Article

Membrane lytic antimicrobial lipopeptides

Gayan Heruka DeZoysa and Viji Sarojini*

*School of Chemical Sciences, University of Auckland, Private Bag 92019, Auckland 1142
(email: *v.sarojini@auckland.ac.nz*)

Keywords: *antibiotic resistance*, *antimicrobial peptides*, *membrane lysis*, *therapeutic potential*, *colorimetric assay*

Dr Sarojini received her PhD degree in chemistry from the Indian Institute of Science, specialising in peptide chemistry under the supervision of Prof. Padmanabhan Balaram. This was followed by a postdoctoral position with Prof. Lars Baltzer at Linkoping, Sweden. She has worked at academic and research institutions in India, Sweden, the UK and USA and came to New Zealand in 2004, taking up a research position at Plant and Food Research Ltd. working on bacterial diseases of plants with Dr Robin Mitchell. She has worked at the University of Auckland since 2006 and is currently a Senior Lecturer in chemistry. Her research interests lie in the areas of fundamental peptide chemistry as well as applications of peptides in medicine and food technology.

Gayan Heruka De Zoysa was born in Sri Lanka and migrated to New Zealand in 2001. He graduated with a BSc Hons, specialising in medicinal chemistry, from the University of Auckland in 2011. He is presently completing his PhD research under the supervision of Dr Viji Sarojini. His research focuses on the synthesis of novel cyclic lipopeptides and their application as broad spectrum antimicrobials. His specific research interests include the use of lipopeptides to inhibit bacterial biofilm formation and disruption of mature biofilms.

Introduction

The development of antibiotics to treat life-threatening infections has been one of the greatest achievements of modern medicine. However, the appearance of penicillin resistant *Staphylococci* almost concomitantly with the introduction of penicillin into the market makes the problem of antibiotic resistance as old as antibiotics. Since then several more bacteria have become resistant to old and newer generations of antibiotics. In the context of antibiotic resistance, multidrug-resistance (MDR) refers to "non-susceptibility to at least one agent in three or more antimicrobial classes", whereas extensively drug-resistant (XDR) is defined as "nonsusceptibility to at least one antimicrobial agent in all but two or less antimicrobial classes".[1] The lack of new antibiotics to treat fatal infections caused by MDR bacteria, particularly *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* has become a serious global concern. The World Health Organisation has referred to antimicrobial resistance as a "problem so serious that it threatens the achievements of modern medicine".[2] There is an urgent need to fill the gap created because of the difference in pace between the rapid emergence of "superbugs" and the lack of new antibiotics to combat these. Antimicrobial agents with novel chemical structures and mechanism of action different to conventional antibiotics are important in this regard. Antimicrobial peptides (AMPs) are promising therapeutics to address the challenge of antibiotic resistance. AMPs are part of the innate immune system in almost all forms of life from microbes to humans. The majority of AMPs are cationic in nature which make them selective to microbial cell membranes having high concentrations of anionic phospholipids as opposed to mammalian and plant cell membranes which are mostly zwitter-ionic in nature. AMPs show broad spectrum antimicrobial activity, including against Gram positive and Gram negative bacteria, fungi, viruses and parasites. Recent research also highlights the anticancer activities of AMPs.[3,4] The therapeutic potential of peptides is now receiving the pharmaceutical industry's attention with focus shifting towards the development of these as marketed drugs[5] (Table 1). Unlike conventional antibiotics, such as streptomycin, development of resistance to AMPs is thought to be highly improbable, mainly because of their unique mechanism of antibacterial action. Since the main target of AMPs is the microbial membrane, bacteria (or other microbes) would have to significantly alter the structure of their membrane, to gain resistance to AMPs, which is highly unlikely.

This is a short review of a sub-class of AMPs, namely the lipopeptide antibiotics, particularly daptomycin and polymyxins (currently in clinical use) and octapeptins which have the potential to follow, based on literature evidence and our own recent research.

Lipopeptides

Natural lipopeptides are synthesised via non-ribosomal peptide synthases (NRPS) and carry long-chain fatty acids on their N-termini.[6] The peptide component of lipopeptides can be cyclic or linear chains of amino acids, generally ranging in length to about 15 residues. The ring can be a lactone, a lactam or mediated by a disulphide bridge. In principle, lactam formation can happen between the N- and C-termini (head-to-tail, in which case the lipidation would involve side chain amine functionality); between the carboxyl terminus and a side chain amine group or the N-terminus and side chain carboxyl group. Lactonisation usually involves the side chain hydroxyl groups in either Ser or Thr and, either the side chain or the C-terminal, COOH. The different possibilities for macrocyclisation in lipopeptides are shown schematically in Fig. 1. Primary structures of polymyxin B and daptomycin, as examples of macrolactam and macrolactone rings, are shown in Fig. 2.

Table 1. Peptide-based antimicrobials already approved for clinical use or as food preservatives as well as those in different stages of clinical trials

Name	Structural Classification	Application	Stage of Development
Gramicidin S	Cyclic peptide	Used topically against Gram positive and negative pathogens	Clinically used
Oritavancin	Glycopeptide	Used topically against Gram positive pathogens	Approved in 2014
Dalbavancin	Lipoglycopeptide	Used in intravenous form against Gram positive skin infections	Approved in 2014
Vancomycin	Glycopeptide	Used in intravenous form against Gram positive pathogens	Clinically used
Daptomycin	Cyclic lipopeptide	Used in intravenous form against Gram positive pathogens	Clinically used
Polymyxin B	Cyclic lipopeptide	Used as treatment against Gram negative pathogens	Clinically used
Colistin	Cyclic lipopeptide	Treatment against lung infection in cystic fibrosis	Clinically used
Nisin	Lantibiotic	Used as a food preservative	Commercially used food preservative
Pexiganan acetate	Cationic peptide	Diabetic foot ulcers	Phase III
CB-315	Lipopeptide	Treatment against *C. difficile*	Phase III
P-113	Cationic peptide (Histatin derivative)	Mouthwash against candidiasis	Completed Phase II
IB-367	Cationic peptide (Protegrin analogue)	Used as aerosol in cystic fibrosis patients with chronic respiratory infections	Phase II

