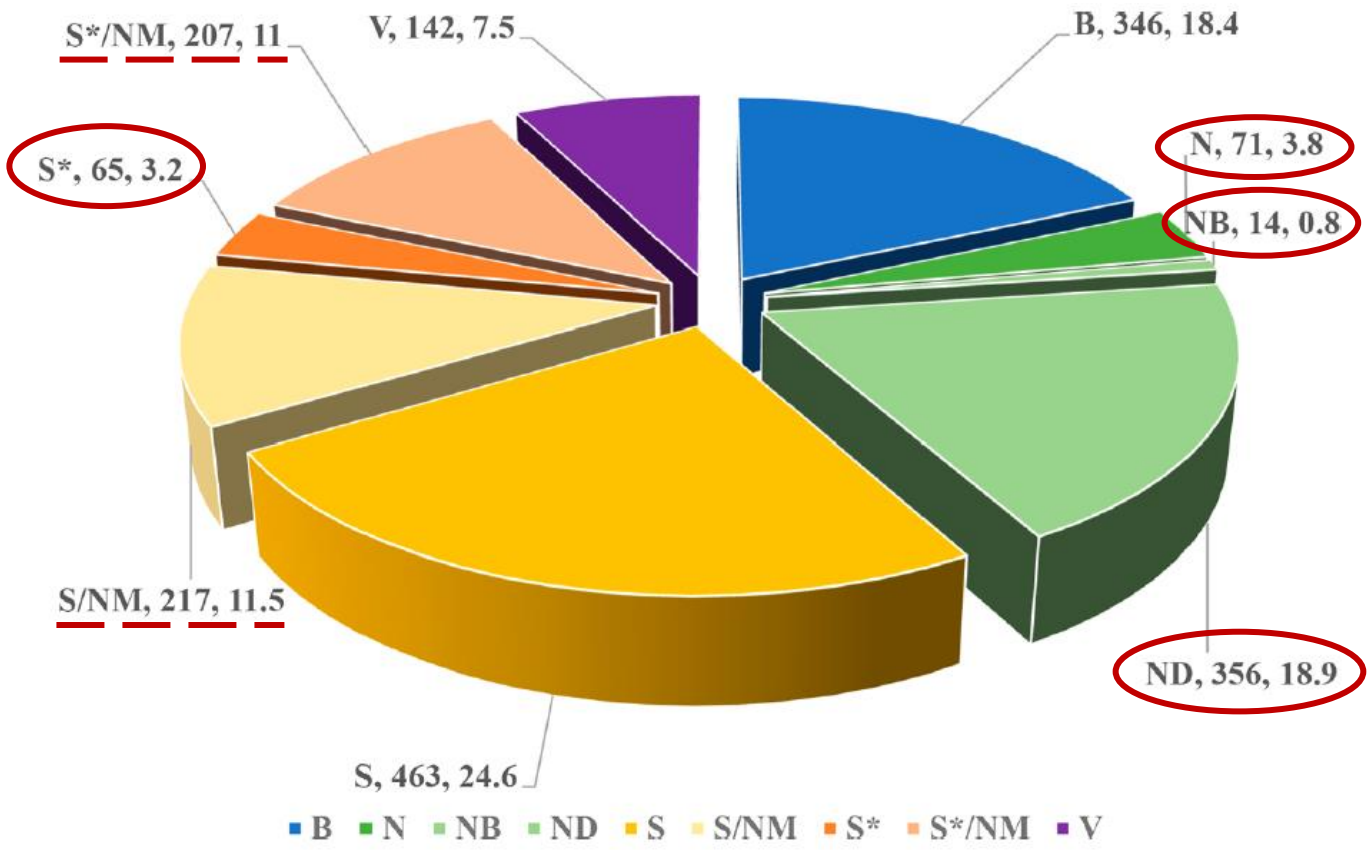
Isolement, structure et évaluation de substances marines à visée pharmacologique et thérapeutique.

Nouvelles stratégies pour l'obtention de séries d'analogues naturels originaux

Olivier Grovel

Institut des Substances et Organismes de la Mer

Nearly 50% of marketed drugs used today are of natural origin or are inspired by natural products.

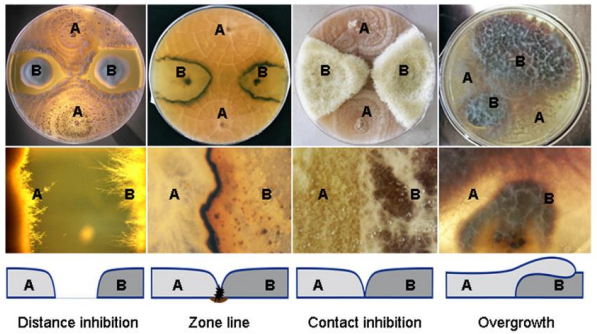


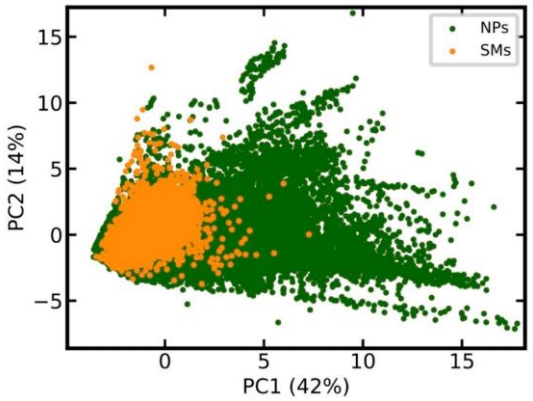
code	brief definition/year
B	biological macromolecule, 1997
N	unaltered natural product, 1997
NB	botanical drug (defined mixture), 2012
ND	natural product derivative, 1997
S	synthetic drug, 1997
S*	synthetic drug (NP pharmacophore), 1997
V	vaccine, 2003
/NM	mimic of natural product, 2003

Newman D.J. and Cragg G.M., *J. Nat. Prod.*, **2020**, 770-803

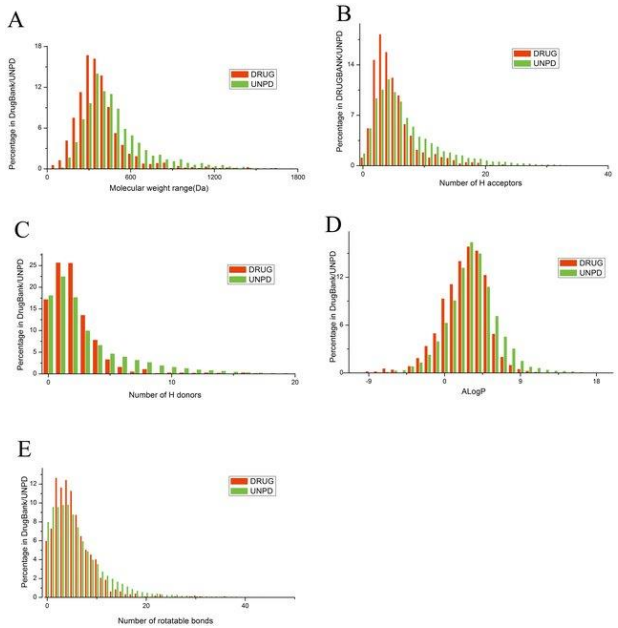
Are natural products still good drug candidates?

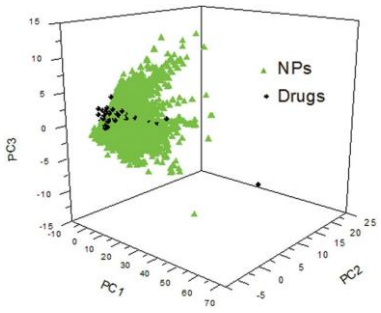
➔ Determinism / chemical ecology





➔ Diversity / Stereoselectivity





➔ Druglikeness (Lipinski)

J. Chem. Inf. Model. 2018, 58, 1518−1532

Bertrand S. *et al.*, *Biotechnol. Adv.*, **2014**, 1180-1204

Gu J. *et al.*, *Plos One*, **2013**, e62839

Chen Y. *et al.*, *Biomolecules*, **2019**, 43

The chemical space of natural products: origins, prefered phyla, chemical distribution

≈ 400,000 natural products described

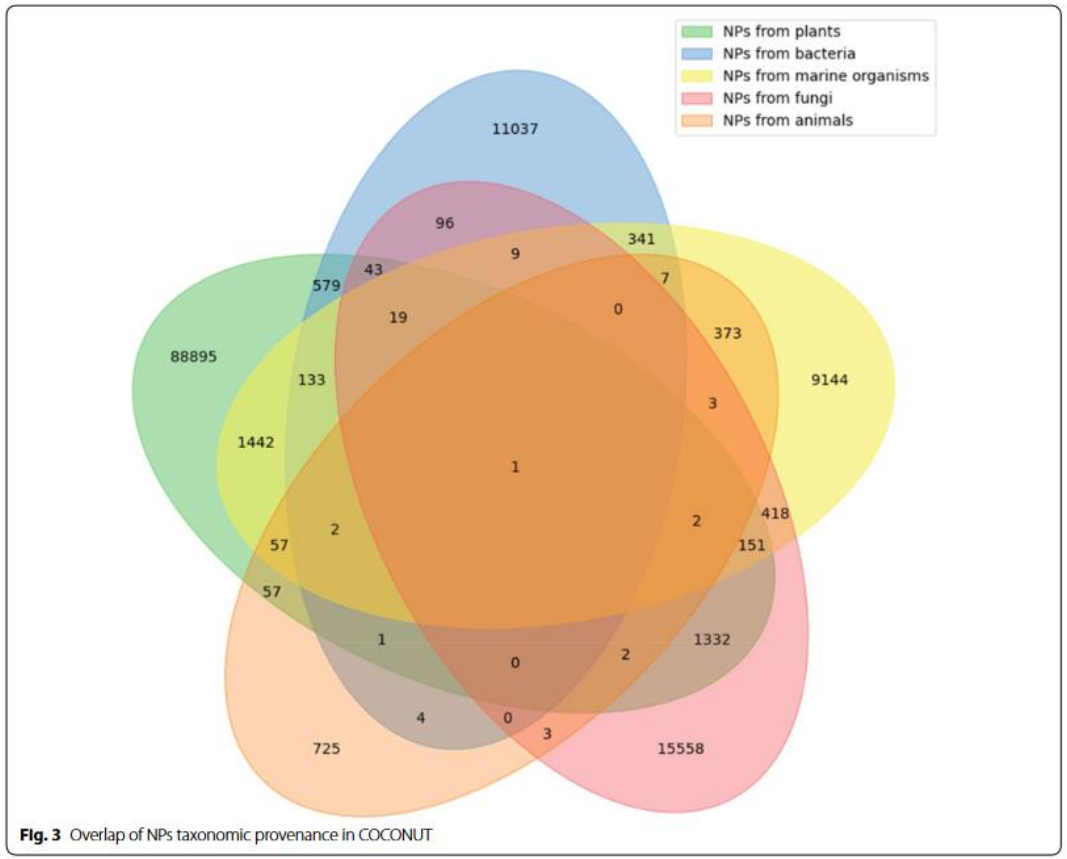
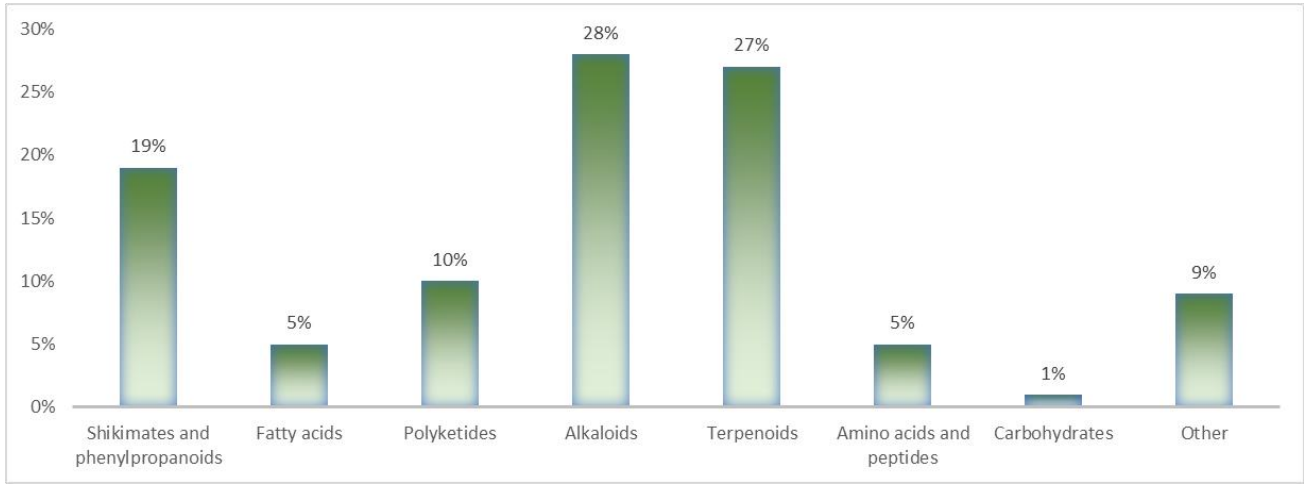


Fig. 3 Overlap of NPs taxonomic provenance in COCONUT



Tay D.W.P. *et al.*, *Sci. Data*, **2023**, 10:296
Sorokina M. *et al.*, *J. Cheminform.* **2021**, 13, 2

Terrestrial and Marine biodiversities: an immense reservoir of chemical diversity

500 000 plants on earth

Estimation of 10^{12} microbial species

270 000 described and classified

20 000 medicinal plants

≈5.6·10⁶ inventoried

Bacteria

Fungi

Archae

Microalgae

Only a few thousands studied and chemically assessed

Locey K.J. and Lennon J.T., *PNAS*, **2016**, 5970-5975

The « silent » world, source of chemodiversity

Origin of life; 71 % of earth surface

The highest reservoir for biodiversity : ca. 10^6 plants and animals and 10^9 microorganisms

High competition (up to 1000 species per m^2); extreme environments (salinity, pressure, UV)

Diversity of microbial communities (holobionts)

→ Special features: - unique pathways
- halogenated compounds

But: No history of traditional use in medicine

Issues: access, culture, sustainibility

→ First chemical investigations in early 1960's

2024: 20 marine-derived drugs marketed

The « silent » world, source of chemodiversity

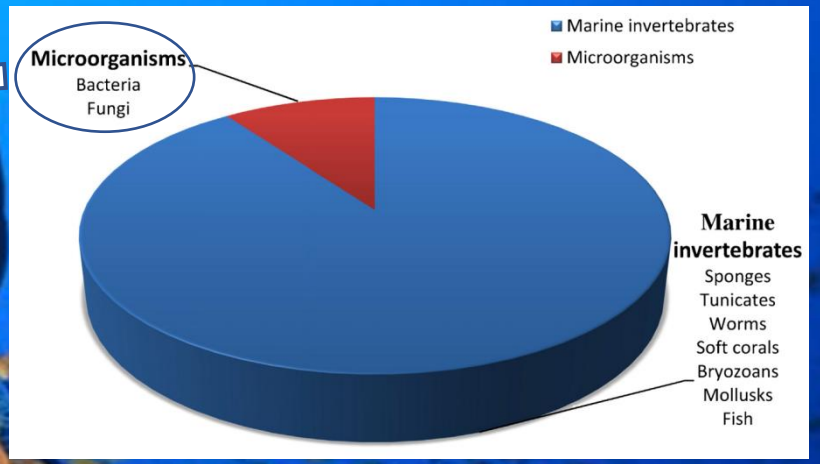
The invisible world

Fungi

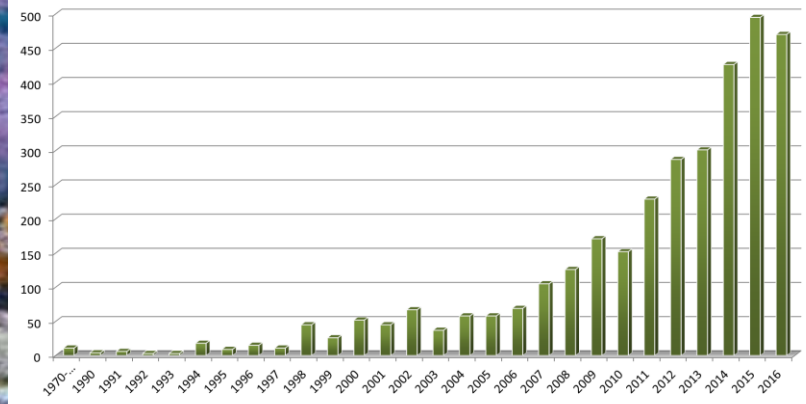
To date only 1% of fungal biodiversity has been « observed »

