Supporting Information

Group 9 metal-based inhibitors of β-amyloid (1–40) fibrillation as potential therapeutic agents for Alzheimer's disease

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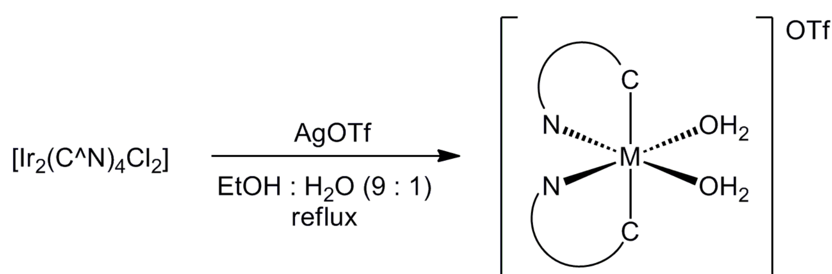
Materials. Reagents were purchased from Sigma Aldrich and used as received. Iridium chloride hydrate ($IrCl_3.xH_2O$) and rhodium chloride hydrate ($RhCl_3.xH_2O$) were purchased from Precious Metals Online.

General Experimental. Mass spectrometry was performed at the Mass Spectroscopy Unit at the Department of Chemistry, Hong Kong Baptist University, Hong Kong (China). Melting points were determined using a Gallenkamp melting apparatus and are uncorrected. Deuterated solvents for NMR purposes were obtained from Armar and used as received.

^{1}H and ^{13}C NMR were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz (^{1}H) and 100 MHz (^{13}C). ^{1}H and ^{13}C chemical shifts were referenced internally to solvent shift (^{1}H MeOD: δ 3.30; ^{13}C δ 49.0; d_6-DMSO: ^{1}H δ 2.50, ^{13}C δ 39.5). Chemical shifts (δ) are quoted in ppm, the downfield direction being defined as positive. Uncertainties in chemical shifts are typically ± 0.01 ppm for ^{1}H and ± 0.05 for ^{13}C. Coupling constants are typically ± 0.1 Hz for ^{1}H–^{1}H and ± 0.5 Hz for ^{1}H–^{13}C couplings. The following abbreviations are used for convenience in reporting the multiplicity of NMR resonances: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. All NMR data was acquired and processed using standard Bruker software (topspin).

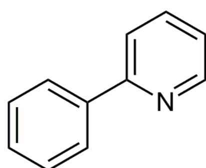
The following compounds were prepared using literature methods: $[Ir_2(ppy)_4Cl_2]$,[1] $[Ir_2(bzq)_4Cl_2]$[1] and $[Ir_2(phq)_4Cl_2]$[2] and $[Ir(ppy)_2(H_2O)_2]OTf$ (**1a**).[3]

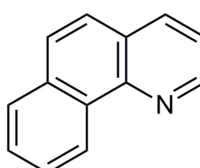
General synthesis of [Ir(C^N)$_2$(H$_2$O)$_2$]OTf (1–3)

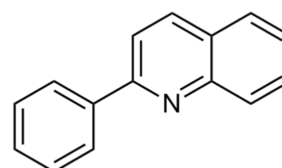


1a: M = Ir, C^N = ppy
1b: M = Rh, C^N = ppy

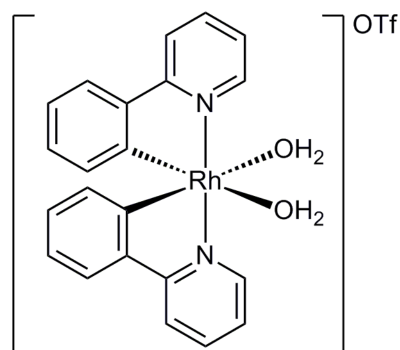
2: M = Ir, C^N = bzq
3: M = Ir, C^N = phq







A suspension of the metal dimer and silver triflate in a mixture of ethanol : water (9 : 1) was refluxed under a nitrogen atmosphere overnight. The resulting suspension was then allowed to cool to room temperature, where the solvent was removed *in vacuo*. The solvato complex **1**–**3** was then extracted from the resulting residues using dichloromethane, and the organic solvent was then removed *in vacuo* to yield the solvato complex as a crystalline solid.

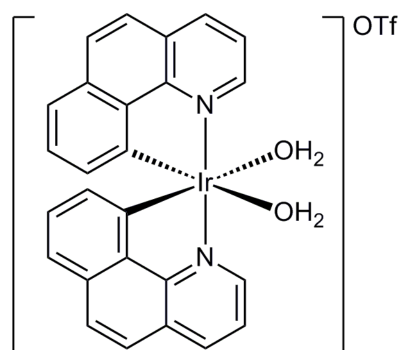


[Rh(ppy)$_2$(H$_2$O)$_2$]OTf (1b). [Rh$_2$(ppy)$_4$Cl$_2$] (49.5 mg, 0.06 mmol) and silver triflate (78.2 mg, 0.30 mmol) was refluxed overnight to yield the titled compound as a yellow solid (35.2 mg, 53 % yield) m.p. dec. > 300 ^{o}C.

Maldi-TOF-HRMS: Calcd. for $C_{22}H_{16}RhN_2$ [M–2H$_2$O]$^+$: 411.2824 Found: 411.3287.

