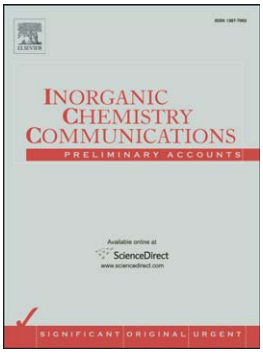


Appraisal of DNA obligatory, DNA cleavage and in vitro anti-biogram efficiency of 9,10-phenanthrenequinone based metal complexes

Natarajan Raman, Thesingu Rajan Arun, Rajkumar Mahalakshmi, Seemon Packianathan, Rajendran Antony

PII: S1387-7003(14)00266-4
DOI: doi: 10.1016/j.inoche.2014.06.011
Reference: INOCHE 5627

To appear in: *Inorganic Chemistry Communications*

Received date: 6 May 2014
Revised date: 7 June 2014
Accepted date: 13 June 2014

Please cite this article as: Natarajan Raman, Thesingu Rajan Arun, Rajkumar Mahalakshmi, Seemon Packianathan, Rajendran Antony, Appraisal of DNA obligatory, DNA cleavage and in vitro anti-biogram efficiency of 9,10-phenanthrenequinone based metal complexes, *Inorganic Chemistry Communications* (2014), doi: 10.1016/j.inoche.2014.06.011

Appraisal of DNA obligatory, DNA cleavage and *in vitro* anti-biogram efficiency of 9,10-phenanthrenequinone based metal complexes

Natarajan Raman* Thesingu Rajan Arun[a], Rajkumar Mahalakshmi[a], Seemon Packianathan[a], Rajendran Antony[b]

[a]*Research Department of Chemistry, VHNSN College, Virudhunagar-626 001, Tamilnadu, India*
[b]*Centre for Scientific and Applied Research, PSN College of Engineering and Technology, Tirunelveli-627152, Tamilnadu, India*

Email: ramchem1964@gmail.com; Tel.: +91-9245165958

List of Abbreviations Used

DNA= Deoxyribonucleic acid

CT DNA= Calf thymus deoxyribonucleic acid

pBR322 DNA = plasmid Bolivar and Rodriguez 322 deoxyribonucleic acid

Tris-HCl = Tris(hydroxymethyl)aminomethane hydrochloride

L= Ligand

M = Metal

OC = Open circular

SC= Supercoiled

DMF= Dimethylformamide

DMSO= Dimethylsulfoxide

IIT = Indian Institute of Technology

CDRI= Central Drug Research Institute

Appraisal of DNA obligatory, DNA cleavage and *in vitro* anti-biogram efficiency of 9,10-phenanthrenequinone based metal complexes

Natarajan Raman* Thesingu Rajan Arun[a], Rajkumar Mahalakshmi[a], Seemon Packianathan[a], Rajendran Antony[b]

[a]*Research Department of Chemistry, VHNSN College, Virudhunagar-626 001, Tamilnadu, India.*
[b]*Centre for Scientific and Applied Research, PSN College of Engineering and Technology (Autonomous), Tirunelveli-627152, Tamilnadu, India.*

Email: ramchem1964@gmail.com; Tel.: +91-9245165958

ABSTRACT

This work presents the synthesis and characterization of four new metal(II) complexes of 9,10-phenanthrenequinone based Schiff base precursor. From UV-Vis, FT-IR, ^{1}H NMR, EPR and Raman spectral data, an octahedral geometry has been assigned to the complexes. The molar conductivity and magnetic moment measurements indicate the non-electrolytic and monomeric nature of the complexes. DNA binding ability of these complexes has been explored using diverse techniques *viz* UV-Vis. absorption, cyclic voltammetry, fluorescence spectroscopy and viscometry. Comparative DNA oxidative cleavage ability of the complexes has been accomplished under UV photo radiation on pBR322 DNA. These studies reveal that they are good metallointercalators and chemical nucleases. In addition, the biocidal action of the complexes is investigated against few pathogenic bacteria and fungi by well diffusion method. The screening data reveal that the complexes exhibit more efficient antimicrobial activity than the free ligand. These findings may lead to develop novel metal based drugs.

Keywords: Schiff base• Metal complexes• Metallointercalators• DNA cleavage• Methionine•.

**Corresponding author: Tel.: +91-9245165958; fax: +91-4562281338*

Email : ramchem1964@gmail.com (N.Raman)

Medicinal inorganic chemistry is one of the rapidly intensifying curiosities in the intend of small novel molecules that can interact with DNA since the interaction of small molecules with DNA helps us to recognize how biomolecules function in biological systems [1]. The interaction of small molecules with DNA is also interested due to its involvement in the development of artificial endonucleases and anticancer drug therapies. DNA offers several potential binding sites for transition metals including the anionic phosphate backbone, electron-rich nitrogenous bases, and the major or minor grooves [2]. The bio-interested transition metal complexes can interact with such DNA binding sites either by covalent or non-covalent binding (intercalation, groove binding, electrostatic forces and hydrogen bonding) [3]. These kinds of interactions displayed by metal complexes toward DNA are currently fascinated owing to the role of metal complexes as DNA structural probes, DNA foot printers, sequence-specific cleavage agents and potential anticancer drugs [4–5]. Intercalative mode has been reported as the predictable mode when metal complexes are under operation with DNA [6].

In the field of inorganic chemistry, contribution of Schiff base metal complexes has been extensive and inevitable because of their diverse functions as catalysts [7], fluorescent indicators, and oxygen carriers [8]. Besides, the imine (-C=N) group of Schiff base ligands escorts to the astounding biological activities including antitumor, antibacterial, antifungal and herbicidal activities [9]. The transition metal complexes of amino acids are also important owing to their functions as models for a number of imperative biological systems [10]. Furthermore, metal complexes of amino acids or their derivatives based ligands are key intermediates in a variety of metabolic reactions such as decarboxylation, transamination, recemization and C-C bond cleavage, which are catalyzed by enzymes [11]. The over specifics put forward that structure of such complexes of amino acid based ligands would be the prolific attitude both in structural and biological aspects.

In this context, we are impelled to synthesize a new Schiff base derived from 9,10-phenanthrenequinone and *p*-nitroaniline and its Cu(II), Co(II), Ni(II) and Zn(II) complexes using methionine as coligand. Methionine amino acid is well known to make coordination bonds with metal ion through COOH and/or NH_2 groups which have potential donor sites [12]. The synthesized complexes have been characterized by physicochemical and various spectroscopic techniques. Besides, their communal biological properties have been analyzed by antimicrobial, DNA cleavage (using gel electrophoresis) and DNA binding (by UV-Vis., viscometry, fluorescence and cyclic voltammetry) studies. The communal biological properties obtained

from our study would be helpful in perceptive of DNA interaction exposed by metal complexes and it also would be assisted in advancing potential probes of DNA structure and conformation and forecasting new therapeutic reagents for some uncommon diseases.

