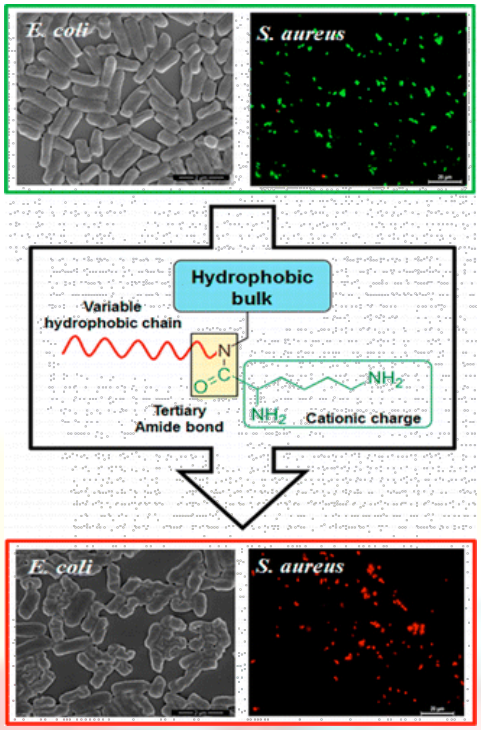


Human subtlety will never devise an invention more beautiful, more simple, or more direct than does Nature—because in her inventions, nothing is lacking—and nothing is superfluous. . .

Leonardo da Vinci

Small Molecular Antibacterial Peptoid Mimics: The Simpler the Better!

Chandradhish Ghosh, Goutham B. Manjunath, Padma Akkapeddi, Venkateswarlu Yarlagadda, Jiaul Hoque, Divakara S. S. M. Uppu, Mohini M. Konai, and Jayanta Haldar

Chemical Biology and Medicinal Chemistry Laboratory, New Chemistry Unit, Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Jakkur, Bengaluru 5600064, Karnataka, India

Marina Kovaliov 1/3/15

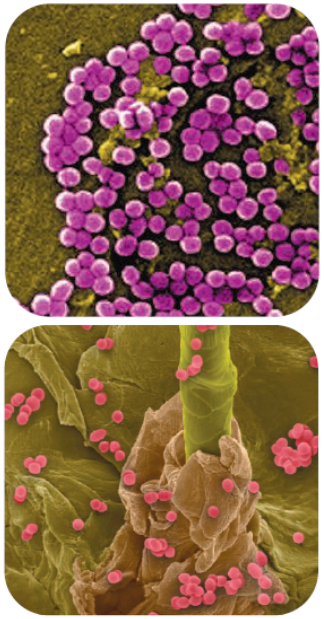
Antibiotic resistance

- 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.

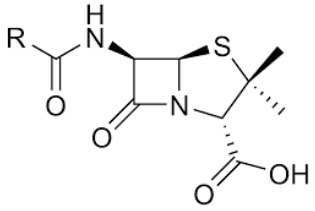
Estimated cases of hospital-acquired infections*	
Antibiotic-Resistant Bacteria	Estimated Cases
Methicillin/*S. aureus*	102,000
Methicillin/CNS	130,000
Vancomycin/enterococci	26,000
Ceftazidime/*P. aeruginosa*	12,000
Ampicillin/*E. coli*	65,000
Imipenem/*P. aeruginosa*	16,000
Ceftazidime/*K. pneumoniae*	11,000

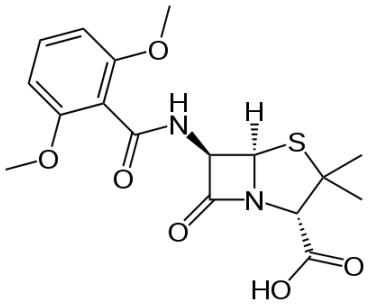
* Selected resistant bacteria, U.S., 2002

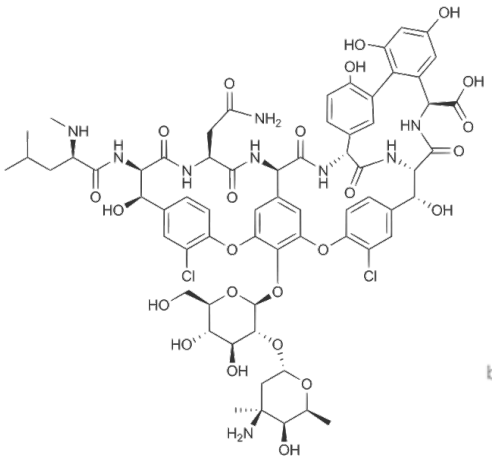
G. Taubes, Science 2008, 321, 356–361



Bad actors. Methicillin-resistant *S. aureus* (*above*) and vancomycin-resistant *Enterococcus*.





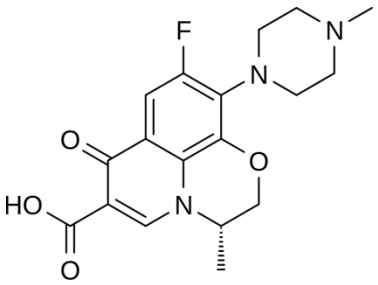


ANTIBIOTICS INTRODUCED

ANTIBIOTIC RESISTANCE IDENTIFIED

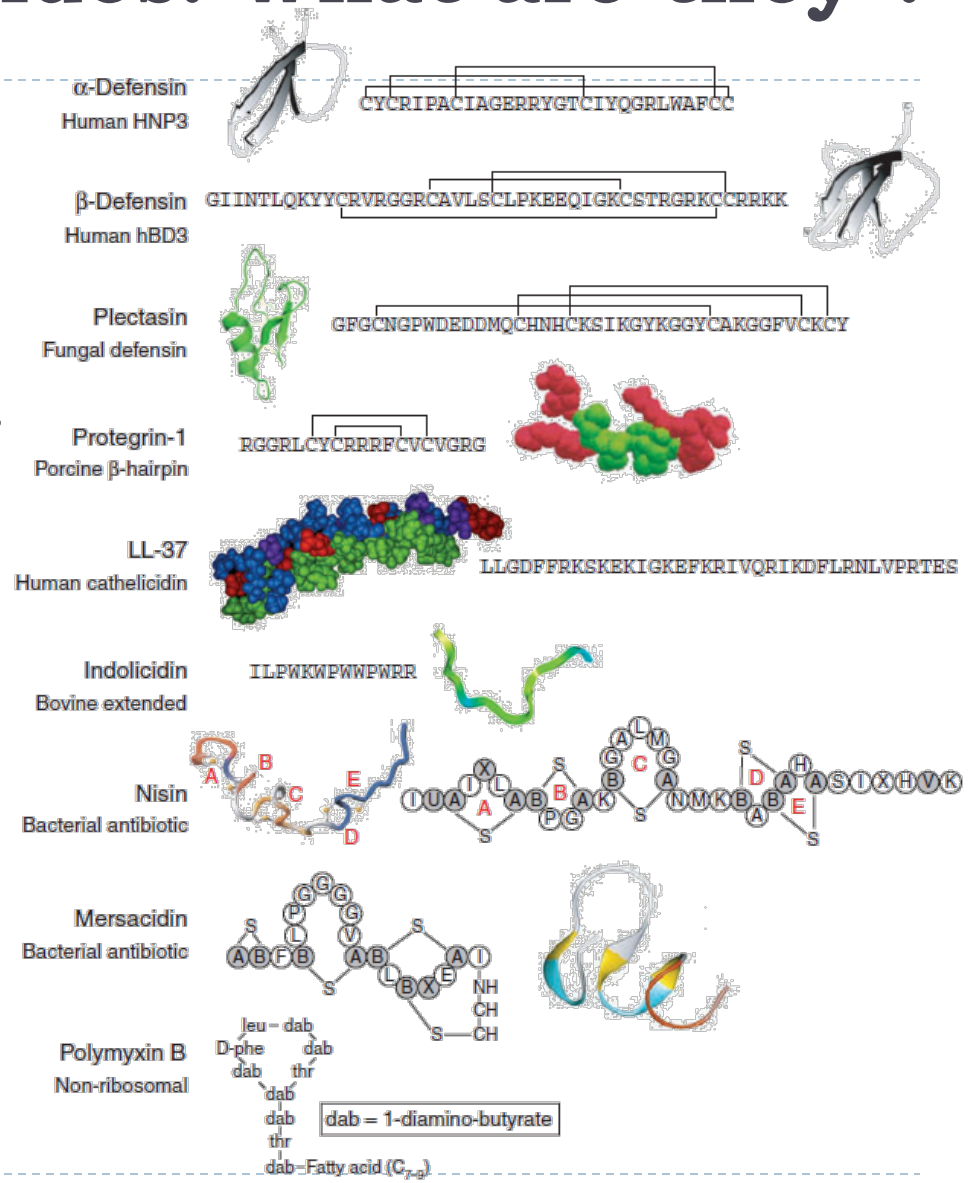
Treats pneumonia; meningitis; skin, bone, joint, stomach, blood, and heart valve infections.	*penicillin	1943	1959	tetracycline-R Shigella
Treats pneumonia; acne; infections of respiratory tract, genitals, urinary systems and stomach ulcers.	tetracycline	1950	1962	methicillin-R Staphylococcus
Treats bronchitis; diphtheria; Legionnaires' disease; whooping cough; pneumonia; rheumatic fever; venereal disease, and many others.	erythromycin	1953	1965	penicillin-R pneumococcus
Treats staph infections.	methicillin	1960	1968	erythromycin-R Streptococcus
Treats infections of lung, skin, bone, joint, stomach, blood, and urinary tract.	gentamicin	1967	1979	gentamicin-R Enterococcus
Treats colitis.	vancomycin	1972	1987	ceftazidime-R Enterobacteriaceae
Treat infections of lung, skin, bone, joint, stomach, blood and gynecological and urinary tracts.	imipenem and ceftazidime	1985	1988	vancomycin-R Enterococcus
Treats pneumonia, chronic bronchitis and sinus, urinary tract, kidney, prostate, and skin infections.	levofloxacin	1996	1996	levofloxacin-R pneumococcus
			1998	imipenem-R Enterobacteriaceae
Treats pneumonia, and infections of the skin and blood.	linezolid	2000	2001	linezolid-R Staphylococcus
			2002	vancomycin-R Staphylococcus
Injection is used to treat some types of skin infections and pneumonia.	ceftaroline	2010	2011	ceftaroline-R Staphylococcus