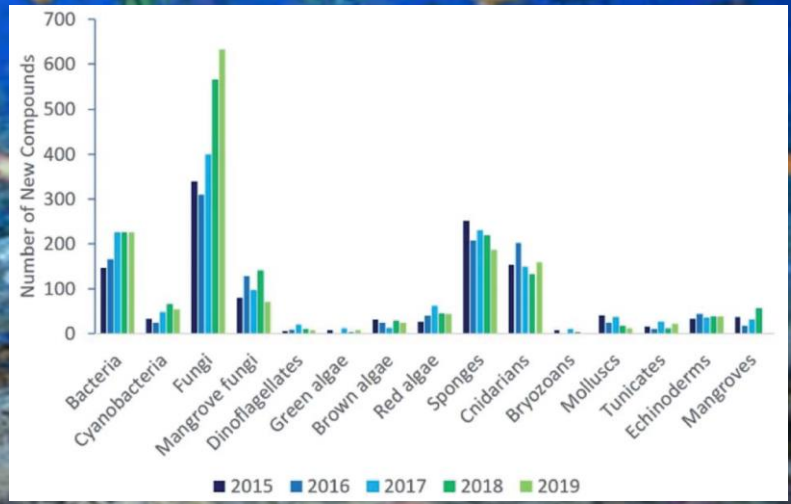
→ penicillins, cephalosporins, pleuromutilins,…

MNPs: *ca.* 27,000 novel natural products



Annual description of original MNPs from marine-sourced fungi (incl. mangroves)





Carroll. A.R. *et al.*, *Nat. Prod. Rep.*: annual reviews

UR 2160 - Institut des Substances et Organismes de la Mer

Marine Mycotheca Strains Collection - MMS

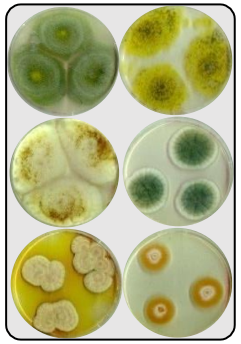
→ ~1700 marine fungal strains stored, from coastal environment samples: sediments, seawater, invertebrates, seaweeds, marine microbiomes

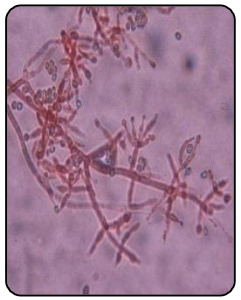
Direction: Nicolas Ruiz nicolas.ruiz@univ-nantes.fr
Curator: Thibaut Robiou du Pont thibaut.robiou@univ-nantes.fr

Isolation of bioactive NPs for
- therapeutics
- nutrition (lipids)
- biocontrol

Biosynthesis of fungal NPs and elicitation

Chemical ecology in marine environments: fungi *vs*
- fungi
- bacteria
- microalgae
- invertebrates



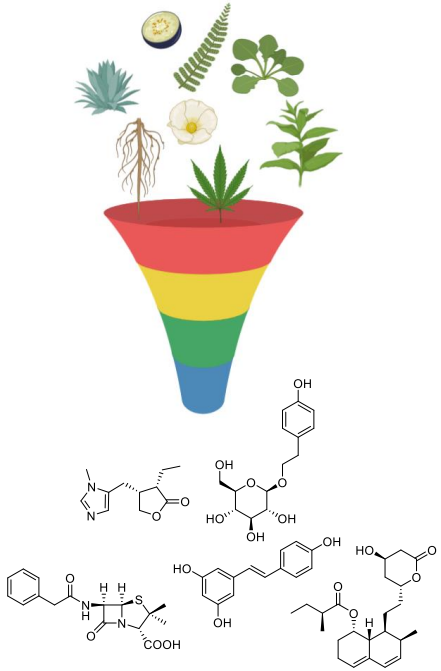


Strategies for researching natural products for therapeutics

« Top-down » strategies

Traditional use of plants and animals: ethnopharmacology

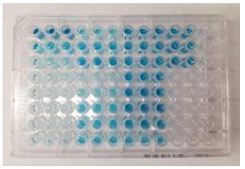
Screening natural extracts libraries



→ Bioguided fractionation

A historical gold-standard pipeline: bioguided purification

Multi target screening

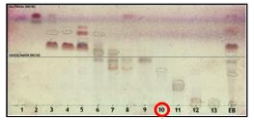


Hits selection

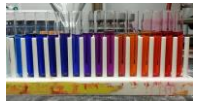
Bioguided fractionation

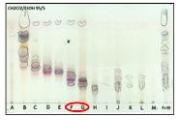
Principle of bioguided fractionation and purification

Bioactive crude extract

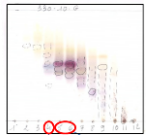


Fr. 1 Fr. 2 Fr. 3 Fr. 4 Fr. 5 Fr. 6 Fr. 7 Fr. 8 Fr. 9

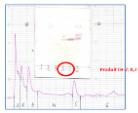




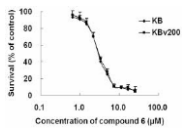
Fr. 4-1 Fr. 4-2 Fr. 4-3 Fr. 4-4 Fr. 4-5 Fr. 4-6 Fr. 4-7



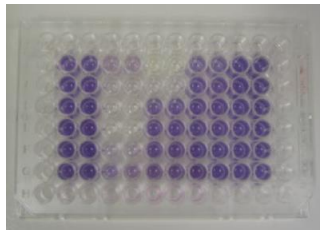
Fr. 4-6-1 Fr. 4-6-2 Fr. 4-6-3 Fr. 4-6-4 Fr. 4-6-5 Fr. 4-6-6 Fr. 4-6-7 Fr. 4-6-8 Fr. 4-6-9 Fr. 4-6-10

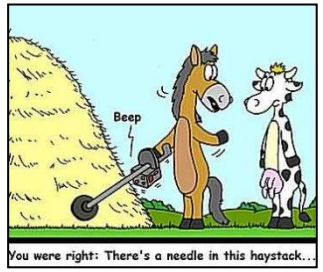


Purified bioactive compound



Structure?
New/known compound?

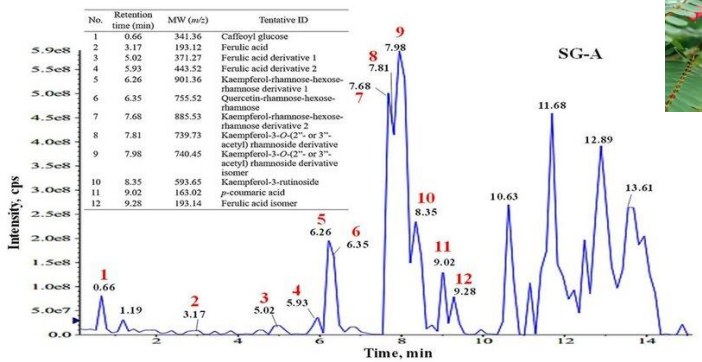




Strategies for researching natural products for therapeutics

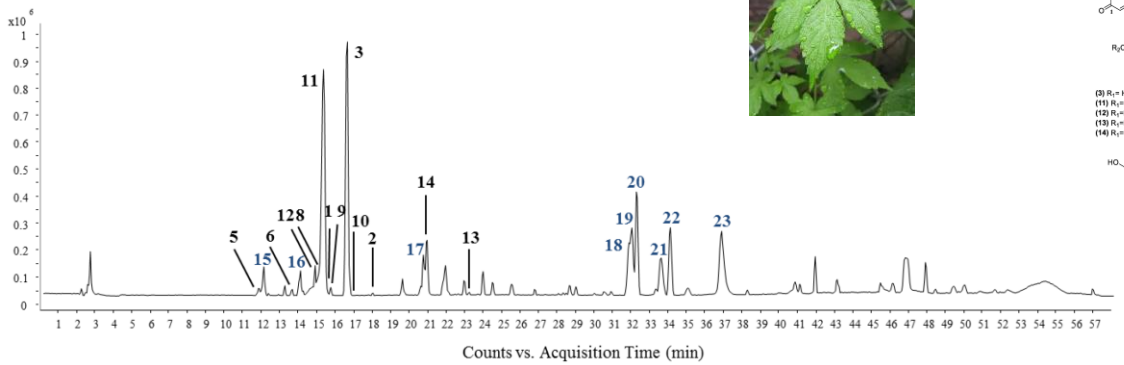
« Top-down » strategies main issues: lack of novelty / few compounds in a series

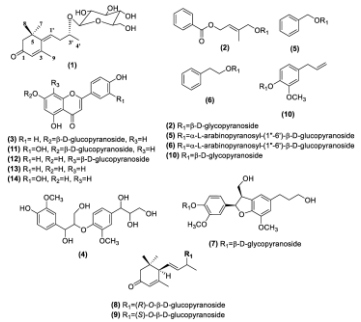
Sesbania grandiflora leaf extract



Mahmud N. *et al*., *Mal. J. Microbiol*., **2020**, 382-385

Humulus japonicus leaf extract



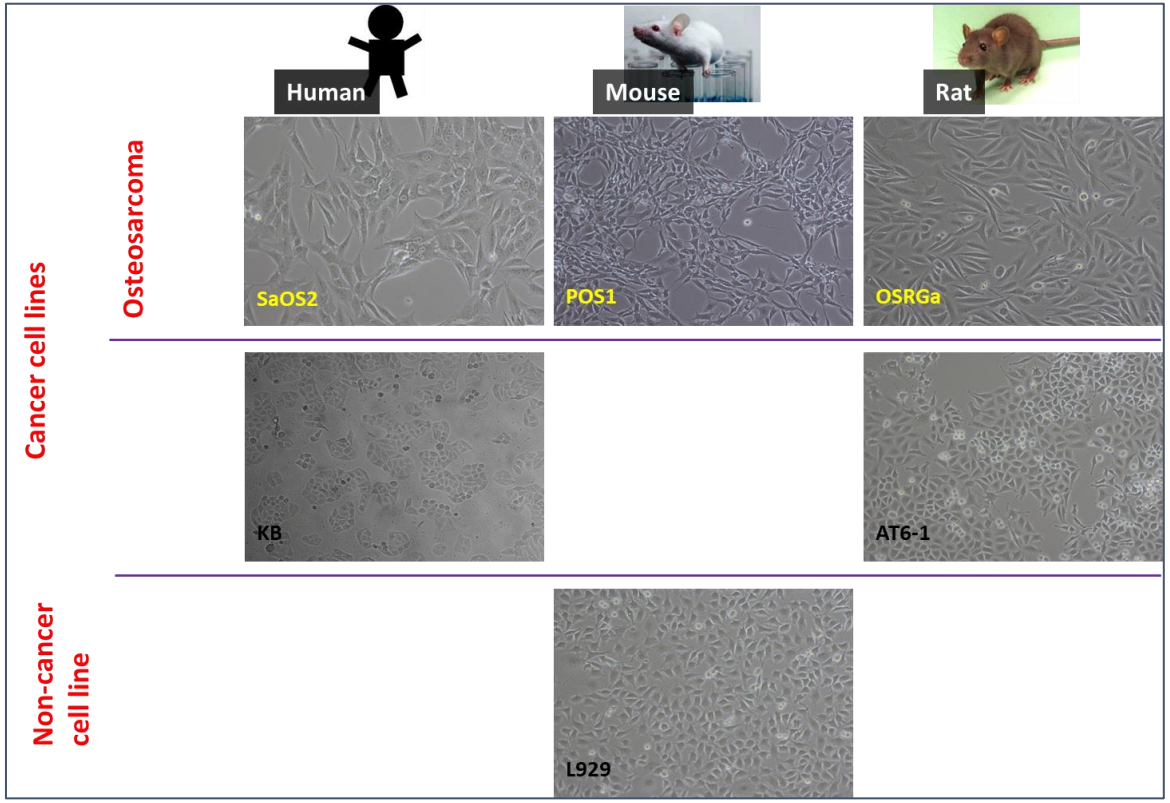


H.J. Lee *et al*., *Molecules*, **2019**, 1435

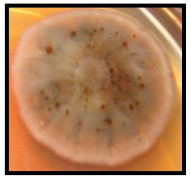
Screening of marine-sourced *Penicillium* extracts for osteosarcoma proliferation inhibition

⇨ Screening for cytotoxicity: EtOAc and/or EtOAc/CH_2Cl_2 (1:1) extracts of cultures on sea water media

⇨ **≈ 500 fungal culture extracts screened**



Strain MMS351

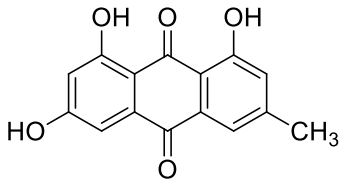


11 days culture
YES/sea water medium
27°C
EtOAC total extract

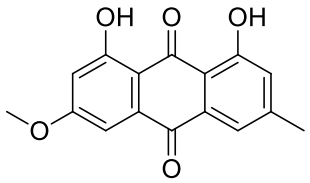
POS1: 21% inhibition at 50 ng/mL
AT6-1: 11% inhibition at 50 ng/mL
KB : IC_{50} = 57 µg/mL

