A Penotypic Screening Toolbox Permits the Identification of Novel Compounds with Anti-Cancer Properties Derived from Marine Fungi

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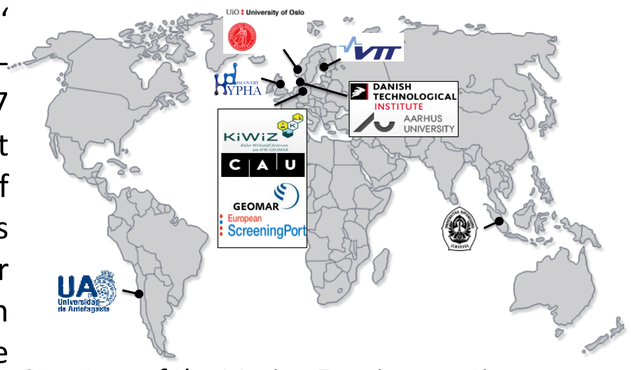
Introduction

The ‚Marine Fungi' project, an inter-national FP7 program, aims at identification of novel compounds with anticancer properties from marine fungi. The projects spans from the isolation and characterisation of the marine fungi to fermentation, activity guided purification and screening of extracts and compounds. The 3 most interesting natural products are now being analysed *in vivo*.



Structure of the Marine Fungi consortium

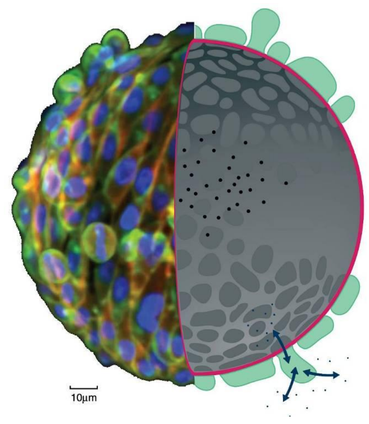
Source	Extracts	Organisms	No. hits	Hit Rate (%)
Mediterranean sponge fungi	754	206	78	10.3
Chilean macro-algal fungi	125	125	48	38.4
Indonesian coral fungi	331	105	47	16.5
Totals	1210	436	173	14.3

Summary of the collected fungi, their natural products and the corresponding bioactivity

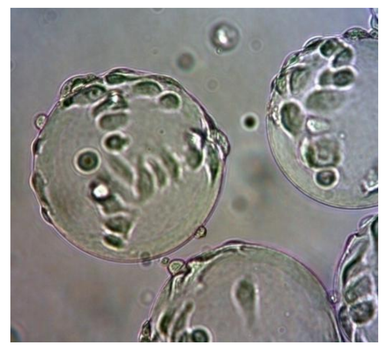
Bead Technology

BioLevitator™; 3D cell culture on Microcarriers

Schematic representation of the incubation chamber with magnetic separator



Schematic representation of the 3D Dextran Microcarriers



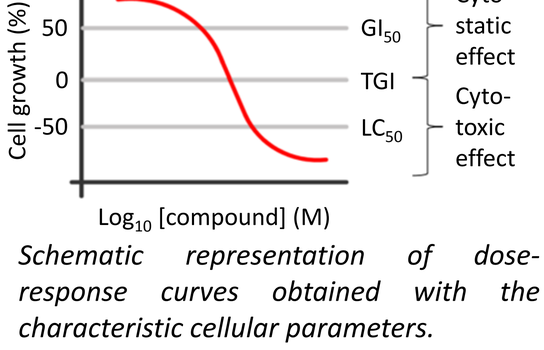
M14 cells (melanoma) on Dextran Microcarriers

