Supplementary Information

Materials and Methods

Western blot analysis

The mice brain tissue was lysed in lysis buffer (50 mM Tris, pH 7.4, 40 mM NaCl, 1 mM EDTA, 0.5% Triton X-100, 1.5 mM Na_3VO_4, 50 mM NaF, 10 mM sodium pyrophosphate, 10 mM sodium β-glycerophosphate, supplemented with protease inhibitors cocktail), and centrifuged for 15 min at 16 000 g. The supernatant was boiled in SDS loading buffer. After SDS-PAGE, the samples were transferred to a nitrocellulose membrane. Western blotting analysis was performed with a variety of antibodies.

Immunostaining

Paraffin-embedded mouse brain sections went through dewaxing and rehydration process by incubating the slides in xylene first and then immerse them into decreasing percentage of ethanol. The sections were boiled in 10mM citric acid for 20min followed by room temperature cooling down for antigen retrieval. Then sections were treated with 3% H_2O_2 for 10 min followed by 3 times wash in PBS and 30 min blocking in 1% RIA-BSA,0.3% Triton X-100 as well as the overnight incubation with p-TrkB (Y816, homemade, 1:300) and Aβ antibody (Sigma-Aldrich, 1:500) at 4℃. The signal was developed using Histostain-SP kit (Invitrogen). To detect the localization of AEP, AEP-derived Tau fragment and phosphorylated Tau in mouse brain section, the slides were incubated with AEP antibody（11B7）(1:500, from Dr. Colin Watts, University of Dundee）, Tau N368 (homemade,1:1000), AT-8 (Thermo, MN1020, 1:500) at 4℃. After overnight incubation, the slides were washed three times in PBS and incubated with Texas Red Red-conjugated anti-rabbit IgG or FITC-conjugated anti-mouse IgG for 1h at room

temperature. The slides were washed three times in PBS, then covered with a glass cover using mounting solution and examined under a fluorescence microscope (Olympus).

Golgi staining

Mice brains were fixed in 10% formalin for 24 h, and then immersed in 3% potassium bichromate for 3 days in the dark. The solution was changed each day. Then the brains were transferred into 2% silver nitrate solution and incubate for 24 h in the dark. Vibratome sections were cut at 60 μm, air dried for 10 minutes, dehydrated through 95% and 100% ethanol, cleared in xylene and coverslipped. For measurement of spine density, only spines that emerged perpendicular to the dendritic shaft were counted.

Aβ plaque staining

Amyloid plaques were stained with Thioflavin-S. The deparaffinized and hydrated sections were incubated in 0.25% potassium permanganate solution for 20 min, rinsed in distilled water, and incubated in bleaching solution containing 2% oxalic acid and 1% potassium metabisulfite for 2 min. After rinsed in distilled water, the sections were transferred to blocking solution containing 1% sodium hydroxide and 0.9% hydrogen peroxide for 20 min. The sections were incubated for 5 s in 0.25% acidic acid, then washed in distilled water and stained for 5 min with 0.0125% Thioflavin-S in 50% ethanol. The sections were washed with 50% ethanol and placed in distilled water. Then the sections were covered with glass cover using mounting solution.

Aβ ELISA

The mice brains were homogenized in 8X mass of 5 M guanidine HCl / 50 mM Tris HCl (pH 8.0), and incubated at room temperature for 3 h. Then the samples were diluted with cold reaction buffer (phosphate buffered saline with 5% BSA and 0.03% Tween 20, supplemented with protease inhibitor cocktail), and centrifuged at 16 000 g for 20 min at 4 ° C. The supernatant was analysed by human Aβ40 and Aβ42 ELISA kit according to the manufacturer's instructions (KHB3481 and KHB3441, respectively, Invitrogen). The Aβ concentrations were determined by comparison with the standard curve.

Morris Water maze

Wild-type and 5XFAD mice maintained on oral administration of vehicle or R13 dissolved in 5% DMSO/0.5% methylcellulose at a dose of 7.25, 21.8 or 43.6 mg/kg/day were trained in a round, water-filled tub (52 inches in diameter) in an environment rich with extra maze cues. An invisible escape platform was located in a fixed spatial location 1 cm below the water surface independent of a subject start position on a particular trial. In this manner, subjects needed to utilize extra maze cues to determine the platform's location. At the beginning of each trial, the mouse was placed in the water maze with their paws touching the wall from 1 of 4 different starting positions (N, S, E, W). Each subject was given 4 trials/day for 5 consecutive days with a 15-min inter-trial interval. The maximum trial length was 60 s and if subjects did not reach the platform in the allotted time, they were manually guided to it. Upon reaching the invisible escape platform, subjects were left on it for an additional 5 s to allow for survey of the spatial cues in the environment to guide future navigation to the platform. After each trial, subjects were dried and kept in a dry plastic holding cage filled with paper towels to allow the subjects to dry off. The temperature of the water was monitored every hour so that mice were tested in water that was

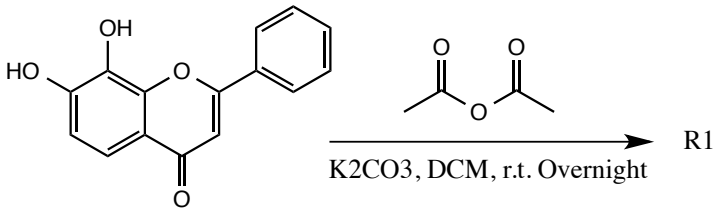
between 22 and 25° C. Following the 5 days of task acquisition, a probe trial was presented during which time the platform was removed and the percentage of time spent in the quadrant which previously contained the escape platform during task acquisition was measured over 60 s. All trials were analysed for latency, swim path length, and swim speed by means of MazeScan (Clever Sys, Inc.).

