SUPPORTING INFORMATION

Aranciamycins I and J: Antimycobacterial anthracyclines from an Australian marine-derived *Streptomyces* sp.

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1 Experimental

1.1 DNA Taxonomic Analysis of *Streptomyces* sp. (CMB-M0150)

Genomic DNA extraction was performed using the DNeasy blood and tissue kit as per the manufacturers protocol. The 16S rRNA genes were amplified from genomic DNA by PCR (PCT-100 Thermal cycler), (cycle conditions, 95 °C for 5 min, 30 cycles of 95 °C for 30 s, 55 °C for 45 s, 72 °C for 30 s), using primers FC27 (5′-AGAGTTTGATCCTGGCTCAG-3′) and RC1492 (5′-TACGGCTACCTTGTTACGACTT-3′). The 50 μL PCR mixture contained 25 to 45 ng of DNA, 250 pmol of each primer, 2.5 U of *Taq* DNA polymerase and 100 μM deoxynucleoside triphosphate mixture. Amplification products were examined by agarose gel electrophoresis.

1.2 Gene Sequence of *Streptomyces*

16S rRNA sequence

GGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTTCGGGACAAGCCC
TGGAAACGGGGTCTAATACCGGATACGACCTGCTCAGGCATCTGAGCGGGTGGAAAGCTCCGGCGG
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AGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCA
GCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCC
TTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCCTTAAAAAGTGACGGTACCTGCAGAAGAAGC
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CCCGCAACGAGCGCAACCCTTGTCTCGTGTTGCCAGCAACTCTTCGGAGGTTGGGGACTCACGGGA
GACTGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGG
GCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGCGAAGCCGTGAGGTGGAGCGAATCTCAAA
AAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCG
CAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAA
GTCGGTAACACCCGAAGCCG

1.3 BLAST search (closest match):

```
LOCUS       NR_112841               1473 bp    DNA     linear   BCT 23-APR-2014
DEFINITION  Streptomyces marinus strain Sp080513GE-26 16S ribosomal RNA gene,
            partial sequence.
ACCESSION   NR_112841
VERSION     NR_112841.1  GI:631251643
DBLINK      Project: 33175
            BioProject: PRJNA33175
KEYWORDS    RefSeq.
SOURCE      Streptomyces marinus
  ORGANISM  Streptomyces marinus
            Bacteria; Actinobacteria; Actinobacteridae; Actinomycetales;
            Streptomycineae; Streptomycetaceae; Streptomyces.
REFERENCE   1
  AUTHORS   Khan,S.T., Tamura,T., Takagi,M. and Shin-Ya,K.
  TITLE     Streptomyces tateyamensis sp. nov., Streptomyces marinus sp. nov.
            and Streptomyces haliclonae sp. nov., isolated from the marine
            sponge Haliclona sp.
  JOURNAL   Int. J. Syst. Evol. Microbiol. 60 (PT 12), 2775-2779 (2010)
   PUBMED   20061489
  REMARK    DOI:10.1099/ijs.0.019869-0
REFERENCE   2  (bases 1 to 1473)
  CONSRTM   NCBI RefSeq Targeted Loci Project
  TITLE     Direct Submission
  JOURNAL   Submitted (23-APR-2014) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
REFERENCE   3  (bases 1 to 1473)
  AUTHORS   Khan,S.T., Takagi,M., Shin-ya,K. and Komaki,H.
  TITLE     Direct Submission
  JOURNAL   Submitted (15-DEC-2008) Contact:Shams T Khan Japan Biological
            Information research Center, Japan Biological Informatics
            consortium; Aomi 2-42, Koto-ku, Tokyo, Tokyo 135-0064, Japan
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1.4 HPLC-DAD chromatogram of CMB-M0150

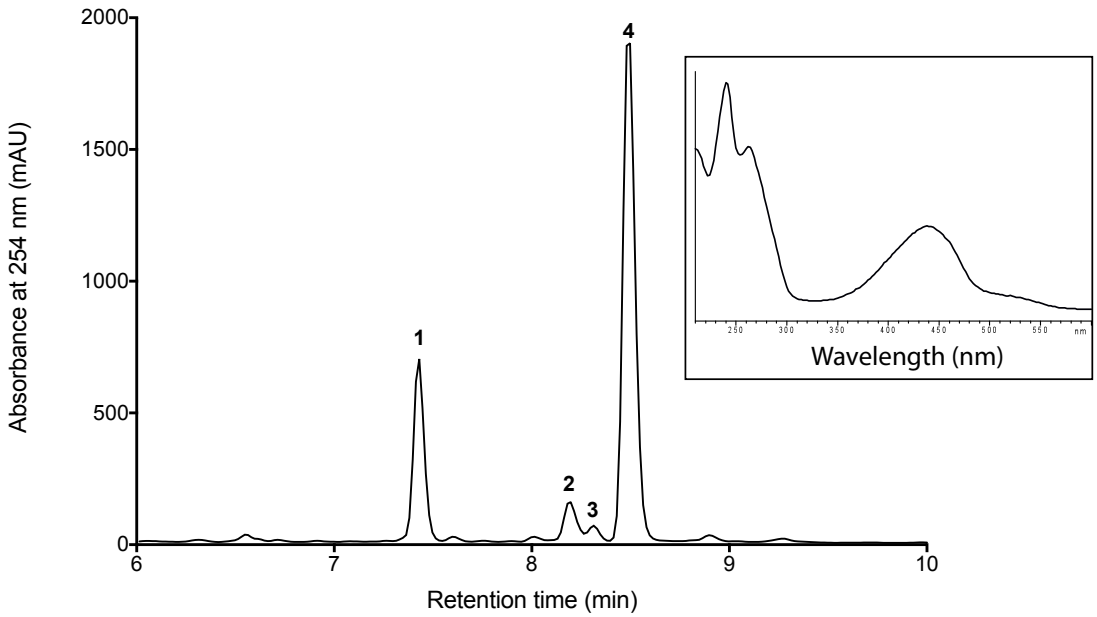


Figure S1. Expansion (6–10 min) of the HPLC-DAD chromatogram of the crude extract of *Streptomyces* sp. (CMB-M0150) and a UV-vis spectrum of aranciamycins **1**–**4**.

2 Biological Assays

2.1 Cytotoxicity (MTT) Assays

The MTT assay was modified from that previously described[1] using adherent cell lines SW620 (adherent epithelial like, human colorectal carcinoma) and NCIH-460 (adherent epithelial like, human lung carcinoma) were cultured in RPMI medium 1640[2] and KB-3-1[3] (adherent epithelial like, human cervix carcinoma) and HepG2[4] (adherent human hepatocellular carcinoma) were cultured in DMEM medium as adherent mono-layer in flasks supplemented with 10% foetal bovine serum, 2 mM L-glutamine, 100 unit/mL penicillin and 100 μg/mL streptomycin in a humidified 37 °C incubator supplied with 5% CO_2. Briefly, cells were harvested with trypsin and dispensed into 96-well microtiter assay plates at 2,000 cells/well for SW620, KB-3-1, HepG2 and NCIH-460, then incubated for 18 h at 37 °C with 5% CO_2 (to allow cells to attach). Testing compounds were dissolved in 5% DMSO in PBS (v/v) and aliquots (20 μL) tested over a series of final concentrations ranging from 10 nM to 30 μM. Control wells were treated with 1% aqueous DMSO. After 48 h incubation at 37 °C with 5% CO_2 an aliquot (20 μL) of MTT in PBS (5 mg/mL) was added to each well (final concentration of 0.5 mg/mL), and the microtiter plates incubated for a further 4 h at 37 °C with 5% CO_2. After this final incubation the medium was aspirated and precipitated formazan crystals dissolved in DMSO (100 μL/well). The absorbance of each well was measured at 580 nm with a PowerWave XS Microplate Reader from Bio-Tek Instruments Inc. (Vinooski, VT). IC_{50} values were calculated using Prism 5.0 (GraphPad Software Inc., La Jolla, CA), as the concentration of analyte required for 50% inhibition of cancer cell growth (compared to negative controls). All experiments were performed in duplicate.

