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Luminescent europium(III)-platinum(II) heterometallic complex as theranostic agent: a proof-of-concept study†

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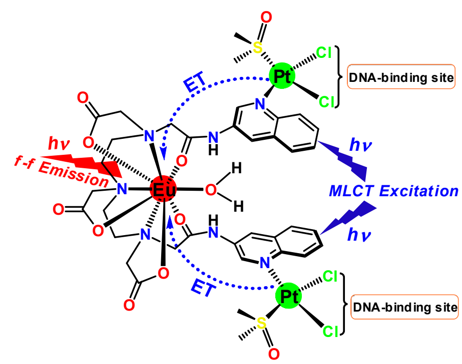
A luminescent heterometallic multifunctional theranostic Eu-Pt_2 complex, [{*cis*-$PtCl_2$(DMSO)}$_2$Eu(L)(H_2O)] has been synthesized, possessing two therapeutic Pt-centers as covalent DNA binders and one emissive Eu^{3+}-center which is sensitized by platinum-based metal-to-ligand charge-transfer excited 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-Pt_2 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 Gd^{3+}-based platinum complexes reported[4c] in which Gd^{3+} and conjugated *cis*-[Pt(NH_3)$_2$Cl]$^+$-moieties act as MRI contrast and therapeutic centers, respectively. These complexes showed therapeutic efficiency along with improved MR imaging capability. However, their DNA-binding ability is limited because of only 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-Pt_2 conjugate possessing four DNA binding sites which can effectively target nuclear DNA along with highly luminescent Eu^{3+} 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



Scheme 1 Molecular structure of Eu-Pt_2 complex **1** showing Pt→ Eu energy transfer pathway upon MLCT photo-excitation leading to *f-f* emission from Eu^{3+} and dissociable Pt-Cl bonds as DNA-cross linking sites.

unique photophysical properties like narrow emission bands, large Stokes' shift and long-lived excited state lifetimes.[6,7] Since direct excitation of the Ln^{3+} *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 Ln^{3+} ion leading to bright luminescence compared to the direct excitation of Ln^{3+} ions.[8,9] 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 Ln^{3+}, excellent photochemical stability and kinetic inertness.[10,11]

Herein, we demonstrate a multifunctional Eu-Pt_2 complex, [{*cis*-$PtCl_2$(DMSO)}$_2$Eu(L)(H_2O)] (**1**) (Scheme 1) which has cytotoxic *cis*-[$PtCl_2$(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 5*d*-4*f* 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

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†Electronic Supplementary Information (ESI) available: Experimental details; synthesis and characterization of compounds; ESI-MS analysis; DNA and BSA binding experiments; cytotoxicity and cellular internalization studies. See DOI: 10.1039/c000000x
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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-Pt_2 complex **1** was prepared in a sequential manner (Scheme S1, ESI†) starting with a multidentate DTPA-bisamide ligand, H_3L = *N,N''*-Bis(3-amidoquinolyl)diethylenetriamine-*N,N',N''*-triacetic acid derived from acylation of 3-aminoquinoline by DTPA-bis(anhydride). [Eu(L)(H_2O)] was synthesized by reacting 1:1 molar ratio of deprotonated ligand and $Eu(NO_3)_3 \cdot 6H_2O$ in water. The [{*cis*-$PtCl_2$(DMSO)}$_2$Eu(L)(H_2O)] (**1**) was isolated on reaction of EuL with freshly prepared *cis*-[Pt$(DMSO)_2Cl_2$] in 1:2 molar ratio. Detailed ESI-MS studies of Eu-Pt_2 complex reveal *m/z* 714.52 corresponding to $\{M-2Cl\}^{2+}$ with matching isotopic distribution profile unequivocally allows attribution of Eu-Pt_2 formulation alongwith other physicochemical data (Fig. S1, ESI†). We observed dissociable chloride ligands in aqueous solution which is crucial towards forming cross-link with base pairs in nuclear DNA.

