SUPPORTING INFORMATION

for

Metal-Chelating Benzothiazole Multifunctional Compounds for the Modulation and ^{64}Cu PET Imaging of Aβ Aggregation

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I. Experimental Procedures

General Methods. All animal studies were performed in strict accordance with the NIH guidelines for the care and use of laboratory animals (NIH Publication No. 85-23 Rev. 1985) and were in compliance with the Guidelines for Care and Use of Research Animals established by the Division of Comparative Medicine and the Animal Studies Committee of Washington University in St. Louis. The animal protocol #20190073 including all the animal studies performed herein was reviewed and approved by the Institutional Animal Care and Use Committee of Washington University in St. Louis. No experimentation with human subjects was performed in these studies. All reagents were purchased from commercial sources and used as received unless stated otherwise. 1,4-dimethyl-1,4,7-triazacyclononane (Me_2Htacn) and 1-methyl-1,4,7-triazacyclononane (MeH_2tacn) were synthesized according to reported procedures.[1-2] Solvents were purified prior to use by passing through a column of activated alumina using an MBRAUN SPS. All solutions and buffers were prepared using metal-free Millipore water that was treated with Chelex overnight and filtered through a 0.22 μm nylon filter. ^1H (300.121 MHz) and ^{13}C (151 MHz) NMR spectra were recorded on a Varian Mercury-300 spectrometer. Chemical shifts are reported in ppm downfield from tetramethylsilane. UV−visible spectra were recorded on a Varian Cary 50 Bio spectrophotometer and are reported as λ_{max}, nm (ε, M^{-1} cm^{-1}). TEM analysis was performed at the Nano Research Facility (NRF) at Washington University. **HYR-1**, **-4**, **-14**, **-16**, **-17** and **-18** were dissolved in DMSO to prepare 10.0 mM stock solutions.

Amyloid *β* Peptide Experiments. A*β* powder was prepared by dissolving commercial A*β* peptide (AnaSpec) in ammonia hydroxide solution (1%, v/v). The solution was then aliquoted out and lyophilized overnight. The resulting aliquoted powder was stored at –80 °C. A*β* monomers were generated by dissolving A*β* powder in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, 1 mM) and incubating for 1 h at room temperature.[3] The solution was then evaporated overnight and dried by vacuum centrifuge to result monomeric films. A*β* fibrils were generated by dissolving monomeric A*β* films in DMSO, diluting into the appropriate buffer, and incubating for 24 h at 37 °C with continuous agitation (final DMSO concentration was < 2%).

Fluorescence Measurements. All fluorescence measurements were performed using a SpectraMax M2e plate reader (Molecular Devices). For ThT fluorescence studies, samples were

diluted to a final concentration of 2.5 μM Aβ in PBS containing 10 μM ThT and the fluorescence measured at 485 nm (λ_{ex} = 435 nm). For ThT competition assays, a 5 μM Aβ fibril solution with 2 μM ThT was titrated with tiny amounts of compound and the ThT fluorescence measured ($\lambda_{ex}/\lambda_{em}$ = 435/485 nm). For calculating K_i values, a K_d value of 1.16 μM was used for the binding of ThT to Aβ fibrils.

Histological Staining of 5×FAD Mice Brain Sections. Eight-month-old 5×FAD transgenic mice brain sections were blocked with bovine serum albumin (2% BSA in PBS, pH 7.4, 10 min) and covered with a PBS solution of compound and Congo Red (5 μM) for 60 min. The sections were treated with BSA again (4 min) to remove any compound non-specifically bound to the tissue. Finally, the sections were washed with PBS (3 × 2 min), DI water (2 min), and mounted with non-fluorescent mounting media.[4] For antibody staining, the brain sections were incubated with CF594-conjugated anti-Aβ antibody (CF594-6E10antibody) solution (1:1000 dilution in blocking solution) at room temperature for 1 h instead of Congo Red. The brain sections were then washed with PBS (3 × 2 min) and mounted with mounting media. The stained brain sections were imaged using a Zeiss LSM 7010 confocal fluorescent microscope.

Native Gel Electrophoresis and Western Blotting. All gels, buffers, membranes, and other reagents were purchased from Invitrogen and used as directed except where otherwise noted. Samples were separated on 10−20% gradient Tris-tricine mini gels. The gel was transferred to a nitrocellulose membrane in an ice bath and the protocol was followed as suggested except that the membrane was blocked overnight at 4 °C. After blocking, the membrane was incubated in a solution (1:2000 dilution) of 6E10 anti-Aβ primary antibody (BioLegend) for 3 h followed by an alkalinephosphatase antimouse secondary antibody. The results were visualized using Invitrogen's Western Breeze Chemiluminescent kit and the protein bands were imaged using a FUJIFILM Luminescent Image Analyzer LAS-1000CH.

Transmission Electron Microscopy (TEM). Glow-discharged grids (Formar/Carbon 300-mesh, Electron Microscopy Sciences) were treated with Aβ_{42} samples (25 μM, 7 μL) for 5 min at room temperature. The excess solution was removed using filter paper and the grids were rinsed with H_2O (7 μL, 2 × 1 min). Then the grids were stained with uranyl acetate (1% w/v in H_2O, 7 μL) for 2 min, rinsed with H_2O (7 μL, 1 min), blotted with filter paper, and dried under vacuum overnight. Images were captured using a FEI G2 Spirit Twin microscope (60−80 kV, 6500−97000×

magnification). TEM analysis was performed at the Nano Research Facility (NRF) at Washington University in St. Louis.

Trolox equivalent antioxidant capacity (TEAC) Assay. The antioxidant ability of multifunctional compounds was investigated by TEAC assay with the established protocol. ABTS (8.2 mg, 2,7 eq.) and $K_2S_2O_8$ (1.6 mg, 1 eq.) were dissolved in 2 ml de-ionized water to prepare $ABTS^{+\bullet}$ cation radicals. The reaction was proceeded overnight in the dark at room temperature. The $ABTS^{+\bullet}$ solution was aliquoted (3-42 μL) into a transparent 96-well plate and diluted with methanol to 300 μL that each volume was monitored at 470 nm via UV-vis spectroscopy to determine the concentration of the radical solution displaying an absorbance around 0.7. The absorbance of the $ABTS^{+\bullet}$ radicals increased linearly in this range and a volume of 36 μL was selected for the following TEAC assay led an approximate absorbance around 0.7. Trolox, glutathione, **HYR-14** and **HYR-16** were dissolved in methanol to prepare 1.5 mM stock solutions. Each solution in different final concentrations (25, 50, 75 ,100 μM) were added into 96-well plate and diluted with methanol to a volume of 270 μL. After adding 36 μL ABTS solution into each well, the absorbance at 470 nm was recorded at different time points (1, 3, 6, 15 min) immediately.

Coumarin-3-carboxylic acid (CCA) antioxidant assay. According to previous reports,[5] CCA was used to measure hydroxy radical production induced by Cu ions. The stock solutions (10 mM) of CCA, CuSO4, ascorbic acid, **HYR-14** and **HYR-16** were prepared in water. Each compound was added into 96-well plate and diluted with PBS (1×, pH 7.4) to make final solution (final concentration: CCA [100 μM], CuSO4 [40 μM], ascorbic acid [400 μM], **HYR-14** [0-160 μM] and **HYR-16** [0-160 μM], final volume: 200 μL). Then, the fluorescence of 7-hydroxycoumarin-3-carboxylic acid was monitored under excitation at 395 nm and emission at 450 nm for 1 h using a SpectraMax M2e plate reader (Molecular Devices, USA).

Cytotoxicity Studies (Alamar Blue Assay). Mouse neuroblastoma Neuro2A (N2A) cell lines were purchased from the American Type Culture Collection (ATCC). Cells were grown in DMEM/10% FBS, which is the regular growth media for N2A cells. N2A cells were plated to each well of a 96 well plate (2.5×10^4/well) with DMEM/10% FBS. The media was changed to DMEM/N2 media 24 h later. After 1 h, the reagents (20 μM $A\beta_{42}$ species, compounds, and metals) were added. Due to the poor solubility of compounds in water or media, the final amount of DMSO used was 1% (v:v). After an additional incubation of 40 h, the Alamar blue solution was added in

each well and the cells were incubated for 90 min at 37 °C. Fluorescence intensity was measured at 590 nm (excitation wavelength = 560 nm).

Radiolabeling. ^{64}Cu was produced by a (p,n) reaction on enriched ^{64}Ni on a TR-19 biomedical cyclotron (Advanced Cyclotron Systems Inc, British Columbia, Canada) at Mallinckrodt Institute of Radiology, Washington University School of Medicine, and purified with an automated system using standard procedures.[6] A stock solution of $^{64}CuCl_2$ was diluted with a 10-fold excess of 0.1 M ammonium acetate (NH_4OAc), pH 7 for radiolabelling. Labelling of MFCs with ^{64}Cu was achieved by adding 1 mM of compounds to 7.4 MBq (200 μCi) of $^{64}CuCl_2$ in 100 μL of 0.1 M NH_4OAc, pH 7. The reactions were incubated on a thermomixer with 800 rpm agitation at 45 °C for 20 - 60 min. Radiolabelled complexes were analysed by high-performance liquid chromatography (HPLC, Shimadzu 10Avp system) with a mobile phase of water (0.1% TFA) and acetonitrile (0.1% TFA), 0-100% acetonitrile over 10 min with a 1 mL/min flow rate. A radiochemical yield of greater than 95% was achieved for all labelled compounds and therefore they were used without further purification.

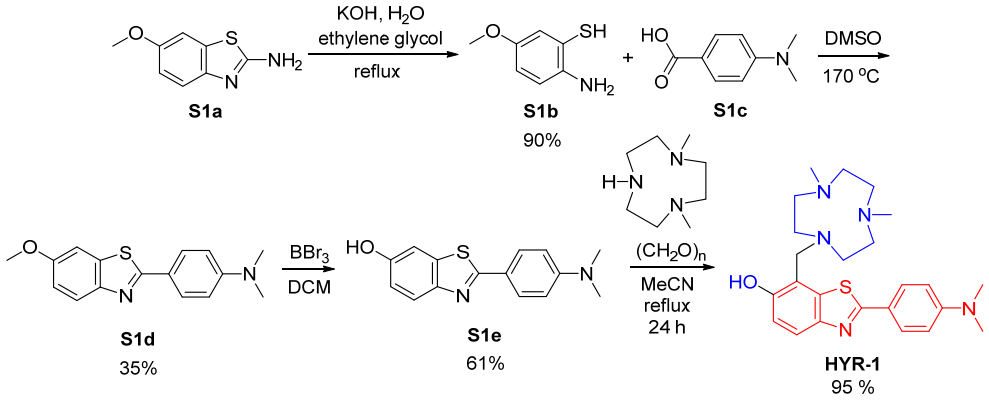
Lipophilicity Studies. The ^{64}Cu-labeled complexes (5 μL, 0.37 MBq, 10 μCi) were added into an 1:1 (v:v) mixture of n-octanol and pH 7.4 PBS (500 μL/ea). The samples were vortexed at 1,000 rpm for 1 h, and then allowed for 30 min for the layers to separate. Aliquots (100 μL) from the aqueous and the n-octanol layers were removed and counted separately in an automated gamma counter. The distribution coefficients were calculated using the ratio of (activity detected in n-octanol)/(activity detected in aqueous layer) to get the *log* D_{oct} values. The experiment was conducted in six replicates, and the average of the different measurements was recorded as the final *log* D_{oct} value for each compound.