^{1}H NMR (400 MHz, MeOD) δ 8.85 (d, *J* = 5.6 Hz, 2H), 8.20–8.10 (m, 4H), 7.74 (d, *J* = 7.7 Hz, 2H), 7.54–7.51 (m, 2H), 6.97 (t, *J* = 7.4 Hz, 2H), 6.78 (t, *J* = 7.4 Hz, 2H), 6.14 (d, *J* = 7.8 Hz, 2H).

^{13}C NMR (100 MHz, MeOD) δ 164.9, 149.2, 144.6, 139.1, 133.8, 129.4, 124.2, 123.2, 123.0, 119.6, 53.4.

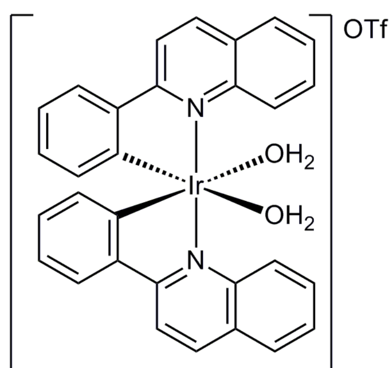


[Ir(bzq)$_2$(H$_2$O)$_2$]OTf (2). [Ir$_2$(bzq)$_4$Cl$_2$] (56.2 mg, 0.05 mmol) and silver triflate (54.2 mg, 0.21 mmol) was refluxed overnight to yield the titled compound as a yellow solid (48.9 mg, 67 % yield) m.p. dec. > 300 ^{o}C.

Maldi-TOF-HRMS: Calcd. for $C_{26}H_{16}IrN_2$ [M–2H$_2$O]$^+$: 549.0943. Found: 549.2301.

^{1}H NMR (400 MHz, MeOD) δ 9.24 (d, *J* = 4.8 Hz, 2H), 8.58 (d, *J* = 8.0 Hz, 2H), 7.91–7.88 (m, 2H), 7.82 (s, 4H), 7.31 (d, *J* = 8.0 Hz, 2H), 6.91 (t, *J* = 7.6 Hz, 2H), 6.02 (d, *J* = 7.2 Hz, 2H).

^{13}C NMR (100 MHz, MeOD) δ 158.5, 150.0, 143.0, 139.1, 135.4, 134.6, 131.5, 130.6, 129.9, 128.2, 124.8, 123.0, 121.3.



$[Ir(phq)_2(H_2O)_2]OTf$ (3). $[Ir_2(phq)_4Cl_2]$ (50.0 mg, 0.04 mmol) and silver triflate (86.0 mg, 0.33 mmol) was refluxed overnight to yield the titled compound as a red solid (41.8 mg, 66 % yield) m.p. dec. 200 °C.
Maldi-TOF-HRMS: Calcd. for $C_{30}H_{20}IrN_2$ $[M–2H_2O]^+$: 601.1256. Found: 600.9451.
^{1}H NMR (400 MHz, d_6-DMSO) δ 9.12 (d, J = 9.0 Hz, 2H), 8.68 (d, J = 8.5 Hz, 2H), 8.46 (d, J = 8.8 Hz, 2H), 8.16 (d, J = 7.8 Hz, 2H), 7.96 (d, J = 8.1 Hz, 2H), 7.86 (t, J = 7.8 Hz, 2H), 7.73 (t, J = 7.2 Hz, 2H), 6.86 (t, J = 7.7 Hz, 2H), 6.54 (t, J = 7.0 Hz, 2H), 6.03 (d, J = 7.7 Hz, 2H). ^{13}C NMR (100 MHz, d_6-DMSO) δ 169.9, 148.4, 146.7, 141.1, 140.5, 134.2, 131.4, 128.8, 127.8, 126.9, 126.6, 126.4, 126.0, 121.9, 117.2

Stock Solution Preparation. The complexes **1–3** were dissolved in DMF with a concentration of millimolar range. The complexes were then diluted with phosphate buffer (50 mM sodium phosphate, 100 mM sodium chloride, pH 7.4, filtered with 20 μm Nylon membrane before use) to the required concentrations before use.

Preparation of $A\beta_{1-40}$ fibrils for seeding. Monomeric $A\beta_{1-40}$ (Anaspec, CA) was used without further purification. The stock $A\beta_{1-40}$ was prepared by dissolving 1 mg of $A\beta_{1-40}$ monomers in 400 μL ice-cold 0.02 % ammonia solution and stored at –80 °C until use. The preparation of amyloid fibrils was similar to those mentioned elsewhere.[4,5] Briefly, 50 μL of stock solution was diluted to 50 μM with phosphate buffer. The mixture was incubated in water bath at 37 °C with gentle shaking for 20 hours, and stored at -18 °C afterwards. Aliquot of stock fibrils was used for the second generation of Aβ seedings. In general, stock fibrils were sonicated for 5 seconds thrice in order to break into small fragmented fibrils. In consequence, 2 μL of the as-prepared fibrils were added into 5 μL of monomeric $A\beta_{1-40}$ and diluted to 50 μL with phosphate buffer solution. The mixture was incubated at 37 °C for 1 hour. The resultant fibrils were sonicated for 5 seconds thrice and used as the second generation of seedings for the seed-mediated fibrillation.

