Supporting information

The Optimized TrkB Agonist Ameliorates Alzheimer's Disease Pathologies and Improves Cognitive Functions via Inhibiting Delta-secretase

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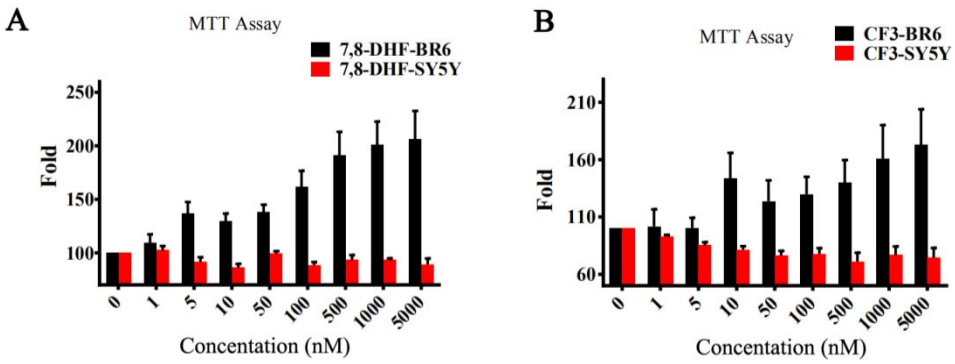
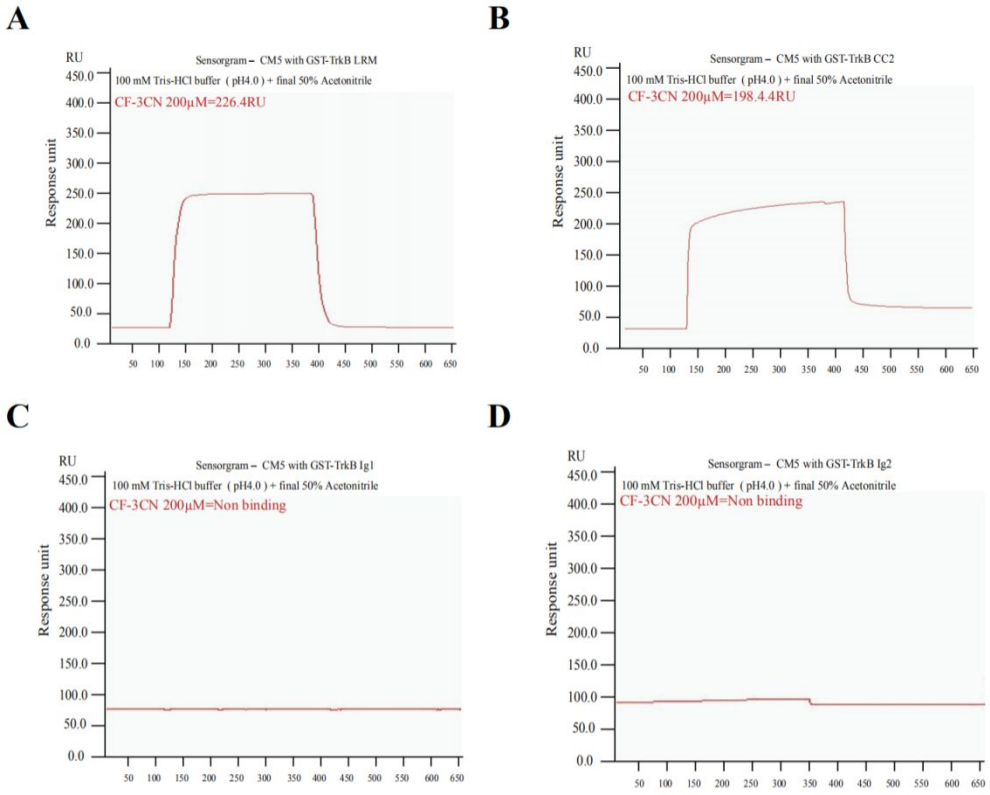


