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Antiinfective therapy with a small molecule inhibitor of *Staphylococcus aureus* sortase

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Edited by Hidde L. Ploegh, Whitehead Institute for Biomedical Research, Cambridge, MA, and accepted by the Editorial Board July 30, 2014 (received for review May 9, 2014)

Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most frequent cause of hospital-acquired infection, which manifests as surgical site infections, bacteremia, and sepsis. Due to drug-resistance, prophylaxis of MRSA infection with antibiotics frequently fails or incites nosocomial diseases such as *Clostridium difficile* infection. Sortase A is a transpeptidase that anchors surface proteins in the envelope of *S. aureus*, and sortase mutants are unable to cause bacteremia or sepsis in mice. Here we used virtual screening and optimization of inhibitor structure to identify 3-(4-pyridinyl)-6-(2-sodiumsulfonatephenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole and related compounds, which block sortase activity in vitro and in vivo. Sortase inhibitors do not affect in vitro staphylococcal growth yet protect mice against lethal *S. aureus* bacteremia. Thus, sortase inhibitors may be useful as antiinfective therapy to prevent hospital-acquired *S. aureus* infection in high-risk patients without the side effects of antibiotics.

nosocomial infection | LPXTG motif | antivirulence | computational screening

The gram-positive bacterium *Staphylococcus aureus* colonizes the human skin and nares yet also causes invasive diseases such as skin and soft tissue infections, osteomyelitis, pneumonia, bacteremia, sepsis, and endocarditis (1). Methicillin-resistant *S. aureus* (MRSA) acquired resistance against many different drugs, including β-lactam, cephalosporin, fluoroquinolone, aminoglycoside, tetracycline, macrolide, trimethoprim-sulfamethoxazole, and vancomycin antibiotics (2). In the United States, MRSA isolates are responsible for >50% of *S. aureus* infections in hospitals and long-term care facilities (3). Individuals at high risk of MRSA infection include very-low-birth-weight neonates, elderly, and patients with indwelling catheters, endotracheal intubation, medical implantation of foreign bodies (prosthetic joints, implants and heart valves), trauma, surgical procedures, diabetes, dialysis, and immunosuppressive or cancer therapy (4). Antibiotic prophylaxis is designed to mitigate the risk of *S. aureus* infection, especially in surgical patients; however, this frequently fails due to drug resistance (5). Importantly, antibiotic therapy suppresses human microbiota and promotes *Clostridium difficile* infection, which is also associated with increased morbidity and mortality (6, 7). Several trials for vaccines and immune therapeutics were designed to prevent MRSA infection in hospital settings; these efforts have thus far failed to meet their study end points (4).

Surface proteins of *S. aureus* are secreted as precursors with C-terminal sorting signals that are cleaved by sortase A (SrtA) between the threonine (T) and the glycine (G) residues of their LPXTG motif (8, 9). The active site cysteine residue of sortase forms an acyl enzyme intermediate that is relieved by the nucleophilic attack of the amino group (pentaglycine crossbridge) in peptidoglycan synthesis precursors (10). Surface proteins attached to peptidoglycan precursors are subsequently incorporated into the cell wall envelope and displayed on the staphylococcal surface (9). Genome sequencing revealed that all *S. aureus* isolates encode 17–21 surface proteins with LPXTG sorting signals, which fulfill diverse functions during the infectious process (11). SrtA mutants cannot assemble surface proteins into their envelope and are unable to form abscess lesions in organ tissues or cause lethal bacteremia when inoculated into the bloodstream of mice (12, 13). In contrast, mutations that abrogate the expression of secreted virulence factors may cause attenuation but do not abrogate the ability of *S. aureus* to cause infectious diseases (12).

We reasoned that small molecule inhibitors blocking SrtA may be useful as antiinfectives to prevent *S. aureus* infection without affecting the growth of other bacteria. If so, such compounds could be used to reduce the incidence of MRSA infections without the side effects of antibiotics.

Results

Identifying Sortase Inhibitors. We used the structural coordinates from the SrtA substrate complex [SrtA/LPAT*; Protein Data Bank (PDB) ID code 2KID] to model the enzyme active site as a target for computational screening (14). The scaffold of topsentin A, a natural product that inhibits sortase A in vitro (15),

Significance

Antiinfectives, drugs that inhibit virulence strategies of microbial pathogens without affecting bacterial growth, may prevent hospital-acquired infections caused by antibiotic-resistant *Staphylococcus aureus*. We used virtual screening and synthetic optimization to identify 3,6-disubstituted triazolothiadiazole compounds as inhibitors of sortase, an enzyme that incorporates surface proteins into the staphylococcal envelope. Other Gram-positive bacteria also use sortase for protein assembly in the envelope and disease pathogenesis, suggesting that sortase inhibitors could protect high-risk patients against infection with many nosocomial pathogens.

