A side-chain tripeptide based PEGylated block copolymer: A potential drug candidate in Alzheimer's disease translational therapeutics

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ABSTRACT

More than 100 years have passed since the discovery of AD by Alois Alzheimer, but no remedial medication against AD is available till date. Here in this study, we designed a PEGylated, side-chain tripeptide (leu-val-phe) based block copolymer targeting anti-aggregation of misfolded amyloid beta peptide ($A\beta_{1\text{-}42}$) which is considered as the seed in amyloid cascade hypothesis.

It was assumed that this block copolymer would act as a competitive inhibitor of the aggregation prone 'KLVFFA' hydrophobic sequence in misfolded $A\beta_{42}$. This peptidic inhibitor was evaluated for the effectiveness against $A\beta_{42}$ fibrillization at an early stage of the oligomer to fibril formation as well as preformed fibril clearance *via* thioflavin T (ThT) assay, dynamic light scattering (DLS) analyses, atomic force microscopy (AFM) and electron microscopic techniques. A significant demolition of the characteristic β-sheet structure of growing $A\beta_{42}$ fibril chain in presence of the inhibitors was observed by the circular dichroism (CD) spectroscopy, Fourier transform infrared (FTIR) spectroscopy and molecular dynamic (MD) simulations. Moreover, the *in silico* MD simulations and isothermal calorimetric (ITC) studies successfully proved our hypothesis of drug action mechanism correct. The IC_{50} value of the inhibitor at a higher concentration of 55 µM in MTT assay and higher survivability of SHSY5Y cells upon treatment with the inhibitor projected a promising drug candidate in the premise of new age translational therapeutics for AD.

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BACKGROUND

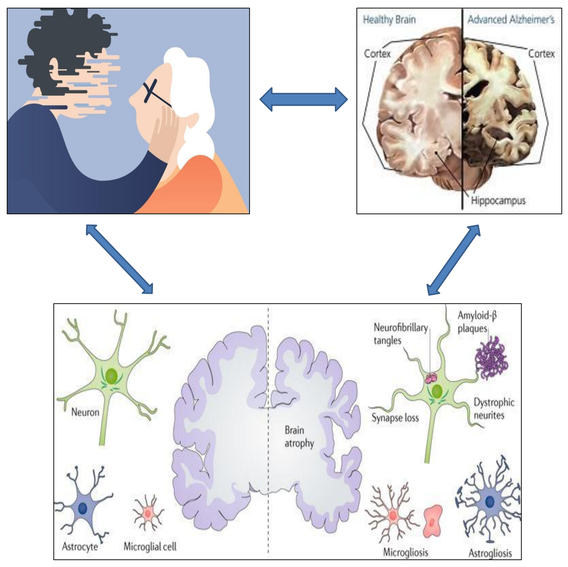


Fig 1: The debilitating memory loss in AD, the pathophysiology and the predicted hypotheses of AD viz. amyloidosis, tauopathy and mitochondrial cascade hypothesis.

- Current Food and Drug Administration approved medications e.g. cholinesterase inhibitors, memantine provide symptomatic relieve only but do not cure the disease.

