Antibacterial Activity of REP8839, a New Antibiotic for Topical Use

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REP8839 is a novel methionyl-tRNA synthetase (MetS) inhibitor with potent antibacterial activity against clinical isolates of *Staphylococcus aureus*, *Streptococcus pyogenes*, and other clinically important gram-positive bacteria but little activity against gram-negative bacteria. All isolates of *S. aureus*, including strains resistant to methicillin, mupirocin, vancomycin, and linezolid were susceptible to REP8839 at concentrations of ≤0.5 μg/ml. REP8839 was also active against *Staphylococcus epidermidis*, including multiply resistant strains (MIC, ≤0.25 μg/ml). All *S. pyogenes* isolates were susceptible to REP8839 at concentrations of ≤0.25 μg/ml, suggesting that MetS2, a second enzyme previously identified in *Streptococcus pneumoniae*, was not present in this organism. REP8839 was highly bound to the protein of human serum, and activity was not greatly influenced by inoculum size but was affected by pH, exhibiting optimal antibacterial activity in a neutral medium rather than a weak acidic medium. Like mupirocin, REP8839 exhibited bacteriostatic activity against key pathogens. The emergence of mupirocin resistance in *S. aureus* highlights the need for a new topical antibiotic with the ability to inhibit high-level mupirocin-resistant strains and other emerging phenotypes, such as vancomycin-resistant and community-acquired methicillin-resistant isolates.

The ongoing emergence of resistance in clinically important bacteria continues to drive the search for new antibiotics directed against previously unexploited drug targets (1, 35). One major pathogen of concern is *Staphylococcus aureus* where methicillin resistance, which encompasses resistance to all currently available β-lactams, is a common phenotype among multiresistant nosocomial isolates (2, 33). The incidence of methicillin-resistant *S. aureus* (MRSA) in U.S. hospitals has increased from 2.5% in 1975 to 43.7% in 2000 (10, 22). More recently, MRSA has been recognized as a community-acquired pathogen causing disease in hosts with few of the risk factors associated with nosocomial MRSA (5, 6, 16, 20). Vancomycin has been widely used to treat MRSA infections, and unsurprisingly, this has led to the identification of vancomycin-intermediate *S. aureus* in 1997 (4, 21) and fully vancomycin-resistant *S. aureus* in 2002 (7–9). *S. aureus* continues to be a significant health problem in the hospital and other settings, such as nursing homes (15). Nasal carriage of *S. aureus* is an important risk factor in the hospital setting, particularly in patients requiring surgery, implanted devices, or hemodialysis (30, 40, 43).

The isoleucyl-tRNA synthetase (IleS) inhibitor mupirocin has potent activity against *S. aureus* and is indicated as a topical agent for primary and secondary skin infections and for the eradication of nasal colonization of MRSA (19). Extensive clinical use of mupirocin has resulted in the emergence of resistance in *S. aureus* and the coagulase-negative staphylococci, although no resistance has been detected in the other major skin pathogen *Streptococcus pyogenes* (11, 14). Mupirocin resistance in *S. aureus* consists of two phenotypes: low-level resistance (MICs, 8 to 256 μg/ml) and high-level resistance (MICs, ≥512 μg/ml) (36). Low-level mupirocin resistance results from spontaneous mutations in the IleS enzyme, whereas high-level resistance results from the acquisition of a transferable plasmid containing *mupA* that encodes for an evolutionarily divergent mupirocin-resistant isoleucyl-tRNA synthetase (38). Until recently there was little data on the clinical significance of resistance to mupirocin, although there are now reports of clinical and microbiological failures in the clinic (13, 41).

The emergence of mupirocin-resistant *S. aureus* warrants the need for new agents with the ability to evade existing resistance mechanisms. The clinical success of mupirocin has demonstrated the utility of aminoacyl-tRNA synthetases as a viable target for the development of topical antibiotics. The bacterial methionyl-tRNA synthetase (MetS; encoded by the gene *metS*) enzyme has not been previously targeted by antibiotics; therefore, novel MetS inhibitors would not be expected to show cross-resistance to currently marketed drug classes. A series of novel compounds with inhibitory activity against the *S. aureus* MetS has previously been described and shown to have potent antibacterial activity against gram-positive pathogens (24–27). REP8839 (Fig. 1) is a novel diaryldiamine-containing MetS (18) inhibitor that has not been previously disclosed but is related to the previously described compounds and is currently being evaluated as a topical antibiotic for the treatment of skin infections and eradication of *S. aureus* from the anterior nares.

