

Discovery of psoralen as a quorum-sensing inhibitor suppresses *Pseudomonas aeruginosa* virulence

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Supplementary Figures

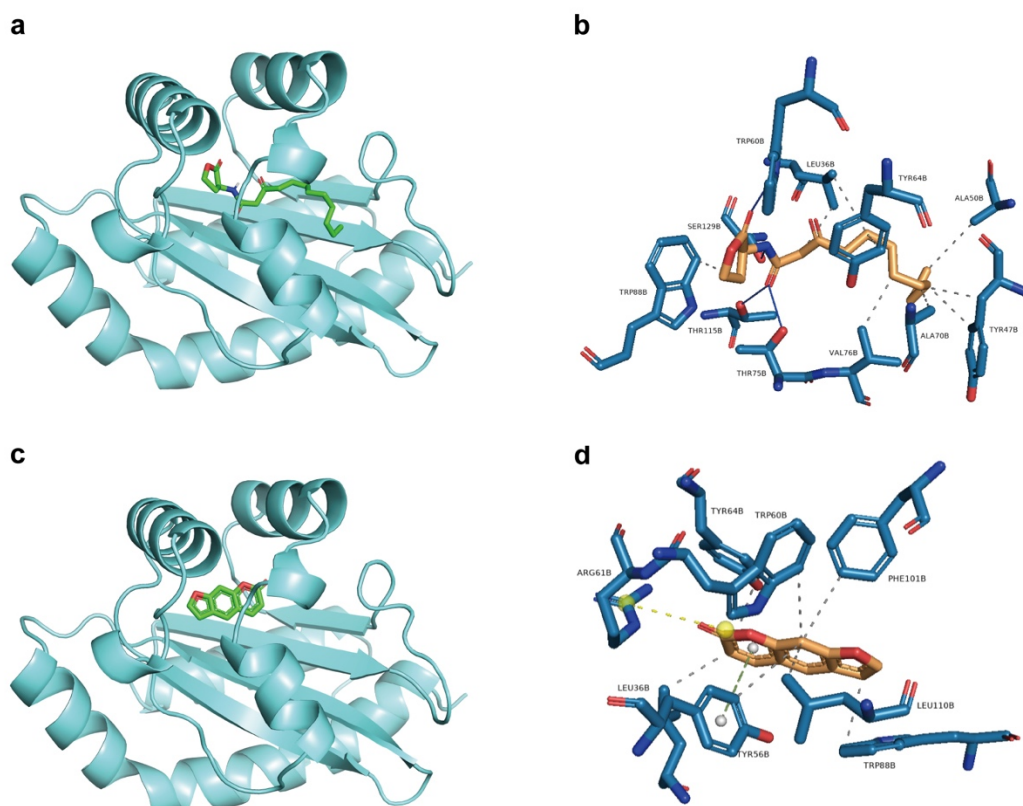


Fig. S1 Molecular docking of LasR protein binding to 3-oxo-C12-HSL (**a**, **b**) and psoralen (**c**, **d**).

