

## Supporting information

# **Metabolic Blockade-Based Genome Mining of Sea Anemone-Associated *Streptomyces* sp. S1502 Identifies Atypical angucyclines WS-5995A-E: Isolation, Identification, Biosynthetic Investigation and Bioactivities**

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**Table S1.** Strains and plasmids used or constructed in this study

Strains or plasmids	Characterization	Reference or source
<b>Strains</b>		
<i>E. coli</i> BW25113/pIJ790	Host strain for homologous reorganization during PCR-targeting process	<sup>1</sup>
<i>E. coli</i> DH5 $\alpha$ /pIJ773	Host strain of plasmid pIJ773	<sup>1</sup>
<i>E. coli</i> ET12567/pUZ8002	Donor strain for conjugation with <i>Streptomyces</i>	<sup>1</sup>
<i>E. coli</i> ET12567/pUB307	Donor strain for conjugation with <i>Streptomyces</i>	<sup>1</sup>
<i>Streptomyces</i> sp. S1502	The wide-type strain, producer of streptopyrroles	This study
$\Delta stp1$	<i>stp1</i> gene deletion mutant	This study
$\Delta stp1/\Delta wsmA$	<i>wsmA</i> gene in-frame deletion mutant	This study
$\Delta wsmO/P/Q/R/O_3/X/Y/R_1/R_2/R_3/R_4/XY$	$\Delta wsmO/P/Q/R/O_3/X/Y/R_1/R_2/R_3/R_4/XY$ gene deletion mutant	This study
<i>S. atratus</i> SCSIO ZH16NSEP	Derived from <i>S. atratus</i> SCSIO ZH16; host strain for heterologous expression	This study
<i>S. lividans</i> SBT5	Host strain for heterologous expression	This study
<b>Plasmids</b>		
SuperCosI	Amp <sup>r</sup> , Kan <sup>r</sup> , cosmid vector	
pIJ790	Cm <sup>r</sup> , including $\lambda$ -RED ( <i>gam</i> , <i>bet</i> , <i>exo</i> ) for PCR-targeting	<sup>1</sup>
pIJ773	Apr <sup>r</sup> , source of <i>acc(3)IV</i> and <i>oriT</i> fragment	<sup>1</sup>
pUZ8002	Kan <sup>r</sup> , including <i>tra</i> for conjugation	<sup>1</sup>
pUB307	Apr <sup>r</sup> , including <i>tra</i> for conjugation	<sup>2</sup>
Cosmid 10-11F	Amp <sup>r</sup> , Kan <sup>r</sup> , a cosmid which contains partial streptopyrrole biosynthetic gene cluster	This study
Cosmid 13-4D; 13-5D	Amp <sup>r</sup> , Kan <sup>r</sup> , a cosmid which contains $\Delta ilaMN$ deleted partial WS-5995 biosynthetic gene cluster	This study
pBAC/1-9A	a cosmid which contains intact <i>wsm</i> biosynthesis cluster	This study

Abbreviations: Amp<sup>r</sup>, ampicillin resistance; Kan<sup>r</sup>, kanamycin resistance; Apr<sup>r</sup>, apramycin

resistance, Cm<sup>r</sup>, chloramphenicol resistance

**Table S2.** Primers used in this study

Name	Sequence (5'-3')	Purpose
For screening the cosmid library and BAC library		
Orf-wsmAa-scF	CGGATCTACCTCGGTGCGG	For screening the cosmid library and BAC library; and for verifying right exconjugants
Orf-wsmAa-scR	GGTAGATGTTGCCGTGGGCC	
Orf-wsmAb-scF	GGGGCTCGAGGTTCTCGCAC	
Orf-wsmAb-scR	CCGGCTCGGTCAGCACCATC	
Orf-wsmAc-scF	GAGTTCACCGGGCTGGAGGC	
Orf-wsmAc-scR	GGTTGACGGCGTCCAGGATG	
Orf-wsmAd-scF	GCTAGGGCTCATCACGTTCG	
Orf-wsmAd-scR	CGGCTGTACGGTGA CTTCGG	
Orf-wsmAe-scF	GCATCGAGCGGGACCTGCAC	
Orf-wsmAd-scR	CCTGAGGGGCTGTGGACGTG	
For gene replacement		
Del_stp1-F	CCGGACGACCGGCTCTCCAACCACGCGCCGT	For in-frame disrupting <i>stp1</i>
	TCAACTTCactagtATTCCGGGGATCCGTCGACC	
Del_ stp1-R	GACGCAGACGGA A C C C G G T C G A C G A T C A T G	
	TAGGTGGGactagtTGTAGGCTGGAGCTGCTTC	
Del_wsmA-F	GCCAAGAACTTCTGGAGCCTGCTGAGTGAGG	For in-frame disrupting <i>wsmA</i>
	GACGCACGactagtATTCCGGGGATCCGTCGACC	
Del_wsmA-R	GCTGCCGACGGTGAGTACCGTGTCGGTGAGCT	
	GGTCGCGactagtTGTAGGCTGGAGCTGCTTC	
del_wsmX-F	GCCTGGTACACCGTCGGCGAGCACC ACTTC	For disrupting <i>wsmX</i> and <i>wsmXY</i>
	GGTGAGCGGATTCCGGGGATCCGTCGACC	
del_wsmX-R	CAGGTGCAGGTCGTGGCCCCACAGCTCGTG	
	CAGGCGCCCTGTAGGCTGGAGCTGCTTC	
del_wsmY-F	GTCTGGGTCGACGTCGACACGGTCCTGTCC	For disrupting <i>wsmY</i> and <i>wsmXY</i>
	GCCCCGCCCATTCGGGGATCCGTCGACC	

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del_ <i>wsmY</i> -R	GCAGGCGGTGACTCCGCTGCCGCAGTAGAC GACGACGTCTGTAGGCTGGAGCTGCTTC	
del_ <i>wsmO</i> <sub>3</sub> -F	GCGGTGATCGCCGCGTCCACCACGCGTGTA CGGGTCTTCATTCCGGGGATCCGTCGACC	For disrupting <i>wsmO</i> <sub>3</sub>
del_ <i>wsmO</i> <sub>3</sub> -R	CAGGCTCAGCCGCTGGATCTCCGCTCGCGC GTCCTGTAGGCTGGAGCTGCTTC	
del_ <i>wsmR</i> <sub>1</sub> -F	CTGCCGTCGTACCTCGCCGGGCACGTGGCG CGCATCGGCATTCCGGGGATCCGTCGACC	For disrupting
del_ <i>wsmR</i> <sub>1</sub> -R	CCGTTCCCTCGGGGGTGAAGCAGCCGAGGA AGGAGTCCTGTGTAGGCTGGAGCTGCTTC	<i>wsmR</i> <sub>1</sub>
del_ <i>wsmR</i> <sub>2</sub> -F	GACGGCCTCGAGGTGTGCAAGGCCATCCGC GCCGTCAGCATTCGGGGATCCGTCGACC	For disrupting
del_ <i>wsmR</i> <sub>2</sub> -R	GCGGAGGCTGCTGACGTGGGTGTCGACGGT CCGCCGGGATGTAGGCTGGAGCTGCTTC	<i>wsmR</i> <sub>2</sub>
del_ <i>wsmO</i> -F	CCCTTTCCCGGCAAGGTGGTGATCCGGTCG GTGGCGCTCATTCGGGGATCCGTCGACC	For disrupting
del_ <i>wsmO</i> -R	GGCGTAGTAGCCGTCGGGGCGGATCAGGAG CGCCTCGTCTGTAGGCTGGAGCTGCTTC	<i>wsmO</i>
del_ <i>wsmP</i> -F	GCGGACGTCGCGGACGACACGGCGAAGGC GGCCGCCGAGATTCCGGGGATCCGTCGACC	For disrupting
del_ <i>wsmP</i> -R	CTGGCTCACGTCCTGCGGAGTACCGAGGCG CGGGATCAGTGTAGGCTGGAGCTGCTTC	<i>wsmP</i>
del_ <i>wsmQ</i> -F	GCGGCCCTGCGCGGGCTGGTCGACGAGGA ATGGGCCCGGATTCCGGGGATCCGTCGACC	For disrupting
del_ <i>wsmQ</i> -R	CGAGGCACCGCCGGTCGAGCCCAGCGGCA CGAGCACCGCTGTAGGCTGGAGCTGCTTC	<i>wsmQ</i>
del_ <i>wsmR</i> -F	GGGGTCAACCCGGTCGACTGGAAACGCCGT TCGGGCCGCATTCCGGGGATCCGTCGACC	For disrupting <i>wsmR</i>

