Perspectives in Medicinal Chemistry

Antimicrobial Peptides and Their Analogs: Searching for New Potential Therapeutics

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ABSTRACT: Antimicrobial peptides (AMPs) are an essential part of innate immunity. These compounds have been considered as potential therapeutics because of their broad-spectrum activities and proven ability to avoid antimicrobial resistance, but their clinical and commercial developments have some limitations, such as susceptibility to proteases and a high cost of peptide production. To overcome these problems, many researchers have tried to develop short active peptides, their modifications and mimics with better properties while retaining their basic features of natural AMPs such as cationic charge and the amphipathic structure.

KEYWORDS: antimicrobial peptides, antibacterial activity, peptide analogs, peptidomimetics, peptoids

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Introduction

Antimicrobial peptides (AMPs), also called host defense peptides, are biologically active molecules produced by a wide variety of organisms as an essential component of their innate immune response.[1] The major part of AMPs is formed from high molecular precursors as a result of post-translational modification.[2] AMPs can be classified according to their structure, origin, biosynthesis mechanism, localization, biological function, mechanism of action, activity, and specificity. In the vast majority of cases, AMPs have the cationic structure[1,3] but anionic AMPs have also been described in literature.[4] Based on their structures, AMPs can be grouped into four classes: α-helical peptides, β-sheet peptides, extended peptides, and loop peptides.[5] The α-helical AMPs constitute a representative class of antibacterial peptides that have the most well-determined structure–activity relationships. Lack of cysteine residues in the molecule is characteristic of peptides of this group. These peptides have a disordered structure in aqueous solutions, but in the presence of trifluoroethanol or liposomes, these molecules are converted into α-helix forms.[2] The β-sheet AMPs are stabilized by disulfide bridges and form relatively rigid structures. The extended AMPs, which are predominantly rich in specific amino acids, such as proline, tryptophan, arginine, and histidine, have no regular secondary structure elements. The loop AMPs adopt a loop formation with one disulfide bridge (Table 1). AMPs show great structural diversity, but some features common to most of them are: a relatively small size (generally between 12 and 50 amino acid residues), their cationic nature because of multiple Arg or/and Lys residues, and the amphipathic structure because of the presence of both hydrophobic and hydrophilic regions.

Mechanism of Action

AMPs show a broad spectrum of antimicrobial activities against various microorganisms. Many of these peptides are effective against multidrug-resistant bacteria and possess a low propensity for developing resistance.

Table 1. Examples of antibacterial peptides isolated from different sources.

PEPTIDES	ORIGIN	NUMBER OF AMINO ACIDS IN MOLECULE	LITERATURE
α-helical			
magainin 2	frog	23	6
cecropin A	insects	37	7
LL-37 (cathelicidin)	human	37	8
β-sheet			
α-defensins and β-defensins (containing three disulphide bonds)	human	29–35 and 36–47	9, 10
protegrin 1 (containing two disulphide bonds)	pig	18	11, 12
extended structure			
indolicin (tryptophan and proline rich peptide)	bovine	13	13
loop			
(containing one disulphide bond)			
bactenecin	bovine	12	14
lactoferricin	bovine	25	15

The precise mechanism of the action of AMPs is yet to be explained. The electrostatic interaction of cationic AMPs with negatively charged molecules on the membrane of bacteria cells seems to be the primary mechanism for antimicrobial activity. Currently, there are three most popular models explaining the increase in membrane permeability because of AMP action: the barrel-stave model, the toroidal pore model, and the carpet model (Fig. 1).[16–19] According to the barrel-stave model, a variable number of channel-forming peptides are positioned in a "barrel-like" ring around an aqueous pore. In the toroidal pore model, the peptides induce membrane depolarization and form toroidal-shaped transmembrane pores. According to the carpet model, the peptides first bind to the target membrane and cover it in a "carpet-like" manner, then they disintegrate the membrane by disrupting the bilayer curvature.
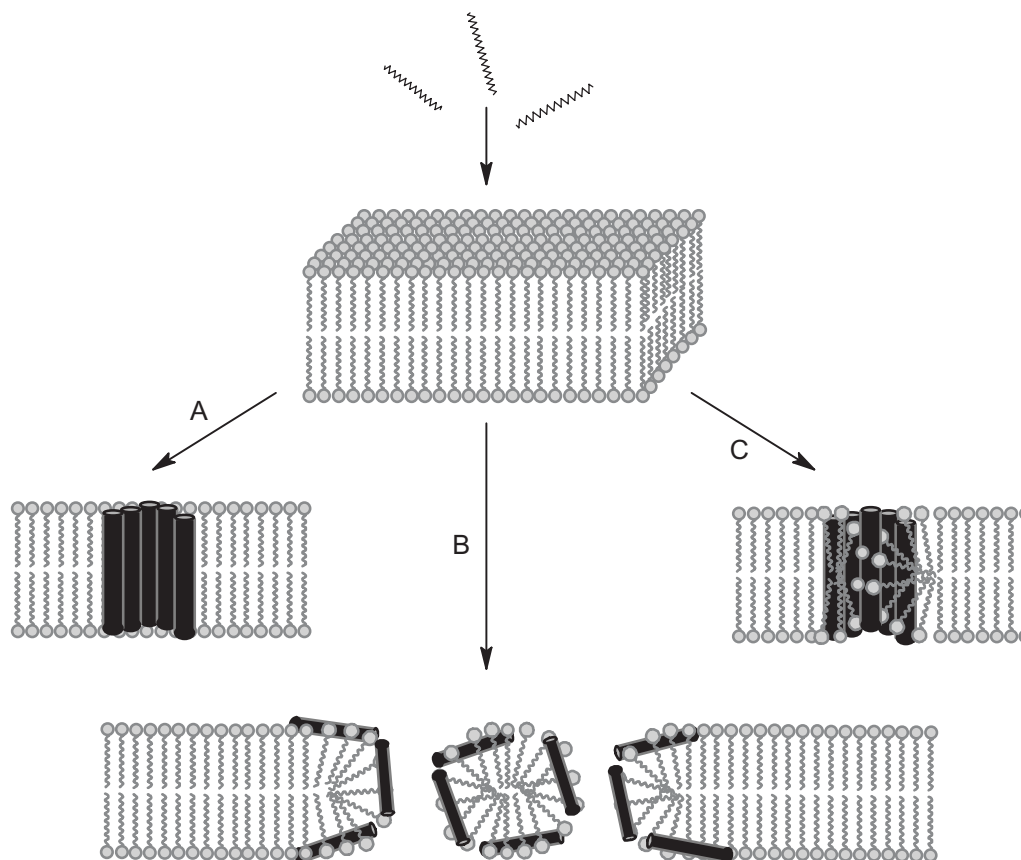
Figure 1. Three most popular modes of action of antimicrobial peptides against cytoplasmic membranes. (**A**) Barrel-stave model; (**B**) carpet model (**C**) toroidal pore model according to Ref. 19.

