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ORIGINAL PAPER

Synthesis, Characterization, Antibacterial, DNA Binding and Cleavage Studies of Mixed Ligand Cu(II), Co(II) Complexes

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Abstract Mixed-ligand Cu(II), Co(II) complexes of formulae [Co(NSALT)(A.A)(H_2O)](1), [Co(OHAPT)(A.A)H_2O](2), and [Cu(ESALT)(ABPH)H_2O] (3) were obtained by refluxing methanol solutions of copper, cobalt chlorides with the appropriate ligands. The complexes were characterized by the ESI-MASS, vibrational spectroscopy (Fourier transform-IR), ^{1}H-NMR spectroscopy, UV–vis spectroscopy, TGA, ESR, SEM and powder XRD. The preliminary DNA-binding activity of the complexes was studied by recording electronic absorption spectra of the complexes in presence of CT-DNA. The binding constants of three complexes towards calf thymus DNA (CT-DNA) [1.2×10^4 M^{-1} for 1, 2.5×10^4 M^{-1} for 2, and 3.0×10^4 M^{-1} for 3] indicate strong interaction of 3. Changes in the fluorescence of ethidium bromide in the presence of DNA suggest intercalation into or electrostatic interactions with CT DNA. The quenching constants, K_{SV} towards-DNA calculated through fluorescence spectra are 2.9×10^4 $M^{-1}$for 1, 1.8×10^4 M^{-1} for 2, and 3.2×10^4 M^{-1} for 3. Docking studies on DNA complexes confirm the binding of 1 and 2 in the major groove of CT-DNA (CTP-1 Endonuclease). Moreover, the antibacterial effect of 1–3 against the five bacterial species was evaluated. The metal complexes have cleavage affinity towards PBR322 plasmid. Furthermore, the antioxidant activities of the complexes were determined by DPPH scavenging activity method.

Keywords Metal complexes · Anti oxidant · Antimicrobial activity · DNA binding · Cleavage studies

Introduction

Transition-metal complexes have been widely studied as antitumor drugs because of their various spectral, chemical properties.[1–5].Thiosemicarbazides and their metal complexes are display a wide range of biological activities such as antitumor, antibacterial, antiviral and anti-malarial activities [6–8].Organic compounds having oxygen, nitrogen and sulfur as donors are widely studied [9] to their complexes cover many areas spacing from effect of sulfur and electron delocalization in transition metal complexes (Scheme 1).

The Schiff bases have more applications because of their various chemical and structural characteristics and also have biologically active molecules [10]. According to earlier reports metal complexes have the ability to bind and notch ds-DNA in physiological environment [11-13]. Binding studies of complexes to DNA are very important in the investigation of DNA molecular probes and new therapeutic reagents [14].Transition metal complexes have the good attention as catalytic systems for use in the oxidation of organic compounds [15].The binding between DNA and transition metal complexes is an significant primary issue in life sciences. These complexes can bind to DNA in various modes such as electrostatic, intercalative and groove binding. The above applications have need of that the complex binds to DNA through an intercalate mode in which, the planar aromatic group is stacked between the base pairs of DNA. In this paper, we have studied on the Synthesis, characterization, DNA

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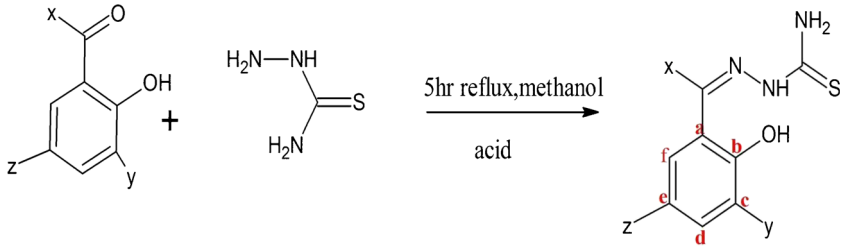
Scheme 1 Synthetic route and structures for compounds L_1–L_3



L_1(X=H,Y=H,Z=NO_2) L_2(X=CH_3,Y=H,Z=H) L_3(X=H,Y=OEt,Z=H)

binding and cleavage studies of mixed-ligand Cu(II), Co(II) complexes. DNA binding studies were studied by absorption spectra, Fluorescence spectra, and viscosity measurements. The nuclease activity was studied with PBR322 plasmid. Metal complexes were characterized by different spectral methods and were subjected to biological activity, antioxidant studies.

