This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal’s standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.

www.rsc.org/dalton
Luminescent europium(III)-platinum(II) heterometallic complex as theranostic agent: a proof-of-concept study†

Anirban Chandra,a Khushbu Singh,a, b Swati Singh,b Sri Sivakumarb and Ashis K. Patra*a

A luminescent heterometallic multifunctional theranostic Eu-Pt2 complex, [[cis-PtCl2(DMSO)]2Eu(L)(H2O)] has been synthesized, possessing two therapeutic Pt-centers as covalent DNA binders and one emissive Eu3+ center which is sensitized by platinum-based metal-to-ligand charge-transfer excited states.

Multifunctional cancer theranostic agents having multiple therapeutic and diagnostic centers in a single platform gains popularity in recent years.1-3 Recently, there are few reports on heterometallic hairpin-shaped lanthanide-Pt2 complexes for DNA recognition and magnetic resonance imaging (MRI) based theranostic agents or selectively delivering gadolinium to tumor cell nuclei.4 In this regard, theranostic Gd3+-based platinum complexes reported5,6, 7, 8, 9, 10, 11 have been synthesized, possessing two available DNA-cross linking sites. In addition, the clinical use of platinum drugs is severely affected by drug resistance mediated by inadequate levels of platinum reaching to critical target DNA.5 Notwithstanding with this progress, it is highly desirable to increase the DNA binding sites that significantly enhances platinum content at target site along with tagging of bright luminescent center as diagnostic probe. To this end, we report the design of multimodal targeted theranostic Eu-Pt2 conjugate possessing four DNA binding sites which can effectively target nuclear DNA along with highly luminescent Eu3+ center to enable interference-free live-tracking of the drug through fluorescence microscopy. Luminescent lanthanide complexes were widely exploited in various bioassays since they offer unique photophysical properties like narrow emission bands, large Stokes’ shift and long-lived excited state lifetimes.6, 7 Since direct excitation of the Ln3+ f-f transition is very inefficient, chemists have designed a variety of chelating agents conjugated to a sensitizing organic chromophore called antenna which can transfer its excited state energy efficiently to the emissive Ln3+ ion leading to bright luminescence compared to the direct excitation of Ln3+ ions.8, 9, 10, 11 Our approach exploit the advantages of transition metal complexes as they exhibit many desirable properties as sensitizer than organic chromophores such as tunable absorption bands, long-lived excited states to maximize the ET to Ln3+ ions.

Herein, we demonstrate a multifunctional Eu-Pt2 complex, [[cis-PtCl2(DMSO)]2Eu(L)(H2O)] (1) (Scheme 1) which has cytotoxic cis-[PtCl2(DMSO)] moieties enabling the DNA binding whereas, EuL unit acts as luminescent reporter. Thus, a combination of a luminescent imaging probe and a conjugated therapeutic agent in a single hybrid 5d-4f complex can provide real-time feedback on drug delivery, distribution and target site localization in a non-invasive manner through fluorescence microscopy. Other key design feature of this complex is having...
four potential DNA cross-linking sites through labile Pt-Cl bonds, thus higher level of activated platinum reaching DNA, a possible way to lower the drug resistance. Eu-Pt₂ complex 1 was prepared in a sequential manner (Scheme S1, ESI† starting with a multidentate DTPA-bisamide ligand, H₂L = N,N'-Bis[3-amidoquinolyl]diethylenetriamine-N,N’,N’’-triacetic acid derived from acylation of 3-aminoquinoline by DTPA-bis(anhydride). [Eu(L)₂(H₂O)] was synthesized by reacting 1:1 molar ratio of deprotonated ligand and Eu(NO₃)₃·6H₂O in water. The [[cis-PtCl₂(DMSO)]₂Eu(L)₂(H₂O)] (1) was isolated on reaction of EuL with freshly prepared cis-[Pt(DMSO)Cl₂] in 1:2 molar ratio. Detailed ESI-MS studies of Eu-Pt₂ complex reveal m/z 714.52 corresponding to (M-2Cl)⁺ with matching isotopic distribution profile unequivocally allows attribution of Eu-Pt₂ formulation along with other physicochemical data (Fig. S1, ESI†). We observed dissociable chloride ligands in aqueous medium. The hairpin-shaped complex with DNA. The hairpin-shaped complex reveals spectrophotometric titration of [Eu(L)(H₂L)]⁰ with [EuL] in presence of DNA shows significant enhancement of Eu-base d fluorescence (Kₚₒₒᵖ = 4.9 x 10⁶ M⁻¹), circular dichroism (CD) and isothermal titration calorimetry (ITC) (Fig. S6-S8, ESI†). ITC titration plot.

