Screening for anticancer compounds in cyanobacteria

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Why cyanobacteria?

-right after Actinobacteria Cyanobacteria are predicted as the most richest source of new chemical entities in the biosphere

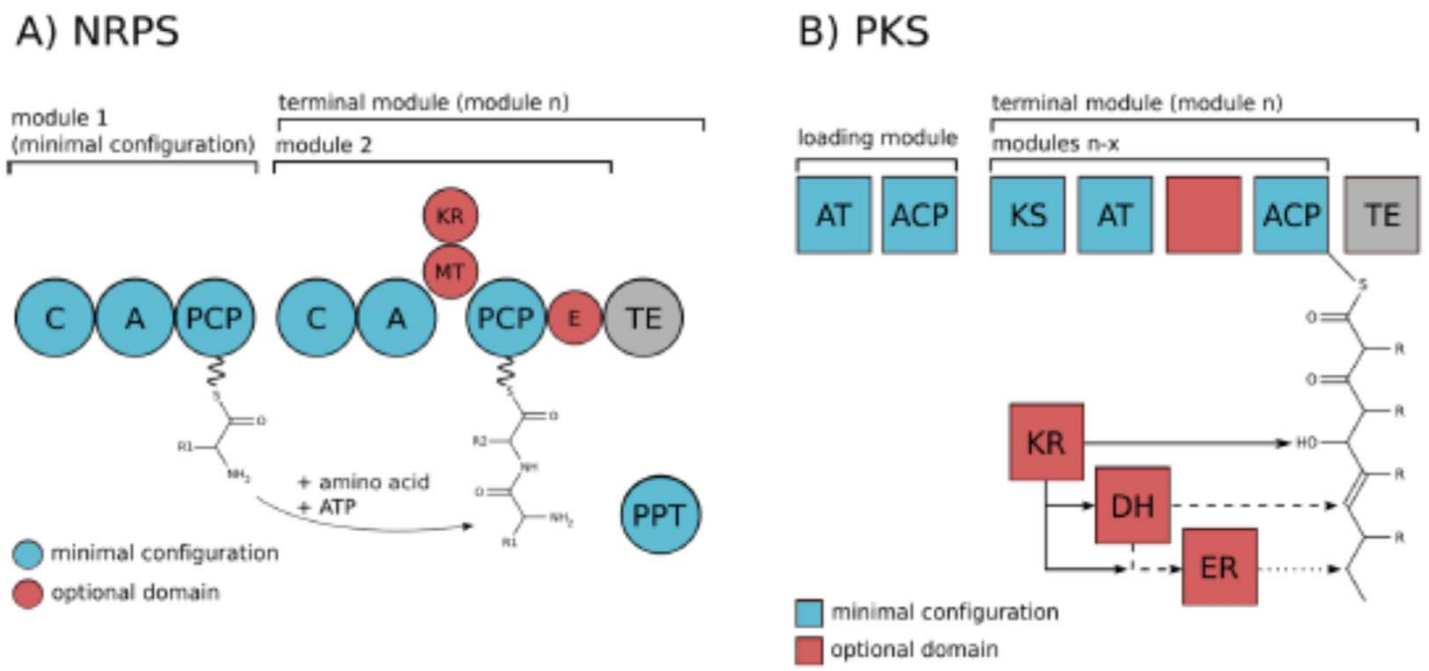
-hundreds of unique chemical scaffolds and thousands of their structural variants exists

-90% of the real diversity is estimated to be undiscovered

Where does it occur?

Biosynthetic pathway of NRPS and PKS

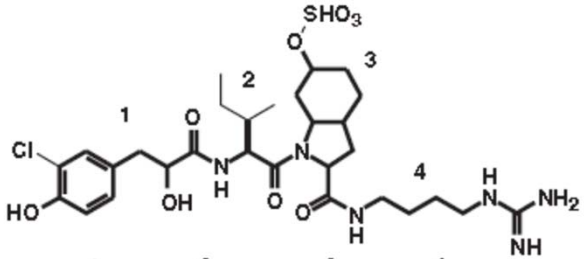
- **hybrid biosynthetic pathways**
- **tailoring domains – methylation, halogenation, transamination, oxygenases etc.**



(Kehr et al. 2011)

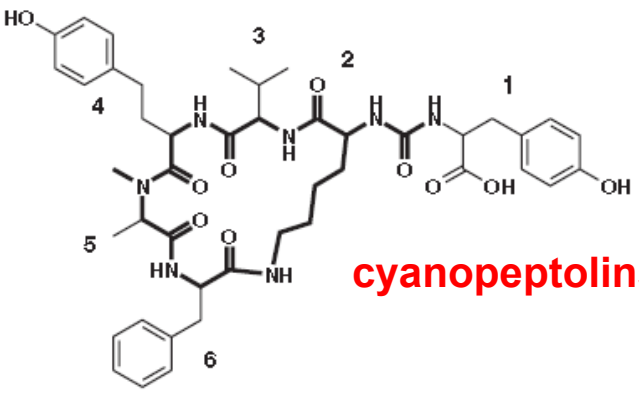
Combinatorial setup results in enormous structural diversity of highly moddified structures

Examples of cyanobacterial peptides



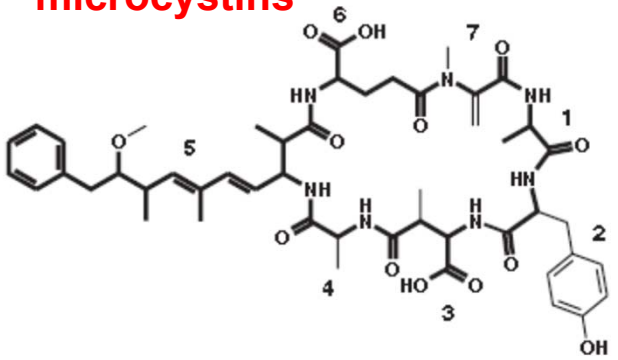
1	2	3	4
*Cl*Hpla	Ile	*Su*Choi	Agmatine
Br	*m*Hty	NH_2	Arg
Su	*Pent*Leu	*Cl*	Aeap
	Tyr		Argininol
			*Ac*Argininal

aeruginosin

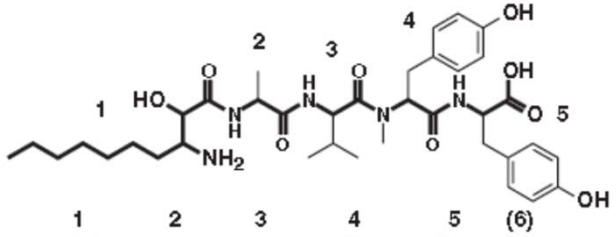


cyanopeptolins

microcystins

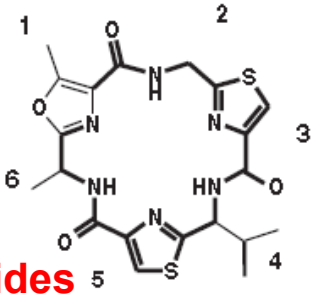


1	2	3	4	5	6	7
Ala	Tyr	*m*Asp	Ala	Adda	Glu	*M*Dha
Leu	Ala		Aba	*Ac*Adda	*m*Glu	*M*Dhb
Ser	Arg		Arg			*M*Ser
	Glu		Glu			
	H_4Tyr		Har			
	Hil		Met			
	Hph		Phe			
	Hty		Tyr			
	Leu		Val			
	Phe					
	Trp					



