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Reaction-based indicator (RIA) displacement hydrogel for the development of a triggered release system capable of biofilm inhibition

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1. General Protocols

Biological materials

Media, buffers and stock solutions

Medium or buffer	Reagents
Luria-Bertani (LB)	ThermoFisher Scientific U.K
Luria-Bertani agar (LBA)	ThermoFisher Scientific U.K
Tryptic soy bean broth (TSB)	ThermoFisher Scientific U.K
Tryptic soy bean agar (TSA)	ThermoFisher Scientific U.K
Phosphate buffered saline (PBS)	Sigma Aldrich U.K

*All media was sterilised using an autoclave prior to use

Bacterial strains and overnight cultures

Pseudomonas aeruginosa PAO1 (*P. aeruginosa* PAO1), *Staphylococcus aureus* H560 (*S. aureus* H560) and *Staphylococcus aureus* MRSA252 (*S. aureus* MRSA252) were obtained from a strain collection belonging to the Biophysical Chemistry Research Group at the University of Bath. *Escherichia coli* NCTC 10418 (*E. coli* NCTC 10418) was obtained from Professor Maillard's group at Cardiff University. All strains were stored in a 30% (v/v) glycerol solution at -80 °C. Overnight cultures of bacterial strains were routinely grown in 10 mL of LB medium for *P. aeruginosa* PAO1 and *E. coli* NCTC 10418, and TSB medium for *S. aureus* MRSA252 and H560 at 37 °C with shaking (225 rpm) for 24 hours.

Bacterial Biofilm Formation

Bacterial biofilms were formed in 96 well microplates (Nunc, UK). Overnight bacterial cultures were sub-cultured into fresh medium, supplemented with 1% (w/v) D-(+)-glucose for TSB and 50% (w/v) D-(+)-glucose for LB, to a starting bacterial concentration of 10⁶ Colony Forming Units per mL (CFU/mL). An aliquot of 100 µL of subculture was added to selected wells, before an equal volume of known drug (or drug combination) concentration was added at desired stage of growth. Plates were then incubated at 37 °C for 24 hours with no shaking. Supplemented TSB or LB was used as a negative control.¹

Crystal Violet Biofilm Staining

After incubation, planktonic bacteria were removed from the microtiter plate, and the remaining biofilm was washed three times with PBS. Next, 250 µL of 0.1% (w/v) Crystal Violet (CV) was added to each well and left to incubate at room temperature for 15 min. After staining, the wells were rinsed three times in PBS and left to dry at room temperature for 3 – 4 h. To quantify the biofilm biomass, 250 µL of 33% (v/v) acetic acid (AcOH) was added to each well to dissolve the CV stain that was bound to the biofilm, and left to incubate at room temperature for 15 min. Finally, 125 µL of dissolved dye solution was transferred into a fresh 96 well microtiter plate and absorbance was measured at 570 nm (Spectrostar Omega plate reader, BMG Labtech).^{2, 3}

General protocol for the evaluation of Alizarin Red S (ARS) Release and for use in Bacterial Biofilm inhibition

Each gel was placed into a PBS solution (1 mL, pH 7.3) containing a specific H₂O₂ concentration. The gel was then incubated at 25°C. At each specified time point, an aliquot was taken to carry out the required UV-Vis experiments. After each measurement, the aliquot was returned to the solution containing the gel to maintain a constant volume.

Biofilm experiments: a PBA gel was placed into a PBS solution (1 mL, pH 7.3) containing 2 mM H₂O₂ and incubated for 3 hours. Next, 100 µl of the solution was then added to 100 µl of sub-cultured MRSA252 in 96-well round bottomed plate, and incubated at 37 °C for 24 hours to allow biofilm formation. After incubation, planktonic bacteria were removed from the microtiter plate, and the remaining biofilm was washed three times with PBS. Next, 250 µL of 0.1% (w/v) CV was added to each well and left to incubate at room temperature for 15 min. After staining, the wells were rinsed three times in PBS and left to dry at room temperature for 3 – 4 h. To quantify the biofilm biomass, 250 µL of 33% (v/v) acetic acid (AcOH) was added to each well to dissolve the CV stain that was bound to the biofilm, and left to incubate at room temperature for 15 min. Finally, 125 µL of the dissolved dye solution was transferred into a fresh 96 well microtiter plate and absorbance was measured at 570 nm. (Spectrostar Omega plate reader, BMG Labtech).

Statistical analysis

All experiments were repeated using three biological replicates and plotted as the mean ± standard deviation. All statistical analysis was carried out on GraphPad 7.0 using a one-way ANOVA to assess statistical significance.

2. Photographs of PBA and BOB-based hydrogels with and without ARS

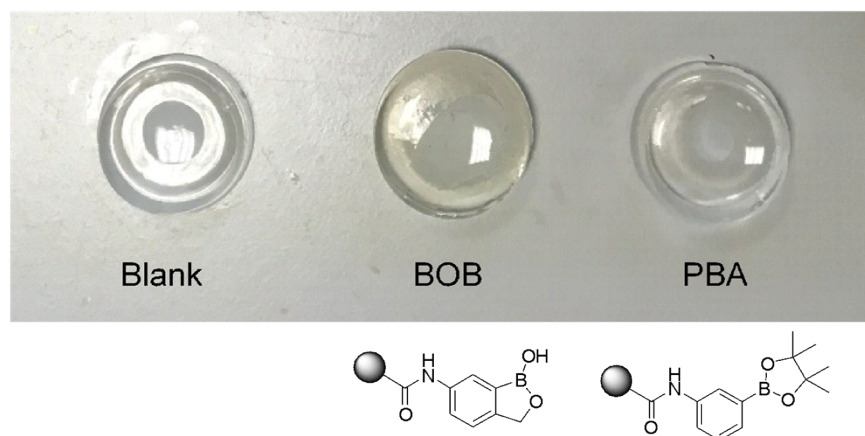


Figure S1 – Photograph of hydrogel slabs, which includes blank hydrogel, **BOB** gel and **PBA** gel.

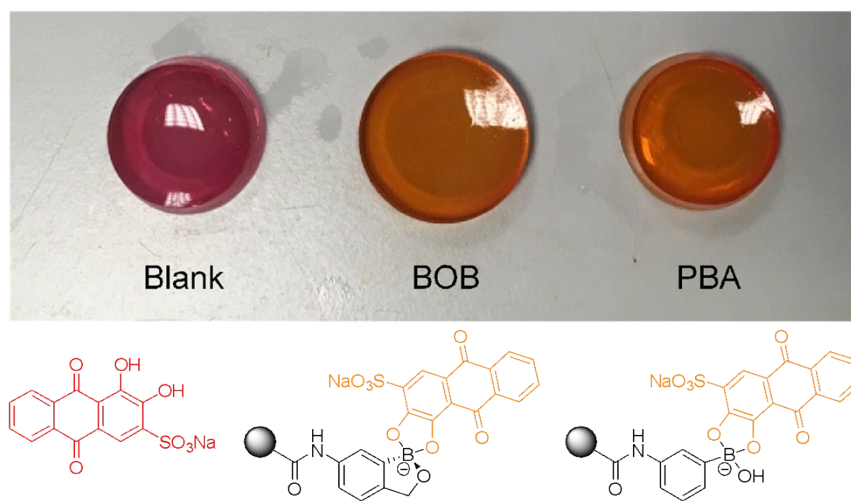
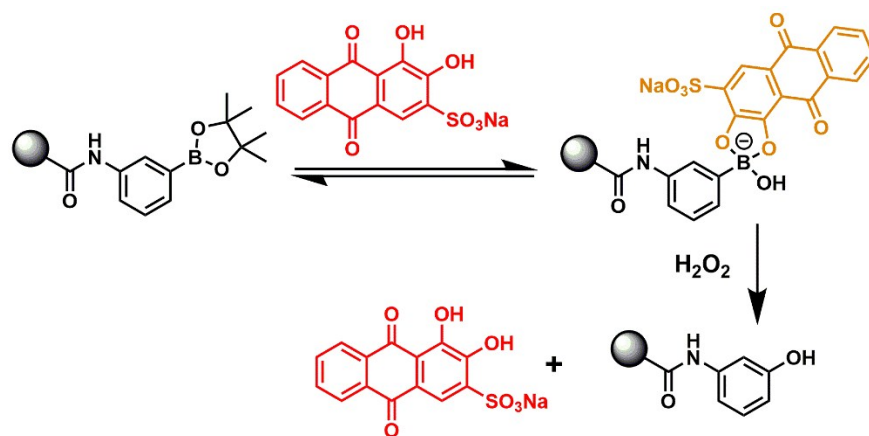
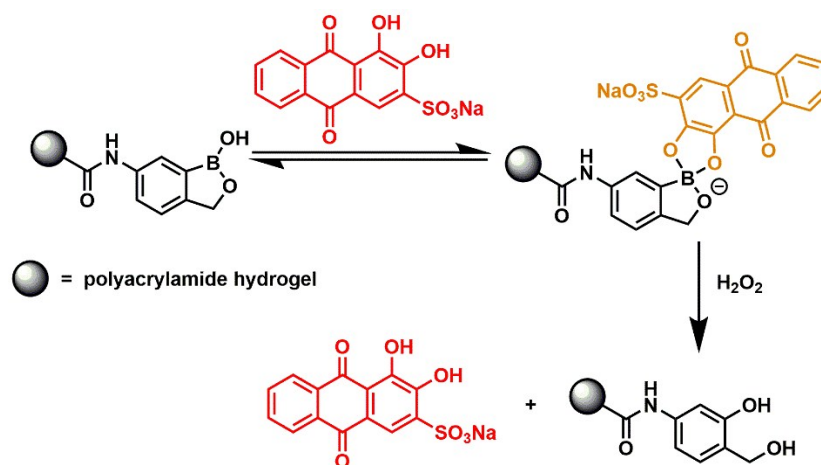


Figure S2 – Photograph of hydrogel slabs after treatment with Alizarin Red S, which includes blank hydrogel (**Red**), **BOB** gel (**Orange**) and **PBA** gel (**Orange**).

3. Mechanism of Triggered-release



Scheme S1 - PBA hydrogel system with H_2O_2 -mediated release of ARS



Scheme S2- BOB hydrogel system with H_2O_2 -mediated release of ARS.

4. UV Analysis

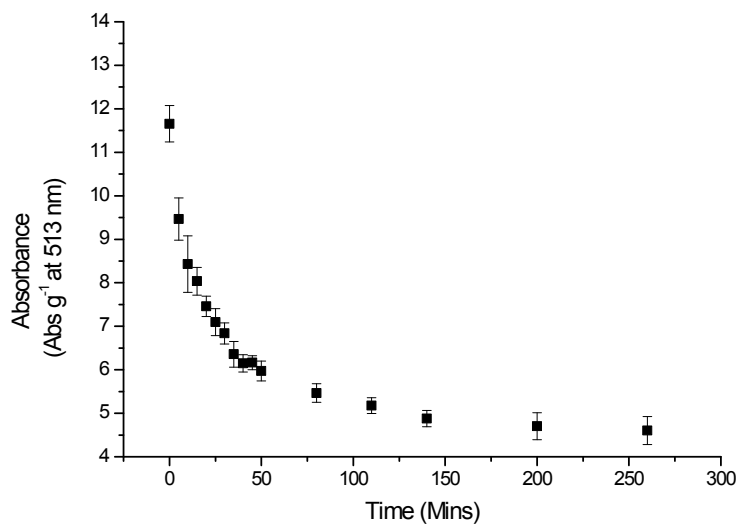


Figure S3 – UV-Vis absorption per gram of **PBA**-based hydrogel at 513 nm over time in PBS (pH 7.3) at 25 °C. Error bars indicate standard deviation (n = 3).

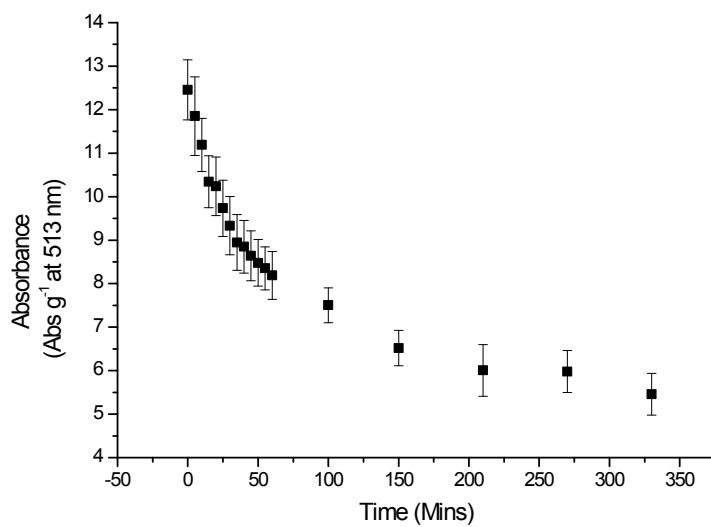


Figure S4 – UV-Vis absorption per gram of **BOB**-based hydrogel at 513 nm over time in PBS (pH 7.3) at 25 °C. Error bars indicate standard deviation (n = 3).

