Designing an ultra-short antibacterial peptide with potent activity against Mupirocin-resistant MRSA.

Giovinna Arfan^, Chu Yang Fann Ong^, Siew Mei Samantha Ng, Qiu Ying Lau, Fui Mee Ng, Esther Hong Qian Ong, Jeffrey Hill, C. S. Brian Chia*

Experimental Therapeutics Centre, Agency for Science, Technology and Research (A*STAR), 31 Biopolis Way, Nanos #03-01, Singapore 138669.

^These authors contributed equally to this paper

*Corresponding author
Tel.: +65 64070348
E-mail: cschia@etc.a-star.edu.sg

Abstract

Staphylococcus aureus is the pathogen responsible for the majority of human skin infections. In particular, the Meticillin-resistant variety, MRSA, has become a global clinical concern. The extensive use of Mupirocin, the first-line topical antibacterial drug of choice, has led to the emergence of Mupirocin-resistant MRSA globally, resulting in the urgent need for a replacement. Antimicrobial peptides are deemed plausible candidates. Herein, we describe a structure-activity relationship approach in the design of an ultra-short peptide with potent anti-MRSA activity with a rapid, bactericidal mode of action. Coupled to a low cytotoxic activity, we believe our lead compound can be developed into a topical antibacterial agent to replace Mupirocin as the first-line drug for treating MRSA skin infections.

Key words

antimicrobial peptide; MRSA; skin infection; topical antibiotic

Introduction

Staphylococcus aureus is a Gram-positive bacteria primarily responsible for human skin and soft tissue infections.[1-3] Of particular clinical concern are the Meticillin-resistant strains known as Meticillin-resistant *S. aureus* (MRSA), prompting the American Centers for Disease Control and Prevention to flag them as a 'serious threat' in their 2013 antibiotic resistance threat report.[4] Mupirocin, the first-line topical drug of choice, has been in use since 1985.[5] This has inevitably lead to the global emergence of Mupirocin-resistant MRSA through selective pressure and cross-transmission.[5-12] For example, a Swiss hospital study revealed Mupirocin-resistant MRSA clinical isolates increased from 0% to 80% between 1999 to 2008,[13] resulting in the urgent need for the development of new topical antibacterials.[14,15]

Antimicrobial peptides (AMPs) are deemed a promising class of antibacterial agents due to their rapid bactericidal activities as they directly target and disrupt bacterial cell membranes.[16-18] Their rapid mechanism of action can significantly reduce the probability of resistance development compared to traditional antibiotics which target bacterial cell-wall components, proteins or enzymes.[16-18] However, two major impediments in developing peptides into drugs are their lack of oral bioavailability and high manufacturing cost.[18-20] The first hurdle can be circumvented by developing peptide drugs for topical applications. For example, a 12-residue tryptophan-rich AMP, Omiganan, entered phase 2 clinical trials in 2015 as a topical antibacterial agent for the treatment acne vulgaris (ClinicalTrials.gov identifier: NCT02571998). To address the second hurdle, manufacturing cost can be minimized by identifying and selecting very short peptides with potent antibacterial activities for development. Towards this goal, we conducted a head-to-head anti-MRSA activity comparison on 30 short peptides up to 9

residues in length that were reported in the literature.[21] Experimental results revealed that nonapeptide **P9-4** (KWRRWIRWL-NH_2) possessed the most potent activity against hospital-acquired, mupirocin-susceptible MRSA (ATCC-BAA-1681) with a minimum inhibitory concentration (MIC) of 3.125 μM.[21] **P9-4** is a rationally-designed, tryptophan-rich synthetic peptide developed by Qi and co-workers from a hit peptide identified from a synthetic peptide library.[22] **P9-4** was also found to have very low hemolytic activity towards human erythrocytes (HC_{50} 400 μg/mL), suggesting that it can potentially be developed as an antibacterial drug.[22]

In this paper, we describe our efforts to enhance the potency of **P9-4** against Mupirocin-resistant MRSA *via* a structure-activity relationship (SAR) study while retaining its low-hemolytic property. At the same time, we sought to reduce manufacturing cost by peptide truncation and the replacement of expensive arginine residues with cheaper amino acids. The novel lead peptide was then screened against a panel of clinical MRSA isolates, followed by a bactericidal/static determination assay, a time-kill assay and a human epidermal keratinocyte viability assay to gauge its potential for development as a topical antibacterial to replace Mupirocin for the treatment of MRSA skin infections.

