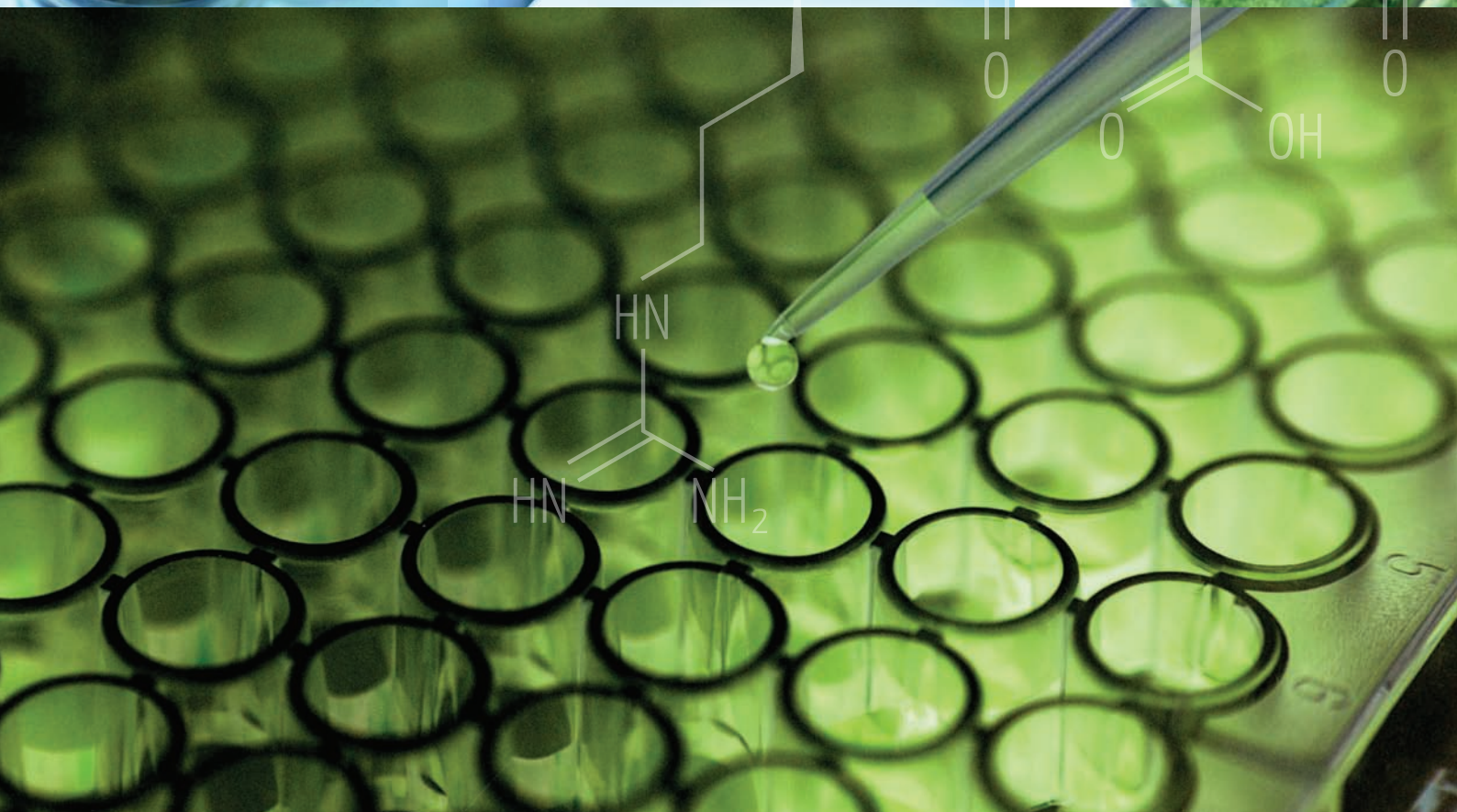
