Staphylococcus aureus is one of the most important human bacterial pathogens, responsible for infections of the skin, soft tissues, bones, lungs, as well as systemic infections. Methicillin-resistant strains (*methicillin-resistant Staphylococcus aureus*, MRSA), which exhibit resistance to many commonly used antibiotics, pose a particular threat. According to the World Health Organization (WHO), antimicrobial resistance is among the most serious threats to global public health. The increasing prevalence of multidrug-resistant strains limits the effectiveness of available therapies and highlights the need to search for new chemotherapeutic agents. Peptidomimetics represent a promising group of compounds that may exhibit high activity against antibiotic-resistant bacteria.

One of the peptidomimetics active against Gram-positive bacteria, including *S. aureus*, is compound A-20 (Cystapep 1). It was developed based on the *N*-terminal fragment of human cystatin C (Arg^{8}-Leu^{9}-Val^{10}-Gly^{11}), a protein with antimicrobial properties.

The main objective of my doctoral research was the design, synthesis, and comprehensive characterization of novel analogs of the Cystapep 1 (A-20) molecule as a potential class of peptidomimetic antibacterial compounds active against MRSA strains.

This objective included determining the relationships between the chemical structure of peptidomimetics, their physicochemical properties, and antibacterial activity using chemometric methods and structure-activity relationship (SAR) analysis, as well as evaluating the antibacterial activity, stability, and cytotoxicity of the obtained compounds. An important part of the dissertation was also investigation of the possible mechanisms of action of selected peptidomimetics against MRSA cells, including the assessment of their effects on cell membrane integrity and macromolecules biosynthesis. Furthermore, potential molecular targets of the investigated compounds in MRSA cells were explored using proteomics-based approaches.

The novel analogs of compound A-20 were designed based on previous studies conducted at the Department of Biomedical Chemistry and with the use of chemometric methods and SAR analysis performed in collaboration with Dr. Hab. Agnieszka Gajewicz-Skrętna from the Department of Physical Chemistry, Faculty of Chemistry, Gdańsk University of Technology. Selected compounds were synthesized using classical solution-phase synthesis, followed by purification and characterization. Their antibacterial activity was evaluated by determining minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values against *S. aureus* and *S. pyogenes* strains. Nearly all obtained compounds exhibited antimicrobial activity against the tested strains. Stability studies demonstrated that selected derivatives were stable both in aqueous environments and in human plasma.

The efficacy of compound A-20 and its selected derivatives (A-75, A-76, A-238, A-241, and A-243) was further evaluated against a broader panel of MRSA strains, including both clinical and laboratory isolates. Bacterial growth studies conducted by measuring optical density (OD_{600}) over time confirmed the high activity of these compounds regardless of the origin of the strains.

For compound A-20 and its selected synthesized analogs (A-75, A-76, A-238, A-241, and A-243), I performed cytotoxicity studies using human keratinocytes and assessed their hemolytic activity. The analyzed peptidomimetics did not exhibit cytotoxic or hemolytic effects within the concentration range corresponding to their antibacterial activity.

The mechanism of action of the investigated peptidomimetics (A-20, A-76, and A-241) in MRSA cells was analyzed by assessing their effects on replication, transcription, translation, and the integrity of the inner cytoplasmic membrane. Using radiolabeled precursors, I demonstrated the inhibition of DNA and RNA synthesis in MRSA cells. The propidium iodide uptake assay and observations performed using transmission electron microscopy (TEM) indicated destabilization of the cytoplasmic membrane. The obtained results suggest a complex mechanism of action involving both interactions of the compounds with the cell membrane and effects on intracellular processes.

Due to the significant role of biofilms in the pathogenesis of *S. aureus* infections, I also evaluated the ability of selected compounds (A-20, A-76, and A-241) to reduce MRSA biofilms. Their efficacy was confirmed by colony-forming unit (CFU) determination, crystal violet staining, and observations using scanning electron microscopy (SEM). All tested compounds demonstrated the ability to reduce bacterial biofilms.

Furthermore, potential molecular targets of the investigated peptidomimetics (A-20, A-220, A-235, A-76, and A-241) were explored using PISA and FITExP approaches. Despite the application of advanced proteomic tools, the identification of a molecular target proved challenging, which may result from the complex and multifaceted mechanism of action of this group of compounds.

The conducted studies expand current knowledge on peptidomimetics as potential candidates for novel antibacterial drugs. The obtained derivatives of compound A-20 are characterized by high activity against MRSA strains, a promising safety profile, and high stability under physiological conditions. The results demonstrate that selected analogs of compound A-20 (A-75, A-76, A-238, A-241, and A-243) are promising candidates for further development as antibacterial agents against MRSA infections.