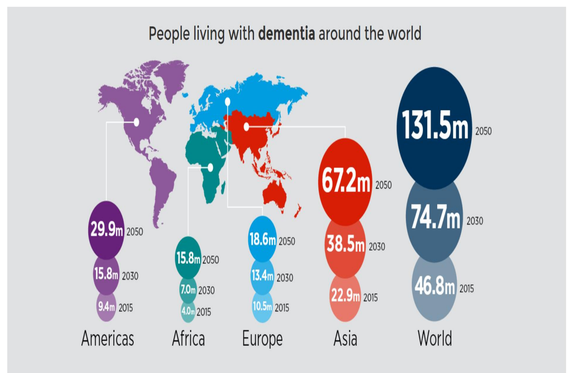
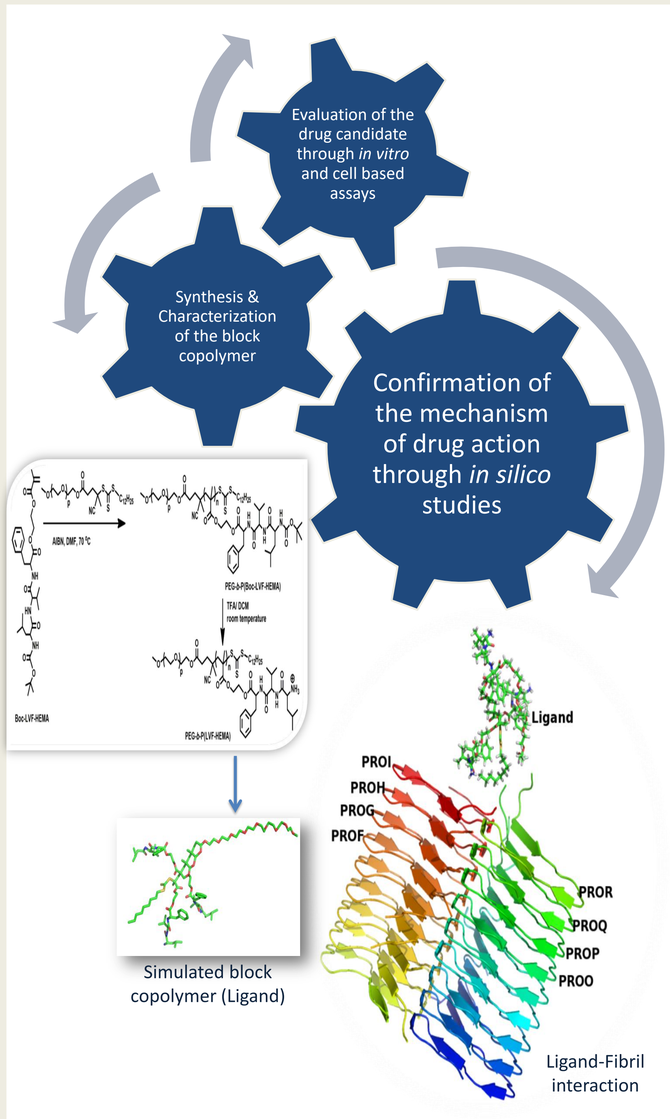


Fig 2: The world map of AD predicting 131.5 million individual by 2050.

OBJECTIVES

Synthesis, characterization, and evaluation of a side-chain tripeptide based PEGylated block copolymer targeted to inhibit the aggregation of misfolded $A\beta_{42}$ peptide of Alzheimer's disease.

METHODS AND MATERIALS



RESULTS AND DISCUSSION

A. Characterization of the drug candidate:

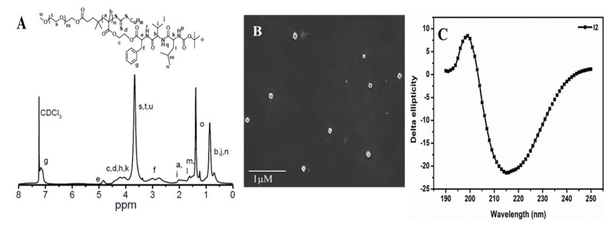


Fig 3: A: NMR of PEG5K-*b*-P(LVF-HEMA)6K, **B:** SEM images of the block copolymers, **C:** The β-sheet conformation of the block copolymer reflected by the CD study.

B. In vitro and cell based assays:

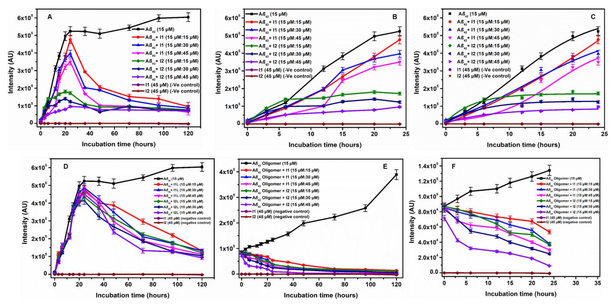


Fig 4: A: $A\beta_{42}$ fibril degradation assed by Thioflavin-T (ThT) assay, **B:** Closer look to 24 h fibril incubation, **C:** Fitted curve of B, **D:** Degradation of preformed fibril at a later stage of drug addition, **E:** Oligomer degradation, **F:** Closer look to 24 h oligomer incubation.

