Designed Inhibitors to Reduce Amyloid Virulence and Cytotoxicity and Combat Neurodegenerative and Infectious Diseases

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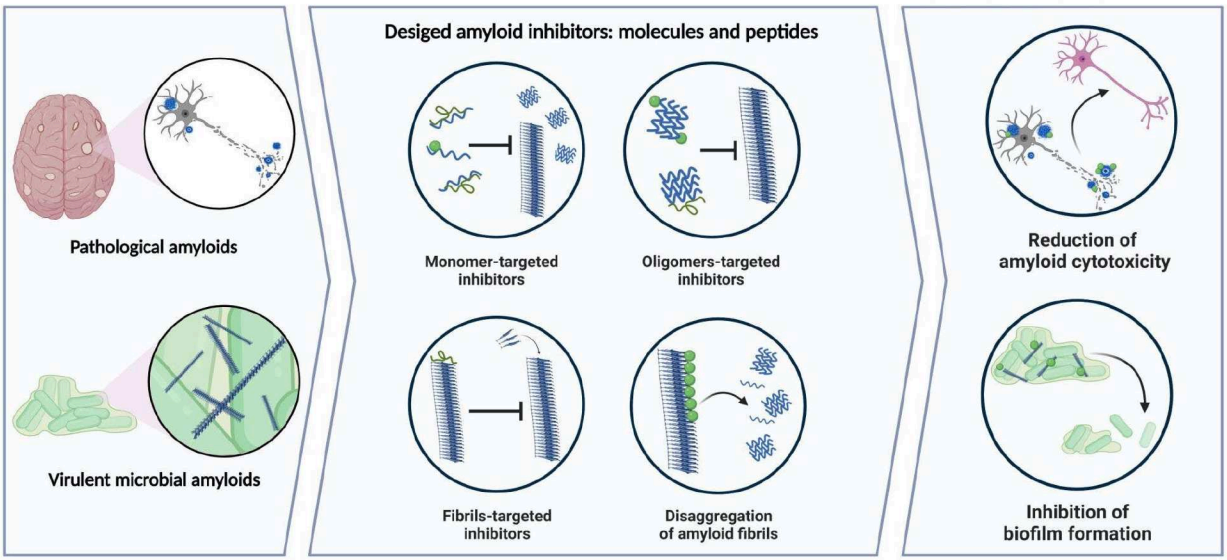
Abstract

The review highlights the role of amyloids in various diseases and the challenges associated with targeting human amyloids in therapeutic development. However, due to the better understanding of microbial amyloids' role as virulence factors, there is a growing interest in repurposing and designing anti-amyloid compounds for antivirulence therapy. The identification of amyloid inhibitors has not only significant clinical implications but also provides valuable insights into the structure and function of amyloids. The review showcases small molecules and peptides that specifically target amyloids in both humans and microbes, reducing cytotoxicity and biofilm formation, respectively. The review emphasizes the importance of further research on amyloid structures, mechanisms, and interactions across all life forms to yield new drug targets and improve the design of selective treatments. Overall, the review highlights the potential for amyloid inhibitors in therapeutic development for both human diseases and microbial infections.



Amyloid aggregation is often associated with fatal neurodegenerative and systemic diseases. However, amyloids also play a crucial role in various physiological processes across all kingdoms of life and hold significant value for biomedical and technological applications. These processes include storage of peptide hormones, memory formation, regulation of transcription and translation, and serving as virulence factors in microbes [1,2]. Structurally, amyloids differ from other protein fibrils in that they are composed of subunits stacked in sheets perpendicular to the fibril axis, which pair up to form stable structures. The mated sheets are often composed of β-strands, forming a configuration known as cross-β [3,4]. Fibrils composed of α-helices forming a cross-α configuration have also been discovered [5–10], and some peptides can switch between cross-β and cross-α [5–7,11]. Although there is a wealth of information available regarding the structure, function, and physiology of amyloids, differentiating between functional and pathogenic aggregation can be challenging due to some amyloids having the ability to perform both roles [12–19]. The term "pathological" implies that there is something abnormal about the protein's function, which may not always be the case in amyloid-related diseases. Some amyloids aggregate as a part of their function, such as in the phase separation of RNA binding proteins [12–19] or fibrillation of those used to scaffold biofilms, which may be physiological from the perspective of bacteria but pathological in

terms of human infections [1,2]. In certain instances, amyloid proteins may carry out their normal functions in either their native or misfolded state, but accumulate in harmful ways, leading to disease. Additionally, amyloid fibrils can also serve as a storage site for various hormones or toxins [1,2,20–22]. Therefore, the mere occurrence of misfolding or aggregation does not provide a definitive way to distinguish between physiological and pathophysiological effects.