1	2	3	4	5	(6)
Ahda	Ala	Val	*M*Tyr	Tyr	Tyr
*Cl*Ahda	Ile	*M*Ile	*M*Hty	Pro	
*M*Ahda	Leu	*M*Leu	Pro	Trp	
	Thr	*M*Pro		Hty	
	Tyr				
	Ser				
	Val				

microginin



(nosto)-cyclamides

1	2	3	4	5	6
*dh*Thr	Gly	*dh*Cys	Val	*dh*Cys	Ala
*dh*Ser	Ala		Ala		Phe
	Ile		*m*His		Val
	Val		Ile		
			*O*Met		
			Phe		

Bioactivites associated with cyanobacterial secondary metabolites:

Highly unusual chemical structures predisposes cyanobacterial secondary metabolites to interact with various biological targets. Wide range of bioactivities is reported:

Anti-bacterial

Anti-viral

Anti-fungal

Anti-protozoal

Cytotoxic and cytostatic

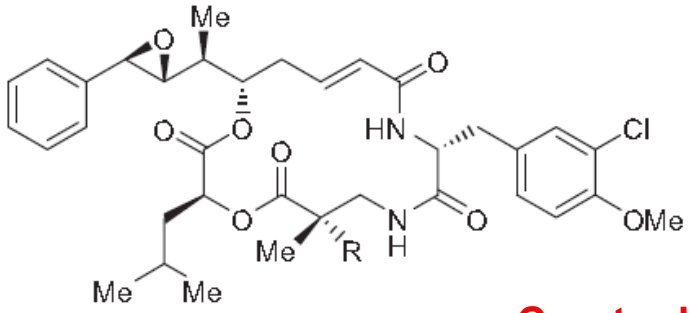
Anti-inflamatory

Imunomodulatory

Very heterogeneous compounds within bioactivity classes

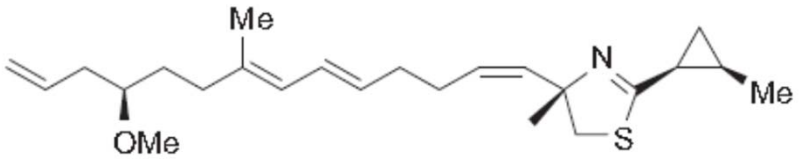
Examples of bioactive secondary metabolites isolated from cyanobacteria

Potential anticancer compounds.

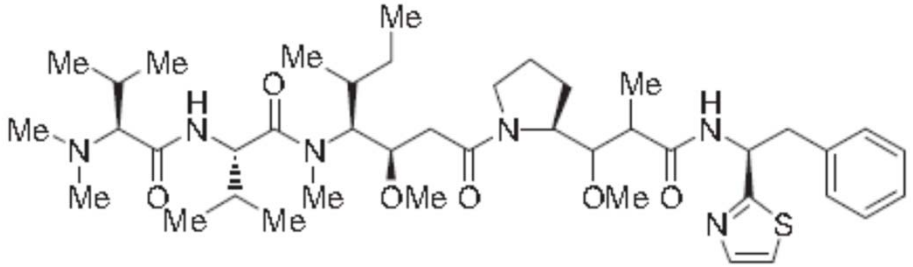


Cryptophycin - (tubulin inhibitor)

Did not passed clinical trials

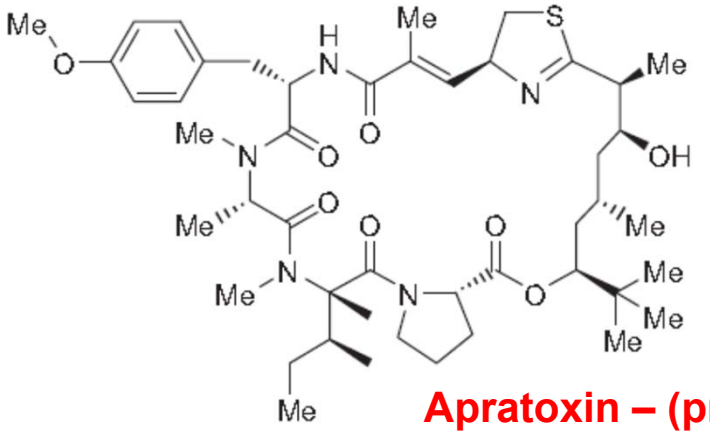


Curacin A - (tubulin inhibitor)



Dolastatin - (tubulin inhibitor)

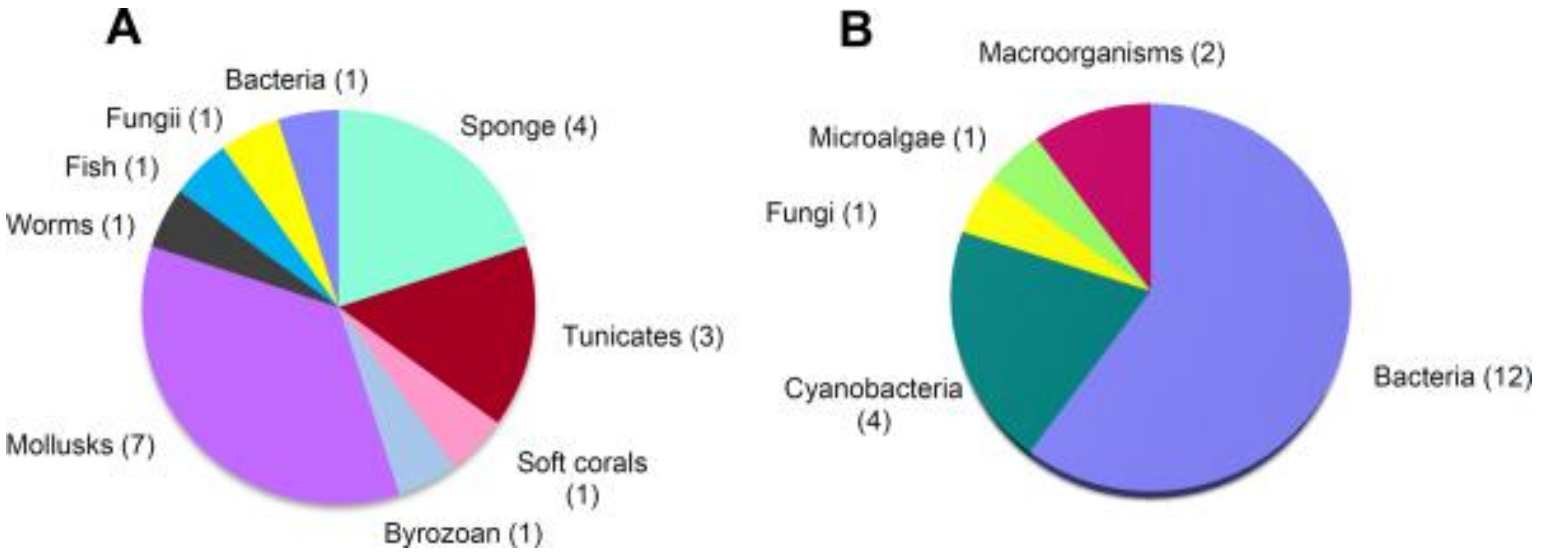
antibody-drug conjugate of a dolastatin derivate approved in 2012 by FDA for Hodkins lymphoma treatment



