Strategies to Combat Bacterial Resistance: Towards Development of Future Antibacterial Drugs

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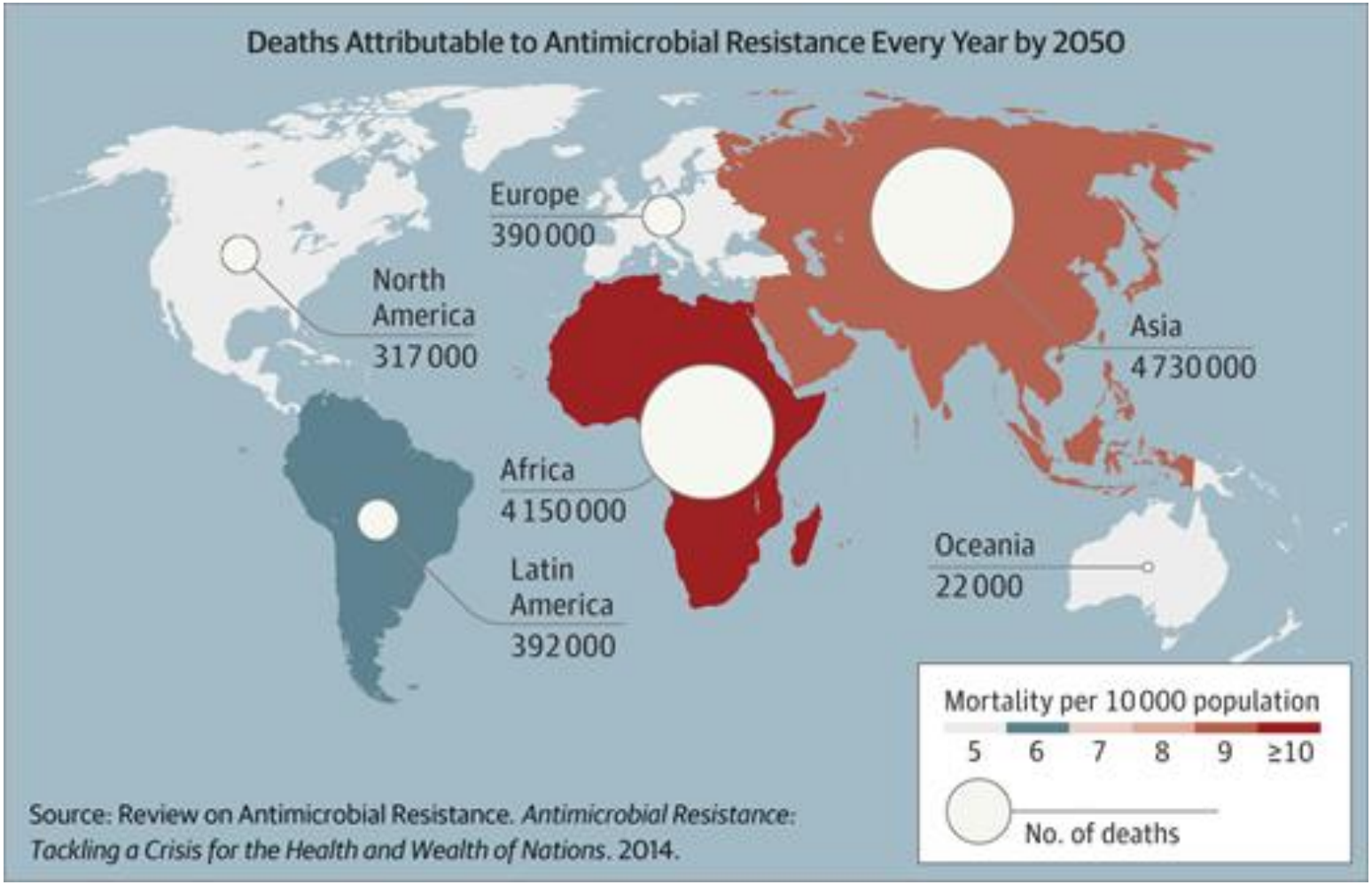
Bangalore, INDIA

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Email-2: jayanta.jnc@gmail.com

World Congress and Exhibition on Antibiotics, September 14-16, 2015- Las Vegas

Antimicrobial Resistance–Global Threat and Need

Antimicrobial Resistance (AMR) causes an estimated 700,000 deaths annually and is predicted to cause 10 million deaths annually by 2050



AMR, if left unchecked, will cost the world $100 trillion by 2050

Strategies to Combat Drug Resistance and Infection

Prevention

- **Stop infections from spreading (Polymeric Paints)**
  - **Publication:** ACS Appl Mater Interfaces 2015
  - **Patent:** WO2015092520 A1

Treatment

- **Developing new drugs with novel targets and novel modes of action (Small Peptoids)**
  - **Publication:** ChemMedChem 2015; ACS Infec Dis 2015; J Med Chem 2015; Chem Commun 2015, J Med Chem 2014; J Med Chem 2014; Chem Commun 2013; Langmuir 2012
  - **Patent:** Invention-I: PCT/GB2015/050750, Invention-II: WO 2014 097178 A1

- **Strategies to make existing drugs better and overcome drug resistance**

a) Development of novel class of semi-synthetic glycopeptides

- **Publication:** Int J Antimicrob Agents 2015; J Antibiot 2015; Int J Antimicrob Agents 2015; J Med Chem, 2014
- **Patent:** Invention-I: WO 2013072838 A1, US 2014 0308347, EP2780359 B1; . CA2855753 A1; Korea. 10-2014-7016259, Australia, India; Invention-II: PCT 2014/10085; Invention-III PCT/IB2014/001835; Invention-IV: 6565/CHE/2014

b) Combination therapy: obsolete antibiotics + novel efflux pump inhibitor

- **Publication:** Microb Pathog 2015; PLoS One 2015
- **Patent:** WO 2014 006601 A2 (India, Europe, USA, S. Korea)

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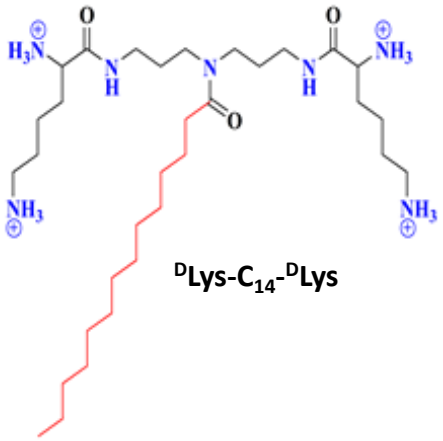
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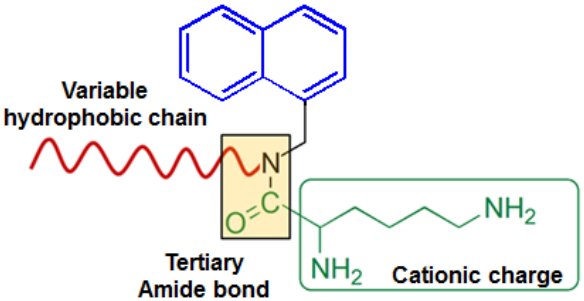
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Synthetic Lipopeptoids



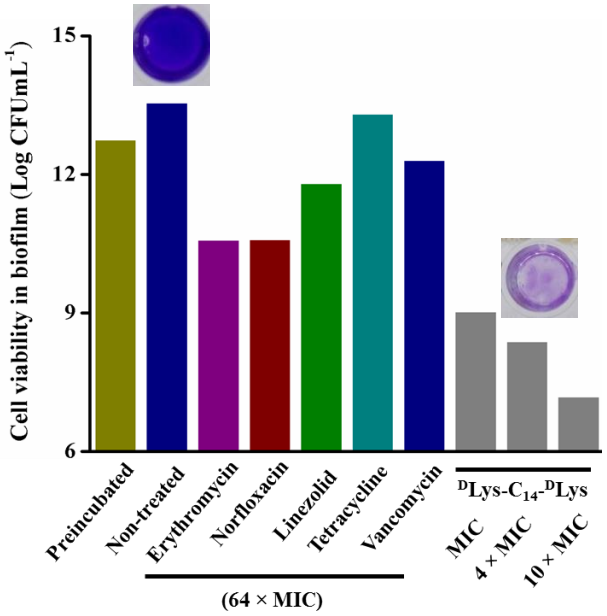
^{D}Lys-C_{14}-^{D}Lys



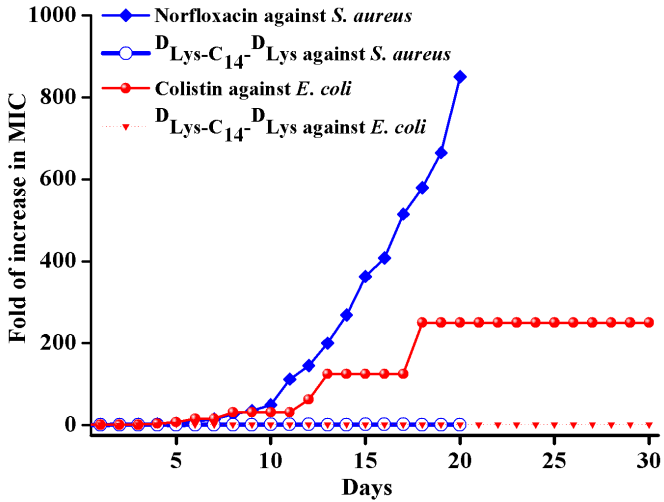
Antibacterial Activity

Clinical Isolates (18 strains)	MIC (μM)
Enterococcus faecium	2.2-2.7
Staphylococcus aureus	1.4-4.2
Klebsiella pneumoniae	5.0-6.4
Acinetobacter baumanii	2.8-3.0
Pseudomonas aeruginosa	2.8-7.0
Enterobacter species	3.0-4.8

Biofilm Disruption of *S. aureus*



Bacteria do not develop resistance



- Kill bacteria rapidly (within 15-30 min)
- Selectively toxic towards bacteria (>350 fold)
- Retained antibacterial efficacy in presence of plasma
- Active against persister cell

J.Med.Chem 2014, 57, 9409; J. Med. Chem., 2014, 57, 1428; ChemMedChem, 2015; Acs Infec. Dis 2015; *Chem. Commun.* 2015, *51*, 13670; *J. Med. Chem.* 2015, *58*, 5486; *Langmuir* 2012, *28*, 12225

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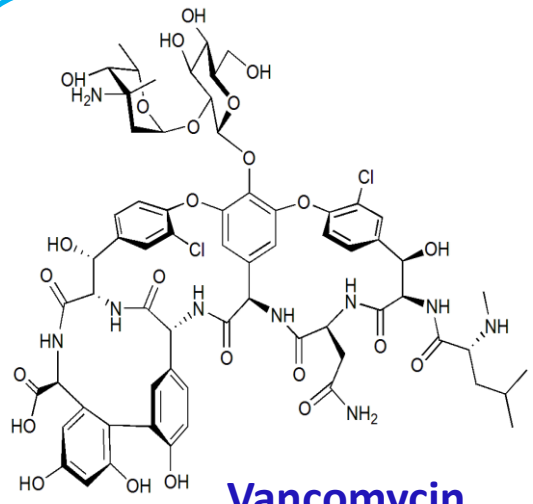
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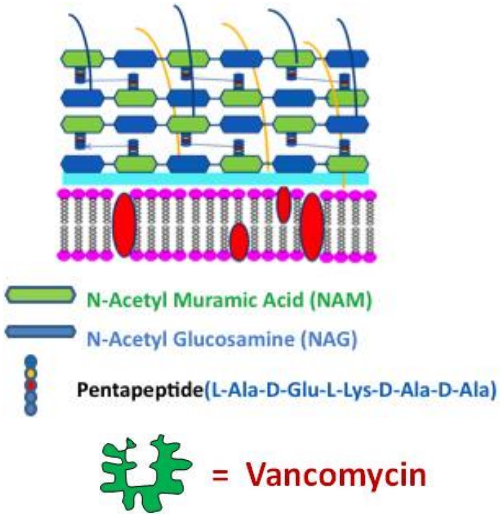
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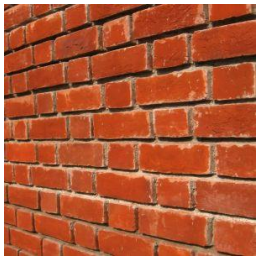
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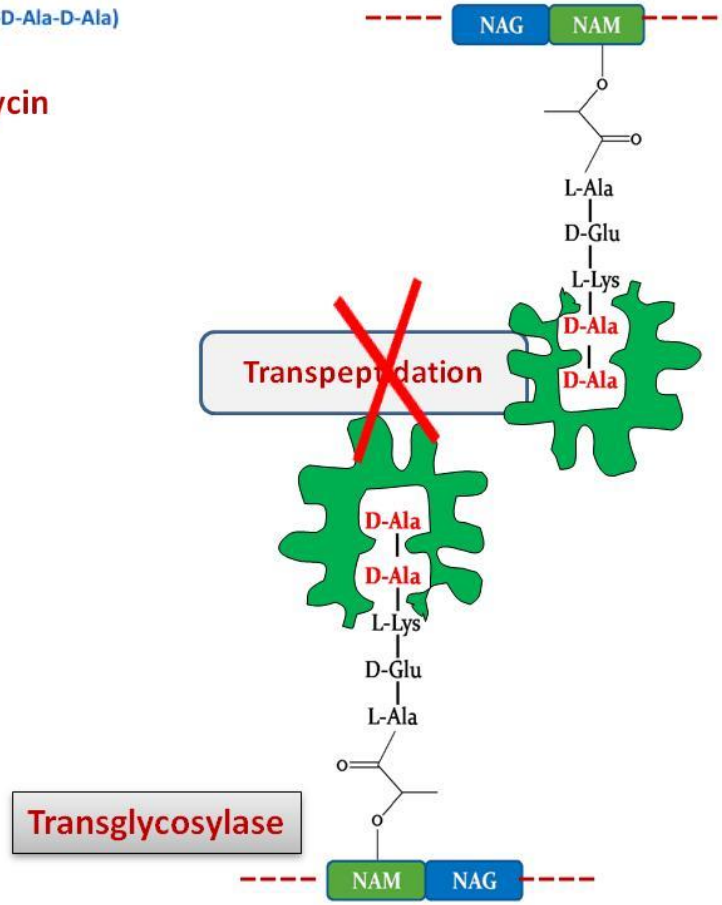
- **Publication:** Microb Pathog 2015; PLoS One 2015
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Vancomycin: A Cell Wall Biosynthesis Inhibitor