Organic synthesis of prodrugs

General. All reagents were commercial and were used without further purification. Silica gel TLC plates (Qing Dao Marine Chemical Factory, Qingdao, China) were used to monitor the progression of the reactions. Flash column chromatography was performed using silica gel (300−400 mesh size, Qing Dao Marine Chemical Factory, Qingdao, China). All reactions were monitored by thin-layer chromatography (TLC) and LCMS. ^{1}H NMR spectra were recorded on Bruker Avance III 400 MHz and Varian Mercury Plus 300 MHz and TMS was used as an internal standard. LCMS was taken on a quadrupole Mass Spectrometer on Agilent LC/MSD 1200 Series (Column: BP-C18 (50 × 4.6 mm, 5 μm) operating in ES (+) or (-) ionization mode; T = 30 ^{o}C; flow rate = 1.5 mL/min; detected wavelength: 214 nm or 254 nm.

1. Preparation of compound R1

7,8-Dihydroxyflavone (100 mg, 0.4 mmol) was added to a suspension of K2CO3 (342 mg, 2.5 mmol) and acetic anhydride (0.1 mL, 0.8 mmol) in DCM. After stiring at r.t. overnight, the mixture was filtered and evaporated under reduced pressure. The residue was washed by ethyl ether to afford the product as a white solid (71 mg, yield: 53.3%, Lot#: MC0777-26-1). 1H NMR (400 MHz, CD3OD): δppm 8.06 (d, J = 8.8 Hz,1H), 7.93-7.95 (m, 2H), 7.58-7.61(m, 3H), 7.38 (d, J = 8.4 Hz, 1H), 6.93 (s, 1H), 2.48(s, 3H), 2.37(s, 3H). Purity: 99.8% (254 nm); MS: 339.0 [M+1]+



2. Preparation of compound R3

7,8-Dihydroxyflavone (200 mg, 0.8 mmol) was added to a suspension of K2CO3 (458 mg, 3.3 mmol) and ethyl chloroformate (0.3 mL, 1.7 mmol) in DCM. After stirring at r.t. overnight, the mixture was filtered and evaporated under reduced pressure. The residue was washed by ethyl ether to afford the product as a white solid (50 mg, yield: 15.9%,Lot#: MC0777-59-1). 1H NMR (400 MHz, CDCl3):δppm 8.14 (d, J = 8.8 Hz,1H), 7.85-7.87 (m, 2H), 7.50-7.56(m, 3H), 7.35-7.38 (m, 1H), 6.83 (s, 1H), 4.35-4.43 (m, 4H), 1.41-1.44(m, 6H). Purity: 96.0%(254 nm); MS: 399.0 [M+1]+

3. Preparation of compound R4

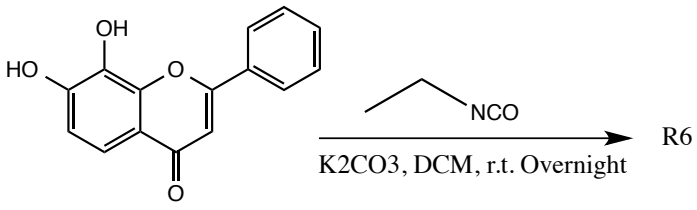
7,8-Dihydroxyflavone (200 mg, 0.8 mmol) was added to a suspension of K2CO3 (458 mg, 3.3 mmol) and Propionyl chloride (0.3 mL, 1.7 mmol) in DCM. After stirring at r.t. overnight, the mixture was filtered and evaporated under reduced pressure. The residue was washed by ethyl ether to afford the product as a yellow solid (51 mg, yield: 17.4%, Lot#: MC0777-37-1). 1H NMR (400 MHz, CDCl3): δppm 8.13 (d, J =8.8 Hz, 1H), 7.79-7.81 (m, 2H), 7.51-7.55(m, 3H), 7.25-7.27 (m, 1H), 6.79 (s, 1H), 2.75 (q, J = 7.6 Hz, 2H), 2.65(q, J = 7.6 Hz, 2H), 1.38(t, J= 7.6 Hz, 3H), 1.31(t, J =7.6 Hz, 3H). Purity: 95.5% (254 nm); MS: 367.0 [M+1]+

4. Preparation of compound R5

7,8-Dihydroxyflavone (150 mg, 0.6 mmol) was added to a suspension of K2CO3 (341 mg, 2.48 mmol) and pivaloyl chloride (0.2 mL, 1.2 mmol) in DCM. After stiring at r.t. overnight, the mixture was filtered and evaporated under reduced pressure. The residue was washed by ethyl ether to afford the product as a red solid (52 mg, yield: 20.9%, Lot#: MC0777-30-1). 1H NMR (400 MHz, CDCl3):δppm 8.12 (d, J = 8.8 Hz, 1H), 7.80-7.82 (m, 2H), 7.50-7.55(m, 3H), 7.20 (d, J = 8.4 Hz, 1H), 6.76 (s, 1H), 1.45(s, 9H), 1.35(s, 9H). Purity: 99.6% (254 nm); MS: 445.1 [M+1]+

5. Preparation of compound R6

7,8-Dihydroxyflavone (200 mg, 0.8 mmol) was added to a suspension of K2CO3 (458 mg, 3.3 mmol) and ethyl isocyanate (0.3 mL, 1.7 mmol) in DCM. After stirring at r.t. overnight, the mixture was filtered and evaporated under reduced pressure. The residue was washed by ethyl ether to afford the product as a white solid (70 mg, yield: 22.5%, Lot#: MC0777-58-1). 1H NMR (400 MHz, CDCl3): δppm 8.05 (d, J = 8.8 Hz, 1H), 7.84-7.86 (m, 2H), 7.47-7.53(m, 3H), 7.28-7.30 (m, 1H), 6.79 (s, 1H), 5.45-5.46 (m, 1H), 5.22-5.23 (m, 1H), 3.32-3.54 (m, 4H), 1.23-1.30(m, 6H). Purity: 99.8% (254 nm); MS: 397.1 [M+1]+.



