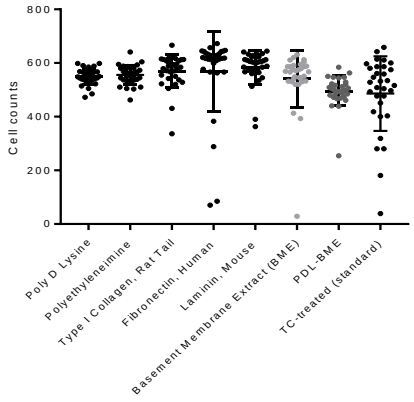
Supporting Information

A.



B.

Plate	HDTA	CTG
1	0.57	0.43
2	0.52	0.55
3	0.67	0.59
4	0.51	0.52
5	0.53	0.50
6	0.50	0.57
7	0.43	0.50
8	0.57	0.54
9	0.69	0.53
10	0.56	0.41

C.

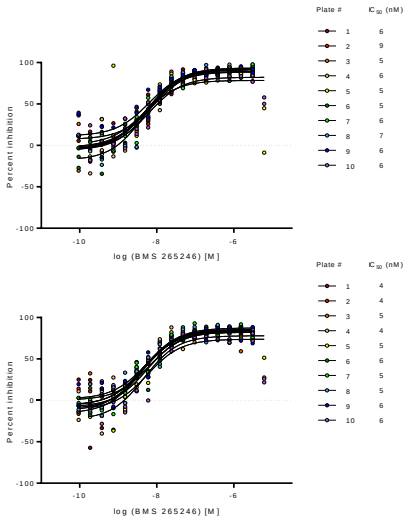


Figure S1. A) Comparison of cell numbers per well using different well coatings, or no coating (TC-treated), in the 1536-well T40PL-GFP^{agg} cell tau inclusion assay after HDTA-extraction and PFA fixation. Several coatings reduced well-to-well variability compared to uncoated wells, with poly-D-lysine selected for compound screening. **B**) A listing of the individual plate Z'-scores from the screening of the NCATS Pharmaceutical Collection in the T40PL-GFP^{agg} cell tau inclusion assay after HDTA or CTG extraction of soluble tau. **C**) A concentration-response profile was obtained for the positive-control compound, BMS-265246, in each 1536-well plate utilized in the screening of the NCATS Pharmaceutical Collection in the T40PL-GFP^{agg} cell tau inclusion assay. Top; BMS-265246 inhibition curves obtained from each screening plate after extraction with HDTA. Bottom; BMS-265246 inhibition curves obtained from each screening plate after extraction with CTG.

