Supporting Information

HYDROPHOBICITY AND HELICITY REGULATE ANTIFUNGAL ACTIVITY OF 14-HELICAL β-PEPTIDES

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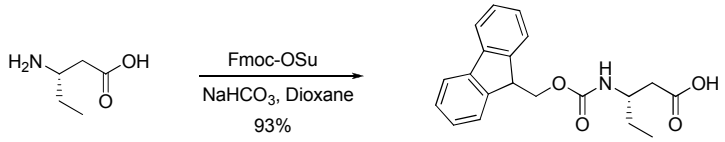
Synthesis of (S)-3-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-pentanoic acid

Scheme S1. Synthesis of Fmoc-β^3-Et-OH.

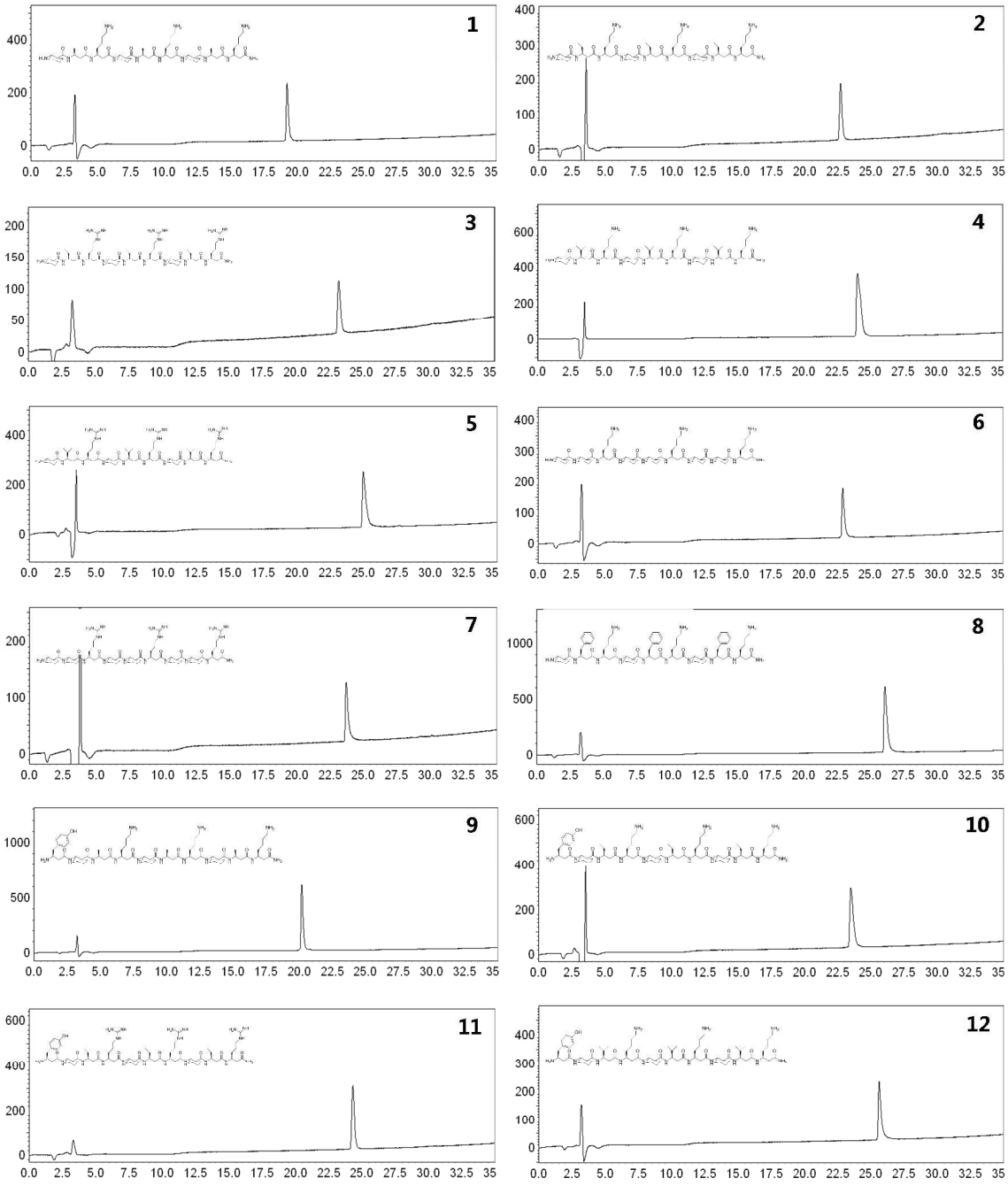


S1

(S)-3-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-pentanoic acid (Fmoc-β^3-Et-OH) (S1) To a stirred solution of (S)-3-aminopentanoic acid (1 g, 8.53 mmol) in 10% aqueous $NaCO_3$ (36 mL) and dioxane (15 mL) was added a solution of Fmoc-OSu (3.45 g, 10.24 mmol) in dioxane (15 mL). After 12 h at room temp, the reaction mixture was diluted with 200 mL water and washed 3 times with ether. The pH of the reaction mixture was adjusted to 2 and extracted with EA. The combined organic solution was washed with H_2O and brine, dried ($MgSO_4$), and concentrated *in vacuo* to give Fmoc-β^3-Et-OH in 93% yield as a white solid. The product was used without further purification.: ^{1}H NMR (300 MH CD_3OD) δ 12.12 (s, 1 H), 7.89 (d, 2 H, J = 9.0 Hz), 7.74-7.65 (m, 2 H), 7.45-7.26 (m, 4 H), 7.19 (d, 2 H, J = 9.0 Hz), 4.40-4.14 (m, 3 H), 3.80-3.60 (m, 1 H), 2.42-2.25 (m, 2 H), 1.56-1.25 (m, 2 H), 0.81 (t, 3 H, J = 6.0 Hz); ^{13}C NMR (75 MHz, CD_3OD) δ 172.5, 155.6, 143.9, 140.7, 127.6, 127.0, 125.2, 120.1, 65.1, 49.3, 46.8, 39.4, 27.1, 10.2; HRMS (m/z, ESI) calculated for $C_{20}H_{21}NO_4$ $[M+H]^+$ 340.1544, found 340.1540.

Table S1. β-Peptide sequences were validated by MALDI-TOF with α-cyano-4-hydroxycinnamic acid.