***Ex vivo* Autoradiography Studies.** Brain sections of 10-month-old 5×FAD transgenic mice and aged-matched WT mice were immersed into a cryo-protectant solution. These sections were sorted and carefully removed using phosphate buffer in saline (PBS) with 1 % tween-20 solution and mounted onto an adhesive glass slide (CFSA 1X, Leica Bio Systems). Each section was washed with 100% PBS three times, and ~0.925 MBq (25 μCi) of ^{64}Cu-labeled MFC in a 100 μL total volume was added to completely cover the brain section and incubate for 1 h at room temperature in a shielded bunker. After the incubation, brain sections were washed using PBS with five 1-minute cycles and briefly air-dried. The imaging slides were mounted on to a phosphor imaging

screen plate (GE Healthcare Life Sciences) and were exposed for 1–5 minutes. The plates were then scanned using a biomolecule imager (Typhoon FLA 9500, GE) and the resulting images were processed using ImageQuant TL 8.1 (GE Healthcare Life Sciences).

Biodistribution Studies. All animal experiments were performed in compliance with the Guidelines for Care and Use of Research Animals established by the Division of Comparative Medicine and the Animal Studies Committee of Washington University School of Medicine. Initial biodistribution studies were conducted in wild type CD-1 female mice (Charles River Laboratories) of age 5-7 weeks weighing 25.8 ± 2.1 g. The injection dose was prepared by diluting in to a 90 % saline solution. The uptake of ^{64}Cu-labeled compounds was evaluated in mice that were injected via the tail vein with 0.22-0.37 MBq (6-10 μCi) of each compound per animal in 100 μL saline solution. After each time point (2, 60, and 240 min), mice were anesthetized with 1-2 % isoflurane and euthanized by cervical dislocation. Brain, blood, kidney, liver and other organs of interest were harvested and amount of radioactivity in each organ was counted on a gamma counter containing a NaI crystal. The data was corrected for radioactive decay and percent injected dose per gram (%ID/g) of tissue was calculated. All samples were calibrated against a known standard. Quantitative data were processed by Prism 7 (GraphPad Software, v 6.03, La Jolla, CA) and expressed as Mean ± SEM. Statistical analysis performed using one-way analysis of variance and Student's *t* test. Differences at the 95% confidence level ($p < 0.05$) were considered statistically significant.

II. Synthesis and characterization of multifunctional compounds



Scheme S1. Synthesis of **HYR-1**.

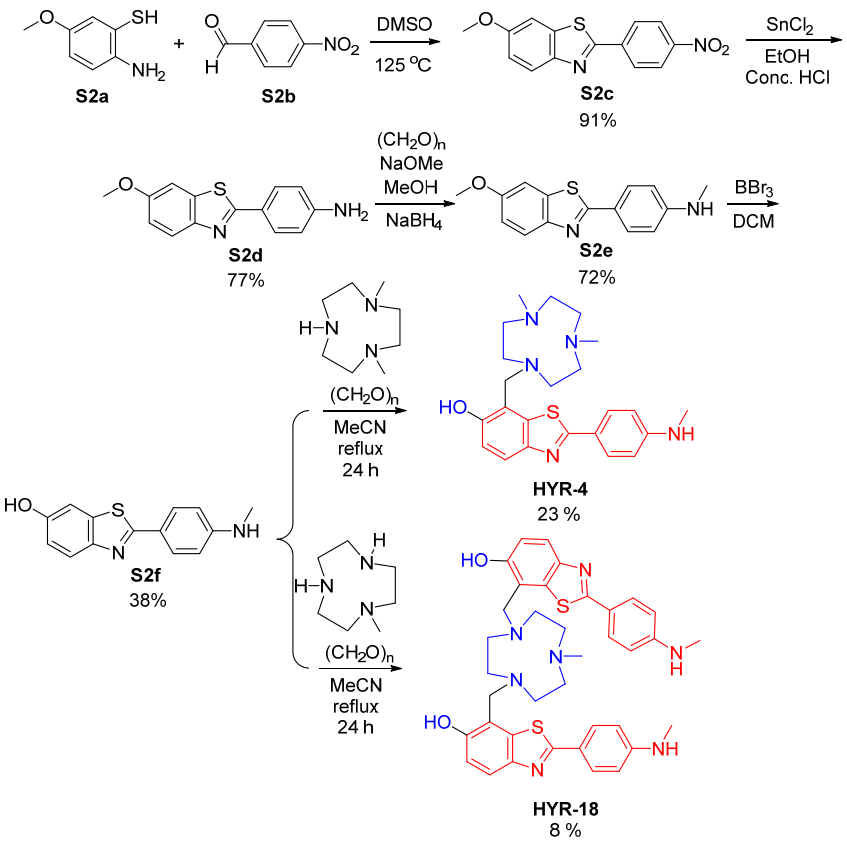
S1b. 2-Amino-6-methoxy-benzothiazole **S1a** (10.0 g, 57.3 mmol) was suspended in 50% KOH (60 g KOH dissolved in 60 mL water) and ethylene glycol (13.3 mL). The suspension was heated to reflux for 48 h. Upon cooling to RT, toluene (100 mL) was added and the reaction mixture was neutralized with acetic acid (60 mL). The organic layer was separated and the aqueous layer was extracted with another 70 mL of toluene. The toluene layers were combined and washed with water and dried over $MgSO_4$. Evaporation of the solvent gave **S1b** as yellow solid (8.0 g, 90%). ^{1}H NMR (acetone-d_6): δ(ppm): 6.78 (d, 1H, J = 2.7 Hz), 6.67 (d, 1H, J = 8.7 Hz), 6.47 (dd, 1H, J = 8.7, 3.0 Hz), 3.77 (s, 3H).

S1d. A mixture of **S1b** (0.75 g, 4.84 mmol) and **S1c** (0.72 g, 4.84 mmol) in DMSO (5 mL) was heated to 170 °C for 30 min. The reaction mixture was cooled to RT and poured into water. The organic compound was extracted with 50 mL of ethyl acetate 3 times. The combined layers were washed with water and dried over $MgSO_4$. The solvent was removed to give a yellowish residue that was purified by silica gel column chromatography using hexane/ethyl acetate (6:1) to yield a yellow solid **S1d** (0.49 g, 35%). ^{1}H NMR (acetone-d_6): δ(ppm): 7.90 (d, 2H, J = 9.0 Hz), 7.78 (d, 1H, J = 9.0 Hz), 7.53 (d, 1H, J = 2.5 Hz), 7.06 (dd, 1H, J = 8.9, 2.6 Hz), 6.83 (d, 2H, J = 9.0 Hz), 3.88 (s, 3H), 3.06 (s, 6H).

S1e. To a suspension of **S1d** (200 mg, 0.7 mmol) in CH_2Cl_2 (20 mL) was injected BBr_3 (1 M in CH_2Cl_2, 2.3 mL, 2.3 mmol). The reaction mixture was stirred at room temperature for 16 h. The reaction was quenched with water (20 mL) and the pH was adjusted to 4-7 with NaOH solution.

Precipitate was isolated by vacuum filtration, washed with water (3 × 5 mL), methanol (5 mL), CH_2Cl_2 (5 mL) and anhydrous ethanol (3 × 5 mL), and dried under vacuum to give a yellow solid **S1e** (116 mg, 61%). ^{1}H NMR (DMSO-d_6): δ (ppm): 9.72 (s, 1H), 7.78 (d, 1H, J = 8.7 Hz), 7.71 (d, 2H, J = 9.0 Hz), 7.31 (d, 1H, J = 2.4 Hz), 6.90 (dd, 1H, J = 8.7, 2.5 Hz), 6.78 (d, 2H, J = 9.0 Hz), 3.01 (s, 6H).

HYR-1. Paraformaldehyde (8 mg, 0.27 mmol) was added to a solution of 1,4-dimethyl-1,4,7-triazacyclononane (28 mg, 0.18 mmol) in MeCN (5 mL) and the resultant mixture was heated to reflux for 30 min. Then **S1e** (50 mg, 0.18 mmol) in MeCN (5 mL) was added, the solution was refluxed for an additional 24 h, and then cooled to room temperature. The solvent was removed to give a yellowish residue that was purified by silica gel column chromatography using CH_2Cl_2/MeOH/NH_4OH (100:15:2) to yield a yellow solid (75 mg, yield 95%). ^{1}H NMR ($CDCl_3$): δ (ppm): 7.89 (d, 2H, J = 9.0 Hz), 7.75 (d, 1H, J = 8.7 Hz), 7.02 (d, 1H, J = 8.7 Hz), 6.73 (d, 2H, J = 9.0 Hz), 4.02 (s, 2H), 3.04 (s, 6H), 2.95 (m, 4H), 2,68 (m, 4H), 2.59 (s, 4H), 2.41 (s, 6H). ^{13}C NMR ($CDCl_3$): δ (ppm): 167.34, 157.96, 154.41, 150.46, 137.78, 131.03, 124.50, 124.45, 119.35, 117.47, 114.41, 62.65, 60.21, 59.91, 55.67, 48.78, 42.86. ESI-MS: Calcd for $[M+H]^+$, 440.2479; Found, 440.2507.



Scheme S2. Synthesis of **HYR-4** and **HYR-18**.

S2c. A mixture of **S1a** (2.97 g, 19 mmol) and **S2b** (2.89 g, 19 mmol) in DMSO (40 mL) was heated to 125 °C overnight. The reaction mixture was cooled to r.t. and poured into water (60 mL). The precipitate was filtered, washed with water, EtOH and dried to give a yellow solid **S2c** (4.9 g, 91%). ^{1}H NMR (DMSO-d_6): δ (ppm): 8.37 (d, 2H, J = 8.7 Hz), 8.29 (d, 2H, J = 9.0 Hz), 8.02 (d, 1H, J = 9.0 Hz), 7.79 (d, 1H, J = 2.6 Hz), 7.19 (dd, 1H, J = 9.0, 2.6 Hz), 3.86 (s, 3H).

S2d. $SnCl_2$ (6.6 g, 35 mmol) was added to a solution of **S2c** (2 g, 7 mmol) in EtOH (60 mL) followed by the addition of Conc. HCl (3 mL). The solution was brought to reflux for 3 h and cooled to r.t. $NaHCO_3$ was added to adjust the pH to 8-9. The mixture was extracted with ethyl acetate (3 × 20 mL). Then the combined layers were washed with water and dried over $MgSO_4$. The solvent was removed to give a yellowish solid **S2d** (1.38 g, 77%). ^{1}H NMR (acetone-d_6): δ (ppm): 7.76 (d, 2H, J = 8.7 Hz), 7.75 (d, 1H, J = 8.7 Hz), 7.50 (d, 1H, J = 2.7 Hz), 7.02 (dd, 1H, J = 9.0, 2.7 Hz), 6.73 (d, 2H, J = 8.7 Hz), 5.26 (s, 2H), 3.86 (s, 3H).

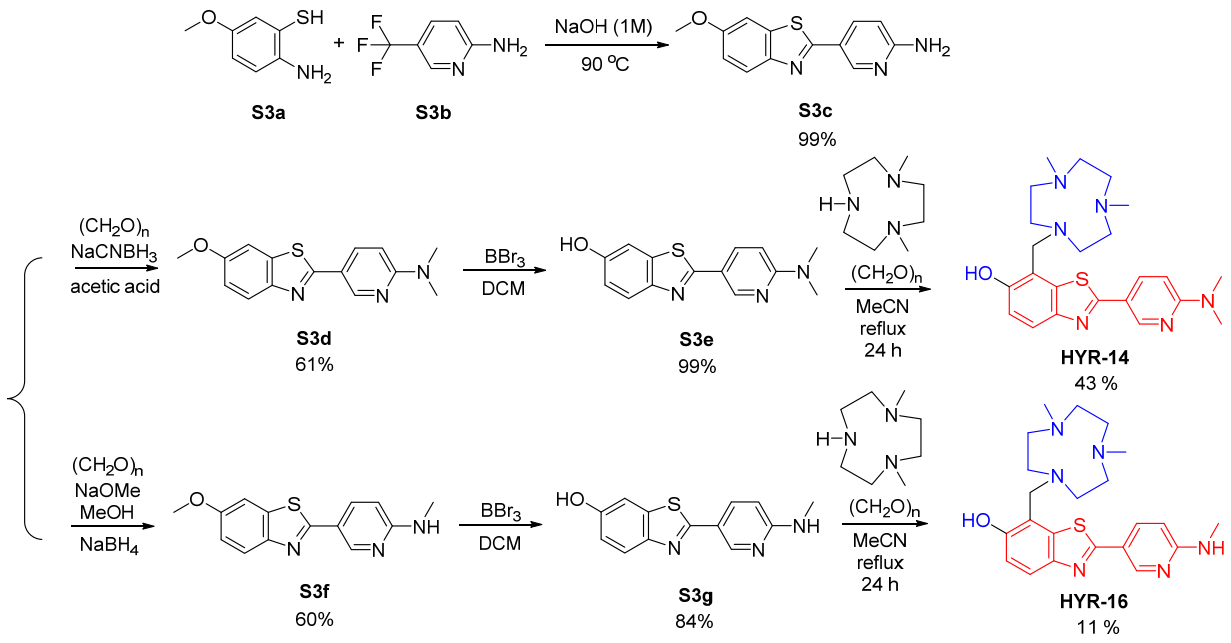
S2e. NaOMe (0.65 g, 13 mmol) was added to the mixture of **S2d** (0.57 g, 2.2 mmol) and paraformaldehyde (0.4 g, 13 mmol) in methanol (60 mL). The solution was refluxed for 2 h. Ice bath was used to cool the mixture to 0 °C, then sodium borohydride (0.5 g, 13 mmol) was added. The mixture was refluxed again for 1 h to give an orange solution. The solvent was removed to give a yellowish residue that was purified by silica gel column chromatography using hexane/ethyl acetate (4:1) to yield a yellow solid **S2e** (0.43 g, 72%).

