European Journal of Medicinal Chemistry 186 (2020) 111901

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Antibacterial activity of lipo-α/sulfono-γ-AA hybrid peptides

Lulu Wei, Minghui Wang, Ruixuan Gao, Rojin Fatirkhorani, Jianfeng Cai*

Department of Chemistry, University of South Florida, 4202 E. Fowler Ave, Tampa, FL, 33620, USA

ARTICLE INFO

Article history:
Received 15 April 2019
Received in revised form
15 November 2019
Accepted 18 November 2019
Available online 19 November 2019

Keywords:
AA peptides
Peptidomimetics
Antimicrobial
Bacterial resistance
Solid-phase synthesis

ABSTRACT

Development of novel antimicrobial agents combating drug resistance is in an urgent need. Herein we report the design and synthesis of a series of short lipo-α/sulfono-γ-AA hybrid peptides. Several short peptides exhibit potent and broad-spectrum antimicrobial activity toward both Gram-positive and Gram-negative bacteria. Membrane depolarization and fluorescence microscopy studies indicate that these short lipo-α/sulfono-γ-AA hybrid peptides can mimic the mechanisms of HDPs to kill bacteria by disrupting bacterial membranes. In addition, these short peptides also show capability to eradicate the biofilm formation of *E. coli* even at very low concentration. The further development of lipidated α/sulofono-γ-AA hybrid peptides may lead to a new class of antibiotic agents to combat drug resistance.

1. Introduction

Antimicrobial resistance is one of the greatest threats to public health [1]. Among them, multidrug-resistant bacterial strains, such as Gram-positive bacteria methicillin-resistant *Staphylococcus epidermidis* (MRSE), methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus faecalis* (*E. faecalis*), Gram-negative bacteria *Escherichia coli* (*E. coli*), and *Pseudomonas aeruginosa* (*PA*), have caused severe and even lethal infections [2,3]. Thus, the development of new generations of antibiotics is of considerable significance.

Host-defense peptides (HDPs) developed in recent decades have been recognized as a potential approach to combat bacterial resistance [1,4,5]. Due to their antimicrobial mechanism of action which selectively compromises bacterial membranes, HDPs have shown good selectivity for bacteria and are believed to have lower tendency to induce antibiotic resistance than traditional antibiotics [2,6]. However, some inherent drawbacks still remain in the development of HDPs, such as susceptibility to proteolytic degradation and low-to-moderate activity [7,8]. New generations of antibiotic agents with minimal probability for resistance selection, as well as enhanced activity and stability, are still in an urgent need [3]. Among them, mimicry of the mechanism of action of HDPs by peptidomimetics is one of the viable strategies [8]. To date antimicrobial peptidomimetics, including β-peptides [9], peptoid-peptide hybrids [10], peptoids [11], acrylamide oligomers [12], and others, are reported [13]. Recently, our group has developed a new class of peptidomimetics termed "γ-AApeptides," as they are oligomers of N-acylated-N-aminoethyl amino acids (Fig. 1) [13,14]. We have designed a variety of γ-AApeptide derivatives to mimic the amphipathic structure of HDPs, and some of them exhibited excellent and broad activity against both Gram-positive and Gram-negative bacteria [15–22]. Among them, it is interesting that short antimicrobial peptide hybrids, containing α-amino acid units and γ-AA residues, exhibit potent antimicrobial activity and selectivity [22]. It is noted that compounds with small molecular weight are expected to be more drug-like molecules. As such, we intended to further explore the potential of this type of peptide hybrids for antimicrobial applications. Our previous findings [23] and other research groups [24,25] reveal that peptides/peptidomimetics with addition of a hydrophobic tail could enhance the interaction between peptides and bacterial membranes because the hydrophobic tail increases the lipophilicity of peptides. Herein we report the design and synthesis of short lipo-α/sulfono-γ-AA hybrid peptides, as well as their antimicrobial activity [26,27].

2. Results and discussion

We synthesized a series of short lipo-α/sulfono-γ-AA hybrid peptides on the solid phase and purified by HPLC adapted from previously reported protocols [28]. (see Supporting Information for details) These peptides contain one or two amphiphilic sulfono-γ-

* Corresponding author.
E-mail address: jianfengcai@usf.edu (J. Cai).

https://doi.org/10.1016/j.ejmech.2019.111901
0223-5234/

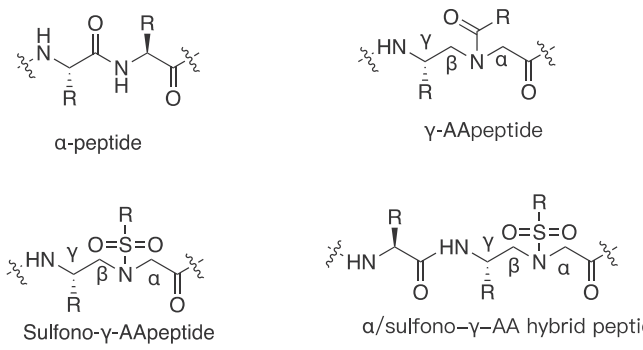

Fig. 1. General structures of canonical α-peptides, γ-AApeptides, sulfono-γ- AApeptides, and α/sulfono-γ-AA hybrid peptides.

