Biosynthetic Potential-based Strain Prioritization for Natural Product Discovery – a Showcase for Diterpenoid Producing Actinomycetes

Pengfei Xie,[†,‡] Ming Ma,[†,‡] Mostafa E. Rateb,[†] Khaled A. Shaaban,[†] Zhiguo Yu,[†] Sheng-Xiong Huang,[†] Li-Xing Zhao,[§] Xiangcheng Zhu,[⊥,ǀ] Yijun Yan,[†] Ryan M. Peterson,[†,∇] Jeremy R. Lohman,[†] Dong Yang,[†] Min Yin,[†] Jeffrey D. Rudolf,[†] Yi Jiang,[§] Yanwen Duan,[⊥,ǀ] and Ben Shen[⊥,∇,¤,♦]*

[†]Department of Chemistry, The Scripps Research Institute, Jupiter, FL 33458, USA; [§]Yunnan Institute of Microbiology, Yunnan University, Kunming, Yunnan 650091, China; [⊥]Hunan Engineering Research Center of Combinatorial Biosynthesis and Natural Product Drug Discovery, Changsha, Hunan 410329, China; [ǀ]Xiangya International Academy of Translational Medicine, Central South University, Changsha, Hunan 410013, China; [∇]Division of Pharmaceutical Sciences, University of Wisconsin-Madison, Madison, WI 53705, USA; [¤]Department of Molecular Therapeutics, The Scripps Research Institute, Jupiter, FL 33458, USA; and [♦]Natural Products Library Initiative, The Scripps Research Institute, Jupiter, FL 33458, USA

[‡]These authors contributed equally

*****To whom correspondence should be addressed: Ben Shen, The Scripps Research Institute, 130 Scripps Way, #3A1, Jupiter, FL; Tel: (561) 228-2456, Fax: (561) 228-2472, E-mail: shenb@scripps.edu

Supporting Information

Table S1. Ten media selected for fermentation of *Streptomyces griseus* CB00830.

mediums	ingredients
medium A[a] (g/L)	Soluble starch 20, glucose 10, peptone 5, yeast extract 5, NaCl 4, K_2HPO_4 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, $CaCO_3$ 2, pH 7.
medium B (g/L)	Dextrin 40, tomato paste 7.5, NZ Amine A 2.5, primary yeast 5, pH 7.
medium C (g/L)	Glycerol 50, corn meal 25, ardamine 5, pH 7.
medium D (g/L)	Lactose 20, glucose 10, primary yeast 4, cotton seed flour 7.5, pH 7.
medium E (g/L)	Soluble starch 30, soybean flour 10, yeast extract 5, $CaCO_3$ 3, pH 7.
medium F[a] (g/L)	Sucrose 100, glucose 10, casamino acids 0.1, yeast extract 5, MOPS 21, trace elements[b] 1 mL, K_2SO_4 0.25, $MgCl_2 \cdot 6H_2O$ 10, pH 7.
medium G (g/L)	Mannitol 20, malt extract 10, ardamine 2.5, pH 7.
medium H (g/L)	Glucose 15, soluble starch 10, beef extract 3, malt extract 2, yeast extract 2, dried yeast 15, corn steep liquor 3, K_2HPO_4 0.5, NaCl 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, pH 7.
medium I (g/L)	Glucose 20, yeast extract 5, soytone 10, $CoCl_2 \cdot 6H_2O$ 0.001, $CaCO_3$ 2, pH 7.
Medium J (g/L)	Glycerol 20, malt extract 4, yeast extract 4, NZ Amine A 2, trace elements 1mL, pH 7.

[a] The two media used in constructing the natural product library at TSRI.

[b] Trace elements solution was prepared according to a literature protocol.[23]

Table S2. The 16 strains used to develop and optimize the method (see Fig. S1 for structures of the four classes of natural products).

classes	natural products	producers	genes targeting each of the biosynthetic machineries				Ref
			type I PKS	type II PKS	NRPS	DTS	
reduced polyketides	Erythromycin	*Saccharopolyspora erythraea*	+				1
	FK506	*Streptomyces sp.*	+				2
	Tylosin	*Streptomyces fradiae*	+				3
	Geldanamycin	*Streptomyces autolyticus*	+				4
	Avermectin	*Streptomyces avermitilis*	+				5
	Rapamycin	*Streptomyces hygroscopicus*	+				6
aromatic polyketides	Tetracenomycin	*Streptomyces glaucescens*		+			7
	Daunorubicin	*Streptomyces peucetius*		+			8
	Actinorhodin	*Streptomyces coelicolor*		+			9
	Fredericamycin	*Streptomyces griseus*		+			10
nonribosomal peptides	Vancomycin	*Amycolatopsis orientalis*			+		11
	Bleomycin	*Streptomyces verticillus*			+		12
	Tallysomycin	*Streptoalloteichus hindustanus*			+		13
	Zorbamycin	*Streptomyces flavoviridis*			+		14
diterpenoids	Platensimycin	*Streptomyces platensis* MA7327				+	15
	Platencin	*Streptomyces platensis* MA7339				+	15

Table S3. ^{1}H (700 MHz) and ^{13}C (175 MHz) NMR data for viguiepinol (**1**), oxaloterpin E (**2**) and oxaloterpin C (**3**) in $CDCl_3$.[a]

	viguiepinol (**1**)		oxaloterpin E (**2**)		oxaloterpin C (**3**)	
position	δ_C, type	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)
1	38.9, CH_2	1.89, dt (13.5, 3.4), 1.32, m*	38.5, CH_2	1.89, dt (13.6, 3.4), 1.42, m*	38.4, CH_2	1.95, dt (13.8, 3.1), 1.44, m*
2	28.2, CH_2	1.64, m*	24.9, CH_2	1.70, m*	24.5, CH_2	1.81, m*, 1.73, m*
3	79.2, CH	3.23, dd (10.1, 5.8)	81.8, CH	4.38, dd (11.4, 4.6)	85.4, CH	4.70, dd (11.8, 4.3)
4	39.1, C		38.2, C		38.3, C	
5	44.8, CH	1.41, m*	45.0, CH	1.51, m*	45.1, CH	1.52, dd (12.4, 5.9)
6	18.4, CH_2	1.79, m*, 1.56, m	18.2, CH_2	1.79, m, 1.56, m*	18.2, CH_2	1.83, m*, 1.60, m*
7	26.8, CH_2	1.67, m*, 1.22, m	26.7, CH_2	1.66, m*, 1.22, m	26.6, CH_2	1.68, m*, 1.25, m
8	29.4, CH	2.28, m	29.4, CH	2.29, m	29.4, CH	2.29, m
9	151.1, C		150.8, C		150.3, C	
10	37.6, C		37.6, C		37.6, C	
11	115.9, CH	5.36, m	116.1, CH	5.36, m	116.5, CH	5.37, m
12	37.6, CH_2	2.02, br d (17.3), 1.76, m	37.5, CH_2	2.02, br d (15.6), 1.77, m*	37.5, CH_2	2.03, br d (17.8), 1.72, m*
13	34.9, C		34.9, C		34.9, C	
14	41.6, CH_2	1.39, m*, 1.02, m	41.6, CH_2	1.39, m*, 1.02, m	41.5, CH_2	1.40, m*, 1.04, m*
15	150.3, CH	5.82, dd (17.5, 10.7)	150.3, CH	5.82, dd (17.5, 10.7)	150.2, CH	5.82, dd (17.5, 10.7)
16	109.1, CH_2	4.93, dd (17.5, 1.0), 4.86, dd (10.8, 1.0)	109.1, CH_2	4.93, dd (17.5, 1.4), 4.86, dd (10.7, 1.4)	109.2, CH_2	4.93, dd (17.5, 1.4), 4.87, dd (10.7, 1.4)
17	22.5, CH_3	0.97, s	22.4, CH_3	0.96, s	22.5, CH_3	0.96, s
18	25.3, CH_3	1.07, s	25.3, CH_3	1.09, s	25.3, CH_3	1.11, s
19	27.8, CH_3	0.99, s	27.7, CH_3	0.92, s*	27.8, CH_3	1.04, s
20	15.3, CH_3	0.87, s	16.4, CH_3	0.92, s*	16.5, CH_3	0.90, s
1'			157.0, C		158.2, C	
2'					154.2, C	
NH_2				4.57, br s		
N-OH						9.60, br s

[a] Assignments were based on COSY, HMBC and HSQC experiments.

* These signals overlapped with others.

