Supporting Information

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SI Materials and Methods

Preparations of Monomeric and Oligomeric Aβ42 Peptides. The preparation procedure was described previously (1). Synthetic and recombinant wild-type Aβ42 (AnaSpec) was initially dissolved to 5 mg/mL in hexafluoroisopropanol (Sigma). Hexafluoroisopropanol was removed under vacuum in a Speed Vac, and the peptide film was stored at −20 °C. For monomer preparation, the peptide film was first resuspended in DMSO to a concentration of 12.5 mg/mL and then diluted with DMEM/F-12 (phenol red-free; Invitrogen) to a final concentration of 500 μg/mL. Oligomer preparation was obtained by incubating the monomer preparation at 4 °C for 24 h. Prepared monomers and oligomers were detected by Western blotting.

Cell Culture, Transfection, and Aβ42 Treatments. COS-7 cells were provided by the James Tissue Culture Facility of Cold Spring Harbor Laboratory. Cells were cultured in DMEM containing 10% FBS (Invitrogen) at 37 °C with 5% CO_2. Human wild-type Aβ42 sequence was amplified by PCR from human APP cDNA and constructed into pcDNA vector. Human wild-type EGFR plasmid was kindly provided by Sarah Parsons (University of Virginia, Charlottesville, VA). Cells were transfected with Lipofectamine 2000 (Invitrogen). At 48 h after transfection, cells were washed with fresh medium and incubated with 25 μg/mL of Aβ42 oligomers for 15 min. Human EGF (Sigma), 0.5 μg/mL, was used as a positive control. For gefitinib (Ge), erlotinib (Er), and synthetic compounds treatments, COS-7 cells were pretreated with or without drugs for 15 min, then oligomeric Aβ42 preparation was added to a final concentration in medium and incubated for another 15 min. Cells were washed three times with PBS before being collected for further experiments.

Western Blotting Analysis. Cell or tissue lysates were prepared using RIPA buffer containing 0.3% SDS, 50 mM Tris·HCl (pH 7.4), 0.5% Nonidet P-40, 1% sodium deoxycholate, 150 mM NaCl, 5 mM EDTA, one tablet per 50 mL complete protease inhibitor mixture (Roche Diagnostics), and 1× phosphatase inhibitor mixture set III (Calbiochem). Lysates were diluted in SDS sample buffer and separated by 10–20% Tris-Tricine gels (Invitrogen) and transferred to nitrocellulose membranes (Invitrogen). The membranes were blocked with 3% BSA and blotted with primary antibody overnight at 4 °C (for testing Aβ, membranes were boiled in PBS for 3 min before blocking). Primary antibodies used in this study were mouse anti-Aβ42 (6E10; Covance Research Products), rabbit anti-EGFR (Cell Signaling Technology), mouse anti–phospho-EGFR (Cell Signaling Technology), and rabbit anti-Actin (Sigma). After washing in Tris-buffered saline with Tween (TBST) three times, membranes were incubated with secondary antibodies for 1 h at room temperature. After three washes in TBST, membranes were incubated with ECL working solution (GE Healthcare) and developed with films (GE Healthcare). Imaging data were analyzed with ImageJ (National Institutes of Health).

Immunoprecipitation. Protein G-agarose (Roche Diagnostics) beads were washed with PBS and RIPA buffer then conjugated with rabbit anti-EGFR for 4 h at 4 °C. Beads were washed and then incubated with cell lysates overnight at 4 °C. Cell lysates were removed after three washes with RIPA. Beads were boiled at 100 °C for 5 min and loaded into 10–20% Tris-Tricine gels following standard Western blotting protocols.

Thioflavin-S Staining. Mice brains were fixed by transcardial perfusion using 4% paraformaldehyde in PBS, pH 7.4. Brain sections (30 μm) were incubated with a solution including Thioflavin-S (2 μg/mL) and Hoechst in PBS for 20 min at room temperature. Images were acquired with a NIKON Ti-E microscope using a 10× objective. An intensity threshold was set and was kept constant for all of the images analyzed with Image-Pro Plus 5.0 software (Media Cybernetic). Plaques within the cerebral cortex were selected and measured by standardized fluorescence intensity. The plaque area [(total plaques area/cortex area) × 100%] was analyzed. The results were obtained from four 10-mo-old male animals in each group (three slices per brain).

1. Dahlgren KN, et al. (2002) Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability. *J Biol Chem* 277:32046–32053.

