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Supplementary Materials for

Targeting the phosphatidylglycerol lipid: An amphiphilic dendrimer as a promising antibacterial candidate

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Table S1. Bacterial strains used in this study

Strains	Description	References
Gram-positive bacteria		
Enterococcus faecium ATCC 70021	VRE	ATCC
Staphylococcus aureus ATCC 25904	Non-MRSA	ATCC
Staphylococcus aureus ATCC 43300	MRSA	ATCC
Gram-negative bacteria		
Escherichia coli MG1655	Wildtype	(*78*)
Escherichia coli MG1655/pACYC184-*mcr-1*	*mcr-1*	This study
Escherichia coli Y8	multidrug-resistant UPEC	This study
Escherichia coli AR Bank 0349	*mcr-1*	(*40*)
Escherichia coli ATCC 25922	CLSI control strain	ATCC
Acinetobacter baumannii ATCC 17978	Wildtype	ATCC
Acinetobacter baumannii ATCC BAA-1791	Multidrug-resistant	ATCC
Acinetobacter baumannii ATCC BAA-1800	Multidrug-resistant	ATCC
Acinetobacter baumannii W47919	Carbapenem-resistant	(*41, 42*)
Klebsiella pneumoniae ATCC BAA-2146	NDM-1	ATCC
Klebsiella pneumoniae ATCC 13883	Wildtype	ATCC
Klebsiella pneumoniae ATCC BAA-1705	KPC	ATCC
Klebsiella pneumoniae ATCC BAA-43816	Wildtype	ATCC
Pseudomonas aeruginosa PAO1	Wildtype	(*78*)
Pseudomonas aeruginosa PAK	Wildtype	(*79*)
Enterobacter cloacae 111	Clinical strain	This study
Enterobacter cloacae 119	Clinical strain	This study
Vibrio parahaemolyticus RIMD 2210633	Wildtype	(*80*)
Vibrio cholerae SCE223	Wildtype	This study
Salmonella enteritidis ATCC 13076	Wildtype	ATCC
Citrobacter rodentium DBS100	Wildtype	ATCC
E. coli WO153	AB1157; *recJ asmB1 ΔtolC*::Kan^r	This study
A. baumannii Δ*lpxC*	deletion within *lpxC gene*	This study

ATCC, American Type Culture Collection; CLSI, Clinical Laboratory Standards Institute; VRE, vancomycin resistant *Enterococcus*; MRSA, Methicillin-resistant *Staphylococcus aureus*; *mcr-1,* mobilized colistin resistance 1; UPEC, uropathogenic *Escherichia coli*; NDM-1, New Delhi metallo-β-lactamase; KPC, *Klebsiella pneumoniae* carbapenemase; *E. coli* WO153, mutant strain expresses less LPS; *A. baumannii* Δ*lpxC*, isogenic mutant strain lpxC of *Acinetobacter baumannii* which is LPS deficient.

Table S2. Antibacterial activity of AD1b

Strains	AD1b MIC (μg/mL)
Gram-positive bacteria	
S. aureus ATCC 25904 (MSSA)	> 96
S. aureus ATCC 43300 (MRSA)	> 96
E. faecium ATCC 700221 (VRE)	48
Gram-negative bacteria	
E. coli MG1655	6.0
E. coli MG1655/pACYC184-*mcr-1* (MCR-1)	6.0
E. coli Y8 (MDR)	6.0
E. coli ATCC25922	6.0
A. baumannii ATCC 17978	6.0
A. baumannii ATCC BAA-1791(MDR)	6.0
A. baumannii ATCC BAA-1800 (MDR)	6.0
K. pneumoniae ATCC 13883	48
K. pneumoniae ATCC 2146 (NDM-1)	> 96
K. pneumoniae ATCC BAA-1705 (KPC)	> 96
K. pneumoniae ATCC BAA-43816	> 96
P. aeruginosa PAO1	> 96
P. aeruginosa PAK	> 96
E. cloacae 111	> 96
E. cloacae 119	> 96
V. parahaemolyticus RIMD 2210633	6.0
V. cholerae SCE223	6.0
S.enteritidis ATCC 13076	6.0
C. rodentium DBS100	6.0
E. coli AR Bank 0349	6.0
A. baumannii W47919	6.0

MSSA, methicillin-sensitive *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin resistant *Enterococcus*; *mcr-1,* mobilized colistin resistance 1; MDR, multidrug-resistant; NDM-1, New Delhi metallo-β-lactamase; KPC, *Klebsiella pneumoniae* carbapenemase.

