

## Electronic Supplementary Information

### **5-Benzylidene-4-oxazolidinones potently inhibit biofilm formation in Methicillin-resistant *Staphylococcus aureus***

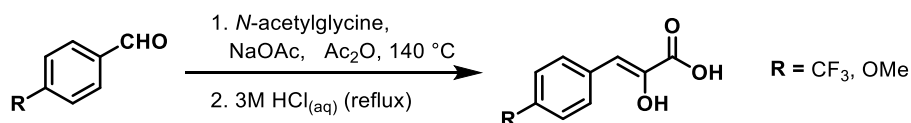
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#### **Contents**

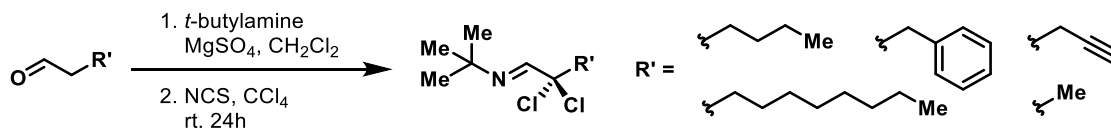
General Information – Synthesis .....	S2
Preparation of 2-Oxo-3-arylpropanoic acids.....	S2
Preparation of <i>N-tert</i> -butyl-2,2-dichloroimines.....	S2
Preparation of 5-arylidene-4-oxazolidinones.....	S3
Characterization of 5-arylidene-4-oxazolidinones .....	S3
References .....	S6
General Information – Biology.....	S7
Growth curves .....	S9
Biofilm Inhibition Data.....	S10
Biofilm Dispersion Data .....	S17
<sup>1</sup> H and <sup>13</sup> C NMR Spectra.....	S24

**General information:** THF was purified using an alumina filtration system. Aldehydes were purchased from a commercial chemical company and used as received. Reactions were monitored by TLC analysis (pre-coated silica gel 60 F<sub>254</sub> plates, 250 mm layer thickness) and visualization was accomplished with a 254 nm UV light and by staining with a KMnO<sub>4</sub> solution (1.5 g of KMnO<sub>4</sub>, 10 g of K<sub>2</sub>CO<sub>3</sub>, and 1.25 mL of a 10% NaOH solution in 200 mL of water). Reactions were also monitored by LC-MS (2.6 mm C18 50 x 2.10 mm column). Flash chromatography on SiO<sub>2</sub> was used to purify the crude reaction mixtures and performed on a flash system utilizing pre-packed cartridges and linear gradients. Melting points were determined using a capillary melting point apparatus. Infrared spectra were determined on a FT/IR spectrometer. <sup>1</sup>H and <sup>13</sup>C NMR spectra were obtained on a 400 or 700 MHz instrument in CDCl<sub>3</sub> or (CD<sub>3</sub>)<sub>2</sub>CO as indicated. Chemical shifts were reported as observed in parts per million with the residual solvent peak used as an internal standard (CDCl<sub>3</sub> = 7.26 ppm for <sup>1</sup>H and 77.16 ppm for <sup>13</sup>C). <sup>1</sup>H NMR spectra were run at 300, 400, or 700 MHz and are tabulated as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, bs = broad singlet, dt = doublet of triplet, tt = triplet of triplet), number of protons, and coupling constant(s). <sup>13</sup>C NMR spectra were run at 100 or 175 MHz using a proton-decoupled pulse sequence with a d<sub>1</sub> of 1 second unless otherwise noted, and are tabulated by observed peak. High-resolution mass spectra were obtained on an ion trap mass spectrometer using heated electrospray ionization (HESI).



**Figure S1.** Synthesis of 2-oxo-3-arylpropanoic acids

2-oxo-3-arylpropanoic acids were prepared according to established literature procedures<sup>1,2,3</sup>. **In brief:** A slurry of aldehyde (1 equiv.), sodium acetate (1.3 equiv.) and *N*-acetylglycine (1.3 equiv.) in acetic anhydride (5 equiv.) was heated to 140 °C for 1 h. The reaction mixture was quenched with ice (~20 mL) with vigorous stirring and cooled in an ice bath. The resulting precipitate was collected by vacuum filtration to afford the azlactone, which was used without further purification. The azlactone was suspended in 3 M HCl and heated at reflux until complete hydrolysis was observed by LC-MS (typically ~3 h). The reaction mixture was cooled in an ice bath to facilitate crystallization. The resulting solid was isolated by vacuum filtration and dried extensively under high vacuum to provide the desired acid as a solid. This solid can be recrystallized from benzene if necessary. Analytical data was consistent with that previously reported.

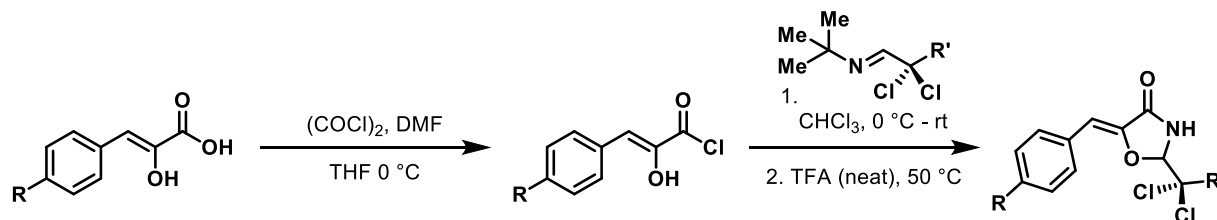


**Figure S2.** Synthesis of *N*-*tert*-butyl-2,2-dichloroimines

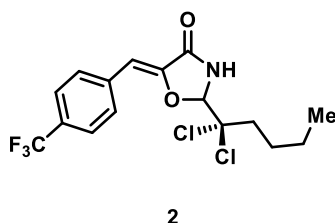
*N*-*tert*-butyl-2,2-dichloroimines were prepared according to an established literature procedure<sup>4</sup>. **In brief:** To a solution of aldehyde in dry dichloromethane (0.2 M) was added dropwise *t*-butyl amine (1.0 equiv.) with vigorous stirring. To this solution was added anhydrous magnesium sulfate (~3 equiv.) and the mixture was stirred vigorously for ~12 h at room temperature. The MgSO<sub>4</sub> was removed by vacuum filtration and the filtrate was concentrated *in vacuo*. The residue was dissolved in CCl<sub>4</sub> (0.5 M) and cooled to 0 °C, before addition of *N*-chlorosuccinimide (2.1 equiv) in four equal portions over the course of 2 h. This mixture was stirred at room temperature for 24 h. The mixture was cooled to 0 °C and filtered through a fine (4-5.5 μm) sintered glass frit funnel to remove succinimide (it may take several filtrations). The filtrate was concentrated *in vacuo* and dried extensively under high vacuum. The resulting α,α-dichloroimines were used without purification and can be stored for several months under inert atmosphere at -30 °C. *N*-*tert*-butyl-2,2-

dichloroimines can also be purified by bulb-to-bulb distillation using a Kugelrohr apparatus (bp ( $R' = C_4H_9$ ) = 55-60 °C at ~0.1 mbar); however, purified dichloroimines did not participate in the oxazolidinone formation reaction, thus the imines were subsequently used without purification.

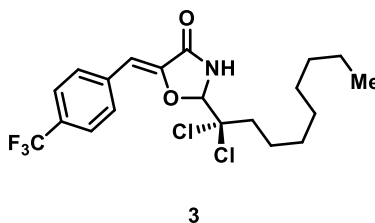
**Figure S3. General procedure for the synthesis of 5-arylidene-4-oxazolidinones**



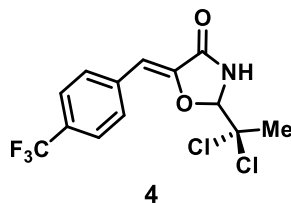
To a solution of 2-oxo-3-arylpropanoic acid (0.25 or 0.5 mmol) in dry THF (~ 0.5 M) at 0 °C was added dropwise oxalyl chloride (1.15 equiv) followed by *N,N*-dimethylformamide (1 drop). The solution was stirred at 0 °C for 2 h before the reaction mixture was concentrated under reduced pressure (directly through the septum using a needle). The crude acid chloride was dissolved in dry  $CHCl_3$  (0.5 M) and added dropwise under inert atmosphere to a solution of crude *t*-butyl  $\alpha,\alpha$ -dichloroimine in dry  $CHCl_3$  (0.5 M) at 0 °C. This mixture was warmed slowly to room temperature and stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo*. The residue was dissolved in trifluoroacetic acid (~ 1 M) and heated to 50 °C until complete conversion was observed by LC-MS (typically 2-4 h). The reaction mixture was concentrated *in vacuo* and purified by reverse-phase flash chromatography ( $SiO_2$ -C18;  $CH_3CN/H_2O$ ). Yields reported are over two steps, based on moles of the appropriate 2-oxo-3-arylpropanoic acid.



