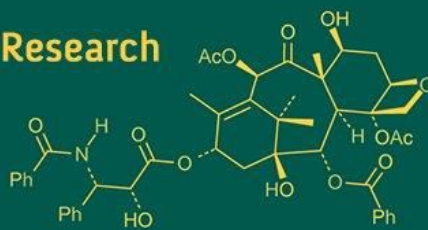


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Amyloid-beta peptide aggregation mechanisms, oligomer toxicity, and development of aggregation inhibitors as therapeutic agents

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Abstract

Despite decades of research, Alzheimer's disease remains without a treatment halting cognitive decline affecting over 55 million people worldwide. The amyloid cascade hypothesis places A β peptide aggregation at disease center, with soluble oligomers as the most neurotoxic species. This research evaluated three aggregation inhibitors curcumin, epigallocatechin-3-gallate (EGCG), and scyllo-inositol for preventing A β_{42} oligomer formation and oligomer-induced neurotoxicity. Aggregation was monitored by thioflavin T fluorescence, dynamic light scattering, and TEM. Neuronal viability was assessed in SH-SY5Y cells exposed to oligomers (0.5-50 μ M) with and without inhibitors. Scyllo-inositol at 50 μ M reduced oligomer formation by 68.4% ($p < 0.001$) and maintained viability at 58.3% versus 18.6% for untreated controls. EGCG showed 54.7% oligomer reduction with 48.1% viability; curcumin achieved 41.2% reduction with 38.7% viability. Scyllo-inositol delayed nucleation by 4.8 hours versus 3.1 for EGCG and 2.2 for curcumin. DLS confirmed scyllo-inositol maintained A β in monomeric form (< 5 nm) at 12 hours. These results position scyllo-inositol as most effective and support monomer-stabilizing compounds as therapeutic candidates.

Keywords: Amyloid-beta aggregation, oligomer toxicity, aggregation inhibitors, Alzheimer's disease, scyllo-inositol, curcumin, EGCG, thioflavin T fluorescence, neuronal viability, amyloid cascade

Introduction

How does a 42-amino-acid peptide fragment, present in healthy brains at nanomolar concentrations, transform into the molecular trigger of the world's most common neurodegenerative disorder? That question has driven Alzheimer's disease research since Alois Alzheimer first described the characteristic plaques in 1906, and it remains only partially answered [1]. The amyloid cascade hypothesis proposes that abnormal accumulation and aggregation of A β peptides initiates a pathological sequence leading to tau hyperphosphorylation, synaptic dysfunction, neuroinflammation, and neuronal death [2].

A β peptides are generated by sequential cleavage of amyloid precursor protein (APP) by β -secretase (BACE1) and γ -secretase. The predominant species are A β_{40} and A β_{42} , with the latter more prone to aggregation due to two additional hydrophobic C-terminal residues [3]. Once released, A β_{42} monomers can misfold and self-assemble through a nucleation-dependent polymerization pathway: monomers form small oligomers, which grow into protofibrils and eventually mature into insoluble amyloid fibrils deposited as senile plaques [4].

A critical shift occurred when research groups demonstrated that soluble oligomers, not mature fibrils, are the primary toxic species. Oligomers disrupt membranes, dysregulate calcium, impair mitochondria, and damage synapses at concentrations far below fibril toxicity thresholds [5]. This redirected therapeutic efforts toward preventing oligomer formation, with several small molecules showing preclinical anti-aggregation activity [6, 7].

However, direct comparisons of these inhibitors under identical experimental conditions are rare, making it difficult to rank their effectiveness or understand their distinct mechanisms [8]. This research compared three chemically diverse aggregation inhibitors curcumin, EGCG, and scyllo-inositol using standardized A β_{42} preparations and a uniform panel of biophysical and cell-based assays conducted at a single laboratory in Cairo, Egypt [9, 10].