Table S3. MIC values of *E. coli* ATCC25922 under different conditions

Culture condition	MIC (μg/ml)
LB media	6.0
500 mM glutathione added in LB media	6.0
Anaerobic conditions	6.0

Table S4. MIC values of *E. coli* and *A. baumannii* LPS mutants

Bacteria strains	MIC (μg/ml)
E. coli WO153	6.0
A. baumannii Δ*lpxC*	6.0

E. coli WO153: mutant strain expresses less LPS; *A. baumannii* Δ*lpxC*: isogenic mutant strain lpxC of *Acinetobacter baumannii* which is LPS deficient.

Table S5. Summary of thermodynamic parameters in ITC assays

Parameters	**AD1b** titrate into PG	BMS titrate into PG	**AD1b** titrate into PG + BMS
n (drug/PG)	/	1.04 ± 0.08	0.049 ± 0.004
K_B (mol L^{-1})	/	$2.44 \pm 0.70 \times 10^{5}$	$2.98 \pm 0.58 \times 10^{8}$
K_D (mol L^{-1})	/	$4.45 \pm 1.24 \times 10^{-6}$	$3.51 \pm 0.79 \times 10^{-9}$
ΔH (kcal mol^{-1})	-44.9 ± 0.4	-7.94 ± 0.31	-49.2 ± 1.1
ΔS (cal mol^{-1} deg^{-1})	48.1 ± 6.2	-2.06 ± 1.32	/

The experiments were carried out in triplicate and the mean values ± standard deviation (SD) were shown. n, number of binding sites; K_B, binding constant; K_D, equilibrium dissociation constant; ΔH, binding enthalpy; ΔS, binding entropy.

Fig. S1. Time dynamics measurement of inner membrane permeability after **AD1b** treatments. The membrane permeability of *E. coli* MG1655 treated with **AD1b** was measured by 1.0 μg mL^{-1} propidium iodide (PI). All data represent average values of three biological replicates ± s.d, n = 3. CT8, colistin 8.0 μg mL^{-1}.

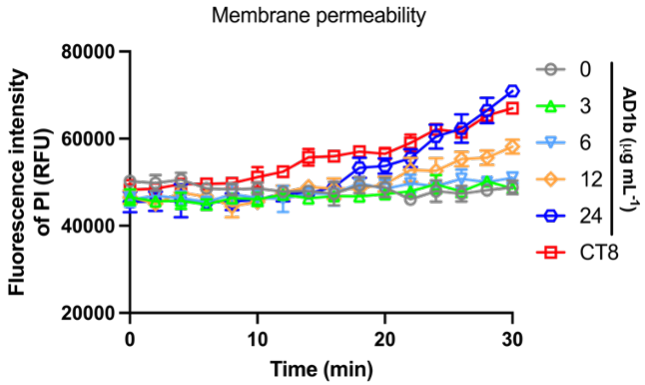


Fig. S2. Time dynamics measurements of ΔΨ and ΔpH after **AD1b** treatments. (A) Time dynamics measurement of ΔΨ in *E. coli* MG1655 treated with increased concentration of **AD1b** and positive control valinomycin (5.0 μmol L^{-1}). (B) Time dynamics measurement of ΔpH in *E. coli* MG1655 treated with increased concentration of **AD1b**. All data represent average values of three biological replicates ± s.d, n = 3.

