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EXPERIMENTAL THERAPEUTICS

In Vivo Targeting of *Escherichia coli* with Vancomycin-Arginine

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ABSTRACT The ability of vancomycin-arginine (V-r) to extend the spectrum of activity of glycopeptides to Gram-negative bacteria was investigated. Its MIC towards *Escherichia coli*, including β-lactamase expressing Ambler classes A, B, and D, was 8 to 16 μg/ml. Addition of 8 times the MIC of V-r to *E. coli* was acutely bactericidal and associated with a low frequency of resistance ($<2.32 \times 10^{-10}$). *In vivo*, V-r markedly reduced *E. coli* burden by >7 $\log_{10}$ CFU/g in a thigh muscle model. These data warrant further development of V-r in combatting *E. coli*, including resistant forms.

KEYWORDS *Escherichia coli*, Gram-negative bacteria, antibiotic resistance, arginine, cationic peptides, multidrug resistance, vancomycin conjugate

Novel antibiotics are desperately needed to combat priority 1 or urgent-threat pathogens (1–3). With only four new classes of antibiotics introduced into the market since the early 1960s (4), structural modifications of current antibiotics provide an attractive and possibly speedier approach to fulfill this significant unmet clinical need. Vancomycin is a standard-of-care glycopeptide antibiotic for the treatment of Gram-positive infections (5). Numerous reports have demonstrated augmentation of its antimicrobial activity against resistant strains via different chemical modifications (6–9). Furthermore, its molecular structure has been successfully manipulated to create a broader spectrum of activity in the targeting of Gram-negative bacteria via adjuvant, formulation, and cationic/lipophilic interventions (10, 11) or synergy with existing Gram-negative antibiotics (12, 13). Recently, the covalent conjugation of L-arginine to vancomycin, to produce vancomycin-L-arginine (V-R), led to promising Gram-negative properties via a cell wall mode of action (14). These findings encouraged us to further characterize the corresponding diastereomer vancomycin-D-arginine (V-r) in animal models of *E. coli* infection using the D-isomer of arginine to reduce the risk of conjugate hydrolysis (Fig. 1).

V-r was synthesized in a single chemical step from commercially available vancomycin HCl (StruChem, Wujiang City, China) and D-arginine amide dihydrochloride (Aladdin Chemical Co., Shanghai, China). The crude compound was purified and isolated as the corresponding HCl salt at 95% purity by high-performance liquid chromatography based on a previously described procedure (14). Identity was confirmed by ^{1}H nuclear magnetic resonance and time of flight mass spectrometry, and HCl content was quantified by ion-exchange chromatography. In various physicochemical screens, V-r behaved similarly to vancomycin, including no observed cellular cytotoxicity at concentrations ranging from 100 to 750 μM on human erythrocytes, HepG2, and primary renal proximal tubule epithelial cells employing fetal bovine serum-deficient media to negate compound quenching (15) (Table 1).

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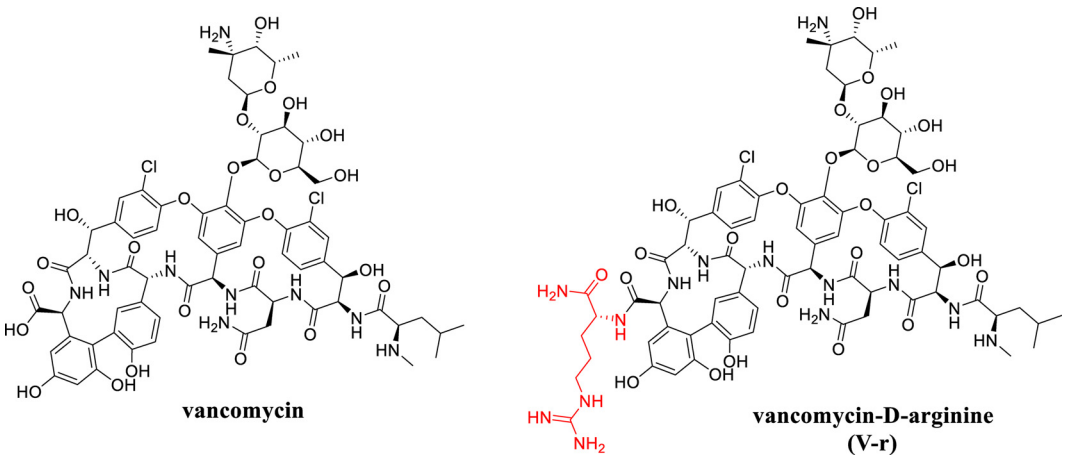

FIG 1 Vancomycin and vancomycin-D-arginine (V-r).

