

Supporting Information

Inhibition of Beta-Amyloid Fibrillation by Luminescent Iridium(III) Complex Probes

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Experimental methods

Photophysical measurement. Emission spectra and lifetime measurements for complexes were performed on a PTI TimeMaster C720 Spectrometer (Nitrogen laser: pulse output 337 nm) fitted with a 380 nm filter. Error limits were estimated: λ (± 1 nm); τ ($\pm 10\%$); ϕ ($\pm 10\%$). All solvents used for the lifetime measurements were degassed using three cycles of freeze-vac-thaw.

Luminescence quantum yields were determined using the method of Demas and Crosby¹ [Ru(bpy)₃][PF₆]₂ in degassed acetonitrile as a standard reference solution ($\Phi_r = 0.062$) and calculated according to the following equation:

$$\Phi_s = \Phi_r(B_r/B_s)(n_s/n_r)^2(D_s/D_r)$$

where the subscripts s and r refer to sample and reference standard solution respectively, n is the refractive index of the solvents, D is the integrated intensity, and Φ is the luminescence quantum yield. The quantity B was calculated by $B = 1 - 10^{-AL}$, where A is the absorbance at the excitation wavelength and L is the optical path length.

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 μ L of minimal essential medium (MEM-Eagle, Sigma) containing 10% fetal bovine serum (Invitrogen) and 1% antibiotic and

antimycotic Solution (Sigma). **14** were dissolved in DMSO and mixed with the growth medium (final DMSO concentration $\leq 1.6\%$). Serial dilutions of each complex were added to each well. The microplate was incubated at 37 °C, 5% CO₂, 95% air in a humidified incubator for 2/6/24 h. After incubation, 10 μ L MTT reagent (5 mg/mL) was added to each well. The microplate was re-incubated at 37 °C in 5% CO₂ for 4 h. Solubilization solution (10% SDS, 0.01 M HCl) (100 μ L) 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 IC₅₀ values of **14** (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 **14** for 2/6/24 h.

Synthesis. The following complexes were prepared according to (modified) literature methods. All complexes are characterized by ¹H-NMR, ¹³C-NMR, high resolution mass spectrometry (HRMS) and elemental analysis.

Complex **1**. [Ir(ppy)₂(bpy)]PF₆ (where ppy = 2-phenylpyridine, bpy = 2,2'-bipyridine) Reported²

Complex **2**. [Ir(ppy)₂(biq)]PF₆ (where biq = 2,2'-biquinoline) Reported³

Complex **3**. [Ir(ppy)₂(dmdpphen)]PF₆ (where dmdpphen = 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline) Reported⁴

Complex **4**. [Ir(ppy)₂(dbbpy)]PF₆ (where dbbpy = 4,4'-diphenyl-2,2'-bipyridine) Reported⁵

Complex **5**. [Ir(phq)₂(dpbpy)]PF₆ (where phq = 2-phenylquinoline) Reported⁵

Complex **6**. [Ir(phq)₂(4,7-dpphen)]PF₆ (where dpphen = 4,7-diphenyl-1,10-phenanthroline) Reported⁴

Complex **7**. [Ir(bzq)₂(chlorophen)]PF₆ (where bzq = benzoquinone, chlorophen = 5-chloro-1,10-phenanthroline) Reported⁶

Complex **8**. [Ir(bzq)₂(phen)]PF₆ (where phen = 1,10-phenanthroline) Reported⁷

Complex **9**. [Ir(bzq)₂(2,9-dpphen)]PF₆ (where 2,9-dpphen = 2,9-diphenyl-1,10-phenanthroline) Reported⁶

Complex **10.** $[\text{Ir}(\text{bzq})_2(\text{aminephen})]\text{PF}_6$ (where aminephen = 1,10-phenanthroline-5-amine) Reported⁸

Complex **11.** $[\text{Ir}(\text{bzq})_2(\text{dnbpy})]\text{PF}_6$ (where dnbpy = 4,4'-dinonyl-2,2'-bipyridine) Reported⁷

