

Introduction

Historically, natural products have been an important source of anticancer drugs. Approximately 50% of all anticancer drugs are natural compounds or derived from natural compounds. Examples are doxorubicin, mitomycin C, vinorelbine, actinomycin D and taxol.

In the 1990's drug discovery efforts in oncology were moving away from conventional cytotoxic agents and focusing on compounds targeting molecular pathways. In addition, the availability of large synthetic compound libraries in combination with modern drug discovery has led to highly selective and target specific small molecules on the market, e.g. the tyrosine kinase inhibitor imatinib.

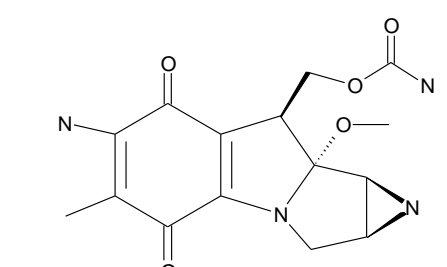
Does nature provide secondary metabolites acting on such specific tumorigenic pathways and targets?

Many examples of novel chemical entities based on natural products with highly specific target profiles are under investigation, have recently entered clinical evaluation or have entered the market (see below).

In this poster we will present three further examples for natural compounds targeting specific tumor pathways: geldanamycin, borrelidin and fredericamycin. We have used microbial producers to generate gram quantities of these natural compounds and employed semi-synthesis to generate focused libraries of natural compound analogs. The analogs have been tested in relevant assays to identify leads with improved profiles.

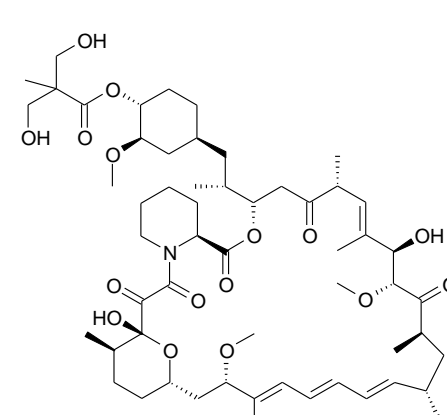
Natural products in use and in development

Some examples of anticancer natural products from microbial sources and some information regarding their mechanism of action are shown below.



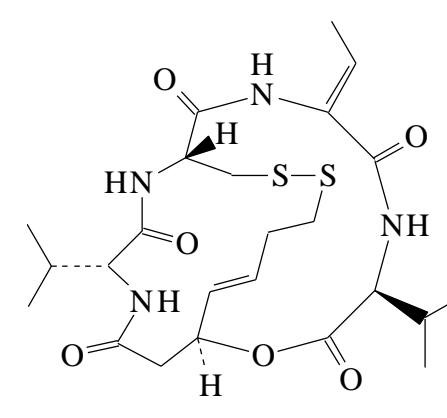
Mitomycin C

- alkylating - DNA crosslinking
- source: *Streptomyces caespitosus*
- on the market use since the 60's



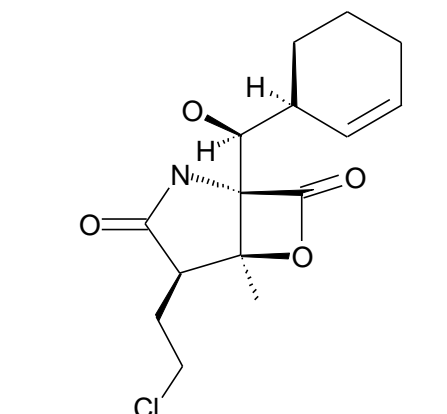
Temsirolimus

- mTOR Kinase inhibitor - PI3Kinase/Akt pathway
- semisynthetic, derivative of rapamycin, source: *Streptomyces hygroscopicus*
- on the market since 2007



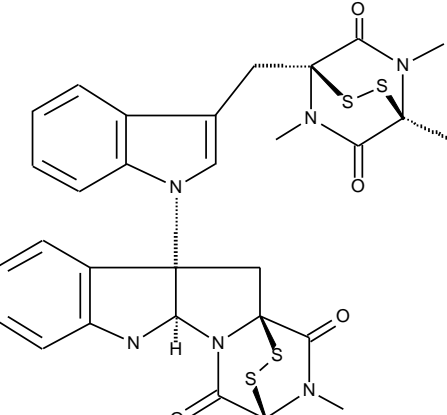
Depsipeptide FK-228

- HDAC inhibitor
- source: *Chromobacterium violaceum*
- on the market (CTCL)