MICs were determined in alignment with CLSI guidelines as previously described for V-R and cationic antimicrobial peptides (14, 16). The MIC range of V-r against 29 different *E. coli* strains was 8 to 16 μg/ml (MIC_{90}, 16 μg/ml), including those with multiple resistance mechanisms (Table 2). The MIC of V-r against the efflux pump mutant strain JW0451-2 was 8 μg/ml, suggesting that V-r is unlikely to be a substrate for efflux in this pathogen. Notably, the MIC of V-r was also 8 μg/ml against two out of five of the *Acinetobacter baumannii* strains tested. In comparison, the MICs of vancomycin were significantly higher, at 64 to 256 μg/ml, against all *E. coli* and *A. baumannii* strains tested. Importantly, the antimicrobial potency of V-r towards a number of Gram-positive bacteria remained intact (Table 2). In frequency-of-resistance (FoR) assays at 8 times the MIC of V-r (128 μg/ml), *E. coli* ATCC 25922 demonstrated an extremely low FoR, at $<2.32 \times 10^{-10}$, which is similar to or lower than those with standard-of-care therapies, such as ciprofloxacin (17, 18). Time-kill assays were performed against uropathogenic *E. coli* strains, including the sequence type 131 (ST131) NCTC 13341 isolate. V-r, but not vancomycin, demonstrated rapid bactericidal activity to limits of detection (i.e., 100 CFU/ml) within 1 or 4 h of exposure, and this was maintained up to 24 h (Fig. 2).

Plasma pharmacokinetics (PK) of V-r after subcutaneous (s.c.) administration (20 and 121 mg/kg) was determined in naive male CD-1 mice ($n = 3$/group) using liquid chromatography-tandem mass spectrometry for analysis with a lower limit of quantitation of 5 ng/ml (Table 3). V-r displayed first-order elimination, similar to vancomycin, after s.c. administration (19, 20). Prior to efficacy studies, a single s.c. administration of V-r

TABLE 1 Physicochemical properties of vancomycin-arginine (V-r) and vancomycin

Physicochemical properties[a]	V-r	Vancomycin
Mol wt (free base)	1,604	1,449
LogD (octanol/buffer)	Less than −4.01	−5.14[b]
TD solubility in saline (mg/ml)	373	> 50
PPB (mouse/human % bound)	65/76	50/50
Red blood cell lysis (CC_{50}, μM)	>750	>750
HepG2 cell cytotoxicity (CC_{50}, μM)	>750	>750
hRPTEC biomarkers[c] (CC_{50}, μM)	>100	>100
FoR (at 8× MIC)	$<2.32 \times 10^{-10}$	Not determined

[a]TD, thermodynamic; PPB, plasma protein binding; hRPTEC, human renal proximal tubular epithelial cells; CC_{50}, concentration at which 50% cytotoxicity is observed; FoR, frequency of resistance.
[b]LogD vancomycin reported according to Dave and Morris (29).
[c]Includes cell count, nuclear size, DNA structure, mitochondrial mass, mitochondrial membrane potential, phospholipidosis, and glutathione content.