Material and methods

Peptide synthesis, antibiotics, chemicals and bacteria

All peptides were synthesized and purified to > 95% purity commercially by GL Biochem (China). Mupirocin calcium and propylene glycol was purchased from Sigma-Aldrich (USA). MRSA were purchased from ATCC (USA).

Minimum inhibitory concentration (MIC) assay

The MICs of test compounds were determined using the broth microdilution protocol from the Clinical and Laboratory Standards Institute guidelines.[23] Briefly, bacteria were grown fresh from frozen stock on Mueller Hinton 2 (MH2) agar at 37 °C. After overnight incubation, five colonies were selected to grow in cation-adjusted MH2 broth in a shaking incubator (220 RPM) at 37 °C. Cells were grown to an optical density (OD_{600}) of 0.13–0.17 determined using a spectrophotometer (Molecular Devices Spectra Max Plus), which corresponds to ~1×10^8 CFU/mL. Test compounds were constituted into 4 mM DMSO stock solutions and then subjected to twofold serial dilution in a 96-well plate with concentrations ranging from 100 to 0.195 μM in duplicates. 50 μL of microbial culture containing ~1×10^6 CFU/mL of microbes in the respective broths was introduced into each well containing 50 μL of compound solution. After an overnight incubation at 35 °C, OD_{600} measurements were conducted using the microplate spectrophotometer. MIC was defined as the lowest test compound concentration (μM) required to inhibit visible microbial growth.

Bactericidal/static determination assay

After the MICs of the test compounds were determined using the microdilution method described earlier on, the entire content of the well (100 μL) corresponding to 1x, 2x and 4x MIC of the test compounds were transferred and spread on sterile MH2 agar plates and incubated overnight at 37 °C. The number of colony forming units (CFU) was counted the next day. A test compound was classified as bactericidal if less than 0.1% of the original inoculum was observed on the plate.

Time-kill assay

Bacteria cells were grown to 1 x 10^8 CFU/mL in MH2 broth and then diluted 200-fold into 5 mL aliquots to a density of 5 x 10^5 CFU/mL in a flask. Test compounds pre-dissolved in DMSO corresponding to 4x their MICs were then added into each flask with a final DMSO concentration of 2.5% v/v. The negative control was 2.5% DMSO. The flasks were incubated in a shaking incubator (220 RPM) at 37 °C. Samples (100 μL) were taken between t = 0, 0.25, 0.5, 1, 2, 3, 4, 5 and 6 h. The samples were serially diluted in MH2 broth before plating on MH2 agar and incubated overnight at 37 °C. A bacteria count for each plate was conducted the next day. The Log CFU/mL was plotted against time to obtain a time-kill graph using GraphPad Prism software.

Human epidermal keratinocyte viability assay

Normal human epidermal keratinocytes (NHEK) were purchased from Lonza (catalog #00192627) and cultured in keratinocyte growth medium (Lonza KGM-Gold BulletKit) containing growth supplements (Lonza Reagent Pack) at 5% CO_2 (37 °C). The cell viability assay was performed using the CellTiter-Glo Luminescent Cell Viability Assay protocol from Promega.[24] Briefly, NHEK were treated with test compounds in keratinocyte growth medium in 96-well plates (Thermo Scientific Nunclon Delta). Treated cells were incubated for 5 days at 5% CO_2 (37 °C). Promega CellTiter-Glo Reagent was added and plates were shaken on an orbital shaker for 2 h. Next, well contents (100 μL) from each well were transferred into opaque 96-well plates (PerkinElmer OptiPlate-96) and luminescence measured by a microplate reader (Tecan Safire 2).

Results and Discussion

The MICs of clinical AMP candidate Omiganan and **P9-4** against Mupirocin-resistant MRSA (ATCC-BAA-1556) are summarized in Table 1. The FDA-approved first-line antibacterial drug Mupirocin was included for comparison.

[Place Table 1 here]

Omiganan was found to be active against Mupirocin-resistant MRSA with an MIC of 12.5 μM, in agreement with the reported MIC range of 16–32 μg/mL (8–16 μM) against 31 MRSA strains.[25] Interestingly, the 9-residue **P9-4** displayed an MIC of 6.25 μM (Table 1), 2-fold more active than 12-residue Omiganan, suggesting that with proper design, shorter peptides can be made more potent than Omiganan. As expected, Mupirocin was found to be impotent against Mupirocin-resistant MRSA (MIC >100 μM).

