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Studies on the antimicrobial activity of new compounds from the group of boraheterocyclic derivatives and arylboronic acids.

Abstract

Key words: antimicrobial resistance, boraheterocycles, arylboronic acids, antimicrobial activity, resistant mutants selection, MPC, MSW

The growing multidrug resistance among microbial strains has led to the urgent need for new, original drugs active against pathogens, primarily against so-called priority pathogens, for which alarmingly few therapeutic options are left. According to the WHO, the number of such drugs marketed in recent years and currently under clinical trials is insufficient. Simultaneously, there are abundant compounds with promising activity against bacteria and fungi in preclinical trials. However, *in vitro* parameters that can predict at this stage the concentration range in which resistant mutants would likely be selected under *in vivo* conditions (so-called mutant selection window - MSW) are lacking. The mutant prevention concentration (MPC) value marking the upper boundary of the MSW frequently exceeds the drug toxicity threshold, is insufficiently repeatable *in vitro*, and can not always be reproduced *in vivo*. This impedes choosing the compound with the lowest potential to select resistance of bacterial strains *in vivo* for further drug development and the establishment of novel, resistance-restricting dosing regimens.

The aims of this thesis were: (**I**) to search for substances active against bacteria and yeasts among new boraheterocyclic derivatives and arylboronic acids, and (**II**) to develop a new approach to assess the potential of compounds to select resistance among bacterial strains, possible to implement at the early stage of *in vitro* preclinical research.

To realize aim **I**, extensive microbiological research was conducted on 44 benzosiloxaboroles and 33 arylboronic acids, mainly di- and triboronic acids. It was evaluated: (**A**) their direct activity against bacteria and yeasts (by the MIC, MBC, and MFC determination for reference and clinical strains); (**B**) the contribution of MDR efflux pumps of Gram-negative bacteria in the active removal of compounds from bacterial cells (by determining the MIC value in the presence of an efflux pumps inhibitor), (**C**) compounds' ability to inhibit β-lactamases (by phenotypic combination disc tests, determining β-lactams' MICs in the presence of tested compounds for KPC-, AmpC- and VIM-positive strains, and confirming the molecular target in microbiological and biochemical assays), and (**D**) the correlation: structure – strength of the

synergy between arylboronic acids and β-lactams (by determining the FICI values and the type of the interaction in the checkerboard as well as by performing statistical analysis).

High antistaphylococcal activity, also against clinical strains of methicillin-resistant *Staphylococcus aureus* (MRSA) was found for 18 benzenesulfonate and sulfonamide benzosiloxaboroles (MICs 0.39-6.25 mg/L), whereas high antienterococcal activity for 3 benzenesulfonate benzosiloxaboroles (MICs 6.25 mg/L). The ability to inhibit KPC/AmpC β-lactamases at high concentrations (30-300 μg/disc) was demonstrated for 25 arylboronic acids and at low concentrations (4-16 mg/L) for 17 arylboronic acids. *ortho*-Phenylenediboronic acid most effectively restored the sensitivity to carbapenems of *Escherichia coli* and *Klebsiella pneumoniae* KPC-positive strains (up to 64-fold MIC reductions) and moderately increased ceftazidime activity against *E. coli* and *Pseudomonas aeruginosa* AmpC-positive strains, without restoring the sensitivity of these strains. *para*-Phenylenediboronic acids restored the susceptibility to carbapenems of KPC-positive strains and the susceptibility to ceftazidime of AmpC-positive strains, whereas *meta*-phenylenediboronic acids restored ceftazidime activity against *E. coli* CMY-2-positive. Studies with the whole cells and total proteins extract of the *E. coli* DH5α transformant carrying the bla_{KPC-3} gene confirmed that KPC-type enzymes are indeed molecular targets for *ortho*- and *para*-, but not for *meta*-phenylenediboronic acids. The statistical analysis revealed that the presence of two boronic groups in the *ortho* position significantly increases the synergy of phenylenediboronic acids with carbapenems (p=0.0001) but weakens their synergy with ceftazidime (p=0.04). In turn, fluorine in the molecule weakens the synergy of these acids with carbapenems (p=0.036) but increases their synergy with ceftazidime (p=0.005). Also, no significant contribution of MDR efflux pumps in their active removal from bacterial cells was detected.

To realize aim **II** it was developed: (**A**) new *in vitro* parameters marking the upper boundary of the MSW, *i.e.*, parameter MPC-D (related to the so-called dominant mutants with high selection potential *in vivo*) and parameter MPC-F (related to mutants with impaired fitness), (**B**) a new broth-dilution method for determining MPC-Ds and MPC-Fs, and (**C**) a new, multi-stage method for obtaining a high-density bacterial inoculum (>10^{11} CFU/mL), which increases the precision of determining the proposed parameters. Another novelty of the work is linking the classical MPC value with the frequency of resistant mutant selection in the agar-dilution method, which takes place in the developed methodology for determining the value of the new MPC-D parameter. The

research was conducted on the *S. aureus* ATCC 29213 strain for three compounds representing different groups of antimicrobials, i.e., ciprofloxacin, linezolid, and benzosiloxaborole No37. MPC and MPC-D values were determined by the agar-dilution method, while MPC-D and MPC-F values were determined by the broth-dilution method. The initial bacterial inocula obtained by the new method had densities of 5–7.5x10^{11} CFU/ml, which is crucial when determining the MPCs parameters. All determined parameters had the same value in the linezolid and benzosiloxaborole No37 case. In the case of ciprofloxacin, the result was as follows: MPC-D<MPC-F<MPC. Its MPC-Ds determined by both methods were comparable. Therefore, the study showed that (**I**) MPC-D may be lower than MPC for some antibacterials, making it more clinically acceptable as a basis for dosage regimens, (**II**) determining the MPC-D value based on the frequency of spontaneous mutant selection is crucial to increase its *in vitro* repeatability compared to the MPC, and (**III**) the proposed broth dilution method allows for the differentiation of mutants generated *in vitro* into dominant mutants and mutants with impaired fitness, which makes likely that the MPC-D would be better reproducible *in vivo* compared to the MPC.

The obtained results of the antimicrobial activity of the tested organoboron compounds, combined with their lack of cytotoxicity, allow to conclude that (**I**) benzenesulfonate and sulfonamide benzosiloxaboroles can be considered a potential source of new drugs active against priority Gram-positive cocci and (**II**) phenylenediboronic acids are interesting scaffolds for the future development of novel KPC/AmpC β-lactamases inhibitors. It has also been shown that the proposed MPC-D parameter may be lower, better repeatable *in vitro*, and likely better reproducible *in vivo* than the MPC parameter, which may improve preclinical testing of candidates for new antimicrobial drugs and facilitate the development of novel, resistance-restricting dosing regimens.

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