

Review

Small molecule inhibitors of amyloid β peptide aggregation as a potential therapeutic strategy for Alzheimer's disease

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Amyloid β (A β) peptides have long been viewed as a potential target for Alzheimer's disease (AD). Aggregation of A β peptides in the brain tissue is believed to be an exclusively pathological process. Therefore, blocking the initial stages of A β peptide aggregation with small molecules could hold considerable promise as the starting point for the development of new therapies for AD. Recent rapid progresses in our understanding of toxic amyloid assembly provide a fresh impetus for this interesting approach. Here, we discuss the problems, challenges and new concepts in targeting A β peptides.

Keywords: Alzheimer's disease; amyloid β peptide; protein-protein interaction; small molecule

Acta Pharmacologica Sinica (2011) 32: 545–551; doi: 10.1038/aps.2011.14; published online 18 Apr 2011

Introduction

More than 100 years have passed since Alzheimer's disease (AD) was first characterized. However, due to the lack of effective treatment, AD remains pandemic in the 21st century, imposing enormous social, and economic burdens on patients and their families^[1]. Modern demographic trends compound the problem; our aging global population has led to a steep increase in the number of individuals with AD. In the United States alone, more than 13 million individuals are predicted to be afflicted with AD by the year 2050^[2], leading to an overburden of scarce healthcare resources. Some studies estimate that the present availability of a treatment that can delay disease onset by 6.7 years would decrease the prevalence of AD 38% by 2050^[3]. Such disease-modifying treatments would lower the annual cost of individual patient care by up to \$24 000, thereby reducing the national cost of AD by trillions of dollars through the year 2050^[4, 5].

Unfortunately, currently available treatments, *eg*, Aricept and Memantine, usually provide at best only temporary and incomplete symptomatic relief. The marginal benefits provided by current therapies emphasize the urgent need to

develop effective disease-modifying AD treatments.

Amyloid β -targeting strategies

Disease-modifying strategies currently being pursued for AD mainly focus on two AD-related proteins, amyloid β (A β) and Tau. Of these, A β has attracted the most attention by far.

Substantial data derived from genetics, animal modeling, and biochemical studies support the idea that A β , the major component of senile plaques, plays a central role in AD pathophysiology^[6, 7]. Paradoxically, A β peptides – the seeds of AD – are present at birth, and these neurotoxic peptides are continuously produced throughout life. Thus, the most direct target in anti-A β therapy is reduction of A β production, which has led naturally to a focus on β - and γ -secretase inhibitors^[8–13]. However, despite considerable effort on this front, few novel chemical compounds based on this strategy have reached clinic trials. Safety issues are the overriding reason; because γ -secretase has many physiologically essential substrates, undesirable side effects are inevitable. One such substrate is the Notch signaling protein, which plays an intermediate and essential role in the differentiation and proliferation of many cell types. The consequences of γ -secretase inhibition include impaired lymphocyte differentiation and altered intestinal goblet cell structure^[14–16]. Thus, although the use of small-molecule γ -secretase modulators is receiving increasing attention as a promising therapeutic approach^[17], many com-

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Received 2010-09-07 Accepted 2011-02-16

panies have abandoned γ -secretase as a potential target.

In theory, inhibiting β -secretase should not carry the same risk of toxicity as γ -secretase inhibition. However, designing β -secretase inhibitors has been challenging. The β -secretase protein contains a large catalytic pocket; thus, the β -secretase inhibitors that have been developed to date are too large to penetrate the blood-brain barrier^[18–20]. There may also be conceptual drawbacks to targeting β -secretase. The development of A β secretase inhibitors is based on the hypothesis that A β deposition in the brain is due to overproduction or poor clearance of A β . However, this is likely only true for cases of familial AD caused by genetic mutation. Non-genetic AD cases, which represent the majority of AD patients, do not carry mutations and do not necessarily have overproduction of amyloid precursor protein (APP)^[21]. More importantly, a deeper understanding of A β has revealed that A β isoforms also serve as endogenous positive regulators of release probability at hippocampal synapses, with some studies suggesting that monomeric A β is beneficial for neurons^[22, 23]. Thus, because A β production may be important for physiological health, inhibiting A β generation may not necessarily be a sound strategy. An alternative tactic is to focus on A β clearance. One approach is to enhance the peripheral A β “sink action” by sequestering plasma A β , for example using A β immunotherapy. The AN1792 A β vaccine was designed for this purpose; however, it failed in Phase II clinical trials due to the development of meningoencephalitis^[24]. Nevertheless, it might be possible to circumvent such complications by targeting the proteolytic machinery in the brain. However, from a therapeutic point of view, it remains to be seen whether any of the relevant proteases will serve as a viable AD drug target^[25].

