Drug Design Hot Paper

Deutsche Ausgabe: DOI: 10.1002/ange.201507567
Internationale Ausgabe: DOI: 10.1002/anie.201507567

Membrane Disruption and Enhanced Inhibition of Cell-Wall Biosynthesis: A Synergistic Approach to Tackle Vancomycin-Resistant Bacteria

*Venkateswarlu Yarlagadda, Sandip Samaddar, Krishnamoorthy Paramanandham, Bibek R. Shome, and Jayanta Haldar**

Abstract: *Resistance to glycopeptide antibiotics, the drugs of choice for life-threatening bacterial infections, is on the rise. In order to counter the threat of glycopeptide-resistant bacteria, we report development of a new class of semi-synthetic glycopeptide antibiotics, which not only target the bacterial membrane but also display enhanced inhibition of cell-wall biosynthesis through increased binding affinity to their target peptides. The combined effect of these two mechanisms resulted in improved in vitro activity of two to three orders of magnitude over vancomycin and no propensity to trigger drug resistance in bacteria. In murine model of kidney infection, the optimized compound was able to bring bacterial burden down by about 6 logs at 12 mg kg^{-1} with no observed toxicity. The results furnished in this report emphasize the potential of this class of compounds as future antibiotics for drug-resistant Gram-positive infections.*

Vancomycin, a glycopeptide antibiotic, has been long considered as the "Antibiotic of Last Resort" for the treatment of lethal infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA).[1] The extensive use of vancomycin for MRSA infections resulted in reduced susceptibility to vancomycin, which may be associated with vancomycin treatment failure against vancomycin intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA).[2] Additionally, the emergence of vancomycin-resistant Enterococci (VRE) is a growing threat worldwide.[3] At present, only a few drugs such as daptomycin, ceftaroline, quinupristin/dalfopristin, and linezolid are available for the treatment of infections caused by VISA, VRSA, and VRE but resistance to even these last line antibiotics in bacteria has been reported in clinical settings.[4,5] This persistent threat of drug resistance has triggered a lot of interest in the scientific community to develop various strategies to tackle the problem.[6–12]

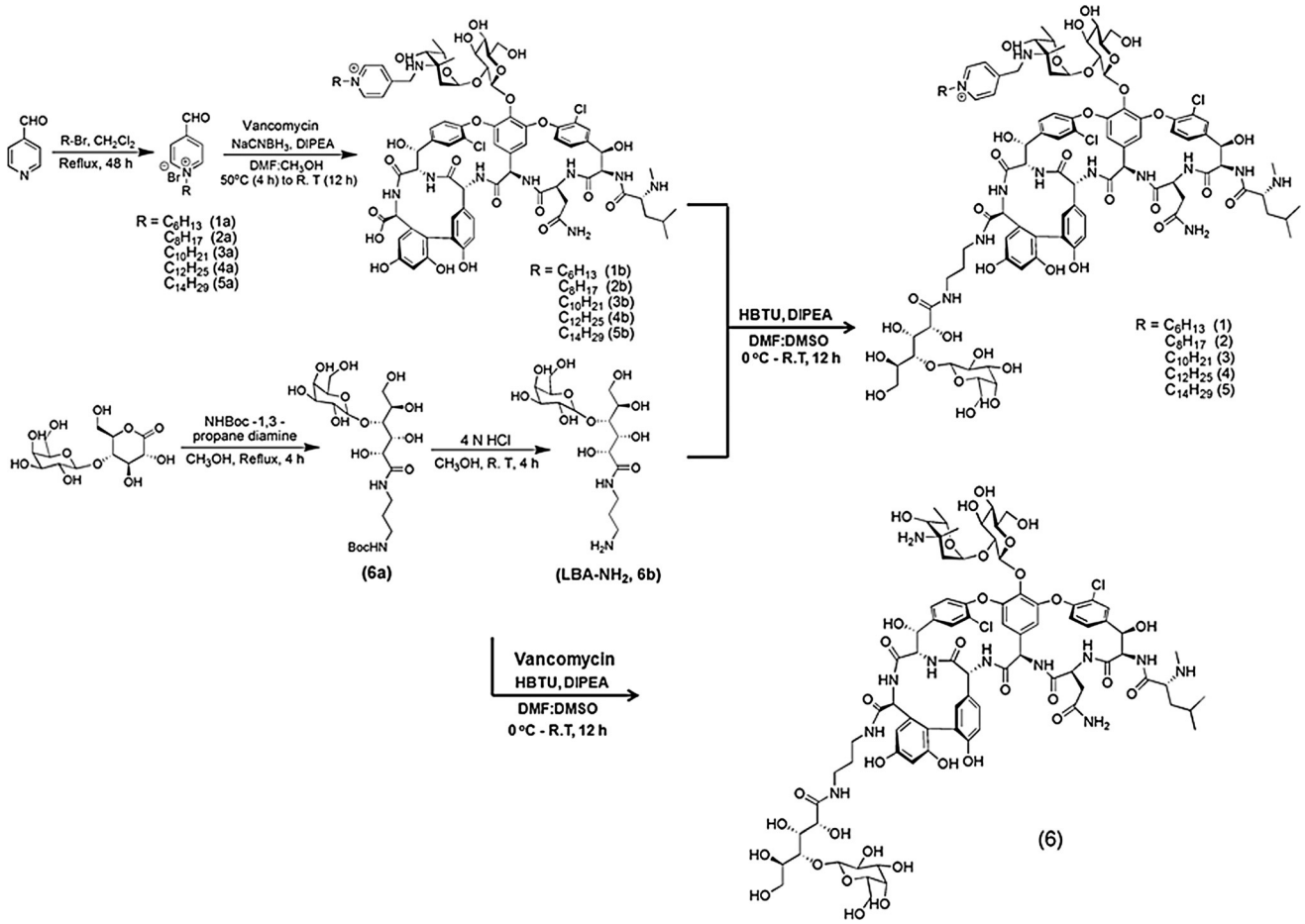
Vancomycin binds to D-Ala-D-Ala terminus of peptidoglycan pentapeptide of the bacterial cell wall through five hydrogen bonds, sequesters the substrate from transpeptidases, and inhibits cell-wall crosslinking.[1] Over the time, bacteria developed resistance to the drug by alteration of cell-wall precursors from D-Ala-D-Ala to D-Ala-D-Lac (depsipeptide, VanA and VanB phenotypes of vancomycin-resistant bacteria; VRB) which leads to manifold reduction in the binding constant of vancomycin to its target and results in >1000-fold loss of antibacterial activity.[13] Divalent, trivalent, and polyvalent vancomycin derivatives have been shown to have improved binding affinity towards resistant bacterial ligand (D-Ala-D-Lac) but these derivatives did not have appreciable activity against more virulent VanA phenotypic VRB.[14] Boger and co-workers developed vancomycin aglyconamidine exhibiting dual binding affinity to both D-Ala-D-Ala and D-Ala-D-Lac and the derivative showed potent antibacterial activity against VanA-resistant VRE strain.[15] Recently, we developed various vancomycin derivatives to enhance the overall binding constant with the target peptide, thereby reinforcing the activity of the drug against VRB.[16]

