

## Supplementary Materials

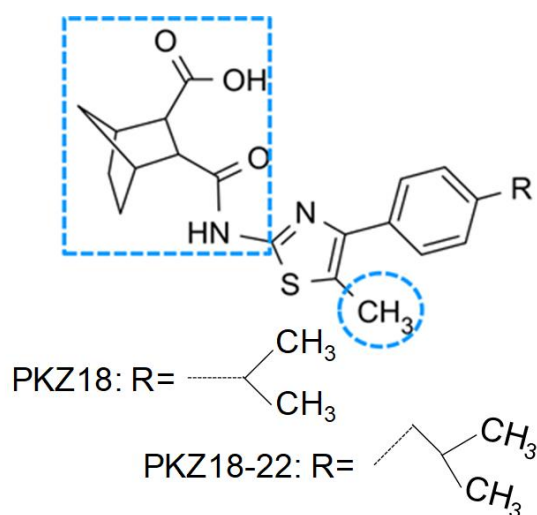
# A New Promising Anti-Infective Agent Inhibits Biofilm Growth by Targeting Simultaneously a Conserved RNA Function that Controls Multiple Genes

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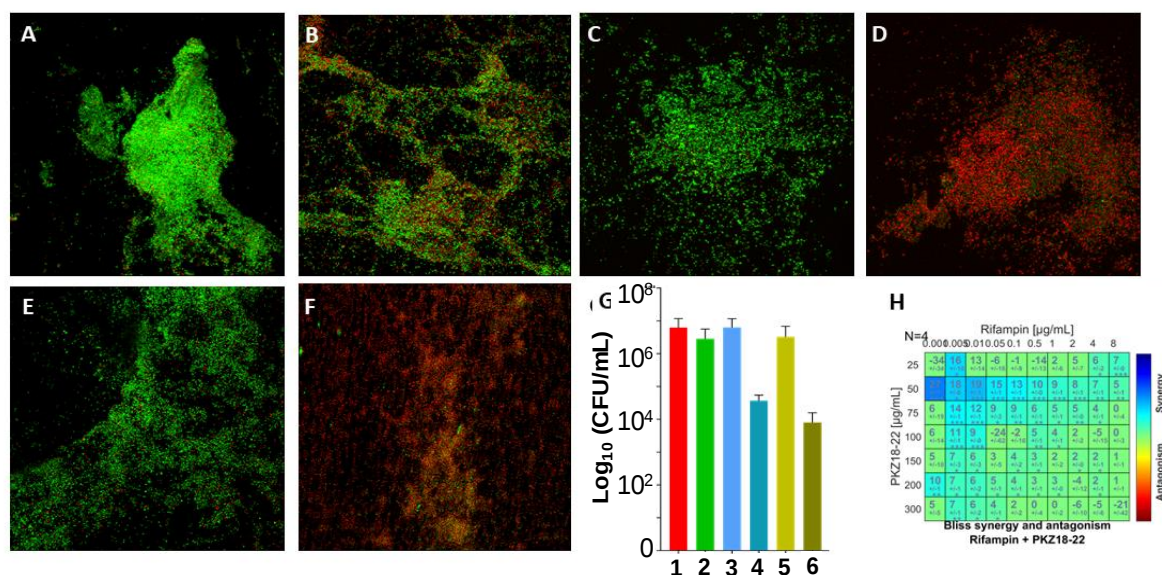
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**Figure S1. Chemical structures of PKZ18 and analog PKZ18-22.** Features identified by structure activity relationships as increasing activity (PKZ18 isobutyl substitute for PKZ18-22 isopropyl). The norbornane is necessary for Gram-positive specificity (dashed box); the methyl is not necessary for activity (dashed circle). (United States Patent 10,266,527.).



**Figure S2. Synergistic activity of PKZ18-22 with rifampin.** **A.** Biofilm grown for 24 h with no treatment. **B.** Biofilm growth after 24 h exposure to 25  $\mu\text{g/mL}$  PKZ18-22. **C.** Growth after 24 h exposure to 0.001  $\mu\text{g/mL}$  rifampin. **D.** Growth after 24 h exposure to 0.001  $\mu\text{g/mL}$  rifampin + 25  $\mu\text{g/mL}$  PKZ18-22. **E.** Growth after 24 h exposure to 0.005  $\mu\text{g/mL}$  rifampin. **F.** Growth after 24 h exposure to 0.005  $\mu\text{g/mL}$  rifampin + 25  $\mu\text{g/mL}$  PKZ18-22. **G.** 1, red *S. aureus* biofilm colony forming units (CFU/ $\text{log}_{10}$ ) growth not treated; 2 green, after 24 h of a single dose PKZ18-22 (25  $\mu\text{g/mL}$ ); 3, blue 0.001  $\mu\text{g/mL}$  rifampin; 4, teal, 0.001  $\mu\text{g/mL}$  rifampin and PKZ18-22 (25  $\mu\text{g/mL}$ ); 5, mustard 0.005  $\mu\text{g/mL}$  rifampin; 6, brown, 0.005  $\mu\text{g/mL}$  rifampin and PKZ18-22 (25  $\mu\text{g/mL}$ ). After single dose 24 h challenge, the combination of rifampin and PKZ18-22 produced a significant greater reduction when compared to each treatment alone. **H.** Synergy score matrix for drug combination of PKZ18-22 and rifampin using a drug interaction Bliss model. Highest drug synergy observed with PKZ18-22 of 50  $\mu\text{g/mL}$  and rifampin concentrations of 0.001–0.005  $\mu\text{g/mL}$  ( $p < 0.001$ ).