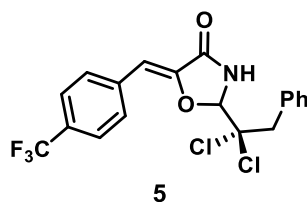
**(Z)-2-(1,1-dichloropentyl)-5-(4-(trifluoromethyl)benzylidene)oxazolidin-4-one (2)**<sup>6</sup>. 25 mg (22%) isolated as a white crystalline solid. Analytical data consistent with that previously reported. mp (previously unreported) 116-117 °C ( $CDCl_3$ ).



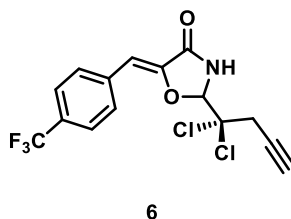
**(Z)-2-(1,1-dichlorononyl)-5-(4-(trifluoromethyl)benzylidene)oxazolidin-4-one (3)**. 23 mg (24%) isolated as an off-white, waxy solid.  $^1H$  NMR (400 MHz,  $CDCl_3$ ):  $\delta$  8.94 (s, 1H), 7.74 (d,  $J = 7.84$  Hz, 2H), 7.64 (d,  $J = 7.88$  Hz, 2H), 6.35 (s, 1H), 5.80 (s, 1H), 2.33 (m, 1H), 2.20 (m, 1H), 1.79 (m, 2H), 1.41 (m, 2H), 1.35-1.29 (m, 8H), 0.99 (t,  $J = 6.61$ , 3H).  $^{13}C$  NMR: (175 MHz,  $CDCl_3$ )  $\delta$  165.0, 144.4, 136.5, 129.6, 125.7, 103.6, 93.6, 91.36, 41.7, 32.0, 29.5, 29.3, 29.2, 24.4, 22.8, 14.2. IR  $\nu_{max}$  ( $cm^{-1}$ ): 3177, 3087, 2926, 2859, 1727, 1614, 1428, 1359, 1324, 869, 838. mp 89-90 °C ( $CDCl_3$ ). HRMS (HESI)  $m/z$  calculated for  $C_{20}H_{24}Cl_2F_3NO_2$  [M-H] 436.10634, found 436.10618.



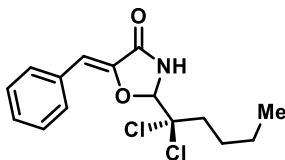
**(Z)-2-(1,1-dichloroethyl)-5-(4-(trifluoromethyl)benzylidene)oxazolidin-4-one (4).** 18 mg (25%) isolated as a fine white powder.  $^1\text{H NMR}$ : (400 MHz,  $(\text{CD}_3)_2\text{CO}$ )  $\delta$  9.44 (s, 1H), 7.93 (d,  $J = 8.07$  Hz, 2H), 7.75 (d,  $J = 7.76$  Hz, 2H), 6.28 (s, 1H), 6.07 (s, 1H), 2.28 (s, 3H).  $^{13}\text{C NMR}$ : (175 MHz,  $(\text{CD}_3)_2\text{CO}$ )  $\delta$  163.2, 145.7, 137.8, 129.7, 125.7, 125.6, 101.0, 91.3, 89.9, 30.9. IR  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3181, 3083, 2922, 2852, 1720, 1324, 1110, 862. mp 211-212 °C ( $\text{CDCl}_3$ ). HRMS (HESI)  $m/z$  calculated for  $\text{C}_{13}\text{H}_{10}\text{Cl}_2\text{F}_3\text{NO}_2$  [M-H] 337.99679, found 337.99648.



**(Z)-2-(1,1-dichloro-2-phenylethyl)-5-(4-(trifluoromethyl)benzylidene)oxazolidin-4-one (5).** 18 mg (10%) isolated as a white crystalline solid.  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.76 (d,  $J = 8.35$  Hz, 2H), 7.67 (d,  $J = 8.39$  Hz, 2H), 7.43 (m, 2H), 7.38 (m, 3H), 6.38 (s, 1H), 5.71 (s, 1H), 3.69 (s, 2H).  $^{13}\text{C NMR}$ : (175 MHz,  $\text{CDCl}_3$ )  $\delta$  164.0, 144.1, 136.5, 132.5, 131.4, 129.6, 128.8, 125.8, 103.8, 92.1, 89.4, 49.6, 48.4. IR  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3157, 3072, 3040, 2852, 1720, 1490, 1450, 1414, 1328; mp 218-219 °C ( $\text{CDCl}_3$ ). HRMS (HESI)  $m/z$  calculated for  $\text{C}_{19}\text{H}_{14}\text{Cl}_2\text{F}_3\text{NO}_2$  [M-H] 414.02809, found 414.02829.

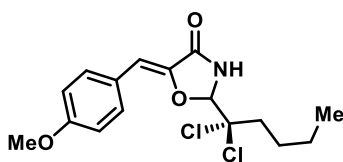


**(Z)-2-(1,1-dichlorobut-3-yn-1-yl)-5-(4-(trifluoromethyl)benzylidene)oxazolidin-4-one (6).** 46 mg (51%) isolated as an off-white, crystalline solid.  $^1\text{H NMR}$ : (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.69 (s, 1H), 7.74 (d,  $J = 7.85$  Hz, 2H), 7.64 (d,  $J = 7.86$  Hz, 2H), 6.38 (s, 1H), 6.06 (s, 1H), 3.37 (s, 2H), 2.34 (s, 1H).  $^{13}\text{C NMR}$ : (175 MHz,  $\text{CDCl}_3$ )  $\delta$  164.8, 144.0, 136.3, 129.6, 125.8, 104.1, 89.7, 75.8, 74.2, 34.4. IR  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3304, 3174, 3076, 2969, 1720, 1409, 1323, 1110, 1017. mp 167-168 °C ( $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ ). HRMS (HESI)  $m/z$  calculated for  $\text{C}_{15}\text{H}_{10}\text{Cl}_2\text{F}_3\text{NO}_2$  [M-H] 361.99679, found 361.99668.



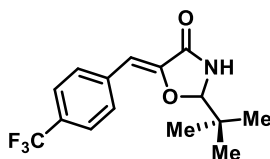
7

**(Z)-5-Benzylidene-2-(1,1-dichloropentyl)oxazolidin-4-one (7).** 19 mg (28%) isolated as a white solid.  $^1\text{H}$  NMR: (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.83 (s, 1H), 7.65 (d,  $J = 7.19$  Hz, 2H), 7.39 (m, 2H), 7.30 (m, 1H), 6.34 (s, 1H), 5.77 (s, 1H), 2.32 (m, 1H), 2.20 (m, 1H), 1.77 (m, 2H), 1.44 (m, 2H), 0.99 (t,  $J = 7.34$ , 3H).  $^{13}\text{C}$  NMR: (100 MHz,  $\text{CDCl}_3$ )  $\delta$  165.4, 142.7, 133.0, 129.6, 128.8, 128.3, 105.3, 93.9, 91.1, 41.3, 26.5, 22.4, 14.0. IR  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3169, 3063, 3028, 2958, 2930, 1712, 1676, 1359, 1328. mp 166-167 °C ( $\text{CDCl}_3$ ). HRMS (HESI)  $m/z$  calculated for  $\text{C}_{15}\text{H}_{17}\text{Cl}_2\text{NO}_2$  [M-H] 312.05636, found 312.05638.



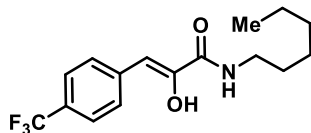
8

**(Z)-2-(1,1-di-Chloropentyl)-5-(4-methoxybenzylidene)oxazolidin-4-one (8).** 47.5 mg (27%) isolated as a brown crystalline solid.  $^1\text{H}$  NMR: (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.21 (bs, 1H), 7.61 (d,  $J = 7.59$ , 2H), 6.92 (d,  $J = 6.91$ , 2H), 6.29 (s, 1H), 5.73 (s, 1H), 3.83 (s, 3H), 2.31 (dt,  $J = 15.89$ , 7.33, 1H), 2.20 (dt,  $J = 14.32$ , 7.51, 1H), 1.76 (dt,  $J = 15.72$ , 7.71, 2H), 1.43 (m, 2H) 0.98 (t,  $J = 7.33$ , 3H).  $^{13}\text{C}$  NMR: (175 MHz,  $\text{CDCl}_3$ )  $\delta$  165.2, 159.7, 141.1, 131.1, 114.3, 105.3, 94.0, 90.8, 55.5, 41.1, 26.5, 22.4, 14.0. IR  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3199, 3069, 2959, 2931, 2872, 2836, 1717, 1606, 1511, 1349, 1252, 1176, 1030. mp 146-148 °C ( $\text{CDCl}_3$ ). HRMS (HESI)  $m/z$  calculated for  $\text{C}_{16}\text{H}_{19}\text{Cl}_2\text{NO}_3$  [M-H] 344.08148, found 344.08179.