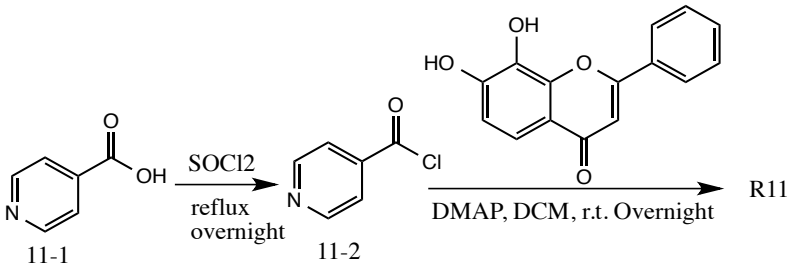
6. Preparation of compound R7

7,8-Dihydroxyflavone (200 mg, 0.8 mmol) was added to a suspension of K2CO3 (458 mg, 3.3 mmol) and dimethylcarbamoyl chloride (0.3 mL, 1.7 mmol) in DCM. After stirring at r.t. overnight, the mixture was filtered and evaporated under reduced pressure. The residue was washed by ethyl ether to

afford the product as a white solid (53 mg, yield: 15.9%, Lot#: MC0777-38-1). 1H NMR (400 MHz, CDCl3): δppm 8.07 (d, J = 8.8 Hz, 1H), 7.82-7.84 (m, 2H), 7.49-7.54(m, 3H), 7.32-7.34 (m, 1H), 6.79 (s, 1H), 3.24 (s, 3H), 3.15(s, 3H), 3.11(s, 3H), 3.05(s, 3H). Purity: 98.2% (254 nm); MS: 397.0[M+1]+

7. Preparation of compound R11

A solution of compound 11-1 (600 mg, 4.9 mmol) in SOCl2 (20 mL) was refluxed overnight under Ar atmosphere. Then the resulting mixture was evaporated under reduced pressure to remove the SOCl2, the residue was used for the next step directly without further purification. Compound 1 (200 mg, 0.8 mmol) was added to a solution of DMAP (1.2 g, 4.8 mmol) and Compound 11-2 (690 mg, 4.9 mmol) in DCM (5 mL), and the mixture was stirred at r.t. overnight. The mixture was filtered and evaporated under reduced pressure. The residue was washed by ethyl ether to afford the product as a white solid (80 mg, yield: 21.9%, Lot#: MC0777-94-1). The product was confirmed by HPLC, LCMS and 1H NMR. 1H NMR (400 MHz, CDCl3): δppm 8.89 (d, J = 6.0 Hz, 2H), 8.83 (d, J = 6.0 Hz, 2H), 8.28 (d, J = 8.8 Hz, 1H), 7.99-8.00 (m, 2H), 7.91-7.92 (m, 2H), 7.67-7.69 (m, 2H), 7.46-7.48 (m, 2H), 7.36-7.40 (m, 2H), 6.86(s, 1H). Purity: 93.7% (254 nm); MS: 465.0 [M+1]+



8. Preparation of compound R12

7,8-Dihydroxyflavone (150 mg, 0.6 mmol) was added to a suspension of K2CO3 (341 mg, 2.48 mmol) and isovaleryl chloride (0.2 mL, 1.2 mmol) in DCM. After stiring at r.t. overnight, the mixture was

filtered and evaporated under reduced pressure. The residue was washed by ethyl ether to afford the product as a white solid (57 mg, yield: 23.1%, Lot#: MC0777-34-1). 1H NMR (400 MHz, CDCl3): δppm 8.12 (d, J = 8.8 Hz, 1H), 7.80-7.82 (m, 2H), 7.48-7.55(m, 3H), 7.23-7.26 (m, 1H), 6.78 (s, 1H), 2.59 (d, J = 6.8 Hz, 2H), 2.49 (d, J = 6.8 Hz, 2H), 2.24-2.32(m, 2H), 1.09-1.11(m, 12H). Purity: 99.6% (254 nm); MS: 445.0 [M+1]+

9. Preparation of compound R13

(4-Oxo2-phenyl-4H-chromene-7,8-diyl bis(methylcarbamate))

1H NMR (300 MHz, d-DMSO): δ ppm 8.21 (q, J = 4.50 Hz, 1H), 7.95-8.02 (m, 3H), 7.90 (d, J = 9.0 Hz, 1H), 7.58-7.64 (m, 3H), 7.38 (d, J = 8.70 Hz, 1H), 7.10 (s, 1H), 2.77 (d, J = 4.80 Hz, 3H), 2.69 (d, J = 4.80 Hz, 3H). Purity: 99.9% (254 nm); MS: 369.1 $[M+1]^+$.

10. Preparation of compound R14

(4-Oxo2-phenyl-4H-chromene-7,8-diyl bis(iso-propylcarbamate))

1H NMR (300 MHz, d-DMSO): δ ppm 8.26 (d, J = 7.80 Hz, 1H), 8.04 (d, J = 8.40 Hz, 3H), 7.90 (d, J = 9.0 Hz, 1H), 7.60 (m, 3H), 7.36 (d, J = 8.70 Hz, 1H), 7.12 (s, 1H), 3.68 (m, 2H), 1.20 (m, 12 H). Purity: 99.4% (254 nm); MS: 425.1 $[M+1]^+$.

11. Preparation of compound R15

(4-Oxo2-phenyl-4H-chromene-7,8-diyl bis(t-butylcarbamate))

1H NMR (300 MHz, d-DMSO): δ ppm 8.09 (d, J = 8.40 Hz, 3H), 7.89 (d, J = 8.70 Hz, 2 H), 7.55-7.64 (m, 3H), 7.31 (d, J = 8.70 Hz, 1H), 7.11 (s, 1H), 1.32 (s, 18 H). Purity: 97.5% (254 nm); MS: 453.5

$[M+1]^+$.

12. Preparation of compound R16

(4-oxo-2-phenyl-4H-chromene-7,8-diyl bis(4-methylpiperazine-1-carboxylate))

^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 8.08 (d, *J* = 9.0 Hz, 1H), 7.84 (d, *J* = 6.3 Hz, 2H), 7.53-7.49 (m, 3H), 7.30 (d, *J* = 9.0 Hz, 1H), 6.79 (s, 1H), 3.85-3.58 (m, 8H), 2.53-2.45 (m, 8H), 2.39 (s, 3H), 2.36 (s, 3H); purity >98% at 214 nm, MS (ESI) m/z = 507.2 $[M+H]^+$.

