

Supporting information

Synthesis and Antimicrobial Activity of Short Analogues of the Marine Antimicrobial Peptide Turgencin A: Effects of SAR Optimizations, Cys-Cys Cyclization And lipopeptide Modifications

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Contents:

Figure S1. UPLC chromatogram of purified cyclic peptide **cTurg-1**.

Figure S2. UPLC chromatogram of purified cyclic peptide **cTurg-2**.

Figure S3. UPLC chromatogram of purified cyclic peptide **cTurg-3**.

Figure S4. UPLC chromatogram of purified cyclic peptide **cTurg-4**.

Figure S5. UPLC chromatogram of purified cyclic peptide **cTurg-5**.

Figure S6. UPLC chromatogram of purified cyclic peptide **cTurg-6**.

Figure S7. UPLC chromatogram of purified cyclic peptide **cTurg-7**.

Figure S8. UPLC chromatogram of purified linear lipopeptide **C₈-Turg-1**.

Figure S9. UPLC chromatogram of purified linear lipopeptide **C₁₀-Turg-1**.

Figure S10. UPLC chromatogram of purified linear lipopeptide **C₁₂-Turg-1**.

Figure S11. UPLC chromatogram of purified linear lipopeptide **C₈-Turg-2**.

Figure S12. UPLC chromatogram of purified linear lipopeptide **C₁₀-Turg-2**.

Figure S13. UPLC chromatogram of purified linear lipopeptide **C₁₂-Turg-2**.

Figure S14. UPLC chromatogram of purified linear lipopeptide **C₈-Turg-6**.

Figure S15. UPLC chromatogram of purified linear lipopeptide **C₁₀-Turg-6**.

Figure S16. UPLC chromatogram of purified linear lipopeptide **C₁₂-Turg-6**.

Figure S17. UPLC chromatogram of purified cyclic lipopeptide **C₈-cTurg-1**.

Figure S18. UPLC chromatogram of purified cyclic lipopeptide **C₁₀-cTurg-1**.

Figure S19. UPLC chromatogram of purified cyclic lipopeptide **C₁₂-cTurg-1**.

Figure S20. UPLC chromatogram of purified cyclic lipopeptide **C₈-cTurg-2**.

Figure S21. UPLC chromatogram of purified cyclic lipopeptide **C₁₀-cTurg-2**.

Figure S22. UPLC chromatogram of purified cyclic lipopeptide **C₁₂-cTurg-2**.

Figure S23. UPLC chromatogram of purified cyclic lipopeptide **C₈-cTurg-6**.

Figure S24. UPLC chromatogram of purified cyclic lipopeptide **C₁₀-cTurg-6**.

Figure S25. UPLC chromatogram of purified cyclic lipopeptide **C₁₂-cTurg-6**.

Figure S26. Kinetic of the effect on viability as measured by relative luminescence in *B. subtilis* (pCGLS11) treated with different concentrations of **cTurg-2**, **cTurg-3**, **cTurg-4**, **cTurg-5**, **cTurg-6** and **cTurg-7**.

Figure S27. Kinetic of the effect on viability as measured by relative luminescence in *B. subtilis* (pCGLS11) treated with different concentrations of **C₈-cTurg-1**, **C₁₀-cTurg-1**, **C₁₀-cTurg-2**, **C₁₂-cTurg-2**, **C₈-cTurg-6**, **C₁₀-cTurg-6**, and **C₁₂-cTurg-6**.

Figure S28. Kinetic of the effect on membrane integrity as measured by relative luminescence in *B. subtilis* (pCSS962) treated with different concentrations of **cTurg-1**, **cTurg-2**, **cTurg-3**, **cTurg-4**, **cTurg-5**, **cTurg-6** and **cTurg-7**.

Figure S29. Kinetic of the effect on membrane integrity as measured by relative luminescence in *B. subtilis* (pCSS962) treated with different concentrations of **C₈-cTurg-1**, **C₁₀-cTurg-1**, **C₁₀-cTurg-2**, **C₁₂-cTurg-2**, **C₈-cTurg-6**, **C₁₀-cTurg-6**, and **C₁₂-cTurg-6**.

Figure S30. Kinetic of the effect on viability as measured by relative luminescence in *E. coli* (pCGLS-11) treated with 50 µg/mL of **cTurg-1-7** and 25 µg/mL of **chlorhexidine**.

Figure S31. Kinetic of the effect on viability as measured by relative luminescence in *E. coli* (pCGLS-11) treated with different concentrations of **C₈-cTurg-1**, **C₁₀-cTurg-1**, **C₁₀-cTurg-2**, **C₁₂-cTurg-2**, **C₈-cTurg-6**, **C₁₀-cTurg-6**, and **C₁₂-cTurg-6**.

Figure S32. Kinetic of the effect on membrane integrity as measured by relative luminescence in *E. coli* (pCSS962) treated with different concentrations of cyclic peptides **cTurg-1**, **cTurg-2**, **cTurg-3**, **cTurg-4**, **cTurg-5**, **cTurg-6** and **cTurg-7**.

Figure S33. Kinetic of the effect on membrane integrity as measured by relative luminescence in *E. coli* (pCSS962) treated with different concentrations of **C₈-cTurg-1**, **C₁₀-cTurg-1**, **C₁₀-cTurg-2**, **C₁₂-cTurg-2**, **C₈-cTurg-6**, **C₁₀-cTurg-6**, and **C₁₂-cTurg-6**.

Table S1. Theoretical and measured monoisotopic mass (Da), and theoretical and observed m/z ions during HRMS of the synthesised peptides.

Table S2. Purity of synthesized peptides (%) and retention time (min), determined by UPLC using a reversed phase column.

Table S3. Selectivity index (SI) calculated as the ration between haemolytic activity (EC₅₀, in µg/mL) and the geometric mean (GM) of the MIC values (in µg/mL) against bacteria and fungi, i.e., SI = EC₅₀ / GM.

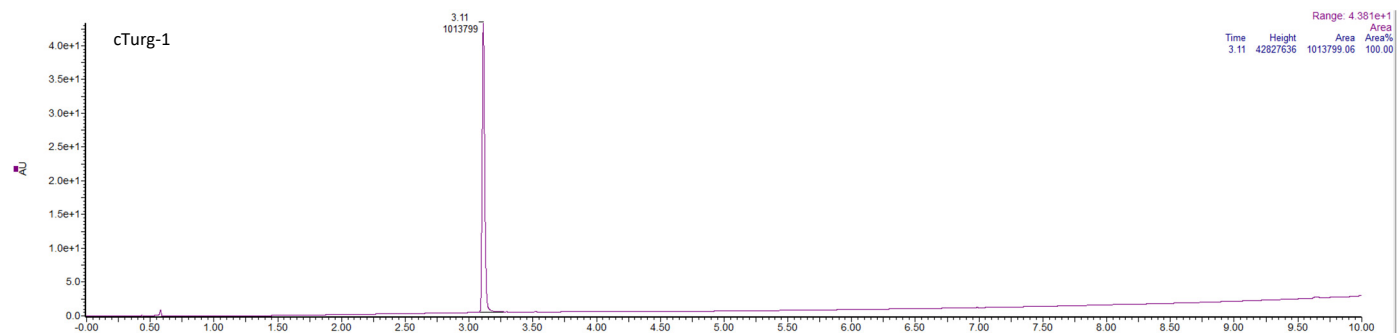


Figure S1. UPLC chromatogram of purified cyclic peptide **cTurg-1**. The peptide purity is 100 % based on the UPLC calculated area under the curves.

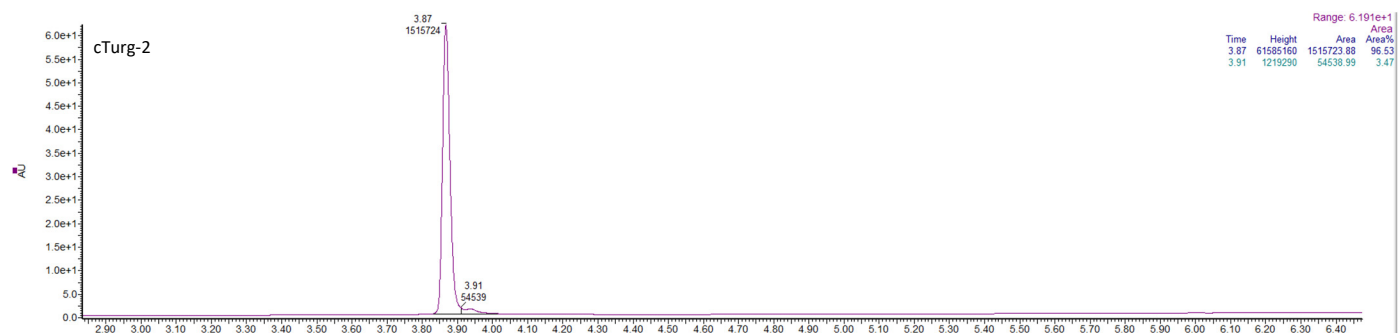


Figure S2. UPLC chromatogram of purified cyclic peptide **cTurg-2**. The peptide purity is 96.53 % based on the UPLC calculated area under the curves.

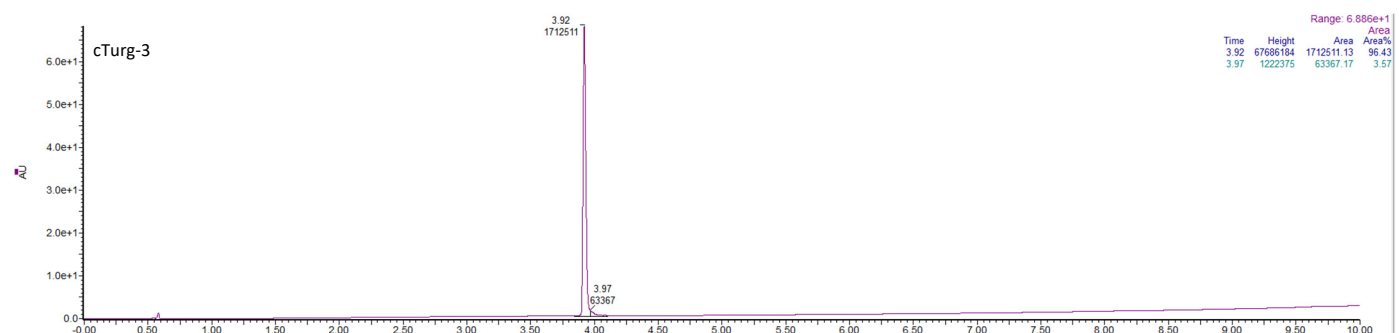


Figure S3. UPLC chromatogram of purified cyclic peptide **cTurg-3**. The peptide purity is 96.43 % based on the UPLC calculated area under the curves.