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del_ <i>wsmR</i> -R	CACGACCGCGCCCAGGATCGCCGGGTCGCG CACCGGCCCTGTAGGCTGGAGCTGCTTC	
del_ <i>wsmR</i> <sub>3</sub> -F	GCGAAACGCCGGGCCATCACCCAAGGCGC GCGTGCCGTCATTCCGGGGATCCGTCGACC	For disrupting
del_ <i>wsmR</i> <sub>3</sub> -R	GACGAAGTGGAGGGTGGCCCCGGGCGGGGC TGGAGATGTGTGTAGGCTGGAGCTGCTTC	<i>wsmR</i> <sub>3</sub>
del- <i>wsmR</i> <sub>4</sub> -F	GACAAGGGCTACGACGCCATGACCGTCGGC GACATCGCCATTCCGGGGATCCGTCGACC	For disrupting
del- <i>wsmR</i> <sub>4</sub> -R	GGCGTGGTAGAAGGTCCGTTCGATCATCCA GCACAGGGCTGTAGGCTGGAGCTGCTTC	<i>wsmR</i> <sub>4</sub>
<b>For verifying the mutants</b>		
ID_stp1-F	GAACCTCCGACATCCAGCGC	For verifying the
ID_stp1-R	TGACCAGCCCACGCCTG	disruption of $\Delta$ <i>stp1</i>
ID-wsmX-F	GCTAGGGCTCATCACGTTTCG	For verifying
ID-wsmX-R	GCGGCGGTCAGTTCGGTG	$\Delta$ <i>wsmX</i> and $\Delta$ <i>wsmXY</i>
ID-wsmY-F	GACGCCGGATGGGTGAGCG	For verifying
ID-wsmY-R	GGACAGGTCCGTGGACCAGG	$\Delta$ <i>wsmY</i> and $\Delta$ <i>wsmXY</i>
ID- <i>wsmR</i> <sub>4</sub> -F	GGCCACCACCCCGCACGAAG	For verifying
ID- <i>wsmR</i> <sub>4</sub> -R	GATGTGTTTCGCATGTCGCCG	$\Delta$ <i>wsmR</i> <sub>4</sub>
ID- <i>wsmO</i> <sub>3</sub> -F	CGTGCCGGACCACCCCTAC	For verifying
ID- <i>wsmO</i> <sub>3</sub> -R	CAGCACCTGGATGACGTGGTG	$\Delta$ <i>wsmO</i> <sub>3</sub>
ID- <i>wsmR</i> <sub>1</sub> -F	CGGTCCGCTGTTTCGGAGC	For verifying
ID- <i>wsmR</i> <sub>1</sub> -R	GTGGTCACTCGGCGCACG	$\Delta$ <i>wsmR</i> <sub>1</sub>
ID- <i>wsmR</i> <sub>2</sub> -F	GCGTGGACCTCGTCCTGATGG	For verifying
ID- <i>wsmR</i> <sub>2</sub> -R	GCCGCGTACGGTGACGACC	$\Delta$ <i>wsmR</i> <sub>2</sub>
ID- <i>wsmO</i> -F	CGAAGGGCATCAGCAACCTG	For verifying
ID- <i>wsmO</i> -R	GGGATGGTCTGCGTTCGGCC	$\Delta$ <i>wsmO</i>

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ID- <i>wsmP</i> -F	GTCGAACGGAACGCAGTCCC	For verifying
ID- <i>wsmP</i> -R	GGCCGAACGCAGACCATCCC	$\Delta wsmP$
ID- <i>wsmQ</i> -F	CGGATACGGAGGGTCTGCAC	For verifying
ID- <i>wsmQ</i> -R	CACGGGGCTTGTGGAAGGTC	$\Delta wsmQ$
ID- <i>wsmR</i> -F	GCCAGACAGAGACGTCGAGG	For verifying
ID- <i>wsmR</i> -R	GCCCGCTCGAACGGGATCAC	$\Delta wsmR$
ID- <i>wsmR</i> <sub>3</sub> -F	GCTGCATGGCAATTCGGCCG	For verifying
ID- <i>wsmR</i> <sub>3</sub> -R	GGAGCGCGGCTGGTGTAGG	$\Delta wsmR_4$
ID- <i>wsmR</i> <sub>4</sub> -F	GGCCACCACCCCGCACGAAG	For verifying
ID- <i>wsmR</i> <sub>4</sub> -R	GATGTGTTTCGCATGTCGCCG	$\Delta wsmR_{44}$

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**Table S3.** Media used in this study

Medium name	Ingredient
ISP-4	1% soluble starch, 0.1% bacteria peptone, 0.05% yeast extract, 0.1% K <sub>2</sub> HPO <sub>4</sub> , 0.1% MgSO <sub>4</sub> ·7H <sub>2</sub> O, 0.2% (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , 0.1% NaCl, 0.01% trace element solution, 3% artificial sea salt, 2% CaCO <sub>3</sub> , pH 7.2–7.4
MS	2% mannitol, 2% soybean powder, 1.5% agar, 3% artificial sea salt, and 0.2% CaCO <sub>3</sub> , pH 7.2–7.4
RA	2% soluble starch, 0.5% corn flour, 1% malt extract, 1% glucose, 1% maltose, 0.01% trace elements, 3% artificial sea salt, and 0.2% CaCO <sub>3</sub> , pH 7.2–7.4 (3% XAD-16 resins)
LB	0.5% yeast extract, 1% Tryptone, 1% NaCl, pH 7.2–7.4



**Table S4.** antiSMASH prediction of biosynthetic gene clusters in *Streptomyces* sp. S1502.

BGC	BGC type	Compounds	Similarity (%)
1	T1PKS	limazepine	11
2	NRPS	coelibactin	100
3	indole	7-prenylisatin	33
4	terpene	isorenieratene	62
5	NRPS	paenibactin	83
6	T3PKS	germicidin	100
7	T1PKS	sceliphrolactam	56
8	T3PKS	herboxidiene	8
9	ectoine	ectoine	100
10	melanin	melanin	60
11	T1PKS	pyrrolomycin	37
12	siderophore	desferrioxamin	83
13	RRE-containing	naphthomycin A	9
14	terpene	albaflavenone	100
15	T2PKS	spore pigment	66
16	siderophore	-	-
17	T2PKS; NRPS	WS-5995/friulimicin	69/75
18	Ripp-like	-	-
19	terpene	geosmin	100
20	siderophore	-	-
21	NRPS; T1PKS	$\alpha$ -lipomycin	100
22	PKS	leinamycin	15
23	lanthipeptide-class-iii	SapB	100
24	terpene	hopene	100
25	Ripp-like	informatipeptin	42
26	NRPS	coelichelin	100
27	ranthipeptide	-	-

**Table S5.** Deduced functions of ORFs of *wsm* gene cluster in *Streptomyces* sp. SCSIO S1502.

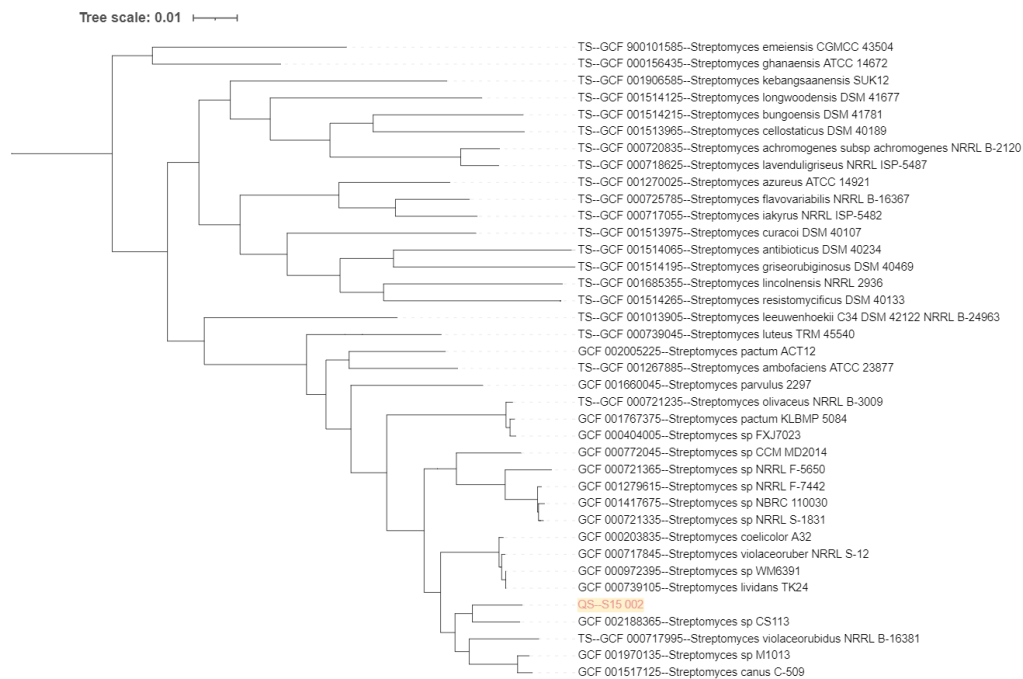
ORF	size <sup>a</sup>	Proposed function	Protein homologue and origin	ID/SI <sup>b</sup>
<i>wsmR<sub>4</sub></i>	202	TetR family transcriptional regulator	ARP51756.1 ( <i>Streptomyces</i> sp. SCSIO 03032)	39/67
<i>wsmO<sub>1</sub></i>	280	KR; short-chain dehydrogenase/reductase SDR	TtmK ( <i>Streptomyces afghaniensis</i> )	42/53
<i>wsmU</i>	107	Polyketide synthesis cyclase	WsdU ( <i>Streptomyces yanglinensis</i> CGMCC 4.2023)	77/83
<i>wsmA</i>	421	Beta-ketoacyl synthase	WsdA ( <i>Streptomyces yanglinensis</i> CGMCC 4.2023)	84/89
<i>wsmB</i>	405	Beta-ketoacyl synthase/CLF	WsdB ( <i>Streptomyces yanglinensis</i> CGMCC 4.2023)	74/82
<i>wsmC</i>	87	ACP	WsdC ( <i>Streptomyces yanglinensis</i> CGMCC 4.2023)	65/75
<i>wsmD</i>	260	KR; C9	WsdD ( <i>Streptomyces yanglinensis</i> CGMCC 4.2023)	80/86
<i>wsmE</i>	313	CYC C7-C12	WsdE ( <i>Streptomyces yanglinensis</i> CGMCC 4.2023)	67/76
<i>wsmF</i>	442	monooxygenase FAD-binding	WsdF ( <i>Streptomyces yanglinensis</i> CGMCC 4.2023)	55/62
<i>wsmI</i>	227	putative_anthrone_monooxygenase	WsdI ( <i>Streptomyces yanglinensis</i> CGMCC 4.2023)/JadG/GilOII	69/81 (71/85)
<i>wsmO<sub>2</sub></i>	494	monooxygenase FAD-binding	WsdT ( <i>Streptomyces yanglinensis</i> CGMCC 4.2023)	59/69
<i>wsmG</i>	148	nuclear transport factor 2 family protein	WsdG ( <i>Streptomyces yanglinensis</i> CGMCC 4.2023)	40/53
<i>wsmH</i>	338	O-methyltransferase	WsdH ( <i>Streptomyces yanglinensis</i> CGMCC 4.2023)	65/76
<i>wsmJ</i>	302	oxidoreductase	WsdJ ( <i>Streptomyces yanglinensis</i> )	60/73

