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Supplementary Materials for

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

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The PDF file includes:

Figs. S1 to S6
Tables S1 to S3

Other Supplementary Material for this manuscript includes the following:

Data S1

SUPPLEMENTARY FIGURES

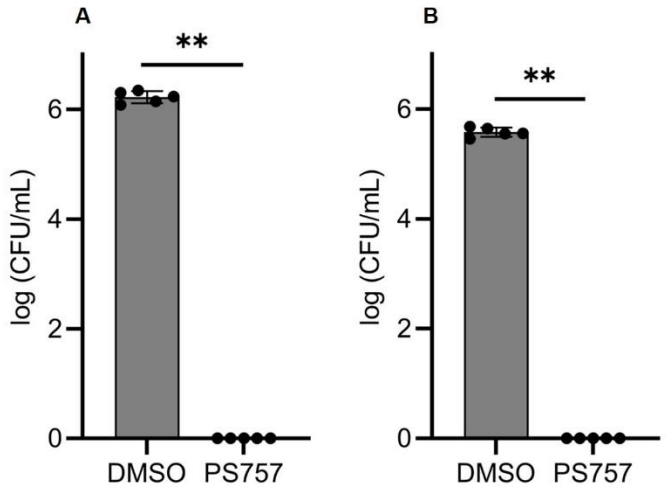

Fig. S1. GmPcide PS757 demonstrates robust bactericidal activity against the exponential- and stationary-phase of *S. pyogenes* HSC5 cells. (A) Exponential-phase (7 hours post inoculum) *S. pyogenes* HSC5 cells treated under bactericidal (20 µM) concentration of PS757 for 12 hours were observed with > 6.0 logCFU reduction. **(B)** Stationary-phase (14 hours post inoculum) *S. pyogenes* HSC5 cells treated under bactericidal (20 µM) 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.

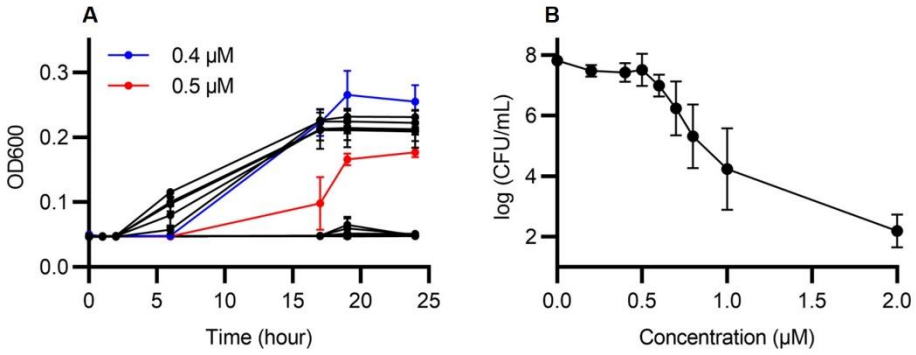

Fig. S2. Sublethal concentration of GmPcide PS757 against *S. pyogenes* HSC5 was determined in microplate assay using C medium by measuring both **(A)** OD600 and **(B)** CFU, which identified 0.4 μM as the sublethal concentration of PS757 against *S. pyogenes* HSC5.

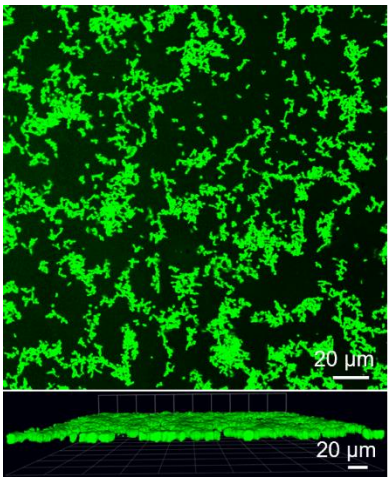

Fig. S3. Immunofluorescent confocal microscopy characterization of *S. pyogenes* HSC5 biofilm at 24 hrs post-inoculation. A dense biofilm with an approximate thickness of 9.5 µm was observed.

