



Generation and Exploitation of Marine Natural Product Extract Collections as Source of New Bioactive Molecules

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Fundación MEDINA

November 2018

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10 YEARS
2008-2018

FUNDACIÓN
CENTRO DE EXCELENCIA
EN INVESTIGACIÓN DE
MEDICAMENTOS INNOVADORES
EN ANDALUCÍA



Outline

- Introduction of Fundación MEDINA and our collection
- Creation of Natural Product Extracts Collections
- Exploitation of collections in search of new bioactive molecules

ABOUT US



**Independent, Non-profit
Research Organization**
established as a Private-public
partnership between:

- Government of Andalucía (Spain)
- University of Granada (Spain)
- Merck Sharp and Dohme de España S.A.



Our Mission

*Discovery of new bioactive compounds and innovative therapies
for unmet medical and industrial needs.*

HEALTH SCIENCES TECHNOLOGY PARK, GRANADA, SPAIN





MEDINA: Microbial Natural Products Drug Discovery



- Multidisciplinary team of scientists (28 permanent staff , up to 40 employees)
- 2300 m² fully equipped facilities
- One of the largest microbial collections and libraries for natural products drug discovery
- High capacity center for high throughput screening
- Drug discovery research programs in infectious diseases, cancer and neurodegeneration





MEDINA at a glance

MEDINA

Leveraging:

- 60 years expertise in Drug Discovery
- 5 Drugs in the market
- Collaborations with industry & academia

RESEARCH

SERVICES

MEDiversity

- Microbial collections (190.000 strains)
- Microbial Genome Banks
- Natural Products Libraries (200.000 extracts)

MEDiscovery

- High Throughput Screening
- Natural Products Chemistry
- Compound Structural Elucidation
- Bioanalytics
- Preclinical Safety
- Genome mining

MEDexpert

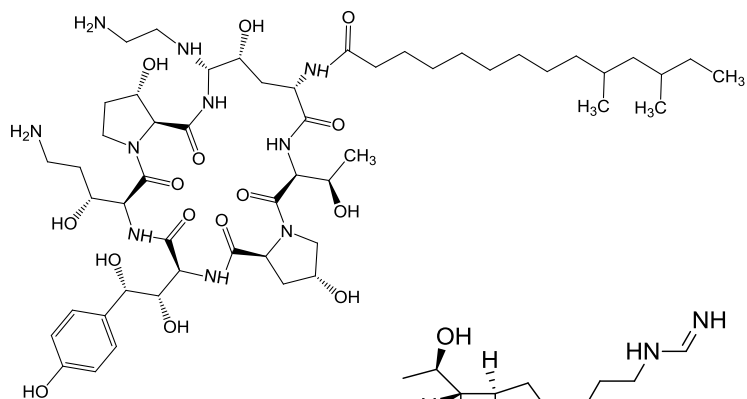
- New Compound Discovery
- Patents
- Collaborations
- Publications

Academia & Industry

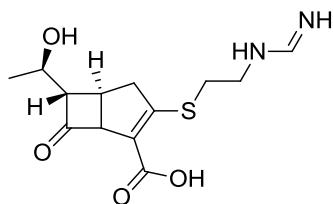


Contribution of CIBE-MRL Spain to Merck Natural Product Drug Discovery

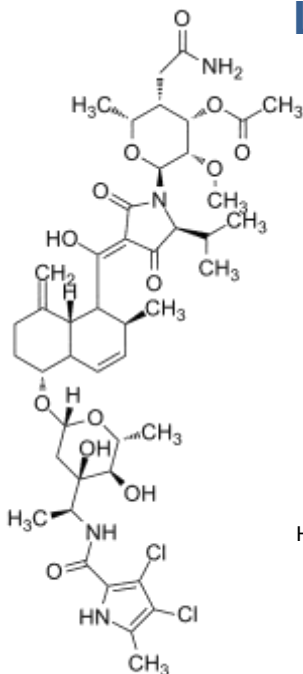
Drugs in the market



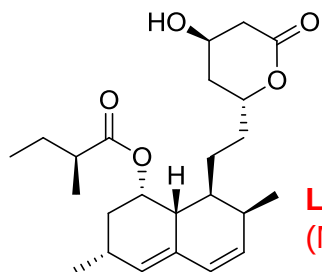
Caspofungin
(Cancidas, Merck)



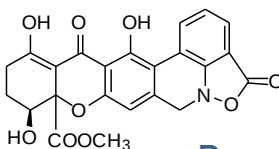
Thienamycin
(Imipenem, Merck)



Kibdelomycin*, antibacterial
Topo II isomerase inhibitor

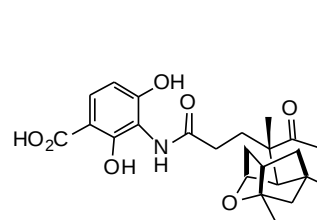


Lovastatin
(Mevacor, Merck)

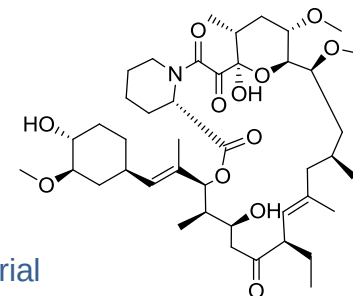


Parnafungins*
mRNA polyadenylation inhibitors

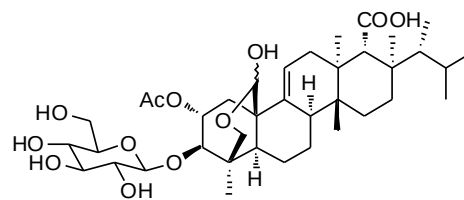
Leads and clinical candidates



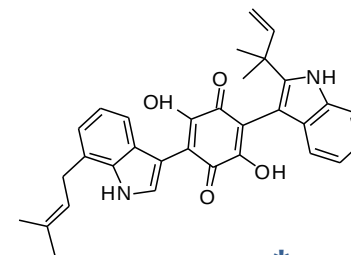
Platensimycin *, antibacterial
Fatty acid synthesis inhibitor



Immunomycin*
immunosuppressor



Enfumafungin*, antifungal

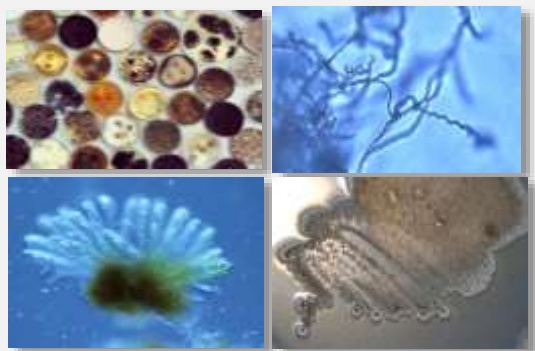


DMAQ-B1*
Insulin mimetic

MICROBIAL NATURAL PRODUCTS



Maximizing chemical diversity by maximizing biodiversity



MEDiversity



○ Microbial Strain Collections

190,000 strains*

Fungi, Actinomycetes & unicellular Bacteria

○ Natural Products Libraries

>200,000 extracts

from a large diversity of well-identified microbial strains

Proven genetic and chemical diversity

Geographical, Ecological & Taxonomical Diversity

* Recent donation of new 74,000 strains from former Cubist-Merck & Co. Inc Collection

LARGEST MICROBIAL COLLECTION WORLDWIDE WITH 190.000 STRAINS



Geography

Marine & Terrestrial

Ecology

Plants, roots, sediments,
rocks, water...

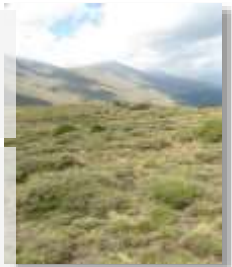
Taxonomy

Fungi, Actinomycetes &
unicellular bacteria

Sources:

- Terrestrial
- Marine

Fungal plant endophytes: 32%
Soil & plant rhizosphere actinomycetes: 86%
Marine actinomycetes: < 10%





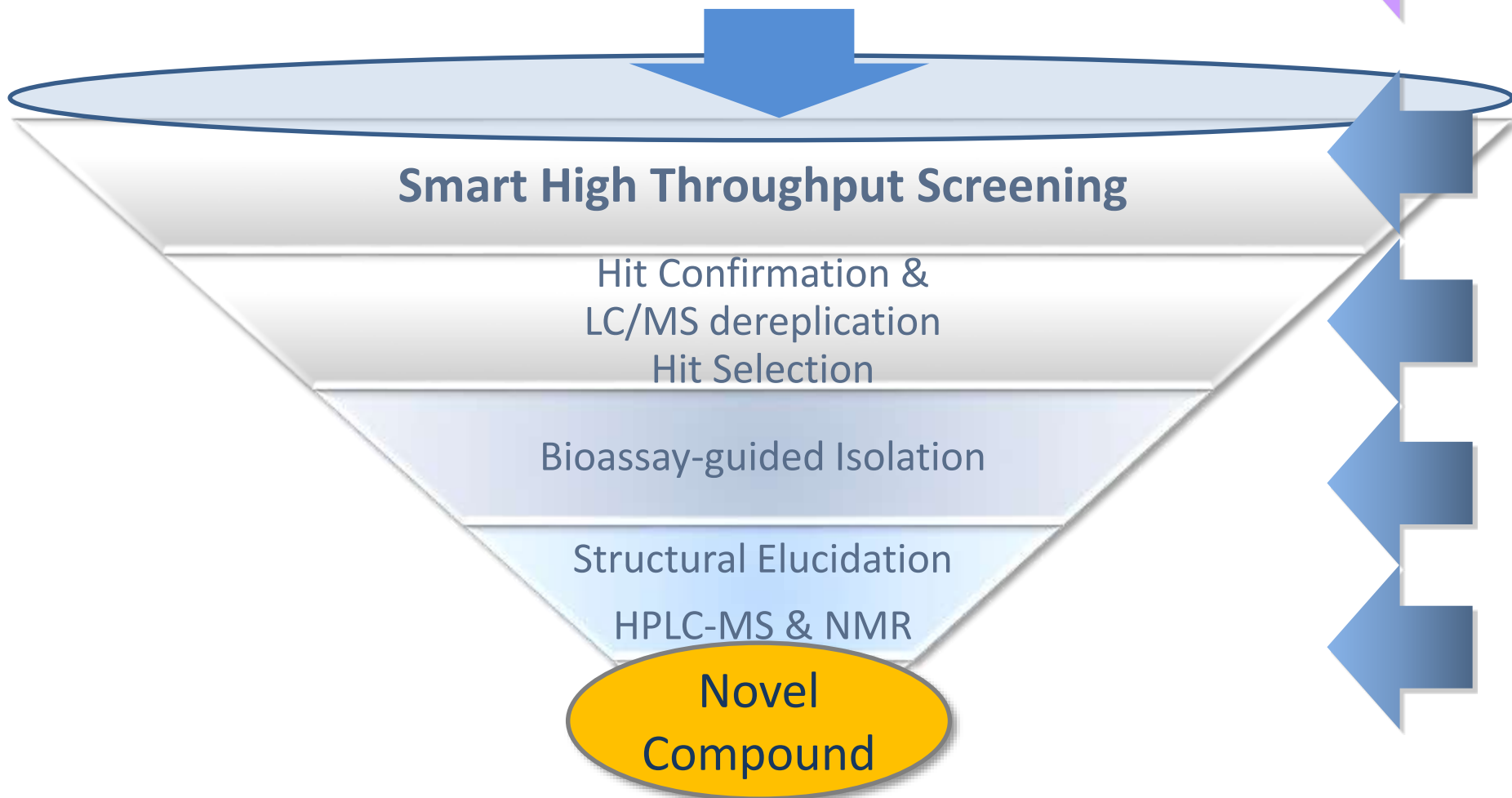
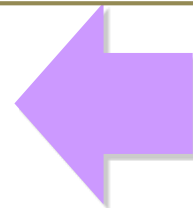
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- **Creation of Natural Product Extracts Collections**
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Natural Products Discovery

