Supporting Information
for
Synthetic Flavonoids, Aminoisoflavones: Interaction and Reactivity with Metal-Free and Metal-Associated Amyloid-β Species

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Experimental Section

Materials and Methods

All reagents and solvents were commercially available and used as received unless otherwise stated. The compounds 2-amino-7-hydroxy-3-phenyl-4*H*-chromen-4-one (**1**), 2-amino-3-(3,4-dihydroxyphenyl)-4*H*-chromen-4-one (**2**), 2-amino-6-chloro-3-(3,4-dihydroxyphenyl)-4*H*-chromen-4-one (**3**), and 2-amino-3-(3,4-dihydroxyphenyl)-7-hydroxy-4*H*-chromen-4-one (**4**) were synthesized from the previously reported compounds (**1a**-**4a**) (Scheme 1).[1] The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra of these molecules were recorded on a Varian Inova 500 spectrometer (Palo Alto, CA, USA) in DMSO-d_6. Infrared (IR) spectra were measured on a Bruker Vector 22 spectrophotometer (Billerica, MA, USA). High-resolution mass spectra were obtained on an Agilent Technologies 6520 Accurate-Mass Q-TOF LC/MS (Santa Clara, CA, USA). Crude compounds (**1**-**4**) were purified by preparative reverse-phase HPLC (Waters Delta Prep 4000 system with Waters Prep LC 40 mm Assembly column C18 (30 cm x 4 cm, 15 μm particle, Milford, MA, USA) and eluted at a flow rate of 20 mL/min with mobile phase solvent A (10% CH_3CN + 0.1% trifluoroacetic acid (TFA) in H_2O, v/v), and a linear gradient from 0 to 50% B (60%, CH_3CN + 0.1% TFA in H_2O, v/v) in 25 min. Analytical HPLC analyses were performed with a Beckman System Gold (Beckman Ultrasphere ODS column, 250 mm x 4.6 mm, 5 μm particle, Brea, CA, USA). Analytical determinations of the products were carried out by HPLC in solvents A and B programmed at flow rate of 1 mL/min with linear gradients from 0 to 100% B in 25 min.

$A\beta_{40}$ and $A\beta_{42}$ were purchased from AnaSpec (Fremont, CA, USA) ($A\beta_{42}$ = DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA). An Agilent 8453 UV-Visible (UV-Vis) spectrophotometer (Santa Clara, CA, USA) was used to measure the optical spectra. Isothermal calorimetric titrations were performed using a VP isothermal titration calorimeter (MicroCal, Northampton, MA, USA). Transmission electron microscopy (TEM) images were recorded on a Philips CM-100 transmission electron microscope in the Microscopy and Imaging Laboratory at the University of Michigan (Ann Arbor, MI, USA). A Molecular Devices SpectraMax M5 microplate reader (Sunnyvale, CA, USA) was employed for the measurement of absorbance for Trolox equivalence antioxidant capacity (TEAC) and 3-(4,5-dimethylthiazol-2-yl)-2,5-

diphenyltetrazolium bromide (MTT) assays.

General Procedure for the Preparation of Hydroxylated 2-Amino-3-aryl-4*H*-chromen-4-ones (1-4). To a solution of the corresponding methoxylated 2-amino-3-aryl-4*H*-chromen-4-one precursor (**1a**-**4a**, 1 mmol)[1b] in acetic acid (2.5 mL) and acetic anhydride (2.5 mL), 57% v/v hydroiodic acid (5 mL) was added dropwise (Scheme 1). The mixture was refluxed for 6 h. After solvent evaporation in vacuo, water (10 mL) was added to the residue; the solid obtained was filtered and washed with water (2 x 5 mL) (76-86% yield). Each compound was purified by preparative reverse-phase HPLC as described above.

2-Amino-7-hydroxy-3-phenyl-4H-chromen-4-one (**1**). Yield: 192 mg, 0.76 mmol, 76%. EtOAc:Hx = 1:1; R_f = 0.56 (TLC). ^{1}H NMR (500 MHz, DMSO-d_6)/δ (ppm): 6.84 (s, 1H, Ar), 6.95 (d, J = 8.5 Hz, 1H, Ar), 7.44 (m, 5H, Ar and NH_2), 7.54 (m, 2H, Ar), 7.94 (d, J = 8.5 Hz, 1H, Ar), 10.58 (brs, 1H, OH). ^{13}C NMR (125 MHz, DMSO-d_6)/δ (ppm): 170.6, 164.1, 162.4, 154.4, 132.1, 131.7, 129.2, 128.0, 127.0, 114.5, 113.1, 102.1, 99.2. HRMS (ESI+): Calcd for $C_{15}H_{11}NO_3$ $[M+H]^+$, 254.0817; found, 254.0863. IR (Nujol)/ν (cm^{-1}): 3465, 3319, 1628, 1603.

2-Amino-3-(3,4-dihydroxyphenyl)-4H-chromen-4-one (**2**). Yield: 221 mg, 0.82 mmol, 82%. EtOAc:Hx = 1:1; R_f = 0.51 (TLC). ^{1}H NMR (500 MHz, DMSO-d_6)/δ (ppm): 6.67 (m, 1H, Ar), 6.81 (s, 1H, Ar), 6.90 (m, 1H, Ar), 7.04 (s, 2H, NH_2), 7.48 (m, 2H, Ar), 7.73 (m, 1H, Ar), 7.94 (d, J = 8.5 Hz, 2H, Ar), 8.88 (s, 1H, OH), 8.94 (s, 1H, OH). ^{13}C NMR (125 MHz, DMSO-d_6)/δ (ppm): 173.1, 162.8, 153.0, 145.6, 114.8, 132.4, 125.7, 124.8, 124.4, 123.5, 122.4, 119.1, 116.8, 116.3, 99.7. HRMS (ESI+): Calcd for $C_{15}H_{11}NO_4$ $[M+H]^+$, 270.0766; found, 270.0765. IR (Nujol)/ν (cm^{-1}): 3453, 3165, 1642, 1601.

2-Amino-3-(3,4-dihydroxyphenyl)-6-chloro-4H-chromen-4-one (**3**). Yield: 261 mg, 0.86 mmol, 86%. EtOAc:Hx = 1:1; R_f = 0.48 (TLC). ^{1}H NMR (500 MHz, DMSO-d_6)/δ (ppm): 6.67 (s, 1H, Ar), 6.81 (s, 1H, Ar), 6.88 (m, 1H, Ar), 7.22 (s, 2H, NH_2), 7.55 (m, 1H, Ar), 7.76 (m, 1H, Ar), 7.98 (s, 1H, Ar), 8.91 (s, 1H, OH), 8.95 (s, 1H, OH). ^{13}C NMR (125 MHz, DMSO-d_6)/δ (ppm): 168.9, 164.7, 151.1, 145.9, 145.6, 132.9, 129.7, 124.4, 122.7, 122.4, 122.0, 119.3, 118.8, 116.6, 101.3. HRMS (ESI+): Calcd. for $C_{15}H_{10}ClNO_4$

$[M+H]^+$, 304.0377; found, 304.0368. IR (Nujol)/ν (cm^{-1}): 3420, 3311, 1644, 1598.

2-Amino-3-(3,4-dihydroxyphenyl)-7-hydroxy-4H-chromen-4-one (**4**). Yield: 228 mg, 0.80 mmol, 80%. EtOAc:Hx = 1:1; R_f = 0.53 (TLC). ^{1}H NMR (500 MHz, DMSO-d_6)/δ (ppm): 6.66 (m, 1H, Ar), 6.82 (m, 1H, Ar), 6.89 (m, 1H, Ar), 6.95 (m, 1H, Ar), 7.33 (s, 2H, NH_2), 7.76 (m, 1H, Ar), 7.93 (s, 1H, Ar), 8.87 (s, 1H, OH), 8.90 (s, 1H, OH), 10.67 (brs, 1H, OH). ^{13}C NMR (125 MHz, DMSO-d_6)/δ (ppm): 170.5, 164.4, 162.1, 154.2, 145.8, 145.4, 126.9, 122.6, 119.0, 116.5, 114.4, 113.2, 102.0, 99.4. HRMS (ESI+): Calcd. for $C_{15}H_{11}NO_5$ $[M+H]^+$ 286.0715; found, 286.0706. IR (Nujol)/ν (cm^{-1}): 3419, 3327, 1631, 1609.