Apratoxin – (protein traficking in ER)

Anticancer compounds form marine sources in clinical trials

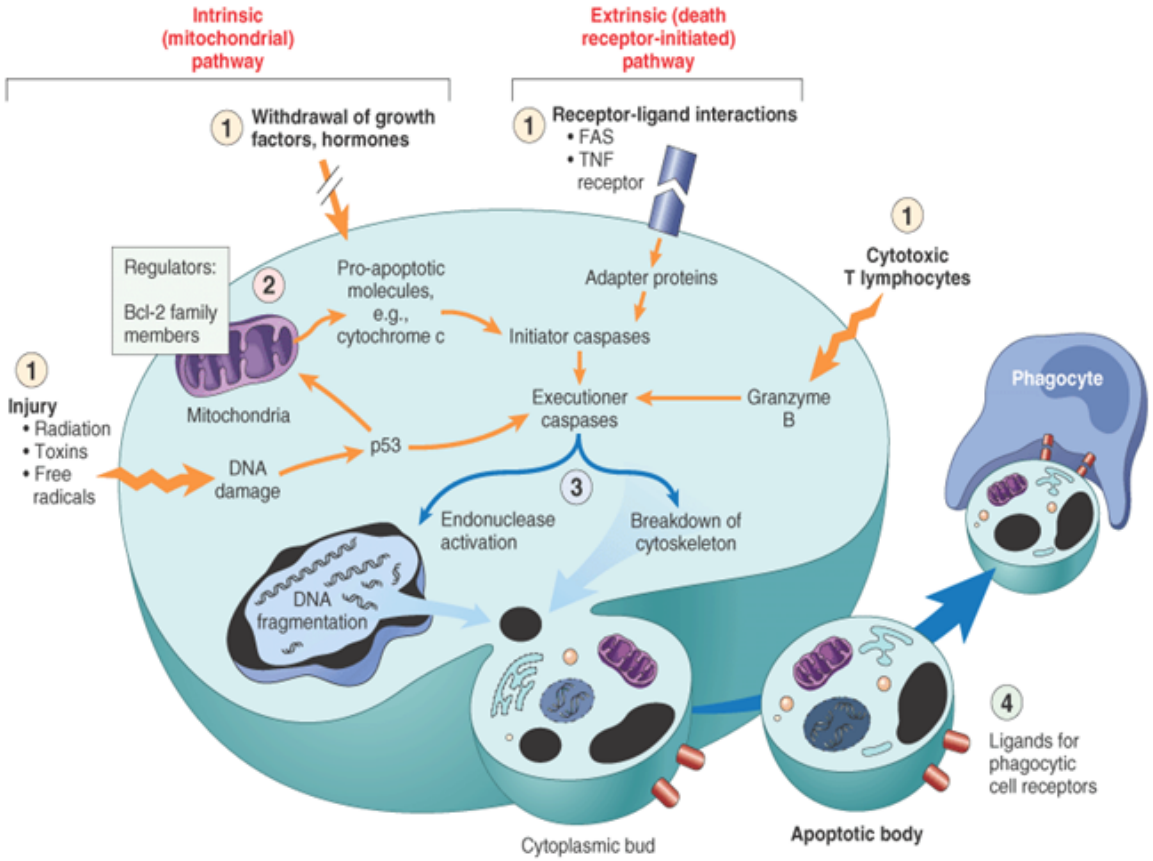
Compounds from marine sources recently in clinical trials (no. 20)

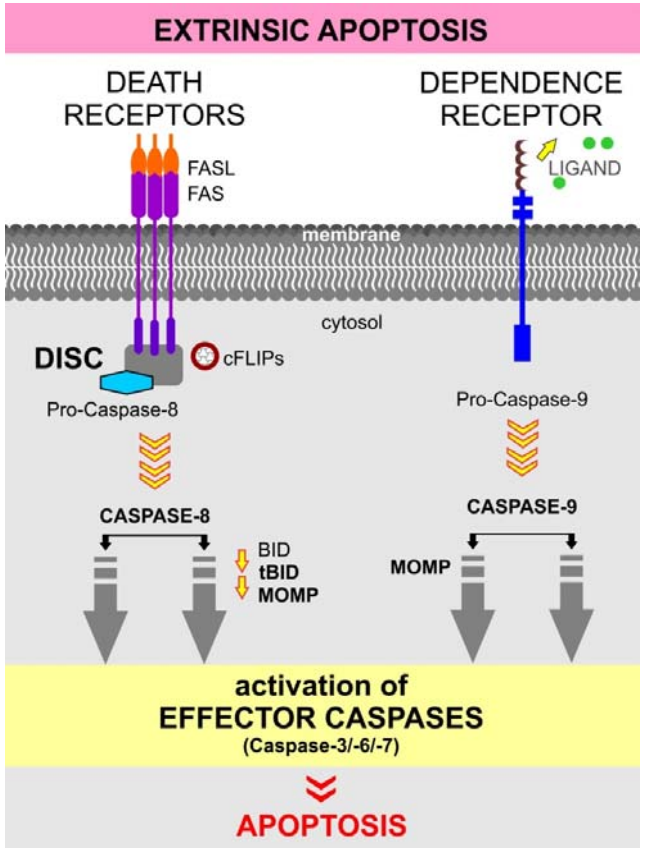


Gerwick & Moore *Chem. Biol.* 2012.

The mode of cell death is crucial for potential pharmacological application

Why to screen for apoptotic inducers?





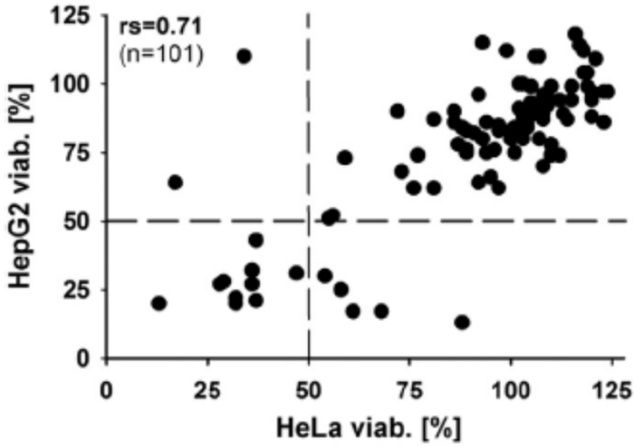
Immunologically silent – prevents inflamation in the tissue

One of the key feature of potential anticance drug – induction of apoptosis

How to screen for apoptotic inducers?