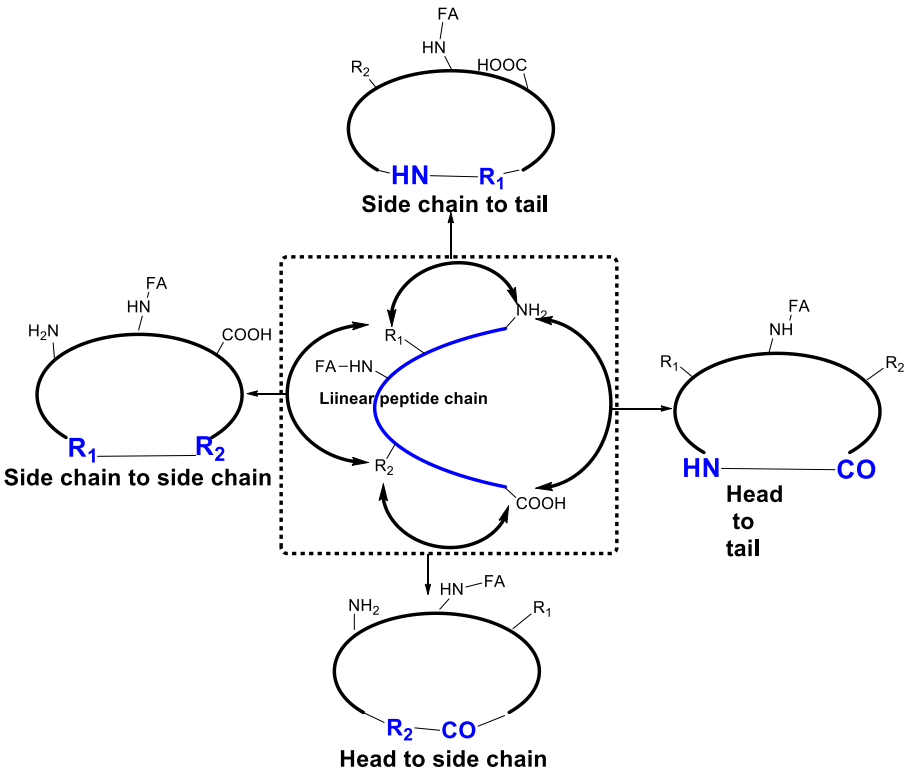


Fig. 1. Four possible ways a peptide/lipopeptide can be constrained into a macrocycle.[30]

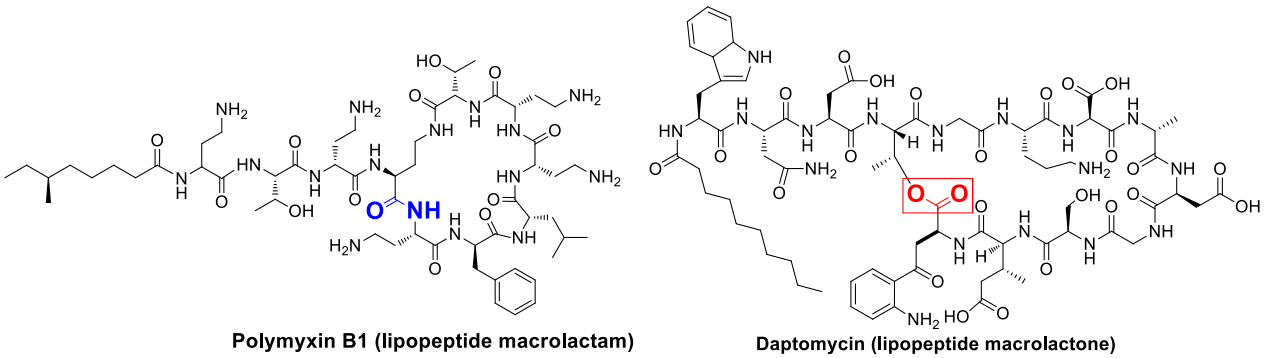


Fig. 2. Chemical structures of polymyxin B1 and daptomycin, depicting the lactam and lactone modes of cyclisation.

Lipopeptides are structurally diverse and are produced by a wide range of bacterial genera such as *Bacillus*, *Streptomyces* and *Pseudomonas* as well as certain fungi, e.g. *Aspergillus*. [6,7] Their structural diversity implies their varied roles are also unique to the producing organism. Most naturally occurring lipopeptides are secondary metabolites produced by soil bacteria. Examples of naturally occurring lipopeptides include polymyxins,[8,9] octapeptins,[10] brevistin,[11] stendomycin,[12] polypeptin,[13] cerexin,[14] iturins,[15] surfactins,[16] fengycins,[17] fusaricidins,[18] tridecaptin,[19,20] etc. The oldest amongst these are polymyxins produced by the soil bacterium *Bacillus polymyxa.*[8,9,21,22] Lipopeptides have entered the stage for the fight against antimicrobial diseases with the FDA approval of daptomycin[23] for the treatment of serious infections caused by Gram positive bacteria and the revival of interest in the previously abandoned polymyxins for clinical use to treat life threatening infections caused by Gram negative bacteria.[24] Lipopeptides are also known for their surfactant activities.[16,25] Table 2 lists the widely known lipopeptides and the microorganisms susceptible to them.

Brief discussion on synthesis of cyclic peptides

The use of orthogonal protecting groups and linkers cleavable under mild conditions are paramount to the solid phase syntheses of cyclic peptides. The specific strategy for a particular synthesis will depend on the nature of the ring (lactone, lactam or disulphide bridge), length of the peptide chain, and, for lipopeptides, the nature and position of the lipid group(s). 2-chlorotritylchloride resin generates protected peptides following mild acid cleavage which can then be used in solution phase cyclisation reactions.[26] Macrocyclisations need to be carried out under high dilution to facilitate intramolecular cyclisation against intermolecular polymerisation. Syntheses of linear and cyclic lipopeptides have been carried out following solid and solution phase strategies in the literature.[27,28,29] There are several articles in recent literature where the syntheses of cyclic peptides, cyclic depsipeptides as well cyclic lipopeptides (lactam, lactone and disulphide bridge) are discussed.[28,30,31] A general strategy for the synthesis of cyclic lipopeptides with a macrolactam ring, using a combination of solid and solution phase techniques is shown in Scheme 1.

