Supplementary Information

for

Leveraging heterocycle-fused 1,4-benzoquinone to design chemical modulators for both metal-free and metal-bound amyloid-β

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Materials and Methods. All chemical reagents for the syntheses of compounds were purchased from commercial suppliers [Sigma-Aldrich (St. Louis, MO, USA), Tokyo Chemical Industry (Tokyo, Japan), Alfa Aesar (Morecambe, UK), Acros Organics (Brookline, MA, USA), Samchun Chemicals (Seoul, Republic of Korea), and Duksan Chemicals (Incheon, Republic of Korea)] and used without further purification. All glassware was thoroughly dried in a convection oven. Chemical reactions for the syntheses of compounds were monitored using thin-layer chromatography (TLC). The spots were visualized in commercial TLC plates (silica gel 60 F_{254}, Merck Co., Rahway, NJ, USA) under ultraviolet (UV) light at 254 nm or 365 nm or by dye such as potassium permanganate solution. Silica gel column chromatography was performed with silica gel 60 (particle size 0.040−0.063 mm, Merck Co.). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were collected on JNM-ECZ400S (JEOL, Tokyo, Japan; Kyung Hee University, Gyeonggi-do, Republic of Korea) or AVANCE III HD 400 NMR spectrometer (Bruker, Billerica, MA, USA; Department of Chemistry, KAIST, Daejeon, Republic of Korea). Chemical shifts in ^{1}H NMR spectra were expressed in parts per million (ppm) downfield from tetramethylsilane, and coupling constants were reported in Hertz (Hz). Splitting patterns are indicated: s, singlet; d, doublet; t, triplet; and m, multiplet. ^{13}C NMR spectra were reported in ppm, referenced to $CDCl_3$ or DMSO-d_6. Melting points (m.p.) of compounds were determined on a Barnstead Electrothermal 9100 instrument (Essex, UK) and uncorrected. Measurements by high-resolution mass spectrometry (HRMS) were performed by a JMS-700 with an electron ionization (EI) or fast atom bombardment (FAB) source (JEOL, Japan) or a Xevo G2-XS QTof with an ESI source [Waters, Milford, MA, USA; KAIST Analysis Center for Research Advancement (KARA), Daejeon, Republic of Korea]. Synthetic $A\beta_{40}$ (DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV) and $A\beta_{42}$ (DAEFRHDSGYEVHH-QKLVFFAEDVGSNKGAIIGLMVGGVVIA) were obtained from Peptide Institute (Osaka, Japan) and purified by high-performance liquid chromatography using Imtakt Cadenza CD-C18 (Imtakt, Portland, OR, USA), Zorbax 300SB-C18 (Agilent), or Xbridge peptide BEH-C18 (Waters) columns. Electronic absorption (Abs) spectra were recorded on an Agilent 8453 UV–visible spectrophotometer (Agilent, Santa Clara, CA, USA). HEPES [2-(4-(2-hydroxyethyl)piperazin-1-yl)ethanesulfonic acid] and ammonium acetate were purchased from Sigma-Aldrich. The buffered solution was prepared in doubly distilled water [ddH_2O; Milli-Q Direct 16 system (18.2 MΩ·cm; Merck KGaA, Darmstadt, Germany)]. Trace metal contamination was removed from all solutions used for experiments by treating with Chelex (Sigma-Aldrich) overnight. The samples were prepared using Eppendorf tubes (Eppendorf, Hamburg, Germany) unless otherwise stated. Images gained by gel/Western blot were visualized by a ChemiDoc MP imaging system (Bio-Rad,

Hercules, CA, USA). Morphologies of peptide aggregates produced from the aggregation experiments were taken on a Tecnai F20 transmission electron microscope (FEI Company, Eindhoven, Netherlands; KARA). ESI–MS and tandem MS (MS^2) experiments were performed by a Xevo G2-XS QTof mass spectrometer with an ESI source (Waters). The human neuroblastoma SH-SY5Y (5Y) cell line was purchased from the American Type Culture Collection (Manassas, VA, USA). A microplate reader (SpectraMax M5; Molecular Devices, Sunnyvale, CA, USA) was used to measure the absorbance for the MTT assay [MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Biosesang, Seoul, Republic of Korea].

Synthesis of 2a.

1b (7-Methoxy-1,2-dihydro-3*H*-indazol-3-one). 1b was synthesized following procedures described in the patent literature.[1] 2-Amino-3-methoxybenzoic acid (2.5 g, 15.0 mmol) was dissolved in HCl (8.4 M, aq, 30 mL) at 0 °C and stirred for 5 min. Sodium nitrite ($NaNO_2$; 1.0 g, 15.0 mmol) was dissolved in H_2O (10 mL) and then slowly added dropwise to the solution at 0 °C for 30 min. $SnCl_2 \cdot 2H_2O$ (16.9 g, 74.8 mmol) was dissolved in HCl (11.3 M, aq, 20 mL) and, subsequently, added dropwise to the mixture at 0 °C. After 30 min, the solution was warmed to room temperature and stirred for 24 h. The precipitated product was filtered, washed with HCl (3.0 M, aq) and CH_2Cl_2, and dried under the vacuum. The mixture was dissolved in HCl (1.0 M, aq, 100 mL) at room temperature and heated and stirred at 100 °C for 24 h. After completion of the reaction, the mixture was cooled at room temperature and neutralized with the saturated aqueous solution of sodium carbonate (100 mL) at 0 °C. The precipitated product was filtered, washed with H_2O, and dried in a vacuum oven. The crude product was recrystallized in ethanol to yield **1b** [R_f = 0.30 [hexanes:ethyl acetate (EtOAc) = 1:2]; white solid; 2.1 g, 12.9 mmol (yield = 86%); m.p.: 196–197 °C]. ^{1}H NMR [400 MHz, DMSO-d_6, δ (ppm)]: 11.50 (s, 1H), 10.42 (s, 1H), 7.12 (d, J = 8.0 Hz, 1H), 6.84 (t, J = 7.8 Hz, 1H), 6.75 (d, J = 7.6 Hz, 1H), 3.86 (s, 3H). ^{13}C NMR [100 MHz, DMSO-d_6, δ (ppm)]: 156.52, 145.53, 134.28, 119.82, 114.34, 112.48, 106.50, 55.75. HRMS (*m/z*): $[M + H]^+$ Calcd. for $C_8H_9N_2O_2$: 165.0659, found: 165.0658.

1c (1-Benzyl-7-methoxy-1,2-dihydro-3*H*-indazol-3-one). 1b (1.9 g, 11.9 mmol) and sodium hydroxide (0.47 g, 11.9 mmol) were dissolved in H_2O (12 mL) at room temperature. Benzyl chloride (1.4 mL, 11.9 mmol) was then added and heated at 70 °C for 3 h. Upon completion of the reaction, the mixture was cooled at room temperature, and H_2O was added to precipitate the product. The precipitated product was filtered, washed with H_2O, and dried in a vacuum oven. The crude product was purified by recrystallization in benzene/*n*-heptane to obtain **1c** [R_f = 0.75 (hexanes:EtOAc = 1:1); ivory solid; 2.1 g, 8.3 mmol (yield = 70%); m.p.: 257–259 °C]. ^{1}H NMR

[400 MHz, DMSO-d_6, δ (ppm)]: 10.62 (s, 1H), 7.16–7.23 (m, 3H), 7.09–7.13 (m, 3H), 6.88 (t, J = 8.0 Hz, 1H), 6.80 (d, J = 8.0 Hz, 1H), 5.43 (s, 2H), 3.86 (s, 3H). ^{13}C NMR [100 MHz, DMSO-d_6, δ (ppm)]: 155.35, 145.99, 139.40, 132.86, 128.83, 127.78, 127.67, 120.34, 115.50, 112.62, 107.47, 56.12, 54.10. HRMS (*m/z*): $[M]^+$ Calcd. for $C_{15}H_{14}N_2O_2$: 254.1055, found: 254.1051.

1d [1-Benzyl-7-methoxy-2-(*p*-tolyl)-1,2-dihydro-3*H*-indazol-3-one]. 1c (1.1 g, 4.3 mmol), *p*-tolylboronic acid (1.8 g, 13.0 mmol), and $Cu(OAc)_2$ (0.8 g, 4.3 mmol) were dissolved in CH_2Cl_2 (8.6 mL) and pyridine (7.3 mL) at room temperature and stirred for 24 h. The reaction was quenched with HCl (1.0 M, aq), and the mixture was extracted with EtOAc (three times, 100 mL). The organic layer was separated, washed with brine, and dried over anhydrous sodium sulfate (Na_2SO_4). After filtration, the mixture was concentrated under reduced pressure, and the crude product was purified by column chromatography (SiO_2; hexanes:EtOAc = from 9:1 to 4:1) to obtain **1d** [R_f = 0.40 (hexanes:EtOAc = 2:1); white solid; 1.3 g, 3.8 mmol (yield = 84%); m.p.: 143–144 °C]. ^{1}H NMR [300 MHz, DMSO-d_6, δ (ppm)]: 7.48 (d, J = 8.4 Hz, 2H), 7.40 (d, J = 8.4 Hz, 2H), 7.29 (d, J = 7.3 Hz, 1H), 7.20 (d, J = 7.7 Hz, 1H), 7.08–7.17 (m, 4H), 6.71 (d, J = 7.9 Hz, 2H), 4.86 (s, 2H), 4.09 (s, 3H), 2.39 (s, 3H). ^{13}C NMR [100 MHz, $CDCl_3$, δ (ppm)]: 162.43, 147.11, 138.77, 136.06, 133.03, 132.52, 129.78, 129.00, 128.00, 127.93, 124.09, 123.75, 122.43, 116.19, 112.65, 55.73, 52.85, 21.11. HRMS (*m/z*): $[M]^+$ Calcd. for $C_{22}H_{20}N_2O_2$: 344.1525, found: 344.1525.

