Detection and disaggregation of amyloid fibrils by luminescent amphiphilic platinum(II) complexes†

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Cyclometallated Pt(II) complexes possessing hydrophobic 2-phenylpyridine (ppy) ligands and hydrophilic acetonyleacetone (acac) ligands have been investigated for their ability to detect amyloid fibrils via luminescence response. Using hen egg-white lysozyme (HEWL) as a model amyloid protein, Pt(II) complexes featuring benzanilide-substituted ppy ligands and ethylene glycol-functionalized acac ligands demonstrated enhanced luminescence in the presence of HEWL fibrils, whereas Pt(II) complexes lacking complementary hydrophobic/hydrophilic ligand sets displayed little to no emission enhancement. An amphiphilic Pt(II) complex incorporating a bis(ethylene glycol)-derivatized acac ligand was additionally found to trigger restructuring of HEWL fibrils into smaller spherical aggregates. Amphiphilic Pt(II) complexes were generally non-toxic to SH-SY5Y neuroblastoma cells, and several complexes also exhibited enhanced luminescence in the presence of Aβ1-42 fibrils associated with Alzheimer’s disease. This study demonstrates that easily prepared and robust (ppy)Pt(II)(acac) complexes show promising reactivity toward amyloid fibrils and represent attractive molecular scaffolds for design of small-molecule probes targeting amyloid assemblies.

Introduction

Luminescent transition metal complexes are uniquely suited for applications in bioimaging and as biochemical probes. Bioprobes incorporating d6 and d8 metal complexes of Re, Ru, and Pt possess photophysical properties that offer particularly distinct advantages over purely organic fluorophores. For example, these complexes often display long-lived emission through triplet metal–ligand charge transfer (3MLCT) excited states that can be tuned to longer or shorter wavelength as a function of ancillary ligands. Additionally, the physical properties of heteroleptic complexes (e.g., biocompatibility, solubility) can be readily modified by employing appropriately functionalized ligands without adversely affecting desirable photophysical properties. Square planar d8 Pt(II) complexes possess several other attractive characteristics that render their use in biological applications especially appealing. Depending on the coordinated ligands, emission from planar Pt(II) complexes can reflect mixing of ligand-centered and metal-centered charge transfer transitions to produce long emission lifetimes with large Stokes shifts. Additionally, the square planar geometry leaves axial positions on the Pt(II) centers available for intermolecular interactions, such as with solvent or, upon stacking, with other Pt(II) centers. In turn, Pt–Pt interactions can give rise to significantly red-shifted metal–metal–ligand charge transfer emission (MMLCT). Many Pt(II) complexes, especially cyclometallated complexes (e.g., 2-phenylpyridine Pt complexes) are easily prepared, robust, and display excellent photostability.

Amyloid oligomers and fibrils are formed from self-assembly of misfolded or denatured proteins and polypeptides. Scores of amyloidogenic proteins have been identified, and, despite differences in primary sequence, all amyloid assemblies exhibit similar topological features. Mature amyloid fibrils are characterized by a cross-β-sheet structure in which interstrand β-sheet hydrogen bonding is propagated parallel to the fibril axis. Hydrophobic interactions and other non-covalent attractions mediate association of parallel β-sheets to complete the cross-β-sheet structure and deliver protofibrils. Intertwining of protofibrils then gives rise to mature fibrils. Protofibrils result from dynamic self-assembly of smaller soluble amyloid oligomers. The structure of oligomers is highly variable (dimers, trimers, tetramers, larger structured oligomers, etc.) according to the identity and concentration of the amyloid protein monomer. Oligomers and fibrils both feature exposed hydrophobic surfaces which provide potential binding sites for hydrophobic probes (such as Thioflavin T).
and, in the case of oligomers, may contribute to biological activity (for example, neurotoxicity of amyloid oligomers assembled from Aβ peptides in Alzheimer’s disease).\textsuperscript{12,13}

