Philippine Seas: Frontier of Pharmaceutical Discovery and Development

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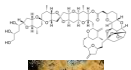
NAST PHL RSM
Camp John Hay
Convention Center
Baguio City
15-16 May 2017

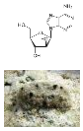
FDA Approved Marine Derived Pharmaceuticals

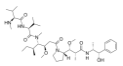

Yondelis (Cancer)
Janssen Prods
Sea Squirt



Eribulin (Cancer)
Eisai Inc
Sponge


Prialt (Pain)
Jazz Pharms Intl
Conus Magus


Vira-A (Herpes Simplex)
Parkedale
Sponge



Adcetris (Lymphoma)
Seattle Genetics
Wedge Sea Hare



Cytosar-U (Cancer)
Teva Pharms USA
Sponge

Philippine Mollusk Symbiont International Cooperative Biodiversity Group

www.pmsicbg.org


Margo Haygood *(Principal Investigator)*
Diverse Drug Lead Compounds from Bacterial Symbionts in Philippine Mollusks


Gary Rosenberg *(Leader)*
AP 1: Philippine marine mollusk biodiversity inventory


Eric Schmidt *(Leader)*
AP 2: Bacterial symbionts of gastropod molluscs: cultivation, drug discovery, and neurological activity


Margo Haygood *(Leader)*
AP 3: Microbes associated with shipworms as sources of drug leads


Gisela Concepcion *(Leader)*
AP 4: Screening of mollusk isolates for biological activity

THE ACADEMY OF NATURAL SCIENCES of DREXEL UNIVERSITY


Baldomero Olivera *(Participant)*


Daniel Distel *(Participant)*

THE UNIVERSITY OF UTAH

Northeastern University
The Ocean Genome Legacy Center of New England Biolabs

Discovery and Development of Health Products (DDHP) Marine Component


Arturo O. Lluisma
Program Leader


Arturo O. Lluisma
Project Leader
Project 1: High-throughput Multi-omics Discovery of Bioactive Marine Compounds and Establishment of a Marine Chemical Library and Informatics Facility


Gisela P. Concepcion
Project Leader
Project 2: Anti-Pain and Anti-Neurodegeneration Drug Candidates: Discovery and Development


Lilibeth S. Reyes
Project Leader
Project 3. Anti-Infective and Anticancer Drug Candidates: Discovery and Development


Lilibeth S. Reyes
Project Staff

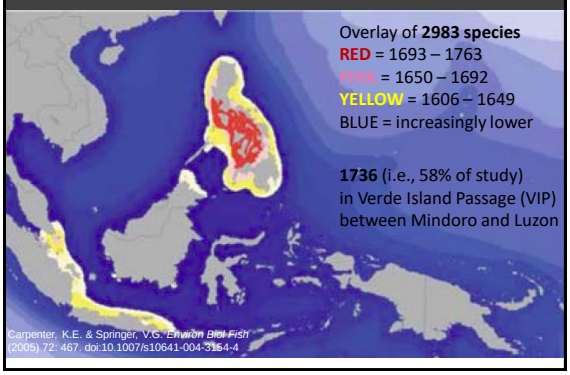

Aaron Joseph L. Villaraza
Project Staff


Ma. Auxilia T. Siringan
Project Staff


Porfirio M. Aliño
Project Staff


Cynthia P. Saloma
Project Staff

Philippines is the "center of the center" of marine biodiversity.



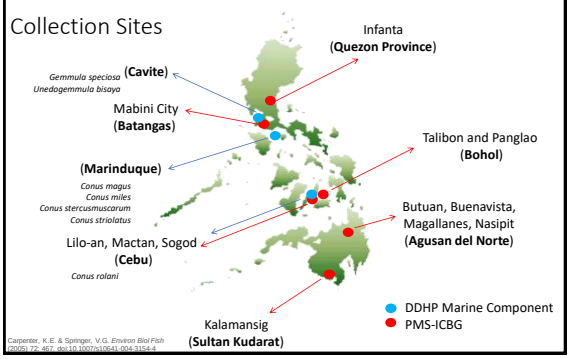
Overlay of **2983 species**
RED = 1693 – 1763
PINK = 1650 – 1692
YELLOW = 1606 – 1649
BLUE = increasingly lower

1736 (i.e., 58% of study) in Verde Island Passage (VIP) between Mindoro and Luzon

Carpenter, K.E. & Springer, V.G. *Environ Biol Fish* (2005) 72: 467. doi:10.1007/s10641-004-3154-4

Philippines is the "center of the center" of marine biodiversity.

