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Discovery of small molecule benzothiazole and indole derivatives tackling Tau 2N4R and α-synuclein fibrils

Small molecules C46 and C48 inhibit tau and alpha-synuclein aggregation and reduce inclusions in cell models.

Researchers at Purdue have developed small molecule inhibitors (dubbed C46 and C48) that can inhibit the growth of 2N4R tau isoform (2N4R-TI) and alpha-synuclein (a-syn) aggregates and a-syn oligomers at 100 micro-molar concentration in vitro. Among the most common diseases for the elderly population are Alzheimer's disease (AD), Parkinson's disease (PD), and dementia, which impact millions of people each year. The most well accepted hypothesis for AD is that the symptoms originate from deposition of amyloid-beta (AB) protein in the neurons of a patient's brain, however, recent findings have realized that AB may not be the origin of the disease, which may instead be the fault of tau proteins. Additionally, it has been found that tau proteins such as 2N4R-TI, synergistically work with a-syn, another protein of interest, to cause aggregation and oligomerization, leading to AD or PD. This demonstrates the need to design small molecule inhibitors that can inhibit both proteins simultaneously.

Using rational design, the researchers developed a series of possible inhibitors, and found that two (C46 and C48) showed remarkable inhibitory effect on the aggregation of both the 2N4R-TI and a-syn proteins, as well as C46 showing a strong inhibitory effect on the formation of oligomers of a-syn. Additionally, the researchers discovered that C48 showed a strong anti-inclusion ability for a-syn aggregates at concentrations 10-40 uM in vivo, in a neuroblastoma M17D cell model.

Technology Validation:

The anti-aggregation ability of the compounds was evaluated using a thioflavin (ThT) fluorescence assay. The researchers added C46 and C48 to a final concentration of 100 uM and added ThT to a final concentration of 20 uM. They then added a-syn protein to the wells, at a concentration of 6 uM

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and measured the fluorescence of the ThT over time using a Synergy HT multi-mode microplate reader for a total of n = 3 trials. C46 and C48 were found to have decreased the ThT fluorescence from 100% (the max fluorescence from the control) to 4.0 and 14.8%, respectively. The anti-oligomer formation ability of the compounds was evaluated by conducting a photo-induced crosslinking of unmodified proteins (PICUP) assay. This involved testing of a-syn alone (60 uM), a-syn with C46 and C48 (at 50, 100, and 200 uM), 2N4R-TI alone (10 uM), 2N4R-TI treated with C46 and C48 (at 50, 100, and 200 uM), and a control with no crosslinking agents or light. The resulting proteins after the assay was complete were compared using gel electrophoresis, with C46 showing the strongest anti-oligomer activity against a-syn by its significant reduction of 35 – 45 kDa a-syn oligomers.

The researchers measured the anti-inclusion ability of their compounds against M17D neuroblastoma cells transformed with the inducible alpha-synuclein protein fused with yellow fluorescent protein (YFP) for imaging. They induced formation of the a-syn::YFP fusion protein at 48 hours after inoculation and treated the cells with a range of concentrations (from 10-40 micro-molar of C46 and C48) 96 hours after inoculation (n = 6). C48 was found to have significantly reduced the intensity of the fluorescence of a-syn::YFP fusion proteins from 10 – 40 uM, indicating successful inhibition of inclusions made from a-syn.

Advantages:

- Duel targeting of a-syn and 2N4R-TI

- Significant inhibitory effect on a-syn and 2N4R-TI aggregation and a-syn oligomers

- Reduction in a-syn-based inclusions

Applications:

- Treatment of Alzheimer's, Parkinson's, and dementia with Lewi bodies

- Biological study of 2N4R-TI and a-syn proteins

- Medical diagnostics

Publications:

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