Supplemental Materials for

Communication

Production of a Novel Tetrahydroxynaphthalene (THN) Derivative from *Nocardia* sp. CS682 by Metabolic Engineering and its Bioactivities

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Figure Captions

Figure S1. Confirmation of deletion by PCR of (A) PCR probe for confirmation of deletion of PKS using probe 1 (~1.2kb) D1: *Nocardia* sp. CS682DR, W1: *Nocardia* sp. CS682 and probe 2 (~0.9kb) D2: *Nocardia* sp. CS682DR, W2: *Nocardia* sp. CS682, M: ladder; (B)PCR probe for amplification of marker gene tsr^r (1034 bp), M: DNA ladder, W: *Nocardia* sp. CS682, D: *Nocardia* sp. CS682DR (C) PCR probe for amplification of apr^r (777bp) V: pULVK2A used as positive control, M: DNA ladder, W: *Nocardia* sp. CS682, D: *Nocardia* sp. CS682DR

Figure S2. ^{1}H of NMR of compound IBR-3

Figure S3. ^{13}C NMR of compound IBR-3

Figure S4. COSY of compound IBR-3

Figure S5. ROESY of compound IBR-3

Figure S6. HSQC-DEPT of compound IBR-3

Figure S7. HMBC of compound IBR-3

Figure S8. HR-QTOF mass analysis of nargenicin A1

Figure S9. HR-QTOF mass analysis of compound IBR-1

Figure S10. HR-QTOF mass analysis of compound IBR-2

Figure S11. HR-QTOF mass analysis of compound IBR-3

Figure S12. HR-QTOF mass analysis of compound IBR-4

Figure S13. The anticancer activities of compound IBR-3 against various cancer cell lines

