Exploring the repository of *de novo* designed bifunctional antimicrobial peptides through deep learning

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Abstract

Antimicrobial peptides (AMPs) are attractive candidates to combat antibiotic resistance for their capability to target bio-membranes and restrict a wide range of pathogens. It is a daunting challenge to discover novel AMPs due to their sparse distributions in a vast peptide universe, especially for peptides that demonstrate potencies for both bacterial membranes and viral envelopes. Here we establish a *de novo* AMP design framework by bridging a deep generative module and a graph-encoding activity regressor. The generative module learns hidden 'grammars' of AMP features and produces candidates sequentially pass antimicrobial predictor and antiviral classifiers. We discover 16 bifunctional AMPs and experimentally validated their abilities to inhibit a spectrum of pathogens *in vitro* and in animal models. Notably, P076 is a highly potent bactericide with the minimal inhibitory concentration of 0.21 μM against multidrug-resistant *A. baumannii*, while P002 broadly inhibits five enveloped viruses. Our study provides feasible means to uncover sequences that simultaneously encode antimicrobial and antiviral activities, thus bolstering the function spectra of AMPs to combat a wide range of drug-resistant infections.

Keywords

Antimicrobial peptides, Antiviral peptides, Deep learning, Dual-function, Generative model, Sequence design

Introduction

In the post-antibiotic era, microbes rapidly evolve to become antimicrobial-resistant under the pressure of antibiotic abuse and through horizontal transfer of resistance genes.[1] Due to increasing cases of untreatable bacterial infections, the World Health Organization (WHO) has projected a looming health threat of 10 million casualties per year by 2050.[2] Specifically, the WHO has identified six antimicrobial-resistant pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) as ESKAPE. The ESKAPE pathogens have severely limited the treatment options for serious infections and impaired the conditions of immunodeficient patients.[3] In sharp contrast, the majority of clinically available antibiotics were developed more than 30 years ago.[4] Hence, there are urgent needs for targeted generation and screening of novel molecules with desired therapeutic properties for the next-generation broad-spectrum antimicrobial agents.

As a naturally evolved defense line of innate immune system, antimicrobial peptides (AMPs) are cationic macromolecules (usually 10-60 amino acids) widely present in all living organisms.[5] AMPs are attractive alternatives to conventional antibiotic treatment because they effectively inhibit a wide range of pathogens, such as the Gram-positive and Gram-negative bacteria, fungi, and viruses.[5] The majorities of AMPs featured amphiphilic patterns of amino acid sequences and enriched positive charges (e.g., lysine and arginine residues), which allowed AMPs to disrupt the membrane components and exert antimicrobial effects.[6] Due to the relative constant compositions of biological membranes as the essential AMP targets, development of resistance to AMPs were rare events even after 100 passages of lab evolutions.[7, 8]

Notably, the naturally evolved and rationally designed AMP sequences (~20 000) are housed in several databases (APD,[9] DBAASP,[10] DRAMP,[11] etc.) and paired with experimentally validated activities (minimal inhibitory concentration, or MIC), thus facilitating data mining endeavors for more diverse sequences and more effective peptides.[12] Indeed, deep learning models have led to the discovery of highly efficient AMPs (e.g., MIC of 2 μM against *E. coli*). Specifically, predictive models like Deep-AMPEP30,[13] AMPlify,[14] TransImbAMP,[15] and sAMPpred-GAT[16] utilized deep convolutional, recurrent, attention-based, and graph neural networks to learn and recognize latent

representations of meaningful peptides. These models recently enabled comprehensive mining of the human gut microbiome,[17] modern and ancient human proteomes,[18, 19] or even the entire sequence space of hexapeptides.[8] Generative models like PepVAE,[20] CLaSS,[21] and HydrAMP[22] reconstructed the sequences from latent space according to the encoder-decoder architecture of variational autoencoders (VAE), while PepGAN[23] and AMPGAN v2[24] utilized the generative adversarial network (GAN) to approximate the antimicrobial sequences by deceiving the discriminator.

Despite the recent advances of data-driven AMP classifiers and generators, the exploration of a vast AMPs repertoire is still hindered by several factors: (1) The sparsity of AMPs in the whole peptide space, which demands accurate prediction of MIC values for efficient sampling and filtering of AMP analogues.[22, 25] The current machine learning predictive models usually perform the binary classification task and label the generated sequences as active or not, instead of giving specific activity values. To overcome this shortcoming, a popular pipeline combines independent modules of filtering, classification, and regression, but it faces risks of step-wise bias accumulation.[8] (2) The impact of different phospholipid species on the efficacy of AMPs. Due to the data incompleteness of antimicrobial measurements, a majority of AMP classifiers or MIC predictions were specific to *E. coli* (Gram-negative). Nevertheless, these models were applied to find peptides that inhibit other Gram-positive bacteria (e.g., *S. aureus*), based on the assumption that the shared membrane components are potential targets of AMPs. More importantly, the enveloped virus also possesses a targetable membrane, which closely mimic the phospholipid components of cellular organelles. Even though the AMP database recorded antiviral activities of at least 2 000 antiviral peptides (AVPs), whether the current models can readily generate antiviral peptides remain elusive. (3) Lack of rigorous pre-clinical models. To advance the designed AMPs towards clinical trials, the experiments should reflect the efficacy of AMPs in tissue samples and require the determination of safe doses of a particular sequence before practical applications. To our knowledge, more than 120 AMPs have been suggested by deep learning models in the past three years, only a fraction of these studies demonstrated animal results.

In the current study, we addressed these shortcomings and presented a new deep learning-based *de novo* AMP design framework, consisting of a GAN generator and an antimicrobial activity

regressor named AMPredictor. The graph convolution network (GCN) of AMPredictor allowed us to effectively train a regression model for the task of MIC predictions. Through in-depth investigations of generated sequences (e.g., antiviral possibilities), we selected three top-ranking sequences (P001, P002, and P076) and evaluated their efficacies against different pathogens (four drug-resistant bacteria and five enveloped virus). Out of the three candidates, we found P076 is a potent bactericide with MIC of 0.21 μM against multidrug-resistant bacterium. It also outperformed a clinically relevant antibiotic polymyxin B in terms of safety and efficacy, as evaluated in mouse models. Another peptide P002 broadly neutralized viruses in cellular infection models. We challenged our computational models to generate bifunctional AMPs and our results demonstrated that these peptides inhibited a spectrum of pathogens, but with delicate preferences towards bacterial or viral species.

Results

Bridging the generator and regressor for *de novo* AMP design

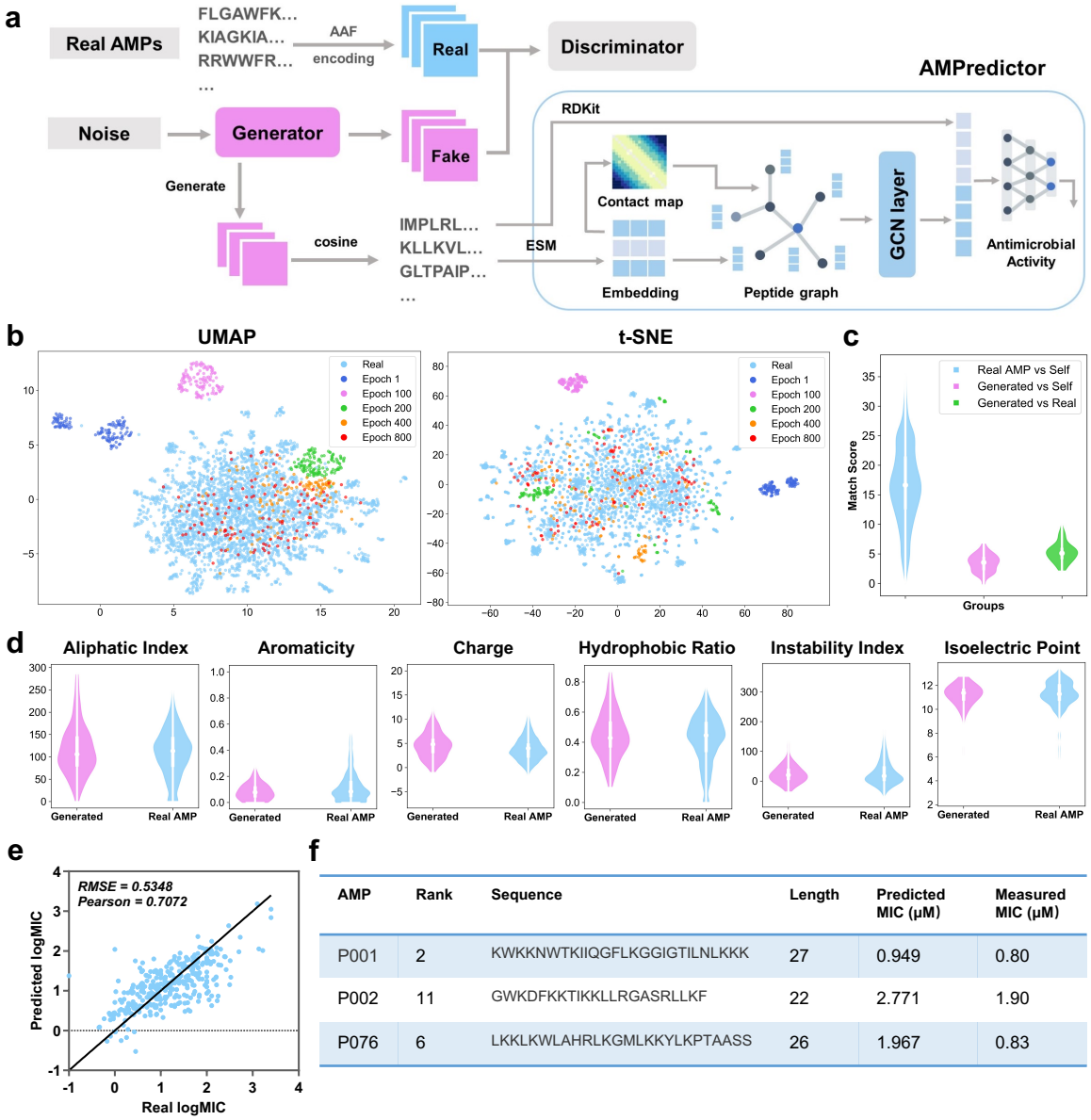
The overall architecture for designing functional peptides fuses a generative adversarial network and an antimicrobial activity predictor (AMPredictor, Figure 1a). We aim to explore beyond the known antimicrobial templates or accessible proteomics space. We trained the GAN module with 3280 AMP sequences to capture the pattern of antimicrobial peptides through few-shot learning. The training goal of GAN is to reach a relative balance between the generator and discriminator (see Methods Equation 1). The generator takes random noise as input to produce fake samples, which are fed into the discriminator along with the real data. The discriminator tries to distinguish between the real and fake data, then returns a probability to update the weights of the networks.[26] Here, we employed a low-dimensional vector called amino acid factors (AAF) to encode the peptide sequences and describe their physicochemical features.[27] We monitored the generated sequences at different training stages (epoch 1, 100, 200, 400, 800) and visualized the optimization process using two common dimension reduction approaches, uniform manifold approximation and projection (UMAP) and t-distributed stochastic neighbor embedding (t-SNE). We clearly observed the distributions of newly sampled sequences among the existing AMPs after mapping peptide

