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

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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


- **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+
- **Fidaxomicin**
  - C. difficile infections, 2010
- **Linezolid**, 2000
  - Systemic, Gram+
- **Retapamulin**, 2007
  - topical, Gram+
- **Bedaquiline**
  - Systemic, 2012
  - Fast-track to treat TB in combination

**Fidaxomicin**

**Bedaquiline**

**Daptomycin**, 2003

**Linezolid**, 2000

**Retapamulin**, 2007
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
Non-profit Research Organization

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 needs*
Natural product antimicrobials, clinical candidates and leads

Caspofungin (Cancidas, Merck)

Thienamycin (Imipenem, Merck)

Enfumafungin, antifungal

Platensimycin, antibacterial
Fatty acid synthesis inhibitor

Kibdelomycin, antibacterial
Topo II isomerase inhibitor

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

- High Throughput Screening
- High Content Bioimaging
- Isolation of Natural Products
- Analytical Chemistry Services
- Structural Elucidation
- Microbiology
- Fermentation
- Access to genomes
- Bioanalysis
- Metabolomics
- Toxicology profiling & functional screening
- 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:
  \[ P.\ aeruginosa, A.\ baumannii, E.\ coli \text{ and } K.\ pneumoniae \]
• 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

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

3-6 months

Novel Compound
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*

68 Primary Hits

Early LC/MS Dereplication: known compounds

17 compounds

Discovery of novel scaffolds

>70% inhibition
Novel compounds from fungal endophytes

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

Known as potent antioxidants

MDN-0075
4,5,6-Trihydroxy-7-methylphthalide; Epicoccene;

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

rosigeninone
rosigenin
rosigenin epimer
massarigenin D

A. baumannii MIC50 8-16 ug/ml
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-0075
MIC50
A. baumannii 8-64 ug/ml
P. aeruginosa 64-128 ug/ml
E. coli > 128 ug/ml
Klebsiella 32-64 ug/ml

MDN-0115

MDN-0114

MDN-0116

MDN-0119

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

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

<table>
<thead>
<tr>
<th>STRAINS</th>
<th>DESCRIPTION</th>
<th>MIC (µg/mL) MDN-0057</th>
<th>MIC (µg/mL) MDN-0058</th>
<th>MIC (µg/mL) MDN-0059</th>
<th>MIC (µg/mL) MDN-0060</th>
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<tbody>
<tr>
<td><em>E. coli</em> ATCC 25922</td>
<td>wild type</td>
<td>16-64</td>
<td>16</td>
<td>nd</td>
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<tr>
<td><em>E. coli</em> 219</td>
<td>ΔAcrAB::Tn903kan</td>
<td>0.125-0.5</td>
<td>0.25-0.5</td>
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<td>nd</td>
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<tr>
<td><em>A. baumannii</em> ATCC BAA-747</td>
<td>Clinical isolate</td>
<td>2-4</td>
<td>1-16</td>
<td>nd</td>
<td>nd</td>
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<tr>
<td><em>A. baumannii</em> ATCC 19606</td>
<td>Clinical isolate</td>
<td>2-4</td>
<td>2-8</td>
<td>nd</td>
<td>nd</td>
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<tr>
<td><em>A. baumannii</em> 2443</td>
<td>Clinical isolate</td>
<td>4</td>
<td>2</td>
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<td>nd</td>
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<tr>
<td><em>A. baumannii</em> 2444</td>
<td>ΔadelIJK</td>
<td>0.25</td>
<td>0.25-1</td>
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<td>nd</td>
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<tr>
<td><em>A. baumannii</em> 2445</td>
<td>ΔadeABC</td>
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<tr>
<td><em>A. baumannii</em> 2446</td>
<td>ΔadeABC, ΔadelIJK</td>
<td>&lt;0.06</td>
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<td><em>A. baumannii</em> 5973</td>
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<td>0.25</td>
<td>0.25</td>
<td>0.5-1</td>
<td>1-2</td>
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<td><em>P. aeruginosa</em> ATCC 27853</td>
<td>wild type</td>
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<td>32-64</td>
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<td><em>P. aeruginosa</em> 839</td>
<td>ΔMexAB-OprM</td>
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<td><em>K. pneumoniae</em> 847</td>
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<td><em>K. pneumoniae</em> 21</td>
<td>permeable strain</td>
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</tr>
</tbody>
</table>
• 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.
THANK YOU FOR YOUR ATTENTION

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