TABLE 2 Antimicrobial susceptibility profiles of V-r and vancomycin

Organism	Strain	Source, resistance mechanism or genotype[a]	Ambler class	MIC (μg/ml) of:	
				V-r	Vancomycin
E. coli	ATCC 25922	CLSI susceptible reference strain		16	128
E. coli	UTI89	Clinical isolate from patient with acute bladder infection		16	128
E. coli	NCTC 13441	Uropathogenic *E. coli* ST131, $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-1}}$, $bla_{\text{TEM-1}}$, *aac6'-Ib-cr*, *mph*(A), *catB4*, *tet*(A), *dfrA7*, *aadA5*, *sul1*	A, D	16	128
E. coli	NCTC 13462	$bla_{\text{CTX-M-2}}$	A	16	128
E. coli	NCTC 13846	Clinical isolate, bacteremia, UK 2013, EUCAST reference isolate, *mcr-1*		8	64
E. coli	AR055	$bla_{\text{NDM-1}}$, *mph*(A), $bla_{\text{CMY-6}}$, *dfrA17*, *sul1*, *tet*(A), *rmtC*, *aac(3)-IIa*, $bla_{\text{OXA-1}}$, *aadA5*	B, C, D	16	128
E. coli	AR089	*strB*, $bla_{\text{CMY-2}}$, *tet*(B), *strA*, *sul2*	C	16	128
E. coli	AR0114	*strB*, $bla_{\text{TEM-1B}}$, $bla_{\text{KPC-3}}$, *aadB*, *dfrA5*, *sul1*, *strA*, *sul2*, *cmlA1*	A	16	256
E. coli	AR0137	$bla_{\text{NDM-6}}$, $bla_{\text{OXA-9}}$, *mph*(A), $bla_{\text{TEM-1A}}$, $bla_{\text{CMY-42}}$, $bla_{\text{CTX-M-15}}$, *dfrA17*, *qnrS1*, *sul1*, *tet*(B), *aadA1*, *aac(3)-IIa*, $bla_{\text{OXA-1}}$, *aadA5*	B	16	128
E. coli	AR0150	$bla_{\text{NDM-5}}$, *mph*(A), $bla_{\text{TEM-1B}}$, $bla_{\text{CMY-42}}$, *dfrA17*, *sul1*, *tet*(A), *aadA5*	A, B, C	8	128
E. coli	AR0346	*mcr-1*, ESBL	A	16	256
E. coli	AR0349	*mcr-1*, ESBL	A	16	128
E. coli	AR0350	*mcr-1*	-	16	128
E. coli	AR0493	*mcr-1*, ESBL	A	16	256
E. coli	AR0494	*mcr-1*	-	8	128
E. coli	B096a	Clinical isolate (UK) 2016, AmpC	C	16	128
E. coli	B808	Clinical isolate (UK) 2016, $bla_{\text{TEM-1}}$, $bla_{\text{CTX-M-15}}$	A	16	256
E. coli	ATCC BAA-2340	bla_{KPC}	A	16	128
E. coli	ATCC BAA-2469	$bla_{\text{NDM-1}}$	B	16	128
E. coli	ExPEC H5	Clinical isolate (UK)		8	128
E. coli	H4/5	Clinical isolate, $bla_{\text{TEM-1}}$, $bla_{\text{CTX-M-15}}$	A	16	256
E. coli	IR3	Clinical isolate, $bla_{\text{NDM-1}}$	B	8	128
E. coli	IR45	Clinical isolate, $bla_{\text{NDM-1}}$	B	16	128
E. coli	IR57	Clinical isolate, $bla_{\text{NDM-1}}$	B	16	256
E. coli	Swiss 2 (AF45)	Clinical isolate (South Africa) ST101, *mcr-1*		16	128
E. coli	Swiss 13	Clinical isolate (France) ST69, *mcr-1*		16	128
E. coli	Swiss 15	Clinical isolate (Switzerland) ST446, *mcr-1*, $bla_{\text{CTX-M}}$	A	16	128
E. coli	BW25113	Parent strain of BW25113Δ*acrB::kan* mutant		8	128
E. coli	JW0451-2	BW25113Δ*acrB::kan,* AcrB-deficient mutant, defective in ArcAB-TolC multidrug efflux system		8	128
A. baumannii	ATCC 19606	Isolated from urine, genome-sequenced strain		32	128
A. baumannii	ACC00527	Clinical respiratory isolate (USA) 2012, $bla_{\text{OXA-24}}$	D	8	128
A. baumannii	B803	Clinical isolate (UK) 2016		32	128
A. baumannii	GS2AB1	Multiresistant clinical isolate (southern Europe) 2017		16	128
A. baumannii	Naval-81	Clinical isolate (USA) 2006		8	128
S. aureus	ATCC 29213	CLSI susceptible reference strain		2	2
S. aureus	NRS 384	USA300-0114 MRSA, community associated		0.5	2
E. faecalis	ATCC 29212	CLSI QC strain		1	2
E. faecalis	B575	Clinical isolate (northwest UK)		1	2
S. agalactiae	B057	Clinical isolate (northwest UK)		0.06	0.5
S. agalactiae	B063	Clinical isolate (northwest UK)		0.06	1
S. pneumoniae	ATCC 49619	Reference strain		0.25	0.5
S. pneumoniae	3259-03	Clinical isolate (northwest UK)		0.5	0.5

