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Structural and Mechanistic Insights into Development of Chemical Tools to Control Individual and Inter-Related Pathological Features in Alzheimer's Disease

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Experimental Section

Synthesis of L2-b1. L2-b1 was synthesized with slight modifications of a previously reported method.[1] Aniline (150 mg, 1.6 mmol) was dissolved in 15 mL of EtOH (treated with molecular sieves overnight) followed by addition of 5-dimethylamino-2-pyridinecarboxaldehyde (285 mg, 1.9 mmol). The reaction solution was allowed to stir at 45 °C for 10 min and its temperature was increased to 90 °C. After 1 h, the solution was cooled down to room temperature and concentrated until precipitates were formed. The resulting solid precipitates were then dissolved in dry MeOH and cooled to 0 °C in a N_2-purged round-bottom flask. To the solution, sodium borohydride ($NaBH_4$; 307 mg, 8.1 mmol) was slowly introduced at 0 °C for 5 min. After warming up to room temperature, the resulting solution was further stirred for 45 min. The reaction mixture was then quenched with water, extracted three times with dichloromethane (CH_2Cl_2), washed once with brine, and concentrated. The crude products were purified by column chromatography (SiO_2; EtOAc:Et_3N 100:0.1; R_f = 0.54) followed by recrystallization with Et_2O and hexanes (260 mg, 1.1 mmol, 71%). ^{1}H NMR (400 MHz, CD_2Cl_2) / δ (ppm): 8.10 (2H, d, J = 3.0 Hz), 7.15 (3H, m), 7.00 (2H, dd, J = 8.6, 3.0 Hz), 6.66 (3H, m), 4.75 (1H, s (broad)), 4.30 (2H, d, J = 5.3 Hz), 2.95 (6H, s). ^{13}C NMR (100 MHz; CD_2Cl_2) / δ (ppm): 149.0, 146.2, 146.0, 134.7, 129.6, 122.0, 120.0, 117.5, 113.4, 49.0, 40.6. HRMS: Calcd for $[M+H]^+$, 228.3190; found, 228.3192.

Synthesis of L2-b2. L2-b2 was synthesized with slight modifications of a previously reported method.[1] **DMPD** (50 mg, 0.4 mmol) was added into a flame-dried flask under N_2 (g) and then dissolved in 10 mL of EtOH (treated with molecular sieves overnight). 5-Dimethylamino-2-pyridinecarboxaldehyde (70 mg, 0.5 mmol) was introduced and stirred at 45 ºC for 10 min and

90 ºC for 1 h. The resulting solution was allowed to cool to room temperature, and the solvent was removed *in vacuo*. Dry MeOH was added to the flask under N_2 (g) and cooled down to 0 °C. A portion of $NaBH_4$ (70 mg, 1.9 mmol) was slowly introduced at 0 °C for 5 min followed by stirring for 45 min at room temperature. The reaction mixture was then quenched with water, extracted three times with CH_2Cl_2, washed once with brine, and concentrated. The crude products were purified by column chromatography (SiO_2; EtOAc:Et_3N 100:1; R_f = 0.29; 68 mg, 0.3 mmol, 70%). ^{1}H NMR (400 MHz, CD_2Cl_2) / δ (ppm): 8.09 (1H, d, J = 3.0 Hz), 7.15 (1H, d, J = 8.6 Hz), 6.99 (1H, dd, J = 8.6, 3.0 Hz), 6.69 (2H, d, J = 8.9 Hz), 6.62 (2H, d, J = 8.9 Hz), 4.25 (1H, s (broad)), 2.95 (6H, s), 2.79 (6H, s). ^{13}C NMR (100 MHz; CD_2Cl_2) / δ (ppm): 147.0, 145.8, 14.6, 141.4, 134.8, 122.1, 120.0, 116.0 114.8, 50.1, 42.4, 40.6. HRMS: Calcd for $[M+H]^+$, 271.3880; found, 271.3879.

Synthesis of DPA2. DPA2 was synthesized with slight modifications of a previously reported method.[2] 5-(Dimethylamino)picolinonitrile (100 mg, 0.7 mmol) was added into a flame-dried round-bottom flask (100 mL) containing dry MeOH (20 mL). Pd/C (10 wt %; 150 mg, 1.6 mmol) was added to the resulting mixture at room temperature. The solution was stirred under N_2 (g) for 10 min and then H_2 (g) for 5 or 6 h at room temperature. The Pd/C residues were filtered through the Celite and washed with cold MeOH (2 x 15 mL). To collected MeOH solution was slowly treated with 4 M HCl (0.4 mL) affording the light yellow solution. The mixture was concentrated *in vacuo* showing light yellow precipitates that were purified by column chromatography (SiO_2; MeOH:CH_2Cl_2 = 1:10; R_f = 0.70) followed by recrystallization with MeOH and Et_2O. The final product was washed with CH_2Cl_2 and Et_2O (65.9 mg, 0.2 mmol, 66%). ^{1}H NMR (400 MHz, CD_3OD) / δ (ppm): 8.25 (2H, d, J = 3.1 Hz), 8.07 (2H, d, J = 9.2 Hz), 7.83 (2H, dd, J = 4.6, 1.6

Hz), 4.68 (4H, s), 3.18 (12H, s). ^{13}C NMR (100 MHz; CD_3OD) / δ (ppm): 149.7, 130.7, 130.4, 127.4, 126.4, 47.8, 40.3. HRMS: Calcd for $[M+H]^+$, 286.2032; found, 286.2030.

Parallel Artificial Membrane Permeability Assay for the Blood-Brain Barrier (PAMPA-BBB). PAMPA-BBB experiments of compounds were carried out using the PAMPA Explorer kit (*p*ION, Inc. Billerica, MA, USA) with modifications to previously reported protocols.[1-3] Each stock solution was diluted with Prisma HT buffer (pH 7.4, *p*ION) to a final concentration of 25 μM (1% v/v final DMSO concentration). The resulting solution was added to the wells of the donor plate (200 μL, number of replicates = 12). BBB-1 lipid formulation (5 μL, *p*ION) was used to coat the polyvinylidene fluoride (PVDF, 0.45 μM) filter membrane on the acceptor plate. This acceptor plate was placed on top of the donor plate forming a sandwich. Brain sink buffer (BSB, 200 μL, *p*ION) was added to each well of the acceptor plate. The sandwich was incubated for 4 h at room temperature without stirring. UV−vis spectra of the solutions in the reference, acceptor, and donor plates were measured using a microplate reader. The PAMPA Explorer software [v. 3.8 (*p*ION)] was used to calculate the value of $-\log P_e$ for each compound. CNS± designations were assigned by comparison to compounds that were identified in previous reports.[4]