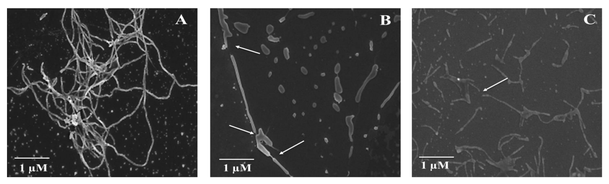


Fig 5: The scanning g electron micrograph showing the $A\beta_{42}$ fibril degradation on treatment with the PEGylated block copolymer.

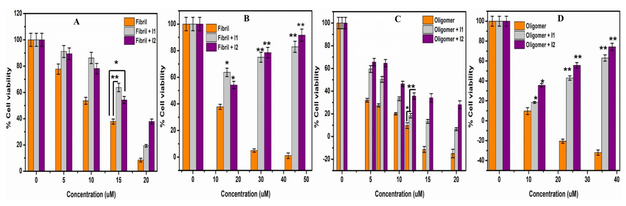


Fig 6: Cell based MTT assays showed the increase in % cell viability for drug treated cells with respect to the $A\beta_{42}$ fibril and oligomer; **A:** For fibril in 1:1 ratio treatment, **B:** For fibril in 1:3 ratio, **C:** For oligomer in 1:1 ratio and **D:** Oligomers in 1:3 ratio. The *P* value significances are $*P< 0.05$ and $**P< 0.001$.

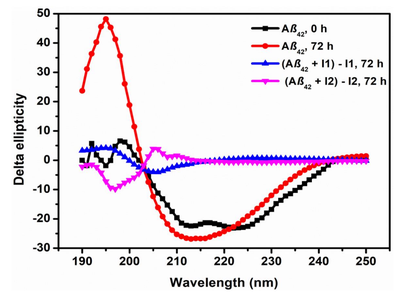


Fig 7: Change in the conformation of the $A\beta_{42}$ fibril observed in CD studies.

Wave no. (cm^{-1})	$A\beta_{42}$ at Initial stage	Fibril (% values)	Fibril + I1	Fibril + I2
1610-1640 (β-sheet/aggregated strands)	21.47	52.37	19.38	19.73
1648-1660 (α-helix/ unordered)	42.71	19.44	11.05	10.30
1660-1685 (Turns)	9.75	11.76	12.22	7.02
1675-1695 (Antiparallel β-sheet, aggregated strands, Arg, Asn, Gln side chains)	0.90	2.72	4.63	2.22

Table 1: Percentage values of different conformations of $A\beta_{42}$, before and after treatment with the inhibitors, as measured by FTIR study.

- The ThT assay has shown the fibril degradation from an early stage of $A\beta_{42}$ incubation. The scanning electron microscopic (SEM) images demonstrated the fibril degrading capability of the PEGylated block copolymer. Also, a change in the secondary structure of the $A\beta_{42}$ fibril was found by the CD and FTIR studies. The cell-based assays proved the drug-likeliness of the inhibitor.

C. Mechanism of drug action evaluated through in silico studies:

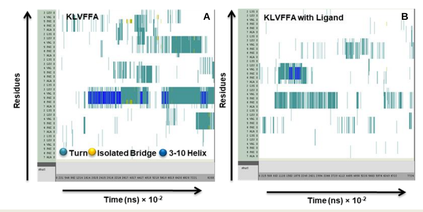


Fig 8: A detailed secondary structure composition of six KLVFFA units in an 80 ns simulation, (A) in absence of ligand and (B) in the presence of ligand *i.e.* the inhibitor.