Amyloid proteins have been targets for the development of inhibitors and modulators for decades [23,24]. This direction has been pursued mainly for the treatment of neurodegenerative and systemic aggregation diseases, which are increasingly prevalent in aging societies and are associated with pathological amyloids that form toxic oligomers and eventually protein plaques [23]. There are several pharmacological approaches aimed at the treatment of neurodegenerative diseases, including alleviation of symptoms by targeting the cholinergic system or other neurotransmitters, but anti-amyloid compounds remain a major approach. Groups of amyloid modulators include natural compounds such as polyphenols, amino compounds, and vitamins, as well as peptides or peptidomimetics, polymeric compounds, antibodies, antibiotics, metal ions, RNA aptamers, proteoglycans and glycosaminoglycans, lipids, lipid rafts and gangliosides, chaperones and other means of inducing refolding, such as nanoparticles [24–29].

Alzforum's therapeutics database, accessed on April 2, 2023, reports that out of the 364 therapeutics listed, 162 are related to amyloid. Among these, only two medications, aducanumab and lecanemab-irmb, which are antibodies against amyloid-β (Aβ), have received approval for treating Alzheimer's disease. Six small molecules have also been approved for Alzheimer's disease, as well as other conditions such as Parkinson's disease and dementia. These small molecules target the cholinergic system or other neurotransmitters. Clinical trials have been completed for 100 compounds, while 164 compounds are currently in various stages of FDA clinical trials, ranging from phase 0 to phase 4. Out of these, 32 are related to amyloid, and only seven are in phase 3, with none in phase 4. ClinicalTrials.gov [30], indicates that there are numerous clinical trials focusing on the diagnosis and treatment of amyloid-related diseases such as

neuropathies, angiopathy, amyloidosis, Alzheimer's disease, mild cognitive impairment, Down syndrome, and Parkinson's disease. These trials include not only therapeutic interventions but also various diagnostic tools, including measuring amyloid levels in different tissues. However, only a small number of these trials have progressed to phase 4, with most being diagnostic in nature. Some trials are currently in phase 3, including trials testing the effectiveness of the antibodies donanemab and remternetug for treating Alzheimer's disease. Overall, devastatingly, despite a multitude of clinical trials with drugs against a variety of targets, there has been very limited and still controversial success in the treatment of Alzheimer's and other neurodegenerative diseases [31]. This is probably due to the fact that the disease process starts many years before the onset of symptoms forcing a late start of treatment, inappropriate dosage and route of administration, as well as to the multifactorial and unclear mechanism of the neurodegenerative diseases [32]. Hopefully, ongoing and future research and clinical trials will address disease prevention, aversion and symptoms.

Applications of amyloid modulation have taken another important turn in recent years, to combat infectious diseases, by targeting amyloids that serve as virulence factors in microbes. These proteins exploit the inherent properties of the amyloid fold, including high stability and adhesive properties and regulation via self-assembly, to act as toxins, attack host cells or other microbes, support adhesion and biofilm scaffolding, and more [33]. Biofilms pose environmental and health risks by damaging equipment, also promoting metal corrosion on surfaces, and increasing resistance to infection [34]. For example, *Staphylococcus aureus* is a major cause of hospital-acquired infections [35], which forms biofilms on implanted medical devices and causes severe and persistent infections [36]. Overall, the development of effective anti-biofilm compounds is of paramount importance to public health, industry and the environment, and microbial amyloids represent a prominent target [26].

The proposed structural and biophysical similarity between human and microbial amyloids suggested that they might be inhibited by the same compounds, providing a route to repurpose existing drugs for new therapies [37,38]. Here we list examples of designed peptide and small molecule inhibitors that have been tested for their ability to

affect fibrillation as well as to reduce the toxicity of pathogenic human amyloids (Table 1), or to prevent biofilm formation by microbial functional amyloids (Table 2). The chemical structures of several designed amyloid inhibitor compounds are shown in Figure 1. The sequences and net charge of the designed peptide amyloid inhibitors are shown in Table 3. It is noteworthy that these peptides all have a neutral or positive net charge, which may indicate their mechanism of inhibition. Some of them contain D-amino acids, which may provide greater resistance to in-vivo degradation.

