Identification of FDA-approved antivirulence drugs targeting the *Pseudomonas aeruginosa* quorum sensing effector protein PqsE

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SUPPLEMENTAL MATERIAL

Table S1. Strains used in this study

Strain	Description	References
PAO1	wild type strain.	ATCC15692
PAO1 Δ*pqsE*	PAO1 derivative carrying an in-frame deletion of the *pqsE* gene.	[36]
PAO1 PqsE-Rep (*pqsE*IND P*pqsA*::*lux*)	PAO1 derivative in which *pqsE* expression is IPTG inducible and containing the P*pqsA*::*luxCDABE* transcriptional fusion integrated into the chromosome at the *attB* neutral site; TCR.	[36]
PAO1 P*pqsA::lux*	PAO1 derivative containing the P*pqsA*::*luxCDABE* transcriptional fusion integrated into the chromosome at the *attB* neutral site; TCR.	[98]
PAO1 Δ*pqsE* P*pqsA*::*lux*	PAO1 Δ*pqsE* derivative containing the P*pqsA*::*luxCDABE* transcriptional fusion integrated into the chromosome at the *attB* neutral site; TCR.	[36]
PAO1 mini-CTX-*lux*	PAO1 derivative containing the mini-CTX-*lux* empty vector integrated into the chromosome at the *attB* neutral site; TCR.	[98]
PAO1 Δ*pqsE* mini-CTX-*lux*	PAO1 Δ*pqsE* derivative containing the mini-CTX-*lux* empty vector integrated into the chromosome at the *attB* neutral site; TCR.	[36]

Table S2. Plasmids used in this study

Plasmid	Relevant characteristics	References
pUCP18	pUC18-derivative containing a stabilising fragment for maintenance in *Pseudomonas* spp.; Ap^R, *E. coli*/Cb^R, *P. aeruginosa*.	[64]
pUCP-*pqsE*	pUCP18 derivative for *pqsE* complementation; Ap^R, *E. coli*/Cb^R, *P. aeruginosa*.	[36]
pMRP9-1	pUC18 derivative allowing constitutive expression of the *Aequorea victoria* GFP protein; Cb^R.	[59]
mini-CTX-*lux*	Promoter-probe vector containing the *luxCDABE* operon as reporter system; Tc^R.	[99]
mini-CTX-P*pqsA*::*lux*	mini-CTX-*lux* derivative used for the insertion of the P*pqsA*::*luxCDABE* transcriptional fusion into PAO1 chromosome; Tc^R.	[80]

References not included in the main text

[98] Fletcher MP, Diggle SP, Crusz SA, et al. A dual biosensor for 2-alkyl-4-quinolone quorum-sensing signal molecules. Environ Microbiol. 2007;9:2683-2693.

[99] Becher A, Schweizer HP. Integration-proficient *Pseudomonas aeruginosa* vectors for isolation of single-copy chromosomal *lacZ* and *lux* gene fusions. Biotechniques. 2000;29:948-950.

Table S3. MIC of selected antibiotics

	Ciprofloxacin		Colistin		Tobramycin		Piperacillin	
Strain	**MHB**	**M9**	**MHB**	**M9**	**M**	**M9**	**MH**	**M9**
P. aeruginosa PAO1	0.125	0.03125	2	4	0.5	0.5	8	2
P. aeruginosa Δ*pqsE*	0.125	0.03125	2	4	0.5	0.25	8	2

Figure S1. Set up of the PqsE-Rep biosensor system

(A) Activity of the P*pqsA* promoter in the PqsE-Rep strain grown in LB supplemented with the indicated concentrations of IPTG, after 3 h (white bars), 5 h (light-grey bars) and 7 h (dark-grey bars) of incubation at 37°C. **(B)** Activity of the P*pqsA* promoter in the PqsE-Rep strain inoculated at starting optical density (OD_{600}) of 0.08 (white bars), 0.03 (light-grey bars) and 0.01 (dark-grey bars), after 5 h of incubation at 37°C in LB supplemented with the indicated concentrations of IPTG. **(C)** Activity of the P*pqsA* promoter in the PqsE-Rep strain inoculated at a starting OD_{600} of 0.08 after 5 h of incubation in LB (white bars) or in LB supplemented with 50 µM IPTG (grey bars) at 30°C or 37°C, in static or shaking (120 rpm) conditions. For **(A)-(C)**, biosensor activity is reported as relative light units (RLU) normalized to cell density (OD_{600}); the average of three independent experiments is reported with SD.

