Research Article

DNA Interactions, Antibacterial and Antioxidant Studies of Newly Synthesized Lanthanum(III) Complexes Using N,N'-Bis(3-Pyridylmethyl) oxamide and N,N-Heterocyclic bases.

P. R. Chetana[1*], Vibha Vinayakumar Bhat[1], Mohan A. Dhale[2]

[1]Department of Chemistry, Central College Campus, Bangalore University, Bengaluru, Karnataka, India.

[2]Microbiology and Fermentation Technology Department, Central Food Technological Research Institute, Mysore, Karnataka, India.

***Corresponding author's E-mail:** pr.chetana@gmail.com

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ABSTRACT

Six new lanthanum(III) complexes $[La(3PMO)(NO_3)_2](NO_3)$ (**2**), $[La(3PMO)_2(B)(NO_3)](NO_3)_2$ (**3-7**) were synthesized using N,N'-bis(3-pyridylmethyl)oxamide (3PMO, **1**) and N,N-heterocyclic bases (*B*). The complexes were well characterized by various physical and spectroscopic methods. The binding propensities of the complexes **2-7** towards CT-DNA were determined by UV-Visible absorption spectroscopy, cyclic voltammetry and viscosity methods, confirm the intercalation mode of binding. Complexes **4** and **6** have shown maximum cleavage of SC pUC 19 DNA into nick circular form in the presence of oxidizing agent H_2O_2 at 10 µM concentration. The involvement of hydroxyl radicals in the oxidative cleavage was confirmed by the inhibition of cleavage in the presence of hydroxyl radical quencher DMSO. The antimicrobial activities of complexes **2-7** were tested against Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram negative (*Escherichia coli* and *Klebsiella pneumonia*) bacteria and it was shown that complex **5** had shown the highest antibacterial activity against all the bacterial strains at 50 µg/mL. The antioxidant activities of complexes **2-7** were determined by DPPH radical scavenging method, which revealed that complexes **3**, **4** and **5** had shown good DPPH radical scavenging abilities with complex **5** has EC_{50} = 56.35 µg/mL. The ferrous ion chelation method showed that complex **3** has the highest chelating ability towards ferrous ions with EC_{50} = 19.42 µg/mL followed by complexes **4** and **5** respectively. The structure-activity relationships determined by these studies prove that the newly synthesized La (III) complexes can be used as potential therapeutic and diagnostic tools.

Keywords: Antibacterial activity, Antioxidant activity, N,N'-bis(3-pyridylmethyl)oxamide, Lanthanum(III) complexes, DNA binding, DNA cleavage.

INTRODUCTION

The interactions of metal complexes with biomolecules such as nucleic acids, proteins, enzymes are the key factors in developing advanced materials in the bioinorganic field that have high pharmaceutical values. Though *cis*-platin and carboplatin are being used as anticancer drugs, their use is limited as they are found to be highly effective only against ovarian, testicular and head and neck cancers. Also, their side effects such as nephrotoxicity, neurotoxicity which are severe make the usage of this drug with limited dose.[1, 2] This provides a way to develop new drugs which can interact through DNA and proteins which are the principal targets and in turn possessing antitumor activities. Synthesizing new drugs needs a thorough understanding of mode of interactions of metal complexes with biomolecules. Metal complex interacts with DNA in two major ways: covalent interaction or non-covalent interaction. The non-covalent interaction of metal complex with DNA includes groove binding, intercalation and electrostatic interaction. Among these, the most common method is intercalation. The flexible geometry of the metal complex, variable coordination number, planarity of the ligand facilitate intercalation mode of binding.[3] In this newly emerging field, lanthanum(III) complexes are having much importance as their biological properties are rarely explored and can be modified by using suitable ligand system. The nuclease activity of the lanthanum (III) complexes can be mimicked efficiently because they possess very high Lewis acidity, high charge density, flexible coordination number and fast ligand exchange rates.[4] Lanthanum (III) complexes having Schiff bases[5-7] are greatly explored and screened for their antitumor activities. In this view, O and N donor ligands are beneficial in synthesizing stable lanthanum (III) complexes. Oxamide is one such ligand that has both O and N donor atoms in the same molecule. This bidentate ligand is used in tuning the structure of metal complexes by its *cis-trans* conformational changes and also by changing amide substituent, thereby changing the activities of the metal complexes.[8] There are many reports on transition metal complexes containing oxamides as ligands which possess various biological properties such as antitumor,[9] antimicrobial and antagonist abilities[10], binding propensities towards DNA and proteins,[11-12] enzyme inhibition properties.[13] Thus, oxamides are the best suited ligands that can be coordinated with lanthanum ion, on which there are no reports. The biological properties of lanthanum complexes can be tuned by using two-ligand system of which one ligand is with extended planar structure. 1,10-phenanthroline is one such ligand which is known to be a good intercalator with its rigid planar aromatic ring system and can bind to biomolecules such as DNA and proteins. The derivatives of 1,10-phenanthroline using amino acids,[14] aliphatic diamines[15] when complexed with

lanthanum(III) ions were found to be more effective comparable to *cis*-platin against few cancerous cell lines. In this direction, lanthanum (III) complexes having O-donor ligands also gained much attention. Lanthanum(III) complexes are found to possess antitumor as well as antioxidant activities,[16-17] photophysical properties such as luminescence that can be employed as labeling agents in MRI and immunohybridization techniques.[18] Few of the lanthanum(III) complexes are reported to possess antimicrobial activities against *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus, Klebsiella pneumonia* and so on.[19-21]. The lanthanum(III) complexes were also found to have significant antioxidant properties.[22]

With all information from the literature, it can be observed that mononuclear lanthanum (III) complexes with oxamides are rarely explored. To induce and enhance their biological properties, we were succeeded in synthesizing mixed-ligand complexes of lanthanum (III) using N, N'-bis (3-pyridylmethyl)oxamide and derivatives of 1,10-phenanthroline. These newly synthesized complexes (**2-7**) were tested for their binding propensities towards calf-thymus DNA (CT-DNA), ability to cleave super coiled (SC) pUC 19 DNA, antibacterial and antioxidant activities.

MATERIALS AND METHODS

Materials

All the reagents and chemicals were obtained from Sigma Aldrich (USA) and Fluka (USA) and used as obtained. Super coiled pUC 19 DNA (cesium chloride purified) was purchased from Bangalore Genei (India). Calf-thymus DNA (CT-DNA), Agarose (molecular biology grade), Ethidium bromide (EB) was obtained from Sigma Aldrich (USA). Tris-(hydroxymethyl) aminomethane-HCl (pH=7.2) buffer solution was prepared using deionized, double distilled water. Bacterial media was purchased from Himedia. The bacterial strains were procured from IMTECH, Chandigarh, India.

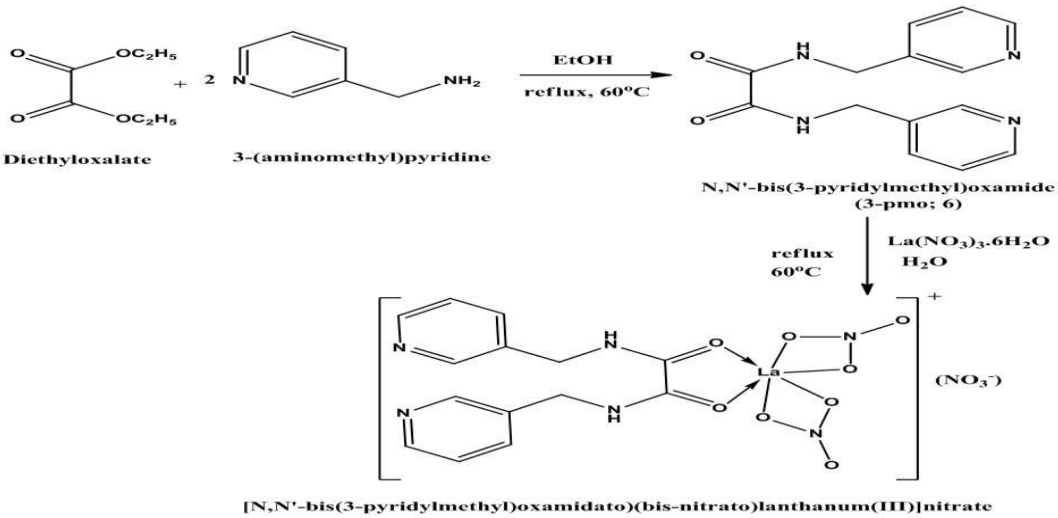
Syntheses

The ligand N,N'-bis (3-pyridylmethyl)oxamide (3PMO, (**1**)) was synthesized by modifying reported procedure[22] for the comparison of its activities with those of lanthanum(III) complexes. The lanthanum (III) complexes were synthesized by following reported method with slight modification.[23]

Synthesis of [La (3pmo)$(NO_3)_2$](NO_3) (2)

Diethyloxalate (0.408 mL, 0.1 M) and 3-aminomethylpyridine (0.432 mL, 0.2 M) were dissolved in 20 mL ethanol. The solution was refluxed at 60 ^{o}C for 1 h. To this, aqueous solution of La $(NO_3)_3.6H_2O$ (0.433 g, 0.1 M) was added and refluxing was continued for 2 h. The clear solution obtained was kept for slow evaporation, yielded white solid. The solid obtained was washed with warm ethanol: water mixture three times and dried.

