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RAPID COMMUNICATION

Dehydrozaluzanin C, a novel type of anti-bacterial agent which targets transporting proteins, Opp and OpuC

To the Editor:

The evolution and widespread distribution of antibiotic-resistant elements in bacterial pathogens is eroding our ability to control infections with existing antibiotics. *Staphylococcus aureus* (*S. aureus*) and Methicillin-resistant *S. aureus* (MRSA) have acquired alarming broad-spectrum antibiotic resistance resulting in hospital- and community-associated infections, which has been responsible for significant morbidities and deaths worldwide.[1] It is estimated that the number of deaths caused by antibiotic resistance may exceed 10 million per year by 2050.[2] This antibiotic-resistance crisis has promoted an urgent demand for finding new medicines. Unfortunately, antimicrobial drug development is uniquely difficult.[3] Almost all antibiotics in clinical use today were derived from soil microorganisms where they were used for fending off competing bacteria and these were discovered by screening cultivable microbes more than 30 years ago. Here we report a new inhibitor named SLs 5-29 (Dehydrozaluzanin C, DHZ, Fig. S1B, S2 and Tables S1, S2). It inhibits the Opp and OpuC transport systems of *S. aureus* (ATCC® 25904™)[4] and is a sesquiterpene lactone with a guaianolide skeleton isolated from *Vernonia novebaracensis*.[5]

By primary antibacterial activities screening (Fig. 1A, S3A), SLs 5-29 showed an excellent bactericidal activity against *S. aureus* (ATCC® 25904™) (Fig. S3A). The resulting liquid medium in SLs 5-29 and *S. aureus* (ATCC® 25904™) co-culture system was extremely clear (Fig. 1B, left panel). The *S. aureus* (ATCC® 25904™) proliferated twice after 2 μM SLs 5-29 treatment 5 h, while no proliferation being observed with 5 and 10 μM administration (Fig. 1B, middle panel). SLs 5-29 was found ineffective against gram-negative bacteria such as *Escherichia coli* (*E. coli*) and Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) (Fig. S3B). Furthermore, the growth of MRSA (ATCC® BAA1717™) was also inhibited by SLs 5-29 administration and the numbers of bacteria were reduced by half at 5 and 10 μM dosages of SLs 5-29 (Fig. 1C). We also investigated the potential of SLs 5-29 as a therapeutic compound *in vivo* (Fig. 1D, S3D). MRSA (ATCC® BAA1717™)-infected mice administrated with SLs 5-29 survived the controls (Fig. 1E). These data demonstrated that SLs 5-29 had strong inhibition on the *Staphylococcus* species.

Transmission electron microscopy (TEM) was used to investigate the morphology of *S. aureus* (ATCC® 25904™) after SLs 5-29 treatment. A low-density bright swelling was seen between the cell wall and cell membrane, and there were also low-density bright spots in the nuclear region in *S. aureus* (ATCC® 25904™) treated with SLs 5-29 (Fig. S3H). The K^+ concentration in the culture medium of *S. aureus* (ATCC® 25904™) after SLs 5-29 treatment sharply increased, while the pH value was decreased (Fig. S3J). To test the DNA integrity of *S. aureus* (ATCC® 25904™) and MRSA (ATCC® BAA1717™) co-cultured with SLs 5-29, terminal deoxynucleotidyl transferase dUTP nick-end labeling and flow cytometry were used. The DNA of *S. aureus* (ATCC® 25904™) and MRSA (ATCC® BAA1717™) was partially damaged in the treated group, and the bacterial death rate was also increased in both *S. aureus* and MRSA with SLs 5-29 (Fig. S3I). Taken together, these data implicated that the integrity of bacterial membrane was destroyed by SLs 5-29.

The ATP-binding cassette (ABC) transporters constitute a ubiquitous superfamily of integral membrane proteins that are present in all living organisms. They are responsible for the ATP-powered translocation of substrates across cell membranes. From the modulated transcripts of RNA sequencing, we found genes in that the ABC transporter pathway, including *oppA*, *oppB*, *oppC*, *oppD*, *oppF*, *opuCB*, *opuCC* and *opuCD* were down-regulated (Fig. 1F, left panel). Q-PCR confirmed that *oppB*, *oppC*, *oppD*,