Collection Sites

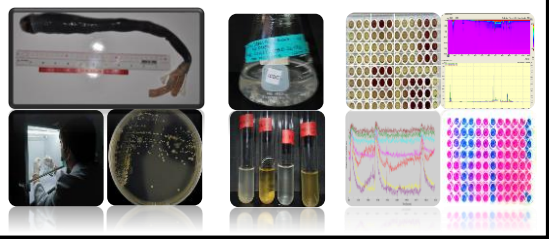


Carpenter, K.E. & Springer, V.G. Environ Biol Fish (2005) 72: 467. doi:10.1007/s10641-004-3154-4

Philippine Mollusk Symbiont International Cooperative Biodiversity Group

Pipeline

Collection and Isolation ⇨ **Small Scale Culture** ⇨ **Assay and HPLC Profiling** ⇨



Philippine Mollusk Symbiont International Cooperative Biodiversity Group

Pipeline

Dereplication ⇨ **Large Scale Culture and Purification** ⇨ **Structure Elucidation**

Philippine Mollusk Symbiont International Cooperative Biodiversity Group

In House Screening Campaign leads to identification of bioactive isolates for PHASE I (2008-2013)

from **7554** *assay events*

161 **Antimicrobial isolates**

86 **Cytotoxic isolates**

5 **Neuroactive isolates**

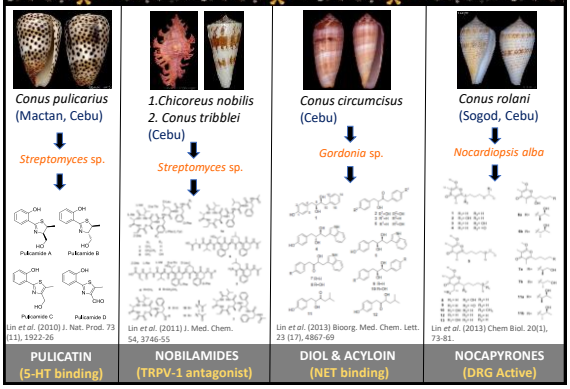
Antimicrobial activity
- *Pseudomonas aeruginosa*
- *Klebsiella pneumoniae*
- *Staphylococcus aureus*

Mamallian cell cytotoxicity
- MCF-7 (breast)
- A549 (lung)
- HCT 116 (colorectal)
- MDCK (normal kidney)

Neuroactivity
- Dorsal root ganglion assay

* Active at low dose

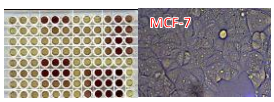
Philippine Mollusk Symbiont International Cooperative Biodiversity Group



Philippine Mollusk Symbiont International Cooperative Biodiversity Group

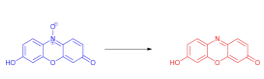
Screening for Cytotoxic Properties

- MCF-7 (breast),
- MDCK (normal kidney cells)
- A549 (lung), HCT 116 (colorectal)
- MTT Assay



Screening for Antimicrobial Properties

- *S. aureus, K. pneumoniae,* and *P. aeruginosa*
- Resazurin-based Microdilution Method



NATIONAL GEOGRAPHIC

Watch: Bizarre Deep-Sea 'Worm' As Long As an Arm Revealed

Scientists studying the giant shipworm for the first time made a truly strange discovery.

By Carrie Arnold

PUBLISHED APRIL 17, 2017

theguardian

Monday 17 April 2017 20.00 BST

Bizarre bivalve: first living giant shipworm discovered in Philippines

BBC

Live, long and black giant shipworm found in Philippines

18 April 2017

RAPPLER

The story behind the giant shipworm discovery that broke the internet

While locals in Sultan Kudarat, Philippines have known the giant shipworm for quite a while, the scientific world is yet to understand it

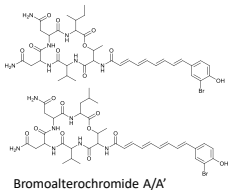
Shaira Panela

GMA NEWS ONLINE
YOUR NEWS AUTHORITY

Giant shipworm discovered in PHL

Published April 18, 2017 6:32pm

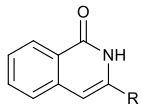
By MICHAEL LOGARTA



Bromoalterochromide A/A'