Many of the potential amyloid inhibitors under investigation are based on natural compounds, such as polyphenols, which are effective in inhibiting both human and microbial amyloid fibrils [39–42]. For example, the green tea epigallocatechin-3-gallate (EGCG) inhibits fibrillation and disaggregates α-synuclein fibrils associated with Parkinson's disease and protects cells from α-synuclein induced toxicity [43]. Recently, the cryoEM structures of brain-extracted tau fibrils on the kinetic pathway to EGCG-induced disaggregation were reported, revealing EGCG molecules stacked in polar clefts between the paired helical protofilaments of tau [44]. In addition, a complex of EGCG with the transthyretin V30M mutant involved in amyloidosis was determined by a crystal structure, revealing binding sites distinct from the thyroxine binding site, suggesting a mode of action distinct from compounds that were shown to bind and stabilize the transthyretin tetrameric structure [45]. Similarly, EGCG acts as an anti-biofilm agent based on its anti-amyloidogenic properties [46]. For example, EGCG inhibits amyloid formation and disaggregates fibrils of the *S. aureus* functional amyloids PSMα1 and PSMα4, which are involved in biofilm scaffolding [40]. In addition, EGCG inhibits the ability of the FapC amyloid secreted by *Pseudomonas* to form fibrils, while remodeling existing fibrils into non-amyloid aggregates. Accordingly, EGCG reduced the stiffness of Fap-containing biofilms [47]. Similarly, EGCG inhibits *E. coli* biofilm formation by interfering with curli amyloid fibrils [48]. EGCG also inhibits the amyloid-forming *E. coli* pleiotropic regulator Hfq, thereby acting as an antibacterial agent [49]. Another polyphenolic compound, myricetin, reduced the formation of Aβ fibrils associated with Alzheimer's disease in a cell culture and reduced Aβ oligomer-induced synaptic toxicity [50]. Myricetin and other plant flavonoids also prevented the assembly of the curli CsgA subunits into biofilm-associated functional amyloid fibrils in enteric bacteria [51].

Unfortunately, many of the natural compounds have pitfalls that reduce their druggability. For example, polyphenolic compounds often have poor solubility and stability in water, leading to low absorption and bioavailability when administered orally, and many are not expected to cross the blood-brain barrier, limiting their site of action [52]. Another concern is the lack of selectivity against a variety of targets, which argues against their clinical application. Therefore, derivatives of the natural compounds have been extensively studied to overcome some of these pitfalls. An example is curcumin, a well-known natural compound with many proposed beneficial properties, including activity against human amyloids including Aβ, tau and α-synuclein [53]. Novel curcumin derivatives have been developed to overcome its poor solubility and low bioavailability, and some have reduced the levels of toxic tau oligomers and decreased its cytotoxicity [54]. In addition, through the development of high-throughput screening assays that measure chemical kinetics, both natural and synthetic amyloid modulators have been discovered. These assays use fluorescence dyes such as thioflavins to measure the kinetics of amyloid fibrillation and have identified many compounds that affect this process. The assays can distinguish between different types of inhibitors, such as those that reduce fibrillation kinetics, those that reduce the final amount of fibrils without affecting kinetics, disaggregators, fibril or monomer stabilizers, and oligomer binders [55–60]. A different method for high-throughput screening involves ion mobility spectrometry-mass spectrometry. This technique quickly identifies small molecules that can bind to amyloid precursors, determining the protein species involved in the interaction and characterizing the inhibitory mechanism [61].

