Supplementary data

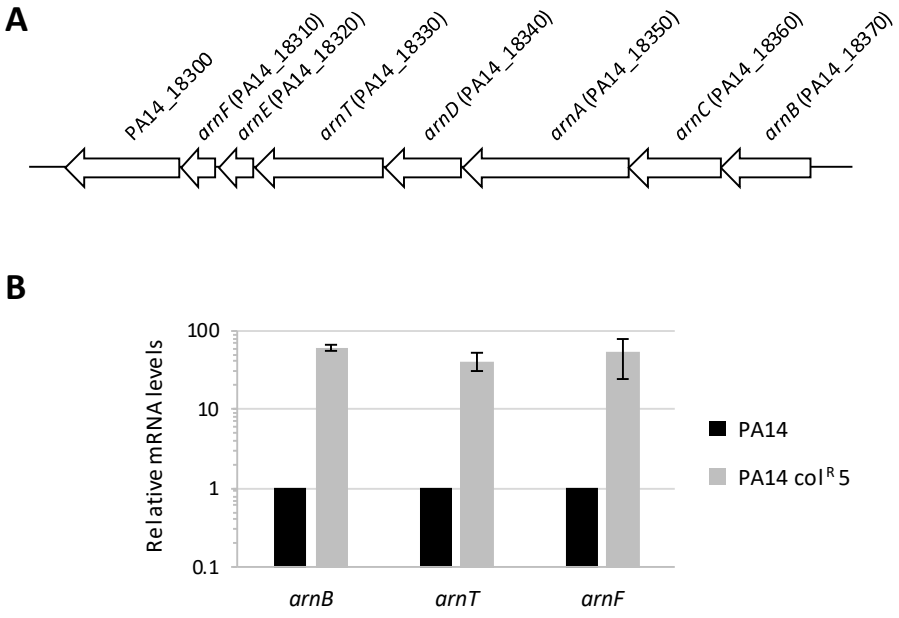


Figure S1. (A) Schematic representation of the *arn* operon in the *P. aeruginosa* PA14 strain (www.pseudomonas.com). (B) Relative mRNA levels of selected *arn* genes, determined by quantitative RT-PCR, in the colistin-resistant derivative PA14 col^R 5 with respect to its parental strain PA14. Data are the mean (±SD) of three assays.

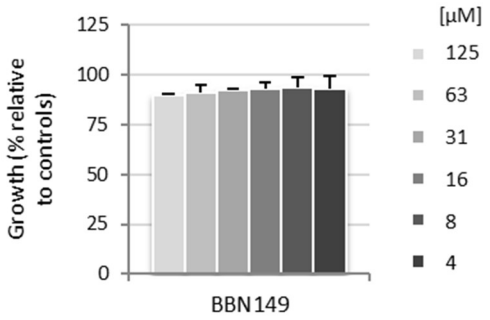


Figure S2. Dose-dependent effect of BBN149 on PA14 col^{R} 5 growth after 24 h at 37°C in MH (in the absence of colistin). Growth values are expressed as percentage relative to the cultures treated with equivalent concentrations of DMSO, and represent the mean (±SD) of three independent experiments.

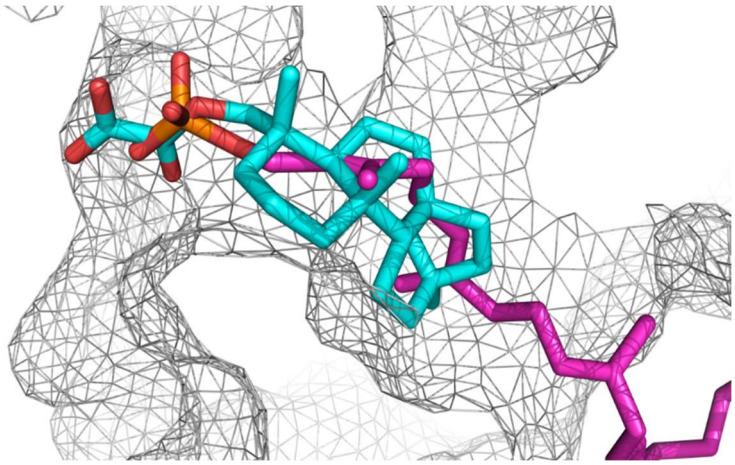


Figure S3. Overlapping between the binding mode of BBN149 (cyan sticks) and the crystallographic pose of undecaprenyl phosphate (magenta sticks) in the crystallographic structure coded by PDB ID 5F15 (light-grey mesh).