2.2 Antibacterial assay

The bacterium to be tested was streaked onto a tryptic soy agar plate and was incubated at 37 °C for 24 h. One colony was then transferred to fresh tryptic soy broth (15 mL) and the cell density was adjusted to 10^4–10^5 cfu/mL. The compounds to be tested were dissolved in DMSO and diluted with H_2O to give 300 μM stock solutions (10% DMSO). The stock solutions were then serially diluted with 10% DMSO to give final concentrations of 30 μM to 0.01 μM in 1% DMSO. An aliquot (20 μL) of each dilution was transferred to a 96-well microtiter plate and freshly prepared microbial broth (180 μL) was added to each well. The plates were incubated at 37 °C for 24 h and the optical density of each well was measured spectrophotometrically at 600 nm using POLARstar Omega plate (BMG LABTECH, Offenburg, Germany). Each test compound was screened against the

Gram-negative bacteria *Escherichia coli* (ATCC 11775) and *Pseudomonas aeruginosa* (ATCC 10145) and the Gram-positive bacteria *Staphylococcus aureus* (ATCC 9144 and ATCC 25923) and *Bacillus subtilis* (ATCC 6633 and ATCC 6051). The IC_{50} value was calculated as the concentration of the compound or antibiotic drug required for 50% inhibition of the bacteria using Prism 6 from GraphPad Software Inc. (La Jolla, CA).

2.3 Antifungal assay

The fungus to be tested was streaked onto a Sabouraud agar plate and was incubated at 26.5 °C for 48 h. One colony was then transferred to fresh Sabouraud broth (15 mL) and the cell density was adjusted to 10^4–10^5 cfu/mL. Test compounds were dissolved in DMSO and diluted with H_2O to give a 300 μM stock solution (10% DMSO). The stock solution was then serially diluted with 10% DMSO to give final concentrations of 30 μM to 0.01 μM in 1% DMSO. An aliquot (20 μL) of each dilution was transferred to a 96-well microtiter plate and freshly prepared microbial broth (180 μL) was added to each well. The plates were incubated at 26.5 °C for 48 h and the optical density of each well was measured spectrophotometrically at 600 nm using POLARstar Omega plate (BMG LABTECH, Offenburg, Germany). Each test compound was screened against the fungus *Candida albicans* (ATCC 90028). The IC_{50} value was calculated as the concentration of the compound or antifungal drug required for 50% inhibition of the fungal cells using Prism 6 from GraphPad Software Inc. (La Jolla, CA)

2.4 *Mycobacterium bovis* (BCG) assay

Mycobacterium bovis, Bacille Calmette Guerin (BCG) was grown until early-mid log phase in 7H9 liquid medium (Difco) containing 0.2% glycerol, 0.05% Tween80, 0.5% bovine serum albumin (BSA), 0.2% dextrose, and 0.085% sodium chloride. Single cell suspensions of independent cultures were prepared by centrifuging the bacterial cultures for 10 min, at room temperature, 130 × g and then diluted to an optical density (OD; 600 nm) of 0.02. Bacterial growth in the presence of araciamycins **1**–**4** (200 μL aliquots) was monitored in 96 well plates by OD measurements. Isoniazid (INH) was used as a positive control (20 μg/mL in H_2O).

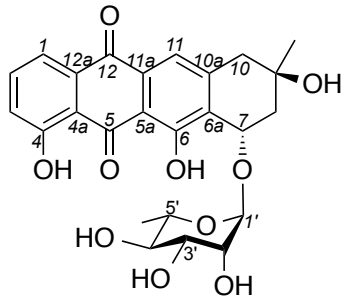


Table S1. NMR (600 MHz, DMSO-d_6) data for aranciamycin I (**1**)

pos	δ_H, mult, (*J* in Hz)	δ_C[a]	COSY	$^1H - ^{13}C$ HMBC	ROESY
1	7.71, d, (*7.7*)	118.9	2	3, 4a, 12	
2	7.79, dd, (*8.3, 7.7*)	137.0	1, 3	4, 12a	
3	7.37, d, (*8.3*)	124.8	2	1, 4a, 4	
4		160.8			
4a		116.2			
5		191.3			
5a		113.7			
6		161.9			
6a		131.9			
7	4.91, dd *(6.4, 5.3)*	72.1	8α/β	1′, 6, 6a, 9, 10a	
8α	2.12, dd, (*13.8*, *6.4*)	43.2	7, 8β	6a, 7, 9, 9-Me, 10a, 10	
8β	1.98, dd, (*13.8*, *5.3*)		7, 8α	6a, 7, 9, 9-Me, 10a, 10	5′
9		67.4			
10α	2.96, d, (*17.0*)	44.5	10β	6a, 8, 9, 9-Me, 10a, 11	11
10β	2.76, d, (*17.0*)		10α	6a, 8, 9, 9-Me, 10a, 11	11
10a		146.7			
11	7.45, s	119.7		5a, 6a, 10, 11a, 12	10α/β
11a		131.9			
12		181.5			
12a		133.5			
1′	5.03, d *(1.1)*	103.5	2′	3′, 5′, 7	
2′	3.60[b], m	70.6	1′, 2′-OH, 3′		
3′	3.29[e], m	70.7	2′, 3′-OH, 4′	4′	
4′	3.22, ddd (*9.2*, *9.2*, *5.3*)	71.8	3′, 4′-OH, 5′	3′, 5′, 5′-Me	
5′	3.59[b], m	69.3	4′, 5′-Me	1′, 4′	8β
5′-Me	1.18, d (*6.2*)	17.9	5′	4′, 5′	
9-Me	1.27, s	28.6		8, 9, 10	
2′-OH	4.73[c]		2′		
3′-OH	4.45, d (*5.8*)		3′	3′	
4′-OH	4.73[c]		4′		
4-OH	[d]				
6-OH	[d]				
9-OH	4.72[c]			8, 9, 9-Me, 10	

*(a) assignments supported by HSQC and HMBC. (b - c) resonances are overlapping. (d) resonance not observed. (e) assignments obscured by water peak.