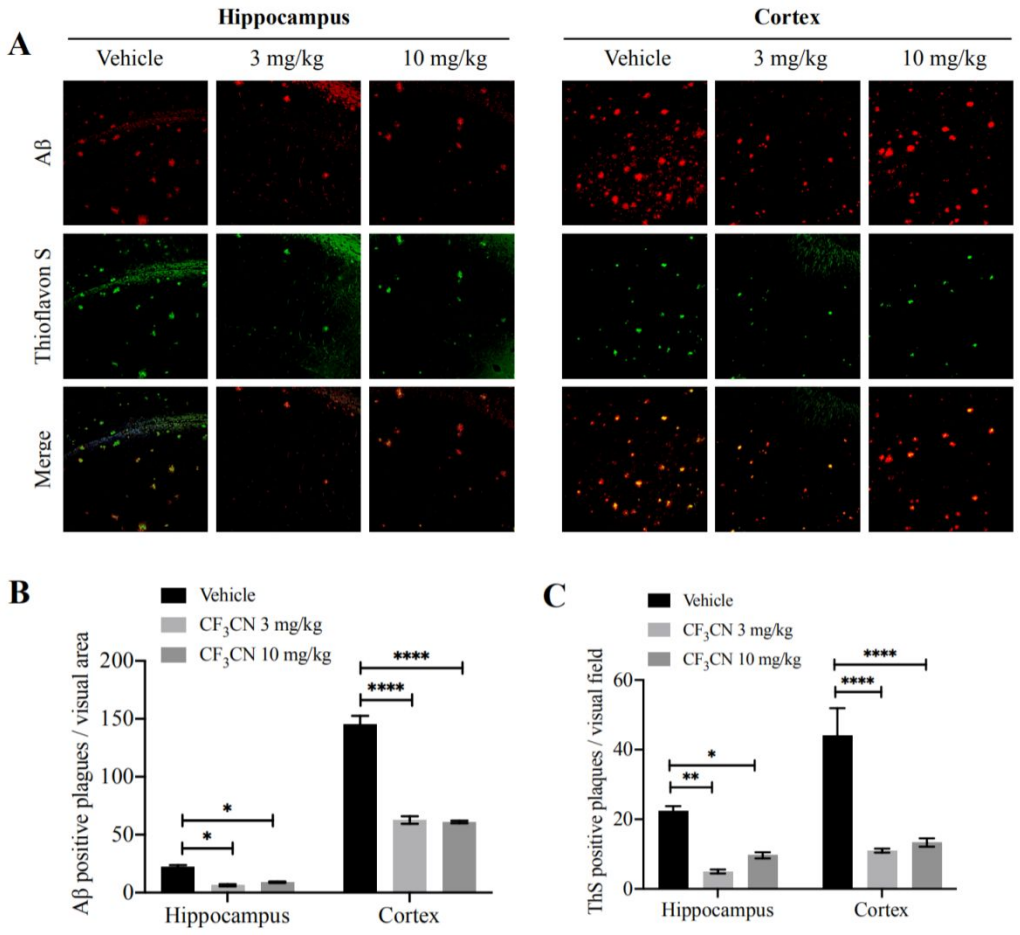
Supplementary Figure 1. CF3CN selectively protects human TrkB stably transfected BR6 cells but not SH-SY5Y cells

A & B. MTT assay. 7,8-DHF and CF3CN selectively protect BR6 but not SH-SY5Y cells in a TrkB-dependent manner.



Supplementary Figure 2. CF3CN selectively binds to TrkB LRM and CC2 domains.

