

Bimetallic Synergy in a Ru(II)–Eu(III) DO3A Complex: Optical Response, Protein-Enhanced Relaxivity and Antibacterial Activity

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Abstract.

A Ru(II)–Eu(III) heterobimetallic complex, (RuII(bpy)₂)(EuIII(DO3A-4ABA))(2Cl), was synthesized and examined for its optical, relaxometric and antibacterial characteristics. The DO3A-4ABA ligand showed an absorption maximum at 274 nm, whereas coordination with the Ru(II) and Eu(III) centres resulted in absorption bands at 278 and 477 nm. The latter band is consistent with a charge-transfer transition involving the Ru(II) centre and the coordinated π -acceptor ligand framework. Relaxation measurements in aqueous solution gave $r_{1\rho}$ and $r_{2\rho}$ values of 9.57 and 14.40 mM⁻¹ s⁻¹, respectively, giving an r_2/r_1 ratio of 1.64. The complex also showed a distinct variation in relaxation behaviour over the pH range of 2.5–11, indicating that protonation and exchange processes influence its interaction with water protons. A notable increase in relaxivity was observed in the presence of 4.5% human serum albumin, where r_1 and r_2 reached 24.33 and 35.12 mM⁻¹ s⁻¹, respectively. This enhancement is consistent with an increase in the effective rotational correlation time following association with the protein. Antibacterial experiments further showed inhibition zones of 13–18 mm against *Clostridium difficile*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The combined optical response, pH-dependent relaxation behaviour, protein-associated relaxivity enhancement and antibacterial activity make this complex a useful platform for further investigation in multimodal imaging and related biomedical applications.

Keywords: Ru(II)–Eu(III) heterobimetallic complex; DO3A-4ABA; Bipyridine; MRI relaxivity; HSA interaction; Antibacterial activity.

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1. INTRODUCTION

Magnetic resonance imaging (MRI) is an established non-invasive diagnostic modality that provides high-resolution anatomical information and excellent soft-tissue contrast without exposure to ionizing radiation. Despite these advantages, the intrinsic contrast between normal and pathological tissues may be insufficient for the reliable identification of certain lesions, creating a continuing need for efficient contrast-enhancing systems. MRI contrast agents principally enhance image contrast by modifying the longitudinal (T_1) and/or transverse (T_2) relaxation rates of surrounding water protons. Among paramagnetic metal ions, Gd(III) has been extensively investigated as a T_1 -weighted MRI contrast centre because of its favourable electronic configuration, high magnetic moment and ability to efficiently influence proton relaxation when incorporated into an appropriate ligand framework [1–3]. Relaxivity, expressed as the longitudinal (r_1) and transverse (r_2) relaxivity, represents one of the principal parameters used to assess the efficiency of a paramagnetic contrast agent. High r_1 values are

desirable for T_1 -weighted contrast enhancement and depend on efficient interaction between the paramagnetic centre and surrounding water protons. The Solomon–Bloembergen–Morgan framework provides a fundamental basis for understanding the relationship between proton relaxivity and molecular parameters such as the number of inner-sphere water molecules, metal–proton distance, water-exchange rate and rotational dynamics [1,4]. Consequently, rational control of ligand architecture and metal coordination is essential for developing efficient molecular MRI probes. Although Eu(III) is not a conventional substitute for Gd(III) in clinically established T_1 contrast agents, Eu(III) coordination chemistry has attracted interest in MRI-related applications, particularly through exchangeable coordinated water and chemical-exchange saturation transfer (CEST) mechanisms [5].

Optical imaging provides a complementary approach to MRI because of its high sensitivity and capability for cellular and subcellular visualization. Integration of optical and

magnetic-resonance responses within a common molecular framework can therefore provide complementary anatomical and molecular information and potentially improve the accuracy of biological imaging [6,7]. Eu(III), in particular, displays characteristic red emission arising from its f–f transitions and can be sensitized through suitable ligand-based antenna systems [8,9]. Transition-metal complexes, especially Ru(II) polypyridyl compounds, provide another attractive component for multifunctional molecular probes. The strong chelating properties of 2,2'-bipyridine (bpy) provide kinetically robust coordination environments around Ru(II), while the resulting complexes commonly exhibit metal-to-ligand charge-transfer (MLCT) transitions, tunable excited-state properties and relatively long-lived luminescence [10,11]. Modification of bipyridine ligands can further influence the charge, lipophilicity, cellular uptake and photophysical characteristics of Ru(II) complexes. These features have resulted in extensive investigation of Ru(II) polypyridyl compounds for biological imaging, sensing, phototherapy and medicinal applications [11,12]. The biomedical importance of Ru(II) complexes extends beyond imaging. Their ability to interact with DNA, proteins and cellular membranes, together with their photochemical and redox properties, has generated considerable interest in their anticancer and antimicrobial applications [12,13]. In particular, bipyridine-containing Ru(II) complexes have demonstrated both antineoplastic and antibacterial activities. Mazumder et al. reported Ru(II) complexes containing 2,2'-bipyridine that exhibited antitumour activity in an Ehrlich ascites carcinoma model together with antibacterial activity against Gram-positive and Gram-negative organisms [14]. More recent investigations have further demonstrated that structural modification of Ru(II) polypyridyl frameworks can influence cytotoxicity and antimicrobial activity.