Table S4. ^{1}H (700 MHz) and ^{13}C (175 MHz) NMR data for 4-chloroanthranilamide (**8**) in d_6-DMSO and methyl 4-chloroanthranilate (**9**) in $CDCl_3$.[a]

position	4-chloroanthranilamide (**8**)		methyl 4-chloroanthranilate (**9**)	
	δ_C, type	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)
1	112.8, C		109.3, C	
2	151.9, C		151.2, C	
3	115.5, CH	6.73, d (2.2)	115.9, CH	6.66, d (2.0)
4	136.8, C		140.0, C	
5	114.5, CH	6.48, dd (8.5, 2.2)	116.7, CH	6.60, dd (8.6, 2.0)
6	131.0, CH	7.53, d (8.5)	132.6, CH	7.77, d (8.6)
7	170.9, C		168.0, C	
8			51.7, CH_3	3.86, s
NH_2		6.82, br s		5.80, br s
$CONH_2$		7.77, br s, 7.14 br s		

[a] Assignments were based on COSY, HMBC and HSQC experiments.

Table S5. ^{1}H (700 MHz) and ^{13}C (175 MHz) NMR data for seco-dinactin (**12**) in comparison with dinactin (**10**) and feigrisolide C (**11**) in CDCl$_3$.[a]

position	dinactin (**10**)		feigrisolide C (**11**)		seco-dinactin (**12**)	
	δ_C, type	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)
1	174.3, C		176.8, C		175.9, C	
1′	174.3, C				174.5, C	
2	45.1, CH	2.52, m*	45.5, CH	2.55, m*	45.7, CH	2.55, m*
2′	45.1, CH	2.52, m*			45.6, CH	2.55, m*
3	79.7, CH	4.03, m*	80.7, CH	4.04, m*	80.3, CH	4.03, m*
3′	79.7, CH	4.03, m*			80.2, CH	4.03, m*
4	27.9, CH$_2$	1.95, m*	29.2, CH$_2$	1.99, m*, 1.64, m*	29.2, CH$_2$	2.00, m*
4′	27.9, CH$_2$	1.47, m*			28.6, CH$_2$	1.62, m*
5	31.4, CH$_2$	1.97, m*	31.1, CH$_2$	2.06, m*, 1.63, m*	31.4, CH$_2$	2.02, m*
5′	31.4, CH$_2$	1.61, m*			31.3, CH$_2$	1.56, m*
6	76.1, CH	3.83, m*	76.5, CH	4.04, m*	76.5, CH	3.87, m
6'	76.1, CH	3.83, m*			76.5, CH	3.87, m
7	42.2, CH$_2$	1.75, m*	42.3, CH$_2$	1.80, m	42.5, CH$_2$	1.85, m*
7'	42.2, CH$_2$	1.75, m*			42.3, CH$_2$	1.85, m*
8	68.9, CH	4.92, m*	68.9, CH	5.07, m	69.6, CH	5.05, m
8'	68.9, CH	4.92, m*			68.8, CH	5.00, m
9	174.0, C		174.4, C		174.4, C	
9′	174.0, C				174.2, C	
10	45.0, CH	2.52, m*	45.1, CH	2.55, m*	45.6, CH	2.55, m*
10′	45.0, CH	2.52, m*			45.5, CH	2.55, m*
11	79.9, CH	4.03, m*	81.1, CH	4.04, m*	80.9, CH	4.03, m*
11′	79.9, CH	4.03, m*			80.9, CH	4.03, m*
12	28.1, CH$_2$	1.95, m	28.8, CH$_2$	1.99, m*, 1.64, m*	28.4, CH$_2$	1.97, m*
12′	28.1, CH$_2$	1.47, m*			28.2, CH$_2$	1.60, m*
13	31.2, CH$_2$	1.97, m	30.5, CH$_2$	2.06, m*, 1.63, m*	31.1, CH$_2$	2.05, m*
13′	31.2, CH$_2$	1.61, m*			30.7, CH$_2$	1.63, m*
14	76.2, CH	3.83, m*	77.1, CH	4.17, m	76.6, CH	4.17, m
14'	76.2, CH	3.83, m*			76.6, CH	4.05, m*
15	39.8, CH$_2$	1.75, m*	40.5, CH$_2$	1.73, m*	40.8, CH$_2$	1.78, m*
15'	39.8, CH$_2$	1.75, m*			40.2, CH$_2$	1.78, m*
16	73.1, CH	4.92, m*	70.3, CH	3.77, m	73.5, CH	4.87, m
16'	73.1, CH	4.92, m*			70.3, CH	3.78, m
17	12.5, CH$_3$	1.08, m*	13.7, CH$_3$	1.14, d (7.0)	13.3, CH$_3$	1.19, d (7.0)
17′	12.5, CH$_3$	1.08, m*			13.1, CH$_3$	1.12, d (6.9)
18	20.3, CH$_3$	1.21, br s	20.3, CH$_3$	1.27, d (6.3)	20.6, CH$_3$	1.27, d (6.3)
18′	20.3, CH$_3$	1.21, br s			20.4, CH$_3$	1.25, d (5.8)
19	13.1, CH$_3$	1.08, m*	13.6, CH$_3$	1.19, d (7.0)	13.4, CH$_3$	1.11, d (5.6)
19'	13.1, CH$_3$	1.08, m*			13.4, CH$_3$	1.10, d (5.6)
20	27.3, CH$_2$	1.60, m*	30.0, CH$_2$	1.55, m	30.0, CH$_2$	1.64, m*
20'	27.3, CH$_2$	1.45, m*			27.5, CH$_2$	1.54, m*
21	9.2, CH$_3$	0.86, br s	10.1, CH$_3$	0.95, t (7.4)	9.3, CH$_3$	0.90, t (7.4)
21'	9.2, CH$_3$	0.86, br s			10.1, CH$_3$	0.95, t (7.4)

[a] Assignments were based on COSY, HMBC and HSQC experiments.

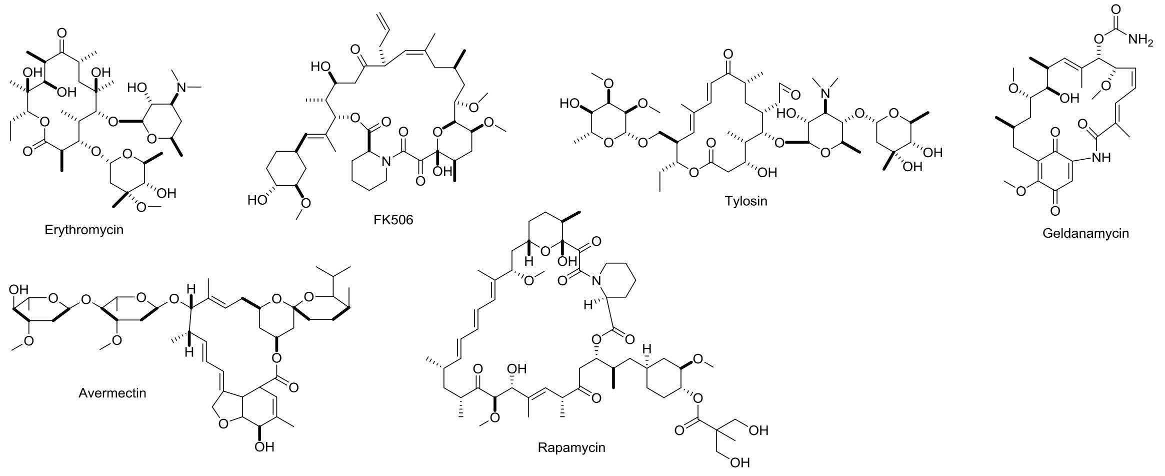
* These signals overlapped with others.

Table S6. Primers used for the amplification and sequencing of the diterpene synthases and the housekeeping genes from *S. griseus* CB00830.