*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

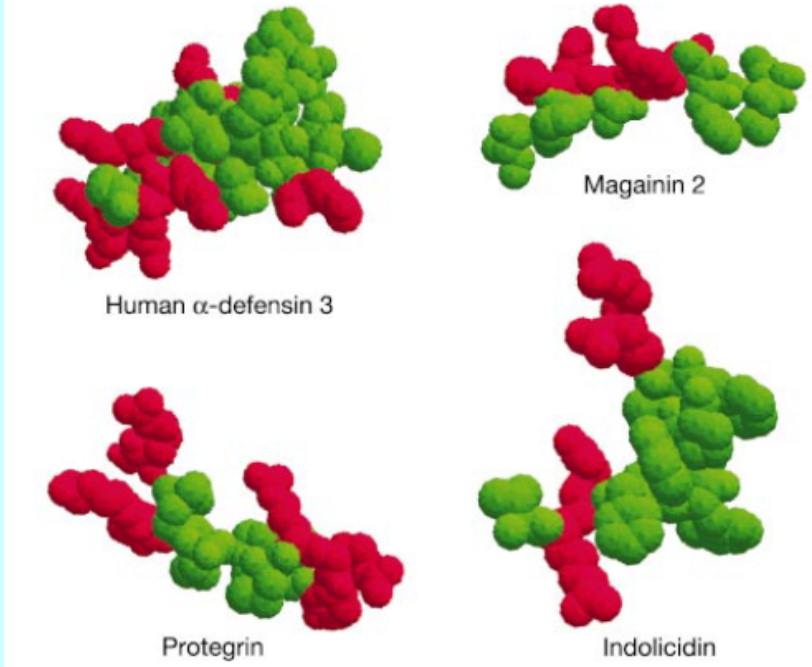
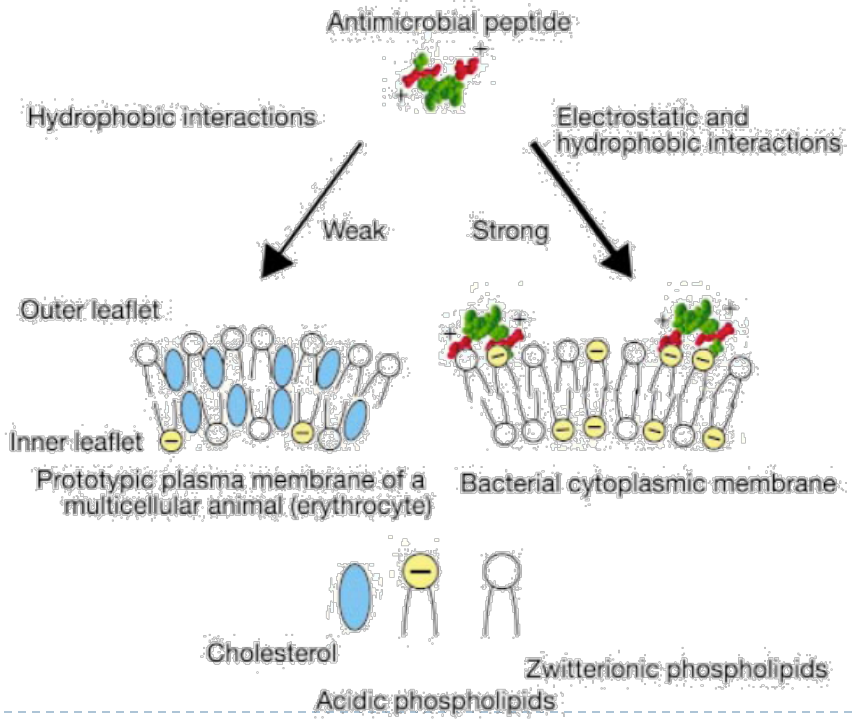


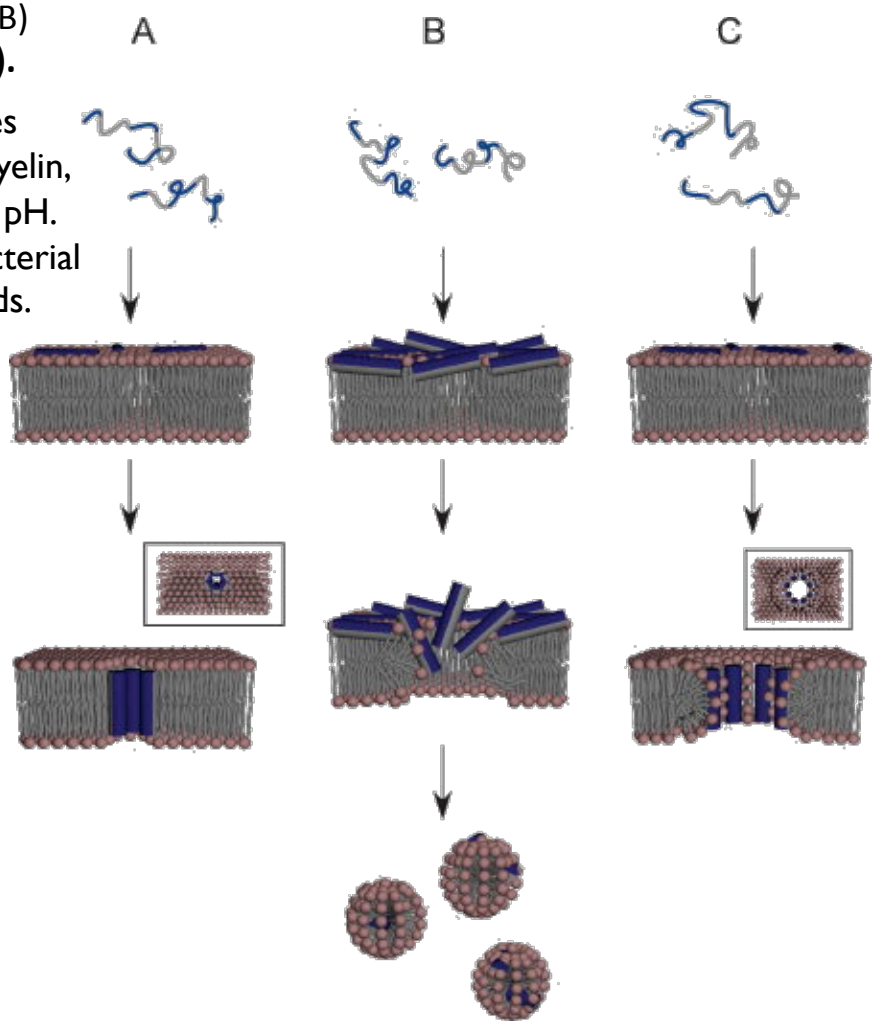
Figure 1 Clustering of cationic and hydrophobic amino acids into distinct domains in several antimicrobial peptides of different structural classes. This 'amphipathic' design is evident in many, but not all, antimicrobial peptides. Red, basic (positively charged) amino acids; green, hydrophobic ('oily') amino acids. Other amino acids are not shown. Magainin is depicted in its α-helical configuration.