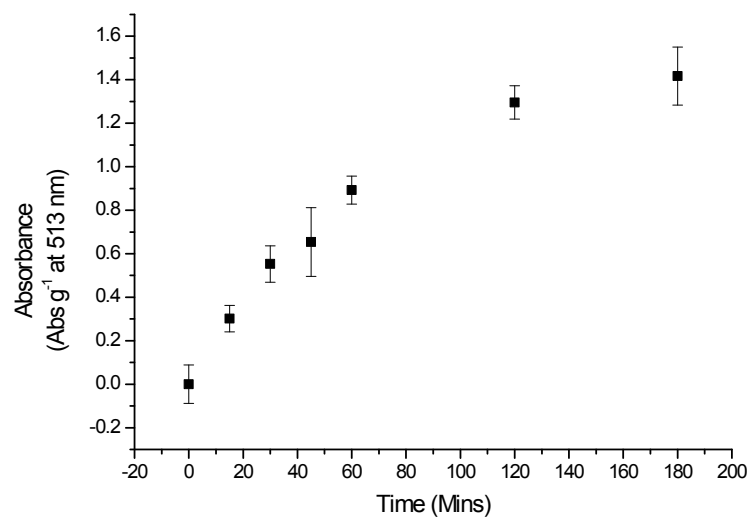


Figure S5 – UV-Vis absorption per gram of **PBA**-based hydrogel at 513 nm over time in PBS (pH 7.3) at 25 °C. Error bars indicate standard deviation (n = 3).

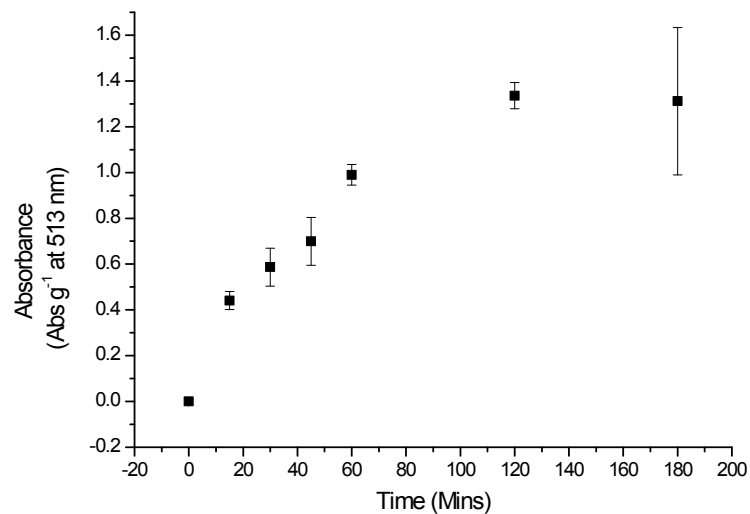


Figure S6 – UV-Vis absorption per gram of **BOB**-based hydrogel at 513 nm over time in PBS (pH 7.3) at 25 °C. Error bars indicate standard deviation (n = 3).

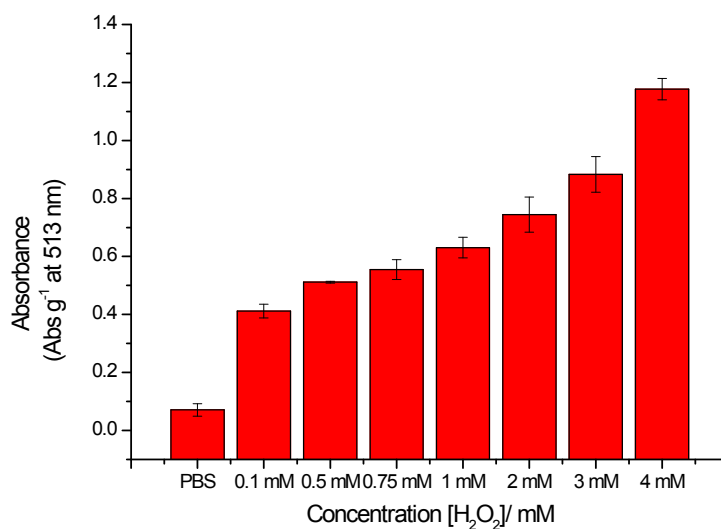


Figure S7 – UV-Vis absorption per gram of **PBA**-based hydrogel in the presence of various concentrations of hydrogen peroxide (0.1 – 4 mM) in PBS (pH 7.3) after 3 hours. Absorbance was measured at 513 nm at 25 °C; error bars indicate standard deviation (n = 3). Increase in absorbance correlates with release of ARS from the hydrogel.

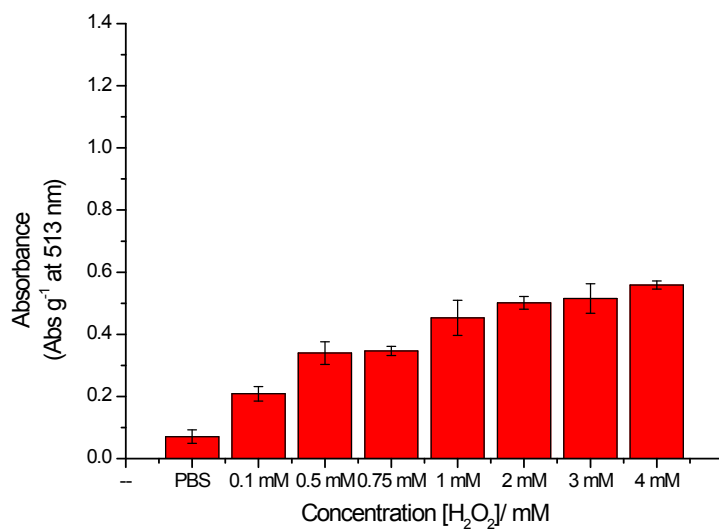


Figure S8 – UV-Vis absorption per gram of **BOB**-based hydrogel in the presence of various concentrations of hydrogen peroxide (0.1 – 4 mM) in PBS (pH 7.3) after 3 hours. Absorbance was measured at 513 nm at 25 °C; error bars indicate standard deviation (n = 3). Increase in absorbance correlates with release of ARS from the gel

5. Minimum Inhibitory Concentration for H₂O₂

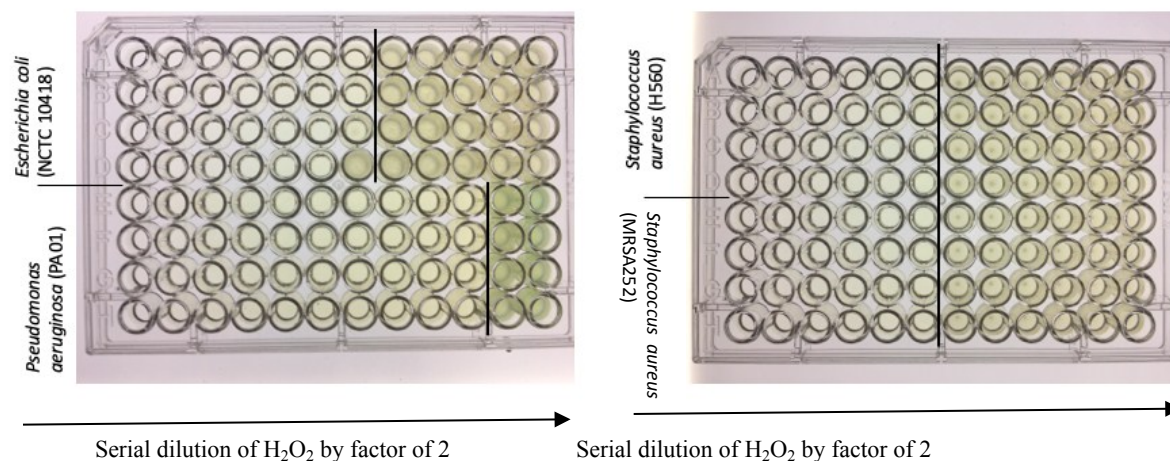


Figure S9 –Minimum Inhibitory Concentration (MIC) of hydrogen peroxide determined through 2-fold serial dilutions (Starting concentration in row 1 was 445 mM H₂O₂) using an established microtiter plate method.⁵ The MIC was defined as the lowest concentration able to inhibit bacterial growth (shown in image with black line). Data was collected by measuring the absorbance at 600 nm and plotted as shown in **Figure S10**⁶

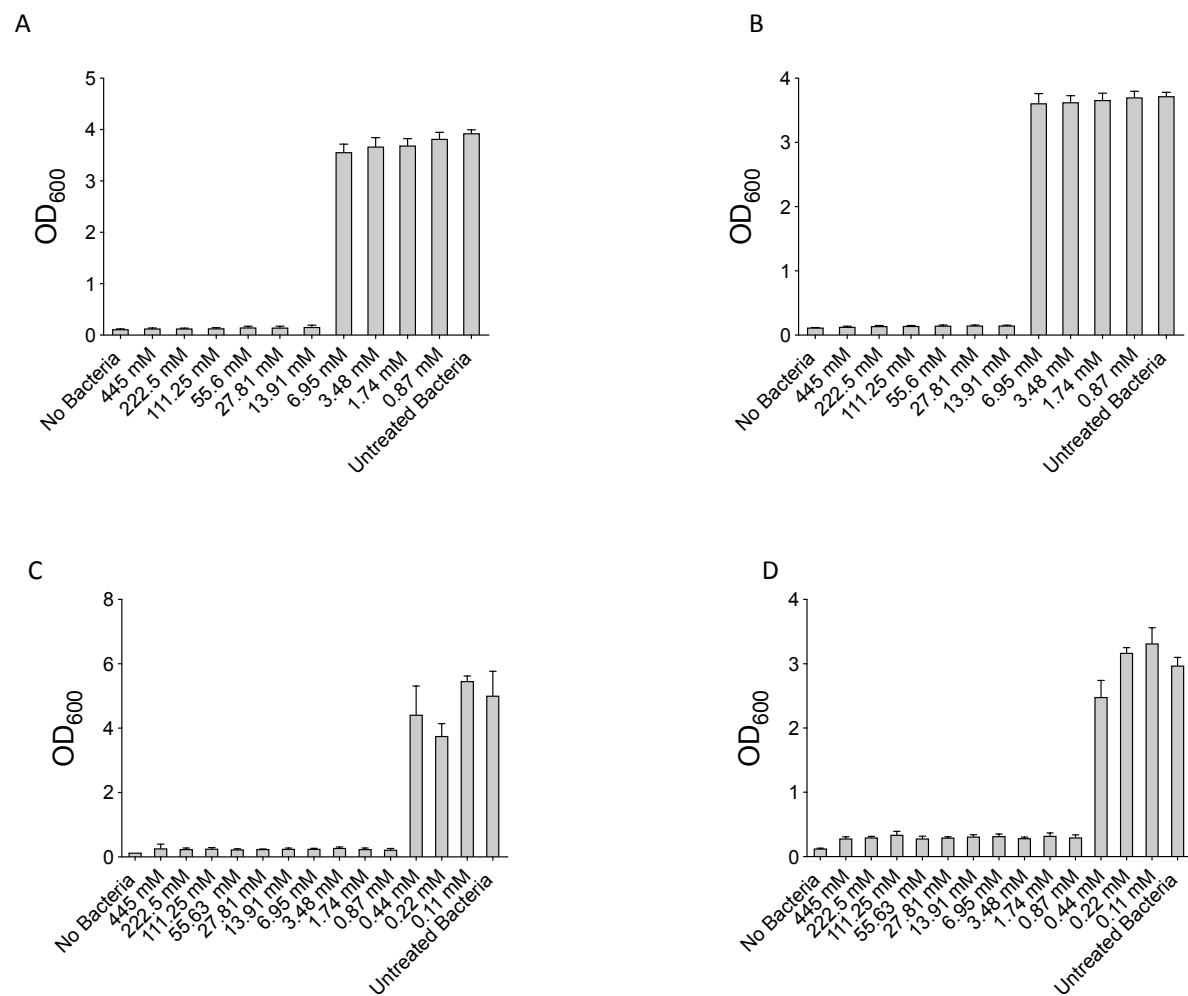


Figure S10 – Determination of the Minimum Inhibitory Concentration (MIC) of hydrogen peroxide (H₂O₂) for (A) *Staphylococcus aureus* MRSA252, (B) *Staphylococcus aureus* H560, (C) *Pseudomonas aeruginosa* PAO1 and (D) *Escherichia coli* NCTC 10418. Data was collected by measuring absorbance at 600 nm every 4 min over 18 hours at 37 °C using a Flurostar Plate reader. Error bars indicate standard deviation (n = 3).