Preparation of control and inhibited $A\beta_{1-40}$ fibrils by seed-mediated growth. In general, stock monomeric $A\beta_{1-40}$ was diluted with phosphate buffer into 50 μM in concentration. While inhibition experiments were performed, complex **1–3** was added to the peptide solution and diluted into a final concentration of 1, 5, 10, 17.5, 25, and 50 μM respectively. Seeding fibrils in 12 μg/mL were added to the mixture and incubated at 37 °C for 1 hour. The resultant $A\beta_{1-40}$ peptides and fibrils were diluted 20 times and labeled with thioflavin T (ThT, λ_{ex} = 450 nm, λ_{em} = 482 nm) for visualization.

Cleaning of cover glasses. All coverslips were pre-washed prior to experiments. Briefly, No.1 22 x 22 square mm cover glasses (Corning, NY) were successively sonicated in absolute ethanol for 5 minutes, sodium hydroxide solution for 40 minutes, glacial acetic acid for 15 minutes, distilled water for 5 minutes thrice and absolute ethanol for 5 minutes thrice. In between each solvent replacement, the coverslips were rinsed with distilled water thoroughly. The cleaned coverslips were dried completely at 140 °C oven for approximately 15 minutes and stored for future usage.

Total Internal Reflection Fluorescence Microscopy (TIRFM) imaging system. An inverted Olympus IX-71 microscope (Olympus, Tokyo, Japan) was equipped with a high numerical-aperture 60× (1.45 NA) oil-immersion objective (PlanApo, Olympus). The sample coverslip was located in-between the fused-silica Isosceles Brewster Prism (CVI Melles Griot, Carksvadm, CA) and the 60× objectives with immersion oil (η = 1.52, Non-fluorescence, Olympus). Besides, 445 nm diode laser (50 mW, LQC445-40E, Newport, USA) was used for the excitation of thioflavin T (ThT) labeled $A\beta_{1-40}$. The incident angle of the laser beam was adjusted to 66 ° in order to achieve the total internal reflection (TIR) and generates evanescent field for the excitation of the fluorophores. A band pass filter HQ 480/40x (Chroma Technology Corp., USA) was coordinated with the use of 445 nm in the imaging correspondingly. Fluorescence images were captured by an electron-multiplying charge coupled device (EMCCD) camera (PhotonMax 512, Princeton Instrument, Princeton NJ, USA) incorporated with a Uniphase mechanical shutter (model LS2Z2, Vincent Associates, Rochester, NY) and a driver (model VMM-T1, Vincent Associates) in external synchronization mode and frame-transfer mode. The exposure time of both the camera and the shutter driver were set at 100 ms, while the multiplication gain of the camera and the delay of the driver was set at 4000 and 100 ms respectively. Images were obtained with the WinSpec/32 software (Version 2.5.22.0, Downingtown, PA) provided by Princeton Instruments.

Data analysis. All fluorescence image data analysis was performed with the use of a public-domain image-processing programme, ImageJ (Version 1.43n). The length of fibrils was determined manually. In general, the length of fibrils was outlined with the *Freehand* function of ImageJ. The length of fibrils was automatically defined with the *Measure* function in the unit of pixels. With the aid of the pixel size of the EMCCD and the magnification of the microscope, it was calculated that each pixel represents approximately 0.26 µm. The length of a hundred of fibrils was measured for each independent experiment and frequency histogram was established with the software *Prism*.

Transmission electron microscopy (TEM). Diluted sample solution of 8 µL was dropped on a carbon-coated copper grid (CF200-Cu Electron Microscopy Sciences, Washington, USA) and then it was negatively stained with 2% uranyl acetate. The dried sample was examined by a JEM 2100 TEM (JEOL, Japan) with an acceleration voltage of 210 kV for high-resolution TEM images.

Mass spectrometry experiments. A solution of containing the solvato complex **1**–**3** (10 µM, final concentration) and $A\beta_{1-40}$ peptide monomers (10 µM, final concentration) (Anaspec) were mixed and diluted to 100 µL with MilliQ water and injected directly into the ESI-TOF-MS at a rate of 3 µL/min. ESI-TOF-MS experiments were conducted in the negative-ion mode with a Bruker MicrOTOFQ mass spectrometer. The capillary voltage was set at +3500V, and the dry N_2 gas flow was 4.0 L/min at 100 °C. Data were analyzed by the software Bruker Daltonics DataAnalysis.

Emission measurements. Stock solution of the complexes **1**–**3** was diluted (10 µM, final concentration) into phosphate buffer (20 mM, pH 7.0) with or without histidine (1 mM). The emission spectra were recorded in the 400–750 nm range, after equilibration at 25 °C for 5 min at the appropriate excitation wavelength. For the emission enhancement experiments, complex **1**–**3** was added to monomeric $A\beta_{1-40}$ and 1 h-incubated fibrillar $A\beta_{1-40}$ respectively. The fibrillar $A\beta_{1-40}$ was diluted to appropriate concentration with respect to its initial monomer concentration. The final concentration of both the complexes and monomeric and fibrillar $A\beta_{1-40}$ peptides were 25 µM, i.e. a complex to peptide ratio of 1 : 1. Two microliters of the solution was pipetted and emission spectra were recorded with the NanoDrop 3300 Fluorospectrometer (Thermo Scientific) from 400–700 nm range. The excitation wavelength was set as 365 nm. In order to minimize deviation and bias, five replicates of spectrum recording were performed for each sample and data was averaged to produce the final fluorescence emission spectrum.

Cytotoxicity test (MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-tetrazolium bromide) assay).

