PROJECT AND WORKPLAN

The massive consumption of antibiotics and the growth of antimicrobial resistant bacteria (ARBs) constantly highlight the urgent need for identifying new therapeutic approaches to handle and resolve bacterial infections. The growing prevalence of infections caused by drug-resistance pathogens has spurred diagnostic and therapeutic advancements, alongisde government initiatives, fueling the antimicrobial resistance surveillance market projected to expand to 7.7 billion USD by 2028 from 5.9 billion USD in 2023 with a compound annual growth rate of 5.6% during the forecast period. In this context, in the past three decades antimicrobial peptides (AMPs) have emerged as promising alternative to traditional antibiotic treatments based on conventional small molecules. They display robust antimicrobial activity and a reduced propensity for resistance development. Naturally produced in livings, including bacteria, plants, insects, and humans, or chemically synthesized, AMPs contribute to specific and not-specific response employing antimicrobial mechanisms able to elude the emergence of drug resistance phenomena. Structurally, these molecules are peptide sequences with less than 50 amino acids, with total cationic charge between +2 and +9 that facilitates the establishment of electrostatic interaction with negatively charged bacterial membrane. At the same time, the presence of highly hydrophobic residues ensures optimal membrane permeability and disruption. Thanks to their structural peculiarities, these peptides are effective also as antifungal, antiviral and anticancer applications. To improve their therapeutic potential, synthetic analogs of AMPs (SAMPs) have been developed, incorporating structural modifications to reduce toxicity and manufacturing costs, and enhance in vivo stability to protease degradation, making them competitive with conventional antibiotics. Anyway, their clinical application remains rather limited. As synthetic analogs of AMPs, SAMPs are polypeptides of 3 to 50 residues, consisting of cationic, hydrophilic and hydrophobic amino acids, able to self-organize into amphipathic structures. The cationic amino acids - typically histidine (His), lysine (Lys), arginine (Arg) - are responsible for the positive charges which destabilize the negatively charged cytoplasmic membranes of bacteria. At the same time, phenylalanine (Phe) and tryptophan (Trp) are essential for the formation of helices in long sequence and for the hydrophobic contribution, impacting on the antimicrobial and hemolytic activity. Further structural modifications to these peptide backbones such as cyclization, conjugation strategies, terminal/side chain modifications and introduction of modifications on amino acids residues, have lead to a new class of antimicrobial peptidomimetics (AMPMs). Among AMPMs entering clinical trials, lytixar (LTX-109) is one of the shortest peptidomimetics, deriving from the longer sequence of bovine lactoferrin. It is a synthetic molecule, developed by Lytix Biopharma, active against both Gram-positive and Gram-negative bacteria, including *methicillin-resistant* *S. aureus* (MRSA), *daptomycin-nonsusceptible* *S. aureus* (DNSSA), *vancomycin-intermediate* *S. aureus* (VISA), *vancomycin-resistant* *S. aureus* (VISA), *linezolid-nonsusceptible S. aureus* (LNSSA). Thus, LTX-109 reached phase II clinical trials for the treatment of Gram-positive bacteria induced eczema and dermatitis. The aim of the project is to demonstrate the utility of LTX-109 in managing chronic wounds. Indeed, thanks to its broad spectrum of antimicrobial activity, LTX-109 has been explored also for conditions like *S.aureus* induced intranasal infections and hidradenitis suppurativa, and even for antiviral applications against SARS-CoV-2.

Starting from LTX-109 structure, some synthetic analogues have been proposed as antibacterial agents. The structural modifications primarily focused on altering the hydrophobicity of the second residue and the terminal amine (or ester), as well as the hydrophilicity of the first and third residues. However, this strategy often required the incorporation of unnatural amino acids, which despite the cost-effectiveness and synthetic efficiency of short peptidomimetic sequences, poses challenges for commercialization of the products due to the limited availability of these modified amino acids.

In this study, a series of LTX-109 derivatives will be synthesized by introducing modifications to peptide sequence and the phenyl ring, to assess their antimicrobial activity and provide insights into their mechanism of action, looking for a correlation of structural changes and biological activity.