Supporting Information

Brain Permeable Tafamidis Amide Analogs for Stabilizing TTR and Reducing APP Cleavage.

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1. General Methods. All commercial chemicals, including acids **1** and **3-9**, and solvents were reagent grade and used without further purification. All reactions were performed under Argon atmosphere. Column chromatography was performed using CombiFlash and pre-packed Silica gel columns. Analytical thin layer chromatography was performed on Merck 250 μM silica gel F_{254} plates, and preparative thin layer chromatography on Merck 1000 μM silica gel F_{254} plates obtained from EMD Millipore corporation. The identity of each product was determined using Agilent 6200 Series TOF LC/MS and NMR using Bruker 400 or 600 MHz instrument. Chemical shifts are reported in δ values in ppm downfield from TMS as the internal standard. ^{1}H data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, br s = broad singlet, m = multiplet, ABq = AB quartet), coupling constant (Hz), integration.

2. Synthesis and spectral data of the amide derivatives of acid 1 and of 3-9. All amide derivatives of **1** and of **3-9** were prepared using Method A and the Boc-protected amide products were deprotected using Method B. All compounds were characterized by performing ^{1}HNMR and/or MS.

Method A (amide formation). HATU (456 mg, 1.2 mmol) was added to a solution of acid (1 mmol) and amine or N-Boc protected diamine (1.1 mmol) in DMF (3 ml) followed by DIEA (0.35 ml, 2 mmol). After the reaction mixture was stirred overnight at room temperature, it was quenched with water to afford the corresponding amide derivatives.

Method B (Boc deprotection). 4M HCl in Dioxane (2 ml) was added to a solution of the amide derivatives of Boc-protected diamine compound (1 mmol) in Dioxane (2 ml), and the reaction mixture was stirred at room temperature overnight. Solvents were removed under reduced pressure and, the solid was triturated using EtOAc and filtered to afford products as HCl salt.

2.1. Compound 1a. Prepared by HATU coupling of acid **1** with mono-Boc-piperazine, followed by Boc deprotection of resulting intermediate **1a**-Boc (or **1a'**) under acidic condition.

1a-Boc: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16 (d, *J* = 1.7 Hz, 2H), 7.82 (d, *J* = 8.2 Hz, 1H), 7.69 (s, 1H), 7.55 (s, 1H), 7.44 (d, *J* = 8.2 Hz, 1H), 3.75 and 3.49 (br, 8H), 1.48 (s, 9H). MS (ESI/TOF) m/z: $[M+Na]^+$ Calcd for $C_{23}H_{23}Cl_2N_3O_4Na$ = 498.1 $(M+Na)^+$; Found 498.1.

1a-HCl: ^{1}H NMR (600 MHz, DMSO): δ 9.48 (s, 1H), 8.15 (s, 2H), 7.97 (s, 1H), 7.95 (s, 1H), 7.93 (d, *J* = 8.1 Hz, 1H), 7.55 (d, J = 8.1 Hz, 1H), 3.72 (br, 4H), 3.17 (br s, 4H). HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{18}H_{16}Cl_2N_3O_2$ = 376.0620; Found 376.0618.

2.2. Compound 1b. Prepared by HATU coupling of acid **1** with N-methylpiperazine.

1b: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.15 (s, 2H), 7.80 (d, J = 8.1 Hz, 1H), 7.69 (s, 1H), 7.54 (s, 1H), 7.45 (d, J = 8.1 Hz, 1H), 3.83 (br, 2H), 3.50 (br, 2H), 2.50 (br, 2H), 2.48 (br, 2H), 2.34 (s, 3H). HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{19}H_{18}Cl_2N_3O_2$ = 390.0776; Found 390.0757.

2.3. Compound 1c. Prepared by HATU coupling of acid **1** with N-Boc-4-(piperazin-1-yl)piperidine, followed by Boc deprotection of resulting intermediate **1c**-Boc (or **1c'**) under acidic condition.
1c-Boc: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.16 (s, 2H), 7.80 (d, J = 7.8 Hz, 1H), 7.69 (s, 1H), 7.55 (s, 1H), 7.44 (d, J = 7.8 Hz, 1H), 4.15 (br, 2H), 3.81 (br, 2H), 3.48 (br, 2H), 2.71 (br, 4H), 2.53 (br, 2H), 2.44 (t, J = 12.0 Hz, 1H), 1.78 (d, J = 12.0 Hz, 2H), 1.55 and 1.45 (2 x s, 9H), 1.41 (m, 2H). HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{28}H_{33}Cl_2N_4O_4$ = 559.1879; Found 559.1884.
1c-HCl: ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.98 (br, 1H), 8.82 (br, 1H), 8.17 (s, 2H), 7.98 (d, J = 8.2 Hz, 1H), 7.97 (s, 1H), 7.95 (d, J = 8.0 Hz, 1H), 7.58 (br, 1H), 3.43 (m, 9H), 3.19 (br, 2H), 2.93 (m, 2H), 2.28 (br, 2H), 1.95 (m, 2H). HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{23}H_{25}Cl_2N_4O_2$ = 459.1355; Found 459.1345.