β-Peptides	Peptide Sequence	Molecular Formula	$[M+H]^+$ Calcd.	MALDI-TOF Found
1	NH_2-(ACHC-β^3hAla-β^3hLys)$_3$	$C_{54}H_{99}N_{13}O_9$	1074.8	1074.6
2	NH_2-(ACHC-β^3Et-β^3hLys)$_3$	$C_{57}H_{105}N_{13}O_9$	1116.8	1116.7
3	NH_2-(ACHC-β^3Et-β^3hArg)$_3$	$C_{57}H_{105}N_{19}O_9$	1200.8	1200.6
4	NH_2-(ACHC-β^3hVal-β^3hLys)$_3$	$C_{60}H_{111}N_{13}O_9$	1158.9	1158.9
5	NH_2-(ACHC-β^3hVal-β^3hArg)$_3$	$C_{60}H_{111}N_{19}O_9$	1242.9	1242.9
6	NH_2-(ACHC-ACHC-β^3hLys)$_3$	$C_{63}H_{111}N_{13}O_9$	1194.9	1194.7
7	NH_2-(ACHC-ACHC-β^3hArg)$_3$	$C_{63}H_{111}N_{19}O_9$	1278.9	1279.0
8	NH_2-(ACHC-β^3hPhe-β^3hLys)$_3$	$C_{72}H_{111}N_{13}O_9$	1302.9	1302.7
9	NH_2-β^3hTyr-(ACHC-β^3hAla-β^3hLys)$_3$	$C_{64}H_{110}N_{14}O_{11}$	1251.9	1252.0
10	NH_2-β^3hTyr-(ACHC-β^3Et-β^3hLys)$_3$	$C_{67}H_{116}N_{14}O_{11}$	1293.9	1294.1
11	NH_2-β^3hTyr-(ACHC-β^3Et-β^3hArg)$_3$	$C_{67}H_{116}N_{20}O_{11}$	1377.9	1377.8
12	NH_2-β^3hTyr-(ACHC-β^3hVal-β^3hLys)$_3$	$C_{70}H_{122}N_{14}O_{11}$	1335.9	1336.1
13	NH_2-β^3hTyr-(ACHC-β^3hVal-β^3hArg)$_3$	$C_{70}H_{122}N_{20}O_{11}$	1420.0	1420.0
14	NH_2-β^3hTyr-(ACHC-ACHC-β^3hLys)$_3$	$C_{73}H_{122}N_{14}O_{11}$	1371.9	1372.0
15	NH_2-β^3hTyr-(ACHC-ACHC-β^3hArg)$_3$	$C_{73}H_{122}N_{20}O_{11}$	1456.0	1455.9
16	NH_2-β^3hTyr-(ACHC-β^3hPhe-β^3hLys)$_3$	$C_{82}H_{122}N_{14}O_{11}$	1479.9	1480.1
17	NH_2-(β^3hVal-β^3hVal-β^3hLys)$_3$	$C_{57}H_{111}N_{13}O_9$	1122.9	1123.0
18	NH_2-(β^3hVal-β^3hVal-β^3hArg)$_3$	$C_{57}H_{111}N_{19}O_9$	1206.9	1206.8
19	NH_2-β^3hTyr-(β^3hVal-β^3Et-β^3hArg)$_3$	$C_{64}H_{116}N_{20}O_{11}$	1341.9	1341.8
20	NH_2-β^3hTyr-(β^3hVal-β^3hVal-β^3hLys)$_3$	$C_{67}H_{122}N_{14}O_{11}$	1299.9	1299.8
21	NH_2-β^3hTyr-(β^3hVal-β^3hVal-β^3hArg)$_3$	$C_{67}H_{122}N_{20}O_{11}$	1384.0	1384.1
22	NH_2-(β^3hIle-β^3Et-β^3hLys)$_3$	$C_{57}H_{111}N_{13}O_9$	1122.9	1123.0
23	NH_2-(β^3hIle-β^3Et-β^3hArg)$_3$	$C_{57}H_{111}N_{19}O_9$	1206.9	1207.1
24	NII_2-β^3hTyr-(β^3hIle-β^3Et-β^3hLys)$_3$	$C_{67}H_{122}N_{14}O_{11}$	1299.9	1300.1
25	NH_2-β^3hTyr-(β^3hIle-β^3Et-β^3hArg)$_3$	$C_{67}H_{122}N_{20}O_{11}$	1384.0	1384.1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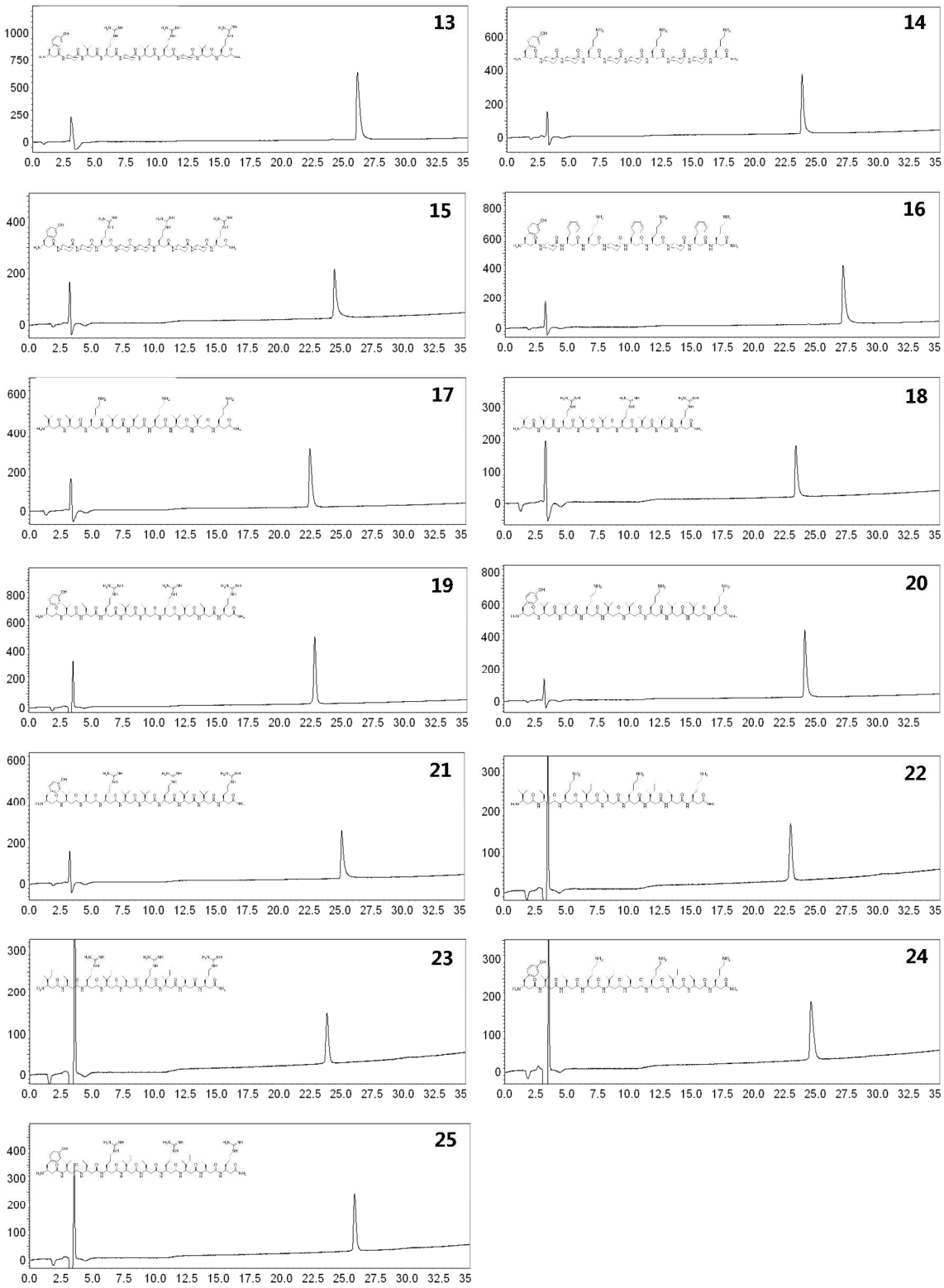
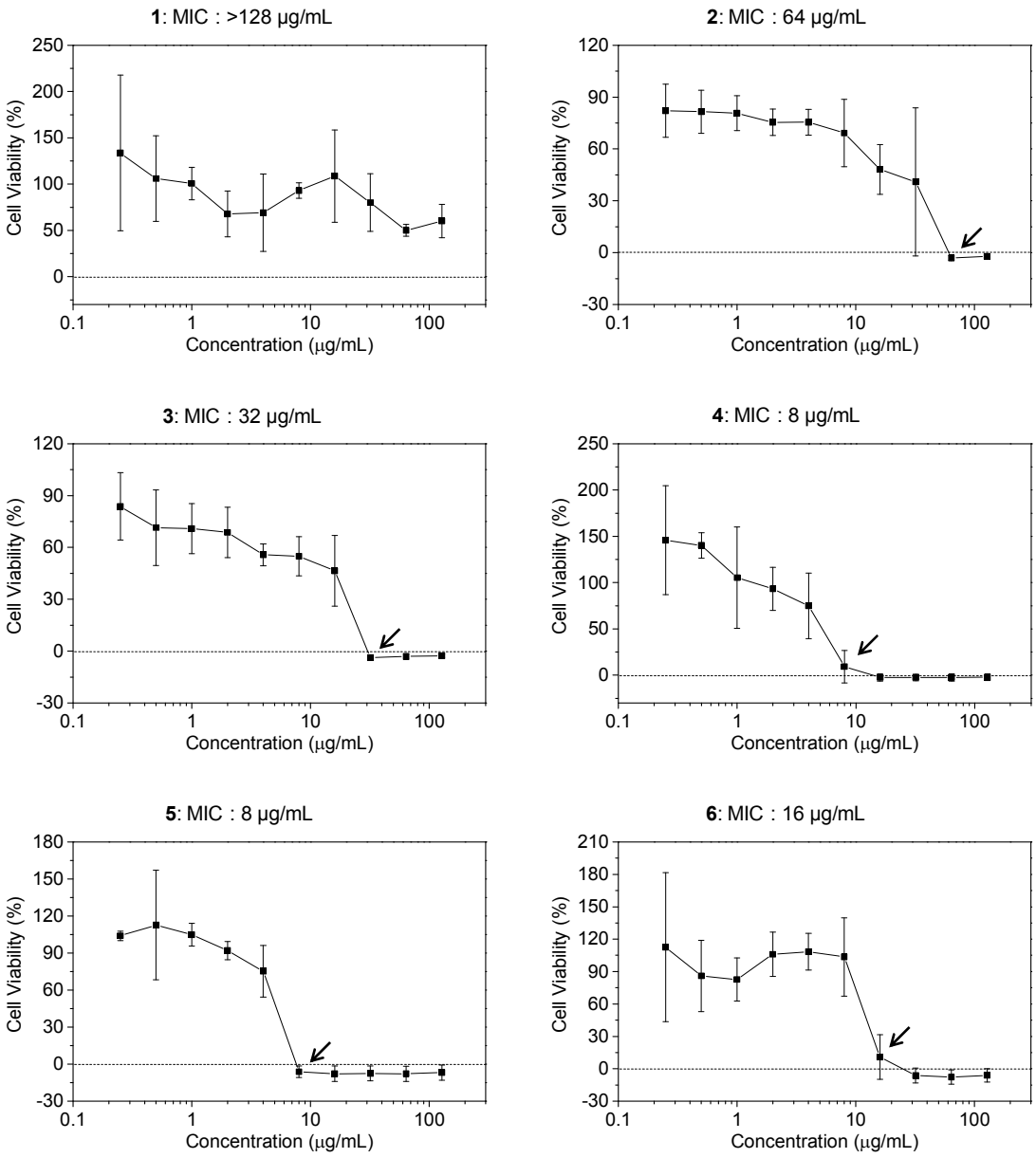
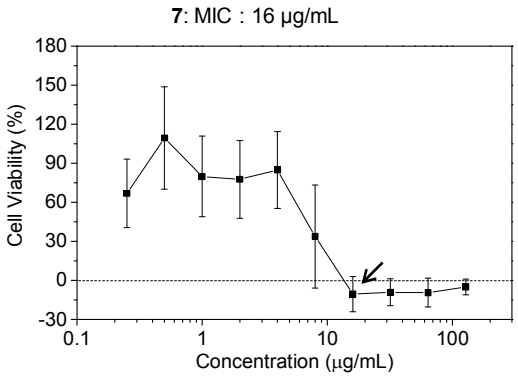
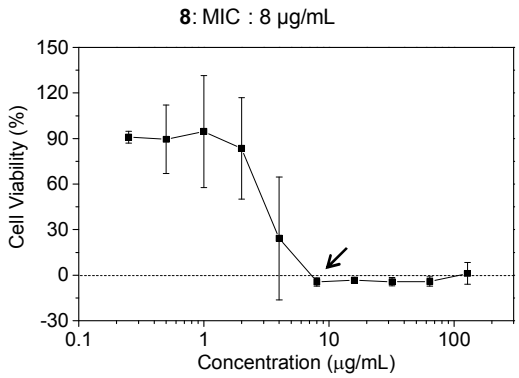
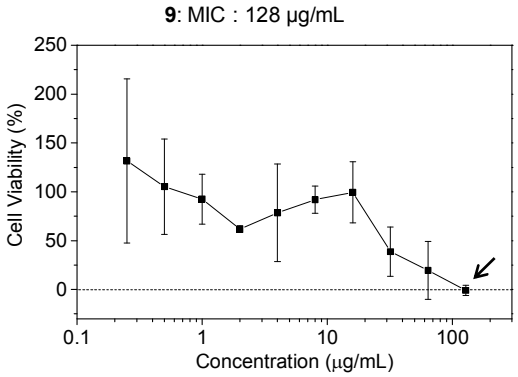