13. Preparation of compound R17

(4-oxo-2-phenyl-4H-chromene-7,8-diyl bis(piperazine-1-carboxylate) dihydrochloride)

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 9.78-9.45 (br, 4H), 7.99-7.95 (m, 3H), 7.67-7.63 (m, 3H), 7.51 (d, *J* = 8.7 Hz, 1H), 7.11 (s, 1H), 4.05-3.70 (m, 8H), 3.27-3.14 (m, *8*H); purity >98% at 214 nm, MS (ESI) m/z = 479.1 $[M+H]^+$.

14. Preparation of compound R18

(4-oxo-2-phenyl-4H-chromene-7,8-diyl bis(4-((dimethylamino)methyl)benzoate)

R18 was obtained as a pale yellow solid with purity 97.8%. ^{1}H NMR (600MHz，DMSO-d6): d 8.10 (m, 3H), 8.01 (d, 2H), 7.84 (d, 2H), 7.71 (d, 1H), 7.52-7.41 (m, 7H), 7.17 (s, 1H), 3.50 (d, 4H), 2.17 (d, 12H) and LC-MS [MW=576.6].

15. Preparation of compound O-8806

(4-Oxo-2-phenyl-4H-chromene-7,8-diyl-bis(2-aminoacetate) Dihydrochloride).

^{1}H NMR (300MHz, D2O) δ7.95 (d, 1H), 7.74 (d, 2H), 7.74 (m, 5H), 6.81 (s, 1H), 4.79 (s, 15H), 4.45 (d, 2H), 4.34 (d, 2H), 3.70 (s, 1H). HPLC 90%; MS: calculated: 368.34; found: 396.1 (M + 1). Elemental Analysis: calculated C: 49.69, found C: 49.62; MS: calculated H: 4.39, found H: 4.45, LC: calculated N 6.10, found: 6.10, calculated Cl: 15.44, found Cl: 15.36.

16. Preparation of compound O-8807

((2S, 2'S)-4-Oxo-2-phenyl-4H-chromene-7,8-diyl-bis(2-aminopropanoate))

^{1}H NMR (300MHz, D2O) δ7.94 (d, 1H), 7.71 (d, 2H), 7.46 (m, 4H), 6.78(s, 1H), 4.77 (m, 2H), 1.83 (d, 3H), 1.76 (d, 3H), 1.45 (m, 1H). HPLC: 0.13 alanine. MS: calculated: 396.36, found: 397.1 (M+1). Elemental Analysis: calculated C: 52.73, found C: 52.83; MS: calculated H: 4.85, found H: 4.79, LC: calculated N 5.86, found: 5.82, calculated Cl: 14.82, found Cl: 14.90.

17. Preparation of compound O-8806

(4-Oxo-2-phenyl-4H-chromene-7,8-diyl-bis(3-aminopropanoate) Dihydrochloride)

^{1}H NMR (300MHz, D2O) δ7.73 (d, 1H), 7.71 (m, 2H), 7.50(m, 3H), 7.40 (d,1H), 6.81 (s, 1H), 4.83 (m, H), 3.39 (m, 4H), 3.374 (d, 2H), 3.37 (m, 2H). LC: 100%. MS: calculated: 396.36, found: 397.1 (M+1). Elemental Analysis: calculated C: 52.73, found C: 52.55; MS: calculated H: 4.85, found H: 4.80, LC: calculated N 5.86, found: 5.92, calculated Cl: 14.82, found Cl: 14.99.

18. Preparation of compound O-8809

((2S, 2'S)-4-Oxo-2-phenyl-4H-chromene-7,8-diyl-bis(2-amino-3-methylbutanoate) Dihydrochloride)

^{1}H NMR (300MHz, D2O) δ7.88 (d, 1H), 7.58 (m, 3H), 7.41 (m, 3H), 6.68 (s, 1H), 4.77 (s, H), 4.49 (d,

1H), 4.44 (d, 1H), 2.65 (m, 2H), 1.19 (m, 9H), 1.06 (m, 4H). HPLC: 0.15 eq. valine. MS: calculated: 452.5, found: 453.2 (M+1). Elemental Analysis: calculated C: 55.07, found C: 54.87; MS: calculated H: 5.95, found H: 45.82, LC: calculated N 5.14, found: 5.22, calculated Cl: 13.00, found Cl: 13.08.

19. Preparation of compound O-8810

(4-Oxo-2-phenyl-4H-chromene-7,8-diyl-bis(3-methylaminopropanoate) Dihydrochloride).

^{1}H NMR (300MHz, D2O) δ7.94 (d, 1H), 7.70 (d, 2H), 7.47(m, 3H), 7.39 (d, 1H), 6.80 (s, 1H), 4.83 (m, H), 3.43 (m, 4H), 3.28 (d, 2H), 3.21 (m, 2H). 2.77(s, 3H), 2.72 (s, 3H). HPLC: 100%. MS: calculated: 424.45, found: 425.2 (Parent+1). Elemental Analysis: calculated C: 52.85, found C: 53.09; MS: calculated H: 5.44, found H: 5.18, LC: calculated N 5.36, found: 5.30, calculated Cl: 14.92, found Cl: 14.96.

20. Preparation of compound O-8811

(4-Oxo-2-phenyl-4H-chromene-7,8-diyl-bis(2-(methylamino)acetate) Dihydrochloride)

^{1}H NMR (300MHz, D2O) δ7.94 (d, 1H), 7.75 (d, 2H), 7.48(m, 5H), 6.80 (s, 1H), 4.80 (m, H), 4.55 (s, 2H), 4.53 (d, 2H), 2.93 (d, 7H). HPLC: 90%. MS: calculated: 396.36, found: fragmented. NMR: Elemental Analysis: calculated C: 53.74, found C: 53.58; calculated H: 4.72, found H: 4.69, LC: calculated N 5.97, found: 5.90, calculated Cl: 15.11, found Cl: 15.02.

