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

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We report herein the application of kinetically inert luminescent iridium(III) complexes as dual inhibitors and probes of beta-amyloid fibrillogenesis. These iridium(III) complexes inhibited Aβ1–40 peptide aggregation in vitro, and protected against Aβ-induced cytotoxicity in neuronal cells. Furthermore, the complexes differentiated between the aggregated and unaggregated forms of Aβ1–40 peptide on the basis of their emission response.

Over the past few years, vast efforts have been dedicated to the development of imaging probes and inhibitors for the diagnosis and treatment of Alzheimer’s disease, which is a highly common neurodegenerative disorder characterized by the progressive loss of cognitive ability1,2. One of the strategies for the treatment of Alzheimer’s disease is the development of inhibitors that prevent the misfolding and self-assembling aggregation of monomeric Aβ peptides into neurotoxic fibrils1. Many inhibitors have been reported to inhibit Aβ peptide aggregation3–6, such as short peptides7,8, organic molecules9–16, supramolecular cucurbit[7]uril17, polyoxometalates18,19, nanoparticles20 and ligand-functionalized quantum dots21. Meanwhile, the development of new diagnostic probes for Aβ fibrillation has also been an important goal. The early diagnosis of Alzheimer’s disease may allow for palliative treatment to alleviate symptoms or to slow down the progression of the disease. A number of luminescent probes have been developed for the specific labeling and imaging of Aβ plaques, such as luminescent conjugated polythiophenes22 and common fluorescent dyes23–31. Dual-role imaging agents and aggregation inhibitors of Aβ may serve a bifunctional purpose for Alzheimer’s disease, as the inhibition of Aβ fibrillogenesis may be monitored without the need for an extraneous labeling agent. However, to our knowledge, only a few examples of dual-function inhibitors and probes of Aβ have been reported in recent years. Organic dyes commonly used for labeling Aβ fibrils, including Congo Red and Thioflavine T (ThT), have been shown to inhibit Aβ aggregation at high concentrations of dyes32.

Luminescent transition metal complexes have found emerging use for the design of chemical and biological sensors33,34 in view of their useful photophysical properties, such as tunable excitation and emission wavelengths (from blue to red), high luminescent quantum yields, and relatively long phosphorescent lifetimes33. At the same time, transition metal complexes have been increasingly regarded as a promising alternative to organic compounds as therapeutic agents for the treatment of human diseases35–41. Due to the well-defined three-dimensional structure of transition metal complexes, highly specific interactions between metal complexes and biomolecules can be obtained through modification of the steric and electronic nature of the organic ligands surrounding the metal centre. In the context of Alzheimer’s disease, metal complexes such as Pt(II)42–48, binuclear Ru(II)–Pt(II)49, Ru(II)50–52, Co(III)53 and Ir(III) or Rh(III) solvato54 complexes have been reported to inhibit the aggregation of Aβ peptide55–57. Furthermore, luminescent metal complexes such as Ru(II)58,59, Re(I)60 complexes have also
been used to monitor the Aβ fibrillation process\cite{30,55}. However, few transition metal complexes have been reported to be dual inhibitors and luminescent probes of Aβ. Moreover, while iridium(III) complexes have been widely used as probes for various biomolecules, their application as dual inhibitors and probes of beta-amyloid fibrillogenesis has not been previously described.

Our group has previously reported the application of Ir(III) and Rh(III) solvato complexes as inhibitors of amyloid fibrillogenesis and as luminescent probes for Aβ peptide\cite{54}. Mass spectrometry experiments indicated that the Rh(III) solvato complex formed 1:1 covalent adducts with Aβ1–40, presumably through coordination of the N-donor histidine residue of Aβ1–40 to the Rh(III) center via displacement of the aqua ligands. Encouraged by our previous results with Group 9 solvato compounds, we sought to investigate the ability of kinetically inert Ir(III) complexes to function as dual imaging agents and inhibitors of Aβ peptide aggregation. We report herein the synthesis of a series of luminescent Ir(III) complexes containing various C^N and N^N ligands, and their ability to detect and inhibit Aβ fibrillation. We envisage that these kinetically inert Ir(III) complexes may be developed as a novel class of dual-purpose probes and inhibitors of Aβ aggregation for the effective diagnosis and/or treatment of Alzheimer’s disease.