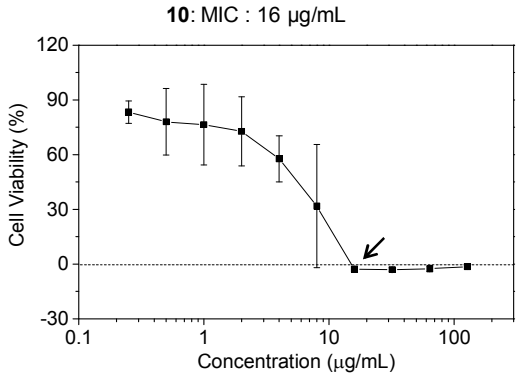
Figure S1. Analytical RP-HPLC profiles to measure retention time of 14-helical β-peptides.

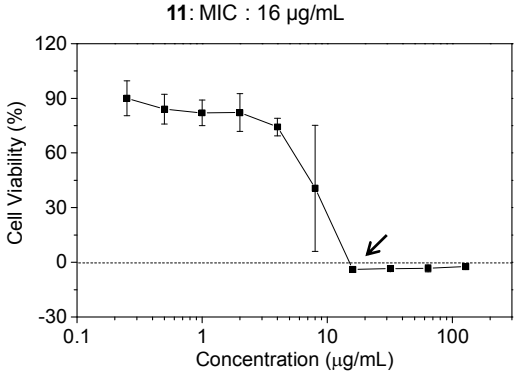

1: MIC : >128 μg/mL
2: MIC : 64 μg/mL
3: MIC : 32 μg/mL
4: MIC : 8 μg/mL
5: MIC : 8 μg/mL
6: MIC : 16 μg/mL
Cell Viability (%)
Concentration (μg/mL)

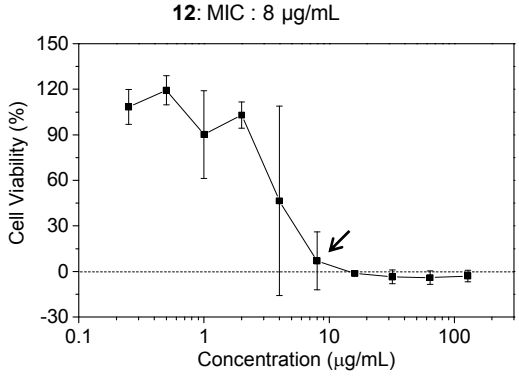

7: MIC : 16 μg/mL
Cell Viability (%)
Concentration (μg/mL)

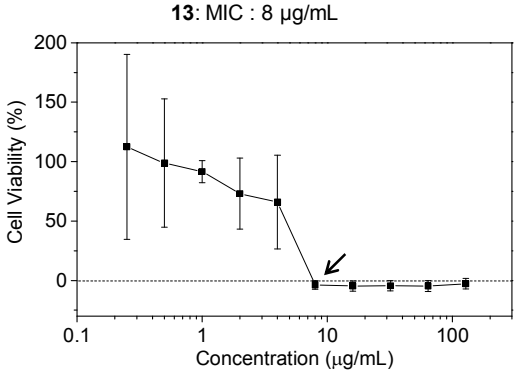

8: MIC : 8 μg/mL
Cell Viability (%)
Concentration (μg/mL)

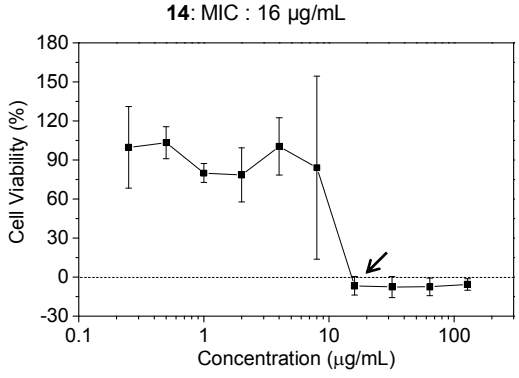

9: MIC : 128 μg/mL
Cell Viability (%)
Concentration (μg/mL)


10: MIC : 16 μg/mL
Cell Viability (%)
Concentration (μg/mL)

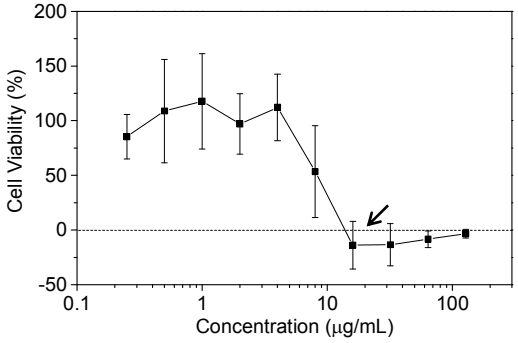

11: MIC : 16 μg/mL
Cell Viability (%)
Concentration (μg/mL)


12: MIC : 8 μg/mL
Cell Viability (%)
Concentration (μg/mL)

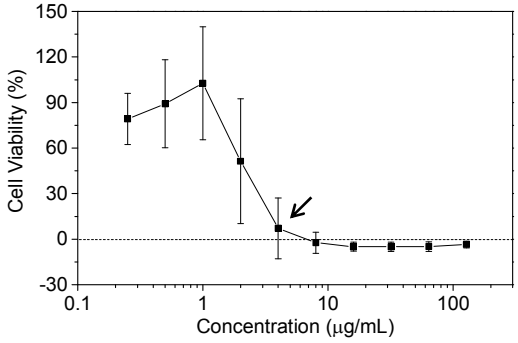

13: MIC : 8 μg/mL
Cell Viability (%)
Concentration (μg/mL)


