In vitro screen for inhibitors of Tau filament formation

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BACKGROUND

Tau protein stabilizes microtubules and contributes to key structural and regulatory cellular functions as axonal transport and signaling. Neurofibrillary tangles, mainly composed of bundles of Tau are implicated in the pathogenesis of neurodegenerative diseases (ND) such as tauopathies including Alzheimer's disease (AD). Accumulation of Tau aggregates correlates well with nerve cell loss and the severity of dementia. The identification of factors involved in Tau fibrillization is of great importance to clarify the etiology of such ND.

MATERIALS AND METHODS

To identify inhibitors of Tau aggregation, a Heparin/ Sodium Octadecylsulfate induced Tau fibril formation assay was used. Fibrillization of recombinant Tau441 (2N4R) P301L was monitored by two ways: Thioflavine S (ThioS) fluorescence read out and transmission electron microscopy (TEM). In a first round, Gradient Biomodeling's computational platform for quantum molecular modeling was used to propose 26 prospective compounds. These compounds were analyzed by ThioS fluorescence resulting in 9 potential Tau aggregation inhibitors. In the Tau-aggregation assay compounds were incubated in parallel with Tau for 15h at 37° C. Fluorescence intensity was measured at 465/510 nm.

Five out of these 9 compounds were selected for IC50 determination in a Tau aggregation and dis-aggregation assay. In the Tau dis-aggregation assay compounds were incubated with pre-aggregated Tau (18h) for another 20h at 37° C.

TEM analysis was performed with the two most promising candidates. Samples were placed onto carbon-coated grids. Negative staining was performed by 1% Uranylacetat. Samples were viewed with a FEI Tecnai G2 20 transmission electron microscope (FEI Eindhoven) with a Gatan ultrascan 1000 ccd camera. Acceleration voltage was 120 kV.

RESULTS

In a first screen, 26 compounds (2 water-soluble, 24 DMSO-soluble) were analyzed for their potential to inhibit Tau fibrillization using a fluorescent read out. Test items (T.I.) were incubated in parallel with Tau441 (2N4R) P301L for 15h at 37° C. Nine out of the 26 compounds significantly reduced ThioS fluorescence intensity and thus were identified as potential inhibitors of Tau fibrillization.

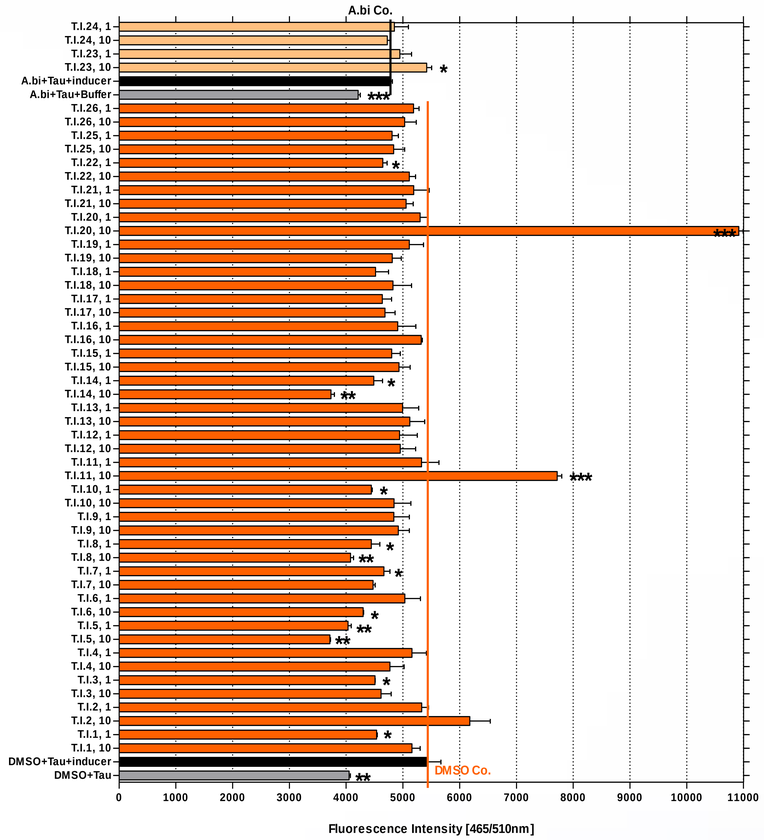
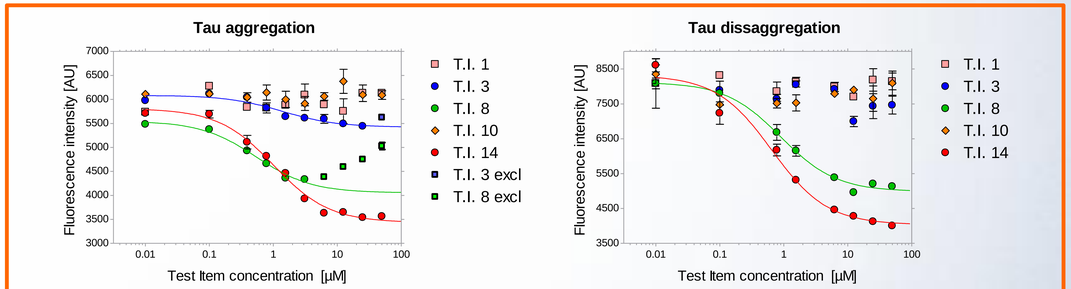


