Published in final edited form as:

Expert Opin Drug Discov. 2018 August ; 13(8): 727–739. doi:10.1080/17460441.2018.1487950.

Developments with investigating descriptors for antimicrobial AApeptides and their derivatives

Olapeju Bolarinwa and Jianfeng Cai*

*Department of Chemistry, University of South Florida, 4202 E. Fowler Ave, Tampa, FL 33620

Abstract

Introduction: The development of multi-drug resistant strains of bacteria resulting from prolonged treatment with conventional antibiotics has necessitated the need for continuous research for better antibiotic strategies. One of these alternatives is evolutionary antimicrobial peptides also known as Host defense peptides (HDPs). HDPs are an integral part of the innate defense system in multicellular eukaryotes. Although HDPs can largely circumvent the persistent problem of antibiotic resistance due to their bacteriolytic membrane mechanism, they have some drawbacks including a low activity profile and protease instability. AApeptides have recently been introduced as a new class of peptidomimetics with resistance to proteolysis, improved activity profile, and limitless possibilities for structural diversity. Furthermore, they have shown excellent antimicrobial activity.

Areas Covered: This review updates the reader on the latest developments of antimicrobial AApeptides, the various derivatizations, and their development for antimicrobial applications. The most recent findings on the heterogeneous γ -AA backbone are also outlined.

Expert opinion: AApeptides, have found diverse applications in antimicrobial studies. AApeptides are believed to exhibit bactericidal properties by imitating the membranolytic action of HDPs. They have shown broad-spectrum antimicrobial activity and are active against medically relevant drug-resistant pathogens. AApeptides and their derivatives could gain therapeutic relevance in the design and development of antibiotic agents.

Keywords

AApeptides; Antibiotic resistance; Host-defense peptides (HDPs); antimicrobial activity; antimicrobial peptides; antimicrobial peptidomimetics

1.0: Introduction

Even though conventional antibiotics have found immense antibacterial applications, their excessive use has led to a major global health threat: antibiotic resistance. However, the

Declaration of Interest:

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer Disclosures:

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose

rising rate of untreatable infections, coupled with the dwindling efforts of pharmaceutical companies in the pursuit of the development of new anti-infectives have further contributed to this global menace [1]. Particularly, the pharmaceutical drug repositories lacked novel antibiotics against severe infections caused by multi-drug resistant bacterial strains such as Vancomycin-resistant *Enterococci* (VRE), Methicillin-resistant *Staphylococcus aureus* (MRSA), Methicillin-resistant *Staphylococcus epidermis* (MRSE) and *Clostridium difficile* [2–5]. A major reason for this dilemma is the high-risk nature of antibiotic developments due to low returns resulting from single short term prescriptions and use restrictions[6]. This urgency has necessitated the need for continuous search for potential alternatives to combat antimicrobial resistance.

Antimicrobial Peptides (AMPs), also known as Host defense peptides (HDPs) have been studied for their intriguing antimicrobial properties. HDPs are short cationic amphiphilic peptides with antimicrobial and/or immunomodulatory activities and forms an essential component of the innate immune system in multicellular organisms [7]. With broad spectrum antimicrobial activity against bacteria, fungi, protozoans, and enveloped viruses, HDPs are dispatched at the first line of the defense system for quicker intervention than the adaptive immune system[8]. HDPs show broad spectrum antimicrobial activity against bacteria, fungi, protozoans and even enveloped viruses[9]. The amino acid composition of HDPs typically ranges from 10–50 residues and are mostly basic and hydrophobic. These residues are arranged to form peptide amphiphiles that can easily interact with bacterial membranes[10]. The antimicrobial activity of HDPs is largely dependent on electrostatic interactions with the negatively charged bacterial membranes or cell walls. [10, 11]. However, the outer layers of cell membranes in eukaryotes have zwitterionic components, which make them interact poorly with HDPs [12–14]. As such, HDPs display considerable selectivity toward bacteria instead of mammalian cells. In addition to targeting bacterial membranes, HDPs could have other mechanisms of action including targeting multiple bacterial proteins and nucleic acids, and inhibition of bacterial cellular pathways and gene expression [15–17]. The diverse cellular and membrane targets of HDPs have made a widespread of bacterial resistance less possible[15].

Despite their promising therapeutic potential, the development of HDPs into antibiotics has faced enormous challenges. The problem of systemic toxicity posed by HDPs has limited their parenteral and oral application; therefore, all clinical trials to date have mostly been approved for topical applications [18,19]. Also, their inherent susceptibility to proteases has so far resulted in unfavorable pharmacokinetics. Furthermore, they exhibit moderate antimicrobial activity and their optimization is challenging. Their high production cost has also limited their derivatization potential [20,21].

These bottlenecks have fostered the development of non-natural antimicrobial peptide mimics based on naturally occurring HDPs templates. These peptidomimetic design mimics the structure and function of HDPs and incorporate unnatural amino acid residues, and backbone structure in the hope to enhance stability, improve bioavailability, and achieve similar or better antimicrobial activity [22]. Examples of antimicrobial peptidomimetics that have been previously reported include β -peptides [23–27], peptoids [28–30], arylamide

oligomers [31–34], β -turn mimetics [35,36], synthetic polymers [37–42], and others [43–46].

Previous reports have suggested that potent antimicrobial activity and great selectivity require flexible backbones that enable the alignment of side chains on opposing faces. This arrangement allows antimicrobial HDPs to adopt global amphipathic conformation upon interaction with bacteria membranes [41,47].

Relevant to the aforementioned approaches, our group has recently reported the design of a new peptidomimetic scaffold termed “AApeptides” [48,49]. AApeptides offer many advantages including convenient solid phase preparation, and limitless potential for derivatization. Importantly, this scaffold is able to project equal number of side chains as a conventional peptide of interest and it exhibits increased in-vivo stability and reduced toxicity profile compared to canonical α -helical HDPs. In the previous review articles [50–54], we have summarized the application of AApeptides and their derivatives as novel HDP-mimicking antimicrobial agents, and for other biological interventions. In this article, we present all the subclasses of antimicrobial AApeptides, including our recently published work on antimicrobial AApeptides with heterogeneous backbone. In addition, we present our perspective on the future development of this class of antimicrobial agents.

2.0: Design and Synthesis of AApeptides

To fully appreciate AApeptides, an understanding of their design is imperative. AApeptides are oligomers of N-Acylated-N-Aminoethyl amino acid and are derived from chiral peptide nucleic acid (PNA) backbones (Figure 1) [48]. They can be sub-classified into α -AApeptides and γ -AApeptides depending on the positioning of the chiral side chains. The AApeptide monomer bears the chiral side chain on the α -C or γ -C (with respect to the carbonyl group), while the other side chain is attached to the central N through acylation reactions. This feature enables each AApeptide monomeric unit to project the same number of functional group as a conventional dipeptide residue. Also, the possibility of introducing half of the side chains through common acylation reactions gives them the potential for unlimited chemical diversity.

AApeptides have found various biological applications [48,49,55–58] including antimicrobial applications [50–52] and similar to other reported peptidomimetic scaffolds, they are highly resistant to enzymatic hydrolysis by proteases [48,49].