			CGMCC 4.2023)	
<i>wsmK</i>	531	extracellular solute-binding protein family 5	WsdK ( <i>Streptomyces yanglinensis</i> CGMCC 4.2023)	37/50
<i>wsmL</i>	317	binding-protein-dependent transport systems	WsdL ( <i>Streptomyces yanglinensis</i> CGMCC 4.2023)	44/60
<i>wsmM</i>	288	binding-protein-dependent transport systems	WsdM ( <i>Streptomyces yanglinensis</i> CGMCC 4.2023)	41/58
<i>wsmN</i>	611	ABC transporter ATP-binding protein	WsdN ( <i>Streptomyces yanglinensis</i> CGMCC 4.2023)	54/66
<i>wsmX</i>	347	luciferase family protein/LLM class flavin-dependent oxidoreductase	OvmZ ( <i>Streptomyces olivaceus</i> SCSIO T05)	41/51
<i>wsmY</i>	268	putative rhodanese domain- containing protein	AWR88429.1 ( <i>Streptomyces</i> <i>filamentosus</i> )	51/61
<i>wsmO<sub>3</sub></i>	538	oxidoreductase	HrbZ1 ( <i>Streptomyces</i> sp. 2238- SVT4)	41/52
<i>wsmZ</i>	480	Drug resistance transporter, EmrB/QacA	BAB69179.1 ( <i>Streptomyces</i> <i>avermitilis</i> )	42/60
<i>wsmR<sub>1</sub></i>	161	transcriptional regulator, MarR family	StfRIII ( <i>Streptomyces</i> <i>steffisburgensis</i> )	39/50
<i>wsmR<sub>2</sub></i>	249	response regulator	WP_237282926.1 ( <i>Streptomyces</i> sp. PKU-MA00045)	71/82
<i>wsmO</i>	476	monooxygenase FAD-binding	WsdO ( <i>Streptomyces yanglinensis</i> CGMCC 4.2023)	77/83
<i>wsmP</i>	265	KR	WsdP ( <i>Streptomyces yanglinensis</i> CGMCC 4.2023)	84/88
<i>wsmQ</i>	362	pyridine nucleotide-disulfide oxidoreductase	WsdQ ( <i>Streptomyces yanglinensis</i> CGMCC 4.2023)	74/84
<i>wsmR</i>	300	crotonyl-CoA reductase / alcohol dehydrogenase	WsdR ( <i>Streptomyces yanglinensis</i> CGMCC 4.2023)	82/86

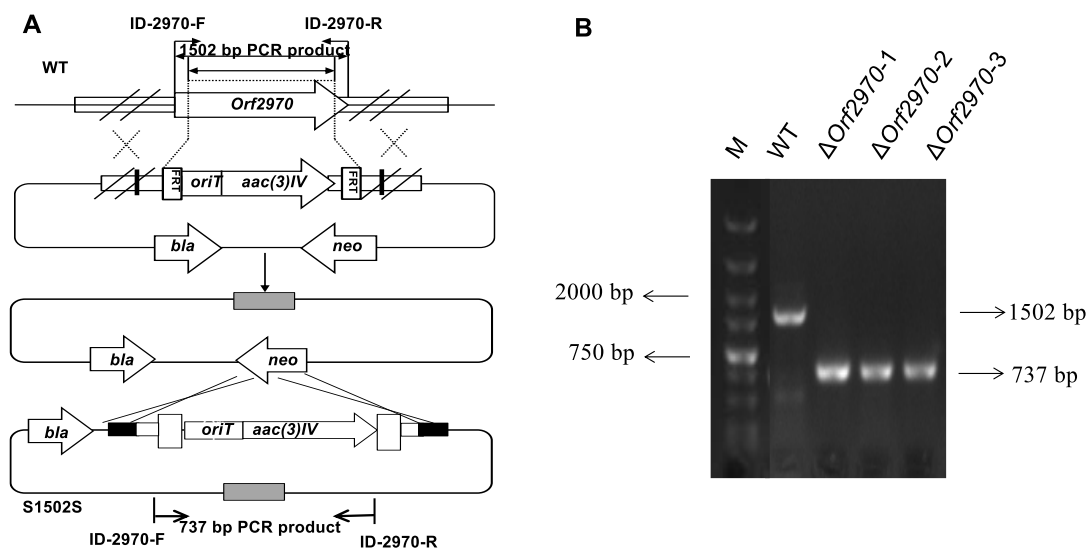
<i>wsmR</i> <sub>3</sub>	241	TetR family transcriptional regulator	BexR2 ( <i>Amycolatopsis orientalis</i> subsp. <i>vinearia</i> )	48/59
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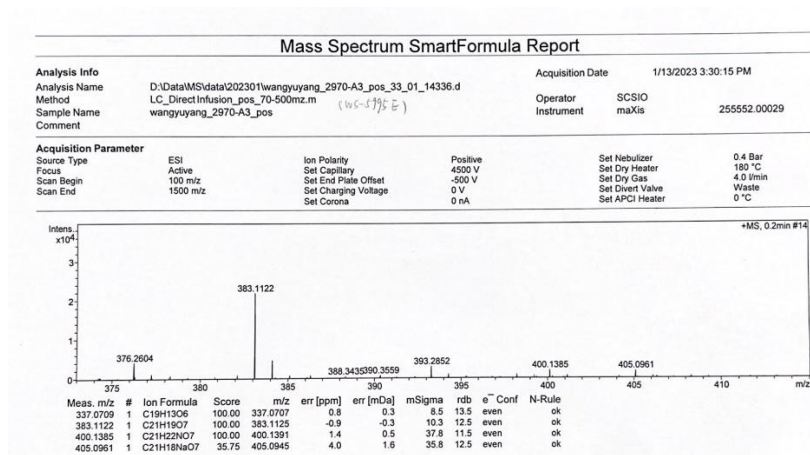
a: amino acid length; b: identity/similarity



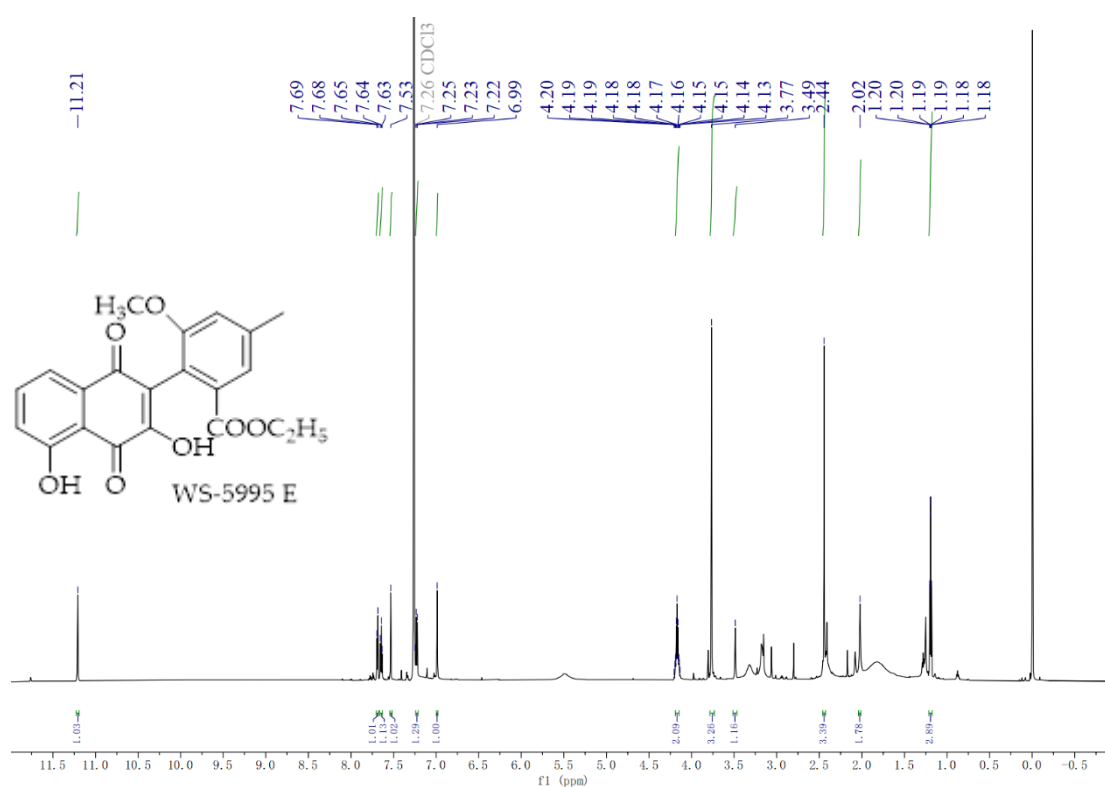
**Figure S1** Phylogenetic analysis of *Streptomyces* sp. S1502 using autoMLST



