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Cyclic Peptides

Cyclic Peptides as Inhibitors of Amyloid Fibrillation

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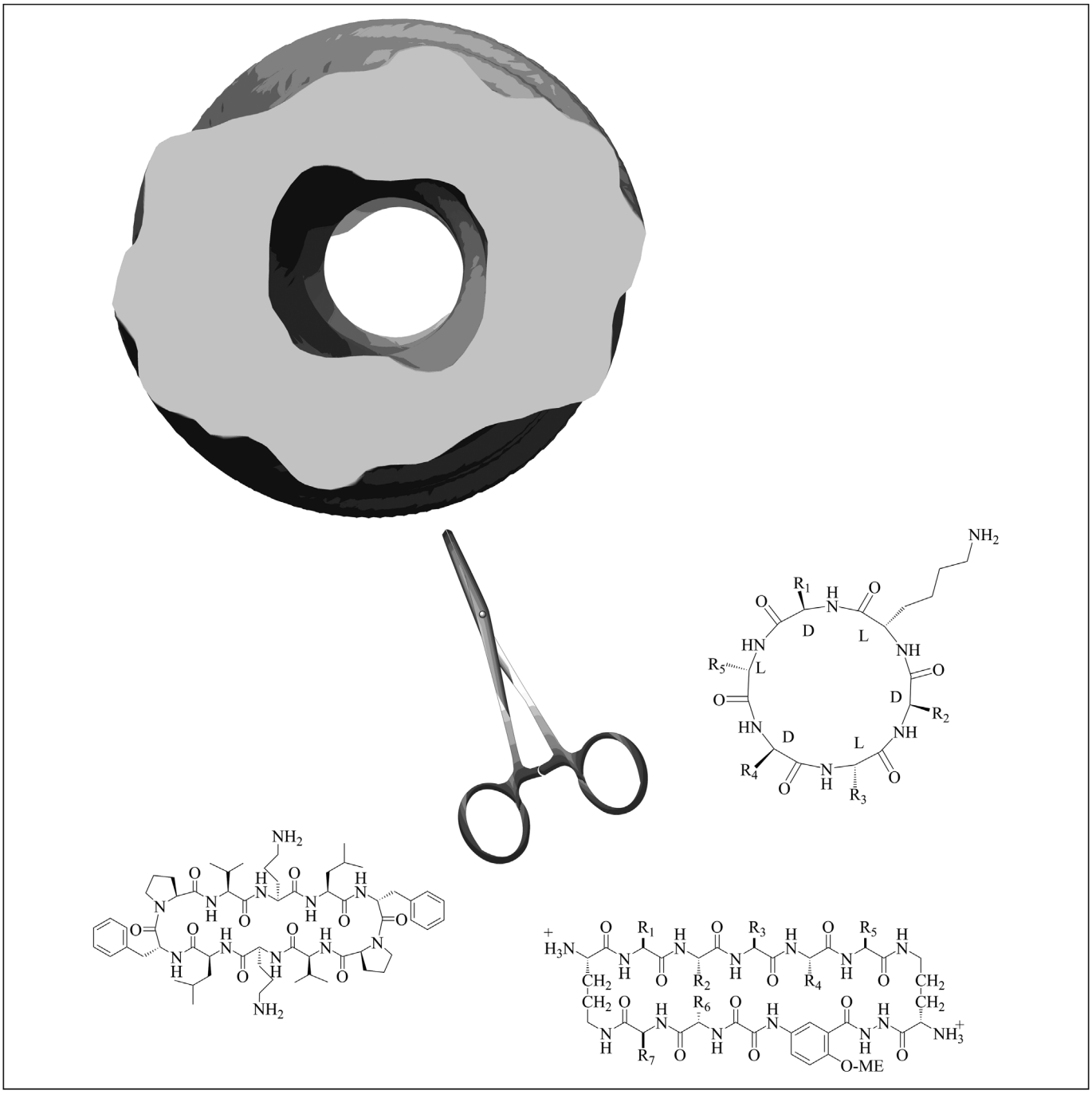

Abstract: Many neurodegenerative diseases, like Parkinson's, Alzheimer's, or Huntington's disease, occur as a result of amyloid protein fibril formation and cell death induced by this process. Cyclic peptides (CPs) and their derivatives form a new class of powerful inhibitors that prevent amyloid fibrillation and decrease the cytotoxicity of aggregates. The strategies for designing CPs are described, with respect to their amino acid sequence and/or conformational similarity to amyloid fibrils. The implications of CPs for the study and possible treatment of amyloid-related diseases are discussed.

Introduction

Many neurodegenerative diseases have been recognized as proteopathies which are caused by abnormal protein fibrillation and subsequent disruption of cells and tissues.[1–3] For instance, in Alzheimer's disease, once the tau protein starts to form fibrillary tangles, the microtubule network in neuronal cells disintegrates, and causes a collapse of the neuron's transport system.[4] In Parkinson's disease, α-synuclein aggregates into insoluble fibrils as Lewy bodies in the substantia nigra, the vagus nerve, the hypothalamus and other regions in brain, inducing the degeneration of substantia nigra and the loss of nerve cell function.[5] The accumulated aggregates of mutant huntington protein form inclusion bodies within cells and interfere with neuron function in Huntington's disease (HD).[6] It is therefore crucial to understand the formation of these aggregates and factors regulating their toxicity, as interference with this process is likely to affect the development of disease.

