INFECTIOUS DISEASE

TarO-specific inhibitors of wall teichoic acid biosynthesis restore β-lactam efficacy against methicillin-resistant staphylococci

Sang Ho Lee,[1]* Hao Wang,[1]* Marc Labroli,[2] Sandra Koseoglu,[1] Paul Zuck,[2] Todd Mayhood,[1] Charles Gill,[1] Paul Mann,[1] Xinwei Sher,[3] Sookhee Ha,[1] Shu-Wei Yang,[1] Mihir Mandal,[1] Christine Yang,[1] Lianzhu Liang,[1] Zheng Tan,[1] Paul Tawa,[1] Yan Hou,[1] Reshma Kuvelkar,[1] Kristine DeVito,[1] Xiujuan Wen,[1] Jing Xiao,[1] Michelle Batchlett,[2] Carl J. Balibar,[1] Jenny Liu,[1] Jianying Xiao,[1] Nicholas Murgolo,[1] Charles G. Garlisi,[1] Payal R. Sheth,[1] Amy Flattery,[1] Jing Su,[1†] Christopher Tan,[1†] Terry Roemer[1†]

The widespread emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) has dramatically eroded the efficacy of current β-lactam antibiotics and created an urgent need for new treatment options. We report an *S. aureus* phenotypic screening strategy involving chemical suppression of the growth inhibitory consequences of depleting late-stage wall teichoic acid biosynthesis. This enabled us to identify early-stage pathway-specific inhibitors of wall teichoic acid biosynthesis predicted to be chemically synergistic with β-lactams. We demonstrated by genetic and biochemical means that each of the new chemical series discovered, herein named tarocin A and tarocin B, inhibited the first step in wall teichoic acid biosynthesis (TarO). Tarocins do not have intrinsic bioactivity but rather demonstrated potent bactericidal synergy in combination with broad-spectrum β-lactam antibiotics against diverse clinical isolates of methicillin-resistant staphylococci as well as robust efficacy in a murine infection model of MRSA. Tarocins and other inhibitors of wall teichoic acid biosynthesis may provide a rational strategy to develop Gram-positive bactericidal β-lactam combination agents active against methicillin-resistant staphylococci.

INTRODUCTION

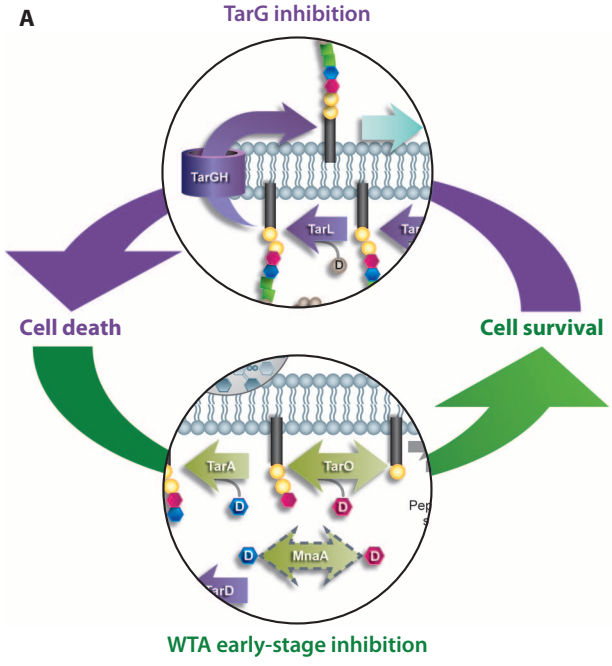
Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *S. epidermidis* (MRSE) are a major cause of bloodstream infections in hospitals and in the community (*1*–*4*). Indeed, MRSA has recently been reported as the second leading cause of mortality by drug-resistant bacterial pathogens in the United States (www.cdc.gov/drugresistance/biggest_threats.html). Consequently, we have witnessed a dramatic erosion in the therapeutic efficacy of ostensibly the entire class of β-lactam antibiotics, which include penicillins such as methicillin, cephalosporins, and the more powerful carbapenems (for example, imipenem, ertapenem, and meropenem) (*5*–*8*). Combating antibacterial drug resistance highlights a need to broaden our focus from exclusively adopting a single-agent antibiotic therapeutic strategy to one more aligned with combination agents (*9*–*11*), as successfully used with emerging therapies for hepatitis C virus as well as historically emphasized by cancer treatment strategies (*12*, *13*). Indeed, such a combination agent strategy of pairing β-lactamase inhibitors with β-lactams has proven highly successful in restoring β-lactam efficacy against Gram-negative bacteria that have aquired diverse β-lactamase enzymes that otherwise inactivate this class of antibiotic (*14*). Clinically relevant β-lactam resistance in MRSA and MRSE, however, is not primarily mediated by β-lactamases. Instead, it is achieved by a horizontally acquired alternative penicillin-binding protein, Pbp2a, with reduced affinity for all classes of β-lactams, thus buffering bacteria from the bactericidal effect of β-lactams that normally inhibit endogenous and essential Pbps (*15*–*19*). A rational β-lactam combination agent strategy applied to methicillin-resistant staphylococci therefore requires the identification of new targets and pathways that normally buffer the drug-resistant pathogen against the effects of β-lactams (either directly or indirectly) and that, if inactivated by genetic or small-molecule inhibition, result in a restored susceptibility to this important class of antibiotics (*9*, *11*, *20*–*22*).

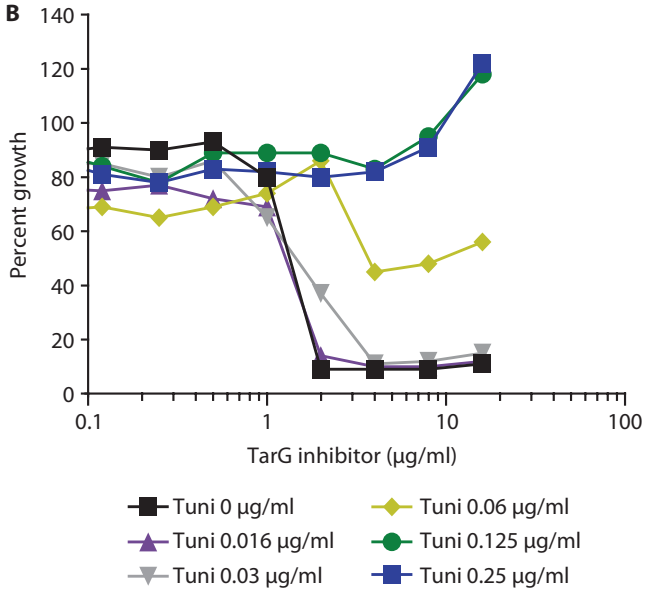
Recently, a series of reports has shown that inactivation or alteration of wall teichoic acid biosynthesis renders MRSA highly susceptible to β-lactam antibiotics both in vitro and in a murine infection setting (*9*, *23*–*27*). Wall teichoic acid is a Gram-positive–specific anionic glycophosphate polymer comprising nearly 50% of the dry weight of the *S. aureus* cell wall that is cross-linked to the peptidoglycan and plays a critical role in cell growth, division, morphology, and in vivo virulence (*24*, *28*–*30*). The wall teichoic acid biosynthetic pathway is uniquely categorized into two phenotypically distinct groups: the nonessential wall teichoic acid early-stage genes *tarO*, *tarA*, and *mnaA*, and the conditionally essential wall teichoic acid late-stage genes *tarB*, *tarD*, *tarF*, *tarI*, *tarJ*, *tarL*, *tarG*, and *tarH*, which are responsible for wall teichoic acid synthesis and eventual transport to the extracellular environment (see fig. S1A) (*27*–*29*, *31*–*36*). Although staphylococci lacking wall teichoic acid polymers are viable under laboratory growth conditions, inactivation of late-stage wall teichoic acid biosynthetic steps results in a growth inhibitory bacteriostatic effect (*24*, *27*, *32*, *34*, *37*). The essentiality of the late-stage genes can be suppressed by concomitant inactivation of one of the nonessential wall teichoic acid early-stage genes; this has been referred to as an essential gene paradox (*31*, *32*, *34*, *35*, *38*). We and others have exploited this phenomenon to develop phenotypic screens and to identify late-stage wall teichoic acid inhibitors whose antibacterial activity recapitulates the conditional essentiality of late-stage wall teichoic acid genes (*27*, *37*). Specifically, such compounds are expected to inhibit wild-type *S. aureus* growth, but their bioactivity is

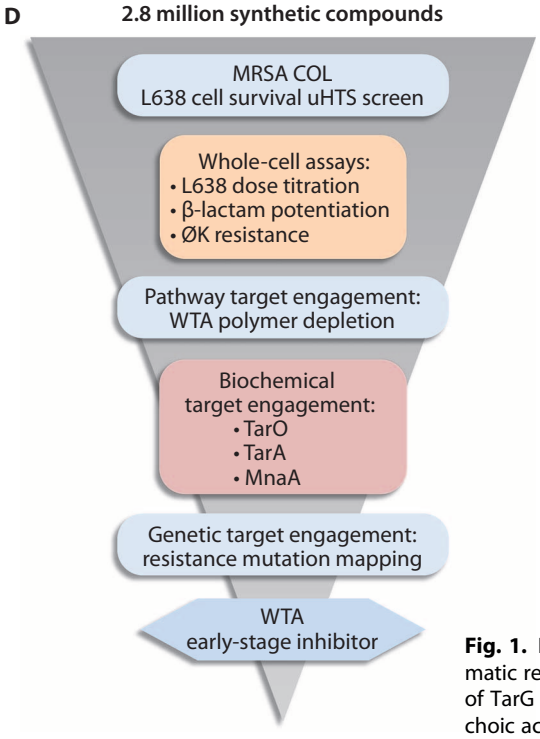
[1]Merck Research Laboratories, Kenilworth, NJ 07033, USA. [2]Merck Research Laboratories, West Point, PA 19486, USA. [3]Merck Research Laboratories, Boston, MA 02115, USA.
*These authors contributed equally to this work.
†Corresponding author. E-mail: jing.su2@merck.com (J.S.); christopher_tan@merck.com (C.T.); terry_roemer@merck.com (T.R.)

specifically suppressed, provided that an early nonessential step in wall teichoic acid biosynthesis is also inactivated (Fig. 1A). In this way, multiple classes of late-stage wall teichoic acid inhibitors have been identified, including targocil and L-638, which both inhibit polymer biogenesis by targeting the wall teichoic acid integral membrane transporter subunit, TarG (*27*, *37*, *39*, *40*).