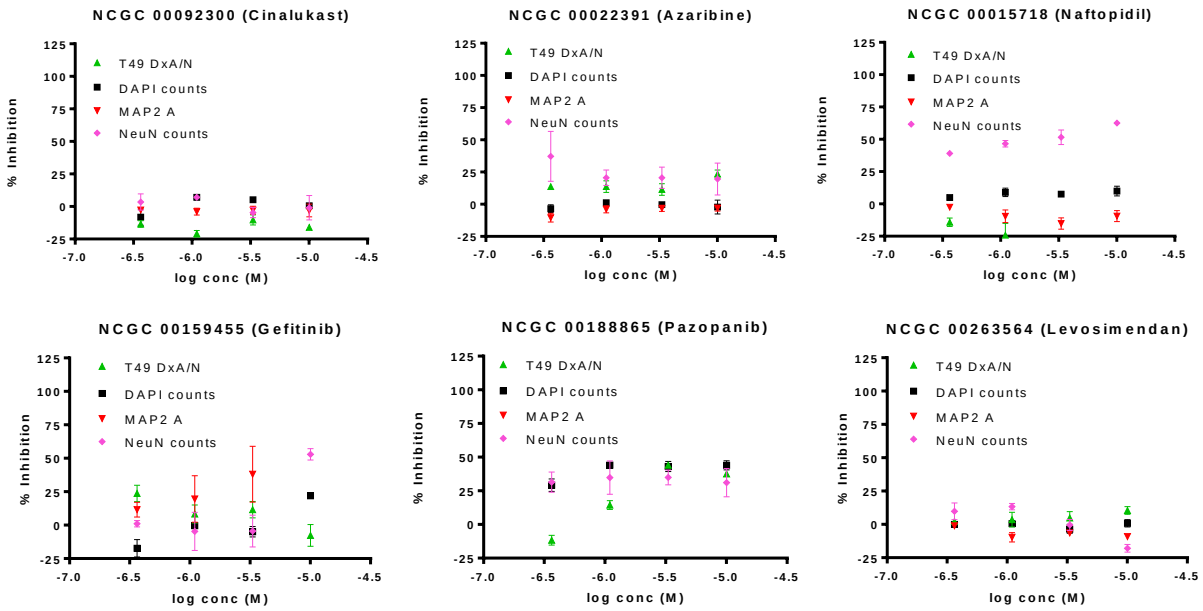
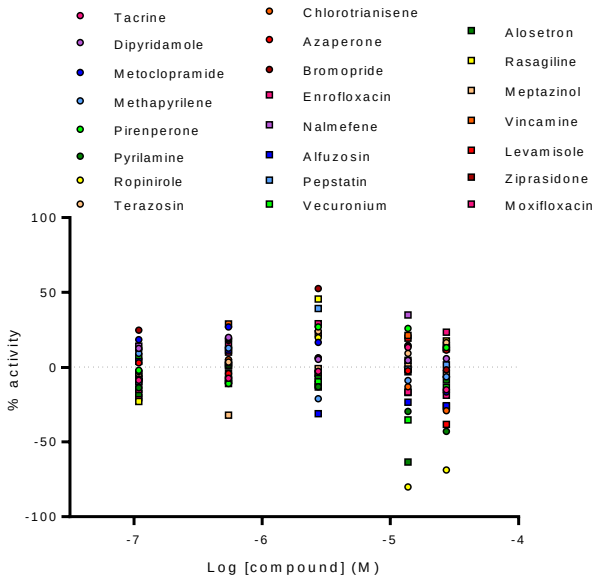


Figure S2. Testing of NCATS Pharmaceutical Collection screening hits from the T40PL-GFP^{agg} cell assay in the rat cortical neuron tau inclusion assay. None of the compounds except SNC-80 (Figure 4B) showed evidence of a concentration-dependent inhibition of tau aggregates. All endpoints are expressed as percent inhibition relative to vehicle-treated neurons. T49 DxA/N = integrated mouse tau signal per DAPI nuclei; DAPI counts = total number of DAPI-positive nuclei; MAP2 A = MAP2 (dendritic) area; NeuN counts = total number of NeuN-positive nuclei.