9

**(Z)-2-(tert-Butyl)-5-(4-(trifluoromethyl)benzylidene)oxazolidin-4-one (9).** Synthesized via a previously reported procedure<sup>5</sup>. 46 mg (96%) isolated as a white solid.  $^1\text{H}$  NMR: (400 MHz,  $\text{CDCl}_3$ )  $\delta$  9.02 (s, 1H), 7.74 (d,  $J = 8.31$  Hz, 2H), 7.62 (d,  $J = 8.33$  Hz, 2H), 6.22 (s, 1H), 5.31 (s, 1H), 1.04 (s, 9H).  $^{13}\text{C}$  NMR: (175 MHz,  $\text{CDCl}_3$ )  $\delta$  165.4, 145.8, 137.5, 129.2, 125.6, 101.3, 95.3, 29.9, 23.6. IR  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3151, 3022, 2063, 2923, 1713, 1377, 1326, 1168, 1106. mp 214-215 °C ( $\text{CDCl}_3$ ). HRMS (HESI)  $m/z$  calculated for  $\text{C}_{15}\text{H}_{16}\text{NO}_2$  [M-H] 298.10604, found 298.10604.



11

**N-Hexyl-2-oxo-3-(4-(trifluoromethyl)phenyl)propanamide (11).** To a solution of 2-oxo-3-(4-(trifluoromethyl)phenyl)propanoic acid (0.232 g, 1.0 mmol) in THF (10 mL, 0.1 M) at 0 °C was added *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrichloride (0.232 g, 1.20 mmol, 1.2 equiv), 1-hydroxybenzotriazole hydrate (0.184 g, 1.20 mmol, 1.2 equiv) and *N,N*-diisopropylethylamine (0.355 mL, 2.0 mmol, 2.0 equiv). This mixture was stirred at 0 °C for several minutes before hexylamine (0.146 mL, 1.0 mmol, 1.0 equiv) was added and the resulting solution was allowed to warm to room temperature with vigorous stirring. After 14 h the reaction mixture was diluted with Et<sub>2</sub>O and washed successively with 3 M HCl (10 mL x 2), sat. NaHCO<sub>3</sub> (10 mL x 3), H<sub>2</sub>O (20 mL) and brine. The organic layer was dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO<sub>2</sub>, EtOAc/hexanes). 116 mg (43%) isolated as an off-white solid: <sup>1</sup>H NMR: (400 MHz, CDCl<sub>3</sub>) δ 7.87 (d, *J* = 7.82 Hz, 2H), 7.67 (d, *J* = 7.81 Hz, 2H), 4.58 (s, 2H), 3.60 (m, 2H), 2.33 (m, 1H), 2.20 (m, 1H), 1.83 (m, 2H), 1.58 (m, 4H), 1.17 (m, 3H). <sup>13</sup>C NMR: (175 MHz, CDCl<sub>3</sub>) δ 195.6, 159.7, 137.4, 130.4, 125.7, 43.1, 39.7, 31.5, 29.3, 22.6, 14.1. IR ν<sub>max</sub> (cm<sup>-1</sup>): 3316, 3060, 2927, 2852, 1724, 1657, 1323, 1174, 1119, 1068. mp 86-88 °C (CDCl<sub>3</sub>). HRMS (HESI) *m/z* calculated for C<sub>16</sub>H<sub>20</sub>F<sub>3</sub>NO<sub>2</sub> [M-H] 314.13734, found 314.13745.

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## Bacterial strains and growth conditions, assay protocols and biological data

### General information

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains were obtained from the Laboratory of Professor Christian Melander (NCSU) ATCC (33591, BAA 44, BAA 1685, BAA 1770, BAA 43 300) and colonies were grown on solid media as instructed. Mueller-Hinton broth (MHB, 211443-BD), tryptic soy broth (TSB, Remel: R455052) and D-glucose (CAS: 492-62-6) were purchased from Fisher Scientific. Tryptic soy agar (TSA, cat. # 22091) and Linezolid (cat. # P70014) were purchased from Sigma-Aldrich. Bacteria for biofilm inhibition and dispersion assays were cultured overnight in TSBG (tryptic soy broth with 0.5% glucose supplement). All assays were run in duplicate and repeated at least two separate times for MIC assays and at least four separate times for biofilm inhibition and dispersion assays. All compounds were dissolved molecular biology grade DMSO as 100 mM stock solutions and further diluted to 10 and 1 mM stock solutions as needed. Optical densities were measured using a Thermo Scientific Genesys 20 spectrophotometer. Data for biofilm inhibition and dispersion assays was collected using a BioTek ELx808 Microplate Reader. All graphs were generated and analyzed using GraphPad Prism 7.

### Broth microdilution method for determination of minimum inhibitory concentrations

As prescribed by the Clinical and Laboratory Standards Institute (CLSI) M07-A8, Vol. 29 (2)

MRSA (ATCC 33591 or BAA 44) was grown in MHB for 6-8 h; this culture was used to inoculate fresh MHB ( $5 \times 10^5$  CFU/mL). The resulting bacterial suspension was aliquoted (1 mL) into 1.5 mL tubes and compound was added from a 100 mM or 10 mM DMSO stock to achieve the desired initial starting concentration (typically 128  $\mu$ g/mL). Linezolid (from a 10 mM DMSO stock) was used as a positive control. Inoculated media not treated with compound served as the negative control. Rows 2-12 of a 96-well microtiter plate were filled at 100  $\mu$ L/well from the remaining inoculated media, allowing the concentration of compound to be kept uniform throughout the dilution procedure. The samples containing test compounds and linezolid were then aliquoted (200  $\mu$ L) into the corresponding first row wells of the microtiter plate (two wells for each compound and two negative controls). Row 1 wells were mixed 6 to 8 times, then 100  $\mu$ L was transferred to row 2. Row 2 wells were mixed 6 to 8 times, followed by a 100  $\mu$ L transfer from row 2 to row 3. This procedure was repeated to serially dilute the rest of the rows of the microtiter plate. The plate was then covered and sealed with GLAD Press'n Seal<sup>®</sup> and incubated under stationary conditions at 37 °C. After 16 h, minimum inhibitory concentration (MIC) values were recorded as the lowest concentration of compound at which no visible growth of bacteria was observed.

### Determination of the inhibitory effect of test compounds on MRSA biofilm formation

(S. A. Rogers and C. Melander, *Angew Chem Int Ed*, 2008, **47**, 5229-5231.)

Inhibition assays were performed by subculturing an overnight culture of MRSA (ATCC BAA 44 or BAA 1685, BAA 1770, BAA 43 300) to an OD<sub>600</sub> of 0.01 in TSBG (tryptic soy broth with a 0.5% glucose supplement). Stock solutions of predetermined concentrations of the test compound were then made using the inoculated TSBG. These stock solutions were aliquoted (100  $\mu$ L) into the wells of the 96-well PVC microtiter plate. Sample plates were then sealed and wrapped in GLAD Press'n Seal<sup>®</sup> and incubated under stationary conditions 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 110  $\mu$ L 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  $\mu$ L of 95% ethanol. A sample of 125  $\mu$ L of solubilized CV stain from each well was transferred to the corresponding wells of a polystyrene microtiter dish. Biofilm inhibition was quantified by measuring the OD<sub>540</sub> of each well and calculated as a percentage of the control (no compound); a negative control lane wherein no biofilm was formed served as a background and was subtracted out. Percent inhibition was then plotted against concentration in Prism 7. Each of the four experiments were plotted separately and analyzed by a normalized nonlinear regression. The IC<sub>50</sub> values

reported represent an arithmetic mean of the four IC<sub>50</sub> values. The graphs on the following pages were generated from an average of the total data set. Biofilm data represent four separate experiments, with each experiment performed in duplicate (average of 8 data points for each concentration tested, unless otherwise noted).

### **Determination of the dispersal effect of test compounds on pre-formed MRSA biofilms**

(J. J. Richards, T. E. Ballard and C. Melander, *Org Biomol Chem*, 2008, **6**, 1356-1363.)