Structure of Amyloid and Its Precursor Aggregates

More than 30 proteins with quite different sequences and structures have been found to form amyloid fibrils. These fibrils share features both in the process of their formation and in the structure of the ultimately formed fibrils.[7] Three phases can be distinguished in amyloid fibrillation: the lag phase, the transition phase and the saturation phase (Figure 1 a). It was found that the most toxic, unstable, and heterogeneous oligomers are produced in the lag phase and that the emergence of less toxic, stable fibrils characterize the saturation phase.[8–10]

The structure of these most toxic, early aggregates has not yet been fully uncovered. It has been proposed to be similar to the cylindrin barrel of the chaperone protein crystalline, in which six antiparallel β-strands are stabilized into a β-barrel (Figure 1 b).[11] A fragment from human prion protein (DBPrP) solved by X-ray crystallography is in line with the model of cylindrin barrel. It is a β-sheet-rich assembly of hexameric oligomers in which strands are connected to each other by disulfide bridges and hydrogen bonds (Figure 1 c). In spite of differences in structure, both presumed precursors of amyloid fibrils are characterized by the β-barrel structure, and the cylindrin and DBPrP barrels consist of six and twelve strands, respectively.[12]

Amyloid fibrils are structurally different from these β-barrel oligomers. In the past decade, Eisenberg characterized the core structure of amyloid on the basis of the aggregation motifs as present in ten amyloid-forming proteins. The crystal structures of these amyloid-core variants reveal a steric zipper-in-register motif. They consist of a pair of tightly interacting β-sheets and each sheet is formed from parallel segments in a stack forming the "cross-β spine" of the fibril.[13–17] The relevance of this observation for the structure of amyloid is confirmed by the fibril structure observed by solid-state NMR spectroscopy that is characterized by a parallel, in-register β-hairpin arrangement (Figure 1 d).[18] Cryo-EM and molecular dynamic simulation confirm the interprotofilament interactions and the hollow core of the fibril (Figure 1 e, f).[19,20]

Toxicity of Amyloid and Its Precursor Aggregates

Soluble oligomers contribute to, or are responsible for, amyloid cytotoxicity resulting in synaptic dysfunction. Conversely, large, insoluble, stable fibrils might function as sources of the toxic oligomers.[21] It has been reported that Aβ(1–42) oligomers reduce neuronal viability 40-fold more than unaggregated peptide whereas for fibrils only a fourfold increase was reported, compared to the unaggregated peptide. However, Aβ(1–42) fibrils of the Dutch (E22Q) or Arctic (E22G) mutants result in a stronger reduction of neuronal viability compared to wild-type fibrils.[10] When soluble oligomers of Aβ(1–42) are prepared exogenously and introduced into neuron and muscle cells, this leads to a reduction in synaptic responses. If Aβ(1–42) aggregates that mainly include fibrils are introduced, an opposite effect occurs: synaptic transmission is enhanced and long-term depression[1] is observed.[22] Apparently, amyloid oligomers are much more harmful than amyloid fibrils. This view has been modified by recent reports that show that accumulated fibrils in low concentration efficiently promote the nucleation of toxic oligomeric species from monomers.[23] Hence, it remains vital to develop inhibitors not only for reducing the toxicity of oligomers but also for preventing formation of fibrils that catalyze the nucleation of the toxic oligomers.

Strategies for Interfering with Amyloid Aggregation

A number of small molecules have been found to prevent amyloid fibrillogenesis. Several classes can be distinguished according to their interfering mechanisms:[24] native-structure stabilizers, monomer sequesterers, protein-aggregation inhibitors,

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[1] *Activity-dependent reduction in the efficacy of neuronal synapses lasting hours or longer following a long-patterned stimulus.*

antibody-mediated inhibitors, molecular chaperones and peptide mimics, native-structure stabilizers, such as ionic liquids (choline dihydrogenphosphate), which enhance thermal stability of the native structure and thereby prevent the unfolding of protein.[25] Protein-aggregation inhibitors, such as thioflavin T (ThT) and curcumin, inhibit oligomerization but do not inhibit fibrillization of Aβ(1–42). On the other hand, lacmoid and phenol red inhibit both Aβ(1–42) oligomerization and fibrillation, whereas Orange G only inhibits fibrillation of Aβ(1–42).[26] The crystal structure of the amyloid fragment (KLVFFA) in complex with Orange G reveals that the latter binds to the hydrophobic core of KLVFFA and forms a π-stacking interaction with phenylalanine.[27]

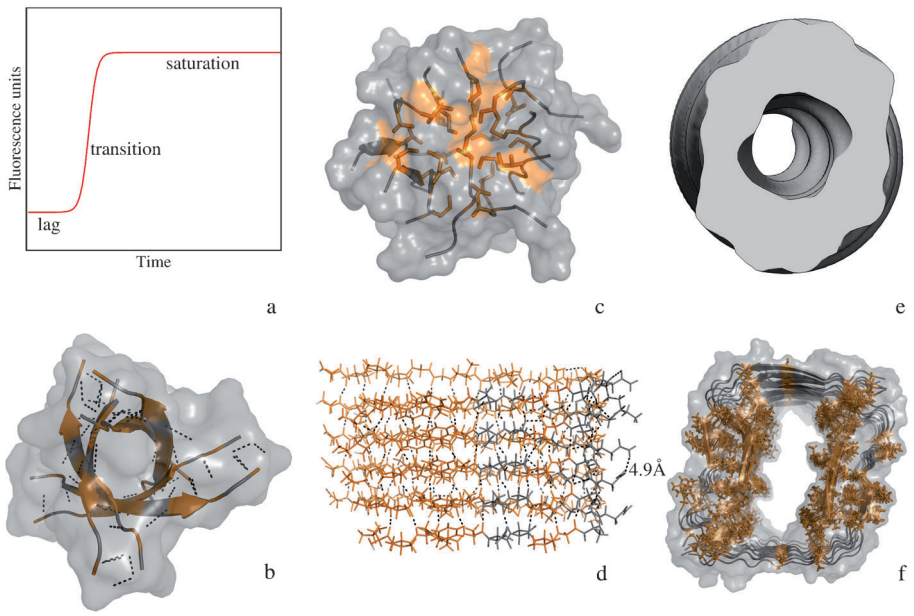

Figure 1. Dynamics of amyloid formation and structure of amyloid fibril and its precursors. a) ThT fluorescence kinetics of amyloid fibrillation. The atomic models of b) α-crystallin B and c) prion fragment oligomers. Hydrophobic and hydrophilic residues are labeled with orange and gray colors, respectively. d) Amyloid fibril hairpin structure determined by solid-state NMR spectroscopy. Dotted lines are hydrogen bonds. The atomic model of Aβ peptide (1–42) fibril built from e) cryo-EM and f) molecular dynamics simulation.

