Project 07: Characterization of the role of hydrolases in the resistance of *Mycobacterium tuberculosis* against hydroxamic acid derivatives
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Background: *Mycobacterium tuberculosis*, the causative agent of tuberculosis in humans, is the most important bacterial pathogen on a global perspective, causing around 1.5 million deaths each year. A particular clinical problem in the fight against the tuberculosis epidemic is the emergence of multidrug resistant (MDR) or even pan-resistant drug resistant (XDR) pathogens as they are difficult to treat with the existing range of antibiotics (1). Therefore, there is an urgent need for novel antitubercular chemotherapeutics, which differ from existing antibiotics in their mechanisms of action and molecular targets to enable effective control of resistant strains. In recent years, our working group has already identified some first interesting antitubercular lead structures and deciphered the underlying mechanisms of action and resistance (2-6).

Own previous work: During the screening of a structurally diverse library of synthetic substances in a collaborative effort, several hydroxamic acid derivatives were identified that exhibit a very strong antibacterial activity but only low cytotoxicity against various human cells (selectivity index IC_{50} cytotoxicity/MIC >100). Interestingly, these compounds are also active against clinical XDR-TB isolates in the low micromolar range, indicating that these molecules must have a different mode of action than the anti-TB antibiotics currently used. In combination with clinically used anti-TB drugs, additive effects result in prolonged inhibition of mycobacterial growth while effectively suppressing resistance formation. Spontaneously resistant *M. tuberculosis* mutants occur with a resistance rate of only $1x10^{-10}$. Genome sequencing of independent resistant mutants suggest that two different hydrolases with currently unknown function are involved in the mode of action and resistance mechanism, possibly by hydrolysis of the inactive prodrugs after uptake into the cells and intracellular release of the actual active drug forms.

Aim of the project: The role of mutant forms of the identified hydrolases in the resistance of *M. tuberculosis* to hydroxamic acid derivatives will be investigated in detail. The findings are an important step in assessing the clinical suitability of the substances in the development of new chemotherapeutic treatment options for tuberculosis and could provide important information for the directed medicinal chemical optimization of the substances.

Work program: The elucidation of the mechanisms of action and resistance will be approached by combining various complementary methods. These include, among others methods, the generation of site-directed *M. tuberculosis* gene deletion mutants employing phage transduction, heterologous expression of His-tagged wild type and mutant versions of the identified hydrolases, chromatographic protein purification and enzymatic characterization of the potential hydrolysis of the compounds, detection of potential hydrolysis products via HPLC.

References:

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