Anal. Cald. (%) for $C_{14}H_{14}LaN_7O_{11}$ (**2**): C, 28.25, H, 2.37, N, 16.47. found: C, 28.28, H, 2.40, N, 16.49. ^{1}H-NMR (400 MHz, DMSO-d^6): 4.34-4.36 (d, 2H), 7.35-7.38 (m, 4H), 7.69-7.71 (d, 2H), 8.47-8.51 (d, 4H), 9.41-9.45 (t, 2H). FT-IR (KBr disc, cm^{-1}): 3281, 1654, 1527, 1473 and 1301, 1427, 1384, 1033, 769, 709, 646, 518. λ_{max} (1.0 mM, DMF/Tris-HCl): 229.97 (ε = 13,300 cm^{-1}mol^{-1}) nm and 259.84 (ε = 8,000 cm^{-1}mol^{-1}). ESI-MS (m/z): 532.92 [M-(NO_3^-)]$^+$. Λ_M in DMF (Scm^{2}mol^{-1}): 91.



Scheme 1: Preparation scheme for [La(3PMO)$(NO_3)_2$](NO_3) (**2**).

Synthesis of [La(3PMO)$_2$(*B*)(NO_3)]$(NO_3)_2$ (*B*= bpy (3), phen (4), dione (5), dpq (6) and dppz (7))

Diethyloxalate (0.408 mL, 0.1 M) and 3-aminomethylpyridine (0.432 mL, 0.2 M) were dissolved in 20 mL ethanol. The solution was refluxed at 60 ^{o}C for 1 h. To this, aqueous solution of La$(NO_3)_3.6H_2O$ (0.433 g, 0.1 M) was added and refluxing was continued for 2 h. To the above reaction mixture, ethanolic solution of 2,2'-bipyridyl (bpy, **3**) (0.468g, 0.1 M), 1,10-phenanthroline (phen, **4**) (0.594, 0.1 M), 1,10-phenanthroline-5,6-dione

(dione, **5**) (0.594, 0.1 M), dipyrido(3,2-d:2',3'-f)quinoxaline (dpq, **6**) (0.696 g, 0.1 M) and dipyrido[3,2-a:2',3'-c]phenazine (dppz, **7**) (0.846 g, 0.1) were added respectively and refluxing was continued for further 2 h. The respective clear solutions were kept for slow evaporation. The solids obtained were washed with ethanol: water mixture three times and dried.

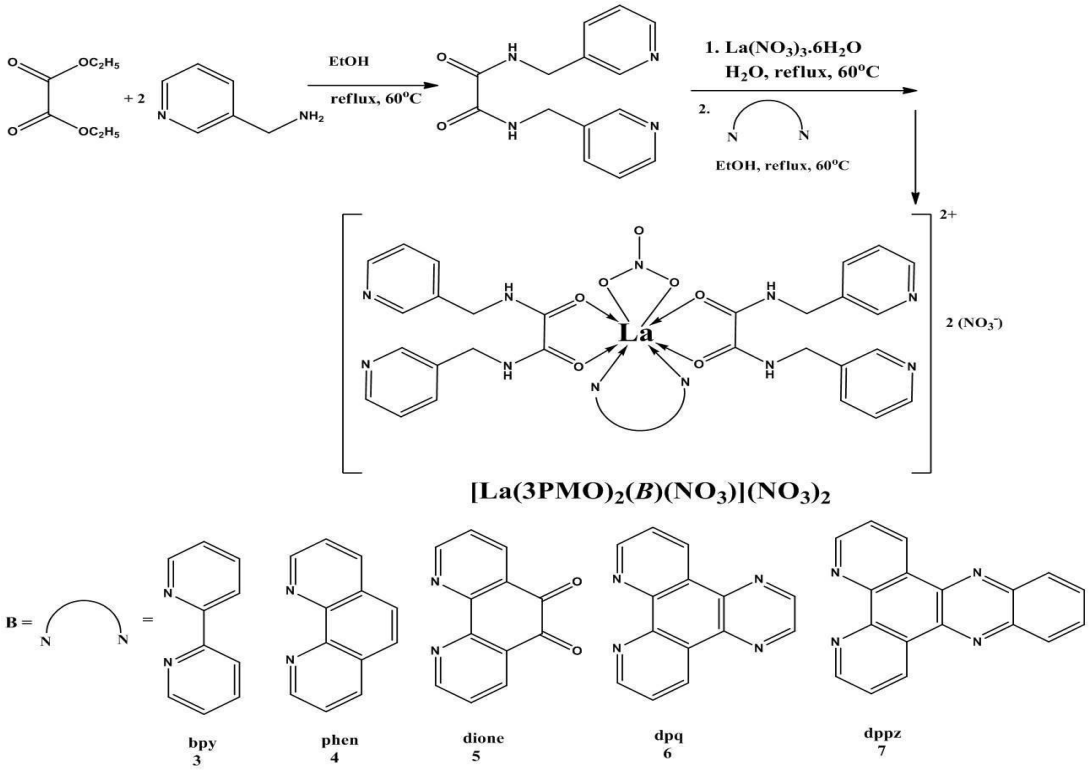
Anal. Cald. (%) for $C_{38}H_{36}LaN_{13}O_{13}$ (**3**): C, 44.67, H, 3.55, N, 17.82. found, C, 44.70, H, 3.58, N, 17.84. ^{1}H-NMR (400 MHz, DMSO-d^6): 4.35-4.37 (d, 2H), 7.38-7.41 (q, 2H), 7.46 (s, 1H), 7.72-7.73 (t, 2H,) 7.74-7.75(t, 2H), 8.38-8.39 (s, 1H), 8.47-8.52 (m, 3H), 8.69 (s, 1H), 9.42-9.45 (t, 2H). FT-IR (KBr disc, cm^{-1}): 3270, 1657, 1527, 1479 and 1301, 1365, 1289, 1015, 755, 695, 505. λ_{max} (1.0 mM, DMF/Tris-HCl): 260.47 (ε = 13,233 $cm^{-1}mol^{-1}$) nm and 319.76 (ε = 6,966 $cm^{-1}mol^{-1}$). ESI-MS (m/z): 448.8 a.m.u $[M-2(NO_3^-)]^+$. Λ_m in DMF (Scm^2mol^{-1}): 153.

Anal. Cald. (%) for $C_{40}H_{36}LaN_{13}O_{13}$ (**4**): C, 45.94, H, 3.47, N, 17.41. found, C, 45.96, H, 3.48, N, 17.43. ^{1}H-NMR (400 MHz, DMSO-d^6): 4.34-4.36 (d, 8H), 7.33-7.36 (q, 3H), 7.66-7.69 (dt, 3H), 7.80-7.83 (s, 4H), 8.03 (s, 4H), 8.44-8.46 (dd, 3H), 8.49-8.50 (d, 2H), 8.53-8.55 (d, 4H), 9.12 (s, 4H), 9.41-9.44 (t, 4H). FT-IR (KBr disc cm^{-1}): 3282, 1654, 1518, 1458 and 1319, 1384, 1105, 1030, 848, 731, 646, 518. λ_{max} (1.0 mM, DMF/Tris-HCl): 279.42 (ε = 13,066 $cm^{-1}mol^{-1}$) nm and 339.34 (ε = 5,166 $cm^{-1}mol^{-1}$). ESI-MS (m/z): 460.78 a.m.u $[M-2(NO_3^-)]^+$ Λ_m in DMF (Scm^2mol^{-1}): 155.

Anal. Cald. (%) for $C_{40}H_{34}LaN_{13}O_{15}$ (**5**): C, 44.66, H, 3.19, N, 16.93. found, C, 44.68, H, 3.20, N, 16.95. ^{1}H-NMR (400 MHz, DMSO-d^6): 4.46-4.48 (d, 2H), 7.76-7.80 (q, 2H), 8.17-8.19 (d, 1H), 8.69-8.72 (dd, 2H), 9.49-9.52 (t, 1H). FT-IR (KBr disc, cm^{-1}): 3286, 3047, 2761, 1656, 1627 and 1612, 1558, 1519, 1465 and 1312, 1380, 1257, 1118, 987, 794, 678, 624, 524, 493. λ_{max} (1.0 mM, DMF/Tris-HCl): 280.60 (ε = 12,833 $cm^{-1}mol^{-1}$) nm and 339.34 (ε = 4,033 $cm^{-1}mol^{-1}$). ESI-MS (m/z): 522.60 a.m.u $[M+2Na-2(NO_3^-)]^+$ Λ_m in DMF (Scm^2mol^{-1}): 154.

Anal. Cald. (%) for $C_{42}H_{36}LaN_{15}O_{13}$ (**6**): C, 45.95, H, 3.31, N, 19.14. found, C, 45.97, H, 3.32, N, 19.16. ^{1}H-NMR (400 MHz, DMSO-d^6): 4.34- 4.36 (d, 4H), 7.34- 7.37 (q, 2H), 7.67-7.70 (dt, 2H), 7.80-7.84 (q, 1H), 8.04 (s, 1H), 8.44-8.46 (q, 2H), 8.49-8.50 (d, 2H), 8.54-8.57 (t, 2H), 9.12 (s, 1H), 9.38-9.41 (t, 2H). FT-IR (KBr disc, cm^{-1}): 3282, 1660, 1527, 1479 and 1315, 1373, 1229, 1171, 1015, 779, 707, 625, 517. λ_{max} (1.0 mM, DMF/Tris-HCl): 280. 05 (ε = 13,133 $cm^{-1}mol^{-1}$) nm and 340.43 (ε =6,433 $cm^{-1}mol^{-1}$). ESI-MS (m/z): 579.0 a.m.u $[M+CH_3OH-2(NO_3^-)]^+$. Λ_m in DMF (Scm^2mol^{-1}): 153.

