Supporting Information

In Silico Discovery and Subsequent Characterization of Potent 4R-tauopathy Positron Emission Tomography Radiotracers

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Purity of compounds delivered by Enamine:

ID	Paper ID	Formula	MW	HPLC %Purity
Z3777013540	1	C17H17FN4O	312.341	100
Z5555972024	2	C16H17N5O	295.339	96
Z3790652493	3	C16H17N5O	295.339	100
Z3347399002	4	C14H15N7O	297.315	96
Z4387103711	5	C18H19N3OS	325.427	94
Z2739855509	6	C17H17N3O2S	327.400	100
Z3831636212	7	C15H15N5OS	313.377	90
Z3505917044	8	C16H16N4OS	312.389	98
Z2905421604	9	C16H16N4O2	296.323	95
Z3831636329	10	C16H17N5O2	311.338	100
Z2998207028	11	C18H17FN4O	324.352	97
Z3777013334	12	C17H17N5O	307.349	100

Z3505916830	13	C19H20N4O	320.388	100
Z5555971761	14	C19H20N4O	320.388	91
Z2723085787	15	C15H19N5O	285.344	94
Z2905421695	16	C15H19N5O2	301.343	100
Z2905421723	17	C15H19N5O	285.344	96
Z3347398375	18	C15H18N4OS	302.394	100
Z5555972217	19	C17H17N5O	307.349	100
Z5555971887	20	C17H16FN3O	297.326	95
Z4169252340	21	C16H15FN4O	298.314	98

Table S1: Details on HPLC purities of compounds delivered by Enamine.

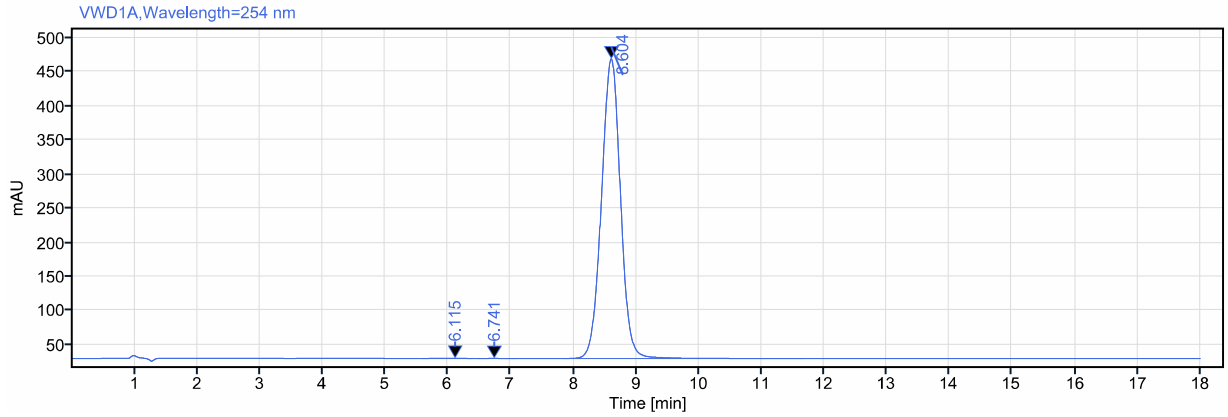

Figure S1. HPLC chromatogram of **1** (Z3777013540). Phenomenex Gemini 5 mm, NX-C18, 110 Å, 100 x 4.6 mm; 35:65 v/v CH_3CN/0.1 M NH_4HCO_2 pH 4.2; 1 mL/min; 254 nm.

