ÚOCHB AV ČR
IOCB PRAGUE

Fantastic Natural Products and Where to Find Them

Dr. Olena Mokshyna | 31.01.2023

IOCB Prague

Tomáš Pluskal Group

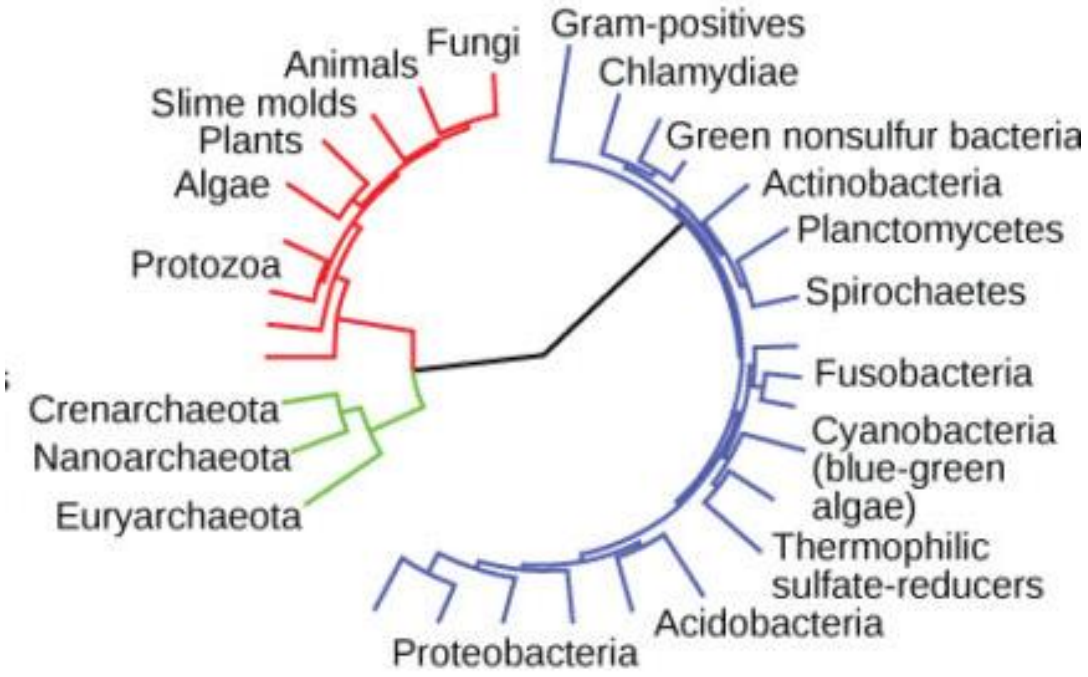
Biochemistry of Plant Specialized Metabolites

Experiments Computations
for bioactive molecules discovery

MZmine 3

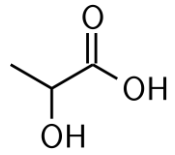
transXpress

Natural Products (NPs): what are they?

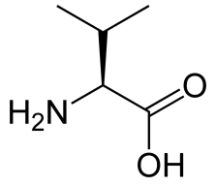


Any compound produced by **living organisms**

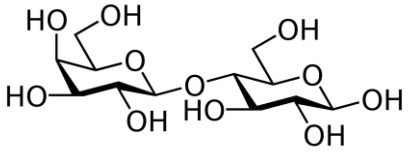
Primary metabolites



Lactic acid



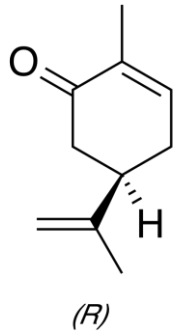
Valine



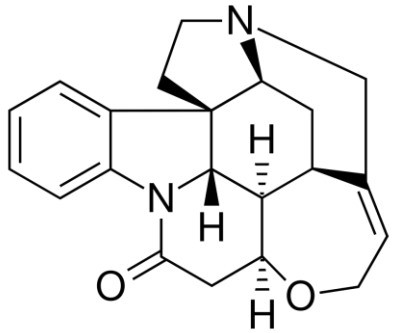
Lactose

No pharmacological activity!

Secondary metabolites

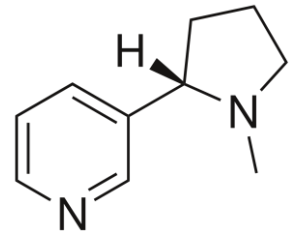


(R)-carvone



Strychnine

Biologically active



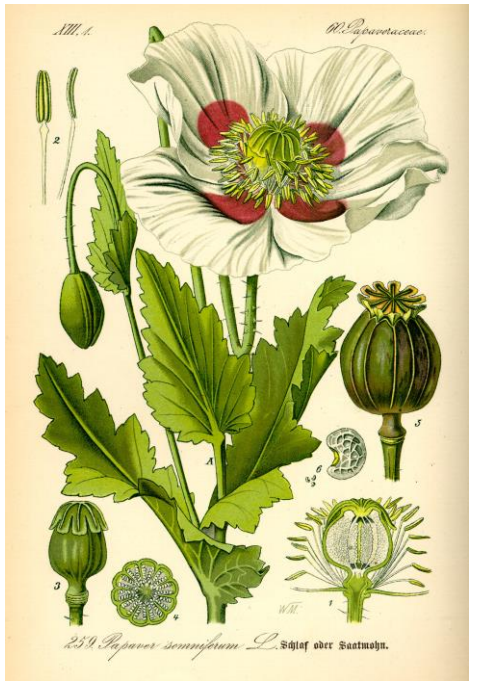
Nicotine

Plants with unusual properties become first medicines

Sumerian clay tablet,
c. 3000 BC

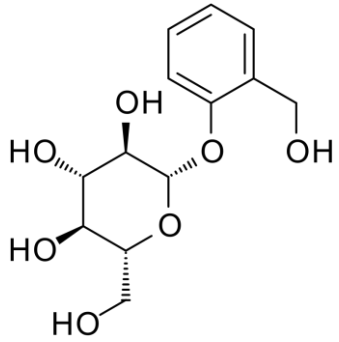
Ebers papyrus, Ancient Egypt
c. 1550 BC

Arabic Dioscorides,
1224

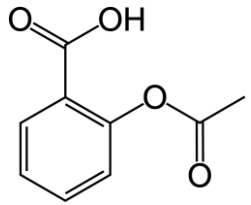


Papaver somniferum

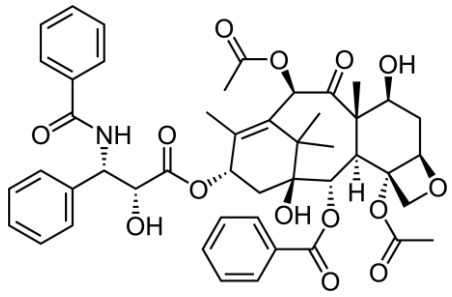
Since then, NPs became a Prominent Source of Drugs



Salicin

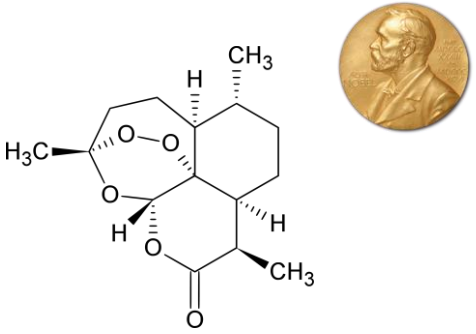


Aspirin



Taxol
Anticancer

Taxus brevifolia,
the Pacific yew



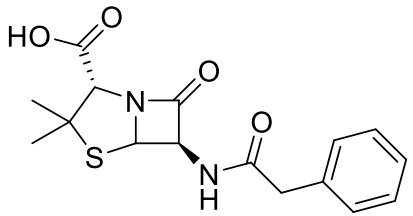
Artemisinin

Artemisia annua,
sweet wormwood

Plants (25%), microorganisms (13%) and animals (about 3%)

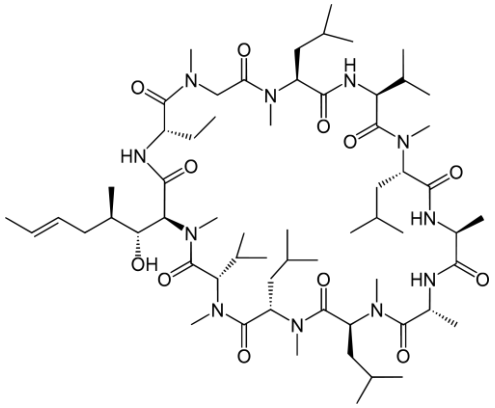
Drugs from bacterial and fungal NPs

Penicillium fungus



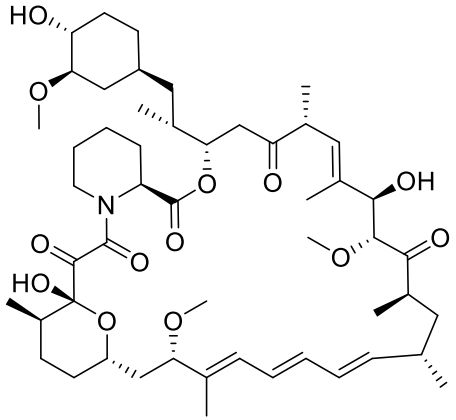
Penicillin G, antibiotic

Tolypocladium inflatum fungus



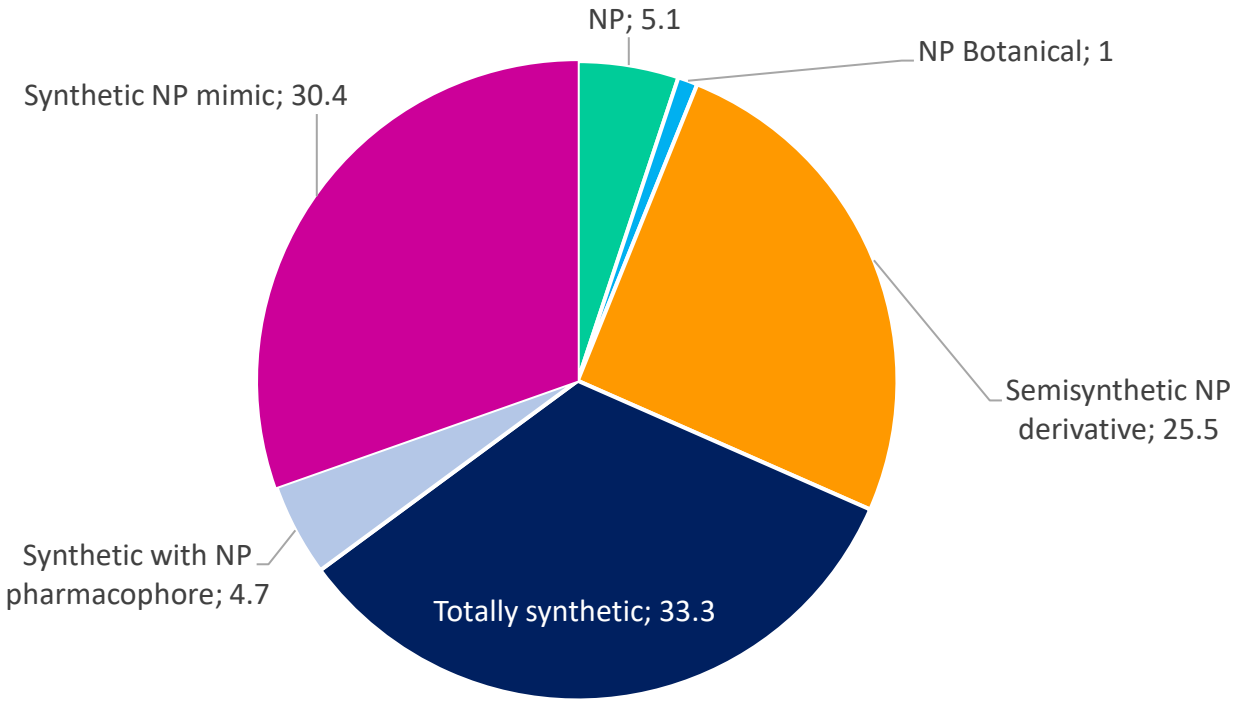
Ciclosporin, immunosuppressant

Streptomyces hygroscopus



Rapamycin, immunosuppressant

Many modern drugs originate from natural products (NP)