Table 2. Antibacterial activity of lactoferricin and its truncated analogs, reported as minimal inhibitory concentration (MIC) according to Ref. 48.

PEPTIDE	NUMBER OF AMINO ACIDS	MIC [μM]	
		S. aureus	E. coli
Cyclic lactoferricin	25	2–10	2–10
Lactoferricin 1–15	15	48–150	20–14
RWRNHBn	3	124	>150
RBipRNHBn	3	16	>150

Abbreviation: Bip, 4,4′-biphenylalanine.

Owing to a high level of cholesterol and low anionic charge, eukaryotic cells become out of the target range of many AMPs.[20] Understanding the selectivity of different AMPs for mammalian and bacterial membranes is of obvious interest in the development of these peptides as novel antibacterial agents. Molecular dynamic simulations are used to visualize in detail the interactions between AMPs and a variety of membrane mimics. This helps to understand the molecular mechanisms of the antimicrobial activity of these compounds and their toxicity.[21–23] Several reviews connected with the structures, potential mechanisms of action, and biological activities of AMPs have recently been published.[24–34]

The AMPs have been considered promising and potential drug candidates for the future because of their broad range of activity, lesser toxicity, and decreased resistance development by their target cells.

Structure Modification

The widespread use of antibiotics has led to the development of numerous multidrug resistant strains, resulting in an urgent need to develop new effective antimicrobial agents capable of being established as therapies for bacterial infections. The AMPs have a proven ability to avoid antimicrobial resistance, a phenomenon that has rendered many pharmacologically derived antibiotics ineffective. Probably one advantage of AMPs is the generality of their mechanism of action, which involves either breaking the bacterial membrane integrity or disrupting their essential components inside their cells.[35,36] This differs from the specific receptors targeted by conventional antibiotics, which allows the pathogenic bacteria to develop resistance more rapidly. AMPs are also fast-acting and potent compounds. They can be metabolically and rapidly cleared from the body. Although AMPs possess considerable benefits, their clinical and commercial developments have some limitations. The disadvantage of the AMPs is the fact that most peptides cannot be administered orally as they are rapidly inactivated by gastrointestinal enzymes, so that subcutaneous or intravenous administration is required.[37–39] Although peptides exhibit significant in vitro activity against bacteria, for many peptides this activity appears to be lost under physiological salt and serum conditions. Salt-dependent

inactivation of human β-defensins is an example.[40] Another problem is the size of molecules. They are small in comparison with proteins but large from the point of view of the chemical peptide synthesis. This is connected with high costs of their production. The price of synthetic peptides is considerably higher in comparison with conventional antibiotics. It can be a reason that the pharmaceutical industry has been reluctant to promote the clinical use of this class of antibacterial therapeutics.[33,40] Because of this problem, the investigations have been centered on searching for smaller peptides with antimicrobial activity.

Lactoferrin Derivatives

An example of an approach to this problem was the investigation of short fragments of complex AMP, lactoferrin or its 25-residue N-terminal fragment, lactoferricin[41] (Fig. 2), for establishing minimal structural requirements for antimicrobial activity (Table 2).[42–48] An 11-residue linear peptide portion of bovine lactoferricin has been reported to have similar antimicrobial activity to lactoferricin itself, but with lower hemolytic activity.[47] The synthetic AMP hLF1–11, derived from the first 11 amino acids of human lactoferrin, was evaluated in both preclinical and clinical trials (Table 3), and it is an interesting candidate for further exploration.[49] The antibacterial active fragment of bovine lactoferricin, hexapeptide RRWQWR, was determined by Tomita et al.[42] Many fragments of lactoferricin were synthesized and examined,[43–50] from the 15 residue peptide LFB, consisted of 17–31 residues of bovine lactoferrin,[43] to the modified dipeptides.[44] According to the pharmacophore model of short AMPs,[51] cationic charged as well as bulky and lipophilic moieties are necessary in the structure of such compounds. Charged moieties generally consisted of a side chain of arginine or lysine. Bulk units were represented by an indol, phenol, or phenyl group. Other studies suggest that the antimicrobial activity of peptides containing arginine is higher than those of peptides containing lysine, while peptides containing tryptophan are more potent than those with either phenylalanine or tyrosine.

