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Structure and Function of AApeptides

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Abstract

The intrinsic drawbacks encountered in bioactive peptides in chemical biology and biomedical sciences have diverted research efforts to the development of sequence-specific peptidomimetics that are capable of mimicking the structure and function of peptides and proteins. Modifications in the backbone and/or the side chain of peptides have been explored to develop biomimetic molecular probes or drug leads for biologically important targets. To expand the family of oligomeric peptidomimetics to facilitate their further application, we recently developed a new class of peptidomimetics, AApeptides based on a chiral peptide nucleic acid backbone. AApeptides are resistant to proteolytic degradation and amenable to enormous chemical diversification. Moreover, they could mimic the primary structure of peptides and also fold into discrete secondary structure such as helices and turn-like structures. Furthermore, they have started to show promise in applications in material and biomedical sciences. Herein, we highlight the structural design and some function of AApeptides and present our perspective on their future development.

The last three decades have witnessed a blooming era of the discovery and characterization of biologically active peptides. Some of these bioactive peptides have been prepared on a large scale and evaluated pharmacologically and clinically, thereby fostering the emergence of new therapies for various disease pathologies.^{1–4} However, the development of peptides for therapeutic or biological applications faces bottlenecks, including proteolytic susceptibility, poor absorption and diffusion in certain tissue organs, and side effects due to nonspecific interaction of peptides with multiple receptors.⁵ As a result, biomedical research is constantly geared toward the improvement of peptide-based therapeutics via the

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introduction of specific and/or random structural modifications in peptides while still retaining the motifs responsible for bioactivity. These motives and requirements formed the basis for peptidomimetics, which are developed as the structural modifications of peptides and proteins but with improved stability and bioactivity.

Sequence-specific peptidomimetics could present alternative approaches to circumvent challenges in chemical biology and biomedical sciences. Biomimetic scaffolds developed in the past, including β -peptides,^{6,7} α/β -peptides,⁸ peptoids,^{9,10} azapeptides,¹¹ oligoureas,¹² aromatic oligoamides,¹³ etc., are excellent examples. Because of their unnatural backbones, they hold potential greater than that of natural peptides with regard to their resistance to enzymatic hydrolysis, improved bioavailability, and great chemodiversity. However, the need for new biomimetic scaffolds is still urgent as proteins show virtually endless structure and function. To enrich the peptidomimetic family, we have recently developed a new class of peptide mimics termed AApeptides.^{14,15} The backbones of AApeptides are derived from the chiral PNA backbone. They consist of *N*-acylated-*N*-aminoethyl amino acid units from which their name originates. They can have at least two subclasses, α -AApeptides and γ -AApeptides, based on the positions of their chiral side chains along the peptide backbone (Figure 1). In fact, half of the side chains in both α -AApeptides and γ -AApeptides are introduced through acylation. While chiral side chains are present at the γ position in γ -AApeptides, in α -AApeptides chiral side chains are linked to the α position (Figure 1).^{16–18} Because half of the side chains can be introduced through acylation of the backbone nitrogen with various carboxylic acids or acyl chlorides or even other classes of functional groups, both α -AApeptides and γ -AApeptides could have limitless potential for functional group diversification.¹⁹

AApeptides have shown excellent stability toward proteolytic hydrolysis akin to other classes of peptide mimetics,¹⁵ augmenting their potential as good candidates for biological applications. Meanwhile, the chiral chains of AApeptides could impose conformational bias on their folding conformation, which allows the rational design of AApeptides to mimic the primary and secondary structure of bioactive peptides. To date, γ -AApeptides have been shown to disrupt protein–protein interactions¹⁵ and recognize nucleic acids with specificity and affinity.²⁰ They have also been developed to mimic host-defense peptides (HDPs) to combat antibiotic resistance.^{21–23} Herein, we sketch our journey of the design, development, and application of AApeptides. We also give our perspective on the future of this class of peptidomimetics.

DESIGN OF AAPEPTIDES

Both α -AApeptides and γ -AApeptides can be prepared on the solid phase efficiently, allowing the generation of AApeptides with great diversity.

