ERA-MBT Stakeholder meeting. Brussels, 13th Oct

Marine Biodiscovery

The experience of PharmaMar

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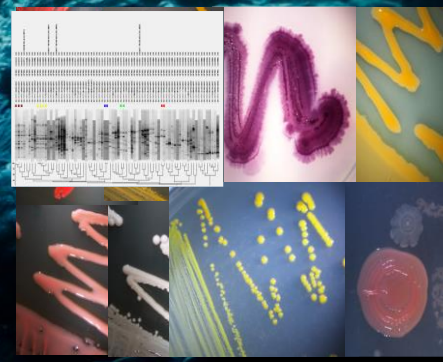
MarineBiotech

Unión Europea

Fondo Europeo de Desarrollo Regional
"Una manera de hacer Europa"

Enabling future innovations



Marine Biodiversity

As source of novel biomaterials

Scope of action: almost-unlimited forms of life (Marine genetic resources)

Opportunity: Marine exploration for pharmaceutical purposes < 30 years old.

Competition for Survival & Environmental pressure

Biodiversity → **Biomass**

Defence, Attack, Signalling

Chemical diversity

Potential new human applications

Industrial enzymes.
Pharmaceuticals.
Functional foods & Nutraceuticals
Cosmetics.
Biomaterials.
Agricultural products.

Biotechnology

Marine-derived approved medicines for the tratment of cancer and pain

Drug discovery

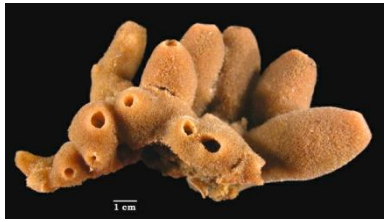
Origin of marine samples

Which are the most promising MARINE organisms as a source of metabolites for application in human health?

Macro-Organisms (mainly invertebrates)

Wide range of chemical defenses

Industrial supply



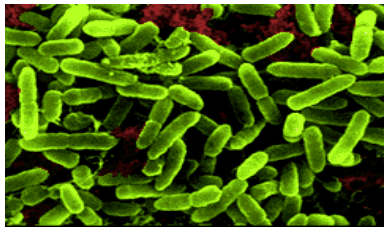
Sponges, tunicates, corals, bryozoans, mollusks

Micro-Organisms

Abundance

Industrial supply

> 99% non-cultivable



Actinomycetes, fungi, dinoflagellates, cyanobacteria

Environmental DNA (Metagenomes)

Directly to the genes

Heterologous expression

From non-cultivable organisms (symbionts)

PharmaMar

From Marine Biodiversity to Medicines

Founded in 1986
Over 350 employees in oncology
Headquarter in Colmenar (Madrid)

"To advance Cancer Care through the discovery, development and commercialization of new marine derived drugs "

FULLY INTEGRATED:
Drug Development Capabilities

FROM MARINE EXPEDITIONS

- Marine-derived products
- New drug candidates
- Molecule optimization
- Build a library (165,000 samples)

TO COMMERCIALIZATION

- Clinical Trials
- Production
- Licensing
- Commercialization

Fully integrated business

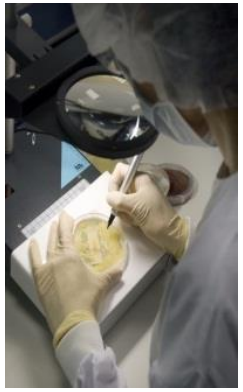
R&D capabilities to bring cancer drugs to the market

Fully integrated drug development capabilities

Marine expeditions → Sample library → Screening & Synthesis → Clinical Trials → Commercialization

- Marine derived products
- Global expeditions

- New drug candidates
- Molecule optimization
- 165,000 samples



- Patent protection
- Synthesis
- FDA approved production facility

- Pre-clinical trials
- Clinical trials

- Own sales force in Europe
- Licensing
- Strong, committed partners

Marine-derived compounds with novel mechanisms of action

Pipeline

Overview

30 years of marine research activities exploring marine biodiversity

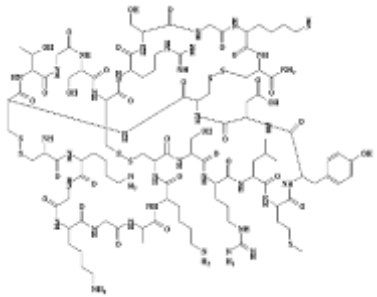
- **1 marketed marine-derived medicine in oncology (Yondelis)**
- **1 application for registration (Aplidin)**
- **4 drugs in clinical phases (II-III)**

Clinical Program / Indication		Phase I	Phase II	Phase III	Registration Application	Market	Partners
Yondelis®							
Soft Tissue Sarcoma 2nd/3rd line; EU/others	Single agent						
Ovarian Cancer 2nd/3rd line; EU/others	(Yondelis®+Doxil)						
Soft Tissue Sarcoma 2nd/3rd line; US	Single agent						janssen
Ovarian Cancer 3rd line US	(Yondelis®+Doxil)						janssen
Soft Tissue Sarcoma 2nd/3rd line; Japan	Single agent						TAIHO
Mesothelioma EU/Others	Single agent						
Aplidin®							
R/R multiple myeloma 4th line; EU/others	Aplidin® + Dexameth.				Specialised Therapeutics ASIA	TTY BIOPHARM 台灣東洋藥品	CHUGAI
R/R T-cell lymphoma	Single agent						
R/R multiple myeloma	Aplidin® + Bortezom+ Dexameth.						
PM1183							
Plat. Resistant ovarian cancer	Single agent						
SCLC 2nd line	1183 + Doxorrubicin						
BRCA 1/2 Breast cancer	Single agent						
Solid tumors	Combinations						
PM184							
Advance Breast Cancer 3rd/4th line	Single agent						
Solid tumors	Single agent and combinations						