AAF features onto the two-dimensional space (Figure 1b). With iterating and updating, the generator learns the feature distribution of actual data and generates diverse sequences.

We checked the novelty and diversity of generated sequences at epoch 1000 by aligning them with our training set. The sequence similarities were evaluated by global alignment using BLOSUM62 matrix. We found the identity of each generated sequence was around 0.35 to the known database (Figure 1c), indicating a low level of similarity to the existing AMPs. With distinct sequence compositions, generated peptides still hold similar physiochemical features to real AMPs, as evidenced by six descriptors of aliphatic index, aromaticity, net charge, the ratio of hydrophobic amino acids, instability index, and isoelectric point (Figure 1d).[28] The distribution of these descriptors closely resembled the physiochemical features of AMPs in the training set. Hence, adopting residue features as the input of GAN generator can capture the functional properties of actual AMPs.

We then developed an independent module AMPredictor to estimate the MIC values of arbitrary peptide sequences. AMPredictor is a GCN-based regression model, equivalent to the 'score function' in virtual drug screen. Unlike the low-dimensional descriptor adopted by GAN generator, we designed distinct representations of peptides in AMPredictor for model accuracy and orthogonal verification of GAN-generated sequences. We chose graph as the representation of peptides, with their amino acid residues as graph nodes and the predicted contact maps as graph edges. The node features were embeddings from the protein language model ESM.[29] The peptide graphs were then fed into a three-layer GCN to learn the hidden 'grammars' of AMP features. In addition to the sequence-level and structural-level features, we transformed the input amino acid sequences into Morgan fingerprints to encode the chemical features at each residual position. This atomic-level information was concatenated with GCN representations before entering a three-layer fully-connected networks to regress on MIC datasets (Figure 1a). AMPredictor obtained a low root mean squared error (RMSE) of 0.5348 and a high Pearson correlation coefficient (PCC) of 0.7072 on the test set (10% size of the dataset) (Figure 1b). The output of AMPredictor is the logMIC value of an AMP sequence.

By integrating the representations of residual-level, sequence-level, and protein structural level information, AMPredictor obtained the best performance in comparison to its ablating versions (Table S1). The ablation study indicated that the context embeddings from ESM play vital roles in predicting MICs, and the contact map assisted in improvement despite it being a simplified representation of protein structure. We also compared the performance of AMPredictor with five typical deep neural networks as baselines, including the multilayer perceptron (MLP), convolutional neural network (CNN), gated recurrent unit (GRU), long short-term memory (LSTM), and Transformer. AMPredictor outperforms these methods in terms of accuracy at the task of predicting antimicrobial activity values (scatter plots in Figure S2). Hence, a well-trained generator could produce a variety of AMP candidates and then AMPredictor ranked the candidates by MIC values. Together with coarse to fine representations of AMP samples, the GAN and AMPredictor facilitated *de novo* design and *in silico* evaluation of AMPs.



AMP	Rank	Sequence	Length	Predicted MIC (μM)	Measured MIC (μM)
P001	2	KWKKNWTKIIQGFLKGGIGTILNLKKK	27	0.949	0.80
P002	11	GWKDFKKTIKKLLRGASRLLKF	22	2.771	1.90
P076	6	LKKLKWLAHRLKGMLKKYLKPTAASS	26	1.967	0.83

Figure 1. Deep learning-based design of novel antimicrobial peptides.

a. The overall framework of generative adversarial network (GAN) generator and AMPredictor.

b. Visualization of GAN training process via UMAP and t-SNE. The newly generated sequences fit the distribution of known AMP space gradually.

c. Global alignment match scores between the generated sequences and itself and AMPs in the training set.

d. Six physiochemistry properties of generated peptides and real AMP sets.

e. Regression results of AMPredictor model on its test set.

f. The sequences of three selected peptides as well as their predicted and experimentally validated MIC values against *E. coli* ATCC 25922.

Designing bifunctional antimicrobial peptides

Our study was based on the hypothesis that both bacterial membranes and viral envelopes were potential targets of AMPs, and the dual-function of antibacterial and antiviral could be encoded into one peptide sequence. We directly compared the outcome of two different training sets for two versions of the GAN generator: with or without adding AVP sequences, for their efficiencies of designing bifunctional peptides (Figure 2a). The generator v1 was trained solely on collected AMP sequences, and generator v2 was trained on the AMP set and known 1788 AVPs from AVPdb. To establish a sequential workflow and filter out antimicrobial peptides with antiviral capabilities, we then considered assembling several AVP classifiers subsequent to the AMPredictor. As the source data, the AVPdb provided ~2000 sequences with IC_{50} labels.[30] However, data curation resulted in only 759 unique AVPs. We determined that the quantitative data of AVPs were insufficient for training another GCN-based regression model. Instead, binary classification should exhibit robustness against noise of a relatively small dataset. Hence, we merely made yes/no decisions using a collective of five published AVP classification tools (AVPpred,[31] DeepAVP,[32] Deep-AVPpred,[33] ENNAVIA,[34] and AI4AVP,[35] details in Table S2). We obtained 1024 new peptides from each generator and kept the sequences with a predicted MIC value of less than 10 μM by AMPredictor. More than one hundred peptides passed the ensemble filter of all five AVP classifiers. Interestingly, generator v2 did not feature a higher passing ratio, although it was trained with AVPs. Based on their hemolysis and other physicochemical properties, we selected 12 peptides from each generator for experimental validation. We also checked their novelty by BLAST with the training set or DRAMP database and all their E-values are greater than 0.1 or no hits found (Table S3).

All 24 peptides exhibited effective antibacterial capability in inhibiting six different strains involving three multidrug-resistant species (Figure 2b). Several peptides obtained remarkable MIC values of less than 1 μM, such as P026 and P036, indicating the effectiveness of our generator for *de novo* designing AMPs. Then we used a stricter rule of 'antibacterial' to define bifunctional peptides. If the MIC value of a peptide was less than 10 μM against at least one strain, we described