In addition to Alzheimer’s disease, amyloid aggregates have been implicated in several other widespread human diseases, including Parkinson’s disease and type II diabetes.\textsuperscript{14} Consequently, luminescent probes capable of detecting amyloid fibrils/oligomers and influencing the course of amyloid aggregation may offer new approaches for disease diagnosis and therapy.\textsuperscript{15,16} The organic dye Thioflavin T (ThT) is the laboratory standard for \textit{ex vivo} detection of mature fibrils, but ThT is not responsive to soluble amyloid oligomers and is not selective for amyloid fibrils in the presence of other proteins and biomolecules. As a result, other organic fluorophores have been investigated as probes for amyloid fibrils,\textsuperscript{17–20} and a much smaller subset of compounds has been shown to respond to the presence of soluble amyloid oligomers.\textsuperscript{21–22} From these studies common structural features of probe molecules that promote binding to amyloid aggregates have been identified. The most important of these is the presence of extended hydrophobic surfaces, such as found in biaryl, polaryl, polyaromatic, and stilbene-like fragments, that can interact with exposed hydrophobic sites on amyloid fibrils and oligomers.

Construction of luminescent amyloid probes from transition metal complexes represents an attractive alternative to use of purely organic-derived materials, however this approach has not been extensively explored.\textsuperscript{33–35} The metal center in amyloid-sensitive complexes can be utilized not only to impart desirable photophysical properties but also as a synthetic linchpin around which one or more functionalized ligands can be organized in a well-defined geometry. For example, Martí and coworkers have investigated a series of Ru(bpy)\textsubscript{2}\textsuperscript{2+} and Ru(phen)\textsubscript{2}\textsuperscript{2+} complexes bearing dipyridophenazine (1, X = CH) and dipyrazinophenanthroline (2, X = N) ligands as probes for Aβ and α-synuclein amyloid aggregates implicated in neurodegenerative diseases (Fig. 1).\textsuperscript{36–46} Carlos, \textit{et al.} have examined related Ru(phen)\textsubscript{2}(aminopyridine)\textsubscript{2} complexes for monitoring Aβ and insulin amyloid aggregation.\textsuperscript{41–43} While Zhong, \textit{et al.} have reported a tris(heteroleptic) Ru(dipyridophenazine)\textsuperscript{2+} complex that exhibits dual emission for ratiometric monitoring of amyloid aggregation.\textsuperscript{44} Several rhenium(II) complexes have been used for luminescence detection of Aβ fibrils. These include a (dipyridophenazine)Re(CO)\textsubscript{2}py complex reported by Martí (3, Fig. 1),\textsuperscript{45,46} alkoxycarded binuclear Re(vinylpyridine) complexes reported by Rajagopal,\textsuperscript{47} and Re(benzothiazole)\textsubscript{2} polythiophene hybrids prepared and studied in our group (4, Fig. 1).\textsuperscript{48} Finally, there has been only a single report describing detection of amyloid fibrils using a luminescent Pt(II) complex. Yam and coworkers found that the Pt(bzimpy) complex 5 (Fig. 1) exhibited enhanced long wavelength emission (650 nm) in the presence of amyloid fibrils obtained from bovine insulin.\textsuperscript{49} This response was ascribed to binding of the aryl alkynyl ligands to hydrophobic surfaces of the fibril leading to Pt–Pt interactions and long wavelength emission through \textsuperscript{3}MMLCT excited states.

We now report the design and synthesis of new luminescent cyclometalated Pt(II) complexes that show enhanced emission in the presence of amyloid fibrils prepared from hen egg-white lysozyme (HEWL). In particular, complexes displaying amphiphilic properties from a combination of hydrophobic 2-phenylpyridine ligands (ppy) and hydrophilic acetonolactone (acac) ligands have been investigated. Amphiphilic fluorophores have shown promising activity toward amyloid fibrils,\textsuperscript{50–52} and assembling complementary hydrophobic/hydrophilic ligands around square planar Pt(II) metal centers offers attractive opportunities to discover new luminescent amyloid probes. The complex displaying the greatest enhanced emission in the presence of fibrils (13) features a benzanilide-substituted ppy ligand and an acac ligand incorporating a bis(diethylene glycol) amide moiety. Complex 13 not only displays 12-fold emission enhancement when treated with HEWL fibrils but also shows the ability to alter the morphology of mature fibrils as determined by atomic force microscopy (AFM) imaging studies.