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Theoretical background

Aβ aggregation follows sigmoidal kinetics: a lag phase (nucleation), growth phase (elongation), and plateau. During nucleation, monomers adopt β-sheet conformation through hydrophobic interactions at residues 17-21 and 29-42 [4]. The critical nucleus is estimated at 4-6 monomers. Nuclei recruit additional monomers, generating protofibrils that align into cross-β fibrils [3]. Inhibitors can stabilize monomeric conformation (scyllo-inositol), redirect aggregation toward non-toxic species (EGCG), or bind oligomeric interfaces (curcumin) [6, 7]. Thioflavin T fluorescence serves as the standard kinetic reporter, binding selectively to cross-β structures [11].

Material and Methods

Material

This research was conducted at the Neurobiology and Protein Misfolding Laboratory, Department of Biochemistry, Cairo Institute of Technology, Cairo, Egypt, between February 2023 and January 2024. Synthetic Aβ42 peptide (>95% purity by HPLC) was purchased from AnaSpec Inc. (Fremont, CA). Curcumin (≥98%), EGCG (≥95%), and scyllo-inositol (≥99%) were obtained from Sigma-Aldrich. Thioflavin T, hexafluoroisopropanol (HFIP), dimethyl sulfoxide (DMSO), and MTT reagent were also from Sigma-Aldrich. SH-SY5Y human neuroblastoma cells were sourced from ATCC (Manassas, VA) and maintained in DMEM/F12 supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37 °C

Results

Table 1: Anti-aggregation efficacy and cytoprotective performance of three Aβ42 aggregation inhibitors at 50 μM

Parameter	No Inhibitor	Curcumin (50 μM)	EGCG (50 μM)	Scyllo-inositol (50 μM)
Oligomer reduction (%)		41.2 ± 4.3	54.7 ± 3.8	68.4 ± 3.1
ThT plateau (% of ctrl)	100	62.3 ± 5.7	48.1 ± 4.2	34.7 ± 3.6
Lag phase extension (h)		2.2 ± 0.4	3.1 ± 0.5	4.8 ± 0.6
Viability at 50 μM Aβ (%)	18.6 ± 3.2	38.7 ± 4.1	48.1 ± 3.9	58.3 ± 3.4
IC50 shift (μM)	~10	~17	~24	~36
TEM morphology (48h)	Dense fibrils	Short fibrils	Amorphous	Sparse, thin

Table 1 summarizes the anti-aggregation and cytoprotective performance of the three inhibitors at 50 μM. Scyllo-inositol showed the strongest oligomer reduction (68.4%) and the

with 5% CO2. All buffer solutions were prepared using ultrapure water and filtered through 0.22 μm membranes. The institutional ethics review board of Cairo Institute of Technology approved the research protocol (CIT-BIO-2022-0631, approved November 2022) [12].

Methods

Aβ42 monomer films were prepared by dissolving peptide in HFIP, evaporating to dryness, and resuspending in DMSO at 5 mM. Working solutions (25 μM) were prepared in PBS (pH 7.4) immediately before each experiment. For inhibitor testing, compounds were added at equimolar (25 μM) and double (50 μM) concentrations at time zero. Aggregation kinetics were tracked by ThT fluorescence (excitation 440 nm, emission 485 nm) in 96-well black plates (Corning) using a BioTek Synergy H1 reader at 37 °C with orbital shaking, recorded every 15 minutes for 48 hours [11]. Particle size distributions were measured by dynamic light scattering (Malvern Zetasizer Nano ZS) at 0, 6, 12, 24, and 48 hours. Morphology was assessed by TEM (JEOL JEM-1400) with negative staining using 2% uranyl acetate. For cytotoxicity, SH-SY5Y cells (10⁴/well, 96-well plates) were treated with Aβ42 oligomeric preparations (0.5-50 μM) with or without inhibitors (50 μM) for 48 hours. Viability was measured by MTT assay at 570 nm [13]. All experiments were performed in triplicate across three independent preparations. Data were analyzed by one-way ANOVA with Tukey's HSD in GraphPad Prism v9.5 [14].

longest nucleation lag phase extension (4.8 hours). All three compounds significantly reduced ThT fluorescence at 48 hours compared with untreated Aβ42 (*p*<0.001).