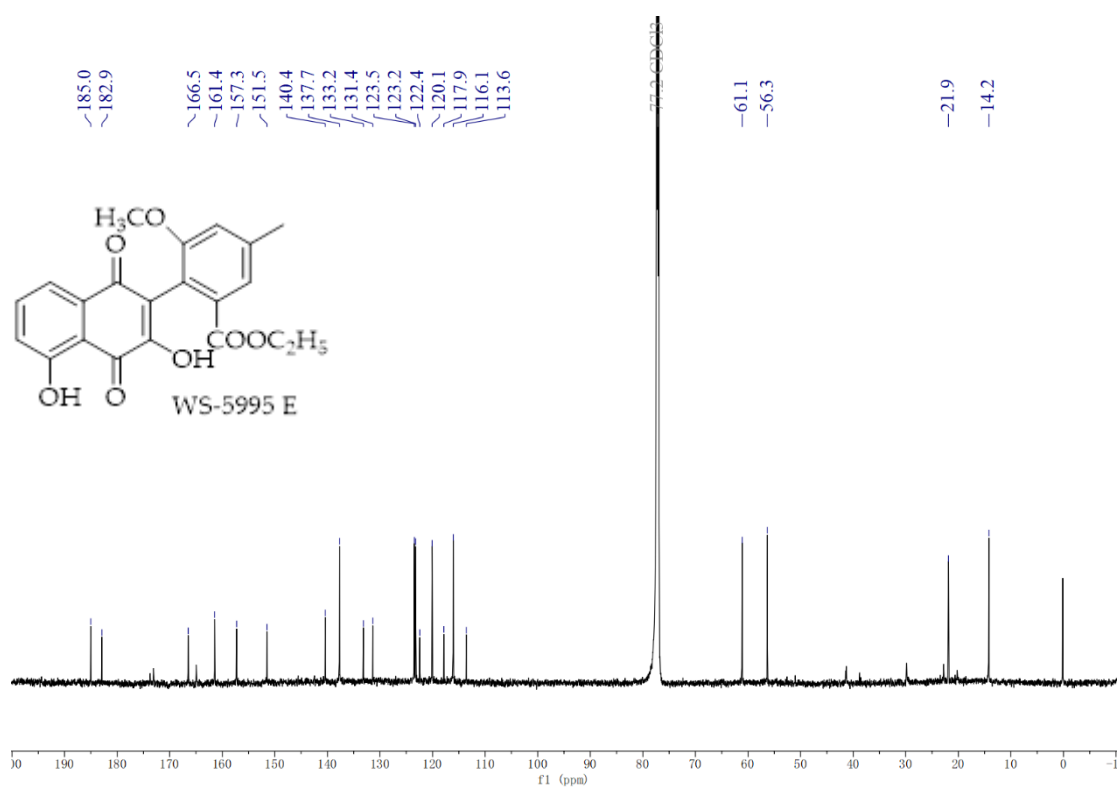
**Figure S2.** Disruption of *stp1* in wild type *S. sp.* S1502 via PCR-targeting. (A) Schematic representation for disruption of *stp1*. (B) PCR analyses of the WT strain and the *stp1* double-cross mutant carried out using the primers listed in Table S2. M: DNA molecular ladder; WT: using the genomic DNA of *S. sp.* S1502 as template;  $\Delta$ Orf2970-1-3: using the genomic DNA of *stp1* mutant as template.



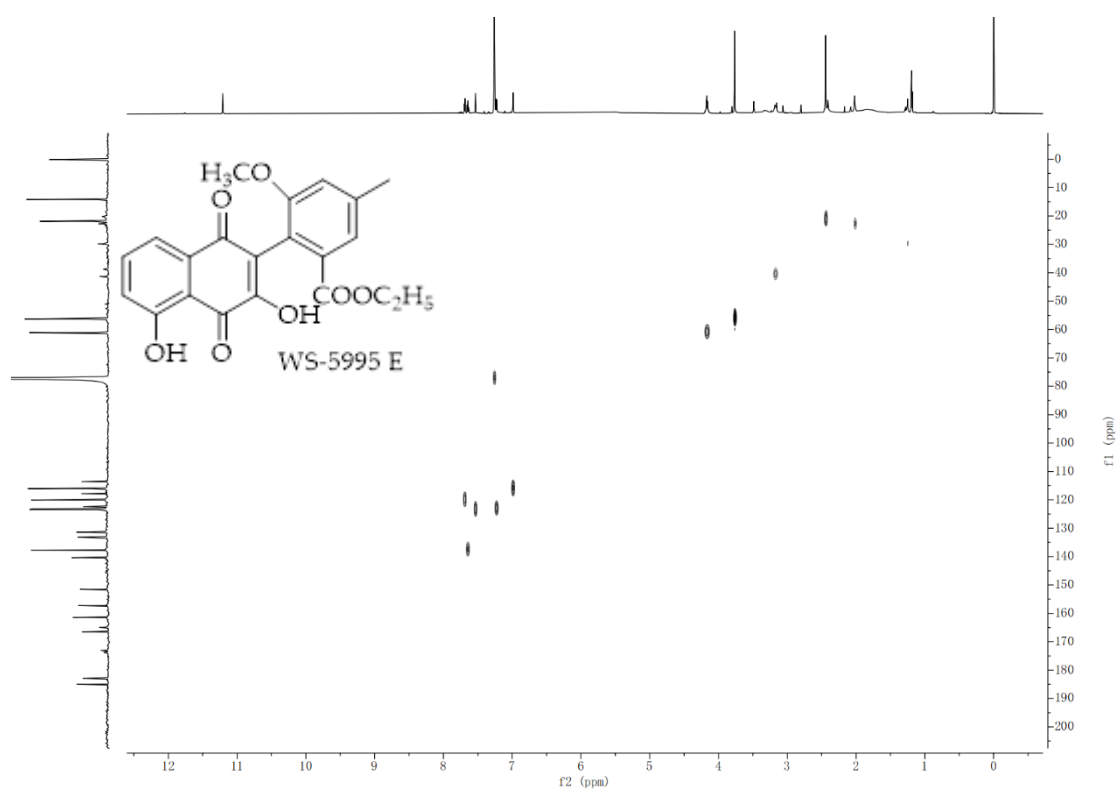
**Figure S3.** HE-ESI-MS spectrum of WS-5995 E (1)



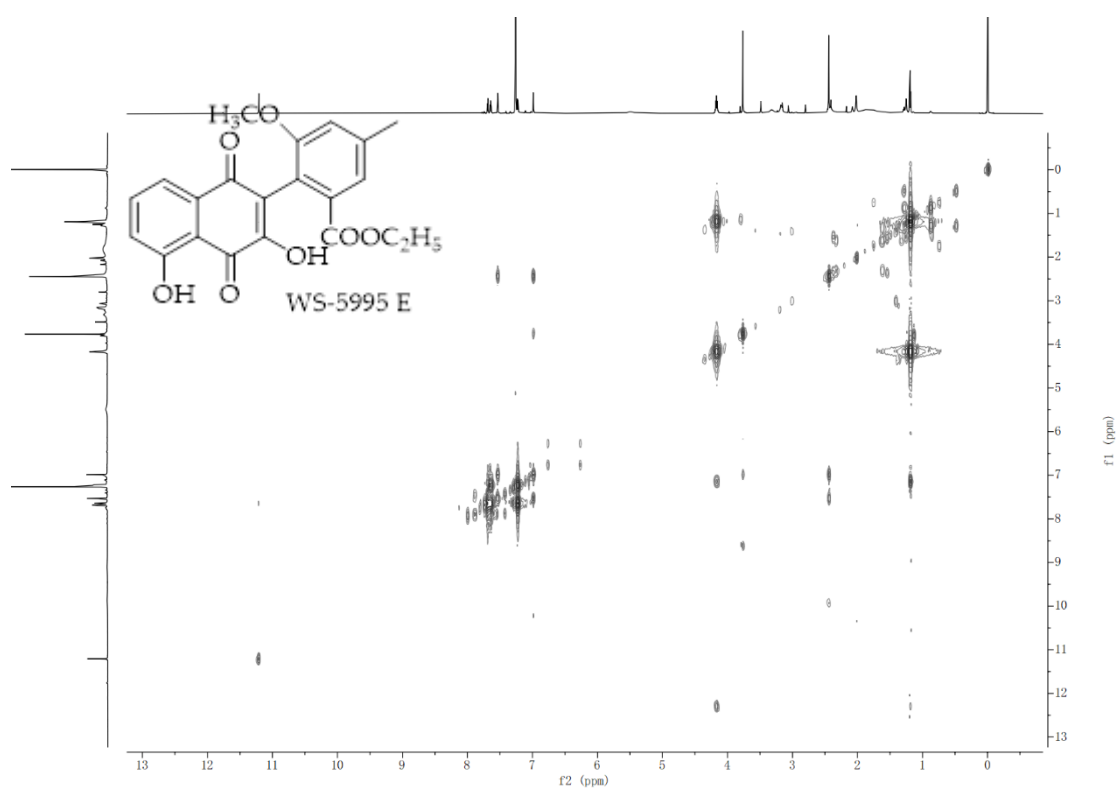
**Figure S4.** <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) spectrum of WS-5995 E (1).



