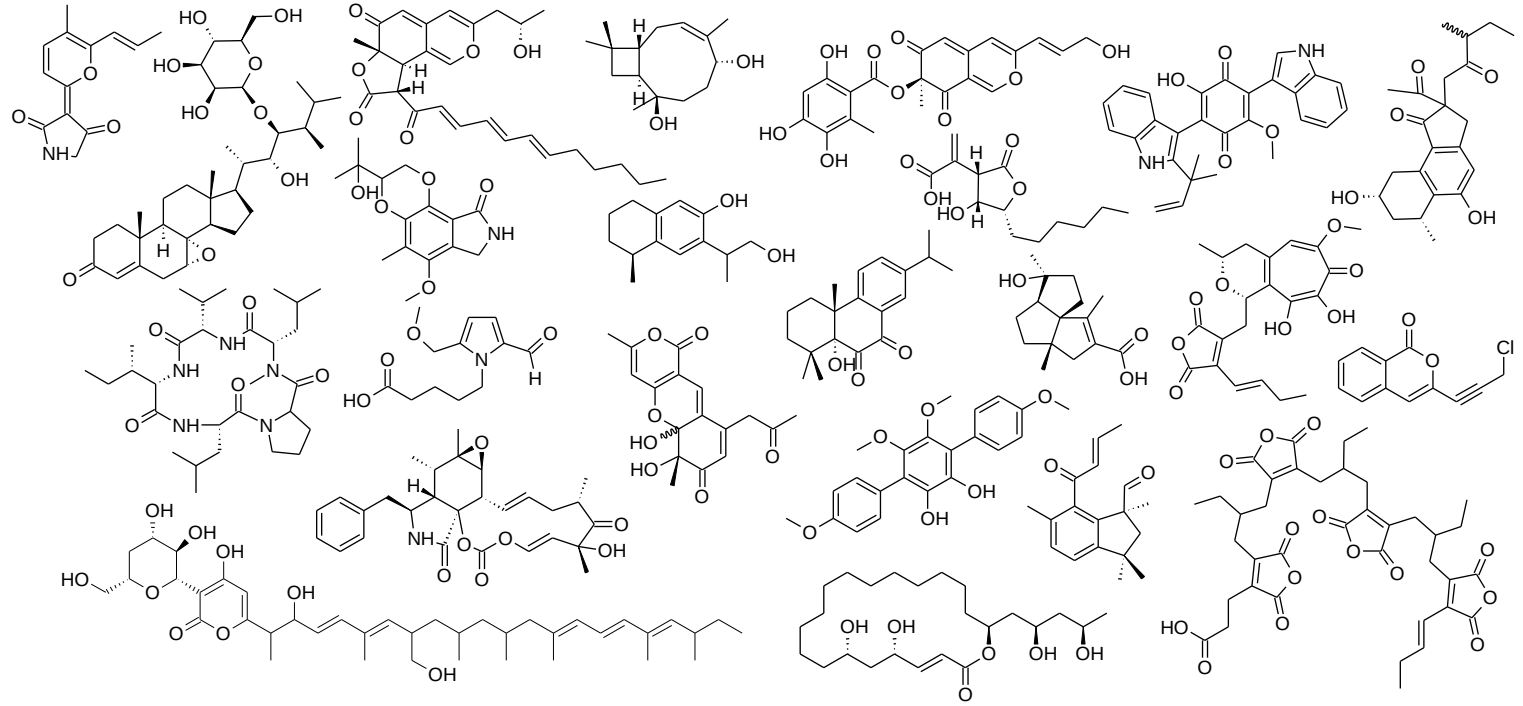
Current Natural Product Approaches in Drug Discovery



Frank Surup et al.

Helmholtz Center for Infection Research, German Center for Infection Research

19th of April, 2024; Cape Town

frank.surup@helmholtz-hzi.de

HZI HELMHOLTZ Zentrum für Infektionsforschung

Natural products in drug development

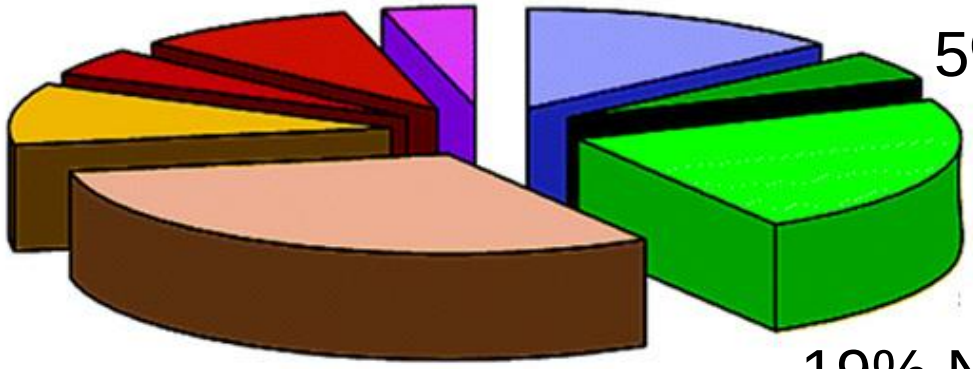
1981-2019: 1881 new drugs approved

11% pharmacophore NP; natural product mimic

8% Vaccines

18% “Biological“ (peptide/protein)

3% synthetic; pharmacophore NP



5% Natural product

12% Natural product mimic

19% Natural product derived

25% Synthetic

50% based upon natural products

Newman DJ, Cragg GM. Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. *J. Nat. Prod.* **2020**, *83*, 770–803.

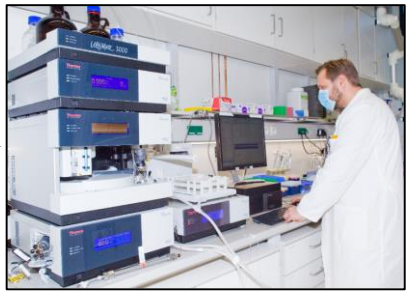
Identification of new lead structures

Fieldwork

Strain isolation

Fermentation

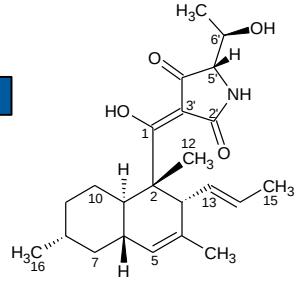
Extraction



Activity screening

Large scale fermentation

Isolation of Natural products



Structure Elucidation

Optimization

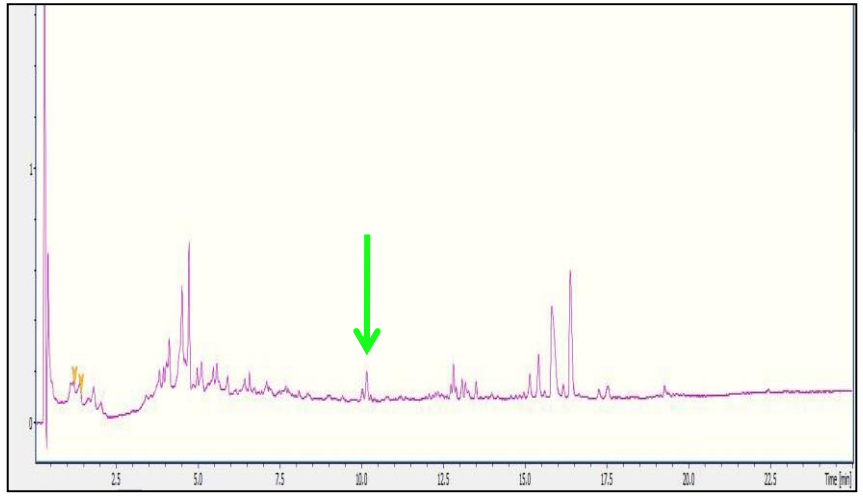
Activity testing

Total synthesis

Disciformycins from *Pyxidicoccus fallax*

- Rescreening of myxobacterial strain collection (8500 strains)
- *P. fallax* AndGT8: Activity against Gram positive bacteria

Chemical Screening (HPLC-MS):
retention time, UV, MS

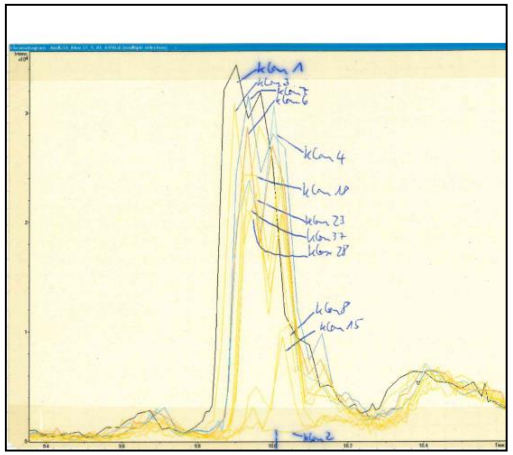


Biological screening:
Inhibition pattern of test strains

- Identification of a „hit“

Disciformycins – Workflow structure elucidation

- Fermentation in 10L scale
- Bioassay-guided fractionation by preparative HPLC
- HRESIMS: → molecular formular: $C_{27}H_{40}O_{10}$
  → number of unsaturations: 7
- Dereplication → unknown metabolite
- Media optimization
- Selection of single cells
- Fermentation in 70L scale



Surup F, Viehrig K, Mohr KI, Herrmann J, Jansen R, Müller R,
Angew. Chem. Int. Ed. **2014**, *53*, 13588–13591.

Disciformycins – Workflow structure elucidation

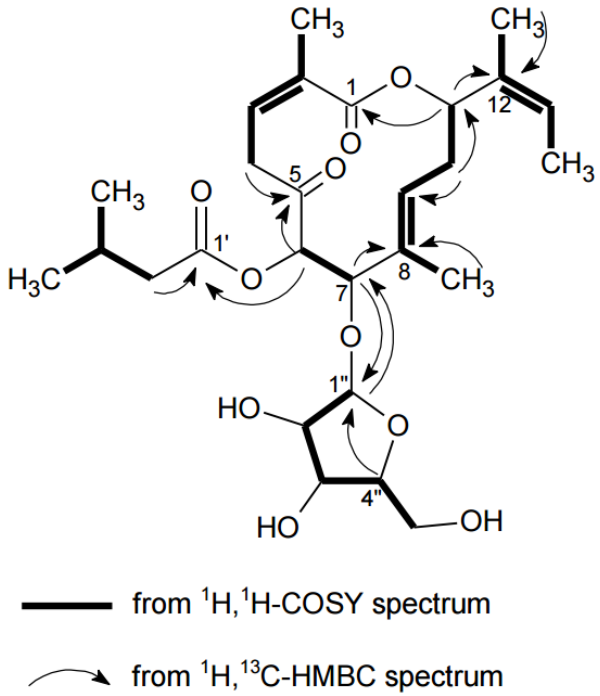
- Structure elucidation is like putting together a puzzle

- HRESIMS: → Molecular formula: $C_{27}H_{40}O_{10}$
  → number of unsaturations: 7

- NMR:

<u>I Determining the connectivity</u>

a) ^{1}H, ^{13}C: functional groups
b) HSQC: connectivity H - C
c) COSY: build chains of carbons
d) HMBC: connect chains & quaternary C



Surup F, Viehrig K, Mohr KI, Herrmann J, Jansen R, Müller R, *Angew. Chem. Int. Ed.* **2014**, *53*, 13588–13591.

Disciformycins – Workflow structure elucidation

II Relative configuration

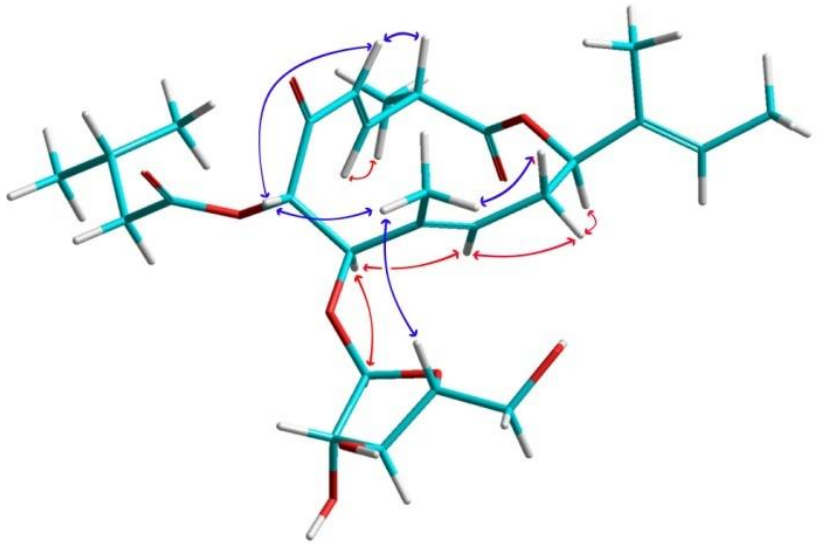
J-based configurational analysis:
ROESY correlations, coupling constants

Identification of the sugar moiety
by chemical shift analysis

III Absolute configuration:

Degradation reaction to release the sugar

GCMC comparison with
authentic standards

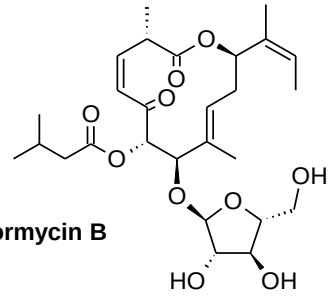


MM+ model of disciformycin B

Surup F, Viehrig K, Mohr KI, Herrmann J, Jansen R, Müller R,
Angew. Chem. Int. Ed. **2014**, *53*, 13588–13591.