Primer	Nucleotide Sequence (5'-3')	Function
830T2_F	GAATGCGACGACCACCGATCTC	Amplification/sequencing of *ent-cpps*
830T2_R	CAGATCGGCCAGGACCTTCAG	Amplification/sequencing of *ent-cpps*
830T2_MF	CGGGCATCACCTCGATCAACTAC	Sequencing of *ent-cpps*
830PMDS_F	CGAGGACGATCGCGAGAATCC	Amplification/sequencing of *pmds*
830PMDS_R	TCAGTCGTTCGT CGA TCA GGC	Amplification/sequencing of *pmds*
830GGDP_F	GTGGTCACTTGGTGAACACCAGG	Amplification/sequencing of *ggps*
830GGDP_R	CGGTCACCTTCTACAGCGGC	Amplification/sequencing of *ggps*
16SrRNA_for	AGAGTTTGATCCTGGCTCAG	Phylogenetic analysis
16SrRNA_rev	ACGGCTACCTTGTTACGACTT	Phylogenetic analysis
recA_for	TAATACGACTCACTATAGGGCCGCRCTCGCACARATTGAACG	Phylogenetic analysis[18]
recA_rev	CGATCAATAACGAGTCGCCGGTCGGGGTTGTCCTTSAGGAAG	Phylogenetic analysis[18]
rpoB-2	CATCGACCACTTCGGCAAC	Phylogenetic analysis
ActRpoB3303R	GAANCGCTGDCCRCCGAACTG	Phylogenetic analysis[16]
trpBfor	TAATACGACTCACTATAGGGGCGCGAGGACCTGAACCACAC	Phylogenetic analysis[18]
trpBrev	CGATCAATAACGAGTCGCCGATGGCCGGGATGATGCCC	Phylogenetic analysis[18]

Figure S1. Structures of the 16 natural products chosen as representatives for the four major classes of natural products (see Table S2 for each of the producers).

Reduced polyketides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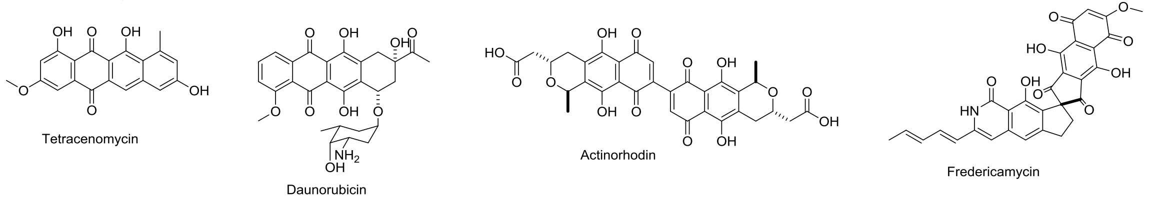
FK506
Tylosin
Geldanamycin
Avermectin
Rapamycin

Aromatic polyketides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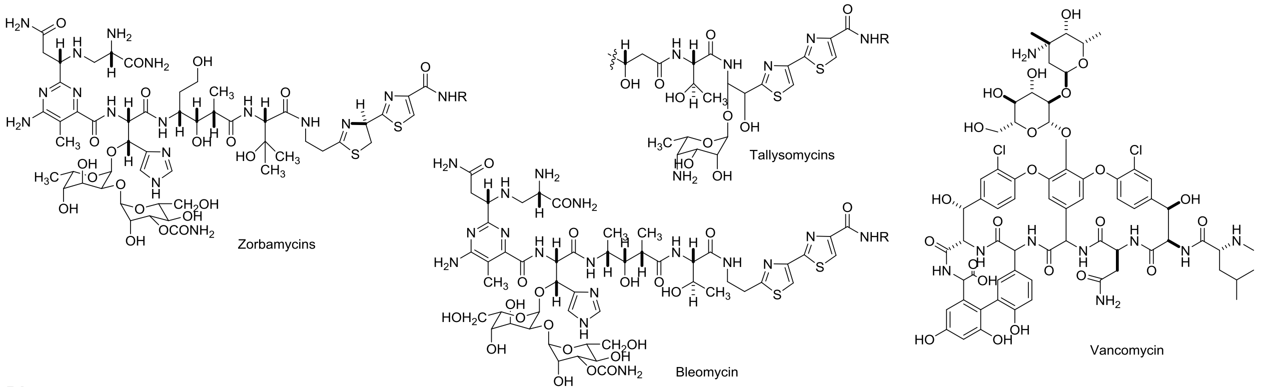
Daunorubicin
Actinorhodin
Fredericamycin

Nonribosomal peptides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Tallysomycins
Bleomycin
Vancomycin

Diterpenes

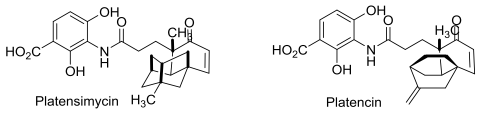
Platensimycin
Platencin

Figure S2. Validation of the method using 16 known producers. (A) Sequence alignments of genes encoding the selected type I PKSs, type II PKSs, NRPSs, and DTSs from the 16 strains. Asterisks (*) indicate complete conservation among the selected protein sequences; double dots (:) indicate conserved substitutions; single dots (.) indicate semi-conserved substitutions; underlined asterisks ($\underline{*}$) indicate the protein sequences selected for degenerate primer design. The number of amino acids between two underlined sequences are shown below each of the sequence alignments. Protein sequences used in Type I PKS sequence alignment: Aves1 (NP822113), GdmAI (ADY00167), FkbC (ADX99523), RapB (CAA60459) and TylG (AAB66504); protein sequences used in Type II PKS sequence alignment: Fdm1 (KS_{α} AAQ08916, KS_{β} AAQ08917), Act1 (KS_{α} NP_629237, KS_{β} NP_629238), Dauno1 (KS_{α} AAA65206, KS_{β} AAA65207) and Tetra1 (KS_{α} P16538, KS_{β} P16539); protein sequences used in NRPS sequence alignment: ZbmX (ACG60782), BlmVI (AAG02359), TlmVI (ABL74940) and Vcm2 (YP_008010469); protein sequences used in DTS sequence alignment: ent-CPPS (BAD86797), PtmT2 (ACO31276) and PtnT2 (ADD83015). (B) Agarose gel electrophoresis showing distinct products with the predicted size of the pool of PCR products; lane 1, type I PKS; lane 2, type II PKS; lane 3, NRPS; lane 4, DTS; 5, DNA marker. (C) Dot blot of the 16 strains; (1) type I PKS; (2) type II PKS; (3) NRPS; (4) DTS. All the known producers for the four representative classes of natural products are highlighted with red boxes. (D) PCR confirmation for the dot blot results; (1) type I PKS; (2) type II PKS; (3) NRPS; (4) DTS. All the known producers for the four representative classes of natural products are highlighted with red boxes.

A

Type I PKS

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Aves1  (59) QGGGFLHDAAHFDAGFFGISPREALAMDPQQRLLLETSWE-----RLSDAVRLGHRVLAVVRGSAVNQDGASNGLTAPNGPAQERVIRQALANAG (298)
GdmAI  (61) REGGFLTDAGGFDAAFFGISPREALAMDPQQRLLLETAWE-----RLSDARRNGHEVLAVVRGTAVNQDGASNGLTAPNGPSQERVIRQALANAG (296)
FkbC   (59) ---GALRGAGDFDAAFFGISPREATALDPQQRILLEIAWE-----RLSDAERHGHSVLAVVRGSAVNQDGASNGLSAPNGPAQQRVIRGALERAG (286)
RapB   (58) VQGGFLDSAGGFDASFFGISPREALAMDPQQRLVLEASWE-----RLSDAQRKGHQVLAVVRGSAVNQDGASNGLSAPNGPSQQRVIRAALSNAG (288)
Ery1   (50) REGGFLHDAAEFDPSVFGISPREALAMDPQQRLLLEVSWE-----RLSDARRLGHPVLAVVRGSAVNQDGASNGLTAPNGPSQQRVILQALTNAR (285)
TylG   (61) REGGFLHDAAEFDAEFFGISPREAVAMDPQQRLLLETSWE-----RLSDARRLGHPVLAVVCGSAVNQDGASNGLTAPSGPSQERVIRQALGNAR (296)
               * * .*. **. .******** *:*****::**  **     ***** * ** ***** *:***********:**.**:*:***  ** .*
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≈204 aa

Type II PKS

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                                 KSα                                                 KSβ
Fdm1   (291) ALDEARINPDEVDYANAHGSGTKQNDRHETAAYKRSLGEHA (332)-----(139)AATTGQLSIRHGMRGPCGVLCSEQAGGLDVLAQARRVLAKGT (180)
Act1   (335) ALDESRTDATDIDYINAHGSGTRQNDRHETAAYKRALGEHA (375)-----(137)AVNTGQISIRHGMRGPSSALVAEQAGGLDALGHARRTIRRGT (179)
Dauno1 (292) ALDEARRDPSDVDYVNAHGTATKQNDRHETSAFKRSLGEHA (332)-----(180)AVNTGQIAIRHDLRGPVGVVVAEQAGGLDALAHARRKVRGGA (201)
Tetra1 (295) ALDQARRTGDDLHYINAHGSGTRQNDRHETAAFKRSLGQRA (336)-----(142)AVNTGQISIRHGMRGHSSVFVTEQAGGLDAAAHAARLLRKGT (183)
             ***::*    :: * ****:.*.*******:*:**:**:.*                *..***::***.:**  ... :*******. .:* * :  *:
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≈300 aa

