Development of metallo-enzyme inhibitors to overcome Pseudomonas aeruginosa antibiotic-resistance in cystic fibrosis patients

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Background. Pseudomonas aeruginosa is the most dominant pathogen associated with adverse clinical outcomes in patients with cystic fibrosis (CF). Anti-pseudomonal β-lactams are the mainstay of therapy for the treatment of chronic P. aeruginosa lung infections CF patients. Unfortunately, this pathogen becomes increasingly resistant to available antibiotics and the global dissemination of multidrug resistant P. aeruginosa strains with acquired carbapenemase genes is a public health concern.

Hypothesis and objectives. One important mechanism of resistance to β-lactams is the production of one or more β-lactamase(s), belonging to either the active serine enzymes or the metallo-β-lactamases (MBLs), which efficiently inactivate these antibiotics. Acquired MBLs are important resistance factors in P. aeruginosa, conferring resistance to most β-lactams, including the last-resort carbapenems. Considering the increasing prevalence of MBL-producing strains in CF patients (as high as >50% in some settings), our goal is to perform the preclinical investigation and optimization of MBLs inhibitors to be used in combination with currently-available β-lactam antibiotics.

Methods. Preclinical investigation of our hit compound and selected novel compounds was performed through assessment of a series of drug-like properties including solubility and chemical stability that was assessed through chromatographic methodologies. Toxicity was evaluated on the mouse fibroblasts NIH3T3 cells and genotoxicity was evaluated using a classical Ames test. The compounds synthesized were tested against purified enzyme IMP-1, VIM-2 and NDM-1 MBL enzymes using a spectrophotometric assay. In addition, selected serine-β-lactamases were also tested to evaluate the spectrum of inhibition. The potential synergistic activity of the tested compounds was evaluated by the agar disk diffusion method, using laboratory strains producing the MBL of interest.

Results. From our structure-based high-throughput docking (HTD) campaign on three clinically-relevant acquired MBLs (IMP-1, NDM-1 and VIM-2) we investigated novel classes of potential MBL inhibitors. Through a concerted effort of computational and synthetic chemistry, we studied the structure-activity relationships of the compounds, and we also analyzed selected drug-like properties such as solubility, chemical stability and toxicity. The initial hit NF1810 was optimized providing a broad-spectrum inhibitor, able to potentiate the in vitro activity of cefoxitin on a VIM-2-producing E. coli strain. However, specific solubility and toxicity issues need to be solved in order to identify inhibitors suitable for in vivo evaluation.

Spin-off for research & clinical purposes. No MBL inhibitors have been approved in therapy today. Novel hits were identified in this project and their further optimization could lead to the selection of preclinical candidates to establish the proof-of-concept for novel combinations therapies for the treatment of lung infections in CF patients.

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References:

1. Zemanick ET, Emerson J, Thompson V, et al. Clinical outcomes after initial pseudomonas acquisition in cystic fibrosis. Pediatr Pulmonol 2015; 50: 42-8. https://doi.org/10.1002/ppul.23036 PMid:24644274

2. Li Y, Zhang X, Wang C, et al. Characterization

by phenotypic and genotypic methods of metallo-beta-lactamase-producing Pseudomonas aeruginosa isolated from patients with cystic fibrosis. Mol Med Rep 2015; 11: 494-8. PMid:25323940

3. Brindisi M, Brogi S, Giovani S, et al. Targeting clinically-relevant metallo-beta-lactamases: from high-throughput docking to broad-spectrum inhibitors. J Enzyme Inhib Med Chem 2016; 1-12.

4. Vallone A. Different Scaffold Decoration Patterns of 2-Arylfurans: from Antiplasmodial to Metallo-β-Lactamase Inhibitors. ESMEC 2016-European School of Medicinal Chemistry; Poster presentation.