Synthesis of α -AApeptides

α -AApeptides are synthesized on the solid phase using Fmoc-protected α -AApeptide building blocks (Figure 2). The building blocks are prepared by reacting Fmoc glycine aldehyde with the benzyl ester of the amino acid to form the secondary amine, which is acylated by carboxylic acids or acyl chlorides, followed by hydrogenation to remove the

benzyl protecting group. The desired building blocks are then assembled on the solid phase to provide various α -AApeptide sequences.

Synthesis γ -AApeptides

The synthesis of γ -AApeptides is achieved through an approach combining building-block and submonomeric methods.¹⁹ The building-block approach, similar to the strategy for the synthesis of α -AApeptides (Figure 2) in which each monomeric building block was synthesized separately and eventually coupled on the solid phase, has proven to be imperfect for quick derivatization of multiple sequences despite its reliability and impressive product yields.²⁴ On the other hand, the submonomeric approach, which bypasses the need to generate γ -AApeptide monomeric building blocks, is tedious as several steps are required for each monomer addition cycle.²⁴ This diminishes the overall yield, especially in the preparation of long peptidomimetic sequences.²⁴

However, the combined synthetic approach seems to overcome the bottlenecks. This synthetic strategy requires the synthesis of just a few *N*-alloc γ -AApeptide building blocks by one of the two routes shown in Figure 3 to make γ -AApeptides with immense functional diversity. The on-resin synthesis of γ -AApeptides using *N*-alloc γ -AApeptide building blocks proves to be versatile, as *N*-alloc deprotection is highly efficient, which allows the subsequent acylation of the backbone nitrogen with a variety of groups to give the desired γ -AApeptide sequence (Figure 4).¹⁹ Overall, the combined building-block and submonomeric approach significantly shortens the number of steps and the duration of synthesis, leading to γ -AApeptides with good purity and yield. In addition, *N*-alloc building blocks have stabilities greatly enhanced compared to those of Fmoc-amino aldehydes that are the basic units utilized in the submonomeric approach. Therefore, these building blocks could be prepared in large batches for long-term usage. The versatility of this synthetic strategy has widened the scope of potential biological applications of γ -AApeptides and also facilitated the development of combinatorial libraries for ligand screening purposes.

FOLDING STRUCTURE OF γ -AAPEPTIDES

After synthetic approaches were developed for AApeptides, our focus has been shifted to their folding structures, as the structure–function relationship in biomacromolecules cannot be overemphasized. Biomimetic scaffolds with an unnatural backbone have been studied and evaluated for their folding propensities in an effort to understand and identify new functions. Therefore, we set out to study the folding propensity of γ -AApeptides. Canonical γ -AApeptides have tertiary amide bonds that foster cis–trans isomerization, which complicates solution structural elucidation by two-dimensional nuclear magnetic resonance (2D NMR). Nonetheless, this problem is diminished in a subclass of γ -AApeptides termed sulfono- γ -AApeptides (Figure 1).²⁵ In sulfono- γ -AApeptides, the tertiary amide groups are substituted with tertiary sulfonamide moieties, and as such, they still possess limitless functional diversities because of the good accessibility to a wide range of functionalized sulfonyl chlorides. Additionally, the tertiary sulfonamide groups are sufficiently bulky to bring about curvature in the sulfono- γ -AApeptide backbone, and the chiral side chains also enforce conformational bias, which synergistically induces the formation of specific secondary

structure. To date, we have investigated the folding conformations of both homogeneous sulfono- γ -AApeptides²⁵ and 1:1 α /sulfono- γ -AA heterogeneous peptide hybrids²⁶ that contain alternating α - and sulfono- γ -AApeptide residues (Figure 1). Both sulfono- γ -AApeptides and chimeric 1:1 α /sulfono- γ -AApeptide oligomers were shown to adopt helical conformations in solution by 2D NMR (Figure 5). Their helical pitches and diameters are slightly different from those of the α -helix, and their folding conformation was also corroborated by results from circular dichroism (CD) and small-angle X-ray scattering (SAXS).^{25,26} The fact that sulfono- γ -AApeptides can fold into helical structures will undoubtedly strengthen their potential in biological applications through the rational design of new helical mimetics. However, because of the short lengths of sulfono- γ -AApeptides, their solution structures are dynamic and have rather low resolution, and as a result, their precise arrangement of side chains could not yet be determined. Crystal structures are expected to provide more insight in the near future.