Experimental

Materials and Chemicals

All the chemicals and solvents were of analytical reagent grade and were used as received unless otherwise noted. Thiosemicarbazide, metal salts, 3-ethoxy salicylaldehyde, o-hydroxy acetophenone, 5-Nitro salicylaldehyde, Tris–HCl, Ethidium bromide (EB) and CT- DNA were purchased from Sigma-Aldrich chemicals. Millipore water was used for preparing buffer.

All the experiments involving with the interaction of the ligand and complexes with CT-DNA were carried out in Millipore water buffer containing 5 mM Tris - Hcl[Tris(hydroxymethyl)-aminomethane], 50 mM Nacl. The solution of DNA in the buffer gave a UV absorbance ratio A260/A280 of about 1.8/1.9 indicating that the DNA was sufficiently free from protein [16].The ligands and complexes were dissolved in a solvent mixture of DMSO and Tris–HCl buffer at the concentration 1.0×10^{-5} M. The stock solution was stored at 4 °C and used within 4 days.

Synthesis of Ligands

(2Z)-2-(2-Hydroxy-5-Nitrobenzylidene) Hydrazine Carbothioamide (NSALT) (L_1)

The ligand NSALT (L_1) has been synthesized by the method that has been reported earlier by Aamer Saeed, Najim et.al [17]. Methanolic solution of (40 ml) thiosemicarbazide (1.82 g, 0.02 mol) and few drops of sulphuric acid was added to methanolic solution of 5-NO_2 salicylaldehyde (3.34 g, 0.02 mol). The resultant mixture was refluxed for 3 h, the solid product formed was filtered and recrytastallized from methanol. Yield: 80 %.Anal calc for $C_{10}H_{13}N_4O_3S$ (%) C, 50; H, 5.41; N, 23.33; Found (%): C, 50.8; H, 5.3; N, 23.29; ESI-MS (DMSO): m/z=240 (calcd 240.4): IR (KBr, cm^{-1}): 1,627 (C=N), 1,489 (C=C),1,635 (−CH=N), 3,249 (−NH), 3,380 (−OH). ^{1}H NMR (400 MHz, DMSO-d_6, ppm):7.8–8.0 (d, Hc, 1H), 8.1–8.5 (d, Hd, 1H), 8.4–8.2 (s, Hf, 1H), 4.5 (s, −OH, 1H), 3.98–3.5 2.0–2.3 (s, −NH, 1H), 2.0–2.3 (s,-NH_2, 2H).

(2Z)-2-[1-(2-Hydroxyphenyl)Ethylidene] Hydrazinecarbothioamide (OHAPT) (L_2)

The ligand OHAPT (L_2) has been synthesized by the method that has been reported earlier by Sukriye Guveli et.al [18]. Methanolic solution of (40 ml) thiosemicarbazide (1.82 g, 0.02 mol) and few drops of sulphuric acid was added to methanolic solution of O-hydroxy acetophenone (2.723 g, 0.02 mol). The resultant mixture was refluxed for 3 h, the solid product formed was filtered and recrytastalized from methanol. Yield: 70 %.Anal calc for $C_{11}H_{16}N_3OS$ (%) C, 63.15; H, 7.65; N, 20.09; Found (%): C, 64.15; H, 7.15; N, 20.29; ESI-MS (DMSO): m/z=209 (calcd 209.3) :IR (KBr, cm^{-1}):1,620 (C=N), 1,485 (C=C),1,635 (−CH=N), 3,240 (−NH), 3,350 (−OH). ^{1}H NMR (400 MHz, DMSO-d_6, ppm):6.8–7.0 (t, He, 1H), 7.4–8.0 (d, Hf, 1H), 6.8–7.0 (d, Hc, 1H) 4.3 (s, −OH, 1H), 0.8−1.2 (s, −CH_3, 3H), 2.0–2.3 (s, −NH, 1H), 2.0−2.3 (s,-NH_2, 2H).

(2Z)-2-(3-Ethoxy-2-(2Z)-2-[1-(2-Hydroxyphenyl)Ethylidene] Hydrazine Carbothioamide (ESALT) (L_3)

The ligand ESALT (L_3) has been synthesized by the method that has been reported earlier by Ling-Wei Xue et.al [19]. Methanolic solution of (40 ml) thiosemicarbazide (1.82 g, 0.02 mol) and few drops of sulphuric acid was added to methanolic solution of 3-ethoxy salicylaldehyde (3.32 g, 0.02 mol). The resultant mixture was refluxed for 3 h, the solid product formed was filtered and recrytatallized from methanol. Yield: 77 %.Anal calc for $C_{10}H_{13}N_3O_2S$ (%) C, 50.2; H, 5.43; N, 17.57 Found (%): C, 50.15; H, 5.15; N, 17.29; ESI-MS (DMSO):m/z=239 (calcd 239.8): IR (KBr, cm^{-1}): 1,610 (C=N), 1,445 (C=C),1,600 (−CH=N), 3,250

(−NH), 3,390 (−OH). ^{1}H NMR (400 MHz, DMSO-d_6, ppm): 6.8–7.0 (d, Hd, 1H), 6.7–7.1 (d, He, 1H), 6.8–7.3 (d, Hf, 1H), 4.5 (s, −OH, 1H), 0.9–1.2 (t, $-CH_3$, 3H),3.98–3.5(q, $-CH_2$, 2H), 2.0–2.3 (s, −NH, 1H), 2.0–2.3 (s,$-NH_2$, 2H).