Here, we leveraged the wall teichoic acid essential gene paradox to identify new wall teichoic acid inhibitors of early nonessential steps that







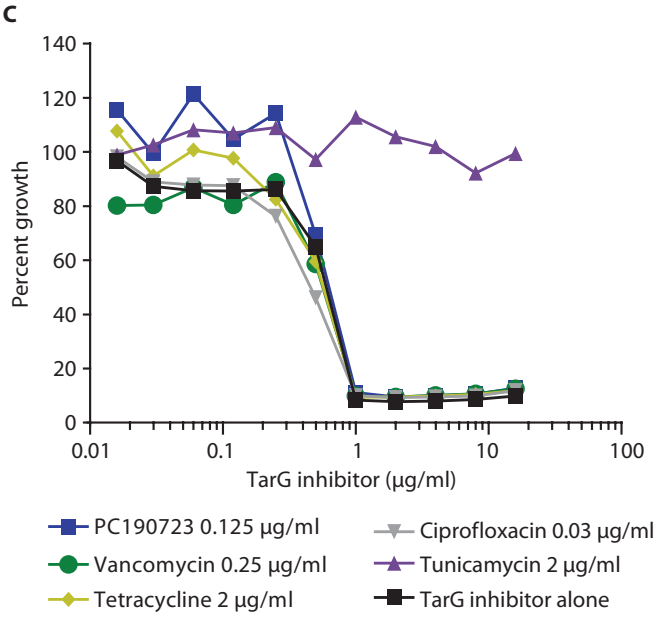


Fig. 1. Phenotypic whole-cell screen to identify wall teichoic acid inhibitors. (**A**) Schematic representation of targeted phenotypic screening strategy. Pharmacological inhibition of TarG leads to cell killing, which can be suppressed by concomitant inhibition of wall teichoic acid early step proteins. This led to the identification of inhibitors of the early steps of the wall teichoic acid (WTA) biosynthetic pathway. (**B**) Dose-response suppression of MRSA COL killing by the TarG inhibitor L-638 alone (black square) or with supplementation with increasing concentrations of tunicamycin (Tuni; 0.016 to 0.25 μg/ml). (**C**) Specificity of L-638 suppression when supplemented with various antibiotic classes as designated on the bottom; subinhibitory concentrations (0.25× minimum inhibitory concentration) of the antibiotics were used in combination with L-638. (**D**) A flow chart guideline established to triage potential hits.

can suppress the inhibitory bioactivity of the wall teichoic acid late-stage TarG inhibitor L-638. We provide mechanistic characterization of two inhibitor classes named, tarocin A and tarocin B, and demonstrate that they block TarO, the enzyme responsible for the first step in wall teichoic acid biosynthesis. Finally, we show that tarocins demonstrate robust in vitro bactericidal chemical synergy with multiple β-lactams against a variety of methicillin-resistant clinical staphylococci isolates as well as efficacy in a murine infection model of MRSA.

RESULTS

Chemical suppression screen for early-stage wall teichoic acid inhibitors

Recent studies have shown that genetic or pharmacological inactivation of early steps in wall teichoic acid biosynthesis renders MRSA highly susceptible to β-lactam antibiotics (*9*, *23*–*27*). To independently corroborate these conclusions, we evaluated the phenotypic consequences of genetically inactivating wall teichoic acid early-stage genes *tarO* and *tarA* in MRSA strain COL (fig. S1A). As expected, genetic null mutations in *tarO* or *tarA* completely abrogate wall teichoic acid polymer production (fig. S1B, left panel). Similarly, this phenotype is faithfully reproduced pharmacologically in a concentration-dependent manner using sublethal concentrations of tunicamycin, a known natural product inhibitor of TarO (*24*, *41*) (fig. S1B, right panel). Consistent with previous reports, genetic or pharmacological inactivation of TarO in MRSA COL restored in vitro susceptibility to diverse β-lactams (*24*, *25*, *27*) (fig. S1C). Extending this evaluation using a previously published murine deep-thigh infection model of MRSA (*11*, *42*), an MRSA COL Δ*tarO::emtA* null mutant demonstrated an attenuated virulence reflecting ~1.5 $\log_{10}$ lower bacterial burden compared to the wild-type MRSA COL parent (fig. S1D), supporting the requirement of wall teichoic acid for virulence during infection. Additionally, mice infected with the Δ*tarO::emtA* mutant and treated with a subefficacious dose of imipenem (10 mg/kg) showed >3 $\log_{10}$ reduction in bacterial burden compared to the isogenic MRSA parent strain (fig. S1D). Collectively, these results corroborate previous studies demonstrating that *tarO* (and presumably other early nonessential steps in wall teichoic acid biosynthesis) serves as an attractive β-lactam potentiation target.

To identify inhibitors to any of the early steps in the wall teichoic acid biosynthetic pathway, we designed a phenotypic screen that exploits the conditional essentiality of late-stage wall teichoic acid genes (Fig. 1A) and uses the previously described TarG inhibitor L-638 (*27*). L-638 (and other TarG inhibitors) is bacteriostatic (*27*, *37*), implying that cells treated with this compound could resume growth (that is, the effects of L-638 would be suppressed) specifically in the presence of a second compound that selectively inhibits one of the early nonessential steps in wall teichoic acid biosynthesis, such as TarO, TarA, or MnaA. Such a chemical suppression of L-638 bioactivity recapitulates the essential gene paradox and mirrors the demonstrated conditional essentiality of late-stage wall teichoic acid genes (*32*, *38*). Further, the readout of this phenotypic whole-cell target-selective screen is restored cell growth (Fig. 1A).

To demonstrate the screening approach, MRSA COL growth was inhibited at a 2× minimal inhibitory concentration (2 μg/ml) of L-638 and concomitantly supplemented with tunicamycin at increasing drug concentrations that resulted in a correspondingly greater depletion of wall teichoic acid (fig. S1B). Indeed, a striking dose-dependent suppression of the growth inhibitory effect of L-638 was observed at tunicamycin concentrations of 0.125 to 0.25 μg/ml, which completely abolished wall teichoic acid synthesis (Fig. 1B). The suppression of L-638 bioactivity was also highly specific to tunicamycin and its cognate target TarO because known antibiotics that target other biological processes, such as PC190723 (cell division), vancomycin (peptidoglycan synthesis), tetracycline (protein synthesis), and ciprofloxacin (DNA synthesis), do not suppress L-638 (Fig. 1C). Adapting this chemical suppression screen to a 1536-well format (see Materials and Methods), we performed a single-point ultrahigh-throughput screen (uHTS) against the complete corporate synthetic chemical library to identify compounds (screened at 20 μM) that specifically suppress L-638 activity against MRSA COL.

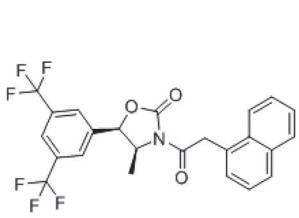
Identification of TarO inhibitors

Our chemical suppression screen resulted in an extremely low (<0.16%) hit rate from a collection of 2.8 million synthetic small molecules. A series of additional wall teichoic acid pathway-specific secondary screens performed in MRSA COL was required to identify authentic early-stage wall teichoic acid inhibitors (Fig. 1D). Hit compounds were prioritized for follow-up studies provided that they (i) demonstrated a dose-dependent suppression of inhibitor L-638, (ii) conferred resistance to cell lysis mediated by the staphylococcal-specific lytic bacteriophage ØK that utilizes wall teichoic acid as its receptor for bacteriophage entry into the cell (*43*), and (iii) were synergistic in combination with imipenem below its clinical breakpoint (4 μg/ml). Two synthetic compounds structurally distinct from each other as well as the positive control tunicamycin met all pathway-specific criteria, herein designated as an oxazolidinone series (named tarocin A) and a benzimidazole series (named tarocin B) (Table 1). Both series reflected chemical classes of existing therapeutically efficacious drugs (including antimicrobial) in clinical use (*44*, *45*). Whereas tunicamycin demonstrated potent whole-cell wall teichoic acid pathway-specific inhibitory effects in these assays at ≤0.1 μM, tarocin A and B were notably less active (3 to 26 μM) in each of the above assays. Neither tarocin A nor tarocin B demonstrated any intrinsic growth inhibitory activity against MRSA COL or other bacterial or fungal pathogens tested (minimum inhibitory concentration, >200 μM), whereas tunicamycin exhibited broad antimicrobial activity (Table 1). Further, tarocins lacked cytotoxicity against HeLa cells [inhibitory concentration (IC_{50}), >100 μM] versus tunicamycin (IC_{50}, 0.2 μM) (Table 1). As an analog of uridine diphosphate–*N*-acetylglucosamine (UDP-GluNAc), tunicamycin is a highly cytotoxic and promiscuous inhibitor of many bacterial glucosyltransferases involved in not only wall teichoic acid biosynthesis (TarO) (*24*) but also peptidoglycan biosynthesis (MraY) (*24*) as well as N-linked glycosylation in yeast (Alg7) (*46*) and in human (DPAGT1) (*47*, *48*). Therefore, whereas tunicamycin's cytotoxicity precluded it as a viable chemical starting point to consider as a β-lactam potentiation agent, tarocins provided an attractive alternative therapeutic candidate because they lacked cytotoxicity and intrinsic bioactivity as a single agent.