Much of the effort in inhibitor design is based on high-resolution structures of amyloids, which have accumulated enormously in recent years due to various technological applications and advances, especially in micro-electron diffraction (microED) and cryogenic electron microscopy (cryo-EM) [62]. Structures of amyloids in complex with inhibitors, can be particularly useful in screening for further compounds. For example, the cryoEM structure of brain-extracted tau fibrils complexed with EGCG was used to computationally screen drug-like compounds for pharmacophore compatibility, and several were discovered that experimentally disaggregated brain-derived tau fibrils in vitro [44]. In addition, based on available structural and biophysical information,

together with computational simulation and medicinal chemistry approaches, a number of synthetic small molecules have been designed to inhibit amyloids. Many are based on modification of natural compounds to improve selectivity, chemical stability and solubility, bioavailability, and overall druggability. In addition, rational design of anti-amyloid drugs is also often based on peptide-based inhibitors, derived from amyloid protein sequences and structures, and is aimed at targeting different stages of polymerization and polymorphs of the fibrils. The designed peptides are often enhanced with additional chemical scaffolds to increase potency, selectivity, and stability such as resistance to degradation [23,63–70].

Amyloid inhibitor design attempts to modulate amyloid fibril formation and activity by different mechanisms, targeting different binding sites and different amyloid species on the monomer to fibril pathway. Many of the designs have focused on targeting the region that serves as the backbone of the fibril, with various modifications to disrupt the β-sheet formation. For example, the KLVFF motif within the core of Aβ42 fibrils was enhanced with modification to D-amino acids and conjugation to D-tryptophan coupled to a C-terminal Aib β breaking moiety, which was previously suggested to interfere with monomer addition to the fibril [71]. This conjugated peptide showed inhibition of aggregation and reduction of Aβ42 cytotoxicity [72] (Table 1&3). In addition, two designed pentapeptides, named P4 and P5, restricted the elongation process, disaggregated the preformed mature fibrils, and reduced the haemolytic effect of insulin fibrils, presumably by affecting β-sheet formation and interactions with tyrosine residues critical for self-assembly [73] (Tables 1&3). The high-resolution structures of Aβ42 fibrils determined by cryo-EM were used to design macrocyclic peptide sequences that are based on core regions in the fibril [23] (Tables 1&3). Using this approach, selectivity for Aβ42 over Aβ40, which lacks two residues at the C-terminus, was achieved by partial mimicry of the C-terminus. In addition, specificity was achieved with no detectable inhibition against α-synuclein and the K19 variant of tau [23]. Other structure-based designed peptides inhibited the fibrillation of Aβ42 and reduced the toxicity, and were also effective against tau, but did not inhibit fibrillation of hIAPP and α-synuclein [74] (Tables 1&3).

In the small molecule field, one approach has been to develop tryptophan-galactosylamine hybrid molecules, such as the compound named WGalNAc (Table 1), which targets the aromatic residues in the hydrophobic core of the amyloid, and has shown inhibition and degradation of Aβ42 and hIAPP fibrils and reduced their toxicity [75]. Another compound was based on the conjugation, via a click or PEG linker, of mannitol and naphthoquinone-tryptophan, both of which can individually inhibit α-synuclein aggregation, while one of the conjugated molecules, termed M3N, was found to be more potent than the mixture of the two [76] (Table 1). Importantly, the conjugates showed low intrinsic toxicity, and reduced α-synuclein toxicity to neuroblastoma cells [76]. In contrast to the focus on inhibitors targeting a single protein, broad-spectrum inhibition of the fibrillation process without interfering with the normal function of the amyloids has been employed with compounds known as molecular tweezers. These compounds, such as CLR01, target lysines that often play a role in the fibrillation of different amyloids, and indeed inhibited a wide range of pathogenic amyloids, including the ability to disassemble fibrils and reduce amyloid toxicity [77]. Moreover, CLR01 successfully suppressed α-synuclein aggregation in neurons, reduced α-synuclein-induced apoptosis, and improved zebrafish phenotype and survival. The proposed mechanism was mitigation of α-synuclein inhibition of the ubiquitin-proteasome system [78]. In addition to human amyloids, molecular tweezers inhibited PSMα1 fibrillation and reduced *S. aureus* biofilm formation [79], and displayed a potent and broad-spectrum antiviral activity against enveloped viruses [80,81].