Despite a number of reports on the inhibition of protein aggregation by small inhibitors or ionic liquids, the inhibition mechanism is still under debate and small inhibitors show a broad spectrum against aggregation of many proteins. Their lack of specificity poses practical problems in their application.

However, there are also more specific inhibitors of amyloid formation. For instance, chemically engineered affibody molecules were reported to sequester the aggregation-prone Aβ regions by hydrophobic interaction.[28] Indeed, immunotherapy is one of most promising methods, as three species of antibodies have been identified to bind to monomeric Aβ, oligomeric Aβ and fibrillar Aβ.[29–32] In vivo, chaperones, but also proteins not only usually recognized for their chaperone activity (for instance, HSP90, clusterin, lysozyme) play an important role in regulating amyloid fibrillation in a proteostasis network.[33–36]

In a separate development, β-sheet breaking peptides have been designed that target the central hydrophobic core of amyloid fibrils. Hydrophobic fragments, such as KLVFF and LVFFA, which were selected from a screen of 31 decapeptide fragments of the Aβ40 sequence, were reported to be highly efficient in binding to Aβ40.[37,38] By analyzing the peptide–peptide interaction of the crystal structure of VQIYVK with the Rosetta software, Eisenberg generated effective inhibitors (d-TLKIVW peptide) of the fibrillation of the tau protein core fragment.[39] In summary, developing specific amyloid inhibitors is an important new trend for combating amyloid-related diseases.

Cyclic Peptides as Competitive Inhibitors of Fibrillation

Cyclic peptides (CPs) have been developed as a new class of amyloid inhibitor over the past five years. They have been shown to be powerful and specific inhibitors of amyloid formation. CPs are metabolized more slowly than their noncyclic equivalents because of their resistance to enzymatic degradation. Many natural compounds, such antibiotics, toxins and hormones are cyclic peptides. However, CPs can self-assemble as a result of hydrophobic interactions, which decreases their inhibition efficiency (Figure 2). It has been shown that sequence and conformational mimics are good starting points for the design of specific inhibitory CPs against amyloid formation.[40–42] However, new technologies based on chemical genetics have also been used for screening CPs that reduce toxicity of α-synuclein in a yeast system.[43] We have engineered and optimized Gramicidin S analogues (primarily known for their antibiotic activity) as inhibitors of amyloid β peptide fibrillation, making use of structure–activity relationships.

Here we review the general concepts of CP fibrillation inhibitors (CPFIs) in the prevention of amyloid formation and discuss how CPs inhibit the formation of fibrils and reduce the toxicity of amyloid aggregates.

Strategies for Designing CPFIs

Sequence mimics

The tau protein-derived ac-peptide VQIVYK (AcPHF6) aggregates into paired helical filaments that have a similar morphol-

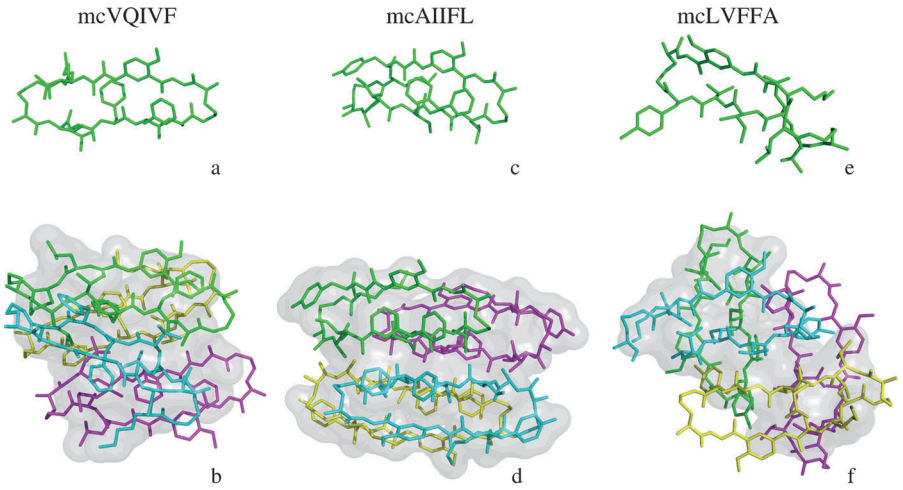

Figure 2. Crystal structures of cyclic peptides derived from amyloid proteins (a, c, e) and oligomeric assemblies of cyclic peptide monomers (b, d, f). The figure was generated with PyMOL (PDB IDs: 3Q9H, 3Q9J and 3Q9G).

ogy to tau-formed neurofibrillary tangles.[44] The AcPHF6 crystal structure shows that VQIVYK adopts an in-register parallel β-sheet conformation (Figure 3).[13] Zheng et al. used the structure of AcPHF6 to design a series of macrocyclic β-sheet peptides containing an artificial amino acid called "Hao", to interfere with the formation of the in-register parallel β-sheet.[45] The macrocyclic β-peptide has two parts: a recognition pentapeptide mimicking the tau-aggregation motif, through which it latches onto growing fibrils, and a β-sheet blocking motif containing the Hao amino acid that prevents further growth of the fibril. The functional groups are connected to each other by two δ-linked ornithine turn units (Figure 4a). It was reported that AcPHF6 blocks fibril growth in a specific direction. These CPFIs inhibit the fibrillation of AcPHF6 by capping the leading front edge of the β-sheet and interfering with further growth by displaying a different configuration of hydrophobic groups and donors/acceptors of hydrogen bonds at the capped leading end.