Eu(III) also possesses biological relevance beyond its optical properties. A systematic evaluation of Eu-containing biomaterials has identified antibacterial and potential anti-tumour activities in several Eu-based systems, although these effects are strongly dependent on the chemical form, ligand environment, concentration and biological model [15–17]. Importantly, antibacterial activity has also been reported for luminescent Eu(III) complexes, demonstrating that optical functionality and biological activity can coexist within a single coordination platform [18]. The combination of bipyridine, Ru(II) and Eu(III) therefore provides a rational d–f heterometallic architecture in which different components can contribute complementary functions. The bipyridine ligand provides coordination stability and electronic conjugation;

Ru(II) contributes MLCT-based photophysical and potentially biological properties; and Eu(III) provides characteristic long-lived red luminescence and additional lanthanide-centred functionality [6,19,20]. However, the simultaneous optimization of luminescence, metal–ligand stability, water interaction, relaxometric behaviour and biological activity remain challenging. The present work focuses on the synthesis and characterization of a bipyridine–Ru(II)–Eu(III) DO3A complex as a potential multifunctional molecular imaging platform. Particular emphasis is placed on establishing its structural and spectroscopic characteristics and assessing its potential biomedical significance through consideration of its luminescence, relaxation behaviour and antibacterial and anticancer properties. Such an integrated approach may provide useful insight into the design of d–f heterometallic coordination compounds capable of combining diagnostic and biological functions within a single molecular framework.

2. Literature Review

The development of multifunctional metal complexes has increasingly focused on combining optical, magnetic and biological properties within a single molecular architecture. Caravan et al. [1] established that the relaxivity of lanthanide-based MRI agents depends strongly on hydration, water-exchange kinetics and molecular rotational dynamics, providing the fundamental framework for rational contrast-agent design. Siriwardena-Mahanama and Allen further emphasized that optimization of water-exchange rates is critical for improving lanthanide-based proton relaxivity [4]. Caravan et al. [5] subsequently reviewed the relationship between water exchange and MRI performance and highlighted the relevance of lanthanide coordination environments in controlling relaxation behaviour. These principles are important when evaluating heterometallic systems in which ligand coordination must simultaneously accommodate photophysical and magnetic requirements. Pushparaj & Alexander [6] reported polymetallic lanthanide systems designed for combined MRI and optical imaging and demonstrated that ligand architecture, hydration, molecular size and rotational dynamics are decisive factors in determining the performance of bimodal probes. Related work on DTPA-bisamide lanthanide complexes demonstrated that modification of the ligand framework can produce complexes with both appreciable relaxivity and characteristic Eu(III)/Tb(III) luminescence [19]. Dehaen et al. [21] reported Ru–lanthanide architectures in which transition-metal-centred optical properties could be combined with lanthanide-based magnetic functionality. These studies provide an important conceptual foundation for the design of d–f heterometallic imaging

systems. Ru(II) polypyridyl chemistry is particularly attractive because 2,2'-bipyridine and related diimine ligands generate stable coordination frameworks with tunable MLCT excited states. Shum et al. [11] reviewed the extensive biological applications of luminescent Ru(II) polypyridine complexes and emphasized their potential for cellular imaging, sensing and therapeutic applications [12,13]. Pushparaj, et al. [14] reported mononuclear Ru(II) complexes containing bipyridine that exhibited antitumour activity against Ehrlich ascites carcinoma and mild-to-moderate antibacterial activity, establishing an early connection between bipyridine–Ru coordination chemistry and dual biological activity. Levina, et al. [21] identified ruthenium complexes among metal-containing scaffolds with potential for antibiotic development and highlighted the ability of metal complexes to generate biological mechanisms distinct from conventional organic antibiotics. Eu-containing systems have also demonstrated antibacterial activity. A systematic review by Zhang et al. [15] and Marydasan et al. [19] demonstrated that Eu(III) complexes possessing strong luminescence could exhibit antibacterial activity against clinically relevant bacterial strains, supporting the possibility of combining optical functionality with antimicrobial behaviour. Ru(II) polypyridyl compounds have been investigated as anticancer agents through mechanisms involving DNA interaction, protein binding, membrane perturbation and reactive oxygen species generation [12,13]. Eu-containing systems have likewise been explored for potential antitumour activity, including interactions with nucleic acids and biological membranes [16,17]. These observations suggest that appropriate ligand design may allow the photophysical and biological properties of Ru(II) and Eu(III) to be integrated within a common molecular framework. The literature demonstrates that bipyridine–Ru(II) chemistry provides a versatile platform for optical imaging and biological applications, while Eu(III) offers distinctive luminescence and additional biomedical functionality. The combination of these components therefore represents an attractive strategy for constructing multifunctional *d-f* coordination systems. Nevertheless, systematic studies simultaneously correlating their structural features, luminescence, relaxometric behaviour, antibacterial activity and anticancer performance remain comparatively limited. The present bipyridine–Ru(II)–Eu(III) system is consequently of interest as a candidate multifunctional molecular platform for investigating the relationship between coordination structure, optical response, magnetic-relaxation behaviour and biological activity.

