Antibacterial, Anti-inflammatory and Anti-biofilm Activities of Novel Short Antimicrobial Peptides Designed from the Cecropin A-Melittin Hybrid Antimicrobial Peptide, BP100

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Abstract

BP100 (KKLFKKILKYL-NH_2) is a short hybrid antimicrobial peptide (AMP) derived from cecropin A and melittin, two potent AMPs. We designed and synthesized several Trp-substituted BP100 analogs to enhance their antimicrobial activity therapeutic index. The therapeutic index represents the ratio of HC10 (the concentration causing 10% hemolysis of sheep red blood cells) to the geometric mean of MIC (minimal inhibitory concentration). Compared to BP100, the analogs BP5, BP6, BP7, BP8, BP11 and BP13 exhibited 1.4- to 5.8-fold higher therapeutic index. At a non-toxic concentration of 4 μM, both BP100 and some analogs (BP1, BP5, BP6, BP8, BP11, BP12 and BP13) suppressed the release of inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) in LPS-stimulated RAW 264.7 cells. This indicates that their potent LPS scavenging activity, similar to the control AMP LL-37. Furthermore, analogs BP5, BP6, BP7, BP8, BP11 and BP13 inhibited the biofilm formation of multidrug-resistant *Pseudomonas aeruginosa* (MDRPA) and eradicated preformed MDRPA by more than 90% at 8 μM. Thus, BP6, BP8, BP11 and BP13 exhibit higher potential than BP100 in terms of therapeutic index, anti-inflammatory and anti-biofilm activities. Additionally, we investigated the synergistic effects of BP100 and its analogs with conventional antibiotics against MDRPA, evaluating their potential as adjuvants in drug combination therapies.

Peptide Design

Table 1. Amino acid sequences and physicochemical properties of BP100 and its analogs

Peptides	Amino acid sequences [a]	Molecular mass (Da)		Net charge	R_t[b]	μH[c]
		Calculated	Observed			
BP100	KKLFKKILKYL-NH_2	1421.87	1420.74	+5	19.399	0.417
BP1	KKLWKKILKYL-NH_2	1460.91	1459.91	+5	20.091	0.469
BP2	KKLFKKWLKYL-NH_2	1494.93	1493.80	+5	19.157	0.468
BP3	KKLFKKIWKYL-NH_2	1494.93	1493.77	+5	19.329	0.477
BP4	KKLFKKILKWL-NH_2	1444.91	1443.87	+5	20.714	0.545
BP5	KKWWKKILKYL-NH_2	1533.96	1532.86	+5	21.373	0.519
BP6	KKWFKKILKWL-NH_2	1517.96	1516.80	+5	21.424	0.595
BP7	KKLWKKIWKYL-NH_2	1533.96	1532.98	+5	19.185	0.519
BP8	KKLWKKILKWL-NH_2	1483.95	1482.94	+5	20.947	0.586
BP9	KKLFKKWWKYL-NH_2	1567.98	1566.80	+5	18.485	0.518
BP10	KKLFKKWLKYW-NH_2	1567.98	1566.78	+5	18.859	0.518
BP11	KKLFKKIWKWL-NH_2	1517.96	1516.99	+5	19.807	0.595
BP12	KKLFKKILKWW-NH_2	1517.96	1516.96	+5	18.335	0.595
BP13	KKWFKKWLKWL-NH_2	1591.02	1589.89	+5	21.349	0.635
BP14	KKLWKKWLKYW-NH_2	1607.02	1605.80	+5	19.263	0.560
BP15	KKLWKKIWKYW-NH_2	1607.02	1605.82	+5	18.513	0.569
BP16	KKLFKKWWKWL-NH_2	1591.02	1589.69	+5	20.229	0.635