Recent studies suggest that transient oligomeric species are more toxic than mature fibrils [82,83]. Therefore, inhibitors that target fibrils and aim to degrade them may potentially increase toxicity. Instead, amyloid modulators can reduce cellular toxicity by targeting monomers or oligomers, stabilizing fibrils, or promoting a benign aggregation pathway to convert toxic species into less harmful ones [27,64,84]. However, designing drugs specific to the dynamic and transient nature of oligomers has been challenging due to their elusive structures. Researchers have employed chemical kinetic studies to identify monomer stabilizers and oligomer binders [55–58,77]. In addition, hits from high-throughput screening can be further optimized using a "structure-kinetic-activity relationship" approach. For example, a rhodanine compound was developed using this

approach to reduce the production of Aβ oligomers [85]. An alternative method was to use in silico studies, for example, one that proposed that a helical portion of monomeric Aβ40 interacts with the side chains on the surface of the fibril. Using the sequence of this fragment as a foundation, an α-helical peptide inhibitor was designed with mutations and modifications [86] (Tables 1&3). The resulting inhibitor, termed cHASI-1, showed micromolar affinity for the fibril surface, inhibited oligomerization of Aβ40, and mitigated its cytotoxicity. A series of investigations, beginning with a mirror image phage display to select all-D peptides that bind Aβ42, and subsequent optimizations, resulted in the development of several promising D-peptides designed to bind to monomers, eliminate oligomers, inhibit fibril formation, disassemble preformed aggregates, and reduce Aβ-induced cytotoxicity [87–89][90,91] . Among these D-peptides, DB3DB3 and ANK6 (Tables 1&3) also demonstrated the ability to inhibit the formation of curli CsgA amyloid, which is involved in biofilm stabilization in enterobacteria, and reduce the biofilm biomass of Salmonella typhimurium [92]. Another D-peptide resulting from the same optimization efforts, known as RD2 or PRI-002, is among the few compounds that have advanced to clinical trials for the treatment of Alzheimer's disease [93]. More recently, to improve the oral absorption of RD2, it was linked to folic acid [94].

In the context of targeting the oligomeric state, Daggett and co-workers proposed, based on molecular dynamics simulations, that toxic oligomeric intermediates of several amyloid proteins adopt α-pleated sheet structures [95], and designed peptide inhibitors accordingly [96]. The α-sheet structures correspond to the ‘polar pleated sheet’ predictions of Pauling and Corey in 1951 [97]. In these α-sheets, the strand spacing is ~4.8 Å as in β-sheets, but each strand is composed of an extended chain with alternating main chain dihedral angles between the right-handed and left-handed helical regions of the Ramachandran space [95]. Pauling and Corey classified the α-sheets as energetically unfavorable compared to the β-sheet structure, which fits a transient state. These α-sheets have been observed in crystal structures of designed synthetic peptides (e.g. [98]), and have been suggested to exist in a truncated mutant transthyretin [99]. Notably, the α-pleated sheets are fundamentally different from the cross-α structure, which consists of canonical α-helices, rather than extended chains. However, it is possible that the unstable α-sheet configuration represents an intermediate species towards the formation of the

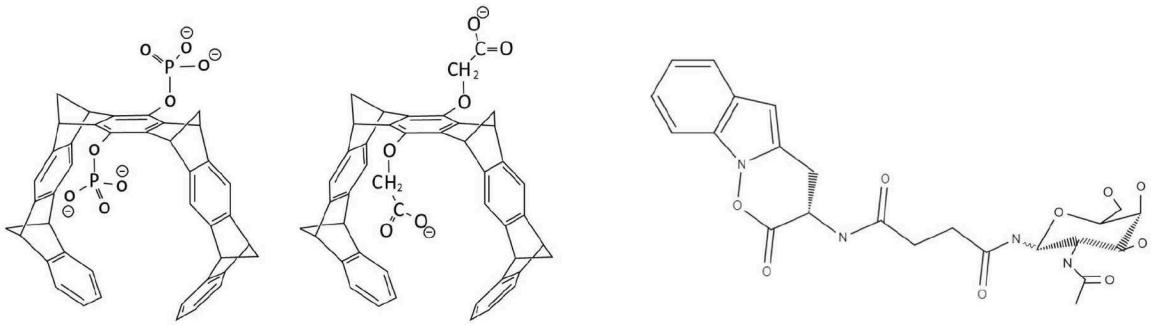
stable β-sheets or α-helical sheets. As inhibitors, designed α-sheet based peptides, such as AP90, inhibited the fibrillation of the virulent *S. aureus* PSMα1, based on the proposed formation of α-sheet structures in its oligomeric species, prior to the conversion into β-sheet rich fibrils [100] (Table 2). Similarly, the designed α-sheet peptides AP5 and AP421 target the toxic oligomers of Aβ and have shown inhibition of fibrillization and reduction of toxicity, including in-vivo, as demonstrated in mouse and *Caenorhabditis elegans* models [101] (Tables 1&3). Other designed α-sheet peptides inhibit *Streptococcus mutans* biofilm formation, but the exact biofilm-associated component targeted by these inhibitors is not yet known [102].

