


# New Gram-negative Antibacterial Agents obtained from a Screening of Fundacion MEDINA's Microbial Natural Products Collection

Fundación MEDINA, Granada

Caridad.diaz@medinaandalucia.es

06-08-2015

# Antibiotic resistance threats and the urgent need for new antibiotics

**Core actions to fight the spread of antibiotic resistance**:

- Preventing infections and preventing resistant bacteria from spreading
- Tracking resistant bacteria
- Improving the use of antibiotics
- **Promoting the development of new antibiotics and diagnostic tests for resistant bacteria**

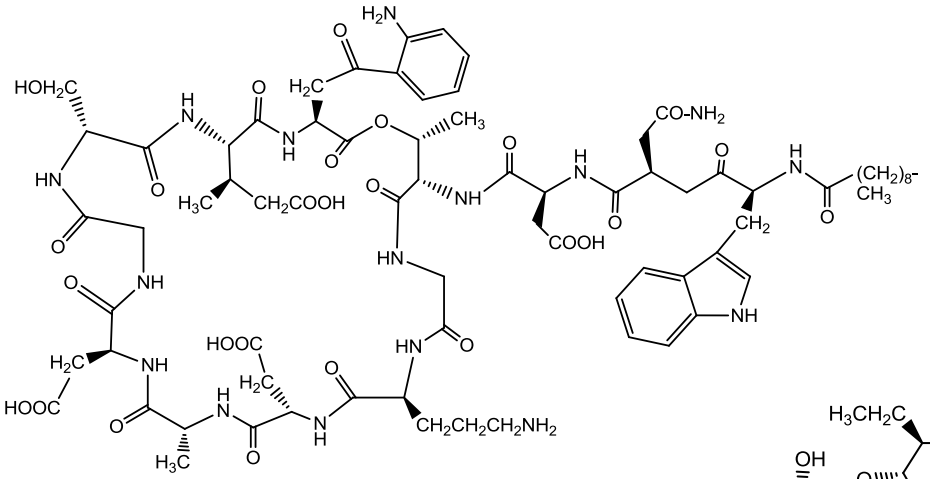
# Recent improvements in the Antibiotic Research landscape

- **US Generating Antibiotic Incentives Act** (GAIN act): automatic priority review and an additional 5–7 years of market exclusivity for qualified infectious disease products)
- **Infectious Diseases Society of America** (2010): Initiative to develop 10 new safe and efficacious systemic antibiotics by 2020.
- **Public–private partnership US government Biomedical Advanced Research and Development Authority (BARDA) - GSK** to study potential new drugs to treat both conventional pathogens and potential bio weapons
- **Innovative Medicines Initiative New Drugs for Bad Bugs** (IMI ND4BB):
  - support the clinical development of new antibiotics
  - basic research in Gram-negative bacteria drug penetration

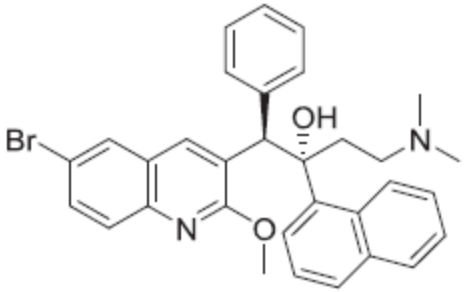


# Five new antibiotic classes in the clinic after 2000

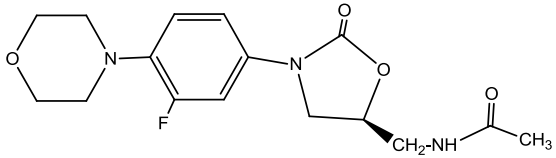
## Limited to treat Gram positive infections