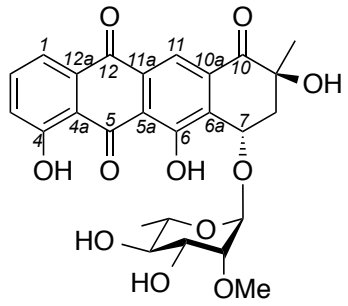


Table S2. NMR (600 MHz, DMSO-d_6) data for aranciamycin J (**2**)

pos	δ_H, mult, (*J* in Hz)	δ_C[a]	COSY	$^1H - ^{13}C$ HMBC	ROESY
1	7.75, dd (*7.6*, *1.1*)	119.3	2	3, 4a, 12	
2	7.84, dd (*8.3*, *7.6*)	137.5	1, 3	4, 12a	
3	7.42, dd (*8.3*, *1.1*)	124.4	2	1, 4, 4a	
4		160.9			
4a		116.8			
5		[d]			
5a		118.2			
6		[d]			
6a		135.8			
7	5.14, dd (*5.1*, *3.6*)	69.4	8α/β	1′, 6a, 9	8α/β
8α	2.50, dd *(14.6, 5.1)*	41.2	7, 8β	7, 6a, 9, 9-Me, 10	7
8β	2.25, dd (*14.6*, *3.6*)		7, 8α	7, 6a, 9, 10	5′, 7
9		72.1			
10		199.7			11
10a		[d]			
11	8.09, s	115.5		5a, 6a, 10, 12	9-Me
11a		[d]			
12		181.2			
12a		133.0			
1′	5.28, d, (*1.6*)	100.1	2′	2′, 5′, 7	
2′	3.27, dd, (*3.2*, *1.6*)	80.5	1′, 3′	1′, 2′-OMe, 3′, 4′	
3′	3.40[b], m	70.1	2′, 3′-OH, 4′	2′, 4′	
4′	3.19, ddd, (*9.4*, *9.4*, *5.5*)	71.7	3′, 4′-OH, 5′	2′, 3′, 5′	
5′	3.63, dq, (*9.4*, *6.2*)	69.5	4′, 5′-Me	3′, 4′	8β
5′-Me	1.20, d, (*6.2*)	17.6	5′	4′, 5′	
9-Me	1.42, s	25.4		8, 9, 10	
2′-OMe	3.40[b], s	58.2		2′	
3′-OH	4.63, d, (*5.8*)		3′	2′, 3′, 4′	9-OH
4′-OH	4.86, d, (*5.5*)		4′	4′, 5′	9-OH
4-OH	[c]				
6-OH	[c]				
9-OH	5.66, s			8, 9, 9-Me	3′-OH, 4′-OH

*(a) assignments supported by HSQC and HMBC. (b) resonances are overlapping. (c - d) resonance not observed

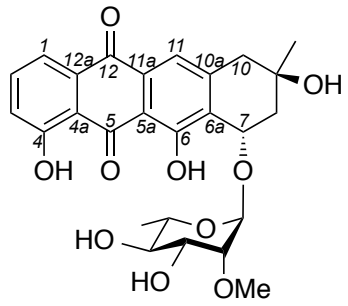


Table S3. 1D and 2D NMR (600 MHz, DMSO-d_6) data for aranciamycin A (**3**)

pos	δ_H, mult, (*J* in Hz)	δ_C	COSY	$^1H - ^{13}C$ HMBC	ROESY
1	7.69, d, (*7.3*)	119.1	2	3, 4a, 12	
2	7.79, dd, (*8.0, 7.3*)	137.0	1, 3	4, 12a	
3	7.37, d, (*8.0*)	124.1	2	1, 4a, 4	
4		160.9			
4a		115.8			
5		[b]			
5a		113.4			
6		161.2			
6a		130.9			
7	4.93, dd, (*6.6, 5.0*)	72.4	8α/β	1′, 6, 6a, 8, 9, 10a	1′, 8α/β
8α	2.14, dd, (*13.6*, *6.6*)	42.7	7, 8β	6, 6a, 7, 9, 9-Me, 10	5′, 7
8β	1.98, dd, (*13.6*, *5.0*)	42.7	7, 8α	6a, 7, 9, 9-Me, 10	5′, 7
9		66.9			
10α	2.96, d, (*17.0*)	44.1	10β	6a, 8, 9, 9-Me, 10a, 11	11
10β	2.76, d, (*17.0*)		10α	6a, 8, 9, 9-Me, 10a, 11	11
10a		146.8			
11	7.44, s	119.7	13	5a, 6a, 10, 12	10α/β
11a		[b]	12		
12		180.9			
12a		133.4			
1′	5.19, s	99.9	2′	2′, 3′, 5′, 7	2′, 7
2′	3.26, m	80.7	1′, 3′	1′, 2′-OMe, 3′, 4′	1′
3′	3.36, m	70.1	2′, 3′-OH, 4′		
4′	3.17, m	71.8	3′, 4′-OH, 5′		5′-Me
5′	3.58, dq *(9.2, 6.1)*	69.2	4′, 5′-Me	4′	5′-Me 4′-OH, 9-Me
5′-Me	1.17, d, (*6.1*)	17.6	5′	4′, 5′	4′, 5′
9-Me	1.28, s	28.3		8, 9, 10	5′
2′-OMe	3.39, s	58.2		2′	
3′-OH	4.58, d, (*5.6*)		3′	3′, 4′	
4′-OH	4.81, d, (*5.2*)		4′	2′, 3′, 4′, 5′	5′
4-OH	12.63[c], br s				
6-OH	12.01[c], br s				
9-OH	4.75, s			8, 9, 9-Me, 10	

*(a) assignments supported by HSQC and HMBC. (b) resonance not observed. (c) assignments are interchangeable

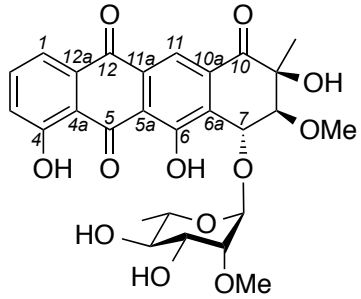


Table S4. 1D and 2D NMR (600 MHz, $CDCl_3$) data for aranciamycin (**4**)

Pos	δ_H, mult, (*J* in Hz)	δ_C[a]	COSY	$^1H - ^{13}C$ HMBC
1	7.88, d *(7.4)*	120.8	2	3, 4a, 12
2	7.74, dd *(8.4, 7.4)*	138.3	1, 3	4, 12a
3	7.34, d *(8.4)*	125.3	2	1, 4a
4		163.2		
4a		115.7		
5		[c]		
5a		118.8		
6		162.6		
6a		133.1[d]		
7	5.19, br s	72.2	8	1′, 6, 6a, 8, 9, 10a
8	3.77, br s	85.6	7	7, 6a, 8-OMe, 9, 9-Me, 10
9		76.6		
10		198.7		
10a		135.9		
11	8.41, s	117.7		5a, 6a, 12
11a		133.4[d]		
12		180.4		
12a		133.5		
1′	5.64, s	100.6		3′, 5′, 7
2′	3.52, m	80.1	3′	2′-OMe
3′	3.54, m	71.5	2′, 4′	4′
4′	3.45, dd *(9.7, 5.6)*	73.6	3′, 5′	3′, 5′-Me
5′	3.82, dq *(9.7, 6.2)*	69.6	4′, 5′-Me	
5′-Me	1.39, d *(6.2)*	17.6	5′	4′, 5′
9-Me	1.52, s	22.7		8, 9, 10
2′-OMe	3.56, s	58.9		2′
8-OMe	3.55, s	60.2		8
3′-OH	[b]			
4′-OH	[b]			
4-OH	11.85, s			3, 4, 4a
6-OH	12.85, s			5a, 6, 6a
9-OH	[b]			

*(a) assignments supported by HSQC and HMBC, (b - c) resonance not observed, (d) assignments are interchangeable within the same letter

Table S5. NMR (600 MHz, DMSO-d_6) data for aranciamycin (**4**)

Pos	δ_H, mult, (*J* in Hz)	δ_C[a]	COSY	$^1H - ^{13}C$ HMBC
1	7.74, d, (*7.3*)	119.2	2	3, 4a, 12
2	7.82, dd, (*8.3*, *7.3*)	137.4	1, 3	4, 12a
3	7.40, d, (*8.3*)	124.6	2	1, 4, 4a
4		162.7		
4a		116.5		
5		191.0		
5a		119.1		
6		161.6		
6a		133.5		
7	5.07, d, (*2.4*)	71.4	8	1′, 6, 6a, 8, 9, 10a
8	3.62, d, (*2.4)*	86.0	7	6a, 7, 8-OMe, 9, 9-Me, 10
9		76.1		
10		199.1	10β	
10a		135.3		
11	8.03, s	114.6	13	5a, 6a, 10, 12
11a		133.4	12	
12		181.1		
12a		133.8		
1′	5.44, d *(1.2)*	100.8	2′	2′, 3′, 5′, 7
2′	3.30[b]	80.6	1′, 3′	1′, 2′-OMe, 3′, 4′
3′	3.39, m	70.3	2′, 4′	1′, 2′
4′	3.22, ddd, (*9.4*, *9.0*, *6.0*)	71.8	3′, 5′	2′, 3′, 5′, 5′-Me
5′	3.66, dq, (*9.4*, *6.2*)	70.2	4′, 5′-Me	4′
5′-Me	1.24, d, (*6.2*)	17.8	5′	4′, 5′
9-Me	1.37, s	23.4		8, 9, 10
2′-OMe	3.42, s	58.6		2′
8-OMe	3.43, s	59.6		8
3′-OH	4.69, d *(6.0)*		3′	
4′-OH	4.94, d, (*6.0*)		4′	
4-OH	[c]			
6-OH	[c]			
9-OH	5.68, s		9-Me	8, 9, 9-Me, 10

*(a) assignments supported by HSQC and HMBC. (b) resonances obscured by water peak. (c) resonance not observed. (d) assignments are interchangeable

Table S6. NMR (600 MHz, DMSO-d_6) data comparison for aranciamycin I (**1**) and aranciamycin A (**3**)

pos	δ_H, mult, (*J* in Hz) **1**	δ_C[a]	δ_H, mult, (*J* in Hz) **3**	δ_C[a]
1	7.71, d, (*7.7*)	118.9	7.69, d, (*7.3*)	119.1
2	7.79, dd, (*8.3, 7.7*)	137.0	7.79, dd, (*8.0, 7.3*)	137.0
3	7.37, d, (*8.3*)	124.8	7.37, d, (*8.0*)	124.1
4		160.8		160.9
4a		116.2		115.8
5		191.3		[g]
5a		113.7		113.4
6		161.9		161.2
6a		131.9		130.9
7	4.91, dd *(6.4, 5.3)*	72.1	4.93, dd, (*6.6, 5.0*)	72.4
8α	2.12, dd, (*13.8*, *6.4*)	43.2	2.14, dd, (*13.6*, *6.6*)	42.7
8β	1.98, dd, (*13.8*, *5.3*)		1.98, dd, (*13.6*, *5.0*)	42.7
9		67.4		66.9
10α	2.96, d, (*17.0*)	44.5	2.96, d, (*17.0*)	44.1
10β	2.76, d, (*17.0*)		2.76, d, (*17.0*)	
10a		146.7		146.8
11	7.45, s	119.7	7.44, s	119.7
11a		131.9		[g]
12		181.5		180.9
12a		133.5		133.4
1′	5.03, d *(1.1)*	103.5	5.19, s	99.9
2′	3.60[b], m	70.6	3.26, m	80.7
3′	3.29[e], m	70.7	3.36, m	70.1
4′	3.22, ddd (*9.2*, *9.2*, *5.3*)	71.8	3.17, m	71.8
5′	3.59[b], m	69.3	3.58, dq *(9.2, 6.1)*	69.2
5′-Me	1.18, d (*6.2*)	17.9	1.17, d, (*6.1*)	17.6
9-Me	1.27, s	28.6	1.28, s	28.3
2′-OMe			3.39, s	58.2
2′-OH	4.73[c]			
3′-OH	4.45, d (*5.8*)		4.58, d, (*5.6*)	
4′-OH	4.73[c]		4.81, d, (*5.2*)	
4-OH	[d]		12.63[f], br s	
6-OH	[d]		12.01[f], br s	
9-OH	4.72[c]		4.75, s	

*(a) assignments supported by HSQC and HMBC. (b - c) resonances are overlapping within the same letter. (d, g) resonance not observed. (e) assignments obscured by water peak. (f) assignments are interchangeable.