Commercialized marine-derived medicines

Most recent cases

2004

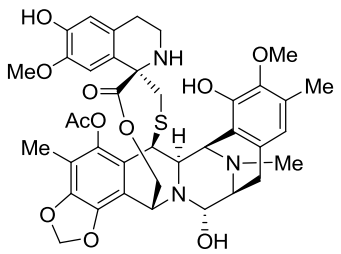


Elan
(Chronic pain)

Conus magus
(Cone snail)

PRIALT
ZICONOTIDE

2007

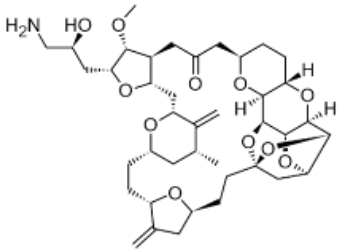


PharmaMar
(Cancer)

E. turbinata
(tunicate)

Yondelis

2010

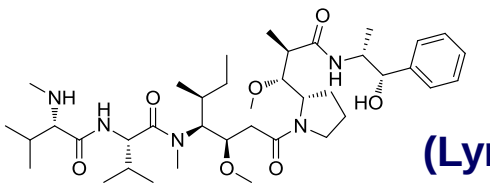


Eisai
(Breast cancer)

Halicondrin B
(marine sponge)

Halaven
(eribulin mesylate) injection

2011



Seattle Genetics
(Lymphoma)

ADC containing MMAE,
(dolastatin analog)

D. auricularia
(mollusc)

ADCETRIS
brentuximab vedotin

First european marine-derived compound to reach the oncology market

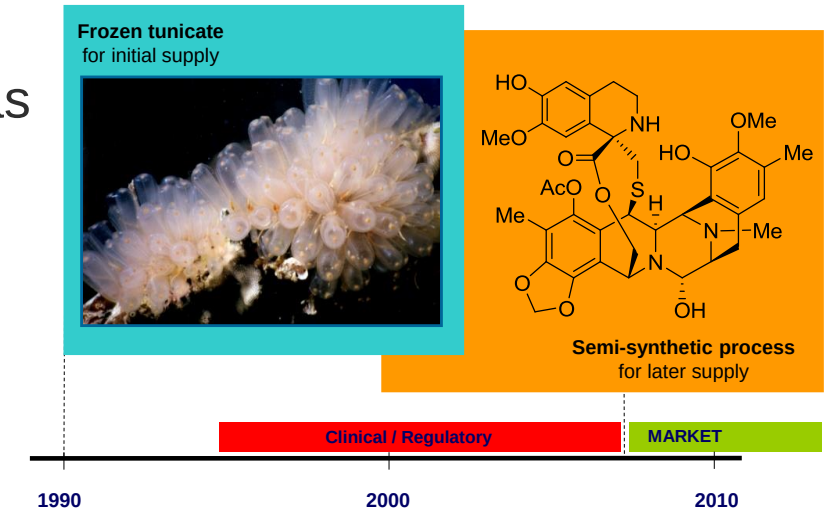
The case of Yondelis

Ecteinascidin 743 was isolated and elucidated from the tunicate *Ecteinascidia turbinata* by Prof. K.L. Rinehart in 1990, member of the scientific broad of directors of PharmaMar.

The drug (Yondelis) was approved in Europe for the treatment of **soft tissue sarcoma** (2007) and platinum sensitive **ovarian cancer** (2009) in combination with doxorubicin.

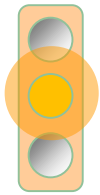
In 2015 was approved for STS in USA (Ortho Biotech, J&J) and Japan (Taiho Pharm.).

The definition of a **robust supply** was the key issue for the industrial development of the compound



The problem of the supply of Marine Natural Products

The case of Yondelis:



No robust manufacture

Industrial supply by MARINCULTURE

The first grams were extracted from several metric tons using marine cultures.

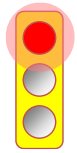
Sea farms for culture *E. turbinata* in Formentera (Balearic Islands, Spain). 1995-2000

However, the **low yield** (1mg/Kg frozen ascidian) and the **uncertain production** control and logistics, we have decided to reject this alternative for further supply.