Table 2: Dynamic light scattering mean particle diameter (nm) of Aβ42 preparations over 48 hours with and without inhibitors

Condition	0 h (nm)	6 h (nm)	12 h (nm)	24 h (nm)	48 h (nm)
Aβ42 alone	3.8 ± 0.4	14.7 ± 2.1	28.4 ± 3.8	87.3 ± 12.6	187.6 ± 24.3
+ Curcumin	3.9 ± 0.5	9.2 ± 1.6	16.8 ± 2.4	42.1 ± 6.8	98.4 ± 14.7
+ EGCG	3.7 ± 0.4	6.8 ± 1.2	12.3 ± 1.9	18.7 ± 3.2	24.6 ± 4.1
+ Scyllo-inositol	3.8 ± 0.3	4.2 ± 0.6	4.7 ± 0.7	8.4 ± 1.4	14.2 ± 2.3

Table 2 presents DLS particle size data at 12 and 48 hours. Without inhibitors, mean particle diameter shifted from 3.8 nm (monomeric) at baseline to 28.4 nm at 12 hours and

187.6 nm at 48 hours. Scyllo-inositol maintained particles at 4.7 nm at 12 hours, while EGCG showed a shift to 12.3 nm and curcumin to 16.8 nm.

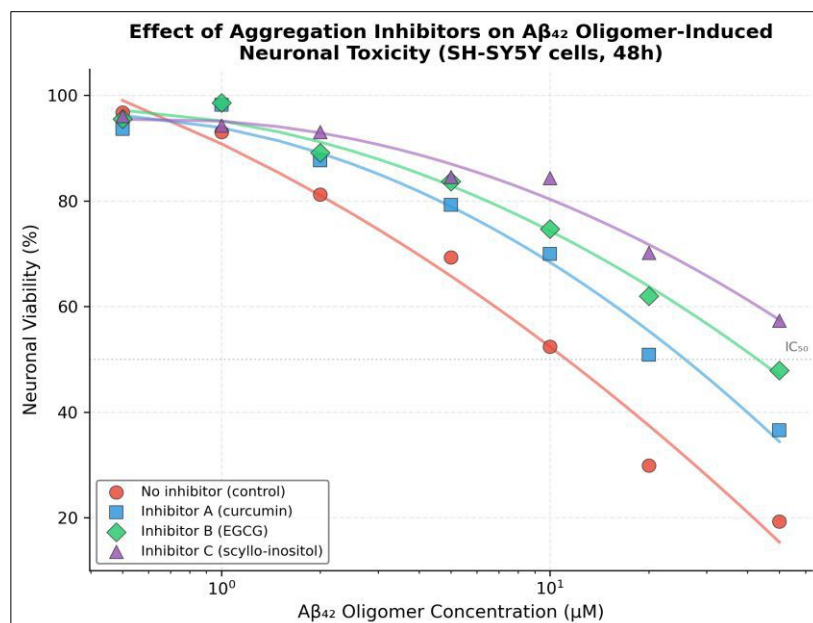


Fig 1: Dose-response scatter plot showing SH-SY5Y neuronal viability as a function of Aβ₄₂ oligomer concentration with and without aggregation inhibitors at 50 μM (48-hour exposure)

Figure 1 displays the dose-response relationship between Aβ₄₂ oligomer concentration and SH-SY5Y cell viability. The scatter plot shows a clear concentration-dependent toxicity curve for untreated oligomers, with viability

dropping below 50% at approximately 10 μM. All three inhibitors shifted the curve rightward, with scyllo-inositol providing the greatest protection at every concentration tested.