2.1: α -AApeptides

α -AApeptides are synthesized using Fmoc-solid phase synthesis. First, Fmoc- α -AApeptides building blocks are prepared in solution by reacting Fmoc-glycine aldehyde with benzyl ester of the amino acid to form the secondary amine. This is then followed by the acylation step which allows respective carboxylic acids or acyl chlorides to introduce the same functionality borne on the chiral side chain (Figure 2a). Lastly, the benzyl protecting group is removed by hydrogenation and the building block is purified. The building blocks are coupled on solid phase to make the desired α -AApeptides sequences (Figure 2c).

2.2: γ -AApeptides

γ -AApeptides synthesis is achieved through a combination of two methods: (i) the building block method (similar to the method described above for the synthesis of α -AApeptides) [49], and (ii) the submonomeric method (Figure 2b) [59]. Each of these methods has its limitations, but a combination of both synthetic approaches has proven effective in circumventing the challenges. In the building block approach, each γ -AApeptide monomer is synthesized separately and subsequently assembled on the solid phase. Briefly, Fmoc-amino aldehyde is reacted with glycine benzyl ester in a reductive amination process. The product is then reacted with the suitable carboxylic acid in an acylation reaction, followed by hydrogenation to give the desired γ -AApeptide monomeric unit (similar to Figure 2a). Using Fmoc chemistry, this is then assembled on a Rink amide resin to produce the γ -AApeptide sequence of interest [49].

Although this method is very reliable and gives good product yield, it is not a good option for easy derivatization. In sharp contrast to this, the submonomeric approach obviates the generation of γ -AApeptides monomers composed of two pre-determined side chains (Figure 2b), and thus allowing the introduction of different functional groups. It involves several steps of monomer addition cycle (Figure 2d) and mostly gives worse yield [59,60].

3.0: Antimicrobial α -AApeptides

AApeptides have better flexibility than conventional α -peptides due to their higher dihedral angles. Their inherent stability, coupled with enormous derivatizing potentials, are desirable for the design of HDP mimics [61–63]. As a result, amphipathic AApeptides could be designed by joining AApeptide monomeric units comprising equal distribution of hydrophobic and cationic side chains. This distribution pattern encourages the adoption of global amphiphilic conformation on interacting with bacterial membranes.

Based on this notion, a diverse range of AApeptide sequences, including α -AApeptides, γ -AApeptides and their cyclic, and lipidated variants were developed and studied. In this review, we provide an overview of the antimicrobial applications of AApeptides, including our recently reported antimicrobial α /sulfono γ -AApeptide hybrids.

3.1: Linear antimicrobial α -AApeptides

We began with the design of a specific library of linear α -AApeptide sequences based on the structural motif of naturally occurring linear HDPs. Although sequences with building blocks less than five were inactive, sequence **α -1** with five amphiphilic monomers was active against *Bacillus subtilis* and *Staphylococcus epidermis* (Figure 3, Table 1). **α -2** composed of seven amphiphilic building blocks was more potent and exhibited a broad spectrum activity. In addition to these, both **α -1** and **α -2** were non-hemolytic [63]. The fluorescence microscopy and SEM studies suggested that **α -2** killed bacteria by membrane disruption. Interestingly, **β -1**, an α -peptide (Figure 3), which is made up of alternating Phe and Lys residues, showed poor antibacterial activity at the tested concentrations.

This further reveals the importance of global amphipathic structure for good antimicrobial activity. The antimicrobial activities of these α -AApeptides were clearly better than that of

Magainin 2 (a natural HDP), and **b-1**, a 14-mer α -peptide composed of similar cationic and hydrophobic groups. This study gave insight to the importance of length and amphiphilicity on antimicrobial profile, and also demonstrated the facile design and development of α -AApeptides as potential antimicrobial agents.

3.2: Lipo antimicrobial α -AApeptides

Lipopeptides, in contrast to HDPs, are produced in bacteria and fungi during their cultivation on various carbon sources, and have proven to be active against Gram-positive and Gram-negative bacteria [65]. They comprise specific lipophilic groups attached to ionic peptides, which are mostly six or seven amino acids long. The lipid moiety is mostly found at the peptide N-terminus and can be saturated or unsaturated, linear/branched [65]. These lipid tails have been previously reported to be the main feature responsible for the antibacterial activity of lipopeptide antibiotics [66]. Lipopeptide antibiotics are able to penetrate and disrupt bacterial membranes with those alkyl chains.

Motivated by the broad-spectrum activity shown by linear antimicrobial α -AApeptides, we decided to probe the effects of lipid tails on the activity of α -AApeptides. We developed an array of linear α -AApeptide sequences bearing C16 lipid tails at the N-terminus [67]. Their amphiphilicity were retained through the correct positioning of cationic, and hydrophobic side chains along the sequence. As shown in Figure 3, **α -3** with two amphiphilic building blocks, and a C16 lipid tail was active against Gram-positive bacteria, and fungus. Increasing the amphiphilic subunit by one produced **α -4** and **α -5** respectively (Figure 3). **α -5** has additional two hydrophobic α -AApeptide units on its N-terminus just before the C16 tail, and it showed better broad spectrum activity than **α -4**. We believe that the lipid tails foster stronger interaction with bacterial membranes. The Fluorescence microscopy studies revealed that the lipidated linear antimicrobial α -AApeptides also mimicked the mechanism of HDPs by disrupting bacterial membranes.

3.3: Lipo-cyclic antimicrobial α -AApeptides

Cyclic lipopeptide antibiotics include the clinically relevant and structurally diverse array of natural product compounds. They consist of peptide macrocycles that are acylated with lipid moieties. Cyclization confers these compounds with resistance to peptidases. The amphipathicity of these lipo-cyclic compounds depends on the length of the lipid chain, and mostly determines the quality of the antimicrobial profile [65]. Examples include Polymyxin B [68,69] and Daptomycin [70], which are used to treat infections caused by Gram-positive and Gram-negative bacteria respectively.

We adopted the cyclization strategy to improve upon our lipidated linear α -AApeptide sequence. We designed some lipo-cyclic α -AApeptides to examine the effects of cyclization on the antimicrobial profile of our already active lipidated linear α -AApeptide sequence. Our findings revealed that the lipo-cyclic α -AApeptide sequence that was appended with a C16 tail, **α -6**, (Figure 3) was active against Gram-positive and Gram-negative bacteria, while those bearing short alkyl chains were inactive (Table 1) [62]. This suggests that the length of the alkyl tail strongly determines the depth of penetration through the bacterial membrane. Also, the ring size is independent of the antimicrobial activity but an optimal ring size is

important for antimicrobial activity. Our findings for antimicrobial α -AApeptides corroborate the hypothesis from previous works done by other researchers that the adoption of global amphipathic conformation rather than defined secondary structures are important for antimicrobial activity [41,47]. This desirable amphiphilic motif adopted by HDP mimics is believed to be induced by the bacterial membrane.

4.0: Antimicrobial γ -AApeptides

Thrilled by the promising antimicrobial profile of α -AApeptides, we proceeded to the design of antimicrobial γ -AApeptides using the strategy discussed above for α -AApeptides since their molecular scaffold is similar. We designed and studied the antimicrobial activities of different variants including linear, lipidated, cyclic, lipo-cyclic, sulfono- γ -AApeptides, and α/γ lipidated- and α /sulfono- γ -AApeptides chimera (Figure 1).

