, 0.5% SDS, 0.1 M LiCl) and extracted twice with phenol/chloroform. Genomic DNA was precipitated with ethanol, and resuspended in H_2O. The genotypes of all mutants were verified by PCR.

4. Chemical Analysis and Compound Isolation

For small-scale analysis, the *P. thymicola* wild-type and transformants were grown in SDA agar for 7 days at 24°C. 1 cm × 1 cm agar was extracted with 2 ml acetone, and then evaporated to dryness. The dried extracts were dissolved in 300 μl methanol for LC-MS analysis. LC-MS analyses were performed on a Shimadzu 2010 EV LC-MS (Phenomenex® Luna, 5μ, 2.0 × 100 mm, C18 column) using positive and negative mode electrospray ionization with a linear gradient of 5-95% MeCN-H_2O in 30 minutes followed by 95% MeCN for 15 minutes with a flow rate of 0.1 ml/min.

For large-scale analysis, the acetone extract from a 2 L SDA solid agar extract of mutant was evaporated to dryness and partitioned between ethyl acetate/H_2O three times. After evaporation of the organic phase, the crude extracts were separated by silica chromatography. The purity of each compound was checked by LC-MS, and the structure was confirmed by NMR. ^{1}H, ^{13}C and 2D NMR spectra were obtained using d_6-DMSO as solvent on Bruker AV500 spectrometer with a 5 mm dual cryoprobe at the UCLA Molecular Instrumentation Center.

5. Protein Expression and Purification

Intron-free *penI* and *penG* were cloned from cDNA and inserted into plasmids pET44a and pET28a to yield the pET44a-*penI* and pET28a-*penG* respectively. The plasmids were transformed into the *E. coli* BL21(DE3) strain for protein expression. The *E. coli* BL21(DE3) cell harboring pET44a-*penI* or pET28a-*penG* were cultured in LB medium supplemented with 100 μg/ml ampicillin or 50 μg/ml kanamycin (final concentration) at 37°C and 250 rpm to an OD_{600} of 0.5. The cultures were then incubated on ice for 10 min before addition of 0.2 mM (final concentration) IPTG to induce protein expression. The cells were further cultured at 16°C for 20 hours, and were harvested by centrifugation (3,500 rpm, 15 min, 4°C), re-suspended in 40 mL buffer A (50 mM Tris-HCl, pH 7.9, 0.5 M NaCl, and 10% glycerol) and lysed by sonication on ice for 40 min. Cellular debris was removed by centrifugation (14,000

rpm, 45 min, 4°C), and the supernatant was used to purify the protein by nickel-affinity chromatography using standard protocols. The protein was eluted with increasing gradient of buffer B (500 mM imidazole in buffer A). Purified proteins were concentrated and exchanged into buffer C (50 mM Tris-HCl, pH 7.9, 50 mM NaCl, and 5% glycerol) with Centriprep filters (Amicon). The protein was stored in buffer C at -80°C. Protein concentration was determined by Bradford assay using bovine serum albumin as a standard.

6. *In vitro* characterization of PenG and PenI

1) Assays for PenG and PenI activity with **2** and GPP or DMAPP in 50 mM Tris-HCl (pH 7.5) buffer were performed at 50 μl scale with10 μM PenG or PenI, 2 mM GPP, or 0.2 mM DMAPP, 0.2 mM **2** (stock in DMSO), 1 mM Mg^{2+}, 28°C for 10 hours.

2) Assays for the PenG and PenI activity with **9** and DMAPP in 50 mM Tris-HCl (pH 7.5) buffer were performed at 50 μl scale with 10 μM PenG or PenI, 0.2 mM DMAPP, 0.2 mM **9** (stock in DMSO), 1 mM Mg^{2+}, 28°C for 10 hours. The ^{18}O labeling assays was performed in 50 mM Tris-HCl (pH 7.5) buffer prepared with 40%$H_2{}^{18}O$.

The reaction mixtures were quenched and extracted with 200 μl ethyl acetate (EA). The resultant organic extracts were evaporated to dryness, re-dissolved in methanol, and then analyzed on LC-MS. LC-MS analyses were performed on a Shimadzu 2020 EV LC-MS (Kinetex™ 1.7 μm C18 100 Å, LC Column 100 x 2.1 mm) using positive and negative mode electrospray ionization with a linear gradient of 5-95% MeCN-H_2O in 15 minutes followed by 95% MeCN for 3 minutes with a flow rate of 0.3 ml/min.

7. Biotransformation of 2 to 6

penI cDNA was amplified by RT-PCR using SuperScript® II Reverse Transcriptase (Invitrogen) and Phusion® High-Fidelity DNA Polymerase, and was cloned into the pET44a and transformed into the *E. coli* BL21(DE3) strain. The transformant with PenI-expression plasmid was grown in LB medium supplemented with 100 μg/ml ampicillin at 37°C to an OD_{600} of 0.5, at which time the cultures were cooled to 16°C and then induced with 0.2 mM IPTG at 250 rpm and grown at 16°C 20 hours. To increase cell density, the *E. coli* cells were concentrated 10-fold before addition of **2**. 1L culture was collected by centrifugation (4°C, 3500rpm, 10 min). The cell pellet was gently resuspended in 100 mL of fresh LB medium supernatant, followed by addition of 200 μM **2** (final concentration), and then shaken at 28°C, 250 rpm for two days. For product isolation, cell culture was extracted with 200 ml ethyl acetate three times. After evaporation of the organic phase, the crude extracts were separated by silica chromatography. The purity of **6** was checked by LC-MS, and the structure was

confirmed by NMR.

penG, *penE* and *penJ* cDNA were amplified by RT-PCR using SuperScript® II Reverse Transcriptase (Invitrogen) and Phusion® High-Fidelity DNA Polymerase. Intron-free *penG* and *penJ* genes were cloned into uracil drop-out plasmid pXW55 to yield the yeast-expression plasmids pXW55-*penG* and pXW55-*penJ* respectively. *penE* was cloned into L-tryptophan drop-out plasmid pXW06 and to yield the yeast-expression plasmid pXW06-*penE*. pXW55-*penG*, and pXW06-*penE*/pXW55-*penJ* were transformed into *S. cerevisiae* BJ5464-NpgA strain. The transformants with PenG-expression plasmid or PenEJ co-expression plasmids were grown in uracil, or uracil and L-tryptophan drop-out medium at 28°C 2 days, at which time the cultures were transferred into 1L fresh YPD medium and were shaken at 28°C, 250 rpm for three days. To increase cell density, the yeast cells were concentrated 10-fold before addition of **9** or **10**. 1L culture was collected by centrifugation (4°C, 2000rpm, 10 min). The cell pellet was gently resuspended in 100 ml of fresh YPD medium, followed by addition of 200 μM **9** or **10** (final concentration), and then shaken at 28°C, 250 rpm for days. For product isolation, cell culture was extracted with 200 ml ethyl acetate three times. After evaporation of the organic phase, the crude extracts were separated by silica chromatography. The purity of each compound was checked by LC-MS, and the structure was confirmed by NMR. ^{1}H, ^{13}C and 2D NMR spectra were obtained using DMSO-d_6 as solvent on Bruker AV500 spectrometer with a 5 mm dual cryoprobe at the UCLA Molecular Instrumentation Center.

Supplementary Tables and Figures

1. Supplementary Tables

Table S1. Primers used in this study

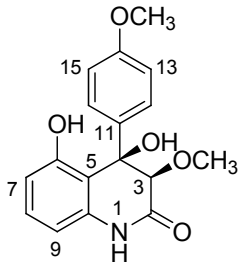
Primer	Sequences of primer (5'-3')
hph-up F	ACCTGGCGGCCGCTACAACGACCATCAAAGTC
hph-up R	ACTGACCGCGGTACCGTCTGCTGCTCCATACAA
hph-dn F	CAGGGAGCTCGGTACCTCGGAGGGCGAAGAATC
hph-dn R	GAATGCGGCCGCAGGATTACCTCTAAACAAGTG
Vhphr	AGTTTATCCAGGAGATGTTG
Vhphf	ACAGGTACACTTGTTTAGAG
KOpenNupf	GGACTCGGTACCCTTGACGATATC
KOpenNupr	ATAAGAATGCGGCCGCCCTTGTGACCTGTGGAAGATGCAG
KOpenNdnf	ATAAGAATGCGGCCGCCAATGTGGGAAGAGTTTGCTGGTG
KOpenNdnr	CGGGGTACCCATACTAGATCTCATCCTCTTG
VKOpenNf	CTTCTTGCACATGGAGAATCAGC
VKOpenNr	GTCTTGGATGCCCAATGCTAAGG
KopenEupf	CGGGGTACCGAATGTCAATGGTCGACGTTTCCAG
KopenEupr	ATAAGAATGCGGCCGCCCAAGGTCAATCCAGCAATAGATC
KopenEdnf	ATAAGAATGCGGCCGCCTCCCACACGTCTGATTCAGACATC
KOpenEdnr	CGGGGTACCCTAAGAACGTTGTCATTATCTTATTC
VKOpenEf	CTGCATCAGCTCCTGGAAGTTTTC
VKOpenEr	GGATAAAATACCACTTGAGATATG
KOpenG-Iupf	CGGGGTACCCACTTGAGGCTTCGGTAAAGGCCTG
KOpenG-Iupr	ATAAGAATGCGGCCGCCCATGTTCATCCTGTAGCAAGGTGC
KOpenG-Idnf	ATAAGAATGCGGCCGCGAGGCTGACAGCAGTCGCAATTGG
KOpenG-Idnr	CGGGGTACCGCATTATTAGTTTTATCAATTAAC
VKOpenG-If	CTATTAACGGAAAGACTTTAAAG
VKOpenG-Ir	GTATATAATCCCCTGCATTATTAG
KopenGupf	CGGGGTACCCACTTGAGGCTTCGGTAAAGGCCTG
KopenGupr	ATAAGAATGCGGCCGCCCATGTTCATCCTGTAGCAAGGTGC
KopenGdnf	ATAAGAATGCGGCCGCCTGTGTCACAGTGGTTCAACCTTC
KopenGdnr	CGGGGTACCCTATTCGGAGATCTCTATGTCCAC
VKOpenGf	CTATTAACGGAAAGACTTTAAAG
VKOpenGr	CGTATCACAGGATCCAACACATC
KopenHupf	CGGGGTACCGTATGCATGCTTTGATAGTGAAGC
KopenHupr	ATAAGAATGCGGCCGCCGTAAGCCTCGTGGTCAATGTAGATAC
KopenHdnf	ATAAGAATGCGGCCGCGGACTTCCTGCGTCGACTCAACCAC