AA peptide building blocks, and some of them have one additional lysine amino acid residue (Fig. 2). The lysine side chains provide positive charges whereas aromatic side chains are used as hydrophobic groups. Meanwhile, A lipid tail was conjugated at N terminus or C terminus to presumably increase their antibacterial activity.

Table 1 displays antimicrobial activity and hemolytic activity for each short lipo-α/sulfono-γ-AA hybrid peptide. As shown in Table 1, the first sequence **1**, containing just one sulfono-γ-AA building block and a C16 lipid tail, already exhibited good activity toward Gram-positive bacteria. The sequence **2**, with one lysine residue, displayed activity toward both Gram-positive and Gram-negative bacteria, suggesting the importance of peptide hybrid backbone [26,29]. Introduction of the C16 lipid tail at the C-terminus of both **1** and **2** leading to sequences **3**, and **4**. Interestingly, these two lipidated sequences show similar antibacterial activity to **1** and **2**. This result suggested that lipidation on either N or C terminus would not affect antibacterial activity of sequences.

To investigate the impact of the length of hydrophobic lipid tails, we next synthesized sequences **5**–**8**. The sequence **5** bearing a C10 lipid tail did not show any antibacterial activity at all, which is consistent to our previous findings that short lipid tails won't interact with bacterial membranes effectively [26,28]. As expected, the sequence **6** started to show antibacterial activity, whereas **7**, bearing a C14 lipid tail, show the most potent and broad-spectrum antimicrobial activity toward all tested Gram-positive or Gram-negative bacteria strains. It is more potent than **2** and **8** that bear C16 and C18 tails respectively, which suggests that this class of peptidomimetics has an optimal length of lipid tail to interact with bacterial membranes most strongly. To test if extra sulfono-γ-AA building block could enhance the activity, we set out to synthesize sequences **9**–**11.** Intriguingly, none of these sequences show improved activity compared to **7**, possibly due to the different ratio of cationic/hydrophobic groups. In order to assess the selectivity of these short lipo-α/sulfono-γ-AA hybrid peptides, we also tested hemolytic activity for the most potent compounds **7, 10 and 11** which exhibited broad-spectrum antimicrobial activity against almost all tested strains. As shown in Table 1, These compounds