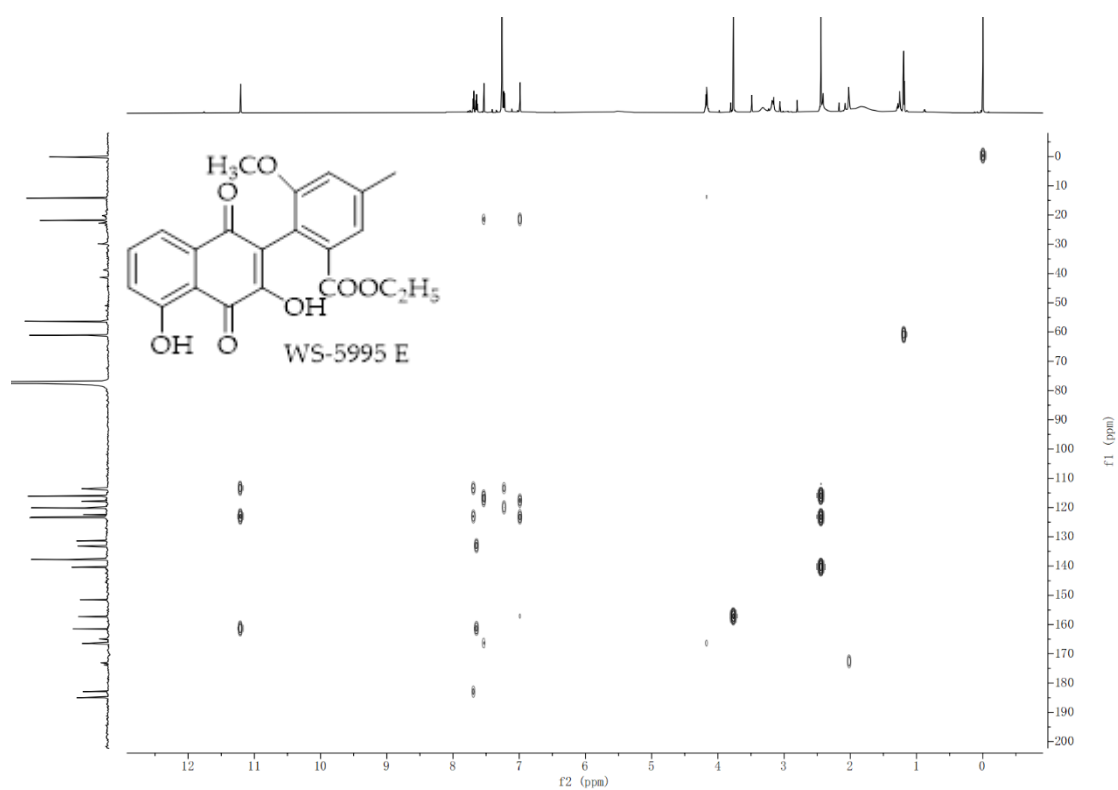
**Figure S5.** <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) spectrum of WS-5995 E (1).



**Figure S6.** HMBC (500 MHz, CDCl<sub>3</sub>) spectrum of WS-5995 E (1).

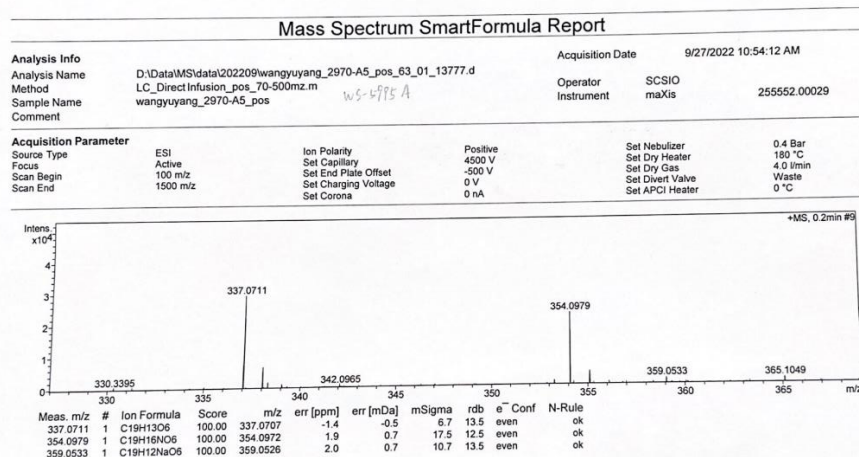


**Figure S7.** COSY (500 MHz, CDCl<sub>3</sub>) spectrum of WS-5995 E (1).

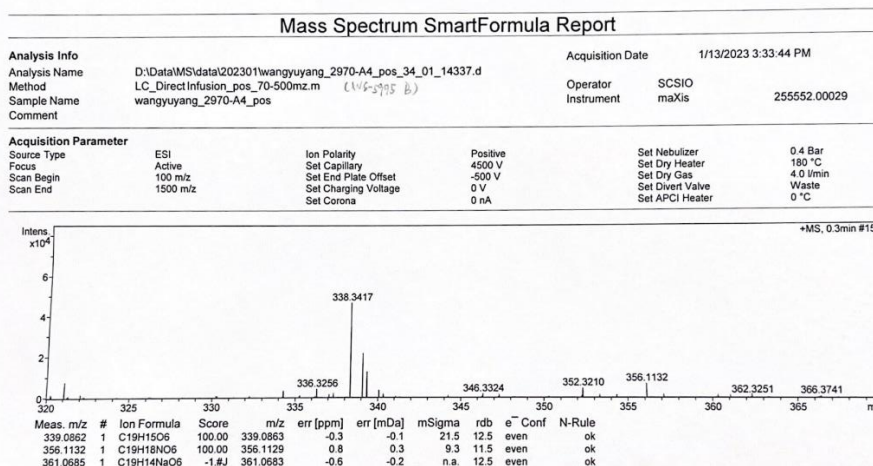


**Figure S8.** HSQC (500 MHz, CDCl<sub>3</sub>) spectrum of WS-5995 E (1).

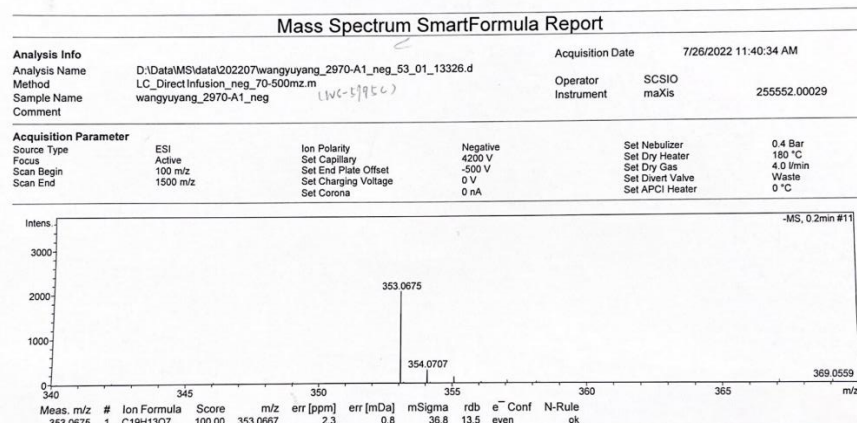




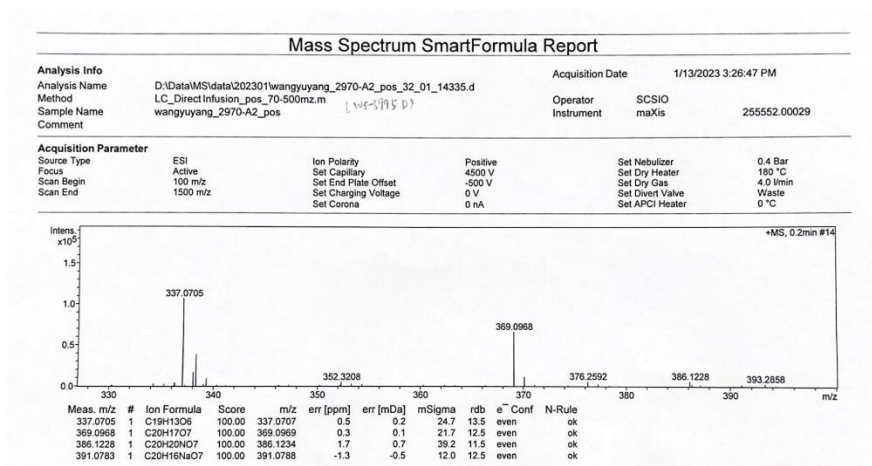
**Figure S9.** HE-ESI-MS spectrum of WS-5995 A (2)



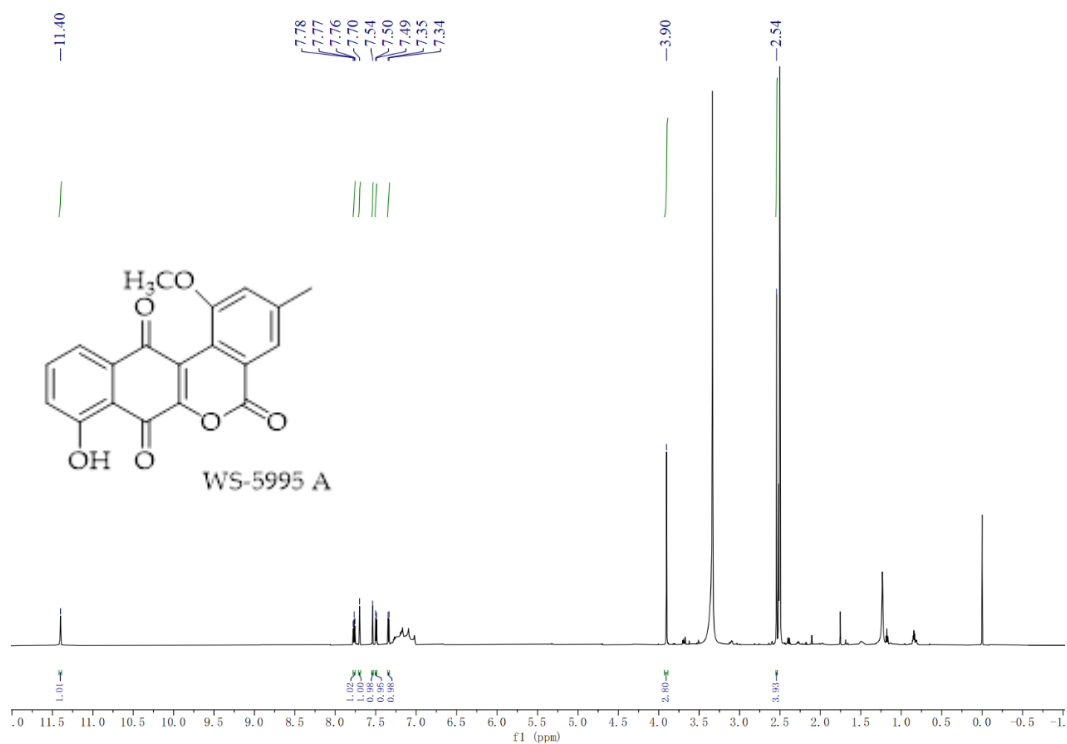
**Figure S10.** HE-ESI-MS spectrum of WS-5995 B (3)