Aβ Aggregation Studies. Experiments with Aβ were conducted according to previously published methods.[1,2,3a,3b] To prepare Aβ peptides, either $Aβ_{40}$ or $Aβ_{42}$ was dissolved in ammonium hydroxide (NH_4OH, 1% v/v, aq), aliquoted, lyophilized overnight, and stored at –80 °C. For the experiments, a stock solution of Aβ was prepared by dissolving the lyophilized peptide in 1% NH_4OH and diluting with ddH_2O. The concentration of Aβ peptides in the

solution was determined by measuring the absorbance of the solution at 280 nm (ε = 1450 $M^{-1}cm^{-1}$ for $A\beta_{40}$; ε = 1490 $M^{-1}cm^{-1}$ for $A\beta_{42}$). The peptide stock solution was diluted to a final concentration of 25 μM in the chelex-treated buffered solution containing HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] (20 μM) (pH 6.6 for Cu(II) samples; pH 7.4 for metal-free and Zn(II) samples) and NaCl (150 μM). For inhibition studies, **L2-b1**, **L2-b2**, **PMA1**, **PMA2**, **DPA1**, or **DPA2** [50 μM; 1% v/v DMSO] was added to the sample of Aβ (25 μM) in the absence and presence of a metal chloride ($CuCl_2$ or $ZnCl_2$; 25 μM) followed by incubation at 37 °C with constant agitation for 24 h. For disaggregation studies, Aβ with and without metal ions was incubated for 24 h at 37 °C with constant agitation. **L2-b1**, **L2-b2**, **PMA1**, **PMA2**, **DPA1**, or **DPA2** (50 μM; 1% v/v DMSO) was added afterward to the solution containing Aβ aggregates, and incubated for additional 24 h at 37 °C.

Gel Electrophoresis and Western Blot. Each sample (10 μL) from both inhibition and disaggregation experiments was separated on a 10–20% Tris-tricine gel (Invitrogen, Grand Island, NY, USA) and transferred onto nitrocellulose membrane which was blocked with bovine serum albumin (BSA) solution (3% w/v; Sigma) in Tris-buffered saline containing 0.1% Tween-20 (TBS-T) for 2 h at room temperature. Then, the membranes were incubated with a primary antibody (6E10, Covance, Princeton, NJ, USA; 1:2,000) in a solution of 2% w/v BSA (in TBS-T) overnight at 4 °C. After washing with TBS-T three times (10 min each), the horseradish peroxidase-conjugated goat anti-mouse secondary antibody (1:5,000; Cayman Chemical Company) in 2% BSA (in TBS-T) was added to the membrane and incubated for 1 h at room temperature. SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific, Rockford, IL, USA) was used to visualize protein bands.

Transmission Electron Microscopy (TEM). Samples for TEM were prepared according to previously reported methods.[1,2,3a,3b] Glow-discharged grids (Formar/Carbon 300-mesh, Electron Microscopy Sciences, Hatfield, PA, USA) were treated with samples from inhibition and disaggregation experiments (5 μL) for 2 min at room temperature. Excess sample was removed carefully with filter paper and washed twice with ddH_2O. Each grid was treated with uranyl acetate (1% w/v ddH_2O, 5 μL) for 1 min. Excess stain was blotted off and the grids were air dried for at least 20 min at room temperature. Images from each sample were taken on a JEOL JEM-2100 TEM (200 kV) at 25,000x magnification.

Calculation of Ionization Potentials (IPs). First-principles calculations using Gaussian09[5] were carried out. The geometry optimization was performed using the M06/6-31G(d) level of theory for both neutral and ionized forms of each molecule. Thermodynamic parameters were only considered to calculate ionization potentials due to the difficulty of computing the kinetics of electron transfer steps. The thermodynamic parameters were calculated at the M06/6-311+G(2df,2p) level of theory at gas and solvent (water) phases (using polarizable continuum model), respectively.

2D NMR. The interaction of $A\beta_{40}$ with **L2-b1** or **L2-b2** was monitored by 2D ^{1}H-^{15}N band-selective optimized flip-angle short transient heteronuclear multiple quantum correlation (SOFAST-HMQC) NMR at 10 °C.[6] Uniformly ^{15}N-labeled $A\beta_{40}$ (rPeptide, Bogart, GA, USA) was first dissolved in 1% NH_4OH and lyophilized. The peptide was redissolved in 3 μL of DMSO-d_6 (Cambridge Isotope, Tewksbury, MA, USA) and diluted with phosphate buffer, NaCl, D_2O, and ddH_2O to a final peptide concentration of 80 μM (20 mM PO_4, pH 7.4, 50 mM NaCl; 7%

v/v D_2O). Each spectrum was obtained using 64 complex t_1 points and a 0.1 s recycle delay on a Bruker Avance 600 MHz NMR spectrometer. The 2D data were processed using TOPSPIN 2.1 (from Bruker). Resonance assignment was performed with SPARKY 3.1134 using published assignments for $A\beta_{40}$ as a guide.[6,7]

Saturation Transfer Difference (STD) NMR. For STD NMR experiments, a solution of fibrillar $A\beta_{42}$ (150 μM) was prepared through incubation for 24 h at 37 °C with constant agitation in 10 mM Tris-DCl at pD 7.4 (corrected for the isotope effect) containing 95% D_2O with or without $ZnCl_2$ (150 μM). The samples for STD experiments were prepared by diluting fibrils to 2 μM (effective monomer concentration) into 10 mM deuterated Tris-DCl to which 200 μM of compound (0.5% DMSO-d_6) was added. STD experiments were acquired with a train of 50 dB Gaussian-shaped pulses of 0.049 sec with an interval of 0.001 sec at either –1.0 ppm (on resonance)[8] or 40 ppm (off resonance) with a total saturation time of 2 sec on a Bruker 600 MHz NMR spectrometer.[3b,9] 1024 scans were recorded for the STD spectrum, and 512 scans were recorded for the reference spectrum at 25 °C. An inter-scan delay of 1 sec was used for both the STD and the reference experiments.

