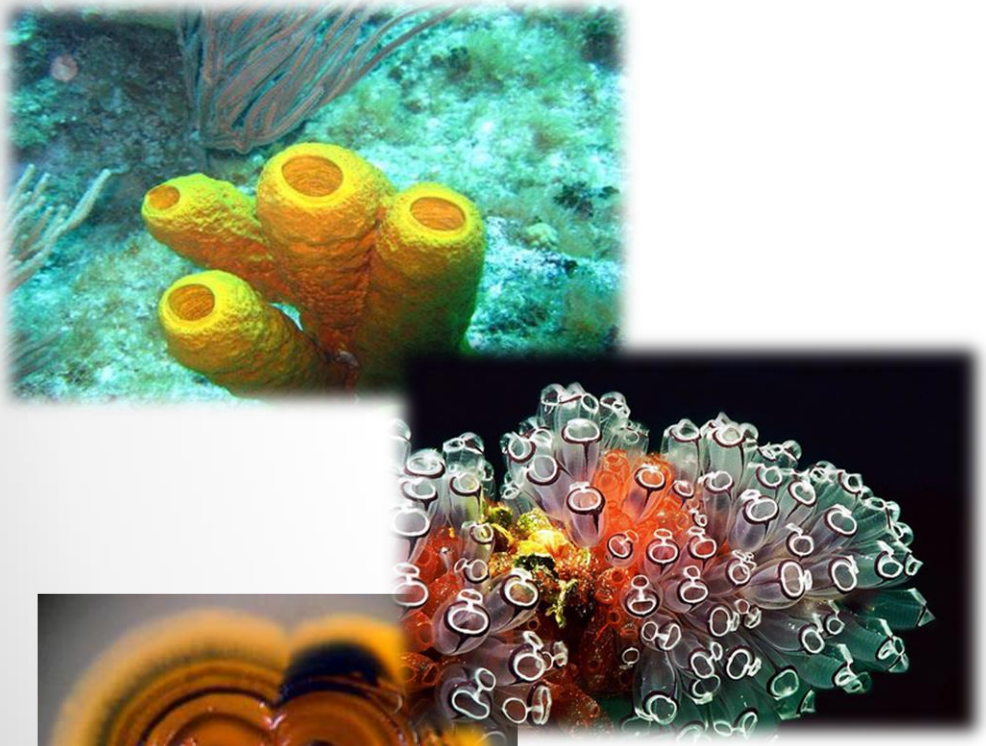
université
PARIS-SACLAY

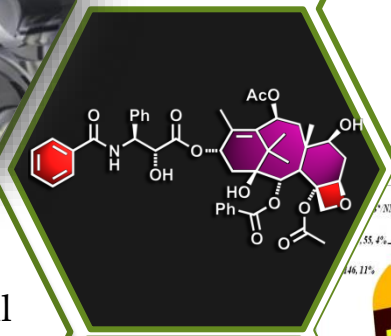
Drug Discovery from Natural Products



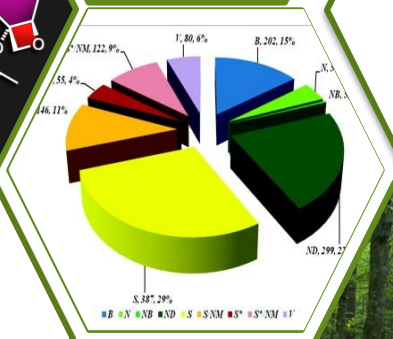
✓Mehdi Beniddir
✓@BeniddirM

Plan

Research

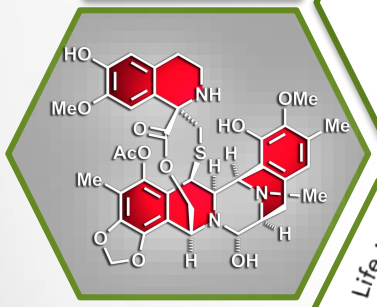


Renewing interest



Pharmaceutical Industry

Natural substances



Biodiversity sourcing

Conclusions

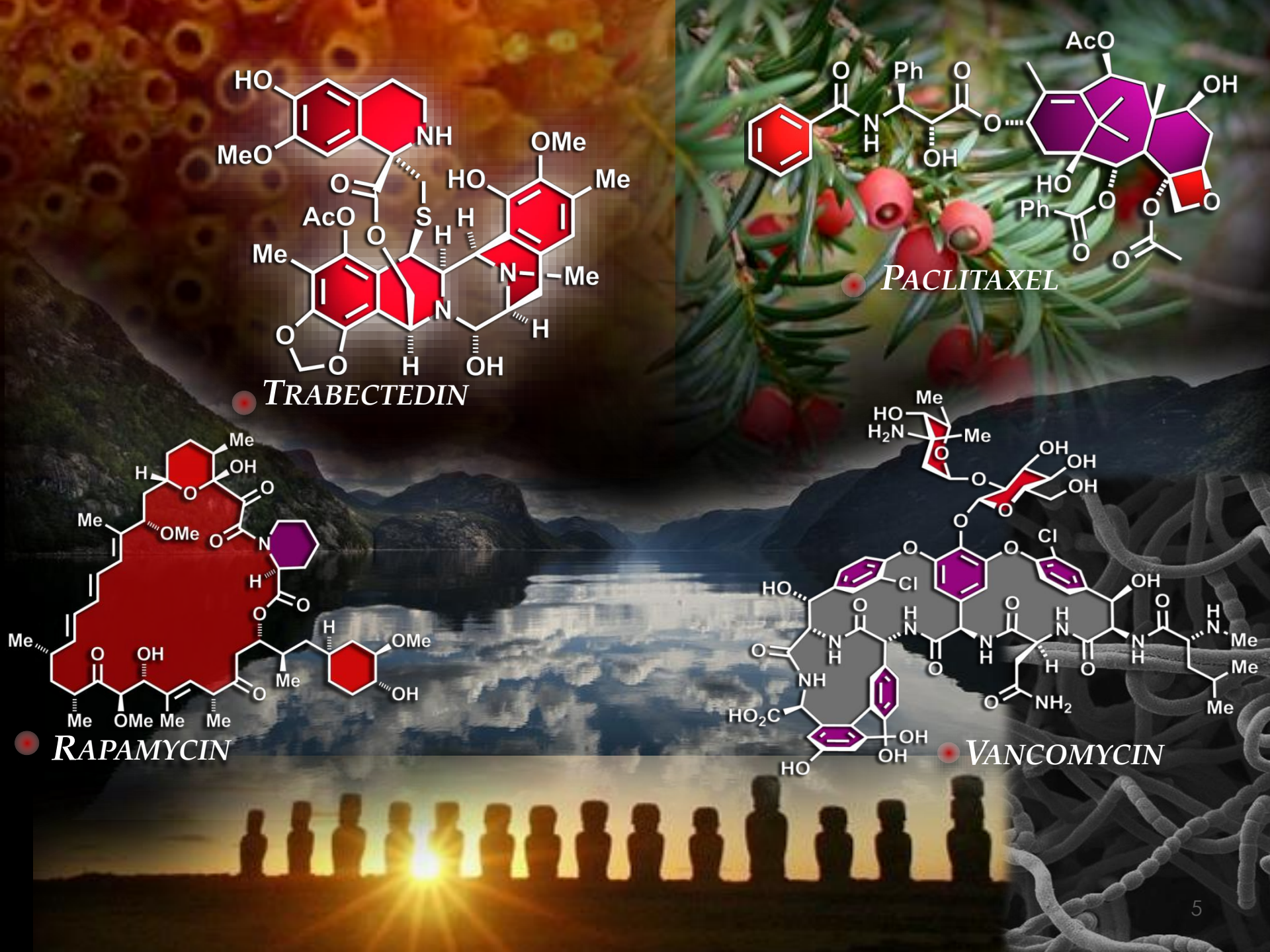
Evolution, a Grand Diversity-Generating Machine

Diversity-generating machine

Angew. Chem. Int. Ed. **2019,** *58,* 2–9

« A primeval tropical jungle, full of the most remarkable things, an amazing thicket, without escape or end, into which one would not dare to enter »

Friedrich Wöhler, 1835

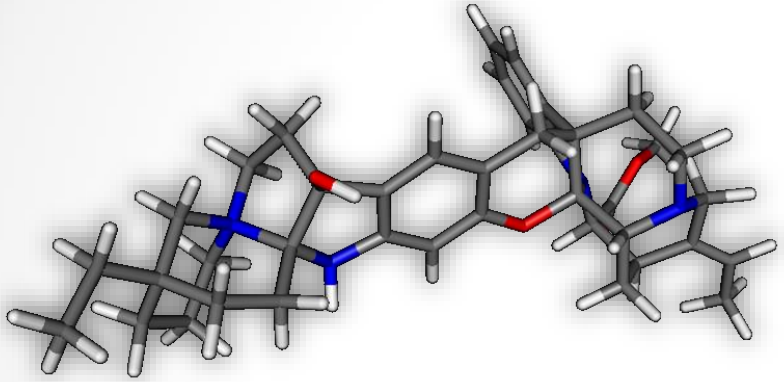

TRABECTEDIN
PACLITAXEL
RAPAMYCIN
VANCOMYCIN

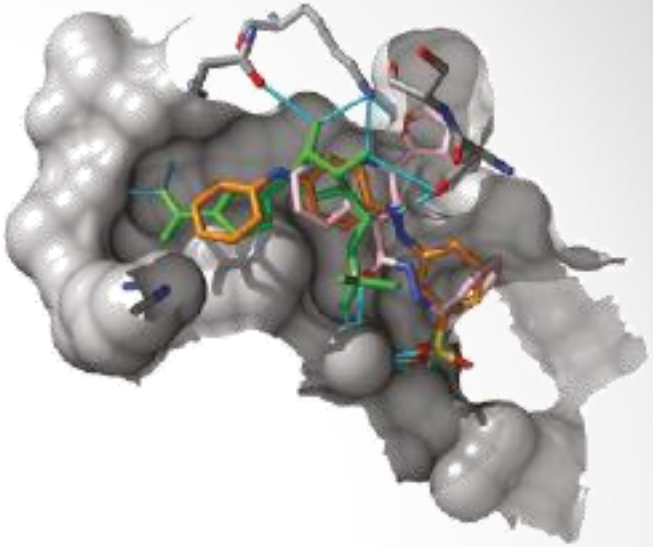
Natural products

A chemical substance produced by a living organism. This term is often used in reference to small chemical substances found in nature that have distinct pharmacological effects, such as the antibiotic penicillin.

Natural products

- ✓ « *biological validation* » concept
- ✓ Natural selection





- ✓ Privileged scaffolds
- ✓ Biochemical specificity
- ✓ Stuctural complexity
- ✓ Highly diversified
- ✓ Efficient chemical space coverage

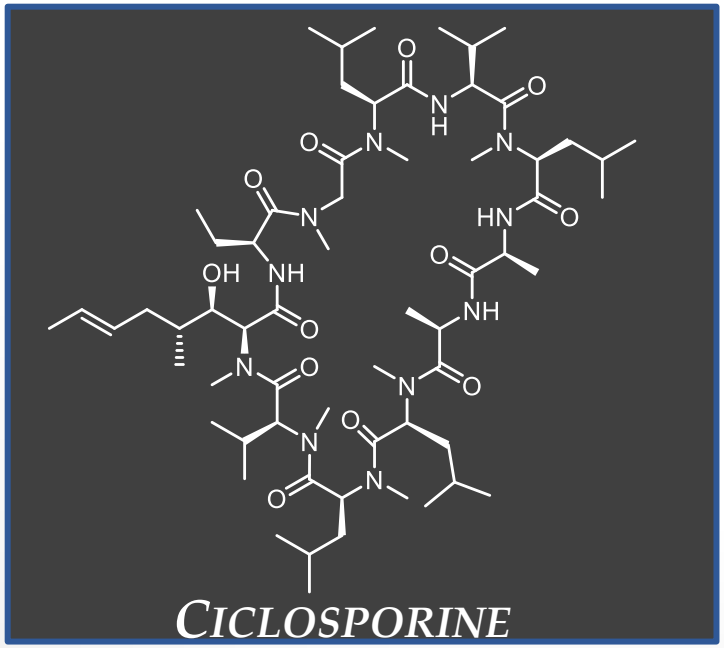
✓Discovered 200 years ago by a german pharmacist named Friedrich Sertürner.

Natural products as exceptions

« LIPINSKI'S RULE OF FIVE » [1997]

THE GUIDLINE FOR THE LIKELIHOOD OF A COMPOUND HAVING ORAL BIOAVAILABILITY (AT LEAST 3 OF THESE CHARACTERISTICS):

- *MOLECULAR WEIGHT < 500*
- *< 5 H-BOND DONORS*
- *< 10 H-BOND ACCEPTORS*
- *LOG P < 5*



Sourcing

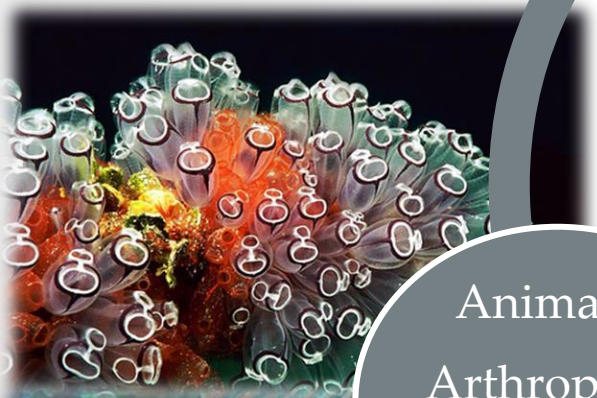
Plants

Natural products

Animals :

Arthropods, marine organisms

Micro-organisms



Sourcing

- ✓ Plants have a rich history of use by humans as therapeutics

Plants

Ebers papyrus
(1600 before J.C.)

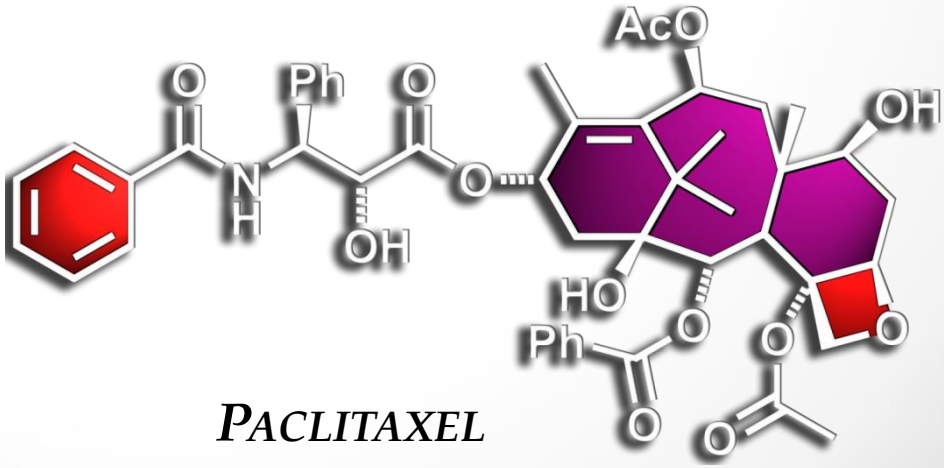
- ✓ … 80% of the world population depend either totally or partially on traditional or alternative medicine [WHO]

Sourcing

Plants

- ✓ 260 000 plants
- ✓ Easily accessible (≠ insects and marine organisms)
- ✓ Traditional uses
- ✓ 60000 studied plants