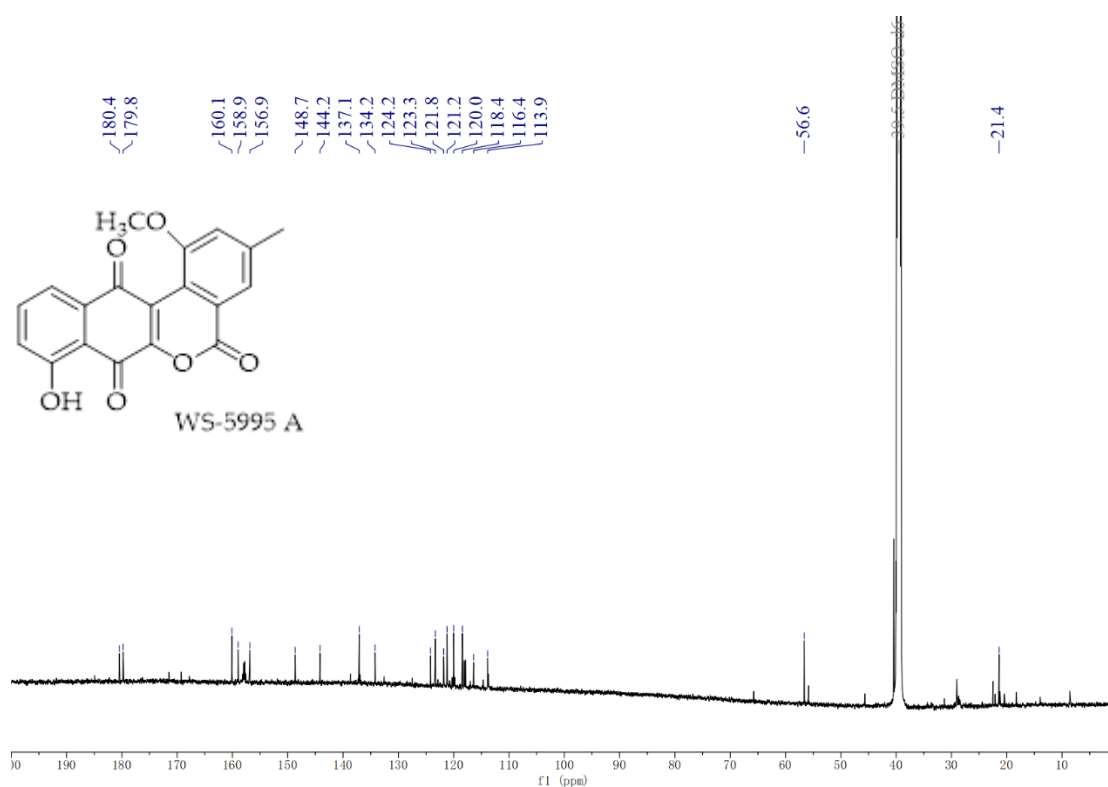
**Figure S11.** HE-ESI-MS spectrum of WS-5995 C (4)



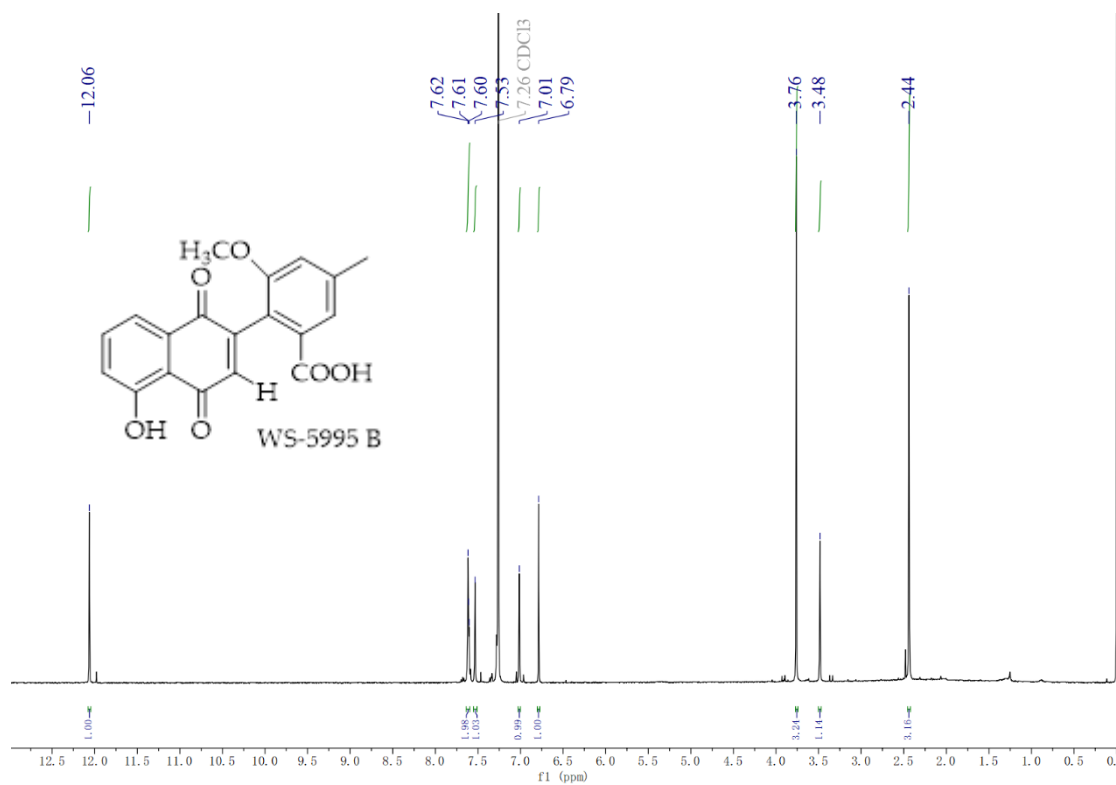
**Figure S12.** HE-ESI-MS spectrum of WS-5995 D (5)



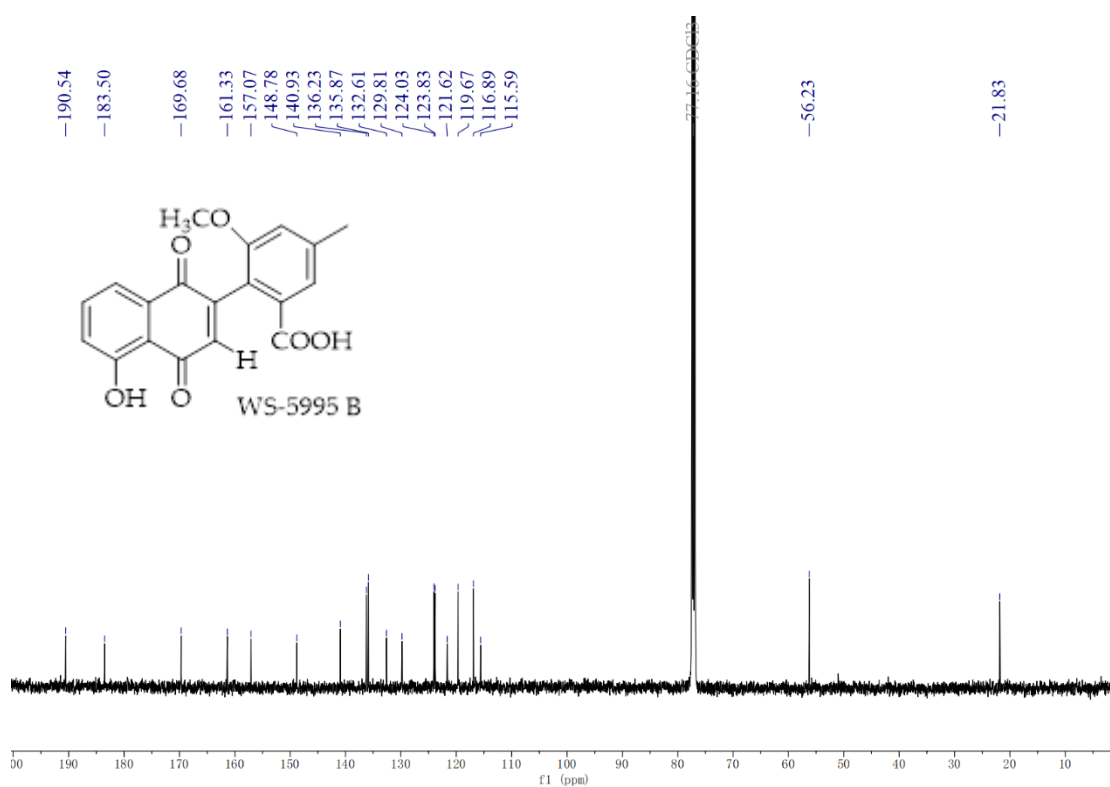
**Figure S13.** <sup>1</sup>H NMR (700 MHz, DMSO-*d*<sub>6</sub>) spectrum of WS-5995 A (2).