Complex **12.** $[\text{Ir}(\text{ppy})_2(\text{phenyl-imidazo-phen})]\text{PF}_6$ (where phenyl-imidazo-phen = 2-phenyl-1*H*-imidazo[4,5-*f*][1,10]phenanthroline) Reported⁹

Complex **13.**

$[\text{Ir}(\text{ppy})_2(\text{phenol-imidazo-phen})]\text{PF}_6$ (where phenol-imidazo-phen = 4-(1*H*-imidazo[4,5-*f*][1,10]phenanthroline-2-yl)phenol) Yield: 61%. ¹H NMR (400 MHz, Acetone-*d*₆) δ 9.31 (d, *J* = 7.6 Hz, 1H), 9.23 (d, *J* = 8.0 Hz, 1H), 8.99 (s, 1H), 8.34 (d, *J* = 8.0 Hz, 2H), 8.24 (d, *J* = 8.0 Hz, 4H), 8.07 (s, 1H), 8.00 (s, 1H) 7.95-7.88 (m, 4H), 7.73 (d, *J* = 4.0 Hz, 2H), 7.10-7.04 (m, 4H), 7.00-6.96 (m, 4H), 6.47 (d, *J* = 8.0 Hz, 2H); ¹³C NMR (100 MHz, Acetone-*d*₆) δ 168.7, 160.6, 154.5, 151.4, 150.4, 149.6, 145.4, 145.2, 139.4, 133.0, 132.6, 131.2, 129.4, 127.4, 125.8, 124.4, 123.4, 121.9, 120.7, 116.8. HRMS: Calcd. for C₄₁H₂₈IrON₆ [M-PF₆]⁺: 813.1954 Found: 813.1867 Anal. (C₄₁H₂₈IrON₆OIrPF₆ + 2H₂O) C, H, N: calcd. 49.55, 3.25, 8.46; found 49.31, 3.13, 8.62.

Complex **14.** $[\text{Ir}(\text{bzq})_2(\text{phenol-imidazo-phen})]\text{PF}_6$ Yield: 52%. ¹H NMR (400 MHz, Acetone-*d*₆) δ 9.22 (d, *J* = 7.6 Hz, 1H), 9.09 (s, 1H), 9.01 (s, 1H), 8.51 (d, *J* = 8.0 Hz, 2H), 8.34 (s, 2H), 8.21-8.18 (m, 4H), 8.01 (d, *J* = 8.0 Hz, 4H), 7.88 (d, *J* = 8.0 Hz, 2H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.46 (s, 2H), 7.24 (t, *J* = 4.0 Hz, 2H), 7.08 (d, *J* = 8.0 Hz, 2H), 6.48 (d, *J* = 8.0 Hz, 2H); ¹³C NMR (100 MHz, Acetone-*d*₆) δ 159.6, 156.4, 153.3, 148.8, 147.2, 140.3, 137.5, 133.7, 129.7, 129.5, 128.5, 128.4, 128.1, 126.8, 126.7, 124.2, 122.7, 120.4, 120.3, 115.9, 115.8. HRMS: Calcd. for C₄₅H₂₈OIrN₆[M-PF₆]⁺: 861.1954 Found: 861.1963 Anal. (C₄₅H₂₈ON₆IrPF₆ + 4H₂O): calcd. C 50.99, H 3.23, N 7.93; found 50.99, 3.18, 8.22.

Table S1 Photophysical properties of **1–14**.