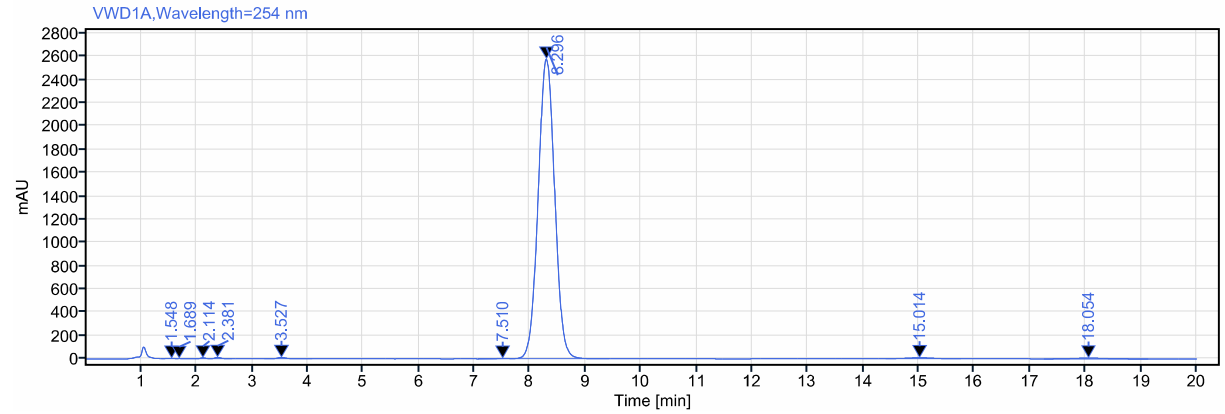

Figure S2. HPLC chromatogram of **21** (Z4169252340). Phenomenex Gemini 5 mm, NX-C18, 110 Å, 100 x 4.6 mm; 45:55 v/v CH_3CN/0.1 M NH_4HCO_2 pH 4.2; 1 mL/min; 254 nm.

HPLC Traces for radiotracer precursors used in this study:

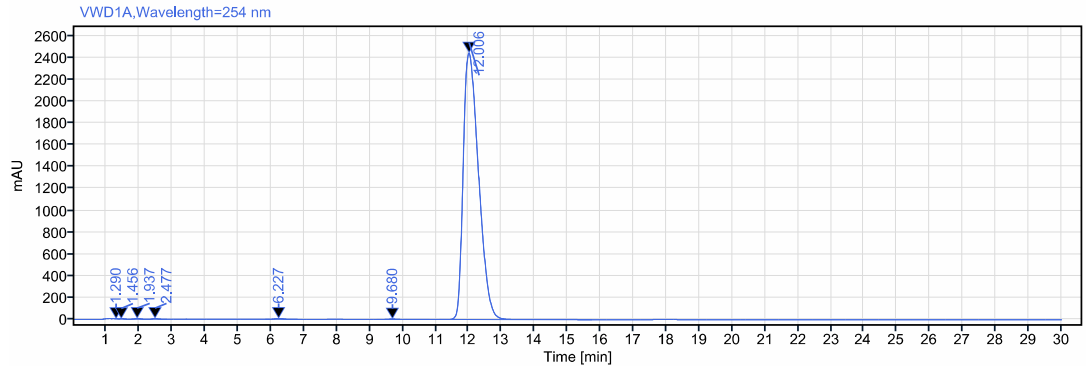

Figure S3. HPLC chromatogram of [^{18}F]**1** radiolabeling precursor, *tert*-butyl 2-(2-(4-((*tert*-butoxycarbonyl)oxy)piperidin-1-yl)pyrimidin-5-yl)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indole-1-carboxylate. Phenomenex Gemini 5 mm, NX-C18, 110 Å, 100 x 4.6 mm; 85:15 v/v CH_3CN/0.1 M NH_4HCO_2 pH 4.2; 1 mL/min; 254 nm.