3. Experimental Section

3.1 Chemicals and Solvents

p-Amino benzoic acid, 2,2'-Bipyridine, 1,4,7,10-Tetraazacyclododecane-1,4,7-Triacetic acid (DO3A), 2-chloroacetyl chloride, Europium chloride, Ruthenium chloride, Lithium chloride, Human Serum Albumin (HSA), celite, Deuterium oxide (99.9 atom % pure), dmsO-d₆ (99.99 atom % pure), CDCl₃ (100 atom % D), Dowex 50WX8 ion exchange resin, Dowex 1X8-200 ion exchange resin, Amberlite IR-120 (H⁺, acidic), and molecular sieves (4A) were purchased from Sigma Aldrich. Sodium carbonate, acetonitrile (AR), Ethanol (AR), N,N-Dimethyl Formamide (DMF) (AR), Xylenol orange indicator, and triple distilled water was purchased from Merk, India and used as such.

3.2. Methods

3.2.1. Physical Measurements.

CHNS/O microanalyses were carried out using a Perkin-Elmer 2400 Series II CHNS/O Elemental Analyzer interfaced with a Perkin-Elmer AD 8 Autobalance. Argon (99.99%) was used as the carrier gas. FT-IR spectra were recorded on a Perkin-Elmer FT-IR Spectrometer in the range of 4000–400 cm⁻¹. Spectra of solid samples were recorded by making transparent KBr pellets. ¹H and ¹³C NMR spectra were recorded on a Bruker AVANCE III 500 MHz multi nuclei solution NMR spectrometer. Broad band gradient probe head 'BBO' 5 mm probe was used for ¹H NMR measurements. Inverse Quad Probe head 5mm 'QXI' was used for ¹³C NMR measurements.

The ESI mass spectra were recorded on a micromass Quattro II triple quadrupole mass spectrometer. The sample dissolved in a suitable solvent was introduced into the ESI source through a syringe pump at the rate of 5 μL per min. The ESI capillary was set at 3.5 kV and the cone voltage at 40 V. The average spectrum of 7–9 scans was printed.

The electronic absorption spectra were recorded on Perkin-Elmer UV-Visible spectrophotometer controlled by the M. Wave Professional 2.0 software. The spectra were recorded in the region 200–900 nm in 0.1 M at 25 °C using a matched pair of Teflon stoppered quartz cell of path length 1 cm.

3.3. Relaxivity Measurements

3.3.1. Longitudinal relaxivity (r_{1p}) measurements.

The longitudinal relaxivity of the complexes were determined from the spin-lattice relaxation time (T₁). The T₁ measurements were carried out on a Bruker Minispec mq 20 NMR Analyzer operating at a frequency of 20 MHz using the standard inversion recovery pulse sequence (180°–□–90°) with a phase sensitive detection and □ values ranging from 50 μs to 6 s for each concentration of the complex. The temperature was maintained using a temperature console at 37±0.1 °C. The instrument parameters were optimized for

each T_1 measurement. Solutions of 0.5, 1.0, 1.5, 2.0, and 2.5 mM concentrations were prepared in phosphate buffer saline (PBS) (pH = 7.4) using HPLC grade water (Merck, India) in 5 mL standard measuring flask (vensil, class “A”). The presence of free europium (III) ion in the solution was checked by xylene orange test. The T_1 values for all concentrations were measured by taking the solution in 180 x 10 mm stoppered glass tubes. The computer program “winfit” was used to plot the time versus signal intensity to get a monoexponential plot and T_1 was calculated from the plot. The T_1 curves for all concentrations have a monoexponential decay character. The longitudinal relaxivity was calculated from the slope of the regression line, obtained by the plot of the concentration of the complex versus $1/T_1$, by least square fitting method. The instrument was calibrated by measuring the relaxivity of $[\text{Gd}(\text{DO3A})(\text{H}_2\text{O})_2]$ in aqueous solution ($r_{1p} = 4.80 \text{ mM}^{-1}\text{s}^{-1}$, 20 MHz, 40 °C).

The transverse relaxation time (T_2) was determined using the standard Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence, consisting of an initial 90° radiofrequency pulse followed by a series of 180° refocusing pulses. The resulting spin echoes were recorded at defined time intervals, and the progressive decrease in echo intensity was used to determine T_2 from the exponential decay profile. Measurements were carried out at six different concentrations of each complex under identical experimental conditions. The transverse relaxivity (r_2) was obtained from the slope of the linear regression plot of $1/T_2$ versus complex concentration, using the least-squares fitting method.

3.3 Preparation of 4.5% (w/v) HSA and Ru(II)–Eu(III)L/HSA samples.

HSA was dissolved in 10 mM sodium phosphate and 150 mM sodium chloride (pH = 7.4) solution. Two HSA solutions (4.5% w/v) were made: one containing the Ru(II)(Bpy)₂–Eu(III)–DO3A complex with a concentration of 1.0 mM and the other without the complex.

3.4. Synthesis and Characterization of ligand and complex.

The Ru(II)–Eu(III) heterobimetallic complex was synthesized through a controlled coordination approach involving the functionalized DO3A-4ABA ligand and the corresponding metal precursors (Figure-1). The synthetic strategy was designed to achieve stable coordination, effective integration of the two metal centres, and preservation of the ligand framework for subsequent optical, relaxometric, and antibacterial investigations.