Isolation of bioactive metabolites from *P. ligerum strain* MMS351

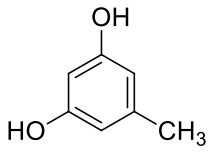
Isolated compounds



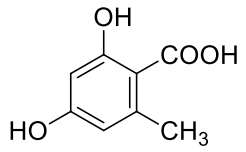
Emodine



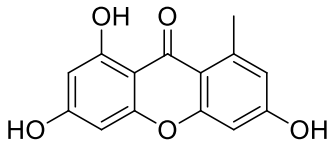
Physcion



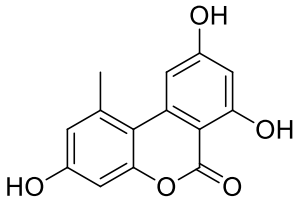
Orcinol



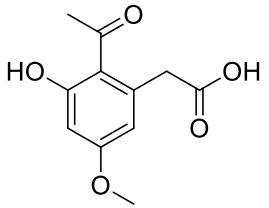
Orsellinic acid



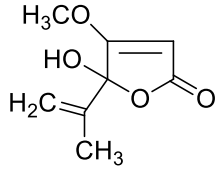
Norlichexanthone



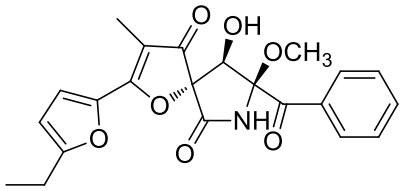
Alternariol



3-O-methylcurvulinic acid



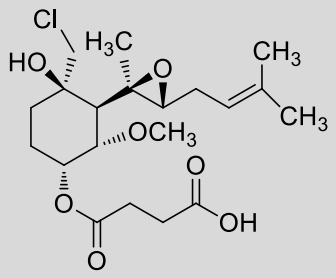
Penicillic acid



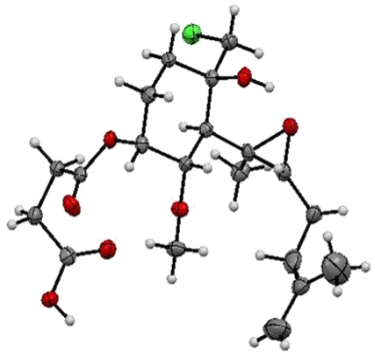
FD-838

LIGERIN

IC_{50} (POS1) = 78 nM

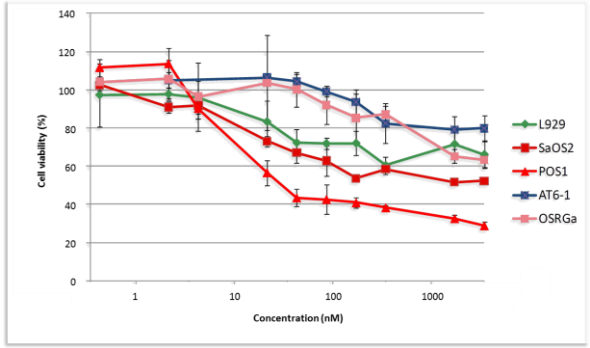


1*S*,2*S*,3*R*,6*R*,13*R*,14*R*

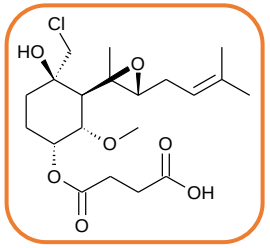


Vansteelandt *et al.*, *J. Nat. Prod.*, 2013,7 6, 297-301

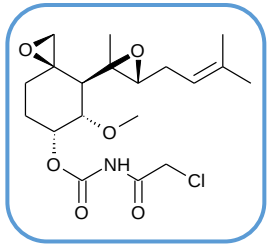
In vitro comparative evaluation of ligerin *vs* TNP-470 and standard anticancer compounds



Ligerin



TNP-470



Takeda Pharm.

	Murine cell lines			Human cell lines		
	IC_{50} (nM)		SI	IC_{50} (nM)		SI
	POS1	**L929**		**SaOS2**	**HFF2**	
Ligerin	**78**	**>2300**	**>29**	**137**	**>2300**	**>17**
TNP470	2	>2300	>961	508	1979	4
Paclitaxel	95	521	5	52	NT	
Vincristine	75	419	6	11	NT	
Doxorubicine	43	161	4	48	NT	
Irinotecan	6300	6500	1	NT	NT	
Fludarabine	5700	17500	3	NT	NT	

Ligerin: **- activity higher on osteosarcoma cell lines**

- similar activity with vincristine and doxorubicine on POS1 cell line

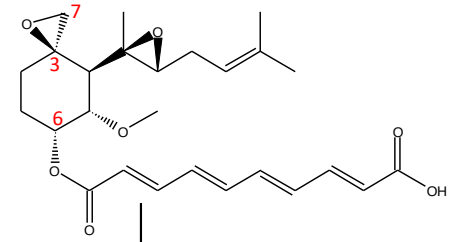
- lowest toxicity on non-tumor cell lines (L929 and HFF2)

- highest selectivity on human cells

Ligerin analogs library construction: semisynthesis of new C6-branched derivatives

3-steps optimized semisynthesis from commercial fumagillin:

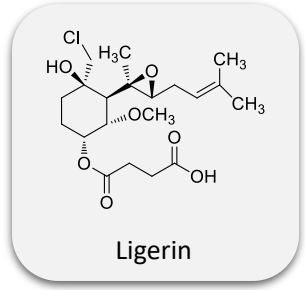
Fumidil B®
Dicyclohexylamine fumagillin



NaOH/H_2O

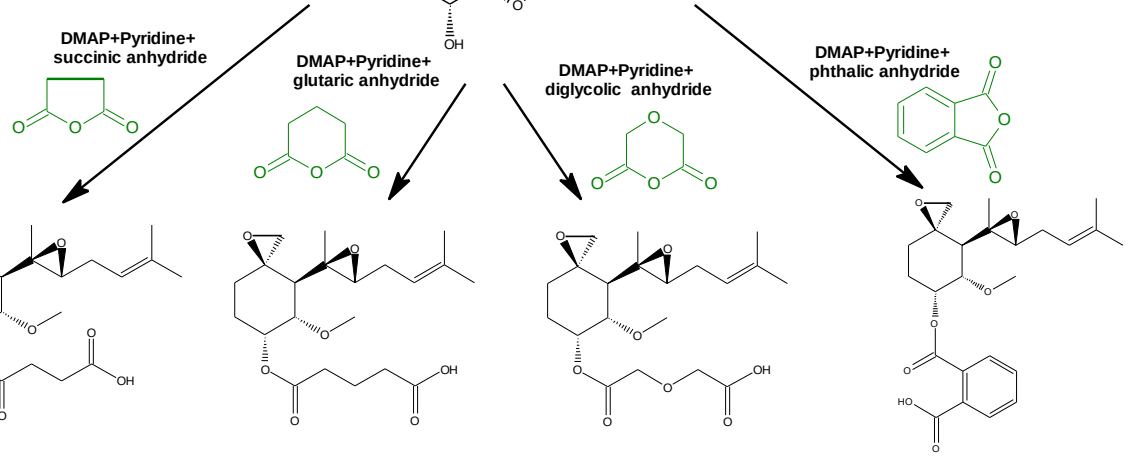
1/ Alkaline hydrolysis

rt, 18 h



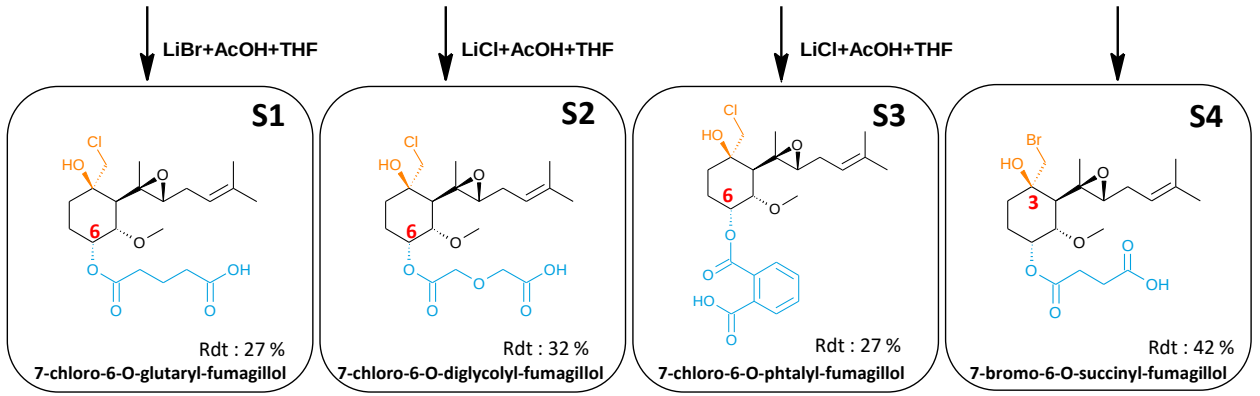
2/ Esterification

rt, 24 h

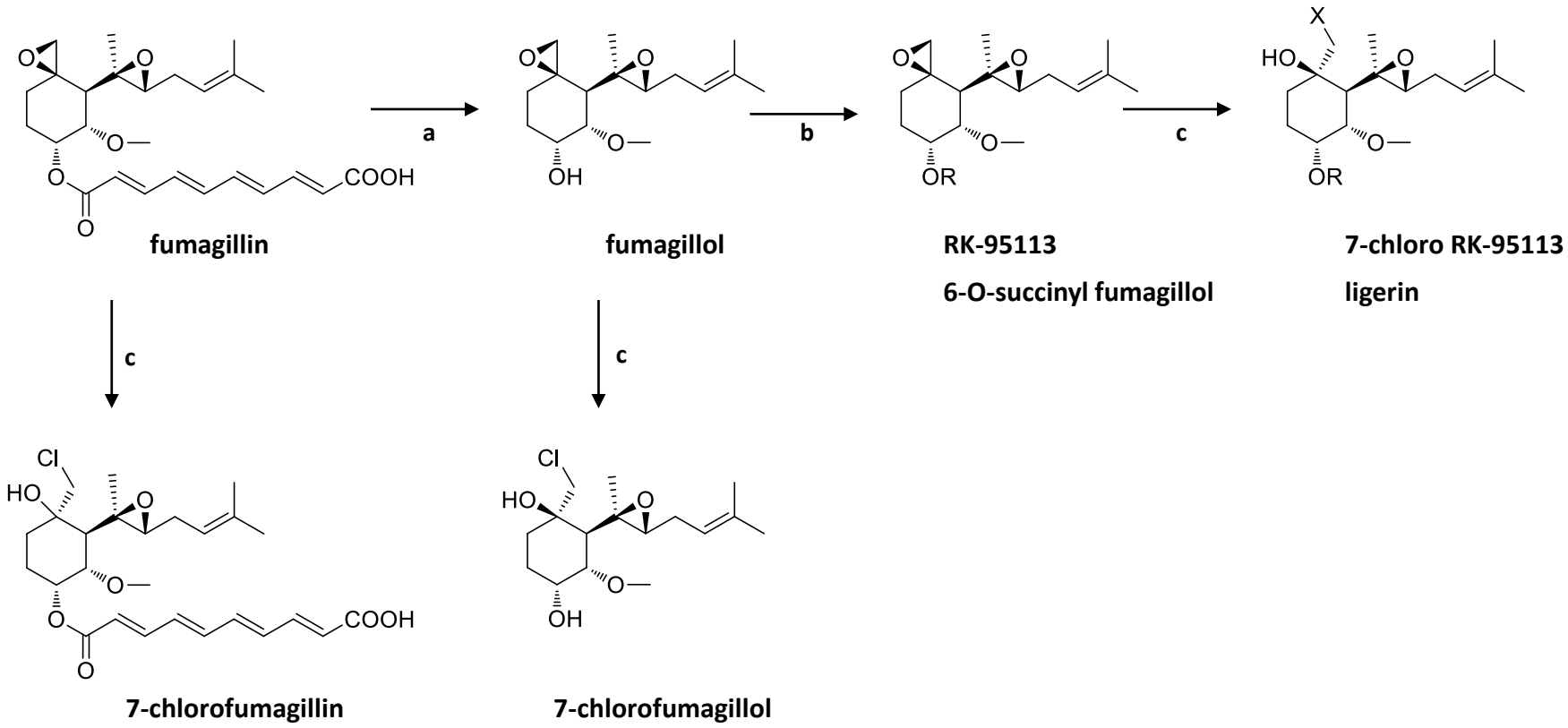


3/ Halogenation

rt, 24 h



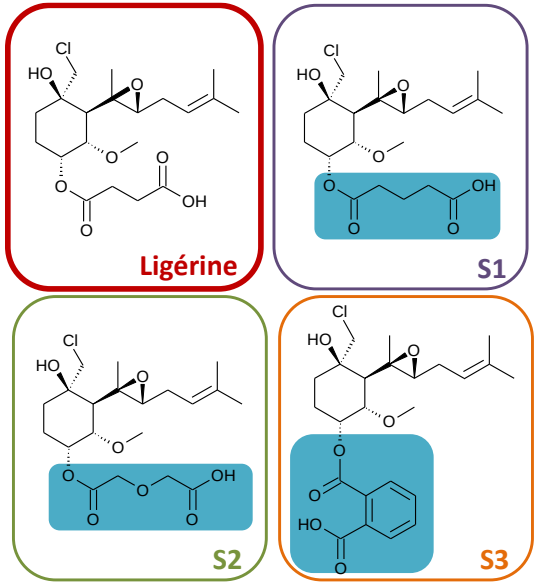
Ligerin analogs library construction: semisynthesis of new halogenohydrin derivatives



a) Hydrolysis (0.5 N NaoH, rt, 18 h); **b)** Esterification (anhydride, DMAP/dry pyridine, rt, 24 h); **c)** Halogenation (LiCl or LiBr, THF, acetic acid, rt, 24 h).

Blanchet E. *et al.*, *Eur. J. Med. Chem.*, **2014**, 79C, 244–250 ; Egorov *et al.*, FR 2973376 B1 (2013), WO 2012130906 A1(2012)