Results and discussion

Design and synthesis

Cyclometalated Pt(II) acac complexes have been investigated in a number of settings due to their luminescent properties.\textsuperscript{53} In
the context of probes for amyloid aggregates, we were also attracted to desirable structural properties evident in the parent (ppy)Pt(acac) complex 6 (Scheme 1). Specifically: (1) substituted ppy ligands that provide increased hydrophobic surfaces known to be important for amyloid-binding probes are easily accessible; (2) acac ligands modified with hydrophilic groups are equally easily accessible through straightforward routes; (3) (ppy)Pt(acac) complexes are assembled in highly modular fashion so that ppy and acac ligands can be independently varied; and (4) hydrophobically-driven binding of amphiphilic complexes to amyloid aggregates may result in positioning of hydrophilic acac ligands along the fibril/oligomer periphery, thereby altering the course of further amyloid self-assembly.

A two-step route adapted from established procedures was employed to prepare Pt(II) complexes 6–14 (Scheme 1, see ESI† for experimental details). In the first step, ppy or substituted ppy ligand L1–L4 was treated with an equimolar amount of K2PtCl4 in aqueous 2-ethoxyethanol and heated to 100 °C. The resulting cyclometalated platinum chloride dimers that precipitated from reaction mixtures were then treated with acac or functionalized acac ligand L5–L8 and base in either 2-ethoxyethanol or 1,4-dioxane at 100 °C. The desired complexes were isolated in the indicated overall yields as air- and moisture-stable materials after purification by flash column chromatography. All new complexes were fully characterized by NMR and mass spectrometry. In addition, single-crystal X-ray structures of complexes 7–9 were successfully obtained (see ESI†).

The hydrophobic ppy ligands examined in this study include a trans-stilbene derivative L2 (7, 10), benzanilide substituted ppy derivative L3 possessing a 2° amide linkage (8, 11–13), and benzanilide L4 possessing an N-methyl 3° amide linkage (9). The stilbene ligand was selected as various stilbene-like molecules are known to exhibit amyloid binding activity.5,5 Functionalized acac ligands incorporating ethylene glycolate methyl ether L6 (10, 11),58 diethylene glycol amide L7 (12), and bis(diethylene glycol) amide L8 (13, 14) were used to provide the hydrophilic sites of the amphiphilic complexes spatially separated from the hydrophobic ligands via the square planar Pt(II) center.

Absorption and emission spectra

Absorption spectra for complexes 6–14 were obtained in several solvents of differing polarities and are shown in the ESI (Fig. S1–S9†). In general, all spectra exhibit similar features and UV absorption of individual complexes was largely insensitive to solvent. Higher energy absorptions (<300 nm) are assigned to intraligand transitions, while broad longer wavelength absorptions centered at ~375 nm for the stilbene-ppy hybrid complexes and ~315 nm for the amide-functionalized ppy complexes are attributed to mixed ligand-centered transitions and MLCT. Amide-substituted ppy complexes exhibited a weaker lower energy shoulder that extended to ~425 nm. The identity of the acac ligand does not influence the absorption spectra.