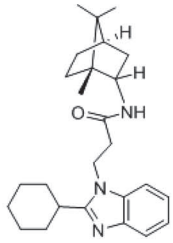
Confirmation of genetic TarO target engagement

To directly demonstrate that tarocin A and B inhibited wall teichoic acid production, MRSA COL was incubated in the presence of each agent, and wall teichoic acid polymers were extracted and visualized on an Alcian blue/silver–stained polyacrylamide gel electrophoresis (PAGE) gel. Tarocin A and B depleted the wall teichoic acid polymer, mirroring the wall teichoic acid depletion effects of tunicamycin-treated cells

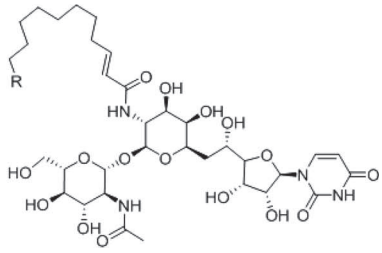
Table 1. Microbiological whole-cell profiles of leading wall teichoic acid inhibitors identified in the uHTS. Shown on top are the chemical structures of oxazolidinone series (tarocin A), benzimidazole series (tarocin B), and natural product tunicamycin. Minimum inhibitory concentrations were determined by the broth microdilution method in accordance with the recommendations of CLSI in 96-well plates and were assayed visually. TarG inhibitor suppression represents effective concentration of wall teichoic acid inhibitor required to suppress L-638 (4 μg/ml). Phage K resistance represents effective concentration of wall teichoic acid inhibitor required to prevent Phage K–induced lysis. Imipenem potentiation represents effective concentration of wall teichoic acid inhibitor required to synergize imipenem below the clinical susceptibility breakpoint (4 μg/ml). HeLa cell cytotoxicity represents concentrations at which 50% of the cell count is reduced within 24 hours of treatment. All concentrations are in micromolar, unless otherwise denoted as microgram per milliliter. NA, no activity; ND, not determined; EC_{50}, median effective concentration; SIC, synergistic inhibitory concentration; MSSA, methicillin-susceptible *S. aureus*.



Tarocin A:
oxazolidinone series



Tarocin B:
benzimidazole series



Tunicamycin

Phenotypic assays (μM)	Tunicamycin	Tarocin A	Tarocin B	Linezolid
MRSA COL	32	>200	>200	1
TarG inhibitor suppression (EC_{50})	<0.1	26	6	NA
Phage K resistance (EC_{50})	0.1	14	3	NA
Imipenem potentiation (SIC)	<0.1	3	3	NA
Intrinsic bioactivity (μM)				
MSSA RN4220	32	>200	>200	1
MRSA COL	32	>200	>200	1
MRSE CLB27217	16	>200	>200	1
Candida albicans (MY1055)	1.19	>200	>200	ND
Haemophilus influenzae (ATCC 49247)	0.595	>200	>200	16
Streptococcus pneumoniae (MB6357)	>2.38	>200	>200	0.5
Streptococcus pyogenes (CL10253)	1.19	>200	>200	1
Moraxella catarrhalis (ATCC 25238)	>2.38	>200	>200	8
HeLa cell cytotoxicity (IC_{50})	0.197	>100	>100	ND

(Fig. 2A). In addition, a highly potent tarocin A derivative (tarocin A1; see fig. S2 for structure and microbiological data) displayed complete depletion of the wall teichoic acid polymer with similar potency to tunicamycin (Fig. 2A).

Forward genetics–based drug resistance and mutation mapping remains the most powerful means of linking an antibiotic to its cognate target (*11*, *27*, *37*, *49*). However, tarocin A or B lacked intrinsic antibacterial activity as a single therapeutic agent. Therefore, to identify their wall teichoic acid pathway target by genetic means, we exploited their synergistic activity in combination with the β-lactam antibiotic, imipenem, to select for MRSA COL mutants that were refractory to the tarocin A–imipenem chemical synergy (Fig. 2B, middle panel). Provided such mutants yielded a target-based drug resistance mechanism, they were also expected to reverse the antagonism between tarocin A and L-638 observed in wild-type treated cells (Fig. 2B, lower panel). In addition, such mutants were expected to be resistant to the wall teichoic acid depletion effects of tarocin A (Fig. 2C). On the basis of these criteria, multiple (n = 22) independently derived target-based resistant mutants were isolated and subjected to whole-genome sequencing (Fig. 3A). Indeed, all predicted target-based drug-resistant mutants contained a single nonsynonymous mutation mapping to *tarO* (Fig. 3A). Because no additional nonsynonymous mutations were identified in any of these resistant isolates, we concluded that the *tarO* mutations were causal for the resistance to the tarocin A–imipenem synergy. Similarly, tarocin B–imipemen drug resistance selections and follow-up studies also revealed TarO to be the drug target, establishing it as a second structurally

distinct inhibitor to this enzyme (Fig. 3A). Unexpectedly, although tarocin A and B are structurally unrelated to one another, multiple *tarO* drug-resistant mutations selected against each series were also cross-resistant to the other series (Fig. 3B), implying that both tarocin A and B shared a common mechanism of action in inhibiting TarO. Conversely, none of the *tarO* tarocin-resistant (tarocin^R) mutations were cross-resistant with tunicamycin (Fig. 3B), indicating that the mechanism of TarO inhibition by tarocin A and B is distinct from that of tunicamycin, which is believed to act as a competitive inhibitor of UDP-GlcNAc and bind to the catalytic active site of TarO (*47*, *50*).

To independently demonstrate that tarocin A and B directly and specifically inhibited TarO, in vitro biochemical assays to monitor the enzymatic activity of TarO, TarA, and MnaA were established, and tarocin inhibition studies were performed (fig. S2). Both tarocin A and B effectively inhibited TarO enzymatic activity, demonstrating IC_{50} of 0.2 and 0.4 μM, respectively (Fig. 3C). Consistent with the improved whole-cell potency of tarocin A1, its inhibitory activity against TarO in this cell-free assay was sevenfold lower (IC_{50}, 0.03 μM) than that of tarocin A, achieving an in vitro potency similar to that of tunicamycin (IC_{50}, 0.01 μM) (Fig. 2B). In contrast, tarocin A, A1, and B did not inhibit TarA or MnaA in vitro at the highest (200 μM) concentration tested (fig. S2), demonstrating their highly selective inhibitory activity toward TarO within the wall teichoic acid biosynthetic pathway.

TarO resistance mapping by three-dimensional protein modeling

To further investigate the potential mechanism of inhibition of TarO by tarocin A and B, we generated a three-dimensional (3D) protein model