-plethora of chemical bioassays available, not a universal criterion exists

PRIMARY SCREENING (~200 strains)



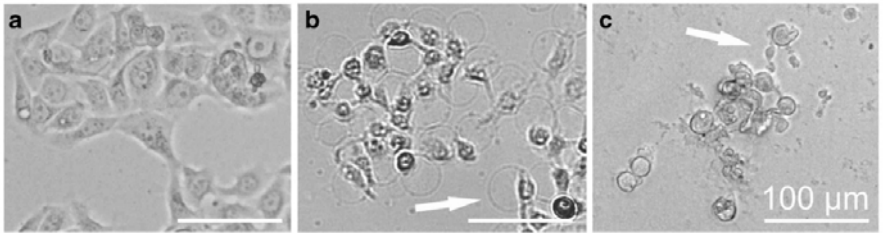
CANDIDATES (50)

24/48 h timepoints

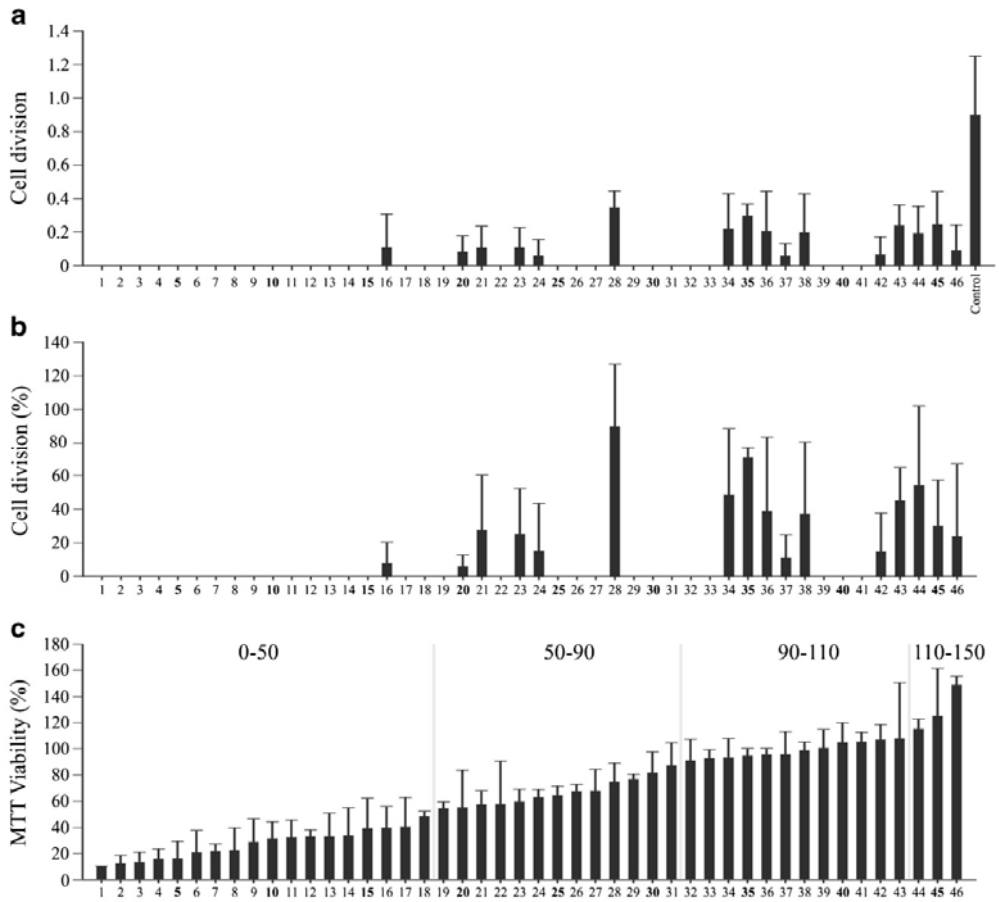
Caspase 3/7 luminiscence assay (96-well plate, end-point measurement)

MTT viability assay

TIME LAPSE MICROSCOPY



Screening for pro-apoptotic inducers

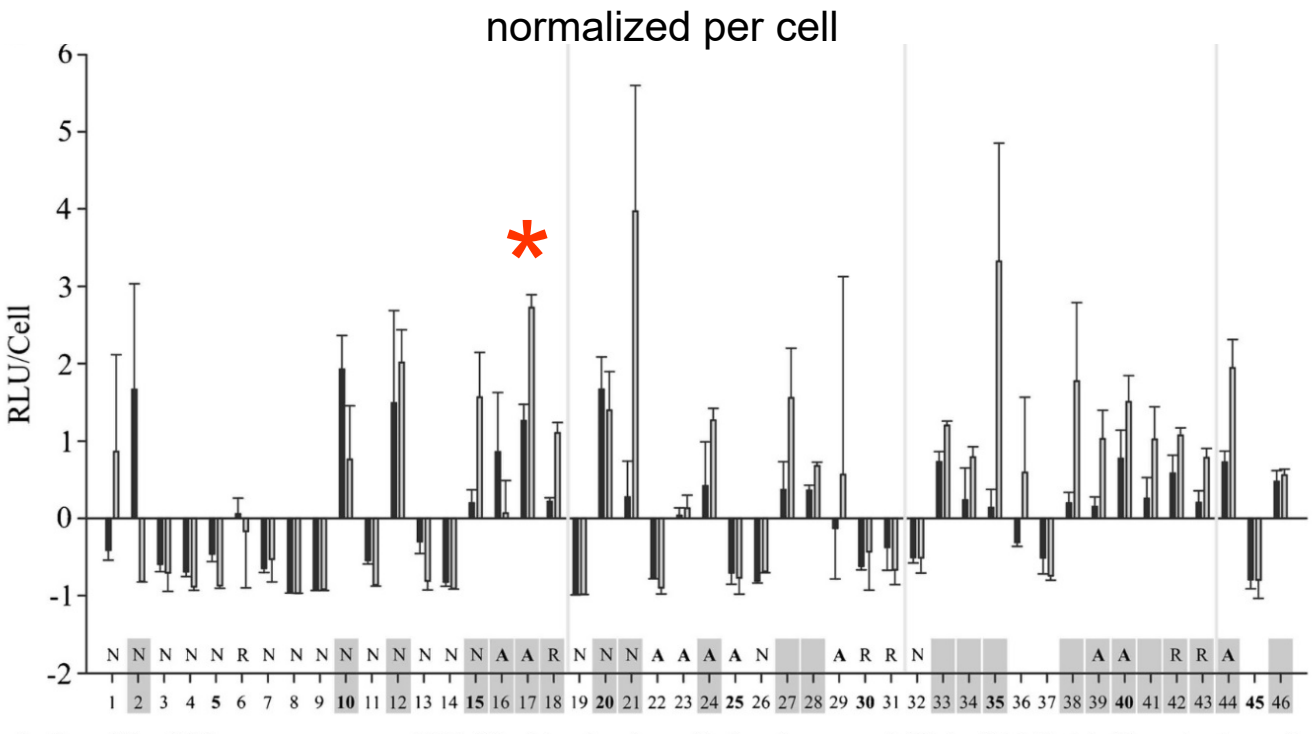


Metabolic activity vs. cell division rates

Majority of extracts affected the cell division to high degree – coctail effects

Screening for pro-apoptotic inducers

Caspase 3/7 assay
(24/48 h)

