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Inhibitors of protein aggregates as novel drugs in neurodegenerative diseases

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Abstract

Neurodegenerative diseases commonly present misfolding and aggregation of one or more proteins, including primarily β-Amyloid, α-synuclein and tau. Over the last decades, several efforts have been made to understand the molecular processes at the basis of such pathological aggregation, as well as in the development of therapeutic strategies targeting these diseases. Among these, the inhibition of protein aggregation with natural compounds, peptides or small molecules has been considered a promising pharmacological approach. Here in, the most promising molecules developed to reduce protein aggregation will be summarised and discussed on the light of their effects in pre-clinical and clinical studies.

Introduction

Neurodegenerative diseases (NDs) are characterised by the misfolding and aggregation of specific proteins [1]. For example, the pathological hallmarks of Alzheimer's disease are represented by β-Amyloid (Aβ) peptide-containing plaques and intraneuronal neurofibrillary tangles composed of hyperphosphorylated protein tau. Meanwhile, Parkinson's disease (PDs) presents brain inclusions of α-synuclein (α-syn), which constitutes the major component of Lewy bodies and Lewy neurites [2-4]. Such pathological protein aggregation relates to a complex self-assembly process involving the formation of small oligomers, larger protein complexes, and mature β-sheet-rich fibrils. Whether prefibrillar aggregates (oligomers, protofibrils) or fibrils are accountable for neuronal death in NDs remains to be established [1,5].

Different therapeutic strategies attempting to reduce brain burden of protein aggregates have been developed [5] including: i) direct targeting of misfolded proteins; ii) drawing the protein excess out of the brain by peripheral administration of oligomer-binding agents (the so-called "sink effect") [6-8]; iii) upregulating molecular chaperones or proteins involved in aggregate clearance [5]; iv) targeting post-translational modifications that promote protein misfolding and aggregation [5]; v) the use of nanotechnological devices, and in particular multifunctional liposomes [9,10]. Herein, current literature on compounds able to prevent/disrupt protein aggregation, thus removing toxic oligomers, will be summarised [11].

α-Syn aggregation inhibitors

The α-syn oligomerization inhibitors have been deeply investigated (Table 1). Among synthetic compounds able to counteract α-syn oligomerization in preclinical studies, NPT200-11 [12] and ANLE138b [13,14] are rising as promising tool for the PD treatment. In particular, NPT200-11 has been shown to cross the blood–brain barrier with low toxicity in control subjects, completing successfully a phase I clinical trial (ClinicalTrials.gov Identifier NCT02606682).

Small peptides able to control oligomerization have been developed as β-sheet breakers, taking into account that the aggregation site involves the 71-82 region of α-syn. In this respect, unmodified peptides [15] and N-methylated peptides [16] have been recently discovered and demonstrated their efficacy *in vitro*. Studies are ongoing to collect clinical data and additional information on the use of peptide able to induce a correct protein folding [17,18].

Among natural-based compounds, polyphenols such as baicalin [19], EGCG [20,21], tannic acid (TA) [22], resveratrol [23] and curcumin [24] have been emerging as potent molecules able to decrease α-syn assembling into oligomers. However, almost all the studies on these compounds are limited to the pre-clinical phase.

Another pharmacological approach is represented by active or passive immunization based on α-syn antibodies [25]. Such immunogenic peptides mimic the C-terminus of α-syn [26], or can direct bind the oligomeric form of α-syn, or the Ser129 phosphorylated site of α-syn [27], which is a crucial site for α-syn accumulation [28].

Tau aggregation inhibitor

Tau aggregates have been targeted using several approaches, which have been reviewed elsewhere [29,30]. In the last decade, different classes of tau aggregation inhibitors (TAIs) have been reported, including polyphenols [31], porphyrins [32], phenothiazines such as Methylene blue [32], benzothiazoles/cyanines such as N744 and Riluzole [33], thioxothiazolidinones (rhodanines), phenylthiazole-hydrazides, anthraquinones, and aminothienopyridazines (ATPZs) [30,33] (Table 2).