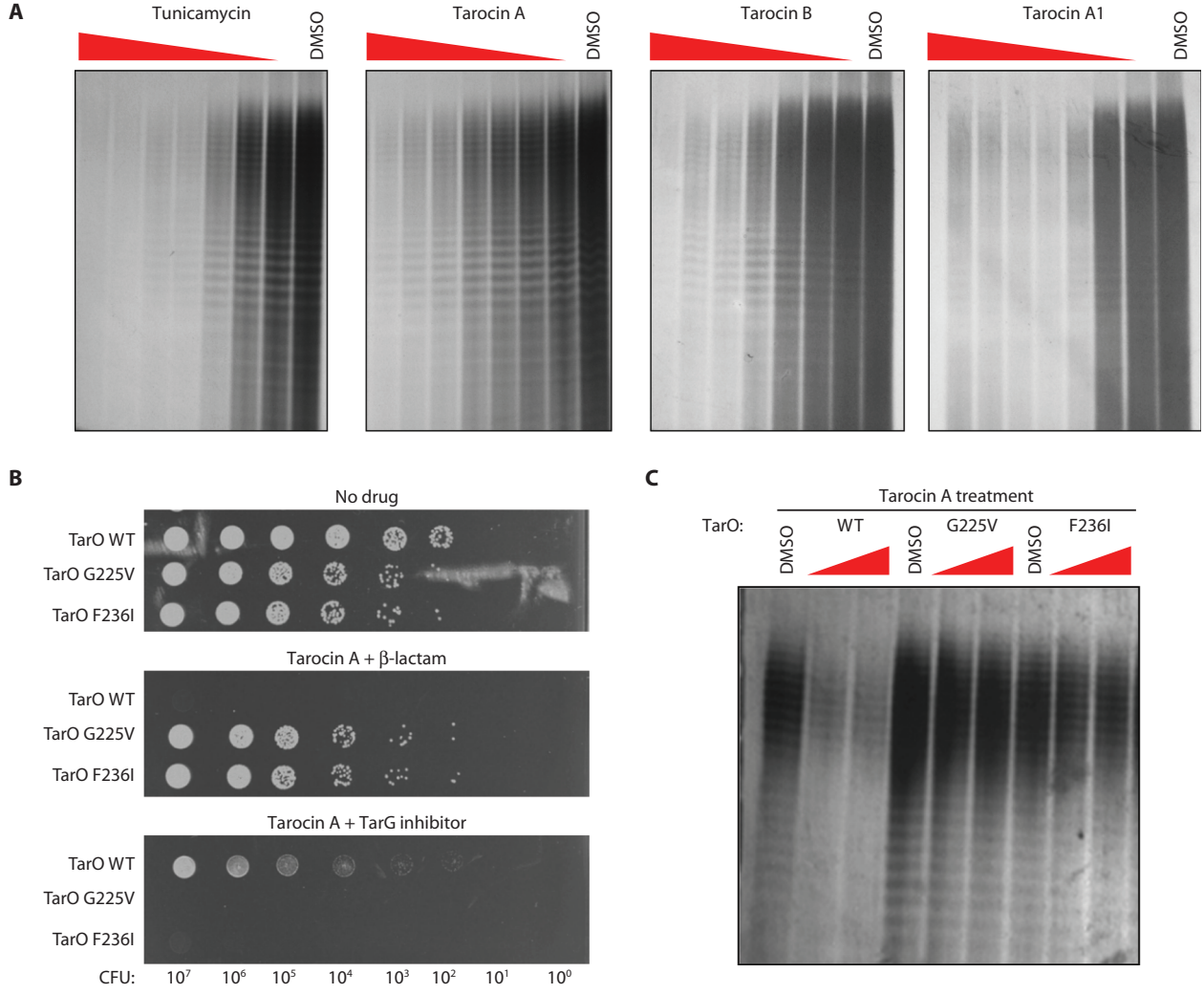


Fig. 2. Confirmation of wall teichoic acid pathway engagement and isolation of tarocin-resistant mutants. (**A**) Depletion of wall teichoic acid polymer from inhibitor-treated MRSA COL strain was visualized on an Alcian blue/silver–stained PAGE gel (*27*). Inhibitors were serially titrated twofold, and the highest tested concentration is designated in parentheses: tunicamycin (0.5 μg/ml), tarocin A (8 μg/ml), tarocin B (8 μg/ml), and tarocin A1 (0.5 μg/ml). DMSO, dimethyl sulfoxide. (**B**) Phenotypic characterization of tarocin A–resistant mutants. Tenfold serial dilutions of wild-type (WT) MRSA COL or isogenic TarO mutants (G225V or F236I) were spotted on agar plates containing no drug (top panel), tarocin A (20 μg/ml) in combination with imipenem (4 μg/ml) (middle panel), and tarocin A (20 μg/ml) in combination with L-638 (4 μg/ml) (lower panel). (**C**) Depletion of wall teichoic acid polymer of MRSA COL or isogenic TarO mutants (G225V or F236I) treated with DMSO alone or with tarocin A (10 or 20 μg/ml).

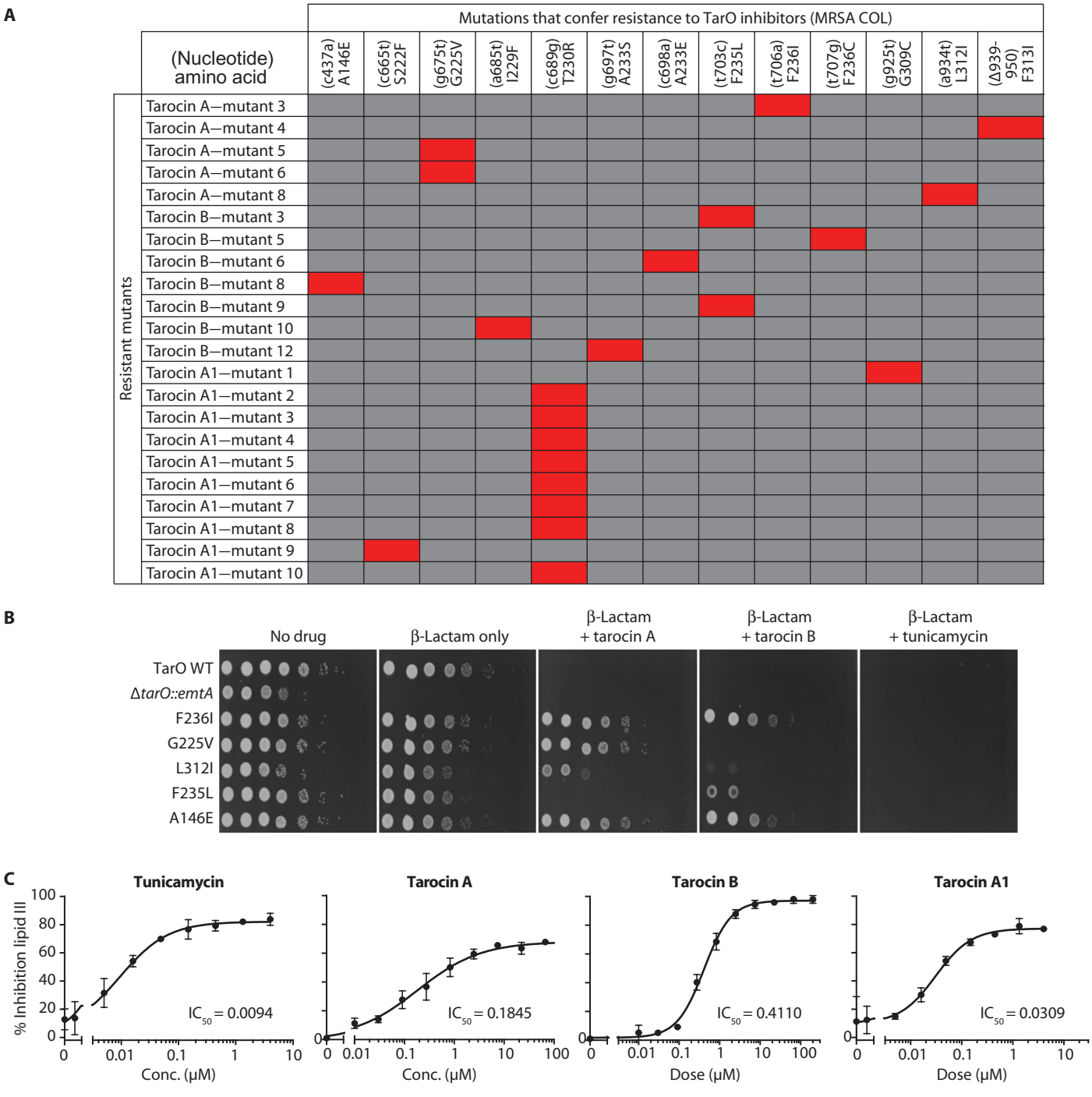


Fig. 3. Genetic and biochemical analyses of TarO target engagement. (**A**) Heat map of 22 tarocin-resistant MRSA COL mutants and corresponding mutations that conferred resistance to tarocin inhibitors. The corresponding nonsynonymous single-nucleotide polymorphisms and amino acid substitutions are shown (top). (**B**) Cross-resistance study of tarocin A and B–resistant mutants. Tenfold serial dilutions of WT MRSA COL or isogenic mutants containing nonsynonymous mutations in *tarO* were spotted onto agar plates containing DMSO, imipenem (4 μg/ml) alone, tarocin A (20 μg/ml) in combination with imipenem (4 μg/ml), tarocin B (20 μg/ml) in combination with imipenem (4 μg/ml), or tunicamycin (2 μg/ml) in combination with imipenem (4 μg/ml). (**C**) TarO biochemical assay measuring inhibition of wall teichoic acid lipid III (C_{55}-P-P-GluNAc) production after treatment with tunicamycin or tarocin A, A1, or B. IC_{50} is the average of two independent experiments.