Extracts, fractions & compound Libraries





Types of HTS Libraries

Extracts/Fractions NP Libraries

- Unknown Composition/
- Potentially new chemistry
- Unknown concentration [WBE]
- vehicle DMSO/AQ
- Dereplication required LC-MS/NMR
- Hit follow-up required for identification

Purified Natural Products Libraries

- Known/unknown Composition
- Know/unknown concentration [1 - 5 mg/mL]
- vehicle DMSO
- Three freeze-thaw cycles maximum
- Degradation analyses required LC-HRMS

Synthetics/Combinatorial Libraries

- Known Composition
- Known concentration [10 mM]
- Vehicle DMSO
- Three freeze-thaw cycles maximum
- Recurrent Degradation analyses required LC-HRMS (QC)



Collections of extracts-General Rules

- Cover a **wide and representative range of biological species**
- Created using **robust and reproducible protocols**
- Must **represent the most significant biodiversity** (ideally all) included in the microbial strains or macroorganism sample collection
- Ideally a **back-up sample or a protocol to generate it must be available** for scaling-up purposes
- Extracts must be **stored in appropriate conditions** and in a format suitable for the rapid generation of multiple aliquots
- Associated **information managed through a Laboratory Information Management System (LIMS)**
- Samples included in the collection **must have been legally collected** in compliance with Nagoya protocol and other national and international legislation



Expeditions, Samples and Extract Collections



200.000 extracts!!!

CLASIFICACION

COLLECTION

EXPEDITIONS



Harvesting and Logistics



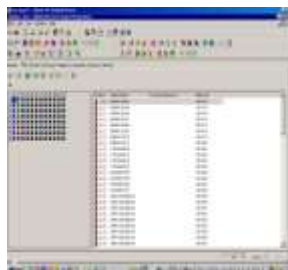
Taxonomy & Labelling



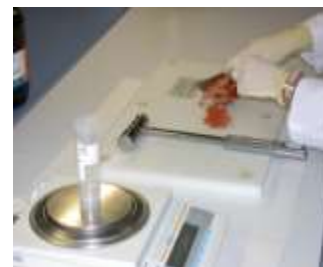
Storage, Safety



GPS, Images



DATA MANAGEMENT



Preparation of extracts



EXTRACTION
Water/MeOH:DCM

MARINE BIODISCOVERY IN IRELAND



Providers

- Academics
- Private

Marine
Organisms



NMBLI

- Taxonomy
- Chemical screening
- Biological screening

Vouchers
Biomass
Fractions
Chem and Biol
Profiling

Users

- Academics
- Privates

Marine Natural
Products



FUNCTIONING OF THE NMBLI

Collection/Sampling SOP MB-100/101



Picture

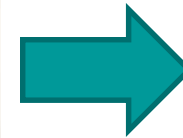
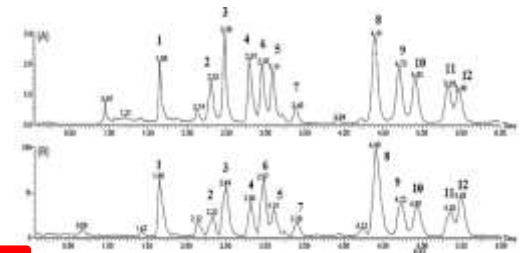


Voucher



Biomass

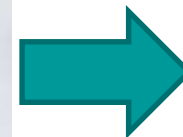
Chemical Screening SOP MB-103



Fractions



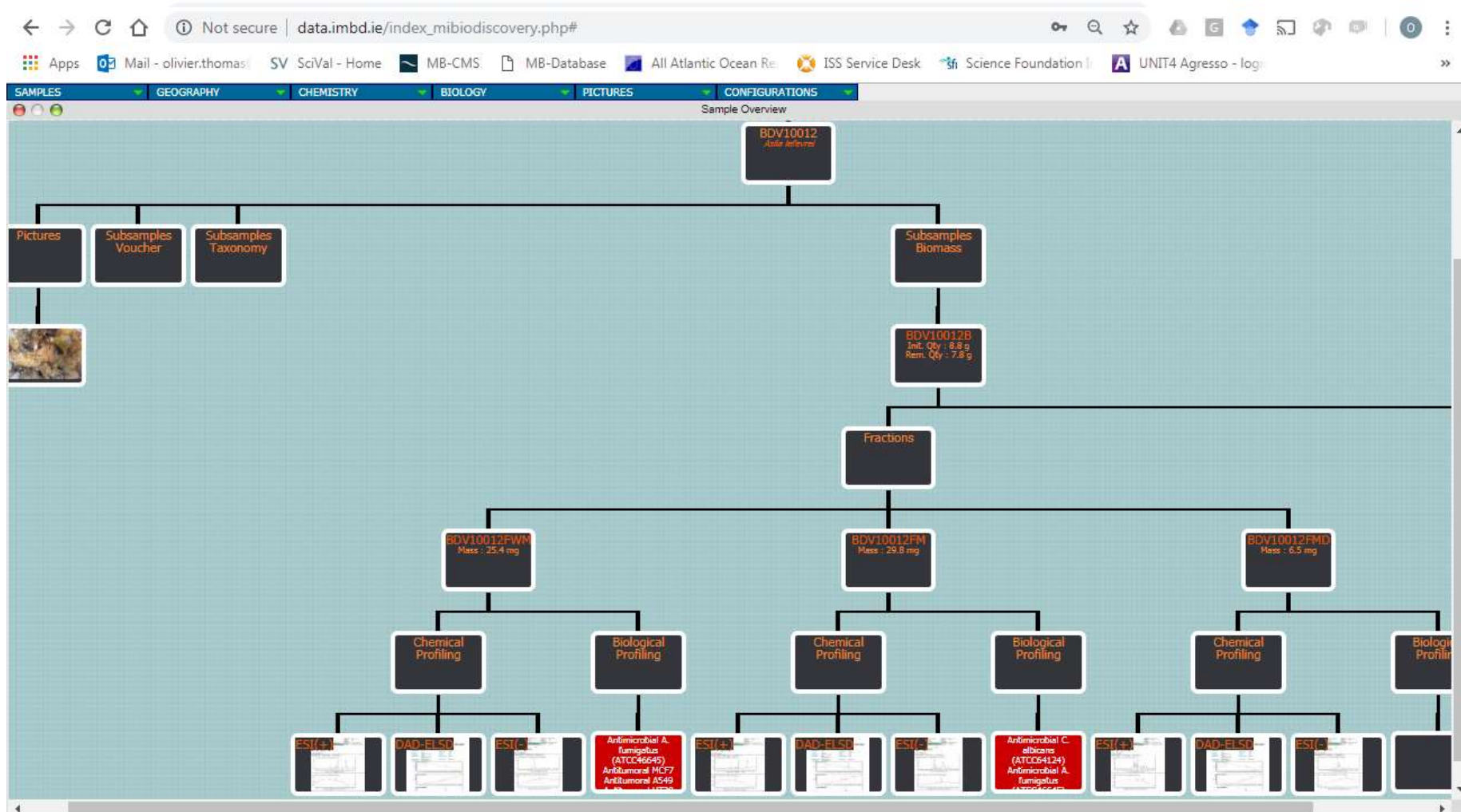
Sample preparation SOP MB-102



Biological Screening SOP MB-104

WEBDATABASE: RESTRICTED ACCESS

End 2018: 141 Irish Invertebrates. Voucher and Biomass
Help to organise and structure. 423 Fractions to be tested. Tracking



CMS: OPEN ACCESS www.imbd.ie

Location, species (link WORMS), natural products (link METABOLIGHTS)

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📱 Apps 📧 Mail - olivier.thomas@sv SciVal - Home 🖨 MB-CMS 📄 MB-Database 🌐 All Atlantic Ocean Re 🛒 ISS Service Desk 📡 Science Foundation I 📄 UNIT4 Agresso - logi »

Marine Biodiscovery Ireland

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Biodiscovery Events: Maps Species Natural products Bioactivities

Biological groups: 141 samples collected

- ☐ Annelid (3) ☐ Arthropod (1) ☐ Brown Seaweed (6)
- ☐ Bryozoan (3) ☐ Cnidarian (11) ☐ Echinoderm (17)
- ☐ Green Seaweed (2) ☐ Hydroid (1) ☐ Jellyfish (1)
- ☐ Mollusc (5) ☐ Peanut Worm (1) ☐ Red Seaweed (21)
- ☐ Sea anemone (1) ☐ Sea Squirt (12) ☐ Sponge (54)
- ☐ Starfish (2)

Species:

Natural products:

Species list:

- Styela clava
- Sponge
- Amphilectus fuorum
- Aplysilla rosea
- Axinella dissimilis
- Clathria (Microciona) strepsitoxa**
- Clathrina coriacea
- Ciona celata
- Crella rosea
- Dercitus bucklandi
- Dysidea fragilis
- Grantia compressa

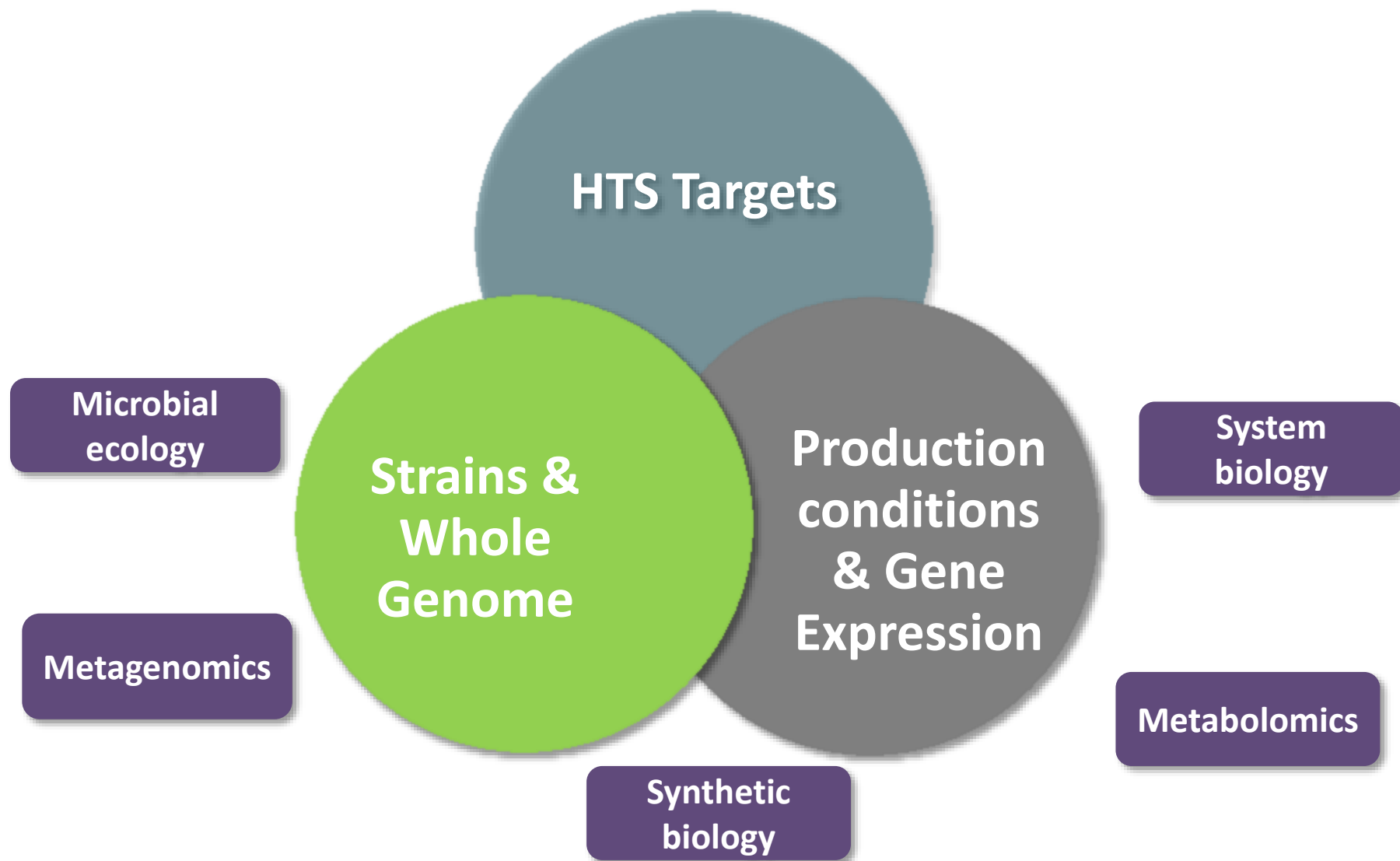
Classification: Porifera (Phylum) > Demospongiae (Class) > Poecilosclerida (Order) > Microcionidae (Family) > Clathria (Genus) > *Clathria (Microciona) strepsitoxa* (Species)

[Link to World Register of Marine Species \(WoRMS\)](#)





Maximizing probabilities of success in NPs drug discovery from microorganisms



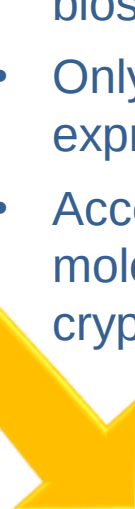


Microbiological Approaches to Maximize Chemical Diversity



- Genes encoding biosynthetic enzymes in bacteria and fungi outnumber known secondary metabolites
- We are missing large part of NP biosynthetic capacity
- Only a subset of pathways is expressed in laboratory conditions
- Access to this reservoir of molecules needs activation of cryptic genes

- **Culture conditions (OSMAC)**
- **Stress factors**
- **Addition chemical inducers**
- **Interspecies crosstalk: Co-culturing**
- **Epigenetic modifiers**



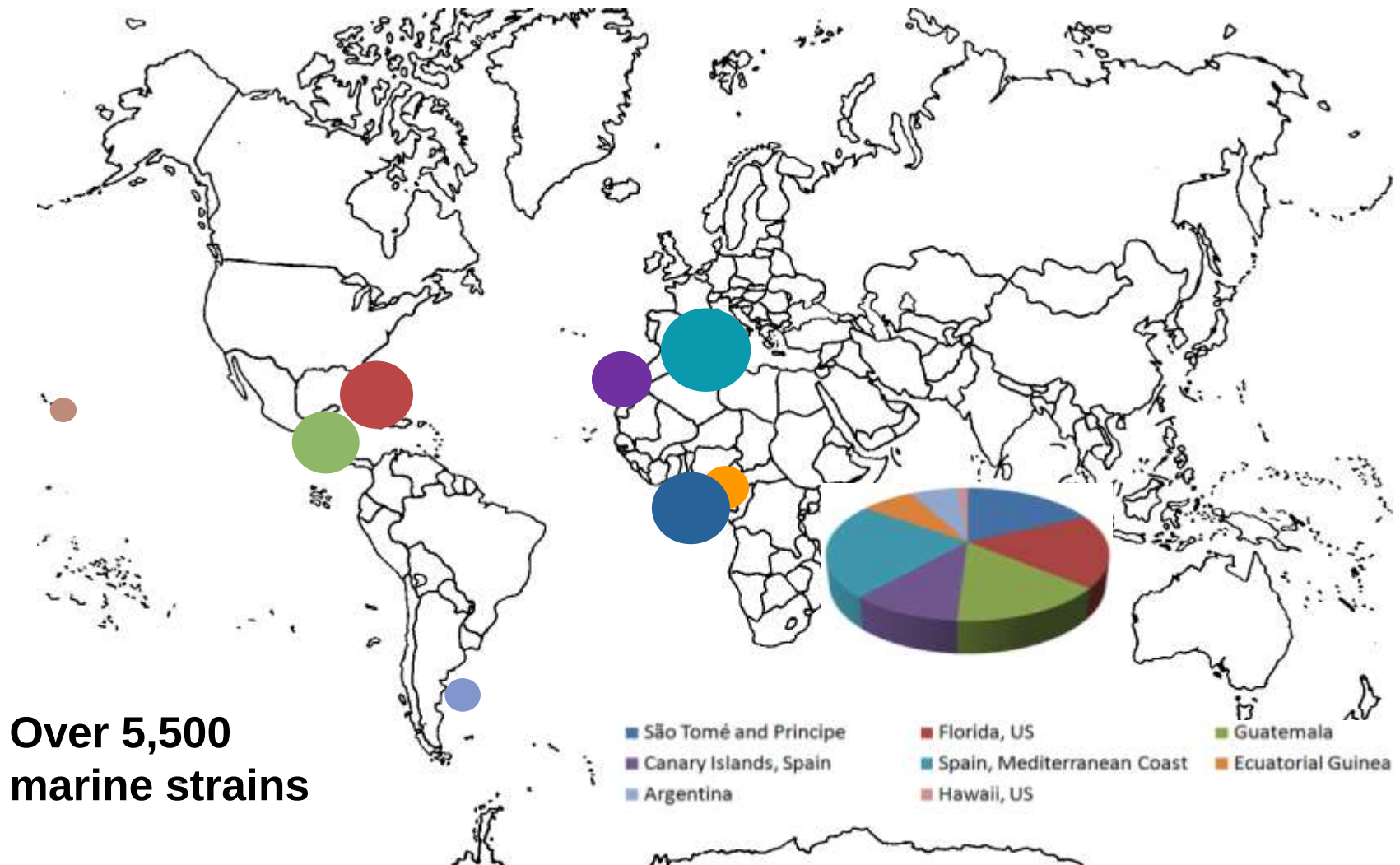
PHARMASEA



“The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013 under grant agreement n ° 312184)”

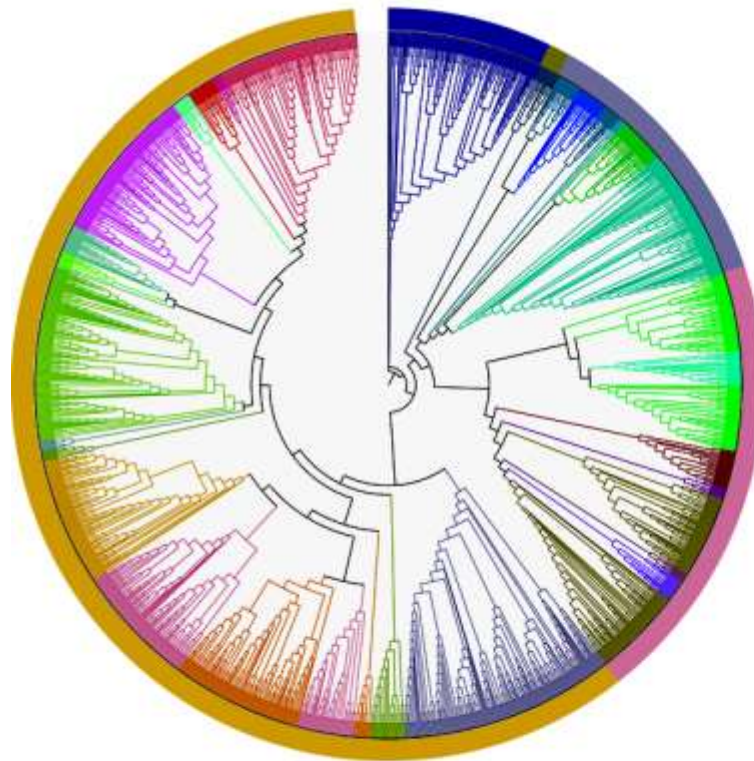


Geographical Origin of MEDINA's Marine Strains





Taxonomic Diversity of the Marine Collection



Bacillaceae
Flavobacteriaceae
Pseudomonadaceae
Alteromonadaceae
Pseudoalteromonadaceae
Vibrionaceae
Erythrobacteraceae
Sphingomonadaceae
Caulobacteraceae
Phyllobacteriaceae
Rhodobacteraceae
Streptomycetaceae
Nocardaceae
Mycobacteriaceae
Dietziaceae
Nocardioidaceae
Micromonosporaceae
Streptosporangiaceae
Nocardiopsaceae
Micrococcaceae
Promicromonosporaceae
Intrasporangiaceae
Microbacteriaceae

Bacilli
Flavobacteria
Gammaproteobacteria
Alphaproteobacteria
Actinobacteria

High taxonomic diversity within the collection, which includes obligate marine bacteria such as *Salinispora* spp.