Table S7. ^{1}H NMR (600 MHz, DMSO-d_6) data comparison for aranciamycin J (**2**) and aranciamycin (**4**)

pos	δ_H, mult, (*J* in Hz) **2**	δ_C[a]	δ_H, mult, (*J* in Hz) **4**	δ_C[a]
1	7.75, dd (*7.6*, *1.1*)	119.3	7.74, d, (*7.3*)	119.2
2	7.84, dd (*8.3*, *7.6*)	137.5	7.82, dd, (*8.3*, *7.3*)	137.4
3	7.42, dd (*8.3*, *1.1*)	124.4	7.40, d, (*8.3*)	124.6
4		160.9		162.7
4a		116.8		116.5
5		[c]		191.0
5a		118.2		119.1
6		[c]		161.6
6a		135.8		133.5
7	5.14, dd (*5.1*, *3.6*)	69.4	5.07, d, (*2.4*)	71.4
8α	2.50, dd *(14.6, 5.1)*	41.2	3.62, d, (*2.4)*	86.0
8β	2.25, dd (*14.6*, *3.6*)			
9		72.1		76.1
10		199.7		199.1
10a		[c]		135.3
11	8.09, s	115.5	8.03, s	114.6
11a		[c]		133.4
12		181.2		181.1
12a		133.0		133.8
