A Clerodane Diterpene from *Callicarpa americana* Resensitizes Methicillin-Resistant *Staphylococcus aureus* to β-Lactam Antibiotics

Micah Dettweiler, Roberta J. Melander, Gina Porras, Caitlin Risener, Lewis Marquez, Tharanga Samarakoon, Christian Melander,* and Cassandra L. Quave*

**ABSTRACT:** The rise of antibiotic resistance presents a significant healthcare challenge and precludes the use of many otherwise valuable antibiotics. One potential solution to this problem is the use of antibiotics in combination with resistance-modifying agents, compounds that act synergistically with existing antibiotics to resensitize previously resistant bacteria. In this study, 12(S),16β-dihydroxycleroda-3,13-dien-15,16-olide, a clerodane diterpene isolated from the medicinal plant *Callicarpa americana*, was found to synergize with oxacillin against methicillin-resistant *Staphylococcus aureus*. This synergy was confirmed by checkerboard (fractional inhibitory concentration index (FICI) = 0.125) and time-kill assays, with a subinhibitory dose of 12(S),16β-dihydroxycleroda-3,13-dien-15,16-olide causing the effective concentration of oxacillin to fall below the susceptibility breakpoint for *S. aureus*, a >32-fold decrease in both cases.

**KEYWORDS:** MRSA, oxacillin, terpene, synergy, botanical, natural product

Antibiotic resistance is a growing problem in modern healthcare, threatening human health directly through difficult-to-treat infections and indirectly by eroding the safety of surgical procedures. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major pathogen in both healthcare-associated and community-associated outbreaks of infection, and new drugs and treatment approaches are needed. One promising tactic to deal with antibiotic-resistant infections is combination therapy. Compared to traditional single-drug treatments, using mixtures of drugs can reduce the risk of resistance developing and increase the therapeutic window via drug–drug interactions, e.g., synergy in efficacy or antagonism in toxicity. The emergence of combination therapy as an answer to drug resistance is more correctly a re-emergence; for the bulk of human history, mixtures of drugs have been at the core of medicine, frequently derived from single or multiple species of plants and fungi. Working from this basis, plants used in traditional medicine for the treatment of infections can be studied as a fruitful source of compounds active in antibiotic combination therapy.

Several small molecules are known to potentiate the activity of β-lactam antibiotics against MRSA, including a number of plant derived natural products. The sesquiterpene farnesol increases the susceptibility of MRSA to β-lactam antibiotics by interfering with cell wall biosynthesis through reduction of free CPS lipid carrier, while the flavonoid baicalein has been reported to potentiate the effects of β-lactam antibiotics including oxacillin, cefmetazole, and ampicillin against MRSA.

Herein, we report that phytochemicals from *Callicarpa americana* L. (Lamiaceae) leaves synergize with oxacillin against multiple MRSA strains. *C. americana*, commonly known as the American beautyberry, is native to the southeastern United States and has a history of traditional use by several American Indian tribal nations to treat fever, stomachache, dysentery, and diarrhea. Extracts of *C. americana* leaves have been shown in lab tests to deter mosquitos and ticks and inhibit the growth of the Gram-positive bacterium *Cutibacterium acnes*. The study was initiated by screening 401 extracts of the Quave Natural Product Library (QNPL) at a concentration of 25 μg/mL alone and in combination with 8 μg/mL oxacillin (1/4 MIC) against the MRSA strain ATCC BAA-1556. From these initial screens, we identified extract 649, an ethanol extract of *C. americana* leaves as a an exceptionally potent modifier of oxacillin.