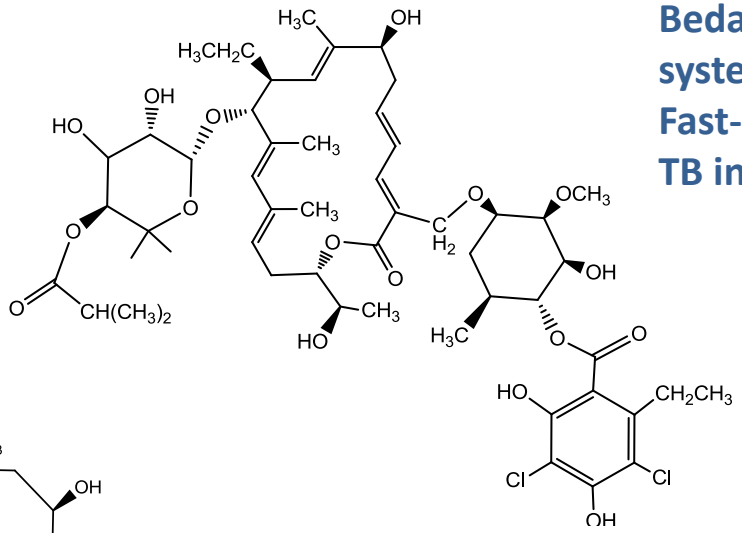
**Daptomycin, 2003**
**Systemic, Gram+**



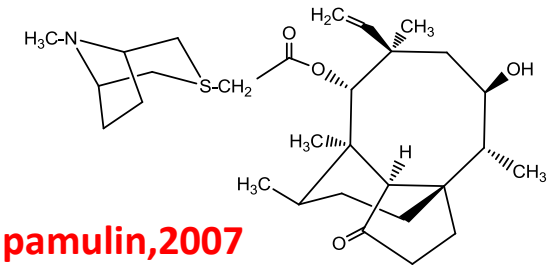
**Bedaquiline**
**systemic, 2012**
**Fast-track to treat TB in combination**



**Linezolid, 2000**
**Systemic, Gram+**



**Fidaxomicin**
***C.difficile* infections, 2010**



**Retapamulin,2007**
**topical, Gram+**

# Natural Products: Untapped sources of novel drugs

## Pivotal Role of Natural Products in modern Drug Discovery

- **Unique chemical space** compared to synthetic libraries
- **Potency and selectivity** derived from extended evolution selection
- **Underexplored** microbial sources of novel compounds
- **Outstanding scaffold starting points** for new drug candidate development
- **Privileged structures**: excellent templates for the synthesis of novel, biologically active, natural product-like molecules

***53% of new chemical entities***
*in the last 20 yrs are **naturally derived** or related with natural product scaffolds*
(Newman and Cragg, 2012)



# Current Discovery of new drugs from Natural Products

- Reduced interest of big pharmas in the last decade to invest internally in microbial natural products discovery programs that were finally abandoned.
- Decades of industrial experience and resources ( extract collections and microbial strains) were lost or disseminated in many cases.
- Natural Products research is currently pursued by academic groups or small biotechs.
- Renovated interest in Natural Products: inspirational source of novel scaffolds



# MEDINA:
# Microbial Natural Products Drug Discovery

**Non-profit Research Organization**

**Private-Public Partnership** between:

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



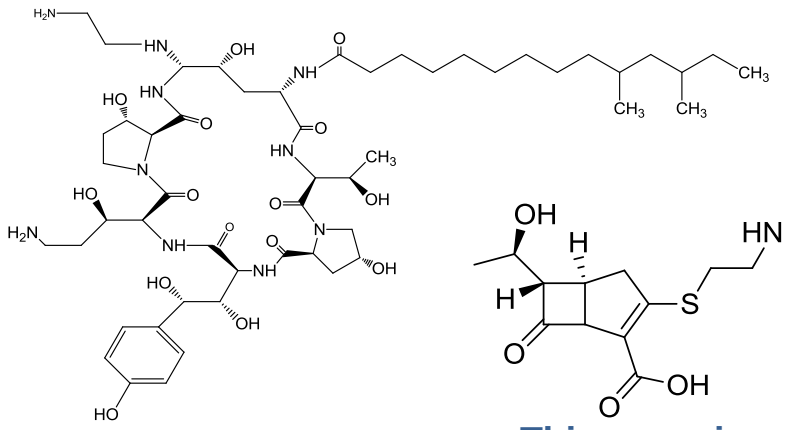
Health Sciences Technology Park, Granada, Spain