Amyloid-β (Aβ) Experiments. Aβ experiments were performed according to the previously published methods.[2-5] Prior to experiments, $A\beta_{40}$ or $A\beta_{42}$ was dissolved in ammonium hydroxide (NH_4OH, 1% v/v, aq), aliquoted, lyophilized overnight, and stored at −80 °C. For experiments described herein, a stock solution of Aβ was prepared by dissolving the peptide in 1% NH_4OH (10 μL) and diluting with ddH_2O. The concentration of Aβ in solution was determined by measuring the absorbance of the solution at 280 nm (ε = 1450 $M^{-1}cm^{-1}$ for $A\beta_{40}$ and ε = 1490 $M^{-1}cm^{-1}$ for $A\beta_{42}$). The peptide stock solution was diluted to a final concentration of 10 μM in a Chelex-treated buffered solution containing HEPES (20 mM, pH 7.4) and NaCl (150 mM). For the inhibition studies,[2-5] an aminoisoflavone or catechol (20 μM, 1% v/v DMSO) was added to the sample of Aβ (10 μM) in the absence and presence of $CuCl_2$ or $ZnCl_2$ (10 μM) followed by incubation at 37 °C with constant agitation for 24 h. For the disaggregation studies,[2-5] Aβ (10 μM) with and without metal ions (10 μM) was incubated for 24 h at 37 °C with constant agitation before adding the aminoisoflavone (20 μM) to the sample. The resulting samples were incubated at 37 °C with constant agitation for 24 h.

Gel Electrophoresis and Western Blot. Samples from the inhibition and disaggregation experiments were analyzed by gel electrophoresis and visualized by Western blot using an anti-Aβ antibody (6E10).[2-5] Each sample was separated on a 10-20% Tris-tricine gel (Invitrogen, Grand Island, NY, USA) and transferred onto

nitrocellulose. The nitrocellulose was blocked with bovine serum albumin (BSA, 3% w/v, Sigma-Aldrich) in Tris-buffered saline (TBS) containing 0.1% Tween-20 (TBS-T) overnight. The membranes were incubated with 6E10 (1:2000, Covance, Princeton, NJ, USA) in a solution of 2% BSA (w/v in TBS-T) for 4 h at room temperature. After washing, the horseradish peroxidase-conjugated goat anti-mouse secondary antibody (1:5000, Cayman Chemical, Ann Arbor, MI, USA) in 2% BSA was added for 1 h at room temperature. The ThermoScientific SuperSignal West Pico Chemiluminescent Substrate (Rockford, IL, USA) was used to visualize the protein bands.

Transmission Electron Microscopy (TEM). TEM samples were prepared according to the previously reported method.[2-5] Glow-discharged grids (Formar/Carbon 300-mesh, Electron Microscopy Sciences, Hatfield, PA, USA) were treated with the Aβ samples from both inhibition and disaggregation experiments (5 μL) for 2 min at room temperature. Excess sample was removed using filter paper followed by washing with ddH_2O twice. Each grid was incubated with uranyl acetate (1% w/v ddH_2O, 5 μL, 1 min) and upon removal of excess was dried for 15 min at room temperature. Images from each sample were taken by a Philips CM-100 transmission electron microscope (80 kV, 25,000x magnification).

Determination of Solution Speciation from Acidity Constants for Aminoisoflavones and Stability Constants for Cu(II)–4 Complexes. The acidity constants (pK_a) for **1**-**4** were determined by spectrophotometric variable-pH titrations with modifications to the previously described methods.[2,3,6,7] A solution (10 mM NaOH, 100 mM NaCl, pH 12) containing **1** (30 μM) was titrated with small aliquots of HCl to acquire at least 30 spectra in the range from pH 1.5-12. Solutions of **2** (20 μM), **3** (15 μM), and **4** (30 μM) in 200 mM HCl, 100 mM NaCl, pH 0.6 were titrated with small aliquots of NaOH and at least 30 spectra were acquired from pH 0.6-12.4. To measure the stability constants ($\log\beta$) for the Cu(II)–**4** complexes, **4** (30 μM) and $CuCl_2$ in a ratio of 2:1 were added to a solution (10 mM NaOH, 100 mM NaOH) at pH 8. Following overnight incubation, the solution was titrated with small aliquots of HCl in the range from pH 4-8. The pK_a and $\log\beta$ values were calculated using the program HypSpec

(Protonic Software, UK).[8] Speciation diagrams were generated from the calculated values by the program HySS2009 (Protonic Software, UK).[9]

Metal Binding Studies. The ability of the aminoisoflavones to bind to Cu(II) or Zn(II) was investigated by UV-Vis in the absence and presence of $A\beta_{40}$.[2,3,5,6] A solution of the aminoisoflavone (20 μM, 1% v/v DMSO) in 20 mM HEPES [4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid], pH 7.4, 150 mM NaCl was incubated with 0.5-5 equiv $CuCl_2$ for up to 2 h. Additionally, the interaction of **4** with $CuCl_2$ in the presence of $A\beta_{40}$ was also studied by measuring changes in the optical spectra. In the same buffered solution, Aβ (10 μM) was pre-incubated with $CuCl_2$ (5 or 10 μM) for 5 min, followed by the addition of 0.5, 1, or 2 equiv of **4** (20 μM) was pre-incubated with $CuCl_2$ for 5 min, followed by addition of Aβ, resulting in final [Aβ]:[$CuCl_2$]:[**4**] ratios of 1:0.5:2, 1:1:2, 1:2:2, or 1:4:2. The solution containing Cu(II), Aβ, and **4** were incubated at room temperature for up to 4 h. To determine Zn(II) binding properties, a solution of 100 μM aminoisoflavone in 20 mM HEPES, pH 7.4, 150 mM NaCl was incubated with $ZnCl_2$ (10 μM) for 12-24 h and monitored by UV-Vis.

Isothermal Titration Calorimetry (ITC) for Measurement of Thermodynamic Parameters for the Interaction of 1, 2, or 4 with Aβ. Experiments were conducted with solutions of **1**, **2**, or **4** (200 μM, 10% v/v DMSO) and $A\beta_{40}$ (20 μM) in 20 mM HEPES buffer (pH 7.4, 150 mM NaCl).[3c] All solutions were degassed for 10 min before each titration. The ligand solution (10 μL) was titrated into the $A\beta_{40}$ solution (1.43 mL) at 25 °C over 1 s with 25 repetitions at a constant interval of 200 s *via* a 310 rpm rotating stirrer syringe using a motor driven 250 μL syringe. In a control experiment, the identical titrant solution was injected into the same buffer used with $A\beta_{40}$ to measure the heat of dilution. A reasonable heat of binding value was measured by subtracting the heat of dilution value from the experimental results. Titration data were analyzed with the MicroCal Origin (v. 7.0) software. The K_A (binding constant) of each ligand and ΔH (binding heat exchange) upon binding interaction between each ligand and $A\beta_{40}$ were determined from a proper fitting model. The binding curves were best fit to a sequential

binding site model with one identical site. ΔG and $-T\Delta S$ were calculated *via* Gibb's free energy relationship ($\Delta G = \Delta H - T\Delta S$; $\Delta G = -RT\,ln(K_A)$).

