Allosteric Heat Shock Protein 70 Inhibitors Rapidly Rescue Synaptic Plasticity Deficits by Reducing Aberrant Tau

Supplemental Information

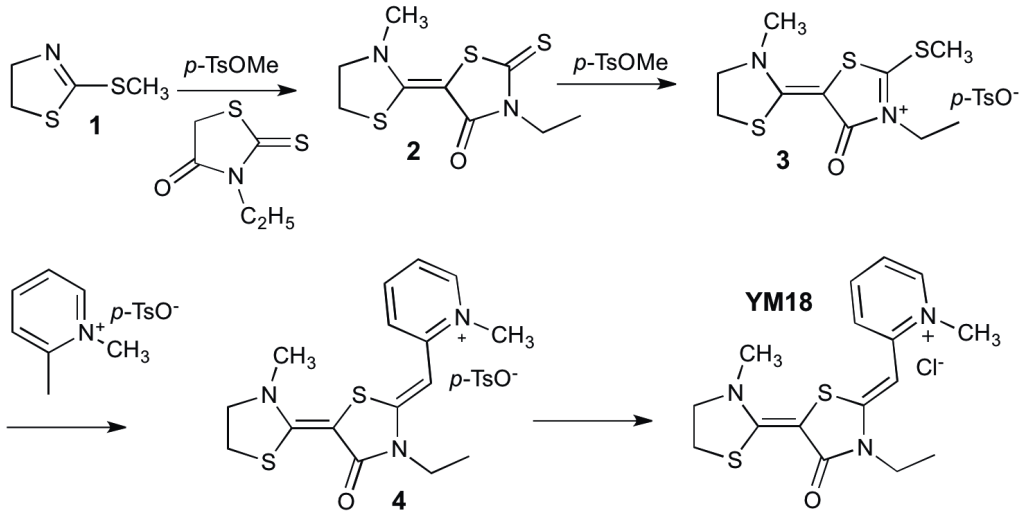

Figure S1. Synthetic Scheme for YM18. 2-((*Z*)-((*E*)-3'-ethyl-3-methyl-4'-oxo-3',4,4',5-tetrahydro-2'*H*,3*H*-[2,5'-bithiazolylidene]-2'-ylidene)methyl)-1-methylpyridin-1-ium (**YM-18**). To 55 mmol of 2-(methylthio)-4,5-dihydrothiazole (**1**) dissolved in 14 mL of anisole was added 82 mmol of methyl-*p*-toluenesulfonic acid. The reaction mixture was stirred at 130°C for 4 hours, cooled to room temperature and 55 mmol of 3-ethylrhodanine dissolved in 200 mL of acetonitrile was added. To the reaction mixture was added dropwise 91 mmol of triethylamine and this mixture was stirred at room temperature overnight. The precipitate was filtered, washed with acetonitrile and dried *in vacuo* to afford (*E*)-3'-ethyl-3-methyl-2'-thioxo-4,5-dihydro-2'*H*,3*H*-[2,5'-bithiazolylidene]-4'(3'*H*)-one (**2**) as a yellow solid in ~90% yield. Intermediate **2** was dissolved in dry DMF and treated with methyl-*p*-toluenesulfonic acid to yield (*E*)-3-ethyl-5-(3-methylthiazolidin-2-ylidene)-2-(methylthio)-4-oxo-4,5-dihydrothiazol-3-ium (**3**), which was then treated with 1,2-dimethylpyridin-1-ium *p*-toluenesulfonate and 1 equiv. triethylamine in 10 mL acetonitrile at 50°C for 3 hrs to yield 2-((*Z*)-((*E*)-3'-ethyl-3-methyl-4'-oxo-3',4,4',5-tetrahydro-2'*H*,3*H*-[2,5'-bithiazolylidene]-2'-ylidene)methyl)-1-methylpyridin-1-ium (**4**) in 40% yield. Anion exchange on Amberlite IRA-400 provided the chloride salt (**YM-18**) in ~15% overall yield. Purity was >95% by ^{1}H nuclear magnetic resonance. ^{1}H *d6*-DMSO 8.63 (1H, d), 8.20 (1H, t), 7.98 (1H, d), 7.40 (1H, t), 5.87 (1H, t), 4.10 (3H, s), 4.0 (2H, q), 3.8 (2H, q), 3.40 (3H, s), 3.2 (2H, q), 1.21 (3H, t). Electrospray ionization mass spectrometry: calculated for $C_{16}H_{19}N_3OS_2^{2+}$ $[M\text{-}Cl^-]^+$ m/z 369.9, found 369.7.