		Singlet Screen (% inhibition)		Triplicate Confirmation Testing (% Inhibition)							
		DAPI counts	Tau per nuclei	DAPI counts	DAPI counts	Tau per nuclei	Tau per nuclei	MAP2 area	MAP2 area	NeuN counts	NeuN counts
Name	Putative Target			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Atracurium	nAchR antagonist	16.5	66.7	-3.4	2.3	52.8	7.9	2.0	7.9	-3.6	-3.6
Dizocilpine	NMDA receptor antagonist	-3.3	76.4	5.9	10.6	54.0	9.4	-11.1	13.4	5.9	5.9
Levamisole	nAchR agonist	-8.2	51.0	-1.6	6.6	55.2	4.1	-18.8	2.2	-5.9	-5.9
Metoclopramide	D2R antagonist	-2.1	54.8	-3.9	3.3	74.5	2.4	24.6	30.8	11.0	11.0
Vincamine	unknown	-5.1	57.8	-5.8	7.9	63.6	4.6	-2.4	6.0	-3.6	-3.6
Tacrine	AchE inhibitor	-5.8	64.7	-18.7	5.1	69.0	6.6	20.4	10.4	10.9	10.9
Alfuzosin	A1 adrenergicR antagonist	-2.3	73.5	-3.5	6.8	57.9	5.0	1.7	19.0	1.6	1.6
Pepstatin A	Acid protease inhibitor	14.7	86.3	2.5	9.1	59.3	3.8	17.8	30.6	10.1	10.1
Alizapride	D2R antagonist	-12.8	66.7	-2.0	5.6	56.9	5.8	-5.9	23.0	0.6	21.3
Pirenperone	5HT2R antagonist	-4.5	54.3	-7.3	2.6	54.2	4.4	5.4	5.5	1.3	9.0
Ropinirole	DAR agonist	12.4	50.5	3.9	6.2	50.4	5.7	4.6	4.3	-6.6	4.9
Lanatoside C	Na+/K+ ATPase	20.5	54.9	8.1	4.5	53.4	5.4	-32.7	16.5	11.0	17.1
Bromopride	D2R antagonist	-1.2	65.5	0.2	3.7	48.5	6.9	8.1	15.2	-4.3	5.6
Meptazinole HCl	u-opioid receptor agonist	-11.4	51.1	-10.2	6.5	57.5	5.9	4.6	8.6	-11.1	2.4
Azaperone	DAR antagonist	1.4	70.8	-12.9	3.6	52.3	10.9	12.1	15.4	-9.3	6.8
Nalmefine HCl	u-opiod receptor antagonist	-7.6	54.1	-0.7	4.5	54.0	15.2	19.1	9.6	-5.5	13.4
Alosetron	5HT3R antagonist	-4.4	53.5	-8.5	2.5	58.7	8.0	2.0	14.7	-0.3	8.3
Cisatracurium	AchR antagonist	14.1	59.4	-6.9	7.9	55.8	8.3	17.3	5.1	-9.5	9.0
Gatifloxacin	bacterial DNA gyrase/topoisomerase inhibitor	4.2	55.0	-4.2	7.9	55.6	9.3	4.6	7.5	-10.1	8.4
Moxifloxacin	bacterial DNA gyrase/topoisomerase inhibitor	-13.7	74.9	-9.6	1.4	63.0	4.6	4.3	3.3	-11.4	1.7
Chlorotrianisene	Estrogen receptor	-15.5	88.8	-5.5	8.8	72.5	6.7	18.8	11.9	7.3	6.5
Alfacalcidol	Vitamin D metabolite	-8.3	86.5	-5.6	45.7	80.2	2.7	30.4	4.7	24.9	7.6
Ziprasidone	D2R/5HT2aR antagonist	-34.9	85.2	-11.6	3.5	48.2	6.0	-1.0	7.9	-1.9	5.2
Alfuzosin	A1 adrenergicR antagonist	5.3	56.1	-10.1	5.1	47.1	7.4	14.6	2.5	4.6	4.6
Dipyridamole	Adenosine uptake inhibitor, PDE inhibitor	10.7	82.8	-6.4	20.9	43.9	1.5	10.1	21.9	9.9	9.9
Thioperamide	H3R antagonist	5.3	75.0	-3.0	1.2	39.9	4.2	-7.9	11.1	-7.4	10.9
Terazosin	A1 AdrenergicR antagonist	-17.0	70.6	5.5	17.6	39.8	11.6	10.9	10.2	-4.2	7.1
Rasagiline	MAO B inhibitor	-3.4	53.3	-1.0	6.3	37.1	1.7	-1.5	12.1	-6.2	6.1
Enrofloxacin	Antibacterial	-15.1	46.0	-12.0	4.2	35.6	3.6	7.5	7.4	-19.2	6.9
Vecuronium bromide	nAchR antagonist	-26.4	51.1	-4.4	3.4	35.5	2.6	2.9	8.6	-1.8	7.2
Pyrilamine	H1R antagonist	3.1	55.5	-7.5	5.7	34.5	12.5	21.9	16.6	2.0	2.0
Methapyrilene	H1R antagonist	-4.1	63.9	4.2	3.2	33.5	13.1	-25.2	8.3	-11.9	-11.9

Table S1. List of confirmed, non-toxic hits from the Prestwick library screen in the rat cortical tau inclusion assay. Data are provided showing the inhibitory activity of each compound on tau inclusions and DAPI-nuclei from the original screen (10 µM compound), and tau, DAPI, MAP2 and NeuN inhibitory activity in the triplicate confirmation analyses (10 µM compound). Compounds highlights in yellow underwent concentration-response testing.