**Received:** May 12, 2020  
**Published:** June 24, 2020
Next, we screened extract 649 against two additional MRSA strains, LAC and MW2, as a first step to determine if activity was isolate specific. This LAC strain recorded a slightly higher stand-alone oxacillin MIC (64 μg/mL), while the MW2 strain had an identical MIC to BAA-1556 (32 μg/mL), and both were potently sensitized to the effects of oxacillin in the presence of 649. A concentration of 4 μg/mL 649 lowered the oxacillin MIC of LAC to 1 μg/mL; a concentration of 16 μg/mL extract reduced the oxacillin MIC in the MW2 strain to 2 μg/mL, while a concentration of 10 μg/mL reduced the oxacillin MIC to 1 μg/mL in BAA-1556. Each resulting oxacillin MIC is at or below the breakpoint of resistance (≤ 2 μg/mL).

Extract 649 was partitioned to create 649B-E (overall isolation scheme depicted in Figure 1), and each partition was subjected to a checkerboard analysis against LAC (shown in Figures S1 and S2 and Table S1). Of the four partitions, 649C proved to be the most potent, showing activity essentially equivalent to the crude extract. A similar result was observed when 649C was tested against BAA-1556, resulting in an oxacillin MIC of 0.5 μg/mL at a concentration of 10 μg/mL. This extract was then subjected to further purification via HPLC, and the resulting fractions were tested for activity with oxacillin against BAA-1556. Of these fractions, fraction 9 (649C-F9) returned the highest level of potentiation, reducing the oxacillin MIC 64-fold (0.5 μg/mL) at a concentration of 5 μg/mL, while fraction 13 (649C-F13) showed 32-fold reduction at 5 μg/mL. All fractions were then tested against LAC (activity of all fractions summarized in the Supporting Information) in checkerboard format, where 649C-F13 showed the highest level of synergy, returning a fractional inhibitory concentration index (FICI) of 0.094. Two further rounds of purification and bioassay of this extract eventually yielded 649C-F13-PF4-SF7, a purified compound that lowered the MIC of oxacillin against BAA-1556 to 0.25 μg/mL at a concentration of 5 μg/mL.

The purified compound was then subjected to structure elucidation studies. Analysis of 1D (Figures S3 and S4) and 2D NMR experiments and accurate mass measurement (Figure S5) allowed us to establish the compound’s structure. Databases searches (SciFinder and Reaxys) enabled verification of this structure by comparisons of NMR and MS data with published values. The purified compound was identified as 12(S),16ξ-dihydroxycleroda-3,13-dien-15,16-olide (1). Compound 1 is a clerodane diterpene previously isolated as a mixture of epimers from a chloroform-soluble
C. americana moderately inhibits the growth of MRSA, supporting the traditional use of C. americana in Infective. This diterpene also potently synergizes with oxacillin for S. aureus oxacillin MIC 16 but no *vitro* speci- de Compound 1 μ at the 24 h time point. Moreover, a combination of 4 S. aureus oxacillin-susceptible notably, 1 μ (1/4 MIC) and 2 μ bactericidal activity against MRSA (LAC). oxacillin, combinations of 1 μ against 5 cell lines *in vitro* but no *in vivo* cytotoxicity up to 50 mg/kg in a hollow fiber mouse model. In our study, 1 exhibited an IC50 of 64 μg/mL (191 μM) against human keratinocytes *in vitro* (Figure 3), mirroring the lower toxicity seen in the mouse study.

Figure 2. Structure of 12(S),16β-dihydroxycleroda-3,13-dien-15,16-olide (1).

<table>
<thead>
<tr>
<th>% Cytotoxicity</th>
<th>Concentration (μg/mL)</th>
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<tbody>
<tr>
<td>100</td>
<td>1000</td>
</tr>
<tr>
<td>75</td>
<td>1000</td>
</tr>
<tr>
<td>50</td>
<td>1000</td>
</tr>
<tr>
<td>25</td>
<td>1000</td>
</tr>
<tr>
<td>0</td>
<td>1000</td>
</tr>
</tbody>
</table>

Figure 3. Cytotoxicity of 1 and 649C-F13 against human keratinocytes (HaCaTs). DMSO was the vehicle control.