Dispersion assays were performed by taking an overnight culture of MRSA (ATCC BAA-44 or BAA 1685, BAA 1770, BAA 43 300) and subculturing it at an OD<sub>600</sub> of 0.01 into TSBG. 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 and incubated under stationary conditions at 37 °C to allow formation of 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 TSBG. 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 under stationary conditions 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 110 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 quantified by measuring the OD<sub>540</sub> of each well and calculated as a percentage of the control (no compound), a negative control lane wherein no biofilm was formed served as a background and was subtracted out. Percent dispersion was then plotted against concentration in Prism 7. Each of the four experiments were plotted separately and analyzed by a normalized nonlinear regression. The IC<sub>50</sub> values reported represent an arithmetic mean of the four IC<sub>50</sub> values. The graphs on the following pages were generated from an average of the total data set. Biofilm data represent four separate experiments, with each experiment performed in duplicate (average of 8 data points for each concentration tested, unless otherwise noted).

### **Growth curves**

MRSA was cultured in TSBG overnight and this culture was used to inoculate fresh TSBG to an OD<sub>600</sub> of 0.01. Inoculated media was aliquoted (3 mL) into culture tubes and compound was added from DMSO stocks; inoculated media with only DMSO added served as the control. Tubes were incubated at 37 °C with shaking at 200 rpm. Samples were taken at time points of 2, 4, 8 and 24 hours; 100 µL aliquots were diluted 1:10:100:100 with PBS. The diluted samples were then plated on TSA and incubated for 24 hours at 37 C under stationary conditions. Colonies were then enumerated and plotted against time.

### **Hemolysis assay**

Hemolysis assays were performed on mechanically defibrinated sheep blood (Hemostat Labs: DSB50). Defibrinated blood (1.5 mL) was placed into a microcentrifuge tube and centrifuged for 10 min at 10,000 rpm. The supernatant was then removed and then the cells were resuspended in 1 mL of phosphate-buffered saline (PBS). The suspension was centrifuged, the supernatant was removed and cells were resuspended two additional times. The final cell suspension was then diluted 10-fold. Test compound solutions were made in PBS in small culture tubes and then added to aliquots of the 10-fold suspension dilution of blood. PBS was used as a negative control and a zero hemolysis marker. Triton X-100 (a 1%



sample) was used as a positive control serving as the 100% lysis marker. Samples were then placed in an incubator at 37 °C while being shaken at 200 rpm for one hour. After one hour, the samples were transferred to microcentrifuge tubes and centrifuged for 10 min at 10,000 rpm. The resulting supernatant was diluted by a factor of 40 in distilled water. The absorbance of the supernatant was then measured with a UV spectrometer at a 540 nm wavelength.

Figure S4. Growth Curves

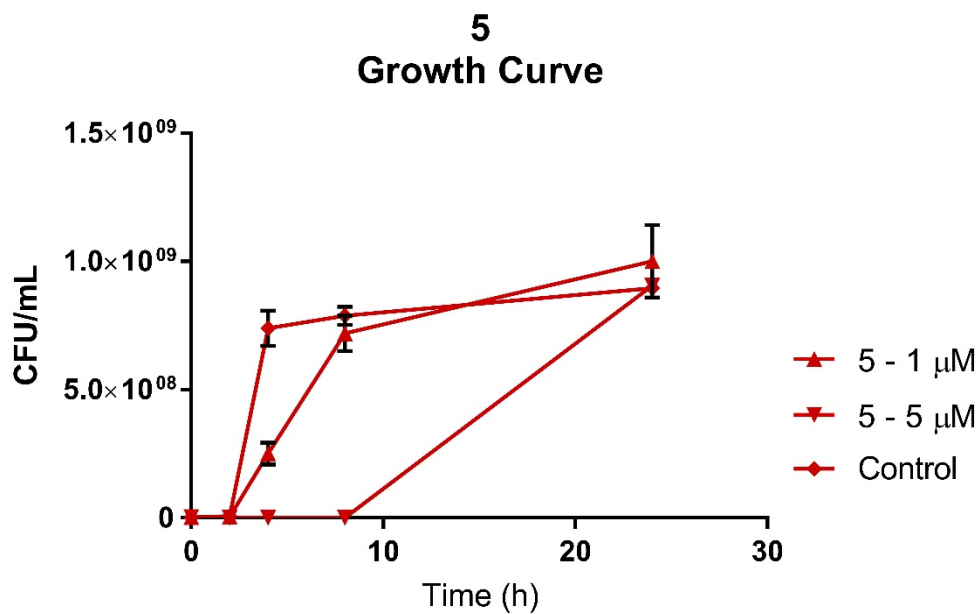
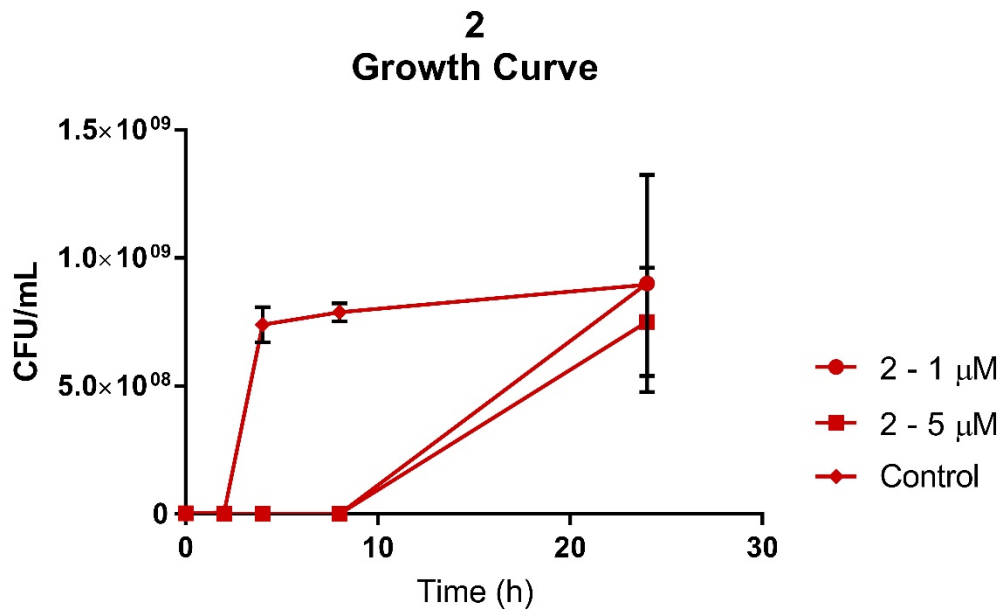
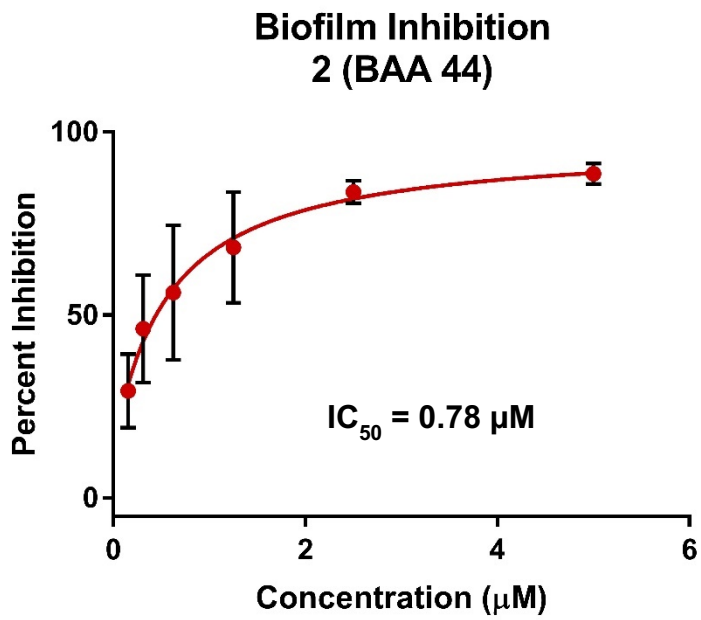
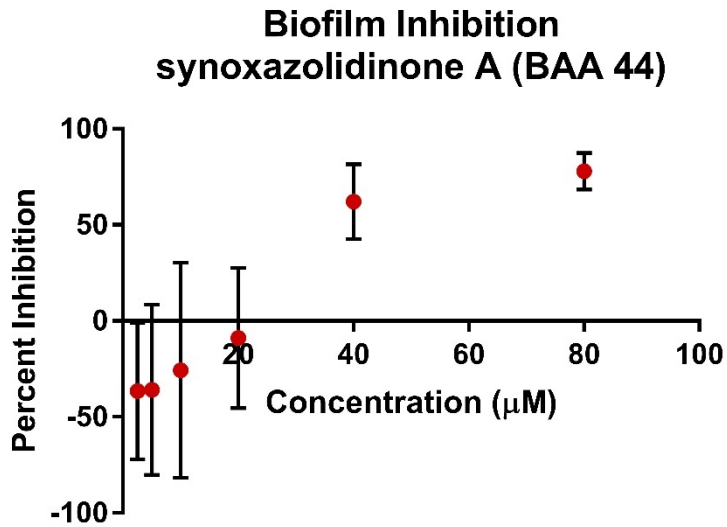
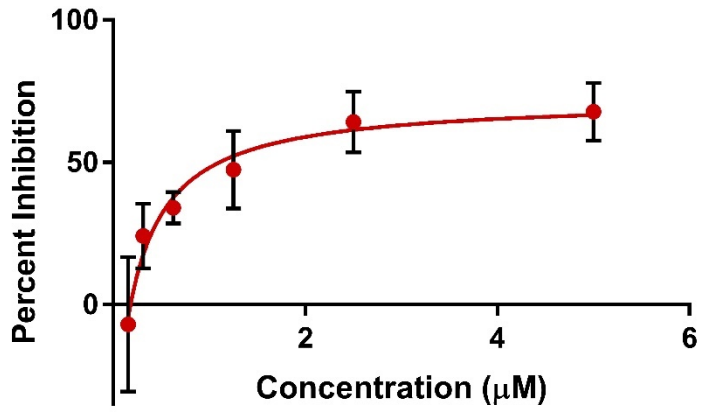