Author contributions: O.S., C.L., and C.-G.Y. designed research; J.Z., H.L., K.Z., S.G., S.D., Y.-T.W., J.L., F.C., R.Z., L.Z., L.L., H.J., O.S., C.L., and C.-G.Y. performed research; J.Z., H.L., K.Z., S.G., R.Z., L.L., H.J., O.S., C.L., and C.-G.Y. analyzed data; and H.L., O.S., and C.-G.Y. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission. H.L.P. is a guest editor invited by the Editorial Board.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1408601111/-/DCSupplemental.

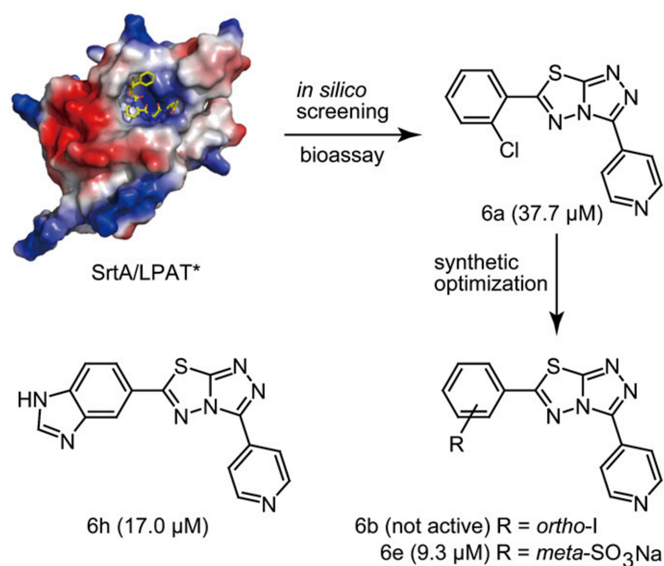

Fig. 1. Screening and optimization of sortase inhibitors. Structure-based in silico screening of small molecule library for compounds that bind the active site of *S. aureus* SrtA identifies hit compound 6a (IC_{50} value in parentheses). Synthetic optimization of the 3,6-disubstituted triazolothiadiazole scaffold generated 14 different compounds including **6b**, **6h**, and **6e**.

was used as a model ligand. Scaffold hopping and molecular docking were combined for the virtual screening of the drug-like Specs database (www.specs.net), which contains about 300,000 compounds, for compounds that bind the active site (Fig. 1). After virtual screening, 105 compounds were selected for experimental validation using purified recombinant sortase ($SrtA_{\Delta N24}$) (10). The K_m of sortase-catalyzed hydrolysis of an internally quenched fluorescent peptide substrate (abz-LPATG-dnp) was 17.5 μM, and percent inhibition of sortase activity was measured at 100 μM compound concentration (Fig. S1 *A* and *B*). Compounds with ≥50% inhibition were analyzed with an orthogonal HPLC assay to quantify $SrtA_{\Delta N24}$ cleavage of abz-LPATG-dnp substrate, and IC_{50} values were calculated. The hit compound **6a** exhibited an IC_{50} of 37.7 μM for *S. aureus* sortase (Fig. 1 and Table S1). To improve the inhibitory activity, we performed synthetic optimization of the chemical structure of compound **6a** (Scheme S1) (16). This synthesis afforded compound **6e** [3-(4-pyridinyl)-6-(2-sodiumsulfonatephenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole] with an IC_{50} of 9.3 μM (Fig. 2*A*), which represents a fourfold improvement over the screening hit, compound **6a** (Fig. 1 and Table S1).

Inhibition of Sortase-Catalyzed Transpeptidation. Sortase-mediated anchoring of surface proteins involves a transpeptidation reaction (17) but is not associated with the release of cleaved surface proteins into the extracellular medium (18). We therefore asked whether the inhibitors identified above also block sortase-catalyzed transpeptidation. $SrtA_{\Delta N24}$ cleavage of the abz-LPATG-dnp peptide and amide bond formation with the NH_2-Gly_3 nucleophile generates products abz-LPAT-Gly_3 and G-dnp, which can be quantified by HPLC and MS (Fig. S2) (19). Compound **6e** is active in a dose-dependent manner with 10.8–93.6% inhibition at 6.25–50 μM, respectively (Fig. 2*B*). The calculated IC_{50} (17.7 μM) is in agreement with the IC_{50} (9.3 μM) derived from the fluorescence-based assay (Fig. S1*C*). Surface proteins IsdA and SasX are expressed by Chinese MRSA isolates (20). Incubation of affinity-purified $IsdA_{64-323}$ (P) or $SasX_{30-178}$ precursor (P) with purified $SrtA_{\Delta N24}$ and NH_2-Gly_3 nucleophile resulted in sorting signal cleavage to yield the transpeptidation product (M), which could be blocked with the noncompetitive inhibitor *N*,*N*,*N*-trimethyl-2-(methylsulfonylthio)ethanaminium chloride (MTSET) (Fig. 2*C* and Fig. S3) (21). Sortase cleavage of sorting signals was blocked in a dose-dependent manner by compounds **6h** and **6e**, but not by compound **6b** (Fig. 2*C* and Fig. S3).