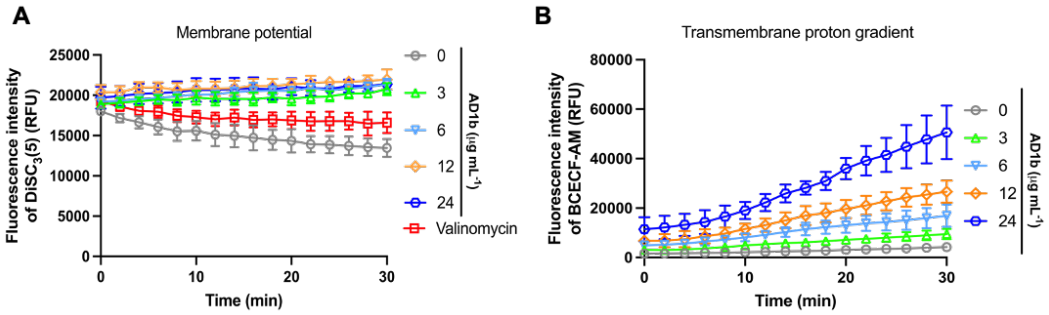


Fig. S3. AD1b causes protein efflux. The intracellular protein level of *E. coli* MG1655 treated with an increased concentration of **AD1b** and the positive control colistin (8.0 μg mL^{-1}) were measured by the BCA assy. Data were shown as average values ± SD (n = 3 per group) and analysed using a one-way ANOVA, ** p <0.01, **** p <0.0001. CT8, colistin 8.0 μg mL^{-1}.

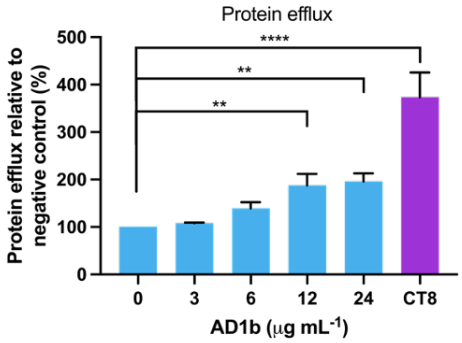


Fig. S4. Membrane phospholipids inhibit the antibacterial activity of **AD1b**. Representative checkerboard assay of PG (A), CL (B), PE (C), PC (D), PGN (E), and LPS (F) inhibiting **AD1b** activity. PG, phosphatidylglycerol; CL, cardiolipin; PE, phosphatidylethanolamine; PC, phosphatidylcholine. PGN, peptidoglycan; LPS, lipopolysaccharide. The dark color represents bacterial growth. These checkerboards were repeated at least two times independently, yielding similar results.