6. Minimum Inhibitory Concentration data for Alizarin Red S (ARS)

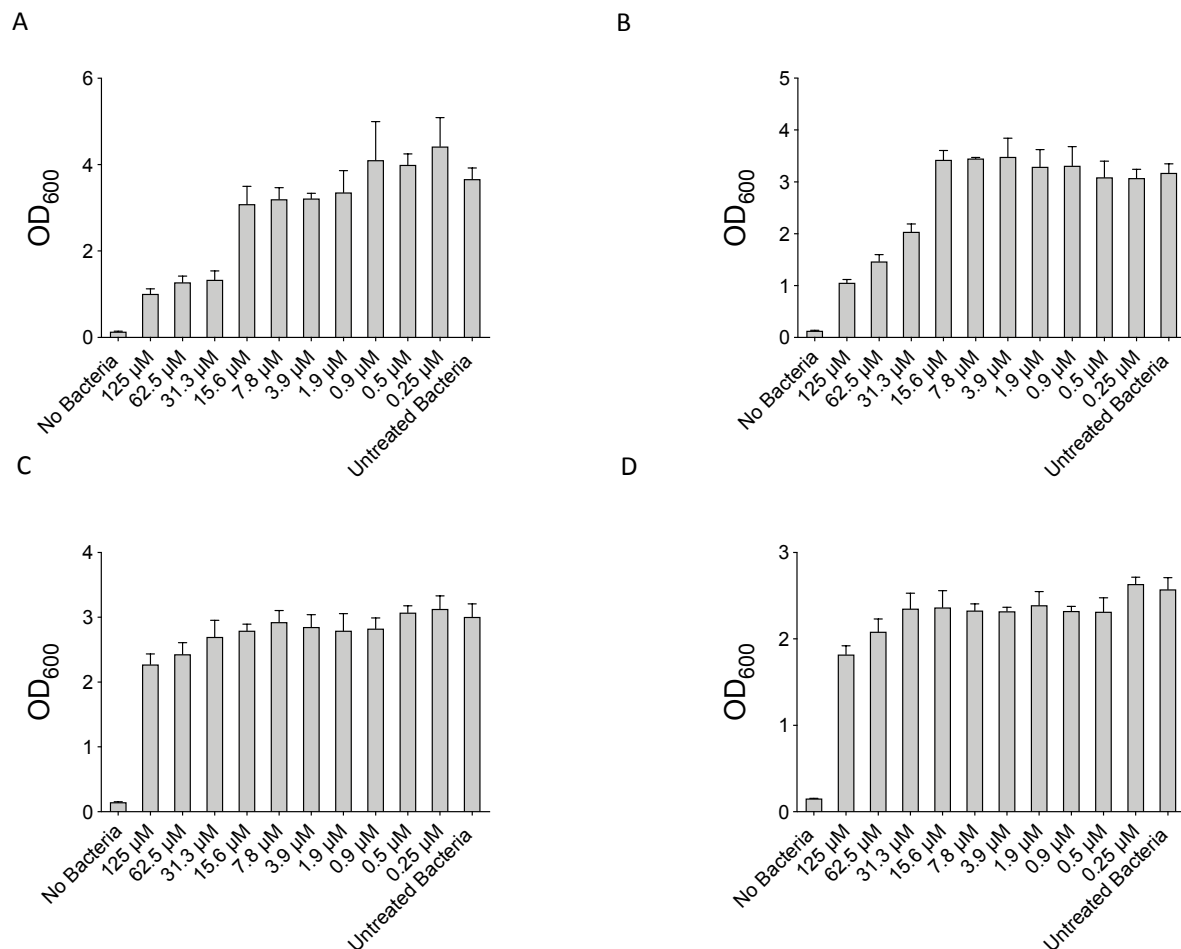


Figure S11 – Determination of the Minimum Inhibitory Concentration (MIC) of Alizarin Red S (ARS) for (A) *Staphylococcus aureus* MRSA252, (B) *Staphylococcus aureus* H560, (C) *Pseudomonas aeruginosa* PAO1 and (D) *Escherichia coli* NCTC 10418. It is believed that the MIC for ARS is above 125 μM for all bacterial strains tested, although the OD₆₀₀ for 31.3 μM - 125 μM was found to be significantly smaller than for lower concentrations including the untreated control, the OD₆₀₀ is higher than that for no bacteria thus the MIC is greater than 125 μM. Untreated control was bacteria grown for 18 h without any treatment. Data was collected by measuring absorbance at 600 nm every 4 min over 18 hours at 37 °C using a Flurostar Plate reader. Error bars indicate standard deviation (n = 3). ARS concentration range was limited due to solubility issues.

7. Growth curves for bacterial strains used

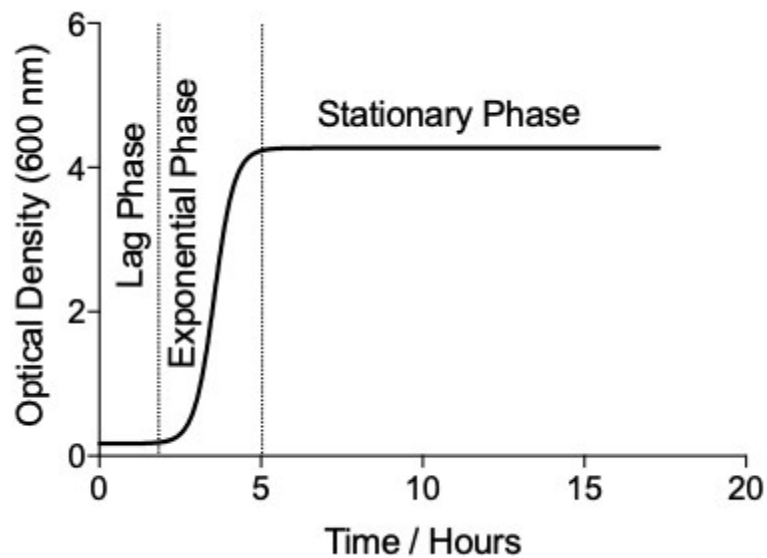


Figure S12 – Schematic of a bacterial growth curve outlining the three phases of bacterial growth over an 18 hours period at 37 °C. Three key phases: lag phase, exponential phase and stationary phase.

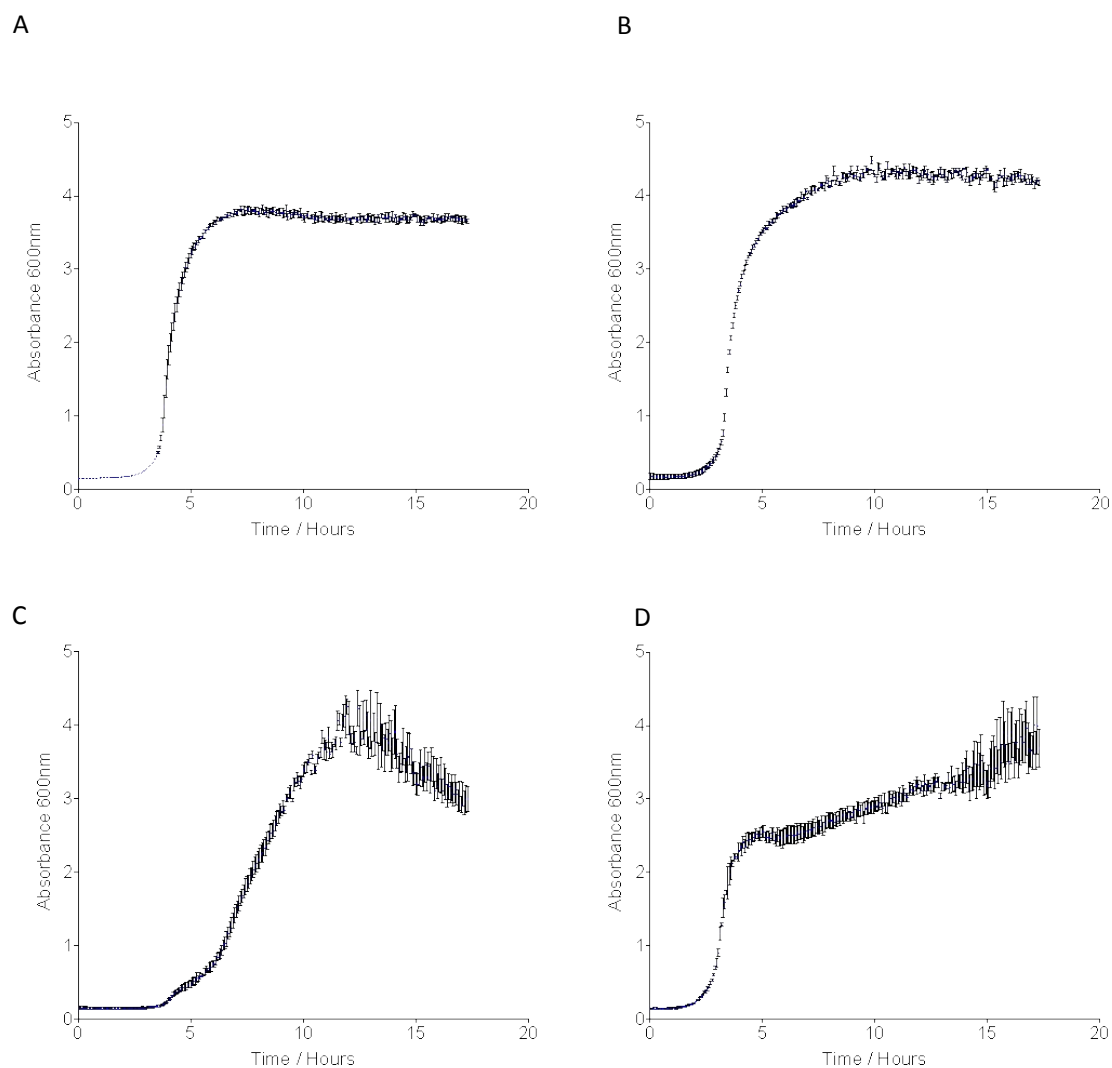


Figure S13 - Growth curves of (A) *Staphylococcus aureus* MRSA252, (B) *Staphylococcus aureus* H560, (C) *Pseudomonas aeruginosa* PAO1 and (D) *Escherichia coli* NCTC 10418 to attain time points for the lag phase, exponential phase, and stationary phase. Data was collected by measuring absorbance at 600 nm every 4 min over 18 hours at 37 °C using a Flurostar Plate reader. Error bars indicate standard deviation (n = 4). Time points for each phase of growth per bacterial strain are shown in **Table S1**.

Table S1 – Time points for each phase of growth per bacterial strain obtained from **Figure S15**.

Bacterial Strain	Lag Phase (hrs)	Exponential Phase (hrs)	Stationary Phase (hrs)
Methicillin-resistant <i>Staphylococcus aureus</i> 252	0 – 2.5	3-6	6<
<i>Staphylococcus aureus</i> H560	0-2	2.5-10	10<
<i>Pseudomonas aeruginosa</i> PAO1	0-1	1-4	4<
<i>Escherichia coli</i> NCTC 10418	0-3	3-10	10<