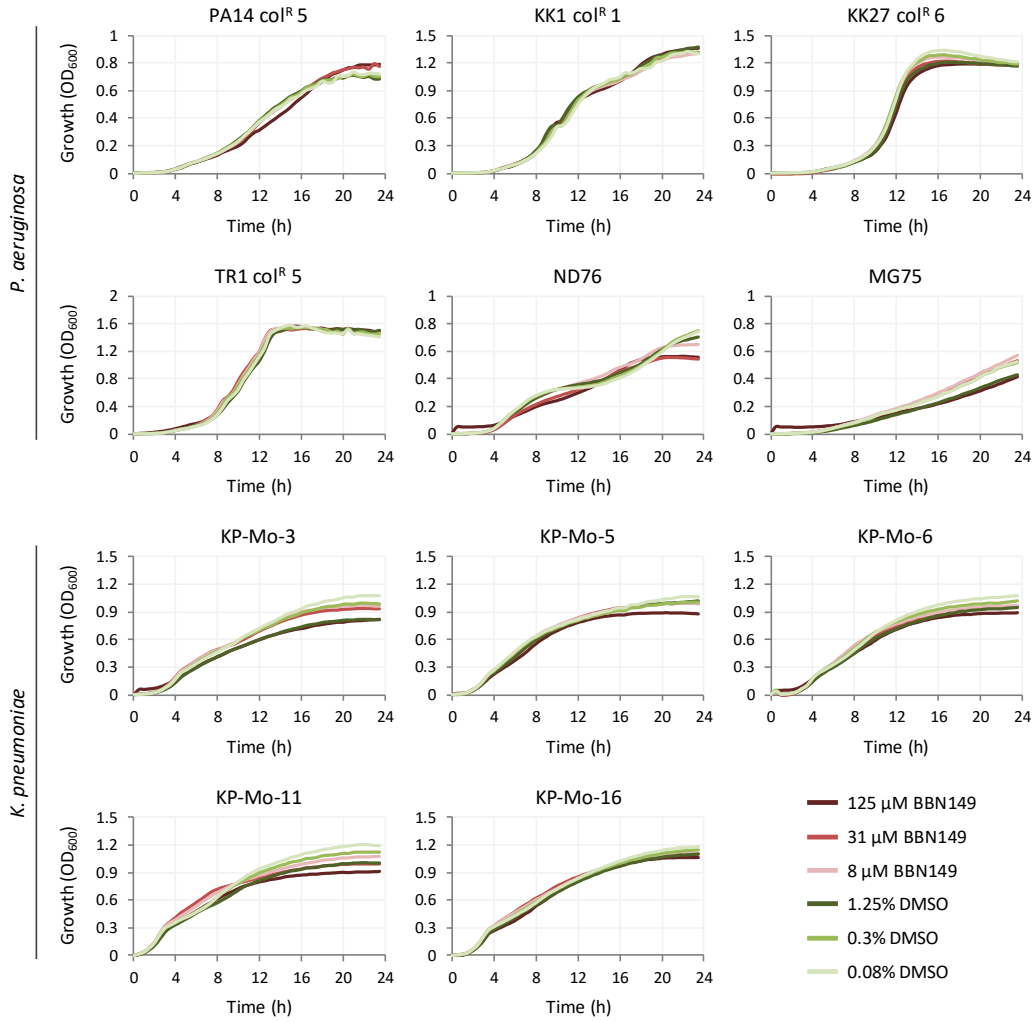


Figure S4. Growth curves of colistin-resistant *P. aeruginosa* and *K. pneumoniae* strains in MH at 37°C in the presence of different concentrations of BBN149 (8, 31 or 125 µM) or equivalent concentrations of DMSO (0.08, 0.3 or 0.125%) as control. Data are the mean of two independent assays, each performed in triplicate.