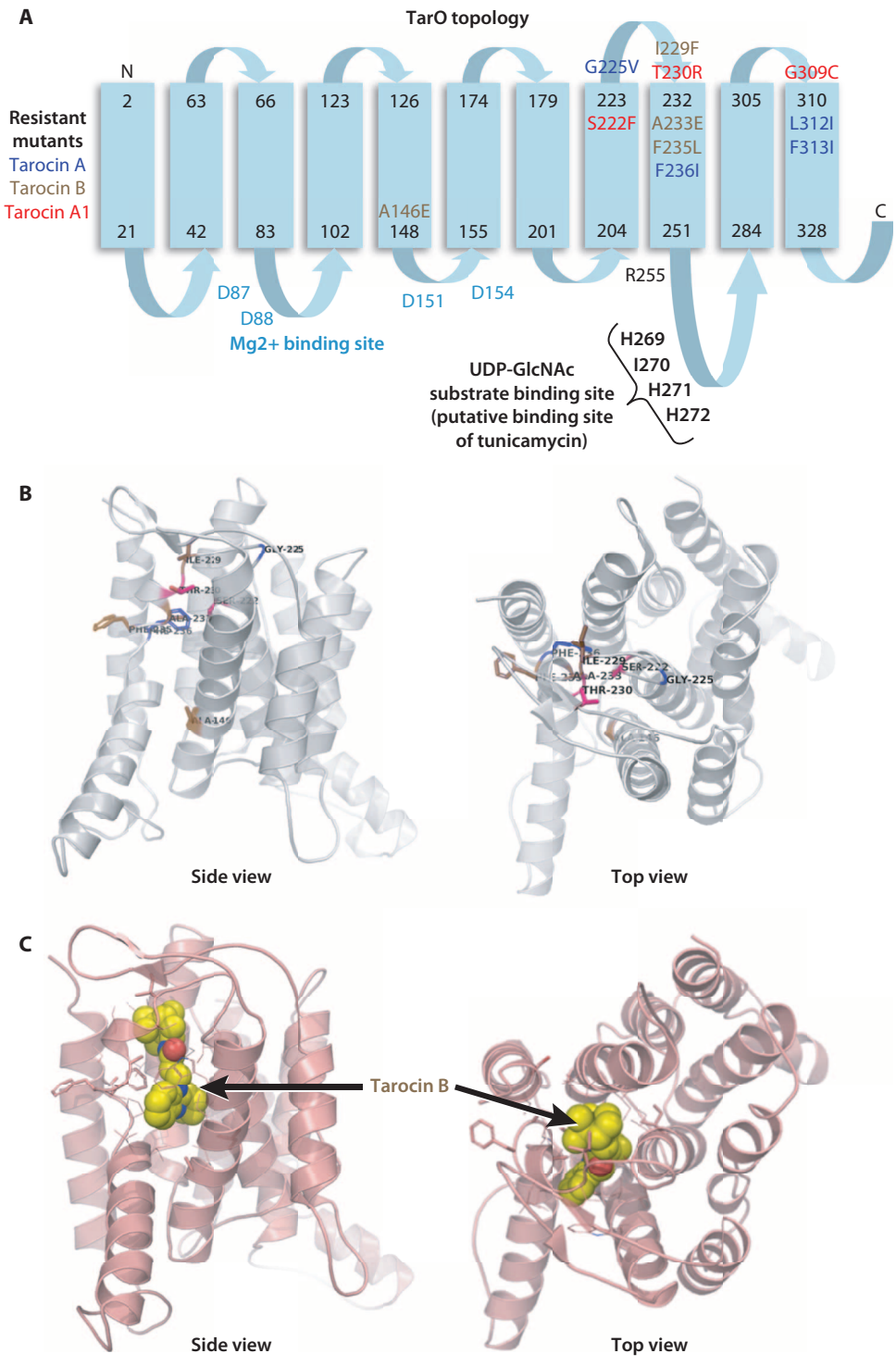


Fig. 4. 3D TarO protein modeling to elucidate mechanism of inhibition. (**A**) TarO protein predicted topology with amino acid residues that confer resistance to wall teichoic acid inhibitors are highlighted (tarocin A, blue; tarocin B, brown; tarocin A1, red). Putative UDP-GlcNAc substrate binding site (black) and Mg^{2+} binding site (light blue) are designated. (**B**) Side and top views of predicted TarO 3D protein modeling based on homology to MraY crystal structure (*51*). Residues that confer resistance to specific inhibitors are highlighted (tarocin A, blue; tarocin B, brown; tarocin A1, red). (**C**) Side and top views of predicted tarocin B (yellow) binding to surface-exposed hydrophobic sites composed of TM5, TM8, TM9, and TM11.

of the TarO structure using the recent x-ray crystal structure of the *Aquifex aeolicus* MraY (*51*) protein as template. Our rationale for selecting MraY to model the TarO protein structure was based on the significant structural and functional similarities of the two plasma membrane glycosyltransferases. They share (i) a similar number of transmembrane (TM) domains (fig. S3; 10 TM domains for MraY and 11 TM domains for TarO), (ii) a conserved predicted membrane topology (extracellular N-terminal domain/cytoplasmic C-terminal domain) (*51*), (iii) conserved Mg^{2+} ion (D87 and D88) and UDP-GlcNAc (H269, L270, H271, and H272) substrate binding sites (fig. S4), and (iv) a common lipid carrier (bactoprenol) cosubstrate that each enzyme requires in their glycosyltransferase reaction to contribute to peptidoglycan and wall teichoic acid polymer synthesis, respectively. Mapping TarO mutations that conferred resistance to tarocin A, A1, and B into the protein structural model of TarO revealed that all mutations resided within TM5, TM8, TM9, and TM11 of the protein (Fig. 4A). These mutations clustered around an extracellularly exposed hydrophobic pocket formed by the affected transmembrane helices (Fig. 4B) with sufficient spacing (~500 $Å^3$) between the helices for either tarocin A or B to presumably bind through multiple hydrophobic interactions (Fig. 4C). MraY crystal structure data predicted that this hydrophobic pocket likely serves as the bactoprenol binding site of the enzyme (*51*) (fig. S5). Tarocin A and B are predicted to inhibit TarO interactions with the bactoprenol cosubstrate. In contrast, tunicamycin has been proposed to compete with UDP-GlucNAc binding to the cytosol-exposed catalytic site of TarO (Fig. 4A). Consistent with these proposed mechanistic differences between TarO inhibitor classes, none of the *tarO* mutations conferring resistance to tarocin A and B were mapped to the proposed TarO catalytic site of the enzyme, and none of these mutations conferred cross-resistance to tunicamycin (Fig. 3B), consistent with tarocins and tunicamycin having distinct mechanisms of action.

Tarocin A and B: Therapeutic proof-of-concept studies

To evaluate the β-lactam potentiation and spectrum that TarO inhibitors exhibited

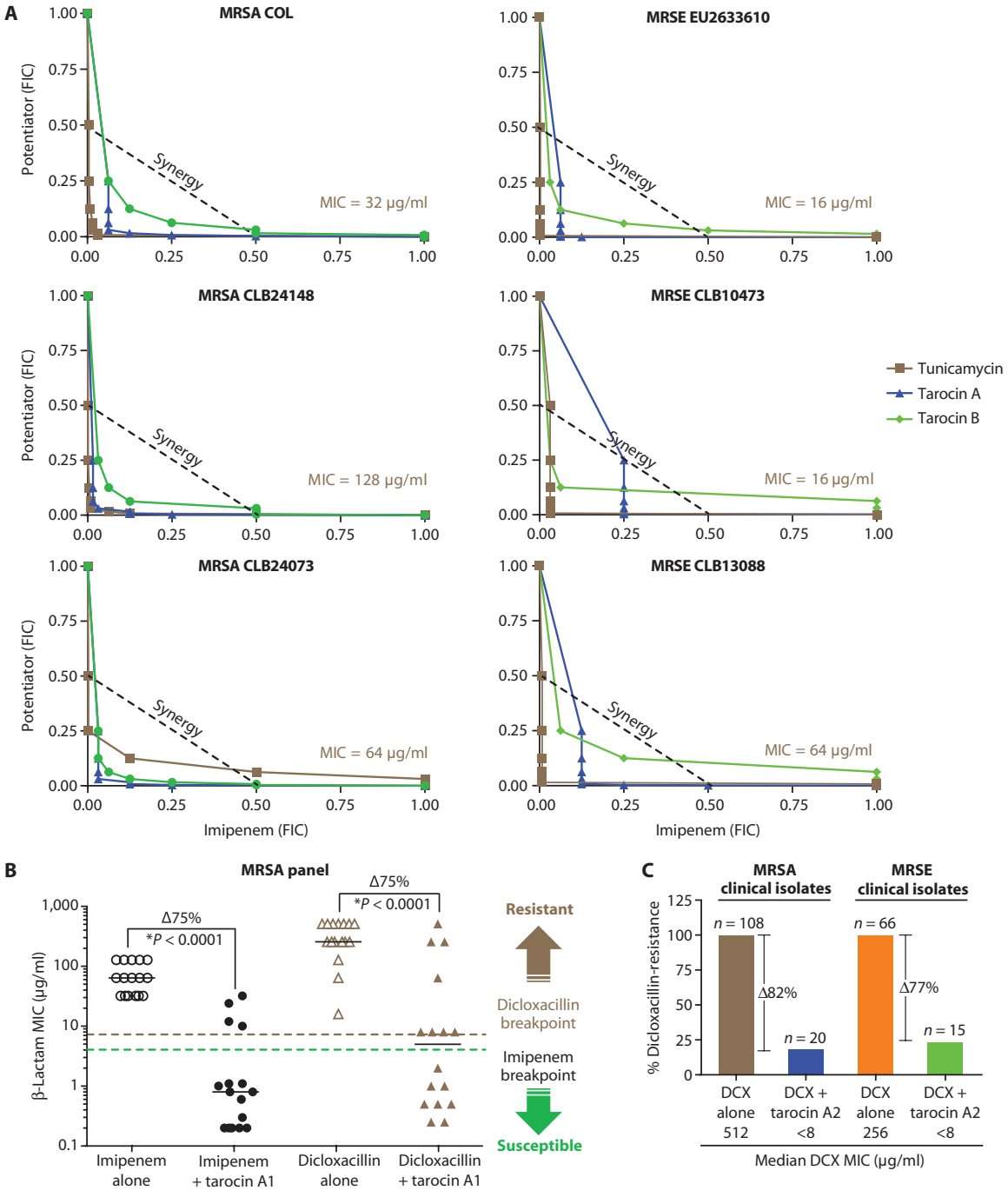


Fig. 5. Tarocin A and B restore β-lactam susceptibility to clinical MRSA and MRSE isolates. (**A**) Synergistic antibiotic susceptibility checkerboard analyses of tunicamycin and tarocin A and B in combination with imipenem against MRSA clinical isolates (left panel) and MRSE clinical isolates (right panel). The FIC of each antibiotic combination pair is plotted, and synergy is achieved with a combination of the two agents fully inhibiting growth with individual FIC values yielding a sum FIC index <0.5 (indicated by diagonal dashed line); an FIC index ≥0.5 indicates additive or no synergistic activity. (**B**) β-Lactam susceptibility coverage of expanded clinical panel of MRSA isolates ($n = 17$) with tarocin A1 combined with either imipenem or dicloxacillin (DCX) and the corresponding median minimum inhibitory concentration (MIC) indicated by a black line. Percent change (top) represents percentage of isolates that were susceptible to β-lactams. *$P < 0.0001$, two-tailed unpaired t test. (**C**) Susceptibility coverage of tarocin A2 in combination with dicloxacillin against an expanded panel ($n = 174$) of MRSA ($n = 108$) and MRSE ($n = 66$) clinical isolates. The y axis represents percent of β-lactam–resistant strains with minimum inhibitory concentration of dicloxacillin (>8 μg/ml; CLSI clinical breakpoint), and the x axis includes the median β-lactam minimum inhibitory concentration of dicloxacillin alone or in combination with tarocin A2. The percent change represents the percent of formerly dicloxacillin-resistant strains that are now susceptible to the tarocin A2–dicloxacillin combination.

