Review

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Development of small molecules for disrupting pathological amyloid aggregation in neurodegenerative diseases

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Abstract

Neurodegenerative diseases (NDs), encompassing Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS), are often characterized by the formation of pathological amyloid aggregates, predominantly composed of proteins like amyloid-β, tau, α-synuclein, TDP-43, and others. These amyloid aggregates inflict significant neuronal harm and incite inflammation. This review underscores the potential of small molecules as innovative therapeutic interventions, designed to influence the formation, stability, and breakdown of these pathological amyloid aggregates, which could potentially modify the disease's progression and minimize its neurotoxic effects. This review first sketches the pathways and mechanisms involved in amyloid aggregation, followed by an in-depth analysis of recent advances in formulating small molecules that directly target these damaging aggregates. This includes various strategies such as inhibiting fibril formation, fostering off-pathway non-toxic oligomers or amorphous aggregates, disaggregating established pathological amyloid fibrils, and enhancing the protein quality control system to combat amyloid aggregation. In the end, this review identifies the challenges and opportunities involved in transitioning these molecules into effective treatments, focusing on critical factors such as penetration of the blood-brain barrier, target specificity, and safety considerations. This review, thus,

presents a comprehensive overview of the potential role of small molecules in tackling NDs typified by amyloid aggregation.

Keywords: Neurodegenerative diseases, amyloid aggregation, small molecules

INTRODUCTION

Neurodegenerative diseases (NDs), including Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS), represent a wide array of neurological conditions that are marked by the gradual deterioration of neurons in the brain and spinal cord[1]. A significant factor in the onset and advancement of these diseases involves the accumulation of pathological amyloid fibrils—an abnormal aggregated form of proteins including amyloid-β (Aβ), tau, α-synuclein (α-syn), TDP-43, and others [Figure 1A][2,3]. These amyloid fibrils can inflict various harmful effects, including cellular membrane disruption[4,5], incitement of neuroinflammation[6], mitochondrial function impairment[7], and disruption of protein homeostasis [Figure 1B][8,9].In addition, amyloid fibrils demonstrate a prion-like attribute, facilitating their own propagation and transmission between cells, a critical process in the spread of disease within the patient's brain [Figure 1B][10]. For instance, AD is characterized by the aggregation of Aβ and Tau into fibrils within the brains of affected patients, representing the central events in AD pathology, known as the amyloid cascade hypothesis[11]. Recent breakthroughs in disease-modifying therapies have shown promising results in slowing AD progression by utilizing specific antibodies that target Aβ aggregates, thus providing strong support for amyloid as a crucial target in combating the disease. These observations underline the significant role of amyloid fibrils in NDs, making them a promising focus for the diagnosis and treatment of related diseases.

The development of small molecules and the molecular chaperone that can disrupt pathological amyloid aggregation and the molecular chaperone represents a promising tool for therapeutic interventions and treatment of NDs[12]. By targeting the formation, stability, or degradation of amyloid fibrils, small molecules may modulate disease progression and alleviate the associated neurotoxic effects. In this review, we will explore recent advancements in the design and development of small molecules as well as small molecular chaperones targeting pathological amyloid aggregation. We will discuss the strategies employed to inhibit amyloid aggregation, as well as approaches aimed at disaggregating preformed pathological amyloid fibrils. In the end, we will highlight the challenges and opportunities in translating these small molecules into effective therapeutics.

