Lipoxazolidinone Natural Product Derivative Displays Potent *in vitro* Antibiofilm Activity

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Bacterial biofilms are major contributors to the rise of multi-drug resistance and are heavily implicated in the incidence of hospital-acquired infections. *Staphylococcus aureus* biofilms can tolerate anywhere from ten to one-thousand times the concentration of frequently prescribed antibiotics required to inhibit planktonic cell growth, *in vitro*. The increasing number of procedures resulting in biofilm-mediated hospital-acquired infections combined with complications caused by biofilm-mediated bacterial infections warrant investigation into antimicrobial therapeutics with potent activity against pathogenic biofilms. It has been previously demonstrated that the 4-oxazolidinone family of natural products, particularly those derived from lipoxazolidinone A, display high degrees of antimicrobial activity against both methicillin-susceptible (MSSA) and methicillin-resistant *S. aureus* (MRSA). Despite this, there has been no significant evaluation of the lipoxazolidinones' activity against bacterial biofilms. Our objective was to further characterize the *in vitro* antimicrobial and antibiofilm activities of a leading 4-oxazolidinone analog (SYNX_0001) against a broader panel of relevant species and their respective biofilms.

Methodology: To assess the activity, we performed minimum inhibitory concentration (MIC) and minimum biofilm eradication concentration (MBEC) assays against a panel of biofilm-forming pathogens from the WHO and CDC's ESKAPE group (*S. aureus, E. faecium, E. faecalis, E. gallinarum, P. aeruginosa, K. pneumoniae*). The antibacterial activity of SYNX_0001 was further assessed by the authors in a series of time-dependent inhibition experiments against *S. aureus* in both planktonic and pre-formed biofilm states. In a more robust model, mature *S. aureus* biofilms were treated with various doses of SYNX_0001 and visualized utilizing a fluorescent live/dead staining assay.

Results: SYNX_0001 displayed a high degree of inhibition against the selected gram-positive species in planktonic and biofilm states, evident by MIC ranges of 0.25 to 2 μg/ml and MBEC ranges of 1 to 64 μg/ml. While the observed planktonic activity of SYNX_0001 vs a *S. aureus* testing strain (ATCC 25923) was similar to vancomycin (both 0.5 μg/ml), SYNX_0001 displayed superior antibiofilm activity compared to vancomycin (32 μg/ml and 1024 μg/ml, respectively). This activity was not reflected in the gram-negative species evaluated, as MICs ranged anywhere from 64 to over 256 μg/ml; while MBECs exceeded 1024 μg/ml. 24 hours post-treatment of *S. aureus* planktonic cultures with SYNX_0001, SYNX_0001 achieved a significant reduction (>2log10) in viable bacterial cells relative to the initial inoculum. In a time-dependent biofilm-eradication model, treatment of pre-formed *S. aureus* (ATCC 25923) biofilms with SYNX_0001 at twice the MBEC (a concentration of 64 μg/ml) resulted in the complete elimination of viable bacterial cells after 24 hours. Fluorescent live/dead staining of mature *S. aureus* biofilms treated with SYNX_0001 revealed a dose-dependent increase in biofilm-embedded cell death associated with increasing concentrations of SYNX_0001.

These *in vitro* results indicate that the lipoxazolidinone A derivative, SYNX_0001, is a potent inhibitor of both planktonic and biofilm-embedded cells for the gram-positive bacterial species evaluated. Future work will focus on understanding the mechanism(s) of action by which SYNX_0001 eradicates *S. aureus* biofilms. The completion of this work may result in the identification of a novel antibiofilm target for the development of future treatments for *S. aureus* biofilm-mediated infections.