To further probe the effects of peptide length versus bioactivity, we truncated **P9-4** from the N- and C-termini to see if the shortened analogs remained MRSA-active (peptides **1** to **8**; Table 1). MIC data revealed that just a single amino acid removal from either the N- or C-terminus resulted in 2 to 4-fold bioactivity reduction (peptides **1** and **5**; Table 1). Further truncation resulted in total bioactivity loss (MICs >100 μM; Table 1), suggesting that a minimum peptide length of 9 residues was required for potent (single-digit micromolar) activity against MRSA. An advantage of designing short peptides over long ones is that the former tend to possess less cytotoxic effects against human cells.

Next, an alanine scan was conducted to elucidate which amino acid residues were crucial for antibacterial activity (peptides **9** to **17**; Table 1). MIC results revealed every

158 amino acid residue in **P9-4** was important because a single alanine mutation resulted in 2
159 to 4-fold bioactivity loss (MICs 12.5 to 25 μM; Table 1). Particularly important were
160 tryptophan W2, W5 and W8 where alanine substitution resulted in a 4-fold activity
161 reduction (peptides **10**, **13** and **16**; MICs 25 μM). This was not surprising as tryptophan's
162 indole side-chain has been shown to possess a high affinity for a phospholipid's carbonyl
163 (C=O) moiety and the ability to disrupt the cohesive hydrophobic interactions between
164 lipids in a phospholipid bilayer and thus imbibing tryptophan-rich peptides with
165 membrane-disrupting properties.[26-31] Indeed, tryptophan residues constituted a third of
166 the residues found in Omiganan and **P9-4** (4 of 12 and 3 of 9 respectively) which serves
167 to explain their ability to lyse bacterial membranes.[22,32] The alanine scan also indicated
168 that the positively-charged residues K1, R3, R4 and R7 played important antibacterial
169 activity roles as a single alanine substitution caused a 2-fold potency reduction (peptides
170 **9**, **11**, **12** and **15**; MICs 12.5 μM; Table 1). Cationic residues are crucial for antibacterial
171 properties due to the anionic nature of bacterial membranes caused by the prevalence of
172 negatively-charged teichoic acids and phospholipids.[32-34] The electrostatic attraction
173 formed between the anionic membrane and the cationic arginine or lysine residues of **P9-**
174 **4** facilitates peptide-membrane binding followed by membrane insertion and subsequent
175 disruption.[22] Interestingly, substituting the aliphatic hydrophobic I6 and L9 with alanine
176 also resulted in a 2-fold activity reduction, suggesting that aliphatic side-chains also
177 played an important role (peptides **14** and **17**; MICs 12.5 μM; Table 1). Indeed, the
178 aliphatic leucine side-chains in AMPs have been observed to insert into model
179 phospholipid membranes[35] while another study showed that leucine increases the
180 propensity of peptides to form amphiphilic helices in solution, a structural requirement for
181 antibacterial activity.[36] Based on this, we decided to conduct a leucine scan to identify
182 which residue can be replaced with leucine without bioactivity loss. This serves to reduce

peptide manufacturing cost as Fmoc-protected leucine is 8 and 6-fold cheaper than Fmoc-protected arginine and tryptophan respectively based on prices in the Sigma-Aldrich website (www.sigmaaldrich.com). Leucine scanning revealed that the majority of leucine-substituted analogs suffered bioactivity loss (peptides **18** to **25**; Table 1). For example, replacing the cationic K1, R4 and R7 residues with leucine resulted in 2-fold bioactivity losses (peptides **18**, **21** and **24**; MICs 12.5 μM; Table 1) while substituting tryptophans W2, W5 and W8 with leucine resulted in 4-fold bioactivity losses (peptides **19**, **22** and **25**; MICs 25 μM; Table 1). These observations support the importance of cationic residues and tryptophan towards the antibacterial activities of AMPs for reasons stated earlier. However, two exceptions were observed. For peptide **20**, replacing the cationic R3 with leucine did not alter the peptide's potency when compared to **P9-4** (MIC 6.25 μM; Table 1), suggesting that it was possible to reduce the number of expensive arginine residues in peptide **P9-4** with no activity loss. For peptide **23**, substituting I6 with leucine surprisingly improved bioactivity 2-fold (MIC 3.125 μM; Table 1). This may be due to isoleucine being more hydrophobic than leucine based on their hydropathy scores of 4.5 and 3.8 respectively.[37]