Disciformycins – Antibacterial activity

	DscA	DscB	VAN
Bacillus subtilis DSM-10	4.2	0.83	0.25
Paenibacillus polymyxa DSM-36	16.6	16.6	n.d.
Staphylococcus aureus DSM-346	16.6	3.3	0.5
Staphylococcus aureus Newman	8.0	1.2	0.5
***Staphylococcus aureus* DSM-11822 (MRSA)**	4.0	0.6	1.0
***Staphylococcus aureus* N315 (MRSA)**	8.0	1.2	1.0
***Staphylococcus aureus* Mu50 (MRSA/VISA)**	2.0	0.6	16.0
Staphylococcus carnosus DSM-20501	7.8	2.4	0.25
Mycobacterium sp. DSM-43270	> 64	> 64	1.7
Mycobacterium diernhoferi DSM-43524	33.3	33.3	3.3
Micrococcus luteus DSM-20030	67	> 64	0.13
Nocardioides simplex DSM-20130	33.3	16.6	0.42



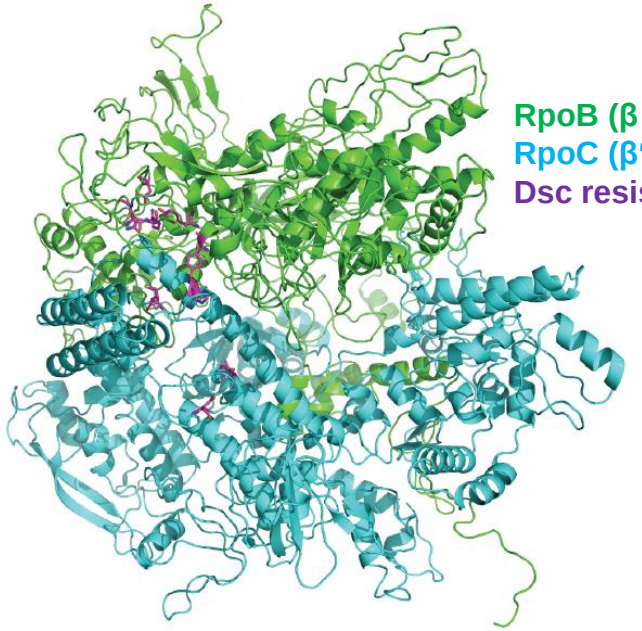
Disciformycin B

- No cross-resistance with methicillin, vancomycin (erythromycin, quinolones)
- No activity against GN, Yeast/Fungi, Mammalian cell lines

Surup F, Steinmetz H, Mohr K, Viehrig K, Müller R, Nett M, Schiefendecker S, Dahse HM, Wolling M, Kirschning A, Novel macrolide antibiotics, **WO 2016/005049 A1**.

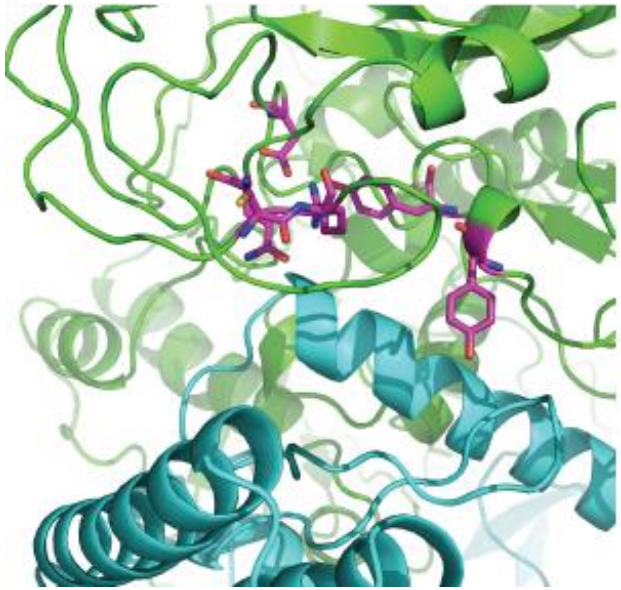
Disciformycins - Binding site on RNAP

***S. aureus* N315 RNAP (homology model)**
template: *E. coli* RNAP (PDB id 4IGC)



RpoB (β subunit)
RpoC (β‘ subunit)
Dsc resistance mutations

Dsc binding pocket (docking)



higher calculated average binding energies for resistant mutants compared to WT (-7.3 kcal/mol vs. -8.6 kcal/ml)

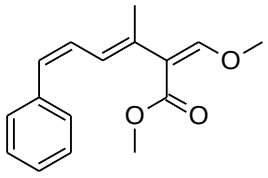
- no cross-resistance with RNAP inhibitors
- Increase of production rate nedeed

Biological data: Herrmann J, Müller R.

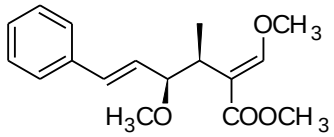
Basidiomycota

- about 35,000 fruiting body-forming species
- > 95% of species unknown
- relatively few are studied for their secondary metabolites (SM)

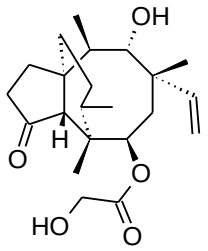
Hericium erinaceus



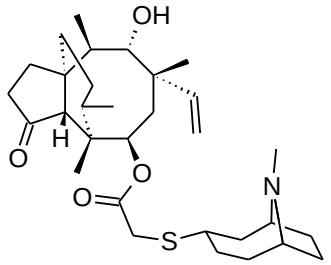
Strobilurin A



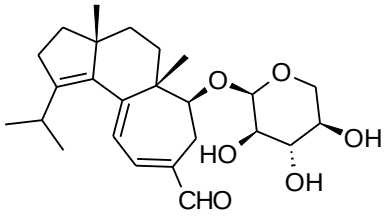
Oudemansin A



Pleuromutilin

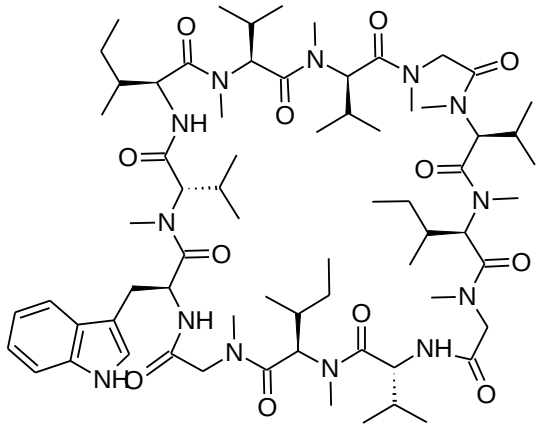


Retapamulin

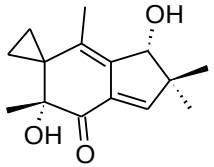


Erinacine A

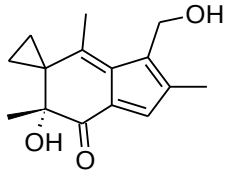
Omphalotus illudens



Omphalotin A



Illudin M



Irofulven

Clitopilus passeckerianus

Rhodotus palmatus

- even German forests harbour basidiomycetes untapped for secondary metabolites

  - pioneer fungus on rotting hardwoods (elm)
  - circumboreal distribution, endangered
  - antimicrobial activity

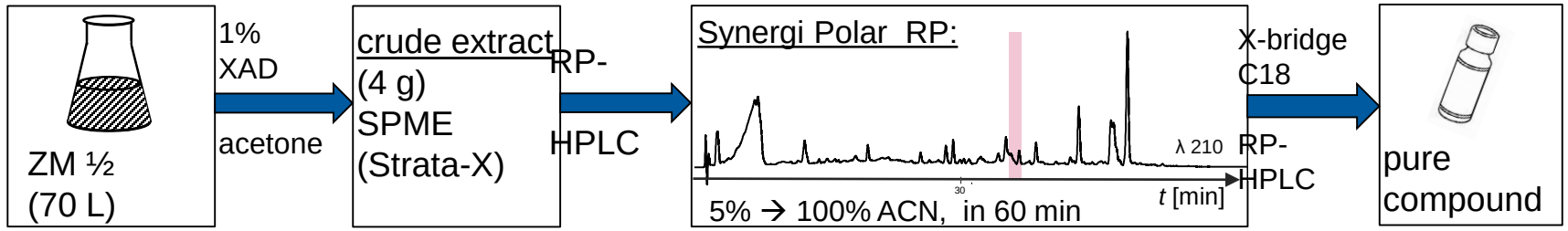
R. palmatus

Elm forest

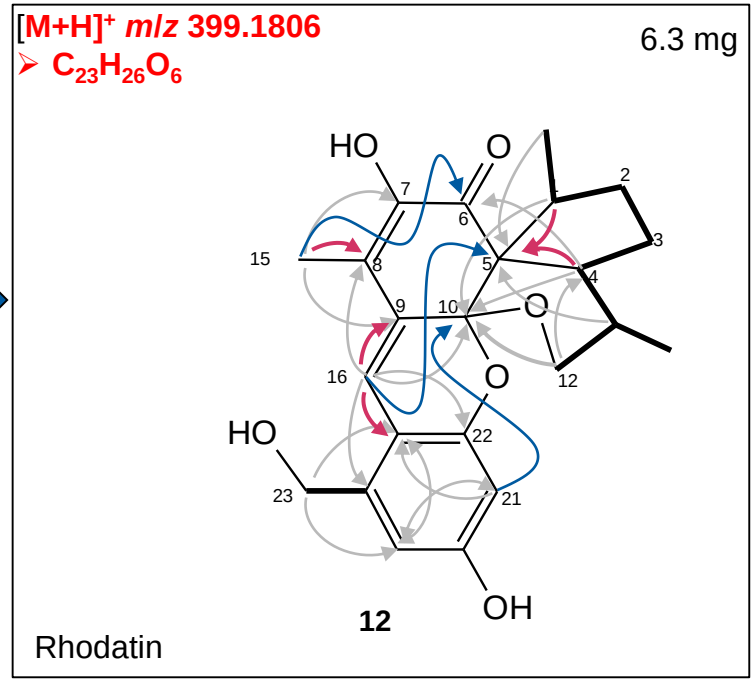
- Chemical Screening approach
- 12 novel secondary metabolites

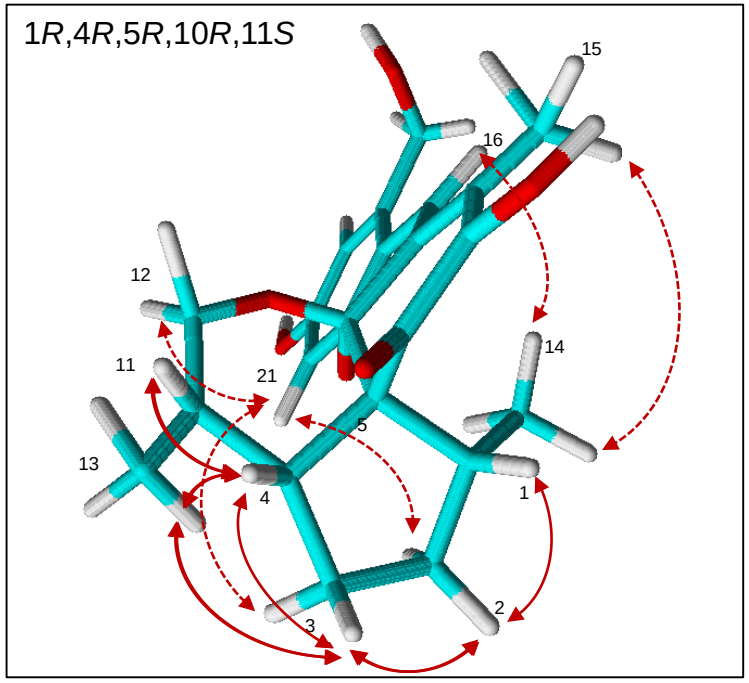
Sandargo B, Michehl M, Praditya D, Steinmann E, Stadler M, **Surup F**. *Org. Lett.* **2019**, *21*, 3286–3289; Sandargo B, Michehl M, Stadler M, **Surup F**. *J. Nat. Prod.* **2020**, *83*, 720–724.

Rhodatin & Rhodocoranes – SM from a neglected fungus



MS / NMR



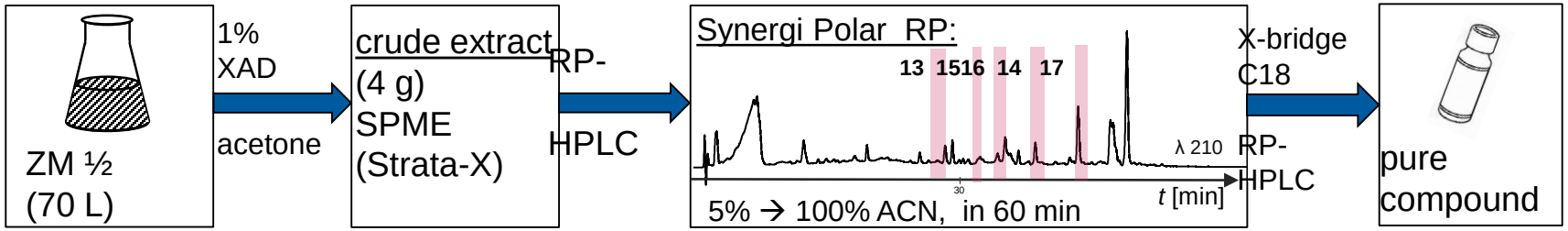


COSY
1,1 ADEQUATE
HMBC (8 Hz)
HMBC (2 Hz)

ROESY (α)
ROESY (β)

1) Dereplication
2) Constitution
3) Relative configuration
4) Absolute configuration

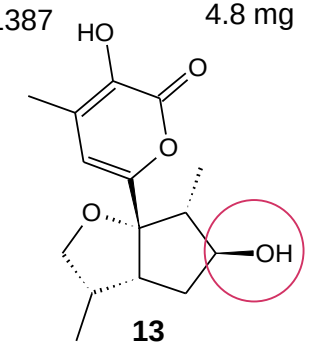
Rhodatin & Rhodocoranes – SM from a neglected fungus