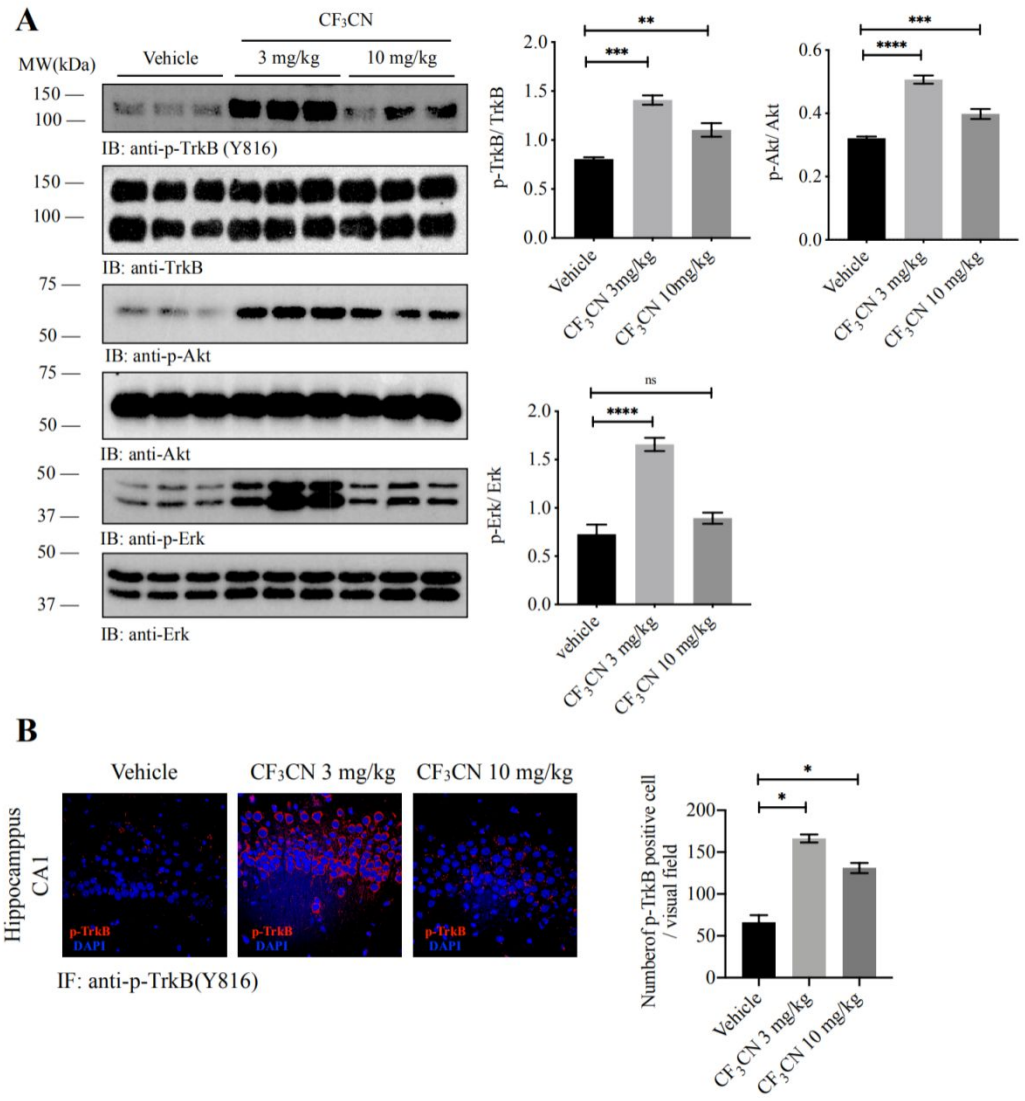
A-D, Biacore analysis of the interaction between CF3CN and various purified TrkB ECD motif recombinant proteins.



Supplementary Figure 3. CF3CN decreases Aβ plaque deposition in 5xFAD mice.

A, Immunofluorescence and Thioflavin-S co-staining of amyloid plaque in the hippocampus and cortex of 5xFAD mice brain sections. Scale bar, 100 μm.

B & C, Quantitative analysis of amyloid plaques. Amyloid deposition in 5xFAD mice was significantly decreased by orally administrated CF3CN. (n=5 per group, Data are shown as mean ± SEM. **p* <0.05, ***p* <0.01, *****p* <0.0001, two-way ANOVA)

