Water soluble, cyclometalated Pt(II)–Ln(III) conjugates towards novel bimodal imaging agents

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Facile conjugation of a luminescent cyclometalated Pt(II) complex with a DO3A-derived Gd(III) moiety yields a hybrid species with visible luminescence and enhanced relaxivity.

A number of imaging techniques (MRI, CT, ultrasound, PET, SPECT, optical) are available at a biomedical level with various pros and cons for each regarding image resolution, depth of tissue penetration, acquisition time, and sensitivity. Therefore combining two or more imaging modalities into a single molecule agent can circumvent many limitations associated with a particular technique, whilst simplifying aspects of the agent administration and biodistribution characteristics (pharmacodynamics). It is very well known that trivalent lanthanide ions (Ln(III)) offer remarkable opportunities in the design of biological imaging agents. With dual applications in optical (luminescence) and magnetic resonance imaging (MRI), Ln(III) complexes have been extensively studied and a range of ligand systems investigated. Therefore Ln(III) moieties are particularly useful as a component of a bi- or multimodal single molecule imaging agent. In this context, systems based on Gd(III) have attracted most attention, with covalent coupling of organic fluorophores (MR/optical), labelling with 18F (MR/PET) and 99mTc chelation (MR/SPECT) providing prospective agents.

We are particularly interested in the use of phosphorescent metal-based luminophores as components of dual MR/optical imaging agents. Such species offer distinct photophysical advantages (tunable emission wavelengths, large Stokes' shifts, long emission lifetimes) over fluorescent variants and can be very effective in cellular imaging studies using confocal fluorescence microscopy. Cyclometalated Pt(II) complexes can possess many of these beneficial luminescent properties and have been successfully applied to biological imaging. In tandem with such physical attributes, interest in the use of Pt(II) complexes with therapeutic benefits also continues. Indeed one of the challenges of probing the (non)-specific biological action of Pt(II) therapeutics often lies in the difficulty of directly imaging the biological action. In this context, Pt(II) complexes can be tagged with fluorophores, such as anthraquinone, providing a means for identifying the intracellular fate of such compounds and understanding the role of targeting vectors. Recent studies into Pt(II) complexes of 2-phenylpyridine (ppy), have also explored binding with amyloid β peptide. The influences of the Pt complexes on protein aggregation, via the inhibition of Cu and Zn peptide complex formation, have been suggested as an approach to the study of Alzheimer’s disease.

In this work, we describe the development of water soluble Pt(II)–Ln(III) hybrid systems wherein the cyclometalated Pt(II) moiety is luminescent. Previous work has described the synthesis of mixed Pt(II)/Ln(III) species and their photophysical properties, where the Pt(II) chelate acts as an antenna for sensitised Ln(III) emission within donor (Pt)-acceptor (Ln) assemblies. However, such systems are often not water soluble or water stable, and thus, to the best of our knowledge, there are no reports on the water relaxometric properties of Pt(II)–Gd(III) heterometallic species. A number of other water stable d–f hybrids employing Ru(II), Re(III), and Os(III) as MLCT-based sensitisers have also been reported, with Re(III)/Ru(II)–Gd(III) hybrids demonstrating the physical properties for potential in dual MR/optical imaging. The aim of this work was to synthesise a water-soluble Pt(II)–Ln(III) complex with favourable luminescence and relaxivity properties. Two new ligands were required for the assembly of the Pt(II)–Ln(III) targets. Firstly, for the macrocyclic complex (Ln–py), a route was utilised to give the ethylamine amide derivative (P1, ESI†), which subsequently reacted with 4-pyridinecarboxaldehyde using a reductive amination procedure (P2, ESI†);
deprotection of the tert-butyl esters with trifluoroacetic acid and complexation with either Gd(OTf)₃ or Yb(OTf)₃ gave the monometallic LnIII complexes, Ln-py (Scheme 1) possessing a pendant pyridine donor. Formation of the lanthanide complexes was confirmed by HRMS with additional ¹H NMR data for Yb-py (ESI† Fig. S1) showing the pronounced chemical shifts of the azamacrocyle and arm protons between +135 and −80 ppm. For the hydrophilic PtII component, two 2-phenylquinoline derivatives incorporating a PEG-like functionality were synthesised, yielding both amide (pq-1, ESI†) and ester linked (pq-2, ESI†) derivatives from either 2-(2-aminoethoxy)ethanol or 2-(2-methoxyethoxy)ethanol, respectively.