Figure S14. Melanin formation assay of compound IBR-3 using B16F10 melanoma cell

Table Legends

Table S1. List of strains and vectors used in the study

Table S2. Oligonucleotides used in the study

Fig. S1

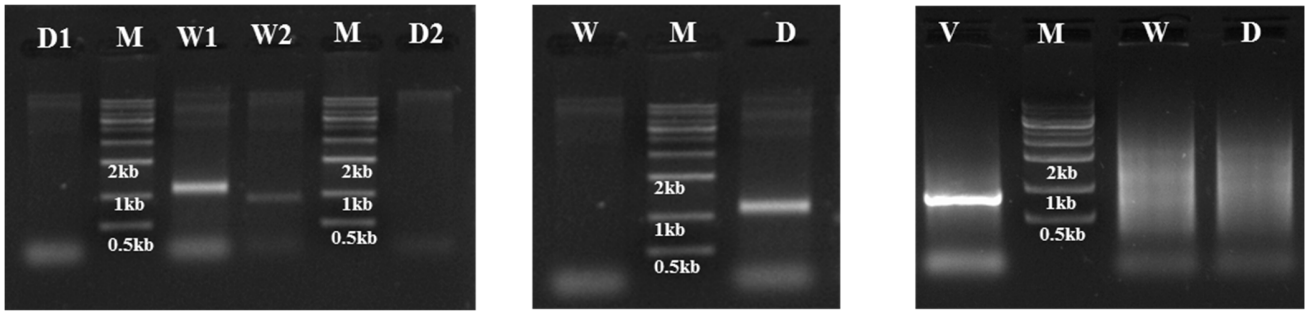
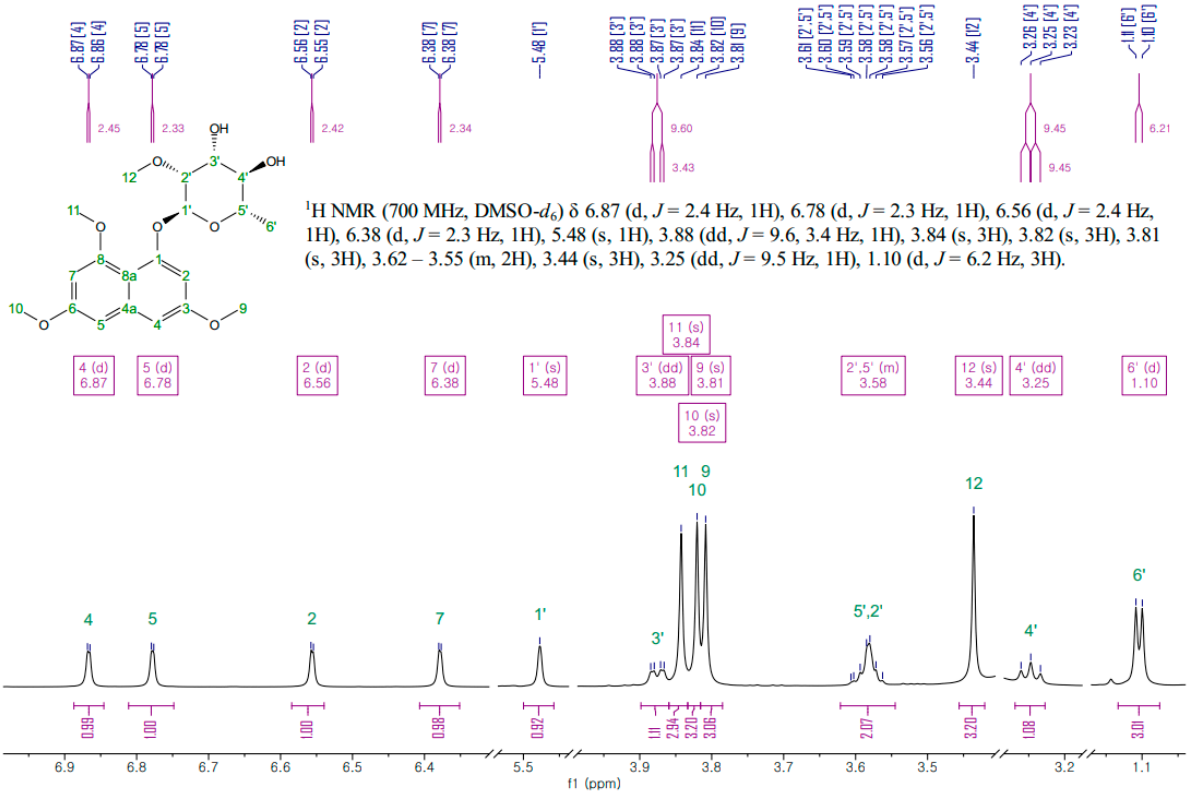
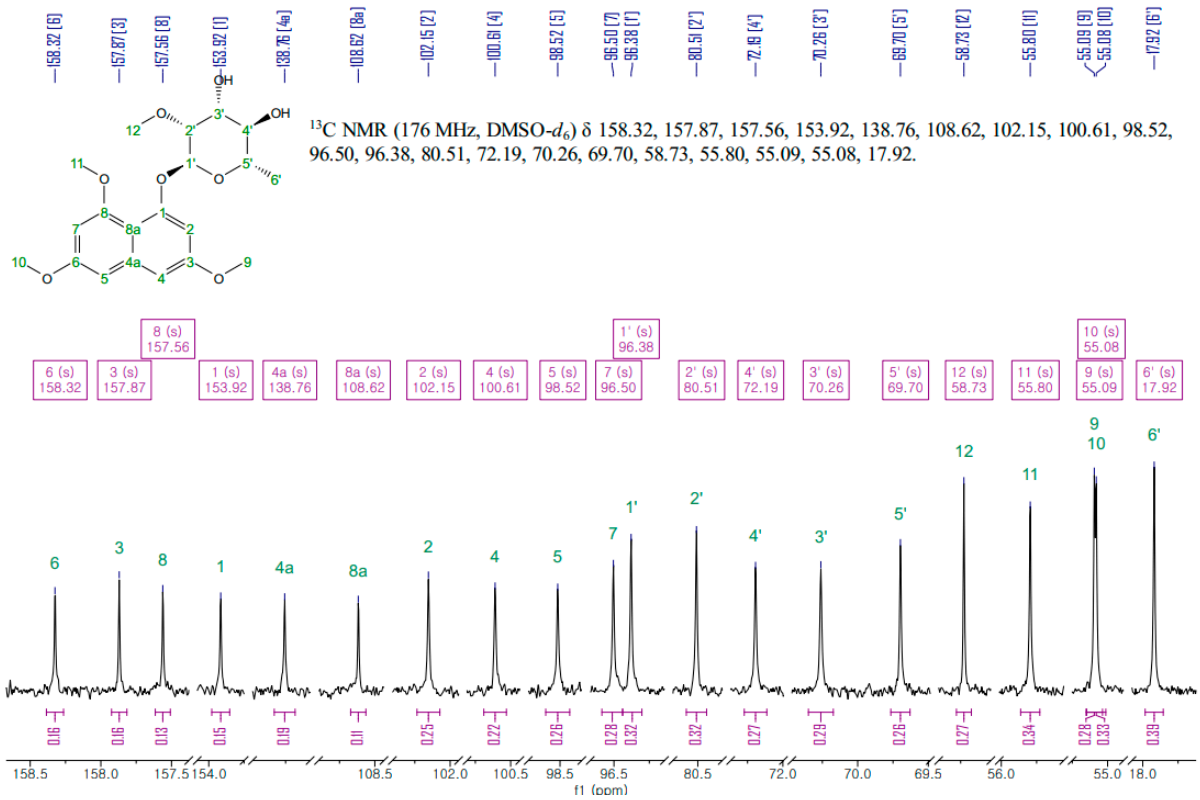

D1
M
W1
W2
M
D2
2kb
1kb
0.5kb
2kb
1kb
0.5kb
W
M
D
2kb
1kb
0.5kb
V
M
W
D
2kb
1kb
0.5kb

Fig. S2



^{1}H NMR (700 MHz, DMSO-d_6) δ 6.87 (d, J = 2.4 Hz, 1H), 6.78 (d, J = 2.3 Hz, 1H), 6.56 (d, J = 2.4 Hz, 1H), 6.38 (d, J = 2.3 Hz, 1H), 5.48 (s, 1H), 3.88 (dd, J = 9.6, 3.4 Hz, 1H), 3.84 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.62 – 3.55 (m, 2H), 3.44 (s, 3H), 3.25 (dd, J = 9.5 Hz, 1H), 1.10 (d, J = 6.2 Hz, 3H).