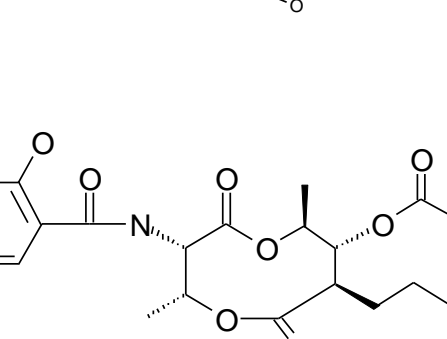
Salinosporamide A

- proteasome inhibitor
- source: marine *Salinispora sp.*
- in clinical development, phase 1



Chetomin

- HIF-1 pathway inhibitor - angiogenesis
- source: *Chaetomium cochlioides*
- research compound



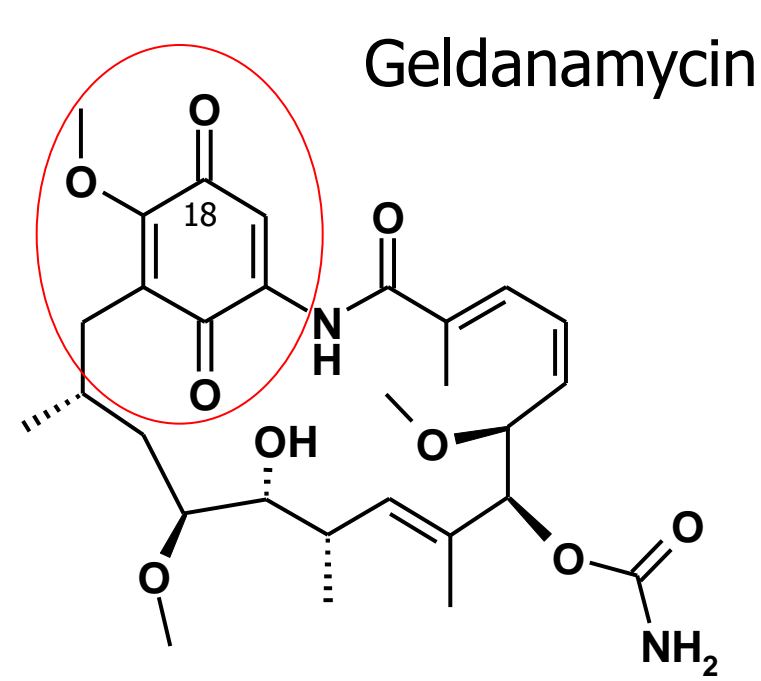
Antimycin A3

- Bcl-2 ligand - apoptosis induction
- source: *Streptomyces sp.*
- research compound

Geldanamycin targeting HSP90

Background

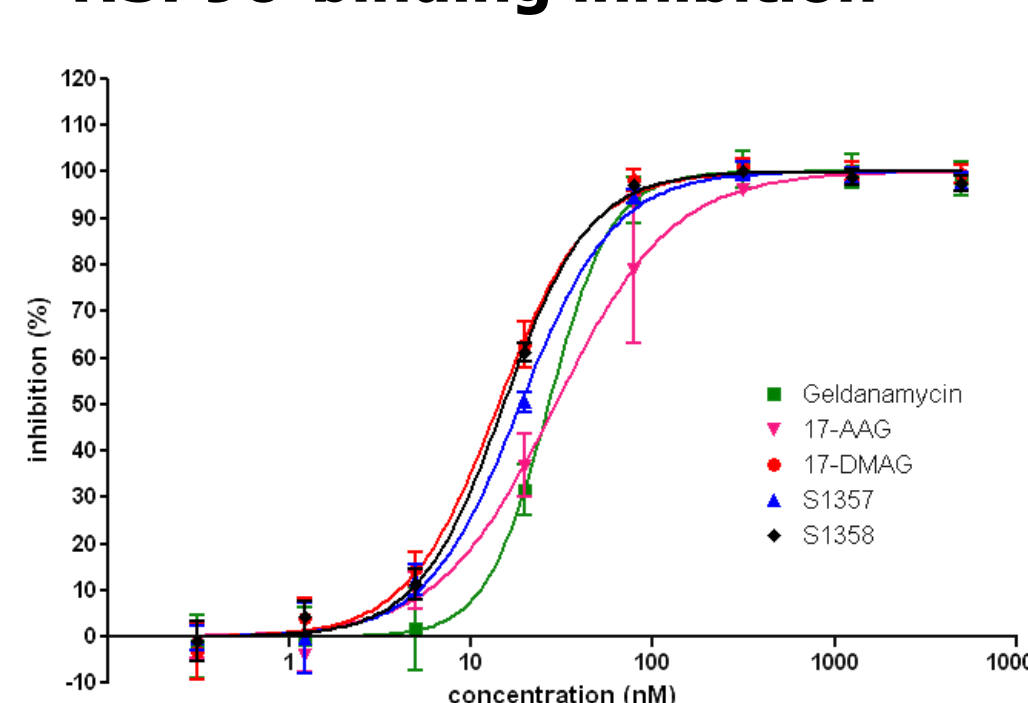
- Geldanamycin (GM) inhibits the chaperone HSP90
- HSP90 assists in the maturation of client proteins that are components of oncology pathways
- GM derivatives (17-AAG, 17-DMAG) are in clinical development, but have hepatotoxic side effects
- Objective of the project: GM derivatives with good HSP90 inhibition but reduced hepatotoxicity.