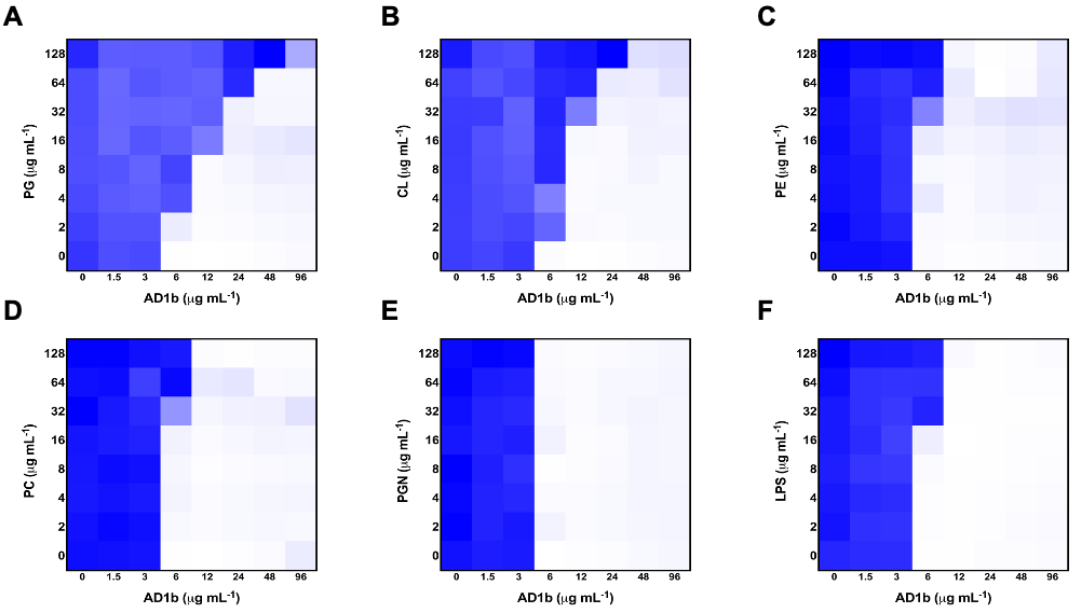


Fig. S5. ITC analysis of the interaction between **AD1b** and PG, PC, PE, and HEPES buffer. PG, phosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine. (A) Direct ITC analysis of **AD1b** and PG. 50 μmol L^{-1} **AD1b** dissolved in 20 mmol L^{-1} HEPES buffer (pH 7.0) was sequentially injected into calorimetric cells filled with 100 μmol L^{-1} PG at 25°C. (B) Direct ITC analysis of small molecule BMS-833923 (BMS) and PG. 300 μmol L^{-1} BMS was sequentially injected into the calorimetric cells containing 40 μmol L^{-1} PG. Relevant thermodynamic parameters were determined: equilibrium dissociation constant ($K_D = 4.33 \times 10^{-6}$ mol L^{-1}), number of binding sites ($n = 1.07$), molar binding enthalpy (ΔH = -8.35 kcal mol^{-1}), molar binding entropy (ΔS = -3.48 cal mol^{-1} deg^{-1}). (C) Repeat of the competitive ITC assay of **AD1b** and PG. 50 μmol L^{-1} of **AD1b** was titrated into 100 μmol L^{-1} PG + 10 μmol L^{-1} BMS in HEPES buffer at 25°C. Relevant thermodynamic parameters were determined: equilibrium dissociation constant ($K_D = 2.94 \times 10^{-9}$ mol L^{-1}), number of binding sites ($n = 0.05$), molar binding enthalpy (ΔH = -50.74kcal mol^{-1}). Direct ITC analysis of **AD1b** and PC (D), PE (E), and 20 mmol L^{-1} HEPES buffer (F). 50 μmol L^{-1} **AD1b** was sequentially injected into calorimetric cells filled with 100 μmol L^{-1} lipid at 25°C. All injections were repeated 20 times at an equilibrium interval of 150 s and the experiments were conducted in triplicate.