Neuroblastoma cells (SH-SY5Y) were seeded in a 96-well flat-bottomed microplate at 8000 cells/well in 100 μL of minimal essential medium (MEM-Eagle, Sigma) containing 10% fetal bovine serum (Invitrogen) and 1% antibiotic and antimycotic Solution (Sigma). Complex **1b** were dissolved in DMSO and mixed with the growth medium (final DMSO concentration ≤ 4%). Serial dilutions of each complex were added to each well. The microplate was incubated at 37 °C, 5% CO_2, 95% air in a humidified incubator for 24 h. After incubation, 10 μL MTT reagent (5 mg/mL) was added to each well. The microplate was re-incubated at 37 °C in 5% CO_2 for 4 h. Solubilization solution (10% SDS, 0.01 M HCl) (100 μL) was added to each well. The microplate was further incubated for 18 h. The absorbance at 570 nm was measured using a microplate reader. The IC_{50} values of **1b** (concentration required to reduce the absorbance by 50% compared to the controls) were determined by the dose-dependence of surviving cells after exposure to the metal complex for 24 h.

Figure S1: Fluorescence images and the frequency histogram (length, microns) of 20-times diluted 50 μM Aβ peptide incubated at 37 °C for 1 h in the presence of: (a) 0 μM; (b) 1 μM; and (c) 5 μM of the complex **1a** visualized using the organic dye thioflavin T (ThT). The scale bar for all fluorescence images is 20 μM.

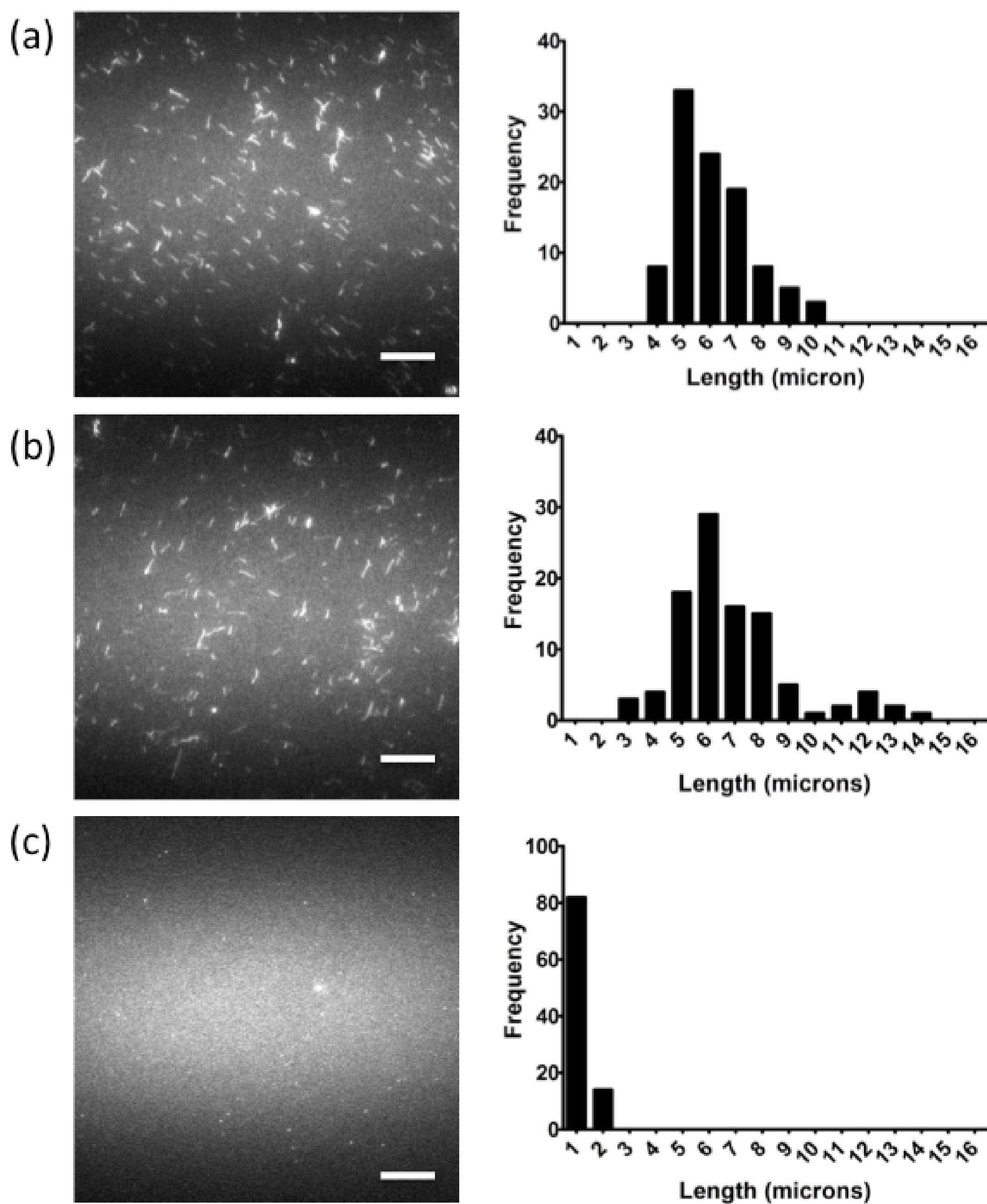


Figure S2: Fluorescence images and the frequency histogram (length, microns) of 20-times diluted 50 μM Aβ peptide incubated at 37 °C for 1 h in the presence of: (a) 0 μM; (b) 25 μM; and (c) 50 μM of the complex **2** visualized using the organic dye thioflavin T (ThT). The scale bar for all fluorescence images is 20 μM.