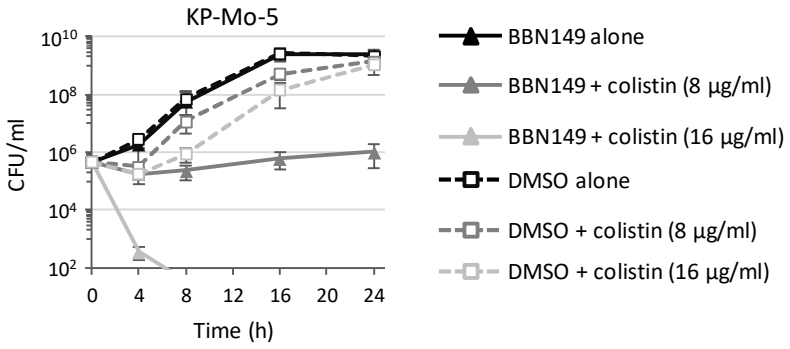


Figure S5. Time-killing curves of *K. pneumoniae* KP-Mo-5 exposed to 30 µM BBN149 in the presence or absence of colistin at 8 or 16 mg/L, corresponding to 1× or 2×MIC, respectively (Table 1). As control, the strains were incubated in the presence of 0.3% DMSO and the same concentrations of colistin. The results are the mean (±SD) of two independent assays.

Table S1. Bacterial strains used in this study.

Species	Strain	Relevant features	Reference/source
P. aeruginosa	PA14	Reference clinical strain	Rahme *et al.*, 1995
	PA14 col^R 5	*In vitro* evolved colistin-resistant PA14 derivative	20
	KK1	Cystic fibrosis isolate	Bragonzi *et al.*, 2009
	KK1 col^R 1	*In vitro* evolved colistin-resistant KK1 derivative	20
	KK27	Cystic fibrosis isolate	Bragonzi *et al.*, 2009
	KK27 col^R 6	*In vitro* evolved colistin-resistant KK27 derivative	20
	TR1	Cystic fibrosis isolate	Bragonzi *et al.*, 2009
	TR1 col^R 6	*In vitro* evolved colistin-resistant TR1 derivative	20
	ND76	Colistin-resistant cystic fibrosis isolate	Strain collection of the CF Center at the G. Gaslini Institute, Genoa (Italy)
	MG75	Colistin-resistant cystic fibrosis isolate	Strain collection of the CF Center at the G. Gaslini Institute, Genoa (Italy)
K. pneumoniae	KP-Mo-3	Colistin-resistant clinical isolate	39
	KP-Mo-5	Colistin-resistant clinical isolate	39
	KP-Mo-6	Colistin-resistant clinical isolate	39
	KP-Mo-11	Colistin-resistant clinical isolate	39
	KP-Mo-16	Colistin-resistant clinical isolate	39
	KP-Mo-26	Colistin-sensitive clinical isolate	39
	KP-Mo-27	Colistin-sensitive clinical isolate	39
A. baumannii	5615	Colistin-resistant clinical isolate	Morroni *et al.*, 2019
	12316	Colistin-resistant clinical isolate	Morroni *et al.*, 2019
	12384	Colistin-resistant clinical isolate	Morroni *et al.*, 2019
E. coli	4451	Colistin-resistant clinical isolate carrying the *mcr-1* gene	Cannatelli *et al*., 2016
	4531	Colistin-resistant clinical isolate carrying the *mcr-1* gene	Cannatelli *et al*., 2016
	4592	Colistin-resistant clinical isolate carrying the *mcr-1* gene	Cannatelli *et al*., 2016

References not included in the main text:

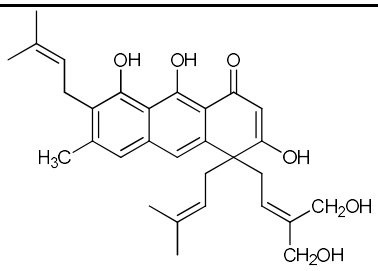
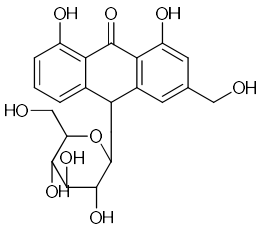
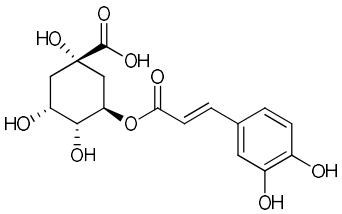
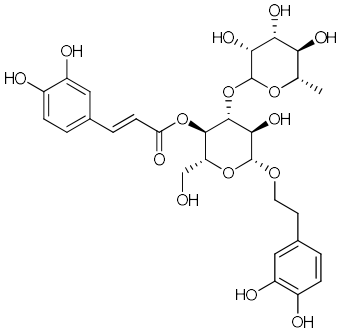
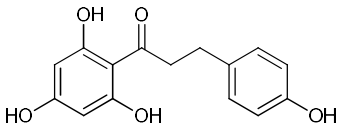
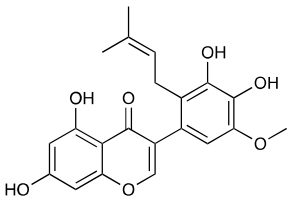
Bragonzi A, Paroni M, Nonis A, *et al*. *Pseudomonas aeruginosa* microevolution during cystic fibrosis lung infection establishes clones with adapted virulence. *Am J Respir Crit Care Med* 2009; **180**: 138-45.

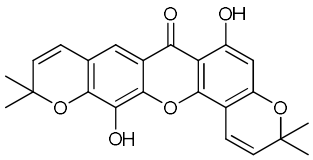
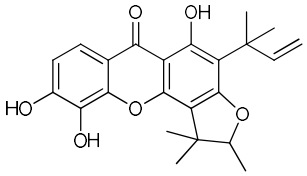
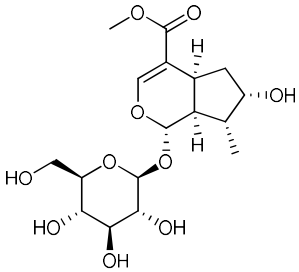
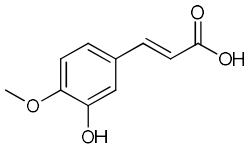
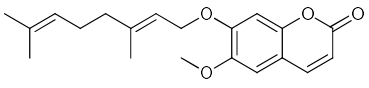
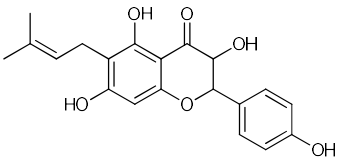
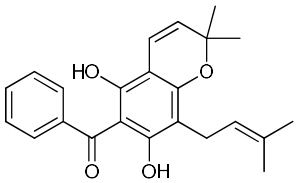
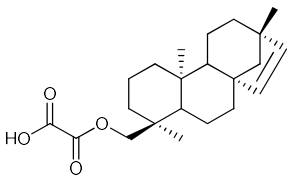
Rahme LG, Stevens EJ, Wolfort SF, *et al*. Common virulence factors for bacterial pathogenicity in plants and animals. *Science* 1995; **268**: 1899-902.

Morroni G, Simonetti O, Brenciani A, *et al.* In vitro activity of Protegrin-1, alone and in combination with clinically useful antibiotics, against *Acinetobacter baumannii* strains isolated from surgical wounds. *Med Microbiol Immunol* 2019; **208**: 877–83.

Cannatelli A, Giani T, Antonelli A, *et al*. First Detection of the *mcr-1* colistin resistance gene in *Escherichia coli* in Italy. *Antimicrob Agents Chemother* 2016; **60**: 3257–8.

Table S2. List of compounds selected and tested in this study.