Compounds & semi-synthesis

- GM isolated in gram quantities from the fermentation broth of the microbial strain GW16569
- 90 derivatives generated by semi-synthesis, targeting the group indicated by a red circle
- goal: reduce reactivity of the quinone moiety by introduction of an oxime function at position 18.

HSP90-binding inhibition



Compound	IC ₅₀ (nM)
Geldanamycin	27
17-AAG	29
17-DMAG	14
S1357	19
S1358	15

Figure 1: Displacement of labelled GM from HSP90 by GM and derivatives, measured using an HTRF assay (displacement of labelled GM from HSP90)

Hepatotoxicity *in vivo*

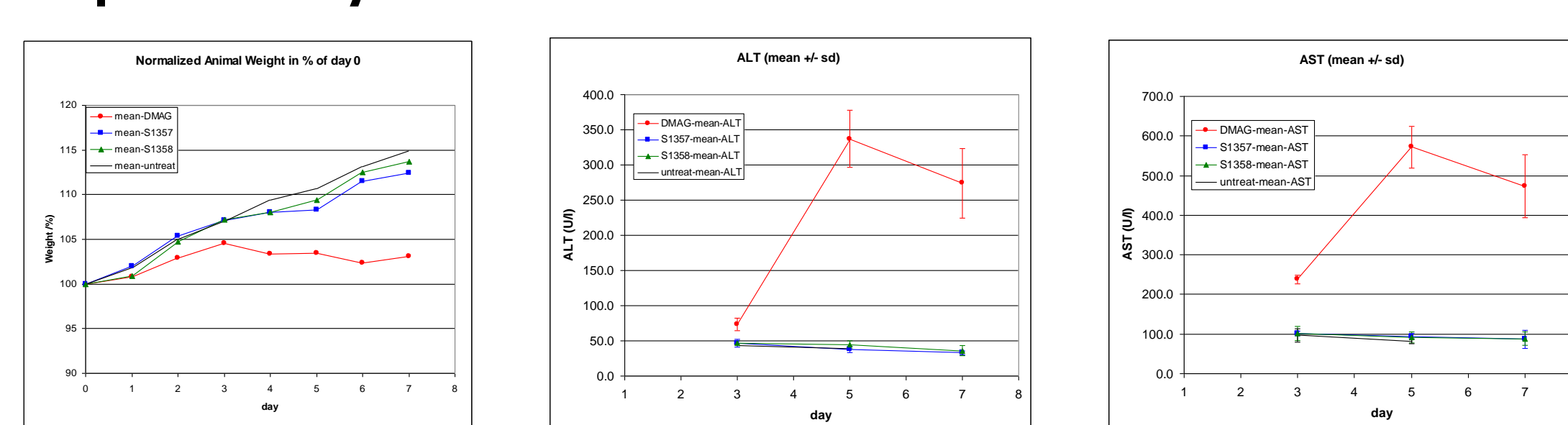


Figure 2: Toxicity of GM derivatives in comparison to 17-DMAG measured in rats. Treatment with two GM derivatives (S1357, S1358) at 4 mg/kg, compared to 17-DMAG at 4 mg/kg and vehicle.