>30%

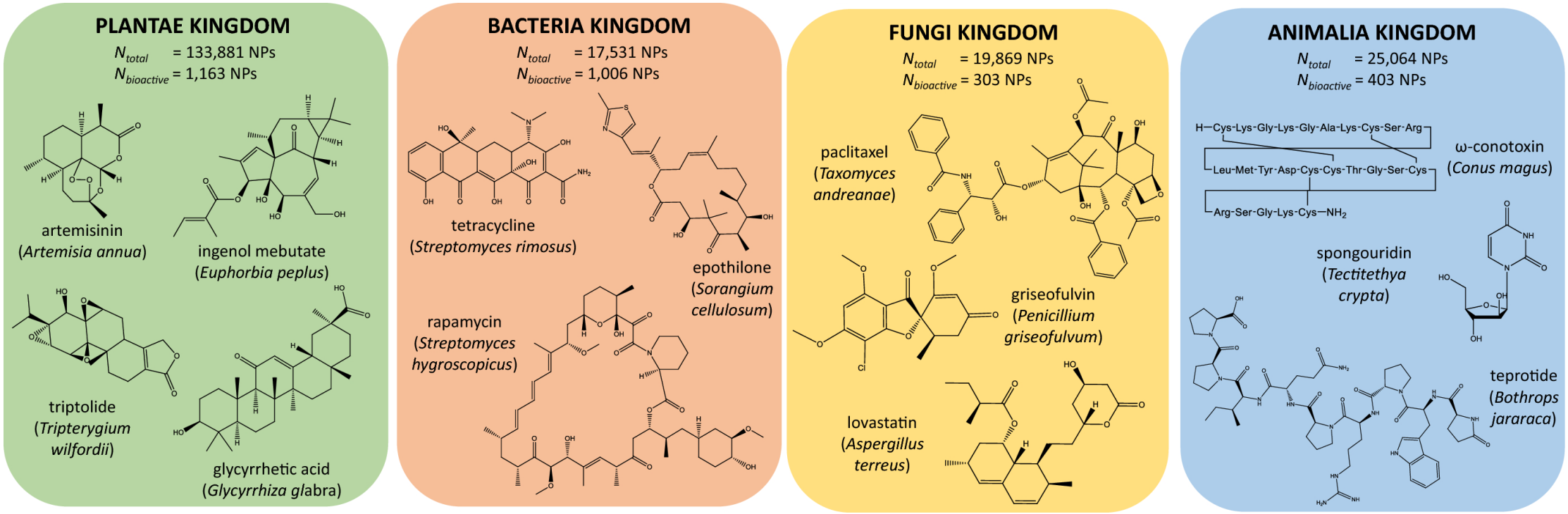
All small-molecule drugs 1981-2019

Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019

David J. Newman* and Gordon M. Cragg

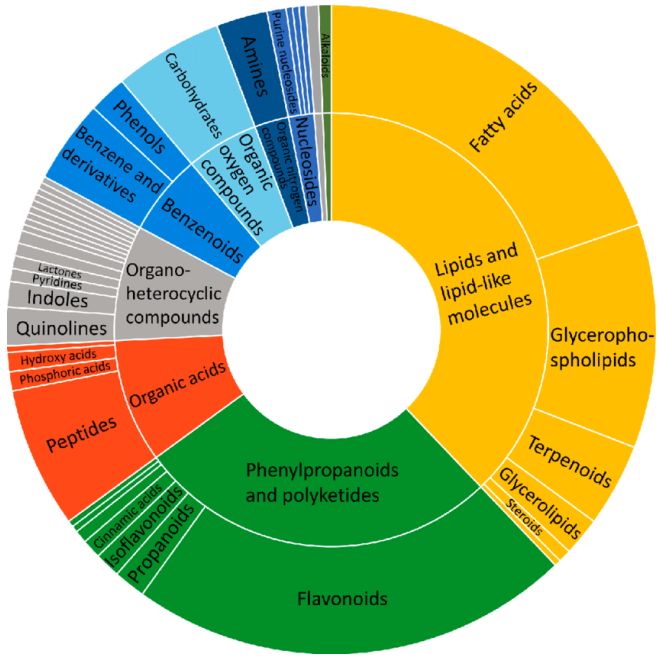
J. Nat. Prod. 2020, 83, 3, 770–803, 10.1021/acs.jnatprod.9b01285

NPs exhibit unique diversity

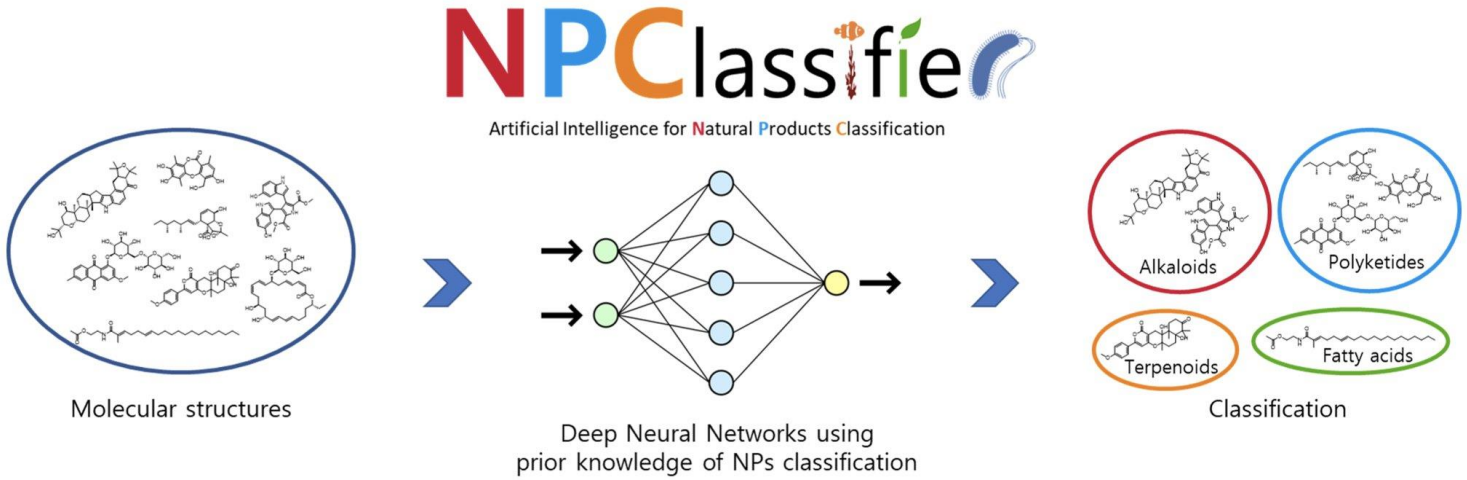


Chassagne, F., Cabanac, G., Hubert, G. *et al.* The landscape of natural product diversity and their pharmacological relevance from a focus on the *Dictionary of Natural Products*®. *Phytochem Rev* **18**, 601–622 (2019). https://doi.org/10.1007/s11101-019-09606-2

Even classifying NPs is a non-trivial task



Example of the ClassyFire classification
Inner circle: superclass level, outer circle: class level.



NPs Structures differ from those of Synthetic Drugs

"Plants don't run"

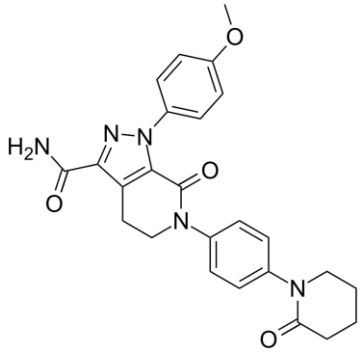
"Natural products differ from synthetic molecules by having evolutionary history"

Structurally more complex:

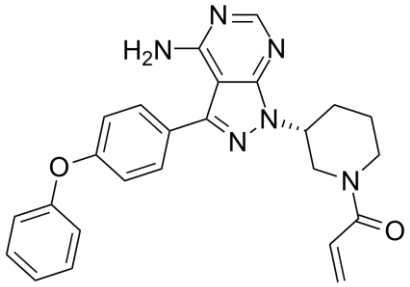
- Higher molecular mass
- More sp^3 C
- Less N & halogens
- More H-bond acceptors & donors
- Lower logP
- Greater rigidity but "more 3D"

primarily recognized as **privileged structures** to interact with protein drug targets

Synthetic drugs

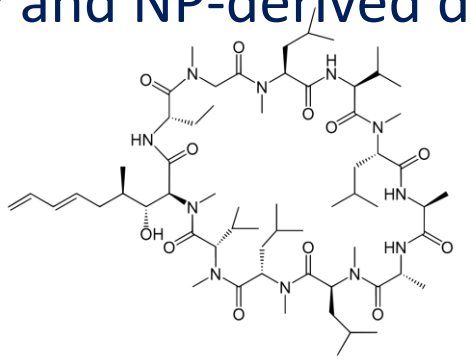


Apixaban



Ibrutinib

NP and NP-derived drugs



Voclosporin

Decline of NP in pharma

Science

Drug Discovery and Natural Products: End of an Era or an Endless Frontier?

JESSE W.-H. LI AND JOHN C. VEDERAS DOI: 10.1126/science.1168243

Table 1 Big/medium Pharma Companies which have currently ceased (between 2000 and 2013) or are still bioprospecting

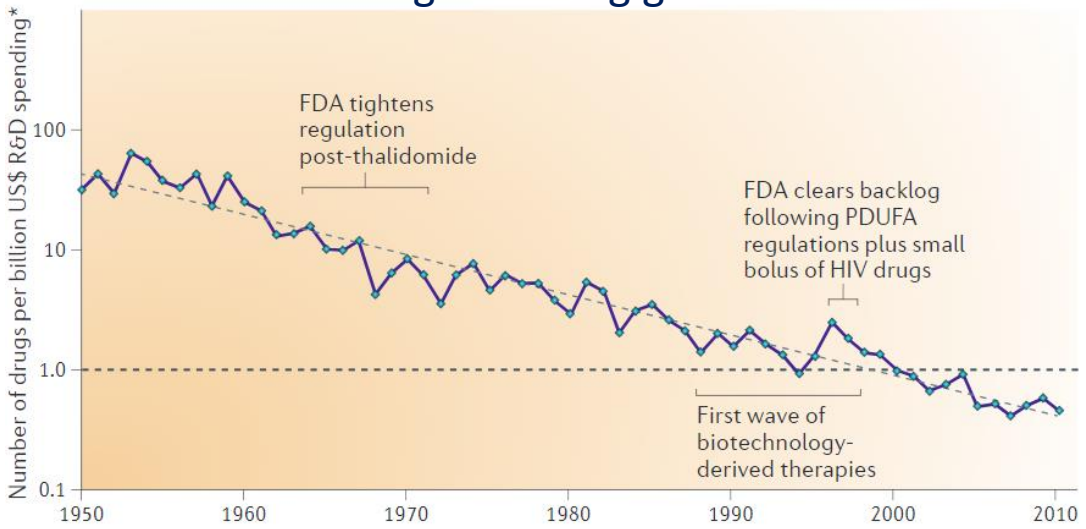
Arrest	Continuation
Abbott	Dabur
Astellas	Eisai
Bayer	Novartis
Boehringer Ingelheim	Otsuka
Bristol-Myers Squibb	Pierre Fabre
Daiichi Sankyo	Piramal
Eli Lilly	
GlaxoSmithKline	
Johnson and Johnson	
Kyowa Hakko	

Diagnosing the decline in pharmaceutical R&D efficiency

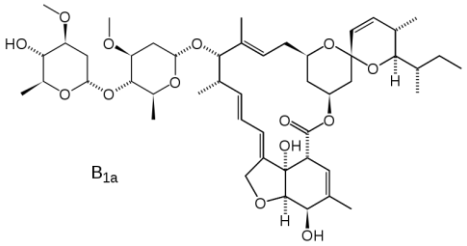
Jack W. Scannell, Alex Blanckley, Helen Boldon & Brian Warrington