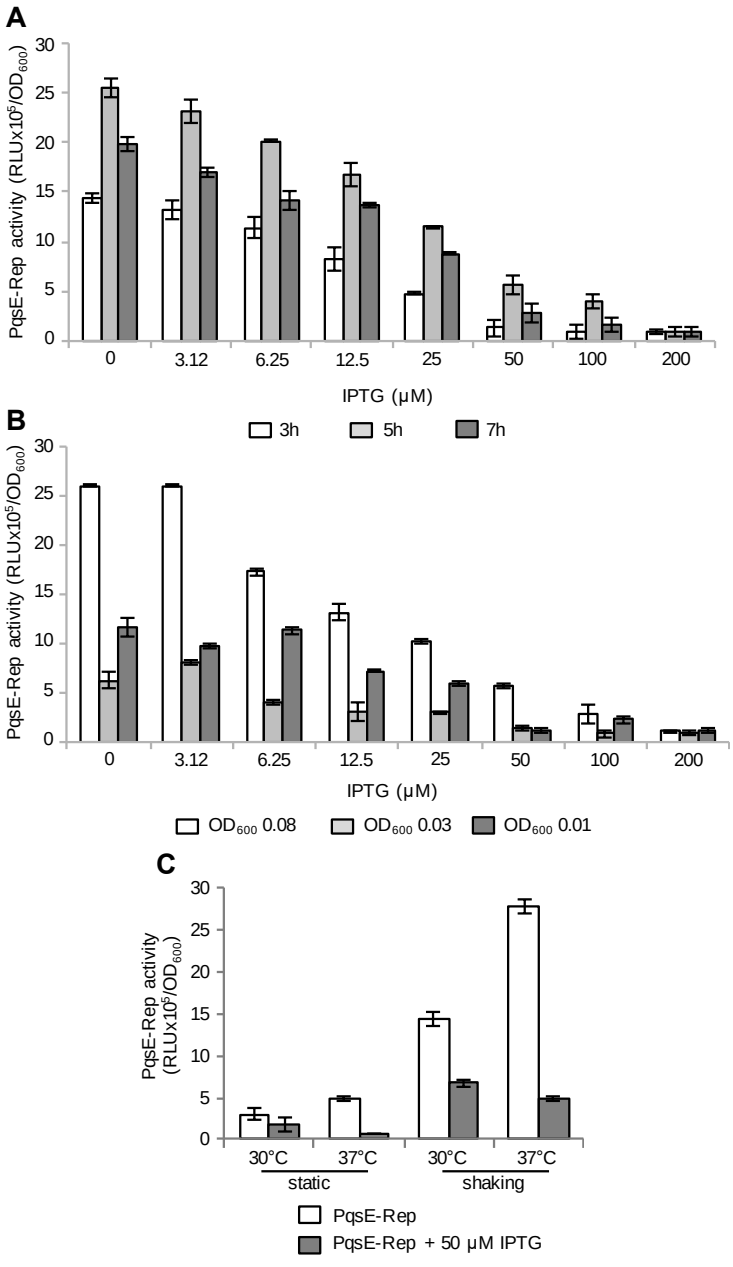

Figure S2. Primary and secondary screens of the PHARMAKON library

(A) Activity of the P*pqsA* promoter (bars) and cell density (diamonds) measured in the PqsE-Rep strain after 5 h incubation at 37°C in shaking conditions in LB supplemented with 50 µM IPTG and with the molecules of the PHARMAKON library, indicated with codes from inhibitor 1 (I-1) to inhibitor 24 (I-24), at 20 µM (white bars and diamonds) or 200 µM (grey bars and diamonds) concentration. PqsE-Rep activity and cell density measured in the presence of 0.2% (v/v) and 2% (v/v) DMSO were considered as 100%, respectively. **(B)** Pyocyanin production measured in supernatants of the PqsE-Rep biosensor strain supplemented with 50 µM IPTG and treated with the PHARMAKON library compounds nitrofurazone (I-2), erythromycin estolate (I-3) and diminazene aceturate (I-8) at 20 µM (white bars) and 200 µM (grey bars) concentration.