1e [1-Benzyl-7-hydroxy-2-(*p*-tolyl)-1,2-dihydro-3*H*-indazol-3-one]. 1d (1.0 g, 2.9 mmol) was dissolved in CH_2Cl_2 (25 mL). Boron tribromide (BBr_3; 1.0 M in CH_2Cl_2, 5.8 mL, 5.8 mmol) was gradually added at 0 °C for 1 h. The reaction was quenched with HCl (1.0 M, aq), and the mixture was extracted with EtOAc (three times, 150 mL). The organic layer was separated, washed with brine, and dried over anhydrous Na_2SO_4. After filtration, the crude product was recrystallized in hexanes/EtOAc to obtain **1e** [R_f = 0.25 (hexanes:EtOAc = 2:1); white solid; 0.9 g, 2.8 mmol (yield = 98%); m.p.: 224–225 °C]. ^{1}H NMR [400 MHz, DMSO-d_6, δ (ppm)]: 10.78 (s, 1H), 7.44 (d, J = 8.8 Hz, 2H), 7.36 (d, J = 8.8 Hz, 2H), 7.00–7.10 (m, 6H), 6.73 (d, J = 8.0 Hz, 2H), 4.86 (s, 2H), 2.35 (s, 3H). ^{13}C NMR [100 MHz, DMSO-d_6, δ (ppm)]: 162.24, 145.67, 138.31, 135.97, 133.32, 132.91, 130.30, 129.46, 128.51, 128.46, 125.12, 123.68, 122.16, 118.23, 114.18, 52.19, 21.17. HRMS (*m/z*): $[M]^+$ Calcd. for $C_{22}H_{20}N_2O_2$: 330.1368, found: 330.1367.

2a [1-Benzyl-2-(*p*-tolyl)-1*H*-indazole-3,4,7(2*H*)-trione]. 1e (0.1 g, 0.3 mmol) was dissolved in acetonitrile (CH_3CN; 3.0 mL) and tetrahydrofuran (THF; 3.0 mL). Ceric ammonium nitrate (CAN) was dissolved in H_2O (3.0 mL) and then slowly added dropwise to the mixture at 0 °C. The mixture was warmed to room temperature and stirred for 1 h. The reaction was quenched with H_2O, and the mixture was then extracted with EtOAc (three times, 50 mL). The

organic layer was separated, washed with brine, and dried over anhydrous Na_2SO_4. After filtration, the mixture was concentrated under reduced pressure, and the crude product was purified by column chromatography (SiO_2; hexanes:EtOAc = from 2:1 to 1:9) to obtain **2a** [R_f = 0.20 (hexanes:EtOAc = 2:1); red-orange solid; 75 mg, 0.2 mmol (yield = 72%); m.p.: 180–181 °C]. ^{1}H NMR [400 MHz, DMSO-d_6, δ (ppm)]: 7.21–7.30 (m, 5 H), 7.08 (d, J = 8 Hz, 2H), 6.82–6.92 (m, 4H), 5.46 (s, 2H), 2.35 (s, 3H). ^{13}C NMR [100 MHz, DMSO-d_6, δ (ppm)]: 179.15, 178.96, 157.53, 140.37, 139.97, 139.84, 135.65, 133.91, 129.95, 128.69, 128.63, 128.40, 128.11, 126.70, 103.75, 50.35, 20.82. HRMS (*m/z*): $[M]^+$ Calcd. for $C_{21}H_{16}N_2O_3$: 344.1161, found: 344.1162.

Synthesis of 1 [2-(*p*-Tolyl)-1*H*-indazole-3,4,7(2*H*)-trione]. 2a (0.2 g, 0.5 mmol) and palladium hydroxide on carbon [$Pd(OH)_2$/C; 0.033 g, 0.046 mmol] were dissolved in CH_3OH (18 mL). The mixture was stirred at room temperature for 2 h under H_2 gas. After completion of the reaction, the mixture was filtered through Celite and washed with EtOAc (200 mL). The filtrate was concentrated under reduced pressure, and the crude product was purified by column chromatography (SiO_2; CH_3OH:EtOAc = 1:9) to obtain **1** [R_f = 0.35 (CH_3OH:EtOAc = 1:9); red-violet solid; 112 mg, 0.4 mmol (yield = 95%); m.p.: decomposed over 300 °C]. ^{1}H NMR [400 MHz, DMSO-d_6, δ (ppm)]: 7.89 (d, J = 8.0 Hz, 2H), 7.19 (d, J = 8.0 Hz, 2H), 6.49–6.61 (m, 2H), 6.46 (d, J = 10.3 Hz, 1H), 2.30 (s, 3H). ^{13}C NMR [100 MHz, DMSO-d_6, δ (ppm)]: 181.22, 176.53, 161.62, 143.88, 142.97, 137.52, 136.19, 133.59, 129.01, 119.26, 20.56. HRMS (*m/z*): $[M]^+$ Calcd. for $C_{14}H_{10}N_2O_3$: 254.0691, found: 254.0692.

Preparation of Aβ. Aβ was dissolved in ammonium hydroxide [NH_4OH; 1% w/w, aq; Sigma-Aldrich). The solutions were aliquoted, lyophilized overnight, and stored at –80 °C. A stock solution of Aβ was prepared by dissolving the lyophilized peptide with NH_4OH (1% w/w, aq; 10 μL) followed by addition of ddH_2O. The concentration of the solution containing Aβ was determined by measuring the absorbance at 280 nm (ε = 1,450 $M^{-1}cm^{-1}$ for $A\beta_{40}$; ε = 1,490 $M^{-1}cm^{-1}$ for $A\beta_{42}$).[2]

Aβ Aggregation Experiments. Aβ samples were prepared in the buffered solution (20 mM HEPES, pH 6.8 or pH 7.4, 150 mM NaCl) following previously reported procedures.[2,3] For the inhibition studies, compounds (final concentration, 25 μM; 1% v/v DMSO) were added to the samples of Aβ (25 μM) in the absence and presence of $CuCl_2$ or $ZnCl_2$ (25 μM) followed by incubation for 24 h at 37 °C with constant agitation. For the disaggregation studies, Aβ (25 μM)

was incubated with and without $CuCl_2$ or $ZnCl_2$ (25 μM) for 24 h at 37 °C with constant agitation to produce preformed Aβ aggregates. The resultant Aβ aggregates were then treated with compounds (25 μM; 1% v/v DMSO) and incubated for an additional 24 h with constant agitation. For the experiments under anaerobic conditions, all samples were prepared following the procedure described above for the aerobic samples under quiescent conditions in a N_2 (g)-filled glove box.

Gel/Western Blot. The resultant Aβ species from the inhibition and disaggregation experiments were analyzed by gel/Western blot using an anti-Aβ antibody (6E10; Covance, Princeton, NJ, USA).[2,3] Each sample (10 μL) was separated on a 10–20% tricine gel (Invitrogen). Following separation, the peptides were transferred onto nitrocellulose membranes and blocked with bovine serum albumin (BSA; 3% w/v; Sigma-Aldrich) in Tris-buffered saline (TBS) containing 0.1% v/v Tween-20 (Sigma-Aldrich) (TBS-T) overnight at 4 °C. The membranes were incubated with 6E10 (1:2,000) in the solution of BSA (2% w/v in TBS-T) for 4 h at room temperature. After washing with TBS-T (three times, 10 min), a horseradish peroxidase-conjugated goat anti-mouse secondary antibody (1:5,000 in 2% w/v BSA in TBS-T; Cayman Chemical Company, Ann Arbor, MI, USA) was added for 1 h at room temperature. A homemade ECL kit[4] was used to visualize gel/Western blots on a ChemiDoc MP Imaging System (Bio-Rad).

TEM. Samples for TEM were prepared according to the procedures described in Aβ aggregation experiments. Glow-discharged grids (Formvar/Carbon 300-mesh; Electron Microscopy Sciences, Hatfield, PA, USA) were treated with the samples (5 μL) for 2 min at room temperature. Excess samples were removed using filter paper followed by washing with ddH_2O three times. Each grid incubated with uranyl acetate (1% w/v, aq; 5 μL; Polysciences, Warrington, PA, USA) for 2 min was blotted off and dried overnight at room temperature. Images for each sample were taken on a transmission electron microscope (200 kV; 29,000x magnification). The location of the samples on the grids was randomly picked, taking more than 20 images from each grid.

ESI–MS. Compounds (25 μM; 1% v/v DMSO) were incubated with and without $A\beta_{42}$ (25 μM) in the absence and presence of $CuCl_2$ or $ZnCl_2$ (25 μM) in 20 mM ammonium acetate, pH 6.8 or pH 7.4 for 6 h or 24 h at 37 °C with constant agitation. The samples were diluted 25-fold with LC-grade H_2O and then injected into the mass spectrometer unless otherwise stated. The capillary voltage, sampling cone voltage, source temperature, desolvation temperature, cone gas flow, and

desolvation gas flow were set to 2.5 kV, 40 V, 150 °C, 250 °C, 30 L/h, and 500 L/h, respectively.

ESI–MS^2. The covalent cross-link between metal-free monomeric Aβ_{42} and **2a** as well as singly oxidized Aβ_{42} species generated with **1**, **2a**, and **3** in the presence of Cu(II) were further analyzed by ESI–MS^2. Mass spectra for the samples prepared under metal-free conditions were obtained without dilution with LC-grade H_2O before injection into the mass spectrometer. The ESI parameters and experimental conditions were the same as those used for the ESI–MS experiments. Collision-induced dissociation was performed by applying the collision energy at 58–65 eV.

Docking Studies. MM2 energy minimization in Chem3D 21.0.0 was used to optimize the structure of **2a** and **2b**. The structures of Aβ_{42} monomer (xx98156[5]) with **2a** and **2b** were prepared using AutoDock Tools,[6] imported into PyRx,[7] and used to run AutoDock Vina 1.5.7 software.[8] The exhaustiveness for the docking runs was set at 1,024. Docked models of compounds with the peptide were visualized using Pymol 2.5.4.

Chemical Transformation of 2a. 2a (25 μM; 1% v/v DMSO) was incubated for 24 h at 37 °C in 20 mM HEPES, pH 7.4, 150 mM NaCl (for Abs spectroscopy) or 20 mM ammonium acetate, pH 7.4 (for ESI–MS). For the analysis of its chemical transformation, spectral changes were monitored by Abs spectroscopy and ESI–MS. For comparison with spectral features of **2b**, the Abs spectrum of **2b** (25 μM; 1% v/v DMSO) was obtained in 20 mM HEPES, pH 7.4, 150 mM NaCl. The isotope patterns of the peaks corresponding to **2a** and its transformed species shown in the mass spectra were simulated according to their chemical formula using enviPat Web.[9]

Synthesis of 2b.