Pseualteromonas sp. isolated from the gill of *Teredo somersi.*

Active against *S. aureus* and methicilin-resistant *S. aureus*



C-3 substitute 1-(2)-isoquinolinone

Pseudomonas sp. isolated from the stomach of *Kuphus polythalamia.*

Active against *S. aureus* and A549 Lung Carcinoma

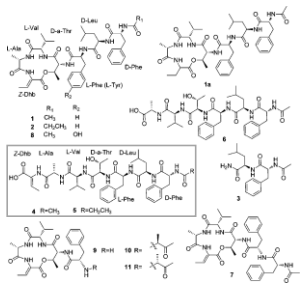
Nobilamides A–H

2011: Isolated from bacteria cultured in *Chicoreus nobilis* and *Conus tribblei*

(Cebu, Philippines)

DRG assay

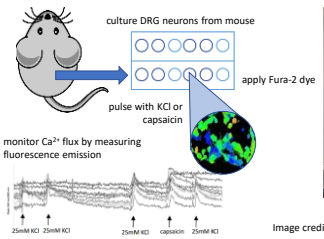
- Neurons express TRPV1
- Ca^{2+} uptake induced by capsaicin
- Fluorescence detection

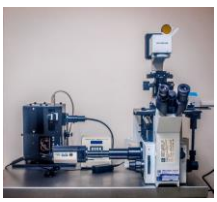


The Nobilamides

Lin *et al.* (2011) Journal of Medicinal Chemistry 54, 3746-55

DRG Assay





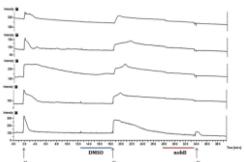
DRG Assay Setup

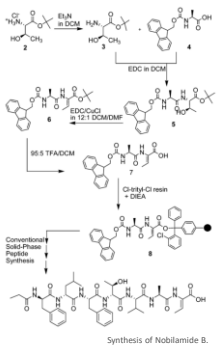
Image credits: Jortan O. Tun (UP Marine Science Institute)

(Supplementary Info) Lin, *et al.* (2011) J. Med. Chem. 54, 3746-55.

Total Synthesis and Neuroactivity

Nobilamide B inhibits capsaicin response in a primary culture of mouse DRG neurons.

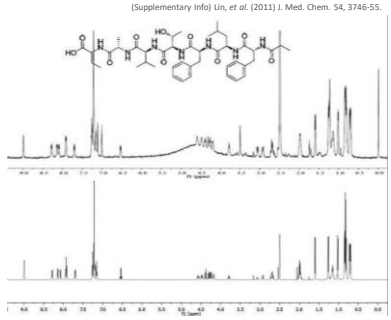




Synthesis of Nobilamide B.

Jacinto *et al.* (2014) RSC Advances *4*, 37609-12.

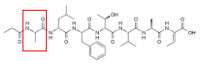
(Supplementary Info) Lin, *et al.* (2011) J. Med. Chem. 54, 3746-55.



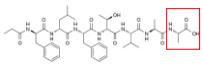
^{1}H NMR spectrum of natural (top) and synthetic (bottom) Nobilamide B (500 MHz, DMSO-d_6)

Synthetic Nobilamides

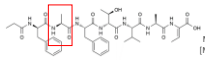
Synthesis of alanine-substituted analogues enables structure-activity relationship studies that may aid in identifying amino acid residues essential to activity.



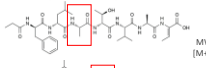
[A1]-Nobilamide B
MW_{calc} = 759.42 g/mol; $[M+H]^+_{obs}$ = 760.50 m/z



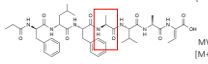
[A7]-Nobilamide B
MW_{calc} = 823.45 g/mol; $[M+H]^+_{obs}$ = 824.55 m/z



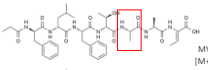
[A2]-Nobilamide B
MW_{calc} = 793.40 g/mol
$[M+H]^+_{obs}$ = 794.50 m/z



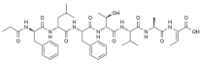
[A3]-Nobilamide B
MW_{calc} = 759.42 g/mol
$[M+H]^+_{obs}$ = 760.43 m/z



