Discovery of novel polymyxin E adjuvants against *Acinetobacter baumannii* guided by a stacking-based machine learning model

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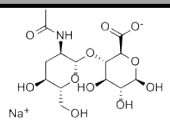
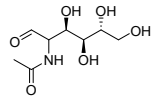
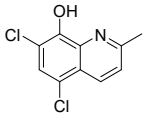
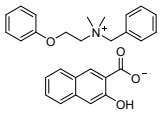
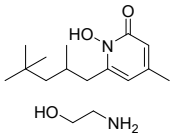
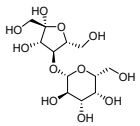
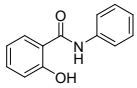
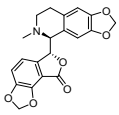
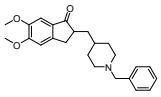
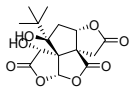
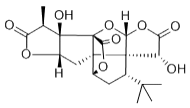
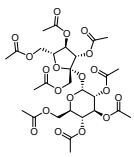
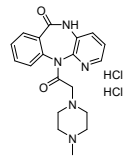
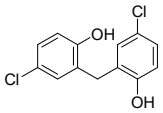
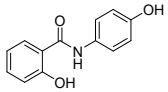
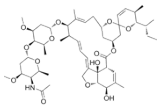
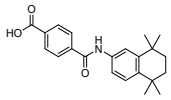
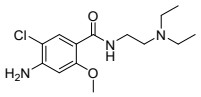
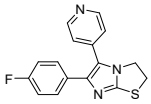
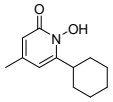
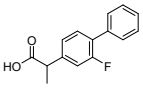
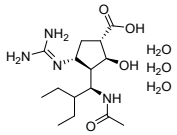
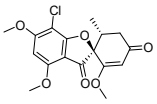
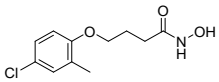
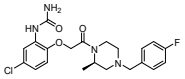
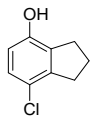
Table S1. General information of target compounds **F1-F31**

ID	Structure	CAS	Name	Purity
F1		9004-61-9	hyaluronic-acid	≥ 95%
F2		7772-94-3	N-acetylmannosamine	≥ 95%
F3		72-80-0	chlorquinaldol	≥ 95%
F4		3818-50-6	bephenium-hydroxynaphthoate	≥ 95%
F5		68890-66-4	piroctone-olamine	≥ 95%
F6		4618-18-2	Lactulose	≥ 95%
F7		87-17-2	salicylanilide	≥ 95%
F8		485-49-4	bicuculline-(+)	≥ 95%
F9		120011-70-3	donepezil	≥ 95%
F10		33570-04-6	bilobalide	≥ 95%
F11		15291-75-5	Ginkgolide A	≥ 95%
F12		126-14-7	sucrose-octaacetate	≥ 95%

F13		29868-97-1	Pirenzepine dihydrochloride	≥ 95%
F14		97-23-4	dichlorophen	≥ 95%
F15		526-18-1	osalmid	≥ 95%
F16		123997-26-2	eprinomectin	≥ 95%
F17		94497-51-5	Tamibarotene	≥ 95%
F18		364-62-5	metoclopramide	≥ 95%
F19		72873-74-6	SKF-86002	≥ 95%
F20		29342-05-0	ciclopirox	≥ 95%
F21		5104-49-4	flurbiprofen	≥ 95%
F22		1041434-82-5	peramivir	≥ 95%
F23		126-07-8	griseofulvin	≥ 95%
F24		99873-43-5	droxinostat	≥ 95%
F25		217645-70-0	ZK811752	≥ 95%
F26		145-94-8	chlorindanol	≥ 95%

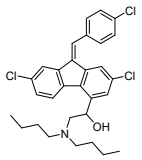
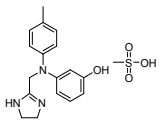
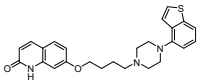
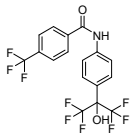
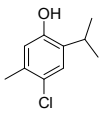
F27		82186-77-4	lumefantrine	≥ 95%
F28		65-28-1	Phentolamine mesylate	≥ 95%
F29		913611-97-9	brexpiprazole	≥ 95%
F30		1246525-60-9	SR1078	≥ 95%
F31		89-68-9	Chlorthymol	≥ 95%

Table S2. Bacterial strains used in this study.