An alternate approach to tackle vancomycin resistance is to incorporate bacterial membrane disruption properties to vancomycin.[17,18] Semi-synthetic lipoglycopeptides such as oritavancin, dalbavancin, and telavancin were shown to have bacterial membrane disruption property at high concentrations (10-fold higher than MIC) due to which they exhibited high activity against resistant strains.[18,19] Recently we have developed vancomycin derivatives having strong membrane disruption properties at sub-MIC value (<0.5 μM) and were shown to have high activity against various VRB.[20]

In the present report, with an aim to effectively combat VRB infections, we have developed permanent positively charged lipophilic vancomycin–sugar conjugates (Scheme 1), wherein a sugar moiety is appended to the carboxylic group of vancomycin in order to enhance the binding affinity towards target depsipeptide and a permanent positively charged lipophilic moiety is conjugated to the amine group of vancosamine in order to confer strong membrane disruption properties. We have demonstrated that the two effects act synergistically and with this combined approach, we could achieve >8000-fold more activity than vancomycin against VRE. An optimized compound showed high in vivo antibacterial activity against VRE kidney infection in mouse models.

[*] V. Yarlagadda, Dr. S. Samaddar, Dr. J. Haldar
Chemical Biology and Medicinal Chemistry Laboratory
New Chemistry Unit
Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR)
Jakkur, Bengaluru 560064, Karnataka (India)
E-mail: jayanta@jncasr.ac.in
Dr. K. Paramanandham, Dr. B. R. Shome
National Institute of Veterinary Epidemiology and
Disease Informatics (NIVEDI)
Yelahanka, Bengaluru 560064 (India)

Supporting information for this article, containing synthetic procedures, characterization data, and experimental details, is available on the WWW under http://dx.doi.org/10.1002/anie.201507567.



Scheme 1. Synthesis of permanent positively charged lipophilic vancomycin conjugates (**1b**–**5b**), vancomycin–sugar conjugate (**6**) and permanent positively charged lipophilic vancomycin–sugar conjugates (**1**–**5**). DIPEA = *N*,*N*-diisopropylethylamine; HBTU = *O*-(1*H*-benzotriazoyl-1-yl)-*N*,*N*,*N*′,*N*,′-tetramethyluronium hexafluorophosphate.