2.4. Compound 1d. Prepared by HATU coupling of acid **1** with 1-(1-methylpiperidin-4-yl)piperazine.
1d: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.15 (s, 2H), 7.80 (d, J = 8.0 Hz, 1H), 7.68 (s, 1H), 7.54 (s, 1H), 7.44 (d, J = 8.0 Hz, 1H), 3.82 (br, 2H), 3.49 (br, 2H), 2.94 and 2.89 (ABq, J_{AB} = 15.0 Hz, 2H), 2.67 (br, 2H), 2.53 (br, 2H), 2.30 (t, J = 12.0 Hz, 1H), 2.27 (s, 3H), 1.94 (t, J = 11.4 Hz, 1H), 1.78 (d, J = 12.0 Hz, 1H), 1.61 (m, 2H). HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{24}H_{27}Cl_2N_4O_2$ = 473.1511; Found 473.1533.

2.5. Compound 1e. Prepared by HATU coupling of acid **1** with N,N-dimethylethylenediamine.
1e: ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.84 (s, 1H), 8.27 (s, 1H), 8.18 (s, 2H), 7.98 (m, 3H), 3.64 (q, J = 5.4 Hz, 2H), 3.25 (br, 2H), 2.84 (s, 6H). HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{18}H_{18}Cl_2N_3O_2$ = 378.0776; Found 378.0774.

2.6. Compound 1f. Prepared by HATU coupling of acid **1** with neopentylamine.
1f: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.14 (s, 2H), 8.07 (s, 1H), 7.80 and 7.76 (ABq, J_{AB} = 8.1 Hz, 2H), 7.54 (s, 1H), 3.39 (s, 1H), 3.29 (s, 2H), 0.99 (s, 9H). HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{19}H_{19}Cl_2N_2O_2$ = 377.0824; Found 377.0829.

2.7. Compound 1g. Prepared by HATU coupling of acid **1** with admantanylamine.
1g: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.16 (d, J = 1.8 Hz, 2H), 8.04 (s, 1H), 7.78 (d, J = 8.4 Hz, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.55 (s, 1H), 5.88 (s, 1H), 2.22 (m, 1H), 2.00 (s, 2H), 1.83 and 1.79 (ABq, J_{AB} =

12.0 Hz, 4H), 1.46 and 1.36 (ABq, J_{AB} = 12.0 Hz, 4H), 1.26 and 1.20 (ABq, J_{AB} = 12.0 Hz, 2H), 0.91 (s, 6H). HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{26}H_{27}Cl_2N_2O_2$ = 469.1450; Found 469.1436.

2.8. Compound 1h. Prepared by HATU coupling of acid **1** with 4-amino-N-Boc-piperidine, followed by Boc deprotection of resulting intermediate **1h**-Boc (or **1h'**) under acidic condition.

1h-Boc: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.16 (s, 2H), 8.08 (s, 1H), 7.81 and 7.75 (ABq, J_{AB} = 8.4 Hz, 2H), 7.56 (s, 1H), 6.01 (d, J = 7.8 Hz), 4.17 (br m, 4H), 2.94 (m, 3H), 1.54 and 1.48 (2 x s, 9H).

1h-HCl: ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.80 (br, 2H), 8.68 (d, J = 8.2 Hz, 1H), 8.32 (s, 1H), 8.16 (s, 2H), 8.00 and 7.92 (ABq, J_{AB} = 8.4 Hz, 2H), 7.97 (s, 1H), 4.11 (m, 1H), 3.33 (br, 2H), 3.03 (t, J = 11.4 Hz, 2H), 2.01 (d, J = 12.0 Hz, 2H), 1.81 (q, J = 10.8 Hz, 2H). HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{19}H_{18}Cl_2N_3O_2$ = 390.0776; Found 390.0763.

2.9. Compound 1i. Prepared by HATU coupling of compound **1** with 1-methylpiperidin-4-amine.

1i: ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.43 (d, J = 7.8 Hz, 1H), 8.27 (s, 1H), 8.17 (s, 2H), 7.97 and 7.91 (ABq, J_{AB} = 8.4 Hz, 2H), 7.97 (s, 1H), 3.79 (m, 1H), 2.83 (d, J = 10.2 Hz, 2H), 2.21 (s, 3H), 2.04 (br s, 2H), 1.81 (d, J = 11.8 Hz, 2H), 1.63 (q, J = 9.6 Hz, 2H). HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{20}H_{20}Cl_2N_3O_2$ = 404.0933; Found 404.0940.