S2f. To a solution of **S2e** (0.43 g, 1.6 mmol) in CH_2Cl_2 was added neat BBr_3 (1 M in CH_2Cl_2, 5 mL, 5 mmol) dropwise at r.t. The reaction was quenched with water and the pH was adjusted to 7 with saturated $NaHCO_3$ solution. Yellowish precipitate was isolated by vacuum filtration and then purified by silica gel column chromatography using hexane/ethyl acetate (2:1) to yield a yellow solid **S2f** (0.16 g, 38%). ^{1}H NMR (DMSO-d_6): δ (ppm): 9.66 (s, 1H), 7.71 (d, 2H, J = 8.7 Hz), 7.67 (d, 1H, J = 9.0 Hz), 7.29 (d, 1H, J = 2.4 Hz), 6.88 (dd, 1H, J = 8.7, 2.4 Hz), 6.60 (d, 2H, J = 8.7 Hz), 6.35 (q, 1H), 2.72 (d, 3H, J = 5.0 Hz).

HYR-4. Paraformaldehyde (6 mg, 0.20 mmol) was added to a solution of 1,4-dimethyl-1,4,7-triazacyclononane (31 mg, 0.20 mmol) in MeCN (5 mL) and the resultant mixture was heated to reflux for 30 min. Then **S2f** (50 mg, 0.20 mmol) in MeCN (5 mL) was added, the solution was refluxed for an additional 24 h, and then cooled to room temperature. The solvent was removed to give a yellowish residue that was purified by CombiFlash (reverse-phase) using MeCN/H_2O/TFA (30:70:0.1) to yield a yellow solution, which was then neutralized with saturated $NaHCO_3$ solution, extracted by $CHCl_3$ and dried to give a yellow solid (19 mg, yield 23%). ^{1}H NMR (CD_3CN): δ (ppm): 7.81 (d, 2H, J = 9.0 Hz), 7.66 (d, 1H, J = 8.7 Hz), 7.00 (d, 1H, J = 8.7 Hz), 6.67 (d, 2H, J = 9.0 Hz), 4.98 (m, 1H), 4.04 (s, 2H), 2.84-2.89 (m, 4H), 2.82 (d, 3H, J = 6.0 Hz), 2,63-2.67 (m, 4H), 2.62 (s, 4H), 2.38 (s, 6H). ^{13}C NMR ($CDCl_3$): δ (ppm): 167.33, 157.84, 153.75, 150.37, 138.00, 131.25, 125.57, 124.60, 119.38, 117.57, 114.70, 62.23, 59.48, 59.41, 55.16, 48.40, 33.02. HR-ESI-MS: Calcd for $[M+H]^+$, 426.2322; Found, 426.2352.

HYR-18. Paraformaldehyde (11 mg, 0.36 mmol) was added to a solution of 1-methyl-1,4,7-triazacyclononane (16 mg, 0.12 mmol) in MeCN (5 mL) and the resultant mixture was heated to reflux for 30 min. Then **S2f** (60 mg, 0.23 mmol) in MeCN (5 mL) was added, the solution was refluxed for an additional 24 h, and then cooled to room temperature. The solvent was removed to give a yellowish residue that was purified by CombiFlash (reverse-phase) using MeCN/H_2O/TFA

(30:70:0.1) to yield a yellow solution, which was then neutralized with saturated $NaHCO_3$ solution, extracted by $CHCl_3$ and dried to give a yellow solid (7 mg, yield 8%). ^1H NMR (CD_3CN): δ (ppm): 7.82 (d, 4H, J = 9.0 Hz), 7.78 (d, 2H, J = 9.0 Hz), 7.05 (d, 2H, J = 9.0 Hz), 6.63 (d, 4H, J = 9.0 Hz), 3.99 (s, 4H), 3.05 (s, 4H), 2.90 (s, 6H), 2.83-2.85 (b, 4H), 2.66-2.68 (b, 4H), 2,50 (s, 3H). HR-ESI-MS: Calcd for $[M+H]^+$, 680.2841; Found, 680.2850.



Scheme S3. Synthesis of **HYR-14** and **HYR-16**.

S3c. A solution of **S3a** (1.0 g, 6.4 mmol) and **S3b** (1.6 g, 9.7 mmol) in NaOH (1 M in water, 18 mL) was stirred at 90 °C for 3 h. After the mixture was cooled to room temperature, the precipitate was collected by filtration, and **S3c** (1.6 g, 99%) was obtained as a green solid.

S3d. To a solution of **S3c** (515 mg, 2 mmol) and paraformaldehyde (300 mg, 10 mmol) in acetic acid (50 mL) was added $NaCNBH_3$ (189 mg, 6 mmol) in one portion at room temperature. The resulting mixture was stirred at room temperature overnight. After neutralization with NH_4OH, water was added, and the precipitate was collected by filtration to give **S3d** as a white solid (347 mg, 61%).

S3e. Intermediate **S3e** was synthesized according to the literature.[7] To a solution of **S3d** (347 mg, 1.2 mmol) in CH_2Cl_2 (30 mL) was added neat BBr_3 (1 M in CH_2Cl_2, 4 mL, 4 mmol) dropwise at r.t. The reaction was quenched with water and the pH was adjusted to 7 with saturated $NaHCO_3$ solution. **S3e** was isolated by vacuum filtration as a yellow solid (325 mg, 99%). ^{1}H NMR (DMSO-d_6): δ (ppm): 9.79 (b, 1H), 8.63 (d, 1H, J = 2.5 Hz), 8.07 (dd, 1H, J = 9.1, 2.5 Hz), 7.75 (d, 1H, J = 8.8 Hz), 7.35 (d, 1H, J = 2.4 Hz), 6.93 (dd, 1H, J = 8.8, 2.5 Hz), 6.81 (d, 1H, J = 9.1 Hz), 3.12 (s, 6H).

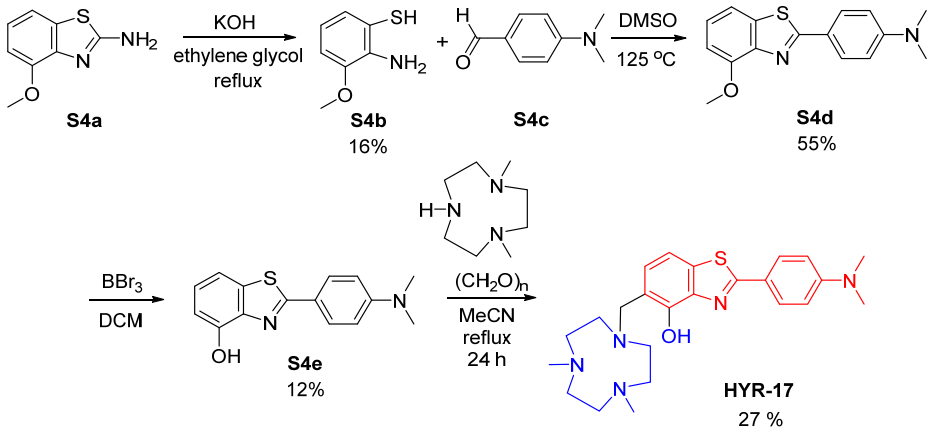
S3f. To a mixture of **S3c** (763 mg, 3 mmol) and paraformaldehyde (356 mg, 12 mmol) in MeOH (30 mL) was added CH_3ONa (1.6 g, 30 mmol). The mixture was stirred under reflux for 1 h. After the mixture was cooled, $NaBH_4$ (238 mg, 6 mmol) was added, and the mixture was brought to reflux again for 2 h. The reaction mixture was poured onto ice−water, and the precipitate was collected by filtration to obtain **S3f** as a white solid (484 mg, 60%). ^{1}H NMR (DMSO-d_6): δ(ppm): 8.63 (d, 1H, J = 2.4 Hz), 7.96 (dd, 1H, J = 8.7, 2.5 Hz), 7.83 (d, 1H, J = 9.0 Hz), 7.65 (d, 1H, J = 2.6 Hz), 7.23 (d, 1H, J = 5.1 Hz), 7.07 (dd, 1H, J = 8.9, 2.6 Hz), 6.57 (d, 1H, J = 8.8 Hz), 3.83 (s, 3H), 2.85 (d, 3H, J = 4.8 Hz).

S3g. To a solution of **S3f** (484 mg, 1.8 mmol) in CH_2Cl_2 (32 mL) was added neat BBr_3 (1 M in CH_2Cl_2, 4.1 mL, 4.1 mmol) dropwise at r.t. The reaction was quenched with water and the pH was adjusted to 7 with saturated $NaHCO_3$ solution. **S3g** was isolated by vacuum filtration as a yellow solid (383 mg, 84%). ^{1}H NMR (DMSO-d_6): δ (ppm): 9.75 (s, 1H), 8.53 (d, 1H, J = 2.4 Hz), 7.97 (dd, 1H, J = 9.0, 2.5 Hz), 7.71 (d, 1H, J = 8.7 Hz), 7.60 (b, 1H), 7.36 (d, 1H, J = 2.4 Hz), 6.93 (dd, 1H, J = 8.8, 2.5 Hz), 6.68 (d, 1H, J = 8.9 Hz), 2.83 (s, 3H).

HYR-14. Paraformaldehyde (8 mg, 0.27 mmol) was added to a solution of 1,4-dimethyl-1,4,7-triazacyclononane (28 mg, 0.18 mmol) in MeCN (5 mL) and the resultant mixture was heated to reflux for 30 min. Then **S3e** (50 mg, 0.18 mmol) in MeCN (5 mL) was added, the solution was refluxed for an additional 24 h, and then cooled to room temperature. The solvent was removed to give a yellowish residue that was purified by CombiFlash (reverse-phase) using MeCN/H_2O/TFA (30:70:0.1) to yield a yellow solution, which was then neutralized by saturated $NaHCO_3$ solution, extracted by $CHCl_3$ and dried to give a yellow solid (34 mg, yield 43%). ^{1}H NMR ($CDCl_3$): δ (ppm): 8.70 (d, 1H, J = 2.4 Hz), 8.08 (dd, 1H, J = 9.0, 2.4 Hz), 7.74 (d, 1H, J = 8.7 Hz), 7.09 (d, 1H, J = 8.7 Hz), 6.55 (d, 1H, J = 9.0 Hz), 4.00 (s, 2H), 3.15 (s, 6H), 2.88-2.91 (m, 4H), 2.67-2.69 (m, 4H), 2,66 (s, 4H), 2.43 (s, 6H). ^{13}C NMR ($CDCl_3$): δ (ppm): 164.82, 162.42, 157.74, 150.18, 150.11, 138.11, 138.05, 124.74, 120.95, 119.52, 117.90, 108.13, 61.49, 58.49, 58.14, 54.36, 47.77, 40.78. HR-ESI-MS: Calcd for $[M+H]^+$, 441.2431; Found, 441.2472.