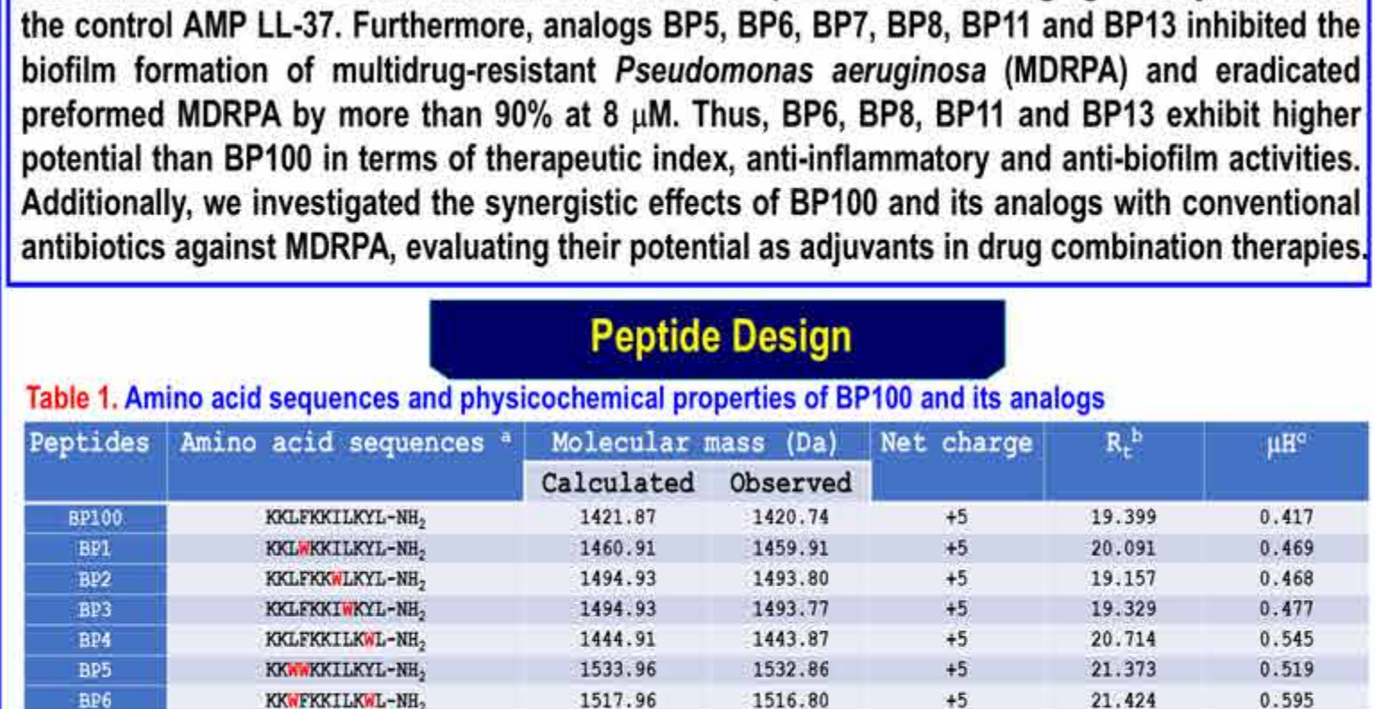

Figure 1. The α-helical wheel diagrams of BP100 and its analogs

Antimicrobial Activity & Cell Selectivity

Table 2. Antimicrobial Activities of BP100 and its analogs against bacterial strains

