Defining the Mode of Action of Tetramic Acid Antibacterials Derived from *Pseudomonas aeruginosa* Quorum Sensing Signals

Colin A. Lowery[1,2], Junguk Park[1,2], Christian Gloeckner[1,2], Michael M. Meijler[1,2,3], Ryan S. Mueller[4], Helena I. Boshoff[5], Ricky L. Ulrich[6], Clifton E. Barry 3rd, 5, Douglas H. Bartlett[4], Vladimir V. Kravchenko[2], Gunnar F. Kaufmann[1,2], Kim D. Janda[1,2,7]**

[1]The Skaggs Institute for Chemical Biology and Departments of Chemistry and [2]Immunology, The Scripps Research Institute, La Jolla, California. [3]Current Address: Department of Chemistry, Ben-Gurion University of Negev, Be'er Sheva, Israel. [4]Marine Biology Research Division, Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego, La Jolla, California. [5]Tuberculosis Research Section, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland. [6]Bacteriology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland 21704. [7]Worm Institute for Research and Medicine (WIRM), The Scripps Research Institute, La Jolla, California.

General Procedure for the Synthesis of Tetramic Acids. The synthesis of the tetramic acids was performed according to the reported procedures. The synthesis of (*S*)-3-(1-hydroxydecylidene)-5-(2-hydroxyethyl)pyrrolidine-2,4-dione **(C_{12}-TA)** is described as a representative example. To a round bottom flask containing 3-oxo-C_{12}-AHL (37 mg, 0.125 mmol) in dry MeOH (0.5 ml), freshly prepared NaOMe in MeOH (0.5 M, 0.249 ml, 0.125 mmol) was added at room temperature under argon. The solution was stirred at 55°C for 3 h, at which point the reaction mixture was passed through acidic ion-exchange

resin (Dowex 50WX2-200, ≈2 cm^3) and eluted with MeOH (20 ml). The filtrate was concentrated under reduced pressure, and the tetramic acids were purified by reverse-phase HPLC on a dual-pump Rainin Dynamax HPLC system (Rainin Instruments) equipped with a Vydac 214TP101522 column (Hesperia, CA) at a flow rate of 10 ml/min with detection at 230 nm. In each preparative separation, the crude material (40 mg) was dissolved in 5 ml of 45:45:10 acetic acid/water/DMSO and filtered through a 0.45-μm poly(vinylidene difluoride) filter. The HPLC purifications used a gradient of 40-60% solvent B (0.085% trifluoroacetic acid in acetonitrile) in solvent A (0.1% trifluoroacetic acid in water) over 30 min. Fractions containing product were confirmed by electrospray ionization-MS on a Sciex API-150EX single quadrupole mass spectrometer (DP 10 V, FP 50 V) operated in multichannel analysis mode, pooled, and lyophilized. Yield: 65% (24 mg). ^{1}H NMR (600 MHz, CD_3OD): δ 0.90 (t, *J* = 7.0 Hz, 3 H), 1.23-1.43 (m, 12 H), 1.66 (qt, *J* = 7.4 Hz, 2 H), 1.71-1.82 (m, 1 H), 1.96-2.08 (m, 1 H), 2.72-2.94 (m, 2 H), 3.62-3.78 (m, 2 H), 3.97 (br s, 1H); $[\alpha]^{25}_d$ = +14.0 (*c* = 7.20, MeOH). [Previously characterized by Kaufmann et al. Proc Natl Acad Sci USA **2005**, *102*, 309-14.] (*R*)-C_{12}-TA: $[\alpha]^{25}_d$ = -13.8 (*c* = 6.9, MeOH).

Disruption of biofilms

Staphylococcus aureus biofilm was grown in a polystyrene 96-well plate using 150 uL CYGP medium as described previously. After 24 h incubation at 37°C, the planktonic cells were removed by pipetting and the wells were washed gently three times with 200 uL of sterilized PBS (pH = 7.4). Freshly prepared CYGP medium containing C12-TA (200 ug/mL) was added to the wells and incubated 24 h at 37 degree. No planktonic cells were grown after 24 h, and the remaining biofilm was quantified using crystal violet. Gentamycin was used as a standard.

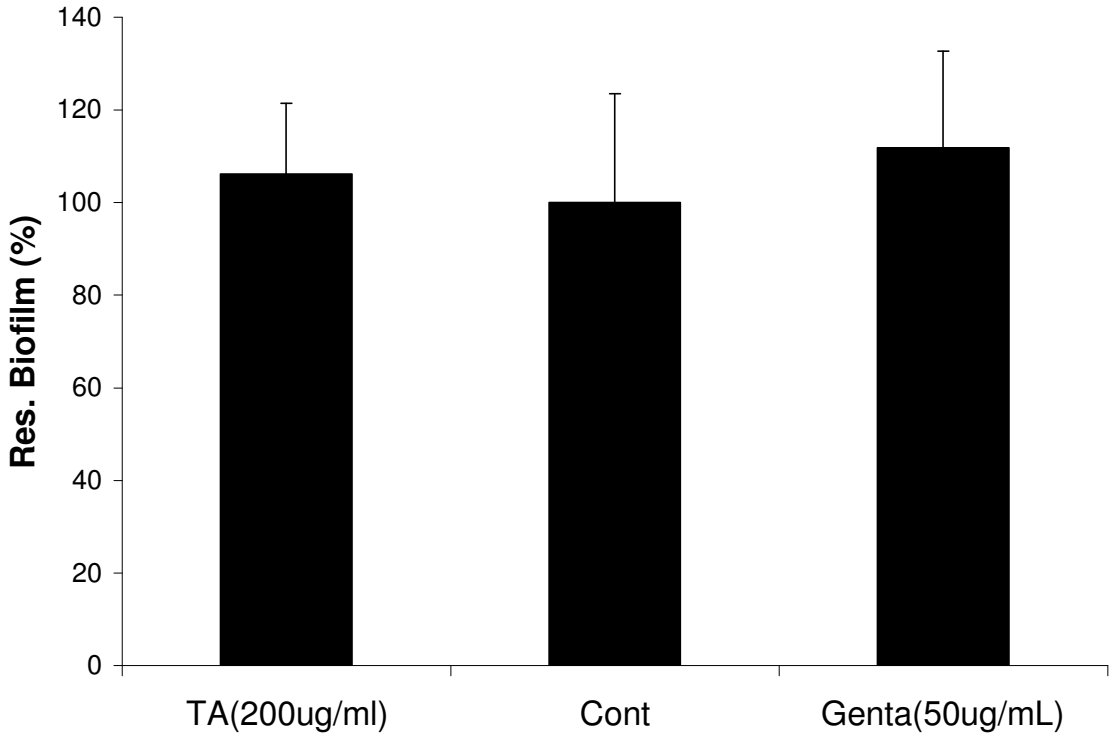

Figure S1. Biofilm dispersal by C_{12}-TA (Cont = control, Genta = Gentamycin).