HYR-16. Paraformaldehyde (6 mg, 0.20 mmol) was added to a solution of 1,4-dimethyl-1,4,7-triazacyclononane (28 mg, 0.20 mmol) in MeCN (5 mL) and the resultant mixture was heated to reflux for 30 min. Then **S3g** (50 mg, 0.20 mmol) in MeCN (5 mL) was added, the solution was refluxed for an additional 24 h, and then cooled to room temperature. The solvent was removed to

give a yellowish residue that was purified by CombiFlash (reverse-phase) using MeCN/H_2O/TFA (30:70:0.1) to yield a yellow solution, which was then neutralized by saturated $NaHCO_3$ solution, extracted by $CHCl_3$ and dried to give a yellow solid (9 mg, yield 11%). ^{1}H NMR ($CDCl_3$): δ (ppm): 8.69 (d, 1H, J = 2.1 Hz), 8.11 (dd, 1H, J = 8.7, 2.4 Hz), 7.76 (d, 1H, J = 8.7 Hz), 7.03 (d, 1H, J = 8.6 Hz), 6.46 (d, 1H, J = 8.7 Hz), 4.89 (m, 1H), 4.03 (s, 2H), 3.00 (d, 3H, J = 5.2 Hz), 2.93-2.96 (m, 4H), 2,67-2.70 (m, 4H), 2.61 (s, 4H), 2.42 (s, 6H). ^{13}C NMR ($CDCl_3$): δ (ppm): 164.47, 162.96, 158.41, 150.45, 150.12, 138.52, 137.49, 124.61, 122.49, 119.67, 117.51, 108.68, 62.76, 60.41, 60.03, 55.78, 48.85, 31.69. HR-ESI-MS: Calcd for $[M+H]^+$, 427.2275; Found, 427.2309.



Scheme S4. Synthesis of **HYR-17**.

S4b. S4a (5 g, 27.7 mmol) was suspended in 50% KOH (29 g KOH dissolved in 29 mL water) and ethylene glycol (6.5 mL). The suspension was heated to reflux for 48 h. Upon cooling to room temperature, toluene (50 mL) was added and the reaction mixture was neutralized with acetic acid (30 mL). The organic layer was separated, and the aqueous layer was extracted with another 35 mL of toluene. The toluene layers were combined and washed with water and dried over $MgSO_4$. The solvent was removed to give a yellowish residue that was purified by silica gel column chromatography using CH_2Cl_2/MeOH/NH_4OH (100:10:0.1) to yield **S4b** as an yellow oil (674 mg, 16%). ^{1}H NMR (DMSO-d_6): δ (ppm): 6.84 (d, 1H, J = 8.0 Hz), 6.68 (d, 1H, , J = 7.9 Hz), 6.43 (t, 1H), 4.98 (s, 2H), 3.77 (s, 3H).

S4d. A mixture of **S4b** (200 mg, 1.3 mmol) and **S4c** (192 mg, 1.3 mmol) in DMSO was heated to 170 °C for 35 min. The reaction mixture was cooled to room temperature and poured into water. The organic compound was extracted with ethyl acetate. The combined organic layers were washed with water and dried over $MgSO_4$. The solvent was removed to give a yellowish residue that was purified by silica gel column chromatography using hexane/ethyl acetate (4:1) to yield a yellow solid **S4d** (204 mg, 55%). ^{1}H NMR (DMSO-d_6): δ (ppm): 7.85 (d, 2H, J = 8.9 Hz), 7.57 (d, 1H, J = 8.0 Hz), 7.30 (t, 1H, J = 8.0 Hz), 7.01 (d, 1H, J = 8.1 Hz), 6.81 (d, 2H, J = 8.9 Hz), 3.96 (s, 3H), 3.01 (s, 6H).

S4e. To a solution of **S4d** (204 mg, 0.7 mmol) in CH_2Cl_2 (25 mL) was added neat BBr_3 (1 M in CH_2Cl_2, 2.5 mL, 2.5 mmol) dropwise at r.t. The reaction was quenched with water and the pH was adjusted to 7 with saturated $NaHCO_3$ solution. The organic compound was extracted with ethyl

acetate. The combined organic layers were washed with water and dried over $MgSO_4$. The solvent was removed to give a yellowish residue that was purified by silica gel column chromatography using hexane/ethyl acetate (3:1) to yield a yellow solid **S4e** (22.5 mg, 12%). ^{1}H NMR ($CDCl_3$): δ (ppm): 7.92 (d, 2H, J = 9.0 Hz), 7.34 (d, 1H, J = 8.0 Hz), 7.19 (t, 1H, J = 7.8 Hz), 6.93 (d, 1H, J = 7.9 Hz), 6.74 (d, 2H, J = 8.7 Hz), 3.06 (s, 6H).

HYR-17. Paraformaldehyde (4 mg, 0.10 mmol) was added to a solution of 1,4-dimethyl-1,4,7-triazacyclononane (11 mg, 0.07 mmol) in MeCN (5 mL) and the resultant mixture was heated to reflux for 30 min. Then **S4e** (19 mg, 0.07 mmol) in MeCN (5 mL) was added, the solution was refluxed for an additional 24 h, and then cooled to room temperature. The solvent was removed to give a yellowish residue that was purified by CombiFlash (reverse-phase) using MeCN/H_2O/TFA (30:70:0.1) to yield a yellow solution, which was then neutralized by saturated $NaHCO_3$ solution, extracted by $CHCl_3$ and dried to give a yellow solid (8 mg, yield 27%). ^{1}H NMR ($CDCl_3$): δ (ppm): 7.97 (d, 2H, J = 9.0 Hz), 7.24 (d, 1H, J = 8.1 Hz), 6.99 (d, 1H, J = 8.1 Hz), 6.73 (d, 2H, J = 9.0 Hz), 3.96 (s, 2H), 3.05 (s, 6H), 2.99-3.02 (m, 4H), 2.83-2.86 (m, 4H), 2,82 (s, 4H), 2.45 (s, 6H). ^{13}C NMR ($CDCl_3$): δ (ppm): 170.54, 154.84, 151.78, 146.58, 137.74, 131.51, 129.70, 123.74, 121.59, 114.63, 114.31, 60.35, 57.11, 56.19, 54.22, 46.74, 42.81. HR-ESI-MS: Calcd for $[M+H]^+$, 440.2479; Found, 440.2503.

III. UV-vis and emission spectra of MFCs

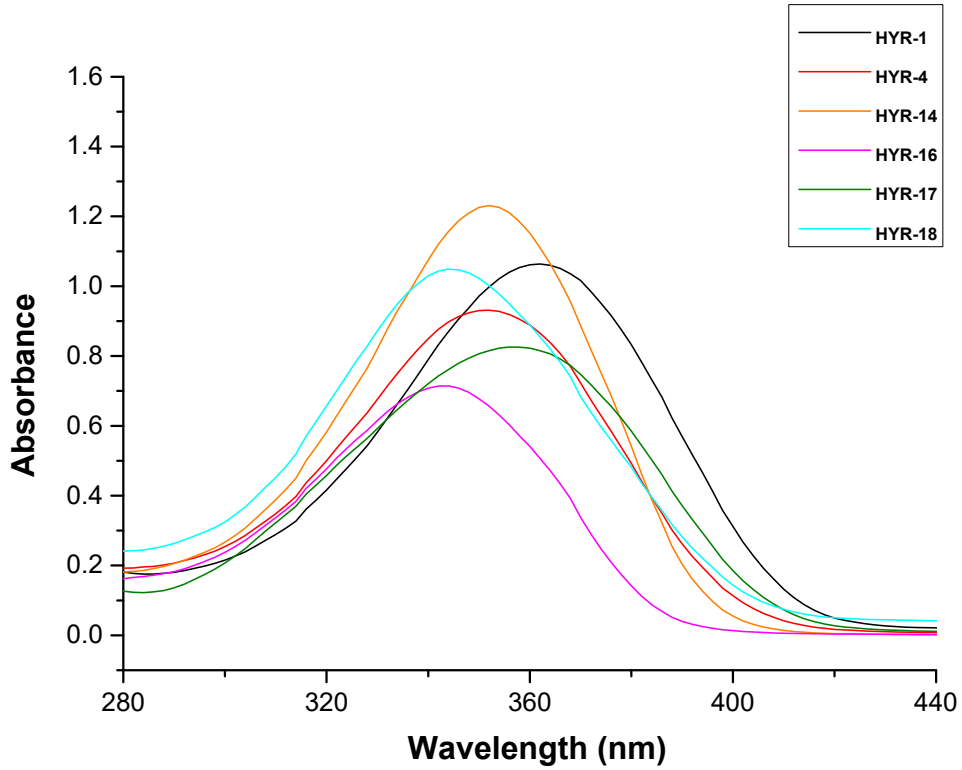


Figure S1. UV-vis spectra of investigated MFCs (50 μM in ultrapure water with 0.5% DMSO).

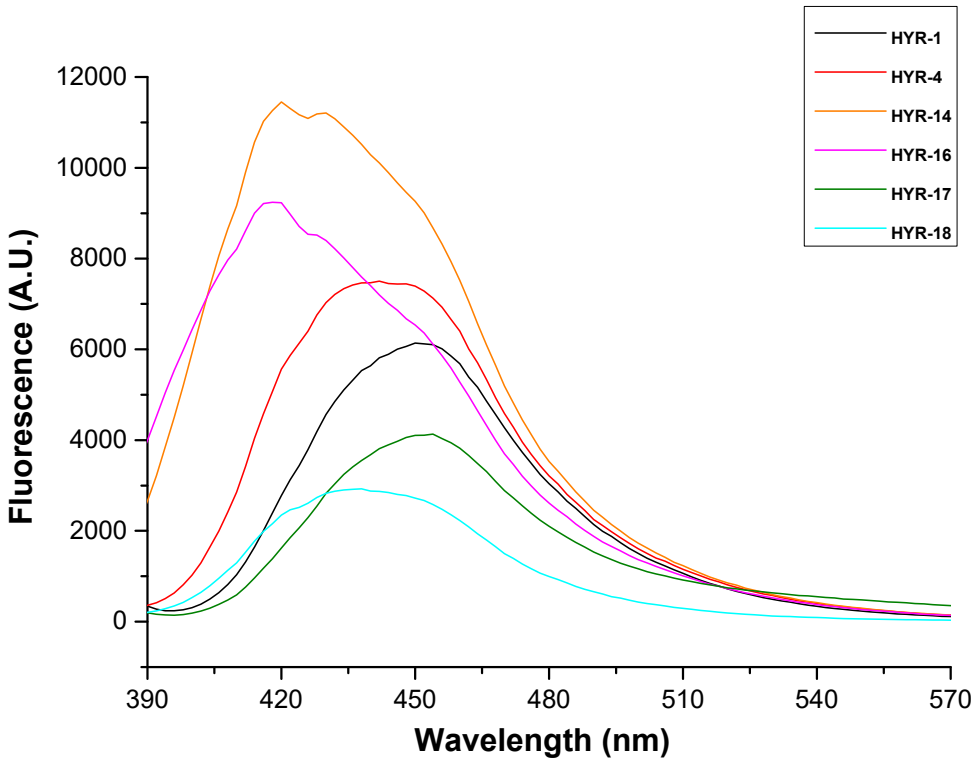


Figure S2. Emission spectra of investigated MFCs in ultrapure water with <0.1% DMSO.

Table S1. Excitation and emission wavelengths of investigated MFCs.

