Experimental Section

Synthesis Experimental

All reagents used for chemical synthesis were purchased from commercially available sources and used without further purification. Chromatography was performed using 60 Å mesh standard grade silica gel from Sorbtech. NMR solvents were obtained from Cambridge Isotope Labs and used as is. All $^1$H NMR (300 MHz or 400 MHz) and $^{13}$C NMR (75 MHz or 100 MHz) spectra were recorded at 25ºC on Varian Mercury spectrometers. Chemical shifts (δ) are given in ppm relative to tetramethylsilane or the respective NMR solvent; coupling constants (J) are in hertz (Hz). Abbreviations used are s = singlet, bs = broad singlet, d = doublet, dd = doublet of doublets, t = triplet, dt = doublet of triplets, bt = broad triplet, qt = quartet, m = multiplet, bm = broad multiplet, p = pentet, and br = broad. Mass spectra were obtained at the NCSU Department of Chemistry Mass Spectrometry Facility. Funding was obtained from the North Carolina Biotechnology Center and the NCSU Department of Chemistry. Infrared spectra were obtained on a FT/IR-4100 spectrophotometer ($v_{\text{max}}$ in cm⁻¹). UV absorbance was recorded on a Genesys 10 scanning UV/visible spectrophotometer ($\lambda_{\text{max}}$ in nm).
Procedure to Determine the Inhibitory Effect of Test Compounds on *S. aureus*, PA14, and *A. baumannii* Biofilm Formation: Inhibition assays were performed by taking an overnight culture of bacterial strain and subculturing it at an OD$_{600}$ of 0.01 into the necessary medium (tryptic soy broth with a 0.5% glucose supplement (TSBG) for MRSA (ATCC # BAA-44) and *S. aureus* (ATCC # 29213), Luria-Bertani (LB) medium for *A. baumannii* (ATCC # 19606) and Luria-Bertani medium without NaCl (LBNS) for PA14. Stock solutions of predetermined concentrations of the test compound were then made in the necessary medium. These stock solutions were aliquoted (100 µL) into the wells of the 96-well PVC microtiter plate. Sample plates were then wrapped in GLAD Press n’ Seal® followed by an incubation under stationary conditions for 24 h at 37 °C (6 h for PA14). After incubation, the medium was discarded from the wells and the plates were washed thoroughly with water. Plates were then stained with 100 µL of 0.1% solution of crystal violet (CV) and then incubated at ambient temperature for 30 min. Plates were washed with water again and the remaining stain was solubilized with 200 µL of 95% ethanol. A sample of 125 µL of solubilized CV stain from each well was transferred to the corresponding wells of a polystyrene microtiter dish. Biofilm inhibition was quantitated by measuring the OD$_{540}$ of each well in which a negative control lane wherein no biofilm was formed served as a background and was subtracted out.
S. aureus

MRSA

Percent inhibition of biofilm inhibition

Concentration (µM)

Percent inhibition of biofilm inhibition

Concentration (µM)
Procedure to Determine the Dispersal Effect of Test Compounds on *S. aureus*, Preformed Biofilms: Dispersion assays were performed by taking an overnight culture of bacterial strain and subculturing it at an $\text{OD}_{600}$ of 0.01 into the necessary medium (tryptic soy broth with a 0.5% glucose supplement (TSBG) for *S. aureus*). The resulting bacterial suspension was aliquoted (100 µL) into the wells of a 96-well PVC microtiter plate. Plates were then wrapped in GLAD Press n’ Seal® followed by an incubation under stationary conditions at 37 °C to establish the biofilms. After 24 h, the medium was discarded from the wells and the plates were washed thoroughly with water. Stock solutions of predetermined concentrations of the test compound were then made in the necessary medium. These stock solutions were aliquoted (100 µL) into the wells of the 96-well PVC microtiter plate with the established biofilms. Medium alone was added to a subset of the wells to serve as a control. Sample plates were then incubated for 24 h at 37 °C. After incubation, the medium was discarded from the wells and the plates were washed thoroughly with water. Plates were
then stained with 100 µL of 0.1% solution of crystal violet (CV) and then incubated at ambient temperature for 30 min. Plates were washed with water again and the remaining stain was solubilized with 200 µL of 95% ethanol. A sample of 125 µL of solubilized CV stain from each well was transferred to the corresponding wells of a polystyrene microtiter dish. Biofilm dispersion was quantitated by measuring the OD$_{540}$ of each well in which a negative control lane wherein no biofilm was formed served as a background and was subtracted out.

**Growth Curve Analysis**

**Procedure to Determine the Effect of Test Compounds on PA14 and A. baumannii**

**Planktonic Viability via Growth Curve Analysis:** Growth curves were performed by taking an overnight culture of bacterial strain and subculturing it at an OD$_{600}$ of 0.01 into the necessary medium (Luria-Bertani (LB) medium for A. baumannii and Luria-Bertani medium without NaCl (LBNS) for PA14. The resulting bacterial suspension was then aliquoted (3.0 mL) into culture tubes. The test compound was then added at a predetermined concentration to the medium of the test samples. Controls were employed in which no test compound was added to the bacterial suspension. Samples were then placed in an incubator at 37 °C and shaken at 200 rpm. The OD$_{600}$ of the samples was measured at time intervals starting at 2 hours and ending at 24 hours.
Broth microdilution method for antibiotic resensitization: Mueller–Hinton broth (MHB) was inoculated (5x10^5 CFU/mL) with MRSA (BAA-44). Aliquots (4 mL) of the resulting bacterial suspension were distributed to culture tubes and compound, from 100 mm DMSO stock, was added to give the final testing concentration. Bacteria not treated with compound served as the control. After sitting for 30 min at room temperature, 1 mL of each sample was transferred to a new culture tube and oxacillin sodium salt was added from 128 mg/mL H₂O stock to give a final concentration of 128 mg/mL. Rows 2–12 of a 96-well microtiter plate were filled (100 mL per well) from the remaining 3 mL bacterial subcultures, allowing the concentration of compound to be kept uniform throughout the antibiotic dilution procedure. After standing for 10 min, aliquots (200 mL) of the samples containing antibiotic were distributed to the corresponding first-row wells of the microtiter plate. Row 1 wells were mixed six to eight times, and then 100 µL were transferred to row 2. Row 2 wells were mixed six to eight times, followed by a 100 µL transfer from row 2 to row 3. This procedure was repeated to serially dilute the rest of the rows of the
microtiter plate, with the exception of the final row, to which no antibiotic was added (to check for growth of bacteria in the presence of compound alone). The plate was then covered and incubated under stationary conditions at 37°C. After 16 h, MIC values were recorded as the lowest concentration of antibiotic at which no visible growth of bacteria was observed. To ensure that the compounds were non-toxic growth curve analysis was also performed at the tested concentrations in MHB media.

![MRSA Graph](image-url)
MRSA

![Graph showing bacterial growth over time with control and 38 50 uM treatments.](image)

**Y-axis:** OD₆₀₀ (abs)

**X-axis:** Time (Hr)

- **Control** (blue diamonds)
- **38 50 uM** (red squares)
NMR Spectra