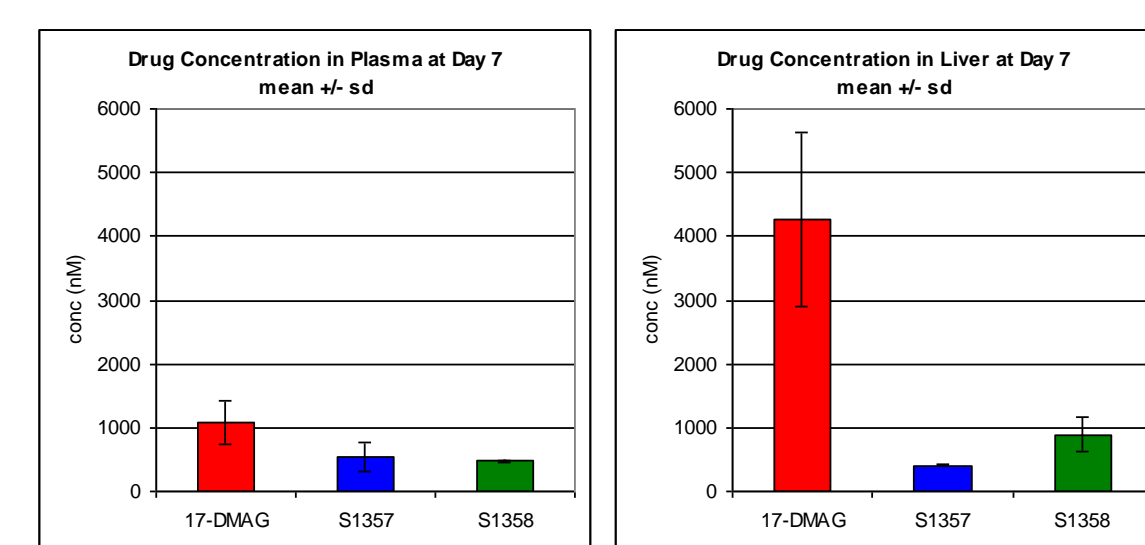


Figure 3: Drug concentration at day 7 in rats, measured at the end of the experiment shown in figure 2.

Results and conclusions

- Oxime derivatives of GM bind potently to HSP90, comparable to GM and 17-AAG, 17-DMAG.
- Improved tox profile: +/- normal weight gain, no effect on liver enzymes
- Compounds show *in vitro* antitumor activity in a cell panel (data not shown)
- Our GM derivatives represent highly attractive starting points for further development.

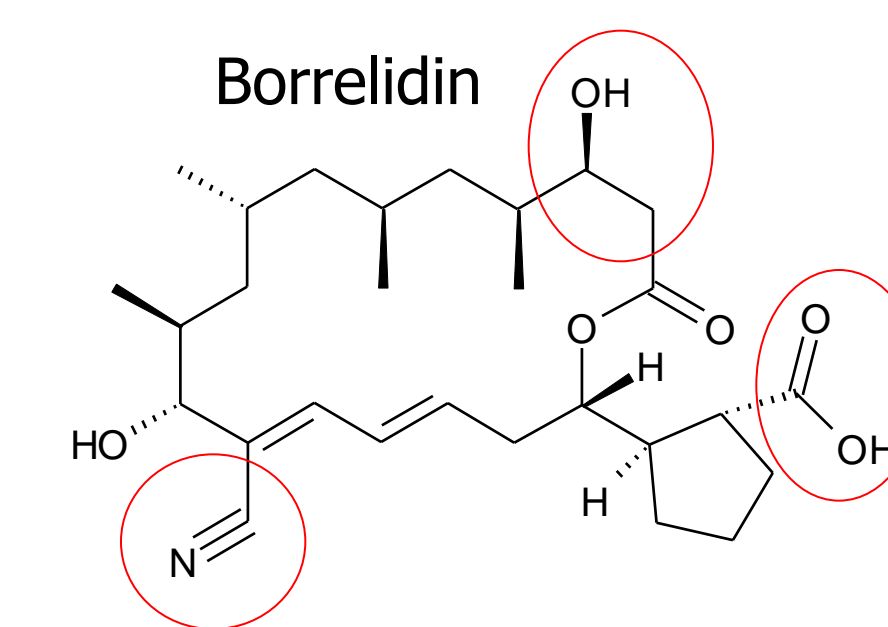
Conclusions

- Natural products from microbial origin represent a valuable source for compounds targeting specific molecular pathways relevant for anticancer therapy
- Appropriate assays, good natural product sources and an efficient platform to identify and supply active natural compounds needed to deliver novel molecules
- BioFocus has established a natural product platform based on pre-purified microbial extracts (see poster: Are Natural Products Delivering? Experiences and Recommendations from 20 Screening Campaigns)
- BioFocus' natural product library has not yet been exploited for oncology applications
- Re-discovery of known natural compounds leads to the identification of novel specific mechanisms of action and such makes them good candidates for further lead optimization and drug development as shown in this poster.

Borrelidin targeting angiogenesis

Background

- Borrelidin (BN) inhibits angiogenesis *in vitro* and *in vivo*
- Exact mechanism of BN action unclear
- BN is cytotoxic against human tumor cell lines
- Objective of the project: BN derivatives which inhibit the proliferation of HUVEC cells (surrogate for angiogenesis inhibition) but show reduced cytotoxicity against other cell lines.



