Natural product discovery pipeline to identify antidotes against amatoxin toxicity: Targeting OATP1B3-mediated uptake

Supervisors: This is a multidisciplinary project and the student will be supervised by both Anima Schäfer (Biopharmacy) and Eliane Garo (Pharmaceutical Biology). This project is therefore advertised in both groups.

The poisonous mushroom *Amanita phalloides*, commonly known as the death cap mushroom, contains amatoxins (e.g. α-amanitin), potent hepatotoxins leading to severe liver damage and potential fatal liver failure. The natural product silibinin, from milk thistle (*Silybum marianum*), is one of the antidotes (Legalon SIL®) available to treat amatoxin intoxications. Nevertheless, the mechanism of action and efficacy of silibinin has yet to be fully determined. Limited studies suggest that one hepatic uptake transporter, the organic anion-transporting polypeptide 1B3 (OATP1B3), may play a crucial role. Further investigations are therefore needed to first confirm these data and clarify the mechanism of this antidote. Finally, it would be highly relevant to delve deeper into the inhibition of OATP1B3 as a treatment of *Amanita phalloides* by identifying other natural inhibitors.
The master's thesis project is a collaboration between the Pharmaceutical Biology and Biopharmacy research groups. Within the project we intend to screen our in-house library of crude extracts from plants and fungi readily available. A previously established cell-based assay using MDCKII cells stably expressing OATP1B3 transporter will be utilized to screen the extracts library. Selected extract hits will be further applied to an HPLC-based activity profiling approach to localize active compounds in these extracts. In brief, extracts will be fractionated by analytical HPLC, micro-fractions will be collected, and tested in the *in vitro* assay to generate the so-called activity profile. Scale-up extraction of selected plant extracts will enable to isolate the active natural products and to determine the affinity profile of these compounds on OATP1B3. Finding new OATP1B3 inhibitors is an important endeavor as it may pave the road for new therapeutic treatments for *Amanita phalloides* poisoning.

Methods: assay screening, cell culture, molecular biology methods to confirm protein expression, HPLC-MS, micro-fractionation, various chromatographic techniques to isolate pure compounds from crude extracts.