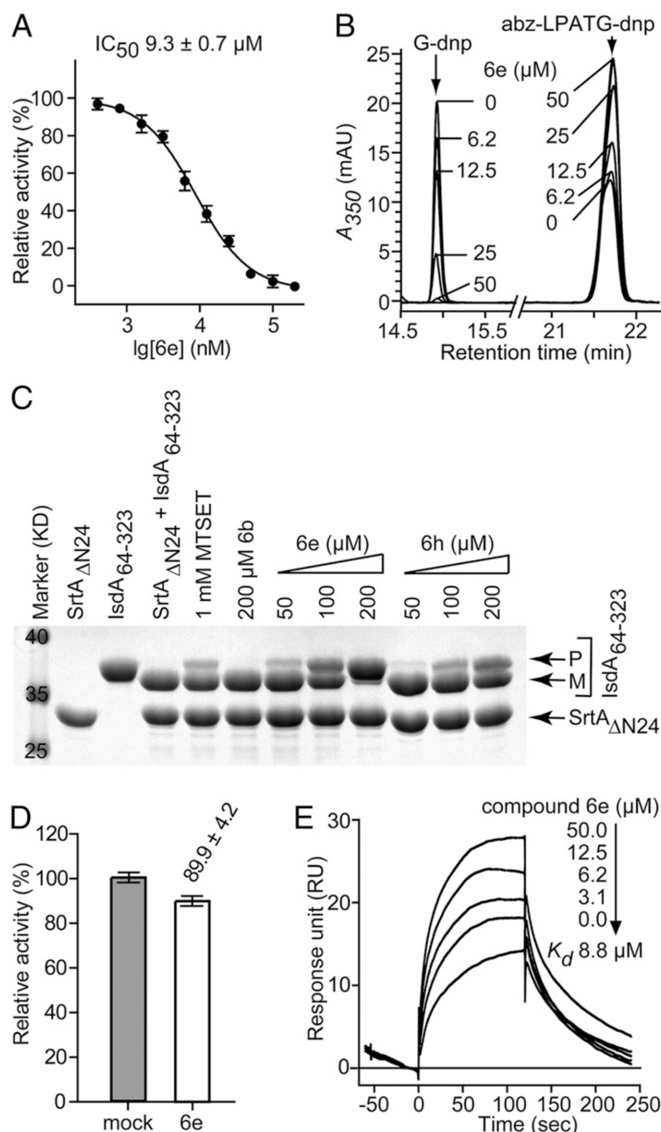

Fig. 2. Inhibition of sortase function in vitro. (*A*) Purified recombinant *S. aureus* sortase ($SrtA_{\Delta N24}$) was incubated with fluorogenic substrate abz-LPATG-dnp and relative activity, i.e., substrate cleavage, measured in the presence of variable concentrations of compound **6e**. Each reaction condition was assayed in triplicate, and average values and SEMs were determined. (*B*) $SrtA_{\Delta N24}$ catalyzed transpeptidation with abz-LPATG-dnp and Gly_3 generates abz-LPAT-Gly_3 and G-dnp was perturbed with increasing concentrations of compound **6e** and relative inhibitory rates calculated. Representative HPLC trace shows the substrate and the dnp-containing product. (*C*) SDS/PAGE analysis of transpeptidation reactions; 10 μg $SrtA_{\Delta N24}$, 10 μg $IsdA_{64-323}$, and 3 mM Gly_3 were incubated for 2 h at 37 °C with variable concentrations of compound **6b**, **6h**, or **6e**. Migratory positions of $IsdA_{64-323}$ substrate precursor (P) and mature transpeptidation product (M) are indicated. (*D*) $SrtA_{\Delta N24}$ was incubated with buffer alone or with compound **6e** at 10 × IC_{50} concentration and diluted, and sortase activity was measured as abz-LPATG-dnp cleavage. Control (mock) sample was assigned 100% activity. Eighty-nine percent (±4.2) activity were recovered from $SrtA_{\Delta N24}$ treated with **6e** inhibitor. (*E*) Compound **6e** binding to $SrtA_{\Delta N24}$ was analyzed with surface plasmon resonance, and the dissociation constant (K_d = 8.8 μM) was calculated.