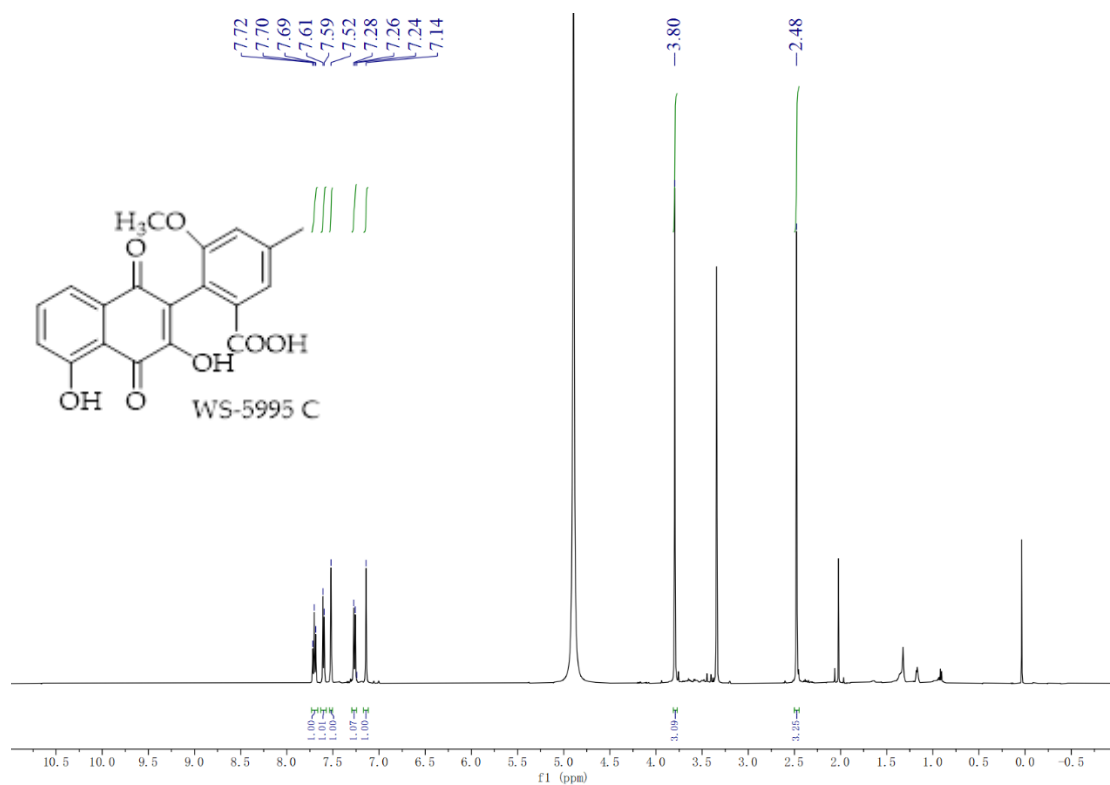
**Figure S14.** <sup>13</sup>C NMR (175 MHz, DMSO-*d*<sub>6</sub>) spectrum of WS-5995 A (2).



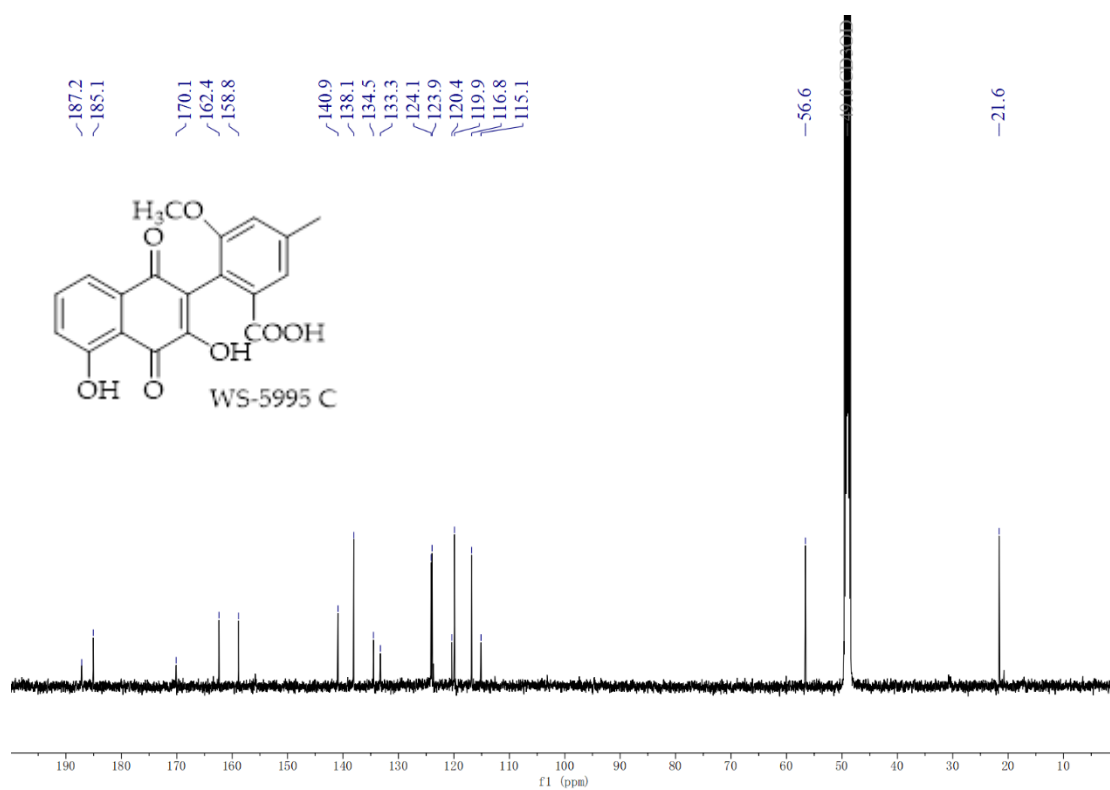
**Figure S15.** <sup>1</sup>H NMR (700 MHz, CDCl<sub>3</sub>) spectrum of WS-5995 B (3).



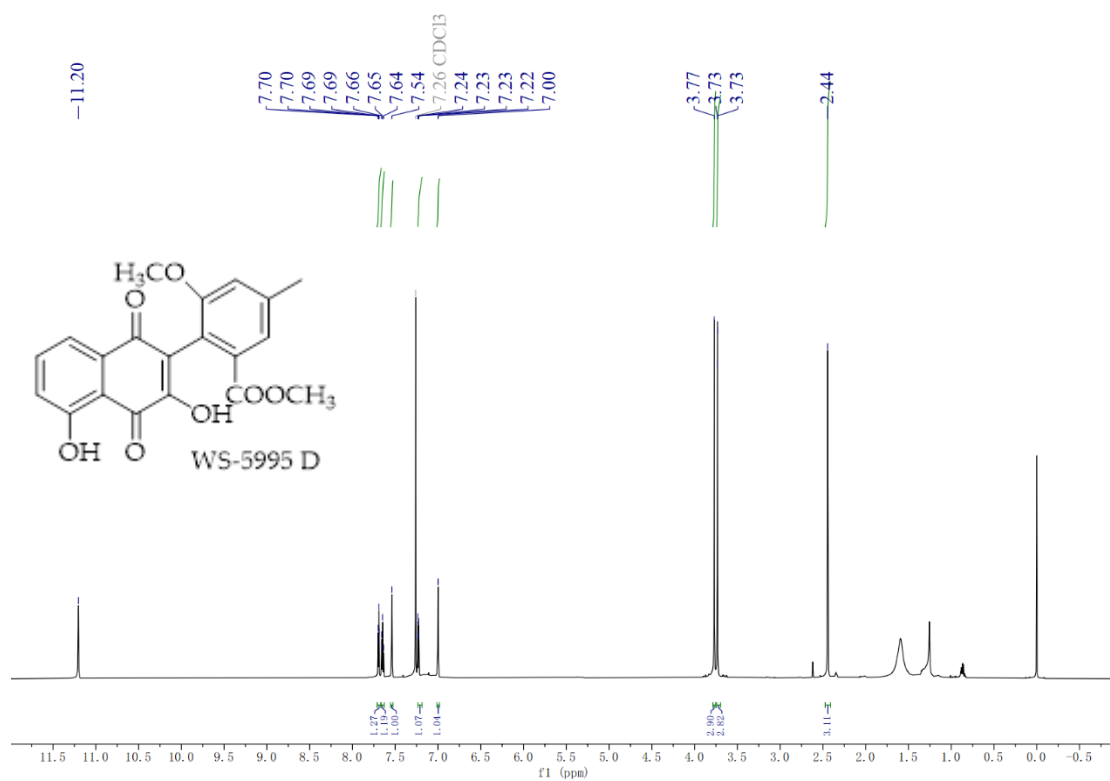
**Figure S16.** <sup>13</sup>C NMR (175 MHz, CDCl<sub>3</sub>) spectrum of WS-5995 B (3).