222 it as antibacterial. We also tested whether these peptides can inhibit Herpes simplex virus 1 (HSV-
223 1) at the concentration of 10 μM. The 'antiviral' feature of a peptide was checked when significant
224 inhibition was observed in immunofluorescence experiments. There were six and seven
225 bifunctional AMPs out of 12 from generators v1 and v2, respectively (Figure 2c). Given that we
226 did not screen on more virus species, the success rate of being antiviral is relatively lower than
227 being antibacterial. This result indicates the capability of our design method for bifunctional AMPs.
228 Through the comparison between generators v1 and v2, we demonstrated that the antiviral feature
229 was 'naturally' encoded in AMP sequences.
230
231 We then paired small-scale design with more comprehensive experimental characterization, using
232 four drug-resistant bacteria, five enveloped viruses, and animal infection models, focusing on
233 generator v1. We acquired 104 novel peptides via the *de novo* design framework. Among them, 42
234 and 26 were predicted to own MIC values below 10 μM and 5 μM, respectively (supplemental
235 spread sheet). The small-scale design procedure did not sacrifice the efficiency of discovering
236 novel peptides and featured a comparable success rate with AMPredictor. We regarded a peptide
237 as an antiviral candidate if four or more positive votes were recorded and 32 peptides met our
238 criteria.
239
240 We also conducted a comprehensive evaluation of efficacy and safety *in silico*, to select novel
241 AMPs before experimental validations. Considering the membrane corruption mechanism of most
242 AMPs, we predicted their hemolytic activity as a quick measure of cellular toxicity.[36] Through the
243 ranking of high activity and low toxicity, we picked three potent peptides P001, P002, and P076
244 (Figure 1f), all of which featured amphiphilic signatures of sequence and enriched cationic regions
245 (e.g., helical wheel projections in Figure S3), for *in vitro* and *in vivo* experimental assessment.
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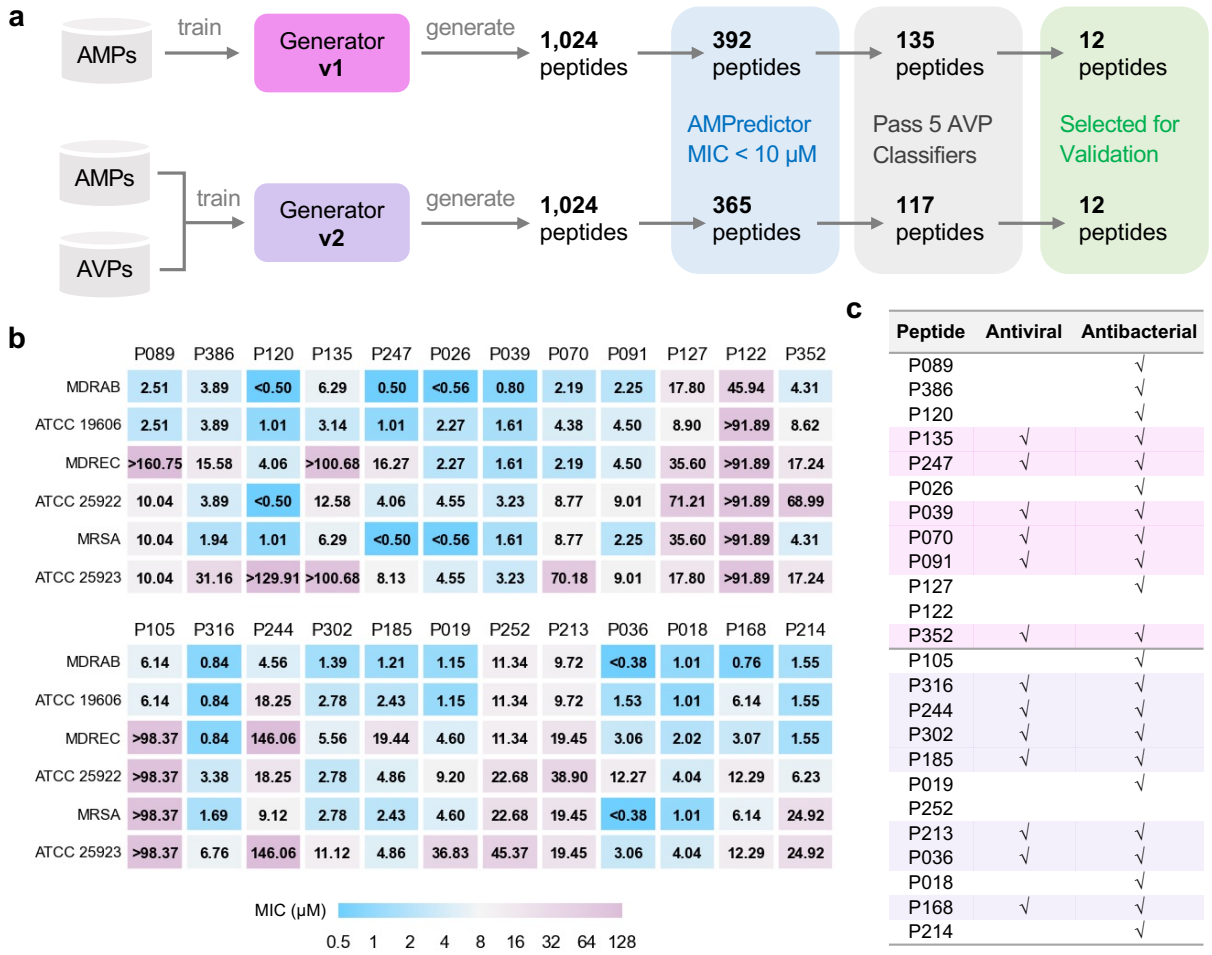

Figure 2. Large-scale design and validation of bifunctional AMPs.

a. Schemes of designing bifunctional AMPs from two versions of generators. 12 designed peptides from each generator were selected for experimental validation.

b. MIC test of 24 designed peptides against six bacterial strains. The first group included 12 peptides from generator v1, and the other 12 peptides were from generator v2.

c. Antiviral and antibacterial results for bifunctional peptides. The rows with pink or purple backgrounds are bifunctional AMPs acquired from generators v1 or v2, respectively.

In vitro antibacterial and hemolytic assays

We examined the antibacterial activity of P001, P002, and P076 against one Gram-positive (*S. aureus*) and three Gram-negative (*A. baumannii*, *E. coli*, and *P. aeruginosa*) bacteria, including three ESKAPE strains. For each species, we cultured a standard strain and a multidrug-resistant

262 (MDR) strain, and measured the MIC values of individual peptides using the broth microdilution
263 method. All the peptides exhibited inspiring bactericidal properties with MICs ranging from 0.20
264 to 15.18 μM (corresponding to 0.625 to 40 μg/mL, Figure 3a). P001 showed broad-spectrum
265 antibacterial capability with MICs less than 4 μM across all the eight strains of four bacteria species.
266 P002 featured lower MIC values in inhibiting Gram-positive bacteria *S. aureus*, while P076 mainly
267 inhibits Gram-negative bacteria *A. baumannii*, *E. coli*, and *P. aeruginosa*. Notably, the
268 experimentally measured MICs against *E. coli* agreed well with the predictions of AMPredictor
269 (Figure 1f), indicating the accuracy of our regression models.
270
271 The hemolytic analysis revealed that both P001 and P002 displayed dose-dependent toxicity to
272 mouse erythrocytes (Figure 3b), with average hemolysis higher than 50% at high concentrations
273 (20 μM, or 100 μg/mL). Nevertheless, the hemolysis of P076 was lower than 1% at concentrations
274 up to 70 μM. We also inspected the apparent cytotoxicity (CC_{50}) of three peptides on three
275 mammalian cell lines (Vero-E6, A549, Huh-7) using CCK8 assays. For the cell cultures, CC_{50}
276 values of all three peptides were well above 30 μM (Figure S8a). The CC_{50} of P076, specifically,
277 were in the range of 35.5 μM (for A549 cells from lung tissues) to 66.8 μM (for Huh-7 cells from
278 liver tissues). The *in vitro* safety assays pointed out thresholds of concentration for administrating
279 these peptides, which were adapted in subsequent animal experiments for estimation of safe dosage.
280
281 Our data suggest that all three peptides are potential candidates for inhibiting the clinically relevant
282 *A. baumannii*, which is a top-ranking pathogen with critical priority for development of new
283 antibiotics, as defined by WHO.[3] The MIC of P076 at eliminating the persistent MDRAB reached
284 0.21 μM, a value half of P001 and P002 (0.40 and 0.47 μM, respectively). Meanwhile, P076 was
285 comparable with P001 but more efficient than P002 against *E. coli* and *P. aeruginosa*. Despite a
286 slight inferiority in killing *S. aureus*, P076 owned a better biosafety. Taken together, the sequence
287 of P076 likely leveraged the balance between efficacy and safety, we therefore focused the
288 following *in vivo* evaluation and animal infection models on P076.
289
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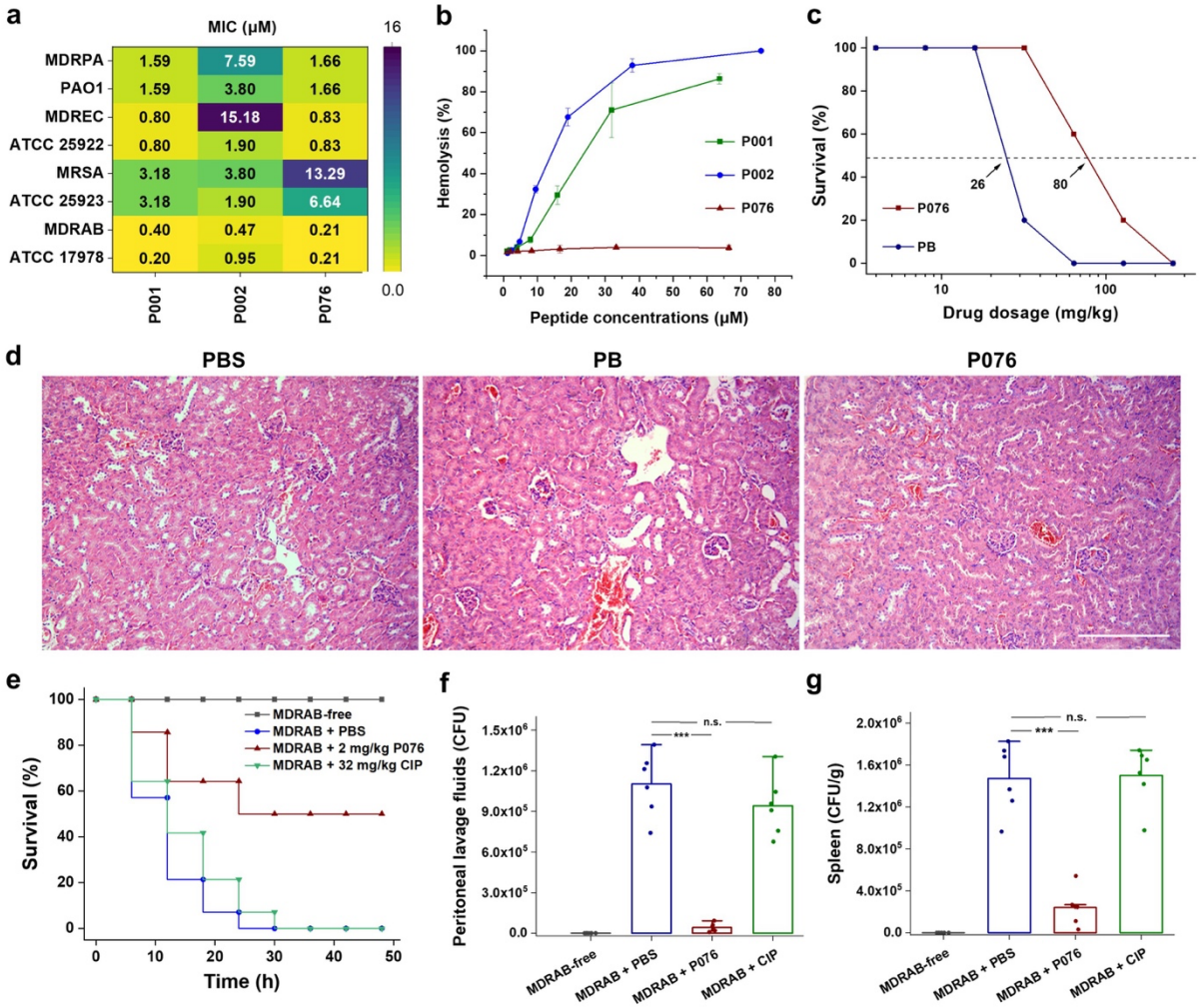