Anal. Cald. (%) for $C_{46}H_{38}LaN_{15}O_{13}$ (**7**): C, 48.14, H, 3.34, N, 18.30. found, C, 48.15, H, 3.36, N, 18.31. ^{1}H-NMR (400 MHz, DMSO-d^6):4.34-4.35 (d, 8H), 7.34-7.37 (q,4H), 7.39-7.41 (q, 1H), 7.67-7.70 (dt, 4H), 7.961-7.992 (q, 1H), 8.06-8.09 (q, 1H), 8.44-8.46 (m, 4H), 8.49-8.50 (d, 3H), 9.231-9.239 (d, 1H), 9.41-9.44 (t, 4H), 9.57-9.59 (d,1H). FT-IR (KBr disc, cm^{-1}): 3281, 1653, 1521, 1458 and 1338, 1384, 1215, 1033, 736, 709, 642, 518. λ_{max} (1.0 mM, DMF/Tris-HCl): 289. 42 (ε = 12,900 cm^{-1} mol^{-1}) nm and 343.90 (ε = 3,500 $cm^{-1}mol^{-1}$). ESI-MS (m/z): 627.1 a.m.u $[M+CH_3OH+Na-2(NO_3^-)]^+$. Λ_m in DMF (Scm^2mol^{-1}): 152.



Scheme 3: Preparation scheme of complexes [La $(3PMO)_2(B)(NO_3)](NO_3)_2$ (3-7).

General Methods

The elemental analyses of carbon, hydrogen and nitrogen of the synthesized La(III) complexes were carried out using a Thermo Finnigan FLASH EA 1112 CHNS analyzer. 1H NMR spectra of the ligand (**1**) and La(III) complexes (**2-7**) were recorded on 400 MHz liquid state Bruker NMR spectrometer obtained in DMSO-d^6 solution at approximately 25°C using tetramethyl silane (TMS) as internal standard. The IR spectra of the ligand and complexes were recorded using KBr pellets over the region 4000 cm^{-1} - 400 cm^{-1} on a Shimadzu FT-IR spectrophotometer. UV-Visible absorption spectra of the ligand and complexes were recorded on SpectraMax Plus 384 spectrophotometer. 10^{-3} M solutions of the ligand and complexes were prepared in DMF/ 5 mM Tris-HCl buffer (pH 7.2) and recorded in the wavelength ranging from 200 to 800 nm. The time-dependent stability of the complexes 2-7 in solution state (DMF/ 5 mM Tris-HCl buffer (pH 7.2)) were measured over 6 h at pH 7.4, 25 °C using UV-Visible absorption spectroscopic method using SpectraMax Plus 384 spectrophotometer. Full scan mass spectra (ESI-MS) of the methanolic solutions of complexes were recorded in positive ion mode on Shimadzu-LCMS equipped with APCI and ESI probes. The corresponding m/z values were recorded in the expected range. Conductivity measurements were carried out using 10^{-3} M solutions in DMF on Control Dynamics (India) conductivity meter with a dip-type cell, calibrated with a (0.1 M) solution of AnalaR potassium chloride. The molar conductivity values were used to determine the electrolytic nature of the complexes. Electrochemical experiments were performed on a CHI 600E Electrochemical analyzer controlled by PC. The cyclic voltammograms of 10^{-3} M solutions of ligand and La(III) complexes in DMF solvent were recorded in the potential range of -0.3 to 0.7 V at the scan rate of 25 – 200 mV/s at 25 °C using 0.1 M tetra butyl ammonium perchlorate (TBAP) as supporting electrolyte. The ligand and complex solutions were degassed with N_2 atmosphere for 10 mins prior to the electrochemical measurements.

DNA binding experiments

UV-Visible absorption titrations

UV-Visible absorption titration experiment was carried out to study the interaction of La (III) complexes with CT-DNA using SpectraMax Plus 384 spectrophotometer. Absorbance was measured at 260 and 280 nm for CT-DNA in 5 mM Tris-HCl (pH=7.2) gave the ratio of 1.9:1, which indicates that the DNA was free of protein. The concentration of CT-DNA was calculated using the intensity of the absorbed band at 260 nm with a known ε value (6600 $M^{-1}cm^{-1}$). The absorption titrations were carried out at constant concentration of the complexes (30 µM) while varying concentration of CT-DNA. Stock solutions (0.5 mM) of La(III) complexes (**2-7**) were prepared by dissolving the complexes in DMF and diluted further to 30 µM using 5 mM Tris-HCl buffer. The absorbance of DNA while measuring the spectra was eliminated by adding equal amounts of DNA to both complex and reference solutions. The complex solution was allowed to get equilibrated for 5 mins after the addition of known aliquots of CT-DNA solution to allow the complex to bind to the DNA.[23] The changes in the absorption intensities were monitored at regular intervals and used to calculate the intrinsic equilibrium binding constant (K_b) values by McGhee-von Hippel (MvH) method using the following equation.[24]

$$(\varepsilon_a - \varepsilon_b) / (\varepsilon_b - \varepsilon_f) = (b - (b^2 - 2 K_b^2 C_t [DNA] / s)^{1/2}) / 2 K_b C_t$$

where b is $(1 + K_bC_t + K_b[DNA]/2s)$, ε_a is the extinction coefficient observed for the charge transfer absorption band at a given concentration of DNA (at each addition), ε_f is the extinction coefficient observed for the absorption band of the complex in the absence of DNA, ε_b is the extinction coefficient of the complex in fully bound form with DNA, K_b is the intrinsic equilibrium constant, C_t is the total concentration of the metal complex in solution, [DNA] is the concentration of DNA in nucleotides and s is the binding site size in base pairs. The non-linear least square analyses were carried out using the software Origin Lab, version 6.1.[25]

Electrochemical titrations

The mode of interaction of the 3PMO (**1**) and the La(III) complexes (**2-7**) with CT-DNA were further confirmed by carrying out electrochemical experiments. Cyclic voltammetric measurements recorded using Tris-HCl (5 mM)/NaCl (50 mM) buffer (pH=7.2) as supporting electrolyte at 25 °C. The reaction solutions were degassed in N_2 atmosphere for 10 mins before carrying out the experiment. The redox behaviour of the 30 µM 3PMO and the La(III) complexes (**2-7**) were studied in the absence and presence of CT-DNA with increasing concentration of CT-DNA at the scan rate of 200 mV/s in the potential range -0.3 to 0.7 V.[26] The shift in the peak potential values indicates the mode of interaction of the ligand and complexes with CT-DNA. The formal potentials of the ligand and complexes in the free form ($E_f^{0'}$) and fully bound form ($E_b^{0'}$) were used to calculate the binding constant ratios using the following formula:

$$E_b^{0'} - E_f^{0'} = 0.0591 \log [K_R/K_O)]$$

where K_R/K_O is the ratio of binding constants of the complexes in the reduced form to the oxidized form.

Viscosity measurements

Viscometric titrations were carried out using Micro-Ubbelohde Viscometer which was thermo-stated at 37 °C in a constant temperature bath. The concentration of CT-DNA was kept constant (100 µM, NP) while concentration of complex solutions were increased at regular aliquots. The flow times were measured with automated times and each sample was measured three times for accuracy and average time flow was calculated for each complex solution. A graph of $(\eta/\eta_0)^{1/3}$ vs. [complex]/[DNA] was plotted where η is the viscosity of CT-DNA in the presence of complex solution and η_0 is the viscosity of CT-DNA

alone. Viscosity values were calculated using observed flow times of DNA containing solutions (*t*) corrected to that of buffer alone (t_0). $\eta = (t-t_0)/t_0$.[27]

DNA cleavage studies

The agarose gel Electrophoresis method was used to determine the ability of the 3PMO (**1**) and the La(III) complexes (**2-7**) to cleave super coiled (SC) pUC 19 DNA to the nick circular (NC) form. The gel electrophoresis was carried out using 16-toothed combed gel trays of 210-150 mm. A constant voltage of 60 V at 10 mA was supplied using an Electrophoresis Power Supply Bangalore-Genei (India) system during electrophoresis. The cleavage experiment was carried out using SC pUC 19 DNA (33.3 µM, 2 µg, NP) in 50 mM Tris-HCl/NaCl (pH=7.2). 1X TAE buffer was used as working buffer and the same was used to prepare 0.8% agarose gel. The concentration of the ligand (**1**) and complexes (**2-7**) were corresponding to the final volume of the reaction mixture in the gel-well. The band intensities were used to measure the extent of cleavage of SC pUC 19 DNA using UVITEC Gel Documentation System. Due corrections were made for the low level of nicked circular (NC) form present in the original super coiled (SC) DNA sample and for the low affinity of EB binding to SC compared to NC and linear forms of DNA.[28] Mechanistic studies were carried out to study the involvement of free radicals in the oxidative cleavage. Agarose gel electrophoresis was carried out in presence of radical quenchers such as DMSO, NaN_3 and Methyl green to study the oxidative cleavage mechanism by La (III) complexes (**4** and **6**) at 10 µM concentration.[29]

Antibacterial activities

The antibacterial properties of the ligand 3PMO (**1**) and La (III) complexes (**2-7**) were determined by following standard protocol of disc diffusion method.[30-32] The test organisms *Escherichia coli, Klebsiella pneumonia, Bacillus subtilis and Staphylococcus aureus* were maintained on nutrient agar slants overnight. The bacterial cultures were then centrifuged at 8000 rpm for 10 min. The bacterial cells were suspended in saline to make a suspension of 10^5 CFU/mL and used for assay. The bacterial culture was then spread over the nutrient agar plate using sterile swab and the petri plates were allowed to dry. The test samples 3PMO (**1**) and its La(III) complexes (**2-7**) were dissolved in DMSO (1 mg/mL). The discs were prepared from Whatman filter paper and had been impregnated with 3PMO (1) and La(III) complex solutions (**2-7**) respectively were placed on agar surface. Each test plate consists of 5 discs; one is standard antibiotic tetracycline (30 µg/mL) and the other four test samples (50 µg/mL and 100 µg/mL). The discs were placed equidistant to one another. The plates were then incubated at 37 °C for 24 h. After the incubation, the plates were checked for zone of inhibition. The inhibition zones were measured and compared with the standard, tetracycline. DMSO was used as negative control.

