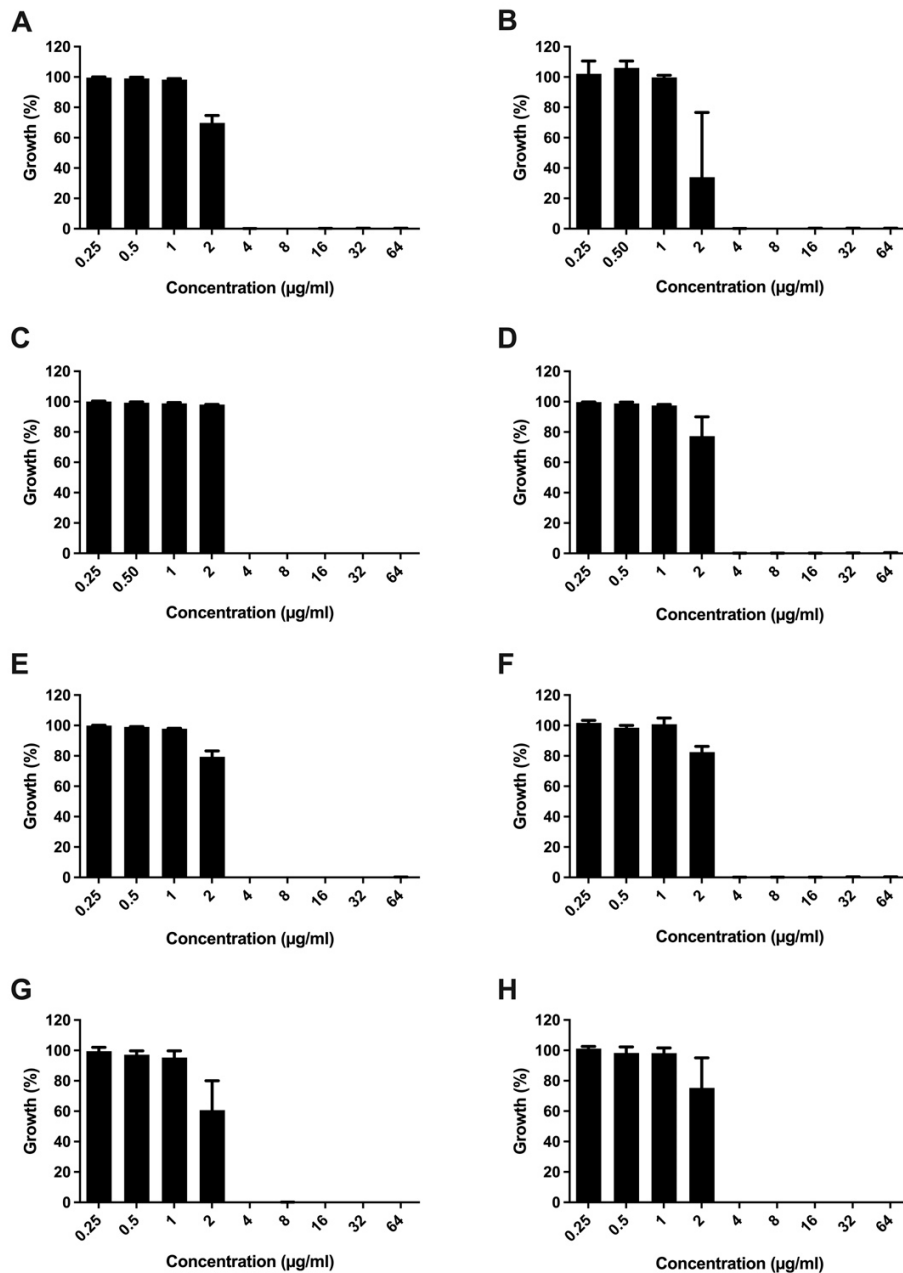