Supplementary Figures

Supplementary Figure 1. Organic Synthesis of 7,8-DHF prodrug via ester or carbamate groups.

Chemical structure of side groups modified on the catechol group in the parent compound 7,8-

DHF.

Supplementary Figure 2. R13 alleviates Aβ deposition, improving the spatial learning and memory of 5XFAD mice.

A, Thioflavin-S staining of amyloid plaques in 5XFAD mice brain sections. Scale bar, 100 μm.

B & C, Quantitative analysis of amyloid plaques and plaque fraction. The density of plaques and plaque fraction in 5XFAD mouse brain were decreased by R13 at both doses of 7.25, 21.8 and 43.6 mg/kg (* P <0.01).

5XFAD mice (n = 8-10/group) orally administrated with control vehicle or R13 (7.25, 21.8 and 43.6 mg/kg/d, respectively) dissolved in 5% DMSO/95% Methylcellulose (0.5%, w/v) were trained in the water maze over five days. Shown are mean ± SEM Swim Path Distance (D), the area under curve of latency (AUC latency) (E), Swim speed (F), *p< 0.05 compared to vehicle-treated 5XFAD mice.

Supplementary Figure 3. R13 inhibits δ-secretase activation and reduces AEP-derived APP fragments and Tau fragments

A, Immunofluoresence staining of hippocampus from 5XFAD mice brain with δ-secretase derived APP fragment antibody (red), MAP2 antibody (green) and DAPI (blue).

B, Immunofluoresence staining of hippocampus from 5XFAD mice brain with δ-secretase derived Tau fragment antibody (red), AT8 antibody (green) and DAPI (blue).

Supplementary Figure 4. 12 weeks treatment with R13 demonstrates no toxic effect on body weight and tissues.

Supplementary Table 1. Intestinal Microsomal Stability Screen

Compound	Species	Mean Remaining Prodrug with NADPH (%)	Mean Remaining Prodrug with NADPH-free (%)	Mean Appearing of 7,8-DHF with NADPH (%)	Mean Appearing of 7,8-DHF with NADPH-free (%)
Verapamil	Human	96.4	100.0		
	Mouse	66.5	97.3		
Warfarin	Human	100.0	103.0		
	Mouse	96.3	107.0		
R1	Human	0.2	0.1	58.2	65.5
	Mouse	0.0	0.0	62.8	61.4
R3	Human	0.1	0.1	31.5	32.5
	Mouse	0.1	0.0	42.8	40.3
R4	Human	0.0	0.1	38.4	37.3
	Mouse	0.1	0.0	39.1	37.9
R5	Human	11.2	17.1	9.6	10.1
	Mouse	17.6	23.0	11.9	12.6
R6	Human	0.0	0.0	5.0	7.0
	Mouse	0.0	0.0	6.1	8.7
R7	Human	102.0	96.5	0.2	0.1
	Mouse	72.5	89.4	0.3	0.2
R11	Human	0.0	0.0	59.2	54.8
	Mouse	0.0	0.0	60.9	58.9
R12	Human	0.2	0.3	32.9	29.3
	Mouse	0.4	0.4	37.1	36.9
R13	Human	0.0	0.0	55.4	58.1
	Mouse	0.0	0.1	58.9	55.4
R14	Human	0.0	0.0	99.5	96.7
	Mouse	0.0	0.0	94.9	93.8
R15	Human	0.0	0.0	55.4	56.4
	Mouse	0.0	0.0	63.3	58.9
R16	Human	53.0	100	No 7,8-DHF	
	Mouse	53.6	100	No 7,8-DHF	
R17	Human	100	100	No 7,8-DHF	
	Mouse	100	99.5	No 7,8-DHF	
R18	Human	N/A	N/A	No 7,8-DHF	
	Mouse	N/A	N/A	No 7,8-DHF	
7,8-DHF	Human	98.9	83.5		
	Mouse	89.2	84.8		

Supplementary Table 2. Plasma Stability Screen

Compound	Species	Mean Remaining Prodrug (%)	Mean Appearing of Parent 7,8-DHF (%)
Propantheline	human	61.7	NA
	Mouse	76.0	NA
Warfarin	human	84.0	NA
	Mouse	124.0	NA
R1	human	0.0	73.3
	Mouse	0.0	46.3
R3	human	0.0	65.6
	Mouse	0.0	67.1
R4	human	0.0	53.3
	Mouse	0.0	62.7
R5	human	20.6	36.7
	Mouse	0.1	61.4
R6	human	0.0	62.5
	Mouse	0.0	79.5
R7	human	97.4	0.2
	Mouse	9.6	0.4
R11	human	0.0	88.0
	Mouse	0.0	101.0
R12	human	2.6	142.0
	Mouse	0.0	162.0
R13	human	0.0	54.0
	Mouse	0.0	63.6
R14	human	0.0	66.4
	Mouse	0.0	82.6
R15	human	0.0	71.2
	Mouse	0.0	79.8
R16	human	96.3	No 7,8-DHF
	Mouse	0.1	No 7,8-DHF
R17	human	97.2	No 7,8-DHF
	Mouse	0.02	No 7,8-DHF
R18	human	0.0	1.3
	Mouse	15.1	20.8
7,8-DHF	human	80.4	NA
	Mouse	104.0	NA
8806	human	<LLOQ	98.1
	Mouse	<LLOQ	104.0
8807	human	<LLOQ	104.0
	Mouse	<LLOQ	111.0
8808	human	<LLOQ	105.0
	Mouse	<LLOQ	107.0
8809	human	<LLOQ	105.0
	Mouse	<LLOQ	123.0
8810	human	<LLOQ	103.0
	Mouse	<LLOQ	117.0
8811	human	<LLOQ	99.1
	Mouse	<LLOQ	76.7