The series of peptides containing simple repeating sequence: $(RW)_n$-NH$_2$ (where $n = 1–5$) were synthesized, and their antimicrobial and hemolytic activity was determined. The hexapeptide ($n = 3$) represents an optimal chain length in terms of the efficacy of peptide synthesis and antibacterial activity and selectivity (evaluated by the hemolytic index).[52] According to the authors,[51] the minimal anti-staphylococcal motif can be defined as a combination of two bulky moieties and two charged groups. An example of such a peptide is RW-OBzl (Fig. 3), where the bulky units are the indole side chain of tryptophan and the benzyl ester group, and where the charged units are the guanidinium group of arginine and the free N-terminal amino group.[51] To improve the properties of the synthesized peptides, different bulky aromatic amino acids were
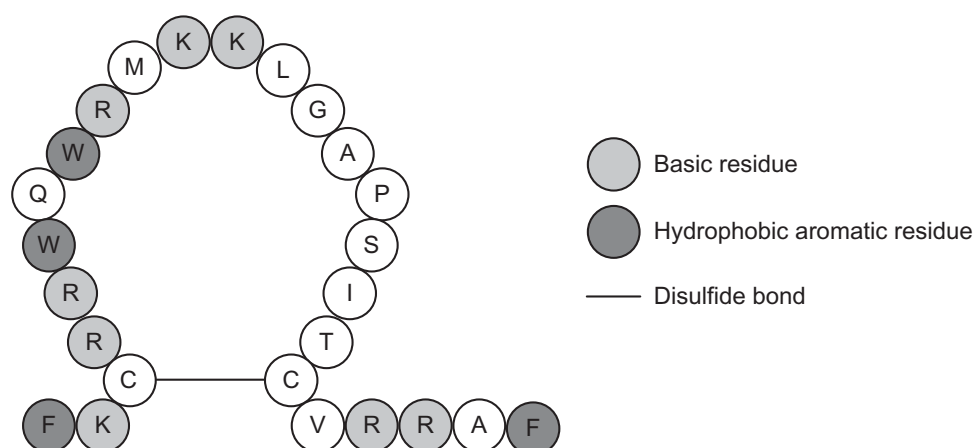
Figure 2. Amino acid sequence of cyclic bovine lactoferricin according to Ref. 46.

used instead of tryptophan.[43,44,48,53] Replacing the indole side chain of Trp with bulkier, more hydrophobic groups has shown to yield peptides with increased antibacterial activity. Antimicrobial tripeptides with a modified structure such as L-arginyl-L-4,4′-biphenylalanyl-L-arginine benzylamide, where L-4,4′-biphenylalanine was used to replace tryptophan residue (Fig. 3, Table 2),[48] belong to extremely truncated versions of lactoferrin. The modified tryptophan molecule (2,5,7-tri(tert-butyl)tryptophan-Tbt) was also applied in the synthesis of the modified tripeptides with general structure RTbtR-X, where X was −OMe, unsubstituted and substituted amides.[54,55] The most promising structure seems to be the tripeptide with C-terminal amide substituted by the ethylphenyl group (LTX-109) (Fig. 3, Table 3).[55]
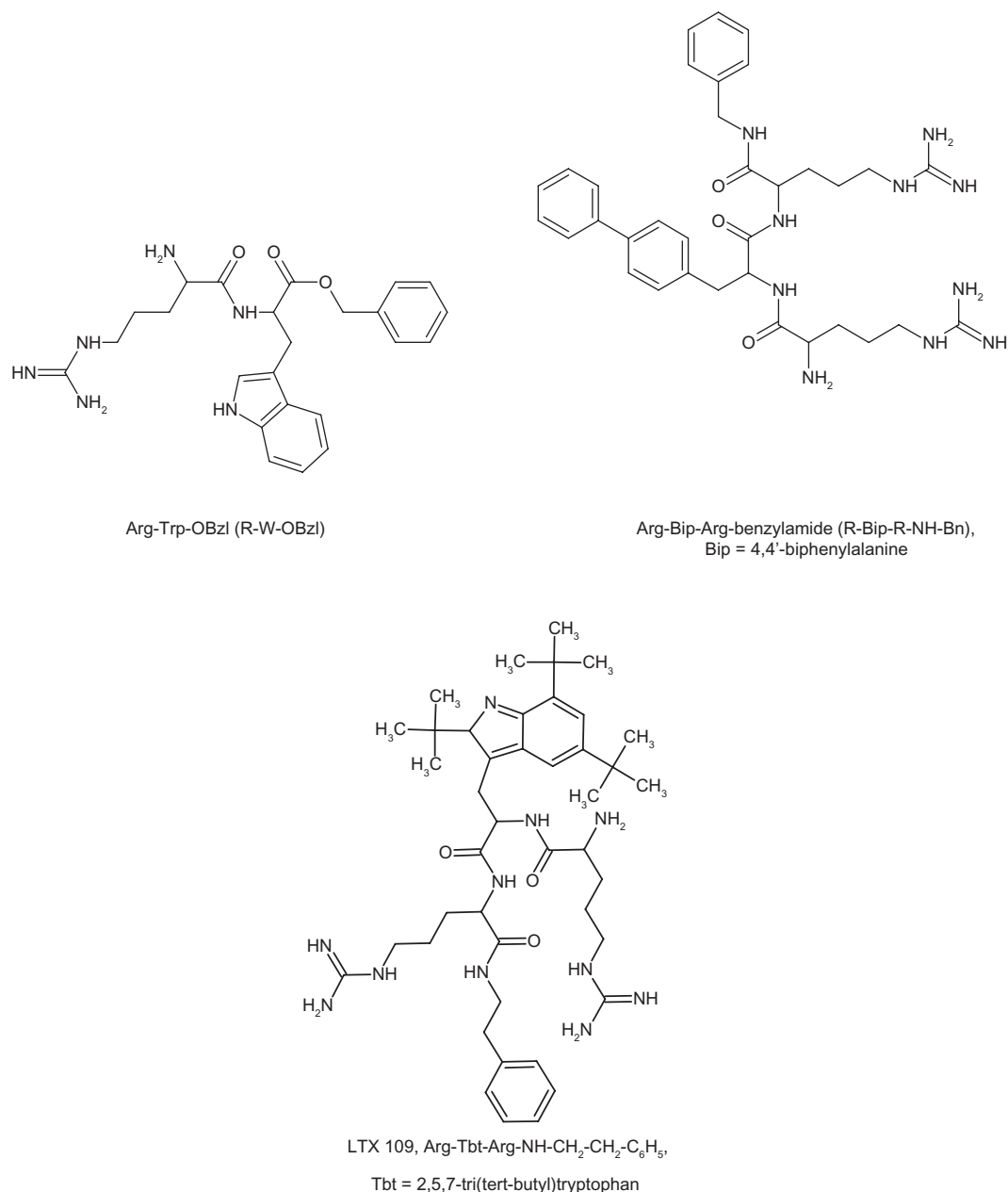
AMPs Derivatives

