Supplementary Figures for 'Forging new antibiotic combinations under iron-limiting conditions'

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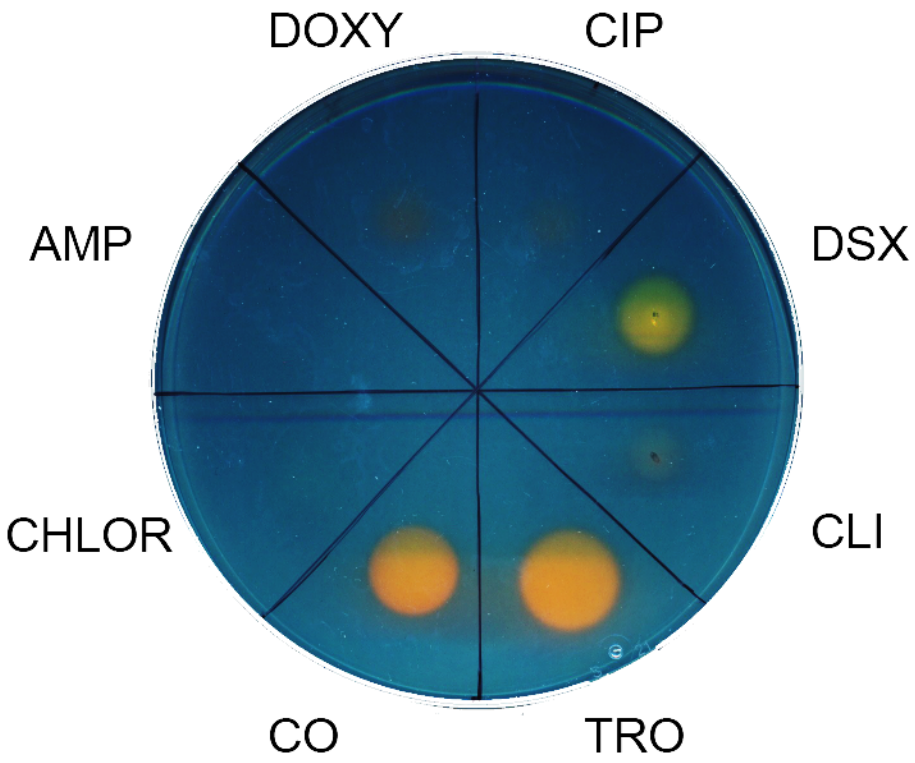
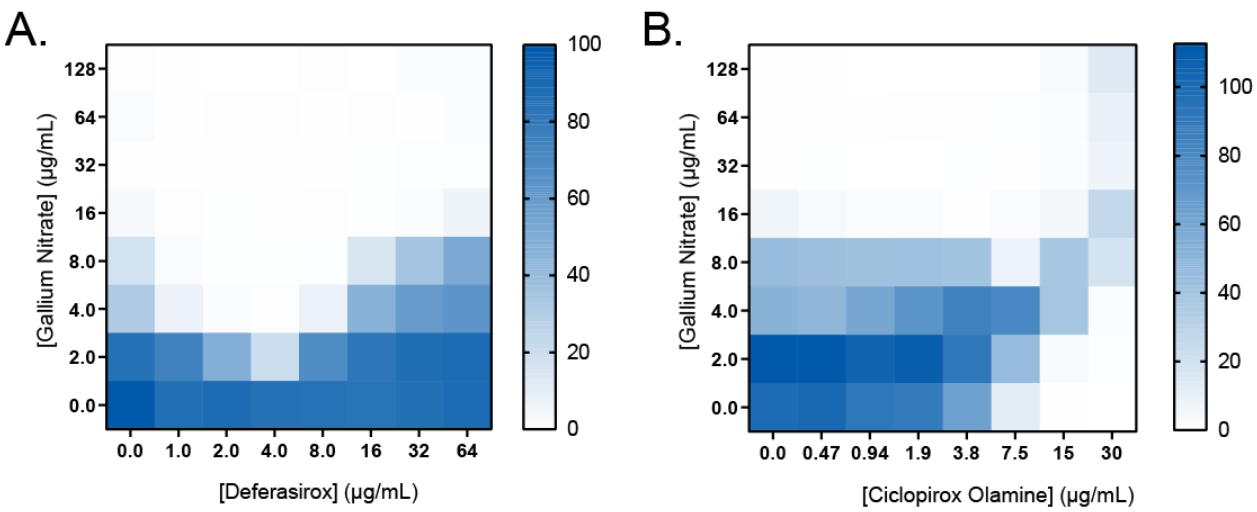
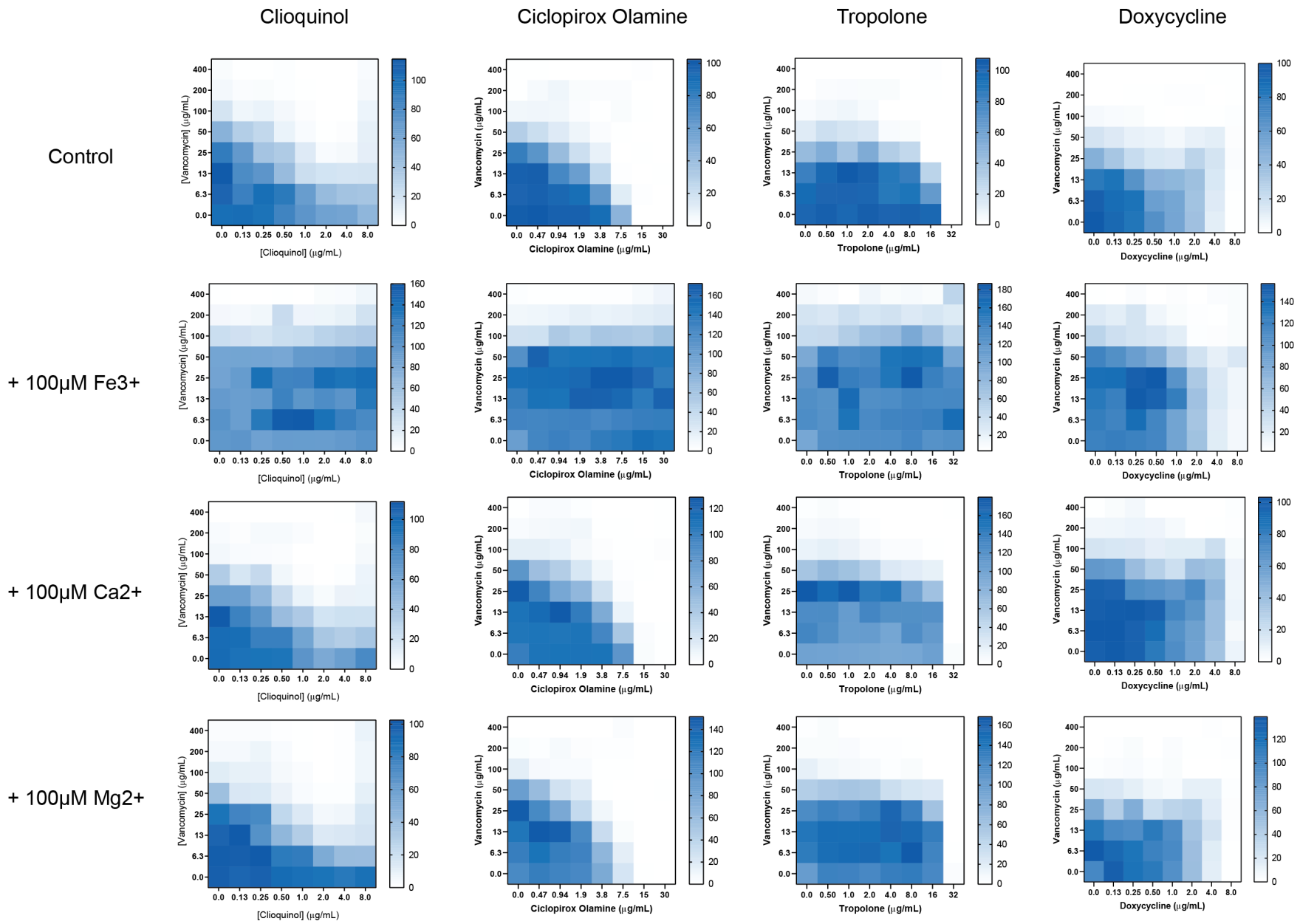