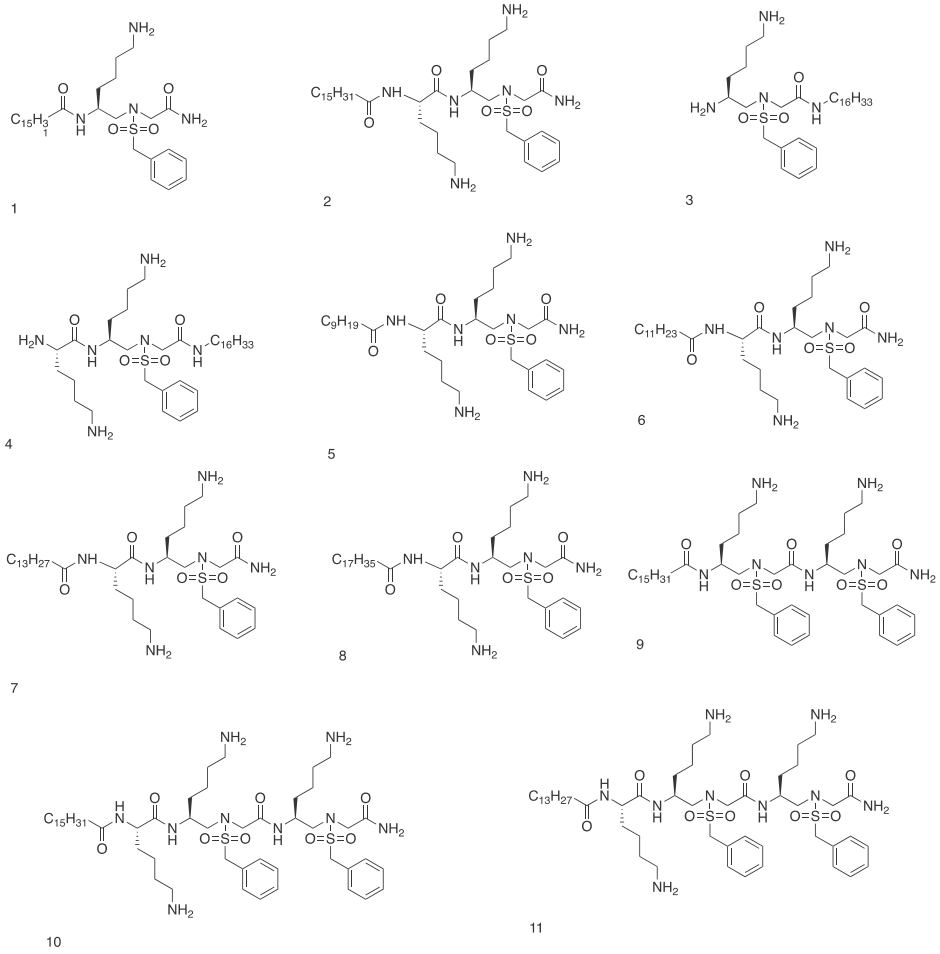

Fig. 2. The structures of compounds **1**–**11.**

Table 1
Antibacterial activity of compounds **1**–**11**
The bacteria used were methicillin-resistant *S. aureus* (MRSA) (ATCC 33591), methicillin-resistant *S. epidermidis* (MRSE) (RP62A), vancomycin-resistant *E. faecalis* (ATCC 700802), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853). Hemolysis activity was not measured for peptidomimetics that did not show antimicrobial activity for all tested strains.

Compound	MIC (μg/mL)					Hemolysis (HC_{50}, μg/mL)	Selectivity (HC_{50}/MIC_{MRSA})
	Gram-positive			Gram-negative			
	MRSA	MRSE	*E. faecalis*	*E. coli*	*P. aeruginosa*		
1	5	8	5	>50	>50	NT	
2	5	5	5	5	20	NT	
3	5	20	5	5	>50	NT	
4	5	20	5	5	>50	NT	
5	>50	>50	>50	>50	>50	NT	
6	20	20	>50	5	8	NT	
7	5	5	5	2	5	125	25
8	2	20	5	>50	>50	NT	
9	8	20	8	>50	>50	NT	
10	2	5	5	8	8	50	25
11	2	5	8	5	5	100	50
Ciprofloxacin	0.5	0.5	0.5	1	1	NT	NT

show very good selectivity.

It is known that HDPs could eradicate bacteria rapidly due to their membrane-disruptive bactericidal mechanism [30]. To determine the time of action and efficacy of our newly synthesized compounds, the time-kill study was conducted to investigate if the most potent compound **7** could rapidly kill MRSA and *E. coli* [31]. As shown in Fig. 3, at 50 μg/mL,7 could eradicate all MRSA in just 10 min. Killing *E. coli* by **7** is comparatively slower, even though, all bacteria were completely killed in 60 min at 50 or 25 μg/mL. This observation suggests that the compound could kill both Gram-positive and Gram-negative pathogens rapidly, in a manner similar to HDP.

According to a recent public statement from the National Institutes of Health, more than 65% of all microbial infections are caused by biofilms [30]. It is known that bacteria in the biofilm are commonly more arduous to destroy than independent cells. Also, biofilms can clog pipes, watersheds, storage areas, and contaminate food products. In order to assess the possibility of the hybrid peptides in the biofilm prevention, Compound **7** was evaluated for its capability to suppress the biofilm formation of *E. coli*. As shown in Fig. 4, at just a concentration of 0.05 μg/mL, compound 7 could eradicate almost 50% of biofilm formation of E. coli.

To further confirm that compound **7** disrupts the bacterial cell membrane integrity, we used spectroscopic methods against both Gram-positive and Gram-negative bacteria to investigate the antimicrobial mechanism. In the assay of membrane depolarization, we utilized 3,3′dipropylthiadicarbocyanine iodide (DiSC35) which is a membrane-potential sensitive dye [32]. Generally, the dye would become fluorescent when the membrane potential is lost. In Fig. 5, Both MRSA and *E. coli* shows fluorescence intensity increase

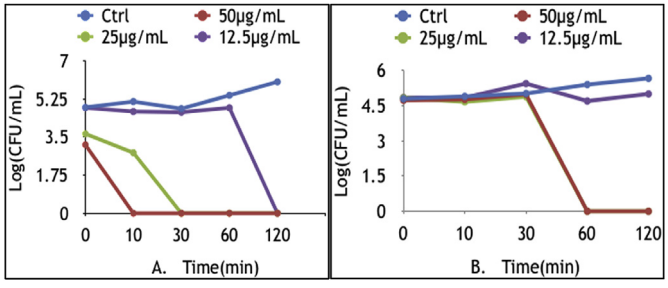

Fig. 3. Time-kill plots of 7 against MRSA (a) and *E. coli* (b).