=> 135 drugs



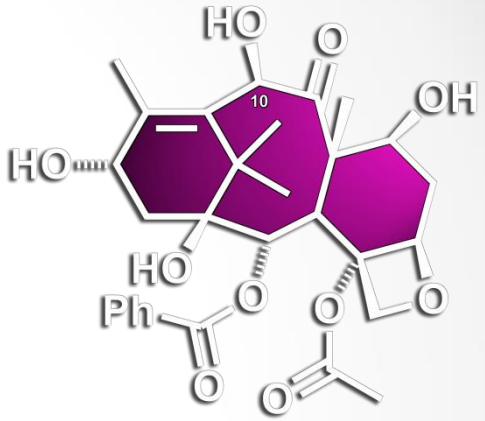
PACLITAXEL

SOLVING THE CHALLENGE OF TAXOL SUPPLY

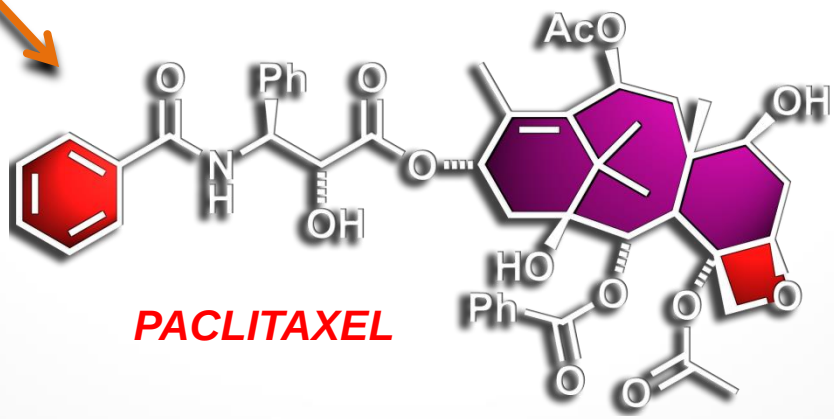
PACIFIC YEW
(TAXUS BREVIFOLIA)

~ 0.02 %
[3 TREES (100 YEARS) = 1 G OF PACLITAXEL]

EUROPEAN YEW
(TAXUS BACCATA)

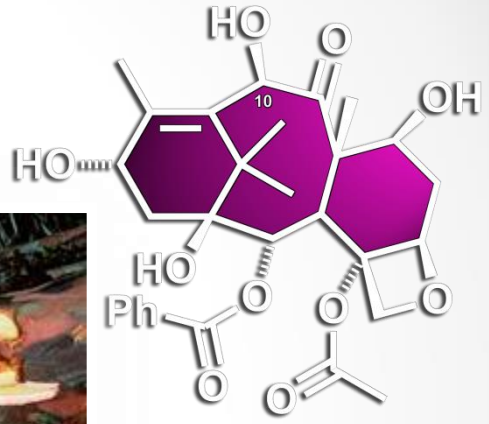


10-DESACETYLBACCATINE III (10-DAB) : PRECURSOR
~ 0.2 %



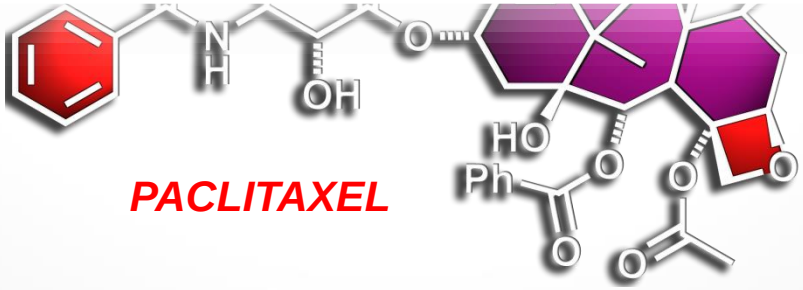
PACLITAXEL

SOLVING THE CHALLENGE OF TAXOL SUPPLY



ACETYLBACCATINE III
(10-DAB) : PRECURSOR
~ 0.2 %

PACIFIC YEW
(TAXUS BREVIFOLIA)



PACLITAXEL

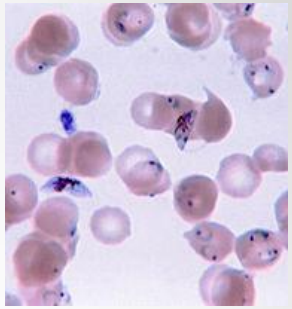
~ 0.02 %
[3 TREES (100 YEARS) = 1 G OF PACLITAXEL]

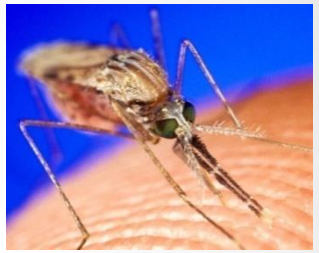
INDUSTRIAL PRODUCTION OF TAXOL:

- *SEMISYNTHESIS FROM 10-DAB*
- *PRODUCED BY PLANT-CELL FERMENTATION*

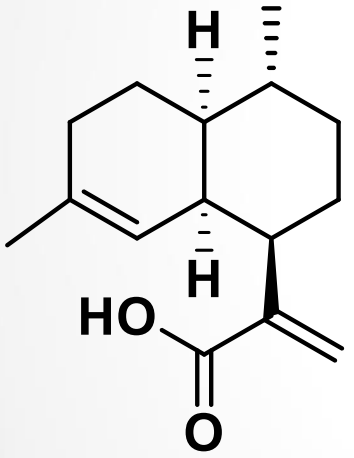
ARTEMISININ SUPPLY

- ✓ ACT « artemisinin combination therapy » WHO
- ✓ Estimated needs: 150 tons/year => 400 millions of ACTs (<1$/day/adult and 0,5$/day/child)
- ✓ Total synthesis: not profitable
- ✓ Semisynthesis from artemisinic acid

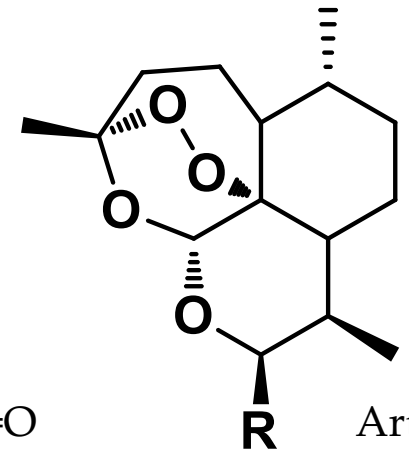




✓*Artemisia annua*

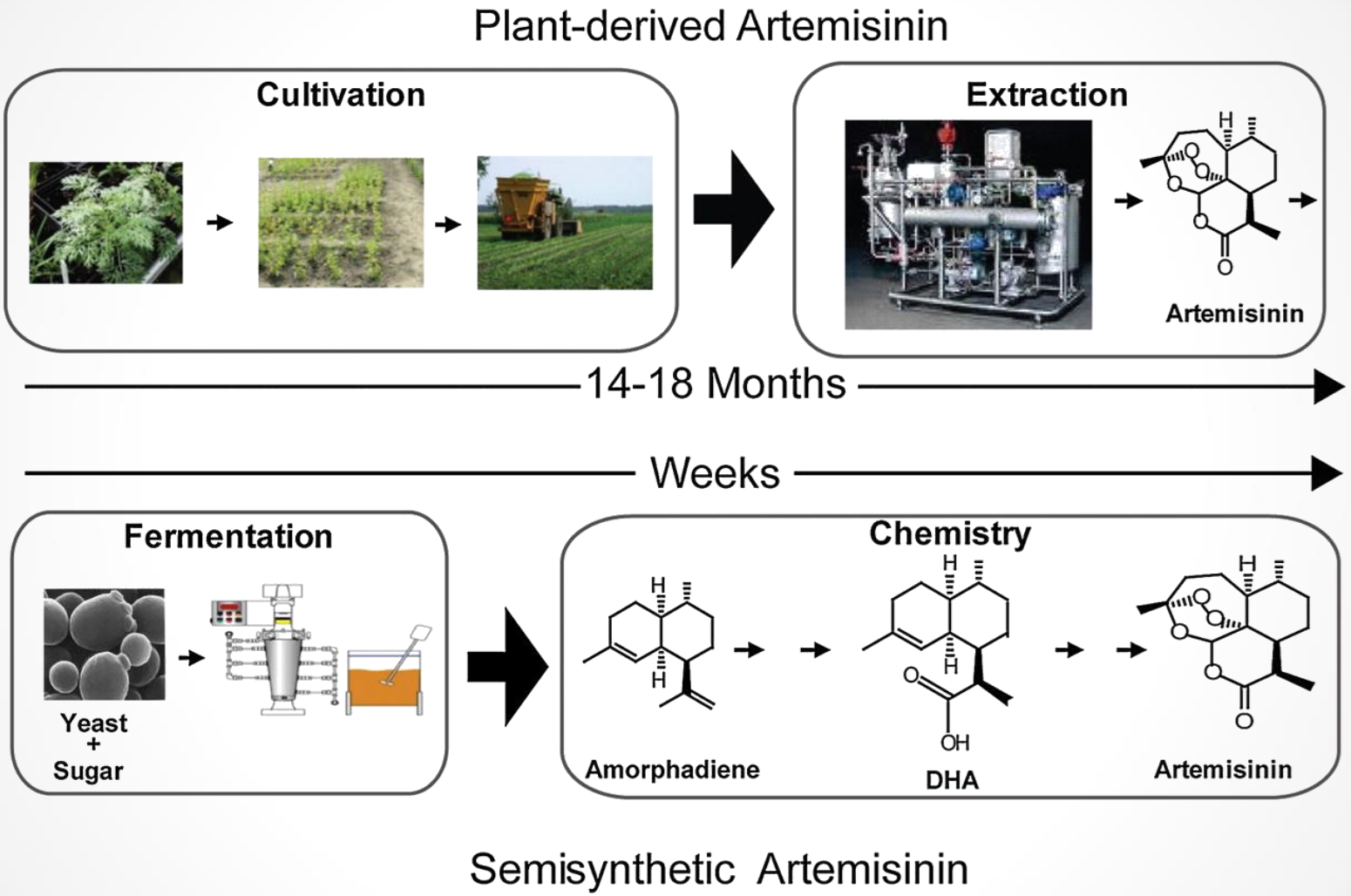


✓artemisinic acid



R = =O	Artemisinin
R = OMe	Artemether
R = OEt	Arteether
R = $OCO(CH_2)_2 CO_2H$	Artesunate
R = OH	Dihydroartemisinin

Production of plant-derived artemisinin compared to semisynthetic artemisinin.

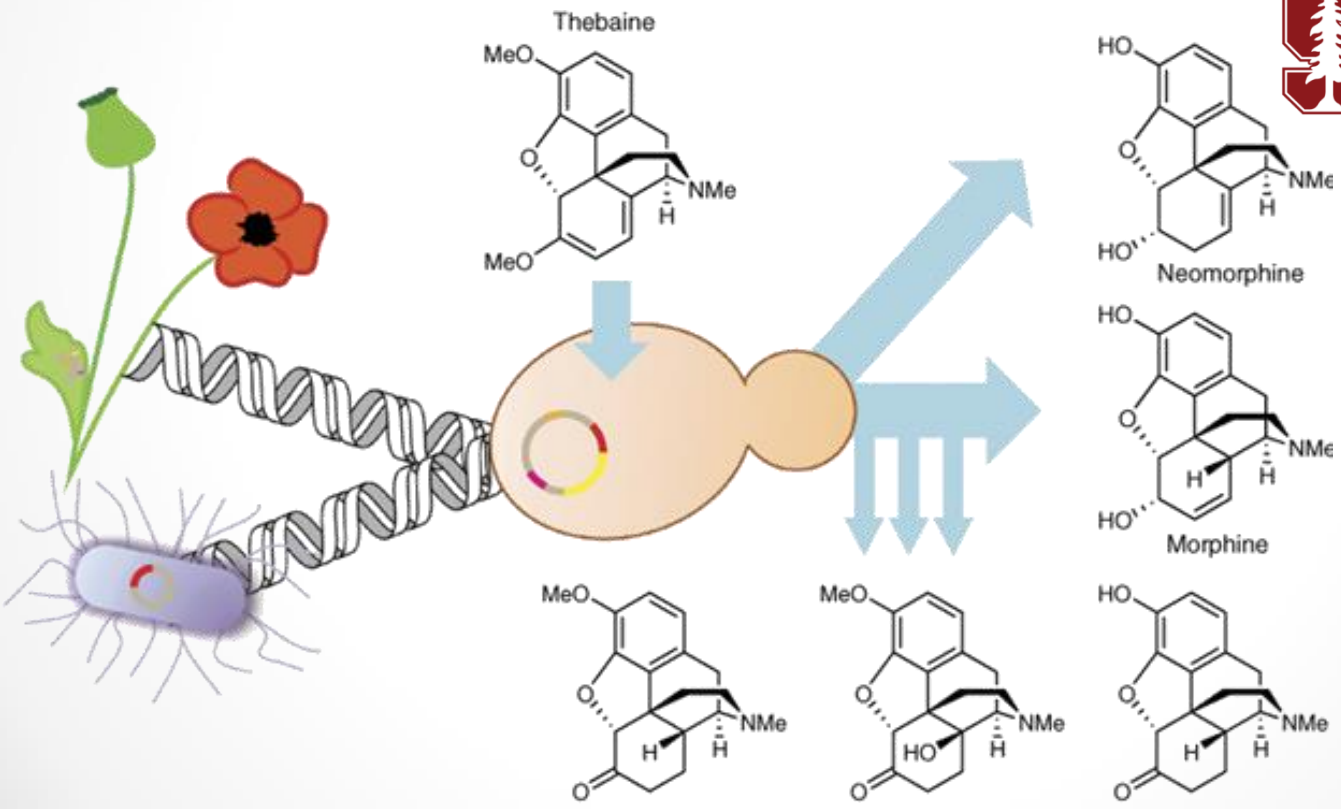


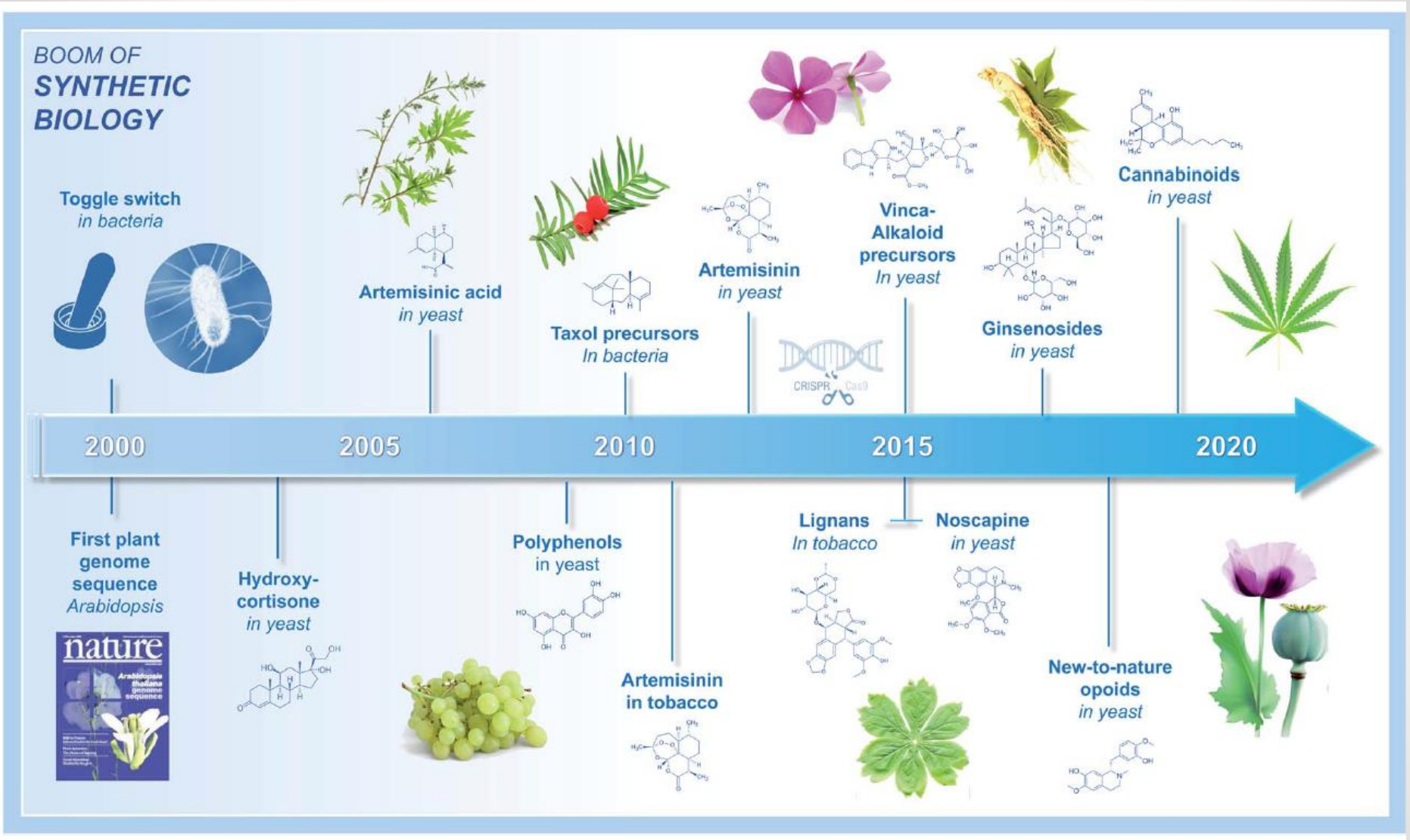
Patrick J. Westfall et al. PNAS 2012;109:655-656