Molecular mechanism of pathological amyloid aggregation

Abnormal protein aggregation involves the misfolding and subsequent aggregation of amyloid proteins, leading to the formation of amyloid fibrils[13]. The process begins when a protein undergoes conformational changes and misfolds[14]. Under normal circumstances, cellular mechanisms efficiently clear such misfolded proteins. However, certain conditions can impair this process, resulting in the accumulation of misfolded proteins[15]. As the concentration of these misfolded proteins increases, they begin to interact, establishing abnormal protein-protein interactions that promote amyloid aggregation[12]. These misfolded proteins initially form soluble oligomers, which are small clusters of misfolded proteins[16]. Importantly, these oligomers have been shown to be toxic to cells and are believed to be the primary pathogenic species in certain diseases[16]. Over time, these oligomers can arrange themselves into larger, insoluble fibrillar structures known as amyloid fibrils[17]. This arrangement is characterized by a specific cross-β structure, where the protein's β-strands are perpendicular to the fibril axis, resulting in a highly stable, non-native protein state[18]. The fibrils can further condense and deposit in brain tissues, forming amyloid deposits which physically disrupt the structure and function of the nervous system[12].
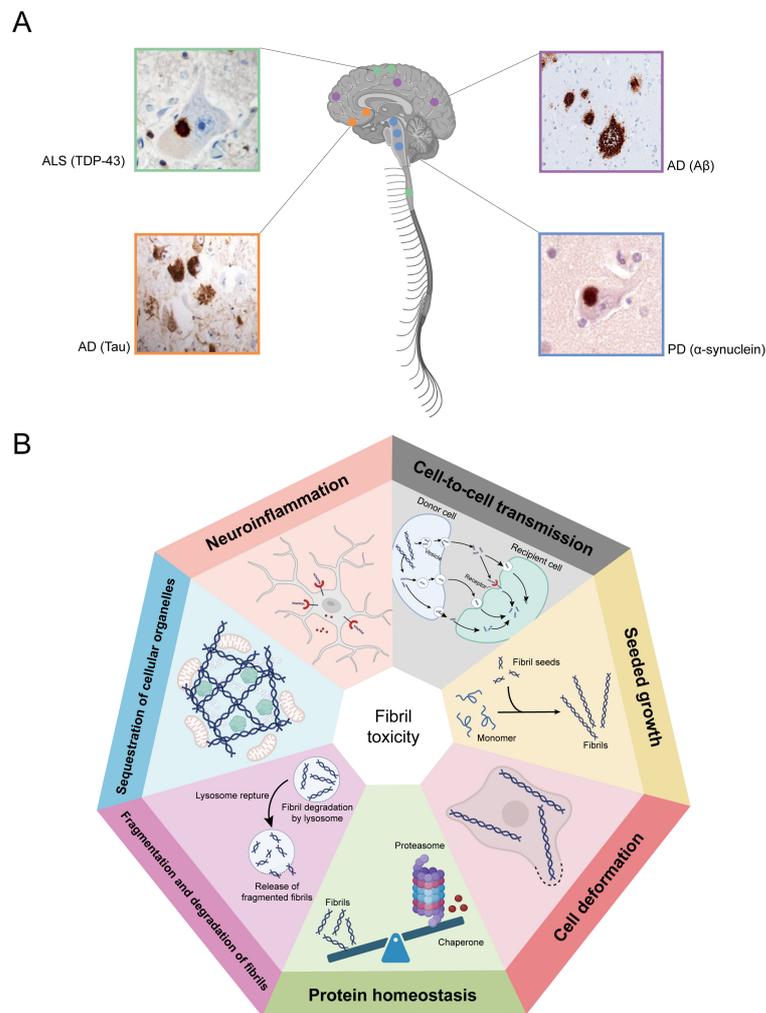
Figure 1. Amyloid fibril pathology in neurodegenerative disease. (A) Illustration of amyloid fibril deposition in different neurodegenerative diseases. TDP-43 inclusions in the motor cortex and the spinal cord in ALS patients, Aβ plaques in the neocortex of AD, tau inclusions as neurofibrillary tangles in neocortical neurons of AD, and α-syn inclusions (Lewy bodies) in neocortical neurons of PD. (B) Overview of the documented pathological effects linked to amyloid fibrils in brain cells. These effects include neuroinflammation, cellular truncation and fragmentation of amyloid fibrils, cell deformation, seeded growth and cell-to-cell transmission of amyloid fibrils, sequestration of cellular organelles, and disruption of protein homeostasis. AD: Alzheimer's disease; ALS: amyotrophic lateral sclerosis; PD: Parkinson's disease.

The pathway of protein amyloid aggregation comprises an intricate process, frequently demonstrated as a nucleation-dependent polymerization mechanism [Figure 2][19]. The early stages involve the generation of misfolded monomers, which have a higher tendency to self-associate, forming oligomeric nuclei through primary nucleation[18]. This stage is typically slow and is often referred to as the "lag phase" due to the low concentration of misfolded proteins and the thermodynamically unfavorable state of early aggregation [Figure 2][18]. Once a critical concentration is reached, the oligomeric nuclei serve as templates for further monomer addition, resulting in the elongation and formation of amyloid fibrils[20]. This is known as the "growth phase" and is marked by an exponential increase in aggregate size and number [Figure 2]. The kinetics during this phase is significantly faster than the primary nucleation stage as the process is
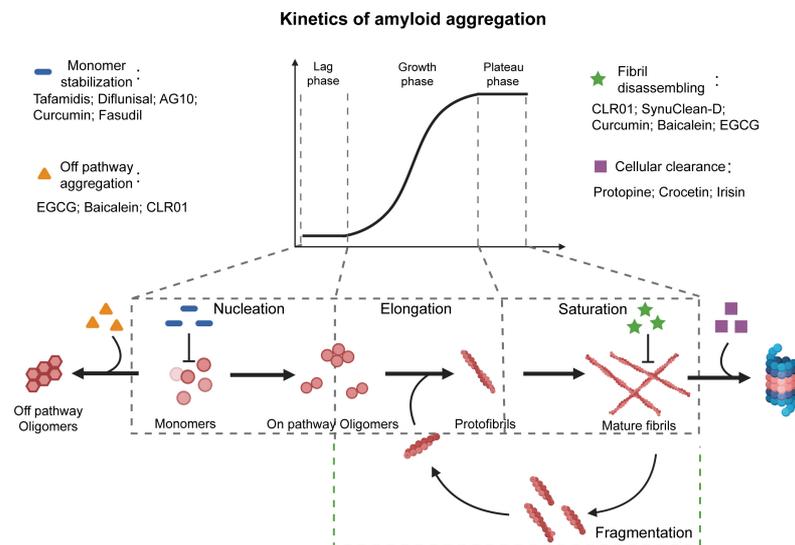
Figure 2. Schematic representation of small molecule inhibitors targeting specific stages of amyloid aggregation. Steps involved in the formation of amyloid fibrils. The process of amyloid fibril formation exhibits sigmoid kinetics, progressing through successive stages of nucleation, elongation, and saturation. The colored symbols represent the specific stages of α-syn aggregation that are targeted by small molecules.

energetically more favorable. During elongation, the surface of the pre-existing fibrils offers a catalytic "milieu" for increasing amyloid toxicity dramatically and accelerating the fibril formation, which refers to secondary nucleation[21,22]. Over time, these fibrils may undergo fragmentation, generating new fibril ends, which can further contribute to the growth phase[23]. Fragmentation dramatically increases the number of active growth sites and thereby enhances the aggregation rate. Finally, once the pool of misfolded monomers is depleted, the system enters the "saturation stage" or "plateau phase", where the total amount of amyloid fibrils remains constant[Figure 2][24,25]. Understanding the kinetics and pathway of protein aggregation is vital for developing small molecules for therapeutic interventions of amyloid-related diseases. These interventions could aim to slow down or inhibit specific stages of the aggregation process, such as preventing the generation of on-pathway oligomeric nuclei to block primary nucleation, inhibiting the formation of catalytic "milieu" for secondary nucleation, or disaggregating preformed amyloid fibrils to limit the formation and accumulation of toxic amyloid aggregates[Figure 2][17].