Zasloff, M. Nature 2002, 415, 389–395

Mechanism of Action

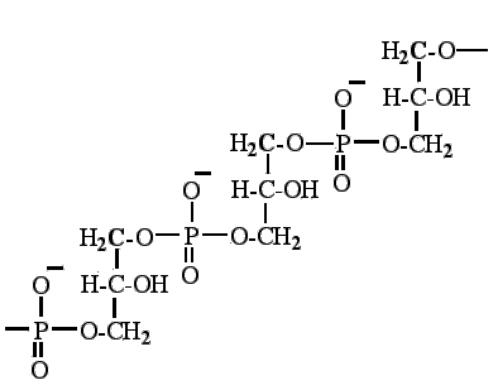
- Mechanisms of antimicrobial peptides: (A) **barrel-stave,** (B) **carpet (detergent-like),** and (C) **toroidal pore (wormhole).**
- The outer mammalian cell membranes is mainly comprises phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, and cholesterol, which are charge-neutral at physiological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

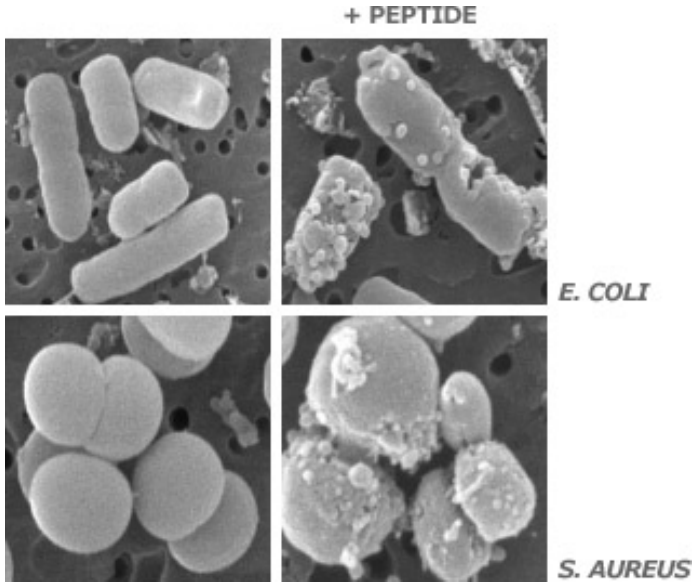


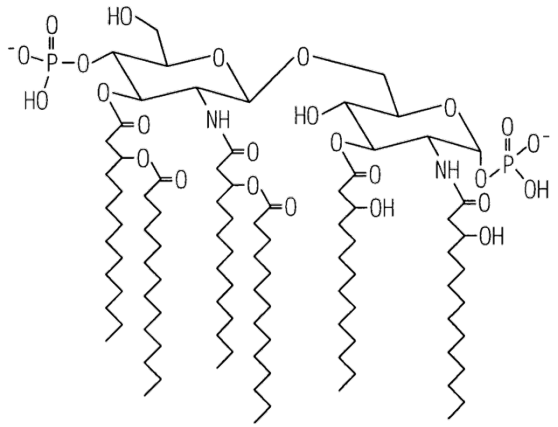


Mechanism of Action

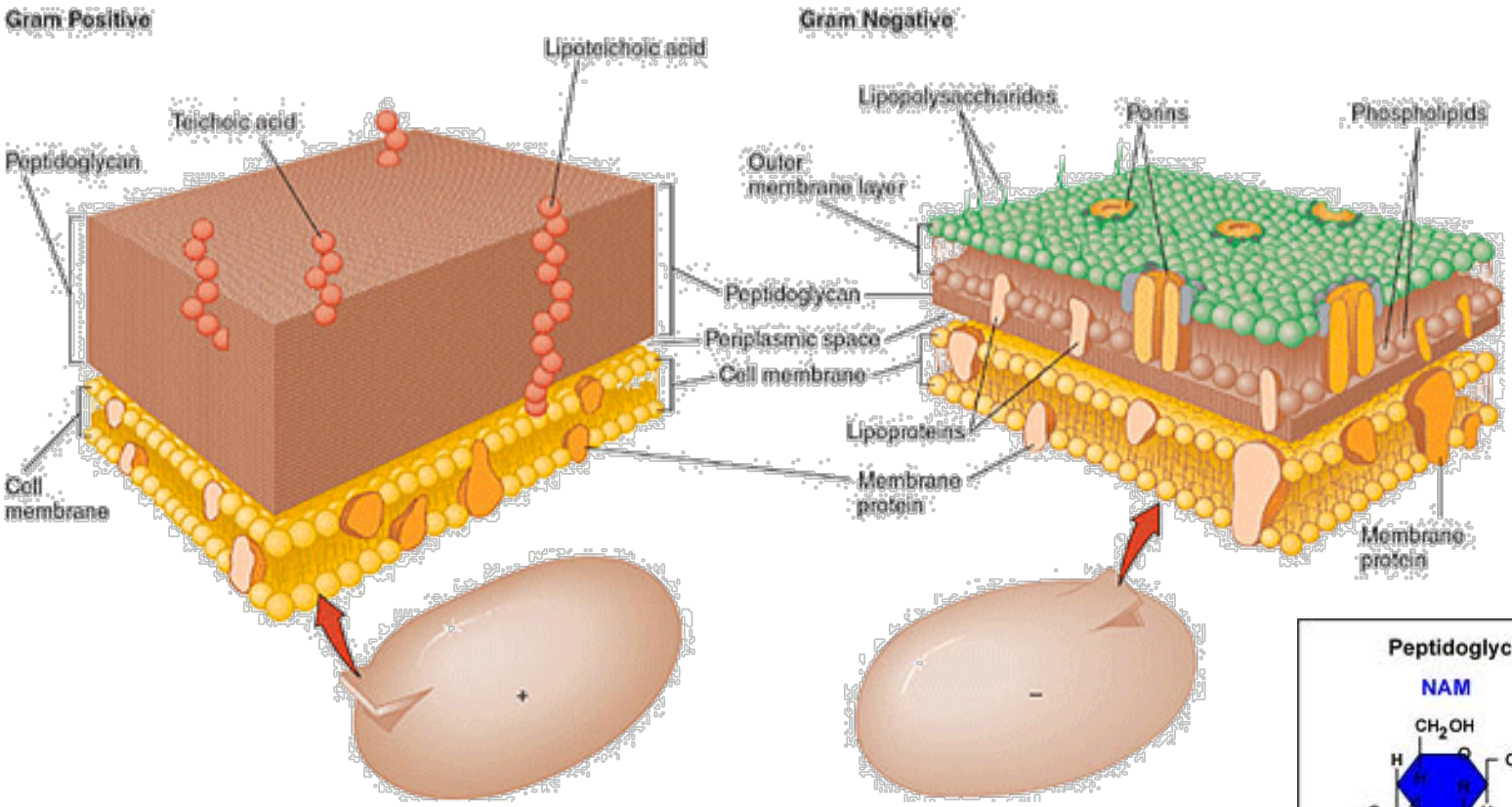
- The frequent occurrence of positively charged residues is a important feature of lytic peptides. It is thought to help the peptide to reach its target, which for most AMPs is believed to be the cytoplasmic membrane.
- This involves binding to LPS and teichoic acid by the displacement of divalent cations, such as **Mg^{2+}** and **Ca^{2+}**, that are essential for the stability of the cell surface and cross-bridging the negative charges of LPS.