The Schiff base ligand and its mixed ligand metal complexes were synthesized by following the previously reported typical procedure [13-19]. Prior to demonstrate, the synthetic procedures of all compounds and discussion of their characterization are provided as supplementary data (S1 and S2). The ligand and complexes were found to be air stable at room temperature. The ligand was soluble in common organic solvents but the complexes were soluble in DMF and DMSO only. The schematic representation of the synthesis of ligand and the complexes is illustrated in Scheme 1. The expected geometry and stoichiometry of complexes ($[ML(met)_2]$) have been affirmed by various spectral techniques such as FT-IR, UV-Vis., ^{1}H NMR, Raman and EPR. A few physicochemical characterizations including elemental analysis, molar conductance and magnetic susceptibility were also employed in the prediction of geometry and stoichiometry.

(Kindly insert Scheme1 here)

Electronic absorption spectroscopy is a valuable technique to examine the binding modes of metal complexes with DNA [20]. The absorption titration of the Cu(II) complex in the absence and presence of CT DNA is illustrated in Fig.1. Upon increasing amounts of DNA, the absorption bands of the complex are disturbed. In specific, hypochromism and red shift (bathochromism) are observed in all the complexes after mounting DNA concentration. While increasing DNA concentration, the observed hypochromisms are 11.4%, 7.3%, 4.5% and 8.1% for Cu(II) (319 nm), Co(II) (327 nm), Ni(II) (338 nm) and Zn(II) (326 nm), respectively. These bands are attributed to intra-ligand π–π^* transition available in the corresponding complexes. The intensities of hypochromism and bathochromism were suggested to occur due to the interaction between the electronic state of aromatic chromophores of azomethine ligand and those of the nitrogenous DNA bases [21, 22].

(Kindly insert Fig.1 here)

The intrinsic DNA binding constants (K_b) of complexes have been measured using Eq. (1) to collect the details regarding binding affinity of complexes with CT-DNA. The calculated values are given in Table 1. The determined intrinsic binding constants for $[CuL(met)_2]$, $[CoL(met)_2]$, $[NiL(met)_2]$ and $[ZnL(met)_2]$ are 7.9×10^5 M^{-1}, 3.4×10^5 M^{-1}, 4.6×10^5 M^{-1} and 5.2×10^5 M^{-1},

respectively. These outcomes reveal that the complexes may bind with DNA through an intercalation mode into the double helix structure of DNA.

(Kindly insert Table.1 here)

To further investigate the interaction mode of the complex with CT DNA, the emission spectrum was studied. Fluorescence titrations were conducted by preset amount of metal complexes which were titrated with escalating amount of CT DNA concentration. The emission spectrum of Cu(II) complex with CT DNA is illustrated in Fig.2. The addition of CT DNA into the complex causes the obvious raise in fluorescence intensity which clearly delegates that binding of metal complexes with DNA might be intercalation [23]. Perhaps, the fortification of fluorescence intensity is due to changes in metal center of complexes. Subsequently it implies that the complex has strong interaction between the base pairs of the CT DNA which is supposed to be one of the criteria for intercalative binding [24]. From Fig.2, it is observed that there is an enrichment of the fluorescence intensity of metal complex in the presence of CT DNA. During the Cu(II) complex formation, the florescence intensity is increased and there is an extension of the π system of the intercalated ligand due to the coordination of the Cu(II) ion. Since the metal complexes have greater planar area than that of the ligand, the metal complexes penetrate more deeply into, and stacking more strongly with the base pairs of the DNA [25].

(Kindly insert Fig.2 here)

The viscosity of DNA solution is sensitive to its length changes and its viscosity measurement upon addition of complexes may give important support to clarify the interaction mode of complexes with DNA [26]. Viscosity measurements were carried out on CT DNA solutions by increasing the amount of the complexes. In classical intercalation, an insertion of the complexes in between the DNA base pairs most probably leads to an increase in the separation of base pairs (intercalation site) to accommodate the bound complexes which again results in the augmentation of DNA viscosity. Conversely, a partial non-classical intercalation causes a bend (or kink) in DNA helix and reduces its effective length and thereby its viscosity. Eventually, the increase of DNA viscosity noted upon addition of complexes can confirm the existence of an intercalative binding mode between DNA and each complex [27]. The understandings and outcomes of this viscosity study are depicted in Fig.3. It apparently shows that the viscosity of DNA is found to be increased when it interacts with metal complexes. The upshot of this study

clearly proposes the intercalative mode of binding between the complexes with CT DNA as evidenced by UV-Vis and fluorescence spectroscopic results.

(Kindly insert Fig.3 here)

Cyclic voltammetry is the widely handled technique to study the binding modes of metal complexes with DNA which greatly withstands the results obtained from the methods *viz* UV Vis spectroscopy, fluorescence titrations and viscosity measurements. In recent years, there is an upward concern on the electrochemical investigation of interaction between metal complexes and DNA [28]. The cyclic voltammograms of $[CuL(met)_2]$ in buffer (pH = 7.2) at 25 ^{0}C in the presence of increasing amount of DNA are illustrated in Fig.4.

(Kindly insert Fig.4 here)

In the absence of DNA, cyclic voltammogram of Cu(II) complex reveals four characteristic peaks which are allotted to two anodic peaks E_{pa} (-0.365 and -0.031 V) and two cathodic peaks E_{pc} (-0.556 and -0.259 V) at a scan rate of 0.1 V s^{-1}. The first reduction and oxidation potentials are noticed at E_{pc} = -0.556 V and E_{pa} = -0.365 V whereas the second reduction and oxidation potentials are identified at E_{pc} = -0.259 V and E_{pa} = -0.031 V. In the same environment, Zn(II) complex shows the redox couple with E_{pc} =−0.804 V and E_{pa} = -0.593 V; Co(II) complex shows the redox couple with E_{pc} = -1.037 V and E_{pa} = -0.044 V; and Ni(II) complex shows redox couple with E_{pc} = -0.374 V and E_{pa} = -0.244 V. The cyclic voltammograms of all studied complexes show approximately unity peak current ratio which is credited that the reaction of the complex on the glassy carbon is quasi-reversible redox process. The incremental addition of CT DNA to the complex reduces the anodic and cathodic peak current of values of the complexes. This consequence shows that complex stabilizes the duplex (GC pairs) by intercalating way. The addition of CT DNA to the complex causes shift in the potential of peak in cyclic voltammogram. Both cathodic and anodic peaks show positive or negative shift which indicates the intercalation of complex with base pairs of DNA [29]. The electrochemical data for all complexes in DMSO and the obtained shifts in cathodic (E_{pc} V) and anodic (E_{pa} V) potentials as a result of DNA inclusion are shown in Table 2. It is concluded that cyclic voltammetry studies here effectively suggest the intercalative mode of binding between metal complexes and binding sites of CT DNA.