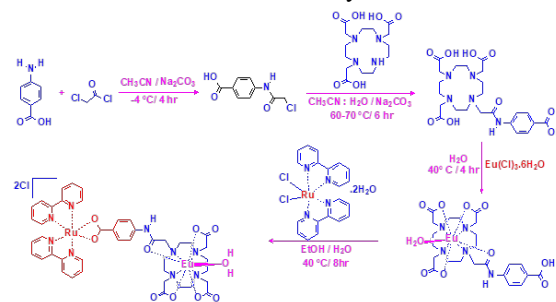


Figure-1. Scheme for the synthesis of *d-f* heterometallic complex

3.4.1 Synthesis of Linker p-Amidobenzoic acid [NC-4AB].

The *N*-alkylation of *p*-amino benzoic acid with 2-chloroacetyl chloride in a 1:1 mole ratio in the presence of sodium carbonate at –4 °C in Acetonitrile for 4 h yielded the amide as pale-yellow crystals. The fraction was concentrated and dried in vacuum. Yield: 2.03 g (95%). The linker molecule melts at 182–184 °C. Anal. calcd. For C₉H₈ClNO₃ ($M_r = 213.62$): C, 50.6; H, 3.77; N, 6.56; O, 22.47. Found: C, 50.42; H, 3.69; N, 6.39; O, 22.15. FT-IR (KBr, cm^{–1}): 3300–3500 (N–H), 3193 (C–H), 2950 (O–H), 1785 (C=O), 1723 (C=O), 1473 (C=C), and 850 (C–Cl). ESI MS (m/z): 214 [M]⁺ (C₉H₈ClNO₃)⁺, 163 [M – CH₂Cl]⁺.

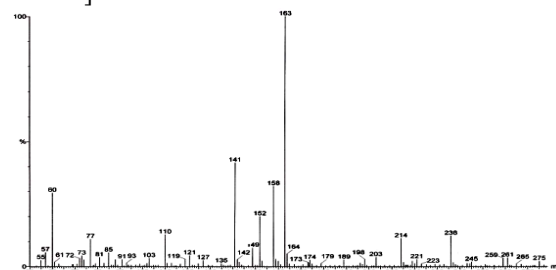


Figure-2. ESI- Mass Spectrum of the linker [NC-4AB]

3.4.2. Synthesis of DO3A appended Amido Benzoic Acid [DO3A-4ABA].

The ligand was synthesised by the condensation of DO3A and 2-chloro-*N*-(pyridine-4-yl) acetamide at 1:1 ratio in acetonitrile/water (1:1, v/v) in the presence of anhydrous sodium carbonate under heating at 60–70 °C for 6 h followed by purification yields the compound as a pale white solid. The fraction was concentrated and dried in vacuum.

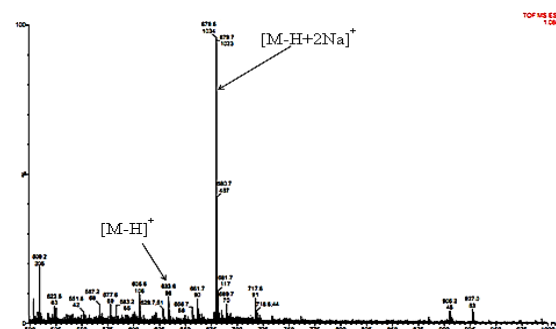


Figure-3. ESI- Mass Spectrum of the ligand [DO3A-4ABA]

Yield: 5.12 g (96 %). Anal. calcd. For $C_{23}H_{33}N_5O_9$ ($M_r = 523.54$): C, 52.77; H, 6.35; N, 13.38; O, 27.50. Found: C, 52.59; H, 6.32; N, 13.26; O, 27.70. FT- IR (KBr, cm^{-1}): 3424 cm^{-1} ν (O–H), 3291 cm^{-1} ν (N–H), 2947 cm^{-1} ν (C–H), 1713 and 1659 cm^{-1} ν (C=O), 1586 and 1394 cm^{-1} ν (C–O), 1252 and 1173 cm^{-1} ν (C–N and C–O), and 850 cm^{-1} δ (C–H). ESI MS (m/z): 524 $[M]^+$ ($C_{23}H_{33}N_5O_9$) $^+$, 463 $[M-CH_2Cl]^+$. 1H NMR (500 MHz, D_2O , 298 K): $\square$ 8.05 (d, 2H, $J=8.5$, 1.7, 0.5 Hz (Aromatic ring), 9.6 (s, OH, hydroxy), 3.56 (m, 16H), 3.27 (m, 8H) ppm. ^{13}C NMR (125 MHz, D_2O , 298 K): $\square$ 175.1–167.1, 137.7–118.5, 44.3, 54.4, and 54.1–9.8 ppm.

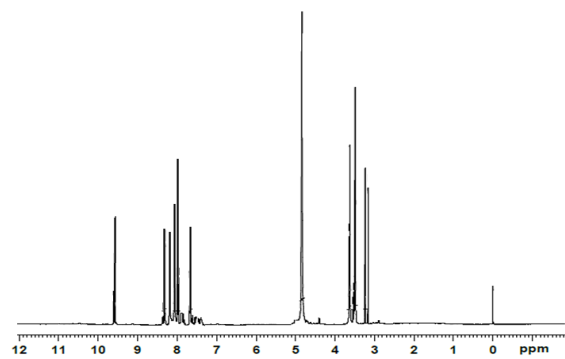


Figure-4. 500 MHz 1H NMR spectrum of the ligand [DO3A-4ABA] in D_2O .