MS / NMR

[M+H]⁺ *m/z* 281.1387 — 4.8 mg

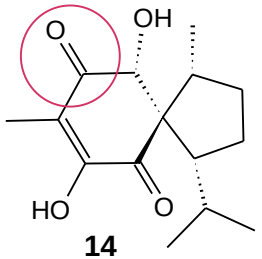
➢ $C_{15}H_{20}O_5$



Rhodocorane A **13**

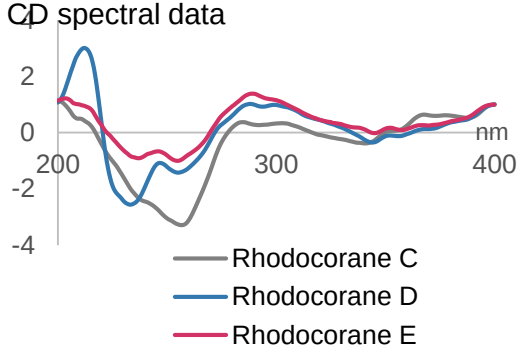
[M+H]⁺ *m/z* 249.1483 — 7.4 mg

➢ $C_{15}H_{22}O_4$



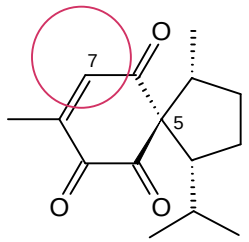
Rhodocorane B **14**

CD spectral data



[M+H]⁺ *m/z* 249.1486 — 2.0 mg

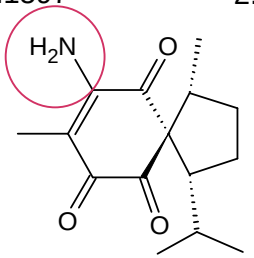
➢ $C_{15}H_{20}O_3$



Rhodocorane C **15**

[M+H]⁺ *m/z* 264.1597 — 2.5 mg

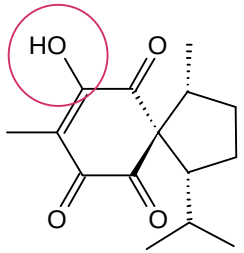
➢ $C_{15}H_{21}NO_3$



Rhodocorane D **16**

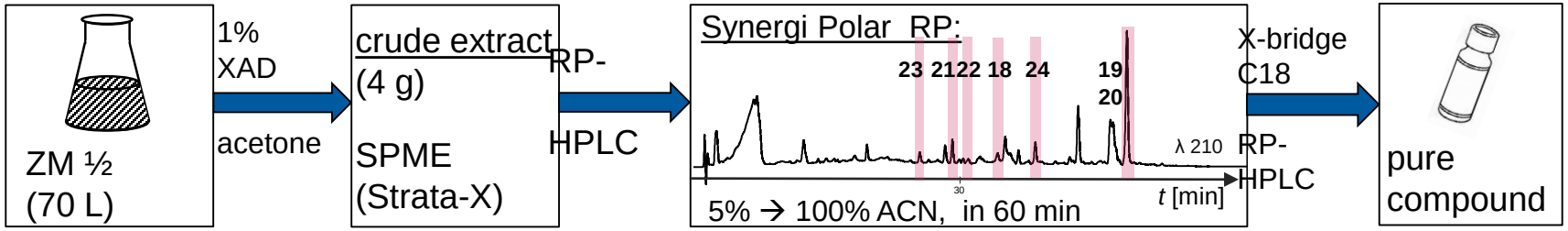
[M+H]⁺ *m/z* 265.1435 — 7.7 mg

➢ $C_{15}H_{20}O_4$



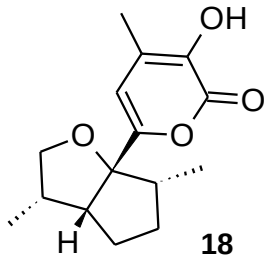
Rhodocorane E **17**

Rhodatin & Rhodocoranes – SM from a neglected fungus



MS / NMR

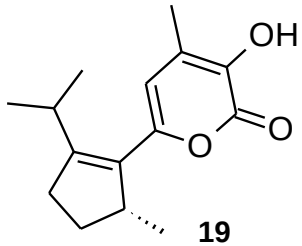
[M+H]⁺ *m/z* 265.1433 — 4.0 mg
- $C_{15}H_{20}O_4$



18

Rhodocorane F

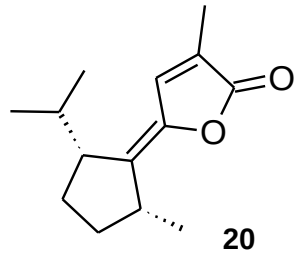
[M+H]⁺ *m/z* 249.1486 — 0.5 mg
- $C_{15}H_{20}O_3$



19

Rhodocorane G

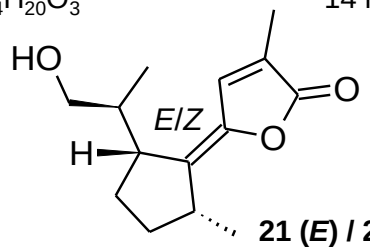
[M+H]⁺ *m/z* 221.1532 — 10 mg
- $C_{14}H_{20}O_2$



20

Rhodocorane H

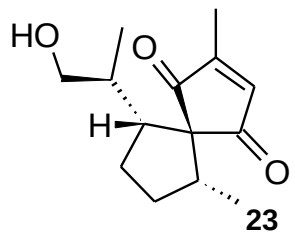
[M+H]⁺ *m/z* 237.1485 — 4.0 mg (*E*), 14 mg (*Z*)
- $C_{14}H_{20}O_3$



21 (*E*) / 22 (*Z*)

Rhodocorane I (*E*) / J (*Z*)

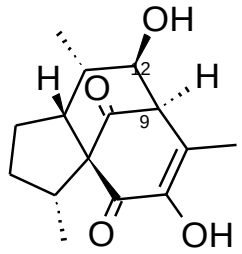
[M+H]⁺ *m/z* 237.1483 — 5.0 mg
- $C_{14}H_{20}O_3$



23

Rhodocorane K

[M+H]⁺ *m/z* 265.1436 — 2.9 mg
- $C_{15}H_{20}O_4$

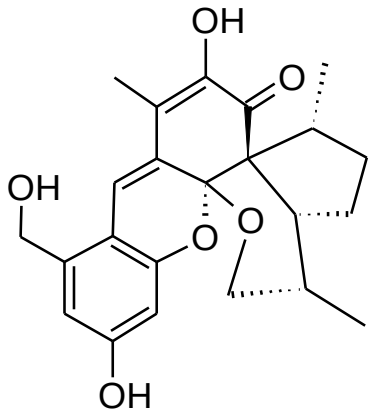


24

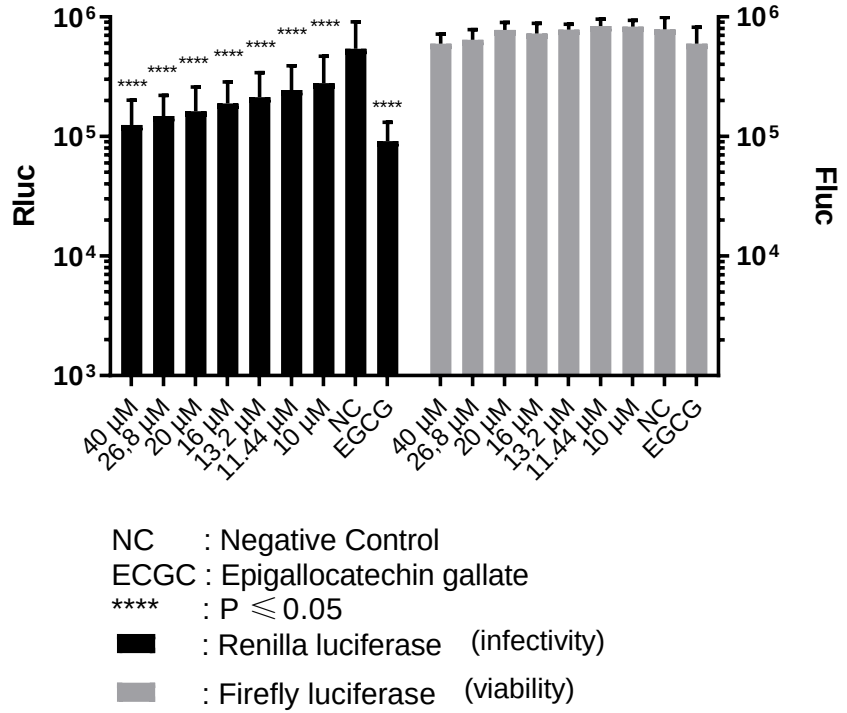
Rhodocorane L

Rhodatin & Rhodocoranes – SM from a neglected fungus

- Chemical Screening approach
- 12 novel secondary metabolites
- antiviral (HCV) activity of rhodatin



rhodatin



Sandargo B, Michehl M, Praditya D, Steinmann E, Stadler M, **Surup F**. *Org. Lett.* **2019**, *21*, 3286–3289;
Sandargo B, Michehl M, Stadler M, **Surup F**. *J. Nat. Prod.* **2020**, *83*, 720–724.

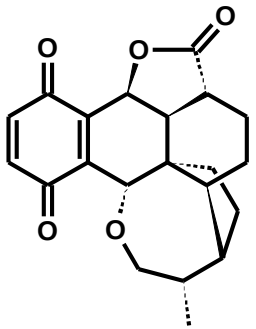
Impressions from field work in Thailand

Post IMC10 Foray, Mushroom Research Centre, Chiang Mai Prov., Thailand (2014)

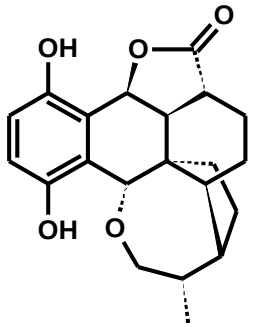
HOHENBUEHELIA – Nematode trapping fungi

Basidiomycete from Northern Thailand, identified as *Hohenbuehelia grisea*

- Parasitic and/or parasitoid
- Nematode-trapping with adhesive knobs, nematotoxins
- strong antibiotic activities in the culture



Pleurotin

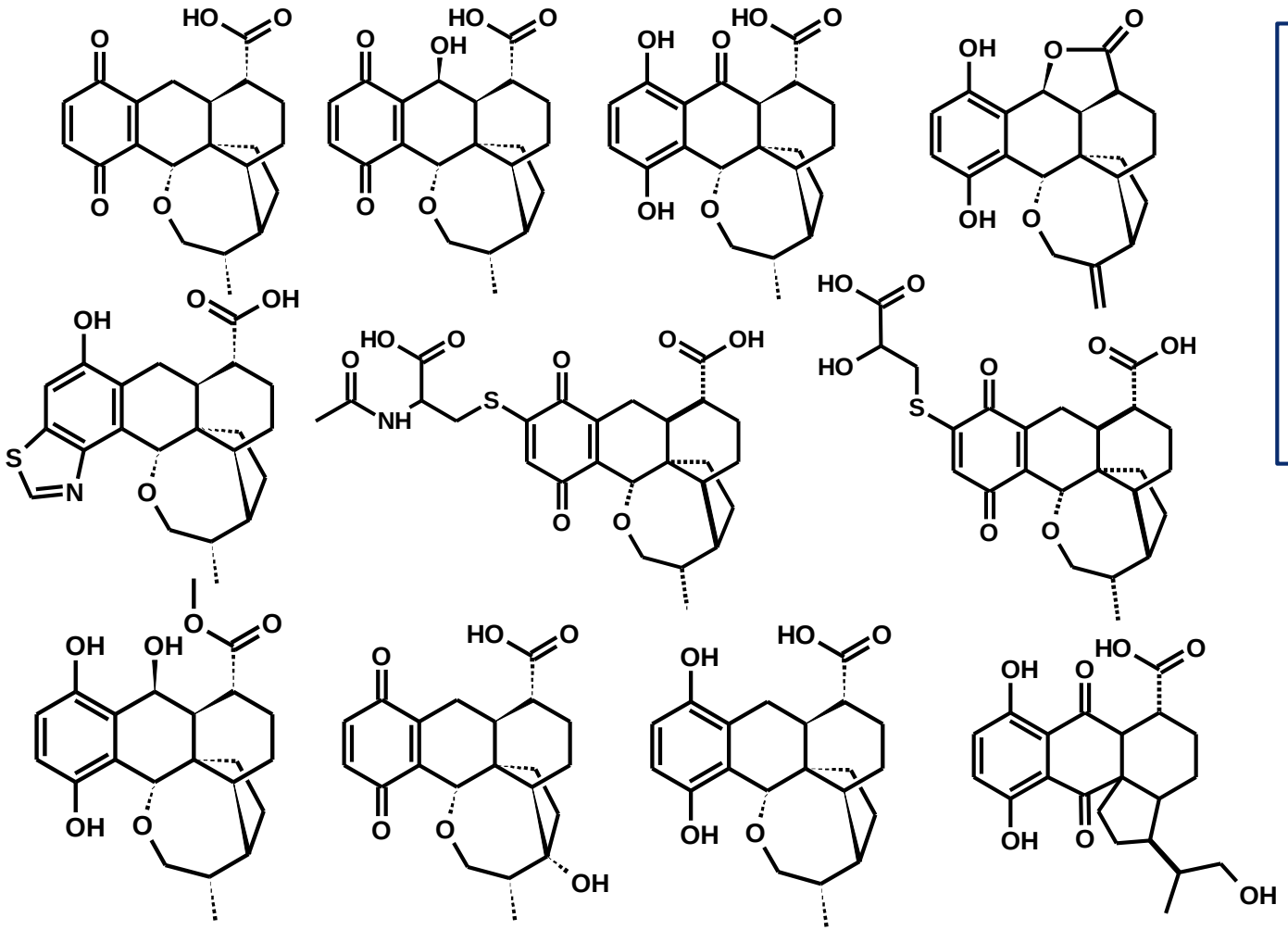


Dihydropleurotin

(Photo: credit to Benjarong Thongbai)

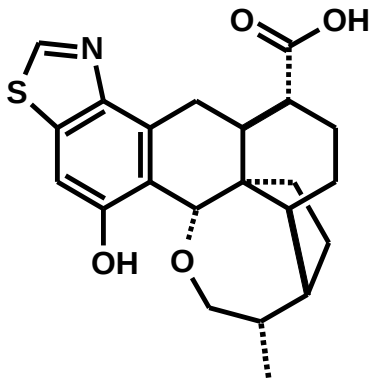
New pleurotin derivatives

Scale up and exhaustive studies of its bioactive metabolites



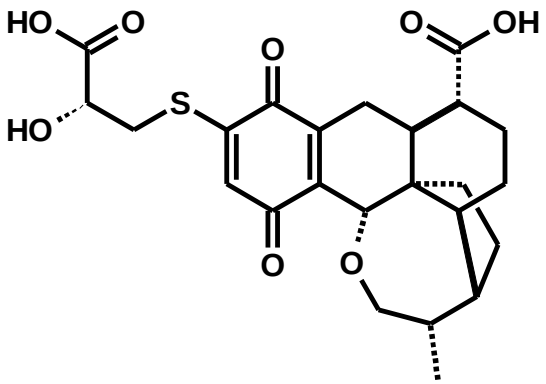
Some compounds known from PhD theses:
Schelling 1969
Vogt 1982
Erb 1986
Capaul 1992
Kaufmann 1996

New cystein-derived pleurotin derivatives



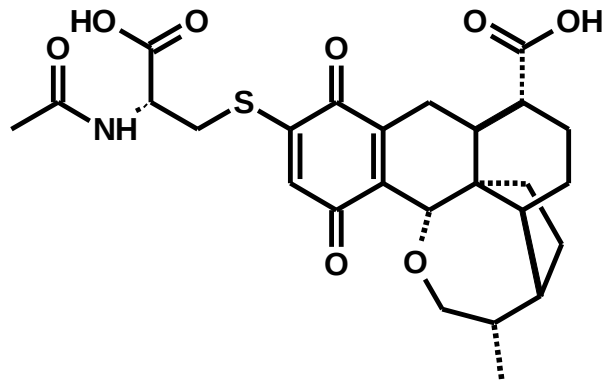
pleurothiazole

Candida tenuis 100
P. anomala 66.7
Rhodot. glutinis 33.3
B. subtilis 100



thiopleurotinic acid A

No bioactivity



thiopleurotinic acid B

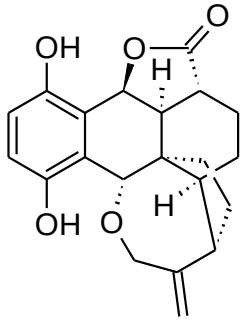
C. Tenuis 100
P. anomala 66.7
Rhodot. 33.3

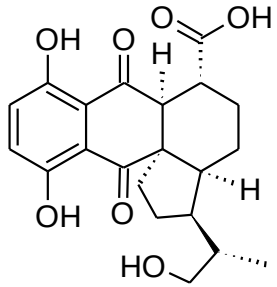
Glutathione detoxification in filamentous fungi

Sandargo B, Thongbai B, Stadler M, Surup F, *J. Nat. Prod.* **2018**, *81*, 286–291.