Strain Name	Species	Relevant Characteristics	Source / Provider	Use in Study
ATCC 19606	*Acinetobacter baumannii*	Wild-type, polymyxin-susceptible reference strain	ATCC (Manassas, VA, USA)	Screening, *in vivo* model, mechanistic studies
ATCC 43300	*Staphylococcus aureus*	Methicillin-resistant (MRSA) reference strain	Shanghai Jiao Tong University	Antibacterial spectrum analysis
ATCC 51299	*Enterococcus faecium*	Vancomycin-resistant (VRE) reference strain	Shanghai Jiao Tong University	Antibacterial spectrum analysis
ATCC 20678	*Bacillus subtilis*	Gram-positive reference strain	Shanghai Jiao Tong University	Antibacterial spectrum analysis
Ab2092	*Acinetobacter baumannii*	Clinical isolate; polymyxin E MIC = 2 μg/mL	Shanghai Institute of Pharmaceutical Industry	Synergy testing
Ab070311	*Acinetobacter baumannii*	Clinical isolate; polymyxin E MIC = 1 μg/mL	Shanghai Institute of Pharmaceutical Industry	Synergy testing
Ab7997	*Acinetobacter baumannii*	Clinical isolate; polymyxin E MIC = 4 μg/mL	Shanghai Institute of Pharmaceutical Industry	Synergy testing
Ab7998	*Acinetobacter baumannii*	Clinical isolate; polymyxin E MIC = 2 μg/mL	Shanghai Institute of Pharmaceutical Industry	Synergy testing
Ab7966	*Acinetobacter baumannii*	Clinical isolate; polymyxin E MIC = 4 μg/mL	Shanghai Institute of Pharmaceutical Industry	Synergy testing
Ab7967	*Acinetobacter baumannii*	Clinical isolate; polymyxin E MIC = 2 μg/mL	Shanghai Institute of Pharmaceutical Industry	Synergy testing
Ab7845	*Acinetobacter baumannii*	Clinical isolate; polymyxin E MIC = 4 μg/mL	Shanghai Institute of Pharmaceutical Industry	Synergy testing
Ab7939	*Acinetobacter baumannii*	Clinical isolate; polymyxin E MIC = 2 μg/mL	Shanghai Institute of Pharmaceutical Industry	Synergy testing

Analysis of antibacterial activity of selected compounds

Our machine learning-based screening project, initially focused on identifying potential synergists for polymyxin E against Gram-negative bacteria, was expanded to assess antibacterial activity against Gram-positive bacteria. We evaluated three Gram-positive strains: methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC 43300), vancomycin-resistant *Enterococcus faecalis* (VRE, ATCC 51299), and *Bacillus subtilis* (ATCC 20678) (**Table S3**). MRSA and VRE were selected due to their clinical significance as antibiotic-resistant pathogens. Most target compounds showed minimal antibacterial activity against the Gram-positive strains, with Minimum Inhibitory Concentrations (MICs) ≥256 μg/mL. However, seven compounds exhibited notable antibacterial activity. Compounds **F5**, **F16**, **F19**, and **F31** demonstrated moderate potency, with MICs ranging from 16 to 32 μg/mL against all three Gram-positive strains. Compounds **F13** and **F20** showed higher potency, with MICs of 4-8 μg/mL. Compound **F3** exhibited the highest potency, with MICs ranging from 0.5 to 2 μg/mL against all three Gram-positive strains. These results revealed that our machine learning-screened compounds possess dual antimicrobial properties: synergistic activity with polymyxin E against Gram-negative bacteria and direct antibacterial activity effects against Gram-positive bacteria, including antibiotic-resistant strains.

Table S3. The MIC of target compounds **F1**-**F31** against Gram-positive bacteria.

No.	MRSA (ATCC43300)	VRE (ATCC51299)	*Bacillus subtilis* (ATCC20678)
F1	>256	>256	>256
F2	>256	>256	>256
F3	2	4	0.5
F4	>256	>256	256
F5	16	32	16
F6	>256	>256	>256
F7	>256	>256	>256
F8	128	>256	>256
F9	>256	>256	>256
F10	>256	>256	>256
F11	>256	>256	>256
F12	>256	>256	>256
F13	4	4	8
F14	>256	>256	>256
F15	64	32	>256
F16	16	32	16
F17	>256	>256	>256
F18	>256	>256	16
F19	16	32	16
F20	64	>256	>256
F21	>256	>256	>256
F22	>256	>256	>256
F23	64	>256	32