Figure 3. Antibacterial and toxicological evaluations of P076 peptide.

a. *In vitro* antibacterial assessment of peptides by determination of MICs.

b. Hemolysis of increasing concentrations of peptides. Results are shown as the means ± standard deviation (SDs).

c. Survival curves of mice given P076 and PB. Arrows indicate the drug concentration inducing a 50% death.

d. H&E staining of mouse kidneys. The scale bar indicates 200 μm.

e. Survival of infected mice treated with P076 and CIP.

f. g. Bacterial colonization of MDRAB in mouse peritoneal lavage fluid and spleen. Results are shown as the means ± SDs. ***, P<0.001. n.s., no significance.

304 ***In vivo* antibacterial and toxicological evaluations**

305

306 We first determined a safe dosage for administrating P076 on mouse models. A total of 140 C57
307 mice (randomly divided into 14 groups) were given increasing concentrations of P076 through
308 intraperitoneal injection, and the cyclic peptide antibiotic polymyxin B (PB) was employed as a
309 positive control. In spite of nephrotoxicity, PB is one of the polymyxin antibiotics that is
310 considered "the last ditch" for the treatment of infections caused by extensively drug-resistant
311 Gram-negative bacteria in clinic.[37] Animal survival was monitored for 48 h after a single drug
312 administration. The P076 concentration to induce a 50% death was calculated to be 80 mg/kg,
313 approximately 3-fold higher than that of PB (26 mg/kg, Figure 3c). Hematoxylin-eosin (HE)
314 staining further verified that no pathological changes were observed in the mice liver, spleen and
315 kidneys of mice given 32 mg/kg P076 after 12 h, whereas an apparent renal tubular injury was
316 discovered in PB-treated mouse (Figure 3c and S5a).

317

318 Encouraged by the biocompatibility of P076, we further conducted an *in vivo* antibacterial assay
319 using a mouse peritoneal infection model.[38] A total of 3×10^7 CFU of MDRAB was
320 intraperitoneally injected in C57 mouse to establish infection symptoms. P076 was administered
321 at 2 mg/kg at 0.5 and 2.5 h after bacterial invasion. As the positive control treatment, we employed
322 a single injection of 32 mg/kg ciprofloxacin (CIP) at 0.5 h after infection. The mice given PBS
323 (blank buffer) or CIP all deceased after 48 h, while 50% of the mice treated with P076 remained
324 alive (Figure 3e). Additional investigations revealed that the bacterial colonization in the peritoneal
325 lavage fluid of PBS-treated mouse was averagely 1.1×10^6 CFU, which was 25 times higher than
326 that in the mice treated with P076 (4.2×10^4 CFU, $P<0.001$), whereas CIP featured less of effect
327 on the number of bacteria (Figure 3f). Similarly, P076 dramatically decreased the bacterial loads
328 in the mouse spleen (about seven times lower, Figure 3g and S5b), thus demonstrating the prospect
329 of P076 to be developed into a new antibiotic.

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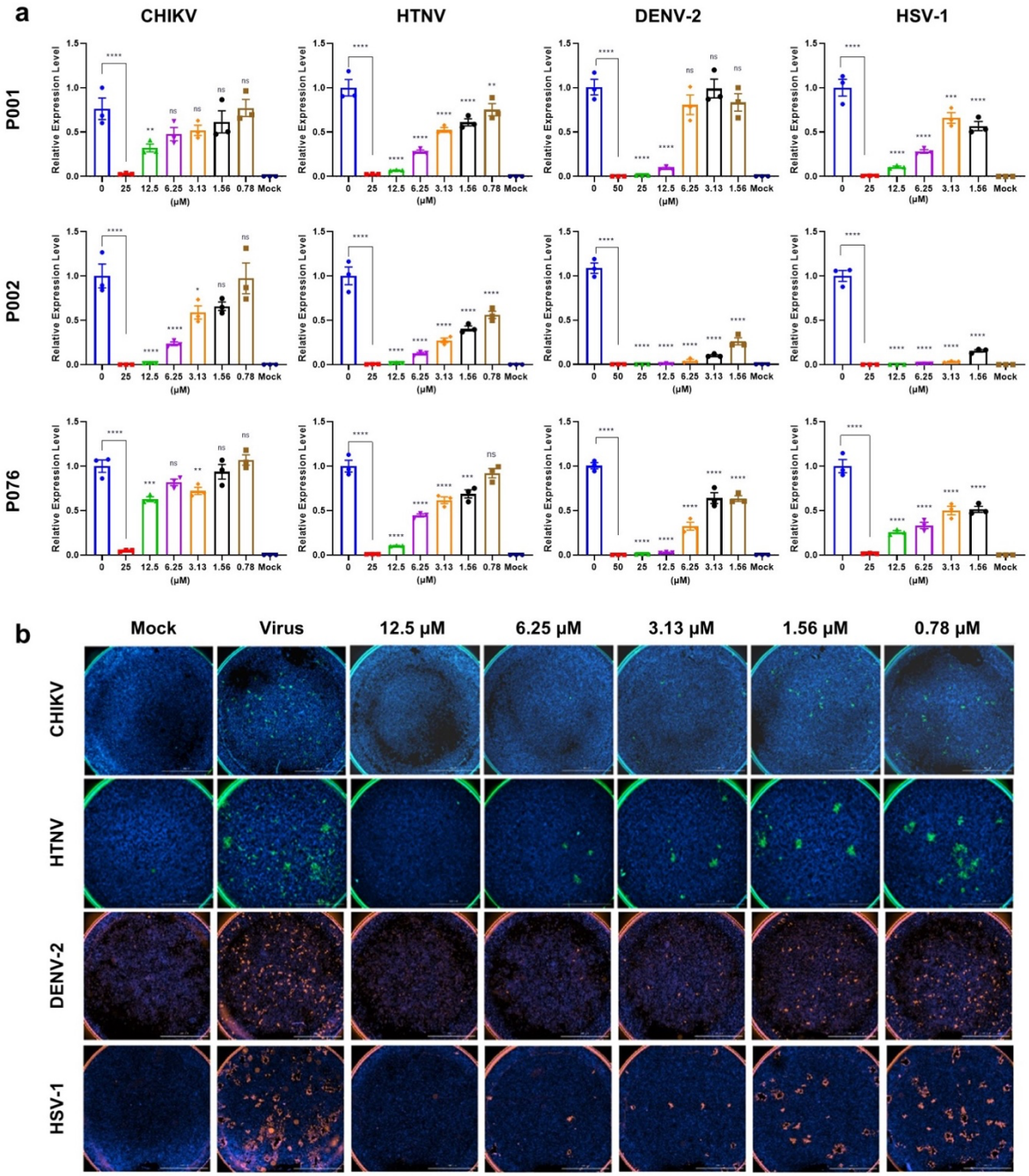

Figure 4. Antiviral assays of AMPs against CHIKV, HTNV, DENV-2, and HSV-1.

a. Quantitative real-time PCR of virus RNA with gradient concentrations of P001, P002, and P076.

b. Immunofluorescence results of four viruses with gradient concentrations of P002.