Compounds	λ_{ex} *(nm)*	λ_{em} *(nm)*
HYR-1	362	450
HYR-4	352	442
HYR-14	352	420
HYR-16	344	425
HYR-17	356	450
HYR-18	344	438

IV. UV-vis and emission spectra of MFC-Cu(II) complexes

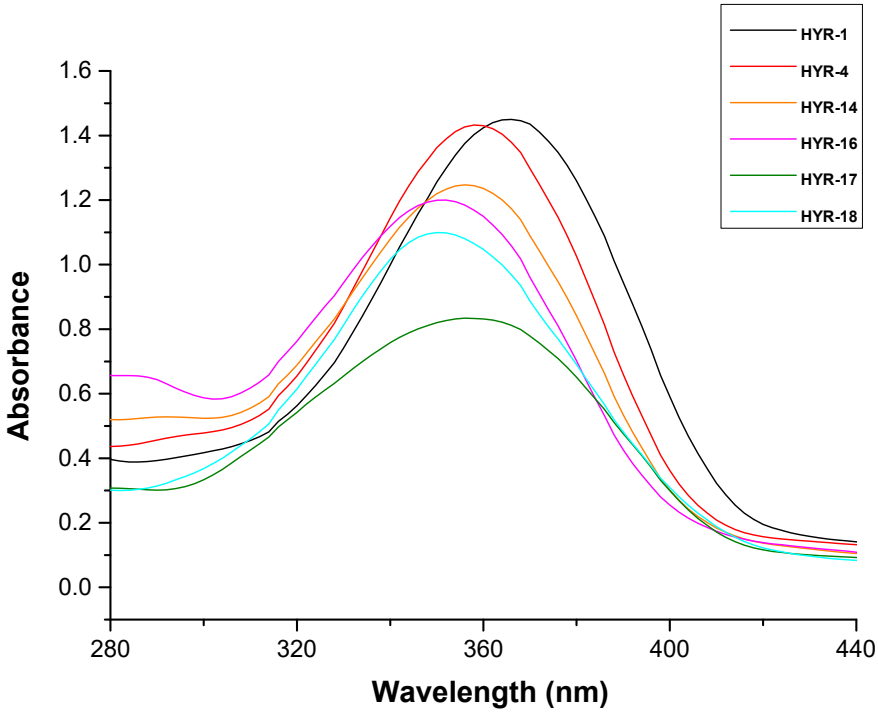


Figure S3. UV-vis spectra of MFC-Cu(II) complexes (50 μM in ultrapure water with 0.5% DMSO).

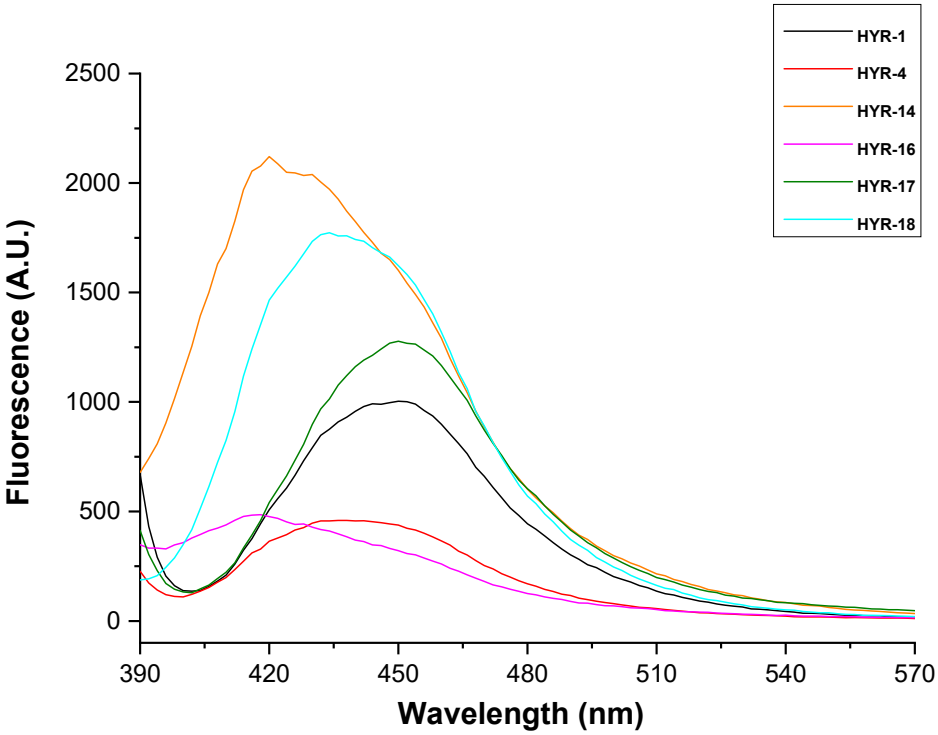


Figure S4. Emission spectra of MFC-Cu(II) complexes in ultrapure water with <0.1% DMSO.

Table S2. Excitation and emission wavelengths of MFC-Cu(II) complexes.

Cu(II) complexes	λ_{ex} *(nm)*	λ_{em} *(nm)*
Cu-HYR-1	366	450
Cu-HYR-4	358	436
Cu-HYR-14	356	420
Cu-HYR-16	352	418
Cu-HYR-17	356	450
Cu-HYR-18	350	434

V. Fluorescence staining of WT mouse brain sections

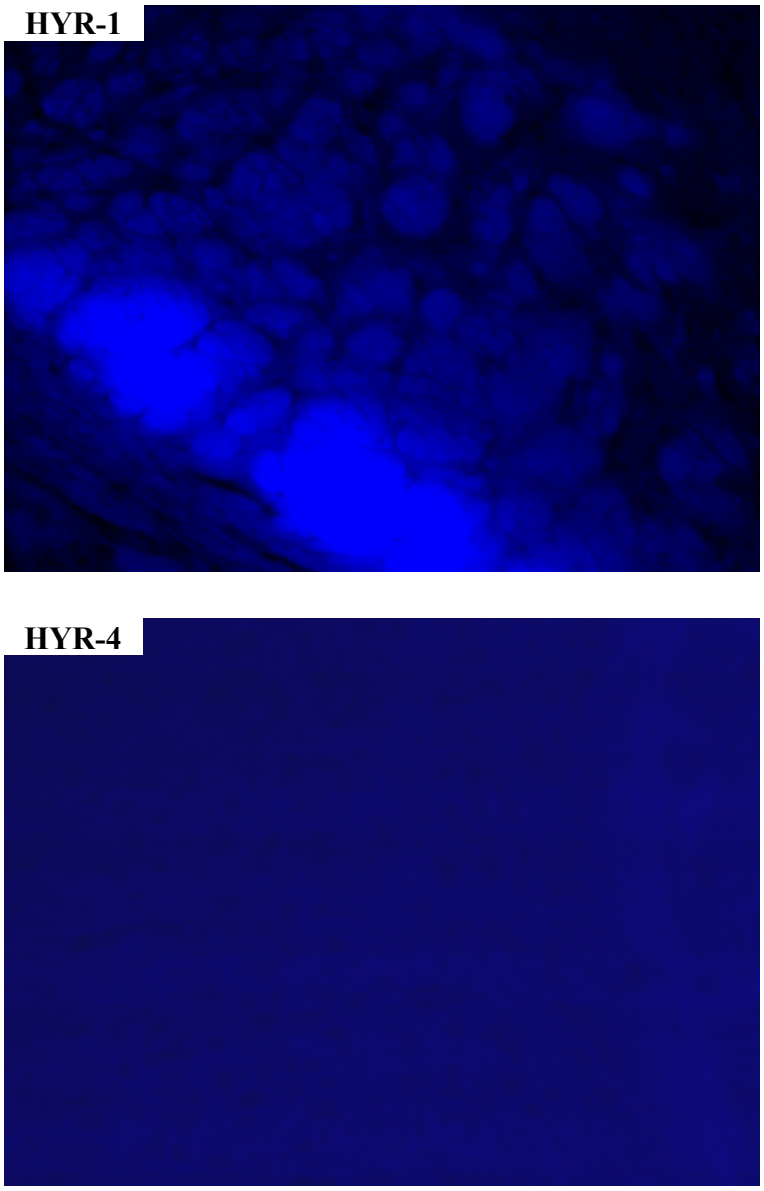
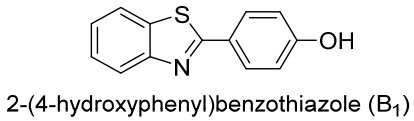


Figure S5. Representative fluorescence microscopy images of WT mouse brain sections stained with **HYR-1**/**HYR-4** -. **HYR-1** shows non-specific binding to various biomolecules.

VI. Structure and Aβ fibril binding assays of blocking agent B1



2-(4-hydroxyphenyl)benzothiazole (B_1)

Figure S6. Structure of non-radiolabeled compounds used for blocking studies.

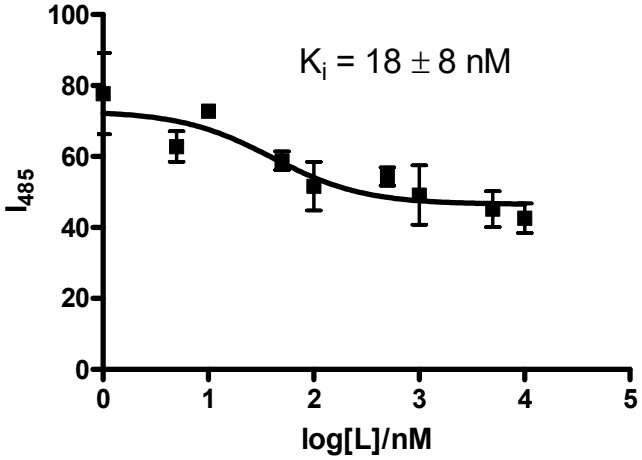


Figure S7. ThT competition assay of **B_1** with Aβ_{40} fibrils ([Aβ] = 2 μM, [ThT] = 1 μM).