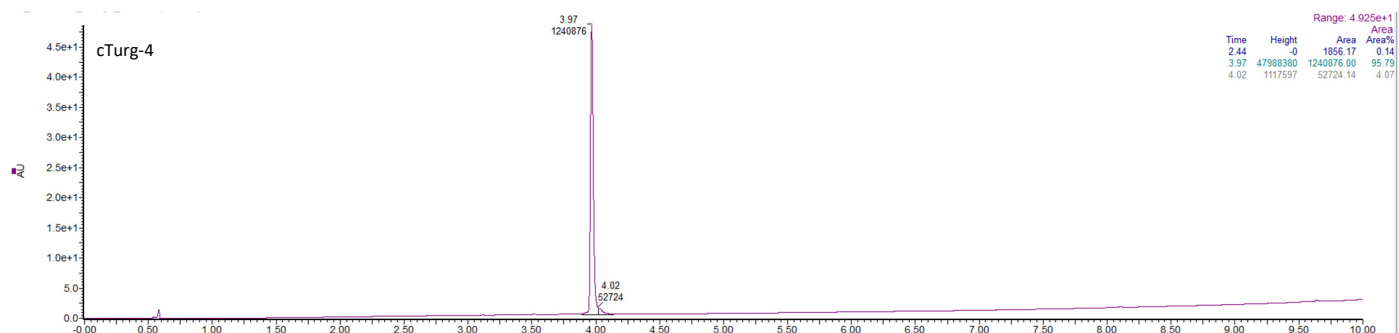


Figure S4. UPLC chromatogram of purified cyclic peptide **cTurg-4**. The peptide purity is 95.79 % based on the UPLC calculated area under the curves.

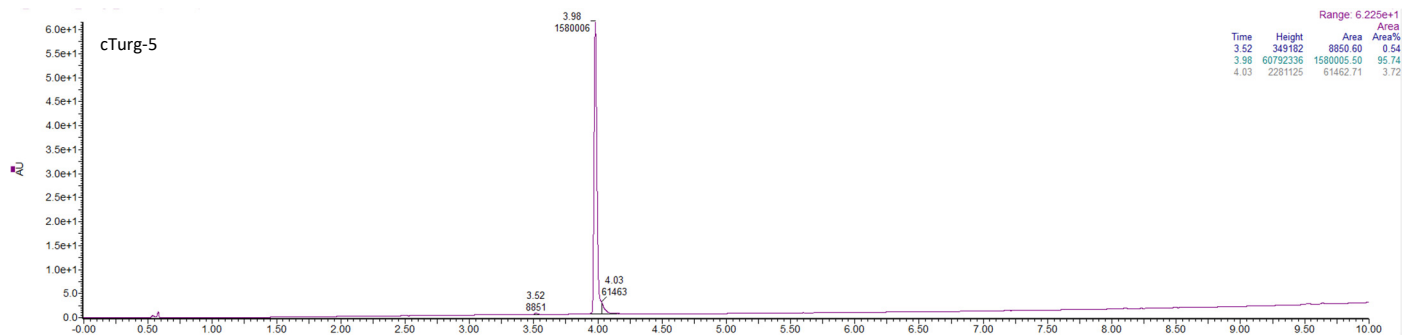


Figure S5. UPLC chromatogram of purified cyclic peptide **cTurg-5**. The peptide purity is 95.74 % based on the UPLC calculated area under the curves.

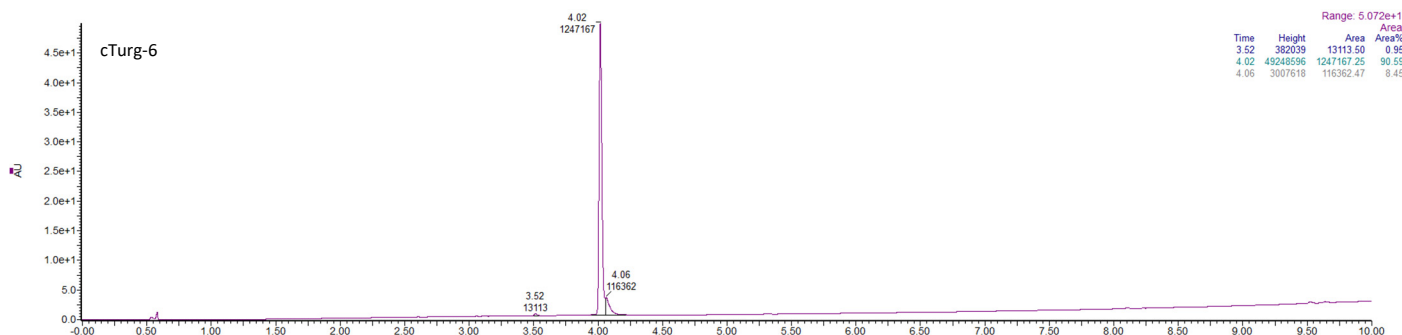


Figure S6. UPLC chromatogram of purified cyclic peptide **cTurg-6**. The peptide purity is 90.59 % based on the UPLC calculated area under the curves.

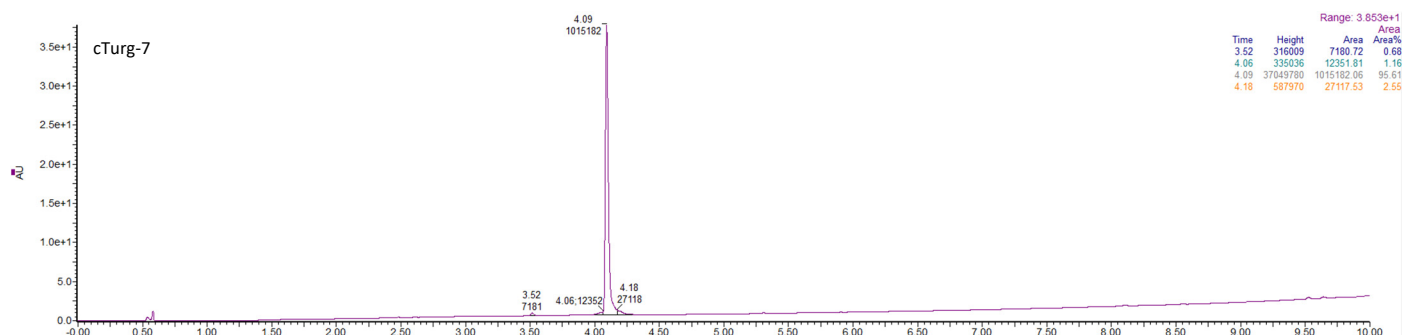


Figure S7. UPLC chromatogram of purified cyclic peptide **cTurg-7**. The peptide purity is 95.61 % based on the UPLC calculated area under the curves.

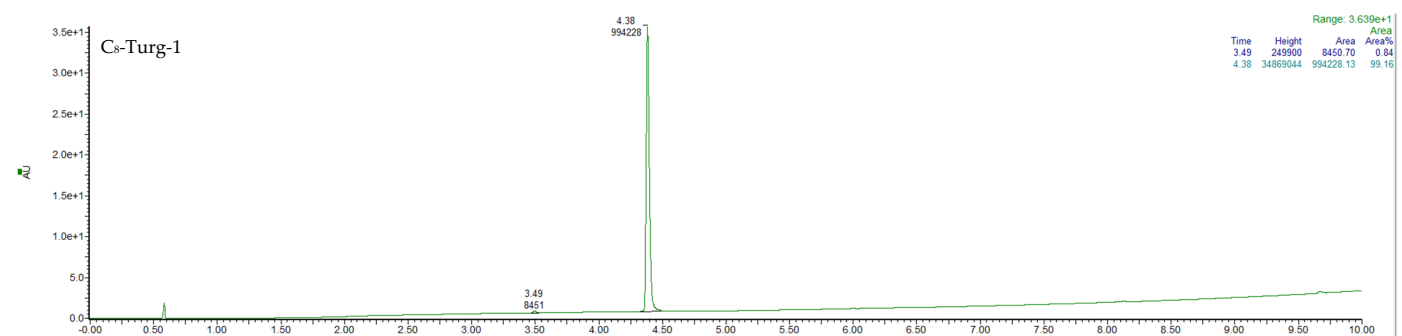


Figure S8. UPLC chromatogram of purified linear lipopeptide **C₈-Turg-1**. The peptide purity is 99.16 % based on the UPLC calculated area under the curves.

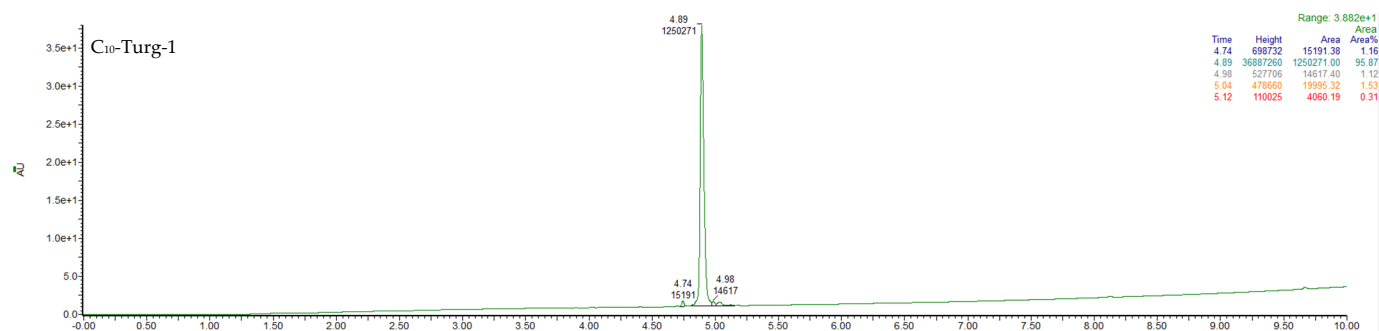


Figure S9. UPLC chromatogram of purified linear lipopeptide **C₁₀-Turg-1**. The peptide purity is 95.87 % based on the UPLC calculated area under the curves.

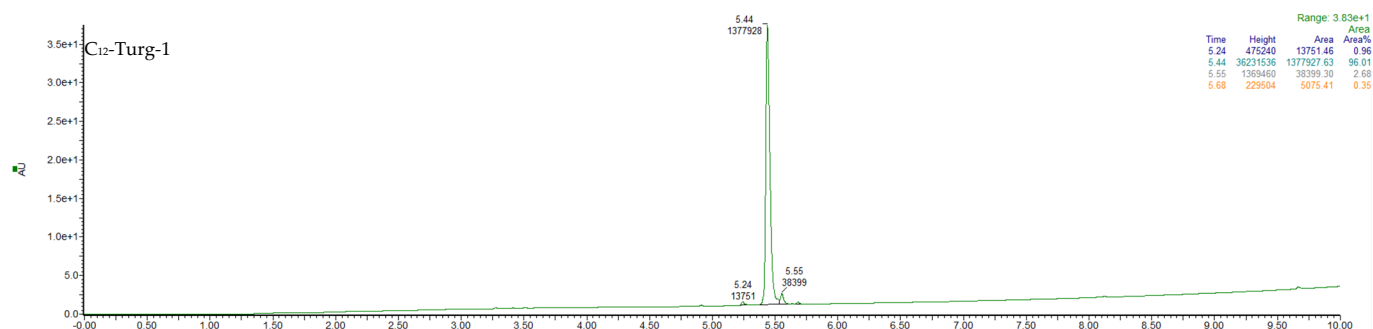


Figure S10. UPLC chromatogram of purified linear lipopeptide **C₁₂-Turg-1**. The peptide purity is 96.01 % based on the UPLC calculated area under the curves.