Results

A library of twelve luminescent Ir(III) complexes (1–12, Fig. 1) were initially examined for their ability to interact with different forms of Aβ1–40 by emission titration. Of these twelve complexes, 12 bearing the 2-phenyl-1H-imidazo[4, 5-f][1,10]phenanthroline (phenyl-imidazo-phen) N^N ligand displayed the $I_{\text{fibril}}/I_{\text{monomer}}$ ratio (>1.5), indicating that it possessed the highest distinguishing ability for detecting Aβ1–40 fibrils over Aβ1–40 monomers (Figure S1). However, 1–11 were unable to effectively discriminate

**Figure 1.** Chemical structures of the luminescent Ir(III) complexes 1–14 which were synthesized and evaluated in this study.
between A\(\beta\)\(_{1-40}\) monomers and fibrils. Based on the structure of 12, we designed and synthesized 13 and 14. The characterization and photophysical properties of 1–14 are given in the ESI (Table S1).

Complexes 12–14 were investigated for their ability to inhibit the fibrillogenesis of A\(\beta\)\(_{1-40}\). Given that these complexes could detect A\(\beta\)\(_{1-40}\) fibrils over A\(\beta\)\(_{1-40}\) monomers, the kinetics of A\(\beta\)\(_{1-40}\) aggregation in the presence of the complexes could be monitored without the need for an external labeling agent. The results revealed typical sigmoidal growth curves in seed-mediated A\(\beta\) fibrillation when A\(\beta\)\(_{1-40}\) monomers was incubated with ThT or 12–14 (Figure S2a and S2b). However, fibrillogenesis was partially retarded by 13 and completely inhibited by 14, indicating that these two complexes were able to inhibit A\(\beta\)\(_{1-40}\) peptide aggregation. As both 13 and 14 contain the phenol-imidazo-phen N\(^\text{N}\) ligand, the effect of that ligand alone on A\(\beta\)\(_{1-40}\) peptide aggregation was investigated. The results showed that the presence of the N\(^\text{N}\) ligand alone had no inhibitory effect on A\(\beta\)\(_{1-40}\) peptide aggregation, highlighting the importance of the iridium(III) metal center in maintaining the octahedral structure of the active complexes (Figure S2c). Moreover, total internal reflection fluorescence microscopy (TIRFM) with laser excitation was used to observe the ThT-labelled fibrils with high sensitivity. In the absence of Ir(III) complexes, the fluorescence images of the A\(\beta\)\(_{1-40}\) peptides incubated by the seed-mediated method showed dense spots, corresponding to newly generated A\(\beta\)\(_{1-40}\) fibrils (Fig. 2a). In contrast, these spots partially or completely disappeared when the samples were treated with 13 (Fig. 2b) or 14 (Fig. 2c), indicating that 13 and 14 could partially or completely inhibit the fibrillogenesis of amyloid peptides, respectively. Transmission electron microscopy (TEM) images further confirmed the ability of the Ir(III) complexes to inhibit seed-mediated A\(\beta\) aggregation. In the control experiment (Fig. 3a), A\(\beta\)\(_{1-40}\) grew into thick, dense and long hair-like fibrils. However, when the sample was incubated with 13, the TEM image showed short flake-like fibrils (Fig. 3b), indicating that 13 was able to partially inhibit the A\(\beta\)\(_{1-40}\) aggregation. Furthermore, incubation of the sample with 14 resulted in the complete absence of elongated fibrils, and only the preformed fibril seeds were observed (Fig. 3c), indicating the complete inhibition amyloid fibrillogenesis by 14. Moreover, the fibrillogenesis kinetics of A\(\beta\)\(_{1-40}\) in the presence of various concentrations of 14 showed that the inhibition of A\(\beta\)\(_{1-40}\) aggregation by 14 was concentration-dependent (Figure S3). At 50 \(\mu\)M of 14, the inhibition of 25 \(\mu\)M of A\(\beta\)\(_{1-40}\) peptides was nearly completely inhibited.