(Kindly insert Table 2. here)

From differential pulse voltammogram of the complex ([CuL(met)$_2$]), it is apparent that current intensity gets decreased upon the incremental addition of DNA. The shift in potentials both in presence and absence of DNA is related to the ratio of binding constant by the following equation:

$$E_b - E_f = 0.0591 \log (K_{[red]}/K_{[oxd]})$$

where E_b and E_f are peak potentials of complex in presence (bound) and absence (free form) of DNA, respectively. The result of the present study is that all metal(II) complexes show one electron transfer during the redox process. The ip_c/ip_a value of such redox process is less than unity which peculiarly supports the quasi-reversible nature of redox process which occurs on the surface of glassy carbon working electrode. The synthesized complexes give both the anodic and cathodic peak potential shifts which are either positive or negative (Table 2). These shifts specify the intercalating mode of DNA binding with metal(II) complexes.

(Kindly insert Fig.5 here)

As the metal(II) complexes showed maximum binding affinity with DNA, thus the DNA cleavage activity was evaluated by agarose gel electrophoresis using supercoiled (SC) plasmid pBR322 DNA in a medium of 5 mM Tris HCl/50 mM NaCl buffer solution (pH 7.2). The reaction mixture was subjected to agarose gel electrophoresis with incubated for 45 min. The agarose gel electrophoresis experiments were carried out in the presence of activating agent (H_2O_2) under aerobic conditions and are illustrated in Fig. 6. When supercoiled (SC) plasmid pBR322 DNA is conducted by electrophoresis, the fastest migration will be observed for the super coiled form-I (SC). If one strand is cleaved, the SC form will relax to produce a slow-moving open circular form-II (OC). If both strands are cleaved, a nicked form-III (NC) will be generated in the presence of oxidizing agent H_2O_2 [30]. As seen from Fig.6, there is no cleavage observed in the case of control DNA (lane 1). However, all the metal complexes expose good DNA cleaving properties. In detail, Cu(II) and Ni(II) complexes have shown complete DNA cleavage as confirmations identified from diminishing of lanes′ intensity. On the other hand, other two complexes (Zn(II) and Co(II)) have shown only partial DNA cleavage. These observations announced that a combination of metal(II) complexes and activating agent (H_2O_2) are very essential to show effectual cleavage of plasmid DNA. The DNA cleavage activity of metal complexes may be allocated to the liberation of metal ion to the DNA helix in the locality of stimulated oxygen or hydroxide free radicals [31].

(Kindly insert Fig.6 here)

The antimicrobial bustle of the ligand and its metal complexes was examined against certain sensitive organisms such as two Gram-positive bacteria (*N. asteroides* and *P. putida*), two Gram- negative bacteria (*E. coli* and *L. lactis*) and few fungi (*A. niger, A. flavus* and *C. albicans*). The minimal inhibitory concentrations of tested complexes against chosen sensitive bacteria and fungi are shown in Tables 3 and 4, respectively. From Tables 3 and 4, it is observed that the ligand functioned feebly against both bacteria and fungi. But after metallation, the same ligand was found to have higher activity in the form of complexes. The presence of imine group may be one of the reasons for the biocidal action of both the ligand and metal complexes [32]. In addition, it has been already proved that the electron-withdrawing substituent such as nitro group on the benzene ring exhibited a comparable growth-inhibitory activity against micro-organisms. The presence of nitro group in our complexes may also be the reasonable for the better antimicrobial activity of metal complexes. The acquired consequences designate that the complexes have higher activity than the ligand against the same microorganisms under indistinguishable experimental conditions. This can be explained by Overtone's concept and Tweedy's chelation theory [33]. Chelation considerably reduces the polarity of the metal ion because of partial sharing of its positive charge with donor groups and possible electron delocalization over the whole chelate ring. This fact could enhance the lipophilic character of the central metal atom, which subsequently favors its permeation through the lipid layer of the cell membrane. The variation in the effectiveness of different compounds against different organisms depend either on the impermeability of the cells of microbes or on differences in ribosome of microbial cells. From the data, it is observed that the Gram-positive bacteria are more sensitive to metal complexes than Gram-negative bacteria. It is due to the presence of cell wall in Gram-negative bacteria which is impermeable lipid based bacterial outer membrane.

(Kindly insert Tables 3 and 4 here)

In the present cram, we have attempted to synthesize and characterize few novel mixed ligand Schiff base metal complexes of Cu(II), Co(II), Ni(II) and Zn(II) in the motive to reach new bioinorganic agents with better biological properties. The complexes have been also employed to bind CT DNA and such binding ability has been explored using diverse techniques such as UV-Vis., viscosity, fluorescence and cyclic voltammetry. It is concluded that all these studies proved the CT DNA binding of the complexes through intercalation mode. The antimicrobial screening data reveal that complexes exhibit higher activity than the free ligand.

The communal biological action of the complexes may be helpful for the designing of metal based drugs.

Acknowledgments

The authors express their heartfelt thanks to the College Managing Board, Principal, and Head of the Department of Chemistry, VHNSN College for providing necessary research facilities to complete this work successfully. The characterization facilities afforded by IIT Bombay and CDRI, Lucknow are gratefully acknowledged by all authors.

References

[1] P.P. Netalkar, S.P. Netalkar, S. Budagumpi, V.K. Revankar, Synthesis, crystal structures and characterization of late first row transition metal complexes derived from benzothiazole core: Anti-tuberculosis activity and special emphasis on DNA binding and cleavage property, Eur.J. Med. Chem. 79 (2014) 47-56.

[2] L.J. Boerner, J.M. Zaleski, Metal complex-DNA interactions: from transcription inhibition to photoactivated cleavage, Curr. Opin. Chem. Biol. 9 (2005) 135-144.

[3] M. Shebl, Synthesis, spectroscopic characterization and antimicrobial activity of binuclear metal complexes of a new asymmetrical Schiff base ligand: DNA binding affinity of copper(II) complexes, Spectrochim. Acta 117A (2014) 127–137.

[4] C. Metcalfe, J. Thomas, Kinetically inert transition metal complexes that reversibly bind to DNA, Chem. Soc. Rev. 32 (2003) 215–733.

[5] W. Huilu, K. Fan, J. Fei, L. Bin, Y. Jingkun,, Y. Ying, A V-shaped ligand 1,3-bis(1-methylbenzimidazol-2-yl)-2-oxapropane and its Cu(II) complex: Synthesis, crystal structure, antioxidation and DNA-binding properties, J. Photochem. Photobiol. B, Biol. 105 (2011) 190-197.

[6] T.R. Arun, N. Raman, Antimicrobial efficacy of phenanthrenequinone based Schiff base complexes incorporating methionine amino acid: Structural elucidation and in vitro bio assay, Spectrochim. Acta 127A (2014) 292–302.

[7] R. Antony, S.T. David, K. Saravanan, K. Karuppasamy, Synthesis, spectrochemical characterisation and catalytic activity of transition metal complexes derived from Schiff base modified chitosan, Spectrochim. Acta 103A (2013) 423-430.

[8] D. Lahiri, R. Majumdar, D. Mallick, T.K. Goswami, R.R. Dighe, A.R. Chakravarty, Remarkable photocytotoxicity in hypoxic HeLa cells by a dipyridophenazine copper(II) Schiff base thiolate, J. Inorg. Biochem. 105 (2011) 1086–1094.

