Human subtlety will never devise an invention more beautiful, more simple, or more direct than does Nature—because in her inventions, nothing is lacking—and nothing is superfluous. . .

Leonardo da Vinci
Antibiotic resistance

- Bacterial resistance to conventional antibiotics is one of the most serious problems facing world health today.
- Each year in the United States, at least 2 million people become infected with bacteria that are resistant to antibiotics and at least 23,000 people die each year as a direct result of these infections.
- Antibiotic-resistant infections can happen anywhere. Data show that most deaths related to antibiotic resistance happen in healthcare settings such as hospitals and nursing homes.

<table>
<thead>
<tr>
<th>Antibiotic-Resistant Bacteria</th>
<th>Estimated Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methicillin/S. aureus</td>
<td>102,000</td>
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<tr>
<td>Methicillin/CMN5</td>
<td>130,000</td>
</tr>
<tr>
<td>Vancomycin/enterococci</td>
<td>26,000</td>
</tr>
<tr>
<td>Ceftazidime/P. aeruginosa</td>
<td>12,000</td>
</tr>
<tr>
<td>Ampicillin/E. coli</td>
<td>65,000</td>
</tr>
<tr>
<td>Imipenem/P. aeruginosa</td>
<td>16,000</td>
</tr>
<tr>
<td>Ceftazidime/K. pneumoniae</td>
<td>11,000</td>
</tr>
</tbody>
</table>

*Selected resistant bacteria, U.S., 2002

Treats pneumonia; meningitis; skin, bone, joint, stomach, blood, and heart valve infections.

Treats pneumonia; acne; infections of respiratory tract, genitai, urinary systems; and stomach ulcers.

Treats bronchitis; diphtheria; Legionnaires’ disease; whooping cough; pneumonia; rheumatic fever; venereal disease; and many others.

Treats staph infections.

Treats infections of lung, skin, bone, joint, stomach, blood, and urinary tract.

Treats colitis.

Treat infections of lung, skin, bone, joint, stomach, blood and gynecological and urinary tracts.

Treats pneumonia, chronic bronchitis and sinus, urinary tract, kidney, prostate, and skin infections.

Treat pneumonia, and infections of the skin and blood.

Injection is used to treat some types of skin infections and pneumonia.

*Penicillin-resistant Staphylococcus appeared in 1940, three years before widespread use of the drug.
Antimicrobial Peptides: What are they?

- Gene-encoded, ribosomally synthesized antimicrobial peptides (AMPs), are an ancient and ubiquitous component of innate defense, found in bacteria, protozoa, plants, and animals ranging from insects to fish, amphibians and mammals.

- In the last two decades, several hundreds of peptides have been isolated in almost all groups of animals.

Hancock, R. E. W.; Sahl, H. G. Nature Biotechnol. 2006, 24, 1551–1557
AMPs Structure

- AMPs molecules are composed of hydrophilic, hydrophobic and cationic amino acids arranged in a molecule that can organize into an amphipathic structure.

Mechanism of Action

- Mechanisms of antimicrobial peptides: (A) **barrel-stave**, (B) **carpet (detergent-like)**, and (C) **toroidal pore (wormhole)**.
- The outer mammalian cell membranes is mainly comprises phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, and cholesterol, which are charge-neutral at physiological pH. The surfaces of both gram-negative and gram-positive bacterial cell walls contain large amounts of negatively charged lipids.
Mechanism of Action

- The frequent occurrence of positively charged residues is an important feature of lytic peptides. It is thought to help the peptide to reach its target, which for most AMPs is believed to be the cytoplasmic membrane.

- This involves binding to LPS and teichoic acid by the displacement of divalent cations, such as $\text{Mg}^{2+}$ and $\text{Ca}^{2+}$, that are essential for the stability of the cell surface and cross-bridging the negative charges of LPS.
Comparison of the Gram positive and Gram negative bacterial cell walls
# AMPs in current clinical studies