NRPS

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ZbmX  (94)  QAGPGNAAYILFTSGSTGEPKGVVVEHRNVVASLR-----ERHYVTGDVVRCLSGGDLEFVGRDDQQVKIRGFRVELSEIEAVLDAHPDVHKA—- (382)
BlmVI (84)  SVHPEQLAAVMATSGSTGTPKTIGVPQRALAGYLR-----ARRYRTGDLARRLPDGTLLLAGRADRQVKIRGHRVEPGEVEQVLGGHPGVRE--- (364)
TlmVI (88)  RTHRDALACVMPTSGSTGVPKLVGVPHRGMVNYLR-----ARRYRTGDLARRLPHGDLVFVGRADRQVKIRGHRVELGELEHTLAAHPAVRQVAA (380)
Vcm2  (111) RVDPRSAAYVIYTSGSTGTPKGVVVTHTGLGNLAL-----ERMYRTGDLAYWTDQGELVSAGRADDQVKIRGFRVEPREIEFALSAYPGITQATV (391)
             .     * :: ****** ** : * :  :           * * ***:.     * *  .** * ******.***  *:* .* .:* : :
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≈263 aa

DTS

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ent-CPPS (57) AQQHEDGTWGGPAAYGLLPTLSAVDALLSVAGTQDARR-----RGQGDTRYDHAAERVGGWLVEQQQPDGRWMDKWHASPYYATACG (391)
PtmT2    (61) KEQNQDGSWGAPDGYGLVPTLSAVEALLTELARTDSGA-----RPDDAGRYGAEIRMISDWLLDNQLPDGSWMDKWHASPYYATACC (414)
PtnT2    (61) EEQNQDGSWGAPDGYGLVPTLSAVEALLTELARPESGA-----RPDDAGRYGAEIRMISDWLLDNQLPDGSWMDKWHASPYYATACC (414)
               :*::**:**.* .***:******:***:  .  ::       * :.  **.     :..**:::* *** ***************
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≈335 aa

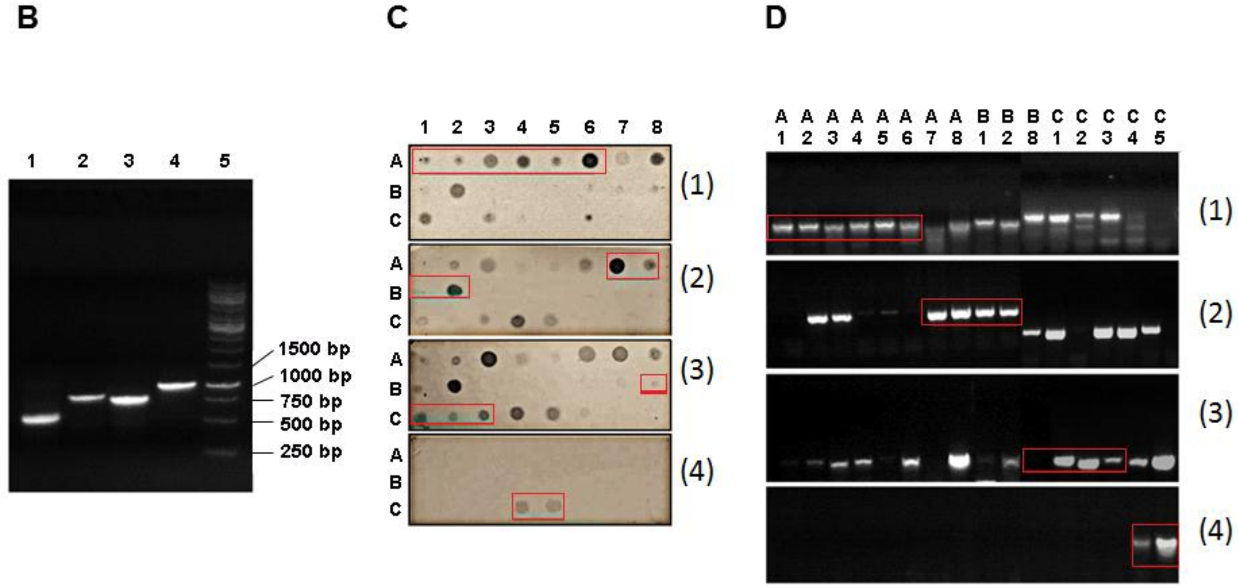


Figure S3. Dot blot survey of the randomly selected 100 strains for their biosynthetic potential of four targeted classes of natural products. (1) Type I PKS; (2) type II PKS; (3) NRPS; (4) DTS. The strain selected to do large scale fermentation and isolation, *Streptomyces griseus* CB00830, is highlighted with a red box.

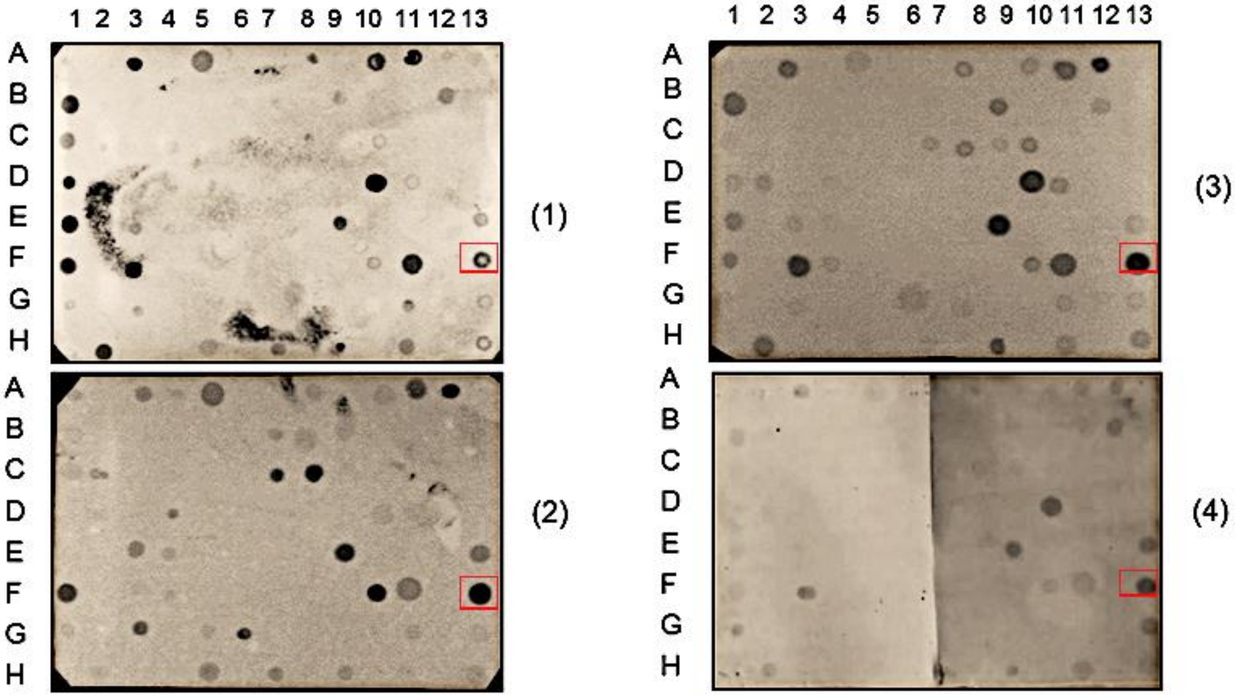
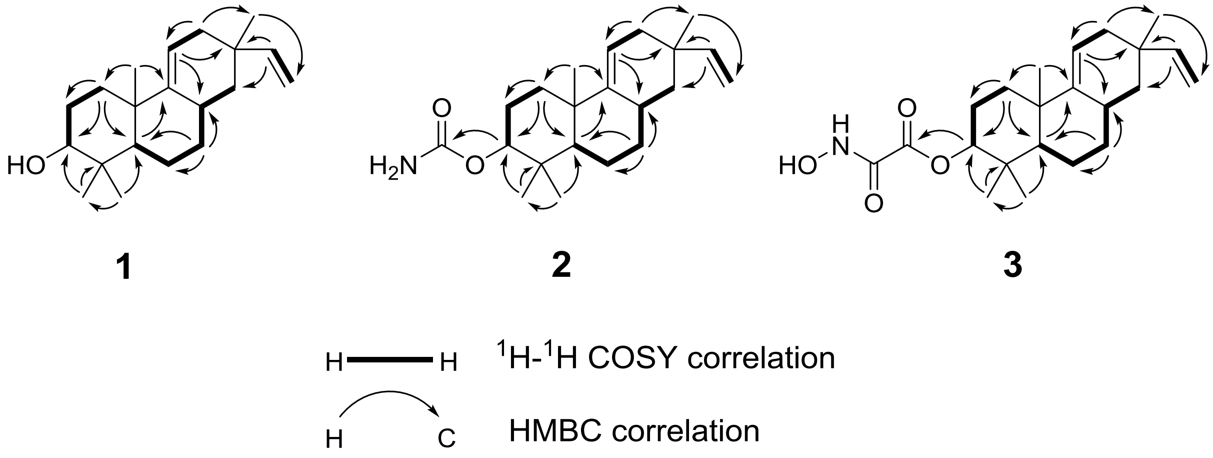
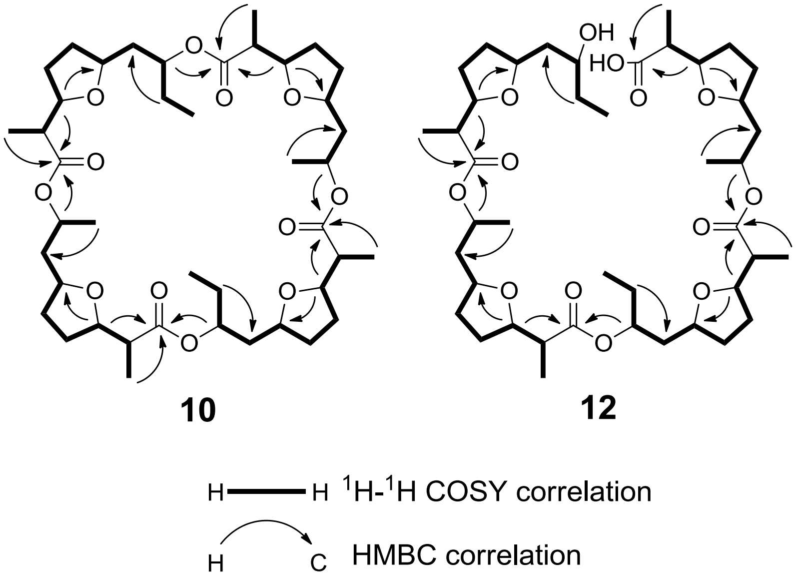