The attempted synthesis of the PtII complexes was undertaken via the splitting of dimeric [(i)Ptμ-Cl]₂Pt(i)] with DMSO to form the monometallic species [PtCl[pq]](DMSO)]. In our hands, the use of the hydroxyl terminated PEG derivative (pq-1) was not amenable to this synthetic pathway, giving a poor yield of the desired PtII complex. However, the methoxy analogue [PtCl[pq-2](DMSO)] proved more amenable to this synthetic pathway, yielding both amide (pq-1, ESI†) and ester linked (pq-2, ESI†) derivatives from either 2-(2-aminoethoxy)ethanol or 2-(2-methoxyethoxy)ethanol, respectively.

The X-ray crystal structure (Fig. 1) of [PtCl[pq-2](DMSO)] was also obtained (crystal structure data and refinement parameters are contained in the ESI†) and revealed the anticipated coordination environment for PtII, with typical Pt-L bond lengths and angles for the coordinated atoms (ESI† Table S1) and a distortion of ca. 7° in the phenylquinoline unit. The packing structure also revealed a head-to-toe arrangement, with some π-stacking between the phenylquinoline moieties; there are no intermolecular Pt-Pt interactions.

Finally, the two complexes Ln-py and [PtCl[pq-2](DMSO)] were dissolved in a minimum volume of acetone and reacted at 40 °C for 48 h. The resultant Pt-Ln dimeric complexes were obtained as extremely hygroscopic orange powders and the formation confirmed using HRMS (ESI† Fig. S2), which revealed the distinct and appropriate isotopic distribution corresponding to the loss of the chloride ligand to give the cationic dimer as [M−Cl]⁺.

The electronic properties of [PtCl[pq-2](DMSO)] show that the complex absorbs in the UV region, with ¹π-π* transitions associated with the phenylquinoline unit, and in the visible region with λmax at 424 nm, which probably corresponds to a ¹MLCT type transition. Supporting theoretical (TD-DFT) calculations on the model complexes [PtCl(epqc)](DMSO)] (where epqc = 4-ethyl-2-phenylquinoline carboxylate) and [PtCl(epqc)[py)] (where py = pyridine) show that the majority of the electron density in the HOMO lies across both the phenyl moiety of the cyclo-metalated ligand and the 5d-orbitals of the platinum. The orbital representations of the calculated lowest energy HOMO-LUMO transitions are shown in the ESI† (Fig. S3). The percentage contribution to the energy levels for the PtII 5d-orbitals (ESI† Table S2) were calculated from the theoretical data showing that for both model complexes the HOMO comprises ca. 25% 5d-orbital character and is consistent, therefore, with a MLCT contribution.

The UV-vis spectra (Fig. 2) of the Pt-Ln hybrids are closely comparable to [PtCl[pq-2](DMSO)], with the visible MLCT absorption characteristics retained for both Pt-Gd and Pt-Yb. The luminescence properties of [PtCl[pq-2](DMSO)] revealed a broad, featureless emission band at 625 nm (λex = 425 nm) with a corresponding lifetime of 116 ns, which are attributed to an excited state of triplet character which is likely to encompass a strong ³MLCT component. The properties of the corresponding
Table 1. UV-vis and luminescence properties of the complexes

| Compound        | λ_{abs} | λ_{em}/nm | t/NS  |
|-----------------|---------|-----------|------|
| [PdCl2(pq-2)](DMSO) | 291, 362, 424 | 625 | 116* |
| Pt–Gd         | 285, 364, 426 | 617 | 59*  |
| Pt–Gd         | 284, 364, 427 | 619 | 99**  |
| Pt–Gd         | 284, 364, 427 | 619 | 99**  |
| Pt–Yb         | 284, 364, 427 | 619 | 99**  |
| Pt–Yb         | 284, 364, 427 | 619 | 99**  |

* CHCl3 solution. † H2O solution. ‡ λ_{em} = 425 nm. □ Yb-based emission.