Permanent positively charged lipophilic vancomycin–sugar conjugates (Scheme 1, **1**–**5**) were synthesized by appending a positively charged lipophilic moiety to the amine group of vancosamine and further by conjugating a lactobiono sugar moiety to the C-terminal of vancomycin. Initially, *N*-alkylation of 4-pyridine carboxaldehyde was performed with various alkyl (hexyl, octyl, decyl, dodecyl, and tetradecyl) bromides to form compounds **1a**–**5a**, which were coupled to vancomycin through Schiff's base formation followed by reduction to give permanent positively charged lipophilic vancomycin analogs (**1b**–**5b**). Subsequently, lactobionolactone was reacted with *N*-Boc-1,3-propanediamine to yield compound **6a** followed by deprotection of *N*-Boc, to give compound **6b**, which were finally coupled to compounds **1b**–**5b** using *O*-(1*H*-benzotriazoyl-1-yl)-*N*,*N*,*N*′,*N*,′-tetramethyluronium hexafluorophosphate (HBTU) to afford compounds **1**–**5**. All the compounds were purified by reverse phase HPLC to more than 95% purity and characterized by ^{1}H NMR spectroscopy and HRMS. A vancomycin–sugar conjugate (compound **6**, Scheme 1) was also prepared wherein vancomycin was conjugated to a sugar moiety, **6b**, which does not have a permanent positively charged lipophilic moiety.

The antibacterial activities of all compounds were determined against vancomycin-sensitive and vancomycin-resistant bacterial strains including drug-resistant clinical isolates. The results are summarized in Table 1, and in Tables S1 and S2 in the Supporting Information. Compounds **1b**–**5b** bearing a permanent positively charged lipophilic moiety, were found to be 2 to 3-fold more active than vancomycin against vancomycin-sensitive strains with the MICs varying from 0.1–0.4 μM ($p < 0.01$). Further these compounds showed high activity against MRSA with the MIC of 0.1 to 0.2 μM. In case of VISA, most of these compounds were found to be 40 to 130-fold more active than vancomycin, the lowest MIC being 0.1 μM ($p < 0.01$). When tested against VRE (VanA phenotype) and VRE (VanB phenotype), these compounds exhibited MIC in the range of 0.8–15 μM and 1.0-12 μM respectively. The activity increased with increase in chain length of the lipophilic moiety.

In our previous study,[16] wherein lactobiono sugar moiety was conjugated to vancomycin, compound **6** exhibited an MIC of 36 μM, which is about 25-fold more active than vancomycin against VRE. This increase in activity was attributed to higher binding affinity towards the target peptide of VRB. Therefore, it was envisioned that incorporation of a lactobiono sugar moiety to compounds **1b**–**5b** also might aid in higher binding affinity towards bacterial target peptides. Thus, compounds **1**–**5** bearing both a permanent positively charged lipophilic moiety and a sugar moiety were

Table 1: In vitro antibacterial activity of the compounds.

Compound	MRSA[a]	VISA[b]	VSE[c]	VREm[d]	VREs[e]
Vancomycin	0.78 ± 0.02	13.5 ± 2.1	0.60 ± 0.02	750 ± 8.3	250 ± 3.6
1b	0.22 ± 0.03	0.25 ± 0.05	0.40 ± 0.03	14.5 ± 1.9	12.3 ± 1.65
2b	0.10 ± 0.02	0.11 ± 0.02	0.31 ± 0.03	2.9 ± 0.1	6.2 ± 0.4
3b	0.12 ± 0.01	0.13 ± 0.01	0.20 ± 0.02	1.8 ± 0.4	3.3 ± 0.6
4b	0.22 ± 0.03	0.25 ± 0.03	0.22 ± 0.04	1.0 ± 0.1	1.5 ± 0.4
5b	0.10 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.75 ± 0.05	1.2 ± 0.2
1	0.10 ± 0.01	0.20 ± 0.04	0.20 ± 0.04	3.3 ± 0.43	2.5 ± 0.5
2	0.09 ± 0.02	0.10 ± 0.01	0.11 ± 0.01	0.09 ± 0.02	1.3 ± 0.25
3	0.20 ± 0.04	0.09 ± 0.02	0.12 ± 0.02	0.30 ± 0.03	1.3 ± 0.3
4	0.29 ± 0.02	0.31 ± 0.01	0.13 ± 0.02	0.41 ± 0.02	1.1 ± 0.2
5	0.10 ± 0.01	0.20 ± 0.03	0.15 ± 0.01	0.55 ± 0.05	0.95 ± 0.15
6	0.44 ± 0.05	0.31 ± 0.04	0.32 ± 0.03	36.3 ± 3.5	30.7 ± 2.3
Dalbavancin	0.04	N.D.[f]	0.08	> 18	0.12
Telavancin	0.3	N.D.[f]	0.15	4.0	1

[a] Methicillin-resistant *S. aureus*, [b] vancomycin-sensitive *E. faecium*, [c] vancomycin-intermediate-resistant *S. aureus*, [d] vancomycin-resistant *E. faecium*, VanA and [e] vancomycin-resistant *E. faecalis*, VanB. Data [MIC_{90}] for dalbavancin and telavancin was taken from Ref. [18]. [f] No data available.