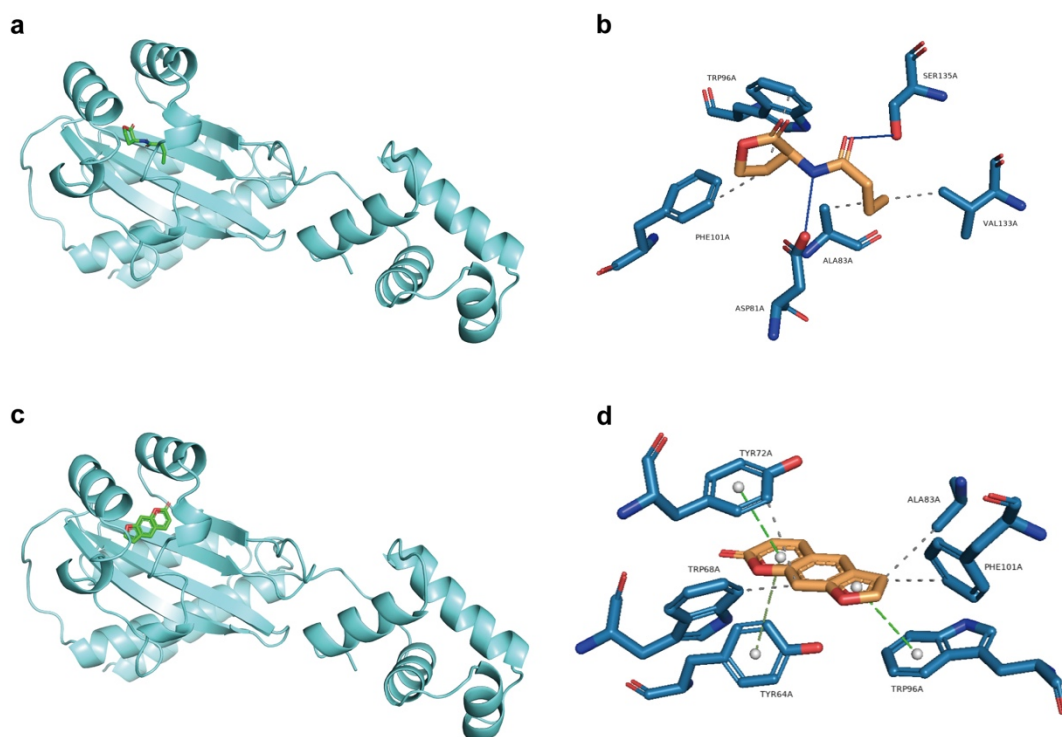


Fig. S2 Molecular docking of RhIR protein binding to C4-HSL (a, b) and psoralen (c, d).

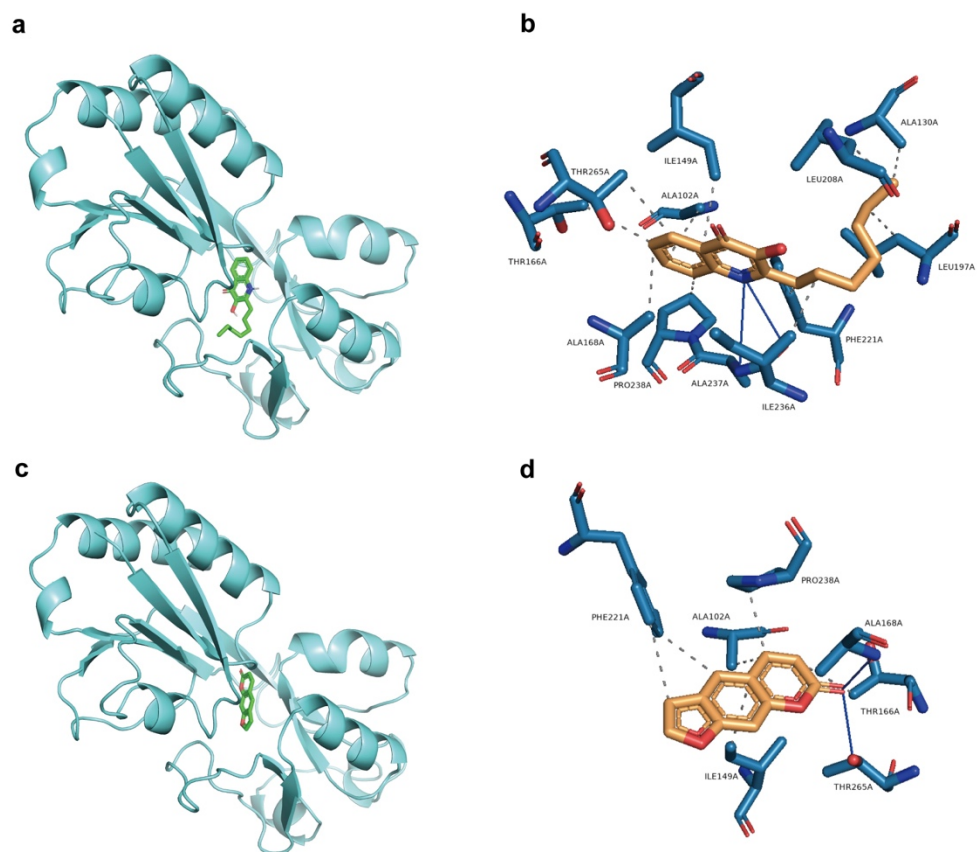


Fig. S3 Molecular docking of PqsR protein binding to PQS (**a, b**) and psoralen (**c, d**).