Nature Reviews Drug Discovery **11**, 191–200 (2012) | Cite this article

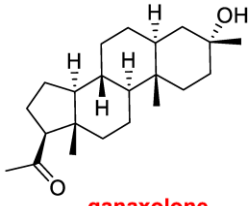
Finding new drug got harder



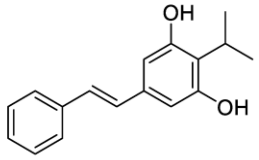
2015



Avermectin and derivatives

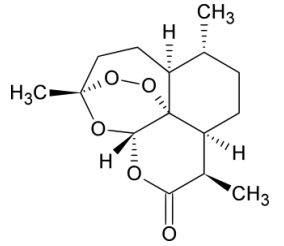


ganaxolone



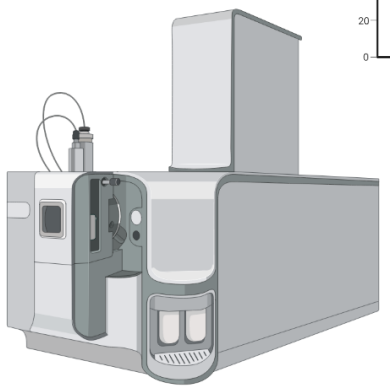
tapinarof

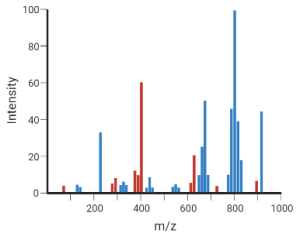
37 new drugs approved by FDA
2022



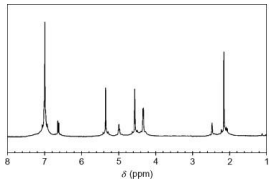
Milestones in the NP-driven drug discovery

LC(GC)-MS

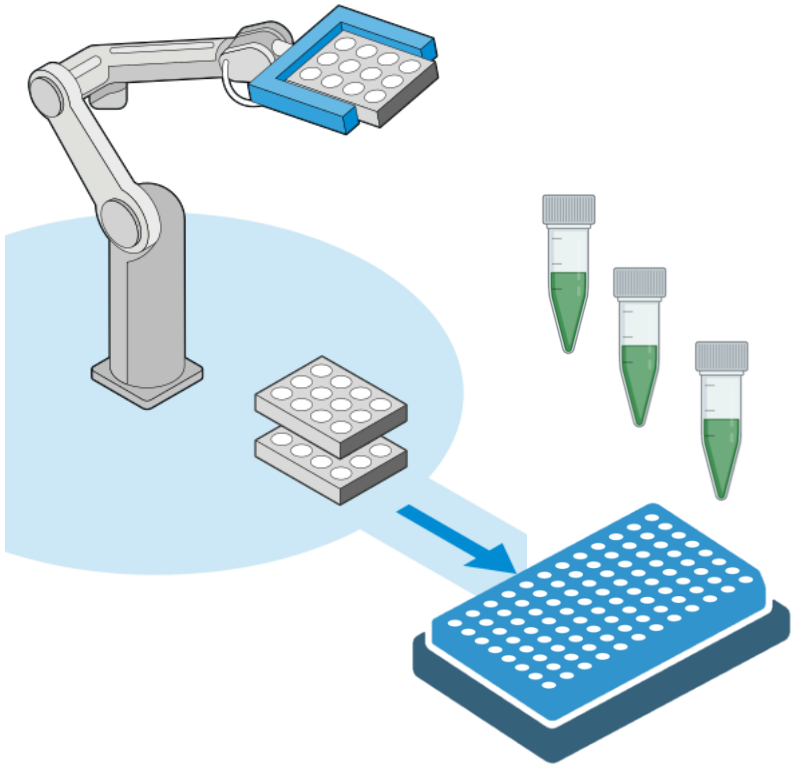




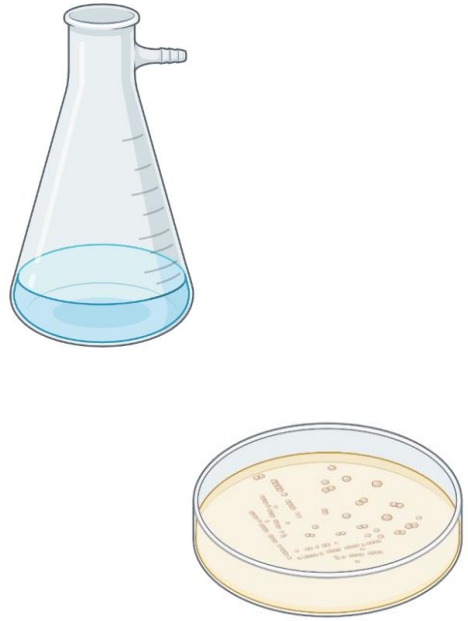
NMR



Discovery of a novel compound



High-throughput screening (HTS)



Synthesis

Grind-and-find approach

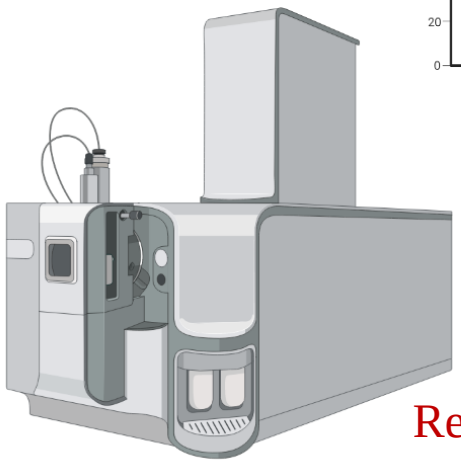
doesn't work that well

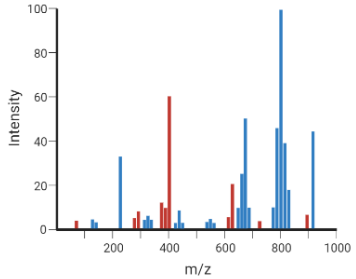
And largely relies on serendipity

Dereplication:
- Time
- Money
- Repeated efforts

Not enough biological material to isolate and characterize a bioactive NP

Extraction
Fractination





Identification
and annotation

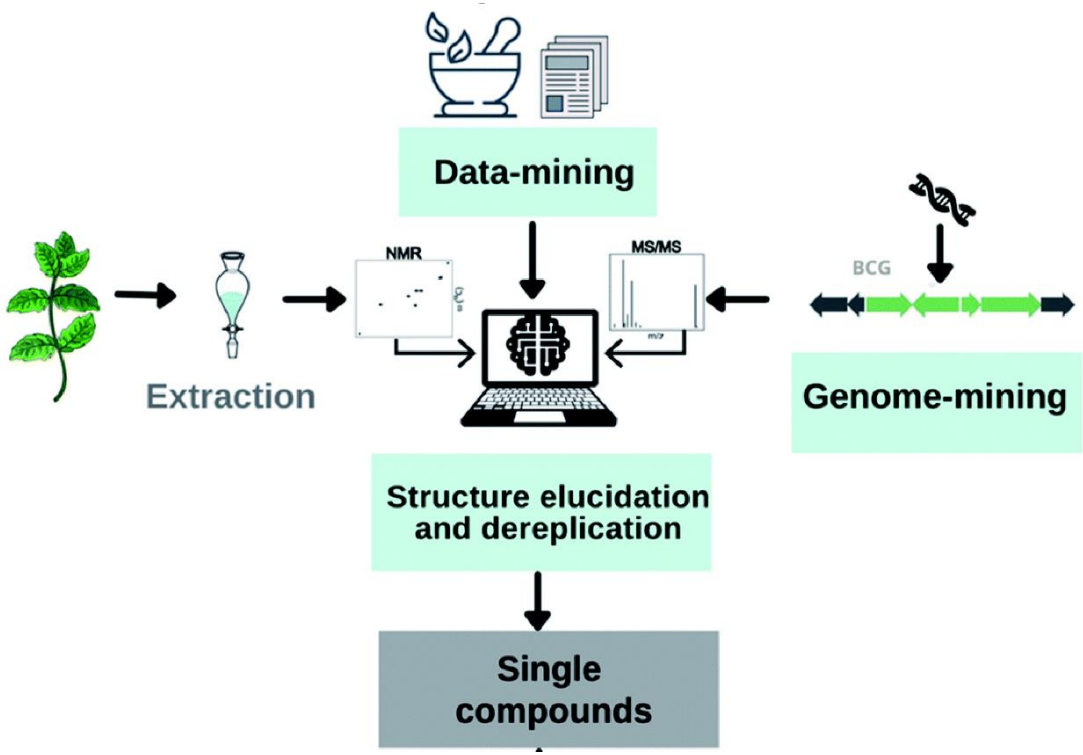
Rediscovery of already known NPs

Searching for biological activity in crude extracts, higher hit rates in HTS

Single compound or extract

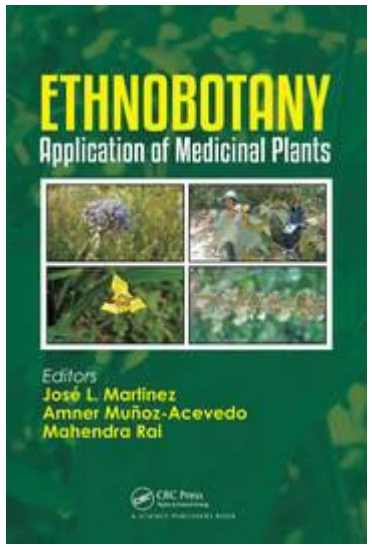
Computer-assisted discovery of NPs

– Data mining into traditional medicine, papers, and plant databases
– Predicting chemical structures from microbial genomes
– Automating NP dereplication process



Saldívar-González, F. I., et al. "Natural product drug discovery in the artificial intelligence era." *Chemical Science* 13.6 (2022): 1526-1546. 10.1039/d1sc04471k
Atanasov et al. “Natural products in drug discovery: advances and opportunities” *Nature Rev.* 20 (2021) https://doi.org/10.1038/s41573-020-00114-z