Current clinically used lipopeptide antibiotics

Daptomycin

Daptomycin (Fig. 2) belongs to A21798C, a complex of acidic lipopeptides produced by *Streptomyces roseosporus* using non-ribosomal peptide synthetases.[32] Closely related to daptomycin are the cyclic lipodepsipeptides A54145[33] and calcium-dependent antibiotic (CAD)[34] as well as the cyclic lipopeptides amphomycin,[35] firulimycin,[36] lasportomycins[37] and glycinocins[38] produced by various streptomyces strains.

Daptomycin was approved for the treatment of infections caused by Gram positive bacteria, particularly methicillin resistant *Staphylococcus aureus*.[23] The presence of several aspartic acids and one glutamic acid makes daptomycin anionic in nature. However it binds to Ca^{2+} and functions as a cationic peptide through electrostatic interactions with the negatively charged phospholipids on cytoplasmic membranes of Gram positive bacteria.[39-41] Literature reports are contradictory about the exact sequence in which bacterial cell death occurs, with views presented of membrane depolarisation being the cause of cell death as well as a consequence of cell death.[39,42] The spectrum of activity of daptomycin includes several clinical isolates belonging to methicillin resistant *S. aureus* strains, penicillin resistant *S. pneumonia* as well as vancomycin resistant *enterococci*.[43] However, daptomycin does not penetrate the cell membranes of Gram negative bacteria, and hence cannot be used against such infections.[41,44]

Polymyxins

Polymyxins, discovered almost 7 decades ago from the soil bacteria *Paenibacillus* (*Bacillus) polymyxa*, are bactericidal cyclic lipopeptides active against multidrug resistant Gram negative bacteria.[8,21,45] Polymyxin B (PMB) and polymyxin E (colistin) are the two clinically used polymyxins. Both have a decapeptide core with 5 copies of a,g-diamino butyric acid. They are N-terminally acylated with (*S*)-6-methyloctanoic acid and (*S*)-6-methylheptanoic acid in polymyxin B and E respectively. Additionally D-Phe^6 in polymyxin B is replaced by D-Leu^6 in polymyxin E. Dab^4 side chain NH forms an intramolecular cyclic lactam with the carboxy terminal Thr^{10} resulting in a 23-membered ring (Fig. 2). Despite entering the clinic decades ago, polymyxins were abandoned in the early 80s because of nephrotoxicity concerns and re-

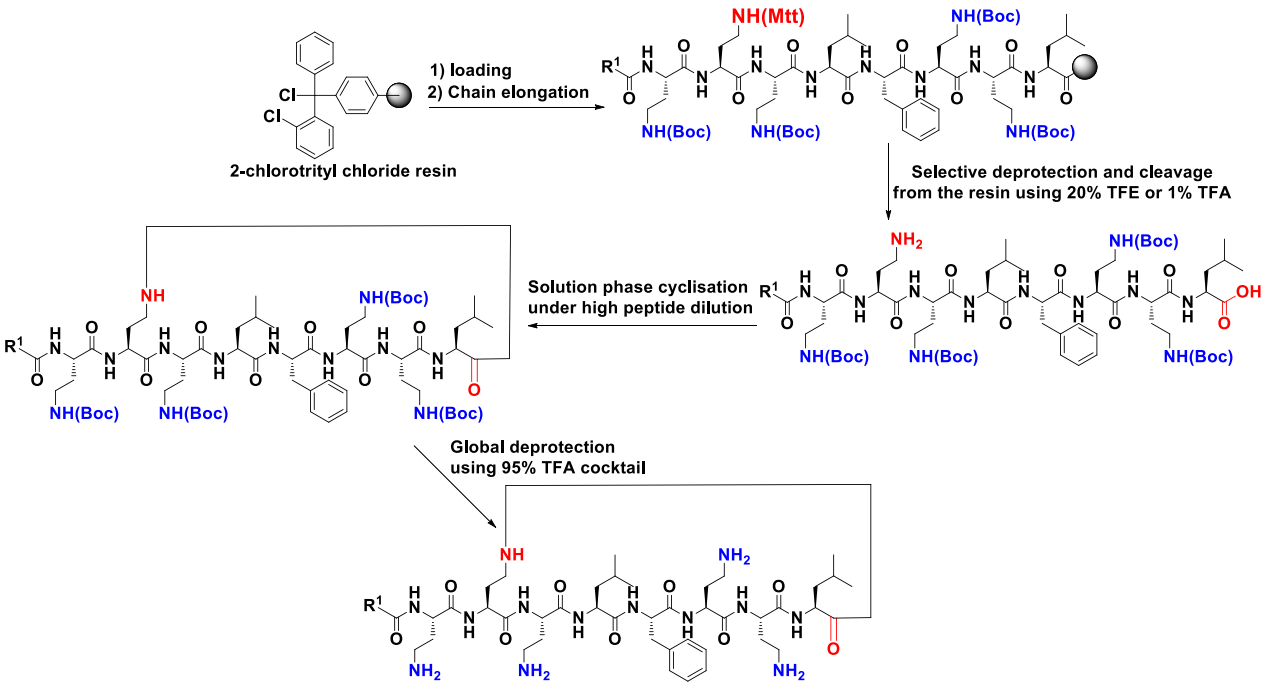
Table 2. Common naturally occurring lipopeptides