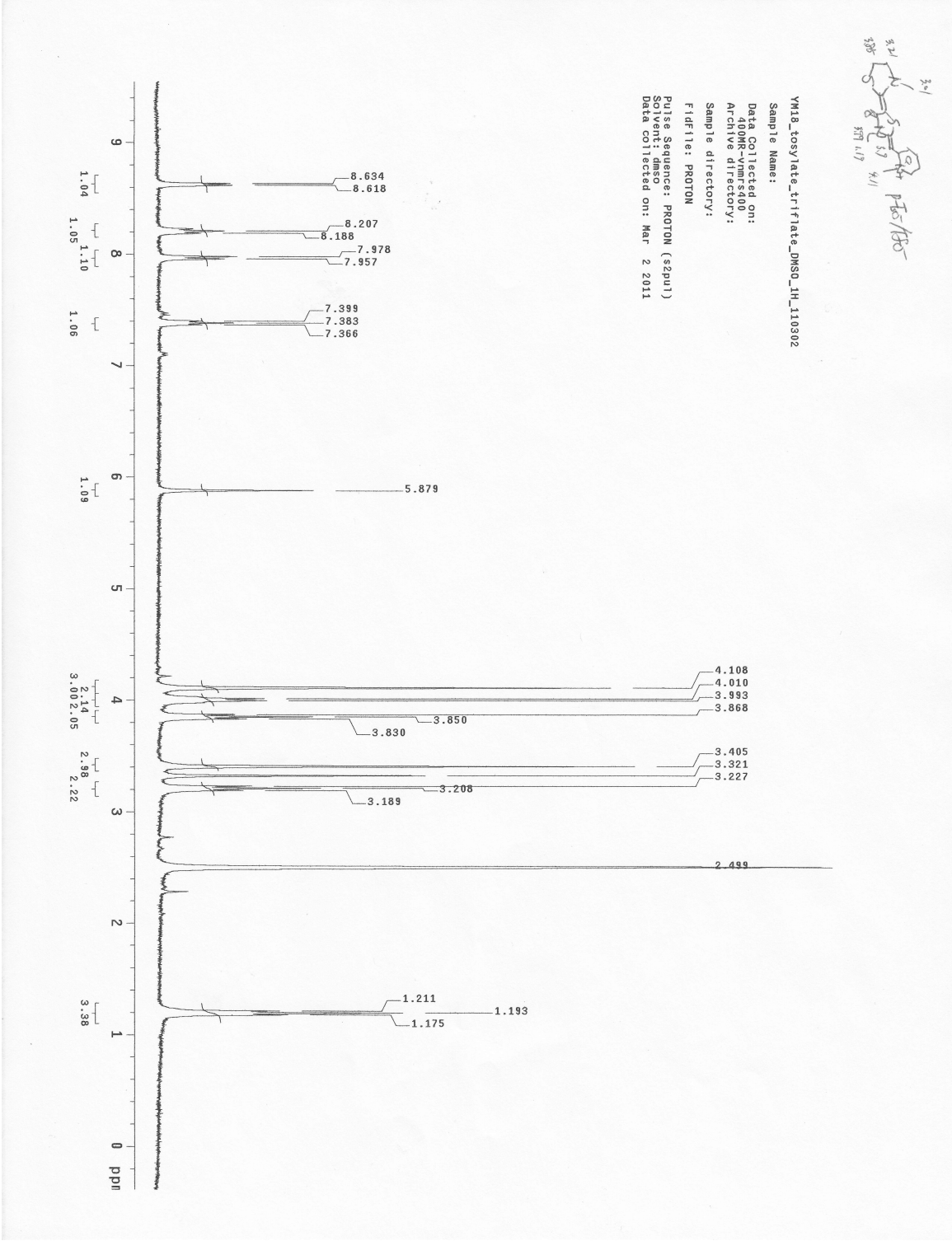

Figure S2. Nuclear magnetic resonance (NMR) characterization of YM18. ^{1}H NMR spectra of YM-18.