[a]ESBL, extended-spectrum β-lactamase.

was shown to be well tolerated in male CD-1 mice ($n = 3$) at the highest dose tested (800 mg/kg).

Using a screening-based strategy, preliminary proof-of-concept studies with V-r employed an abbreviated 9-h thigh muscle infection model in male CD-1 mice rendered neutropenic (21). To that end, an *E. coli* ATCC 25922 isolate was inoculated at 9.7×10^4 CFU into both thigh muscles per mouse ($n = 5$ per experimental group). V-r was administered s.c. every 2 h (110 to 880 mg/kg total dose) starting 1 h postinfection. At 9 h, thigh homogenates were prepared, and CFU were enumerated after culture on CLED (cystine-, lactose-, and electrolyte-deficient) agar. Compared to pretreatment and

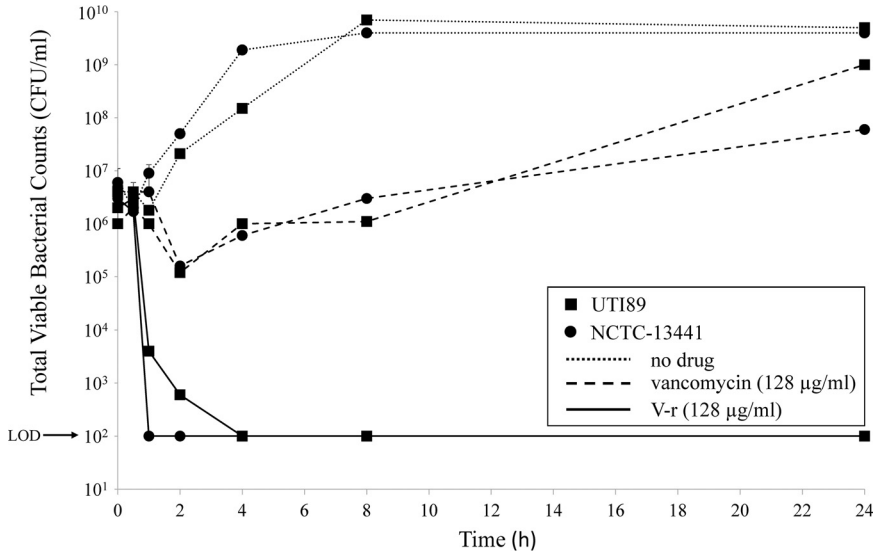

FIG 2 Time-kill of vancomycin-arginine (V-r) and vancomycin against *E. coli* uropathogens UTI89 and NCTC 13441.