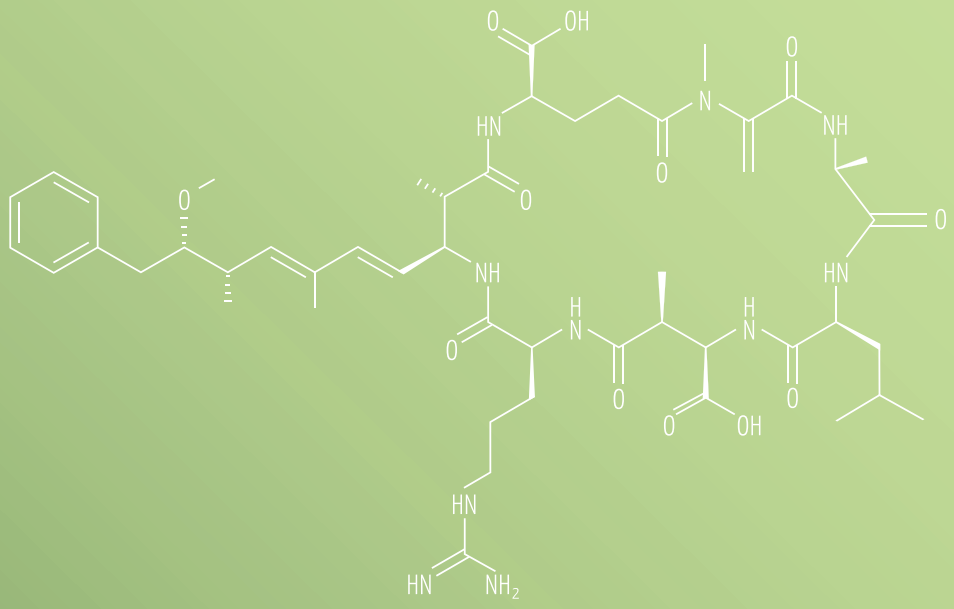
cyano Biotech GmbH