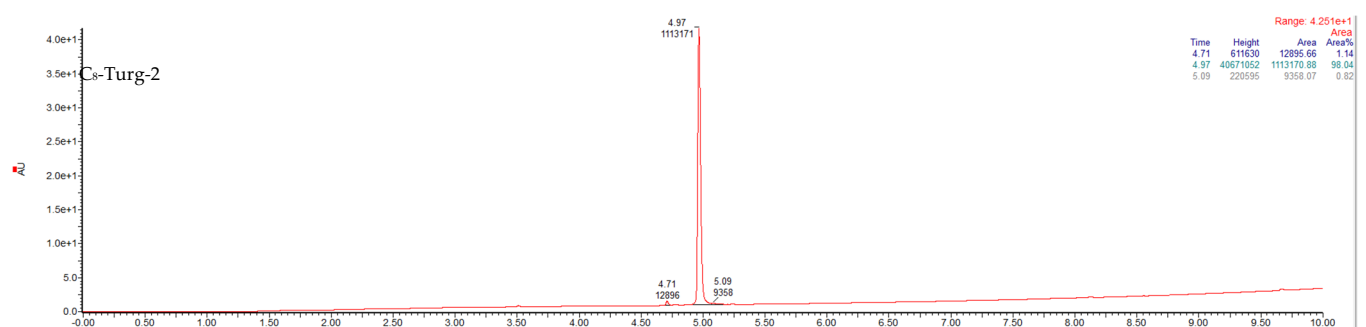


Figure S11. UPLC chromatogram of purified linear lipopeptide **C₈-Turg-2**. The peptide purity is 98.04 % based on the UPLC calculated area under the curves.

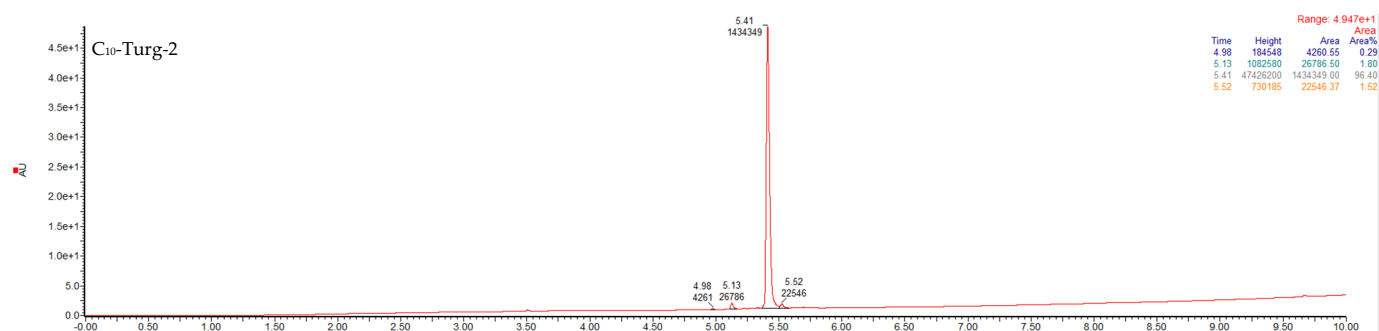


Figure S12. UPLC chromatogram of purified linear lipopeptide **C₁₀-Turg-2**. The peptide purity is 96.40 % based on the UPLC calculated area under the curves.

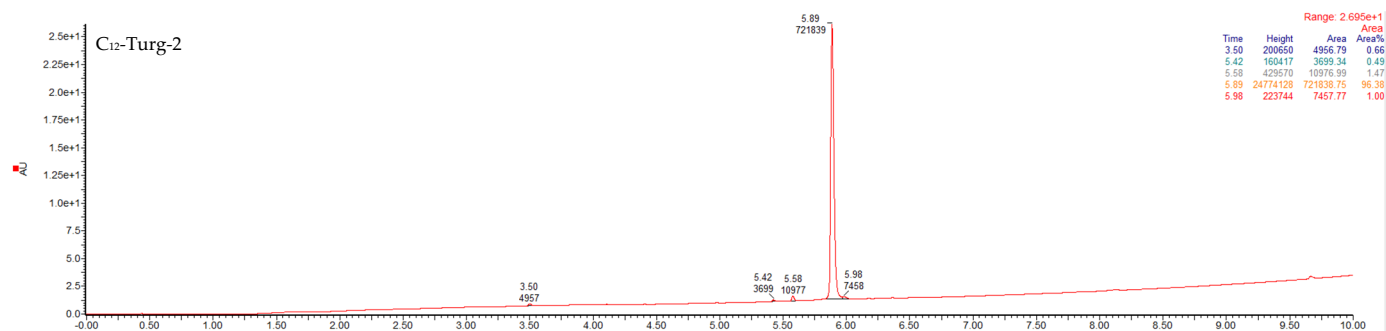


Figure S13. UPLC chromatogram of purified linear lipopeptide **C₁₂-Turg-2**. The peptide purity is 96.38 % based on the UPLC calculated area under the curves.

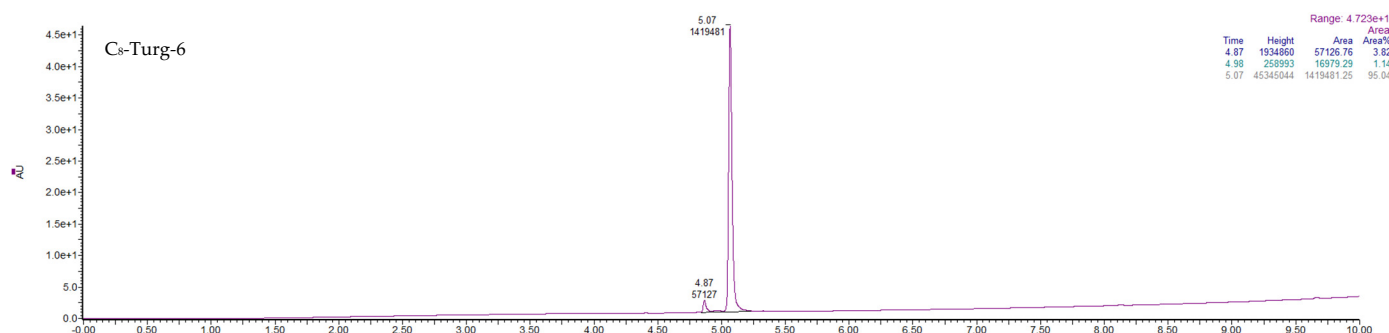


Figure S14. UPLC chromatogram of purified linear lipopeptide **C₈-Turg-6**. The peptide purity is 95.04 % based on the UPLC calculated area under the curves.

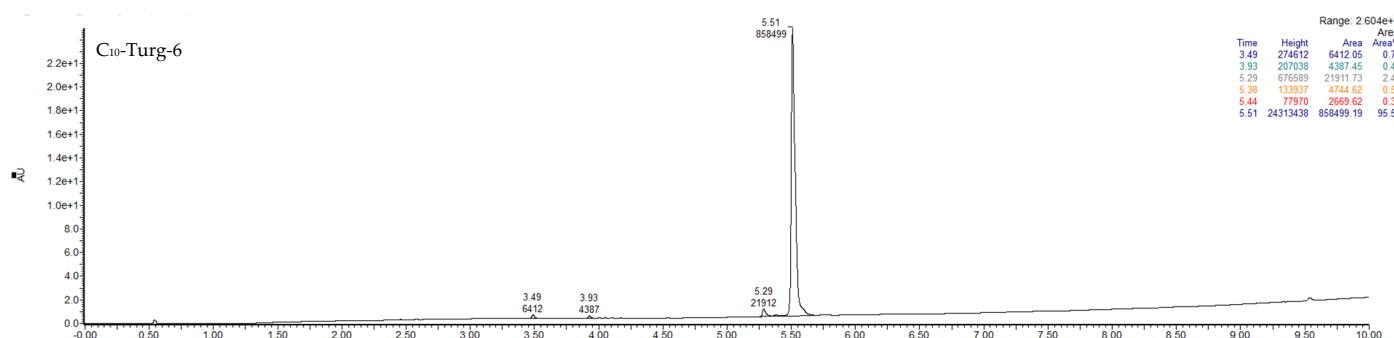


Figure S15. UPLC chromatogram of purified linear lipopeptide **C₁₀-Turg-6**. The peptide purity is 95.53 % based on the UPLC calculated area under the curves.

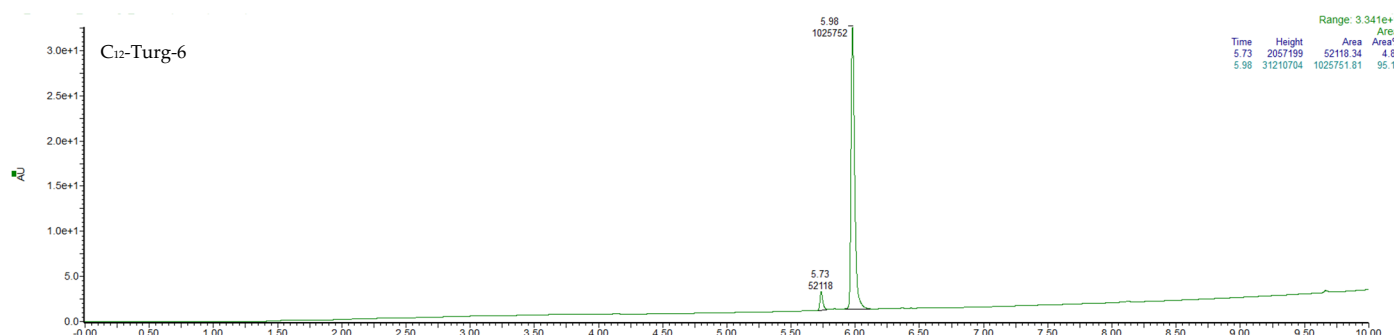


Figure S16. UPLC chromatogram of purified linear lipopeptide **C₁₂-Turg-6**. The peptide purity is 95.16 % based on the UPLC calculated area under the curves.