VII. HPLC traces of ^{64}Cu-radiolabeled MFCs

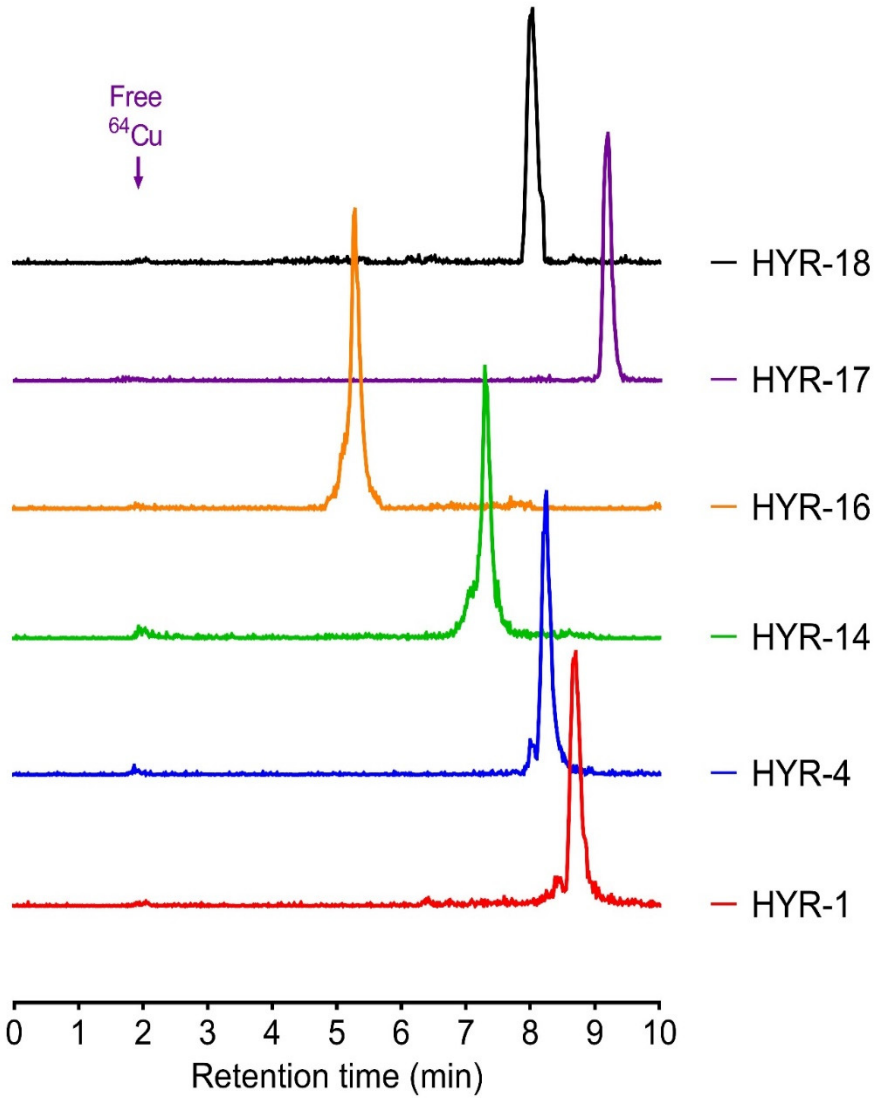
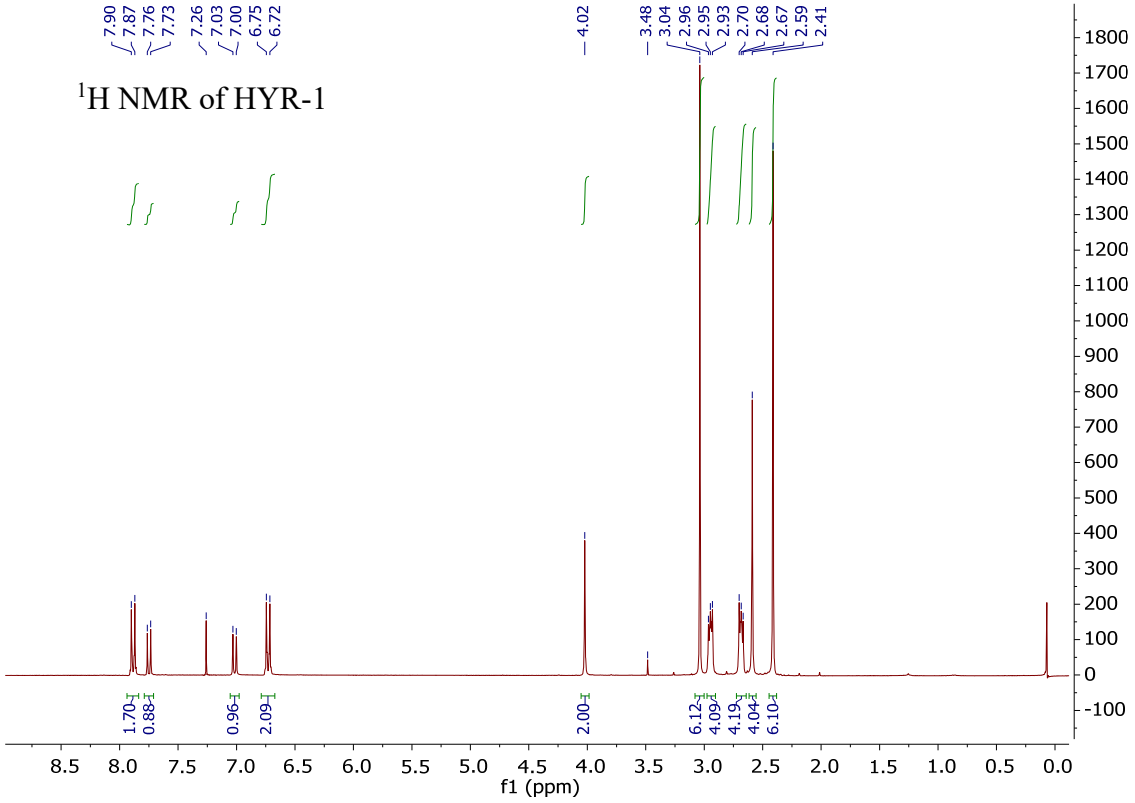
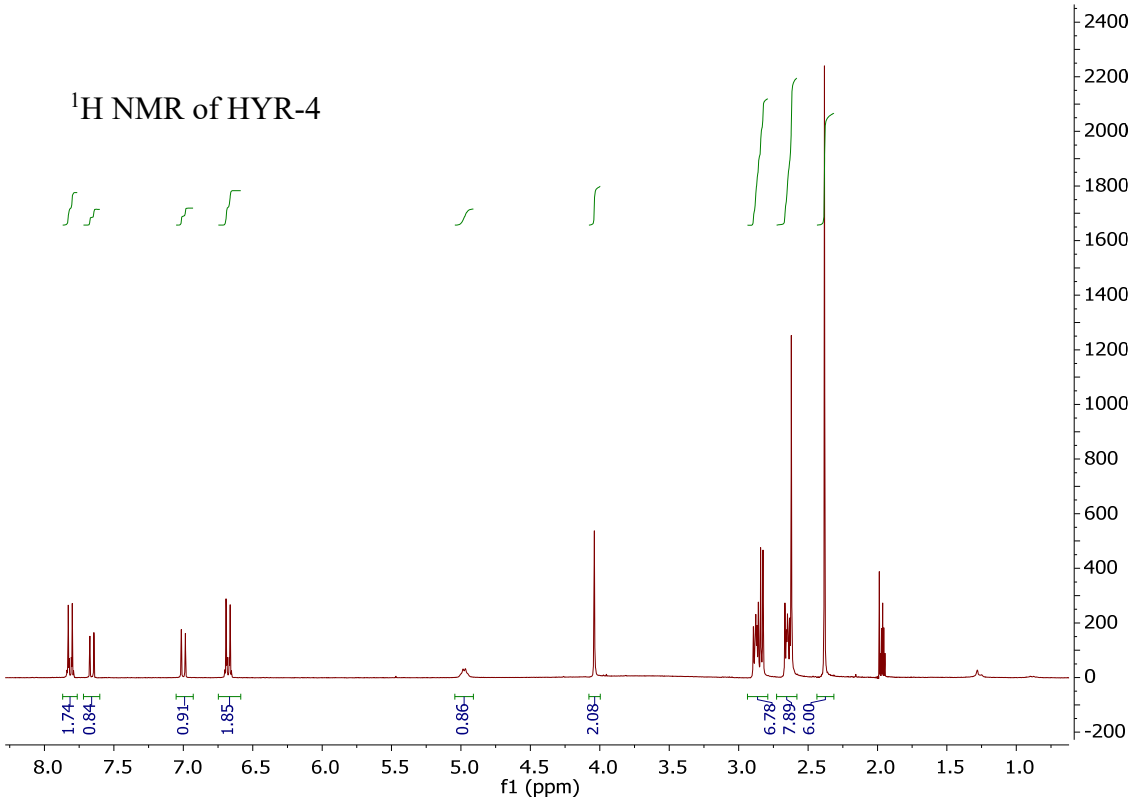


Figure S8. HPLC chromatographic profiles from ^{64}Cu radiolabeling. The retention times were 8.7, 8.2, 7.3, 5.3, 9.2, and 8.0 min for ^{64}Cu-**HYR-1**, ^{64}Cu-**HYR-4**, ^{64}Cu-**HYR-14**, ^{64}Cu-**HYR-16**, ^{64}Cu-**HYR-17** and ^{64}Cu-**HYR-18**, respectively.