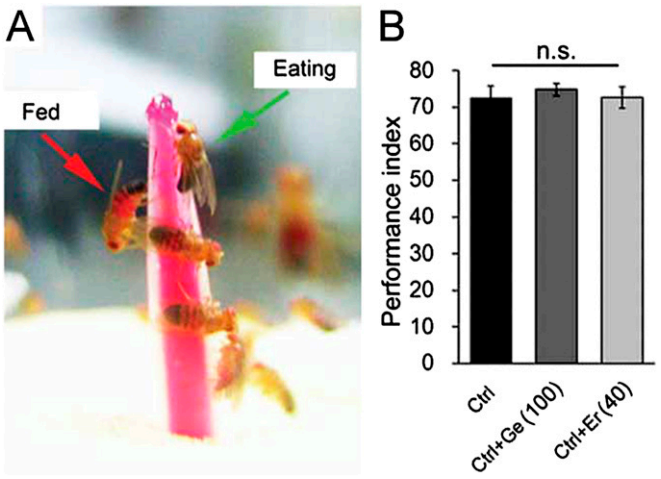


Fig. S1. Toxicity of EGFR inhibitors on memory ability of fruit flies. (*A*) A red food colorant was dissolved in drug solution for indicating the fed flies. Green arrow points to a fly eating the drug; red arrow points to a fly finished eating (red abdomen). (*B*) No significant memory toxicity of two EGFR inhibitors was found compared with control flies (*+/Y;UAS-Aβ42/+*) treated with sucrose. Concentrations (in μg/mL): 100 for gefitinib (Ge) and 40 for erlotinib (Er). $n = 8$. Data in this and following figures, unless otherwise indicated, are expressed as means ± SEM; n.s., not significant.

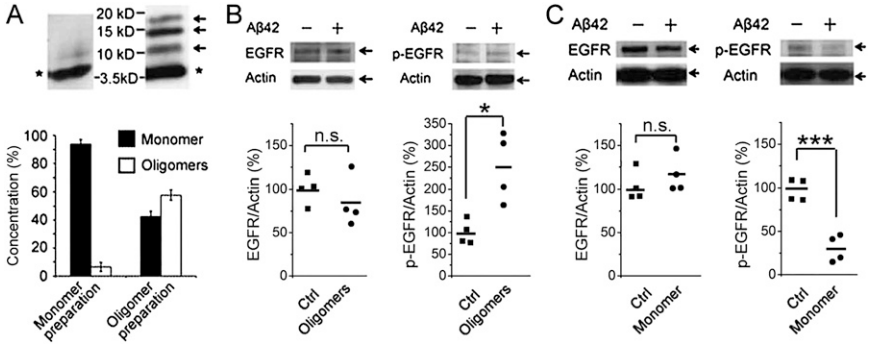


Fig. S2. Effects of monomeric and oligomeric Aβ42 peptides on endogenous EGFR phosphorylation. (*A*) Prepared Aβ42 peptides were separated by 10–20% Tricine gels and immunoblotted with 6E10 antibody (*Upper*). Star shows monomer and arrows indicate oligomers. Statistical results indicate the concentrations of monomers and oligomers in different preparations (*Lower*). (*B* and *C*) COS-7 cells were not transfected with human $EGFR^{wt}$ and Aβ42 plasmids. Endogenous p-EGFR and EGFR levels were detected. Incubation with 25 μg/mL monomeric or oligomeric Aβ42 for 15 min induced opposite effects on EGFR activation (*Right*), whereas total EGFR levels were not influenced (*Left*). $n = 3$–4. For Western blotting results in this and following figures, individual data points and means (horizontal lines) are shown.

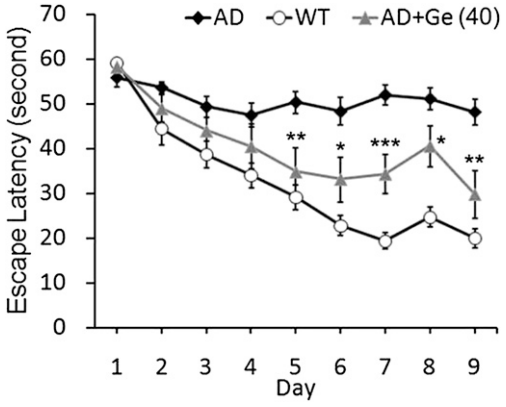


Fig. S3. Treatment with Ge rescues the memory loss of APP/PS1 mice. Oral administration of 40 $mg \cdot kg^{-1} \cdot d^{-1}$ Ge for 2 mo (beginning at 6 mo of age) showed a moderate rescue of memory compared with that of APP/PS1 mice treated with vehicle. $n = 11$–20. *t* test: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