Figure 2. Inhibition of seed-mediated A\(\beta\)\(_{1-40}\) fibril growth by Ir(III) complexes. TIRFM images of A\(\beta\)\(_{1-40}\) fibrils grown in the (a) absence and presence of (b) 13, (c) 14 after incubation at 37°C for 1 h. The scale bar for TIRFM is 20 \(\mu\)m.
Encouraged by the promising activity of 13 and 14 against Aβ aggregation, we investigated that the luminescence behaviour of 13 and 14 towards different forms of Aβ_{1–40}. The results showed that both complexes displayed a significantly enhanced luminescence response in the presence of the Aβ_{1–40} monomers or fibrils (Fig. 4). 13 exhibited ca. 6- and 12-fold emission enhancements at λ_{max} = 540 nm in the presence of comparable mass concentrations of Aβ_{1–40} monomers and fibrils, respectively. On the other hand, 14 showed ca. 11- and 18-fold emission enhancements at λ_{max} = 540 nm in the presence of Aβ_{1–40} monomers and fibrils. Taken together, these two Ir(III) complexes showed luminescence enhancement to the presence of Aβ_{1–40} monomers and fibrils to different extents. We presume that this behaviour of the Ir(III) complexes towards the Aβ_{1–40} peptide may be due to the ability of the complexes to bind to a hydrophobic region within the peptide, thus protecting the complexes from non-radiative decay by solvent quenching and thereby giving rise to an enhanced luminescence response. The differential
luminescence response of the complexes towards Aβ_{1-40} monomers and fibrils may be due to the different microenvironments experienced by the Ir(III) complexes upon binding to the Aβ_{1-40} monomers or the fibrils.

ESI-TOF mass spectrometry experiments were performed to examine the binding of the Ir(III) complexes to Aβ_{1-40} peptide. The mass spectrum of the Aβ_{1-40} monomer in the absence of the Ir(III) complexes shows two characteristic peaks at 1083 and 1444, corresponding to the 4+ and 3+ ionization states of the Aβ_{1-40} monomer, respectively (Figure S4a). However, incubation of the Aβ_{1-40} peptide with 13 (Figure S4b) or 14 (Figure S4c) produced no new peaks in the mass spectra besides those corresponding to the free complex (813 for 13 and 861 for 14), suggesting that the Ir(III) complexes were not covalently bound to the Aβ_{1-40} peptide.

The cytotoxicity of the most potent Ir(III) complex 14 was examined using the 3-(4,5-dime thylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Figure S5). Neuroblastoma cells (SH-SY5Y) were incubated in the presence of different concentrations for 24 h and cell viability was examined using the MTT assay. The IC_{50} value of 14 was estimated to be >100 μM at 24 h of exposure. Notably, these IC_{50} values are significantly higher than the concentration of 14 required for complete inhibition of Aβ_{1-40} peptide aggregation, suggesting the presence of a therapeutic window whereby Aβ_{1-40} peptide aggregation can be controlled without significant damage to brain cells.

The effect of 14 on Aβ_{1-40}-induced cytotoxicity in SH-SY5Y cells and mouse primary cortical cells was also investigated. The cytotoxicity of three different forms of Aβ_{1-40} peptide in the presence and the absence of 14 were examined: Aβ_{1-40} peptide monomer (M), Aβ_{1-40} peptide monomer with seeded fibrils (MS) and Aβ_{1-40} fibril (F) (Fig. 5). The results showed that treatment of cells with different forms of Aβ_{1-40} peptides caused toxicity to SH-SY5Y cells and mouse primary cortical cells (Fig. 4a,c,e,g). Encouragingly, 14 exhibited a neuroprotective effect against the cytotoxicity induced by all three forms of Aβ_{1-40} peptide at [Aβ_{1-40}]/[14] ratios of 0.2, 1.0, or 5.0 for SH-SY5Y cells (Fig. 5a,b) or mouse primary cortical cells (Fig. 5e,f) after 2 h of incubation. The neuroprotective effects of 14 were still observable after 24 h of incubation of 14 (Fig. 5c,d,g,h). As a negative control, we also investigated the effect of 12, which showed no effect against amyloid aggregation, on Aβ_{1-40}-induced toxicity. The results showed that 12 had no neuroprotective effect against cytotoxicity induced by all three forms of Aβ_{1-40} peptide at [Aβ_{1-40}]/[12] ratios of 0.2, 1.0, or 5.0 in SH-SY5Y cells (Figure S6). Taken together, these data indicate that 14 displays neuroprotective effects against Aβ-mediated cytotoxicity when administered at a low enough dosage in SH-SY5Y cells and mouse primary cortical cells.