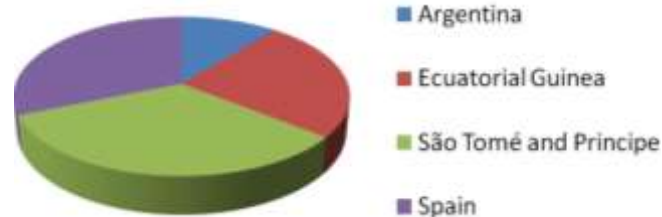
over **5,500 marine bacteria**, mainly actinobacteria (~75%), non-actinobacteria taxa (~25%).

Diversity tree: partial 16S rRNA sequences of 1,600 strains

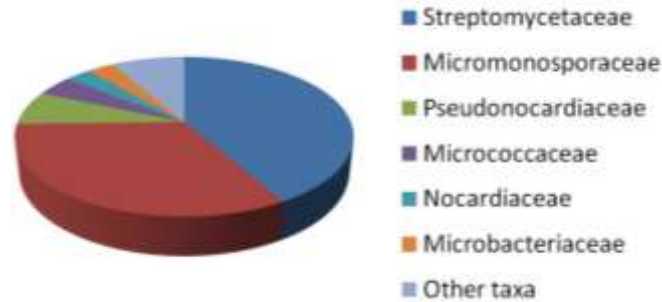


PharmaSea: Selection of Strains

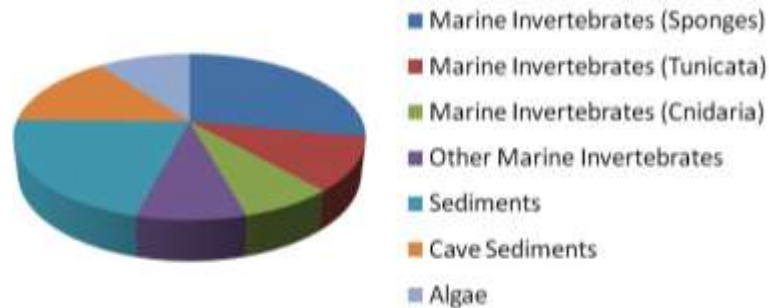
Geographic Origin



Taxonomy



Microbial Source



Strains were selected in order to maximize the diversity in geographic origin, taxonomy and isolation source

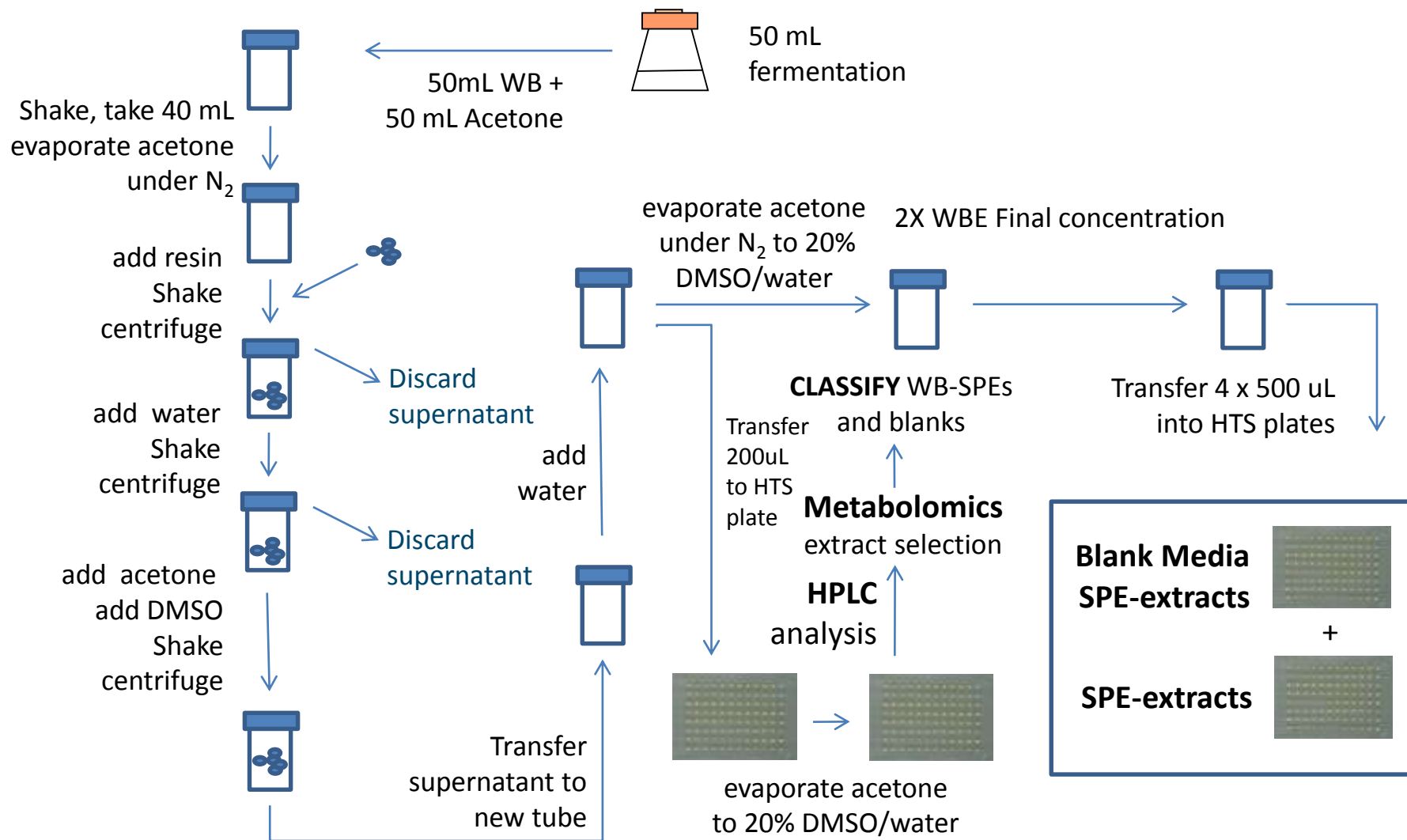


Fermentation of each strain in 4 different media

Components (in g/L)	DEF15S-M	APM9-M	R358	FPY12-M
Amicase				5
Bacto Peptone			2	5
Bacto Yeast Extract			4	
Fructose				20
Glucose		50		10
Maltose				10
Saccharose	5			
Soluble Starch (potato)	20	12	10	
Soybean Flour		30		
CoCl ₂ .6H ₂ O		2 mg		
FeSO ₄ .7H ₂ O			5 mL (stock 8 g/L)	
KBr			5 mL (stock 8 g/L)	
KH ₂ PO ₄	1			
MgCl ₂ .6H ₂ O	1			
Na ₂ SO ₄	2			
NaCl	1			
NH ₄ Cl	2			
CaCO ₃	2	7		
Sigma Sea Salts	40	40	40	40
Trace Elements	1 mL			1 mL
pH	7.0 (before adding CaCO ₃)	7.0 (before adding CaCO ₃)	7	7



PharmaSea Extraction Procedure Details



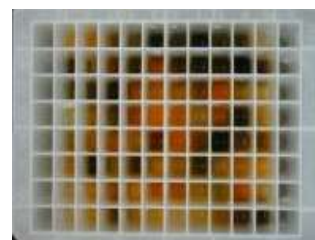


Pharma Sea Collection generated at MEDINA (6K)

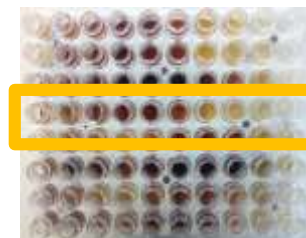
Module	Type of Sample	# Samples
PCA-000001 to PCA-000022	Crudes + blanks	1696
PFA-000001 to PFA-000054	Fractions + blanks	4320