Complex	Quantum yield	$\lambda_{\text{em}}/\text{nm}$	Lifetime/ μs	UV/vis absorption $\lambda_{\text{abs}}/\text{nm}$ ($\epsilon/\text{dm}^3\text{mol}^{-1}\text{cm}^{-1}$)
1	0.1278	583.5	4.325	265 (1.13×10^5)
2	0.20	656	0.75 ³	275 (1.9×10^5), 355 (6.8×10^4), 376 (7.2×10^4)
3	0.2152	568	3.692 ⁴	255 (3.8×10^4), 326 (1.6×10^4)
4	0.1674	591 ⁵	4.367	205 (9.3×10^4), 255 (7.9×10^4), 305 (3.5×10^4)
5	0.2512	560	4.586	278 (1.34×10^5), 355 (1.92×10^4), 454 (4.0×10^3)
6	0.058	565	2.39 ⁴	278.5 (4.2×10^4), 332 (1.8×10^4)
7	0.054	580	4.492	267(3.8×10^4), 320(1.6×10^4),
8	0.2573	569	186 ⁷	326(2.06×10^4), 417(5.7×10^3)
9	0.13	580	4.843	231 (3.9×10^4), 257 (3.15×10^4), 304 (2.3×10^4)
10	0.015	567	25.02 ⁸	215 (1.4×10^5), 252 (8.6×10^4), 278 (6.3×10^4), 396 (1.6×10^4)
11	0.04179	566	4.7 ⁷	330 (6.9×10^3), 419 (1.89×10^3)
12	0.24	587	0.704 ⁸	227 (6.4×10^4), 278 (1.5×10^5), 478 (1.0×10^4)
13	0.10146	583	4.515	218 (5.9×10^4), 277 (2.8×10^5), 416 (8.8×10^3)
14	0.0806	586	4.502	280 (2.7×10^5), 422 (1.2×10^4)

Figure S1. Luminescence response of **1–12** (2 μ M) in the absence or presence of 25 μ M A β _{1–40} monomer or fibril in phosphate buffer (50 mM Na₂HPO₄, 100 mM NaCl, pH 7.4).

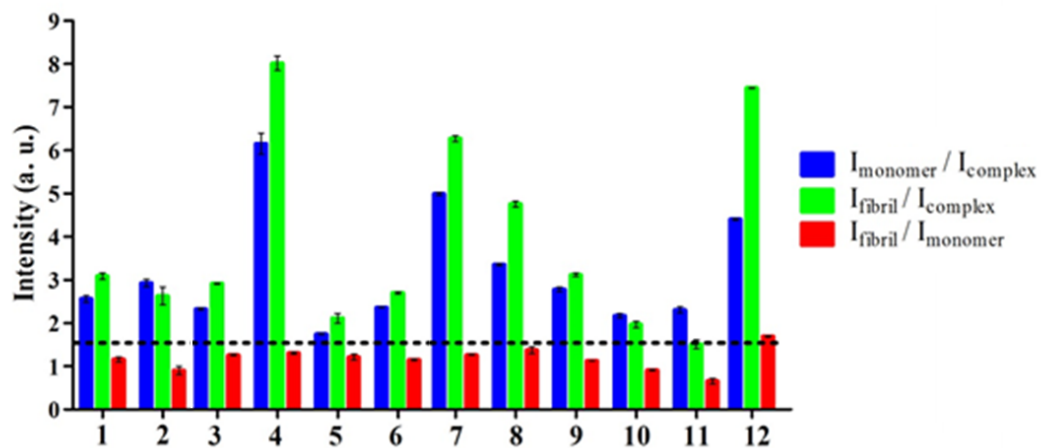


Figure S2. Seed-mediated fibril growth of 25 μM $\text{A}\beta_{1-40}$ in the presence of 50 μM of (a) ThT, **12** and (b) ThT, **13**, **14** and (c) ThT, phenol-imidazo-phen, DMSO as measured by luminescence spectroscopy. $\lambda_{\text{Ex}} = 310$ nm for **12–14**. $\lambda_{\text{Ex}} = 435$ nm for ThT.