New pleurotin derivatives

- Antimicrobial Pleurotin Derivatives



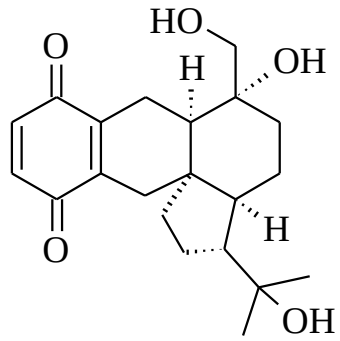


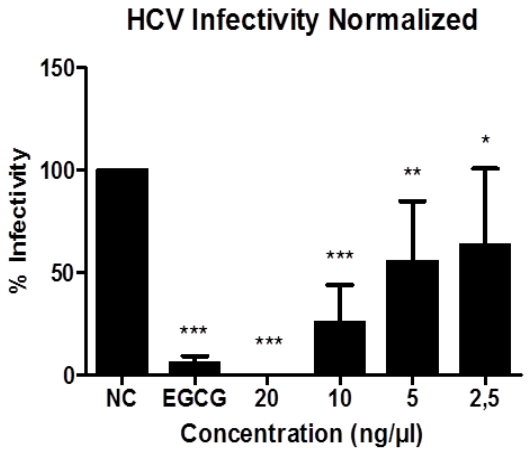
- Cytotoxicity: IC_{50} 6-7.5 µg/mL (KB3.1 & L929)
- Minimum Inhibitory concentrations:

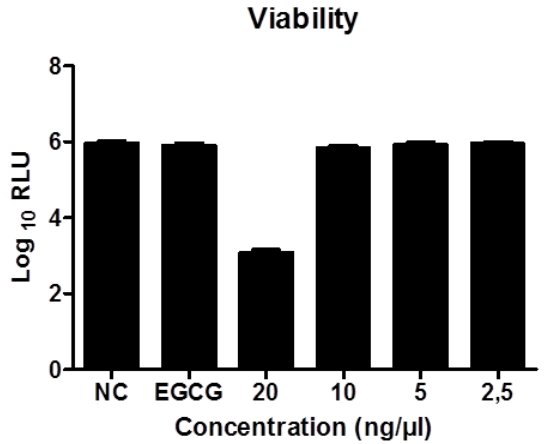
  Gram-positiv bacteria: 33-66 µg/mL

  yeasts: 12-50 µg/mL

- Antiviral (HCV) activity of 4-Hydroxypleurogrisein





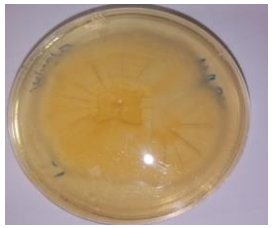


Sandargo B, Thongbai B, Praditya D, Steinmann E, Stadler M, **Surup F**. *Molecules* **2018**, *23*, 2697.

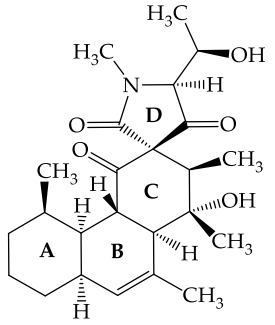
Ascomycota – Simplicilones A and B from the endophytic fungus *Simplicillium subtropicum*

Bark of *Allanblackia floribunda* is used in traditional African medicine for treatment of upper respiratory tract infections, dysentery, diarrhoea, and toothache

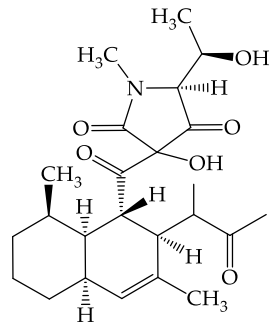
D. staudtii



After 10 days of growth



1



2

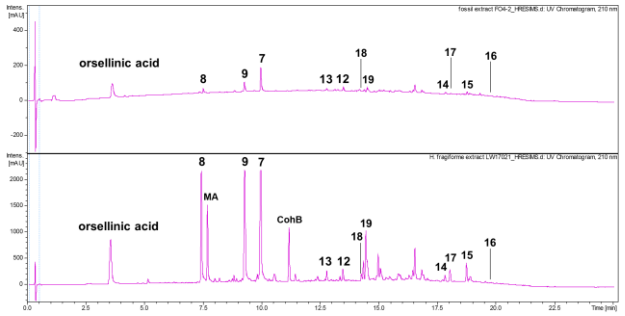
Endophyte: endosymbiont, that lives within a plant for at least part of its life cycle without causing apparent disease

- Cytotoxic activities against KB3.1 cells

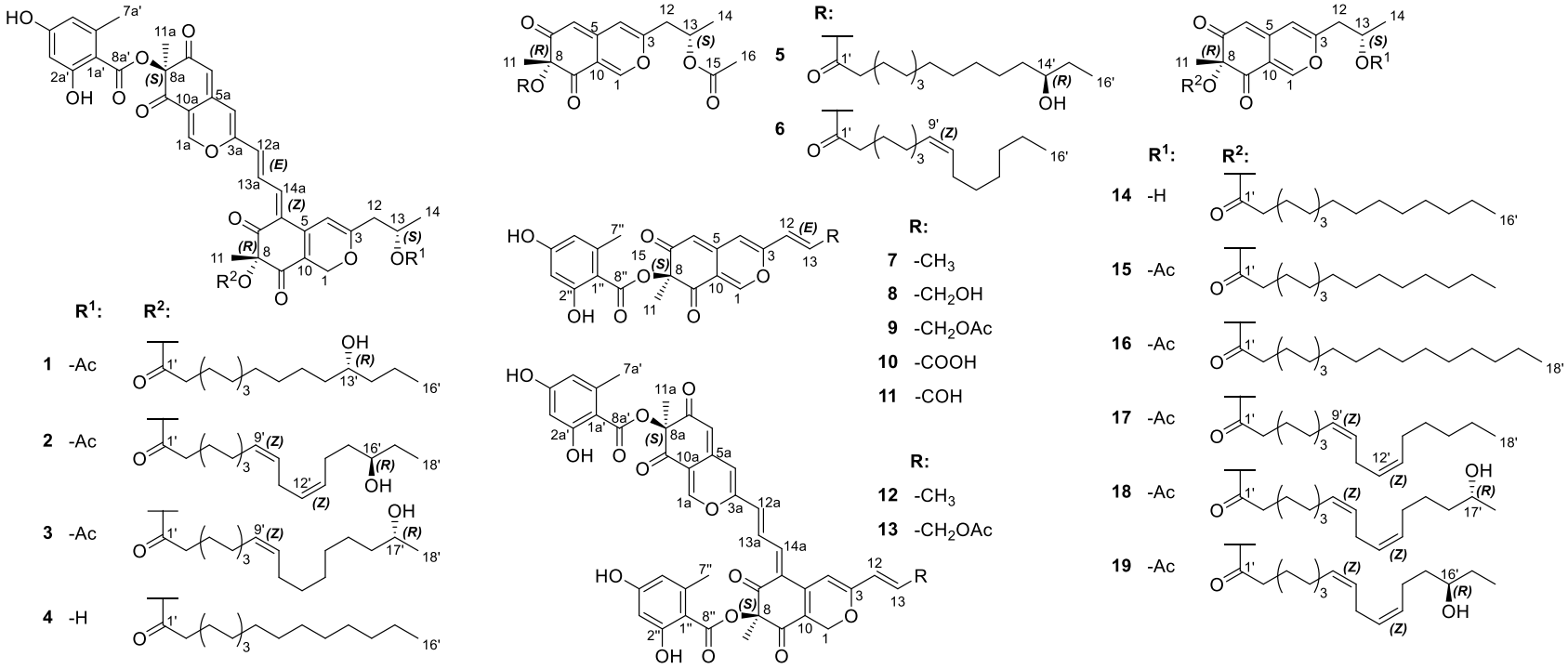
Anoumedem EGM, Mountessou BYG, Kouam SF, Narmani A, **Surup F**,*Antibiotics* **2020**, *9*, 753.

Ascomycota – Bisazaphilones from *Hypoxylon fragiforme*

- Wood samples from the medieval period (738 - 1411 AD)

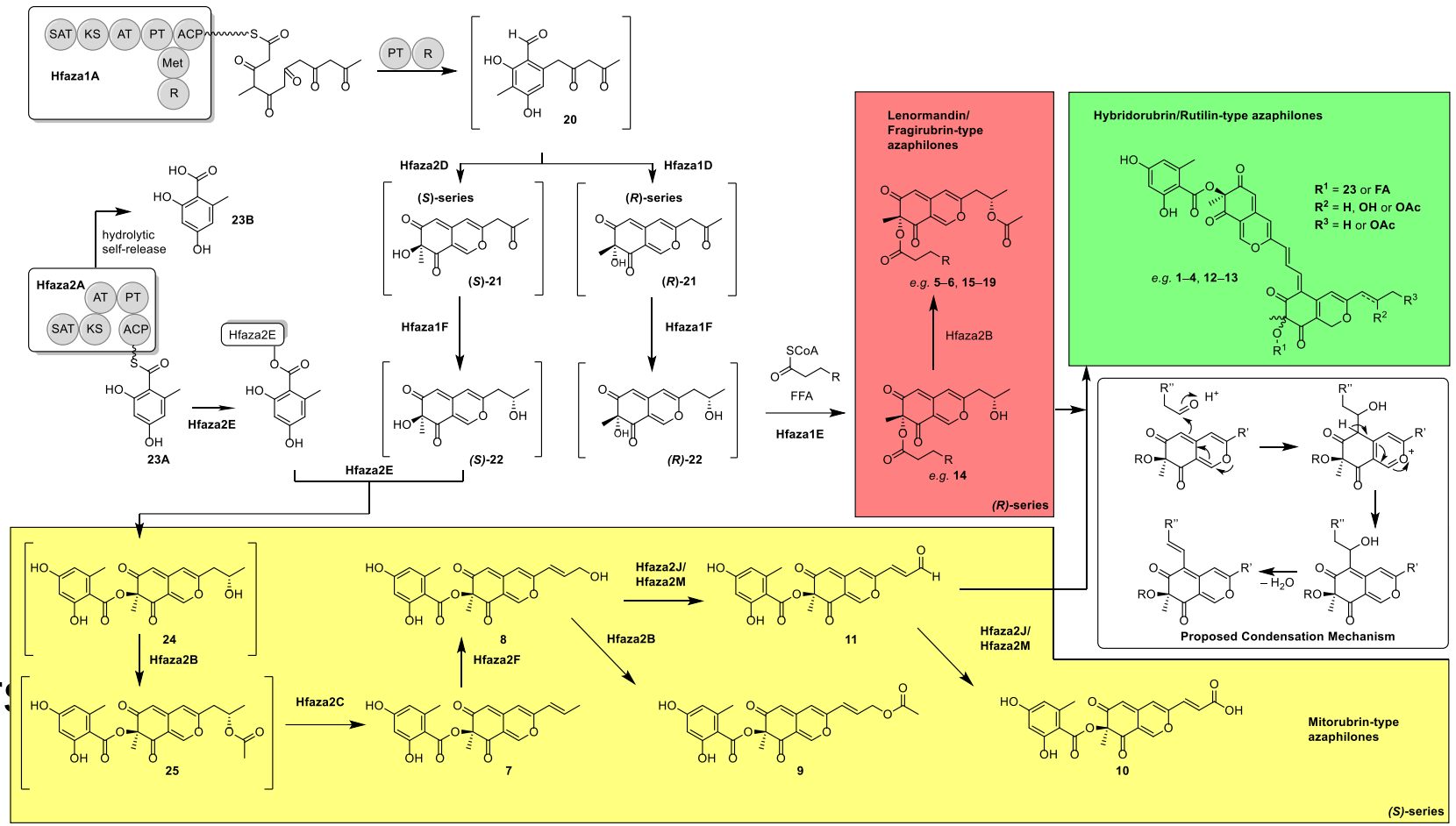


Comparison of HPLC-UV/VIS chromatograms (at 330nm) of the fossil sample (upper part) and fresh stromatal material (lower part).



Surup F, Narmani A, Wendt L, Pfütze S, Kretz R, Becker K, Menbrivès C, Giosa A, Elliott M, Petit C, Rohde M, Stadler M. *Fungal Div.* **2018**, *92*, 345–356; Becker K, Pfütze S, Kuhnert E, Cox R, Stadler M, Surup F. *Chem. Eur. J.* **2021**, 27(4):1438-1450.