14: MIC : 16 μg/mL
Cell Viability (%)
Concentration (μg/mL)

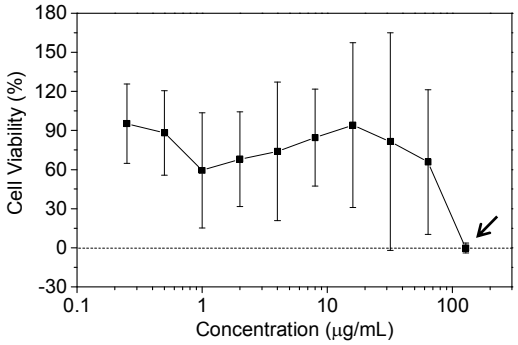
15: MIC : 16 μg/mL



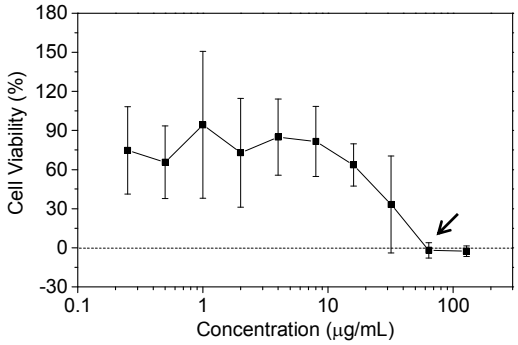
16: MIC : 4 μg/mL



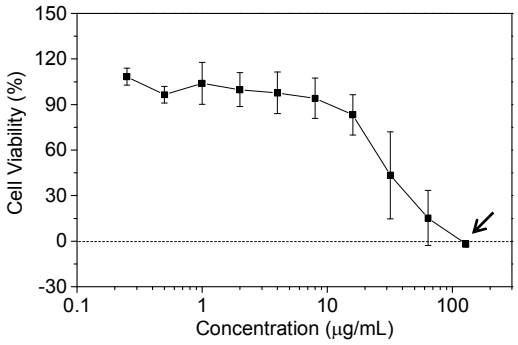
17: MIC : 128 μg/mL



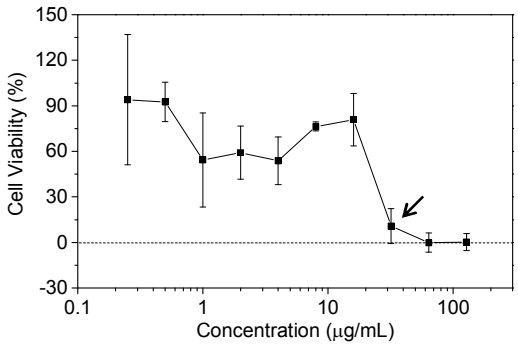
18: MIC : 64 μg/mL



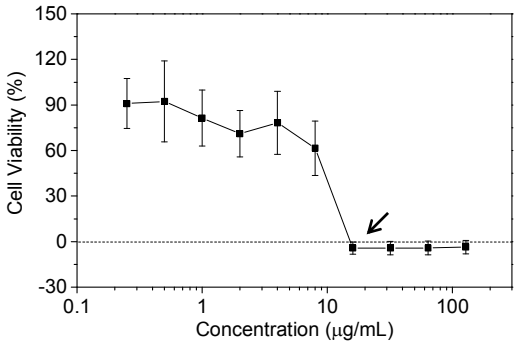
19: MIC : 128 μg/mL



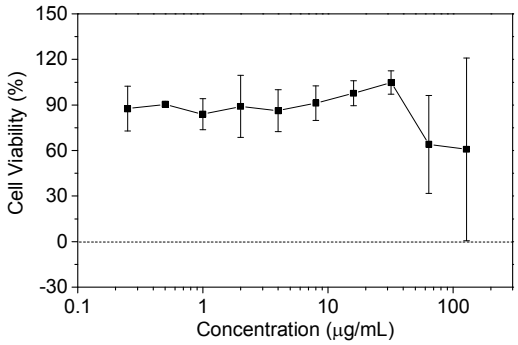
20: MIC : 32 μg/mL



21: MIC : 16 μg/mL



22: MIC : >128 μg/mL



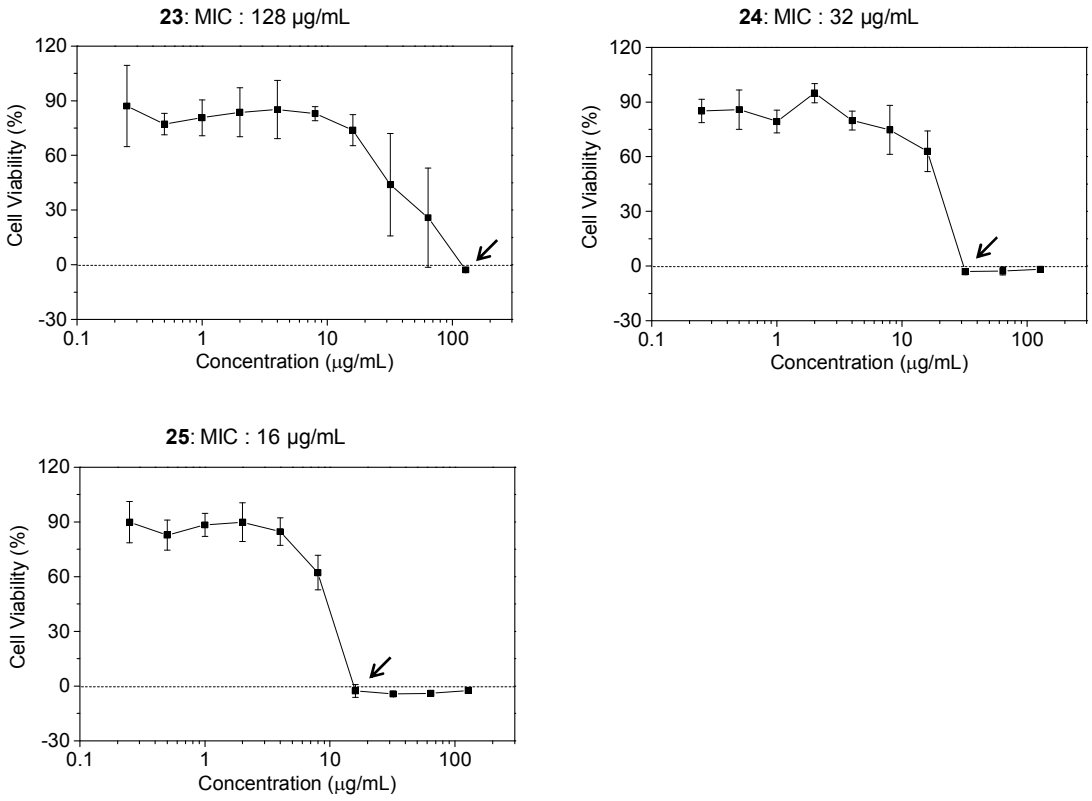
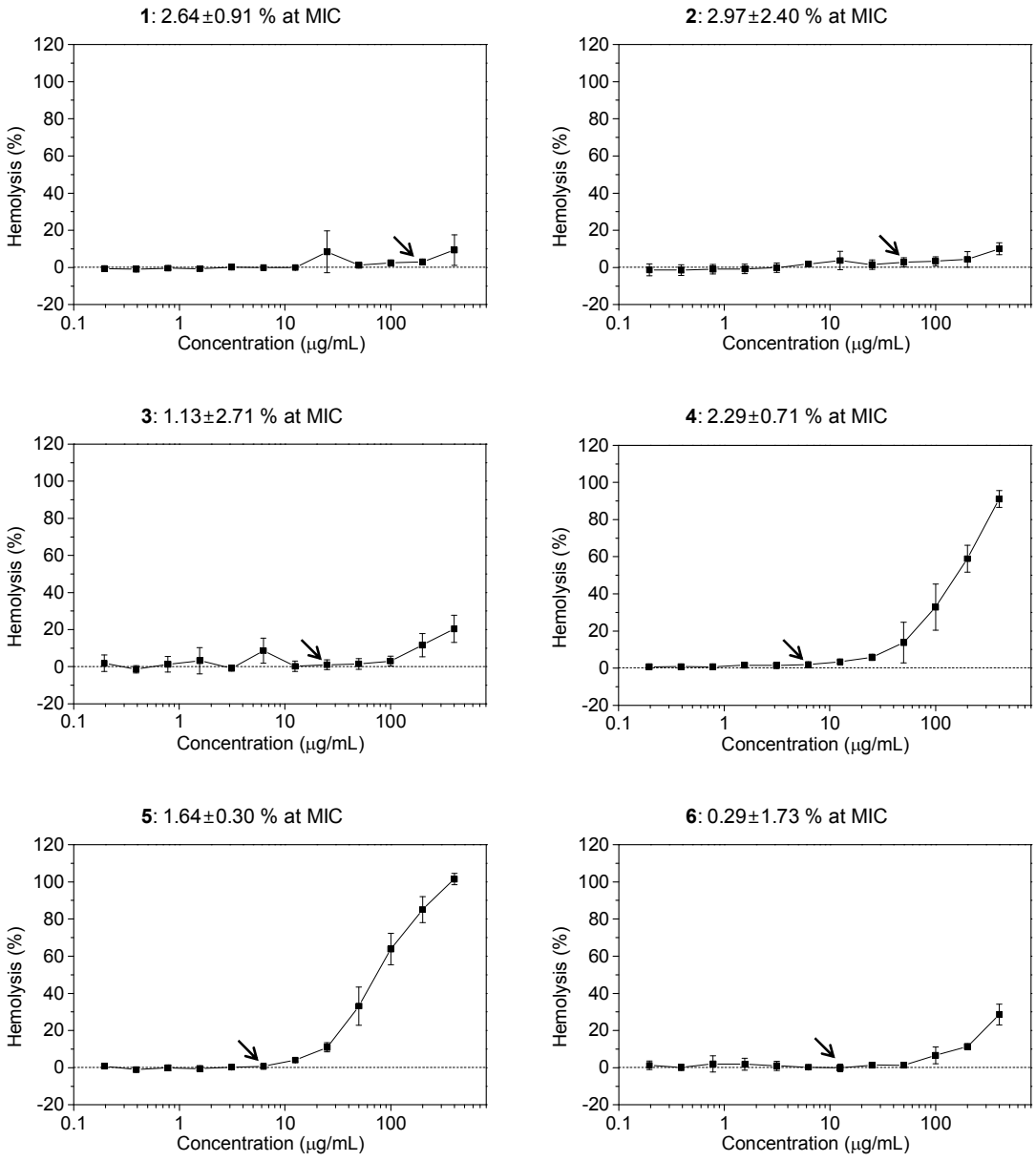
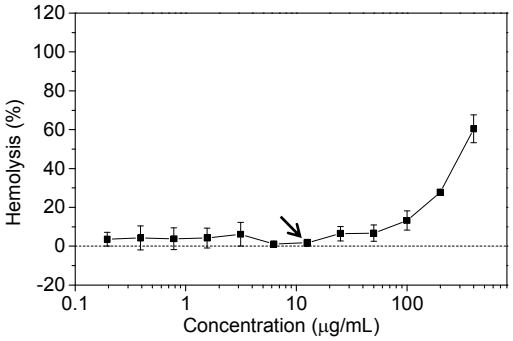