4.1: Linear γ -AApeptides

We designed a library of linear γ -AApeptide sequences based on the template used for antimicrobial α -AApeptides and explored their antimicrobial activity. As anticipated, γ -1, (Table 2 and Figure 4) composed of seven amphiphilic γ -AApeptide monomers, showed broad-spectrum antibacterial activity [61]. γ -AApeptide sequences comprising less amphiphilic monomers showed a trend of reduced activity akin to α -AApeptides. This implies that the amount of cationic and hydrophobic residues is critical for successful bacterial membrane interaction and disruption. We also noticed that an increase in the overall hydrophobicity improved antimicrobial activity at the expense of the hemolytic and cytotoxic activities.

However, an introduction of more cationic charges could fine-tune these selectivity indices. Therefore, we envisaged that a potent and selective antimicrobial γ -AApeptides would be one with an optimized ratio of cationic and hydrophobic groups. Furthermore, γ -2, with two hydrophobic monomers and similar length to γ -1, showed broad-spectrum antibacterial activity, although, it was inactive against *Pseudomonas aeruginosa* bacteria (Table 1). Also, γ -2 was able to inhibit the growth of the antibiotic-resistant USA 100 lineage MRSA strain and *Bacillus anthracis*.

These findings indicate that antimicrobial γ -AApeptides are promising candidates for bio-defense in the near future. γ -1 and γ -2 (Figure 4) were not hemolytic as revealed by their HC50s ($\sim 300\mu\text{g/mL}$). We rationalized that bacterial membrane surface is grafted with negative charges that promotes electrostatic interactions with γ -AApeptides unlike the zwitterionic mammalian cell membranes with zero net charge. Fluorescence microscopy and drug resistance studies both demonstrated that γ -2 may exert bactericidal effects by membrane disruption similar to HDPs [61].

4.2: Antimicrobial Lipo- γ -AApeptides

Akin to lipo α -AApeptides, we conjectured that lipo γ -AApeptides may be investigated for potential antimicrobial activity. We therefore designed a focused library of lipo- γ -AApeptides. As expected, results showed that almost all the cationic lipo γ -AApeptides exhibited broad-spectrum antimicrobial activity and were also active against the fungus

Candida albicans. It is possible that the lipophilicity of the alkyl tail takes precedence over cationicity as far as membrane interaction is concerned. The lipo γ -AApeptides with longer tails were more active than those with more cationic charges. This reiterates the importance of fine-tuning the ratio of hydrophobic and cationic groups for good antimicrobial and selectivity profiles.

Lipo γ -AApeptides, γ -3 and γ -4 [71], emerged to be the most potent leads identified in our studies as they both showed broad-spectrum activities that were better than the most active γ -AApeptides, γ -2, from previous studies [61]. They both proved to be potent against Gram-negative *Pseudomonas aeruginosa* and fungus *Candida albicans* (Table 2). γ -3 and γ -4 have great structural similarity except that the alkyl chain in γ -4 is unsaturated. (Figure 4). Highly lipophilic sequences show a corresponding increase in hemolytic activity, thereby, resulting in undesirable selectivity profile. γ -4 is more potent with wider activity spectrum and less hemolysis than γ -3.

The result may suggest that bacterial membranes are more sensitive to unsaturated hydrophobic tails. Mechanistic studies suggested that lipo γ -AApeptides could compromise the integrity of the cell membrane and cause cell death just like HDPs [71].

4.3: Cyclic Antimicrobial γ -AApeptides

Motivated by the antimicrobial activity of linear γ -AApeptides, we investigated the effects of cyclization on their antimicrobial activity. Macrocyclic peptide antibiotics including gramicidin S, protegrin I, tyrocidine, and Polymyxin B are products of nature [36]. These class of peptide antibiotics generally possess semi-rigid backbones that favor the proper positioning of substituents due to reduced rotational freedom.

We predicted that amphipathic cyclic γ -AApeptides may have enhanced antimicrobial activity due to the ability of the covalent constraints to hold side chains in adequately defined positions for good interaction. On this basis, amphiphilic γ -AApeptide monomers were joined together and cyclized [72]. γ -5, in which two adjacent amphiphilic units were substituted with hydrophobic γ -AApeptide monomers, emerged to be the most potent one (Figure 4). γ -5 exhibited better antimicrobial activity against two of the most clinically relevant strains: *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*, than pexiganan and the previously reported active γ -AApeptide sequence, γ -2.

However, γ -5 is more hemolytic due to its increased hydrophobicity and enhanced rigidity. As revealed by computational studies, γ -5 adopted a global amphipathic structure with clusters of cationic and hydrophobic side chain groups on opposing faces of the ring upon interaction with bacterial membranes [72].

4.4: Lipo-cyclic antimicrobial γ -AApeptides

Cyclic lipopeptide antibiotics such as polymyxin [68,69] and daptomycin [70] have been used as the last resort options for the treatment of broad-spectrum antibacterial infections, respectively. Cyclization enhances structural rigidity, which favors the disruption of bacterial membranes, whereas introduction of alkyl tails in antimicrobial agents enhances membrane interaction.

Having had success with the combined strategies optimized for antimicrobial α -AApeptides, we sought to explore γ -AApeptides for the synergistic effects of these structural modifications on antimicrobial activity. As such, we designed a series of lipo-cyclic γ -AApeptides with up to six amphiphilic γ -AApeptide units [73]. Some of these cyclic lipo γ -AApeptides contain a hydrophobic building block with a lipid tail attached directly to the ring structure, while others majorly comprise amphiphilic γ -AApeptide units with the lipid tail appended on the monomer at the external face of the ring. In this study, γ -6, possessing a small amphipathic ring and a C16 alkyl tail anchor on a monomer outside the ring (Figure 4), showed very potent antimicrobial activity against all tested drug-resistant Gram-positive and Gram-negative strains (Table 2 and Figure 3).

In addition to having a better activity profile than Pexiganan, γ -6 also displayed superior activity to the previously reported cyclic γ -AApeptide, γ -5, which has a bigger ring size especially towards Gram-negative pathogens. Mechanistic studies as revealed by fluorescence microscopy implied that γ -6 exerts bactericidal effects by membrane disruption. Our more recent reports revealed that lipidated cyclic γ -AApeptides may prove better in the prevention of biofilms than conventional antibiotics due to the ability of their lipid tails to inhibit the growth of biofilms [74].

5.0 Helical Antimicrobial Sulfono- γ -AApeptides

One of our latest developments is the design of a new subclass of γ -AApeptides termed sulfono- γ -AApeptides. Sulfono- γ -AApeptides are oligomers of *N*-sulfono-acylated-*N*-aminoethyl amino acids derived from the γ -AApeptide scaffold [75]. This new class of unnatural foldamer has been shown to form stable helices in solution with characteristic pitches and diameters akin to an α -helix. Sulfono- γ -AApeptides are able to resist enzymatic hydrolysis and may be able to circumvent the problems faced by helical HDPs like Magainin 2 [76] for antibiotic development. Similar to γ -AApeptides, they have large chemical diversity due to the possibility to attach half of their side chains through the reaction of the γ -AA backbone with various sulfonyl chlorides [49,75]. Interestingly, Circular Dichroism studies showed that sulfono- γ -AApeptides have very high helical propensity even with a short sulfono- γ -AApeptide pentamer and 2D-NMR revealed that a typical sulfono- γ -AApeptides helix bears four side chains per turn unlike α -helix, which has 3.6 residues per turn. This unique feature favors the design of amphipathic helices with great potential for antimicrobial application.