Discussion
In conclusion, a library of 12 luminescent Ir(III) complexes containing various C^N and N^N ligands were initially screened as luminescent probes for Aβ_{1-40} peptide. Based on the ability of 12 for distinguishing Aβ_{1-40} fibrils over monomers, 13 and 14 were further synthesized and tested. The novel Ir(III) complex 14 emerged as the most potent candidate and was shown to inhibit Aβ_{1-40} peptide aggregation as revealed by a luminescence assay, as well as TIRFM and TEM imaging. Notably, Aβ_{1-40} peptide aggregation was nearly completely inhibited at 50 μM of 14. A neuroprotective effect of 14 against Aβ_{1-40}-induced cytotoxicity in SH-SY5Y cells and mouse primary cortical cells was also demonstrated. Using ESI-TOF mass spectrometry, we also showed 14 was not covalently bound to the Aβ_{1-40} peptide. Non-covalent probes may have a better safety index, lower cross-reactivity, and lower immunogenicity compared to covalently-binding molecules61-63. We envision that this work would open up new avenues for the development of dual-role imaging agents and aggregation inhibitors of Aβ for the treatment of Alzheimer’s disease.

Methods
Chemicals and materials. Reagents, unless specified, were purchased from Sigma Aldrich (St. Louis, MO) and used as received. Iridium chloride hydrate (IrCl₃·xH₂O) was purchased from Precious Metals Online (Australia).

General experiment. Mass spectrometry was performed at the Mass Spectroscopy Unit at the Department of Chemistry, Hong Kong Baptist University, Hong Kong (China). Deuterated solvents for NMR purposes were obtained from Armar and used as received.

¹H and ¹³C NMR were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz (¹H) and 100 MHz (¹³C). ¹H and ¹³C chemical shifts were referenced internally to solvent shift (acetone-δ_d: ¹H δ 2.05, ¹³C δ 29.8). Chemical shifts (δ) are quoted in ppm, the downfield direction being defined as positive. Uncertainties in chemical shifts are typically ±0.01 ppm for ¹H and ±0.05 for ¹³C. Coupling constants are typically ±0.1 Hz for ¹H-¹H and ±0.5 Hz for ¹H-¹³C couplings. The following abbreviations are used for convenience in reporting the multiplicity of NMR resonances: s, singlet; d, doublet; t, triplet; m, multiplet. All NMR data was acquired and processed using standard Bruker software (Topspin).

Photophysical measurement. Emission spectra and lifetime measurements for complexes were performed as previously reported method64.
Transmission electron microscopy (TEM) imaging.  8 μL of diluted sample solution was applied to a carbon-coated copper grid (T200H-Cu Electron Microscopy Sciences, Washington, USA), dried, and was then negatively stained with 2% uranyl acetate. The stained sample was then allowed to be dried. Transmission electron micrographs were recorded using a Technai G2 Transmission Electron Microscope (FEI, USA) with an acceleration voltage of 200 kV.

Total internal reflection fluorescence microscopy (TIRFM) imaging.  This experimental system and operation was as previously described\textsuperscript{12}. After the incubation period, the organic dye ThT (the mole ratio of Aβ\textsubscript{1–40} and ThT is 1:2) was added to label the Aβ\textsubscript{1–40} fibrils. Then, a 445 nm diode laser (50 mW, LQC445-40E, Newport, USA) was used for the excitation of the ThT-labeled Aβ\textsubscript{1–40}. Images were obtained by using the WinSpec/32 software (Princeton Instruments, Version 2.5.22.0, Downingtown, PA).
Preparation of stock solution of tested Ir(III) complexes. The stock solution of all the tested Ir(III) complexes are 10 mM in DMSO.

Emission measurement in buffered solution. Ir(III) complexes (2 μM) and different concentrations of Aβ1-40 monomer/fibril were added into phosphate buffer (50 mM Na2HPO4, pH 7.4). The mixtures were allowed to equilibrate at room temperature for 3 min without degassing. Emission spectra were recorded on PTI TimeMaster C720 Spectrometer.

Preparation of Aβ3-40 fibrils for seeding. The incubation of Aβ3-40 fibrils and seeds used the previously reported method[52].