[9] N. Raman, A. Selvan, S. Sudharsan, Metallation of ethylenediamine based Schiff base with biologically active Cu(II), N i(II) and Zn(II) ions: Synthesis, spectroscopic characterization, electrochemical behaviour, DNA binding, photonuclease activity and in vitro antimicrobial efficacy, Spectrochim. Acta 79A (2011) 873-883.

[10] J. Zuo, C. Bi, Y. Fan, D. Buac, C. Nardon, K.G. Daniel, Q. Ping Dou, Cellular and computational studies of proteasome inhibition and apoptosis induction in human cancer cells by amino acid Schiff base–copper complexes, J. Inorg. Biochem. 118 (2013) 83–93.

[11] J. Zuo, C. Bi, Y. Fan, D. Buac, C. Nardon, K.G. Daniel, Q. Ping Dou, Cellular and computational studies of proteasome inhibition and apoptosis induction in human cancer cells by amino acid Schiff base–copper complexes, J. Inorg. Biochem. 118 (2013) 83–93.

[12] C.M. Metcalfe, S. Spey, H. Adams, J.A.J. Thomas, Extended terpyridyl and triazine complexes of d6-metal centres, Chem. Soc. Dalton Trans. 24 (2002) 4732-4739.

[13] The Schiff base ligand was synthesized by ethanolic solution of 9,10-phenanthrenequinine (0.01 M) was added drop wise to an ethanolic solution of *p*-nitroaniline (0.01 M). Few drops of glacial acetic acid were added to the reaction mixture and were refluxed for 4 h. The solid product formed was filtered, washed, dried and recrystallized from ethanol, dried *in vacuo.*

[14] [L] Yield: 74%; yellow colour; Anal.Calc. (%): C (69.6), H (3.6) and N (12.5); Found (%): C (69.3) H (3.3) and N (12.2); FT-IR (KBr) (cm^{-1}): 1631(C=N), 2900-2950 (C-H) and 1400-1600 (C=C); ^{1}H NMR (DMSO-d_6) δppm: 6.9-7.4 (aromatic protons); UV-Vis. In DMSO, cm^{-1} (transition): 38,314 (π-π*) and 34,742 (n-π*).

[15] For the synthesis of mixed ligand complexes, initially an equimolar ethanolic mixture of L (0.01 M) and the metal chloride salt (0.01 M) was heated at 60 °C under magnetic stirring for 4 h. Then, methionine (0.02 M) in 1:1 water-ethanol was added to the above reaction mixture and allowed to reflux for 6 h. Finally the resultant product was washed with ethanol and then recrystallized. The obtained solid product was filtered, dried *in vacuo* at 60 °C and kept in desiccator.

[16] [$CuL(met)_2$]**:** Yield: 72%; brown colour; Anal.Calc.(%): C (53.7), H (4.7), N (10.7), S (8.2) and Cu (8.1); Found (%): C (53.4), H (4.4), N (10.3), S (7.9) and Cu (7.9); FT-IR (KBr) (cm^{-1}): 3372 (NH_2), 1622 (C=N), 1447 ($\upsilon_{asy}(COO^-)$), 1365 ($\upsilon_{sy}(COO^-)$), 536 (M-O) and 424 (M-N); Λ_m ($\Omega^{-1}mol^{-1}cm^2$) 16.21; μ_{eff} (BM) 1.81; UV-Vis. in DMSO, cm^{-1} (transition): 31,746 (LMCT) and 12,236 (d-d).

[17] [$CoL(met)_2$]: Yield: 68%; green colour; Anal.Calc (%): C (53.9), H (4.9), N (10.7), S (8.1) and Co (7.6); Found (%): C (53.7), H (4.5), N (10.4), S (7.9) and Co (8.2); FT-IR (KBr) (cm^1): 3360 (NH_2), 1623 (C=N), 1440 (υ_{asy} (COO^-)), 1358 ($\upsilon_{sy}(COO^-)$), 532 (M-O) and 424 (M-N); Λ_m ($\Omega^{-1}mol^{-1}cm^2$) 22.30; μ_{eff} (BM) 2.57; UV-Vis. in DMSO, cm^{-1} (transition): 33,493 (LMCT) and 21,420 (d-d).

[18] [NiL(met)$_2$]: Yield: 71%; yellow colour; Anal.Calc (%): C (53.9), H (4.9), N (10.7), S (8.1), Ni (7.5); Found (%): C (53.8), H (4.5), N (10.4), S (7.9) and Ni (7.3); FT-IR (KBr) (cm^{-1}) 3358 (NH_2), 1625 (C=N), 1443 (υ_{asy} (COO)), 1358 (υ_{sy}(COO)), 527 (M-O) and 432 (MN); $\wedge_m$ (Ω^{-1}mol^{-1}cm^2) 19.65; μ_{eff} (BM) 3.18; UV-Vis. in DMSO, cm^{-1} (transition): 29,585 (LMCT) and 17,456 (d-d).

[19] [ZnL(met)$_2$]: Yield: 78%; greenish yellow colour; Anal.Calc (%) C (53.7), H (4.7); N (10.7), S (8.2) and Zn (8.3); Found (%) : C (53.3), H (4.3), N (10.3), S (7.9) and Zn (8.1); FT-IR (KBr) (cm^{-1}): 3361 (NH_2), 1621 (C=N), 1448 (υ_{asy}(COO$^-$)), 1304 (υ_{sy}(COO$^-$)), 534 (M-O) and 438 (M-N); ^{1}H NMR (DMSO-d_6) δppm: 6.5-7.8 (phenyl multiplet),3.4 (s, (−NH_2)) 3.9–4.2 (C−H) (mn- H) δ; $\wedge_m$ (Ω $^{-1}$mol^{-1}cm^2) 18.70; μ_{eff} (BM) diamagnetic; UV-Vis. in DMSO, cm^{-1} (transition): 30,674 (LMCT).

[20] V.G. Vaidyanathan, B. U. Nair, Synthesis, characterization and DNA binding studies of a chromium(III) complex containing a tridentate ligand, Eur. J. Inorg. Chem. 19 (2003) 3633-3638.

[21] M. Navarro, E.J. Cisneros-Fajardo, A. Sierralta, M. Fernández-Mestre, P. Silva, D.Arrieche, E. Marchán, DNA-based Asymmetric Organometallic Catalysis in Water, J. Biol. Inorg. Chem. 8 (2003) 401-408.

[22] H. M. Berman, P. R. Young, The interaction of intercalating drugs with nucleic acids, Annu. Rev. Biophys. Bioeng. 10 (1981) 87–114.

[23] S. Tabassum, M. Zaki, F. Arjmand, I. Ahmad, Synthesis of heterobimetallic complexes: In vitro DNA binding, cleavage and antimicrobial studies, J. Photochem. Photobiol. B, Biol. 114 (2012) 108–118.

[24] Z.C. Liu, B.D. Wang, B.Li, Q. Wang, Z.Y.Yang, T.R. Li , Y. Li, Crystal structures, DNA-binding and cytotoxic activities studies of Cu(II) complexes with 2-oxo-quinoline-3 carbaldehyde Schiff-bases, Eur.J. Med. Chem. 45 (2010) 5353-5361.