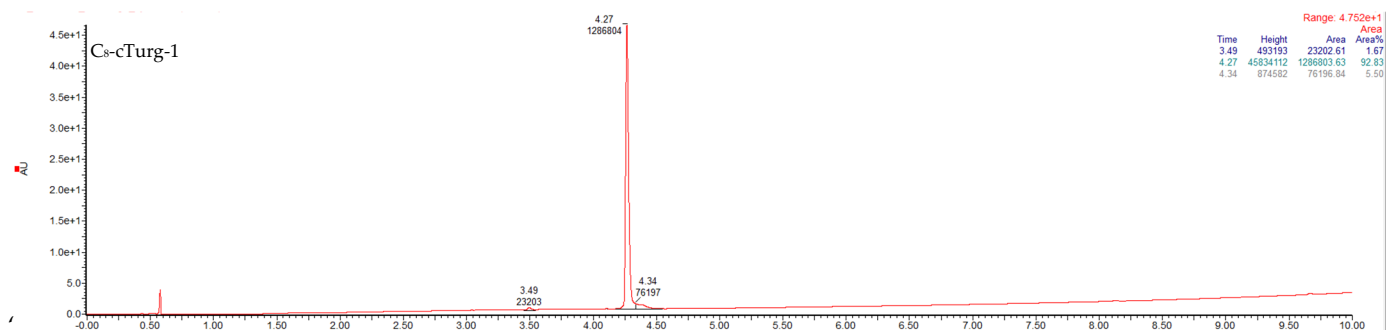


Figure S17. UPLC chromatogram of purified cyclic lipopeptide **C₈-cTurg-1**. The peptide purity is 92.83 % based on the UPLC calculated area under the curves.

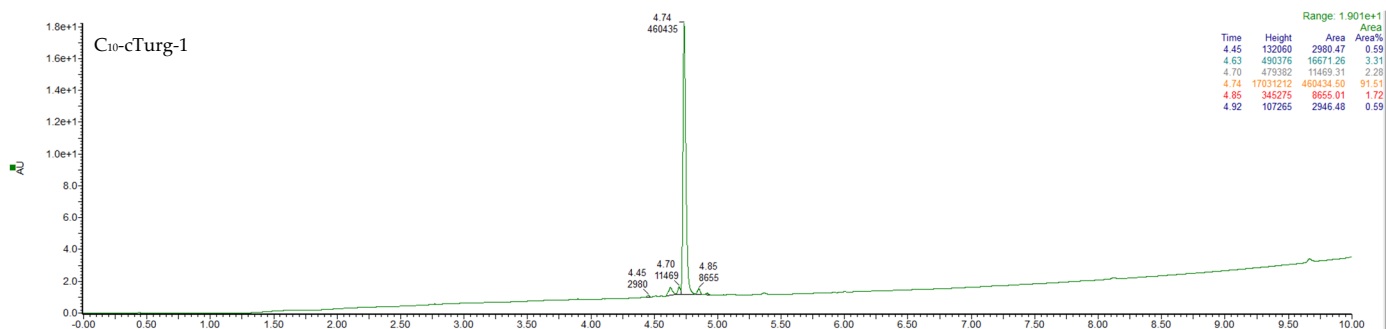


Figure S18. UPLC chromatogram of purified cyclic lipopeptide **C₁₀-cTurg-1**. The peptide purity is 91.51 % based on the UPLC calculated area under the curves.

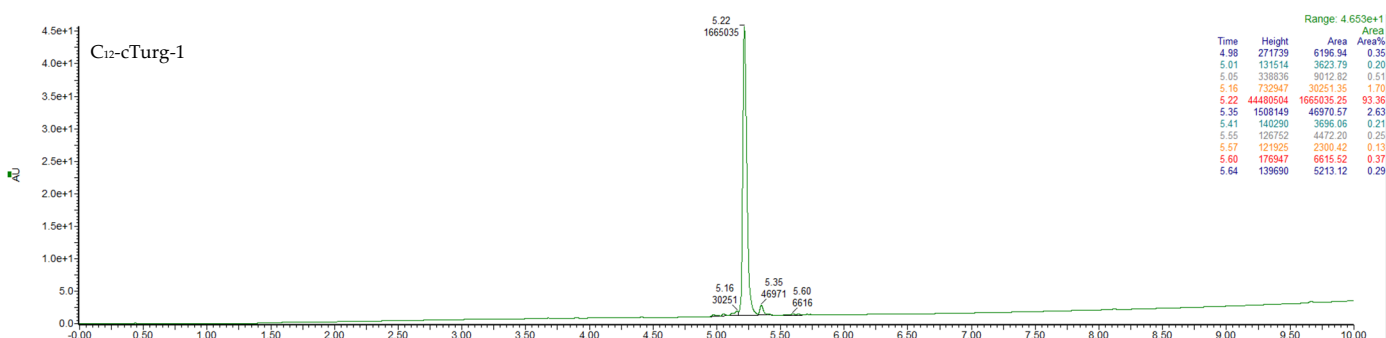


Figure S19. UPLC chromatogram of purified cyclic lipopeptide **C₁₂-cTurg-1**. The peptide purity is 93.36 % based on the UPLC calculated area under the curves.

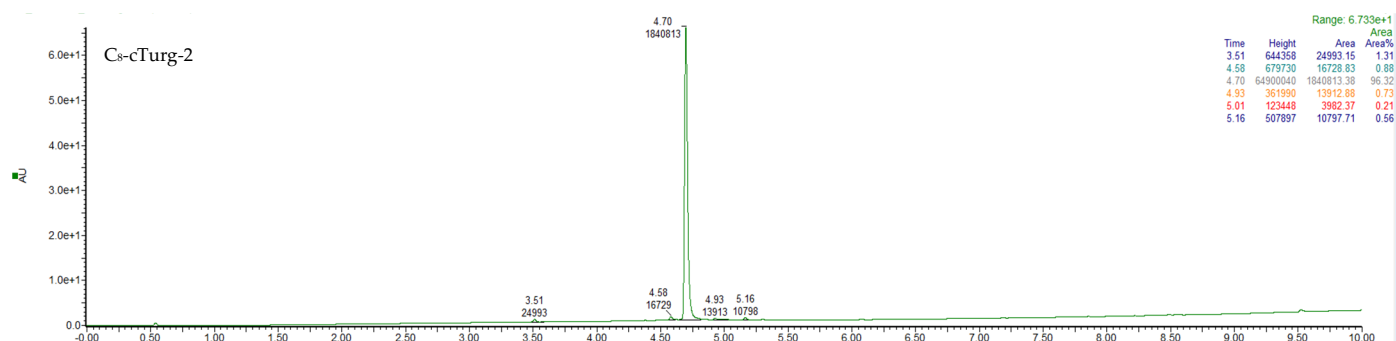


Figure S20. UPLC chromatogram of purified cyclic lipopeptide **C₈-cTurg-2**. The peptide purity is 96.32 % based on the UPLC calculated area under the curves.

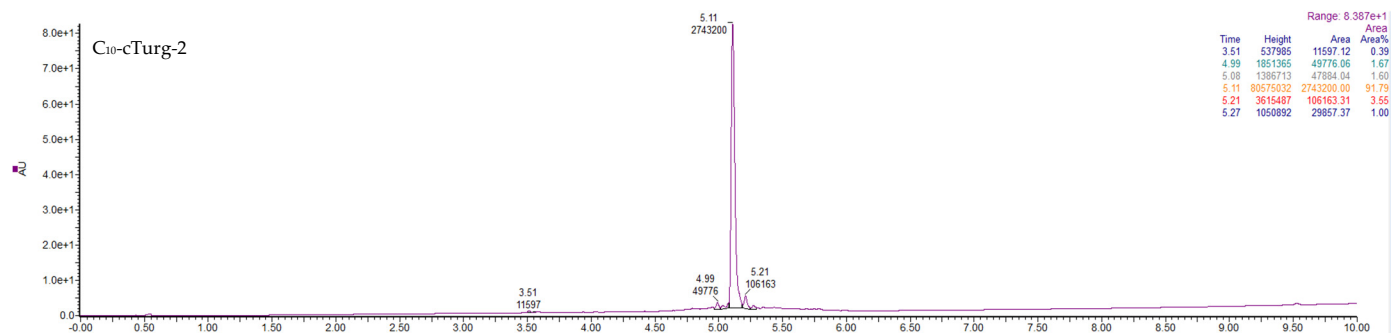


Figure S21. UPLC chromatogram of purified cyclic lipopeptide **C₁₀-cTurg-2**. The peptide purity is 91.79 % based on the UPLC calculated area under the curves.

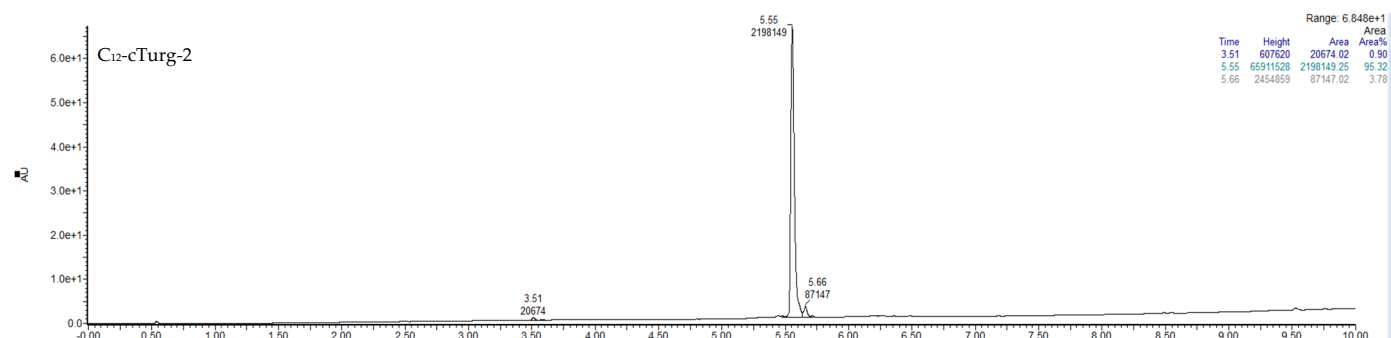


Figure S22. UPLC chromatogram of purified cyclic lipopeptide **C₁₂-cTurg-2**. The peptide purity is 95.32 % based on the UPLC calculated area under the curves.