Magainin 2, a naturally occurring HDP can adopt amphipathic helical conformation that promoted good interaction with phospholipids upon interaction with bacterial membrane[76]. Using Magainin as a template, we designed some sulfono- γ -AApeptides with distinct length, amphipathicity, and hydrophobicity to enable us understand the structure-function correlation in this new class of helical foldamer as regards their antimicrobial application [77]. In line with our expectation, γ -7 and γ -8, with the same charge distribution and hydrophobicity, showed excellent antimicrobial activity even better than Pexiganan (Figure 4). As a matter of fact, they are among the best antibacterial helical peptidomimetics reported to date.

We could also infer from the Small-angle X-ray scattering (SAXS) studies that N-terminus acetyl capping significantly enhanced the folding propensity of the sequence as seen in γ -7, whereas highly dense positive charges disrupt the helix. γ -8 is not acetylated at the N-terminus and has lower helical content than γ -7, however, it exhibits better antimicrobial activity and lower hemolytic and cytotoxic activities. This is consistent with the findings that secondary structures are not the sole factor for antimicrobial activity [41,64].

We also discovered that the antimicrobial activity of the sulfono- γ -AApeptides is dependent on their respective lengths, amphipathicity and nature of hydrophobic groups. For example, sequences with isopropyl groups were poorly active compared to γ -7 and γ -8, which had phenyl hydrophobic groups. This could be due to the lower hydrophobicity of isopropyl groups compared to the strongly hydrophobic phenyl group, which can interact better with bacterial membranes. Furthermore, mechanistic studies revealed that γ -8 was able to compromise the membrane integrity of Gram-positive and Gram-negative bacteria. Moreover, sulfono- γ -AApeptides are also resistant to degradation by proteases, and thus could be a promising candidate for new generation antimicrobial agents.

6.0: Chimeric antimicrobial AA peptides with heterogenous backbones

6.1: Antimicrobial lipo- α/γ -AApeptides

We also explored the antimicrobial effects of short α/γ -AApeptide chimeric sequences. Previous findings showed that oligomeric antibacterial peptide hybrids display good potency and selectivity [29,78], possibly due to their enhanced diversity. These short sequences comprise conventional α -peptide units and γ -AApeptide building blocks. Previous reports also suggested that peptides or peptidomimetic oligomers with lipid tails may improve antimicrobial activity due to increased lipophilicity of the sequence, which consequently facilitates good interaction with bacterial membranes [66,79].

Building upon previous studies, we designed a focused library of oligomeric α/γ -AApeptides hybrids comprising γ -AApeptide building blocks, and α -Lysine residue [80]. The γ -AApeptide units are composed of either two cationic groups or one cationic group and one hydrophobic group. Hence, they are expected to adopt global amphipathic structures akin to HDPs. One or two C16 alkyl tails were anchored to the α - or both α - and ϵ -NH₂ groups in the Lysine residue (Figure 5). To our surprise, $\alpha\gamma$ -1 showed excellent activity towards a various pathogenic strains and was superior to Pexiganan in their antimicrobial activity and toxicity (Table 3 and Figure 5). Most importantly, $\alpha\gamma$ -1 showed great selectivity towards many bacteria strains better than the lead lipo- γ -AApeptide, γ -4, which we reported previously [71].

Additionally, the antibacterial and hemolytic activities of the short oligomeric hybrids could be fine-tuned by varying the composition ratio of cationic charges and hydrophobicity. This is evidenced by $\alpha\gamma$ -2, which is composed of one more hydrophilic γ -AApeptide monomer. $\alpha\gamma$ -2 exhibited improved selectivity than $\alpha\gamma$ -1 (Table 3). Even though $\alpha\gamma$ -2 has a reduced ratio of hydrophobicity to hydrophilicity, its antibacterial activity did not diminish and no hemolysis was observed. This is in consistence to previous findings that hydrophobicity is directly related to antimicrobial activity, but at the expense of toxicity.

It is noteworthy that most hybrid oligomers appended with a C16 lipid tail showed poor antibacterial activity across the strains tested. We hypothesized that the chimeric peptides may form stable micelle structures which are difficult to dissociate upon interaction with bacterial membranes. Mechanistic studies revealed that the lipo- α / γ -AA hybrid oligomers mimic HDPs by penetrating and compromising the integrity of bacterial membranes.

6.2: Antimicrobial α /sulfonyl- γ -AApeptides

We have recently reported on the ability of sulfonyl- γ -AApeptide to adopt helical conformation in solution[75]. We examined the helical propensity of our heterogeneous backbone and the results suggested that 1:1 α /sulfonyl- γ -AA heterogeneous peptides are also able to form helices in solution[81]. It is therefore imperative to evaluate the antimicrobial activity of this newly reported heterogeneous backbone using the analogous design. Even though their helical pitch and diameter are not identical to α -peptides, it is believed that helical 1:1 α /sulfonyl- γ -AApeptide oligomers with cationic amphipathic structures could be designed through side chain manipulation. Thus, we designed some 1:1 α /sulfonyl- γ -AApeptides with different ratios of positive charges and hydrophobic groups on the heterogeneous helical backbone to enable us identify new antimicrobial agents (Figure 5) [82].

Interestingly, amphipathic 1:1 α /sulfonyl- γ -AApeptide sequence with an extra hydrophobic sulfonyl- γ -AApeptide building block (projecting two hydrophobic side chains) appended at the C-terminus displayed a greatly improved antibacterial activity, especially towards Gram-positive bacteria, MRSA and MRSE. This may imply that Gram-positive bacteria, which have one layer of plasma membranes, are more sensitive to hydrophobic interaction of antimicrobials than Gram-negative bacteria with both inner and outer membranes.

Consistent with previous reports [77], N-terminus acetyl capping greatly increased hemolytic activity of the 1:1 α /sulfonyl- γ -AApeptides oligomers [82]. Indeed, both **$\alpha\gamma$ -3** (with N-terminus acetyl capping) and **$\alpha\gamma$ -4** (no N-terminal capping) displayed outstanding broad-spectrum antimicrobial activity. (Table 3 and Figure 5). The most potent 1:1 α /sulfonyl- γ -AApeptides oligomer, **$\alpha\gamma$ -4**, exhibited better potency than Pexiganan [83,84] with no observed hemolytic activity. Fluorescence microscopy and Time kill analysis suggested that the active antimicrobial 1:1 α /sulfonyl- γ -AApeptides oligomers exhibit bactericidal mechanism of action similar to magainin.

7: Conclusion

The application of peptidomimetics to drug discovery research has witnessed great developments in the past 25 years and has expanded into a multidisciplinary research area [85]. The continuous research efforts on the development of potent peptide mimics is critical to pharmacology as they are able to circumvent the challenges limiting the therapeutic applications of HDPs [86]. Some of the remarkable breakthroughs include the antimicrobial arylamides developed by DeGrado et al. [32], which is currently in Phase III clinical trials, Lytixar TM(also known as LTX-109), a synthetic antimicrobial peptidomimetic developed by Lytix Biopharma AS currently in Phase I/II clinical trials(<http://www.lytixbiopharma.com>), Polymedix's PMX-30063 currently in Phase II clinical trials

[87], hLF1–11, an antimicrobial peptide derived from N-terminus of human lactoferrin the intravenous treatment of bacterial and fungal infections in haematopoietic stem cell transplant patients ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00509938): NCT00509938) [19], MBI-226 (Omiganan) developed by BioWest Therapeutics and Migenix proved effective against catheter colonization and infections ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00231153) identifier: NCT00231153) [18] among others.