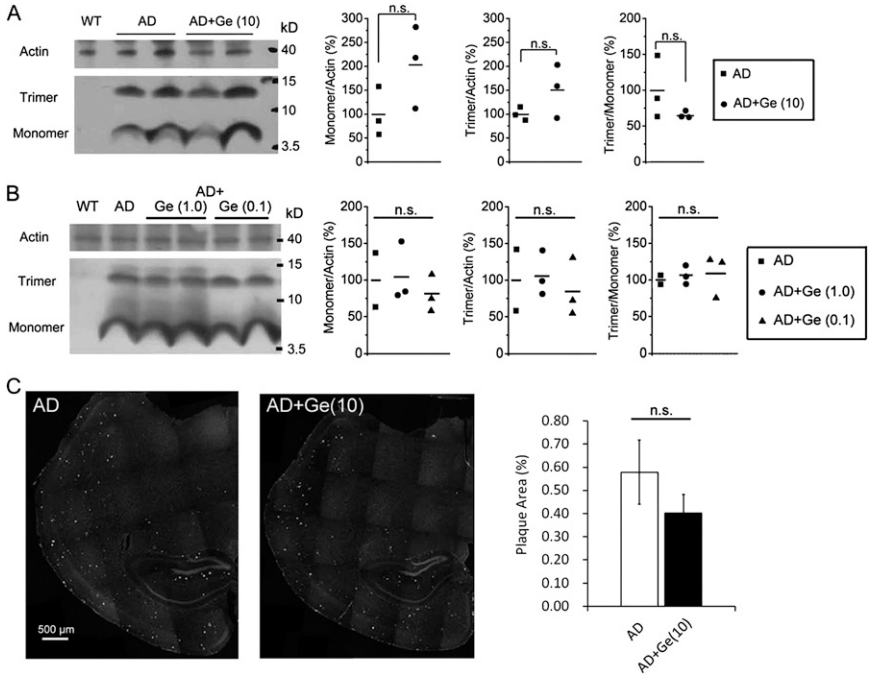
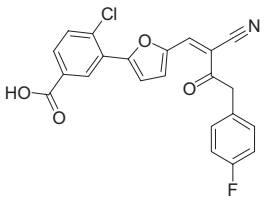
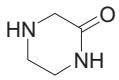
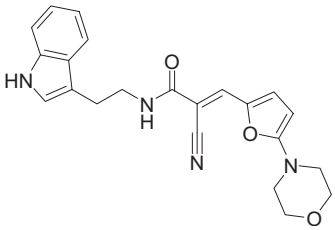


Fig. S4. Short-term Ge treatment does not influence the Aβ42 aggregation in APP/PS1 mice. Ge treatment paradigm was consistent with that used for behavior tests. Drug doses were indicated in milligrams per kilogram per day. Representative results of Western blotting for detecting the Aβ42 monomer and oligomer levels in hippocampus from 11-mo-old mice (*A*) or 15-mo-old mice (*B*). Ratios of monomer/actin, trimer/actin, and trimer/monomer were shown. n = 2–3. (*C*) Brain sections from 10-mo-old male mice treated with vehicle or Ge. Thioflavin-S staining was used for detecting the amyloid plaques. n = 4. n.s., not significant.

Table S1. Information on screened synthetic compounds

JKF ID/CAS	Molecular formula	M_r	TimTec ID	Structure	Chemical name
JKF-006 330861–11-5	$C_{21}H_{12}ClFN_2O_4$	410.79	ST026864		Benzoic acid, 4-chloro-3-[5-[2-cyano-3-[(4-fluorophenyl)amino]-3-oxo-1-propen-1-yl]-2-furanyl]-
JKF-011 5625–67-2	$C_4H_8N_2O$	100.12	ST025752		2-Piperazinone
JKF-027 335207–38-0	$C_{22}H_{22}N_4O_3$	390.44	ST052637		2-Propenamide, 2-cyano-*N*-[2-(1H-indol-3-yl) ethyl]-3-[5-(4-morpholinyl)-2-furanyl]-

Through behavioral screening and western blotting assay, 3 synthetic compounds were found from 2,000 kinase modulating candidates. JKF ID is labeled by JoeKai Biotech, LLC. CAS is registry number at Chemical Abstracts Service.