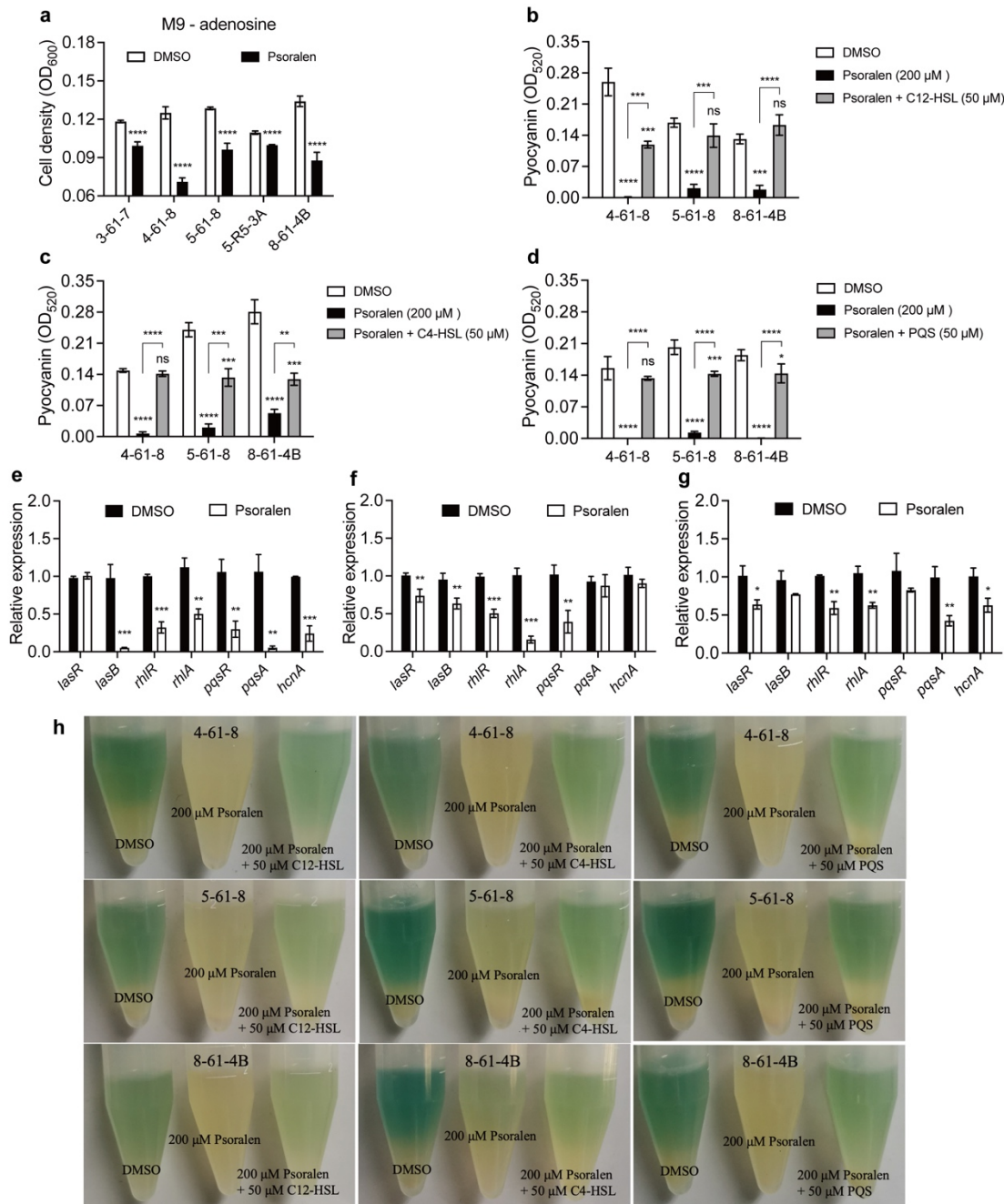


Fig. S4 Effects of psoralen on clinical isolates. **a** The growth of clinical isolates in M9-adenosine (0.1%, w/v) medium containing psoralen (200 μ M). The pyocyanin production of clinical isolates in LB broth containing psoralen supplementation with different AIs (**b-d**). **b** Psoralen supplementation with C12-HSL. **c** Psoralen supplementation with C4-HSL. **d** Psoralen supplementation with PQS. Expression of main QS genes of psoralen-treated clinical isolates was determined by qPCR(**e-g**). **e** Clinical isolate 4-61-8. **f** Clinical isolate 