Antioxidant activities

The antioxidant activities of synthesized 3PMO (*1*) and its La (III) complexes (**2-7**) were tested by DPPH free radical scavenging method and metal chelating activity.

DPPH free radical scavenging activity

The most widely used method to check the antioxidant activity is the DPPH radical scavenging activity method. DPPH being a stable nitrogen based free radical gives violet colour in its oxidized form, changes to yellow colour on reduction either by electron transfer or hydrogen transfer. The compounds which facilitate this reaction are considered to be radical scavengers or antioxidants. DPPH has an absorbance of 517 nm in its radical form that disappears after accepting hydrogen radical or electron from the antioxidant and becomes a stable diamagnetic substance.[33] The DPPH radical scavenging ability of the synthesized ligand 3PMO (**1**) and its La (III) complexes (**2-7**) were determined by modifying the method reported by Brand-Williams *et al*.[34] 1 mg/ mL stock solutions were prepared by dissolving 3PMO (**1**) and its La(III) complexes (**2-7**) in DMF. Test solutions (0-70 µg/mL) were taken in 96-well plate and the volumes were made up to 200 µL using 5 mM Tris-HCl buffer (pH 7.4). Positive controls were prepared using BHT and Ascorbic acid respectively in a manner exactly same as test samples. 200 µL DPPH in ethanol (500 µM) was added to each well. The 96-well plate was then kept for incubation under dark for 30 min. Blank solution contained same experimental solutions except that of test samples. Absorbance was then measured at 517 nm against the blank. The decrease in absorbance measures the radical scavenging ability of the complexes. Radical scavenging ability was expressed as EC_{50}, the efficient concentration at which 50% of the DPPH radicals are being scavenged.[35]

Ferrous ion chelating assay

Ferrozine has the ability to chelate Fe^{2+} ions resulting in red colour. When other chelating agents are present, the ferroznie-Fe^{2+} complex formation is disrupted and the red colour will be diminished. The decrease in the red colour formation is a measure of chelating ability of the chelating agents present and the decreased intensity is measured at 562 nm by UV-Visible spectrophotometer. The ability of the 3PMO (**1**) and its La(III) complexes (**2-7**) to chelate Fe^{2+} ions were determined by the reported methods with slight modifications.[36-37] 1 mg/mL stock solutions were prepared by dissolving 3PMO (**1**) and its complexes (**2-7**) in DMF. 0-70 µL from the stock solutions of the tested complexes were added to a 96-well microplate and the volumes were made upto 150 µL using distilled water. To these wells, 2 mM $FeCl_2$ solution (20 µL) was added. The reaction was initiated by adding 5 mM Ferrozine (30 µL) to each well and shaken vigorously. The microplate was then incubated for 10 mins at room temperature. Once the reaction mixture attained equilibrium, absorbance of each well was measured at 562 nm against the blank. EDTA-Na_2 was used as

standard. The reaction mixture without test sample is used as blank. The ferrous ion chelating ability (% inhibition of formation of ferroznie-Fe^{2+} complex) was calculated using the formula:

Ferrous ion chelating ability (%) = $[1-A_s/A_0] \times 100$

where A_s is the absorbance in the presence of test sample and is the absorbance of blank. A graph using %I i.e., % inhibition of ferroznie-Fe^{2+} complex formation vs concentration of sample were plotted. Using the graph, EC_{50}, the efficient concentration at which 50% of the metal chelation occurred was calculated.

RESULTS AND DISCUSSION

Syntheses

A series of six La (III) complexes were newly synthesized by following literature procedure using N,N'-bis-(3-pyridylmethyl)oxamide and N,N-heterocyclic bases under given conditions (**Scheme 1-2**). All the complexes were fully characterized by ^{1}H-NMR, FT-IR, UV-Visible absorption spectroscopic techniques, ESI-Mass spectrometry, molar conductivity measurement, electrochemical measurements and elemental analyses. The complexes are soluble in methanol, ethanol, dichloromethane; DMF, DMSO and Tris-HCl buffer (pH 7.2) and are highly stable in DMF/Tris-HCl buffer solution. All the results were correlated and are in the expected range. Molar conductivity measurements revealed that complex **2** is 1:1 electrolyte whereas complexes **3-7** are 1:2 electrolytes in DMF solvent.

Table 1: Physicochemical data of 3PMO (**1**) and its La (III)-complexes (**2-7**)

Complex	1	2	3	4	5	6	7
IR# cm^{-1} $\nu_{C=O}$	1649	1654	1657	1654	1656	1660	1653
ν_{NH}	3298	3281	3270	3282	3286	3282	3281
ν_{NO3} (coordinated)		1473,1301	1479,1301	1458,1319	1465,1312	1479,1315	1458,1338
ν_{NO3}^- (ionic)	-	1384	1365	1384	1380	1373	1384
λ_{max}* /nm (ε $M^{-1}cm^{-1}$)	223.07 (14,333)	229.97 (13,300)	260.47 (13,233)	279.42 (13,066)	280.60 (12,833)	280.05 (13,133)	279.42 (12,900)
CV$ $E_{1/2}$ (V)	257.83 (8,800)	259.84 (8,000)	319.76 (6,966)	339.34 (5,166)	339.34 (4,033)	340.43 (6,433)	343.90 (3,500)
Λ_M $($\Omega^{-1}cm^2M^{-1}$)	-	0.1714	0.1363	0.1558	0.1861	0.1054	-0.0939
K_b (M^{-1})	-	91 1.20×10^4 ±0.3	153 4.25×10^4 ±0.4	155 2.04×10^5 ±0.1	154 4.19×10^5 ±0.1	153 2.04×10^5 ± 0.2	152 2.29×10^5 ±0.2
K_R/K_O	-	1.02	1.04	1.26	1.06	1.25	1.28

KBr disc
* DMF/Tris-HCl solvent
$ DMF solvent

^{1}H-NMR spectroscopy

^{1}H-NMR spectroscopic method revealed the presence of amide protons in the ligand 3PMO (**1**) and the complexes and aromatic protons in the complexes **2-7**. The ligand 3PMO (**1**) and the La(III) complexes (**2-7**) exhibit NH protons shift in the spectra against TMS as internal standard. The chemical shift in the range 4.34 - 4.48 reveals the presence of CH_2 - protons in the ligand and all the synthesized complexes. The chemical shifts in the range 7.69 – 7.80 ppm in complexes **2-7** indicate the presence of amide NH protons in the complexes.[38] This result confirms that amide NH group of the ligand is not involved in coordination with La(III) ion while forming complexes. The aromatic protons appear in the region 7.38 – 9.57 ppm in all the complexes. These results confirm the presence of CH_2-, NH- and aromatic protons in the compelexes.[22, 39-40]

FT-IR spectroscopy

The FT-IR spectra obtained in the range 4000-400 cm^{-1} reveal the involvement of atoms of groups of atoms in coordination. The obtained FT-IR spectral data of 3PMO and its La(III) complexes (**2-7**) are given in **Table 1**. The asymmetric vibration peak of amide NH appeared at 3298 cm^{-1} in 3PMO (**1**). In all the complexes (**2-7**), the amide NH of oxamide appeared as a single band with medium intensity in the range of 3281- 3286 cm^{-1}. This confirms that NH group of oxamide did not involve in coordination, the result correlated with ^{1}H-NMR result. The C=O of oxamide is basically the amide I band which appears in the region 1649 cm^{-1}.[41] The amide I band is due to N-C-O vibration, but it is viewed as C-O. In all the complexes the amide C=O band has been shifted to higher frequencies. From the literature it can be seen that, if the C=O frequencies are shifted to higher frequencies and then the coordination is through carbonyl oxygen. This confirms the involvement of carbonyl oxygen of oxamide in coordination with La(III) ions.[42] The strong bands

appeared in the region 1479-1458 cm^{-1} and 1338-1301 cm^{-1} in La(III) complexes are attributed to the presence of coordinated nitrate groups. It is well known that if the difference in wavenumbers of two highest frequencies v_4 and v_1 of C_{2v} nitrate is above 150 cm^{-1} then mode of coordination is bidentate. The bands observed near 1380 cm^{-1} in all the complexes confirm the presence of ionic nitrate. These results correlate with molar conductivity measurements and thus presence of both coordinated and ionic nitrate are confirmed.