Carballo, JL; et al, *J.W.Aquaculture* Soc. **2000**, *31*, 481

The problem of the supply of Marine Natural Products

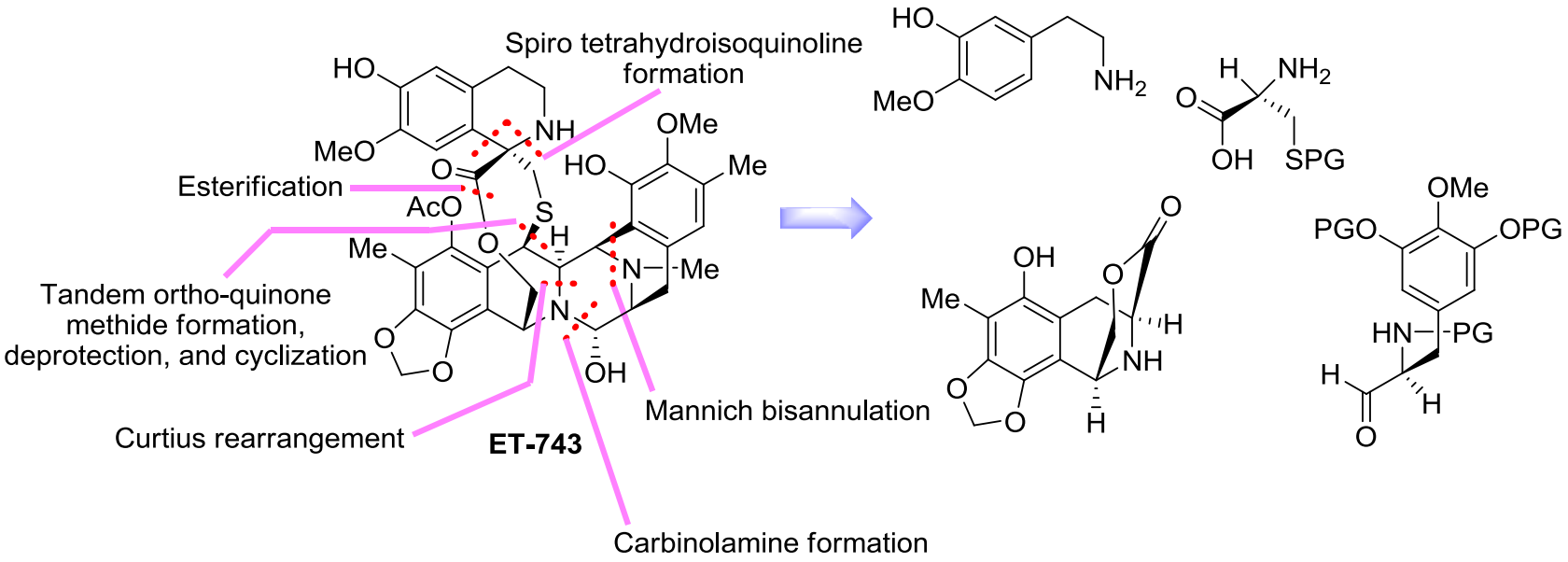
The case of Yondelis:



Not cost-effective

Industrial supply by TOTAL SYNTHESIS

First Total Synthesis (Prof. Corey) published in 1996.....



Retrosynthesis gave four “amino-acid” sub units, > 50 steps in total (longest linear sequence 35 steps). overall yield 0.7% theory. **Not cost effective route**

Corey, EJ; et al, *J. Am. Chem.* Soc. **1996**, *118*, 9202

The role of biotechnology as supply of Marine Drugs

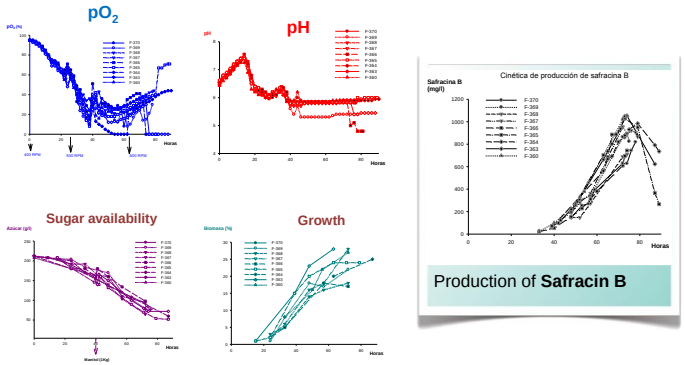
The case of Yondelis: Current Drug Master File

