**AID 1475**

**Quantitative High-Throughput Screen for Inhibitors of Tau Fibril Formation: Summary**

**Description**

* NIH Molecular Libraries Probe Production Network [MLPCN]
* NIH Chemical Genomics Center [NCGC]
* MLPCN Grant: X01 MH083262-01
* Assay Provider: Carlo Ballatore, University of Pennsylvania

**NCGC Assay Overview:**

The microtubule-associated protein tau is an abundant protein in the axons of neurons that stabilizes microtubules. With its ability to modulate microtubule dynamics, tau contributes directly or indirectly, to key structural and regulatory cellular functions. Particularly important is the influence tau exerts on axonal transport, which allows signaling molecules, trophic factors and other essential cellular constituents to travel along the axons. Under pathological conditions, tau becomes sequestered into insoluble aggregates called neurofibrillary tangles. This phenomenon is believed to have pathological consequences by promoting axonal transport deficits that ultimately lead to synaptic dysfunction and neuronal loss.

To identify inhibitors of tau aggregation, a heparin-induced tau fibril formation assay was used that employed a recombinantly expressed fragment of tau, K18 (Q242-E372), bearing a P301L mutation (Gustke et al., 1994; Hong et al., 1998)). This assay monitors tau fibrillization in two ways, Thioflavin T (ThT) fluorescence and fluorescence polarization (FP) of Alexa 594-labeled K18 P301L, which does not fibrillize readily but incorporates into growing filaments of unlabeled tau. The assay was screened against the MLSMR in a quantitative high-throughput screen (qHTS, (Inglese et al., 2006)) of six library concentrations where ThT ([AID 1460](https://pubchem.ncbi.nlm.nih.gov/bioassay/1460)), FP mP ([AID 1468](https://pubchem.ncbi.nlm.nih.gov/bioassay/1468)) and FP total fluorescence ([AID 1463](https://pubchem.ncbi.nlm.nih.gov/bioassay/1463)) readouts were collected. The total fluorescence measurement for the FP assay served as a counterscreen to identify fluorescent compounds. The titration-response data were curve-fit and classified to identify actives and nascent SAR analysis was performed.

For follow-up, 134 compounds were selected based on the following criteria: active with quality curve-fit in one or both of the ThT and FP measurements, little or no activity in the total fluorescence readout, absence of unwanted series bearing reactive or unstable functional groups and lacking promiscuous activity in other screens. The compounds were tested in the original screening assay where ThT ([AID 1558](https://pubchem.ncbi.nlm.nih.gov/bioassay/1558)), FP mP ([AID 1559](https://pubchem.ncbi.nlm.nih.gov/bioassay/1559)) and FP total fluorescence ([AID 1694](https://pubchem.ncbi.nlm.nih.gov/bioassay/1694)) readouts were collected. Assay samples were also tested in a sedimentation assay ([AID 1720](https://pubchem.ncbi.nlm.nih.gov/bioassay/1720)) where tau filaments pellet upon centrifugation while tau monomers remain in the supernatant. Assay samples where the compound inhibited tau sedimentation by 40% or more were examined by transmission electron microscopy ([AID 1719](https://pubchem.ncbi.nlm.nih.gov/bioassay/1719)) to visualize the size and morphology of the remaining tau filaments.

An aminothienopyridazine series that showed good performance in the follow-up assays and suitable chemical tractability was chosen for probe optimization. Analogs from this series were tested in several secondary assays to determine the selectivity of tau filament inhibition. The analogs did not inhibit tau-mediated microtubule assembly ([AID 1709](https://pubchem.ncbi.nlm.nih.gov/bioassay/1709)) or filament formation of the beta-amyloid protein fragment, Abeta 1-42 ([AID 1712](https://pubchem.ncbi.nlm.nih.gov/bioassay/1712)). Series members showed little or no inhibition in a caspase-1 enzymatic assay ([AID 1711](https://pubchem.ncbi.nlm.nih.gov/bioassay/1711)), which is sensitive to nonspecific redox active compounds. These assays indicated the aminothienopyridazine series were selective inhibitors of tau filament formation in vitro. The most active compound (ML103) from this series was nominated as the probe for Tau fibril inhibition.

Gustke, N., B. Trinczek, et al. (1994). "Domains of tau protein and interactions with microtubules." Biochemistry 33(32): 9511-22.

Hong, M., V. Zhukareva, et al. (1998). "Mutation-specific functional impairments in distinct tau isoforms of hereditary FTDP-17." Science 282(5395): 1914-7.

Inglese, J, Auld, DS, Jadhav, A, Johnson, RL, Simeonov, A, Yasgar, A, Zheng, W, Austin, CP (2006). Quantitative high-throughput screening: a titration-based approach that efficiently identifies biological activities in large chemical libraries. Proc Natl Acad Sci U S A 103: 11473-8.