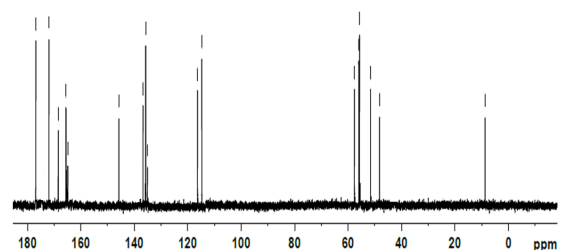


Figure-5. 125 MHz ^{13}C NMR spectrum of the ligand [DO3A-4ABA] in D_2O .

3.4.3. (Dichloro)bis(2,2'-bipyridine)ruthenium(II) dihydrate, $[Ru(bpy)_2.2Cl].2H_2O$.

The reaction of $RuCl_3.3H_2O$ (1 equiv) and 2,2'-bipyridine (2 equiv) in DMF in the presence of lithium chloride as a mild reducing agent gives $[Ru(bpy)_2.2Cl].2H_2O$ as a dark red solid. The fraction was concentrated by vacuum filtration, wash with cold ether to remove residual solvent and unbound ligand, and dry under vacuum. Yield: 5.04 (97%) mp 287°C. Anal. calcd. For $C_{20}H_{20}Cl_2N_4O_2Ru$ ($M_r = 520.38$): C, 46.16; H, 3.87; Cl, 13.63; N, 10.77; O, 6.15; Ru, 19.42. Found: C, 46.26; H, 3.95; N, 10.58; O, 6.11. ESI MS (m/z): 520 $[M]^+$ ($C_{20}H_{20}Cl_2N_4O_2Ru$) $^+$, 489 $[M-Cl]^+$, 449 $[M-2Cl]^+$ ($C_{20}H_{20}N_4O_2Ru$) $^+$.

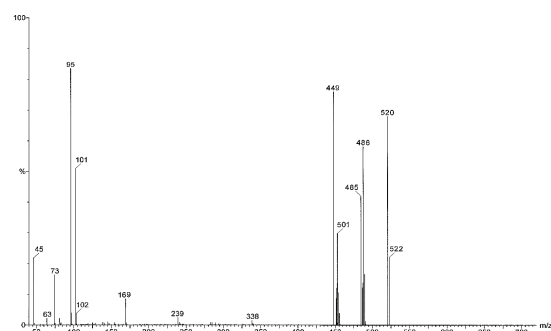


Figure-6. ESI- Mass Spectrum of $[Ru(bpy)_2.2Cl].2H_2O$.

3.4.4. Synthesis of $[Eu(III)(DO3A-4ABA)].H_2O$ complex.

The reaction of the preformed ligand with europium (III) chloride hexahydrate in a 1:1 mole ratio in water at 40 °C (pH = 6.5–7.5) for 4 h followed by purification yields the Eu(III)-DO3A-Amidobenzoic acid complex as a white hygroscopic solid. The fraction was concentrated and dried in vacuum. Yield: 6.91g (98%), mp 228°C. Anal. calcd. For $C_{24}H_{35}EuN_5O_{10}$ ($M_r = 705.53$): C, 40.86; H, 5.00; Eu, 21.54; N, 9.93; O, 22.68. Found: C, 40.56; H, 5.12; Eu, 21.41; N, 9.79; O, 22.13. ESI MS (m/z): 706 $[M+1]^+$ ($C_{24}H_{36}EuN_5O_{10}$) $^+$, 705 $[M]^+$, 688 $[M-OH]^+$ ($C_{24}H_{34}EuN_5O_9$) $^+$, 340 $[M-C_9H_{10}NO_3Eu]^+$ ($C_{15}H_{25}N_4O_7$) $^+$.

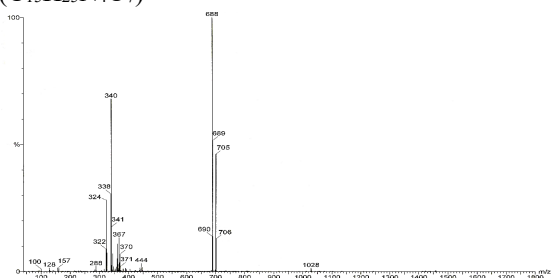


Figure-7. ESI- Mass Spectrum of $[Eu(III)(DO3A-4ABA)].H_2O$

3.4.5 Synthesis of $[(Ru(II)(bpy)_2)(Eu(III)(DO3A-4ABA))](H_2O)(2Cl)$ target complex.

To a solution of $[Ru(bpy)_2.2Cl].2H_2O$ (0.5 g, 0.93 mmol) in water/ethanol (1:1, v/v) (60 mL) mixture a solution of the preformed Europium (III) complex

$[Eu(III)(DO3A-Amidobenzoic acid)(H_2O)]$ (1.21 g, 1.86 mmol) in water was added slowly under reflux condition in an argon atmosphere. The solution was concentrated in a rotavapor and the resulting pale brown solid was purified. The fraction was concentrated and dried in vacuum. Yield: 11.96 g (97%). mp 287°C. Anal. calcd. For $C_{44}H_{51}Cl_2N_9O_{10}EuRu$ ($M_r = 1190.14$): C, 44.41; H, 4.32; Cl, 5.96; N, 10.59; O, 13.45; Eu, 12.77; Ru, 8.49. Found: C, 44.49; H, 4.18; Cl, 5.81; N, 10.63; O, 13.33; Eu, 12.48; Ru, 8.35. FT-IR (KBr): 1596 $\nu_{as}(COO^-)$, 1544 $\delta(N-H)$, 1402 $\nu_s(COO^-)$ cm^{-1} . ESI-MS. Calcd for $[C_{44}H_{51}Cl_2N_9O_{10}EuRu]^+$: (m/z) 1190.14, Found: (m/z) 1190 $[M]^+$, 1076 $[M-H_7O_2Cl_2]^+$.