8. CV data for ARS biofilm inhibition

8.1 Addition of ARS during lag phase

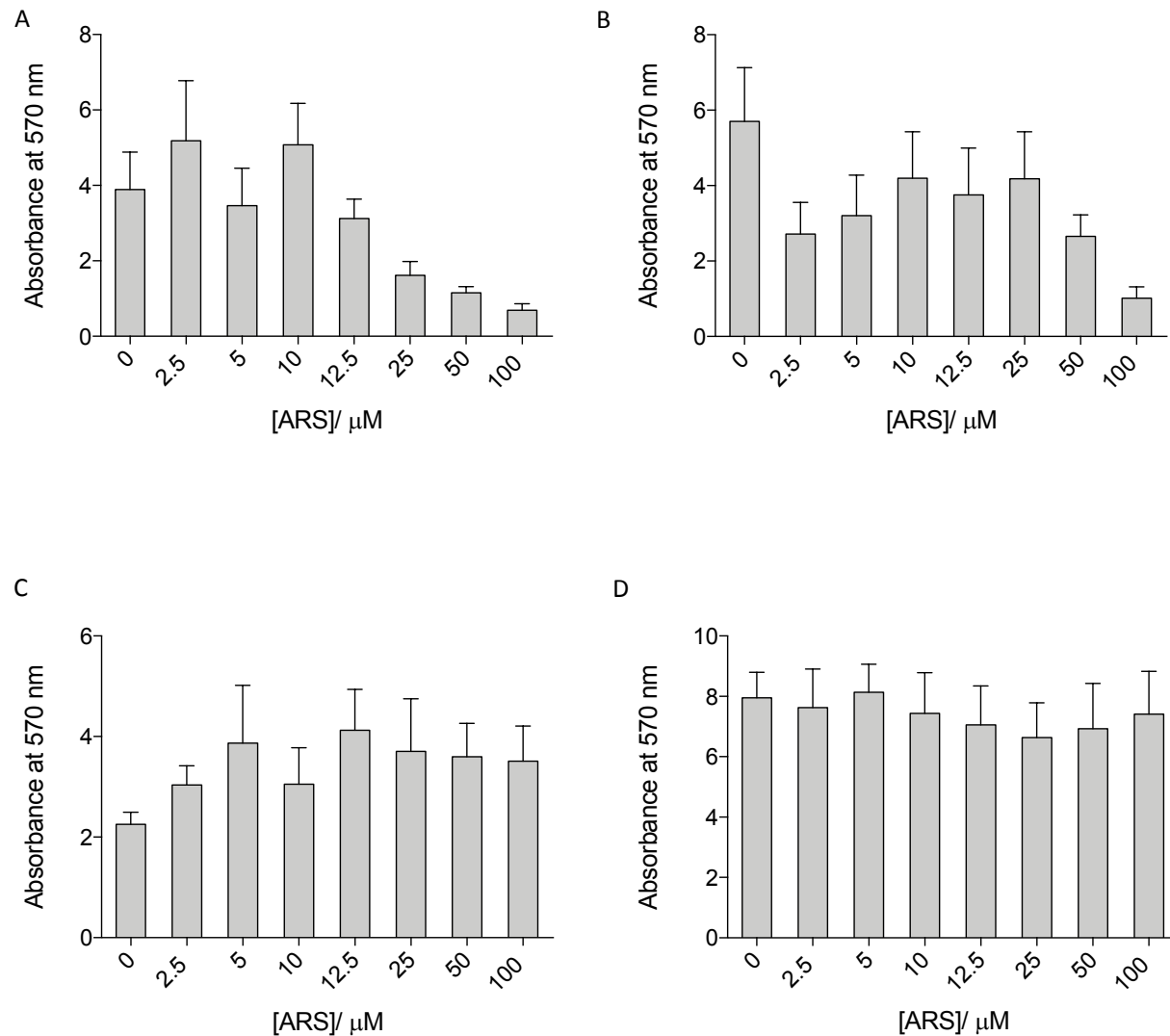


Figure S14 - Varying concentrations of ARS (2.5 – 100 μM) were added at lag phase (time points attained in **Table S1**) to (A) *Staphylococcus aureus* MRSA252, (B) *Staphylococcus aureus* H560, (C) *Pseudomonas aeruginosa* PAO1 and (D) *Escherichia coli* NCTC 10418. After 24 hours growth at 37 $^{\circ}\text{C}$, bacterial biomass was determined by crystal violet staining whereby absorbance was measured at 570 nm. A decrease in absorbance at 570 nm corresponds to decrease in bacterial biofilm. Error bars indicate standard deviation ($n = 4$).

8.2 Addition of ARS during exponential phase

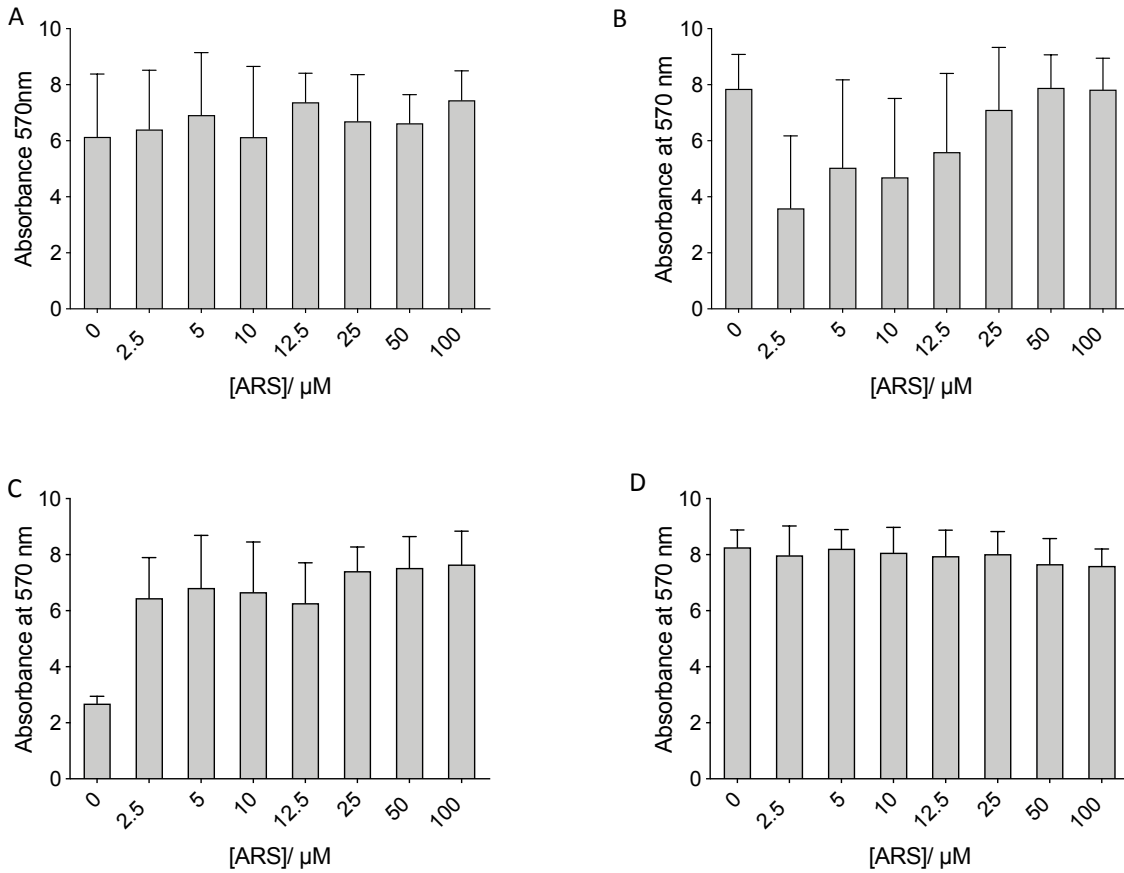


Figure S15- Varying concentrations of ARS (2.5 – 100 μM) were added at exponential phase (time points attained in **Table S1**) to (A) *Staphylococcus aureus* MRSA252, (B) *Staphylococcus aureus* H560, (C) *Pseudomonas aeruginosa* PAO1 and (D) *Escherichia coli* NCTC 10418. After 24 hours growth at 37 $^{\circ}\text{C}$, bacterial biomass was determined by crystal violet staining whereby absorbance was measured at 570 nm. A decrease in absorbance at 570 nm corresponds to decrease in bacterial biofilm. Error bars indicate standard deviation (n = 4).

8.3 Addition of ARS during stationary phase

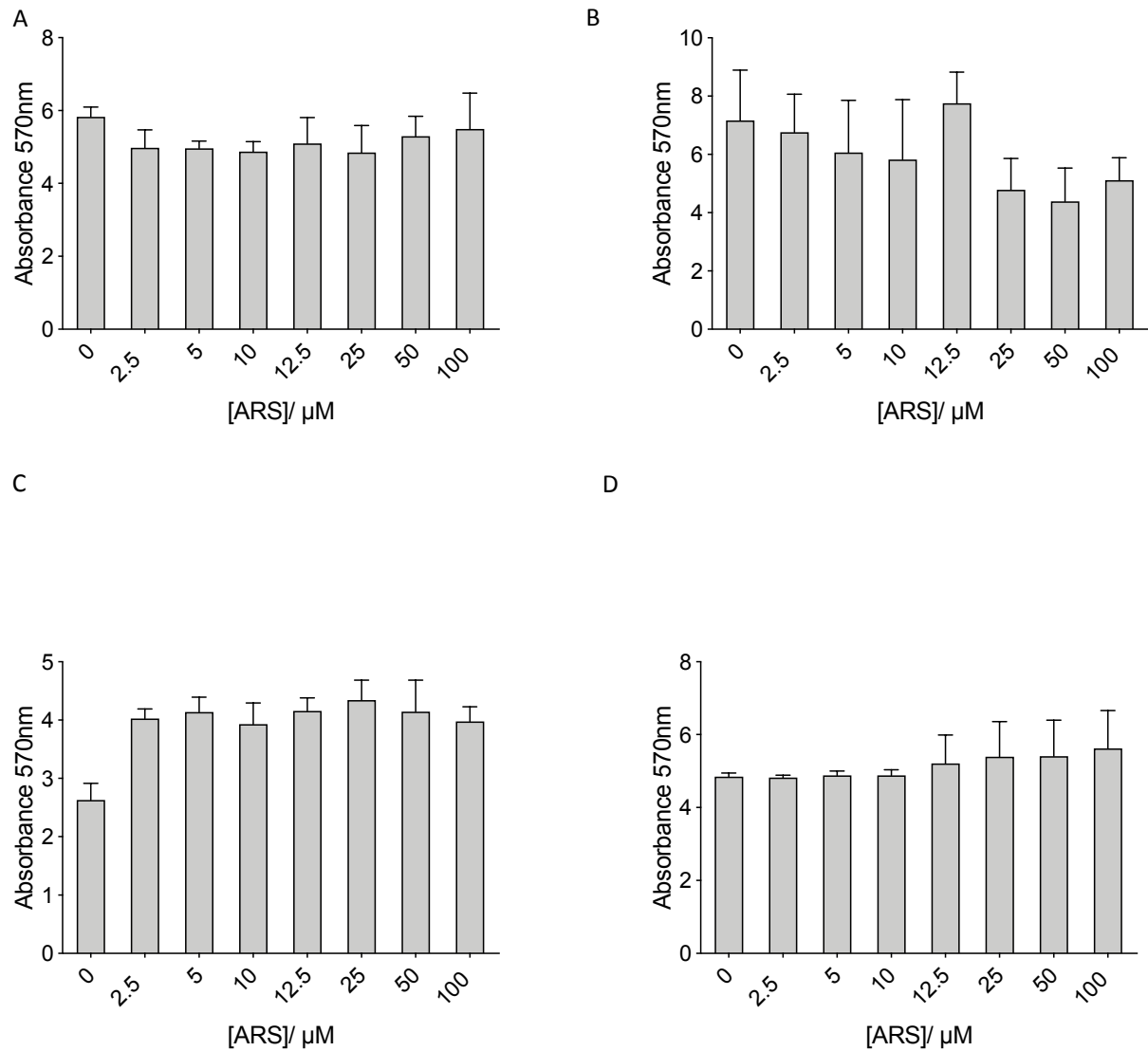


Figure S16 - Varying concentrations of ARS (2.5 – 100 μM) were added at stationary phase (time points attained in **Table S1**) to (A) *Staphylococcus aureus* MRSA252, (B) *Staphylococcus aureus* H560, (C) *Pseudomonas aeruginosa* PAO1 and (D) *Escherichia coli* NCTC 10418. After 24 hours growth at 37 $^{\circ}\text{C}$, bacterial biomass was determined by crystal violet staining whereby absorbance was measured at 570 nm. A decrease in absorbance at 570 nm corresponds to decrease in bacterial biofilm. Error bars indicate standard deviation (n = 4).