KopenHdnr	CGGGGTACCCTCATAGGTAGATAGAGTATTGAC
VKOpenHf	GCACTATCAAAGAGTCAGGCAC
VKOpenHr	CTTGTGACATATAGTGCAGGTC
pET-44a-penI f	CGC*GGATCC*ATGGCCAGCCTGATTGGCGGAAG
pET-44a-penI r	CCG*CTCGAG*CTATCCTGGCAAAAGATAGC
pET-28a-penG f	GGAATTC*CATATG*ACACAAGACGTGGTCACCGTTTC
pET-28a-penG r	CCG*CTCGAG*TCAAAACCAGGTCTTGCGGATGTTG
pXW55-penJf	CATATGGCTAGCGATTATAAGGATGATGATGATAAGACTAGTATGTCTTCTACCGCTCAA
pXW55-penJr	TTTGTCATTTAAATTAGTGATGGTGATGGTGATGCACGTGCTATGCACGAATATCTTCCT
pXW06-penEf	ATCAACTATCAACTATTAACTATATCGTAATACCATCATATGGAAAAGCCAGAGTTCAAG
pXW06-penEr	TTGATAATGGAAACTATAAATCGTGAAGGCATGTTTAAACCTAAGACGAAATTGGTAGAC

Table S2. Deduced functions of the ORFs in *pen* gene cluster

Protein name	Proposed function from bioinformatic analysis
PenA	FAD dependent monooxygenase
PenB	Cytochrome P450
PenC	*O*-Methyltransferase
PenD	Short-chain dehydrogenase/reductase
PenE	FAD dependent monooxygenase
PenF	Unknown function
PenG	Aromatic prenyltransferase
PenH	FAD dependent dehydrogenase
PenI	Aromatic prenyltransferase
PenJ	Hydrolase
PenK	*O*-Methyltransferase
PenL	Unknown function
PenM	α-Ketoglutarate-dependent dioxygenase
PenN	NRPS (A-T-C-A-MT-T-C)

Table S3. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data of compound **2** in DMSO-d_6

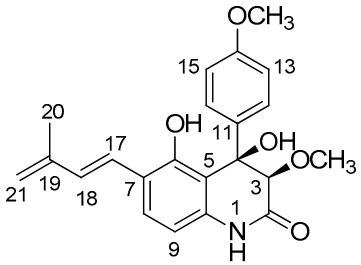


2

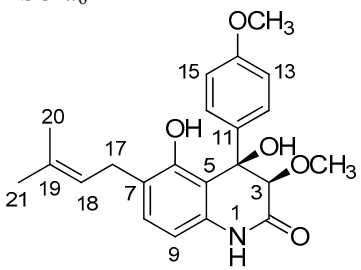
Position	Compound **2**	
	δ_H (mult, *J* in Hz)	δ_C
1	10.21 (1H, s)	-
2	-	166.4
3	3.63 (1H, s)	84.3
4	-	78.1
5	-	111.1
6	-	157.4
7	6.46 (1H, d, 8.5)	106.6
8	7.10 (1H, t, 8.0)	132.2
9	6.43 (1H, d, 8.0)	111.6
10	-	137.1
11	-	129.4
12	7.09 (1H, d, 9.0)	127.6
13	6.89 (1H, d, 9.0)	113.8
14	-	159.2
15	6.89 (1H, d, 9.0)	113.8
16	7.09 (1H, d,9.0)	127.6
3-OCH_3	3.44 (3H, s)	58.4
14-OCH_3	3.73 (3H, s)	55.1

All spectral data for **2** are consistent with those previously reported in the literature.[3]

Table S4. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data of compound **9** and **6** in DMSO-d_6



9

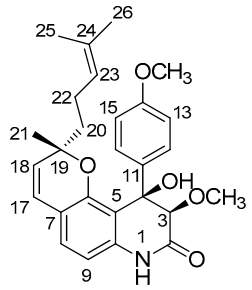


6

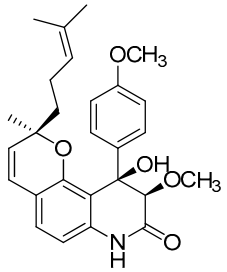
	Compound **9**		Compound **6**	
Position	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)	δ_C
1	10.27 (1H, s)	-	10.13 (1H, s)	-
2	-	166.3	-	166.4
3	3.61 (1H, s)	84.2	3.58 (1H, s)	84.5
4	-	78.3	-	78.5
5	-	111.2	-	111.1
6	-	155.1	-	155.1
7	-	119.5	-	127.9
8	7.44 (1H, d, 8.0)	126.3	6.95 (1H, d, 8.0)	131.8
9	6.42 (1H, d, 8.5)	106.9	6.37 (1H, d, 8.0)	106.4
10	-	136.3	-	135.0
11	-	129.2	-	131.4
12	7.09 (1H, d, 8.5)	127.6	7.08 (1H, d, 7.5)	127.8
13	6.87 (1H, d, 8.5)	113.9	6.88 (1H, d, 7.5)	114.0
14	-	159.4	-	159.5
15	6.87 (1H, d, 8.5)	113.9	6.88 (1H, d, 7.5)	114.0
16	7.09 (1H, d, 8.5)	127.6	7.08 (1H, d, 7.5)	127.8
17	6.86 (1H, d, 16.5)	122.9	3.08 (1H, dd, 15.5, 7.5)	27.6
			3.18 (1H, dd, 15.5, 7.5)	
18	6.70 (1H, d, 16.5)	131.5	5.23 (1H, t, 7.5)	123.0
19	-	142.1	-	123.1
20	1.87 (3H, s)	18.4	1.64 (3H, s)	17.8
21	5.07 (1H, s); 5.00 (1H, s)	116.3	1.67 (3H, s)	25.7
3-OCH_3	3.43 (3H, s)	58.3	3.42 (3H, s)	58.4
14-OCH_3	3.71 (3H, s)	55.1	3.70 (3H, s)	55.3

All spectral data for **9** and **6** are consistent with those previously reported in the literature.[3,4]

Table S5. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data of compound **5a** and **5b** in DMSO-d_6



5a

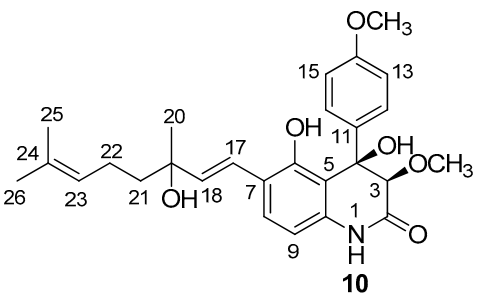


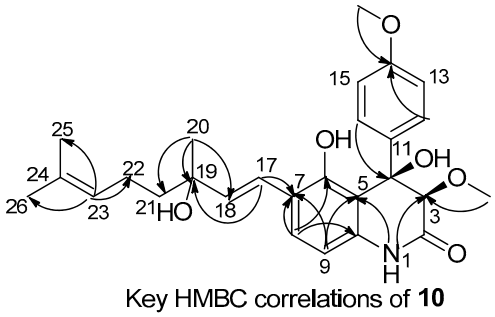
5b

	Compound **5a**		Compound **5b**	
Position	δ_H (mult, J in Hz)	δ_C	δ_H (mult, J in Hz)	δ_C
1	10.20 (1H, s)	-	10.18 (1H, s)	-
2	-	167.6	-	168.3
3	3.93 (1H, s)	84.4	4.04 (1H, s)	84.7
4	-	78.9	-	79.0
5	-	114.6	-	115.4
6	-	151.5	-	151.9
7	-	116.0	-	116.8
8	6.93 (1H, d, 8.0)	127.0	6.93 (1H, d, 8.0)	127.7
9	6.43 (1H, d, 8.0)	108.1	6.43 (1H, d, 8.0)	108.2
10	-	138.0	-	138.7
11	-	135.9	-	137.0
12	7.21 (1H, d, 8.5)	127.0	7.25 (1H, d, 9.0)	127.5
13	6.94 (1H, d, 8.5)	112.9	6.83 (1H, d, 9.0)	113.3
14	-	158.1	-	158.5
15	6.94 (1H, d, 8.5)	112.9	6.83 (1H, d, 9.0)	113.3
16	7.21 (1H, d, 8.5)	127.0	7.25 (1H, d, 9.0)	127.5
17	6.31 (1H, d, 10.0)	122.2	6.30 (1H, d, 10.0)	122.7
18	5.46 (1H, d, 10.0)	131.5	5.45 (1H, d, 10.0)	131.4
19	-	76.6	-	76.8
20	1.13 (2H, m)	40.4	1.28 (2H, m)	40.3
21	1.18 (3H, s)	26.3	0.95 (3H, s)	25.9
22	1.51 (2H, m)	22.1	1.86 (2H, m)	22.8
23	4.77 (1H, m)	124.0	5.03 (1H, m)	124.6
24	-	130.5	-	131.4
25	1.35 (3H, s)	17.3	1.37 (3H, s)	17.8
26	1.56 (3H, s)	25.4	1.41 (3H, s)	25.8
3-OCH_3	3.29 (3H, s)	59.5	3.26 (3H, s)	60.1
14-OCH_3	3.71 (3H, s)	54.8	3.73 (3H, s)	55.5

All spectral data for **5a** and **5b** are consistent with those previously reported in the literature.[5]

Table S6. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data of compound **10** in DMSO-d_6

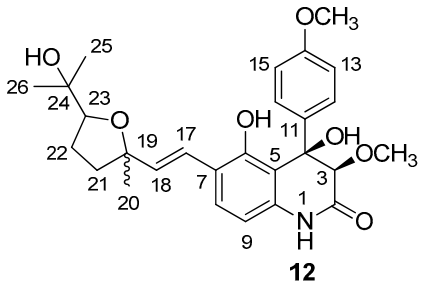




Key HMBC correlations of **10**

Position	Compound **10**	
	δ_H (mult, J in Hz)	δ_C
1	10.21 (1H, s)	-
2	-	166.2
3	3.61 (1H, s)	84.3
4	-	78.4
5	-	111.1
6	-	154.9
7	-	119.8
8	7.31 (1H, d, 8.5)	126.3
9	6.39 (1H, d, 8.5)	106.6
10	-	136.0
11	-	131.5
12	7.09 (1H, d, 9.0)	127.7
13	6.88 (1H, d, 9.0)	113.9
14	-	159.4
15	6.88 (1H, d, 9.0)	113.9
16	7.09 (1H, d, 9.0)	127.7
17	6.76 (1H, d, 16.0)	120.0
18	6.18 (1H, d, 16.0)	135.7
19	-	71.5
20	1.20 (3H, s)	28.4
21	1.44 (2H, m)	42.9
22	1.51 (2H, m)	22.5
23	5.08 (1H, t, 7.5)	125.0
24	-	130.2
25	1.48 (3H, s)	17.5
26	1.62 (3H, s)	25.5
3-OCH_3	3.43 (3H, s)	58.3
14-OCH_3	3.70 (3H, s)	55.1

Table S7. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data of compound **12** in DMSO-d_6