$(C_{44}H_{44}N_9O_8EuRu)$, 954 $[M-(EuH_2OCl_2)]^+$
 $(C_{44}H_{49}N_9O_9Ru)$, 912 $[M-(EuH_7O_3Cl_2)]^+$
 $(C_{44}H_{44}N_9O_7Ru)$, 803 $[(M-1)-(C_{20}H_{22}OCl_2)]^+$
 $(C_{24}H_{29}N_5O_9EuRu)$, 767 $[(M-1)-(C_{20}H_{22}O_2Cl_2)]^+$ $[C_{24}H_{31}N_5O_8EuRu]^+$

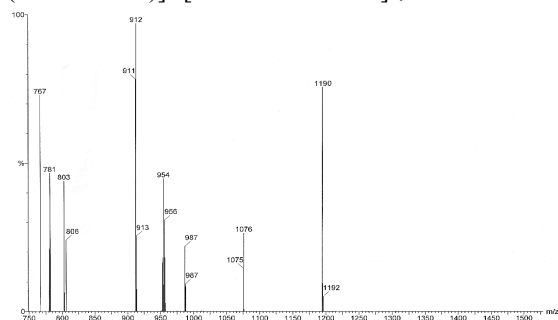


Figure-8. ESI-Mass Spectrum of $[(RuII(bpy)_2)(EuIII(DO3A-4ABA))](H_2O)(2Cl)$

4. Result And Discussion

4.1. Absorptionspectrum of the ligand [DO3A-4ABA].

The UV-Vis spectrum shown exhibits a broad absorbance band around 274 nm attributed to the $\pi \rightarrow \pi^*$ transitions of the aromatic benzene ring, which is commonly observed in substituted aromatic systems (Figure-9). Additionally, while the macrocyclic DOTA structure itself is not inherently UV-active, it can influence electronic transitions due to its electron-withdrawing carboxylate groups. This conjugation enhances the molecule's ability to absorb UV light, making it suitable for photometric detection and indicating potential biomedical applications such as drug conjugation or the development of MRI contrast agents. Thus, the spectrum confirms the electronic properties expected from the structure.

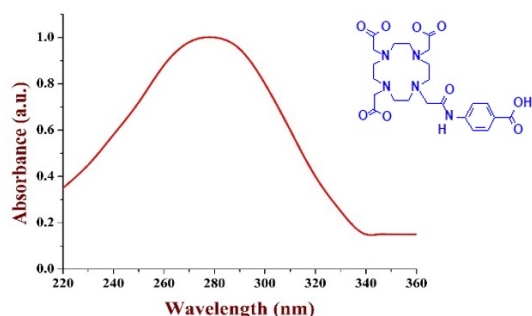


Figure-9. UV-Visible spectrum of the ligand [DO3A-4ABA]

4.2. Absorption spectrum of $[(RuII(bpy)_2)(EuIII(DO3A-4ABA))](H_2O)(2Cl)$.

The UV-Visible spectrum of the Ru(II)–Eu(III) heterometallic complex is expected to exhibit an intense absorption band in the 278 nm region, which can be assigned predominantly to $\pi \rightarrow \pi^*$ transitions associated with the coordinated bipyridyl/pyridyl and aromatic ligand systems (Figure-10). A relatively weak and broad absorption in the 477 nm region can be attributed to a metal-to-ligand charge-transfer (MLCT)

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transition involving the Ru(II) centre and the π -acceptor ligand.

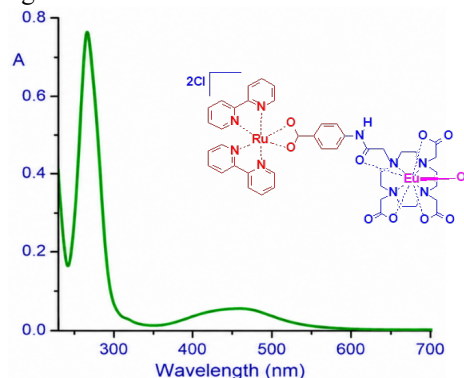


Figure-10. UV-Visible spectrum of the Target complex

The incorporation of Eu(III) is not expected to introduce intense absorption bands in the visible region because of the weak nature of the Laporte-forbidden 4f–4f transitions. Nevertheless, weak characteristic Eu (III) transitions may occur in the red region, particularly around 580–590, 610–615, 650 and 690–700 nm. The presence of both ligand-centred transitions and Ru (II)-based MLCT absorption indicates the preservation of the electronic communication between the coordinated ligand framework and the metal centres.