Current manufacture process

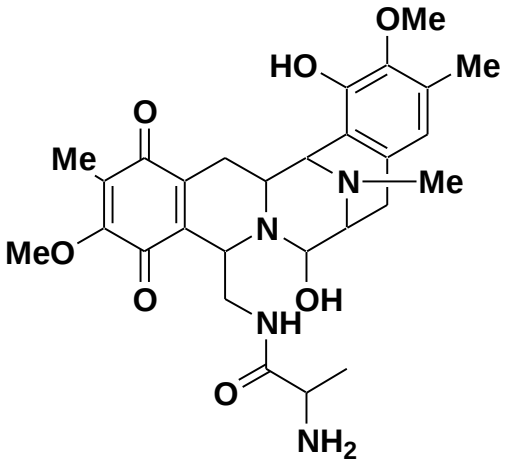
Industrial supply by SEMI_SYNTHESIS

Free living Pseudomonas sp. PHM003

FERMENTATION & DSP scale m^3

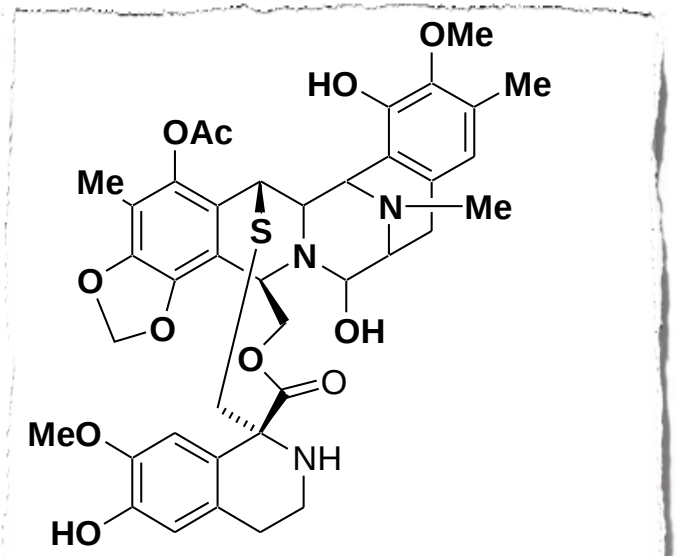


Production of **Safracin B**



Safracin B, produced by *Pseudomonas sp. PHM003* (recently renamed as *P. poae)*

18 Linear synthesis steps



Trabectedin (Ecteinascidin 743)

Current yield (1 g/l of safracin).
Industrial Scale-up under GMP to 15 m^3.

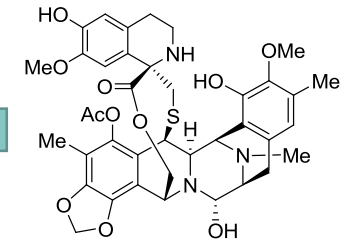
Cuevas, C, de la Calle, F.et al., *Org. Lett.* **2000**, 2, 2545.

The role of marine biotechnology

Marine ascidian *E. turbinata*

Marine culture

Biomass extraction

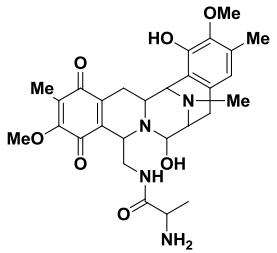


Trabectedin (ecteinascidin ET-743)

Semisynthetic proccess

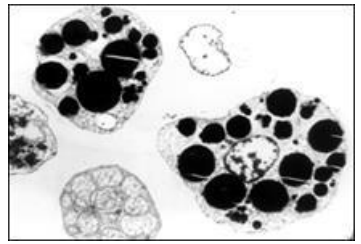
Bacteria

Fermentation



Safracin B

Symbiont Bacteria



Bacteriocytes with vesicles filled of the gammaproteobacteria symbiont: Cand. *Endoecteinascidia frumentensis.*

Moss, C., et. al., (**2003**) *Mar Biol*

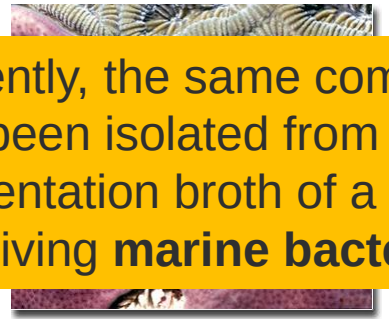
Metagenomic mining for NRPS genes

Identified the putative genes of the biosynthesis of ecteinascidins into an extrictely bacteria symbiont living Into bacteryocite cells of E. turbinata

Rath, C.M et. al., (**2011**) , *Chem. Biol*

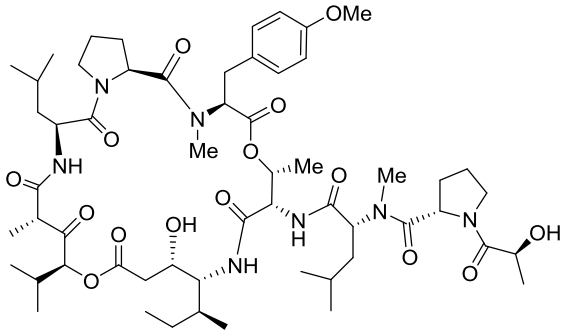
The role of bacteria as suppliers of *ascidian?* Marine Drugs

The case of Aplidin/didemnins



Recently, the same compound has been isolated from the fermentation broth of a free living **marine bacteria**

Caribbean Trididemnum spp



Didemnin B
PM91105

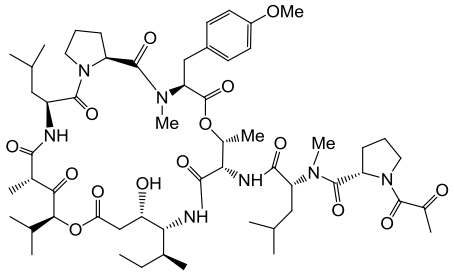
Rinehart, K L, et al, *Science*, **1981,** *221*, 933

First marine compound entering in Clinical trials as an antineoplasic agent by NCI

Chun, HG; et al, *Inv. New Drugs*.. **1986**, 4, 279

..but it was discontinued by cardiotoxicity

Aplidium albicans
Collected by PhM
in Ibiza (Balearic Island)
-60 m in depth



Aplidin (Plitidepsin)
-dehydrodidemnin B
PM90001

Currently in Clinical Phase II/III

Total synthesis (g/scale)