F24	>256	>256	256
F25	64	32	64
F26	>256	>256	>256
F27	>256	>256	>256
F28	>256	>256	>256
F29	>256	>256	>256
F30	8	8	8
F31	32	32	8

Synergistic effects of polymyxin E and novel compounds against *A. baumannii* ATCC 19606

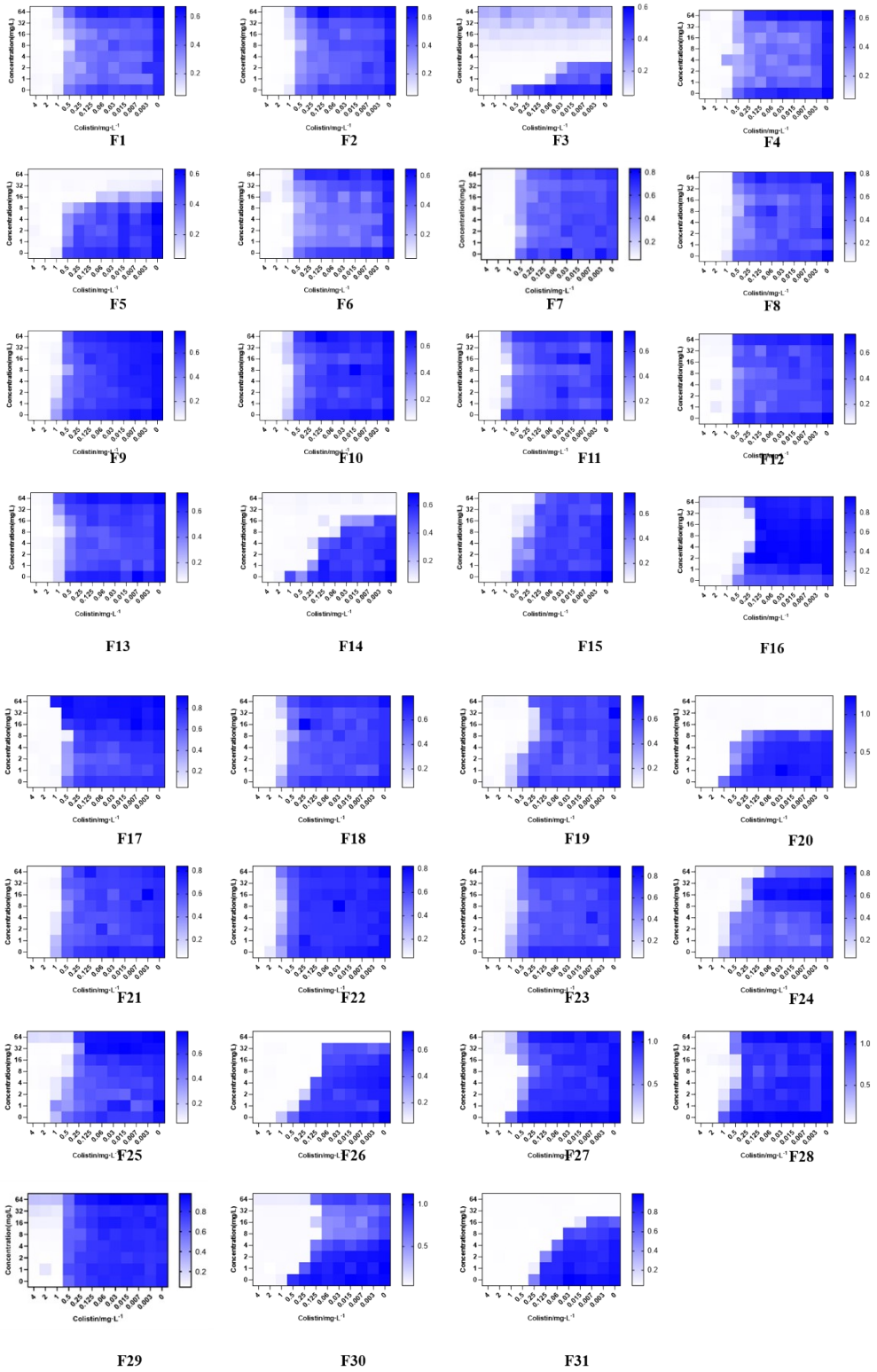


Fig. S1. Synergistic effects of polymyxin E and novel compounds against *A. baumannii* ATCC 19606. Chequerboard broth microdilution assays showing interactions between polymyxin E and

compounds **F1-F31**. Assays were performed at 37°C. Blue regions indicate higher bacterial cell density. X-axis: polymyxin E concentration; Y-axis: compound concentration.