Bacterial strains	Minimal inhibitory concentration (MIC)[a] (μM)																	
	BP100	BP1	BP2	BP3	BP4	BP5	BP6	BP7	BP8	BP9	BP10	BP11	BP12	BP13	BP14	BP15	BP16	Melittin
Gram-positive bacteria																		
S. aureus (KCTC 1621)	4	8	4	8	4	2	2	2	4	8	8	4	4	4	2	2	16	8
S. epidermidis (KCTC 1917)	4	8	2	8	8	1	2	2	2	4	8	4	4	8	2	4	16	32
B. subtilis (KCTC 3068)	8	8	8	16	4	2	4	4	8	16	16	8	8	8	4	4	16	16
Resistant Gram-positive bacteria																		
MRSA[b] (CCARM 3089)	32	32	16	64	64	4	8	8	16	64	64	8	64	32	16	16	16	32
MRSA (CCARM 3090)	64	64	16	64	64	8	16	32	16	64	64	32	64	32	32	64	128	8
VREF[c] (ATCC 51559)	64	64	128	64	16	8	8	32	16	64	128	16	128	16	64	64	64	64
Gram-negative bacteria																		
E. coli (KCTC 1682)	4	8	2	8	4	2	4	4	2	4	8	8	8	8	4	4	16	8
P. aeruginosa (KCTC 1637)	16	16	8	32	16	4	16	8	16	16	8	8	16	16	8	8	8	16
S. typhimurium (KCTC 1926)	4	8	8	4	2	2	4	2	4	4	8	2	4	8	8	4	16	16
Resistant Gram-negative bacteria																		
MDRPA[d] (CCARM 2095)	32	16	8	16	8	8	8	8	8	16	16	8	16	8	16	16	32	32
MDRPA (CCARM 2109)	16	16	4	16	16	4	16	4	16	16	16	16	16	16	8	8	32	32
GM[e]	22.5	22.5	18.5	27.3	18.7	4.1	8.0	9.6	9.8	25.1	31.3	10.4	30.2	14.2	14.9	17.6	32.7	24.0
HC_{10}[f]	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	2.0
TI[g]	22.7	22.7	27.7	18.8	27.4	124.9	64.0	53.3	52.2	20.4	16.4	49.2	17.0	36.1	34.4	29.1	15.7	0.08

Resistance to Salts and Human serum

Peptides	Control	150 mM NaCl	4.5 mM KCl	6 μM NH_4Cl	1 mM $MgCl_2$	2.5 mM $CaCl_2$	4 μM $FeCl_3$	10% Human Serum
E. coli (KCTC 1682)								
BP100	4	16	16	16	16	16	16	16
BP5	2	8	4	4	4	8	8	4
BP6	4	8	4	16	8	16	16	4
BP8	2	8	4	8	8	16	4	4
BP11	8	16	8	4	8	16	4	8
BP13	8	16	8	8	8	16	8	16
S. aureus (KCTC 1621)								
BP100	4	16	16	16	16	16	16	32
BP5	2	4	4	4	4	4	4	4
BP6	2	4	4	4	4	4	8	4
BP8	4	4	4	4	4	4	4	4
BP11	4	8	4	4	4	16	4	8
BP13	4	8	8	8	8	8	8	8