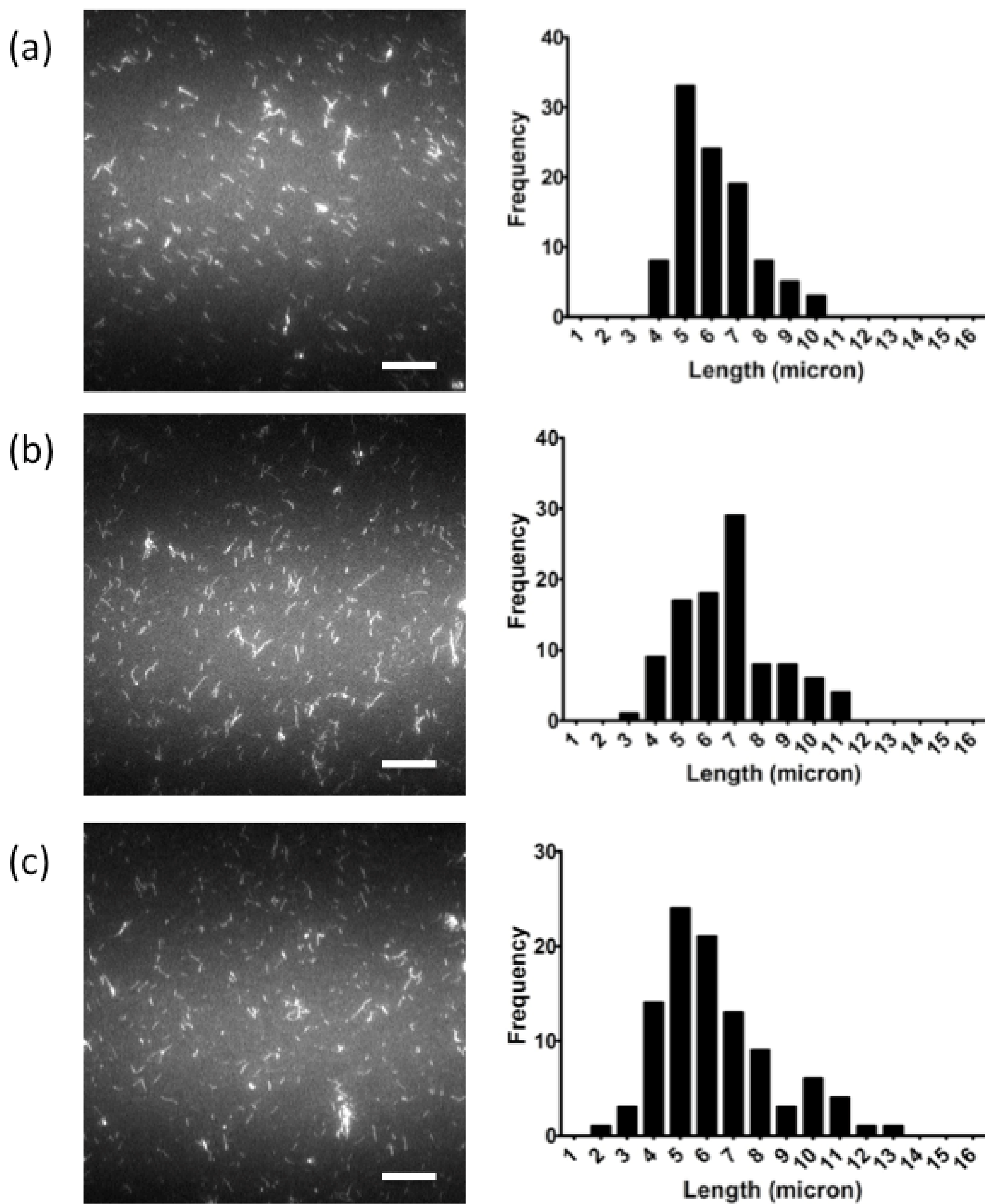


Figure S3: Fluorescence images and the frequency histogram (length, microns) of 20-times diluted 50 μM Aβ peptide incubated at 37 °C for 1 h in the presence of: (a) 0 μM; (b) 10 μM; and (c) 17 μM of the complex **3** visualized using the organic dye thioflavin T (ThT). The scale bar for all fluorescence images is 20 μM.