2.10. Compound 3a. Prepared by HATU coupling of acid **3** with mono-Boc-piperazine, followed by Boc deprotection of resulting intermediate **3a**-Boc under acidic condition.

3a-Boc: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.40 (d, J = 8.0 Hz, 2H), 7.83 (d, J = 7.8 Hz, 1H), 7.82 (d, J = 8.0 Hz, 2H), 7.71 (s, 1H), 7.45 (d, J = 7.8 Hz, 1H), 3.76 and 3.50 (2 x br s, 8H), 1.55 and 1.48 (2 x s, 9H). HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{24}H_{25}F_3N_3O_4$ = 476.1797; Found: 476.1804.

3a-HCl: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.38 (d, J = 8.4 Hz, 2H), 7.85 (d, J = 8.0 Hz, 1H), 7.81 (d, J = 8.4 Hz, 2H), 7.73 (s, 1H), 7.44 (d, J = 8.0 Hz, 1H), 4.01 (br s, 4H), 3.24 (br s, 4H), (NH obscure). HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{19}H_{17}F_3N_3O_2$ = 376.1273; Found: 376.1289.

2.11. Compound 3b. Prepared by HATU coupling of compound **3** with N-methylpiperazine.

3b: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.39 (d, J = 8.1 Hz, 2H), 7.85-7.81 (m, 3H), 7.71 (s, 1H), 7.44 (d, J = 8.1 Hz, 1H), 3.84 (br, 2H), 3.52 (br, 2H), 2.46 (br, 4H), 2.35 (s, 3H). HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{20}H_{19}F_3N_3O_2$ = 390.1429; Found: 390.1437.

2.12. Compound 3e. Prepared by HATU coupling of compound **3** with N,N-dimethylethylenediamine.

3e: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.40 (d, J = 8.4 Hz, 2H), 8.14 (s, 1H), 7.82 (m, 4H), 6.94 (br s, 1H),

3.57 (q, J = 5.4 Hz, 2H), 2.56 (t, J = 5.4 Hz, 2H), 2.30 (s, 6H). HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{19}H_{19}F_3N_3O_2$ = 378.1429; Found: 378.1422.

2.13. Compound 3f. Prepared by HATU coupling of acid **3** with neopentylamine.

3f: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.40 (d, J = 8.4 Hz, 2H), 8.12 (s, 1H), 7.84 (d, J = 8.2 Hz, 1H), 7.84 (d, J = 8.4 Hz, 2H), 7.77 (d, J = 8.2 Hz, 1H), 6.21 (br s, 1H), 3.34 (d, J = 6.0 Hz, 1H), 1.03 (s, 9H). HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{20}H_{20}F_3N_2O_2$ = 377.1477; Found: 377.1480.

2.14. Compound 3g. Prepared by HATU coupling of acid **3** with admantanylamine.

3g: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.40 (d, J= 8.2 Hz, 2H), 8.06 (s, 1H), 7.83-7.80 (m, 3H), 7.70 (d, J = 8.2 Hz, 1H), (5.90, s, 1H), 2.22 (m, 1H), 2.00 (s, 2H), 1.83 and 1.80 (ABq, J_{AB} = 12.5 Hz, 4H), 1.46 and 1.35 (ABq, J_{AB} = 12.2 Hz, 4H), 1.26 and 1.20 (ABq, J_{AB} = 12.5 Hz, 2H), 0.90 (s, 6H). HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{27}H_{28}F_3N_2O_2$ = 469.2103; Found: 469.2108.

2.15. Compound 4b. Prepared by HATU coupling of acid **4** with N-methylpiperazine.

4b: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.54 (s, 1H), 8.45 (d, J = 7.6 Hz, 1H), 7.82 (d, J = 8.0 Hz, 2H), 7.72-7.68 (m, 2H), 7.45 (d, J = 8.0 Hz, 1H), 3.84 (br s, 2H), 3.54 (br s, 2H), 2.47 (br s, 4H), 2.34 (s, 3H). HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{20}H_{19}F_3N_3O_2$ = 390.1429; Found: 390.1443.

2.16. Compound 5a. Prepared by HATU coupling of acid **5** with mono-Boc-piperazine, followed by Boc deprotection of resulting intermediate **5a**-Boc under acidic condition.

5a-Boc: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (d, J = 7.2 Hz, 1H), 7.87 (m, 2H), 7.71 (m, 3H), 7.44 (d, J = 8.2 Hz, 1H), 3.50 (br m, 8H), 1.45 (s, 9H).