Common name (library code)	Chemical structure	MW	Molecular formula	Reference
γ-γ'-OH Ferruginin A (BBN35)		492.6	$C_{30}H_{36}O_6$	Delle Monache *et al.*, 1979
Aloin (BBN36)		418.4	$C_{21}H_{22}O_9$	Peng *et al.*, 2019
Chlorogenic acid (BBN53)		354.3	$C_{16}H_{18}O_9$	Leitão *et al.*, 2008
Verbascoside (BBN79)		624.6	$C_{29}H_{36}O_{15}$	Scarpati & Delle Monache, 1963
Phloretin (BBN101)		274.3	$C_{15}H_{14}O_5$	Hu *et al.*, 2018
Piscidone (BBN118)		384.4	$C_{21}H_{20}O_7$	Tahara *et al.*, 1992

Rheediaxanthone A (BBN119)		392.4	$C_{23}H_{20}O_6$	Delle Monache *et al.*, 1981
Rheediaxanthone B (BBN120)		396.4	$C_{23}H_{24}O_6$	Delle Monache *et al.*, 1981
Loganin (BBN139)		390.4	$C_{17}H_{26}O_{10}$	Garaev *et al.*, 2014
3-hdroxy-4-methoxycinnamic acid (BBN145)		194.2	$C_{10}H_{10}O_4$	Gießel *et al.*, 2019
6-methoxy-7-O-geranyl-coumarin (BBN146)		328.4	$C_{20}H_{24}O_4$	Torres *et al.*, 1979
6-prenyl-aromadendrin (BBN147)		356.4	$C_{20}H_{20}O_6$	Harborne, 1993
Vismiaphenone B (BBN148)		364.4	$C_{23}H_{24}O_4$	Delle Monache *et al.*, 1980
ent-beyer-15-en-18-O-oxalate (BBN149)		360.5	$C_{22}H_{32}O_4$	24,25

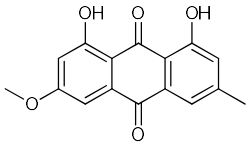
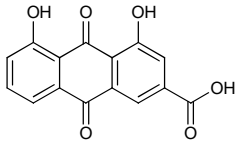
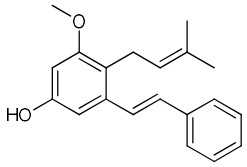
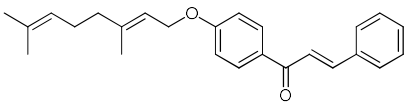
Physcion (BBN151)		284.3	$C_{16}H_{12}O_5$	Camele *et al.*, 1982
Rhein (BBN152)		284.2	$C_{15}H_8O_6$	Lee *et al.*, 2002
Longistilin C (BBN153)		294.4	$C_{20}H_{22}O_2$	Delle Monache, 1979
Chalcon 19 4'-O-geranyl-chalcon (BBN154)		360.5	$C_{25}H_{28}O_2$	Guglielmi *et al.*, 2019

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Table S3. Primers used in this study.

Primer name	Sequence (5'→3')	Application
*arnB*_RT_FW	TCCTTCCACGCGATCAAG	qRT-PCR
*arnB*_RT_RV	GTTGTATTTGAAACCGGGCTC	
*arnT*_RT_FW	GCTACTGGATGATCGCCCTC	
*arnT*_RT_RV	AAGCCGAAGCTGGCGTAG	
*arnF*_RT_FW	GCTCGCGGTAGCCCTG	
*arnF*_RT_RV	CCAGGGTCTTGCTGGTACTG	
*rpoD*_RT_FW	GGGCGAAGAAGGAAATGGTC	
*rpoD*_RT_RV	CAGGTGGCGTAGGTGGAGAA	

Table S4. MIC of ofloxacin, gentamicin and meropenem for *P. aeruginosa* PA14 and the colistin-resistant derivative PA14 col^R 5 in the presence of 30 μM BBN149 or 0.3% DMSO as the control.

Strain	**MIC (mg/L)**					
	Ofloxacin		**Gentamicin**		**Meropenem**	
	BBN149	**DMSO**	**BBN149**	**DMSO**	**BBN149**	**DMSO**
PA14	0.25	0.25	0.5	0.25	0.25	0.5
PA14 col^R 5	0.5	0.5	4	4	1	1