SHIFTING TOWARDS SYNTHETIC BIOLOGY

- ✓ Gene discovery from opium poppy *Papaver sominferum*
- ✓ Gene cloning into *Saccharomyces cerevisiae*

Stanford University




BOOM OF
SYNTHETIC
BIOLOGY
Toggle switch
in bacteria
First plant
genome
sequence
Arabidopsis
nature
Hydroxy-
cortisone
in yeast
Artemisinic acid
in yeast
Polyphenols
in yeast
Taxol precursors
In bacteria
Artemisinin
in tobacco
Artemisinin
in yeast
CRISPR Cas9
Lignans
In tobacco
Vinca-
Alkaloid
precursors
In yeast
Noscapine
in yeast
Ginsenosides
in yeast
New-to-nature
opoids
in yeast
Cannabinoids
in yeast
2000
2005
2010
2015
2020

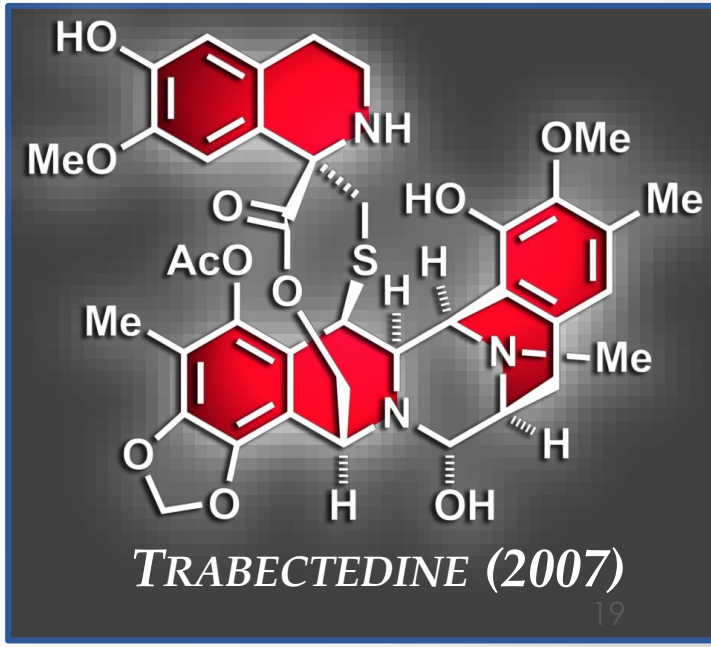
Sourcing

Animals:
Arthropods, marine organisms

Arthropods
- ✓ 1 million described species
- ✓ Insects, spiders
- ✓ Vastly unexplored chemical space

Marine organisms
- ✓ 500 000 described species
- ✓ A unique chemistry (pressure, salinity, temperature, halogenation)

ECTEINASCIDIA TURBINATA



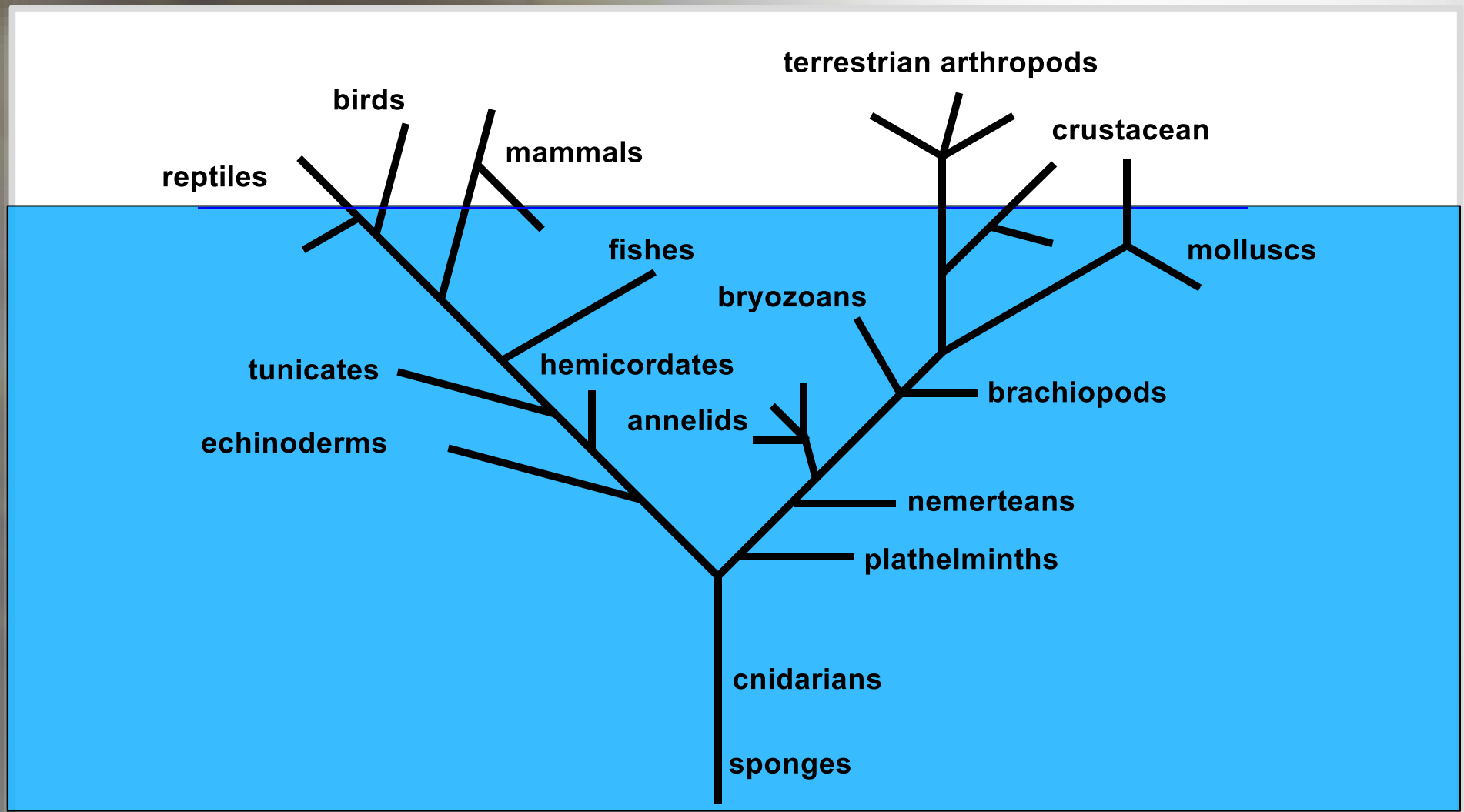
TRABECTEDINE (2007)

Drugs from the sea

- **1967: Symposium Rhode Island (USA)**
  « Drugs from the Sea »
- **1970: SCUBA diving advances**
- **50 years later… 40 000 marine compounds discovered and 6 drugs on the market**

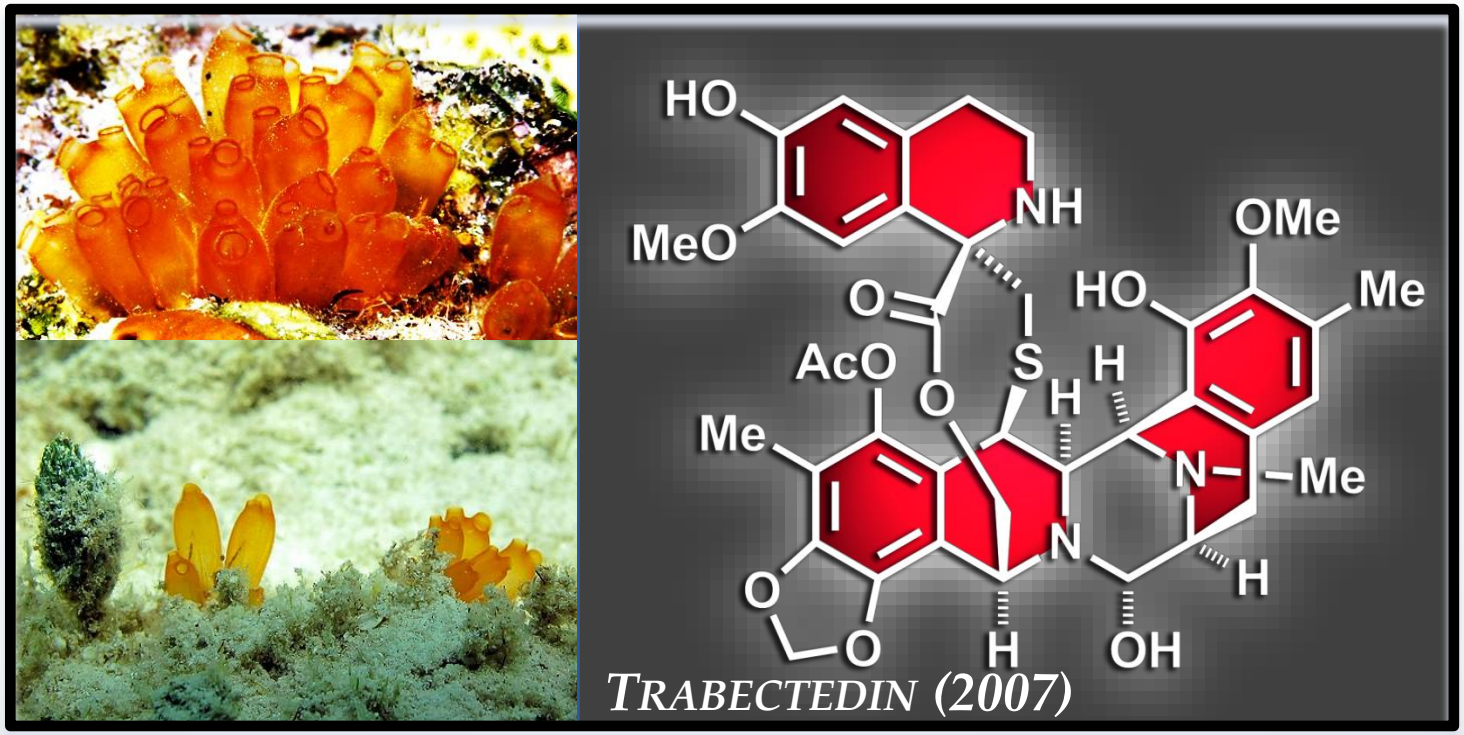
85 % of the known species

15 % of the known species
(80 % of the zoological orders)

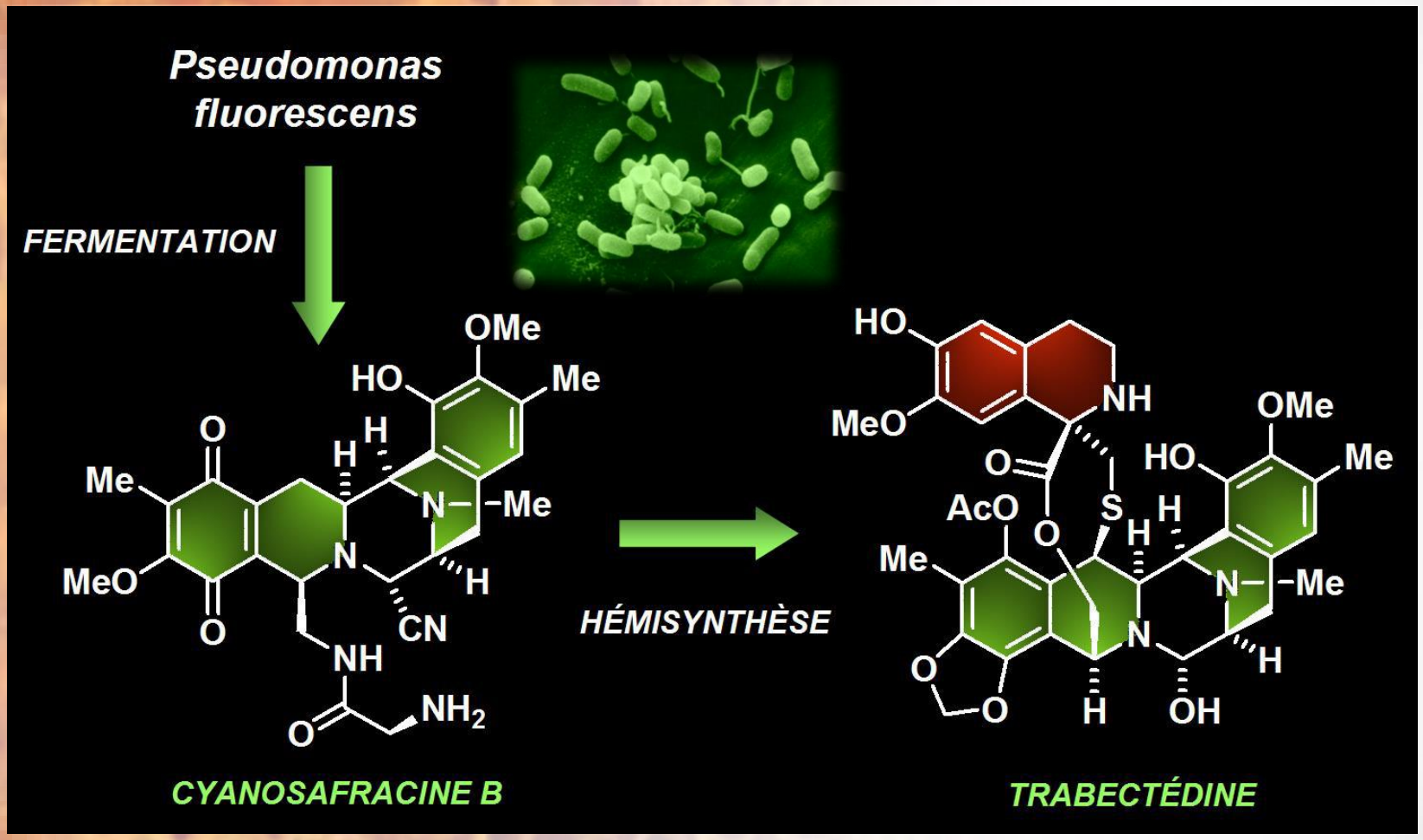

terrestrian arthropods
birds
crustacean
mammals
reptiles
fishes
molluscs
bryozoans
tunicates
hemicordates
brachiopods
annelids
echinoderms
nemerteans
plathelminths
cnidarians
sponges

ECTEINASCIDINE (TRABECTEDINE DCI)

- *STRUCTURE: POLYCYCLIC ALKALOID.*
- *ANTITUMORAL*
- *SUPPLY: ISOLATED FROM ECTEINASCIDIA TURBINATA, TUNICATE (FLORIDA KEYS, BAHAMAS, MEDITERRANEAN SEA).*