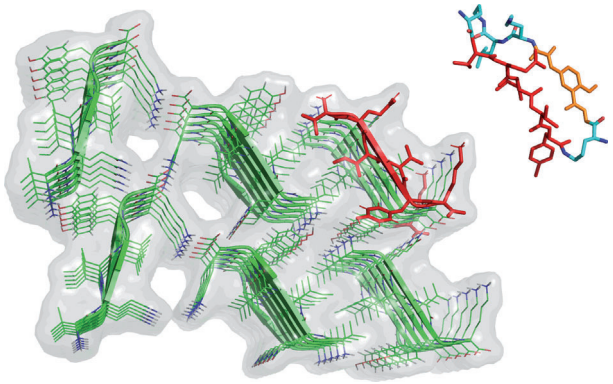

Figure 3. Crystal structure of the tau fragment VQIVYK (PDB ID: 2ON9) and crystal structure of the corresponding cyclic peptide mcVQIVY (PDB ID: 3Q9G). The residues at the recognition site are in red, the HAO blocker is in orange.

Two different pentapeptides were incorporated into the recognizing part of the CPFI: VQIVY-K6L7 and QIVYK-L6K7. At equal molar ratio, the fibrillation of AcPHF6 was completely suppressed only by VQIVY-K6L7. However, the inhibition of AcPHF6 fibrillation by VQIVY-R6L7 was greatly diminished if the hydrophobic side chain of L7 was replaced by a hydrophilic side-chain. Inversing the stereochemistry from L- to D-amino acids at R^6 and R^7 showed a more pronounced inhibitory effect in VQIVY-L6K7 than in VQIVY-K6L7. This confirmed the importance of facial hydrophobicity of the engineered CPFIs for the recognition of the AcPHF6 parallel β-sheet at the growing fibril edge.[45]

Zheng et al. also examined the roles of the other side chains of the macrocyclic template. Reducing the hydrophobicity of R^1, R^3 and R^7 reduces the inhibition of AcPHF6 fibrillation. Although the hydrophobicity in R^5 could be reduced without penalty, enhancing hydrophobicity at this position clearly increases the inhibition efficiency against AcPHF6 fibrillation.[40] The hydrophobic surface of the macrocyclic peptide to which residues R^1, R^3 and R^7 contribute, is crucial for inhibitory activity.

Cheng et al. investigated a robust amyloid β-sheet mimic (ABSM) as a platform for engineering fibrillation inhibitors of full length amyloid proteins (Figure 4b). ABSM not only is capable of antagonizing the aggregation of amyloid proteins, amyloid β peptide, β2-microglobulin, α-synuclein, islet amyloid polypeptide, prion and tau proteins, but also reduces the toxicity of amyloid aggregates. Importantly, ABSM delays the aggregation of Aβ peptides already at very low, substoichiometric concentrations, even at molar ratios of 0.05.[41] This inhibition efficiency is much higher than the linear fragment derived from the Aβ peptide.[46] ABSM is assumed to bind to early β strands of oligomers and block further nucleation towards the formation of fibrils. The recognition between ABSM and amyloid β oligomer most likely induces the formation of Aβ–ABSM complexes. This interaction between ABSM and full-length amyloid β peptide is likely to share important characteristics with the tau-derived macrocyclic peptide inhibitory mechanism, namely, face-to-face hydrophobic interaction and edge-to-edge hydrogen bonds between ABSM and the Aβ peptide.

Furthermore, Cheng et al. engineered a series of heterodivalent-linked macrocyclic peptides (Figure 4e–g) based on a monovalent template (Figure 4d). The N- and C-terminal regions of Aβ were merged as shown in Figure 4c and d. Two different sites, I and II, in the above macrocyclic β-sheet components were connected to generate heterodivalent or homodivalent

