Project Details	
Project Code	IIAR27Ba Lovell
Title	Designing Cell-Accumulating Covalent Inhibitors to Combat Gram-Negative Antimicrobial Resistance
Research Theme	Infection, Immunity & Antimicrobial Resistance
Project Type	Wet lab
Summary	Antimicrobial resistance is one of the greatest global health challenges, with Gram-negative bacteria particularly difficult to treat because their protective cell envelope prevents many antibiotics from reaching their targets. This PhD will develop a new generation of covalent antibiotics designed to overcome these barriers. The student will combine medicinal chemistry, microbiology, protein biochemistry and chemical proteomics to design, synthesise and evaluate novel compounds that accumulate inside bacteria and irreversibly engage essential proteins. The project offers outstanding multidisciplinary training while addressing one of the biggest challenges in modern antibiotic discovery and the fight against drug-resistant infections.
Description	Background: Antimicrobial resistance is one of the greatest threats to global health, with Gram-negative bacteria presenting a particular challenge because their outer membrane and efflux pumps prevent many drugs from reaching intracellular targets. Recent studies have identified physicochemical features that promote compound accumulation in Gram-negative bacteria, including molecular rigidity and appropriately positioned ionisable amines. However, these principles have largely been applied to reversible inhibitors. In parallel, covalent inhibitors have transformed drug discovery by enabling prolonged target engagement, increased potency and inhibition of challenging proteins. Very little is currently known about how to design covalent inhibitors that efficiently accumulate within Gram-negative bacteria. This PhD will address this gap by developing cell-accumulating covalent inhibitors. The project will use the essential cell-wall biosynthesis enzyme MurA as a model intracellular target. Unlike most bacterial proteins, MurA possesses a chemically validated ligandable cysteine (Cys115), which is covalently modified by the clinically used antibiotic fosfomycin, making it uniquely suited to study intracellular covalent target engagement. MurA therefore provides an ideal model system to determine whether rationally designed covalent compounds can enter Gram-negative bacteria, overcome permeability and efflux barriers, and irreversibly engage an intracellular cysteine target. Aims and overview: The aim of this PhD is to establish design principles for Gram-negative accumulating covalent inhibitors by synthesising and evaluating a library of acrylamide-containing compounds based on rigid amino-acid scaffolds. The project will determine how the three-dimensional presentation of a primary amine governs bacterial accumulation, efflux susceptibility and intracellular covalent target engagement. While MurA provides the experimental model, the broader objective is to generate medicinal chemistry principles that can be applied to covalent inhibitors targeting intracellular proteins across Gram-negative pathogens. Objective 1: Development of a Gram-negative accumulating covalent library.

	The student will synthesise an 800-member covalent library using commercially available rigid amino-acid scaffolds and structurally diverse amines. Each compound will incorporate an acrylamide electrophile for cysteine targeting while maintaining molecular properties associated with Gram-negative accumulation, including rigidity and a primary ionisable amine. The scaffold set has been deliberately designed to systematically vary ring topology, stereochemistry and, critically, the three-dimensional presentation and steric environment of the primary amine while maintaining broadly comparable molecular properties. This will enable the student to directly test the hypothesis that reducing steric congestion around the primary amine improves bacterial accumulation and productive intracellular target engagement. Direct-to-biology workflows developed in the Lovell laboratory will enable rapid parallel synthesis and biological evaluation of the library. Objective 2: Biochemical validation of MurA covalent target engagement. Recombinant E. coli MurA will be expressed and purified to establish a biochemical screening platform. The library will be screened for MurA inhibition using well-established enzyme activity assays, with time-dependent inhibition used to prioritise putative covalent inhibitors. Hit compounds will be validated by intact-protein mass spectrometry to confirm covalent modification of MurA. A C115A mutant protein will be used as a counter-assay to determine whether inhibition is dependent upon the known ligandable cysteine. Objective 3: Defining outer membrane permeability, efflux and intracellular covalent target engagement. Lead compounds will be evaluated in a panel of genetically defined E. coli strains, including wild-type cells, the outer membrane-permeable imp4213 mutant and a ΔtolC efflux-deficient mutant. Initial antibacterial activity will be assessed using growth inhibition and MIC assays. To determine whether whole-cell activity results from productive MurA engagement, competitive activity-based protein profiling (ABPP) experiments will be performed using a broad-spectrum cysteine-reactive iodoacetamide probe. Cells pre-treated with lead compounds will subsequently be labelled with the probe, enabling quantitative chemoproteomic analysis of cysteine occupancy across the bacterial proteome. This approach will quantify MurA Cys115 engagement while simultaneously identifying off-target cysteine residues and defining proteome-wide selectivity. Comparison of chemoproteomic profiles across wild-type, imp4213 and ΔtolC strains will reveal how outer membrane permeability and efflux influence intracellular covalent target engagement, distinguishing permeability-, efflux- and target affinity-limited compounds. Outcomes: This project will establish a systematic framework for developing cell-accumulating covalent inhibitors of Gram-negative bacteria. Beyond validating MurA as a model intracellular target, the project will define how scaffold topology, stereochemistry and the three-dimensional presentation of a primary amine influence bacterial accumulation, efflux susceptibility and productive intracellular covalent target engagement. The integrated datasets generated will provide broadly applicable design principles for developing covalent inhibitors

	against intracellular proteins in Gram-negative pathogens. The chemical library, screening cascade and target-engagement workflows established during this project will provide a versatile platform for future antibacterial drug discovery. Student Ownership: The student will receive interdisciplinary training in medicinal chemistry, microbiology and chemical biology. The project offers substantial opportunities for student ownership. The student could steer the project towards development of improved bacterial accumulation assays, quantitative modelling of structure-accumulation relationships, biochemical characterisation of MurA covalent inhibition, or expansion to other intracellular antibacterial targets, such as FabH, FabA and DsbA. The project provides an outstanding training platform at the interface of chemical biology, antimicrobial discovery and translational drug development.
Can be completed part time?	No
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