REP8839 has been shown to be a potent inhibitor of *S. aureus* MetS enzyme, and its activity has been confirmed in macro-molecular synthesis assays (34). The objective of the current study was to determine the microbiological activity of REP8839 against recent clinical isolates, including emerging resistant phenotypes.

MATERIALS AND METHODS

Organism collection. The recent clinical isolates of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, and *Enterococcus faecium* used in the antimicrobial pro-
filing studies were obtained from Focus Bio-Inova (Herndon, VA) and included resistant phenotypes. S. aureus, S. epidermidis, and S. pyogenes were obtained from skin and wound specimen sources and were collected from a geographically distributed network of sites in the United States between 2001 and 2003. The low-level mupirocin-resistant clinical isolates (LZ8, 014-354, 031-1334, and 036-1298) and high-level mupirocin-resistant isolates (LZ10, LZ6, 010-420, and 87-2797) of S. aureus were obtained from Carol A. Kauffman (University of Michigan Medical School, Ann Arbor, MI). The vancomycin-intermediate, vancomycin-resistant, linezolid-resistant, community-acquired MRSA, and other mupirocin-resistant clinical isolates of S. aureus were obtained from NARSA (Network on Antimicrobial Resistance in S. aureus; Focus Bio-Inova).

Antimicrobial testing. REP8839 was used as the acetate salt and was synthesized in the laboratories of Replidyne, Inc. Mupirocin was used as the free acid and was obtained from Carol A. Kauffman (University of Michigan Medical School, Ann Arbor, MI). The vancomycin-intermediate, vancomycin-resistant, linezolid-resistant, community-acquired MRSA, and other mupirocin-resistant clinical isolates of S. aureus were obtained from NARSA (Network on Antimicrobial Resistance in S. aureus; Focus Bio-Inova).

RESULTS

Activity against contemporary clinical isolates of gram-positive bacteria. The results in Table 1 show the comparative activities of REP8839 and mupirocin against recent U.S. clinical isolates of clinically important gram-positive pathogens. The S. aureus isolates that were used to profile the activity of REP8839 included strains that were resistant to currently marketed agents, such as oxacillin and mupirocin. REP8839 demonstrated potent activity against all 82 isolates of S. aureus (MIC_{90} 0.12 μg/ml). Methicillin-resistant S. aureus isolates were slightly less susceptible to REP8839 than their methicillin-susceptible counterparts with MIC_{90} of 0.25 and 0.06 μg/
ml, respectively. All *S. epidermidis* isolates tested were inhibited by REP8839 at concentrations of ≤0.12 μg/ml. REP8839 maintained activity against both methicillin-susceptible and resistant *S. epidermidis* with MIC<sub>90</sub> of 0.06 and 0.12 μg/ml, respectively. In contrast, mupirocin was active against methicillin-susceptible strains (MIC<sub>90</sub> 0.12 μg/ml) but exhibited poor activity against methicillin-resistant *S. epidermidis* (MIC<sub>90</sub> >8 μg/ml).

All 48 isolates of *S. pyogenes* tested were susceptible to both REP8839 and mupirocin with MIC<sub>90</sub> of 0.12 and 0.25 μg/ml, respectively. In the case of REP8839, all *S. pyogenes* strains were inhibited by concentrations of ≤0.25 μg/ml. In addition to profiling the activity of REP8839 against skin pathogens, we also determined its activity against other important gram-positive bacteria, such as *S. pneumoniae* and enterococci, to confirms its spectrum of activity and identify any outliers with elevated MICs. Among *S. pneumoniae* strains, REP8839 was less active than mupirocin with MIC<sub>90</sub> of >8 and 4 μg/ml, respectively. REP8839 exhibited potent antibacterial activity against recent clinical isolates of *E. faecalis* and *E. faecium* (MIC<sub>90</sub> 0.015 and ≤0.004 μg/ml, respectively). Among *E. faecalis* isolates, REP8839 exhibited potent activity against both vancomycin-susceptible and -resistant strains (MIC<sub>90</sub> 0.008 and 0.015 μg/ml, respectively). Similarly, the activity of REP8839 against *E. faecium* was unaffected by the vancomycin resistance status of the organism with MIC<sub>90</sub> of ≤0.004 μg/ml for both vancomycin-susceptible and -resistant isolates.