Figure S4. Key COSY and HMBC correlations of compounds **1-3**.



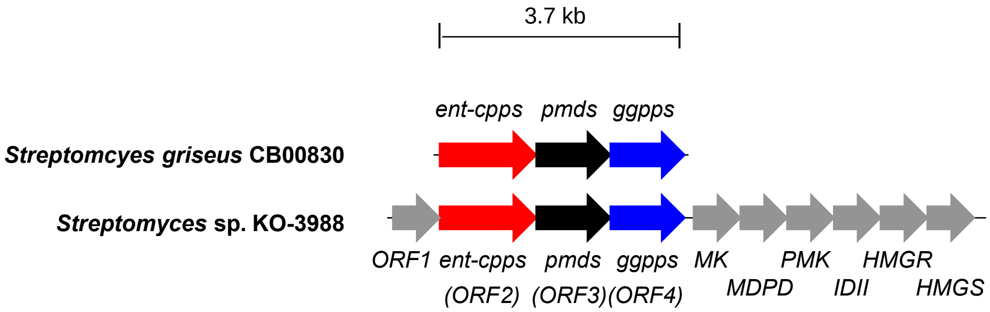
1 **2** **3**

Figure S5. Key COSY and HMBC correlations of compounds **10** and **12**.



10 **12**

Figure S6. The biosynthetic loci of compounds **1-3** in *S. griseus* CB00830 and *S*. sp. KO-3988. The diterpene synthase genes proposed for diterpene biosynthesis in *S. griseus* CB00830 (accession ##) are aligned with the viguiepinol and mevalonate biosynthetic gene cluster from *S*. sp. KO-3988 (AB183750).[16] DNA and protein identities are listed below each corresponding gene. The three diterpene synthase genes sequenced were *ent*-copalyl diphosphate synthase (*ent-cpps*), pimaradiene synthase (*pmds*), and geranylgeranyl diphosphate synthase (*ggpps*).



Identity			
DNA	95	95	94
Protein	94	89	94

Figure S7. Phylogenetic analysis assigns CB00830 (highlighted in red box) as a *Streptomyces griseus* species. The phylogenetic tree was generated from the alignment of concatenated partial sequences[18,19] of the four housekeeping genes 16S rRNA, *recA*, *rpoB*, and *trpB* (2975 bp total) using the Tamura-Nei evolutionary distance method[20] and the neighbor-joining algorithm. Bootstrap[21] values >60% (based on 1000 resampled trials) are given at nodes. Bar, 0.02 substitutions per nucleotide position. Sequence alignment and phylogenetic tree construction were conducted with MEGA 5.05.[22] *S. coelicolor* A3(2) (AL645882), *S. avermitilis* MA-4680 (BA000030), *S. rimosus* ATCC 10970 (ANSJ00000000), *S. griseus* NBRC 13350 (AP009493), *S. scabies* 87.22 (NC_013929), *S. venezuelae* ATCC 10712 (FR845719), and *S. clavuligerus* ATCC 27064 (ADWJ00000000) were obtained from the NCBI database and used for representative *Streptomyces* spp. *Mycobacterium tuberculosis* H37Rv (AL123456) was used as an outgroup.

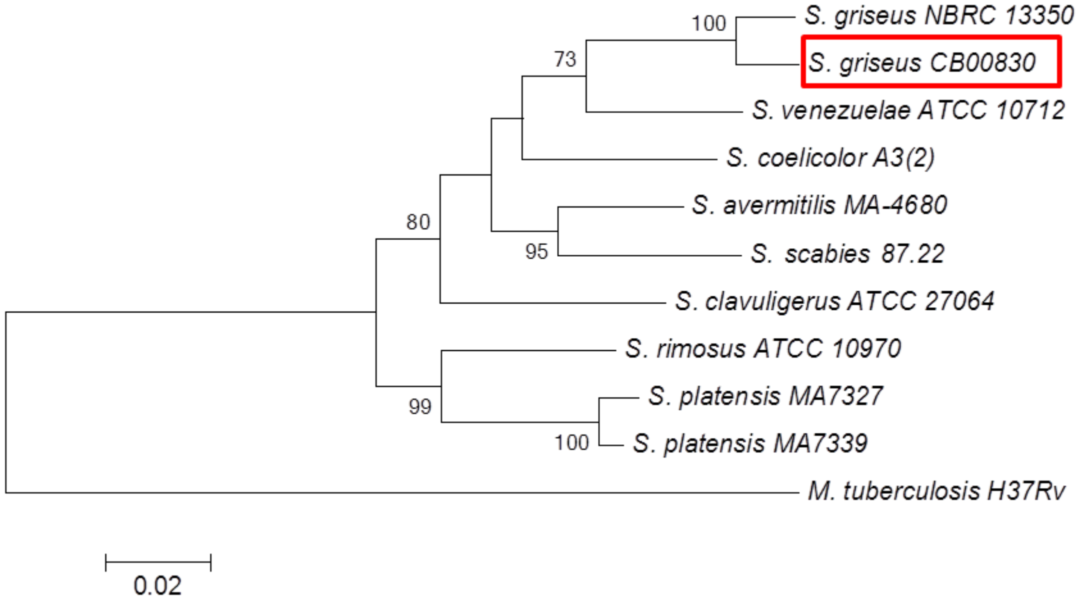


Figure S8. ^{1}H NMR (700 MHz) and ^{13}C (175 MHz) spectra of grisechelin A (**4**) in d_6-DMSO.