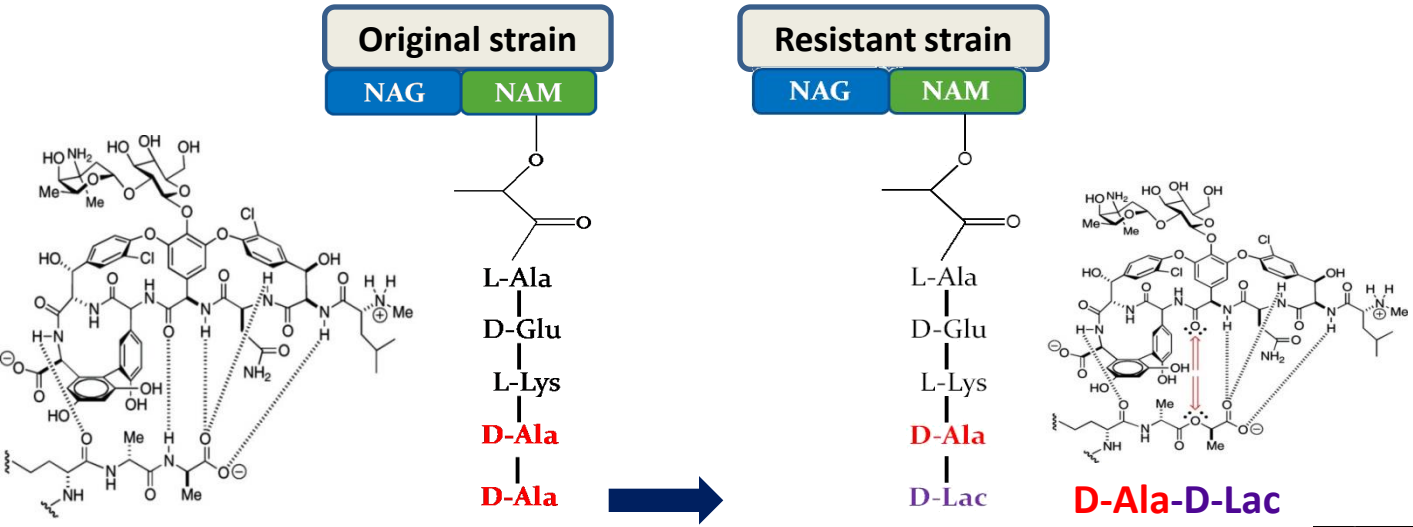
DRUG OF LAST RESORT

- Used to treat ONLY Gram-positive lethal bacterial infections such as
- Methicillin resistant Staphylococcal infections
- Enterococcal infections
- Diseases: sepsis, endocarditis, skin infections, pneumonia

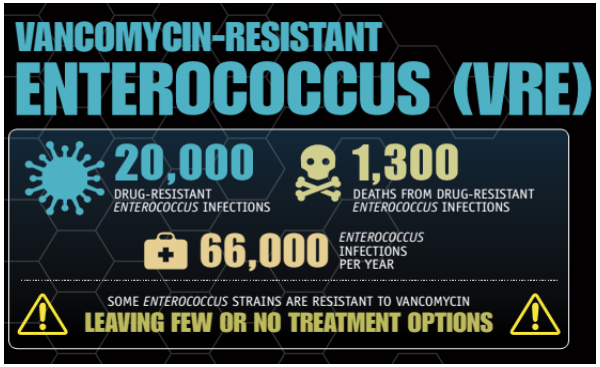
Concern of Vancomycin Resistant Bacteria

Lack of one Hydrogen bond and the presence of lone pair repulsions:

- **The binding constant decreases by 1000 fold**
- **The antibacterial activity decreases by more than 100–1000 fold**

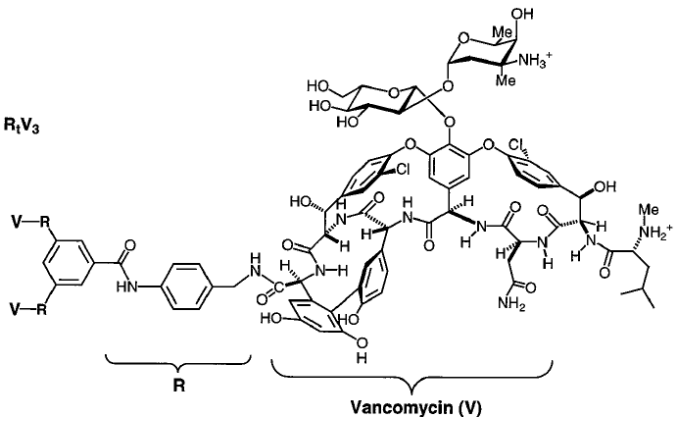


- *Vancomycin-resistant Enterococci (VRE).....1986*
- *Vancomycin-intermediate-resistant Staphylococcus aureus (VISA)...1996*
- *Vancomycin-resistant Staphylococcus aureus (VRSA)....2001*

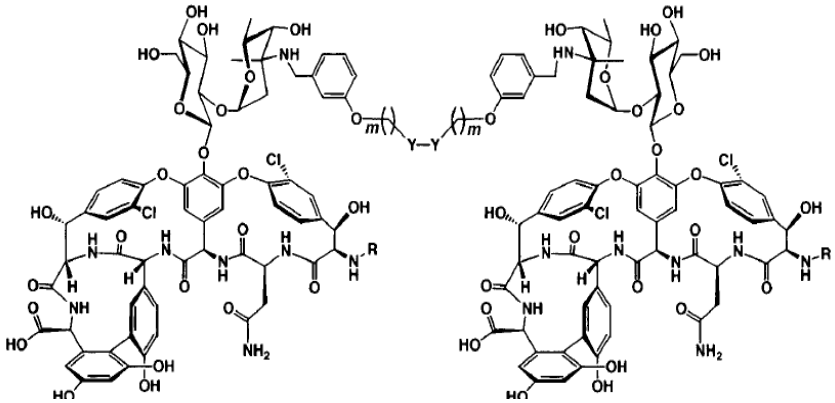


Semi-synthetic Glycopeptides to Enhance the Binding Affinity

Multivalency Approach



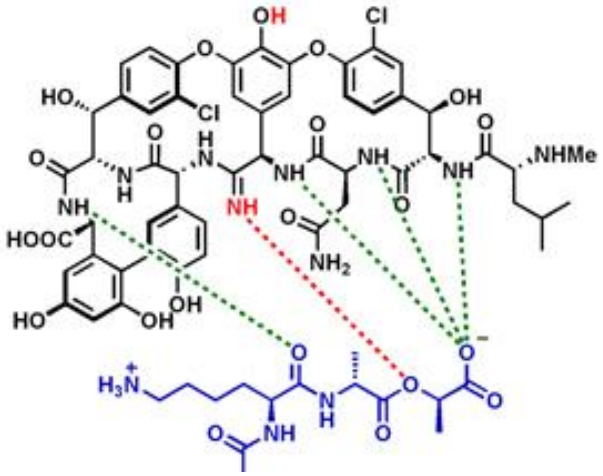
G. M. Whitesides *et al. (Harvard University)*
Science, 1998, 280,708