A: SPE EXTRACTION

B: POLARITY CHROMATOGRAPHY



**Module of
CRUDES**



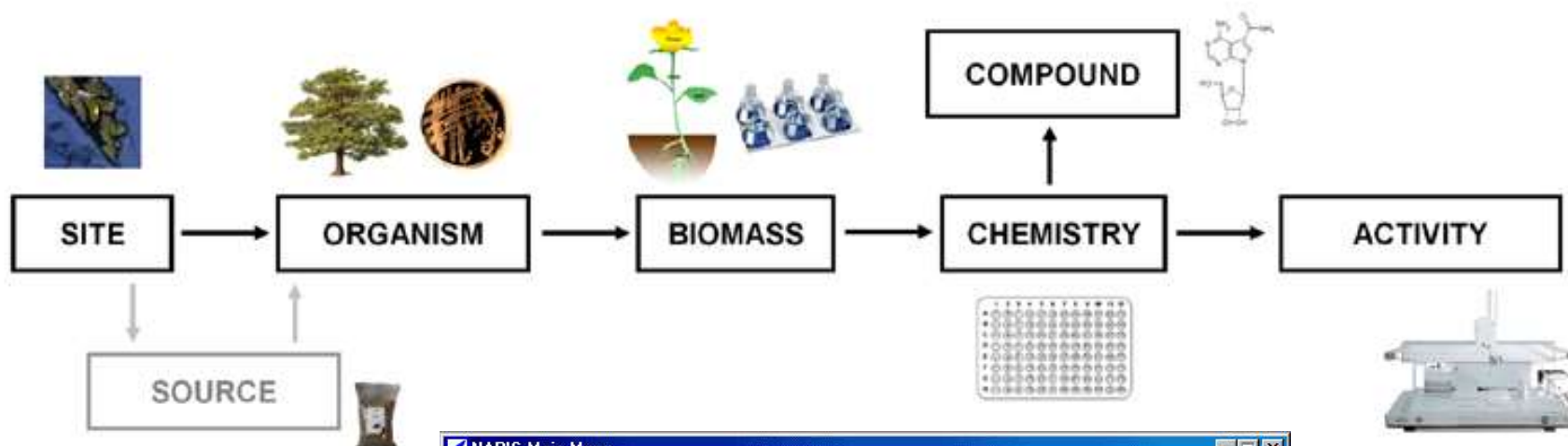
**Module of
FRACTIONS**

Storage - Labeling

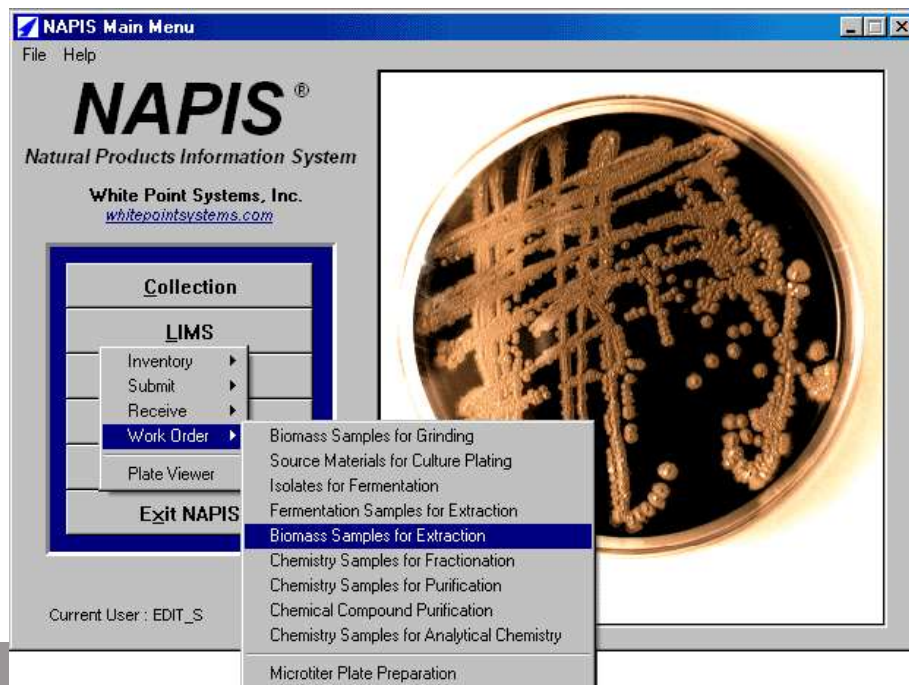




LIMS Tracking of Samples: NAPIS



OpenNAPIS™
Functional Design



White Point Systems, Inc.
20100526



LIMS Tracking of Samples: Nautilus

Thermo
SCIENTIFIC

- Medina_Carambolo - [Hierarchy - CA-253965-a01-MR016-EC01-FS004-FH175-FH001]

View Window Help

Strains

- CA-253963
- CA-253964
- CA-253965
 - CA-253965-a01
 - CA-253965-a01-MO001
 - CA-253965-a01-MO002
 - CA-253965-a01-MO003
 - CA-253965-a01-MO004
 - CA-253965-a01-MO005
 - CA-253965-a01-MO006
 - CA-253965-a01-MO007
 - CA-253965-a01-MO008
 - CA-253965-a01-MR009
 - CA-253965-a01-MR010
 - CA-253965-a01-MR011
 - CA-253965-a01-MR012
 - CA-253965-a01-MR013
 - CA-253965-a01-MR014
 - CA-253965-a01-MR015
 - CA-253965-a01-MR016
 - CA-253965-a01-MR016-EC01
 - CA-253965-a01-MR016-EC01-FS001
 - CA-253965-a01-MR016-EC01-FS002
 - CA-253965-a01-MR016-EC01-FS003
 - CA-253965-a01-MR016-EC01-FS004
 - CA-253965-a01-MR016-EC01-FS004-a01
 - CA-253965-a01-MR016-EC01-FS004-a02
 - CA-253965-a01-MR016-EC01-FS004-a03
 - CA-253965-a01-MR016-EC01-FS004-FH001
 - CA-253965-a01-MR016-EC01-FS004-FH002
 - CA-253965-a01-MR016-EC01-FS004-FH003
 - CA-253965-a01-MR016-EC01-FS004-FH004

Small scale Fermentations

Large scale Fermentations

Extracts

Fractions

Assay Aliquots

Sub-Fractions

Field Definition Table:

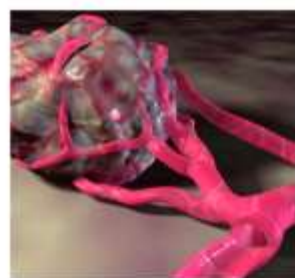
Field	Value
Platename	PL-HPF-000659
PI Order	24
PI Row	8
Crow	H
PI Column	3
Sampleid	PL-HPF-000659-H3
Identifyer	(None)
Parent Name	CA-253965-a01-MR016-EC01-FS004-FH
External Id	(None)
Clients	(None)
Requesters	(None)
Category	SAMPLE
IDENTIFICATION	CA-253965-a01-MR016-EC01-FS004-FH
medAliquot Type	aF
Extraction Type	FRACTION
Extraction Solvent	ACN/AQ
CTS_ID	(None)
NEXUS_ID	(None)



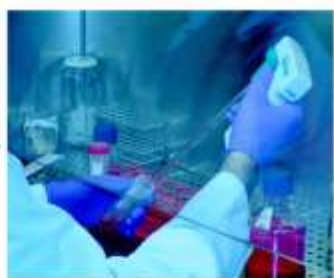
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- **Exploitation of collections in search of new bioactive molecules**

Antitumor Screening -Cytotoxicity



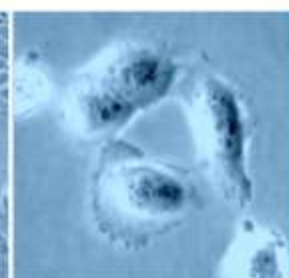
Human tumour



Cell culture



Immortalized cell lines



In vitro cell culture



Sampling

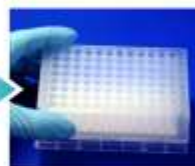


Macros

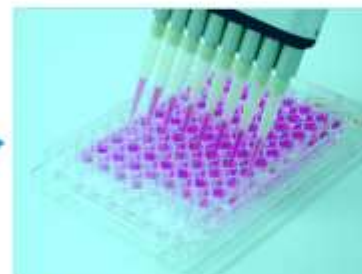
Micros



Extracts



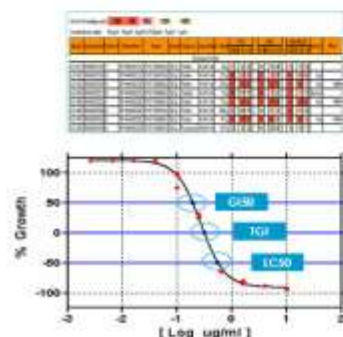
Sample supply
(96-well format)