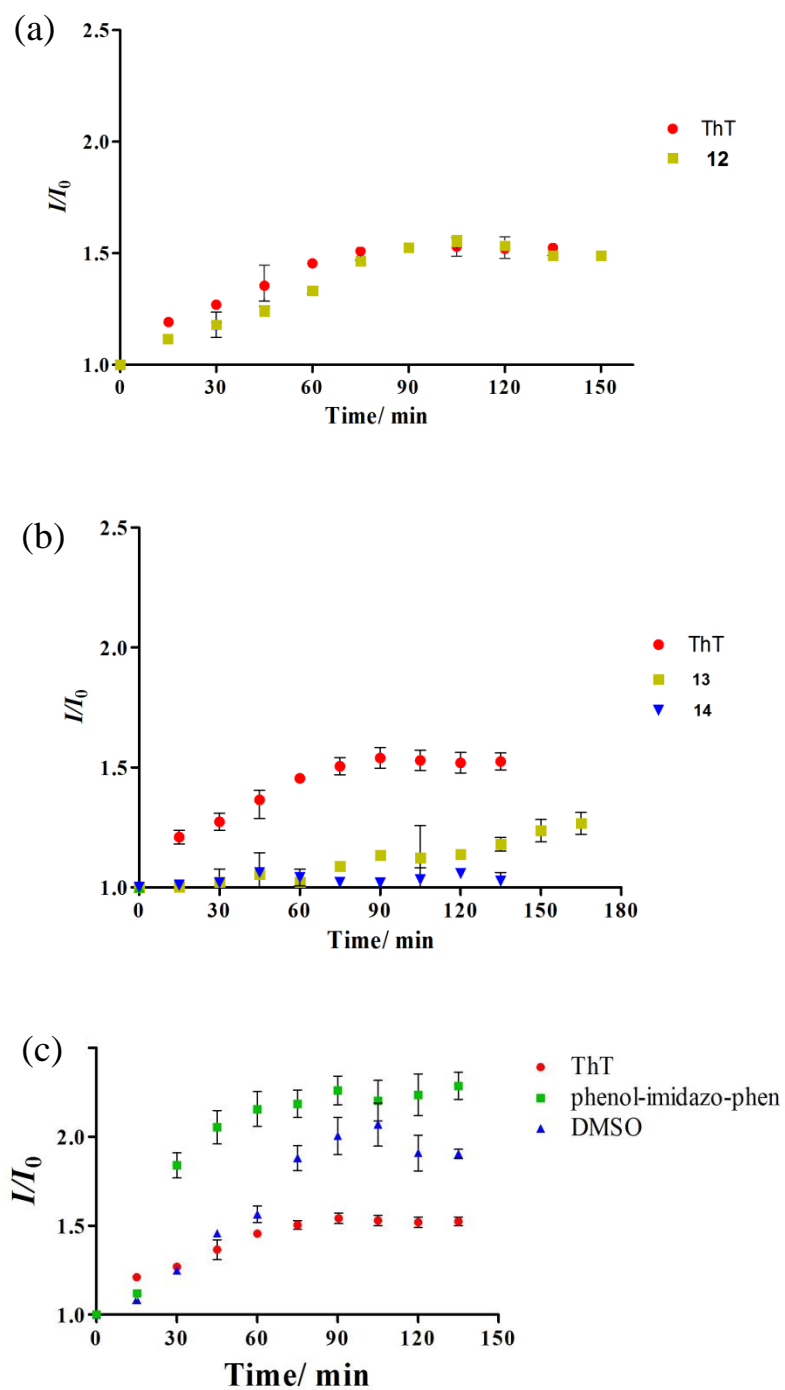


Figure S3. Seed-mediated fibril growth of 25 μM $\text{A}\beta_{1-40}$ in the presence of various concentrations of **14** as measured by luminescence spectroscopy.

