Novel Amide Derivatives as Modulators of Amyloid-Beta Aggregation

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Abstract

Purpose: Alzheimer's Disease (AD), the misfolding and aggregation of amyloid-beta 42 (Aβ42) is one of the hallmarks underlying its pathophysiology. Small molecule modulators that target various protein species of Aβ42 aggregation can reduce the overall burden of toxic Aβ. The purpose of this study was to design, synthesize and evaluate novel amide derivatives' ability to modulate the aggregation of Aβ42 and to understand the mechanism of aggregation.

Methods: The small molecule library was designed based on the structure of curcumin and chalcone. To rule out pan-assay interfering, the α, β-unsaturated system was replaced by amide bond and various bioisosteres were included. The target compounds were synthesized, purified and characterized by chromatography, NMR, LCMS and UPLC. The modulatory activity on Aβ42 aggregation was screened by thioflavin T (ThT) based kinetic study. The morphology of aggregates was obtained by transmission electron microscopy (TEM) study. Computational modeling was used to investigate the binding interactions and to understand the structure-activity relationship.

Results: The small molecule library was synthesized by coupling acid chlorides and amines in good-to-high yields (71-96%) and purity (96.1% to 99.6%). Aβ42 kinetic assay showed that nearly all compounds exhibit moderate ability to inhibit Aβ42 (5 μM) aggregation. The inhibition rate ranges from 11-36% at 10 μM and 41-71% at 25 μM. TEM studies showed a significant reduction in the formation of Aβ42 in the presence of some compounds. Molecular docking study indicates that these small molecules undergo interactions with the core ^{16}KLVFF^{21}A region to stabilize the aggregation process.

Conclusion: A library of novel amide derivatives were designed, synthesized and characterized. The primary biological screening showed that these derivatives can moderately inhibit the Aβ42 aggregation. These studies demonstrate that this class of compounds is capable of binding to Aβ42 and preventing their aggregation. The results suggest their application to study the mechanisms of aggregation and to design novel therapeutic agents.

Introduction

The mechanism and modulation of amyloidogenesis are closely associated with human diseases. In Alzheimer's Disease (AD), the misfolding and aggregation of amyloid-beta 42 (Aβ42) is one of the hallmarks underlying its pathophysiology. The accelerated approval of Aβ mono-antibody Aducanumab further validates the fundamental role of Aβ aggregation in disease development.

Figure 1 Amino acid sequence of Aβ42

Small molecule modulators that target various protein species of Aβ42 aggregation can reduce the overall toxic burden of aggregation. The purpose of this study is to design and synthesize a novel small molecule library and evaluate its ability to modulate the aggregation of Aβ42 and thus as potential therapeutic agents.

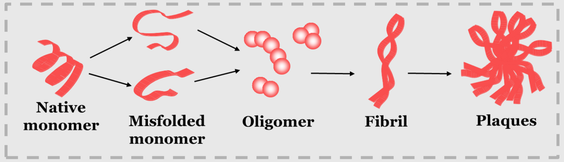
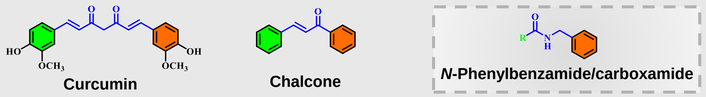


Figure 2 Aggregation cascade of Aβ42

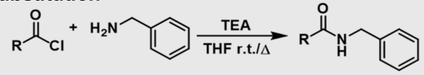
Design and Synthesis of Modulators

- Small molecule modulators were designed based on the structure of curcumin and chalcone which shows extensive biological activity spectrum, including anti-Aβ42 aggregation.

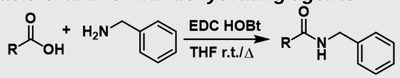


- The design in this study includes replacing the α,β unsaturated system with amide bonds and various bioisoteres were incorporated into the modulator library.

Nucleophilic acyl substitution



Coupling carboxylic acid & amine with dehydrating agents



Purification and characterization

Reaction mixtures were purified by column chromatography.
Structure characterization conducted by NMR, LCMS.
The purity of probes for assays was tested by UPLC, LCMS.

✓ Yield: 71% - 96%; Purity: 96.1% - 99.6%

Thioflavin T (ThT) Aggregation Kinetic Study

ThT selectively binds to Aβ fibrils to give fluorescent changes that can be monitored (excitation 440 nm, emission 490 nm). By incubating Aβ42 (5 μM) in the presence and absence of small molecule modulators for 24 h at 37 ℃, the fluorescence to time curve was plotted. The inhibition rate was calculated based on the readings at 24 h.

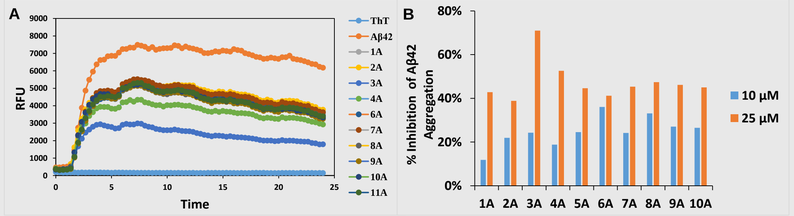


Figure 3 A) Florescence to time curve of ThT kinetic study of selected modulators B) Inhibition rate of selected compounds at 24 h. Data were measured in triplicates (n=2).

Transmission Electron Microscopy (TEM) Study

Samples were taken after incubation at 37 ℃ for 24 h with Aβ40/42 in the presence and absence of small molecule probes. TEM study was used to determine the morphology of aggregates and further validate the results from kinetic study.

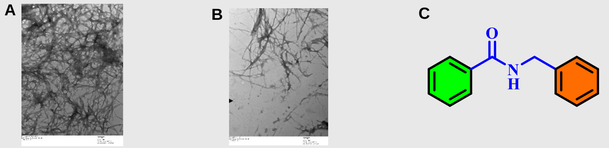
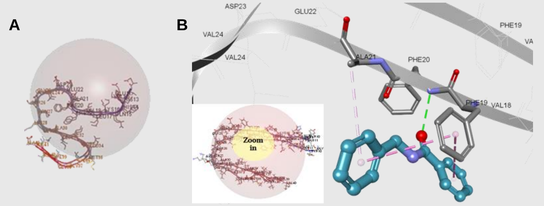


Figure 3 A) TEM image of Aβ42 control in the absence of modulator B) TEM image of Aβ42 in the presence of lead compound 3A at 25 μM. C) Chemical structure of lead modulator 3A.

Molecular Docking Study

Molecular docking were used to either predict the potential binding interactions of small molecule modulators with Aβ42 or used to analyze and build the pharmacophore model. Discovery Studios, *Structure-Based-Design* program from BIOVIA Inc. CDOCKER algorithm were used for docking and ranking.



Category	Distance(Å)
Hydrophobic	5.4
Hydrophobic	5.2
Hydrophobic	5.1
Hydrogen Bond	2.1

Figure 4 A) Aβ42 dimer (PDB id: 5KK3) model and the binding site (red ball) B) Docking results of lead compound 3A to Aβ42 dimer C) Binding interactions and distance

Conclusion

A library of novel amide derivatives were designed, synthesized and characterized. The primary biological screening showed that these derivatives can moderately inhibit the Aβ42 aggregation. These studies demonstrate that this class of compounds is capable of binding to Aβ42 and preventing their aggregation. The results suggest their application to study the mechanisms of aggregation and to design novel therapeutic agents.

References:
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