12

Position	Compound **12**	
	δ_H (mult, J in Hz)	δ_C
1	10.21 (1H, s)	-
2	-	166.2/166.1
3	3.61 (1H, s); 3.62 (1H, s)	84.2
4	-	78.3
5	-	111.1
6	-	154.6
7	-	119.4
8	7.34 (1H, d, 8.5)	126.6/126.5
9	6.42 (1H, d, 8.5)	106.8
10	-	135.9/135.7
11	-	131.3
12	7.09 (1H, d, 9.0), 7.10 (1H, d, 9.0)	127.6
13	6.89 (1H, d, 9.0)	113.9
14	-	159.4
15	6.89 (1H, d, 9.0)	113.9
16	7.09 (1H, d, 9.0), 7.10 (1H, d, 9.0)	127.6
17	6.45 (1H, d, 16.0), 6.69 (1H, dd, 16.0, 3.5)	120.0
18	6.18 (1H, dd, 16.0, 7.0), 6.30 (1H, dd, 16.0, 7.0)	134.2/135.0
19	-	84.2/84.7
20	1.30 (3H, s)	27.2/27.3
21	1.76 (2H, m)	37.5
22	1.78 (2H, m)	26.0/26.1
23	3.73 (1H, m), 3.68 (1H, m)	85.2/85.0
24	-	70.2
25	1.05 (3H, s)	26.8/26.9
26	1.02 (3H, s)	25.0/24.9
3-OCH_3	3.43 (3H, s)	58.3
14-OCH_3	3.71 (3H, s)	55.1

All spectral data for **12** are consistent with those previously reported in the literature.[4]

2. Supplementary Figures

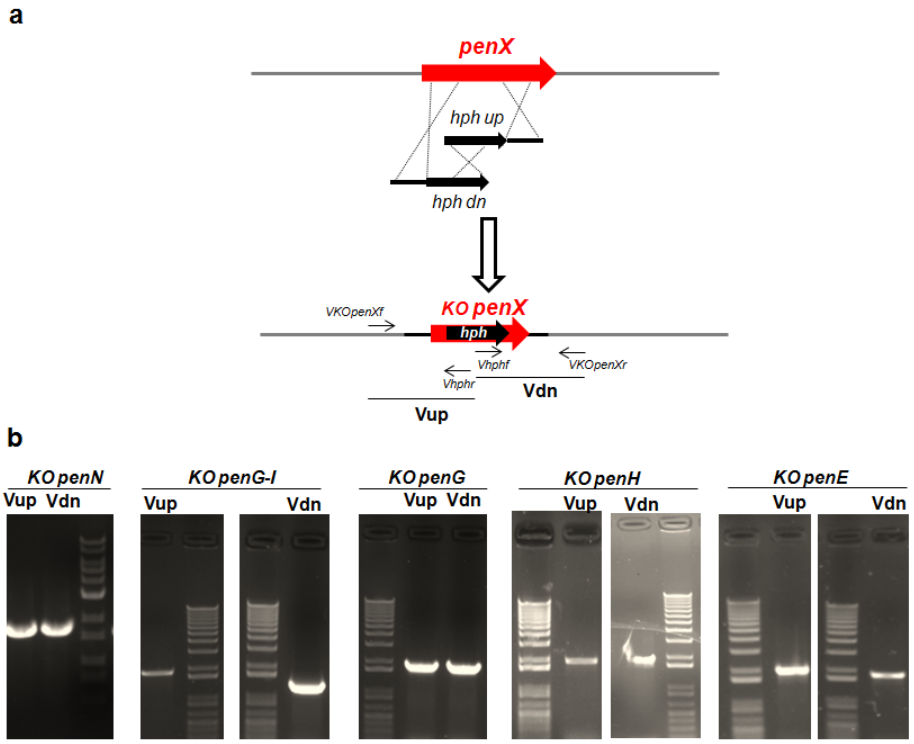


Figure S1. Genes knock-out of *pen* gene cluster in *P.thymicola*. **a.** Scheme of hygromycin-resistance split-marker approach for gene knock-out**. b.** Genotypical verification of each mutant by PCR.

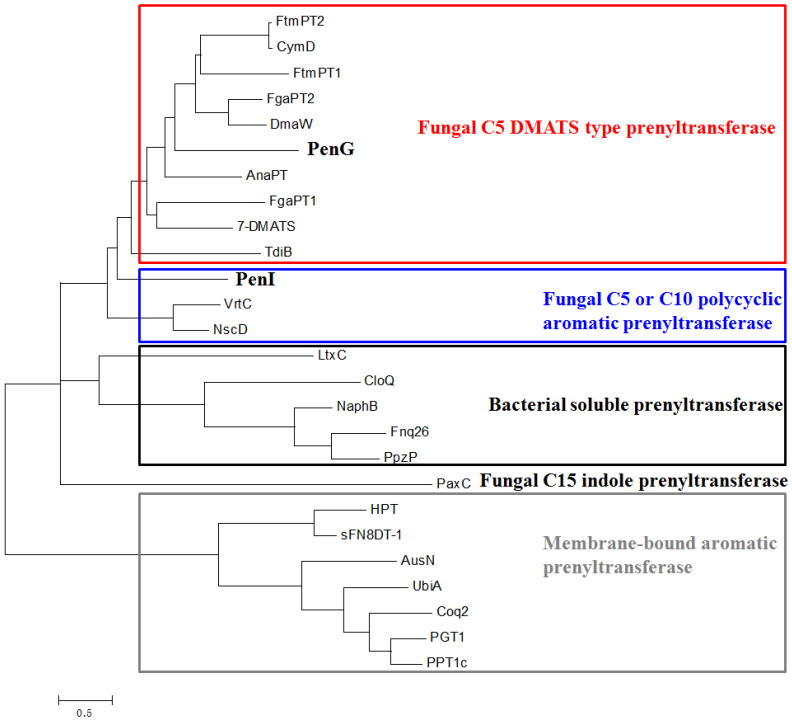


Figure S2. Phylogenetic relationship between known prenyltransferases and PenG, PenI. MEGA 6.06 version was used for alignment of amino acid sequences, Maximum likelihood was used as statistical method. FgaPT2 (Accession no. AAX08549), FgaPT1 (XP_756136), FtmPT1 (AAX56314), FtmPT2 (EU622826), 7-DMATS (ABS89001) are from *Aspergillus fumigatus*, CymD (NFIA_093760), AnaPT (EAW16181) and NscD (NFIA_112230) are from *Neosartorya fischeri*, TdiB (ABU51603) and AusN (XP_682528) are from *Aspergillus nidulans*, DmaW (Q6X2E0) is from *Claviceps purpurea*, VrtC (ADI24928.1) is from *Penicillium aethiopicum*, PaxC (AAK11529) is from *Penicillium paxilli*, LtxC (AAT12285) is from *Lyngbya majuscula*, NaphB (BAE00106) is from *Streptomyces sp. CL 190*, Fnq26 (CAL34104) is from *Streptomyces cinnamonensis*, PpzP(C4PWA1) is from *Streptomyces anulatus*, CloQ (AAN65239) is from *Streptomyces roseochromogenes*, PGT1 (BAB84122) is from *Lithospermum erythrorhizon*, PPT1c (BAE96574) is from *Oryza sativa*, Coq2 (P32378) is from *Saccharomyces cerevisiae*, UbiA (BAB38446) is from *E. coli*, HPT (NP849984) is from *Arabidopsis thaliana* and SFN8DT-1 (BAG12671) is from *Sophora flavescens*.

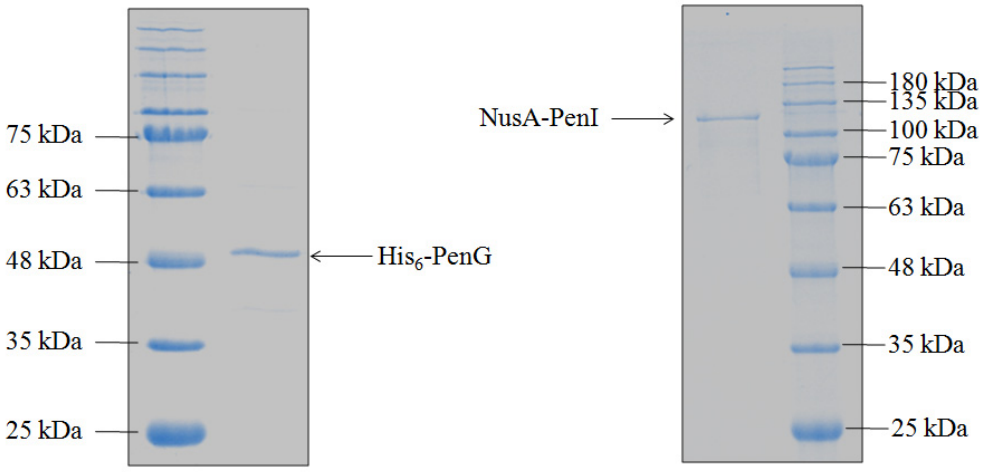


Figure S3. SDS-PAGE of the heterogeneously expressed PenG and PenI from *E. coli* BL21(DE3). PenG contains an *N*-terminal His_6-Tag (~51.8 kDa), PenI contains an *N*-terminal NusA-Tag (~113.2 kDa). These proteins were purified using Ni-NTA agarose affinity resin.

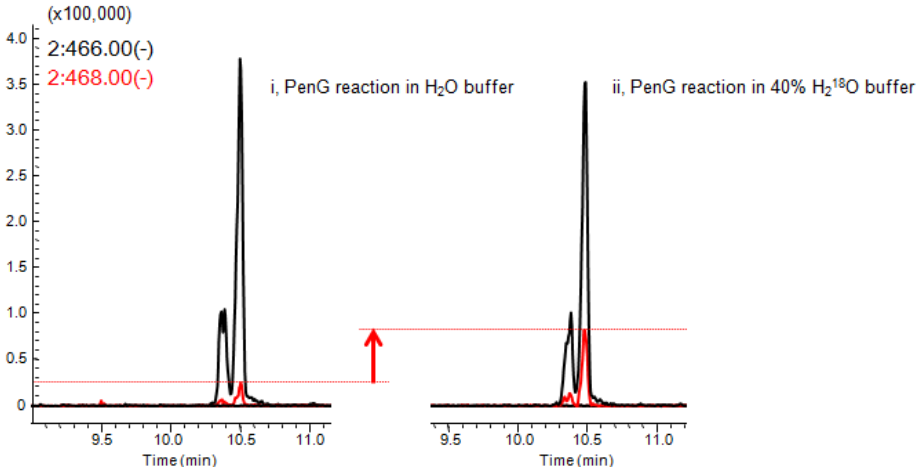


Figure S4. ^{18}O labeling assays in the PenG-catalyzed conversion of **9** to **10**. Reactions performed in buffer prepared with i) H_2O; ii), 40% $H_2{}^{18}O$.