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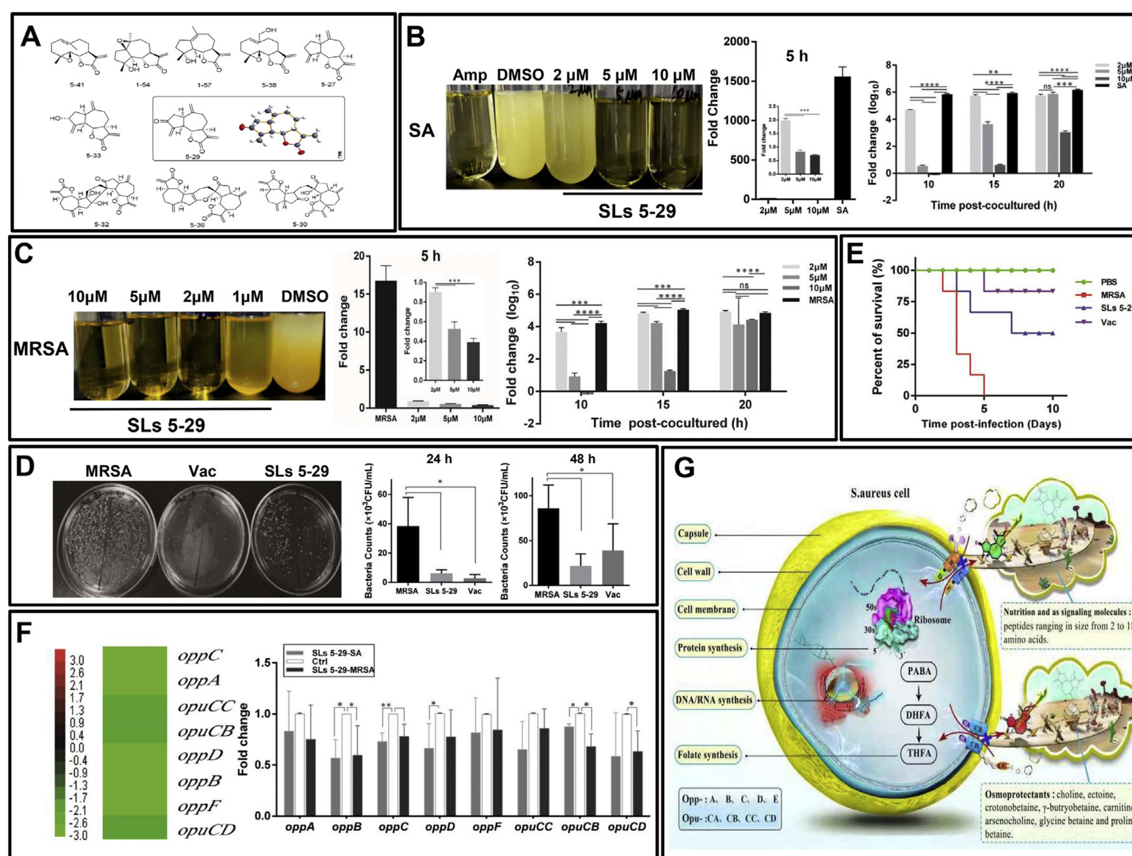

Figure 1 The growth of *S. aureus* (ATCC® 25904™) and MRSA (ATCC® BAA1717™) was inhibited by SLs 5-29. **(A)** The X-ray crystallographic structure of SLs 5-29 and the screened SLs compounds. **(B)** Left panel: The transparency of *S. aureus* (ATCC® 25904™), showed as SA, co-cultured with different concentrations of SLs 5-29 (2, 5 and 10 μM) for 10 h, with DMSO and ampicillin (50 μg/mL) as control; Middle panel: The growth of *S. aureus* (ATCC® 25904™) after cultured with SLs 5-29 for 5 h by plate counting; Right panel: The growth of *S. aureus* (ATCC® 25904™) after being cultured with SLs 5-29 for 10, 15 and 20 h as assessed by plate counting. **(C)** Left panel: The transparency of MRSA (ATCC® BAA1717™) co-cultured with different concentrations of SLs 5-29 (1, 2, 5 and 10 μM) for 10 h, with DMSO as control; Middle panel: The growth of MRSA (ATCC® BAA1717™) after cultured with SLs 5-29 for 5 h by plate counting; Right panel: The growth of MRSA (ATCC® BAA1717™) after being cultured with SLs 5-29 for 10, 15 and 20 h as assessed by plate counting. **(D)** The numbers of bacteria in the peripheral blood after being infected with MRSA (ATCC® BAA1717™) for 24 and 48 h as assessed by plate counting (Vac, vancomycin). **(E)** The survival times were monitored. **(F)** Left panel: RNAseq data of *S. aureus* (ATCC® 25904™) co-cultured with SLs 5-29 for 10 h showing the *oppA*, *oppB*, *oppC*, *oppD*, *oppF*, *opuCB*, *opuCC* and *opuCD* genes were down-regulated; Right panel: Q-PCR was used to verify the results of RNAseq data. **(G)** Diagrammatic representation of the action mechanism of SLs 5-29 inhibition of *S. aureus* bacteria: SLs 5-29 blocked the transport channels of nutrients or signaling molecules and osmoprotectants by binding to the oligopeptide permeases, oppB, oppC and oppD, and the osmoprotectant uptake of opuCB and opuCD in gram-positive bacteria. All results were carried out at least in triplicates and the Student's *t*-test was used for two-group comparisons; *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$; mean ± SEM. $n = 8$.

opuCB and *opuCD* were significantly down-regulated in both *S. aureus* (ATCC® 25904™) and MRSA (ATCC® BAA1717™) after SLs 5-29 treatment (Fig. 1F, right panel). Thus, the down-regulation of ABC transporter genes suggested that SLs 5-29 possibly regulated the transportation of nutritional substances.

To further reveal the binding mechanism, simulated docking of SLs 5-29 with five potential target proteins was performed (Fig. S4–6 and Table S3). The results indicated that SLs 5-29 can effectively block the transport channels of nutrients or signaling molecules and osmoprotectants by binding to the oligopeptide permeases OppB, OppC and OppD and the osmoprotectant uptake of OpuCB and OpuCD in *S. aureus* (Fig. 1G). Blocking of the Opp and OpuC transport transmembrane channels can be used to cut off the supply of nutrients to cells and disrupt the osmotic equilibrium of the membrane, and then bacteria would be incapable of maintaining their normal physiological functions leading to their demise. The results are consistent with RNA transcription sequencing experiments. Other compounds in Figure 1A were also subjected to simulated binding with target proteins (Table S4).

This study characterized a promising therapeutic candidate, SLs 5-29. It is effective for inhibiting the growth

of MRSA. This novel compound is a natural product which targets the transmembrane channel proteins of the bacteria (Fig. 1G). The blockage of nutrition-associated transport could be a potential new strategy to help in the development of novel antibiotics.

Author contributions

X. Q. and X. H. designed the project and drafted the manuscript. X. Q. synthesized the compounds. J. X. and D.Y. performed the functional experiments. F. F. partially sponsored and provided help for experiments. X. F. Z helped with some experiments. B. N. carried out the biological simulations.

Conflict of interests

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2021.11.009.

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