From the leucine scan results, the most potent compound, peptide **23** (KWRRWLRWL-NH_2; MIC 3.125 μM) was selected for further SAR studies. In the next series of analogs, R3 was substituted for the cheaper leucine as this did not affect bioactivity (peptide **20**; Table 1). We also decided to explore replacing residues K1 and R4. The rationale for replacing the sole lysine (K1) with either leucine or tryptophan was to lower manufacturing cost. We also wanted to see of we could reduce the number of expensive arginine residues by substituting R4 with either leucine or tryptophan. Hence, the next four peptide analogs containing substituted K1 and R4 (**26** to **29**) were synthesized. MIC

208 results revealed that all four analogs were more potent than reference peptide **P9-4**, with
209 tryptophan-rich peptide **29** (WWLWWLRWL-NH_2) displaying the highest potency against
210 Mupirocin-resistant MRSA (MIC 1.56 μM; Table 1), 2- to 4-fold more potent than peptides
211 **23** and **P9-4** respectively.
212
213 Next, lead peptide **29** was subjected to further SAR studies. Sequence reversal resulted
214 in an unexpected 2-fold activity reduction (peptide **30**; LWRLWWLWW-NH_2; MIC 3.125
215 μM; Table 1). A possible reason for the observed MIC difference could be that the
216 peptides' primary sequences may be playing a role in their abilities to disrupt bacterial
217 membranes. Further studies involving molecular dynamic simulations of peptide-model
218 membrane interaction may shed some light into this and is beyond the scope of this
219 paper. Next, an attempt to further enhance peptide **29**'s antibacterial potency was made
220 by attaching a palmitoyl (C16) fatty acid moiety to its N-terminus (peptide **31**). This
221 technique has been reported to enhance the antibacterial activities of some AMPs as the
222 hydrophobic alkyl C16 chain facilitates peptide-membrane insertion and subsequent
223 disruption.[38,39] Strangely, our MIC results revealed lipopeptide **31** to be impotent (MIC
224 >100 μM; Table 1). We suspected that the peptide's positively-charged N-terminal amine
225 may be important for antibacterial activity and could have reduced lipopeptide **31**'s ability
226 to bind to the anionic bacterial membrane. Unsurprisingly, N-terminal acetylated peptide
227 **32** also displayed poor anti-MRSA activity (MIC 25 μM; Table 1), suggesting that the N-
228 terminal positive charge was important for antibacterial activity. To test this hypothesis
229 further, peptide **33** with a negatively-charged C-terminal carboxylate group also exhibited
230 significant activity loss (WWLWWLRWL-OH; MIC 25 μM; Table 1). From these results,
231 we concluded that the minimum number of positive charges that a peptide must possess
232 for antibacterial activity is +2. In an attempt to reduce manufacturing cost further, the sole

arginine (R7) of lead peptide **29** was substituted with leucine and tryptophan (peptides **34** to **35** respectively; Table 1). MIC results revealed both peptides to be impotent (MICs >100 μM; Table 1), supporting our earlier deductions that a peptide must possess a minimum two positive charges for antibacterial activity.

Intrigued by the lone arginine in peptide **29**, we relocated it at various positions along the peptide chain (peptides **36** to **43**; Table 1). Surprisingly, experimental results revealed peptides with MICs ranging between 3.125 to 6.25 μM, none of which were more potent than lead peptide **29** (MIC 1.56 μM). This suggested that the position of the arginine in the peptide sequence played an important role in antibacterial activity.

Lastly, we truncated lead peptide **29** to see if we could further reduce peptide length (peptides **44** to **47**; Table 1). Unfortunately, MIC results revealed that a single residue removal, either at the N- or C-termini, resulted in a 4-fold bioactivity reduction (peptides **44** and **46**; MICs 6.25 μM; Table 1) and the removal of two residues caused 8-fold bioactivity losses (peptides **45** and **47**; MICs 12.5 μM; Table 1), indicating that the shortest peptide length required for potent anti-MRSA activity was 9 residues.