Antiproliferative evaluation of new analogs of ligerin

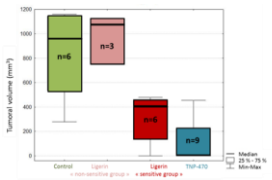


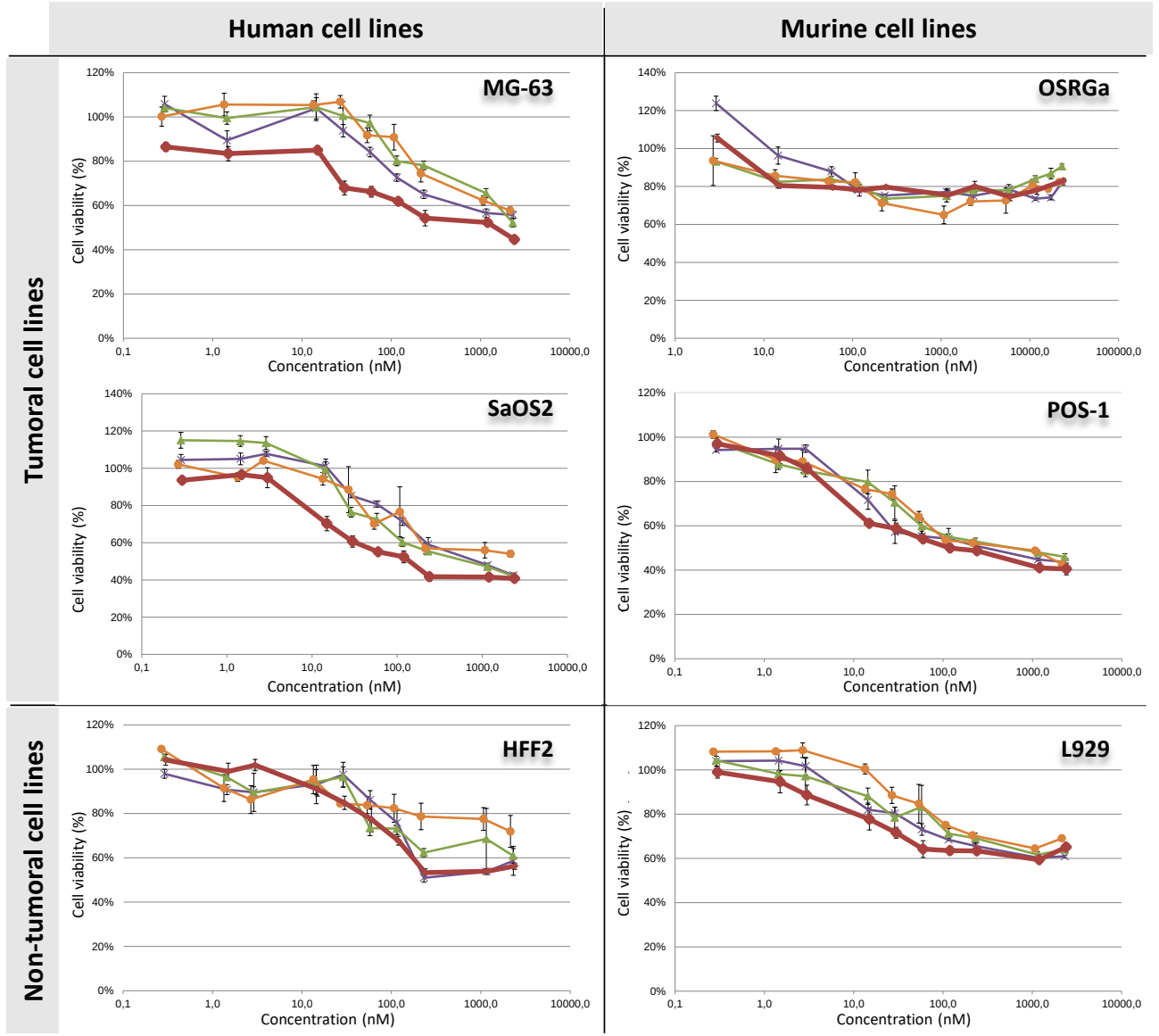
→ no enhancement of the activity

→ Ligerin exhibits the highest activity and selectivity

Ligerin (30mg/kg), SC

CTL

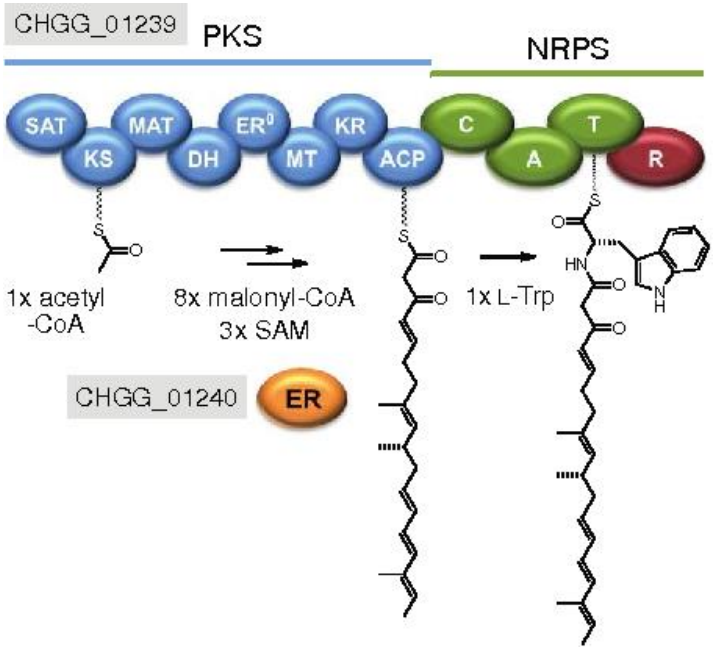


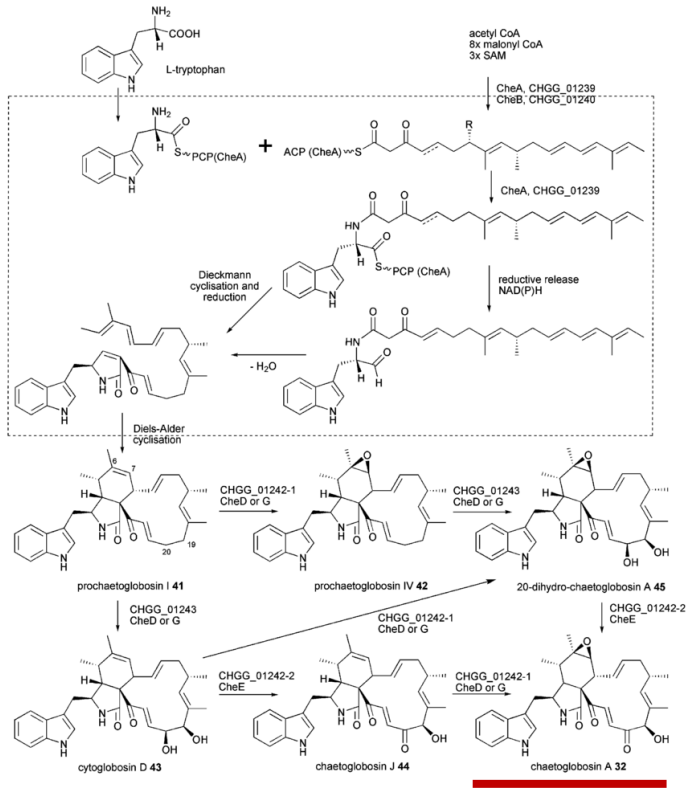


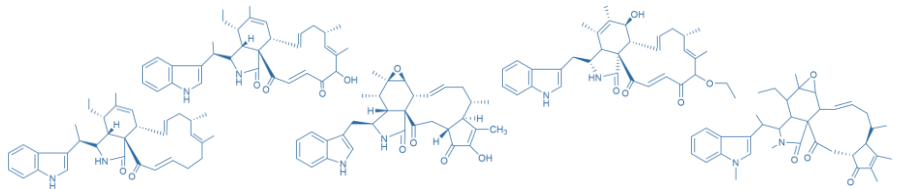
Blanchet E. *et al.*, *Eur. J. Med. Chem.*, **2014**, 79C, 244–250 ; Egorov *et al.*, FR 2973376 B1 (2013), WO 2012130906 A1(2012)

Nature is a source of large under-explored chemical libraries

The chaetoglobosins metabolic pathway







1 Biosynthetic Gene Cluster

↓

1 pathway

↓

1 main final product

↓

Many co-metabolites

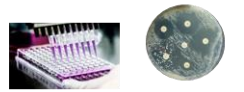
↓

- > 80 chaetoglobosins
- from > 25 publications

Ishiuchi K. *et al.*, *JACS*, **2013**, 7371-7377

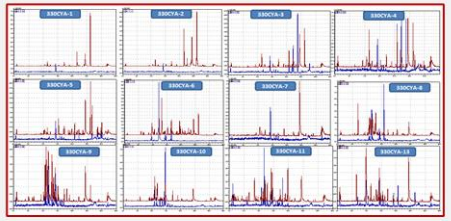
« Bottom-up » strategies for enriching NPs libraries from *de novo* dereplication of extracts

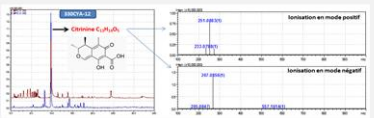
Crude extracts library



Multi target screening

Metabolite profiling and dereplication



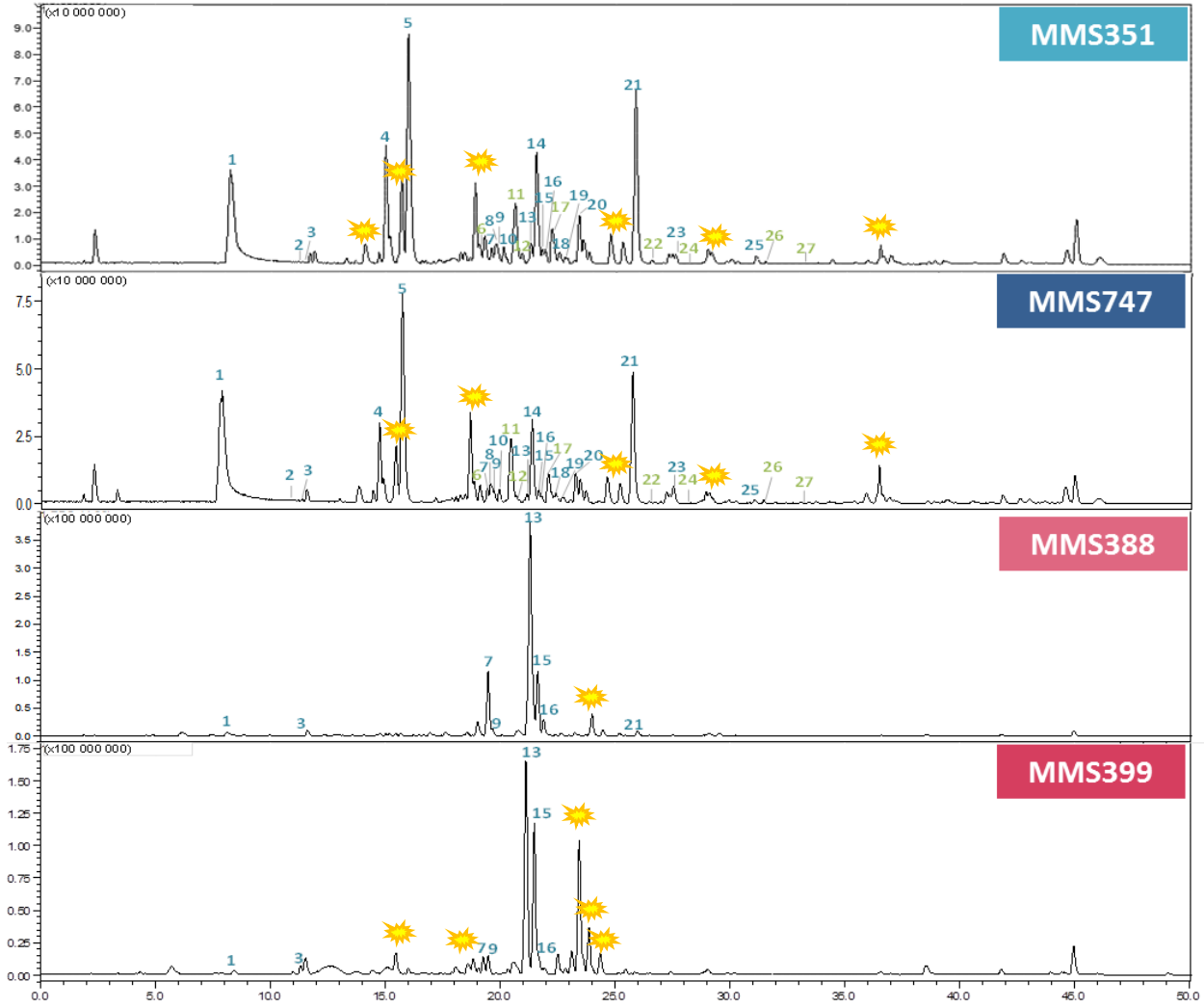


Hits selection

MS-guided purification of targeted compounds

Library of new (bioactive) compounds

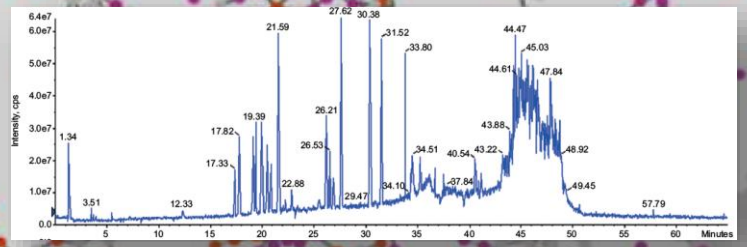
« Bottom-up » strategies for enriching NPs libraries from *de novo* dereplication of extracts



1. Acide pénicillique
2. Acide orsellinique
3. Acide 3-O-methyl-curvulinique
4. Agroclavine
5. Festuclavine
6. Fumagillol
7. Dé ou épidéchloro-griséofulvine
8. Fiscaline A ou un de ses stéréosisomère
9. Alternariol
10. FD-838
11. 6-O-succinyl-fumagillol
12. 7-chloro-fumagillol
13. Griséofulvine
14. Fiscaline A ou un de ses stéréosisomère
15. 5',6'-Didéhydro-griséofulvine
16. Norlichéxanthone
17. Ligerine
18. Fiscaline C ou Epifiscaline C
19. Fiscaline B
20. Fiscaline C ou Epifiscaline C
21. Nortryptoquivaline
22. Fumagilline
23. Tryptoquivalone
24. 7-chloro-fumagilline
25. Physcion
26. RK-95113
27. 7-chloro-RK-95113

Unknown compounds

Exploration of expressed chemical diversity, dereplication and classification: molecular networks



Mixture of molecules

5-*OH*-Tryptophan
[M+H]$^+$ *m/z* 221.09

Tryptophan
[M+H]$^+$ *m/z* 205.10

N-Acetyl-tryptophan
[M+H]$^+$
m/z 247.11

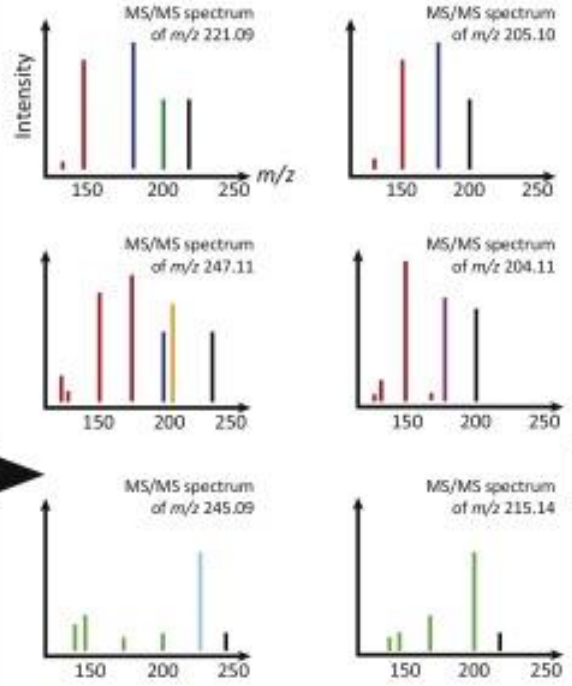
Tryptophamide
[M+H]$^+$ *m/z* 204.11

Biotin [M+H]$^+$
m/z 245.09

Desthiobiotin [M+H]$^+$
m/z 215.14

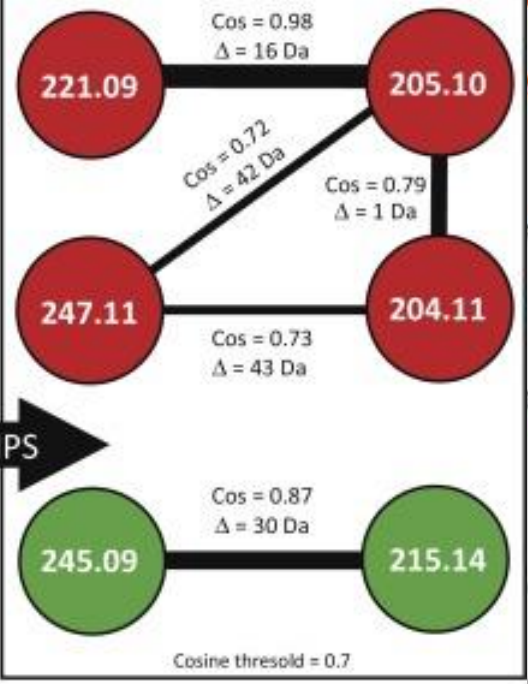
(LC-)MS/MS

Tandem mass spectrometry



GNPS

Molecular networks



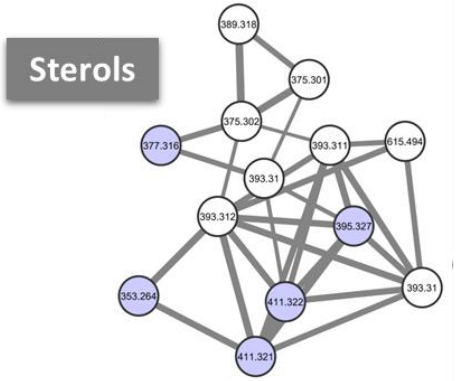
Trends in Pharmacological Sciences

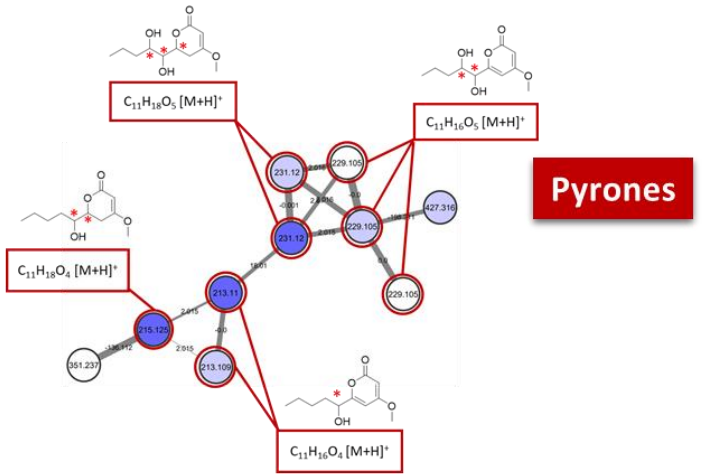
Exploration of expressed chemical diversity, dereplication and classification: molecular networks