Electronic spectroscopy

The UV-Visible absorption spectra of 10^{-3} M DMF/Tris-HCl buffer (5 mM, pH 7.2) solutions of 3PMO (**1**) and the La(III) complexes (**2-7**) exhibit the characteristic carbonyl absorption bands and intensed π-π* transitions of pyridyl and phenanthroline bases. The physicochemical data from **Table 1** shows that, the maximum absorption band appears for 3PMO (**1**) is at 223.07 nm which is due to n-π* transition of carbonyl group of oxamide and the π-π* transition of pyridyl aromatic group appeared at 257.83 nm. These absorption bands are shifted to higher wavelength and the molar absorptivity coefficient values are diminished in La(III) complexes (**2-7**). The intensed bands in the region 319-343 nm in complexes **3-7** are due to the coordination of N,N-donor heterocyclic bases.[23] The enhanced red shift in π-π* transitions in complexes are due to the presence of increased number of chromophoric groups, meanwhile the hypochromism of these transitions are due to the involvement of phenanthroline bases in coordination with La(III) ions. The time-dependent stability of the complexes **2-7** by UV-Visible absorption spectroscopy over 6 h at pH 7.2, 25 °C in DMF/Tris-HCl buffer (5 mM, pH 7.2) showed that all the complexes **2-7** were stable beyond 6 h in solution state which facilitated all the biological experiments to be carried out in this medium.

ESI-Mass spectrometry

The formation of ionic complexes was confirmed by the ESI-Mass spectra of the complexes measured in positive ion mode. Major peaks of each complex are discussed here. Complex **2** has two major m/z values at 532.92 and 555.07 a.m.u. which are corresponding to the formation of $[M-(NO_3^-)]^+$ ion and $[M+Na-(NO_3^-)]^+$ species i.e., $[La(3PMO)(NO_3)_2]^+$. This confirms that complex **2** is six coordinated complex having molecular formula $[La(3PMO)(NO_3)_2](NO_3)$. The molecular ion peak complex **3** is appeared at m/z 448.8 a.m.u. corresponds to the formation of $[M-2(NO_3^-)]^+$ ion i.e., $[La(3PMO)_2(bpy)(NO_3)]^+$. The major peak in the mass spectrum of complex **4** appeared at m/z 460.78 a.m.u is due to $[M-2(NO_3^-)]^+$ ion i.e., $[La(3PMO)_2(phen)(NO_3)]^+$. The molecular ion peak for complex **5** appeared at 522.6 which is due to $[M+2Na-2(NO_3^-)]^+$ ion. Complex 6 contains 579.0 a.m.u. which is corresponding to $[M+CH_3OH-2(NO_3^-)]^+$ confirms the formation of $[La(3PMO)_2(dpq)(NO_3)](NO_3)_2$ complex. Finally, mass spectrum of complex **7** consists of molecular ion peak at 627.1 a.m.u. due to the formation of the complex ion $[M+CH_3OH+Na-2(NO_3^-)]^+$. It is accepted that ESI-MS+ mode needs extra positively charged ions for interaction such as H^+, Na^+, K^+ or any species that depends on experimental conditions.[43] These results reveal that complex **2** has coordination number 6 whereas complexes **3-7** have coordination number 8, the results are correlating with molar conductivity measurements.

Electrochemical studies

The electrochemical responses of 10^{-3} M solutions of **1** and the La(III) complexes (**2-7**) in DMF solvent were scanned in the potential range of -0.3 to 0.7 V in the scan range 25 mV to 200 mV. The formal potentials of the complexes were calculated at the scan rate 200 mV/s as the recognizable change in potential and current was observed at that scan rate. No measurable changes were observed for the ligand **1**. The formal potential values $E_{1/2}$ of all the complexes (**2-7**) are given in **Table 1**. The lower potentials are due to greater basicity of the amide-N and carbonyl-O atoms.[8] These changes in potential values also suggest that these complexes are redox active and this property can be employed in carrying out chemical nuclease activity.[23]

DNA binding studies

Determination of the ability of the complexes to bind to DNA is the prerequisite to evaluate their biological properties. In this view, mode of binding of La(III) complexes to CT-DNA is determined using UV-Visible absorption, electrochemical methods and viscosity methods. Using UV-Visible absorption method, binding constants of the complexes were determined. Higher the binding constant value, greater is the ability to bind to DNA.

Electronic absorption studies were carried out to study the DNA binding propensities of the complexes **2-7** to the calf-thymus (CT) DNA in 5 mM Tris-HCl/50 mM NaCl buffer (pH 7.2). The changes in the absorption intensities of the spectral bands of the complexes as a function of increased concentration of CT-DNA were determined (**Figure 1**). The intrinsic binding constants (K_b) of the complexes to the CT-DNA were determined by monitoring changes in the absorption spectral band with increasing CT-DNA concentration, keeping complex concentration constant (30 µM). The intrinsic binding constant values are given in **Table 1**. The intrinsic binding constant values of the complexes are in the order **7** > **6**=**4** > **5** > **3** > **2**. The order revealed that complexes **3-7** have phenanthroline bases with extended planar rings which facilitate non covalent interactions of complexes into the DNA.[44] The absorption spectral changes can be observed in **Figure 1**. The significant hypochromism and very small bathochromic shift (~4 nm) are the characteristic spectral changes in intercalative mode. These changes are due to the strong interaction between the chromophore of the complexes and the base pairs of the DNA. This may be due to the coupling of π*-orbital of the intercalated

complex with that of π-orbital of the base pairs of DNA which in turn results in decreasing the π-π* transition energy, in turn bathochromic shift. Also, the π*-orbital is partially filled by electrons due to coupling and thereby transition probabilities will be decreased and in turn results in hypochromism.[45]

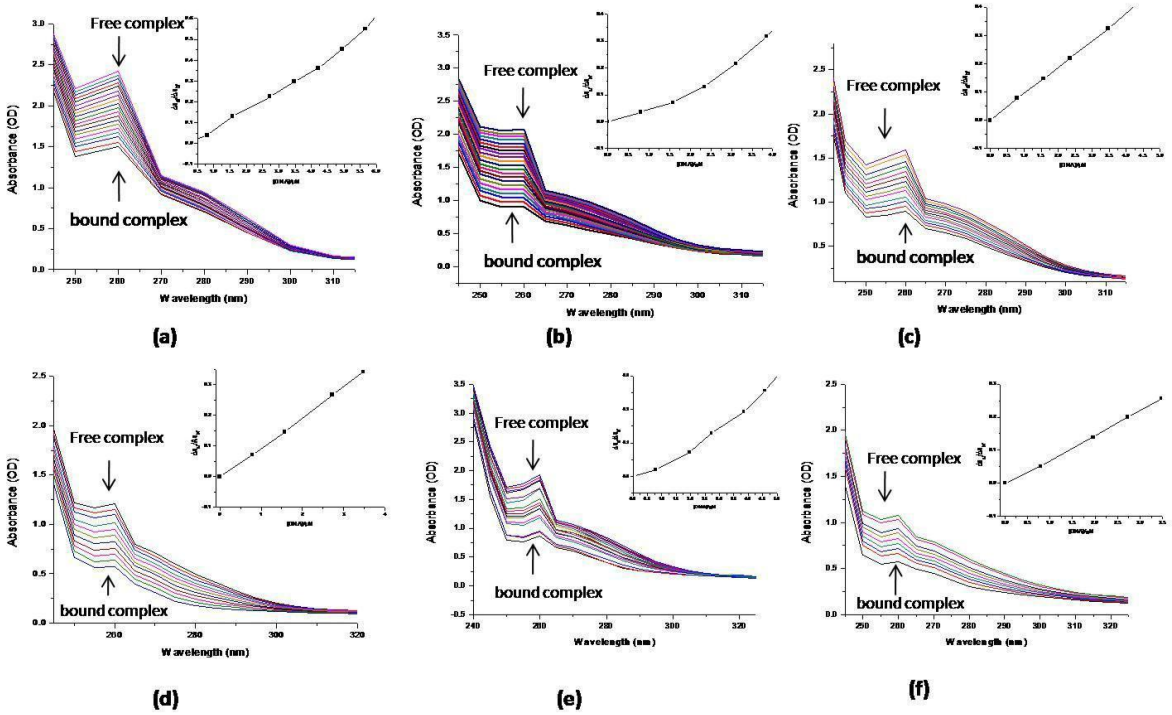


Figure 1: UV-Visible absorption titration spectra of La(III) complexes (**2-7**) (**a-f**) in the absence (free complex) and presence (bound complex) of CT-DNA in 5 mM Tris-HCl/50 mM NaCl buffer (pH 7.2) at 25°C. The inset plots show [DNA]/μM vs. $\Delta\varepsilon_{af}/\Delta\varepsilon_{bf}$, that is used to calculate intrinsic binding constant K_b (M^{-1}) for each complex.