Like a number of other peptidomimetic scaffolds, there is convincing evidence of the antimicrobial potential of AApeptides and their derivatives. AApeptides have exhibited enhanced stability, potential for chemodiversity and facile design for ease of optimization. Their ability to exert bactericidal effects by mimicking membrane permeation and disruption method of HDPs make them suitable alternatives for combating the rapidly increasing antibiotic drug resistance.

8: Expert Opinion

In order to expand the scope of antimicrobial AApeptides application, a few research opinions need to be considered. First, there is the need to investigate the effects of different functional groups on antimicrobial activity. To date, we have only studied a limited number of functional groups. The straightforward design of AApeptides allows easy chemical derivatization and this task could be carried out in the near future. We anticipate that the introduction of an array of functional groups including bulky, hydrophobic and cationic groups could improve the biological activity and selectivity of antimicrobial AApeptides. Indeed, more structure-activity relationship studies are desirable because they could provide insight into the design of antimicrobial peptidomimetic libraries comprising an array of diverse potential leads. Therefore, this may be help to resolve the problem of hemolysis and cytotoxicity of some active AApeptides. Resolving these problems would pave way for future *in vivo* application. Obviously, computer modeling will be able to provide good guidance for the rational design.

Although solution structures of sulfono- γ -AApeptides are known, there is the need to investigate more sequences with diverse functional groups in order to fully ascertain their folding propensity. The crystal structures may be more helpful in proposing and designing different derivatives. Meanwhile, since the development of antimicrobial agents against Gram-negative bacteria is exigent, there is an urgent need to direct more research focus on the development of antimicrobial AApeptides that exert potent bactericidal effects on Gram-negative bacteria.

In addition to the aforementioned, the *in vivo* efficacy of AApeptides need to be evaluated to fully understand the pharmacokinetic properties and antimicrobial mechanism of the active peptides. In general, mechanistic studies are required to assess the possibility for the induction of drug resistance by antimicrobial peptidomimetics, and the development of potential drug delivery systems for lead antimicrobial peptidomimetics. In line with this, nanoformulation strategies may be critical in enhancing delivery and stability of antimicrobial peptidomimetics. To this end, nanocarriers with inherent antimicrobial function may be used to boost the effects and functions of the antimicrobials [88,89].

Currently in-vitro and experimental animal model experiments have been designed to evaluate nanoformulations for the delivery of HDPs [89]. It may be one of the most promising strategies to advance antimicrobial AApeptides into clinical trials.

Although our preliminary studies have shown that antimicrobial activity of antimicrobial peptidomimetics is strongly correlated to increased hydrophobicity, tweaking the balance between cationic charges and overall hydrophobicity to obtain a pharmacologically safe peptidomimetic for systemic use is a daunting challenge. To date, most works including ours have reported several strategies to modulate the hydrophobicity, but these strategies have mostly led to a concomitant loss of cell selectivity, and augmented hemolytic properties. If antimicrobial AApeptides must scale through clinical trials, this hurdle has to be overcome.

In addition, rarely explored approaches including non-membrane-based mechanism of action, and immunomodulatory activities could provide alternative directions in the development of antimicrobial AApeptides. For example, antibacterial peptidomimetics targeting virulent gene expression is very scarce [16,17]. Antivirulent peptidomimetics that can potentially modulate the expression of virulence factors regulated by the accessory gene regulator (*agr*) system could be designed. This approach does not interfere with the viability of the bacteria, but it inactivates the bacteria and makes it susceptible to the host immune defense. It is not likely to find any class of antibiotic agents that can completely prevent the development of resistant strains. However, it is believed that this strategy has great potential in combating the rising menace of resistance development since it will put less pressure on bacterial populations than antibiotics.

There is reluctance of pharmaceutical companies to invest in antibiotic development. In a bid to encourage pharmaceutical companies to venture into antibiotic development, the 21st Century Cures Act was recently (2015) passed in the US to speed up the development and market availability of antibiotics especially for severe bacterial or fungal infections without compromising safety and effectiveness standards. Such regulatory incentives will make the development of novel antibiotics attractive to willing investors, since the approval of new antibiotics depends on studies in limited patient populations instead of the rigorous and full clinical trials. This may also strengthen research collaboration between the academia and the pharmaceutical industry as they work hand-in-hand to translate lead compounds from research findings to new products.

Even though the awareness of the major hurdles preventing the translation process for nonclinical potential HDP candidates into successful clinical and marketable products is overwhelming, continuous research on the design and development of new generation antimicrobial peptides and peptidomimetics, profound understanding of their action and resistance mechanisms, and enhanced delivery systems could make this feat achievable.

Acknowledgments

Funding:

The authors are supported by NIH grant 1R01GM112652-01A1.

References

1. Projan SJ. Why is big Pharma getting out of antibacterial drug discovery? *Curr Opin Microbiol* 2003;6(5):427–30. [PubMed: 14572532]
2. Endres BT, Bassères E, Alam MJ, et al. Cadazolid for the treatment of *Clostridium difficile*. *Expert Opin Investig Drugs* 2017;26(4):509–14.
3. Tomasz A Multiple-Antibiotic-Resistant Pathogenic Bacteria -- A Report on the Rockefeller University Workshop. *N Engl J Med* 1994;330(17):1247–51. [PubMed: 8139650]
4. Murray BE. Vancomycin-Resistant Enterococcal Infections. *N Engl J Med* 2000;342(10):710–21. [PubMed: 10706902]
5. Aires de Sousa M, de Lencastre H. Evolution of Sporadic Isolates of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Hospitals and Their Similarities to Isolates of Community-Acquired MRSA. *J Clin Microbiol* 2003;41(8):3806–15. [PubMed: 12904393]
6. Dodds DR. Antibiotic resistance: A current epilogue. *Biochem Pharmacol* 2017;134(Supplement C): 139–46. [PubMed: 27956111]
7. Hemshekhar M, Anaparti V, Mookherjee N. Functions of Cationic Host Defense Peptides in Immunity. *Pharmaceuticals* 2016;9(3):40.
8. Hancock REW, Haney EF, Gill EE. The immunology of host defence peptides: beyond antimicrobial activity. *Nat Rev Immunol* 2016;16:321. [PubMed: 27087664]
9. Hancock REW. Cationic peptides: effectors in innate immunity and novel antimicrobials. *Lancet Infect Dis* 2001;1(3):156–64. [PubMed: 11871492]
10. Zhang L-J, Gallo RL. Antimicrobial peptides. *Curr Biol* 2016;26(1):R14–R19. [PubMed: 26766224]
11. Lehrer RI, Lu W. α -Defensins in human innate immunity. *Immunol Rev* 2012;245(1):84–112. [PubMed: 22168415]
12. Mohanram H, Bhattacharjya S. Resurrecting Inactive Antimicrobial Peptides from the Lipopolysaccharide Trap. *Antimicrob Agents Chemother* 2014;58(4):1987–96. [PubMed: 24419338]
13. Bhattacharjya S, Ramamoorthy A. Multifunctional host defense peptides: functional and mechanistic insights from NMR structures of potent antimicrobial peptides. *FEBS J* 2009;276(22): 6465–73. [PubMed: 19817858]
14. Domadia PN, Bhunia A, Ramamoorthy A, et al. Structure, Interactions, and Antibacterial Activities of MSI-594 Derived Mutant Peptide MSI-594F5A in Lipopolysaccharide Micelles: Role of the Helical Hairpin Conformation in Outer-Membrane Permeabilization. *J Am Chem Soc* 2010;132(51):18417–28. [PubMed: 21128620]
15. Bechinger B, Gorr SU. Antimicrobial Peptides: Mechanisms of Action and Resistance. *J Dent Res* 2016;96(3):254–60. [PubMed: 27872334]
16. Welsh MA, Blackwell HE. Chemical probes of quorum sensing: from compound development to biological discovery. *FEMS Microbiol Rev* 2016;40(5):774–94. [PubMed: 27268906]
17. Karathanasi G, Bojer MS, Baldry M, et al. Linear peptidomimetics as potent antagonists of *Staphylococcus aureus* agr quorum sensing. *Sci Rep* 2018;8(1):3562. [PubMed: 29476092]
18. Fjell CD, Hiss JA, Hancock REW, et al. Designing antimicrobial peptides: form follows function. *Nat Rev Drug Discov* 2011;11:37. [PubMed: 22173434]
19. Van der Velden WJ, Van Iersel TM, Blijlevens NM, et al. Safety and tolerability of the antimicrobial peptide human lactoferrin 1–11 (hLF1–11). *BMC Med* 2009;7(1):44. [PubMed: 19735580]
20. Marr AK, Gooderham WJ, Hancock REW. Antibacterial peptides for therapeutic use: obstacles and realistic outlook. *Curr Opin Pharmacol* 2006;6(5):468–72. [PubMed: 16890021]
21. Hancock REW, Sahl H-G. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nat Biotechnol* 2006;24:1551. [PubMed: 17160061] ** A comprehensive review of host-defense peptides
22. Rinaldi AC, Scorciapino M. Antimicrobial Peptidomimetics: Reinterpreting Nature to Deliver Innovative Therapeutics. *Front Immunol* 2012;3(171).