The UV-vis spectra of **1** exhibits high energy band at 273 nm are due to ligand centered $\pi \rightarrow \pi^*$ transitions and a broad band ranging from 332-350 nm corresponding to $\pi \rightarrow \pi^*$ MLCT transitions of quinoline bound Pt^{2+} moiety (Fig. 1a). Here we have judiciously utilized this ^{3}MLCT excited state as means to populate $^5D_0$ emissive states of europium through efficient energy transfer. The ^{3}MLCT$\rightarrow f$ energy transfer through such photosensitization is shown in few Eu-Pt complexes.[12] Addition of *cis*-[Pt$(DMSO)_2Cl_2$] to EuL resulted in appearance of new band at 350 nm with isobestic point at ~270 nm indicative of formation of Eu-Pt_2 complex **1** during titration (Fig. S2 in ESI†). Upon excitation at MLCT band, the Eu-Pt_2 complex under time-gated mode displayed narrow emission bands spanning from 575-700 nm characteristic of the $^5D_0 \rightarrow {}^7F_J$ (J = 0-4) *f-f* transitions of Eu^{3+} (Fig. 1b). The luminescence spectra demonstrates efficient photosensitized energy transfer from MLCT excited state of quinoline bound Pt^{2+} moiety to emissive $^5D_0$ excited state of Eu^{3+} with a overall quantum yield ($\phi_{overall}$) of 0.04. Spectrophotometric titration of *cis*-[Pt$(DMSO)_2Cl_2$] with [Eu(L)(H_2O)] at λ_{ex} = 330 nm showed the formation of [{*cis*-$PtCl_2$(DMSO)}$_2$Eu(L)(H_2O)] (**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 $^5D_0$ excited state was measured at 616 nm results in monoexponential decay curve with a lifetime (τ_{obs}) of 0.65(±10%) ms in aqueous buffer medium, indicating the presence of single chemical environment. The enhancement of τ_{obs} in presence of DNA (τ_{obs} = 0.89 (±10%) ms) indicate minimization of nonradiative relaxation pathways in DNA bound form for Eu-Pt_2 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)(H_2O){*cis*-Pt$(OH_2)_{n/2}$(DMSO)}$_2$]$^{n+}$ (n = 1-4), which can readily cross-link with nucleobases of ds-DNA (Scheme S2, ESI†). The binding interaction of Eu-Pt_2 complex with calf-thymus DNA (CT-DNA) were studied using UV-vis titration, competitive displacement of ethidium bromide by fluorescence (K_{app} = 4.9 x 10^5 M^{-1}) , circular dichroism (CD) and isothermal titration calorimetry (ITC) (Fig. S6-S8, ESI†). ITC titration plot

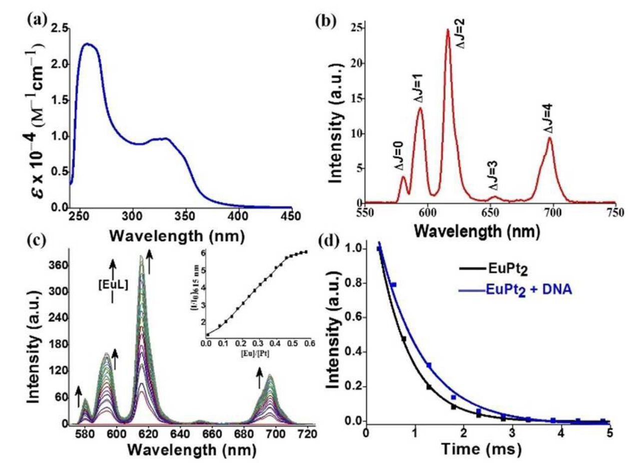

Fig. 1 (a) UV-vis and (b) emission spectrum (λ_{ex} = 330 nm) of Eu-Pt_2 complex **1** in Tris-HCl buffer. (c) Evolution of emission spectra with increasing [EuL] to a solution of [$PtCl_2(DMSO)_2$] in DMF (λ_{ex} = 330 nm), Inset: the plot of relative emission intensity at 615 nm *vs.* [Eu]/[Pt] ratio. (d) Luminescence decay profile from $^5D_0$ state of Eu^{3+} in complex **1** at λ_{em} = 616 nm (λ_{ex} = 330 nm) (black) and in presence of CT-DNA (blue) in Tris-HCl buffer (pH=7.2). [**1**] = 90 μM, [DNA] = 450 μM, delay time and gate time = 0.1 ms. The solid lines are the best fits considering single-exponential behaviour of the decay.