NPs Databases



TM-MC Database of medicinal plants

CHEMnetBASE

Dictionary of Natural Products
>200k NPs

npatlas

>20k NPs

COCONUT
COllecTion of Open Natural ProdUcTs

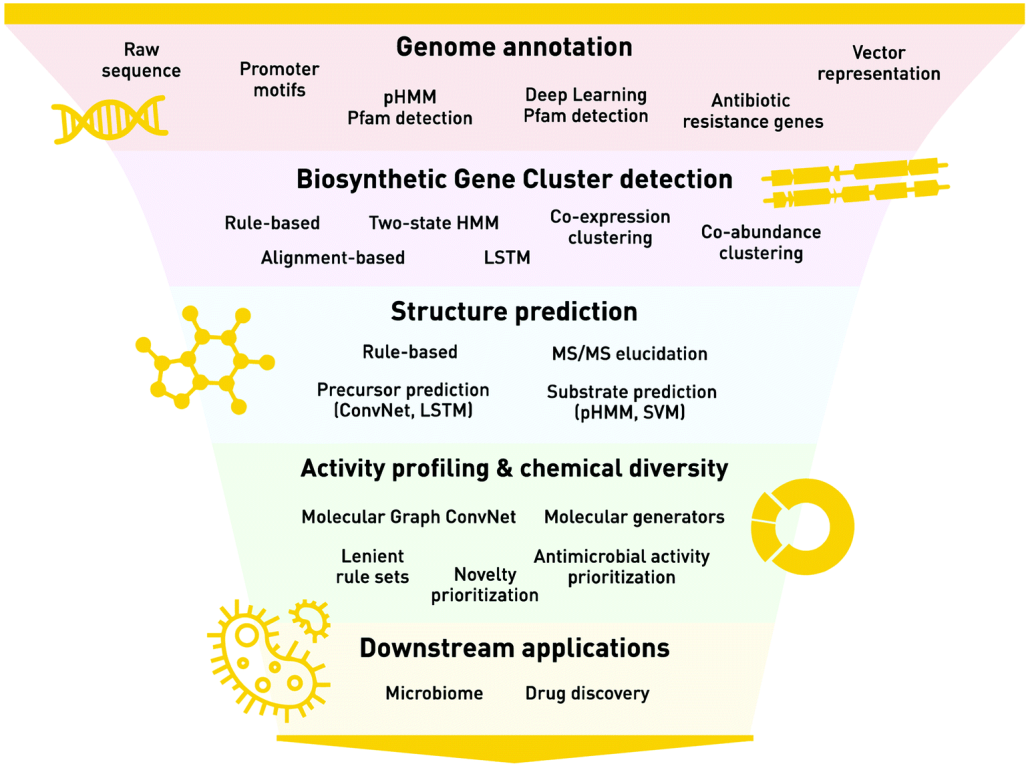
>400k NPs

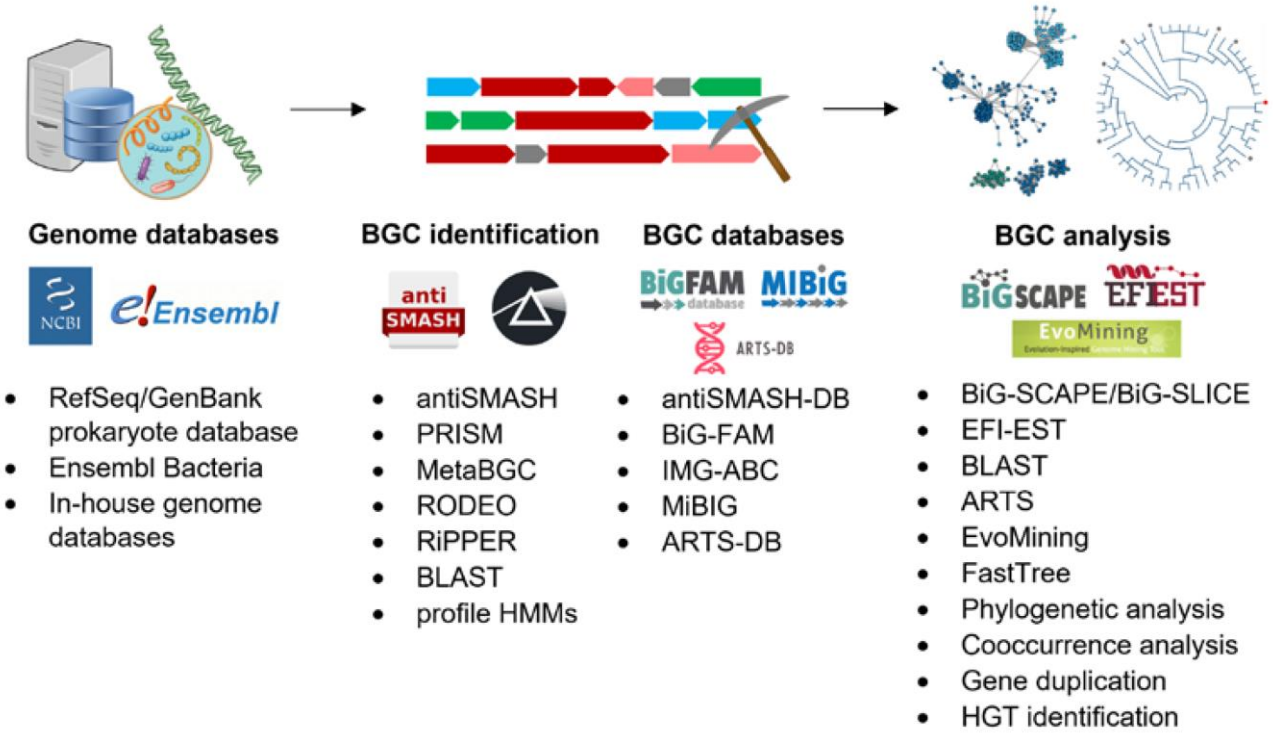
LOTUS

>200k NPs

Chen, (2017) Data Resources for the Computer-Guided Discovery of BioactiveNatural Products. J. Chem. Inf. Model. 2017. 10.1021/acs.jcim.7b00341
Rutz, (2022) The LOTUS initiative for open knowledge management in natural products research. eLife 11:e70780. https://doi.org/10.7554/eLife.70780

Genomic mining



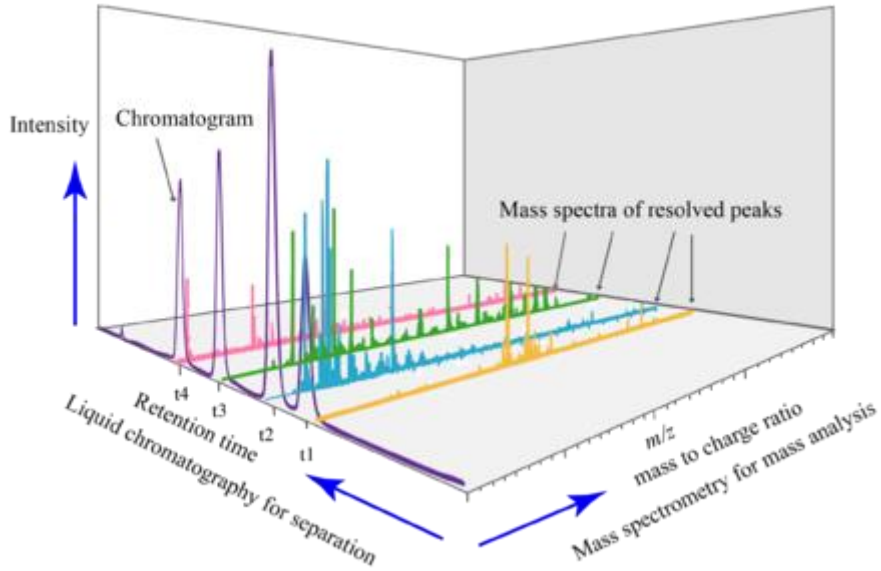


The application potential of machine learning and genomics for understanding natural product diversity, chemistry, and therapeutic translatability. Prihoda et al. *Natural Products Reports, 2021.* https://doi.org/10.1039/D0NP00055H

Targeted Large-Scale Genome Mining and Candidate Prioritization for Natural Product Discovery. Malit et al. *Mar. Drugs* 2022, *20*(6), 398; https://doi.org/10.3390/md20060398

MS-based Untargeted Metabolomics

Tandem mass spectra (MS/MS) are of crucial importance



Chromatographic feature detection and alignment

MZmine 3

LC-IMS-MS and imaging

MS-DIAL

GC/MS

MetaboAnalyst 5.0

LC-MS,
joint pathway analysis

Feature annotation

SIRIUS

CSI:FingerID
Multiple kernel learning supported by fragmentation trees

CASMI

Critical Assessment of Small Molecule Identification

www.casmi-contest.org/

Spectral data from MS as an additional tool in dereplication

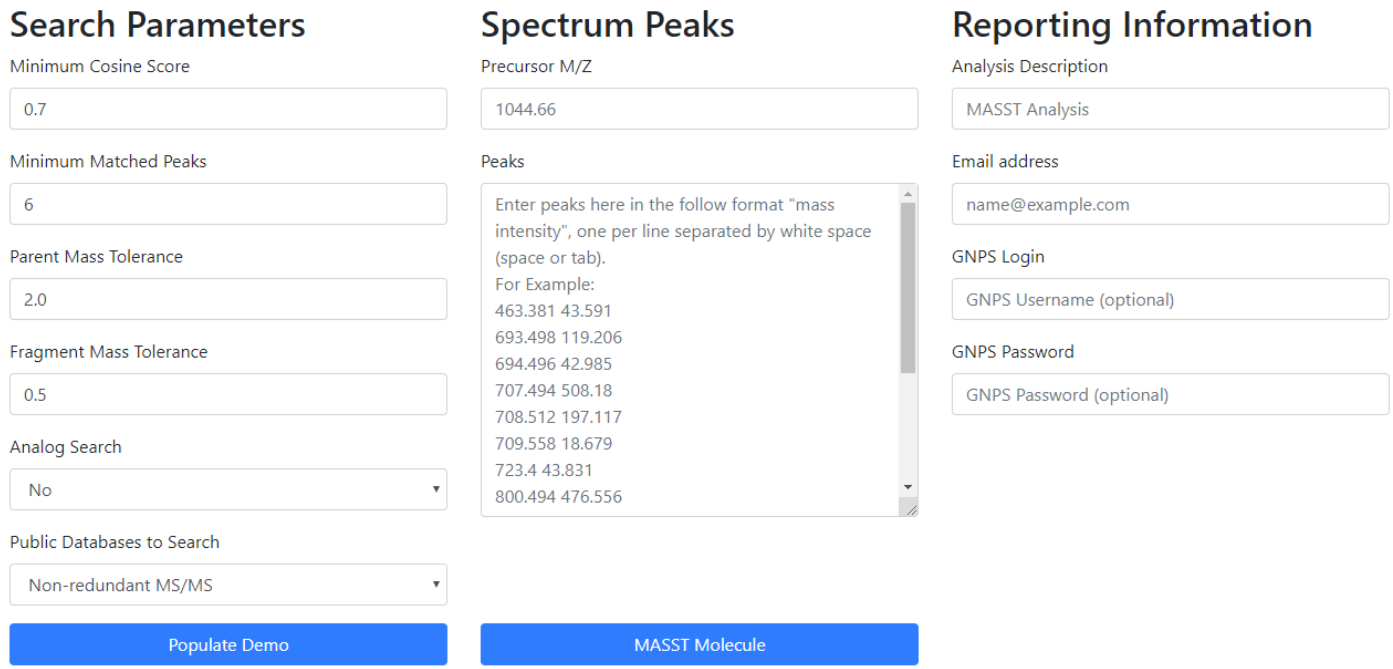
Public repositories

GNPS

MassIVE

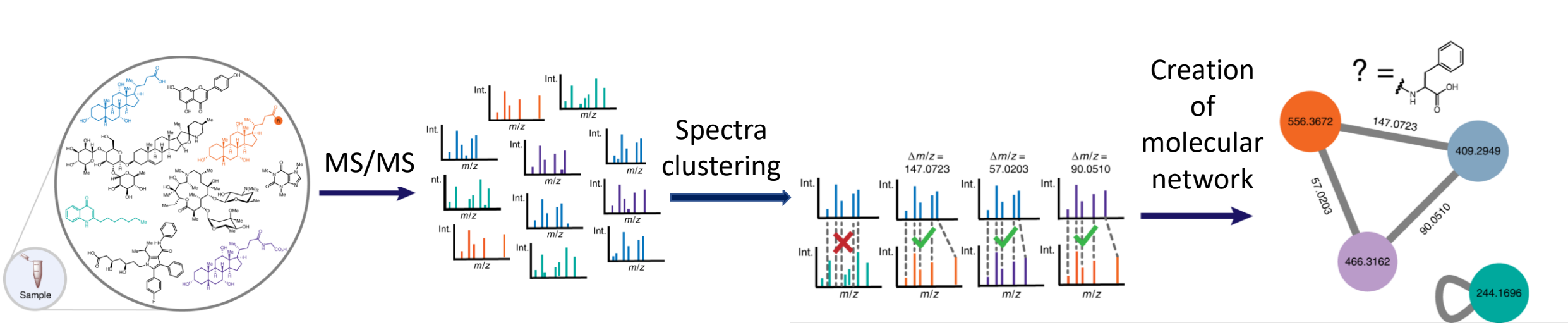
Mass Spectrometry
Interactive **V**irtual **E**nvironment

MASST



Wang, M., Jarmusch, A.K., Vargas, F. *et al.* **Mass spectrometry searches using MASST**. *Nat Biotechnol* **38**, 23–26 (2020). https://doi.org/10.1038/s41587-019-0375-9