2b-i [1-Benzyl-6-bromo-4,7-dihydroxy-2-(*p*-tolyl)-1,2-dihydro-3*H*-indazol-3-one] and 2b-ii [1-benzyl-6-bromo-2-(*p*-tolyl)-1*H*-indazole-3,4,7(2*H*)-trione]. 1e (0.2 g, 0.6 mmol) was dissolved in CH_2Cl_2 (6.0 mL). Br_2 (0.034 mL, 0.7 mmol) was dissolved in CH_2Cl_2 (0.6 mL) and then slowly added dropwise to the solution at 0 °C. The mixture was warmed to room temperature and stirred for 15 min. This process was repeated with an additional Br_2 (0.034 mL, 0.7 mmol) dissolved in CH_2Cl_2 (6.0 mL). The reaction was quenched with H_2O, and the mixture was extracted with EtOAc (three times, 50 mL). The organic layer was separated, washed with brine, and dried over anhydrous Na_2SO_4. After filtration, the mixture was concentrated under reduced pressure,

and the crude product was purified by column chromatography (SiO_2; hexanes:EtOAc = from 4:1 to 1:4) to yield **2b-i** [R_f = 0.70 (hexanes:EtOAc = 1:1); pale pink solid; 116 mg, 0.3 mmol (yield = 45%); m.p.: 174–175 °C] and **2b-ii** [R_f = 0.10 (hexanes:EtOAc = 1:1); purple solid; 107 mg, 0.3 mmol (yield = 42%); m.p.: 178–179 °C]. For **2b-i**, ^{1}H NMR [400 MHz, DMSO-d_6, δ (ppm)]: 9.85 (s, 1H), 9.57 (s, 1H), 7.37–7.39 (m, 4H), 7.12–7.15 (m, 4H), 6.74 (d, J = 7.2 Hz, 2H), 6.61 (s, 1H), 4.83 (s, 2H), 2.35 (s, 3H). ^{13}C NMR [100 MHz, $CDCl_3$, δ (ppm)]: 162.87, 149.02, 137.06, 136.31, 132.80, 132.70, 131.62, 130.16, 129.12, 128.48, 128.36, 123.76, 115.11, 111.17, 108.55, 52.69, 21.23. HRMS (*m/z*): $[M]^+$ Calcd. for $C_{21}H_{16}N_2O_3$: 424.0423, found: 424.0420. For **2b-ii**, ^{1}H NMR [400 MHz, DMSO-d_6, δ (ppm)]: 7.42 (s, 1H), 7.19–7.25 (m, 5H), 6.99 (d, J = 8.4 Hz, 2H), 6.88 (d, J = 7.6 Hz, 2H), 5.44 (s, 2H), 2.31 (s, 3H). ^{13}C NMR [100 MHz, $CDCl_3$, δ (ppm)]: 176.26, 172.34, 157.89, 141.94, 140.83, 139.36, 134.22, 133.32, 130.61, 129.12, 129.07, 128.66, 127.89, 127.09, 106.62, 51.61, 21.43. HRMS (*m/z*): $[M]^+$ Calcd. for $C_{21}H_{16}N_2O_3$: 422.0266, found: 422.0267.

Conversion of 2b-i to 2b-ii. 2b-i (0.4 g, 0.9 mmol) was dissolved in a mixture of CH_3CN (8.0 mL) and THF (8.0 mL). CAN (1.2 g, 2.1 mmol) was dissolved in H_2O (8.0 mL) and then added dropwise to the solution at 0 °C. The mixture was subsequently warmed to room temperature and stirred for 30 min. The reaction was quenched with H_2O, and the mixture was extracted with EtOAc (three times, 100 mL). The organic layer was separated, washed with brine, and dried over anhydrous Na_2SO_4. Following filtration, the mixture was concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2; hexanes:EtOAc = from 1:1 to 1:4) to yield **2b-ii** (78 mg, yield = 98%).

2b-iii [1-Benzyl-6-methoxy-2-(*p*-tolyl)-1*H*-indazole-3,4,7(2*H*)-trione]. 2b-ii (0.1 g, 0.2 mmol) was dissolved in CH_3OH (4.7 mL). The solution of sodium methoxide (4.4 M in CH_3OH, 0.2 mL, 0.8 mmol) was added dropwise to the solution of **2b-ii** at 0 °C. The mixture was then warmed to room temperature and stirred for 1 h. The reaction was quenched with HCl (1.0 M, aq), and the mixture was extracted with EtOAc (three times, 50 mL). The organic layer was separated, washed with brine, and dried over anhydrous Na_2SO_4. After filtration, the mixture was concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2; hexanes:EtOAc = from 2:1 to 1:1) to obtain **2b-iii** [R_f = 0.45 (hexanes:EtOAc = 1:2); red solid; 34 mg, 0.091 mmol (yield = 49%); m.p.: 227–228 °C]. ^{1}H NMR [400 MHz, DMSO-d_6, δ (ppm)]: 7.25 (d, J = 8.0 Hz, 2H), 7.20–7.22 (m, 4H), 7.02 (d, J = 8.0 Hz, 2H), 6.85 (d, J = 7.6 Hz, 2H), 5.46 (s, 2H), 4.24 (s, 3H), 2.32 (s, 3H). ^{13}C NMR [100 MHz, $CDCl_3$, δ (ppm)]: 172.45, 172.15, 160.31, 140.81, 139.77, 133.36, 130.57, 129.04, 128.98, 128.68, 128.10, 127.23, 115.17, 104.18, 63.07, 51.43, 21.43. HRMS (*m/z*): $[M]^+$ Calcd. for $C_{21}H_{16}N_2O_3$: 374.1267, found: 374.1268.

2b [1-Benzyl-6-hydroxy-2-(*p*-tolyl)-1*H*-indazole-3,4,7(2*H*)-trione]. 2b-iii (0.03 g, 0.1 mmol) was dissolved in CH_2Cl_2 (2.4 mL). BBr_3 (1.0 M in CH_2Cl_2, 0.1 mL, 0.1 mmol) was then added to the solution at 0 °C for 1 h. The reaction was quenched with HCl (1.0 M, aq), and the mixture was extracted with EtOAc (three times, 50 mL). The organic layer was separated, washed with brine, and dried over anhydrous Na_2SO_4. Following filtration, the mixture was concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2; CH_3OH:EtOAc = from 1:9 to 1:3) to obtain **2b** [R_f = 0.15 (CH_3OH:EtOAc = 1:9); gray solid; 24 mg, 0.067 mmol (yield = 83%); m.p.: decomposed over 300 °C]. ^{1}H NMR [400 MHz, DMSO-d_6, δ (ppm)]: 7.24–7.28 (m, 6 H), 7.05 (d, J = 8.0 Hz, 2H), 6.82–6.84 (m, 2H), 5.71 (s, 2H), 2.30 (s, 3H), 2.35 (s, 3H). ^{13}C NMR [100 MHz, DMSO-d_6, δ (ppm)]: 175.13, 171.06, 165.09, 159.10, 146.54, 139.06, 134.95, 129.83, 129.63, 128.62, 128.15, 127.92, 126.70, 103.50, 101.01, 49.70, 20.80. HRMS (*m/z*): $[M + Br]^-$ Calcd. for $C_{21}H_{16}N_2O_4$: 439.0293, found: 439.0142.

Solution Speciation Studies. The acidity constant (K_a) of **2b** was determined through Abs variable-pH titration spectroscopy.[2] The solution of **2b** (50 μM; 1% v/v DMSO; 10 mM NaOH, pH 12, 100 mM NaCl) was titrated with small aliquots of HCl to obtain at least 30 spectra in the pH range from 0.3 to 12. Using the measured pK_a values, the metal-binding properties of the compound were analyzed through Abs variable-pH titration spectroscopy. Small aliquots of HCl were added into the solution of **2a** (50 μM; 1% v/v DMSO) with $CuCl_2$ or $ZnCl_2$ (25 μM). At least 30 spectra were acquired in the range of pH 0.9–7.8. The values of pK_a and stability constants were calculated by the HypSpec program (Protonic Software, Leeds, UK).[10] Solution speciation diagrams were modeled in the Hyss2009 program (Protonic Software).[11]

Metal-binding Studies. The binding stoichiometry and affinity of **1** for Cu(II) were determined by Job's method of continuous variation[12] and Cu(II)-titration experiments, respectively, employing Abs spectroscopy. A mixture of **1** (0.63, 1.88, 5, 7.5, 10, 11.25, 13.75, 15, 16.25, 18.75, 20, 21.25, 22.5, 23.13, 23.75, 24.38, and 25 μM; 1% v/v DMSO) and $CuCl_2$ (0.63, 1.88, 5, 7.5, 10, 11.25, 13.75, 15, 16.25, 18.75, 20, 21.25, 22.5, 23.13, 23.75, 24.38, and 25 μM) was prepared in H_2O with a fixed total concentration (25 μM). The changes in Abs at 250 nm were plotted as a function of the mole fraction of Cu(II) to obtain a Job plot. To estimate the Cu(II)-binding affinity of **1**, the Abs values of **1** (25 μM; 1% v/v DMSO) upon titration with various concentrations of $CuCl_2$ (0.75, 1, 1.5, 1.75, 5, 12.5, 17.5, 30, and 45 μM) in H_2O were measured. The alteration in Abs at 250 nm upon increasing the concentration of Cu(II) was plotted and fitted using the online software

BindFit.[13] The 1:2 Cu(II)-to-ligand (L) system constructed based on equations 1 and 2 was chosen in the program.[14,15] Note that $\varepsilon_{\Delta Cu(L)}$ and $\varepsilon_{\Delta Cu(L)_2}$ represent the change in the molar absorption coefficients of Cu(L) and $Cu(L)_2$ complexes, respectively, and $[Cu(II)]_0$ indicates the total concentration of Cu(II).

$$\Delta Abs_{280\ nm} = \frac{\varepsilon_{\Delta Cu(L)}[Cu(II)]_0 K_{a1}[L] + 2\varepsilon_{\Delta Cu(L)_2}[Cu(II)]_0 K_{a1}K_{a2}[L]^2}{1 + K_{a1}[L] + K_{a1}K_{a2}[L]^2} \quad \text{(equation 1)}$$

$$K_d = \frac{1}{K_a} \quad \text{(equation 2)}$$

In addition, to investigate the Zn(II)- and Cu(II)-binding properties of **1** and **6**, respectively, the changes in Abs of **1** (25 μM; 1% v/v DMSO; 20 mM HEPES, pH 7.4, 150 mM NaCl) and **6** (25 μM; 1% v/v DMSO; H_2O) upon treatment of $ZnCl_2$ (25 μM) or $CuCl_2$ (25 μM) were monitored.

