

SUPPLEMENTARY MATERIALS

Dihydrothiazolo ring-fused 2-pyridone antimicrobial compounds treat *Streptococcus pyogenes* skin and soft tissue infection

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SUPPLEMENTARY FIGURES

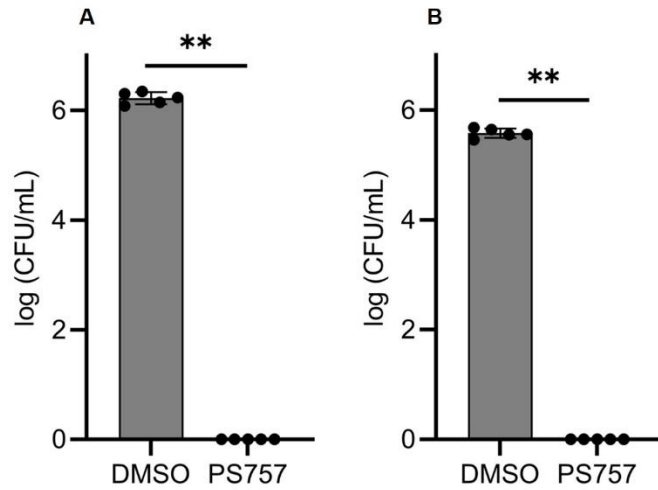


Fig. S1. GmPcide PS757 demonstrates robust bactericidal activity against the exponential- and stationary-phase of *S. pyogenes* HSC5 cells. (A) Exponential-phase (7 hours post inoculum) *S. pyogenes* HSC5 cells treated under bactericidal (20 μ M) concentration of PS757 for 12 hours were observed with > 6.0 logCFU reduction. **(B)** Stationary-phase (14 hours post inoculum) *S. pyogenes* HSC5 cells treated under bactericidal (20 μ M) concentration of PS757 for 12 hours were observed with > 5.0 logCFU reduction. Statistics were performed with Mann-Whitney U test. $P \leq 0.05$ is considered as statistically significant. * $P \leq 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, ns indicates not significant.

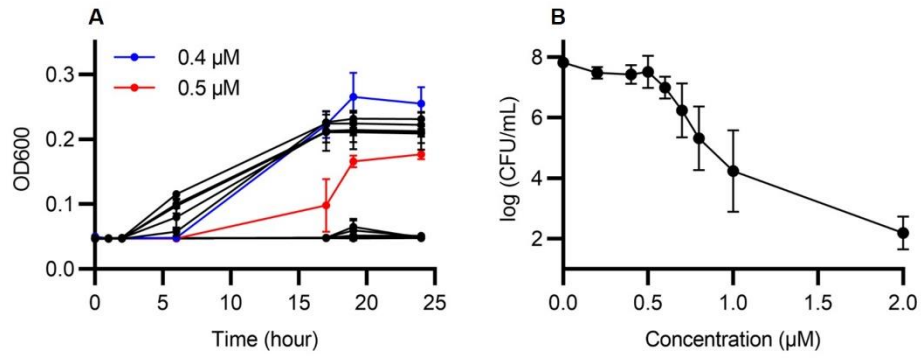


Fig. S2. Sublethal concentration of GmPcide PS757 against *S. pyogenes* HSC5 was determined in microplate assay using C medium by measuring both **(A)** OD600 and **(B)** CFU, which identified 0.4 μM as the sublethal concentration of PS757 against *S. pyogenes* HSC5.

