

## Supplementary Materials

### *In Vitro & In Vivo* studies on Identifying and Designing Temporin-1CEh from the Skin Secretion of *Rana chensinensis* as the Optimized Antibacterial Prototype Drug

**Table S1.** Two-way ANOVA analysis of permeability of Temporin-1CEh and its analogues against *S. aureus*. The significance was presented by the symbol ns (non-significant difference), \*\* (P<0.01) and \*\*\*\* (P<0.0001).

Tukey's multiple comparisons test					
Peptide concentration (μM)	Peptide name	Mean Diff.	95.00% CI of diff.	Summary	Adjusted P Value
4	Temporin-1CEh vs. T1CEh-KKPW	-5	-11.90 to 1.898	ns	0.2269
	Temporin-1CEh vs. T1CEh-KKPWW	-4.333	-11.23 to 2.564	ns	0.3454
	Temporin-1CEh vs. T1CEh-KKPWW2	-60	-66.90 to -53.10	****	<0.0001
	T1CEh-KKPW vs. T1CEh-KKPWW	0.6667	-6.231 to 7.564	ns	0.9938
	T1CEh-KKPW vs. T1CEh-KKPWW2	-55	-61.90 to -48.10	****	<0.0001
	T1CEh-KKPWW vs. T1CEh-KKPWW2	-55.67	-62.56 to -48.77	****	<0.0001
8	Temporin-1CEh vs. T1CEh-KKPW	57	50.10 to 63.90	****	<0.0001
	Temporin-1CEh vs. T1CEh-KKPWW	46.67	39.77 to 53.56	****	<0.0001
	Temporin-1CEh vs. T1CEh-KKPWW2	-32.67	-39.56 to -25.77	****	<0.0001
	T1CEh-KKPW vs. T1CEh-KKPWW	-10.33	-17.23 to -3.436	**	0.0014
	T1CEh-KKPW vs. T1CEh-KKPWW2	-89.67	-96.56 to -82.77	****	<0.0001
	T1CEh-KKPWW vs. T1CEh-KKPWW2	-79.33	-86.23 to -72.44	****	<0.0001
16	Temporin-1CEh vs. T1CEh-KKPW	92.67	85.77 to 99.56	****	<0.0001
	Temporin-1CEh vs. T1CEh-KKPWW	56	49.10 to 62.90	****	<0.0001
	Temporin-1CEh vs. T1CEh-KKPWW2	14.67	7.769 to 21.56	****	<0.0001
	T1CEh-KKPW vs. T1CEh-KKPWW	-36.67	-43.56 to -29.77	****	<0.0001

	T1CEh-KKPW vs. T1CEh-KKPWW2	-78	-84.90 to -71.10	****	<0.0001
	T1CEh-KKPWW vs. T1CEh-KKPWW2	-41.33	-48.23 to -34.44	****	<0.0001
32	Temporin-1CEh vs. T1CEh-KKPW	90.67	83.77 to 97.56	****	<0.0001
	Temporin-1CEh vs. T1CEh-KKPWW	29	22.10 to 35.90	****	<0.0001
	Temporin-1CEh vs. T1CEh-KKPWW2	66.33	59.44 to 73.23	****	<0.0001
	T1CEh-KKPW vs. T1CEh-KKPWW	-61.67	-68.56 to -54.77	****	<0.0001
	T1CEh-KKPW vs. T1CEh-KKPWW2	-24.33	-31.23 to -17.44	****	<0.0001
	T1CEh-KKPWW vs. T1CEh-KKPWW2	37.33	30.44 to 44.23	****	<0.0001
64	Temporin-1CEh vs. T1CEh-KKPW	78.67	71.77 to 85.56	****	<0.0001
	Temporin-1CEh vs. T1CEh-KKPWW	20	13.10 to 26.90	****	<0.0001
	Temporin-1CEh vs. T1CEh-KKPWW2	80	73.10 to 86.90	****	<0.0001
	T1CEh-KKPW vs. T1CEh-KKPWW	-58.67	-65.56 to -51.77	****	<0.0001
	T1CEh-KKPW vs. T1CEh-KKPWW2	1.333	-5.564 to 8.231	ns	0.9543
	T1CEh-KKPWW vs. T1CEh-KKPWW2	60	53.10 to 66.90	****	<0.0001

**Table S2.** Two-way ANOVA analysis of permeability of Temporin-1CEh and its analogues against *E. coli*. The significance was presented by the symbol ns (non-significant difference), \*\* (P<0.01) and \*\*\*\* (P<0.0001).