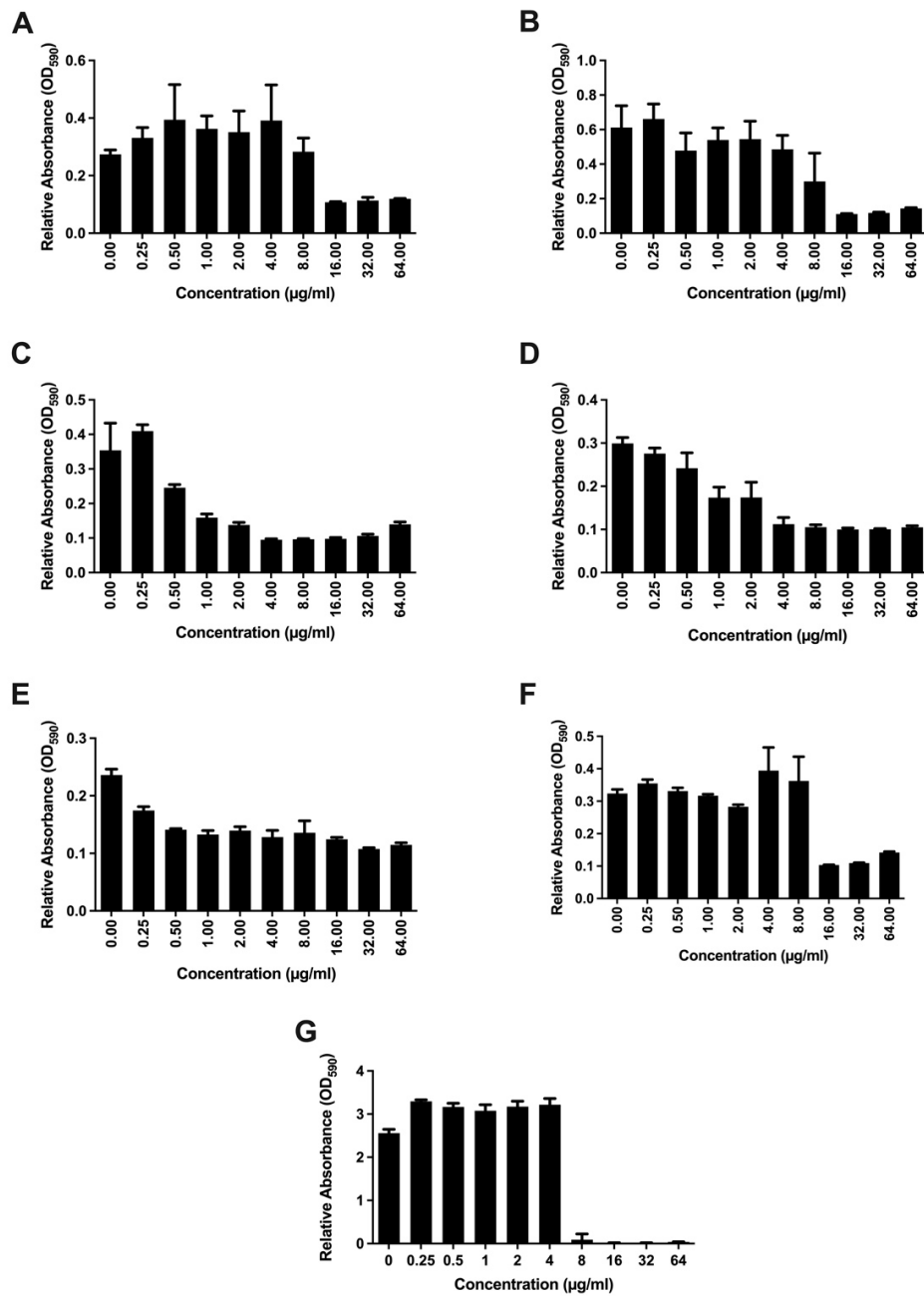