Cell Culture. 5Y cell line was maintained in media containing minimum essential medium (MEM; 50% v/v; GIBCO, Grand Island, NY, USA) and F-12 (50% v/v; GIBCO) supplemented with fetal bovine serum (FBS, 10% v/v; Sigma-Aldrich) and penicillin (100 U/mL) with streptomycin (100 mg/mL; GIBCO). The cells were grown and maintained at 37 °C in a humidified atmosphere with 5% CO_2. The cells used for our studies did not indicate mycoplasma contamination.

Cell Viability Studies. Cell viability was determined by the MTT assay. Compounds (125 μM; 1% v/v DMSO) with and without Aβ (125 μM) were preincubated in the absence and presence of $CuCl_2$ or $ZnCl_2$ (125 μM) for 24 h at 37 °C with constant agitation in 20 mM HEPES, pH 6.8 or pH 7.4, 150 mM NaCl. Cells were seeded in a 96-well plate (20,000 cells/100 μL; SPL Life Sciences, Pocheon, Gyeonggi-do, Republic of Korea) and treated with the samples (final concentrations, 25 μM for compounds, Aβ, and metal ions). After 24 h of incubation, the cells were washed once with PBS (pH 7.4). MTT [5 mg/mL in PBS (pH 7.4); 25 μL] was added to each well, and the plate was incubated for 4 h at 37 °C. Formazan produced by cells was solubilized using an acidic solution of *N*,*N*-dimethylformamide (pH 4.5, 50% v/v, aq; Daejung Chemicals, Busan, Republic of Korea) and sodium dodecyl sulfate (20% w/v; Wako Chemicals, Richmond, VA, USA) overnight at room temperature in the dark. The absorbance was measured at 600 nm by a microplate reader (Molecular Devices). Cell viability was calculated, compared to that of the cells treated with an equivalent amount of the buffered solution. Data are presented as mean ± s.e.m. for n = 6 examined over three independent experiments. For the statistical analysis, a two-sided unpaired

Student's *t*-test was used. Statistical difference was considered significant at $*P < 0.05$, $**P < 0.01$, or $***P < 0.001$.

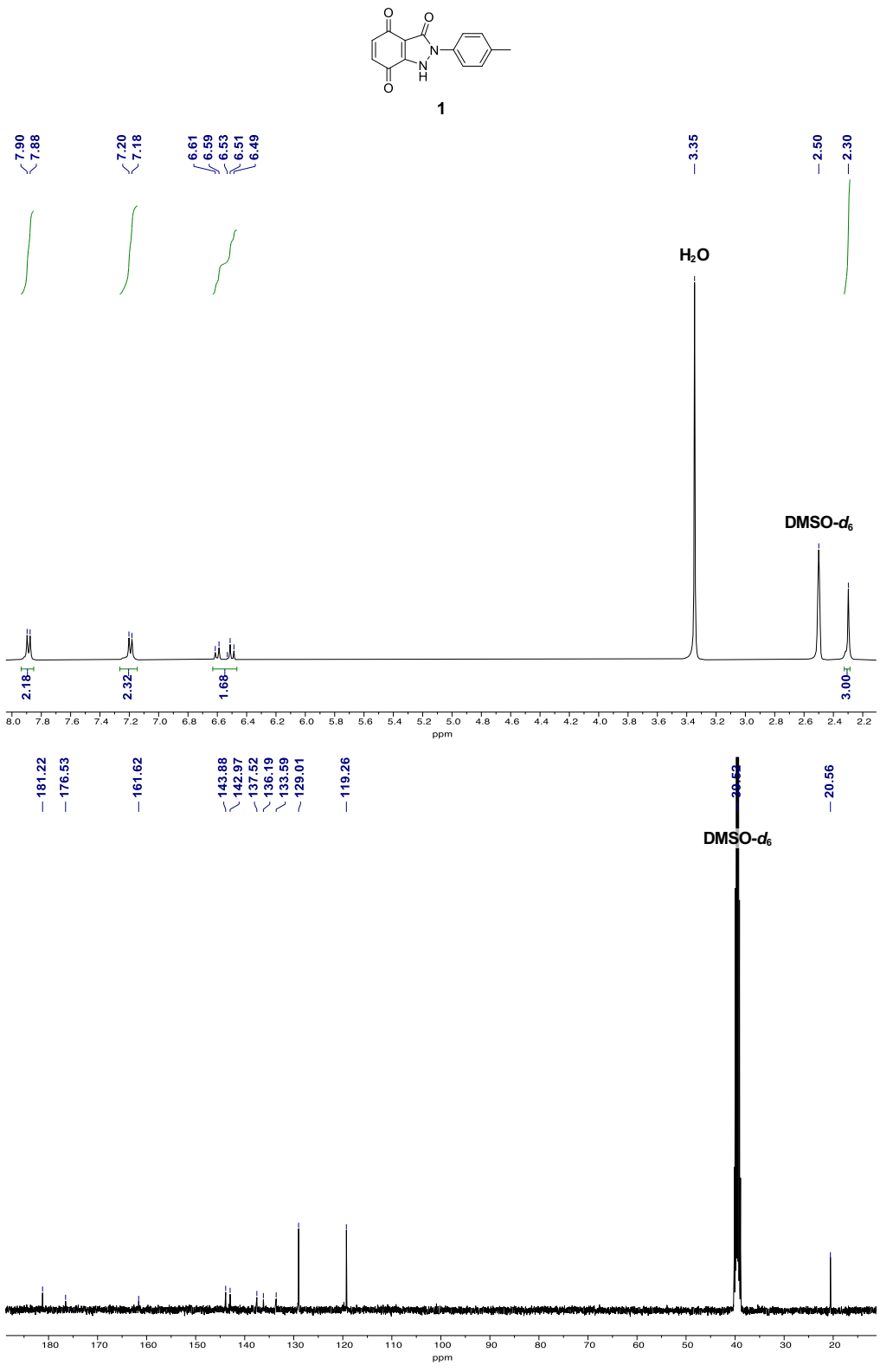


Fig. S1 ^{1}H (400 MHz, top) and ^{13}C (100 MHz, bottom) NMR spectra of **1** in DMSO-d_6.

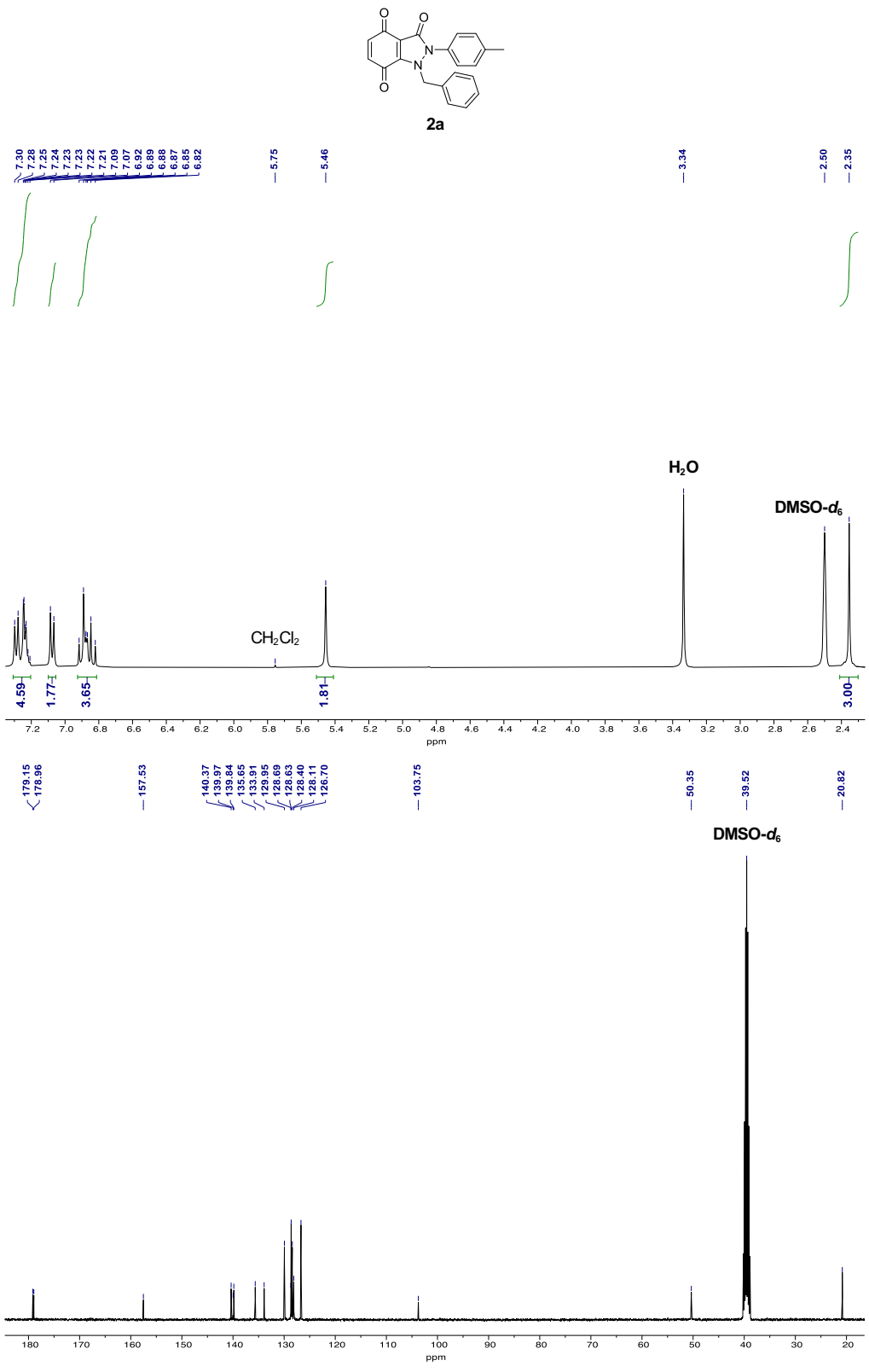


Fig. S2 ^{1}H (400 MHz, top) and ^{13}C (100 MHz, bottom) NMR spectra of **2a** in DMSO-d_6.