All experiments are performed in triplicates. Column bars are means ± SEMs. * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001. ns, no significance.

338 **Broad-spectrum antiviral assays**

339

340 A cohort of traditional antimicrobial peptides have been rediscovered with antiviral abilities.[39] One
341 significant reason is that their membrane-disrupting capacity can adapt to both bacterial
342 membranes and viral envelopes, which may be captured by AMPredictor and AVP classifier filters.
343 Thus, we tested the antiviral activities of P001, P002, and P076, which passed our dual filters. Our
344 experiments included five typical enveloped viruses. Chikungunya virus (CHIKV), Hantaan virus
345 (HTNV), Dengue virus 2 (DENV-2), Herpes simplex virus 1 (HSV-1), and severe acute respiratory
346 syndrome coronavirus 2 (SARS-CoV-2, and the BA.2. omicron variant) are all pathogens of the
347 past or ongoing epidemics, such as the chikungunya fever, hemorrhagic fever with renal syndrome,
348 dengue, encephalitis, and COVID-19. Hence, the peptide drugs may be repurposed as preventions
349 against viral infections.

350

351 We incubated the peptides with different viruses before they invaded the cultured cells and self-
352 amplified. We analyzed the potential antiviral effects of P001, P002, P076 at the mRNA and protein
353 levels, respectively. At the mRNA level, the quantitative real-time PCR suggested dose-dependent
354 inhibition of viral-specific gene expressions (E1 gene for CHIKV, S gene for HTNV, VP16 gene
355 for HSV-1, NS5a gene for DENV-2, ORF for SARS-CoV-2, Figure 4a and Figure S6b). At the
356 protein level, we applied fluorescence-conjugated antibodies to visualize trace amounts of virus
357 populations. Corroborating with the RT-PCR results, P002 nearly abolished all viral infections at
358 12.5 μM (Figure 4b, Figure S6a and Figure S7). To quantify the immunofluorescence results, we
359 plotted the log-scale fluorescent focus forming unit (FFU) against varying peptide concentrations
360 (Figure S8b). Among the three peptides, P002 exhibited the highest potential with EC_{50} of 0.37
361 μM (against CHIKV) to 2.08 μM (against HTNV) (Figure 4b, Table S4). The EC_{50} of P076 and
362 P001 were in the range of 1.62 to 2.67 μM (except for inhibiting DENV-2).

363

364 Additionally, we tested the antiviral effects of P135 and P244 from our second round of generation.
365 Both the immunofluorescence and qRT-PCR results showed that these AMPs were active broad-
366 spectrum antiviral peptides against four viruses (Figure S9, S10). In particular, these two AMPs
367 showed a significant inhibition on DENV-2, for which P135 obtained an EC_{50} of 0.09 μM (Table

368 S3). Hence, we demonstrated the effectiveness of GAN and AMPredictor combined pipeline on
369 discovering new antiviral peptides.
370
371 Combining the EC_{50} and CC_{50} calculations, we calculated the selectivity index (SI = CC_{50}/ EC_{50})
372 as the experimental guides of safety versus efficacy (Table S5). The SI of P002 against four viruses
373 are 149.21, 31.75, 51.05, and 59.01, three of which outperformed the SI of P001 and P076,
374 especially for the case of HTNV. Hence, we further observed the performance of P002 with
375 transmission electron microscopy. For the control set, the viruses were allowed to propagate in
376 cells for days and identified as assembled particles in cytosol (Figure 5a, b). When the viruses were
377 mixed with 10 μM P002 before addition to the cells, the viral particles became absent while the
378 cell morphology kept intact, indicating the broad-spectrum inhibition effects of P002 (Figure 5c,
379 d).
380
381 Because we observed a gradient of antimicrobial and antiviral activities from P001, P002, and
382 P076 with their individual preferences against bacteria and viruses, we aimed to probe the
383 interactions between peptides and different phospholipid assemblies, thus deriving feasible means
384 judging their underlying preferences. We performed all-atom molecular dynamics simulations with
385 molecular models of bacterial membranes and viral envelopes, placed each peptide adjacent to the
386 phospholipid bilayers, and simulated them attaching to the membrane (Figure S11a, b). For the
387 model of Gram-negative bacteria systems, the MM/PBSA calculations and ΔG_{bind} distinguished
388 P002 from P076 and P001 (Table S10). Evaluated by ΔG_{bind} (-465.79 ± 11.39 kJ/mol), P076 would
389 associate with the membrane of Gram-negative bacteria tighter than others. For the model of viral
390 envelopes, all binding energies were weaker than that to bacteria. The ranking of $\Delta G_{nonpolar}$
391 corroborated with the antiviral potential of P002. Combining this energy term with a geometrical
392 parameter (average distance between the COM of peptide and phosphorus atoms in the top leaflet
393 on z-axis) may predict the behavior of P002 better than $\Delta G_{nonpolar}$ alone (Figure S11c).
394

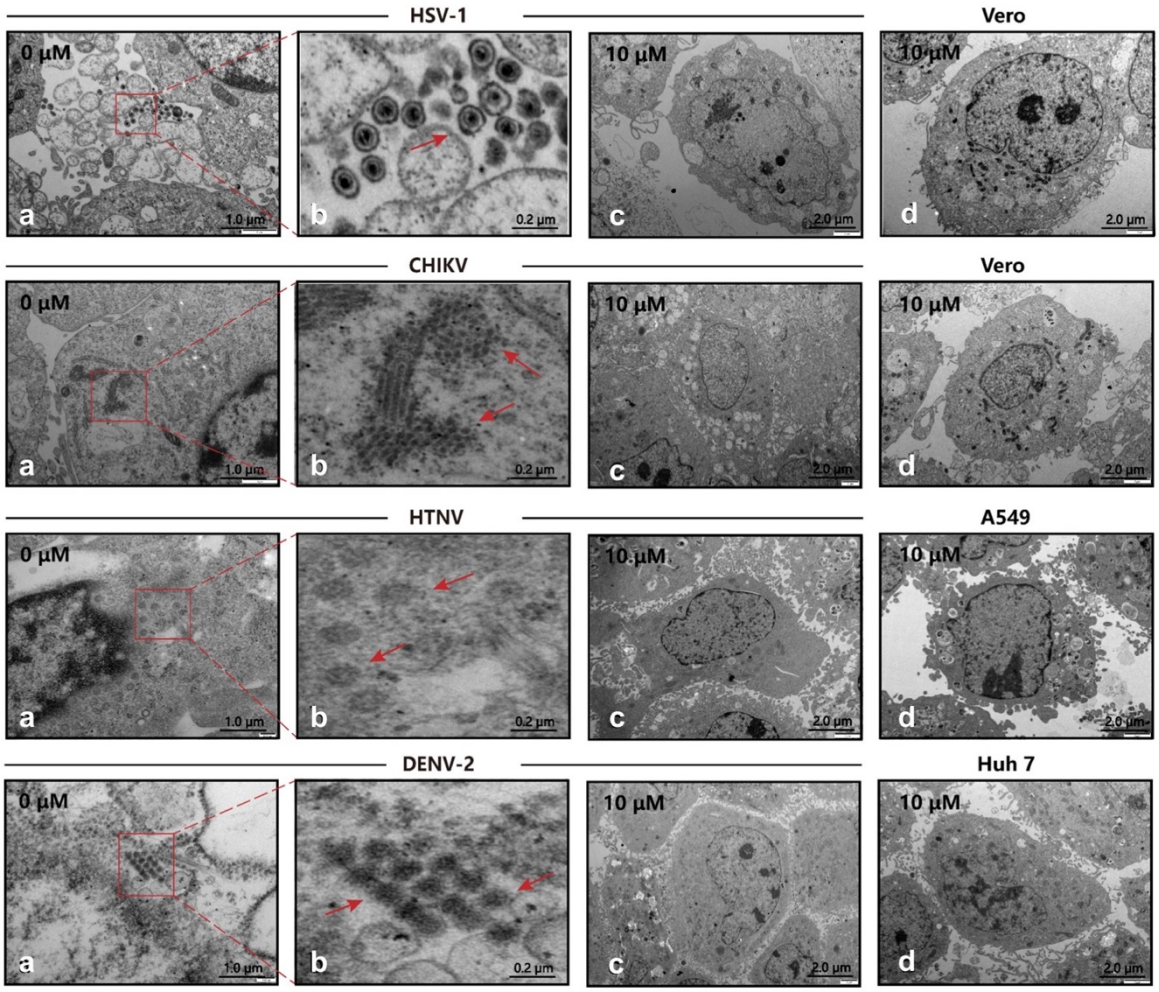

Figure 5. Transmission electron microscopy (TEM) of viral infection with P002.

a. b. Cells infected with virus, where the red arrows mark the viral particles.

c. Cells are treated with 10 μM P002 and infected with virus (MOI=1), where no viral proliferation is observed.

d. Control of intact cells with 10 μM P002.

All experiments are performed in triplicates.