Electrochemical titrations were carried out to study the mode of interaction of La(III) complexes with CT-DNA at the scan rate of 200 mV/s by keeping the complex concentration constant (30 μM) while increasing the concentration of CT-DNA. The incremental addition of CT-DNA to the ligand and complexes causes the anodic peak potential to shift towards more positive values as shown in **Figure 2**. These results indicate that the La (III) complexes stabilize the CT-DNA through intercalation. It is well known that if both potential shifts to either positive values or to negative values, then the mode of binding is intercalation. At the scan rate of 200 mV/s, in the absence of CT-DNA, the formal potentials ($E_f^{0\prime}$) of the complexes 2-7 are, 0.0932V, 0.0366 V, 0.1318 V, 0.066 V, 0.1082 V and 0.1030 V respectively. On incremental addition of CT-DNA to the complexes, the formal potential values shift to more positive values i.e., $E_b^{0\prime}$ of the complexes **2-7** were found to be 0.0942 V, 0.0386 V, 0.1444 V, 0.0691 V, 0.1158 V and 0.1157 V respectively. Using these formal potential values in the free state and fully bound state of the complexes, binding constant ratio K_R/K_O were calculated and were found to be 1.02, 1.04, 1.26, 1.06, 1.25 and 1.28 for complexes **2-7** respectively.[46] The order follows: **7** > **6** > **4** > **5** > **3** > **2**, correlates with the intrinsic binding constant values obtained by UV-Visible absorption spectroscopy. The ratio K_R/K_O demonstrates that La(III) complexes bound to CT-DNA more strongly in the reduced state than in oxidized state. It was also observed that the voltammetric current have been decreased when the complexes are bound to CT-DNA. This indicates that, when complexes are bound to DNA through intercalation, the molecule becomes bigger in size and it is difficult for it to diffuse into the electrolyte. This confirms the intercalation mode of binding of La (III) complexes into CT-DNA.

The binding mode of the 3PMO (**1**) La (III) complexes (**2-7**) with CT-DNA was further clarified by viscosity method. It is known to be the least ambiguous methods to study the mode of interactions of metal complexes when crystallographic data are unavailable. In principle, a classical intercalator lengthens the DNA by intercalating between the base pairs and results in increase of DNA viscosity. While in partial or non-classical mode of intercalation, complex bends or kinks DNA resulting in decreasing the length of DNA and in turn decrease in its viscosity.[47] The effects of La (III) complexes (**2-7**) on viscosity of CT-DNA are shown in Figure 3. From the figure it is observed that, the viscosities of the DNA increases with increase in the concentration of La (III) complexes. This result confirms the intercalation mode of binding of La (III) complexes with CT-DNA.

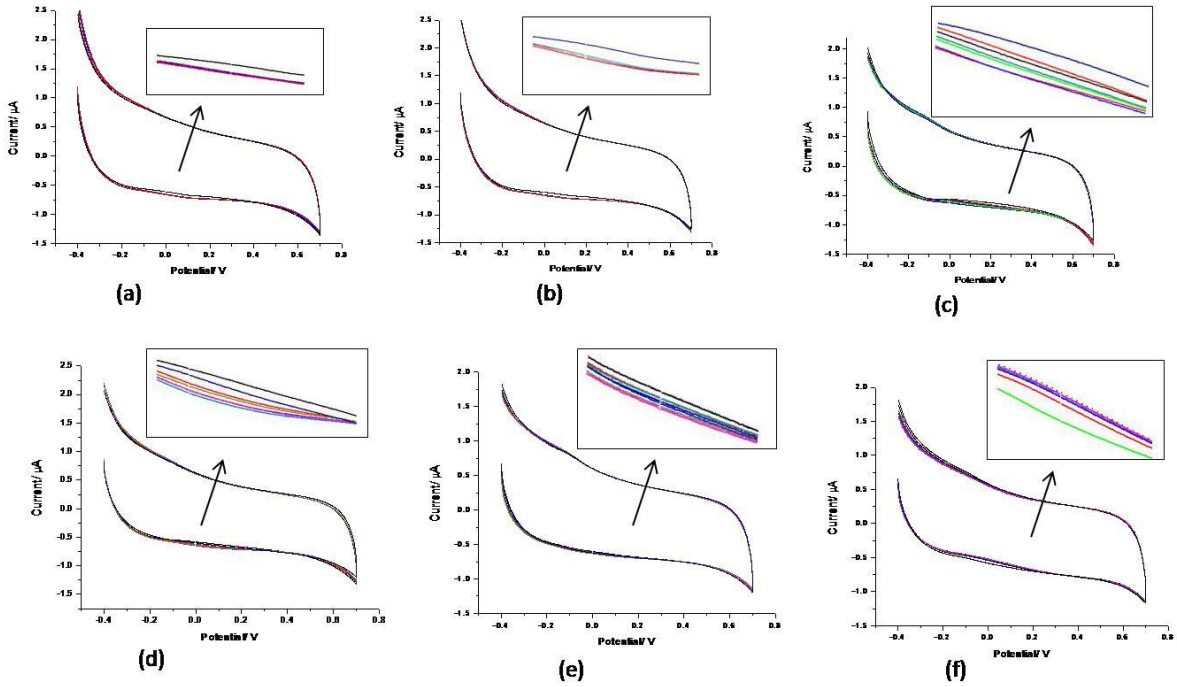


Figure 2: The cyclic voltammograms (**a-f**) showing changes in redox potentials and voltammetric currents of 30 µM La(III) complexes (**2-7**) in the absence and presence of CT-DNA in 5 mM Tris-HCl/ 50 mM NaCl buffer (pH 7.2) which acts as supporting electrolyte at 25^{O}C. The insets show the decrease in voltammetric current once CT-DNA is added in aliquots to the complex solutions.

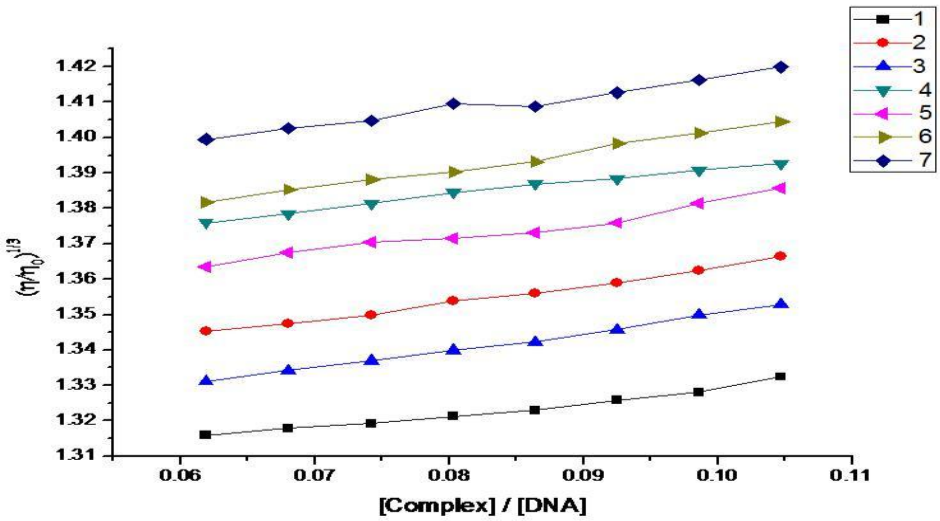


Figure 3: A plot of [complex]/[DNA] vs. $(\eta/\eta_0)^{1/3}$ showing effect of 30 µM 3PMO (**1**) and La(III) complexes (**2-7**) on relative viscosity of CT-DNA (200 µM) in 5 mM Tris-HCl buffer (pH = 7.2) at 37 °C.

DNA cleavage studies

The synthesized La(III) complexes (**2-7**) in comparison with 3PMO (**1**) were screened for their ability to cleave SC pUC19 DNA in hydrolytic and oxidative conditions by agarose gel electrophoresis method using plasmid super coiled (SC) pUC19 DNA (33.3 µM, 0.2 µg) in 50 mM Tris-HCl buffer/50 mM NaCl (pH7.2). Oxidative cleavage method was carried out using oxidizing agent H_2O_2 and reducing agent MPA. From the pilot experiment it has been shown that, in the presence of H_2O_2, at 10 µM, complexes **4** and **6** were shown to cleave SC pUC 19 DNA into nick circular (NC) form to the extent of 98% and 97% respectively while complex **7** has shown moderate cleavage of pUC 19 DNA, to the extent of 50% (**Figure 4**).

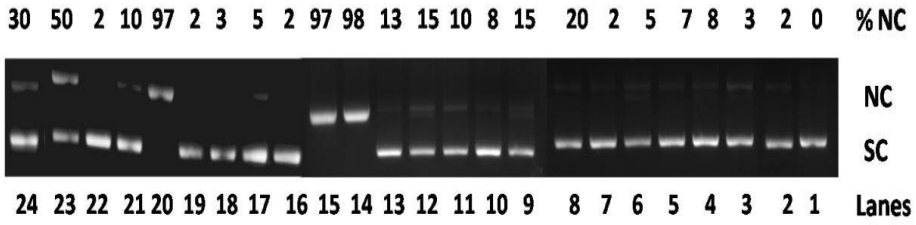


Figure 4: Agarose gel electrophoresis diagram showing the cleavage of SC pUC19 DNA by 10 µM 3PMO (**1**) and its La (III) complexes (**2-7**) in 50 mM Tris-HCl/NaCl buffer (pH 7.2) in the absence and presence of H_2O_2 and MPA.

Table 2: SC pUC 19 DNA cleavage data of 3PMO (**1**) and its La (III) complexes (**2-7**).

