Supporting information

Peptide/β-peptoid hybrids with activity against vancomycin-resistant enterococci: Influence of hydrophobicity and structural features on antibacterial and hemolytic properties

Martin Vestergaard[1], Bolette Skive [1], Ilona Domraceva,[3] Hanne Ingmer[1] & Henrik Franzyk[2*]

1. Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Stigbøjlen 4, DK-1870 Frederiksberg C, Denmark.
2. Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, DK-2100 Copenhagen, Denmark.
3. Latvian Institute of Organic Synthesis, Aizkraukles 21, 1006, Riga, Latvia

Supplementary Table S1. Screening of 20 peptidomimetics against Enterococci.

Initial screening of peptidomimetics at a single concentration (32 µg/ml) against multiple *E. faecium* and *E. faecalis* strains. Compounds marked in blue were selected for further studies.

Inhibition at 48 h	Growth after 48 h	Growth after 24 h

		E. faecium				***E. faecalis***		
No.	**Sequence**	ATCC 19434	3978	2961	1798	ATCC 29212	37216	38262
1	Ac-[hArg-βNPhe]$_8$-NH_2							
	Ac-[hArg-βNspe]$_6$-NH_2							
2	Ac-[hArg-βNsce]$_6$-NH_2							
3	SpermineAc-[hArg-βNspe-Lys-βNspe]$_3$-NH_2							
	NDab-[hArg-βNspe-Lys-βNspe]$_3$-NH_2							
	TODA-[hArg-βNspe-Lys-βNspe]$_3$-NH_2							
4	H-[hArg-βNspe-Lys-βNspe]$_4$-NH_2							
	Ac-[hArg-Nspe-Lys-Nspe]$_3$-NH_2							
5	Ac-[hArg-βNsce-Lys-βNspe]$_3$-NH_2							
6	Cinn-[hArg-βNspe-Lys-βNspe]$_3$-NH_2							
	H-(NLys-Phe)$_8$-NH_2							
	SpermineAc-(Lys-βNPhe)$_6$							
	Ac-(Lys-βNspe)$_8$-NH_2							
7	H-[Lys-βNPhe(F)]$_8$-NH_2							
8	H-[Lys-βNPhe(F_3)]$_6$-NH_2							
9	H-[Lys-βNCha]$_8$-NH_2							
10	Oct-[Lys-βNspe]$_6$-NH_2							
11	Lau-[Lys-βNPhe]$_6$-NH_2							
	Ac-[NLys-Trp]$_8$-NH_2							
	Ac-[βNLys-Trp]$_8$-NH_2							

Abbreviations: Ac = acetyl; Cinn = cinnamoyl; hArg=homoarginine; Lau = lauroyl; βNCha = N-cyclohexylmethyl-β-alanine; NLys = N-(4-aminobutyl)-glycine; βNLys = N-(4-aminobutyl)-β-alanine; βNPhe = N-benzyl-β-alanine; βNPhe(F) = N-(4-fluorobenzyl)-β-alanine; βNPhe(F3) = N-(3,4,5,-trifluorobenzyl)-β-alanine; βNsce=N-(S)-1-cyclohexylethyl-β-alanine; βNspe=N-(S)-1-phenylethyl-β-alanine; Oct = octanoyl; SpermineAc = $H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NHCH_2(C=O)$-; TODA = $H_3CO(CH_2)_2O(CH_2)_2OCH_2(C=O)$-.

Supplementary Table S2. Drug combinations of peptidomimetics with conventional antibiotics. N.D.: Not determined

Antibiotic	Peptidomimetic (+/- 0.5x MIC)	MIC µg/ml			
		E. faecium ATCC 19434	*E. faecalis* ATCC 29212	*E. faecium* 2961	*E. faecium* 1798
Vancomycin	-	1-2	2-4	>256	>256
	+ **2**	2	4	>256	>256
	+ **5**	2	4	>256	>256
	+ **10**	2	2	>256	>256
Gentamicin	-	64	16	N.D.	N.D.
	+ **2**	64	16	N.D.	N.D.
	+ **5**	32	16	N.D.	N.D.
	+ **10**	64	16	N.D.	N.D.
Ciprofloxacin	-	4-8	0.5-1	N.D.	N.D.
	+ **2**	4-8	1	N.D.	N.D.
	+ **5**	4-8	0.5-1	N.D.	N.D.
	+ **10**	4-8	0.5-1	N.D.	N.D.
Linezolid	-	2	2	N.D.	N.D.
	+ **2**	2	2	N.D.	N.D.
	+ **5**	2	2	N.D.	N.D.
	+ **10**	2	2	N.D.	N.D.
Rifampicin	-	8	1	N.D.	N.D.
	+ **2**	8	1	N.D.	N.D.
	+ **5**	8	1	N.D.	N.D.
	+ **10**	1-2	0.5-1	N.D.	N.D.
Azithromycin	-	4-8	1-2	N.D.	N.D.
	+ **2**	4-8	1	N.D.	N.D.
	+ **5**	4-8	1-2	N.D.	N.D.
	+ **10**	4-8	1-2	N.D.	N.D.

Supplementary Table S3. Minimum bactericidal concentration for three selected peptidomimetics.

Compound	MV388 (μg/ml)	MV269 (μg/ml)
2	4-8	8
5	128	16
10	16	32

Supplementary Table S4. Strains used in this study.

Identity no.	Strain name	Source
MV270	*E. faecium* D344R	[1]
MV388	*E. faecium* ATCC 19434	ATCC
MV389	*E. faecium* 3978	Human clinical isolate
MV390	*E. faecium* 2961	Human clinical isolate
MV391	*E. faecium* 1798	Human clinical isolate
MV269	*E. faecalis* ATCC 29212	ATCC
MV392	*E. faecalis* 37216	Veterinary isolate
MV393	*E. faecalis* 38262	Veterinary isolate
MV394	*E. faecalis* 39002	Veterinary isolate

Reference:

1. Williamson, R.; Le Bouguénec, C.; Gutmann, L.; Horaud, T. One or two low affinity penicillin-binding proteins may be responsible for the range of susceptibility of *Enterococcus faecium* to benzylpenicillin. *Microbiology* **1985**, *131*, 1933-1940, doi:10.1099/00221287-131-8-1933.