Figure S5. Biofilm Inhibition – Dose Response Curves

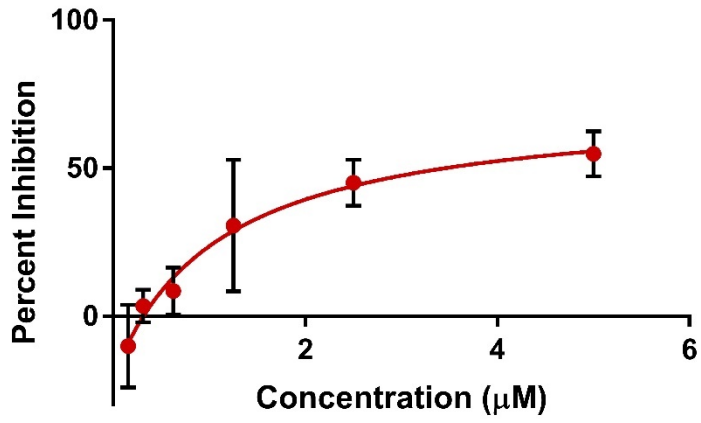
MRSA BAA 44



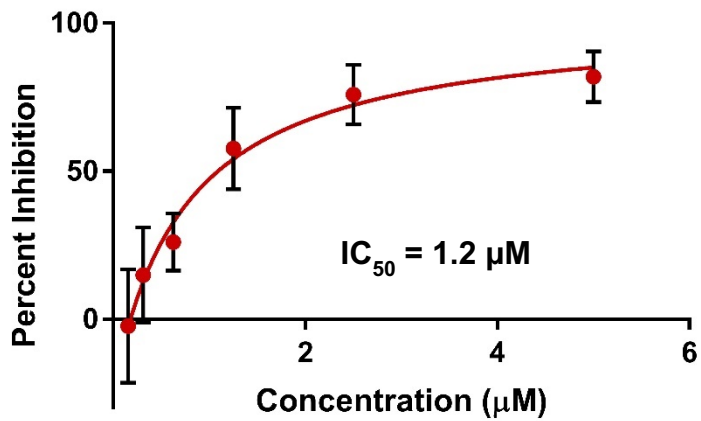
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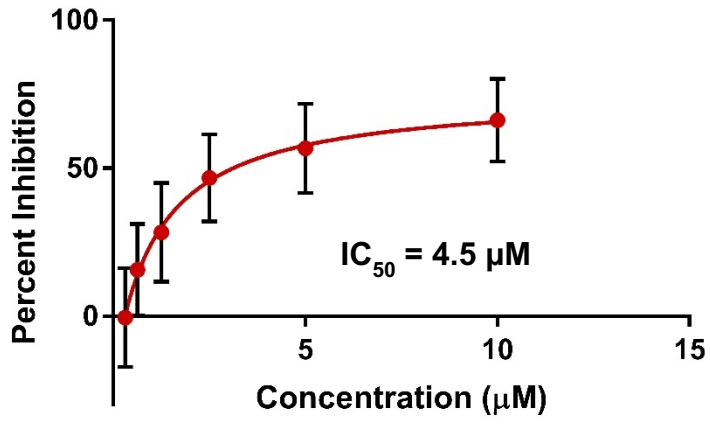
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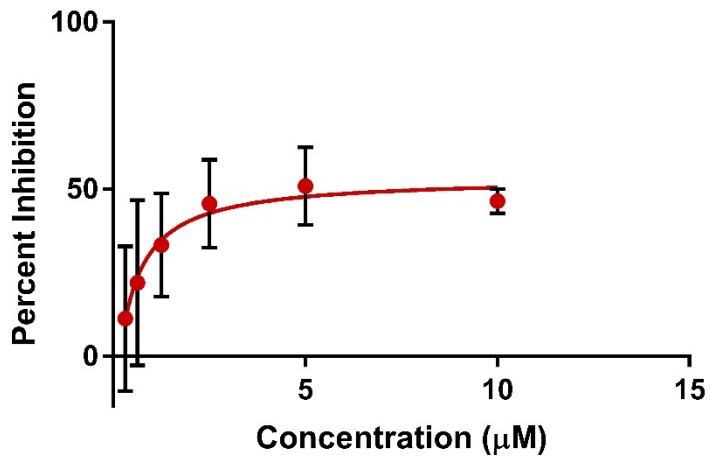
**Biofilm Inhibition  
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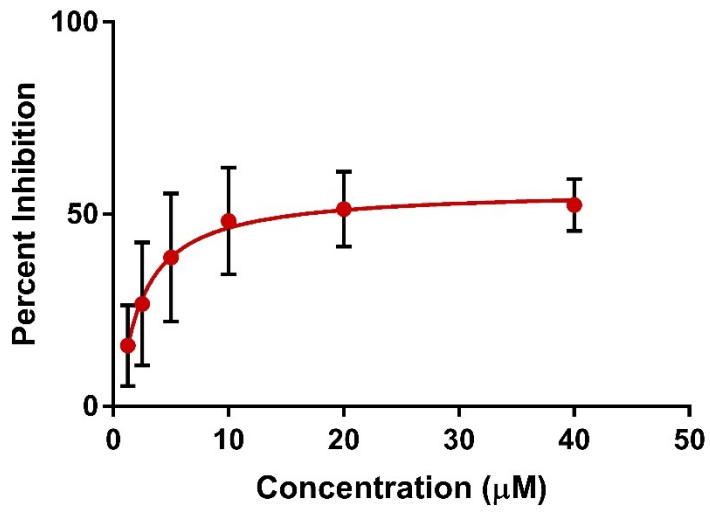
### Biofilm Inhibition 6 (BAA 44)