Another possibility to design small peptides with antimicrobial activity was using combinatorial libraries for the examination of potential short active peptide sequences.[56–58] These libraries were not only used to discover novel short AMPs[56] but also to improve the activity of already known compounds.[58] The result of such investigations was the synthesis of arginine and

tryptophan-rich hexapeptides called by the authors combi-1 (Ac-RRWWRF-NH$_2$) and combi-2 (Ac-FRWWHR-NH$_2$). These hexapeptides possess antibacterial activities similar to natural peptides.[35] Smaller AMPs like cationic containing 13 amino acid peptide amide – indolicin[59] or dodecapeptide – bactenecin[60,61] were used as a lead structure to develop potential new antimicrobial drugs, for example, omiganan (ILRWPWWPWRRK-NH$_2$) (Table 3), a synthetic cationic peptide derived from indolicin.[62,63] To obtain short cationic peptides with enhanced antimicrobial activity, fluorine atoms or trifluoromethyl groups were introduced in the structure.[64] Another reason for the structure modification of AMPs is searching for compounds with a potential for oral administration. It is connected with the problem of their stability toward the enzymatic degradation. Series of short cationic peptides (generally, tripeptides), with the presence of unnatural amino acids in the structure, were synthesized by the application of combinatorial libraries and its antibacterial activity, and their stability for tryptic[65,66] and chymotryptic[67] degradation was examined. The use of Trp and Arg analogs can minimize the interaction of antimicrobial tripeptides with binding pockets of proteolytic enzymes and increase their stability.[48] The

Table 3. Selective antimicrobial peptides in clinical trial according to Refs. 5, 92, and 93.

NAME	NUMBER OF AMINO ACIDS	DESCRIPTION	INTENDED USE	COMPANY	TRIAL PHASE
Omiganan (MBI-226, MX-226, CSL-001)	12	Synthetic analog of indolicidin	Topical antiseptic prevention of catheter infection, severe acne and rosacea	Migenix/BioWest therapeutics Cutanea Life Sciences	III/II
Pexiganan (MSI-78)	22	Synthetic analog of magainin 2	Topical antibiotic – diabetic ulcers	MacroChem	III
Iseganan (IB-367)	17	Protegrin 1 derivative	Prevention of oral mucositis	Ardea Biosciences	III
LTX-109	3	Peptidomimetic	Topical antibiotic	Lytic Biopharma	I/II
hLF1–11	11	Lactoferrin derivative	Prevention of bacteraemia and fungal infections	AM Pharma	I/II
OP-145	24	LL-37 derivative	Treatment of chronic middle ear infection	Octoplus	I/II

Arg-Trp-OBzl (R-W-OBzl)

Arg-Bip-Arg-benzylamide (R-Bip-R-NH-Bn),
Bip = 4,4'-biphenylalanine

LTX 109, Arg-Tbt-Arg-NH-CH$_2$-CH$_2$-C$_6$H$_5$,

Tbt = 2,5,7-tri(tert-butyl)tryptophan

Figure 3. Short peptides derived from lactoferrin with minimal antibacterial motif.

replacement of arginine with Agp (α-amino-3-guanidino-propionic acid) in AMP Sub-3 (NH$_2$-RRWRIVVIRVRR-CONH$_2$) protects this compound from its fast degradation in serum.[68] Also the cyclization of short tryptophan and arginine-rich AMPs generally brings about significant stabilization against serum proteases.[50]

Another Antibacterial Compounds with Peptide Structure

Antibacterial activity was also observed in the case of peptides that are not fragments or analogs of natural AMPs. Several compounds with the linear or cyclic dipeptide structure belong to this group. Antimicrobial dipeptide β-alanyl-tyrosine was isolated from insects.[69] Series of antimicrobial analogs of Trp-His

and His-Arg[70] as well as dipeptide-based amphiphiles[71] were synthesized. Antimicrobial cyclic dipeptides (diketopiperazines) were isolated from a *Bacillus* sp. strain.[72,73] Peptide dendrimers – branched polymers with peptides attached centrally to the core matrix – were developed for a number of different applications.[74] Antimicrobial activity was observed in the case of dendrimeric peptides containing a lysine core with attached two or eight copies of tetrapeptide R4 (RLYR) or octapeptide R8 (RLYRKVYG).[75] Low molecular dendrimers containing basic amino acid lysine and hydrophobic fragments also showed antibacterial activity. According to the authors,[76] such amphiphilic dendrimeric peptides can be non-sequential pharmacophores, which mimic the active conformation of linear AMPs. Lipopeptides are native antimicrobial agents produced

in bacteria and fungi. They are composed of aliphatic fatty acid attached to N-terminus of a short peptide.[74] While searching for new antibacterial compounds, a series of short lipopeptides constructed from natural L and D amino acids was synthesized.[77,78] A non-genetically coded amino acid ornithine was also used to synthesize short peptides covalently attached to fatty acids of different chain lengths.[79,80] The obtained results demonstrate a strong potential of lipopeptides as a class of novel antimicrobial therapeutics.[80]

Peptoids and Peptidomimetics

The antimicrobial β-peptides have been designed as oligomers mimicking the structure of AMP magainin.[81] They can adopt a variety of different helical conformations and are resistant toward the degradation by trypsin and chymotrypsin.[82] A series of short, highly potent β-peptidomimetics based on the pharmacophore model of short AMPs[51] were obtained by coupling of achiral lipophilic 3-amino-2,2-disubstituted propionic acid to a C-terminal L-arginine amide residue.[83]