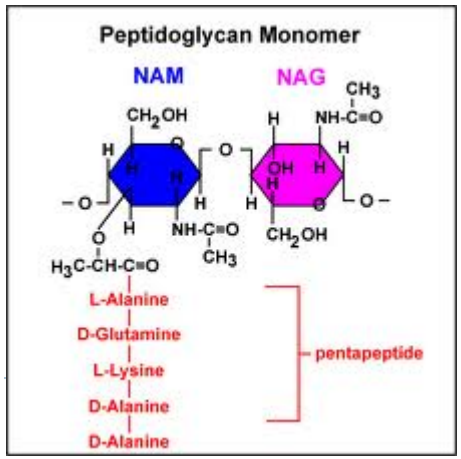






Comparison of the Gram positive and Gram negative bacterial cell walls



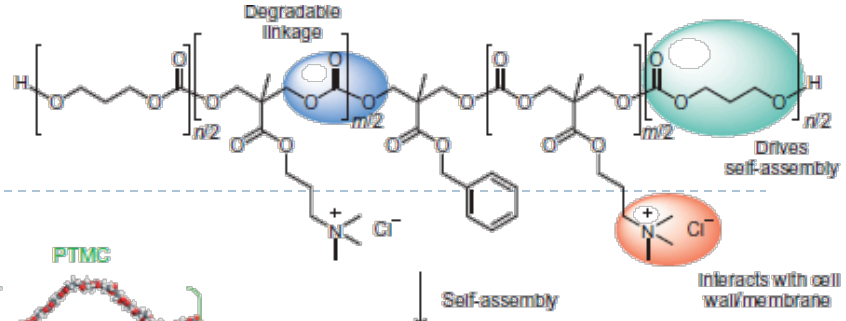


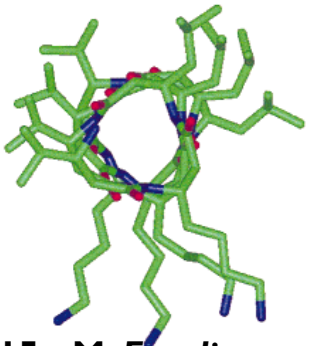
AMPs in current clinical studies

Name	Sequence	Company	Description	Application	Trial phase	Comments	Clinical trial identifiers and further information
PMX-30063	Structure not disclosed	PolyMedix	Arylamide oligomer mimetic of a defensin	Acute bacterial skin infections caused by *Staphylococcus* spp.	II	Mimetic rather than peptide; currently in Phase II trials	NCT01211470; PolyMedix website
Delmitide (RDP58)[211]	RXXXRX XXGY (X = norleucine)	Genzyme	Semisynthetic D-amino acid decapeptide derived from HLA class I B2702	Inflammatory bowel disease	II (completed)	A protease-resistant, D-amino acid-containing peptide with similar efficacy to asacol; attempting to improve activity through formulation	Genzyme website; ISRCTN84220089
Plectasin[212]	GFGC$_1$NG PWDEDD MQC$_2$HNH C$_3$KSIKGYK GGYC$_1$AKG GFVC$_2$KC$_3$Y)	Novozymes	Fungal defensin; candidate in development is an amino-acid substitiution variant	Bacterial diseases	Pre-clinical	Excellent efficacy demonstrated in animal models	Novozymes website
HB1345	Decanoyl-KFKWPW	Helix BioMedix	Synthetic lipohexapeptide	Acne; broad-spectrum antibiotic	Pre-clinical	Looks promising as this is a very small lipopeptide	Helix BioMedix website
Pexiganan acetate (MSI 78)	GIGKFLKK AKKFGKAF VKILKK	MacroChem	Synthetic analogue of magainin 2 derived from frog skin	Topical antibiotic	III	No advantage demonstrated over existing therapies	NCT00563433 and NCT00563394

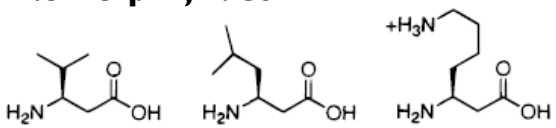
Examples

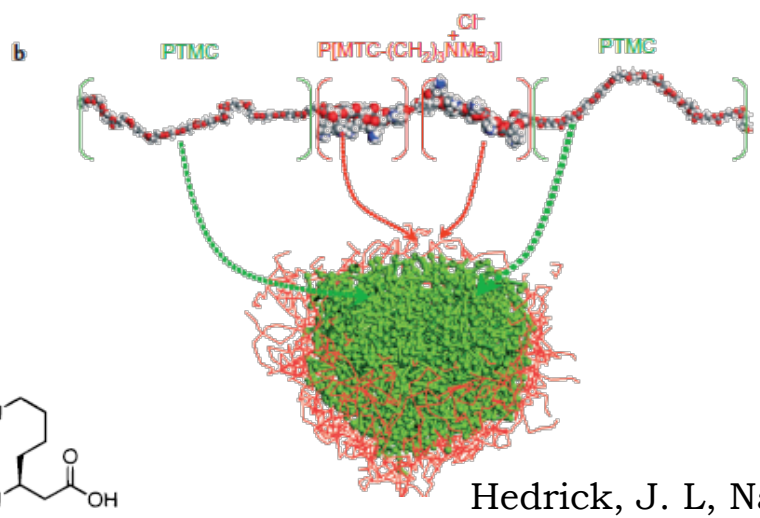
Polymer 1: $m = 10$, $n = 30$, $M_n = 6{,}200$, PDI = 1.15
Polymer 2: $m = 10$, $n = 45$, $M_n = 7{,}800$, PDI = 1.26
Polymer 3: $m = 20$, $n = 30$, $M_n = 9{,}200$, PDI = 1.25

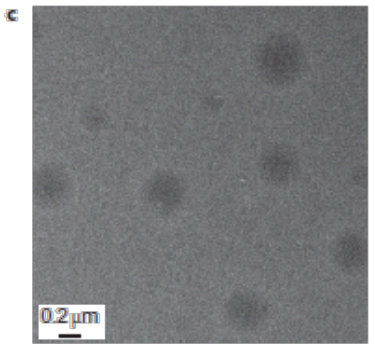




1.5-15 μM, *E. coli*



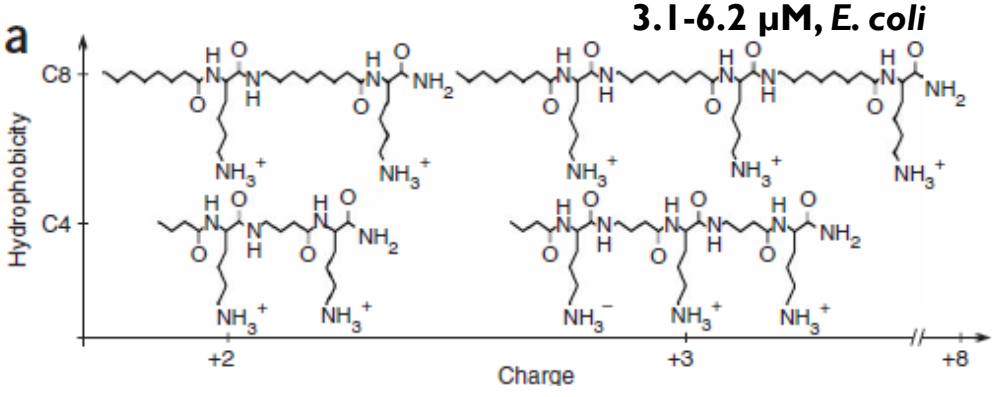




Hedrick, J. L, Nature Chemistry 2011, 3, 409

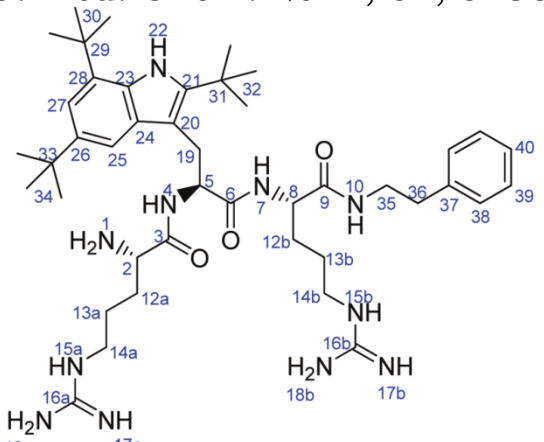
DeGrado, W. F., JACS 1999, 121, 12200

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



3.1-6.2 μM, *E. coli*

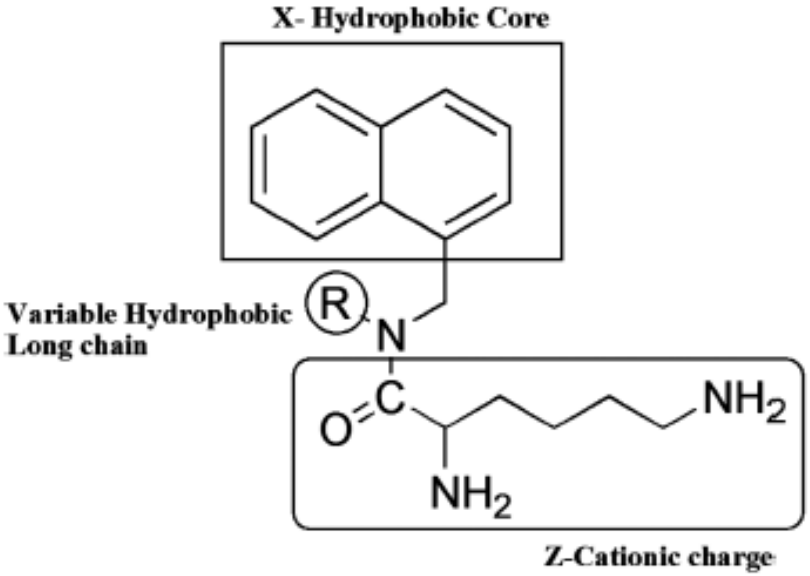
Mor, A., Nature Biotechnol. 2007, 25, 657