Gutierrez, M; et al, *J. Med. Chem*.. **2004**, 47, 5700

Rinehart, KL, *Med. Res. Rev*. **2000**, *20*, 1

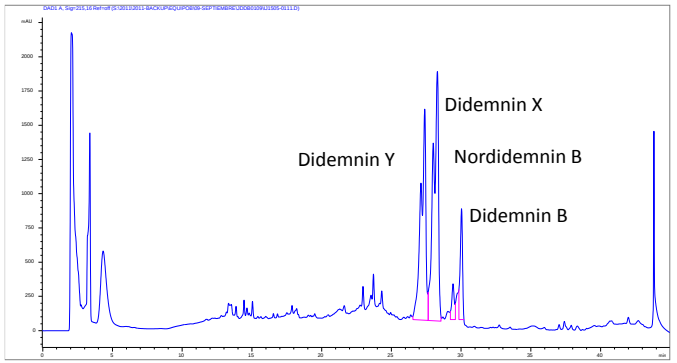
The role of bacteria as suppliers of *ascidian?* Marine Drugs

The case of Aplidin/didemnins

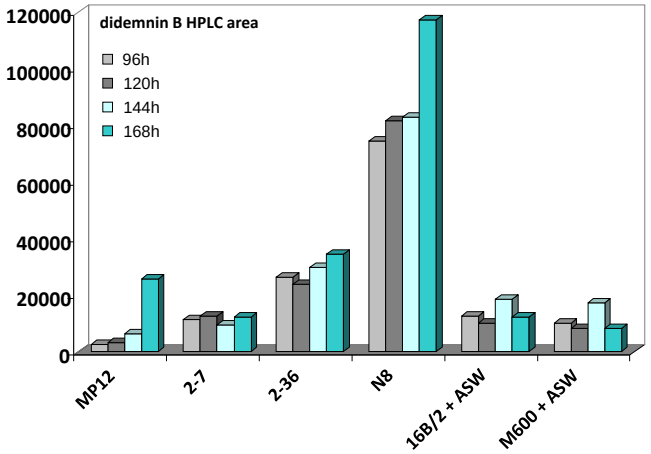
April, 2011

Polychaete *Seballastarte*
Tanzania -45m

Tistrella mobilis PM396
(alphaproteobacteria)



HPLC chromatogram of Tistrella PM396
In N8 medium. 168h. 215nm

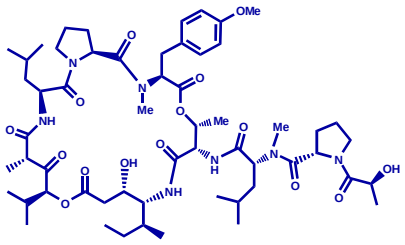


Kinetics of production of didemnin B in different media

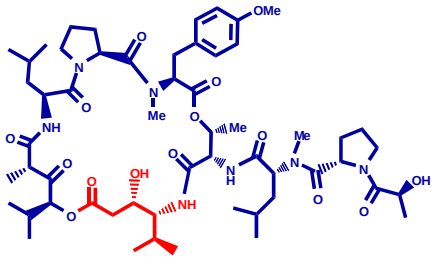
MaNaPro 2013 XIV International Symposium on Marine Natural Products

8th European Conference on Marine Natural Products

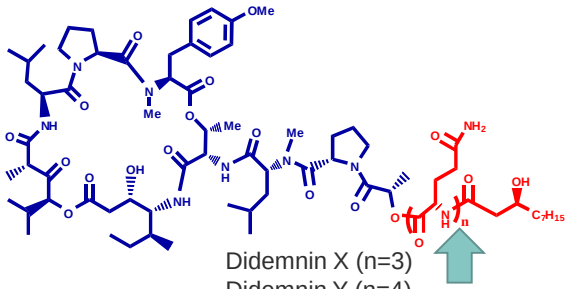
Schleissner , C. et al., Poster ManaPro 2013



Didemnin B



Nordidemnin B



Didemnin X (n=3)
Didemnin Y (n=4)

Several strains of the free living *Tistrella (alphaproteo)* produce didemnins.

Since 2011 -2016 Other three *Tistrella* spp. have been isolated at PharmaMar from different samples (sponges, polichaetes, tunicates in different geographical areas . All bacteria have de-replicated (HPLC-MS) didemnins but no **aplidin**.

Examples of the most Relevant **Marine Natural Products**

All chemical structures are derived from **polyketide** and/or **peptide** scaffolds (***PKS&NRPS gene clusters***)