Docking Studies of Aminoisoflavones against Aβ$_{40}$ Monomer and fibrils. Flexible ligand docking studies of aminoisoflavones were conducted using AutoDock Vina[10] against the Aβ$_{40}$ monomer and fiber structures from the previously determined aqueous solution NMR (PDB 2LFM) and solid-state NMR models (PDB 2LMO and 2LMN).[11] Representative conformations were selected for docking from those within the PDB file (1, 3, 8, 10, 12, 13, 16, 17, 20 for 2LFM, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 for 2LMN and 2LMO). The MMFF94 energy minimization function in ChemBio3D Ultra 11.0 was used to optimize the structure of aminoisoflavones for the docking studies. Individual structures of Aβ and aminoisoflavones were prepared using AutoDock Tools.[12] The search space was constrained to the dimensions of the peptide. The exhaustiveness for the docking runs was set at 1024. Docked models of the aminoisoflavones were visualized with Aβ$_{40}$ using Pymol.

NMR Sample Preparation. Prior to the preparation of the Aβ$_{40}$ sample for NMR, the peptide was dissolved in ammonium hydroxide (NH_4OH, 2% v/v aq) at 1 mg/ml to remove any preformed aggregates.[13] Volumes equivalent to 0.1 mg (100 µL) were then aliquoted and lyophilized. The lyophilized peptide was initially dissolved in 1 mM NaOH and then allowed to solubilize at 4 °C until the solution became clear (*ca.* 1 h). At this point the concentrated buffer was added to the solution and the pH was adjusted to the final value to generate freshly dissolved, low MW Aβ$_{40}$ samples. The final sample contained 20 mM deuterated Tris (pD 7.0 or a calculated pH of 7.4) with 50 mM NaCl and 10% v/v D_2O. Immediately prior to the experiment, compounds **1** and **4** were dissolved in deuterated DMSO at a concentration of 19.5 mM. The 1D ^{1}H NMR and 2D ^{15}N-^{1}H SOFAST-HMQC titrations were performed directly using the freshly dissolved, low MW Aβ$_{40}$ samples. For STD NMR experiments on ligand binding to the amyloid fibrils, samples were prepared as above except that all of the solutions were prepared in D_2O and the sample was allowed to incubate for 48 h at 37 °C in a shaker (model MS3 from IKA) at 1000 rpm to generate amyloid fibrils.

NMR Measurements. A 900 MHz Bruker Avance NMR spectrometer equipped with a triple-resonance z-gradient cryogenic probe (SOFAST-HMQC experiments) or a 500 MHz without cryoprobe was used to collect the spectra. SOFAST-HMQC spectra of 80 μM $A\beta_{40}$ in solution (20 mM Tris, pH 7.4, 50 mM NaCl) were recorded at 4 °C with 128 t_1 experiments, 16 scans, and a 100 ms recycle delay.[14] The 2D ^{15}N-^{1}H SOFAST-HMQC data were processed using TOPSPIN 2.1 (from Bruker) and Sparky.[15] Resonances were assigned based on previous assignments under similar conditions.[16] For the STD experiments, both **1** and **4** were kept at a molar ratio of 10:1 relative to the fibril (200 μM to 20 μM).[13] For the STD experiment, a 3 s train of Gaussian-shaped 50 ms pulses was employed to selectively saturate the amyloid fibril.[17] The saturation pulses were centered on −2 and 100 ppm for on and off resonance respectively. The magnitude of the STD effect for each peak was calculated by dividing the normalized signal intensity in the on resonance spectrum by its corresponding intensity in the off resonance reference spectrum.

Trolox Equivalence Antioxidant Capacity (TEAC) Assay. The TEAC assay was used to determine the antioxidant ability based on the extent of decolorization of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) cation radical relative to that of the vitamin E analogue, Trolox.[18a] The assay was performed according to the previously reported method with slight modifications.[3b,3d,18] First, $ABTS^{\bullet+}$ was generated by dissolving ABTS (19 mg, 7.0 mM) with potassium persulfate (3.3 mg, 2.5 mM) in 5 mL water and incubating for 16 h in the dark at room temperature. The resulting solution of $ABTS^{\bullet+}$ was diluted with EtOH to an absorbance of *ca.* 0.7 at 734 nm. The assay was conducted in a clear 96 well plate to which 200 μL diluted solution of $ABTS^{\bullet+}$ was added and incubated at 30 °C for 5 min in the plate reader. Each ligand was added from a stock solution prepared in DMSO (1% v/v) or in EtOH (for Trolox) and was incubated with the solution of $ABTS^{\bullet+}$ at 30 °C for different time periods (1, 3, 6, 10, 15, and 30 min). The percent inhibition was calculated according to the measured absorbance at 734 nm (% Inhibition = $100 \times (A_0 - A)/A_0$) and was plotted as a function of ligand concentration. The TEAC value of compounds for each time point was

calculated as a ratio of the slope of the standard curve of the compound to that of Trolox. The measurements were carried out in triplicate.

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List of Tables and Figures

4a. Experimental conditions: [$A\beta_{40}$ or $A\beta_{42}$] = 10 μM; [$CuCl_2$ or $ZnCl_2$] = 10 μM; [compound] = 20 μM; 1% v/v DMSO; 20 mM HEPES, pH 7.4, 150 mM NaCl; 24 h; 37 °C; agitation.

Fig. S4. Cu(II) binding studies of the aminoisoflavones. Treatment of (a) **1**, (b) **2**, (c) **3**, or (d) **4** (black) with 0.5 equiv of $CuCl_2$ (light gray, incubated for 1-2 h), 1 equiv of $CuCl_2$ (medium gray, incubated for 10-30 min), or 5 equiv of $CuCl_2$ (dark gray, incubated for 5-30 min). Experimental conditions: [aminoisoflavone] = 20 μM; 1% v/v DMSO; 20 mM HEPES, pH 7.4, 150 mM NaCl; room temperature.

Fig. S5. Zn(II) binding properties of aminoisoflavones [(a) **1**, (b) **2**, (c) **3**, or (d) **4** (black) with $ZnCl_2$ (dark green, 2 h incubation; gray, 12 h incubation; light green, 24 h incubation)]. Experimental conditions: [aminoisoflavone] = 100 μM; [$ZnCl_2$] = 10 μM; 1% v/v DMSO; 20 mM HEPES, pH 7.4, 150 mM NaCl; room temperature.

Fig. S6. Interaction of **4** with Cu(II) in the presence of $A\beta_{40}$. (a-d) A solution containing $A\beta_{40}$ (green) was treated with $CuCl_2$ (red) for 5 min followed by addition of **4** (blue). Note that (d) immediate precipitation was observed upon treatment of Aβ with excess $CuCl_2$; thus, **4** was not added. (e-h) A solution containing **4** was added with $CuCl_2$ (red) for 5 min followed by treatment with $A\beta_{40}$ (green). All solutions containing Aβ, $CuCl_2$ and **4** were incubated at 20, 40, 60, 120, 180 (black), and 240 min (purple). The ratios of [$A\beta_{40}$]:[$CuCl_2$]:[**4**] were (a and e) 1:0.5:2, (b and f) 1:1:2, (c and g) 1:2:2, and (d and h) 1:4:2. Experimental conditions: [**4**] = 20 μM; 1% v/v DMSO; 20 mM HEPES, pH 7.4, 150 mM NaCl; room temperature.

Fig. S7. Docking studies of **1**, **2**, and **4** with metal-free $A\beta_{40}$ (PDB ID 2LFM). Top: Cartoon (left) and surface (right) representations of the peptide are depicted with **1** (pink), **2** (light blue), and **4** (yellow). Bottom: Summary of predicted binding energies for the ligands docked with $A\beta_{40}$.

