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Triazolyl-bridged peptides with enhanced antimicrobial activity and potency against pathogenic bacteria

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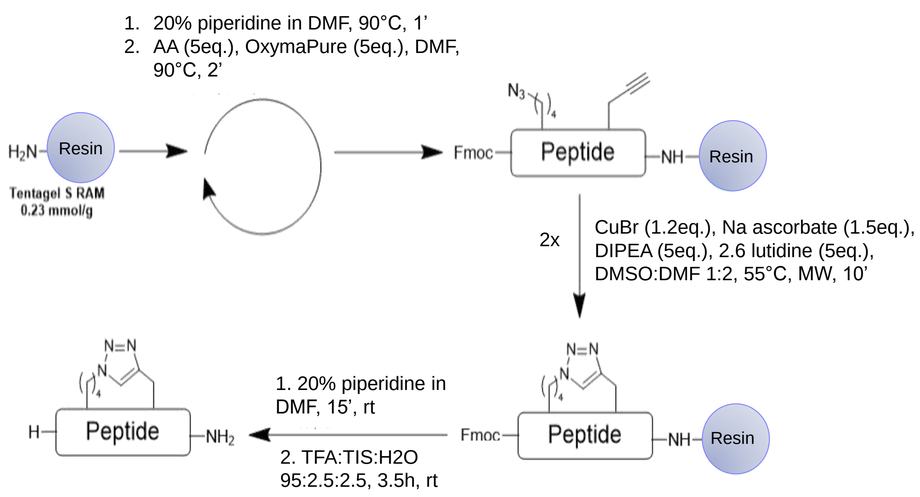
Abstract

Still, there are no linear antimicrobial peptides (AMPs) available as treatment option against bacterial infections. This is caused by several drawbacks that come with AMPs as for instance their limited proteolytic stability and low selectivity against human cells. In this work, we screened a small library of rationally designed new peptides based on the cell-penetrating peptide sC18* towards their antimicrobial activity.[1] We identified several effective novel AMPs and chose one out of this group to further develop its potency. Therefore, we introduced a triazolyl-bridge at different positions to provide a preformed helical structure assuming that this modification would (i) improve proteolytic stability and (ii) membrane activity. Indeed, placing the triazolyl-bridge within the hydrophilic part of the linear version highly increased membrane-activity as well as stability against proteolytic degradation. The new peptides 8A and 8B demonstrated high activity against several bacterial species tested including pathogenic *N. gonorrhoeae* and methicillin-resistant *S. aureus*. Since they exhibited significantly lower activity against human cells, these novel peptides offer true alternatives for future clinical applications and are worth to study in more detail.[2]

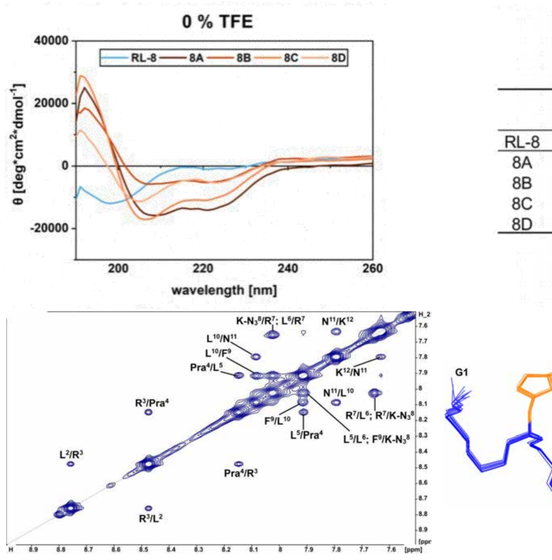
1 Synthesis of triazolyl-bridged novel AMPs



- Synthesis scheme of triazolyl-bridged peptides
- Cupper catalyzed alkyne azide click (CuAAC) reaction on solid support
- Bridge built between azidolysine and propargylglycine
- Reaction supported by using microwave
- Aim to improve stability and biological activity