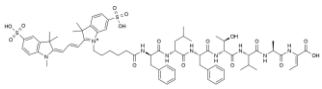
[A4]-Nobilamide B
MW_{calc} = 805.44 g/mol
$[M+H]^+_{obs}$ = 806.45 m/z



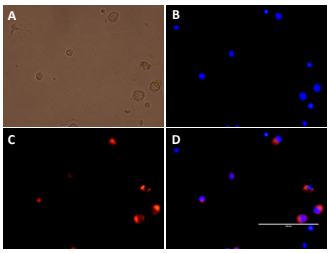
[A5]-Nobilamide B
MW_{calc} = 807.42 g/mol
$[M+H]^+_{obs}$ = 808.50 m/z



Nobilamide B
MW_{calc} = 835.45 g/mol; $[M+H]^+_{obs}$ = 836.50 m/z



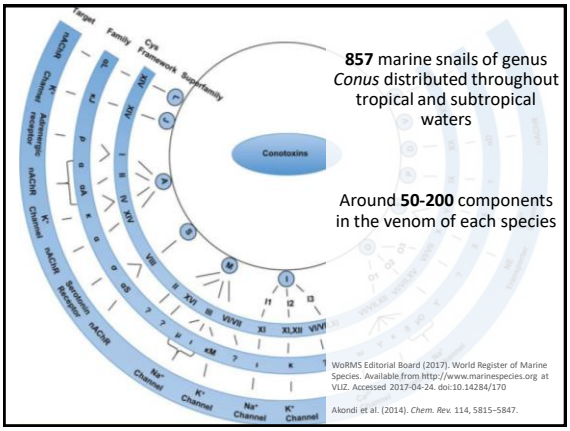
Cy3-labeled Nobilamide B (Cy3-Nob B)



Fluorescence Labeling

DRG neurons incubated with Cy3-Nob B (200 µM) and DAPI (4.4 µM) at RT for 5 minutes. (A) Bright-field image (B) labeled with DAPI, (C) labeled with Cy3-Nob B and (D) overlay image.
All images were taken at 40x magnification using Evos FL Cell Imaging System.
Scale bar = 100 µm.

Image credits:
Malem S. Flores (UP Institute of Biology)



857 marine snails of genus *Conus* distributed throughout tropical and subtropical waters

Around **50-200** components in the venom of each species

WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017-04-24. doi:10.14284/170

Akondi et al. (2014). *Chem. Rev.* 114, 5815–5847.

Discovery and Development of Health Products (DDHP) Marine Component

PRIALT® (Ziconotide)

- Only FDA approved conotoxin
- ω-conotoxin MVIIA from *Conus magus*
  - 25 residues, MW: 2637.10 Da
- Mediates its activity through $Ca_V2.2$ channels
- Cannot cross the blood brain barrier
  - Administered intrathecally
- Side effects
  - Whole body shakes driven by possible cerebellar motor defects in rats
  - Hallucinations in humans
- Remains as last resort for chronic pain sufferers

Jazz Pharms International

CKGKGAKCSRLMYDCCTGSCRSGKC(NH_2)

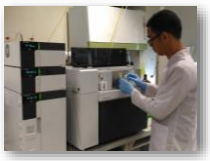
Conus magus

Wermeling DP (2005) *Pharmacotherapy* 25(8):1084–1094

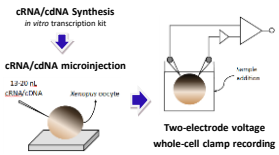
Discovery and Development of Health Products (DDHP) Marine Component

Aapptec Focus XC Peptide Synthesizer

Electrophysiology Work Station (MSI, UP Diliman)



Shimadzu Protein/Peptide Sequencer-31B



Discovery and Development of Health Products (DDHP) Marine Component

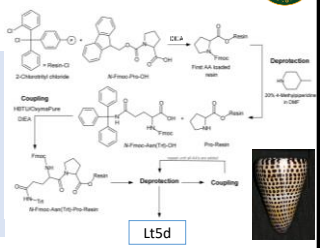
The total synthesis of the ff. peptides has been undertaken