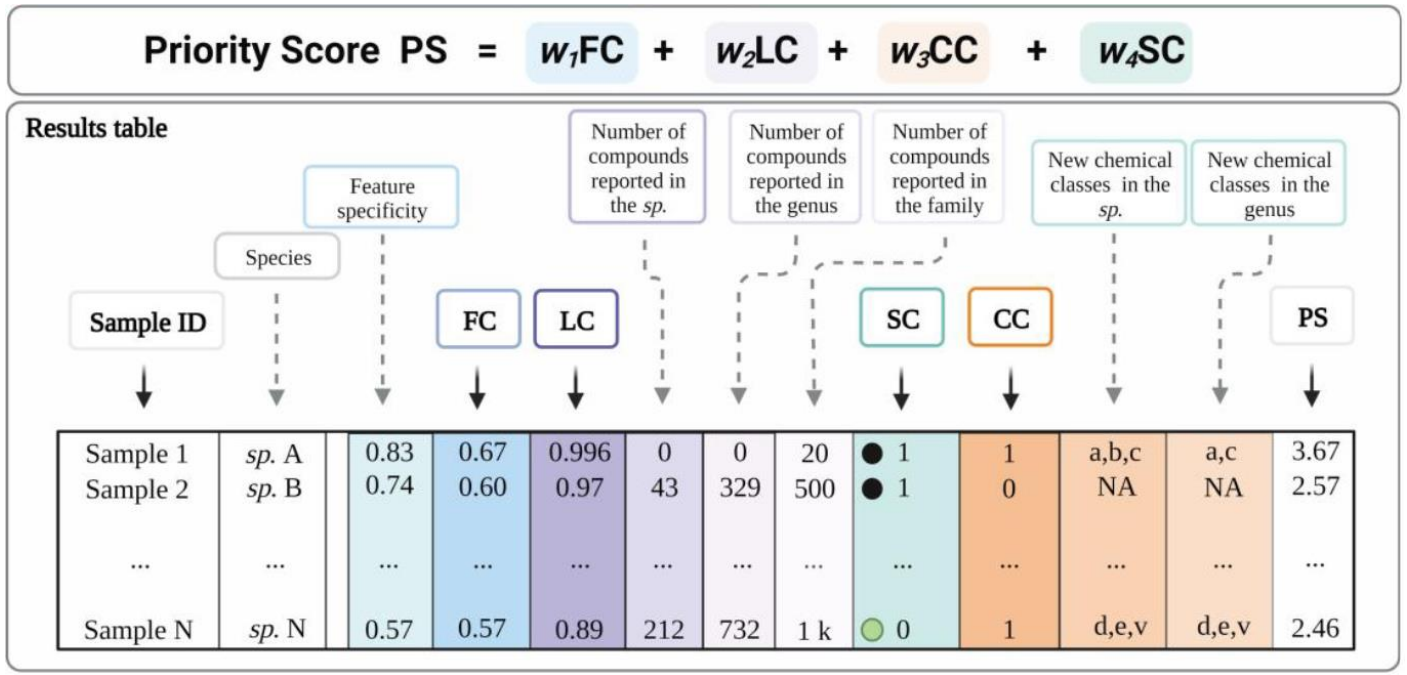
GNPS Molecular Networking



Querying of nodes against public datasets

Reproducible molecular networking of untargeted mass spectrometry data using GNPS
Allegra T. Aron et al. *Nature Protocols* **volume 15**, pages1954–1991 (2020) DOI
https://doi.org/10.1038/s41596-020-0317-5

Using public data to prioritize extracts

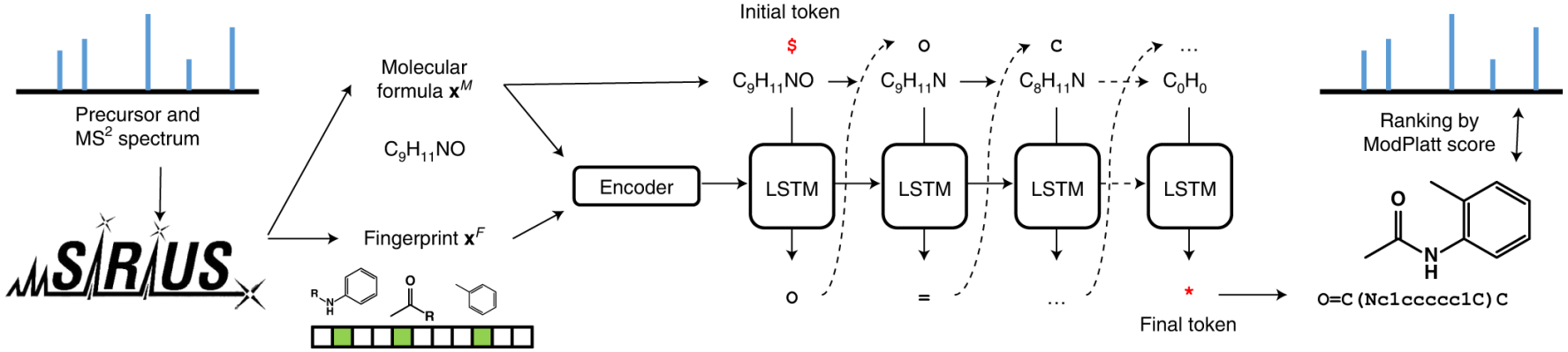


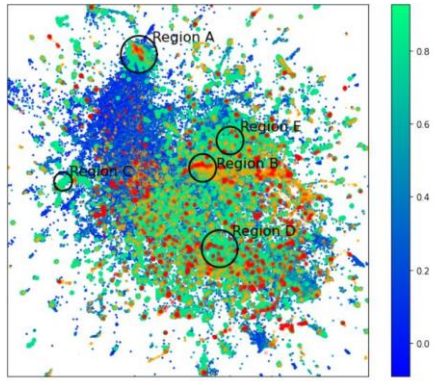
FC - Feature component
LC - Literature component
CC - Class component
SC - Similarity component
w - User-defined weights

Quiros-Guerrero LM, Nothias LF, Gaudry A, Marcourt L, Allard PM, Rutz A, David B, Queiroz EF, Wolfender JL. ***Inventa*: A computational tool to discover structural novelty in natural extracts libraries**. Front Mol Biosci. 2022 Nov 11;9:1028334. doi: 10.3389/fmolb.2022.1028334

Deep learning applications to accelerate metabolomic research

'inverse problem' of mass spectrometric molecular identification

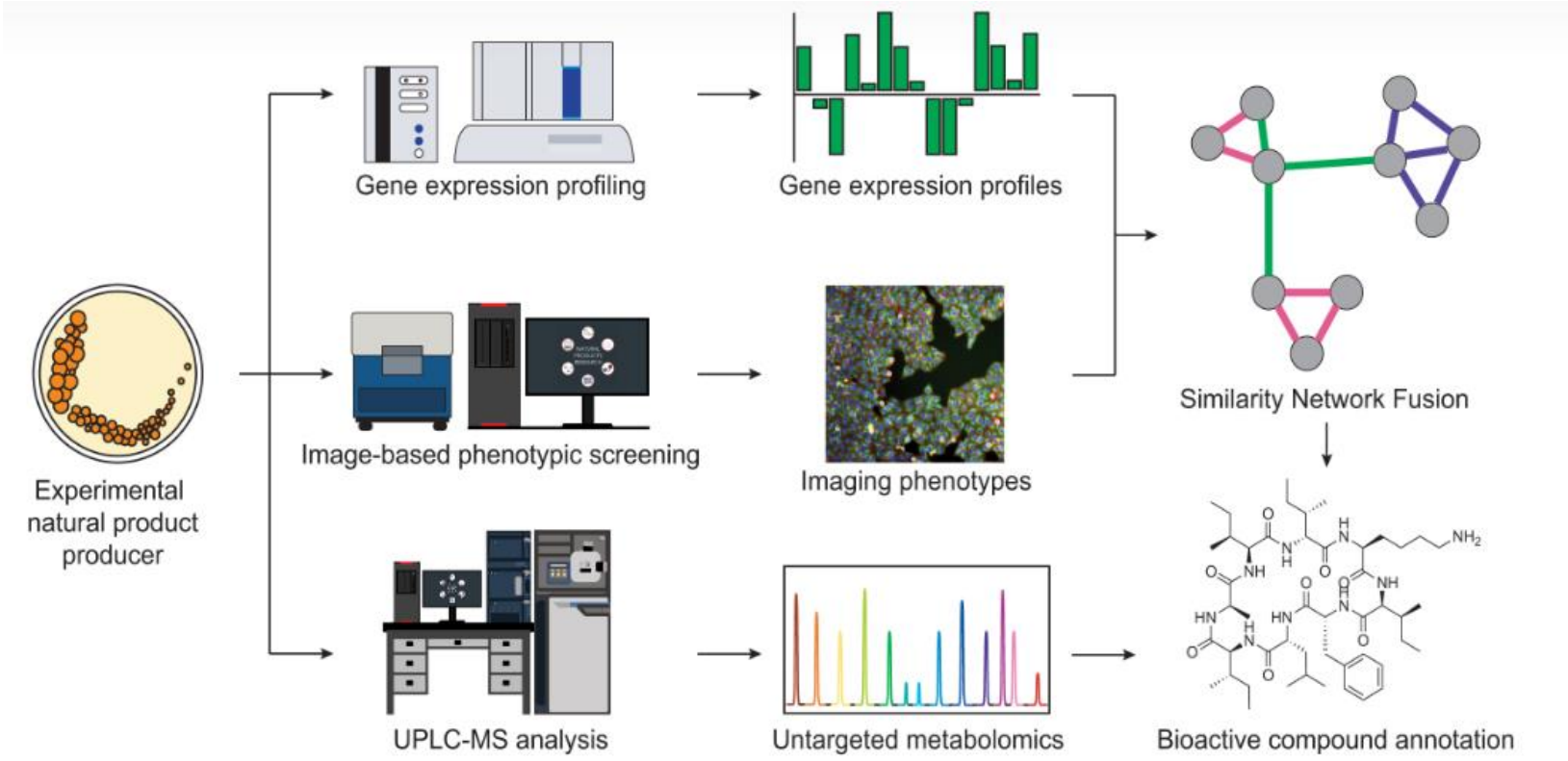




UMAP of a sub-sample of unlabeled MS/MS spectra from GNPS. Red points – MS/MS spectra of FDA-approved drugs (not NPs)

MSNovelist: de novo structure generation from mass spectra. Stravs et al. *Nature Methods* **volume 19**, pages 865–870 (2022). https://doi.org/10.1038/s41592-022-01486-3

MS2Prop: A machine learning model that directly predicts chemical properties from mass spectrometry data for novel compounds. *bioRxiv preprint.* Voronov et al. https://doi.org/10.1101/2022.10.09.511482

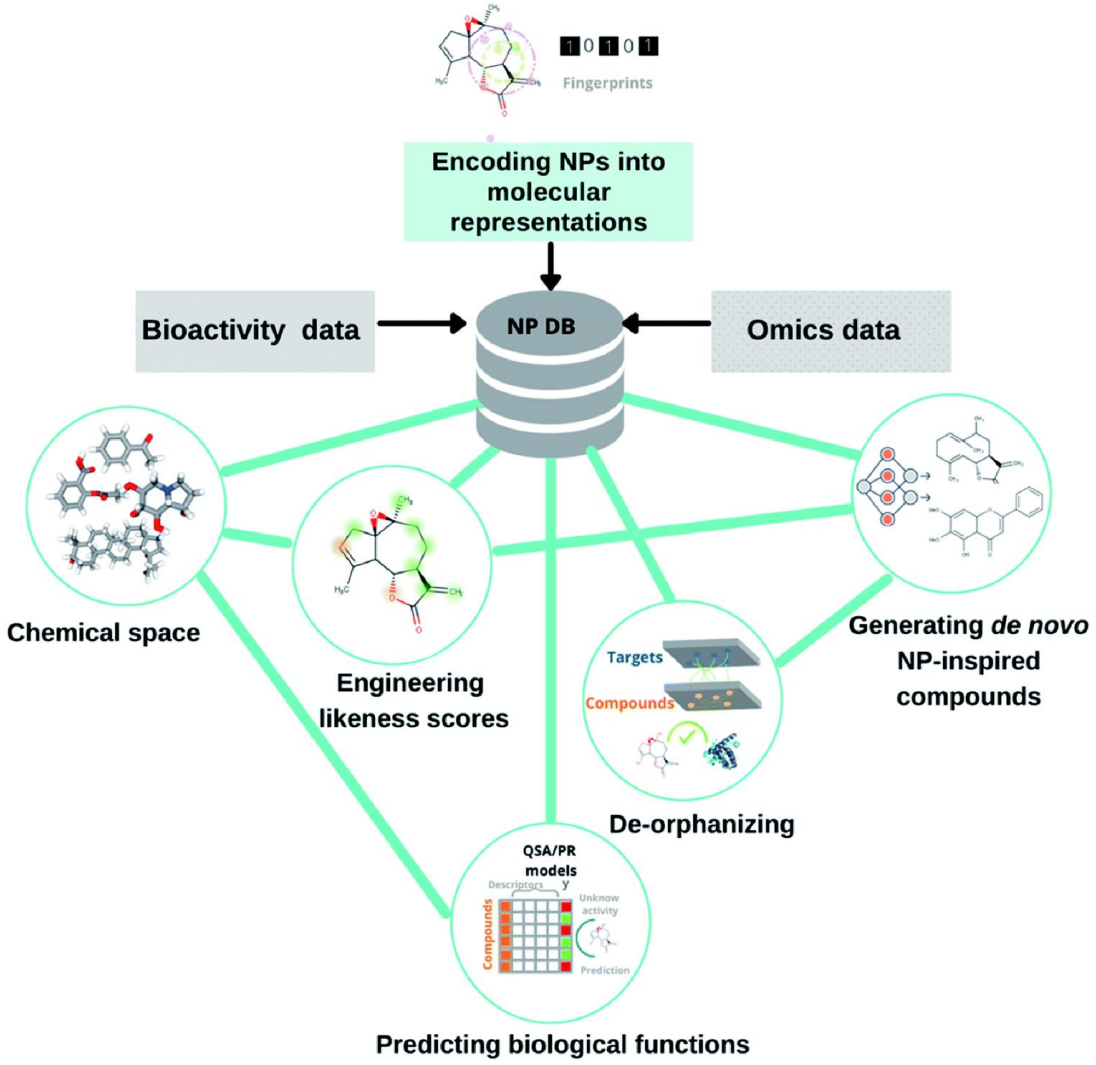


High-throughput functional annotation of natural products by integrated activity profiling