**Supplementary Materials:****Table S1.** List of MDR ESKAPE pathogens tested.

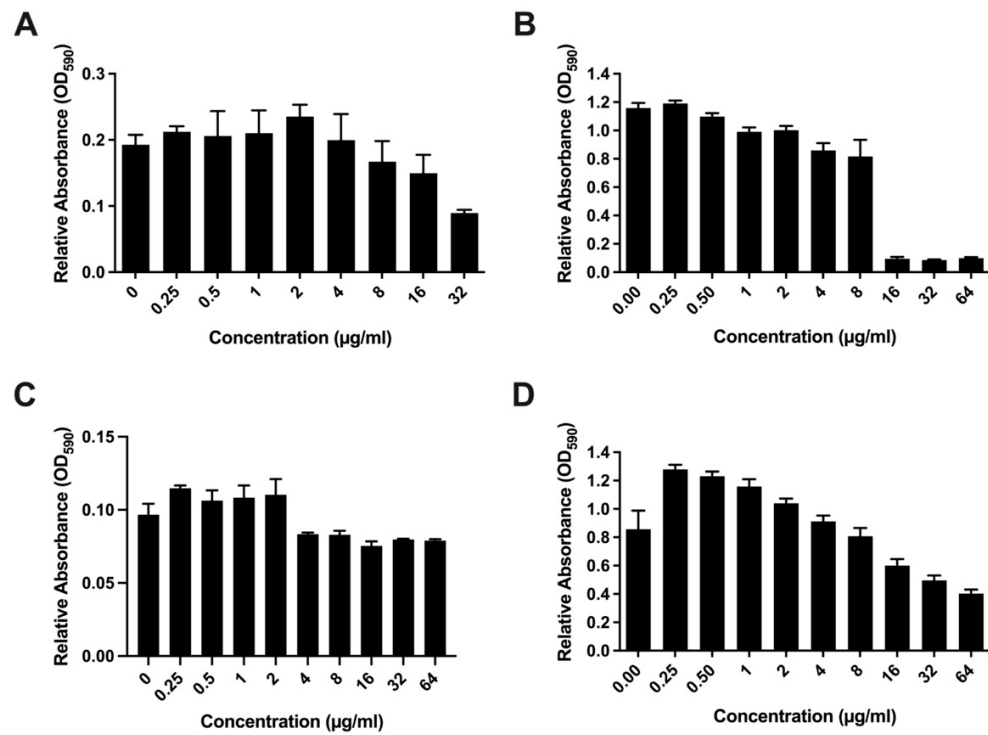
<b>Strain</b>	<b>Source</b>	<b>Source information</b>	<b>MDR</b>
<i>Enterococcus (E.) faecalis</i> 51299	ATCC	Human peritoneal fluid	VRE
<i>S. aureus</i> 33592	ATCC	Human blood	MRSA
<i>K. pneumoniae</i> BAA-1705	ATCC	Human urine	KPC
<i>P. aeruginosa</i> BAA-2110	ATCC	Human sputum	Yes
<i>Enterobacter (E.) asburiae</i> BAA-3043	ATCC	Human wound	Yes
<i>A. baumannii</i> AB5075 (MRSN 959)	BEI Resources	Human tibia/osteomyelitis	Yes
<i>A. baumannii</i> BAA-1710	ATCC	Human blood	Yes
<i>A. baumannii</i> BAA-1794	ATCC	Human sputum	Yes
<i>A. baumannii</i> BAA-1795	ATCC	Human nasotracheal aspirate	Yes
<i>A. baumannii</i> BAA-1797	ATCC	Human blood	Yes
<i>A. baumannii</i> BAA-1799	ATCC	Human blood	Yes
<i>A. baumannii</i> BAA-1605	ATCC	Human sputum	Yes
<i>A. baumannii</i> BAA-1800	ATCC	Human deep trachea	Yes