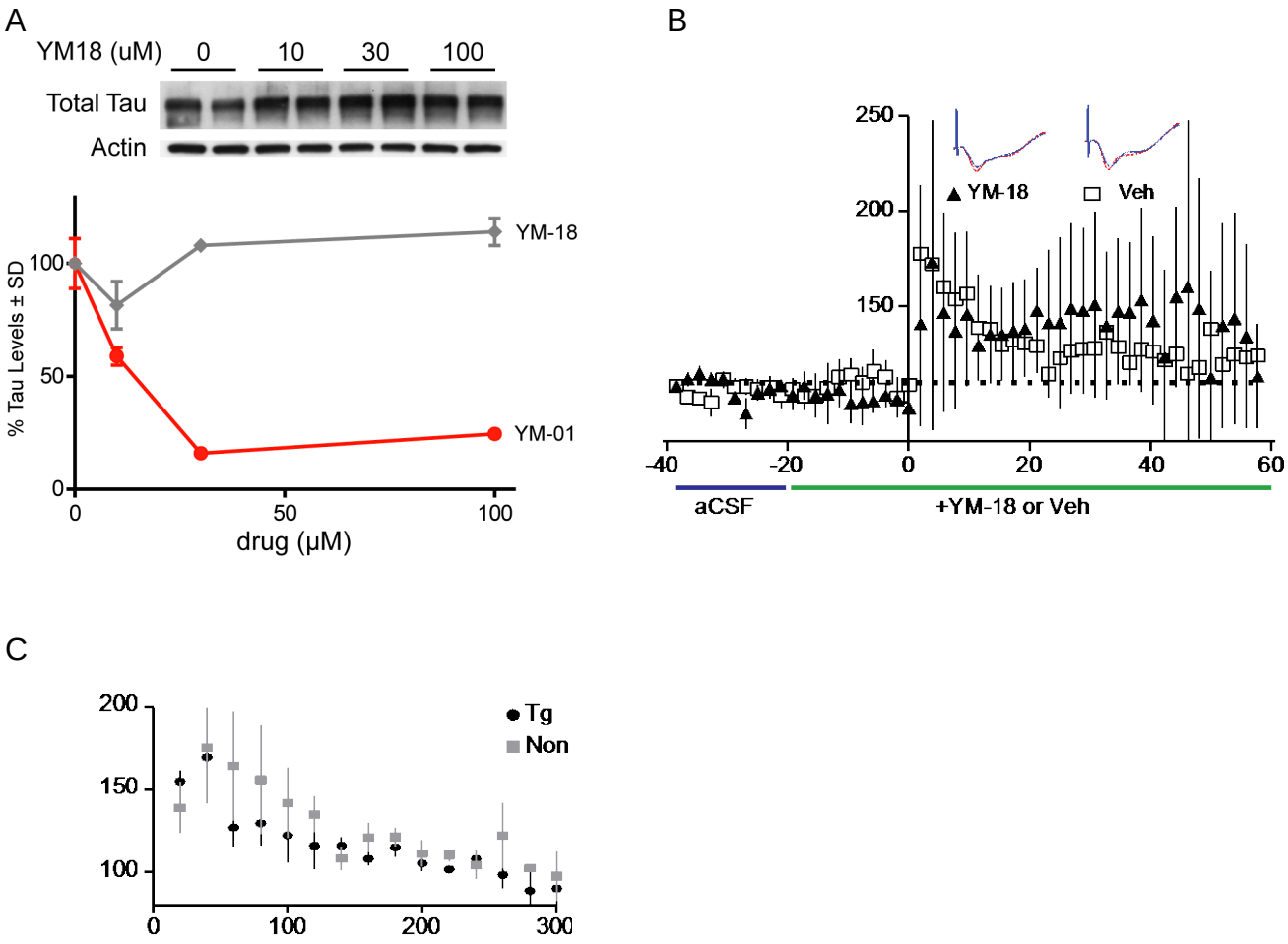

Figure S3. YM-18 does not reduce tau levels in HeLaC3 cells and does not rescue long-term potentiation (LTP) deficits in tau transgenic mice. (**A**) Cells were treated with YM-18 or vehicle control for 4 hrs. Tau levels were assessed by western blot, quantified and graphed next to YM-01-treated cells for comparison. (**B**) Graphical representation of electrophysiology experiments in hippocampal slices of rTg4510 mice. After recording baseline signal for 20 min, hippocampal slices were perfused with 30 μm YM-18 or vehicle (Veh) control in artificial cerebrospinal fluid (aCSF) continuously for the remainder of the experiment (green line). Baseline signal was recorded for 20 min, LTP was induced with theta-burst stimulation (5 bursts of 200 Hz separated by 200 ms, repeated 6 times with 10 s between the 6 trains), and LTP was recoded for 60 min. Changes in field excitatory post-synaptic potentials (fEPSP) slope are expressed as a percentage of baselines. Black triangles and white boxes represent fEPSP traces of YM-18- (n = 4) or vehicle-treated (n = 6) hippocampal slices from 3-4 month old tau transgenic mice. Statistical analysis was performed using a 2-way ANOVA with Bonferroni post-test, which showed no difference in the slopes between both conditions (p = 0.98). Blue traces represent baseline recordings, while red traces correspond to representative LTP recordings of each condition. (**C**) Pre-pulse facilitation (PPF) graph of the same mice used for LTP. There was no significant difference in PPF between Non and Tg mice.