Supporting Information

Controlling resistant bacteria with a novel class of β-lactamase inhibitor peptides: from rational design to *in vivo* analyses

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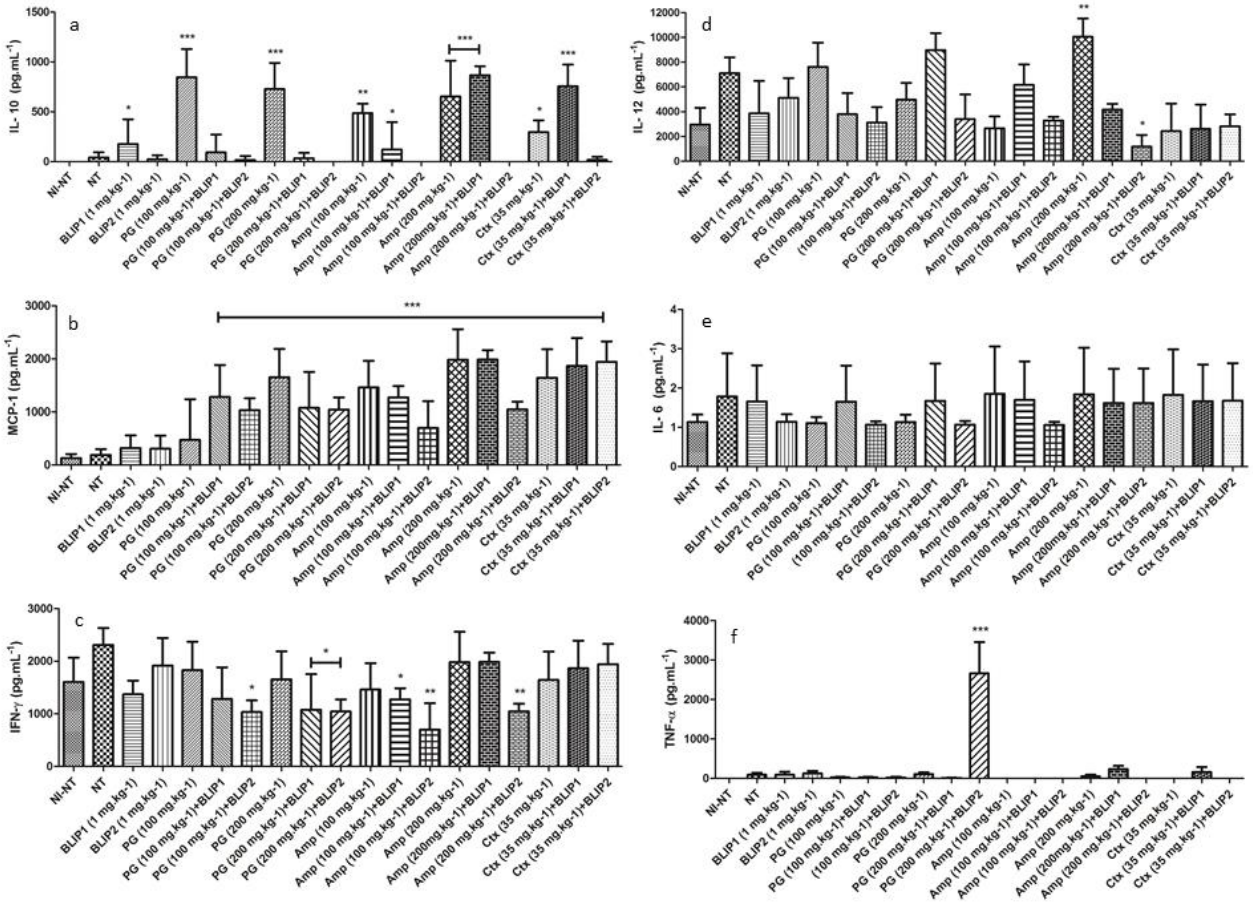
Supplementary Table 1. Data derived after fitting the raw heat associated data with nonlinear regression.