Suzie K. Hight et al., PNAS, **2022**

https://doi.org/10.1073/pnas.220845811

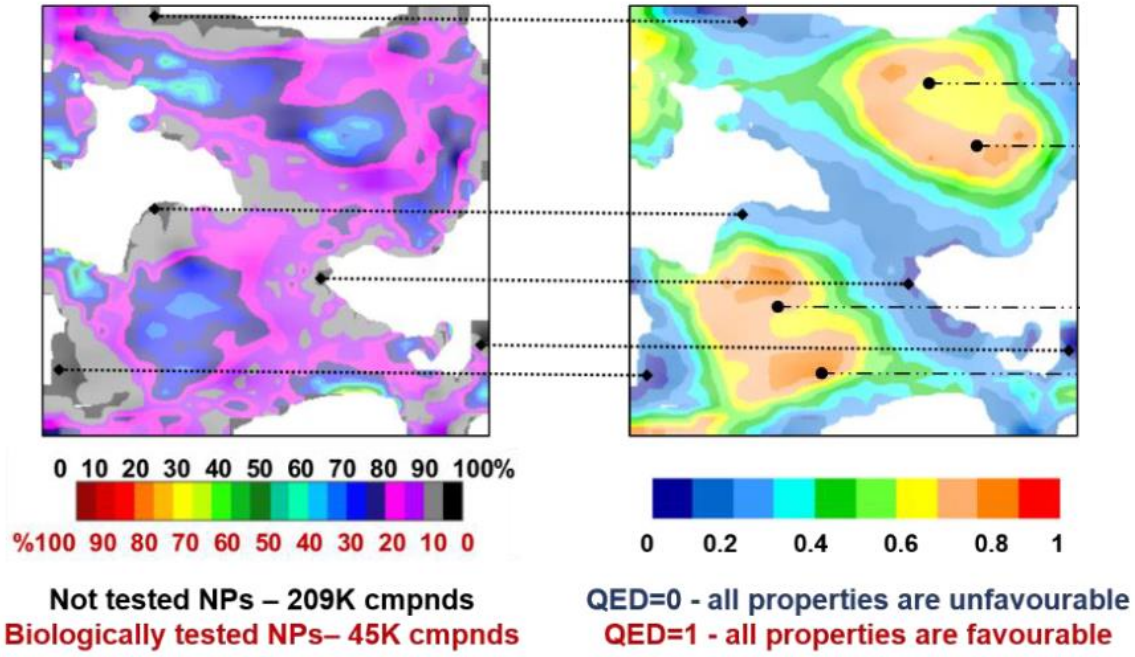
After NP identification, what's next?

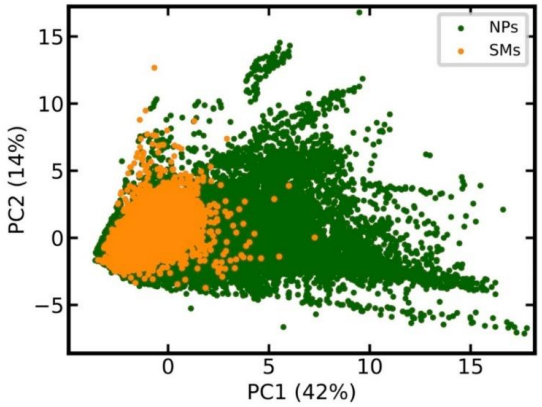


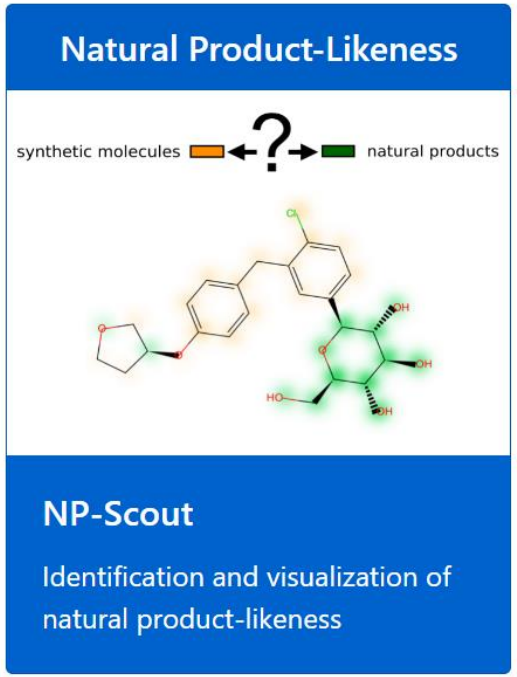
- Mapping NPs in the chemical space
- De-orphanizing
- Generating *de novo* NP-inspired compounds

Saldívar-González, F. I., et al. "Natural product drug discovery in the artificial intelligence era." *Chemical Science* 13.6 (2022): 1526-1546. 10.1039/d1sc04471k

Chemical space of NPs





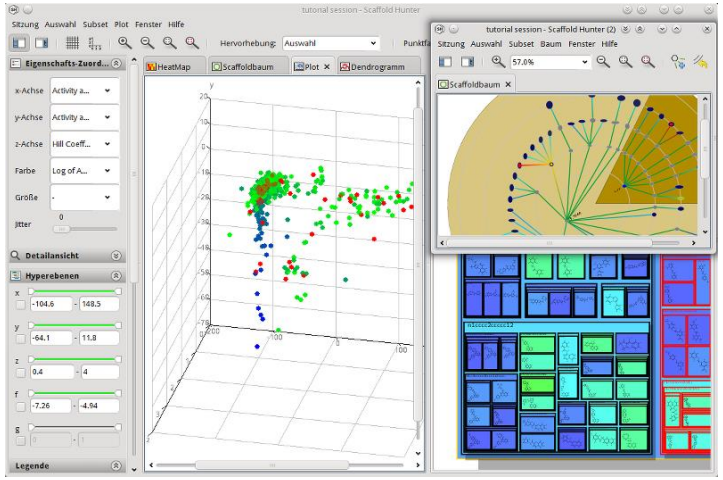


NP Navigator: A New Look at the Natural Product Chemical Space. Zabolotna et al, *J.Mol.Inf*, 2021. 10.1002/minf.202100068

NP-Scout: Machine Learning Approach for the Quantification and Visualization of the Natural Product-Likeness of Small Molecules. Chen et al. Biomolecules. 2019 Feb; 9(2): 43. 10.3390/biom9020043

Identifying scaffolds for further structural modifications

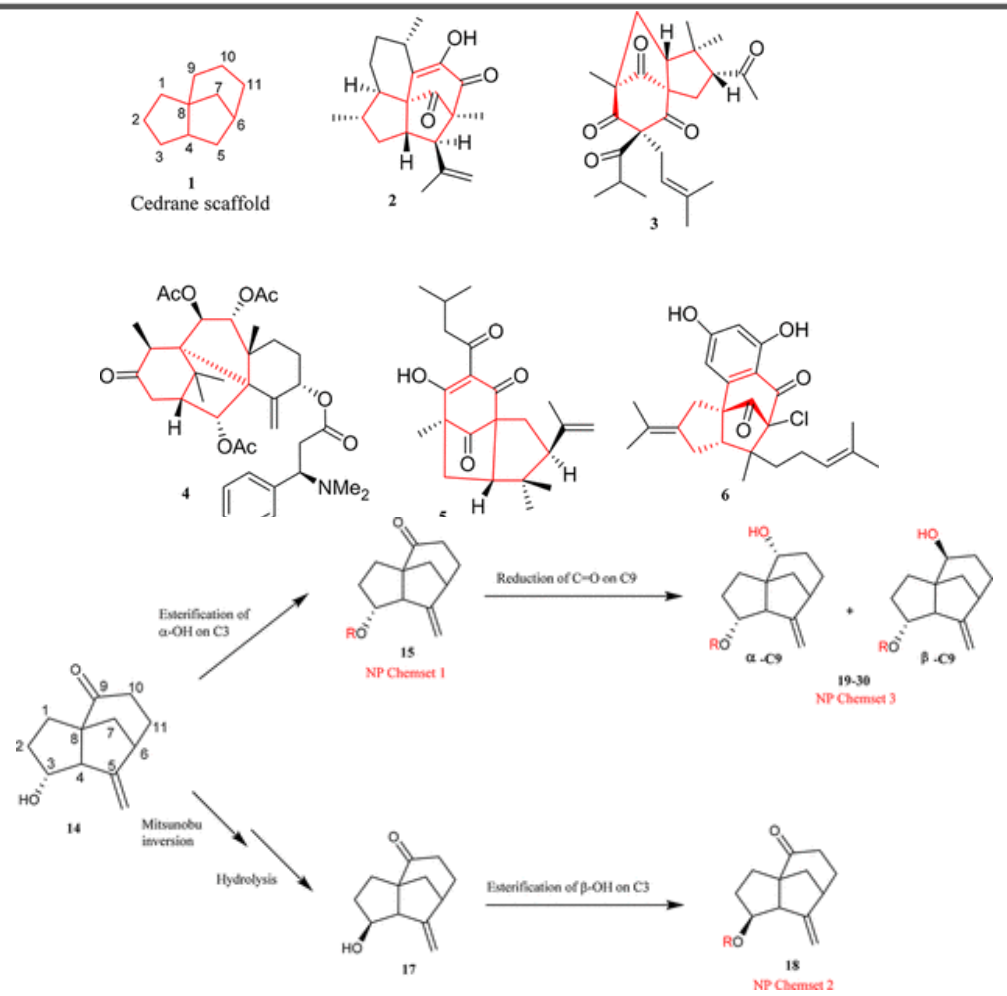
Scaffold Hunter



RDKit

Open-Source Cheminformatics and Machine Learning

rdScaffoldNetwork
rdkit.Chem.Scaffolds



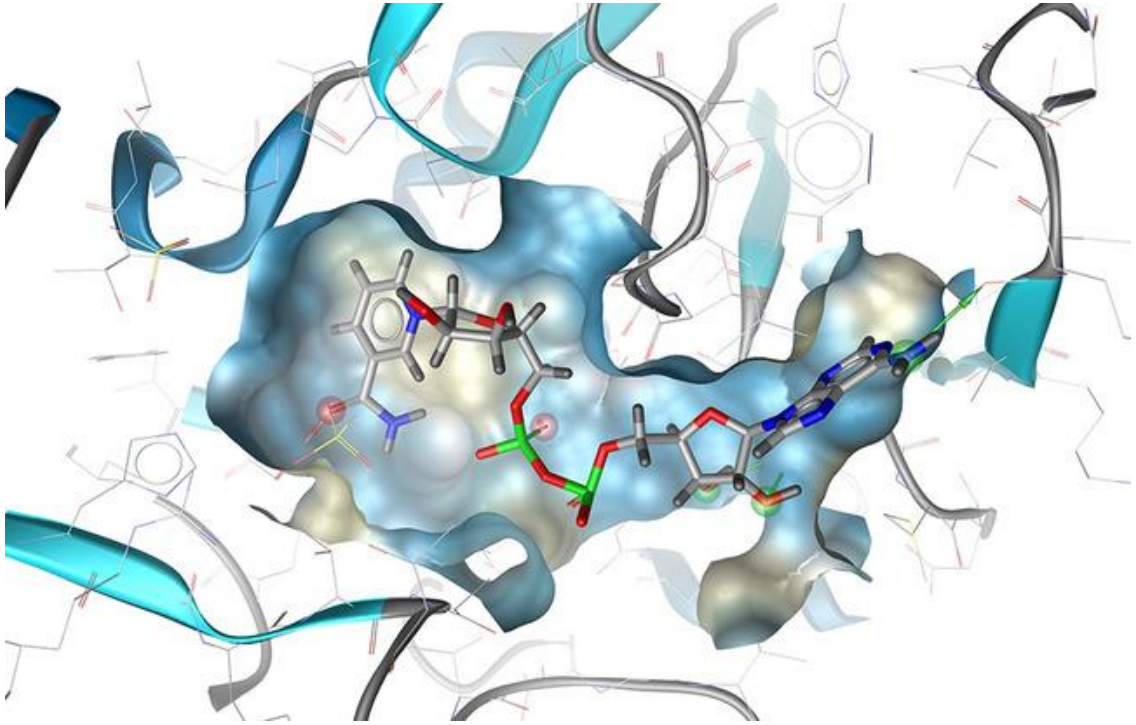
Design and Synthesis of Natural Product Inspired Libraries Based on the Three-Dimensional (3D) Cedrane Scaffold: Toward the Exploration of 3D Biological Space
Tajabadi et al, *J. Med. Chem.* 2018
https://doi.org/10.1021/acs.jmedchem.8b00194