ThT and Ir(III) complexes luminescence binding assays. 50 μM of ThT/complex solution (1%) was incubated with 25 μM Aβ1-40 monomer solution in phosphate buffer at 37 °C. The luminescence intensity at different time points was measured on a PTI TimeMaster C720 Spectrometer.

Mass spectrometry experiments. A solution of containing 13 or 14 (25 μM, final concentration) (final DMSO concentration ≤ 1%) and Aβ1-40 peptide monomers (50 μM, final concentration) were mixed in 1 mM ammonium acetate (pH 7.6), and injected into the ESI-TOF-MS at a rate of 3L/min. ESI-TOF-MS experiments were conducted in the negative-ion mode with a Bruker MicroTOFQ mass spectrometer. The capillary voltage was set at +3500 V, and the dry N2 gas flow was 4.0 L/min at 100 °C. Data were analyzed by the software Bruker Daltonics Data Analysis.

Cell viability analysis. The cell viability analysis is referenced from a reported method[52]. Specifically, SH-SY5Y cells or primary cortical cells were plated into 96-well plates at a density of 5 × 104 cells/well in cell culture medium. At the next day, the culture medium was replaced with the same medium with 0.2% serum and containing 10 μM 12 and 14 together with monomer, mixture of monomer and seed, and seeding fibril respectively in 1-to-0.2, 1-to-1 or 1-to-5 ratio (final concentration of DMSO ≤ 1.6%). Cells were incubated at 37 °C for 2 and 24 h, respectively. The medium of each well was replaced with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL) and further incubated for 3 h at 37 °C. The MTT solution was aspirated off and then 100 μL of DMSO was added to each well to dissolve the formazan crystals. The plates were agitated on a plate shaker for 15 min and the absorbance was measured at 570 nm using a SpectraMax M5 microplate reader (Molecular Devices). Wells without cells were used as blanks and were subtracted as background from each sample. Results were expressed as a percentage of control.