1′	5.28, d, (*1.6*)	100.1	5.44, d *(1.2)*	100.8
2′	3.27, dd, (*3.2*, *1.6*)	80.5	3.30[c]	80.6
3′	3.40[b], m	70.1	3.39, m	70.3
4′	3.19, ddd, (*9.4*, *9.4*, *5.5*)	71.7	3.22, ddd, (*9.4*, *9.0*, *6.0*)	71.8
5′	3.63, dq, (*9.4*, *6.2*)	69.5	3.66, dq, (*9.4*, *6.2*)	70.2
5′-Me	1.20, d, (*6.2*)	17.6	1.24, d, (*6.2*)	17.8
9-Me	1.42, s	25.4	1.37, s	23.4
2′-OMe	3.40[b], s	58.2	3.42, s	58.6
8-OMe			3.43, s	59.6
3′-OH	4.63, d, (*5.8*)		4.69, d *(6.0)*	
4′-OH	4.86, d, (*5.5*)		4.94, d, (*6.0*)	
4-OH	[d]		[e]	
6-OH	[d]		[e]	
9-OH	5.66, s		5.68, s	

*(a) assignments supported by HSQC and HMBC. (b) resonances are overlapping within the same letter. (c) assignments obscured by water peak. (d - e) resonance not observed.