Furthermore, small molecules belonging to TAIs have already been developed and tested in humans [29,34,35], even if with discrepancy

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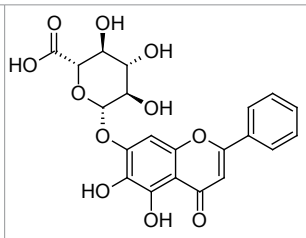
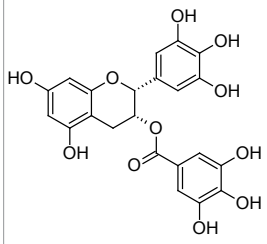
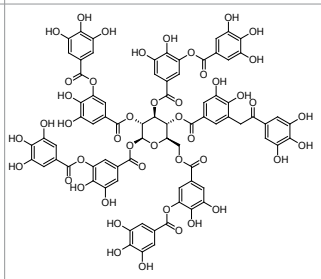
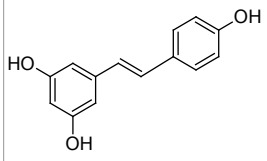
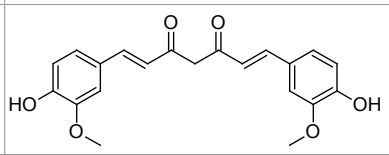
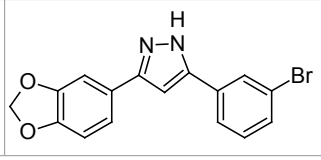
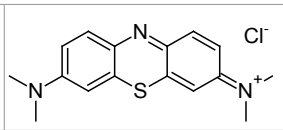
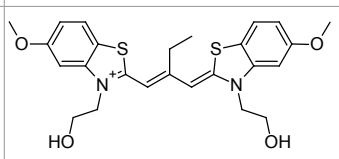
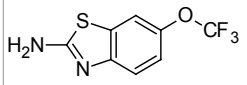
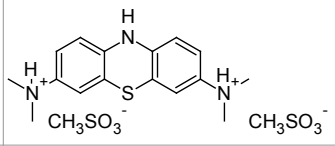
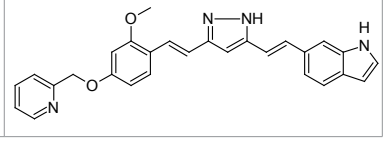
Table 1. Structures of the mainα-syn aggregate inhibitors.

Protein	Compound	Structure
α-syn	*Small molecules*	
	Baicalin	
	Epigallocatechin gallate (EGCG)	
	Tannic acid (TA)	
	Resveratrol	
	Curcumine	
	ANLE138b	

between the cell-based and/or *in vitro* data and the *in vivo* efficacy. Important pharmaceutical implications have been rising from the possibility to distinguish the tau–tau binding interaction from the tau–tubulin binding one with new aggregation inhibitors [36,37]. Among these, the most promising compound is leucomethylthioninium (LMT, leucomethylene blue (MB), LMTX, TRx0237), developed by TauRxTherapeutics Ltd., Republic of Singapore, which is a second-generation TAI for AD treatment. The numerous clinical trials that are currently ongoing for this compound and its derivatives (ClinicalTrials.gov Identifier NCT01626391, NCT01689233, NCT01689246, NCT01626378, NCT02245568) are failing, probably due to the subject advanced stage of the pathology.

Several other chemical entities and compounds have been reported

Table 2. Structures of the main Tau aggregate inhibitors.

Protein	Compound	Structure
Tau	*Small molecules*	
	Methylene blue	
	N744	
	Riluzole	
	TRx0237 (LMT, leucomethylene blue, LMTX)	
	PE859	

[38-40]. A new compound, PE859 (3-[(1E)-2-(1H-indol-6-yl)ethenyl]-5-[(1E)-2-[2-methoxy-4-(2-pyridylmethoxy)phenyl]ethenyl]-1H-pyrazole) has been shown to inhibit *in vitro* tau aggregation and to delay the onset and progression of motor dysfunction *in vivo* [38]. Moreover, 1,2-dihydroxybenzene-containing compounds have been shown to reduce tau oligomerization [39] *in vitro* or *in vivo*.

Aβ aggregation inhibitor

Small derivatives have been developed that are able to interfere with Aβ aggregation by decreasing the oligomerization process and/or by inducing a conformational change in β-sheet assembly and/or by inducing quick conversion of soluble aggregates into less toxic fibrils (Table 3) [1,41].

Among natural compounds, most Aβ inhibitors present a polyphenolic core [42], including resveratrol [43], myricetin [44], curcumin [45], caffeine [46].