References
1. Hardy, J. & Selkoe, D. J. The Amyloid Hypothesis of Alzheimer’s Disease: Progress and Problems on the Road to Therapeutics. Science 297, 353–356 (2002).
2. Ballard, C., Lee, V. M. Y. & Trojanowski, J. Q. Tau-mediated neurodegeneration in Alzheimer’s disease and related disorders. Nat. Rev. Neurol. 8, 663–672 (2007).
3. Wang, Q., Yu, X., Li, L. & Zheng, J. Inhibition of Amyloid-β Aggregation in Alzheimer’s Disease. Curr. Pharm. Des. 20, 1223–1243 (2014).
4. Cheng, B. et al. Inhibiting toxic aggregation of amyloidogenic proteins: A therapeutic strategy for protein misfolding diseases. Biochim. Biophys. Acta 1830, 4860–4871 (2013).
5. Zhang, M. et al. Nanomaterials for Reducing Amyloid Cytotoxicity. Adv. Mater. 25, 3780–3801 (2013).
6. Belluti, F., Rampa, A., Gobbi, S. & Bisi, A. Small-molecule inhibitors/modulators of amyloid-β peptide aggregation and toxicity for the treatment of Alzheimer’s disease: a patent review (2010–2012). Expert Opin. Ther. Pat. 23, 581–596 (2013).
7. Tamin, G., Ruchala, P. & Teplow, D. B. A Peptide Hairpin Inhibitor of Amyloid β-Protein Oligomerization and Fibrogenesis. Biochemistry 48, 11329–11331 (2009).
8. Taylor, M. et al. Development of a Proteolitically Stable Retro-Inverso Peptide Inhibitor of β-Amyloid Oligomerization as a Potential Novel Treatment for Alzheimer’s Disease. Biochemistry 49, 3261–3272 (2010).
9. Cohen, T., Frydman-Marom, A., Rechter, M. & Gazit, E. Inhibition of Amyloid Fibril Formation and Cytotoxicity by Hydroxyindole Derivatives. Biochemistry 45, 4727–4735 (2006).
10. Reinke, A. A. & Gestwicki, J. E. Structure-activity Relationships of Amyloid Beta-aggregation Inhibitors Based on Curcumin: Influence of Linker Length and Flexibility. Chem. Biol. Drug Des. 70, 206–215 (2007).
11. Hong, H.-S. et al. Inhibition of Alzheimer’s amyloid toxicity with a tricyclic pyrone molecule in vitro and in vivo. J. Neurochem. 108, 1097–1108 (2009).
12. Reinke, A. A., Ung, P. M. U., Quintero, J. J., Carlson, H. A. & Gestwicki, J. E. Chemical Probes That Selectively Recognize the Earliest Aβ Oligomers in Complex Mixtures. J. Am. Chem. Soc. 132, 17655–17657 (2010).
13. Mishra, R. et al. Small-Molecule Inhibitors of Islet Amyloid Polypeptide Fibril Formation. Angew. Chem. Int. Ed. 47, 4679–4682 (2008).
14. Arai, T. et al. Rational Design and Identification of a Non-Peptidic Aggregation Inhibitor of Amyloid-β Based on a Pharmacophore Motif Obtained from cyclo-[Lys-Leu-Val-Phe-Pro-]. Angew. Chem. Int. Ed. 53, 8236–8239 (2014).
15. Razavi, H. et al. Benzoxazoles as Transthyretin Amyloid Fibril Inhibitors: Synthesis, Evaluation, and Mechanism of Action. Angew. Chem. Int. Ed. 42, 2758–2761 (2003).
16. Sharma, A. K. et al. Bifunctional Compounds for Controlling Metal-Mediated Aggregation of the Aβ42 Peptide. J. Am. Chem. Soc. 134, 6625–6636 (2012).
17. Lee, H. H. et al. Supramolecular Inhibition of Amyloid Fibrillation by Cucurbit[7]uril. Angew. Chem. Int. Ed. 53, 7461–7465 (2014).
18. Geng, J., Li, M., Ren, J., Wang, E. & Xu, Q. Polyoxometalates as Inhibitors of the Aggregation of Amyloid β Peptides Associated with Alzheimer’s Disease. Angew. Chem. Int. Ed. 50, 4184–4188 (2011).
19. Gao, N. et al. Transition-metal-substituted polyoxometalate derivatives as functional anti-amyloid agents for Alzheimer's disease. Nat. Commun. 5, 5422 (2014).
20. Yoo, S. I. et al. Inhibition of Amyloid Peptide Fibrillation by Inorganic Nanoparticles: Functional Similarities with Proteins. Angew. Chem. Int. Ed. 50, 5110–5115 (2011).
21. Xiao, L., Zhao, D., Chan, W.-H., Choi, M. M. F. & Li, H.-W. Inhibition of beta 1–40 amyloid fibrillation with N-acetyl-L-cysteine capped quantum dots. Biomaterials 31, 91–98 (2010).
22. Herland, A., Thomsson, D., Mirzov, O., Schelbykin, I. G. & Inganas, O. Decoration of amyloid fibrils with luminescent conjugated polymers. J. Mater. Chem. 18, 126–132 (2008).
23. Nesterov, E. E. et al. In Vivo Optical Imaging of Amyloid Aggregates in Brain: Design of Fluorescent Markers. Angew. Chem. Int. Ed. 44, 5452–5456 (2005).
24. Chang, W. M. et al. ANCA: A Family of Fluorescent Probes that Bind and Stain Amyloid Plaques in Human Tissue. ACS Chem. Neurosci. 2, 249–255 (2011).
25. Li, M. et al. In Situ Monitoring Alzheimer's Disease β-Amyloid Aggregation and Screening of Aβ Inhibitors Using a Perylene Probe. Small 9, 52–55 (2013).
26. Mishra, R., Sjölander, D. & Hammerstrom, P. Spectroscopic characterization of diverse amyloid fibrils in vitro by the fluorescent dye Nile red. Mol. Biosyst. 7, 1232–1240 (2011).
27. Hong, Y. et al. Monitoring and Inhibition of Insulin Fibrillation by a Small Organic Fluorogen with Aggregation-Induced Emission Characteristics. J. Am. Chem. Soc. 134, 1680–1689 (2011).
28. Sarthiv, A. et al. Smart Near-Infrared Fluorescence Probes with Donor–Acceptor Structure for in Vivo Detection of β-Amyloid Deposits. J. Am. Chem. Soc. 136, 3388–3394 (2014).