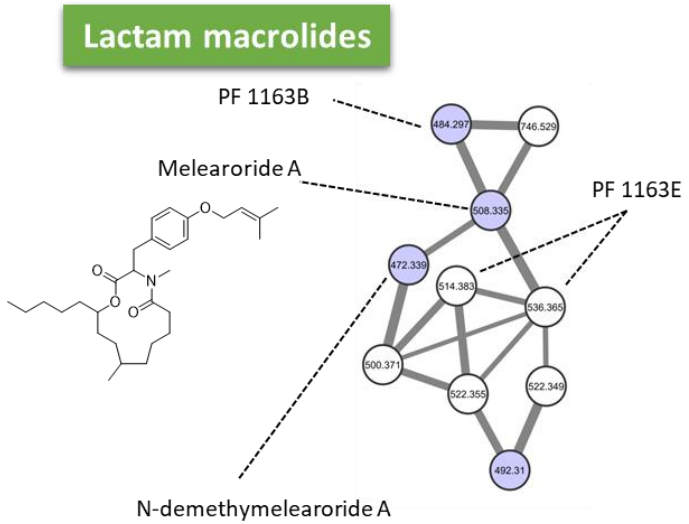
Marine *Penicillium restrictum*
MMS417

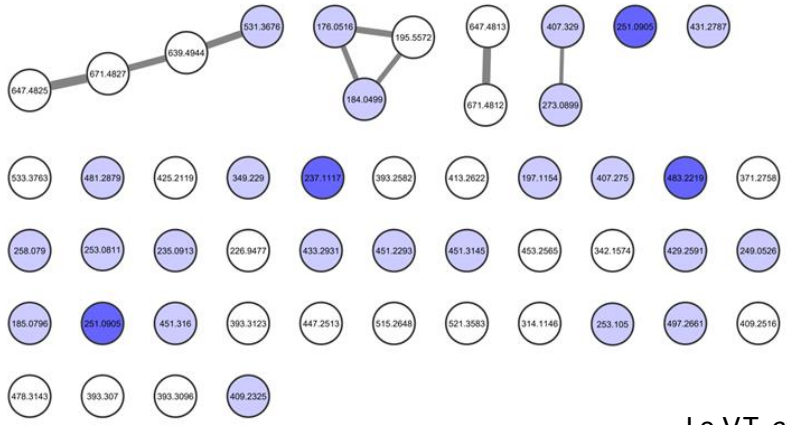
UHPLC-(+/-)HRESIMS/MS data → MZmine 2 → **Feature Based MN** GNPS

Dictionary of Natural Products
LOTUS
ChromAnnot









Le V.T. *et al. Mar. Drugs.* **2021**, 19, 378

Revisiting previously studied plants: targeted isolation of antiplasmodial indole monoterpenoids alkaloids of the serpentine series

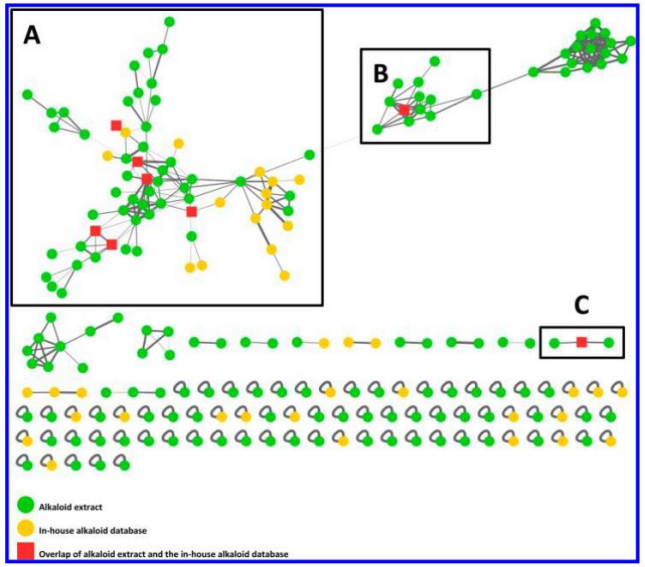
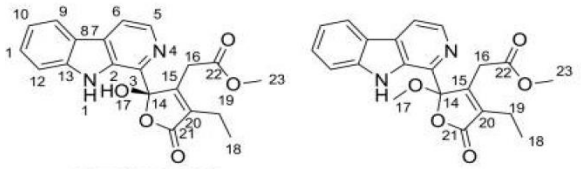


Figure 1. Full molecular network realized using MS/MS data from the alkaloid extract from the bark of *Geissospermum laeve* and the reference compounds from the in-house alkaloid database. The cosine similarity score cutoff for the molecular network was set at 0.6. Details for clusters **A**–**C** are presented in Figures S3 and S4, Supporting Information.

LC-HRMS/MS
Mzmine 2
GNPS

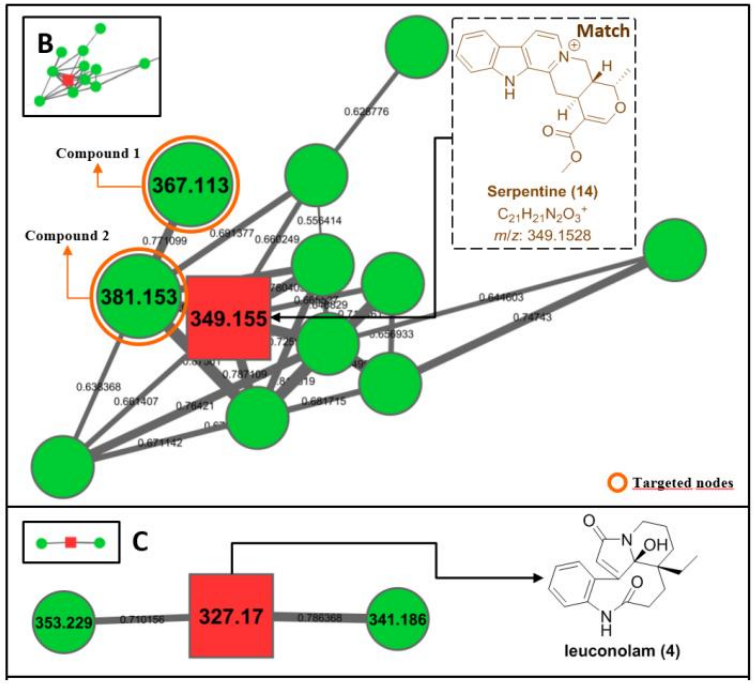
Geissospermum laeve

MS-targeted isolation



geissolaevine (**1**)

O-methylgeissolaevine (**2**)

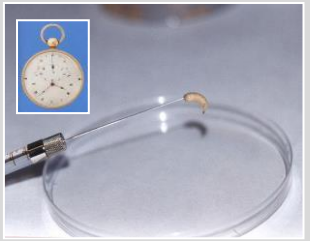


Fox-Ramos *et al*. *J. Nat Prod*., **2017**, 80-1007-1014

Penicillium expansum MMS42: neuroactive rare complex alkaloids

Marine sediments
Le Croisic, France

***Penicillium expansum* MMS42**
DCA medium , 10 days

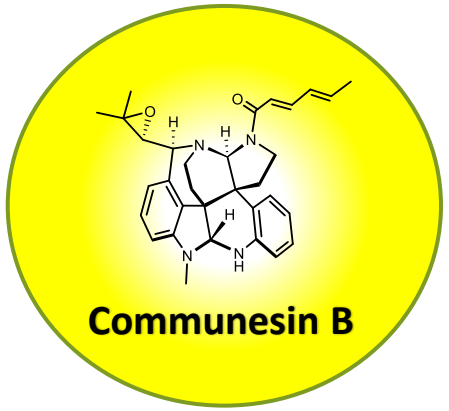


Neuroactivity on Diptera larvae

Bioguided purification

LC-HRMS/MS analyses of extracts

MED = 40 µg/g

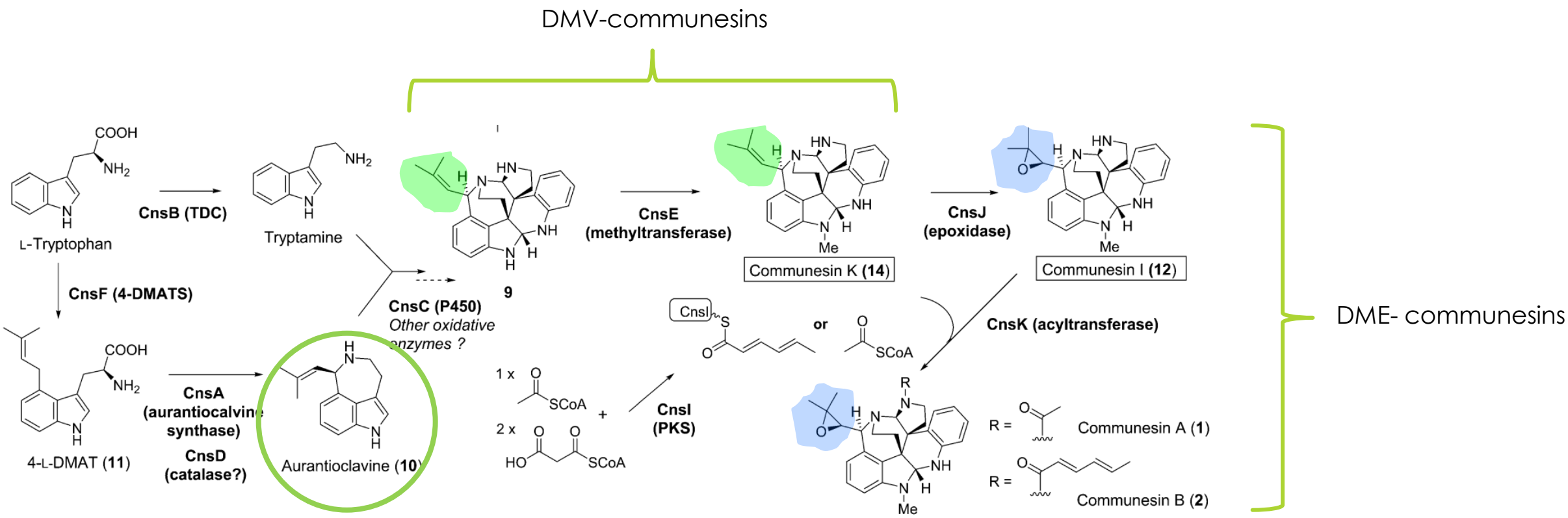


dereplication of 5 other known communesins

structure prediction for 7 putatively new communesins

Biological activities:
Few data :
- antiproliferative (cytoskeleton?)
- antimicrobial, antiviral, insecticide
- cardiovascular effects (zebra fish)

Biosynthesis of communesins: a common precursor and 2 equivalent series ?

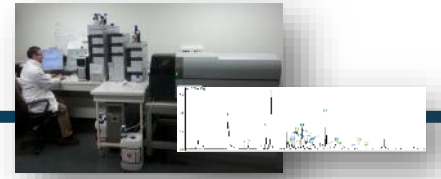


Alternative hypothesis: Com K → acyltransferase → DMV→ epoxydase → DME

Diversity of DME ⇔ diversity of DMV?

Adapted from Lin *et al., Angew. Chem. Int. Ed*. **2015**, 54, 3004-3007.

Molecular network of *P. expansum* MMS 42



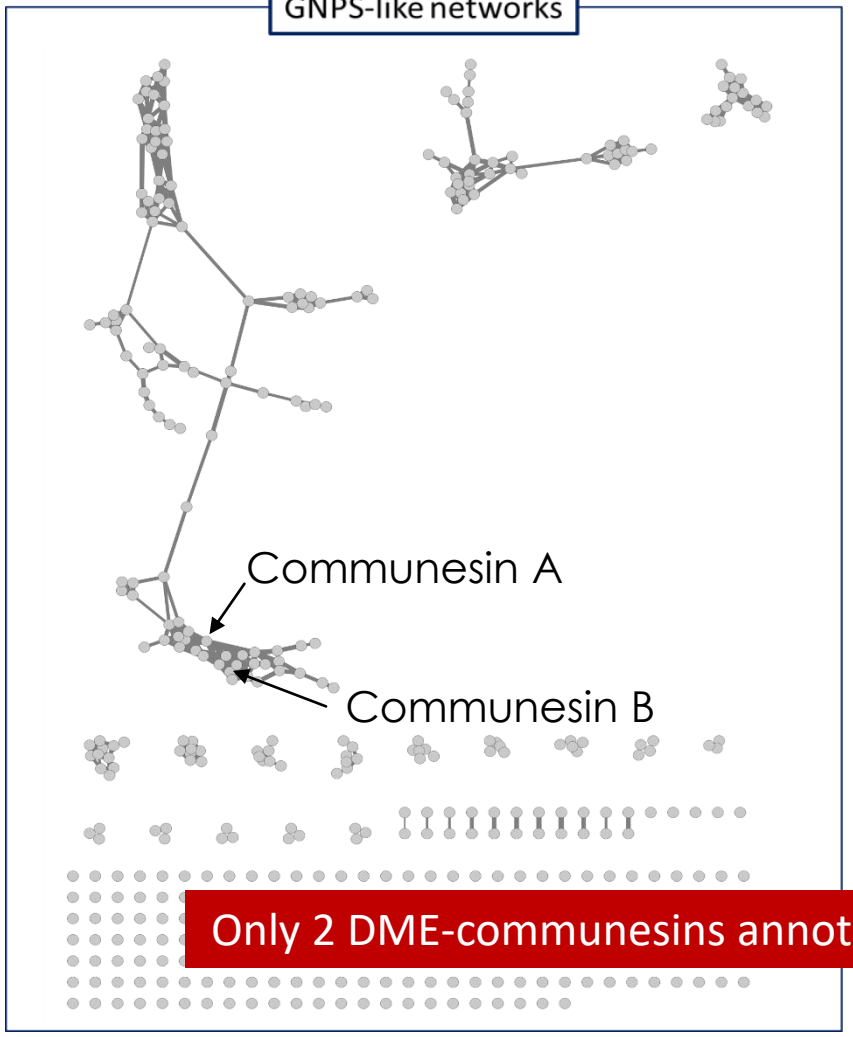
MMS42 *P. expansum*
DCA medium , 10 days