Pharmacophores & NPs

Identification of natural inhibitors of *Trypanosoma brucei* Glyceraldehyde-3-phosphate-dehydrogenase (*Tb*GAPDH)

Human African trypanosomiasis (HAT), or “sleeping sickness”

4803 NPs from MEGx database
3 structures (PDB-IDs: 2X0N, 3IDS and 1GYP)
4 pharmacophore models by MOE
Docking
Experimental testing
5 cmpds w/ >50 % inhibition at 50 µM



Pharmacophore models by LigandScout

F. C. Herrmann, M. Lenz, J. Jose, M. Kaiser, R. Brun, T. J. Schmidt, *Molecules* 2015, 20, 16154–16169.
doi.org/10.3390/molecules200916154

Applications of the Pharmacophore Concept in Natural Product inspired Drug Design
Seidel et al. J.Mol.Inf., 2020.
https://doi.org/10.1002/minf.202000059

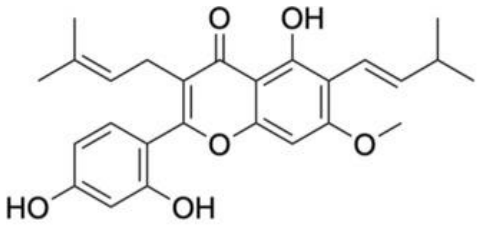
More successful examples of NP screening

DOI: 10.1002/minf.202000171

Cheminformatics in Natural Product-based Drug Discovery

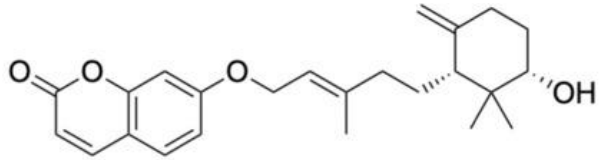
Ya Chen[a] and Johannes Kirchmair*[a, b]

3D molecular shape-based screening, antiviral

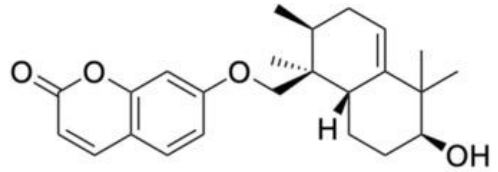


1 (artocarpin)

Pharmacophore + shape-base, activators of the G protein-coupled bile acid receptor 1

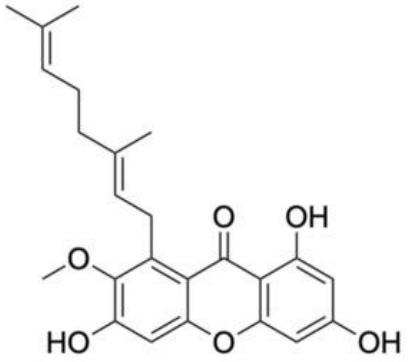


2 (farnesiferol B)



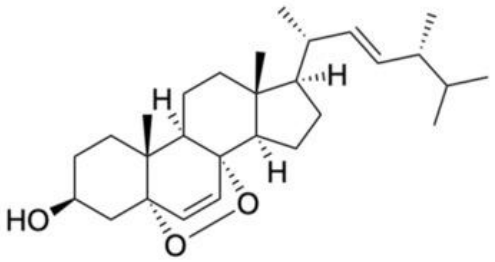
3 (microlobidene)

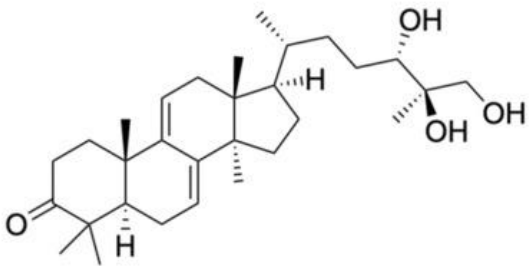
Synthetic compounds with novel scaffolds



4 (rubraxanthone)

Docking, antiinfluenza

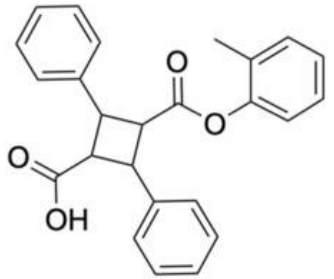




Lanostane triterpenes from the mushroom Ganoderma lucidum.

5 (ergosterol peroxide) **6** (ganodermanontriol)

Pharmacophore, FXR induction / anti-inflammatory



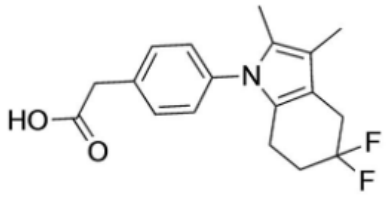
7 (truxillic acid derivative)

Gaussian process model, PPARγ activator

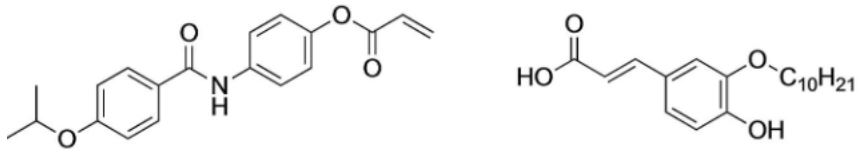
De novo design of NP-inspired molecules

- Diversity-oriented synthesis (DOS)
- Biology-oriented synthesis (BIOS)
- Design of Genuine Structures (DOGS)
- Deep Neural Networks (variety of approaches

DOGS



DNN

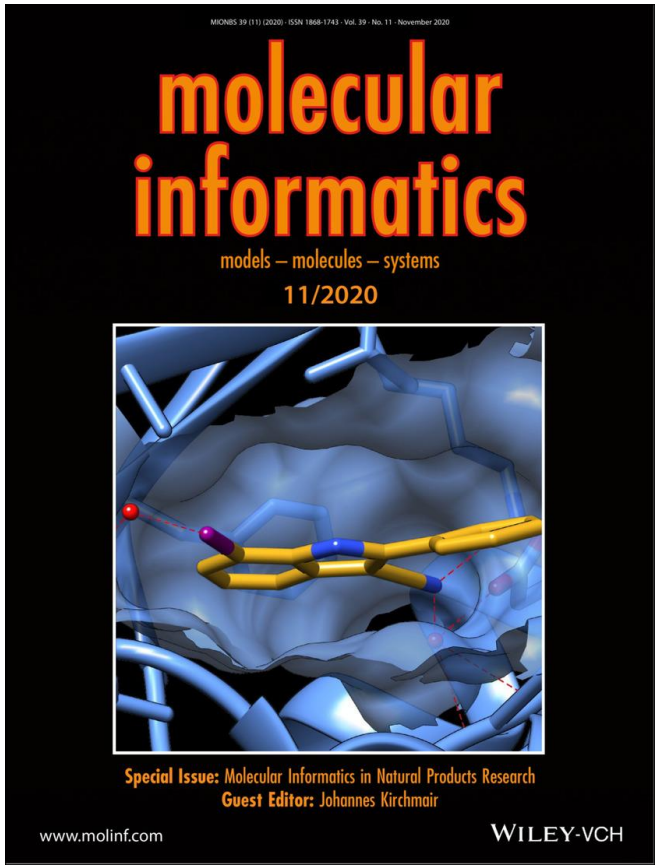


DOI: 10.1002/minf.202000171

Cheminformatics in Natural Product-based Drug Discovery

Ya Chen[a] and Johannes Kirchmair*[a, b]

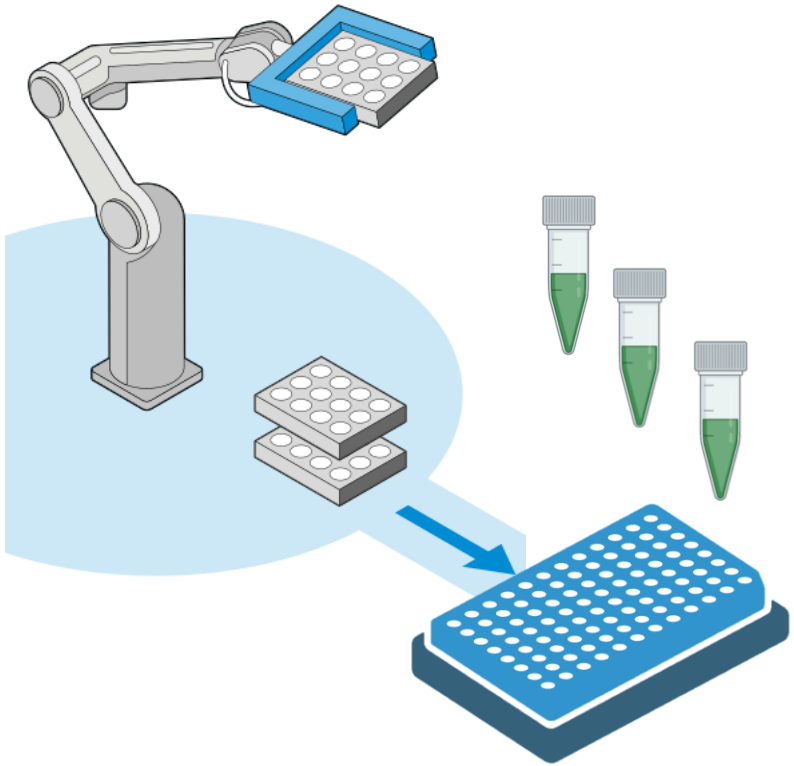
Recommended chemoinformatics read



Volume 39, Issue 11
Special Issue:Molecular Informatics in Natural Products Research
November 2020