synthesized and their antibacterial activities were determined. These compounds showed improved antibacterial activities compared to their respective homologous compounds (**1b–5b**) against all the bacteria tested. In case of vancomycin-sensitive bacteria, compounds **1–5** showed slightly better activity compared to compounds **1b–5b** whereas enhanced activity was observed against VRB. Compound **2** bearing an octyl chain showed the best activity against MRSA with the MIC of 0.09 μM. In case of VISA, compounds **2** and **3** displayed good activity with the MIC of about 0.1 μM. When checked against VRE (VanA phenotype), compound **2** showed high activity with the MIC of 0.09 μM whereas vancomycin was active at 750 μM. However, further increase in lipophilic chain length, did not enhance the activity significantly against VRE as seen for compounds **3–5** (MIC = 0.3–0.4 μM). Compounds **1–5** also showed good activity against VRE (VanB phenotype) with MIC in the range of 0.9–2.5 μM. Further, these compounds (**1-5**) displayed high activity against multidrug-resistant clinical isolates of Staphylococci (*S. aureus*, *S. haemolyticus*, and *S. epidermidis*) with the MIC of < 0.3 μM. Compound **2** displayed the best activity exhibiting the MIC of about 0.1 μM (Table S2).

The glycopeptide (compound **6)** which bears a sugar moiety but is devoid of permanent positively charged lipophilic moiety and compound **2b** comprising permanent positively charged lipophilic (octyl) moiety but lacks sugar moiety showed MIC of 36 μM and 2.9 μM, respectively, against VREm, which are about 25 and 300-fold more active than vancomycin. Glycopeptide **2**, on the other hand, (comprising both sugar moiety and permanent positively charged lipophilic moiety) was > 8000-fold more active than vancomycin. Therefore, this enhanced activity of compound **2** is attributed to the synergistic action of incorporated sugar moiety and permanent positively charged lipophilic moiety. On the other hand, the MIC_{90} values of telavancin and dalbavancin against VREm (VanA phenotype) were found to be 4 and 18 μM, respectively (Table 1).

To check our hypothesis, we had evaluated the binding constants of compound **6** and the best compound of this series (**2**) using UV-difference spectroscopy against model ligands which represent the target peptides found in sensitive and resistant bacteria: *N,N′*-diacetyl-Lys-D-Ala-D-Ala and *N,N′*-diacetyl-Lys-D-Ala-D-Lac, respectively (Figures S1–S4). The binding affinities of compounds **2** and **6** were found to be 2-fold higher than vancomycin against *N,N′*-diacetyl-Lys-D-Ala-D-Ala. When evaluated against *N,N′*-diacetyl-Lys-D-Ala-D-Lac, the binding affinity of compounds **2** and **6** were ≥ 150-fold ($5.7 \times 10^4\,M^{-1}$ and $8.8 \times 10^4\,M^{-1}$) higher than vancomycin ($5 \times 10^2\,M^{-1}$). This result suggests that conjugation of sugar moiety improved the binding affinity of the compound towards target peptides and this was irrespective of the presence of the permanent positively charged lipophilic moiety.

Next, we investigated membrane disruptive properties of compounds **1–6** (Figure 1 A–C), compounds **1b-5b** (Figure S5) and vancomycin at 5 μM, using fluorescence spectroscopy against VRE. First, the ability of the compounds to depolarize VRE membranes has been examined using a membrane-potential-sensitive dye $DiSC_3(5)$ (3,3′-dipropylthiadicarbocyanine iodide). An increase in fluorescence was observed upon dissipation of the membrane potential after addition of test compounds. Unlike in case of vancomycin and compound **6**, all the new compounds were able to dissipate the bacterial membrane potential (Figure 1 A and Figure S5 A). Bacterial cytoplasmic membrane permeabilization was studied using the fluorescent probe propidium iodide. Unlike vancomycin and compound **6**, which did not cause significant membrane permeability, compounds **1–5** and compounds **1b–5b** showed strong ability to permeabilize the cytoplasmic membrane of VRE. Then, we investigated the release of intracellular potassium ion caused by the compounds using a potassium-ion-sensitive fluorophore, PBFI-AM compared with a positive control, valinomycin. Compounds **1–5** (Figure 1 C) and compounds **1b–5b** (Figure S5C) caused significant leakage of potassium ion similar to valinomycin, whereas vancomycin and compound **6** were completely ineffective. Hence, bacterial exposure to these new compounds resulted in increased membrane permeability, perturbation of cell membrane potential, and finally leakage of intracellular K^+ ions, while vancomycin showed no such effect. Further, we observed a gradual increase in membrane disruption with increase in lipophilic chain length.