5a-HCl: ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.18 (br, 1H), 8.19 (d, J = 7.8 Hz, 1H), 8.05 (d, J = 7.8 Hz, 1H), 7.99 (s, 1H), 7.97 (d, J = 7.8 Hz, 1H), 7.97 (t, J = 7.2 Hz, 1H), 7.91 (t, J = 7.2 Hz, 1H), 7.55 (d, J = 7.8 Hz, 1H), 3.74 (br, 4H), 3.18 (br s, 4H). HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{19}H_{17}F_3N_3O_2$ = 376.1273; Found: 376.1286.

2.17. Compound 5b. Prepared by HATU coupling of acid **5** with N-methylpiperazine.

5b: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16 (d, J = 7.1 Hz, 2H), 7.90 (d, J = 8.1 Hz, 1H), 7.87 (d, J = 8.1 Hz, 1H), 7.75-7.71 (m, 3H), 7.47 (d, J = 8.1 Hz, 1H), 3.83 (br, 2H), 3.49 (br, 2H), 3.46 (br, 4H), 2.35 (s, 3H). HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{20}H_{19}F_3N_3O_2$ = 390.1429; Found: 390.1428.

2.18. Compound 6a. Prepared by HATU coupling of acid **6** with mono-Boc-piperazine, followed by Boc deprotection of resulting intermediate **6a**-Boc under acidic condition.

6a-Boc: MS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{23}H_{24}Cl_2N_3O_4$ = 476.2 $(M+H)^+$; Found 476.2.
6a-HCl: ^{1}H NMR (600 MHz, DMSO-*d*$_6$): δ 8.87 (br, 1H), 8.18 (s, 2H), 7.99 (s, 1H), 7.98 (s, 1H), 7.93 (d, *J* = 8.4 Hz, 1H), 7.59 (d, *J* = 8.4 Hz, 1H), 3.75 (br, 4H), 3.19 (s, 1H). HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{18}H_{16}Cl_2N_3O_2$ = 376.0620; Found 376.0615.

2.19. Compound 7a. Prepared by HATU coupling of acid **7** with mono-Boc-piperazine, followed by Boc deprotection of resulting Boc intermediate under acidic condition.
7a-HCl: ^{1}H NMR (600 MHz, DMSO-*d*$_6$) of **7a**: δ 9.18 (br, 1H), 8.03 (s, 1H), 8.02 (d, *J* = 8.4 Hz, 1H), 7.75 (m, 3H), 7.60 (d, *J* = 8.4 Hz, 1H), 3.74 (br, 4H), 3.17 (br s, 4H). HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{18}H_{16}Cl_2N_3O_2$ = 376.0620; Found: 376.0626.

2.20. Compound 8a. Prepared by HATU coupling of acid **8** with mono-Boc-piperazine, followed by Boc deprotection of resulting **8a**-Boc intermediate under acidic condition.
8a-Boc: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.21 (d, *J* = 8.4 Hz, 2H), 7.75 (d, *J* = 7.8 Hz, 1H), 7.65 (s, 1H), 7.39 (d, *J* = 7.8 Hz, 1H), 7.05 (d, *J* = 8.4 Hz, 2H), 3.72 and 3.49 (br and br s, 8H), 1.55 and 1.48 (2 x s, 9H). HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{24}H_{27}N_3O_5Na$ = 460.2029; Found: 460.2042.
8a-HCl: ^{1}H NMR (600 MHz, $CDCl_3$+CD_3OD): δ 8.19 (d, *J* = 8.4 Hz, 2H), 7.75 (d, *J* = 8.4 Hz, 1H), 7.66 (s, 1H), 7.39 (d, *J* = 7.8 Hz, 1H), 7.04 (d, *J* = 8.4 Hz, 2H), 4.01 (br s, 4H), 3.24 (br s, 4H). HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{19}H_{20}N_3O_3$ = 338.1505; Found: 338.1517.

2.21. Compound 9a. Prepared by HATU coupling of acid **9** with mono-Boc-piperazine, followed by Boc deprotection of resulting **9a**-Boc intermediate under acidic condition.
9a-Boc: ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.81 (d, *J* = 8.2 Hz, 1H), 7.80 (s, 1H), 7.79 (s, 1H), 7.69 (s, 1H), 7.44 (d, *J* = 8.2 Hz, 1H), 7.02 (t, *J* = 8.2 Hz), 3.79 and 3.49 (2 x br, 8H), 1.55 and 1.48 (2 x s, 9H).
9a-HCl: ^{1}H NMR (600 MHz, $CDCl_3$+CD_3OD): δ 7.81 (br d, *J* = 7.8 Hz, 1H), 7.75 (br s, 2H), 7.70 (s, 1H), 7.43 (d, *J* = 8.4 Hz, 1H), 7.00 (t, *J* = 7.6 Hz), 3.96 (br s, 4H), 3.19 (br s, 4H). HRMS (ESI/TOF) m/z: $[M+H]^+$ Calcd for $C_{18}H_{16}F_2N_3O_2$ = 344.1211; Found: 344.1209.