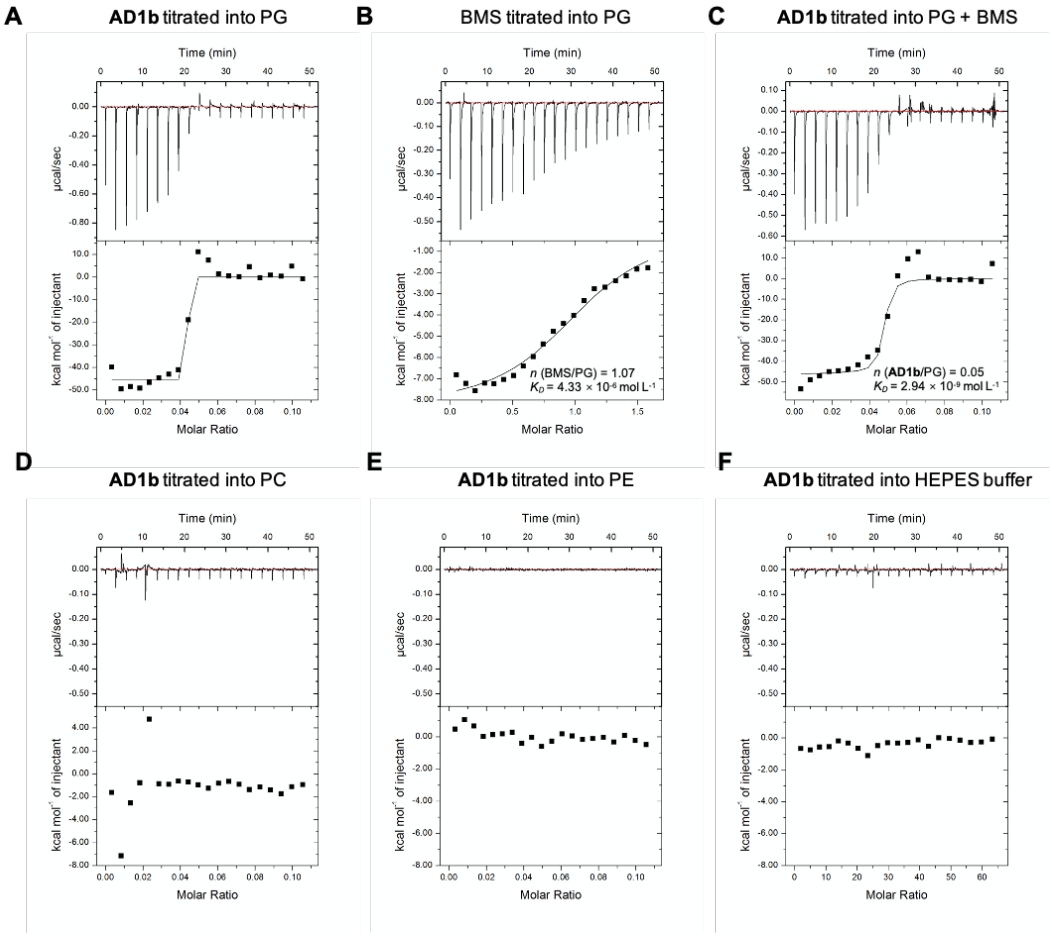


Fig. S6. Safety evaluation of **AD1b**. Hemolytic toxicity evaluation of **AD1b** using sheep red blood cells. Quantitative analysis of hemolysis determined by UV absorption at 576 nm. 0.2% Triton X-100 was used as a positive control (mean ± SD, n = 3).

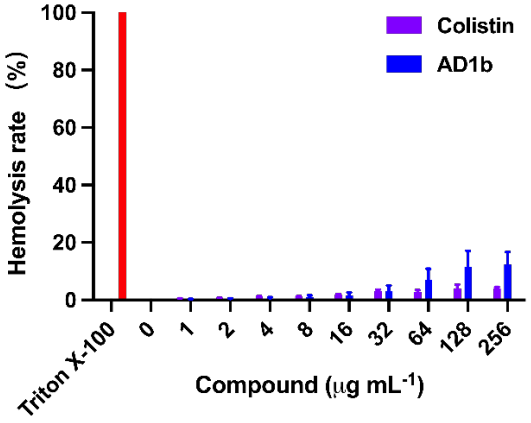


Fig. S7. The weight of CD-1 mice after 7 days of intraperitoneally-injected **AD1b** (5 mg/kg, twice daily). Error bars represent SD for n=5.