To investigate the effect of newly installed properties on inhibition of peptidoglycan biosynthesis, we determined the accumulation of UDP-linked peptidoglycan precursor, UDP-*N*-acetyl-muramyl-pentadepsipeptide (UDPMurNAc-pp) after treating VRE with compounds **2**, **6** and vancomycin at 5 μM. On treatment with compounds **2** and **6**, a more intense peak was observed at 260 nm, compared to vancomycin, indicative of greater accumulation of UDPMurNAc-pp; which was confirmed by HRMS (m/z = 1150.94 (cal), 1150.90 (obs) for $[M+H]^+$) (Figure 1 D and Figure S6). The higher accumulation of UDPMurNAc-pp indicated increased

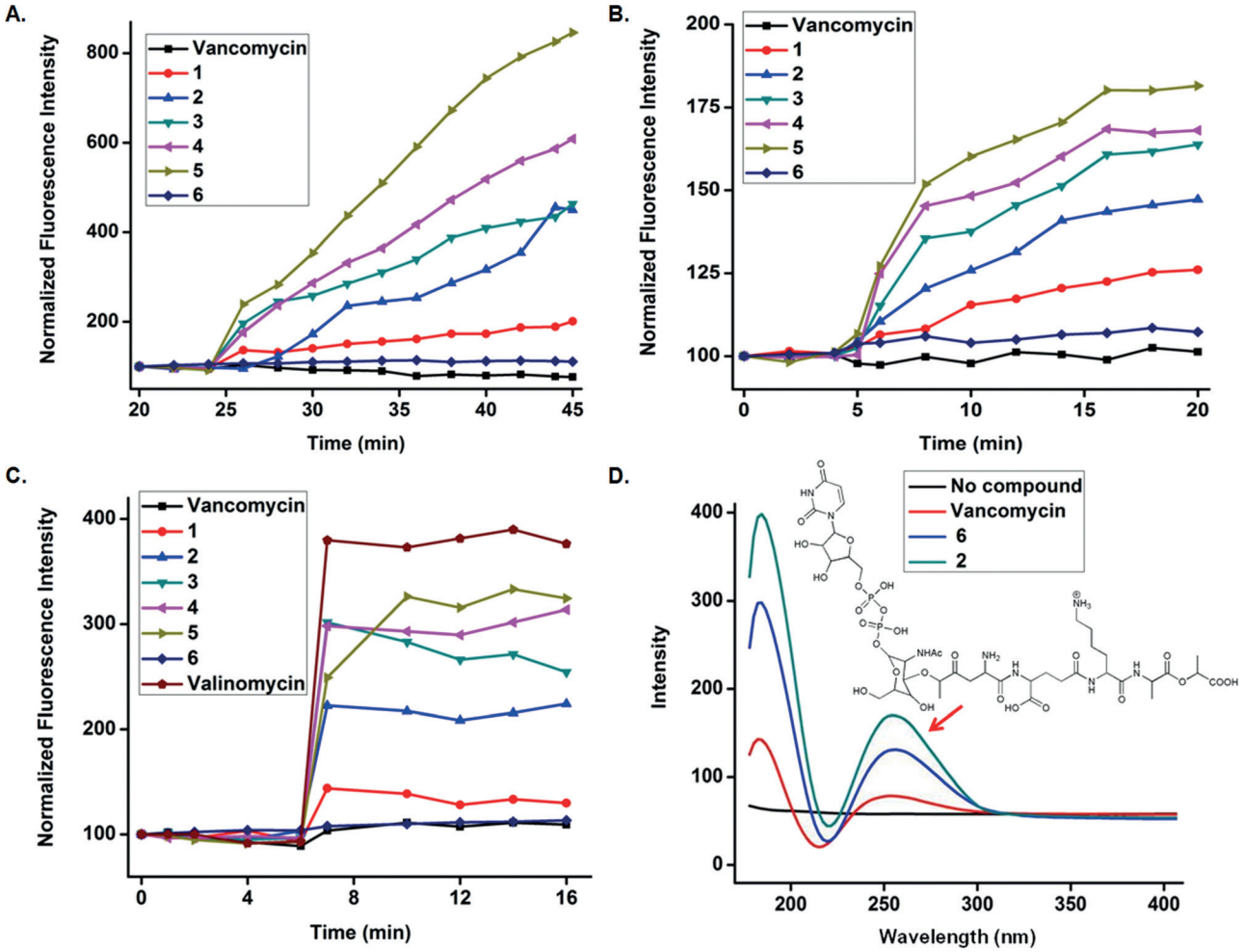


Figure 1. Mechanistic studies: Disruption of bacterial cell membrane integrity of vancomycin and compounds **1**–**6**, at 5 μM against VRE (A–C). A) Cytoplasmic membrane depolarization, B) cytoplasmic membrane permeabilization, C) intracellular potassium ion leakage, and D) intracellular accumulation of the cell-wall precursor UDPMurNAc-pp after treatment of VRE with vancomycin, compounds **2** and **6** at 5 μM by monitoring absorbance at 260 nm wavelength.

inhibition of cell-wall biosynthesis by compounds **2** and **6** over vancomycin. The enhanced ability of compound **2** to inhibit the cell-wall biosynthesis can be attributed to its higher binding affinity to the target peptide and its membrane-disruptive properties which may also indirectly affect the cell-wall biosynthesis.