Fig. S3



^{13}C NMR (176 MHz, DMSO-d_6) δ 158.32, 157.87, 157.56, 153.92, 138.76, 108.62, 102.15, 100.61, 98.52, 96.50, 96.38, 80.51, 72.19, 70.26, 69.70, 58.73, 55.80, 55.09, 55.08, 17.92.

Fig. S4

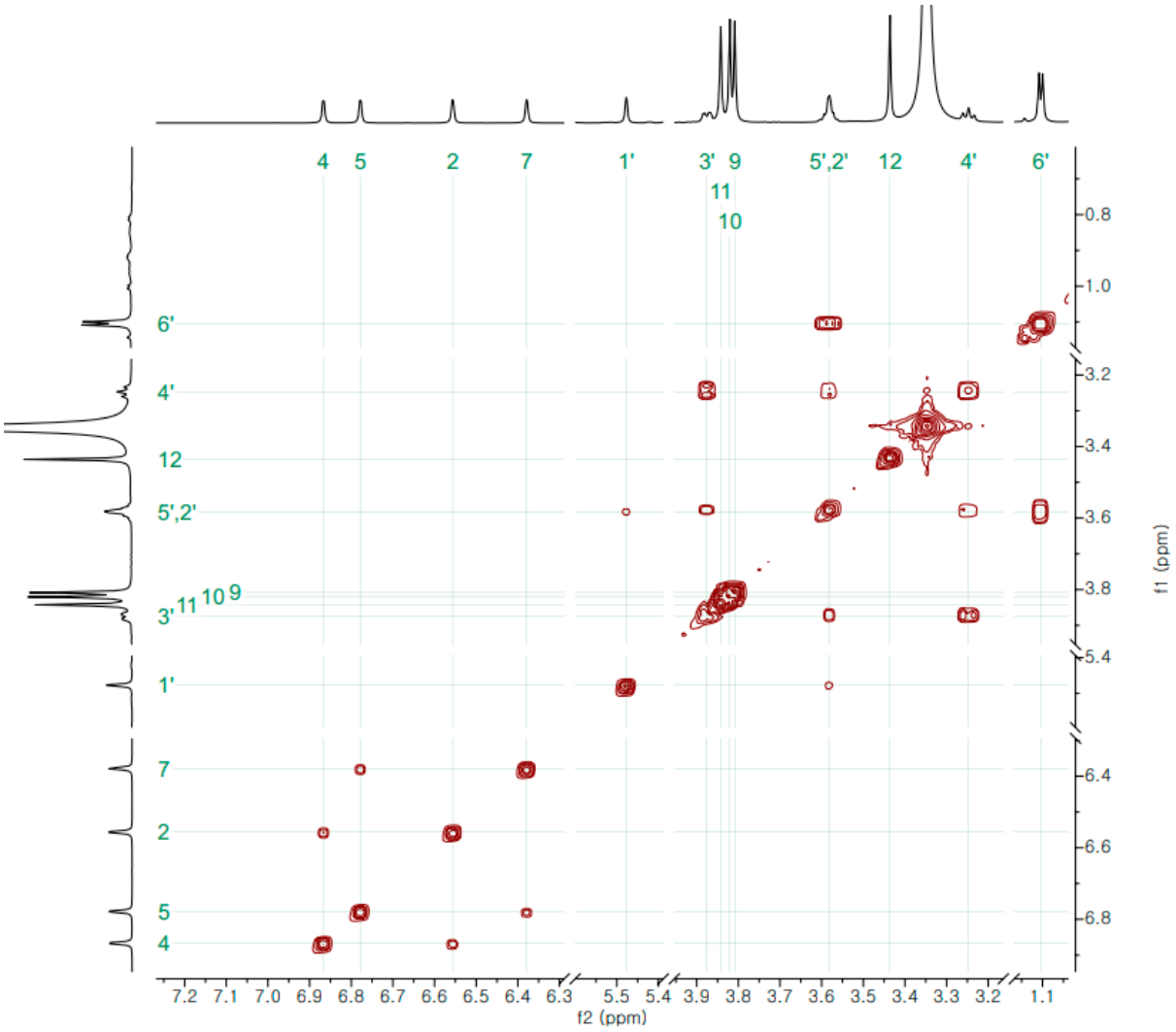


Fig. S5