Mass Spectrometric Analyses. All mass spectrometric experiments with Aβ were carried out on a Synapt G2 (Waters, Manchester, UK). Two different ionization methods, electrospray (ESI) and nano-electrospray ionization (nESI), were applied. (a) By ESI, $A\beta_{40}$ (100 μM) was prepared with a compound [**L2-b** or **L2-b2** (500 μM)] in 20 mM ammonium acetate (pH 7.5) with and without the addition of $CuCl_2$ (100 μM). Prepared samples were incubated at 37 °C for 6 h (for metal-free samples) and 1 h [for Cu(II)-containing samples] without agitation. Incubated samples

were diluted by 10-fold before mass spectrometric analysis. The capillary voltage, sampling cone voltage, and source temperature were adjusted to 2.8 kV, 70 V, and 40 °C, respectively. (b) Samples were ionized using a nano-electrospray source operated in the positive ion mode. MS instrumentation was operated at a backing pressure of 2.7 mbar and sample cone voltage of 40 V. The *m/z* scale was calibrated using aqueous cesium iodide (20 mg/mL). For peptide-derivative-metal ligation studies, aliquots of $A\beta_{40}$ peptides (final concentration, 20 μM) were sonicated for 5 sec prior to incubation with or without a source of Cu(II) [copper(II) acetate; 20 μM] at 37 °C for 10 min. After the initial incubation, samples were titrated against a source of the ligand (final concentration: 0, 20, 40 and 120 μM) and incubated at 37 °C for 30 min prior to analysis. Solution conditions were 100 mM ammonium acetate (pH 7.5) with 1% v/v DMSO. Accurate mass values for covalently modified complexes were calculated using the monoisotopic peak difference between apo and modified states with errors reported as a function of two times the standard deviation. All other conditions are consistent with previously published methods.[10]

Molecular Dynamics (MD) Simulations. Molecular docking procedures were performed using the Autodock Vina 1.5.6[11] software to investigate the binding of **L2-b**, **L2-b1**, and **L2-b2** to the $A\beta_{40}$ monomer and fibril. All three molecular docking methods were utilized: (i) rigid docking, (ii) flexible docking, and (iii) rigid docking on different conformations of fibrils. Since the flexibility of the receptor (the Aβ monomer and fibril) was absent in rigid docking, multiple structures of the Aβ fibrils derived from short-term 5 ns molecular dynamics (MD) simulations in an aqueous solution were used in the third method of docking. Furthermore, due to the flexibility of the monomer, a long 100 ns MD simulation was run on the peptide alone. The size of the grid was chosen to cover the whole ligand−protein complex, and the spacing was kept to

1.00 Å which is a standard value for Autodock Vina. Each docking trial produced 20 poses with an exhaustiveness value equal to 20. Snapshots were taken every nanosecond to allow for docking to different monomeric Aβ_{40} conformations. 20 poses were obtained for each snapshot taken, totaling 2000 poses. Starting structures for MD simulations were chosen based on residue binding abundances obtained by careful analysis of the docked poses.

The MD simulations of **L2-b**, **L2-b1**, or **L2-b2** bound to Aβ monomers or fibrils were performed using the GROMACS program utilizing the GROMOS force field GROMOS96 53A6.[12] For the monomer, unrestrained 25 ns, all-atom MD simulations were performed, where the first 5 ns of the simulation was part of the pre-production phase followed by 20 ns of a production phase. For the fibril, unrestrained 50 ns, all-atom MD simulations were carried out. For all simulations, the starting structures were placed in a cubic box with dimensions of 40 × 40 × 40 Å for monomeric Aβ_{40} (PDB 1BA4[13]) and 74 × 60 × 50 Å for the 2-fold fibrils (PDB 2LMN[14]). This dismisses unwanted effects that may arise from the applied periodic boundary conditions (PBC). The box was filled with single point charge (SPC) water molecules. Some water molecules were replaced by sodium and chloride ions to neutralize the system. The starting structures were subsequently energy-minimized with a steepest descent method for 3,000 steps. The results of these minimizations produced the starting structures for the MD simulations. The MD simulations were then carried out with a constant number of particles (N), pressure (P), and temperature (T) (NPT ensemble). The SETTLE algorithm[15] was used to constrain bond lengths and angles of the water molecules, while the LINCS algorithm[16] was used to constrain the bond lengths of the peptide. The long-range electrostatic interactions were calculated by the particle-mesh ewald (PME) method.[17] A constant pressure of 1 bar was applied with a coupling constant of 1.0 ps; peptide, water molecules, and ions were coupled separately to a bath at 300 K with a

coupling constant of 0.1 ps. The equation of motion was integrated at each 2 fs time steps. The tools available in GROMACS were utilized to analyze the MD trajectories. We used the most representative structures for the structural elucidation which were derived from the cluster analysis, where the trajectories are analyzed by grouping structurally similar frames [root-mean-square deviation (rmsd) cutoff of 0.30 nm], while the frame with the largest number of neighbors is denoted as a middle structure that represents that particular cluster. YASARA program[18] was used for visualization and preparation of the structural diagrams presented in this study.

Cell Viability Studies. Human neuroblastoma SK-N-BE(2)-M17 (M17) cells (ATCC, Manassa, VA, USA) were cultured in media containing 1:1 Minimum Essential Media (MEM; GIBCO, Grand Island, NY, USA), Ham's F12K Kaighn's Modification Media (F12K; GIBCO), 10% (v/v) fetal bovine serum (FBS; Atlanta Biologicals, Flowery Branch, GA, USA), 100 U/mL penicillin (GIBCO), and 100 mg/mL streptomycin (GIBCO). The cells were grown and maintained at 37 °C in a humidified atmosphere with 5% CO_2. M17 cells were seeded in a 96-well plate (150,000 cells in 100 μL per well) and treated with various concentrations of compounds (0–50 μM, 1% v/v DMSO) with and without $CuCl_2$ or $ZnCl_2$ (1:1 or 1:2 metal/ligand ratio) with and without $A\beta_{40}$ (Aβ:metal:compound = 10:10:20 μM). After 24 h incubation at 37 °C, 25 μL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; 5 mg/mL in phosphate buffered saline (PBS), pH 7.4, GIBCO] was added to each well and the plates were incubated for 4 h at 37 °C. Formazan produced by the cells was dissolved in a solution containing *N*,*N*-dimethylformamide (DMF, 50% v/v aq) and sodium dodecyl sulfate (SDS, 20% w/v) overnight at room temperature. The absorbance at 600 nm was measured on a microplate reader.