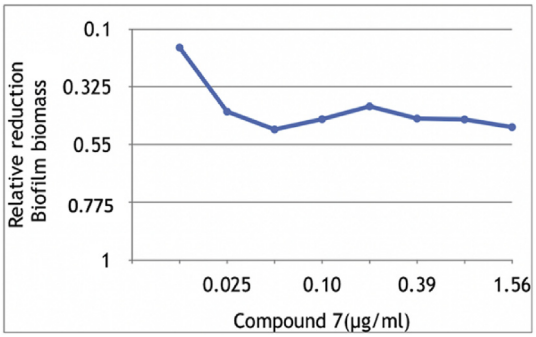

Fig. 4. Biological activity of 7 in the inhibition of a biofilm by *E. coli*.

dramatically upon treatment of **7** The result indicated that the membrane potential was lost due to the disruption of the bacterial membranes.

Subsequently, we carried out fluorescent microscopic study to further assess the compounds' impact on bacterial membranes. As we know, HDPs can compromise bacterial membranes to let cell death. The compounds should also compromise bacterial membranes if they mimic the mechanism of HDPs. As such, compound 7, the most potent compound, was chosen in the study. 4′,6-diamidino-2-phenylindole (DAPI) and propidium iodide (PI) were used as dyes in the assay (Fig. 6). The control, which was just MRSA or *E. coli* bacteria, shows blue fluorescence in the DAPI channel (a1 and a3). However, they were not observed in the PI channel (b1 and b3) because their membranes were intact [33].After MRSA and *E. coli* were incubated with compound **7** for 2h, they fluoresced in both DAPI and PI (a2,b2, a4, b4) channels. The result suggests the membranes of both MRSA and *E. coli* were disrupted.

3. Experimental procedure

3.1. General information

Rink-amide resin (0.7 mmol/g, 200–400 mesh), 2-Chlorotrityl chloride resin(0.97 mmol/g,100–200 mesh) and Fmoc protected α-amino acids were purchased from Chem-Impex Int'l Inc. Solvents and other chemicals were purchased from either Fisher Scientific or Sigma-Aldrich and used without further purification. Solid-phase synthesis of the compounds in a peptide reaction vessel on a

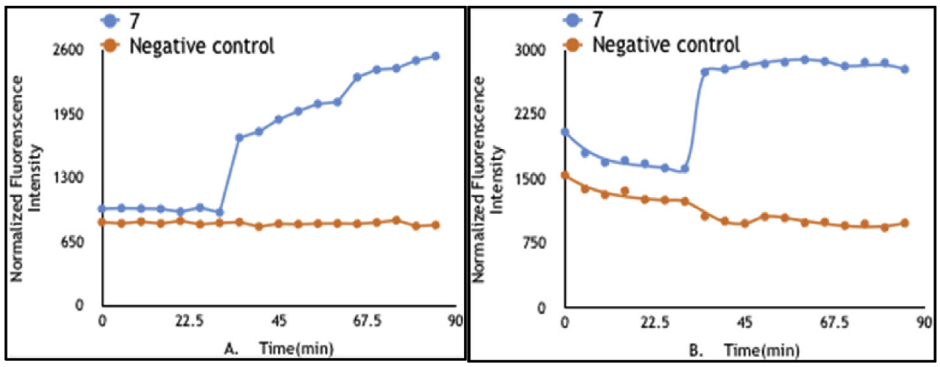

Fig. 5. Membrane depolarization of MRSA (A) and *E. coli* (B).Negative control is the culture without antibacterial treatment. The experiment was repeated three times with duplicates each time.