Inhibiting protein aggregation by stabilizing the native conformation
The fundamental principle of this approach is aimed at the primary phase of the protein amyloid aggregation pathway, specifically by augmenting the stability of amyloid proteins in their natural and functional conformations. This, in turn, curtails the propensity of proteins to misfold and initiate amyloid aggregation. Small molecules can be utilized to accomplish this[26]. By adhering to the protein's native state, these small molecules can bolster its stability, thereby diminishing the presence of misfolded proteins that could trigger the aggregation process and slow the rate of amyloid fibril formation by increasing the kinetic stability of native conformation[27].

A successful case of this strategy is the development of the small molecule drug tafamidis, achieved by Kelly and colleagues, which stabilizes the transthyretin (TTR) tetramer[28,29]. Tafamidis, a benzoxazole derivative lacking nonsteroidal anti-inflammatory drug activity[30], was approved by the FDA in 2019 as a therapeutic

treatment for transthyretin amyloidosis (ATTR), a debilitating condition characterized by the aggregation and deposition of TTR protein in various tissues[31,32]. Typically, TTR exists as a tetramer in the bloodstream, but its destabilization can lead to disassembly into monomers, which then misfold and aggregate into amyloid deposits[33]. Small molecules devised to tackle ATTR operate by binding to and stabilizing the TTR tetramer, thereby forestalling its disassembly into monomers and, ultimately, impeding the onset of the amyloidogenic process. Tafamidis exerts its effect by binding to the thyroxine-binding sites of the TTR tetramer, which fortifies it against dissociation into monomers [Figure 3A][30]. The cocrystal structure shows that tafamidis engages a combination of charged and hydrophobic interactions to bridge adjacent dimers and kinetically stabilize the TTR tetramer [Figure 3B]. The benzoxazole ring of tafamidis is positioned in a hydrophobic environment. The 3,5-dichloro groups form halogen bonds with Ser117 of TTR. In addition, the carboxylate engages in water-mediated hydrogen bonds with the Lys15 and Glu54 of TTR.

In addition to tafamidis, several other small molecules, including diflunisal and acoramidis (AG10), have been investigated for their ability to stabilize TTR. Diflunisal, a nonsteroidal anti-inflammatory drug (NSAID), was repurposed for TTR amyloidosis after it was found to bind and stabilize TTR tetramers[34]. AG10, a newer molecule, has been designed to mimic the natural mechanism for TTR stabilization, binding to the same sites as tafamidis but with greater selectivity and potency[35]. In summary, the development of small molecules for stabilizing protein native conformation exemplifies a targeted approach for treating amyloid-related diseases. This strategy inhibits the initial step of protein aggregation, offering a promising avenue for therapeutic intervention.

Redirecting amyloid aggregation towards off-pathway oligomers or amorphous aggregates
The development of small molecules to redirect amyloid proteins towards forming off-pathway oligomers or amorphous aggregates is another effective approach to managing amyloid-related diseases. The premise behind this strategy is to intervene in the protein aggregation process, diverting proteins from forming the toxic amyloid oligomer, and highly ordered β-sheet-rich amyloid fibrils, to instead forming less-structured and generally less-toxic aggregates. Effective small molecules may function by binding to specific regions of the amyloid proteins, altering their aggregation pathway. Instead of the usual nucleation, elongation, and fibril formation stages, small molecules may promote alternative pathways, leading to the formation of amorphous aggregates or off-pathway oligomers[36]. Notably, these off-pathway aggregates are often more soluble and less resistant to proteolysis compared to their amyloid counterparts[17], allowing them to be more easily managed by the cellular protein quality control systems. This can limit the amount of protein aggregation and reduce the formation and deposition of amyloid plaques, thereby mitigating the disease pathology[37].