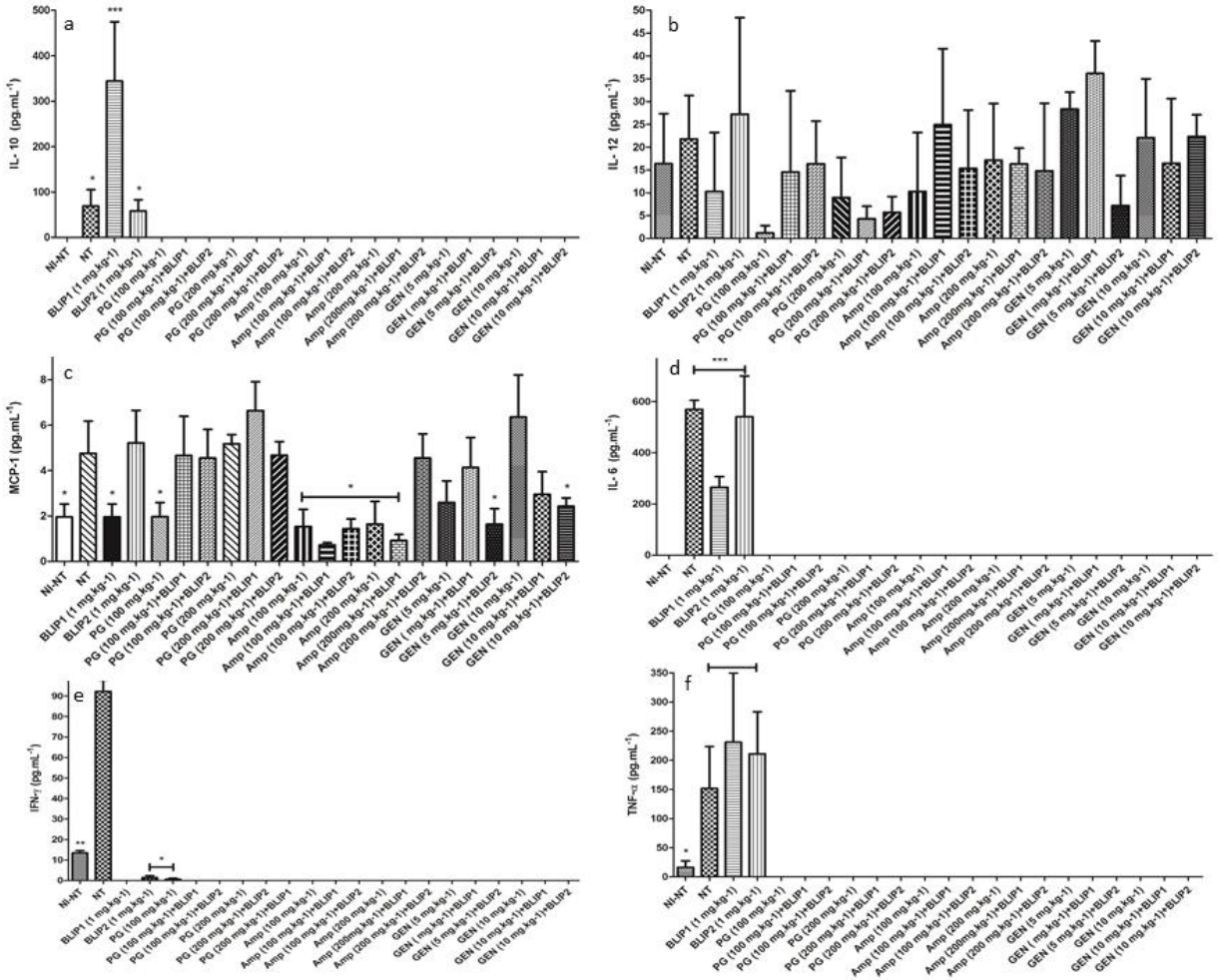
Parameters	dBLIP-1	dBLIP-2
K	6.33± 0.52E^{4} M^{-1}	4.5± 0.18E^{4} M^{-1}
ΔH	-12.9 ±0.56E^{4} cal.mol^{-1}	-35.7 ±0.82E^{3} cal.mol^{-1}
ΔS	-415 cal/mol/deg	-98.6 cal/mol/deg