From the SAR study involving peptides **1** to **47**, peptide **29** was found to be the most potent against Mupirocin-resistant MRSA (MIC 1.56 μM; Table 1). Compared to reference peptide **P9-4**, peptide **29** was 4-fold more potent (MIC 1.56 vs. 6.25 μM). We suspect this was probably due to the increased number of membrane-active tryptophan residues in peptide **29** compared to **P9-4** (five vs. three respectively). Interestingly, the reduction in the number of cationic residues in peptide **29** compared to **P9-4** (one vs. four respectively) did not affect antibacterial potencies.

Peptide **29** was next tested against a panel of clinical MRSA strains with Mupirocin and **P9-4** as comparators (Table 2).

[Place Table 2 here]

As expected, Mupirocin was found to be inactive against Mupirocin-resistant strains while displaying high activity against susceptible strains (MICs ≤ 0.39 μM; Table 2). Table 2 also revealed peptide **29** to be highly potent against all nine MRSA strains (MICs 1.56 μM; Table 2), up to 4-fold more potent than **P9-4** (MICs 3.125 to 6.25 μM; Table 2). This suggested peptide **29** has a high potential for further development as a topical antibacterial. Lead peptide **29** was subsequently subjected to a bactericidal/static determination assay against Mupirocin-resistant MRSA with **P9-4** as a comparator (Fig. 1). A bactericidal drug is advantageous over a bacteriostatic one as the former can significantly reduce the chances of resistance development.

[Place Figure 1 here]

The bactericidal/static determination assay revealed both peptides to be bactericidal, with peptide **29** exhibiting a minimum bactericidal concentration (MBC) of 1.56 μM (1x MIC) while **P9-4** possessed an MBC of 12.5 μM (2x MIC), suggesting that MRSA was more susceptible towards peptide **29** compared to **P9-4**.

Next, both peptides were subjected to a time-kill assay using Mupirocin-resistant MRSA (Fig. 2). A compound with rapid-killing bactericidal activity is generally preferred as it can potentially reduce the chance of resistance development and treatment duration.

[Place Figure 2 here]

The time-kill graph (Fig. 2) revealed both peptides to be rapidly bactericidal, causing a 6-log reduction in bacteria count within one hour. This extremely rapid bactericidal mechanism suggested both peptides were acting *via* a membrane-lytic mechanism.

The membrane-disrupting nature of AMPs have raised concerns that they may be too cytotoxic to be used as drugs. For example, Melittin, a 26-residue AMP isolated from bee venom, is extremely hemolytic towards human erythrocytes at low micromolar concentrations.[40] However, short cationic tryptophan-rich AMPs up to 10 residues in length have been reported to be non-cytotoxic.[22,30,41-43] Hence, peptide **29** was subjected to a human epidermal keratinocyte viability assay with clinical candidate Omiganan as comparison (Fig. 3). Propylene glycol, an FDA-approved solvent used in many topical creams, gels and lotions, was used as a control. Experimental results revealed peptide **29** was relatively less cytotoxic than Omiganan (GI_{50} 18.1 vs. 57.5 μM respectively; Fig. 3). Based on this, we believe that peptide **29** can be safely developed as a topical drug.

[Place Figure 3 here]

Conclusions

By utilizing an SAR approach, we designed nonapeptide **29** with high potency against a range of clinical MRSA strains. Peptide **29** possessed a 4-fold activity enhancement over reference synthetic AMP **P9-4** and was found to be rapidly bactericidal with an MBC of 1.56 μM. Peptide **29** was also found to have low cytotoxicity, making it a potential candidate to replace Mupirocin as the first-line topical antibacterial drug for treating skin infections.

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Conflicts of interest

The authors of this paper declare no conflicts of interest.

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Ethical approval

Not required.