Compound 1 alone has an MIC of 16 μg/mL against MRSA (LAC) and FICIs of 0.125 with oxacillin, 0.094 with Meropenem, and 0.625 with vancomycin, and data on the drug combinations is visualized in heatmaps and isobolograms for oxacillin, Meropenem, and vancomycin with 1 (Figure 4). The existence of synergy (FICI ≤ 0.5) between 1 and the β-lactam antibiotics and not vancomycin suggests a possible specificity of 1 for β-lactams. Synergy between 1 and oxacillin was confirmed by a time-kill assay (Figure 5), according to the definition of time-kill synergy as a 2 log10 decrease in CFU/mL at the 24 h time point. Moreover, a combination of 4 μg/mL 1 (1/4 MIC) and 2 μg/mL oxacillin (1/32 MIC) exhibited bactericidal activity against MRSA (LAC).

To further characterize the interactions between 1 and oxacillin, combinations of 1 and oxacillin were tested for growth inhibition against a panel of *S. aureus* clinical isolates, including both oxacillin-resistant and -susceptible strains (Table 1). Against ATCC strains, compound 1 lowered the oxacillin MIC 16–512-fold at a concentration of 5 μg/mL; notably, 1 did not exhibit synergy with oxacillin against the oxacillin-susceptible *S. aureus* AR Bank #0470 and oxacillin-intermediate *S. aureus* AR Bank #0474.

In conclusion, we report that the clerodane diterpene 1 from *C. americana* moderately inhibits the growth of MRSA, supporting the traditional use of *C. americana* as an anti-infecive. This diterpene also potently synergizes with oxacillin and Meropenem against MRSA, suggesting a possible future application of 1 or related compounds as potentiators for treatment of β-lactam-resistant infections. Interestingly, when tested against selected strains from the CDC & FDA Antibiotic Resistance Bank *Staphylococcus* with the Borderline Oxacillin Susceptibility (BORSA) panel (Table 1),* synergy was only observed in one out of three strains. The CDC & FDA AR Bank documents the mechanisms of resistance for the three tested strains (AR Bank #0470, #0474, and #0480) are from the mecA allele, which is responsible for the expression of a modified penicillin-binding protein (PBP-2a), which has a low affinity for oxacillin. Oxacillin inhibits the activity of native PBPs, but PBP-2a remains efficient in cell wall synthesis. Since the BORSA strains tested exhibited varying resistance to oxacillin and different interactions between 1 and oxacillin, there may be additional genetic mechanisms of interest that may influence the PBP-2a structure and function. Further synergy studies using the BORSA panel will help elucidate these mechanisms.

Clerodane diterpenes isolated from plants have been previously reported for their antibacterial activity (Table S2). One of these clerodane diterpenes, 16β-hydroxycleroda-3,13(14)-Z-dien-15,16-olide, has also been shown to synergize with fluoroquinolone antibiotics against MRSA by downregulating genes vital to the expression of multidrug-resistant efflux pumps, such as norA, norB, norC, mdeA, and mepA. The determination of the interactions of compound 1 with other antibiotic classes is therefore an additional future direction of our study with 1. Finally, the toxicity of compound 1 against mammalian cells will be further investigated, particularly in light of previous evidence for anticancer activity *in vitro*. However, our preliminary results coupled with the lack of toxicity in a previous murine model indicate that compound 1 may possess significantly reduced toxicity than initially reported. Routes to access the compound as well as analogues are currently being developed. Given the high yield of compound from extracts, methods to generate analogues through semisynthesis are also currently under development. Future studies involving the synthesis and testing of analogues of 1 against a large panel of *S. aureus* isolates, as well as mutant libraries, will assist in the identification of the mechanism of action for compound 1 and provide a basis to determine the therapeutic potential of compound 1 and its analogues. Current therapies to treat MRSA bacteremia require the use of glycopeptide antibiotics such as vancomycin, which are known to present the risk of nephrotoxicity. Compound 1 may be useful to restore β-lactam sensitivity, therefore allowing β-lactam antibiotics to be a lower risk option for treating MRSA infections.