Supplementary Table 3. Liver Microsomal Stability Screen

Compound	Species	Mean Remaining Prodrug with NADPH (%)	Mean Remaining Prodrug with NADPH-free (%)	Mean Appearing of 7,8-DHF with NADPH (%)	Mean Appearing of 7,8-DHF with NADPH-free (%)
Verapamil	Human	17.2	91.3		
	Mouse	7.4	98.8		
Warfarin	Human	92.2	100.0		
	Mouse	95.0	92.5		
R1	Human	0.0	0.0	67.8	80.9
	Mouse	0.0	0.0	77.3	95.4
R3	Human	0.1	0.4	46.1	57.6
	Mouse	0.3	0.3	55.6	70.0
R4	Human	0.0	0.0	43.1	55.8
	Mouse	0.0	0.0	51.5	67.3
R5	Human	4.1	5.4	25.9	32.7
	Mouse	6.6	7.3	29.0	32.2
R6	Human	0.0	0.0	86.4	10.9
	Mouse	0.0	0.0	110.0	102.0
R7	Human	42.7	78.2	0.6	0.3
	Mouse	2.4	91.2	9.3	0.5
R11	Human	0.0	0.0	60.2	66.0
	Mouse	0.0	0.0	68.7	79.3
R12	Human	0.3	1.2	45.6	50.0
	Mouse	0.7	0.9	49.6	54.0
R13	Human	0.1	0.0	53.1	56.7
	Mouse	0.0	0.1	49.9	57.8
R14	Human	0.0	0.0	86.5	85.9
	Mouse	0.0	0.0	68.3	87.4
R15	Human	0.0	0.0	51.9	61.0
	Mouse	0.0	0.0	15.5	29.4
R16	Human	8.5	100	No 7,8-DHF	
	Mouse	12.3	100	No 7,8-DHF	
R17	Human	75.6	100	No 7,8-DHF	
	Mouse	60.9	84.7	No 7,8-DHF	
R18	Human	2.6	2.6	3.8	5.1
	Mouse	0.7	0.8	0.7	1.0
7,8-DHF	Human	82.2	107.0		
	Mouse	62.1	121.0		
8806	Human	<LLOQ	<LLOQ	82.5	132.0
	Mouse	<LLOQ	<LLOQ	89.5	120.0
8807	Human	<LLOQ	<LLOQ	7.2	18.9
	Mouse	<LLOQ	<LLOQ	33.3	19.0
8808	Human	<LLOQ	<LLOQ	80.5	122.0
	Mouse	<LLOQ	<LLOQ	86.0	115.0
8809	Human	<LLOQ	<LLOQ	14.6	39.0
	Mouse	<LLOQ	<LLOQ	22.5	31.3
8810	Human	<LLOQ	<LLOQ	71.5	121.0
	Mouse	<LLOQ	<LLOQ	86.6	104.0
8811	Human	<LLOQ	<LLOQ	67.9	110.0
	Mouse	<LLOQ	<LLOQ	73.1	113.0

Supplementary Table 4. Caco-2 Permeability

Compound	A ->B P_{app} (10^{-6} cm·S^{-1})	B ->A P_{app} (10^{-6} cm·S^{-1})	R_E
Ranitidine	0.9	2.3	2.7
Warfarin	24.2	10.9	0.4
Talinolol	0.4	10.8	28.8
R1	< LLOQ	< LLOQ	< LLOQ
R1 (7,8-DHF)	12.7	6	0.5
R5	< LLOQ	< LLOQ	< LLOQ
R7	6.5	5.4	0.8
R11	< LLOQ	< LLOQ	< LLOQ
R11 (7,8-DHF)	10.6	2.4	0.2
R12	< LLOQ	< LLOQ	< LLOQ
R13	< LLOQ	< LLOQ	< LLOQ
R13 (7,8-DHF)	10.8	18.1	1.7
R16	3.0	28.9	9.6
R17	0.89	11.5	13.0
R18	0.98	60.40	61.60
8806	< LLOQ	< LLOQ	< LLOQ
8806 (7,8-DHF)	7.9	6.2	0.8
8808	< LLOQ	< LLOQ	< LLOQ
8808 (7,8-DHF)	8	6.9	0.9
8810	< LLOQ	< LLOQ	< LLOQ
8810 (7,8-DHF)	11.9	8.6	0.7
8811	< LLOQ	< LLOQ	< LLOQ
8811 (7,8-DHF)	12.1	8.5	0.7

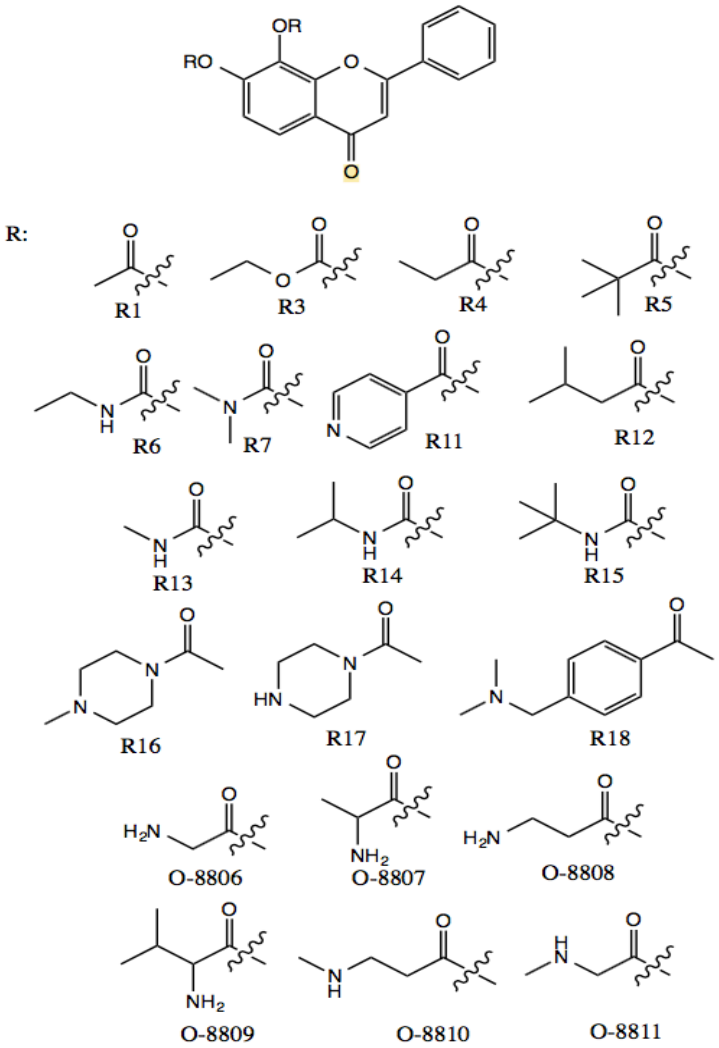
Supplementary Table 5. Chemical Stability