L-L-L (**1**)
D-D-D (**2**)
D-L-L (**3**)
L-D-D (**4**)
L-D-L (**5**)
D-L-D (**6**)
L-L-D (**7**)
D-D-L (**8**)

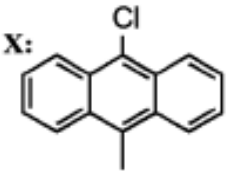
3-10 μg/mL, *E. coli*; 2-4 μg/mL *S.eureus*

Design and Synthesis



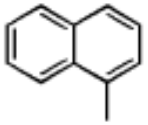
Series I
Z:L-Lysine
R:
C_2H_5: **1**
C_4H_9: **2**
C_6H_{13}: **3**
C_8H_{17}: **4**
$C_{10}H_{21}$: **5**

X:



Series II
Z: L-Lysine
R:
C_4H_9 : **6**
C_6H_{13} : **7**
C_8H_{17} : **8**
$C_{10}H_{21}$: **9**
$C_{12}H_{25}$: **10**

X:

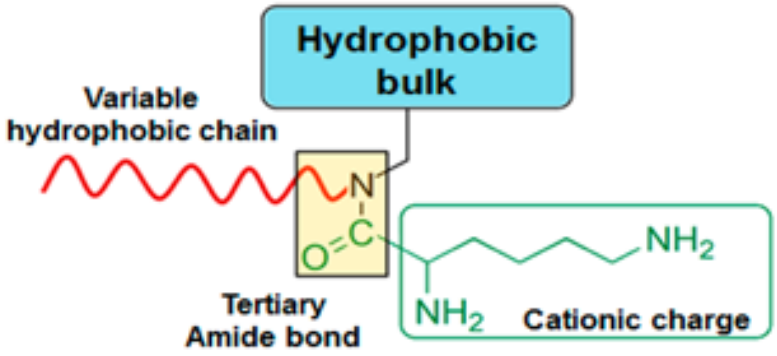


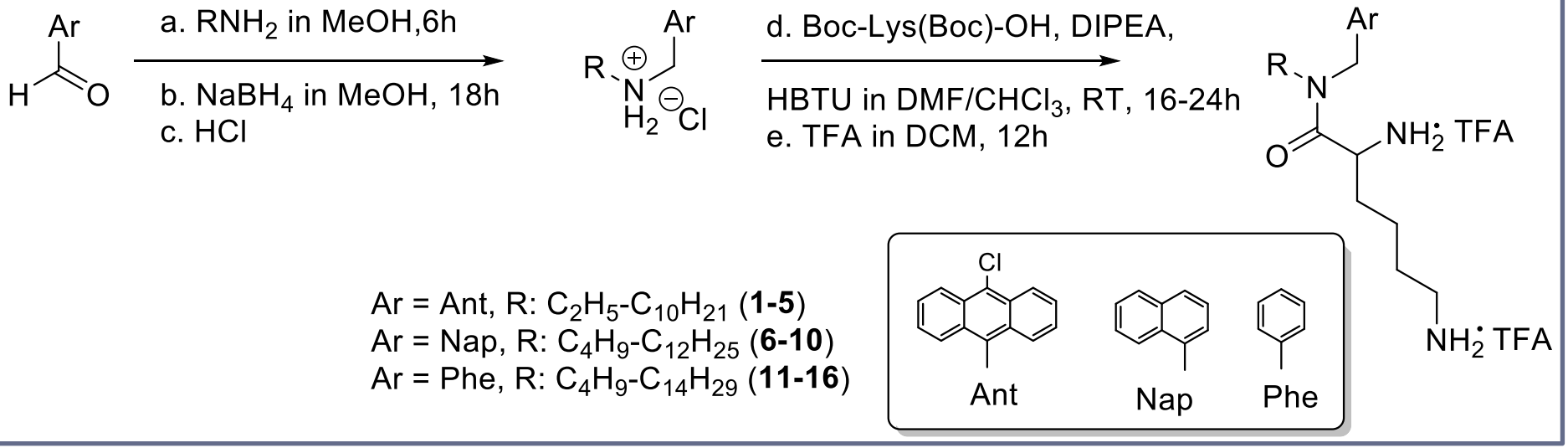
Series III
Z: L-Lysine
R:
C_4H_9 : **11**
C_6H_{13} : **12**
C_8H_{17} : **13**
$C_{10}H_{21}$: **14**
$C_{12}H_{25}$: **15**
$C_{14}H_{29}$: **16**

X:

Design and Synthesis

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



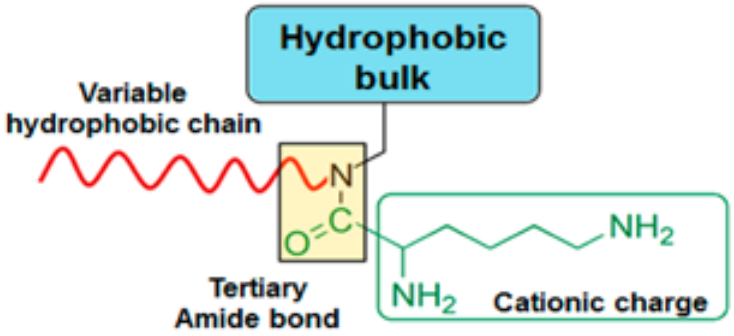


Antibacterial Activity

	minimum inhibitory concentration ($\mu g\ mL^{-1}$)								
	drug sensitive bacteria				drug resistant bacteria				
compd	*S. aureus*	*E. faecium*	*E. coli*	*P. aeruginosa*	MRSA	VRE	*K. pneumoniae*	HC_{50} ($\mu g\ mL^{-1}$)	HPLC retention times (min)
1	11	13.6	25	4	21	7.2	31	118	11.8
2	5.3	4.5	4.8	1.9	6.3	5.3	17	91	12.4
3	2.4	3.3	3.5	1.6	2.8	5.2	16	82	13.6
4	2.2	2.5	2.9	3.8	2.3	3	4.3	64	14.6
5	7.1	4.9	26	11	4.6	5.6	7.6	71	15.6
6	>100	>100	>100	>100	>100	>100	>100	>1000	10.8
7	20	34	25	11	65	54	100	508	11.8
8	6.3	5.5	5	5.4	4.4	7	13	60	12.9
9	2.5	3.5	4	3	2.6	1.6	5.8	54	14.2
10	3	1.6	3.1	3.2	2.7	3.4	4	56	15.1
11	>100	>100	>100	>100	>100	ND[a]	>100	>1000	9.4
12	>100	>100	>100	>100	>100	ND	>100	>1000	10.6
13	46	60	51	60	>100	>100	>100	325	12.1
14	5.7	6.5	6.5	4	15.7	5.8	31	95	13.4
15	2.7	2.6	5	4	2.9	3.3	2.8	45	14.5
16	3.1	2	3.1	2.8	2.5	2.5	4	50	15.8
vancomycin	0.9	0.87	ND	ND	0.9	>100	ND	ND	ND
colistin	20	>100	0.4	0.4	54	>100	1.2	ND	ND
MSI-78	8–16[b]	64[b]*	16–32[b]	8–16[b]	16–32[b]	8[b]	8–16[b]	120[c]	ND

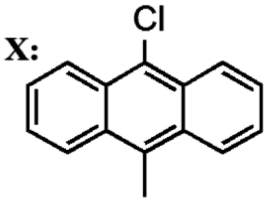
[a]ND stands for "not determined". [b]Literature values obtained from ref 36. * indicates value for *E. faecalis*, [c]Literature value obtained from ref 28. VRE (vancomycin-resistant *E. faecium*) and MRSA (methicillin-resistant *S. aureus*), *K. pneumoniae* is resistant to β-lactam antibiotics.