Trolox Equivalent Antioxidant Capacity (TEAC) Assay. The free organic radical scavenging capacity of compounds was determined by the TEAC assay in (a) EtOH or (b) M17 cell lysates. (a) The assay in EtOH was performed according to a previously reported method with slight modifications.[3a,3b] To generate blue ABTS cation radicals [$ABTS^{\bullet+}$; ABTS = 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; Sigma], ABTS (7.0 mM, Sigma) with potassium persulfate (2.5 mM) was dissolved in 5 mL of water and incubated for 16 h in the dark at room temperature. The resulting solution of $ABTS^{\bullet+}$ was diluted with EtOH to absorbance of *ca.* 0.7 at 734 nm. The solution of $ABTS^{\bullet+}$ (200 μL) was added to the wells of a clear 96 well plate and incubated at room temperature for 5 min in the plate reader. **L2-b1**, **L2-b2**, **PMA1**, **PMA2**, **DPA1**, **DPA2**, or Trolox (Trolox = 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid; dissolved in EtOH) [various concentrations: 0, 1, 2.5, 5, 7.5, 10, 15, and 20 μM] was incubated with the $ABTS^{\bullet+}$ solution at room temperature for 10 min. The percent inhibition was calculated according to the measured absorbance at 734 nm [% inhibition = $100 \times (A_0 - A)/A_0$] and plotted as a function of ligand concentration. The TEAC value of compounds was calculated as a ratio of the slope of the compound to that of Trolox. The measurements were carried out in triplicate. (b) The assay employing cell lysates was conducted following the protocol of the antioxidant assay kit purchased from Cayman Chemical Company (Ann Arbor, MI, USA) with minor modifications.[3a,3b] For the antioxidant assay using cell lysates, cells were seeded in a 6 well plate and grown to approximately 80-90% confluence. Cell lysates were prepared following a previously reported method with modifications.[19] M17 cells were washed once with cold PBS (pH 7.4, GIBCO) and harvested by gently pipetting off adherent cells with cold PBS. The cell pellet was generated by centrifugation (2,000 g for 10 min at 4 °C). This pellet was sonicated on ice (5 sec pulses, 3 times with 20 sec intervals between each pulse) in 2 mL of cold Assay Buffer

(5 mM potassium phosphate, pH 7.4; 0.9% NaCl; 0.1% glucose). The cell lysates were centrifuged at 5,000 g for 10 min at 4 °C. The supernatant was removed and stored on ice until use. For standard and samples in 96 well plates, the supernatant of cell lysates (10 μL) was delivered followed by addition of compound, metmyoglobin, ABTS, and H_2O_2 in order. After 5 min incubation at room temperature on a shaker, absorbance values at 750 nm were recorded. The final concentrations (45, 90, 135, 180, 225, and 330 μM) of compounds and Trolox were used. The antioxidant concentration was calculated according to the measured absorbance [% inhibition = $100 \times (A_0 - A)/A_0$, where A_0 is absorbance of the supernatant of cell lysates]. The measurements were conducted in triplicate.

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Table S1. Values (MW, *c*log*P*, HBA, HBD, PSA, logBB, and $-\log P_e$)[a] for small molecules.

Calculation	L2-b1	L2-b2	PMA1	PMA2	DPA1	DPA2	Lipinski's rules and others
MW	227	270	108	151	199	285	≤ 450
*c*log*P*	2.31	2.47	-0.40	0.31	0.67	2.10	≤ 5.0
HBA	3	4	2	3	3	5	≤ 10
HBD	1	1	2	2	1	1	≤ 5
PSA	28.2	31.4	38.9	42.1	37.8	44.3	≤ 90
logBB	0.064	0.041	–0.507	–0.446	–0.327	–0.206	< –1.0 (poorly)
$-\log P_e$	4.13 ± 0.01	4.52 ± 0.01	5.02 ± 0.06	4.68 ± 0.03	5.18 ± 0.05	4.47 ± 0.01	$-\log P_e$ < 5.4 (CNS+)
CNS± prediction[b]	CNS+	CNS+	CNS+	CNS+	CNS+	CNS+	$-\log P_e$ > 5.7 (CNS–)

[a]MW, molecular weight; *c*log*P*, calculated logarithm of the octanol water partition coefficient; HBA, hydrogen bond acceptor atoms; HBD, hydrogen bond donor atoms; PSA, polar surface area; logBB = –0.0148 × PSA + 0.152 × *c*log*P* + 0.139 (logBB < –1.0, poorly distributed to the brain); $-\log P_e$ values were determined using the Parallel Artificial Membrane Permeability Assay adapted for BBB (PAMPA-BBB) were then calculated by the PAMPA 9 Explorer software v. 3.8. [b]Prediction of a compound's ability to penetrate the central nervous system (CNS) on the basis of literature values. Compounds categorized as CNS+ have the possibility to penetrate the BBB and are available in the CNS.