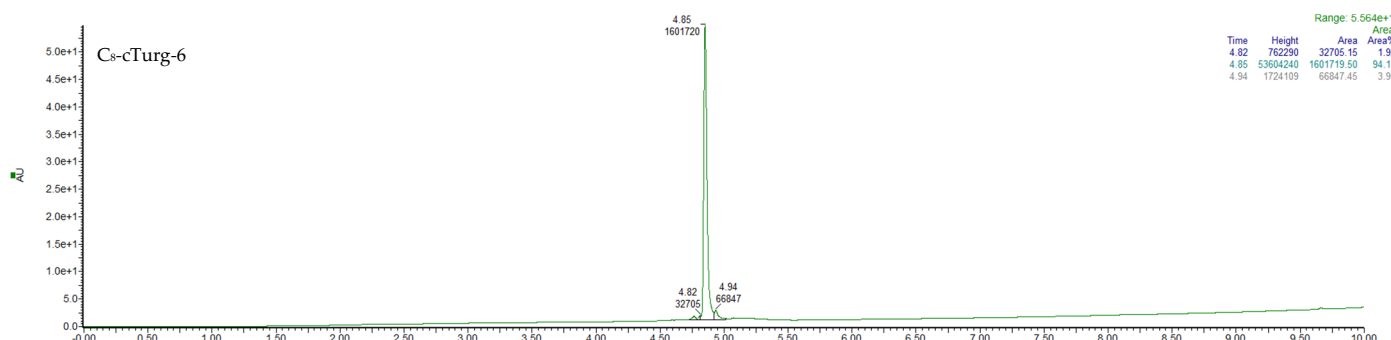


Figure S23. UPLC chromatogram of purified cyclic lipopeptide **C₈-cTurg-6**. The peptide purity is 94.15 % based on the UPLC calculated area under the curves.

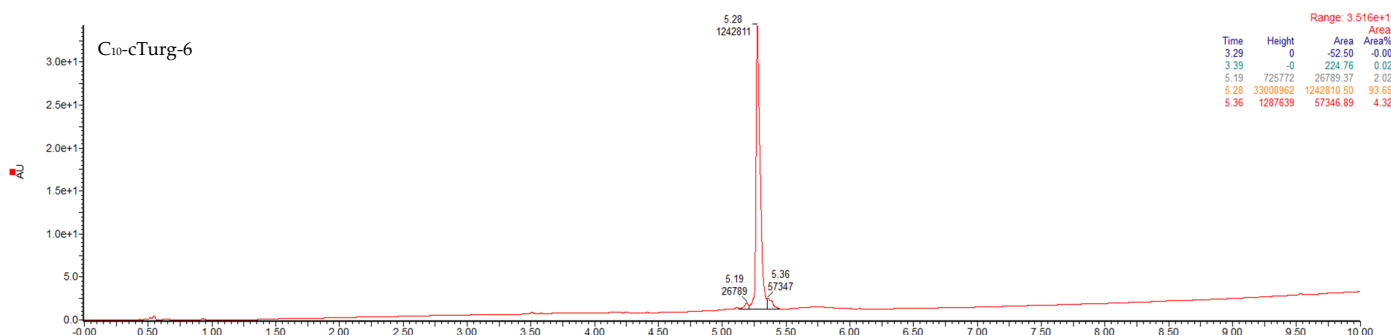


Figure S24. UPLC chromatogram of purified cyclic lipopeptide **C₁₀-cTurg-6**. The peptide purity is 93.65 % based on the UPLC calculated area under the curves.

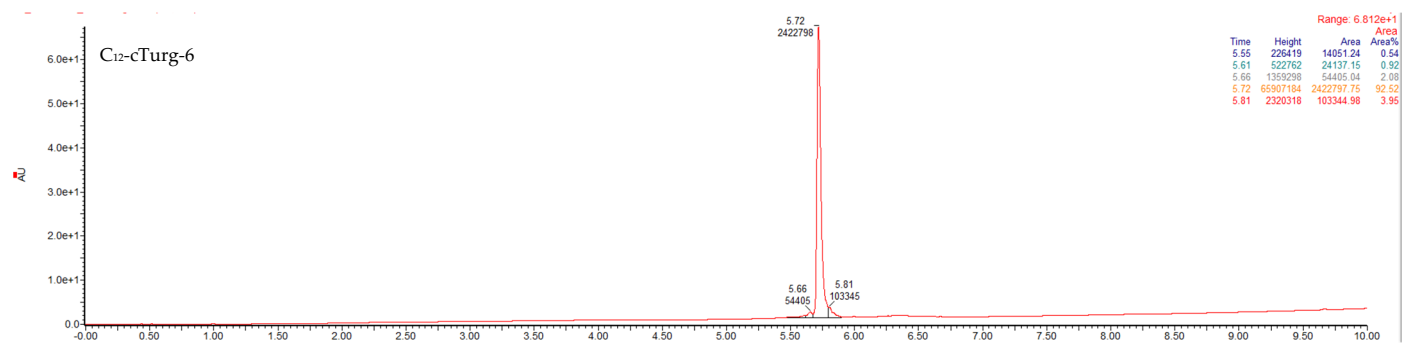


Figure S25. UPLC chromatogram of purified cyclic lipopeptide **C₁₂-cTurg-6**. The peptide purity is 92.52 % based on the UPLC calculated area under the curves.

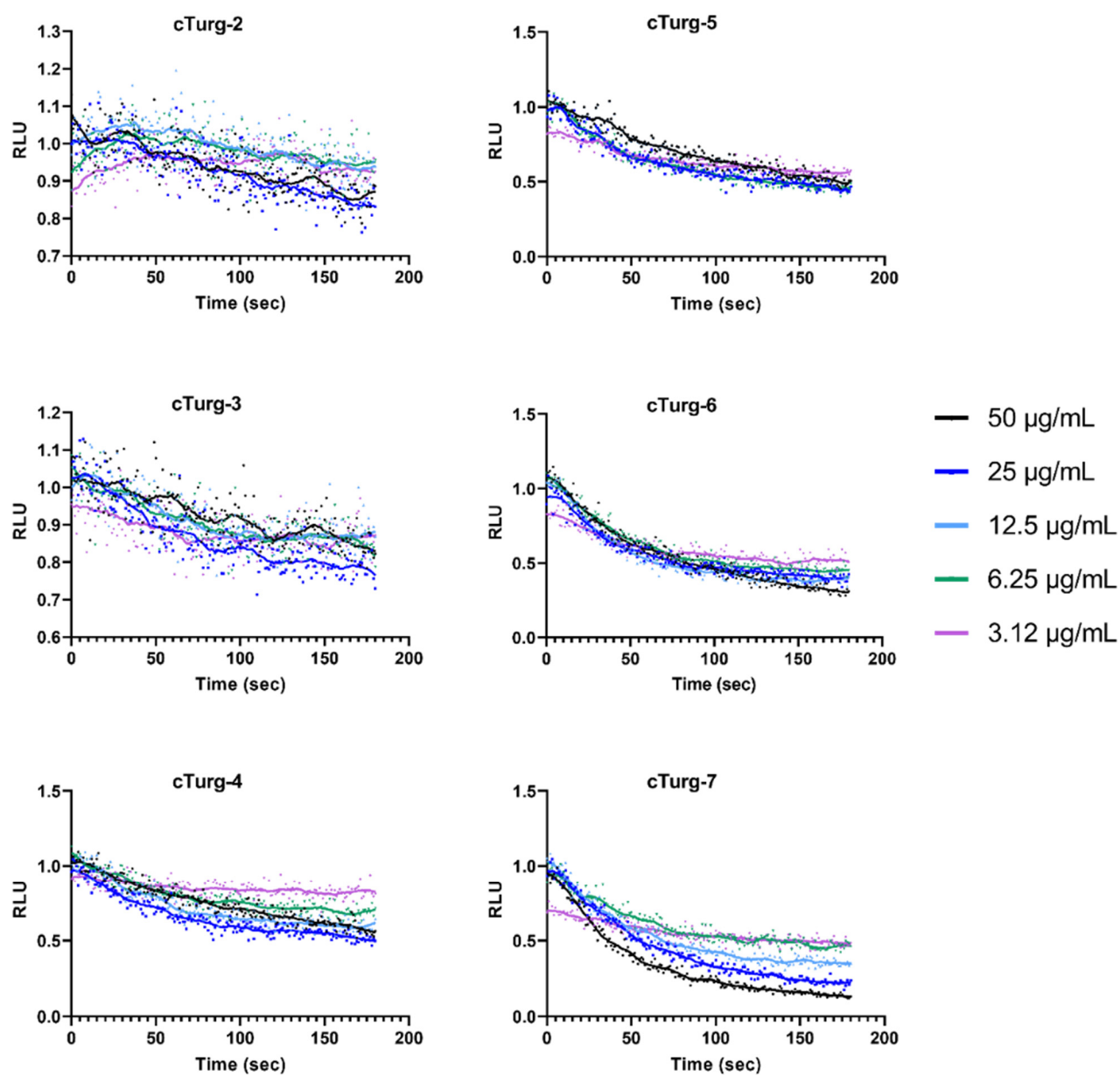


Figure S26. Kinetic of the effect on viability as measured by relative luminescence in *B. subtilis* (pCGLS11) treated with different concentrations of cTurg-2, cTurg-3, cTurg-4, cTurg-5, cTurg-6 and cTurg-7.