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Company</th>
<th>Description</th>
<th>Application</th>
<th>Trial phase</th>
<th>Comments</th>
<th>Clinical trial identifiers and further information</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMX-30063</td>
<td>Structure not disclosed</td>
<td>PolyMedix</td>
<td>Arylamide oligomer mimetic of a defensin</td>
<td>Acute bacterial skin infections caused by <em>Staphylococcus</em> spp.</td>
<td>II</td>
<td>Mimetic rather than peptide; currently in Phase II trials</td>
<td>NCT01211470; PolyMedix website</td>
</tr>
<tr>
<td>Delmitide (RDP58)</td>
<td>RXXXRX XXGY (X = norleucine)</td>
<td>Genzyme</td>
<td>Semisynthetic d-amino acid decapeptide derived from HLA class I B2702</td>
<td>Inflammatory bowel disease</td>
<td>II (completed)</td>
<td>A protease-resistant, d-amino acid-containing peptide with similar efficacy to asacol; attempting to improve activity through formulation</td>
<td>Genzyme website; ISRCTN84220089</td>
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<tr>
<td>Plectasin222</td>
<td>GFGC3NG PWDEDDB MQC3HNH C3KS3GYK GGYC3AKG GFVC3KC3Y</td>
<td>Novozymes</td>
<td>Fungal defensin; candidate in development is an amino-acid substitution variant</td>
<td>Bacterial diseases</td>
<td>Pre-clinical</td>
<td>Excellent efficacy demonstrated in animal models</td>
<td>Novozymes website</td>
</tr>
<tr>
<td>HB1345</td>
<td>Decanoyl-KFKWPW</td>
<td>Helix BioMedix</td>
<td>Synthetic lipohexapeptide</td>
<td>Acne; broad-spectrum antibiotic</td>
<td>Pre-clinical</td>
<td>Looks promising as this is a very small lipopeptide</td>
<td>Helix BioMedix website</td>
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<tr>
<td>Pexiganan acetate</td>
<td>GiGKFLKK AKKFGKAF VKILKK</td>
<td>MacroChem</td>
<td>Synthetic analogue of magainin 2 derived from frog skin</td>
<td>Topical antibiotic</td>
<td>III</td>
<td>No advantage demonstrated over existing therapies</td>
<td>NCT00563433 and NCT00563394</td>
</tr>
</tbody>
</table>
Examples

1.5-15 µM, E. coli

DeGrado, W. F., JACS 1999, 121, 12200

3.1-6.2 µM, E. coli

Hedrick, J. L, Nature Chemistry 2011, 3, 409

Mor, A., Nature Biotechnol. 2007, 25, 657

3-10 µg/mL, E. coli; 2-4 µg/mL S. eureus

Stensen, W., J. Med. Chem. 2011, 54, 5786

Marina Kovaliov 1/3/15
Design and Synthesis

![Chemical structures and formulas](image)

Series I
- Z: L-Lysine
- R:
  - C₂H₅: 1
  - C₄H₉: 2
  - C₆H₁₃: 3
  - C₈H₁₇: 4
  - C₁₀H₂₁: 5

Series II
- Z: L-Lysine
- R:
  - C₆H₁₃: 6
  - C₆H₁₇: 7
  - C₈H₁₇: 8
  - C₁₀H₂₁: 9
  - C₁₂H₂₅: 10

Series III
- Z: L-Lysine
- R:
  - C₆H₁₃: 11
  - C₆H₁₇: 12
  - C₈H₁₇: 13
  - C₁₀H₂₁: 14
  - C₁₂H₂₅: 15
  - C₁₄H₂₉: 16
Design and Synthesis

- No imposed structural rigidity
- Include an \( N \)-disubstituted or tertiary amide bond
- Only three synthetic steps

\[
\text{Ar} \quad \text{H} \quad \text{O} \\
\text{a. RNH}_2 \text{ in MeOH, 6h} \\
\text{b. NaBH}_4 \text{ in MeOH, 18h} \\
\text{c. HCl} \\
\text{R} \quad \text{N} \quad \text{H}_2 \quad \text{Cl} \\
\text{d. Boc-Lys(Boc)-OH, DIPEA,} \\
\text{HBTU in DMF/CHCl}_3, \text{ RT, 16-24h} \\
\text{e. TFA in DCM, 12h} \\
\text{Ar} \quad \text{R} \quad \text{N} \quad \text{O} \quad \text{NH}_2 \quad \text{TFA} \\
\text{Ar} \quad \text{R} \quad \text{N} \quad \text{O} \quad \text{NH}_2 \quad \text{TFA} \\
\text{Cl} \quad \text{Ant} \\
\text{Nap} \\
\text{Phe} \\
\text{Ar} = \text{Ant, R: C}_2\text{H}_5-\text{C}_{10}\text{H}_{21} \quad (1-5) \\
\text{Ar} = \text{Nap, R: C}_4\text{H}_9-\text{C}_{12}\text{H}_{25} \quad (6-10) \\
\text{Ar} = \text{Phe, R: C}_4\text{H}_9-\text{C}_{14}\text{H}_{29} \quad (11-16)
## Antibacterial Activity