LC-HRMS/MS
of EtOAc extracts

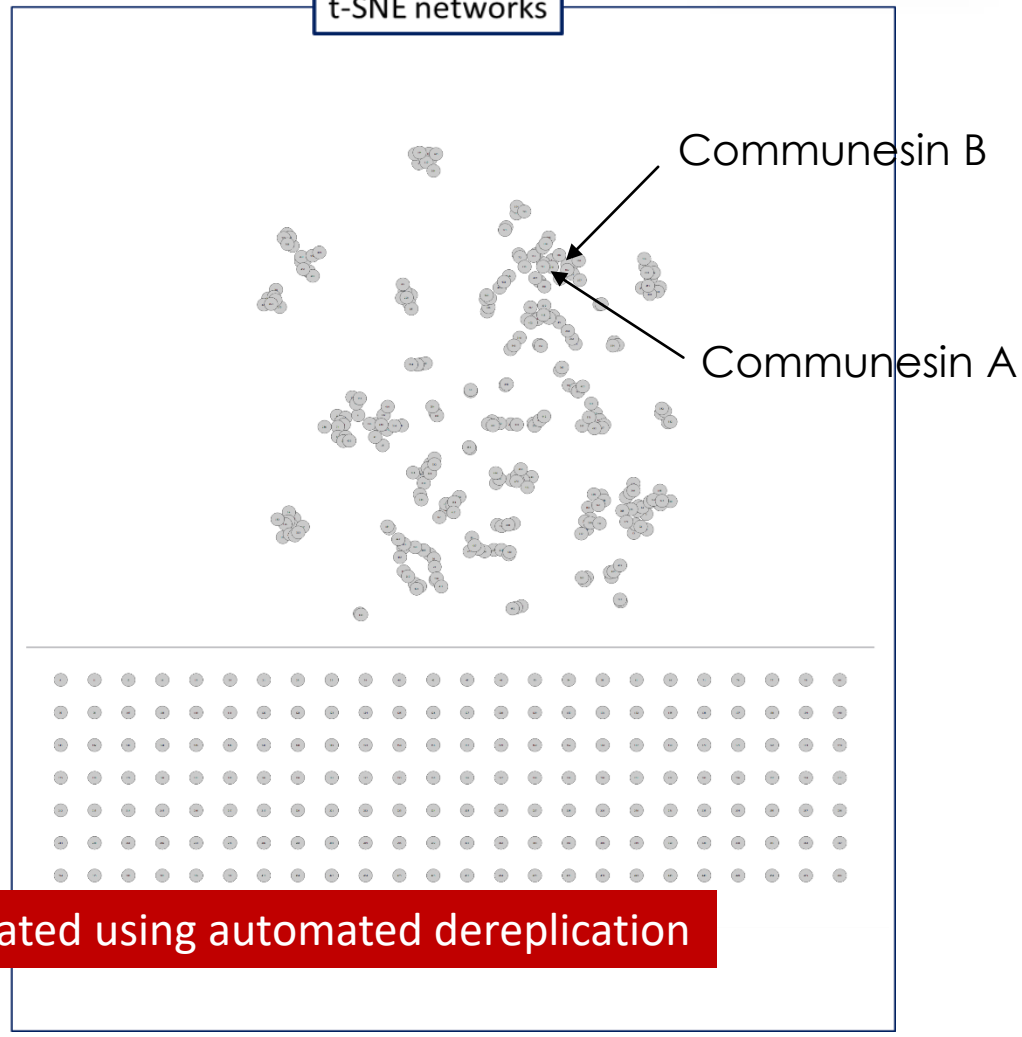
MZmine 2

MetGem

GNPS-like networks



t-SNE networks



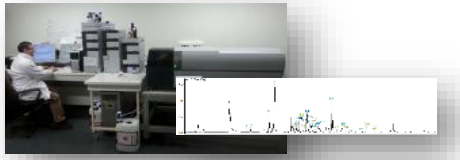
Only 2 DME-communesins annotated using automated dereplication

Min fragments : 4
Cosine: 0.55

At least 1 cosine > 0.5
Iterations: 1000

Olivon *et al.*, *Anal. Chem.*, **2018**, 90, 13900.
https://metgem.github.io

DME-communesins: fragmentation pattern allows dereplication and structure prediction



LC-MS/MS (ESI-IT-TOF)

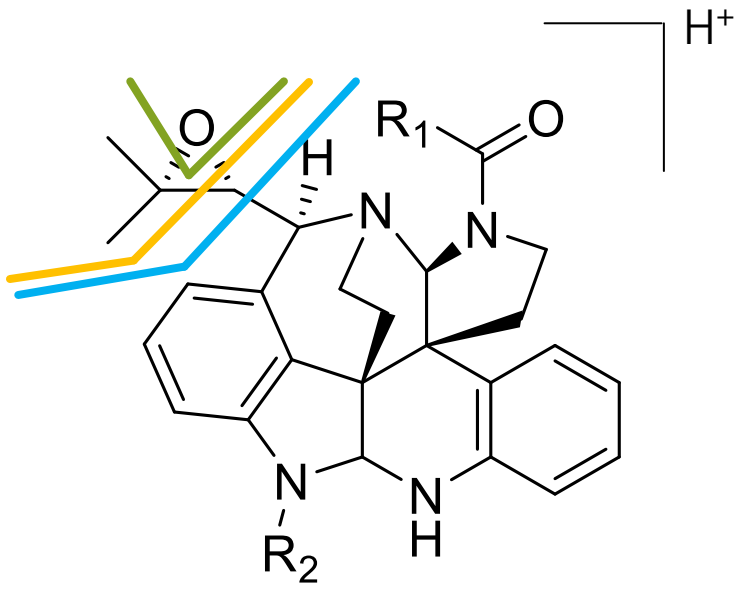
Neutral losses:

- H_2O **- 18**
- $-[(CH_3)_2\text{-}CO]$ **- 58**
- $-[(CH_3)_2\text{-}CO\text{-}CH]$ **- 72**

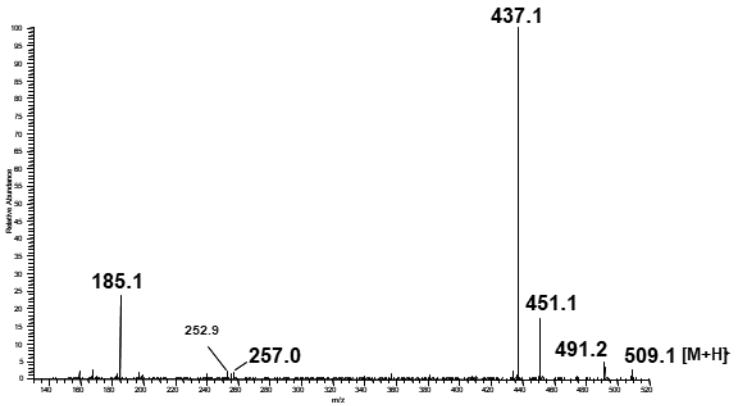
Fragments :

$m/z = 242 + R^2$

$m/z = 170 + R^2$

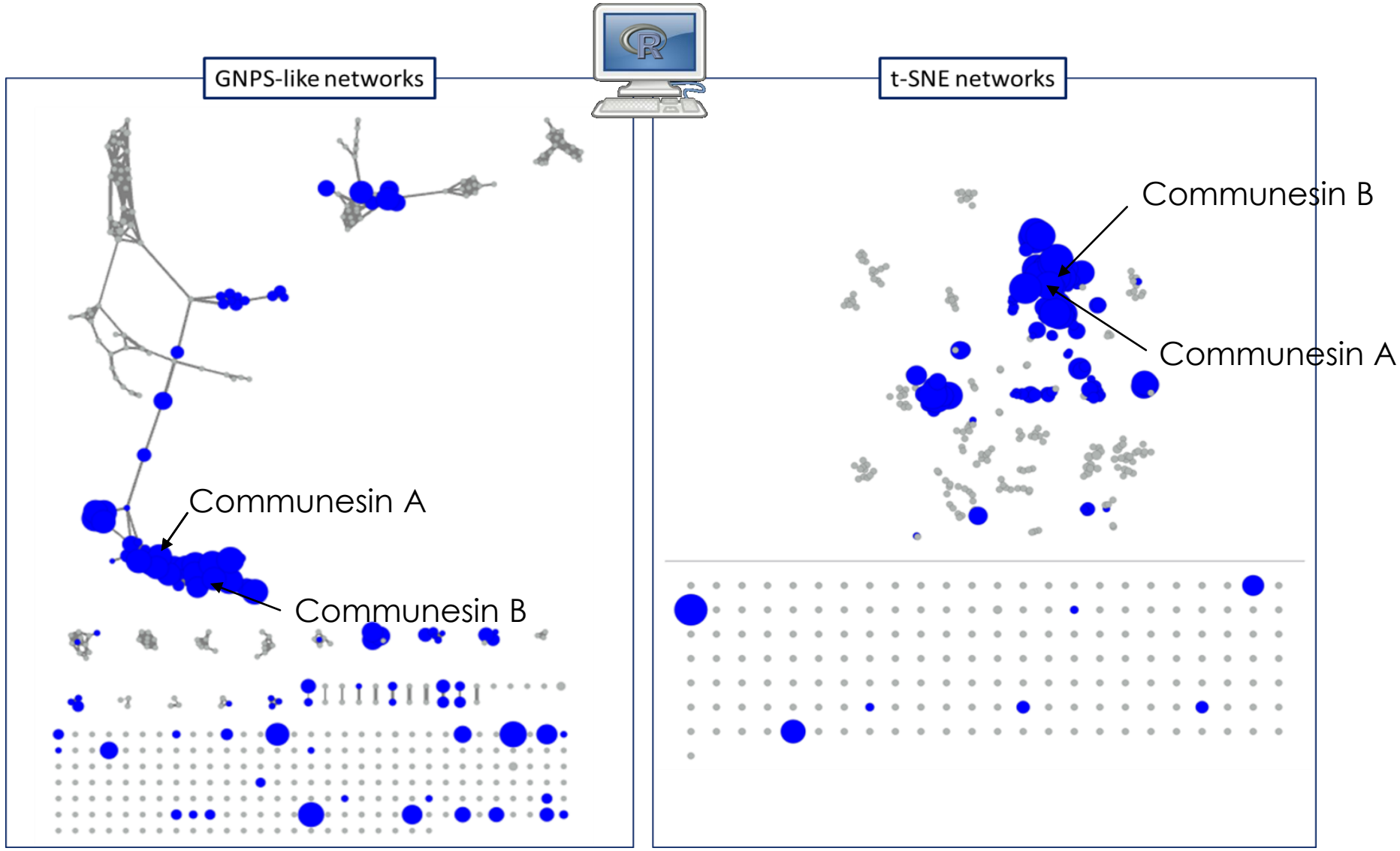


$[M+H]^+ : \ m/z = [427+R^1+R^2]$



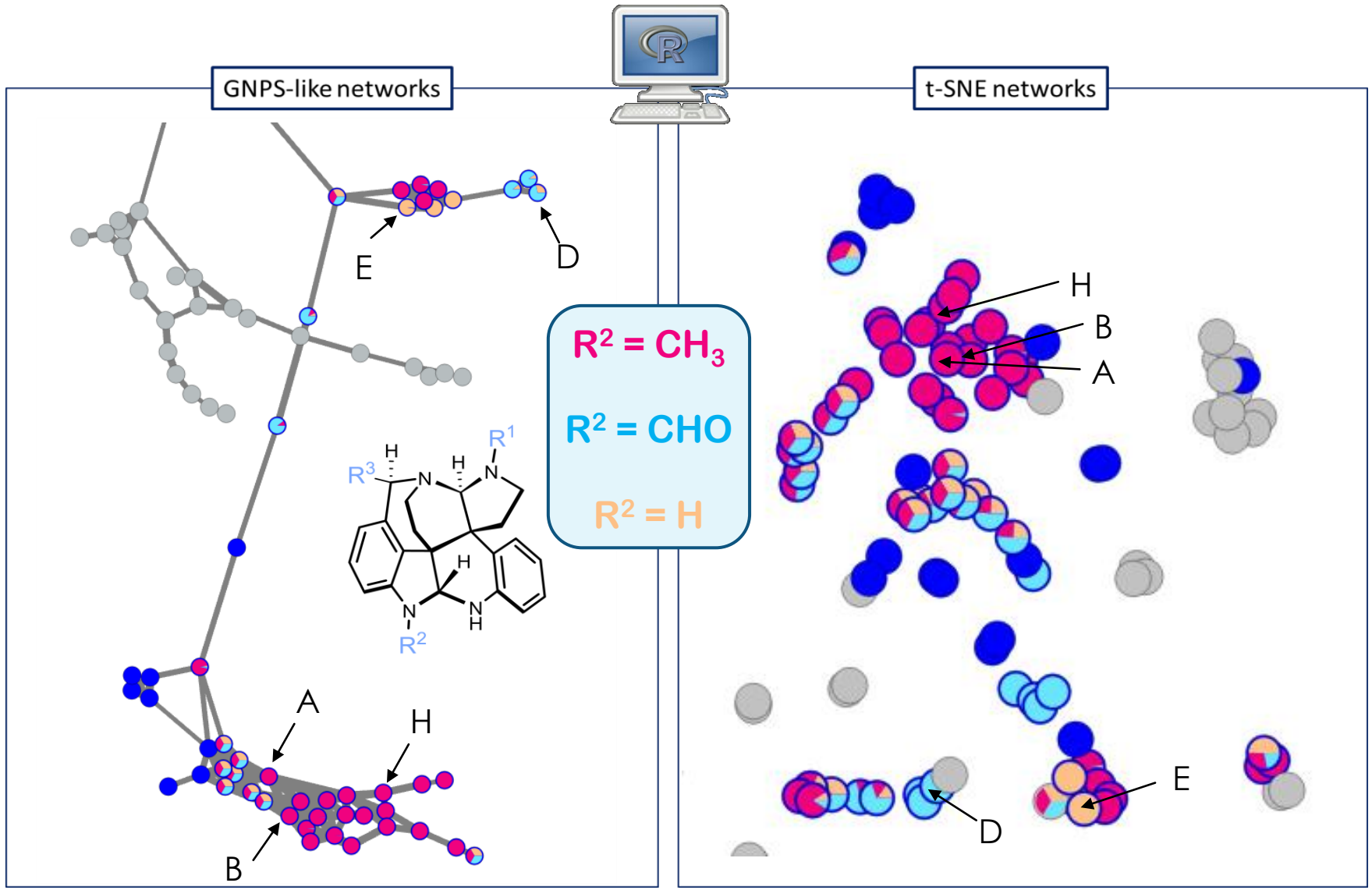
Kerzaon *et al. RCMS.* **2009**, *23*, 3928–3938