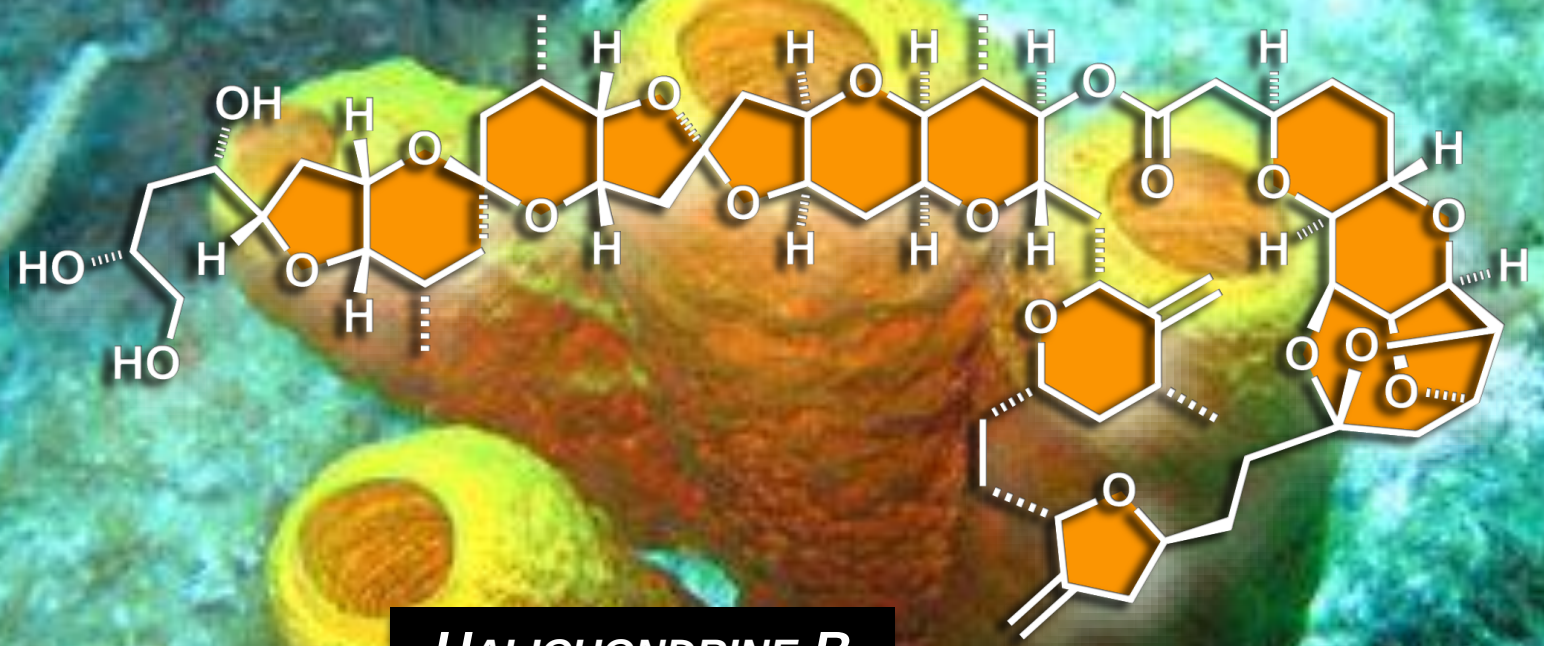
ECTEINASCIDINE : INDUSTRIAL PRODUCTION



PharmaMar

HALICHONDRINES

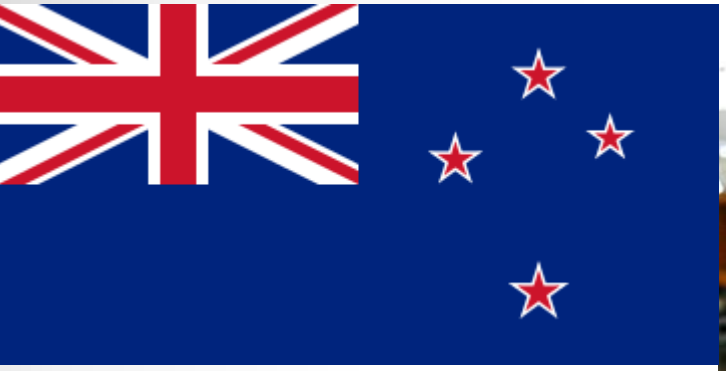
- SOURCE: MARINE SPONGE (*Halichondria okadai*)
- ACTIVE COMPOUND: HALICHONDRINE B
- SUPPLY CHALLENGE ? (10 G FOR CLINICAL TRIALS)



HALICHONDRINE B

Halichondria okadai

HALICHONDRINES

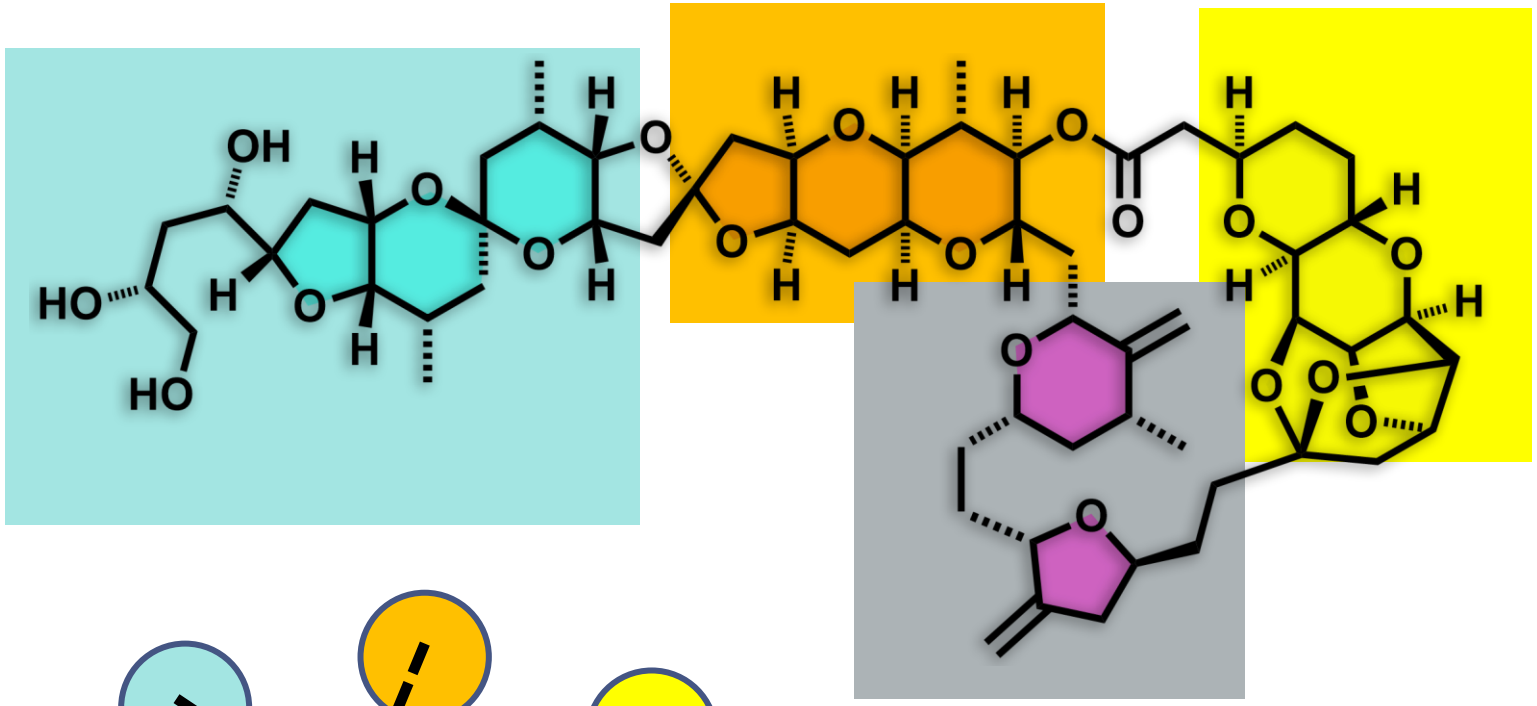


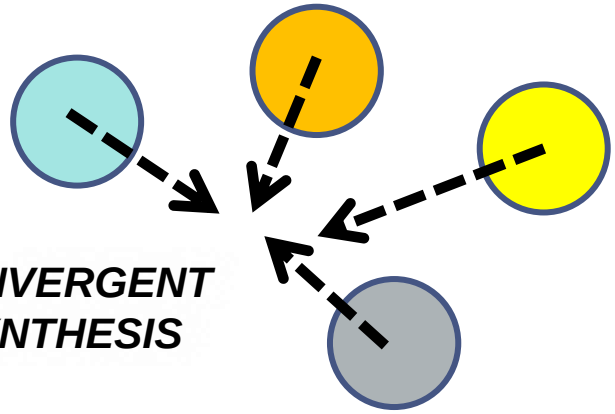
AQUACULTURE: LISSODENDORYX SP.

1 TON = 310 MG

HALICHONDRINES

« THE ART OF TOTAL SYNTHESIS »

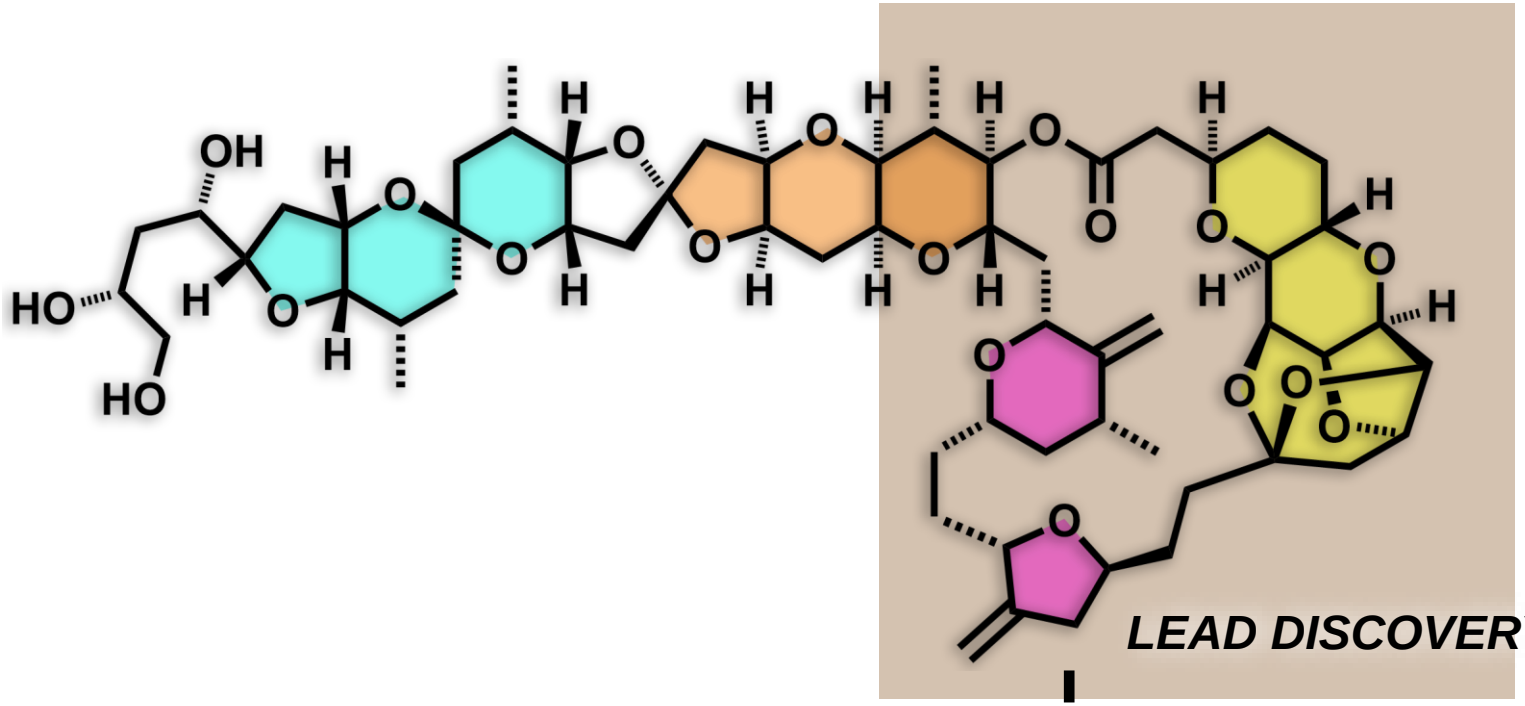




CONVERGENT SYNTHESIS

HALICHONDRINES

« THE ART OF TOTAL SYNTHESIS »

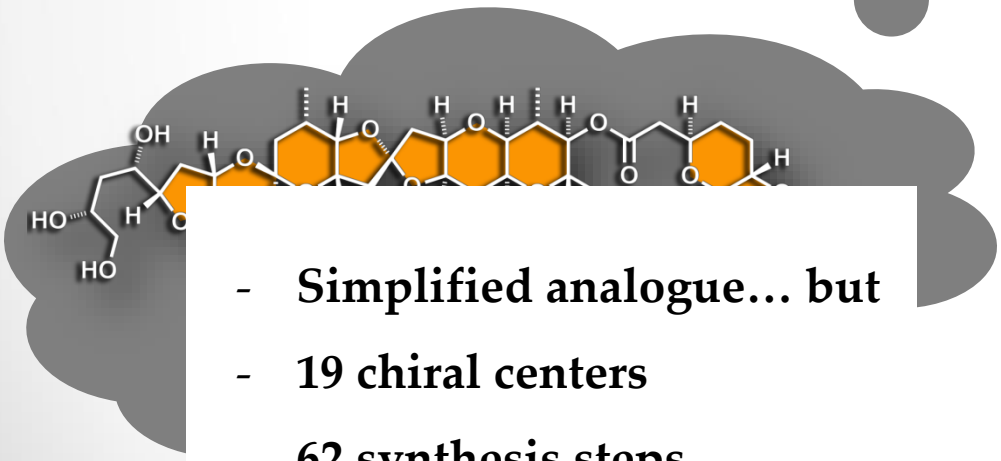


« DRUG DESIGN »

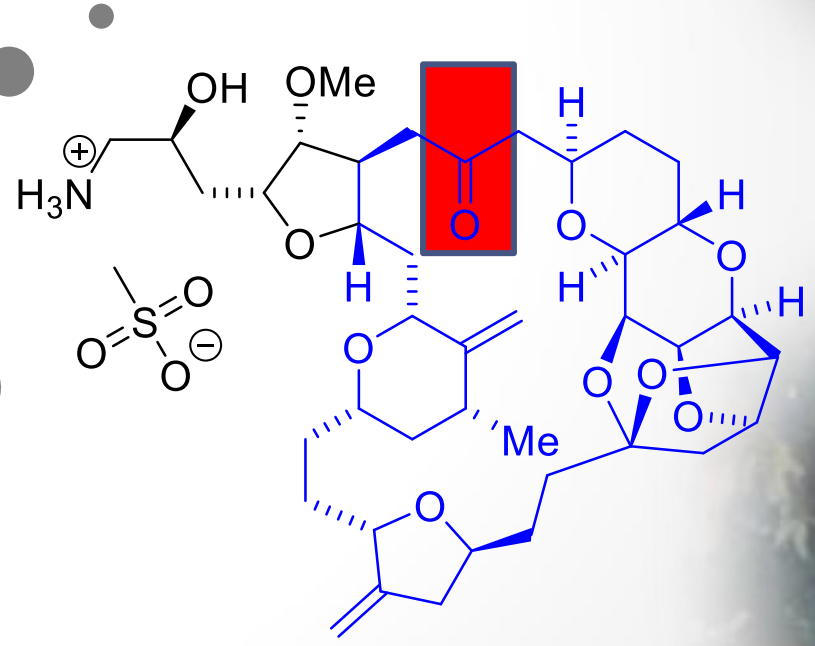
ERIBULINE

HALICHONDRINES: DISCOVERY OF ERIBULINE

- DISCOVERY OF A SIMPLIFIED ANALOGUE: ERIBULINE
- TARGET: TUBULINE
- USES: BREAST CANCER (2ND INTENTION), HALAVEN® (2011)