K. C. Nicolaou *et al. (Scripps Research Institute)*
Angew. Chem. Int. Ed, 2000, 39, 3823

❖ **Not active against more virulent bacteria**

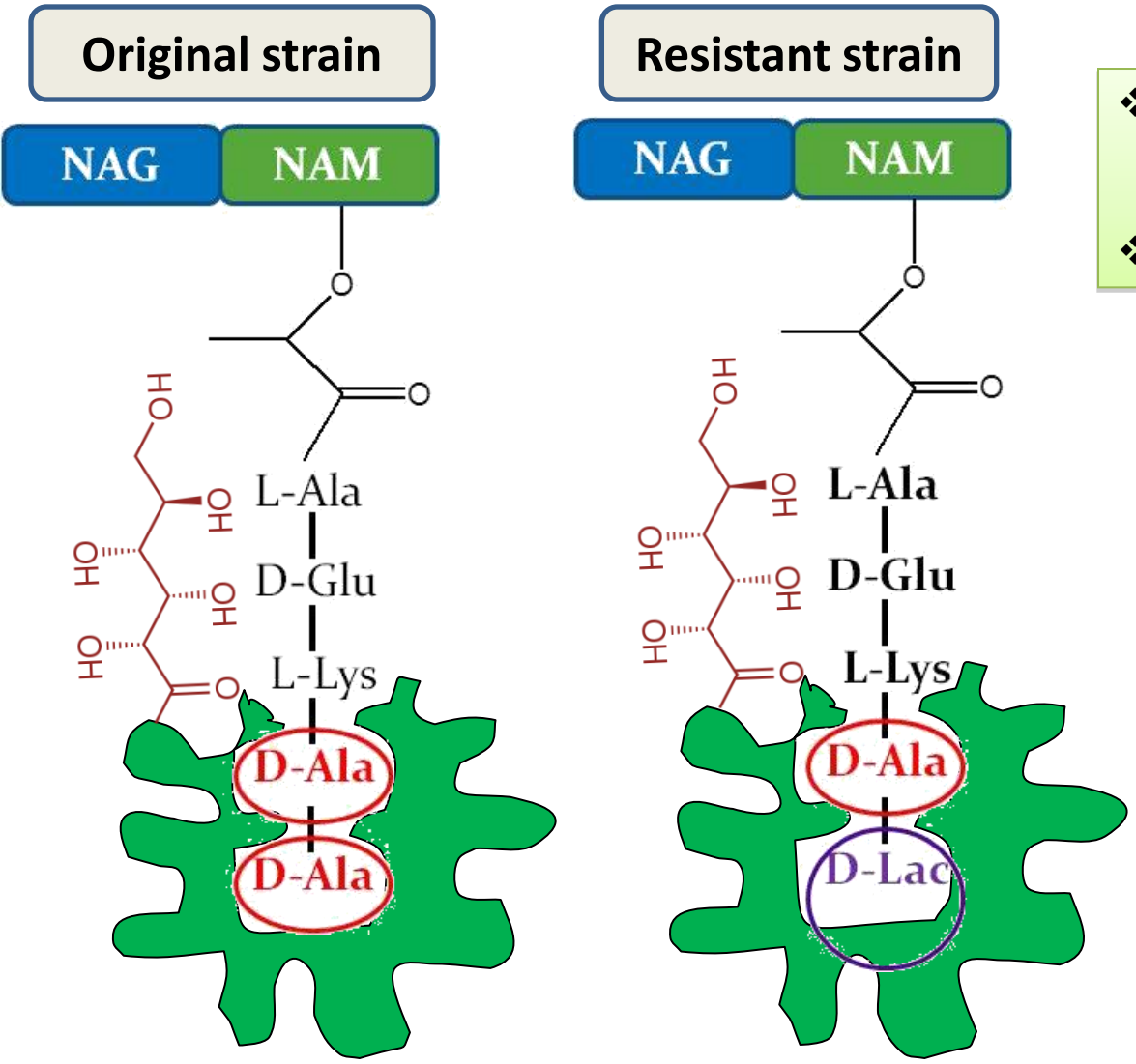
Vancomycin Amidine



D. L. Boger *et al. (Scripps Research Institute)*
JACS, 2011, 133, 13946

~75 step synthesis

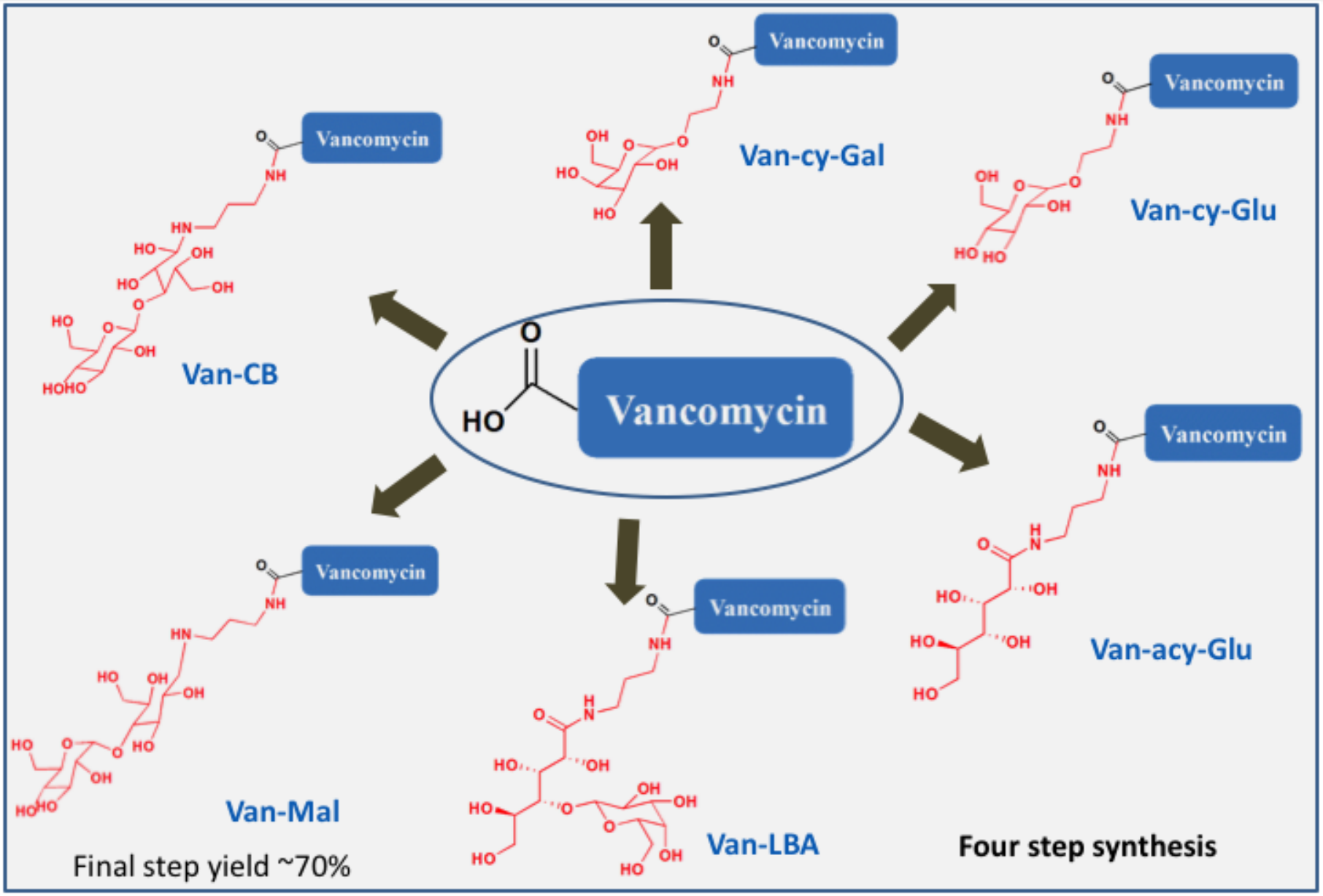
Invention-1: Improved Binding Affinity & Greater Cell Wall Inhibition



- Extra possible H-bonding interactions
- Overall Improved binding affinity

= Vancomycin

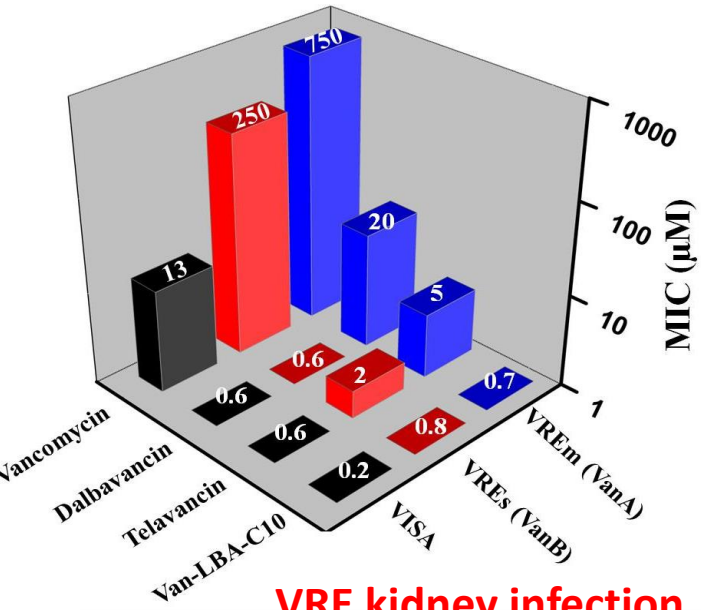
Development of Vancomycin-Sugar Conjugates



V. Yarlagadda *et al. J. Antibiotics 2014, 68, 302; & Patent:* PCT 2014/10085

Combating Acquired Resistance of Gram-positive Bacteria

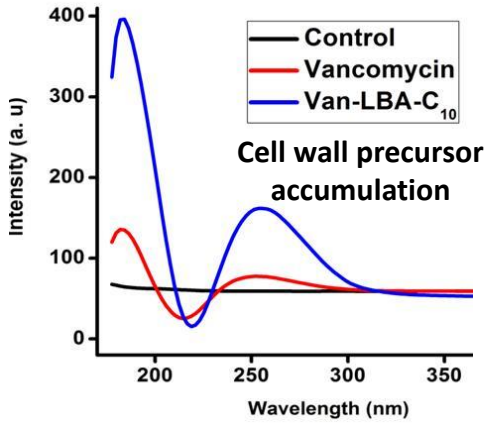
In-vitro antibacterial activity



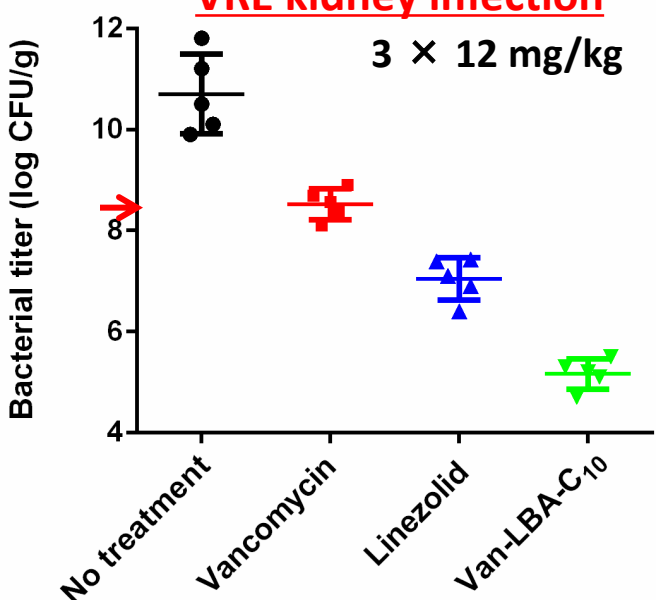
Target peptide binding

Drug	Ligand	K_a (M^{-1})
Vancomycin	Lys-Ala-Ala	1.1×10^5
Van-LBA-C_{10}	Lys-Ala-Ala	2.1×10^5
Vancomycin	Lys-Ala-Lac	5.0×10^2
Van-LBA-C_{10}	Lys-Ala-Lac	6.5×10^4

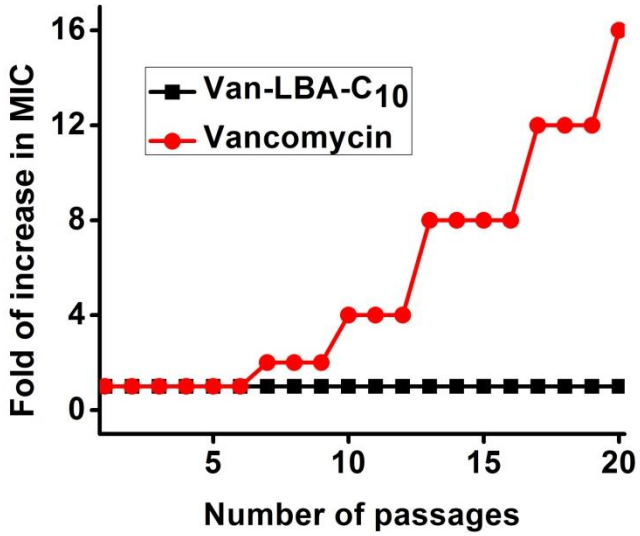
Cell wall inhibition



VRE kidney infection



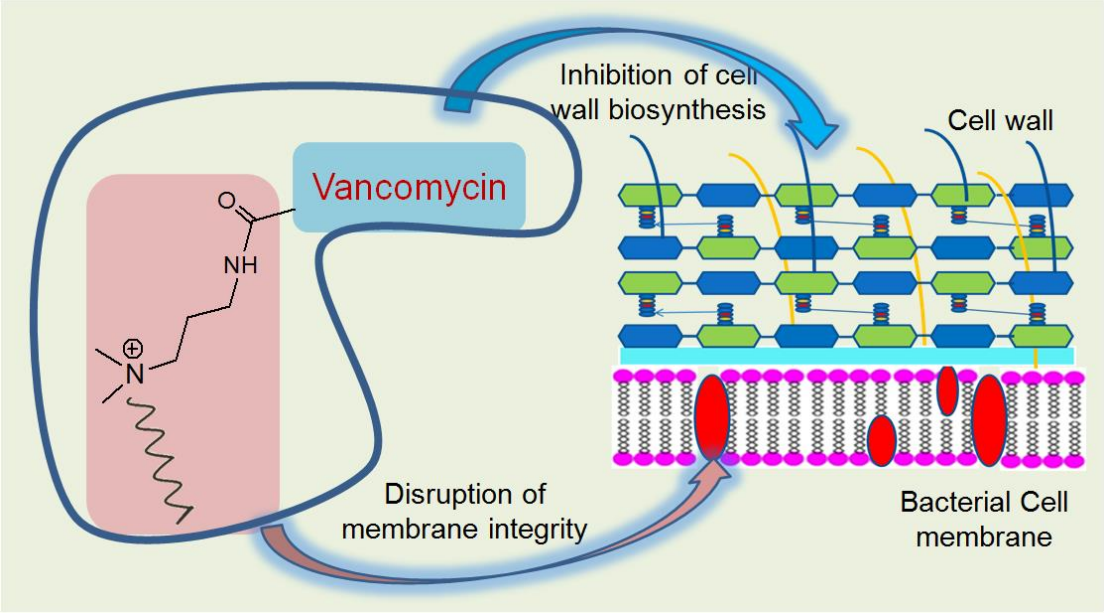
Resistance development in MRSA

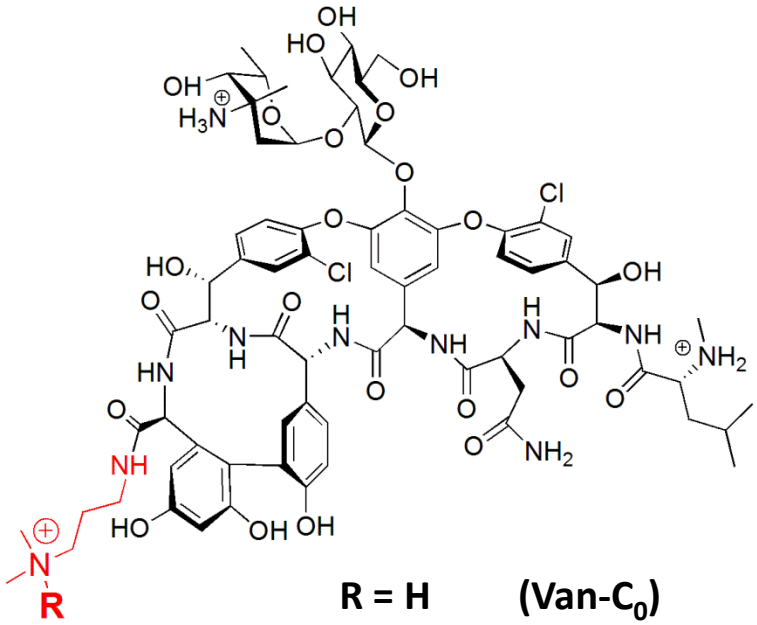


V. Yarlagadda *et al. J. Antibiotics 2014, 68, 302;* . Int. J. Antimicrob. Agents 2015 *& Patent:* PCT 2014/10085

Invention-2: Introducing New Membrane Active Mechanism of Action

Membrane active vancomycin analogues bearing permanently positive charged lipophilic moiety