### METHODS

**General Experimental Procedures.** Nuclear magnetic resonance (NMR) data were recorded in chloroform-*d* (CDCl3) on a Bruker AVANCE III HD 600 (600 MHz for 1H NMR, 150 MHz for 13C, 5 mm CryoProbe) spectrometer. Chemical shifts (δ) are reported in ppm with the solvent (CDCl3: δH 7.26; δC 77.0) peaks used as reference. High resolution/accurate mass-atmospheric pressure chemical ionization (HRMS-APCI) experiments were performed on a Thermo Exactive Plus using the Ion Max Source with APCI probe. Spectra were collected with 140 000 resolution at m/z 200 using Tune software and analyzed with Thermo’s Freestyle software. Flash chromatography was carried out using a...
CombiFlash Rf+ Lumen (Teledyne ISCO) system with a RediSep Rf Gold silica column. Preparative high-performance liquid chromatography (Prep-HPLC) was performed using an Agilent 1260 Infinity II LC system (CA, USA) equipped with an Agilent Technologies 1200 Infinity Series Diode Array Detector detecting at 214 and 254 nm, auto collector, and Agilent XDB-C18 (30 × 250 mm, 5 μm) column.

**Plant Material.** *C. americana* leaves were collected in June and August 2017 from wild individuals in Atlanta, GA, and in June 2018 and 2019 from wild individuals from The Jones Center at Ichuaway in Baker County, Georgia. The identity was verified by botanist Dr. Tharanga Samarakoon, and voucher specimens (accession numbers 22044, 22205, 22848, and 25009) were deposited in the Emory University Herbarium (GEO). Vouchers are available for viewing through the SERNEC portal.29 Leaves were dried in a dehumidified chamber and ground to powder in a Wiley Mill (Thomas Scientific, Swedesboro, NJ) with a 2 mm mesh.

**Extraction and Fractionation.** Plant material was extracted as previously described.14 Briefly, powdered leaves were extracted by double maceration in 95% ethanol for 72 h; the resulting extract was filtered and then dried by rotary evaporation and lyophilization. Dry extract was stored at −20 °C.

Crude extract (649) was suspended in water at 1 g/33 mL and underwent sequential liquid–liquid partitioning with equal volumes of hexane, ethyl acetate, and butanol, yielding hexane (649B), ethyl acetate (649C), n-butanol (649D), and water (649E) partitions. The organic partitions were dried over Na₂SO₄ and filtered. Each partition was concentrated in vacuo at <40 °C. The hexane partition (649B) was dissolved and transferred to a tared scintillation vial and dried under forced air. The remaining partitions were shell frozen and lyophilized, and the resulting powder was stored at −20 °C. The ethyl

![Figure 4](https://dx.doi.org/10.1021/acsinfecdis.0c00307)

**Figure 4.** Checkerboards of 1 and (A) oxacillin (FICI = 0.125), (B) Meropenem (FICI = 0.094), and (C) vancomycin (FICI = 0.625) for growth inhibition of MRSA (LAC) and the corresponding isobolograms of 1 and (D) oxacillin, (E) Meropenem, and (F) vancomycin. In the checkerboards, white indicates 0% growth and dark blue indicates 100% growth relative to vehicle control. Points on the isobolograms represent combinations of 1 and antibiotics (relative to their MICs alone) that exhibit >90% growth inhibition of MRSA.