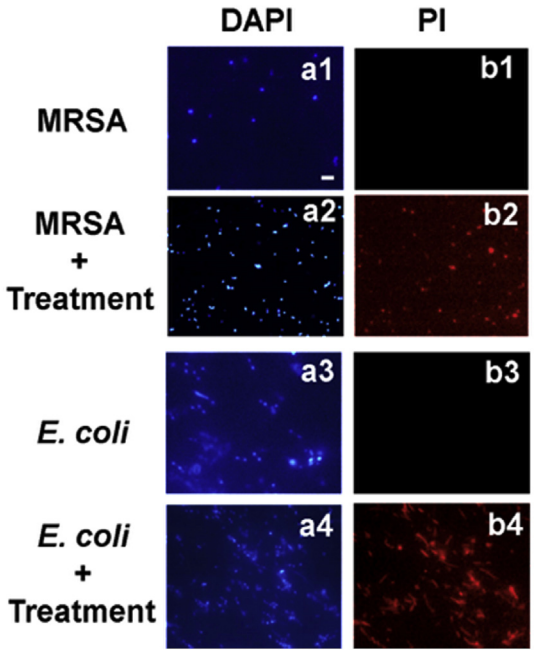

Fig. 6. Fluorescence micrographs of MRSA and *E. coli* that are treated or not treated with 6 μg/mL of compound 7 for 2 h: (a1) control, no treatment, DAPI stained; (b1) control, no treatment, PI stained; (a2) MRSA treatment with 7, DAPI stained; (b2) MRSA treatment with 7, PI stained; (a3) control, no treatment, DAPI stained; (b3) control, no treatment, PI stained; (a4) *E. coli* treatment with 7, DAPI stained; (b4) *E. coli* treatment with 7, PI stained.

Burrell wrist-action Shaker and purified on a Waters Breeze 2 HPLC system with both analytical and preparative functions. HPLC fractions were collected and confirmed using a Bruker AutoFlex MALDI-TOF mass spectrometer. The desired products and lyophilized on a Labcono lyophilizer.

3.2. Synthesis of sulfono-γ-AApeptide building blocks

The syntheses of the sulfono-γ-AApeptide Building Blocks (Fig. S1) was followed the previously reported procedure [34].

3.3. Synthesis of desired α/sulfono-γ-AA hybrid peptides

3.3.1. Synthesis of compound **3** [34]

Building block 2 (0.3 mmol) and 1.5 equivalent of 1- Hexadecanamine were dissolved in DCM in a 50 mL round bottom flask, to which3 equivalent of TBTU, 3 equivalent of HOBt, and 5 equivalent of DMAP were added. The resulted solution was allowed to stir overnight. Then the solution was washed with 0.1 M HCl three times, water, and brine. The organic layer was collected, dried over anhydrous Na_2SO_4, and then the solvent was removed by vacuum. The residue was nexttreated with 2 mL DCM/TFA (1:1) for 1 h to remove Boc protecting group. The crude compound 3 was purified on a Waters HPLC system, followed by lyophilization to give the pure product 3.

3.3.2. Synthesis of compound **4** [34]

2-Chlorotrityl chloride (CTC) resin (200 mg, 0.2 mmol) was allowed to swell in 2 mL of DCM for 15 min. The first attachment was conducted by adding building block 3 (0.3 mmol) and DIPEA (0.3 mmol) to the beads in the reaction vessel, which was allowed to shake at room temperature for 2 h. After that, the reaction solution was drained, followed by washing with DMF three times and DCM three times. The unreacted residues were capped with 2 mL of methanol for 30 min. After washed with DMF and DCM three times, the Alloc protecting group removed treated with Pd(PPh3)4 (0.2 mmol) and Me2NH.BH3 (1.2 mmol) in 2 mL DCM for 10 min twice. After the reaction, the solution was drained. The beads were washed with DCM and DMF three times, then 1.5 equivalent of Boc-Lys(Boc)-OH, 4 equivalent of HOBT and DIC in DMF were added and the mixture was reacted for 4 h. The beads were washed with DMF and DCM three times, then used 4 mL cleavage cocktail (acetic acid: TFE: DCM = 1:1:8) to cleave from resin. After 2 h, the solution was collected and concentrated by vacuum. The residue was then added with1.5 equivalent of 1- Hexadecanamine. followed by the addition of coupling reagents: 3 equivalent of TBTU, 3 equivalent of HOBt, and 5 equivalent of DMAP in DCM. The reaction was allowed to run overnight, and the solution with washed with 0.1 M HCl three times, water, and brine. DCM layer was collected, dried over anhydrous Na_2SO_4, and the solvent was removed by vacuum. The residue was treated with 2 mL DCM/TFA (1:1) for 1 h to remove Boc protecting group. The crude compound 4 was purified on a Waters HPLC system, followed by lyophilization to give the pure product 4.

3.3.3. Synthesis of compound1-**2** and **5**–**11** [27]

Others lipo-α/γ-AA hybrid peptides sequences were synthesized following the standard solid phase peptide synthesis protocol by using Rink-amide resin. For every coupling step, 20% Piperidine in DMF was first used to remove the Fmoc protecting group, then 1.5 equivalent of building block 1/Fmoc-Lys(Boc)-OH/saturated fatty acids, 4 equivalent of HOBT and DIC in DMF were added to react for 4 h. The assembled sequences were cleaved from the resin in 50:48:2 TFA/DCM/TIS (triisopropylsilane) for 2 h. The solvent was

removed by vacuum and the peptide sequences were purified on a Waters HPLC system, followed by lyophilization to give the pure product.