Emission spectra for all complexes were obtained in various solvents using an excitation wavelength of 410 nm. Complexes 7 and 10 bearing the stilbene-like ppy ligand both exhibited broad emission at ~510 nm in CH2Cl2 solution that was quenched in aqueous phosphate buffered saline (PBS) (ESI, Fig. S10 and S11†). The emission spectra of the remaining Pt complexes all resemble the emission spectrum of the parent (ppy)Pt(acac) complex 6 (ESI, Fig. S12†) to varying degrees. For example, emission spectra of 8 in organic solvents (Fig. 2a) exhibit structured emission bands similar to those seen for 6 at 500 and 535 nm. In water or PBS solution, however, these structured emission bands are replaced with broad, unstructured, and red-shifted emissions centered at 600 nm, consistent with excimer formation caused by π-stacking of the complex in aqueous solutions. The N-methyl analogue 9 displayed similar emission spectra (ESI, Fig. S13†). Excimer emission is observed in aqueous solutions of 6 as well (see ESI, Fig. S12†) and in the solid state.59

Fluorescence spectra of 13 in organic solvents also exhibited structured emission bands with λmax ~ 500 nm (Fig. 2b). In
contrast with 8, emission of 13 was quenched in aqueous solutions. This is attributed to the presence of the functionalized hydrophilic acac ligand, rendering 13 more solvated in aqueous environments and impeding hydrophobic stacking interactions. Notably, emission profiles of 11–12 (possessing hydrophilic acac ligands L6 and L7, respectively) and 14 (incorporating an unsubstituted ppy ligand in combination with L8) are very similar to 13 (ESI, Fig. S14–S16).

Dynamic light scattering
To gain insight into Pt(II) complex behaviour in aqueous solutions, dynamic light scattering (DLS) experiments were performed on complexes 8 and 13. Fig. 3a–c illustrate changes in emission of 8 as a function of H2O content in aqueous CH3CN solutions, and Fig. 3d shows the corresponding particle sizes as measured using DLS. Emission at 500 nm was observed to steadily increase with increasing H2O content up to 60% H2O/CH3CN. In aqueous CH3CN with water fraction >60% the emission at 500 nm decreased concomitantly with increased emission at 600 nm. The average particle size of 8 in 10% aqueous CH3CN was determined to be ~320 nm. Particle size decreased to an average size of 110 nm and became more narrowly distributed in solutions containing higher water fraction, up to 60% (corresponding to maximum emission at 500 nm). In 90% H2O/CH3CN (corresponding to 600 nm emission), the particle size of 8 was broadly distributed around an average of ~446 nm.

Amphiphilic complex 13 also exhibited slightly enhanced emission at 500 nm in aqueous CH3CN up to a H2O fraction of 70% (Fig. 3e and f) and an average particle size of ~195 nm (Fig. 3g). At higher water fractions, significantly diminished
emission was observed along with increased aggregate particle size (>800 nm in 90% aqueous CH₃CN).

This data suggests that maximum emission of 8 observed in 60% aqueous CH₃CN (λ_{max} 500 nm) arises from relatively small aggregates, around 100 nm in size. At higher H₂O fractions (above 60%), self-assembly of 8 produces larger particles (average size ~ 446 nm) and red-shifted emission tentatively attributed to excimer formation via π stacking of the complexes. Similarly, maximum emission of 13 at 500 nm (70% aqueous CH₃CN) is observed to occur from relatively small aggregates with average size of 195 nm. At higher water fraction (>70%) aggregates with larger average particle size are observed but, unlike 8, these larger aggregates of 13 are essentially non-emissive. We conclude that the functionalized acac ligand in 13 must influence the structure of supramolecular aggregates to impede π stacking and/or Pt-Pt interactions.

**Interaction of Pt complexes with HEWL amyloid fibrils**

All new Pt complexes were assayed for luminescence response in the presence of amyloid fibrils prepared from hen egg-white lysozyme (HEWL). HEWL is a 129-amino acid protein that forms amyloid fibrils upon denaturation. Moreover, HEWL benefits from wide availability and low cost, making it an attractive model protein for studies of amyloid-binding probes.60,61 Fibrillization of HEWL was performed under acidic conditions and monitored using a standard ThT fluorescence assay (ESI, Fig. S17†). ThT fluorescence revealed the expected sigmoidal growth curve for fibrillation, consisting of a lag phase for the first ~4 h of incubation followed by rapid fibril growth to attain a stationary phase after ~8 h. Fibril formation under these conditions was confirmed using atomic force microscopy (AFM, vide infra).

