Synthese von Q203 analoga, ihre bioanalytische Prüfung und das Verständnis des molekularen Wirkungsmechanismus durch Strukturbestimmung v. Protein-Wirkstoff-Komplexen

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My PhD research project takes place in the context of a long-term collaboration between research groups at the Department of Pharmacy of Martin Luther University Halle (Prof. Imming, Dr. Richter), Hospital for Sick Children and University of Toronto (Prof. Rubinstein) and Division of Infectious Diseases at the University of British Colombia (Prof. Av-Gay, Dr. Richter).

1. Introduction

Our research group at Martin Luther University (MLU) works on the development of new drug candidates targeting mycobacteria like *Mycobacterium tuberculosis* (*Mtb*), a slow-growing acid-fast bacterium that can be transmitted by droplets through the air causing tuberculosis (TB). The continuous emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Mtb* strains emphasizes the need to better understand the mechanism of action of existing anti-TB drugs to optimize their activity, as well as the need for discovering new and better TB targets.[1]

2. My research project

My project focuses on the development of new anti-TB drug candidates targeting the oxidative phosphorylation pathway in mycobacteria; in particular, the respiratory cytochrome *bcc-aa*$_3$ supercomplex ($CIII_2CIV_2$ supercomplex), and testing their activity against *Mtb* and understanding their structure-activity relationships (SAR).

The biological activity of my drug candidates is tested by Dr. Adrian Richter, a postdoc in Prof. Peter Imming's research group at MLU and our collaborator Prof. Yossef Av-Gay, Division of Infectious Diseases at the University of British Colombia (UBC).

The research work is in collaboration with Prof. John Rubinstein at the Hospital for Sick Children and the University of Toronto. His group studies the structure and function of macromolecular assemblies using electron cryo-electron microscopy (cryo-EM), image analysis, molecular biology, and molecular genetics.[2,3] Prof. Rubinstein is a worldwide leader of the methodology as such, and a leading international expert for its application in antitubercular research.

In 2018, Prof. Rubinstein's group were able to determine and characterise the structure of the $CIII_2CIV_2$ supercomplex of *M. smegmatis* (a model of *Mtb*) using cryo-EM.[3]

3. Research work undertaken during the past two years

My chemical work focuses on a class of compounds called imidazopyridineamides (IPAs). IPAs target the cytochrome *bcc* complex in the electron transport chain of mycobacteria include *Mtb* and *M. smegmatis*.

The lead compound of this class, telacebec or "Q203", inhibits the growth of MDR and XDR strains in the nM range (**Figure 1**).[4,5] One drawback of this compound is its high lipophilicity, which greatly affects its solubility and thus uptake by and distribution in the body.

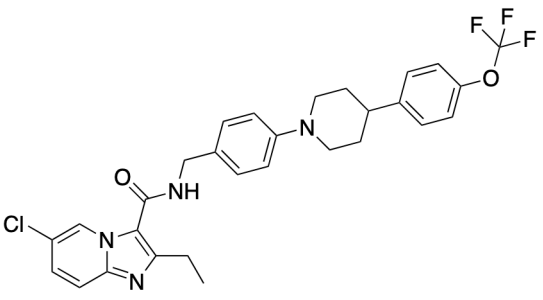

Figure 1. Structure of Q203, the lead compound of imidazopyridineamides (IPAs).

Several analogues were synthesized aiming to decrease the lipophilicity in analogues; for example, by introducing fused ring systems in the side chain.[4,6]

- Synthetic scheme

Through a 6-step synthetic scheme, I was able to synthesize a series of analogues bearing heterocycles like benzoxazole in the side chain. The modifications were specifically designed for the detailed exploration of the drug-target interaction in Prof. Rubinstein's group in Toronto.

Recent progress in 2021

Recently, Prof. Rubinstein's group succeeded in determining a 3D structure of the *M. smegmatis* $CIII_2CIV_2$ supercomplex bound to the inhibitor Q203. I used an atomic model of this structure to study *in silico* the drug-protein interactions in the binding pocket. This work was done using the Schrödinger software package (Schrödinger Release 2019-1: Protein Preparation Wizard; Epik, Schrödinger, LLC, New York, NY, 2019; Impact, Schrödinger, LLC, New York, NY; Prime, Schrödinger, LLC, New York, NY, 2019).

We are also preparing a publication entitled "Structure of the mycobacterial $CIII_2CIV_2$ respiratory supercomplex bound to the inhibitor telacebec (Q203)'' with Mr. David Yanofsky, an MSc student, and Dr. Justin Di Trani, a postdoctoral fellow in Prof. Rubinstein's group as co-authors.

4. Research stay in Canada

4.1. Work plan

As I mentioned above, Prof. Rubinstein is an expert in cryo-EM and the structure of the target ($CIII_2CIV_2$ supercomplex) was determined by his group in 2018. Accordingly, I can only perform the molecular analysis of the imidazopyridine analogues with the target complex in his lab, making this research stay critical to my study.

I plan to stay four months, from November 2021 until the end of February 2022. In these months I will first learn to grow *M. smegmatis. M. smegmatis* is used as a model of *Mtb* because its $CIII_2CIV_2$ supercomplex is highly similar to that of *Mtb*, fast-growing and is non-pathogenic, allowing large volumes of cell culture to be used for protein purification. The next step is the isolation and purification of the supercomplex, which will be used to test the inhibitors' activity and for structural studies.

- Assays planned:

An oxygen-consumption assay was developed in Prof. Rubinstein's group, which includes specific additives to avoid spurious background from the spontaneous autoxidation of MQH_2 (Menaquinol, a membrane-embedded electron carrier).[7]

This stay is a great opportunity for me to be introduced to cryo-EM and gain hands-on experience in biochemical assays.

The results of these assays, as well as the biological assays, will help in understanding the mechanism of action and the development of more effective $CIII_2CIV_2$ inhibitors.

Testing the compounds in whole-cell assays (biological assays) and enzyme-activity (biochemical assays) assays is also important for the development of more effective inhibitors. In general, inactivity of could some analogues could be due to penetration problems, metabolism, or other factors that are not related to the ability to bind to the target.