Series I

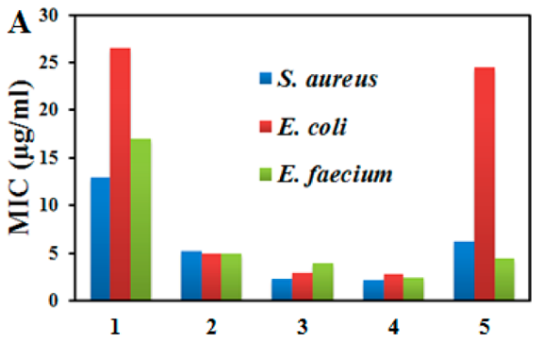


Series I
Z:L-Lysine
R:
C_2H_5: **1**
C_4H_9: **2**
C_6H_{13}: **3**
C_8H_{17}: **4**
$C_{10}H_{21}$: **5**

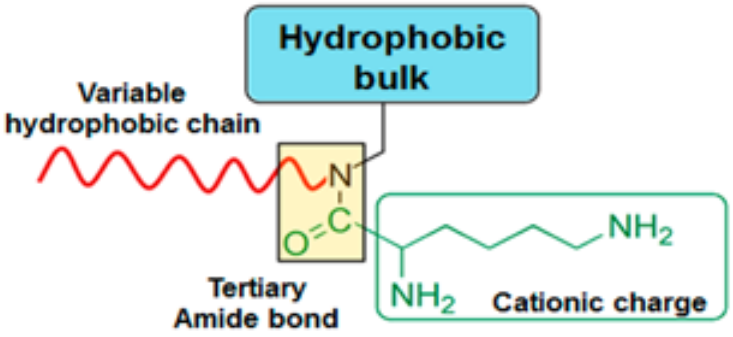
X:



	minimum inhibitory concentration ($\mu g\ mL^{-1}$)						
	drug sensitive bacteria				drug resistant bacteria		
compd	*S. aureus*	*E. faecium*	*E. coli*	*P. aeruginosa*	MRSA	VRE	*K. pneumoniae*
1	11	13.6	25	4	21	7.2	31
2	5.3	4.5	4.8	1.9	6.3	5.3	17
3	2.4	3.3	3.5	1.6	2.8	5.2	16
4	2.2	2.5	2.9	3.8	2.3	3	4.3
5	7.1	4.9	26	11	4.6	5.6	7.6

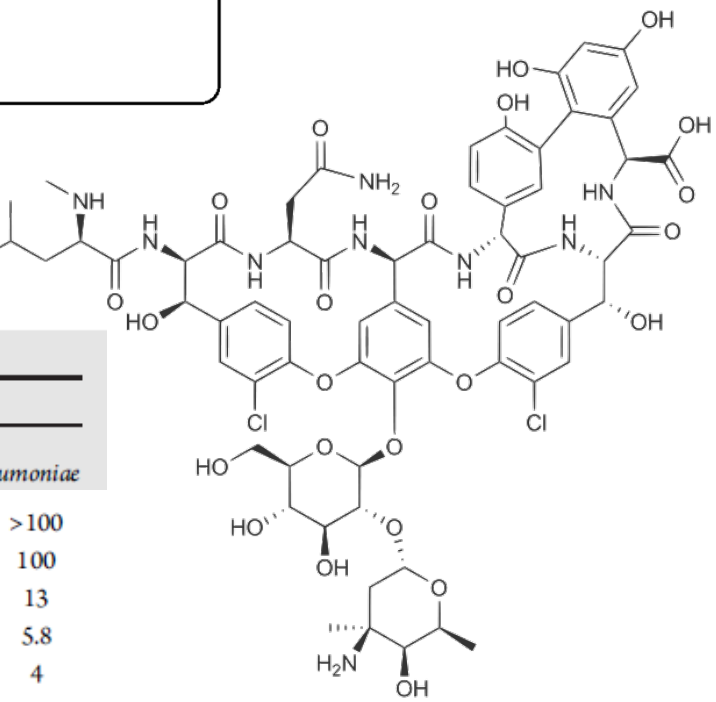


Series II



Series II
Z: L-Lysine
R:
C_4H_9 : **6**
C_6H_{13} : **7**
C_8H_{17} : **8**
$C_{10}H_{21}$: **9**
$C_{12}H_{25}$: **10**

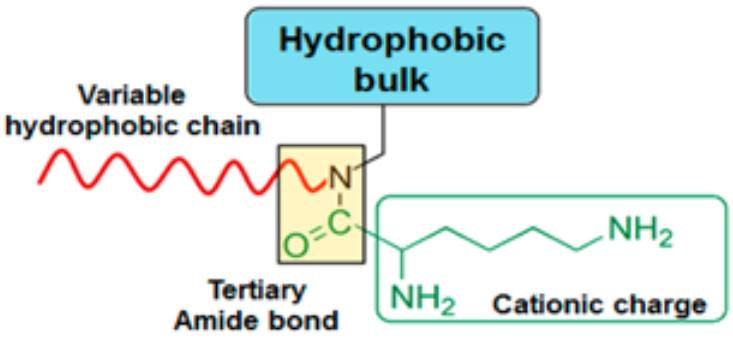
X:



	minimum inhibitory concentration ($\mu g\ mL^{-1}$)						
	drug sensitive bacteria				drug resistant bacteria		
compd	*S. aureus*	*E. faecium*	*E. coli*	*P. aeruginosa*	MRSA	VRE	*K. pneumoniae*
6	>100	>100	>100	>100	>100	>100	>100
7	20	34	25	11	65	54	100
8	6.3	5.5	5	5.4	4.4	7	13
9	2.5	3.5	4	3	2.6	1.6	5.8
10	3	1.6	3.1	3.2	2.7	3.4	4

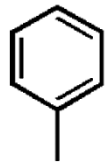
Vancomycin 0.87 μg/mL

Series III



Series III
Z: L-Lysine
R:
C_4H_9 : **11**
C_6H_{13} : **12**
C_8H_{17} : **13**
$C_{10}H_{21}$: **14**
$C_{12}H_{25}$: **15**
$C_{14}H_{29}$: **16**

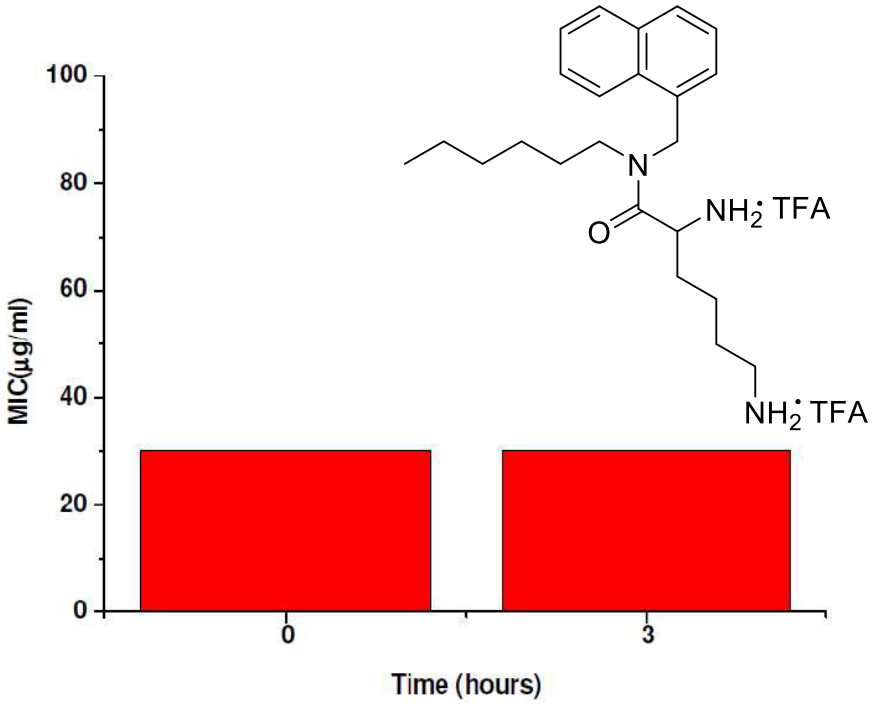
X:



	minimum inhibitory concentration ($\mu g\ mL^{-1}$)						
	drug sensitive bacteria				drug resistant bacteria		
compd	*S. aureus*	*E. faecium*	*E. coli*	*P. aeruginosa*	MRSA	VRE	*K. pneumoniae*
11	>100	>100	>100	>100	>100	ND[a]	>100
12	>100	>100	>100	>100	>100	ND	>100
13	46	60	51	60	>100	>100	>100
14	5.7	6.5	6.5	4	15.7	5.8	31
15	2.7	2.6	5	4	2.9	3.3	2.8
16	3.1	2	3.1	2.8	2.5	2.5	4

Antibacterial Activity in Plasma and Enzyme Stability

- MIC of compound **7** against S. aureus in 50% blood plasma was 30 μ g/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.