3.4. NMR data

Compound 7 ^{1}H NMR (600 MHz, C_2D_6OS) δ 7.80 (d, J = 6 Hz, 1H),7.628 (s,5H),7.41(s,1H), 7.31(dd, J = 23.28,6.37Hz,5H),7.13(s, 1H),4.39(d, J = 1.92 Hz,2H),4.01(dd, J = 13.57, 8.02Hz,1H) 3.81(dd,J = 27.75,17.89Hz,3H),2.66(s,5H),2.04(t,J = 6.8,2H),1.55(s,1H),1.34−1.5(m,8H),1.17(s,27H),0.79 (t, J = 6.8, 3H).

3.5. Minimum inhibitory concentrations (MICs) antimicrobial assays [26]

We tested the antimicrobial activity of the 11 compounds on five different bacteria strains including MRSA (ATCC 33591), MRSE (RP62A), *E. faecalis* (ATCC700802), *P. aeruginosa* (ATCC27853), and *E. coli* (ATCC 25922). A single colony of each bacterial strain was inoculated into 4 mL of TSB buffer at 37 °C overnight. Then the bacteria culture was diluted 100 times and allowed to grow to the mid-logarithmic phase. 50 μL of these bacteria suspension were added into 50 μL of different concentrations of lipo-α/γ-AA hybrid peptides diluted using the same TSB medium. The mixtures were incubated at 37 °C for 12−16 h, the absorption at 600 nm wavelength on a Biotek Synergy HT microtiter plate reader was recorded. MICs are the lowest concentrations of the compounds which can inhibit the bacteria growth. Results were repeated three times with duplicates each time.

3.6. Time-kill assay [26]

The best compound 7 was tested the kinetics of bacteria killing. The bacteria MRSA (Gram-positive) and *E. coli* (Gram-negative) suspensions were grown at 37 °C to the mid-logarithmic phase in TSB medium and diluted to 1 × 106 CFU/mL, the suspension was incubated with the compound 7 at the concentration of 12.5, 25, 50 μg/mL for 10 min, 30 min, 1 h, and 2 h, respectively. The mixtures were diluted by 10^2 to 10^4-fold, the colonies on the plates were counted and plotted against the incubation time after overnight incubation at 37 °C.

3.7. Fluorescence microscopy [26]

DAPI (4',6-diamidino-2-phenylindole dihydrochloride) and PI (propidium iodide) are two dyes used to stain the bacterial membrane and visualized by fluorescence microscopy. As we know, PI only stains dead cells since it can only pass through damaged membranes. However, DAPI can dye any bacterial cells. The compound 7 was incubated with the bacteria at 37 °C, the procedure of 7 is detailed below. Bacteria were grown to mid-logarithmic phase and incubated with the lipo-α/sulfono-γ-AA hybrid peptide 7 at 10 μg/mL for 2 h. The culture centrifuged at 5000g for 15 min. The pellets were washed with PBS, then incubated with PI (5 μg/mL), followed by PBS washing three times, and then DAPI (10 μg/mL) incubation. Each dye incubation was incubated for 15 min on ice in dark. The stained bacteria were observed under a Zeiss Axio Image Zloptical microscope using the 100X oil-immersion objective.

3.8. Inhibition of biofilms [30]

E. coli biofilms was grown at 37 °C for 24 h in 96-well plates in TSB in this assay. The wells were washed three times with TSB to remove unattached or weakly attached biofilms.50μL of the compound 7 solution (prepared by 2:1 serial dilution) in PBS and added fresh TSB to make up the total volume of 100 μL. Then the plate was incubated at room temperature over 24h, the percentage of biofilm detachment was recorded at a wavelength of 600 nm by using a microtiter plate reader, biofilm biomass was presented as CV OD/OD of growth. Experiments were repeated at least three times.

3.9. Bacterial Membrane Depolarization Assay [32]

Mid-log phase MRSA cells were collected and washed by using 5 mM HEPES and 5 mM glucose. The bacteria was re-suspended in 5 mM glucose, 5 mM HEPES buffer, and 100 mM KCl solution in 1:1:1 ratio (10^8 CFU/mL). 200 μL of bacterial suspension and 2 μM DiSC3(5) were incubated in a 96-well plate at 37 °C, and the fluorescence of the suspension was monitored for 30 min at the excitation wavelength of 622 nm and the emission wavelength of 670 nm. The concentration of 7 is 250 μg/mL. After the minimum value of the fluorescence (after 20 min) was reached, the compound 7 was added to the wells, so as to monitor the decrease in potential by the increase in fluorescence. The experiment was repeated at least three times with duplicates each time.