A.



B.

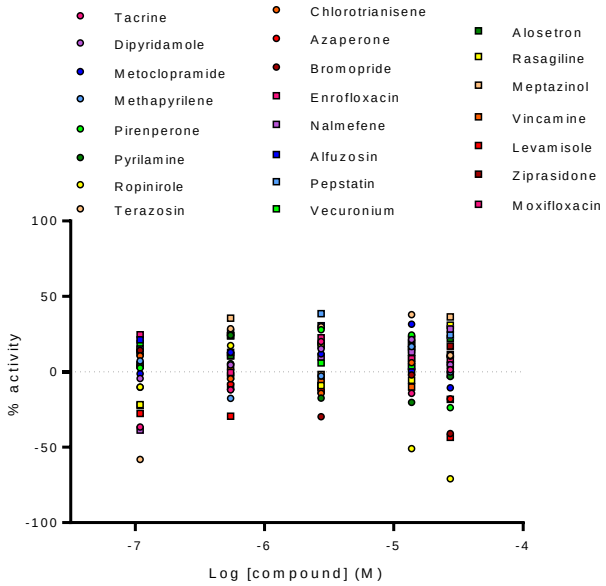


Figure S3. A subset of the compounds identified as confirmed non-toxic hits in the rat cortical neuron tau inclusion assay were also within the NCATS Pharmaceutical Collection, and none showed consistent evidence of a concentration-dependent inhibition of tau inclusions in the T40PL-GFP^{agg} cell assay. **A)** results from the T40PL-GFP^{agg} cell tau inclusion assay screen using HDTA extraction; **B)** results from the T40PL-GFP^{agg} cell tau inclusion assay screen using CTA extraction.

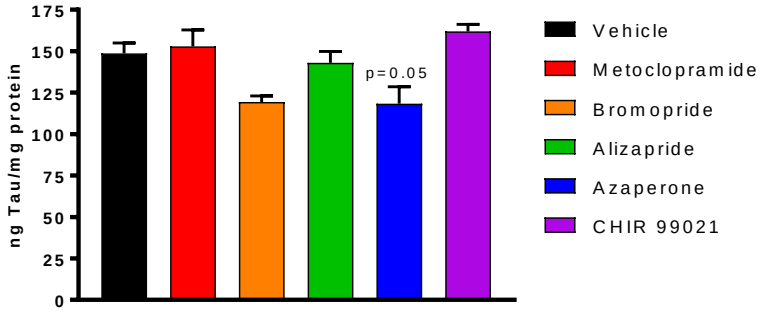


Figure S4. The dopamine D2 receptor antagonists identified in the Prestwick library screen in the rat cortical neuron tau inclusion assay, along with CHIR-99021, were tested for their effects on soluble tau levels in rat cortical neurons. Only azaperone caused a significant decrease of soluble tau as measured by ELISA.