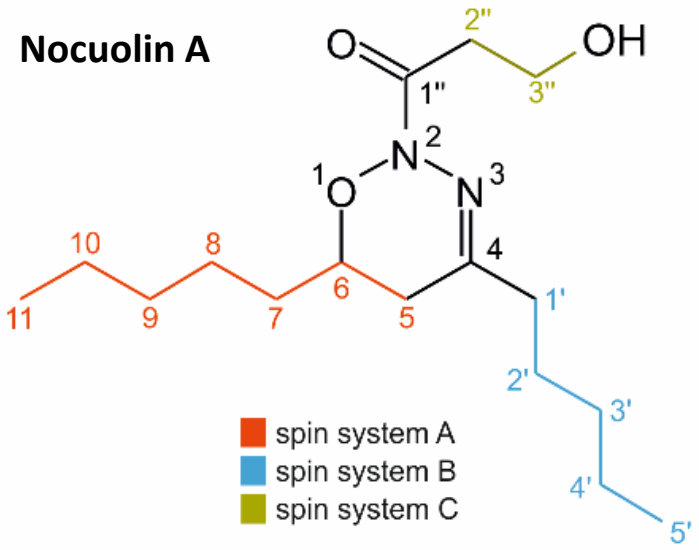


~21 hits

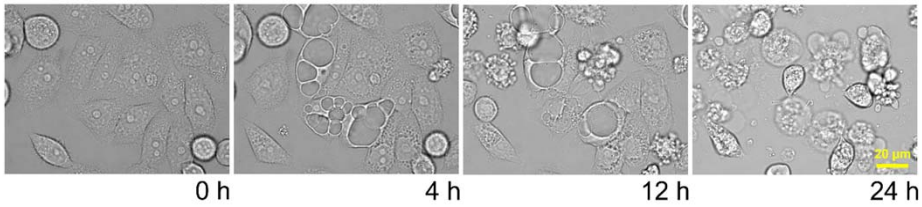
Hunting for potential anticancer compounds with proapoptotic and cytostatic activity in cyanobacteria

Crude cyanobacterial extract → Bioassay – Caspase 3/7 test + time lapse microscopy → Activity guided HPLC fractionation → Compound purrification → HRMS/MS and NMR experiments → Evaluation of compound potency

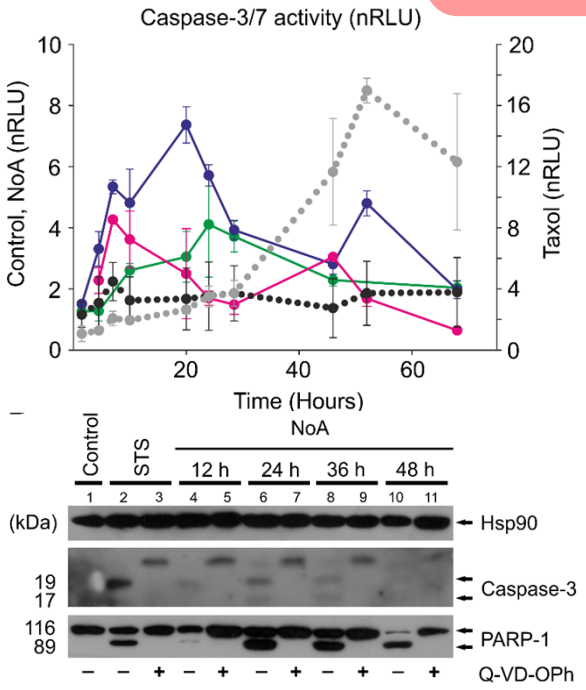
Nocuolin A



Nocuolin A (NoA) established a novel class of bioactive natural metabolites based on oxadiazine heterocycle which was never observed from natural sources before.

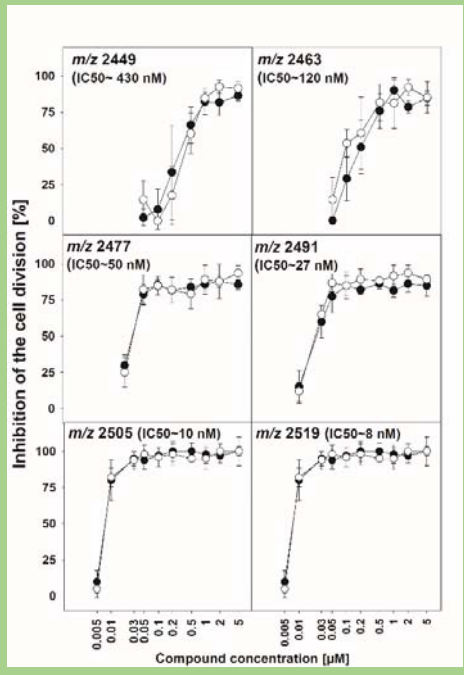


NoA exhibits a potent anti-proliferative activity (IC $_{50}$ 0.7–4.5 µM) against several human cancer lines, with p53-mutated cell lines being even more sensitive than cell lines bearing unchanged p53.



We demonstrated that NoA-induced cell death has attributes of caspase-dependent apoptosis (Caspase 3 activation and PARP cleavage etc.). Since the apoptosis inducers are still the major pilar of anticancer treatment, NoA provides new organic scaffol for SAR studies.

metabolite 2505



The most promissing screening hit – compound 2505 with IC$_{50}$ value of 10 nM is causing cell cycle arrest via novel mechanism of the action

Laboratory summary of the screening for pro-apoptotic inducers

Screening of ~ 200 crude extracts for general cytotoxic activity (Phenotypic screening – microscopy, MTT

Subset of extracts compared with primary human hepatocytes (~50)

Subset of tested of apoptosis inducing activity (~50)= 12 candidate extrats

1 compound showing some degree of selectivity
2 novel candidate proapoptotic scaffolds
3 novel general cytotoxins
2 rediscoveries (scytophycins)
3 profuction compounds during process

Our screening platform provides interesting compounds but with low selectivity and at a small rate

Modern HTS screening in general

Compound libraries ~ 20 000 – 320 000 compounds

~ hits 1 – 20 – (50 ??) in a target screening

90% falls appart within *in vitro, in vivo* tests

Drug (usually moddified scaffold of the original structure)

