Abstract

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Title of diploma thesis: Advanced antibacterial activity testing of candidate newly synthesized compounds

Background: The aim of this thesis was to perform an extended study of the antibacterial activity of selected newly synthesized rhodanine derivatives. In this study, activity against clinical isolates of bacterial strains of the genus *Staphylococcus* and *Enterococcus* was evaluated. The main part of the work also includes the evaluation of the antibacterial activity of one selected substance in combination with commercially available antibiotics using the checkerboard method.

Methods: Evaluation of the antibacterial activity of tested substances was performed using the broth microdilution method according to EUCAST guidelines (with minor modifications). The activity of these substances was evaluated against clinical isolates of bacteria of the genera *Staphylococcus* and *Enterococcus* and one *Staphylococcus aureus* MRSA reference strain (ATCC 43300, CCM 4750). For a selected compound with a demonstrably promising antistaphylococcal effect, the combined effect of this substance was tested with three selected commercially available antibiotics (ciprofloxacin, rifampicin, vancomycin) using the checkerboard method. The *Staphylococcus aureus* MRSA reference strain (ATCC 43300, CCM 4750) was used to test the combined antibacterial effect. In order to determine the MIC value, three methods of reading the results were chosen, namely spectrophotometric detection, reading of the growth of bacteria with the naked eye and reading of the colour change of the metabolic indicator alamarBlue.

Results: Nine substances from the group of rhodanine derivatives were tested. These substances showed activity mainly against *Staphylococcus aureus* strains. On the contrary, the lowest activity was observed against *Enterococcus faecium* VRE strain. The selected substance codenamed RHO-y showed a synergistic effect in combination with ciprofloxacin at a concentration ratio of 0,25*MIC (RHO-y) : 0,125*MIC (ciprofloxacin), as well as an additive effect at a ratio of 0,5*MIC : 0,5*MIC; 0,25*MIC : 0,5*MIC; 0,125*MIC : 0,5*MIC; 0,0625*MIC : 0,5*MIC and 0,03125*MIC : 0,5*MIC. In other concentration ratios, these substances showed only an indifferent effect. In combination with rifampicin, RHO-y showed an additive effect in combinations of 0,5*MIC (RHO-y) : 0,25*MIC

(rifampicin) and 0,5*MIC : 0,5*MIC. In other concentration ratios, these substances showed only an indifferent effect. The combination of RHO-y with vancomycin showed only an indifferent effect in all concentration ratios. None of the combinations of RHO-y with commercially available antibiotics showed an antagonistic effect.

Conclusion: In advanced testing, nine rhodanine derivatives showed antistaphylococcal activity, especially against the *Staphylococcus aureus* strain. Using the checkerboard method, a synergistic effect of RHO-y in combination with ciprofloxacin and an additive effect in combination with rifampicin were found. The combination of RHO-y with vancomycin showed only an indifferent effect. None of the tested combinations of these substances showed evidence of antagonism.

Keywords: antibacterial activity *in vitro*, checkerboard test, rhodanines, resistance, combination therapy