Fig. S8. ^{1}H spectra of low MW $A\beta_{40}$ titrated with **1** and **4**. A solution of freshly dissolved $A\beta_{40}$ (80 μM) in 20 mM deuterated Tris (pH 7.4) with 50 mM NaCl and 10% D_2O was titrated with either **1** or **4** to the indicated molar ratios at 4 °C. New peaks at 7.64, 6.77, 6.83, and 6.89 ppm with 5 equiv of **1** indicate the presence of a new ligand species.

Fig. S9. Docking studies of **4** with positively staggered metal-free $A\beta_{40}$ fibrils (PDB ID 2LMN). Top: Cartoon representations of the peptide are depicted with **4** (yellow). Bottom: Summary of predicted binding energies for the ligands docked with $A\beta_{40}$ fibers.

Fig. S10. Docking studies of **4** with negatively staggered metal-free $A\beta_{40}$ fibrils (PDB ID 2LMO). Top: Cartoon representations of the peptide are depicted with **4** (yellow). Bottom: Summary of predicted binding energies for the ligands docked with $A\beta_{40}$ fibers.

Fig. S11. Radical scavenging ability of the aminoisoflavones (**1**-**4**) determined by the Trolox equivalence antioxidant capacity (TEAC) assay. TEAC values were calculated relative to Trolox, a vitamin E analogue, after 15 min incubation and represent the mean of four independent experiments. The experimental details are described in the main text.

Table S1. Thermodynamic values for the interaction of **1**, **2**, and **4** with $A\beta_{40}$ determined by isothermal titration calorimetry (ITC).

	1	**2**	**4**
K_A **(M^{-1})**	$(6.8 \pm 3.0) \times 10^4$	$(7.9 \pm 2.9) \times 10^3$	$(3.2 \pm 0.7) \times 10^3$
ΔG **(kJ/mol)**	−27.3 ± 1.1	−22.2 ± 0.9	−19.9 ± 0.6
ΔH **(kJ/mol)**	11.3 ± 1.2	−59.2 ± 22.6	−116.3 ± 19.6
$-T\Delta S$ **(kJ/mol)**	−38.7 ± 1.6	36.9 ± 22.6	96.4 ± 19.6

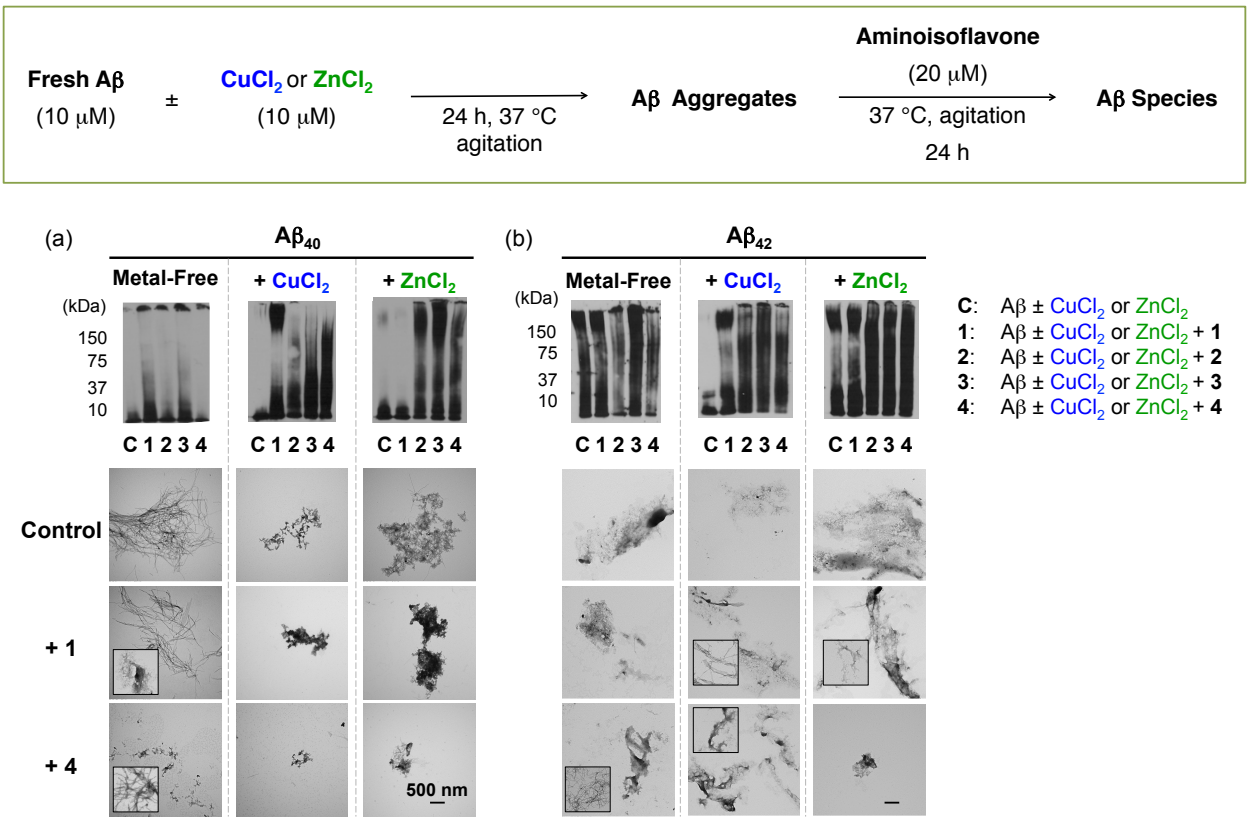


Fig. S1. Disassembly of metal-free and metal-associated $A\beta_{40}$ and $A\beta_{42}$ aggregates by the aminoisoflavones. Top: Scheme of the disaggregation experiment. Middle: Analysis of the samples containing (a) $A\beta_{40}$ and (b) $A\beta_{42}$ by gel electrophoresis followed by Western blot (6E10). Lanes: (C) Aβ ± $CuCl_2$ or $ZnCl_2$, (1) C + **1**, (2) C + **2**, (3) C + **3**, (4) C + **4**. Bottom: TEM images of samples with **1** or **4** for (a) $A\beta_{40}$ and (b) $A\beta_{42}$. Experimental conditions: [$A\beta_{40}$ or $A\beta_{42}$] = 10 μM; [$CuCl_2$ or $ZnCl_2$] = 10 μM; [aminoisoflavone] = 20 μM; 1% v/v DMSO; 20 mM HEPES, pH 7.4, 150 mM NaCl; 24 h; 37 °C; agitation.

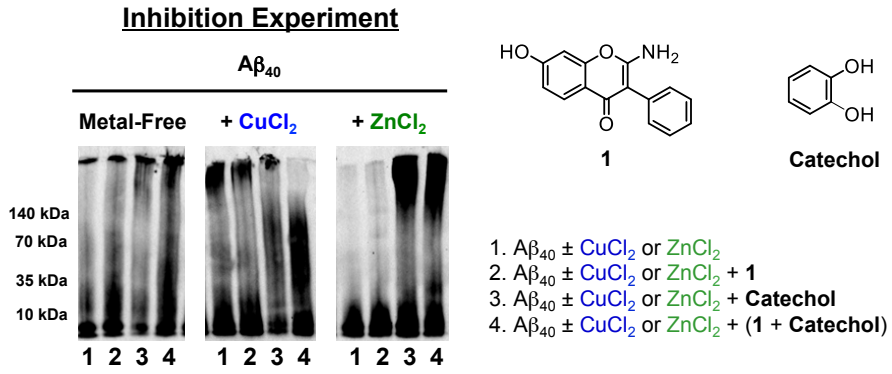


Fig. S2. Inhibition experiments employing **1**, catechol, and a mixture of **1** and catechol (**1** + catechol). Top: Structures of **1** (2-amino-7-hydroxy-3-phenyl-4*H*-chromen-4-one) and catechol (2-hydroxyphenol). Bottom: Analysis of inhibition samples with $A\beta_{40}$ by gel electrophoresis followed by Western blot (6E10). Lanes: (1) Aβ ± $CuCl_2$ or $ZnCl_2$, (2) Aβ ± $CuCl_2$ or $ZnCl_2$ + **1**, (3) Aβ ± $CuCl_2$ or $ZnCl_2$ + catechol, (4) Aβ ± $CuCl_2$ or $ZnCl_2$ + (**1** + catechol). Experimental conditions: [$A\beta_{40}$] = 10 μM; [$CuCl_2$ or $ZnCl_2$] = 10 μM; [compound] = 20 μM; 1% v/v DMSO; 20 mM HEPES, pH 7.4, 150 mM NaCl; 24 h; 37 °C; agitation.