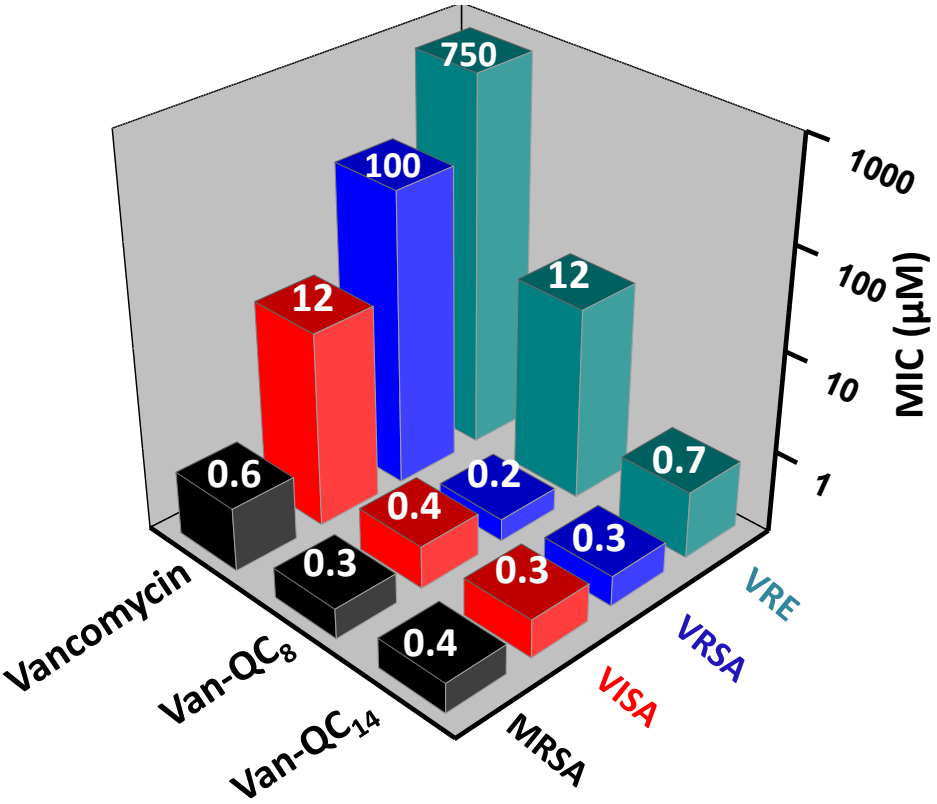
R = H (C_0)
C_2H_5 (QC_2)
C_4H_9 (QC_4)
C_8H_{17} (QC_8)
$C_{10}H_{21}$ (QC_{10})
$C_{14}H_{29}$ (QC_{14})
$C_{18}H_{37}$ (QC_{18})

- Increasing the cationic charges in the molecule: **Electrostatic interaction**
- Increasing the lipophilicity of the molecule: **Hydrophobic interaction**

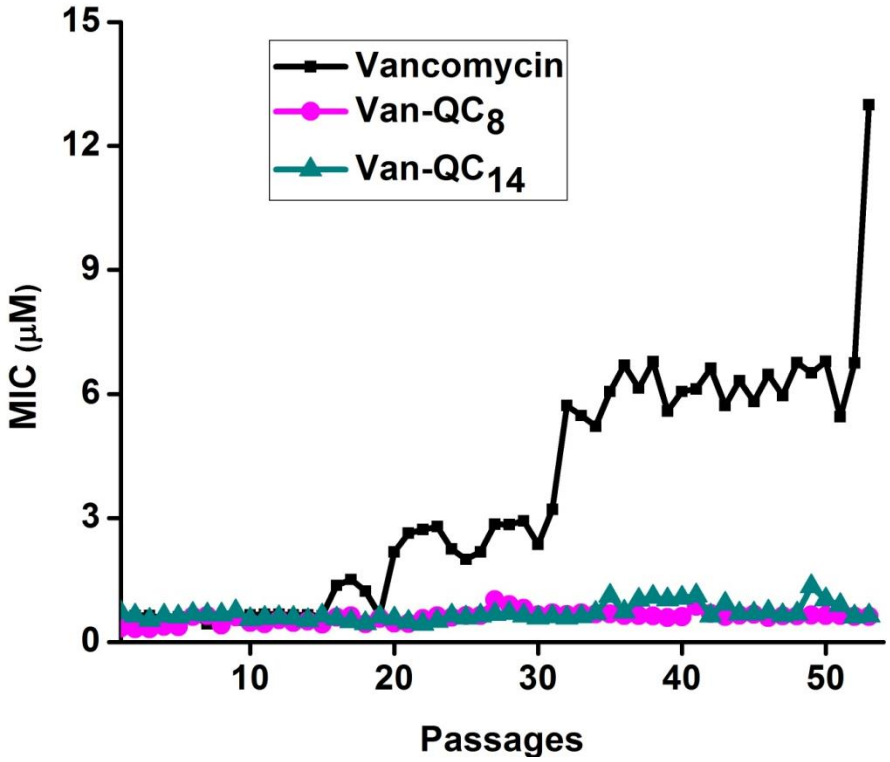
V. Yarlagadda et al. J. Med Chem. 2014, 57, 4558; . Int. J. Antimicrob. Agents 2015 , 45, 627
Patent: WO 2013072838 A1, US 2014 0308347, EP2780359 B1; . CA2855753 A1; Korea. 10-2014-7016259, Australia, India

Combating Acquired Resistance of Gram-positive Bacteria

Antibacterial activity



Resistance development in MRSA



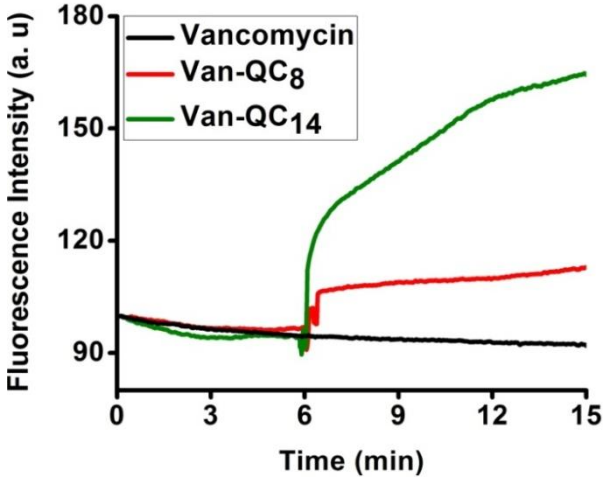
- 1000-fold more activity than vancomycin was achieved against VRE.
- Bacteria were unable to develop resistance even after 50 serial passages.

V. Yarlagadda et al. Int. J. Antimicrob. Agents 2015 , 45, 627.

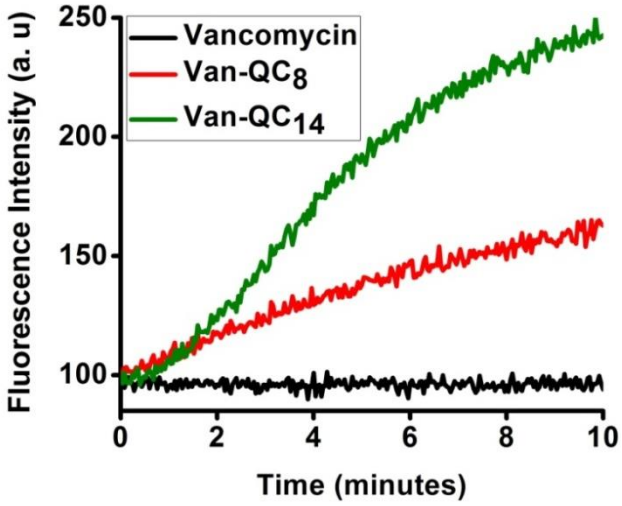
Bacterial Membrane Disruption- A New Mechanism of Action to Vancomycin & Cell Wall Inhibition