FUNCTION OF AAPEPTIDES

Antimicrobial Agents

While we are continuing our investigation of the secondary and tertiary structure of AApeptides, we have started to explore the application of AApeptides in biological sciences. The first area we attempted to tackle is the development of antimicrobial peptidomimetics. Antibiotic resistance has been a well-recognized public concern because of its increasing frequency over the past several decades. This has led to a meaningful demand for the development of antimicrobial peptides (AMPs) as therapeutic treatments.^{27,28} Antimicrobial peptides (AMPs), also known as host-defense peptides (HDPs), are a crucial part of the innate immune system found in a wide range of organisms, including humans. They serve as the first line of defense antibiotics that protect organisms from antimicrobial infection.^{28–33} HDPs have a unique way of interacting with the membranes of bacteria that is mostly dependent on the molecular properties of both the peptides and the composition of the lipid membranes.³⁴ Generally, HDPs have a net positive charge, which makes them selectively attracted to the negatively charged bacterial cell surfaces instead of eukaryotic cell surfaces. After association of peptides with bacterial membranes, numerous membrane flaws such as pore formation, phase separation, facilitation of nonlamellar lipid structure, or membrane bilayer disruption could be induced, thereby making them bactericidal rather than bacteriostatic as observed for conventional antibiotics.³⁵ As a result of the nonspecificity of the interactions between HDPs and the anionic components of microbial membranes, many HDPs are broad-spectrum in their antimicrobial activities against both Gram-positive and -negative bacteria and are less prone to the development of antibiotic resistance in bacteria.^{29,36} These unique features present HDPs as a preferable potential source of future antibiotics. However, HDPs have some shortcomings that may hamper their alternative use as antibiotics. These drawbacks include susceptibility to rapid degradation by proteases, poor to moderate antibacterial activity, and potential immunogenicity leading to resistance of bacteria to the body's own immune system.³⁷ The quest to overcome these challenges posed by innate HDPs has fueled the search for biomimetic oligomers over the past two decades. These oligomeric peptidomimetics such as β -peptides,^{6,38–40} peptoids,^{10,31,41–47} arylamide oligomers,⁴⁸ β -turn mimetics,^{49,50} and others¹² have been able to proffer solutions to the

problems mentioned above because of their unnatural backbone. Inspired by these findings, we have designed and investigated a few types of antimicrobial AApeptides.

Antimicrobial α -AApeptides Linear α -AApeptides—The α -AApeptides were designed on the basis of the amphipathic structural motif of host-defense peptides (HDPs) and the finding that a distinct secondary structure is not necessary for the antimicrobial activity.⁵¹ We hypothesized that α -AApeptides composed of amphiphilic building blocks are able to adopt an amphipathic conformation when interacting with bacterial membranes because of the limited flexibility of their backbone. On the basis of this hypothesis, a series of linear α -AApeptides were initially synthesized (Figure 6).⁵² Sequences consisting of either one, two, three, or four amphiphilic building blocks had no activity against Gram-positive or Gram-negative bacteria,⁵² whereas α_1 -AA and α_2 -AA, containing five and seven amphiphilic building blocks, respectively, were active against *Bacillus subtilis* and *Staphylococcus epidermidis* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria). These results suggested that longer sequences possess more potent antimicrobial activity (Table 1 and Figure 6). The antimicrobial activity of α -AApeptides was found to be superior to that of magainin II (a natural antimicrobial peptide) and a 14-mer conventional peptide bearing similar cationic and hydrophobic groups. In addition, α -AApeptides displayed remarkable selectivity. Both α_1 -AA and α_2 -AA did not show any hemolysis at a concentration of 250 μ g/mL. This early study suggested that α -AApeptides may emerge into a new class of antimicrobial peptidomimetics.