How to aplly modern approach in extract screening

50 cyanobacterial extracts

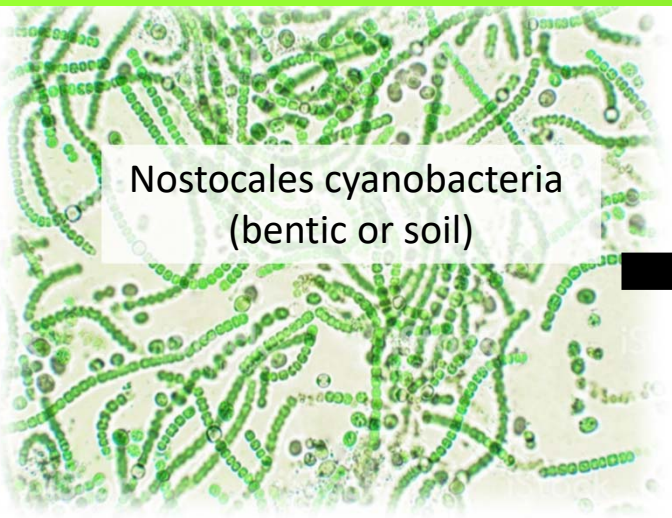
~ 50 extracts x 100 compounds (~ 5 000 tested compounds) including the minor variants

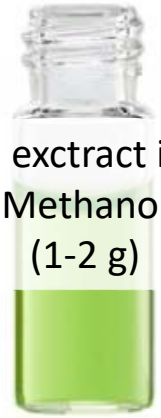
The problem is not the number of compounds tested. But the complexity of the exracts itself (masking of the compound effect)

Simplify the tested sample by fractionation (50 x 40 = 2000 fractions)

HTS

Fraction based screening for cyanobacterial compounds with anticancer activity

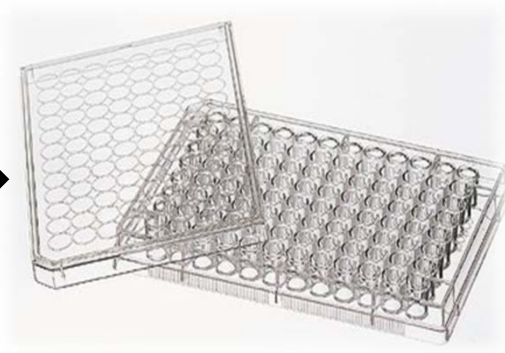




40 fractions

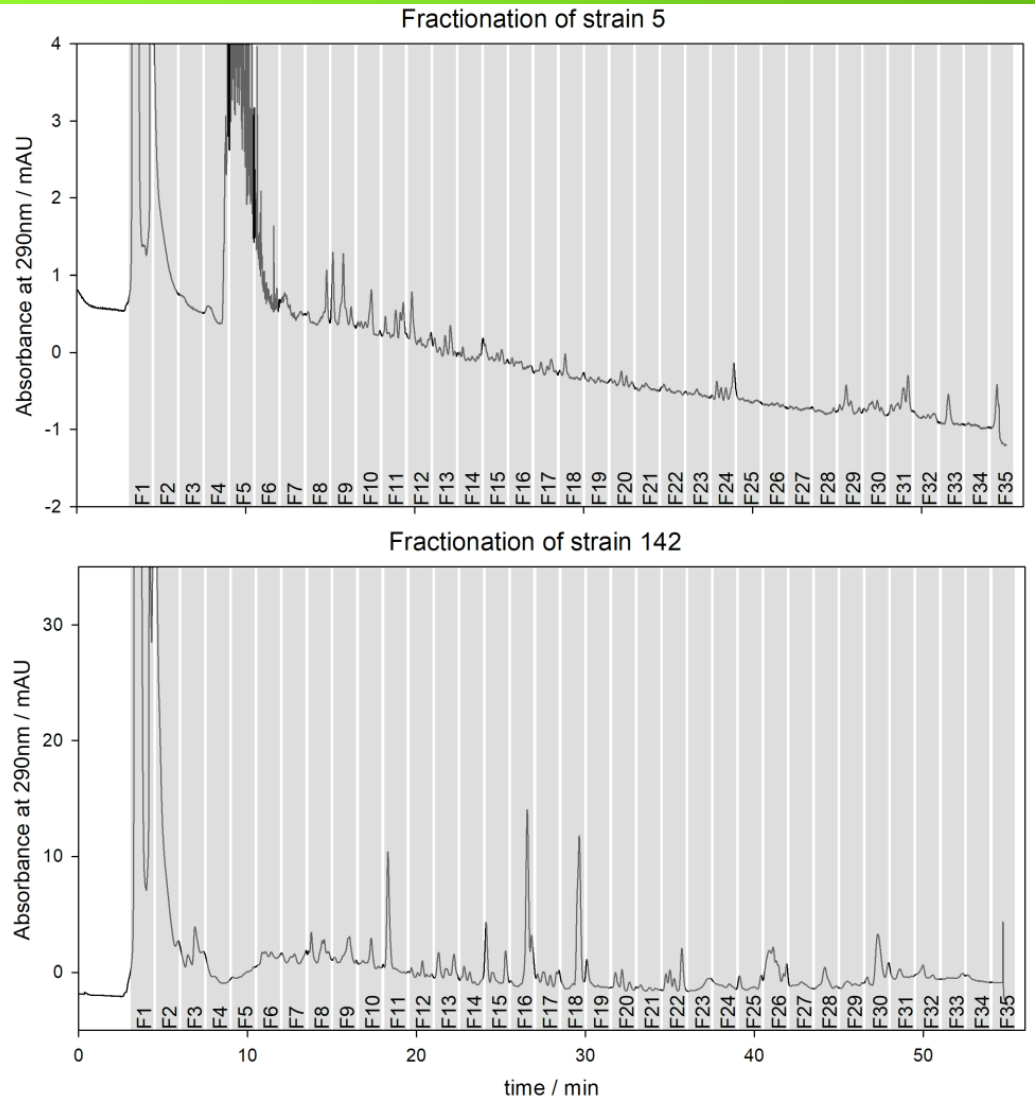
Dried by MiVac

Dilluted in DMSO
(2.5 mg/mL)