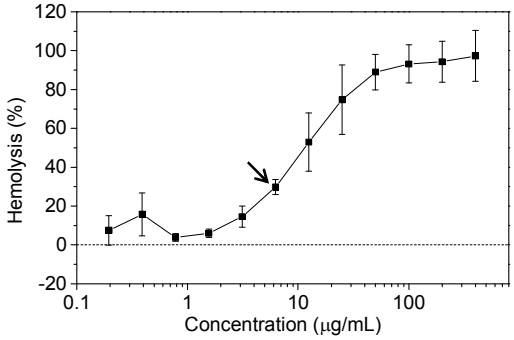
Figure S2. The plots of concentration-dependent growth inhibition of *C. albicans* by β-peptides. *C. albicans* cells (10^3 cells/mL) were incubated with β-peptides for 48 h and β-peptide susceptibility was assessed using an XTT reduction assay to compare the absorbance at 490 nm for β-peptide-treated samples and untreated samples. Data points are the average of three independent experiments of three replicates each. The arrow indicates the MIC value.


1: 2.64±0.91 % at MIC
2: 2.97±2.40 % at MIC
3: 1.13±2.71 % at MIC
4: 2.29±0.71 % at MIC
5: 1.64±0.30 % at MIC
6: 0.29±1.73 % at MIC
Hemolysis (%)
Concentration (μg/mL)
120
100
80
60
40
20
0
-20
0.1
1
10
100

