

Supplementary Information

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S1. Isolation of Chromomycin A3

Attempting to isolate compound **2** in order to compare the results with the ones obtained with compound **1**, the bacterial strain was regrown again under the same conditions. The culture broth (21.9 L), after fermentation, was extracted with EtOAc and subsequently the solvent was removed under reduced pressure to yield 708.6 mg of crude extract. This extract was subjected to a silica gel chromatography column using the solvents hexane/CH₂Cl₂ 1:1, CH₂Cl₂, CH₂Cl₂/EtOAc 1:1, EtOAc, EtOAc/MeOH 8:2, EtOAc/MeOH 1:1 and MeOH to yield fractions A – G. Fractions D and E were combined, since both showed similar cytotoxicity and chemical profile by TLC analysis. Fraction D/E (248.6 mg) was chromatographed on SPE C-18 cartridge (Strata C18-E, 20 g/60 mL, 55 μm, 70 Å), eluting with H₂O/MeOH 8:2, 6:4, 4:6, 2:8, MeOH and EtOAc/MeOH 1:1. After cytotoxic assays and TLC analysis, the three last fractions were combined and rechromatographed on silica gel employing the solvents CH₂Cl₂/MeOH 9:1, 8:2, 7:3 1:1 and MeOH. Fraction CH₂Cl₂/MeOH 7:3 (6.4 mg) was subjected to semipreparative HPLC (Phenomenex C₁₈ 4.6 × 250 mm, gradient H₂O/CH₃OH from 60% to 100% CH₃OH in 20 min, flow rate 4.7 mL/min, UV detection 210 – 400 nm) to yield compound **2** (tr 14.65, 0.4 mg). Compound **2** was identified by HRESIMS, UV data and AntiMarin data base as chromomycin A3 (**2**) λ_{max} 413.87, 277.53 and 228.76 nm; HRESIMS *m/z* 1205.4865 [M + Na]⁺.

Figure S3. HR-ESI-MS spectrum of chromomycin A3 (**2**).

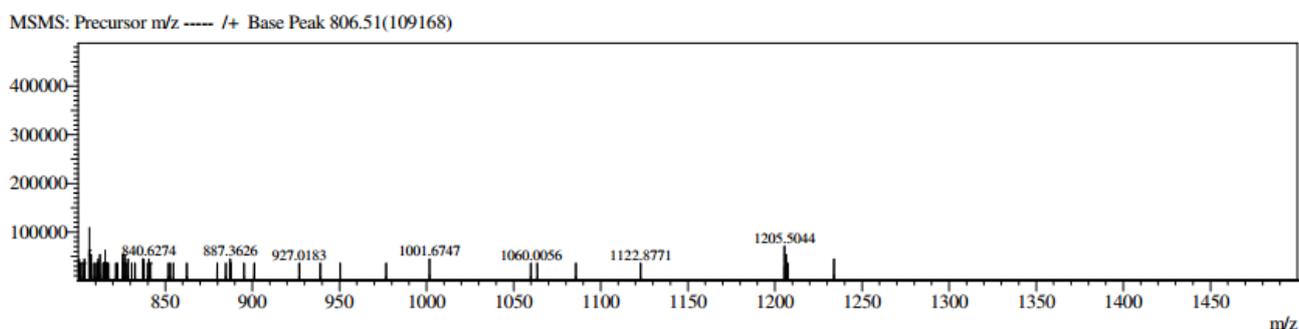


Figure S4. UV spectrum of chromomycin A3 (2).