Compounds and semi-synthesis

- BN isolated in gram quantities from the fermentation broth of the microbial strain G1/1543
- 150 derivatives generated by semi-synthesis targeting the groups indicated by a red circle

Anti-proliferative activity on HUVEC and MCF-7 cells

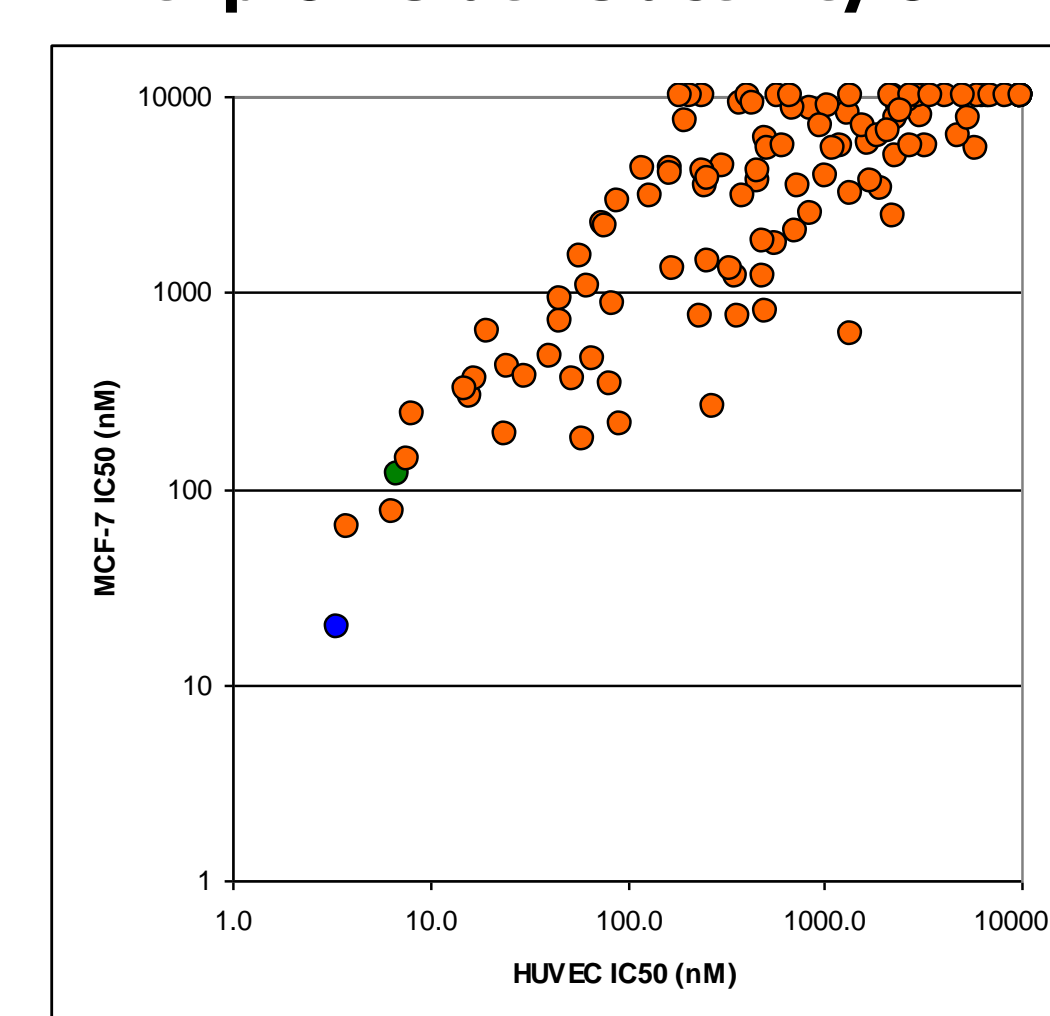


Figure 4: Anti-proliferative activity of Borrelidin (blue) and derivatives (orange); derivative S1253 (green). HUVEC: 1300 cells/well, EGM2 medium, 24 hr pre-incubation, addition of test compound, 48 hr incubation. MCF-7: 2400 cells/well, RPMI-Medium, same protocol. Quantification of cells with Cell-Titer Glo.

