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Incorporation of β-amino acids enhances the antifungal activity and selectivity of the helical antimicrobial peptide aurein 1.2

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Abstract

Antimicrobial peptides (AMPs) are attractive antifungal drug candidates because they kill microbes via membrane disruption and are thus less likely to provoke development of resistance. Low selectivity for fungal vs. human cells and instability in physiological environments have limited the development of AMPs as therapeutics, but peptidomimetic AMPs can overcome these obstacles and also provide useful insight into AMP structure-function relationships. Here, we describe antifungal peptidomimetic α/β-peptides templated on the natural α-peptidic AMP aurein 1.2. These α/β-aurein analogues fold into $i \rightarrow i+4$ H-bonded helices that present arrays of side chain functionality in a manner virtually identical to that of aurein 1.2. By varying charge, hydrophobicity, conformational stability, and α/β-amino acid organization we designed active and selective α/β-peptide aurein analogues that exhibit minimum inhibitory concentrations (MIC) against the opportunistic pathogen *Candida albicans* that are 4-fold lower than that of aurein 1.2 and elicit less than 5% hemolysis at the MIC. These α/β-aurein analogues are promising candidates for development as antifungal therapeutics and as tools to elucidate mechanisms of AMP activity and specificity.

Candida albicans is the most prevalent human fungal pathogen and is the fourth leading cause of bloodstream infections in the U. S.[1] The mortality rate associated with systemic candidemia is approximately 30–50%.[2] The development of new antifungal drugs is critical because of the small number of existing drugs that are effective against *C. albicans* and the emergence of pathogenic strains resistant to current drugs.[3, 4] Antimicrobial peptides (AMPs) are attractive as antifungal agents because the mechanisms of membrane disruption through which they act are less likely to lead to the development of resistance than targeted therapeutics.[5] However, AMPs exhibit low structural stability at physiological pH and ionic strength and are susceptible to proteolytic degradation *in vivo*. Peptidomimetic compounds offer the potential to improve native α-amino acid peptide properties, including structural

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Notes

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and metabolic stability.[6] Peptidomimetics of AMPs have been constructed from β-amino acids, peptoids, cyclic peptoids, and AApeptides (*N*-acetyl-*N*-aminoethyl peptides)[7, 8] to possess similar amphiphilicity, charge, hydrophobicity, and secondary structure as natural AMPs.[7] However, these peptidomimetic AMPs exhibit structural folding patterns that are distinct from those of natural AMPs, preventing conservation of side-chain presentation and often adversely influencing AMP interactions with target cell membranes. In prior work, we generated antifungal β-peptides that were structurally inspired by cationic, amphiphilic AMPs[9, 10]. While these β-peptides exhibit the global amphiphilicity of AMPs, the side chain organization around the 14-helix periphery differs from that of native antimicrobial α-peptides. In contrast, α/β-peptides, consisting of repeating patterns of α- and β-amino acids (e.g. repeating αβ, ααβ, αααβ, αβαβααβ, or ααβαααβ sequences), fold into $i \rightarrow i$+4 H-bonded helices that exhibit similar folding patterns to α-helices, enabling construction of α/β-peptides that exhibit similar spatial side chain presentation to native α-peptides.[11, 12] In addition, protease resistance can be imparted to α/β-peptides by controlling α and β amino acid organization to improve peptide stability *in vivo*.[13]

Here, we report that α/β-peptide analogues of the natural AMP aurein 1.2 maintain the antifungal activity of native aurein 1.2, an AMP produced by Australian Bell Frogs[14], and that more potent and selective α/β-aurein analogues can be designed by altering hydrophobicity, helicity, and charge in these synthetic mimics.[15] Linear regression analysis illustrated that α/β-peptide hydrophobicity is the key determinant of antifungal activity, while helical stability is the primary driver of hemolytic activity, indicating the potential to design active and selective α/β-peptide analogues of native AMPs.[11, 16]