Lane no.	Conditions	%SC	%NC
1	DNA + NaCl + Tris-HCl	100	0
2	DNA + NaCl + Tris-HCl +H_2O_2	98	2
3	DNA + NaCl + Tris-HCl + MPA	97	3
4	DNA + NaCl + Tris-HCl + **1**	92	8
5	DNA + NaCl + Tris-HCl + **1** + H_2O_2	93	7
6	DNA + NaCl + Tris-HCl + **1** + MPA	95	5
7	DNA + NaCl + Tris-HCl + **2**	98	2
8	DNA + NaCl + Tris-HCl + **2** + H_2O_2	80	20
9	DNA + NaCl + Tris-HCl + **2** + MPA	85	15
10	DNA + NaCl + Tris-HCl + **3**	92	8
11	DNA + NaCl + Tris-HCl + **3** + H_2O_2	90	10
12	DNA + NaCl + Tris-HCl + **3** + MPA	85	15
13	DNA + NaCl + Tris-HCl + **4**	87	13
14	DNA + NaCl + Tris-HCl + **4** + H_2O_2	2	98
15	DNA + NaCl + Tris-HCl + **4** + MPA	3	97
16	DNA + NaCl + Tris-HCl + **5**	98	2
17	DNA + NaCl + Tris-HCl + **5** + H_2O_2	95	5
18	DNA + NaCl + Tris-HCl + **5** + MPA	97	3
19	DNA + NaCl + Tris-HCl + **6**	98	2
20	DNA + NaCl + Tris-HCl + **6** + H_2O_2	3	97
21	DNA + NaCl + Tris-HCl + **6** + MPA	90	10
22	DNA + NaCl + Tris-HCl + **7**	98	2
23	DNA + NaCl + Tris-HCl + **7** + H_2O_2	50	50
24	DNA + NaCl + Tris-HCl + **7** + MPA	70	30

[NaCl] = 50 mM; [Tris-HCl] = 50 mM; [H_2O_2] = 200 µM; [MPA] = 500 µM; [Complex] = 10 µM.

The mechanistic pathway of oxidative cleavage was studied using the radical quenchers such as DMSO, NaN_3 and methyl green (MG) in presence of H_2O_2. From the experiment it was observed that, the cleavage ability of complexes was inhibited in presence of hydroxyl radical quencher DMSO. Thus, it was confirmed that oxidative cleavage was mediated by the generation of hydroxyl radicals. It was also confirmed that, major groove binder methyl green inhibited the cleavage ability of complex **4**. This shows that complex **4,** having 1, 10-phenanthroline is a major groove intercalator (**Figure 5** and **Table 3**).

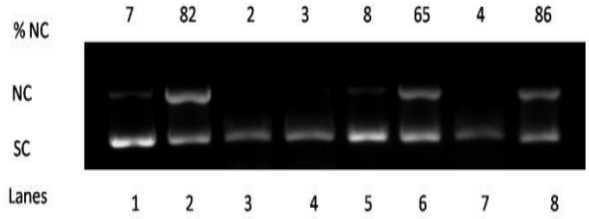


Figure 5: Agarose gel electrophoretic diagram showing cleavage of SC pUC19 DNA in presence of H_2O_2, MPA, 50mM Tris-HCl/NaCl buffer (pH 7.2) and additives (DMSO, NaN_3 and Methyl green (MG)) by complexes **4** and **6**.

Antibacterial activities

The antibacterial activities determined by disc diffusion method for 3PMO (**1**) and its La(III) complexes (**2-7**) revealed that La(III) complexes exhibited better antibacterial activities than their ligand 3PMO against the tested bacteria. Of all the tested complexes, at 50 µg/mL, complex **5** was found to be highly active against all the tested bacteria whereas complex **4** was active against *E. coli*, *K. pneumonia* and *S. aureus*. At 50 µg/mL, complex **6** was active only against Gram-positive bacteria (*B. subtilis* and *S. aureus*). At 100 µg/mL, all the complexes showed zone of inhibition. Among all the complexes tested, complex **5** shows the highest activity against all the tested bacteria. The zone of inhibition exhibited by the test samples were given in the following **table 4**.

Table 3: SC pUC 19 DNA cleavage data of mechanistic studies of complexes **4** and **6** at 10 µM.

Lane no.	Conditions	%SC	%NC
1	DNA + NaCl + Tris-HCl +H_2O_2 + **4** + DMSO	93	7
2	DNA + NaCl + Tris-HCl +H_2O_2 + **4** + NaN_3	18	82
3	DNA + NaCl + Tris-HCl +MPA + **4** + DMSO	98	2
4	DNA + NaCl + Tris-HCl +MPA + **4** + NaN_3	97	3
5	DNA + NaCl + Tris-HCl + H_2O_2 + **6** + DMSO	92	8
6	DNA + NaCl + Tris-HCl + H_2O_2 + **6** + NaN_3	35	65
7	DNA + NaCl + Tris-HCl +H_2O_2 + **4** + MG	96	4
8	DNA + NaCl + Tris-HCl + H_2O_2 + **6** + MG	14	86

[H_2O_2]= 200 µM, [MPA] = 500 µM, DMSO= 4 µL, [NaN_3]= 200 µM, Methyl green (MG) = 100 µM, [Complex]= 10 µM.

Table 4: The zone of inhibition (mm) values measured for the antibacterial activities of 3PMO and its La (III) complexes compared with Tetracycline.

Test samples	Gram negative				Gram positive			
	E. coli		*K. pneumoniae*		*B. subtilis*		*S. aureus*	
	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL
Tetracycline (30 µg/mL)	20		19		17		24	
3PMO (**1**)	-	-	-	6	-	-	-	5
[La(3PMO)$(NO_3)_2$](NO_3) (**2**)	-	5	-	10	-	13	-	6
[La(3PMO)$_2$(bpy)(NO_3)]$(NO_3)_2$ (**3**)	-	9	-	7	-	9	-	7
[La(3PMO)$_2$(phen)(NO_3)]$(NO_3)_2$ (**4**)	10	17	7	10	-	5	8	15
[La(3PMO)$_2$(dione)(NO_3)]$(NO_3)_2$ (**5**)	15	19	20	27	23	26	20	27
[La(3PMO)$_2$(dpq)(NO_3)]$(NO_3)_2$ (**6**)	-	7	-	12	17	20	9	16
[La(3PMO)$_2$(dppz)(NO_3)]$(NO_3)_2$ (**7**)	-	6	-	10	-	7	-	6
DMSO (negative control)	-	-	-	-	-	-	-	-

The order of antibacterial activities is compared at 100 µg/mL as all the complexes are active in this concentration. For *E. coli*: **5** > **4** > **3** > **6** > **7** > **2**, *K. pneumonia*: **5** > **6** > **7**, **4, 2** > **3** > **1**, *B. subtilis*: **5** > **6** > **2** > **3** > **7** > **4** and *S. aureus*: **5** > **6** > **4** > **3** > **7**, **2** > **1**.

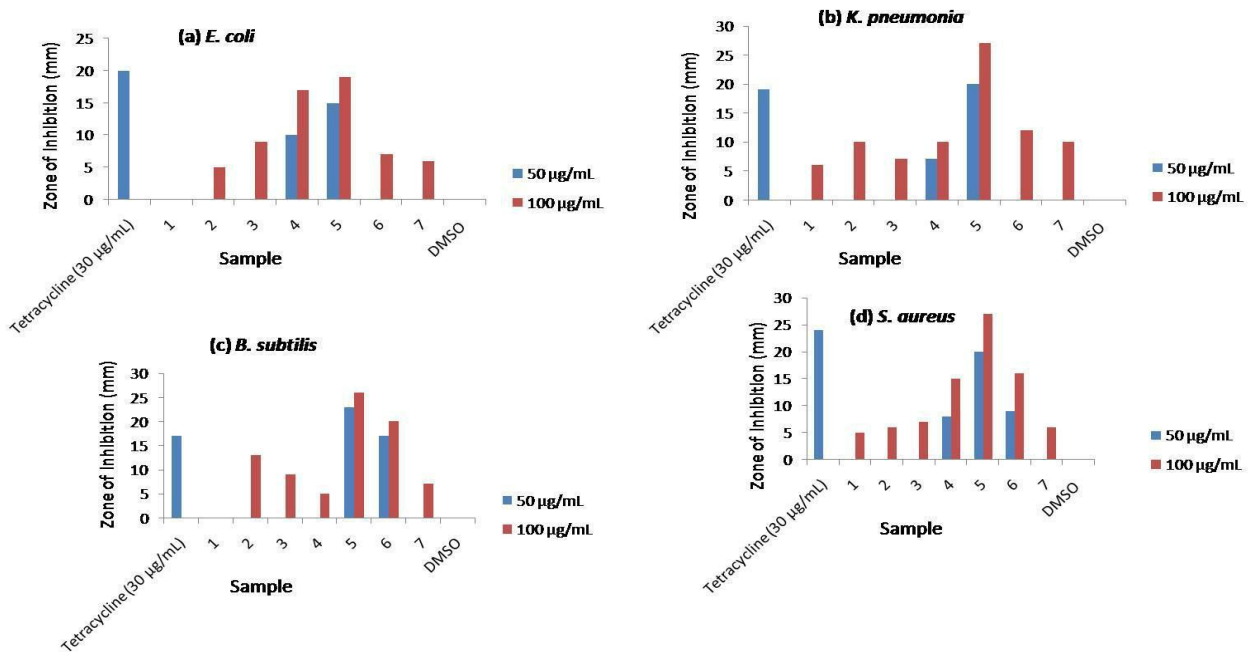


Figure 6: The bar diagram showing antibacterial activities of 3PMO (**1**) and its La (III) complexes (**2-7**) against a) *E. coli*, b) *K. pneumoniae*, c) *B. subtilis* and d) *S. aureus* at concentrations 50 µg/mL and 100 µg/mL. Tetracycline (30 µg/mL) was used as positive control and DMSO was used as negative control.