For example, epigallocatechin gallate (EGCG), a polyphenol found in green tea, has been shown to redirect amyloid aggregation of proteins such as α-syn and Aβ peptide associated with PD and AD, respectively [Figure 3C][36,38]. EGCG interacts with these proteins according to its aromatic rings and a large number of hydroxyl groups; it might induce intermolecular interactions between α-syn monomers that are stabilized by hydrogen bonds involving the hydroxyl groups, and thus action as a molecular zipper or seed, altering their aggregation pathway, leading to the formation of non-toxic, off-pathway oligomers[Figure 3D and Figure 3E][36]. Another small molecule, CLR01, employs a similar strategy. CLR01 uses a process known as molecular tweezers to disrupt the assembly of misfolded proteins into toxic aggregates, redirecting them towards forming non-toxic structures[39,40]. Solution-state NMR experiment elucidated that CLR01 could modulate the structure of early Aβ assemblies in a subtle way but is sufficient to render the resulting assemblies non-toxic and prevent their further aggregation. The highly labile binding of CLR01 for Lys residues supports the hypothesis that this labile binding is sufficient for interfering with the weak molecular interactions that lead to the formation of oligomers or nuclei [Figure 3F and G]. Furthermore, CLR01 was
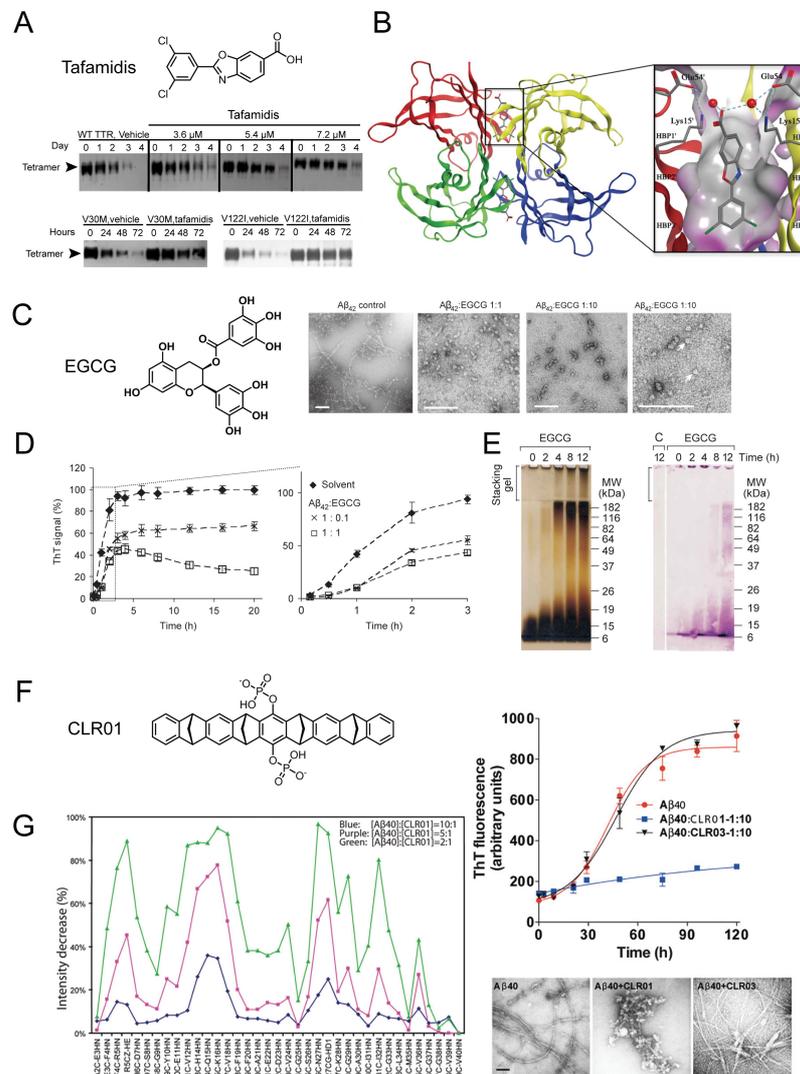
Figure 3. Small molecule to stabilize amyloid monomer and divert to off-pathway aggregates. (A) Tafamidis-mediated stabilization of TTR in plasma obtained from patients with wild-type (WT), V30M, and V122I alleles. Figure reproduced from Bulawa *et al.*[30]. (B) Crystal structure depicting the binding of tafamidis to TTR (PDB ID: 3TCT). The four TTR monomers are shown in distinct colors. Figure reproduced with permission from Bulawa *et al.*[30]. (C) Electron microscopy analysis comparing the aggregation reactions of Aβ_{42} with and without EGCG treatment. Figure reproduced from Ehrnhoefer *et al.*[36]. (D, E) Evaluation of the impact of EGCG on Aβ_{42} aggregation using ThT fluorescence measurements. (D) SDS-PAGE assays. (E) Reproduced from Ehrnhoefer, *et al.*[36]. (F) Inhibition of Aβ aggregation by CLR01. The impact of CLR01 on Aβ_{40} aggregation was evaluated using ThT fluorescence measurements and electron microscopy. The data was reproduced with permission from Sinha *et al.*[40]. (G) Identification of CLR01 binding sites on Aβ through solution-state NMR analysis. Figure reproduced with permission from Sinha *et al.*[40]. EGCG: Epigallocatechin gallate; NMR nuclear magnetic resonance; SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis; TTR: transthyretin.

found to block key interactions in the self-assembly of α-syn oligomer by binding to positively charged Lys residues Lys-10 and Lys-12 and disrupting both hydrophobic and electrostatic interactions, increasing the absolute net charge of the protein and weakening intramolecular long-range associations between N-terminal lysines and C-terminal aspartates and glutamate[39]

Thus, the development of small molecules to redirect amyloid proteins towards forming off-pathway aggregates represents a novel therapeutic approach for amyloid diseases[41]. By manipulating the aggregation pathway, these small molecules offer a potential solution to reduce the toxicity associated with amyloid

oligomers and fibrils[42].