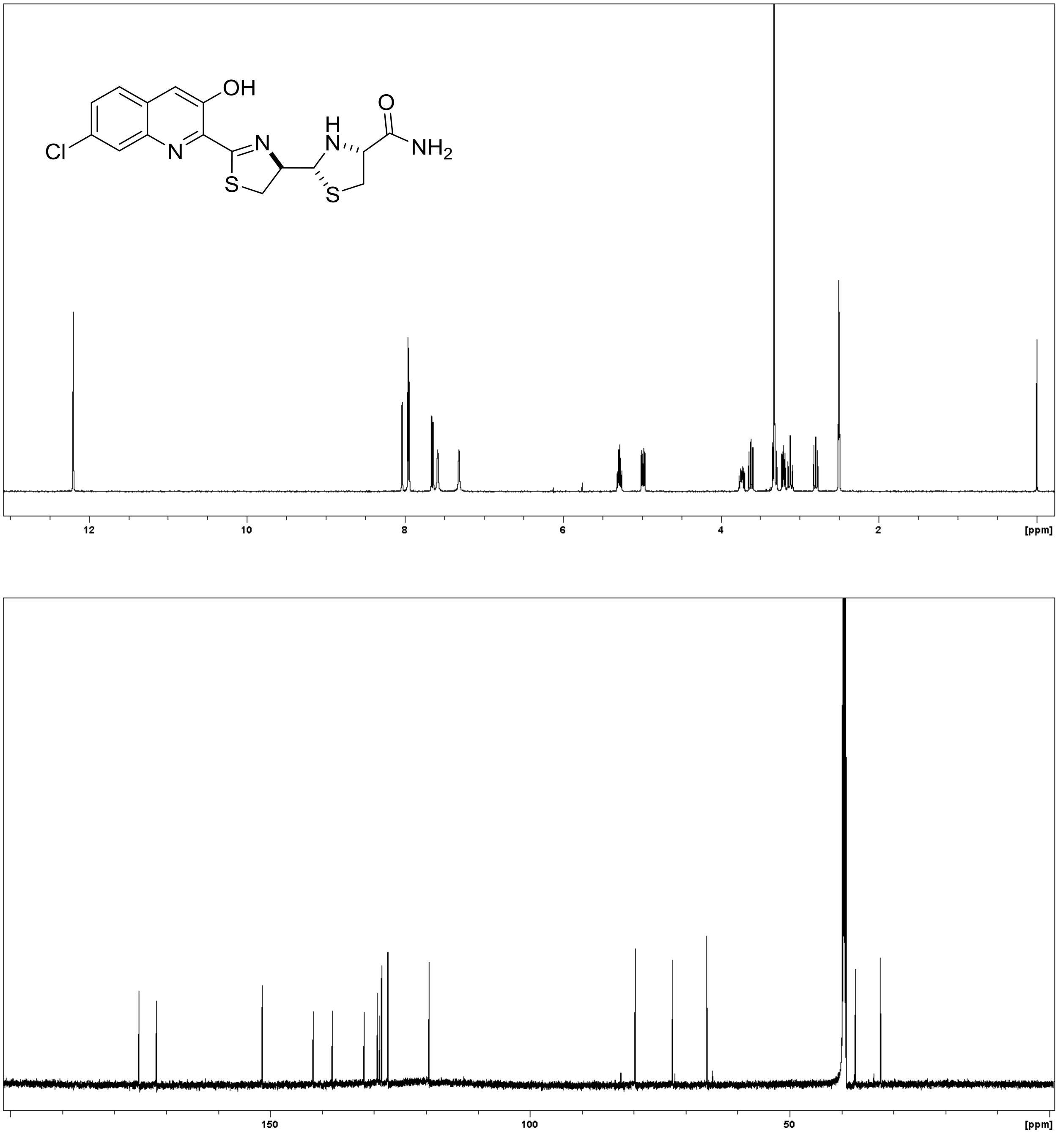


Figure S9. ^{1}H NMR (700 MHz) and ^{13}C (175 MHz) spectra of grisechelin B (**5**) in $CDCl_3$.

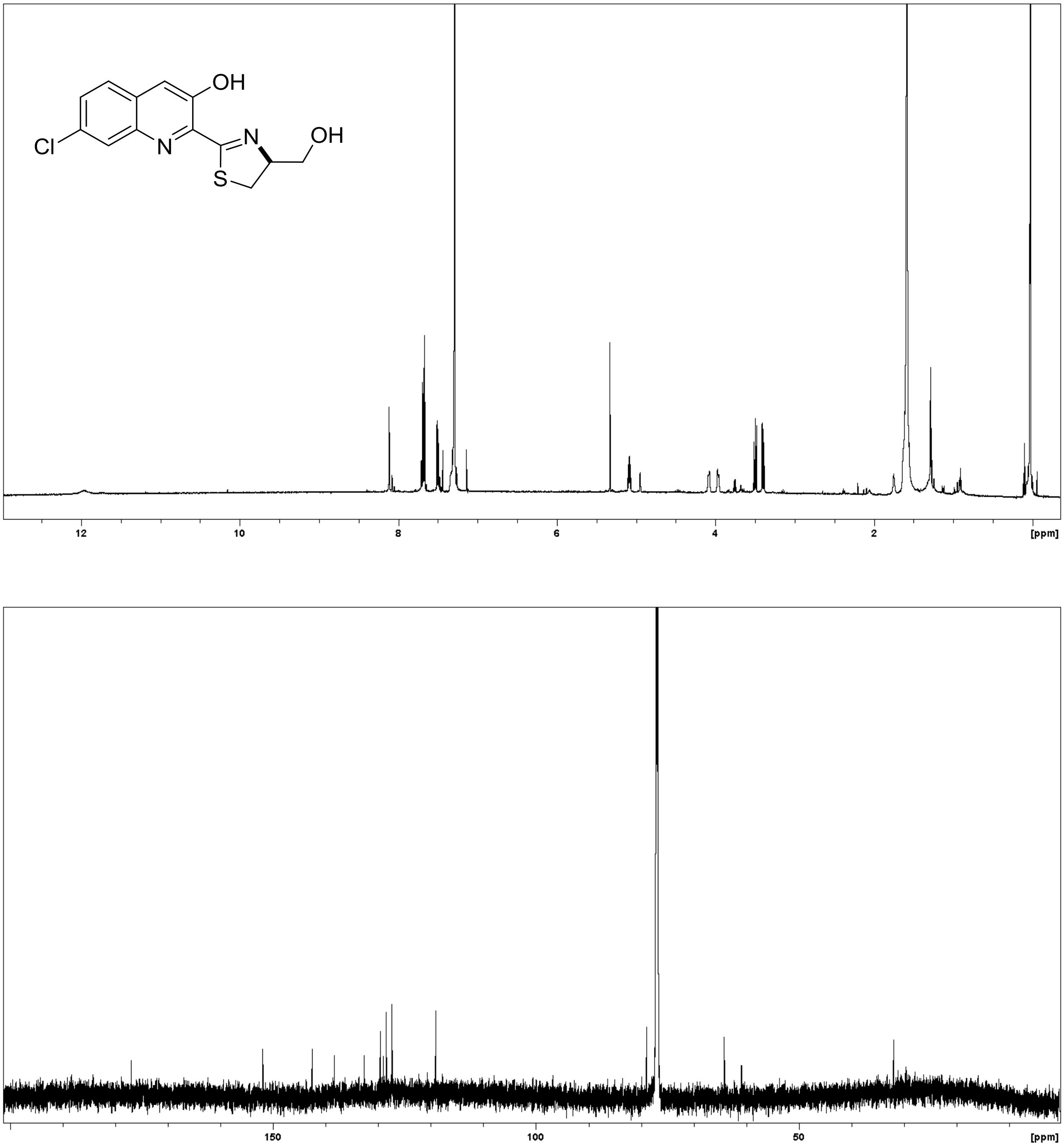


Figure S10. ^{1}H NMR (700 MHz) and ^{13}C (175 MHz) spectra of grisechelin C (**6**) in $CDCl_3$.