**Our Mission**

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



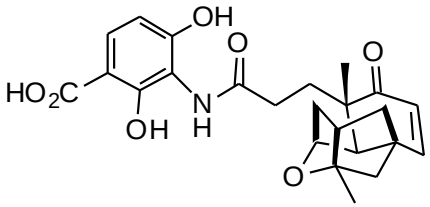
# Natural product antimicrobials, clinical candidates and leads



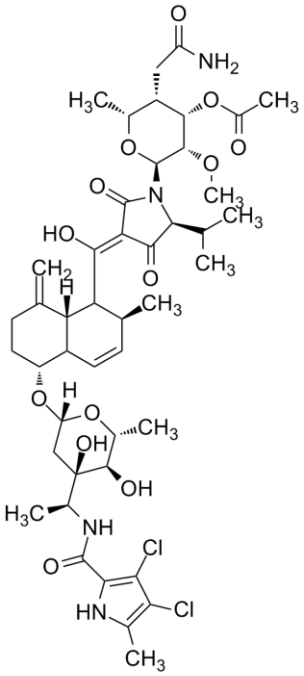
**Caspofungin (Cancidas, Merck)**



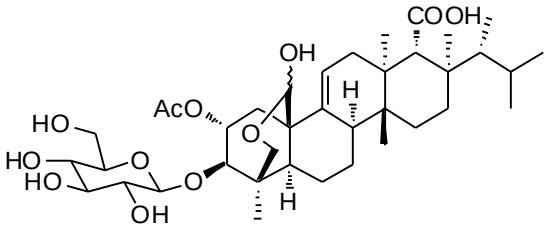
**Thienamycin (Imipenem, Merck)**



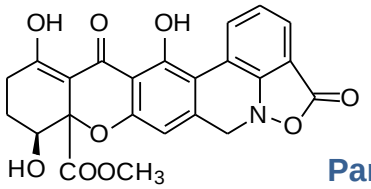
**Platensimycin**, antibacterial
Fatty acid synthesis inhibitor

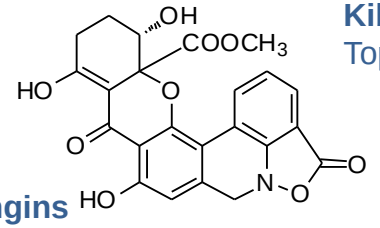


**Kibdelomycin,** antibacterial
Topo II isomerase inhibitor



**Enfumafungin**, antifungal





**Parnafungins**
mRNA polyadenylation inhibitors

Molecules derived from MSD (now MEDINA ) collections



# Technology Platforms for NPs Drug Discovery

- 50 yrs expertise in Drug Discovery
- 5 Drugs in the market
- Collaborations with industry & academia

**MEDiscovery**

| MEDscreen | MEDchemistry | MEDmicro | MEDanalytics | MEDpreclinical |
|---|---|---|---|---|
| ◆High Throughput Screening<br>◆High Content Bioimaging | ◆ Isolation of Natural Products<br>◆Analytical Chemistry Services<br>◆ Structural Elucidation | ◆Microbiology<br>◆Fermentation<br>◆Access to genomes | ◆Bioanalysis<br>◆Metabolomics | ◆Toxicology profiling & functional screening<br>◆Translational / Clinical R&D support |



# Gram negative antibacterial programs

***Sources of novel compounds***:
MEDINA Natural Product Libraries of extracts
(actinomycetes and filamentous fungi )

***Primary Targets:***

- **Broad spectrum Gram negative antibiotics** targeting multidrug resistant strains:
  *P. aeruginosa, A. baumannii, E.coli and K.pneumoniae*
- **Synergists of carbapenem activity**

***Empirical Screening approach***:
Whole cell liquid assays with growth inhibition as end-points (absorbance and fluorescence)