Microcystins as new therapeutic agents for OATP1B3-expressing tumors

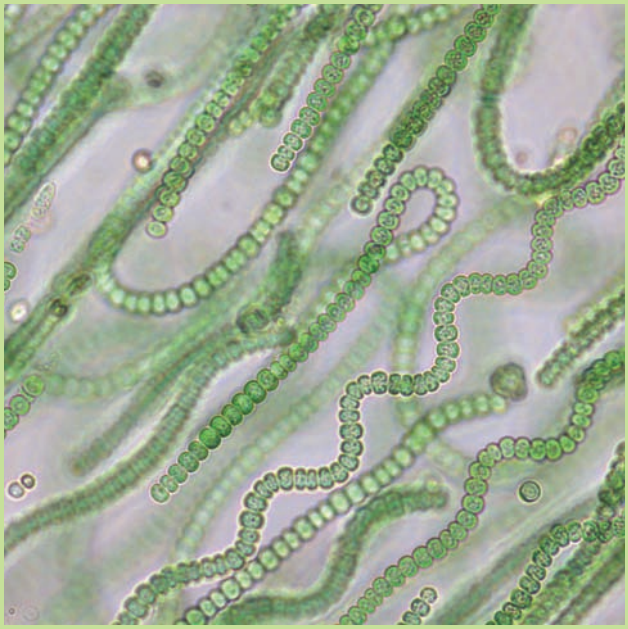


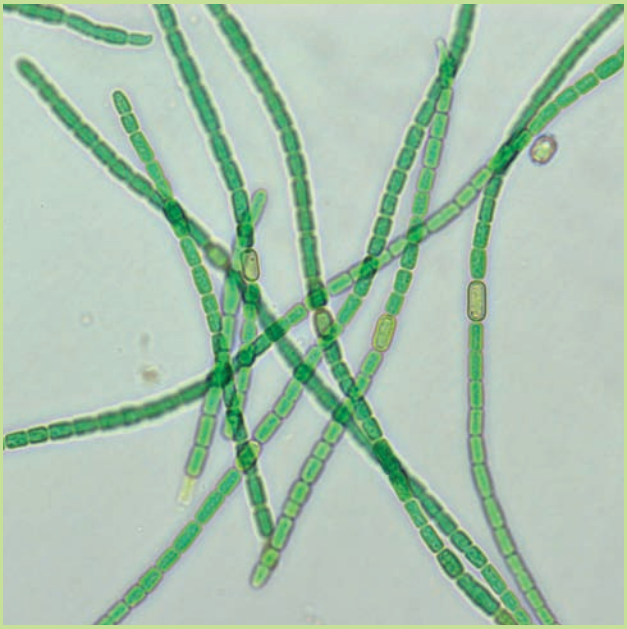
Introduction

Microcystins are cyclic heptapeptide toxins produced by cyanobacteria. They potently inhibit the eukaryotic protein phosphatase families PP1 and PP2A. Microcystins display their toxicity after being actively uptaken by the cell. Three human proteins are thought to be able to mediate this uptake, the organic anion transporting polypeptides OATP1B1, OATP1B3, and OATP1A2. Liver cells express these transporters and are thus able to absorb microcystins from the blood, resulting in a pronounced liver toxicity of microcystins. These OATPs are also expressed in hepatocellular carcinoma (HCC), as well as gastrointestinal and lung tumors. Recent data offer support that the OATP1B3 protein is expressed in a significant percent of breast and colon tumors. Interestingly, compared to OATP1B1, OATP1B3 is found only in low abundance in liver cells. Thus selectivity that favors OATP1B3 over OATP1B1 should lead to a decreased hepatic clearance and increased uptake in OATP1B3-expressing tumors, widening the therapeutic window of the respective compound by decreasing hepatic clearance rate and toxicity.



Microcystin LR , a common microcystin from cyanobacteria





Cyanobacteria (formely known as blue-green algae) belong to the earliest life forms on earth. Their enormous adaptability and vast diversity of species and strains allow them to inhabit almost all ecological niches. Cyanobacteria synthesise diverse natural products with a broad range of biological activities and represent a novel source of lead structures for the pharmaceutical industry.

First Results

Novel microcystins with improved selectivity

Since dozens of naturally occurring microcystins with variations in the 7 constituting amino acids are known and many more structures are not described yet, there is high potential for isolating variants with unique properties. In fact, in our preliminary screening we have found two microcystin structural variants with an IC_{50} in the low nanomolecular range and with transporter selectivity that favors OATP1B3 over OATP1B1 by a factor of ten. One of these two microcystin variants has not been described in the literature before. The dose-activity curve for its toxicity for OATP1B3 and OATP1B1 expressing cells is shown below.

Xenograph models of transporter expression and novel non-apoptotic mode of action

We have developed a xenograft model of the expression of OATP1B1 and OATP1B3 in stably transduced HeLa cells and stably transduced RKO colon cancer cells, and have demonstrated the proof of principle that microcystin LR can decrease the growth of OATP1B1-expressing cancer cells in vivo. Furthermore we found that microcystin-induced cell death occurs by a novel, non-apoptotic pathway, making these agents potentially useful in malignancies that have developed resistance to apoptosis. Therefore we conclude that the identification of a microcystin variant for the development as a novel therapeutic agent for OATP1B3-expressing tumors is promising.