[25] D.S.Raja, N.S.P.Bhuvanesh, K.Natarajan, Synthesis, crystal structure and pharmacological evaluation of two new Cu(II) complexes of 2-oxo-1,2-dihydroquinoline-3-carbaldehyde (benzoyl) hydrazone: A comparative investigation, Eur.J. Med. Chem. 47 (2012) 73-85.

[26] C.Tolia, N. Athanassios, B.Papadopoulos,P. Catherine, C. Raptopoulou, V. Psycharis, C. Garino, L. Salassa, G.Psomas, Copper(II) interacting with the non-steroidal antiinflammatory drug flufenamic acid: Structure, antioxidant activity and binding to DNA and albumins J. Inorg. Biochem. 123 (2013) 53–65.

[27] S. Satyanarayana, J.C. Dabrowiak, J.B. Chaires, Tris(phenanthroline)- ruthenium(II) enantiomer interactions with DNA: mode and specificity of binding, Biochemistry 32 (1993) 2573–2584.

[28] A.M.O. Breet, T.R.A. Macedo, D. Raimundo, M.H. Marques, S.H.P. Serrano, voltametric behavior of mitoxantrone at a DNA-biosensor. Biosensors and Bioelectron.13 (1998) 861-867.

[29] N. Raman, A. Sakthivel, M. Selvaganapathy, L. Mitu, Effect of DNA interaction involving antioxidative 4-aminoantipyrine incorporating mixed ligand complexes having alpha-amino acid as co-ligand, J.Mole.Struc. 1060 (2014) 63-74.

[30] S. Tabassum, M. Afzal, F. Arjmand, New modulated design, docking and synthesis of carbohydrate-conjugate heterobimetallic CuIIeSnIV complex as potential topoisomerase II inhibitor: In vitro DNA binding, cleavage and cytotoxicity against human cancer cell lines, Eur. J. Med. Chem.74 (2014) 694-702.

[31] P. Krishnamoorthy, P. Sathyadevi, R.R. Butorac, A.H. Cowley, N.S.P. Bhuvanesh, N. Dharmaraj, Copper(I) and nickel(II) complexes with 1:1 vs. 1:2 coordination of ferrocenyl hydrazone ligands: Do the geometry and composition of complexes affect DNA binding/cleavage, protein binding, antioxidant and cytotoxic activities, Dalton. Trans. 41 (2012) 4423-4436.

[32] W.I. Rehman, M.K. Baloch, A. Badshah, Synthesis, spectral characterization and bio-analysis of some organotin(IV) complexes, Eur. J. Med. Chem.11 (2008) 2380-2385.

[33] Y. Anjaneyulu, R.P. Rao, Preparation, characterization and antimicrobial activity studies on some ternary complexes of Cu(II) with acetylacetone and various salicylic acids, Synth. React. Inorg. -Org. Chem. 16 (1986) 257-272.

Figure captions

Scheme 1. Schematic route for the synthesis of Schiff base ligand and its metal complexes.

Fig 1. Absorption spectrum of $[CuL(met)_2]$ in buffer pH = 7.2 at 25 ^{0} C in the presence of increasing amount of DNA. Arrow indicates the changes in absorbance upon increasing the DNA concentration.

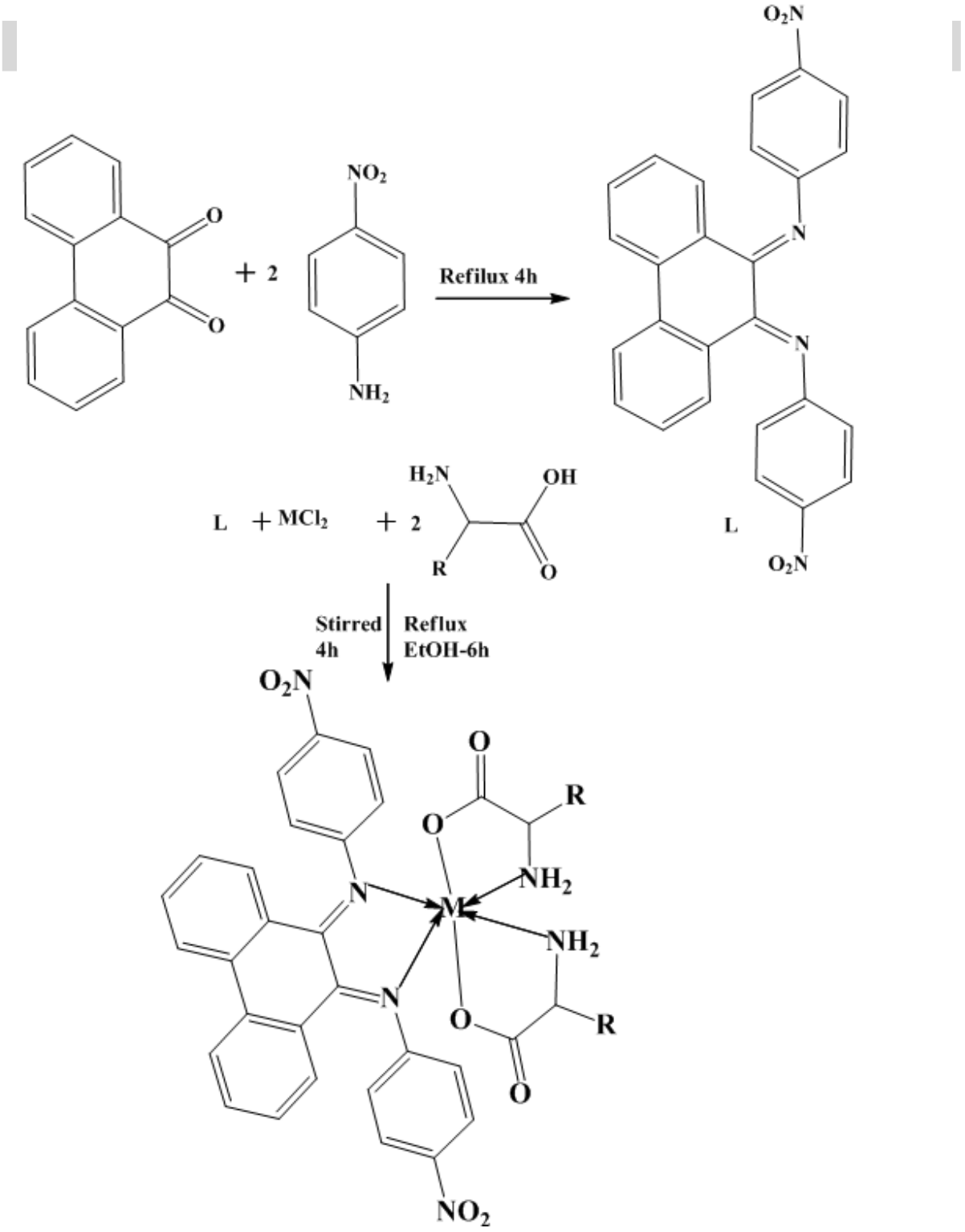
Fig 2. Emission enhancement spectrum of $[CuL(met)_2]$ in the absence and presence of increasing amounts of CT-DNA. Arrow indicates the emission intensity changes of upon the increasing CT-DNA concentration.