Assay Principle

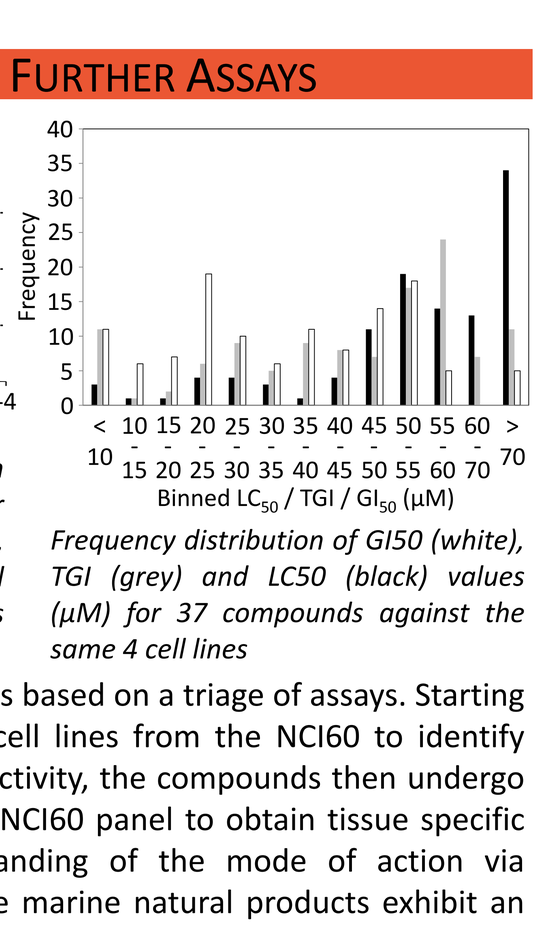
The principle of the NCI60 human tumour cell line anticancer drug screen was developed 15 years ago. We adapted the assay for HTS purposes by changing the read out, from dye based to Lumines-cence, miniaturising it, from 96 to 384 well plates, and reducing the volume per well from 200 µl to 20 µl. The wealth of data available for the cell lines from the NCI60 panel, like COMPARE or the COSMIC database, is an invaluable source of information for the screening process. Parallel to the NIH procedure assays for improved automatisation, using the BioLevitator™ (Hamilton Bonaduz AG), or alternative read outs, using the CellMetric™ (Solentim Ltd), were evaluated.

Tissue origin	No. of cell lines
Leukemia	6
Non-Small Cell Lung	9
Colon	7
CNS	6
Melanoma	9
Ovarian	7
Renal	8
Prostate	2
Breast	6

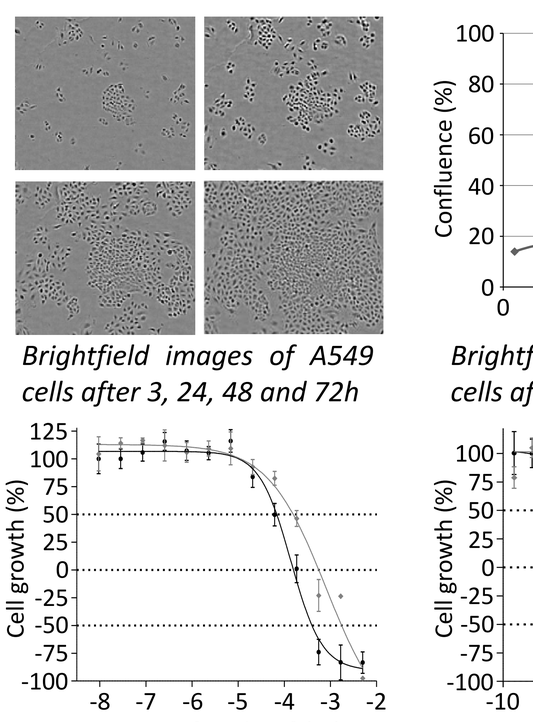
Tissue specific composition of the NCI60 cell line panel.



Schematic representation of dose-response curves obtained with the characteristic cellular parameters.



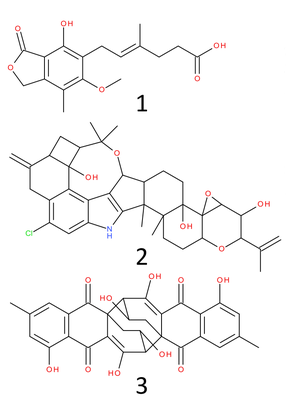
Marine Fungi cytotoxicity assay principle based on the NIH procedure. By detecting viability at day 1 cytostatic and cytotoxic compounds can be identified



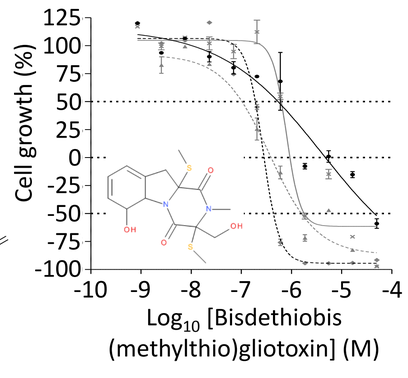
Direct comparison of 258 compounds screened against A549 cells in 2D and 3D (R^2=0.95)

Next to conventional plate based assays dextran microcarriers were evaluated. 56 Hits from the screen were followed up in dose response. No significant differences were observed. 3D data confirm 2D experiments.