Membrane-penetration mechanisms

We hypothesized that a common target among bacteria and viruses was the membrane components. To shed light on the possible membrane-penetration abilities of designed peptides, we applied

408 quantitative measurements, first by focusing on signature components of Gram-negative bacterial
409 outer membranes. We measured the binding of three peptides to lipopolysaccharide (LPS) via
410 biolayer interferometry (BLI). More P076 and P001 peptides were recruited to immobilized LPS
411 than that of P002 at 500 nM (Figure 6b). Further experiments demonstrated that P076 bound to
412 lipid A, the innermost part of LPS, at an equilibrium dissociation constant (K_D) of 56.1 nM. This
413 binding affinity of P076 to lipid A was slightly higher than that of P001 (65.7 nM) and was
414 approximately 2.4-fold less than that of P002 (135 nM, Figure 6a). Then we directly investigated
415 the membrane permeability by 1-N-phenylnaphthylamine (NPN) uptake assay. NPN is a
416 fluorescent dye for detecting the impaired outer membrane of Gram-negative bacteria. P076
417 elicited a stronger membrane penetration relative to P001 and P002 (wavelength scanning in
418 Figure 6c). Together, our results suggested that P076 could efficiently bind to and damage bacterial
419 membranes.
420
421 To gain a more comprehensive understanding of AMP-membrane interactions with fine content of
422 amphiphilic assemblies (both outer membranes and inner membranes), we also observed the
423 membrane attachment and partial insertion of AMPs via all-atom molecular dynamics simulations.
424 The top leaflet of Gram-negative bacterial outer membranes was built as simplified *E. coli* LPS
425 (lipid A and R1 core). With an initial equilibrated position above the membrane surface, the peptide
426 started to approach the oligosaccharides of LPS and then bind to them. During the simulation
427 processes, P002 and P076 gradually inserted into the outer leaflet, and represented poses binding
428 to LPS at 100 ns (Figure 6e). We also measured the distance between the COM of peptide and
429 phosphorus atoms of lipid A on z-axis in the latter 50 ns of trajectories (Figure 6d). Compared with
430 the initial value about 4 nm (showing as dashed line), this metric of P002 and P076 declined to
431 less than 2 nm. Nevertheless, P001 performed weaker interactions with an increasing distance,
432 which was consistent with the membrane-disrupting experiment in Figure 6c. Moreover, three
433 AMPs tended to attach to the membrane in the MD simulations with lipid bilayer system
434 mimicking the inner membranes of Gram-negative bacteria (Figure S11b). Collectively,
435 experimental characterizations along with computational simulations indicate the membrane-
436 acting mechanisms of our AMPs when playing antimicrobial roles. Further explanation of
437 membrane-disrupting details or other potential biomolecular targets remains to be explored in the
438 future.

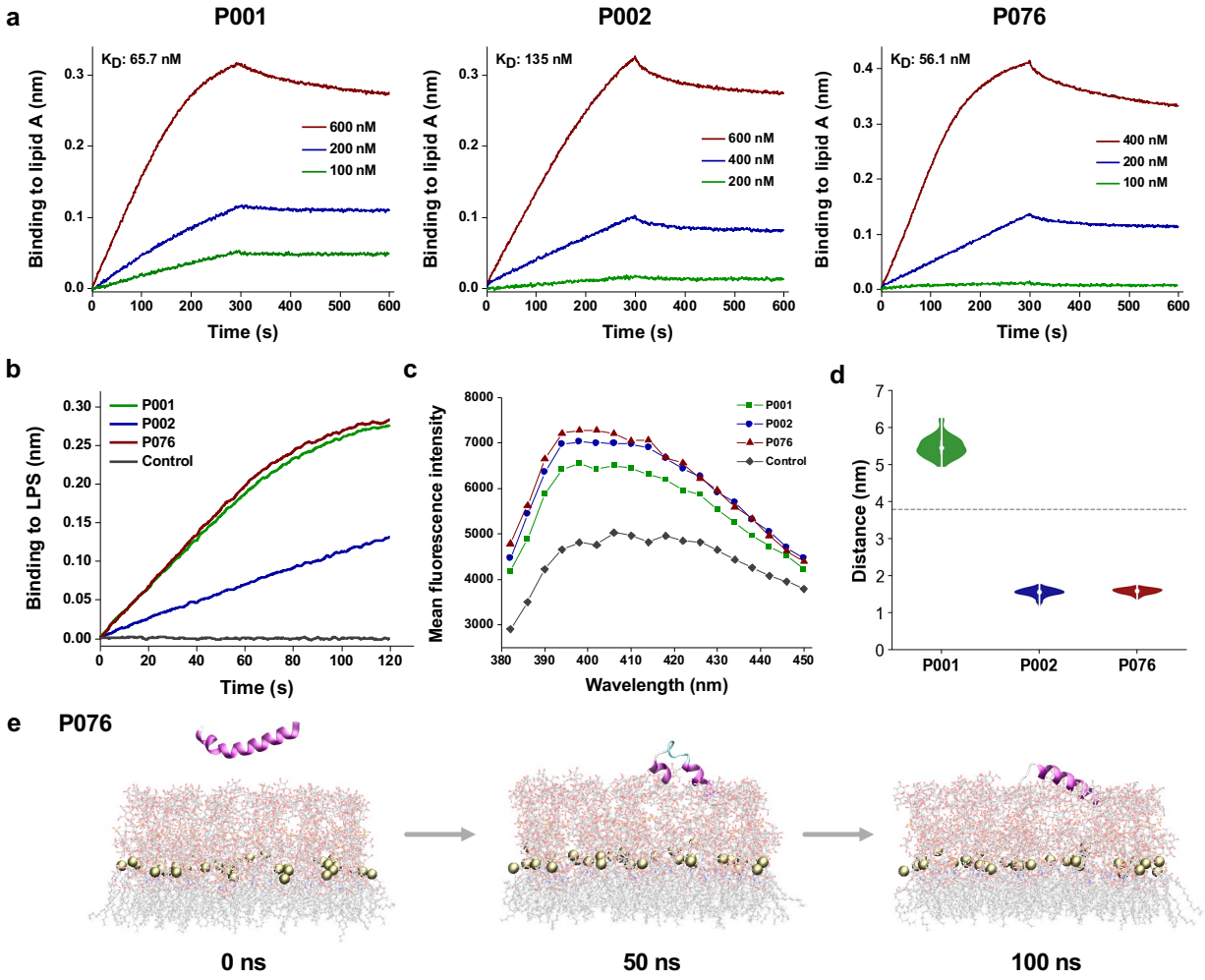

Figure 6. Bacterial membrane-acting assessment of AMPs.

a. Binding kinetics of three AMPs to lipid A measured by biolayer interferometry.

b. Binding thicknesses of 500 nM AMPs to immobilized LPS within 120 seconds.

c. Fluorescence intensity of NPN after excitation at 350 nm. The maximum emission appeared at approximately 396 nm, where P001, P002, and P076 were detected higher intensity than control.

d. The distance between COM of peptides and phosphorus atoms of lipid A (around the midplane of G- bacterial outer membranes) during the last 50 ns of the MD simulation trajectories. The gray dashed line displays the initial distance at 0 ns.

e. Snapshots of P076 with G- bacterial outer membranes at 0 ns, 50 ns, and 100 ns. Only the top leaflet (LPS) is shown. Tan balls represent the phosphorus atoms of lipid A molecules.

452 ## Discussion

453

454 The traditional 'lock and key' model for therapeutic molecule design requires knowledge of target
455 structures.[40] When the targets are biological membranes, the means of docking and ranking by
456 binding affinities (K_D) can easily become futile. The rapid development of deep neural networks,
457 on the other hand, has facilitated self-supervised learning of meaningful molecule representations
458 in hidden dimensions.[29] Combined with vast data of known AMPs, numerous deep learning models
459 have enabled mining of new antibiotics.[18, 19] In the current study, we deployed a new combination
460 of descriptors to extract representations of biological sequences at residual and protein levels. First,
461 we transformed input amino acid sequences into Morgan fingerprints to encode the chemical
462 features of each residue. Second, the scaffolds of AMPs are closely relative to their antimicrobial
463 function, but rarely considered at the structural level in recently reported models (except for
464 sAMPpred-GAT[16]). Instead of using limited amounts of crystal structures, we introduced predicted
465 contact maps of peptides as the edge encoding of peptide graphs. Finally, we added the embedding
466 from ESM as the residue node features to present the associations of a single peptide sequence in
467 the large protein repertoire. ESM was trained on millions of natural protein sequences via mask
468 attention mechanism and assisted in few-shot learning via transferring the related knowledge of
469 protein sequences.[29] The resulting AMPredictor accurately predicted the MICs of three *de novo*
470 design AMPs in our study. As an independent test on 28 new AMPs discovered by different models,
471 AMPredictor also scored well (Table S6).

472

473 Despite the ability of deep learning model to guide targeted generation and screen of functional
474 peptides, it lacks the merit of molecular docking program to explicitly consider subtle
475 environmental features. For a particular sequence, we found it was difficult to tell its preference
476 towards bacterial membranes or viral envelopes, due to slight differences in their lipid
477 compositions. Consequently, we observed broad antimicrobial activity for P001, broad antiviral
478 activity for P002, while P076 demonstrated superior capacity on *A. baumannii*. As a control, we
479 made a tiny change of P076 C-terminal with amide modification but observed loss of functions
480 (Table S7). The change of a few atoms on an intact sequence can lead to dramatic functional
481 consequences, which may be challenging for neural networks to capture.