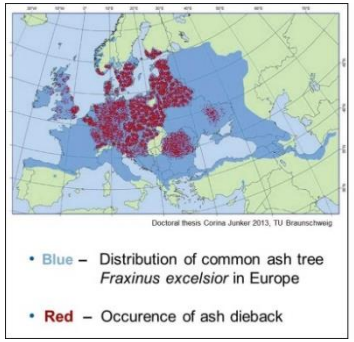
- New bisazaphilones
- Inhibition of the biofilm formation of *S. aureus*, no antibacterial effects (MIC >66.7µg/mL) nor cytotoxic activity (IC_{50} > 10µM)
- Biosynthesis proposal
- Interaction of up to 3 biosynthetic gene clusters



Becker K, Pfütze S, Kuhnert E, Cox R, Stadler M, **Surup F**. *Chem. Eur. J.* **2021**, 27(4):1438-1450. Becker K, Kuhnert E, Cox R, **Surup F**, *Eur. J. Org. Chem.* **2021**, *36*, 5094–5103

Ash dieback

- *Hymenoscyphus fraxineus* causative agent of European Ash Dieback disease
- Detected in 1995, fast distribution

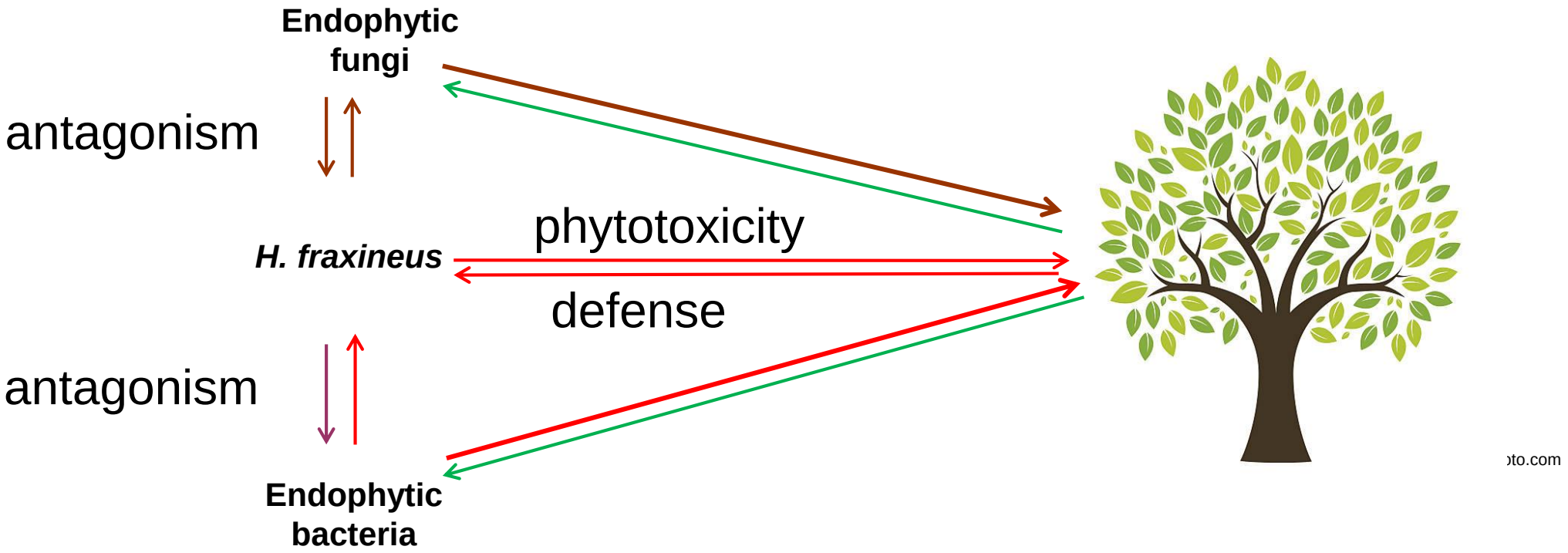


An introduced pathogen disturbs the balances!

- *H. fraxineus:* introduced from Asia

Ash dieback

- Microbiome and host interact metabolically with secondary metabolites



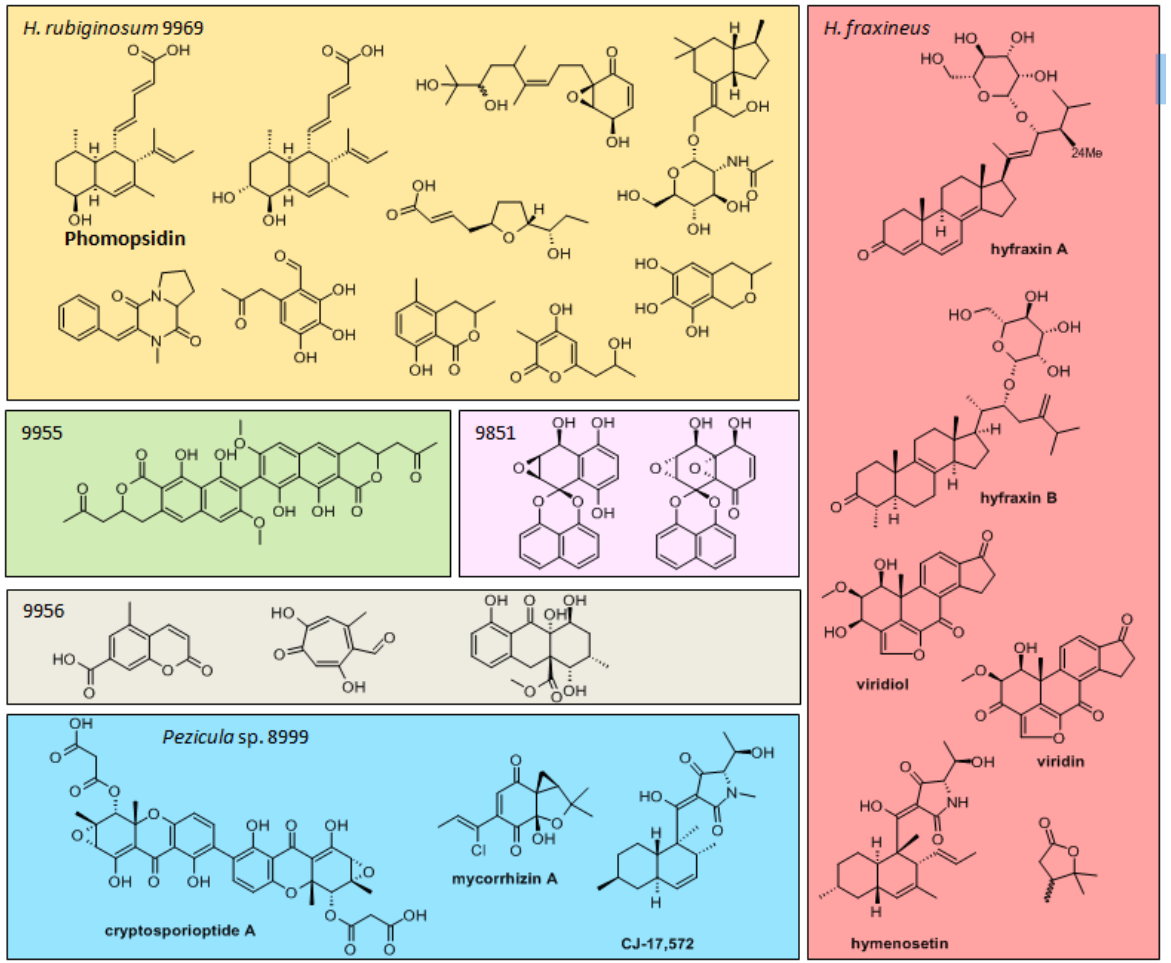
Surup F, Halecker S, Nimtz M, Rodrigo S, Schulz B, Steinert M, Stadler M. *Steroids* **2018**, *135*, 92–97.

Metabolomics - Metabolites from endophytes

- Dual culture experiments

H. fraxineus

Leptosphaeria sp.



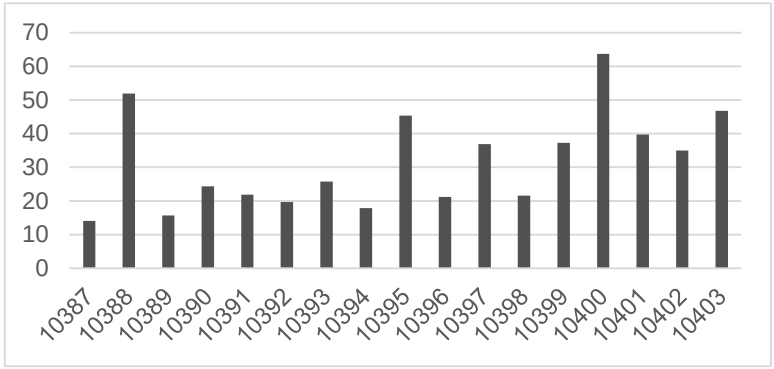
- Aim: Development of endophytes as plant protection agents

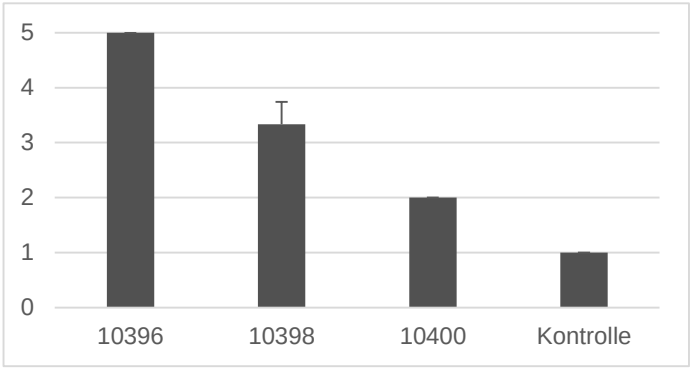
Halecker S, Wennrich J-P, Rodrigo S, Andrée N, Rabsch L, Baschien C, Steinert M, Stadler M**, Surup F**, Schulz B. *Fungal Ecol.* **2020***, 45*, 100918.

Secondary metabolites of *Penicillium manginii* 10400

- Protective effect of *P. manginii* against *H. fraxineus in planta*

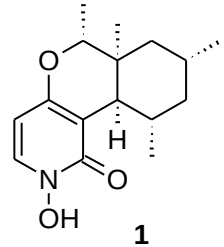
Inhibit.
H.f.
dual culture



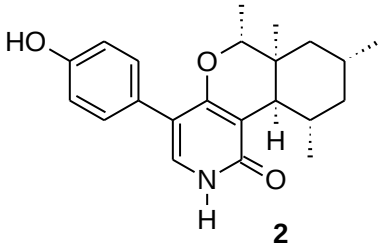


Disease symptoms of ash seedlings inoculated with *H. fraxineus and* endophytes
(1 = no disease symptoms to 5 = dead)

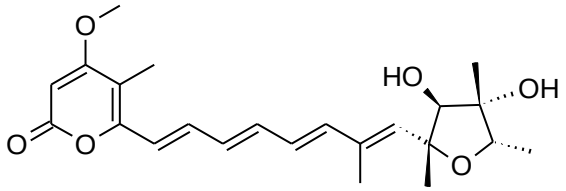
- Bioactivity-guided fractionation led to PF1140 (**1**)
- Metabolites **2** - **7** were isolated and elucidated (NMR & MS data)



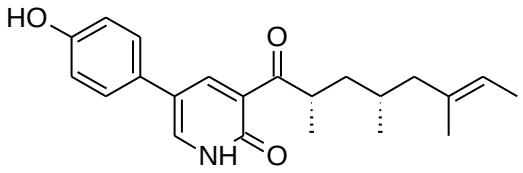
1



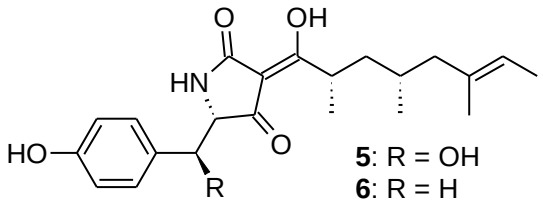
2



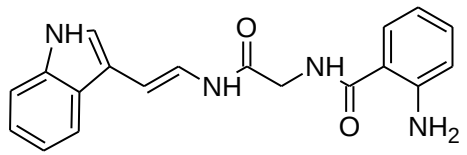
3



4

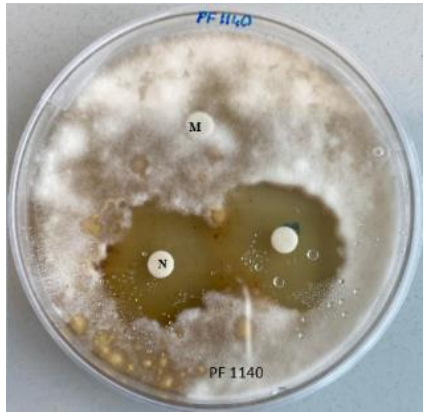


5: R = OH
6: R = H

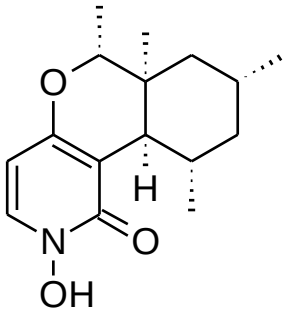


7

PF1140 - key metabolite of *Penicillium manginii* 10400



- Isolated from marine *Penicillium* sp.
- Strong antifungal activity against *H. fraxineus* (> positive nystatin!)

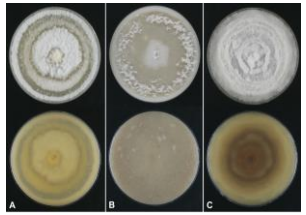


PF1140

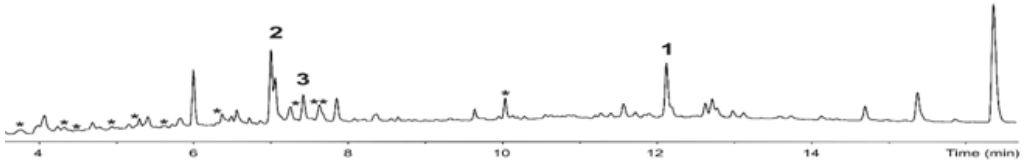
- PF1140 showed phytotoxic activity in leaf puncture assay
- Might explain the phytoxic activity of 10400 against ash seedlings in later experiments

de Silva et al., J. *Nat. Prod.* **2009**, *72*, 477-479
Fujita et al., J. Antibiot. **2005**, *58*, 425-427.