VIII. NMR spectra of MFCs

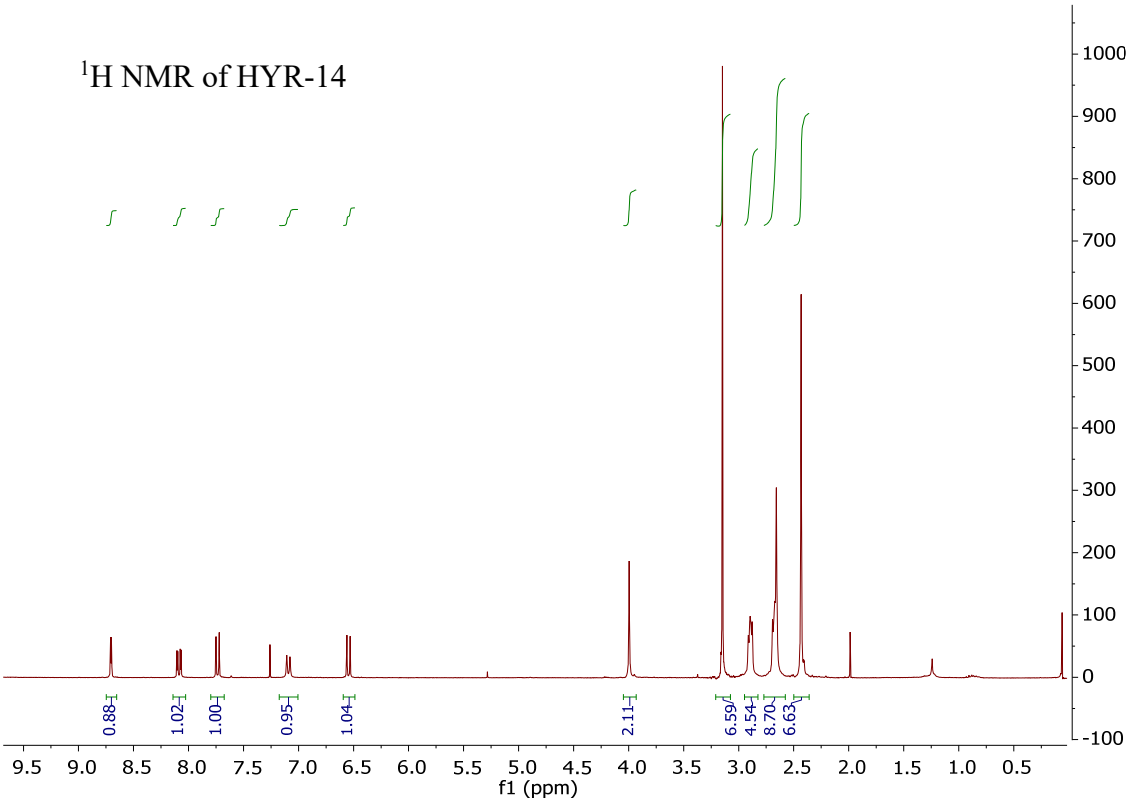


^{1}H NMR of HYR-1

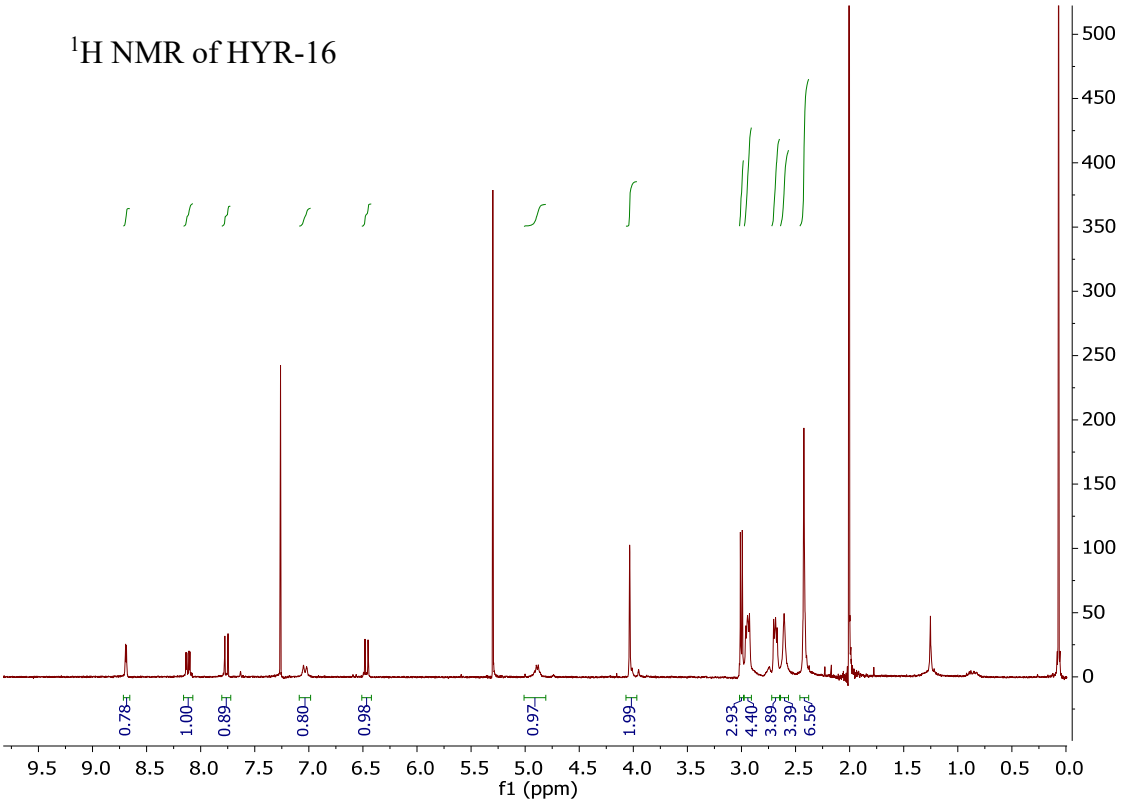


^{1}H NMR of HYR-4

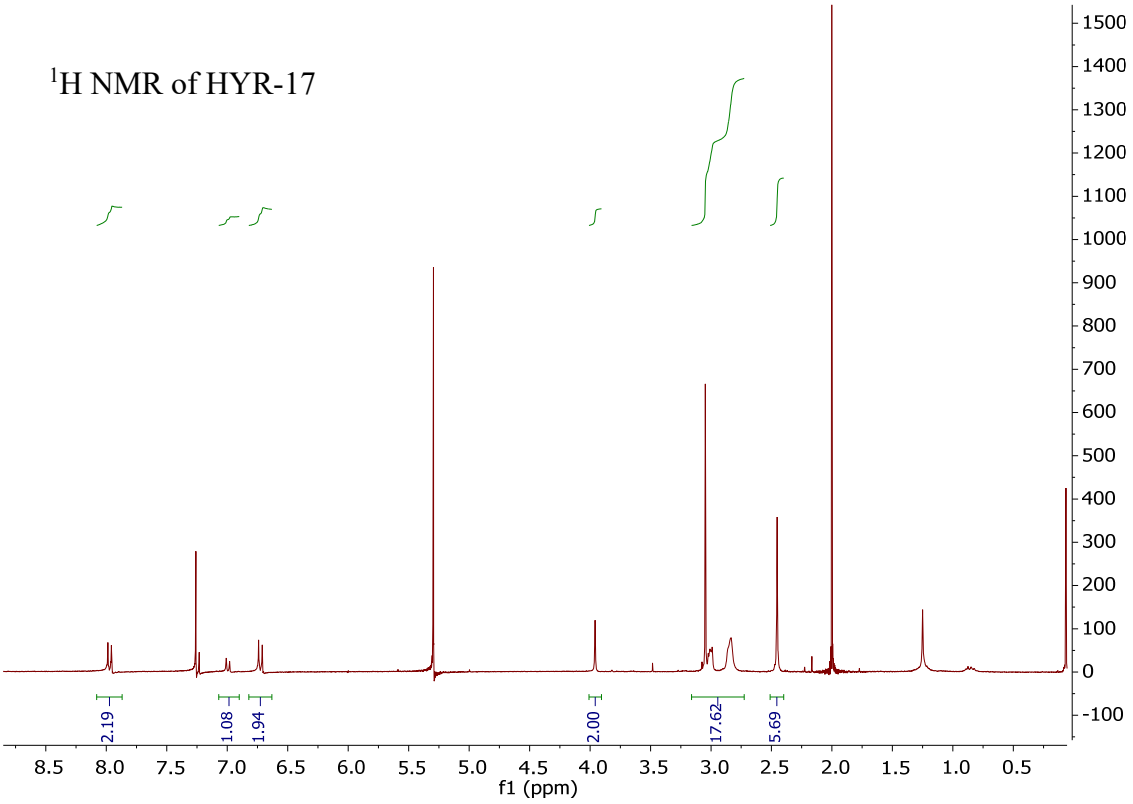
^{1}H NMR of HYR-14



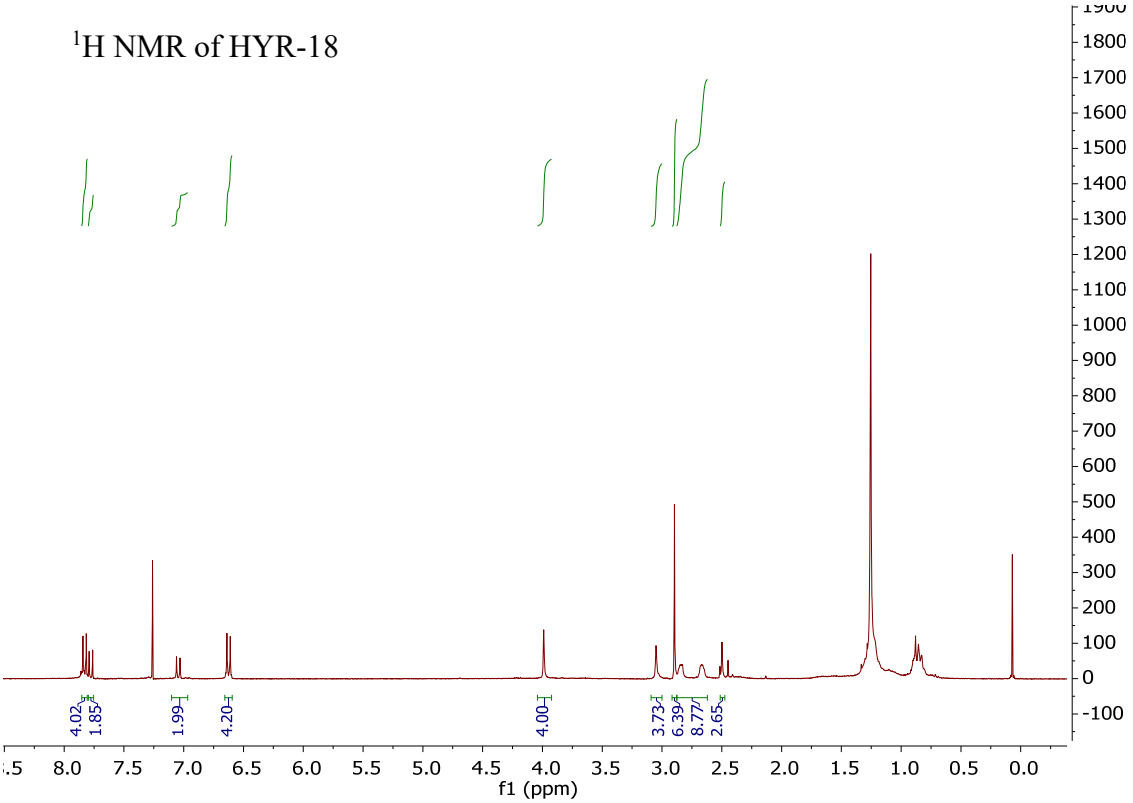
^{1}H NMR of HYR-16

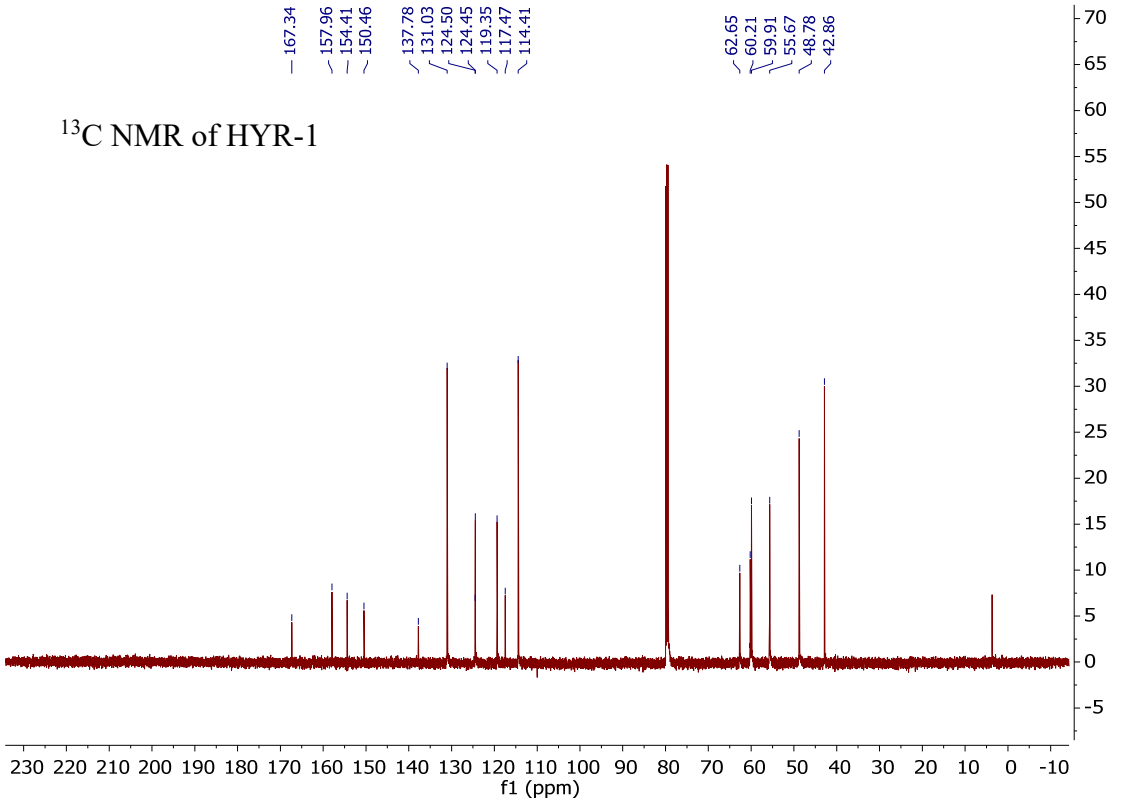


^{1}H NMR of HYR-17

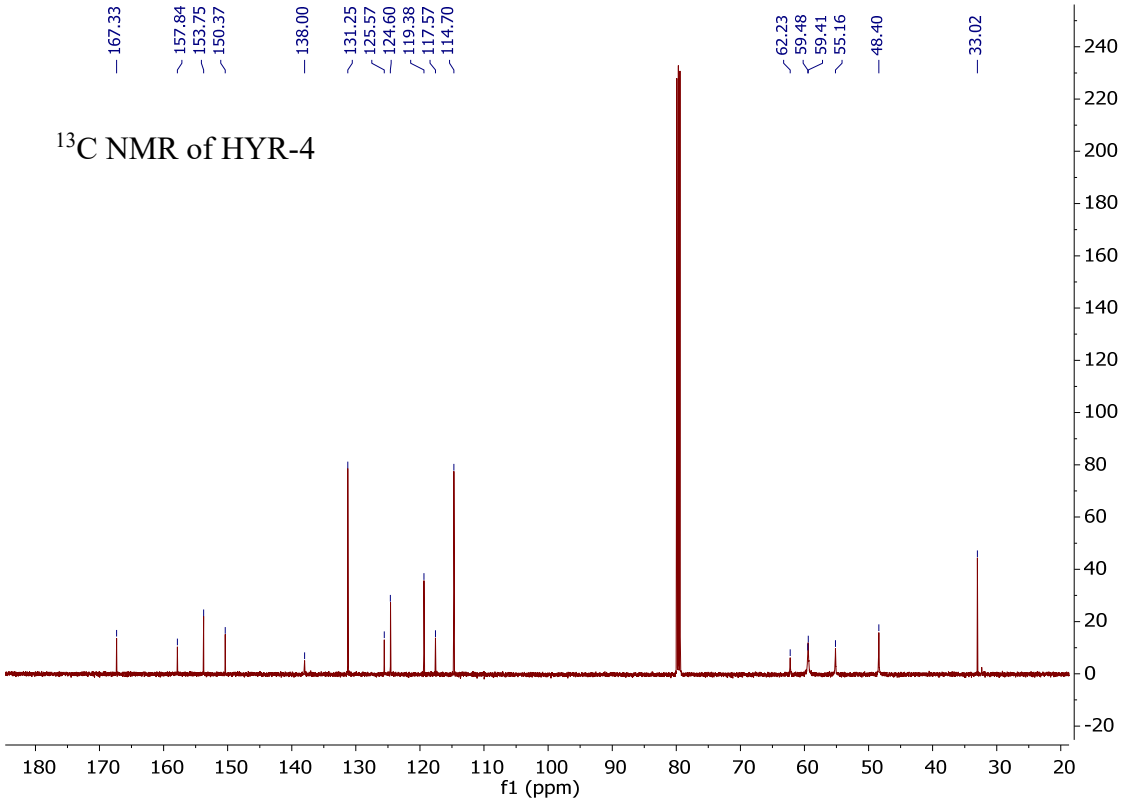


^{1}H NMR of HYR-18

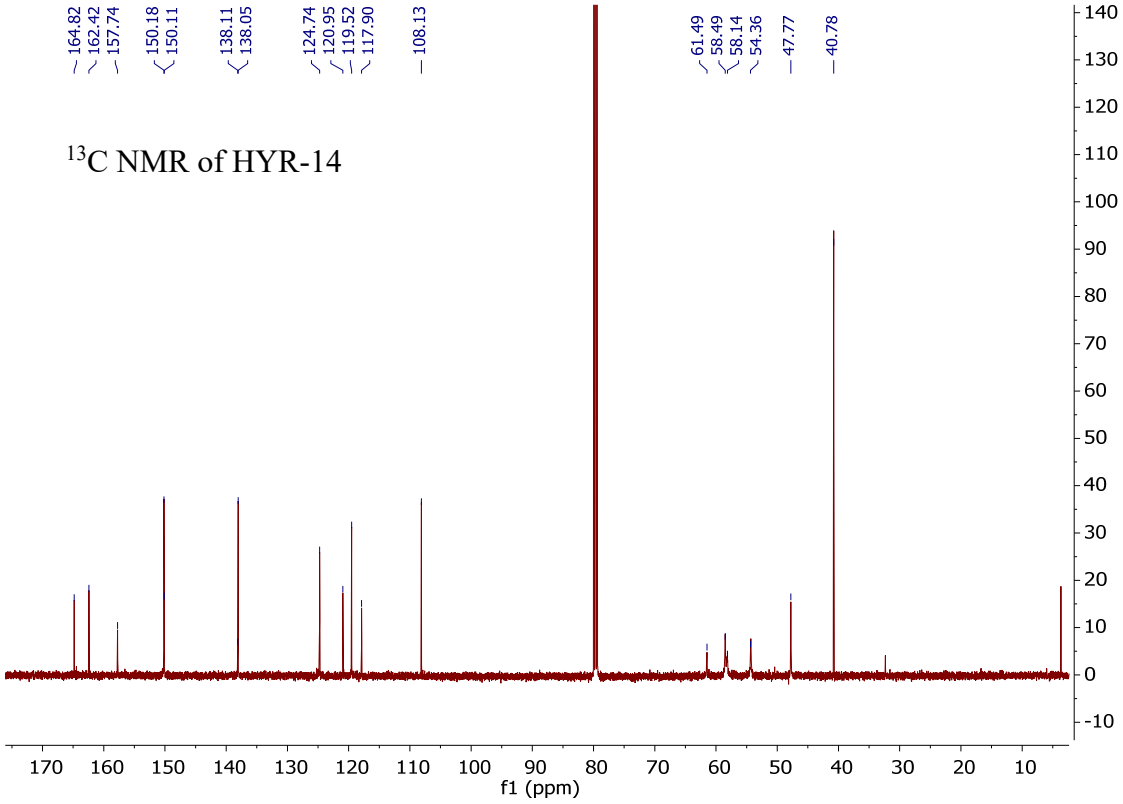




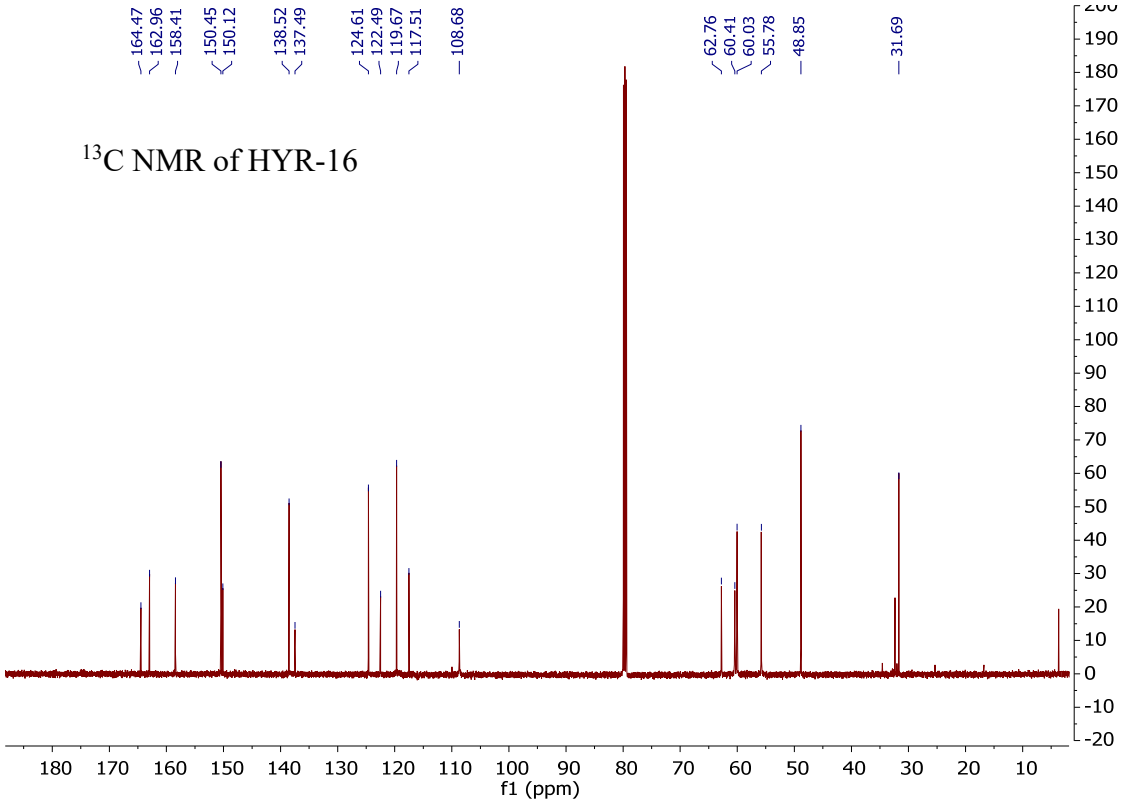
^{13}C NMR of HYR-1



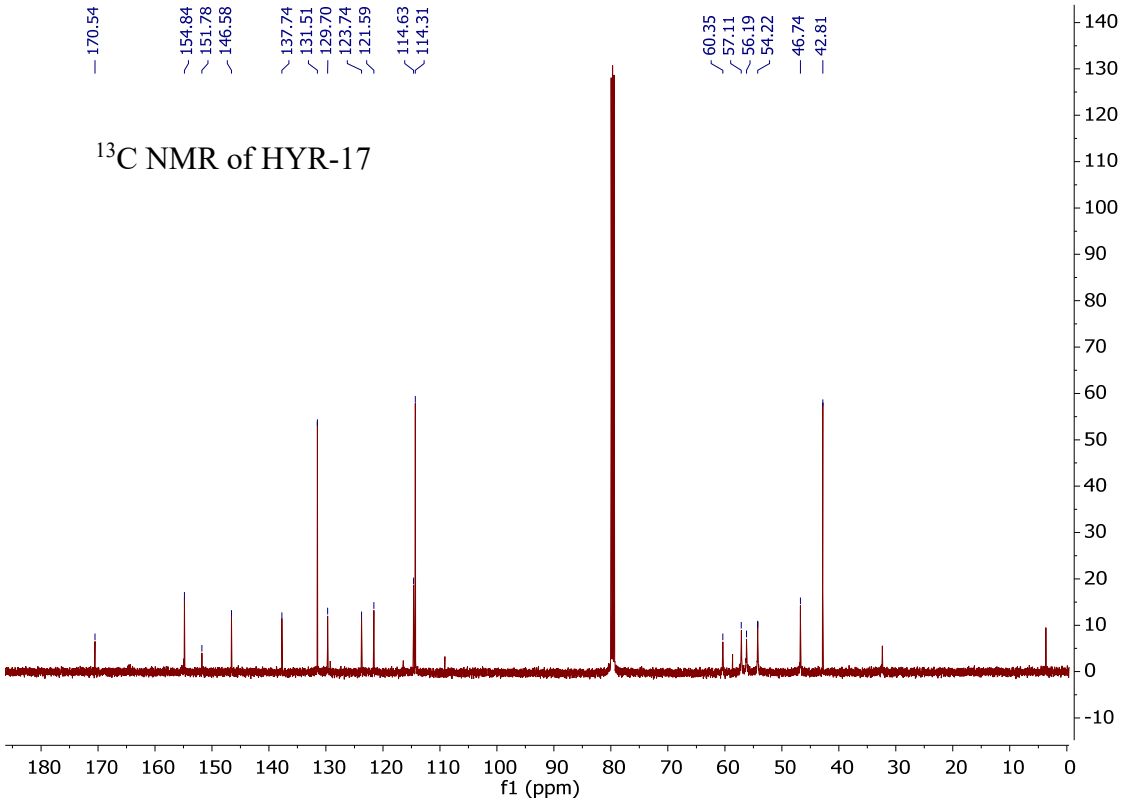
^{13}C NMR of HYR-4



^{13}C NMR of HYR-14



^{13}C NMR of HYR-16


170.54
154.84
151.78
146.58
137.74
131.51
129.70
123.74
121.59
114.63
114.31
60.35
57.11
56.19
54.22
46.74
42.81
13C NMR of HYR-17
f1 (ppm)

IX. References

(1) Bandara, N.; Sharma, A. K.; Krieger, S.; Schultz, J. W.; Han, B. H.; Rogers, B. E.; Mirica, L. M. *J. Am. Chem. Soc.* **2017**, *139*, 12550.