9. CV data for hydrogen peroxide biofilm inhibition

9.1 Addition of hydrogen peroxide during lag phase

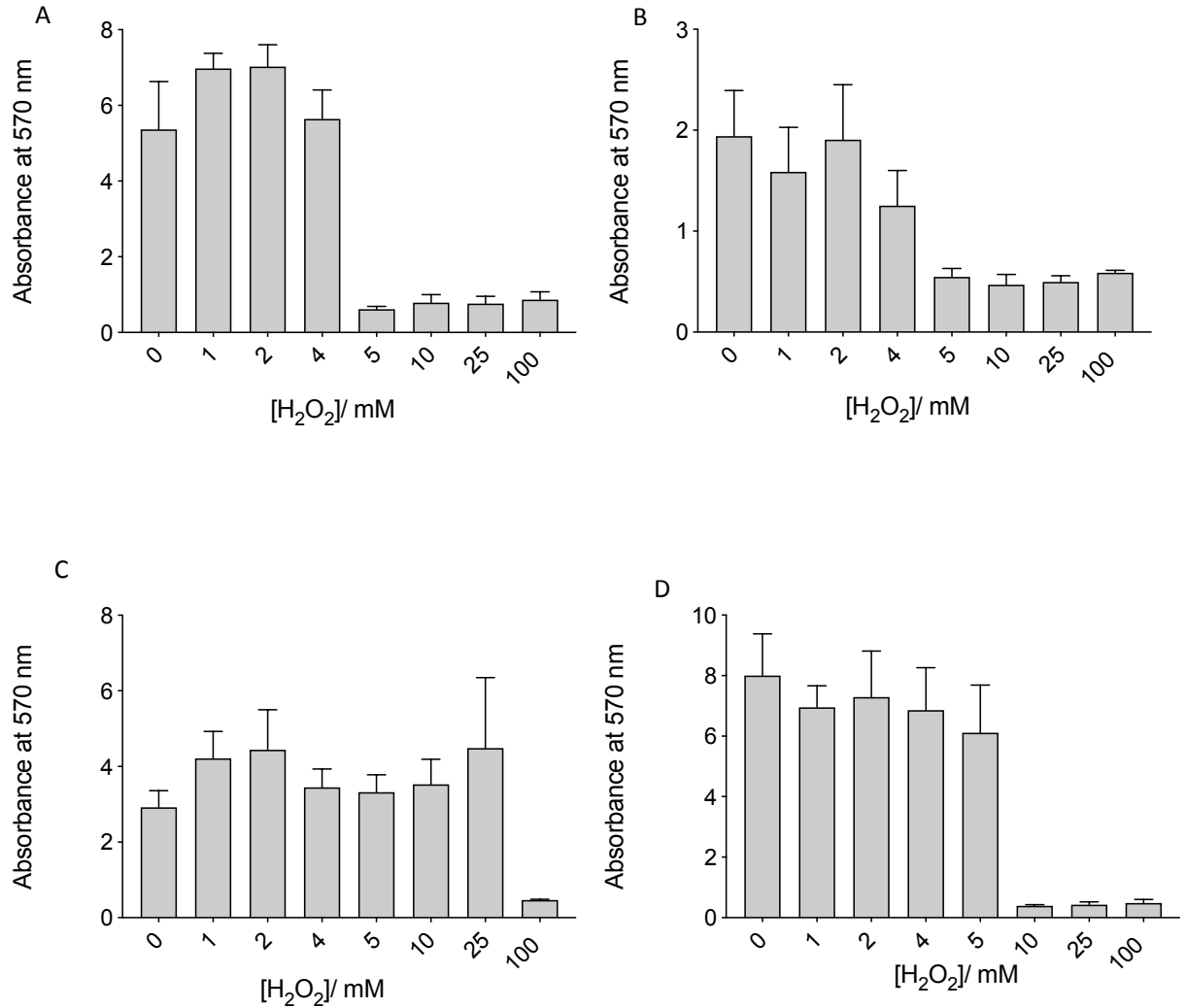


Figure S17 - Varying concentrations of H_2O_2 (1 – 100 mM) were added at lag phase (time points attained in **Table S1**) to (A) *Staphylococcus aureus* MRSA252, (B) *Staphylococcus aureus* H560, (C) *Pseudomonas aeruginosa* PAO1 and (D) *Escherichia coli* NCTC 10418. After 24 hours growth at 37 °C, bacterial biomass was determined by crystal violet staining whereby absorbance was measured at 570 nm. A decrease in absorbance at 570 nm corresponds to decrease in bacterial biofilm. Error bars indicate standard deviation (n = 4).

9.2 Addition of hydrogen peroxide during exponential phase

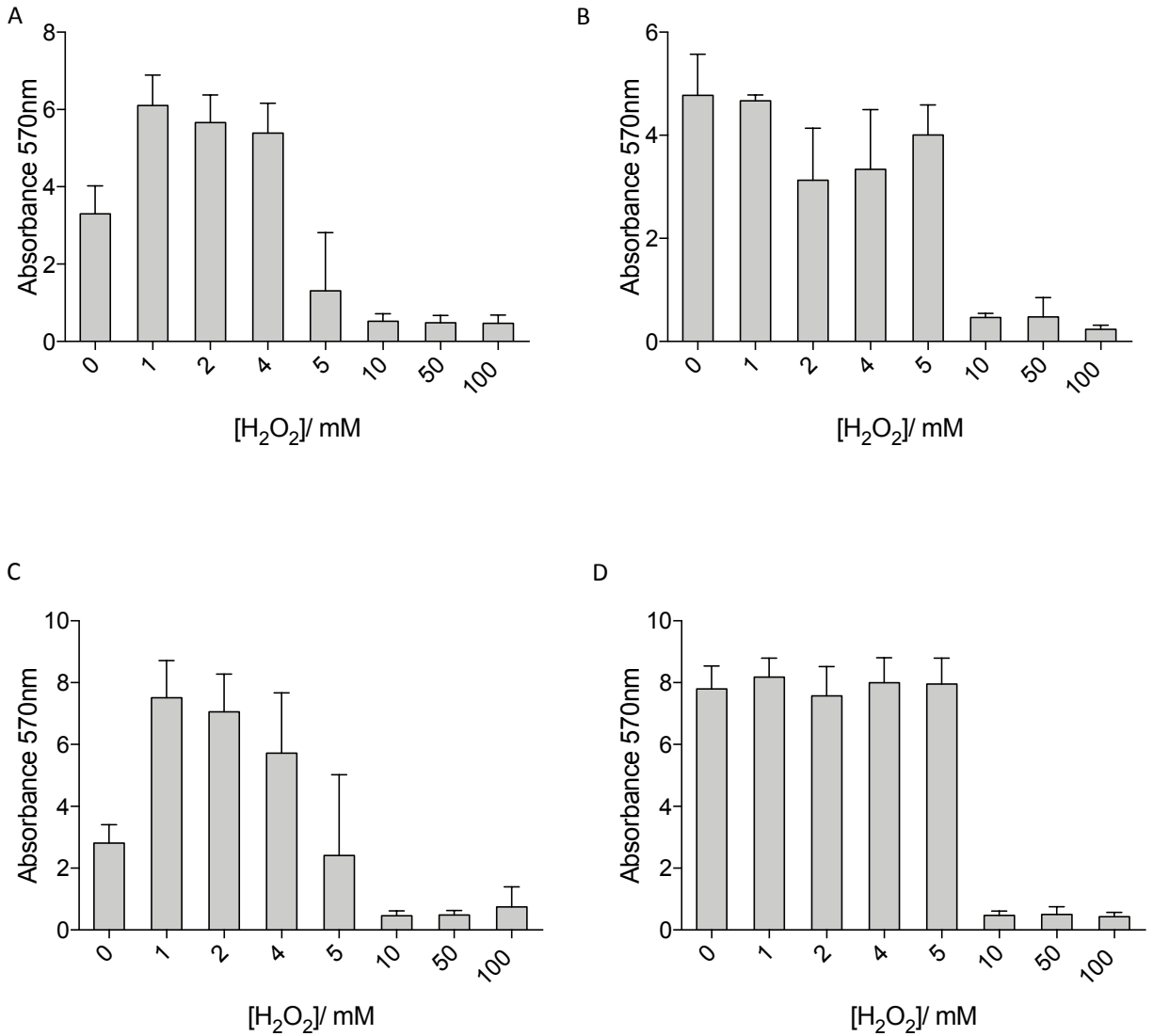


Figure S18 - Varying concentrations of H_2O_2 (1 – 100 mM) were added at exponential phase (time points attained in **Table S1**) to (A) *Staphylococcus aureus* MRSA252, (B) *Staphylococcus aureus* H560, (C) *Pseudomonas aeruginosa* PAO1 and (D) *Escherichia coli* NCTC 10418. After 24 hours growth at 37 °C, bacterial biomass was determined by crystal violet staining whereby absorbance was measured at 570 nm. A decrease in absorbance at 570 nm corresponds to decrease in bacterial biofilm. Error bars indicate standard deviation (n = 4).

9.3 Addition of hydrogen peroxide during stationary phase

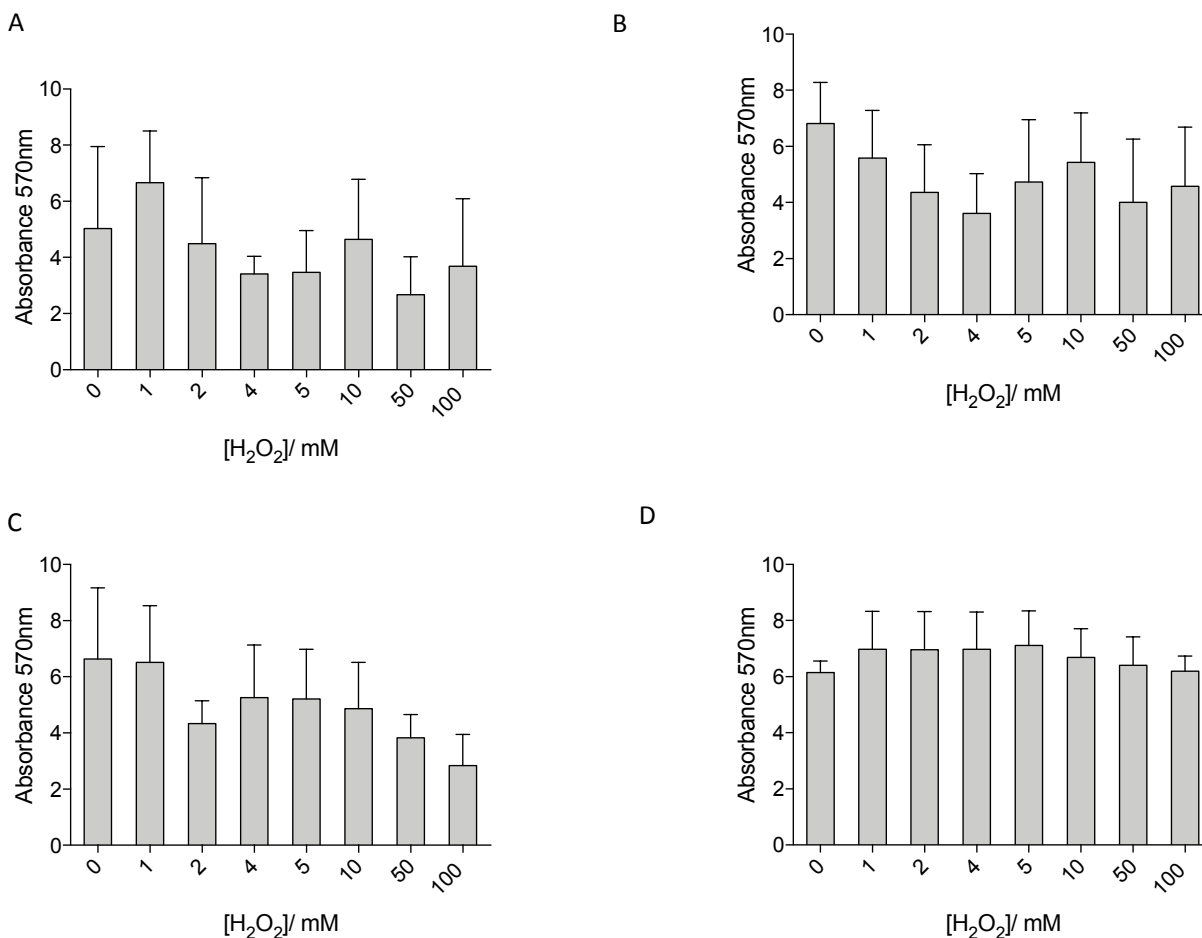


Figure S19 - Varying concentrations of H_2O_2 (1 – 100 mM) were added at stationary phase (time points attained in **Table S1**) to (A) *Staphylococcus aureus* MRSA252, (B) *Staphylococcus aureus* H560, (C) *Pseudomonas aeruginosa* PAO1 and (D) *Escherichia coli* NCTC 10418. After 24 hours growth at 37 °C, bacterial biomass was determined by crystal violet staining whereby absorbance was measured at 570 nm. A decrease in absorbance at 570 nm corresponds to decrease in bacterial biofilm. Error bars indicate standard deviation (n = 4).