Targeting the preformed pathological amyloid fibrils

Although the majority of current research concentrates on slowing or stopping the development of harmful amyloid aggregation, newly emerging studies suggest that disassembling the pathological fibrils could be just as critical in combating amyloid-related diseases. This method aims to break down established fibrillar clusters and counter their potential toxic impacts. Amyloid fibrils, possessing a distinct cross-β structure, offer significant stability, making them resilient to the protein degradation processes in cells, which results in their build-up in tissues[15,43]. Small molecules designed to interrupt these fibrils principally operate by destabilizing their structure, decomposing them into less organized, more soluble, and often less harmful species[44,45]. This is usually accomplished by interacting with the fibrils, which triggers alterations in the protein-protein interactions that maintain the fibril's integrity.

One such example is again EGCG, which has been shown to attach to and break down tau fibrils[46]. Cryo-electron microscopic (Cryo-EM) structure has revealed the mechanism by which EGCG directly binds to the tau paired helical filaments (PHF) extracted from post-mortem brains of AD patients [Figure 4A]. The complex structure shows that EGCG molecules stack in the two symmetrically related crevices created at the juncture of the two protofilaments[46]. Based on the EGCG molecules docking to the extra densities, two types of disruption mechanisms have been proposed [Figure 4A-C]. First, the polyphenolic groups of EGCG form hydrogen bonds with the side chains of tau located in the two clefts, bury the charged Lys340 residues, and therefore disrupt the interaction between the positive charge of Lys340 and the negative charges of Glu338, Glu342 [Figure 4A and B]. The repulsive forces caused by Glu residues in adjacent layers of tau molecules weaken the β-sheet hydrogen-bonding network of the fibril. The second proposed mechanism suggests that the inter-ligand interactions between stacked EGCG molecules further destabilize the fibril integrity. Although the complex structure shows that EGCG molecules stacked about 4.8 Å apart along the fibril axis, the inter-ligand π-π stacking can be further stabilized by reducing the distance between the solvent-facing aromatic rings to around 3.5 Å, falling in the favorable van der Waals contact for parallel π-π stacking [Figure 4C]. The compression of ligand-ligand distance induces a curve in the stack and increases the spacing on the fibril facing surface, possibly allowing water to solvate the separated tau molecules. This mechanism transforms the binding energy between stacked EGCG molecules into a conformational change that separates stacked tau molecules.

Given EGCG's limited suitability for targeting the central neuron system, in silico screening was used to identify new disaggregate molecules with better drug attributes, using the EGCG pharmacophore as a docking site[46]. Four molecules, named CNS-11, CNS-17, CNS-2, and CNS-12, were discovered. Of these, CNS-11 exhibited disaggregation activity similar to that of EGCG, destabilizing the inter-layer spacing through molecular dynamics, with the most significant disturbances centered on Lys340 and additional disruptions seen at Ser341 and Glu342[46]. The same compound was also demonstrated to disassemble α-syn fibrils extracted from multiple system atrophy (MSA) patient brains and prevent their intracellular seeding[47].

Curcumin, a natural polyphenol derived from turmeric, which can exist in an equilibrium between keto and enol tautomers[48], has been discovered to attach to Aβ oligomers and fibrils and disassemble their structures[49,50]. Its properties of fibril-destabilization have positioned it as a potential therapeutic compound against AD. Both in vitro and in vivo studies have demonstrated that curcumin can significantly clear existing amyloid deposits, and remaining plaques are reduced in size when newly formed or completely cleared plaques are excluded from the analysis[51]. Furthermore, *in vivo* studies have observed significantly
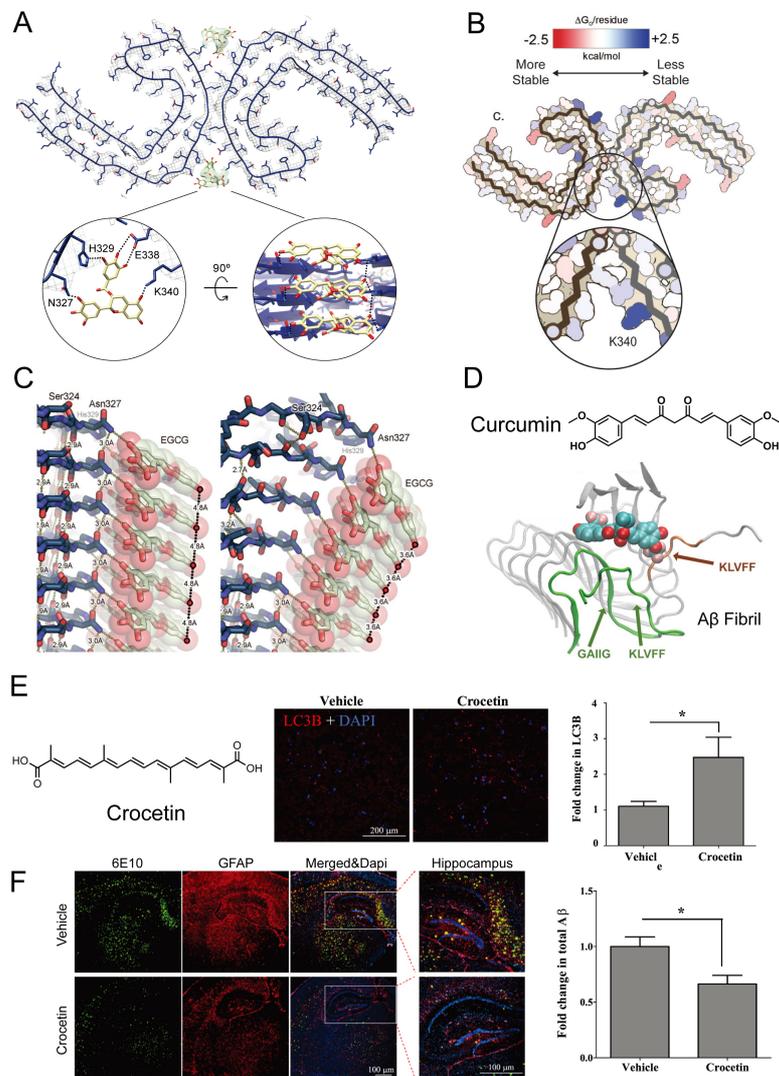
Figure 4. Disaggregating binder and PQC enhancer for protein amyloid aggregates. (A) Cryo-EM structure depicting the complex of EGCG with AD-tau PHF. EGCG assumes a primarily planar conformation, with stabilization facilitated by π-π interactions of stacked aromatic rings, reproduced from Seidler *et al*.[46]; (B) Solvation energy difference maps illustrating the comparison between tau PHF structures in the absence and presence of EGCG. Red residues indicate increased stability, while blue residues indicate decreased stability. A notable increase in the free energy of Lys340 at the EGCG binding site is observed, reproduced from Seidler *et al*.[46]. (C) The stacking of EGCG molecules at an approximate distance of 4.8 Å enables each EGCG molecule to establish hydrogen bonds with specific stacked tau molecules. The curvature of the EGCG stack leads to an expanded spacing on the fibril-facing surface. Figure reproduced from Seidler *et al*.[46]. (D) Molecular docking of curcumin in complex with a hexamer peptide model representing the Aβ$_{1-42}$ fibril. The docking simulations demonstrate the partial dissociation of the outermost peptide, reproduced from Jakubowski *et al*.[53]. (E) Crocetin treatment in 5XFAD mice resulted in significantly elevated levels of LC3B (red), reproduced from Wani *et al*.[64]. (F) Crocetin treatment resulted in a significant reduction of brain Aβ load in 5XFAD mice. Representative brain sections and corresponding quantification analysis show the total Aβ levels (6E10 antibody, green) and activated astrocytes (GFAP antibody, red), reproduced from Wani *et al*.[64]. Cryo-EM: Cryo-electron microscopic; EGCG: epigallocatechin gallate; GFAP: glial fibrillary acidic protein; PQC: protein quality control.