5-61-8. **g** Clinical isolate 8-61-4B. **h** The image of pyocyanin production of clinical isolates containing psoralen supplementation with different AIs. The results were presented as mean $\pm$ SD, and three independent experiments. The analysis methods were used one-way ANOVA and t test, ns, not significant, *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, ****, $P < 0.0001$.

Supplementary Information

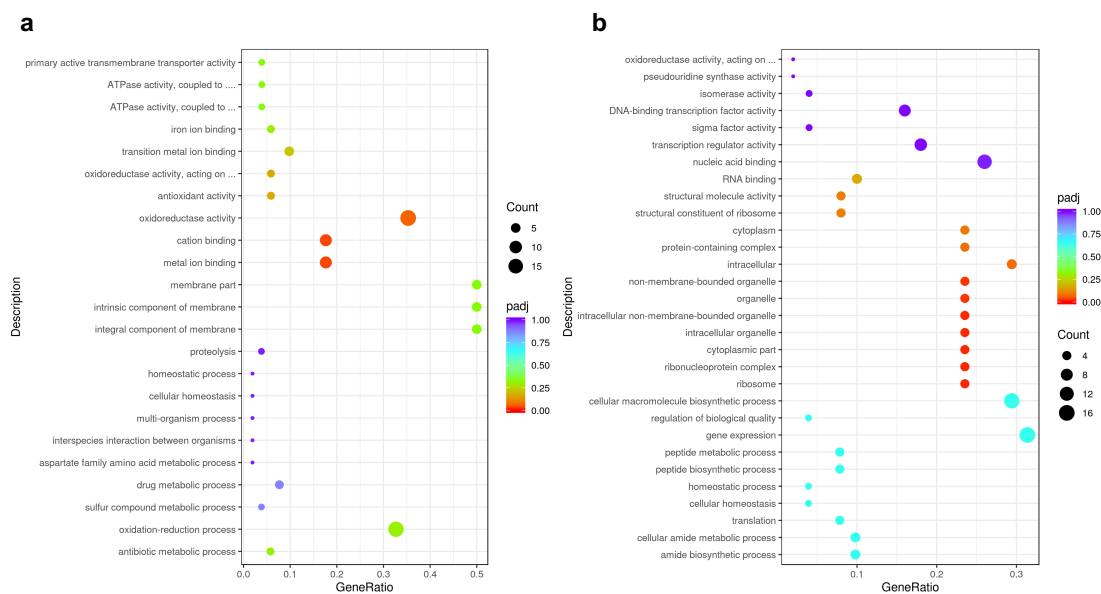


Fig. S5 The mechanism of anti-QS and antibiofilm activity of psoralen. Significantly downregulated (a) and upregulated (b) GO terms of psoralen-treated PAO1.