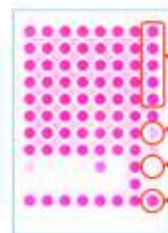
Sample preparation



Cell seeding



Quantification & Evaluation



Inactive
Cytostatic
Cytotoxic
Control



Incubation

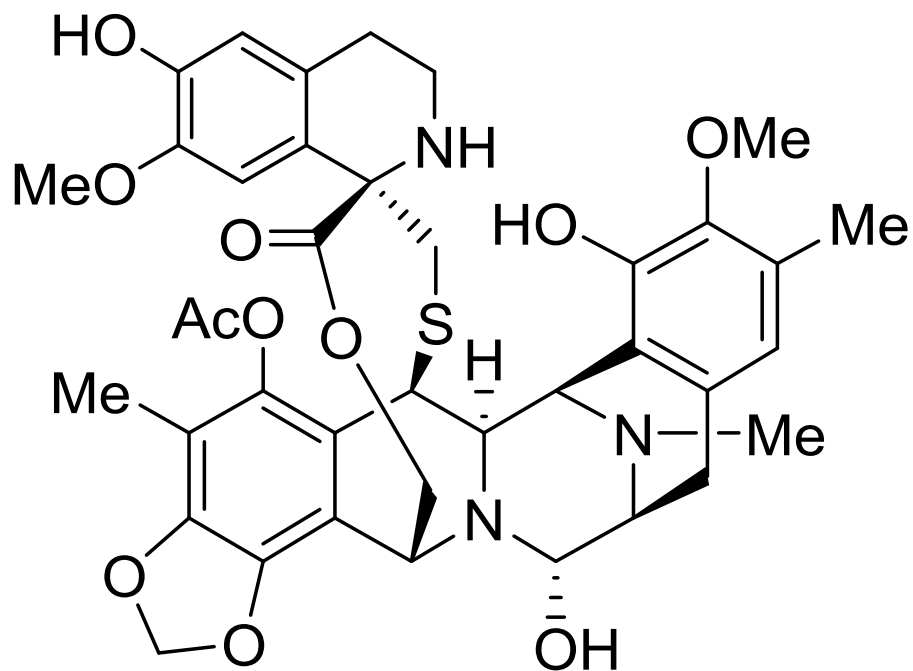


Cell treatment

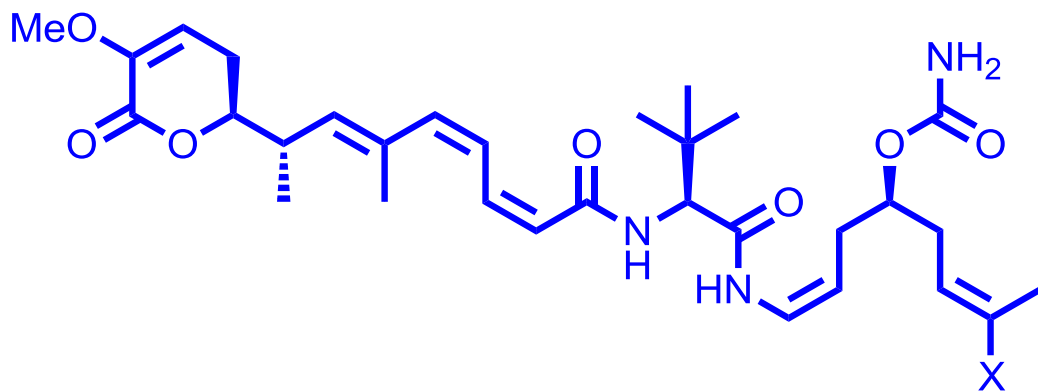
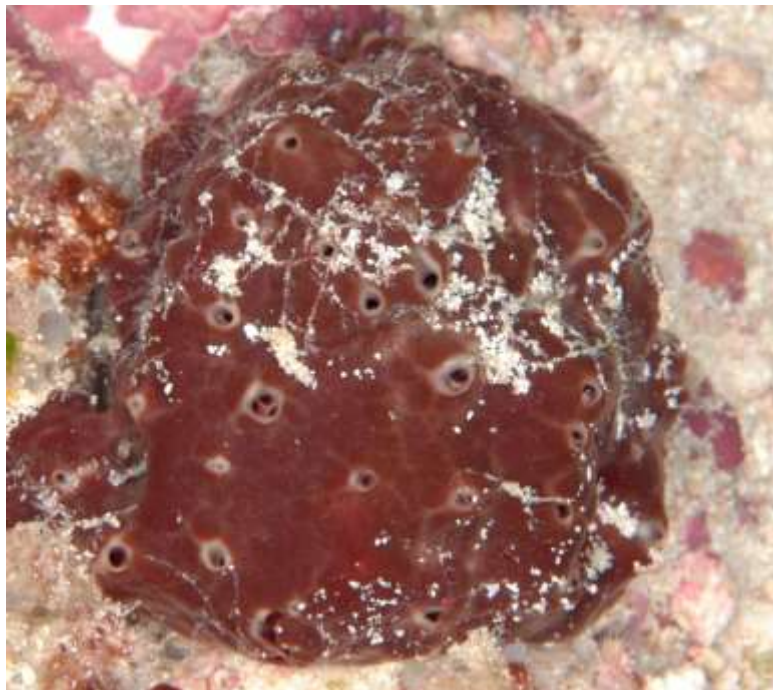
Ecteinascidia turbinata



Ecteinascidia turbinata



Approved for the treatment of Soft
Tissue Sarcoma and Ovarian Cancer



PM050489 X = Cl

PM060184 X = H

Undergoing Phase II clinical trials against colorectal cancer as single agent

Lithoplocamia lithistoides

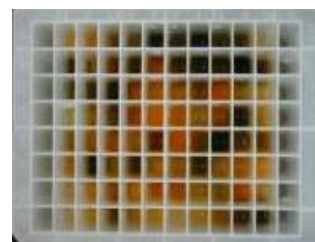


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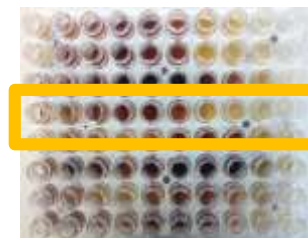
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A: SPE EXTRACTION

B: POLARITY CHROMATOGRAPHY



**Module of
CRUDES**



**Module of
FRACTIONS**



MEDINA - PharmaSea Antimicrobial screening

Antibacterial

- ✓ *Staphylococcus aureus* MRSA
- ✓ *Pseudomonas aeruginosa*
- ✓ *Escherichia coli*
- ✓ β -lactam synergy vs. multi-resistant *Pseudomonas aeruginosa*

Antifungal

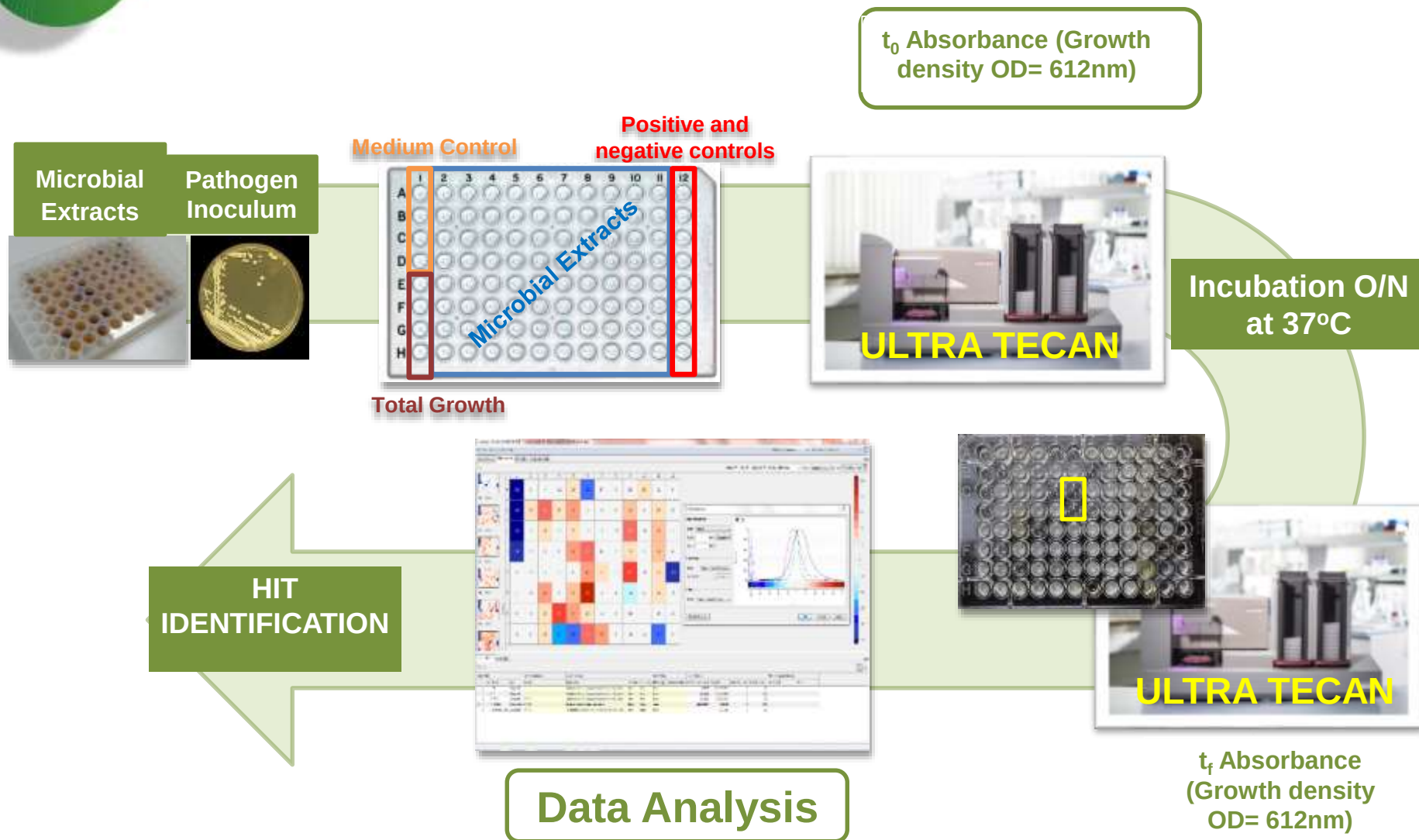
- ✓ *Candida albicans*
- ✓ *Aspergillus fumigatus*

Anti-TB

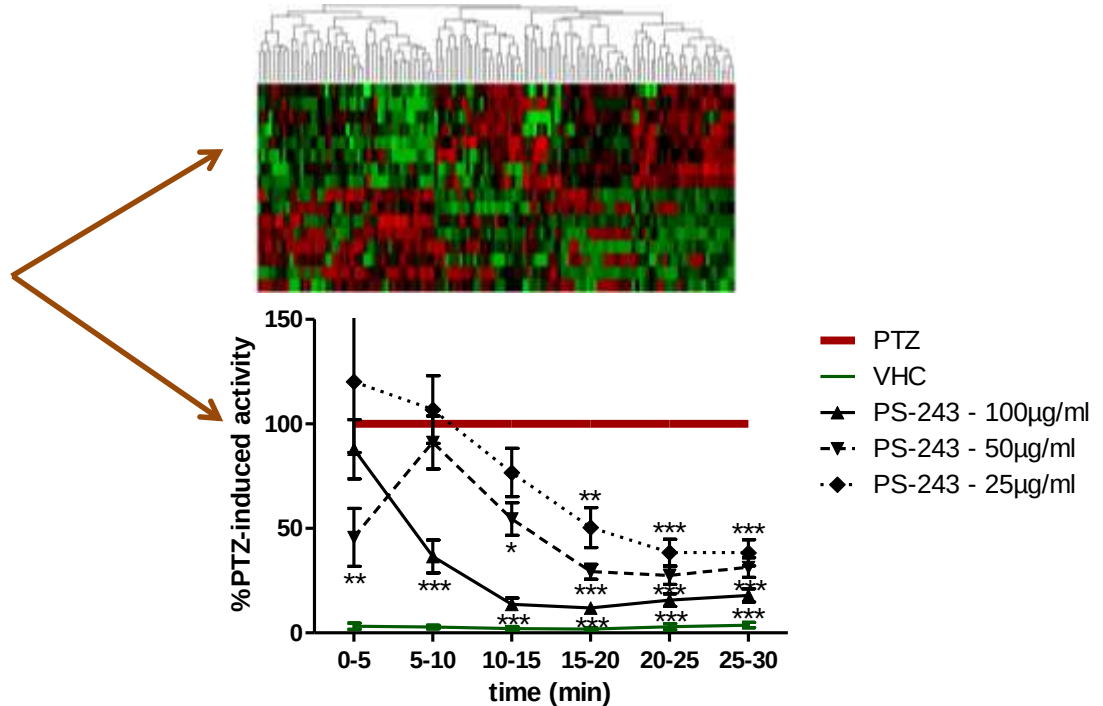
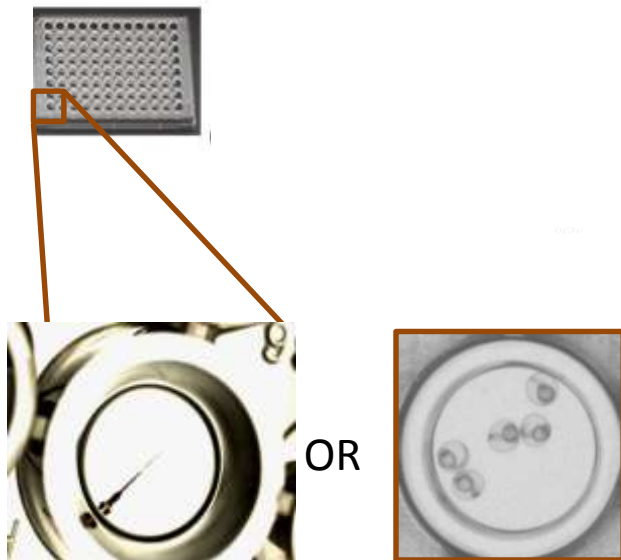
- ✓ *Mycobacterium tuberculosis* H37Ra
- ✓ *Mycobacterium bovis* BCG