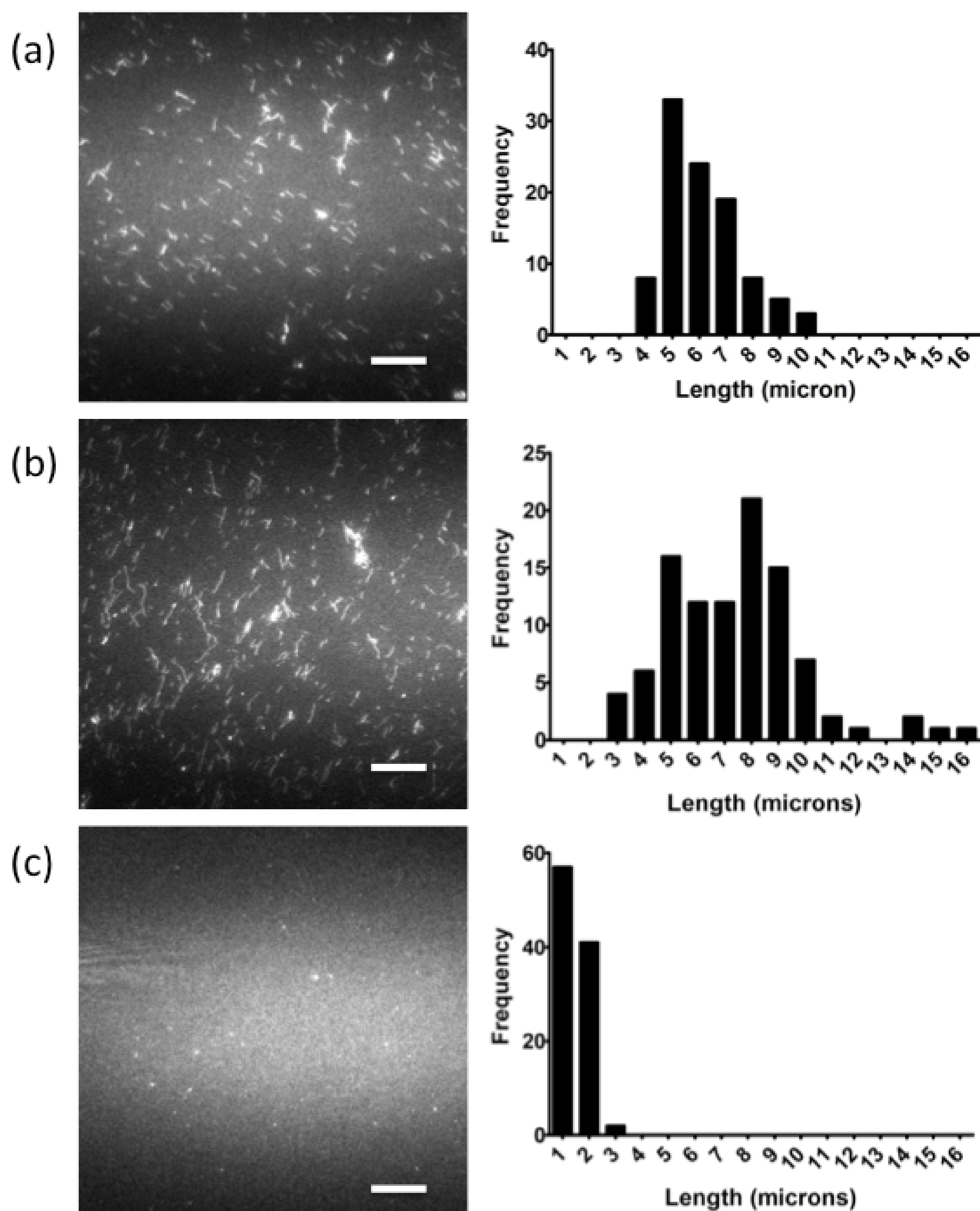


Figure S4: Fluorescence images and the frequency histogram (length, microns) of 20-times diluted (a) 50 μM Aβ peptide incubated at 37 °C for 1 h (control); 50 μM Aβ peptide incubated at 37 °C for 1 h in the presence of (b) 5 μM of rhodium(III) chloride; and (c) 10 μM of 2-phenylpyridine visualized using the organic dye thioflavin T (ThT). The scale bar for all fluorescence images is 20 μM.

No significant deviation in fibril lengths was observed comparing the control and fibrils incubated with rhodium(III) chloride and 2-phenylpyridine, which shows that aromatic scaffold and metal center are both needed for inhibitory activity.

Remarks: The difference in length distribution of control fibrils compared to the previous plots maybe contributed by the use of different batches of monomers and buffer solution.

