Development of the Novel Small Molecule Tau Aggregation Inhibitors PTI-51-CH3 (TauPro™) and PTI-80 for the Treatment of Tauopathies

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Abstract

Accumulation of intracellular neurofibrillary tangles (NFTs) composed of aggregated tau protein is a key pathological hallmark of all tauopathies. PTI-51-CH3 (TauPro™) and PTI-80 have been identified and developed as two potent tau aggregation inhibitors using ProteoTech's proprietary small molecule library. Both compounds target the tau repeat domains (TauRD) that constitute the core of tau fibrils in NFTs, and have the ability to prevent tau fibril formation as well as disaggregate pre-formed tau fibrils. The robust inhibitory activities occur at a compound: tau protein molar ratio of 0.3-0.4 in Thioflavin S fluorometry studies. PTI-51-CH3 and PTI-80 also dose-dependently inhibit tau from forming β-sheet-containing fibrils as determined by circular dichroism (CD) spectroscopy and electron microscopy (EM). The inhibitory potency appears to be superior to those previously reported in the literature. As drug candidates, PTI-51-CH3 and PTI-80 also possess good PK parameters in plasma, and have reasonable brain exposure at the C_{max} exceeding the estimated free-tau concentration range in brain cells (< 10 nM, as previously estimated). Both compounds have safe drugability profiles with no cytotoxicity observed in cell culture studies, and no significant CYP450 inhibition. PTI-51-CH3 and PTI-80 are currently being tested for *in vivo* efficacy in a transgenic mouse model that expresses a human tau isoform with a FTDP-17 P301S mutation, and has commonly been used as a tauopathy animal model. Our results suggest that PTI-51-CH3 (TauPro™) and PTI-80 are top pre-clinical candidates for development as tau aggregation inhibitors for the treatment of Alzheimer's disease, progressive supranuclear palsy and other tauopathies.

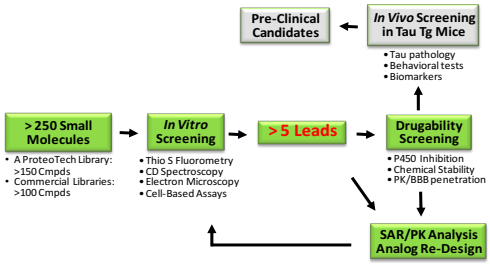


Figure 1. ProteoTech's Program for Identifying Novel Small Molecules Targeting Tau Protein Aggregation

PTI-80

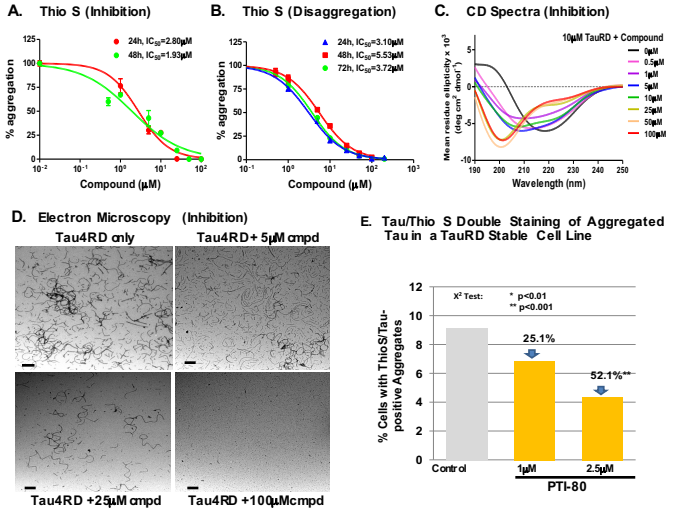
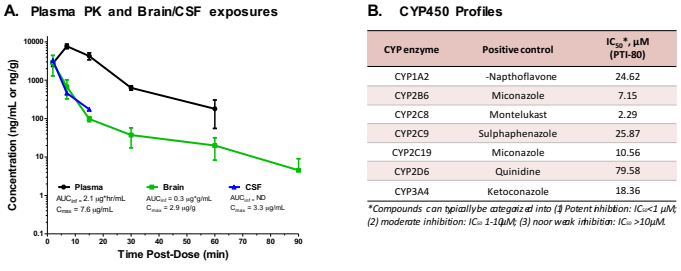