against MRSA and MRSE, we initially examined the in vitro activity of tarocin A or B in combination with imipenem against a modest panel of MRSA and MRSE clinical isolates by standard checkerboard analyses. Both tarocin A and B showed potent synergy with imipenem against MRSA and MRSE clinical isolates similar to what was observed for tunicamycin [fractional inhibitory concentration (FIC) index <0.5] (Fig. 5A). We expanded the spectrum study to include dicloxacillin (a Gram-positive–specific penicillin class β-lactam) against a broader panel of methicillin-resistant staphylococcal clinical isolates (see table S1 for antibiogram). These highly drug-resistant staphylococcal isolates had median MRSA minimum inhibitory concentrations of 64 μg/ml for imipenem and 256 μg/ml for dicloxacillin. Addition of tarocin A1 at a fixed concentration (16 μg/ml) to each of the β-lactams resulted in substantially reduced median β-lactam minimum inhibitory concentrations of 1 and 5 μg/ml (Fig. 5B), respectively, representing >50-fold reductions from their single β-lactam minimum inhibitory concentration values. The observed potentiation resulting in the increased susceptibility of MRSA to β-lactams was achieved by restoring the bactericidal mode of action of the parent β-lactams (fig. S6). In addition, combining tarocin A1 with either β-lactam resulted in a restored β-lactam clinical susceptibility profile as determined by the Clinical and Laboratory Standards Institute (CLSI) standard (<4 μg/ml for imipenem and <8 μg/ml for dicloxacillin) for 75% of the clinical MRSA isolates tested (Fig. 5B). On the basis of these results, an extensive evaluation of the efficacious coverage possible for a TarO inhibitor/β-lactam combination in restoring MRSA and MRSE susceptibility was performed against 174 clinical isolates with median dicloxacillin minimum inhibitory concentrations of 512 and 256 μg/ml (Fig. 5C), respectively. Combining dicloxacillin with tarocin A2, a second analog with improved solubility (fig. S2, structure and biological data), restored susceptibility to dicloxacillin in 82% of MRSA clinical isolates and in 77% of MRSE clinical isolates (Fig. 5C). Further, an acceptable frequency of spontaneous resistance (FOR) was observed in MRSA COL, ranging from 3.2×10^{-7} to 1.6×10^{-8}, using 2× to 8× SIC of tarocin A2 in combination with a fixed concentration of imipenem (4 μg/ml) or dicloxacillin (8 μg/ml) after 24 hours of treatment (fig. S7). Finally, the addition of L-638 at 2× minimum inhibitory concentration (4 μg/ml) as a third component under otherwise identical conditions considerably reduced target-mediated spontaneous resistance to tarocin A2 in combination with dicloxacillin with an FOR value of $<3.9 \times 10^{-9}$ (fig. S7; see Discussion). Collectively, these data provide strong in vitro evidence that a β-lactam potentiation strategy based on inhibition of wall teichoic acid biosynthesis is efficacious and largely unaffected by spontaneous resistance or the substantial heterogeneity observed among clinical isolates of MRSA and MRSE.

On the basis of in vitro data that tarocin A2 is broadly synergistic in combination with dicloxacillin against diverse clinically relevant methicillin-resistant staphylococci and displays an equivalent pharmacokinetic half-life of 1.8 hours compared to its partner antibiotic (*52*) (tables S3 and S4), we evaluated the efficacy of this combination when coadministered to a murine systemic infection model of MRSA (*42*). At all doses studied, neither dicloxacillin (125 mg/kg) nor tarocin A2 (12.5, 25, or 50 mg/kg) administered as a single agent provided any efficacious benefit in treating an MRSA COL infection (Fig. 6) compared with vehicle treatment. Conversely, coadministering tarocin A2 and dicloxacillin at concentrations otherwise nonefficacious when administered singly provided a significant dose-dependent reduction in MRSA bacterial burden after 24 hours of combination therapy [Fig. 6; $P < 0.05$, one-way analysis of variance (ANOVA)]. This reduction of up to 2.5 $\log_{10}$ colony-forming units (CFU) ($P < 0.05$, one-way ANOVA) paralleled that of the control antibiotic linezolid (Fig. 6; $P < 0.05$, one-way ANOVA). Further, no evidence of mortality or morbidity was observed among mice in any of the tarocin A2 single treatment or β-lactam combination cohorts, consistent with the low in vitro cytotoxicity observed for tarocins and proven safety of β-lactam antibiotics. In addition, no tarocin A2–dicloxacillin–resistant mutants were identified among the CFU recovered from the in vivo study. Collectively, these data provide strong evidence supporting the hypothesis that tarocins (or other early-stage wall teichoic acid inhibitors) may provide an important new strategy to develop Gram-positive bactericidal β-lactam combination agents that are active against methicillin-resistant staphylococci.

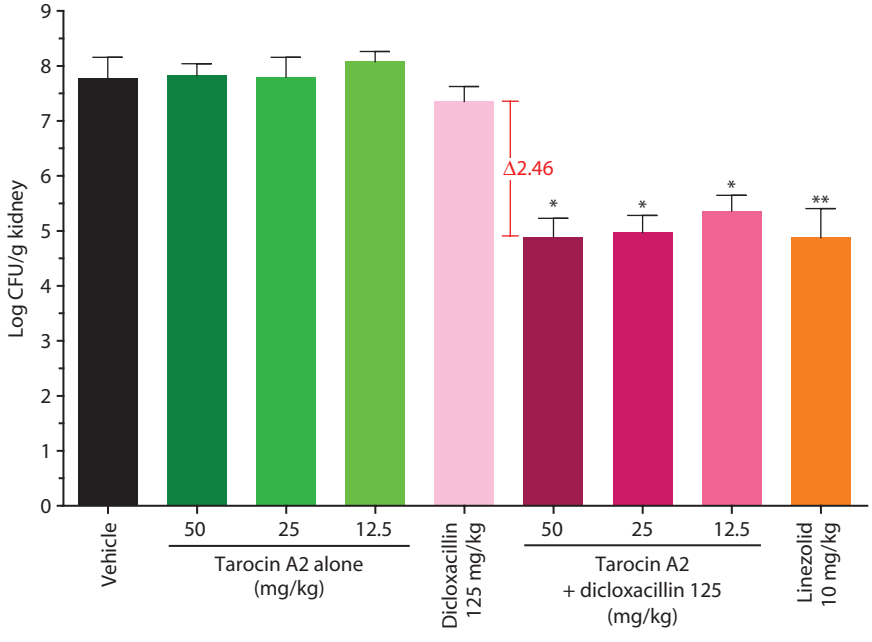


Fig. 6. The tarocin A2–dicloxacillin combination demonstrates in vivo efficacy. Murine systemic in vivo efficacy study (*42*) of the tarocin A2–dicloxacillin combination against MRSA COL (dicloxacillin alone; minimum inhibitory concentration, 256 μg/ml). Treatment was initiated 2 hours after infection [three times a day (2, 5, and 8 hours after challenge)] by subcutaneous injection. Bacterial burden was enumerated 24 hours after infection and compared among five groups: vehicle alone, tarocin A2 alone, dicloxacillin alone, tarocin A2 and dicloxacillin coadministration, and linezolid alone. *$P < 0.05$, one-way ANOVA compared to the dicloxacillin-alone group. **$P < 0.05$, one-way ANOVA compared to the vehicle-alone group.

DISCUSSION

We report the discovery and characterization of two structurally distinct chemical series, the tarocins. Tarocins effectively and specifically abolish staphylococcal

wall teichoic acid biosynthesis by inhibiting TarO, the first committed enzyme in polymer synthesis. Tarocins themselves are nonbioactive against staphylococci because of their selective inhibition of a nonessential enzyme. However, we now show that tarocins provide a highly potent and broad-spectrum synergistic activity specifically in combination with β-lactam antibiotics, which are otherwise inactive among methicillin-resistant staphylococci. Hence, tarocins represent a new class of potential therapeutic adjuvants to restore the bactericidal activity of β-lactams against MRSA and MRSE. This general β-lactam combination approach has proven highly successful in treating multidrug-resistant Gram-negative bacterial pathogens by inhibiting the β-lactamase enzymes responsible for clinical resistance to β-lactams (*14*). In contrast, methicillin-resistant staphylococci resist the effects of β-lactams by a separate mechanism involving the acquisition of an additional penicillin-binding protein, Pbp2a, that is intrinsically refractory to β-lactam inhibition (*15*–*19*). The identification and characterization of tarocins demonstrate that identifying new targets and cognate inhibitors capable of restoring the susceptibility of MRSA and MRSE to β-lactam antibiotics could be a general strategy for addressing β-lactam resistance among Gram-positive bacterial pathogens.