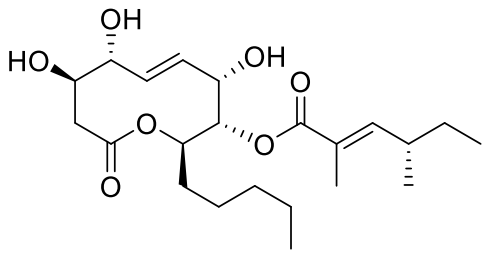
III Metabolomics – Tool for screening



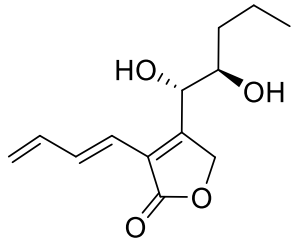
- Metabolites from *Diaporthe caliensis* sp. nov.
- Endophyt of the medical plant *Otoba gracilipes*
- HPLC analysis of the crude extract



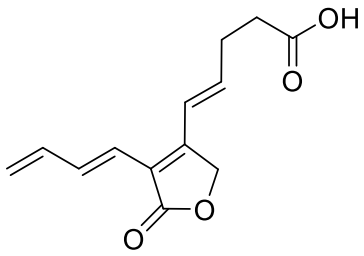
- Isolation & structure elucidation of bioactive metabolites



Phomol



Caliensolid A

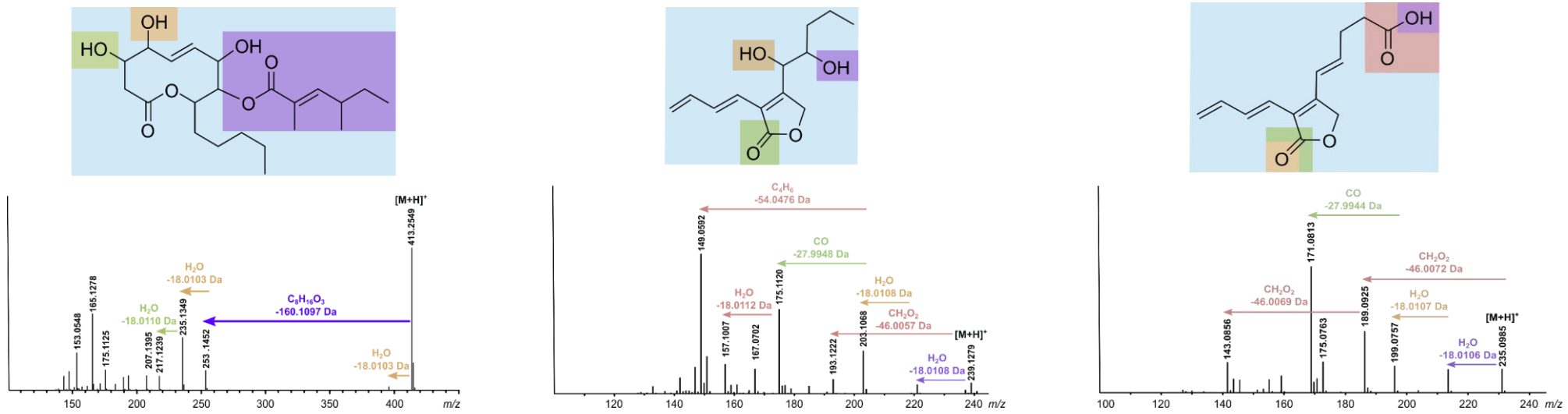


Caliensolid B

Charria-Girón E, Marin-Felix Y, Beutling U, Franke R, Brönstrup M, Vasco-Palacios AM, Caicedo NH, **Surup F**. Metabolomics insights into the polyketide-lactones produced by *Diaporthe caliensis* sp. nov., an endophyte of the medicinal plant *Otoba gracilipes*. *Microbiology Spectrum* **2023**, e02743-23.

Fragmentation patterns

MS2 spectra can be explained by the strctures

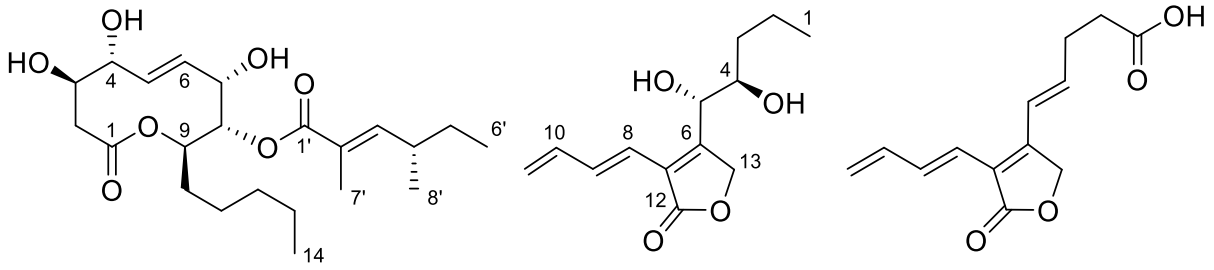


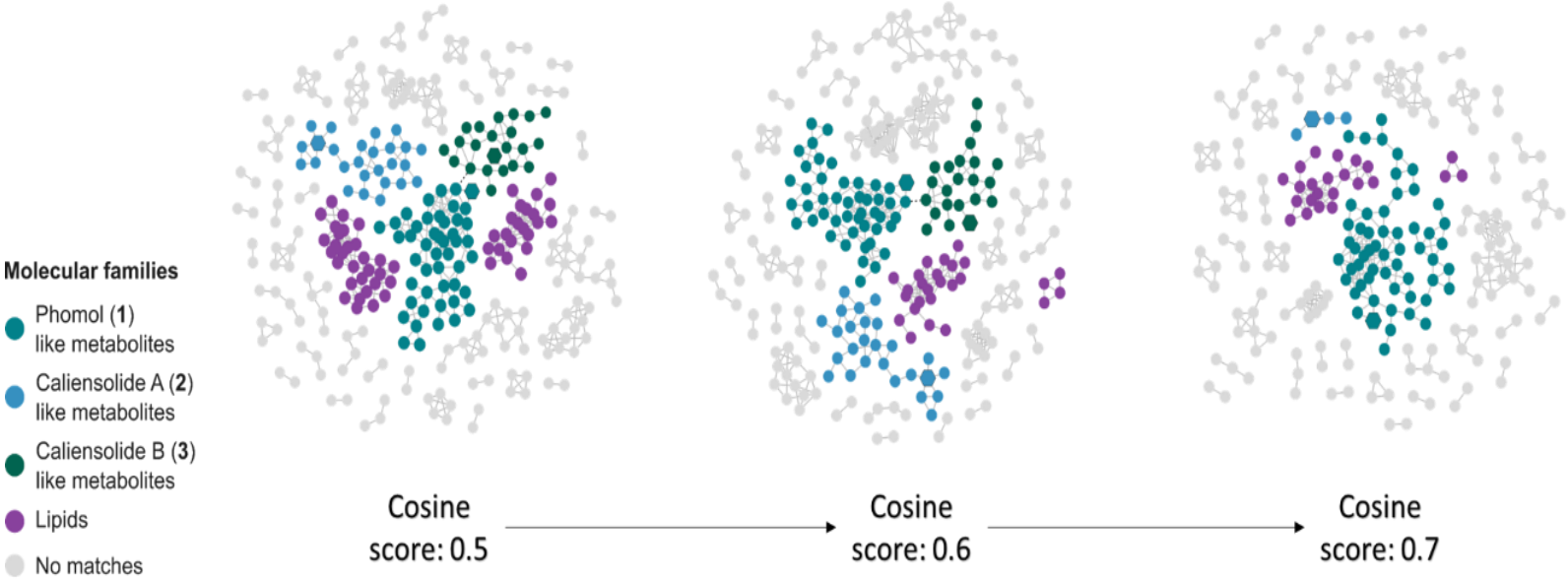
Structures of phomol, caliensolide A and B with highlighted observed fragments and its corresponding representative MS/MS spectrum. Unassigned neutral losses are shown in red.

→ Structural proposal from MS2 and MS3 spectra (sometimes) possible

Feature-based molecular networking

Visualize and annotate the chemical space in non-targeted mass spectrometry data





Global Natural Products Social Molecular Networking (GNPS) infrastructure

Wrong associations with standard parameters!

Nothias L-F, …, Dorrestein PC. Feature-based molecular networking in the GNPS analysis environment Nat Methods 2020,17, 905-908.

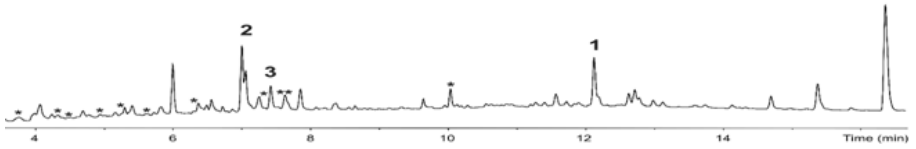
Untargeted Metabolomics Analysis - Dereplication with MS/MS data

Compound	*m/z*	rt	Formula	Annotation*	Database
Trichocladinol C	229.1074 $[M+H]^+$	3.75	$C_{11}H_{16}O_5$	Level 2	NP Atlas (Genus: *Diaporthe*)
Udagawanone B	177.0547 $[M-H2O+H]^+$	4.33	$C_{10}H_{10}O_4$	Level 2	NP Atlas (Genus: *Diaporthe*)
Multiforisin A	207.0651 $[M-H2O+H]^+$	4.51	$C_{11}H_{12}O_5$	Level 2	NP Atlas (Genus: *Diaporthe*)
Gulypyrone A	213.1125 $[M+H]^+$	4.94	$C_{11}H_{16}O_4$	Level 2	NP Atlas (Genus: *Diaporthe*)
Udagawanone A	211.0968 $[M+H]^+$	5.25	$C_{11}H_{14}O_4$	Level 2	NP Atlas (Genus: *Diaporthe*)
Multiforisin A	225.0688 $[M+H]^+$	5.63	$C_{11}H_{12}O_5$	Level 2	NP Atlas (Genus: *Diaporthe*)
Pyrenocine L	267.1226 $[M-H2O+H]^+$	6.35	$C_{14}H_{20}O_6$	Level 2	NP Atlas (Genus: *Phomopsis*)
Caliensolide A (**2**)	239.1280 $[M+H]^+$	7.06	$C_{13}H_{18}O_4$	Level 0	Present study
Diaportheone B	203.0702 $[M-H2O+H]^+$	7.34	$C_{12}H_{12}O_4$	Level 2	NP Atlas (Genus: *Diaporthe*)
Caliensolide B (**3**)	235.0970 $[M+H]^+$	7.44	$C_{13}H_{14}O_4$	Level 0	Present study
Kobifuranone B	195.1023 $[M+H]^+$	7.64	$C_{11}H_{14}O_3$	Level 2	NP Atlas (Genus: *Diaporthe*)
Chaetolactone	253.1442 $[M+H]^+$	7.67	$C_{14}H_{20}O_4$	Level 2	NP Atlas (Genus: *Diaporthe*)
Phomol	395.2435 $[M-H_2O+H]^+$	10.04	$C_{22}H_{36}O_7$	Level 1	NP Atlas (Genus: *Phomopsis*)
(3*R*,4*R*,7*S*,8*S*,9*R*)-phomol (**1**)	413.2538 $[M+H]^+$	12.13	$C_{22}H_{36}O_7$	Level 0	Present study

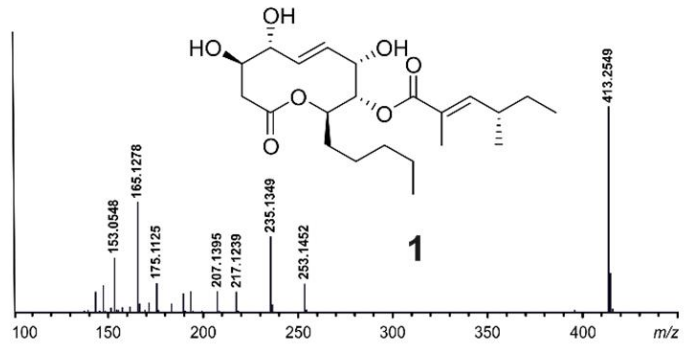
→ **Fast overview of (secondary) metabolome**

Global Natural Product Social Molecular Networking (GNPS)

HPLC chromatogram of crude extract



Straight-forward identification of phomol on MS2 data



New biotechnology platform at HZI (operative since 2021)

Upstream Processing Equipment

Shake flask cultivations

- RAMOS (50 mL; 200 mL)
- Transfer from shake flask to bioreactor
- Media development

Multifermenter

- DASGIP (1.5 L)
- Process development in laboratory scale

Stainless steel bioreactors

- Six vessels (10 L)
- Process implementation
- Optimization for technical scale

Pilot scale bioreactors

- 4 x 150 L; 2 x 350 L
- Material supply for e.g. preclinical studies
- Process transfer to CRO’s

Downstream Processing Equipment

Biomass separati

- Tube centrifuge
- Filtration

Extraction

- Fluidized bed
- Liquid-liquid

Concentration

- Rotary evaporator
- High vacuum

Product separation

- MPLC
- Preparative HPLC

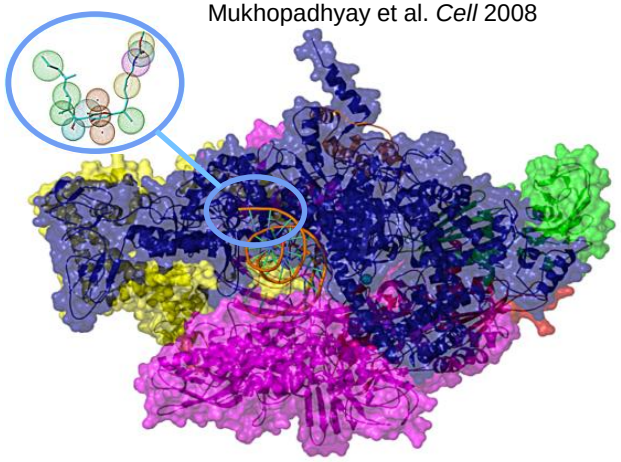
Only facility in European academia that can handle 100 g scale amounts of natural products

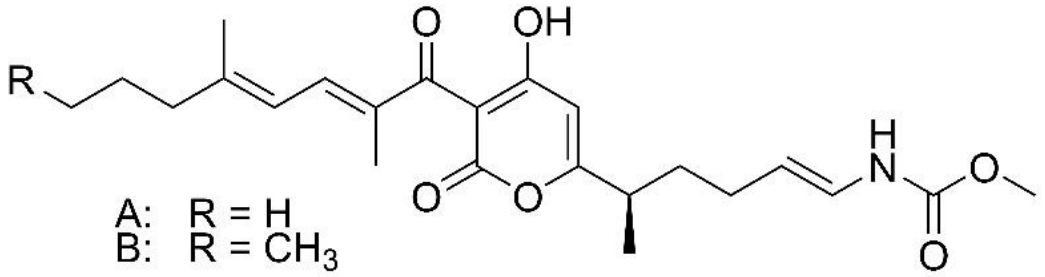
Heterologous Expression of Myxopyronin A