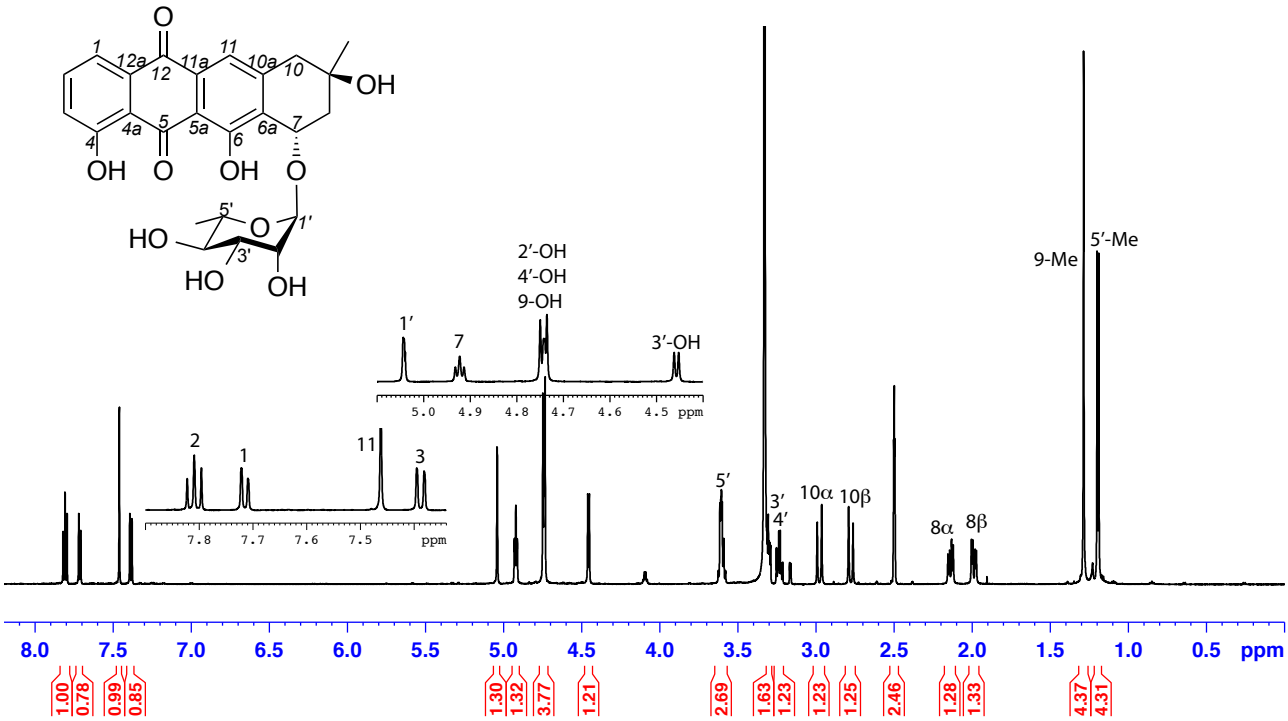


Figure S2. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of aranciamycin I (**1**)

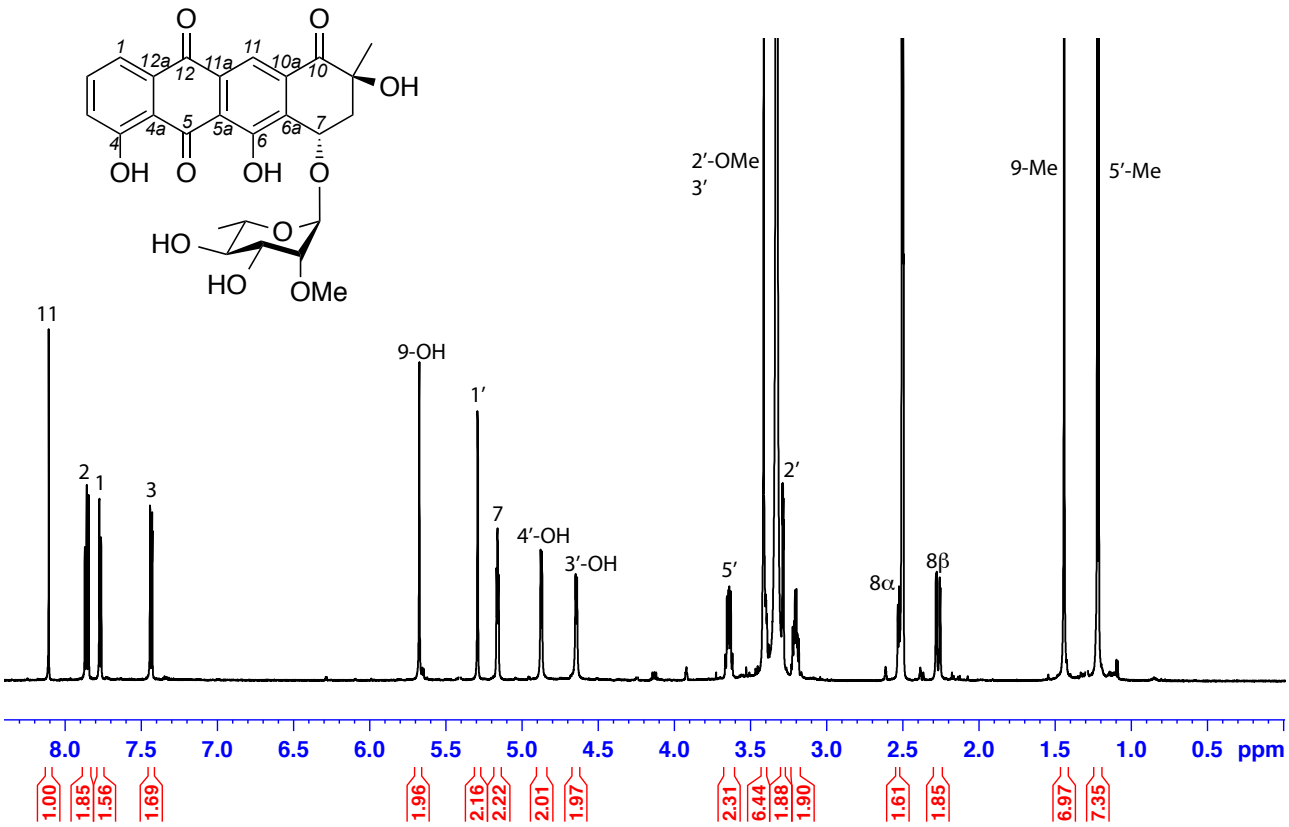


Figure S3. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of aranciamycin J (**2**)

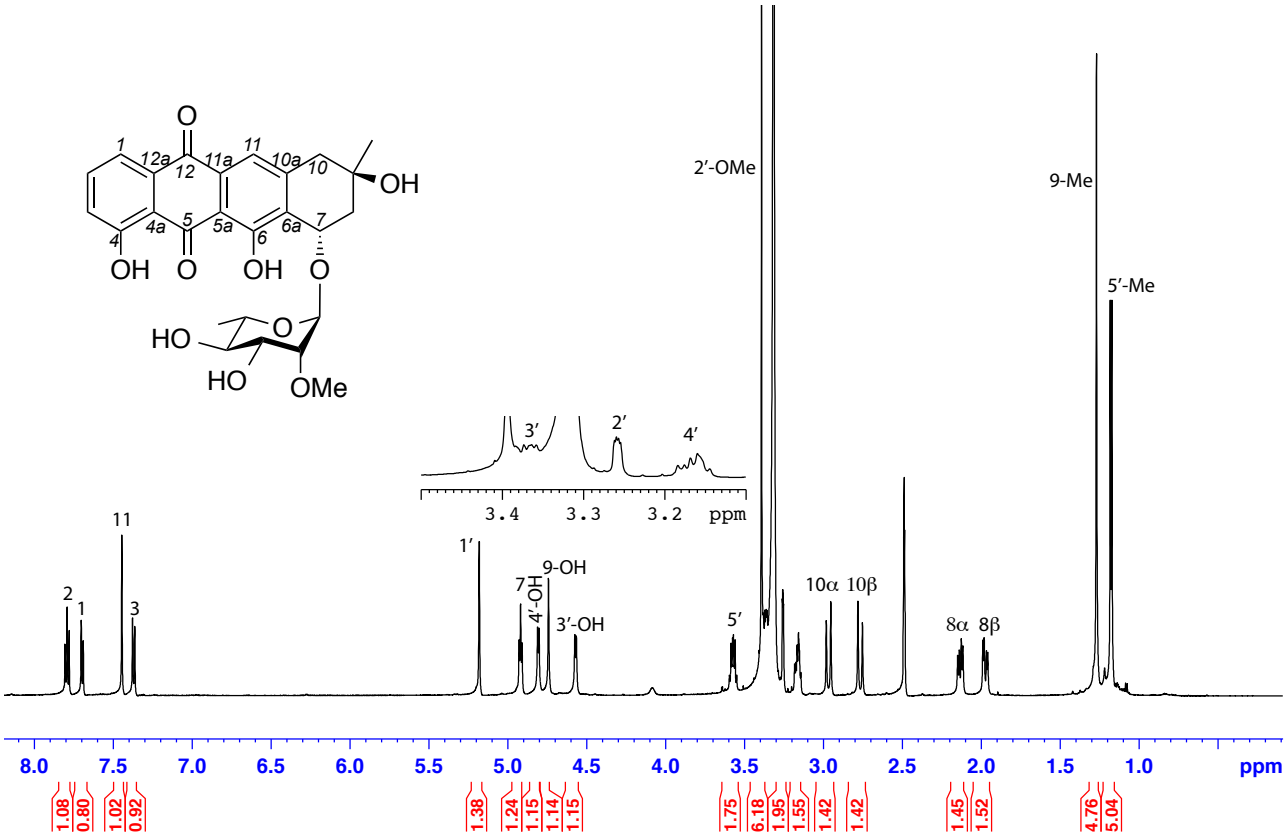


Figure S4. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of aranciamycin A (**3**)

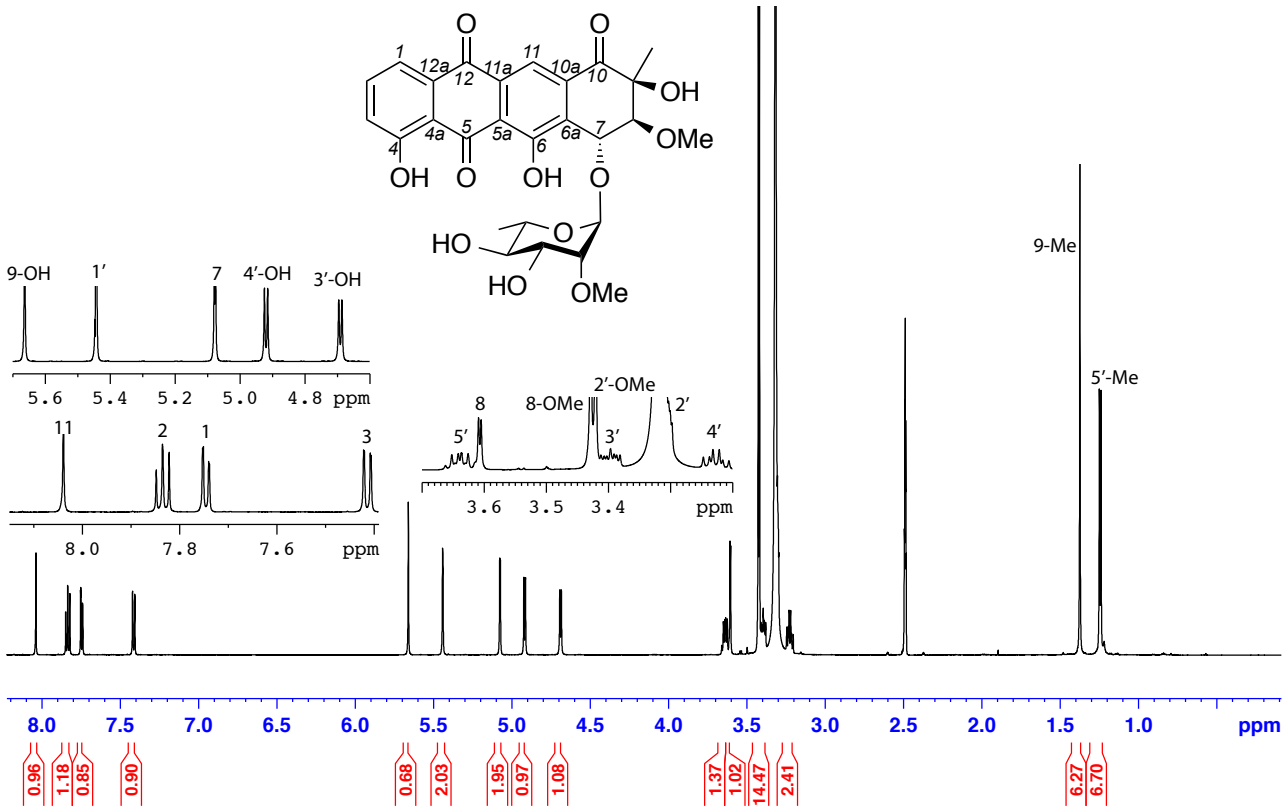


Figure S5. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of aranciamycin (**4**)

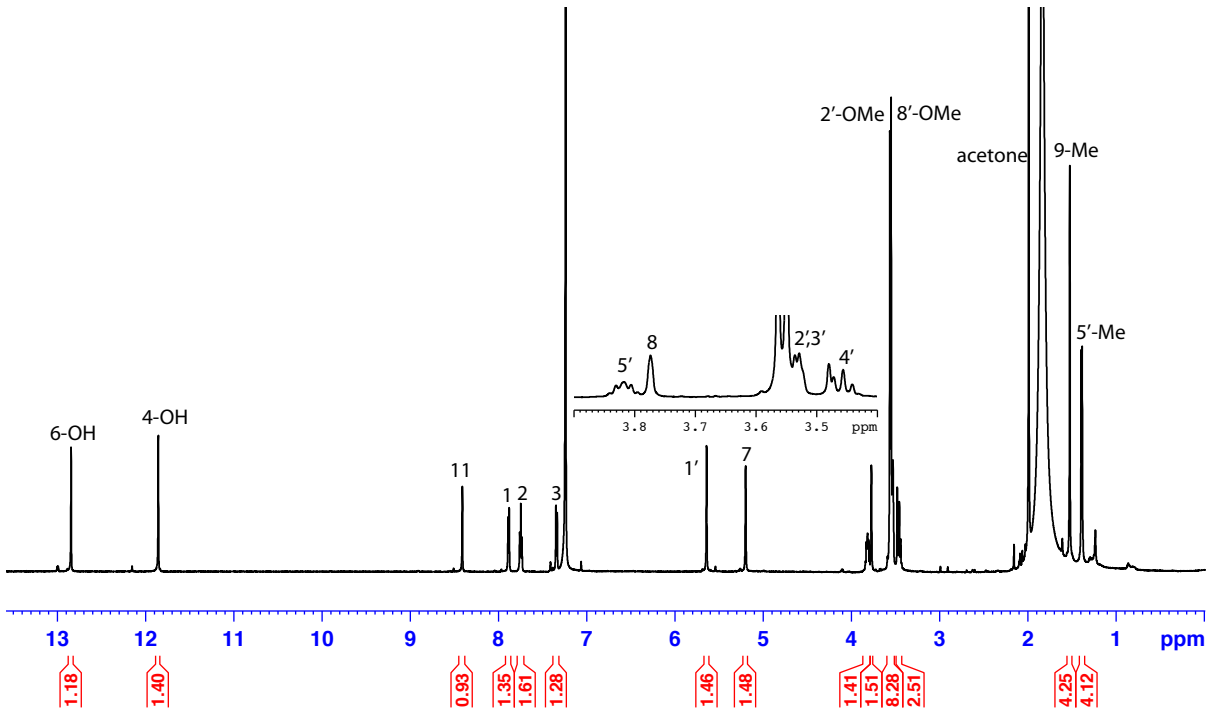


Figure S6. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of aranciamycin (**4**)

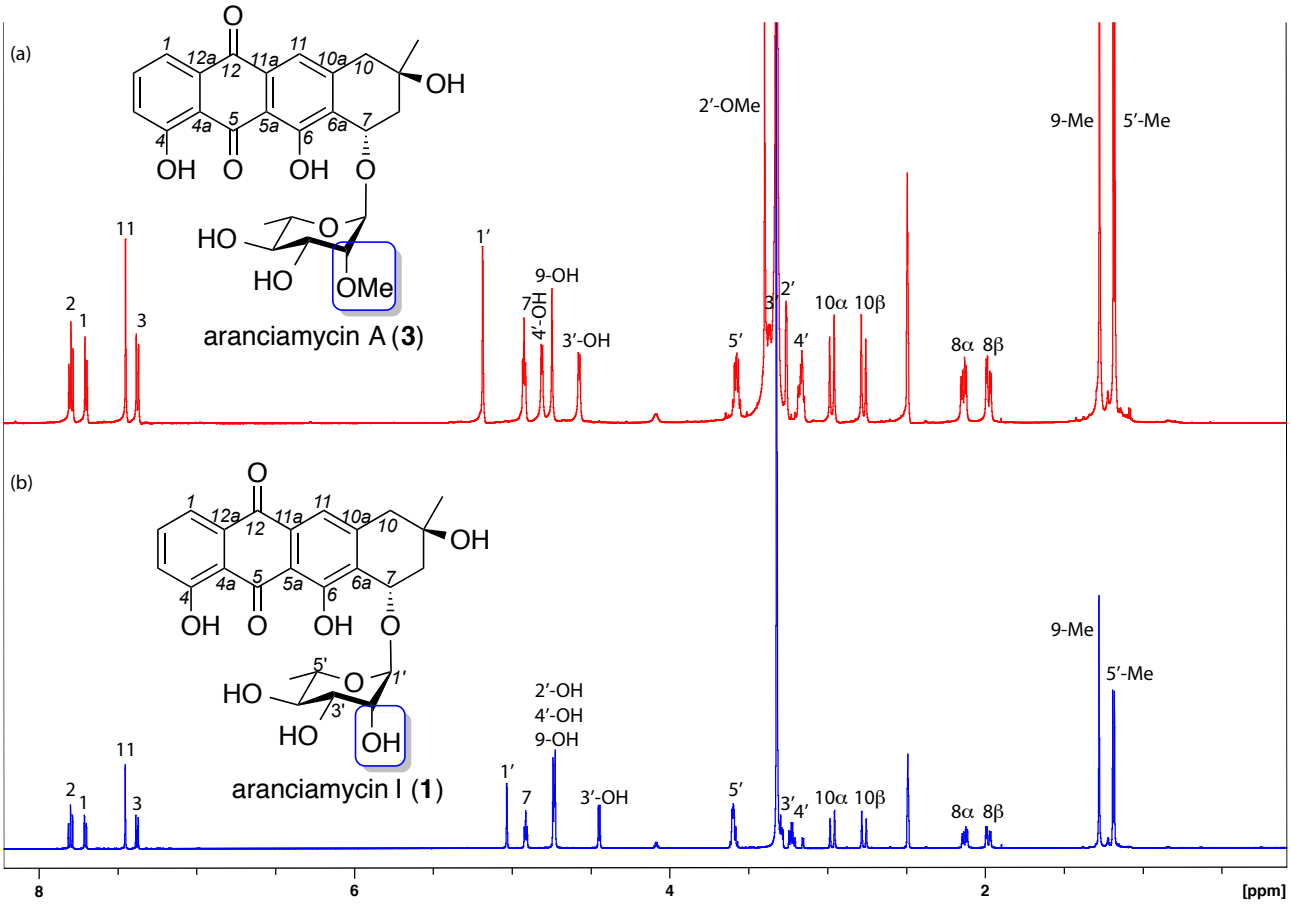