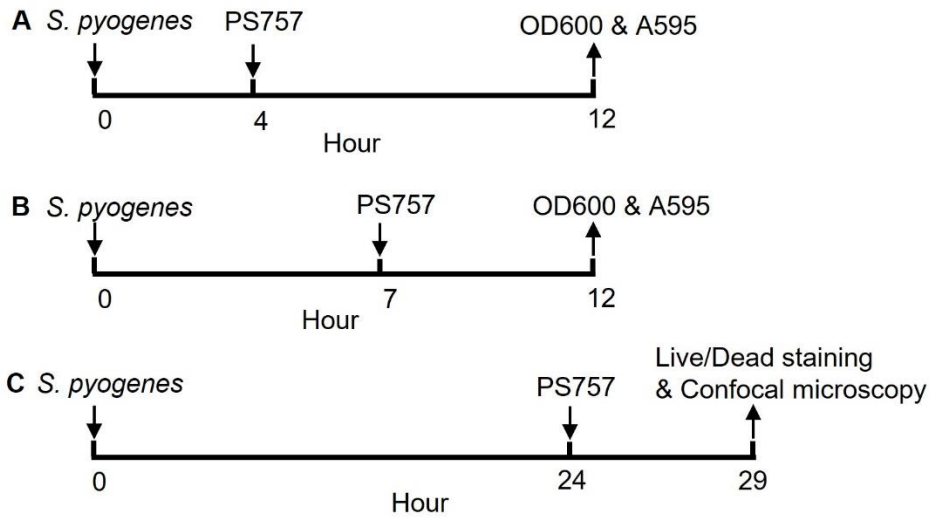

Fig. S4. GmPcide PS757 treatment to different phases of *S. pyogenes* biofilm. (A) GmPcide PS757 treatment to *S. pyogenes* HSC5 biofilm at 4 hrs during initiation phase. **(B)** GmPcide PS757 treatment to *S. pyogenes* HSC5 biofilm 7 hrs during maturing development. **(C)** GmPcide PS757 treatment to mature *S. pyogenes* HSC5 biofilm at 24 hrs.