Figure 2. PTI-80 Inhibits Tau Protein Fibril Formation and Disaggregates Pre-Formed Tau Fibrils. Tau fibril formation was achieved by incubation of 10μM Tau4RD protein with 10μM heparin at 37°C with shaking for 24-72 hrs. For inhibition assays, compound was added into the reactions at time 0. For disaggregation assays, compound was incubated with pre-aggregated tau fibrils. The readouts were measured by Thioflavin S fluorometry **(A-B)**, circular dichroism spectroscopy **(C)**, and electron microscopy (**D**; bar=200nm). **(E)** Treatment of HEK-TauRD-ΔK280 cells (a stable cell line) with PTI-80 for 48 hrs led to a dose-dependent reduction in cells containing tau-/Thio S-positive aggregates as measured by double staining (using a tau specific antibody and Thioflavin S fluorescence).



CYP enzyme	Positive control	IC_{50}*, μM (PTI-80)
CYP1A2	-Napthoflavone	24.62
CYP2B6	Miconazole	7.15
CYP2C8	Montelukast	2.29
CYP2C9	Sulphaphenazole	25.87
CYP2C19	Miconazole	10.56
CYP2D6	Quinidine	79.58
CYP3A4	Ketoconazole	18.36

*Compounds can typically be categorized into (1) Potent inhibition: IC_{50}<1 μM; (2) moderate inhibition: IC_{50} 1-10μM; (3) noor weak inhibition: IC_{50} >10μM.

Figure 3. PTI-80 PK and Drugability Profiles. **(A).** PTI-80 levels in plasma, brain (post transcardial perfusion), and CSF (pooled samples) after a single 50 mg/kg s.c. injection in mice (n=4). Samples were collected at 2-360 min post dose, and analyzed by HPLC/MS. **(B)**. Reversible CYP450 inhibition was determined using Vivid® CYP450 kits (LifeTechnologies).

PTI-51-CH3 (TauPro™)

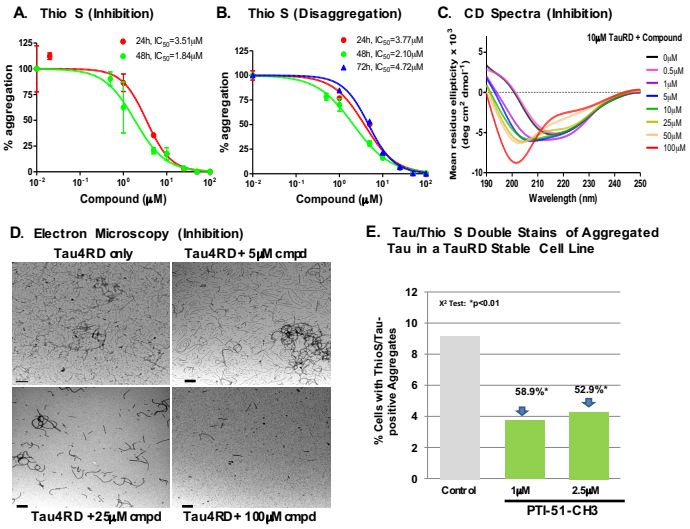
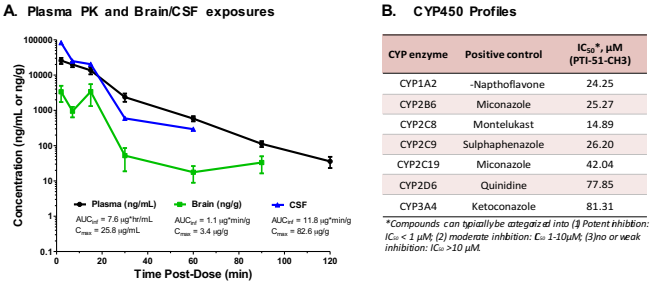