Activity against mupirocin-resistant *S. aureus*. The results in Table 2 show the activity of REP8839 against low- and high-level mupirocin-resistant clinical isolates of *S. aureus*. Against the six low-level mupirocin-resistant isolates (mupirocin MICs, 8 to 256 μg/ml), REP8839 demonstrated MICs that ranged from ≤0.008 to 0.5 μg/ml. REP8839 was also active against the eight high-level mupirocin-resistant strains (mupirocin MICs, >512 μg/ml) with all isolates being inhibited by concentrations of ≤0.06 μg/ml. All high-level mupirocin-resistant *S. aureus* isolates were confirmed to be positive for the acquisition of *mupA* (data not shown).

Activity against other resistant phenotypes of *S. aureus*. The results in Table 3 show the activity of REP8839 against vancomycin-intermediate, vancomycin-resistant, linezolid-resistant, and community-acquired MRSA clinical isolates. The vancomycin-intermediate *S. aureus* isolates included isolates with vancomycin MICs of ≥4 μg/ml but ≤16 μg/ml. REP8839 demonstrated potent activity against all eight isolates tested with MICs ranging from ≤0.008 to 0.06 μg/ml. In contrast, mupirocin was less active against three vancomycin-intermediate isolates with MICs of ≥8 μg/ml. Against the vancomycin-resistant clinical isolates (vancomycin MICs, >32 μg/ml) from Michigan (VRS1), Pennsylvania (VRS2), and New York (VRS3), REP8839 exhibited MICs of 0.03, 0.5, and 0.12 μg/ml, respectively. REP8839 was also active against the laboratory-generated transconjugant strain of *S. aureus* (COLVA) that shows high and homogenous resistance to oxacillin and vancomycin (MIC, ≥0.008 μg/ml). Mupirocin was active against *S. aureus* VRS2 (Pennsylvania), VRS3 (New York), and COLVA with MICs of 0.03, 0.5, and 0.06 μg/ml, respectively, but was considerably less active against *S. aureus* VRS1 (Michigan) with an MIC of >8 μg/ml. Among the linezolid-resistant *S. aureus* isolates (linezolid MICs, ≥64 μg/ml), REP8839 was active against all three isolates with an MIC of 0.12 μg/ml. Mupirocin was also active against linezolid-resistant *S. aureus* with MICs of 0.06 μg/ml. REP8839 was also tested against four community-acquired MRSA isolates from Minnesota and North Dakota and exhibited MICs ranging from ≤0.008 to 0.03 μg/ml. Mupirocin was also active against the same isolates with MICs of 0.12 to 0.25 μg/ml.

**Effects of inoculum, pH, and serum on antibacterial activity.** The activity of REP8839 against *S. aureus* was slightly influenced by varying the size of the bacterial inoculum. For *S. aureus* ATCC 29213, the MIC of REP8839 increased from 0.008 μg/ml at 10<sup>5</sup> CFU to 0.06 μg/ml at 4 × 10<sup>5</sup> CFU/ml. The MIC of mupirocin increased from 0.03 μg/ml at 10<sup>3</sup> CFU to 0.12 μg/ml at 4 × 10<sup>5</sup> CFU against the same organism. The activity of REP8839 against *S. pyogenes* was less affected by changes in the size of the bacterial inoculum than *S. aureus* ATCC 29213 was (data not shown). The activity of REP8839 was also affected by changes in the pH of the growth medium; REP8839 was more active against *S. aureus* ATCC 29213 in medium at neutral pH (MIC of 0.03 μg/ml at pH 7.6) than in mildly acidic medium (MIC of 1 μg/ml at pH 5.7). These results suggest that REP8839 will maintain potent activity when formulated at neutral pH. This was in contrast to mupirocin, which was more active in a mildly acidic medium (Fig. 2). Serum MIC determinations were used to determine the effect of protein binding on the activity of REP8839. REP8839 was highly bound to serum proteins, which results in reduced