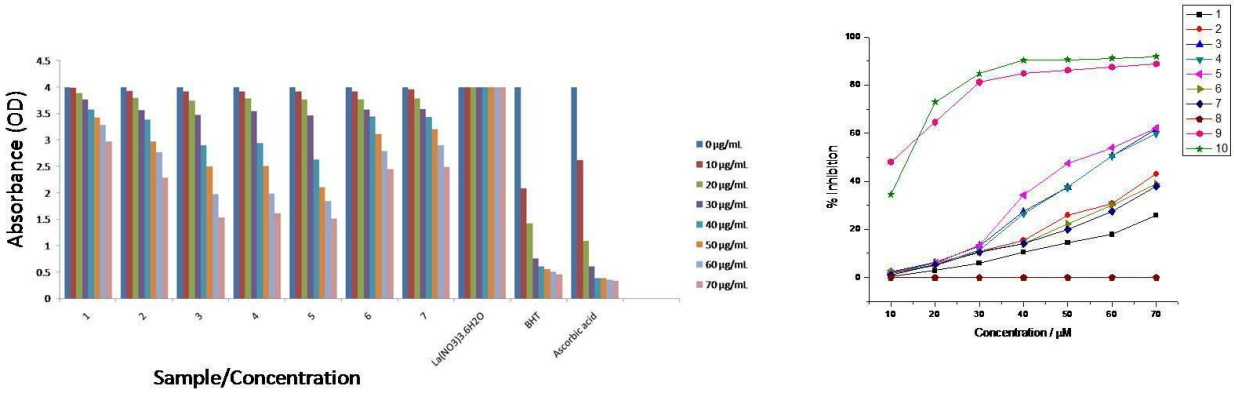
The antibacterial activities of the La (III) complexes are enhanced when compared to ligand is because of increase in the lipophilicity of the complexes. The permeability barrier of the bacterial cell walls would be broken down and their normal physiology would be disturbed since their enzyme metabolism is affected and in turn the bacterial growth. Since aromatic planar ligand systems are used, they induce chelating effect to the metal ion and thereby its polarity will be reduced and hence π-electrons delocalize throughout the chelate ring. Thus, La (III)-complexes with the mixed ligand system show increased antibacterial activities and thus represent a new class of antibacterial drugs with high pharmaceutical values.[20]

Antioxidant activities

DPPH radical scavenging method

It is well known that, many physiological redox processes in our body release free radicals like hydroxyl, superoxide radical etc, which are responsible for oxidative damage of various biomolecules such as nucleic acids, proteins and lipids. These free radicals may also induce carcinogenesis,

aging and various other life threatening diseases. The enzymes in the body to counteract with these radicals are limited.[33, 48] Thus, in a view of developing new antioxidants, we tested our synthesized La(III) complexes and the ligand 3PMO for the DPPH radical scavenging activity, which is the most widely used method to determine the antioxidant activity. All the tested complexes were compared with one natural antioxidant (ascorbic acid) and one synthetic antioxidant (butylated hydroxy toluene, BHT). Of all the tested La(III) complexes in concentration range 0-70 µg/mL, complex **5** has shown good antioxidant activity with EC_{50}=56.35 µg/mL followed by complexes **3** and **4** with EC_{50}=60 µg/mL (**Figure 7**). Other complexes did not show recognizable DPPH radical scavenging activities. The radical scavenging ability of the complexes were better than the ligand 3PMO, but less active than the standards used (EC_{50}=12 µg/mL for BHT and EC_{50}=11 µg/mL for ascorbic acid). The antioxidant activities of these complexes may be due to their electron releasing property of the -CH_2 groups or hydrogen-donating –NH groups present in the complexes, but mechanism is not yet clear. The presence of phenanthroline bases may have synergistic effect with the La(III) ion as metal-ligand charge transfer, in inducing radical scavenging ability.[48] Thus, the La(III) complexes with 3PMO and phenanthroline bases can be good antioxidant activities.



(a) (b)

Figure 7: (a): Bar diagram showing DPPH radical scavenging activity of 3PMO (**1**) and its La (III) complexes (**2-7**) in presence of 5 mM Tris-HCl buffer (pH 7.2). The activities are compared with La $(NO_3)_3.6H_2O$ and positive controls BHT and Ascorbic acid. (b) A plot of concentration vs. % inhibition of DPPH radical by 3PMO (**1**), its La (III) complexes (**2-7**), La$(NO_3)_3.6H_2O$ (8) and positive controls BHT (9) and Ascorbic acid (10).

Ferrous ion chelating assay

The ability of 3PMO and its La (III) complexes to chelate Fe^{2+} ions were determined. According to the results, La (III) complexes have shown better activity than ligand but not as good as standard EDTA-Na_2. The EC_{50} values were calculated and were found to be 19.42 µg/mL, 39.68 µg/mL and 43.01 µg/mL for complexes **3**, **4** and **5** respectively and EC_{50} values could not be calculated for other complexes as 50% of chelation by other complexes were not achieved even at higher concentration. From the literature it can be revealed that compounds containing functional groups such as -C=O, -NR_2, -COOH, -OH , -SH have the ability to chelate ferrous ions.[37] In physiological conditions, lipid peroxidation could be stimulated and enhanced by ferrous ions by the decomposition of lipid hydroperoxides. Hence chelating excess ferrous ions would be the beneficial aspect in developing antioxidants. In this view, our synthesized La(III) complexes can be used as good antioxidants.

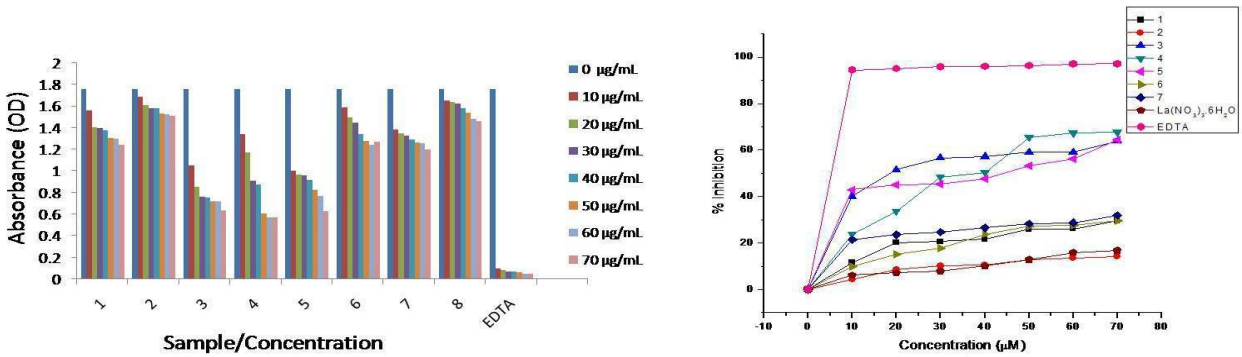


Figure 8: (a) Bar diagram showing ferrous ion chelating ability of 3PMO (**1**) and its La (III) complexes (**2-7**). The activities are compared with La $(NO_3)_3.6H_2O$ (8) and positive control EDTA. (b) A plot of concentration vs. % inhibition of formation of Fe^{2+} - Ferrozine complex by 3PMO (**1**), its La (III) complexes (**2-7**), La $(NO_3)_3.6H_2O$ and EDTA

CONCLUSION

Six new lanthanum (III) complexes using N,N'-bis(3-pyridylmethyl)oxamide (**1**) and N,N, heterocyclic bases were synthesized and well characterized by physical and spectroscopic techniques. The DNA binding experiments confirmed that complexes bind to CT-DNA through intercalation mode with complex **7** having the highest binding constant value K_b= 2.29 x 10^6 M^{-1}. The agarose gel electrophoresis method confirmed that complex **4** and complex **6** have the highest cleaving ability of SC pUC19 DNA to the maximum extent of 97% in the presence of H_2O_2 at 10 µM concentration with of the involvement of hydroxyl radicals in the cleavage. The antibacterial studies revealed that complex **5** has shown the highest antibacterial activity against all the four tested bacterial strains at 50 µg/mL, whereas at 100 µg/mL, all the complexes showed antibacterial activity. The determination of antioxidant activities of the complexes revealed that complexes **3**, **4** and **5** showed better antioxidant activities. Complex 5 has shown the highest DPPH radical scavenging ability with EC_{50} = 56.35 µg/mL whereas complex **3** has shown the highest ability to bind to Fe^{2+} ions with EC_{50} = 19.42 µg/mL. In conclusion, N,N'-bis(3-pyridylmethyl)oxamide along with the phenanthroline bases have synergistic effect in inducing charge transfer to the metal ion and thereby enhance the biological activities. Thus, the structure-activity relationship demonstrated that these newly synthesized La(III) complexes (**2-7**) are proven to be good therapeutic agents in pharmaceutical field.

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