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Small molecular decoys in Alzheimer's disease

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Recent progress in the treatment of Alzheimer's disease (AD) using antibodies against amyloid sustains amyloid generation as a key process in AD. Amyloid formation starts with two amyloid-beta (Aβ) molecules interacting (dimer formation) followed by an accelerating build-up of so-called protofibrils, which turn into fibrils, which accumulate in the characteristic plaques. To interfere with the process at the root we used molecular modeling to define the surfaces of interaction in dimer formation. In a series of small molecules, we identified candidates that changed the course of interactions and generated aggregates with other macrostructures and reduced toxicity. We have introduced the term "decoys" to identify these molecules.

To combat AD is a medical challenge that has just begun to show progress. Although already described by the neuropathologist Alois Alzheimer more than 100 years ago, it has long been considered an incurable condition of aging without remedy. However, the finding of a characteristic deposition of amyloid in the AD brain has been seen as a lead. Although this so-called amyloid cascade hypothesis has been criticized, there is compelling evidence through genetics and experimental work that it is central to the disease. A treatment breakthrough has occurred recently, with the generation of monoclonal antibodies (mabs) against Aβ in early protofibrils which show therapeutic potential (Sims et al., 2023; van Dyck et al., 2023). This raises hopes that finally, this disease is curable, whereas several issues remain. Although lecanemab (Leqembi®) was recently (July 2023) approved fully by the US Food and Drug Administration, the treatment is very costly and potentially harmful and only slows down the course of the disease. From a principal point of view, it seems illogical to attack the already formed toxic Aβ aggregates. A more rational way would be blocking their formation. However, the enzymatic process central to the generation of the 40 to 42 amino acid Aβ peptide by the enzymes presenilin and β-secretase is unspecific, and blocking interferes with the degradation of several other proteins. An additional possibility would be the inhibition of Aβ aggregation. In our earlier work, we developed technology for following Aβ aggregation *in vitro* using fluorescence correlation spectroscopy, a technique that could in real time monitor the aggregation as an increase in molecular weight via reduction of diffusion of fluorescently labeled Aβ peptide aggregates. We identified a central sequence for aggregation, $A\beta_{16-20}$ (KLVFF), and found that by adding this peptide (or an extended variant) we could block the aggregation (Tjernberg et al., 1999). This epitope has been confirmed in several other studies.

By and large, peptides are not ideal drugs, even with high specificity to the target, due to degradation by peptidases and a limited penetrance of blood-brain barriers. Small molecules would be advantageous in such situations. We have taken the initiative to search for potential inhibitors in a library of molecules in the NCI compound depository (Nicklaus MC, 2022; Oasa et al., 2023). We reasoned that by repurposing (Begley et al., 2021), the likelihood of identifying leads would be favorable as compared to random compound libraries. The target would be the first step in the aggregation process, the docking of two $A\beta_{42}$ molecules (dimer formation) acting prophylactically in individuals with known genetic disposition, risk factors or just subjected sporadically to become AD victims.

A further consideration was that the aggregation is built on the combined affinity based on interaction at different sites (epitopes) which might contribute by a zipper mechanism. We further decided to use the classic fluorescent probe, thioflavin T recognizing a β-bend structure of Aβ aggregates to monitor the process of the aggregation using fluorescence correlation spectroscopy. It was immediately clear that a few compounds, particularly with an extended structure and named bifunctional, remarkably affected the aggregation process in this test. In total, five active compounds were identified. By transmission electron microscopy, we observed that there were still aggregates, but their macroanatomy was altered. As shown in the paper (Oasa et al., 2023), we could identify several macrostructural patterns not normally observed, protofibrils with buds and branches, double-stranded aggregates (two thin filaments glued together), or a two-dimensional network. These modifications blocked fibril formation. Importantly, several of the compounds reduced toxicity in cultured cells. Testing was directed both against $A\beta_{40}$ and $A\beta_{42}$ (Figure 5 in Oasa et al., 2023). Based on these data, we have selected 3 compounds for this commentary. Structures are illustrated in **Figure 1**.

Compound #4 is the most potent in the series with an IC50 = 40 nM (concentration at half-maximum effect) against $A\beta_{40}$. The macrostructure of the aggregates is protofibrils with buds and branches, also observed with other compounds. The transformation completely blocked the formation of fibrils.

Compound #5 has no affinity for $A\beta_{40}$ is roughly equipotent with #4 in blocking $A\beta_{42}$ toxicity, but gives another opening by the observation that it is selective against $A\beta_{42}$. It is well-known that $A\beta_{42}$ is more toxic and is used in clinical diagnosis as a biomarker in cerebrospinal fluid or plasma (Olsson et al., 2016; Schindler et al., 2019). Selectivity of action is a hallmark in pharmacotherapy. The aggregate induced by compound #5 has a similar macrostructure as those induced by the elongated structures (such as compound #4) which act on the aggregation of $A\beta_{40}$ and $A\beta_{42}$ indiscriminately.