A β can aggregate into fibrils and oligomers (Figure 1). It was originally believed that only the large fibrils constituting mature neuritic amyloid plaques are toxic. However, in recent years research focus has shifted towards A β oligomers. Currently, A β peptide aggregation into toxic, prefibrillar oligomers is considered the key pathogenic event in the onset of AD^[26, 27]. Consistent with this view, A β oligomers can: i) directly induce synaptic dysfunction and neuronal death, both which are responsible for AD initiation and progression^[28–30]; and ii) trigger events such as oxidative damage and inflammation, which contribute to the progression of AD^[31–33]. Although generation of A β monomers is, in and of itself, a physiologically relevant event, their aggregation into oligomers and

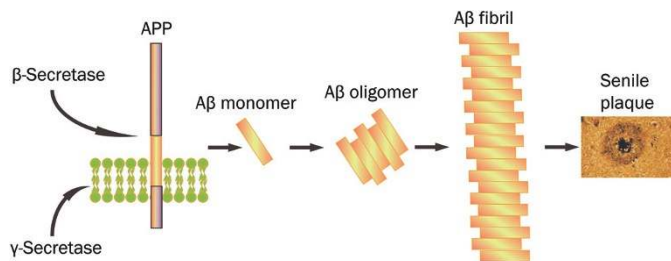


Figure 1. The plaque formation.

fibrils is pathogenic. Thus, blocking aggregation (while sparing A β generation) should not lead to mechanism-based toxicity. Hence, the strategy of inhibiting aggregation of A β , more specifically the A β 42 isoform, has emerged as a valid disease-modifying therapy for AD^[34].

Problems associated with inhibiting A β aggregation

Tremendous effort has been expended in recent years on developing small molecules that are capable of inhibiting A β aggregation. However, to date, no compounds have entered into clinical use. This is because the inhibition of A β aggregation requires blocking interactions between A β monomers, and historically, protein–protein interaction interfaces are particularly difficult drug targets. Thus, inhibiting A β aggregation has been deemed intractable^[35, 36]. The challenges associated with targeting A β aggregation are substantial, with the most obvious obstacle being the sheer size and geometry of the protein interaction surface. First, the regions of protein–protein interactions are approximately 1500–3000 Å²^[37–40], whereas protein–small molecule interaction regions are only about 300–1000 Å²^[41, 42]. Thus, small molecules are often unable to produce sufficient steric hindrance to inhibit A β aggregation^[43]. Second, the regions of protein–protein interactions are often relatively featureless, devoid of grooves or pockets into which a small molecule could dock in an energetically favorable manner^[43–47]. The binding energy that drives protein–protein contacts is typically distributed over a large area that lacks a defined “hotspot” for pharmacological intervention^[48]. Third, the highly plastic nature of protein surfaces can accommodate small molecules, thereby thwarting inhibition^[48]. Together, these problems place severe restrictions on the development of A β aggregation inhibitors.