Anti-biofilm Activity

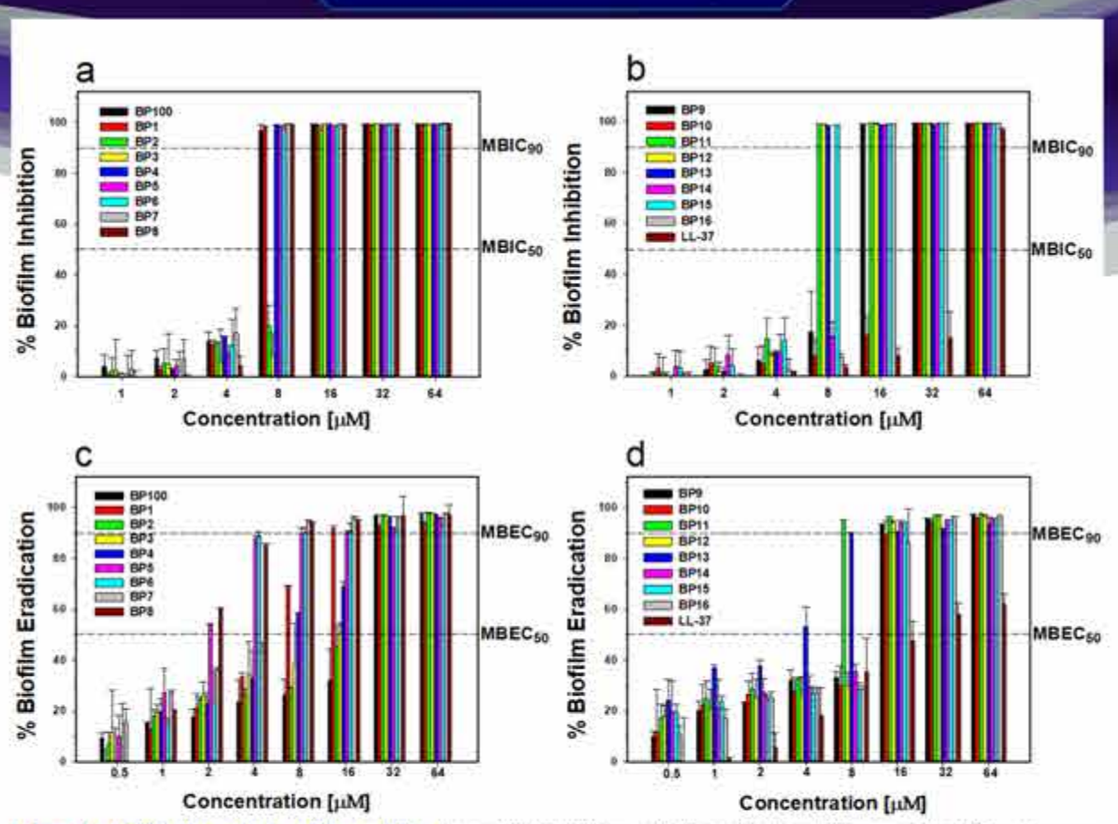

Figure 2. Antibiofilm activity of the peptides. (a and b) Inhibitory activity against multidrug-resistant *Pseudomonas aeruginosa* (MDRPA). (c and d) Eradication activity of preformed MDRPA biofilms. The dotted lines indicate 50% and 90% inhibition and eradication concentration.

Anti-inflammatory Activity

Figure 3. Effects of BP100 and its analogs on the release of TNF-α (a) and IL-6 (b) from LPS-stimulated RAW264.7 cells. The peptides were used at a concentration of 2μM for this experiment. All data represent at least three independent experiments and are expressed as means ± standard error of the mean (SEM). The data were analyzed using one-way analysis of variance (ANOVA) with Bonferroni's post-test. Asterisks indicate statistically significant differences (*P < 0.001 for each agonist).

Synergy Activity

Table 3. Synergistic antimicrobial activity of the peptide with ciprofloxacin (CIP) against MDRPA (CCARM 2095)

Peptides	MIC_A	[A]	FIC_A	MIC_B	[B]	FIC_B	FICI[a]	Interpretation
BP100	32	4	0.125	2048	512	0.25	0.375	synergy
BP5	8	0.5	0.0625	2048	512	0.25	0.3125	synergy
BP6	8	1	0.125	2048	512	0.25	0.375	synergy
BP8	8	0.5	0.0625	2048	512	0.25	0.375	synergy
BP11	8	0.5	0.0625	2048	512	0.25	0.3125	synergy
BP13	8	0.125	0.015625	2048	512	0.25	0.2656	synergy