Oligoacyllysines (OAKs) are a group of antimicrobial compounds, composed of tandem repeats of acyllysines. These simple structures were designed to mimic the primary structure and function of natural AMPs. To study the structure–activity relationships in OAKs, the library composed of 103 OAKs have been synthesized. Only few members of this library displayed the ability to inhibit bacterial growth. Antimicrobial activity was observed in the case of short (dimer) dodecanoyl-based OAK.[84] Further investigations suggested that the minimal requirements for potent and selective antibacterial activity with low hemolytic activity represent the sequence: aminolauryl-[lysyl-aminolauryl-lysyl]$_2$ (designated NC$_{12}$–2β$_{12}$).[85] Peptoids (N-substituted glycines) are mimics of α-peptides in which their side chains are attached to the backbone of N$^\alpha$ amide nitrogen instead of the C$^\alpha$-atom.[74,82,86] A series of peptoids constructed from N-alkylated glycine residues were synthesized as potential mimics of antimicrobial lipopeptides.[87] The head-to-tail macrocyclization of linear peptoids was used to enhance the antimicrobial activity of these compounds.[88] The combinations of the peptoid subunits with lysine residues were also investigated. Several such lysine–peptoid hybrids showed potent antibacterial and low hemolytic activities.[89,90] For example, N,N-disubstituted L-lysine amides were the simplest peptoids with antimicrobial activity.[91] Two positive charges were contributed by α and ε NH$_3^+$ groups of L-lysine; the hydrophobicity was brought about by a substituent of amide nitrogen: alkyl chain and bulky aromatic core.

Clinical Development

In their search for new antimicrobial therapeutics, several pharmaceutical companies have been attempting to introduce AMPs into the market.[29] Recent investigations generally focus on relatively small and cost-effective molecules that contain only the biologically active core region of the natural AMP. AMPs are in various stages of drug development, from early preclinical studies to phase III of clinical studies (Table 3). Compounds with different molecular sizes were examined, from the peptide containing 24 amino acids, OP-145,[67] to the modified tripeptide LTX-109 (Fig. 3),[55] both in phase II of clinical trial (Table 3). There has been limited success with those AMPs that were introduced into clinical trials, especially when the results were compared to conventional antibiotics.[92,93] Two indolicin-based AMPs, MBI-226 and MX-594AN, which have been developed for the treatment of catheter-related infection and acne, respectively, belong to the most advanced potential therapeutics.[23,29,62,63,92] MBI-226 (omiganan) has now completed two separate phase III clinical trials demonstrating safety and significant efficacy in decreasing catheter colonization and reducing microbiologically confirmed tunnel infections (Table 3).[93] The other one has been less successful. The AMP – pexiganan – a broad-spectrum synthetic analog of the African frog peptide magainin, developed for the treatment of diabetic foot ulcers, was denied approval for clinical use.[23,29] This compound did not demonstrate an advantage over existing fluoroquinolone therapy.[23,29,92,93] But it has recently been announced that pexiganan would re-enter phase III trials to enable the resubmission of a new drug application. It has also been reported that the second generation of AMPs based on pexiganan have been developed.[93] None of the AMPs have been granted Food and Drug Administration (FDA) approval for clinical use.[23]

Conclusions

Out of thousands of potential synthetic peptides with antimicrobial activity, only a small number have been systematically studied and tested. Many AMPs and its analogs have been synthesized. Some of them have very small molecules (di- and tripeptides) and improved stability against the enzyme digestion, so they are devoid of certain disadvantages of natural AMPs and can be more suitable for pharmaceutical applications. The field is young, and it would be premature to conclude if AMPs can be clinically used therapeutic agents. Many AMPs have demonstrated antimicrobial activity under controlled experimental conditions *in vitro*, but these results have not yet been transformed into success as therapeutics in clinical trials. Although some early preclinical studies as well as clinical trials have been encouraging, no AMP has yet been approved for clinical use. The question whether AMPs can be a new class of future drugs still remains unanswered.

Author Contributions

Conceived the concepts: KMN. Wrote the first draft of the manuscript: KMN, AM. Contributed to the writing of the manuscript: KMN, AM. Agree with manuscript results and conclusions: KMN, AM. Jointly developed the structure and arguments for the paper: KMN. Made critical revisions

and approved final version: KMN, AM. Both authors reviewed and approved of the final manuscript.

