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Supplementary Data

Regulation of *in vitro* $A\beta_{1-40}$ Aggregation Mediated by Small Molecules

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SYNTHESIS

2-Morpholinoacetic acid (*compound G*)

To a solution of morpholine (1.74 g, 20 mmol) in THF (15 mL) was added triethylamine (3.3 mL, 24 mmol) and ethyl bromoacetate (2.21 mL, 20 mmol) at 0°C under N_2. The reaction mixture was stirred to reach the same temperature for 1 h. The solvent was evaporated *in vacuo*. The mixture was extracted with DCM and water. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*, which was used in the next reaction without further purification. To a solution of ethyl 2-morpholinoacetate (1.6 g, 5.8 mmol) in MeOH (10 mL) was added dropwise Claisen's alkali (1.8 mL) at 0°C. The reaction mixture was stirred to reach room temperature. After the reaction was completed, the solvent was evaporated *in vacuo*. The mixture was diluted with water and washed with DCM. The aqueous layer was concentrated by the freeze dryer to give 1.6 g (65%) of 2-morpholinoacetic acid. ^{1}H-NMR (400 MHz, DMSO-d_6)δ 2.80 (t, $J = 4.5$ Hz, 4H), 3.42 (s, 2H), 3.67 ((t, $J = 4.5$ Hz, 4H), 5.12 (bs, COOH).

2-(*4-*(*2-Hydroxyethyl*)*piperazin-1-yl*)*acetic acid* (*compound J*)

To a solution of 1-(2-hydroxyethyl)piperazine (1.3 g, 10 mmol) in THF (10 mL) was added triethylamine (2.1 mL, 15 mmol) and ethyl bromoacetate (1.1 mL, 10 mmol) at 0°C under N_2. The reaction mixture was stirred to reach the same temperature for 1 h. The solvent was evaporated *in vacuo*. The mixture was extracted with DCM and water. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*, which was used in the next reaction without further purification. To a solution of ethyl 2-(4-(2-hydroxyethyl)piperazin-1-yl)acetate (1.25 g, 5.8 mmol) in MeOH (10 mL) was added dropwise Claisen's alkali (1.8 mL) at 0°C. The reaction mixture was stirred to reach room temperature. After the reaction was completed, the solvent was evaporated *in vacuo*. The mixture was diluted with water and washed with DCM. The aqueous layer was concentrated by the freeze dryer to give 640 mg (58%) of 2-(4-(2-Hydroxyethyl)piperazin-1-yl)acetic acid. ^{1}H-

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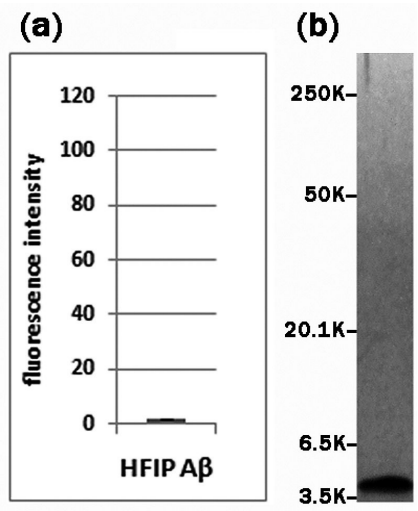

Figure S1. Aβ_{1-40} monomerization by HFIP treatment. HFIP was treated to synthetic Aβ_{1-40} to make homogeneous monomers. Monomerized Aβ_{1-40} was monitored by ThT fluorescence assay (a) and SDS-PAGE with PICUP method (b). Fluorescence shift of ThT was measured using an Envision II spectrofluorometer at 450 nm (ex) and 480 nm (em). Standard deviations were calculated and displayed in bar type graphs with error bars. Aβ_{1-40} was separated by SDS-PAGE on a 10–20% Criterion gradient tris-tricine gel, and visualized by silver staining method. All experiments were carried out in triplicate independently.