Synthesis of Complexes

Synthesis of [Co(NSALT)(A.A)(H_2O)] Complex

This complex was prepared by mixing $CoCl_2.6H_2O$ (1 m.mol) in MeOH(50 ml),and NSALT(L_1)(1 mM) in 15 ml methanol this mixture was refluxed for 2 h. To this anthranilic acid (A.A)(1 m.mol) in 15 ml methanol was added and refluxed at refluxing temperature for 3 h. In the resulting reddish brown solution solvent was slowly evaporated. The products were filtered, washed with cold ethanol and dried under vacuum over calcium chloride. Analytical data: IR ν_{max}: 3,300–3,560 cm^{-1}(ν_{O-H}), 3,184 cm^{-1} (ν_{N-H}), 1,540 cm^{-1} ($\nu_{C=N}$ Im), 740 cm^{-1} (ν_{C-N}), 1,390 cm^{-1}(ν_{C-S}), 536 (ν_{M-O}), 450 (ν_{M-N}), 340 (ν_{M-S}), Anal. Calc.for. C 45.13, H 4.42, N 15.48, O 21.23, S 7.07; found C 43.99, H 4.39, N 15.39, O 20.23,S 6.90 UV–vis (nm):290, 340, 440; ESI-MS: 452 (calc 452.5) [Co(NSALT)(A.A)(H_2O)]

Synthesis of [Co(OHAPT)(A.A)H_2O] Complex

This complex was prepared by mixing $CoCl_2.6H_2O$ (1 m.mol) in MeOH (50 ml), and OHAPT(L_2)(1 m.mol) in 15 ml methanol this mixture was refluxed for 2 h. To this anthranilic acid(1 m.mol) in 15 ml methanol was added and refluxed at refluxing temperature for 3 h. In the resulting brown solution solvent was slowly evaporated. The product was filtered, washed with cold ethanol and dried under vacuum over calcium chloride. *Analytical data*: IR ν_{max} : 3,400–3,500 cm^{-1} (ν_{O-H}), 3,200 cm^{-1} (ν_{N-H}),1,600 cm^{-1} ($\nu_{C=N}$ Im), 750 cm^{-1} (ν_{C-N}), 1,368 cm^{-1} (νc-s), 530 cm^{-1} (ν_{M-O}),445 cm^{-1} (ν_{M-N}), 330 cm^{-1} (ν_{M-S}), Anal. Calc.C 52.42,H 5.58,N 13.5,O 15.53 S 7.76; found C 51.9,H 5.3,N 13.4,O 15.53,S 7.76 UV–vis (nm): 290, 400; ESI-MS: 421 (calc 421.4) [Co(OHAPT)(A.A)H_2O].

Synthesis of [Cu(ESALT)(ABPH)H_2O] Complex

This complex was prepared by mixing $CuCl_2.2H_2O$ (1 m.mol) in MeOH (50 ml), and ESALT (L_3)(1 m.mol) in 15 ml methanol and this mixture was refluxed for 2 h. To this azobenzoyl phenyl hydrazone (ABPH) (1 mM) in 15 ml methanol was added and refluxed at refluxing temperature for 3 h. In the resulting brown solution solvent was slowly evaporated. The products were filtered, washed with cold ethanol and dried under vacuum over calcium chloride. Analytical data: IR ν_{max}: 3,300–3,560 cm^{-1} (ν_{O-H}), 3,194 cm^{-1} (ν_{N-H}),1,548 cm^{-1} ($\nu_{C=N}$ Im), 730 cm^{-1} (ν_{C-N}), 1,369 cm^{-1} (ν_{c-s}), 528 cm^{-1} (ν_{M-O}),435 cm^{-1} (ν_{M-N}), 320 cm^{-1} (ν_{M-S}), Anal. Calc.C 51.97, H 4.89, N 7.90, O 10.16 S 6.02; found C 50.9, H 4.8, N 7.65, O 10.28, S 5.8 UV–vis (nm):290, 350; ESI-MS: 530 (calc 530.7) [Cu(ESALT)(ABPH)H_2O].