Fig 3. Effect of increasing amounts of $[CuL(met)_2]$ (▲), $[CoL(met)_2$ (×), $[NiL(met)_2]$(♦), $[ZnL(met)_2]$(■), on the relative viscosity of CT DNA *vs* [complex]/[DNA].

Fig 4. Cyclic voltammogram of $[CuL(met)_2]$ in buffer (pH = 7.2) at 25 ^{0}C in the presence of increasing amount of DNA. The changes in voltammetric current upon increasing the DNA concentration.

Fig 5. Differential pulse voltammograms of $[CuL(met)_2]$ in buffer (pH = 7.2) at 25 ^{0}C in the presence of increasing amount of DNA. Arrow indicates the changes in voltammetric currents upon increasing the DNA concentration.

Fig 6. The gel electrophoretic separation of plasmid pBR322 DNA treated with $[ML(met)_2]$ complexes. Lane 1; DNA control ; Lane 2: DNA + ligand + H_2O_2; Lane 3: DNA + $[CuL(met)_2]$ + H_2O_2; Lane 4: DNA + $[CoL(met)_2]$ + H_2O_2; Lane 5: DNA + $[NiL(met)_2]$ + H_2O_2; Lane 6: DNA + $[ZnL(met)_2]$ + H_2O_2.



where **M= Cu(II),Co(II),Ni(II) and Zn(II); R= -CH_2-S-CH_3**

Scheme 1. Schematic route for the synthesis of Schiff base ligand and its metal complexes.

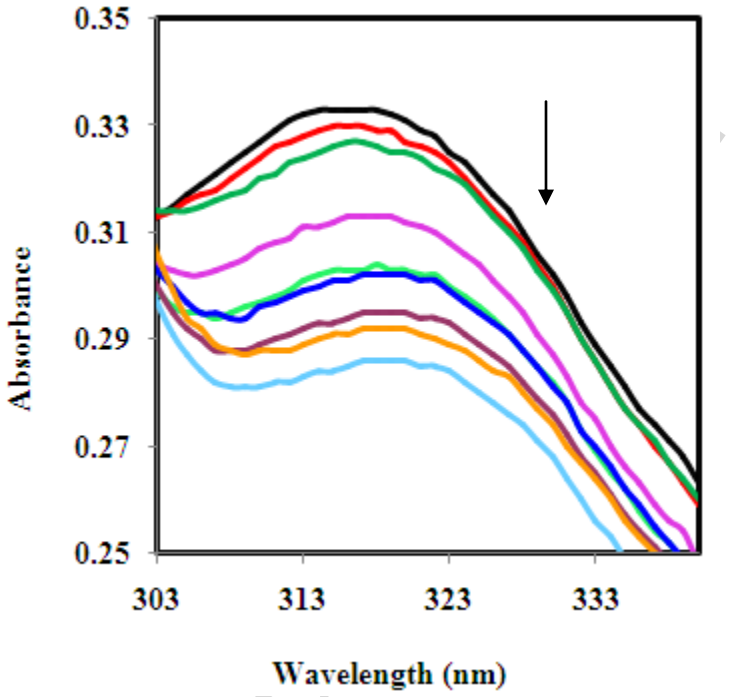


Fig 1. Absorption spectrum of $[CuL(met)_2]$ in buffer pH = 7.2 at 25 ^{0} C in the presence of increasing amount of DNA. Arrow indicates the changes in absorbance upon increasing the DNA concentration.

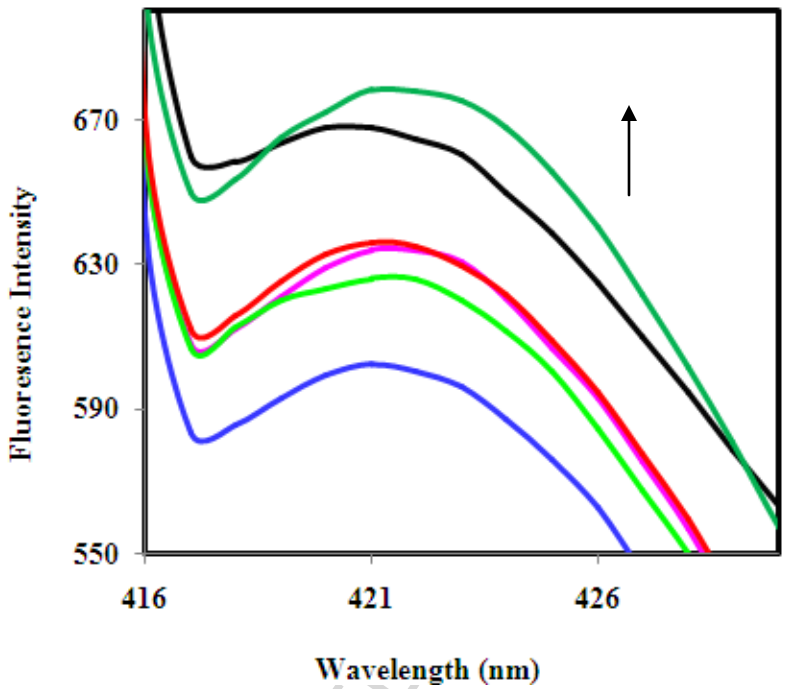


Fig 2. Emission enhancement spectrum of $[CuL(met)_2]$ in the absence and presence of increasing amounts of CT-DNA. Arrow indicates the emission intensity changes of upon the increasing CT-DNA concentration.

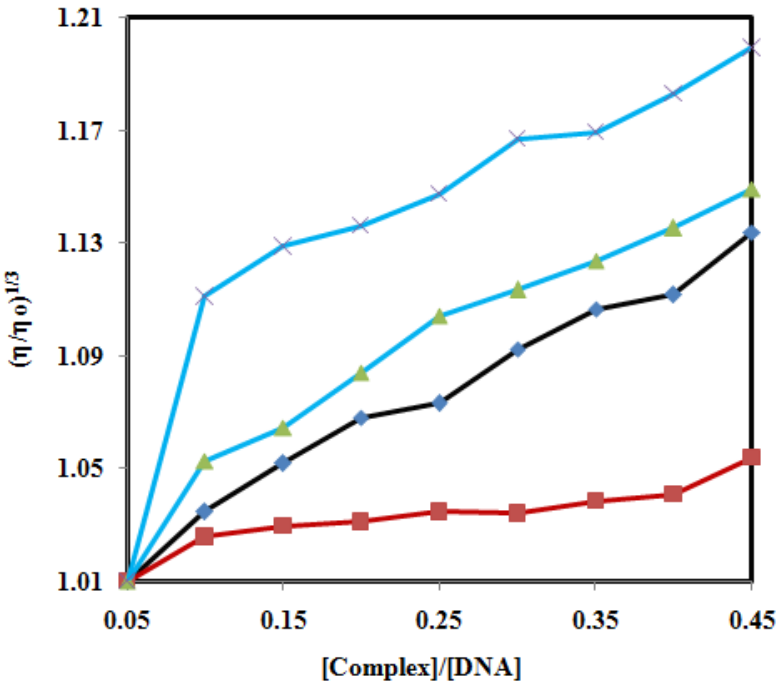


Fig 3. Effect of increasing amounts of [CuL(met)$_2$] (▲), [CoL(met)$_2$ (×)], [NiL(met)$_2$](♦), [ZnL(met)$_2$](■) on the relative viscosity of CT DNA *vs* [complex]/[DNA].