REFERENCES

1. Brown KL, Hancock REW. Cationic host defense (antimicrobial) peptides. *Curr Opin Immunol*. 2006;18:24–30.
2. Gennaro R, Zanetti M. Structural features and biological activities of the cathelicidin-derived antimicrobial peptides. *Biopolimers (Peptide Science)*. 2000;55: 31–49.
3. Bradshaw JP. Cationic antimicrobial peptides: issues for potential clinical use. *BioDrugs*. 2003;4:233–40.
4. Harris F, Dennison SR, Phoenix DA. Anionic antimicrobial peptides from eukaryotic organisms. *Curr Protein Pept Sci*. 2009;10:585–606.
5. Hancock REW, Sahl H-G. Antimicrobial and host-defense peptides as new anti-infective therapeutics strategies. *Nat Biotechnol*. 2006;24:1551–7.
6. Bechinger B, Zasloff M, Opella SJ. Structure and orientation of the antibiotic peptide magainin in membranes by solid-state nuclear magnetic resonance spectroscopy. *Protein Sci*. 1993;2:2077–84.
7. Silvestro L, Weiser JN, Axelsen PH. Antibacterial and antimembrane activities of Cecropin A in *Escherichia coli*. *Antimicrob Agents Chemother*. 2000;44:602–7.
8. Dürr UHN, Sudheendra US, Ramamoorthy A. LL-37, the only human member of the cathelicidin family of antimicrobial peptides. *Biochim Biophys Acta*. 2006;1758:1408–25.
9. Jarczak J, Kościuczuk EM, Lisowski P, et al. Defensins: natural component of human innate immunity. *Hum Immunol*. 2013;74:1069–79.
10. De Smet K, Contreras R. Human antimicrobial peptides: defensins, cathelicidins and histatins. *Biotechnol Lett*. 2005;27:1337–47.
11. Jang H, Ma B, Nussinov R. Conformational study of the protegrin-1 (PG-1) dimer interaction with lipid bilayers and its effect. *BMC Struct Biol*. 2007;7:21.
12. Lazaridis T, He Y, Prieto L. Membrane interactions and pore formation by the antimicrobial peptide protegrin. *Biophys J*. 2013;104:633–42.
13. Ladokhin AS, Selsted MS, White SH. Bilayer interactions of indolicidin, a small antimicrobial peptide rich in tryptophan, proline, and basic amino acids. *Biophys J*. 1997;72:794–805.
14. Wu M, Hancock REW. Interaction of the cyclic antimicrobial cationic peptide bactenecin with the outer and cytoplasmic membrane. *J Biol Chem*. 1999;274:29–35.
15. Zhang TN, Yang W, Liu N. Effect of loop structure of bovine lactoferricin on apoptosis in Jurkat cells. *Biometals*. 2010;23:555–61.
16. Yeaman MR, Yount NY. Mechanism of antimicrobial peptide action and resistance. *Pharmacol Rev*. 2003;55:27–55.
17. Nguyen LT, Haney E, Vogel HJ. The expanding scope of antimicrobial peptide structures and their modes of action. *Trends Biotechnol*. 2011;29:464–70.
18. Wimley WC. Describing the mechanism of antimicrobial peptide action with the interfacial activity model. *ACS Chem Biol*. 2010;5:905–17.
19. Park S-C, Park Y, Hahm K-S. The role of antimicrobial peptides in preventing multidrug-resistant bacterial infections and biofilm formation. *Int J Mol Sci*. 2011;12:5971–92.
20. Jensen H, Hamill P, Hancock REW. Peptide antimicrobial agents. *Clin Microbiol Rev*. 2006;19:491–511.
21. Langham A, Kaznessis YN. Molecular simulations of antimicrobial peptides. *Methods Mol Biol*. 2010;618:267–85.
22. Mátyus E, Kandt C, Tieleman D. Computer simulation of antimicrobial peptides. *Curr Med Chem*. 2007;14:2789–98.
23. Wang Y, Schlamadinger DE, Kim JE, McCammon JA. Comparative molecular dynamics simulations of the antimicrobial peptide CM15 in model lipid bilayers. *Biochim Biophys Acta*. 2012;1818:1402–9.
24. Phoenix DA, Dennison SR, Harris F. Antimicrobial peptides: their history, evolution, and functional promiscuity. In: *Antibacterial Peptides*. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA; 2013. doi:10.1002/9783527652853.
25. Pushpanathan M, Gunasekaran P, Rajendhran J. Antimicrobial peptides: versatile biological properties. *Int J Pept*. 2013;2013:15.
26. Nakatsuji T, Gallo RL. Antimicrobial peptides: old molecules with new ideas. *J Invest Dermatol*. 2012;132:887–95.
27. Guaní-Guerra E, Santos-Mendoza T, Lugo-Reyes SO, Terán LM. Antimicrobial peptides: general overview and clinical implications in human health and disease. *Clin Immunol*. 2010;135:1–11.
28. Lazarev VN, Govorun VM. Antimicrobial peptides and their use in medicine. *Appl Biochem Microbiol*. 2010;46:804–14.
29. Kang SJ, Kim DH, Mishig-Ochir T, Lee BJ. Antimicrobial peptides: their physicochemical properties and therapeutic application. *Arch Pharm Res*. 2012;35:409–13.
30. Seo MD, Won HS, Kim JH, Mishig-Ochir T, Lee BJ. Antimicrobial peptides for therapeutic application: a review. *Molecules*. 2012;17:12276–86.
31. Reddy KVR, Yedery RD, Aranha C. Antimicrobial peptides: premises and promises. *Int J Antimicrob Agents*. 2004;24:536–47.