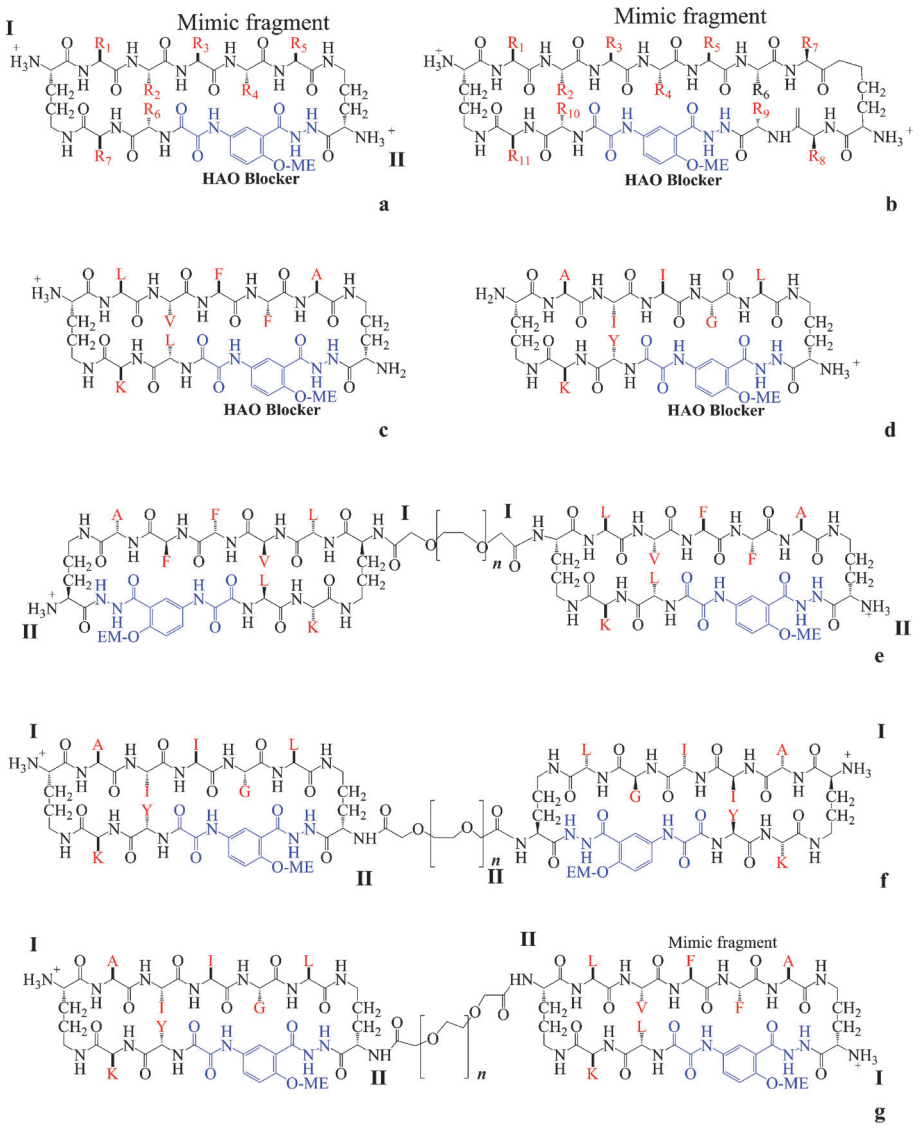

Figure 4. Amyloid mimics. a,b) Amyloid β-sheet mimics (a: macrocycle 1; b: macrocycle 2) consist of a mimic fragment and an HAO Blocker. c,d) Two components of macrocyclic β-sheet mimics with a sequence derived from the Aβ fragment. e–g) Three heterodivalent-linked macrocyclic β-sheet mimics based on c and d. The most active inhibitor is shown in panel g.

derived from ApoC-II is formed by disulfide cross linking of cysteine residues. Cyc[60–70] has been reported to inhibit the fibril formation of ApoC-II peptide. Cyc[60–70] does not self-assemble and delays the fibrillation of the linear peptides ApoC-II[60–70] and ApoC-II[56–76]. The inhibition activity of Cyc[60–70] could be decreased by the reduction of cysteine disulfide bonds or by mutating the amino acid sequence. NMR spectroscopy and molecular dynamics simulation reveal that Cyc[60–70] is very flexible and contains two faces: a hydrophobic surface and a hydrophilic surface. A mechanism was proposed in which the flexible hydrophobic and hydrophilic surfaces of the CPs interact transiently with the fibrillogenic peptide.[49,50] Although the detailed inhibition mechanism of fibrillation of linear peptide Aβ(1–28) by cyclo(17,21)-[Lys17,Asp21] remains to be investigated, we assume that cyclic hydrophobic groups and linear hydrophilic groups in cyclo(17,21)-[Lys17, Asp21]Aβ(1–28) play different roles in amyloid inhibition. In the design of CPFIs, the N and C termini of the functional peptides could be linked by peptide bonds or disulfide cross bridges. The CPFIs linked by disulfide cross bridges might be more dynamic once they interact with amyloid fibrils due to the more flexible nature of the S–S linkages, compared to the peptide bond.

counterparts (Figure 4e–g). It has been shown that the heterodivalent macrocycle (Figure 4g) is more active against amyloid β peptide aggregation than the monovalent and homodivalent-linked macrocycle (Figure 4e, f). Site I in the heterodivalent macrocycle is assumed to interact with the N-terminal based core of Aβ oligomers and site II of the component can bind to the C-terminal based hydrophobic regions.[47] This information suggests that polyvalent CPs could target multiple regions of amyloid aggregate.

Side chain-to-side chain lactam-bridged cyclo(17,21)-[Lys17,Asp21]Aβ(1–28) was found to inhibit the fibrillation of linear peptide Aβ(1–28). Also, cyclo(17,21)-[Lys17,Asp21]Aβ(1–28) reduced the toxicity of Aβ aggregates. It has been suggested that cyclo(17,21)-[Lys17,Asp21]Aβ(1–28) interacts with Aβ(1–28) and that they form a heterodimer to prevent the fibrillation of Aβ(1–28).[48] Another cyclic peptide (Cyc[60–70])

Sequence mimics have provided an efficient strategy for designing inhibitors of amyloid fibrillation. The hydrophobic and hydrophilic surfaces are essential characteristics of the sequence mimics and their characteristics could be tailored to specific amyloid proteins. However, the hydrophobic surface of macrocycles can also lead to the formation of oligomers of macrocycle peptides, as for instance observed in their crystal structures.[42]

Conformational mimics

D,L-α-Cyclic peptides can self-assemble into nanotubes.[51] These cyclic peptides stack on top of each other with an approximately 4.8 Å spacing to form a hollow β-sheet tubular

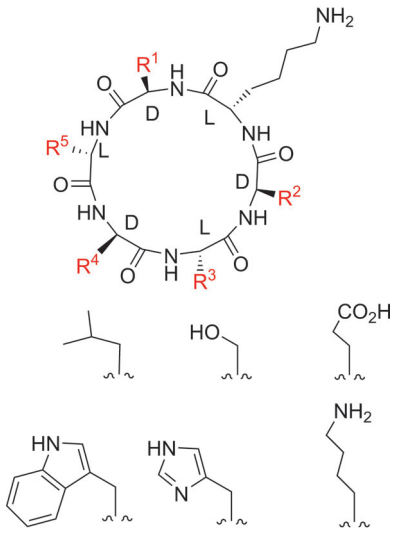

Figure 5. The scaffold of conformational mimics based on a cyclic D,L-α-CP hexapeptide (upper panel) and the substituted groups (lower panel) in R^1–R^5.

