Investigating Proteasome Activity Recovery By Tau Fibril Disaggregants

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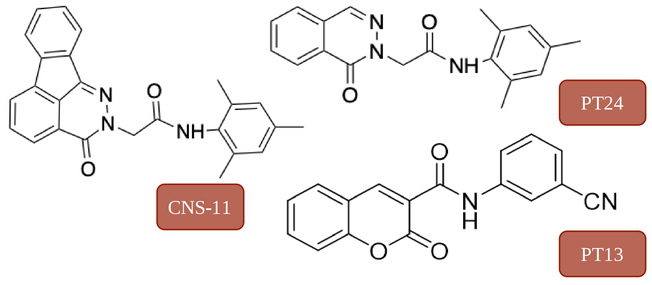
Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the accumulation of misfolded proteins such as tau, leading to the formation of toxic aggregates[1,2]. Abnormal chemical changes cause tau to detach from microtubules in neurons and stick to other tau molecules, forming tangles[1]. In AD, the efficiency of the proteasome, a critical protein complex responsible for protein degradation, is disrupted due to the unhealthy accumulation of fibrils[3]. In response to this challenge, fibril disaggregates have emerged as a promising therapeutic strategy to counteract the detrimental effects of protein aggregation. These compounds target and dismantle the existing protein aggregates, offering hope for potential treatments for AD[2]. This study investigates how PT13 and PT24, both modified versions of the known disaggregant CNS-11, can reverse the impact of harmful protein buildup.

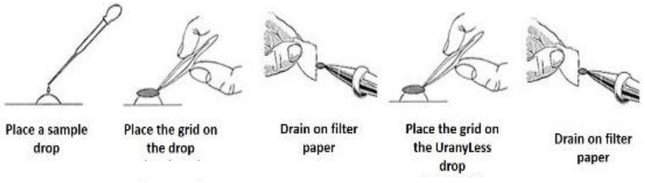
Hypothesis

We hypothesize that fibril disaggregants can alleviate fibril-induced proteasome inhibition.

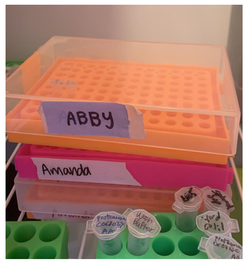
Compound Structures



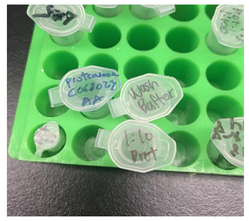
Negative Staining and Electron Microscopy(EM)



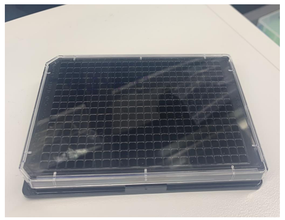
Proteasome Activity Assay

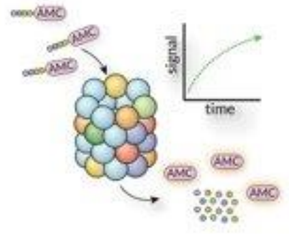


Overnight incubation with compounds and AD-brain purified fibrils



Another overnight incubation after adding purified proteasome





Add buffer and Suc-LLVY-AMC substrate before running the activity assay; Fluorescence is measured by the plate reader to reflect proteasome activity

Results

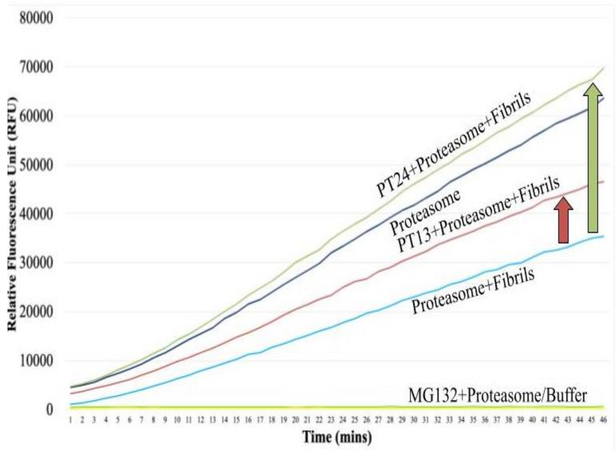


Figure 1: General Trends of Tau Fibril Disaggregants Overtime

When fibrils are added to the proteasome, fluorescence decreases, suggesting proteasome inhibition. However, proteasome activity is recovered partially and completely by adding, respectively, PT13 and PT24 to the fibrils.

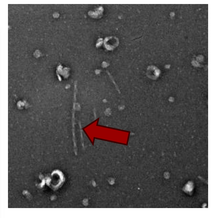


Figure 2: EM Representative Micrograph of Fibrils With No Inhibitor

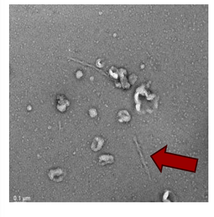


Figure 3: EM Representative Micrograph of Fibrils With PT13

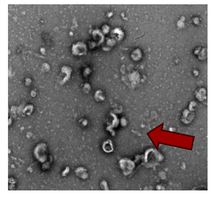
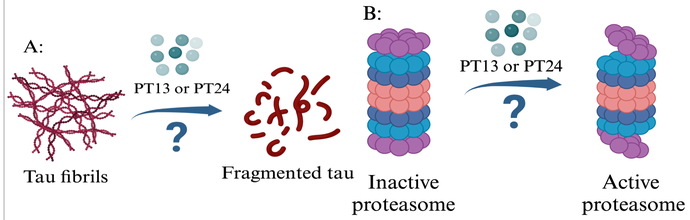


Figure 4: EM Representative Micrograph of Fibrils With PT24

Conclusion

- The activity assay reveals two key findings regarding the role that PT13 and PT24 play in proteasome activity recovery.
  - PT13 alleviates proteasome activity.
  - PT24 recovers and enhances proteasome activity.
- EM micrographs of fibrils before and after incubation with PT13 and PT24 suggest that the compounds might be weak tau disaggergants, fragmenting fibrils moderately.
- We hypothesize two mechanisms of action of PT13 and PT24:
  - A: PT13 and PT24 are disaggregating fibrils, creating more suitable substrates for the proteasome to process, and resulting in enhanced proteasomal activity.
  - B: PT13 and PT24 is to directly act on the proteasome, becoming proteasomal activators.



Summary

From these preliminary studies, PT24 and PT13 emerge as promising compounds that could: **A.** disaggregate tau fibrils and/or **B.** enhance proteasomal activity. This leads to great potential for future therapeutic interventions targeting Alzheimer's disease.

Future Directions

- Quantitative negative-stain electron microscopy (qEM) imaging will reveal the extent to which PT13 and PT24 disaggregate fibrils.
- Additional studies need to be performed to answer whether compounds such as PT13 and PT24 could, in synergy, disaggregate fibrils and potentiate proteasome activity.
- Synthesis of PROTACs (Proteolysis Targeting Chimeras) can be used to enhance selective degradation of tau.

References

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2. Seidler, P.M., Murray, K.A., Boyer, D.R. *et al*. Structure-based discovery of small molecules that disaggregate Alzheimer's disease tissue derived tau fibrils in vitro. *Nat Commun* 13, 5451 (2022). https://doi.org/10.1038/s41467-022-32951-4
3. Oddo, Salvatore. "The ubiquitin-proteasome system in Alzheimer's disease." *Journal of cellular and molecular medicine* vol. 12,2 (2008): 363-73. doi:10.1111/j.1582-4934.2008.00276.x

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