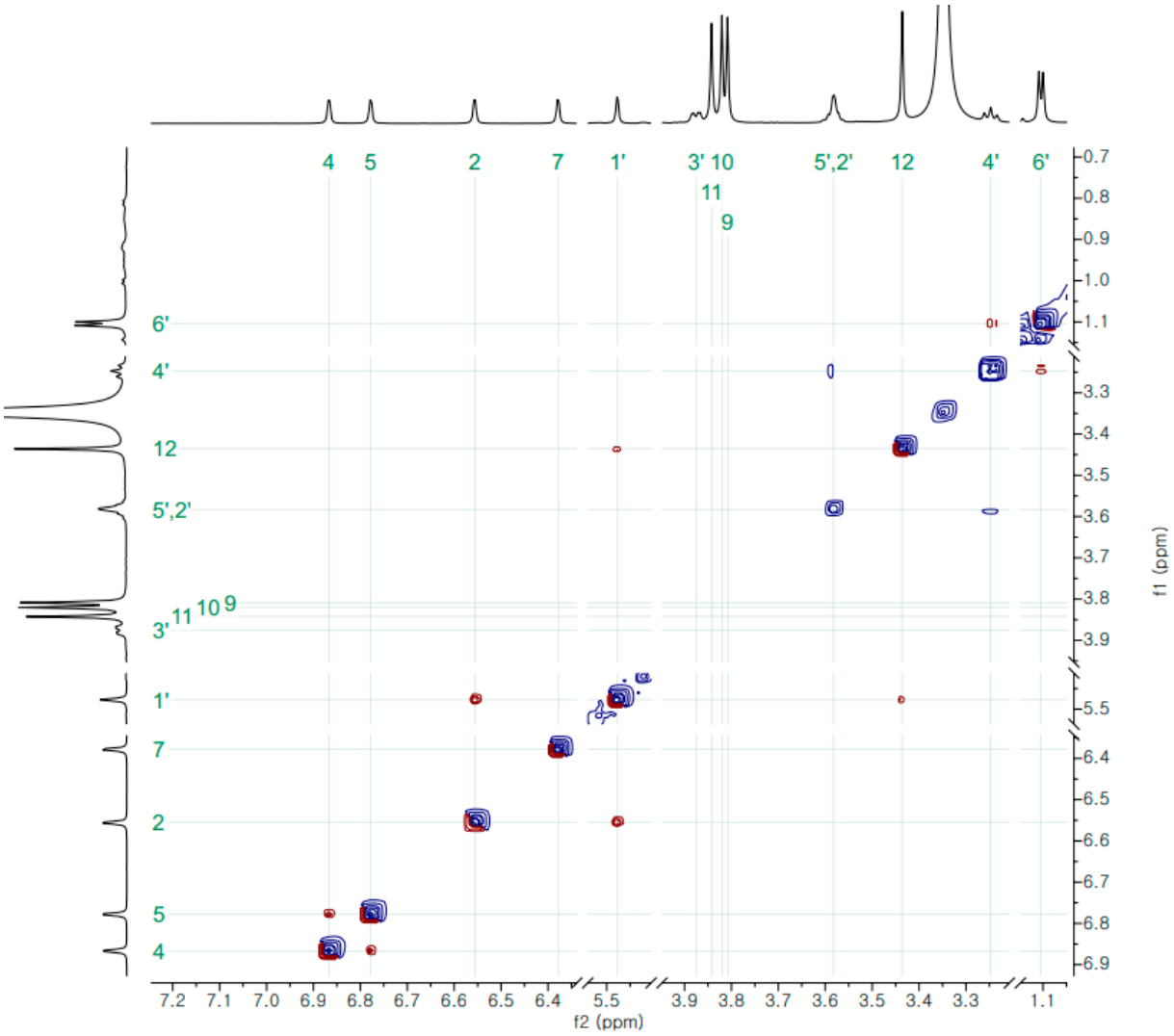

4
5
2
7
1'
3' 10
11
9
5',2'
12
4'
6'
6'
4'
12
5',2'
3' 11 10 9
1'
7
2
5
4
7.2 7.1 7.0 6.9 6.8 6.7 6.6 6.5 6.4
5.5
3.9 3.8 3.7 3.6 3.5 3.4 3.3
1.1
f2 (ppm)
0.7 0.8 0.9 1.0 1.1
3.3 3.4 3.5 3.6 3.7 3.8 3.9
5.5 6.4 6.5 6.6 6.7 6.8 6.9
f1 (ppm)