- Simplified analogue… but
- 19 chiral centers
- 62 synthesis steps



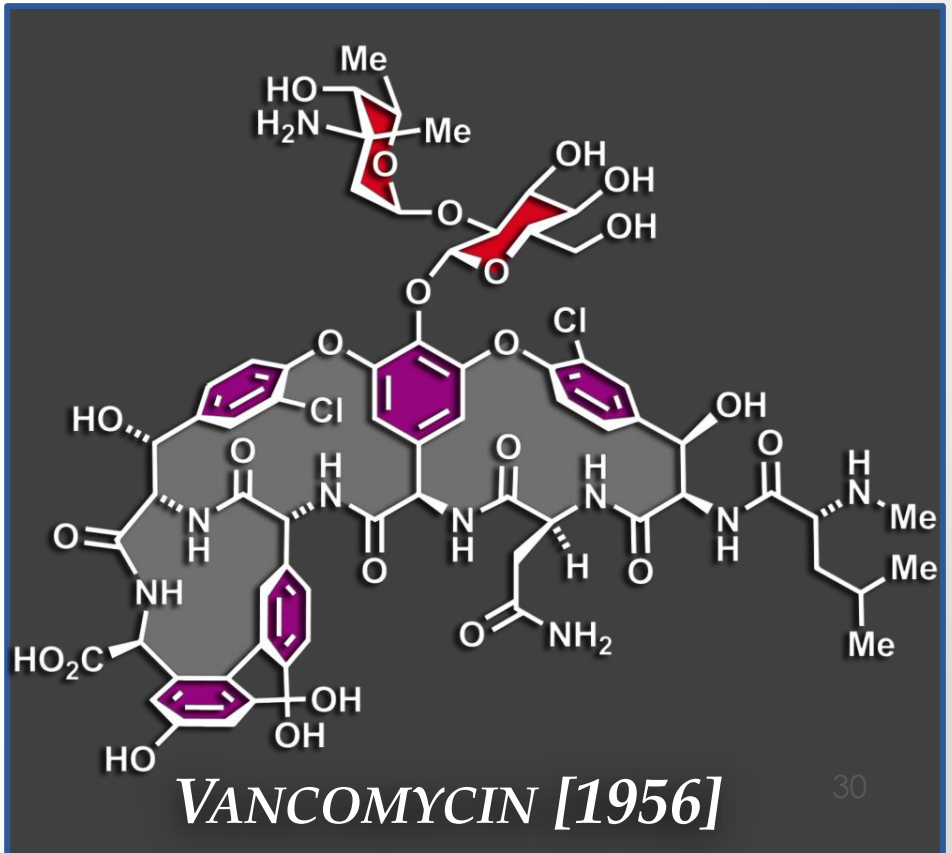
ERIBULINE

Sourcing

Micro-organisms

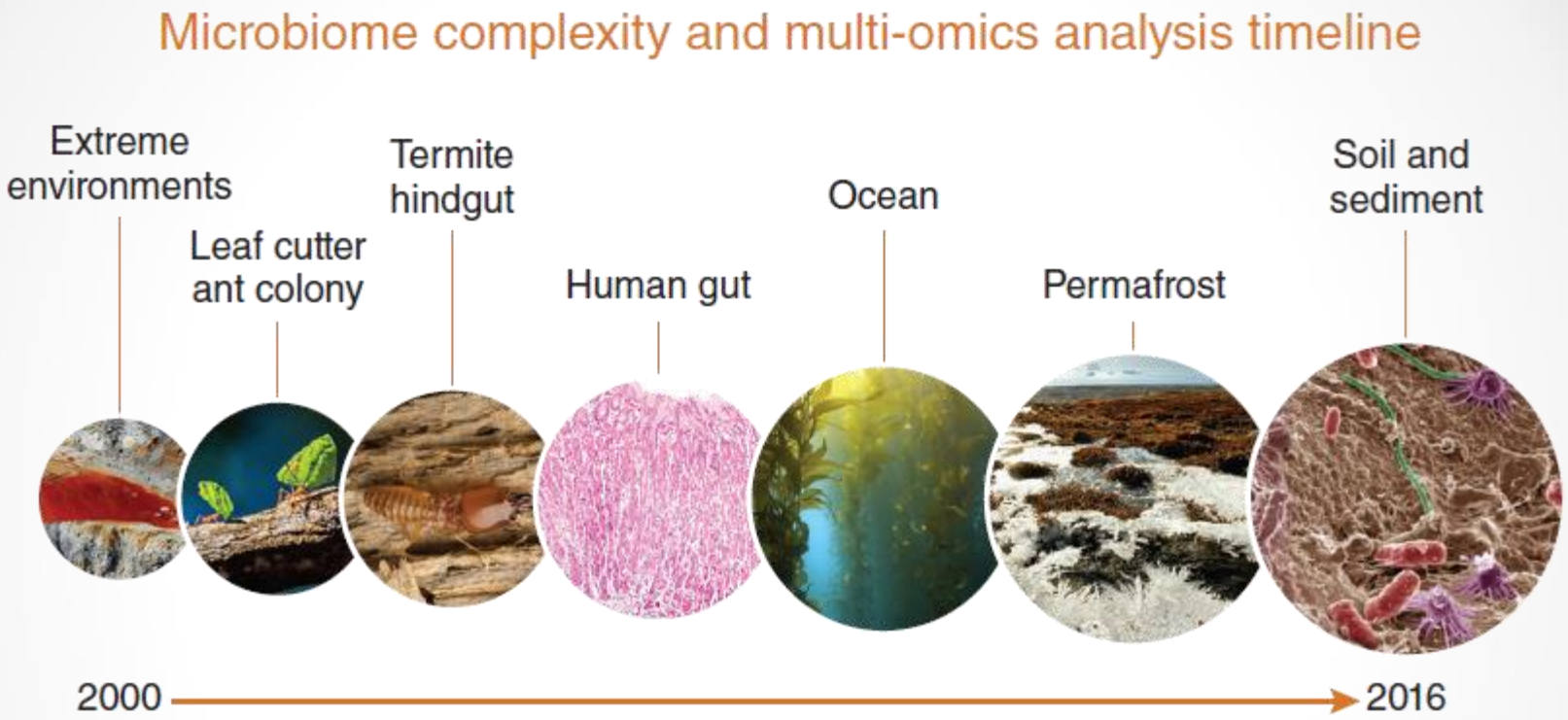
Terrestrian and marine micro-organisms (actinomycetes), fungi and bacteria (myxobacteria)

- ✓ A prolific source of antibiotics
- ✓ Highly complex molecular architecture
- ✓ Cultivable (scale-up)

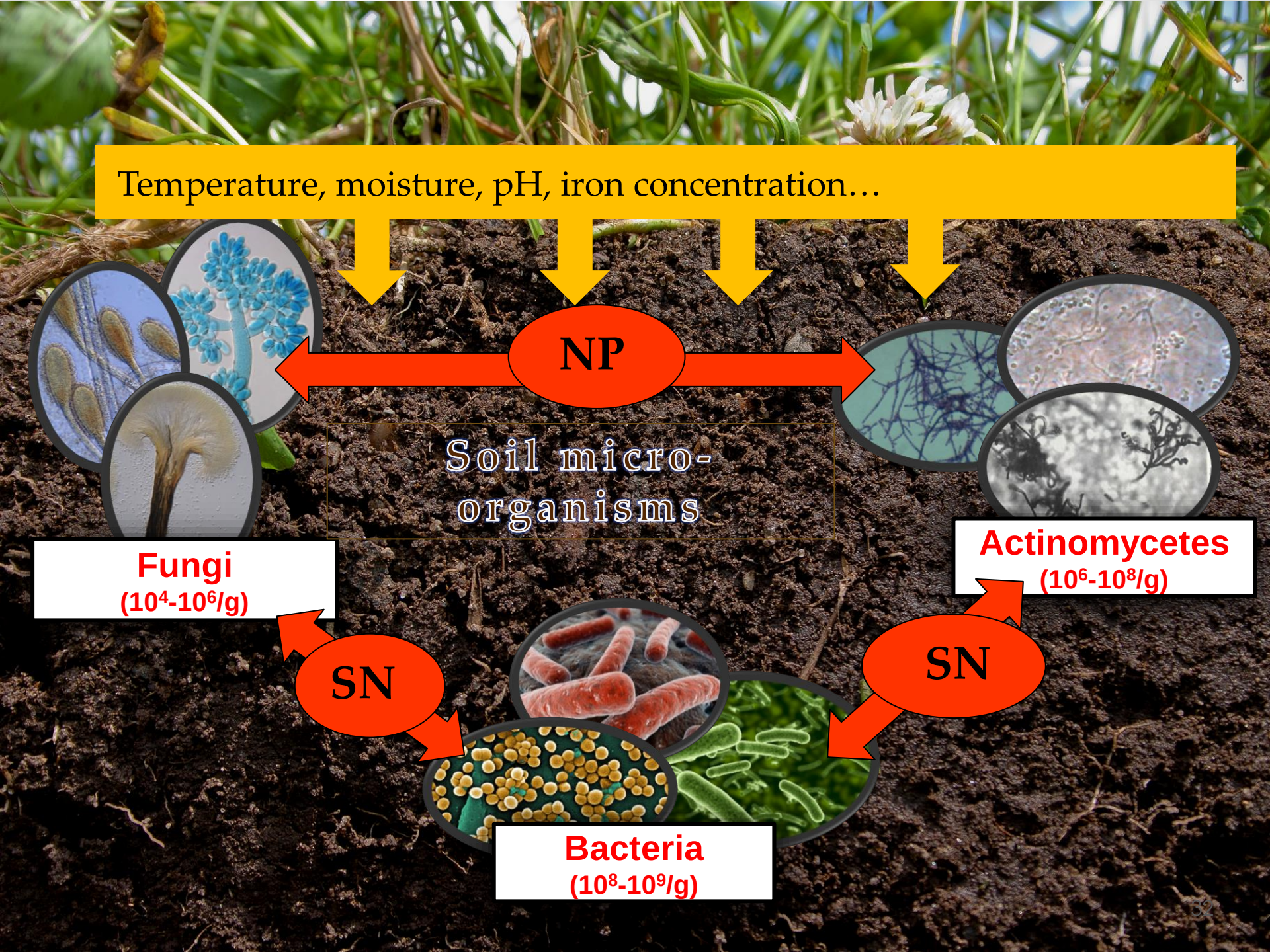


VANCOMYCIN [1956]

Bioprospection

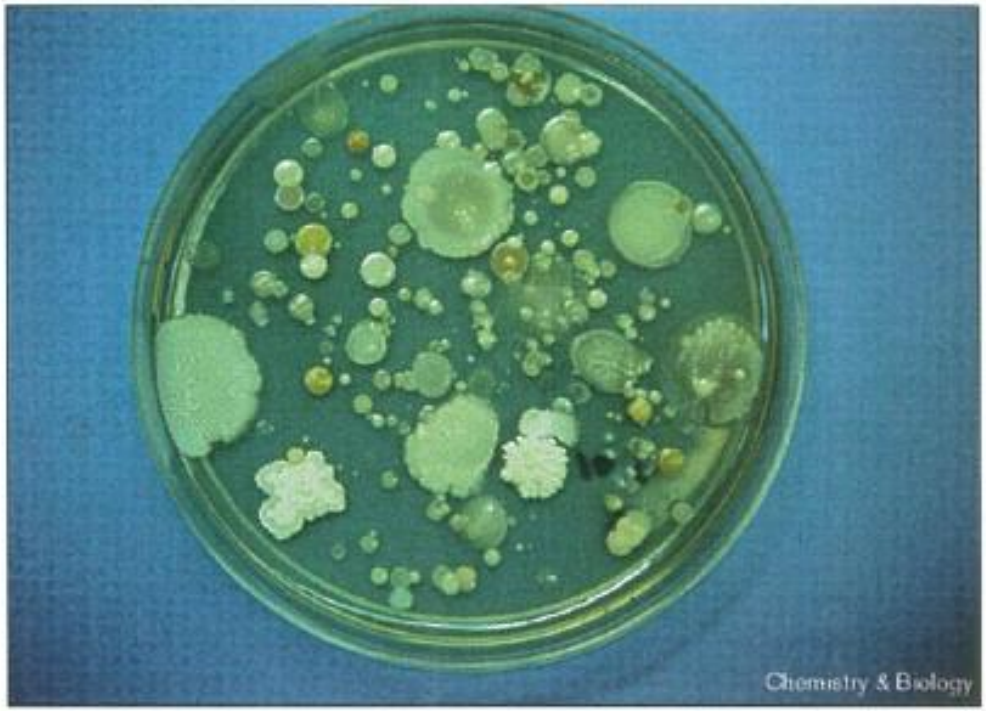


NATURE PROTOCOLS | VOL.11 NO.11 | 2016 |

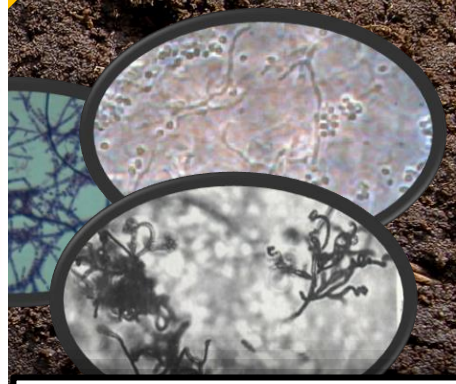

Temperature, moisture, pH, iron concentration…
NP
Soil micro-organisms
Fungi
(10⁴-10⁶/g)
Actinomycetes
(10⁶-10⁸/g)
SN
SN
Bacteria
(10⁸-10⁹/g)

Temperature, moisture, pH, iron concentration…

Fungi
(10^4-10^6/g)



Morphological diversity typical of microorganisms cultured from soil on a broad spectrum medium, tryptic soy agar.



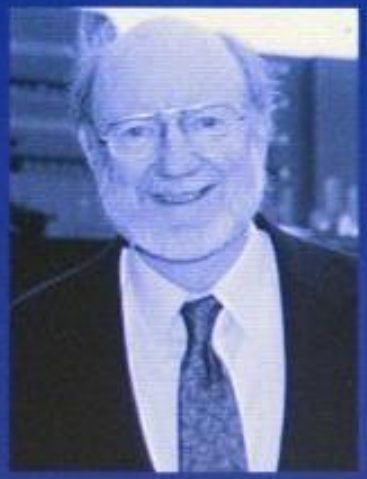
Actinomycetes
(10^6-10^8/g)

N

Bacteria
(10^8-10^9/g)

Actinomycetes

The 2015 Nobel Prize in Physiology or Medicine



William C. Campbell

Born 1930, Ireland
Drew University,
Madison, New Jersey,
USA

Satoshi Ōmura

Born 1935, Japan
Kitasato University,
Tokyo, Japan

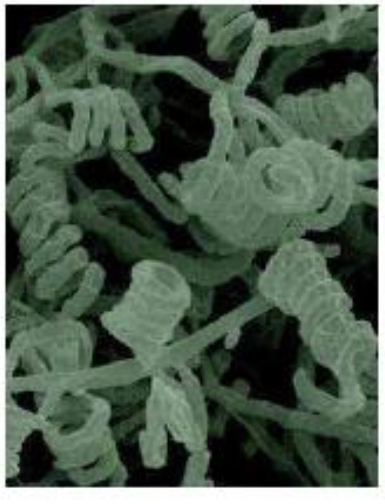
Youyou Tu

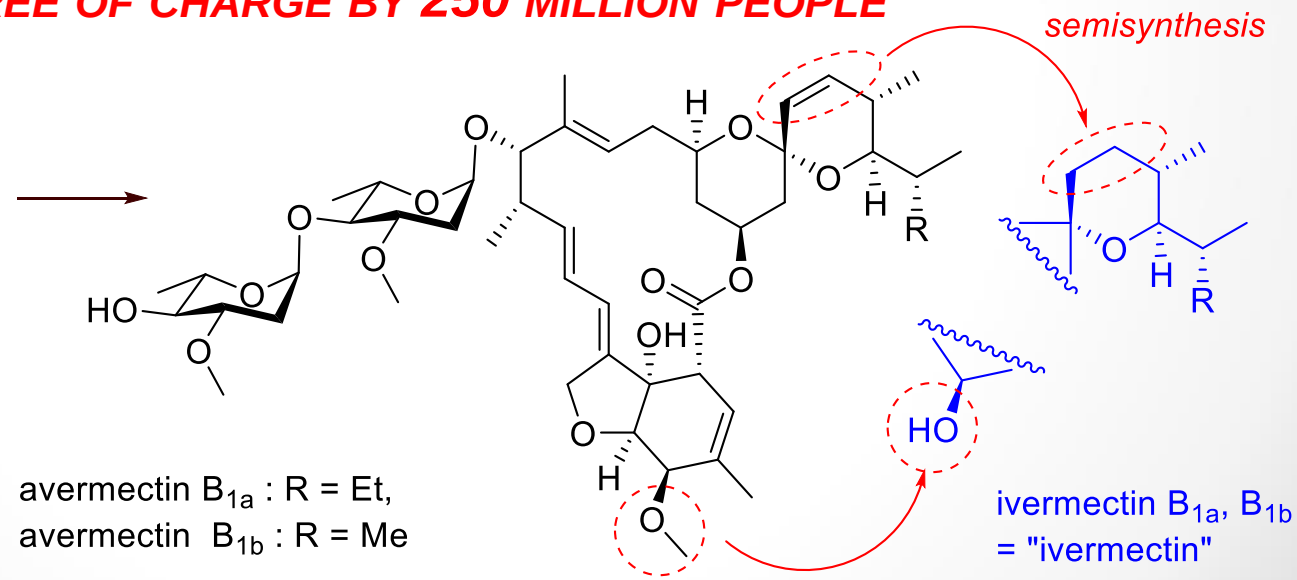
Born 1930, China
China Academy of
Traditional Chinese
Medicine, Beijing, China