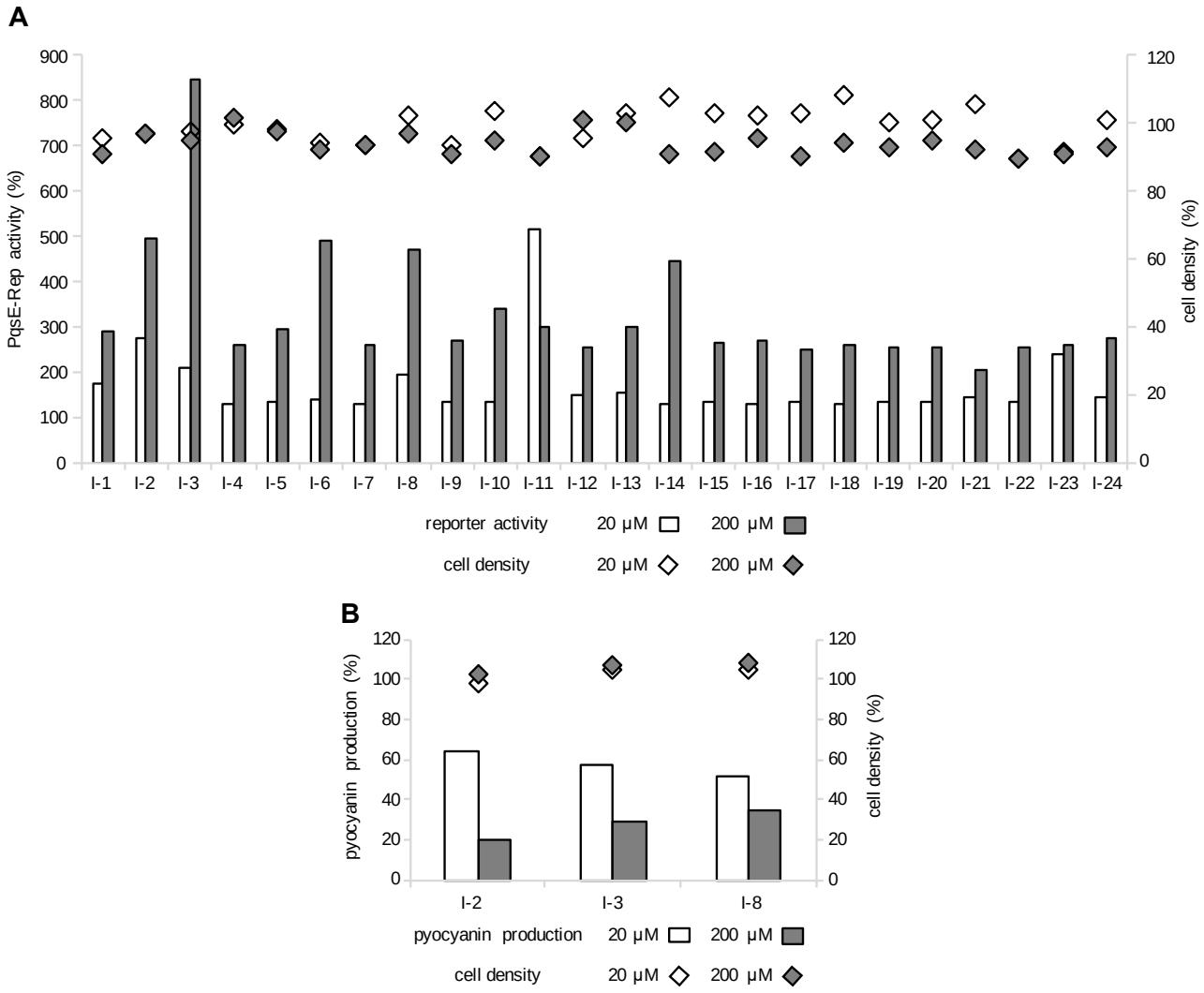

Figure S3. Growth curves of *P. aeruginosa* in the presence of PqsE inhibitors

Growth curves of *P. aeruginosa* PAO1 and its isogenic Δ*pqsE* mutant incubated at 37°C in shaking conditions in LB supplemented with: **(A)** 100 µM nitrofurazone (PAO1, blue; PAO1 Δ*pqsE*, black) or 0.125% (v/v) DMSO (PAO1, red; PAO1 Δ*pqsE*, green); **(B)** 50 µM erythromycin estolate (PAO1, blue; PAO1 Δ*pqsE*, black), or 0.025% (v/v) EtOH (PAO1, red; PAO1 Δ*pqsE*, green). The average of three independent experiments is reported with SD.