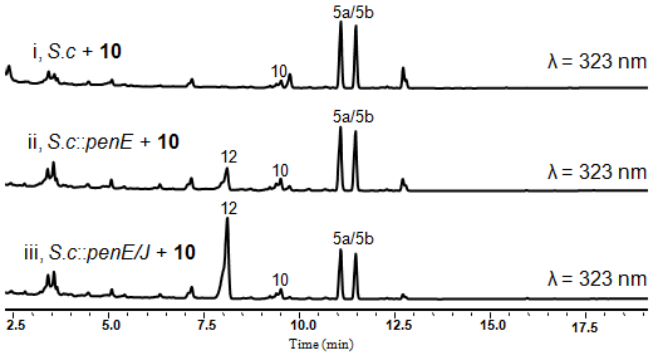


Figure S5. LC analysis of *S.c* biotransformation experiments demonstrating the conversion of **10** to **12** in the presence of PenE, PenE/PenJ expression.

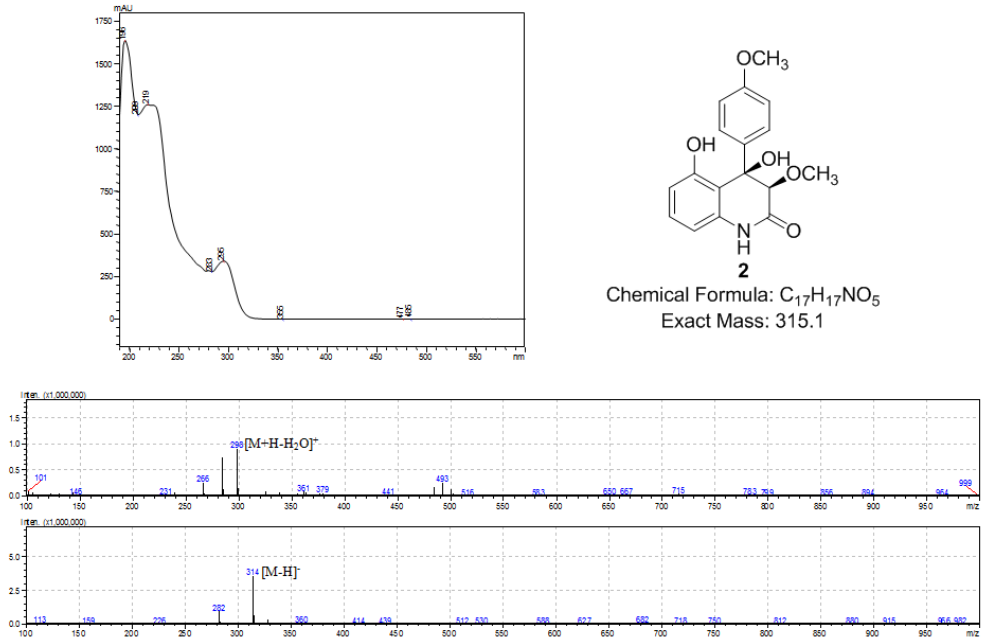


Figure S6. UV and MS spectra of **2**.

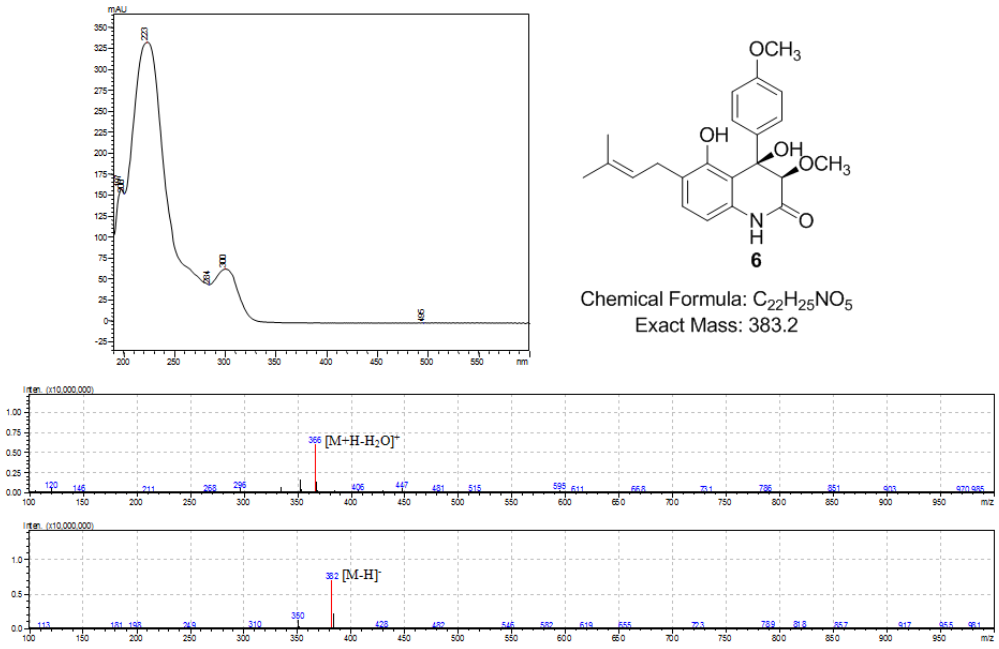


Figure S7. UV and MS spectra of **6**.

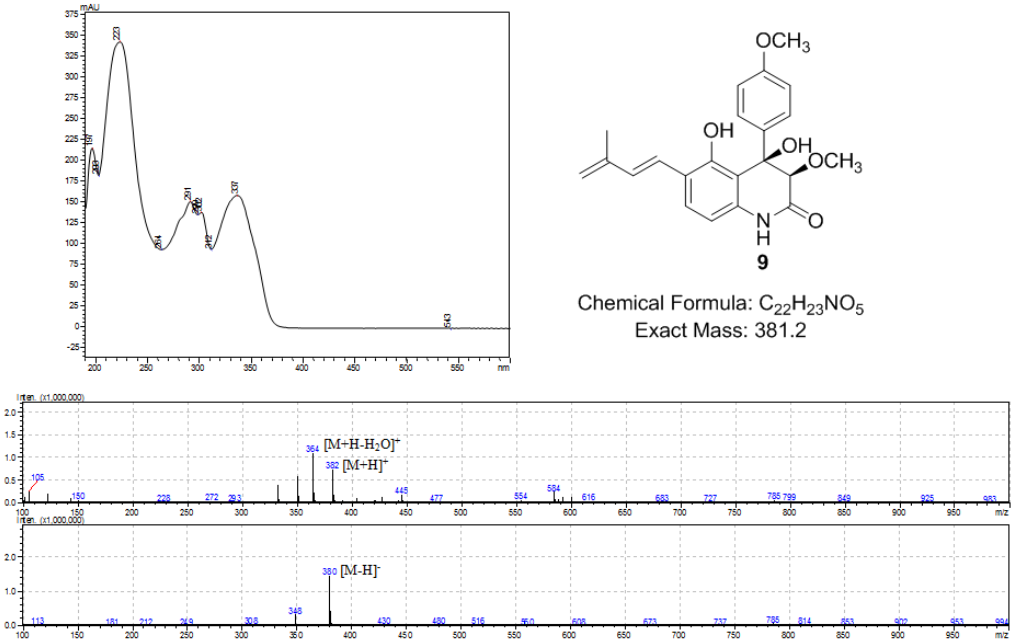


Figure S8. UV and MS spectra of **9**.

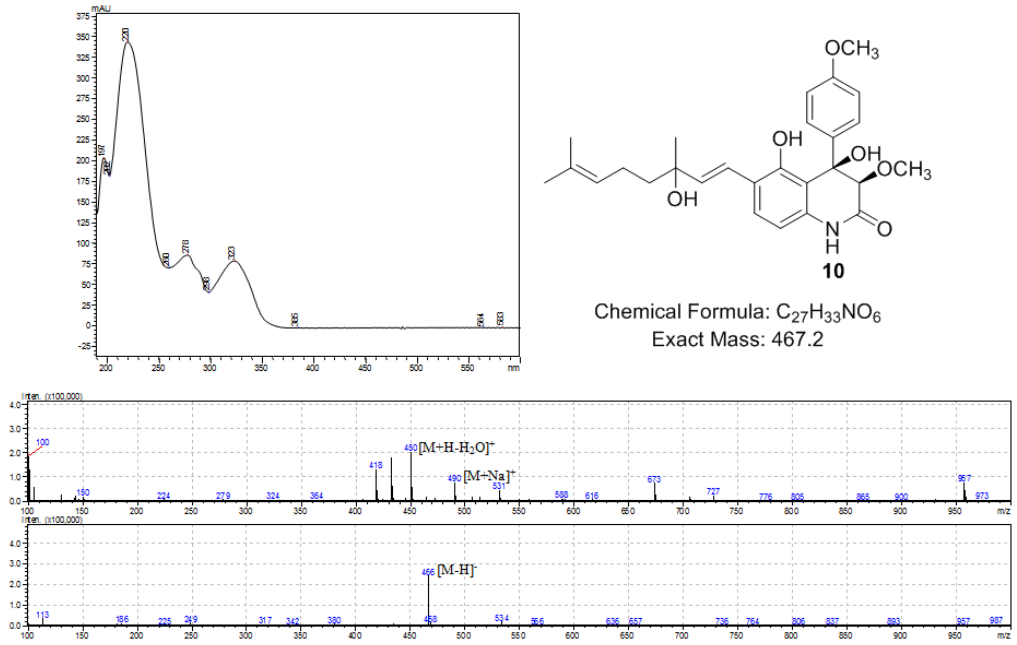


Figure S9. UV and MS spectra of **10**.

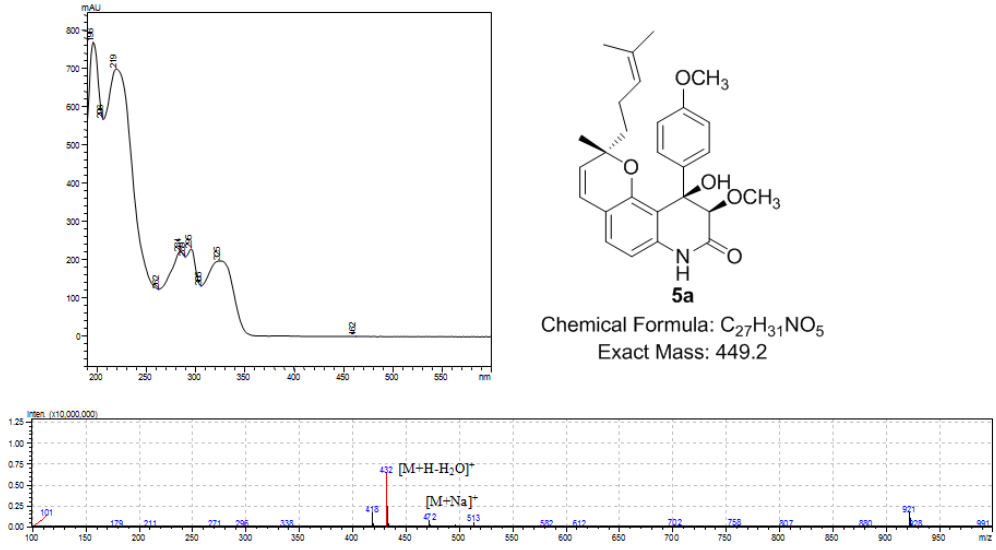


Figure S10. UV and MS spectra of **5a**.