Compound	Test Concentration (μM)	Aqueous Buffer pH	$T_{1/2}$ (day)	Mean Remaining of Prodrug at Last Time Point (%)	Mean Appearing of Parent 7,8-DHF at Last Time Point (%)
R1	10	1.2	2.8	25.8	3.0
	10	7.4	0.9	0.6	11.5
R5	10	1.2	0.4	2.6	ND
	10	7.4	0.5	7.7	1.4
R7	10	1.2	28.7	84.10	0.05
	10	7.4	>100	109	ND
R11	10	1.2	0.5	9.2	75.0
	10	7.4	NA	0.0	15.5
R12	10	1.2	1.2	5.60	ND
	10	7.4	1.2	20.70	10.4
R13	10	1.2	225	97.4	65.0
	10	7.4	NA	0.0	27.1
R16	10	1.2	>200	100	No 7,8-DHF
	10	7.4	136	94.8	No 7,8-DHF
R17	10	1.2	19.4	73.0	No 7,8-DHF
	10	7.4	29.9	87.0	No 7,8-DHF
R18	10	1.2	23.3	87.5	No 7,8-DHF
	10	7.4	0.9	0.5	No 7,8-DHF

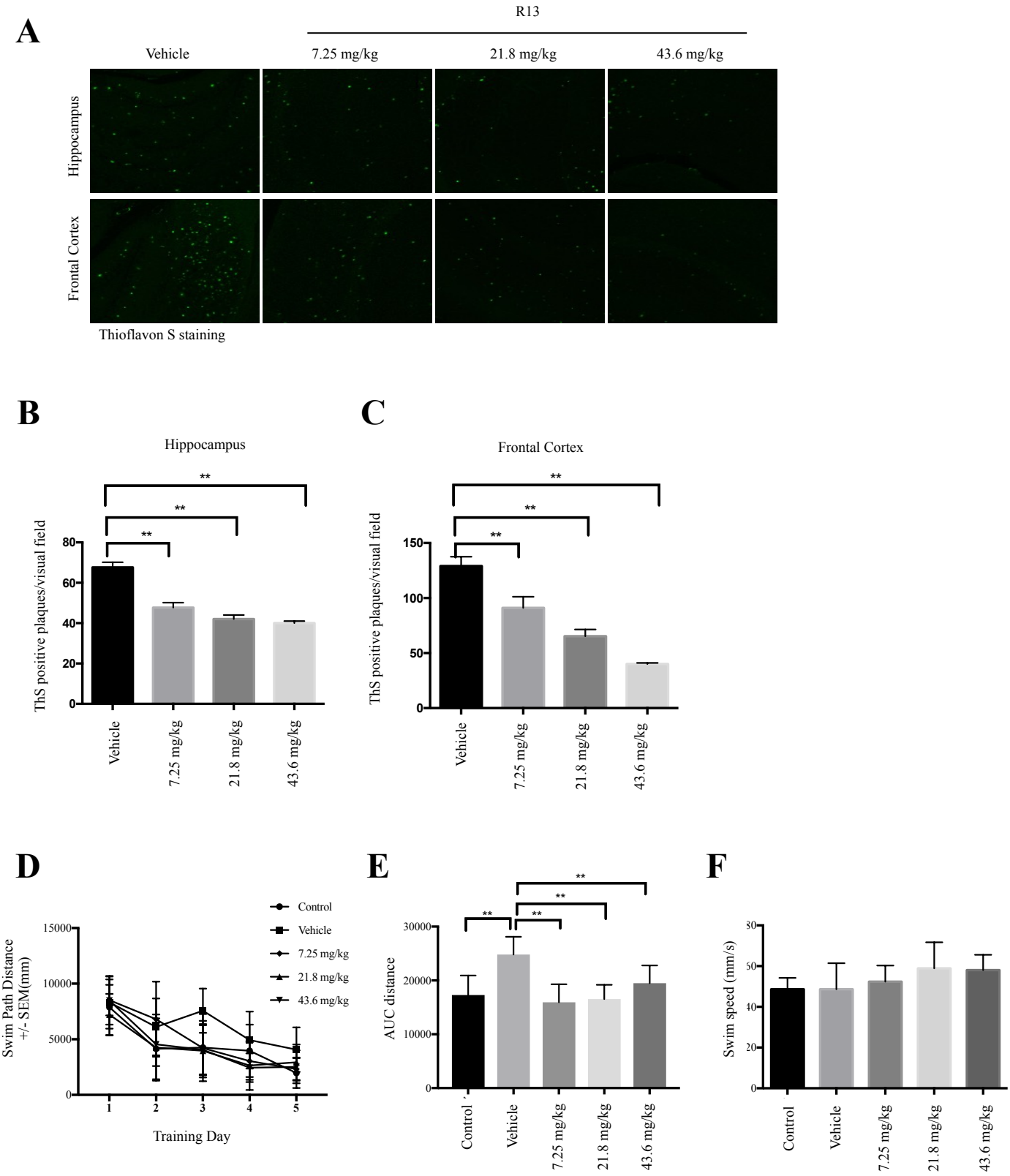
Supplementary Table 6. In vivo pharmacokinetic parameters of 7,8-DHF and prodrug R13