PEPTIDE	SOURCE ORGANISM	BASIS OF SYNTHESIS	SEQUENCE	MASS (Da)	MOLECULAR TARGET
KIIIa	*Conus kinoshitai*	from Conoserver	CCNCSSKWCRDHSKCC(NH_2)	**1882.68**	Na^+ channel
Lt5d	*Conus litteratus*	from Conoserver	DCCPAKLICCNP	**1274.49**	Na^+ channel
Cstri26-13	*Conus striolatus*	isolated	X_2-CC-X_5-C-X_5-C-X_5-CC	**2434.97**	K^+ channel
Contulakin -Ro1	*Conus rolani*	isolated	X_4-C-X_4-C-X_3	**1572.64**	-
CsterL1062 16-1	*Conus stercusmuscarum*	isolated	C-X_6-C-X_6-CC-X_3-C-X_6-C	**2847.19**	-

Discovery and Development of Health Products (DDHP)
Marine Component

Lt5d

- Organism: *Conus litteratus*
- Gene superfamily: T Superfamily
- Cysteine framework: V (CC-----CC)
- Pharmacological family: μ
- Molecular weight: 1274.49 Da
- Isolated but not yet chemically synthesized
- Dose-dependent inhibition of TTX-sensitive sodium currents (IC_{50}=156.16 nM) shown by electrophysiology using adult dorsal root ganglion (DRG)



Lt5d

❖ Conotoxins are translated from mRNA as peptide precursors and cDNA sequencing is now the primary method for identification of new conotoxin sequences

Lt5d Precursor Sequence
(from transcriptome data)

MRCLPVFIILLLLISASVDAQPTKDDVPLA
SLHDNAKR ALQMFWNKR**DCCPAKLLCCNP**

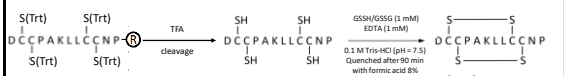
Signal sequence (underlined), Mature peptide (in bold)

Liu, J. et al. (2007). *Peptides* 28:2313-2319
Kaas, Q. et al. *Nucleic Acids Research* (2012) 40 (Database issue):D325-30
Kaas, Q. et al. *Bioinformatics* (2008) 24(3):445-6.

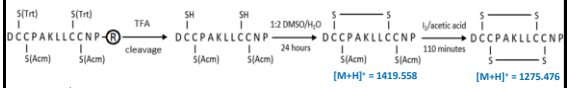
Discovery and Development of Health Products (DDHP)
Marine Component

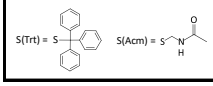
Lt5d Folding Strategies

Glutathione-Assisted Oxidative Folding



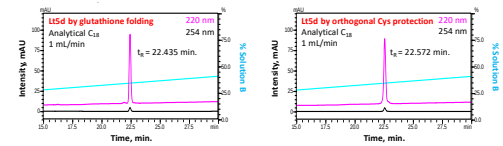
Orthogonal Cysteine Protection

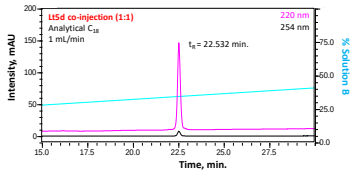




MS Data c/o Monci de Boda using Waters Xevo G2-XS QTof MS

Discovery and Development of Health Products (DDHP)
Marine Component



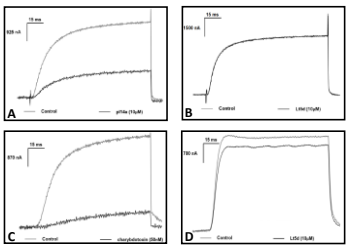


Analytical C_{18} column
15% to 45% Solvent B over 35 minutes at 1 mL/min flow rate

Solvent A: 0.1% TFA in H_2O
Solvent B: 90% MeCN in H_2O

Discovery and Development of Health Products (DDHP)
Marine Component

No significant inhibitory effects of Lt5d were observed on potassium channel subunits $K_v1.3$ (B) and $K_v1.6$ (D) as compared to known $K_v1.3$ (A) and $K_v1.6$ (C) blockers.



Representative positive currents recorded before (control trace, gray) and following exposure to 10 μM Lt5d or positive control (black trace). Each trace represents the average of two responses.

Credits to Iris Bea L. Ramiro from MSI, UP Diliman

Summary

- Marine environments: a very rich resource for new bioactive compounds
- Marine natural products: new carbon skeletons for synthesis of potentially more active, selective compounds
- Assays: antimicrobial, cytotoxic, neuroactive
- Synthetic capability: access new structures for SAR study
- Databases: aid in the screening and selection of compounds to pursue

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Discovery and Development of Health Products (DDHP) Marine Component

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