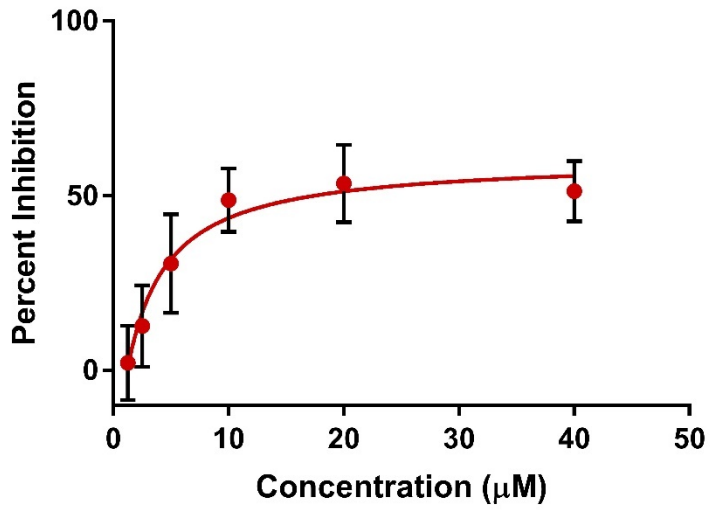
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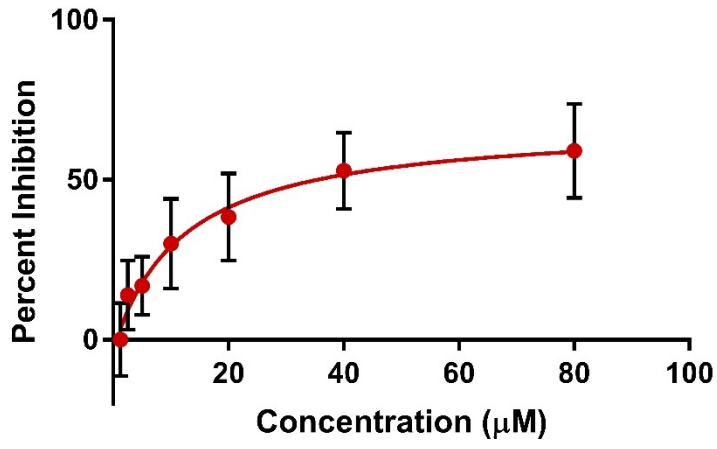
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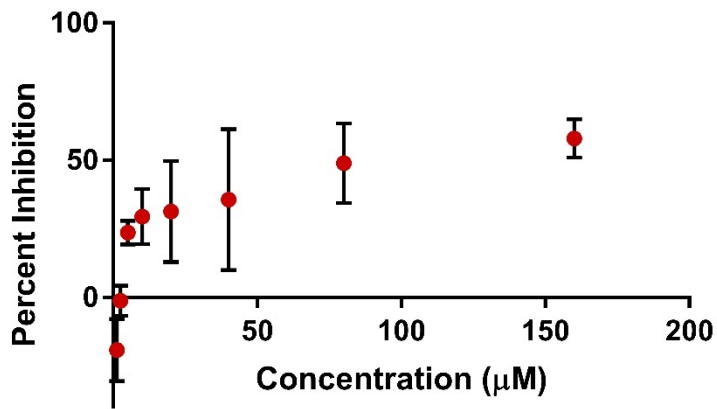
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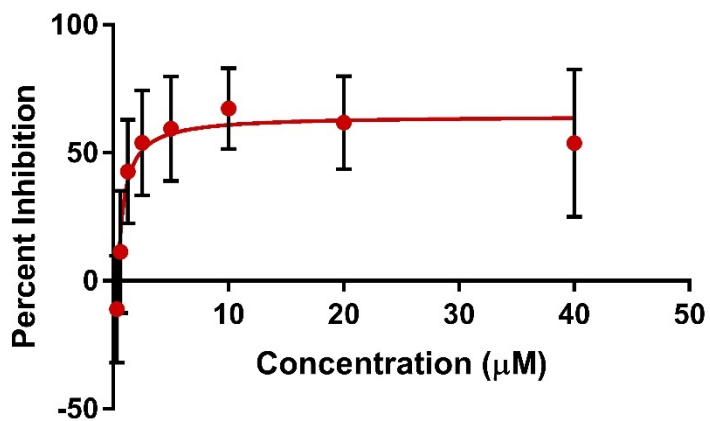


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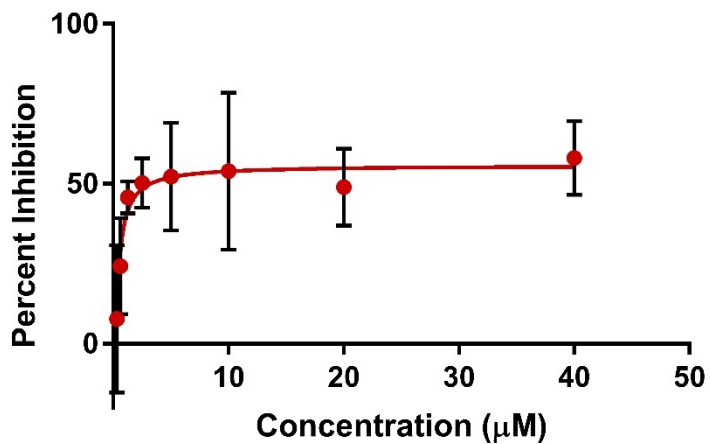


MRSA BAA 43 300 (These data represent two separate experiments, each experiment was performed in duplicate)

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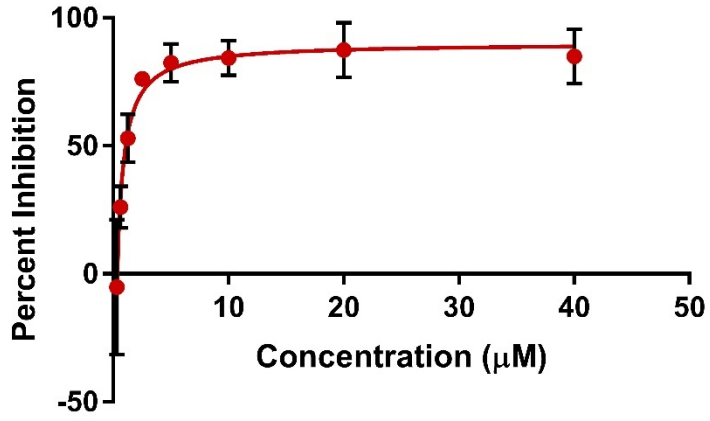


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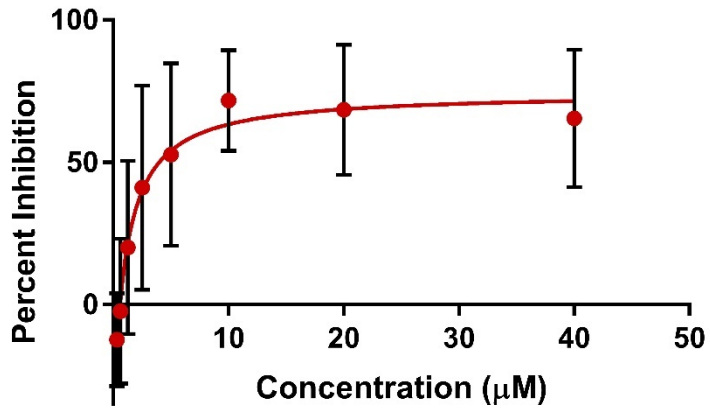


MRSA BAA 1685 (These data represent two separate experiments, each experiment was performed in duplicate)

### Biofilm Inhibition 2 (BAA 1685)



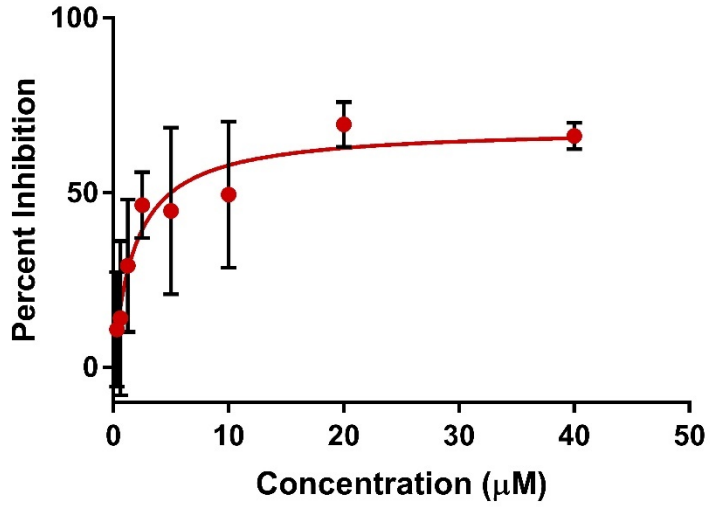
### Biofilm Inhibition 5 (BAA 1685)





MRSA BAA 1770 (These data represent two separate experiments, each experiment was performed in duplicate)