Antimicrobial Mechanism

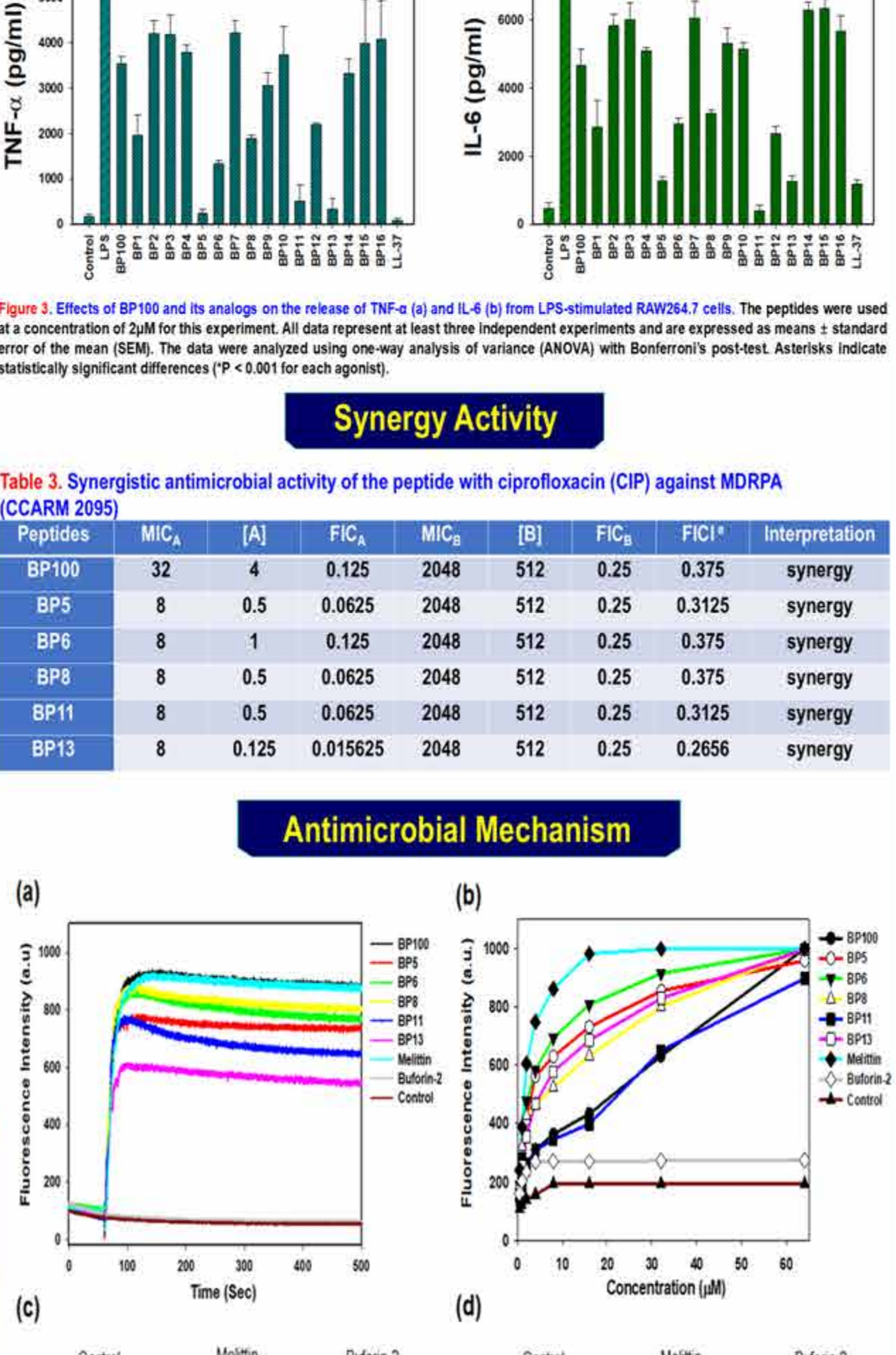

Figure 4. (a) Time-dependent cytoplasmic membrane depolarization of *S. aureus* (KCTC 1621) treated with the peptides (1×MIC), as assessed by the release of the membrane potential-sensitive dye $DiSC_3$-5. (b) Membrane uptake of 1-N-phenylnaphthylamine (NPN) by *E. coli* (KCTC 1682) in the presence of different concentrations of the peptides. (c) Membrane integrity of *E. coli* (KCTC 1682), as observed by flow cytometry. (d) Membrane integrity of *S. aureus* (KCTC 1621), as observed by flow cytometry.