Type	Source	Susceptible strains	Ref.
Lactam			
Polymyxin	*Paenibacillus polymyxa*	Gram negative *(P.aeruginosa*, *A.baunmannii*, and *K.pneumoniae*)	8
Octapeptin	*Bacillus* and *Paenibacillus* sp	Various Gram negative (*P.aeruginosa*, *A.baunmannii* and *K.pneumoniae*), positive (*S.aureus*) and fungi (*C.albicans*)	10
Polypeptin	*Paenibacillus* sp	Various Gram negative (*P.aeruginosa*),positive (methicillin-resistant *S.aureus*) and fungi (*F.graminearum*)	67
Friulimicins	*Actinoplanes friuliensis*	Gram positive (methicillin-resistant *S.aureus* and vancomycin-resistant *Enterococcus*)	36
Marihysin A	*Bacillus marinus B*	Low broad spectrum activity against plant pathogens	68
Laspartomycin	*Streptomyces viridochromogenes*	Gram positive (methicillin-resistant *S. aureus* and vancomycin-resistant *S. aureus*)	37
Echinocandins	*Aspergillus nidulans var*	Antifungal (*C. albicans* and *Aspergillus* sp)	69
Iturins	*Bacillus subtilis* and *Bacillus amyloliquefacien*	Antifungal (*Aspergillus* sp, *Penicillium* and *Pyricularia* spp)	70
Bacillomycin	*B. subtilis*	Antifungal (*Aspergillus* sp, *C. albicans*)	71
Lactone (Depsipeptide)			
Daptomycin	*Streptomyces roseosporus*	Gram positive (methicillin-resistant *S. aureus* and vancomycin-resistant *S. aureus*)	32
Surfactin	*B. subtilis*	Gram positive (*Enterococcus faecalis*) and Gram negative (*E.coli*)	16
Fengycin	*B. subtilis*	Antifungal (*Pyricularia oryzae* and *Curvularia lunata*)	17
Empedopeptin	*Empedobacter haloabium*	Gram positive (methicillin-resistant *S.aureus* and penicillin-resistant *Streptococcus pneumoniae*)	72
Tripropeptin	*Lysobacter* Sp	Gram positive (methicillin-resistant *S. aureus*, penicillin-resistant *Streptococcus pneumoniae* and vancomycin-resistant *Enterococcus*)	73
Calcium dependent antibiotics (CDAs)	*S. coelicolor* A3	Gram positive in presence of calcium (*S. aureus*)	34
Brevistin	*B. brevis*	Gram positive (*S. aureus* and *Streptococcus pneumoniae*)	11
Fusaricidins	*Paenibacillus polymyxa*	Antifungal (*Fusarium* and *Aspergillis*) and Gram positive (*S. aureus*)	74
Linear			
Cerexin	*Bacillus cereus*	*S. aureus* and *S. pneumoniae*	14
Tridecaptin	*Paenbacillus polymyxa*	*K. pneumoniae* and *E. cloacae*	19
Tauramadine	*Brevibacillus laterosporus*	*Enterococcus* sp	75
Dragomide	*Lyngbya majuscule*	*Plasmodium falciparum*, *Leishmania donovani* and *Trypanosoma cruzi*	76

placed by aminoglycosides that were considered to be less toxic. The rapid emergence of *Pseudomonas aeruginosa*, *Acinetobacter baumannii, Klebsiella pneumonia*, and *Enterococci* resistant to b-lactams, fluoroquinolones and aminoglycosides, has led to rejuvenated interest in polymyxins for the treatment of such serious infections.[24] Currently polymyxins are being used as the last line of defence against otherwise untreatable infections caused by Gram negative bacteria.

Mechanism of action and spectrum of antibacterial activity

Lipopolysaccharides (LPS), the structural components of Gram negative outer bacterial membranes, are composed of the highly conserved amphiphilic lipid moiety, "lipid A".[46] PMB binds to the lipid A component of LPS, disrupting the outer membrane which eventually causes inner membrane permeabilisation as well.[47] PMB has been shown to displace Mg^{2+} and Ca^{2+} from cation binding sites.[48] LPS binding has been directly linked to anti-endotoxin activity of PMB.[49] Polymyxin B and colistin are active against all important nosocomial pathogens, namely *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Pseudomonas aeruginosa*, and *Acinetobacter.*[50] PMB is administered as it is, but colistin is administered as a prodrug colistin methanesulfonate.[51] Primary clinical use of colistin is in the treatment of lung infections caused by *P. aeruginosa* in cystic fibrosis patients. *P. aeruginosa* is known to be a metabolically dynamic pathogen. It has been reported that metabolically active *P.*



Scheme 1. General strategy for the synthesis of a cyclic lipopeptide macrolactam formed between a side chain NH_2 group and the C-terminus. Abbreviations: Mtt - 4-methyltrityl; Boc - tert-butyloxycarbonyl; R^1- fatty acid

aeruginosa biofilm cells are resistant to colistin, while metabolically inactive ones are sensitive to the drug.[52] Resistance to polymyxin has been reported in *Acinetobacter baumannii*.[53] Resistant bacteria have been found to alter the lipid A structure leading to weak binding by PMB.[54] The interaction between PMB and LPS has been studied by various biophysical techniques including high resolution NMR and circular dichroism.[55] The five diamino butyric acid residues together with the hydrophobic components (N-terminal fatty acid and the D-Phe-Leu dipeptide moiety have been shown to be important for the antibacterial activity and LPS binding of polymyxin B.[29,56]

Nephrotoxicity of polymyxins

The strong cationic nature of polymyxins contributes to their nephrotoxicity, resulting in damage of renal proximal tubuli. To minimise the nephrotoxic effect, polymyxin analogues with fewer positive charges (the NAB series with 3 positive charges) have been developed.[57] The NAB compounds did not induce necrosis in porcine renal proximal tubular LLC-PK1 cells even at 1 mM, whereas polymyxin B elicited 50% necrosis at 0.5 mM.[58] Table 3 lists the sequence and level of nephrotoxicity observed in PMB, colistin and their synthetic derivatives. Endotoxin (lipopolysaccharide) the structural component of Gram negative outer bacterial membrane is a pathogenicity factor involved in septic shock which leads to organ failure and death in critically ill patients. Unfortunately, the anti-endotoxin activity of PMB is over-shadowed by its nephrotoxicity.