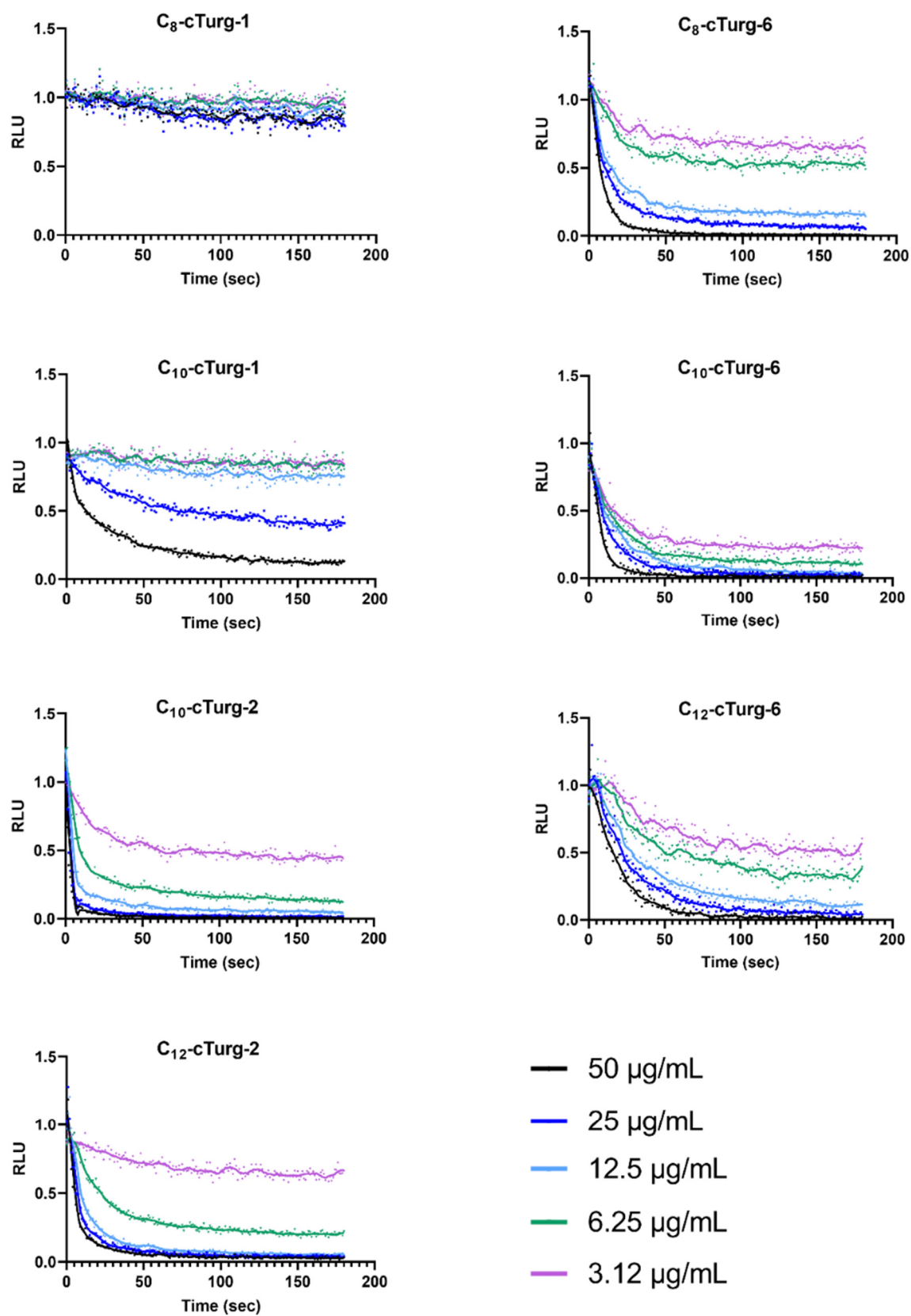


Figure S27. Kinetic of the effect on viability as measured by relative luminescence in *B. subtilis* (pCGLS11) treated with different concentrations of $\text{C}_8\text{-cTurg-1}$, $\text{C}_{10}\text{-cTurg-1}$, $\text{C}_{10}\text{-cTurg-2}$, $\text{C}_{12}\text{-cTurg-2}$, $\text{C}_8\text{-cTurg-6}$, $\text{C}_{10}\text{-cTurg-6}$, and $\text{C}_{12}\text{-cTurg-6}$.

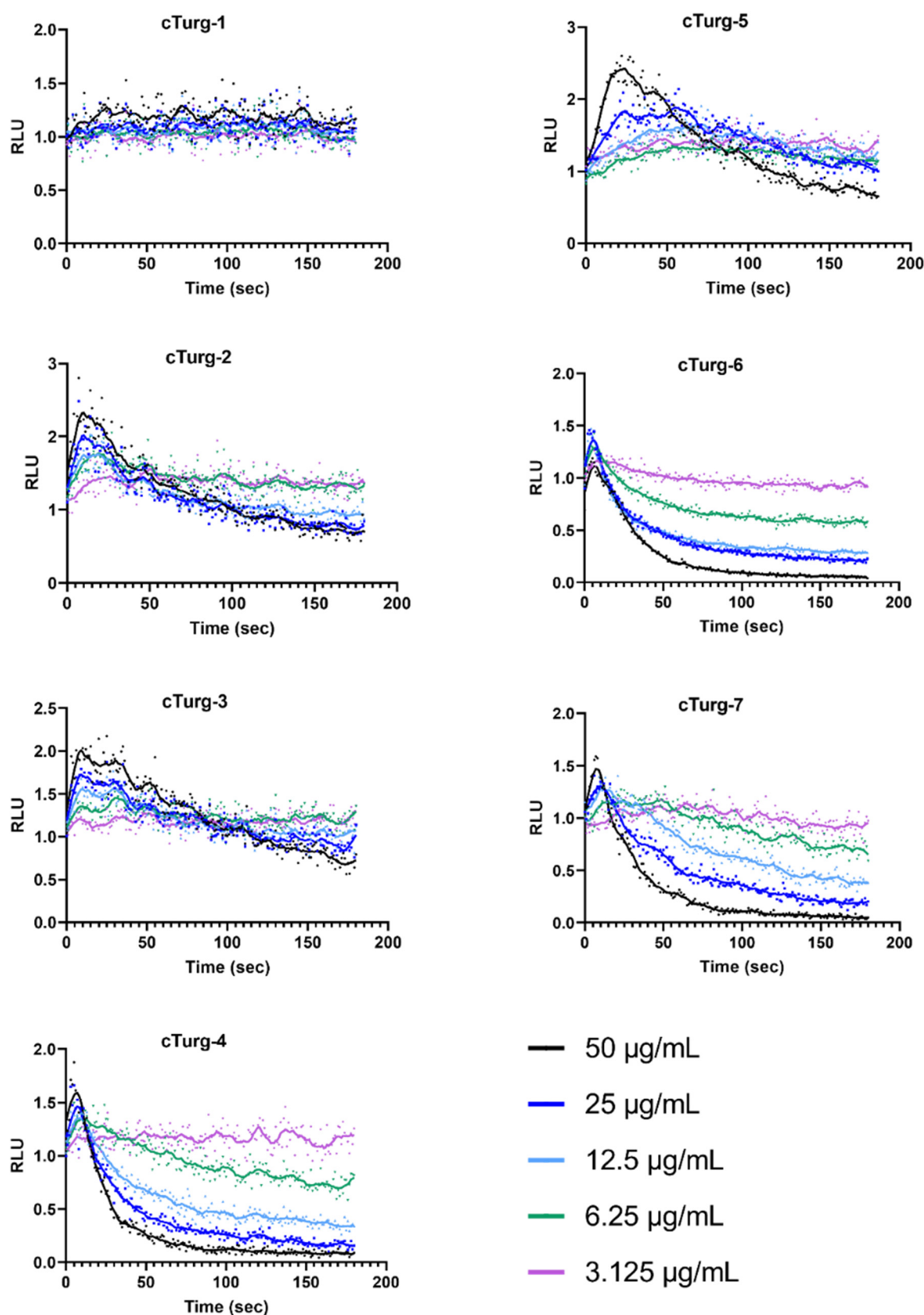


Figure S28. Kinetic of the effect on membrane integrity as measured by relative luminescence in *B. subtilis* (pCSS962) treated with different concentrations of cTurg-1, cTurg-2, cTurg-3, cTurg-4, cTurg-5, cTurg-6 and cTurg-7.

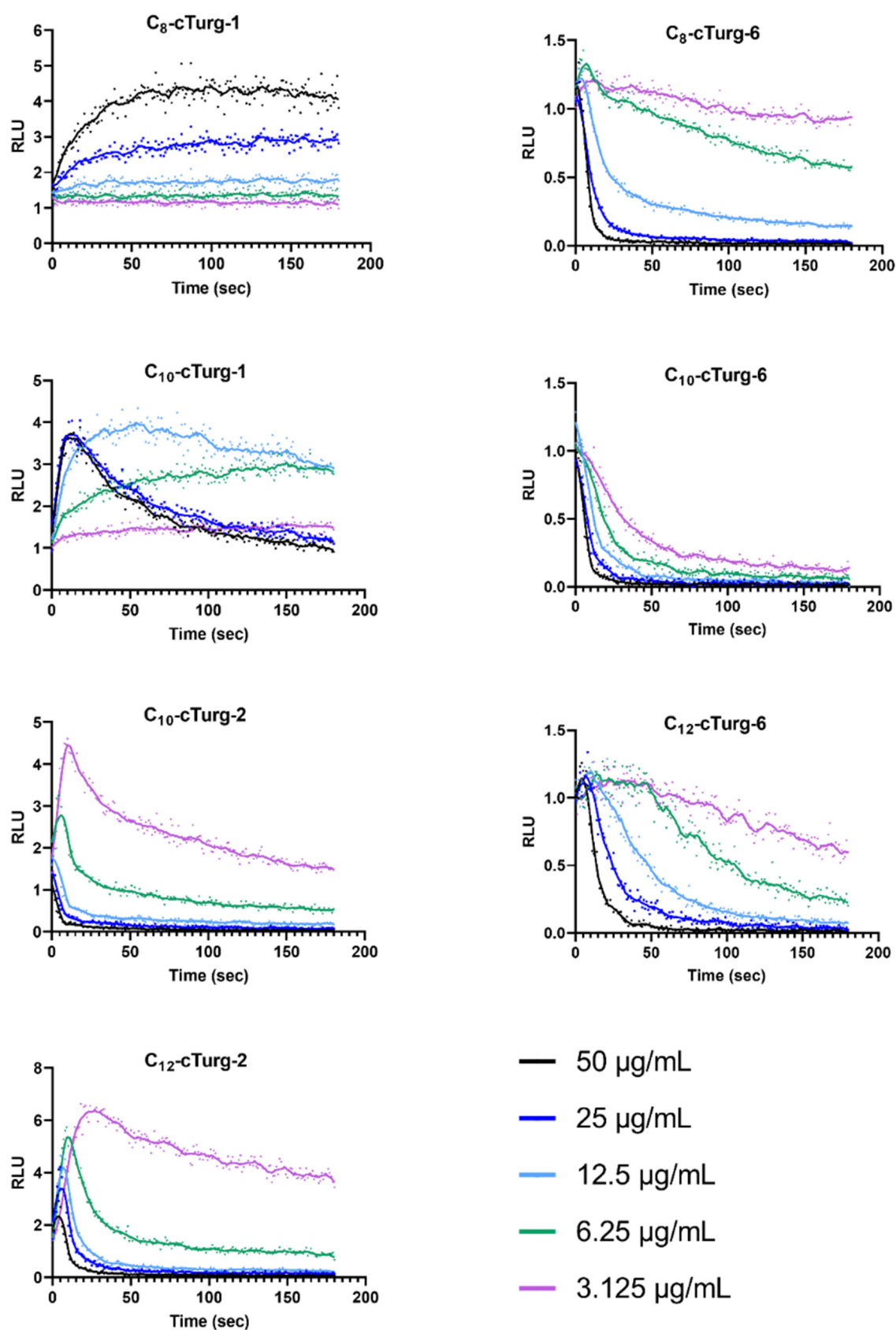


Figure S29. Kinetic of the effect on membrane integrity as measured by relative luminescence in *B. subtilis* (pCSS962) treated with different concentrations of C₈-cTurg-1, C₁₀-cTurg-1, C₁₀-cTurg-2, C₁₂-cTurg-2, C₈-cTurg-6, C₁₀-cTurg-6, and C₁₂-cTurg-6.