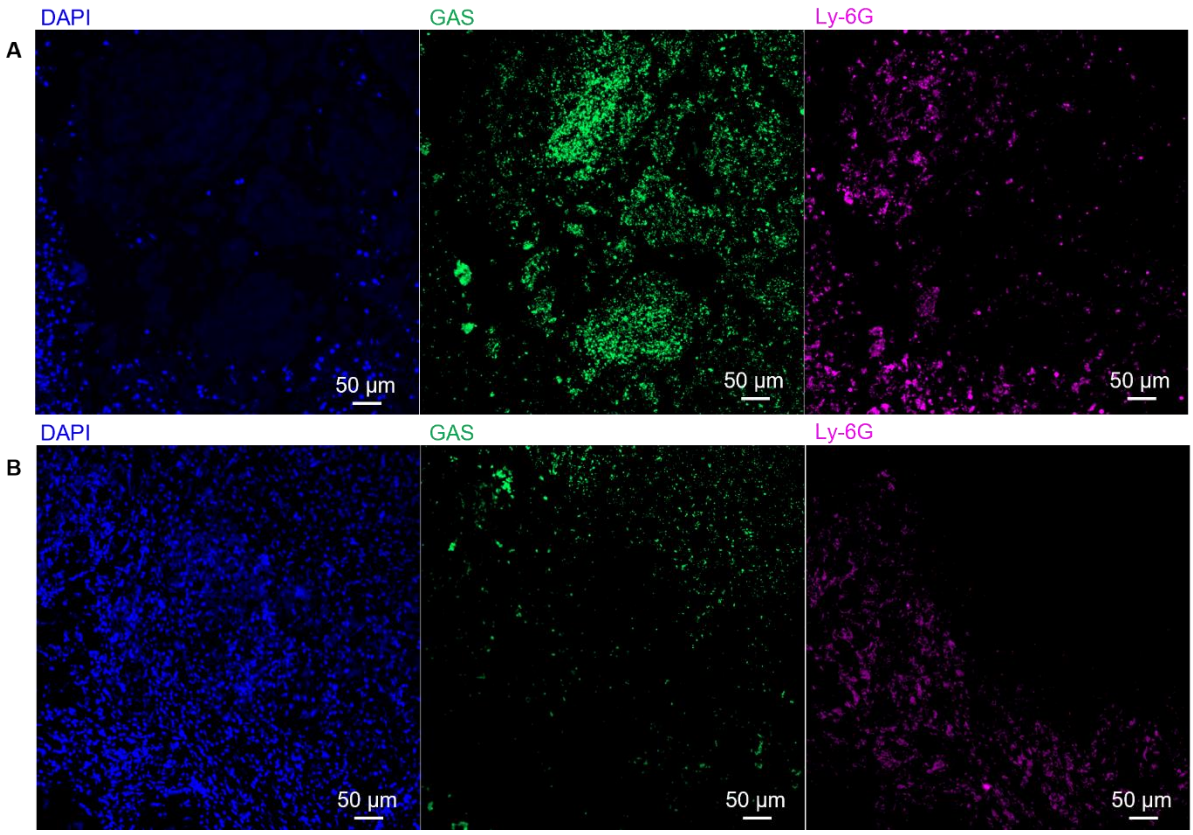

Fig. S5. Immunofluorescence microscopy characterizations of thigh tissue samples from vehicle-treated (DMSO) (A) and PS757-treated (B) mice. Host cell nuclei, *S. pyogenes* HSC5, and polymorphonuclear neutrophils were stained with DAPI, GAS, and Ly-6G antibodies, respectively.

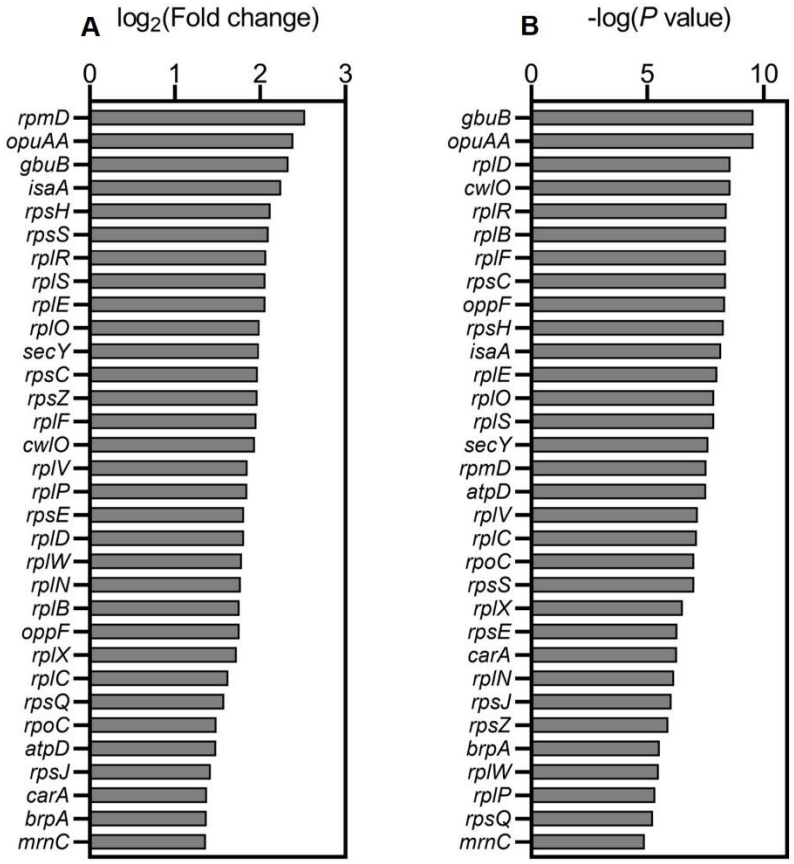

Fig. S6. Differentially expressed genes (DEGs) induced by PS757 treatment were identified as $\log_2(FC) > 0.5$ and $P < 0.05$ in the comparative RNA-seq analysis. Among DEGs, two more stringent selection criteria, $|\log_2(FC)|$ **(A)** and -log(P) **(B)** > 99% confidence intervals (CI) upper limits 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. MIC and MBC concentrations of four standard-of-care antibiotics against *S. pyogenes* HSC5

Strain	Antibiotic	Concentration (μM)	
		MIC	MBC
S. pyogenes HSC5	Penicillin	0.09	0.35
	Cefotaxime	0.13	0.52
	Vancomycin	0.17	0.34
	Azithromycin	0.32	5.10

Table S2. Antimicrobial synergy of GmPcide PS757 with four standard-of-care antibiotics examined by checkerboard method and E test.

Strain	Combined treatment		MIC (μM)		FICI
			Alone	Combined	
S. pyogenes HSC5	PS757 + Penicillin	PS757	0.78	0.39	0.75
		Penicillin	0.09	0.02	
	PS757 + Cefotaxime	PS757	0.78	0.39	0.75
		Cefotaxime	0.13	0.03	
	PS757 + Vancomycin	PS757	0.78	0.39	1.50
		Vancomycin	0.17	0.17	
	PS757 + Azithromycin	PS757	0.78	0.39	1.50
		Azithromycin	0.32	0.32	

Table S3. The list of most down- and up-regulate S. pyogenes genes induced by sublethal PS757 treatment identified by comparative transcriptomic analysis.

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