### Biofilm Inhibition 2 (BAA 1770)



### Biofilm Inhibition 5 (BAA 1770)

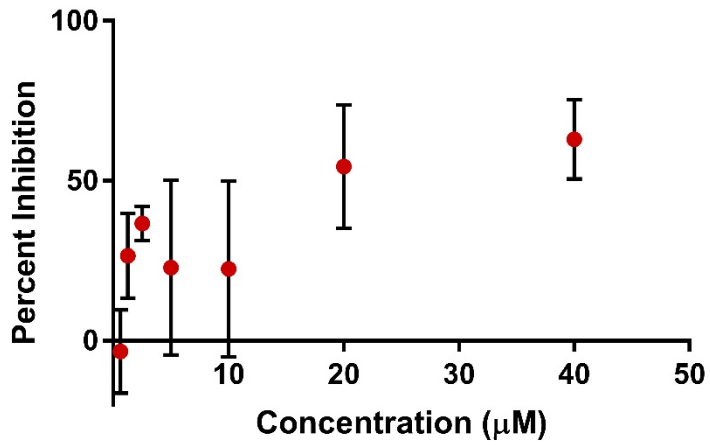
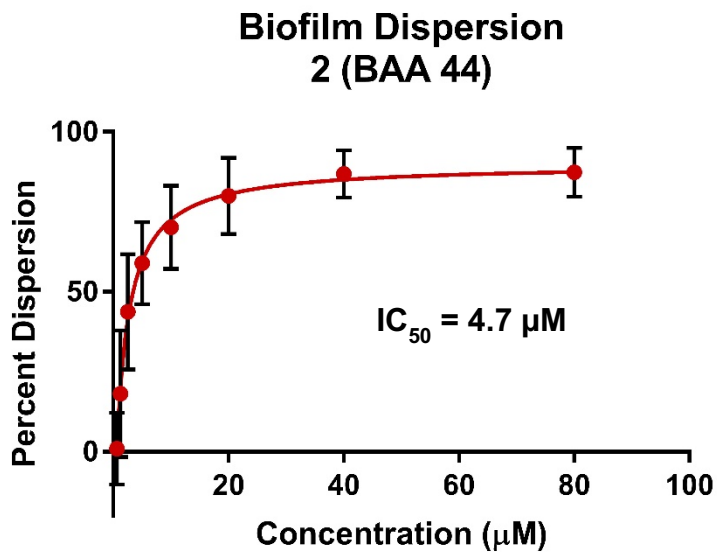
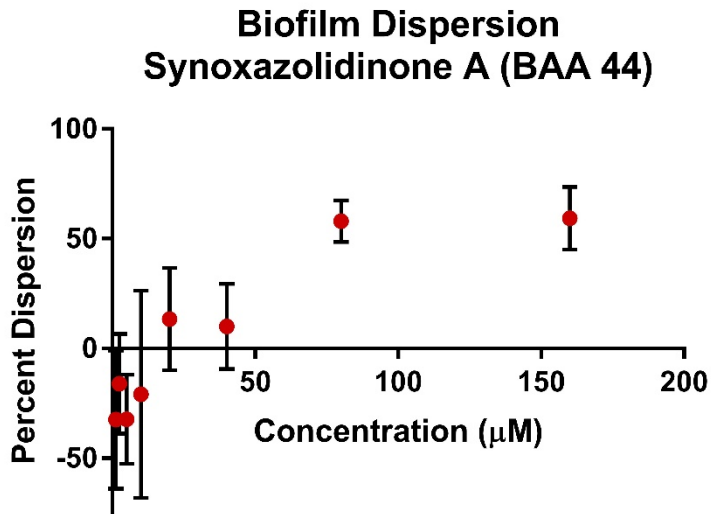
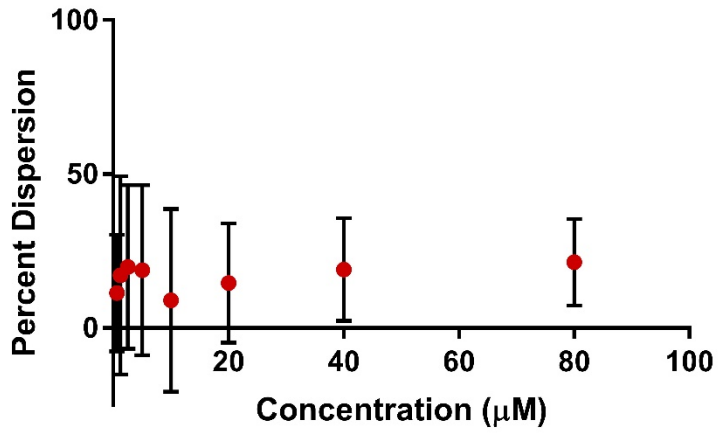


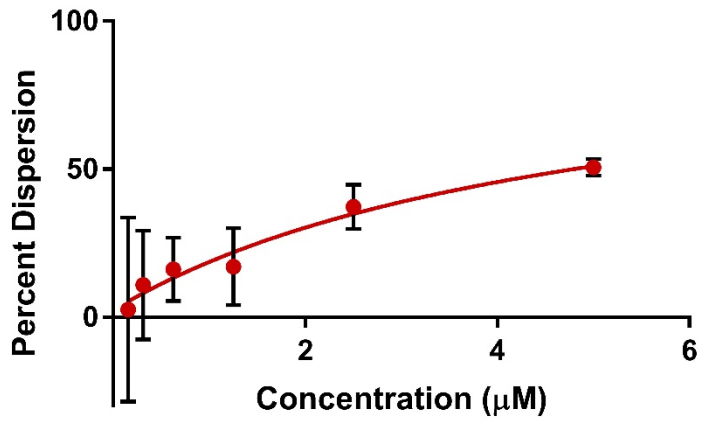
Figure S6. Biofilm Dispersion – Dose Response Curves  
MRSA BAA 44



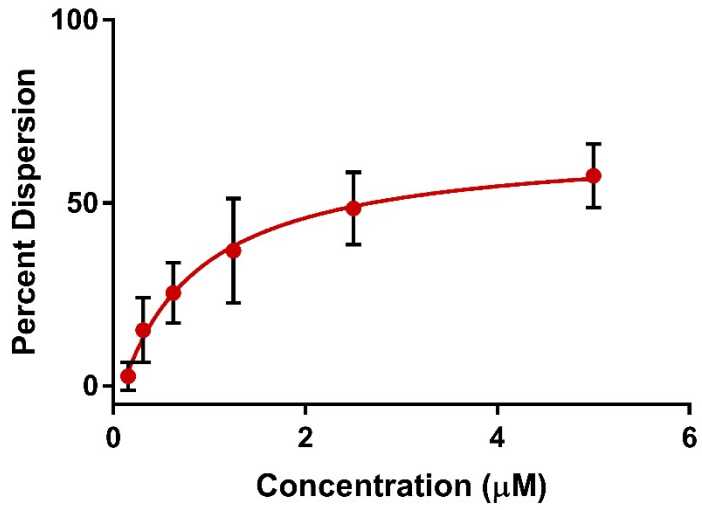
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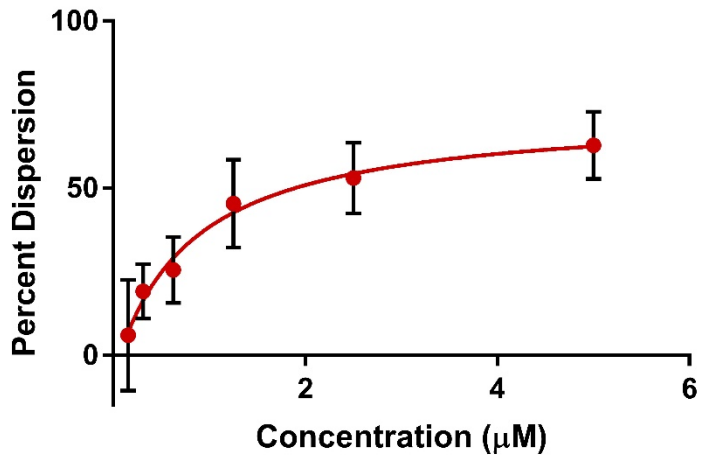
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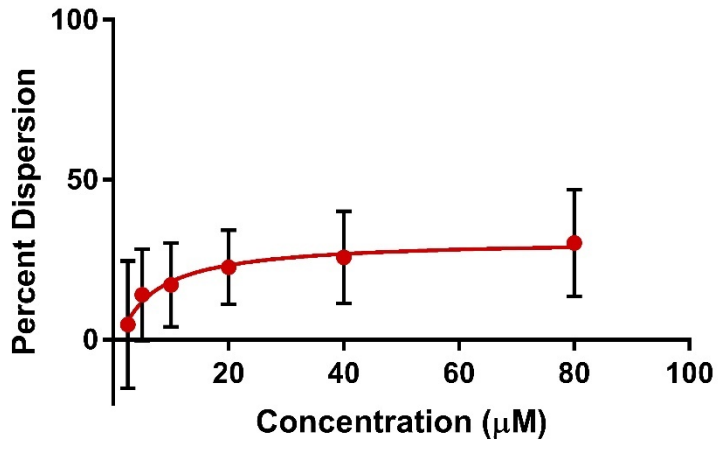
**Biofilm Dispersion  
5 (BAA 44)**