S2

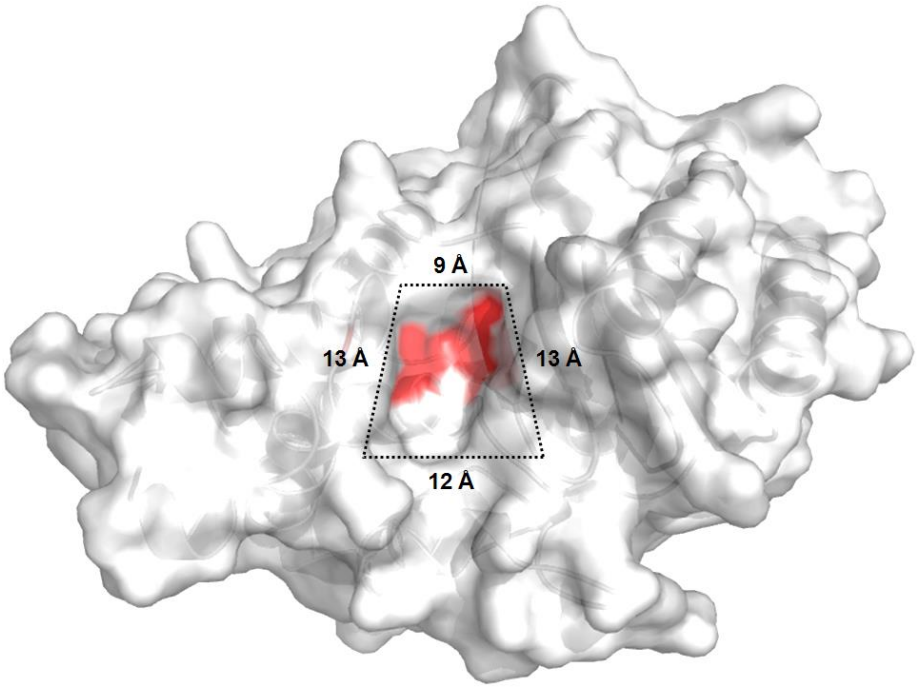


Supplementary Figure 1. dBLIP-1 and dBLIP-2 effects on immune response of mice in *in vivo* model under *Staphylococcus aureus* infection. Determination of IL-10 (A), MCP-1 (B), IFN-g (C), IL-12 (D), IL-6 (E) and TNF-α (F) in *S. aureus* groups treated with dBLIP 1 and 2 alone or in combination with (PG) penicillin, (AMP) ampicillin and (CFX) cefotaxime. Bars represent means and SEs from three to six independent experiments. Results are shown as mean ± SD from triplicate measurements. *p, 0.05; **p , 0.01;***p , 0.001; comparison by ANOVA with Tukey's post hoc test.

S3



Supplementary Figure 2. dBLIP-1 and dBLIP-2 effects on immune response of mice in *in vivo* model under *Escherichia coli* infection. Determination of IL-10 (A), MCP-1 (B), IFN-g (C), IL-12 (D), IL-6 (E) and TNF-α (F) in *E. coli* groups treated with dBLIP 1 and 2 alone or in combination with (PG) penicillin, (AMP) ampicillin and (GEN) gentamicine. Bars represent means and SEs from three to six independent experiments. Results are shown as mean ± SD from triplicate measurements. *p, 0.05; **p , 0.01;***p , 0.001; comparison by ANOVA with Tukey's post hoc test.



Supplementary Figure 3. Three-dimensional structure of *E. coli* β-lactamase firstly utilized for peptide designe. The trapezoid highlight (dotted lines) represents the catalytic pocket area (red region) and neighbour regions used for docking analysis.

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Supplementary Table 2. β-Lactamases present in different clinical isolates here studied. AmpC lactamases correspond to cephalosporinases. ESBLs correspond to extended-spectrum β-lactamases and MBL to metallo-β-lactamase.

Clinical isolates	**Beta-lactamases**	
ID No 2101123 *E. coli*	AmpC	ESBL
ID No 6881 *E. coli*	AmpC	MBL
ID No 1812446 *E. coli (blaKPC)*	AmpC	MBL
ID No 6817 *P. aeruginosa*	AmpC	ESBL
ID No 7314 *S. aureus*	AmpC	ESBL
ID No ATCC33591 *S. aureus*	Ampc	TEM-1 like β-lactamase
ID No 6591 *B. cereus*	AmpC	ESBL

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Supplementary Table 3. Peptides rationally designed evaluated against β-lactamases. Assays were *in vitro* performed toward β-lactamases from *E. coli* and *S. aureus* showing the presence (+)or absence of inhibition activity (-).

Peptides	Sequence	Activity
dBLIP-1	KKGEE	+
dBLIP-2	KQGQE	+
dBLIP-3	KNGNE	-
dBLIP-4	KNPNE	-
dBLIP-5	KQPQE	-
dBLIP-6	KGPGE	-
dBLIP-7	KGPAE	-