Our approach

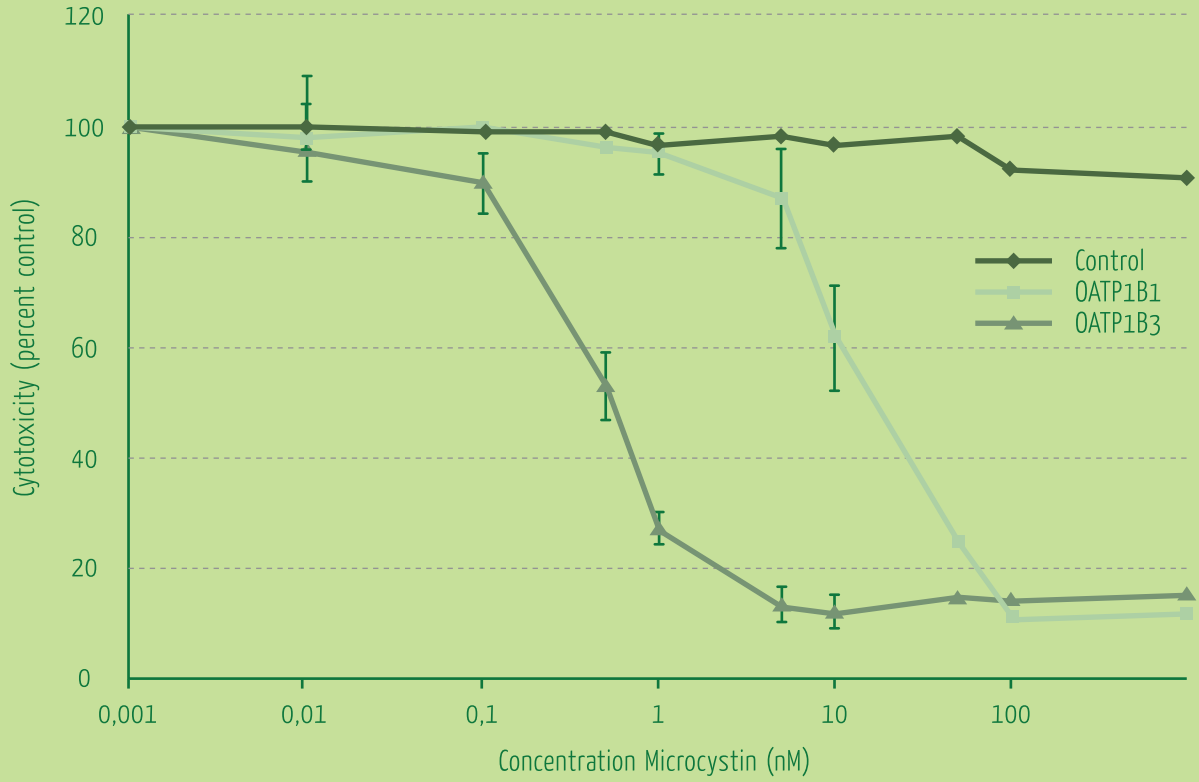
1. Screening of Cyano Biotech's in-house cyanobacterial strain collection using HPLC-DAD/MS as well as degenerate and specific PCR for microcystin producers.

2. Large scale cultivation of selected cyanobacterial strains, isolation of a variety of microcystin variants.

3. Screening of the resulting microcystin library for effectiveness as well as selectivity in OATP1B1 and OATP1B3 transfected HeLa and RKO cells.

4. Structural characterization of the isolated compounds, deduction of structure-activity relationships.

5. Lead optimization by creation and pharmacological examination of "unnatural" natural products based on the most promising hits by Cyano Biotech's biocombinatorial strategies (based on precursor feeding and genetic optimization of producer strains).



Dose-Activity Curve of a Selective Microcystin

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Jeffrey A. Moscow, M.D. is a pediatric oncologist and has pursued an anti-cancer drug discovery strategy by the identification of solute and nutrient transporters on the surface of malignant cells, and targeting them with small molecules that are both selective substrates for those transporters and toxic to the cancer cell.

His lab and others identified of the restricted expression of OATP1B3 in normal cells, but broader expression in malignant tumors, and determined that cancer cells expressing OATP1B1 and OATP1B3 were sensitive to microcystin LR and its structural analogs.

Cyano Biotech

Cyano Biotech GmbH is the worldwide leading company in cyanobacterial R&D and transfers the know-how gained in more than 25 years of cyanobacterial research into an innovative Biotech enterprise as a partner for the pharmaceutical industry. Cyano Biotech's business is focused on natural product based drug discovery and development. For this aim the company exploits its in-house strain collection of 1.000 cyanobacterial strains, its partly exclusive access to 10.000 strains from all over the world, and its collaboration with diverse strain collections and academic partners. Cyano Biotech is known for its extensive knowledge in screening for novel microcystins, their isolation, characterization and structural optimization. Selected microcystin variants are worldwide sold by Cyano Biotech as analytical standard and bioreagent to distributors like Sigma-Aldrich and LGC Standards as well as to individual end-users.

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