29. Wolfe, L. S. et al. Protein-induced photophysical changes to the amyloid indicator dye thioflavin T. Proc. Natl. Acad. Sci. USA 107, 16863–16868 (2010).
30. Ma, D.-L. et al. Current Advancements in Aβ Luminescent Probes and Inhibitors of Aβ Aggregation. Curr. Alzheimer Res. 9, 830–843 (2012).
31. Nilsson, K. P. R. Small organic probes as amyloid specific ligands – Past and recent molecular scaffolds. FEBS Lett. 583, 2593–2599 (2009).
32. Lorenzo, A. & Yankner, B. A. Beta-amyloid neurotoxicity requires fibril formation and is inhibited by congo red. Proc. Natl. Acad. Sci. USA 91, 12243–12247 (1994).
33. Zhao, Q., Huang, C. & Li, F. Phosphorescent heavy-metal complexes for bioimaging. Chem. Soc. Rev. 40, 2508–2524 (2011).
34. Zhao, Q., Li, F. & Huang, C. Phosphorescent chemosensors based on heavy-metal complexes. Chem. Soc. Rev. 39, 3007–3030 (2010).
35. Meggers, E. Exploring biologically relevant chemical space with metal complexes. Curr. Opin. Chem. Biol. 11, 287–292 (2007).
36. Meggers, E. Targeting proteins with metal complexes. Chem. Commun. 1001–1010, doi: 10.1039/b813568a (2009).
37. Meggers, E. From Conventional to Unusual Enzyme Inhibitor Scaffolds: The Quest for Target Specificity. Angew. Chem. Int. Ed. 50, 2442–2448 (2011).
38. Gasser, G., Ott, I. & Metzler-Nolte, N. Organometallic Anticancer Compounds. J. Med. Chem. 54, 3–25 (2010).
39. Ott, I. & Gust, R. Non Platinum Metal Complexes as Anti-cancer Drugs. Archiv. der Pharmazie. 340, 117–126 (2007).
40. Wong, C.-Y., Chung, L.-H., Lu, L., Wang, M., He, B., Liu, L.-J., Leung, C.-H., & Ma, D.-L. Dual inhibition and monitoring of beta-amyloid fibrillation by a luminescent iridium(III) complex. Curr. Alzheimer Res. 12, 439–443 (2015).
41. Chan, S.-L.-F., Lu, L., Lam, T.-L., Yan, S.-C., Leung, C.-H., & Ma, D.-L. A novel tetradentate rhenium(II) complex containing tris(2-pyridylmethyl)methane (tpa) as an inhibitor of beta-amyloid fibrillation. Curr. Alzheimer Res. 12, 434–438 (2015).
42. Sarnham, K. E. J. et al. Platinum-based inhibitors of amyloid-β as therapeutic agents for Alzheimer's disease. Proc. Natl. Acad. Sci. USA 105, 6813–6818 (2008).
43. Kenche, V. B. et al. Development of a Platinum Complex as an anti-Amyloid Agent for the Therapy of Alzheimer's Disease. Angew. Chem. Int. Ed. 52, 3374–3378 (2013).
44. Ma, G. et al. Identification of [PtCl2(phen)] Binding Modes in Amyloid-β Peptide and the Mechanism of Aggregation Inhibition. Chem. Eur. J. 17, 11657–11666 (2011).
45. Ma, G. et al. PtCl2(phen) disrupts the metal ions binding to amyloid-β peptide. Metallomics 5, 879–887 (2013).
46. Streltsov, V. A. et al. Structural insights into the interaction of platinum-based inhibitors with the Alzheimer's disease amyloid-β peptide. Chem. Commun. 49, 11364–11366 (2013).
47. Wang, X., Wang, X., Zhang, C., Jiao, Y. & Guo, Z. Inhibitory action of macrocyclic platiferiniferous chelators on metal-induced Aβ aggregation. Chem. Soc. 3, 1304–1312 (2012).
48. Collin, F. S., Sasaki, L., Eury, H., Fallier, P. & Hureau, C. P(II) compounds interplay with Cu(II) and Zn(II) coordination to the amyloid-β peptide has metal specific consequences on deleterious processes associated to Alzheimer's disease. Chem. Commun. 49, 2130–2132 (2013).
49. Kumar, A. et al. Inhibition of Aβ42 Peptide Aggregation by a Binuclear Ruthenium(II)–Platinum(II) Complex: Potential for Multimetal Organometallics as Anti-amyloid Agents. ACS Chem. Neurosci. 1, 691–701 (2010).
50. Vyas, N. A. et al. Ruthenium(II) polypropyridyl complex as inhibitor of acetylcholinesterase and Aβ aggregation. Eur. J. Med. Chem. 75, 375–381 (2014).
51. Valensin, D. et al. fac-[Ru(CO)3Cl2] Selectively Targets the Histidine Residues of the β-Amyloid Peptide 1-28. Implications for New Alzheimer's Disease Treatments Based on Ruthenium Complexes. Inorg. Chem. 49, 4720–4722 (2010).
52. Messori, L., Camarri, M., Ferraro, T., Gabbiani, C. & Franceschini, D. Promising Metal complexes as inhibitors of β-amyloid aggregation. Perspectives for an innovative metalletherapeutics on Alzheimer's disease. Coord. Chem. Rev. 256, 2357–2366 (2012).
53. Li, M. et al. Chiral metallo-helical complexes enantioselectively target amyloid β for treating Alzheimer's disease. J. Am. Chem. Soc. 136, 11655–11663 (2014).
54. Cook, N. P., Torres, V., Jain, D. & Martí, A. A. Sensing Amyloid-β Aggregation Using Luminescent Dipyridophenazine Ruthenium(II) Complexes. J. Am. Chem. Soc. 133, 11121–11123 (2011).
55. Cook, N. P., Oehl, M., Katsampes, C., Prabhakar, R. & Martí, A. A. Unraveling the Photoluminescence Response of Light-Switching Ruthenium(II) Complexes Bound to Amyloid-β. J. Am. Chem. Soc. 135, 10810–10816 (2013).
56. Sathiv, V. et al. Alkoxys bridged binuclear rhenium(I) complexes as a potential sensor for β-amyloid aggregation. Talanta 130, 274–279 (2014).
61. Singh, J., Petter, R. C., Baillie, T. A. & Whitty, A. The resurgence of covalent drugs. *Nat. Rev. Drug. Discov.* **10**, 307–317 (2011).