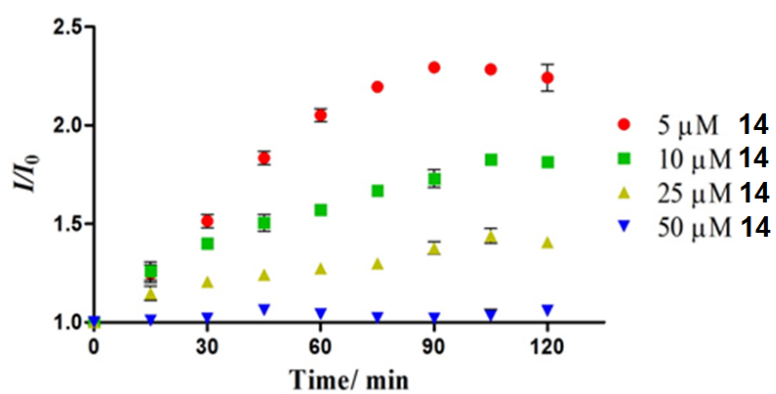


Figure S4. Electrospray ionization time-of-flight mass spectra of 50 μM $\text{A}\beta_{1-40}$ peptide incubated in the (a) absence or presence of 25 μM of (b) **13** and (c) **14** in 1 mM ammonium acetate (pH 7.6).