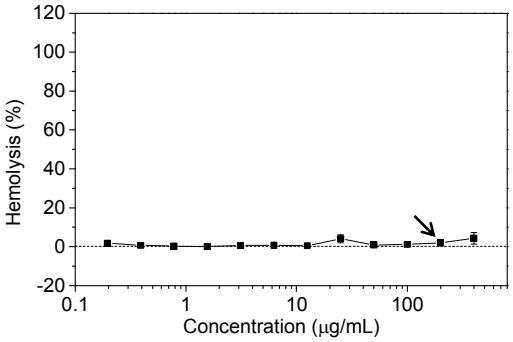
7: 3.04±2.32 % at MIC



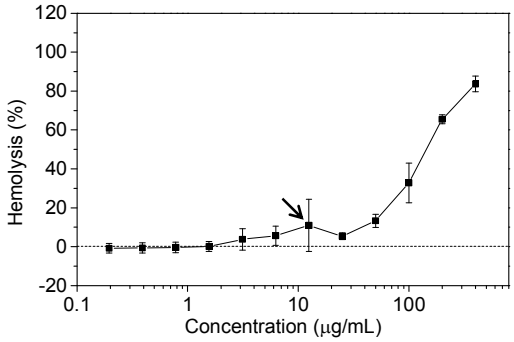
8: 36.26±5.97 % at MIC



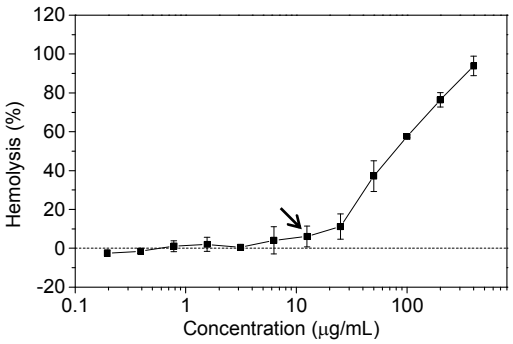
9: 1.36±0.64 % at MIC



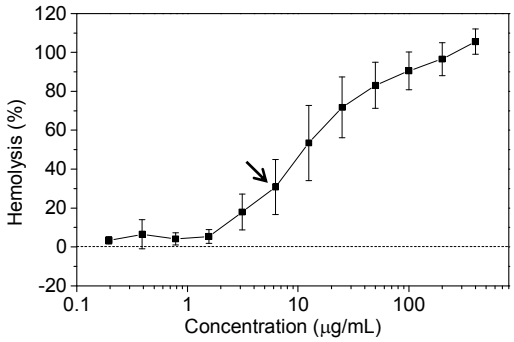
10: 9.36±9.27 % at MIC



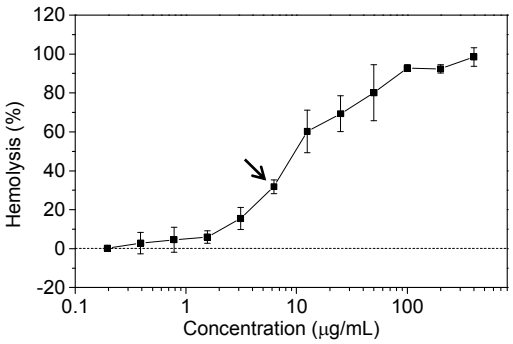
11: 7.50±5.18 % at MIC



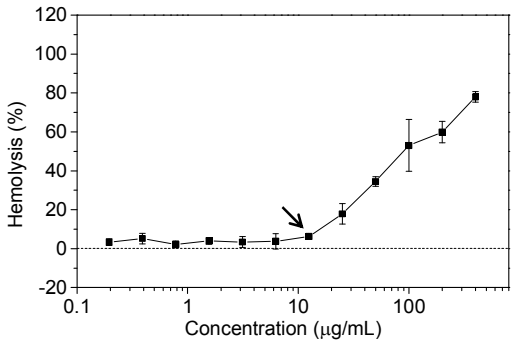
12: 37.16±15.4 % at MIC

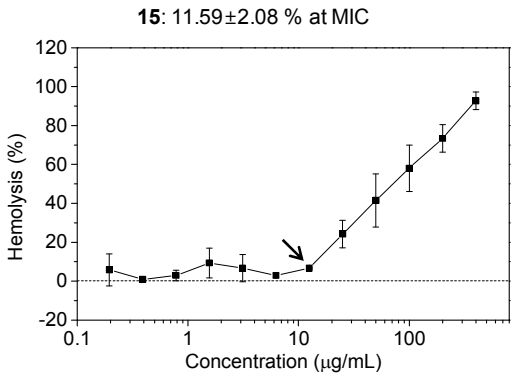


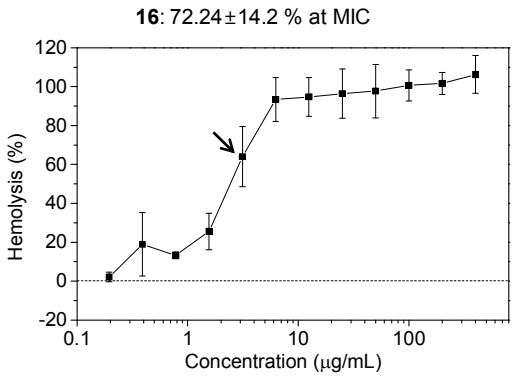
13: 39.78±2.69 % at MIC

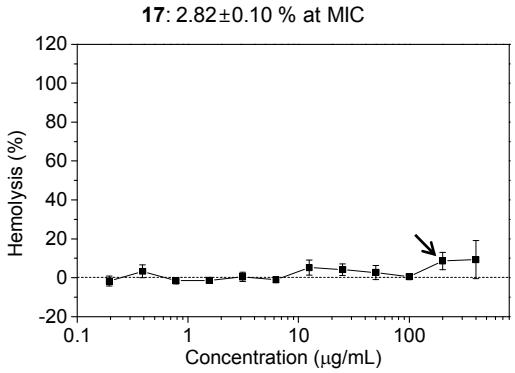


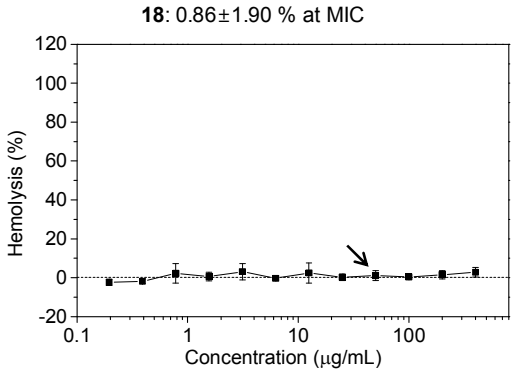
14: 9.46±2.23 % at MIC

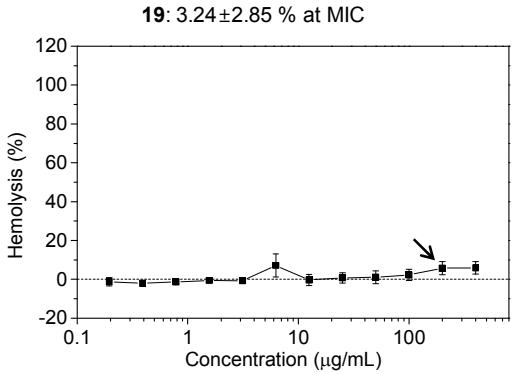


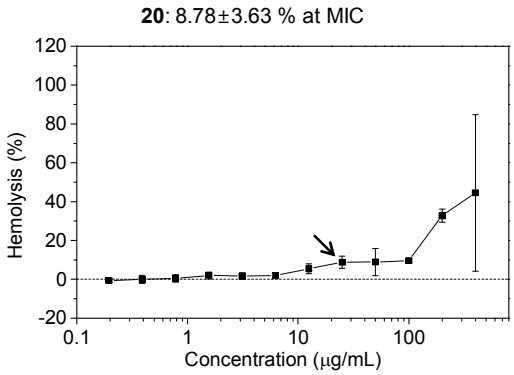

15: 11.59±2.08 % at MIC
Hemolysis (%)
Concentration (μg/mL)

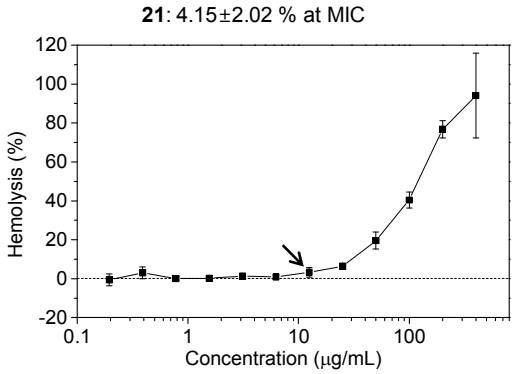

16: 72.24±14.2 % at MIC
Hemolysis (%)
Concentration (μg/mL)

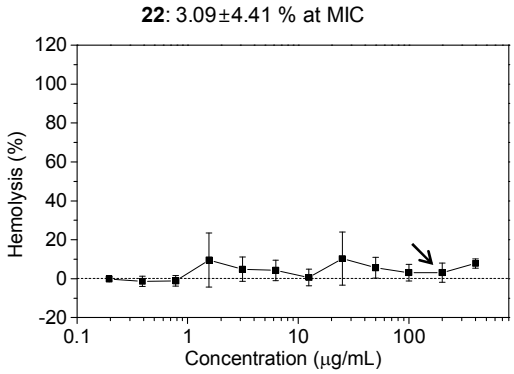

17: 2.82±0.10 % at MIC
Hemolysis (%)
Concentration (μg/mL)


18: 0.86±1.90 % at MIC
Hemolysis (%)
Concentration (μg/mL)


19: 3.24±2.85 % at MIC
Hemolysis (%)
Concentration (μg/mL)


20: 8.78±3.63 % at MIC
Hemolysis (%)
Concentration (μg/mL)


21: 4.15±2.02 % at MIC
Hemolysis (%)
Concentration (μg/mL)


22: 3.09±4.41 % at MIC
Hemolysis (%)
Concentration (μg/mL)

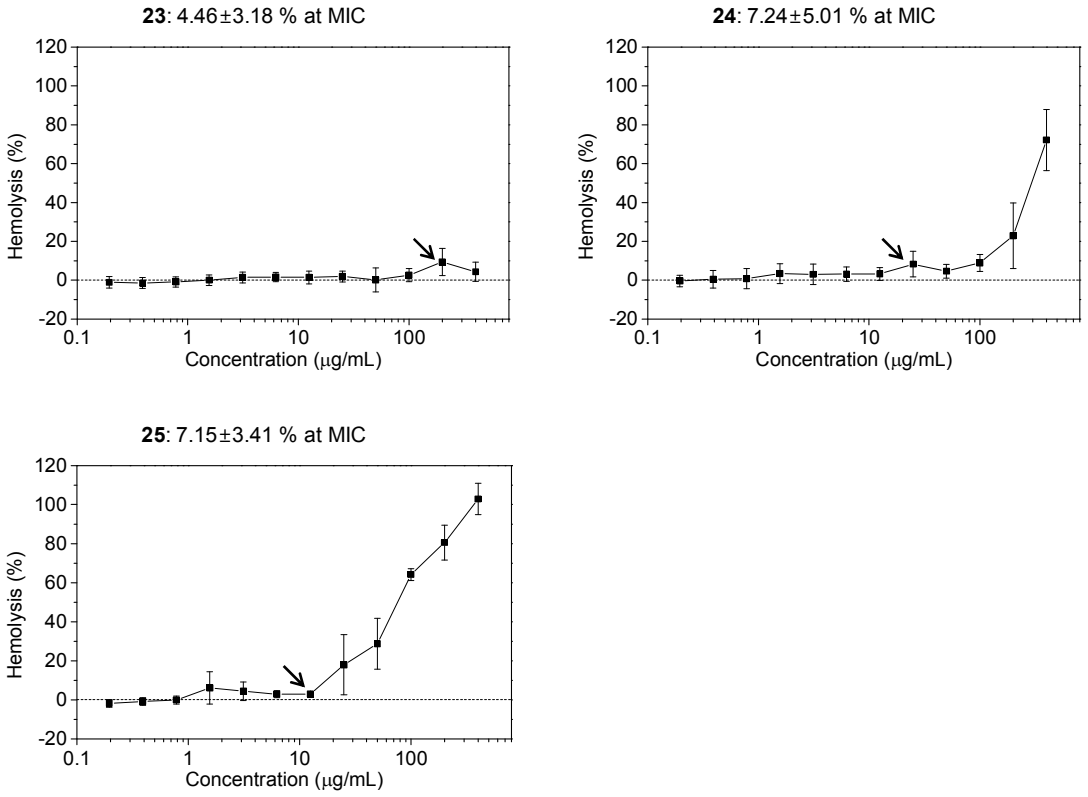


Figure S3. The concentration-dependent hemolysis of human red blood cells by β-peptides. β-peptides were incubated with human red blood cells for 1 h, and the absorbance of the supernatant was measured at 405 nm to calculate the percent of red blood cells lysed. 100% hemolysis was determined using a melittin control. Error bars denote standard deviation (n=3). The arrow indicates the hemolysis at the β-peptide MIC.