The Pt–Gd adduct was obtained in aqueous solution: the UV-vis spectrum shares all of the same features as [PdCl2(pq-2)](DMSO)] with 1MLCT absorption retention ca. 425 nm and visible luminescence observed at 617 nm (Table 1).

In comparison, the steady state luminescence spectrum (λ_{em} = 425 nm) of Pt–Yb displayed dual emission comprised of 1MLCT character at ca. 620 nm and YbIII-centred emission (inset, Fig. 2) in the near-IR region around 980 nm (corresponding to 2F_5/2 → 2F_7/2). Since absorption at 425 nm is dominated by the PtII chromophore this observation suggests that the YbIII emission must be sensitised and therefore confirms the formation of the heterometallic dimer. For completeness, lifetime measurements on the donor component of the two Pt–Ln complexes were recorded in water to estimate the through-space Pt → Yb energy transfer rate, k_{ET} (using k_{ET} = (τ_q)^{-1} – (τ_{ref})^{-1} where τ_q is the 3MLCT lifetime in the presence of YbIII and τref is the lifetime in the presence of GdIII). The value for k_{ET} was calculated to be ca. 3 x 10^6 s^{-1}, which is similar to the rate of 2 x 10^6 s^{-1} for a previously reported PtII–YbIII complex.19

The lifetimes of the YbIII emission were also obtained in H2O (0.90 µs) and D2O (5.46 µs) to give an approximate inner sphere hydration (q = k_{H2O} / k_{D2O} = 0.1)20 of 0.8, which is consistent with an octadentate YbIII complex, implying that the amide carbonyl participates in the coordination sphere of YbIII, as observed in related lanthanide complexes of mono-amide DO3A derivatives.21

The relaxivity properties of the GdIII-containing species were determined using field-cycling relaxometry. 1H NMRD plots (ESI† Fig. S4) were obtained for Gd-py and Pt–Gd (at field strengths between 1 x 10^{-2} – 30 MHz) to give bulk relaxivities, r1, in water per mM of complex per second. The relaxivity of Gd-py was obtained as 3.8 mM^{-1} s^{-1} (30 MHz, 37 °C, pH 6.5), which is in accordance with the literature values for [Gd(DOTA)(H2O)]2−, where r1 was recorded as 3.8 mM^{-1} s^{-1} (20 MHz, 37 °C) and consistent with a q = 1 species.22 Interestingly, the r1 value for Pt–Gd was 7.1 mM^{-1} s^{-1} (30 MHz, 37 °C, pH 6.7), which shows that, upon formation of the dimetallic species, r1 increases by 86%. This is most likely due to the increase in the molecular weight of the dimer resulting in a longer rotational correlation time, τ_{R}. The plots also demonstrate the temperature dependence of r1 with relaxivities at 25 °C approximately 10–25% higher than at 37 °C (1 x 10^{-2} to 30 MHz).

Preliminary optical imaging studies were attempted with Pt–Gd using both MCF7 cells and Schizosaccharomyces pombe (fission yeast). Confocal fluorescence microscopy revealed that MCF7 cells poorly took up the complex with little evidence for internalisation. However, with S. pombe the images (ESI† Fig. S5 and S6) clearly showed characteristic red emission from the cells, particularly those that were dividing, consistent with the PtII-based chromophore, showing that these species are compatible with confocal fluorescence microscopy.

Our studies have shown how a luminescent, amphiphilic cyclometalated PtII chelate can be coupled with a macrocyclic hydrophilic Ln(III) species to give a water soluble dimetallic construct. Such an approach has allowed the study of heterometallic Pt–Yb and Pt–Gd species: sensitised YbIII emission of the former corroborates the integrity of the dimer, while the latter reveals a bimetallic complex with visible luminescence properties and enhanced water relaxivity. Preliminary studies reveal that these complexes can be applied to the confocal fluorescence microscopy of cells, but that a careful balance of charge and amphiphilicity, and an understanding of cytotoxicity, will be required for future studies with different cell lines.

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