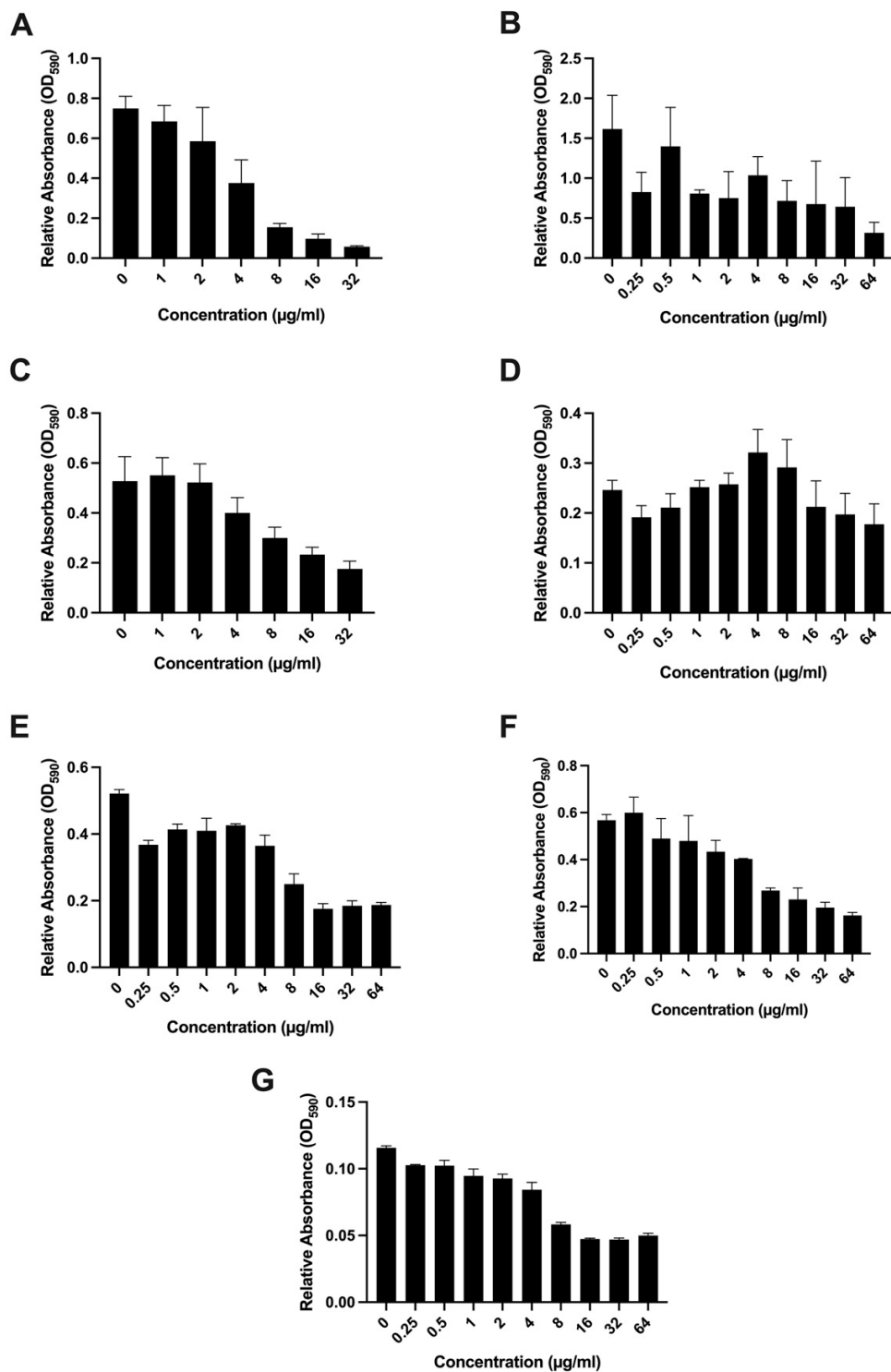
**Figure S1.** Minimum inhibitory concentration (MIC) of GATR-3 against *A. baumannii*: (A) AB5075, (B) BAA-1710, (C) BAA-1794, (D) BAA-1795, (E) BAA-1797, (F) BAA-1799, (G) BAA-1605 and (H) BAA-1800. Growth was measured spectrophotometrically (OD<sub>600</sub>) after 20 – 24 h incubation. The percentage of growth was calculated relative to the untreated bacteria control (100%).



**Figure S2.** Minimum biofilm inhibitory concentration (MBIC) of GATR-3 against *A. baumannii*: (A) AB5075, (B) BAA-1710, (C) BAA-1794, (D) BAA-1795, (E) BAA-1797, (F) BAA-1605 and (G) BAA-1800. Crystal violet stain was read via OD<sub>590</sub>.



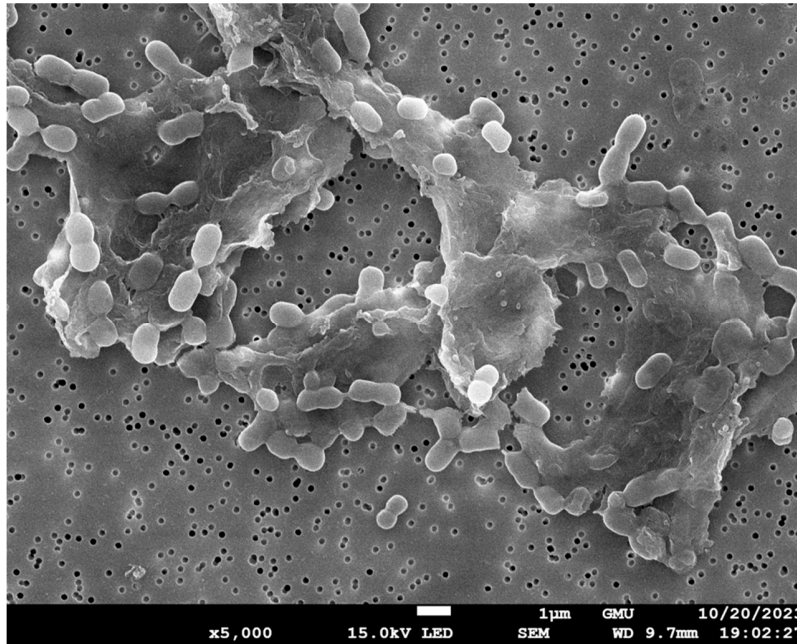
**Figure S3.** Minimum biofilm inhibitory concentration (MBIC) of GATR-3 against (A) *P. aeruginosa* BAA-2110, (B) *K. pneumoniae*, (C) *E. asburiae* BAA-3043, and (D) *E. faecalis* 51299. Crystal violet stain was read via OD<sub>590</sub>.