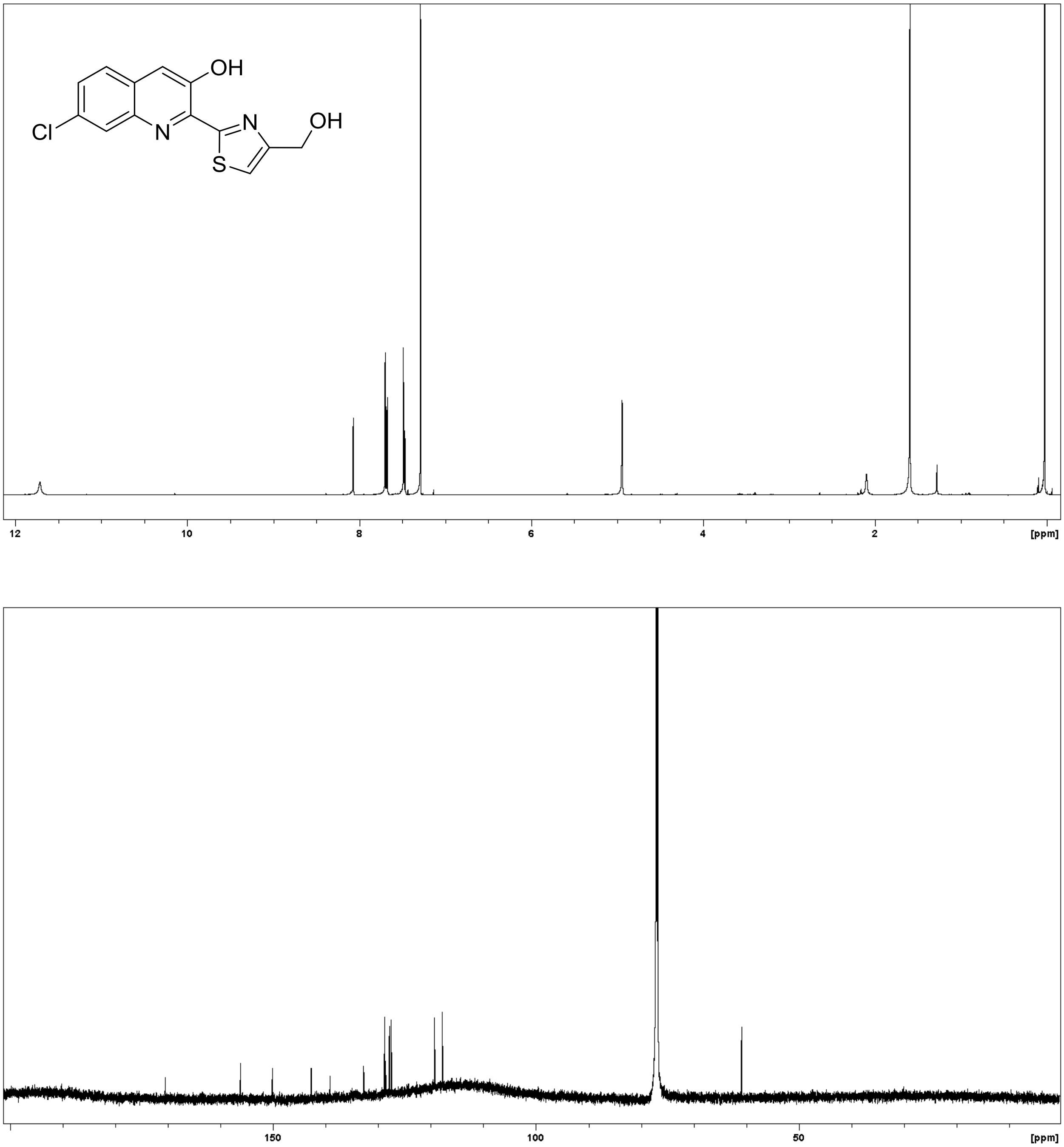


Figure S11. ^{1}H NMR (700 MHz) and ^{13}C (175 MHz) spectra of grisechelin D (**7**) in d_6-DMSO.

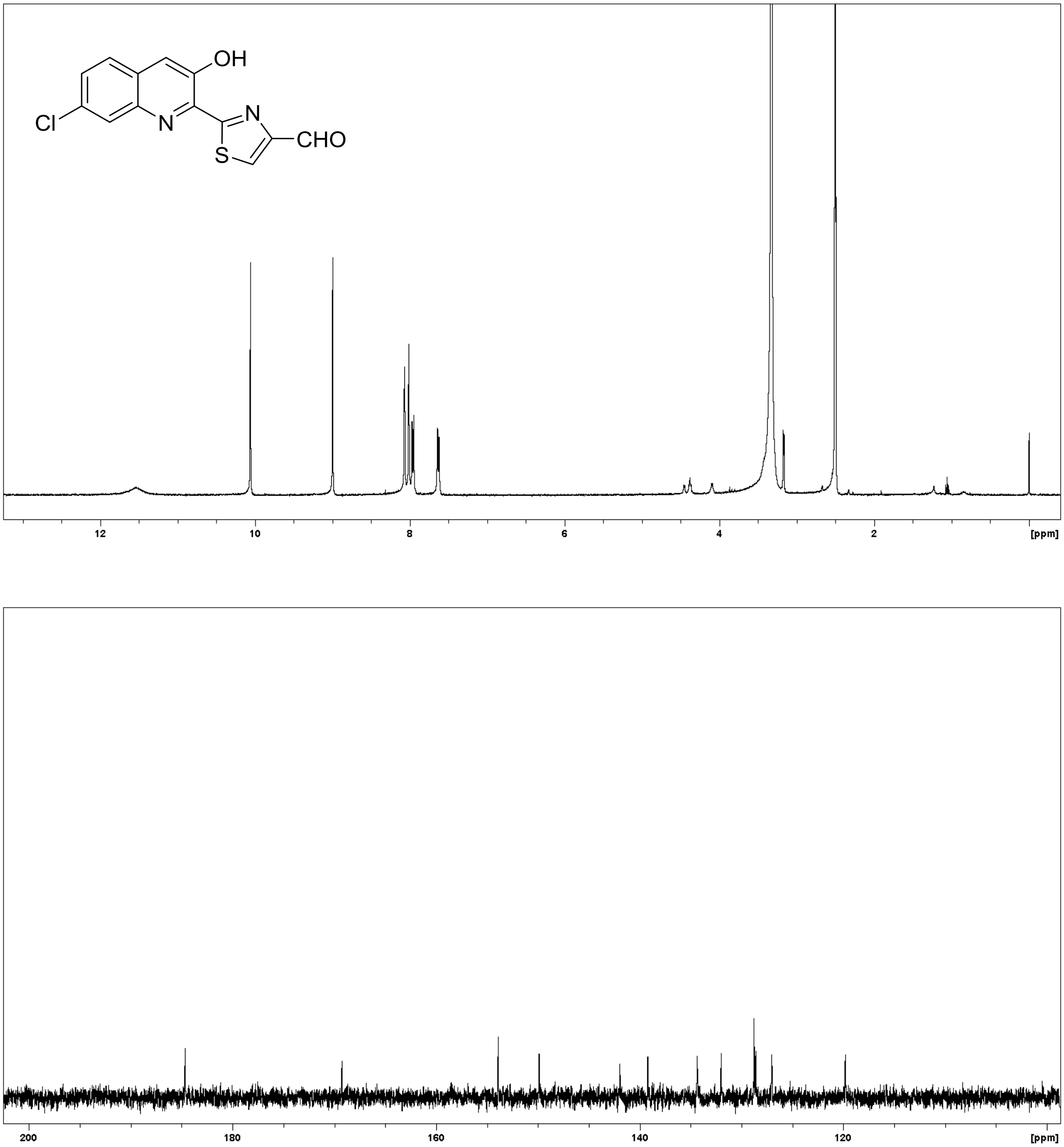


Figure S12. ^{1}H NMR (700 MHz) and ^{13}C (175 MHz) spectra of seco-dinactin (**12**) in $CDCl_3$.