structure.[52,53] These tubular structures share conformational and functional features with amyloid fibrils. For instance, a similar parallel intermolecular distance is observed in the amyloid fibril (Figure 1 d). Moreover, these CPs and amyloid protein both interact with membrane lipids and lead to the loss of membrane integrity and cell death.[54,55]

The structural similarity of amyloid fibrils and D,L-α CP tubes may indicate the cross-reaction of their monomers. By screening a focused library of six residue D,L-α-CPs, Richman et al. found that such CPs interact with monomeric Aβ and inhibit the aggregation of Aβ. D,L-α-CP hexamers were synthesized with varying side chains at positions R^{1-5} (Figure 5). These were occupied by either positively charged Lys, negatively charged Glu, neutral Ser, hydrophobic Leu or aromatic Trp and His resulting in a broad structural diversity. A subsequent screen of this library revealed D,L-α-CPs[1LwHsK] (CP-1) to be the most potent inhibitor of Aβ peptide fibrillation. Subsequent Ala scanning found D,L-α-CPs[1JwHsK] (CP-2) to have an even better antiamyloidogenic activity. This compound not only reduced the toxicity of Aβ aggregates but also dissolved preformed fibrils. CD and NMR spectroscopy studies indicated that CP-2 may promote the conformational transition of the Aβ peptide from the antiparallel β-strand to the parallel β-strand. The parallel oligomeric structure is considered to be less toxic than the antiparallel structure.[51] These results suggest that structural mimics could be used to interfere with the conformational conversion of the nontoxic monomer to toxic oligomers/fibrils. In this strategy, careful placement of the hydrophobic groups within the CPs may be essential to optimally reduce the nucleation.

Optimizing a Lead Compound

There are natural conformational mimics of amyloid fibrils: cyclic antibiotics. For example, D,L-α-CPs (analogues of) the natural cyclic decapeptide gramicidin S (GS), self-assemble into tubular β-sheet structures with flat- and ring-shaped conformations (Figure 6 a–f).[56] GS and its analogues have an antibiotic activity to a broad range of Gram positive and certain Gram negative bacteria and some fungi.[57] The similarity between Aβ and GS (-analogues) prompted the hypothesis that GS might interact with the Aβ peptide. Recently, we found that GS indeed inhibits Aβ amyloid formation in vitro and could redissolve amyloid that had formed in the absence of the antibiotic.[58] Encouraged by this result, we engineered a series of GS analogues (Figure 6 b–f) by modifying different groups A–H (Figure 6 a). We could identify an analogue (Figure 6 f) with a potency that was four-times higher than the natural product. In silico docking suggested a potential binding pocket: gramicidin S (analogues) adopted a β-sheet conformation, binding to the Aβ peptide hairpin-like fibril through β-sheet interactions. In the interaction mechanism that we proposed, the hydrophobic groups Val, Leu and naphthylalanine of GS analogue interacted directly with hydrophobic residues Phe19, Phe20, Ala21, Leu34, Met35 and Val36 of the Aβ(18–42) peptide, and the naphthalene groups of GS analogue formed hydrophobic interactions though π-stacking with Phe19 of the hairpin-like structure (Figure 6 g–i). Additional experiments are required to establish the effects of GS and its analogues on reducing cell toxicity of amyloid aggregates.

Chemical Genetics Screening

Although the compounds discussed so far exhibit in vitro inhibitory activity against amyloid fibrillation and toxicity, screening of analogues may still be optimized. Recently, with the development of chemical genetics, an efficient strategy has been developed to screen CPs for reduction of amyloid toxicity in vivo in a yeast model and in *Caenorhabditis elegans*.

A robust and flexible technology, named SICLOPPS (split intein-mediated circular ligation of peptides and proteins technology) was developed for intracellular synthesis of CPs.[59,60] The method utilizes split-intein chemistry to cyclize randomized peptide sequences in vivo (see Figure 7 for a diagram of the steps involved). With this CP expression system, Lindquist's group constructed a yeast-compatible library to express diverse CPs and combined it with their established yeast synucleinpathy model.[61–63] The expression of CPs was verified by MALDI electrospray mass spectrometry after streptavidin affinity purification. The yeast synucleinopathy model uses the GAL1 promoter to switch on expression of human α-synuclein in a galactose containing medium.[63] Synuclein aggregation is toxic to yeast cells, hence a biological selection for aggregation inhibiting CPs is possible. From five million independent transformants, 31 clones were demonstrated to suppress α-synuclein toxicity in yeast. Filtering assays were performed to exclude promoter effects and other nonspecific modes of action. Mutation assays verified that the toxicity of α-synuclein was prevented by the spliced CPs rather than linear peptides or others.[43] Eventually, two CPs (CP1 and CP2 in Figure 7) were found to significantly suppress α-synuclein toxicity. To study the structure–activity relationships of CP1 and suppression of