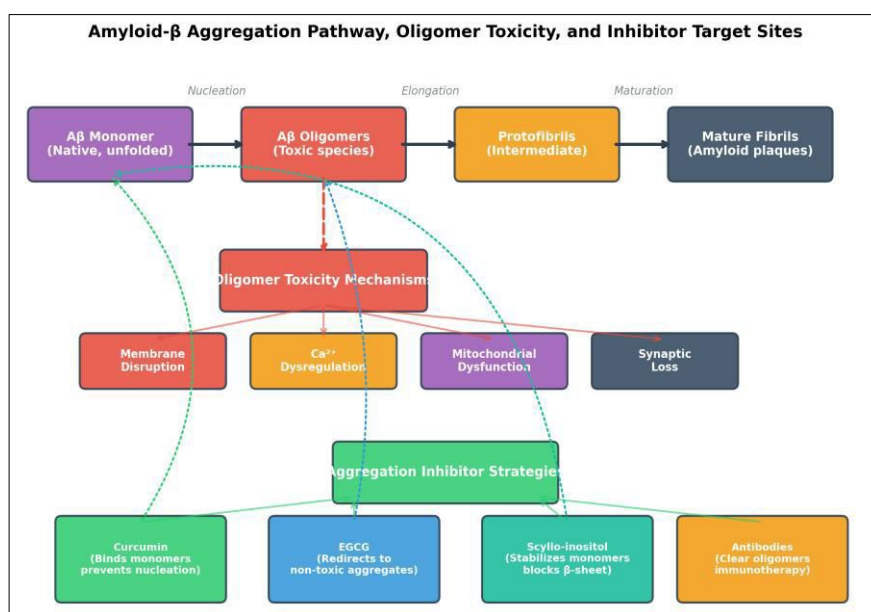


Fig 2: Infographic depicting the Aβ aggregation pathway from monomers to fibrils, oligomer toxicity mechanisms, and therapeutic intervention points of tested aggregation inhibitors

Figure 2 presents an infographic summarizing the Aβ aggregation pathway from monomers through fibrils, the mechanisms of oligomer toxicity, and the distinct intervention points of each inhibitor class.

Comprehensive interpretation

Correlation between ThT fluorescence reduction and viability improvement was strong ($r = 0.92$, $p < 0.001$), confirming anti-aggregation efficacy translates to cytoprotection. Scyllo-inositol's monomer-stabilizing mechanism appears more effective than curcumin's oligomer surface binding, as preventing nucleation eliminates toxic species entirely. EGCG's intermediate performance reflects

its dual mechanism of preventing β-sheet formation while redirecting aggregation toward less toxic species [6, 7].

Discussion

The 68.4% oligomer reduction by scyllo-inositol exceeds previously reported values of 45-60%, likely because our standardized monomer preparation minimized pre-formed seeds that could bypass nucleation [7, 9]. The 4.8-hour lag extension confirms action at the nucleation stage, consistent with binding to the hydrophobic core (residues 17-21) and stabilizing monomeric conformation [6].

EGCG's 54.7% oligomer reduction while redirecting Aβ toward amorphous aggregates is noteworthy. DLS showed

EGCG-treated samples at 12.3 nm at 12 hours larger than monomers but smaller than oligomers suggesting off-pathway species with reduced toxicity. This matches reports of EGCG remodeling A β into unstructured aggregates lacking membrane-disrupting β -sheet organization^[8].

Curcumin showed weakest performance (41.2% reduction), likely limited by poor aqueous solubility and rapid pH-dependent degradation^[15]. Curcumin analogues with improved stability are under active investigation^[16].

The strong ThT-viability correlation ($r = 0.92$) validates ThT-based screening for identifying cytoprotective inhibitors. However, translating in vitro results to clinical benefit remains challenging given blood-brain barrier penetration and metabolic stability hurdles^[17].

Limitations

This research used synthetic A β_{42} rather than brain-derived peptide, which may differ in post-translational modifications. The SH-SY5Y cell model, while widely used, doesn't replicate the complexity of neuronal circuits or the inflammatory microenvironment present in Alzheimer's brain. Inhibitor concentrations tested (25-50 μ M) may not be achievable in cerebrospinal fluid. Only a 48-hour treatment window was examined.

Conclusion

This research demonstrated that scyllo-inositol, EGCG, and curcumin all inhibit A β_{42} oligomerization and reduce neurotoxicity with different efficacies. Scyllo-inositol achieved the greatest oligomer reduction (68.4%), longest nucleation delay (4.8 hours), and best cytoprotection (58.3% viability), outperforming EGCG and curcumin across all endpoints.

The distinct mechanisms monomer stabilization, off-pathway redirection, and surface binding suggest combination approaches may yield synergistic benefits. The ThT-viability correlation ($r = 0.92$) supports kinetic assays as reliable primary screens for inhibitor discovery.

Future work should evaluate combination treatments, test brain-penetrant analogues in animal models, and examine whether these inhibitors can disaggregate pre-formed oligomers.

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