Octapeptins

Octapeptins are another class of cyclic lipopeptides produced by the soil bacteria *Panebacellius* species.[10] As is evident from the name, the peptide component of octapeptins is made up of eight amino acids and the N-terminus carries a β-hydroxy fatty acid (similar to polymyxin B6), which can be either a straight or branched chain of carbon atoms. The protein amino acids, leucine and phenylalanine, together with the non-protein amino acid a,g-diaminobutyric acid, constitute the peptide part. Octapeptins can be considered as truncated polymyxins, however both have similar amino acid compositions with a high percentage of a,g-diaminobutyric acid and an N-terminal fatty acid of similar length. Unlike polymyxins, octapeptins are active against both Gram positive and Gram negative bacteria.[59] Octapeptins have been classified into four sub-classes A-D.[60,61] Battacin (Fig. 3) is a novel cyclic lipopeptide belonging to the octapeptin B class reported in 2012.[61] Similar to other octapeptins, battacin has a high percentage of a,g-diaminobutyric acid. The only protein amino acid found in battacin is leucine (residues 4 and 8). Battacin also has one D-Phe residue (position 5). Battacin has been proposed to have an LD sequence (Leu[4]-DPhe[5])[61], unlike other octapeptins and all polymyxins which are known to have a DL sequence. This is intriguing and will have to be verified us-

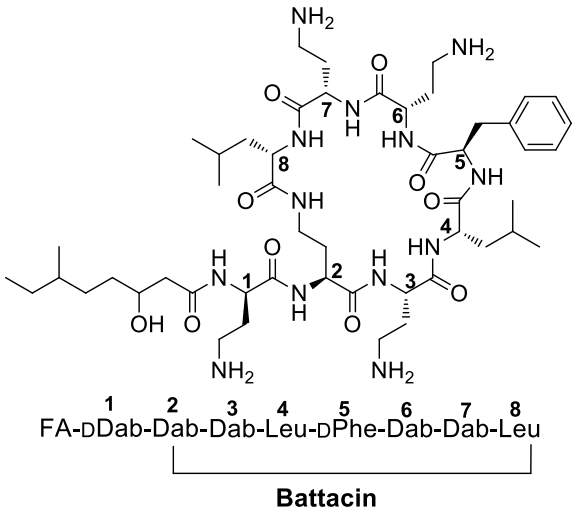


Fig. 3. Chemical structure and sequence of battacin. FA: fatty acid

Table 3. Sequences of polymyxin and related lipopeptides showing net positive charge and nephrotoxicity potential[58]

Lipopeptide	Sequence	Charge	Nephrotoxicity
Polymyxin B	MHA/MOA-Dab-Thr-Dab-cy[Dab-Dab-$_D$Phe-Leu-Dab-Dab-Thr]	5	+++
Collistin	MHA/MOA-Dab-Thr-Dab-cy[Dab-Dab-$_D$Leu-Leu-Dab-Dab-Thr]	5	+++
CB-182,804	2-CPAC- Dab-Thr-Dab-cy[Dab-Dab-$_D$Phe-Leu-Dab-Dab-Thr]	5	++
NAB739	OA-Thr-$_D$Ser- cy[Dab-Dab-$_D$Phe-Leu-Dab-Dab-Thr]	3	+
NAB7061	OA-Thr-Abu- cy[Dab-Dab-$_D$Phe-Leu-Dab-Dab-Thr]	3	+
NAB741	Ac-Thr-$_D$Ser - cy[Dab-Dab-$_D$Phe-Leu-Dab-Dab-Thr]	3	+

Abbreviations and symbols: MHA/MOA - mixture of methyl octanoyl and methyl heptanoyl, 2-CPAC - 2-chloro-phenylaminocarbonyl, Dab - diaminobutyryl, Abu - aminobutyryl, OA -octanoyl, Ac - acetyl.

+++: 50 % necrosis of porcine renal proximal tubular LLC-pk1 cells at 0.5 mM

++: necrosis potential considered high, but no data available on % necrosis

+: <10 % necrosis of porcine renal proximal tubular LLC-pk1 cells at 0.5 mM

ing chemical synthesis. Battacin has a better therapeutic index than polymyxin B with high potency against MDR and XDR pathogens and low level of cytotoxicity. These characteristics make battacin, and probably other octapeptins, ideal candidates for development as future antibiotics against MDR Gram negative pathogens, against which polymyxins are not ideal because of nephrotoxicity and neurotoxicity. Our recent research has identified synthetic battacin derivatives more potent than battacin as well as having a broader spectrum of antimicrobial activity.[62]

The following section describes a simple membrane lytic assay to quickly determine the ability of antimicrobial agents to lyse bacterial membranes and puts this in the context of our own results using a library of battacin peptides.

Membrane lytic assay

Given the rapid and alarming increase in antibiotic resistance and the urgent need for novel antimicrobials with a mechanism of action different to conventional antibiotics, a simple but reliable assay for the detection of such compounds is important. Detection of biological molecules following colour changes with the naked eye is particularly attractive. Vesicles, composed of phospholipids and polymerised diacetylene, mimic cell membranes and serve as a biomimetic sensing platform.[63] Lipid/polydiacetylene (PDA) vesicles have recently been utilised for the detection of bacteria based on colour changes induced by membrane active amphiphilic molecules produced by these organisms.[64] Dimyristoylphosphatidylcholine-polydiacetylene (DMPC-PDA) vesicles provide an excellent system to mimic the bacterial membrane and exhibit an intense blue color upon irradiation with UV light which immediately changes to red when the vesicular structure is disrupted.[65] A representative diagram depicting such a scenario is shown in Fig. 4. Differences in colour changes induced by native and analogue peptides have been correlated to their observed antibacterial activities and the extent of membrane permeabilities.

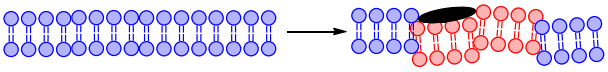


Fig. 4. Representation of membrane perturbation induced by antimicrobial agents being detected as colour changes. An ordered membrane is shown on the left hand side in blue. The black oval represents a potential antimicrobial agent, which upon binding to the membrane causes structural perturbations, detected as immediate colour changes from blue to red.