Figure S7. ^{1}H NMR (600 MHz, DMSO-d_6) spectra comparing (a) aranciamycin A (**3**) and (b) aranciamycin I **(1)**

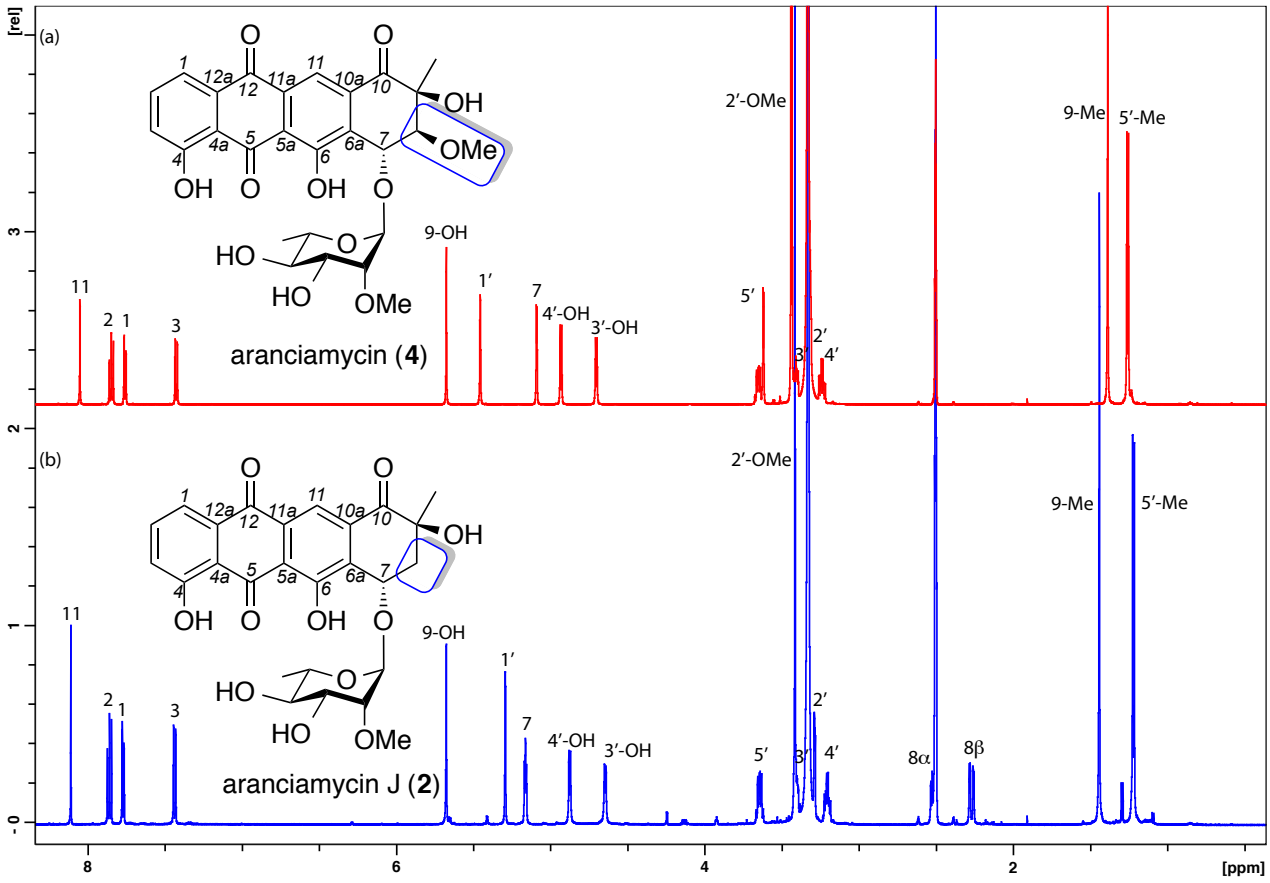


Figure S8. ^{1}H NMR (600 MHz, DMSO-d_6) spectra comparing (a) aranciamycin (**4**) and (b) aranciamycian J (**2**).

3 Biological Data

3.1 Antibiotic Screening/BCG screening data for 1−4

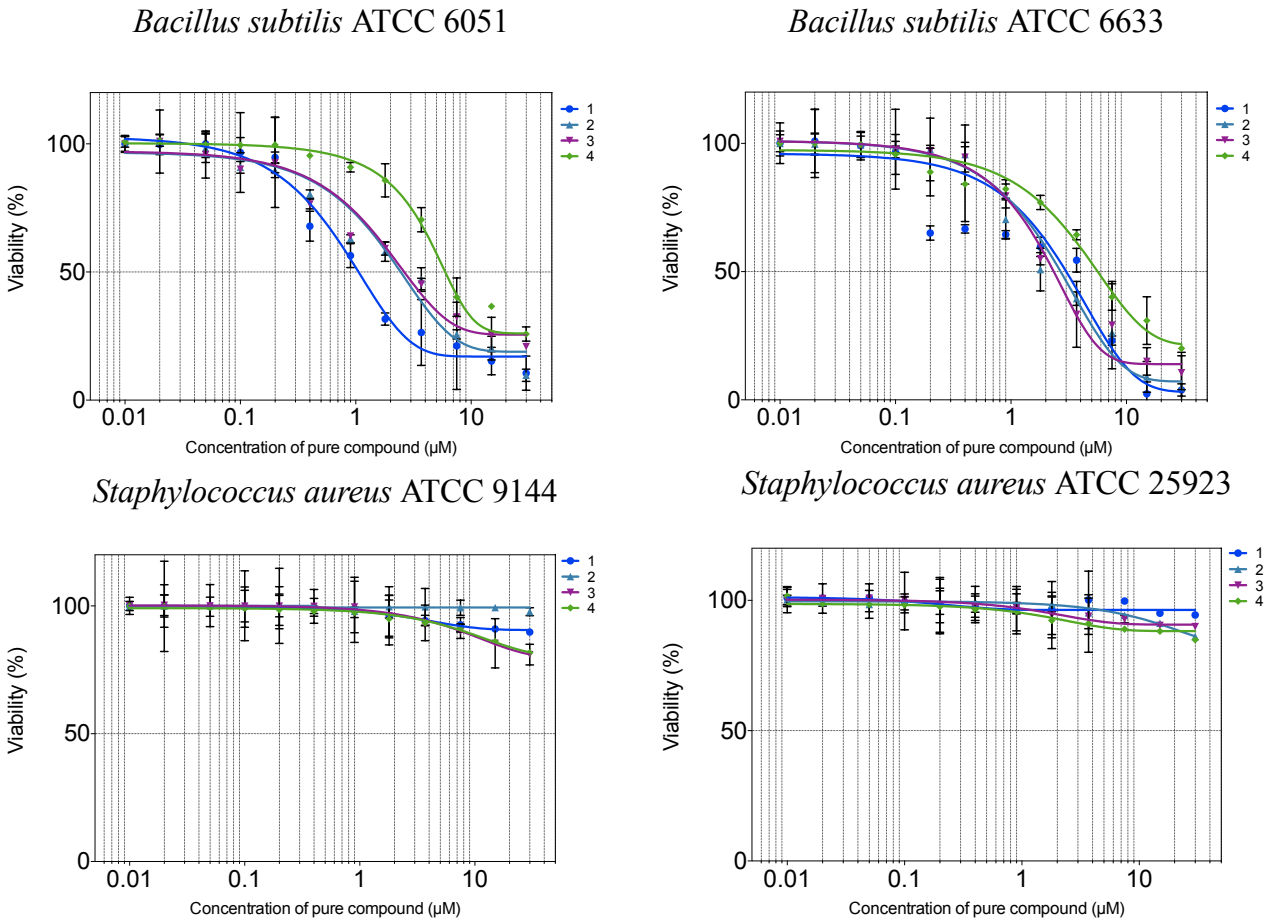
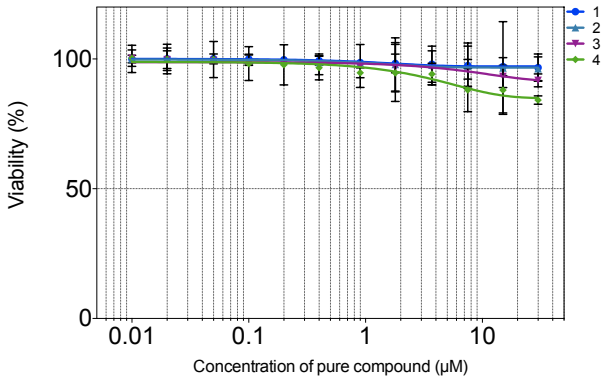
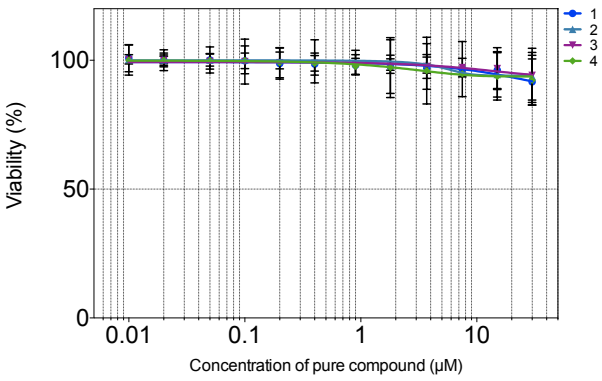


Figure S9. Antimicrobial assay screening graphs of aranciamycins **1**–**4**

Escherichia coli ATCC 11775



Pseudomonas aeruginosa ATCC 10145



Candida albicans ATCC 90028

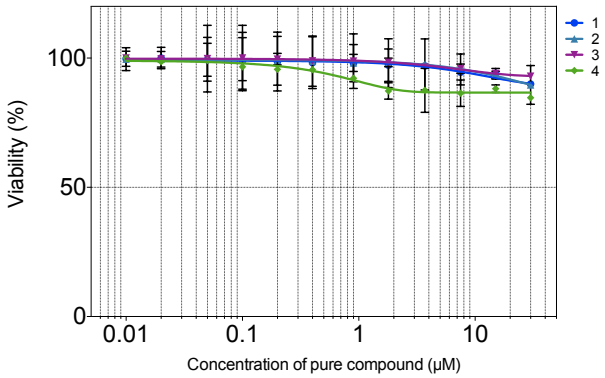