Anticancer bioactivity test
MTT

Reduction of the matrix complexity – blind fractionation by HPLC



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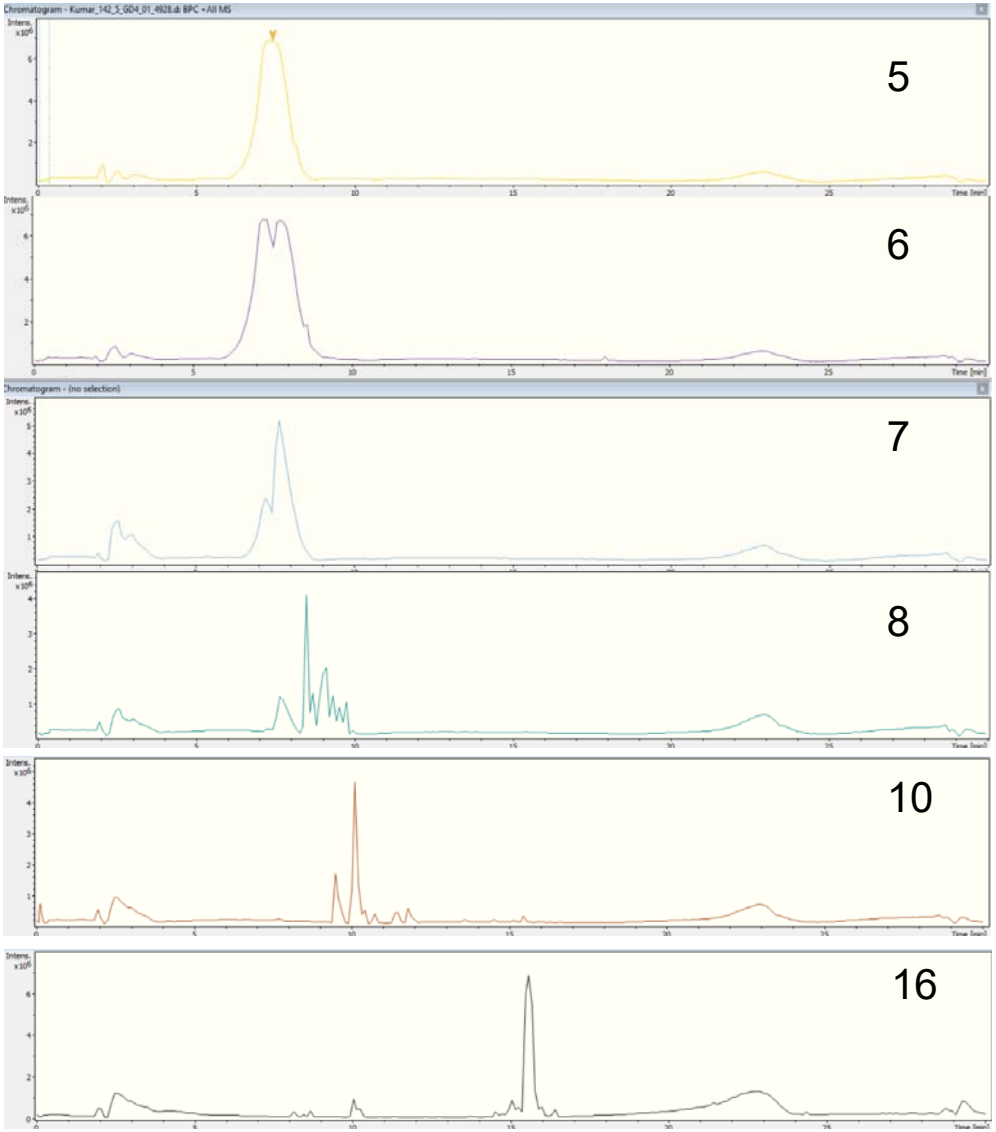
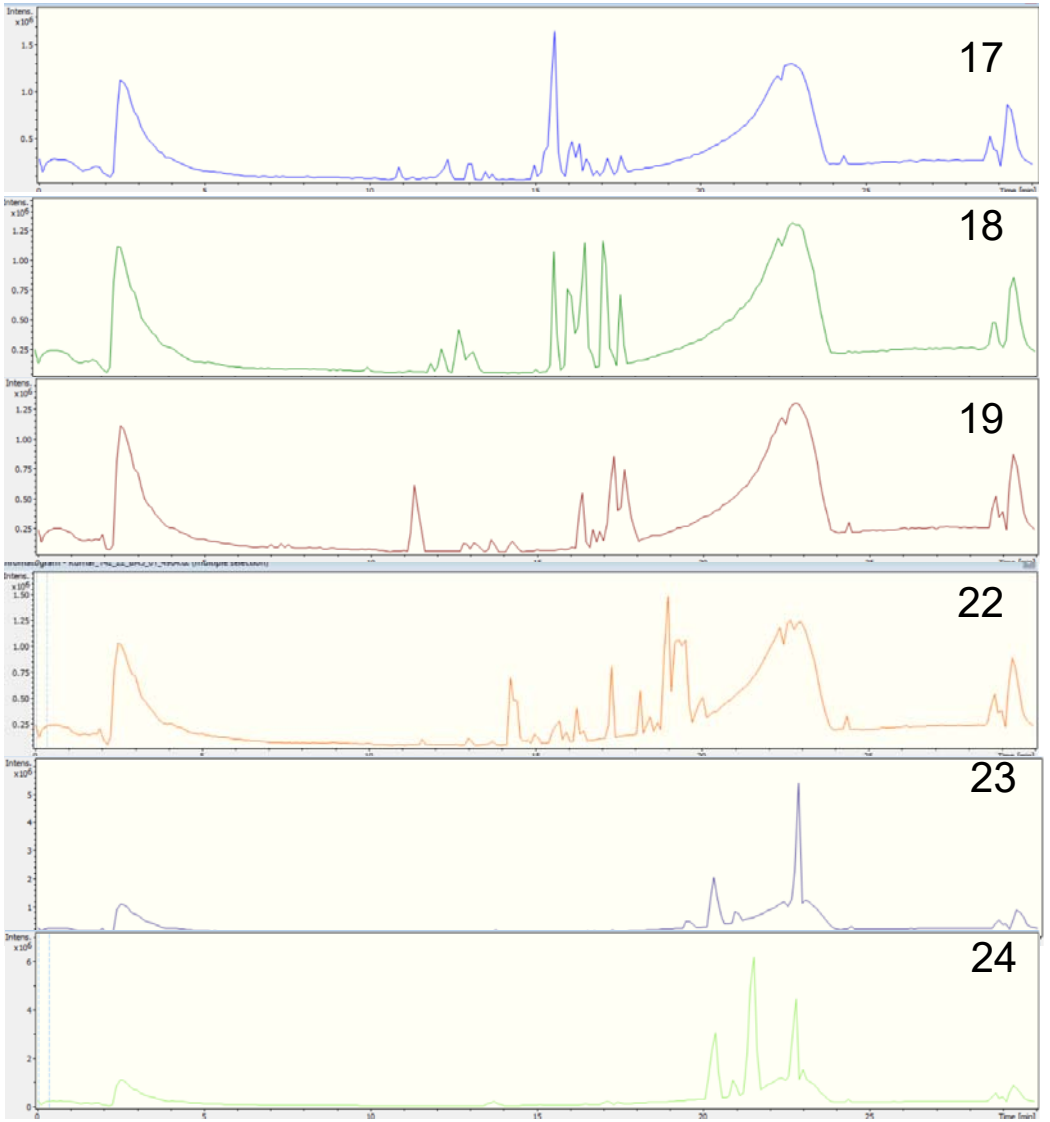
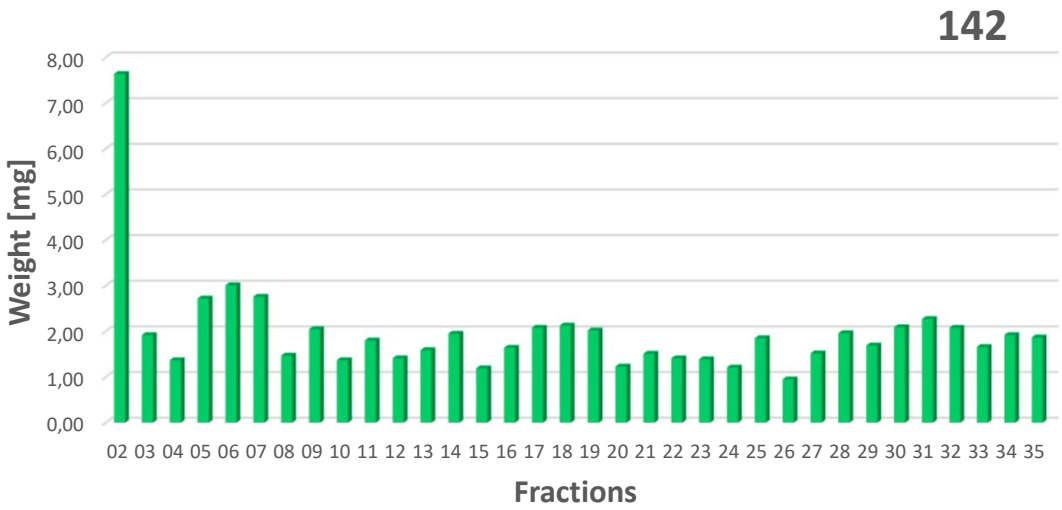
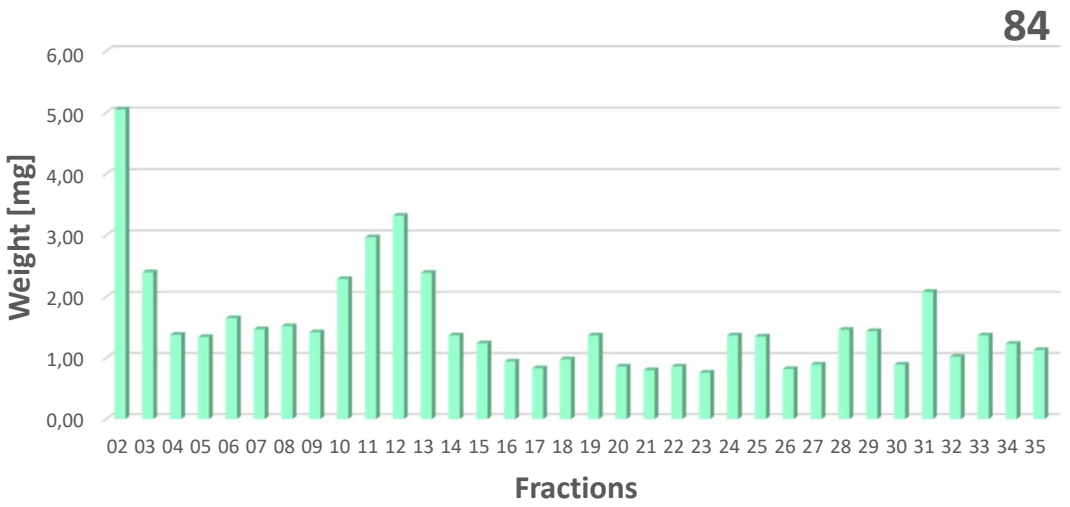
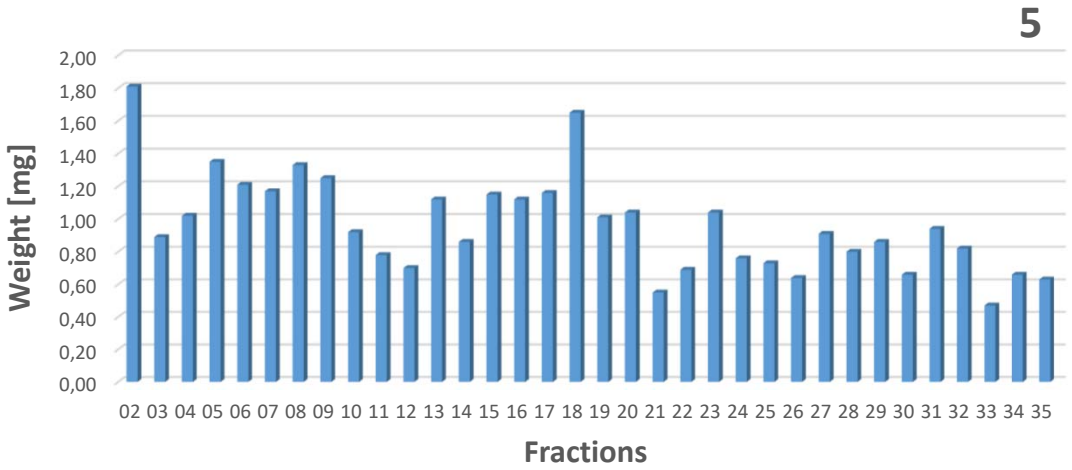