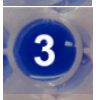
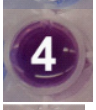
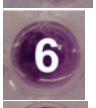
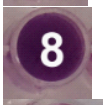
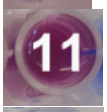
Evaluation of membrane lytic activity of a synthetic battacin library

A library of battacin peptides was synthesised following the general protocol shown in Scheme 1. DMPC-PDA vesicles prepared following literature protocols[63,65] were used to assess the ability of the battacin peptides to cause lysis of bacterial membranes following the assay referred to above. Streptomycin and a known membrane lytic antimicrobial peptide (pexiganan) were used as the controls. All compounds were used at 0.1 mM concentration in the membrane lytic assay. Results are shown in Table 4. As expected, pexiganan, a well-known membrane-lytic peptide showed an intense colorimetric response from blue to red immediately on addition to the vesicles. Streptomycin, despite being bactericidal, did not show any color change, which is in accordance with its known mechanism of action that does not involve membrane perturbation. The observed colorimetric responses of the battacin library correlated well with their observed antimicrobial potencies (minimal inhibitory concentrations: MICs). Battacin analogues with MICs < 100 mM showed clear membrane perturbation as confirmed by the immediate color change observed on addition to the vesicles, whereas those analogues with MICs > 500 mM did not induce any membrane perturbation.

Summary

Development of biologically active peptides is an active

Table 4. Membrane lytic activity of battacin library

Peptide	MIC	Colour of the well[a]	Membrane lysis
GZ3.55	~1000 μM		No
GZ3.40	~500 μM		No
GZ3.19	~500 μM		No
GZ3.15	~50 μM		Yes
GZ3.37	~20 μM		Yes
GZ3.26	~20 μM		Yes
GZ3.38	~100 μM		Yes
Pexiganan	~5 μM		Yes
Streptomycin sulphate	~1 μM		No
Negative control	-		-

[a] The numbers inside the coloured wells in column 3 are the numbers of the corresponding wells in the 96-well assay. Blue indicates unperturbed membrane structure whereas red indicates perturbed membranes. (See Fig. 4 for a schematic representation).

area of research in our laboratory at the University of Auckland. Our previous work in this area has resulted in short synthetic AMPs with potency against PSA, the kiwi fruit pathogen.[66] Work is currently in progress towards the chemical syntheses, characterisation and bioassay analysis of battacin diastereomers in an effort to establish the stereochemistry of the natural product and to develop potent analogues with a broader spectrum of antimicrobial activity.

Acknowledgements

Research reported in this paper has been supported through funding from the University of Auckland.

References

1. Magiorakos, A. P.; Srinivasan, A.; Carey, R. B.; Carmeli, Y.; Falagas, M. E.; Giske, C. G.; Harbarth, S.; Hindler, J. F.; Kahlmeter, G.; Olsson-Liljequist, B.; Paterson, D. L.; Rice, L. B.; Stelling, J.; Struelens, M. J.; Vatopoulos, A.; Weber, J. T.; Monnet, D. L. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **2012**, *18*, 268-281.
2. www.who.int/drugresistance/documents/surveillancereport/en/ (accessed October 5, 2014).
3. Zaiou, M. *J. Mol. Med.* **2007**, *85*, 317-329.
4. Jenssen, H.; Hamill, P.; Hancock, R. E. W. *Clin. Microbiol. Rev.* **2006**, *19*, 491-511.
5. Rossi, L. M.; Rangasamy, P.; Zhan, J.; Qiu, X. Q.; Wu, G. Y. *J. Pharm. Sci.-US* **2008**, *97*, 1060-1070; Marx, V. *Chem. Eng. News* **2005**, *83*, 17; Vlieghe, P.; Lisowski, V.; Martinez, J.; Khrestchatisky, M. *Drug Discov. Today* **2010**, *15*, 40-56.
6. Strieker, M.; Marahiel, M. A. *Chembiochem.* **2009**, *10*, 607-616.
7. Bonmatin, J. M.; Laprevote, O.; Peypoux, F. *Comb. Chem. High T. Scrl.* **2003**, *6*, 541-556; Mandal, S. M.; Barbosa, A. E. A. D.; Franco, O. L. *Biotechnol. Adv.* **2013**, *31*, 338-345.
8. Benedict, R. G.; Langlykke, A. F. *J. Bacteriol.* **1947**, *54*, 24-25.
9. Wilkinson.S; Lowe, L. A. *Nature* **1966**, *212*, 311.
10. Meyers, E.; Parker, W. L.; Brown, W. E.; Linnett, P.; Stroming.Jl. *Ann. New York Acad. Sci.* **1974**, *235*, 493-501.
11. Shoji, J.; Kato, T. *J. Antibiot.* **1976**, *29*, 380-389.
12. Bodanszk.M; Izdebski, J.; Muramats.I. *J. Am. Chem. Soc.* **1969**, *91*, 2351-2358.
13. Sogn, J. A. *J. Med. Chem.* **1976**, *19*, 1228-1231.
14. Shoji, J.; Hinoo, H. *J. Antibiot.* **1975**, *28*, 60-63.
15. Magetdana, R.; Peypoux, F. *Toxicology* **1994**, *87*, 151-174.
16. Arima, K.; Kakinuma, A.; Tamura, G. *Biochem. Bioph. Res. Co.* **1968**, *31*, 488-494.
17. Vanittanakom, N.; Loeffler, W.; Koch, U.; Jung, G. *J. Antibiot.* **1986**, *39*, 888-901.
18. Nakajima, N.; Koyama, Y.; Chihara, S. *J. Antibiot.* **1972**, *25*, 243; Kurusu, K.; Ohba, K.; Arai, T.; Fukushima, K. *J. Antibiot.* **1987**, *40*, 1506-1514; Kajimura, Y.; Kaneda, M. *J. Antibiot.* **1997**, *50*, 220-228.
19. Kato, T.; Hinoo, H.; Shoji, J. *J. Antibiot.* **1978**, *31*, 652-661.
20. Shoji, J.; Hinoo, H.; Sakazaki, R.; Kato, T.; Wakisaka, Y.; Mayama, M.; Matsuura, S.; Miwa, H. *J. Antibiot.* **1978**, *31*, 646-651; Kato,T.; Sakazaki, R.; Hinoo, H.; Shoji, J. *J. Antibiot.* **1979**, *32*, 305-312.