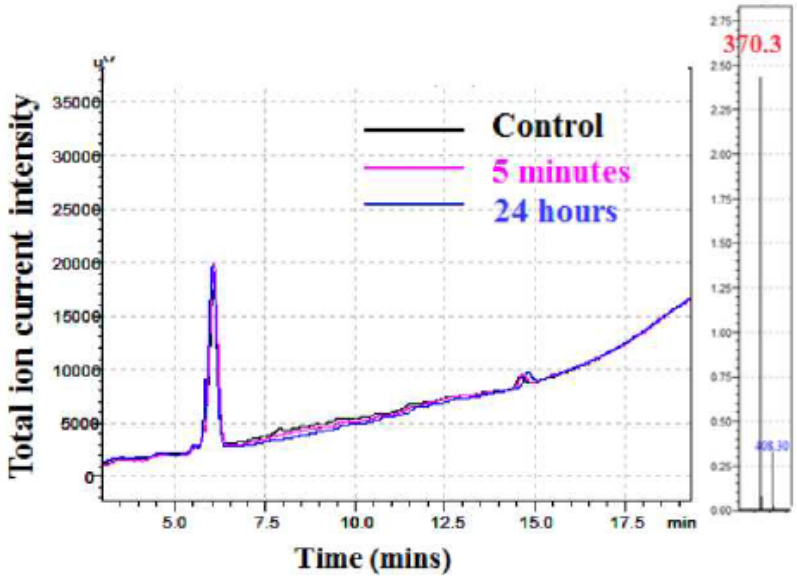
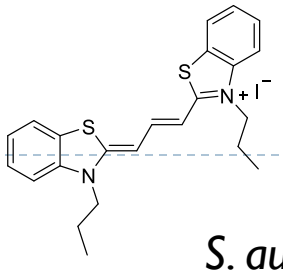


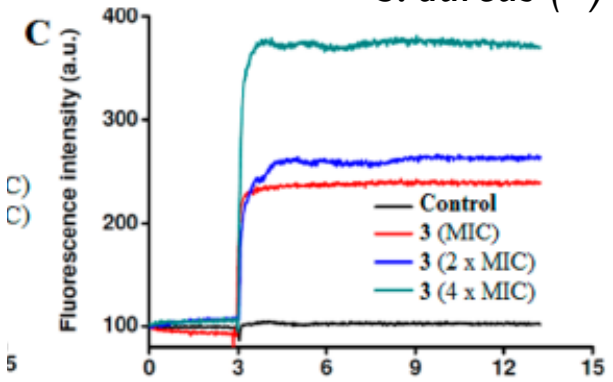
Figure S66: Effect of human plasma on the antibacterial activity of compound 7 in three hours.

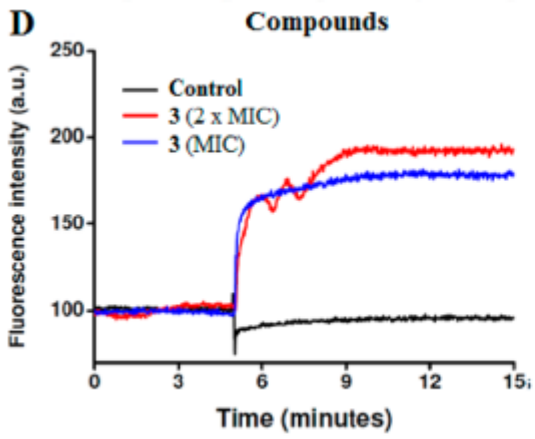
Mechanism of Action



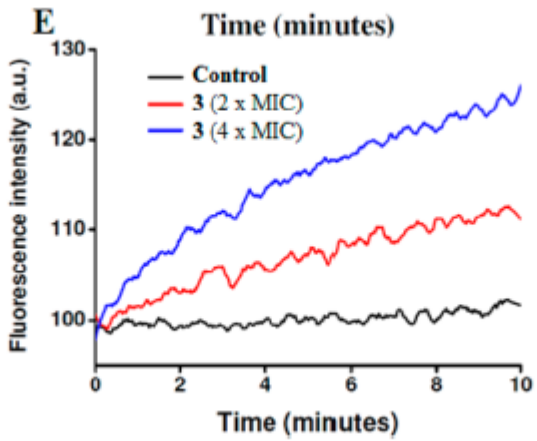
- Experiments with membrane potential sensitive dye reviled that these compounds rapidly depolarize the membrane of both, Gram(+) and Gram(-) bacteria.
- Compounds **3** and **7** could cause permeabilization of Gram(+) and Gram(-) membrane at MIC concentrations.