23. Porter EA, Wang X, Lee H-S, et al. Non-haemolytic β -amino-acid oligomers. *Nature* 2000;404:565. [PubMed: 10766230]
24. Hamuro Y, Schneider JP, DeGrado WF. De Novo Design of Antibacterial β -Peptides. *J Am Chem Soc* 1999;121(51):12200–01.
25. Karlsson AJ, Pomerantz WC, Weisblum B, et al. Antifungal Activity from 14-Helical β -Peptides. *J Am Chem Soc* 2006;128(39):12630–31. [PubMed: 17002340]
26. Karlsson AJ, Pomerantz WC, Neilsen KJ, et al. Effect of Sequence and Structural Properties on 14-Helical β -Peptide Activity against *Candida albicans* Planktonic Cells and Biofilms. *ACS Chem Biol* 2009;4(7):567–79. [PubMed: 19518070]
27. Karlsson AJ, Flessner RM, Gellman SH, et al. Polyelectrolyte Multilayers Fabricated from Antifungal β -Peptides: Design of Surfaces that Exhibit Antifungal Activity Against *Candida albicans*. *Biomacromolecules* 2010;11(9):2321–28. [PubMed: 20831274]
28. Chongsiriwatana NP, Patch JA, Czyzewski AM, et al. Peptoids that mimic the structure, function, and mechanism of helical antimicrobial peptides. *Proc Natl Acad Sci U S A* 2008;105(8):2794–99. [PubMed: 18287037]
29. Olsen CA, Ziegler HL, Nielsen HM, et al. Antimicrobial, Hemolytic, and Cytotoxic Activities of β -Peptoid–Peptide Hybrid Oligomers: Improved Properties Compared to Natural AMPs. *ChemBioChem* 2010;11(10):1356–60. [PubMed: 20503219]
30. Huang ML, Shin SBY, Benson MA, et al. A Comparison of Linear and Cyclic Peptoid Oligomers as Potent Antimicrobial Agents. *ChemMedChem* 2012;7(1):114–22. [PubMed: 21990117]
31. Liu D, Choi S, Chen B, et al. Nontoxic Membrane-Active Antimicrobial Arylamide Oligomers. *Angew Chem Int Ed* 2004;43(9):1158–62.
32. Choi S, Isaacs A, Clements D, et al. De novo design and in vivo activity of conformationally restrained antimicrobial arylamide foldamers. *Proc Natl Acad Sci U S A* 2009;106(17):6968–73. [PubMed: 19359494]
33. Hua J, Yamarthy R, Felsenstein S, et al. Activity of Antimicrobial Peptide Mimetics in the Oral Cavity: I. Activity Against Biofilms of *Candida albicans*. *Mol Oral Microbiol* 2010;25(6):418–25. [PubMed: 21040515]
34. Hua J, Scott RW, Diamond G. Activity of antimicrobial peptide mimetics in the oral cavity: II. Activity against periopathogenic biofilms and anti-inflammatory activity. *Mol Oral Microbiol* 2010;25(6):426–32. [PubMed: 21040516]
35. Srinivas N, Jetter P, Ueberbacher BJ, et al. Peptidomimetic Antibiotics Target Outer-Membrane Biogenesis in *Pseudomonas aeruginosa*. *Science* 2010;327(5968):1010–13. [PubMed: 20167788]
36. Obrecht D, Robinson JA, Bernardini F, et al. Recent Progress in the Discovery of Macrocyclic Compounds as Potential Anti-Infective Therapeutics. *Curr Med Chem* 2009;16(1):42–65. [PubMed: 19149562]
37. Kuroda K, DeGrado WF. Amphiphilic Polymethacrylate Derivatives as Antimicrobial Agents. *J Am Chem Soc* 2005;127(12):4128–29. [PubMed: 15783168]
38. Ilker MF, Nüsslein K, Tew GN, et al. Tuning the Hemolytic and Antibacterial Activities of Amphiphilic Polynorbornene Derivatives. *J Am Chem Soc* 2004;126(48):15870–75. [PubMed: 15571411]
39. Gelman MA, Weisblum B, Lynn DM, et al. Biocidal Activity of Polystyrenes That Are Cationic by Virtue of Protonation. *Org Lett* 2004;6(4):557–60. [PubMed: 14961622]
40. Nimmagadda A, Liu X, Teng P, et al. Polycarbonates with Potent and Selective Antimicrobial Activity toward Gram-Positive Bacteria. *Biomacromolecules* 2017;18(1):87–95. [PubMed: 28064500]
41. Mowery BP, Lee SE, Kissounko DA, et al. Mimicry of Antimicrobial Host-Defense Peptides by Random Copolymers. *J Am Chem Soc* 2007;129(50):15474–76. [PubMed: 18034491]
42. Takahashi H, Caputo GA, Vemparala S, et al. Synthetic Random Copolymers as a Molecular Platform To Mimic Host-Defense Antimicrobial Peptides. *Bioconjug Chem* 2017;28(5):1340–50. [PubMed: 28379682]
43. Su M, Xia D, Teng P, et al. Membrane-Active Hydantoin Derivatives as Antibiotic Agents. *J Med Chem* 2017;60(20):8456–65. [PubMed: 28984451]