<table>
<thead>
<tr>
<th>Compd</th>
<th>S. aureus</th>
<th>E. faecium</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
<th>MRSA</th>
<th>VRE</th>
<th>K. pneumoniae</th>
<th>HPC retention times (min)</th>
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<td>16–32</td>
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<td>8–16</td>
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</table>

*ND stands for “not determined”. †Literature values obtained from ref 36. ‡Indicates value for E. faecalis. §Literature value obtained from ref 28. VRE (vancomycin-resistant E. faecium) and MRSA (methicillin-resistant S. aureus), K. pneumoniae is resistant to β-lactam antibiotics.
Series I

**Hydrophobic bulk**
- Variable hydrophobic chain
- Tertiary Amide bond
- Cationic charge

**Series I**
- Z: L-Lysine
- R:
  - C$_2$H$_5$: 1
  - C$_4$H$_9$: 2
  - C$_6$H$_{13}$: 3
  - C$_8$H$_{17}$: 4
  - C$_{10}$H$_{21}$: 5

<table>
<thead>
<tr>
<th>compd</th>
<th>S. aureus</th>
<th>E. faecium</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
<th>MRSA</th>
<th>VRE</th>
<th>K. pneumoniae</th>
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<td>7.6</td>
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</tbody>
</table>

![Graph showing MIC values](chart.png)

**A**
- 30
- 25
- 20
- 15
- 10
- 5
- 0

**MIC (µg/mL)**
- S. aureus
- E. coli
- E. faecium

---

Marina Kovaliov 1/3/15
Series II

<table>
<thead>
<tr>
<th>Compd</th>
<th>S. aureus</th>
<th>E. faecium</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
<th>MRSA</th>
<th>VRE</th>
<th>K. pneumoniae</th>
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</table>

Vancomycin 0.87 µg/mL
Series III

Z: L-Lysine
R:
C₄H₉ : 11
C₆H₁₃ : 12
C₈H₁₇ : 13
C₁₀H₂₁ : 14
C₁₂H₂₅ : 15
C₁₄H₂₉ : 16

---

<table>
<thead>
<tr>
<th>compd</th>
<th>S. aureus</th>
<th>E. faecium</th>
<th>E. coli</th>
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</tbody>
</table>
Antibacterial Activity in Plasma and Enzyme Stability

- MIC of compound 7 against S. aureus in 50% blood plasma was 30 μg/mL, no loss of activity was observed in physiologically relevant time frame of 3 h.
- 7 was incubated with trypsin and showed that this type of compounds was not a substrate for protease.

Figure S66: Effect of human plasma on the antibacterial activity of compound 7 in three hours.
Mechanism of Action

- Experiments with membrane potential sensitive dye reviled that these compounds rapidly depolarize the membrane of both, Gram(+) and Gram(-) bacteria.
- Compounds 3 and 7 could cause permeabilization of Gram(+) and Gram(-) membrane at MIC concentrations.
Mechanism of Action

Figure 3. Fluorescence microscopy images of *S. aureus* (A) untreated and (B) treated with 3 (10 × MIC) for 1.5 h after staining with SYTO 9 and PI (scale: 20 μm). Scanning electron microscopy (SEM) images of (C) untreated *E. coli* and (D) *E. coli* treated with 3 (10 × MIC).
Conclusions

This report illustrates a systematic way of creating highly potent, broad-spectrum small molecular peptide mimics which emulate the efficiency of AMPs.

Prepared from inexpensive starting materials in only three steps, these compounds are selectively toxic toward bacterial cells (over mammalian cells) at very low concentrations.

Spectroscopic and microscopic studies reveal that depolarization and disruption of bacterial cell membranes are the primary mechanisms of their bactericidal action.

These promising compounds can be developed into a new class of antibiotics against multidrug resistant (MDR) bacteria.
penicillin

gentamicin

tetracycline

erythromycin

vancomycin

imipenem

methicillin

linezolid

ceftaroline

levofloxacin

ceftazidime