The escalating rise of drug resistance in bacteria led us to evaluate the possibility of emergence of bacterial resistance to this class of compounds. Here, we exposed MRSA to vancomycin and compound **2** for serial passages and monitored the changes in MIC values for a period of 25 days. Even after 25 passages, the MIC of compound **2** remained unchanged. However, the MIC of vancomycin started increasing after 7 passages and the value increased to >10-fold after 25 passages (Figure 2 A). Thus bacteria were futile in acquiring resistance against compound **2** and this indicates the potential, long-lasting clinical utility of this class of compounds. Further, the compounds were found to be non-toxic even up to 100 μM concentration against human RBC and HeLa cells (Table S3).

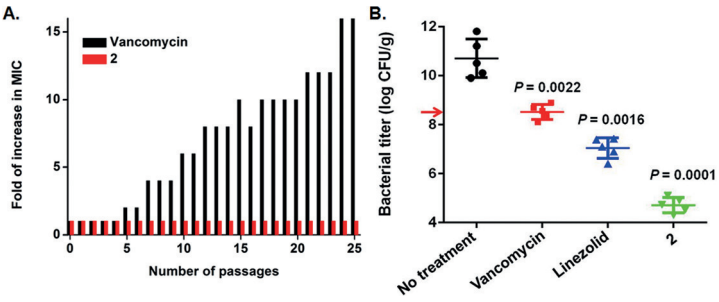


Figure 2. A) Bacterial resistance studies of vancomycin and compound **2** against MRSA. B) In vivo antibacterial activity of vancomycin, linezolid, and compound **2** in renal infection model against VRE. Differences are considered statistically significant from the untreated group with a value of $P<0.05$. Error bars represent 95% confidence intervals. The red arrow in (B) indicates the bacterial pretreatment titer.

Biofilms constitute a protected mode of bacterial growth that allows survival in a hostile environment and are resistant to conventional antibiotics. The biofilm disruption ability of vancomycin, compounds **2** and **2b** was performed against

MRSA. Compounds **2** and **2b** displayed significant reduction (> 5 log colony-forming units per milliliter; CFU mL^{-1}) of cell viability in preformed biofilms at 5 μM whereas vancomycin showed only an about 2 log CFU mL^{-1} reduction compared to nontreated control (Figure S7A). Further, compound **2** was more effective in disrupting the biofilm compared to compound **2b**. Crystal violet staining was used to visually observe the extent of biofilm disruption (Figure S7B).

The in vivo activity of compound **2** was evaluated in a murine kidney infection model against VRE (10^8 CFU per mouse; intravenous, iv). After 4 h of infection, the mice were treated with three doses of vancomycin, linezolid, compound **2** (each at 12 mg kg^{-1}; intraperitoneal, ip), and saline (Figure 2B). After 72 h of the initial treatment, the antibacterial activity was evaluated by determining the bacterial titer in the infected kidneys. In comparison to vancomycin and linezolid, compound **2** reduced the bacterial titer from the infected kidneys more effectively. Linezolid produced an about 4 $\log_{10}$ CFU reduction from vehicle-treated control (saline) whereas compound **2** produced an about 6 $\log_{10}$ CFU reduction (Figure 2B). Further, compound **2** was found to be non-toxic even at 100 mg kg^{-1} (bolus dosage, iv) indicating a good safety profile of the compound required for therapeutic applications.

The clinical impact of such efficient glycopeptides antibiotics is likely to be significant, presenting a rational approach forward in the development of antibiotics for the treatment of drug-resistant bacterial infections. The incorporation of membrane disruption properties into vancomycin along with increased binding affinity makes these new glycopeptides distinct from other existing derivatives in their ability to effectively tackle VRB. This is displayed in the high antibacterial activity of the compounds against MRSA, VISA, VRE, and in curbing the development of bacterial resistance. An optimized compound showed potent activity against VRE, being 2 to 3 orders of magnitude more effective than vancomycin. Further, this compound showed high in vivo antibacterial activity against VRE kidney infection. We firmly believe that our multipronged approach bears high potential and could be a valuable extension to the antibiotic arsenal to combat infections caused by VRB.

Acknowledgements

We thank Prof. C. N. R. Rao FRS (JNCASR) for his constant support and encouragement. J.H. acknowledges Department of Science and Technology (DST), Government of India for a Ramanujan Fellowship. V.Y. thanks CSIR for a research fellowship. S.S. is grateful to the Sheikh Saqr laboratory at JNCASR for a postdoctoral fellowship. We thank Dr. H. Rahman, Director ICAR-NIVEDI for allowing us to use the facilities.