3. Table S-1. Calculated Physicochemical and ADME properties of amide derivatives[a]

Compd No	Mol Wt g/mol	tPSA Å^2	Consensus Log $P_{o/w}$	GI absorption	BBB permeant	P-gp substrate	Synthetic accessibility
1a	376.24	58.37	3.40	High	Yes	Yes	2.91
1b	390.26	49.58	3.66	High	Yes	No	3.02
1h	390.26	67.16	3.88	High	Yes	Yes	2.94
1i	404.29	58.37	4.14	High	Yes	No	3.05
3a	375.34	58.37	3.42	High	Yes	Yes	3.01
3b	389.37	49.58	3.68	High	Yes	No	3.12
3e	377.36	58.37	3.81	High	Yes	No	3.08

[a] Physicochemical data and ADME properties of the active amide derivatives of **1** and **3**, and N-methyl derivatives of the active compounds were calculated using SwissADME (http://www.swissadme.ch/index.php) web tool.[1]

4. Screening and evaluation of 1 and amide derivatives of 1 and 3-9.

Cell lines, antibodies, and reagents and kits for evaluation of compounds. N2a695 cells used to screen and evaluate the amide derivatives of **1** and of **3-9** were available in house. Cells were cultured in 1:1 OptiMem Reduced Serum Media (Life Technologies): Dulbecco's Modified Eagle Medium ([+] 4.5 g/L D-glucose; [+] L-Glutamine; [-] Sodium pyruvate (Life Technologies) supplemented with 5% fetal bovine serum, 0.4% Penstrep and 0.4% Geneticin and incubated at 37 °C in 5% CO_2. 96-Well ELISA plate for human Aβ40 peptide and V-Plex Plus MSD plate for Aβ Peptide Panel 1 (6E10) Kit (Catalog number K15200G) were obtained from Thermo Fischer and Meso Scale Discovery.

4.1. Aβ production assay. 6-Well tissue culture plates (Corning) were seeded with N2a695 cells at $4.0x10^5$ – $4.5x10^5$ N2a695 cells/mL, 2 mL/well. Upon overnight incubation at 37 ˚C under 5% CO_2 atmosphere, media were exchanged with fresh media containing 10 μM solution of compounds (prepared from 10 mM solution in DMSO). After cells (>95% confluent) were further incubated with compounds for 6 h at 37 °C in 5% CO_2, culture media were collected. Subsequently, culture media (50-100 μl, 5-10x dilution for Aβ40 and no dilution for Aβ42 measurement) were transferred to strips of 96-well ELISA plates pre-coated with the human Aβ40 and Aβ42 peptide capture antibodies. Further

processing was carried out per manufacturer instructions, and signals for Aβ were measured using Perkin Elmer Envision ELISA reader.