482

483 Recent studies indicated unexpected side effects of traditional antibiotics. Instead of restraining
484 the growth of carbapenem-resistant *Enterobacteriaceae* in the intestine environment, broad-
485 spectrum antibiotics reduced gut microbial populations and depleted beneficial metabolites, thus
486 facilitated colonization of *Enterobacteriaceae* species.[41] With more limitations of conventional
487 small molecule antibiotics discovered, AMPs are attractive alternatives to conventional antibiotic
488 treatment. Their wide bioactivity spectra can expand to virus, thus minimizing the cost of screen
489 multiple drugs. Our results suggested efficient *in silico* screen tools plus rigorous experimental
490 validations could uncover highly potent peptide drugs without comprising on safety, and large-
491 scale animal experiment could facilitate translation towards clinical research. In summary, we
492 believe that a mature workflow involving sequence generation and evaluation can promote the
493 discovery of ideal AMPs to eventually alleviate the hazard of drug-resistant diseases.
494

Methods

Data Curation. Our AMP dataset is collected from PepVAE,[20] containing 3280 antimicrobial peptide sequences with their minimal inhibitory concentrations (MICs) in logarithmic form. To integrate various AMP sequence and bioactivity data, Witten et al. developed the Giant Repository of AMP Activity (GRAMPA).[42] Since the MIC values change when targeting different microbial strains, only AMPs inhibiting *E. coli* are selected. Also, to simplify the input and reduce accidental effect, peptides without modifications are filtered so that no cysteine appears (amino acid composition in Figure S1a). The sequence length of AMPs are less than 40 amino acids, and the majority of lengths (~ 60%) are between 10 and 20 (Figure S1a). Labels, i.e., logMIC values, are distributed from -1 to 4. During the training process, the dataset is divided into the training, validation, and testing sets in a ratio of 8:1:1 randomly. The entire AMP dataset (3280 sequences) is used for training the generator v1. The AVP dataset for training the generator v2 is collected from AVPdb (accessed in Aug. 2023). AVPs with lengths of more than 60 amino acids are excluded. After removing overlaps with the AMP dataset, 1788 AVPs are kept and added to the training set of generator v2.

Generator. The generative adversarial network (GAN) is composed of a generator network and a discriminator. Through the co-training strategy, the generator evolves to produce ideal sequences from random noises z, fitting the distribution of real samples and faking the discriminator.

$$min_G max_D V(G,D) = E_{x\sim p_{real(x)}}[logD(x)] + E_{z\sim p_{fake(z)}}\left[log\left(1 - D\big(G(z)\big)\right)\right] \quad (1)$$

For the combined training of GAN generator and discriminator, the AMP sequences were encoded with amino acid factors (AAF). AAF encoding represents five physiochemical values, including the polarity, secondary structures, molecular size, codon diversity, and charges.[27] The GAN architecture was utilized to generate novel AMP sequences, where three layers of 2D convolution were inside both the generator and discriminator.[43] Loss function modified with WGAN-GP strategy was taken to avoid mode collapse, and the optimizer was kept as RMSProp. The learning rate was 1e-4. Cross training ratio between generator and discriminator was set as 1:5. Deep learning-based models were trained on an NVIDIA GeForce RTX 2080 Ti GPU. After training for

1000 epochs, string sequences are calculated via cosine similarity. A batch of sequences were generated with the limited length of 30 amino acids. Redundant alanine (A) at the end of every sequence were removed, therefore leaving sequences with variable length.

Predictor. Antimicrobial peptide activity predictor (AMPredictor) was built based on PyTorch and PyTorch Geometric library. All input sequences were treated with padded zeros to reach unified lengths of 60. For chemical fingerprint, RDKit (www.rdkit.org) was used to transform each amino acid into 2048-bit Morgan fingerprint in peptide sequences. For each peptide, the fingerprint vectors were concatenated and contrasted into a 60-dimensional vector by average.[44] Pretrained language model esm1b_t33_650M_UR50S was used to provide the embeddings and the contact maps.

During the training process, the optimizer was Adam, and loss function was the mean squared loss. Batch size and learning rate were decided upon grid-search, whose values were 128 and 0.001, respectively (Figure S1b). Evaluation metrics MSE, RMSE, PCC, and CI are calculated as following:

$$MSE = \frac{1}{n}\sum_{1}^{n}(b_i - a_i)^2 \tag{2}$$

$$RMSE = \sqrt{\frac{1}{n}\sum_{1}^{n}(b_i - a_i)^2} \tag{3}$$

$$PCC = \frac{\sum_{i=1}^{n}(a_i - \bar{a})(b_i - \bar{b})}{\sqrt{\sum_{i=1}^{n}(a_i - \bar{a})^2 \sum_{i=1}^{n}(b_i - \bar{b})^2}} \tag{4}$$

$$CI = \frac{1}{Z}\sum_{a_i > a_j} h(b_i - b_j) \tag{5}$$

where a_i is the real activity label of peptide i, and b_i is the predicted activity value, Z is the scale constant, and $h(b)$ is the step function.

For comparisons in Figure S2, five baseline models were implemented by DeepPurpose package.[45] The amino acid composition encoding was used as the input of MLP, while one hot encoding was

550 for CNN, CNN-GRU, and CNN-LSTM. Fragment partition fingerprint of peptides was fed into
551 the Transformer encoder.
552
553 In ablation study, three encoding approaches (chemical fingerprint, contact matrix, and ESM) and
554 their different combinations were tested individually. It was noted that the peptide graph exists
555 only when both the node embedding and contact matrix are present together. Therefore, a CNN
556 with the same number of layers and output dimension was adopted instead of the GCN in ablation
557 test.
558
559 **Peptide synthesis.** All the peptides were synthesized by DGpeptide Co., ltd. with 95% purity via
560 solid-phase peptide synthesis procedures. Mass spectrometry was used to verify the molecular
561 weights (Figure S4, S12-S35).
562
563 **MIC detection.** The minimum inhibitory concentrations (MICs) of peptides against bacteria
564 including *A. baumannii* (ATCC 19606), multidrug-resistant *A. baumannii* (MDRAB) ,[46] *S.*
565 *aureus* (ATCC 25923), methicillin-resistant *S. aureus* (MRSA, ATCC 43300), *E. coli* (ATCC
566 25922), multidrug-resistant *E. coli* (MDREC),[47] *P. aeruginosa* (PAO1), and multidrug-resistant
567 *P. aeruginosa* (MDRPA),[48] were determined using the broth microdilution method recommended
568 by the Clinical and Laboratory Standards Institute (CLSI) with modifications reported by the
569 Hancock laboratory.[49] Peptides were prepared in 0.01% acetic acid containing 0.2% BSA at
570 concentrations of 6.25, 12.5, 25, 50, 100, 200, 400, 800, 1600, and 3200 μg/mL. Bacteria grown
571 to the mid-logarithmic phase in Meller-Hinton broth (MHB) were diluted to 2×10^5 CFU/mL.
572 Aliquot of 100 μL of the bacterial suspension was then co-incubated with 11 μL of the peptides at
573 37°C in a sterile polypropylene microtiter plate (3365, Corning, Shanghai, China). An M2e
574 microplate reader (Molecular Devices, Silicon Valley, CA, USA) was applied to detect the
575 bacterial absorbance at 600 nm after 18 h. The recorded MIC was the lowest concentration of
576 peptides causing a 70% reduction in bacterial turbidity.
577
578 **Hemolytic assay.** The hemolysis of peptides was evaluated as previously described in Ref.[50]
579 Briefly, the fresh mouse blood was centrifuged at 1000 g for 10 min and washed with cold PBS
580 three times. The erythrocytes were collected and diluted in saline solution to prepare a 2% (v/v)

581 suspension. A total of 75 μL of the erythrocyte suspension was co-incubated with 75 μL of the
582 peptides (6.25, 12.5, 25, 50, 100, 200, and 400 μg/mL) at 37°C for 1 h. After centrifuging at 1000
583 g for 10 min, 60 μL supernatant was obtained, and the absorbance was determined at 414 nm. This
584 experiment was conducted in triplicate and repeated twice.
585
586 ***In vivo* antibacterial evaluation.** The therapeutic efficacy of P076 peptide against drug-resistant
587 bacteria was analyzed using a mouse peritoneal infection model.[38] The 8-week-old female C57
588 mice (n = 10) was intraperitoneally administered with 3×10^5 CFU/mL of MDRAB. P076 (2
589 mg/kg) was given by intraperitoneal injection at 0.5 and 2.5 h after bacterial infection.
590 Ciprofloxacin (CIP, C129896, Aladdin, 32 mg/kg) was employed as the control. Mouse survival
591 was monitored for 48 h. For detection of the bacterial colonization, another 24 mice were randomly
592 divided into four groups (n = 6 for each group). The mice were euthanized 12 h after MDRAB
593 infection, and the peritoneal cavity was rinsed with 2 mL of PBS. Additionally, mouse liver and
594 spleen were obtained by surgery and homogenized in 1 mL of PBS. The lavage fluid and tissue
595 suspension were both diluted for bacterial colony counting using an MHB agar plate as described
596 in MIC detection.
597
598 ***In vivo* toxicological evaluation.** The 8-week-old female mice (18-22 g) were randomly divided
599 into 14 groups (n = 10 for each group). P076 peptide and polymyxin B (PB, P105490, Aladdin,
600 Shanghai, China) were given to the mice by intraperitoneal injection at 4, 8, 16, 32, 64, and 128
601 mg/kg. Mouse survival was monitored for 48 h. For pathologic analysis, mouse liver, spleen, and
602 kidneys were obtained by surgery for hematoxylin-eosin (HE) staining after treatment with 64
603 mg/kg P076 and PB for 12 h. Mice were cared for and treated in accordance with the NIH
604 guidelines for the care and use of laboratory animals (NIH Publication No. 85e23 Rev. 1985).
605 Animal experiments were conducted with approval from the Animal Experimental Ethics
606 Committee of the Army Medical University (AMUWEC20223290).
607
608 **Cell cultures**. Vero-E6 (African green monkey kidney cell line), Huh-7 (Hepatocellular carcinoma
609 cell line), A549 (Human Lung carcinoma cell line) and HeLa-hACE2 were cultured in DMEM
610 (Thermo Fisher Scientific, Waltham, USA) containing 10% fetal bovine serum in a 5% CO_2
611 incubator at 37°C. HSV-1 and CHIKV were propagated in Vero-E6 cells. DENV-2 and HTNV