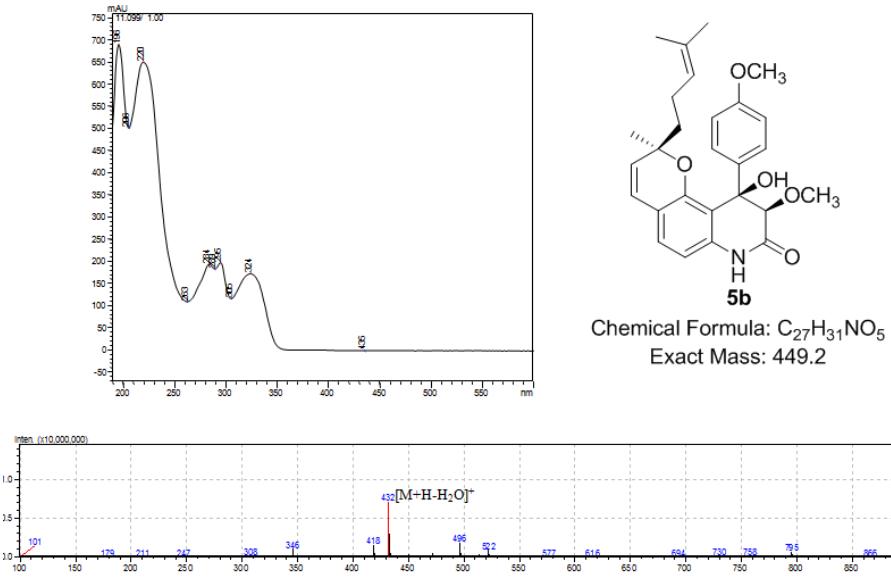


Figure S11. UV and MS spectra of **5b**.

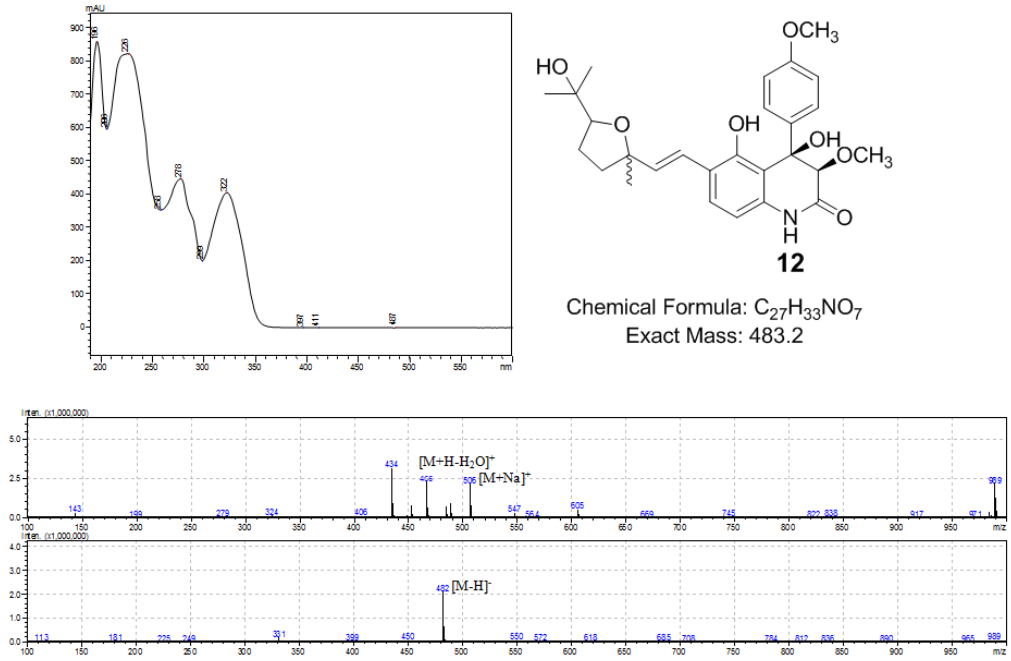


Figure S12. UV and MS spectra of **12**.

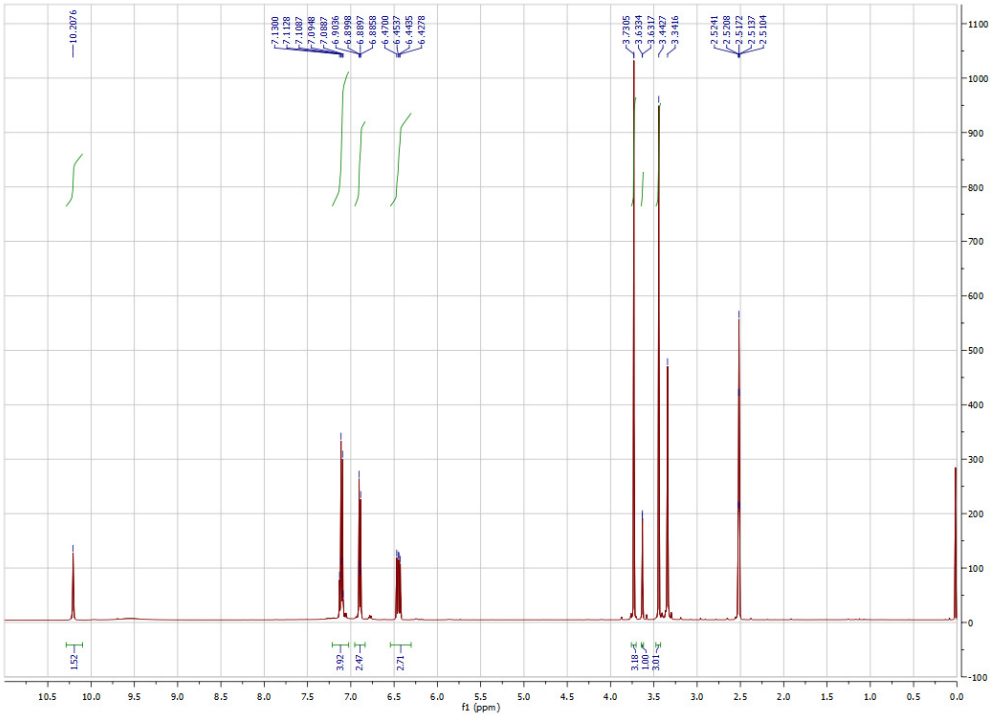


Figure S13. ^{1}H NMR spectrum of **2** (DMSO-d_6, 500 MHz).

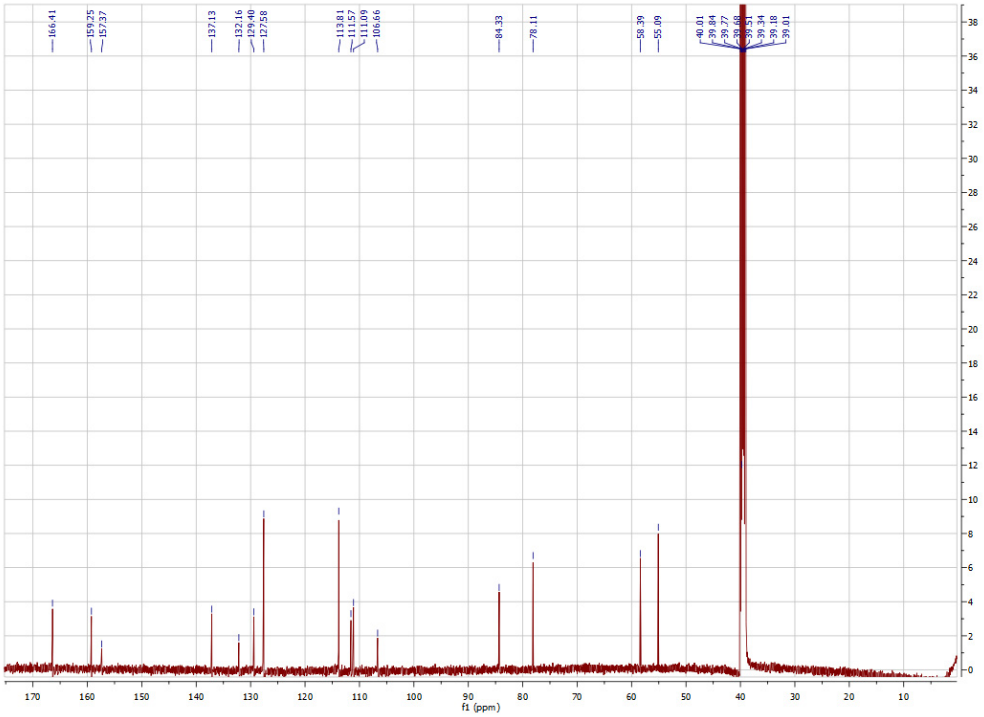


Figure S14. ^{13}C NMR spectrum of **2** (DMSO-d_6, 125 MHz).

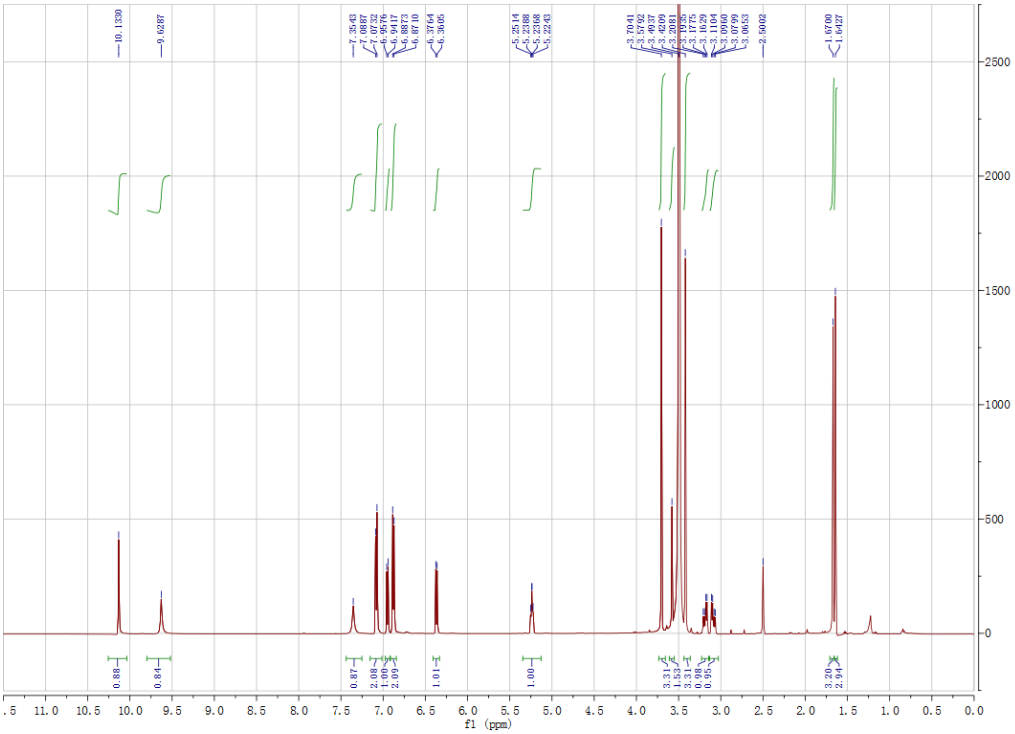


Figure S15. ^{1}H NMR spectrum of **6** (DMSO-d_6, 500 MHz).