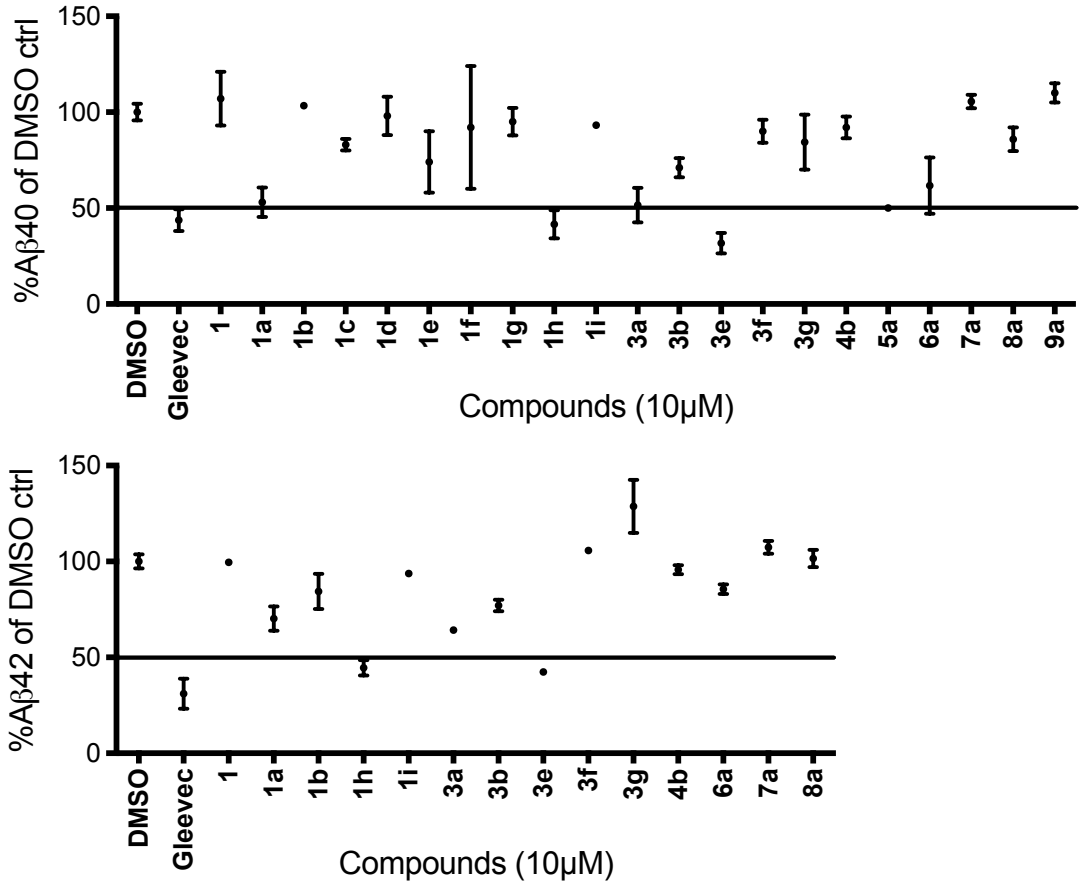

Figure S-1. Screening of the amide derivatives of **1** and of **3**-**9** using N2a695 cells. Shown are average of 2 or 3 (in some case only 1) independent assays in duplicates using DMSO and Gleevec as negative and positive controls.

4.2. Cell viability (MTT) assay. A 96-well plate was seeded with N2a695 cells (100,000 cells/ml, 200 µl) and kept in incubator at 37 ˚C and 5% CO_2 atmosphere overnight. Media of the cells were exchanged with new media (100 µl) containing compounds (10 µM or appropriate concentration) or DMSO and Media alone (for controls) 24 hours later, and the plate was placed back in the incubator for another 5 or 24 h, as needed. MTT reagent (Thermo Fisher) (1:10 dilution using Media, 100 µl) was added to each well. Three hours later, solubilization buffer (100 µl) was added to each well and kept at 37 ˚C overnight to solubilize the formazan crystals, before the plate was cooled to room temperature and the absorbance

was measured at 560 nm and 650 nm wavelength. The data were processed as described in product manual.

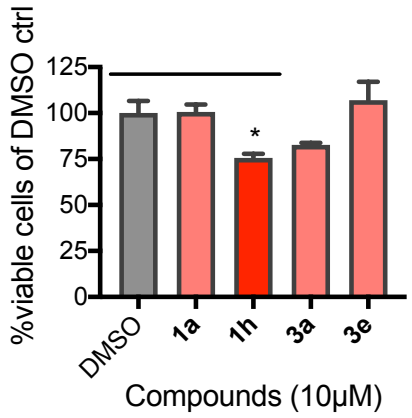

Figure S-2. Cellular toxicity of potent PD173955 analogs. Shown are cell viability of **2** and 9 potent analogs of **2** at 1 and 10 μM concentrations, respectively, in A and B, and dose dependence of 3 potentially toxic compounds in C and D, in 5- and 24-hours assays. Assays were performed in triplicates, and cell viability was determined as % of DMSO control.

4.3. Western Blotting experiments. Cell lysates were obtained using the media-free cells, described above in 5.1. Cells were scrapped in cold Dulbecco's PBS buffer (1 mL) containing EDTA-free protease inhibitor (Roche) and centrifuged for 1 minute at 13,000 rpm at 4 °C to afford cell pellets. The latter were lysed in 3% SDS by sonication for two rounds of 20 seconds on a low setting. Before performing the WBs, protein concentrations were measured using the Pierce BCA Protein Assay (Thermo Fisher) kit in accordance with the manufacturer's instructions, and lysates were appropriately diluted before loading on gels. Samples from cell lysates (30 μl) were loaded and separated on a 16.5 % Tris-Tricine gel (Criterion), and electrotransferred to PVDF membrane (EMD Millipore) overnight at 30V. The membrane was treated with glutaraldehyde (Sigma) solution (0.25% in PBS) for 30 min, blocked using milk PBST (30 minutes), and probed using antibody RU369 (1 hour at room temperature). HRP-linked secondary antibody and enhanced chemiluminescence ECL reagent were used for detection.