Compound I.D.	Route	Dose (mg/kg)	Subject	Tmax (min)	Cmax (ng/mL)	$T_{1/2}$ (min)	AUClast (min*ng/mL)	ACINF (min*ng/mL)	CL (mL/min/kg)	Vz (mL/kg)	Vss (mL/kg)	F (%)
7,8-DHF	IV	10	Mice			248	28072	30696	326	116681	35447	
7,8-DHF	PO	50	Mice	10	70	134	6500	7515				**4.6**
7,8-DHF from R13 (PO)	PO	36 (R13)	Mice	30	129	219.6	11880	15480				**10.5**

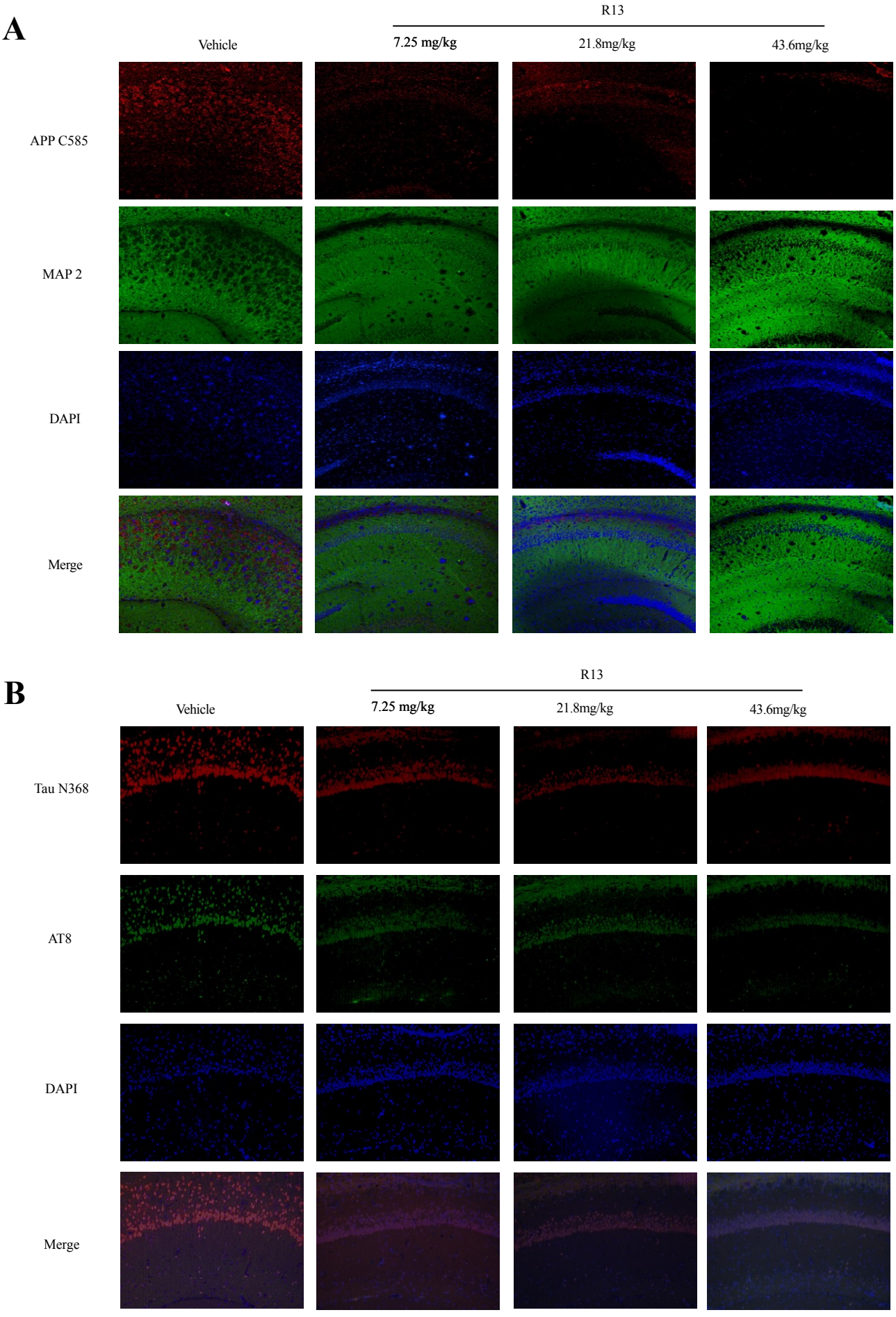
Supplementary Figure 1



Supplementary Figure 2

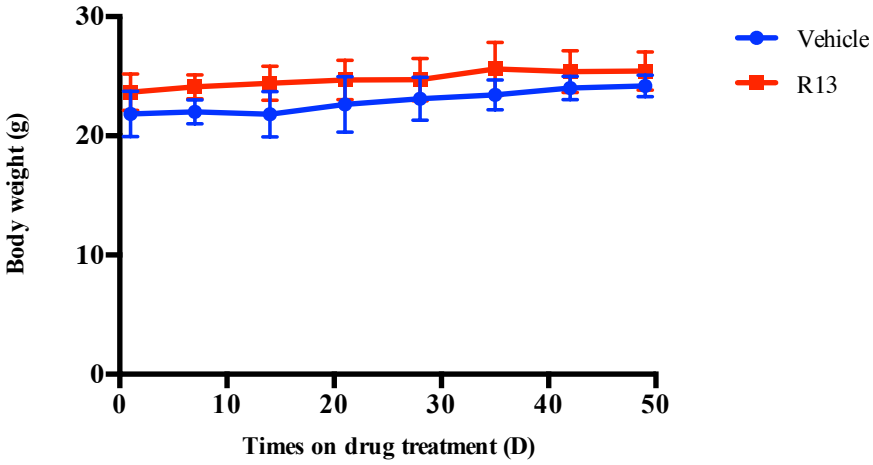


Supplementary Figure 3



Supplementary Figure 4

A



B