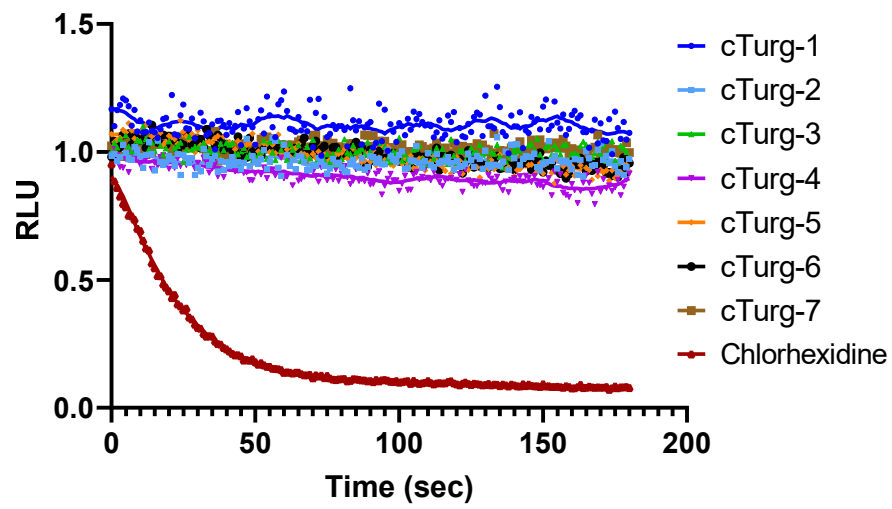


Figure S30. Kinetic of the effect on viability as measured by relative luminescence in *E. coli* (pCGLS-11) treated with 50 $\mu\text{g/mL}$ of cTurg-1-7 or 25 $\mu\text{g/mL}$ of chlorhexidine.

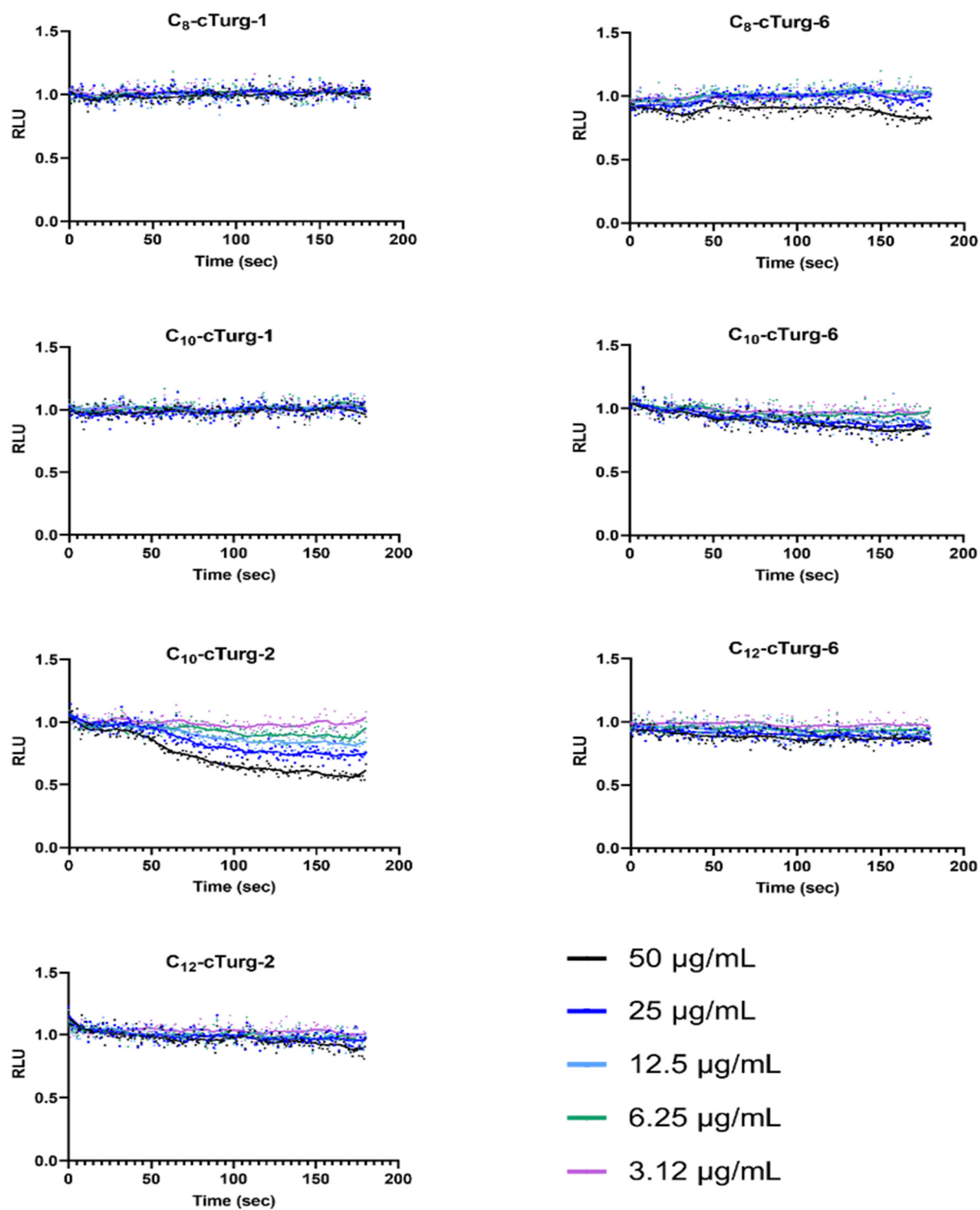


Figure S31. Kinetic of the effect on viability as measured by relative luminescence in *E. coli* (pCGLS-11) treated with different concentrations of $\text{C}_8\text{-cTurg-1}$, $\text{C}_{10}\text{-cTurg-1}$, $\text{C}_{10}\text{-cTurg-2}$, $\text{C}_{12}\text{-cTurg-2}$, $\text{C}_8\text{-cTurg-6}$, $\text{C}_{10}\text{-cTurg-6}$, and $\text{C}_{12}\text{-cTurg-6}$.

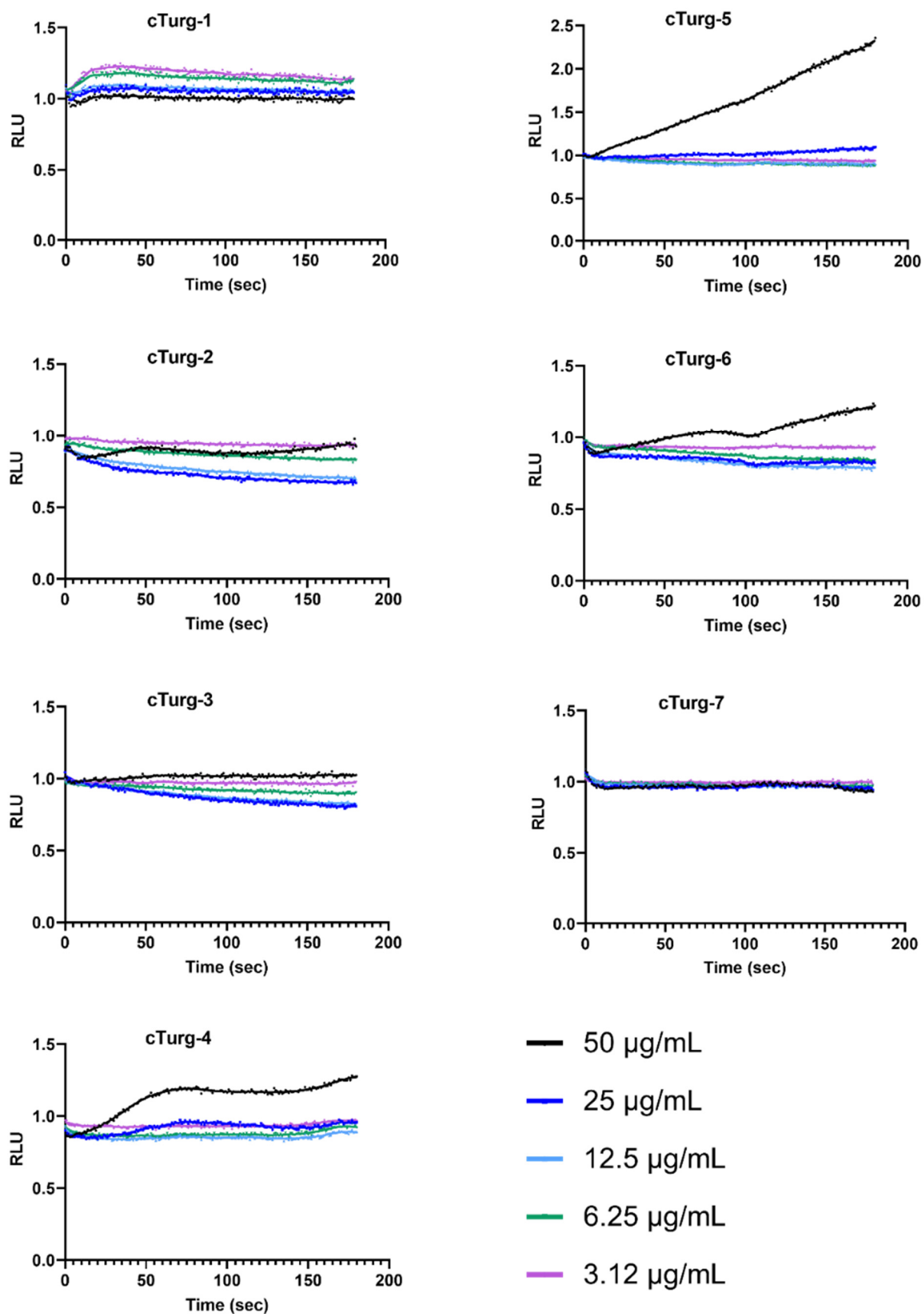


Figure S32. Kinetic of the effect on membrane integrity as measured by relative luminescence in *E. coli* (pCSS962) treated with different concentrations of cyclic peptides **cTurg-1**, **cTurg-2**, **cTurg-3**, **cTurg-4**, **cTurg-5**, **cTurg-6** and **cTurg-7**.