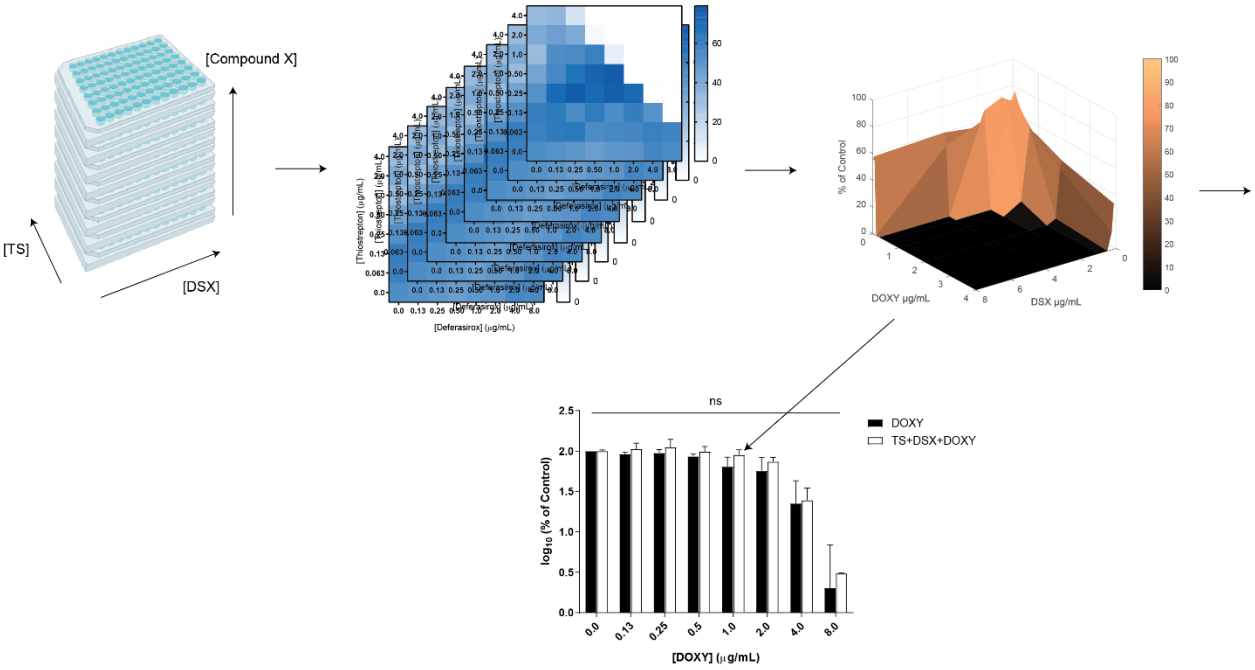
Supplementary Figure S1: **CAS assay of potential iron chelators**. A representative plate is shown. Doxycycline (DOXY), ciprofloxacin (CIP), deferasirox (DSX), clioquinol (CLI), tropolone (TRO), ciclopirox olamine (CO), chloramphenicol (CHLOR), and ampicillin (AMP) were standardized to 2mg/mL. Ten µL was spotted in each sector. The plate was incubated at room temperature for 1 h. CLI precipitated on the surface of the agar. DSX served as a positive control. CHLOR and AMP were negative controls. Decolourization was not observed for CLI due to precipitation.