44. Teng P, Huo D, Nimmagadda A, et al. Small Antimicrobial Agents Based on Acylated Reduced Amide Scaffold. *J Med Chem* 2016;59(17):7877–87. [PubMed: 27526720]
45. Tang H, Doerksen RJ, Tew GN. Synthesis of urea oligomers and their antibacterial activity. *Chem Commun* 2005;(12):1537–39.
46. Tang H, Doerksen RJ, Jones TV, et al. Biomimetic Facially Amphiphilic Antibacterial Oligomers with Conformationally Stiff Backbones. *Chem Biol* 2006;13(4):427–35. [PubMed: 16632255]
47. Schmitt MA, Weisblum B, Gellman SH. Interplay among Folding, Sequence, and Lipophilicity in the Antibacterial and Hemolytic Activities of α/β -Peptides. *J Am Chem Soc* 2007;129(2):417–28. [PubMed: 17212422]
48. Hu Y, Li X, Sebt SM, et al. Design and synthesis of AApeptides: A new class of peptide mimics. *Bioorg Med Chem Lett* 2011;21(5):1469–71. [PubMed: 21292484]
49. Niu Y, Hu Y, Li X, et al. γ -AApeptides: design, synthesis and evaluation. *New J Chem* 2011;35(3):542–45.
50. Bolarinwa O, Nimmagadda A, Su M, et al. Structure and Function of AApeptides. *Biochemistry* 2017;56(3):445–57. [PubMed: 28029249]
51. Sang P, Shi Y, Teng P, et al. Antimicrobial AApeptides. *Curr Top Med Chem* 2017;17(11):1266–79. [PubMed: 27758686]
52. She F, Oyesiku O, Zhou P, et al. The development of antimicrobial γ -AApeptides. *Future Med Chem* 2016;8(10):1101–10. [PubMed: 27284624]
53. Teng P, Shi Y, Sang P, et al. γ -AApeptides as a New Class of Peptidomimetics. *Chem Eur J* 2016;22(16):5458–66. [PubMed: 26945679]
54. Shi Y, Teng P, Sang P, et al. γ -AApeptides: Design, Structure, and Applications. *Acc Chem Res* 2016;49(3):428–41. * A good introduction of background of AApeptides. [PubMed: 26900964]
55. Yang Y, Niu Y, Hong H, et al. Radiolabeled γ -AApeptides: A New Class of Tracers for Positron Emission Tomography. *Chem Commun (Camb)* 2012;48(63):7850–52. [PubMed: 22785080]
56. Niu Y, Jones AJ, Wu H, et al. γ -AApeptides bind to RNA by mimicking RNA-binding proteins. *Org Biomol Chem* 2011;9(19):10.1039/c1ob05738c.
57. Niu Y, Bai G, Wu H, et al. Cellular Translocation of a γ -AApeptide Mimetic of Tat Peptide. *Mol Pharm* 2012;9(5):1529–34. [PubMed: 22413929]
58. Bai G, Padhee S, Niu Y, et al. Cellular uptake of an α -AApeptide. *Org Biomol Chem* 2012;10(6):1149–53. [PubMed: 22193209]
59. Wu H, Amin MN, Niu Y, et al. Solid-Phase Synthesis of γ -AApeptides Using a Submonomeric Approach. *Org Lett* 2012;14(13):3446–49. [PubMed: 22731678]
60. Wu H, Teng P, Cai J. Rapid Access to Multiple Classes of Peptidomimetics from Common γ -AApeptide Building Blocks. *Eur J Org Chem* 2014;2014(8):1760–65.
61. Niu Y, Padhee S, Wu H, et al. Identification of γ -AApeptides with potent and broad-spectrum antimicrobial activity. *Chem Commun* 2011;47(44):12197–99.
62. Padhee S, Smith C, Wu H, et al. The Development of Antimicrobial α -AApeptides that Suppress Proinflammatory Immune Responses. *ChemBioChem* 2014;15(5):688–94. [PubMed: 24677440]
63. Padhee S, Hu Y, Niu Y, et al. Non-hemolytic α -AApeptides as antimicrobial peptidomimetics. *Chem Commun* 2011;47(34):9729–31.
64. Ivankin A, Livne L, Mor A, et al. Role of the conformational rigidity in de novo design of biomimetic antimicrobial compounds. *Angew Chem Int Ed* 2010;49(45):8462–65.
65. Kleijn LHJ, Martin NI. The Cyclic Lipopeptide Antibiotics. Berlin, Heidelberg: Springer Berlin Heidelberg:1–27.
66. Makovitzki A, Avrahami D, Shai Y. Ultrashort antibacterial and antifungal lipopeptides. *Proc Natl Acad Sci U S A* 2006;103(43):15997–6002. [PubMed: 17038500]
67. Hu Y, Amin MN, Padhee S, et al. Lipidated Peptidomimetics with Improved Antimicrobial Activity. *ACS Med Chem Lett* 2012;3(8):683–86. [PubMed: 24900530]
68. Yu Z, Qin W, Lin J, et al. Antibacterial Mechanisms of Polymyxin and Bacterial Resistance. *Biomed Res Int* 2015;2015:11.
69. Olaitan AO, Morand S, Rolain J-M. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. *Front Microbiol* 2014;5:643. [PubMed: 25505462]

70. Kern WV. Daptomycin: first in a new class of antibiotics for complicated skin and soft-tissue infections. *Int J Clin Pract* 2006;60(3):370–78. [PubMed: 16494659]
71. Niu Y, Padhee S, Wu H, et al. Lipo- γ -AApeptides as a New Class of Potent and Broad-Spectrum Antimicrobial Agents. *J Med Chem* 2012;55(8):4003–09. [PubMed: 22475244]
72. Wu H, Niu Y, Padhee S, et al. Design and synthesis of unprecedented cyclic γ -AApeptides for antimicrobial development. *Chem Sci* 2012;3(8):2570–75.
73. Li Y, Smith C, Wu H, et al. Lipidated cyclic γ -AApeptides display both antimicrobial and anti-inflammatory activity. *ACS Chem Biol* 2014;9(1):211–17. [PubMed: 24144063]
74. Padhee S, Li Y, Cai J. Activity of lipo-cyclic γ -AApeptides against biofilms of *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. *Bioorg Med Chem Lett* 2015; 25(12):2565–69. [PubMed: 25977094]
75. Wu H, Qiao Q, Hu Y, et al. Sulfono- γ -AApeptides as a New Class of Nonnatural Helical Foldamer. *Chem Eur J* 2015; 21(6):2501–07. [PubMed: 25504756]
76. Matsuzaki K Magainins as paradigm for the mode of action of pore forming polypeptides. *Biochim Biophys Acta Rev Biomembr* 1998;1376(3):391–400.
77. Li Y, Wu H, Teng P, et al. Helical Antimicrobial Sulfono- γ -AApeptides. *J Med Chem* 2015;58(11): 4802–11. [PubMed: 26020456]
78. Schmitt MA, Weisblum B, Gellman SH. Unexpected Relationships between Structure and Function in α,β -Peptides: Antimicrobial Foldamers with Heterogeneous Backbones. *J Am Chem Soc*;126(22):6848–49. [PubMed: 15174837]
79. Ghosh C, Manjunath GB, Akkapeddi P, et al. Small Molecular Antibacterial Peptoid Mimics: The Simpler the Better! *J Med Chem* 2014;57(4):1428–36. [PubMed: 24479371]
80. Li Y, Smith C, Wu H, et al. Short Antimicrobial Lipo- α/γ -AA Hybrid Peptides. *ChemBioChem* 2014;15(15):2275–80. [PubMed: 25169879]
81. Wu H, Qiao Q, Teng P, et al. New Class of Heterogeneous Helical Peptidomimetics. *Org Lett* 2015;17(14):3524–27. [PubMed: 26153619]
82. She F, Nimmagadda A, Teng P, et al. Helical 1:1 α /Sulfono- γ -AA Heterogeneous Peptides with Antibacterial Activity. *Biomacromolecules* 2016;17(5):1854–59. [PubMed: 27030636]
83. Ge Y, MacDonald DL, Holroyd KJ, et al. In Vitro Antibacterial Properties of Pexiganan, an Analog of Magainin. *Antimicrob Agents Chemother* 1999;43(4):782–88. [PubMed: 10103181]
84. Thaker HD, Som A, Ayaz F, et al. Synthetic Mimics of Antimicrobial Peptides with Immunomodulatory Responses. *J Am Chem Soc* 2012;134 (27):11088–91. [PubMed: 22697149]
85. Sawyer TK. 2.15 - Peptidomimetic and Nonpeptide Drug Discovery: Receptor, Protease, and Signal Transduction Therapeutic Targets A2 - Taylor John B. In: Trigg DJ, ed. *Comprehensive Medicinal Chemistry II*. Oxford: Elsevier 2007:603–47.
86. Méndez-Samperio P Peptidomimetics as a new generation of antimicrobial agents: current progress. *Infect Drug Resist* 2014;7:229–37. [PubMed: 25210467]
87. Brilacidin (PMX-30063) Antibiotic Fact Sheet, February, 2013.
88. d'Angelo I, Casciaro B, Miro A, et al. Overcoming barriers in *Pseudomonas aeruginosa* lung infections: Engineered nanoparticles for local delivery of a cationic antimicrobial peptide. *Colloids Surf B Biointerfaces* 2015;135:717–25. [PubMed: 26340361]
89. Silva JP, Gonçalves C, Costa C, et al. Delivery of LLKKK18 loaded into self-assembling hyaluronic acid nanogel for tuberculosis treatment. *J Control Release* 2016;235:112–24. [PubMed: 27261333]