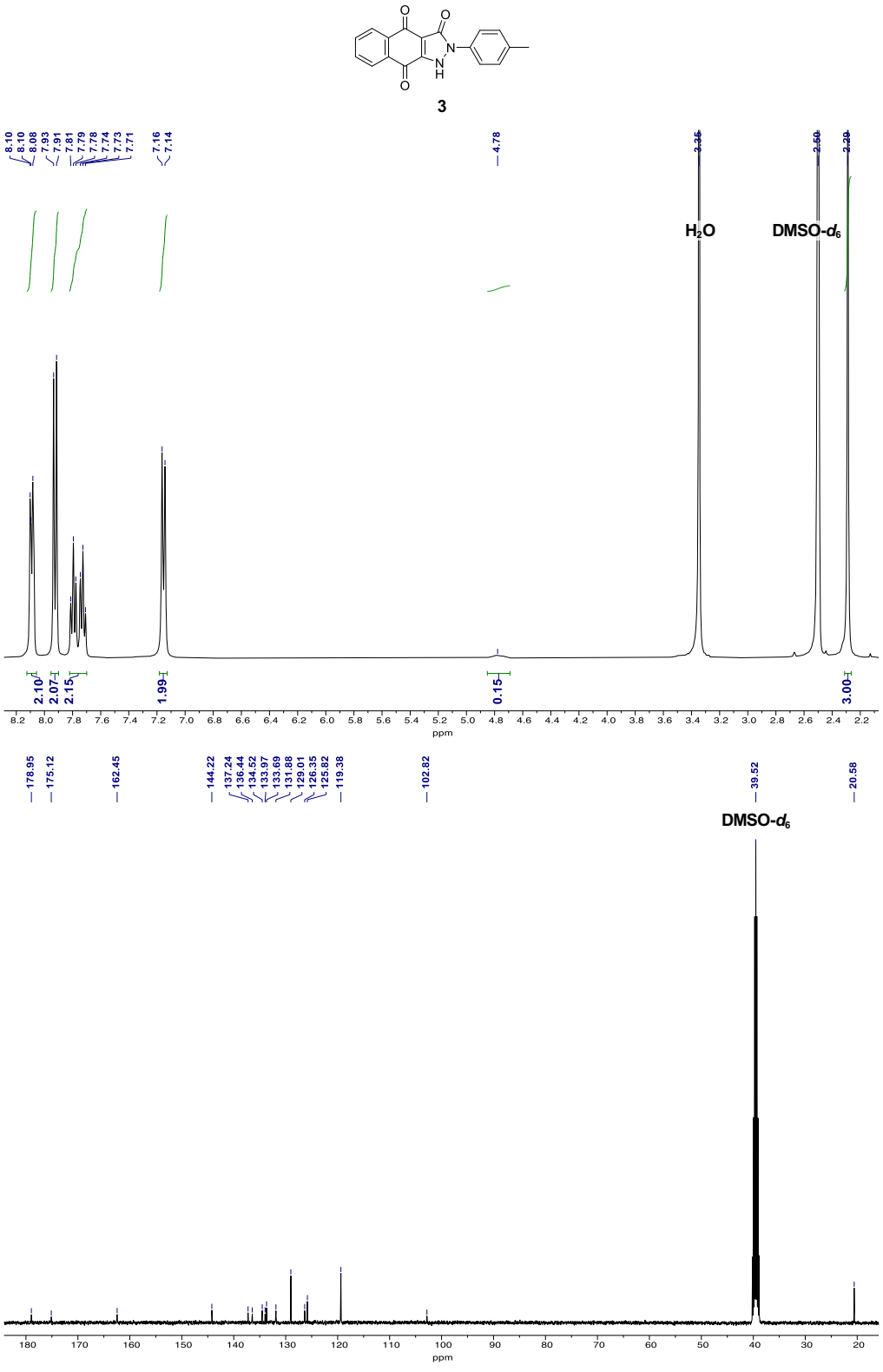


Fig. S3 ^{1}H (400 MHz, top) and ^{13}C (100 MHz, bottom) NMR spectra of **3** in DMSO-d_6.

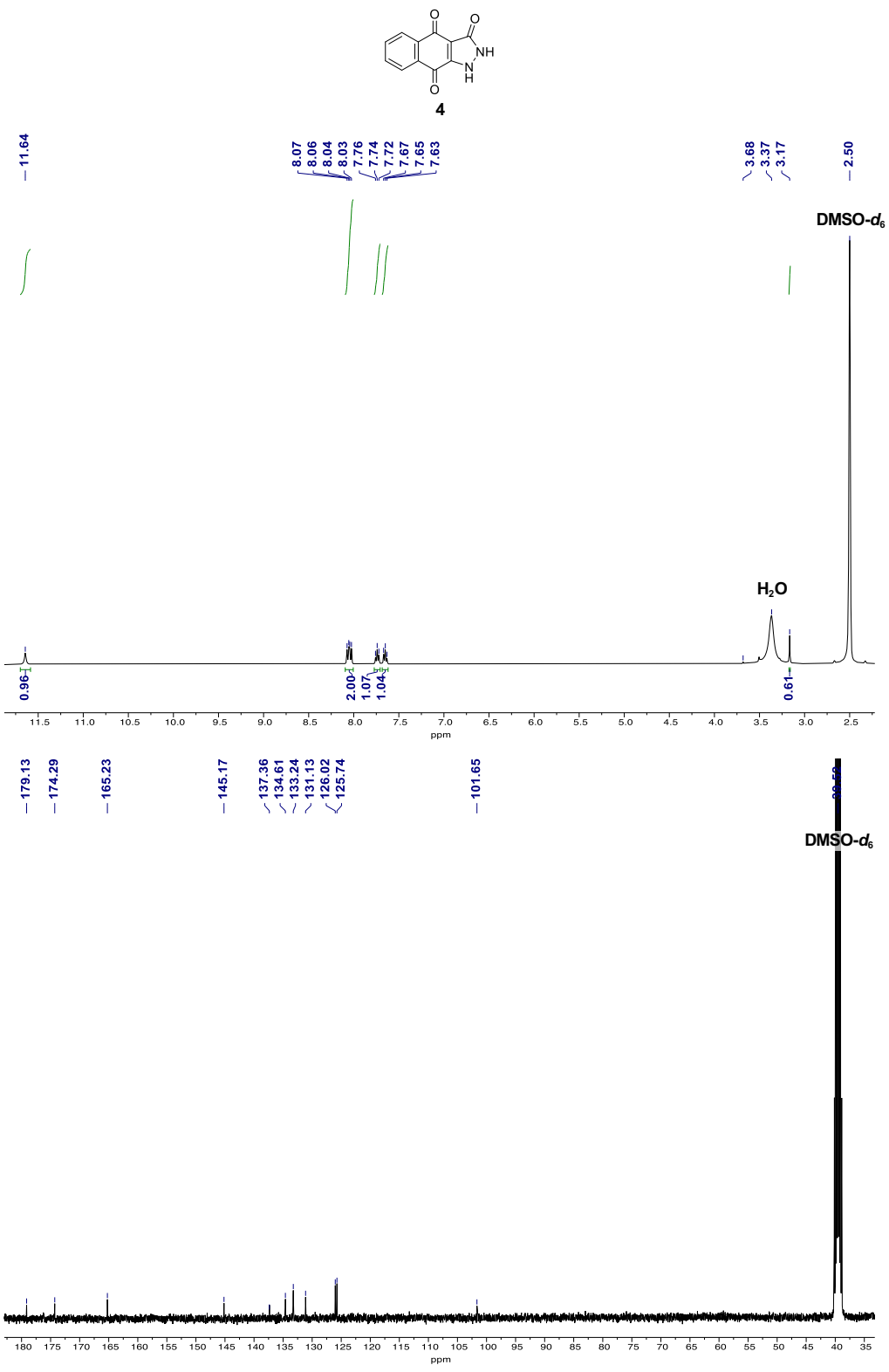


Fig. S4 ^{1}H (400 MHz, top) and ^{13}C (100 MHz, bottom) NMR spectra of **4** in DMSO-d_6.