(2) Sharma, A. K.; Schultz, J. W.; Prior, J. T.; Rath, N. P.; Mirica, L. M. *Inorg. Chem.* **2017**, *56*, 13801.

(3) Klein, W. L. *Neurochem. Int.* **2002**, *41*, 345.

(4) Chan, C. Y.; Noor, A.; McLean, C. A.; Donnelly, P. S.; Barnard, P. J. *Chem. Comm.* **2017**, *53*, 2311.

(5) Jones, M. R.; Mathieu, E.; Dyrager, C.; Faissner, S.; Vaillancourt, Z.; Korshavn, K. J.; Lim, M. H.; Ramamoorthy, A.; Wee Yong, V.; Tsutsui, S.; Stys, P. K.; Storr, T. *Chem. Sci.* **2017**, *8*, 5636.

(6) Kume, M.; Carey, P. C.; Gaehle, G.; Madrid, E.; Voller, T.; Margenau, W.; Welch, M. J.; Lapi, S. E. *Appl. Radiat. Isot.* **2012**, *70*, 1803.

(7) Cui, M. C.; Wang, X. D.; Yu, P. R.; Zhang, J. M.; Li, Z. J.; Zhang, X. J.; Yang, Y. P.; Ono, M.; Jia, H. M.; Saji, H.; Liu, B. L. *J. Med. Chem.* **2012**, *55*, 9283.