reduced amyloid levels in aged mice with established amyloid deposition after treatment with curcumin[49]. The molecular dynamics simulation results show that the aromatic ring of curcumin can interact with amino acids such as histidine, tyrosine, and phenylalanine, thus getting inserted into Aβ fibril [Figure 4D]. Its symmetrical methoxyl and phenyl groups can easily bind to Aβ fibrils via hydrophobic interactions with nonpolar sections. These interactions can be stabilized by curcumin's di-ketone and hydroxyl groups with

polar sections of Aβ fibrils through hydrogen bonding [Figure 4D][52,53].

Recent evidence found that in various in vitro and in vivo models of AD, Resveratrol (3,5,4'-trihydroxy-trans-stilbene, Res), a naturally polyphenolic phytoalexin containing two aromatic rings in its structure with two isomers: trans-Res, which has been most widely studied, and cis-Res, which is less stable and potent than the trans form, abundantly found in grapes, berries, red wine and many other plant species, shows diverse biological activities in attenuating Aβ-induced cytotoxicity, apoptosis and intracellular reactive oxygen intermediate (ROI) through disrupting preformed Aβ aggregation by binding directly to Aβ fibril[54,55].

Considering the structural commonalities of polyphenols, these molecular ligands may share a generally similar mechanism with EGCG, curcumin, and Res, shedding light on their potential to be developed into amyloidosis therapies[56]. The direct insertion of the compounds into the fibril's β-sheets could reveal the disassembly mechanism, whereby these small compounds could interact with the β-turn of fibrils through hydrogen bonding, hydrophobic interaction, or π-π stacking, thus breaking down the fibril structure into smaller fragments[53]. However, the difference among these compounds brings adjustment in the way they disassemble the fibrils to varying degrees, which requires exact structural analysis[51]. Additionally, it is important to acknowledge that while disrupting amyloid fibrils might alleviate disease pathology, the breakdown products, especially smaller oligomers, may remain toxic. Thus, the ideal therapeutic strategy may involve not only fibril disruption but also mechanisms to neutralize or eliminate the resulting potentially harmful species[47].

Other than targeting and disrupting the performed fibril, several researches also focused on the secondary nucleation, which provides a catalytic cycle generating toxic oligomers by the surfaces of high molecular weight fibrillar aggregates. Previous studies have shed light on the effect of the chaperone domain BRICHOS[57], which consists of about 100 amino acid residues, in performing the anti-aggregation function of Aβ42[58]. Through image from transmission electron microscopy (TEM) and kinetic analysis, it has been verified that the BRICHOS domain of human prosurfactant protein C (ProSP-C) interacts specifically with fibrillar but not monomeric Aβ42 through directly binding to the fibril[58]. Suppressing the secondary nucleation process directs the reaction pathway towards elongation events, thereby preventing almost entirely the generation of oligomers, delaying the formation of low molecular weight oligomeric species and hence reducing dramatically the toxicity associated with the aggregation reaction[59]. Furthermore, recent research has uncovered that BRICHOS domain from Bri2 (also known as ITM2B, integral membrane protein 2B) also prevents secondary nucleation and the consequent increase in the neurotoxic water-soluble oligomeric form of Aβ42[60]. The result of immunogold labeling and electron microscopy demonstrated that Bri2 BRICHOS chaperone covers the Aβ42 fibril surface more extensively than ProSP-C BRICHOS, and also binds more efficiently to fibril ends. Additionally, the more efficacious Bri2 BRICHOS chaperone rescued the cellular and neuronal network performance from Aβ42-induced functional impairments, thus showing promising therapeutic potential for Alzheimer's disease[60].