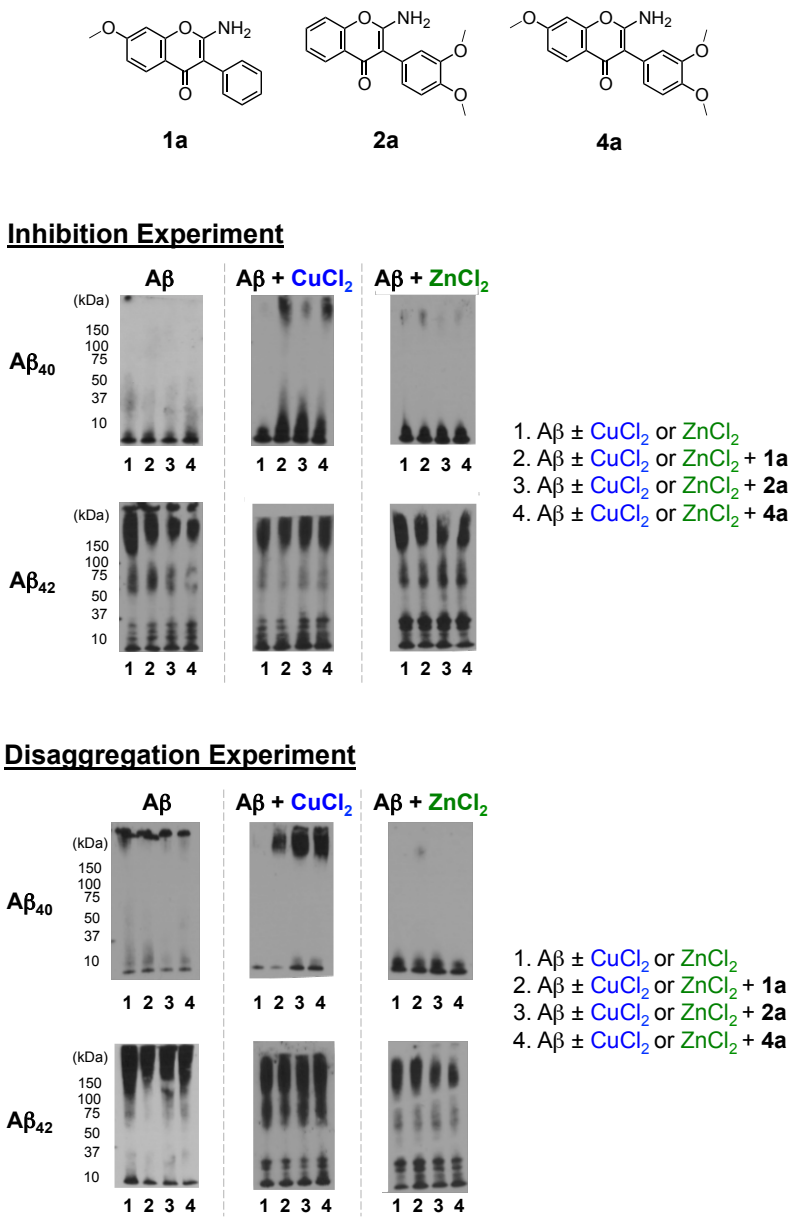


Fig. S3. Inhibition and disaggregation experiments employing the methoxylated aminoisoflavone precursors (**1a**, **2a**, and **4a**). Top: Structures of **1a** (2-amino-7-methoxy-3-phenyl-4*H*-chromen-4-one), **2a** (2-amino-3-(3,4-dimethoxyphenyl)-4*H*-chromen-4-one), and **4a** (2-amino-3-(3,4-dimethoxyphenyl)-7-methoxy-4*H*-chromen-4-one). Analysis of inhibition (middle) and disaggregation (bottom) samples with $A\beta_{40}$ and $A\beta_{42}$ by gel electrophoresis followed by Western blot (6E10). Lanes: (1) Aβ ± $CuCl_2$ or $ZnCl_2$, (2) Aβ ± $CuCl_2$ or $ZnCl_2$ + **1a**, (3) Aβ ± $CuCl_2$ or $ZnCl_2$ + **2a**, (4) Aβ ± $CuCl_2$ or $ZnCl_2$ + **4a**. Experimental conditions: [$A\beta_{40}$ or $A\beta_{42}$] = 10 μM; [$CuCl_2$ or $ZnCl_2$] = 10 μM; [compound] = 20 μM; 1% v/v DMSO; 20 mM HEPES, pH 7.4, 150 mM NaCl; 24 h; 37 °C; agitation.

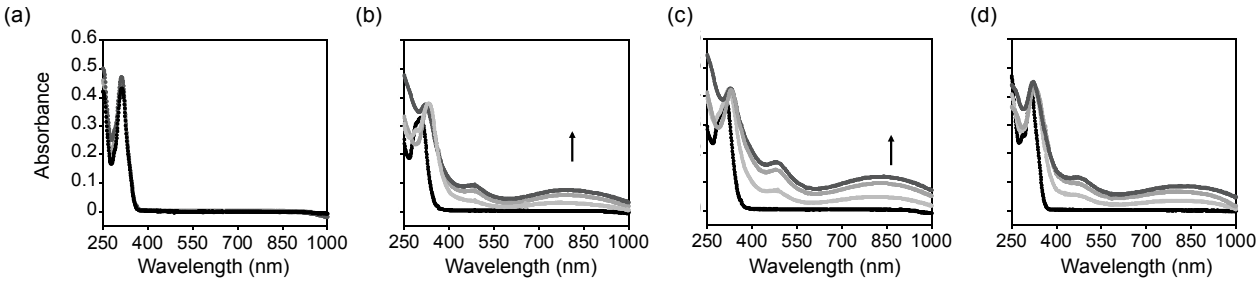


Fig. S4. Cu(II) binding studies of the aminoisoflavones. Treatment of (a) **1**, (b) **2**, (c) **3**, or (d) **4** (black) with 0.5 equiv of $CuCl_2$ (light gray, incubated for 1-2 h), 1 equiv of $CuCl_2$ (medium gray, incubated for 10-30 min), or 5 equiv of $CuCl_2$ (dark gray, incubated for 5-30 min). Experimental conditions: [aminoisoflavone] = 20 μM; 1% v/v DMSO; 20 mM HEPES, pH 7.4, 150 mM NaCl; room temperature.