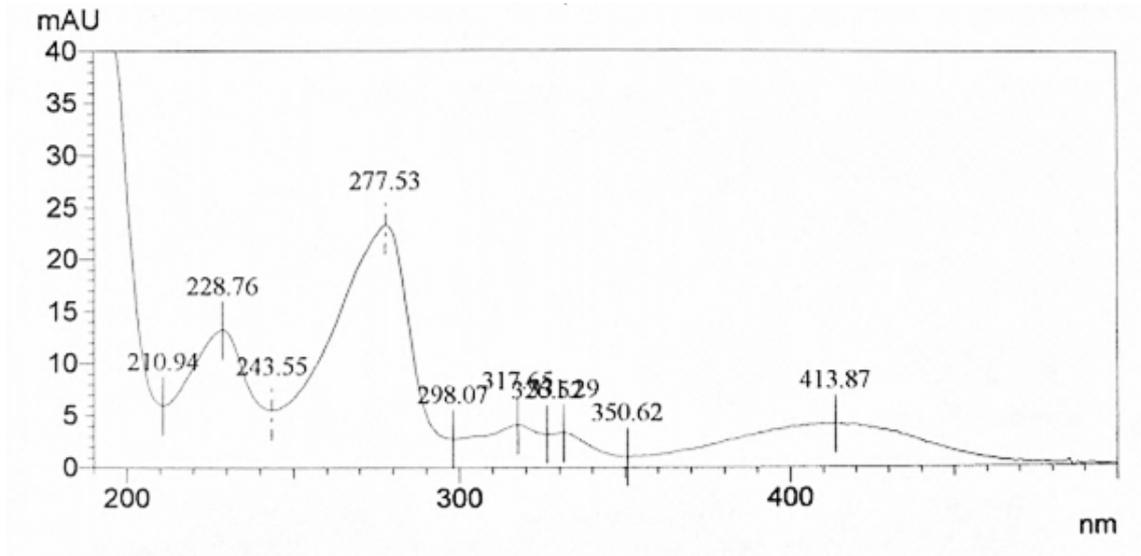


Figure S5. Cell viability analysis of MALME-3M after 48h treatment. The gating strategy for flow cytometry analysis is presented as follows. All cells were identified using SSC-A vs. FCS-A and doublets excluded using FSC-A vs. FSC-H. Non-viable cells show high PI fluorescence in FL3 channel (red fluorescence). Cell density was measured using an additional gate to fit normal cell morphology.

