Searching for Novel Anti-Infective Natural Products – The Junior Research Group "Anti-Infective Compounds from Actinobacteria*"

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Introduction

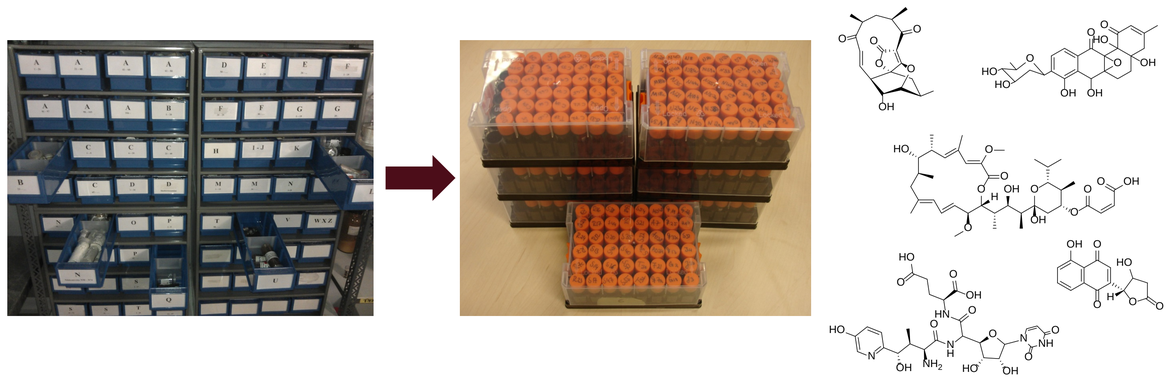
Secondary metabolites from microorganisms have been the major source of lead structures for the development of antibiotics. About 75% of the antibiotics introduced into the clinic over the past 30 years are based on natural products. Actinomycetes are a major source of these lead compounds, and many important compound families such as the macrolides, tetracyclines, glycopeptides, and aminoglycosides have in the past been isolated from these bacteria. Still today they contribute to our arsenal of antibiotics, as the introduction of fidaxomicin into the clinic shows. However, also other groups of bacteria (e.g. myxobacteria) are currently established as novel sources for anti-infective compounds.

The aim of the group "Actinobacterial Anti-Infective Compounds", located at the University Tübingen, is to isolate and characterize novel anti-infective compounds. Using a combination of chemical and biological screening, novel bacterial isolates are chosen for scale-up and subsequent isolation, structure elucidation, and biological characterization of metabolites. In addition to the compounds of the existing Tübingen Compound Collection that are currently being introduced into the TI Natural Compound Library, also these newly isolated compounds will be made available via the TI. While the research will focus on actinomycetes, we will also work with cyanobacteria as a rather untapped source of secondary metabolites.

Projects with Actinomycetes

Making use of the Tübingen Compound Collection

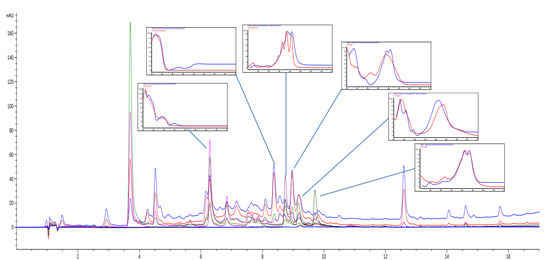
- More than 650 compounds from actinomycetes have been accumulated in the Tübinger Compound Collection over the last decades.
- A subset of ~ **400 compounds** with sufficient purity (~75% > 80%) and high chemical diversity (> 200 singleton scaffolds) has been prepared **to be incorporated into the DZIF Natural Compound Library** and thus be made available for all bioactivity screening partners.



Atacama Desert Actinomycetes

- The **Atacama desert** is the oldest and driest desert on earth. **Actinomycete strains** have been isolated from extreme hyper-arid sites, among them many uncommon genera.
- **Primary screening** of 283 strains against *B. subtilis*, *S. aureus*, *E. coli*, *P. fluorescens*, and *S. cerevisiae* has **indicated antiinfective activity** of several strains (see table below).
- **Chemical screening** is on-going; several strains producing novel compounds have already been identified. Upscaling of these strains is under way, and **isolation of the novel compounds has started**.