In summary, creating small molecules to target amyloid fibrils is a promising new approach in the battle against pathological amyloid aggregation. By actively disassembling these pathological clusters, this strategy has the potential to not only slow the progression of the disease but also possibly reverse it. Instead of targeting the fibrils at the plateau phase, the performed fibrils generating a catalytic "milieu" for secondary nucleation at the elongation state also provide an inspiring target against amyloidosis. Inhibiting the process of secondary nucleation by small molecular chaperones reduces the amount of oligomers, thus alleviating the toxicity. Nonetheless, further research is required to understand the exact structural mechanisms of

targeting performed fibril, and ensure that the products of the breakdown can be effectively managed by the body to minimize potential toxicity.

Enhancing the protein quality control system to combat amyloid aggregation
Small molecules not only have the ability to disaggregate fibrils directly, but they can also work in concert with the protein quality control system to disrupt the formation and facilitate the clearance of pathological amyloid aggregation[61]. The protein quality control system, chiefly constituted of chaperones and the proteostasis network, is critical for preserving cellular protein homeostasis, encompassing aspects such as proper folding, assembly, trafficking, and degradation[62,63]. During conditions marked by amyloid aggregation, this system gets inundated or dysfunctional, resulting in an escalation of misfolded proteins and the creation of toxic amyloid aggregates[64]. Two major pathways for the clearance of misfolded proteins and amyloid aggregates include the autophagy-lysosomal pathway (ALP) and the ubiquitin-proteasomal system (UPS)[65]. Consequently, small molecules that can bolster these related clearance mechanisms could potentially become preclinical candidates for developing new treatments to mitigate pathological amyloid aggregation.

An example of this is crocetin, an active compound found in the flower stigmas of *Crocus sativus*, which has been shown to boost the autophagy clearance of Aβ via the STK11/LKB1-mediated AMPK pathway[64,66], effectively reducing Aβ levels and neuroinflammation in vitro. Crocetin activates STK11 (serine/threonine kinase 11), which further phosphorylates and activates AMPK (AMP-activated protein kinase) [Figure 4E]. With the upregulation of major autophagosome initiation proteins, the ALP was triggered in order to escalate Aβ aggregates. The effect of crocetin as an inducer of autophagy to treat AD is revealed in both cultured neuronal cells and transgenic rodent models of AD [Figure 4F][64].

Another proposed strategy involves small molecules amplifying chaperone activities, indirectly stimulating the clearance process. Recent research has identified the presence of several heat shock proteins (HSPs, part of the chaperone protein family) such as HSP104, HSP70, and HSP40 alongside α-syn amyloid aggregates in Lewy bodies (LBs), the key hallmark of PD[67]. These proteins seem to work in unison with cellular clearance mechanisms or directly target the aggregates to improve the pathological condition[68]. Given that the functions of HSPs range from inhibiting α-syn aggregation and dismantling α-syn oligomers to participating in the ubiquitination and proteasomal degradation of α-syn[69], bolstering their activity offers a promising avenue to mitigate α-syn accumulation. Recently, a small molecule named Protopine-Br (PRO-Br), the brominated derivative of protopine, which is a dibenzazecine alkaloid extracted from Corydalis and Fumaria species, was identified to promote pathological tau fibril degradation by activating chaperone-mediated autophagy (CMA), both *in vitro* and *in vivo*[70]. PRO-Br can enhance the expression of HSC70 and lysosomal-associated membrane proteins 2A (LAMP2A) while mediating the interaction between them, thus clearing tau inside the lysosomal lumen in AD models via the CMA pathway. Furthermore, the in vivo study elucidated that PRO-Br treatment dose-dependently increased the chaperones HSP70 and HSC70, and promoted the acetylation of HSP90, collectively mediating the CMA to clear pathological tau clearance in AD models[70].

However, these promising strides are accompanied by several challenges. A significant concern lies in the selectivity and specificity of these small molecules, with a crucial need to ensure they target only the problematic proteins without perturbing normal cellular protein homeostasis. Furthermore, given the complexity of the protein quality control system, it is crucial to fully comprehend the precise mechanisms and effects of these small molecules to prevent any inadvertent consequences.

CONCLUSION

As we reflect on the advancements in developing small molecules for disrupting pathological amyloid aggregation in NDs, we are confronted with both enormous potential and significant challenges. These agents represent an exciting frontier in the therapeutic landscape of conditions such as AD, PD, and ALS. However, the path to translating these small molecules into effective therapies is fraught with challenges that require careful navigation and diligent research.

A critical barrier in developing effective therapies for NDs is the blood-brain barrier (BBB), which restricts the access of many potential therapeutics into the brain[71]. Small molecules designed to disrupt amyloid aggregation must be capable of crossing this barrier to reach their targets. However, the previous study demonstrated that curcumin and resveratrol exhibit poor BBB permeability, thus limiting their potential for transitioning to clinical treatment, compared to the EGCG which presents high permeability and is more promising for therapy[72,73]. Although Crocetin has been proved to be a promising candidate for AD treatment, its poor water solubility and bioavailability are the major obstacles in pharmaceutical applications[74]. Therefore, future research should focus on optimizing the physicochemical properties of these molecules to enhance their BBB penetration. For example, a novel water-soluble CRT-γ-cyclodextrin inclusion complex suitable for intravenous injection was developed, which significantly increased the bioavailability of Crocetin and facilitated Crocetin crossing the blood-brain barrier to enter the brain[75]. Additionally, alternative strategies, such as nanoparticle-based delivery systems, could also be explored to bypass this obstacle[76].