![Figure 5](https://dx.doi.org/10.1021/acsinfecdis.0c00307)

**Figure 5.** (A) Time-kill assay of 1 and oxacillin against MRSA (LAC). The treatments displayed in this figure are 1/4 MIC of 1 and 1/4 MIC of oxacillin alone and in combination and DMSO, the vehicle control. (B) Time-kill assay repeated with oxacillin concentrations at the susceptibility breakpoint (2 μg/mL).
acetate partition, 649C (15.43 g), was determined to be most active in bioassays and was fractionated using a 330 g silica column (RediSep, Teledyne ISCO) via normal phase flash chromatography (Combi Flash Rf+ Lumen, Teledyne ISCO) utilizing a hexane/ethyl acetate gradient and a methanol/ethyl acetate gradient. A total of 32 fractions were collected with fraction 429C-F13 (2.15 g, 13.9% yield) showing the highest activity. Fraction 469C-F13 was fractionated further via Prep-HPLC starting from a 50:50 mixture of the mobile phase of 0.1% (v/v) formic acid in water (A) and 0.1% (v/v) formic acid in acetonitrile (B) at a flow rate of 42.5 mL/min and monitored for 35 min. A total of 12 fractions were collected with the most bioactive fraction being 649C-F13-PF4 (186.2 mg, 93.1% yield). Fraction 649C-F13 was fractionated further via Prep-HPLC starting from a 30:70 mixture of the mobile phase of 0.1% (v/v) formic acid in water (A) and 0.1% (v/v) formic acid in acetonitrile (B) at a flow rate of 42.5 mL/min and monitored for 45 min. A total of 12 fractions were collected with the most bioactive fraction being 649C-F13-PF4 (186.2 mg, 93.1% yield). Fraction 649C-F13-PF4-PF7 was identified by 1D and 2D NMR and MS data as -dihydroxycleroda-3,13-dien-15,16-olide (1) (see the Supporting Information for spectra).

- **Table 1. Interactions between 1 and Oxacillin for Growth Inhibition of a Panel of *S. aureus* Strains**

<table>
<thead>
<tr>
<th>Strains</th>
<th>MIC alone (μg/mL)</th>
<th>oxacillin</th>
<th>MIC in combination (μg/mL)</th>
<th>oxacillin</th>
<th>FICI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LAC and AR bank strains</strong></td>
<td></td>
<td></td>
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<tr>
<td>LAC</td>
<td>16</td>
<td>64</td>
<td>2</td>
<td>1</td>
<td>0.141</td>
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<tr>
<td>AR Bank #0470</td>
<td>16</td>
<td>4</td>
<td>8</td>
<td>0.625</td>
<td>0.65</td>
</tr>
<tr>
<td>AR Bank #0474</td>
<td>16</td>
<td>8</td>
<td>8</td>
<td>1</td>
<td>0.625</td>
</tr>
<tr>
<td>AR Bank #0480</td>
<td>16</td>
<td>32</td>
<td>4</td>
<td>1</td>
<td>0.283</td>
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<tr>
<td><strong>ATCC strains</strong></td>
<td></td>
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<td></td>
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<tr>
<td>ATCC BAA-1556</td>
<td>64</td>
<td>5</td>
<td>0.25</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>ATCC 43300</td>
<td>32</td>
<td>5</td>
<td>2</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>ATCC BAA-44</td>
<td>256</td>
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<td>2</td>
<td>128</td>
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<tr>
<td>ATCC BAA-811</td>
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<td>5</td>
<td>0.25</td>
<td>512</td>
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<tr>
<td>ATCC BAA-1770</td>
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<td>2</td>
<td>16</td>
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<tr>
<td>ATCC 700799</td>
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<td>5</td>
<td>2</td>
<td>64</td>
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<tr>
<td>ATCC 33591</td>
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<td>5</td>
<td>2</td>
<td>64</td>
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<td>ATCC BAA-1685</td>
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<td>5</td>
<td>8</td>
<td>32</td>
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<tr>
<td>ATCC BAA-1753</td>
<td>256</td>
<td>5</td>
<td>0.5</td>
<td>512</td>
<td></td>
</tr>
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</table>