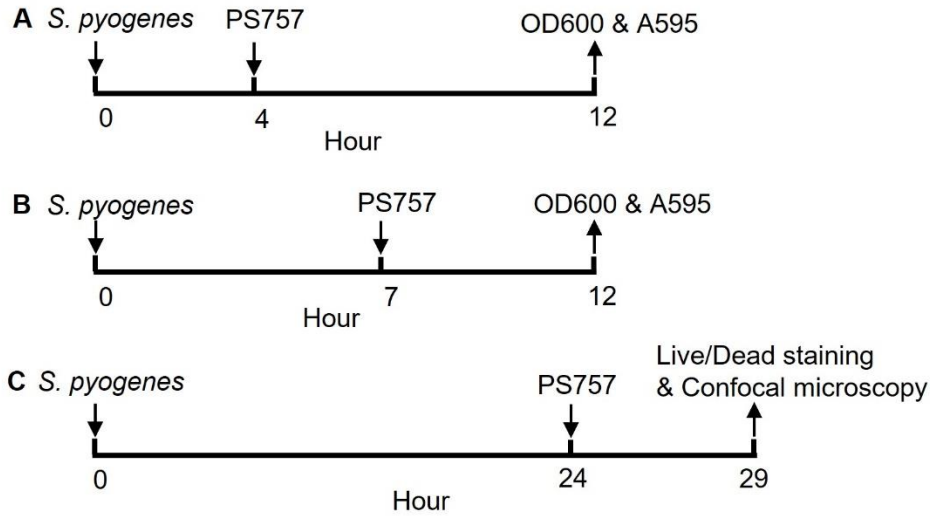


Fig. S3. GmPcide PS757 treatment to different phases of *S. pyogenes* biofilm. (A) GmPcide PS757 treatment to *S. pyogenes* HSC5 biofilm at 4 hrs during initiation phase. **(B)** GmPcide PS757 treatment to *S. pyogenes* HSC5 biofilm 7 hrs during maturing development. **(C)** GmPcide PS757 treatment to mature *S. pyogenes* HSC5 biofilm at 24 hrs.

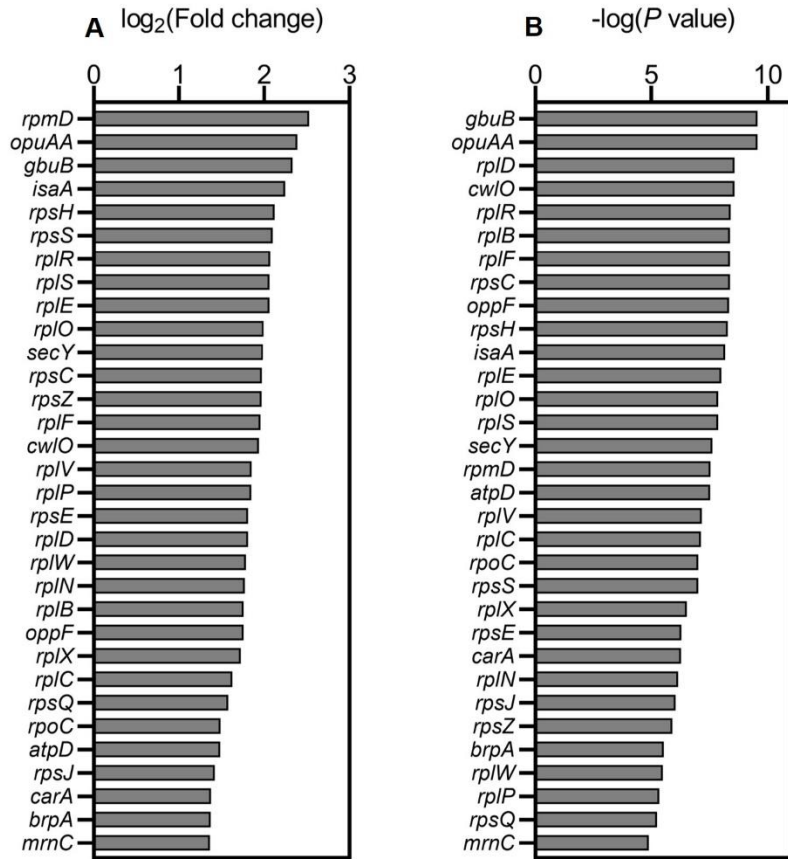


Fig. S4. Differentially expressed genes (DEGs) induced by PS757 treatment were identified as $\log_2(\text{FC}) > 0.5$ and $P < 0.05$ in the comparative RNA-seq analysis. Among DEGs, two more stringent selection criteria, $|\log_2(\text{FC})|$ **(A)** and $-\log(P)$ **(B)** $> 99\%$ confidence intervals (CI) upper limits were applied to select the most up-regulated group of genes, which identified 32 most up-regulated genes featuring the involvement of two ribosomal protein-associated pathways, Rpl and Rps.

SUPPLEMENTARY TABLES

Table S1. The list of most down- and up-regulate *S. pyogenes* genes induced by sublethal PS757 treatment identified by comparative transcriptomic analysis.

Differentially expressed levels (DEs)	Differentially expressed genes (DEGs)
Most down-regulated	<i>emm5, malQ, udp, malX, nupX, ycjP, arlR, NA, NA</i>
Most up-regulated	<i>rpmD, opuAA, gbuB, isaA, rpsH, rpsS, rplR, rplS, rplE, rplO, secY, rpsC, rpsZ, rplF, cwI/O, rplV, rplP, rpsE, rplD, rplW, rplN, rplB, oppF, rplX, rplC, rpsQ, rpoC, atpD, rpsJ, carA, brpA, mrnC, NA</i>