https://onlinelibrary.wiley.com/toc/18681751/2020/39/11

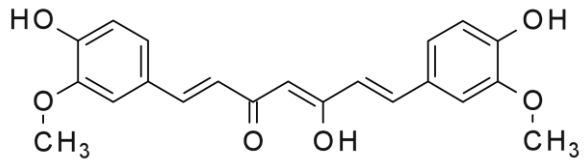
HTS problems



Very high (or low) hit rates in HTS assays

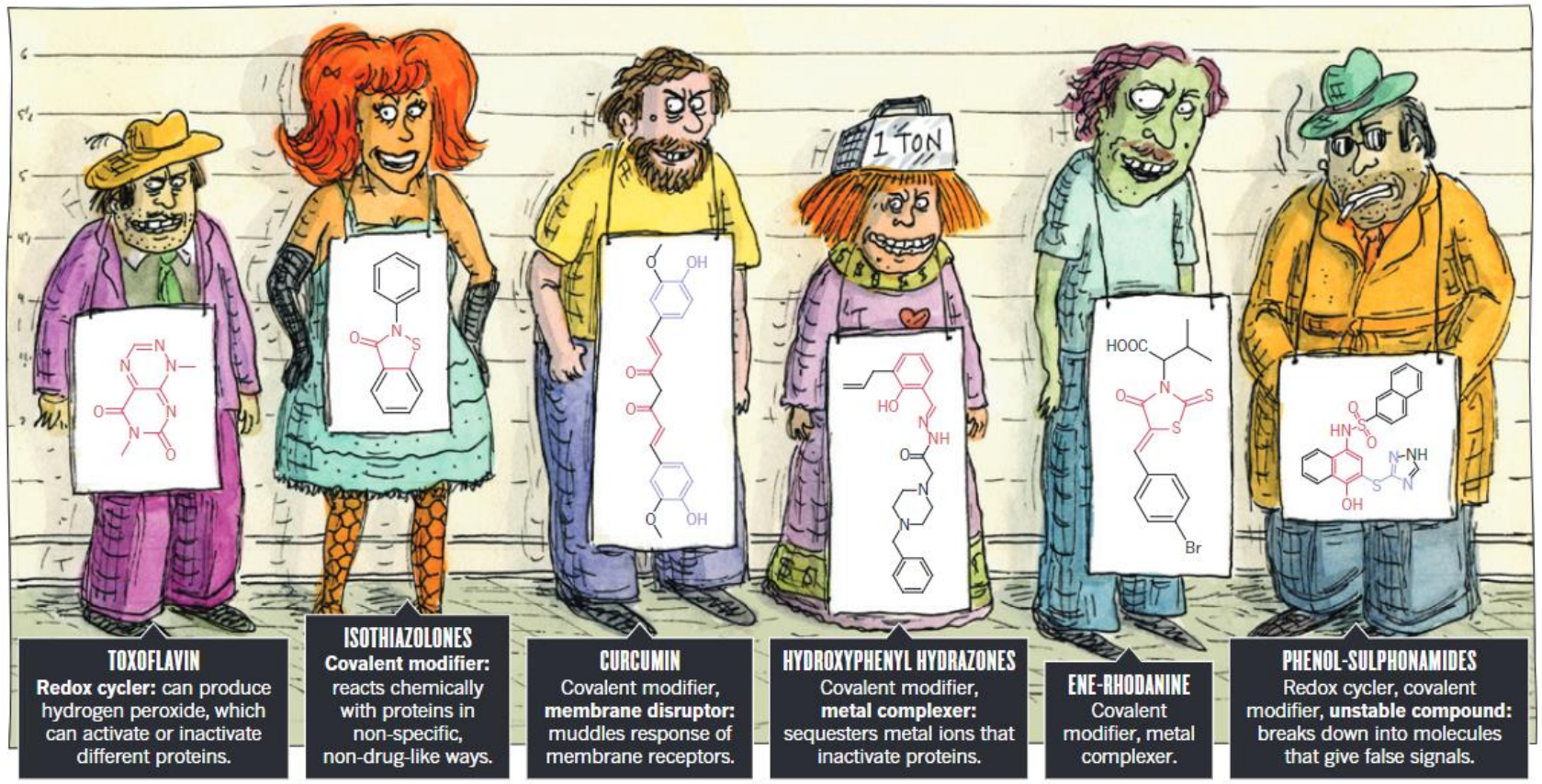
- Polyphenols quench fluorescence
- Highly fluorescent or coloured compounds interfere with colorimetric or fluorescent endpoint

Enol form of curcumin



Creating and screening natural product libraries. Brice A. P. Wilson *Nat. Prod. Rep.*, 2020, 37, 893-918. 10.1039/C9NP00068B

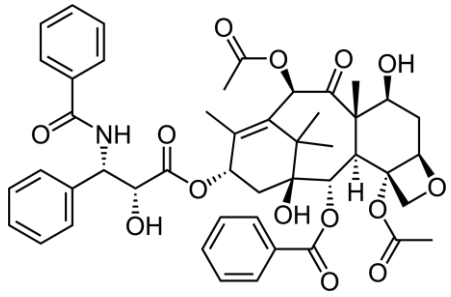
All those PAINS



Chemistry: Chemical con artists foil drug discovery. Baell & Walters. *Nature* volume 513, pages481–483 (2014) https://doi.org/10.1038/513481a

Feeling Nature's PAINS: Natural Products, Natural Product Drugs, and Pan Assay Interference Compounds (PAINS). Jonathan B. Baell, *J. Nat. Prod.* 2016, 79, 3, 616–628. https://doi.org/10.1021/acs.jnatprod.5b00947

Let’s imagine a promising bioactive NP is identified



Taxol
(Paclixatel)

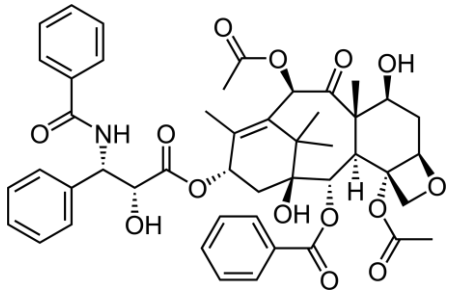
But what about synthesis and scaling up?

News

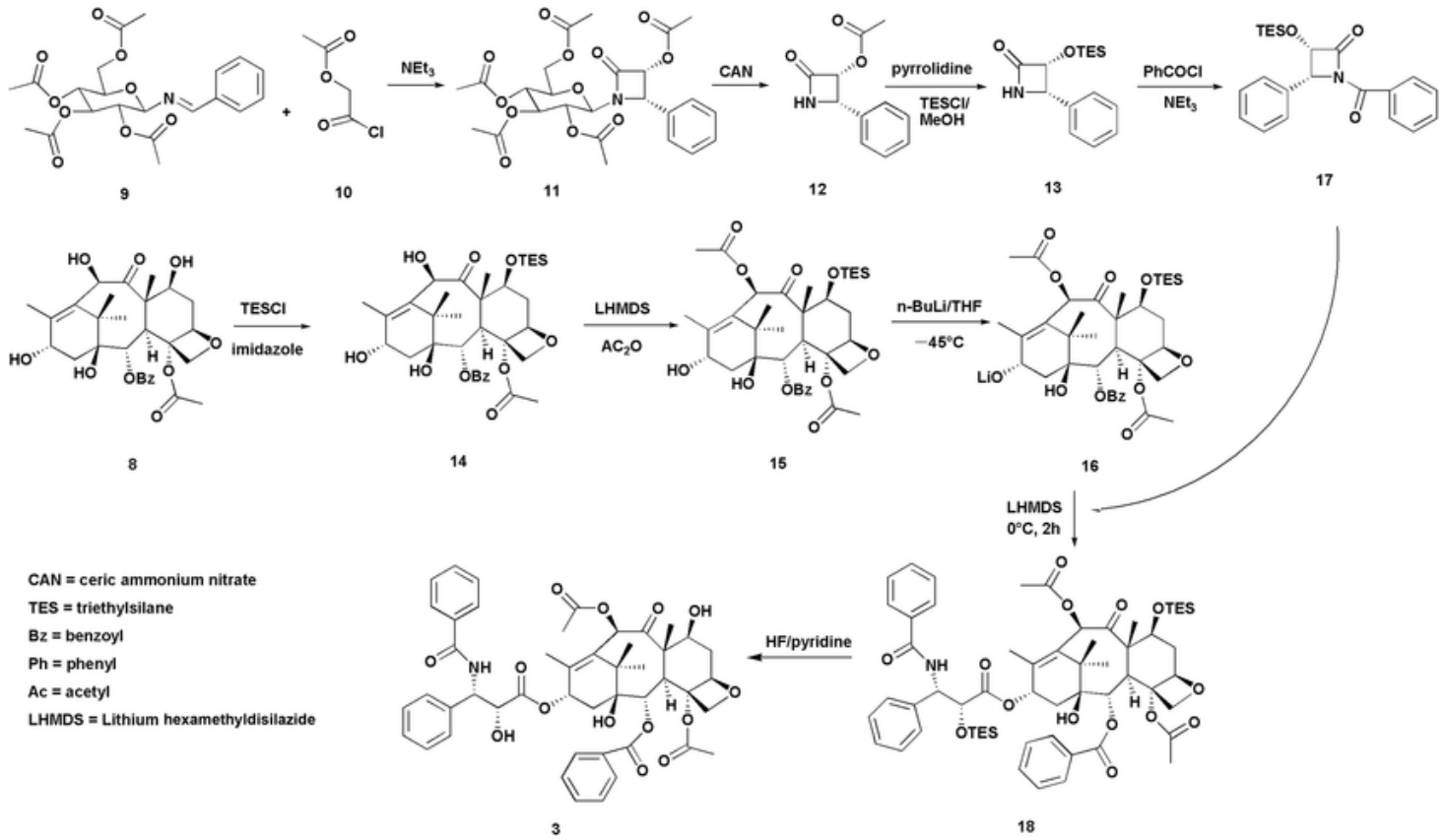
Tree that provides paclitaxel is put on list of endangered species

BMJ 2011 ; 343 doi: https://doi.org/10.1136/bmj.d7411 (Published 15 November 2011)

Synthesis alone is infeasible



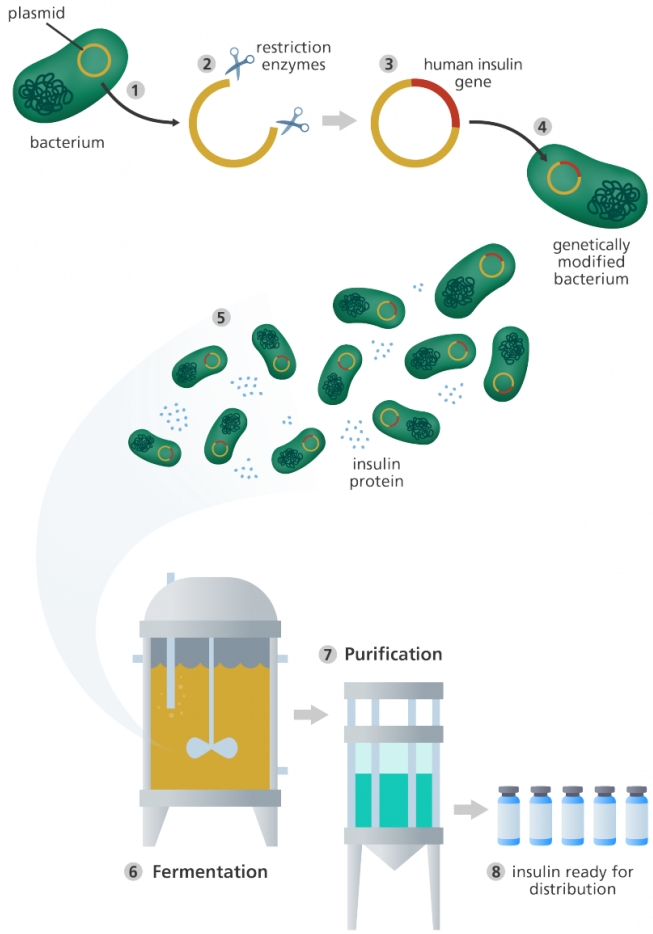
Taxol
(Paclixatel)



- Robert Holton's group suggested complete synthesis
- With ability to produce 11.6 mg of taxol

- Semi-synthesis from deacetylbaccatin extracted from European yew (Liu, Gong, Zhu, *RSC Adv*, 2016)
- 2017: 2600 kg produced

How genomic knowledge can be used?



1951:
10,000 POUNDS OF PIG PANCREASES MAKE 1 POUND OF INSULIN

TODAY:
GENETICALLY ENGINEERED BACTERIA PRODUCE ANIMAL-FREE INSULIN

An Engineered Microbial Platform for Direct Biofuel Production from Brown Macroalgae

Wargacki *et al*., *Science* 2012

High-level semi-synthetic production of the potent antimalarial artemisinin

Paddon *et al*., *Nature* 2013

Metabolic Engineering for the Biosynthesis of Longevity Molecules Rapamycin and Resveratrol

Ye & Bathia, *Industrial Biocatalysis* 2014

A microbial supply chain for production of the anti-cancer drug vinblastine

Zhang *et al*., *Nature* 2022

Perspectives and Sustainability

Are we seeing a resurgence in the use of natural products for new drug discovery?

Feng Li, Yongli Wang, Dapeng Li, Yilun Chen & Q. Ping Dou

Natural Product Research: An Immense Hope and Sustainability in Present Time

Dipankar Ghosh*

THE LANCET

- the higher rigidity of NPs can be valuable in drug discovery tackling protein–protein interactions
- NPs as a source of oral drugs ‘beyond Lipinski’s rule of 5’
- Novel antibiotics able to tackle antibiotic resistance?

Take away messages

- State-of-art analytical and computational methods give a new boost to NP-driven drug discovery
- Problems for the different discovery stages mirror each other, which might provide room for collaboration
- Interest in natural products as drug leads is being actively revitalized

Thank you for your attention!