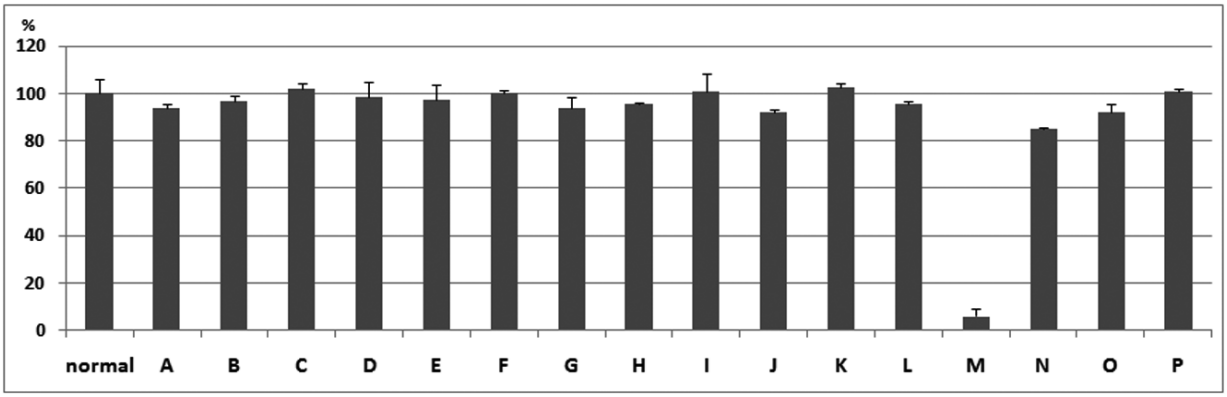

Figure S2. Neuronal cell viability assays (MTT assay) of various assay solutions. The toxicities of fifteen assay solutions and PBS were tested by MTT assays. All solutions were diluted 25 folds by starvation medium (DMEM, 2% FBS, 1% antibiotics). A (glycine), B (taurine), C (tramiprosate), D (tricine), E (TES), F (TAPS), G (2-Morpholinoacetic acid), H (MES), I (MOPS), J (2-[4-(2-hydroxy-ethyl)-piperazin-1-yl]-acetic acid), K (HEPES), L (EPPS), M (N-cyclohexylglycine), N (CHES), O (CAPS), P (PBS), and normal (no treat). Percentage of cell viability is expressed as 100% of normal control. MTT assays were carried out in duplicate independently. Standard deviations were calculated and displayed in bar type graphs with error bars.

NMR (400 MHz, DMSO-d_6): δ 2.37 (t, $j = 6.24$ Hz, 2H), 2.45 (br, 4H), 2.63 (br, 4H), 3.08 (s, 2H), 3.47 (t, $J = 6.27$ Hz, 2H).

N-Cyclohexylglycine (*compound M*)

Cyclohexylamine (0.99 g, 0.01 mol) was dissolved in anhydrous THF under nitrogen and maintained at -78°C. 2-Bromoacetic acid (1.39 g, 0.01 mol) was slowly added and heated to reach room temperature for 2 h. White powder was filtered off and dried (1.51 g, 96%). ^{1}H NMR (400 MHz, D_2O) δ 3.72 (s, 2H), 3.02–2.98 (m, 1H), 1.84 (s, 2H), 1.77–1.75 (s, 2H), 1.53–1.49 (d, $J = 12.6$ Hz, 1H), 1.25–1.02 (m, 5H).