Lipidated Linear α -AApeptides—Lipopeptide antibiotics have proven to be active against both Gram-positive and Gram-negative bacteria.^{53,54} Inspired by their structural motif, we developed a focused library of lipidated α -AApeptides bearing cationic and hydrophobic side chains, as well as lipid tails.⁵⁵ Lipid tails were expected to enhance the activity as they serve as the hydrophobic entities and facilitate bacterial association and disruption. As shown in Figure 7, α_3 -AA, with two amphiphilic building blocks and a C16 lipid tail, was active against Gram-positive bacteria and fungus. The activity against both Gram-positive and Gram-negative bacteria was achieved by α_4 -AA, which contains three amphiphilic building blocks and the C16 lipid tail. The activity was further improved against both Gram-positive and Gram-negative bacteria with lipidated AApeptide α_5 -AA that contains five building blocks and a lipid tail, possibly because of its stronger interaction with bacterial membranes. The subsequent fluorescence microscopy study suggested that the lipidated α -AApeptides could mimic the mechanism of HDPs and kill the bacteria by membrane disruption.

Lipo-Cyclic α -AApeptides—Cyclic lipopeptide antibiotics such as polymyxin B⁵⁶ and daptomycin^{57,58} are drugs marketed for the treatment of infections caused by Gram-positive and Gram-negative bacteria, respectively. Inspired by the structures, we have developed a series of lipo-cyclic α -AApeptides. Cyclization reduces the sequence flexibility, while lipidation enhances bacterial membrane interaction; thus, the antibacterial potency of the sequences is expected to be improved.⁵⁹ Interestingly, the lipo-cyclic α -AApeptide with C₆ lipid tail were not active against any bacteria, whereas α_6 -AA (bearing a C16 lipid tail) was active against both Gram-positive and Gram-negative bacteria (Table 1 and Figure 8).⁵⁹

These data may suggest that a short lipid tail cannot penetrate the bacterial membranes, but as the lipid tail length increases, the depth of penetration becomes effective. In addition, the ring size did not play a determinative role in antimicrobial activity,⁶⁰ suggesting an optimal ring size might be essential for the activity.

Antimicrobial γ -AApeptides—Because our findings for antimicrobial α -AApeptides were consistent with the hypothesis from other research groups that helical and other secondary conformations are unnecessary for antimicrobial activity,^{51,61} we moved forward to develop antimicrobial γ -AApeptides using the strategy for the design of α -AApeptides.

Linear γ -AApeptides—We first studied the antimicrobial activity of linear γ -AApeptides. As expected, their antimicrobial activity data showed a predictable structure–function correlation. γ -AA1, which is the longest amphiphilic sequence ($n = 7$) (Figure 9), demonstrated potency against Gram-positive bacteria better than that of the shorter sequences ($n = 3$ or 5). This implies that a sufficient number of amphiphilic building blocks (composed of hydrophobic and cationic groups) are needed to effectively interact with and disrupt bacterial membranes. γ -AA2, which contains two hydrophobic building blocks (Figure 9), showed broad-spectrum antibacterial activity even though it was inactive against *P. aeruginosa* bacteria (Table 2).²² Additionally, γ -AA2 significantly inhibited the growth of the life-threatening *Bacillus anthracis* and the multi-drug-resistant USA 100 lineage MRSA strain that is commonly identified as the most hospital-acquired infection in the United States.²¹ Similar to that of natural HDPs, the mode of action of γ -AApeptides was through membrane disruption as revealed by fluorescence microscopy and drug resistance studies. Overall, the initial studies suggested that γ -AApeptides could be developed for antimicrobial applications. Their activity and selectivity could be adjusted by the ratio of hydrophobic and hydrophilic building blocks in the sequence.

Antimicrobial Lipo- γ -AApeptides—Lipidation of biologically active peptides has been recommended as a way to improve their tendency for membrane interaction and binding as well as biological activity.^{62,63} Lipidated peptides like polymyxin B⁶⁴ and daptomycin⁶⁵ have fatty acid tails that are important for their bactericidal activities. These aliphatic tails are believed to enhance lipophilicity, which promotes membrane interaction.^{66,67} Even though lipopeptides are active against both Gram-positive and Gram-negative bacteria and fungi, not too much attention has been paid to lipidated peptidomimetics.⁴² We speculated that like lipo- α -AApeptides, lipo- γ -AApeptides could also be designed to imitate the mode of action of HDPs because their overall structures are also cationic and globally amphipathic.²² As such, we synthesized and evaluated the antimicrobial activity of a few lipo- γ -AApeptides sequences.²² The designed lipo- γ -AApeptides include six cationic peptides with saturated alkyl tails, two alkylated anionic peptide sequences (negative controls), one cationic peptide with no alkyl group, and three cationic peptides with unsaturated alkyl tails. These designs were also aimed at structure–activity relationship studies in an effort to understand strategies for further development of antimicrobial lipo- γ -AApeptides.