Aliquots of HEWL incubation solutions taken at different time points were diluted with pH 7.4 PBS solution (to stop fibrillation)60 in a 96 well plate followed by addition of Pt(n) complex. Luminescence response was then measured using a fluorescence plate reader. In experiments involving the parent (ppy)Pt(acac) complex 6 as well as stilbene-derived ppy complexes 7 and 10, little to no luminescence enhancement was observed throughout the fibrillation process at the wavelength of maximum emission for each complex (490 nm for 6, 510 nm for 7 and 10). In contrast, amide-substituted Pt(acac) complex 8 exhibited a luminescence profile for HEWL fibrillation resembling ThT that resulted in ~6-fold emission enhancement at 510 nm in the presence of fully formed HEWL fibrils. This data is illustrated in Fig. 4a in which emission enhancement (I/I₀) at the λ_{max} for each complex as a function of HEWL incubation time is shown for the four Pt complexes discussed above.

As it appears 8 is capable of binding to HEWL fibrils to produce enhanced emission, luminescence responses of the remaining amide-substituted complexes 9 and 11–13 to HEWL fibrillation were measured and the data is shown in Fig. 4b. Complex 9, possessing an N-methyl amide group on the ppy ligand, and complex 11, incorporating functionalized acac ligand 16, were both inferior to 8 as probes of HEWL fibrillization. Amphiphilic complexes 12 and 13, however, displayed greater emission enhancement during HEWL fibrillation relative to 8, an effect attributed to the presence of hydrophilic acac ligands L7 and L8. Moreover, 13 displays enhanced emission at earlier time points of HEWL fibrillation (i.e., after 3 h incubation) compared to other Pt(n) complexes and ThT. This may be indicative of interaction between 13 and pre-fibrillar HEWL aggregates, such as soluble oligomers. Amphiphilicity has been identified as an important characteristic of other amyloid fibril/oligomer probes due to the ability of amphiphilic materials to interact with hydrophobic surfaces of oligomers/fibrils while also engaging with more polar regions of amyloid aggregates.52 Amphiphilic probes might also shield hydrophobic surfaces of fibrils/oligomers from aqueous solvent, in turn influencing the course of further aggregation and fibril stability. Notably, complex 14 which lacks a functionalized ppy ligand but retains hydrophilic acac L8 does not show a significant luminescence response to HEWL fibrils (ESI, Fig. S18†), demonstrating the importance of both the substituted ppy and functionalized acac ligands in 13 for mediating fibril interaction.

Binding assays were performed to quantify the interaction of 8 and 13 with HEWL fibrils. Saturation binding isotherms were generated from luminescence titration experiments in which Pt(n) complex luminescence was measured against a fixed concentration of HEWL fibrils (ESI, Fig. S19 and S20†). The data was analysed by non-linear regression using a one-site binding model to calculate equilibrium dissociation constants (K_{d}).38 The calculated K_{d} for binding of 8 and 13 to HEWL fibrils is 7.9 ± 0.8 µM and 5.5 ± 1.5 µM, respectively. These binding constants reflect the greater luminescence response of 13 toward HEWL fibrils, further highlighting advantages of an amphiphilic probe, although binding is relatively modest in both cases.

Luminescence of 13 (20 µM) in the presence of other biomolecules (10 µM) was briefly examined (ESI, Fig. S21†). The most intense emission response was observed in the presence of HEWL fibrils. Emission to a lesser extent was also noted in the presence of human and bovine serum albumins (HSA, BSA). Little to no luminescence was observed when 13 was...
combined with HEWL monomers, human immunoglobulin G (IgG), pepsin, and calf thymus DNA (CT DNA).

**Atomic force microscopy (AFM) imaging**

Successful formation of HEWL fibrils under incubation conditions used in this study was verified by AFM height imaging. After 8 h of incubation under acidic conditions (the time needed for mature fibril formation according to ThT assay described previously), an HEWL stock solution was diluted with pH 7.4 PBS. An aliquot of this solution was used to prepare a sample for AFM imaging which revealed fully formed amyloid fibrils (Fig. 5a).