Bacterial Membrane Disruption

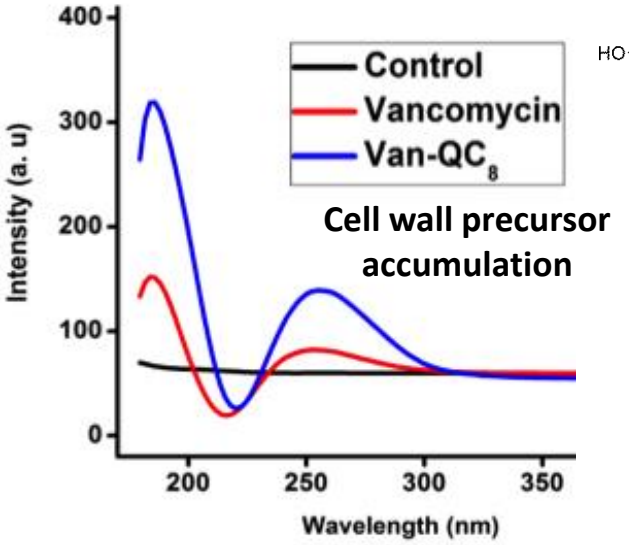
Membrane Depolarization



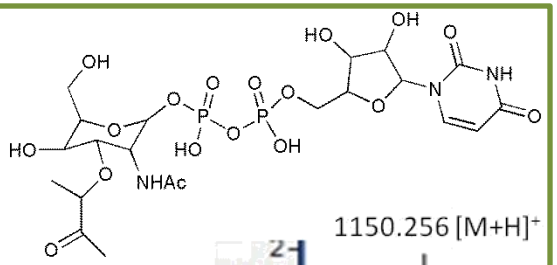
Inner Membrane Permeabilization



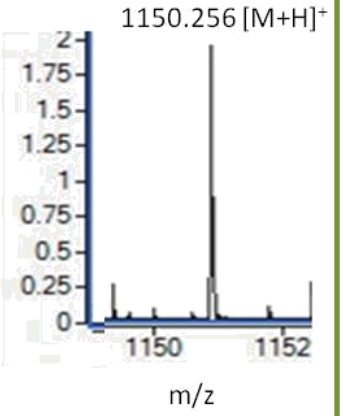
Cell Wall Inhibition

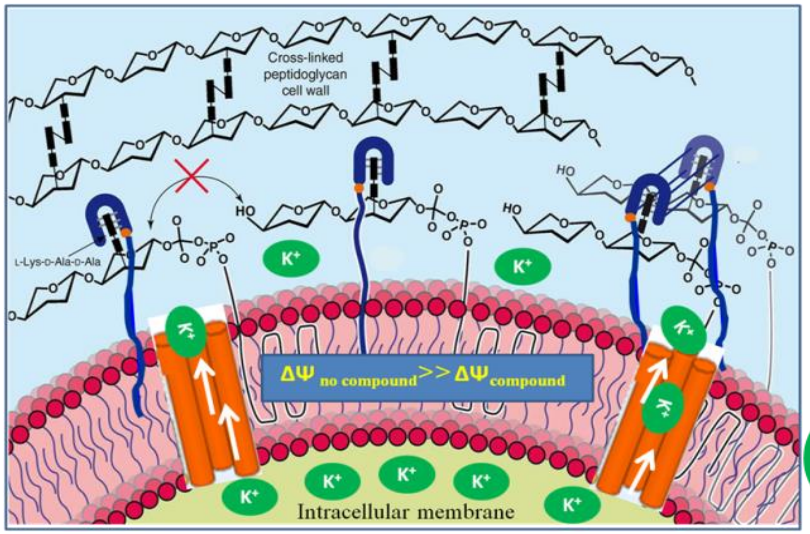


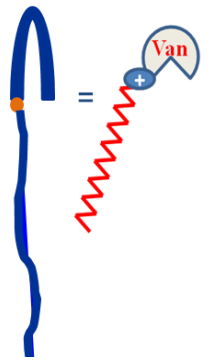
Cell wall precursor accumulation



L-Ala
D-Glu
L-Lys
D-Ala
D-Ala





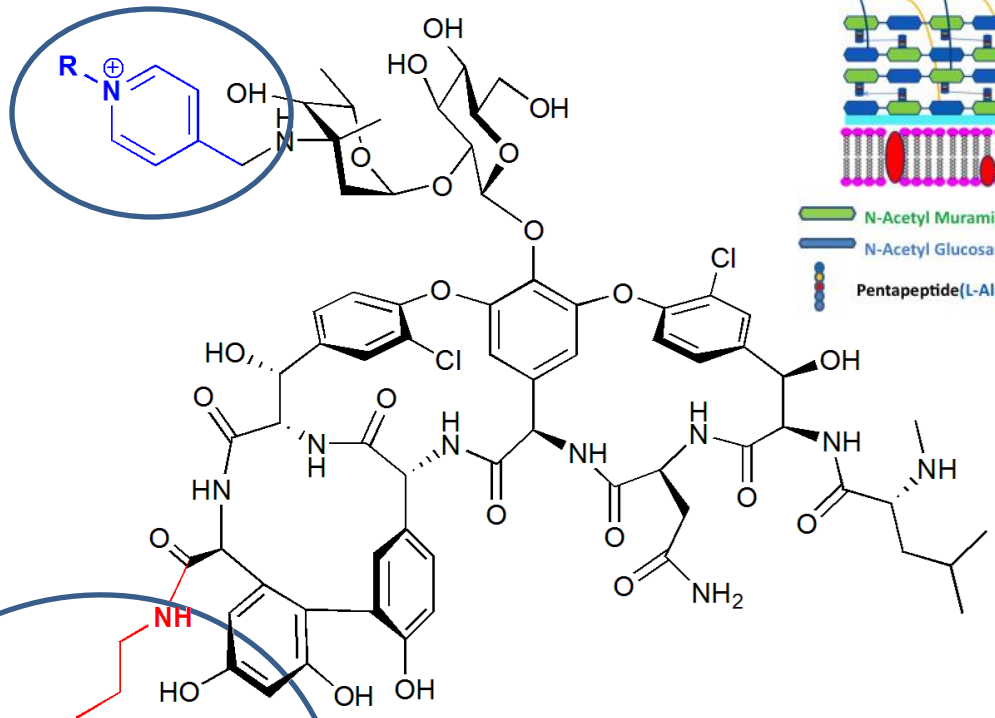


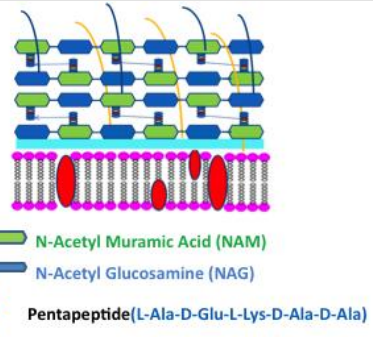
K+ = Potassium ion

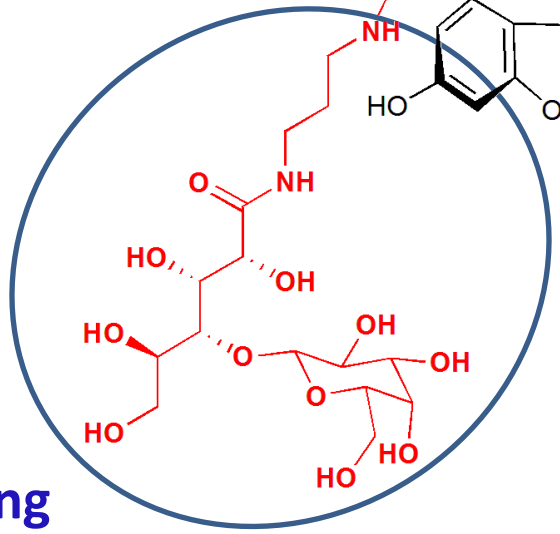
Invention-3: Improved Binding Affinity & New Membrane Disruption Action lead to Greater Cell Wall Inhibition

Permanently positive charged lipophilic moiety-

Strong membrane disruption







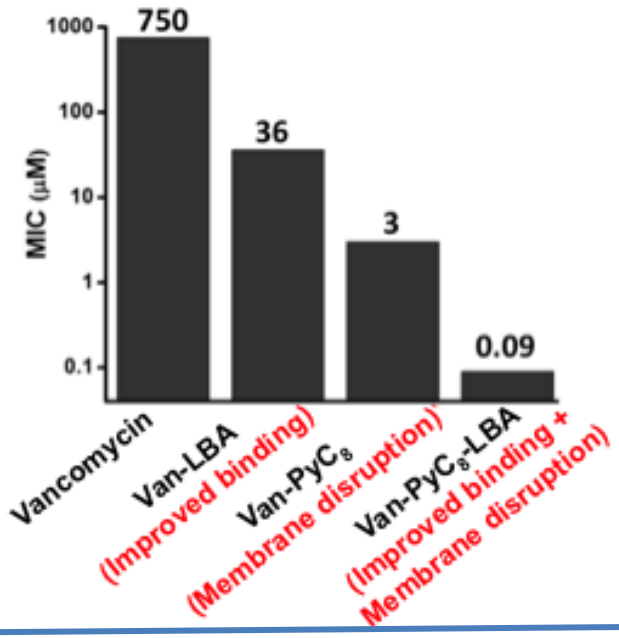
Sugar moiety-

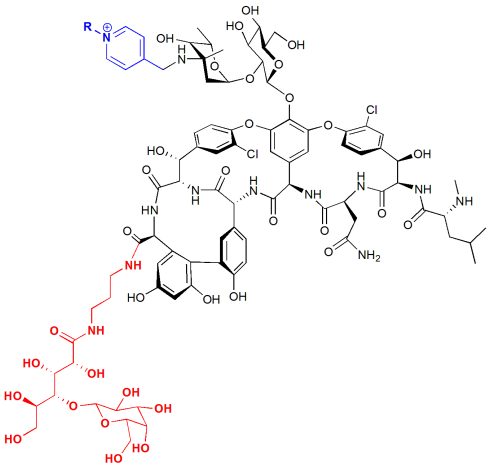
Improved binding affinity

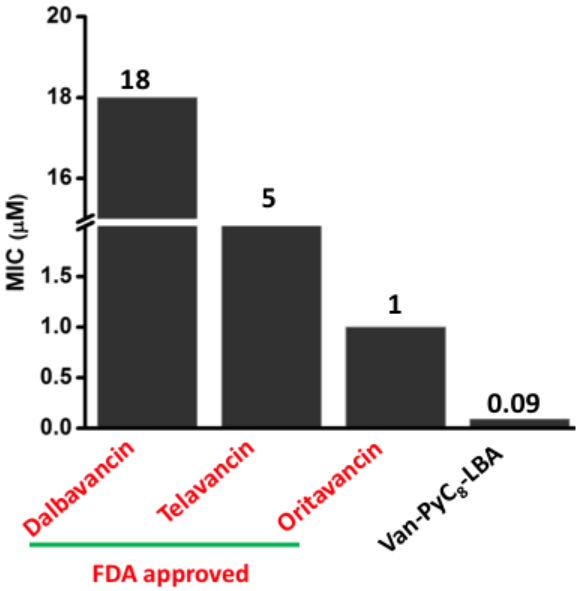
R = C_6H_{13} (Van-PyC_6-LBA)
C_8H_{17} (Van-PyC_8-LBA)
$C_{10}H_{21}$ (Van-PyC_{10}-LBA)
$C_{12}H_{25}$ (Van-PyC_{12}-LBA)
$C_{14}H_{29}$ (Van-PyC_{14}-LBA)

Four step synthesis

In-vitro Antibacterial Activity and Mechanism of Action against VRE

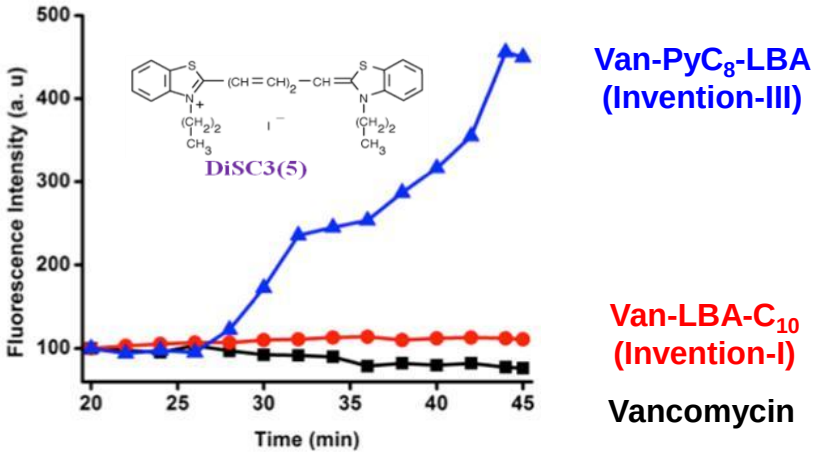






Mechanism of action

1. Membrane depolarization, permeabilization & K^+ ion leakage

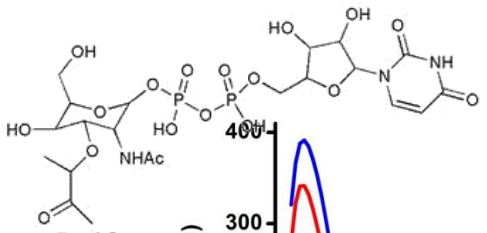


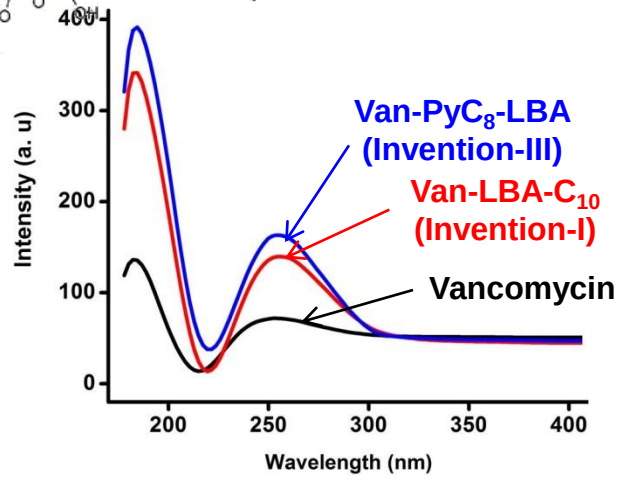
Van-PyC$_8$-LBA
(Invention-III)

Van-LBA-C$_{10}$
(Invention-I)