Reversible Inhibition of Sortase with 3,6-Disubstituted Triazolothiadiazole. Because of its high inhibitory activity and aqueous solubility, compound **6e** was analyzed for its mechanism of sortase inhibition. Following dilution of $SrtA_{\Delta N24}$/inhibitor at 10-fold IC_{50}, 89.9 ± 4.2% of sortase activity was recovered compared with mock-treated sortase (Fig. 2*D*). This result suggested that compound **6e** functions as a reversible inhibitor that does not covalently modify the active site cysteine of sortase (22). Inhibitor binding to sortase was tested with surface plasmon resonance (SPR) experiments, which revealed the direct binding of compound **6e** to $SrtA_{\Delta N24}$ with a K_d of 8.8 μM (Fig. 2*E*). Circular dichroism (CD) spectroscopy was used to monitor changes in sortase structure on binding inhibitor. As expected, $SrtA_{\Delta N24}$ exhibited a negative band near 200 nm, indicative of its mixed β-sheet secondary structure (23, 24) (Fig. S4). Addition of 200 μM compound **6e** to purified $SrtA_{\Delta N24}$ (100-fold excess of inhibitor vs. enzyme) caused minor changes in secondary structure content (Fig. S4), indicating that the inhibitor does not promote protein aggregation. Together these data suggest that compound **6e** binds reversibly to the active site of sortase and likely interferes with the enzyme's ability to recognize and cleave its substrates.

Inhibition of Sortase Activity in Staphylococci. During bacterial growth, sortase catalyzes the assembly of surface proteins into the cell wall at the septal and polar compartments of *S. aureus* (25). Sortase-mediated cleavage of surface protein sorting signals can be measured with pulse-chase experiments, which permit an analysis of precursor processing using [^{35}S]Met/Cys radiolabeling of staphylococcal proteins and immunoprecipitation (26). Immediately following ribosomal synthesis, the P1 precursor species of protein A (SpA) are secreted and their N-terminal signal peptides are removed to generate P2 intermediates. Sortase cleaves the P2 precursor at its LPXTG motif and anchors mature SpA (M) in the cell wall envelope (Fig. 3*A*) (26). As expected, treatment of staphylococci with 2 mM MTSET abolishes sortase-mediated cleavage of P2 precursors (21). Treatment of staphylococci with compound **6e** blocked P2 precursor cleavage by sortase A (Fig. 3*B*). By varying the concentration of the inhibitor, we calculated the IC_{50} 68.7 μM of compound **6e** for the in vivo inhibition of SrtA.

In *S. aureus* Newman, SrtA anchors 19 different surface proteins in the bacterial envelope (27), including SpA, a molecule that binds the Fcγ and Fab domains of host immunoglobulins (28), as well as clumping factor A and B, which bind to the γ- and α-chains of host fibrinogen, respectively (29, 30). Treatment of *S. aureus* cultures with the sortase inhibitor **6e** reduced the incorporation of SpA into the bacterial envelope (Fig. 3 *C* and *D*). Similarly, treatment with compound **6e** reduced staphylococcal association with fibrinogen (Fig. 3*E*), a key mechanism for the pathogenesis of bloodstream infections (13). We noticed a slight reduction in the abundance of Sbi (staphylococcal binder of Ig), a secreted protein that is not cleaved by SrtA, both in the Δ*srtA* mutant and in staphylococci that had been treated with compound **6e** (Fig. 3*D*); the molecular basis for this phenotype is not known.

Antiinfective Therapy with 3,6-Disubstituted Triazolothiadiazole. Deletion of the SrtA gene does not affect the in vitro growth of *S. aureus* Newman (9), a human clinical isolate (31), or of the laboratory strain *S. aureus* RN4220 (32). Unlike MTSET, which reacts with all available thiolate moieties and rapidly kills staphylococci (21), the addition of compound **6e** concentrations inhibitory for SrtA (up to 200 μM) to staphylococcal cultures did not affect the growth of *S. aureus* Newman (Fig. 4*A*). Using the microtiter broth dilution method, we measured the minimal inhibitory concentration of compound **6e** to be >15 mg/mL (>40 mM). These results indicate that compound **6e** selectively inhibits sortase activity and does not function as an antibiotic for *S. aureus*.

