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Pravibismane is a Potent, Broad Spectrum Anti-Infective Small Molecule that Rapidly Disrupts Bacterial Bioenergetics and Halts Bacterial Growth

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ABSTRACT

The rise in resistance to existing antimicrobials has prompted a need for the development of novel antibiotics. Microbion has identified a novel compound, pravibismane, with potent broad spectrum anti-infective and anti-biofilm activity. Here we used a variety of assays, including Bacterial Cytological Profiling (BCP), to analyze pravibismane in *E.coli* to gain insight into its likely mechanism of action (MOA). The BCP profile of pravibismane suggested it rapidly shut down cell growth, potentially by turning off cellular gene or protein expression. This was confirmed using a plasmid based GFP induction assay in *E.coli tolC* that showed pravibismane strongly reduced expression of GFP. The kinetics, reversibility and MOA of pravibismane was further characterized by using time-lapse microscopy, wash out experiments and measurements of both membrane potential and relative intracellular ATP levels. We found that pravibismane acts rapidly (within 30 mins) to completely halt cell growth rather than causing immediate cell lysis such as that observed with non-specific cell damaging agents bleach or detergent. Inhibitor wash out experiments in which cells were exposed to pravibismane for 2 hours, washed to remove the compound, and then observed using time-lapse microscopy revealed that the effect of pravibismane is reversible and that cells recovered 8-12 hrs after removing the compound. Wash out experiments with an *E.coli tolC* strain carrying a plasmid with an IPTG inducible GFP demonstrated that transcription and translation ultimately resumed in most cells after washout. The bioenergetics of the membrane was measured using DiBAC 4(5), a membrane potential sensitive dye which can enter depolarized cells, which revealed that pravibismane caused depolarization of the membrane within 30 mins of exposure in a concentration dependent manner. Finally, a luciferase assay determined pravibismane reduced ATP levels (resulting in decreased luminescence) within 15 mins of exposure in a concentration dependent manner unlike antibiotic controls that had modest or no effect on luminescence.

Conclusion: Our results suggest that pravibismane acts rapidly to disrupt cellular bioenergetics, resulting in the immediate cessation of cell growth and protein expression.

RESULTS

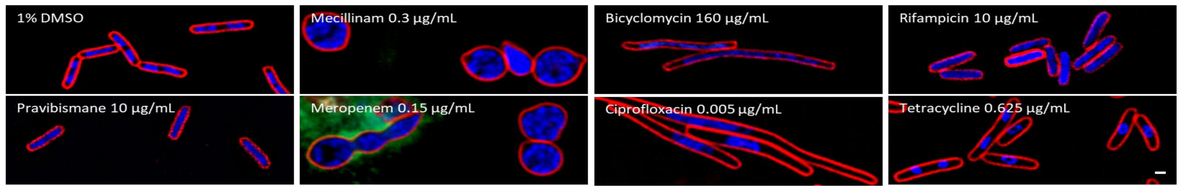


Figure 1. BCP in *E.coli tolC.* The BCP profile of pravibismane did not perfectly match any of our control antibiotics; however, it was most similar to rifampicin. The white scale bar is 1 µm.

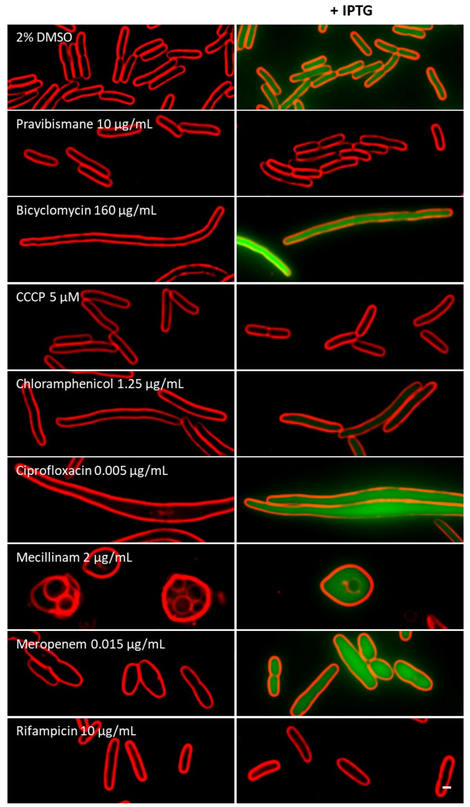


Figure 2. Pravibismane affects bacterial gene expression. A *E.coli tolC* strain carrying a plasmid with an IPTG inducible GFP was treated with various compounds and induced with IPTG. We found pravibismane reduced expression of GFP, similar to transcription inhibitor, rifampicin. The white scale bar is 1 µm.