High Throughput Antibacterial Screening Overview



Zebrafish-based identification of neuroactive and antiepileptic hits (KU Leuven)



- Photomotor response assay: neuroactive hits
- Epilepsy seizure model: anticonvulsant hits
- Maximum Tolerated Concentration (MTC) analysis: general toxicity analysed for hits

3777 screened samples
727 neuroactive hits
43 anticonvulsant hits
5 out of 11 hits validated
→ To be continued



Screening Campaigns Results

Count of Hits per Assay	Qualifier			Extracts Tested
	A	Q	I	
Absorbance_MB2884 (E.coli)	68	137	5761	5966
Absorbance_MB5393 (MRSA)	114	10	5842	5966
Resazurine_A.fum_ATCC46645	196	30	5740	5966
Absorbance_C.alb_MY1055	142	62	3316	3520
Resazurine_C.alb_MY1055	73	35	2338	2446
Resazurine_P.aer_PAO1 + Imipenem	0	0	2480	2480
Resazurine_A.bau_MB5973	0	0	1840	1840
Resazurine_A.bau_MB5973 + Imipenem	25	28	3673	3726
Fluorescence_M.tub_H37Ra	201	44	5721	5966
Grand Total	843	374	41025	42242

1217





PharmaSea Drug Discovery Campaign

6016 samples (blanks+extracts+fractions) from 405 strains

1217 Hits (843 Active, 374 Questionable) selected for CherryPicking
416 confirmed Hits selected for **LCMS Dereplication** of knowns

163 samples with no matches in LCMS DB
71 strain-condition-assay selected for confirmation

71 ferment + extraction + assay confirmation
HPLC fractionation+assay+ **LCMS and NMR dereplication**

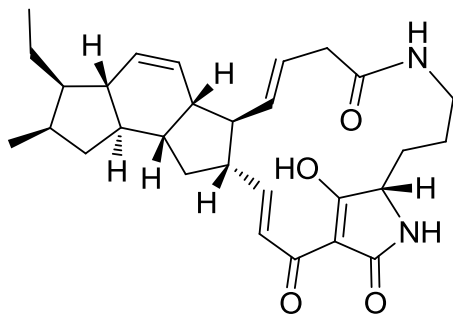
20 Strains selected for Big Scale Purification

52 Compounds



New ikarugamycins

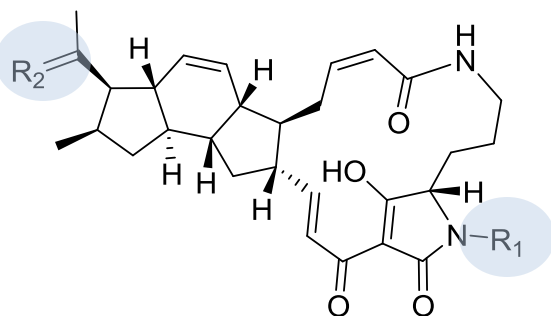
Streptomyces zhaozhouensis CA-185989



Isoikarugamycin

Biological activity

Compound ID	MIC [$\mu\text{g/mL}$]		
	<i>MRSA</i>	<i>C. albicans</i>	<i>A. fumigatus</i>
Isoikarugamycin	2-4	2-4	4-8
Ikarugamycin	2-4	4	4-8
28-N-methylkarugamycin	1-2	4	4-8
30-oxo-28-N-methylkarugamycin	32-64	>64	>64



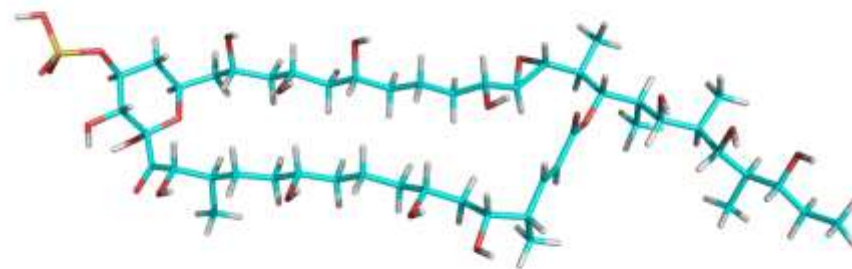
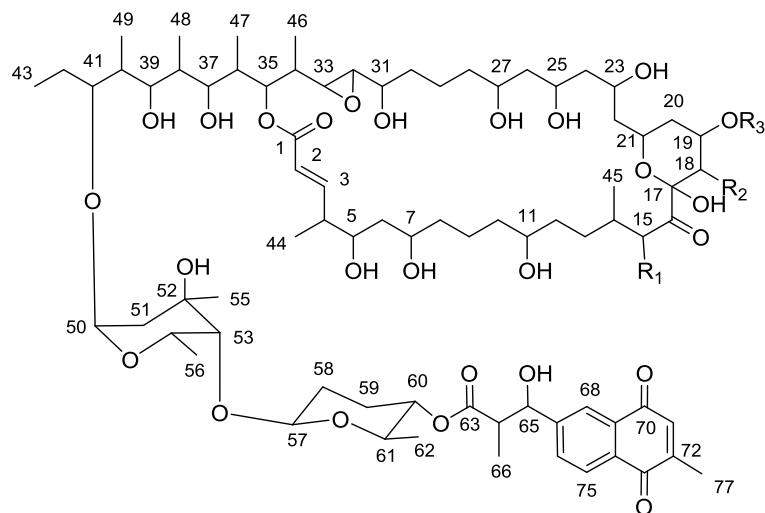
$R_1 = \text{H}$	$R_2 = \text{H}$	Ikarugamycin	
$R_1 = \text{CH}_3$	$R_2 = \text{H}$	28-N-methylkarugamycin	New
$R_1 = \text{CH}_3$	$R_2 = \text{O}$	30-oxo-28-N-methylkarugamycin	New

Mar. Drugs, **2015**, *13*, 128-140.



New antifungal macrolides

Streptomyces caniferus CA-271066

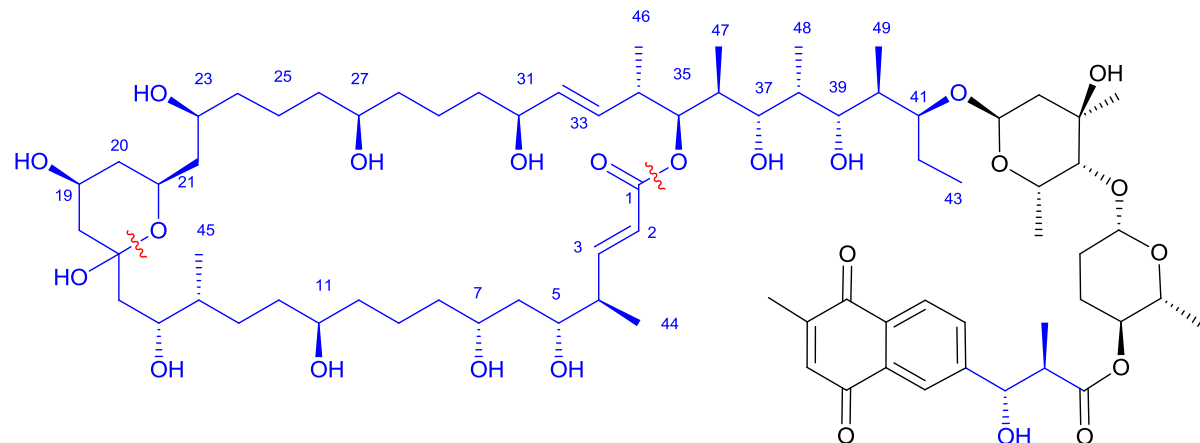


$R_1 = -OH$ $R_2 = -H$ $R_3 = -H$ **Compound 1**
 $R_1 = -OH$ $R_2 = -OH$ $R_3 = -H$ **GT-35**
 $R_1 = -H$ $R_2 = -OH$ $R_3 = -SO_3H$ **Compound 2**
 $R_1 = -OH$ $R_2 = -OH$ $R_2 = -SO_3H$ **Compound 3**