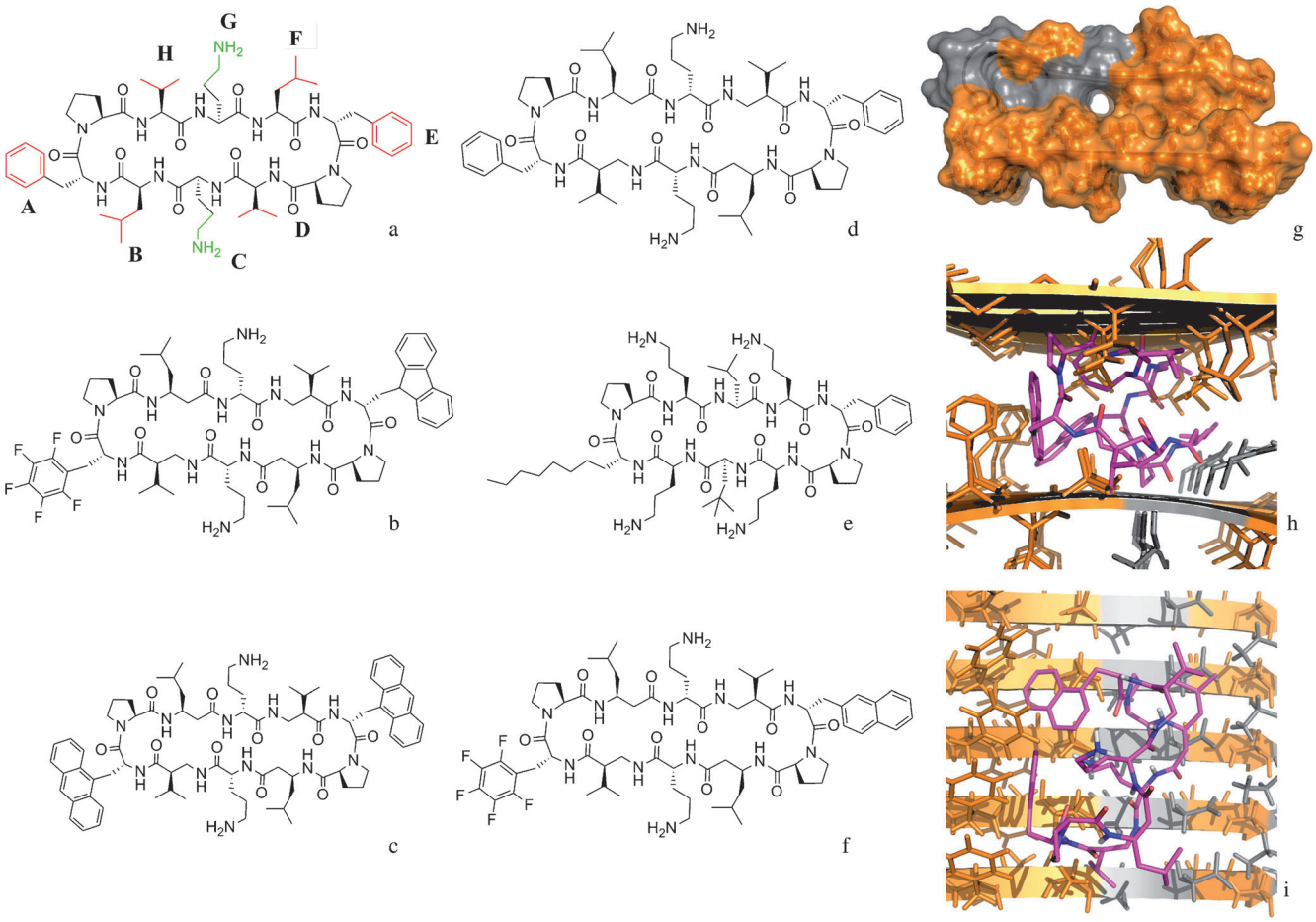

Figure 6. a–f) The chemical structure of gramicidin S and its derivatives. g) Aβ(1–42) hairpin structure determined by solid-state NMR spectroscopy. Hydrophobic groups are in orange, hydrophilic groups in grey. h,i) Docking of gramicidin S into the amyloid hairpin structure.

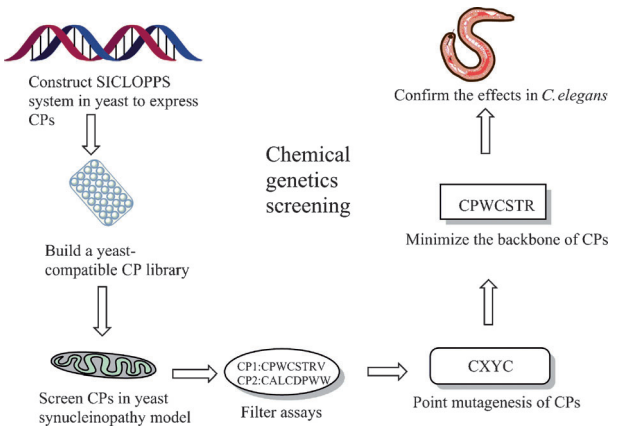

Figure 7. Split-intein mediated circular ligation of peptides (SICLOPPS) allows chemical genetics and the selection and optimization of CP amyloid inhibitors. First, a SICLOPPS library is introduced in yeast, which starts producing CPs. Then, α-1-synnuclein, which is toxic to yeast, is induced. Yeast cells that produce peptides that protect against this toxicity survive this selection. Subsequently, the CPs can be improved and tested in a nematode *C. elegans* system.

α-synuclein toxicity, point mutagenesis demonstrated that CP1 and CP2 have a common scaffold, namely CXYC, wherein X could be any residue, whereas Y is hydrophobic. Iterative design minimized the CP backbone. CP hexamers showed equal activity to suppress α-synuclein toxicity, like CP1 and CP2. In parallel, the selected CPs prevented dopaminergic neuron loss in a *C. elegans* model of Parkinson's disease.[43] Thus, despite limits imposed by the chemistry of the technique (Figure 7), chemical genetics offered a rapid and efficient strategy that could be an attractive alternative to other methods for CPFI screening for inhibiting amyloid toxicity in cellular models.