4.3. Longitudinal and Transverse Relaxivity Studies.

The heterobimetallic complex $[(RuII(bpy)_2)(EuIII(DO3A-4ABA))](H_2O)(2Cl)$ containing Eu (III) and Ru (II) pairs a paramagnetic europium (III) center with a photoactive $Ru(bpy)_2^{2+}$ unit through an amide linker. The nine-coordinate Eu (III) core has a single inner-sphere water molecule directly coordinated to the DO3A ligand. The rapid chemical exchange of this coordinated water with surrounding bulk water molecules facilitates efficient dipole-dipole proton relaxation, allowing it to function as a magnetic resonance contrast agent. The relaxation plot illustrates a linear correlation between the solvent proton relaxation rates (R_1 and R_2) and the complex concentration up to 2.5 mM. The slopes derived from the data indicate high molar relaxivity values: the longitudinal relaxivity (r_{1p}) is $9.57 \text{ mM}^{-1}\text{s}^{-1}$, while the transverse relaxivity (r_{2p}) is $14.4 \text{ mM}^{-1}\text{s}^{-1}$. The increased relaxivity is attributed to both the inner-sphere hydration ($q=1$) and the bulky bimetallic framework, which enhances the rotational correlation time, allowing better alignment with proton Larmor frequencies (Figure-11). With a low r_2/r_1 ratio of 1.64, this system acts primarily as a T_1 -positive contrast agent, providing dual optical and MRI capabilities with strong aqueous stability.

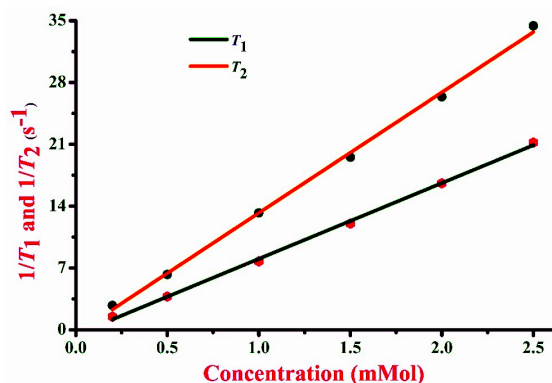


Figure-11. Longitudinal and Transverse Relaxivity of the Target complex in water.

4.4. Longitudinal and Transverse Relaxivity Studies at various pH (2.5-11).

The pH-dependent relaxation profile of the $[(RuII(bpy)_2)(EuIII(DO3A-4ABA))](H_2O)(2Cl)$ complex demonstrates a direct coupling between ligand protonation state and water proton relaxation performance across a 2.5-11 pH domain (Figure-12). In acidic environments (pH = 2.5-6.0), both T_1 and T_2 values experience a pronounced increase, peaking near 29-32 $mM^{-1}s^{-1}$. This enhancement is driven by the protonation of carboxylate oxygen centers, nitrogen atoms on the polypyridine framework, and peripheral hydrogen-bonded water molecules, which accelerates chemical exchange and alters coordination geometry. Transitioning toward neutral conditions (pH = 7-8.5) triggers rapid deprotonation, causing T_1 relaxivity to drop significantly and stabilize near baseline above pH = 8.5. In contrast, T_2 relaxivity diverges sharply upward under strongly alkaline conditions (pH = 8.5-10.6), rising back to 32 $mM^{-1}s^{-1}$ due to base-catalysed proton exchange dynamics and enhanced transverse dephasing.

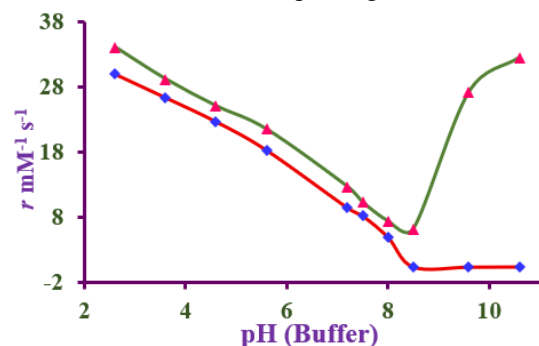


Figure-12. Longitudinal and Transverse Relaxivity of the Target complex in different solution pH 2.5-11.

4.5. Longitudinal and Transverse Relaxivity Studies in 4.5 %HSA.

The interaction between the heterobimetallic $[(RuII(bpy)_2)(EuIII(DO3A-4ABA))](H_2O)(2Cl)$ complex and Human Serum Albumin (4.5% HSA) produces a significant boost in nuclear magnetic relaxation efficiency. In this biomolecular environment, the linear concentration

plots yield a longitudinal relaxivity (r_1) of 24.33 $mM^{-1}s^{-1}$ and a transverse relaxivity (r_2) of 35.12 $mM^{-1}s^{-1}$ (Figure-13). This enhancement is driven by adduct formation between the heterodinuclear contrast agent and HSA, stabilized through polar interactions along the complex framework. Non-covalent binding to the macromolecular protein restricts the complex's rotational tumbling, thereby increasing its rotational correlation time (τ_R). Concurrently, the coordinated aqua ligand on the Eu(III) core provides two exchangeable proton sites that interact efficiently with bulk water molecules, facilitating dipolar relaxation transfer and establishing strong efficacy for macromolecule-targeted MRI applications.