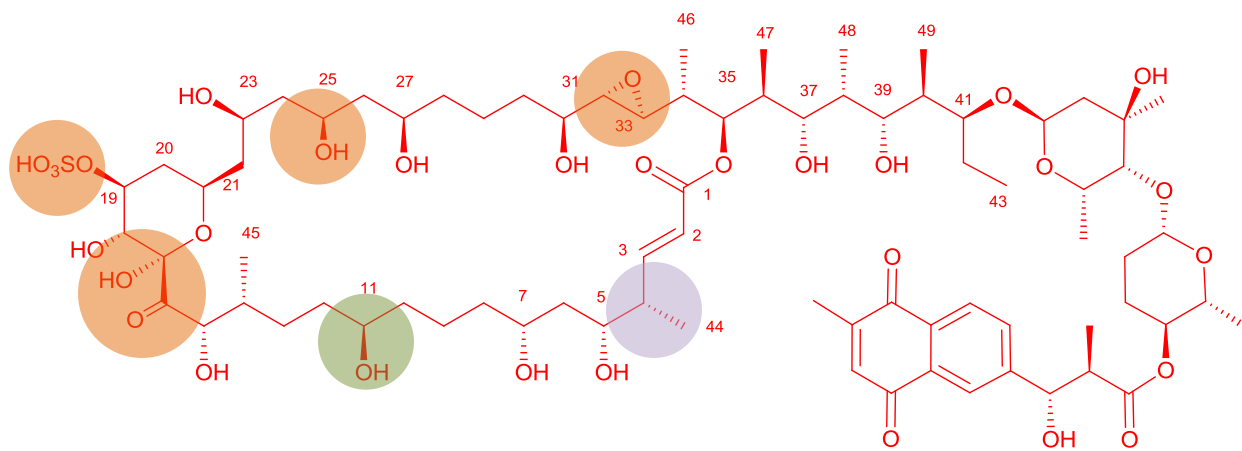
		MIC values ($\mu\text{g/mL}$)			
Fungal strains	Strain number	Compound 1	Compound 2	Compound 3	Compound 4
<i>C. albicans</i>	ATCC64124	0.5-1	2	0.5-1	8
<i>C. tropicalis</i>	ATCC750	0.5-1	2	0.5-1	8
<i>C. glabrata</i>	ATCC90030	0.5-1	2	0.5-1	8
<i>C. krusei</i>	ATCC6258	0.5-1	1	0.5-1	8
<i>C. parapsilosis</i>	ATCC22019	0.5-1	2	0.5-1	16
<i>A. fumigatus</i>	ATCC46645	4	4	4	8



Full absolute configuration of macrolides



Bioinformatic prediction



Bioinformatic + NMR
prediction

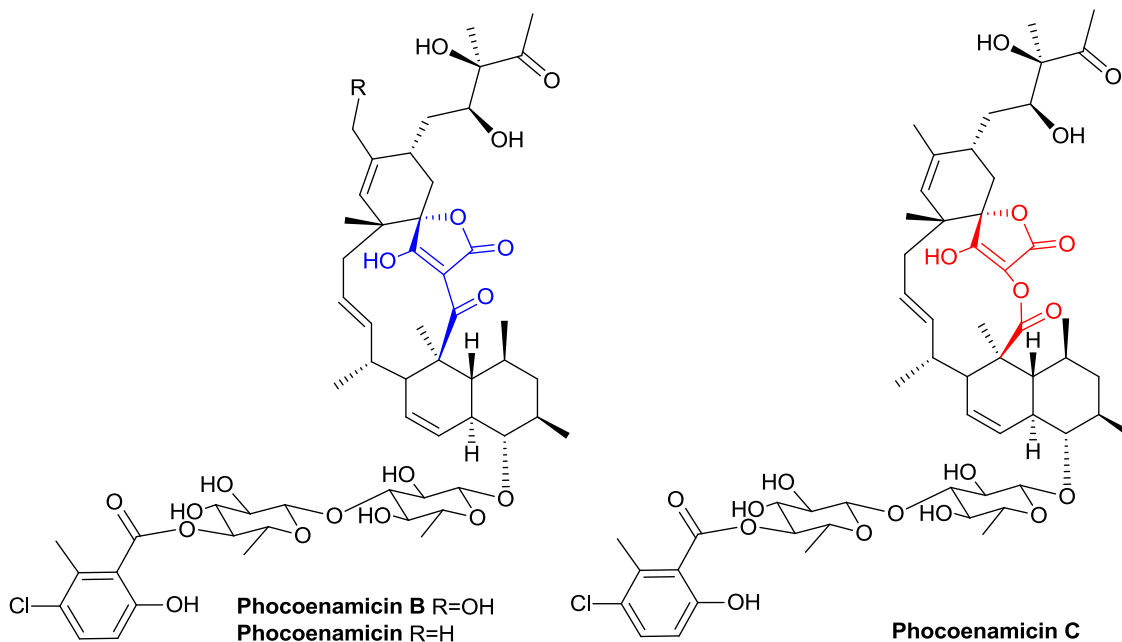
- Post-Tailoring NMR predicted
- Bioinformatic prediction
- DISCREPANCY





New spirotetronate derivatives. Phocoenamicins

Micromonospora chaiyaphumensis CA-214671



compounds	MIC (mg/mL)				ZOI* mm(mg)
	<i>S. Aureus</i> MB5393	<i>M. tuberculosis</i> ATCC 25177	<i>M. bovis</i> ATCC 35734	<i>E. faecium</i> MB5571	<i>B. subtilis</i> MB964
phocoenamicin B	8-16	>128	>128	>128	7 (2)
phocoenamicin C	32-64	32	>128	>128	7 (4)
phocoenamicin	4-8	16-32	>128	32-64	7 (4)
vancomycin	2-4			>128	
streptomycin		1.6-3.2	0.4-0.8		
gentamicin					8 (0.25)
penicillin G					19 (0.06)

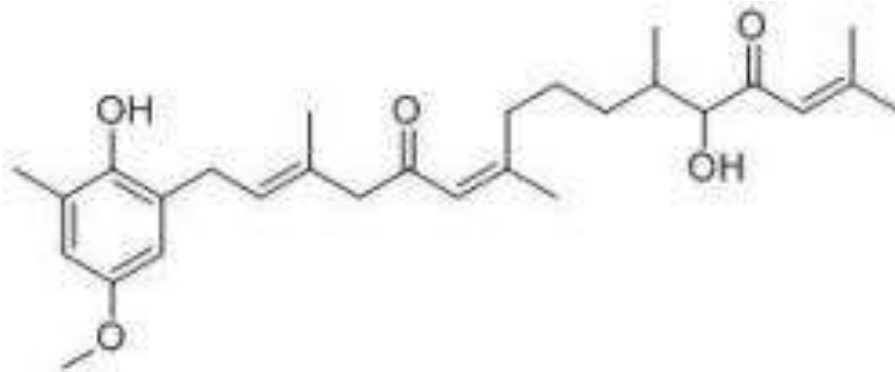
Mar. Drugs, **2018**, *16*, 95.

PRIORITY LIST AND BIOACTIVE COMPOUNDS



Fundación MEDINA

Antibacterial activity

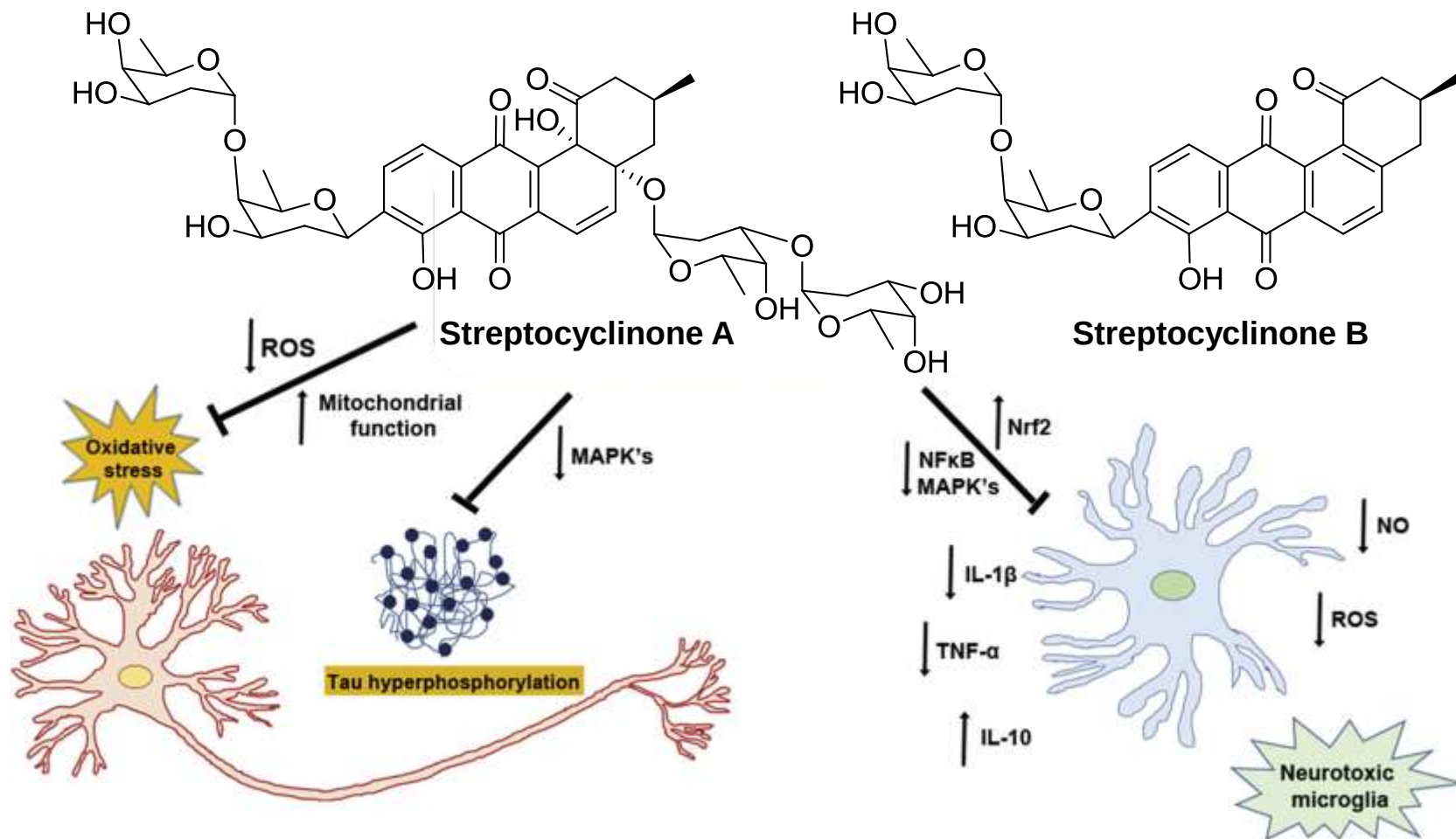


Halidrys siliquosa

New bioactive meroterpenoid derivatives

New C-glycosilated angucycline derivatives

Streptomyces sp. CA-237351



Neuropharmacology, 2018, 141, 283-295



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