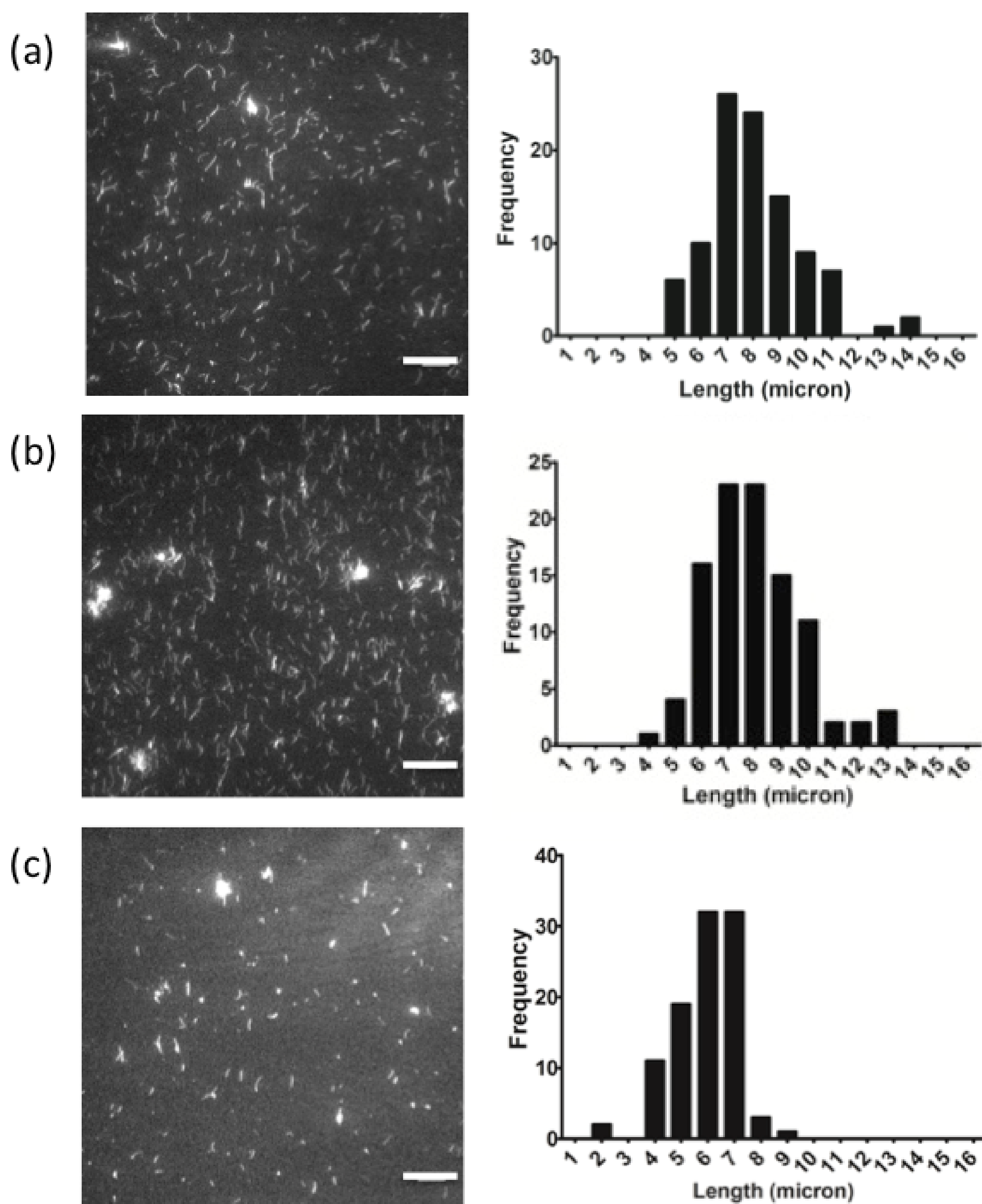


Figure S5: Fluorescence images and the frequency histogram (length, microns) of 20-times diluted (a) seed; (b) 50 μM Aβ peptide incubated at 37 °C for 15 min; and (c) 50 μM Aβ peptide incubated at 37 °C for 1 h with the addition of complex **1b** at t = 15 min. The scale bar for all fluorescence images is 20 μM.

No significant deviation in the fibril length was observed in Fig. S5b and Fig. S5c, which concluded that the fibrillogensis was terminated at any instance addition of complex addition. It is also observed that the addition of complex does not shorten the preformed fibril (Fig. S5a and S5c), supporting that the inhibitory effect was not induced by the disaggregation of fibrils, but instead, may cause by the blockage of amyloid elongation site.