vehicle burdens of 5.1 ± 0.2 and 7.1 ± 0.1 $\log_{10}$ CFU/g tissue, respectively, V-r exhibited a dose-dependent reduction in bacterial burden of 1.2 to 3.4 $\log_{10}$ compared with vehicle (Kruskal-Wallis one-way analysis of variance using StatsDirect Statistical Analysis Software) (Table 4). V-r doses at 440 and 880 mg/kg afforded 1.0- and 1.3-$\log_{10}$ reductions below stasis, respectively, with an extrapolated static dose of 215 mg/kg. As anticipated, vancomycin failed to significantly impact *E. coli* burden at a dose equivalent to the highest dose of V-r. In a 24-h thigh muscle infection model, *E. coli* UTI89 was inoculated at 7.8×10^4 CFU into one thigh muscle per mouse ($n = 5$ to 8 per group) and treated with V-r (total dose, 200 to 1,400 mg) using an every-6-h dosing regimen from 1 h postinfection. All doses of >200 mg/kg significantly reduced burden below stasis by up to 2.7 $\log_{10}$ CFU/g. These bactericidal effects of V-r were statistically superior to those of ciprofloxacin, which induced a 1.4 $\log_{10}$ reduction from stasis (Fig. 3 and Table 5). Overall, V-r caused an ~4 to 7.5 $\log_{10}$ reduction in bacterial burden, compared with vehicle control, over the entire dose range.

The MIC data confirm previous findings that the coupling of arginine with vancomycin bestows significant antimicrobial activity of the V-r conjugate against *E. coli* infection while remaining effective against methicillin-resistant *Staphylococcus aureus* (MRSA) (14). Such *in vitro* findings were effectively translated into thigh muscle infection models, where a total 24-h dose of 250 mg/kg V-r reduced *E. coli* burden to pretreatment (stasis) levels. Since area under the curve over 24 h in the steady state divided by the MIC (AUC/MIC ratio) is the primary PK/pharmacodynamic predictor of vancomycin (5), this static dose corresponds to a total AUC/MIC of 47.3. Based on a free (*f*) fraction of 35%, as determined in plasma protein binding studies (Table 1), the *f*AUC/MIC of V-r was 16.5. As an approximation of exposure using allometric scaling (22), this would be equivalent to a human dose of ~20 mg/kg, with a dose of 28 mg/kg

TABLE 3 PK parameters of V-r in CD-1 mice after s.c. administration

PK parameter[a]	V-r at 20 mg/kg	V-r at 121 mg/kg
Half-life (h)	0.87	1.29
C_{max} (mg/liter)	20.4	98.4
Clearance (ml/min/kg)	7.8	5.4
AUC (mg · h/liter)	42.7	366
V_d (liter/kg)	0.59	0.60

[a]C_{max}, maximum concentration of drug in plasma; AUC, area under the curve; V_d, volume of distribution.

TABLE 4 Efficacy of V-r in an *E. coli* ATCC 25922 thigh muscle infection model (9 h) in neutropenic CD-1 mice

Group, total dose over 9 h (mg/kg)	Log_{10} (group geometric mean ± SD CFU/g)	Log_{10} change from vehicle (CFU/g)	*P* value (versus vehicle)
Pretreatment	5.1 ± 0.18	−2.01	0.0045
Vehicle	7.11 ± 0.12	0	0
V-r, 110	5.87 ± 0.60	−1.24	0.0415
V-r, 440	4.14 ± 0.63	−2.97	<0.0001
V-r, 880	3.76 ± 0.40	−3.35	<0.0001
Vancomycin, 800	6.60 ± 0.66	−0.51	Not significant

required to elicit an additional 1-log_{10} kill. Such allometric doses of V-r are in line with the daily and loading doses of vancomycin in humans (5).

The positive efficacy data support the notion that the cationic feature of arginine within V-r allows for breaching of the stubborn outer membrane of *E. coli* isolates and possibly other Gram-negative bacteria (14). The sequelae of events leading to V-r-mediated *E. coli* eradication likely involve (i) improved cell surface association with negatively charged groups, (ii) effective translocation across the outer membrane leading to enhanced drug uptake, and (iii) disruption of peptidoglycan synthesis within the periplasmic space (6, 14). To our knowledge, the current findings describe the first report of a marked abrogation of *E. coli* burden *in vivo* with a minimally modified vancomycin-cationic transporter conjugate. Previously, it was reported that vancomycin-QC14, a strongly lipophilic/cationic molecule, reduced thigh muscle infection of a carbapenem-resistant *A. baumannii* strain (23). Because V-r was highly effective in time-kill assays against *E. coli* NCTC 13441, a pandemic uropathogenic clone (24), a logical next step would be to evaluate the conjugate in a model of urinary tract infection (UTI). Based on the high renal elimination of vancomycin in humans (25) in a nonmetabolized form (26), it is reasonable to hypothesize that V-r may drive a highly targeted therapeutic intervention to combat *E. coli*-associated UTIs.