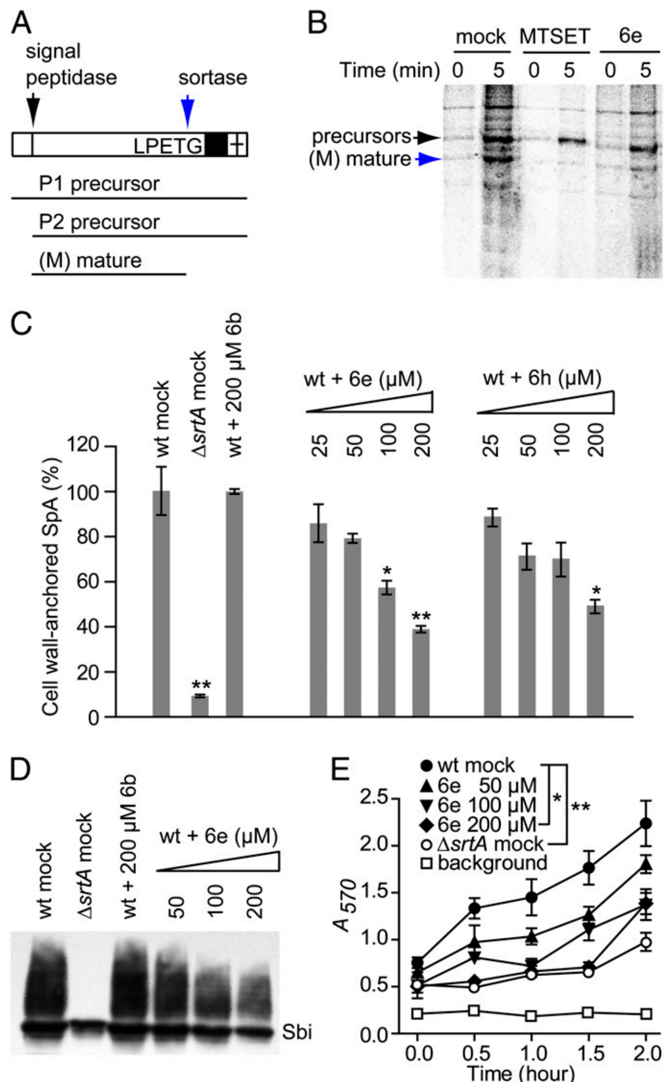

Fig. 3. In vivo inhibition of staphylococcal sortase. (*A*) Diagram illustrating SpA precursors P1 and P2 and the sortase-catalyzed mature anchored product (M). (*B*) Pulse-chase experiment with [^{35}S]Met/Cys (0 and 5 min) reveals the migratory positions of the P1/2 precursors and mature (M) species of *S. aureus* on SDS/PAGE. Treatment of *S. aureus* with the noncompetitive inhibitor MTSET and with compound **6e** causes accumulation of SpA precursors, revealing the inhibition of SrtA activity. (*C*) The abundance of Ig binding to SpA in the bacterial cell wall envelope was quantified with FITC-labeled human IgG and washed *S. aureus* cells from cultures grown in the absence of inhibitor (control) or in the presence of variable concentrations of compounds **6b**, **6h**, and **6e**; values for the *srtA* deletion mutant are included as a control. Statistical significance (*P < 0.05, **P < 0.01) was determined using the unpaired, two-tailed Student *t* test (*n* = 3, brackets identify the mean and the SEMs). (*D*) The abundance of SpA in the bacterial cell wall envelope was quantified by SDS/PAGE immunoblotting with SpA-reactive antibodies using *S. aureus* cultures grown in the absence of inhibitor (*S. aureus* Newman) or in the presence of variable concentrations of compounds **6b** and **6e**; the *srtA* deletion mutant was included as control. The migratory position of cross-reactive Sbi is identified. (*E*) Binding of *S. aureus* Newman cells grown for indicated amounts of time (hours) in the presence of variable concentrations of compound **6e** or mock control to fibrinogen-coated microtiter plates was quantified with crystal violet staining and absorbance measurements (A_{570}). As control, the *srtA* deletion mutant cannot anchor fibrinogen binding surface proteins (ClfA and ClfB) in the bacterial cell wall. Microtiter plate staining without staphylococci was used to determine background signal. Statistical significance (*P < 0.05, **P < 0.01) was determined using the unpaired, two-tailed Student *t* test (*n* = 3, brackets identify the mean and the SEMs).