Actinomycetes

AVERMECTINS, A GIFT FROM THE SOIL

STREPTOMYCES AVERMITILIS (RENAMED AS AVERMICTICINIUS) [1974]
ORIGIN: JAPANESE SOIL (KAWANA)
A MIXTURE OF TWO CLOSE ANALOGUES
BROAD-SPECTRUM ANTIPARASITIC AGENT (ANTIHELMINTIC) NEGLECTED TROPICAL DISEASES
TAKEN ANNUALLY FREE OF CHARGE BY 250 MILLION PEOPLE



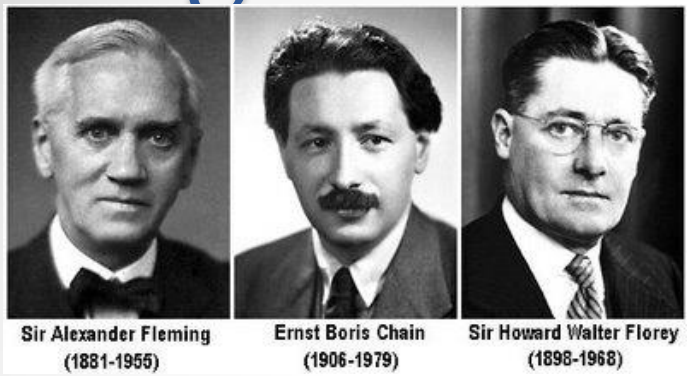


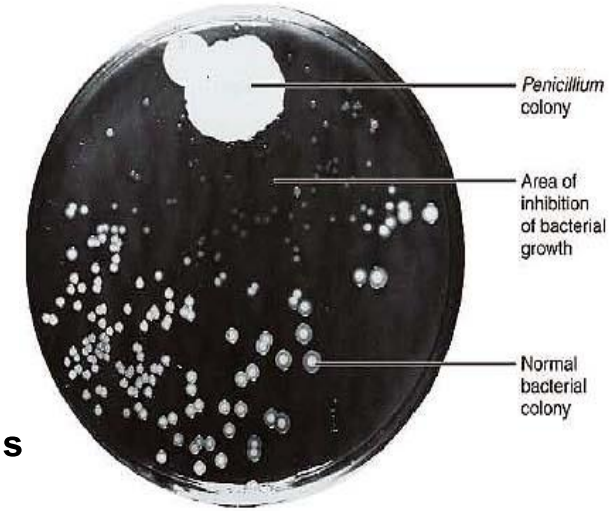
[*Streptomyces avermitilis*]

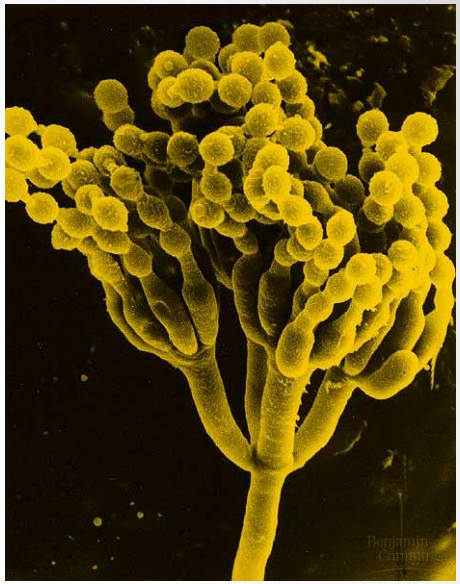
« River blindness » (Onchocerciasis, *Onchocerca volvulus*)

« elephantiasis » (lymphatic filariasis, *Wuchereria bancrofti*)

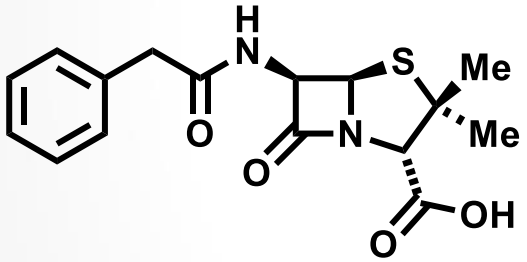
Fungi and antibiotics





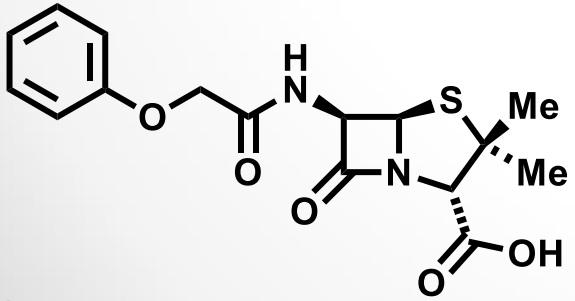


Currently used natural penicillins

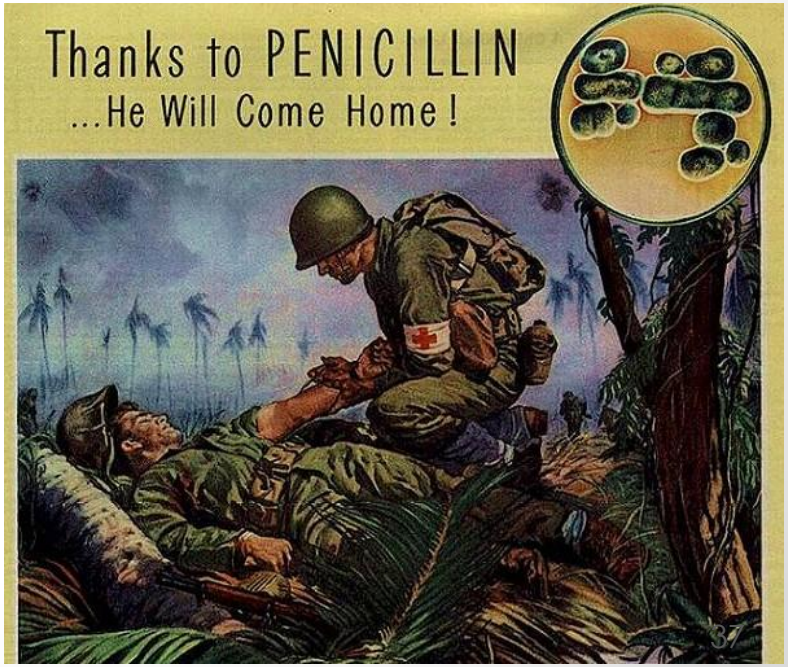


benzylpenicillin = penicillin G

historically *aka* "american penicillin"



phenoxymethylpenicillin = penicilline V



Natural products and pharmaceutical industry

- ✓ 1940-1960 : High scale production of penicillin during the World War II (golden age era)

Antibiotics : Tetracyclins, aminosides, glycopeptides

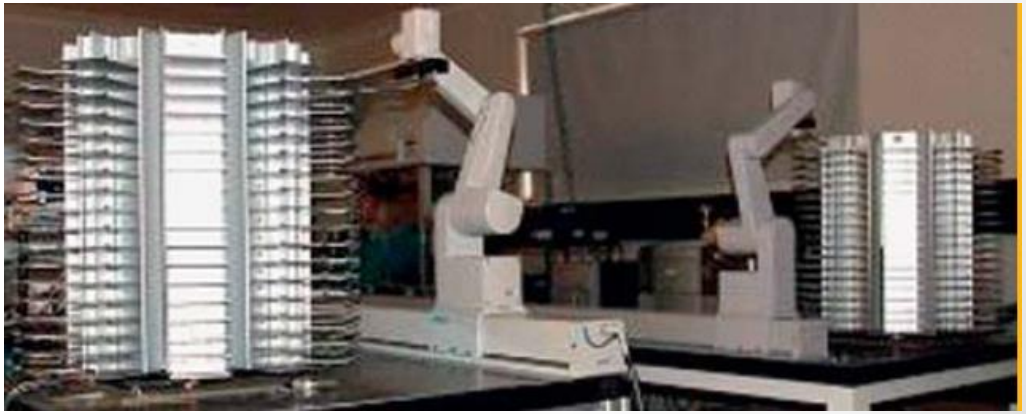
- ✓ 1960-1990 : Lipid-lowering medications (lovastatines), anticancer drugs: vinblastine (1965) , taxol (1967), antimalarials (quinine, artemisinin), immunosuppressants

Life expectancy in much of the world lengthened from about 40 years early in the 20th century to more than 77 years today.

Natural products and pharmaceutical industry

1990 - 2000 :

- ✓ Development of High-throughput Screening (HTS): chemical libraries
- ✓ Emergence of combinatorial chemistry



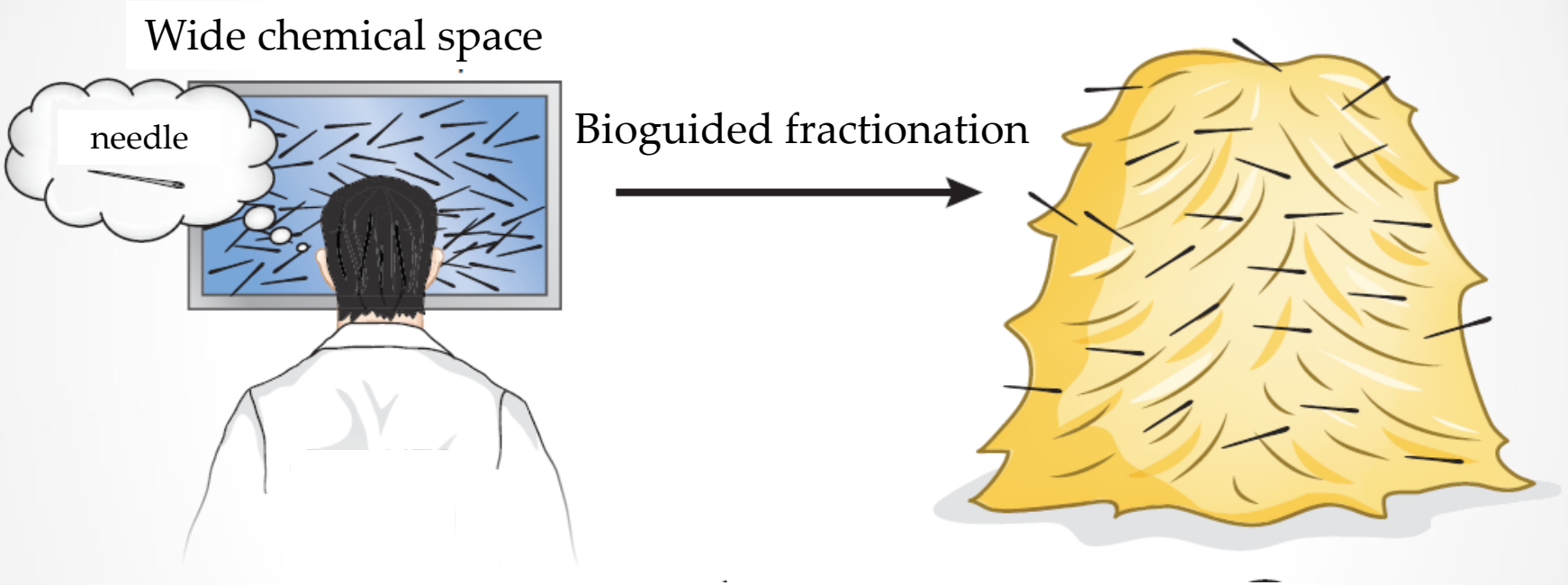
Natural products and pharmaceutical industry

1990-2000:

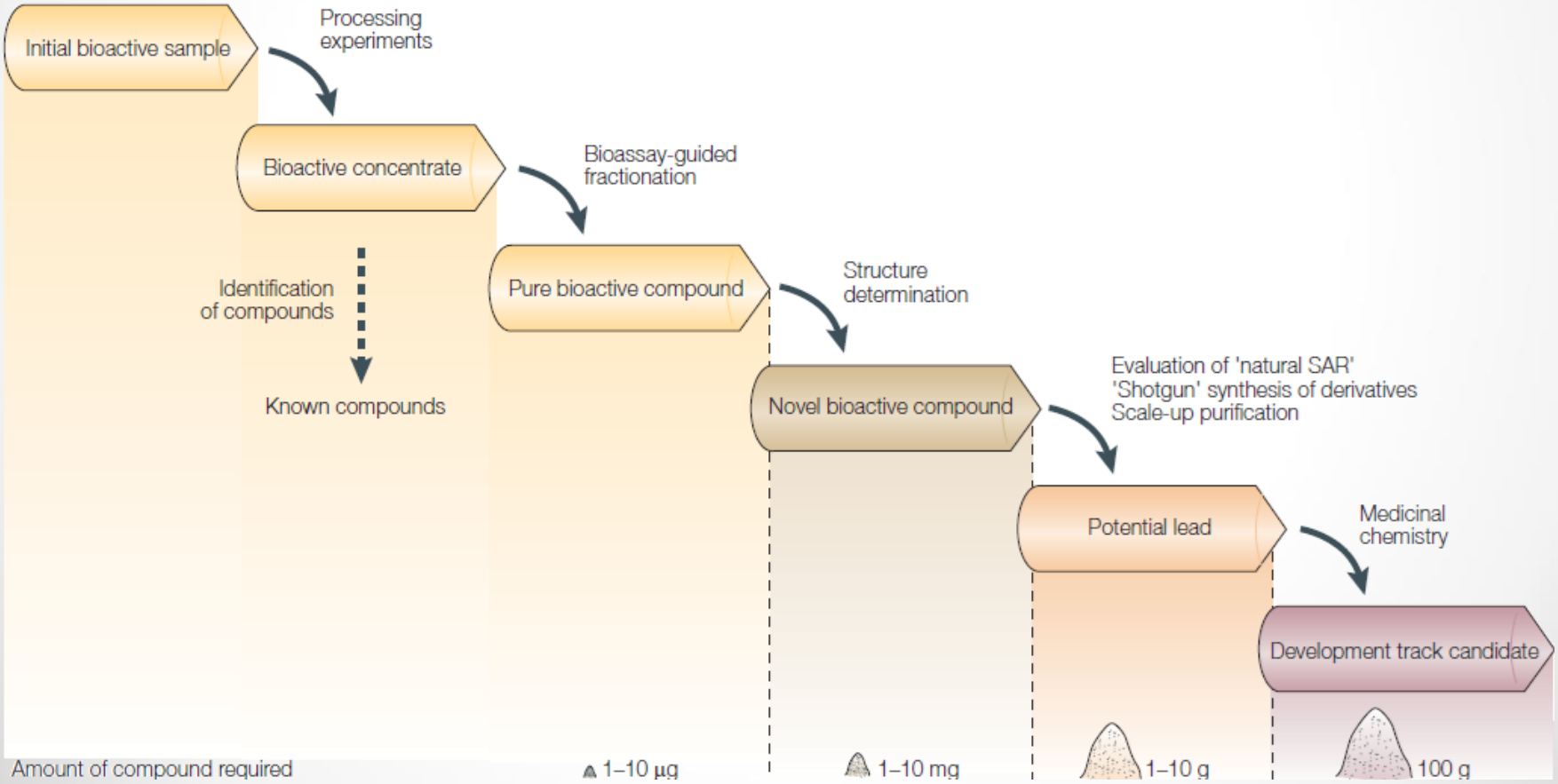
Industry reluctancy to pursue NP-based drug discovery program:

- ✓ Elimination of their NP research programs

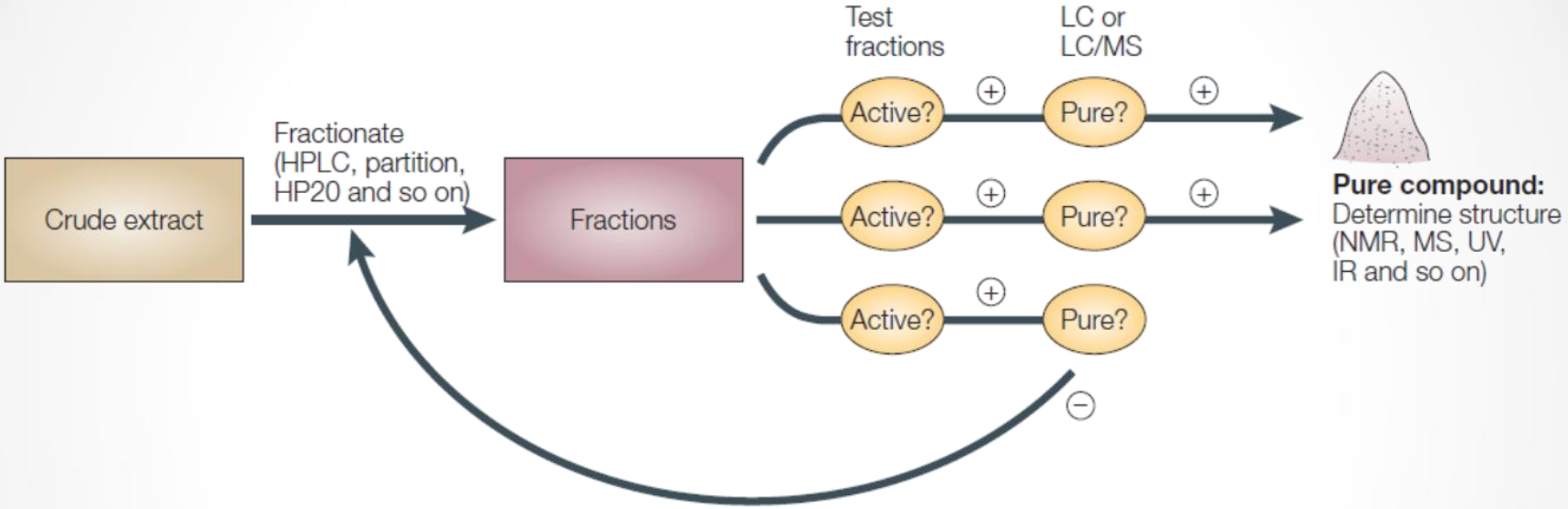
What could be the reasons of this reluctancy ?