suggests a biphasic sequential binding interaction[13] of Eu-Pt_2 with CT-DNA with an initial favorable exothermic binding event (K_1 =1.7x10^5 M^{-1}, ΔH_1 = -26.0 kcalmol^{-1}), followed by a second exothermic event (K_2 = 9.1x10^5 M^{-1}, ΔH_2 = -77.6 kcalmol^{-1}) presumably due to successive sequential covalent cross-link formation with base pairs of duplex-DNA (Fig. 2a). The intrinsic binding constant (K_b=1.5(±0.3) x 10^5 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 EuPt$_2$-DNA adduct formation, strong electrostatic interaction with activated $\{Eu-Pt_2\}^{n+}$ 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-Pt_2 complexes.[4a,4b] Formation of such bis(bifunctional) platinum-DNA cross-links should induce major unrepairable 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.[14] This structural distortion of DNA could be beyond cellular DNA repair machinery and thereby inhibit transcription and replication, triggering cell-death pathways.[5] The decrease in DNA migration rate in presence of Eu-Pt_2 compared to EuL in gel electrophoretic mobility assay using SC pUC19 DNA also suggest unwinding of supercoiled DNA helix by **1** (Fig. 2 c).[15] We have observed significant enhancement of Eu-based luminescence intensity originating from $^5D_0 \rightarrow {}^7F_J$ transitions of Eu^{3+} upon addition of DNA due to efficient energy transfer to Eu^{3+} and enhanced excited state lifetime in a hydrophobic environment created due to binding of Eu-Pt_2 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_2 complex with bovine serum albumin (BSA) studied from tryptophan emission quenching experiment showing high binding propensity (K_{BSA} = 1.50±0.03 x 10^5 M^{-1}) desirable for efficient transport to the pathological site (Fig. S9, ESI†).