Figure S9. *Continued*, antimicrobial assay screening graphs of aranciamycins **1**–**4**

3.2 Cytotoxicity assay

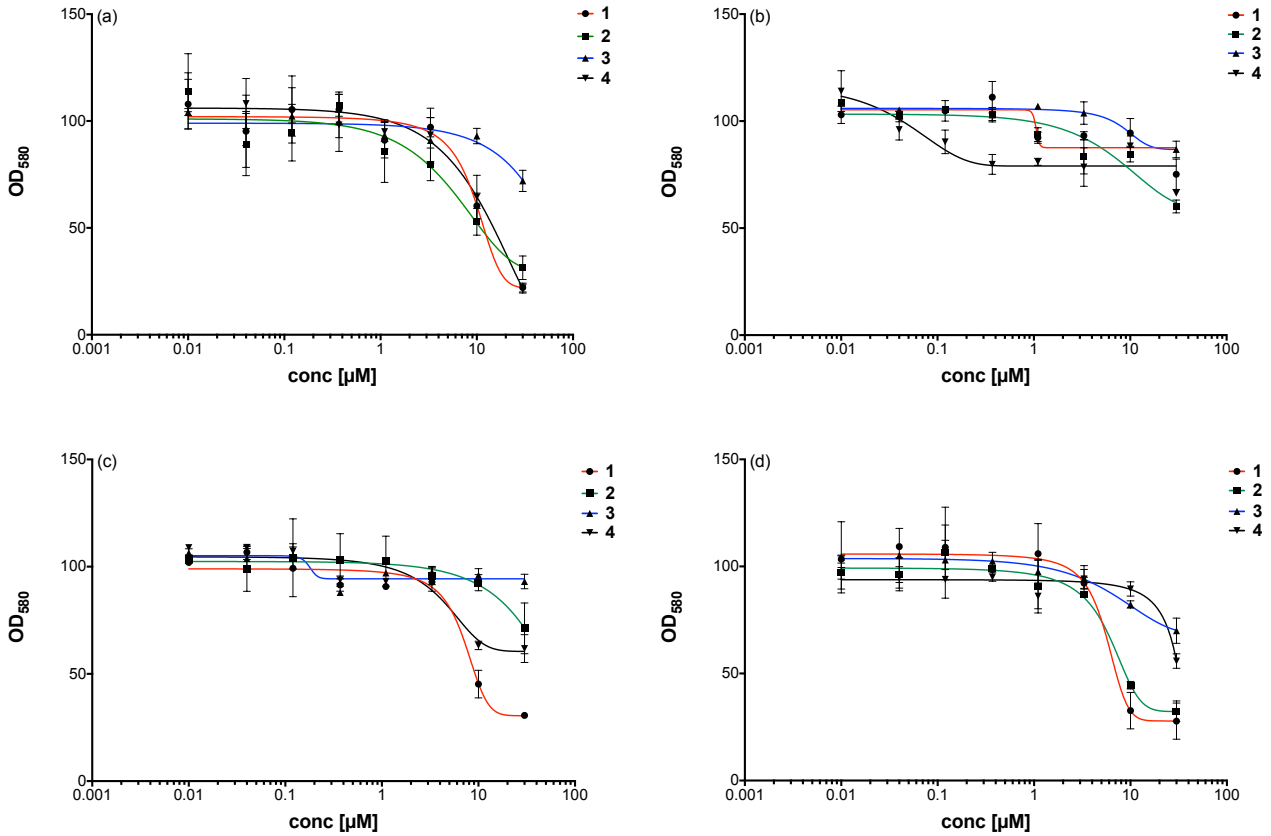


Figure S10. Cytotoxicity assay of the aranciamycins **1**–**4** against (a) HepG2 (human hepatocellular cancer cell line), (b) KB-3-1 (human cervical cancer cell line), (c) NCI-H460 (human lung cancer cell line), SW620 (human colorectal cancer cell line)

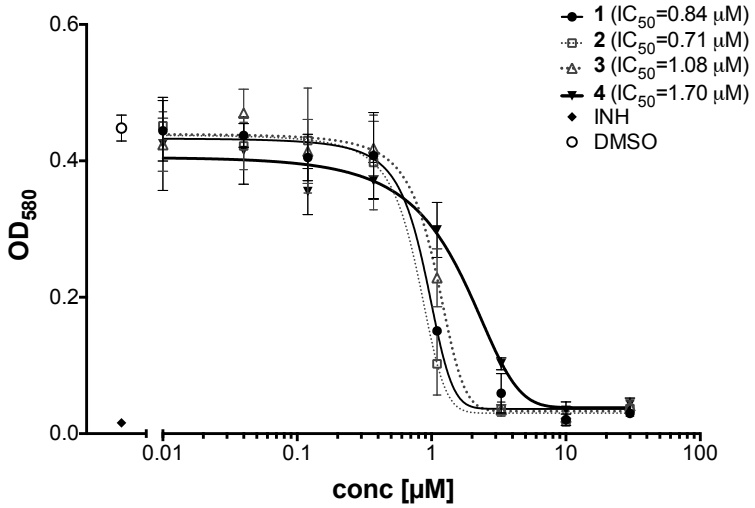


Figure S11. Optical density (OD) of *M. bovis* BCG cultures after 7 days growth in the presence of 0.01–30 μM of aranciamycins **1**–**4**. Isoniazid (INH; 20 μg/mL) and DMSO served as positive and negative controls respectively.

4 References

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