10. CV data for combination of ARS and hydrogen peroxide when added at various stages of bacterial growth

10.1 Addition of ARS and 2 mM hydrogen peroxide during lag phase

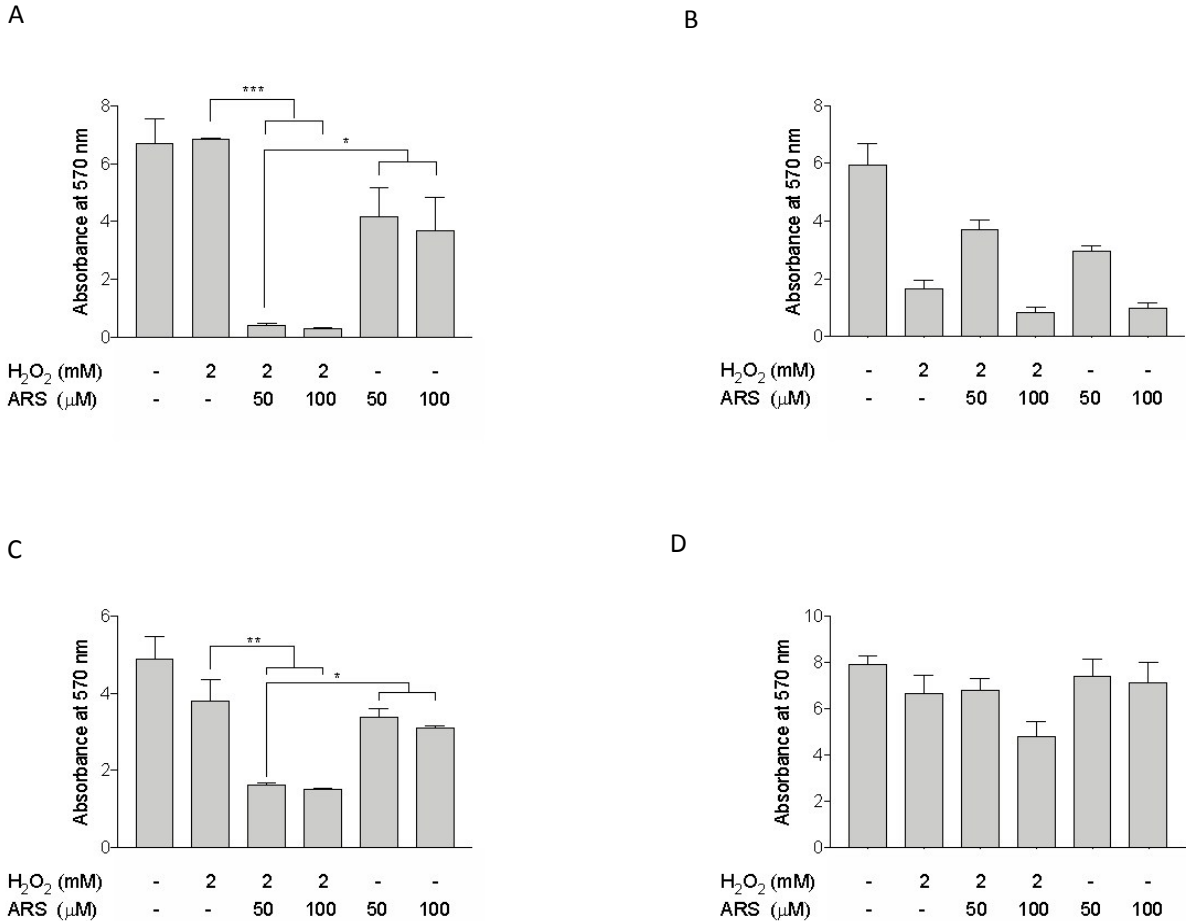


Figure S20 – H₂O₂ (2 mM) was added to ARS (50 and 100 μM) during lag phase (time points attained in **Table S1**) to (A) *Staphylococcus aureus* MRSA252, (B) *Staphylococcus aureus* H560, (C) *Pseudomonas aeruginosa* PAO1 and (D) *Escherichia coli* NCTC 10418. After 24 hours growth at 37 °C, bacterial biomass was determined by crystal violet staining whereby absorbance was measured at 570 nm. A decrease in absorbance at 570 nm corresponds to decrease in bacterial biofilm. Error bars indicate standard deviation (n = 4).

10.2 Addition of ARS and 2 mM hydrogen peroxide during exponential phase

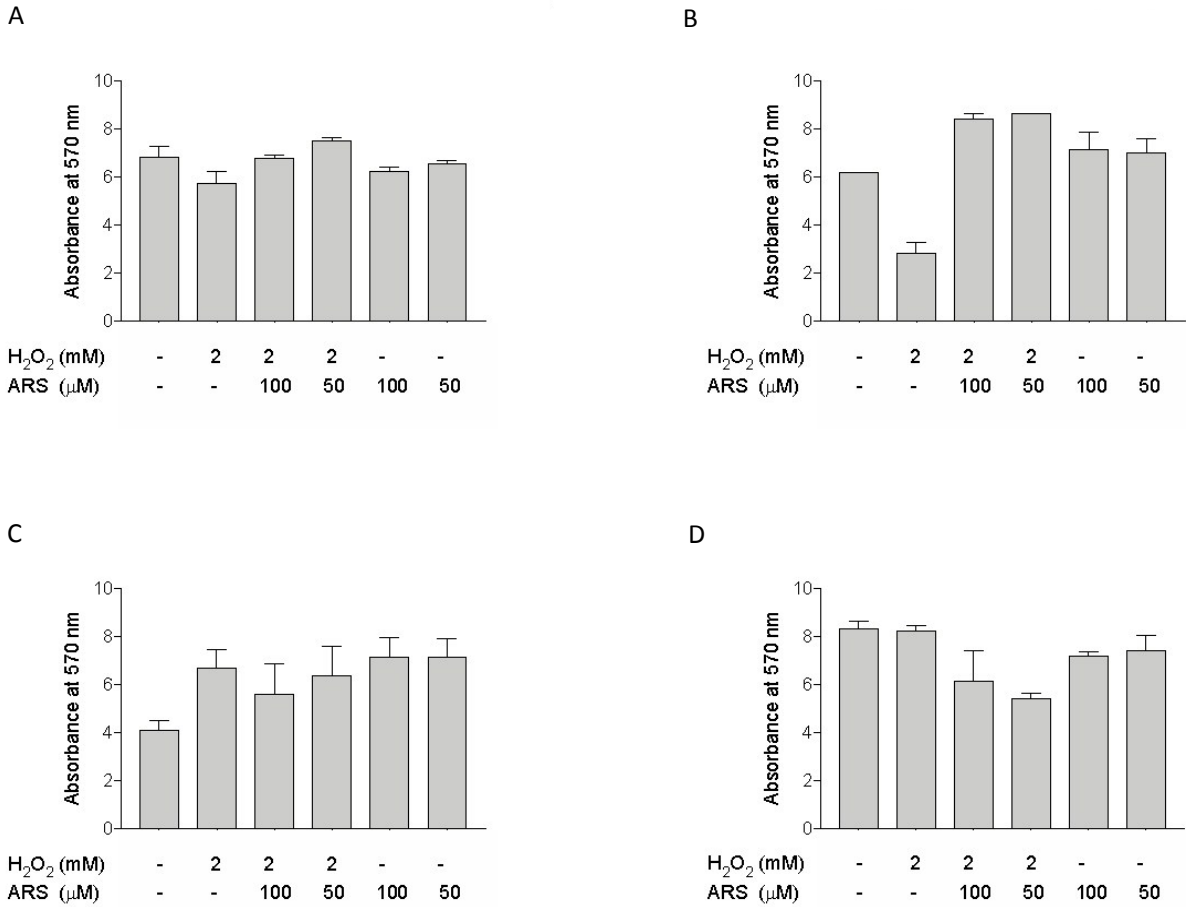


Figure S21 - H₂O₂ (2 mM) was added to ARS (50 and 100 μM) during exponential phase (time points attained in **Table S1**) to (A) *Staphylococcus aureus* MRSA252, (B) *Staphylococcus aureus* H560, (C) *Pseudomonas aeruginosa* PAO1 and (D) *Escherichia coli* NCTC 10418. After 24 hours growth at 37 °C, bacterial biomass was determined by crystal violet staining whereby absorbance was measured at 570 nm. A decrease in absorbance at 570 nm corresponds to decrease in bacterial biofilm. Error bars indicate standard deviation (n = 4).

10.3 Addition of ARS and 2 mM hydrogen peroxide during stationary phase

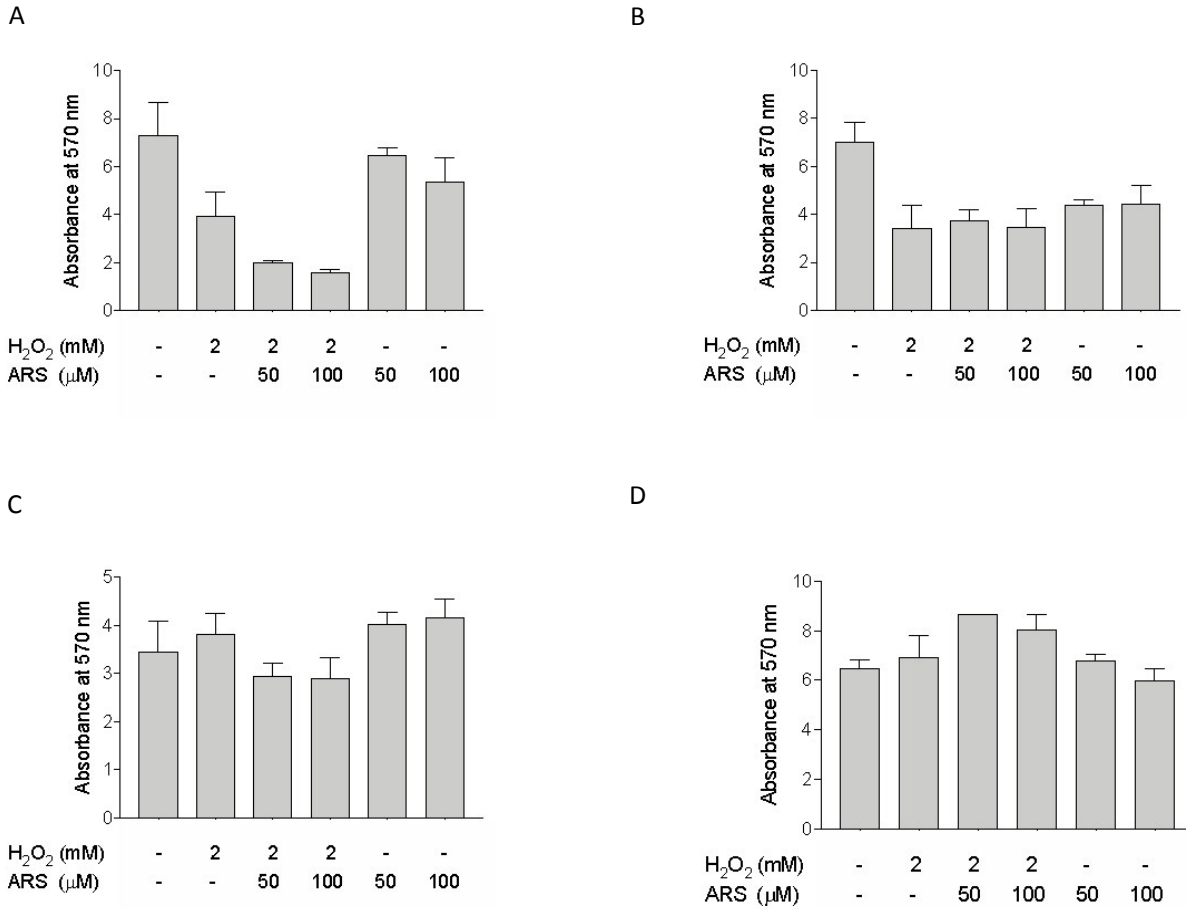


Figure S22 - H₂O₂ (2 mM) was added to ARS (50 and 100 μM) during stationary phase (time points attained in **Table S1**) to (A) *Staphylococcus aureus* MRSA252, (B) *Staphylococcus aureus* H560, (C) *Pseudomonas aeruginosa* PAO1 and (D) *Escherichia coli* NCTC 10418. After 24 hours growth at 37 °C, bacterial biomass was determined by crystal violet staining whereby absorbance was measured at 570 nm. A decrease in absorbance at 570 nm corresponds to decrease in bacterial biofilm. Error bars indicate standard deviation (n = 4).