Name	Sequence	$MW_{calc.}$ [Da]	$MW_{exp.}$ [Da]	Net charge
RL-8	GLRKLLRKFLNK	1485.9	1485.3	+6
8A	GLR-**Pra**-LLR-**Aza**-FLNK	1478.8	1478.2	+4
8B	GLR-**Aza**-LLR-**Pra**-FLNK	1478.8	1478.2	+4
8C	G-**Pra**-RKL-**Aza**-RKFLNK	1508.8	1508.2	+6
8D	G-**Aza**-RKL-**Pra**-RKLNK	1508.8	1508.2	+6

2 Secondary structure

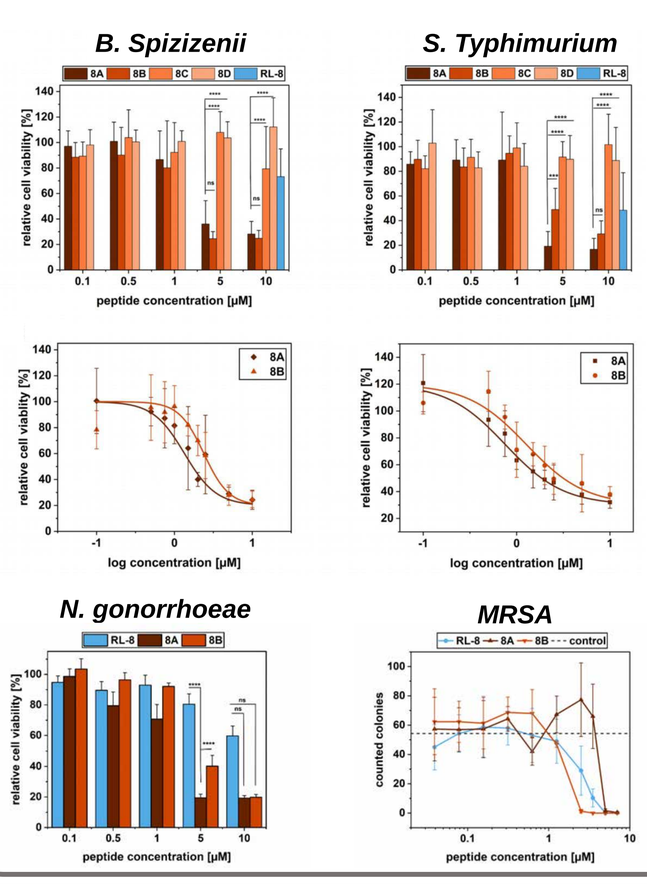


	TFE content	
	0 %	25 %
RL-8	0.22	0.68
8A	0.88	0.77
8B	0.89	0.71
8C	0.63	0.75
8D	0.54	0.80

- CD spectroscopy confirmed an α-helical structure for peptides 8A-8D
- NMR data showed exemplary perfectly formed α-helix of 8A