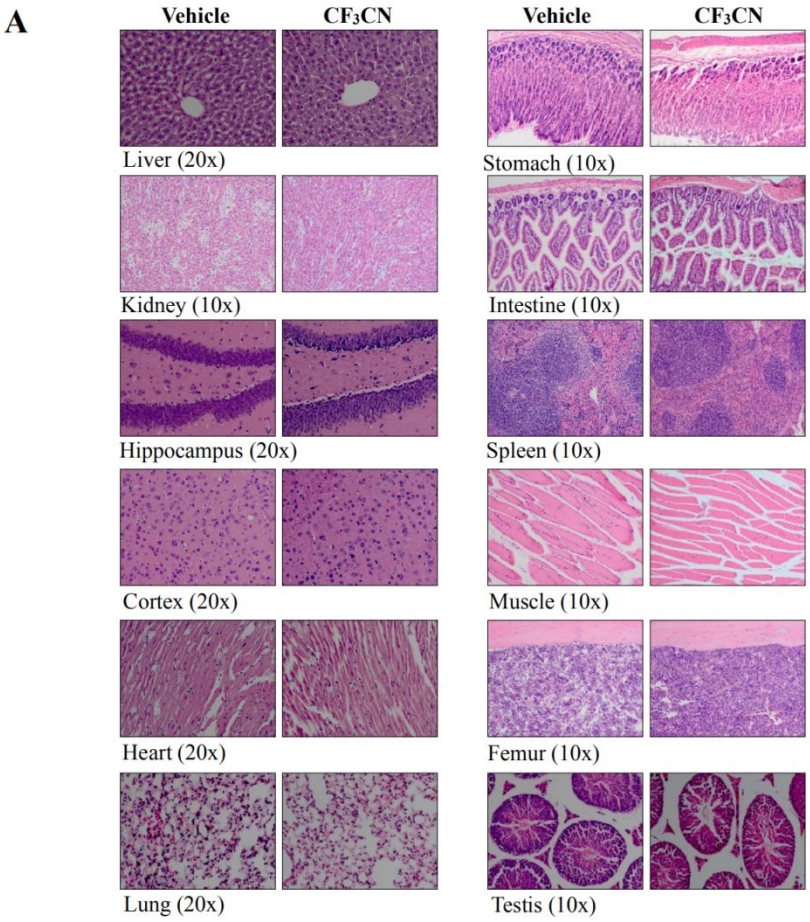


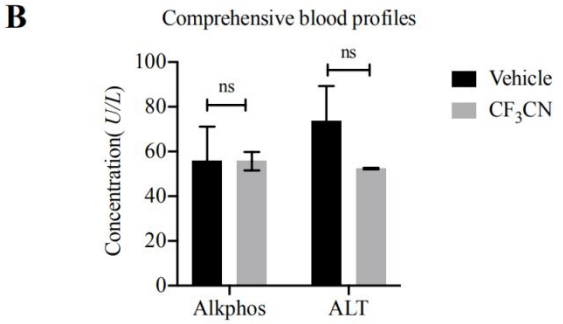
Supplementary Figure 4. CF3CN elicits TrkB and downstream signaling activation in 5xFAD mice.

A, CF3CN activate TrkB signaling cascade in the hippocampus of 5xFAD mice. CF3CN were dissolved in pure DMSO, then suspended in 0.5% methylcellulose at final concentration of 5% DMSO/0.5% methylcellulose. The suspension was orally administrated to 3 months old 5xFAD mice (3mg/kg/d and 10mg/kg/d) consecutively

for 3 months, and the brain lysates were prepared. The p-TrkB and its downstream signals were monitored by immunoblotting, and the ratio of p-TrkB/TrkB, p-Akt/Akt and p-ERK/ERK were quantitatively analyzed. (n=3 per group, Data are shown as mean ± SEM. ** p <0.01, *** p <0.001, **** p <0.0001, one-way ANOVA).

B, Immunofluorescence staining of p-TrkB in 5xFAD brain sections. Three-months old 5xFAD mice were fed with CF3CN or vehicle consecutively for 3 months. The phosphorylation of TrkB in dentate gyrus was detected by immunofluorescence with anti-p-TrkB 816 antibodies. Scale bar, 50 μm. Quantification of p-TrkB positive neurons in the dentate gyrus. Note that CF3CN treatment elicited the phosphorylation of TrkB in 5xFAD mice. (n=5 per group, Data are shown as mean ± SEM. * p <0.05).





Supplementary Figure 5. 12 weeks treatment with CF3CN demonstrates no toxic effect on tissue and blood.

Supplementary Tables

Table S1: Plasma Stability: Half-Life Data Summary

Compound	Species	Half Life (mins)	MRM Transition	Avg % Remaining at Last Point*	Comments
CF3CN	Human	> 480	356.01 > 153.891	94.2	
Propantheline	Human	23.9	369.315 > 182.078	3.15	Control
Warfarin	Human	> 480	309.149 > 163.011	93.6	Control
CF3CN	Mouse	> 480	356.01 > 153.891	107	
Propantheline	Mouse	22.2	369.315 > 182.078	2.56	Control
Warfarin	Mouse	327	309.149 > 163.011	80.8	Control