Vancomycin

2. Cell wall inhibition

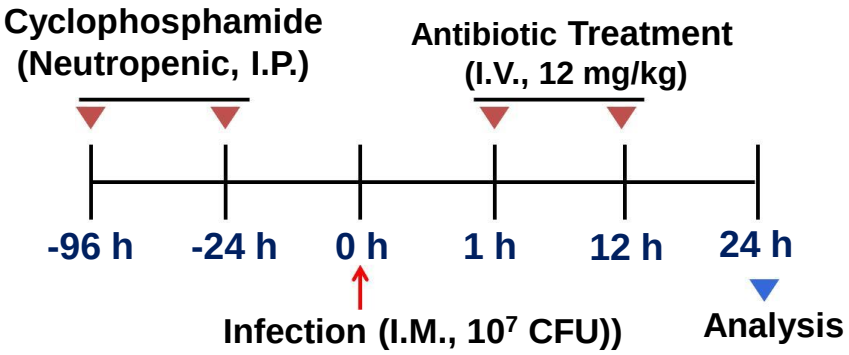


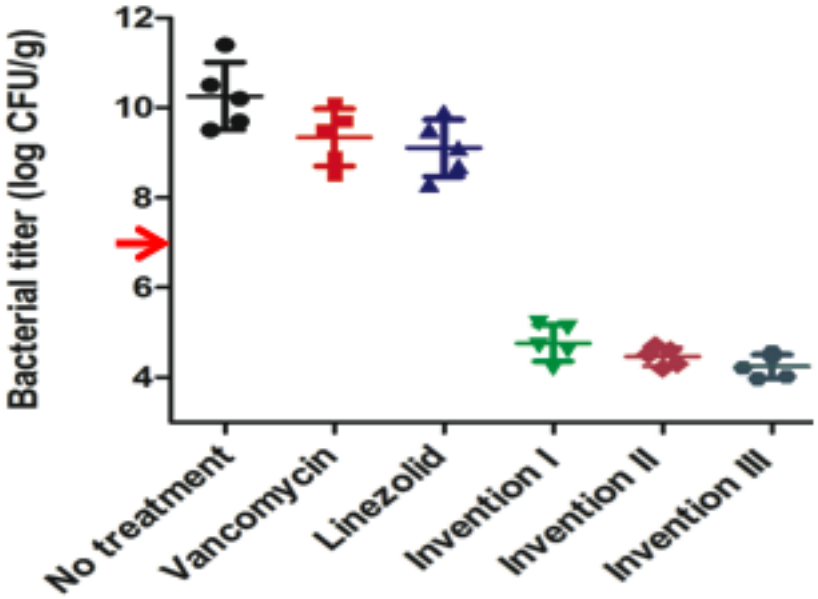


Patent Application no. 6565/CHE/2014

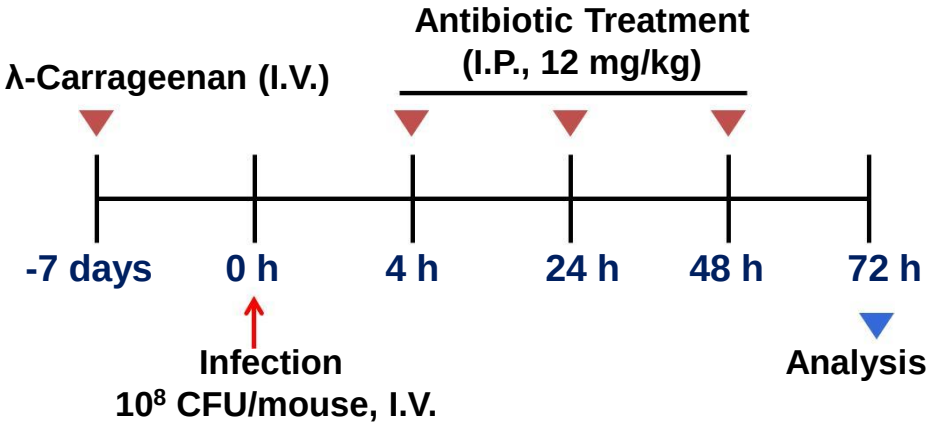
In-vivo Antibacterial Activity

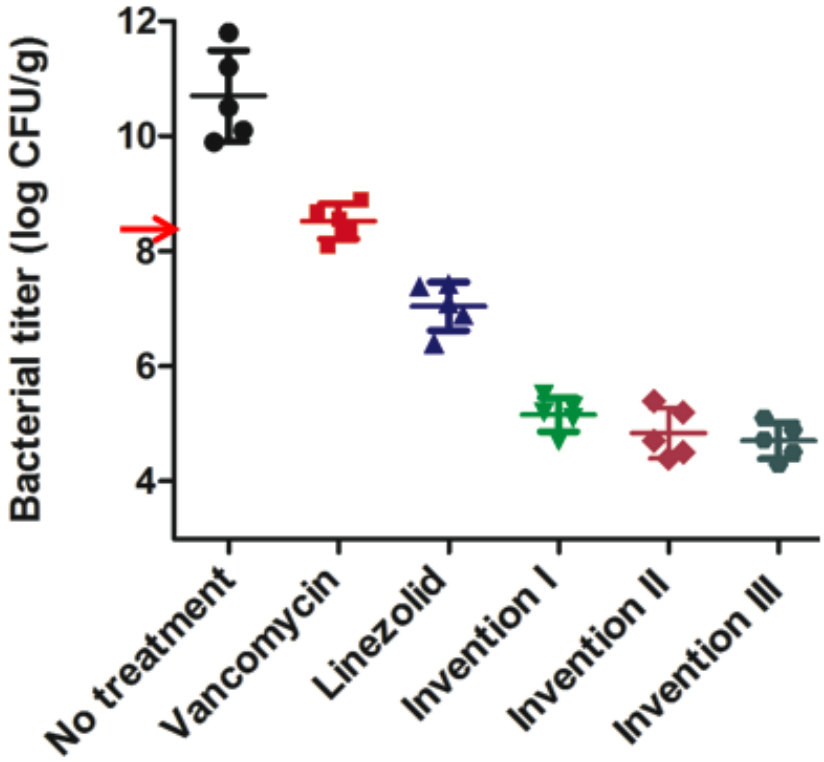
VISA tissue infection





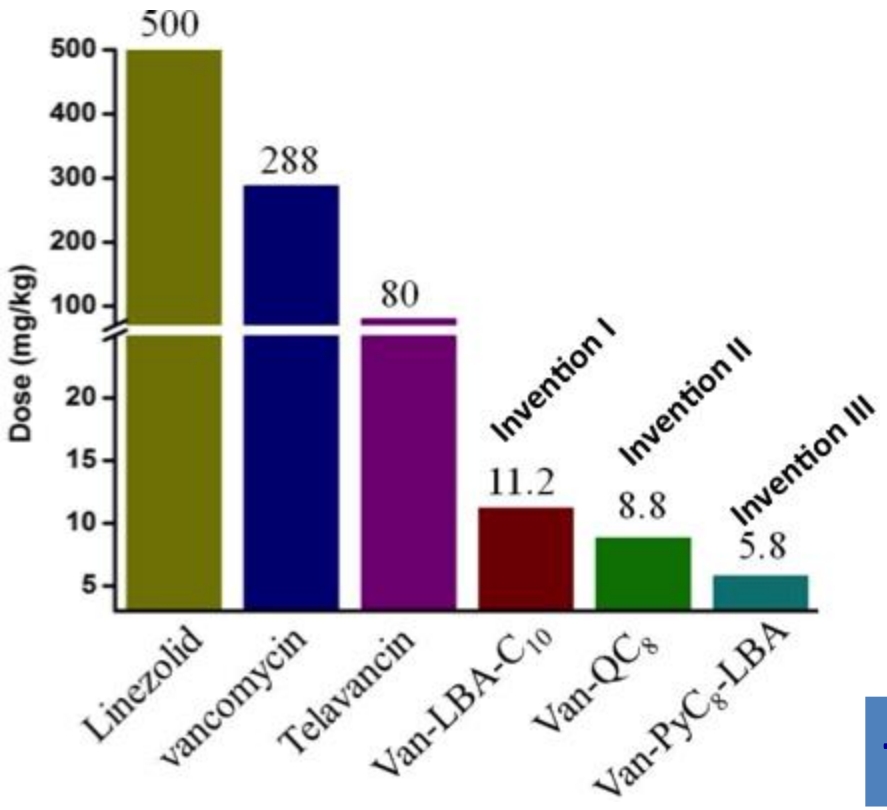
VRE kidney infection





PK-PD & Toxicity

Comparison of $ED_{2\text{-logkill}}$ against VISA



PK & PD study against VISA

- Van-LBA-$C_{10,}$ Van-QC8 and Van-PyC_8-LBA are 25-fold 30-fold and 49-fold more active than vancomycin on the basis of $ED_{2\text{-log kill}}$
- Half-life of Vancomycin, Van-LBA-$C_{10,}$ Van-QC8 and Van-PyC_8-LBA : 20 min, 2.76 h, 2.4 h & 1.7 h

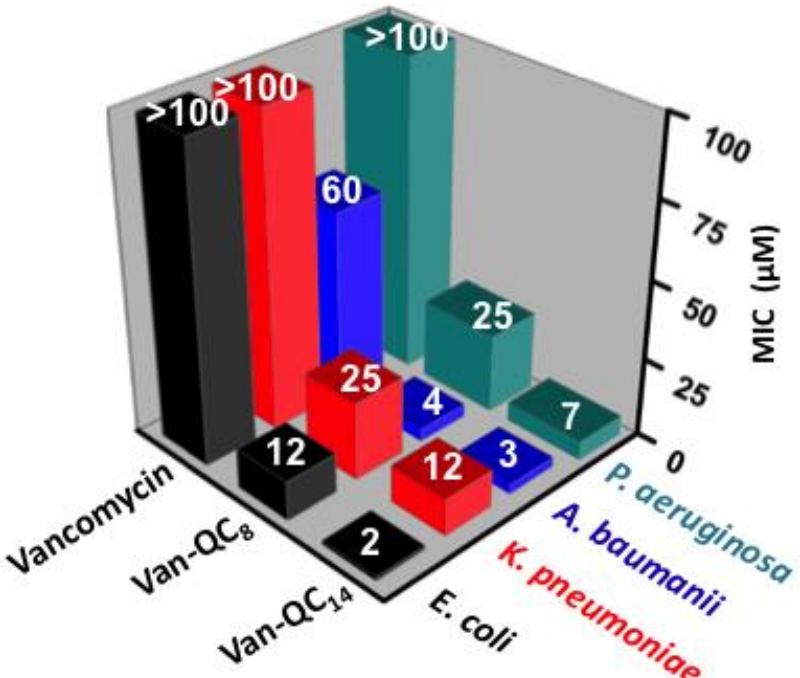
Toxicity

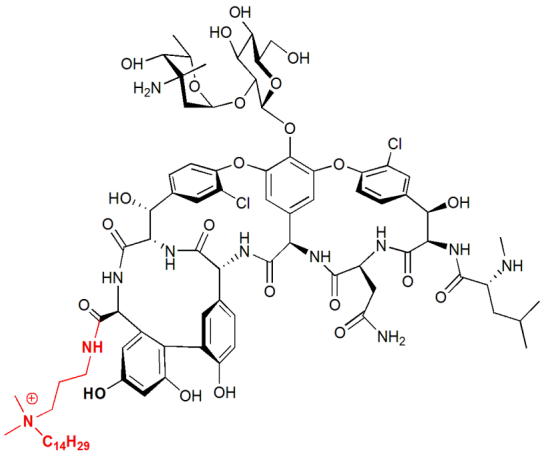
- No hemolysis and cytotoxicity up to 1000 µM
- LD_{50} > 100 mg/kg for Van-LBA-C_{10} and Van-PyC_8-LBA ; LD_{50} = 80 mg/kg for Van-QC8

V. Yarlagadda et al.
Patent: WO 2013072838 A1, US 2014 0308347, EP2780359 B1; . CA2855753 A1; Korea. 10-2014-7016259, Australia, India

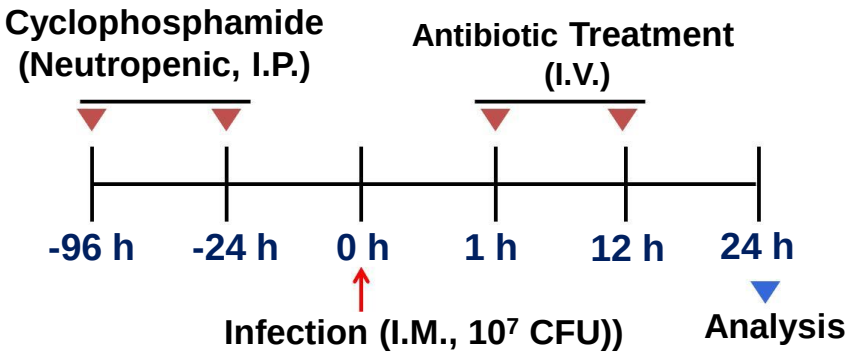
Semi-synthetic Glycopeptides Active against Gram-negative Bacteria

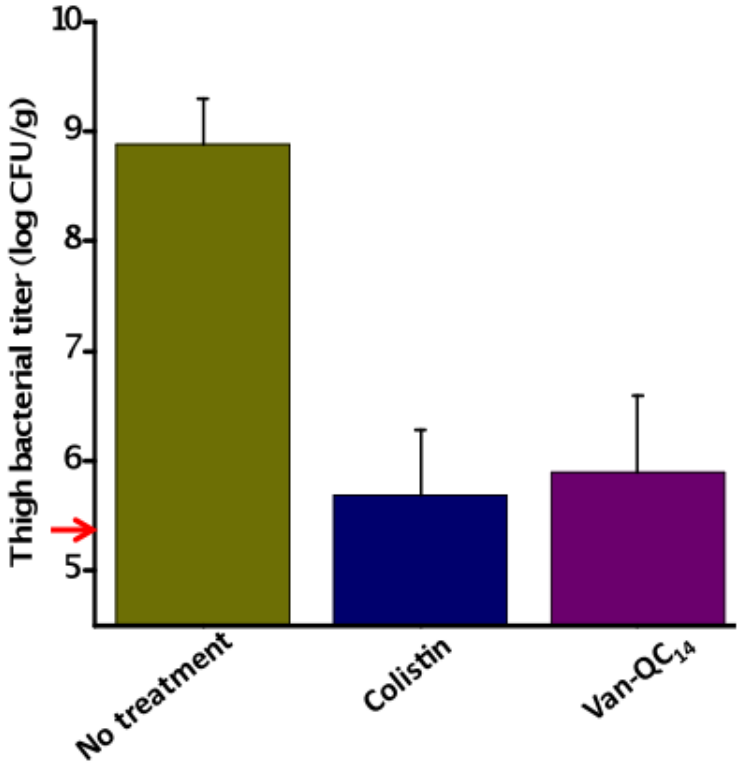
In-vitro Antibacterial activity





In-vivo activity *(A. baumannii)*





Strategies to Combat Drug Resistance and Infection

Prevention

- **Stop infections from spreading (Polymeric Paints)**
  - **Publication:** ACS Appl Mater Interfaces 2015
  - **Patent:** WO2015092520 A1

Treatment

- **Developing new drugs with novel targets and novel modes of action (Small Peptoids)**
  - **Publication:** ChemMedChem 2015; ACS Infec Dis 2015; J Med Chem 2015; Chem Commun 2015, J Med Chem 2014; J Med Chem 2014; Chem Commun 2013; Langmuir 2012
  - **Patent:** Invention-I: PCT/GB2015/050750, Invention-II: WO 2014 097178 A1

- **Strategies to make existing drugs better and overcome drug resistance**

a) Development of novel class of semi-synthetic glycopeptides

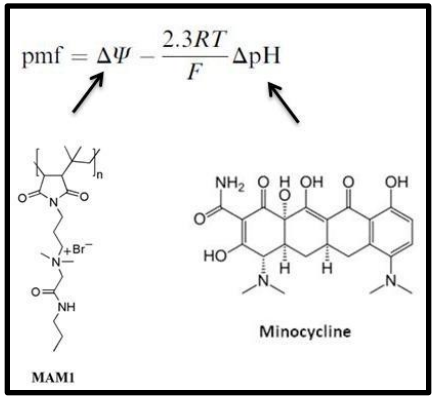
- **Publication:** Int J Antimicrob Agents 2015; J Antibiot 2015; Int J Antimicrob Agents 2015; J Med Chem, 2014
- **Patent:** Invention-I: WO 2013072838 A1, US 2014 0308347, EP2780359 B1; . CA2855753 A1; Korea. 10-2014-7016259, Australia, India; Invention-II: PCT 2014/10085; Invention-III PCT/IB2014/001835; Invention-IV: 6565/CHE/2014