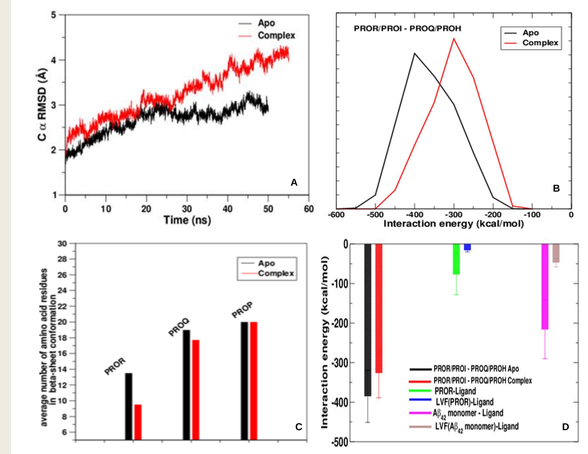


Fig 9: *In silico* modelling to reveal the mechanism of drug action; **A:** Comparison of root mean square fluctuation between the apo ($A\beta_{42}$ fibril) and $A\beta_{42}$ fibril-drug complex, **B:** Change in interaction energy between apo and complex, **C:** Decrease in β-sheet forming amino acids in the outermost exposed fragment of $A\beta_{42}$ fibril when treated with the drug, **D:** Contribution of hydrophobic 'LVF' segment in the interaction energy for the different case of $A\beta_{42}$ and drug.

- The change in interaction energy predicted through in silico studies also proved in isothermal calorimetric (ITC) analyses.

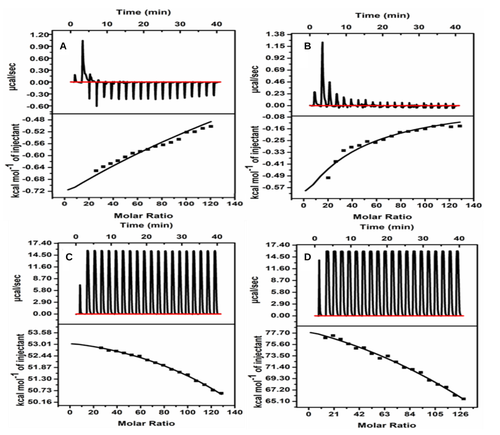


Fig 10: Binding isotherms of $A\beta_{42}$ with I1 & I2 at different stage of incubation; (A & B) prior to incubation; (C & D) at the pre-formed fibrillar stage. Buffer subtracted thermograms are shown here.

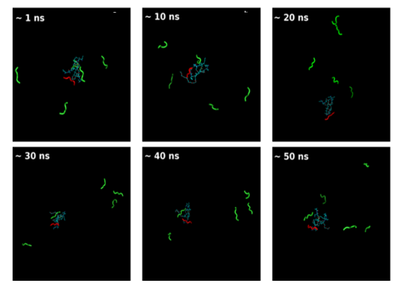
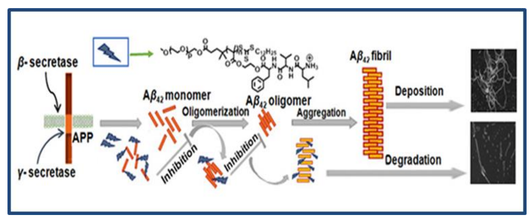


Fig 11: The presence of ligand inhibits the growth of fibril. PROR unit (shown in red cartoon) inhibits further aggregation of other monomers (shown in green) with PROR unit during the entire simulation.

CONCLUSIONS

✓The PEGylated block copolymer is able to inhibit the $A\beta_{42}$ fibril formation from the very initial stage of monomer to oligomer formation.
✓The mechanism of drug action lies in the competitive inhibition of fibrillization of the hydrophobic stretch 'LVFFA' of $A\beta_{42}$ fibril by the side chain tripeptide 'LVF' of the drug.
✓This present compound can be further modified to impregnate with the symptomatic drugs of AD like memantine or acetylcholinesterase inhibitors and tested as new drug vehicle.



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