Angiogenesis inhibition *in vivo*

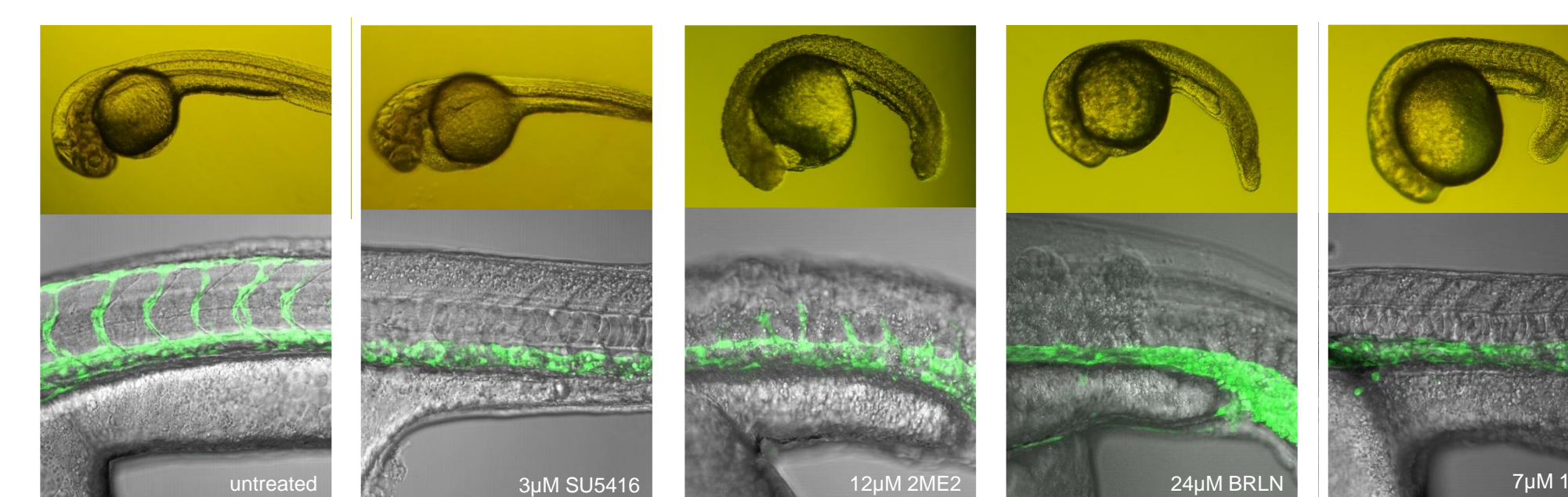


Figure 5: Angiogenesis inhibition in zebrafish; the upper part of each image shows a brightfield image of the whole zebrafish embryo, the lower part shows the fluorescent vessels formed in the developing embryo. Selected compounds were tested at the Katholieke Universiteit Leuven, Belgium.

Compound	HUVEC IC ₅₀ (nM)	MCF-7 IC ₅₀ (nM)	TI	Zebrafish IC ₅₀ (µM)	Zebrafish toxicity*
Reference: Sugen VEGF inhibitor SU5416	nd	nd	nd	2	no
Reference: 2-methoxyestradiol	nd	nd	nd	11	high
Borrelidin	3.4	20	6	18	medium
Borrelidin derivative S1253	6.9	118	17	5	medium-low

Table 2: Antiproliferative and anti-angiogenic activity of selected compounds. * at IC₅₀, nd = not determined

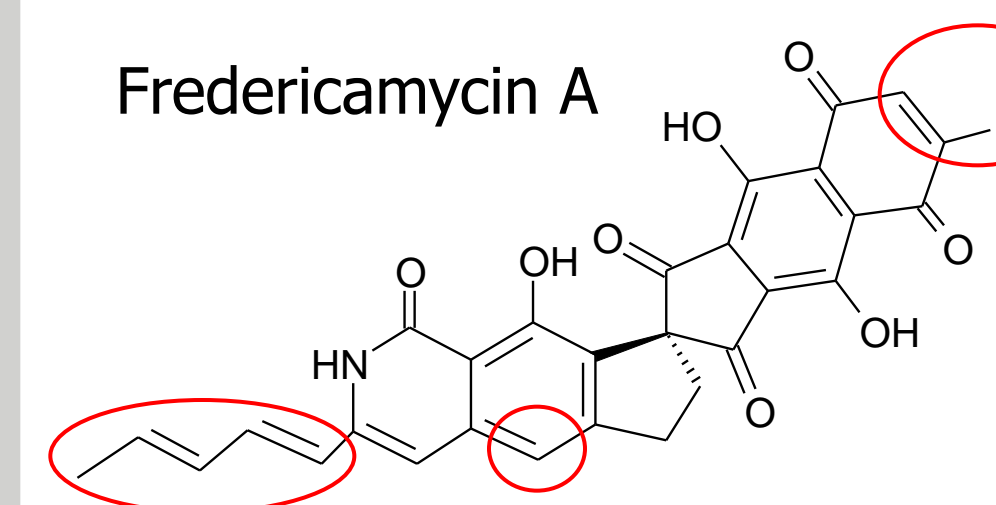
Results and conclusions

- BN derivatives generated with improved therapeutic index (HUVEC/MCF-7 inhibition)
- Selected compounds inhibit angiogenesis *in vivo*. Potency comparable to reference compounds. Toxicity reduced.
- Results achieved so far merit further exploration of this compound class.