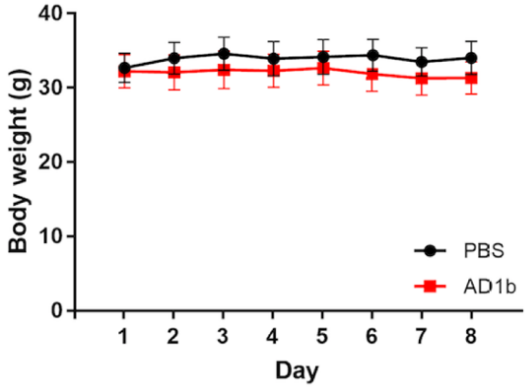
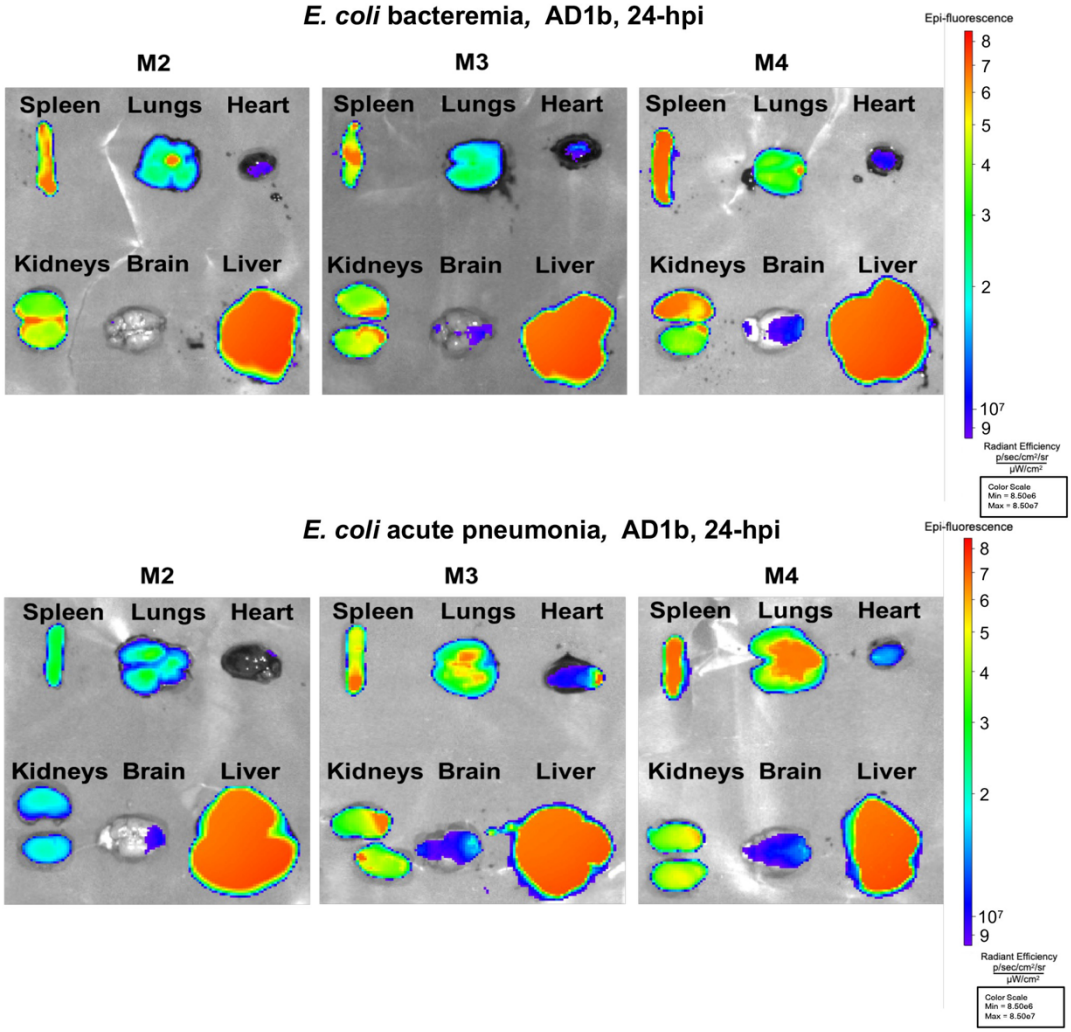


Fig. S8. Pharmacokinetic properties of **Ad1b** in mouse organs infected with *E. coli* AR0349. Biostability and biodistribution of intraperitoneally-injected Cy7.5-**AD1b** (50 µg Cy7.5) in CD-1 mice (n=4) with *E. coli*-mediated bacteremia (IP, 5.2 x 10^7 CFU) and acute pneumonia (1.1 x 10^8 CFU, IN) infection respectively, and imaged by an IVIS SpectrumCT imager. Major organs were excised at 24-hpi, imaged in a rainbow color scale, and quantified. IN- intranasal infection, IP, intraperitoneal injection. Images of organs from mice # 2 - 4 (M2 - M4) in Figure 4E are shown. Organs from the mice # 1 (M1) in Figure 4E can be found in Figure 4F.



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