Designing solutions to these problems

To tackle these challenges, researchers have envisioned a Trojan horse strategy in which a small bifunctional molecule gains access to the relevant biological compartment, binds tightly to a chaperone, and thereby provides the necessary steric bulk to disrupt protein–protein interactions^[48]. A second approach is to directly target the important chaperones, such as metals, in the brain. A growing body of evidence suggests that the presence of excess zinc, iron, copper and/or aluminum within senile plaques exacerbates A β -mediated oxidative damage and acts as a catalyst for A β aggregation in AD^[49]. Thus, chelation therapies aimed at disrupting aberrant interactions between A β and metals have attracted considerable attention; one such example is PBT2, which is currently in Phase IIa clinical trials^[50]. Unfortunately, to date, no new compounds employing this strategy have entered into clinical use.

The discovery that A β fibril formation is controlled by specific amino acids within the A β peptide itself has given renewed life to the idea that small molecules might occlude A β aggregation. Studies have demonstrated that various A β peptide regions contribute differently to A β aggregation and have identified important interactions among specific peptide regions that control this process^[51]. For instance, the N-termi-

nus^[52, 53], hydrophobic core^[54, 55], hinge or turn regions^[51, 56, 57] and C-terminus^[58, 59] of A β 41-42 are all crucial for the peptide's ability to aggregate and promote neurotoxicity.

The importance of the His13-Lys16 (HHQK) region in oligomerization, fibril propagation and neurotoxicity is well known^[60-62]. This four-residue region is also an essential component of the heparin-binding site for glycosaminoglycans (GAGs), which assist the HHQK motif in effecting a change in the A β secondary structure from a soluble, unordered α -helix to a stable β -sheet-rich conformation. These β -sheet-rich structures, in turn, have an affinity for associating with other monomers to form oligomers, protofibrils and fibrils that aggregate into plaques^[60]. The β -hairpin structure serves to nucleate the folding of A β monomers, which is the rate-limiting step in fibril formation^[56, 57, 63]. The formation of hinge or turn regions brings together two hydrophobic segments in space and produces the β -strand structure. In this region, Lys28 forms a salt bridge with Asp23/Glu22, which further stabilizes the structure^[63-65]. Electrostatic and hydrophobic interactions between these non-native side chains bring the peptides together, allowing native backbone-backbone interactions to initiate the assembly of β -sheets, albeit in non-native β -sheet registries^[64]. The hydrophobic segments are the main core of A β aggregates, and hydrophobic interactions are an important driving force in A β aggregate formation^[66, 67]. Based on these observations, a β -sheet breaker was first investigated as a potential inhibitor of A β aggregation^[68]. A substantial body of evidence suggests that the C-terminus of A β 42 plays a key role in controlling oligomerization; indeed, several studies of prefibrillar A β have suggested that the C-terminus of A β 42 is more rigid than the C-terminus of the more abundant but less toxic A β 40^[69-71]. This increased rigidity has been

attributed to interactions involving C-terminal residues Ile41 and Ala42, which stabilize a putative turn conformation^[71]. Although these subregions contribute differently to structure formation, each is important for A β aggregation.

By targeting these subregions for binding, a small molecule will be more likely to directly occlude A β aggregation. In fact, most inhibitors that have entered clinical trials target these specific subregions within the A β sequence (Figure 2). Tramiprosate (3-aminopropanesulfonic acid), a mimic of GAGs, targets the HHQK subregion at the N-terminus. Although the structural simplicity of tramiprosate makes it highly specific to A β , it ultimately failed in the late stages of a Phase III clinical trial^[72]. Despite this setback, the data obtained provided evidence that inhibitors targeting specific A β subregions represent a viable approach for AD treatment^[73]. The agents targeting C-terminus feature one scaffold, the cyclohexanehexol stereoisomers. Among these, scyllo-inositol, which interferes with A β fibril assembly by competing with endogenous phosphatidylinositol for binding to A β 41-42^[59], has shown promise in current Phase II trials. The hydrophobic central region is yet another promising target for A β fibrillation inhibitors, which include RS-0406^[74].