As a first step toward the development of active and selective α/β-peptide AMP mimics, we templated α/β-peptide sequences on aurein 1.2.[14] Aurein 1.2, a member of the aurein family of AMPs, which also includes aurein 2.2, aurein 3.1, citropin 1.1, citropin 1.1, citropin 1.3, and maculatin 1.1,[17–19] is a 13 residue +1 charged peptide (entry **1**, Table 1) secreted from skin glands of Australian Bell Frogs and exhibits activity against both bacteria and fungi.[14, 20, 21] Aurein 1.2 folds into a helix with hydrophobic and charged residues segregated to different faces (Fig. 1).[14] The activity of aurein 1.2 and α/β-aurein analogues against *C. albicans* strain SC 5314 was quantified as the minimum inhibitory concentration (MIC) after 48 h incubation at 35°C in RPMI using a broth microdilution assay (NCCL document M27-A2) (Table 1, and Fig. S1). The cytotoxicity of the peptides was assessed as % hemolysis at the MIC and HC_{10} (the peptide concentration that causes 10% hemolysis) of human red blood cells (Table 1, S1, and Fig. S1). The *in vitro* selectivity index (SI) is defined as the ratio of HC_{10} to MIC ($SI = HC_{10}/MIC$) (Table 1)[22]. Peptide hydrophobicity was measured as the retention time during reversed phase high performance liquid chromatography (RP-HPLC)[23–27] (Table 1 and Fig S2). Helicity was quantified by circular dichroism (CD) spectrometry in trifluoroethanol (TFE) and deionized water containing 15% TFE (Table S1 and Fig. S3). We synthesized +1 charged α/β-peptides templated on aurein 1.2 with different α/β-amino acid organization by replacing key α-amino acids with β-amino acids to generate αβ, ααβ, αααβ, αβαβααβ, and ααβαααβ repeating units. These α/β-aurein analogues possess a C-terminal amide, similar to native aurein 1.2. However, these α/β-peptides exhibited poor solubility in aqueous media. To improve solubility, we

synthesized +5 charged α-peptides by replacing the D4 and E11 residues of aurein 1.2 with the amino acid K. +1 (**1**) and +5 (**2**) aurein 1.2 α-peptides exhibited the same antifungal minimum inhibitory concentration (MIC) of 32 μg/ml (Table 1 and Fig. S1a). However, the hemolysis at the MIC and the selectivity index (SI) of +5 aurein 1.2 were two-fold lower and three-fold higher than +1 aurein 1.2, respectively (Table 1, Fig. S4) suggesting that this was an effective approach for increasing selectivity of α/β-peptides.

Next, we synthesized +5 charged α/β-aurein analogues to create αβ (**3a**), ααβ (**4a**), αααβ (**5a**), αβαβααβ (**6a**), and ααβαααβ (**7a**) repeats (Fig. 1 and Table 1). To investigate the effects of helical stability on antifungal activity and selectivity, we exchanged two hydrophobic β-amino acids located at sites shown in Figure 1 with helix-stabilizing trans-2-aminocyclopentane-carboxylic acid (ACPC) cyclic residues to enhance the conformational stability of the +5 analogue (αβ (**3Ca**), ααβ (**4Ca**), αααβ (**5Ca**), αβαβααβ (**6Ca**), ααβαααβ (**7Ca**)) (Fig. 1 and Table 1).[16] These α/β-aurein analogues (αβ (**3a** and **3Ca**), ααβ (**4a** and **4Ca**), αααβ (**5a** and **5Ca**), αβαβααβ (**6a** and **6Ca**), ααβαααβ (**7a** and **7Ca**)) exhibited RP-HPLC retention times, used here as a measure of hydrophobicity as described above, ranging from 13 to 19 min (Table 1 and Fig. S5a). ACPC incorporation stabilized α/β-peptide helices composed of ααβ, αααβ, and ααβαααβ repeating units, but not αβ and αβαβααβ repeats (Fig. 2a, and Fig. S5a). This finding is consistent with a report that αβ and αβαβααβ GNC4-pLI peptides were unstable compared to other αβ combinations.[11]

The MIC of +5 charged α/β-aurein analogues monotonically decreased with increasing hydrophobicity (Fig. 2b and Table 1). However, ACPC content had little effect on MIC, suggesting that antifungal activities are dominated by α/β-peptide hydrophobicity. In contrast, SI was significantly affected by the inclusion of ACPC, which increased the helical stability of the peptides. The maximum SI value of α/β-aurein analogues containing ACPC residues occurred at a higher hydrophobicity than α/β-aurein analogues lacking ACPC residues (Fig 2c). α/β-Peptide backbone organization also affected the activity and SI of α/β-aurein analogues (Fig. 2b-c (solid line)). By varying hydrophobicity and helicity, we identified α/β-peptides (**4a**, **5Ca**, **7a**, **7Ca**) with two-fold greater activity than the +5 α-amino acid aurein 1.2 analog peptide **2** (Fig. 2b). Significantly, peptides **5Ca**, **7a**, and **7Ca** elicited less than 5% hemolysis at MIC and a SI that was similar (**5Ca**, and **7a**) or 3-fold higher (**7Ca**) than the +5 α-amino acid peptide **2** (Fig. 2c), demonstrating improved selectivity for fungal cells over mammalian cells.