The increasing number of naturally occurring and artificially designed compounds that inhibit amyloid formation in both humans and microbes provide additional evidence of shared structural features. These molecular similarities among amyloids from various species may contribute to the development of prion-like agents via molecular mimicry. This is particularly noteworthy given the diverse array of microbial species in the human microbiome that can produce substantial amounts of secreted amyloids. There are several potential pathways that could explain the hypothesized link between microbes and human neurodegenerative diseases and amyloidosis [103,104]. One pathway involves the ability of seeds of amyloid fibrils from one species to nucleate monomers from another species [105–107]. Another pathway involves the activation of immune receptors, which can lead to neuroinflammation and neurodegeneration [108,109]. Molecular mimicry and structural similarity can be exploited to manipulate the host immune system and inhibitory immune checkpoints [110], representing another possible pathway. Additionally, an aberrant and increasingly dysbiotic innate immune response, as well as the deposition of amyloids with antimicrobial properties, could contribute to the amyloid-antimicrobial link [111–119]. Studies have shown that some antimicrobial peptides secreted by different organisms assemble into amyloid-like fibrils, further supporting this link [5,6,11,38,120–124]. Interestingly, human antimicrobial α-defensins that form amyloids [122] were found to inhibit the aggregation of Aβ, hIAPP, and human calcitonin while maintaining their antibacterial activity and reducing amyloid-induced cell toxicity [38]. In addition, some antibiotics have been shown to inhibit human amyloids,

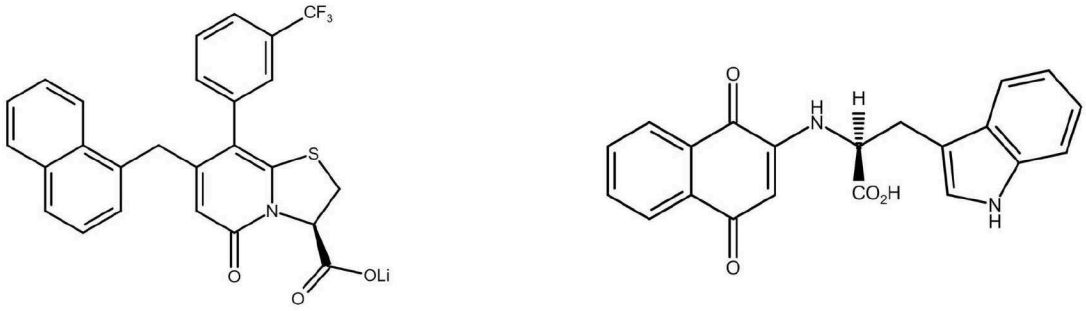
overall suggesting potential bi-directional repurposing of anti-amyloids and antimicrobials [37].

In summary, while past clinical trials for Alzheimer's and other neurodegenerative diseases have failed, the development of new anti-amyloid drugs offers hope for the future. With the rise of antibiotic resistance, novel approaches to treating infectious diseases are necessary, and targeting microbial amyloids presents a promising option due to their involvement in highly virulent pathways. Inhibiting virulent amyloids can decrease the aggressiveness of resistant infections. For example, curlicides have been shown to attenuate virulence in mouse models of urinary tract infections by inhibiting the curli biofilm associated amyloid system [125,126]. Antivirulence compounds have the potential to induce less resistance compared to antibiotics that directly kill microbes. Furthermore, some curlicides can also inhibit Aβ [127], but the lack of selectivity may have both positive and negative consequences. Recent developments in techniques for determining molecular structures, particularly in the field of cryo-EM, have opened up fresh possibilities for designing amyloid modulators that are both highly targeted and efficacious. These advances have also brought into focus antimicrobial peptides that form amyloids, which could potentially serve as therapeutic agents that offer improved stability and controlled activity through self-assembly and specific morphologies [5,11]. Artificial intelligence (AI) methods can help identify new microbial and antimicrobial amyloids, design new drugs, and optimize existing candidates. The discovery of inhibitors not only has potential clinical implications but also provides valuable insights into amyloids' structure and function. As researchers uncover new amyloids and further our understanding of their properties and interactions, amyloids are expected to play a significant role in biomedical and technological applications in the coming years.