In line with our expectations, results showed that virtually all the cationic lipo- γ -AApeptides displayed broad-spectrum antimicrobial activity and were even active against

the fungus *Candida albicans*. Meanwhile, it seemed that lipophilicity of the alkyl tail is more critical for membrane interaction than cationicity. The lipo- γ -AApeptide sequences with longer tails were more active than the lipo- γ -AApeptide oligomers with more cationic charges. This belabors the importance of a good balance between hydrophobicity and cationicity for good antimicrobial activity and selectivity. As shown in Figure 9, γ -AA3 and γ -AA4 are the most potent leads identified in our studies. Sequences γ -AA3 and γ -AA4 also demonstrated potency and broad-spectrum activities that were higher than those of the most active γ -AApeptides from previous studies, γ 5,²¹ most especially against Gram-negative *P. aeruginosa* and fungus *C. albicans*. Compared with γ -AA3, γ -AA4 has an unsaturated bond in its alkyl chain (Figure 9). It is known that the highly lipophilic sequences demonstrate a commensurate increase in the level of hemolysis, thereby making selectivity unfavorable. Interestingly, γ -AA4 shows better potency, a wider activity spectrum, and less hemolysis than γ -AA3 does. This may be an indication of the preference and sensitivity of bacterial membranes for unsaturated hydrophobic tails compared to mammalian cells. The hypothesis that lipo- γ -AApeptides could mimic the mechanism of HDPs was supported by fluorescence microscopy and membrane depolarization studies that suggested that lipo- γ -AApeptides kill bacteria by way of membrane disruption.²²

Cyclic Antimicrobial γ -AApeptides—Inspired by the antimicrobial activity of linear γ -AApeptides, we explored the option of macrocyclization, due to its advantages of enforcing rigidity through the introduction of covalent constraints. This constraint facilitates the proper positioning of side chain groups binding to bacterial membranes and also improves proteolytic stability and potency. Naturally occurring cyclic peptides, including tyrocidine and protegrin I, show good antimicrobial activity.⁵⁰ To ensure a global distribution of cationic and hydrophobic groups, amphiphilic γ -AApeptide building blocks were joined together and the resulting peptide amphiphiles were cyclized.⁶⁸ Results showed that cyclic γ -AApeptides with the largest ring size ($n = 6$) have better antimicrobial activity, and a structure–activity relationship study revealed γ -AA5 as the most potent one. γ -AA5 was designed by substituting two adjacent amphiphilic monomers with hydrophobic building blocks (Figure 9). γ -AA5 showed antimicrobial activity toward two of the most clinically relevant strains, *S. aureus* (MRSA) and *P. aeruginosa* (PA), better than those of Pexiganan and the linear sequence γ -AA2, and it was also active against *C. albicans*. However, in line with our expectations, it was more hemolytic because of increased hydrophobicity and enhanced rigidity. Computational studies of γ -AA5 suggested its global amphipathicity with clustering cationic and hydrophobic side chain groups at different faces of the ring.⁶⁸ We speculate that the antimicrobial profile and druggability of cyclic γ -AApeptides can be further improved through the use of various functional groups.

Lipo-Cyclic Antimicrobial γ -AApeptides—Lipidated cyclic peptides like polymyxin⁶⁴ and daptomycin⁶⁵ have been used as “last-resort” antibiotics in the treatment of infections caused by Gram-negative and Gram-positive bacteria, respectively. Cyclization imposes structural rigidity, which helps to promote the disruption of bacterial membranes, whereas lipidation in antimicrobial agents facilitates interaction with membranes. Hence, a series of lipo-cyclic γ -AApeptides composed of amphiphilic building blocks ($n = 3–6$) were designed.⁶⁹ Some of these AApeptides contain a hydrophobic building block with an