The quest for small molecules that target A β

As the strategy of inhibiting A β aggregation has increasingly gained acceptance, greater numbers of inhibitors have been developed and the structure-activity relationships of potent inhibitors have been systematically explored^[75]. These studies revealed that typical A β aggregation inhibitors such as Congo red (CR), chrysamine G (CG) and curcumin share a similar chemical scaffold. These molecules contain two aromatic groups or inositol groups (with a suitable substituted

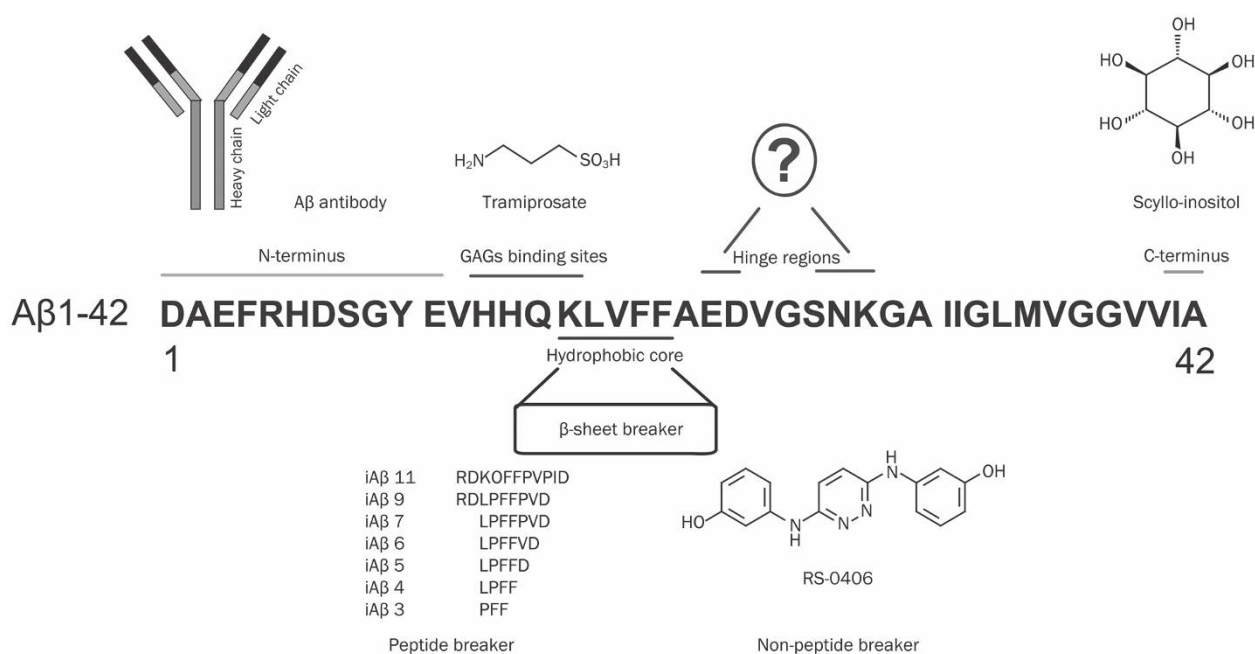


Figure 2. The subregion-targets in A β .

group) separated by a backbone of the appropriate length^[75]. We speculate that the two terminal groups interact with A β protein residues to provide the binding affinity, whereas the linker facilitates binding of inhibitors to specific subregions. Determining which subregion the inhibitors target will be key to future research efforts. A molecule that targets a specific subregion should satisfy the following design criteria: (1) it should contain terminal groups suitable for interacting with residues in, or adjacent to, the subregion; and (2) the linker should be of an appropriate length to join the two terminal groups, span the subregion, and provide sufficient steric hindrance. Molecules designed according to these specifications would therefore be capable of inhibiting A β aggregation by binding to a specific subregion.