Fig. S6

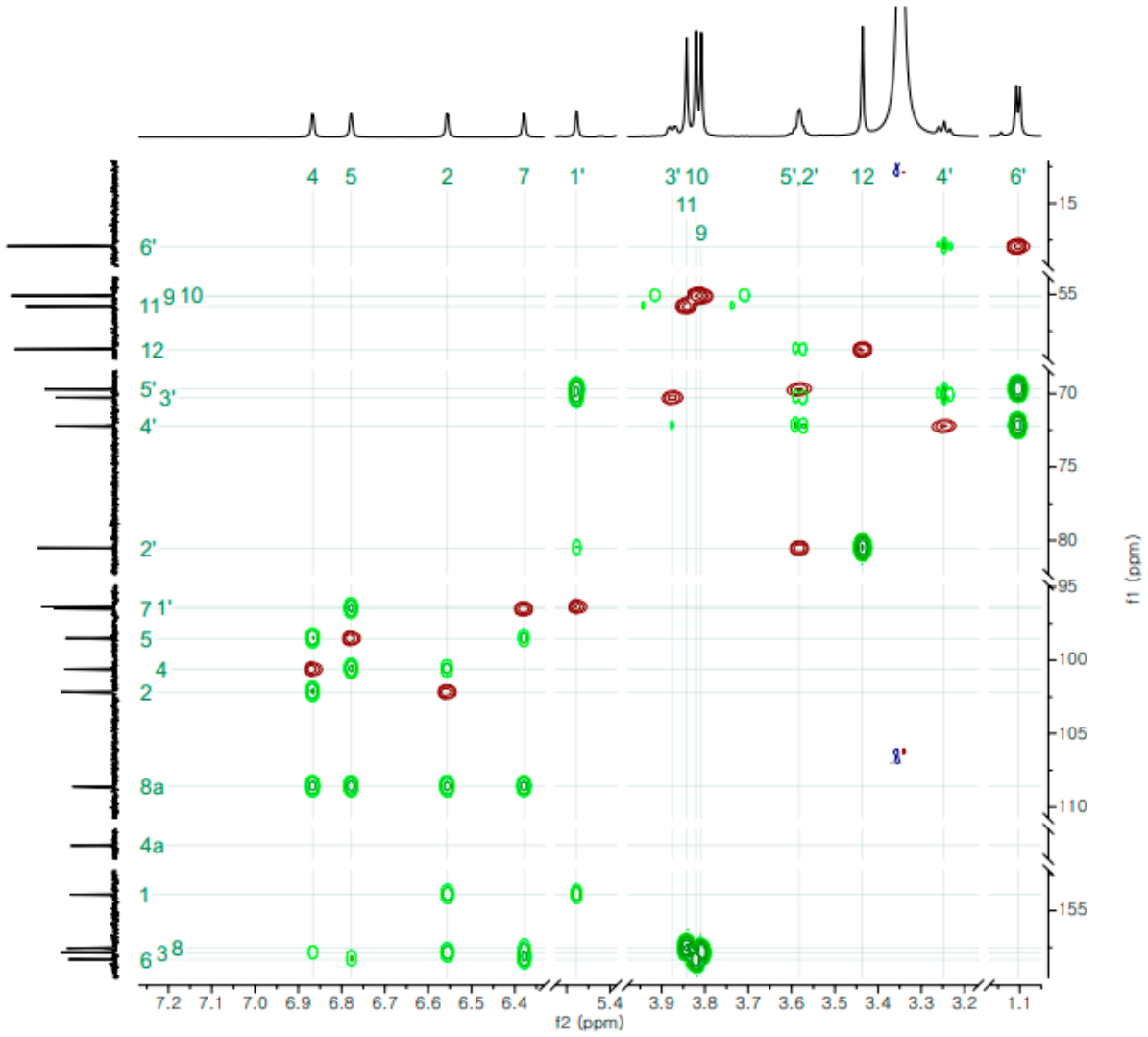

4
5
2
7
1'
3'
10
11
9
5',2'
12
4'
6'
6'
11
9
10
12
5'
3'
4'
2'
7
1'
5
4
2
8a
4a
1
6
3
8
8'
15
55
70
75
80
95
100
105
110
155
f1 (ppm)
7.2
7.1
7.0
6.9
6.8
6.7
6.6
6.5
6.4
5.4
3.9
3.8
3.7
3.6
3.5
3.4
3.3
3.2
1.1
f2 (ppm)