A Lesson from CPFIs

Despite structure and sequence variations of CPFIs, they share some chemical and conformational properties: 1) in all cases there is a logical division in sequence and structure, with a hydrophobic and a hydrophilic region, 2) aromatic groups may be essential for CPFIs, 3) the surface of CPFIs is dominated by hydrophobic groups. Encouraged by these observations, we scanned for yet another class of macrocycle: cyclic antibiotics. Indeed, it has been reported that rifampicin and its analogues prevent the aggregation of several different proteins and suppress the toxicity of aggregates.[64] Rifampicin is a complex cyclic molecule the size of a CP, has a hydrophobic and a hy-

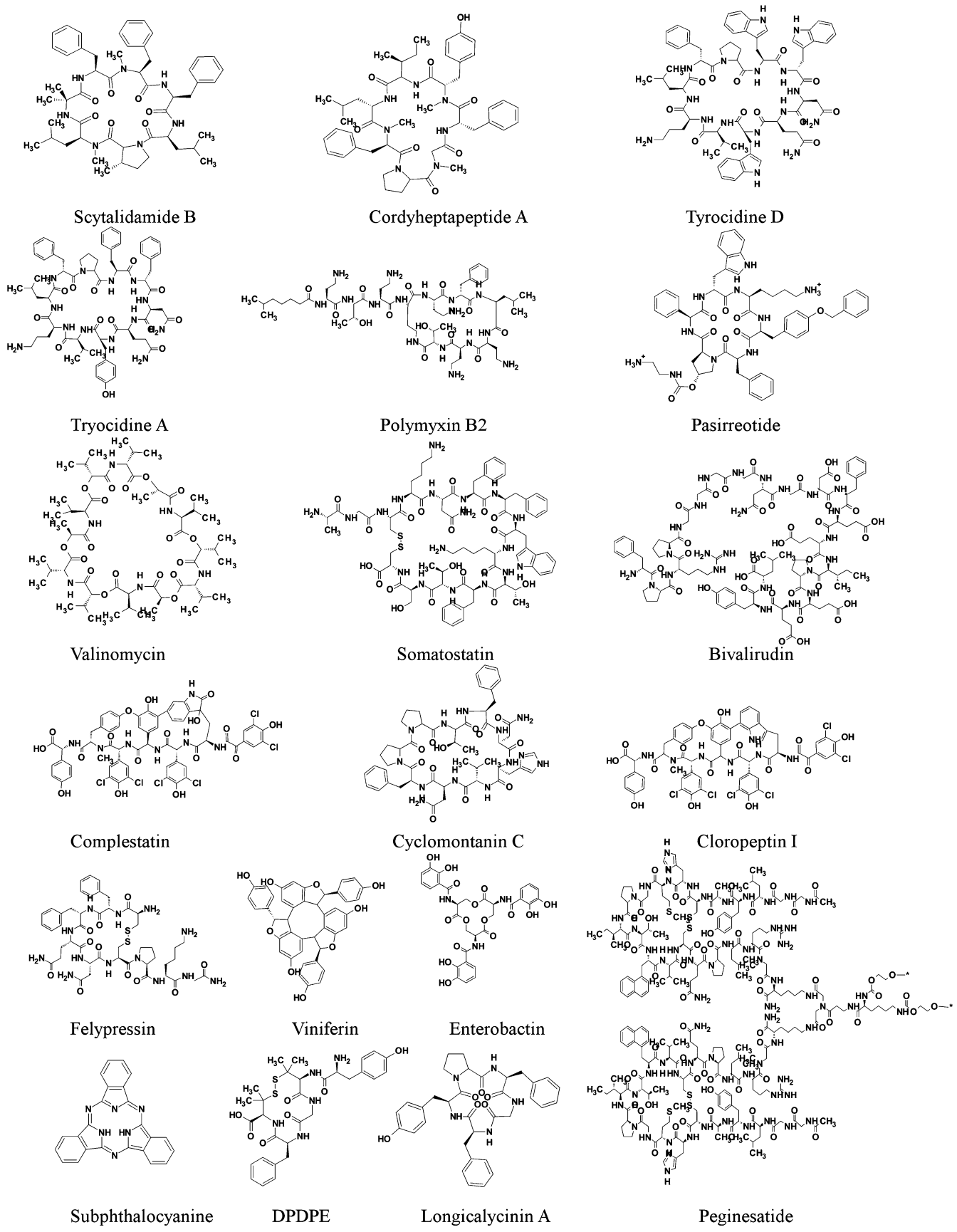

Figure 8. Selection of cyclic antibiotics screened by CHEBI that share relevant structural features with the CPFIs with proven amyloid inhibiting activity (http://www.ebi.ac.uk/chebi/).

drophilic face and a large aromatic group, but it can hardly be called a peptide, as it only has a single peptide bond. One of the advantages of cyclic antibiotics is that many have been thoroughly tested and are already in use for treating bacterial infections. This may lower the threshold preventing fast clinical application. Based on the 1D conformational/chemical similarity, we searched the CHEBI database (http://www.ebi.ac.uk/chebi/). We found a cluster of cyclic antibiotics that share relevant features with the CPFIs discussed above, for instance with respect to the hydrophobic and hydrophilic properties of side chains (Figure 8).

Perspectives

In summary, CPFIs can inhibit fibrillation of amyloid proteins and suppress the toxicity of amyloid precursor aggregates. The fibrils may act as reservoirs for the more toxic oligomers that also characterize the initial stage of fibrillation. Inhibiting the formation of fibrils and suppressing the toxicity of amyloid precursor oligomers are both important targets for the development of drugs against amyloidogenesis. Due to the structural differences between the amyloid fibril and its amyloid precursor aggregates (oligomers), an amyloid inhibitor may interact with both types of complex in distinct ways. Furthermore, amyloid fibrils have different ends and they grow at both ends. Perhaps there may be CPFIs that bind better at one end than at the other due to the heterogeneous structure. In the above two cases, we could even envisage using a pair of CPFIs, dual functional heterodivalent or homodivalent counterparts with different specific binding groups for oligomers and different fibrils. Clearly, developing dual effective inhibitors to suppress oligomer toxicity and prevent amyloid formation might be challenging. However, the CPFIs concept is versatile and there are many strategies for designing and optimizing CPFIs. Hence, this challenge can be faced with confidence.

Keywords: amyloid fibrillation · cyclic peptides · fibrils · inhibitors · mimics · proteins

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