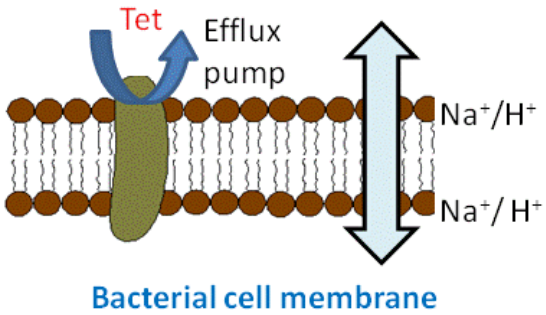
b) Combination therapy: obsolete antibiotics + novel efflux pump inhibitor

- **Publication:** Microb Pathog 2015; PLoS One 2015
- **Patent:** WO 2014 006601 A2 (India, Europe, USA, S. Korea)

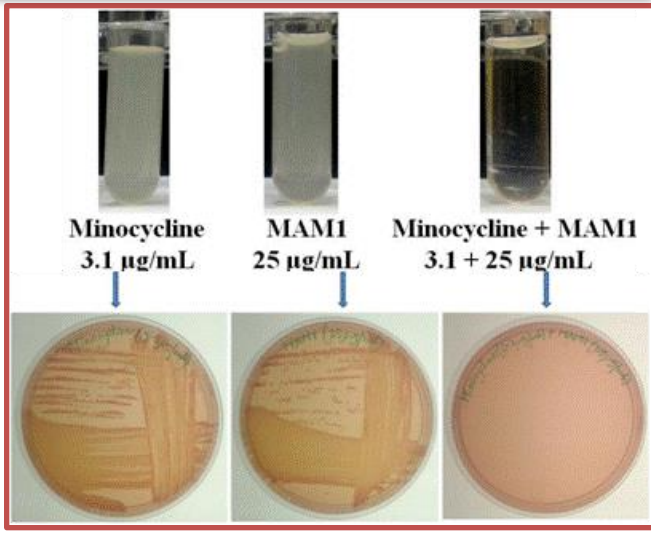
Resensitization of Tetracycline Antibiotics

- Tetracyclines - Protein biosynthesis inhibitors
- Resistance to tetracyclines - due to efflux pumps

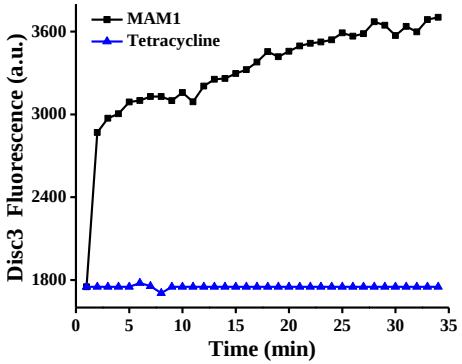




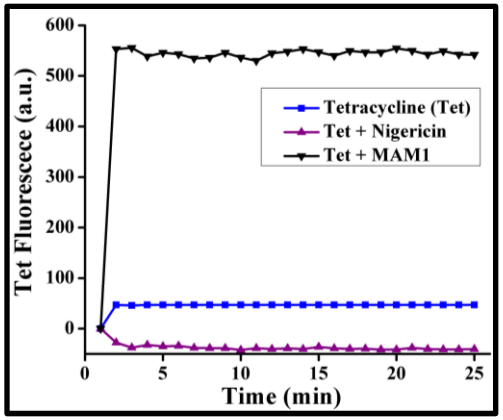
In-vitro activity
against NDM-1 *E. coli*



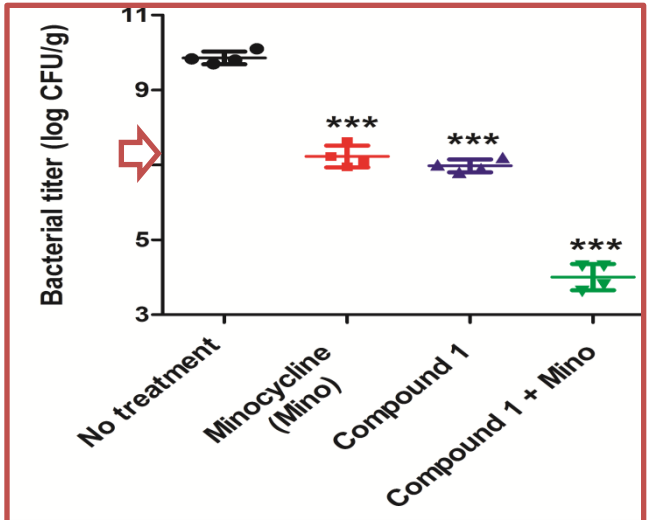
Dissipation of ΔΨ



Tetracycline uptake



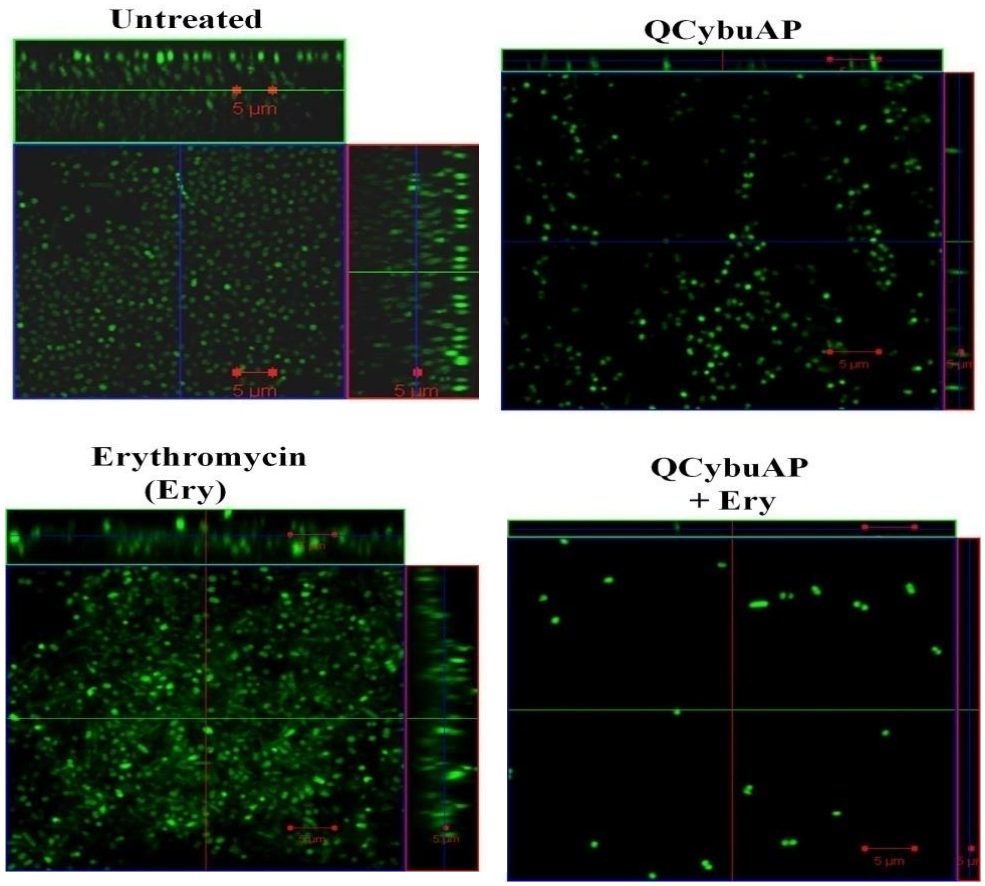
In-vivo activity
skin infection (*A. baumannii*)



Divakara, SSMU et. al. Chem. Commun. 2013, 49, 9389
Divakara, SSMU et. al. PLos One 2015, 10, e0119422
Patent WO 2014 006601 A2 (India, Europe, USA, S. Korea)

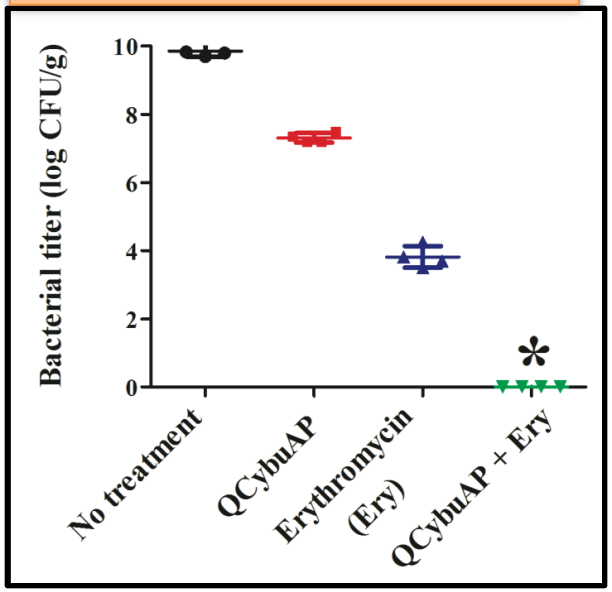
Combination Therapy: Obsolete Antibiotics + Novel Efflux Inhibitor

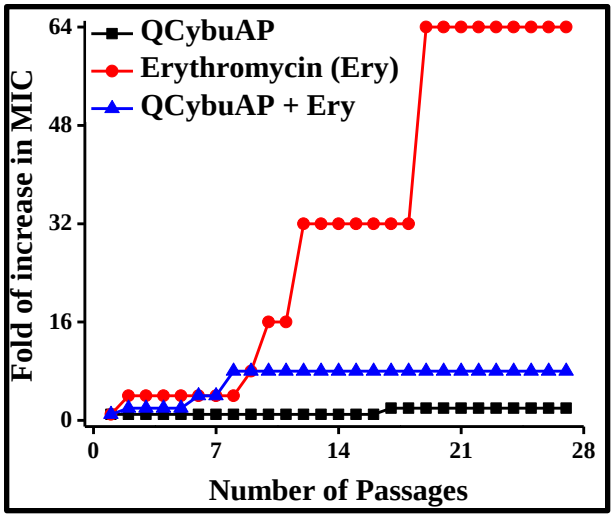
***A. boumanii* Biofilms**



Combination of MAMs and antibiotics is effective even against biofilms whereas antibiotics fail

In-vivo activity
skin infection (*A. baumannii*)





Bacteria do not develop resistance to MAMs and MAMs stall bacterial resistance to antibiotics

Patent WO 2014 006601 A2 (India, Europe, USA, S. Korea)

Acknowledgement

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Sandip Samaddar

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Goutham B M

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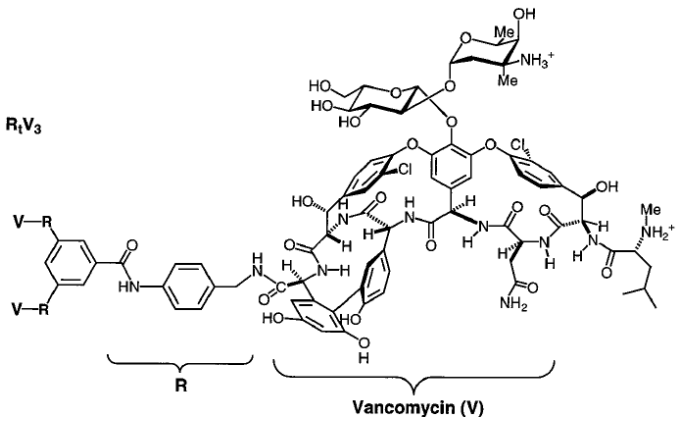
JNCASR, DST, SSL