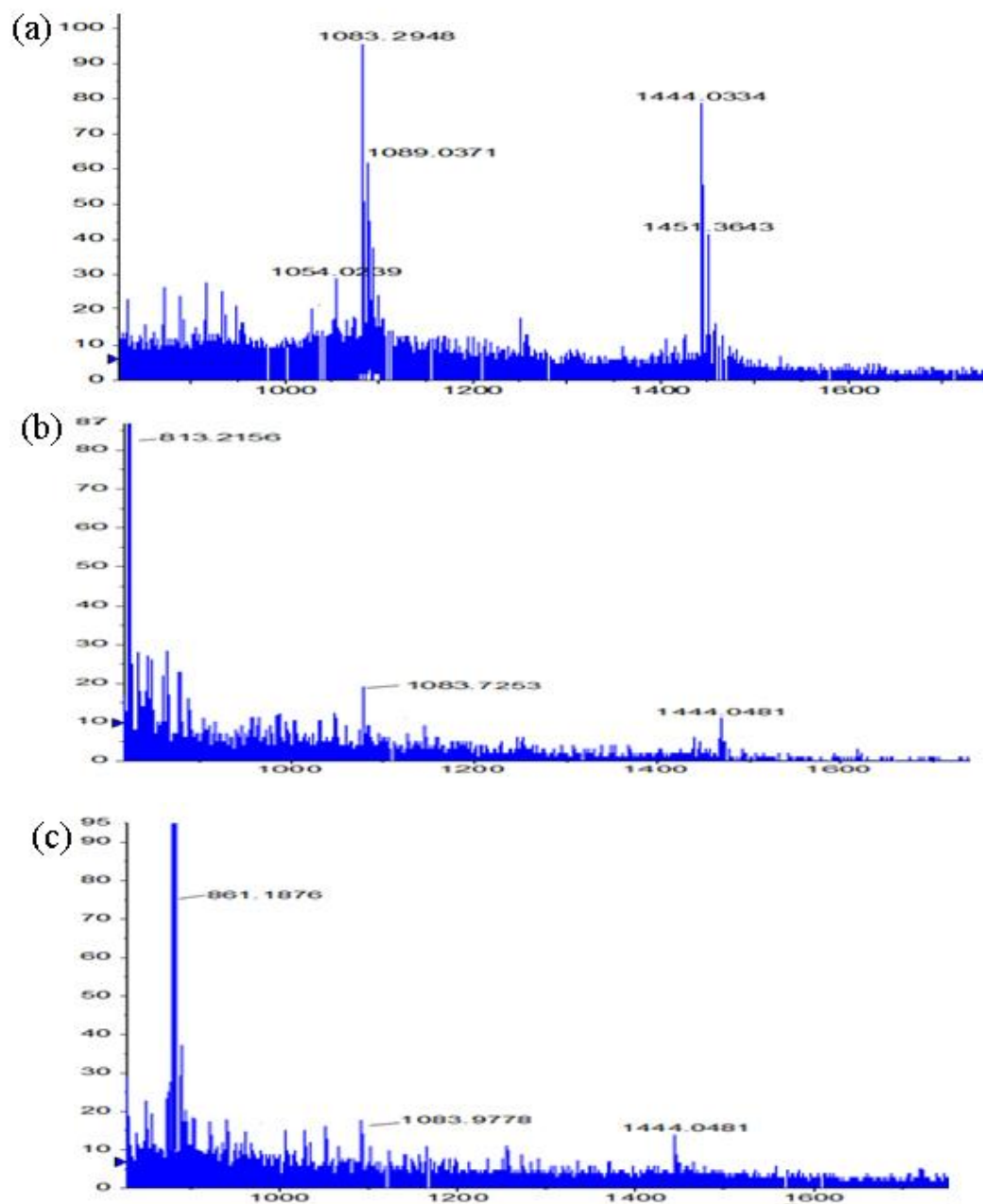


Figure S5. Cytotoxicity studies for **14** with MTT assays. Cytotoxicity of various concentrations of **14** ranged from 1 nM to 100 μ M to SH-SY5Y cells incubated for 24 h were measured with MTT assays.

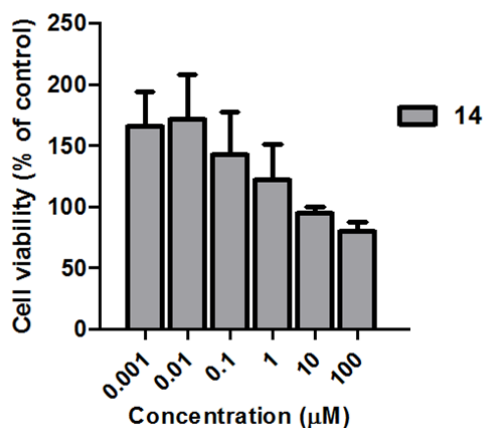
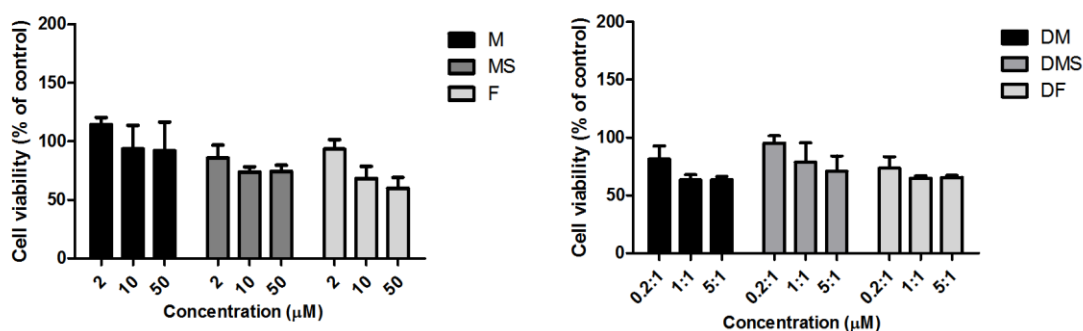


Figure S6. Neuroprotective effect of **12** against $A\beta_{1-40}$ peptide-induced cytotoxicity towards (a-b) human neuroblastoma SH-SY5Y cells. The histograms show the relative cytotoxicity of various concentrations of $A\beta_{1-40}$ peptide monomer (M), $A\beta_{1-40}$ peptide with seeded fibril (MS), and fibrillar $A\beta_{1-40}$ peptide (F), in the presence of **12**. Various forms of $A\beta_{1-40}$ peptide were incubated for 2 h at [$A\beta_{1-40}$]:[**12**] ratios of 0.2:1, 1:1, and 5:1. The relative cytotoxicity was calculated from the cytotoxicity measured for different forms of $A\beta$ peptide with **12** relative to that without, that is, $DM/M=(\mathbf{12}+M)/M$; $DMS/MS=(\mathbf{12}+MS)/MS$; $DF/F=(\mathbf{12}+F)/F$ and $D=\mathbf{12}$.



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