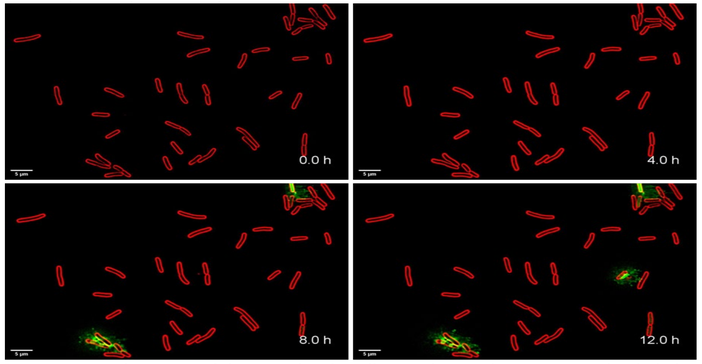


Figure 3. Pravibismane inhibits *E.coli tolC* cell growth. Pravibismane completely halt cell growth but it does not cause membrane damage or immediate cell lysis. Green staining indicates permeabilization of the cell membrane.

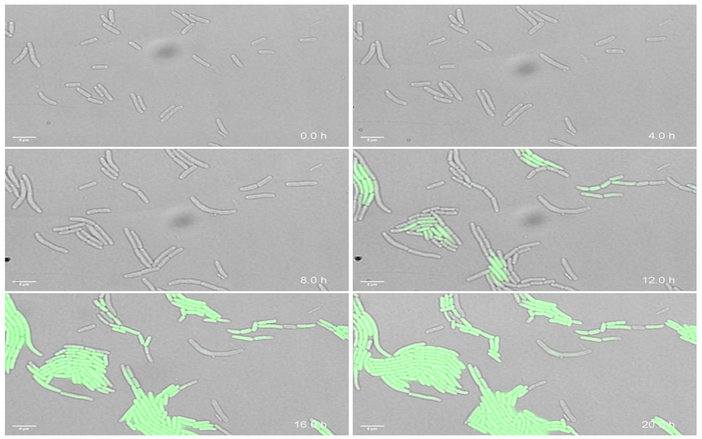
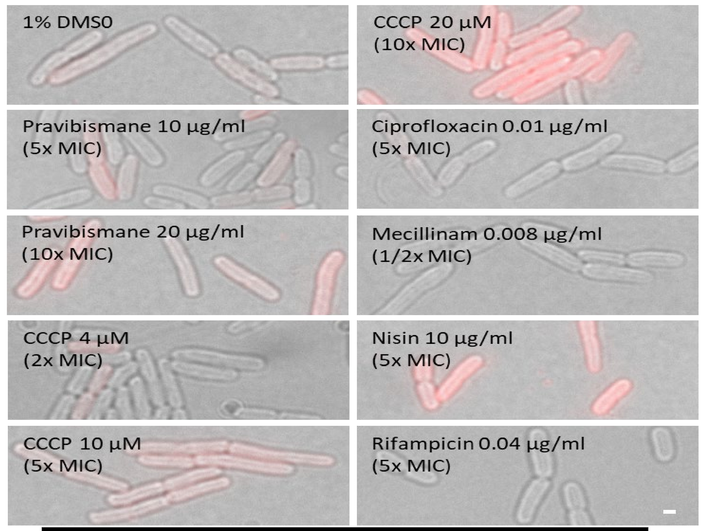


Figure 4. Treatment with pravibismane is reversible. A *E.coli tolC* strain carrying a plasmid with an IPTG inducible GFP was treated with 10 µg/ml pravibismane for 2 hours and then washed 3x with media. Cells recovered 8-12 hours after compound removal and GFP expression demonstrates that transcription had resumed.



	Cells Counted (n)	Mean Intensity (AU)
1% DMSO	1460	414
Pravibismane 10 µg/ml	1228	494
Pravibismane 20 µg/ml	504	865
CCCP 10 µM	1859	965
CCCP 20 µM	218	1124
Ciprofloxacin 0.01 µg/ml	668	297
Mecillinam 0.008 µg/ml	610	260
Nisin 10 µg/ml	146	1310
Rifampicin 0.04 µg/ml	255	146

Figure 5. Pravibismane depolarizes the membrane in a concentration dependent manner. *E.coli lptD5* was treated with the indicated compounds for 30 min and stained with 5 µg/ml DiBAC 4(5) (red), a potential-sensitive dye which can enter depolarized cells. CCCP and nisin were included as positive controls while mecillinam, ciprofloxacin and rifampicin served as negative controls. The white scale bar is 1 µm.

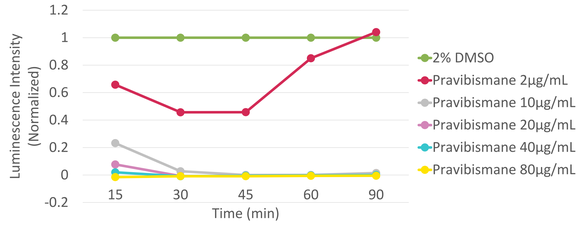


Figure 6. Pravibismane treatment decreased ATP levels in a concentration dependent manner. Pravibismane treatment reduced ATP levels (resulting in decreased luminescence) within 15 mins. Compounds were added at time zero. Luminescence intensity was normalized to the intensity of the DMSO control.