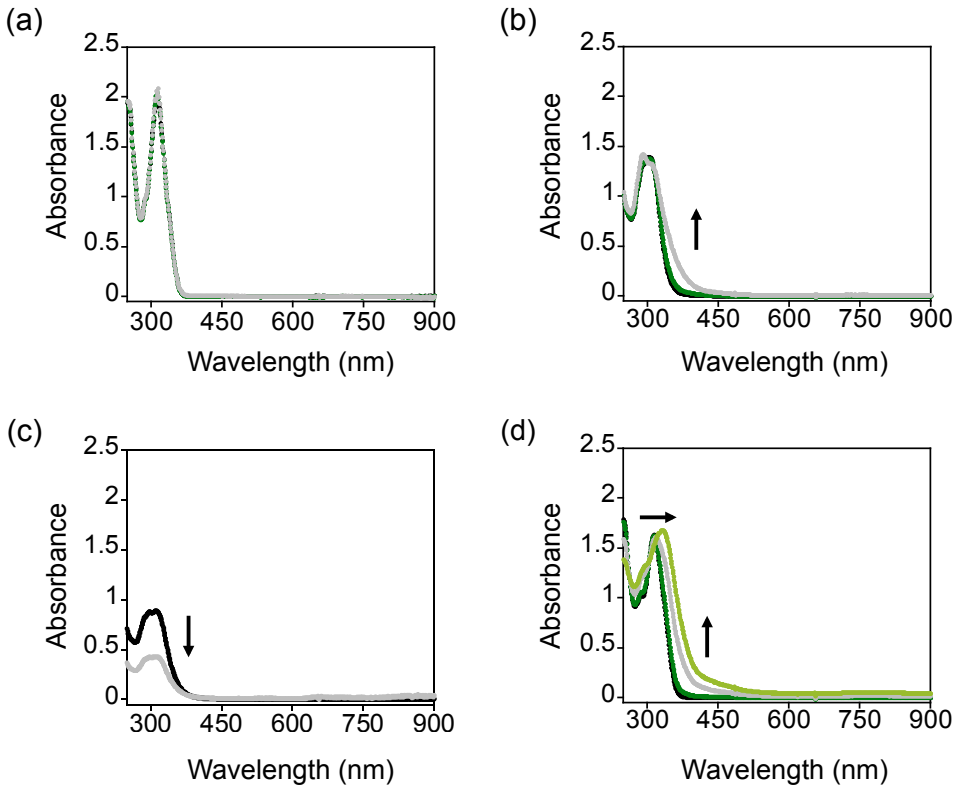


Fig. S5. Zn(II) binding properties of aminoisoflavones [(a) **1**, (b) **2**, (c) **3**, or (d) **4** (black) with $ZnCl_2$ (dark green, 2 h incubation; gray, 12 h incubation; light green, 24 h incubation)]. Experimental conditions: [aminoisoflavone] = 100 μM; [$ZnCl_2$] = 10 μM; 1% v/v DMSO; 20 mM HEPES, pH 7.4, 150 mM NaCl; room temperature.

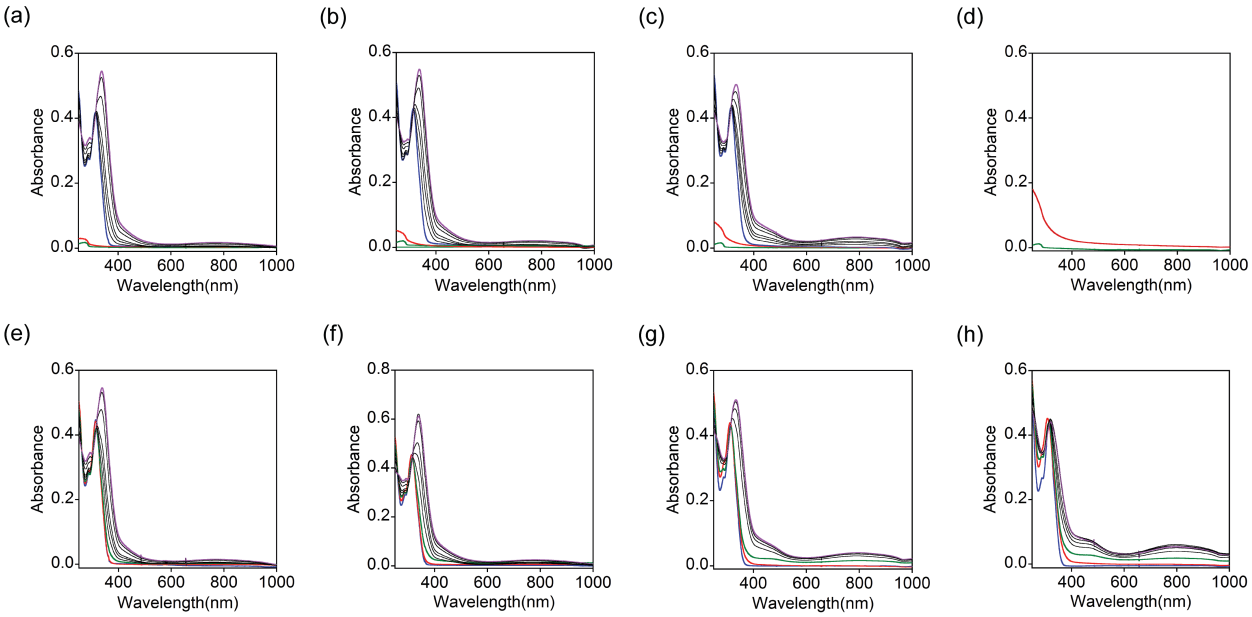
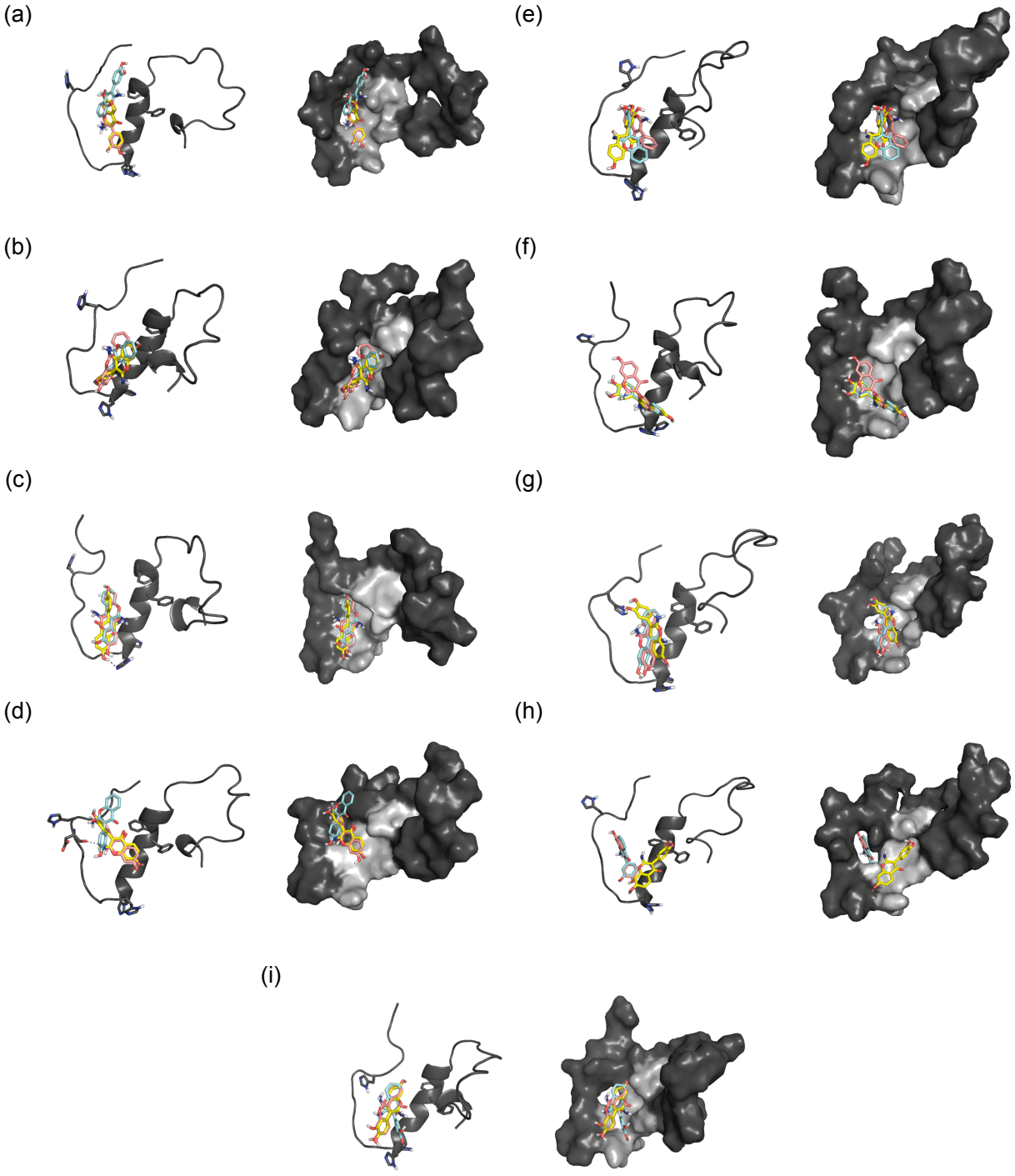