# Discovery of Gram negative broad spectrum antibiotics

MEDiversity

Extracts & fractions collections from microbial fermentations

**High Throughput Screening:**
**Empirical assays on panels of key pathogens**

Hit Confirmation & LC/MS dereplication
Hit Selection

Bioassay-guided Isolation

Structural Elucidation

HPLC-MS & NMR

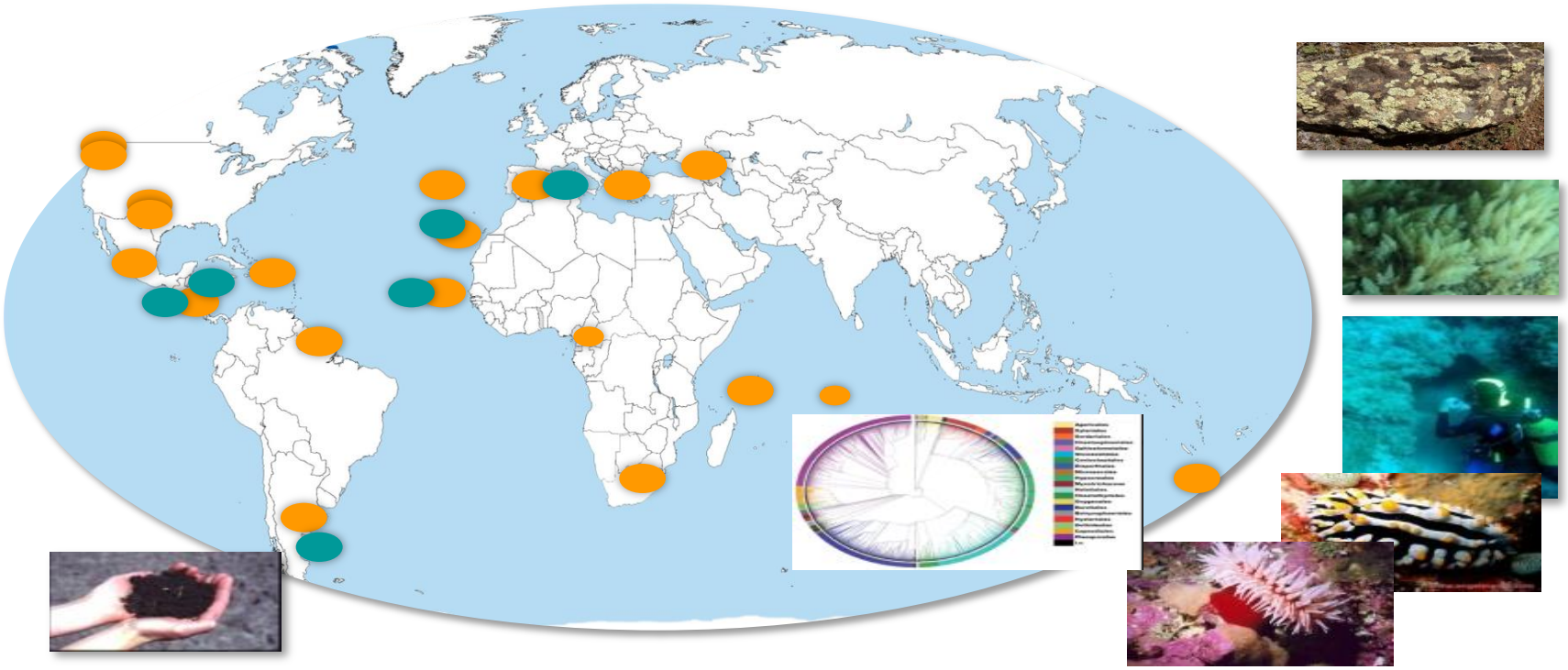
Novel Compound

3-6 months



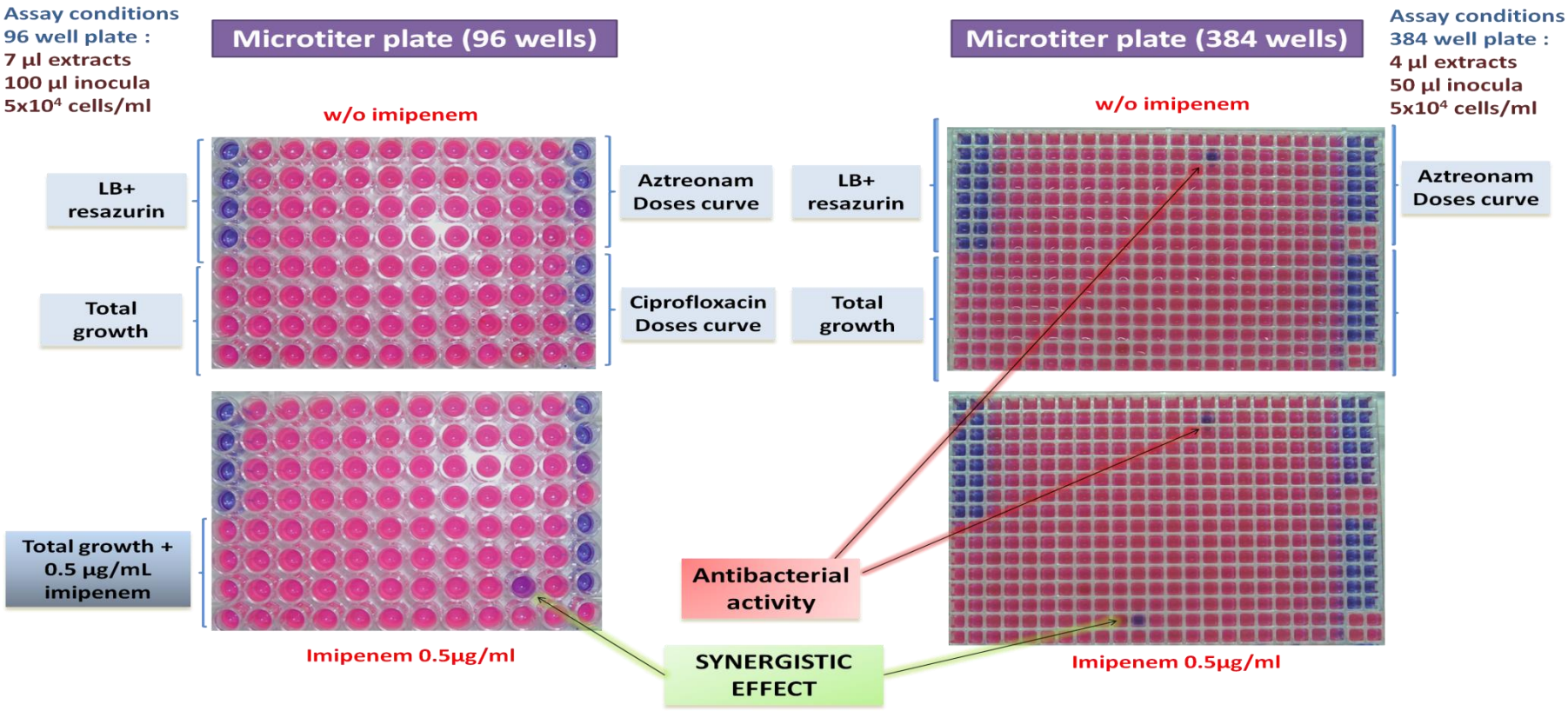
# Natural Products Collection

**Screen Strain Collections for Natural Products Discovery** (MEDINA collection harbors more than 130,000 Microbial Strains)



# High throughput screening approach

**In this liquid growth inhibition assay, rezasurin, is used for identification of antibacterial activity. Rezasurin is a non-fluorescent blue dye that by reduction is converted to the pink colored highly red fluorescent resorufin. Each extract is tested with and without the sublethal dose of imipenem allowing the identification of extracts containing antibacterial activity as well as the extracts presenting synergistic effect with imipenem.**



# High Throughput Antimicrobial screening

NP Library: subset of 28,500 extracts

Primary Screening on *P. aeruginosa* and *A. baumannii*

>70% inhibition

68 Primary Hits

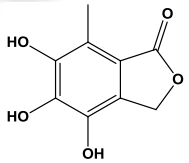
Early LC/MS Dereplication: known compounds

