The polyaminoisoprenyl potentiator NV716 revives old disused antibiotics against intracellular forms of infection by *Pseudomonas aeruginosa*

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Supplementary Materials

Table S1. Relevant properties of the antibiotics used in this study

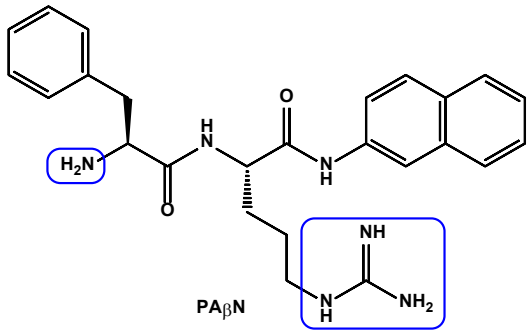
Antibiotics	Efflux pumps shown to increase the antibiotic MIC in *Pseudomonas aeruginosa* (1-3)	Apparent cellular accumulation in eukaryotic cells (at equilibrium) (4)[a]	Human serum C_{min} (mg/L) (5, 6)	Human serum C_{max} (mg/L) (6, 7)
Ciprofloxacin	MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexXY-OprM	5	0.05	5
Doxycycline	MexAB-OprM, MexCD-OprJ, MexXY-OprM	2-10	1	10
Chloramphenicol	MexAB-OprM, MexCD-OprJ, MexEF-OprN	2-5	5	20
Rifampin	Poor substrate for these four efflux pumps	1-4	0.5	20

[a] apparent accumulation factor (C_c/C_E: ratio between (C_C) cellular concentration and (C_E) extracellular concentration).

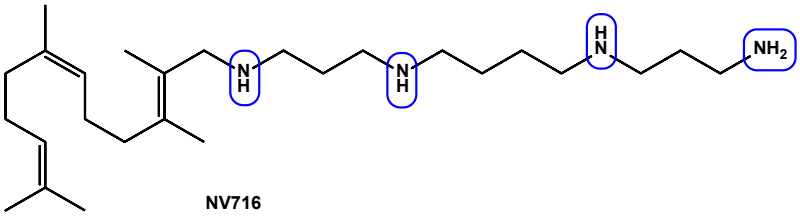
Table S2. IC_{50} values from cytotoxicity tests, for antibiotics alone or combined with potentiators, and expressed in different units (x MIC, mg/L, µM)

Antibiotic	units	THP-1 non-infected				THP-1 infected by PAO1			
		AB alone	+PAβN (20mg/L)	+731 (10µM)	+716 (10µM)	AB alone	+PAβN (20mg/L)	+731 (10µM)	+716 (10µM)
RIF	x MIC	24.6±1.1	27.3±3.3	23.1±2.6	22.0±0.9	27.8±1.9	26.6±0.2	31.9±2.9	28.7±3.4
	mg/L	393.0±18.3	436.1±52.6	369.3±41.5	351.6±14.2	445.5±30.2	426.2±2.8	510.0±47.1	459.0±55.1
	µM	477.5±22.3	529.9±63.9	448.8±50.5	427.2±17.3	541.4±36.7	517.9±3.4	619.7±57.3	557.8±66.9
DOX	x MIC	41.9±2.8	41.6±3.7	38.5±8.7	32.1±2.5	56.7±17.7	53.8±17.9	51.2±16.4	46.0±4.2
	mg/L	334.8±22.6	333.0±29.3	307.9±69.3	256.7±19.9	453.7±141.7	430.5±143.4	409.8±131.0	368.0±34.0
	µM	753.4±50.8	749.3±65.9	692.9±155.9	577.6±44.9	1,020.8±318.8	968.6±322.6	922.2±294.8	828.0±76.4
CHL	x MIC	22.5±0.8	21.0±0.8	20.1±0.5	19.5±4.7	22.3±2.5	20.2±3.0	23.5±3.1	22.1±3.7
	mg/L	719.1±25.3	673.2±27.0	642.0±16.2	624.4±150.2	714.1±80.4	645.9±95.0	752.6±98.4	707.0±118.6
	µM	2,225.6±78.4	2,083.3±83.7	1,986.9±50.1	1,932.4±464.8	2,210.0±248.8	1,998.8±294.1	2,329.2±304.4	2,187.9±366.9

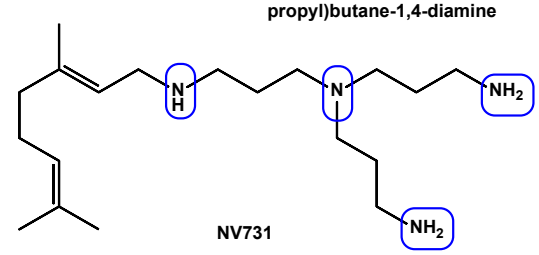
Figure S1: Chemical structures of the potentiators used in this study. The aminated functions that are partially protonated at physiological pH are evidenced by blue squares.