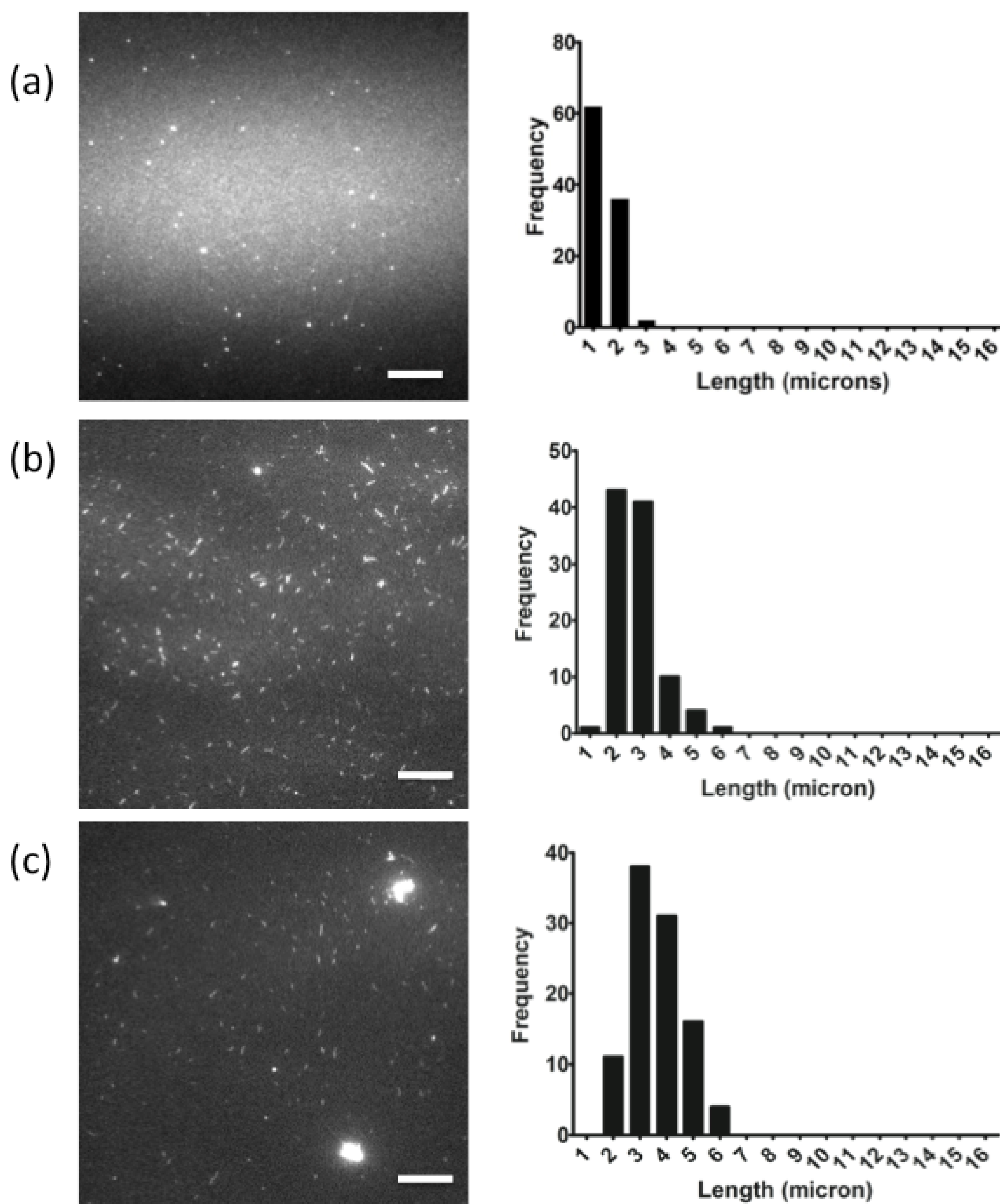
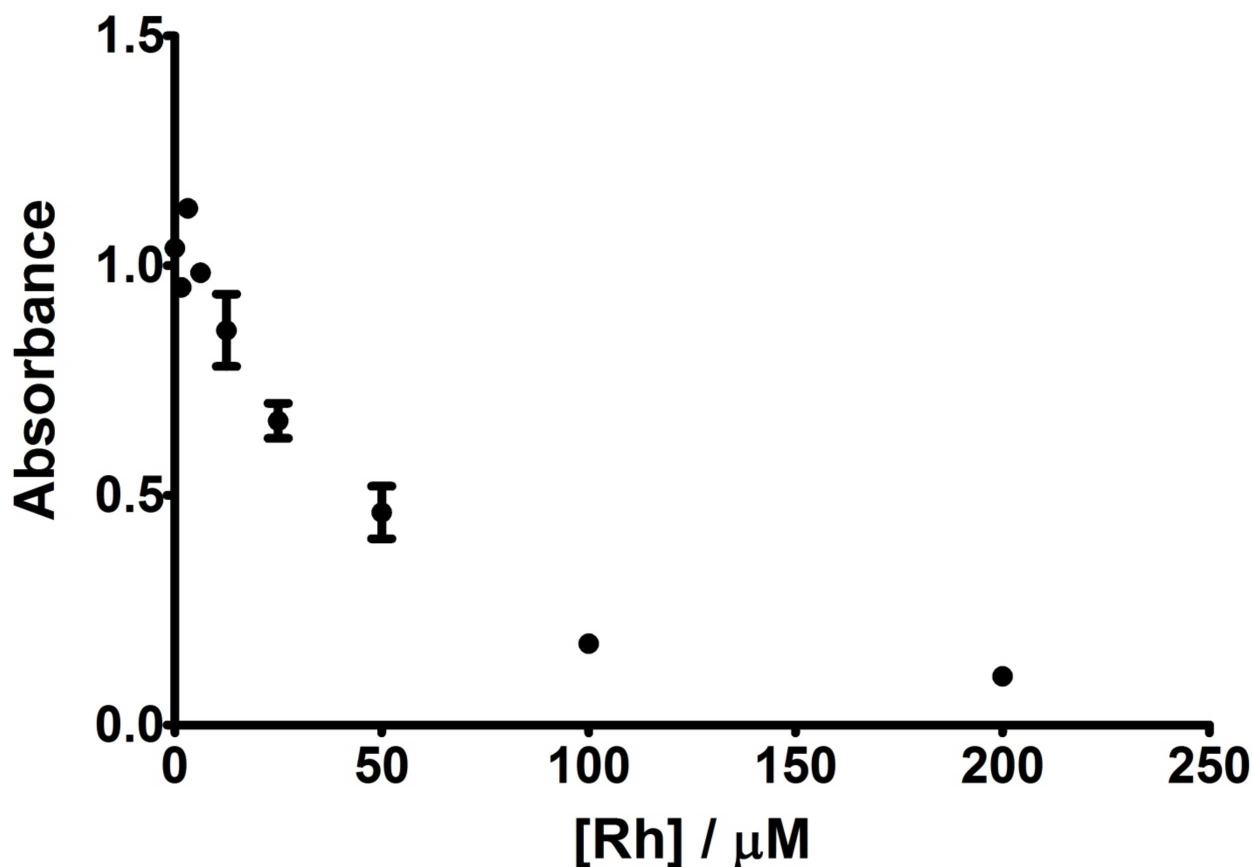


Figure S6: Results of the MTT assay showing the viability of Neuroblastoma cells incubated in varying concentration of the complex **1b** over a period of 24 h.



References:

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