Discovery of novel scaffolds

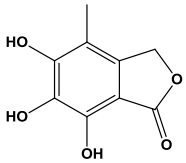
17 compounds



# Novel compounds from fungal endophytes



**MDN-0075**
4,5,6-Trihydroxy-7-methylphthalide;
Epicoccone;

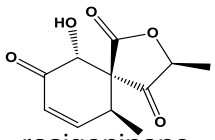


**MDN-0076**
4,5,6-Trihydroxy-7-methyl-3H-isobenzofuran-1-one

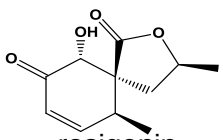
Known as potent antioxidants

**MDN-0075**
**MIC50**
A. *baumannii* 8-16 ug/ml
*P. aeruginosa* < 16 ug/ml

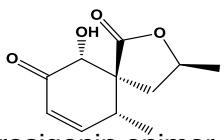




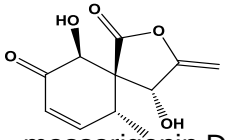
rosigeninone



rosigenin



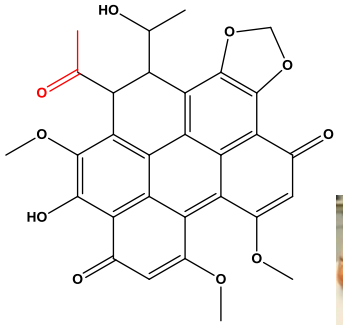
rosigenin epimer



massarigenin D

*A.baumannii* **MIC50** 8-16 ug/ml / **NEW**
Previous activity described against *S.aureus*