The UV-vis spectra of 1 exhibits high energy band at 273 nm are due to ligand centered π→π* transitions and a broad band ranging from 332-350 nm corresponding to π→π* MLCT transitions of quinoline bound Pt⁺⁺ moiety (Fig. 1a). Here we have judiciously utilized this πMLCT excited state as means to populate ⁵D₀ emissive states of europium through efficient energy transfer. The ⁵MLCT→¹f energy transfer through such photosensitization is shown in few Eu-Pt complexes. Addition of cis-[Pt(DMSO)Cl₂] to EuL resulted in appearance of new band at 350 nm with isobestic point at ~270 nm indicative of formation of Eu-Pt₂ complex 1 during titration (Fig. S2 in ESI†). Upon excitation at MLCT band, the Eu-Pt₂ complex under time-gated mode displayed narrow emission bands spanning from 575-700 nm characteristic of the ⁵D₀ → ⁷Fₖ (J = 0-4) f-f transitions of Eu³⁺ (Fig. 1b). The luminescence spectra demonstrates efficient photosensitized energy transfer from MLCT excited state of quinoline bound Pt⁺⁺ moiety to EuL with a overall quantum yield (q) of 0.04. Spectrophotometric titration of cis-[Pt(DMSO)Cl₂] with [Eu(L)₂(H₂O)] at λₐₑₓ = 330 nm showed the formation of [[cis-PtCl₂(DMSO)]₂Eu(L)₂(H₂O)] (1) with gradual increase in europium centered emission until it reached a plateau at Eu/Pt = 0.5 (Fig. 1c). The decay rate of emissive ⁵D₀ excited state was measured at 616 nm results in monoeponential decay curve with a lifetime (τₑₓᵣ) of 0.65±10% ms in aqueous buffer medium, indicating the presence of single chemical environment. The enhancement of τₑₓᵣ in presence of DNA (τₑₓᵣ = 0.89 ±10% ms) indicate minimization of nonradiative relaxation pathways in DNA bound form for Eu-Pt₂ complex (Fig. 1d).

Since DNA is the most important target for therapeutic platinum drugs, we attempted detailed binding studies of 1 with DNA. The hairpin-shaped complex 1 gets activated by aquation through substitution of chlorides by water generating potent cation, [Eu(L)₂(H₂O)][cis-Pt(OH)₂⁴⁺(DMSO)] (n = 1-4), which can readily cross-link with nucleobases of ds-DNA (Scheme S2, ESI†). The binding interaction of Eu-Pt₂ complex with calf-thymus DNA (CT-DNA) were studied using UV-vis titration, competitive displacement of ethidium bromide by suggests a biphasic sequential binding interaction of Eu-Pt₂ with CT-DNA with an initial favorable exothermic binding event (K₁ =1.7x10⁷ M⁻¹, ΔH₁ = -26.0 kcal mol⁻¹), followed by a second exothermic event (K₂ = 9.1x10⁶ M⁻¹, ΔH₂ = -77.6 kcal mol⁻¹) presumably due to sequential sequential covalent cross-link formation with base pairs of duplex-DNA (Fig. 2a). The intrinsic binding constant (Kₙ=1.5±0.3) x 10⁵ M⁻¹ along with a hypochromism and bathochromic shift of the electronic spectral bands suggests favorable binding interaction of the complex 1 with DNA due to EuPt₂-DNA adduct formation, strong electrostatic interaction with activated (Eu-Pt²⁺)⁰⁰⁰ and favorable stacking interactions of the two planar –Pt(L) chromophores with planar base pairs of ds-DNA as observed in similar hairpin shaped Eu-Pt₂ complexes. Formation of such bis(bifunctional) platinum-DNA cross-links should induce major unpairable structural distortion of DNA double-helix. The significant decrease in ellipticity in CD spectra of CT-DNA in the presence of 1 (Fig. 2b) also suggests unwinding of DNA helix and major structural deformation of DNA induced by Pt-DNA cross-links. This structural distortion of DNA could be beyond cellular DNA repair machinery and thereby inhibit transcription and replication, triggering cell-death pathways. The decrease in DNA migration rate in presence of Eu-Pt₂ compared to EuL in gel electrophoretic mobility assay using SC pUC19 DNA also suggest unwinding of supercoiled DNA helix by 1 (Fig. 2c). We have observed significant enhancement of Eu-based luminescence intensity originating from ⁵D₀→⁷Fₖ transitions of Eu³⁺ upon addition of DNA due to efficient energy transfer to Eu³⁺ and enhanced excited state lifetime in a hydrophobic environment created due to binding of Eu-Pt₂ complex with
DNA (Fig. 2d). Serum albumin proteins constitutes a major component in blood plasma proteins and plays important roles in drug transport and metabolism. The interaction of the Eu-Pt<sub>2</sub> complex with bovine serum albumin (BSA) studied from tryptophan emission quenching experiment showing high binding propensity ($K_{BSA} = 1.50 \pm 0.03 \times 10^5 \text{M}^{-1}$) desirable for efficient transport to the pathological site (Fig. S9, ESI†).