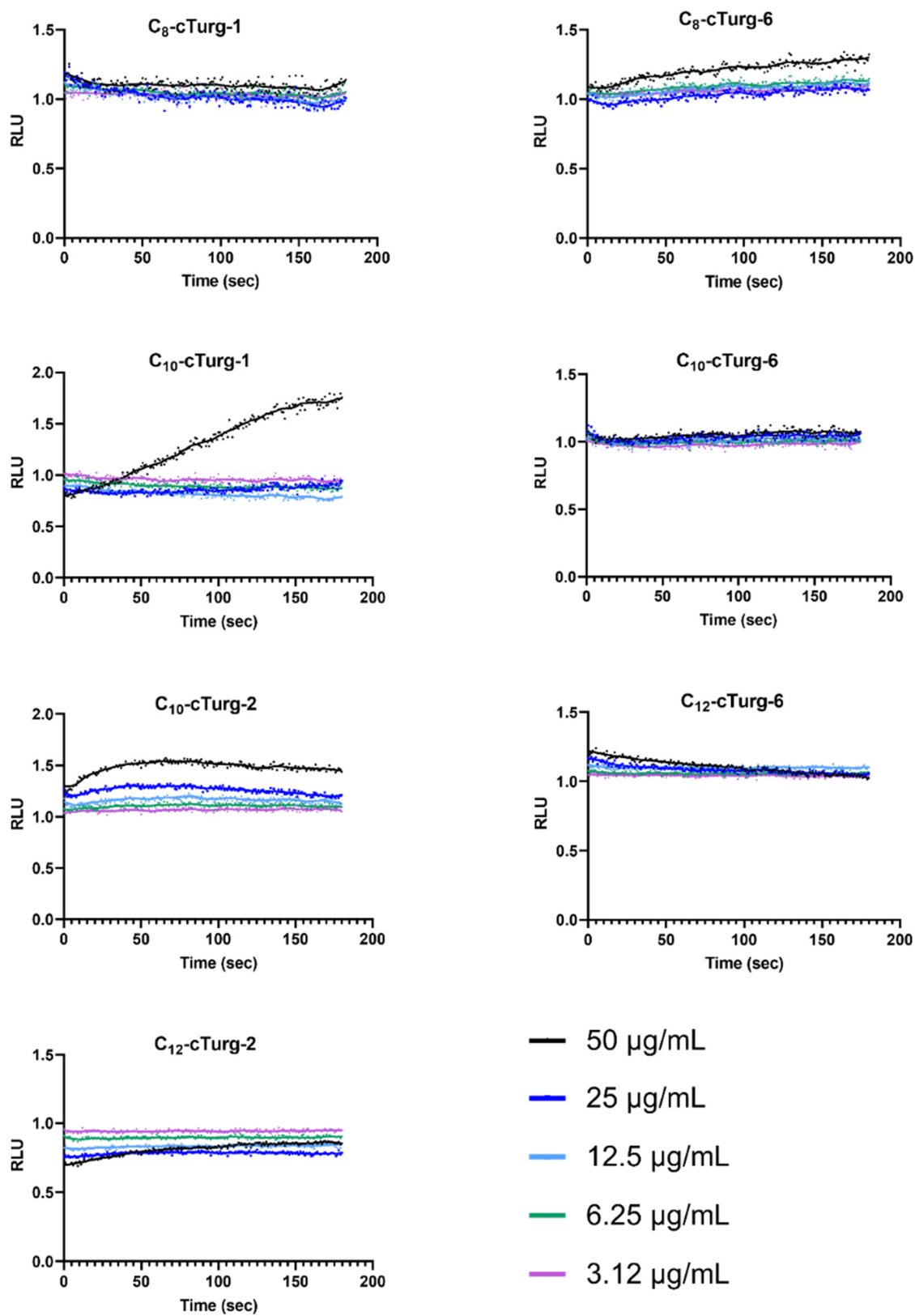


Figure S33. Kinetic of the effect on membrane integrity as measured by relative luminescence in *E. coli* (pCSS962) treated with different concentrations of C₈-cTurg-1, C₁₀-cTurg-1, C₁₀-cTurg-2, C₁₂-cTurg-2, C₈-cTurg-6, C₁₀-cTurg-6, and C₁₂-cTurg-6.

Table S1. Theoretical and measured monoisotopic mass (Da), and theoretical and observed m/z ions during HRMS of the synthesised peptides.

Peptide	Monoisotopic mass (Da)		[M+2H] ²⁺		[M+3H] ³⁺		[M+4H] ⁴⁺	
	Theoretical	Measured	Theoretical	Measured	Theoretical	Measured	Theoretical	Measured
cTurg-1	1300.6897	1300.6915	651.3521	651.3525	434.5705	434.5713	326.1797	326.1803
cTurg-2	1518.7741	1518.7746	760.3943	760.3944	507.2653	507.2656	380.7008	380.7009
cTurg-3	1518.7741	1518.7746	760.3943	760.3944	507.2653	507.2656	380.7008	380.7009
cTurg-4	1558.8054	1558.8061	780.4100	780.4102	520.6091	520.6094	390.7086	390.7088
cTurg-5	1630.7986	1630.7993	816.4066	816.4068	544.6068	544.6071	408.7069	408.7071
cTurg-6	1630.7987	1630.7999	816.4066	816.4068	544.6068	544.6073	408.7069	408.7074
cTurg-7	1670.8299	1670.8308	836.4222	836.4222	557.9506	557.9509	418.7148	418.7152
C₈-Turg-1	1428.8098	1428.8104	715.4122	715.4123	477.2772	477.2775	358.2097	358.2099
C₁₀-Turg-1	1456.8411	1456.8415	729.4278	729.4279	486.6210	486.6211	365.2176	365.2177
C₁₂-Turg-1	1484.8724	1484.8732	743.4435	743.4437	495.9647	495.9651	372.2254	372.2256
C₈-Turg-2	1646.8942	1646.8950	824.4544	824.4544	549.9720	549.9724	412.7308	412.7311
C₁₀-Turg-2	1674.9255	1674.9254	838.4700	838.4695	559.3158	559.3158	419.7387	419.7388
C₁₂-Turg-2	1702.9568	1702.9566	852.4857	852.4851	568.6595	568.6597	426.7465	426.7465
C₈-Turg-6	1758.9188	1758.9195	880.4667	880.4666	587.3135	587.3139	440.7370	440.7373
C₁₀-Turg-6	1786.9501	1786.9515	894.4823	894.4827	596.6573	596.6579	447.7448	447.7452
C₁₂-Turg-6	1814.9814	1814.9829	908.4980	908.4981	606.0011	606.0019	454.7526	454.7531
C₈-cTurg-1	1426.7941	1426.7948	714.4043	714.4045	476.6053	476.6056	357.7058	357.7060
C₁₀-cTurg-1	1454.8254	1454.8257	728.4200	728.4200	485.9491	485.9493	364.7136	364.7137
C₁₂-cTurg-1	1482.8567	1482.8573	742.4356	742.4358	495.2928	495.2931	371.7215	371.7216
C₈-cTurg-2	1644.8785	1644.8805	823.4465	823.4470	549.3001	549.3013	412.2269	412.2273
C₁₀-cTurg-2	1672.9098	1672.9107	837.4622	837.4621	558.6439	558.6446	419.2347	419.2349
C₁₂-cTurg-2	1700.9411	1700.9422	851.4778	851.4775	567.9876	567.9888	426.2426	426.2427
C₈-cTurg-6	1756.9031	1756.9036	879.4588	879.4586	586.6416	586.6420	440.2331	440.2333
C₁₀-cTurg-6	1784.9344	1784.9351	893.4745	893.4743	595.9854	595.9860	447.2409	447.2411
C₁₂-cTurg-6	1812.9657	1812.9685	907.4901	907.4905	605.3292	605.3303	454.2487	454.2498

Table S2. Purity of synthesized peptides (%) and retention time (min), determined by UPLC using a reversed phase column.

Peptide	Sequence	Purity [%]	Retention time [min]
cTurg-1	cyclic	100.00	3.11
cTurg-2	cyclic	96.53	3.87
cTurg-3	cyclic	96.43	3.92
cTurg-4	cyclic	95.79	3.97
cTurg-5	cyclic	95.74	3.98
cTurg-6	cyclic	90.59	4.02
cTurg-7	cyclic	95.61	4.09
C₈-Turg-1	linear	99.16	4.38
C₁₀-Turg-1	linear	95.87	4.89
C₁₂-Turg-1	linear	96.01	5.44
C₈-Turg-2	linear	98.04	4.97
C₁₀-Turg-2	linear	96.40	5.41
C₁₂-Turg-2	linear	96.38	5.89
C₈-Turg-6	linear	95.04	5.07
C₁₀-Turg-6	linear	95.53	5.51
C₁₂-Turg-6	linear	95.16	5.98
C₈-cTurg-1	cyclic	92.83	4.27
C₁₀-cTurg-1	cyclic	91.51	4.74
C₁₂-cTurg-1	cyclic	93.36	5.22
C₈-cTurg-2	cyclic	96.32	4.70
C₁₀-cTurg-2	cyclic	91.79	5.11
C₁₂-cTurg-2	cyclic	95.32	5.55
C₈-cTurg-6	cyclic	94.15	4.85
C₁₀-cTurg-6	cyclic	93.65	5.28
C₁₂-cTurg-6	cyclic	92.52	5.72

Table S3. Selectivity index (SI) calculated as the ration between haemolytic activity (EC₅₀, in µg/mL) and the geometric mean (GM) of the MIC values (in µg/mL) against bacteria and fungi, i.e., SI = EC₅₀ / GM. MIC >256 were set to 256 for bacteria, MIC >128 were set to 128 for fungi, and the values for the highest tested concentration was used for haemolytic activity when calculating the SI.

		GM of MIC				Selectivity index (SI)			
		Peptide	G+	G-	Tot. bact. ¹	Fungi	G+	G-	Tot. Fungi
Cyclic peptides	W	cTurg-1	128	256	161	64	nd ²	nd	nd
		cTurg-2	11	64	20	32	92	16	52
		cTurg-3	10	64	18	32	89	13	47
		cTurg-4	16	128	32	40	67	8	33
	R / W	cTurg-5	8	11	9	32	138	97	123
		cTurg-6	7	11	8	32	164	97	138
		cTurg-7	7	23	10	32	29	9	20
Linear lipopeptides	W	C ₈ -Turg-1	19	64	29	40	50	15	33
		C ₁₀ -Turg-1	7	23	10	25	142	42	95
		C ₁₂ -Turg-1	5	11	6	25	204	86	153
	R / W	C ₈ -Turg-2	7	16	9	40	29	12	22
		C ₁₀ -Turg-2	7	11	8	40	10	6	8
		C ₁₂ -Turg-2	11	23	14	40	5	2	4
		C ₈ -Turg-6	7	16	9	102	8	3	6
		C ₁₀ -Turg-6	16	45	23	102	1	0	1
		C ₁₂ -Turg-6	19	91	32	91	2	0	1
Cyclic lipopeptides	W	C ₈ -cTurg-1	16	64	25	32	59	15	37
		C ₁₀ -cTurg-1	4	16	6	20	239	60	151
		C ₁₂ -cTurg-1	3	8	4	20	77	27	55
	W	C ₈ -cTurg-2	3	6	4	13	155	78	123
		C ₁₀ -cTurg-2	3	8	5	32	32	13	24
		C ₁₂ -cTurg-2	7	16	9	40	5	2	4
	R / W	C ₈ -cTurg-6	5	11	6	51	6	3	5
		C ₁₀ -cTurg-6	6	16	8	51	3	1	2
		C ₁₂ -cTurg-6	10	32	14	81	1	0	1

¹ GM of all bacterial test strains, ² nd: not determined.