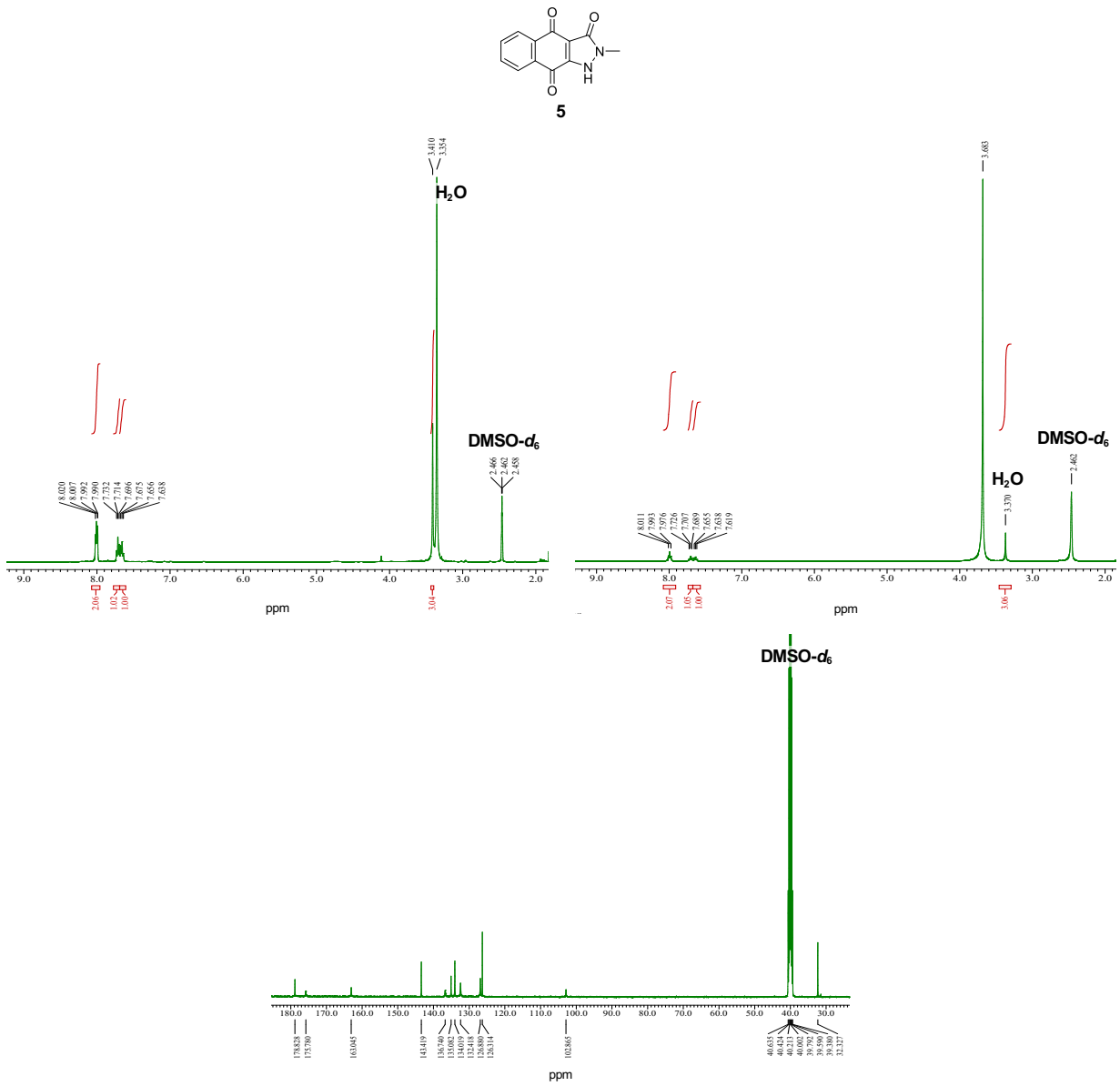


Fig. S5 ^{1}H (400 MHz, top left), ^{1}H (shaking with D_2O, 400 MHz, top right), and ^{13}C (100 MHz, bottom) NMR spectra of **5** in DMSO-d_6.

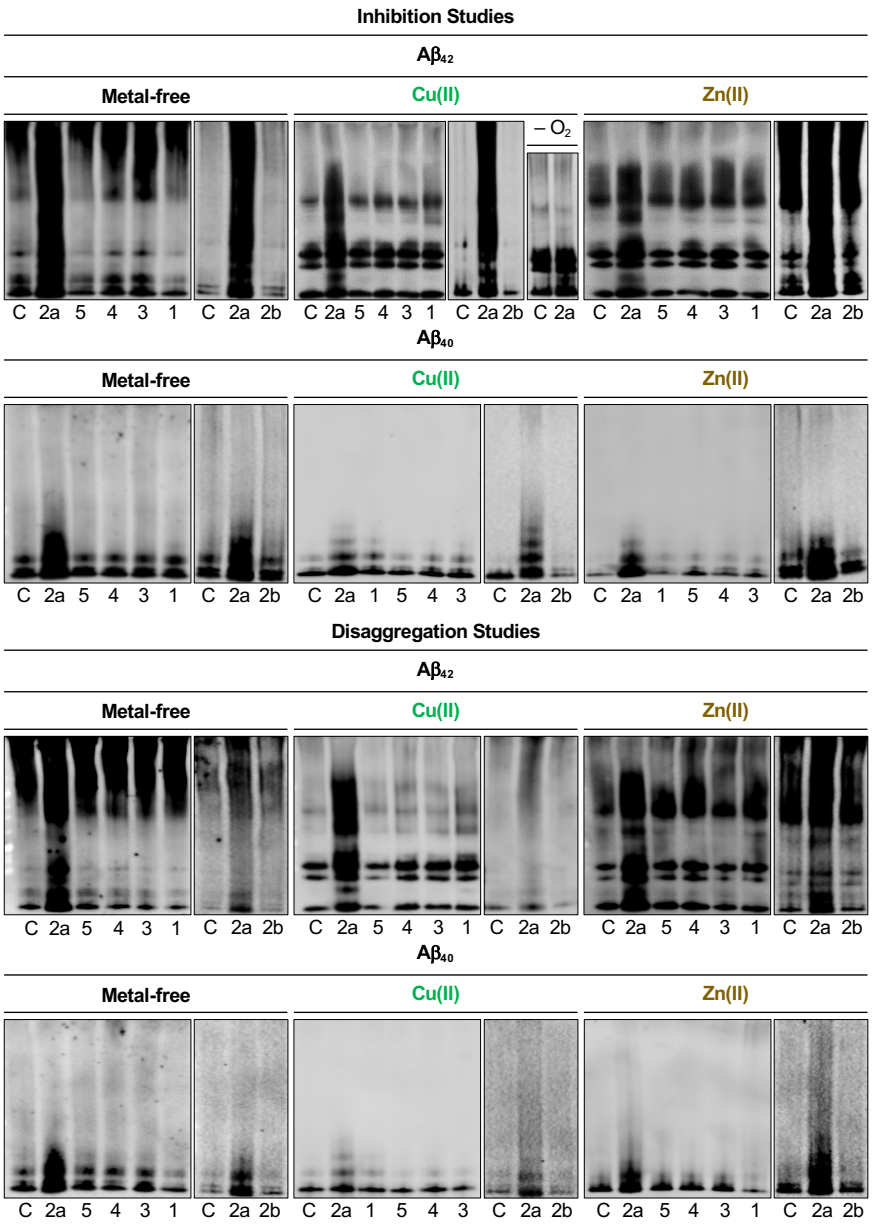


Fig. S6 Original gel images from Fig. 2, 5, 6, and S13.

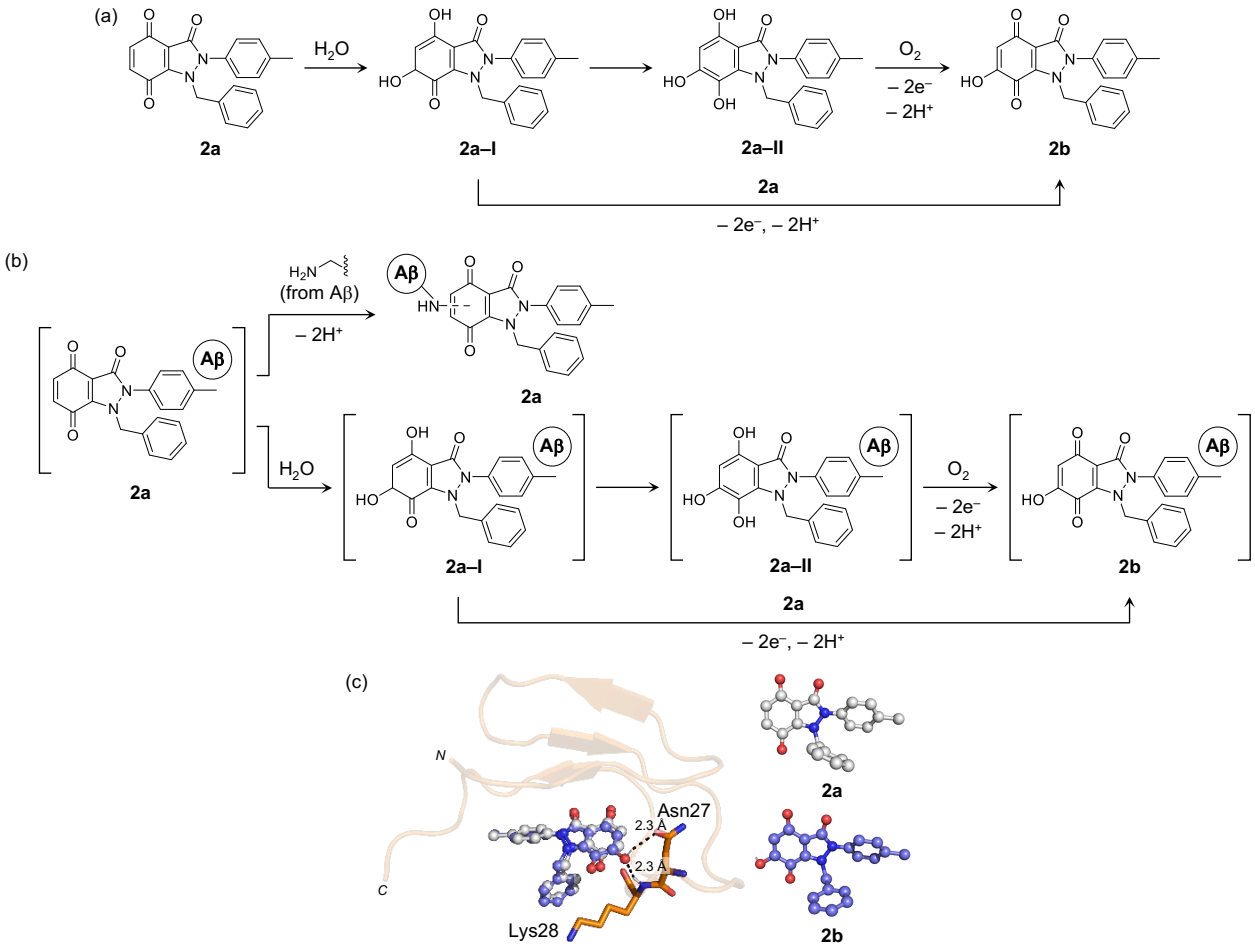


Fig. S7 Proposed reaction pathways between **2a** and Aβ. (a) Proposed chemical transformation of **2a** into **2b**. In aqueous media, the **BQ** moiety in **2a** can be transformed into **BQ(H$_2$O)** in **2a–I**, which then rearranges into **THB** in **2a–II**. This intermediate undergoes auto-oxidation, leading to the formation of **2-hydroxy-BQ** in **2b**. The oxidation of **2a–I** by **2a** can also generate **2b**. (b) Proposed covalent and non-covalent adduct formation of **2a** and its chemically converted forms with Aβ, respectively. Our ESI–MS studies indicate that **BQ** in **2a** forms covalent adducts with Lys residues in Aβ, whereas **2b** produces non-covalent adducts with Aβ. (c) Possible interactions of **2a** and **2b** with metal-free Aβ$_{42}$ (xx98156[5]), as visualized through docking studies. Nine docked models of **2a** and **2b** were obtained with binding energies ranging from –5.9 to –5.5 kcal/mol.