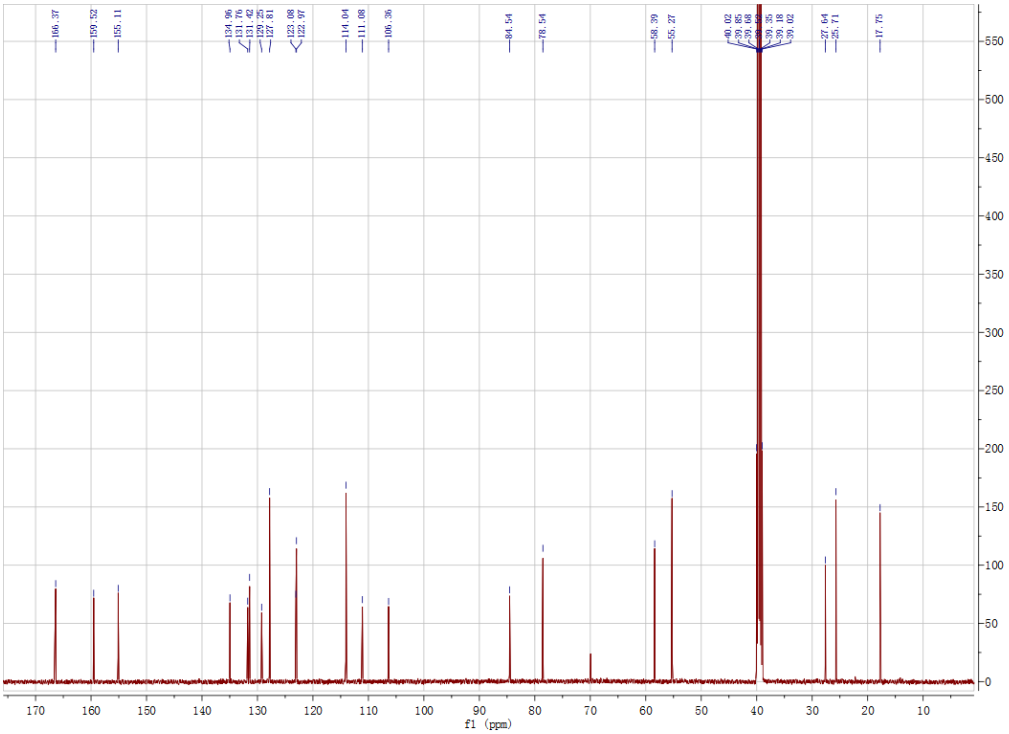


Figure S16. ^{13}C NMR spectrum of **6** (DMSO-d_6, 125 MHz).

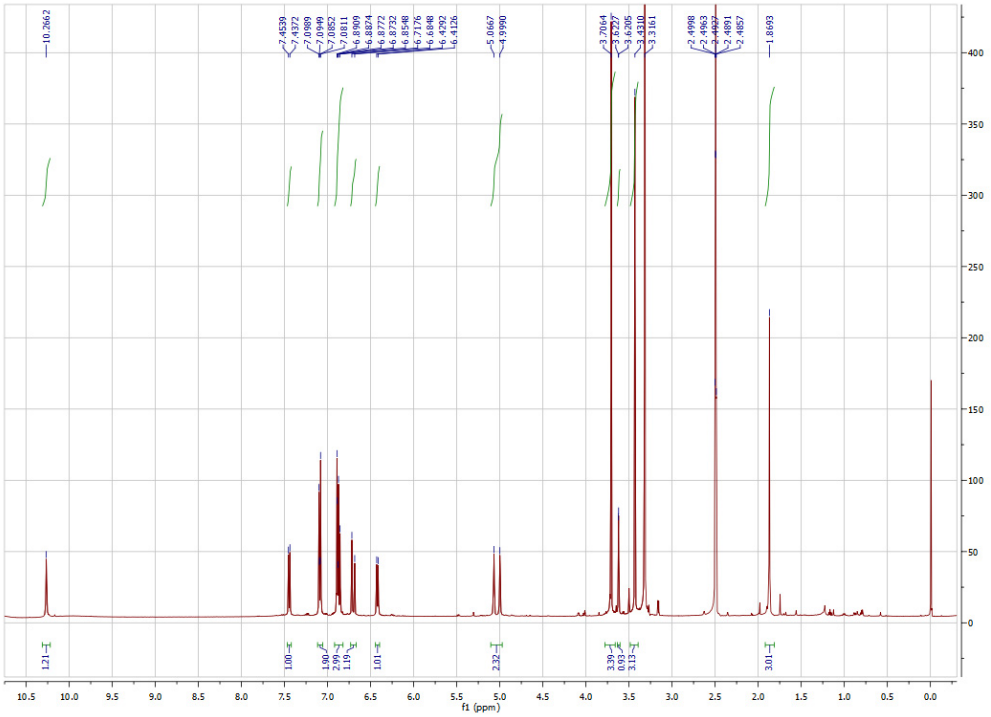


Figure S17. ^{1}H NMR spectrum of **9** (DMSO-d_6, 500 MHz).

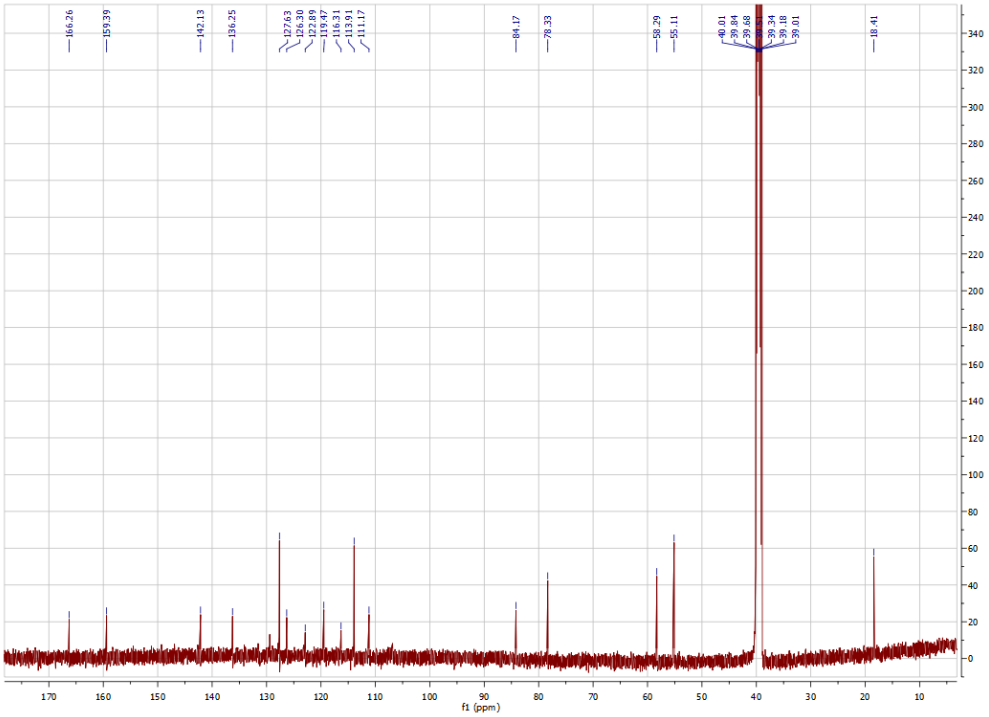


Figure S18. ^{13}C NMR spectrum of **9** (DMSO-d_6, 125 MHz).

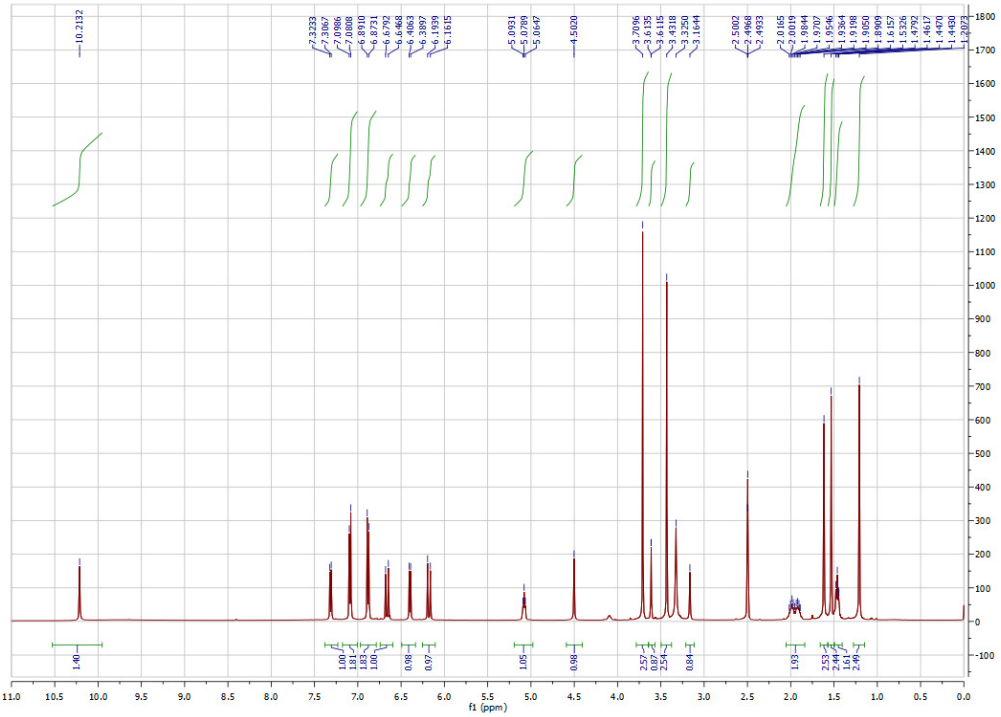


Figure S19. ^{1}H NMR spectrum of **10** (DMSO-d_6, 500 MHz).