- Antibacterial compound published in 1983
- Original producer: *Myxococcus fulvus* Mx f50
- Product titer: 5 mg L^{-1}
- α-pyrone antibiotic with activity against *Mycobacterium tuberculosis*
- Model compound for heterologous expression of polyketides

Target: RNA polym. switch region

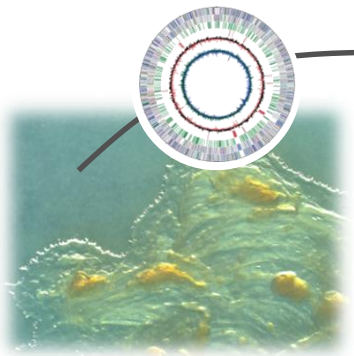
Mukhopadhyay et al. *Cell* 2008



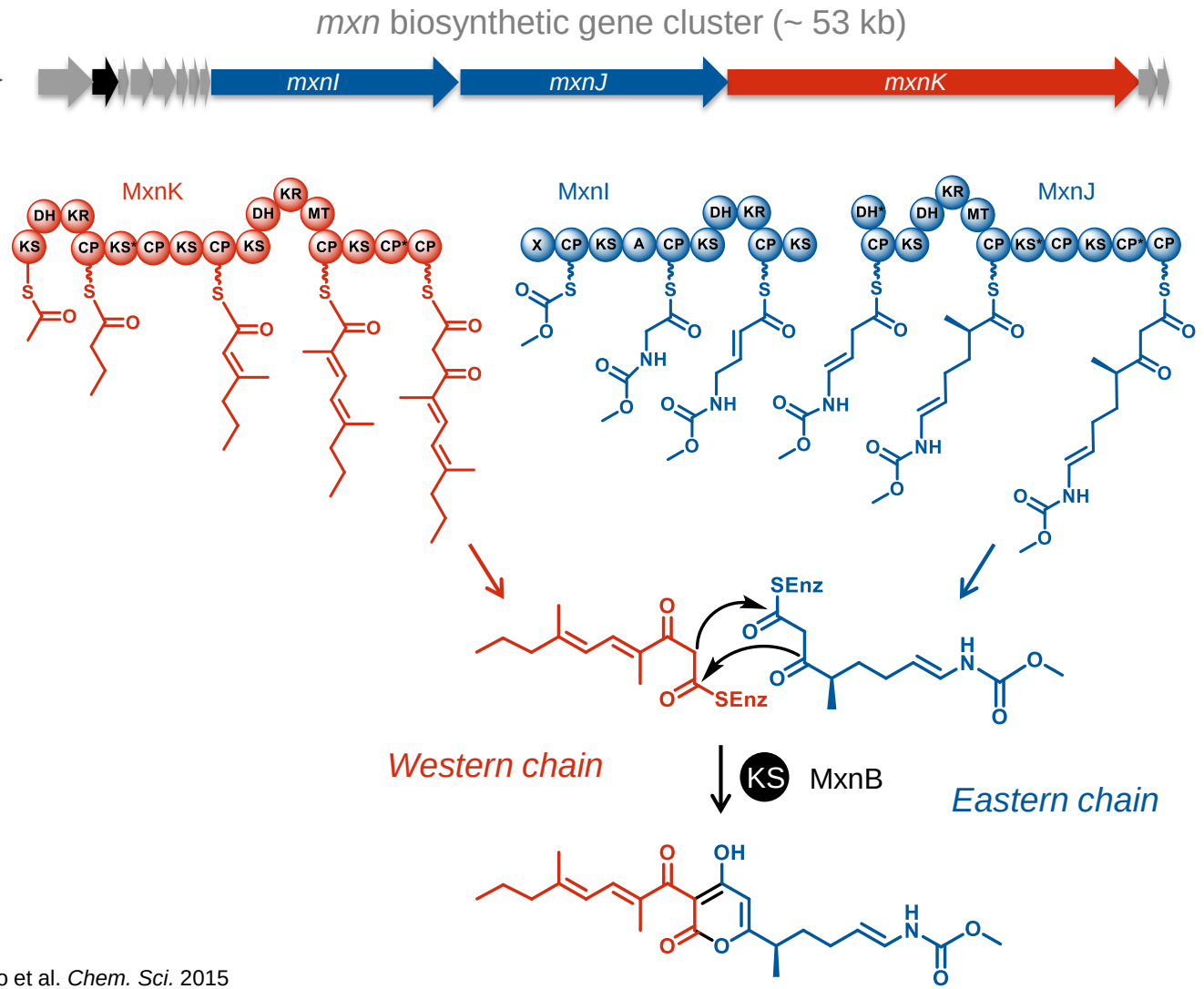


Irschik H, Gerth K, Höfle G, Kohl W, Reichenbach H. The myxopyronins, new inhibitors of bacterial RNA synthesis from *Myxococcus fulvus* (Myxobacterales). *J Antibiot.* **1983**, 36, 1651-1658.

Myxopyronin biosynthesis



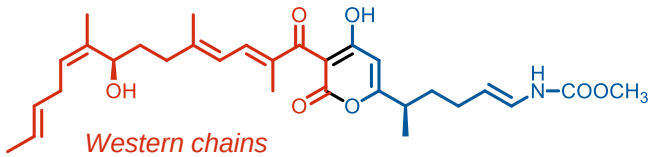
Myxococcus fulvus



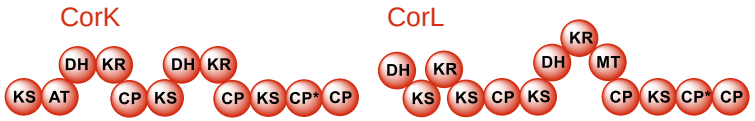
Sucipto et al. *ChemBioChem* 2013; Sucipto et al. *Chem. Sci.* 2015

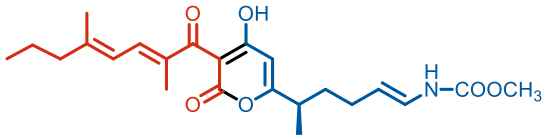
Heterologous corallopyronin production platform

Domen Pogorevc



Corallopyronin A





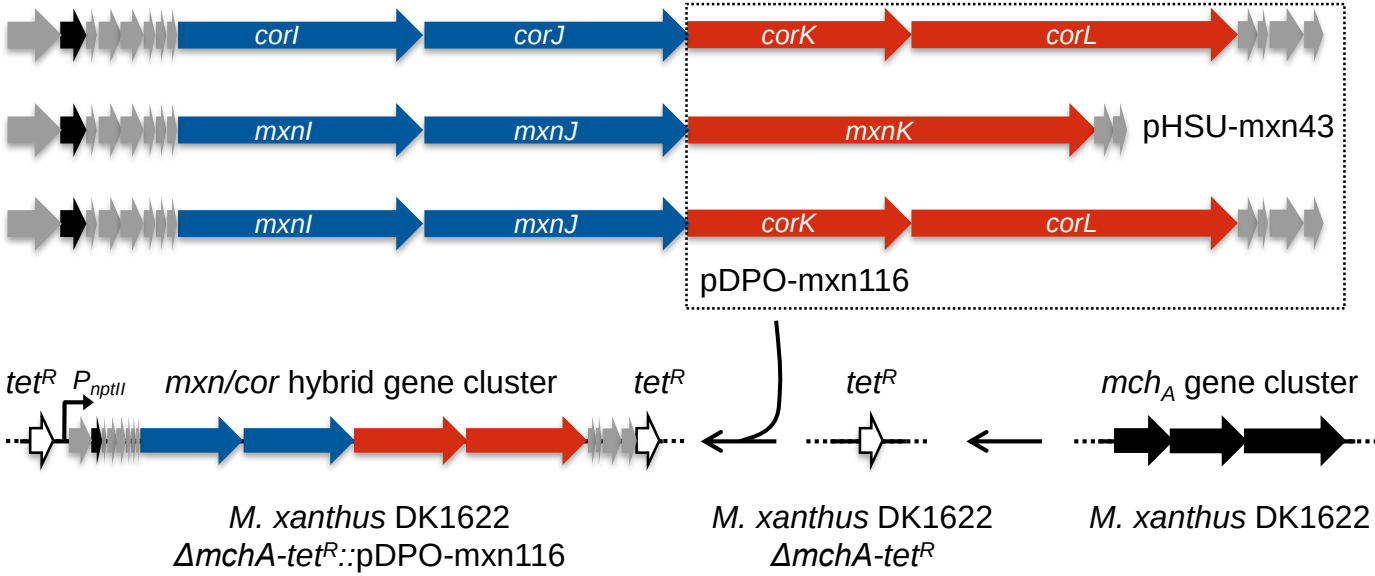
Myxopyronin A

Exchange of western chain biosynthetic genes

cor cluster (~ 65 kb)
Erol et al. *CBC* 2010

mxn cluster (~ 53 kb)
Sucipto et al. *CBC* 2013

mxn/cor hybrid cluster
Sucipto et al. *CBC* 2017

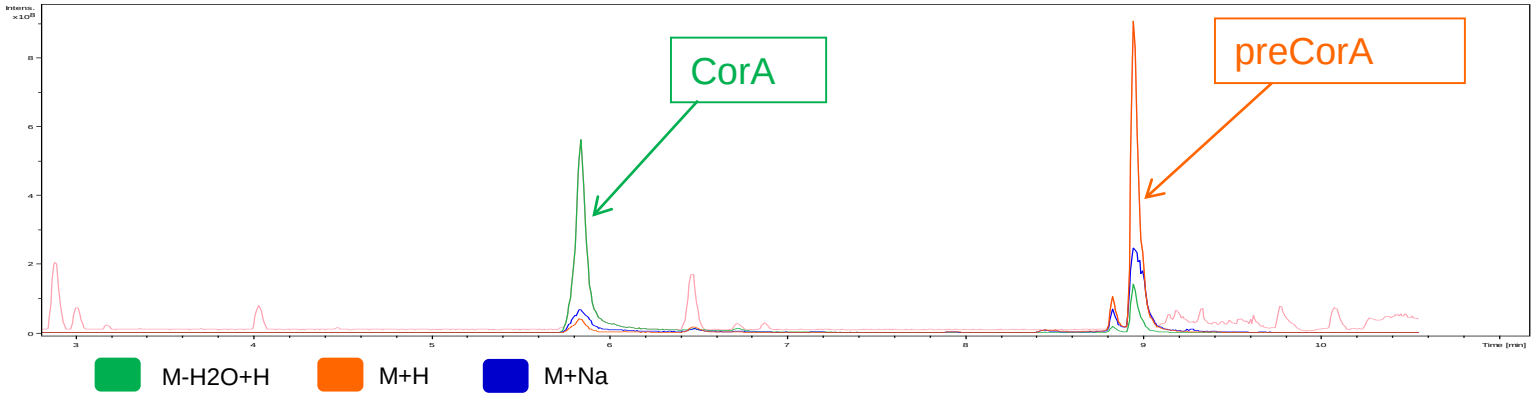


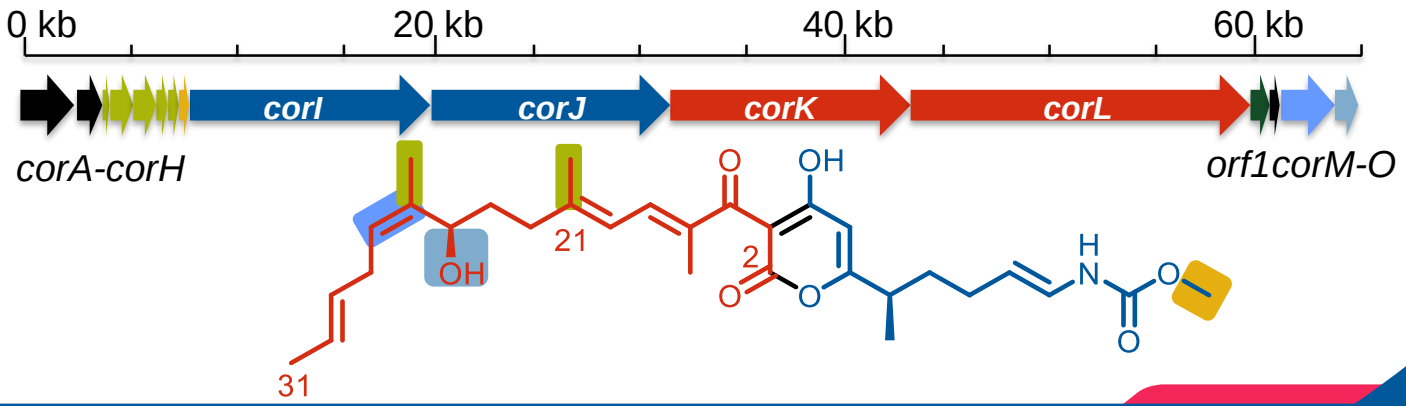
Sucipto et al. *Metabolic Engineering* 2017

Myxopyronin and Corallopyronin yields

MxnA [mg/L]		D (acies plate) I	II	III	Average
CTT	6d	3.8	3.9	3.7	**3.8**
CTT	8d	3.6	3.2	3.4	**3.4**
Mxn	6d	2.9	3.1	3.0	**3.0**
Mxn	8d	2.7	2.9	2.6	**2.8**
M7s/6	6d	169.4	149.3	147.9	**155.5**
M7s/6	8d	152.3	140.6	/	**146.4**