→ **Triazolyl-bridged peptides stabilize secondary structure**

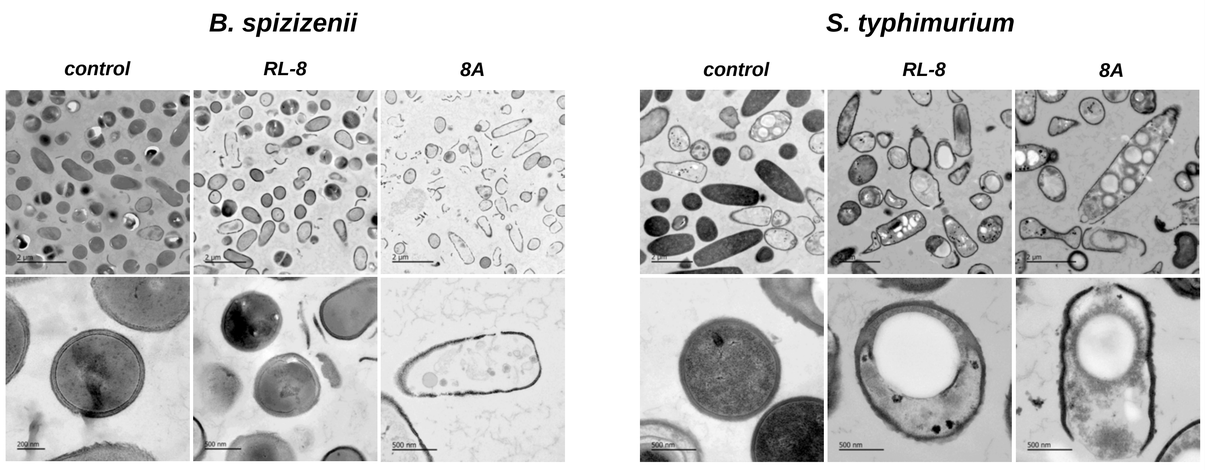
3 Analyzing antimicrobial activity



- Peptides 8A/8B exhibited higher antimicrobial activity compared to 8C/8D in gram-negative and -positive bacteria
- 8A and 8B were also potent against pathogenic species in the lower micromolar range
- High activity is related to the presence of the triazolyl-bridge

→ **Triazolyl-bridged peptides with enhanced antimicrobial activity**

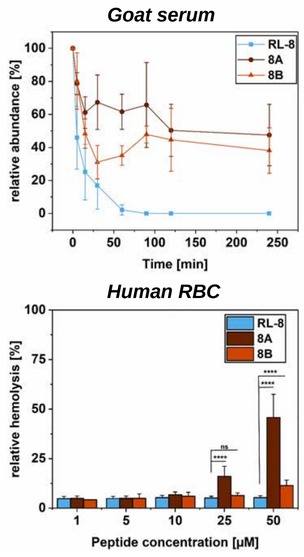
4 Transmission electron microscopy to analyze peptide-membrane interaction



- Lytic activity detectable for both analyzed peptides
- Cyclopeptide 8A showed even stronger impact on membrane integrity

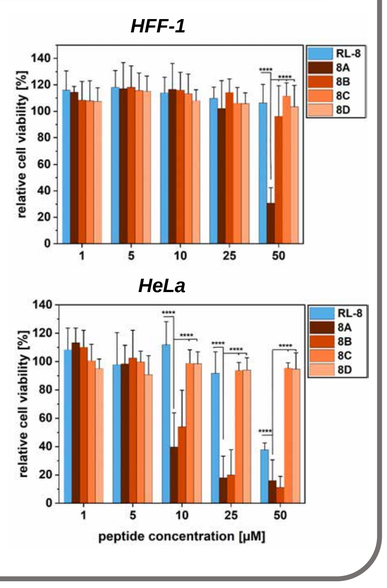
→ **Triazolyl-bridged peptides are highly membrane active**

5 Stability in serum and activity in human cells



- Increased proteolytic stability was detected for 8A/8B
- 8A showed hemolytic activity at higher concentrations
- 8A/8B exhibited good biocompatibility in human fibroblast cells
- 8A/8B demonstrated cytotoxicity against cancerous cells

→ **Increased proteolytic stability and cytotoxic effects on cancerous cells**



Summary and Outlook

Antibiotic resistance is a growing threat, necessitating the development of new drugs to combat resistant bacterial strains. A novel antimicrobial peptide, 8A, demonstrated potent antibacterial activity, stability against proteolytic cleavage, selectivity for bacteria over human cells, and potential cytotoxic effects against cancer cells, making it a promising candidate for further research.

1. Drexelius, M., Reinhardt, A., Grabeck, J., Cronenberg, T., Nitsche, F., Huesgen, P. F., Maier, B., & Neundorf, I. (2021). Multistep optimization of a cell-penetrating peptide towards its antimicrobial activity. Biochemical Journal, 478(1), 63–78. https://doi.org/10.1042/BCJ20200698
2. Grabeck, J., Mayer, J., Miltz, A., Casoria, M., Quagliata, M., Meinberger, D., Klatt, A. R., Wielert, I., Maier, B., Papini, A. M., & Neundorf, I. (2024). Triazole-Bridged Peptides with Enhanced Antimicrobial Activity and Potency against Pathogenic Bacteria. ACS Infectious Diseases. https://doi.org/10.1021/acsinfecdis.4c00078