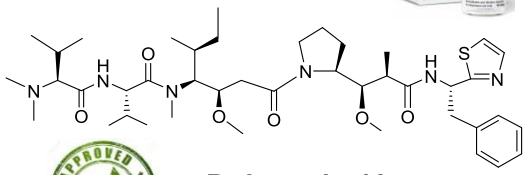
Yondelis

2007

Trabectedin

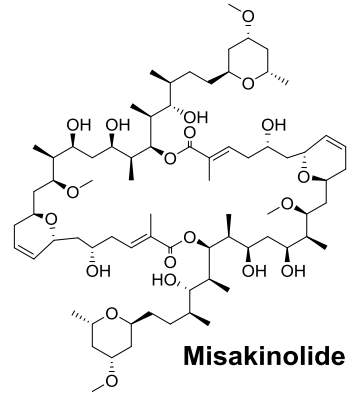
PM92106

ADCETRIS
brentuximab vedotin

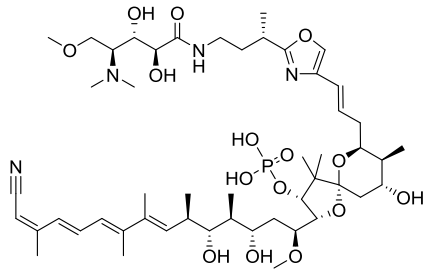


Dolastatin 10

2011

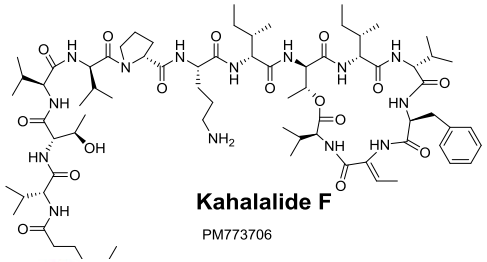


Misakinolide A



Calyculin A

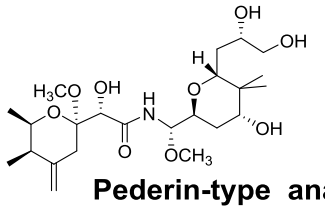
Halaven
(eribulin mesylate) injection



Kahalalide F

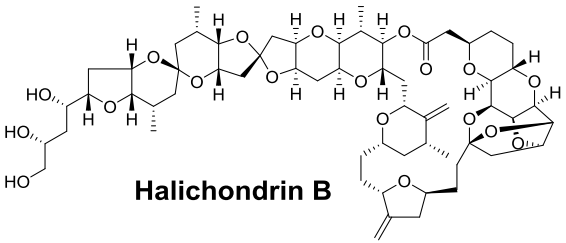
PM773706

CLINICAL TRIAL

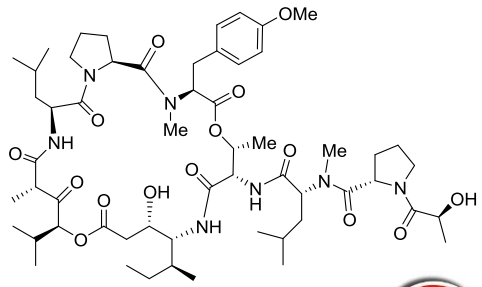


Pederin-type analogs

PM160153



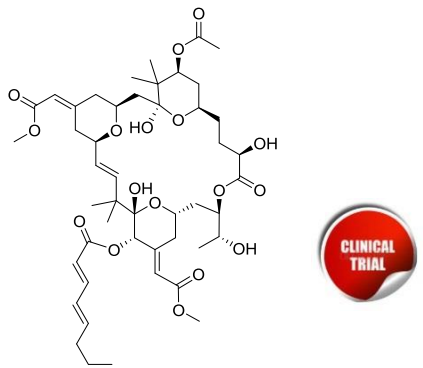
Halichondrin B



Didemnin B

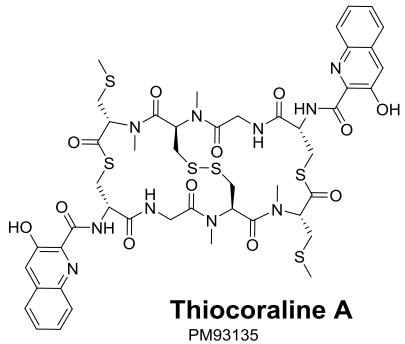
PM91105

CLINICAL TRIAL



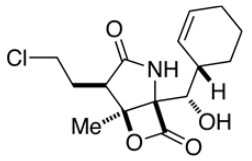
Bryostatin 1

CLINICAL TRIAL



Thiocoraline A

PM93135



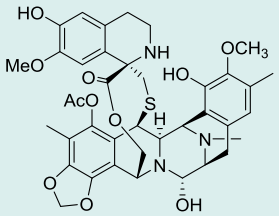
Salinosporamide A

CLINICAL TRIAL

Marine Natural Products

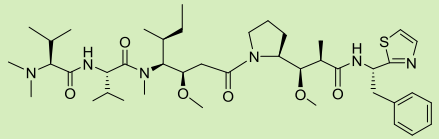
Originally isolated from

Tunicate
Ecteinascidia
Rinerhart, JOC, **1990**



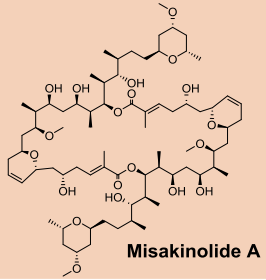
Trabectedin
PM92106

Sea hare
Dolabella
Pettit, JACS, **1987**



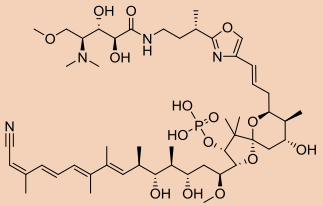
Dolastatin 10

Sponge
Theonella
Sakai, Chem. Lett. **1986**



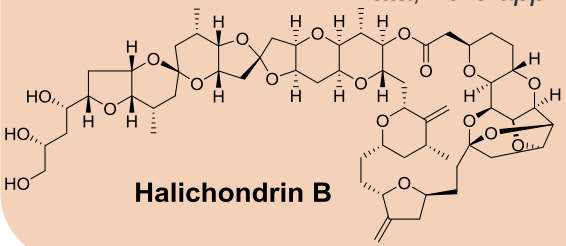
Misakinolide A

Sponge
Discodermia
Kato, JACS **1986**



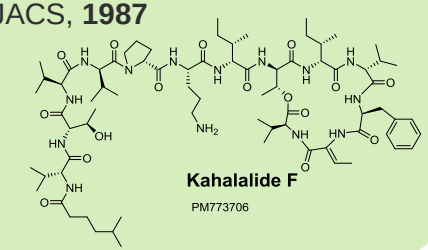
Calyculin A

Sponge
Halichondria
Hirata, *Pure appl. Chem*. **1986**



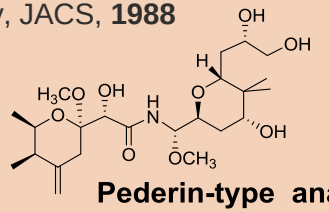
Halichondrin B

Sea hare & alga
Elysia, Bryopsis