### Table 2. Activity of REP8839 against low- and high-level mupirocin-resistant *S. aureus*

<table>
<thead>
<tr>
<th>Phenotype and strain</th>
<th>REP8839</th>
<th>Mupirocin</th>
<th>Oxacillin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mupirocin-susceptible</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LZ9</td>
<td>0.03</td>
<td>0.12</td>
<td>&gt;64</td>
</tr>
<tr>
<td>LZ10</td>
<td>0.03</td>
<td>0.12</td>
<td>&gt;64</td>
</tr>
<tr>
<td>010-100</td>
<td>0.03</td>
<td>0.06</td>
<td>8</td>
</tr>
<tr>
<td>087-2789</td>
<td>0.06</td>
<td>0.12</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Low-level mupirocin resistant&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LZ8</td>
<td>0.03</td>
<td>16</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Miles Hall</td>
<td>≤0.008</td>
<td>32</td>
<td>0.12</td>
</tr>
<tr>
<td>014-354</td>
<td>0.015</td>
<td>16</td>
<td>&gt;64</td>
</tr>
<tr>
<td>031-1334</td>
<td>0.015</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td>036-1298</td>
<td>≤0.008</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>NRS 127</td>
<td>0.5</td>
<td>32</td>
<td>64</td>
</tr>
<tr>
<td>High-level mupirocin resistant&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NRS107</td>
<td>≤0.008</td>
<td>&gt;256</td>
<td>0.12</td>
</tr>
<tr>
<td>LZ10</td>
<td>0.015</td>
<td>&gt;256</td>
<td>&gt;64</td>
</tr>
<tr>
<td>010-420</td>
<td>0.03</td>
<td>&gt;256</td>
<td>8</td>
</tr>
<tr>
<td>87-2797</td>
<td>0.03</td>
<td>&gt;256</td>
<td>&gt;64</td>
</tr>
<tr>
<td>25-670</td>
<td>0.03</td>
<td>&gt;256</td>
<td>&gt;64</td>
</tr>
<tr>
<td>1079101</td>
<td>0.06</td>
<td>&gt;256</td>
<td>0.25</td>
</tr>
<tr>
<td>NRS 54</td>
<td>0.06</td>
<td>&gt;256</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Range</td>
<td>≤0.008–0.5</td>
<td>≤0.06–&gt;256</td>
<td>0.12–&gt;64</td>
</tr>
<tr>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>0.03</td>
<td>32</td>
<td>&gt;64</td>
</tr>
<tr>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>0.5</td>
<td>&gt;256</td>
<td>&gt;64</td>
</tr>
</tbody>
</table>

<sup>a</sup> Isolates with mupirocin MICs of 8 to 256 μg/ml.

<sup>b</sup> Isolates with mupirocin MICs of ≥512 μg/ml.
activity when the drug is tested in the presence of human serum with arithmetic MICs for REP8839 against *S. aureus* increasing from 0.04/μg/ml in serum-free medium to 8/μg/ml in 100% serum.

Bactericidal activity. REP8839, like mupirocin, has bacteriostatic activity against *S. aureus* when tested at its MIC. Typically, the minimal bactericidal concentrations for REP8839 against *S. aureus* were 32-fold higher than their corresponding MICs. When tested in a time-kill kinetic assay against *S. aureus* ATCC 29213 at 4,096-fold the MIC, REP8839 continued to show bacteriostatic activity. Therefore, although some reduction in colony count was observed with both compounds after 24 h, neither REP8839 nor mupirocin was bactericidal at 4,096 times the MIC according to conventional criteria, since neither drug resulted in ≥3 log₁₀ reduction in viable CFU (Fig. 3).

**DISCUSSION**

REP8839 is a novel MetS inhibitor with potent biochemical activity and specificity against the *S. aureus* enzyme (34) as well as antibacterial activity against *S. aureus* and other gram-positive pathogens. REP8839 has been evaluated against a wide collection of gram-negative bacteria where it demonstrated little or no antibacterial activity. However, its potent activity against *S. aureus* and the other important skin pathogen, *S. pyogenes*, warranted its evaluation as a new topical antibiotic. The isoleucyl-tRNA synthetase inhibitor, mupirocin, has proved to be a successful topical antibiotic with coverage against both *S. pyogenes* and *S. aureus*, including methicillin-resistant isolates (29), and serves as a precedent for the successful development of aminoacyl-tRNA synthetases as topical antibiotics.

However, in recent years there have been increasing reports of mupirocin-resistant isolates of *S. aureus*. Unfortunately, few of the major antibiotic resistance surveillance initiatives have monitored mupirocin resistance on a regular basis, making it difficult to determine the true extent or significance of the emergence of resistance. Furthermore, there are no interpretive criteria that have been approved either by the U.S. FDA or CLSI for topical antibiotics such as mupirocin. The SENTRY

![Fig. 3](http://aac.asm.org/) Time-kill curves for REP8839 and mupirocin at 4,096× MIC against *S. aureus* ATCC 29213. The viable cell densities were determined at the indicated time points. Cultures in drug-free medium and in medium containing the bactericidal agent levofloxacin were included as controls.
Antimicrobial Surveillance Program has recently started to track mupirocin resistance on a global basis and reported emerging elevated resistance rates (mupirocin MICs, ≥16 μg/ml) in staphylococcal clinical isolates collected and tested in 2000 (14). In North American clinical isolates of *S. aureus*, mupirocin resistance was detected in 14.1% of methicillin-resistant isolates compared with 1.5% in methicillin-susceptible isolates (14). Among the methicillin-resistant coagulase-negative staphylococci, the mupirocin resistance rates were 43.1% and 6% in methicillin-resistant and -susceptible isolates, respectively.