Tukey's multiple comparisons test					
Peptide concentration (μM)	Peptide name	Mean Diff.	95.00% CI of diff.	Summary	Adjusted P Value
4	Temporin-1CEh vs. T1CEh-KKPW	1.333	-4.825 to 7.491	ns	0.9374
	Temporin-1CEh vs. T1CEh-KKPWW	-0.3333	-6.491 to 5.825	ns	0.9989
	Temporin-1CEh vs. T1CEh-KKPWW2	-69.67	-75.82 to -63.51	****	<0.0001
	T1CEh-KKPW vs. T1CEh-KKPWW	-1.667	-7.825 to 4.491	ns	0.8863
	T1CEh-KKPW vs. T1CEh-KKPWW2	-71	-77.16 to -64.84	****	<0.0001
	T1CEh-KKPWW vs. T1CEh-KKPWW2	-69.33	-75.49 to -63.18	****	<0.0001
8	Temporin-1CEh vs. T1CEh-KKPW	38.33	32.18 to 44.49	****	<0.0001

	Temporin-1CEh vs. T1CEh-KKPWW	33	26.84 to 39.16	****	<0.0001
	Temporin-1CEh vs. T1CEh-KKPWW2	-36.33	-42.49 to -30.18	****	<0.0001
	T1CEh-KKPW vs. T1CEh-KKPWW	-5.333	-11.49 to 0.8245	ns	0.1101
	T1CEh-KKPW vs. T1CEh-KKPWW2	-74.67	-80.82 to -68.51	****	<0.0001
	T1CEh-KKPWW vs. T1CEh-KKPWW2	-69.33	-75.49 to -63.18	****	<0.0001
16	Temporin-1CEh vs. T1CEh-KKPW	39.33	33.18 to 45.49	****	<0.0001
	Temporin-1CEh vs. T1CEh-KKPWW	1	-5.158 to 7.158	ns	0.972
	Temporin-1CEh vs. T1CEh-KKPWW2	-29	-35.16 to -22.84	****	<0.0001
	T1CEh-KKPW vs. T1CEh-KKPWW	-38.33	-44.49 to -32.18	****	<0.0001
	T1CEh-KKPW vs. T1CEh-KKPWW2	-68.33	-74.49 to -62.18	****	<0.0001
	T1CEh-KKPWW vs. T1CEh-KKPWW2	-30	-36.16 to -23.84	****	<0.0001
32	Temporin-1CEh vs. T1CEh-KKPW	21	14.84 to 27.16	****	<0.0001
	Temporin-1CEh vs. T1CEh-KKPWW	-20.33	-26.49 to -14.18	****	<0.0001
	Temporin-1CEh vs. T1CEh-KKPWW2	-54	-60.16 to -47.84	****	<0.0001
	T1CEh-KKPW vs. T1CEh-KKPWW	-41.33	-47.49 to -35.18	****	<0.0001
	T1CEh-KKPW vs. T1CEh-KKPWW2	-75	-81.16 to -68.84	****	<0.0001
	T1CEh-KKPWW vs. T1CEh-KKPWW2	-33.67	-39.82 to -27.51	****	<0.0001
64	Temporin-1CEh vs. T1CEh-KKPW	0	-6.158 to 6.158	ns	>0.9999
	Temporin-1CEh vs. T1CEh-KKPWW	-7.667	-13.82 to -1.509	**	0.0095
	Temporin-1CEh vs. T1CEh-KKPWW2	28	21.84 to 34.16	****	<0.0001
	T1CEh-KKPW vs. T1CEh-KKPWW	-7.667	-13.82 to -1.509	**	0.0095
	T1CEh-KKPW vs. T1CEh-KKPWW2	28	21.84 to 34.16	****	<0.0001
	T1CEh-KKPWW vs. T1CEh-KKPWW2	35.67	29.51 to 41.82	****	<0.0001