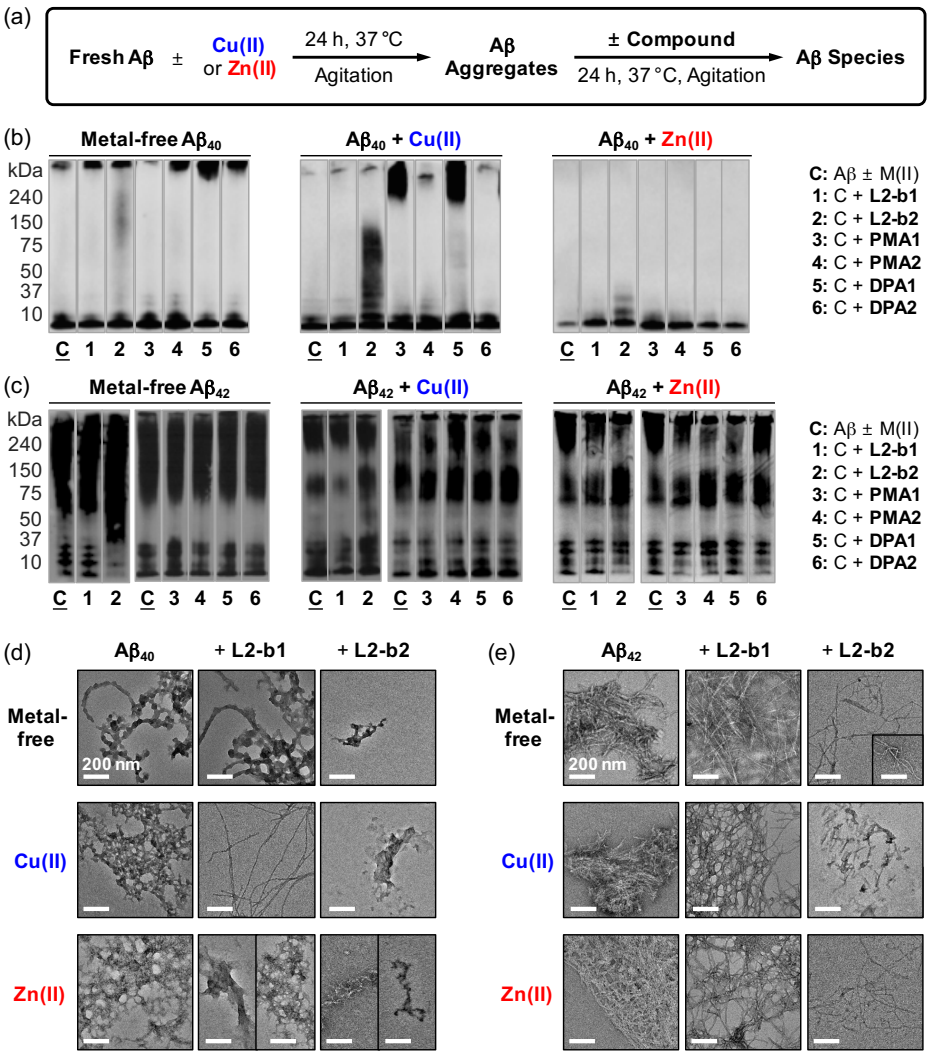


Figure S1. Influence of compounds on disassembly and further aggregation of metal-free Aβ and metal–Aβ aggregates. (a) Scheme of the disaggregation experiment. Analysis of size distributions of the resultant (b) $A\beta_{40}$ and (c) $A\beta_{42}$ by gel/Western blot with an anti-Aβ antibody (6E10). Conditions: [Aβ] = 25 μM; [$CuCl_2$ or $ZnCl_2$] = 25 μM; [compound] = 50 μM; pH 6.6 (for Cu(II) experiments) or pH 7.4 (for metal-free and Zn(II) experiments); 37 ˚C; constant agitation. TEM images of the resultant (d) $A\beta_{40}$ and (e) $A\beta_{42}$ aggregates from (b) and (c), respectively.

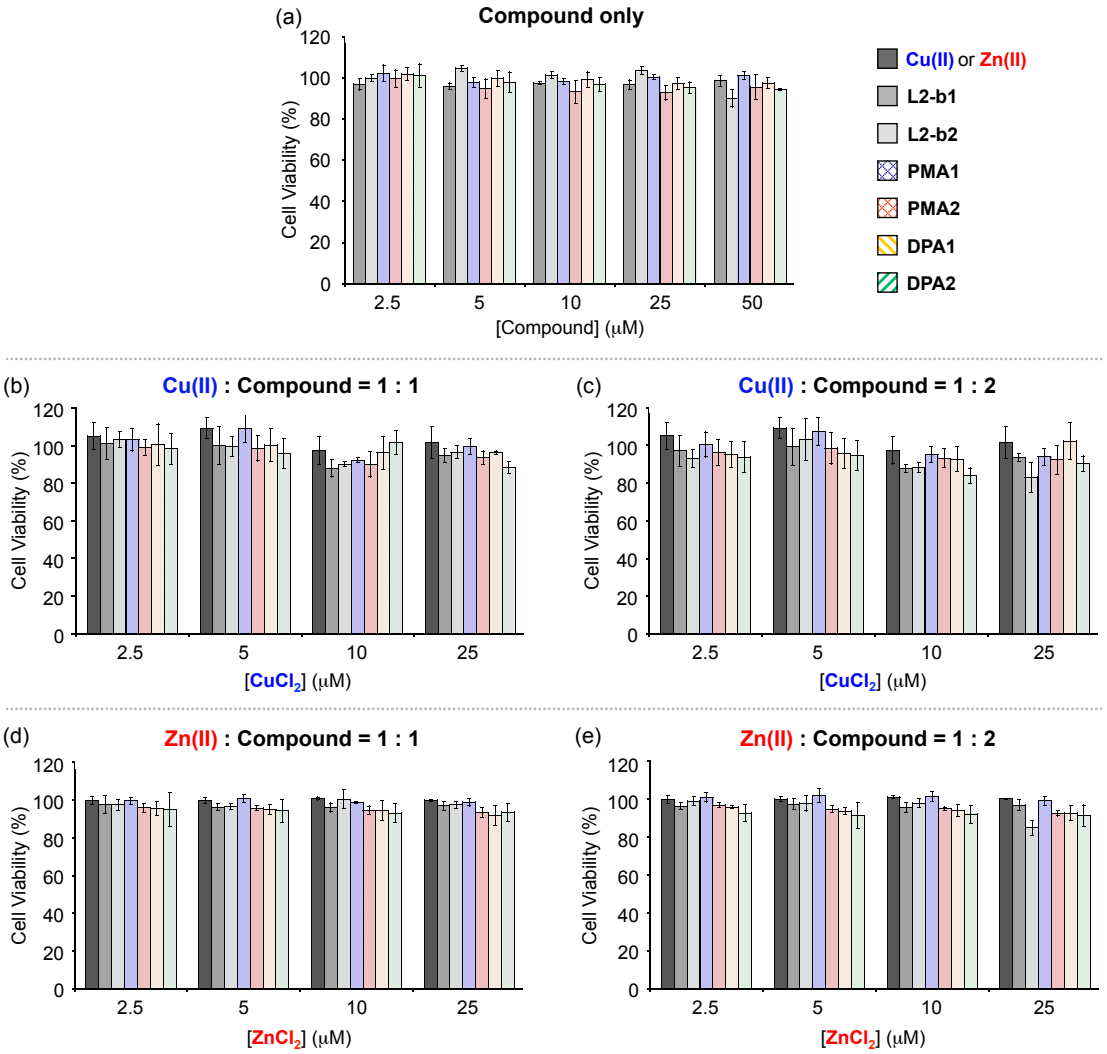


Figure S2. Viability of cells treated with small molecules in both the absence and presence of $CuCl_2$ or $ZnCl_2$. M17 cells were treated with (a) various concentrations of compounds (2.5–50 μM; 1% v/v DMSO) with and without $CuCl_2$ [(b) 1:1 or (c) 1:2] or $ZnCl_2$ [(d) 1:1 or (e) 1:2]. Cell viability (%) was determined by the MTT assay compared to cells treated with DMSO only (0-1%, v/v). Error bars represent the standard error (SE) from three independent experiments.