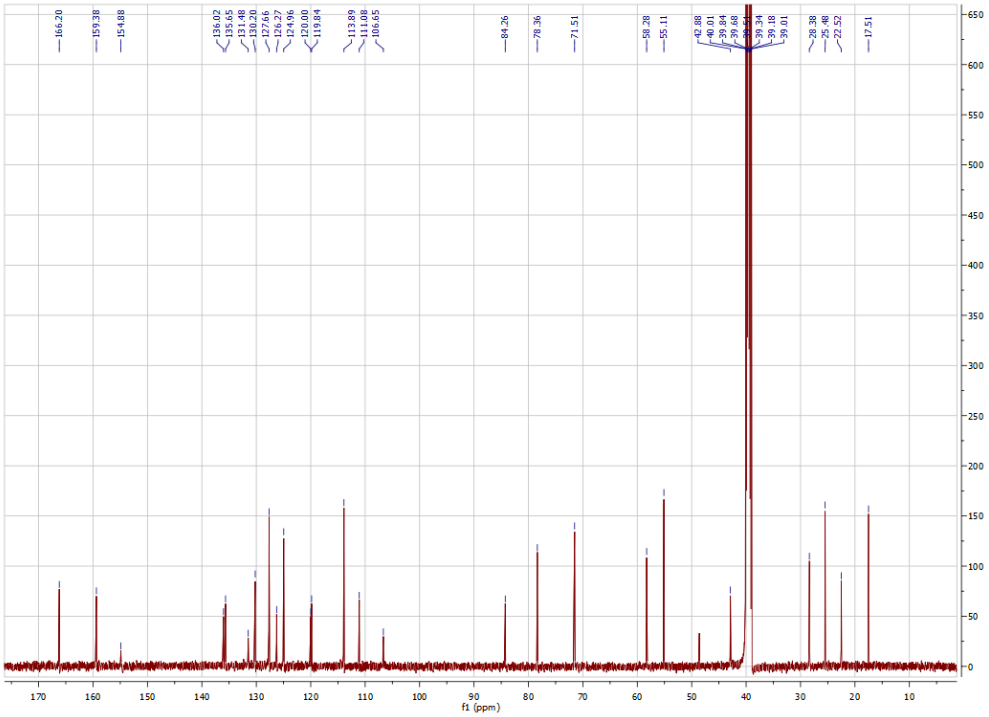


Figure S20. ^{13}C NMR spectrum of **10** (DMSO-d_6, 125 MHz).

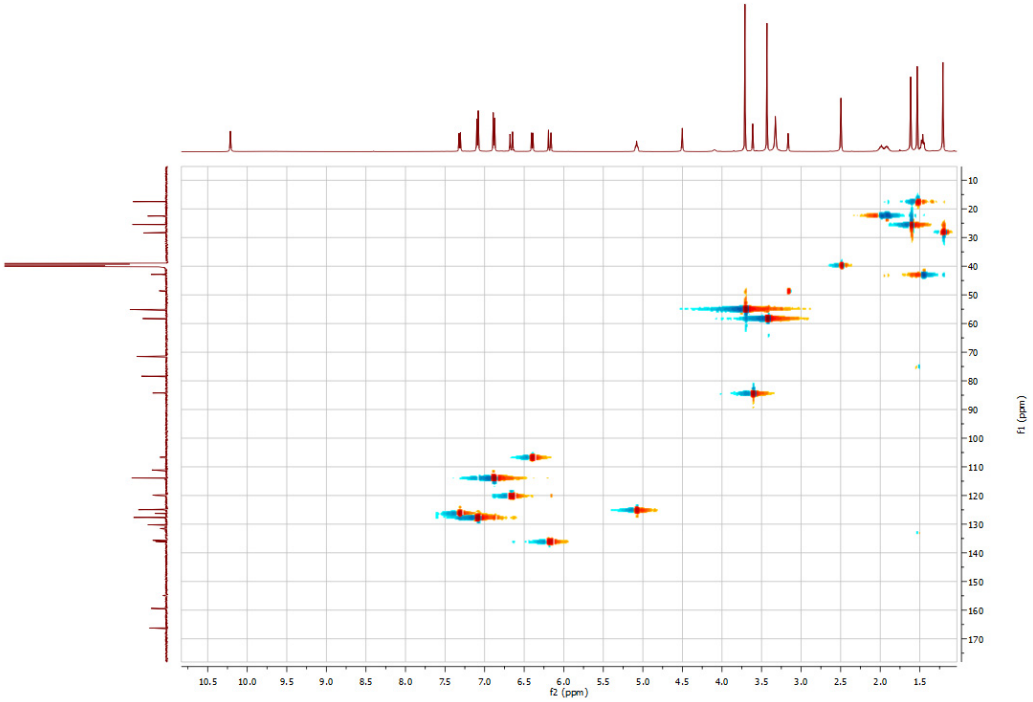


Figure S21. HSQC135 spectrum of **10** (DMSO-d_6, 500 MHz).

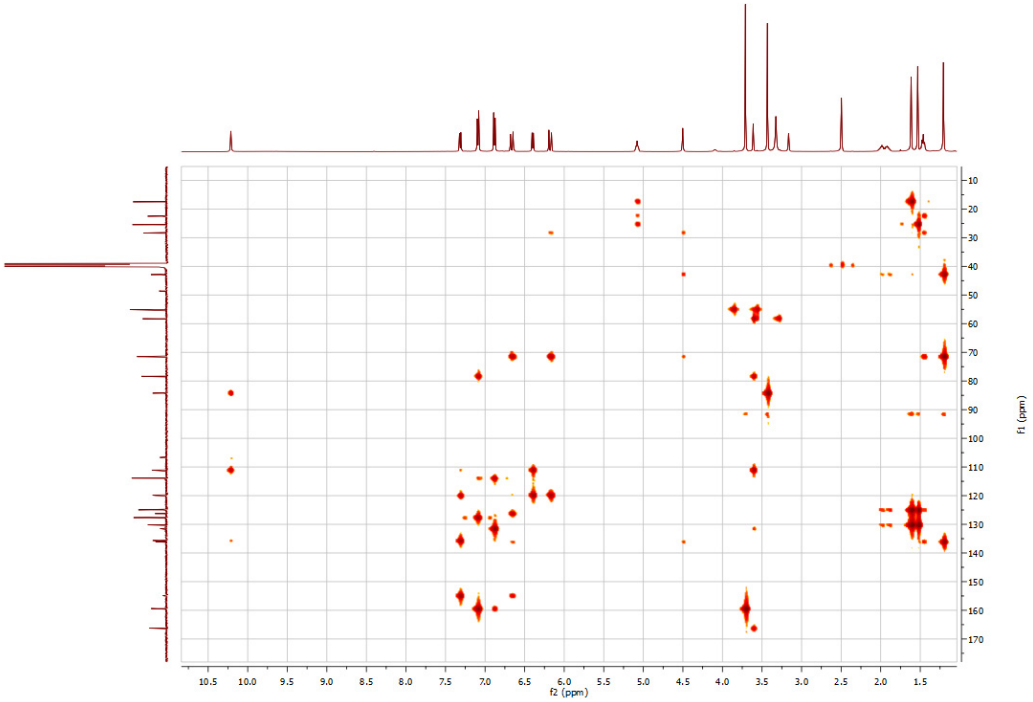


Figure S22. HMBC spectrum of **10** (DMSO-d_6, 500 MHz).

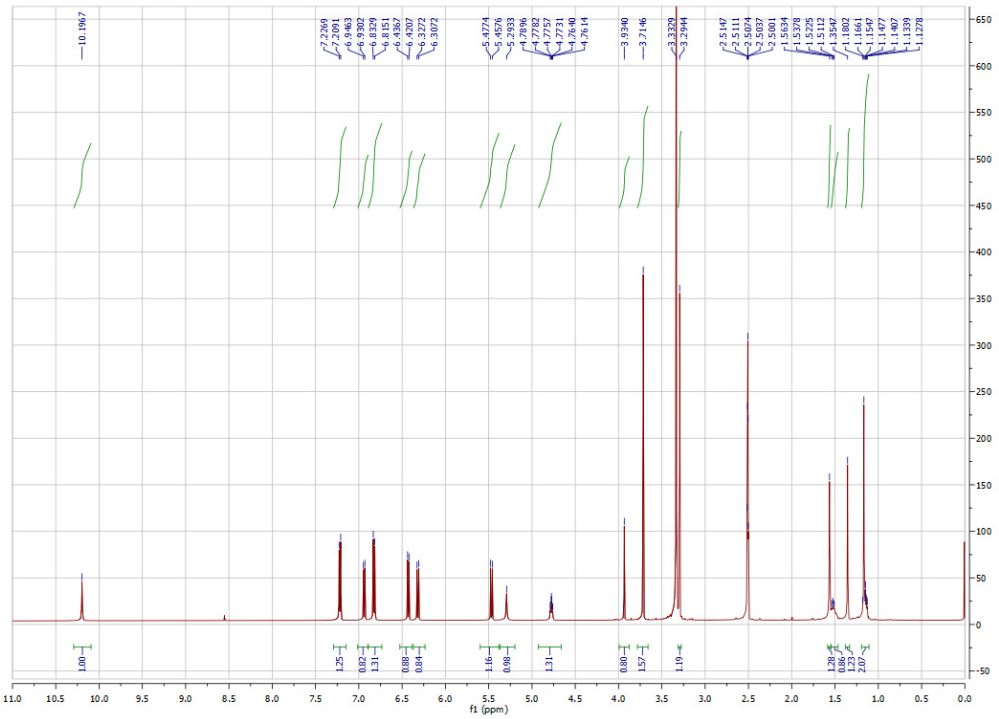


Figure S23. ^{1}H NMR spectrum of **5a** (DMSO-d_6, 500 MHz).

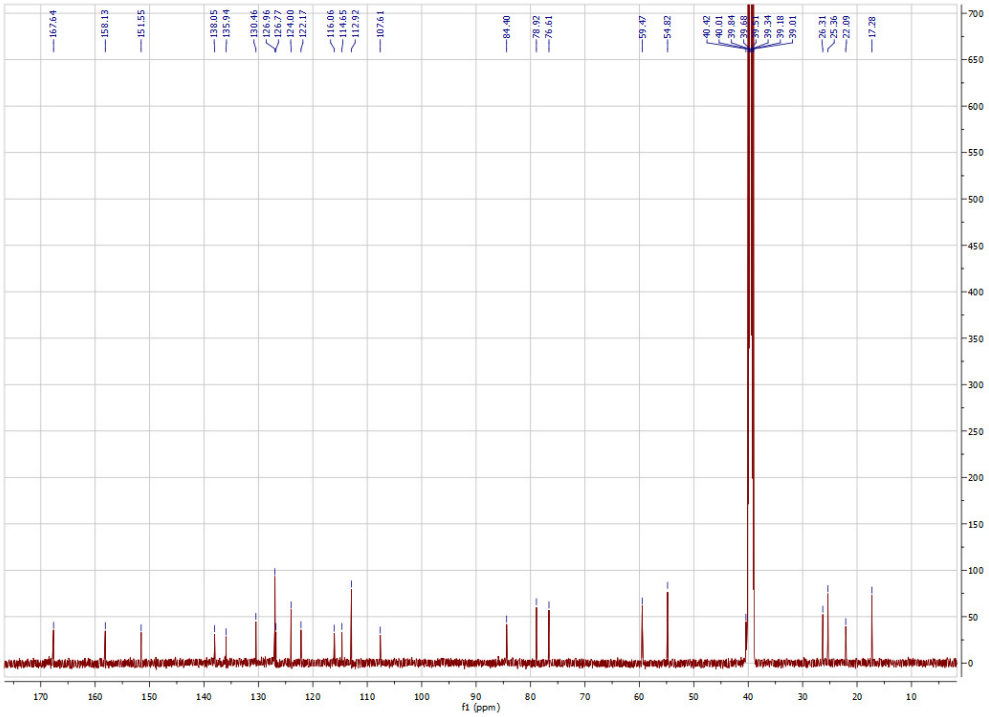


Figure S24. ^{13}C NMR spectrum of **5a** (DMSO-d_6, 125 MHz).