Pettit, JACS, **1987**



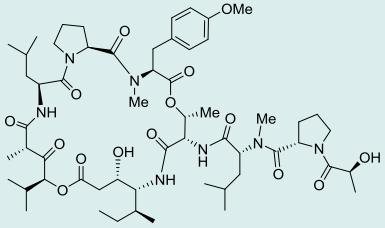
Kahalalide F
PM773706

Sponges, insects
Several genera
Perry, JACS, **1988**



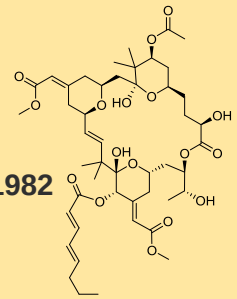
Pederin-type analogs
PM160153

Tunicate
Trididemnum
Rinerhart, JACS, **1981**



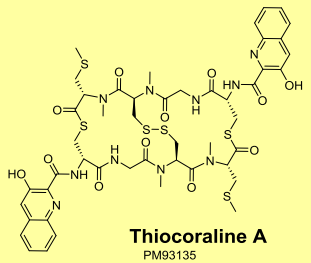
Didemnin B
PM91105

Bryozoan
Bugula
Pettit, JACS, **1982**



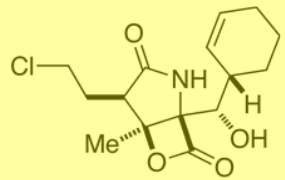
Bryostatin 1

Marine Actinobacteria
Micromonospora
Romero, *J. Antib*, **1997**



Thiocoraline A
PM93135

Marine Actinobacteria
Salinispora
Feling, Ang.Chem.I.E, **2003**

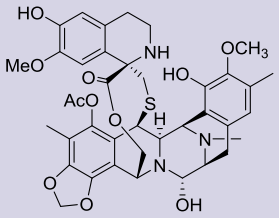


Salinosporamide A

Marine Natural Products

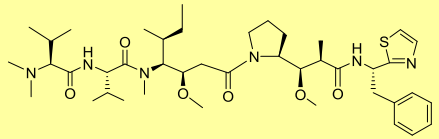
Currently, their biosynthetic genes PKS/NRPS located in bacteria

Bacterial Symbiont
gamma proteo
Rath, ACS Chem Biol. **2011**



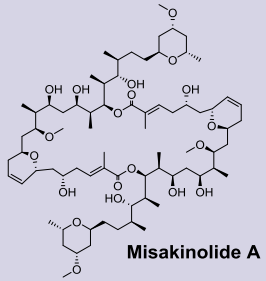
Trabectedin
PM92106

Free living Cyanobacteria
Symploca
Luesch, JNP, **2001**



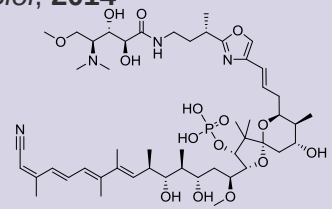
Dolastatin 10

Bacterial Symbiont
New Phylum: ***Tectomicrobia***
Piel, *Nature*, **2014**



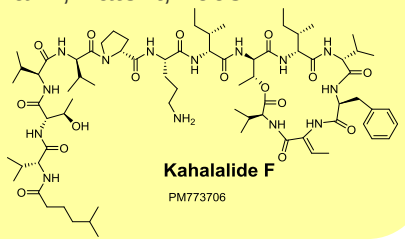
Misakinolide A

Bacterial Symbiont
New Phylum: ***Tectomicrobia***
Yakimoto, *Nat. Che. Biol*, **2014**



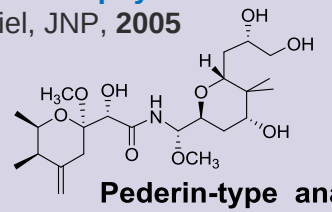
Calyculin A

Free living proteobacteria
gamma proteo
Hamann, Patent, **2005**



Kahalalide F
PM773706

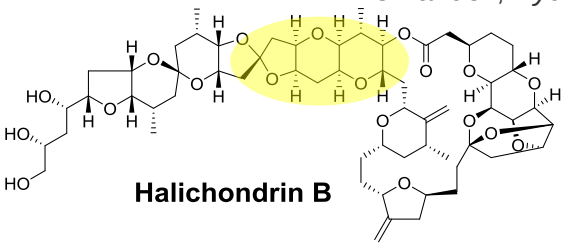
Bacterial Symbionts
Several phyla
Piel, JNP, **2005**