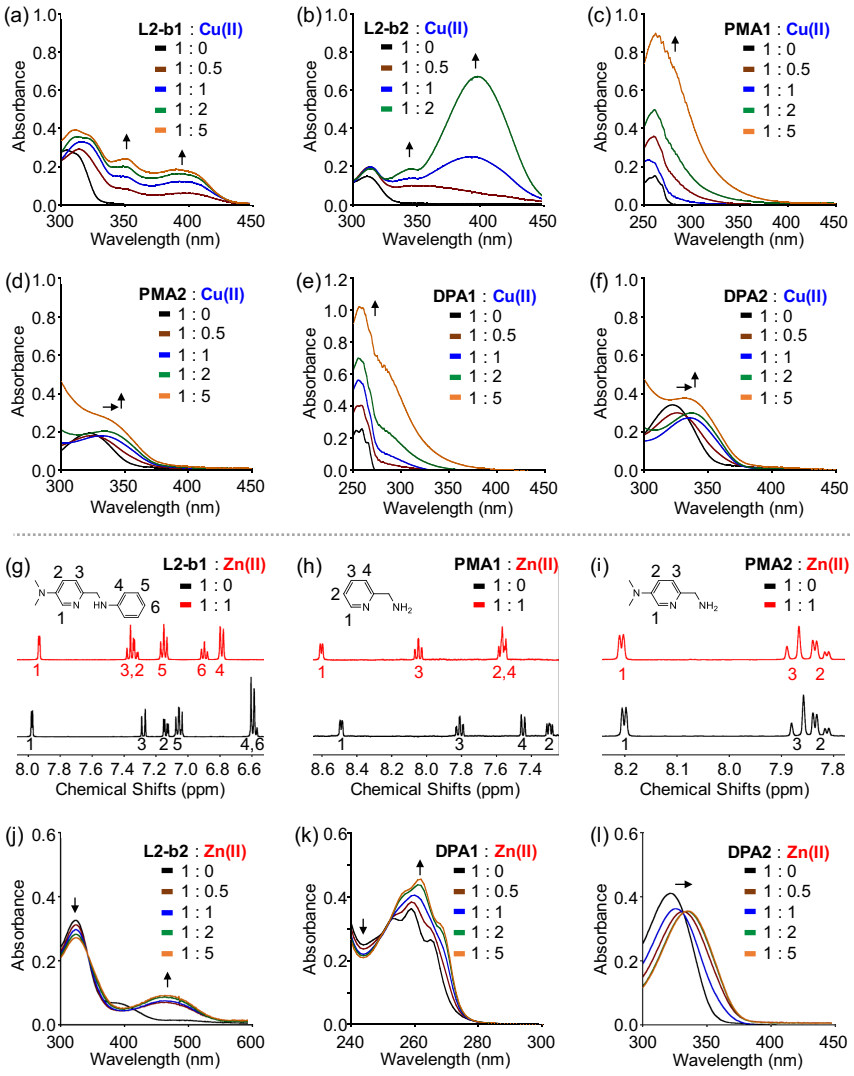


Figure S3. Metal binding studies of small molecules. UV–vis spectra of (a) **L2-b1**, (b) **L2-b2**, (c) **PMA1**, (d) **PMA2**, (e) **DPA1**, and (f) **DPA2** with $CuCl_2$ (up to 2 or 5 equiv) in EtOH. Conditions: [compound] = 25 μM (for **L2-b2**) or 50 μM (for **L2-b1**, **PMA1**, **PMA2**, **DPA1**, and **DPA2**); [$CuCl_2$] = 0–250 μM; room temperature; incubation for 30 min (for **L2-b1** and **L2-b2**) or 10 min (for **PMA1**, **PMA2**, **DPA1**, and **DPA2**). ^{1}H NMR spectra of (g) **L2-b1**, (h) **PMA1** (black) and (i) **PMA2** (black) with $ZnCl_2$ (1 equiv, red) in CD_3CN. Conditions: [**L2-b1**, **PMA1**, or **PMA2**] = 4 mM; [$ZnCl_2$] = 4 mM; room temperature; incubation for 5 min. UV–vis spectra of (j) **L2-b2**, (k) **DPA1**, and (l) **DPA2** with $ZnCl_2$ (up to 5 equiv) in EtOH. Conditions: [**L2-b2**, **DPA1**, and **DPA2**] = 50 μM; [$ZnCl_2$] = 0–250 μM; room temperature; incubation for 30 min (for **L2-b2**) or 10 min (for **DPA1** and **DPA2**).

