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Dithiourea Derivatives Inhibit Alpha-synuclein Oligomer and Fibril Formation

Dithiourea derivatives block alpha-synuclein oligomers and fibrils with strong anti-inclusion effects in cells.

Researchers at Purdue have discovered several small molecule inhibitors (dubbed C1 and C2) of alpha-synuclein (a-syn) that are capable of inhibiting a-syn oligomer and fibril growth, as well as reducing the number of a-syn inclusions in a M17D cell model. Parkinson's disease (PD) is a neurodegenerative disease afflicting 10 million people worldwide. Thus far, it was believed that large inclusions of proteins called Lewy Bodies (LB) were responsible for the symptoms of PD, however, recent experimental evidence points to LBs being inert, and instead formation of smaller oligomers from proteins such as a-syn may be the origin of the pathogen. Currently, there are no FDA-approved drugs to treat PD, only drugs that can manage symptoms.

The researchers found that C2 had the strongest anti-oligomer activity against a-syn in vitro, with C1 having a moderate anti-oligomer effect, both compounds were effective at reducing the amount of mature a-syn fibrils. Additionally, C1 showed the strongest anti-inclusion ability in vivo, significantly reducing the number of a-syn inclusions in a M17D neuroblastoma cell model. Notably, no loss in cell confluence was detected, indicating little to no cytotoxicity.

Technology Validation:

The anti-oligomer activity of the compounds was evaluated by conducting a PICUP assay, this involved treatment of a-syn (60 uM) with DMSO (the control), C1 (50 uM), or C2 (50 uM) and visualizing the resultant oligomers on a Coomassie blue stained polyacrylamide gel. It was found that C1 and C2 reduced the oligomers by 52.6% and 84.2%, respectively, compared to the control. The anti-fibrillary activity of the compounds was observed by incubating a-syn (2 uM) for 22 hours to reach mature a-syn fibrils, the mature fibrils were treated with C1, C2 (both at 100 uM), or DMSO (1.5%) as a control, and viewed with transmission electron microscopy. Samples

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treated with C1 and C2 both showed fewer and shorter a-syn fibrils, compared to the control. This was quantified by conducting a thioflavin (ThT) fluorescence assay, briefly: a-syn (9.4 uM) was allowed to mature for 48 hours, after which the ThT assay was performed by diluting the mature fibrils to 6 uM, and treating them with C1, C2 (both at 100 uM), and DMSO (1.5%) as a control. Both C1 and C2 promoted disaggregation of the mature fibrils, reducing the ThT fluorescence intensity by ~50% each, as compared to the control.

The anti-inclusion ability of the compounds was quantified by treating M17D neuroblastoma cells expressing an inducible alpha-synuclein and yellow fluorescent protein (YFP) fusion protein (a-syn::YFP). The cells were treated with 1.25, 2.5, 5, and 10 uM of C1 and C2, as well as 0.1% DMSO as a control 24 hours after plating the cells (control, n = 18; 10 μM, n = 6; all other concentrations, n = 12). At t = 48 hours, the cells were induced to produce a-syn::YFP and the total fluorescence and confluence of each sample was observed for an additional 48 hours. It was found that C1 reduced the number of a-syn inclusions at 5 and 10 uM, with the strongest anti-inclusion effect at 10 uM. C2 was found to increase the inclusions at 5 and 10 uM concentrations. Neither treatment affected the cell confluence.

Advantages:

- Significantly inhibits oligomer and fibril formation at micro molar concentrations

- Ability to disaggregate mature a-syn fibrils

- Strong anti-inclusion activity

- Compounds did not display cytotoxic effects towards M17D cells

Applications:

- Treatment of Parkinson's disease

- Parkinson's disease medical diagnostics

- Biological investigation of a-syn protein

Publication:

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