Pederin-type analogs
PM160153

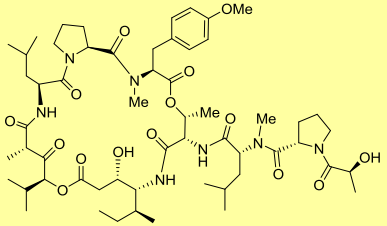
Free living dinoflagellate
Prorocentrum
Hernandez, *Hydrobiol*. **2000**

Okadaic acid



Halichondrin B

Free living proteobacteria
alpha proteo
Tsukimoto, *JNP*, **2011**

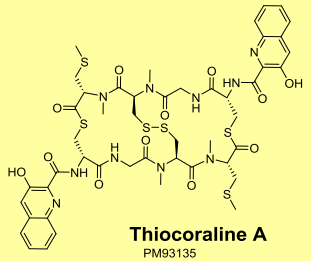


Didemnin B
PM91105

Bacterial Symbiont
gamma proteo
Haygood, JNP, **2007**

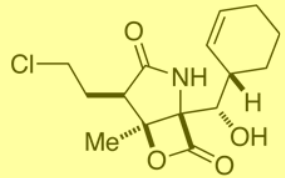
Bryostatin 1

Free living actinobacteria
Micromonospora, Streptomyces
Romero, *J. Antib*, **1997**



Thiocoraline A
PM93135

Free living actinobacteria
Salinispora
Feling, Ang.Chem.I.E, **2003**



Salinosporamide A

New concept in Drug Discovery from marine microorganisms

Post-genomic era

We are moving from the classical "*to isolate and then, to test*" ⇨ "*to test and then, to biotech expression*"

Marine Biodiversity as a whole
(metagenomes)
(microbial consortia)

Genomic mining for targeted genes

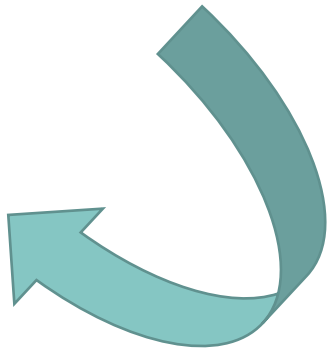
⇨

Identification of secondary metabolism gene clusters (or enzymes)

⇨

Are they new genes?
Can they produce new compounds?
Where are they located?
Are they silent genes?
Could be cultivable the owners?
Can be induced by culture?
Can be expressed by external hosts?

To design innovative drug discovery strategies using marine biotechnology



Marine Biotechnology (as genome mining & culture)

EU initiatives

Marine Metagenomics for new Biotechnological applications

KBBE-2008-32-07
10 Partners, 3M€

Marine Microorganisms: Biodiversity, Bioinformatics, Biotechnology

OCEAN.2011-2
32 Partners, 9M€

Marine Microorganisms: Cultivation Methods for Improving their Biotechnology Applications.

KBBE-2012-3,2-02
24 Partners, 9M€

Marine Bacterial Polyketides Supply by Biotechnology

INNPACTO 2011-2015
4 Partners, 3M€

Industrial Application of Marine Enzymes

H2020 BG-02-2015-2019
23 Partners, 6M€

Awakening silent PKS/NRPS gene clusters in marine bacteria

RETOs program 2016-2020
3 Partners, 3M€

Marine Bacteria Drug Discovery

Summary

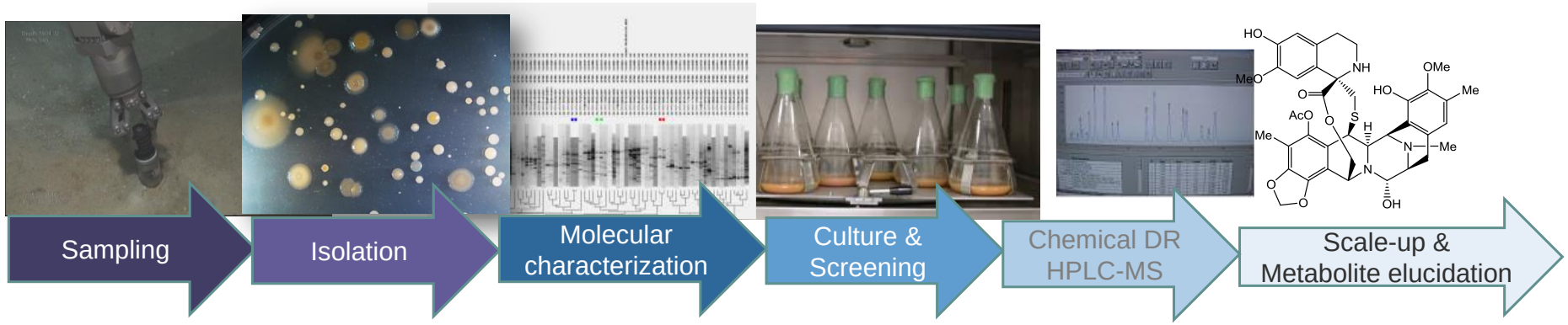
Biodiversity

Metabolite Diversity

Target_ Directed isolation

Induction of targeted genes

Secondary gene clusters
PKS & NRPS



Conclusion

Marine

Biodiversity

Metabolite Diversity

Biotechnology

“*Marine Biotechnology can unlock the pharmaceutical potential of marine origin materials leading to new treatment options*“

(in Marine biotechnology Strategic research and Innovation Roadmap. Sep, 2016)