Chemical process for natural product discovery



Generic scheme for bioassay-guided fractionation



- ✓ Bioguided fractionation using **cell-based assays** (assessment of molecular interactions within the context of a living cellular environment)
- ✓ Bioguided fractionation using **biochemical-assays** has the advantage of providing target-specific information.

What could be the reasons of this reluctancy ?

- ✓ **Incompatibility with the pharmaceutical environment**

Firms involved in drug discovery must hit the target not only accurately, but very quickly and very profitably. However, for reasons outlined below, natural product sources are currently not very amenable to rapid high-throughput screening (HTS).

- ✓ **Difficulties in discovering natural product drug candidates**

In contrast to synthetic libraries, hits from natural sources are likely to have complex structures with numerous oxygen-containing substituents and an abundance of centers of stereochemistry rendering their optimization very difficult.

Dealing with mixtures !

the initial extract of the natural material usually consists of a complex mixture after fractionation. It may contain only very small quantities of a bioactive substance, often as a mixture with structurally related molecules. The initial concentration of an interesting compound may be too low to be effectively detected by HTS, or the assay may be obscured by poor solubility or by fluorescent or colored contaminants. The key compound may be unstable in the mixture. A further complication can be synergistic (or antagonistic) activity of two constituents that may then diminish or disappear upon separation.

What could be the reasons of this reluctancy ?

✓ **Problems of supply and manufacture**

Intellectual property concerns of local governments and the Rio and Nagoya Conventions on Biodiversity.

Seasonal or environmental variations in the composition of living organisms.

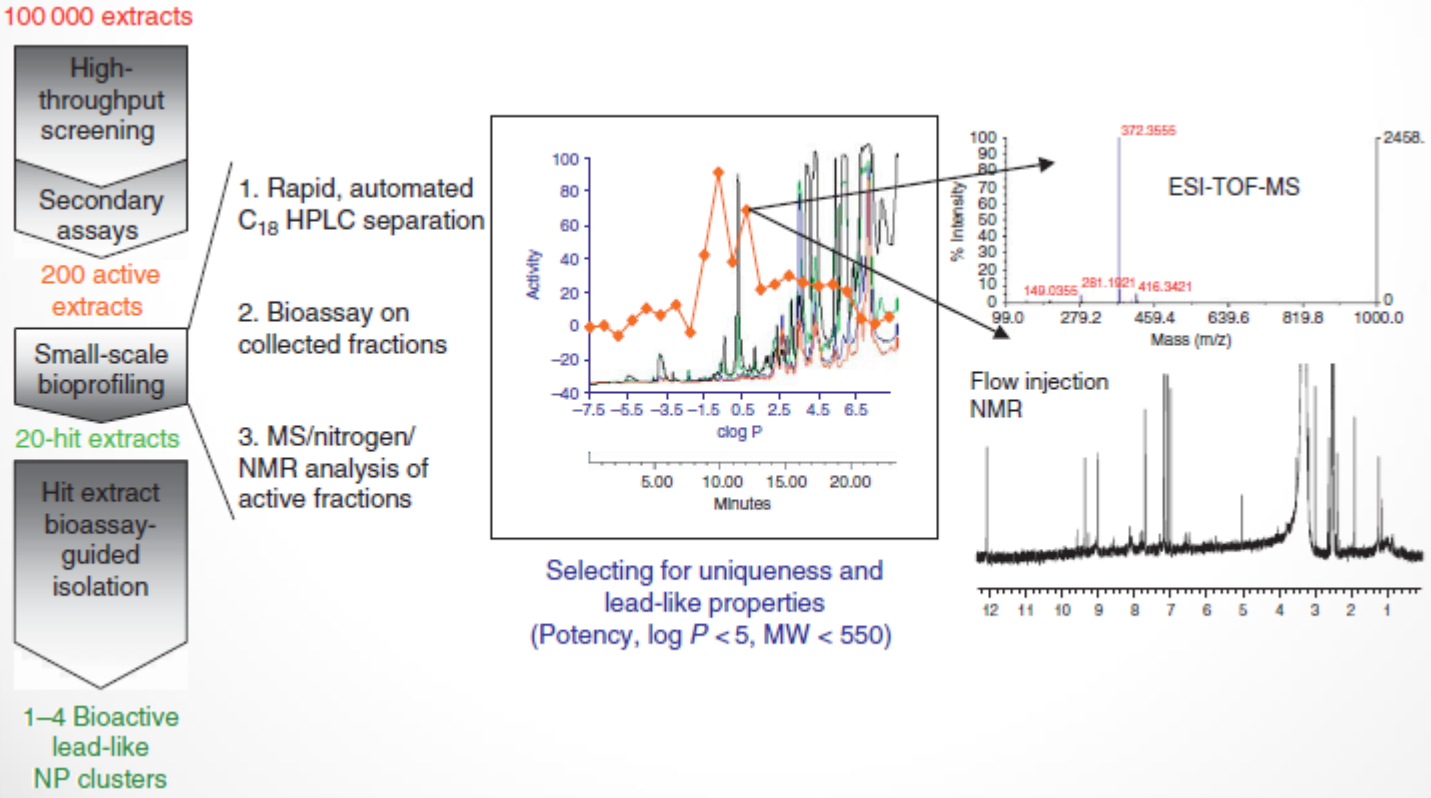
A prevailing sentiment in many pharmaceutical organizations is that screening of natural product sources is a difficult effort with a high probability of duplication; that is, the result may be a known compound that cannot be patented.

However, fewer than 1% of microorganism species are easily cultured, and perhaps fewer than 15% of higher plant species have been examined for bioactivity. Certain insects and other animals have been targeted for specific bioactivities, such as toxins, but are not generally subjected to HTS efforts. Clearly the biological resource is there, but access and examination are problematic, especially if there is pressure for a short time frame for discovery of new leads.

Natural product discovery approaches

- ✓ Development of advanced **analytical techniques** (*hyphenated methods :* LC-MS-NMR) and **dereplication (avoiding the isolation of already known NPs)**

- ✓ Development of natural product databases (DNP, MarineLit)



Natural products in the post-genomic era

Genomic and metabolomic databases

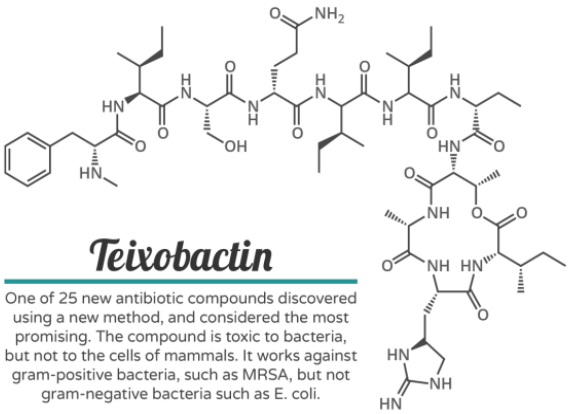
SHIFTING FROM THE GRIND AND FIND MODEL TO THE GENOMIC ERA

« non-cultivable » bacteria

Discovery of teixobactine

TEIXOBACTIN - A NEW ANTIBIOTIC

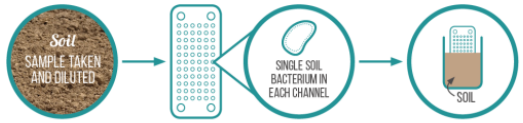
Teixobactin is the first member of a new class of antibiotics - and, more importantly, the method used to to discover it could lead to many more.



Teixobactin

One of 25 new antibiotic compounds discovered using a new method, and considered the most promising. The compound is toxic to bacteria, but not to the cells of mammals. It works against gram-positive bacteria, such as MRSA, but not gram-negative bacteria such as E. coli.

HOW TEIXOBACTIN WAS DISCOVERED



A DEVICE REFERRED TO BY RESEARCHERS AS THE 'iCHIP' WAS USED TO CULTURE SOIL BACTERIA ALLOWING NUTRIENTS & SIGNAL MOLECULES TO PASS TO THE BACTERIA & STIMULATE GROWTH.

Bacteria themselves produce antibacterial compounds to kill off competing bacteria, but 99% of these bacteria cannot be grown in a lab. The discovery of teixobactin is actually less important than the method used to discover it; researchers used a device that allowed them to dilute the bacteria-containing soil samples, sandwich them between two semi-permeable membranes, then immerse them in soil, allowing the bacteria to be grown in the lab; a method which could eventually lead to many more potential antibiotic candidates.

HOW TEIXOBACTIN WORKS

Teixobactin has a unique mechanism of action, targeting lipid molecules bacteria use to build their cell walls. As it's hard for bacteria to alter these molecules, it's expected to take much longer for resistance to develop.

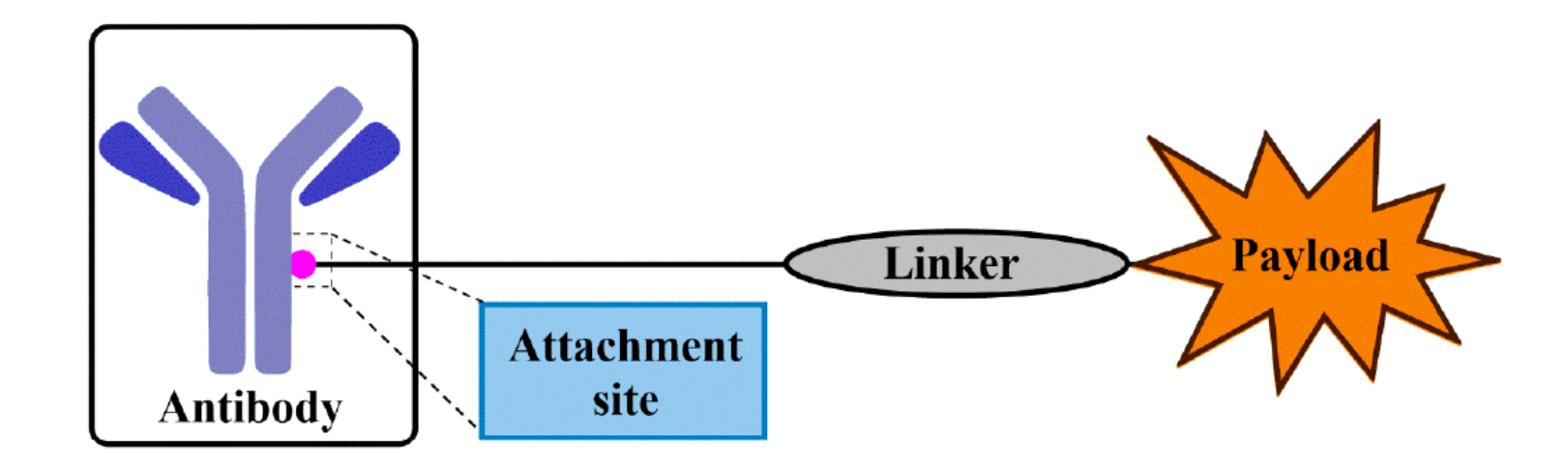
LIMITATIONS OF TEIXOBACTIN

Advanced culture techniques: In-situ cultivation

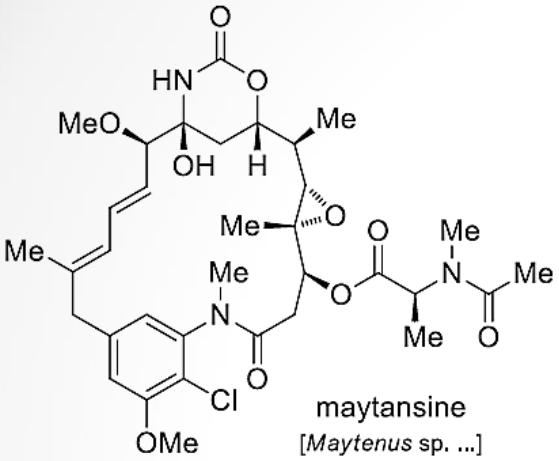
I-chip

Renewing interests

- ✓ **Targeted therapies**: Reusing old toxic natural products as Antibody-drug conjugates (ADC) directed toward identified targets (tumor-specific antigens abundant in tumor tissue but minimally expressed in normal tissues)



Renewing interest

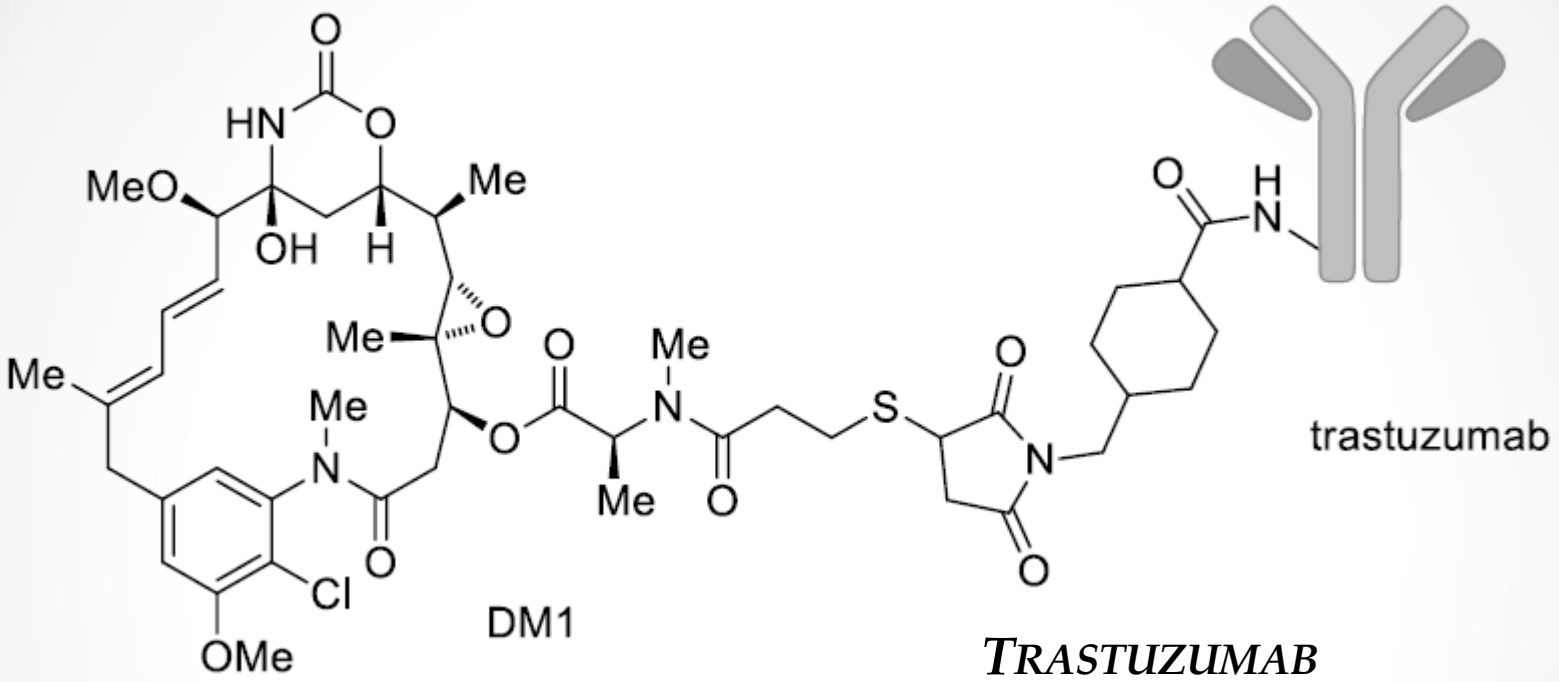


maytansine
[*Maytenus* sp. ...]