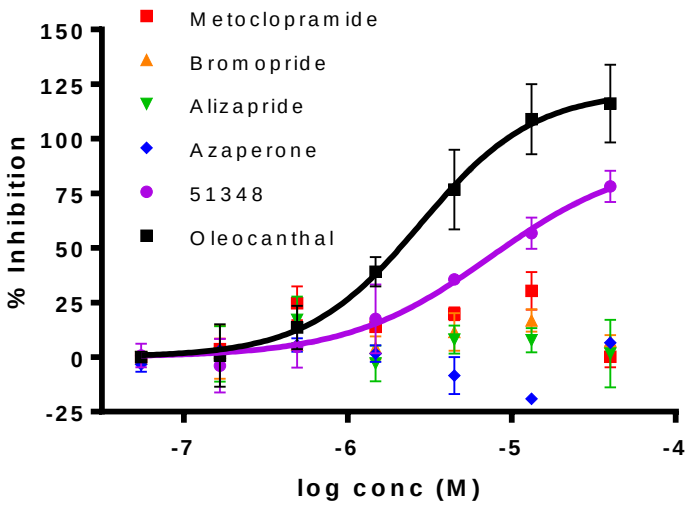
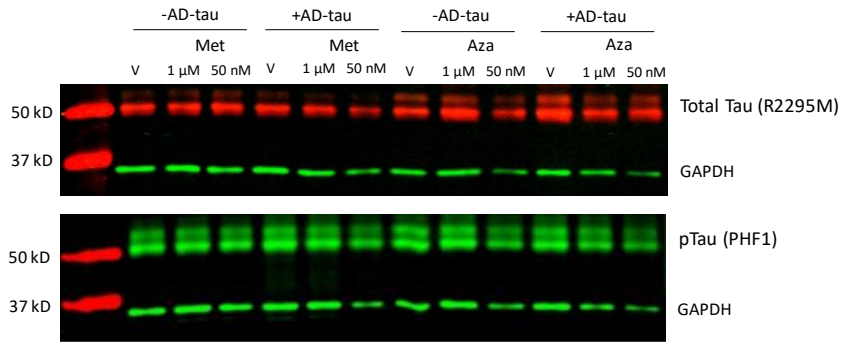


Figure S5. D2 receptor antagonists do not inhibit tau fibrillization. The four D2 receptor antagonists identified in the neuronal tau inclusion assay were tested for their ability to inhibit recombinant tau fibrillization. None of these compounds showed an appreciable inhibition of tau fibril formation, whereas the positive control compounds, CNDR-51348 and oleocanthol, caused a concentration-dependent inhibition of tau fibrillization.

A.



B.

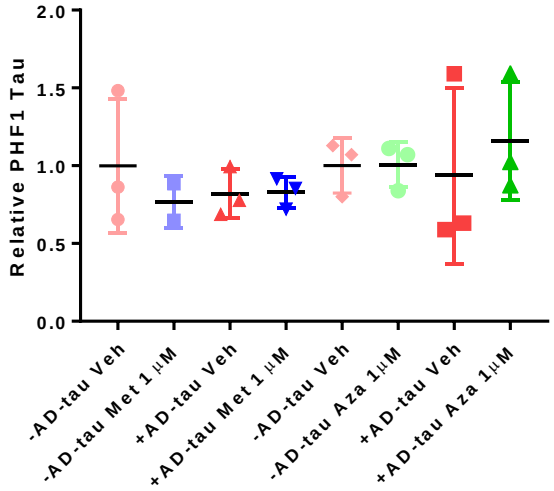


Figure S6. The D2 receptor antagonists metclopromide (Met) and azaperone (Aza) do not reduce rat cortical neuron soluble tau phosphorylation at Ser396/Ser404, as detected by immunoblotting with the PHF1 antibody. Rat cortical neurons were treated with Met or Aza at two concentrations, or vehicle (V) only, followed by addition of AD-tau (+AD-tau) to induce neuronal tau inclusions using the protocol depicted in Fig. 4A. A parallel set of compound- or vehicle-treated neurons did not receive AD-tau (-AD-tau). Cultures were grown for 15 days after compound or vehicle addition, after which neuronal homogenates were prepared in RIPA buffer. **A.** The RIPA-soluble fraction containing soluble tau was examined by immunoblotting, using R2295M antibody to detect total rat tau, PHF1 antibody to visualize tau phosphorylated at Ser396/Ser404, and GAPDH as a loading control. Representative blots are depicted. **B.** The relative PHF1-tau/total-tau ratio is depicted for the neurons treated with D2 receptor antagonists or vehicle in the absence or presence of AD-tau (n=3 except for –AD-tau + 1 µM Met where n=2 due to a loading error). Only the 1 µM concentrations of the compounds are graphed, in which compound activity was confirmed by a reduction of multimeric tau in the neuronal RIPA-insoluble fraction as measured with the mTau8 multimer ELISA.