Fredericamycin targeting PIN1

Background

- Fredericamycin A (FMA) is a structurally unique antitumor antibiotic
- Evidence for inhibition of the peptidyl-prolyl cis/trans isomerase PIN-1 by FMA (WO 2004/002429)
- PIN-1 catalyzes cis/trans isomerization of peptidyl-prolyl bonds in the vicinity of phosphorylated amino acids; role in phosphorylation status dependent modulation of client proteins activity
- Objectives: FMA derivatives with improved cytotoxic properties; determination of PIN-1 inhibition by FMA derivatives



Compounds and semi-synthesis

- FMA isolated in gram quantities from the fermentation broth of a mutant *Streptomyces griseus* strain
- 320 derivatives generated by semi-synthesis targeting the groups indicated by a red circle

Cytotoxicity and selectivity in a human tumor cell line panel

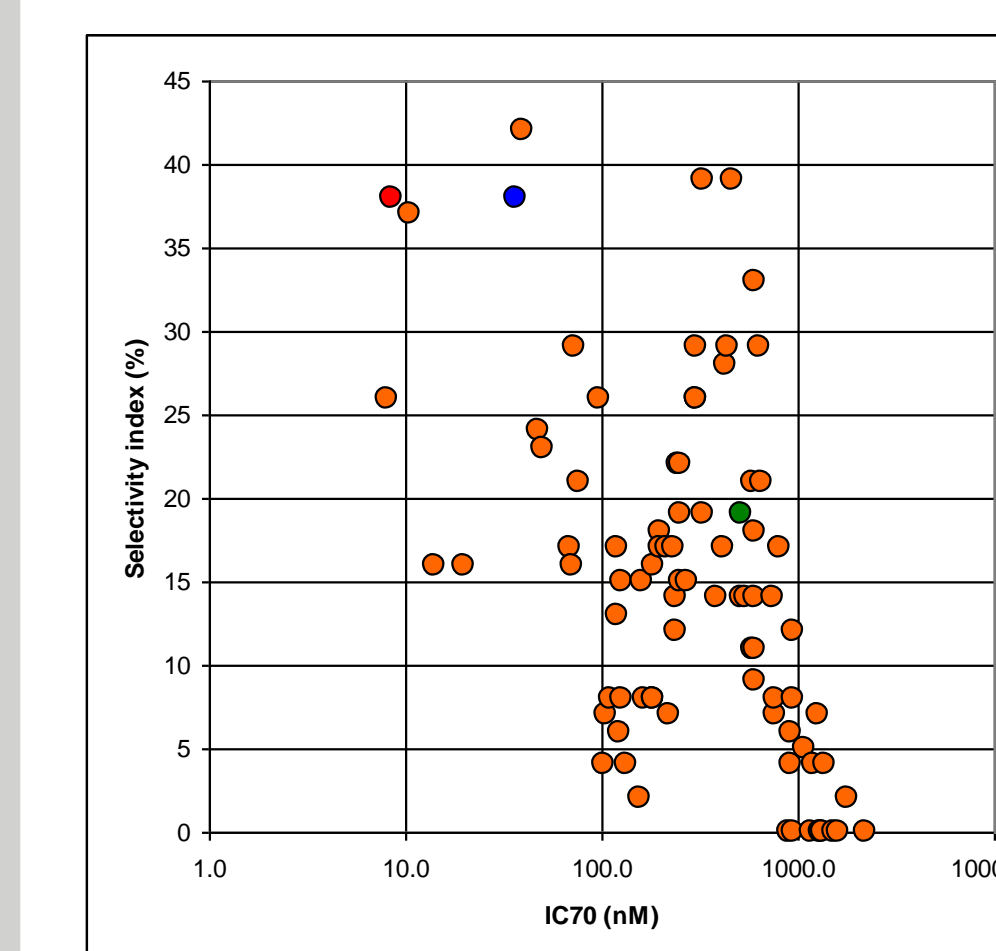


Figure 6: Cytotoxicity and selectivity index of 82 derivatives of FMA. Measured in a panel of 10 human tumor cell lines (as described in Bioorg Med Chem Lett 2006, 16:3292). IC₇₀'s of FMA derivatives (orange) compared to reference compounds (FMA green, adriamycin blue, camptothecin red).