Figure legends

Fig.1

no figure legend

Fig.2

DMSO

Peptide **P9-4** 4x MIC

Peptide **29** 4x MIC

Fig.3

Omiganan

Peptide **29**

propylene glycol

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Drug or Peptide #	Sequence	# residues (# charges)	MIC (μM)
Mupirocin	Not applicable	not applicable	>100
Omiganan	ILRWPWWPWRRK-NH_2	12 (+5)	12.5
P9-4	KWRRWIRWL-NH_2	9 (+5)	6.25
	Truncation		
1	WRRWIRWL-NH_2	8 (+4)	25
2	RRWIRWL-NH_2	7 (+4)	100
3	RWIRWL-NH_2	6 (+3)	>100
4	WIRWL-NH_2	5 (+2)	>100
5	KWRRWIRW -NH_2	8 (+5)	12.5
6	KWRRWIR -NH_2	7 (+5)	>100
7	KWRRWI -NH_2	6 (+4)	>100
8	KWRRW -NH_2	5 (+4)	>100
	Alanine scan		
9	AWRRWIRWL-NH_2	9 (+4)	12.5
10	KARRWIRWL-NH_2	9 (+5)	25
11	KWARWIRWL-NH_2	9 (+4)	12.5
12	KWRAWIRWL-NH_2	9 (+4)	12.5
13	KWRRAIRWL-NH_2	9 (+5)	25
14	KWRRWARWL-NH_2	9 (+5)	12.5
15	KWRRWIAWL-NH_2	9 (+4)	12.5
16	KWRRWIRAL-NH_2	9 (+5)	25
17	KWRRWIRWA-NH_2	9 (+5)	12.5
	Leucine scan		
18	LWRRWIRWL-NH_2	9 (+4)	12.5
19	KLRRWIRWL-NH_2	9 (+5)	25
20	KWLRWIRWL-NH_2	9 (+4)	6.25
21	KWRLWIRWL-NH_2	9 (+4)	12.5
22	KWRRLIRWL-NH_2	9 (+5)	25
23	KWRRWLRWL-NH_2	9 (+5)	3.125
24	KWRRWILWL-NH_2	9 (+4)	12.5
25	KWRRWIRLL-NH_2	9 (+5)	25
	Peptide 23 K1 and R4 substitution		
26	LWLLWLRWL-NH_2	9 (+2)	3.125
27	WWLLWLRWL-NH_2	9 (+2)	3.125
28	LWLWWLRWL-NH_2	9 (+2)	3.125
29	WWLWWLRWL-NH_2	9 (+2)	1.56
	Peptide 29 sequence reversal		
30	LWRLWWLWW-NH_2	9 (+2)	3.125
	Peptide 29 N-capping		
31	Pa-WWLWWLRWL-NH_2	9 (+1)	>100
32	Ac-WWLWWLRWL-NH_2	9 (+1)	25
	Peptide 29 C-terminal -COOH		
33	WWLWWLRWL-OH	9 (+1)	25
	Peptide 29 R7 substitution		
34	WWLWWLLWL-NH_2	9 (+1)	>100
35	WWLWWLWWL-NH_2	9 (+1)	>100

	Peptide 29 arginine repositioning		
36	RWLWWLWWL-NH_2	9 (+2)	6.25
37	WRLWWLWWL-NH_2	9 (+2)	6.25
38	WWRWWLWWL-NH_2	9 (+2)	3.125
39	WWLRWLWWL-NH_2	9 (+2)	6.25
40	WWLWRLWWL-NH_2	9 (+2)	3.125
41	WWLWWRWWL-NH_2	9 (+2)	6.25
42	WWLWWLWRL-NH_2	9 (+2)	3.125
43	WWLWWLWWR-NH_2	9 (+2)	3.125
	Peptide 29 truncation		
44	WLWWLRWL-NH_2	8 (+2)	6.25
45	LWWLRWL-NH_2	7 (+2)	12.5
46	WWLWWLRW -NH_2	8 (+2)	6.25
47	WWLWWLR -NH_2	7 (+2)	12.5

Table 1. MICs (μM) of various drugs and peptides against Mupircocin-resistant MRSA (ATCC-BAA-1556). Ac, acetyl; Pa, palmitoyl (C16).

	Mupirocin	**P9-4**	Peptide **29**
Mupirocin-resistant MRSA			
ATCC-BAA-1556	>100	6.25	1.56
ATCC-BAA-1708	>100	6.25	1.56
ATCC-BAA-1750 (USA200, ST30)	25	6.25	1.56
Mupirocin-susceptible MRSA			
ATCC-BAA-1681 (USA100)	0.39	3.125	1.56
ATCC-BAA-1756 (USA300)	< 0.2	6.25	1.56
ATCC-BAA-1707 (USA400)	< 0.2	6.25	1.56
ATCC-BAA-1689 (USA500, ST8)	0.39	6.25	1.56
ATCC-BAA-2762 (ST22)	< 0.2	6.25	1.56
ATCC-700699 (VISA, Mu50)	< 0.2	6.25	1.56

Table 2. MICs (μM) of peptides against various clinical strains of MRSA.