**Figure S4.** Crystal violet staining of minimum biofilm eradication concentration (MBEC) assay. GATR-3 was challenged with *A. baumannii*: (A) AB5075, (B) BAA-1710, (C) BAA-1794, (D) BAA-1795, (E) BAA-1797, (F) BAA-1605 and (G) BAA-1800. Crystal violet stain was read via OD<sub>590</sub>.

**Table S2.** Sequences of peptides mentioned in this study.

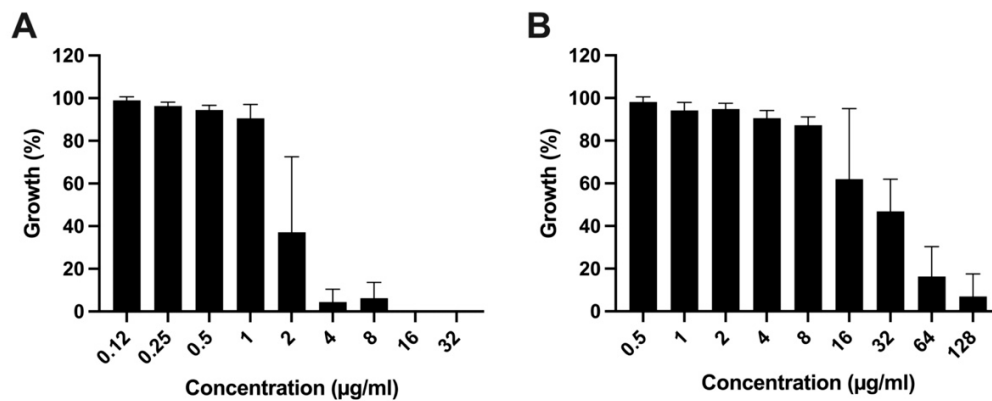
Peptide	Sequence	Reference
HRZN-15	FLPWISKFLGKIL	[39]
SAAP-148	LKRVWKRVPFKLLKRYWRQLKKPVR	[54]
LL-37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLPRTES	[105]
Pexiganan	GIGKFLKKAKKFGKAFVKILKK	[106]



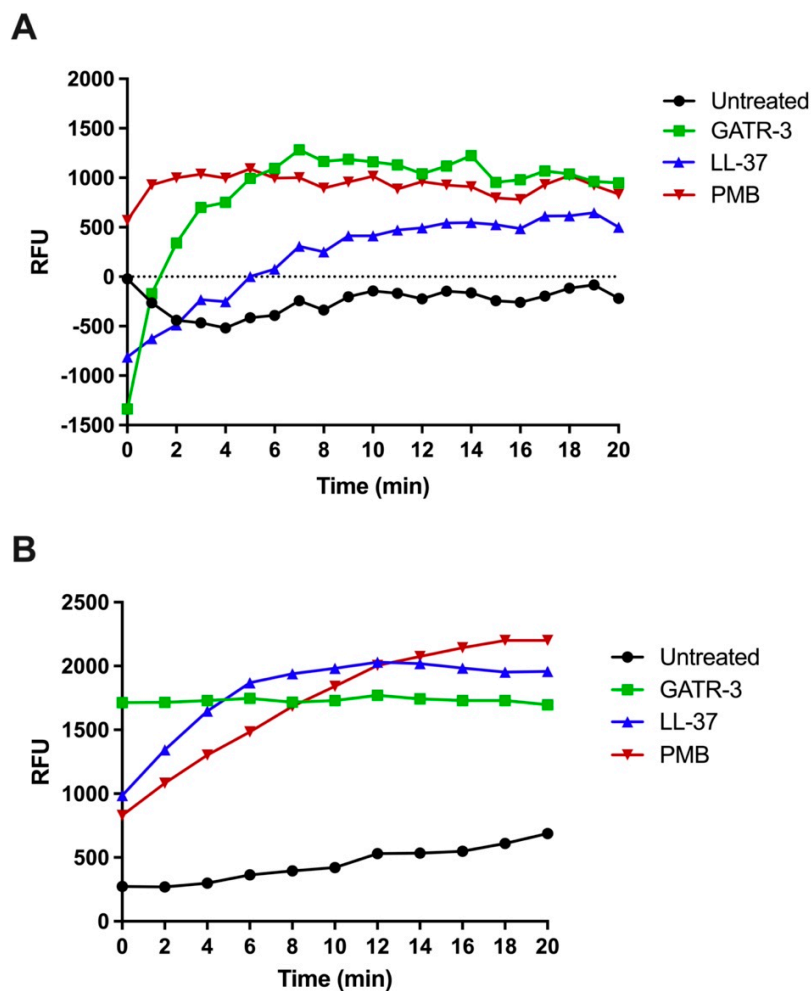
**Figure S5.** Sub-biofilm eradication concentration of GATR-3. Scanning electron micrograph of *A. baumannii* AB5075 treated with 16 µg/ml GATR-3 (in the treatment well). The image is taken at 5,000x with a working distance of 9.7 mm. The white bar represents 1 µm.