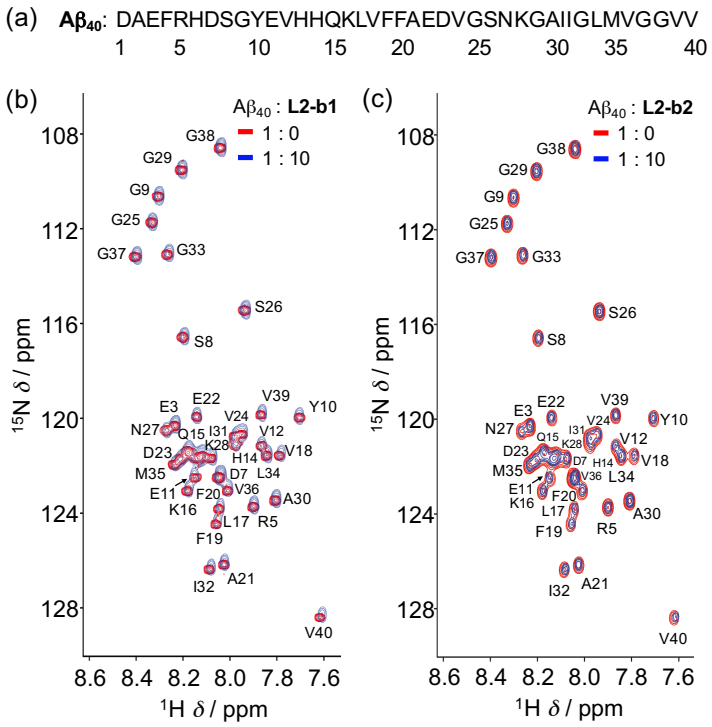
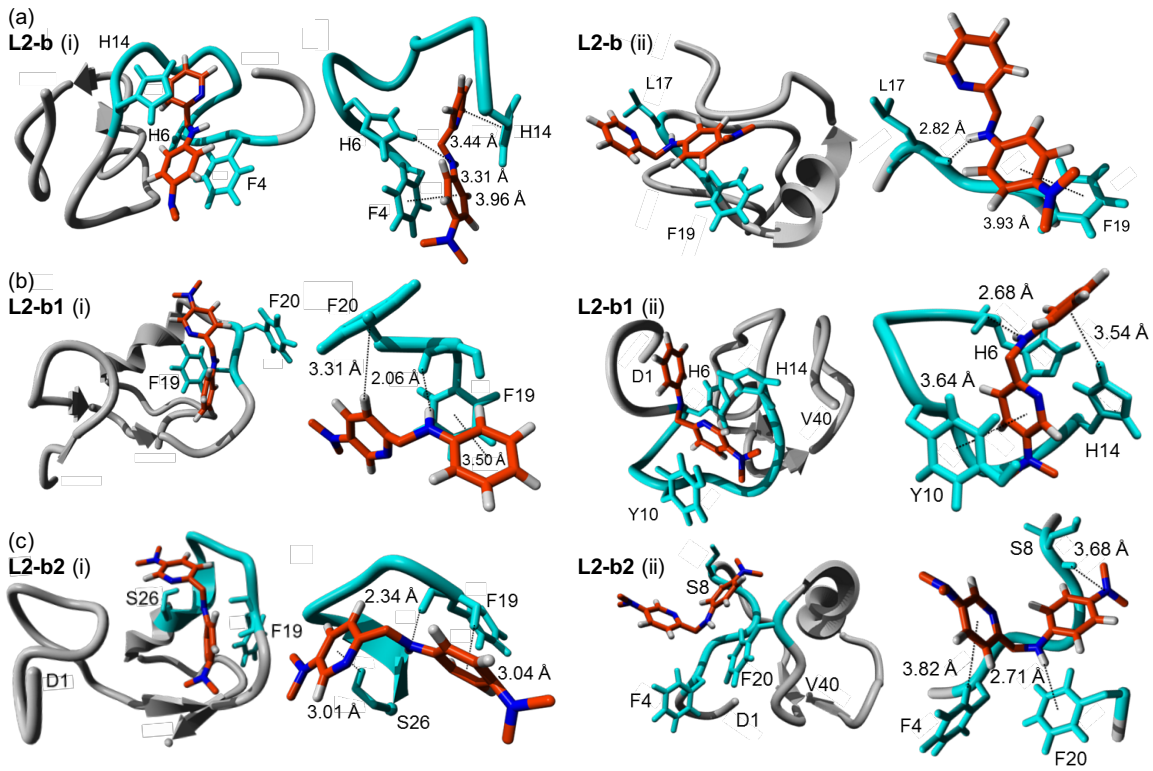


Figure S4. Interactions of **L2-b1** or **L2-b2** with metal-free monomeric $A\beta_{40}$. (a) Amino acid sequence of $A\beta_{40}$. 2D ^{1}H–^{15}N SOFAST-HMQC NMR spectra of a solution of uniformly ^{15}N-labeled monomeric $A\beta_{40}$ with (red) and without (blue) 10 mole % of (b) **L2-b1** or (c) **L2-b2**. Conditions: [$A\beta_{40}$] = 80 μM; [**L2-b1** or **L2-b2**] = 0 or 800 μM; 20 mM PO_4, pH 7.4, 50 mM NaCl; 7% D_2O (v/v); 10 °C.



Energy \ Compound	L2-b (i)	L2-b (ii)	L2-b1 (i)	L2-b1 (ii)	L2-b2 (i)	L2-b2 (ii)
Electrostatic (kcal/mol)	1.105	0.847	1.292	2.581	1.132	0.658
Hydrophobic (kcal/mol)	-2.639	-1.711	-2.439	-2.839	-3.014	-2.053
Binding (kcal/mol)	-1.534	-0.364	-1.147	-0.258	-1.882	-1.368

Figure S5. MD simulations showing interactions of **L2-b**, **L2-b1**, or **L2-b2** with monomeric $A\beta_{40}$. Possible sites and energy of interaction of $A\beta_{40}$ (PDB 1BA4) with (a) **L2-b**, (b) **L2-b1**, or (c) **L2-b2** after all-atom MD simulations are summarized. Right: The zoomed-in view of each binding site with residues showing interaction distances labeled in Å with dashed lines.

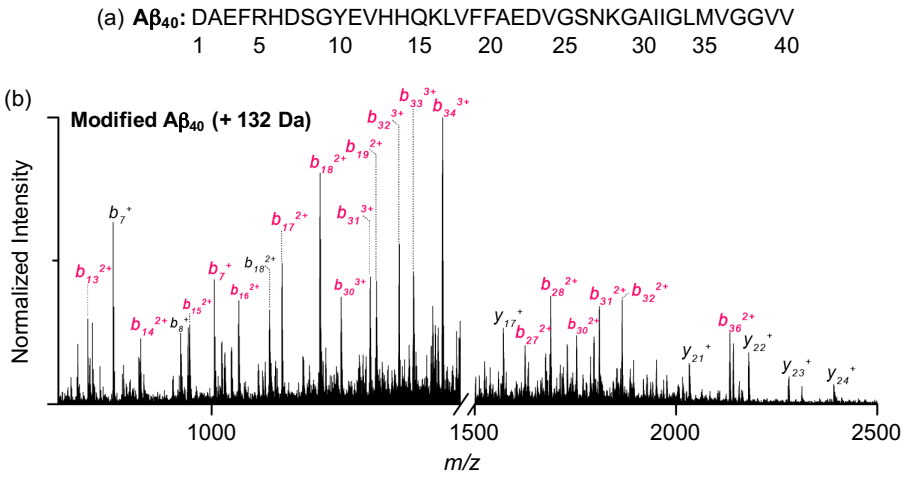


Figure S6. Tandem mass spectrometry (MS/MS) sequencing studies of modified metal-free Aβ by **L2-b2**. (a) Amino acid sequence of $A\beta_{40}$. (b) The MS/MS study of the modified $A\beta_{40}$ generated upon treatment with **L2-b2**. These data support that the amino acid sequence of Aβ is directly interact with structurally transformed **L2-b2**. The Aβ species containing the identified +131.97 Da covalent modification are highlighted in magenta.