11. CV data of PBA-ARS gel in PBS solution only

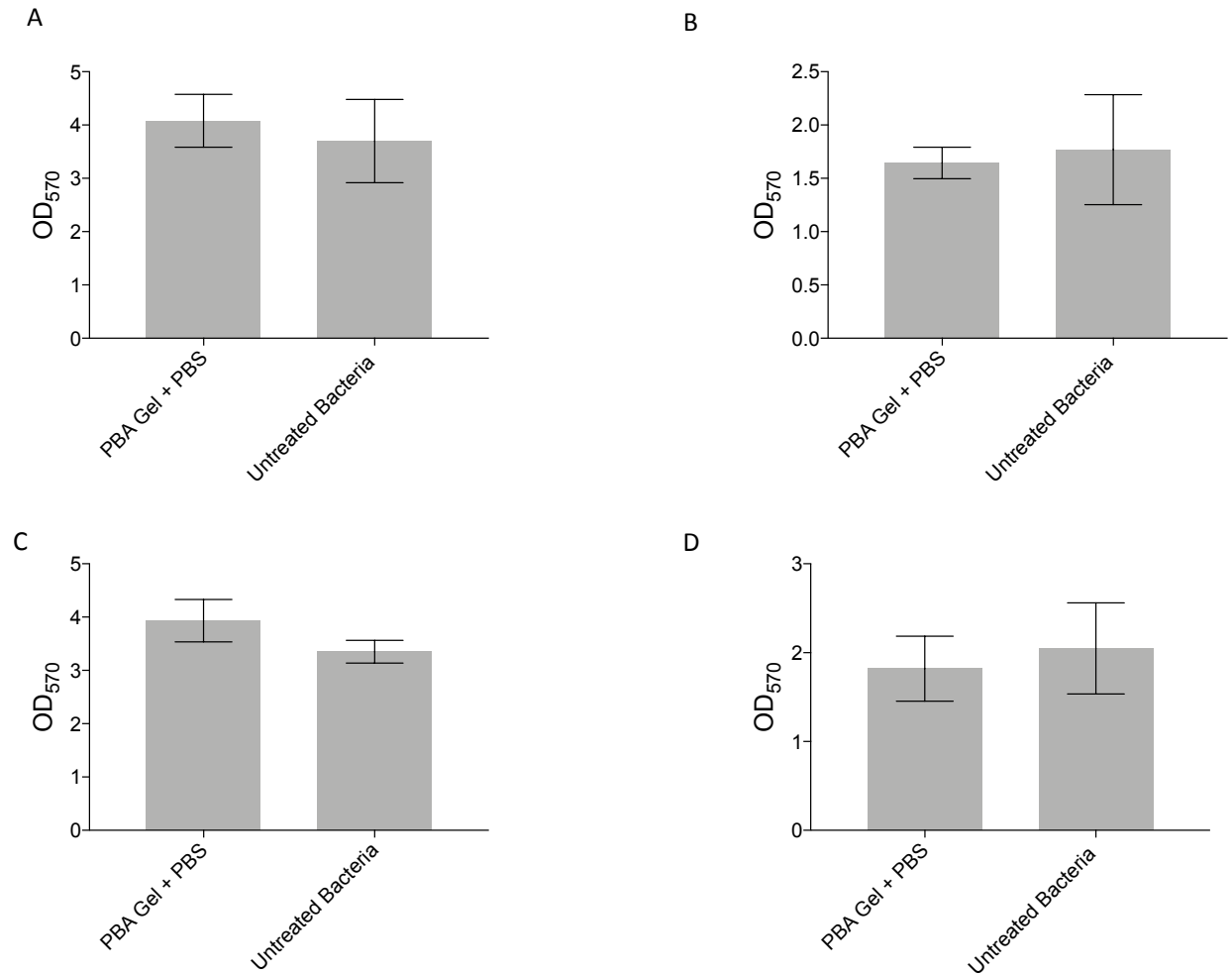


Figure S23 – PBS solution only (pH 7.4) with **PBA** -ARS- gel (incubation 3 h), the resulting solution was assessed for biofilm inhibitory effects. The PBS solution was added to (A) *Staphylococcus aureus* H560, (B) *Staphylococcus aureus* MRSA252, (C) *Pseudomonas aeruginosa* PAO1 and (D) *Escherichia coli* NCTC 10418. After 24 h incubation at 37 °C biofilm formation was assessed using crystal violet staining. Absorbance was recorded at 570 nm. A decrease in absorbance at 570 nm corresponds to decrease in bacterial biofilm. Error bars indicate standard deviation (n = 3).

12. CV data of control acrylamide gel pretreated with ARS

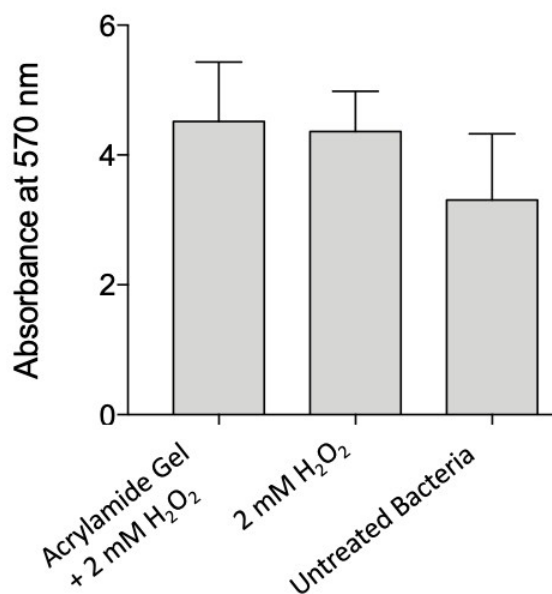
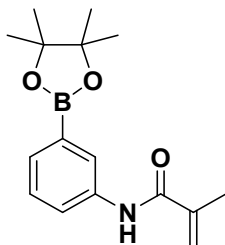


Figure S24 – Acrylamide gels were made according to the same protocol without the use of BPin monomer. Acrylamide gels were then loaded with ARS for 5 h and washed overnight in PBS (pH 7.4). Gels were then added to 1 mL of hydrogen peroxide (2 mM). Gels were incubated at 25 °C for 3 h, the resulting solution was then added to *Staphylococcus aureus* MRSA252. After 24 h incubation at 37 °C biofilm formation was assessed using crystal violet staining. Absorbance was recorded at 570 nm. A decrease in absorbance at 570 nm corresponds to decrease in bacterial biofilm. Error bars indicate standard deviation (n=3) One-way ANOVA was performed. No significant difference was observed between acrylamide gel with 2 mM hydrogen peroxide and 2 mM hydrogen peroxide alone.

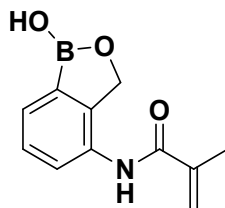
13. Synthetic Experimental

N-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)methacrylamide (PBA)



NEt₃ (0.49 g, 4.88 mmol) and methacryloyl chloride (0.51 g, 4.88 mmol) in DCM (4.0 mL) was added dropwise to a solution of 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (1.00 g, 4.56 mmol) in DCM (60 mL) at 0 °C. The reaction mixture was stirred for 2 h and was allowed to warm to rt, which was then stirred for a further 30 min. It was ensured that the temperature did not rise above 30 °C in order to minimise the occurrence of side polymerisation products. The solution was then washed with H₂O (3 x 40 mL) and dried over MgSO₄. The organic solvent was removed under reduced pressure and the title compound yielded as an off white solid (1.25 g, 95 %). ¹H NMR (δ; 300 MHz; DMSO-*d*₆) 7.98 (1H, dq, *CH*), 7.68 (1H, d, *CH*), 7.56 (1H, d, *CH*), 7.36 (1H, t, AR *CH*), 5.79 (1H, s, *CHH*), 5.44 (1H, s, *CHH*), 2.05 (3H, s, *CH*₃), 1.35 (12H, s, 4 x *CH*₃). ¹³C NMR (δ; 75 MHz; DMSO-*d*₆) 166.54, 140.90, 137.32, 130.73, 128.74, 125.89, 123.23, 119.15, 84.14, 24.98, 18.85. FTIR (thin film) ν; 1622 (C=C), 1662 (C=O), 2977 (C-H), 3355 (N-H). HRMS (FTMS): *m/z* calculated for C₁₆H₂₂BNO₃: requires 288.1768 for [M-H]⁺, found 288.1795.

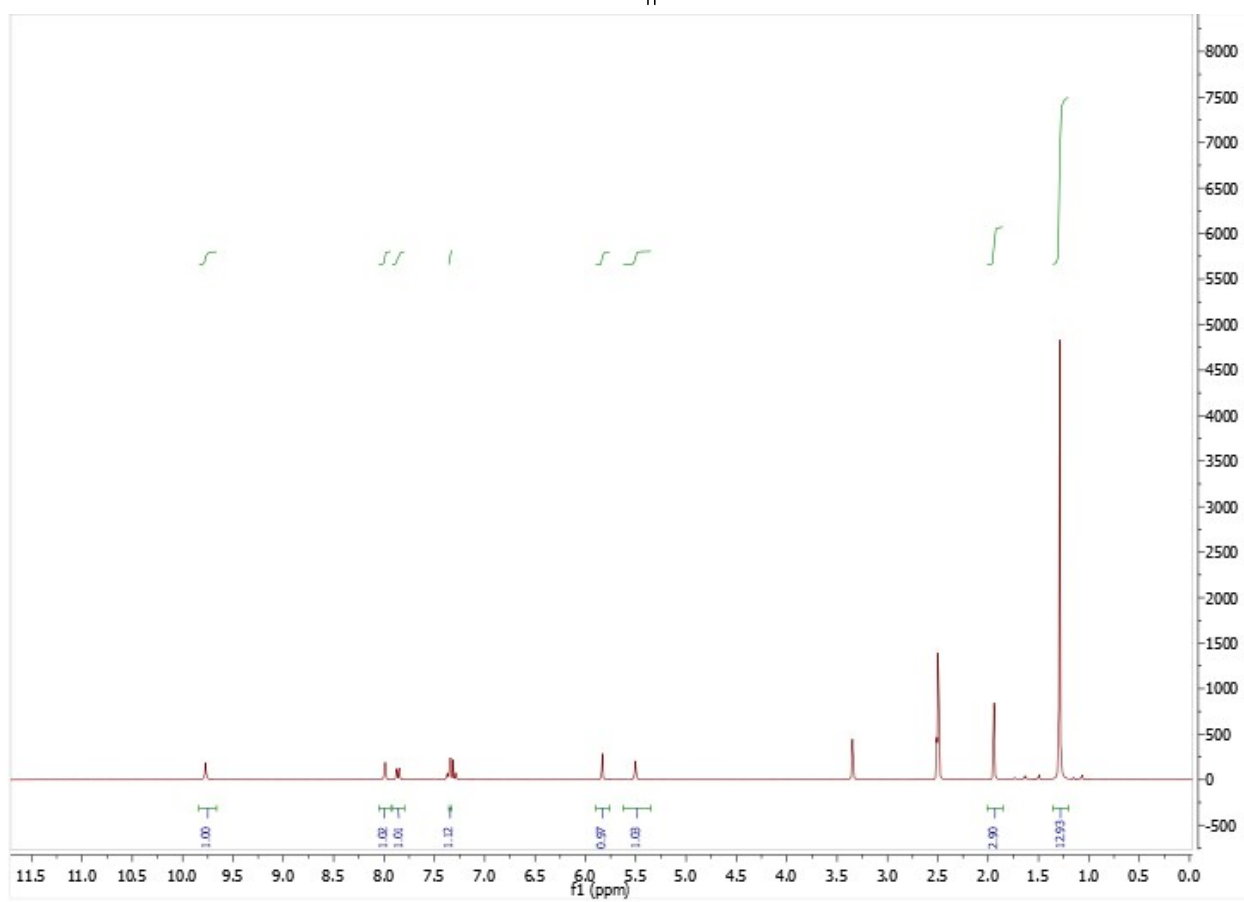
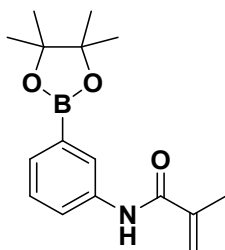
***N*-(1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-6-yl)methacrylamide (BOB)**