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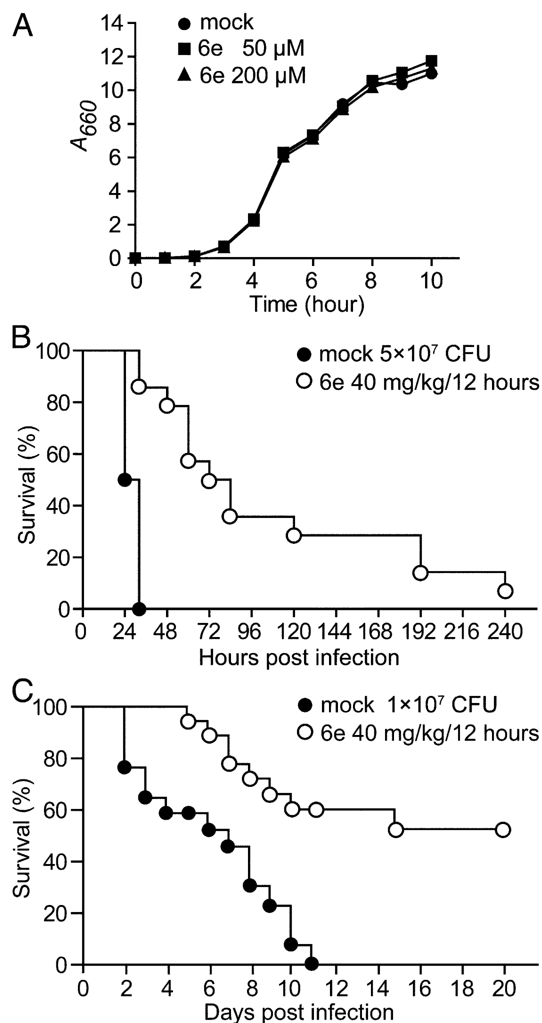

Fig. 4. Antiinfective therapy with 3,6-disubstituted triazolothiadiazole. (*A*) The addition of sortase inhibitors does not affect the growth of *S. aureus*. (*B*) BALB/c mice ($n = 14$) received mock or compound **6e** (40 mg/kg body weight) treatment via i.p. injection at 12-h intervals for 5 d. Four hours after the first injection, animals were challenged by i.v. injection with 5×10^7 CFU *S. aureus* Newman, and survival was recorded. (*C*) BALB/c mice ($n = 15$) were treated as in *B* and challenged with 1×10^7 CFU *S. aureus* Newman. Statistical significance was examined with the log-rank test (mock vs. compound **6e**, $P < 0.001$).

Cohorts of BALB/c mice ($n = 14$) received i.p. injections with either drug vehicle (mock) or with compound **6e** (40 mg/kg body weight) in 12-h intervals for 120 h (5 d). Four hours following the initiation of treatment, mice were infected via i.v. inoculation of 5×10^7 colony-forming units (CFUs) *S. aureus* Newman, a challenge dose that is lethal for healthy animals (33). Mock-treated mice died of staphylococcal bacteremia within 32 h, whereas animals that had received treatment with compound **6e** displayed increased time to death and 10% survival (mock vs. compound **6e**, $P < 0.001$; Fig. 4*B*). This experiment was repeated ($n = 15$) with a lower challenge dose (1×10^7 CFUs) of *S. aureus* Newman. As before, all of the mock-treated animals succumbed to staphylococcal bacteremia, whereas more than half (8/15) of the compound **6e**-treated mice survived staphylococcal bacteremia (mock vs. compound **6e**, $P < 0.001$; Fig. 4*C*). These data suggest that sortase inhibitors may be useful as antiinfective therapy to prevent *S. aureus* bloodstream infections in hospital settings.

Inhibition of Sortase from Different Gram-Positive Bacteria. Most Gram-positive bacteria assemble surface proteins in their cell wall envelope via a sortase-catalyzed mechanism (34). The LPXTG motif is conserved in a wide variety of surface proteins (35); however, the chemical structure of the peptidoglycan crossbridge varies between different bacterial species (36). Although structurally similar (37–40), the amino acid identity between sortases from different Gram-positive bacteria is limited to a few key residues at or near the active site (34). We therefore asked whether compound **6e** can inhibit sortase from three different microbes: *Bacillus anthracis*, *Streptococcus pneumoniae,* and *Streptococcus pyogenes*. Compounds **6e** and **6h** both inhibited purified, recombinant sortase from *S. pyogenes*. Using the fluorescence-based assay, we calculated the compound **6e** IC_{50} of 0.82 μM, revealing a 10-fold higher inhibitory activity for *S. pyogenes* sortase than for the staphylococcal enzyme (Fig. 5*A*); this result was confirmed with the orthogonal HPLC assay (Fig. 5*B*). The structural analogs **6d**, **6h**, **6j**, **6l**, and **6n** also displayed inhibitory activities for sortases from *S. pyogenes*, *S. pneumoniae*, and *B. anthracis* (Table S2). *S. pyogenes* sortase-catalyzed cleavage of the IsdA surface protein from *S. aureus* was inhibited in a dose-dependent manner by compounds **6e** and **6h** (Fig. 5*C*). These data suggest that 3,6-disubstituted triazolothiadiazole inhibit sortase enzymes from different Gram-positive bacteria.