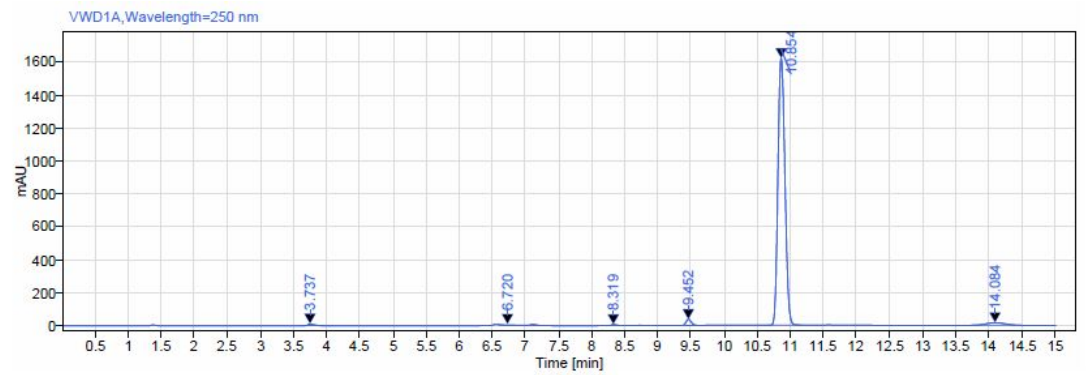

Figure S4. HPLC chromatogram of [^{18}F]**21** precursor, tert-butyl 2-(2-morpholinopyrimidin-5-yl)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indole-1-carboxylate. Chemical purity was confirmed by HPLC. Agilent InfinityLab Poroshell 120 PFP, 4.6 × 150 mm, 2.7 µm) with UV using acetonitrile-NH_4CO_2H (0.05 M) (5-85% gradient over 15 min) at a flow rate of 2 mL/min (4.6 × 150 mm, 3.5 µm); mobile phase A: H2O with NH_4CO_2H (0.05 M); mobile phase B: MeCN; gradient: 5–85% B over 10 min, hold at 85% B for 5 min; flow rate: 2 mL/min; detector wavelength: 254 nmRetention time for tert-butyl 2-(2-morpholinopyrimidin-5-yl)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indole-1-carboxylate (precursor for [^{18}F]**21** radiolabeling) was 10.85 min, and had a purity of 95%.

Radio-HPLC traces of isolated radiotracers co-injected with authentic standards:

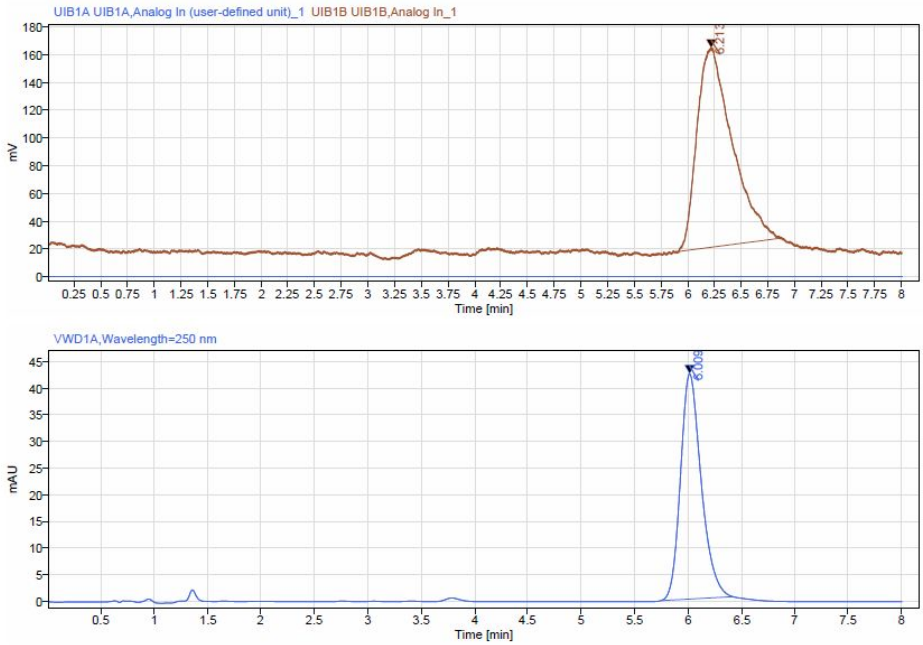

Figure S5. Radio-HPLC chromatogram of [^{18}F]**1** co-injected with reference standard **1**. InfinityLab Poroshell 120 PFP, 4.6 × 150 mm, 2.7 µm, Agilent); acetonitrile-NH_4CO_2H (0.05 M) (50/50, v/v) at a flow rate of 2 mL/min.