To test our original ‘theranostic’ design, we performed in vitro cytotoxicity assay by MTT on a human cervical carcinoma HeLa and lung carcinoma H460 cell lines. The IC<sub>50</sub> values for Eu-Pt<sub>2</sub> complex 1 are 51.0±1.05 µM in HeLa cells and 30.0±1.27 µM in H460 cells (Fig. 2d). It exerts anticancer activity via extensive DNA-adduct formation through conjugated cis-[PtCl<sub>2</sub>(DMSO)] moieties in a similar mechanism to cisplatin.

The cellular internalization of the Eu-Pt<sub>2</sub> complex was investigated to probe diagnostic aspect of [Eu(L)(H<sub>2</sub>O)] utilizing long luminescence lifetime and intrinsic luminescence from Eu<sup>3+</sup> by confocal fluorescence microscopy (panel A, Fig. 3). Theranostic Eu-Pt<sub>2</sub> complex showed significant cellular uptake within 4 h of incubation with HeLa cells. Staining with nuclear staining dye Hoechst 33258 demonstrate both nucleus and cytosolic distribution of the complex (panel C, Fig. 3). The red spots observed in some nuclei originate from luminescence from the Eu<sup>3+</sup> reporter tag in Eu-Pt<sub>2</sub> theranostic conjugate (Fig. S11, ESI†).

In conclusion, we have developed a luminescent multimodal heterometallic Eu-Pt<sub>2</sub> theranostic system using sensitization and energy transfer from a conjugated Pt<sup>2+</sup> based chromophore to Eu<sup>3+</sup>. The complex shows strong binding propensity to DNA via formation of Pt-DNA cross-links through four potential DNA binding sites. The complex exhibit cytotoxicity through DNA damage and nuclear localization observed due to Eu-based f-f transition by fluorescence imaging. Thus, such systems will have great potential towards designing theranostic agents and delivery vehicles for cancer chemotherapy. Further studies are on towards designing potent lanthanide based theranostic agents as efficient drug delivery platform and understanding mechanism of their action.

A.K.P. acknowledges SERB, Govt. of India and IIT Kanpur for funding. A.C. and K.S. acknowledge UGC and CSIR their research fellowships.

**Notes and references**


Synopsis: In this communication, we present a luminescent heterometallic multifunctional theranostic Eu-Pt\textsubscript{2} complex, \([\text{cis-PtCl}_2(\text{DMSO})_2\text{Eu(L(H}_2\text{O)})]_2\), possessing two cytotoxic Pt-centers having four DNA-binding sites showing intracellular Eu-based red luminescence sensitized by platinum based MLCT excited states.