These data further underscore a precedent for creating a novel Gram-negative active agent by transforming a commonly used and selective Gram-positive antibiotic by introducing certain cationic features through a simple and scalable synthesis protocol (14). Such an approach, in consort with effective *in silico* predictions (27, 28), might expedite antibiotic development and increase the overall probability of success of

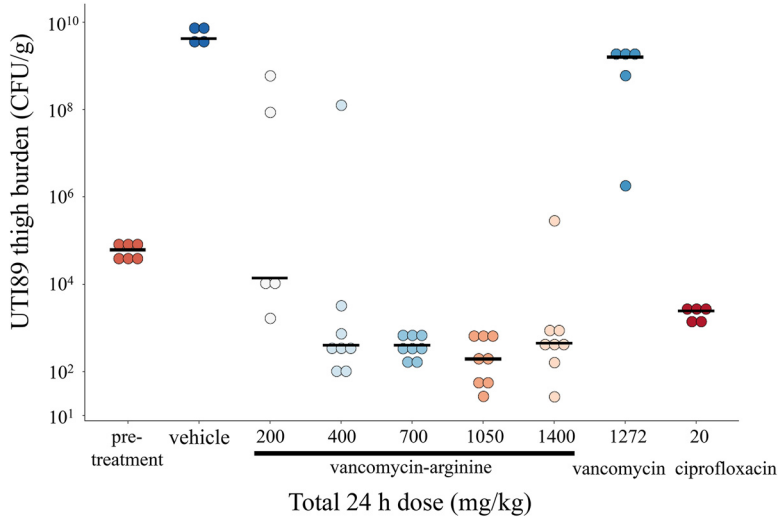

FIG 3 Efficacy of V-r in reducing *E. coli* UTI89 burden in a 24-h thigh muscle infection model in neutropenic CD-1 mice.

TABLE 5 Efficacy of V-r in reducing *E. coli* UTI89 burden in 24-h thigh muscle infection model in neutropenic CD-1 mice

Group, total dose over 24 h (mg/kg)	Log_{10} (group geometric mean ± SD CFU/g)	Log_{10} change from vehicle (CFU/g)	*P* value (versus vehicle)
Pretreatment	4.76 ± 0.18	−4.95	0.0248
Vehicle	9.71 ± 0.17	0	0
V-r, 200	5.60 ± 2.28	−4.11	0.0217
V-r, 400	3.27 ± 1.88	−6.43	<0.0001
V-r, 700	2.58 ± 0.25	−7.13	<0.0001
V-r, 1,050	2.08 ± 0.89	−7.63	<0.0001
V-r, 1,400	2.68 ± 1.38	−7.03	<0.0001
Vancomycin, 1,272	8.48 ± 1.31	−1.23	Not significant
Ciprofloxacin, 20	3.32 ± 0.14	−6.39	<0.0007

drug candidates. Most important, this would help to arrest the insidious pandemic of difficult-to-treat bacterial infections.

