Project Details	
Project Code	MRCIIAR24Ca Serpi
Title	Fighting antimicrobial resistance by stopping bacterial cell division.
Research Theme	Infection, Immunity, Antimicrobial Resistance & Repair
Summary	The continuing rise of antimicrobial resistance has led to the failure of antibiotics to treat common infections. This project proposes to develop novel antimicrobials that target bacterial cell division, a crucial step for bacterial replication and survival. We will combine our expertise in medicinal chemistry and microbiology to design molecules that, acting like “Trojan horses”, cross the bacterial cell envelope and once inside become toxic and kill the bacteria.
Description	Background: Despite antibiotics crucial role in saving millions of lives, they have proven to become significantly less effective in treating common infections due to the spread of Antimicrobial Resistance (AMR). Among the most promising targets for the discovery of novel classes of broad-spectrum antimicrobials is bacterial cell division, a process orchestrated by the filamenting temperature-sensitive mutant Z (FtsZ) protein, whose effective inhibition stops cell division, triggers its enlargement and subsequent lysis, followed by bacteria death. FtsZ guanosine triphosphate (GTP) binding site is widely conserved throughout the bacterial species and no drug resistant GTP binding site mutants have been reported so far, presumably because it is essential for the GTP correct recognition. GTP analogues with small hydrophobic substituents at C8 of the nucleobase have been reported to efficiently inhibit FtsZ polymerization and GTPase activity, without inhibiting its eukaryotic homologue tubulin, likely due to their low sequence similarity. Although potent and selective FtsZ inhibitors, these compounds were devoid of antibacterial activity as the could not pass the bacterial cell envelope, which is practically impermeable for the highly polar, negatively charged phosphates group of the nucleotides. Key research question. Could nucleotide Ftsz inhibitors be developed as antibiotics to fight AMR? One major challenge in antibiotic drug discovery is indeed to develop molecules able to rapidly penetrate the bacterial cell envelope to achieve a lethal intracellular drug accumulation. To enable nucleotide FtsZ inhibitors to cross the bacterial cell envelope, we propose to temporary block the free phosphonic functional group of the molecule, masking its acidic oxygen atoms with metabolically labile and non-toxic protecting groups to produce a charge-neutral compound (prodrug). Such prodrugs with increased lipophilicity, will cross the bacterial cell wall and once inside upon activation will release the antimicrobial drug. As additional advantage these prodrugs having one or more phosphate groups attached to the nucleoside, do not require all the phosphorylation steps decreasing the chance for the bacteria to become resistance through mutation of the phosphorylating enzymes. Phosphate prodrug approaches have been successfully applied to antiviral and anticancer nucleoside analogues with many of these drugs reaching the clinic. It is extremely important to establish if such powerful technologies have the potential to lead to novel therapeutics to fight AMR. Objective 1 is the design and synthesis of prodrugs masking only the last phosphate group while keeping the others partially charged to stabilize the anhydride bonds. The student

	will investigate prodrugs, whose intracellular cleavage is based on an entirely pH-driven chemical hydrolysis or enzymatic activation processes. They will also optimize the nucleoside scaffold using in silico studies to identify more potent inhibitors. Objective 2 is the prodrugs chemical and enzymatic stability and their activation study by bacterial enzymes using HPLC to determine the half-lives and LCMS to identify metabolites. Objective 3 is the evaluation of the in vitro prodrugs antibacterial, antibiofilm activity in planktonic culture as well as biofilm of multiple isolates of multi drug resistant bacteria and toxicity in human cell lines. We will examine cell division using fluorescence microscopy in FtsZ green fluorescent protein labelled bacteria. Objective 4 is the study on the bacterial response to the novel prodrugs to establish if they can escape pre-existing resistance mechanisms. We will examine phenotypic responses to prodrugs, conducting real-time diffusion-mutation assays and microfluidic single-cell experiments. In summary this research will generate new knowledge, insight, and novel antibacterial molecules which will contribute to counteract the continuing emergence and spread of AMR.
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