Although there is little data on the prevalence of high-level mupirocin-resistant *S. aureus* (mupirocin MICs, ≥256 μg/ml), several of the high-level mupirocin-resistant isolates had mupirocin MICs that were as high as 8,000 μg/ml. Few studies have monitored the multidrug resistance profiles of high-level mupirocin-resistant staphylococci, although one study conducted at a Korean hospital identified isolates resistant to three or more different classes of antibiotics (44). Thus, although the formal concentration of mupirocin in currently marketed topical formulations is high (2% [wt/wt] mupirocin or ~20,000 μg/ml), it is possible that local concentrations may not remain above the MIC for the high-level mupirocin-resistant isolates, resulting in microbiological and clinical failure. Although few studies have focused on the pharmacokinetics of topical agents, there is one report of low concentrations of mupirocin in the pharynx of a patient contributing to the development of mupirocin resistance in methicillin-resistant *S. aureus* (42).

REP8839 was active against all *S. aureus* isolates (MIC_{90} 0.12 μg/ml) regardless of their resistance to mupirocin and/methicillin. The least susceptible isolates of *S. aureus* were strains with REP8839 MICs of 0.5 μg/ml that are well below the proposed local concentrations available in a 2% (wt/wt) topical formulation. The low- and high-level mupirocin-resistant *S. aureus* isolates included in the isolate challenge set were historical clinical isolates that have previously been described and characterized (23, 37), and REP8839 was active against all isolates with MICs ranging from ≤0.008 to 0.5 μg/ml. The poor activity of mupirocin (MIC_{90} ≥8 μg/ml) was due to the fact that the *S. aureus* isolate collection included six isolates with low-level resistance to mupirocin and eight isolates with high-level mupirocin resistance.

REP8839 also showed potent activity against *S. epidermidis* (MIC_{90} 0.12 μg/ml) in contrast to mupirocin which was considerably less active (MIC_{90} ≥8 μg/ml). All 25 isolates were recent U.S. clinical isolates from skin and wound specimen sources and were not selected to specifically include mupirocin-resistant isolates. These results are indicative of the increased prevalence of mupirocin resistance in clinical isolates of coagulase-negative staphylococci, an observation that has also been recognized by other investigators (14, 28, 39).

Both REP8839 and mupirocin showed potent activity against the 48 clinical isolates of *S. pyogenes* with MIC_{90} of 0.12 and 0.25 μg/ml, respectively. In contrast, REP8839 was less active against *S. pneumoniae* clinical isolates (MIC_{90} >8 μg/ml); this is presumably due to the fact that subpopulations of *S. pneumoniae* with two distinct methionyl-tRNA synthetase genes (*metS1* and *metS2*) have been identified (17). Since *S. pyogenes* is a key target pathogen for REP8839 as a topical antibiotic, there was concern that *metS2* may also be prevalent in clinical isolates of this organism. The clinical isolates of *S. pyogenes* were procured from a geographically distributed network of sites across the United States to maximize the identification of outliers with elevated MICs to REP8839 indicating the presence of *metS2*. All isolates were susceptible to REP8839 at concentrations of ≤0.25 μg/ml, suggesting that *metS2* does not appear to be present in this organism. Furthermore, phylogenetic analyses of methionyl-tRNA synthetase protein sequences has shown that the *S. pyogenes* enzyme clusters with *S. pneumoniae* MetS1 and not *S. pneumoniae* MetS2 (3).

This study has shown that REP8839 has important coverage against both major skin pathogens: *S. aureus* and *S. pyogenes*. REP8839 is an entirely synthetic compound, rather than a derivative of a natural product, that has potent activity not only against methicillin- and mupirocin-resistant *S. aureus* but also against emerging resistant phenotypes, including vancomycin-intermediate and -resistant strains and community-acquired strains. The compound is currently in preclinical development as a topical antibiotic for the treatment of skin infections and for the eradication of nasal carriage of *S. aureus*.

**ACKNOWLEDGMENTS**

We thank Carol A. Kaufmann of the University of Michigan Medical School for providing the low- and high-level mupirocin-resistant isolates of *S. aureus*. We also thank Hao Le of Focus Bio-Inova for testing REP8839 and mupirocin against vancomycin-resistant *S. aureus* clinical isolates.

**REFERENCES**


