| Project Details | |
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| Project Code | MRCIIAR25Ba Lovell |
| Title | Tackling Antimicrobial Resistance with Targeted Covalent Macrocycles |
| Research Theme | Infection, Immunity, Antimicrobial Resistance & Repair |
| Summary | Infections with drug-resistant bacteria are major threats to human health. Discovering novel druggable targets for antimicrobial development is a pivotal task to guarantee effective future treatments. This interdisciplinary project will target WalK, a protein kinase essential for the viability of S. aureus cells. Large libraries of covalent macrocycles will be screened against WalK to identify an inhibitor. Hit-to-lead optimization will be achieved using a suite of chemical, structural and molecular methods. Finally, lead molecules will be applied in vivo using an invertebrate infection model. Ultimately, this work will provide novel antimicrobial compounds ready for pre-clinical assessment in mice. |
| Description | **Background:** Infections with multidrug-resistant bacteria like methicillin-resistant Staphylococcus aureus (MRSA) are major threats to human health. Discovering novel druggable targets for antibiotic development is a pivotal task to guarantee effective treatment in the future. WalKR is a two-component system present in S. aureus and other gram-positive organisms. It consists of a kinase (WalK) that senses environmental cues (such as cell wall damage) and relays this information to a response regulator (WalR), via phosphorylation, which then activates a suite of genes required to combat stress. WalK possesses a large extracellular PAS domain that must homodimerize for the enzyme to be functionally active. The WalKR system is an attractive antimicrobial target as it is essential for growth of gram-positive bacteria and mutations within the S. aureus walK gene are linked to vancomycin resistance. Despite this, the pharmacology around WalK remains largely unexplored and inhibitors reported to date lack in vivo stability and/or selectivity.<br>Aim and Overview: The aim of this PhD project is to use a phage display screening platform, developed in the Lovell lab, to identify a targeted covalent macrocycle (TCM) inhibitor for WalK, which blocks homodimerization and prevents the formation of a functional WalKR system in S. aureus. TCMs are a novel and powerful chemical entity for protein inhibition, which combine the properties of a macrocyclic peptide and an irreversible inhibitor, binding to proteins with high affinity and selectivity by forming interactions over a large surface area and achieving permanent target engagement by covalent modification of a proximate nucleophilic residue. The Lovell lab have used TCMs to inhibit challenging viral, bacterial and cancer protein targets.<br>**Objective 1:** Identification of a TCM inhibitor – Using intact protein mass spectrometry, we will identify covalent fragments that modify Lysine or Tyrosine residues present at the homodimer interface of WalK by screening libraries available in the Lovell lab. Hit fragments will be resynthesized as chemical 'linchpin' derivatives and reacted with peptide phage display libraries to generate billions of dimer interface-directed TCMs for screening against WalK. After multiple rounds of panning and amplification, enriched TCMs will be synthesized and tested in SPR binding studies and in a size-exclusion chromatography assay to assess inhibition of homodimer formation. Co-crystal structures of hit TCMs bound to WalK will reveal critical interacting residues and guide |

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| | optimisation of key parameters such as selectivity and proteolytic stability. Mass spectrometry-based chemical proteomics techniques, namely activity-based protein profiling (ABPP), will be employed to assess the selectivity of hit TCMs proteome wide. The Lovell lab is a leading group in ABPP.<br>**Objective 2:** Lead TCM Validation – The lead TCM will be applied to a panel of MRSA isolates to assess toxicity and changes in growth rate. The Laabei lab will develop GFP transcriptional fusions for WalR target genes to enable activation of the WalKR system to be monitored upon treatment with our TCM. Further counter screens against E. Coli and human HepG2 cells will validate on-target activity of our lead molecule. Finally, in vivo engagement of WalK will be demonstrated by applying the CCP to Manduca Sexta larvae challenged with S. aureus. After 3 days larvae will be sacrificed, and bacterial titers will be assessed to determine the molecule's effect on infection progression. We will also use ABPP to enable an organism-wide assessment of molecule selectivity.<br>**Outcome:** This research will validate WalK as a therapeutic target for treatment of MRSA infections and will provide an optimized TCM for further pre-clinical assessment.<br>Opportunities for student ownership and steering: This is a highly interdisciplinary project with several opportunities for the student to steer the project based on their interests. This could include, but is not limited to, the following: (A) Focusing on phage display technology development by inventing novel phage-compatible chemistries to allow diverse TCM libraries to be generated. (B) Focusing on structural biology by gaining expertise in X-ray crystallography/cryo-EM during TCM hit-to-lead optimisation. (C) Developing new bioinformatics pipelines to allow in-depth analysis of deep sequencing data from phage display screens. (D) Invertebrate infection model development by working more extensively with Manduca Sexta larvae. |
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