Project Details	
Project Code	MRCIIAR25Br 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) has been described as the silent pandemic, fuelled in part by insufficient antibiotic development. We have identified novel small molecules that target a crucial bacterial component called lipoteichoic acid. Precise understanding of how antibiotics kill bacteria is critical to the safe and effective use of antimicrobials in therapy. By combining molecular microbiology, proteomics, and medicinal and analytical chemistry, this proposal aims to unravel the mechanism of action of a novel class of antibiotic, the oxadiazole based small molecule which we have shown potently inhibits multi-drug resistant bacterial pathogens, most notably methicillin-resistant Staphylococcus aureus.
Description	**Background:** Tackling infectious diseases represents one of the major global societal challenges of the 21st century. The discovery of antibiotics led to a new era of infection medicine and is widely regarded as one of the most significant medical advancements in history. However widespread use of antibiotics in the clinic and in agriculture has led to the rapid emergence of antimicrobial resistant (AMR) pathogens. In the most recent predictive statistical model, bacterial AMR was attributed to the deaths of an estimated 1.27 million people worldwide; worryingly additional models speculate that this number will rise to 10 million deaths per year, with a commensurate cost of around $1 trillion in additional healthcare costs, by 2050. In the UK, 148 severe antibiotic resistant infections and six deaths a day occurred in 2022, inflicting an estimated £180 million in costs to the NHS annually. As a response, UKRI have committed to tackling antimicrobial resistance as one of their immediate strategic aims with 'Transforming Tomorrow Together' outlining AMR as a strategic priority. 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 16) 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 16 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 16, test the activity and stability of a second-generation cohort of derivatives of compound 16 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 16 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 16 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 16 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 a Galleria mellonella invertebrate infection model to determine the activity of novel antimicrobials against S. aureus. 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 the model for mimicking specific S. aureus infections. Following this refinement, the student will use the model to assess the toxicity and antimicrobial activity of 1771 and compound 16 in vivo, using known antibiotics as controls. Lack of toxicity and successful prevention of infection in the Galleria model will direct the testing of compounds in established murine models of infection in collaboration with partners at Trinity College Dublin, Ireland (Prof Rachel McLoughlin). Combined this PhD will determine the molecular mechanism of action of a novel class of antibiotic and confirm in vivo antimicrobial activity using optimised infection models.
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