appended alkyl tail within the ring structure, while others are strictly composed of amphiphilic building blocks with the lipid tail anchored on the monomer outside the ring. γ -AA6, with a small amphipathic ring and a C16 alkyl tail (Figure 9), emerged as the most potent lipo-cyclic γ -AApeptide. γ -AA6 proved to be very potent against all tested drug-resistant Gram-positive and Gram-negative strains (Table 2). Also apart from an antimicrobial activity better than that of Pexiganan, it is also superior to the previously reported cyclic γ -AA5 with a much larger ring size, particularly against Gram-negative pathogens. Fluorescence microscopy results suggested that γ 6 kills bacteria by disrupting their membranes. In addition, our more recent findings showed that lipidated cyclic γ -AApeptides may be more effective for biofilm prevention more effective than conventional antibiotics because of their lipid tails that inhibit the growth of biofilms.⁷⁰

Antimicrobial Sulfono- γ -AApeptides—One of our most recent developments is another subclass of AApeptides, sulfono- γ -AApeptides (Figure 1). Sulfono- γ -AApeptides are oligomers of *N*-sulfono-acylated-*N*-aminoethyl amino acids.²⁵ They adopt stable helical structures in solution with a characteristic pitch and diameter similar to those of an α -helix.²⁵ This makes them a promising scaffold in mimicking the structure and function of helical peptides. In fact, sulfono- γ -AApeptides may be preferable to α -helical peptides because of their unnatural backbone, which confers upon them stability to proteolytic degradation and greater chemical diversity. Naturally occurring HDPs like magainin-2 can form an amphipathic helix in the membrane environment that facilitates effective interaction with phospholipids.⁷¹ This compelled us to study helical sulfono- γ -AApeptides for their ability to mimic magainin-2.⁷² We designed a series of sulfono- γ -AApeptides ($n = 2-8$) with distinct amphipathicity, length, and hydrophobicity to evaluate the structure–function relationship in this foldamer class for their antimicrobial application. The research results showed that sulfono- γ -AApeptides with shorter lengths were inactive, while an increasing length resulted in a noticeable improvement in antimicrobial activity. This may be due to the inability of the very short peptides to adopt amphipathic structures at bacterial membranes. γ -AA7 and γ -AA8 (Figure 9), with the same charge distribution and hydrophobicity, were the most potent antimicrobial sulfono- γ -AApeptides in the library (Table 2).^{12,45,73,74} Small-angle X-ray scattering (SAXS) showed that γ -AA8 is less helical than γ -AA7, suggesting N-terminal acetylation has significant effects on folding. Interestingly, γ -AA8 showed antimicrobial activity that was better than that of γ -AA7 and is less hemolytic and cytotoxic to mammalian cells than γ -AA7 is. This conforms to previous findings that defined secondary structures are unnecessary for potent antimicrobial activity.^{12,61,75}

AApeptides for the Modulation of p53–MDM2 Interaction

One of our long-term goals is to develop oligomeric peptidomimetics that can modulate medically relevant protein–protein interactions. The p53–MDM2 protein–protein interaction has been a testing ground for the development of modulators and therefore was chosen as the target for our study.⁷⁶ We used an enzyme-linked immunosorbent assay (ELISA) to test the ability of both α -AApeptides¹⁴ and γ -AApeptides to antagonize the p53–MDM2 interaction.¹⁵ Interestingly, both types of AApeptides are effective inhibitors of p53–MDM2 interaction. Among them, α 7-AA and γ -AA9 (Figure 10) exhibited IC₅₀s of 38 and 50 μ M, respectively, for the disruption of p53–MDM2 interaction (Table 3). Computer

modeling suggests that their side groups can mimic the side chains of Phe, Trp, and Leu residues in the helical domain of p53 and therefore prevent the binding of p53 to MDM2. Although their activities were moderate, results obtained from this study provided valuable information for the future design and development of bioactive AApeptides.

Combinatorial Library of γ -AApeptides

Combinatorial chemistry is a unique tool in drug discovery.⁷⁸ However, compared to a peptide-based combinatorial library, peptidomimetic libraries are more challenging and less common.⁷⁹ We envisioned that with the ease of synthesis and limitless chemodiversity for AApeptides, they would be excellent candidates for combinatorial screening. Thus, we set out to explore the potential of γ -AApeptides for the development of the combinatorial library.