4.4. GS activity assay, *in vitro*. The γ-secretase assay, *in vitro*, was performed similarly as described previously.[2 3] Briefly, Sb4 substrate (1 μM) was incubated with cell membrane (40 μg/ml) in the presence of 0.25% CHAPSO and PIPES buffer (50mM PIPES, pH 7.0, 150mM KCl, 5mM $CaCl_2$, 5mM $MgCl_2$) and compounds **1**, **1a**, **1h**, **3a**, or **3e** at various concentrations noted later at 37°C for 3 hours. The amount of cleavage product generated was then determined using a detection mixture with cleavage specific antibodies for

Aβ42 (10-G3) ans Aβ40 (G2-10), in combination with AlphaLISA Protein A (for Aβ42) or AlphaLISA anti-mouse (for Aβ40) acceptor beads and streptavidin coated donor beads (Perkin-Elmer). Equal volumes of reaction mixtures were added to detection mixtures in a 384 well plate and incubated at room temperature overnight, and then the AlphaLisa signal was read using the EnVision multilabel plate reader (Perkin-Elmer).

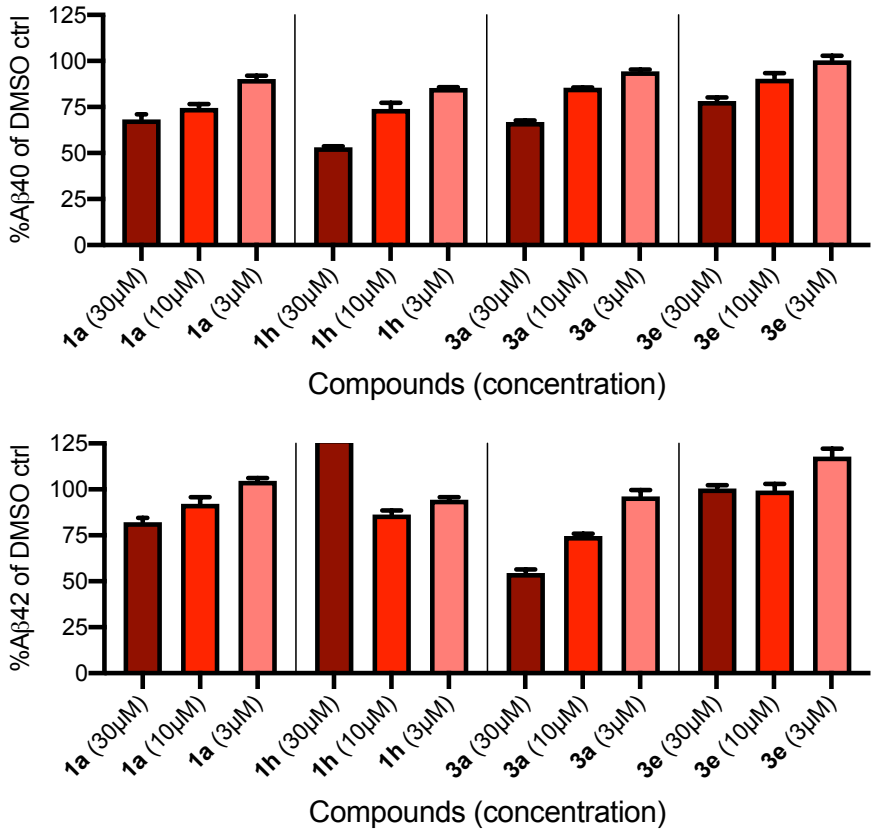

Figure S-3. Effect of compound **1a**, **1h**, **3a**, and **3e** on γ-secretase activity measured *in vitro.*

4.5. Monitoring the oligomerization of Aβ using FCS. FCS measurements were performed on Zeiss LSM 880 confocal microscope equipped with a ConfoCor 2 module (Carl Zeiss, Inc.) as described in the "Materials and Methods" section. Preparation of Aβ solutions used in this study was based on the procedure adapted from Yumin and Teplow (*J. Neurochem.*, 2017, 140, 210-215).[4] Briefly, prior to the FCS experiment 1 mg of Aβ40 and Aβ42 (Anaspec) were freshly dissolved in 100 μL of 60 mM aqueous NaOH, 450 μL of water, and 450 μL of 22.2 mM sodium phosphate buffer, pH 7.5. The resulting solutions, 10 mM sodium phosphate buffer, pH 7.5, were sonicated for 1 min in a bath sonicator (Branson, Model 1510) and filtered through a pre-washed 30 000 molecular weight cut-off Microcon centrifugal filter device (Millipore) for 15 min at 16 000 g under 4°C using a bench top