The unique selectivity of compound #5 against $A\beta_{42}$ can be related to observations using cryo-electron microscopy showing differences in the macroanatomy of $A\beta_{40}$ and $A\beta_{42}$ dimers. Intermediate aggregates are built up with 2n Aβ molecules, which explain why an addition of two amino acids has such pronounced consequences (Schmidt et al., 2015).

The significance of the $A\beta_{42}$ C-terminus was illustrated by a systematic study of C-terminal peptides that could inhibit $A\beta_{42}$-induced toxicity (Fradinger et al., 2008). The smallest active peptides were $A\beta_{31-42}$ and the C-terminal hydrophobic tetrapeptide, $A\beta_{39-42}$ with affinity in the 10–20 μM concentration range. They postulated, based on molecular modeling that the peptide fragments co-assembled with $A\beta_{42}$ to form hetero-oligomers and thereby reduced neurotoxicity. In analogy, compound #5 has a hydrophobic polycyclic moiety which could constitute hydrophobic binding potential and provide selectivity for $A\beta_{42}$.

Compound #2–2 eliminates the toxicity of both $A\beta_{40}$ and $A\beta_{42}$ and induces the formation of double-stranded aggregates with thin protofibrils. It is also very potent, with an IC50 = 120 nM against $A\beta_{40}$. Since toxicity is eliminated, this may be related to the change in aggregate macroanatomy. The same is true with compound #7, which is not discussed here, which by generating a two-dimensional network also eliminates toxicity.

This commentary introduces an intervention strategy that emphasizes the studying of macrostructures of aggregates in neurodegenerative disease and opens a vista to new pharmacotherapies. The transformatory properties of the compounds are predicted to be induced already at the dimerization stage. In a strict sense, they are not inhibitory of aggregation but turn it into non-toxic end-products. The term "decoy" is used to illustrate this action mechanism. Mechanistically it would seem an attractive alternative to antibody adsorption which is directed towards protofibrils/fibrils at a later stage of an aggregation process and would not interact with toxic intermediates in the oligomer state. Essentially a similar approach has been taken earlier, where a series of small molecules were tested (at much higher concentration) for interference of aggregation (5–50 μM) and colloid formation (100–200 μM; Feng et al., 2008). Our toxicity tests show strong activity at 3 μM concentrations.

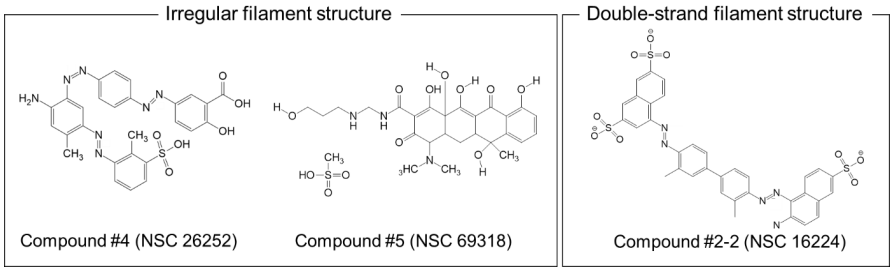

Figure 1 | **Small molecule decoys.**
Compounds were categorized by macrostructure of modified Aβ aggregates. The compound numbering is the same as that in the original publication (modified from Oasa et al., 2023). Compound structures were drawn with ChemDraw software.

Studies using other approaches address pathology characteristics of this disease built on amyloid deposition in the AD brain. Super-resolution microscopy and electron microscopy have characterized the macro-structures of the depositions isolated from disease-affected brains. Several of our observations corroborate with these other studies. What is new is the availability of small molecular tools that address the characteristics of the disease. For example, compound #5 selectively suppresses the $A\beta_{42}$ aggregation which is known to be more toxic than $A\beta_{40}$, thus it may allow us to clarify which Aβ species make a stronger contribution to the disease. The approach can also be complementary to other approaches including immunotherapy, now in the spotlight. AD is a common disease and to win against this scourge will require treatments that can be made accessible.

Final remarks: The pharmatherapy of AD is challenging and many attempts have failed. Recently, monoclonal antibodies against intermediate-size aggregates (protofibrils) have shown therapeutic effects. Our approach is the earliest step in the multistep trajectory and therefore theoretically more able to halt the aggregation at any step. It has repeatedly been shown that different oligomers are the most toxic (De et al., 2019). There is even evidence it might be possible to develop agents selective for $A\beta_{42}$ the most toxic principle, leaving $A\beta_{40}$ to aggregate which might be beneficial. A small molecule in a tablet or a nasal spray would be ideal for self-medication. Future experimentation to optimize the molecular profile, its pharmacokinetics, and the absence of general toxicity will add to further progress using this approach.

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