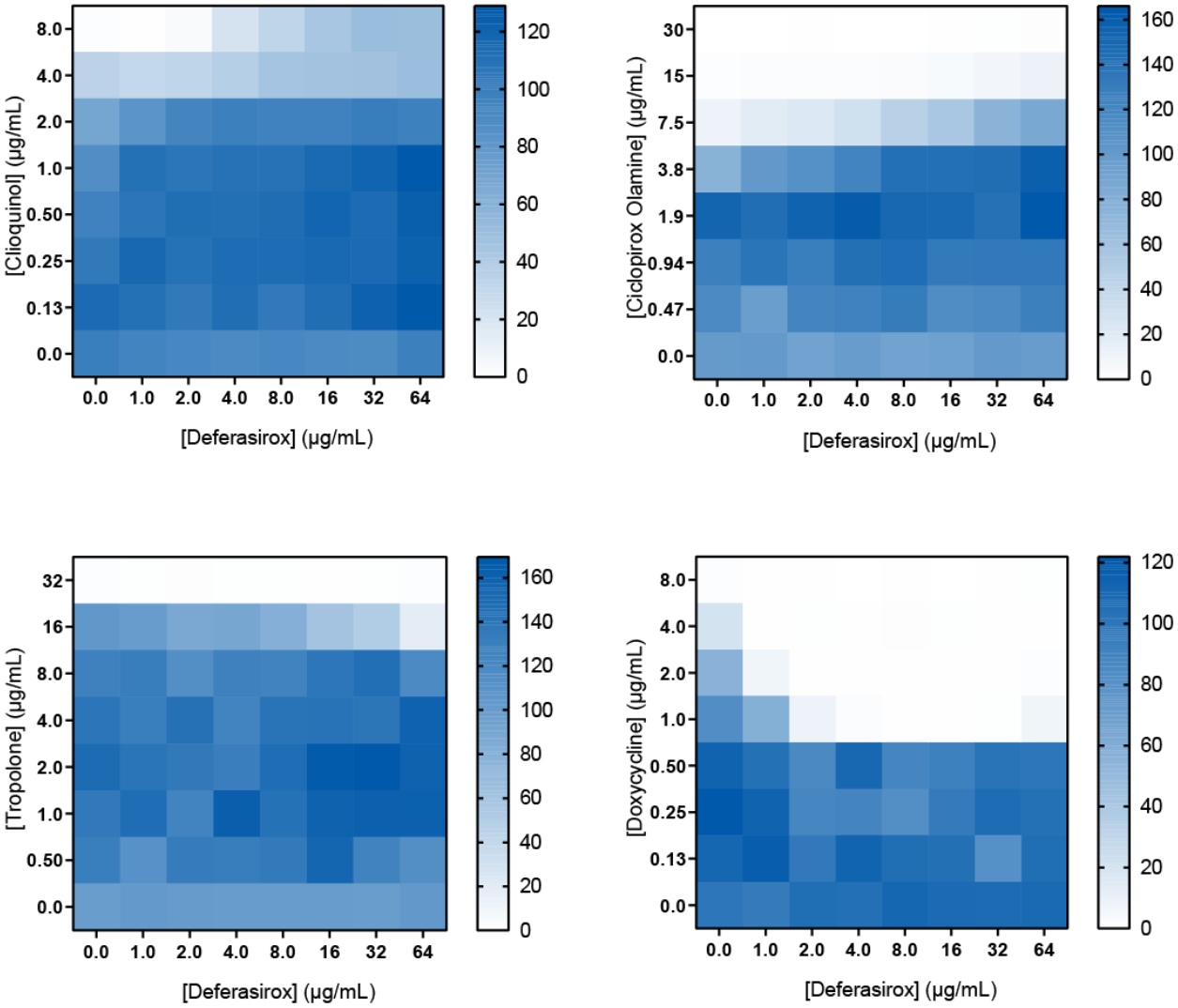
Supplementary Figure S2: Iron chelators antagonise GN. A. GN + DSX and **B.** GN + CO. DSX with GN has an additive effect at low concentrations but antagonizes at higher concentrations. The average of three independent experiments are shown for each checkerboard.