Figure 4. PTI-51-CH3 (TauPro™) Inhibits Tau Protein Fibril Formation and Disaggregates Pre-Formed Tau Fibrils. Tau fibril formation was performed as described in Fig.2. The readouts were measured by Thioflavin S fluorometry **(A-B)**, circular dichroism spectroscopy **(C)**, and electron microscopy (**D**; bar=200nm). **(E)** Treatment of HEK-TauRD-ΔK280 cells with PTI-51-CH3 for 48 hrs led to a reduction in cells containing tau-/Thio S-positive aggregates as measured by double staining (using a tau specific antibody and Thioflavin S fluorescence).



CYP enzyme	Positive control	IC_{50}*, μM (PTI-51-CH3)
CYP1A2	-Napthoflavone	24.25
CYP2B6	Miconazole	25.27
CYP2C8	Montelukast	14.89
CYP2C9	Sulphaphenazole	26.20
CYP2C19	Miconazole	42.04
CYP2D6	Quinidine	77.85
CYP3A4	Ketoconazole	81.31

*Compounds can typically be categorized into (1) Potent inhibition: IC_{50} < 1 μM; (2) moderate inhibition: IC_{50} 1-10μM; (3)no or weak inhibition: IC_{50} >10 μM.

Figure 5. PTI-51-CH3 (TauPro™) PK and Drugability Profiles. **(A).** PTI-51-CH3 levels in plasma, brain (post transcardial perfusion), and CSF (pooled samples) after a single 50 mg/kg s.c. injection in mice (n=4). Samples were collected at 2-360 min post dose, and analyzed by HPLC/MS. **(B).** Reversible CYP450 inhibition was determined using Vivid® CYP450 screening kits.

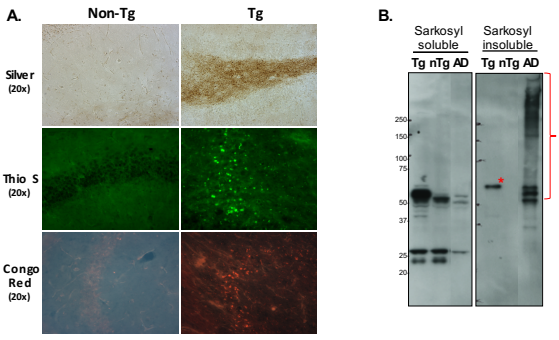


Figure 6. Use of a Tau Transgenic Mouse Model for Testing of PTI-51-CH3 and PTI-80 Efficacy *In Vivo*. The transgenic mice express human tau isoform 1N4R with a FTDP-17 P301S mutation. **(A)** Tau aggregates/NFTs were detected in the hippocampus of 8-month-old Tg mice by Bielschowsky silver (upper panels), Thio S (middle panels), and Congo red (lower panels) staining. **(B)** Aggregated/PHF tau proteins were also detected in Sarkosyl insoluble fractions of the Tg mouse brain lysates (indicated by *) as well as in AD brain (brackets) by Western blotting with Tau mAb AT180.

Conclusions

1) **PTI-51-CH3 (TauPro™) and PTI-80 are two small molecules (representing new chemical entities) developed by ProteoTech that are both (1) a potent inhibitor of tau protein aggregation and tangle formation; (2) a disaggregator and reducer of pre-formed tau fibrils and tangles.**

2) **PTI-51-CH3 and PTI-80 are currently being tested *in vivo* for reduction/inhibition of tangles in a transgenic tau mouse model.**

3) **PTI-51-CH3 and PTI-80 are top pre-clinical candidates as tau aggregation inhibitors/reducers for the treatment of Alzheimer's disease, progressive nuclear palsy and other tauopathies.**

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