Next, the integrity of HEWL fibrils in the presence of Pt(II) complexes 8 and 13–14 was examined. Aliquots of fully formed HEWL fibril solution were combined with the indicated complexes in pH 7.4 PBS and maintained for 24 h. After this time AFM imaging was performed on each sample and the results are shown in Fig. 5b–d.

Fibrils incubated with 8, possessing a benzanilide ppy ligand and an unfunctionalized acac ligand, remained essentially unchanged, exhibiting size and shape comparable to control fibrils (Fig. 5b). Likewise, exposure of fibrils to 14 produced no significant changes to fibril size or shape (Fig. 5d). In contrast, AFM revealed that incubation of fibrils for 24 h with amphiphilic complex 13 resulted in dramatic changes to fibril morphology. Rather than typical hair-like structures, aggregates shown in Fig. 5c exhibit sub-micron spherical shapes with average particle height less than half the height of control fibrils and fibrils exposed to complexes 8 and 14. These results suggest that the combination of ligands in complex 13 not only mediates binding to HEWL fibrils but also promotes the disaggregation and remodelling of fully formed fibrils. Since HEWL fibrillization is performed under strongly acidic conditions, the ability of 13 to inhibit fibril formation was not investigated. However, small molecules capable of disrupting disease-related amyloid aggregates (fibrils and/or oligomers) have been advanced as potential therapeutic agents.62,63 Future work will examine the ability of 13 and related amphiphilic Pt complexes to affect the fibrillization process of toxic amyloid proteins.

**Detection of Aβ42 fibrils**

Luminescence response of selected Pt(II) complexes toward amyloid fibrils prepared from disease-relevant peptides was briefly examined. Specifically, samples of fibrils prepared from Aβ42, an amyloidogenic peptide implicated in Alzheimer’s disease, were separately treated with platinum complexes 12 and 13. In each case, ~7-fold luminescence enhancement over Pt(II) complex emission in buffer (PBS) was observed (Fig. 6a). This result indicates that amphiphilic Pt(II) complexes may be suitable for monitoring and/or influencing the aggregation of peptides linked to neurodegeneration.

**MTT assay**

Experiments were performed to examine the cytotoxicity of selected Pt(II) complexes using MTT cell viability assays. For these experiments, SH-SY5Y neuroblastoma cells were treated with Pt(II) complexes 8 and 11–13 in concentrations ranging from 1 to 100 μM. Untreated cells and cells treated with rotenone (1, 5, and/or 10 μM) were used as positive and negative controls, respectively. assay results for all four Pt(II) complexes are illustrated in Fig. 7. Only complex 11, containing a hydrolytically labile ester linkage in the functionalized acac ligand, exhibited any appreciable cytotoxicity in a dose-dependent manner at concentrations of 5 μM and above. The remaining three complexes exhibited essentially no cytotoxicity up to 100 μM. The compatibility of amphiphilic Pt(II) complexes such as 12–13 with SH-SY5Y cells bodes well for use of these and related compounds in in vivo settings.

**Basis for fibril detection**

Several small organic molecules have been found to exhibit enhanced fluorescence in the presence of amyloid fibrils. The
The basis for fluorescence response is generally ascribed to the ability of non-polar probe molecules to bind hydrophobic fibril surfaces, resulting in restriction of molecular motion and shielding of probe molecules from aqueous solution. A similar rationale is invoked to explain luminescence enhancement of amyloid probes constructed from transition metal complexes (e.g., Fig. 1). The Pt(II) complexes used in this study incorporate hydrophobic cyclometallated 2-phenylpyridine ligands to mediate binding to amyloid surfaces as revealed through enhanced emission. Dynamic light scattering experiments indicate that 8 forms supramolecular aggregates in aqueous solution that exhibit emission at 600 nm. In the presence of HEWL fibrils, however, complex 8 exhibits ~6-fold enhanced emission at shorter wavelength (510 nm) which we ascribe to hydrophobically-driven binding of the Pt(II) complex to exposed non-polar regions of the fibril surface. Thus, introduction of amyloid fibrils to PBS solutions of 8 appears to disrupt 600 nm-emitting Pt(II) complex assemblies in favour of binding to intact fibrils, leading to blue-shifted enhanced emission at 510 nm (Fig. 8a).