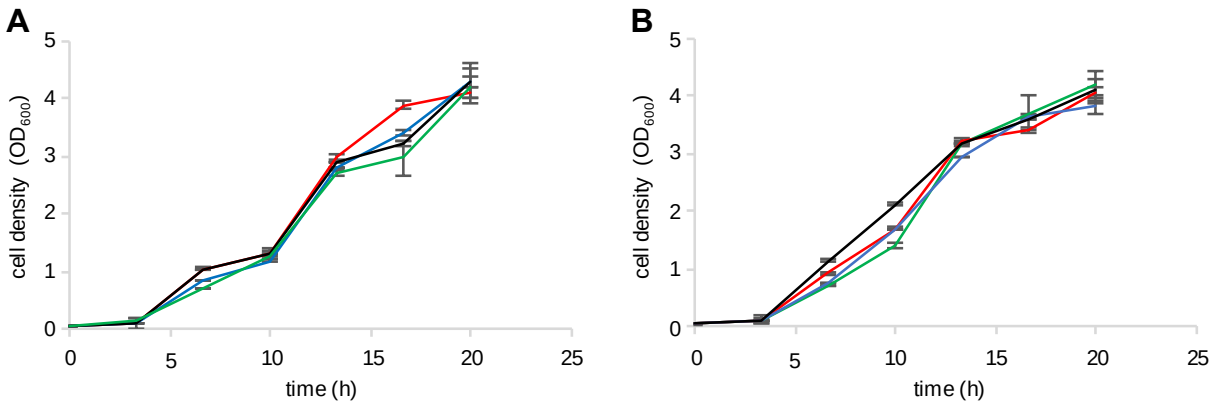

Figure S4. Effect of the PqsE inhibitors on constitutive bioluminescence

Percentage of light emitted by the indicated *P. aeruginosa* PAO1 strains carrying the mini-CTX-*lux* empty vector. The strains were grown at 37°C in shaking conditions in LB supplements with 100 µM nitrofurazone **(A)** or 50 µM erythromycin estolate **(B)**. Bioluminescence emitted by the same strains grown in the presence of 0.125% (v/v) DMSO or 0.025% (v/v) EtOH was considered as 100%. The average of three independent experiments is reported with SD.

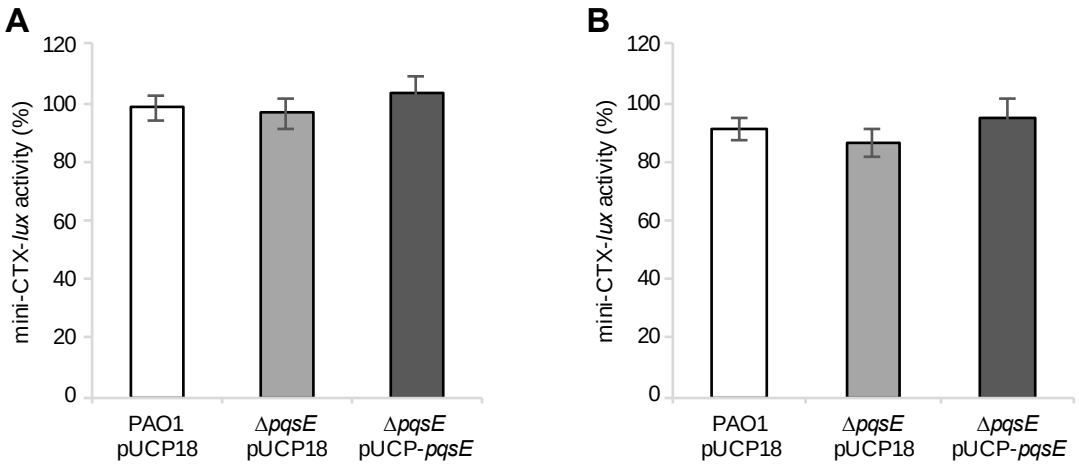

Figure S5. Effect of PqsE inhibitors on *P. aeruginosa* tolerance to tobramycin

Fraction of *P. aeruginosa* PAO1 cells tolerant to 4 µg/mL tobramycin (8x MIC) untreated (white bar) or after the treatment with 100 µM nitrofurazone (light-grey bar) or 50 µM erythromycin estolate (dark-grey bar). The untreated PAO1 Δ*pqsE* strain was used as control (black bar). The tolerant fraction expressed as N-fold change was determined as the ratio between the CFU/mL values measured after antibiotic addition (24 h post-antibiotic) divided by CFU/mL values measured before antibiotic addition. The average of three independent experiments is reported with SD. Similar results were obtained 16 h post-antibiotic treatment.