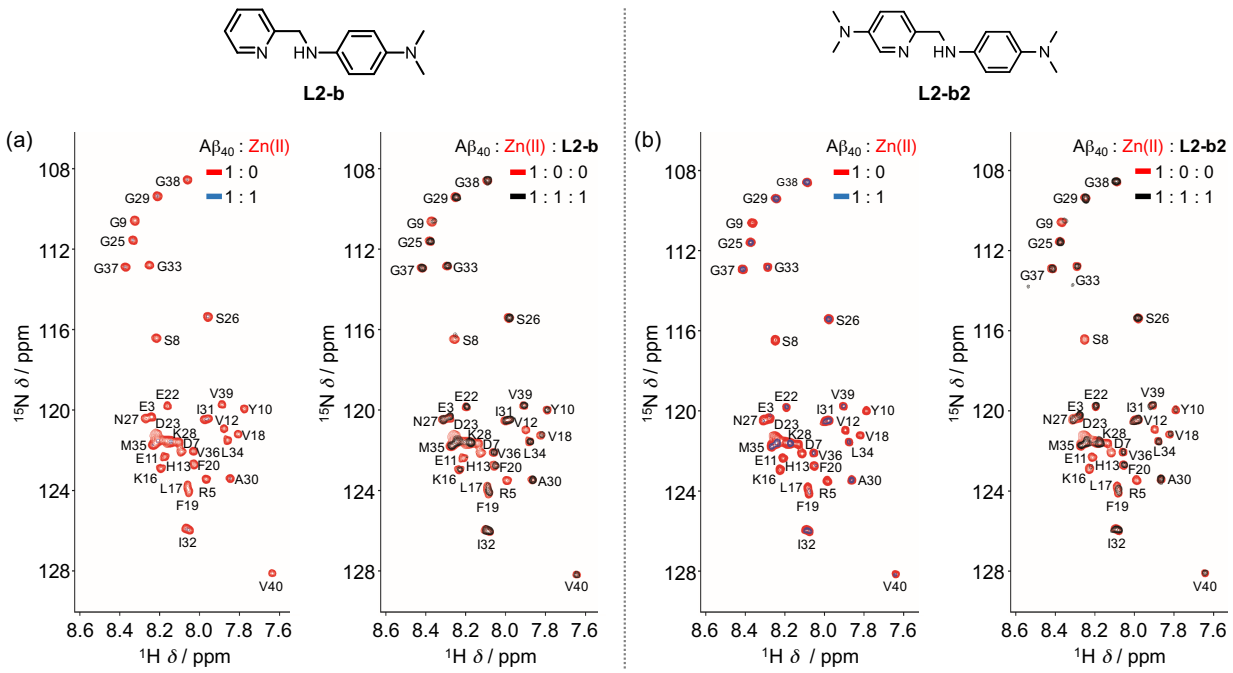
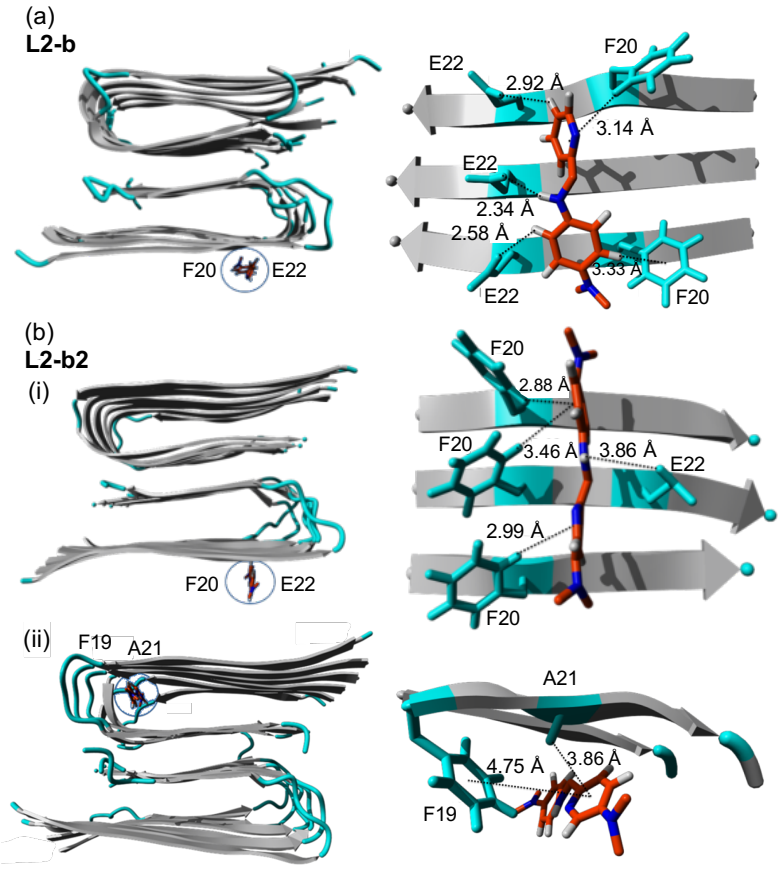


Figure S7. Interactions of **L2-b** or **L2-b2** with Zn(II)-treated monomeric $A\beta_{40}$. 2D ^{1}H–^{15}N SOFAST-HMQC NMR spectra of a solution of uniformly ^{15}N-labeled monomeric $A\beta_{40}$ treated with Zn(II) (left) and (a) **L2-b** or (b) **L2-b2** (right). Conditions: [$A\beta_{40}$] = 80 μM; [$ZnCl_2$] = 80 μM; [**L2-b** or **L2-b2**] = 80 μM; 20 mM PO_4, pH 7.4, 50 mM NaCl; 7% v/v D_2O.



Compound \ Energy	Electrostatic (kcal/mol)	Hydrophobic (kcal/mol)	Binding (kcal/mol)
L2-b	0.164	-2.881	-2.717
L2-b2 (i)	1.879	-3.235	-1.357
L2-b2 (ii)	2.831	-3.982	-1.151

Figure S8. MD simulations showing interactions of **L2-b** or **L2-b2** with metal-free Aβ_{40} fibrils. Potential binding sites and energy of interaction of (a) **L2-b** or (b) **L2-b2** with Aβ_{40} fibrils (PDB 2LMN) after all-atom MD simulations are summarized. Right: The zoomed-in view of each binding site with residues showing interaction distances labeled in Å with dashed lines.