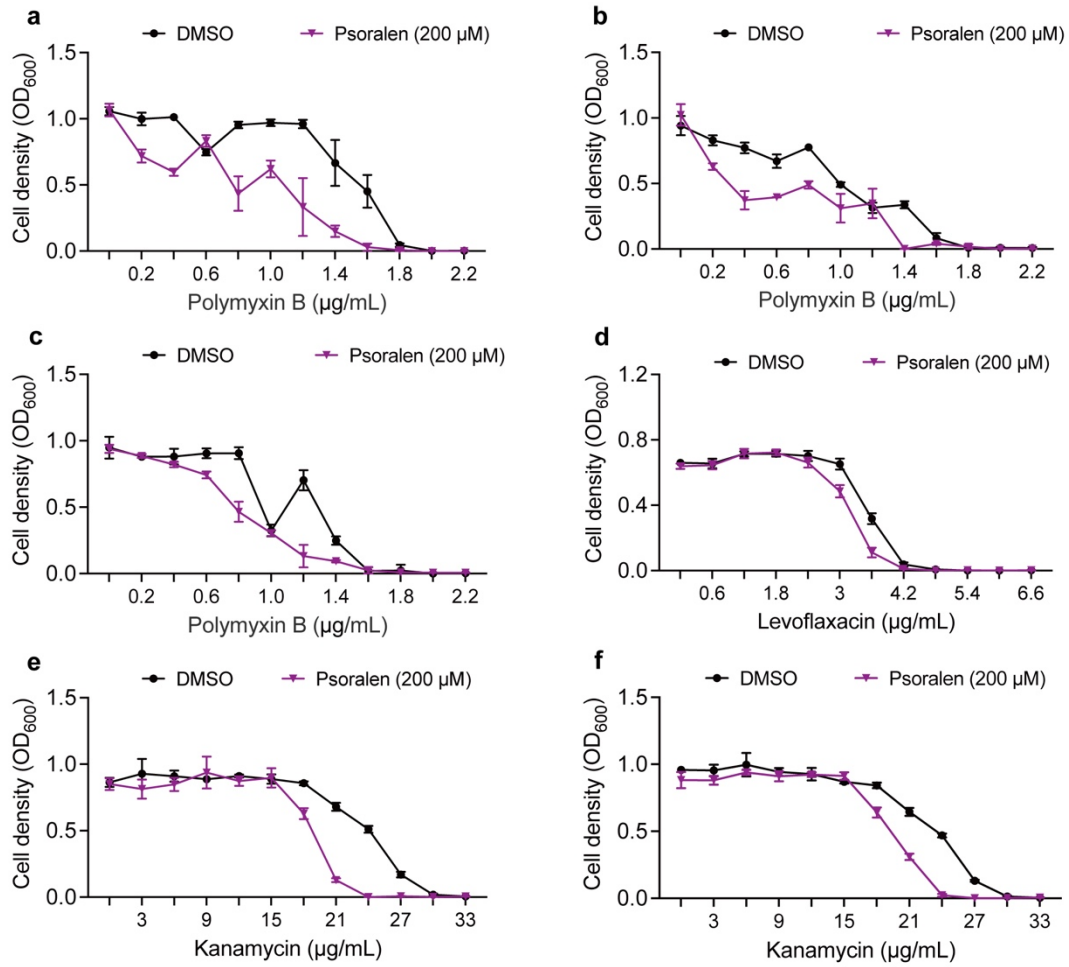


Fig. S6 Effects on growth of clinical isolates combining antibiotics with psoralen. **a** Clinical isolate 3-100-1. **b** Clinical isolate 7-61-28. **c** Clinical isolate 8-61-4B. **d** Clinical isolate 1-65-1. **e** Clinical isolate 3-61-7. **f** Clinical isolate 4-61-8. The results were presented as mean $\pm$ SD, and three independent experiments.

Supplementary Tables

Table S1. Docking scores and binding sites of receptors with ligands

Receptors	Ligands	Docking scores (kcal/mol)	H-bonds	Van der waals	π -stacking	Slat bridges
LasR	3-oxo-C12-HSL	-7.80	Trp-60, Thr-75, Thr-115, Ser-129	Leu-36 , Tyr-47, Ala-50, Tyr-64 , Ala-70, Val-76, Trp-88	/	/
LasR	Psoralen	-8.02	/	Leu-36 , Tyr-56, Trp-60, Tyr-64 , Trp-88, Phe-101, Leu-110	Tyr-56	Arg-61
RhlR	C4-HSL	-5.46	Asp-81, Ser-135	Ala-83 , Trp-96, Phe-101 , Val-133	/	/
RhlR	Psoralen	-6.15	/	Trp-68, Tyr-72, Ala-83 , Phe-101	Tyr-64, Tyr-72, Trp-96	/
PqsR	PQS	-7.62	Ile-236, Ala-237	Ala-102 , Ala-130, Ile-149 , Thr-166 , Ala-168, Leu-197, Leu-208, Phe-221 , Ile-236, Pro-238 , Thr-265	/	/
PqsR	Psoralen	-7.09	Ala-168, Thr-265	Ala-102 , Ile-149 , Thr-166 , Phe-221 , Pro-238	/	/

The **bold** indicated the same binding sites.