centrifuge (model 5415R, Eppendorf, Hamburg, Germany) to remove insoluble material. The concentration of Aβ solutions was determined by UV absorbance, using an estimated extinction coefficient ε280 = 1280 M^{-1} cm^{-1}. The Aβ solutions were then diluted into PBS buffer to a final concentration of 10 μM (Aβ 40 and Aβ 42 in 1:1 ratio), containing 2 nM of tetramethylrhodamine (TMR)-labeled Aβ40, and 2 nM cholesterol with/without compound **1** and **1a** (10 μM). The mixed solution was then monitored by FCS at 0, 2, and 4-hour time point.

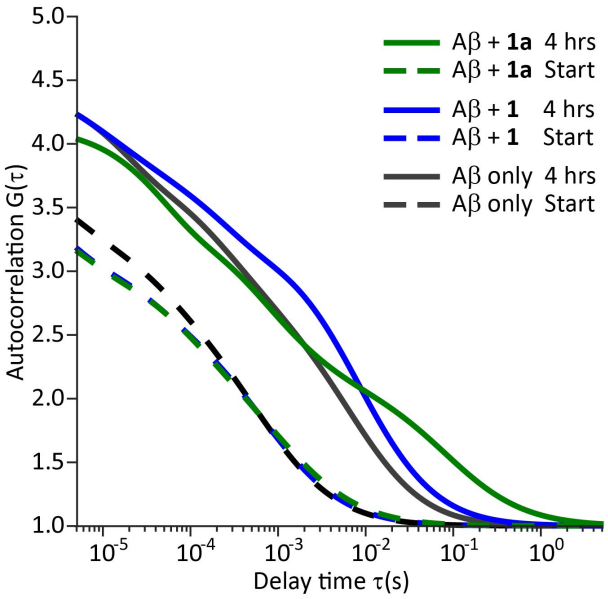

Figure S-4. Compounds **1** and **1a** does not affect Aβ oligomerization, as determined using FCS. Shown are fitted autocorrelation curves for diffusion of decreasing Aβ (Aβ40:Aβ42, 1:1) particle concentrations as a function of time. The amplitude of autocorrelation curve at a given diffusion time increases along the reaction time point (start and 4 hours) represent the decreasing particle numbers as a result of Aβ polymerization. In addition, the autocorrelation functions show increasing contribution from long delay times, indicating the increasing size of Aβ particles. A minimum difference in autocorrelation curves was found when Aβ polymerization was followed in the presence or absence of compounds **1** and **1a**, indicating that neither compounds have no effect on Aβ polymerization.

4.6. *In vivo* brain permeability and retention studies. Compounds **1**, **1a-b**, **1e**, **1h**, and **3a** were administered to mice orally (in drinking water or gavage) for 5 to 15 days or IP for 4 hours. After mice were euthanized, brain hemispheres were harvested and collected in pre-tared tubes and blood (100 μl) in other non-tared tubes.

Concentration in brain. EtOH (200 Proof, 1 ml) and an internal standard (1 μM in DMSO, 10 μL) were added to a pre-weighed brain sample in a tube and sonicated to homogeneity (~2 min). After tubes were shaken at room temperature (1K RPM) for 40 minutes and centrifuged at 13K RPM for 8 minutes, supernatants were transferred to new collection tubes. Pellets were extracted using 0.5 ml EtOH once more and the supernatants were combined with the first collections before LC-MS/MS was performed.

Concentration in blood. Acetonitrile (300 μl) and internal standard (1 μM in DMSO, 10 μL) were added blood samples (100 μl) in tubes, sonicated to homogeneity for 2 mins and shaken for 10 minutes. After tubes were centrifuged at 13K RPM for 9 minutes, supernatants (300 μl) were collected and combined with aqueous ammonium formate solution (5 mM, 500 μL) before LC-MS/MS analysis was performed.

4.7. *In vitro* metabolic stability of 1a. Compound **1a** (1 and 10 μM) was treated with CD-1 mouse liver microsomes (0.5 mg protein/mL) solution in the presence of 2.5 mM NADPH and 3.3 mM $MgCl_2$ in 100 mM phosphate buffer (pH 7.4) in an Eppendorf tube at 37 °C. The incubation contained a final organic solvent concentration of 0.1% DMSO. Reactions was stopped by removing 100 μL aliquots at 0, 30 and 60 min and mixing with 200 μL of acetonitrile, vortexed and centrifuged to obtain an aliquot of the supernatants. The latter were diluted using 10 % acetonitrile containing internal standards and analyzed using LC-MS/MS to determine unmodified **1a**.

5. References.

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