Based on these initial evaluations of α/β-aurein activity and selectivity, we selected α/β-peptide **7Ca**, composed of repeating ααβαααβ peptides, as a lead compound to systematically evaluate the effects of α/β-peptide sequence, structure, and charge on antifungal activity and selectivity. We synthesized +3 charged α/β-peptide **8** by replacing D4 with K, and **9** by replacing E11 with K (Table 1). Peptides **8** and **9** possessed lower and similar MICs compared to **7Ca**, respectively, but exhibited higher hemolysis than **7Ca** (Table 1 and Fig. S5b). Thus, the +5 α/β-aurein analog possesses greater selectivity than these +3 α/β-peptides. Peptides **10** (β^3*h*L2ACPC) and **11** (β^3*h*I9ACPC), containing single ACPC residues, exhibited lower activity and/or higher hemolysis than α/β-peptide **7Ca**

(Table 1 and Fig. S5b). On the basis of these results, we fixed the charge of α/β-aurein analogues at +5 and focused on α/β-peptides containing two ACPC residues.

To investigate how the hydrophobicity of α/β-peptides affects activity and selectivity, we replaced A10 and S12 with the more hydrophobic amino acid L (Table 1). In each α/β combination studied, antifungal MIC decreased and hemolysis at the MIC increased as α/β-peptide hydrophobicity increased (Fig. S6a-e). We defined a hydrophobicity region for each α/β-combination wherein the MIC was ≤ 16 μg/mL and hemolysis at MIC was ≤ 10% (Fig. S6f, green area). This region encompasses α/β-aurein analogues with the greatest therapeutic potential based on activity and selectivity. α/β-Peptides with the repeating αβ-motif were not active and selective at any retention time, while α/β-peptides with repeating αααβ, ααβααβ, and ααβ-motifs that led to greater conformational stability exhibited retention time ranges corresponding to active and selective antifungal behavior (Fig. S6f). We identified 11 active and selective peptides in these hydrophobicity regions: **4a**, **4b**, **4Cb, 5Ca, 5b**, **6c**, **6Cc, 7a**, **7Ca, 7b**, **7Cb** (Table 1, and Table s1). Importantly, peptides **4Cb**, **5Ca**, **6Cc**, **7a**, **7Ca** were superior in terms of activity and/or selectivity (MIC ≤ 16 μg/mL and SI > 3.3, Table 1) compared to +5 charged α-peptide **2**.

A prior report demonstrated that hydrophobicity and helicity synergize to regulate antifungal activity and hemolysis in antifungal 14-helical β-peptides.[9] To determine how the activities and selectivities of α/β-aurein analogues are affected by hydrophobicity and helicity, we plotted MIC, hemolysis at MIC, and SI as a function of retention time and helicity for 28 +5 α/β-peptides (peptides **3** – **7**, Table 1 and S1) with different α/β-amino acid organization. The antifungal activity of +5 charged α/β-peptides correlated with hydrophobicity, independent of ACPC residue content (Fig. 3a). In general, α/β-peptides that displayed higher helix stability exhibited lower MICs than did conformationally unstable α/β-peptides, but some conformationally unstable α/β-peptides were toxic to *C. albicans* (Fig. S7a). The effect of ACPC content on enhancing antifungal activity of α/β-analogues of aurein 1.2 is similar to the effect of an aminocyclohexane carboxylic acid (ACHC) side chain previously reported to increase antifungal activity in 14-helical β-peptides.[10] In addition to stabilizing helical structures of the peptides, ACPC and ACHC residues affect hydrophobicity, which also regulates antifungal activity. Interestingly, the hemolysis of +5 charged α/β-aurein analogues roughly correlated with hydrophobicity (Fig. S7b). Hemolysis was a strong function of α/β-peptide helicity, however, with ACPC-containing peptides eliciting less hemolysis than α/β-peptides lacking ACPC residues (Fig. 3b). In addition, SI of α/β-aurein analogues correlated with hydrophobicity but not helicity (Fig. 3c and S7c). The RP-HPLC retention time value that resulted in the maximum SI was greater in α/β-peptides containing ACPC residues than in α/β-peptides lacking ACPC residues (Fig. 3c). The effect of ACPC on selectivity is primarily driven by the reduction in hemolysis upon ACPC incorporation (Fig 3b) since ACPC content had little effect on the antifungal MIC (Fig. 3a and S7a). Based on these results, we conclude that α/β-peptide hydrophobicity and helicity affect antifungal activity and hemolysis. Furthermore, incorporation of the helix-stabilizing β-amino acid ACPC is a strategy to improve the selectivity of α/β-peptides.