32. Bahar AA, Ren D. Antimicrobial peptides. *Pharmaceuticals*. 2013;6:1543–75.
33. Giuliani A, Pirri G, Nicoletto SF. Antimicrobial peptides: an overview of a promising class of therapeutics. *Cent Eur J Biol*. 2007;2(1):1–33.
34. Brandenburg L-O, Merres J, Albrecht L-J, Varoga D, Pufe T. Antimicrobial peptides: multifunctional drugs for different applications. *Polymers*. 2012;4: 539–60.
35. Chan DI, Prenner EJ, Vogel HJ. Tryptophan- and arginine-rich antimicrobial peptides: structure and mechanism of action. *Biochim Biophys Acta*. 2006;1758:1184–202.
36. Brogden KA. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria. *Nat Rev Microbiol*. 2005;3:238–50.
37. Sun L. Peptide-based drug development. *Mod Chem Appl*. 2013;1:e103.
38. Edwards CMB, Cohen MA, Bloom SR. Peptides as a drugs. *Q J Med*. 1999;92:1–4.
39. Craig DJ, Fairlie DP, Liras S, Price D. The future of peptide-based drugs. *Chem Biol Drug Des*. 2013;81:136–47.
40. Marr AK, Gooderham WJ, Hancock REW. Antibacterial peptides for therapeutic use: obstacles and realistic look. *Curr Opin Pharmacol*. 2006;6:468–72.
41. Bellamy W, Takase M, Yamauchi K, Wakabayashi H, Kawase K, Tomita M. Identification of the bactericidal domain of lactoferrin. *Biochim Biophys Acta*. 1992;1121:130–6.
42. Tomita M, Takase M, Bellamy W, Shimamura S. A review: the active peptide of lactoferrin. *Acta Paediatr Jpn*. 1994;36:585–91.
43. Haug BE, Skar ML, Svendsen JS. Bulky aromatic amino acids increase the antibacterial activity of 15-residue bovine lactoferrin derivatives. *J Pept Sci*. 2001;7:425–32.
44. Haug BE, Strøm MB, Svendsen JSM. The medicinal chemistry of short lactoferrin-based antibacterial peptides. *Curr Med Chem*. 2007;14:1–18.
45. Vogel HJ, Schilbi DJ, Jing W, Lohmeier-Vogel EM, Epand RF, Epand RM. Towards a structure-function analysis of bovine lactoferrin and related tryptophan- and arginine-containing peptides. *Biochim Cell Biol*. 2002;80:49–63.
46. Moriaty LC, Joannou CL, van den Berg JJM, Gorinsky B, Evans RW. Factors contributing to the potency of antimicrobial cationic peptides from the N-terminal region of human lactoferrin. *FEMS Microbiol Lett*. 2004;239:295–9.
47. Nguyen LT, Schibli DJ, Vogel HJ. Structural studies and model membrane interaction of two peptides derived from bovine lactoferricin. *J Pept Sci*. 2005;11:379–89.
48. Svenson J, Vergote V, Karstad R, Burvenich C, Svendsen JS, De Spiegeleer B. Metabolic fate of lactoferricin-based antimicrobial peptides: effect of truncation and incorporation of amino acid analogs on the in vitro metabolic stability. *J Pharmacol Exp Ther*. 2010;332:1032–9.
49. Brouwer CP, Rahman M, Welling MM. Discovery and development of a synthetic peptide derived from lactoferrin for clinical use. *Peptides*. 2011;32:1953–63.
50. Nguyen LT, Chau JK, Perry NA, de Boer L, Zaat SAJ, Vogel HJ. Serum stabilities of short tryptophan- and arginine-rich antimicrobial peptide analogs. *PLoS One*. 2010;5(9):e12684.
51. Strøm MB, Haug BE, Skar ML, Stensen W, Stiberg T, Svendsen JS. The pharmacophore of short cationic antibacterial peptides. *J Med Chem*. 2003;46:1567–70.
52. Liu Z, Brady A, Young A, et al. Length effects in antimicrobial peptides of the (RW)n series. *Antimicrob Agents Chemother*. 2007;51:597–603.
53. Haug BE, Stensen W, Stiberg T, Svendsen JS. Bulky nonproteinogenic amino acids permit the design of very small and effective cationic antibacterial peptides. *J Med Chem*. 2004;47:4159–62.
54. Haug BE, Stensen W, Kalaaji M, Rekdal Ø, Svendsen JS. Synthetic antimicrobial peptidomimetics with therapeutic potential. *J Med Chem*. 2008;51:4306–14.
55. Isaksson J, Brandsdal BO, Engqvist M, Flaten GE, Svendsen JSM, Stensen W. A synthetic antimicrobial peptidomimetics (LTX 109): stereochemical impact on membrane disruption. *J Med Chem*. 2011;54:5786–95.
56. Blondelle SE, Takahashi E, Weber PA, Houghten RA. Identification of antimicrobial peptides by using combinatorial libraries made up of unnatural aminoacids. *Antimicrob Agents Chemother*. 1994;38:2280–6.
57. Blondelle SE, Takahashi E, Dingh KT, Houghten RA. The antimicrobial activity of hexapeptides derived from synthetic combinatorial libraries. *J Appl Bacterol*. 1995;78:39–46.
58. Blondelle SE, Takahashi E, Houghten RA, Pérez-Payá E. Rapid identification of compounds with enhanced antimicrobial activity by using conformationally defined combinatorial libraries. *Biochem J*. 1996;313:141–8.
59. Staubitz P, Peschel A, Nieuwenhuizen WF, et al. Structure-function relationship in the tryptophan rich, antimicrobial peptide indolicidin. *J Pept Sci*. 2001;7:552–64.
60. Hilpert K, Elliott MR, Volkmer-Engert R, et al. Sequence requirements and an optimization strategy for short antimicrobial peptides. *Chem Biol*. 2006;13:1101–7.
61. Hai Nan Y, Jacob B, Kim Y, Yub Shin S. Linear bactenecin analogs with cell selectivity and anti-endotoxic activity. *J Pept Sci*. 2012;18:740–7.
62. Melo MN, Castanho MARB. Omiganan interaction with bacterial membranes and cell wall models. Assigning a biological role to saturation. *Biochim Biophys Acta*. 2007;1768:1277–90.

63. Rubinchik E, Dugourd D, Algara T, Pasetka C, Friedland HD. Antimicrobial and antifungal activities of a novel cationic antimicrobial peptide, omiganan, in experimental skin colonisation models. *Int J Antimicrob Agents*. 2009;34:457–61.

64. Giménez D, Andreu C, del Olmo M, Varea T, Diaz D, Asensio G. The introduction of fluorine atoms or trifluoromethyl groups in short cationic peptides enhances their antimicrobial activity. *Bioorg Med Chem*. 2006;14:6971–8.

65. Svenson J, Stensen W, Brandsdal B-O, Haug BE, Monrad J, Svendsen JS. Antimicrobial peptides with stability towards tryptic degradation. *Biochemistry*. 2008;47:3777–88.

66. Karstad R, Isaksen G, Wynendaele E, et al. Targeting the S1 and S3 subsite of trypsin with unnatural cationic amino acids generates antimicrobial peptides with potential for oral administration. *J Med Chem*. 2012;55:6294–305.