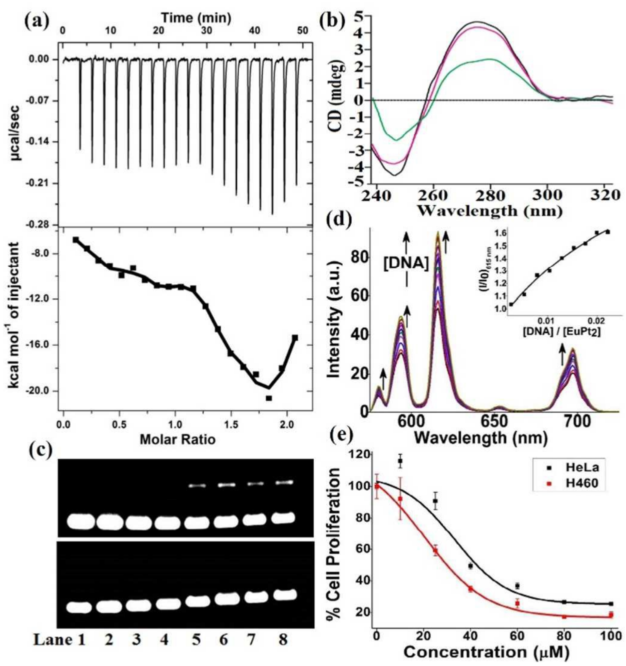

Fig. 2 (a) ITC plot profile for the interaction of Eu-Pt_2 (0.1 mM) with CT-DNA (0.01 mM) in 5 mM Tris-HCl/NaCl buffer (pH 8.5) at 30 ^{0}C. The top panel represents the raw data for 20 injections of 2 μL each. The bottom panel shows the amounts of heat evolved on interaction of the Eu-Pt_2 complex with CT-DNA against molar ratio of Eu-Pt_2 complex to CT-DNA. Data were fitted by sequential binding site model. (b) CD spectra of CT-DNA in the presence of EuL and Eu-Pt_2 in Tris-HCl buffer medium. (c) Agarose gel electrophoresis of SC pUC19 DNA after incubation with EuL and Eu-Pt_2 in 50 mM Tris-HCl buffer at 37 °C for 16 h. Lane 1: DNA control, lanes 2-8 (10, 15, 20, 40, 60, 80, 100 μM complexes respectively) (d) Luminescence spectra of Eu-Pt_2 (λ_{ex} = 330 nm) with addition of CT-DNA in 5 mM Tris-HCl buffer. Inset: the relative emission intensity (I/I_0) of **1** at 615 nm *vs.* [DNA]/[Eu-Pt_2] ratio. (e) Cell viability plots showing the cytotoxicity of Eu-Pt_2 complex with HeLa and H460 cells on 16 h incubation by MTT assay.

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_{50} values for Eu-Pt_2 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*-[$PtCl_2$(DMSO)] moieties in a similar mechanism to *cis*platin.

The cellular internalization of the Eu-Pt_2 complex was investigated to probe diagnostic aspect of [Eu(L)(H_2O)] utilizing long luminescence lifetime and intrinsic luminescence from Eu^{3+} by confocal fluorescence microscopy (panel A, Fig. 3). Theranostic Eu-Pt_2 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^{3+} reporter tag in Eu-Pt_2 theranostic conjugate (Fig. S11, ESI†).

In conclusion, we have developed a luminescent multimodal heterometallic Eu-Pt_2 theranostic system using sensitization and energy transfer from a conjugated Pt^{2+} based chromophore to Eu^{3+}. 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.

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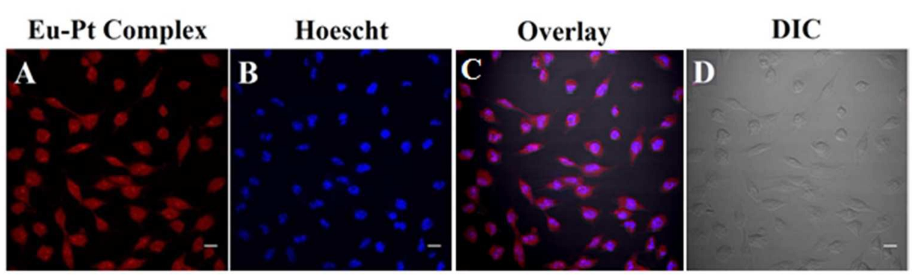

Fig. 3 Confocal laser scanning fluorescence microscopic images of the HeLa cells treated with Eu-Pt_2 complex (25 μM) on 4 h incubation and Hoechst 33258 dye (5 μg mL^{-1}): (A) cells incubated with the Eu-Pt_2 complex; (B) incubated with the Hoechst 33258 dye for staining nucleus showing blue emission; (C) merged images showing nuclear and cytosolic localization of the complex; (D) DIC images. Scale bar = 20 μm.

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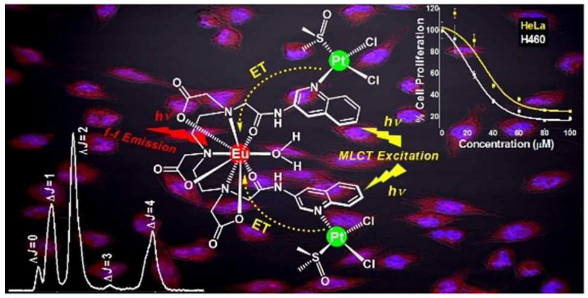
TOC Graphics



Synopsis: In this communication, we present a luminescent heterometallic multifunctional theranostic Eu-Pt_2 complex, [{*cis*-$PtCl_2$(DMSO)}$_2$Eu(L)(H_2O)], possessing two cytotoxic Pt-centers having four DNA-binding sites showing intracellular Eu-based red luminescence sensitized by platinum based MLCT excited states.