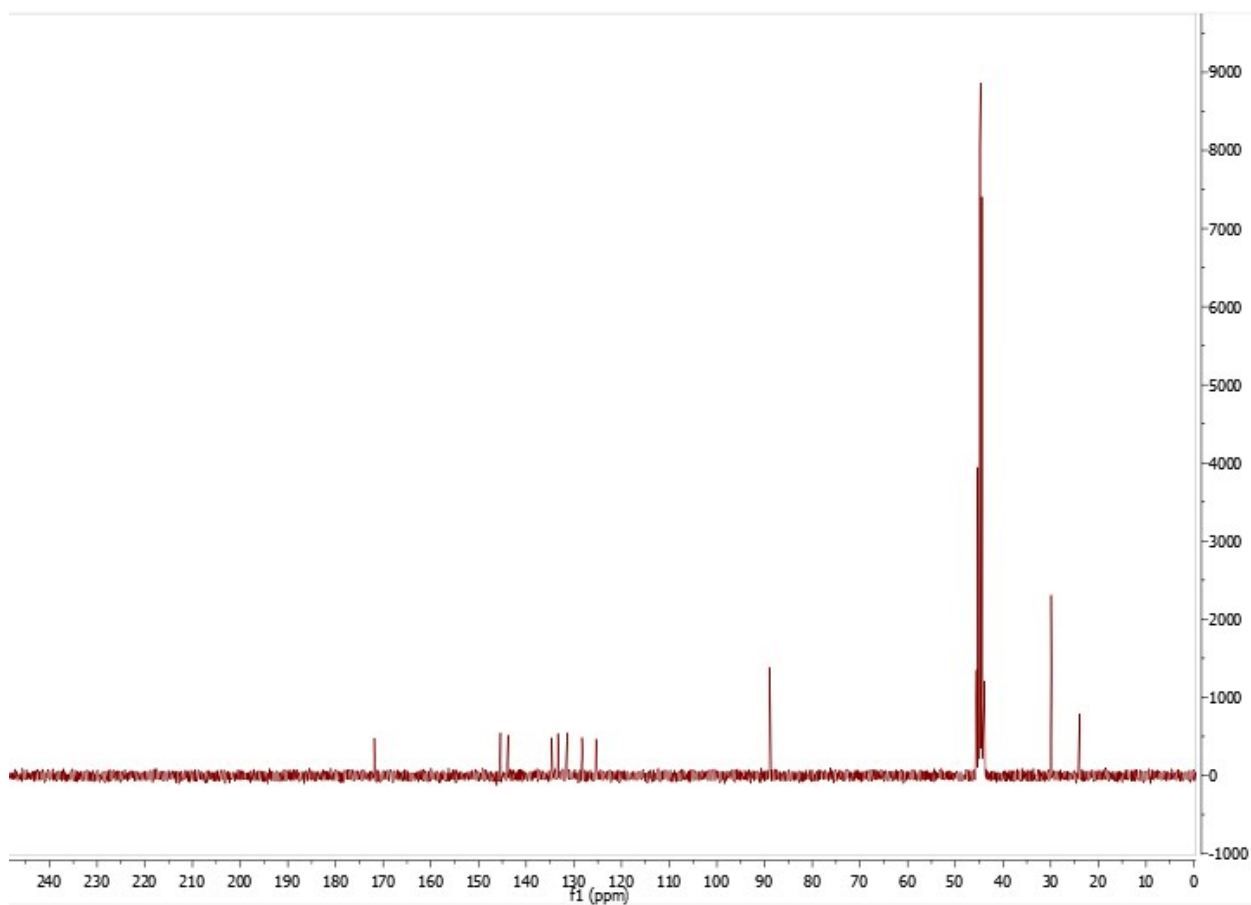
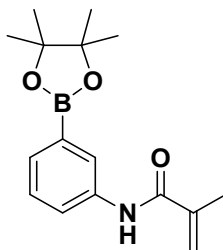
Methacryloyl chloride (1.32 mL, 13.42 mmol) was added dropwise to a solution of 6-Aminobenzoxaborole¹ (1.00 g, 6.71 mmol) and NaHCO₃ (2.26g, 26.84 mmol) in H₂O:THF (1:1, 40 mL) at 0 °C. The reaction was stirred for 2 h at 0 °C, then overnight at room temperature. The organic solvent was removed under reduced pressure and solid product obtained via extraction with EtOAc for 2 h. The organic layer was continuously washed with H₂O, sat. NaHCO₃ solution and brine. The organic layer was dried over MgSO₄ and the solvent removed under reduced pressure, yielding a pale brown solid (1.19 g, 82 %). ¹H NMR (300 MHz, DMSO-d₆): δ 9.81 (1H, NH, s), 9.19 (1H, OH, s), 8.07 (1H, ArH, d, J = 1.6 Hz), 7.68 (1H, ArH, dd, J₁ = 8.2, J₂ = 1.9 Hz), 7.35 (1H, ArH, d, J = 8.2 Hz), 5.82 (1H, CH, s), 5.51 (1H, CH, s), 4.95 (2H, CH₂, s), 1.97 (3H, CH₃, s); ¹³C NMR (75 MHz, DMSO-d₆): δ 167.10, 149.34, 140.77, 138.12, 123.85, 122.60, 121.68, 120.20, 70.05, 18.46; ¹¹B NMR (96 MHz, DMSO-d₆): δ 23.76; FTIR ν (cm⁻¹): 3276, 3086, 2973, 1655, 1214, 979; HRMS (ES) m/z calculated for C₁₁H₁₃BNO₃: [M+H]⁺ 218.0988, found 218.0993; Mp: 167-170 °C

14. NMR

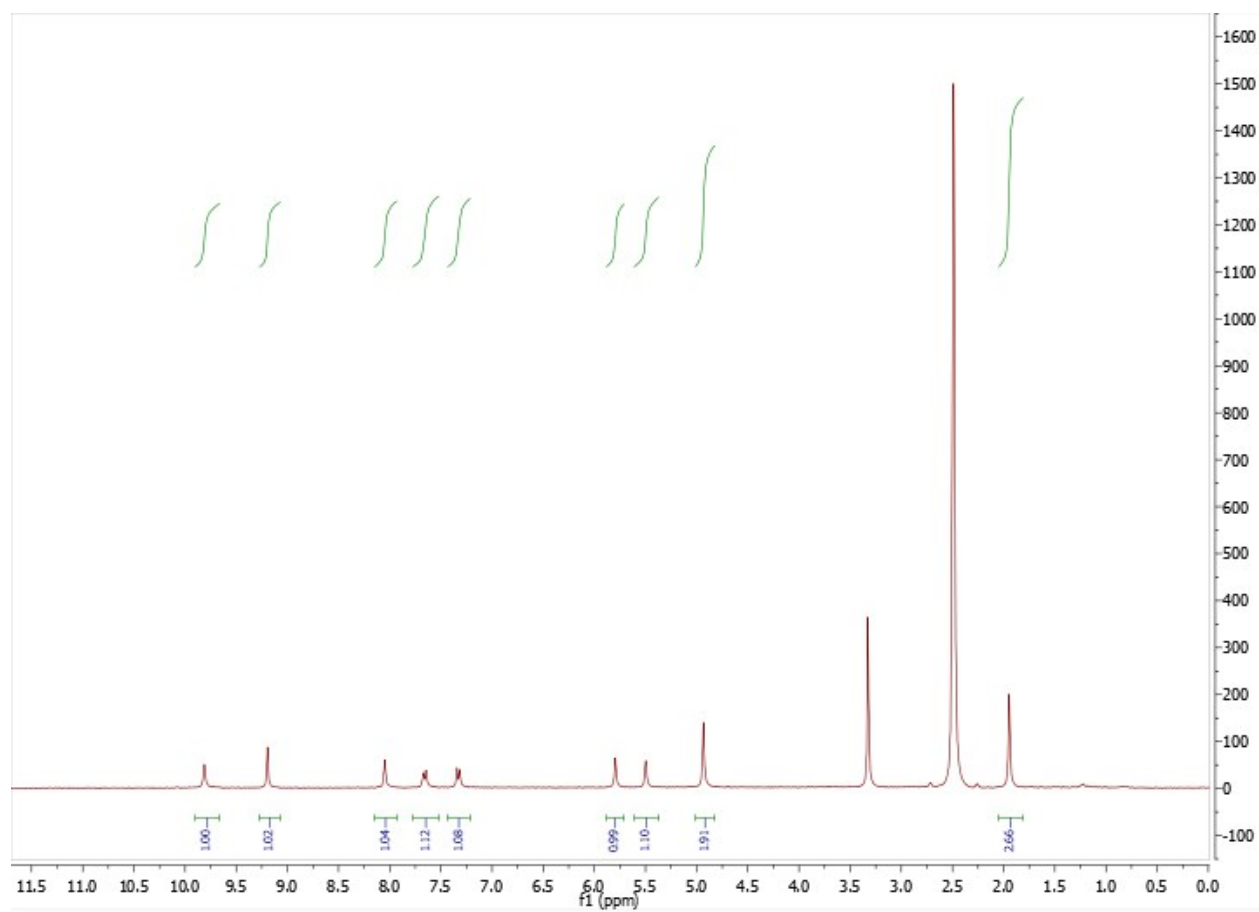
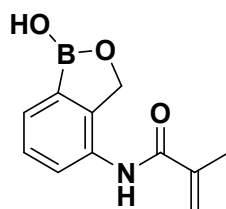
N-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)methacrylamide (PBA) (^1H NMR, 300 MHz, DMSO- d_6)



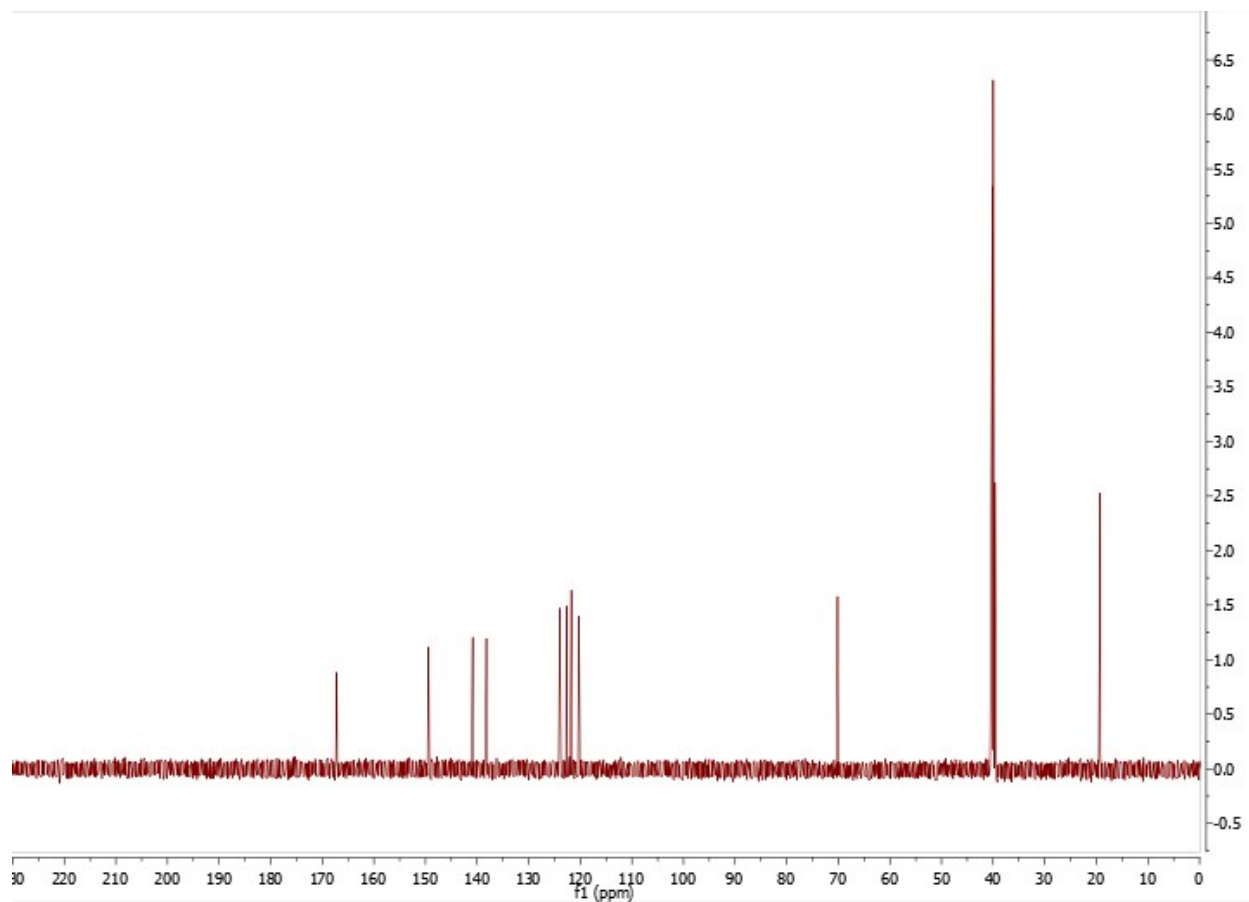
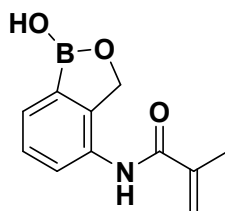
***N*-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)methacrylamide (PBA)** (^{13}C NMR, 75.5 MHz, DMSO- d_6)



***N*-(1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-6-yl)methacrylamide** (¹H NMR, 300 MHz, DMSO-*d*₆)



N-(1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-6-yl)methacrylamide (^{13}C NMR, 75.5 MHz, DMSO- d_6)



15. References

1. J. H. Merritt, D. E. Kadouri and G. A. O'Toole, *Curr Protoc Microbiol*, 2005, **0 1**, Unit-1B 1.
2. D. M. Goeres, L. R. Loetterle, M. A. Hamilton, R. Murga, D. W. Kirby and R. M. Donlan, *Microbiology*, 2005, **151**, 757-762.
3. C. M. Watters, T. Burton, D. K. Kirui and N. J. Millenbaugh, *Infection and Drug Resistance*, 2016, **9**, 71-78.
4. J. S. Oh, E. J. Szili, N. Gaur, S. H. Hong, H. Furuta, H. Kurita, A. Mizuno, A. Hatta and R. D. Short, *Journal of Physics D-Applied Physics*, 2016, **49**.
5. M. E. Olson, H. Ceri, D. W. Morck, A. G. Buret and R. R. Read, *Canadian Journal of Veterinary Research*, 2002, **66**, 86-92.
6. Y. Ge, D. L. MacDonald, K. J. Holroyd, C. Thornsberry, H. Wexler and M. Zasloff, 1999.
7. J. H. Lee, Y. G. Kim, S. Yong Ryu and J. Lee, in *Sci Rep*, 2016, vol. 6.
8. I. Keren, N. Kaldalu, A. Spoering, Y. Wang and K. Lewis, *FEMS Microbiology Letters*, 2004, **230**, 13-18.
9. E. V. Lampard, A. C. Sedgwick, T. Sombuttan, G. T. Williams, B. Wannalerse, A. T. A. Jenkins, S. D. Bull and T. D. James, *Chemistryopen*, 2018, **7**, 266-268.