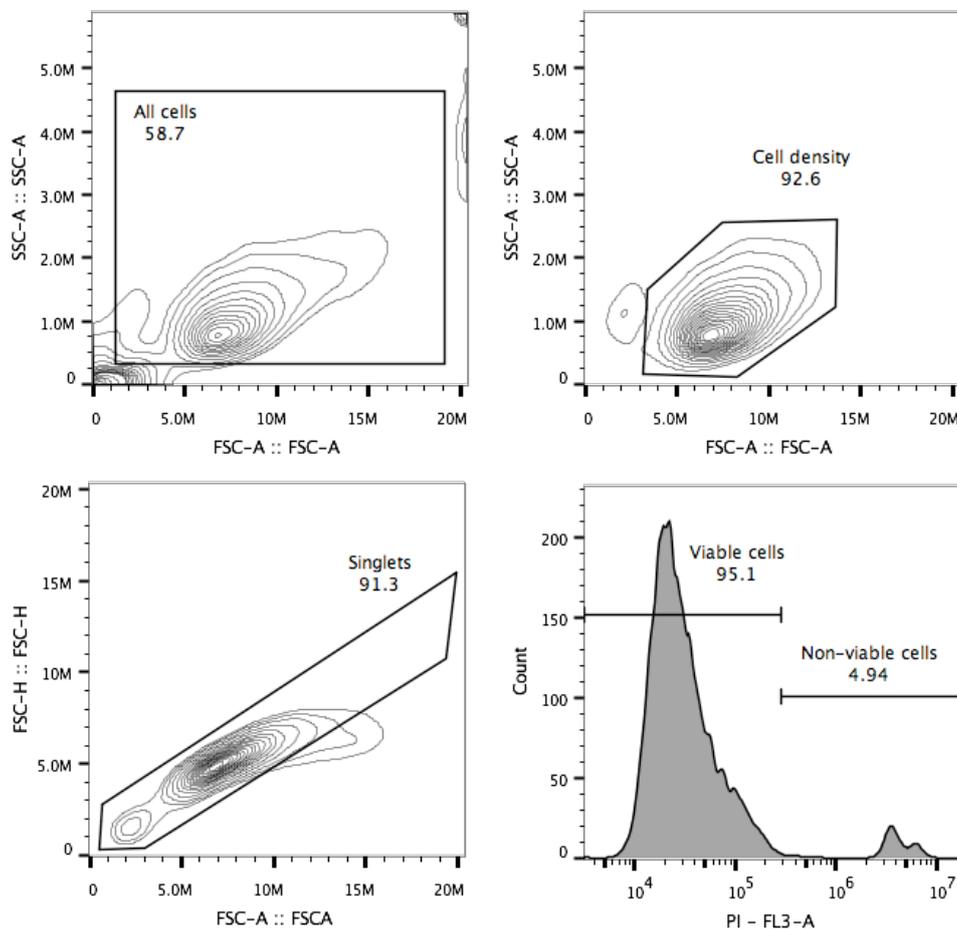


Figure S6. Cell cycle analysis of MALME-3M treated with 10 and 30 nM **1** for 48h. (a) Gating strategy for flow cytometry analysis. Cells were identified using SSC-A vs. FCS-A and gated for singlets using PI-H vs. PI-A; (b) Representative histograms of each treatment analyzed and modeled by Modfit LT 4.0 program. DMSO (0.4% v/v) and doxorubicin (100 nM) and rapamycin (30 nM) were used, respectively, as negative (−) and positive (+) controls.

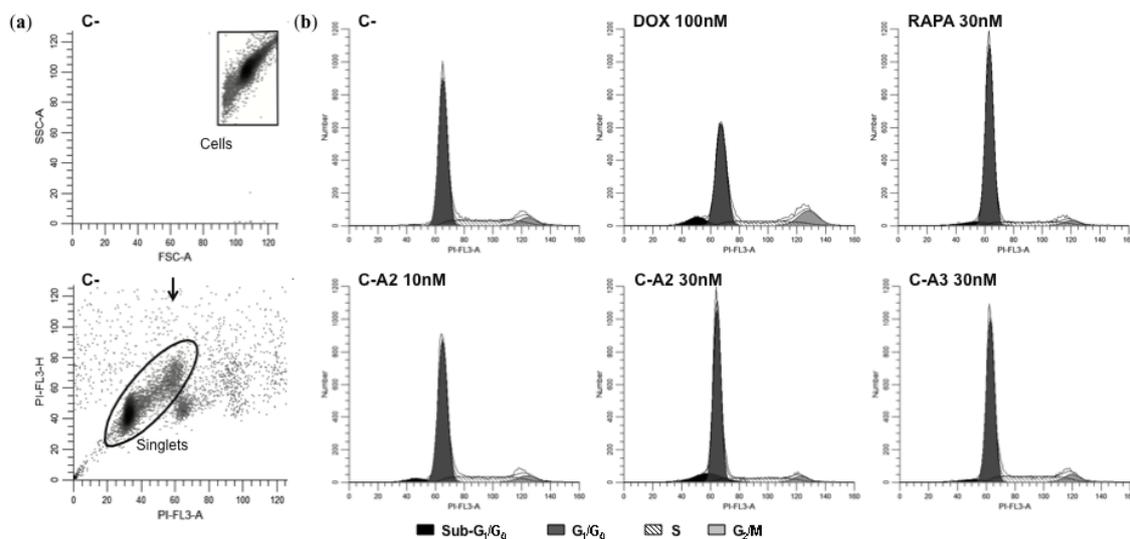


Table S1. Cytotoxicity of doxorubicin on selected cell lines. Activity of doxorubicin was evaluated using the MTT assay after 72 h incubation. IC₅₀ [nM] values and 95% CI were obtained via nonlinear regression.

Cell Line	IC ₅₀ [nM] (CI 95%)
	72 h
HCT-116	42 (30–59)
HL-60	6 (4–8)
MALME-3M	244(179–334)
OVCAR-8	36(25–50)
PC-3M	0.9922 (0.5204–1.892)
SF-295	26 (18–36)
MRC-5	3828 (2105–6960)