To compare the extent to which hydrophobicity and helicity regulate α/β-aurein antifungal activity and selectivity, we employed a linear regression analysis to elucidate key α/β-

peptide descriptors that determine MIC and hemolysis at the MIC. Combinations of retention time, helicity, molar ellipticity, the number of ACPC residues, and β-amino acid ratio in α/β-aurein analogues could account for antifungal activity and hemolysis. MIC exhibited a strong negative correlation with α/β-peptide retention time as shown in Fig 2a, and ACPC content and molar ellipticity in 15% TFE (ME_{15}) significantly affected log_2 MIC ($p<0.05$, Table S2). In contrast, log_2 hemolysis exhibited a more significant correlation with helicity than retention time. Additionally, retention time, ACPC content, β-amino acid ratio, and molar ellipticity in 100% TFE (ME_{100}) significantly affected hemolysis ($p < 0.05$, Table S2). Taken together, the antifungal activity of α/β-aurein analogues was most significantly controlled by peptide hydrophobicity with more minor contributions of other descriptors such as the number of ACPC residues and helicity. On the other hand, α/β-aurein analogue cytotoxicity was predominantly a function of helicity with less statistically significant contributions of hydrophobicity and ACPC content.

In conclusion, we have engineered +5 charged α/β-peptide analogues of aurein 1.2 composed of repeating ααβ, αααβ, and ααβααβ-peptide motifs that fold into $i \rightarrow i$+4 H-bonded helices and exhibit high selectivity for *C. albicans* over mammalian red blood cells. This approach for templating AMP sequences on α/β-peptide backbones that adopt $i \rightarrow i$+4 H-bonded helices provides a novel approach to engineer natural AMPs and improve their properties for therapeutic applications. In addition, we used a panel of α/β-aurein analogues and linear regression analysis to clarify how key α/β-peptide properties confer functionality, including that hydrophobicity is the primary predictor of antifungal activity and that helicity most strongly correlates with hemolysis. These structure-activity relationships provide insights that will expedite the development of α/β-peptides for antifungal applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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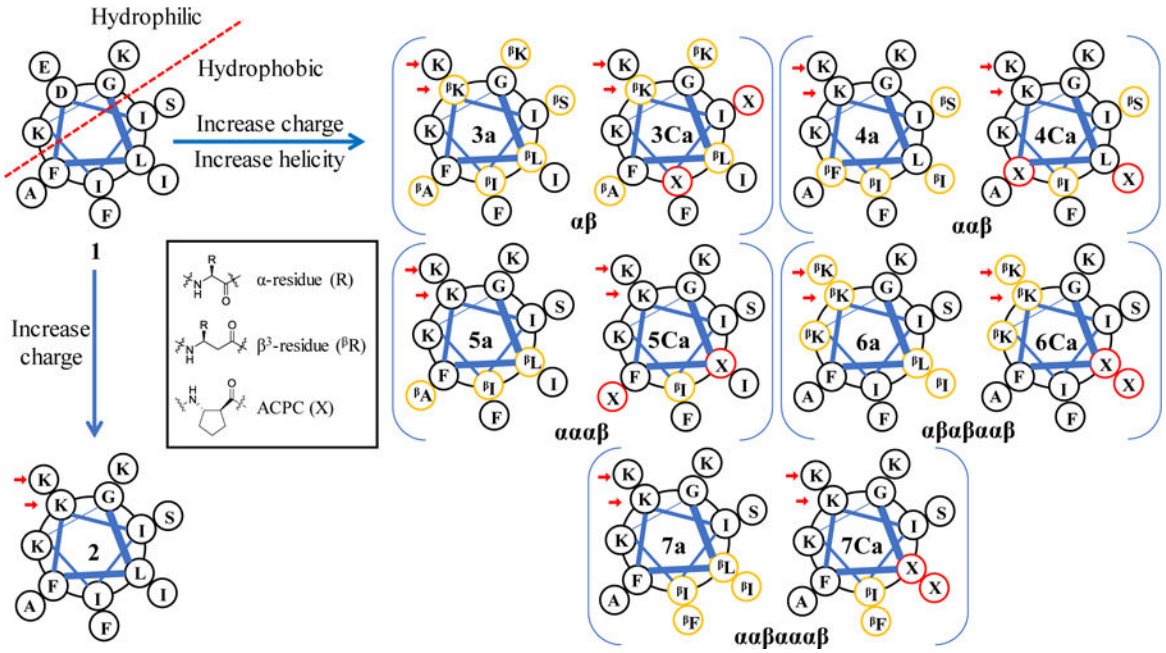