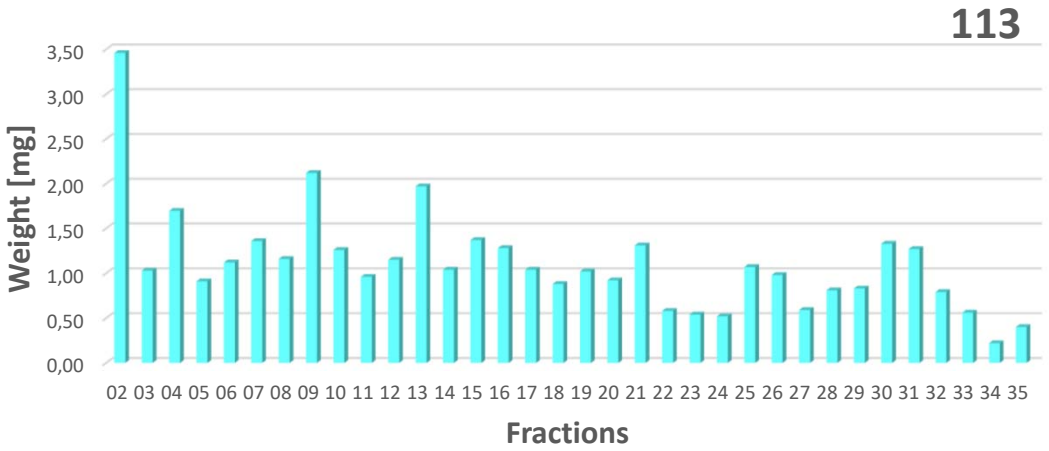


Validation of the fraction weights before HTS









HTS….to be performed

CZ OPENSCREEN
National Infrastructure for Chemical Biology

2000 fraction/3 concentration/20 cancer cell lines = 14-21 days measurement in HTS

Conclusions

Cyanobacteria are rich source of bioactive compounds, tuned to interact with various biological targets. Especially rich are compounds interacting with eucaryotic (incl. human) cells.

Cyanobacterial crude extracts screening for apoptosis induction is a convenient approach to get sufficient amount of hits for characterization of their activity.

Important limitation:

- coctail (synergystic, antagonic) effect of the extract constituents

- unknown concentration - mode of action of extract constituents

- problematic selection of time of endpoint measurrements

-frequent inhibition of the cell division

-Caspase activity detection in combination with time-lapse microscopy can prevent some of these limitation. By this approach we got enough positive hits for future compound

- Fraction base HTS-screening migth be a solution to overcome the complexity of the cyanobacterial extracts and obtain more specific hits in a reasonable time

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Evropská unie
Evropský fond
pro regionální rozvoj

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