Three *S. aureus* strains with mecA-mediated resistance, AR Bank #0470, AR Bank #0474, and AR Bank #0480, were obtained from the CDC & FDA AR Isolate Bank. Strains BAA-1556, 43300, BAA-44, BAA-811, BAA-1770, 700799, 33591, BAA-1685, and BAA-1753 were obtained from the American Type Culture Collection (ATCC). Bacteria were maintained on tryptic soy agar (TSA) plates. Liquid cultures for ATCC strains were grown in Mueller Hinton broth (MHB) incubated for 5 to 6 h at 37 °C with constant shaking (200 rpm). Liquid cultures for LAC and AR Bank strains were grown in tryptic soy broth (TSB) incubated overnight at 37 °C with constant shaking (230 rpm).

**Growth Inhibition.** *S. aureus* growth inhibition was determined by broth microdilution according to CLSI M100-S23 guidelines. Liquid cultures were inoculated in cation adjusted Mueller Hinton broth (CAMHB) to 5 × 10⁶ CFU/mL, and the resulting working cultures were combined with extracts and controls in 96-well microtiter plates to produce a final well volume of 100 μL for ATCC strains and 200 μL for LAC and AR Bank strains. The initial screen of crude extracts was undertaken in Mueller Hinton Broth (MHB), and then, CAMHB was used for all fractions and isolated compound studies. Vehicle (DMSO) controls, media blanks, and antibiotic controls were included in each assay, and all treatments were tested in triplicate. Concentration gradients were created using 2-fold serial dilution. Microwell plates were incubated under stationary conditions at 37 °C for 16 h for ATCC strains and 18 h for LAC and AR Bank strains; optical density (OD₆₀₀) was measured at initial and final time points, and percent growth inhibition was calculated relative to the vehicle control using the change in optical density to account for extract color. The minimum inhibitory concentration (MIC) is defined as the lowest concentration of drug that achieves ≥90% growth inhibition.

**Synergy with β-Lactam Antibiotics.** The interactions between study samples and β-lactam antibiotics were quantified using the fractional inhibitory concentration index (FICI) calculated in the framework of Loewe additivity according to accepted standards in the field of antimicrobial synergy: FICI ≤ 0.5 was defined as synergy, 0.5 < FICI < 4 was defined as noninteraction, and FICI ≥ 4 was defined as antagonism. Constant-ratio combinations of sample and antibiotic were created using 2-fold serial dilution. Microwell plates were incubated under stationary conditions at 37 °C for 16 h for ATCC strains and 18 h for LAC and AR Bank strains; optical density (OD₆₀₀) was measured at initial and final time points, and percent growth inhibition was calculated relative to the vehicle control using the change in optical density to account for extract color. The minimum inhibitory concentration (MIC) is defined as the lowest concentration of drug that achieves ≥90% growth inhibition.

**Bacterial Strains and Conditions.** The primary *Staphylococcus aureus* strain used in these experiments was the Los Angeles County clone (LAC), a CA-MRSA USA300 isolate. Three *S. aureus* strains with mecA-mediated resistance, AR Bank #0470, AR Bank #0474, and AR Bank #0480, were obtained from the CDC & FDA AR Isolate Bank. Strains BAA-1556, 43300, BAA-44, BAA-811, BAA-1770, 700799, 33591, BAA-1685, and BAA-1753 were obtained from the American Type Culture Collection (ATCC). Bacteria were maintained on tryptic soy agar (TSA) plates. Liquid cultures for ATCC strains were grown in Mueller Hinton broth (MHB) incubated for 5 to 6 h at 37 °C with constant shaking (200 rpm). Liquid cultures for LAC and AR Bank strains were grown in tryptic soy broth (TSB) incubated overnight at 37 °C with constant shaking (230 rpm).

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antibiotic were tested in growth inhibition assays, adapting the method described by Berenbaum and Cokol et al. Highly synergistic combinations (FICI < 0.1) were confirmed via the checkerboard assay.