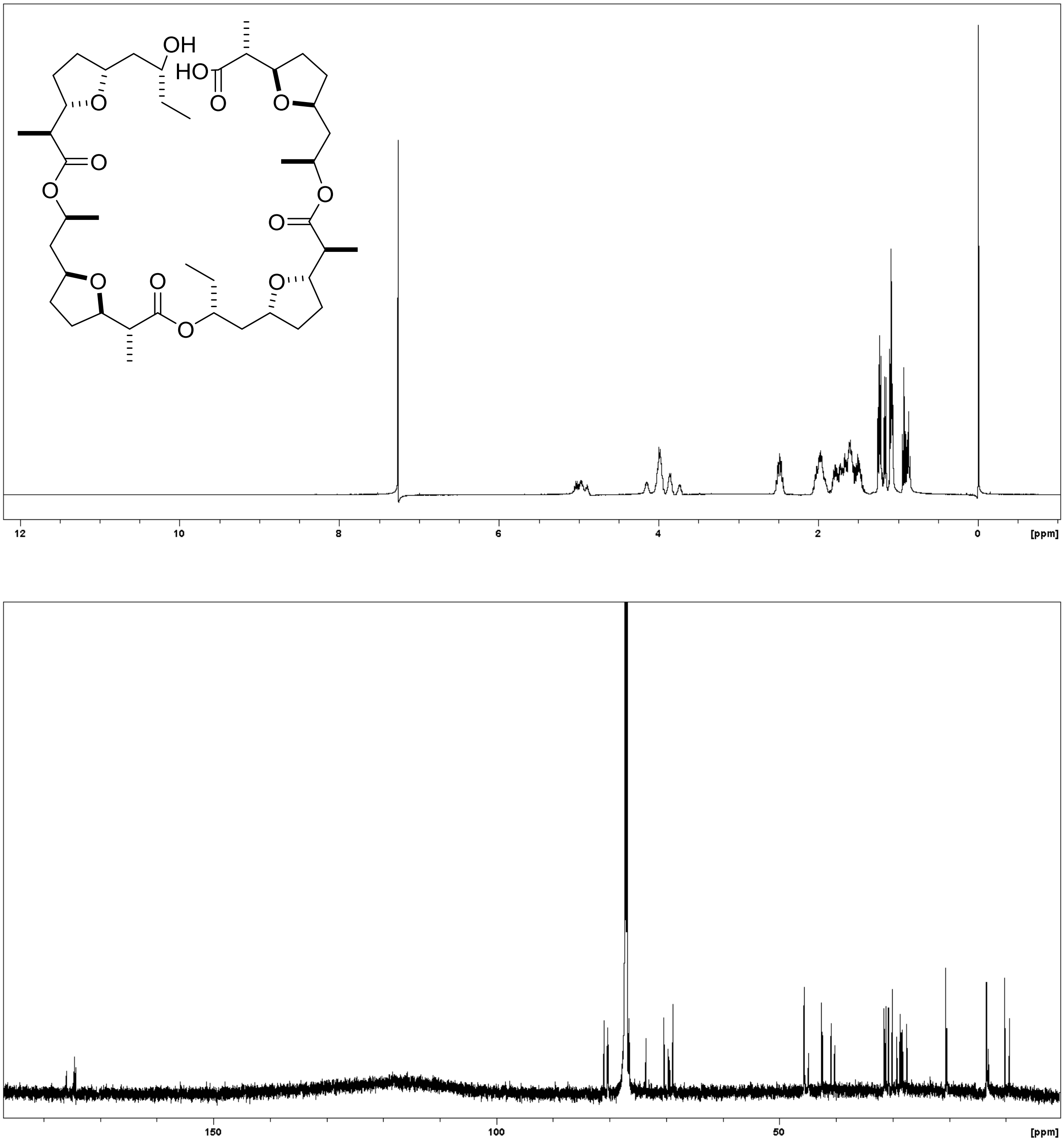
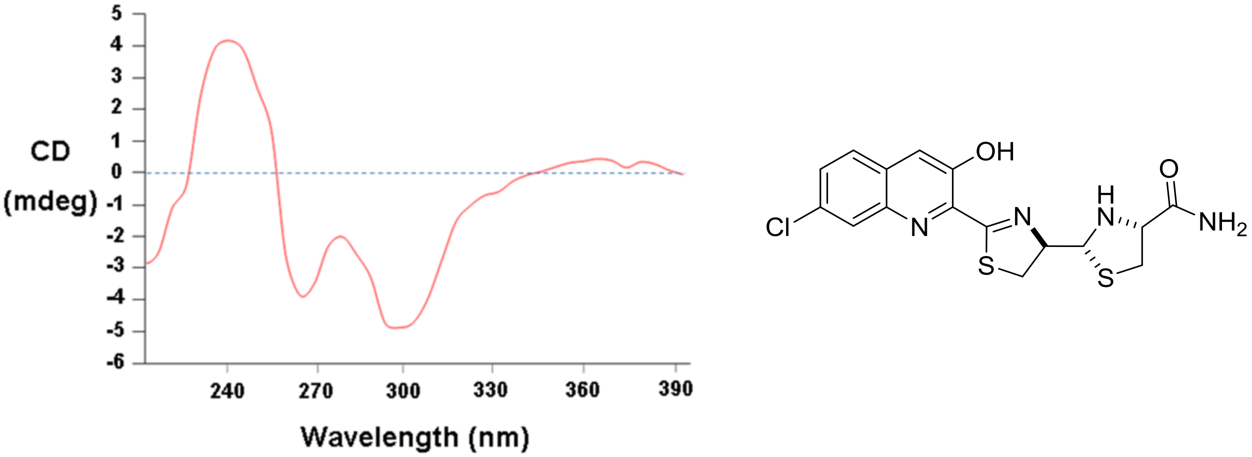


Figure S13. Circular dichroism spectrum of grisechelin A (**4**) (317 μM in $CHCl_3$).



References

1. Caffrey, P.; Bevitt, D. J.; Staunton, J.; Leadlay, P. F. *FEBS Lett.* **1992**, *304*, 225-228.
2. Motamedi, H.; Shafiee, A. *Eur. J. Biochem*. **1998**, *256*, 528-534.
3. Cundliffe, E.; Bate, N.; Butler, A.; Fish, S.; Gandecha, A.; Merson-Davies, L. *Antonie van Leeuwenhoek* **2001**, *79*, 229-234.
4. Dai, S.; Ouyang, Y.; Wang, G.; Li, X. *Curr. Microbiol*. **2011**, *63*, 68-74.
5. Ikeda, H.; Nonomiya, T.; Usami, M.; Ohta, T.; Omura, S. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 9509-9514.
6. Schwecke, T.; Aparicio, J. F.; Molnar, I.; Konio, A.; Khaw, L. E.; Haydock, S. F.; Oliynyk, M.; Caffrey, P.; Cortes, J.; Lester, J. B.; Bohm, G. A.; Staunton, J.; Leadlay, P. F. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 7839-7843.
7. Motamedi, H.; Hutchinson, C. R. *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 4445-4449.
8. Otten, S. L.; Stutzman-Engwall, K. J.; Hutchinson, C. R. *J. Bacteriol*. **1990**, *172*, 3427-3434.
9. Okamoto, S.; Taguchi, T.; Ochi, K.; Ichinose, K. *Chem. Biol*. **2009**, *16*, 226-236.
10. Wendt-Pienkowski, E.; Huang, Y.; Zhang, J.; Li, B.; Jiang, H.; Kwon, H.; Hutchinson, C. R.; Shen, B. *J. Am. Chem. Soc*. **2005**, *127*, 16442-16452.
11. Wageningen, A. A.; Kirkpatrick, P. N.; Williams, D. H.; Harris, B. R.; Kershaw, J. K.; Lennard, N. J.; Jones, M.; Jones, S. J. M.; Solenberg, P. J. *Chem. Biol.* **1998**, *5*, 155-162.
12. Du, L.; Sanchez, C.; Chen, M.; Edwards, D. J.; Shen, B. *Chem. Biol*. **2000**, *7*, 623-642.
13. Tao, M.; Wang, L.; Wendt-Pienkowski, E.; George, N. P.; Galm, U.; Zhang, G.; Coughlin, J. M.; Shen, B. *Mol. BioSyst*. **2007**, *3*, 60-74.
14. Galm, U.; Wendt-Pienkowski, E.; Wang, L.; George, N. P.; Oh, T.; Yi, F.; Tao, M.; Coughlin, J. M.; Shen, B. *Mol. BioSyst*. **2009**, *5*, 77-90.
15. Smanski, M. J.; Peterson, R. M.; Shen, B. *Methods Enzymol*. **2012**, *515*, 163-186.
16. Adekambi, T.; Butler, R. W.; Hanrahan, F.; Delcher, A. L.; Drancourt, M.; Shinnick, T. M. *PLoS ONE* **2011**, *6*, e14792
17. Kawasaki, T; Kuzuyama, T.; Kuwamori, Y.; Matsuura, N.; Itoh, N.; Furihata, K.; Seto, H.; Dairi, T. J. *J. Antibiot.* **2004**, *57*, 739-747.
18. Guo, Y.; Zheng, W.; Rong, X.; Huang, Y. *Int. J. Syst. Evol. Microbiol.* **2008**, *58*, 149-159.
19. Labeda, D. P. *Int. J. Syst. Evol. Microbiol.* **2011**, *61*, 2525-2531.
20. Tamura, K.; Nei, M. *Mol. Biol. Evol.* **1993**, *10*, 512-526.
21. Felsenstein, J. *Evol.* **1985**, *39*, 783-791.
22. Tamura, K.; Peterson, D.; Peterson, N.; Stecher, G.; Masatoshi, N.; Sudhir, K. *Mol. Biol. Evol.* **2011**, *28*, 2731-2739.
23. Kieser, T.; Bibb, M. J.; Buttner, M. J.; Chater, K. F.; Hopwood, D. A. *Practical Streptomyces Genetics*; The John Innes Foundation: Norwich, UK, 2000.