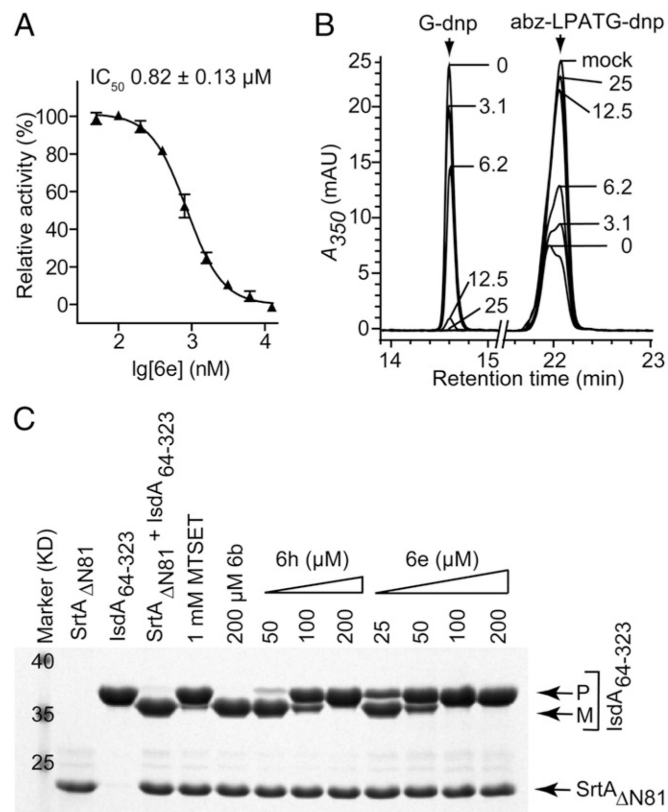

Fig. 5. Inhibition of SrtA from *Streptococcus pyogenes*. (*A*) Purified recombinant *S. pyogenes* sortase ($SrtA_{\Delta N81}$) was incubated with fluorogenic substrate abz-LPATG-dnp, and relative activity was measured in the presence of variable concentrations of compound **6e**. Each reaction condition was assayed in triplicate, and average values and SEMs were determined. (*B*) *S. pyogenes* $SrtA_{\Delta N81}$ catalyzed transpeptidation with abz-LPATG-dnp and Gly_3 generates abz-LPAT-Gly_3 and G-dnp, which was perturbed with increasing concentrations of compound **6e**. The relative inhibitory rate was calculated ($IC_{50} = 7.8$ μM). (*C*) SDS/PAGE analysis of transpeptidation reactions with 10 μg *S. pyogenes* $SrtA_{\Delta N81}$, 10 μg *S. aureus* $IsdA_{64–323}$, and 3 mM Gly_3 were incubated for 2 h at 37 °C with variable concentrations of compound **6b**, **6h**, or **6e**. Migratory positions of $IsdA_{64–323}$ substrate precursor (P) and mature transpeptidation product (M) are indicated.

Discussion

Hospital-acquired infections with multidrug-resistant bacteria represent a global public health threat, and MRSA is currently the most frequent cause of morbidity and mortality (41). The emergence of multidrug-resistant MRSA isolates acquiring glycopeptide resistance during vancomycin therapy documents the urgent need for controlling the use of antibiotics (42). Recent research efforts have been directed at developing antiinfective therapies against *S. aureus*, focusing on small molecules that interfere with virulence gene regulation (43). *S. aureus agr*, a four-gene operon, promotes the constitutive synthesis and secretion of the AgrB-AgrD–derived autoinducing pheromone (AIP) (44), which activates the sensory kinase-response regulator AgrC-AgrA at threshold concentrations to promote staphylococcal expression and secretion of exotoxins (45). AIP-mediated activation of AgrC can be inhibited with peptide analogs and small molecules, which diminishes *S. aureus* colonization and invasion of skin and soft tissues (43, 46). AIP is inactivated by neutrophil Nox2 NADPH oxidase modification and apolipoprotein B binding (47, 48), which interferes with *agr*-mediated quorum sensing when *S. aureus* enters the bloodstream (49, 50). In agreement with this model, AgrC inhibitors do not affect staphylococcal load, abscess formation, or disease outcome when mice are challenged by i.v. inoculation with *S. aureus* (51).

In contrast to *agr*-controlled virulence gene expression, sortase-mediated assembly of surface proteins in the bacterial envelope is essential for the pathogenesis of abscess formation and lethal bacteremia following i.v. inoculation of the pathogen (12, 13). Earlier work used in vitro (inhibition of fluorogenic substrate cleavage) and virtual screening of compound libraries to identify sortase inhibitors (15, 52–56). Although these studies identified both competitive and noncompetitive inhibitors (57, 58), isolated compounds have not yet been shown to inhibit in vivo sortase activity in staphylococci, i.e., the cleavage of sorting signals or the assembly of surface proteins into the bacterial cell wall (54, 59–62). Many of the isolated compounds diminish or block staphylococcal growth, indicating that they cannot function as selective inhibitors of *S. aureus* sortase (53, 61, 63, 64). Thus, the efficacy of sortase inhibitors as antiinfective therapeutics was heretofore not demonstrated (62, 65).