Targeted dereplication of DME-communesins using MN and R-script



Node annotated as a DME-communesin. Node size = DME score

Complementary score calculation allows to map structural prediction on MN



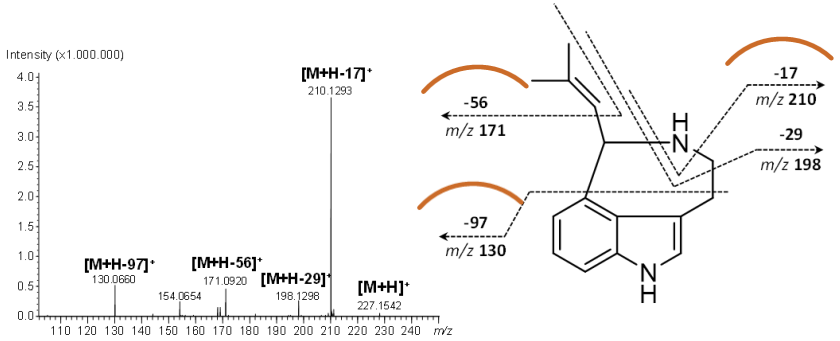
Described communesins

	R^1	R^2	R^3
A	$CO\text{-}CH_3$	CH_3	DME
B	$CO\text{-}C_5H_7$	CH_3	DME
C	$CO\text{-}C_5H_7$	H	DME
D	$CO\text{-}C_5H_7$	CHO	DME
E	$CO\text{-}CH_3$	H	DME
F	$CO\text{-}CH_3$	CH_3	DMV
G	$CO\text{-}C_2H_5$	CH_3	DME
H	$CO\text{-}C_3H_7$	CH_3	DME
I-1	H	CH_3	DME
I-2	$CO\text{-}C_5H_{11}O$	CH_3	DME
J	$CO\text{-}C_5H_7$	CH_3	DMV
K	H	CH_3	DMV

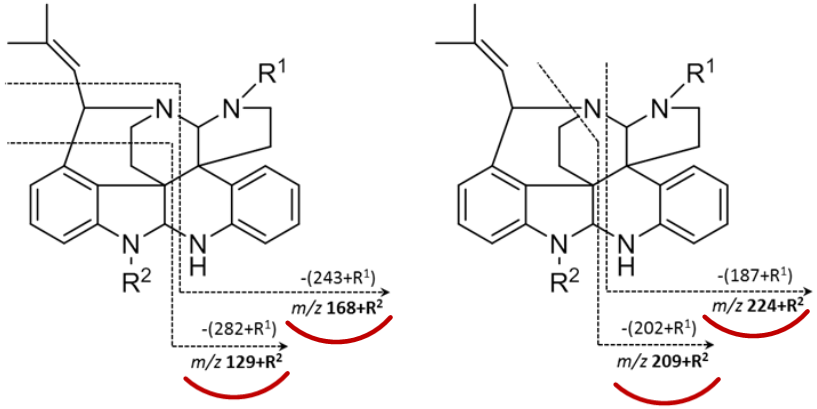
Hoang T.P.T. *et al.*, *Chem. Eur. J.* , **2023**, e202300103

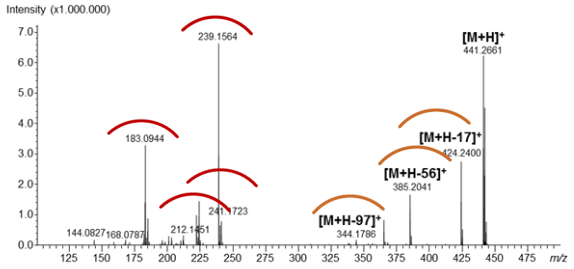
Fragmentation pattern and MS/MS-based structure prediction of DMV-communesins

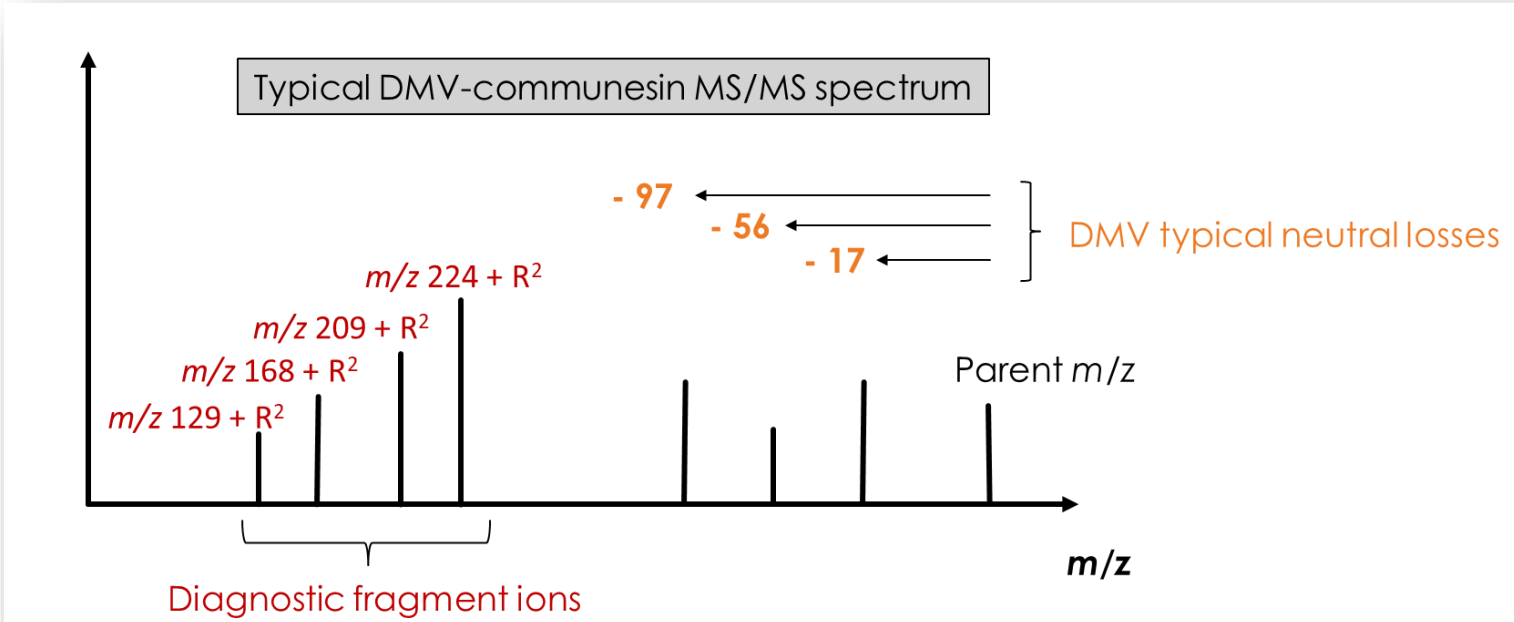
Aurantioclavine MS/MS pattern



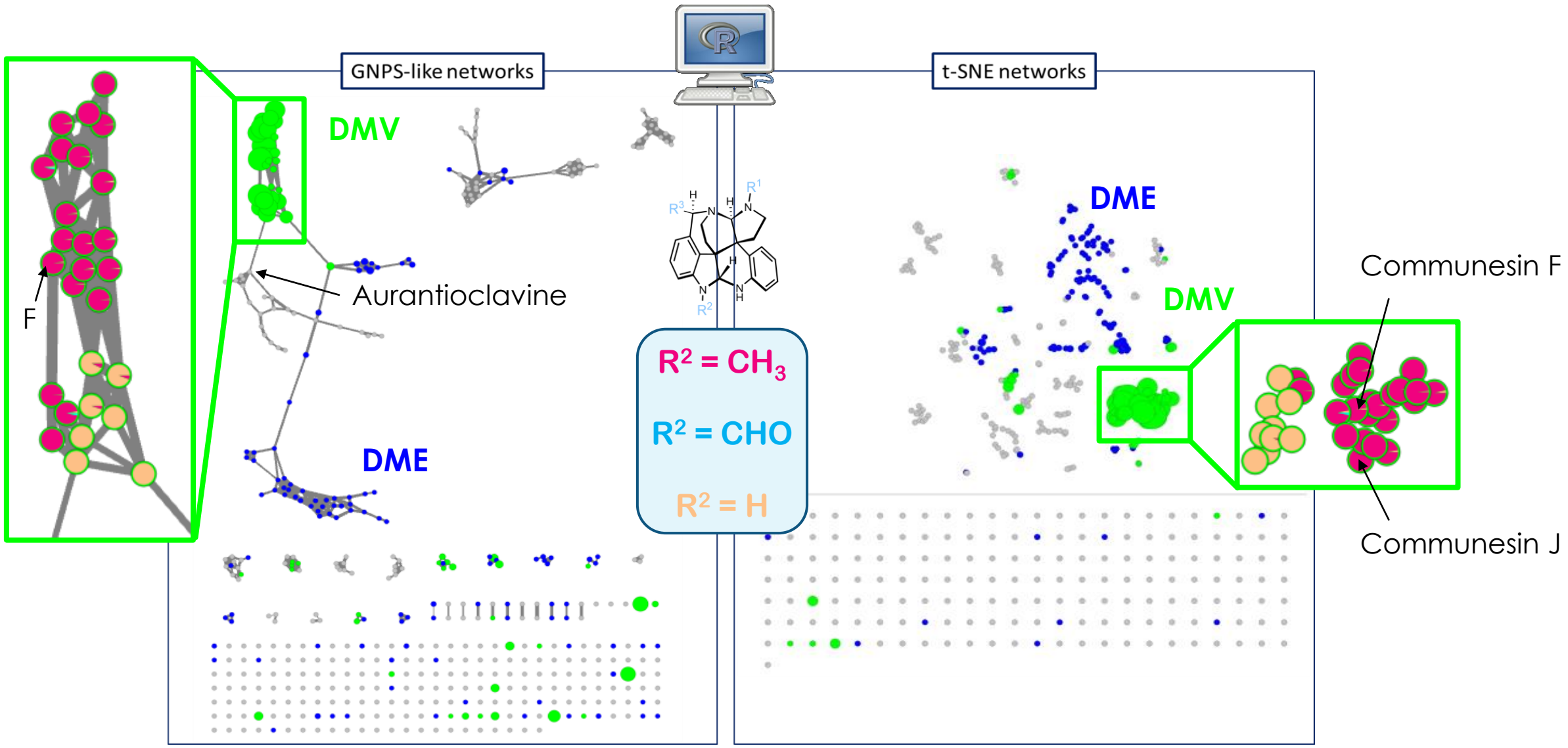
Communesin F MS/MS pattern





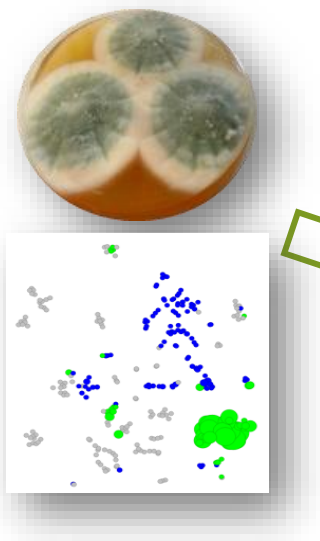


DMV-communesins targeted MN mapped with substituents prediction



Hoang T.P.T. *et al., Chem. Eur. J.* , **2023**, e202300103

Communesins: enhanced targeted MN reveals a huge unexplored chemical diversity



		Communesin	No.*	Exact mass	Formula	Substituent R¹	Substituent R²	Substituent R³
Known compounds	DME	Communesin A	1	456.2525	$C_{28}H_{32}N_4O_2$	CO-CH_3	CH_3	DME
		Communesin B	2	508.2838	$C_{32}H_{36}N_4O_2$	CO-C_5H_7	CH_3	DME
		Communesin D	9	522.2631	$C_{32}H_{34}N_4O_3$	CO-C_5H_7	CHO	DME
		Communesin E		442.2369	$C_{27}H_{30}N_4O_2$	CO-CH_3	H	DME
		Communesin H		484.2838	$C_{30}H_{36}N_4O_2$	CO-C_5H_7	CH_3	DME
		Communesin I-1		414.242	$C_{26}H_{30}N_4O$	H	CH_3	DME
	DMV	Communesin F	4	440.2576	$C_{28}H_{32}N_4O$	CO-CH_3	CH_3	DMV
		Communesin J		492.2889	$C_{32}H_{36}N_4O$	CO-C_5H_7	CH_3	DMV
New compounds produced by MN542	DME	Com400		400.2263	$C_{25}H_{28}N_4O$	H	H	DME
		Com442-1		442.2369	$C_{27}H_{30}N_4O_2$	Not identified		DME
		Com442-2		442.2369	$C_{27}H_{30}N_4O_2$	Not identified		DME
		Com454-1		454.2369	$C_{28}H_{30}N_4O_2$	C_2H_3	CHO	DME
		Com456-1		456.2525	$C_{28}H_{32}N_4O_2$	CO-CH_3	CH_3	DME
		Com456-2		456.2525	$C_{28}H_{32}N_4O_2$	Not identified		DME
		Communesin N	5	470.2318	$C_{28}H_{30}N_4O_3$	CO-CH_3	CHO	DME
		Com470-2		470.2682	$C_{29}H_{34}N_4O_2$	CO-C_2H_5	CH_3	DME
		Com458-1		458.2318	$C_{27}H_{30}N_4O_3$	CO-CH_3O	H	DME
		Com458-2		458.2318	$C_{27}H_{30}N_4O_3$	Not identified		DME
		Com458-3		458.2318	$C_{27}H_{30}N_4O_3$	Not identified		DME
		Com472-1		472.2474	$C_{28}H_{32}N_4O_3$	C_2H_5O	CHO	DME
		Com472-2		472.2474	$C_{28}H_{32}N_4O_3$	C_2H_5O	CHO	DME
		Com474		474.2631	$C_{28}H_{34}N_4O_3$	$C_2H_5O_2$	CH_3	DME
		Com482		482.2682	$C_{30}H_{34}N_4O_2$	CO-C_5H_5	CH_3	DME
		Com484-1		484.2474	$C_{29}H_{32}N_4O_3$	Not identified		DME
		Com486		486.2267	$C_{28}H_{30}N_4O_4$	$C_2H_3O_2$	CHO	DME
		Com488		488.2424	$C_{28}H_{32}N_4O_4$	$C_2H_5O_2$	CHO	DME
		Com494-1		494.2318	$C_{30}H_{30}N_4O_3$	Not identified		DME
		Com494-2		494.2682	$C_{31}H_{34}N_4O_2$	CO-C_5H_7	H	DME
		Com494-3		494.2682	$C_{31}H_{34}N_4O_2$	CO-C_4H_5	CH_3	DME
		Com496-1		496.2474	$C_{30}H_{32}N_4O_3$	CO-C_3H_3O	CH_3	DME
		Com496-2		496.2838	$C_{31}H_{36}N_4O_2$	CO-C_4H_7	CH_3	DME
		Com498		498.2995	$C_{31}H_{38}N_4O_2$	CO-C_4H_9	CH_3	DME
		Com508-1		508.2838	$C_{32}H_{36}N_4O_2$	CO-C_5H_7	CH_3	DME
		Com510-1		510.2631	$C_{31}H_{34}N_4O_3$	CO-C_4H_5O	CH_3	DME
		Com512-1		512.2787	$C_{31}H_{36}N_4O_3$	CO-C_4H_7O	CH_3	DME
		Communesin P	7	512.3151	$C_{32}H_{40}N_4O_2$	CO-C_5H_{11}	CH_3	DME
		Com514		514.2944	$C_{31}H_{38}N_4O_3$	CO-C_4H_9O	CH_3	DME
		Com518		518.2682	$C_{33}H_{34}N_4O_2$	CO-C_6H_5	CH_3	DME
		Com524-1		524.2787	$C_{32}H_{36}N_4O_3$	CO-C_5H_7O	CH_3	DME
		Com524-2		524.2787	$C_{32}H_{36}N_4O_3$	CO-C_5H_7O	CH_3	DME
		Com528		528.31	$C_{32}H_{40}N_4O_3$	CO-$C_5H_{11}O$	CH_3	DME
		Com534		534.2842	$C_{30}H_{38}N_4O_5$	Not identified		DME
		Com536		536.2787	$C_{33}H_{36}N_4O_3$	CO-C_6H_7O	CH_3	DME
		Com542		542.2893	$C_{32}H_{38}N_4O_4$	CO-$C_5H_9O_2$	CH_3	DME
		Com544		544.2474	$C_{34}H_{32}N_4O_3$	CO-C_7H_5O	CH_3	DME
		Com546		546.2631	$C_{34}H_{34}N_4O_3$	CO-C_7H_5O	CH_3	DME
		Com548		548.2787	$C_{34}H_{36}N_4O_3$	CO-C_7H_7O	CH_3	DME
		Com550		550.2944	$C_{34}H_{38}N_4O_3$	CO-C_7H_9O	CH_3	DME
		Com568		568.2686	$C_{33}H_{36}N_4O_5$	CO-CH_3	$C_6H_7O_3$	DME
		Com620		620.2999	$C_{37}H_{40}N_4O_5$	CO-C_5H_7	$C_6H_7O_3$	DME
	DMV	Com412-1		412.2263	$C_{26}H_{28}N_4O$	CHO	H	DMV
		Com412-2		412.2263	$C_{26}H_{28}N_4O$	CHO	H	DMV
		Com426-1		426.242	$C_{27}H_{30}N_4O$	CO-CH_3	H	DMV
		Com426-2		426.242	$C_{27}H_{30}N_4O$	CO-CH_3	H	DMV
		Com426-3		426.242	$C_{27}H_{30}N_4O$	CHO	CH_3	DMV
		Com454-2		454.2733	$C_{29}H_{34}N_4O$	CO-C_2H_5	CH_3	DMV
		Communesin F	6	454,2369	$C_{28}H_{30}N_4O_2$	CO-CH_3	CHO	DMV
		Com468		468.2889	$C_{30}H_{36}N_4O$	CO-C_3H_7	CH_3	DMV
		Com484-2		484.2838	$C_{30}H_{36}N_4O_2$	CO-C_3H_7O	CH_3	DMV
		Com484-3		484.2838	$C_{30}H_{36}N_4O_2$	CO-C_3H_7O	CH_3	DMV
		Com496-3		496.2838	$C_{31}H_{36}N_4O_2$	CO-C_4H_7O	CH_3	DMV
		Communesin C	8	496.3202	$C_{32}H_{40}N_4O$	CO-C_5H_{11}	CH_3	DMV
		Com512-3		512.3151	$C_{32}H_{40}N_4O_2$	CO-$C_5H_{11}O$	CH_3	DMV
		Com536		536.2787	$C_{33}H_{36}N_4O_3$	CO-$C_6H_7O_2$	CH_3	DMV
		Com584		584.2999	$C_{34}H_{40}N_4O_5$	Not identified		DMV