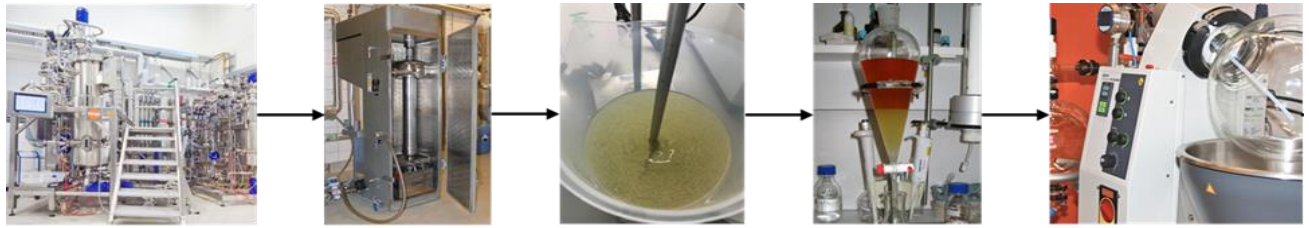
CorA [mg/L]		B (pDPO-mxn116 #3) I	II	III	Average
CTT	6d	1.4	1.4	1.6	**1.5**
CTT	8d	1.3	1.8	1.8	**1.6**
Cor	6d	1.0	1.0	0.9	**1.0**
Cor	8d	1.0	1.0	0.8	**1.0**
M7s/6	6d	38.8	40.1	32.6	**37.2**
M7s/6	8d	41.6	37.7	41.9	**40.4**

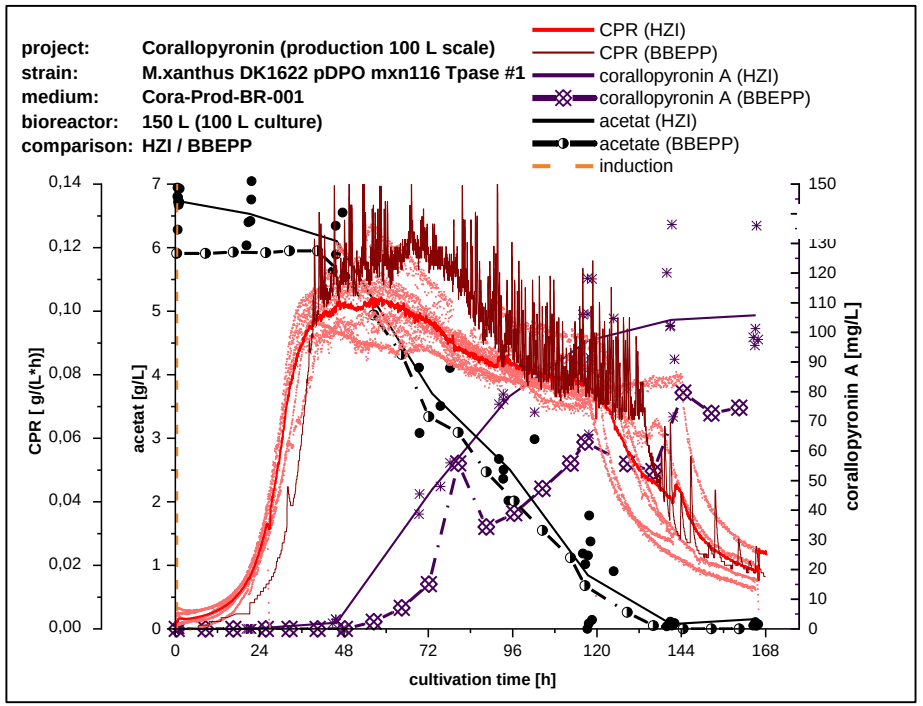




Transfer of CorA production process

Bio Base Europe Pilot Plant

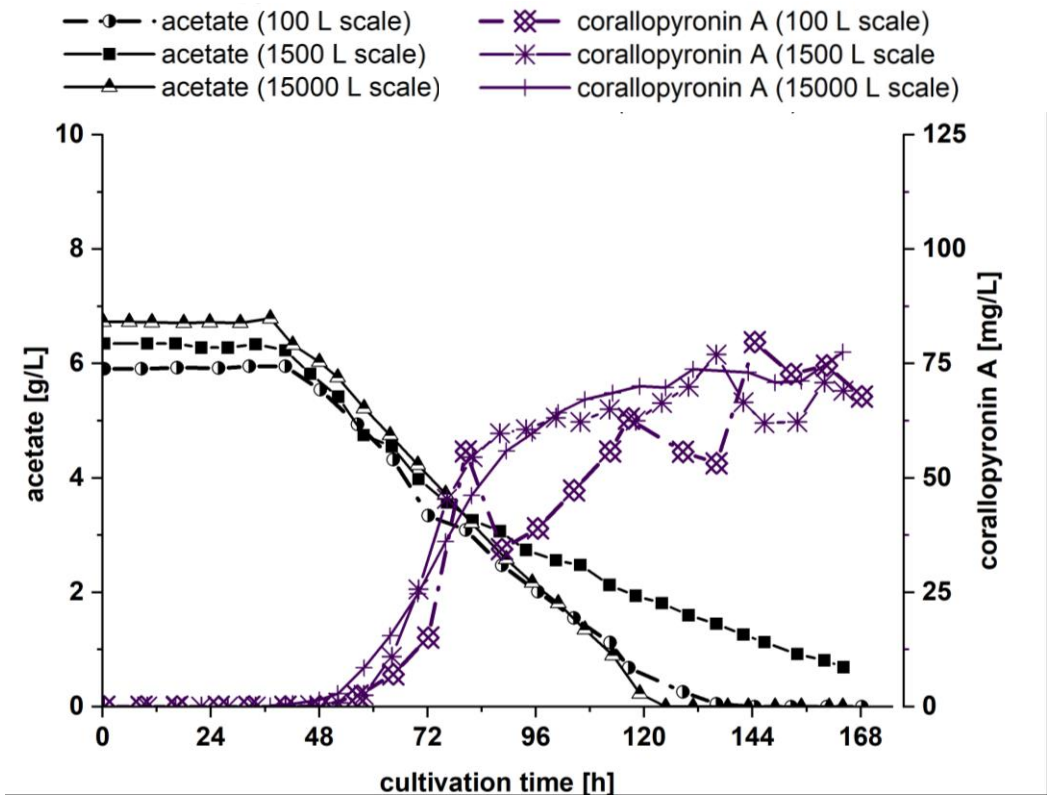




Process Transfer in 2021

- Preparation of SOP‘s for production and purification
- USP & DSP successfully established
- Produced material sent from Belgium to HZI for final purification

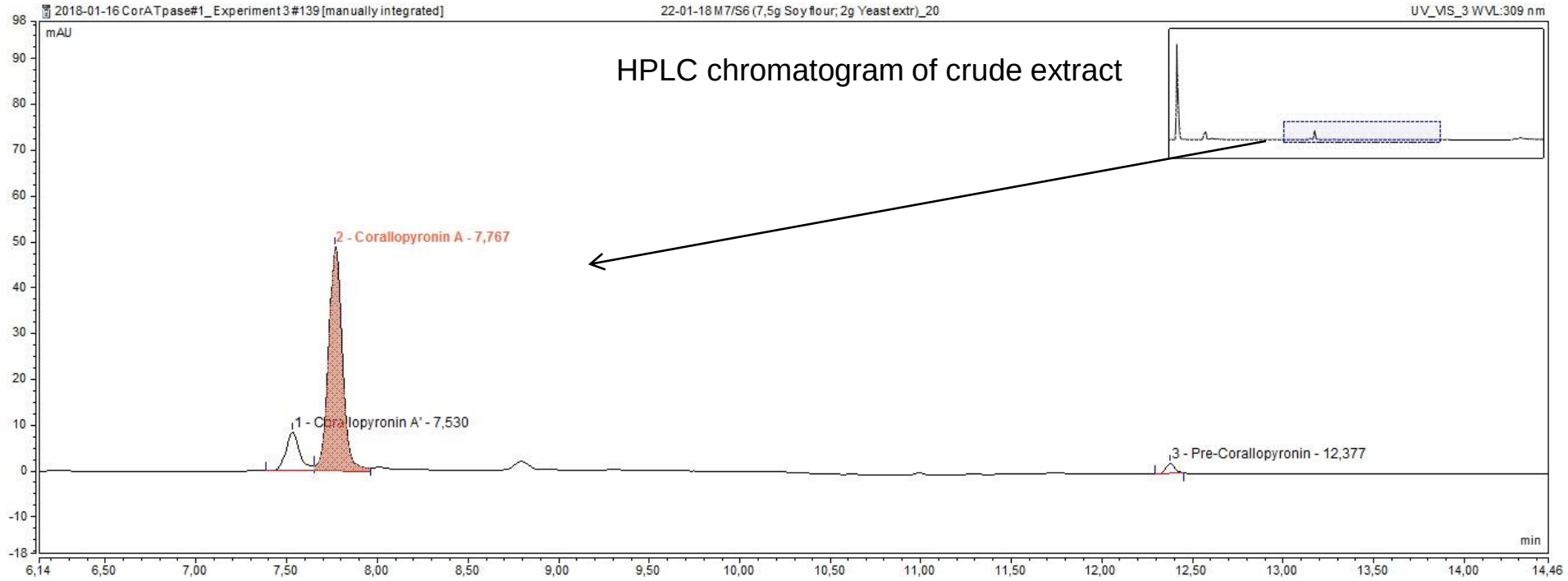
Scale up in Belgium (BBEPP Ghent)



Scale up: USP

- Up to now 5 successful runs in 15 m^3 scale
- Titers equivalent to that observed at HZI
- Process reproducible
- **Successful scale up to industrial scale**

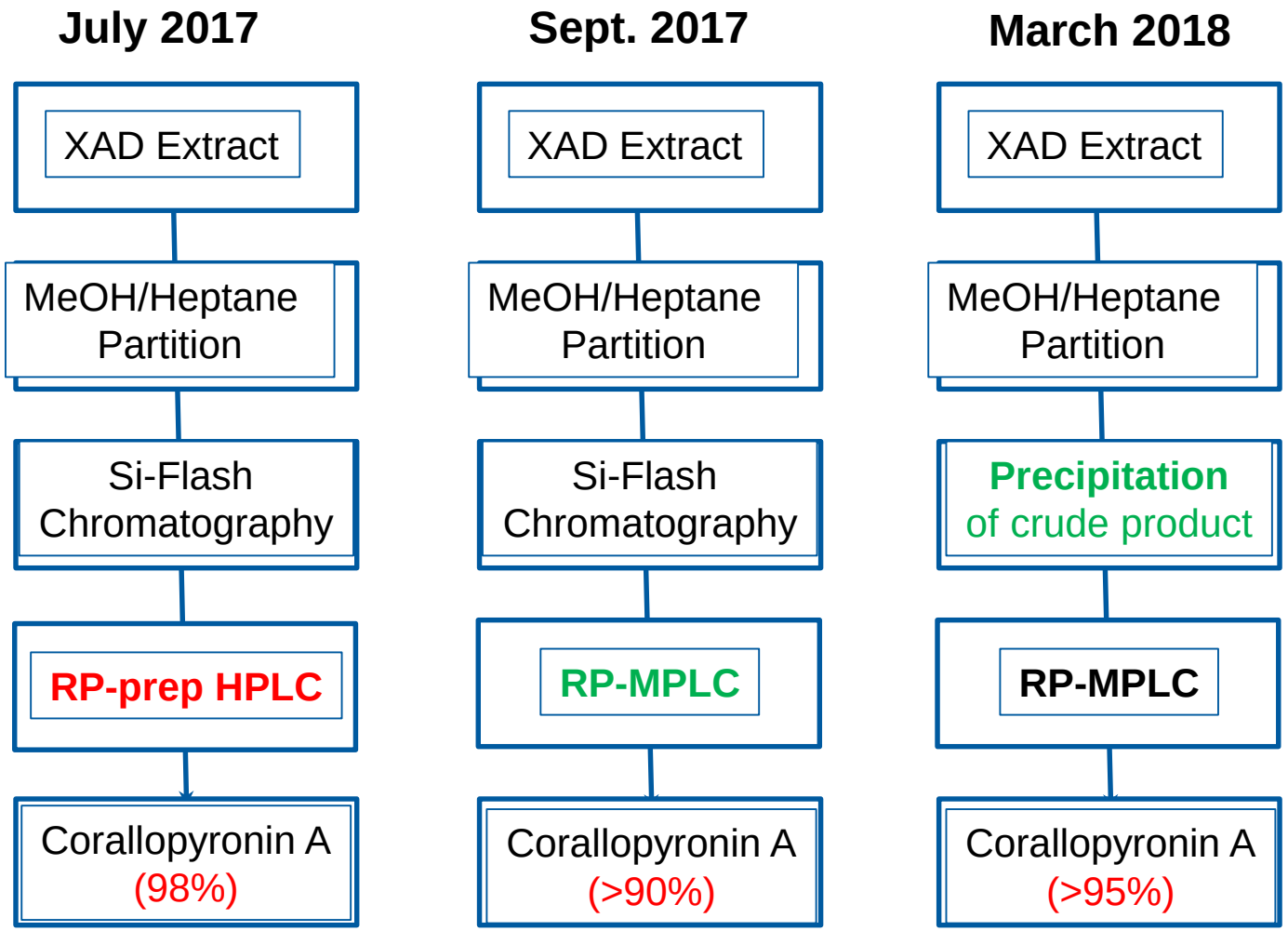
Production of Cor A in 250 L pilot scale – Metabolite profiles in the supernatant crude extract



Almost no other derivatives except for Cor A‘ and Pre Cor A in the crude extract

=> DSP facilitated substantially as compared to wild type strain

CorA Downstream Processing (time course)



Summary: HZI CorA production by BBEPP

7-days repeated dose of dogs

Group (n=4)	Sex	Dose level (mg/kg/day)	CorA need (g)	Treatment start
1	2 m, 2 f	vehicle	-	05.09.2022
2	2 m, 2 f	150	**35**	05.09.2022
3	2 m, 2 f	450	**106**	05.09.2022
4	2 m, 2 f	750	**214**	07.12.2022

Isolated at HZI and delivered to Bonn in 2022

- 22.02.2022 44.04 g
- 27.06.2022 59.40 g
- 15.08.2022 127.99 g
- 25.11.2022 232.00 g

Contract with German CRO for GMP production (including downstream processing!) signed in September 2023!

Summary – Challenging variety of natural products

- **Bioactivity-guided fractionation**:
  → disciformycins: RNA polymerase inhibitors from bacterium *Pyxidicoccus fallax*

- **Screening for novel compunds**
  of *Rhodotus palmatus, Hohenbuehelia grisea, Hypoxylon fragiforme, etc.*

- **Metabolomics**: MS/MS data as additional information for dereplication

- **Corallopyronin**: Development of an old antibiotic metabolite

Acknowledgements

Marc Stadler (MWIS)
& department

Birthe Förster

Karen Harms

Esteban Giron

Özge Demir

Rolf Müller (HIPS)
Michael Steinert (TU BS)
All Coauthors
Corallo team

DZIF

Bundesministerium
für Bildung
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DFG Deutsche Forschungsgemeinschaft

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