The handful of inhibitors that have been developed to date do not possess this chemical scaffold. Scyllo-inositol contains only one terminal group and can therefore target only a very small region. Fortunately, scyllo-inositol targets the C-terminus, which is important for A β aggregation. However, the presence of a single terminal group restricts the area of the targeted subregion to the C-terminus, and the lack of a linker prevents scyllo-inositol from modulating other targeted subregions. Additionally, a single terminal group results in a weaker binding avidity. In the case of tramiprosate, its simple structure provides specificity for A β , yet, for unknown reasons it failed in Phase III clinical trials^[72]. Thus, although these atypical molecules possess inhibitory activity, they are severely limited by their weak binding affinity and small occupied region.

To our knowledge, most inhibitors target only one subregion. In fact, if inhibitors could target multiple specific subregions in concert, they might act synergistically to effectively inhibit A β aggregation. Thus, in our view, the ideal A β -targeting inhibitor should be of appropriate length and suitable flexibility, and should contain multiple groups that interact with A β residues. These multiple groups would both convey tighter binding avidity and permit targeting of multiple subregions. In addition, having sufficient length and flexibility would enable the inhibitor to capture the entire A β peptide and accommodate conformational changes in the target. Unfortunately, our current lack of the A β peptide's detailed molecular structure precludes designing such inhibitors. The successful creation of these new inhibitors will require gaining a better understanding of the modules necessary for activity as well as the key elements in the amyloid surface required for aggregation and toxicity. Alternatively, it might also be possible to employ a combination of multiple molecules.

Future perspectives

Compounds such as peptide-based inhibitors, antibodies and small molecules that target specific A β subregions represent the first generation of amyloid-based therapeutics with the potential to demonstrate disease-modifying activity. Although the results of ongoing clinical trials are inconclusive, these compounds hold the promise of a new day in the development of disease-modifying therapies for AD; some A β aggregation

inhibitors are listed in Table 1. As we gain additional insights into amyloid biology and AD itself, this will likely guide the development of the next generation of inhibitors.


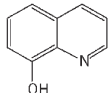
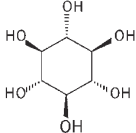
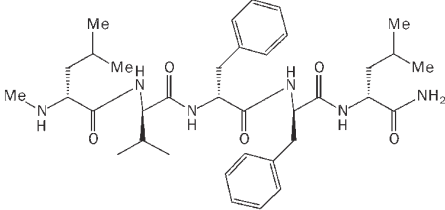
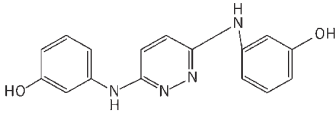
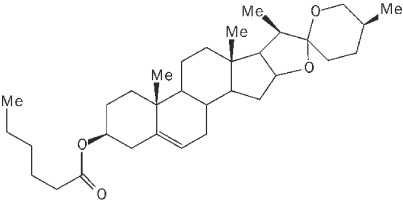
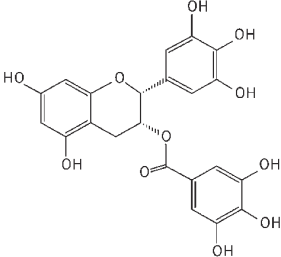
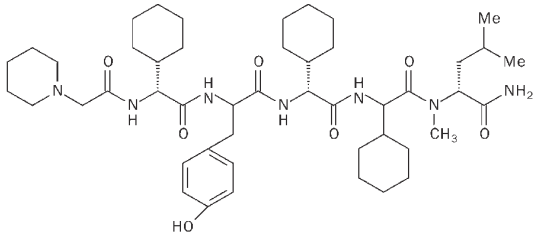
Acknowledgements

This work was funded by grants from the National Natural Science Foundation of China (No 30472029) and the National Natural Science Foundation of China for Distinguished Young Scholars (No 30725046).

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Table 1. Some chemical inhibitors of amyloid beta aggregation.

Chemical compound	Structure	Current trial state
Bapineuzumab	Monoclonal antibody	Phase III
Tramiprosate		Failed in phase III
PTB2		Phase IIa
Scyllo-inositol		Phase II
PPI1019		Completed phase II
RS0406		Phase II
SP-233		Phase I
EGCG		Phase II
Exebryl-1	No structural details are available	Phase I
SEN606		Preclinical

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