We used virtual screening for compounds that bind the active site of sortase and experimental validation to identify 3,6-disubstituted triazolothiadiazole compounds as a new class of sortase inhibitors. SARs were studied to improve the efficacy of sortase inhibitors, which characterized 3-(4-pyridinyl)-6-(2-sodiumsulfonatephenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (compound **6e**) as the most potent inhibitor. Of note, compound **6e** blocked sortase-catalyzed cleavage and transpeptidation reactions with LPXTG substrate peptides and inhibited the incorporation of surface proteins into the staphylococcal envelope. Although not optimized for its pharmacokinetic and pharmacodynamics attributes, compound **6e** displayed efficacy as an antiinfective in preventing the lethal outcome of *S. aureus* bacteremia in mice. Earlier work synthesized a wide variety of 3,6-disubstituted triazolothiadiazole derivatives and examined these compounds for their antibacterial, antifungal, and analgesic attributes (16). Although certain members of this class of compounds can inhibit bacterial or fungal growth, antibiotic or analgesic effects are not universal attributes of 3,6-disubstituted triazolothiadiazole compounds (16), which may be explored further for clinical development of antiinfectives (16).

Sortases and cell wall-anchored surface proteins contribute to the virulence strategies of many different bacterial pathogens (34). Sortases and surface proteins with LPXTG sorting signals are found in other nosocomial pathogens, for example, *Enterococcus faecalis*, *Enterococcus faecium*, and *Clostridium difficile* (66–68). Earlier work revealed the contribution of sortase toward *enterococcal* virulence and the pathogenesis of urinary tract infections or endocarditis (67, 69, 70). Thus, antiinfective therapy with sortase inhibitors may be useful to broadly prevent nosocomial infections with antibiotic-resistant Gram-positive bacteria.

Materials and Methods

In Vivo Inhibition of Staphylococcal Sortase A. *S. aureus* cultures were pulse-labeled with [^{35}S]Met/Cys for 1 min, and all further incorporation of radioactive amino acids into proteins was quenched by the addition of excess unlabeled Met/Cys (chase). At timed intervals, 0 and 5 min after the addition of the chase, culture aliquots were precipitated with trichloroacetic acid, washed in acetone, and dried, and the cell wall peptidoglycan was digested with lysostaphin. MTSET (100 mM in water) was added at a final concentration of 2 mM 10 s after labeling with [^{35}S]Met/Cys had commenced. Compound **6e** (100 mM in water) was added at a final concentration of 200 μM 10 min before pulse-labeling with [^{35}S]Met/Cys. SpA was immunoprecipitated with SpA-specific antibodies, and radiolabeled polypeptides were analyzed by 10% SDS/PAGE and PhosphoImager.

Animal Model of *S. aureus* Infection. Lethal challenge experiments were performed at the Shanghai Public Health Clinical Center following animal care and use protocols that were reviewed, approved, and supervised by the Committee for Animal Experiments at Fudan University. Overnight cultures of *S. aureus* Newman were diluted 1:1,000 into 30 mL fresh tryptic soy broth (TSB) and grown with rotation at 37 °C for 3 h. Bacteria were centrifuged at 3,000 × *g*, washed, and suspended in PBS to A_{600} 0.8 or 1.6. Cohorts of BALB/c mice (6-wk-old females; Shanghai Super-B&K Laboratory Animal Corp.) were randomly assigned into of two cohorts. Water and laboratory chow were provided ad libitum. Compound **6e** was dissolved in sterile double-distilled water (ddH_2O) and administered by i.p. injection at a dose of 40 mg/kg in 12-h intervals. Four hours after the first injection of compound **6e** or mock (ddH_2O) control, animals were challenged by periorbital injection of *S. aureus* Newman; aliquots of the inoculum were plated for enumeration of CFUs, and animals were monitored for survival over a 20-d observation period. The log-rank test was used to analyze mortality data; $P < 0.05$ was deemed statistically significant.

Other Procedures. Detailed procedures are available in *SI Materials and Methods*.

ACKNOWLEDGMENTS. This work was supported by National Science and Technology Major Project "Key New Drug Creation and Manufacturing Program" Grant 2013ZX09507-004, National Natural Science Foundation of China Grants 91313303, 20972173, and 81230076, and Hi-Tech Research and Development Program of China Grants 2012AA020302 and 2012AA020301. Work on sortase in the laboratory of O.S. is supported by National Institute of Allergy and Infectious Diseases Grant AI038897. Computation resources were partially supported by the Computer Network Information Center, Chinese Academy of Sciences, and Shanghai Supercomputing Center.

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