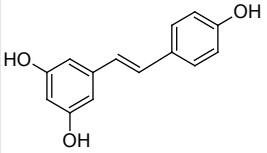
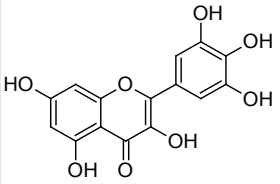
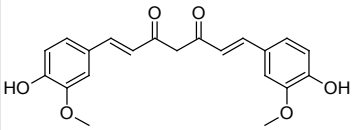
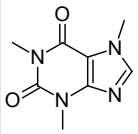
As concern, newly synthesised compounds, ALZ-801 is an orally bioavailable prodrug of Tramiprosate, which has been demonstrated to reduce Aβ oligomers and neurotoxicity.

Moreover, ALZT-OP1 results from the combination of two FDA-approved drugs, cromolyn and ibuprofen. Consequently, it decreases neuronal death by reducing Aβ accumulation and suppressing neuroinflammation in the brain [47].

Another interesting small molecule is CSP-1103, able to inhibit brain deposition of Aβ plaques, reducing tau pathology and neuroinflammation, and reversing memory deficits in AD transgenic mouse model. Additionally, CSP-1103 restores normal microglial function by increasing phagocytosis and decreasing production of pro-inflammatory cytokines.

ELND005 is an orally bioavailable inositol stereoisomer that

Table 3. Structures of the mainAβ aggregate inhibitors.

Protein	Compound	Structure
Aβ	*Small Molecules*	
	Resveratrol	
	Myricetin	
	Curcumine	
	Caffeine	
	New compounds	
	ALZ-801	Prodrug of Tramiprosate
	ALZT-OP1	Combination of cromolyn and ibuprofen
	CSP-1103	
	ELND005	Inositol stereoisomer
	Immunization therapy	
	Aducanumab (BIIB037)	Fully human IgG1 monoclonal antibody
	Crenezumab	
	Solanezumab	Humanized monoclonal IgG1

causes a dose-dependent decrease in amyloid pathology and plaque accumulation in TgCRND8 mice. The completed Phase II AD study demonstrated ELND005 treatment led to reduction in myo-inositol levels in the brain, an effect that is shared by other approved neuropsychiatric drugs such as lithium and valproic acid. In addition, ELND005 has been associated with a reduction in the levels of Aβ and tau proteins in the cerebrospinal fluid.

Another therapeutic approach targets the nucleation site of aggregation. This region, known as the KLVFFA, is an hexapeptide sequence that facilitates monomer-monomer interaction, leading to dimer and oligomer formation [48,49]. Based on these findings, a few compounds have been identified and demonstrated to interact with the KLVFFA region [50].

Among the Aβ-anti-aggregating strategies, an anti-Aβ immunotherapy approach has been emerging. In particular, antibodies that recognize Aβ toxic species have been developed to bind and neutralize them; otherwise, the antibodies can stimulate microglial clearance, or induce Aβ exit from the brain [51-57].

Among antibodies, Aducanumab (BIIB037) is a high-affinity, fully human IgG1 monoclonal antibody against a conformational epitope found on Aβ, which is now in phase III trial. On the other hand, Crenezumab recognizes multiple forms of aggregated Aβ, including oligomeric and fibrillar species and amyloid plaques with high affinity. It has been engineered to clear Aβ excess and stimulate amyloid phagocytosis while limiting release of inflammatory cytokines, as a way to avoid side effects such as vasogenic edema [53].

Finally, Solanezumab is a humanized monoclonal IgG1 antibody directed against the mid-domain of the Aβ peptide, which recognizes soluble monomeric Aβ. It has been designed to sequester Aβ, shifting equilibria between different species of Aβ, and removing small soluble species of Aβ that are directly toxic to synaptic function [58].

Conclusions and future perspectives

Despite several efforts, inhibitors of Aβ, α-syn and tau protein deposition have failed in clinical trials. The inadequacy of the disease-modifying strategy, and the stage of the disease during the drug administration are only two of the reasons at the basis of the clinical trial failure. Indeed, several findings have suggested that the pharmacological treatment of NDs should start prior to the onset of clinical symptoms [59], and that each inhibitor may have a precise temporal window in which to be used, depending on the ND stage [1].

Drug combinations that capitalise on more than one therapeutic strategy will constitute the most effective treatment for NDs. Furthermore, the cooperation of Aβ, tau and α-syn in the pathogenic processes of NDs [60-62] is opening the way to broad-spectrum compounds potentially able to reduce the oligomerization of more than one protein. Rifampicin, a well-known antibiotic, can reduce prevent the aggregation of Aβ, tau and α-syn in vitro [62] and in a mouse model of AD. Future advance may be represented by the development of agents able to interfere with hybrid oligomers.

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