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REFERENCES

1. Centers for Disease Control and Prevention. 2019. CDC's antibiotic resistance threats in the United States, 2019. Centers for Disease Control and Prevention, Atlanta, GA.
2. Tacconelli E, Magrini N. 2017. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. World Health Organization, Geneva, Switzerland. https://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf.
3. Peri AM, Doi Y, Potoski BA, Harris PNA, Paterson DL, Righi E. 2019. Antimicrobial treatment challenges in the era of carbapenem resistance. Diagn Microbiol Infect Dis 94:413–425. https://doi.org/10.1016/j.diagmicrobio.2019.01.020.
4. De Oliveira DMP, Forde BM, Kidd TJ, Harris PNA, Schembri MA, Beatson SA, Paterson DL, Walker MJ. 2020. Antimicrobial resistance in ESKAPE pathogens. Clin Microbiol Rev 33:e00181-19. https://doi.org/10.1128/CMR.00181-19.
5. Rybak MJ, Le J, Lodise TP, Levine DP, Bradley JS, Liu C, Mueller BA, Pai MP, Wong-Beringer A, Rotschafer JC, Rodvold KA, Maples HD, Lomaestro BM. 2020. Therapeutic monitoring of vancomycin for serious methicillin-resistant *Staphylococcus aureus* infections: a revised consensus guideline and review by the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, the Pediatric Infectious Diseases Society, and the Society of Infectious Diseases Pharmacists. Am J Health Syst Pharm 77:835–864. https://doi.org/10.1093/ajhp/zxaa036.
6. Antonoplis A, Zang X, Huttner MA, Chong KKL, Lee YB, Co JY, Amieva MR, Kline KA, Wender PA, Cegelski L. 2018. A dual-function antibiotic-transporter conjugate exhibits superior activity in sterilizing MRSA biofilms and killing persister cells. J Am Chem Soc 140:16140–16151. https://doi.org/10.1021/jacs.8b08711.
7. Umstätter F, Domhan C, Hertlein T, Ohlsen K, Mühlberg E, Kleist C, Zimmermann S, Beijer B, Klika KD, Haberkorn U, Mier W, Uhl P. 2020. Vancomycin resistance is overcome by conjugation of polycationic peptides. Angew Chem Int Ed Engl 59:8823–8827. https://doi.org/10.1002/anie.202002727.
8. Umstätter F, Domhan C, Hertlein T, Ohlsen K, Mühlberg E, Kleist C, Zimmermann S, Beijer B, Klika KD, Haberkorn U, Mier W, Uhl P. 2020. Corrigendum: vancomycin resistance is overcome by conjugation of polycationic peptides. Angew Chem Int Ed 59:17326–17326. https://doi.org/10.1002/anie.202007022.
9. Wu Z-C, Cameron MD, Boger DL. 2020. Vancomycin C-terminus guanidine modifications and further insights into an added mechanism of action imparted by a peripheral structural modification. ACS Infect Dis 6:2169–2180. https://doi.org/10.1021/acsinfecdis.0c00258.
10. Blaskovich MAT, Hansford KA, Butler MS, Jia Z, Mark AE, Cooper MA. 2018. Developments in glycopeptide antibiotics. ACS Infect Dis 4:715–735. https://doi.org/10.1021/acsinfecdis.7b00258.
11. Dhanda G, Sarkar P, Samaddar S, Haldar J. 2019. Battle against vancomycin-resistant bacteria: recent developments in chemical strategies. J Med Chem 62:3184–3205. https://doi.org/10.1021/acs.jmedchem.8b01093.
12. Gordon NC, Png K, Wareham DW. 2010. Potent synergy and sustained bactericidal activity of a vancomycin-colistin combination versus multidrug-resistant strains of *Acinetobacter baumannii*. Antimicrob Agents Chemother 54:5316–5322. https://doi.org/10.1128/AAC.00922-10.
13. Zhou A, Kang TM, Yuan J, Beppler C, Nguyen C, Mao Z, Nguyen MQ, Yeh P, Miller JH. 2015. Synergistic interactions of vancomycin with different antibiotics against *Escherichia coli*: trimethoprim and nitrofurantoin display strong synergies with vancomycin against wild-type *E. coli*. Antimicrob Agents Chemother 59:276–281. https://doi.org/10.1128/AAC.03502-14.
14. Antonoplis A, Zang X, Wegner T, Wender PA, Cegelski L. 2019. Vancomycin-arginine conjugate inhibits growth of carbapenem-resistant *E. coli*