Keywords: antibiotics · bacterial resistance · drugs design · multidrug-resistant bacteria · vancomycin

How to cite: *Angew. Chem. Int. Ed.* **2015**, *54*, 13644–13649
Angew. Chem. **2015**, *127*, 13848–13853

[1] a) K. C. Nicolaou, C. N. C. Boddy, S. Brase, N. Winssinger, *Angew. Chem. Int. Ed.* **1999**, *38*, 2096–2152; *Angew. Chem.* **1999**, *111*, 2230–2287; b) D. Kahne, C. Leimkuhler, W. Lu, C. T. Walsh, *Chem. Rev.* **2005**, *105*, 425–448; c) B. K. Hubbard, C. T. Walsh, *Angew. Chem. Int. Ed.* **2003**, *42*, 730–765; *Angew. Chem.* **2003**, *115*, 752–789.

[2] a) K. Hiramatsu, *Lancet Infect. Dis.* **2001**, *1*, 147–155; b) L. M. weigel, D. B. Clewell, S. R. Gill, N. C. Clark, L. K. McDougal, S. E. Flannagan, J. F. Kolonay, J. Shetty, G. E. Killgore, F. C. Tenover, *Science* **2003**, *302*, 1569–1571.

[3] G. Taubes, *Science* **2008**, *321*, 356–361.

[4] a) R. P. Wenzel, M. B. Edmond, *N. Engl. J. Med.* **2000**, *343*, 1961–1963; b) S. B. Levy, B. Marshall, *Nat. Med.* **2004**, *10*, S122–S129; c) M. A. Fischbach, C. T. Walsh, *Science* **2009**, *325*, 1089–1093.

[5] a) B. Gu, T. Kelesidis, S. Tsiodras, J. Hindler, R. M. Humphires, *J. Antimicrob. Chemother.* **2013**, *68*, 4–11; b) U. Bertsche, et al., *PLoS One* **2013**, *8*, e67398.

[6] a) K. Lewis, *Nat. Rev. Drug Discovery* **2013**, *12*, 371–387; b) K. Bush, et al., *Nat. Rev. Microbiol.* **2011**, *9*, 894–896.

[7] a) K. M. G. O'Connell, J. T. Hodgkinson, H. F. Sore, M. Welch, G. P. C. Salmond, D. R. Spring, *Angew. Chem. Int. Ed.* **2013**, *52*, 10706–10733; *Angew. Chem.* **2013**, *125*, 10904–10932; b) G. L. Thomas, et al., *Angew. Chem. Int. Ed.* **2008**, *47*, 2808–2812; *Angew. Chem.* **2008**, *120*, 2850–2854; c) G. Guchhait, A. Altieri, B. Gorityala, X. Yang, B. Findlay, G. G. Zhanel, N. Mookherjee, F. Schweizer, *Angew. Chem. Int. Ed.* **2015**, *54*, 6278–6282; *Angew. Chem.* **2015**, *127*, 6376–6380.

[8] a) C. Ghosh, G. B. Manjunath, P. Akkapeddi, V. Yarlagadda, J. Hoque, D. S. Uppu, M. M. Konai, J. Haldar, *J. Med. Chem.* **2014**, *57*, 1428–1436; b) M. M. Konai, C. Ghosh, V. Yarlagadda, J. Haldar, *J. Med. Chem.* **2014**, *57*, 9409–9423; c) D. S. S. M. Uppu, P. Akkapeddi, G. B. Manjunath, V. Yarlagadda, J. Hoque, J. Haldar, *Chem. Commun.* **2013**, *49*, 9389–9391; d) H. Wu, Y. Niu, S. Padhee, R. E. Wang, Y. Li, Q. Qiao, G. Bai, C. Cao, J. Cai, *Chem. Sci.* **2012**, *3*, 2570–2575.

[9] a) A. Makovitzki, D. Avrahami, Y. Shai, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 15997–16002; b) S. Choi, A. Isaacs, D. Clements, D. Liu, H. Kim, R. W. Scott, J. D. Winkler, W. F. Degrado, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 6968–6973; c) B. P. Mowery, S. E. Lee, D. A. Kissounko, R. F. Epand, B. Weisblum, S. S. Stahl, S. H. Gellman, *J. Am. Chem. Soc.* **2007**, *129*, 15474–15476; d) M. Wenzel, et al., *Proc. Natl. Acad. Sci. USA* **2014**, *111*, E1409–E1418.

[10] a) J. R. Morones-Ramirez, J. A. Winkler, C. S. Spina, J. J. Collins, *Sci. Transl. Med.* **2013**, *5*, 190ra81; b) T. Zheng, E. Nolan, *J. Am. Chem. Soc.* **2014**, *136*, 9677–9691; c) P. I. O'Daniel, et al., *J. Am. Chem. Soc.* **2014**, *136*, 3664–3672.