Fig. S6. Interaction of **4** with Cu(II) in the presence of $A\beta_{40}$. (a-d) A solution containing $A\beta_{40}$ (green) was treated with $CuCl_2$ (red) for 5 min followed by addition of **4** (blue). Note that (d) immediate precipitation was observed upon treatment of Aβ with excess $CuCl_2$; thus, **4** was not added. (e-h) A solution containing **4** was added with $CuCl_2$ (red) for 5 min followed by treatment with $A\beta_{40}$ (green). All solutions containing Aβ, $CuCl_2$ and **4** were incubated at 20, 40, 60, 120, 180 (black), and 240 min (purple). The ratios of [$A\beta_{40}$]:[$CuCl_2$]:[**4**] were (a and e) 1:0.5:2, (b and f) 1:1:2, (c and g) 1:2:2, and (d and h) 1:4:2. Experimental conditions: [**4**] = 20 μM; 1% v/v DMSO; 20 mM HEPES, pH 7.4, 150 mM NaCl; room temperature.


(a)
(e)
(b)
(f)
(c)
(g)
(d)
(h)
(i)

Conformation	1 (kcal/mol)	2 (kcal/mol)	4 (kcal/mol)
a	−6.4	−6.3	−6.4
b	−5.9	−5.8	−5.9
c	−6.2	−6.2	−6.3
d	−5.8	−5.9	−5.8
e	−5.8	−5.9	−6.0
f	−5.8	−6.0	−5.7
g	−5.7	−5.8	−5.7
h	−6.0	−5.9	−6.0
i	−6.2	−5.9	−6.0

Fig. S7. Docking studies of **1**, **2**, and **4** with metal-free Aβ_{40} (PDB ID 2LFM). Top: Cartoon (left) and surface (right) representations of the peptide are depicted with **1** (pink), **2** (light blue), and **4** (yellow). Bottom: Summary of predicted binding energies for the ligands docked with Aβ_{40}.

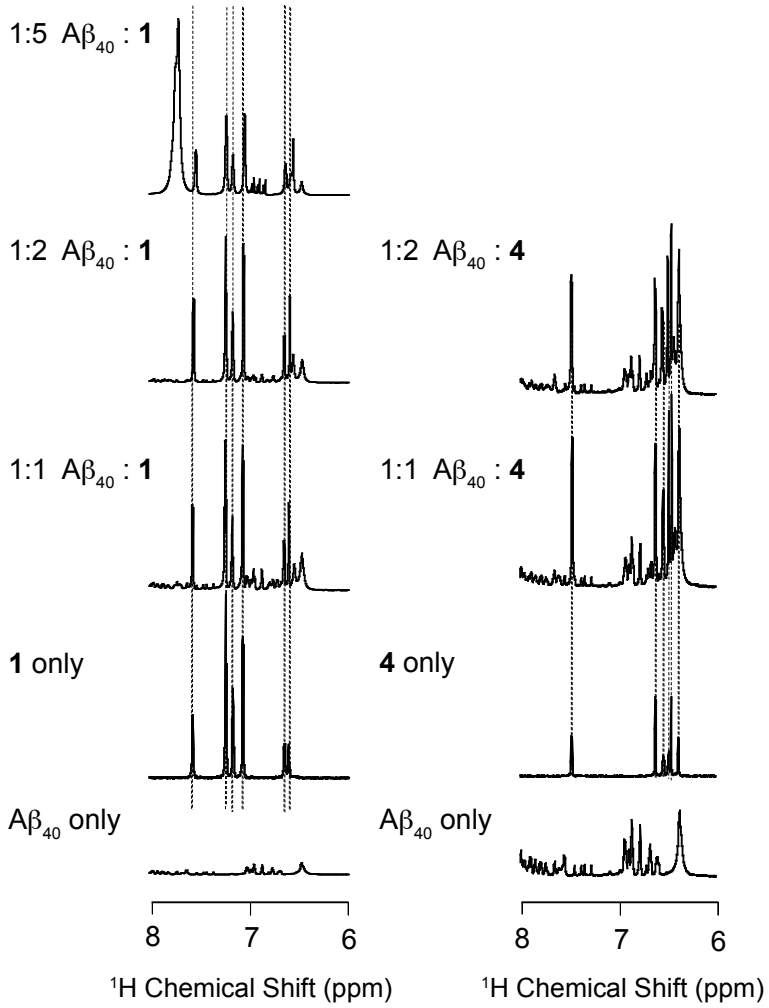
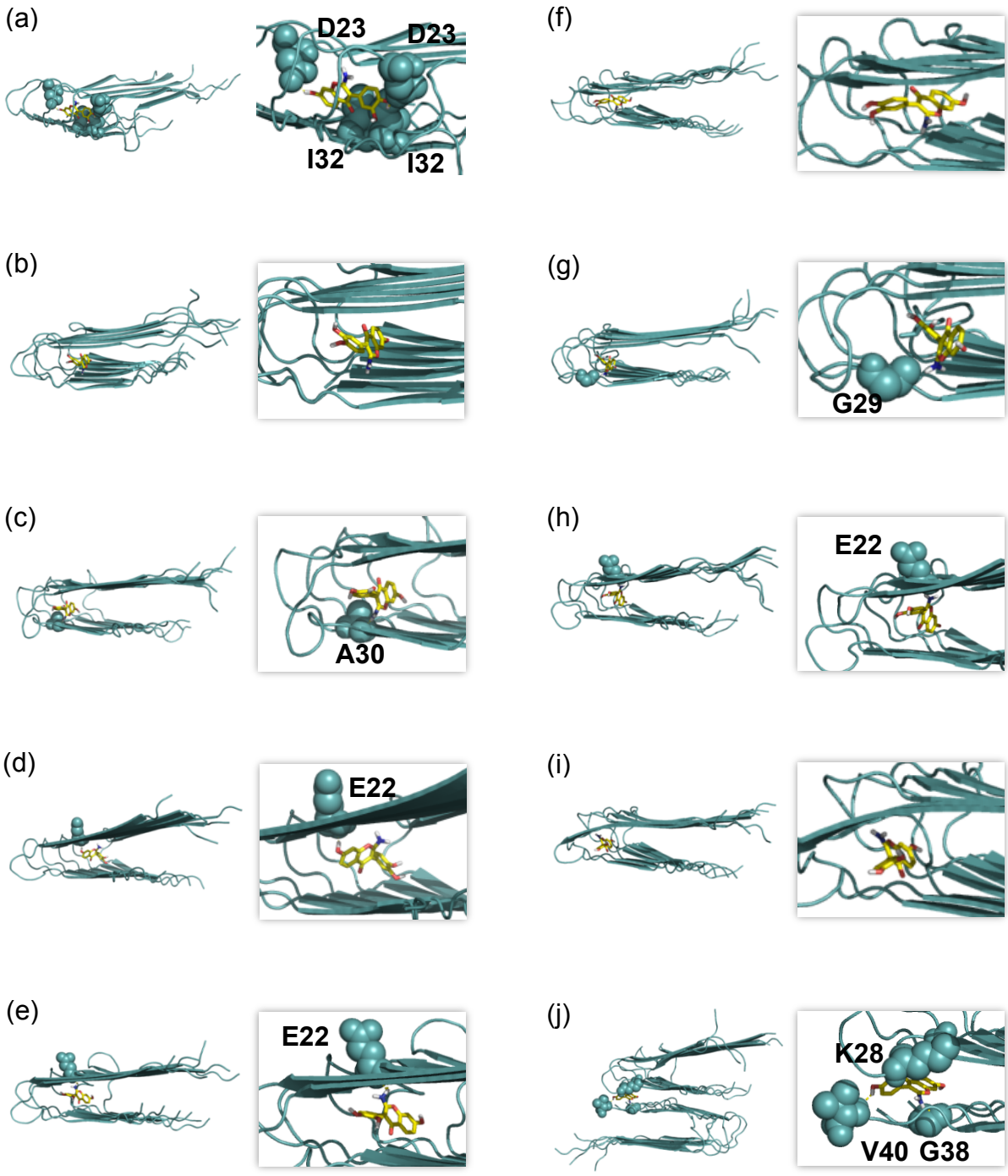
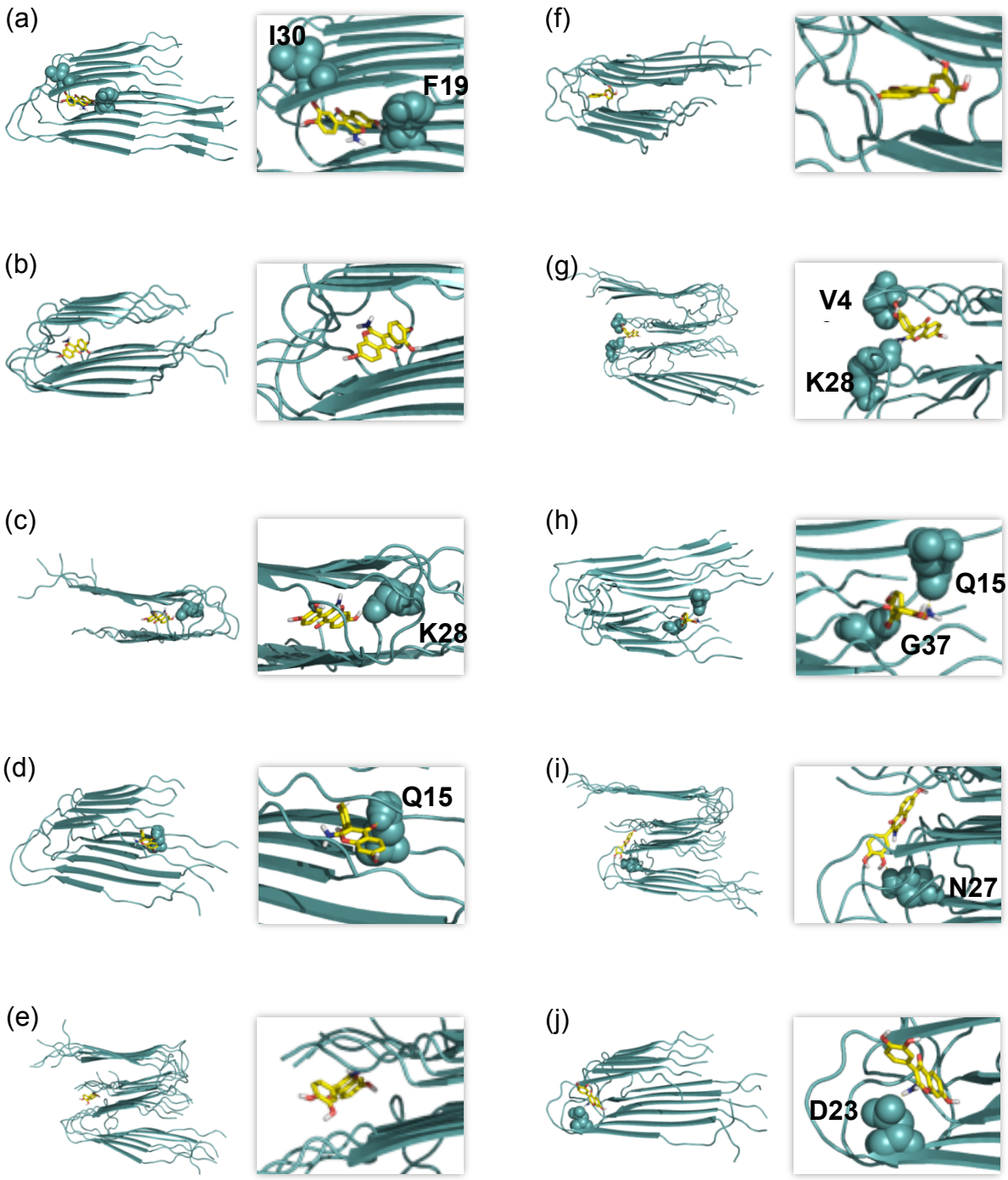