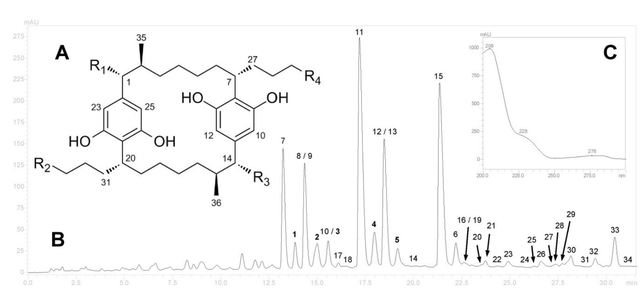
Active against	% of strains
Gram-positive	28
Yeast	18
Gram-negative	9



Projects with Cyanobacteria

Antibacterial Cyclophanes from *Nostoc*

- A **screening of cyanobacteria extracts** revealed an extract from a *Nostoc* sp. as highly **active against Gram-positive pathogens**. The active compounds were isolated and structurally characterized, resulting in 5 new and 11 known **cyclophanes**.
- In addition to a pronounced antibacterial activity **against *S. aureus* and *S. pneumoniae*** (MIC 0.1–2 µM), the compounds displayed **cytotoxicity** (IC_{50} HaCaT 3–12 µM). Presence of a carbamoyl group slightly enhances both antibacterial and cytotoxic activity.



- Presence of bromide in the cultivation medium led to the synthesis of **brominated cyclophanes**. Comparative activity testing showed that bromine substituting chlorine has a neglectable effect on bioactivity.

- After development of a rapid and exhaustive one-step extraction protocol, a **screening** of > 100 cyanobacteria strains for **novel analogs** was conducted. This led to the isolation of the **cylindrofridines**.
- Interestingly, the **antibacterial activity** of two cylindrofridine congeners is **much lower** (MIC > 75 µM), while their **cytotoxicity** is **comparable** (IC_{50} 25 µM). In contrast, a third cylindrofridine still displays antibacterial activity (MIC 8–17 µM), but lower cytotoxicity (IC_{50} 100 µM).

Structures will be disclosed in this online version of the poster as soon as the publication has been submited…

Projects with both groups of organisms

Quorum sensing inhibition

- Inhibition of quorum sensing has been postulated as target for "anti-infective" drugs against which resistance development is unlikely.
- Using several quorum sensing monitor strains, we will **screen** both our cyanobacteria extract library as well as the Tübinger actinomycete strain collection **for compounds interfering with** both Gram-negative and Gram-positive **quorum sensing**.

Screening of cyanobacterial extracts for various anti-infective activities

- A **library of 5700 fractions** (generated from extracts of > 300 individual strains) has been **assayed against *B. subtilis*, *M. luteus*, *E. coli*, and *P. fluorescens***. About 5% of the fractions showed independent activity. In most cases, activity against *B. subtilis* was observed, but 15 fractions were found to be active against Gram-negative microorganisms. Follow-up of the active fractions is currently under way.

- Several cyanobacterial metabolites are potent protease inhibitors. As some proteases are interesting targets in anti-infective therapy, we will **screen a library of about 700 cyanobacteria extracts against selected infection-relevant proteases** such as rhodesain (*T. brucei rhodesiense*; African trypanosomiasis), falcipain (*P. falciparum*; malaria), and LmCPB2.8 (*L. mexicana*, leishmaniosis).

- Some secondary metabolites from cyanobacteria influence epigenetic processes. We will **screen our extract library against sirtuins** (NAD^+ dependent lysine deacetylases) **from *Schistosoma mansoni*** (schistosomiasis).

*and other microorganisms

I thank my **collaboration partners** in these projects: **M. Goodfellow**, University of Newcastle, UK, **A. Bull**, University of Kent, UK, and **J. Asenjo**, University of Chile, Chile (Atacama desert actinomycetes), **M. Preisitsch** and **S. Mundt**, University of Greifswald, Germany (cyclophanes from Nostoc), **Cyano Biotech GmbH**, Berlin, Germany (screening of cyanobacteria extract fractions), **T. Schirmeister**, University of Mainz, Germany, and **T. Steinmetzer**, University of Marburg, Germany (protease inhibitors), **M. Jung**, University of Freiburg, Germany (sirtuin inhibitors)