MAYTENUS SERRATA

- ✓ Isolated in 1960 from *Maytenus serrata*
- ✓ Failure of clinical trials in 1980.

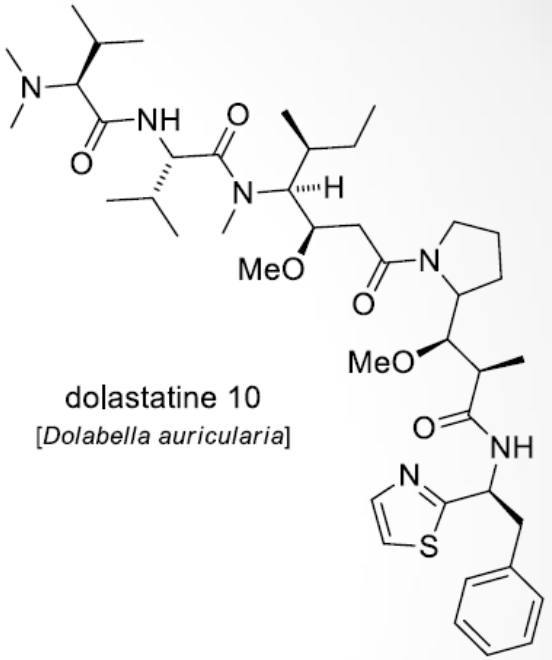
Renewing interest



TRASTUZUMAB EMTANSINE

- ✓ Breast cancer (*Her2* positive)
- ✓ Approved in Europe in 2013.

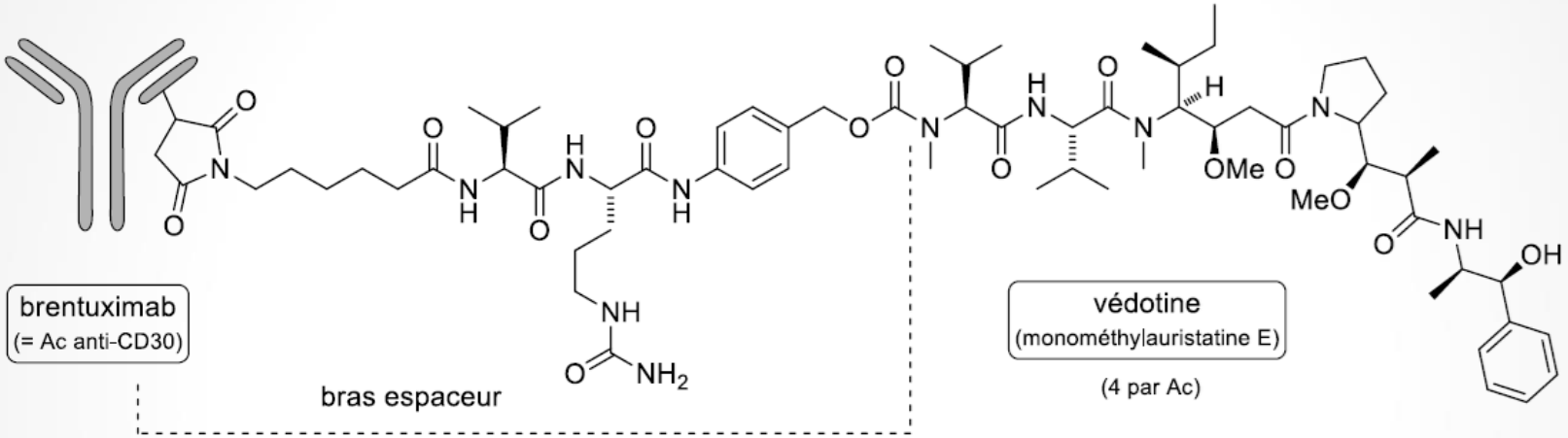
Renewing interest



dolastatine 10
[*Dolabella auricularia*]

- ✓ Isolated in 1980 from *Dolabella auricularia* (very low amounts)
- ✓ Sub-nanomolar cytotoxicity

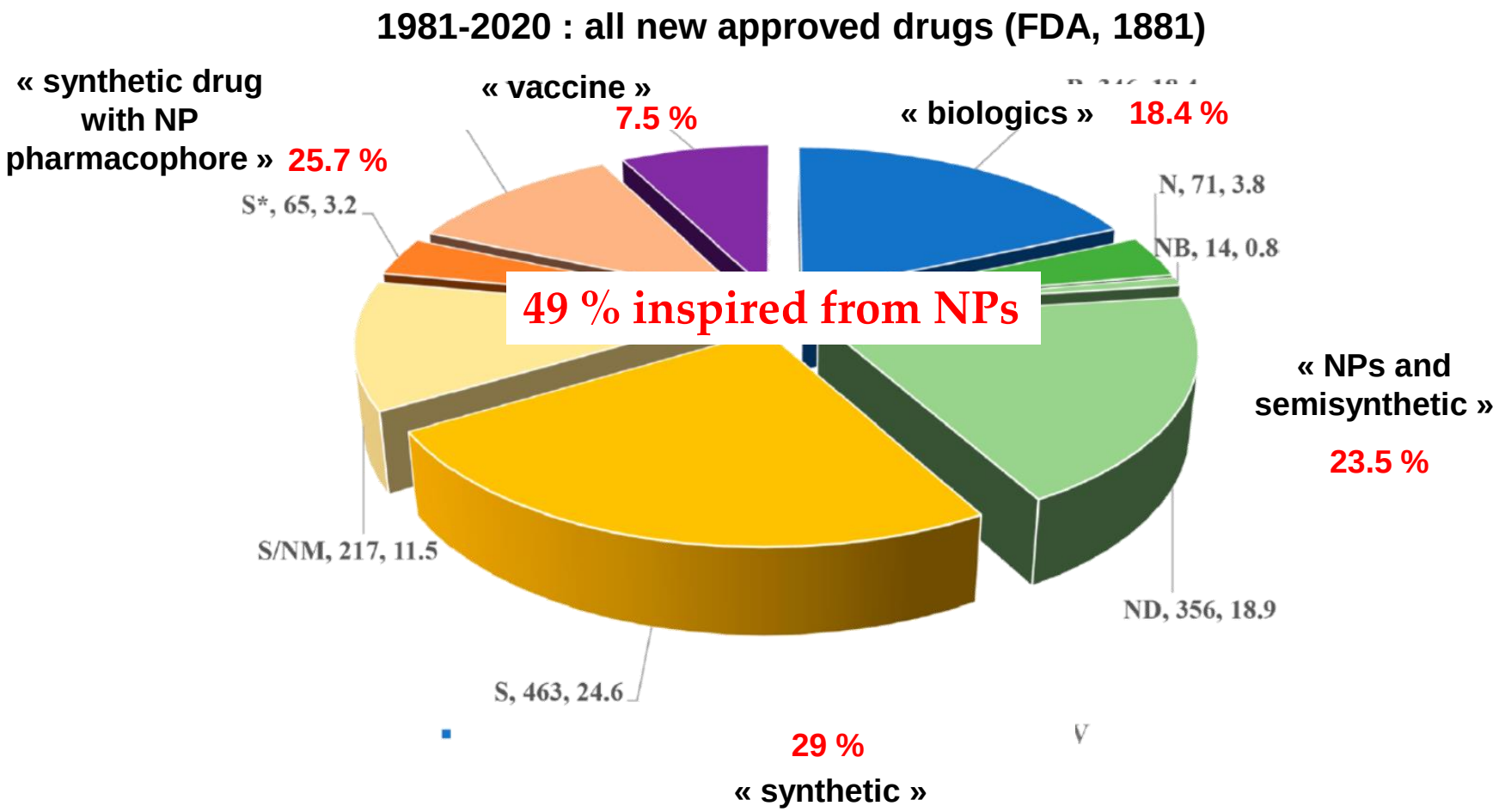
Renewing interest



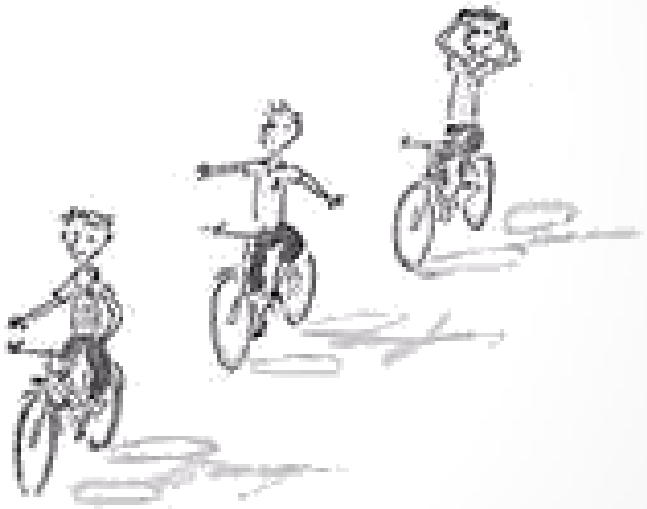
BRENTUXIMAB VEDOTIN

- ✓ Hodgkin lymphoma CD30 positive
- ✓ Marketed in 2013.

Natural products-based approved drugs

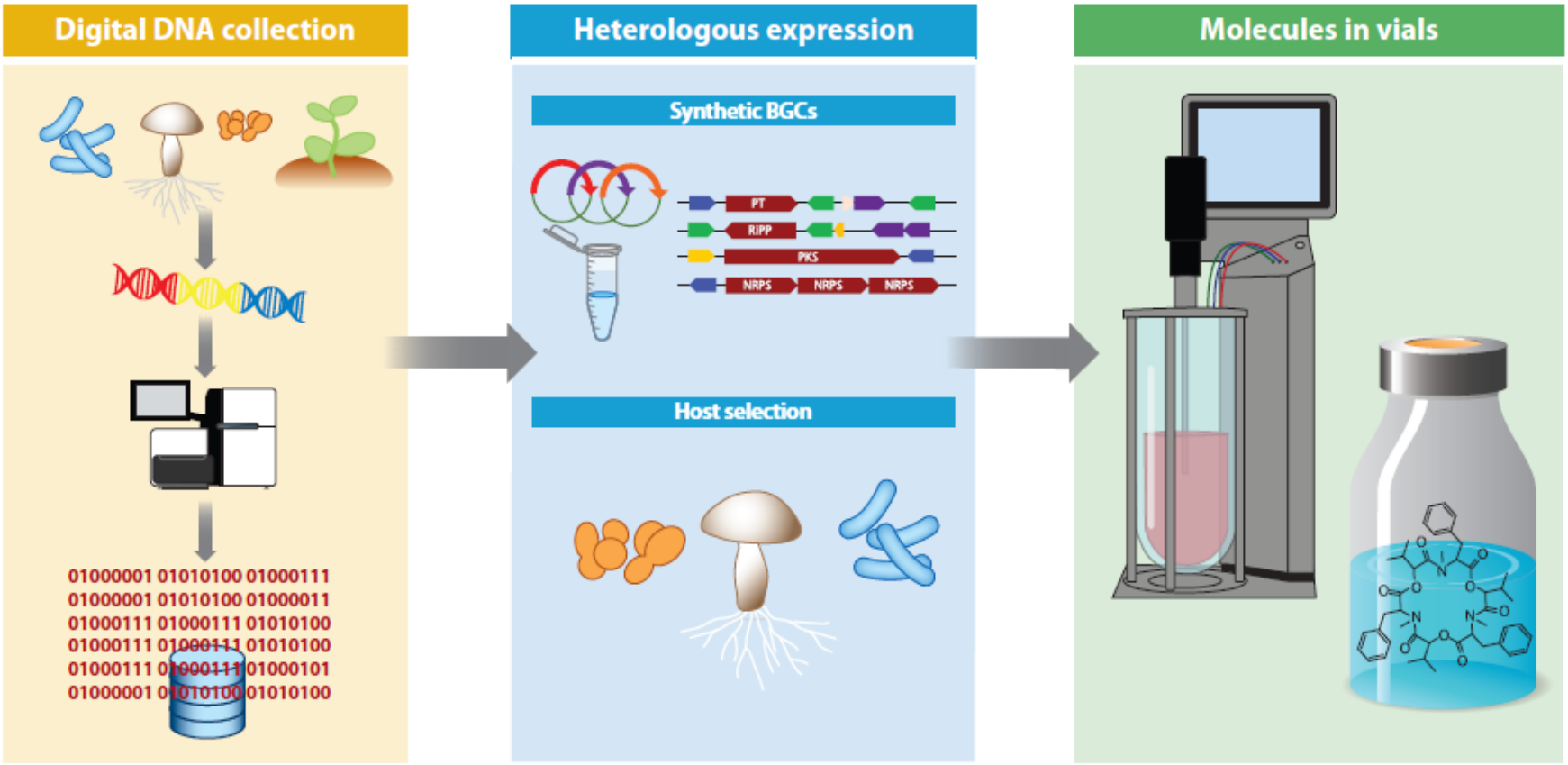


Conclusions



Conclusions

- ✓ NPs remain a promising pool for the discovery of scaffold with structural diversity and various bioactivities that can be directly developed or used as starting points for opitimization into novel drugs.
- ✓ The last 40 years have seen a remarkable exploration of the marine environment.
- ✓ The explosion of numerous « omic » approaches- genomics, proteomics, metabolomics and transcriptomics-has empowered researchers with the ability to address fundamental questions
- ✓ Advances in synthetic biology in order to address supply problems
- ✓ The futur of NP research lies in multidisciplinary project between academia and industry in small biotechnology companies



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Thank you for your attention

Chemical space

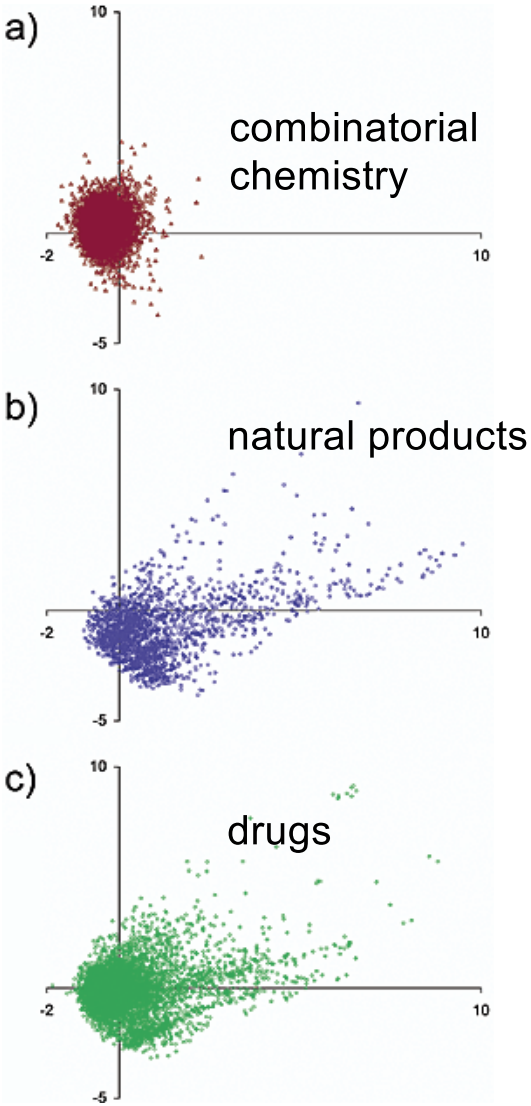
- *A collection of chemical compounds that can be characterized by a wide range of molecular descriptors (50 descriptors: MW, polarity, C,N,O number, …)*
- *Chemical space is defined as the total descriptor space that encompasses all the small carbon-based molecules that could in principle be created.*

- *Drug chemical space:*

MW< 500, < 30 atoms non H, with only C, H, O, N, P, S, F, Cl, Br, stable at room temperature, with water and oxygene

= 10^{63} molecules !!

Natural products and chemical space



Efficient chemical space coverage

Chemical space exploration