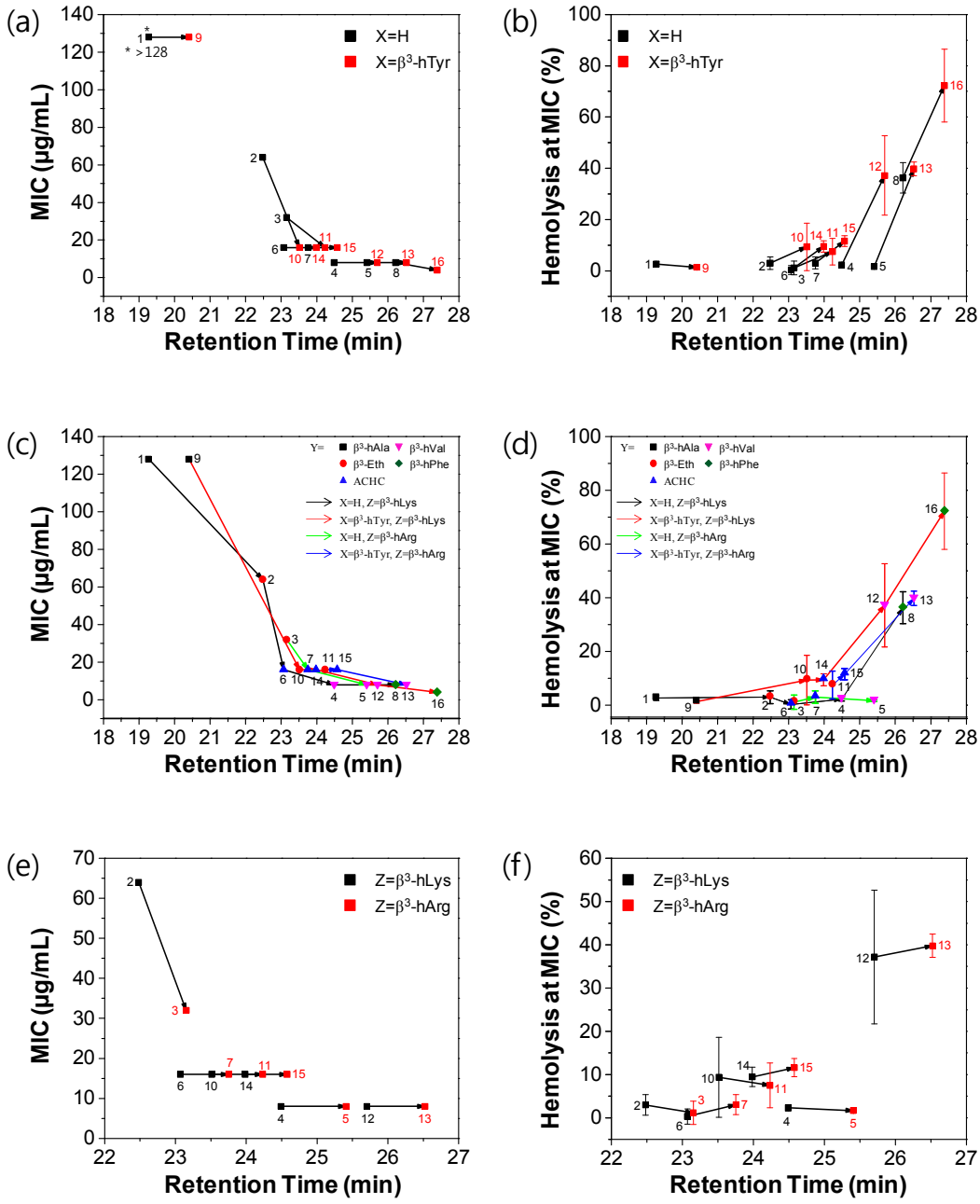


Figure S4. The effect of β-peptide modifications on RP-HPLC retention times, antifungal activity, and hemolytic activity. MIC (a) and hemolysis at the MIC (b) were affected by N-terminal modifications (Figure 1a and 1d, X). MIC (c) and hemolysis at the MIC (d) were affected by hydrophobic side chain variations (Figure 1a and 1d, Y). MIC (e) and hemolysis at the MIC (f) were affected by cationic residue variation (Figure 1a and 1d, Z). Error bars denote standard deviation (n=3). Sequences corresponding to the peptide numbers are provided in Figure 1. Arrows link peptides with a one β-amino acid difference.

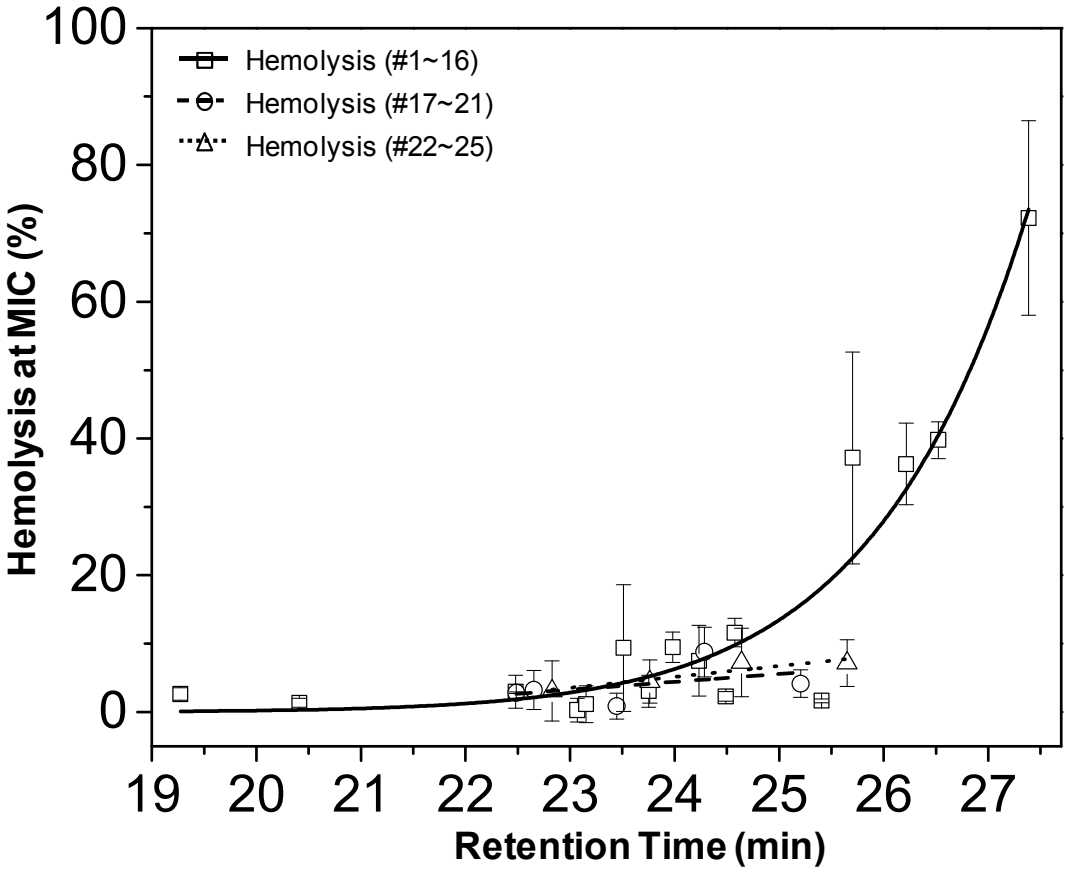


Figure S5. The relationship between hemolysis at the MIC at RT-HPLC retention time for β-peptides containing (1-16) and lacking (17-25) a helix-stabilizing ACHC residue. Sequences corresponding to the peptide numbers are provided in Figure 1. Error bars denote the standard deviation (n=3).