67. Karstad R, Isaksen G, Brandsdal B-O, Svendsen JS, Svenson J. Unnatural amino acid side chains as S1,S1', and S2' probes yield cationic antimicrobial peptides with stability toward chymotrypic degradation. *J Med Chem*. 2010;53:5558–66.

68. Knappe D, Henklein P, Hoffman R, Hilpert K. Easy strategy to protect antimicrobial peptides from fast degradation in serum. *Antimicrob Agents Chemother*. 2010;54:4003–5.

69. Meylaers K, Cerstiaens A, Vierstraete E, et al. Antimicrobial compounds of low molecular mass are constitutively present in insects: characterization of β-alanyl-tyrosine. *Curr Pharm Des*. 2003;9:159–74.

70. Sharma RK, Reddy RP, Tegge W, Jain R. Discovery of Trp-His and His-Arg analogues as new structural classes of short antimicrobial peptides. *J Med Chem*. 2009;52:7421–31.

71. Mitra RN, Shome A, Paul P, Das PK. Antimicrobial activity, biocompatibility and hydrogelation ability of dipeptide-based amphiphiles. *Org Biomol Chem*. 2009;7:94–102.

72. Nishanth Kumar S, Mohandas C, Siji JV, Rajasekharan KN, Nambisan B. Identification of antimicrobial compound, diketopiperazines, from *Bacillus* sp. N strain associated with rhabditid entomopathogenic nematode against major plant pathogenic fungi. *J Appl Microbiol*. 2012;113:914–24.

73. Nishanth Kumar S, Dileep C, Mohandas C, Nambisan B, Jayaprakas CA. Cyclo (D-Tyr-D-Phe): a new antibacterial, anticancer, and antioxidant cyclic dipeptide from Bacillus sp.N strain associated with rhabditid entomopathogenic nematode. *J Pept Sci*. 2014;20:173–85.

74. Guliani A, Rinaldi AC. Beyond natural antimicrobial peptides: multimeric peptides and other peptidomimetic approaches. *Cell Mol Life Sci*. 2011;68:2255–66.

75. Tam JP, Lu Y-A, Yang J-L. Antimicrobial dendrimeric peptides. *Eur J Biochem*. 2002;269:923–32.

76. Janiszewska J, Urbańczyk-Lipkowska Z. Amphiphilic dendrimeric peptides as model non-sequential pharmacophores with antimicrobial properties. *J Mol Microbiol Biotechnol*. 2007;13:220–5.

77. Avrahami D, Shai Y. Bestowing antifungal and antibacterial activities by lipophilic acid conjugation to D,L amino acids-containing antimicrobial peptides: a plausible mode of action. *Biochemistry*. 2003;42:14946–56.

78. Kamysz W, Silvestri C, Cirioni O, et al. In vitro activities of the lipopeptides palmitoyl (Pal)-Lys-Lys-NH$_2$ and Pal-Lys-Lys alone and in combination with antimicrobial agents against multiresistant gram-positive Cocci. *Antimicrob Agents Chemother*. 2007;51:354–8.

79. Laverty G, McLaughlin M, Shaw C, Gorman SP, Gilmore BF. Antimicrobial activity of short, synthetic cationic lipopeptides. *Chem Biol Drug Des*. 2010;75:563–9.

80. Lohan S, Cameotra SS, Bisht GS. Systematic study of non-natural short cationic lipopeptides as novel broad-spectrum antimicrobial agents. *Chem Biol Drug Des*. 2013;82:557–66.

81. Porter EA, Wang X, Lee HS, Weisblum B, Gellman SH. Antibiotics: non-haemolytic β-amino-acid oligomers. *Nature*. 2000;404:565.

82. Godballe T, Nilsson LL, Petersen PD, Jenssen H. Antimicrobial β-peptides and α-peptoids. *Chem Biol Drug Des*. 2011;77:107–16.

83. Hansen T, Alst T, Havelkova M, Strøm MB. Antimicrobial activity of small β-peptidomimetics based on the pharmacophore model of short cationic antimicrobial peptides. *J Med Chem*. 2010;53:595–606.

84. Radzishevsky IS, Kovachi T, Porat Y, et al. Structure relationships of antibacterial acyl-lysine oligomers. *Chem Biol*. 2008;15:354–62.

85. Zaknoon F, Sarig H, Rotem S, et al. Antibacterial properties and mode of action of a short acyl-lysyl oligomer. *Antimicrob Agents Chemother*. 2009;53:3422–9.

86. Dohm MT, Kapoor R, Barron AE. Peptoids: bio-inspired polymers as potential pharmaceuticals. *Curr Pharm Des*. 2011;17(25):2732–47.

87. Chongsiriwatana NP, Miller TM, Wetzler M, et al. Short alkylated peptoid mimics of antimicrobial lipopeptides. *Antimicrob Agents Chemother*. 2011;55:417–20.

88. Huang ML, Shin SB, Benson MA, Torres VJ, Kirshenbaum K. A comparison of linear and cyclic peptoid oligomers as potent antimicrobial agents. *ChemMedChem*. 2012;7:114–22.

89. Rynge TS, Hansen PR. Novel lysine-peptoid hybrids with antibacterial properties. *J Pept Sci*. 2005;11:727–34.

90. Ryge TS, Hansen PR. Potent antibacterial lysine-peptoid hybrids identified from a positional scanning combinatorial library. *Bioorg Med Chem*. 2006;14:4444–51.

91. Ghosh C, Manjunath GB, Akkapeddi P, et al. Small molecular antibacterial peptoid mimics: the simpler the better. *J Med Chem*. 2014;57:1428–36.

92. Fjell CD, Hiss JA, Hancock REW, Schneider G. Designing antimicrobial peptides: form follows function. *Nat Rev Drug Discov*. 2012;11:37–51.

93. Afacan NJ, Yeung ATY, Pena OM, Hancock REW. Therapeutic potential of host defense peptides in antibiotic-resistant infections. *Curr Pharm Des*. 2012;18:807–19.