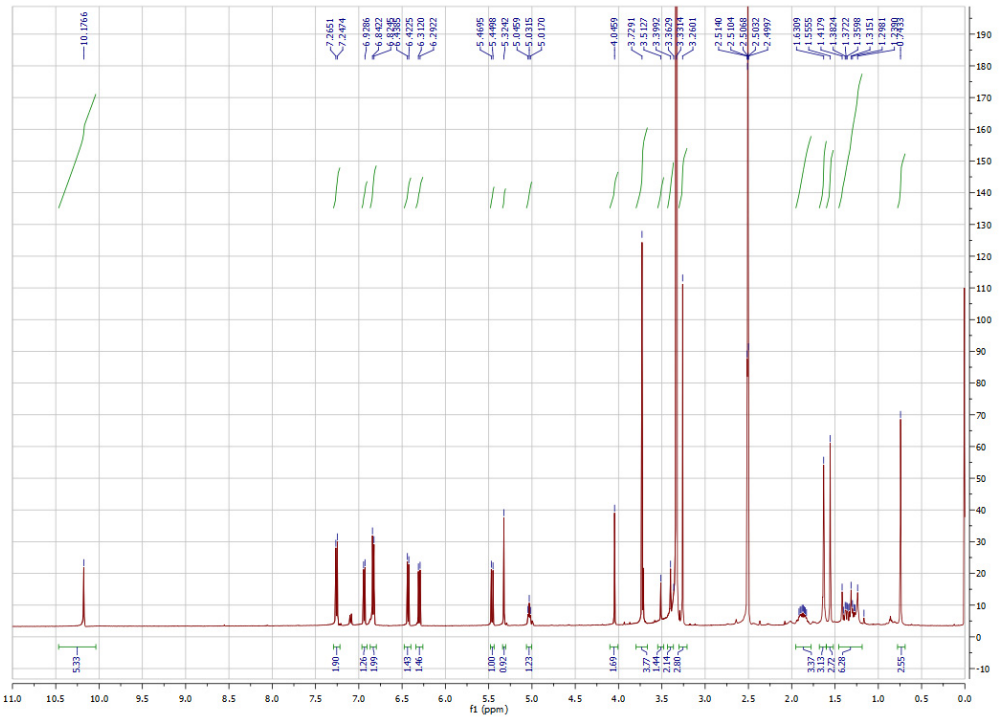


Figure S25. ^{1}H NMR spectrum of **5b** (DMSO-d_6, 500 MHz).

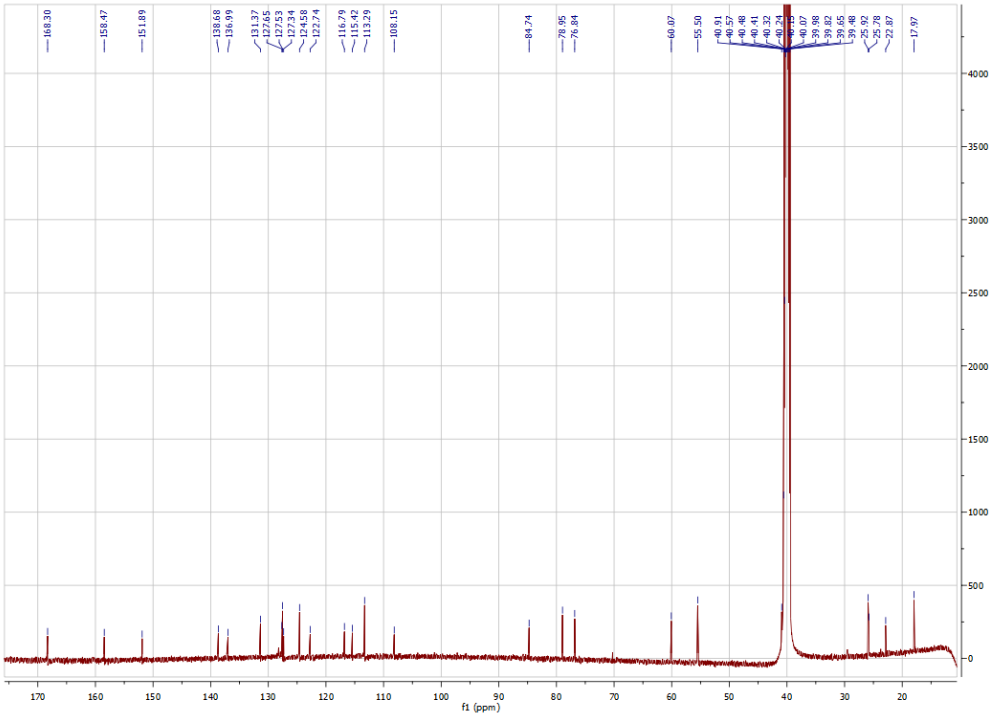


Figure S26. ^{13}C NMR spectrum of **5b** (DMSO-d_6, 125 MHz).

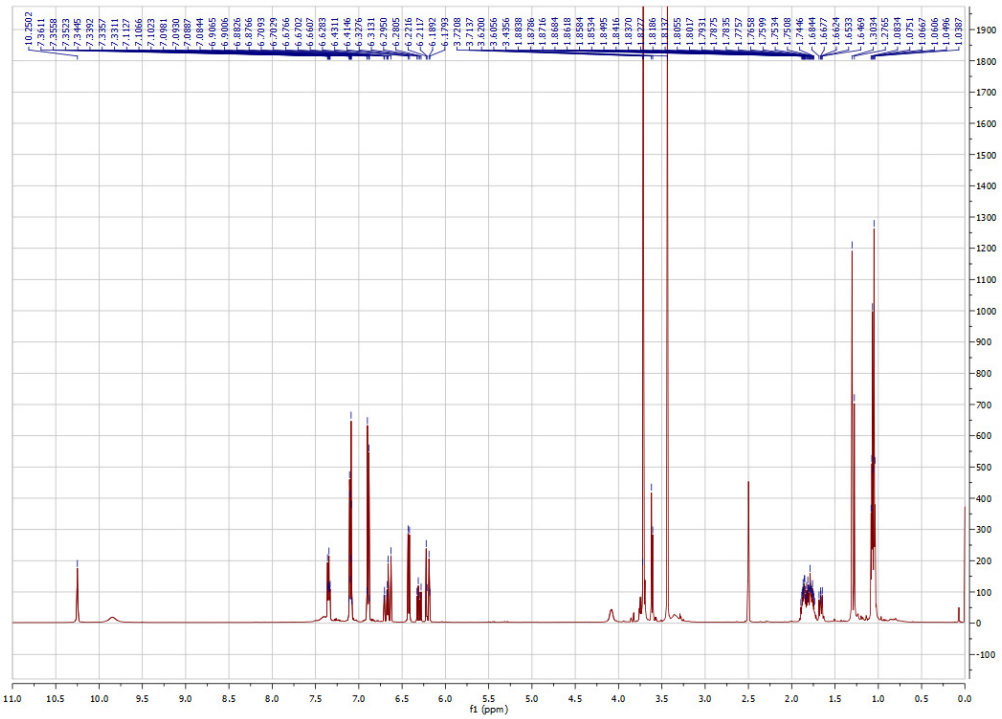


Figure S27. ^{1}H NMR spectrum of **12** (DMSO-d_6, 500 MHz).

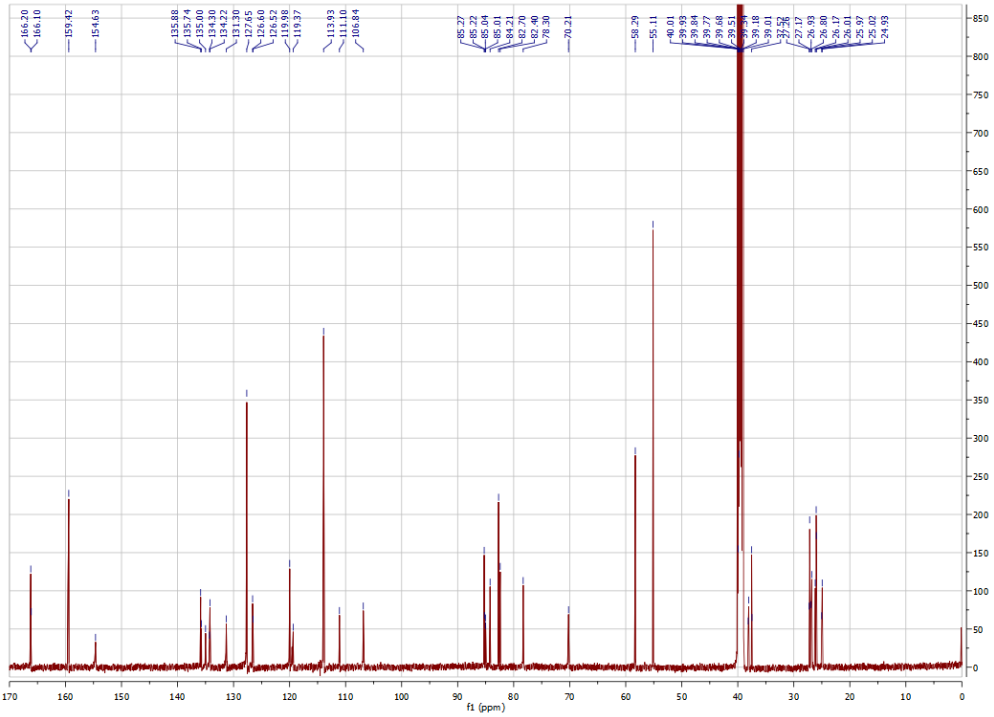


Figure S28. ^{13}C NMR spectrum of **12** (DMSO-d_6, 125 MHz).

Supplementary References

(1) F. N. Gravelat, D. S. Askew, D. C. Sheppard, *Methods Mol Biol* **2012**, *845*, 119-130.

(2) Chooi, Y. H.; Cacho, R.; Tang, Y. *Chem Biol* **2010**, *17*, 483.

(3) Kusano, M.; Koshino, H.; Uzawa, J.; Fujioka, S.; Kawano, T.; Kimura, Y. *Biosci Biotechnol Biochem* **2000**, *64*, 2559.

(4) Uchida, R.; Imasato, R.; Tomoda, H.; Omura, S. *J Antibiot (Tokyo)* **2006**, *59*, 652.

(5) Uchida, R.; Imasato, R.; Shiomi, K.; Tomoda, H.; Omura, S. *Org Lett* **2005**, *7*, 5701.