Figure 1.

Schematic of the approach to develop active and selective α/β-analogues of aurein 1.2 that fold into $i \rightarrow i$+4 H-bonded helices. Wheel diagrams of α-aurein 1.2 and α/β-peptides illustrate amphiphilicity and side chain positions of the helical peptides. Black circles represent α-amino acids, orange circles represent β-amino acids, red circles represent the cyclic β-amino acids ACPC, and red arrows represent the amino acid side chain changes from the sequence of native aurein 1.2.

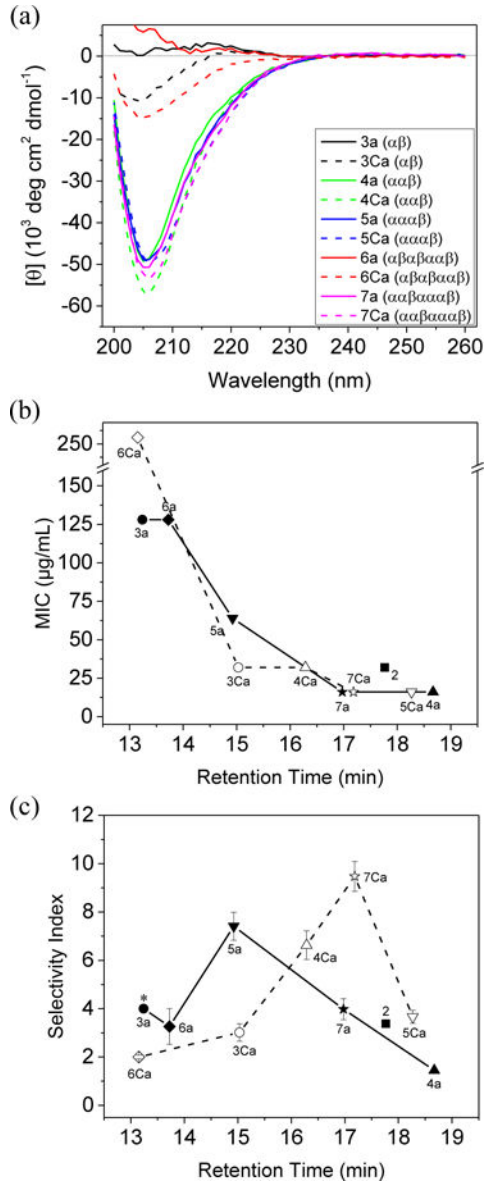

Figure 2.

(a) Circular dichroism spectra of α/β-peptides in 100% trifluoroethanol. Solid lines and dotted lines indicate α/β-peptides lacking (**3a** – **7a**) and containing ACPC residues (**3Ca** – **7Ca**), respectively. Correlations of MIC (b) and SI (c) with α/β-peptide RP-HPLC retention time. In (b) and (c) α/β-amino acid organization is denoted by symbol shape. Peptides numbers are shown near corresponding symbols. * represents the SI of peptide **3a** (SI > 4). Filled symbols and solid lines indicate α/β-peptides lacking ACPC residues, while open symbols and dotted lines indicate α/β-peptides containing ACPC residues. Data are presented as mean ± SD (n = 3).