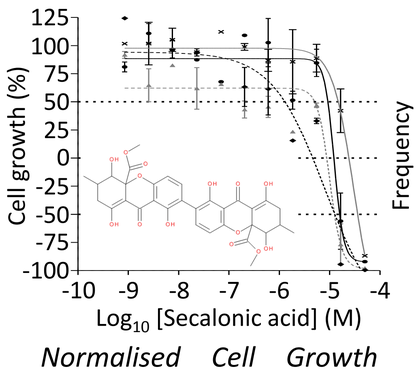
Results of the Cytotoxicity Profiling and Further Assays



Structures of myco-phenolic acid (1), penitrem A (2), rugulosin (3)

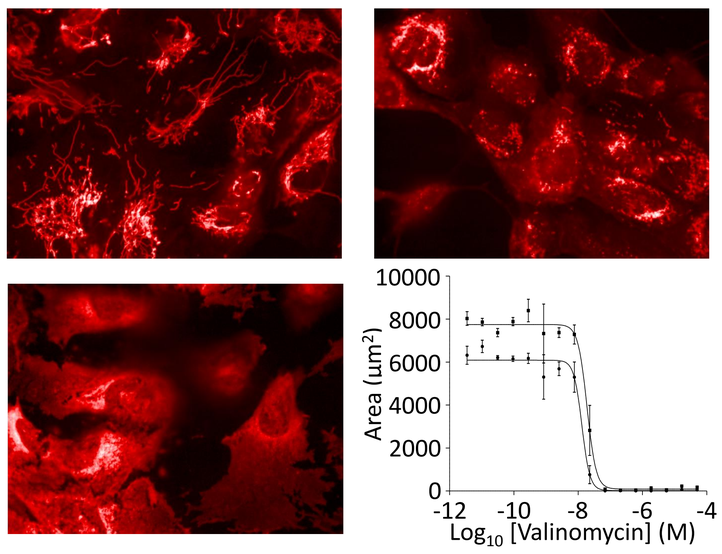


Normalised Cell Growth dose response curves for bisdethiobis- (methylthio)-gliotoxin in 786-0, MCF-7, M14 and HL-60 cell lines



Normalised Cell Growth dose response curves for secalonic acid in 786-0, MCF-7, M14 and HL-60 cell lines showing TGI values between 8 and 30 µM

Frequency distribution of GI50 (white), TGI (grey) and LC50 (black) values (µM) for 37 compounds against the same 4 cell lines



Mitotox assay based on image analysis. Read out is the area of viable Mitochondria. IC_{50} of Valinomycin varies between 13 and 19 nM.

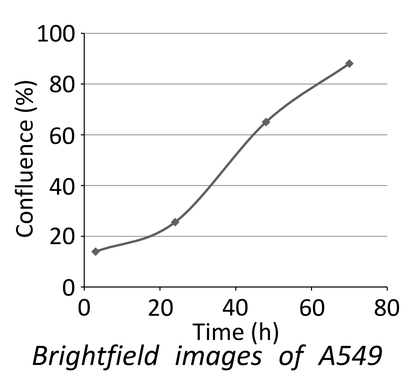
The screening process is based on a triage of assays. Starting with 4 very sensitive cell lines from the NCI60 to identify also weak anticancer activity, the compounds then undergo a full screening in the NCI60 panel to obtain tissue specific data and an understanding of the mode of action via COMPARE. Most of the marine natural products exhibit an activity in the low micromolar range.

Further assays include apoptosis and necrosis assays as well as pathway analysis using PhosphoFlow analysis. ADMET assays like plasma protein binding or Cytochome P450 inhibition help to select the most interesting compounds for *in-vivo* studies.

A research article describing the identification of the first 37 identified natural products has been submitted and will appear in the journal for ASSAY and Drug Development Technologies.

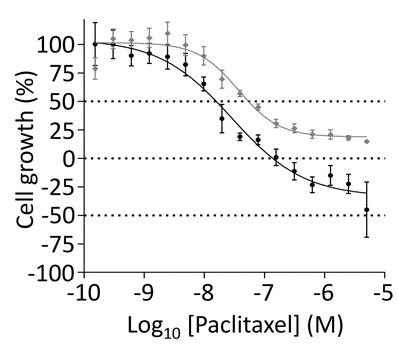
Image Based Cytotoxicity Profiling

Brightfield images of A549 cells after 3, 24, 48 and 72h



Brightfield images of A549 cells after 3, 24, 48 and 72h

Comparison of cell growth using CTG (grey) or image based (black) analyses.



Comparison of cell growth using CTG (grey) or image based (black) analyses.

Acknowledgements

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