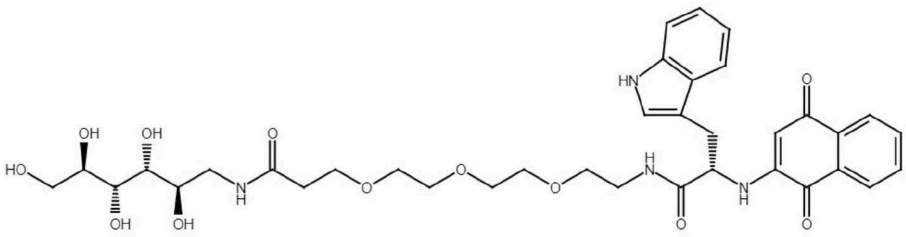
Figure 1 – Chemical structure of selected designed inhibitors of pathological and virulent microbial amyloids.



CLR01 CLR05 WGalNAc



FN075 NQTrp



M3N

The molecular structures are displayed for CLR01 and CLR05 [79], WgalNAc [75], FN075 [125,126], NQTrp [128], and M3N [76].

Table 1 – Selected designed inhibitors of pathological amyloids that reduce their cytotoxicity

Type of inhibitor	Inhibitors[a]	Targeted amyloid	Modulation of aggregation	Disaggregation of amyloid fibrils	Reduction of amyloid cytotoxicity	Inherent toxicity	Citation
Small molecules	WGalNAc	Aβ42 and hIAPP	V	V	V	X	[75]
	NQTrp, 1,4-naphthoquinon-2-yl-L-tryptophan	PAP248–286 (PAP f39)	V	ND	V	X	[129]
		Aβ42 and Aβ40	V	ND	V	X	[130]
		α-synuclein	V	ND	V	X	[76]
		Tau, Tau-derived PHF6	V	V	V	X	[128]
	Mannitol-3G-NQTrp, M3N	α-synuclein	V	ND	V	X	[76]
Curlicides	FN075	Aβ40	V	ND			[127]
Molecular tweezers	CLR01	Aβ42, Aβ40	V	V	V	X	[77]
		Tau	V	ND	ND		
		insulin, β2m, TTR, CT	V	ND	V		
		IAPP	V	V	V		
		α-synuclein	V	V	V		[77,78]
		SEM1(45-107), SEVI and PAP(85-120)	V	V	V (antiviral effect)	X	[131]
Peptides	Peptide named P4 and P5	Insulin	V	V	V	X	[73]

	α-sheet peptides designated AP5 and AP421	Aβ42	V	ND	V	X	[101,132]
	AP5	IAPP	V				
	Macrocyclic peptides: mcK6A1, mcG6A1 and mcG6A2	Aβ42	V	ND	V	V (Low toxicity)	[23]
	Designated β-strand peptide 2 (D-isoform)	Aβ42	V	X	V	X	[72]
	DB3DB3	Aβ42	V	V	V	X	[90]
	ANK6	Aβ42	V	ND	V	X	[91]
	RD2[b]	Aβ42	V	V	V	X	[133]
	Peptides designated D1b and D1d	Aβ42 and tau[c]	V	X	V	X	[74]
	cHASI-1	Aβ40	V	ND	V	X	[86]
	iAβ-H	Aβ42	V	ND	V	ND	[65]
	iαSyn-F	α-synuclein	V	ND	V	ND	

Symbols used in the Table: V indicates an observed effect; X indicates no effect, and ND indicates that the effect was not determined to the best of our knowledge. [a] The inhibitors listed are the most effective compounds among those tested in the referenced manuscript. [b] This compound, also called PRI-002, has been tested in Phase 1 clinical

trials [93]. [c] The referenced manuscript provides more information about the specificity of each compound against Aβ42 and tau.

Table 2- Designed inhibitors of virulent microbial amyloids.