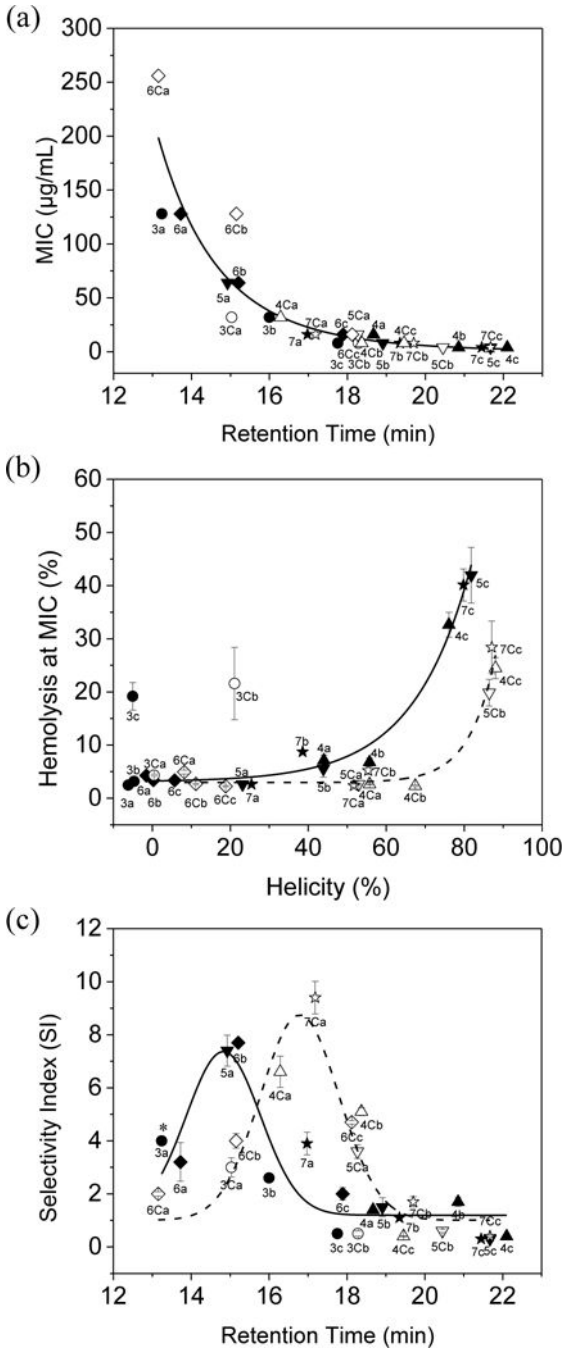

Figure 3.

(a) The relationship between MIC against *C. albicans* and RP-HPLC retention time of α/β-aurein analogues. (b) The relationship between hemolysis at the MIC and helicity of α/β-aurein analogues. (c) The relationship between selectivity index and RP-HPLC retention time of α/β-aurein analogues. Open and filled symbols represent α/β-peptides containing and lacking ACPC residues, respectively. Peptides numbers are shown near corresponding data points. * represents the SI of peptide **3a** (SI > 4). Lines in (a) and (b) show data fits to

allometric relationships and lines in (c) show fits to Gaussian distributions. Data are presented as mean ± SD (n = 3).

Table 1

Retention time, minimum inhibitory concentration (MIC) against *C. albicans*, % hemolysis at MIC, and selectivity index (SI) of α/β-aurein analogues.