[11] a) L. L. Ling, et al., *Nature* **2015**, *517*, 455–459; b) B. P. Conlon, E. S. Nakayasu, L. E. Fleck, M. D. LaFleur, V. M. Isabella, K. Coleman, S. N. Leonard, R. D. Smith, J. N. Adkins, K. Lewis, *Nature* **2013**, *503*, 365–370; c) M. N. Thaker, W. Wang, P. Spanogiannopoulos, N. Waglechner, A. M. King, R. Medina, G. D. Wright, *Nat. Biotechnol.* **2013**, *31*, 922–927.

[12] a) X. Fu, C. Albermann, J. Jiang, J. Liao, C. Zhang, J. S. Thorson, *Nat. Biotechnol.* **2003**, *21*, 1467–1469; b) L. Chen, D. Walker, B. Sun, Y. Hu, S. Walker, D. Kahne, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 5658–5663; c) P. A. Ashford, S. P. Bew, *Chem. Soc. Rev.* **2012**, *41*, 957–978; d) S. Yoganathan, S. J. Miller, *J. Med. Chem.* **2015**, *58*, 2367–2377; e) Y. Nakama, O. Yoshida, M. Yoda, K. Araki, Y. Sawada, J. Nakamura, S. Xu, K. Miura, H. Maki, H. Arimoto, *J. Med. Chem.* **2010**, *53*, 2528–2533.

[13] a) C. T. Walsh, S. L. Fisher, I. S. Park, M. Prahalad, Z. Wu, *Chem. Biol.* **1996**, *3*, 21–28; b) C. C. McComas, B. M. Crowley, D. L. Boger, *J. Am. Chem. Soc.* **2003**, *125*, 9314–9315; c) J.

Pootoolal, J. Neu, G. D. Wright, *Annu. Rev. Pharmacol. Toxicol.* **2002**, *42*, 381 – 408.

[14] a) J. Rao, J. Lahiri, L. Isaacs, R. M. Weiss, G. M. Whitesides, *Science* **1998**, *280*, 708 – 711; b) J. H. Griffin, et al., *J. Am. Chem. Soc.* **2003**, *125*, 6517 – 6531; c) K. C. Nicolaou, R. Hughes, S. Y. Cho, N. Winssinger, C. Smethurst, H. Labischinski, R. Endermann, *Angew. Chem. Int. Ed.* **2000**, *39*, 3823 – 3828; *Angew. Chem.* **2000**, *112*, 3981 – 3986; d) S. K. Choi, et al., *ACS Nano* **2013**, *7*, 214 – 218.

[15] a) R. C. James, J. G. Pierce, A. Okano, J. Xie, D. L. Boger, *ACS Chem. Biol.* **2012**, *7*, 797 – 804; b) J. Xie, J. G. Pierce, R. C. James, A. Okano, D. L. Boger, *J. Am. Chem. Soc.* **2011**, *133*, 13946 – 13949; c) J. Xie, A. Okano, J. G. Pierce, R. C. James, S. Stamm, C. M. Crane, D. L. Boger, *J. Am. Chem. Soc.* **2012**, *134*, 1284 – 1297.

[16] a) V. Yarlagadda, M. M. Konai, G. B. Manjunath, C. Ghosh, J. Haldar, *J. Antibiot.* **2015**, *68*, 302 – 312; b) V. Yarlagadda, M. M. Konai, K. Paramanandham, B. R. Shome, J. Haldar, *Int. J. Antimicrob. Agents*, DOI: 10.1016/j.ijantimicag.2015.05.014.

[17] a) F. Van Bambeke, M. P. Mingeot-Leclercq, M. J. Struelens, P. M. Tulkens, *Trends Pharmacol. Sci.* **2008**, *29*, 124 – 134; b) D. L. Higgins, et al., *Antimicrob. Agents Chemother.* **2005**, *49*, 1127 – 1134; c) O. Domenech, G. Francius, P. M. Tulkens, F. Van Bambeke, Y. Dufrene, M. P. Mingeot-Leclercq, *Biochim. Biophys. Acta Biomembr.* **2009**, *1788*, 1832 – 1840.

[18] a) G. G. Zhanel, et al., *Drugs* **2010**, *70*, 859 – 886; b) M. S. Butler, K. A. Hansford, M. A. Blaskovich, R. Halai, M. A. Cooper, *J. Antibiot.* **2014**, *67*, 631 – 644.

[19] G. A. McKay, S. Beaulieu, F. F. Arhin, A. Belley, I. Sarmiento, T. Parr, Jr., G. Moeck, *J. Antimicrob. Chemother.* **2009**, *63*, 1191 – 1199.

[20] a) V. Yarlagadda, P. Akkapeddi, G. B. Manjunath, J. Haldar, *J. Med. Chem.* **2014**, *57*, 4558 – 4568; b) V. Yarlagadda, et al., *Int. J. Antimicrob. Agents* **2015**, *45*, 627 – 634.

Received: August 13, 2015
Revised: September 11, 2015
Published online: October 16, 2015

