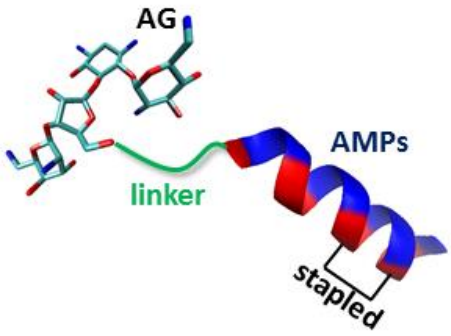
Conjugates of aminoglycosides with amphipathic peptides as antibacterials



Due to limited number of effective antibacterial medications and the challenges related to bacterial multi-drug resistance, scientists are continuously looking for new potent antibacterial compounds. In recent years, interest in antimicrobial peptides (AMP) has increased. Thus, we believe that the use of such peptides as antibiotic carriers is a promising strategy of designing new potential antibacterials. Within this project, we propose the Trojan horse approach (a warhead delivery system) in designing novel antimicrobial compounds. The proposed design uses AMPs and their stapled versions to transport antibiotics into bacteria. We will determine antimicrobial activity of new in-house designed and synthesized bifunctional compounds composed of aminoglycoside antibiotics (AG) and short peptide sequences. We believe that our approach would allow the rebirth of AGs – drugs that have already been known for decades – specifically for aminoglycoside resistant bacterial strains.

The aim of this project is the synthesis of AG-AMP conjugates (see figure) and determination of their antimicrobial activity. The synthesized conjugates will contain one of the four aminoglycoside antibiotics: neomycin B, amikacin, tobramycin, and kanamycin B. The selected antimicrobials are peptides that interact with the bacterial membrane. Due to their structure and physicochemical properties, these peptides penetrate bacteria effectively. Chemical synthesis of peptide and aminoglycoside segments will be carried out separately. In the case of peptides, they will be synthesized on a solid support using the Merrifield method. Purified and characterized derivatives of aminoglycosides and peptides will then undergo a conjugation reaction.

We envisaged a simple possibility of synthesizing AG–peptide conjugates using "click chemistry" (specific cycloaddition reaction between azide and alkyne in the conjugated segments) and by creating a disulphide bond between the segments of the conjugate. These strategies will allow making combinations of conjugates using different substrate derivatives: AMPs and AGs. By using two different conjugation approaches (cleavable and non-cleavable), we will be able to assess the meaning of releasing the transported AG from the conjugate inside the bacterial cell. We will check the impact of the applied linkers and stapled peptides in terms of conjugate stability. Also, we will test the structure and interactions of the AMPs and conjugates with mimetics of bacterial membrane, evaluating the importance of helicity and amphipathicity of the peptides in conjugates. The scope of our research includes examining the activity of the obtained AG-AMP conjugates against selected strains of *Escherichia coli* (*E. coli*) that are resistant to the chosen AGs, as well as determination of toxicity of selected conjugates to eukaryotic cells. We will also examine the permeabilization of the outer and inner membranes of *E. coli* and determine the potential for acquiring bacterial resistance to potential antibiotics. Our preliminary data indicate that the combination of aminoglycosides with suitably designed peptide sequences improves the antibacterial activity of antibiotics, against which *E. coli* strains have become resistant.

The development of an effective method for the delivery of aminoglycosides to resistant bacteria will allow the reuse of these promising compounds as antibacterial substances. The developed strategy can be used in many fields of research: chemistry, biotechnology, molecular biology, and diagnostics. This project can open new ways towards application of peptides as delivery vehicles of biologically active molecules into bacteria cells. It may also lead to the discovery of new antibacterial agents, and new insight for the fight against resistant bacteria.