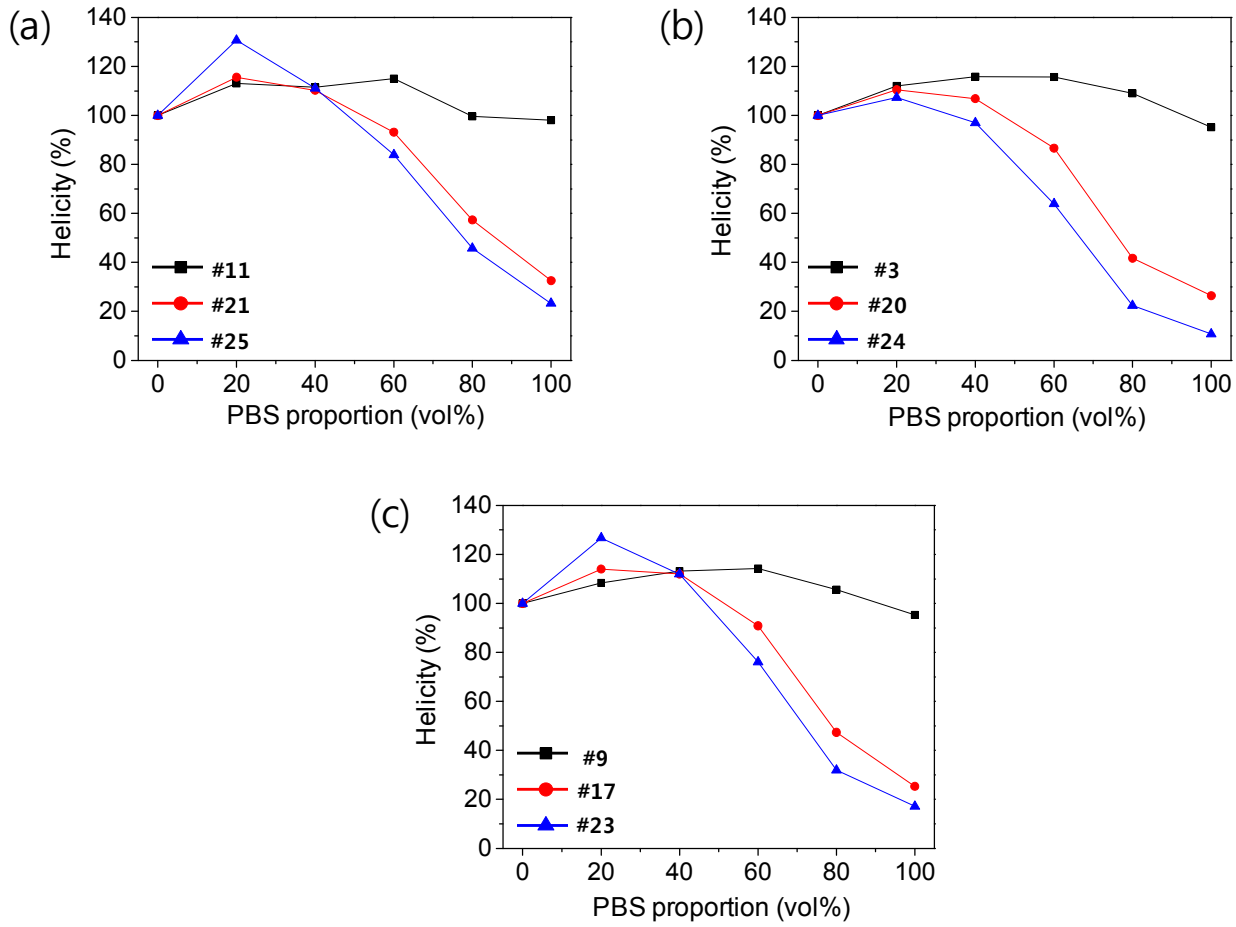


Figure S6. CD titration curves of β-peptides in buffers of different ratios of PBS:methanol for β-peptides with an MIC of (a) 16, (b) 32, and (c) 128 μg/mL. Sequences corresponding to the peptide numbers are provided in Figure 1.

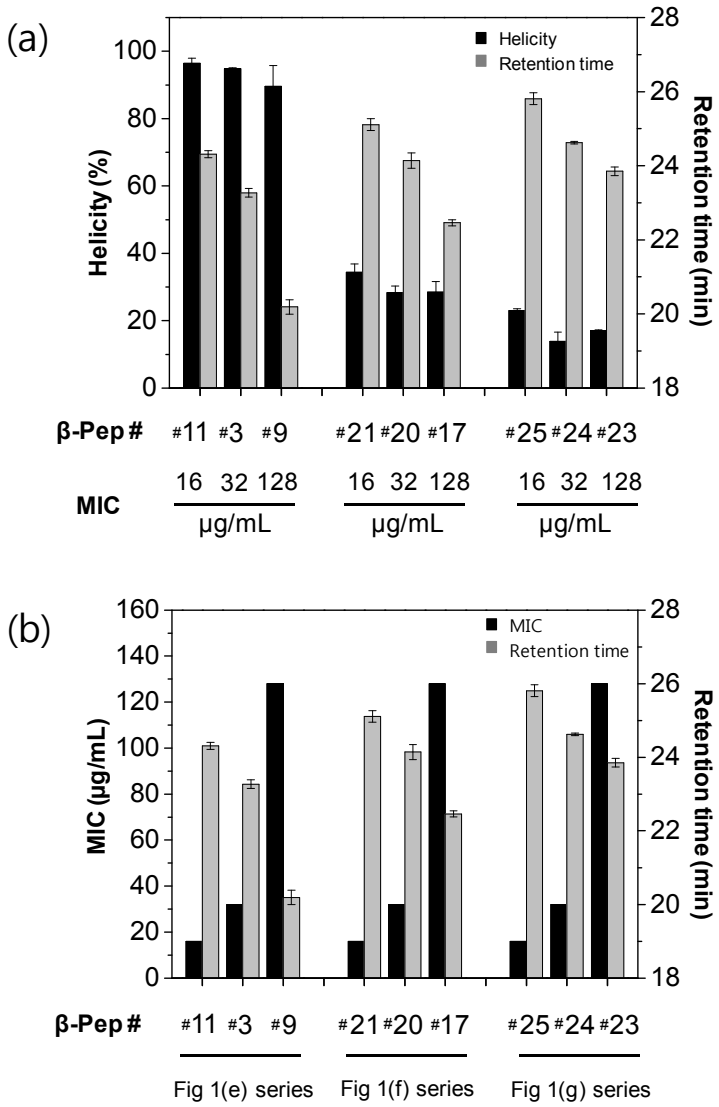


Figure S7. The relationship between RT-HPLC retention time and antifungal MIC of β-peptides possessing similar helicity. (a) β-peptides grouped by similar helicity show antifungal MIC decreases with increasing RT-HPLC retention time. (b) β-peptides possessing an ACHC (series 1(e)), or containing a β^3-hVal (series 1(f)) or β^3-hIle (series 1(g)) in place of the ACHC exhibit a decrease in antifungal MIC with increasing RT-HPLC retention time. Sequences corresponding to the peptide numbers are provided in Figure 1. Error bars denote the standard deviation (n=3).

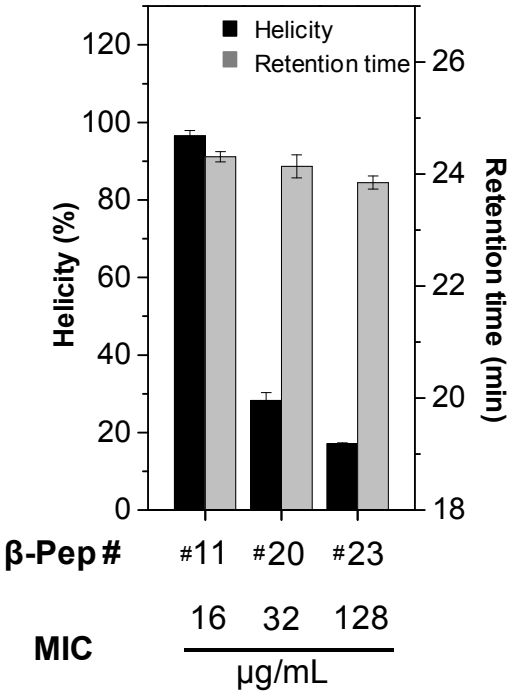


Figure S8. The relationship between helicity and antifungal MIC of β-peptides possessing similar RT-HPLC retention times. Sequences corresponding to the peptide numbers are provided in Figure 1. Error bar denoted standard deviation (n=3).