Type of inhibitor	Inhibitors[a]	Targeted amyloid	Modulation of aggregation	Disaggregation of amyloid fibrils	Inhibition of biofilm formation	Citation
Curlicides	FN075 and BibC6	*Escherichia coli* curli csgA and	V	ND	V	[125,126]
Molecular tweezers	CLR01 and CLR05	*S. aureus* PSMα1	V	V	V	[79]
α-sheet based peptides	AP90	*S. aureus* PSMα1	V	ND	V	[100]
	AP407	*S. aureus* amyloid	V	ND	V	
Peptides	DB3DB3	CsgA	V	ND	V	[92]
	ANK6	CsgA	V	ND	V	

Symbols used in the Table: V indicates an effect; X indicates no effect, and ND indicates that the effect was not determined to the best of our knowledge. [a] The listed inhibitors are the most effective compounds amongst those tested in the referred manuscript.

Table 3 – Sequences and biophysical properties of selected designed peptide amyloid inhibitors

	Inhibitors	Sequence	Net Charge	Citation
Inhibitors of human amyloids	P4	VIFYT	0	[73]
	P5	VVVVV	0	
	AP5	Ac-RGNwNeSkMNEYSGWmLmLtMGR-NH2	1	[101]
	AP421	Ac-RGEcNlSwMNEYSGWtMnLkCGR-NH2	1	
	mcK6A1	TLWYKKY	2	[23]
	mcG6A1	HYFKYKW	2	
	mcG6A2	HYYIKKH	2	
	Peptide 2	klvfw-Aib[a]	-	[72]
	DB3DB3	rpitrlrthqnrrpitrlrthqnr-NH2	9	[90]
	ANK6	rkrirlvtkkkr-NH2	9	[91]
	RD2	ptlhthnrrrrr-NH2	6	[133]
	D1b	lyiwiwrt	1	[74]
	D1d	lyiwiqkt	1	
	cHASI-1	cyclo(isoD-F-R-Dap)-DVRAERAE[b]	0	[86]
	iAβ-H	PKRVTYTLNNRVHVQITHTDQKIVYVESSTG DKDAAMTAVKIADELAKK	2	[65]
	iαSyn-F	PVYHYRYKGRAAAEAAKEAAKIAQKLGGAL VVRVDGDTIRITIAV	4	
Inhibitors of microbial amyloids	AP90	Ac-RGEmNlSwMNEYSGWtMnLkMGR-NH2	1	[100]

The peptide sequences indicate the isomeric form of the amino acid, with upper case letters indicating L-amino acids and lower-case letters indicating D-amino acids. The capping of the termini is indicated by Ac- for N-acetylation and -NH2 for C-amidation. Net charge has been calculated taking into account the full charges of side chains and termini (unless capped) at physiological pH=7.4. [a] Aib is β-breaker moiety, which stands for achiral geminal disubstituted aminobutyric acid. [b] The HASI-1 peptide was mutated in the first and second residue to iso-D (L-isoaspartic acid) and a Dap (2,3-diaminopropionic acid), respectively. The two unnatural amino acids were then cross-linked, yielding a cyclic variant (cHASI-1).

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The article outlines a strategy to enhance the druggability and efficacy of amyloid inhibitors by conjugating tryptophan with galactosylamine. Previous research had demonstrated the ability of tryptophan and its derivatives to target aromatic residues that are crucial for amyloid aggregation (e.g., reference 76). However, molecules with such properties, like natural polyphenols, tend to be insoluble in aqueous media. This obstacle can be overcome by attaching a hydrophilic moiety to enhance solubility, such as derivatives of galactose, one of the most prevalent monosaccharides in the human body. The resulting hybrid molecules of tryptophan-galactosylamine hindered the fibrillation of Aβ42 and hIAPP and decreased their cytotoxicity without displaying inherent toxicity to mammalian cells. Moreover, the molecules also disaggregated amyloid fibrils. These findings suggest a therapeutic approach involving hybrid moieties for the treatment of Alzheimer's disease and type 2 diabetes.

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This paper describes the development of d-peptides that bind with high specificity to monomeric amyloid-beta (Aβ) and prevent its oligomerization. The researchers optimized the peptides through iterative rounds of design and screening, and evaluated their ability to reduce Aβ toxicity in vitro and in vivo. The most promising d-peptide showed improved specificity and efficacy compared to the original design, and significantly reduced Aβ-associated behavioral deficits in a transgenic mouse model of Alzheimer's disease. These results highlight the potential of d-peptides as a therapeutic strategy for Alzheimer's disease and other amyloid-related disorders.

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