Figure 1. Effects of 26 compounds on Tau aggregation *in vitro*. Graph represents effects of 26 compounds (final concentration of 10 and 1 µM) on Tau aggregation *in vitro*. Tau (1 µM) was incubated with the different T.I. for 15h at 37° C. Fluorescence was measured at 465/510 nm. Black bars represent the Vehicle Controls (including DMSO or A.bi. plus Tau and inducers), grey bars are controls missing Tau inducers, dark orange bars are Tau aggregated with compounds in DMSO and light orange bars represent compounds in water aggregated with Tau. Statistical significance is indicated by *<0.05, **<0.01, ***<0.001 as determined by t-test (unpaired, two-tailed compared to either DMSO+Tau+inducer or A.bi+Tau+inducer). Data are shown as group mean + SEM (n=3).

From the 9 potential candidates, 5 compounds were further analyzed for their IC50 in a Tau aggregation and dis-aggregation assay. IC50 was determined showing effective concentrations at 0.54 and 1.02 µM for T.I. 8 and 14 in the Tau aggregation assay. The dis-aggregation assay was used to determine the capability of the compounds to interfere with already aggregated Tau. The effective concentrations of this assay were comparable to the Tau aggregation assay and showed 0.9 and 0.65 µM for T.I. 8 and 14, respectively.

Three compounds in two different concentrations were chosen for TEM analysis (T.I.3 not shown) using the same experimental setup as for the Tau aggregation and dis-aggregation assay (two independent samples per concentration). A tremendous effect on the size and number of Tau fibrils was observed in samples treated with T.I.14 and 8 (30 µM) when compared to Vehicle Control treated samples in the Tau aggregation and dis-aggregation assay.

From 26 compounds, 2 compounds were identified as promising inhibitors of Tau fibrillization identified by two independent assays.



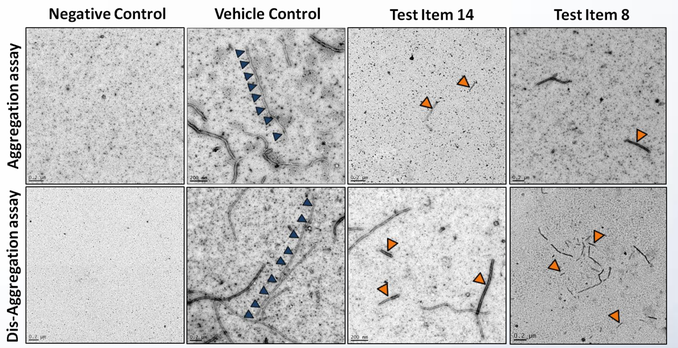


Figure 2. Effects of compounds on Tau aggregation and dis-aggregation *in vitro*.

A) T.I.8 and 14 not only minimized Tau aggregation *in vitro* (left), but were also able to reduce pre-aggregated Tau (right). IC50 was determined showing effective concentrations at 0.52 and 1.02 µM and 0.9 and 0.65 µM in the Tau aggregation and Tau dis-aggregation assay for T.I.8 and T.I.14, respectively.

B) T.I.14 reduced tremendously the size and number of Tau fibrils in the Tau aggregation and dis-aggregation assay, also T.I.8 showed similar effects, but to a less extend. Long Tau fibrils in the Vehicle Control are indicated by blue and short ones after applying T.I.8 and T.I.14 are indicated by orange triangles. Representative images are shown from around 15.

CONCLUSION

The Tau dis-aggregation and aggregation assay in combination with TEM analysis are powerful screening tools for the identification of potential Tau aggregation inhibitors. These physiological inhibitors of Tau fibrillization hold promise for developing new strategies for treatment of AD.

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