4. Conclusion

In conclusion, we designed and synthesized short sulfono lipo-a/sulfono-γ -AA hybrid peptides. These sequences, bearing sulfono-γ-AA building blocks, a lysine amino acid residue and a hydrophobic lipid tail, were able to be synthesized at ease. These compounds could interact with bacteria membrane and kill bacteria in a manner similar to HDPs. The lead sequences display potent and broad-spectrum antimicrobial activity against a series of Gram-positive and Gram-negative bacteria. Additionally, they also show little hemolytic toxicity and good potency to inhibit the formation of biofilms. The further development of this class of peptidomimetic could lead to a new class of antimicrobial agents.

Declaration of competing interest

The authors declare no competing interest.

Acknowledgement

The work was supported by NSF 1708500 and NIH 1R01GM112652.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2019.111901.

References

[1] S.B. Levy, B. Marshall, Antibacterial resistance worldwide: causes, challenges and responses, Nat. Med. 10 (2004) S122.
[2] K.L. Brown, R.E.W. Hancock, Cationic host defense (antimicrobial) peptides, Curr. Opin. Immunol. 18 (2006) 24−30.
[3] D.J. Payne, M.N. Gwynn, D.J. Holmes, D.L. Pompliano, Drugs for bad bugs: confronting the challenges of antibacterial discovery, Nat. Rev. Drug Discov. 6 (2006) 29.
[4] C.D. Fjell, J.A. Hiss, R.E.W. Hancock, G. Schneider, Designing antimicrobial peptides: form follows function, Nat. Rev. Drug Discov. 11 (2012) 37−51.
[5] A.K. Marr, W.J. Gooderham, R.E.W. Hancock, Antibacterial peptides for therapeutic use: obstacles and realistic outlook, Curr. Opin. Pharmacol. 6 (2006) 468−472.
[6] M.A. Fischbach, C.T. Walsh, Antibiotics for emerging pathogens, Science 325 (2009) 1089−1093.
[7] H. Wu, Y. Niu, S. Padhee, R.E. Wang, Y. Li, Q. Qiao, B. Ge, C. Cao, J. Cai, Design and synthesis of unprecedented cyclic gamma-AApeptides for antimicrobial development, Chem. Sci. 3 (2012) 2570−2575.
[8] D.K. Mercer, D.A. O'Neil, Peptides as the next generation of anti- infectives,

