Project Details	
Project Code	MRCIIAR24Ba Laabei
Title	Developing new weapons to fight drug-resistant superbugs – targeting lipoteichoic acid biosynthesis
Research Theme	Infection, Immunity, Antimicrobial Resistance & Repair
Summary	Antimicrobial resistance (AMR) is a major threat to global health. Lack of drug development severely hampers treatment options. We identified novel small molecules that target a crucial bacterial component called lipoteichoic acid. This interdisciplinary project will develop more stable and potent drug derivatives and further define the molecular activity of these compounds using a suite of chemical and molecular methods and invertebrate infection models.
Description	Background: The introduction of antibiotics into medicine was arguably the most important medical development of the last century, however widespread use of antibiotics in the clinic and in agriculture has led to the rapid emergence of antimicrobial resistant (AMR) pathogens. Staphylococcus aureus is classically considered the first 'superbug' owing to its combined ability to rapidly become resistant to antibiotics and express multiple virulence factors linked to severe disease. Worryingly, in the most recent study estimating global AMR, S. aureus caused more than 100,000 deaths in 2019 and was listed as second in the top six pathogens for deaths associated with AMR. Therefore, more concerted efforts are required to identify targets and develop novel antimicrobials to tackle this severe health threat. Aims and overview: Following a structure activity relationship (SAR) analysis, our team has identified a molecule (compound 13) that exhibits potent activity against important Gram-positive pathogens including multi-drug resistant S. aureus. This compound is based on the 1,3,4 oxadiazole-based small molecule named 1771 but displays 16-32-fold increased antimicrobial activity while maintaining low toxicity to mammalian cells. Importantly, 1771 and 13 inhibit the production of lipoteichoic acid (LTA), integral components of the Gram-positive cell envelope. This project will determine the mechanism of action of both 1771 and 13, test the activity and stability of a second-generation cohort of derivatives of compound 13 and establish an invertebrate infection model that will determine the in vivo activity of novel, pre-clinical antimicrobials. Interestingly, we have shown that when combined with an efflux pump inhibitor, 1771 and 13 surprisingly inhibit Gram-negative pathogens, indicating that the target(s) of these compounds is not restricted to the LTA pathway in Gram-positives. Importantly, we could not generate resistant mutants against 1771 or 13 following in vitro serial passage. Therefore, in Objective 1 will employ gold standard multi-omic approaches to determine the proteins, lipids and pathways affected following treatment with these compounds by performing comparative global proteomics and lipidomics using core facilities established at Bath, Bristol and Cardiff. Objective 2 will establish the binding partner(s) of 1771 and 13 using pull down assays and whole cell lysate derived from either S. aureus (Gram-positive) or E. coli (Gram-negative). A combination of biotin-labelled small molecule probes and label free techniques will be used determine protein targets. Our previous SAR analysis will direct the development of labelled probes without

	significant loss of activity and a control probe which exhibits no activity. The supervisory team have a track record in generating labelled probes and expertise in chemical biological techniques required for this analysis. Combined proteomic/lipidomic and pull-down assays will inform on likely protein targets. Here the student will determine the trajectory of future research and identify genes coding for hit proteins that will be subjected to genetic manipulation either via gene deletion using established techniques in the Laabei group or if genes are essential, through RNAi knockdown or over expression of genes to assess impact on compound activity. Objective 3 will establish invertebrate infection models to determine the activity of novel antimicrobials against S. aureus. The student will make use of four invertebrate infection models at Bath; 1) Zebrafish; 2) Galleria, 3) C. elegans and 4) M. sexta. The student will optimise infection conditions and assess the importance of known virulence genes/regulators to cause infection in these models, drawing conclusions on the appropriateness of each model for S. aureus infection analysis. Following this refinement, we will use these models to assess the activity of 1771 and compound 13 in vivo, using known antibiotics as controls.
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