(*S*)-2-((*S*)-2-amino-3-phenylpropanamido)-5-guanidino-*N*-(naphthalen-2-yl)pentanamide



N^1-(3-aminopropyl)-*N*^{4}-(3-(((2*E*,6*Z*)-2,3,7,11-tetramethyldodeca-2,6,10-trien-1-yl)amino) propyl)butane-1,4-diamine



(*E*)-*N*$^{1'}$,*N*^{1}-bis(3-aminopropyl)-*N*^{3}-(3,7-dimethylocta-2,6-dien-1-yl)propane-1,3-diamine

Figure S2. Cytotoxicity of antibiotics alone or combined with potentiators, as assessed by the trypan blue exclusion test. The graphs show the IC_{50} for three antibiotics (doxycycline [DOX], chloramphenicol [CHL] or rifampin [RIF] alone or combined potentiators at the indicated concentrations, calculated based on the Hill equation of concentration-response curves experiments similar to those illustrated in Figure 1, in non-infected THP-1 cells (left) or infected THP-1 cells (right). These IC_{50} values are higher than 400 mg/L in all cases, i.e. similar to the highest concentration tested in our model. The same type of experiment was performed with ciprofloxacin, but 50% cytotoxicity was not reached over the range of concentrations investigated (up to 25 mg/L), so that the IC_{50} could not be calculated. The dotted horizontal dotted lines in the left panel show the IC_{50} of potentiators alone (blue: NV731 [IC_{50}: 354 µM or 115 mg/L]; red: NV716 [IC_{50}: 75 µM or 32 mg/L]). These values are above the concentration used in most experiments (10 µM). All data are shown as means ± SEM (triplicates from 3 experiments). Statistical analysis (1-way ANOVA; Tukey's Multiple Comparison Test): no significant difference was noticed when comparing the different conditions for each antibiotic ($p>0.05$). See further details in Table S2.

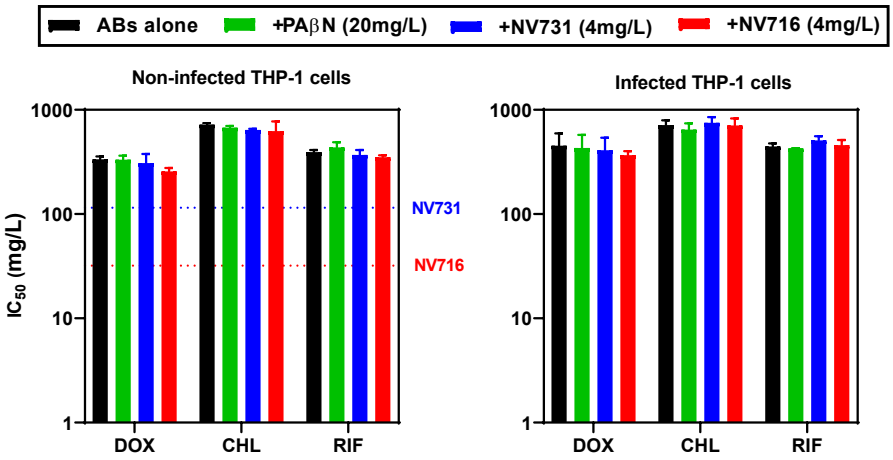
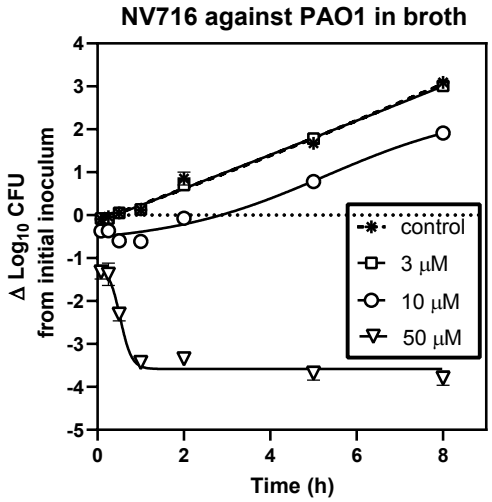


Figure S3. Kill curve of extracellular PAO1 by NV716 at increasing concentrations. The graph shows the changes in the Log_{10} CFU counts from the initial inoculum per mL of broth over 8 h of incubation with NV716 at the indicated concentrations (expressed in µM). All data are means ± SEM (n=3).



Corresponding method: PAO1 was incubated on TSA overnight. A single colony was inoculated in 10 mL MHB-CA and incubated overnight at 37°C with gentle agitation (130 rpm). The density of the culture was adjusted at 0.5 McF (around 10^8 CFU/mL) in PBS, and diluted 100 times in MHB-CA to obtain a starting inoculum of 10^6 CFU/mL. Concentration-kill curves were determined over time with NV716 concentrations ranging from 3 to 50 µM. Fifty µL aliquots were diluted and plated on agar containing 2g/L charcoal to adsorb the residual antibiotic.

References

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