Figure S7

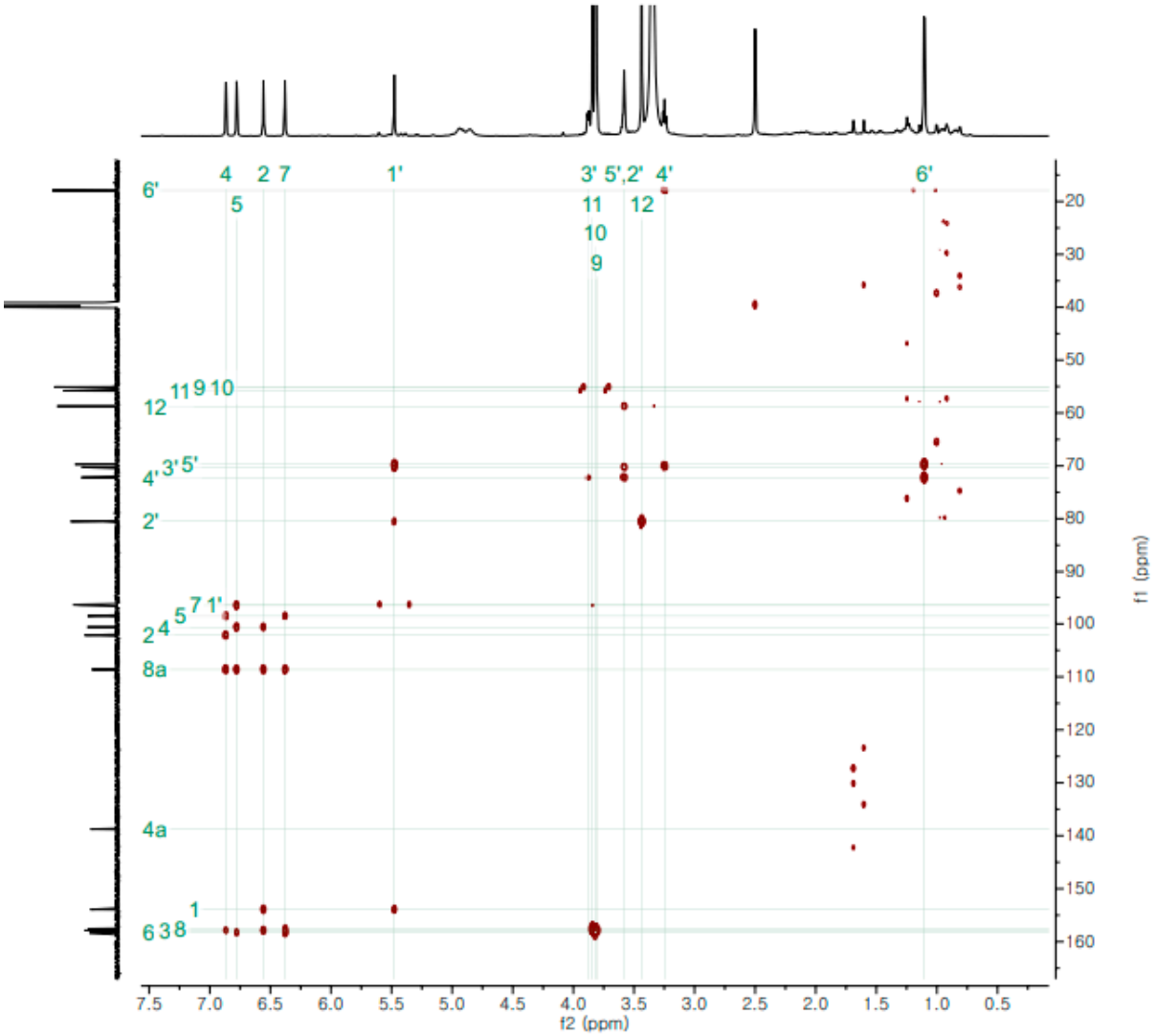

4 2 7
1'
3' 5',2' 4'
6'
5
11
12
10
9
6'
12 11 9 10
4' 3' 5'
2'
2 4 5 7 1'
8a
4a
6 3 8 1
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f2 (ppm)
20 30 40 50 60 70 80 90 100 110 120 130 140 150 160
f1 (ppm)

Fig. S8

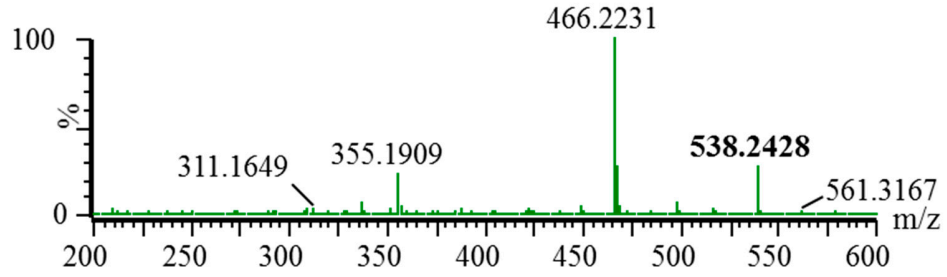
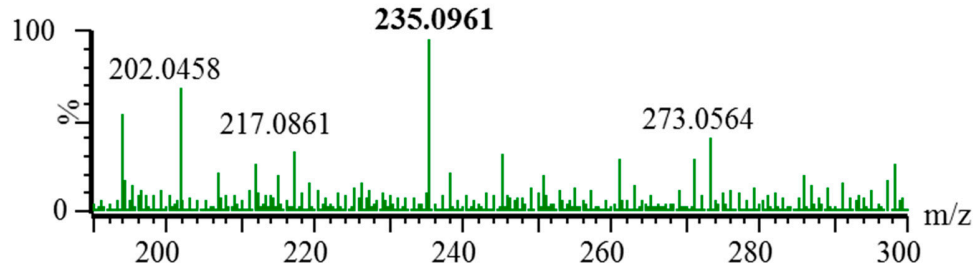

466.2231
100
%
311.1649
355.1909
538.2428
561.3167
0
m/z
200
250
300
350
400
450
500
550
600

Fig. S9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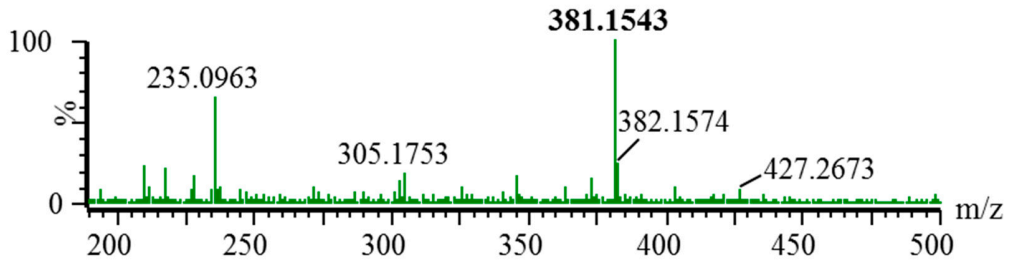
100
202.0458
%
217.0861
273.0564
0
m/z
200
220
240
260
280
300

Fig. S10


381.1543
100
235.0963
%
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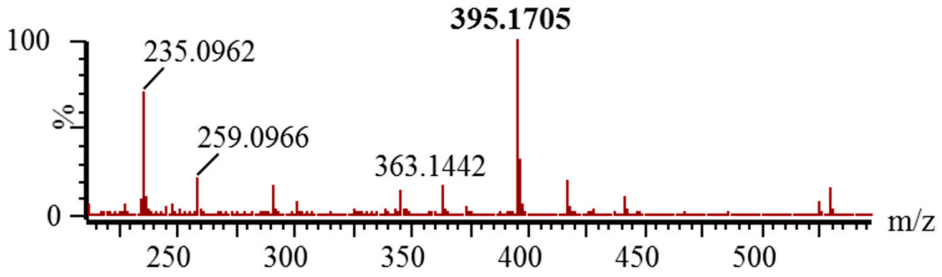
305.1753
427.2673
0
m/z
200
250
300
350
400
450
500