21. Ainsworth, G. C.; Brown, A. M.; Brownlee, G. *Nature* **1947**, *160*, 263-263.
22. Storm, D. R.; Rosenthal, K. S.; Swanson, P. E. *Ann. Rev. Biochem.* **1977**, *46*, 723-763.
23. Kirkpatrick, P.; Raja, A.; LaBonte, J.; Lebbos, J. *Nature rev. Drug discovery* **2003**, *2*, 943-944; Sauermann, R.; Rothenburger, M.; Graninger, W.; Joukhadar, C. *Pharmacology* **2008**, *81*, 79-91.
24. Evans, M. E.; Feola, D. J.; Rapp, R. P. *Annals Pharmacother.* **1999**, *33*, 960-967.
25. Nakano, M. M.; Corbell, N.; Besson, J.; Zuber, P. *Molecular & General Genetics:* **1992**, *232*, 313-321; Raaijmakers, J. M.; De Bruijn, I.; Nybroe, O.; Ongena, M. *FEMS Microbiol. Rev.* **2010**, *34*, 1037-1062.
26. Barlos, K.; Chatzi, O.; Gatos, D.; Stavropoulos, G. *Int. J. Pept. Protein Res.* **1991**, *37*, 513-520.
27. Ludolph, B.; Eisele, F.; Waldmann, H. *J. Am. Chem. Soc.* **2002**, *124*, 5954-5955; Kragol, G.; Lumbierres, M.; Palomo, J. M.; Waldmann, H. *Angew. Chem. Int. Ed.* **2004**, *43*, 5839-5842; Malesevic, M.; Strijowski, U.; Bachle, D.; Sewald, N. *J Biotechnol.* **2004**, *112*, 73-77.
28. Vila, S.; Badosa, E.; Montesinos, E.; Feliu, L.; Planas, M. *Org. Biomol. Chem.* **2013**, *11*, 3365-3374.
29. Tsubery, H.; Ofek, I.; Cohen, S.; Fridkin, M. *J. Med. Chem.* **2000**, *43*, 3085-3092; Tsubery, H.; Ofek, I.; Cohen, S.; Fridkin, M. *Peptides* **2001**, *22*, 1675-1681.
30. White, C. J.; Yudin, A. K. *Nat. Chem.* **2011**, *3*, 509-524.
31. Lam, H. Y.; Zhang, Y. F.; Liu, H.; Xu, J. C.; Wong, C. T. T.; Xu, C.; Li, X. C. *J. Am. Chem. Soc.* **2013**, *135*, 6272-6279; De Vleeschouwer, M.; Sinnaeve, D.; Van den Begin, J.; Coenye, T.; Martins, J. C.; Madder, A. *Chem. Eur. J.* **2014**, *20*, 7766-7775.
32. Debono, M.; Barnhart, M.; Carrell, C. B.; Hoffmann, J. A.; Occolowitz, J. L.; Abbott, B. J.; Fukuda, D. S.; Hamill, R. L.; Biemann, K.; Herlihy, W. C. *J. Antibiot.* **1987**, *40*, 761-777.
33. Boeck, L. D.; Papiska, H. R.; Wetzel, R. W.; Mynderse, J. S.; Fukuda, D. S.; Mertz, F. P.; Berry, D. M. *J. Antibiot.* **1990**, *43*, 587-593; Fukuda, D. S.; Mynderse, J. S.; Baker, P. J.; Berry, D. M.; Boeck, L. D.; Yao, R. C.; Mertz, F. P.; Nakatsukasa, W. M.; Mabe, J.; Ott, J.; Counter, F. T.; Ensminger, P. W.; Allen, N. E.; Alborn, W. E.; Hobbs, J. N. *J. Antibiot.* **1990**, *43*, 623-633.
34. Lakey, J. H.; Lea, E. J. A.; Rudd, B. A. M.; Wright, H. M.; Hopwood, D. A. *J. Gen. Microbiol.* **1983**, *129*, 3565-3573.
35. Heinemann, B.; Kaplan, M. A.; Muir, R. D.; Hooper, I. R. *Antibiot. Chemother.* **1953**, *3*, 1239-1242.
36. Aretz, W.; Meiwes, J.; Seibert, G.; Vobis, G.; Wink, J. *J Antibiot* **2000**, *53*, 807-815.
37. Naganawa, H.; Hamada, M.; Maeda, K.; Okami, Y.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1968**, *21*, 55-62.
38. Kong, F. M.; Carter, G. T. *J. Antibiot.* **2003**, *56*, 557-564.
39. Jung, D.; Rozek, A.; Okon, M.; Hancock, R. E. *Chem. Biol.* **2004**, *11*, 949-957.
40. Baltz, R. H.; Miao, V.; Wrigley, S. K. *Nat. Prod. Rep.* **2005**, *22*, 717-741.
41. Silverman, J. A.; Perlmutter, N. G.; Shapiro, H. M. *Antimicrob. Agents Ch.* **2003**, *47*, 2538-2544.
42. Allen, N. E.; Alborn, W. E.; Hobbs, J. N. *Antimicrob. Agents Ch.* **1991**, *35*, 2639-2642.
43. Tally, F. P.; Zeckel, M.; Wasilewski, M. M.; Carini, C.; Berman, C. L.; Drusano, G. L.; Oleson, F. B., Jr. *Expert opinion investig. drugs* **1999**, *8*, 1223-1238.
44. Carpenter, C. F.; Chambers, H. F. *Clin. Infect. Dis.* **2004**, *38*, 994-1000.
45. Jones, T. S. *Annals New York Acad. Sci.* **1949**, *51*, 909-916.
46. Hancock, R. E. W. *Trends Microbiol.* **1997**, *5*, 37-42.
47. Morrison, D. C.; Jacobs, D. M. *Immunochemistry* **1976**, *13*, 813-818; Velkov, T.; Thompson, P. E.; Nation, R. L.; Li, J. *J. Med. Chem.* **2010**, *53*, 1898-1916.
48. Groisman, E. A.; Kayser, J.; Soncini, F. C. *J. Bacteriol.* **1997**, *179*, 7040-7045.