Fig. S8. ^{1}H spectra of low MW Aβ$_{40}$ titrated with **1** and **4**. A solution of freshly dissolved Aβ$_{40}$ (80 μM) in 20 mM deuterated Tris (pH 7.4) with 50 mM NaCl and 10% D_2O was titrated with either **1** or **4** to the indicated molar ratios at 4 °C. New peaks at 7.64, 6.77, 6.83, and 6.89 ppm with 5 equiv of **1** indicate the presence of a new ligand species.


(a)
D23
D23
I32
I32
(b)
(c)
A30
(d)
E22
(e)
E22
(f)
(g)
G29
(h)
E22
(i)
(j)
K28
V40 G38

Conformation	**4** (kcal/mol)
a	-10.4
b	-10.0
c	-9.3
d	-8.9
e	-8.7
f	-8.7
g	-8.6
h	-8.5
i	-8.4
j	-8.0

Fig. S9. Docking studies of **4** with positively staggered metal-free Aβ_{40} fibrils (PDB ID 2LMN). Top: Cartoon representations of the peptide are depicted with **4** (yellow). Bottom: Summary of predicted binding energies for the ligands docked with Aβ_{40} fibers.


(a)
I30
F19
(b)
(c)
K28
(d)
Q15
(e)
(f)
(g)
V4
K28
(h)
Q15
G37
(i)
N27
(j)
D23

Conformation	**4** (kcal/mol)
a	-10.2
b	-10.0
c	-9
d	-8.4
e	-8
f	-8
g	-7.8
h	-7.7
i	-7.5
j	-7.5

Fig. S10. Docking studies of **4** with negatively staggered metal-free $A\beta_{40}$ fibrils (PDB ID 2LMO). Top: Cartoon representations of the peptide are depicted with **4** (yellow). Bottom: Summary of predicted binding energies for the ligands docked with $A\beta_{40}$ fibers.

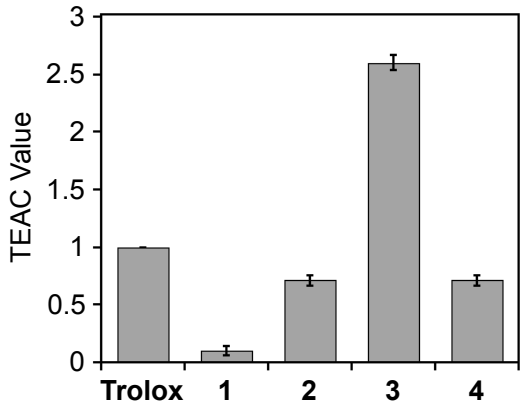


Fig. S11. Radical scavenging ability of the aminoisoflavones (**1**-**4**) determined by the Trolox equivalence antioxidant capacity (TEAC) assay. TEAC values were calculated relative to Trolox, a vitamin E analogue, after 15 min incubation and represent the mean of four independent experiments. The experimental details are described in the main text.