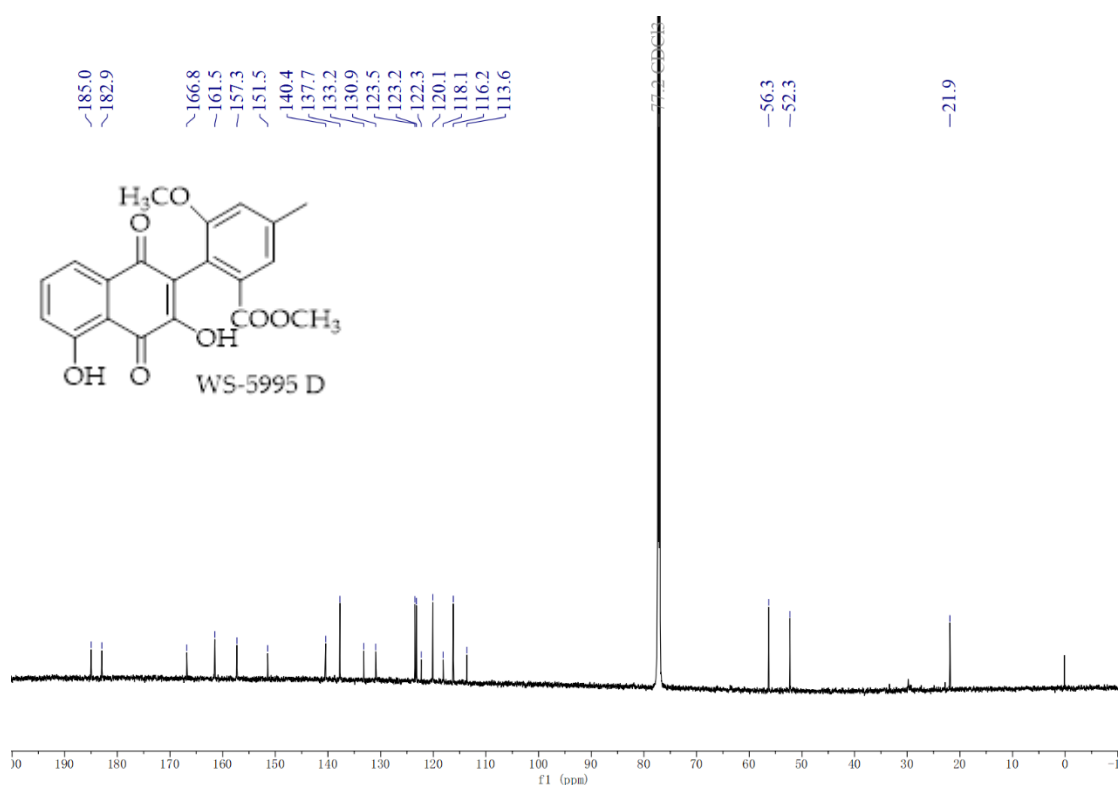
**Figure S17.** <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) spectrum of WS-5995 C (4).



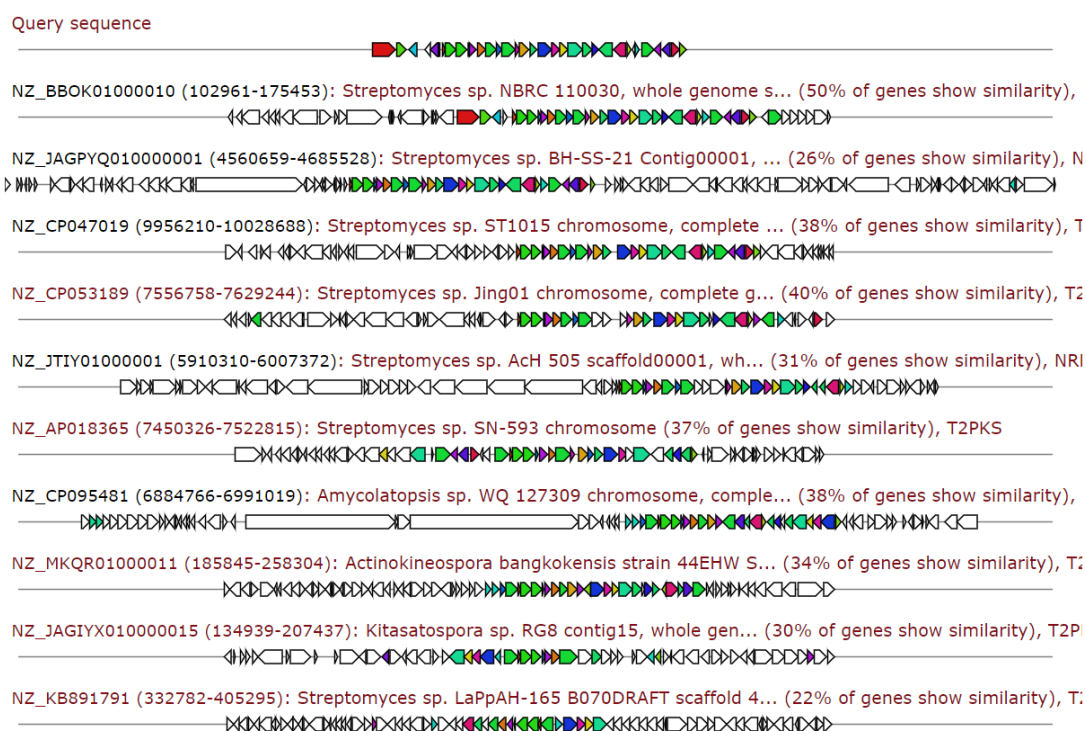
**Figure S18.** <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD) spectrum of WS-5995 C (4).



**Figure S19.** <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) spectrum of WS-5995 D (5).



**Figure S20.**  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ) spectrum of WS-5995 D (5).



**Figure S21** Similar gene cluster to *wsm* in antiSMASH database





Figure S24 transformation of WS-5995 C.

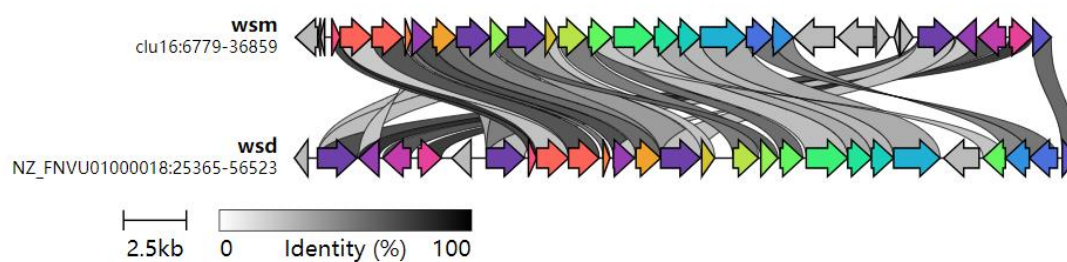


Figure S25 Comparison of *wsm* and *wsd* gene clusters using Clinker.

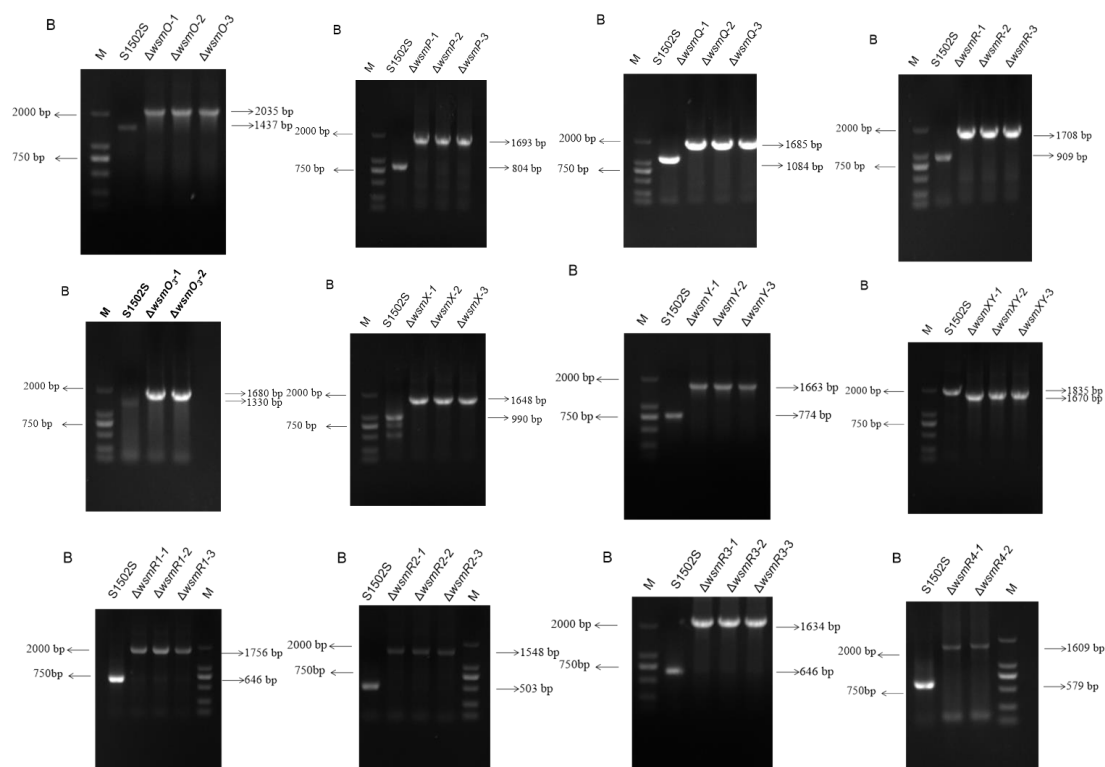


Figure S26 PCR verification of disruption of *genes of wsm* in *S. sp.* S1502/Δ*stp1* via PCR-targeting.



Reference:

1. Wang, Y.; He, J.; Alam, M. S.; Wang, F.; Shang, Z.; Chen, Y.; Sun, C.; Lu, Z.; Gao, Y.; Zhang, T.; Ju, J.; Ma, J., Efficient mutasynthesis of “non-natural” antitubercular ilamycins with low cytotoxicity. *ACS Synthetic Biology* **2024**, 13, (3), 930-941.
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