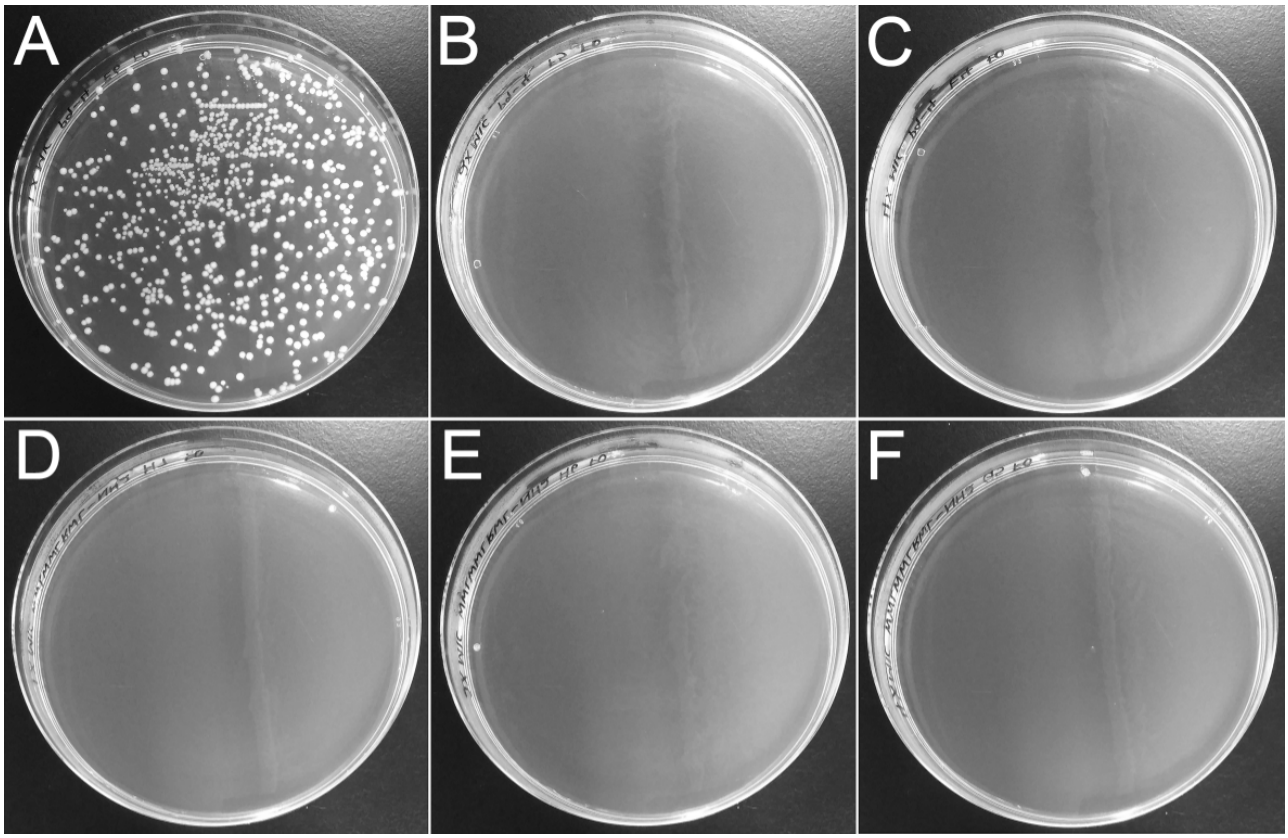


Fig. 1. Bactericidal/static determination assay; (A) **P9-4** 1xMIC; (B) **P9-4** 2xMIC; (C) **P9-4** 4xMIC; (D) peptide **29** 1xMIC; (E) peptide **29** 2xMIC; (F) peptide **29** 4xMIC against Mupirocin-resistant MRSA (ATCC-BAA-1556).