49. Rifkind, D. *J. Bacteriol.* **1967**, *93*, 1463; Warren, H. S.; Kania, S. A.; Siber, G. R. *Antimicrob. Agents Ch.* **1985**, *28*, 107-112.
50. Landman, D.; Georgescu, C.; Martin, D. A.; Quale, J. *Clin. Microbiol. Rev.* **2008**, *21*, 449-465.
51. Bergen, P. J.; Li, J.; Rayner, C. R.; Nation, R. L. *Antimicrob. Agents Chemother.* **2006**, *50*, 1953-1958.
52. Pamp, S. J.; Gjermansen, M.; Johansen, H. K.; Tolker-Nielsen, T. *Mol. Microbiol.* **2008**, *68*, 223-240.
53. Li, J.; Nation, R. L.; Turnidge, J. D.; Milne, R. W.; Coulthard, K.; Rayner, C. R.; Paterson, D. L. *The Lancet. Infectious diseases* **2006**, *6*, 589-601; Li, J.; Rayner, C. R.; Nation, R. L.; Owen, R. J.; Spelman, D.; Tan, K. E.; Liolios, L. *Antimicrob. Agents Chemother.* **2006**, *50*, 2946-2950.
54. Hase, S.; Rietschel, E. T. *Eur. J. Biochem.* **1977**, *75*, 23-34; Sidorczyk, Z.; Zahringer, U.; Rietschel, E. T. *Eur. J. Biochem.* **1983**, *137*, 15-22.
55. Bruch, M. D.; Cajal, Y.; Koh, J. T.; Jain, M. K. *J. Am. Chem. Soc.* **1999**, *121*, 11993-12004; Meredith, J. J.; Dufour, A.; Bruch, M. D. *J. Phys. Chem. B* **2009**, *113*, 544-551; Pristovsek, P.; Kidric, J. *J. Med. Chem.* **1999**, *42*, 4604-4613; Pristovsek, P.; Kidric, J. *Curr. Top. Med. Chem.* **2004**, *4*, 1185-1201
56. Clausell, A.; Garcia-Subirats, M.; Pujol, M.; Busquets, M. A.; Rabanal, F.; Cajal, Y. *J. Phys. Chem. B* **2007**, *111*, 551-563.
57. Vaara, M.; Fox, J.; Loidl, G.; Siikanen, O.; Apajalahti, J.; Hansen, F.; Frimodt-Moller, N.; Nagai, J.; Takano, M.; Vaara, T. *Antimicrobial. Agents Chemother.* **2008**, *52*, 3229-3236; Vaara, M.; Vaara, T. *Peptides* **2010**, *31*, 2318-2321; Vaara, M. *Curr. Opin Microbiol.* **2010**, *13*, 574-581.
58. Mingeot-Leclercq, M. P.; Tulkens, P. M.; Denamur, S.; Vaara, T.; Vaara, M. *Peptides* **2012**, *35*, 248-252.
59. Kato, T.; Shoji, J. *J. Antibiot.* **1976**, *29*, 1339-1340; Meyers, E.; Parker, W. L.; Brown, W. E. *J. Antibiot.* **1976**, *29*, 1241-1242.
60. Terabe, S., Konaka, R., Shoji, J. *J. Chromatography A* **1979**, *173*, 313-320; Sugawara, K.; Yonemoto, T.; Konishi, M.; Matsumoto, K.; Miyaki, T.; Kawaguchi, H. *J. Antibiot.* **1983**, *36*, 634-638; Kato, T.; Shoji, T. *J. Antibiot.* **1980**, *33*, 186-191.
61. Qian, C. D.; Wu, X. C.; Teng, Y.; Zhao, W. P.; Li, O.; Fang, S. G.; Huang, Z. H.; Gao, H. C. *Antimicrob. Agents Chemother.* **2012**, *56*, 1458-1465.
62. De Zoysa, G. H.; Cameron, A. J.; Hegde, V. V.; Raghothama, S.; Sarojini, V. *J. Med. Chem.* **2015,** *58*, 625-639.
63. Jelinek, R.; Kolusheva, S. *Top. Curr. Chem.* **2007**, *277*, 155-180.
64. Rangin, M.; Basu, A. *J. Am. Chem. Soc.* **2004**, *126*, 5038-5039.
65. Kolusheva, S.; Boyer, L.; Jelinek, R. *Nat. Biotechnol.* **2000**, *18*, 225-227.
66. Cameron, A.; De Zoysa, G. H.; Sarojini, V. *Biopolymers* **2014**, *102*, 88-96.
67. Howell, S. F. *J. Biol. Chem.* **1950**, *186*, 863-877.
68. Liu, R. F.; Zhang, D. J.; Li, Y. G.; Tao, L. M.; Tian, L. *Helv. Chim. Acta* **2010**, *93*, 2419-2425.
69. Nyfeler, R.; Keller-Schierlein, W. *Helv. Chim. Acta* **1974**, *57*, 2459-2477.
70. Peypoux, F.; Guinand, M.; Michel, G.; Delcambe, L.; Das, B. C.; Lederer, E. *Biochemistry-US* **1978**, *17*, 3992-3996.
71. Peypoux, F.; Pommier, M. T.; Das, B. C.; Besson, F.; Delcambe, L.; Michel, G. *J. Antibiot.* **1984**, *37*, 1600-1604.
72. Konishi, M.; Sugawara, K.; Hanada, M.; Tomita, K.; Tomatsu, K.; Miyaki, T.; Kawaguchi, H.; Buck, R. E.; More, C.; Rossomano, V. Z. *J. Antibiot.* **1984**, *37*, 949-957.
73. Hashizume, H.; Igarashi, M.; Hattori, S.; Hori, M.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **2001**, *54*, 1054-1059.
74. Kajimura, Y.; Kaneda, M. *J. Antibiot.* **1996**, *49*, 129-135.
75. Desjardine, K.; Pereira, A.; Wright, H.; Matainaho, T.; Kelly, M.; Andersen, R. J. *J. Nat. Prod.* **2007**, *70*, 1850-1853.
76. Balunas, M. J.; Linington, R. G.; Tidgewell, K.; Fenner, A. M.; Urena, L. D.; Della Togna, G.; Kyle, D. E.; Gerwick, W. H. *J. Nat. Prod.* **2010**, *73*, 60-66.