#	α/β	Sequence	RT[a] (min ± SD)	MIC[b] (μg/mL)	% Hemolysis at MIC[c] ± SD	Selectivity Index[d] ± SD
1	α	G-L-F-D-I-I-K-K-I-A-E-S-F-NH2	22.89 ± 0.02	32	7.6 ± 0.9	1.1 ± 0.03
2	α	G-L-F-K-I-I-K-K-I-A-K-S-F-NH2	17.76 ± 0.02	32	2.9 ± 0.1	3.3 ± 0.13
3a	αβ	G –$^{\beta}$L-F –$^{\beta}$K -I –$^{\beta}$I-K –$^{\beta}$K-I –$^{\beta}$A-K –$^{\beta}$S-F-NH2	13.23 ± 0.03	128	2.5 ± 0.1	> 4
3b	αβ	G –$^{\beta}$L-F –$^{\beta}$K-I –$^{\beta}$I-K –$^{\beta}$K-I –$^{\beta}$L-K –$^{\beta}$S-F-NH2	15.99 ± 0.01	32	3.2 ± 0.3	2.6 ± 0.16
3c	αβ	G –$^{\beta}$L-F –$^{\beta}$K-I –$^{\beta}$I-K –$^{\beta}$K-I –$^{\beta}$L-K –$^{\beta}$L-F-NH2	17.75 ± 0.01	8	19.2 ± 2.6	0.5 ± 0.07
3Ca	αβ	G –$^{\beta}$L-F –$^{\beta}$K-I- X-K –$^{\beta}$K-I –$^{\beta}$A-K- X-F-NH2	15.03 ± 0.02	32	4.3 ± 0.2	3 ± 0.36
3Cb	αβ	G –$^{\beta}$L-F –$^{\beta}$K-I- X-K –$^{\beta}$K-I –$^{\beta}$L-K- X-F-NH2	18.28 ± 0.02	8	21.6 ± 6.8	0.5 ± 0.1
4a	ααβ	G-L –$^{\beta}$F-K-I –$^{\beta}$I-K-K –$^{\beta}$I-A-K –$^{\beta}$S-F-NH2	18.66 ± 0.02	16	7.1 ± 1.0	1.4 ± 0.05
4b	ααβ	G-L –$^{\beta}$F-K-I –$^{\beta}$I-K-K –$^{\beta}$I-L-K –$^{\beta}$S-F-NH2	20.85 ± 0.02	4	6.8 ± 0.8	1.7 ± 0.17
4c	ααβ	G-L –$^{\beta}$F-K-I –$^{\beta}$I-K-K –$^{\beta}$I-L-K –$^{\beta}$L-F-NH2	22.11 ± 0.03	4	32.6 ± 2.3	0.4 ± 0.02
4Ca	ααβ	G-L- X-K-I –$^{\beta}$I-K-K- X-A-K –$^{\beta}$S-F-NH2	16.28 ± 0.01	32	2.6 ± 0.1	6.6 ± 0.59
4Cb	ααβ	G-L- X-K-I –$^{\beta}$I-K-K- X-L-K –$^{\beta}$S-F-NH2	18.37 ± 0.02	8	2.3 ± 0.1	5.1 ± 0.15
4Cc	ααβ	G-L- X-K-I –$^{\beta}$I-K-K- X-L-K –$^{\beta}$L-F-NH2	19.45 ± 0.02	8	24.5 ± 2.0	0.4 ± 0.01
5a	αααβ	G –$^{\beta}$L-F-K-I –$^{\beta}$I-K-K-I –$^{\beta}$A-K-S-F-NH2	14.92 ± 0.02	64	2.6 ± 0.3	7.4 ± 0.58
5b	αααβ	G –$^{\beta}$L-F-K-I –$^{\beta}$I-K-K-I –$^{\beta}$L-K-S-F-NH2	18.91 ± 0.02	8	5.5 ± 1.6	1.5 ± 0.35
5c	αααβ	G –$^{\beta}$L-F-K-I –$^{\beta}$I-K-K-I –$^{\beta}$L-K-L-F-NH2	21.67 ± 0.02	4	41.9 ± 5.2	0.3 ± 0.02
5Ca	αααβ	G- X-F-K-I –$^{\beta}$I-K-K-I- X-K-S-F-NH2	18.26 ± 0.02	16	2.6 ± 0.2	3.6 ± 0.26
5Cb	αααβ	G- X-F-K-I –$^{\beta}$I-K-K-I- X-K-L-F-NH2	20.45 ± 0.32	4	19.8 ± 2.5	0.6 ± 0.06