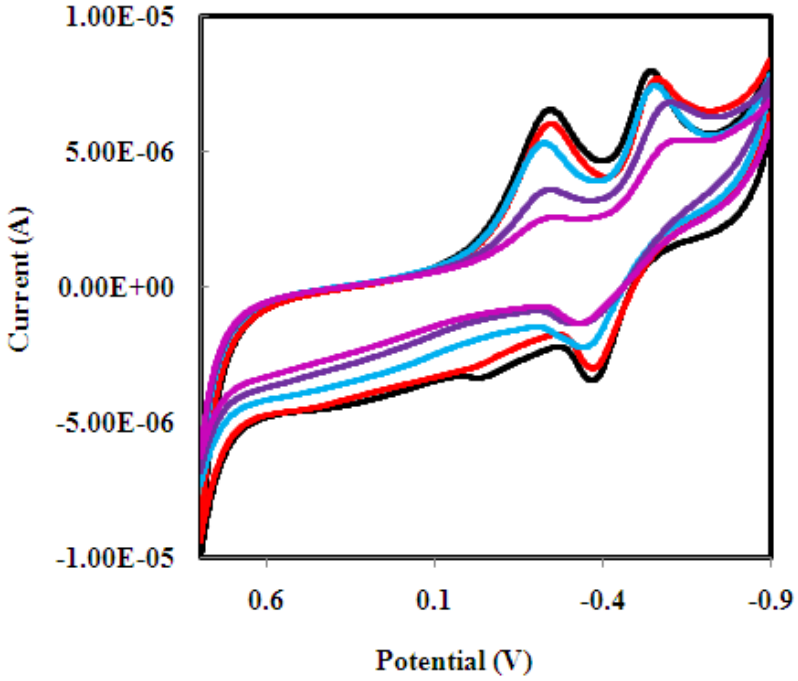


Fig 4. Cyclic voltammogram of $[CuL(met)_2]$ in buffer (pH = 7.2) at 25 ^{0}C in the presence of increasing amount of DNA. The changes in voltammetric current upon increasing the DNA concentration.

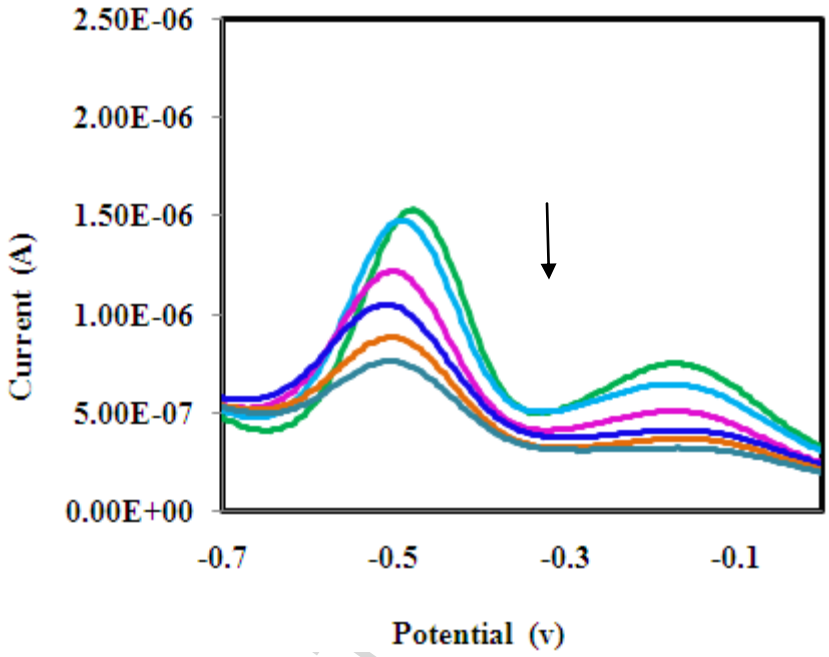


Fig 5. Differential pulse voltammograms of [CuL(met)2] in buffer (pH = 7.2) at 25 0C in the presence of increasing amount of DNA. Arrow indicates the changes in voltammetric currents upon increasing the DNA concentration.