**Average percent remaining at last time point used to determine slope and half-life*

Table S2: Microsomal Intrinsic Clearance: Data Summary

Test Article	Test Species	NADPH- dependent CL_{int}[a] ((µl/min/mg)	NADPH- dependent T1/2[b] (min)	NADPH-free CL_{int}[a] (µl/min/mg)	NADPH-free T1/2[b] (min)	Comment
CF3CN	Human	< 12.8	> 180	< 12.8	> 180	
Midazolam	Human	511	4.52	< 12.8	> 180	Control
Verapamil	Human	142	16.3	< 12.8	> 180	Control
CF3CN	Mouse	< 12.8	> 180	< 12.8	> 180	
Midazolam	Mouse	1099	2.1	< 12.8	> 180	Control
Verapamil	Mouse	247	9.34	< 12.8	> 180	Control

[a] *Microsomal Intrinsic Clearance*
[b] *Half Life*

Table S3: Hepatocyte Stability: Half-Life Data Summary

Compound	Species	Clearance (µl/min/million cells)	Half Life(min)	MRM Transition	Avg % Remaining at Last Point*	Comments
CF3CN	Human	<2.9	>480	356.01 > 153.891	97.2	
7-OH-Coumarin	Human	88.5	15.7	229.061 > 152.96	7.17	Control
Midazolan	Human	38.8	35.7	326.121> 291.203	9.82	Control
Verapamil	Human	62.3	22.3	455.305 > 150.075	2.40	Control
CF3CN	Mouse	<2.9	>480	356.01 > 153.891	94.8	
7-OH-Coumarin	Mouse	63.2	21.9	229.061 > 152.96	38.8	Control
Midazolan	Mouse	35.1	39.4	326.121> 291.203	34.0	Control
Verapamil	Mouse	31.0	44.8	455.305 > 150.075	41.0	Control

**Average percent remaining at last time point used to determine slope and half-life*

Table S4: Caco-2 Permeability: Data Summary

Test article	Test Article Concentration	Assay Duration	Mean A-B Papp 10^{-6}cm/s	Mean B-A Papp 10^{-6}cm/s	Effux Ratio	Comments
CF3CN	10µM	2 hr	13.0	42.6	3.6	
Ranitidine	10µM	2 hr	0.370	5.00	16.7	Low pemeability Control
Talinolol	10µM	2 hr	0.926	9.31	11.7	P-gp Efflux Control
Warfarin	10µM	2 hr	39.5	27.0	0.7	High pemeability Control

Table S5: BBB-PAMPA Permeability: Data Summary

Test article	Pe	Recovery(%)	Test Conc.
Atenolol	NC	84.6	10µM
Verapamil	2.13	26.3	10µM
CF3CN	3.09	89.9	10µM

Supplementary Tables

Table S6: Turbidimetric Solubility Screen: Data Summary

Test article	Buffer	Solubility Limit(μM) 2 Hour	Test Conc.
Reserpine	PBS	31.3	Low solubility control
Tamoxifen	PBS	15.6	Low solubility control
Verapamil	PBS	>500	High solubility control
CF3CN	PBS	>200	

Table S7: Human plasma protein binding: Data Summary

Test Article	Test Species	Test Conc. (μM)	Mean Plasma Fraction Unbound	Mean Plasma Fraction Bound	Post-Assay Recovery	Comment
CF3CN	Human	5	0.101%	99.9%	94.9%	
Propranolol	Human	5	31.30%	68.7%	109%	Control
Warfarin	Human	5	0.826%	99.2%	91.7%	Control

Table S8: Mouse plasma protein binding: Data Summary

Test Article	Test Species	Test Conc. (μM)	Mean Plasma Fraction Unbound	Mean Plasma Fraction Bound	Post-Assay Recovery	Comment
CF3CN	Mouse	5	3.55%	96.4%	99.8%	
Propranolol	Mouse	5	11.8%	88.2%	91%	Control
Warfarin	Mouse	5	17.6%	82.4%	99.7%	Control

Table S9: % reduction of hERG current by the indicated compound

Compound	IC50 (μM)	0.2μM	1μM	5μM	25μM
CF3CN	>25	21.4	30.1	21.3	47.4
		12.0	35.9	38.3	32.3
		2.4	9.9	16.4	27.1
X±SD		16.3±11.8	26.7±11.5	21.7±11.9	35.6±10.5