S. aureus (+)

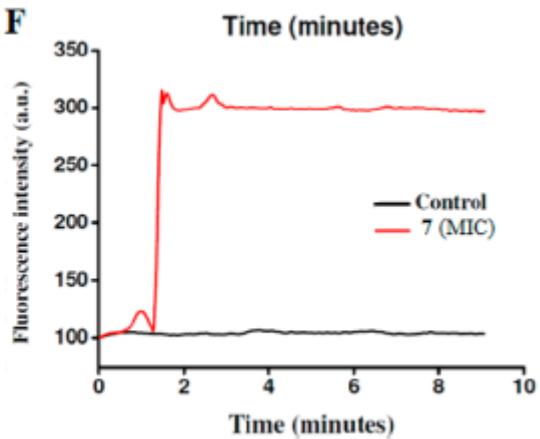




P. aeruginosa (-)

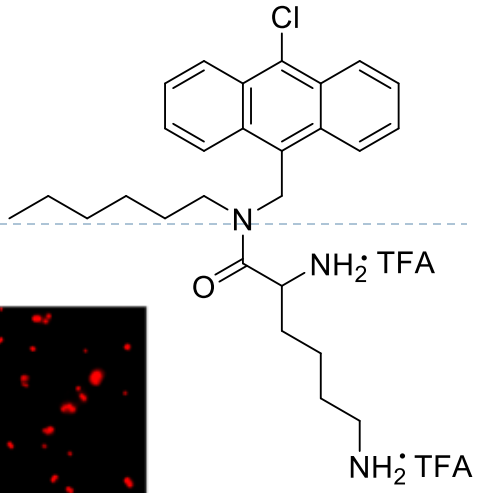


S. aureus (+)



E. coli (-)

Mechanism of Action



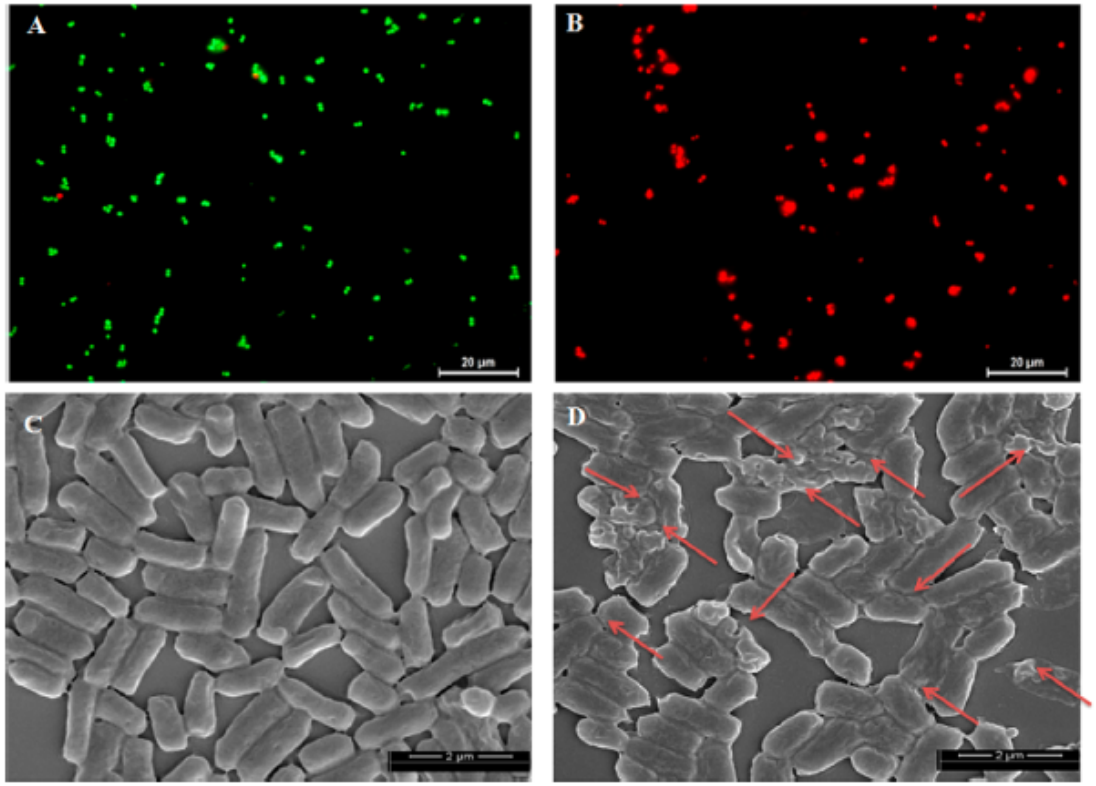
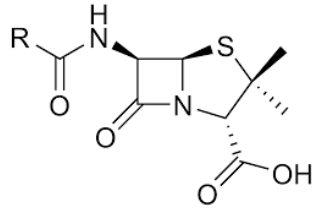


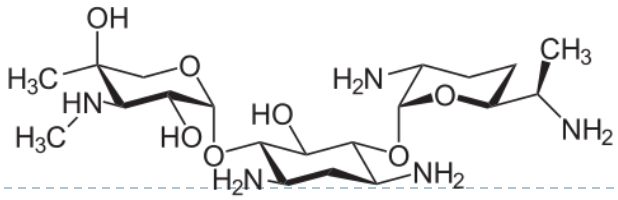
Figure 3. Fluorescence microscopy images of *S. aureus* (A) untreated and (B) treated with 3 (10 × MIC) for 1.5 h after staining with SYTO 9 and PI (scale: 20 μm). Scanning electron microscopy (SEM) images of (C) untreated *E. coli* and (D) *E. coli* treated with 3 (10 × MIC).

Conclusions

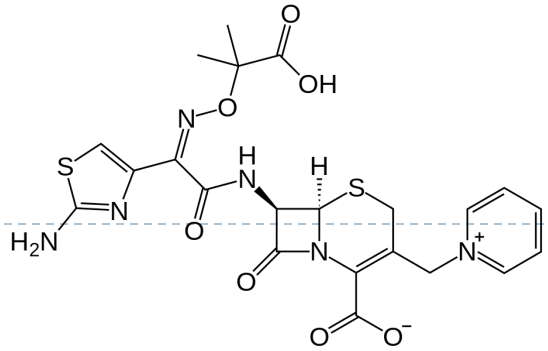
- This report illustrates a systematic way of creating highly potent, broad-spectrum small molecular peptide mimics which emulate the efficiency of AMPs.
- Prepared from inexpensive starting materials in only three steps, these compounds are selectively toxic toward bacterial cells (over mammalian cells) 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.



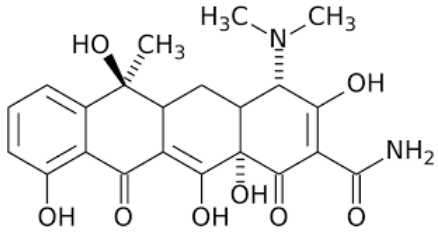
penicillin



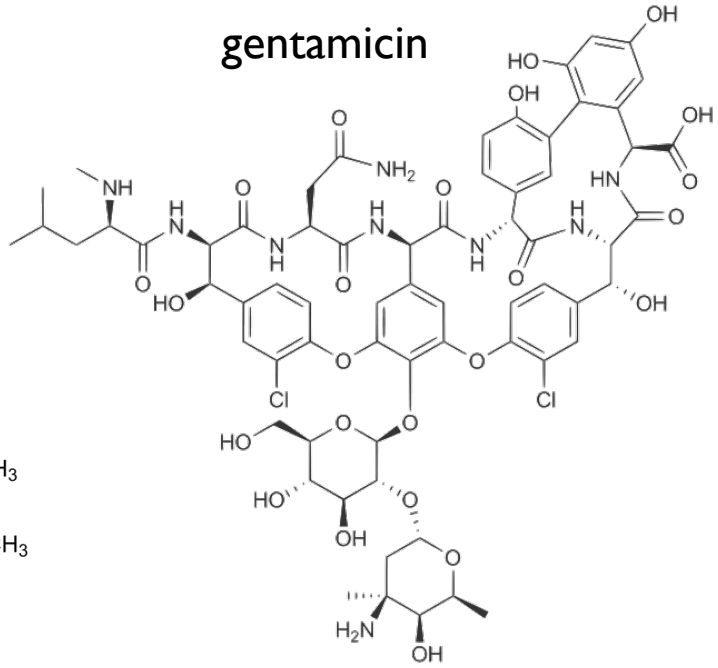
gentamicin



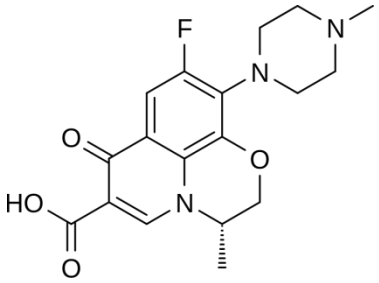
ceftazidime



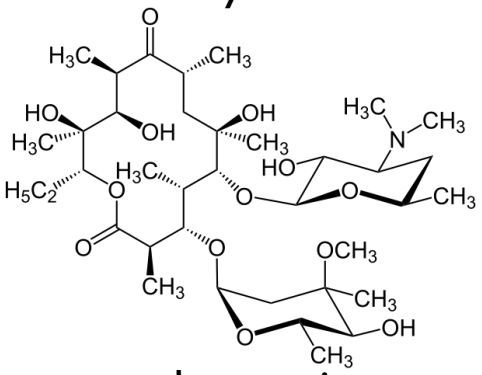
tetracycline



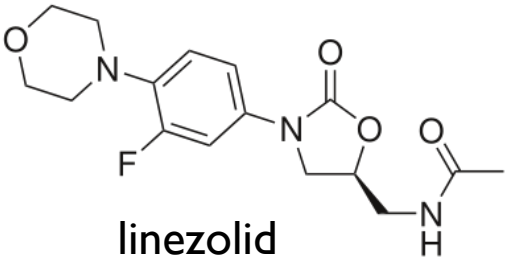
vancomycin



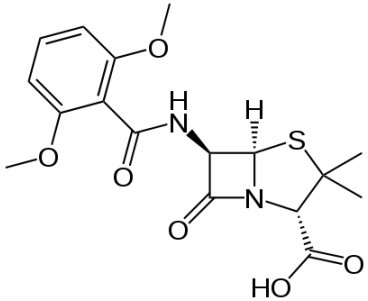
levofloxacin

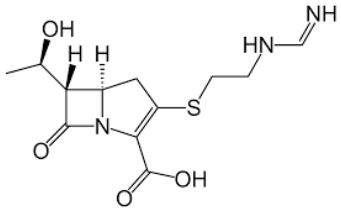


erythromycin

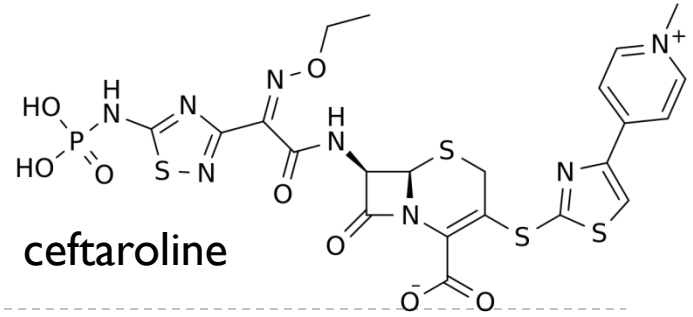


linezolid





imipenem



ceftaroline