Table S2. Primers used in this study

Gene	Primer direction	Sequence (5'-3')	Source
<i>16S</i> RNA	Forward	TCGCATCCTGTTGTCCTCCA	(Yuan et al. 2022)
<i>16S</i> RNA	Reverse	TTAGCCAGGGTCAGCGTCA	
<i>lasR</i>	Forward	CTTCATCGTCGGCAACTAC	(Yuan et al. 2022)
<i>lasR</i>	Reverse	GTCTGGTAGATGGACGGTTC	
<i>lasB</i>	Forward	ATCGGCTACGACATCAAGAAGG	(Yuan et al. 2022)
<i>lasB</i>	Reverse	CCGCTGTTGTAGTTGCTGGTG	
<i>rhlR</i>	Forward	GCTCCTCGGAAATGGTGGT	(Yuan et al. 2022)
<i>rhlR</i>	Reverse	GGAAAGCACGCTGAGCAAAT	
<i>rhlA</i>	Forward	ACTGAACCAGGCGATGCTC	(Yuan et al. 2022)
<i>rhlA</i>	Reverse	GCTCCAGGCAAGCCAAGTA	
<i>pqsR</i>	Forward	CACTGGTTGAAGCGGGAGA	(Yuan et al. 2022)
<i>pqsR</i>	Reverse	TCGTTCTGCGATACGGTGAG	
<i>pqsA</i>	Forward	GCTGAGCGGTCCTTTGGC	(Yuan et al. 2022)
<i>pqsA</i>	Reverse	TGGAACCCGAGGTGTATTGC	
<i>pslA</i>	Forward	TGGGTCTTCAAGTTCCGCTC	This study
<i>pslA</i>	Reverse	ATGCTGGTCTTGCGGATGAA	
<i>pilV</i>	Forward	ACGACGTCAAGGACCAGATG	This study
<i>pilV</i>	Reverse	AGCTGCGGCAGATGTAGTAG	
<i>hcnA</i>	Forward	GCAGACATGACCATCCACCTC	(Yuan et al. 2022)
<i>hcnA</i>	Reverse	CGGTTGCTTTTCGGTTTCCA	
<i>phzA1</i>	Forward	GCAACTGGACCACGGAAAG	(Yuan et al. 2022)
<i>phzA1</i>	Reverse	GCACGCAGTTTCTGTATCGG	

Yuan Y, Yang X, Zeng Q, Li H, Fu R, Du L, Liu W, Zhang Y, Zhou X, Chu Y, Zhang X, Zhao K (2022)

Repurposing Dimetridazole and Ribavirin to disarm *Pseudomonas aeruginosa* virulence by targeting the quorum sensing system. Front Microbiol 13:1–15.

<https://doi.org/10.3389/fmicb.2022.978502>

Supplementary Information

Table S3. The MICs of *P. aeruginosa* PAO1 and clinical isolates to commonly used antibiotics (µg/mL).

<i>P. aeruginosa</i> isolates	Meropenem	Aztreonam	Kanamycin	Polymyxin B	Levofloxacin	Piperacillin
PAO1	3	4	16	2	0.4	4
1-65-1	8	16	16	1	5	20
3-61-7	8	16	64	1.5	0.7	96
3-100-1	16	1	16	2.5	2.4	4
4-61-8	8	16	64	1	0.4	96
5-61-8	8	32	64	1.5	0.5	96
5-R5-3A	16	1	32	1	0.5	8
7-61-28	8	8	32	4	0.2	4
8-61-4B	16	16	64	1	0.8	80

Supplementary Dataset

Dataset S1. Significantly differentially expressed genes of psoralen-treated *Pseudomonas aeruginosa* PAO1 at the concentration of 200 μ M. $p_{adj} < 0.05$.