Clioquinol
Ciclopirox Olamine
Tropolone
Doxycycline
Control
+ 100μM Fe3+
+ 100μM Ca2+
+ 100μM Mg2+
[Vancomycin] (μg/mL)
[Clioquinol] (μg/mL)
Vancomycin (μg/mL)
Ciclopirox Olamine (μg/mL)
Tropolone (μg/mL)
Doxycycline (μg/mL)

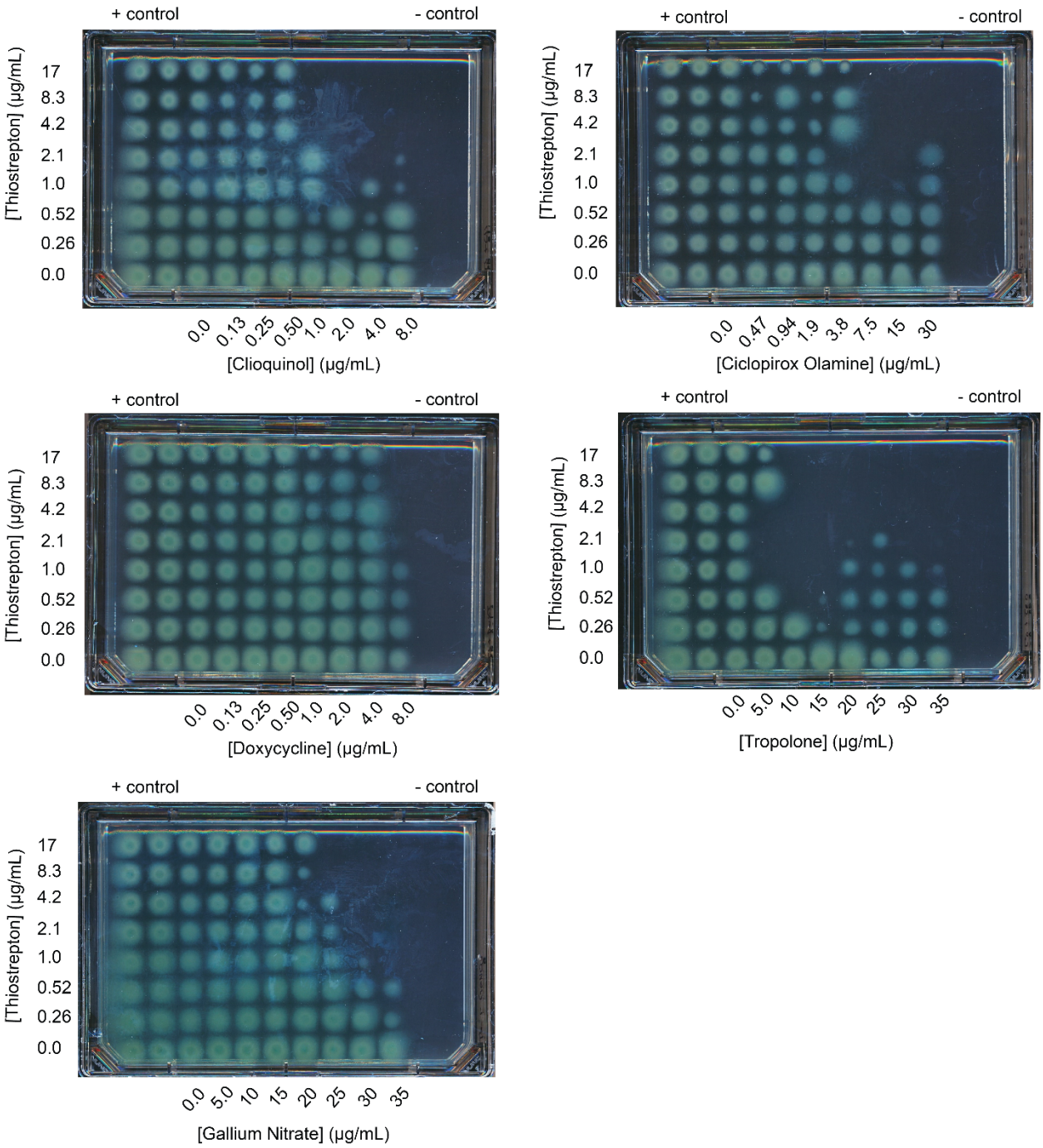
Supplementary Figure S3: Addition of 100 µM $FeCl_3$ reduces the inhibitory activity of potential chelators. Vancomycin (VAN) + compound checkerboards are shown. None of the compounds synergized with VAN although an additive effect was seen with some compounds. The additive effect was abrogated by the addition of $FeCl_3$ but not $MgCl_2$ or $CaCl_2$, indicating that the effects of calcium and magnesium ion chelation from the outer membrane is minimal. The average of three independent repeats are shown for each checkerboard.