Mechanistically, tarocins likely reset the susceptibility of methicillin-resistant staphylococci to β-lactams by antagonizing multiple aspects of cell wall biosynthesis, regulation, and remodeling that ultimately compromise the physical strength of the bacterial cell wall. For example, wall teichoic acid is required to localize Pbp4 to the septal division site, and in its absence, delocalization of Pbp4 impairs peptidoglycan cross-linking and septation (*53*). The major cell wall autolysin Atl is also negatively regulated by its association with wall teichoic acid. In the absence of wall teichoic acid, Atl-mediated peptidoglycan hydrolysis, normally restricted to the division septum, is markedly delocalized through the cell surface and results in a further weakening of the cell wall (*54*, *55*). Finally, wall teichoic acid polymers comprise up to half the dry weight of the staphylococcal cell wall (*28*, *56*), and their absence may directly physically weaken the wall and/or enhance the permeability of β-lactams to more effectively interdict Pbp targets (*57*). Any of these perturbations, in the context of an already fragile cell wall resulting from β-lactam drug exposure, could create a synthetically lethal interaction of detrimental cell wall effects reflecting the restored synergistic bactericidal activity between tarocins and the β-lactam antibiotic.

Notably, the identification of tarocins was achieved by performing a phenotypic whole-cell pathway-specific chemical suppression screening approach similarly performed recently by Farha *et al.* (*58*). This screening rationale is based on a deep understanding of wall teichoic acid biosynthesis (*29*, *35*, *40*), its unorthodox gene dispensability pattern (*31*, *32*, *34*, *35*, *38*), and the expectation that small-molecule inhibitors targeted to wall teichoic acid early and late steps in the pathway should recapitulate genetic findings that abolishing early events in polymer synthesis are nonessential for growth but suppress the otherwise growth inhibitory defects of later steps in the pathway. Accordingly, our screen involved identifying small molecules that effectively suppressed the bacteriostatic effects of the TarG inhibitor L-638, thus restoring growth by inhibiting an early step in wall teichoic acid biosynthesis. Using growth as a readout for a target-specific inhibitor is therefore analogous to a genetic selection (*59*), but one that was performed by pharmacological means, underscoring the potency and selectivity of tarocins in a whole-cell context. A growth readout also seems counterintuitive to the goal of identifying new antibiotics because the TarO inhibitors themselves are nonbioactive. However, the knowledge that TarO (and other early steps in wall teichoic acid biosynthesis) serves as a β-lactam potentiation target provides a compelling rationale for their discovery.

Recently, the U.S. Food and Drug Administration–approved antiplatelet drug ticlopidine has also been reported to impair *S. aureus* wall teichoic acid biosynthesis by inhibiting TarO (*25*). Accordingly, it was examined in direct comparison with tarocin A1 and tunicamycin. We conclude that ticlopidine is synergistic in combination with the β-lactam cefuroxime, although less potent and more restrictive in achieving synergy with β-lactams than tarocin A1 or tunicamycin [fig. S8 and (*25*)]. Tarocins and tunicamycin also display superior in vitro TarO inhibitory activity and depletion of wall teichoic acid polymers among drug-treated cells versus ticlopidine (fig. S8 and see fig. S9 for ticlopidine structure verification). Conversely, tunicamycin and tarocin A1 and A2 display similar in vitro TarO inhibitory activity and potency in depleting cellular wall teichoic acid polymers. An important distinction between these agents, however, is that tarocins inhibit TarO activity by a distinct mechanism versus tunicamycin. Whereas the latter is a natural product analog of UDP-GlcNAc proposed to competitively inhibit the enzyme by binding to its UDP-GlcNAc binding site (*47*), tarocins are likely allosteric inhibitors that interfere with the enzyme's binding to the cosubstrate bactoprenol or glycosylation of the activated sugar substrate to the lipid carrier. Therefore, whereas tunicamycin is generally regarded as a highly toxic and promiscuous glycosyltransferase inhibitor with multiple drug targets, tarocins display similar potency and exquisite selectivity without obvious cytotoxicity. Tarocins also demonstrate robust β-lactam potentiation and efficacy in a murine infection model of MRSA and against diverse methicillin-resistant staphylococci in vitro, key requirements for their therapeutic potential.

The possibility of clinically relevant bacterial drug resistance exists for all antibiotics and similarly could limit the success of developing tarocins as β-lactam potentiation agents. For example, although tarocin A2 and dicloxacillin together provide extensive in vitro efficacy against a broad set of MRSA (82%) and MRSE (77%) clinical isolates, complete coverage was not achieved in combination with dicloxacillin at its in vitro clinical breakpoint drug concentration (8 μg/ml; the drug concentration that defines *S. aureus* resistance to dicloxacillin). This minor group of isolates therefore have varying levels of intrinsic tolerance to the combination agent. However, strong drug synergy was commonly observed against these same isolates, provided that higher dicloxacillin concentrations (for example, 16 to 32 μg/ml) were used. Notably, similar higher dicloxacillin exposure levels in the blood may also be achieved in a clinical setting (*52*) on the basis of pharmacokinetic data (tables S3 and S4). We also note that DNA sequence analysis of these clinical isolates did not identify known *tarO* drug-resistant amino acid substitutions, and therefore, such strains may be vulnerable to more potent tarocins. Indeed, a clear correlation between tarocin potency and achievable coverage of MRSA clinical isolates (fig. S10) provides strong evidence that enhancing tarocin potency may further increase the number of susceptible MRSA and MRSE clinical isolates.

Spontaneous drug resistance to these β-lactam combination agents may also limit their ultimate therapeutic utility. We believe this to be unlikely for several reasons. First, drug resistance appears to be TarO-specific and linked to tarocin rather than the β-lactam. This is not surprising, considering the extremely low FOR to β-lactams, which target multiple Pbps. Conversely, if resistance to the β-lactam were to appear, tarocin-based inactivation of TarO should mimic the extensive genetic studies that demonstrate *tarO* null strains to have markedly attenuated virulence in several different in vivo infection models (*30*, *60*–*63*). Second, recent reports demonstrate that β-lactams such as nafcillin are

efficacious against MRSA in a clinical setting despite its poor in vitro activity against MRSA (*64*). Although perhaps counterintuitive, the molecular basis of its in vivo efficacy appears to be its nonlethal yet dramatic effects on peptidoglycan synthesis and cross-linking as well as indirect consequences on overall cell wall structure. This collectively exacerbates bacterial susceptibility to innate immune responses, including host peptide defensins and macrophage killing (*64*). Accordingly, MRSA suffers a substantial fitness cost in an infection setting when treated with a β-lactam that ultimately provides significantly greater in vivo efficacy than previously appreciated. In this therapeutic context, TarO-mediated resistance to the tarocin–β-lactam synergy would still be vulnerable to the effects of the β-lactam. Consistent with this hypothesis, tarocin-resistant MRSA colonies were not detected during the in vivo efficacy study performed in this work.

Spontaneous resistance to tarocin–β-lactam potentiation agents may also be reduced in combination with L-638, which displays broad and potent growth inhibitory activity against *S. aureus*, *S. epidermidis*, MRSA, and MRSE (*27*). The addition of L-638 in combination with tarocin A2 and dicloxacillin also substantially reduced *tarO*-mediated resistance to the latter two drug combination. The mechanism by which this is achieved, however, appears highly unique because L-638 bioactivity is effectively suppressed in combination with tarocins. Therefore, the reduced FOR of the tarocin-dicloxacillin combination is not simply achieved by adding another antibiotic with a distinct mechanism of action. Instead, in the context of combining these three agents, L-638 is predicted to be inactive against the bacterial population sensitive to the tarocin-dicloxacillin combination (where TarO is inhibited) but specifically bioactive against tarocin-resistant *tarO* mutants that retain functional activity and spontaneously appear during drug selection (*27*, *37*). Further, *tarO* loss-of-function mutations that suppress L-638 activity are highly sensitive to β-lactams (*25*, *28*) and strikingly attenuated in virulence in diverse animal infection studies (*28*, *29*, *31*). Consequently, the addition of L-638 to the tarocin–β-lactam cocktail strongly exploits the genetic interactions and antibiotic hypersensitivities within the cell wall network (*12*, *24*, *25*) as well as the avirulent phenotypes of *tarO* mutants to provide a new interdependent means of mitigating target-based resistance of the β-lactam potentiator. In conclusion, tarocins are an important new class of nonbioactive synthetic chemical inhibitors that are mechanistically suitable to serve as adjuvants when paired with existing β-lactam antibiotics, thus potentially restoring the therapeutic efficacy of this important class of antibiotics against methicillin-resistant staphylococci.

MATERIALS AND METHODS

Study design

The objective of this study was to validate a targeted wall teichoic acid phenotypic strategy to identify a druggable molecule that could restore the susceptibility of methicillin-resistant staphylococci pathogens to β-lactam antibiotics. An additional aim was to confirm both pathway and target engagement of the inhibitors by genetic and biochemical means and to demonstrate therapeutic efficacy in an in vivo murine infection model. All strains were grown in LB or cation-adjusted Mueller-Hinton broth at 37°C with shaking at 250 rpm. All compounds were prepared and stored in DMSO. MRSA COL is a hospital-acquired penicillinase-negative strain commonly used in *S. aureus* studies and has a fully sequenced and annotated genome (*42*). MRSA COL isogenic *tarO* and *tarA* loss-of-function mutants have been previously described (*27*). USA300 (MB6256) is a community-acquired MRSA strain (*9*). The clinical MRSA and MRSE isolates used in this study are listed in tables S1 and S2. Minimum inhibitory concentrations were determined by microdilution methods established by CLSI. MRSA and MRSE susceptibility coverage was calculated as a percentage of strains with a minimum inhibitory concentration below CLSI clinical breakpoint for imipenem (4 μg/ml) or dicloxacillin (8 μg/ml) in combination with TarO inhibitors. A checkerboard synergy technique was used to quantify the relative synergistic interactions between antibiotic agents (*65*) and was performed as previously described (*27*).