Future Med. Chem. 5 (2013) 315–337.
[9] E.A. Porter, B. Weisblum, S.H. Gellman, Mimicry of host-defense peptides by unnatural Oligomers: antimicrobial β-peptides, J. Am. Chem. Soc. 124 (2002) 7324–7330.
[10] N.P. Chongsiriwatana, J.A. Patch, A.M. Czyzewski, M.T. Dohm, A. Ivankin, D. Gidalevitz, R.N. Zuckermann, A.E. Barron, Peptoids that mimic the structure, function, and mechanism of helical antimicrobial peptides, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 2794–2799.
[11] H.L. Bolt, L.H.J. Kleijn, N.I. Martin, S.L. Cobb, Synthesis of antibacterial Nisin-Peptoid hybrids using click methodology, Molecules 23 (2018) 1566.
[12] S. Choi, A. Isaacs, D. Clements, D. Liu, H. Kim, R.W. Scott, J.D. Winkler, W.F. DeGrado, De novo design and in vivo activity of conformationally restrained antimicrobial arylamide foldamers, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 6968–6973.
[13] Y. Shi, P. Teng, P. Sang, F. She, L. Wei, J. Cai, γ-AApeptides: design, structure, and applications, Acc. Chem. Res. 49 (2016) 428–441.
[14] P. Teng, Y. Shi, P. Sang, J. Cai, γ-AApeptides as a new class of peptidomimetics, Chem. Eur J. 22 (2016) 5458–5466.
[15] S. Singh, A. Nimmagadda, M. Su, M. Wang, P. Teng, J. Cai, Lipidated alpha/alpha-AA heterogeneous peptides as antimicrobial agents, Eur. J. Med. Chem. 155 (2018) 398–405.
[16] F. She, O. Oyesiku, P. Zhou, S. Zhuang, D.W. Koenig, J. Cai, The development of antimicrobial gamma-AApeptides, Future Med. Chem. 8 (2016) 1101–1110.
[17] P. Teng, H. Wu, L. Lin, J. Cai, Antimicrobial gamma-AApeptides (WO2013112548): a patent evaluation, Expert Opin. Ther. Pat. 25 (2015) 111–118.
[18] Y.Q. Li, H.F. Wu, P. Teng, G. Bai, X.Y. Lin, X.B. Zuo, C.H. Cao, J.F. Cai, Helical antimicrobial sulfono-gamma-AApeptides, J. Med. Chem. 58 (2015) 4802–4811.
[19] Y. Li, C. Smith, H. Wu, P. Teng, Y. Shi, S. Padhee, T. Jones, A.M. Nguyen, C. Cao, H. Yin, J. Cai, Short antimicrobial lipo-alpha/gamma-AA hybrid peptides, Chembiochem 15 (2014) 2275–2280.
[20] Y. Li, C. Smith, H. Wu, S. Padhee, N. Manoj, J. Cardiello, Q. Qiao, C. Cao, H. Yin, J. Cai, Lipidated cyclic gamma-AApeptides display both antimicrobial and anti-inflammatory activity, ACS Chem. Biol. 9 (2014) 211–217.
[21] H.F. Wu, Y.H. Niu, S. Padhee, R.S.E. Wang, Y.Q. Li, Q. Qiao, G. Bai, C.H. Cao, J.F. Cai, Design and synthesis of unprecedented cyclic gamma-AApeptides for antimicrobial development, Chem. Sci. 3 (2012) 2570–2575.
[22] P. Sang, Y. Shi, P. Teng, A. Cao, H. Xu, Q. Li, J. Cai, Antimicrobial AApeptides, Curr. Top. Med. Chem. 17 (2017) 1266–1279.
[23] Y. Hu, X. Li, S.M. Sebti, J. Chen, J. Cai, Design and synthesis of AApeptides: a new class of peptide mimics, Bioorg, Med. Chem. Lett. 21 (2011) 1469–1471.
[24] A. Makovitzki, D. Avrahami, Y. Shai, Ultrashort antibacterial and antifungal lipopeptides, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 15997–16002.
[25] A. Makovitzki, J. Baram, Y. Shai, Antimicrobial lipopolypeptides composed of Palmitoyl Di- and Tricationic peptides: in vitro and in vivo activities, self-assembly to nanostructures, and a plausible mode of action, Biochemistry 47 (2008) 10630–10636.
[26] Y. Li, C. Smith, H. Wu, P. Teng, Y. Shi, S. Padhee, T. Jones, A.-M. Nguyen, C. Cao, H. Yin, J. Cai, Short antimicrobial lipo-α/γ-AA hybrid peptides, Chembiochem 15 (2014) 2275–2280.
[27] Y. Li, H. Wu, P. Teng, G. Bai, X. Lin, X. Zuo, C. Cao, J. Cai, Helical antimicrobial sulfono-γ-AApeptides, J. Med. Chem. 58 (2015) 4802–4811.
[28] Y. Niu, S. Padhee, H. Wu, G. Bai, Q. Qiao, Y. Hu, L. Harrington, W.N. Burda, L.N. Shaw, C. Cao, J. Cai, Lipo-γ-AApeptides as a new class of potent and broad-spectrum antimicrobial agents, J. Med. Chem. 55 (2012) 4003–4009.
[29] R.E.W. Hancock, H.-G. Sahl, Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies, Nat. Biotechnol. 24 (2006) 1551.
[30] P. Teng, A. Nimmagadda, M. Su, Y. Hong, N. Shen, C. Li, L.-Y. Tsai, J. Cao, Q. Li, J. Cai, Novel bis-cyclic guanidines as potent membrane-active antibacterial agents with therapeutic potential, ChemComm 53 (2017) 11948–11951.
[31] N. Alekhya, S. Yan, C. Jianfeng, γ-AApeptides as a new strategy for therapeutic development, Curr. Med. Chem. 24 (2017) 1–16.
[32] Y. Niu, M. Wang, Y. Cao, A. Nimmagadda, J. Hu, Y. Wu, J. Cai, X.-S. Ye, Rational design of dimeric lysine N-alkylamides as potent and broad-spectrum antibacterial agents, J. Med. Chem. 61 (2018) 2865–2874.
[33] S. Padhee, C. Smith, H. Wu, Y. Li, N. Manoj, Q. Qiao, Z. Khan, C. Cao, H. Yin, J. Cai, The development of antimicrobial a-AApeptides that suppress proinflammatory immune responses, Chembiochem 15 (2014) 688–694.
[34] H. Wu, F. She, W. Gao, A. Prince, Y. Li, L. Wei, A. Mercer, L. Wojtas, S. Ma, J. Cai, The synthesis of head-to-tail cyclic sulfono-γ-AApeptides, Org. Biomol. Chem. 13 (2015) 672–676.