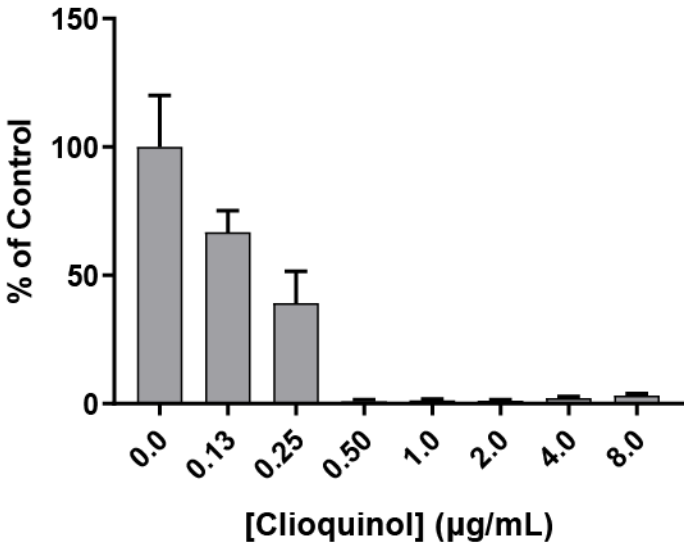
Supplementary Figure S4. Configuration and interpretation of 3D checkerboards. Three-dimensional checkerboards were arrayed in an 8 x 8 x 8 format (plate diagram created with BioRender). Each plate had identical serial dilutions of TS and DSX and a single concentration of the third compound. The direction of the arrows indicates increasing TS, DSX, or chelator concentration. Using MATLAB, each checkerboard was plotted in 3D with the % of control growth in the z-axis. The surface area of the checkerboard was calculated and expressed in terms of % of control, which was plotted against the third drug concentration. These data were compared to the dose-response curve of the third compound alone and the datasets compared using Graphpad Prism to identify statistically significant differences using 2-way ANOVA.



Supplementary Figure S5: DSX and putative iron chelators show no synergy against *P. aeruginosa* PA14. A. DSX + Clioquinol. **B.** DSX + CO. **C.** DSX + TRO. **D.** DSX + DOXY. DSX was additive to DOXY and indifferent with CO and TRO. DSX showed antagonism with CLI at the highest concentration tested. Higher concentrations were not tested because CLI precipitates above 8 µg/mL in aqueous media. The average of three independent experiments are shown for each checkerboard.



Supplementary Figure S6: TS combinations are bactericidal. Checkerboards were pinned to LB agar plates using a sterile 96 pin tool. Plates were incubated overnight at 37°C. Independent experiments were conducted 3 times and a representative plate is shown.



Supplementary Figure S7: *A. baumannii* C0286 is susceptible to CLI at low concentrations. The average of 3 independent experiments is shown.