AApeptides Targeting A β ₄₀—The production and deposition of β -amyloid peptides play a significant role in the pathogenesis of Alzheimer's disease (AD). Given that A β ₄₀ is the major form of β -amyloid peptides produced in AD pathology (~80–90%),⁸⁰ we prepared a γ -AApeptide combinatorial library consisting of 192000 compounds using a split-and-pool method.⁸¹ The library was screened against ligands targeting A β ₄₀, and one-hit **γ -AA10** (Figure 11) was identified and found to be at least 100-fold more potent than the regular peptide KLVFF (control) derived from the hydrophobic core of the A β peptide (Figure 11). Interestingly, **γ -AA10** not only could prevent the aggregation of A β ₄₀ in vitro but also showed inhibitory activity for A β aggregation in cellular assays,⁸¹ in which **γ -AA10** could restore the viability of cells by preventing A β ₄₂ aggregation and its subsequent toxicity.

AApeptides Disrupting STAT3–DNA Interaction—The one-bead–one-compound (OBOC) γ -AApeptide library approach was also applied in the identification of potential anticancer agents antagonizing STAT3-mediated cell signaling. The signal transducer and activator of transcription 3 is a transcription factor (STAT3) that is always activated in many solid tumors and hematological cancers, and its inhibition may be a viable treatment for cancer.⁸² Even though STAT3 signaling can be inhibited by blocking either STAT3 dimerization or STAT3–DNA interaction, the strategic disruption of STAT3–DNA interaction is very uncommon because of challenges posed by the rational design of agents targeting the protein–DNA interface. We prepared an OBOC library that was screened against STAT3 protein to identify ligands that potentially target STAT3–DNA binding. Eventually, four putative hits, including **γ -AA11– γ -AA14** (Figure 12), were discovered and resynthesized for biological study.⁸³ Fluorescent polarization assays were conducted to evaluate the ability of these oligomers to disrupt the binding of STAT3 to a fluorescein-labeled GpYLPQTV phosphotyrosine peptide that is known to bind the STAT3 SH2 domain. We concluded that none of these four γ -AApeptide oligomers could prevent STAT3 dimerization because no inhibitory activity was observed. However, all four γ -AApeptides effectively disrupted STAT3–DNA interaction in a STAT3–DNA binding filter assay. Furthermore, **γ -AA11– γ -AA14** were shown to suppress the expression of STAT3-regulated genes, including survivin and cyclin D1 in whole cells (Figure 13), thereby suggesting that modulators of STAT3–DNA interaction could be an alternative strategy for cancer therapy.

FUTURE PERSPECTIVES

In this review, we have highlighted the structure and applications of γ -AApeptides. Their stability, diversity, and ability to assume defined folding structures make them suitable for a variety of applications. For instance, their straightforward synthetic routes encourage the development of combinatorial libraries, from which molecular probes and potential drug leads can be identified. On the basis of the secondary structure, it is envisioned that AApeptides can be designed to mimic the protein interface and thus disrupt biologically relevant protein–protein interactions. To widen the scope of the biological applications of γ -AApeptides in the nearest future, a few aspects need to be further investigated. First, more studies of the structure of γ -AApeptides with diverse functional groups need to be performed to ascertain the folding consistency in the same class of backbones. It is believed that the adequate distribution of hydrophobic, polar, and charged groups on the peptidomimetic scaffold could improve the stability of the secondary structure. Even though solution structures of sulfono- γ -AApeptides have been obtained, it is important to determine the crystal structures to fully study and understand their folding propensities to guide the future structural design of this class of peptide mimics. Second, in vivo studies need to be performed to elucidate the biological potential of AApeptides. Meanwhile, development of antimicrobial AApeptides needs to move forward. Although many classes of peptidomimetics are potential candidates for new-generation antimicrobial agents, the lingering problem of hemolysis and cytotoxicity has limited their in vivo application. The fine-tuning of the biological activity of AApeptide derivatives as well as their hemolytic and cytotoxic activities may produce potential antimicrobial drug leads. Also, there is a demand for further investigation of active antimicrobial AApeptide mimics for the possibility of the development of drug resistance, and more research should be focused on the development of more potent antimicrobial AApeptides against the hard-to-kill Gram-negative bacteria. Furthermore, to enhance combinatorial screening, the development of a combinatorial library of cyclic γ -AApeptides would be beneficial. In line with our expectation of cyclic peptidomimetics, we hope to see new ligands with improved binding affinity and ligand specificity.