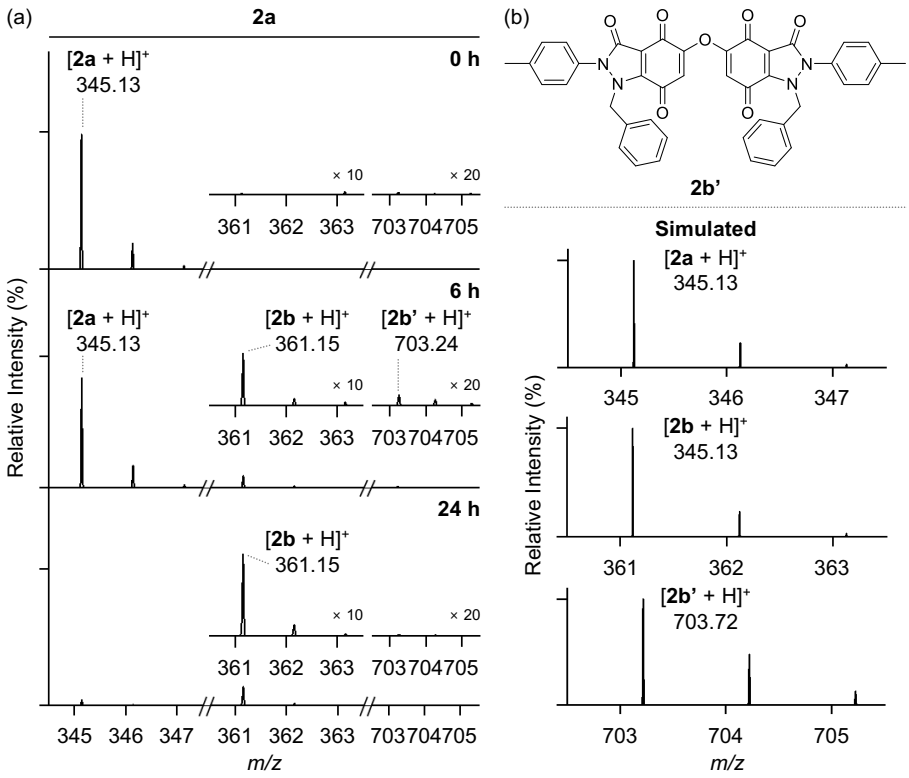


Fig. S8 Chemical transformation of **2a** monitored by ESI–MS. (a) Mass spectra of **2a** upon incubation for 6 h and 24 h. Conditions: [**2a**] = 25 μM (1% v/v DMSO); 20 mM ammonium acetate; 37 °C; 24 h; constant agitation. The samples were diluted 25-fold with H_2O before injection into the mass spectrometer. (b) Simulated mass spectra of **2a** and its transformed species.

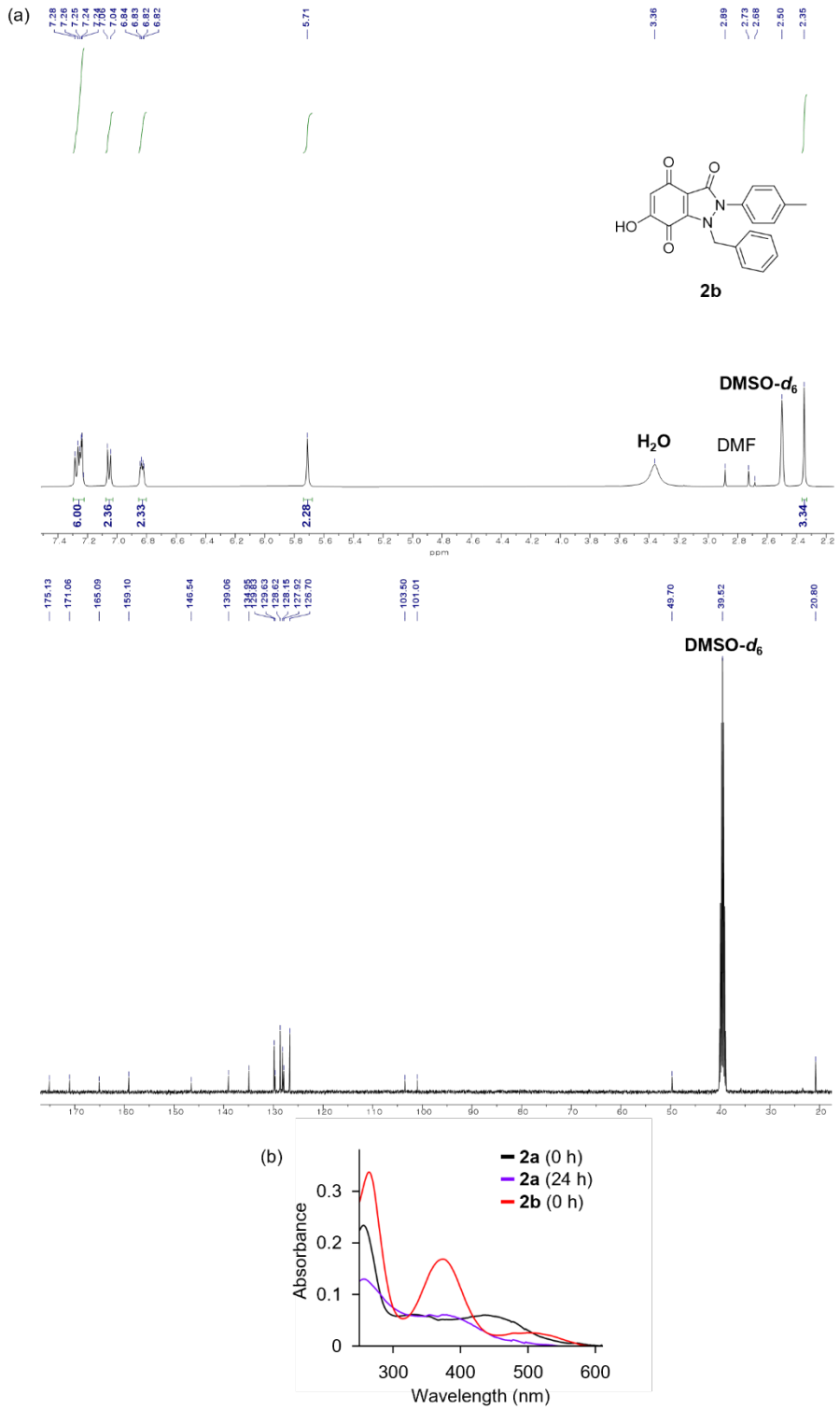
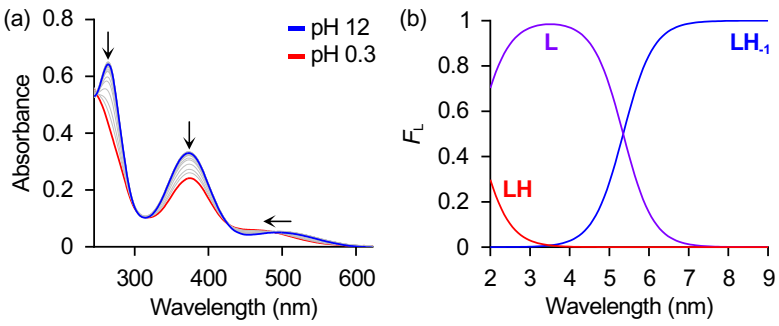


Fig. S9 Characterization of **2b**. (a) ^{1}H (400 MHz, top) and ^{13}C (100 MHz, bottom) NMR spectra of **2b** in DMSO-d_6. (b) Abs spectra of **2b** and **2a**. Conditions: [compound] = 25 μM (1% v/v DMSO); 20 mM HEPES, pH 7.4, 150 mM NaCl; 37 °C; 24 h; no agitation.



(c)

	pK_a
L + H ⇆ LH	0.627(5)
LH_{-1} + H ⇆ L	4.827(5)

Fig. S10 Solution speciation studies of **2b** (L). The Abs variable-pH titration spectra (a), solution speciation diagram (F_L = fraction of species at given pH) (b), and pK_a values (c) of **2b** are depicted. The error in the last digit is shown in parentheses. Charges are omitted for clarity. Conditions: [**2b**] = 50 μM; room temperature; I = 0.1 M NaCl.

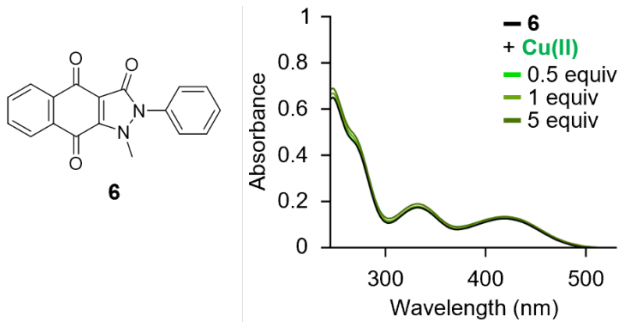


Fig. S11 Abs spectra of **6** upon treatment of Cu(II). Conditions: [**6**] = 25 μM (1% v/v DMSO); [Cu(II)] = 12.5, 25, and 125 μM; H_2O; room temperature.