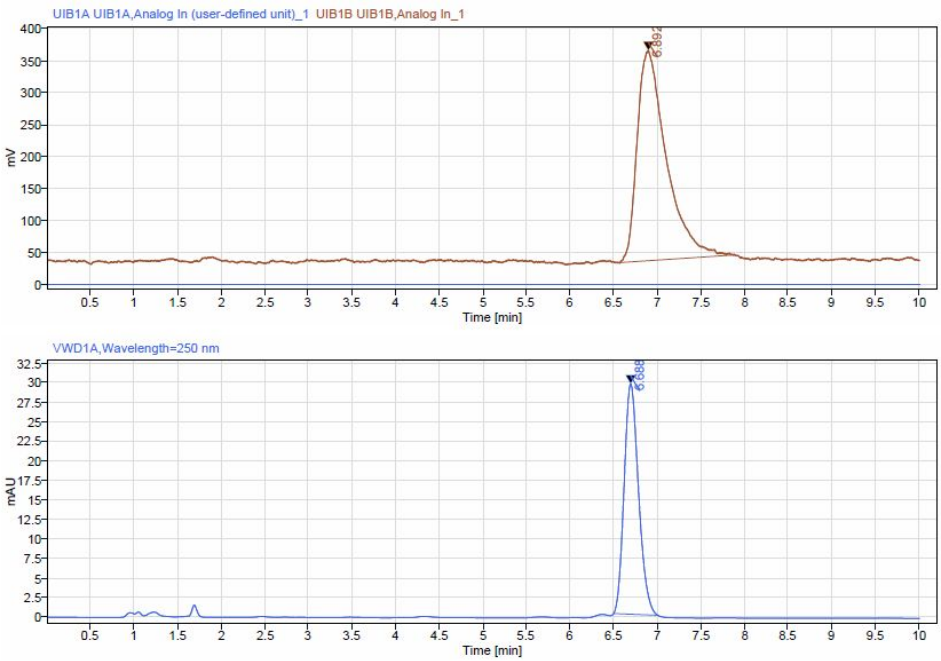

Figure S6. Radio-HPLC chromatogram of [^{18}F]**21** co-injected with reference standard **21**. InfinityLab Poroshell 120 PFP, 4.6 × 150 mm, 2.7 µm, Agilent); acetonitrile-NH_4CO_2H (0.05 M) (50/50, v/v) at a flow rate of 2 mL/min.

Tanimoto similarity scores between purchased compounds and 1 or CBD-2115:

ID	Cpd ID	Tanimoto Similarity Z3540	Tanimoto Similarity CBD-2115
Z4387103711	2	0.424	0.154
Z3347399002	3	0.414	0.173
Z2905421723	4	0.421	0.160
Z2905421695	5	0.400	0.154
Z3505916830	6	0.491	0.189
Z3831636212	7	0.431	0.187
Z2905421604	8	0.424	0.169
Z3347398375	9	0.414	0.189
Z3505917044	10	0.464	0.189
Z2739855509	11	0.448	0.200
Z5555972024	12	0.714	0.286
Z2723085787	13	0.390	0.173
Z2998207028	14	0.588	0.225
Z3790652493	15	0.615	0.250
Z3831636329	16	0.424	0.169

Z3777013334	17	0.439	0.189
Z5555971761	18	0.500	0.192
Z5555971887	19	0.692	0.362
Z5555972217	20	0.458	0.227
Z4169252340	21	0.708	0.294

Table S2: Tanimoto similarity scores between purchased compounds from Enamine and **1** or CBD-2115. The similarity scores were calculated using RDKit Morgan fingerprints (1024 bit, radius=2).