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Figure 1. General structures of α -peptides, α -AApeptides, γ -AApeptides, sulfono- γ -AApeptides, and chimeric α/γ -AApeptides.

Figure 2.
Synthesis of α -AApeptides.

Figure 3.

Synthesis of *N*-alloc γ -AApeptide building blocks is shown in route 1. Route 2 should be adopted when the chiral side chain, R, is protected with acid-labile groups.¹⁹ Alloc denotes allyloxycarbonyl.

Figure 4.
Synthetic scheme for the γ -AApeptide sequence on the solid phase.

Figure 5. Helical conformation of a representative (a) sulfono- γ -AApeptide and (b) α /sulfono- γ -AApeptide. Reproduced with permission from refs 25 and 26. Copyright 2015 Wiley and American Chemical Society, respectively.

Figure 6.
Structures of linear α -AApeptides and control peptides used in the antimicrobial studies.

Figure 7.
Structures of lipitated α -AApeptides.

Figure 8.
Structure of lipo-cyclic α -AApeptides.

Figure 9.
Structures of antimicrobial γ -AApeptides.

Figure 10. AApeptide sequences synthesized for the disruption of p53–MDM2 interaction.^{14,15}

Figure 11.

On-bead screening of the γ -AApeptide library against the A β 40 peptide. Reproduced with permission from ref 81. Copyright 2014 Royal Society of Chemistry.

Figure 12.
Chemical structures of γ -AA11– γ -AA14.

Figure 13.

DNA-STAT3 cell signaling assay, where 1–4 represent γ -AA11– γ -AA14, respectively. Reproduced with permission from ref 83. Copyright 2014 Royal Society of Chemistry.

Table 1

Antimicrobial and Hemolytic Activities of α -AApeptides^a

oligomer	MIC (μ g/mL)							
	Gram-negative				Gram-positive			
	<i>E. coli</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>B. subtilis</i>	<i>S. epidermidis</i> (MRSE)	<i>Enterococcus faecalis</i> (VRE)	<i>Staphylococcus aureus</i> (MRSA)	hemolysis (HC ₅₀)
α_1 -AA	13	>50	>50	8	20	>50	>50	>250
α_2 -AA	5	>50	>50	2	10	>50	>50	>250
α_3 -AA	>50	>50	>50	2	10	1	5	>250
α_4 -AA	>50	>50	>50	2	20	10	8	150
α_5 -AA	30	8	8	2	4	4	4	>400
α_6 -AA	–	5	10	–	1	1	4	150
magainin	40	–	–	40	>50	–	–	>250
14-mer regular peptide	>100	>100	>100	>100	>100	>100	>100	>100

^aThe minimum inhibitory concentration (MIC) is defined as the lowest concentration that can inhibit the growth of the bacteria after 24 h. HC₅₀ is defined as the inhibitory concentration that can cause 50% hemolysis of the human red blood cells or erythrocytes (hRBCs). A dash indicates no test was performed.

Table 2Antimicrobial Activities of γ -AApeptides^a

oligomer	MIC ($\mu\text{g/mL}$)							
	Gram-negative			Gram-positive				
	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>	<i>S. epidermidis</i> (MRSE)	<i>En. faecalis</i> (VRE)	<i>S. aureus</i> (MRSA)	hemolysis (HC ₅₀)
γ -AA1	5	>50	>50	3	8	15	15	>500
γ -AA2	5	5	>50	2	5	5	5	300
γ -AA3	5	5	5	3	4	5	4	>500
γ -AA4	5	3	3	3	3	4	3	>500
γ -AA5	–	8	8	1	2	5	1	100
γ -AA6	2	3	5	–	1	2	1	100
γ -AA7	4	–	6	–	2	2	4	75
γ -AA8	4	–	4	–	2	2	2	100

^a A dash indicates no test was performed.

Table 3ELISA Results for AApeptides for the Disruption of p53–MDM2 Interaction^{14,15}

AApeptide	IC ₅₀ (μM)
γ-AA9	50 ± 8
α ₇ -AA	38 ± 8
p53-derived peptide (Ac-QETFSDLWKLLP)	8.7 ⁷⁷