Fig. S11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235.0962
%
259.0966
363.1442
0
m/z
250
300
350
400
450
500

Fig. S12

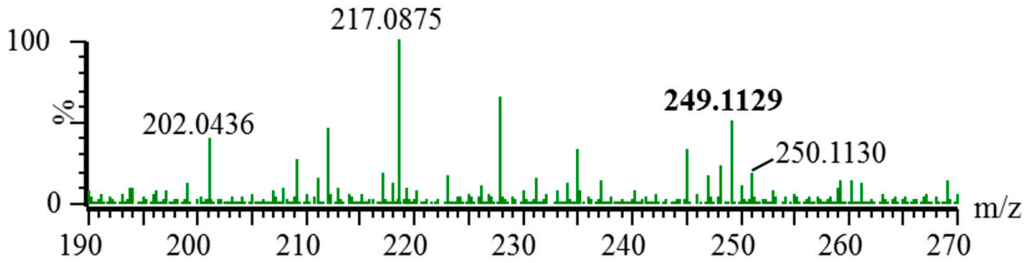
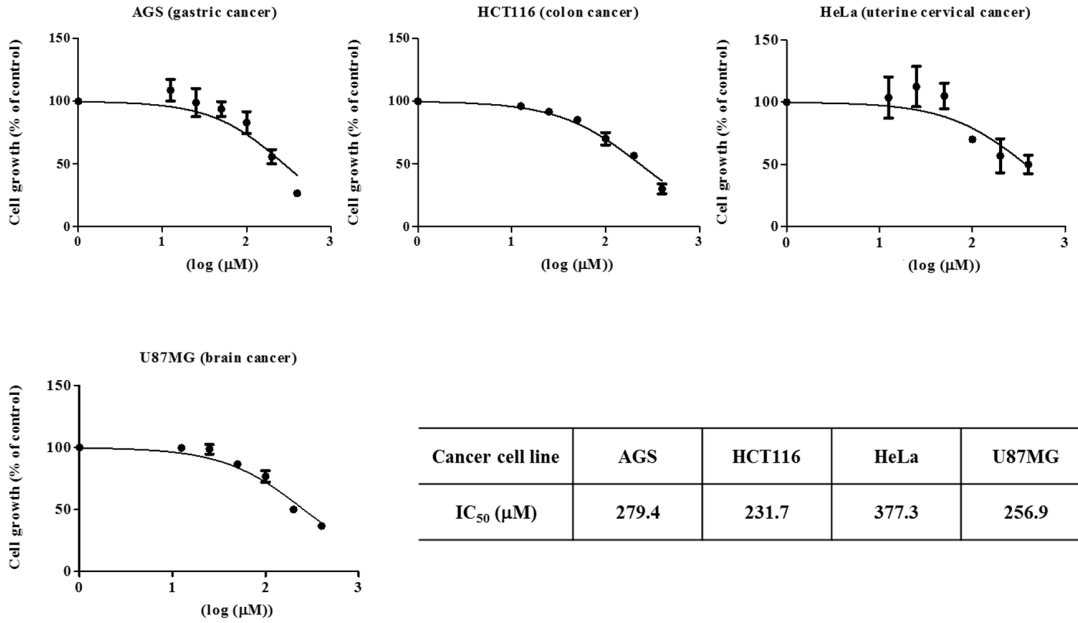

217.0875
100
%
202.0436
249.1129
250.1130
0
m/z
190
200
210
220
230
240
250
260
270

Figure S13.



Cancer cell line	AGS	HCT116	HeLa	U87MG
IC_{50} (μM)	279.4	231.7	377.3	256.9

Figure S14.

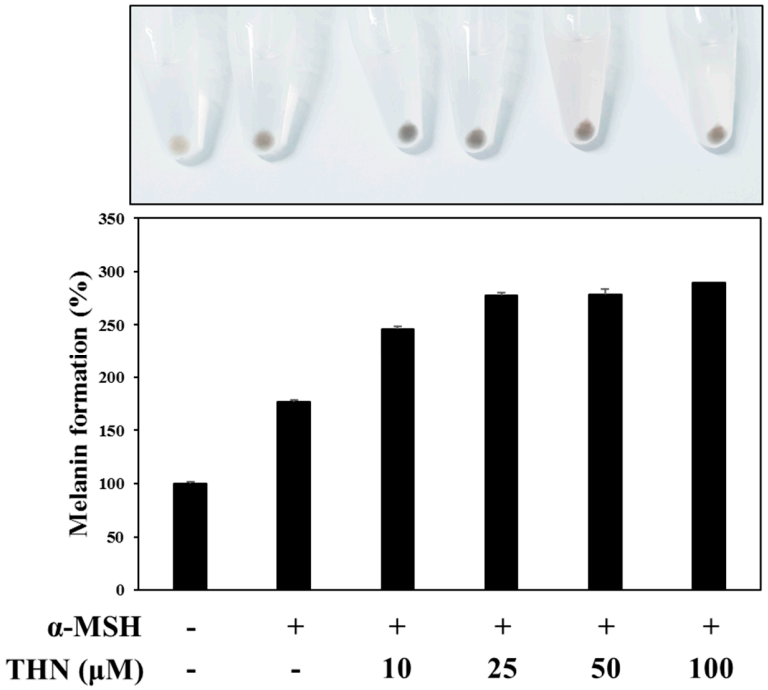

Melanin formation (%)
350
300
250
200
150
100
50
0
α-MSH - + + + + +
THN (μM) - - 10 25 50 100