64 predicted compounds

→ **8** (/12) known communesins

→ **42 undescribed DME-**communesins

R^2 = other detected

R^3 = -CH_3, -C_2H_5, -C_3H_5, -C_4H_5, -C_4H_9, -C_5H_7, -CH_3O, -C_4H_5O, -$C_5H_{11}O$, -$C_5H_9O_2$,…

→ **14 undescribed DMV-**communesins

R^3 = similar diversity as for DME-communesins

Hoang T.P.T. *et al.*, *Chem. Eur. J.* , **2023**, e202300103

Semisynthesis of communesins inspired by natural congeners

Optimized production and purification

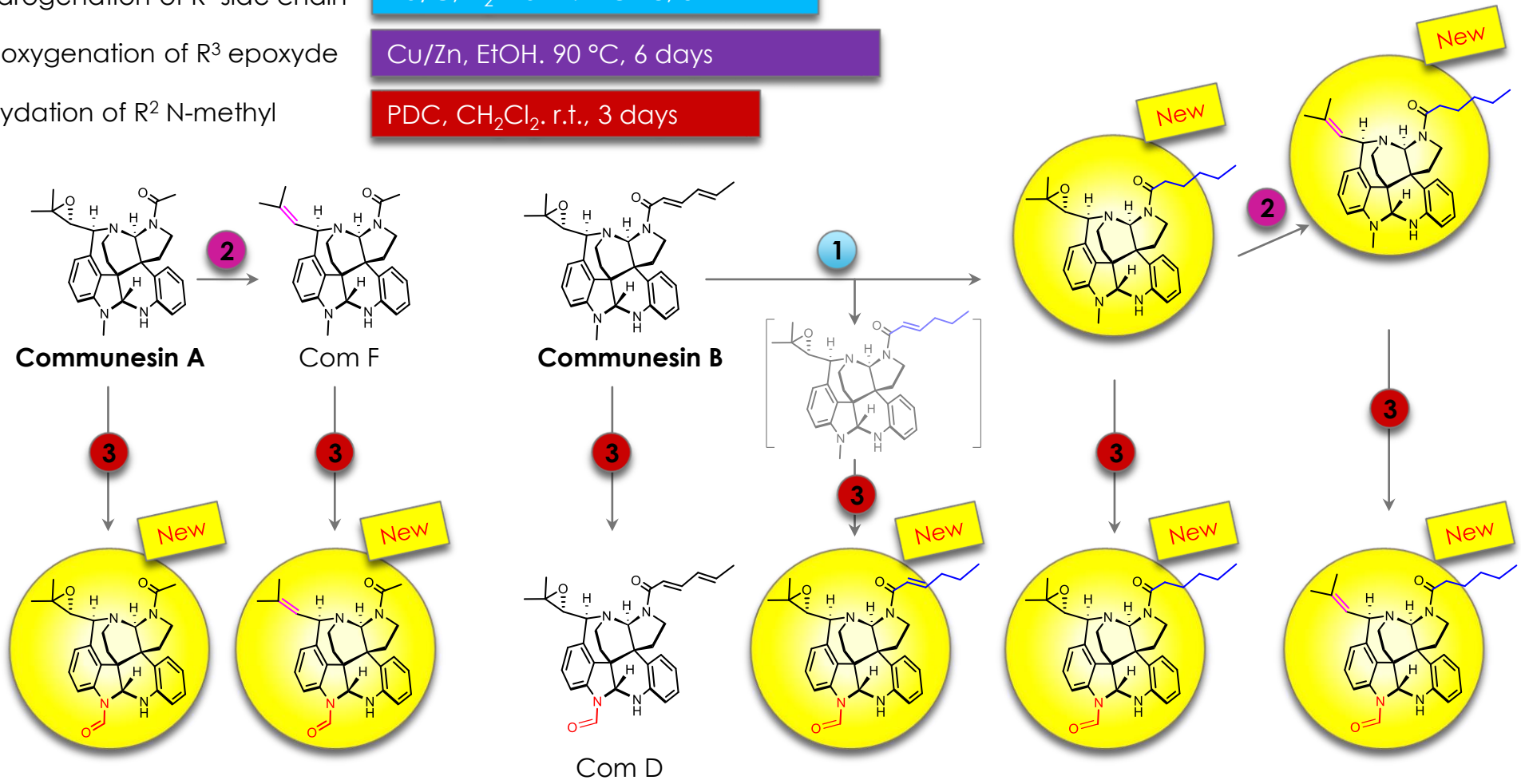
484 flasks

Communesin A
114,8 mg
Communesin B
92,2 mg

1 Hydrogenation of R^1 side chain — Pd/C, H_2 1 atm. EtOAc, 3 h

2 Deoxygenation of R^3 epoxyde — Cu/Zn, EtOH. 90 °C, 6 days

3 Oxydation of R^2 N-methyl — PDC, CH_2Cl_2. r.t., 3 days



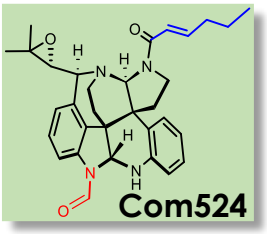
Synthesis of 9 communesins including 7 new

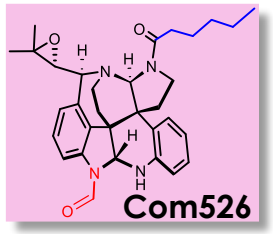
Hoang T.P.T. *et al.*, *Chem. Eur. J.* , **2023**, e202300103

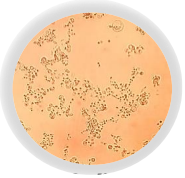
Biological activities and SAR

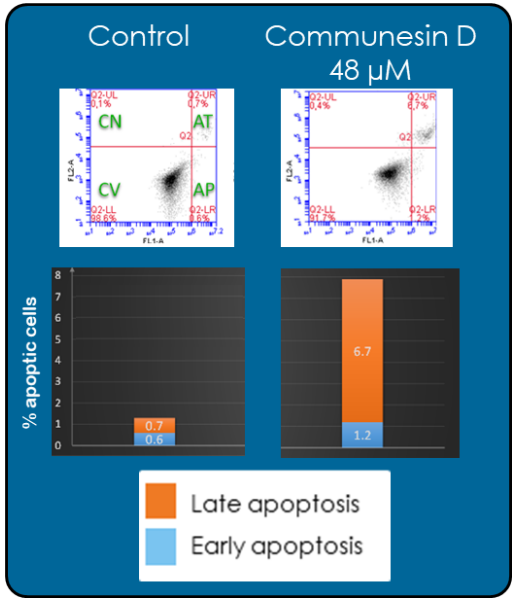
Cytotoxicity on cancer cell lines

IC50 (µM)	M	454	A	D	526	524	F	B	510	N	O
KB	> 50	> 50	> 50	26,6	24,7	**4,8**	40,9	27,5	18,6	17,6	> 50
MCF-7	> 50	> 50	> 50	13,8	**6,3**	**9**	> 50	20,3	13,5	14,4	> 50

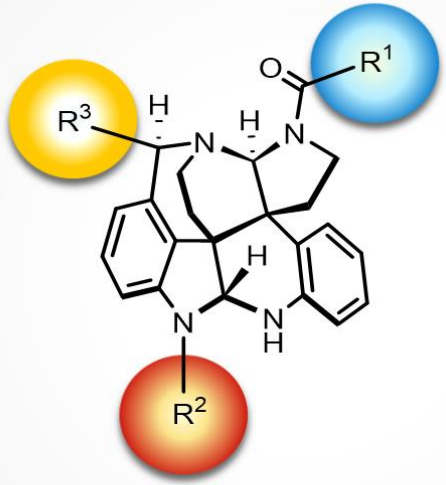








Epoxyde ↗ activity



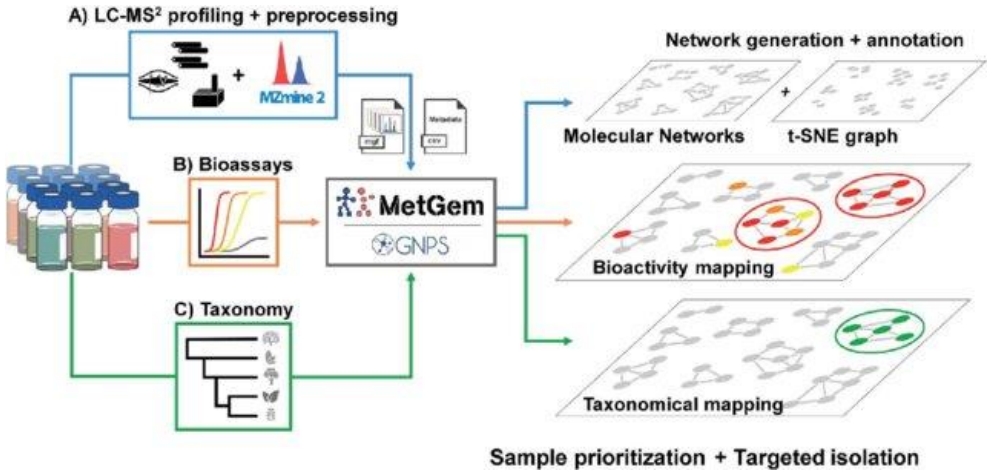
R^1 = methyl → no cytotoxicity
Length and lipophily ↗ activity

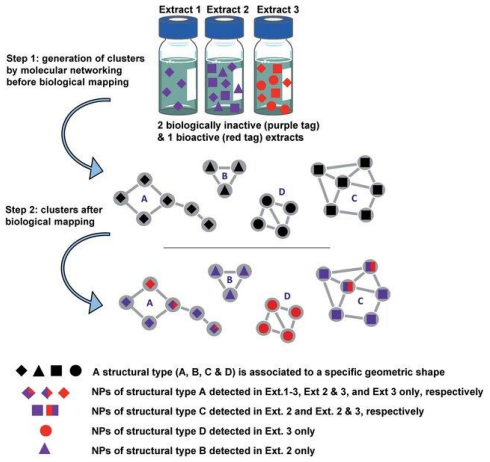
Oxygenated groups ↗ activity

Hoang T.P.T. *et al., Chem. Eur. J.* , **2023**, e202300103

Combining extract fractionation, bioactivity screening and molecular networking for prioritisation and targeted isolation of series of bioactive natural products

Concept

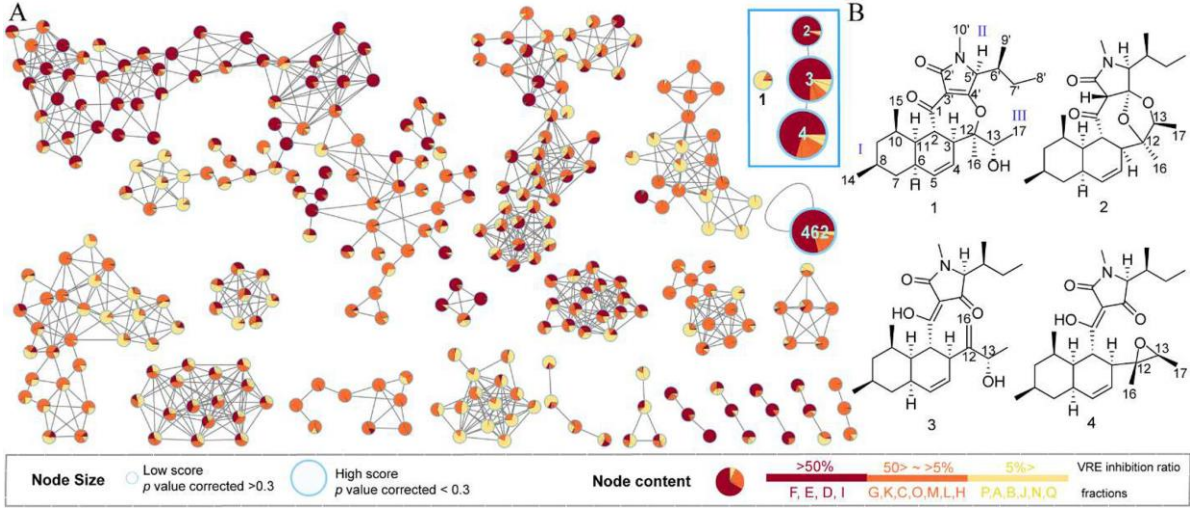




Wolfender J.-L. *et al.*, *Nat. Prod. Rep.*, **2019**, 855-868

Case study

Epicoccum sp.



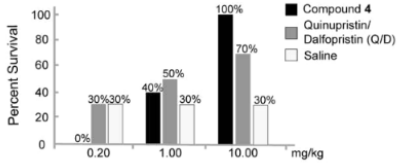


Figure 4. *In vivo* anti-VRE activity of compound **4** in the VRE-*G. mellonella* infection model.

Chang S. *et al.*, J. *Nat. Prod. Rep.*, **2024**, 1582-1590

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