

ABSTRACT

Charles University

Faculty of Pharmacy in Hradec Králové

Department of Biochemical Sciences

Author: Bc. Pavlína Holmanová

Supervisor: RNDr. Miloslav Macháček, Ph.D.

Consultant: RNDr. Klára Konečná, Ph.D.

Title of diploma thesis: Photodynamic inactivation of microorganisms using (aza)phthalocyanine photosensitisers

Background: This work is aimed to optimize methodological approaches for the evaluation of the antimicrobial activity of selected representative (aza)phthalocyanine photosensitizers (PSs) *in vitro*. In addition, the *in vitro* antimicrobial activity of candidate PSs was evaluated against three clinically important pathogens. Namely, gram positive methicillin resistant bacterium *Staphylococcus aureus* (MRSA), gram negative bacterium *Pseudomonas aeruginosa* (PA), and yeast *Candida albicans* (CA) were included in the study.

Methods: Microorganisms were incubated with the selected PSs, which were subsequently washed away, and microorganisms with bound PSs were irradiated for 15 or 30 min. At the same time, the intrinsic antimicrobial activities of the studied compounds without irradiation were determined as well. Three different methodological approaches were used to evaluate the minimum bactericidal concentration (MBC)/minimum fungicidal concentration (MFC). The Bioscreen C instrument was used for an assessment of the dynamics of microbial growth. A methodology for the evaluation of metabolic activities using resazurin was also included. The third method for evaluating the antimicrobial activity of the studied compounds was the spread plate technique.

Results: Of the selected four candidate compounds, the most promising antimicrobial potential showed the amphiphilic cationic compound with the working designation P42. The determined activity against SA was $MBC \leq 0,01 \mu M$, against PA was $MBC = 20 \mu M$, and against CA a compound P42 showed activity $MBC = 0,5 \mu M$. Especially, concerning the technical complexity and evaluation options for screening *in vitro* antimicrobial activities of PSs, the

methodical approach consisted of mapping of growth dynamics was recognized to be the most suitable.

Conclusions: The whole world is facing a severe problem related to the phenomenon of microbial resistance. As the World Health Organization states, if this problem is not addressed effectively, severe socio-economic impacts can be expected shortly.

One alternative approach for the treatment of infectious diseases involves antimicrobial photodynamic therapy, using a drugs called PSs. In this work, a pilot study of the antimicrobial activity of four novel candidate compounds was performed. In three of them, promising activity against the bacterial strain MRSA was revealed. One tested compound also showed a promising effect against the yeast CA, and one compound showed a promising effect against all three microbial strains.

Key words: antimicrobial photodynamic therapy, (aza)phthalocyanine photosensitizers, dynamics of microbial growth, metabolic activity, spread plate method