**Table S3.** Log of mean and standard deviation of MBEC assay.

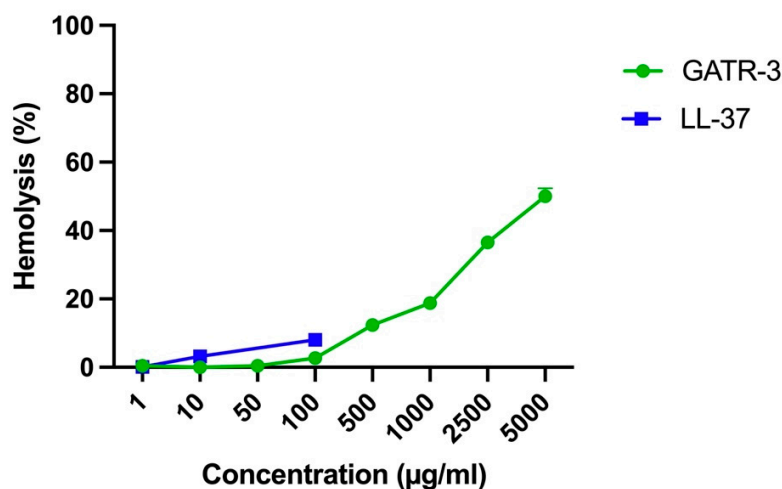
GATR-3			Polymyxin B		
Concentration µg/ml	Mean	SD	Concentration µg/ml	Mean	SD
0	8.253	0.048	0	8.516	0.174
2	7.637	0.012	0.25	7.662	0.093
4	7.563	0.036	0.5	7.801	0.089
8	7.263	0.219	1	7.354	0.154
16	6.249	0.228	2	0	0
32	0	0	4	0	0
64	0	0	8	0	0
-	-	-	16	0	0



**Figure S6.** Evaluation of cross-resistance development of (A) GATR-3 against colistin-exposed *A. baumannii* from the 60<sup>th</sup> passage by minimum inhibitory concentration (MIC) assay. (B) Colistin was tested to show that the bacteria are resistant to colistin.



**Figure S7.** Kinetics of membrane depolarization (A) and disruption (B) of GATR-3, LL-37 and polymyxin B (PMB) against AB5075 by the fluorescent dye DiSC<sub>3</sub>(5) and ethidium bromide. Bacteria were incubated with 50 μg/ml peptide/drug, and the results were obtained after 20 minutes of challenge exposure.



**Figure S8:** Hemolytic activity of GATR-3 and LL-37 against human red blood cells. A 2% RBC suspension was incubated with 5000, 2500, 1000, 500, 100, 50, 10, and 1 µg/ml GATR-3 and 100, 10, and 1 µg/ml LL-37. The release of hemoglobin (n = 3) was measured at OD<sub>540</sub>, and the percentage of hemolysis was calculated relative to the Triton X-100-treated RBCs (100% hemolysis).

**Figure S9:** GATR-3 peptide quality control information. Purity, liquid chromatography (HPLC) and mass-spectrometry (ESI-MS) analysis of GATR-3 peptides used in this study from manufacturer.



**Figure S10:** LL-37 peptide quality control information. Purity, liquid chromatography (HPLC) and mass-spectrometry (ESI-MS) analysis of LL-37 peptides used in this study from the manufacturers.