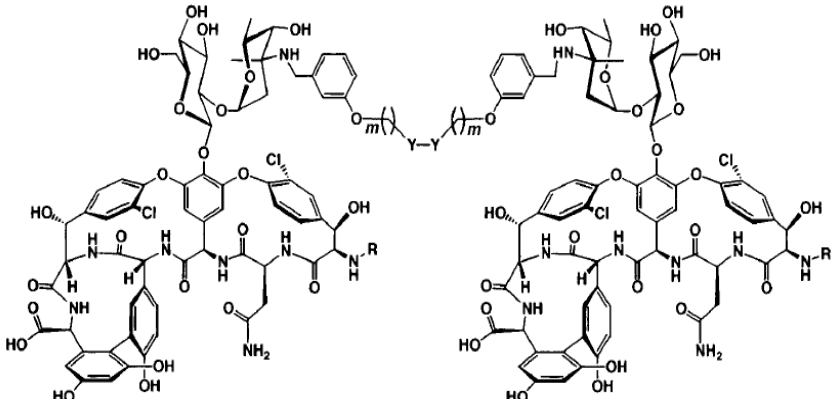
Back Up

Semi-synthetic Glycopeptides to Enhance the Binding Affinity

Multivalency Approach



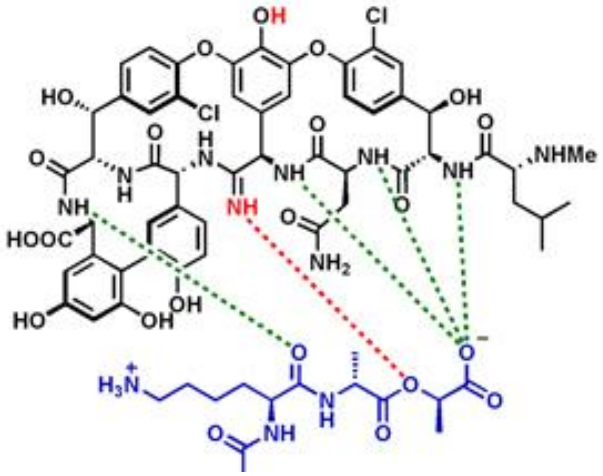
G. M. Whitesides *et al. (Harvard University)*
Science, 1998, 280,708



K. C. Nicolaou *et al. (Scripps Research Institute)*
Angew. Chem. Int. Ed, 2000, 39, 3823

❖ **Not active against more virulent bacteria**

Vancomycin Amidine



D. L. Boger *et al. (Scripps Research Institute)*
JACS, 2011, 133, 13946

~75 step synthesis

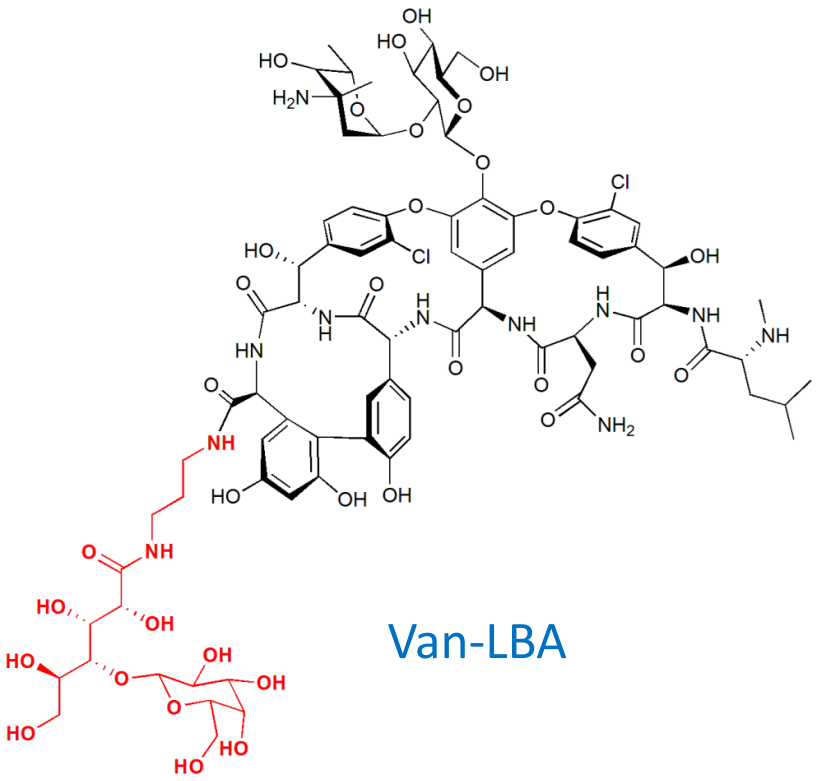
Current Treatment for VISA, VRSA & VRE

- Daptomycin for VISA & VRSA
- Ceftraroline
- Quinupristin/Dalfopristin for VRE
- Linezolid

❖ **Unfortunately resistance to even these last line antibiotics has been reported in clinical settings**

Improved Binding Affinity towards Cell Wall Peptides

Compound	Association Constant (K_a, M^{-1})	
	Sensitive	Resistant
Vancomycin	1.1×10^5	**5.0×10^2**
Van-cy-Glu	1.4×10^5	9.1×10^2
Van-cy-Gal	1.2×10^5	8.5×10^2
Van-CB	0.5×10^5	12×10^2
Van-Mal	0.8×10^5	11×10^2
Van-acy-Glu	**2.2×10^5**	**6.3×10^4**
Van-LBA	**2.1×10^5**	**8.8×10^4**



Van-LBA

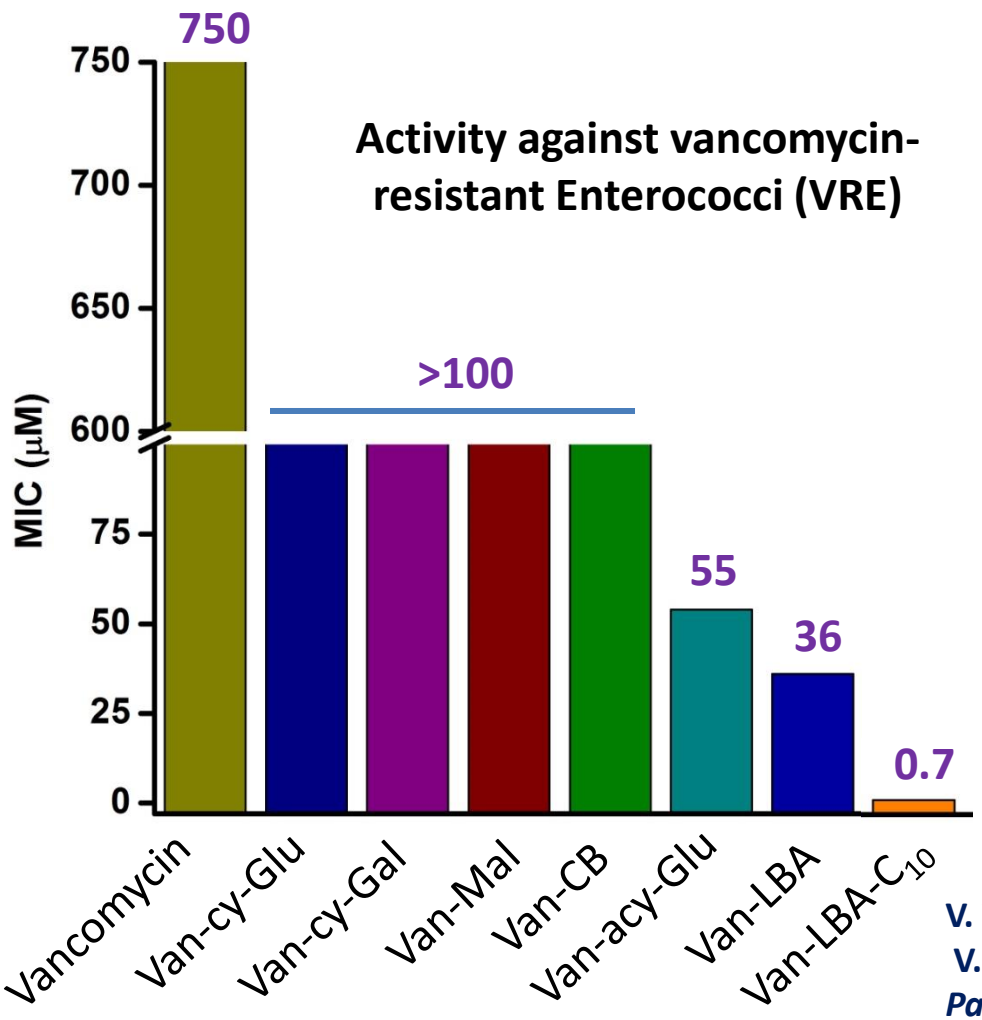
N,N'-diacetyl-Lys-D-Ala-D-Ala: Susceptible bacteria

N,N'-diacetyl-Lys-D-Ala-D-Lac: Resistant bacteria

❖ Improved binding affinity of about 2-orders of magnitude over vancomycin was observed against *N,N*-Diacetyl-Lys-Ala-Lac.

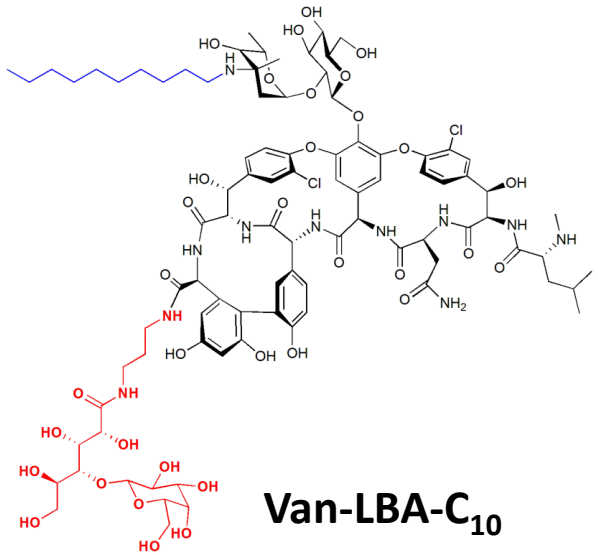
In-vitro Antibacterial Activity

❖ Exhibited slightly better antibacterial efficacy than vancomycin against sensitive strains (Staphylococci and Enterococci)



Activity against vancomycin-resistant Enterococci (VRE)

Addition of hydrophobicity to vancomycin leads to stronger association with bacterial membrane

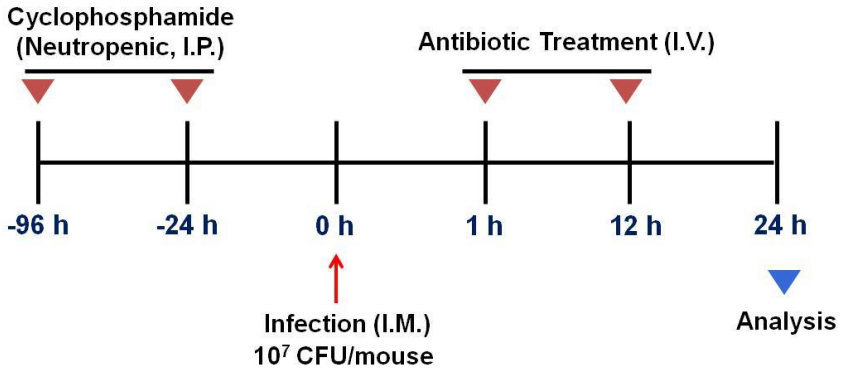


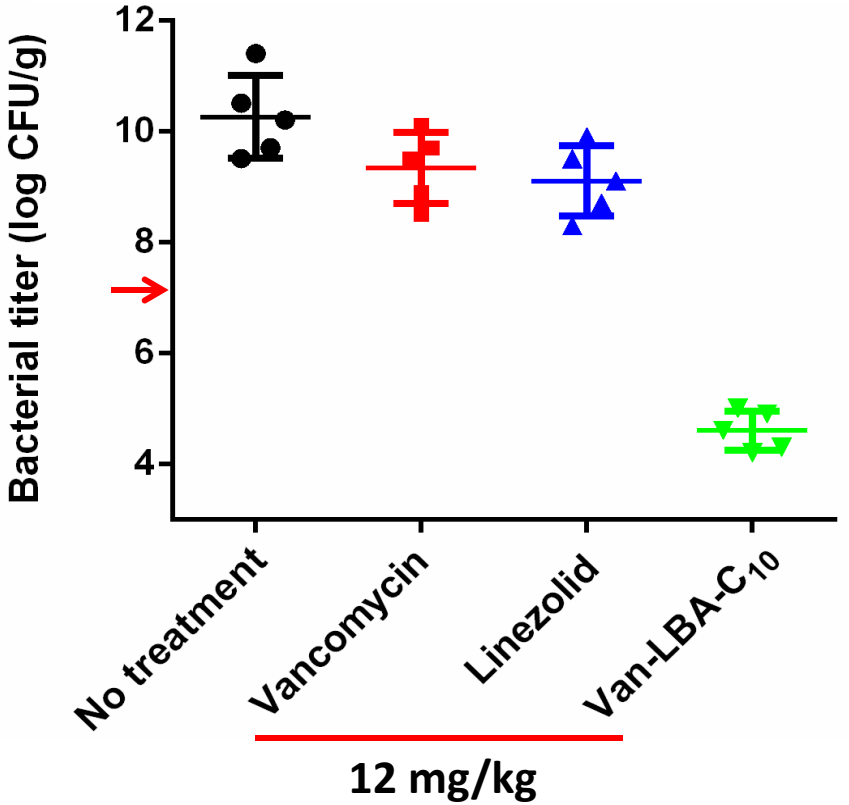
Van-LBA-C_{10}

V. Yarlagadda *et al. J. Antibiotics 2014, 68, 302 &*
V. Yarlagadda *et al. Int. J. Antimicrob. Agents 2015 45, 627*
Patent: **PCT 2014/10085**

In-vivo Antibacterial Activity of Van-LBA-C_{10}

VISA tissue infection





VRE kidney infection