612 were propagated in Huh-7 and A549 cells, respectively. And SARS-CoV-2 were propagated in
613 HeLa-hACE2 cells.
614
615 **Cytotoxicity measurements.** The Vero-E6, Huh-7 and A549 cells (2×10^4 cells/well) were
616 inoculated in the 96-well culture plate and grown overnight to 90% confluence at 37°C. The cells
617 were treated with different concentrations of peptides (P001, P002, and P076) for 1 hour, and the
618 same concentration of PBS was used as a control. After 48 hours of cell culture, 10% CCK8
619 solution was added to each well and incubated at 37°C for 1 hour. The absorbance was measured
620 at 450 nm using a microplate reader. The CC_{50} represents the peptide concentration at the 50% of
621 viability of uninfected cells.
622
623 **Immunofluorescence assay.** The cells were cultured in a 96-well plate (2×10^4 cells/well).
624 Different viruses were mixed with P001, P002, or P076 peptides for 10 min before infecting the
625 cells for 1 h. The infected cells were then cultured with DMEM containing 10% serum for 24-48
626 h. After discarding the supernatant and washing it three times with PBS, it was permeated with
627 PBS containing 0.2% Triton X-100 (containing 0.1% BSA) at room temperature for 30 minutes.
628 Then, the cells were mixed with an antiviral antibody at 4°C overnight. After washing with PBS
629 three times, the cells were incubated with fluorescence-conjugated secondary antibodies at room
630 temperature for 1 hour. Cell nuclei were stained using 4',6'- diamidino-2-phenylindole (DAPI) in
631 the dark for 5 min, and images were obtained by fluorescence microscope.
632
633 **Real-time PCR.** The cells were cultured in a 6-well plate (2×10^5 cells/well) following the same
634 cell culture procedures as in the immunofluorescence assay. Cellular RNAs were extracted and
635 purified using a viral RNA extraction kit (Qiagen, Dusseldorf, Germany), and then cDNA was
636 synthesized and monitored with SuperScript-III kit (Takara, Dalian, China). qRT–PCR analysis
637 was performed using SYBR Green (BIO-RAD, California, USA) according to the manufacturer's
638 protocol. Viral RNA expression was calculated using the $2^{-\Delta\Delta C.T.}$ (cycle threshold) method
639 normalized to GAPDH expression. The qRT–PCR primers sequences for HTNV, CHIKV, HSV-
640 1 and DENV-2 are listed in the Table S8, while the detection kits (Daan gene, Guangzhou, China)
641 were used for SARS-CoV-2 wild-type and BA.2 subtype.
642

643 **Transmission electron microscopy.** The cells were cultured in a 6-well plate (2×10^5 cells/well)
644 following the same cell culture procedures as in the immunofluorescence assay. The virus-infected
645 cells were collected by centrifugation to prepare cell particles and fixed in cacodylate sodium
646 buffer containing 1% glutaraldehyde (0.2 M, pH 7.2). The fixed sample was dehydrated by acetone
647 solution and embedded in epoxy resin. The sample was polymerized at 60°C for 3 days. The resin
648 block was used to prepare ultrathin slices (50-70 nm thick). These sections were supported by
649 copper mesh, negatively stained with uranyl acetate and lead citrate (electron microscopy) and
650 observed with a JEM100SX transmission electron microscope (JEOL, Tokyo, Japan).
651
652 **Biolayer interferometry**. The Octet Red96 BLI platform (Sartorius BioAnalytical Instruments,
653 Bohemia, USA) was utilized to measure the binding kinetics of three peptides to lipid A (L5399,
654 Sigma, Shanghai, China). Peptides were diluted with 75 mM NaCl solution at concentrations of
655 100, 200, 400, and 600 nM. The amine reactive second-generation (AR2G) biosensors were
656 processed with 1-ehtyl-3-(3-dimethylaminopropy) carbodiimide hydrochloride (EDC, E1769,
657 Sigma) and sulfo-N-hydroxysulfosuccinimide (s-NHS, 56485, Sigma). Subsequently, lipid A was
658 loaded onto the activated AR2G biosensors at 30°C for 5 min, and both the processes of association
659 and disassociation were monitored for 5 min to measure the kinetic constants. The equilibrium
660 dissociation constant (K_D) was defined as the division of dissociation rate constant (K_{off}) and the
661 association rate constant (K_{on}). In case of LPS, AR2G biosensors immobilized with 15 μg/mL LPS
662 (L2880, Sigma) were incubated with 500 nM peptides for 2 min in 75 mM NaCl solution. The
663 binding thickness was recorded and analyzed using ForteBio Data Analysis 7.0 software.
664
665 **NPN uptake assay.** MDRAB in mid-logarithmic phase was diluted to 1×10^8 CFU/mL in 10 mM
666 sodium phosphate buffer (pH 7.4). Then 10 μL of each peptide was mixed with 190 μL of MDRAB
667 at 37°C for 1 hour. Afterwards, 1-N-phenylnaphthylamine (NPN, 104043, Sigma, Shanghai, China)
668 was added with a final concentration of 10 μM. The fluorescence intensity was assessed with the
669 wavelength ranging from 380 to 450 nm at 2 nm intervals, after excitation at 350 nm using a Tecan
670 Infinite M1000 Pro microplate reader (Mannedorf, Zurich, Switzerland). Each experiment was
671 conducted in duplicate and repeated three times.
672

673 **Molecular dynamics simulations.** The initial structures of peptides were built via AlphaFold2
674 single sequence version.[51] Lipid bilayer models were constructed by CHARMM-GUI server along
675 with placing parallel peptides approximately 2 nm above the membrane surface.[52] Nine systems
676 were built for three peptides versus three kinds of lipid bilayers (viral envelopes, Gram-negative
677 bacterial inner membranes and outer membranes).[39, 53] Ions of 150 mM NaCl were added to
678 neutralize the system, and the calcium ions were used for LPS in outer leaflet of bacterial outer
679 membranes. Detailed system settings are recorded in Table S9. The standard CHARMM-GUI
680 equilibration protocols including NVT and NPT ensembles were adopted, keeping the temperature
681 at 310K. All-atom MD simulations were performed using GROMACS 2021.2 with the
682 CHARMM36 force field and the TIP3P water model for 100 ns, with a time step of 2 fs.[54, 55] Three
683 independent run was performed for each system. The visual molecular dynamics (VMD) software
684 version 1.9.4 was used for visualization.
685
686 The distance between the center of mass (COM) of peptides and phosphorus atoms in top leaflets
687 was calculated via *gmx distance*. Binding free energy was calculated using the molecular
688 mechanics Poisson-Boltzmann surface area (MM/PBSA) method on average of extracted 20
689 frames from the last 20 ns. The binding free energy of a peptide-membrane complex is calculated
690 as below:

691 $$\Delta G_{bind} = G_{complex} - (G_{peptide} + G_{membrane}) \quad (6)$$

692 $$G_{bind} = G_{polar} + G_{nonpolar} = \left(E_{elec} + G_{psolv}\right) + (E_{vdw} + G_{sasa}) \quad (7)$$

693 where E_{elec} is the Coulomb potential, E_{vdw} is the van der Waals energy, G_{psolv} and G_{sasa} are polar
694 and nonpolar solvation free energy. Following previous study, the dielectric constant was set as 7
695 for phospholipids and the entropy term was neglected.[56]
696

Data Availability

All the python codes of the AMP design model are available at https://github.com/ruihan-dong/GAN-for-AMP-Design and https://github.com/ruihan-dong/AMPredictor. An online version of AMPredictor can be accessed via https://huggingface.co/spaces/ruihan-dong/AMPredictor.

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Author Contributions

C.Z. and R.D. devised the project. C.Z., S.Y., and H.L. conceived the main conceptual ideas and were in charge of overall direction and planning. R.D. and F.G. developed the computational model. J.W., C.W., and G.Z. measured antimicrobial activities and performed animal experiments. X.W., R.L., Z.L., S.H., X.M., H.K., and J.L. carried out antiviral evaluations. Y.L. and P.Z. carried out anti-SARS-CoV-2 evaluations. C.Z. and R.D. analyzed the data and wrote the paper with input from all authors.

Conflict of Interest

There are no conflicts to declare.

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