Amphiphilic Pt complex 13 binds to HEWL fibrils and promotes fibril disaggregation as detected by AFM imaging (see Fig. 5c). Disruption of amyloid fibril integrity by certain transition metal complexes has been noted in other cases. In some instances, this behaviour is attributed to generation of reactive oxygen species (ROS) that result in degradation of fibril assemblies, while in other instances direct coordination of transition metal centers to amyloid amino acid side chains is believed to be important. Given that (ppy)Pt\(^{II}\)(acac) complexes are not especially redox active nor coordinatively labile, neither of these mechanisms appears operative in reactions of fibrils with 13. Instead, we speculate that the amphiphilicity of 13 is important in driving fibril disaggregation (Fig. 8b). Initial hydrophobically-driven binding of 13 to fibril surfaces may allow interaction between hydrophilic acac ligands and adjacent polar regions of the fibril, leading to breakdown of intertwined protofibrils and formation of the smaller spherical particles detected by AFM. We further speculate that positioning of hydrophilic acac ligands on solvent-exposed surfaces may interfere with the ability of these particles to undergo further aggregation. Notably, while we have used HEWL as a model amyloid protein in this study, topologically related amyloid fibrils from other peptides are believed to play important roles in human diseases such as Alzheimer’s and type II diabetes. Utilization of amphiphilic Pt(II) complexes to detect and
disrupt disease-relevant protein oligomers and/or fibrils offers new strategies to develop diagnostic or therapeutic organo-metallic agents targeting amyloid diseases.

Conclusions

This study establishes the ability of amphiphilic cyclometalated Pt(n) acac complexes to detect amyloid fibrils prepared from HEWL via enhanced luminescence. In addition, exposure of HEWL fibrils to Pt(n) complex 13, possessing a hydrophobic benzanilide phenylpyridine ligand and a hydrophilic bis(di-ethylene glycol) acac ligand, resulted in fibril degradation and formation of smaller spherical protein aggregates as determined by AFM imaging. Other Pt(n) complexes lacking the amphiphilic properties of 13 did not alter the integrity or morphology of HEWL fibrils. The Pt(n) complexes investigated in this work were all easily prepared using a modular synthetic route that should facilitate assembly of additional luminescent Pt(n) complexes bearing complementary ppy and acac chelating ligands spatially separated by the square planar metal center. These complexes offer a convenient organometallic platform from which amyloid-responsive small molecules may be designed for detection and/or alteration of disease-related amyloid oligomers and fibrils. In support of this objective, 13 was shown to exhibit enhanced luminescence in the presence of Aβ42 fibrils, an amyloid aggregate associated with Alzheimer’s disease. Amphiphilic Pt(n) complexes were further demonstrated to be non-toxic toward SH-SY5Y neuroblastoma cells in MTT cell viability assays. In current work we seek to build on these results and ultimately develop Pt(n) complexes capable of recognizing and modifying small pre-fibrillar oligomers of Aβ peptides implicated as neurotoxic agents in Alzheimer’s disease.

Author contributions

Z. L. helped conceive the project, performed experimental work, analyzed the data, and wrote the original draft of the manuscript; Z. L., A. B. E., and A. V. T. conducted AFM imaging studies; A. E. B. and J. A. D. performed MTT assays; F. C. P. conceived the project, supervised the work, and revised the manuscript. All authors reviewed the final manuscript.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

1 L. C.-C. Lee and K. K.-W. Lo, Strategic Design of Luminescent Rhenium(I), Ruthenium(II), and Iridium(III) Complexes as Activity-Based Probes for Bioimaging and Biosensing, Chem. – Asian J., 2022, 17, e202200840.