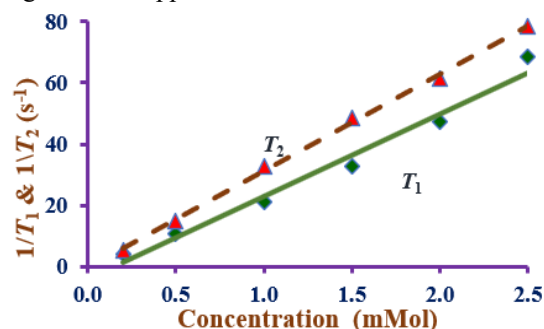


Figure-13. Longitudinal and Transverse Relaxivity of the Target complex in HSA.

4.6. Antibacterial Studies.

The heterobimetallic $[(RuII(bpy)_2)(EuIII(DO3A-4ABA))](H_2O)(2Cl)$ complex displays broad-spectrum antibacterial activity on gram +ve bacteria's like *Clostridium difficile* (C.D), *Staphylococcus aureus* (S.A), and gram -ve bacteria's like *Escherichia coli* (E.C), *Pseudomonas aeruginosa* (P.A) driven by the synergistic interplay between its metal centers and hydrophobic chelating framework. As shown by the disk diffusion assays, the complex exhibits high potency against Gram-positive (C.D), (S.A), (zones of inhibition ranging from 18 mm down to 16 mm) and good efficacy against Gram-negative (E.C), (P.A) (zones of inhibition ranging 15 mm to 13mm) (Table-1, Figure-14, and 15). This antimicrobial activity correlates directly with the complex's structural features: the lipophilic polypyridine ligands reduce metal ion polarity.

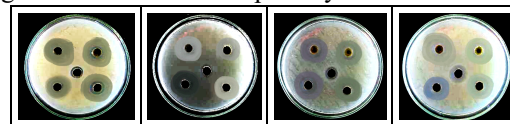


Figure-14. Antibacterial Studies of the Target complex on *Clostridium difficile* (C.D), *Staphylococcus aureus* (S.A), *Escherichia coli* (E.C), and *Pseudomonas aeruginosa* (P.A).

Table.1. Zones of inhibition of the complex on Gram (+ve) and Gram (-ve) bacteria

Bacteria	ZOI (mm)	ZOI (mm)	ZOI (mm)	ZOI (mm)

	@ 0.5 mM	@ 1.0 mM	@ 1.5 mM	Contro l
Clostridium difficile (C.D)	12	18	25	12
Staphylococu s aureus (S.A)	09	16	22	11
Escherichia coli (E.C)	10	15	21	14
Pseudomonas aeruginosa (P.A)	07	13	20	13

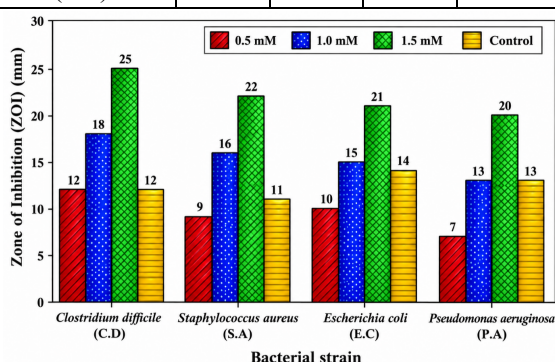


Figure-15. Graph showing the complex Antibacterial efficiency vs Control on Gram (+ve) and (-ve) bacteria

Once inside, the cationic metal centers disrupt bacterial membrane integrity and interfere with intracellular metabolic pathways. The higher susceptibility observed in *S. aureus* reflects its single-layered peptidoglycan cell wall, which allows easier complex penetration.

5. Conclusion

A new small-molecular Ru(II)–Eu(III) heterobimetallic complex, (Ru(II)(bpy)₂(Eu(III)(DO3A-4ABA))(2Cl), demonstrates a promising optical, relaxometric and antibacterial probe for dual-mode fluorescence and MR imaging reported. The ligand exhibits a characteristic absorption at 274 nm, while the heterometallic complex shows intense ligand-centred absorption at 278 nm together with a broad band around 477 nm, consistent with ligand-centred and Ru(II)-based charge-transfer transitions. The complex displays appreciable proton relaxation efficiency in aqueous medium, with $r_{1p} = 9.57$ and $r_{2p} = 14.40 \text{ mM}^{-1} \text{ s}^{-1}$ and an r_2/r_1 ratio of 1.64, indicating predominantly T₁-weighted behaviour. These values are higher than those commonly reported for conventional DO3A-based Gd(III) systems, for which r_1 values are typically approximately 3–6 $\text{mM}^{-1} \text{ s}^{-1}$. Importantly, the relaxivity increased substantially in 4.5% HSA, reaching 24.33 and 35.12 $\text{mM}^{-1} \text{ s}^{-1}$ for r_1 and r_2 , respectively, highlighting the beneficial effect of macromolecular association and restricted molecular rotation. This enhancement is

comparable in concept with reported DO3A-based albumin-binding systems, although some optimized Gd(III)–HSA systems reach even higher bound relaxivities. Furthermore, the pronounced pH dependence and antibacterial activity against *C. difficile*, *S. aureus*, *E. coli* and *P. aeruginosa* (inhibition zones 13–18 mm) demonstrate additional functional versatility. The results establish this Ru(II)–Eu(III) platform as a promising multimodal optical/MR and antimicrobial molecular system, warranting further cellular, mechanistic, stability and in-vivo investigations.

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