62. Beck, P., Dubiella, C. & Groll, M. Covalent and non-covalent reversible proteasome inhibition. *Biol. Chem.* **393**, 1101–1120 (2012).

63. Patonay, G., Salon, J., Sowell, J. & Strekowski, I. Noncovalent labeling of biomolecules with red and near-infrared dyes. *Molecules* **9**, 40–49 (2004).

64. Lu, L., Chan, D.S.-H., Kwong, D.W., He, H.-Z., Leung, C.-H. & Ma, D.-L. Detection of nicking endonuclease activity using a G-quadruplex-selective luminescent switch-on probe. *Chem. Sci.* **5**, 4561–4568 (2014).

Acknowledgements

This work is supported by Hong Kong Baptist University (FRG2/14-15/004), the Health and Medical Research Fund (HMRF/13121482 and HMRF/14130522), the Research Grants Council (HKBU/201811, HKBU/204612 and HKBU/201913), the French National Research Agency/Research Grants Council Joint Research Scheme (A-HKBU201/12), State Key Laboratory of Environmental and Biological Analysis Research Grant (SKLP-14-15-P001), National Natural Science Foundation of China (21575121), Guangdong Province Natural Science Foundation (2015A030313816), Hong Kong Baptist University Century Club Sponsorship Scheme 2015, Interdisciplinary Research Matching Scheme (RC-IRMS/14-15/06), the State Key Laboratory of Synthetic Chemistry, the Science and Technology Development Fund, Macao SAR (103/2012/A3 and 098/2014/A2) and the University of Macau (MYRG091(Y3-L2)-ICMS12-LCH, MYRG2015-00137-ICMS-QRCM and MRG023/LCH/2013/ICMS).

Author Contributions

L.L., H.-J.Z., M.W. and S.-L.H. carried out all the experiments, performed the data analysis and wrote the manuscript. H.-W.L., C.-H.L. and D.-L.M. designed the experiments and analyzed the results.

Additional Information

Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Lu, L. et al. Inhibition of Beta-Amyloid Fibrillation by Luminescent Iridium(III) Complex Probes. *Sci. Rep.* **5**, 14619; doi: 10.1038/srep14619 (2015).

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