Streszczenie rozprawy doktorskiej w języku angielskim

The emergence of drug-resistant bacteria and the spread of antibiotic resistance represent a significant challenge to modern medicine. An example of an antibiotic-resistant bacterium of high clinical relevance is the opportunistic pathogen *Pseudomonas aeruginosa*. *P. aeruginosa* can cause severe and often fatal infections of the respiratory or urinary tract. Furthermore, this species is one of the primary etiological agents responsible for nosocomial infections. The prevalence of drug-resistant strains among *P. aeruginosa* has resulted in a reduction in the efficacy of antibiotic therapy and an increase in the difficulty of infection treatment. Accordingly, there is a need to identify alternative strategies for combating this bacterium. Ionic liquids, a class of low-melting salts with diverse biological activities, including antibacterial properties, represent a promising avenue for developing new approaches to combat *P. aeruginosa*.

The primary objective of this study was to evaluate the antibacterial potential of morpholinium-based ionic liquids with herbicidal anions against drug-resistant strains of *Pseudomonas aeruginosa* and to determine the potential for using these compounds as stand-alone antibacterial agents and as adjuvants in antibiotic therapy. The study was conducted on four strains of *Pseudomonas aeruginosa* of high clinical importance: PAO1, LES B58, 39016, and UCBPP-PA14. These were tested against a set of 12 morpholinium-based ionic liquids synthesized from herbicides.

The conducted studies have determined the inhibitory potential of morpholinium-based ionic liquids to inhibit the growth and survival of *P. aeruginosa*. It was demonstrated that the antibacterial activity of morpholinium-based ionic liquids is contingent upon the structure of the cation, exhibiting a pronounced effect only in compounds with two long alkyl substituents. Furthermore, it was demonstrated that ionic liquids with inhibitory effect also exhibit bactericidal activity. The study identified strain 39016 as insensitive to ionic liquids, thereby demonstrating the existence of strain-specific resistance to ionic liquids among *P. aeruginosa* species. It was also demonstrated that ionic liquids can suppress the metabolic activity of bacteria at concentrations below the MIC, that was largely independent of the ability of these compounds to inhibit bacterial growth.

The results of the virulence studies indicated that subinhibitory concentrations of morpholinium-based ionic liquids inhibit the synthesis of pyocyanin, an important secreted virulence factor. Thus, we were the first to identify the potential of ionic liquids to reduce the pathogenicity of *P. aeruginosa* by inhibiting the synthesis of its virulence factors. The observed inhibitory effect was found to be partly dependent on the structure of the cation, but was not due to a decrease in bacterial culture density.

The results of the biofilm inhibition studies have enabled the identification of four ionic liquids with the capacity to inhibit biofilm formation by all tested strains of *P. aeruginosa*. Inhibition was observed at concentrations below the MIC values, highlighting the potential of the morpholinium-based ionic liquids to combat bacterial biofilms. Conversely, a stimulating effect of low doses of test compounds on biofilm formation by LES B58 and UCBPP-PA14 strains was observed, indicating a hormetic effect. Moreover, it was demonstrated that high concentrations of morpholinium-based ionic liquids with two long alkyl substituents can facilitate biofilm formation due to enhanced bacterial aggregation. To date, no other studies have reported the occurrence of concentration-dependent induction of biofilm formation by ionic liquids.

The study also demonstrated that morpholinium-based ionic liquids can form either additive or synergistic combinations with antibiotics. Positive interactions were the most prevalent in mixtures with colistin and combinations comprising ionic liquids with two long alkyl substituents. Furthermore, we were the first to demonstrate that the addition of ionic liquids can result in the sensitisation of drug-resistant *P. aeruginosa*, thereby providing a clear evidence of the potential utility of ionic liquids as adjuvants in antibiotic therapy.

Conversely, the experiments demonstrated that all tested ionic liquids exhibit high haemolytic activity, which considerably limits the potential applications of morpholinium-based ionic liquids in the control of *P. aeruginosa* infections, confining them to extracorporeal utilisation. The degree of haemolytic activity was found to be higher for compounds with two long alkyl substituents and correlated well with the observed relationship between cation structure and the ability of the tested compounds to inhibit the growth of *P. aeruginosa*. The results provided further evidence that the

antibacterial mechanism of action of morpholinium-based ionic liquids is based on the interaction of these compounds with cell membranes.

In conclusion, the study demonstrated the high potential of morpholinium-based ionic liquids synthesized from herbicides for the control of drug-resistant *P. aeruginosa*, providing insight into the effects of these compounds on bacterial growth and survival, metabolic activity, synthesis of virulence factors and biofilm formation. Moreover, it was demonstrated that ionic liquids can enhance *P. aeruginosa* sensitivity to antibiotics, indicating a potential role for ionic liquids in antibiotic therapy as adjuvants. Conversely, the observed haemolytic activity and promotion of biofilm formation by ionic liquids, due to the occurrence of hormesis and cell aggregation, highlighted significant limitations of ionic liquids in their use against bacterial pathogens.