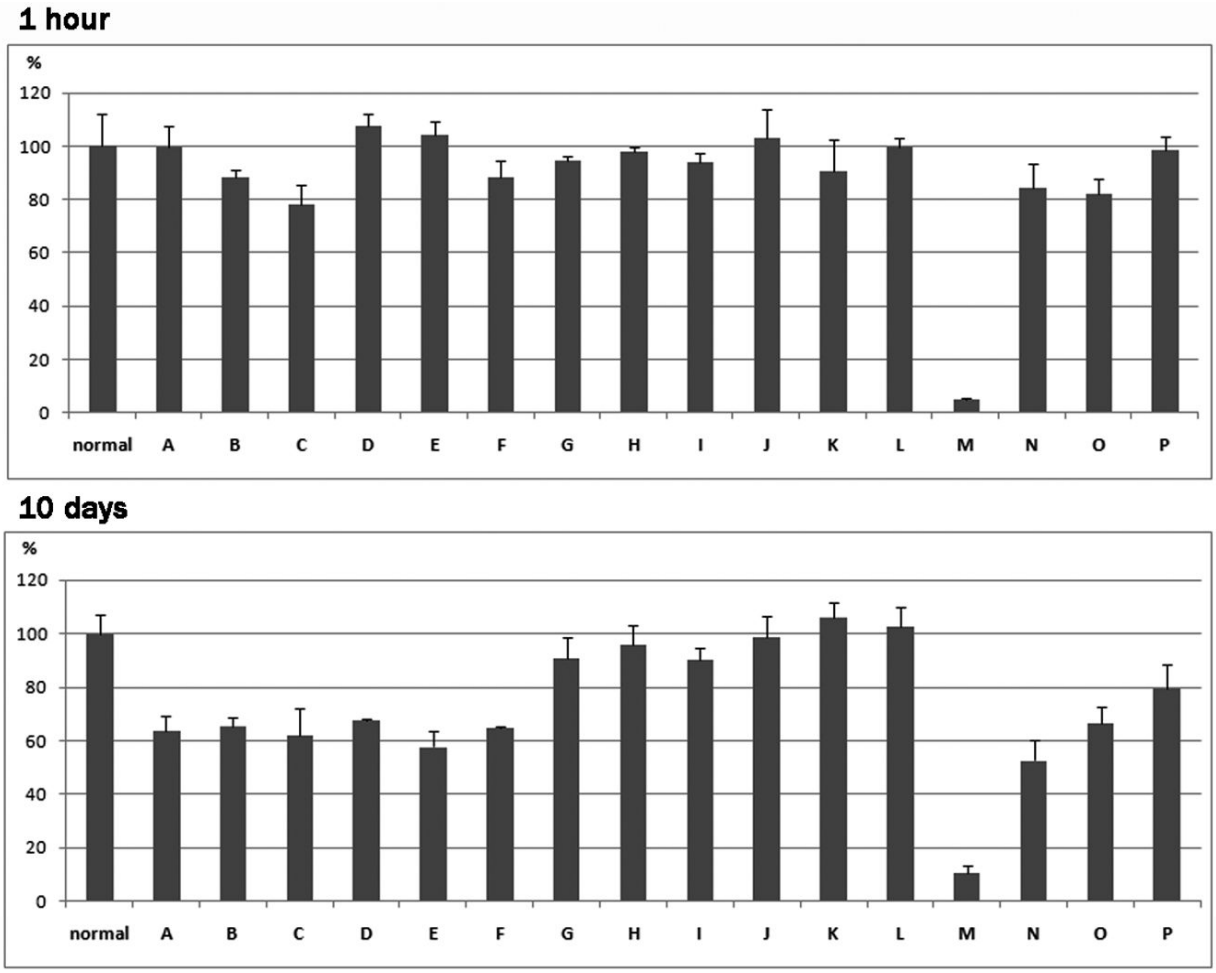

Figure S3. Neuronal cell viability assays (trypan blue exclusion) of $A\beta_{1-40}$ aggregates in various assay solutions. The toxicities of $A\beta_{1-40}$ aggregates in assay solutions were tested by trypan blue exclusion assay (for 1 hour (top) and 10 days (bottom)). All incubated $A\beta$ aggregates were diluted 25 folds by starvation medium (DMEM, 2% FBS, 1% antibiotics). Diluted $A\beta_{1-40}$ aggregates were treated on HT-22 cells for 24 h. All solutions were prepared at 20 mM and pH 5.5 except PBS, which was used as commercially provided. Synthetic $A\beta$ was dissolved to be 5 mM in DMSO and $A\beta$ was incubated in each assay solution at 37°C for 10 days without agitation. A (glycine), B (taurine), C (tramiprosate), D (tricine), E (TES), F (TAPS), G (2-Morpholinoacetic acid), H (MES), I (MOPS), J (2-[4-(2-hydroxy-ethyl)-piperazin-1-yl]-acetic acid), K (HEPES), L (EPPS), M (N-cyclohexylglycine), N (CHES), O (CAPS), P (PBS), and normal (no treat). Percentage cell viability is expressed as 100% of normal control. Trypan blue exclusion assays were carried out in duplicate independently. Standard deviations were calculated and displayed in bar type graphs with error bars.