ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Králové

Department of Biological and Medical Sciences

Candidate: Mgr. Adéla Diepoltová

Supervisor: prof. PharmDr. Petr Nachtigal, Ph.D.

Consultant: RNDr. Klára Konečná, PhD.

Title of Doctoral Thesis: Characterization of the antibacterial potential of recently synthesized compounds and bacterial communities

The theoretical part of this dissertation thesis describes the issue of antimicrobial resistance, which represents one of the greatest current challenges to global health. Many different factors contribute to the criticality of this situation. In addition to undeniable human involvement, microbes themselves, such as bacteria from the ESKAPE group, are responsible for this serious problem. Microbes exhibit a variety of resistance mechanisms. The ability to form microbial biofilms is one of them. As a matter of fact, cells present in biofilm are up to 1000 × more resistant compared to their planktonic form.

To reveal the most relevant data regarding the process of biofilm formation *in vitro*, it is essential to use reproducible methods in order to obtain robust biofilms with all their key attributes that represent the adaptive resistance (presence of an extracellular polymeric matrix, the architecture of a matured biofilm, etc.). These attributes are strongly influenced by cultivation conditions (cultivation media, substrate modification, etc.). Results of the first part of the experimental work show that a trypton soy broth medium supplemented with human plasma and mild shaking during the cultivation period are the most favourable conditions among all tested parameters in the formation of *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms. Another important finding is that the formation of *Staphylococcus epidermidis* biofilm on plastic pegs is considerably positively affected by surface modification with human plasma and foetal bovine serum.

The alarming state of antimicrobial resistance is also reflected in the research of new antimicrobial substances. The combination therapy with adjuvant compounds generates promising results. Therefore, the second part of the experimental work is devoted to the characterization of selected (micro)biological properties of a newly synthesized derivative of 2-aminooxazole labelled AB15. This compound shows promising antibacterial activity against the Gram-negative bacteria, especially *Acinetobacter baumannii.* In the target cell, AB15 affects protein synthesis and reveals bactericidal effect. Compound AB15 does not exhibit toxicity effect *in vitro* or *in vivo*. Checkerboard studies confirmed the potential of AB15 as an adjuvant compound – synergistic effect was revealed in AB15 and colistin combination. AB15 + colistin combination also shows an anti-biofilm activity against biofilm formed by *Acinetobacter baumannii*. In this part of the study, it can be concluded that the newly synthesized compound, AB15, can be considered a suitable adjuvant molecule to clinically relevant antibiotics in antimicrobial drug research.

Another effective therapeutic strategy to fight antimicrobial resistance is an employment of antimicrobial compounds obtained from naturally occurring molecules. In the third part of this experimental work, selected (micro)biological properties of semi-synthetic montanine-type derivatives obtained from alkaloids of the *Amaryllidaceae* family are characterized. Compounds labelled NMA5 and NMA12 show promising activity especially against staphylococci. In addition, NMA12 is non-/low-toxic using an animal model and shows synergistic effect with clinically relevant antibiotics against methicillin-resistant *Staphylococcus aureus.* However, the use of compounds NMA5 and NMA12 in further antimicrobial research is limited due to their solubility issues.

The conclusion of this thesis highlights the importance of conducting extensive study of alternative strategies for antimicrobial drug research and development. This work contributes to the global effort to discover new therapeutic approaches and mitigate antimicrobial resistance.