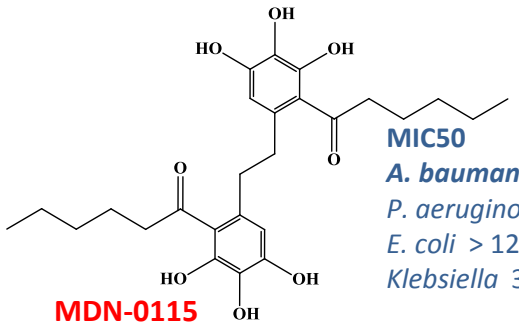


Analog of Eisinochrome D

**MIC50**
*A. baumannii* 40-80 ug/ml
*P. aeruginosa* 160 ug/ml
*E. coli* > 128 ug/ml



**MDN-0119**



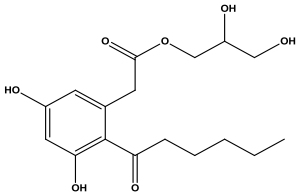
**MDN-0115**

**MIC50**
***A. baumannii* 8-64 ug/ml**
*P. aeruginosa* 64-128 ug/ml
*E. coli* > 128 ug/ml
*Klebsiella* 32-64 ug/ml



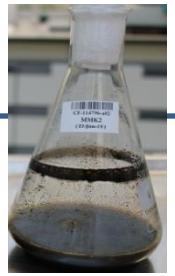
**MDN-0114**

**MIC50**
*A. baumannii* 8-64 ug/ml
*P. aeruginosa* > 128 ug/ml
*E. coli* > 128 ug/ml
*Klebsiella sp.* 32-64 ug/ml



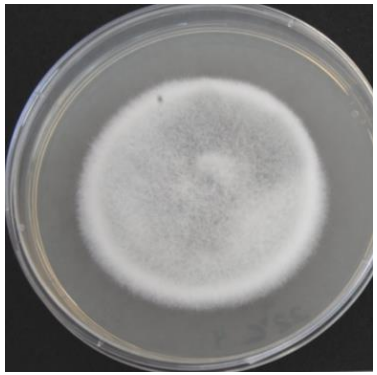
**MDN-0116**

**MIC90**
*A. baumannii* > 128 ug/ml
*P. aeruginosa* > 128 ug/ml
*E. coli* > 128 ug/ml
*Klebsiella* ND

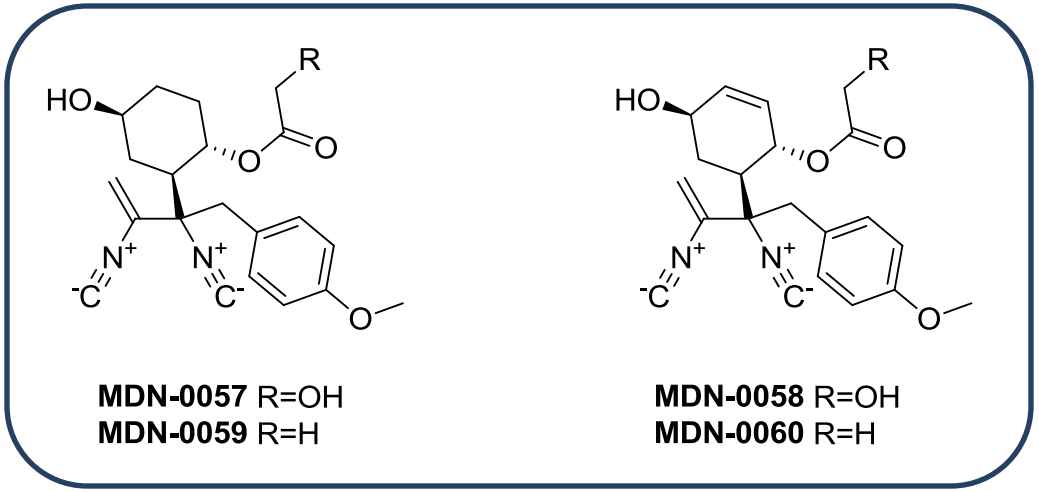




# A Novel Family of Natural Product Antibiotics with Broad Spectrum activity against Gram-negative Pathogens








New family of novel natural products of fungal origin.

- **Family of 4 related compounds** (MDN-0057, -0058, -0059 and -0060) with MW ranging from **366 to 384 Da**)
- Unique novel, previously unreported chemical scaffold , not similar to known antibiotics
- MIC values against wild type strains of *E. coli*, *P. aeruginosa*, and *A. baumannii* of 0.25-16 µg/mL
- Produced in liquid and solid media by **fungal strains** in titers ranging from 2 to 25 mg/L

# Antimicrobial profile of MDN-0057-0060

| | | MIC (µg/mL) | | | |
|---|---|---|---|---|---|
| **STRAINS** | **DESCRIPTION** | **MDN-0057** | **MDN-0058** | **MDN-0059** | **MDN-0060** |
| *E.coli* ATCC 25922 | wild type | **16-64** | 16 | nd | nd |
| *E.coli* 219 | ΔAcrAB::Tn903kan | **0.125-0.5** | 0.25-0.5 | nd | nd |
| *A.baumannii* ATCC BAA-747 | Clinical isolate | **2-4** | **1-16** | nd | nd |
| *A.baumannii* ATCC 19606 | Clinical isolate | **2-4** | **2-8** | nd | nd |
| *A.baumannii* 2443 | Clinical isolate | **4** | 2 | nd | nd |
| *A.baumannii* 2444 | ΔadeIJK | **0.25** | 0.25-1 | nd | nd |
| *A.baumannii* 2445 | ΔadeABC | **2** | **2-8** | nd | nd |
| *A.baumannii* 2446 | ΔadeABC, ΔadeIJK | **<0.06** | <0.06 | nd | nd |
| *A.baumannii* 5973 | Clinical isolate | **0.25** | 0.25 | 0.5-1 | **1-2** |
| *P.aeruginosa* ATCC 27853 | wild type | **2-8** | 64 | 16 | 32-64 |
| *P.aeruginosa* 839 | ΔMexAB-OprM | **1-2** | **1-4** | nd | nd |
| *K.pneumoniae* 847 | Clinical isolate | **64** | 64 | nd | nd |
| *K.pneumoniae* 21 | permeable strain | **0.25** | 0.5 | nd | nd |

# ENABLE_MEDINA Hit to Lead Program: MEDINA's novel antibiotics MDN-0057 – MDN-0060

• MEDINA compounds MDN-0057 –0060 were selected for preclinical hit to lead development within **ENABLE anti-bacterial drug discovery platform**.

• Involves a multidisciplinary core team from the University of Uppsala (Sweden), SERMAS (Spain), CNB-CSIC (Spain), University of Liège (Belgium), Asclepia (Belgium), and MEDINA (Spain)

•**H2L development program** focused primarily on:

- Generation of improved medicinal chemistry series
- MOA determination and potential target
- PK studies for efficacy of mice models infections.



MEDINA

www.medinadiscovery.com

Centro de Excelencia en Investigación de Medicamentos Innovadores en Andalucía



# THANK YOU FOR YOUR ATTENTION

**Caridad.diaz@medinaandalucia.es**

Avda Conocimiento 3, Health Sciences Technology Park - 18016 Granada, SPAIN