and targets cell-wall synthesis. ACS Chem Biol 14:2065–2070. https://doi.org/10.1021/acschembio.9b00565.
15. Zink D, Chuah JKC, Ying JY. 2020. Assessing toxicity with human cell-based *in vitro* methods. Trends Mol Med 26:570–582. https://doi.org/10.1016/j.molmed.2020.01.008.
16. Wiegand I, Hilpert K, Hancock REW. 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc 3:163–175. https://doi.org/10.1038/nprot.2007.521.
17. Martinez JL, Baquero F. 2000. Mutation frequencies and antibiotic resistance. Antimicrob Agents Chemother 44:1771–1777. https://doi.org/10.1128/aac.44.7.1771-1777.2000.
18. Charrier C, Salisbury A-M, Savage VJ, Moyo E, Forward H, Ooi N, Cheung J, Metzger R, McGarry D, Walker R, Cooper IR, Ratcliffe AJ, Stokes NR. 2016. *In vitro* biological evaluation of novel broad-spectrum isothiazolone inhibitors of bacterial type II topoisomerases. J Antimicrob Chemother 71:2831–2839. https://doi.org/10.1093/jac/dkw228.
19. Crandon JL, Kuti JL, Nicolau DP. 2010. Comparative efficacies of human simulated exposures of telavancin and vancomycin against methicillin-resistant *Staphylococcus aureus* with a range of vancomycin MICs in a murine pneumonia model. Antimicrob Agents Chemother 54:5115–5119. https://doi.org/10.1128/AAC.00062-10.
20. Lepak AJ, Zhao M, Andes DR. 2017. Comparative pharmacodynamics of telavancin and vancomycin in the neutropenic murine thigh and lung infection models against *Staphylococcus aureus*. Antimicrob Agents Chemother 61:e00281-17. https://doi.org/10.1128/AAC.00281-17.
21. Everett M, Sprynski N, Coelho A, Castandet J, Bayet M, Bougnon J, Lozano C, Davies DT, Leiris S, Zalacain M, Morrissey I, Magnet S, Holden K, Warn P, De Luca F, Docquier J-D, Lemonnier M. 2018. Discovery of a novel metallo-β-lactamase inhibitor that potentiates meropenem activity against carbapenem-resistant *Enterobacteriaceae*. Antimicrob Agents Chemother 62:e00074-18. https://doi.org/10.1128/AAC.00074-18.
22. FDA. Guidance for industry: estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers. 2005. U.S. Department of Health and Human Services, Food and Drug Administration Center for Drug Evaluation and Research (CDER), Washington, DC. https://www.fda.gov/media/72309/download.
23. Yarlagadda V, Manjunath GB, Sarkar P, Akkapeddi P, Paramanandham K, Shome BR, Ravikumar R, Haldar J. 2016. Glycopeptide antibiotic to overcome the intrinsic resistance of Gram-negative bacteria. ACS Infect Dis 2:132–139. https://doi.org/10.1021/acsinfecdis.5b00114.
24. Nicolas-Chanoine M-H, Bertrand X, Madec J-Y. 2014. *Escherichia coli* ST131, an intriguing clonal group. Clin Microbiol Rev 27:543–574. https://doi.org/10.1128/CMR.00125-13.
25. Matzke GR, Zhanel GG, Guay DRP. 1986. Clinical pharmacokinetics of vancomycin. Clin Pharmacokinet 11:257–282. https://doi.org/10.2165/00003088-198611040-00001.
26. Cao M, Feng Y, Zhang Y, Kang W, Lian K, Ai L. 2018. Studies on the metabolism and degradation of vancomycin in simulated *in vitro* and aquatic environment by UHPLC-triple-TOF-MS/MS. Sci Rep 8:15471. https://doi.org/10.1038/s41598-018-33826-9.
27. Richter MF, Drown BS, Riley AP, Garcia A, Shirai T, Svec RL, Hergenrother PJ. 2017. Predictive compound accumulation rules yield a broad-spectrum antibiotic. Nature 545:299–304. https://doi.org/10.1038/nature22308.
28. Richter MF, Hergenrother PJ. 2019. The challenge of converting Gram-positive-only compounds into broad-spectrum antibiotics: challenges in developing broad-spectrum antibiotics. Ann N Y Acad Sci 1435:18–38. https://doi.org/10.1111/nyas.13598.
29. Dave RA, Morris ME. 2016. A quantitative threshold for high/low extent of urinary excretion of compounds in humans: threshold for high/low urinary excretion. Biopharm Drug Dispos 37:287–309. https://doi.org/10.1002/bdd.2013.