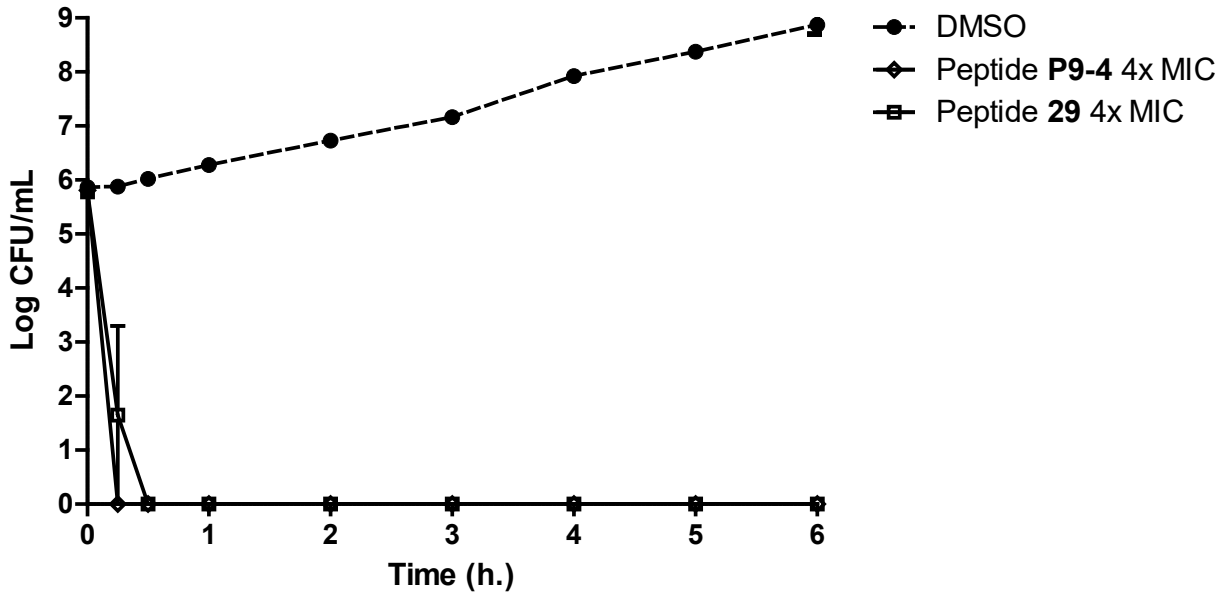
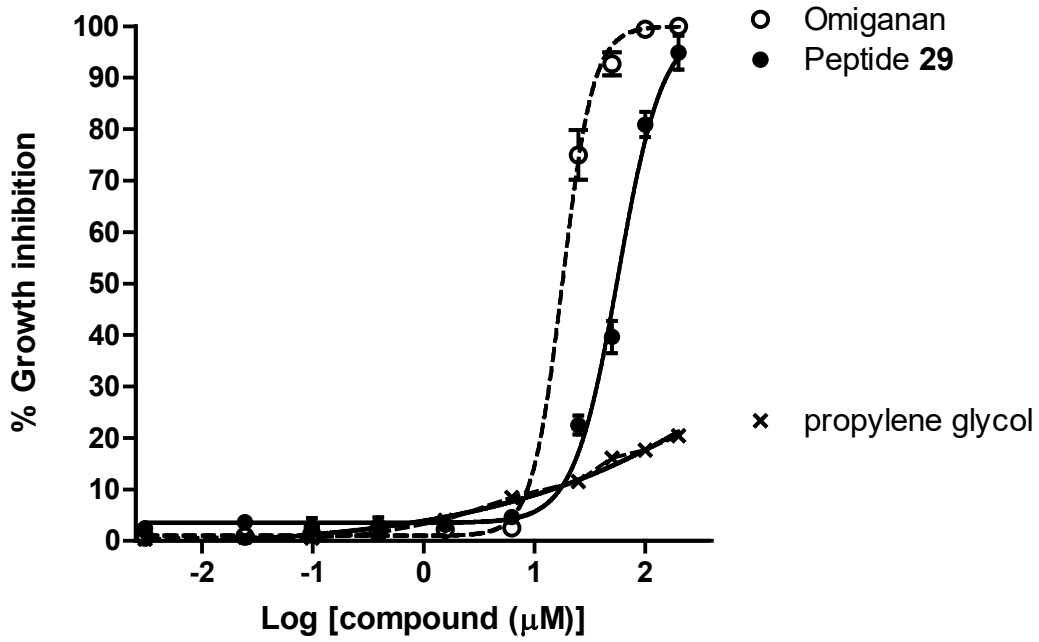


Fig. 2. Time-kill comparison of peptides **P9-4** and **29** at 4x MIC against Mupirocin-resistant MRSA (ATCC-BAA-1556).



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497 **Fig. 3**. Human epidermal keratinocyte viability assay of Omiganan and peptide **29**.

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