Another major challenge is ensuring target selectivity. Given the complex protein network in neurons, small molecules must be designed to specifically target and disrupt the pathological aggregates without interfering with normal protein functions. The potential off-target effects of these molecules could lead to unwanted side effects and complications. Advanced computational modeling and structure-based drug design strategies could be beneficial in improving the selectivity and potency of these molecules[77].

Moreover, the safety profile of these small molecules must be thoroughly evaluated. As these therapeutics may require long-term use, it is crucial to assess their potential toxicities, such as hepatotoxicity or nephrotoxicity, and any other long-term health impacts. Preclinical studies using appropriate animal models, along with careful clinical trial design, will be instrumental in addressing these safety concerns. Furthermore, some molecules mentioned in this review have been tested in patients with amyloidosis, and their clinical outcomes have been reported. For example, diflunisal therapy in transthyretin cardiac amyloidosis was proved to be well tolerated and safe in the major patient population without significant adverse events in previous studies, while there are still a smaller number of patients had to stop treatment due to gastrointestinal side effects and transient renal dysfunction[78]. Chinese Parkinson Study Group (CPSG) evaluated the efficacy and safety of green tea polyphenols therapy including EGCG in 2009, and the procedural result showed that the treatment was well tolerated without severe adverse effects except slight insomnia and positively provided mild symptomatic relief in early untreated PD[79].

Nonetheless, to date, clinical trials have failed to identify most molecular compounds with compelling disease-modifying properties. The major reason for this drawback is the lack of a reliable tracer that can be used to track disease progression and make accurate diagnoses, thus assessing the clinical outcome[79]. Short-term follow-up and the small cohort also result in limitations, and some studies mentioned that the lack of large, randomized controlled trials in amyloidosis treatment is also a vexed question[78]. Additionally, we refrain from covering small molecules that target amyloid oligomers in this review due to the scarcity of available structural information. In certain disease contexts, oligomers are considered pathogenic species of

amyloid proteins, as observed with Aβ oligomers in AD[80,81]. In this regard, amyloid fibrils may serve as a protective mechanism, suggesting that the clearance of amyloid fibrils may be a less efficient strategy for therapeutic intervention.

Despite these challenges, the development of small molecules for disrupting amyloid aggregation provides immense opportunities. With the rapid advancement of technologies such as high-throughput screening, high-resolution structure-based in silico drug design, and precision medicine, we are better equipped than ever to design, screen, and optimize these small molecules[46]. Using the present candidate molecules that can bind to and disrupt specific fibrils as the lead compounds for high-throughput screening, molecules with higher efficiency can be screened in the small molecule compound library. Moreover, rational design of the therapeutic molecules will optimize the clinical effect of small molecules through the introduction of different functional groups and the modification of the scaffold, thus overcoming the challenge and providing a promising future for the treatment of ND. Molecular engineering, such as proteolysis targeting chimera (PROTAC) based on specific small molecule binders, may provide opportunities for selective degradation of amyloid aggregates. Furthermore, the increasing understanding of the molecular mechanisms underlying amyloid aggregation could open new avenues for targeted therapy.

Developing small molecules is on the host spot of the research field searching therapies for neurodegenerative diseases. The capabilities of these molecules extend beyond just targeting amyloid; they may also counteract the dysfunction caused by neurodegeneration[82]. A therapeutic potential for compounds such as EX15 and RE01 has been discovered to modulate Kv3.1/Kv3.2 potassium channels on fast-spiking interneurons (FSN), resulting in faster activation kinetics and increased firing frequency, suggesting improvement for cognition-relevant gamma oscillations (30-80 Hz), particularly in a situation where network activity is pathologically compromised in the presence of neurotoxic Aβ[83]. Additionally, considering the importance of gamma oscillations in cognitive functions and its relationship with AD, some non-targeted strategies, such as gamma frequency (40 Hz) entrainment, have also been proved to recruit both neuronal and glial responses to attenuate AD-associated pathology in the rodent brains, thus avoiding the off-target side effects[84]. Recent research uncovered that optogenetically driving FS-PV-interneurons at gamma (40 Hz), but not other frequencies, reduced levels of Aβ1-40 and Aβ1-42 isoforms in visual cortex of pre-depositing mice and mitigated plaque load in aged mice, which promotes the development of indirect therapy for AD[84].

In conclusion, the development of small molecules to disrupt pathological amyloid aggregation signifies a promising but challenging path in the fight against NDs. As we forge ahead in this exciting area of research, a balanced view of the immense potential and the formidable challenges will help guide the way towards effective therapies. Above all, further research is needed to elucidate the precise mechanisms of fibril disruption, ensure safety, and optimize drug delivery to truly realize the potential of these small molecules in improving the lives of patients afflicted with NDs.

DECLARATIONS
Authors' contributions
Performed data analysis, prepared figures, and wrote the manuscript: Cao T, Li X, Li D, Tao Y

Availability of data and materials
Not applicable.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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