PIN-1 inhibition

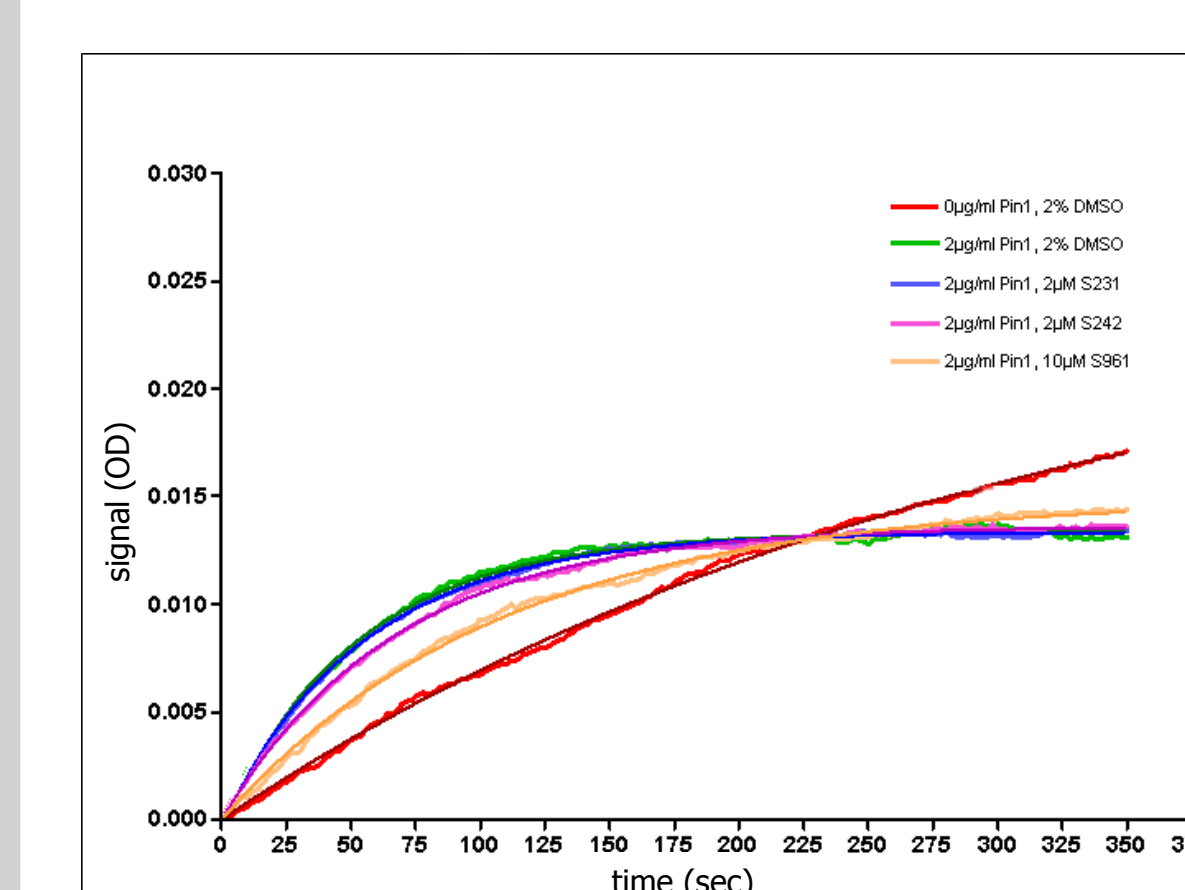


Figure 7: PIN-1 activity and inhibition by FMA (10 µM S961) and derivatives (2 µM); method based on the isomer specific activity of the protease chymotrypsin, which preferentially cleaves the trans conformation of a proline-containing substrate peptide (Biochem Biophys Acta 1984, 791:87); progress and fitted curves shown.

Results and conclusions

- FMA derivatives with improved antitumor potency and increased selectivity index generated
- PIN-1 assay established and inhibition by FMA shown
- We have shown, that FMA derivatives modulate the target PIN-1, but additional work is required to verify the role of PIN-1 in the potent antitumor activity of this compound class.
- FMA derivatives with improved PIN-1 inhibition identified
- Further experiments are planned to determine IC₅₀'s and to correlate PIN-1 inhibition with the cytotoxic activity observed against human tumor cell lines.