Table S1. List of strains and vectors used in the study

Bacterial strains, plasmids and rDNAs	Relevant characteristics	Sources
Bacterial strains		
E. coli XL1Blue MRF	Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac*	Stratagene, USA
E. coli ET-12567	DNA demethylating strain (dam$^-$ dcm$^-$ hsdS cm^R)	John Innes Center, UK
Nocardia sp. CS682	Wild type producer of nargenicin A1	Sohng et al. 2008
Nocardia sp. CS682DR	Mutant strain with inactivation of nargenicin biosynthesis	This study
Plasmids and rDNAs		
pGEM®-T Easy	T_7 and SP6 promoters, *ColE1 ori, lacZ, Amp*^{R}	Promega, USA
pKC1139	Multi copy *E. coli-Streptomyces* shuttle vector	
pULVK2A	*Nocardia-E. coli* shuttle vector, *neo*^{r}, *apr*^{r}	Kelly and Townsend
pDN	pULVK2A containing deletion cassette for nargenicin A1 PKS region	This study.

Table 2. Oligonucleotides used in the study

Gene	Sequence (5′-3′)	Restriction site	Notes
delPKup-F	GGATTCGCCTCGCTGGACCGGGTC	*Bam*HI	For amplification of upstream region for deletion of PKS
delPKup-R	GGTACCGTCGGACGGTTCGTGCAA	*Kpn*I	
delPKdn-F	GGTACCGCTGGCCCGTGGACCACAGCG	*Kpn*I	For amplification of downstream region for deletion of PKS
delPKdnR	AAGCTTGTGCTGGTACTCCTGTGATCTCTCGGT	*Hind*III	
tsrF	GGTACCTGCAGTTAACTCTAGATTTAAATGTATGATCAAGGCGAA	*Kpn*I	For confirmation of double cross over mutant by PCR of thiostrepton resistance gene used as selection marker
tsrR	GGTACCTGCAGTTAACTCTAGATTTAAACAGAGGCGCTTATCGGT	*Kpn*I	
aprF	ATGCAATACGAATGGCGA		For confirmation of loss of vector in double cross over mutant by PCR of apramycin resistance gene
aprR	TCAGCCAATCGACTGGCG		
p1-F	CCCCGTACACCTTCACCGGGTCCC		For confirmation of deletion by PCR amplification of internal segment of PKS of nargenicin biosynthetic gene cluster
p1-R	GCCATGCCGACCCACTGCGAGCCCT		
p2-F	CGCCTCGCGTGCGTCGAGGACCGGA		
p2-R	GTCTTCTCACGGTCTCCTGAGCTGA		
orf1-F	CGAGCAAGCTATCCATCGTG		For qRT-PCR of *orf1*
orf1-R	ACAACGCCAGCATTATGACC		
orf2-F	GGTACGTTGCACCGTGATG		For qRT-PCR of *orf2*
orf2-R	CCTGCGAAATATTGCCTGCT		
thnM1-F	CGATGCTGGTGTTGACTGAG		For qRT-PCR of *thnM1*
thnM1-R	CGAATGGCCCAAATCGAACT		
thnT1-F	GCTCCTCGAAATGGACGATG		For qRT-PCR of *thnT1*
thnT1-R	CGCGTACAGCAGAATCTTGT		
thnG-F	CGGCTGGCCATGAGATATTG		For qRT-PCR of *thnG*
thnG-R	GACGATGCGAAAGCTAGTCC		
thnA-F	GAGTCAGGCTGGAACGAAA		For qRT-PCR of t*hnA*
thnA-R	ATCGTATGTGGGACTCGCTT		
thnM2-F	GTCAACACTGGTCTGACTGC		For qRT-PCR of *thnM2*
thnM2-R	TCGTGTCGATATCACCGGAT		
thnM3-F	CCAAATCGACACCGTAGCAG		For qRT-PCR of *thnM3*
thnM3-R	CTGGATCCCAGTTGTGGAGT		
thnT2-F	GTTTGTTGCTGACCGACCAT		For qRT-PCR of *thnT2*
thnT2-R	GGTGTTCGTATTGGGTCTGC		
orf3-F	TGATCGACAGAGCATTGGGT		For qRT-PCR of *orf3*
orf3-R	TGACCTCCGACGAAGTACAG		
16S-RT-F	AAAGAGCTTGTAGGCGGTCT		For qRT-PCR of *16S RNA*
16S-RT-R	TTCACCGCTACACCAGGAAT	-	