8.1:**Article highlights**

- The rising rate of antibiotic resistance is a menace to global health due to unchecked use of antibiotics. Multidrug-resistant bacterial strains have caused acute infections since they have become unresponsive to most traditional antibiotics.
- Host-defense peptides (HDPs) hold promise for the development of next generation antibiotics since they can largely overcome obstacles of drug resistance, however, they have their own drawbacks that limit their clinical applications including poor bioavailability, potential immunogenicity, and high cost of production.
- AApeptides is a new peptidomimetic scaffold which have found great applications in drug discovery and medicinal chemistry as a result of the derivatization potential of their facile backbone.
- AApeptides mostly showed broad spectrum antimicrobial activity due to their intrinsic ability to adopt global amphipathic conformations upon interaction with bacterial membrane, and mimic HDP in their mechanism of action. They kill pathogenic bacteria by membrane permeation and disruption.
- Secondary structure alone does not determine antimicrobial activity. Length of peptides, nature of functional groups, and a good balance of cationic charge and hydrophobicity are all important factors for antimicrobial AApeptides design.

Figure 1.

General structures of α -peptides and AApeptide derivatives, including α -AApeptides, γ -AApeptides, sulfono- γ -AApeptides, and hybrid α/γ -AApeptides.

Figure 2.

(a) Synthesis of α -AApeptide building block; (b) Synthesis of γ -AApeptide building block; **Route 1** shows the synthesis of *N*-alloc γ -AApeptide building blocks. **Route 2** should be adopted when chiral side chain, R, is protected with acid-labile groups[60]; Alloc: allyloxycarbonyl; (c) Solid Phase Synthesis of α -AApeptides; (d) Solid Phase Synthesis of γ -AApeptides. Adapted with permission from [50] Copyright (2016) American Chemical Society.

Figure 3.

The structures of active α-AApeptides. Adapted with permission from [50] Copyright (2016) American Chemical Society.

Figure 4.
Structures of antimicrobial γ -AApeptides. Adapted with permission from [50] Copyright (2016) American Chemical Society.

Figure 5.

Representative structures of antimicrobial α/γ -AApeptides with heterogeneous backbone. Adapted with permission from [80,82] Copyright (2016) American Chemical Society and Copyright (2014) Wiley-VCH Verlag GmbH & Co. KGaA

Table 1.

Antimicrobial and hemolytic activities of α -AApeptides. Minimum inhibitory concentration (MIC) is defined as the lowest concentration that can inhibit the growth of the bacteria after 24h time period. HC_{50} is defined as the inhibitory concentration that can cause 50% hemolysis of the human red blood cells or erythrocytes (hRBCs). “-” indicates no test were carried out. Adapted with permission from [50] Copyright (2016) American Chemical Society.

Oligomers	MIC (μ g/ml)							
	Gram negative			Gram positive				
	<i>K.pneumoniae</i>	<i>E. coli</i>	<i>P.aeruginosa</i>	<i>E.faecalis</i> (VRE)	<i>B.subtilis</i>	<i>S.aureus</i> (MRSA)	<i>S.epidermidis</i> (MRSE)	Hemolysis (HC_{50})
α-1	>50	13	>50	>50	8	>50	20	>250
α-2	>50	5	>50	>50	2	>50	10	>250
α-3	>50	>50	>50	1	2	5	10	>250
α-4	>50	>50	>50	10	2	8	20	150
α-5	8	30	8	4	2	4	4	>400
α-6	5	-	10	1	-	4	1	150
Magainin 2	-	40	-	-	40	-	>50	>250
b-1	>100	>100	>100	>100	>100	>100	>100	>100

Table 2.

Antimicrobial activity of γ -AApeptides. “-” indicates no test were carried out Adapted with permission from [50] Copyright (2016) American Chemical Society

Oligomers	MIC (μ g/ml)							
	Gram-negative			Gram-positive				
	<i>K. pneumoniae</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. epidermidis</i> (MRSE)	<i>S. aureus</i> (MRSA)	<i>E. faecalis</i> (VRE)	<i>B. subtilis</i>	Hemolysis (HC ₅₀)
γ -1	≥ 50	5	≥ 50	8	15	15	3	≥ 500
γ -2	5	5	≥ 50	5	5	5	2	300
γ -3	5	5	5	4	4	5	3	≥ 500
γ -4	3	5	3	3	3	4	3	≥ 500
γ -5	8	-	8	2	1	5	1	100
γ -6	3	2	5	1	1	2	-	100
γ -7	-	4	6	2	4	2	-	75
γ -8	-	4	4	2	2	2	-	100

Table 3.

Antimicrobial and hemolytic activities of chimeric α/γ -AApeptides oligomers. “-” indicates no test were carried out. Minimum inhibitory concentration (MIC) is defined as the lowest concentration that can inhibit the growth of the bacteria after 16h time period. HC_{50} is defined as the inhibitory concentration that can cause 50% hemolysis of the human red blood cells or erythrocytes (hRBCs). Pexiganan [83,84] is included for comparison. Adapted with permission from [80,82] Copyright (2016) American Chemical Society and Copyright (2014) Wiley-VCH Verlag GmbH & Co. KGaA.

Oligomers	MIC ($\mu\text{g/ml}$)						
	Gram-negative			Gram-positive			
	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>S. epidermidis</i> (MRSE)	<i>E. faecalis</i> (VRE)	<i>S. aureus</i> (MRSA)	Hemolysis (HC_{50})
$\alpha\gamma$ -1	5	-	2	4	5	4	≥ 500
$\alpha\gamma$ -2	3	-	2	2	4	4	≥ 500
$\alpha\gamma$ -3	-	12.5–25	-	3	-	5	230
$\alpha\gamma$ -4	-	4	-	3	-	3	≥ 250
Pexiganan	-	32	-	8	-	16	-