Phenotypic TarG inhibitor suppression screen

MRSA COL (about 2×10^5 CFU/ml) was mixed with increasing concentrations of L-638 (0.125 to 16 μg/ml) (*27*) in LB broth, and then increasing concentrations of tunicamycin (0 to 0.25 μg/ml; cat. no. 654380, Calbiochem) were overlaid to each combination well. The plate was incubated for 20 hours, and optical density at 600 nm (OD_{600}) was read and plotted. Specificity assay was performed as above but with tunicamycin fixed at 2 μg/ml and antibiotics PC190723 (*11*), vancomycin (Sigma-Aldrich, V1130), tetracycline (Sigma, T7660), or ciprofloxacin (Sigma-Aldrich, 17850) fixed at 0.25× minimum inhibitory concentration. The ultrahigh-throughput L-638 suppression screen was executed on a fully integrated robotic platform (GNF Systems). Briefly, the assay was carried out in a 1536-well plate format by the addition of LB broth (5 μl per well) containing L-638 (2.5 μg/ml), and then 100 nl of the test compounds in 75%/25% DMSO/water was added at 20 μM final compound concentration. On top of this solution, 5 μl of MRSA COL (~2.5×10^4 CFU) was added to the plate. The culture was grown to stationary phase by incubating for 20 hours at 37°C, and the OD_{600} was read using a Safire2 microplate reader (Tecan). Tunicamycin was used as positive control throughout the screen at fixed concentration of 0.4 μg/ml.

Secondary confirmation assays

Primary screen hit compounds were validated in a dose-response format in secondary assays for β-lactam potentiation, TarG inhibitor suppression, and bacteriophage ØK resistance (*43*) in MRSA COL. For the β-lactam potentiation assay, MRSA COL (5×10^5 CFU/ml) was mixed with increasing concentrations of the test compound in combination with imipenem (4 μg/ml); after 20 hours of incubation at 37°C, the OD_{600} was read, and percent inhibition was calculated and compared to the DMSO control. For the TarG inhibitor suppression assay, MRSA COL (5×10^5 CFU/ml) was mixed with increasing concentrations of the test compound in combination with L-638 (4 μg/ml; 2× minimum inhibitory concentration) (*27*); after 20 hours of incubation at 37°C, the OD_{600} was read, and percent suppression was calculated and compared to the DMSO control. For the bacteriophage ØK (ATCC 19685-B1) resistance assay, MRSA COL (5×10^5 CFU/ml) was infected with bacteriophage ØK (final multiplicity of infection, 0.4) and mixed with increasing concentrations of test compounds, and EC_{50} was calculated and compared to the DMSO control. Wall teichoic acid extraction analyses (*66*), time-kill curve studies (*11*), agar susceptibility assays (*9*), and mammalian HeLa cell cytotoxicity assays (*49*) were performed as previously described.

TarO liquid chromatography–mass spectrometry biochemical assay

The liquid chromatography–mass spectrometry (LC-MS)–based TarO assay, an end-point assay that measures C55-P-P-GlcNAc production,

was performed in a 384-well microtiter plate (Labcyte) with a reaction volume of 20 µl. The reaction mix contained TarO membrane prep (0.1 µg/µl), 1500 µM UDP-GlcNAc, and 75 µM C55-P substrate in a buffer containing 83 mM tris (pH 8.0), 6.7 mM $MgCl_2$, 6 mM CHAPS, and 8.3% DMSO. Enzyme reactions were quenched by the addition of 40 µl of 1-pentanol, followed by the addition of 0.04 µM 15C C55-PP-GlcNAc, used as an internal standard. A 10-µl volume of the quenched reaction mixture (pH 3) was injected onto a reversed-phase high-performance liquid chromatography, and mass spectrometric detection was carried out in the negative-ion mode using selected reaction monitoring. Additional TarA and MnaA biochemical assays are described further in the Supplementary Materials.

Resistant mutant characterization

About 5×10^8 CFU of late-exponential-phase MRSA COL was spread on LB agar plates containing twofold escalating minimum inhibitory concentrations of TarO inhibitors in combination with imipenem (4 µg/ml) or dicloxacillin (8 µg/ml). After a 24-hour incubation at 37°C, resistant colonies were picked and streaked on identical drug-containing plates. Resistance to the combination was verified by the agar susceptibility method. Briefly, late-exponential-phase MRSA COL or isogenic resistant mutants were 10-fold serially diluted, and 2.5 µl was spotted on LB agar containing tarocin A (8 µg/ml) in combination with either imipenem (4 µg/ml) or L-638 (4 µg/ml) and incubated for 24 hours at 37°C. Cross-resistance study was performed as described above, with LB agar containing tarocin A (8 µg/ml) or tarocin B (8 µg/ml), or tunicamycin (2 µg/ml) in combination with imipenem (4 µg/ml).

Bioinformatic and protein modeling analyses

Whole-genome sequence analyses were performed using paired-end sequence reads of MRSA COL parent strain and resistant strains that were aligned to the NCBI (National Center for Biotechnology Information) MRSA COL genome reference with alignment algorithm BWA (Burrows-Wheeler Aligner), followed by variant calling with GAKT (Genome Analysis Toolkit) pipeline. To detect gene duplication and deletion, read coverage on each base of the reference genome was calculated for all samples. A relative gene copy number was obtained by comparing per-kilobase read coverage of each gene and the whole genome. 3D TarO model building was performed using the Molecular Operating Environment (MOE; Chemical Competing Group, Montreal, Canada) on the basis of the x-ray crystal structure of *A. aeolicus* MraY (*51*) as a reference template using a homology modeling module in the MOE package.

Murine systemic infection model

Female Balb/c mice were rendered neutropenic with a single intraperitoneal dose of cyclophosphamide (250 mg/kg) on day −4. MRSA COL (MB 5393) was grown for16 hours in trypticase soy broth at 37°C, and 0.5 ml of diluted culture (~1×10^4 CFU) was administered intraperitoneally. Tarocin A2 and dicloxacillin were administered alone or in combination at 2, 5, and 8 hours after challenge (subcutaneously, three times a day). Twenty-four hours after challenge, the mice were euthanized, and kidneys were harvested, weighed, homogenized, and plated to determine the CFU remaining in the compound-treated groups as compared to the vehicle or β-lactam alone groups (statistical analyses was determined by one-way ANOVA). All animal procedures were performed according to the protocol reviewed and approved by the Institutional Animal Care and Use Committee of Merck and Co. Inc.

Statistical analysis

Statistical analysis and EC_{50} and IC_{50} determinations were performed with Prism 6 (GraphPad Software). All in vitro data are shown as means ± SD or median value where appropriate, and the in vivo animal study data are shown as means ± SEM. Tests of statistical significance were performed using one-way ANOVA; $P < 0.05$ was considered statistically significant for all in vivo data. Tests for statistical significance for in vitro microbiological data were performed by two-tailed unpaired *t* tests; $P < 0.0001$ was considered statistically significant.

SUPPLEMENTARY MATERIALS

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Materials and Methods
Fig. S1. Validation of wall teichoic acid–targeted phenotypic screen strategy.
Fig. S2. Microbiological and biochemical activity of tarocin A derivatives.
Fig. S3. Comparative protein alignment of TarO and MraY TM domains.
Fig. S4. Alignment of TarO homologs.
Fig. S5. Surface modeling of TarO intracellular binding pocket.
Fig. S6. Bactericidal effect of β-lactam and tarocin combination.
Fig. S7. FOR of tarocin A2 in combination with imipenem or dicloxacillin.
Fig. S8. Comparative analysis of tarocin, tunicamycin, and ticlopidine.
Fig. S9. Structure verification of ticlopidine by LC-MS and nuclear magnetic resonance.
Fig. S10. Comparative analysis of clinical MRSA coverage versus TarO biochemical activities.
Table S1. Antibiogram of clinical MRSA isolates.
Table S2. Dicloxacillin and tarocin A2 synergy profile of MRSA and MRSE clinical isolates.
Table S3. Pharmacokinetic analysis of dicloxacillin by subcutaneous administration.
Table S4. Pharmacokinetic analysis of tarocin A2 by subcutaneous administration.

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TarO-specific inhibitors of wall teichoic acid biosynthesis restore β-lactam efficacy against methicillin-resistant staphylococci

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Editor's Summary

Addressing antibiotic resistance with nonantibiotic adjuvants

Coupled with the crisis in antibiotic drug resistance is a dearth of mechanistically new classes of antibacterial agents. One possible solution to this problem is to improve the efficacy of existing antibiotics against otherwise resistant bacteria using a combination agent approach. Lee *et al.* now describe just such a combination agent strategy to resuscitate the efficacy of β-lactam antibiotics. They identify nonantibiotic adjuvants termed tarocins that restore the killing activity of β-lactams against methicillin-resistant staphylococci, thereby enabling the application of β-lactams to treat Gram-positive bacterial infections.

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