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#	α/β	Sequence	RT[a] (min ± SD)	MIC[b] (μg/mL)	% Hemolysis at MIC[c] ± SD	Selectivity Index[d] ± SD
6a	αβαβααβ	G –$^{\beta}$**L**-F –$^{\beta}$**K**-I-I –$^{\beta}$**K**-K –$^{\beta}$**I**-A –$^{\beta}$**K**-S-F-NH2	13.72 ± 0.01	128	4.3 ± 0.4	3.2 ± 0.73
6b	αβαβααβ	G –$^{\beta}$**L**-F –$^{\beta}$**K**-I-I –$^{\beta}$**K**-K –$^{\beta}$**I**-L –$^{\beta}$**K**-S-F-NH2	15.20 ± 0.01	64	3.4 ± 0.1	7.7 ± 0.1
6c	αβαβααβ	G –$^{\beta}$**L**-F –$^{\beta}$**K**-I-I –$^{\beta}$**K**-K –$^{\beta}$**I**-L –$^{\beta}$**K**-L-F-NH2	17.88 ± 0.01	16	3.4 ± 0.2	2 ± 0.23
6Ca	αβαβααβ	G-**X**-F –$^{\beta}$**K**-I-I –$^{\beta}$**K**-K-**X**-A –$^{\beta}$**K**-S-F-NH2	13.15 ± 0.01	256	4.9 ± 0.4	2 ± 0.08
6Cb	αβαβααβ	G-**X**-F –$^{\beta}$**K**-I-I –$^{\beta}$**K**-K-**X**-L –$^{\beta}$**K**-S-F-NH2	15.15 ± 0.01	128	2.7 ± 0.2	4 ± 0.28
6Cc	αβαβααβ	G-**X**-F –$^{\beta}$**K**-I-I –$^{\beta}$**K**-K-**X**-L –$^{\beta}$**K**-L-F-NH2	18.12 ± 0.01	16	2.3 ± 0.1	4.7 ± 0.06
7a	ααβαααβ	G –$^{\beta}$**L**-F-K-I –$^{\beta}$**I**-K-K –$^{\beta}$**I**-A-K-S-$^{\beta}$**F**-NH2	16.97 ± 0.02	16	2.6 ± 0.3	3.9 ± 0.43
7b	ααβαααβ	G –$^{\beta}$**L**-F-K-I –$^{\beta}$**I**-K-K –$^{\beta}$**I**-L-K-S –$^{\beta}$**F**-NH2	19.34 ± 0.03	8	8.7 ± 0.3	1.1 ± 0.02
7c	ααβαααβ	G –$^{\beta}$**L**-F-K-I –$^{\beta}$**I**-K-K –$^{\beta}$**I**-L-K-L –$^{\beta}$**F**-NH2	21.44 ± 0.03	4	40.1 ± 3.0	0.3 ± 0.05
7Ca	ααβαααβ	G-**X**-F-K-I –$^{\beta}$**I**-K-K-**X**-A-K-S –$^{\beta}$**F**-NH2	17.18 ± 0.01	16	2.4 ± 0.3	9.4 ± 0.61
7Cb	ααβαααβ	G-**X**-F-K-I –$^{\beta}$**I**-K-K-**X**-L-K-S –$^{\beta}$**F**-NH2	19.70 ± 0.01	8	5.2 ± 0.7	1.7 ± 0.19
7Cc	ααβαααβ	G-**X**-F-K-I –$^{\beta}$**I**-K-K-**X**-L-K-L –$^{\beta}$**F**-NH2	21.67 ± 0.03	4	28.4 ± 5.0	0.4 ± 0.04
8	ααβαααβ	G-**X**-F-K-I –$^{\beta}$**I**-K-K-**X**-A-E-S –$^{\beta}$**F**-NH2	19.17 ± 0.03	64	3.1 ± 0.4	2.2 ± 0.17
9	ααβαααβ	G-**X**-F-D-I –$^{\beta}$**I**-K-K-**X**-A-K-S –$^{\beta}$**F**-NH2	19.67 ± 0.01	16	2.3 ± 0.1	2.9 ± 0.1
10	ααβαααβ	G-**X**-F-K-I –$^{\beta}$**I**-K-K –$^{\beta}$**I**-A-K-S –$^{\beta}$**F**-NH2	18.26 ± 0.02	16	4.8 ± 0.2	1.9 ± 0.23
11	ααβαααβ	G –$^{\beta}$**L**-F-K-I –$^{\beta}$**I**-K-K-**X**-A-K-S –$^{\beta}$**F**-NH2	16.10 ± 0.01	32	2.9 ± 0.2	6.2 ± 0.63

[a]The average retention time was obtained from three independent analytical RP-HPLC measurements.

[b]The MIC was obtained from an average of three independent experiments with triplicate measurements in each.

[c]The percent hemolysis at the MIC was obtained from three independent experiments with duplicate measurements in each. SD denotes standard deviation (n = 3).

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[d] SI = HC_{10}/MIC. Black characters represent α-amino acids, orange characters represent β-amino acids, red X represents the cyclic β-amino acid ACPC. SD denotes standard deviation. See Table S1 for additional data describing peptides.