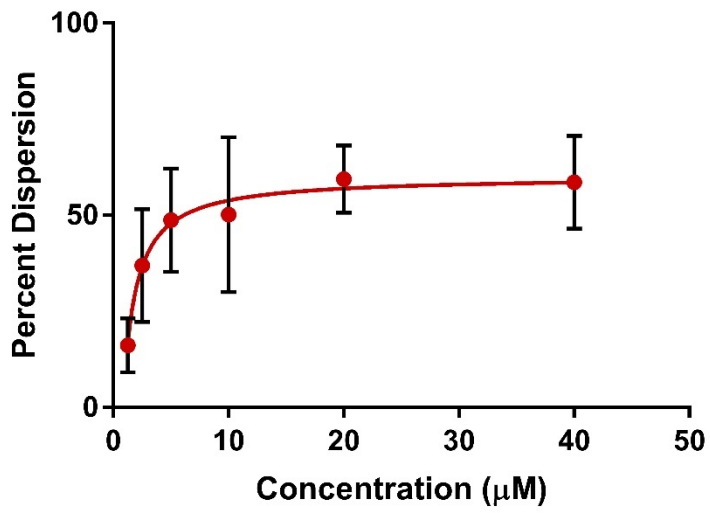
**Biofilm Dispersion  
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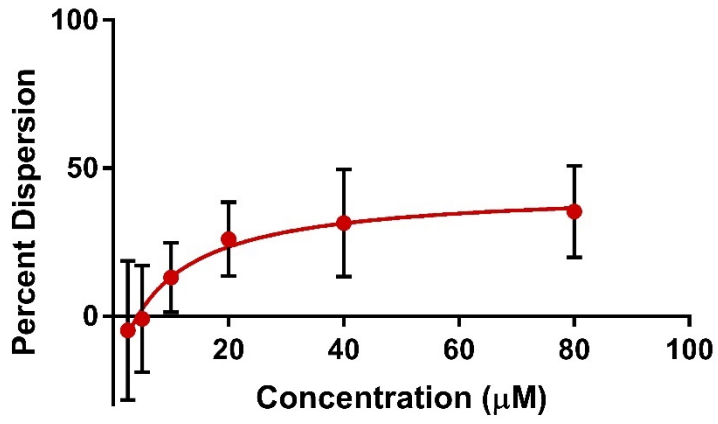
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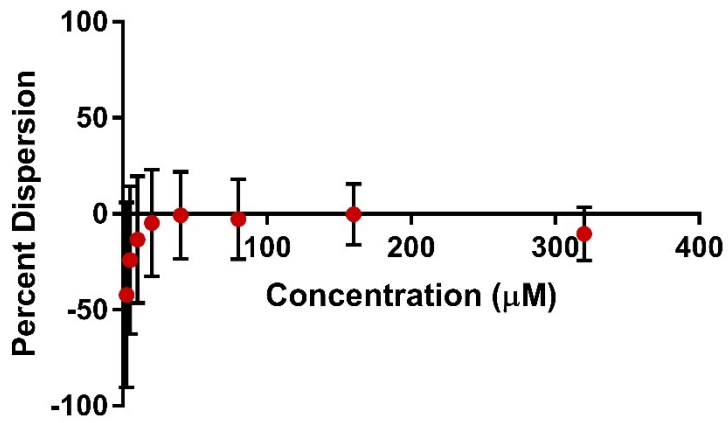
### Biofilm Dispersion 8 (BAA 44)



### Biofilm Dispersion 9 (BAA 44)

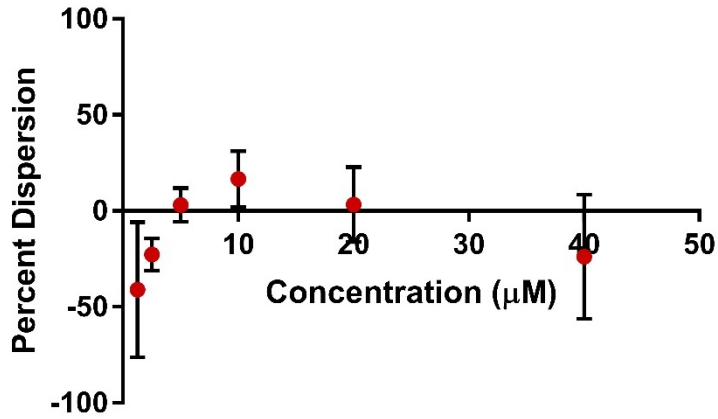


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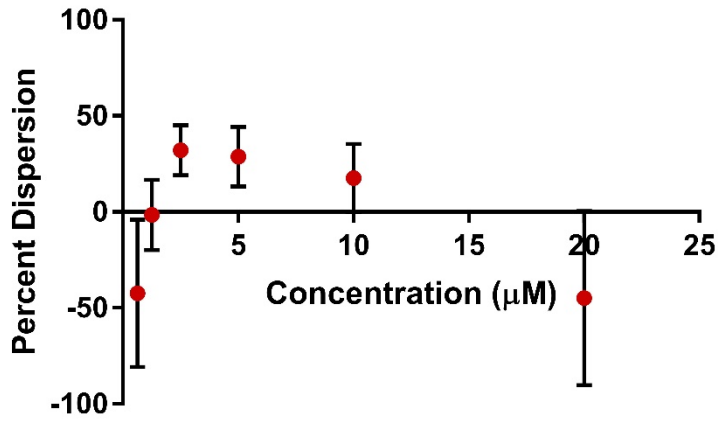


MRSA BAA 43 300

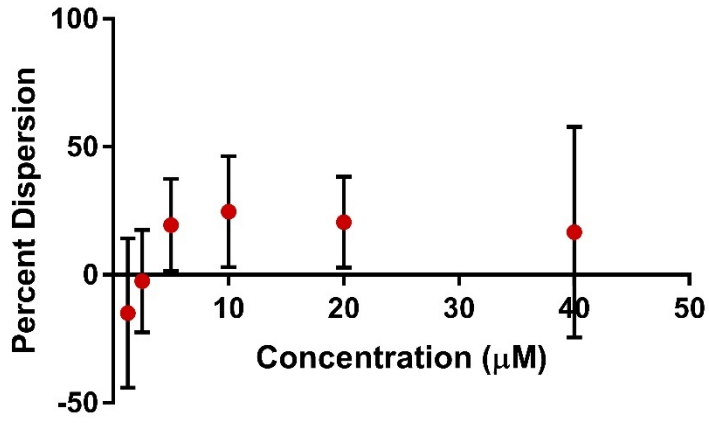
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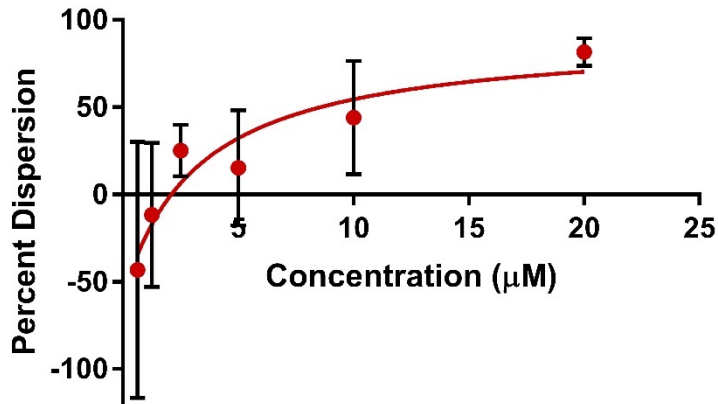
### Biofilm Dispersion 5 (BAA 43 300)



**Biofilm Dispersion  
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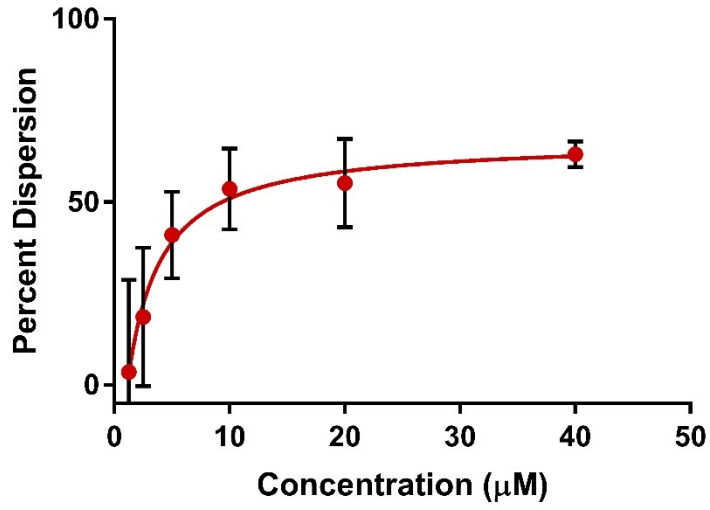


**Biofilm Dispersion  
5 (BAA 1685)**





**Biofilm Dispersion  
2 (BAA 1770)**



**Biofilm Dispersion  
5 (BAA 1770)**