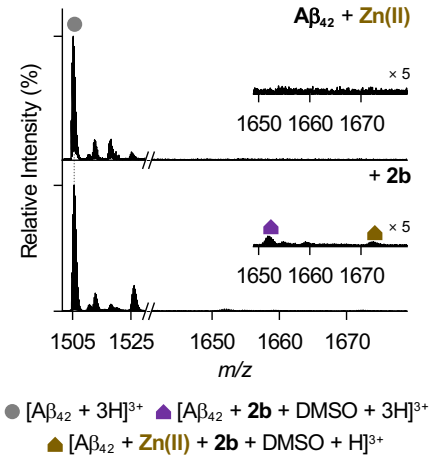


Fig. S12 Interaction of **2b** with Zn(II)–Aβ_{42} investigated by ESI–MS. Conditions: [Aβ_{42}] = 25 μM; [Zn(II)] = 25 μM; [**2b**] = 25 μM (1% v/v DMSO); 20 mM ammonium acetate, pH 7.4; 37 °C; 24 h; constant agitation. The samples were diluted 25-fold with H_2O before injection into the mass spectrometer.

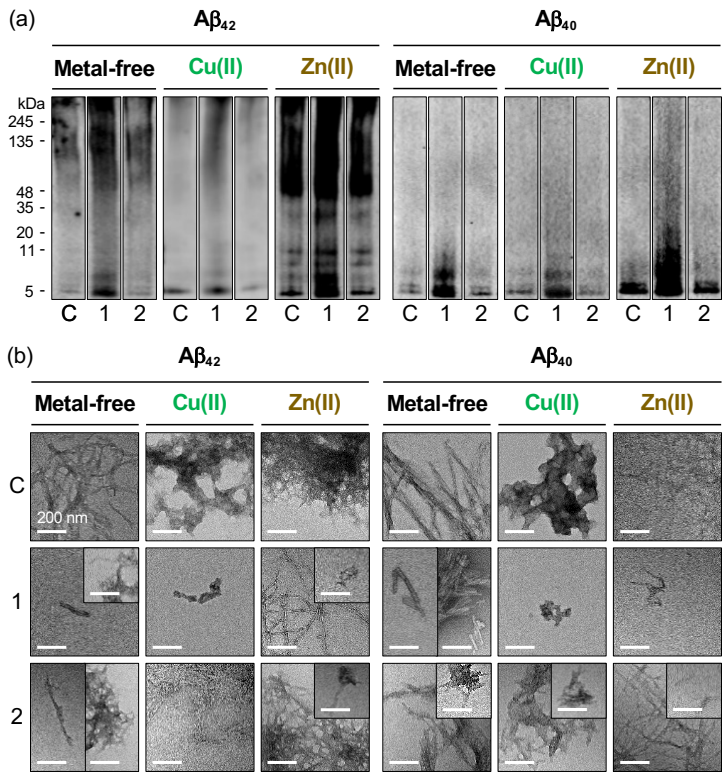


Fig. S13 Influence of **2a** and **2b** on preformed Aβ aggregates generated in the absence and presence of metal ions. (a) Size distribution of the resultant Aβ species analyzed by gel/Western blot using 6E10. Lanes: (C) Aβ ± Cu(II) or Zn(II); (1) C + **2a**; (2) C + **2b**. The original gel images are shown in Fig. S6. (b) Morphologies of the resultant Aβ species investigated by TEM. Conditions: [Aβ] = 25 μM; [Cu(II) or Zn(II)] = 25 μM; [compound] = 25 μM (1% v/v DMSO); 20 mM HEPES, pH 7.4 [for metal-free and Zn(II)-containing samples] or pH 6.8 [for Cu(II)-containing samples], 150 mM NaCl; 37 °C; 24 h; constant agitation. Scale bar = 200 nm.

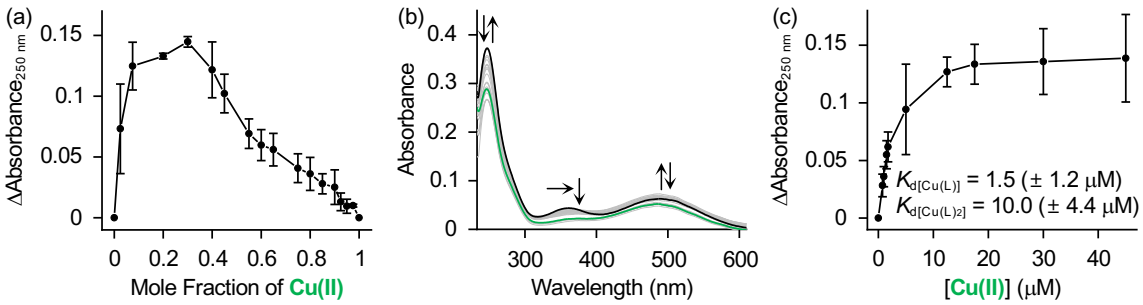


Fig. S14 Cu(II)-S1binding properties of **1** determined by Abs spectroscopy. (a) Job plot of **1** with Cu(II). Conditions: [**1**] = 0.63–25 μM (1% v/v DMSO); [Cu(II)] = 0.63–25 μM; H_2O; room temperature. (b) Abs spectra of **1** upon treatment of Cu(II). Conditions: [**1**] = 25 μM (1% v/v DMSO); [Cu(II)] = 0.75–45 μM; H_2O; room temperature. (c) Change in Abs at 250 nm of **1** fitted as a function of [Cu(II)]. The data were fitted using the online software BindFit[13] to estimate the K_d values for Cu(II) complexes of **1**. Data are presented as mean ± s.e.m. for n = 3.

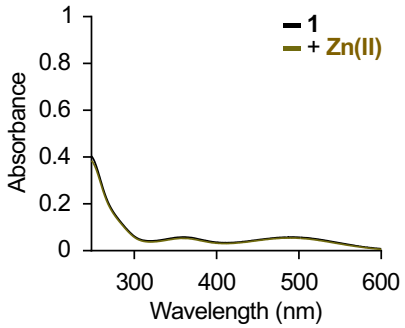


Fig. S15 Abs spectra of **1** upon treatment of Zn(II). Conditions: [**1**] = 25 μM (1% v/v DMSO); [Zn(II)] = 25 μM; 20 mM HEPES, pH 7.4, 150 mM NaCl; room temperature.

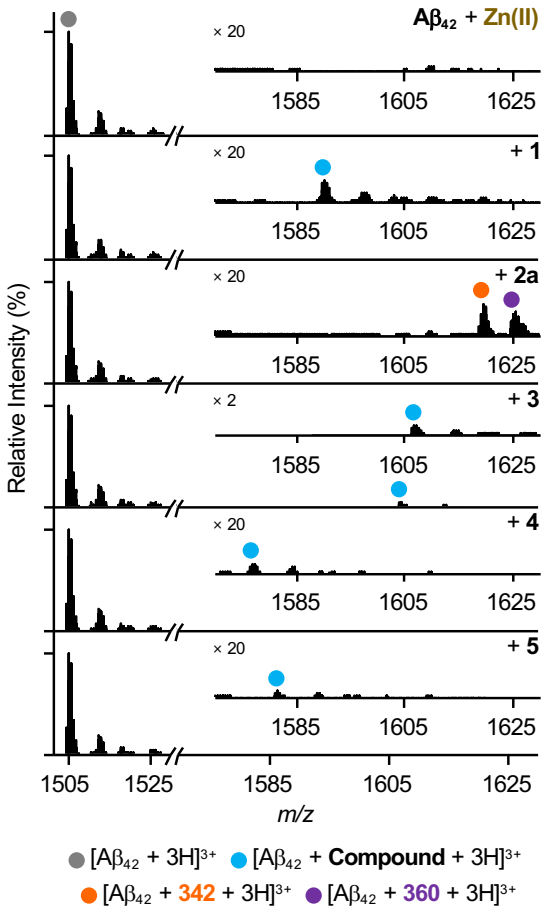


Fig. S16 Interaction of compounds with $A\beta_{42}$ in the presence of Zn(II) analyzed by ESI–MS. Conditions: [$A\beta_{42}$] = 25 μM; [Zn(II)] = 25 μM; [compound] = 25 μM (1% v/v DMSO); 20 mM ammonium acetate, pH 7.4; 37 °C; 24 h; constant agitation. The samples were diluted 25-fold with H_2O before injection into the mass spectrometer.

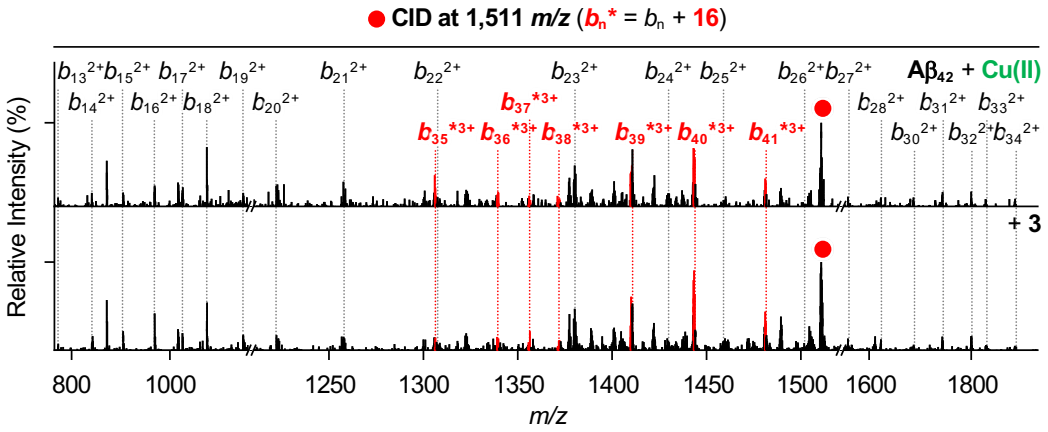


Fig. S17 ESI–MS^2 analysis of singly oxidized $A\beta_{42}$ obtained by incubation with **3**. Conditions: $[A\beta_{42}]$ = 25 μM; [Cu(II)] = 25 μM; [**3**] = 25 μM (1% v/v DMSO); 20 mM ammonium acetate, pH 6.8; 37 °C; 24 h; constant agitation. The samples were diluted 25-fold with H_2O before injection into the mass spectrometer.

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