**Time Kill.** Time-kill assays were carried out according to previously described methods. Briefly, *S. aureus* (LAC) in logarithmic phase was standardized to $5 \times 10^5$ CFU/mL using a BioTek Cytation3 plate reader. Five mL of this working culture was added to test tubes for each of the following treatments: 64, 16, and 2 $\mu$g/mL oxacillin alone, 16 and 4 $\mu$g/mL SF7 alone, a combination of 16 $\mu$g/mL oxacillin with 4 $\mu$g/mL SF7, and a combination of 2 $\mu$g/mL oxacillin with 4 $\mu$g/mL SF7. Tubes were also prepared for the vehicle control (DMSO), a bacterial growth control, and a media blank. All tubes were incubated at 37 °C with shaking at 260 rpm. At 0, 3, 6, and 24 h time points, 10 $\mu$L was taken from each tube, diluted in sterile PBS, and inoculated on TSA plates, which were incubated at 37 °C until colonies were clearly visible (6 to 12 h); colonies on these plates were then used and calculated CFU/mL for each treatment and time point.

**Mammalian Cytotoxicity.** Active fractions and their parents were tested for in vitro mammalian cytotoxicity using immortalized human keratinocytes (HaCaTs) with a lactate dehydrogenase (LDH) assay kit (G-Biosciences, St. Louis, MO) as previously described. Briefly, 200 $\mu$L of cell culture standardized to $4 \times 10^4$ cells/mL was added to wells in 96-well tissue culture microtiter plates (Falcon 35-3075) and incubated for 48 h; then, treatments and fresh media were added, and plates were incubated for 24 more hours and processed according to the LDH kit protocol for chemical-induced cytotoxicity. Samples tested in this assay were sterile-filtered, and the concentration of DMSO in the wells was kept at <1%.

**ASSOCIATED CONTENT**

**Supporting Information**
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsinfecdis.0c00307.

Checkerboards of oxacillin and 649 partitions, synergy between oxacillin and 649C fractions, checkerboard of oxacillin and 649C-F13, 13C NMR spectrum of compound 1, 1H NMR spectrum of compound 1, HRMS-APCI spectra of compound 1, and antibacterial activity of clerodane diterpenes (PDF)

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**Author Contributions**

Conceptualization, data curation, and formal analysis: C.L.Q. and C.M. Investigation: M.D., R.J.M., G.P., C.R., T.S., and C.M. Methodology: C.L.Q., C.M., M.D., and R.J.M. Project administration, resources, supervision, funding, validation, and visualization: C.L.Q. and C.M. Writing, original draft: M.D. Writing, review, and editing: all authors.

**Notes**

The authors declare the following competing financial interest(s): C.M. is a co-founder and a member of the board of directors of Agile Sciences, a biotechnology company seeking to commercialize antibiotic potentiators.

**ACKNOWLEDGMENTS**

This work was supported by the National Institute of Allergy and Infectious Disease (R21 AI136563 to C.L.Q. and RO1 DE22350 to C.M.), a National Institute of General Medical Sciences training grant (T32-GM088602) to C.R., Emory University development funds to C.L.Q., and a graduate student fellowship from The Jones Center at Ichuaway to L.M. Thanks to Monique Salazar and Huaiqiao Yang for assistance with the extractions and Kate Nelson for assistance with the microbiological assays.

**ABBREVIATIONS**

CAMHB, cation adjusted Mueller Hinton broth; CFU, colony forming unit; FICI, fractional inhibitory concentration index; LDH, lactate dehydrogenase; MHB, Mueller Hinton broth; SERNEC, SouthEast Regional Network of Expertise and Collections; TSA, tryptic soy agar; TSB, tryptic soy broth

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https://dx.doi.org/10.1021/acsinfecdis.0c00307 ACS Infect. Dis. 2020, 6, 1667–1673