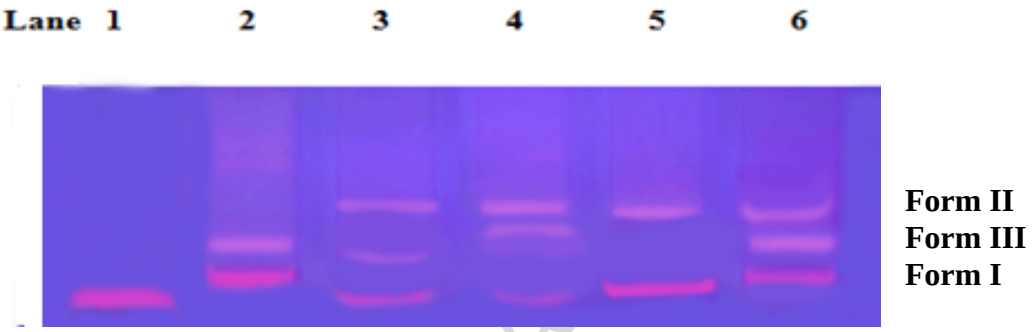
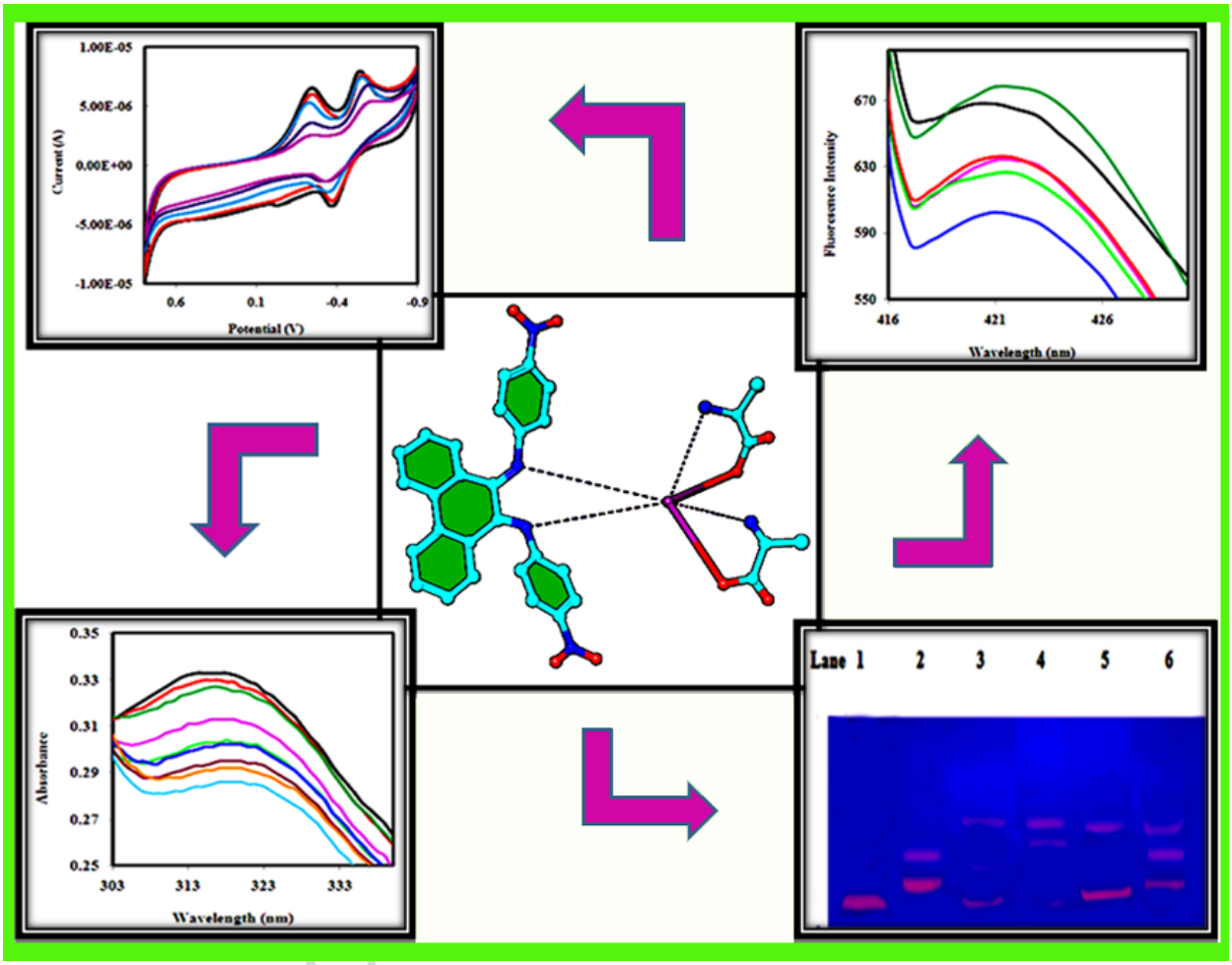


Fig 6. The gel electrophoretic separation of plasmid pUC19 DNA treated with $[M(L)(met)_2]$ complexes. Lane 1; DNA control ; Lane 2: DNA + ligand + H_2O_2; Lane 3: DNA + $[CuL(met)_2]$ + H_2O_2; Lane 4: DNA + $[CoL(met)_2]$ + H_2O_2; Lane 5: DNA + $[NiL(met)_2]$ + H_2O_2; Lane 6: DNA + $[ZnL(met)_2]$ + H_2O_2.

Graphical abstract



Synopsis

The synthesized 9,10-phenanthrenequinone based complexes have good *in vitro* DNA binding and cleavage efficacy. They are efficient antimicrobial and metallointercalators. These findings may lead to develop novel DNA targeting metallonucleases.

Table Captions

Table 1. Electronic absorption parameters for the interaction of DNA with Cu(II), Co(II), Ni(II) and Zn(II) complexes.

Table 2. Electrochemical parameters for the interaction of DNA with Cu(II), Co(II), Ni(II) and Zn(II) complexes.

Table 3. Minimum inhibitory concentration of the synthesized compounds against the growth of bacteria (μM).

Table 4. Minimum inhibitory concentration of the synthesized compounds against the growth of bacteria (μM).

Table 1. Electronic absorption parameters for the interaction of DNA with Cu(II), Co(II), Ni(II) and Zn(II) complexes.

Compound	λ max		Δλ (nm)	H%	$K_b \times 10^5$ (M^{-1})
	Free	Bound			
$[CuL(met)_2]$	315.4	319.2	3.8	11.4	7.9
$[CoL(met)_2]$	327.0	329.3	2.3	7.3	3.4
$[NiL(met)_2]$	338.5	340.6	2.1	4.5	4.6
$[ZnL(met)_2]$	326.2	328.1	1.9	8.1	5.2

Table 2. Electrochemical parameters for the interaction of DNA with Cu(II), Co(II), Ni(II) and Zn(II) complexes

Compound	$E_{1/2}(V)^a$		$^b\Delta Ep(V)$		Ip_a/Ip_c	*K[red]/K[oxd]*
	Free	Bound	Free	Bound		
$[CuL(met)_2]$	-0.460 -0.145	-0.466 -0.135	0.191 0.228	0.201 0.190	0.84 1.26	0.392
$[CoL(met)_2]$	-0.201	0.221	0.065	0.672	1.12	0.346
$[NiL(met)_2]$	-0.663	0.683	-0.033	-0.039	0.89	0.123
$[ZnL(met)_2]$	-0.678	-0.680	0.211	0.165	0.81	0.458

Data from cyclic voltammetric measurements: $^aE_{1/2}$ is calculated as the average of anodic (E_{Pa}) and cathodic (E_{pc}) peak potentials; $E_{1/2}{}^a = E_{Pa} + E_{pc} / 2$; $^b\Delta Ep = E_{pa} - E_{pc}$

Table 3. Minimum inhibitory concentration of the synthesized compounds against the growth of bacteria (μM).

Compound	Minimum inhibitory concentration (MIC) (× 10^4 μM)			
	N.asteroide	*P.putida*	*L.lactis*	*E.coli*
[L]	18.2	18.9	19.5	19.8
[$CuL(met)_2$]	7.6	8.2	8.9	9.2
[$CoL(met)_2$]	9.3	9.7	10.2	10.4
[$NiL(met)_2$]	9.7	10.5	10.2	10.4
[$ZnL(met)_2$]	10.8	10.9	10.4	11.6
Gentamicin	3.1	3..4	3.9	4.1

Table 4. Minimum inhibitory concentration of the synthesized compounds against the growth of fungi (μM).

Compound	Minimum inhibitory concentration (MIC) (× 10^4 μM)		
	A. niger	*A. flavus*	*C.albicans*
[L]	19.7	17.1	18.8
[$CuL(met)_2$]	7.5	6.9	5.8
[$CoL(met)_2$]	7.9	6.2	8.2
[$NiL(met)_2$]	10.2	9.5	8.7
[$ZnL(met)_2$]	12.2	11.3	10.2
Fluconazole	3.2	2.9	2.4

Research Highlights

- The complexes exhibit admirable DNA binding and chemical nuclease activity.
- They have efficient antimicrobial effect against various pathogens.
- They act as potential DNA targeting agents.
- Leading to develop designing and synthesis of novel metal based drugs.