Physical Measurement

The Elemental analysis (C, H, N, O and S) of complexes and ligands were conducted using micro analytical methods on Perkin Elmer 240C (USA) elemental analyzer. FT-IR spectra of metal complexes and ligand were recorded by means of KBr disks with a schimadzu Fourier transform IR spectrometer. Electronic spectra of the ligand and its metal complexes were recorded in DMSO using Elico SL159 spectrophotometer. ^{1}H NMR spectra was recorded on a Bruker 400 MHz

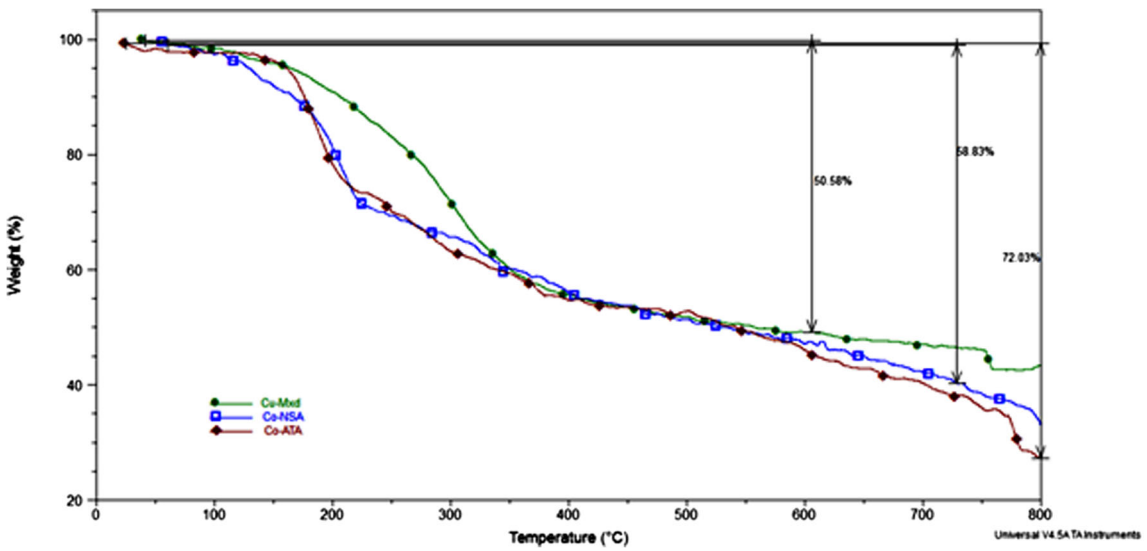


Fig. 1 TGA graphs of *1* [Co(L1)(A.A)(H2O)] *2* [Co(L2)(A.A)(H2O)] *3* [Cu(L3)(ABPH)(H2O)] complexes

spectrometer. The mass spectra were recorded by ESI technique on VG AUTOSPEC mass spectrometer. An ESR spectrum of copper complex was conducted on JEOL JES-FA200ESR spectrometer. TGA of the complexes were recorded on Shimadzu DTG-60H system in the temperature range of 0–1,000 °C. Fluorescence spectra were recorded with an Elico spectrofluorimeter (model SL 174). Viscosity experiments were carried out by means of an Ostwald viscometer maintained at a constant temperature at 28.0±0.1 °C in a thermostatic bath.

DNA Binding and Cleavage Studies

DNA binding and cleavage experiments were carried out according to the procedures reported earlier [20]. Relative binding of metal complexes to CT-DNA was studied by fluorescence spectral method based on the emission properties of metal complex with CT-DNA in Tris – HCl/NaCl buffer (pH 7.2). The cleavage of super coiled (SC) PBR-322 plasmid was monitored by agarose gel electrophoresis. The electrophoresis was carried out at 50 V for 2 h until the bromophenol blue had travelled through 75 % of the gel. Subsequently, the gel was stained for 10 min with EB. The plasmid bands were viewed by gel documentation system and photographed using a Gel doc system (Alpha InfoTech Corporation).

Viscosity Measurements

Viscosity experiments were performed on Ostwald viscometer, maintained in a water bath at 25.0±0.1 °C. Titrations were done for the compound (10 μM), and each compound was added into CT-DNA solution (100 μM) in the viscometer. Data were presented as $(\eta/\eta_0)^{1/3}$ versus [compound]/ [DNA, where η is the viscosity of CT-DNA in the presence of the compound and η_0 is the viscosity of CT-DNA alone. Viscosity values were calculated from the observed flow time of CT-

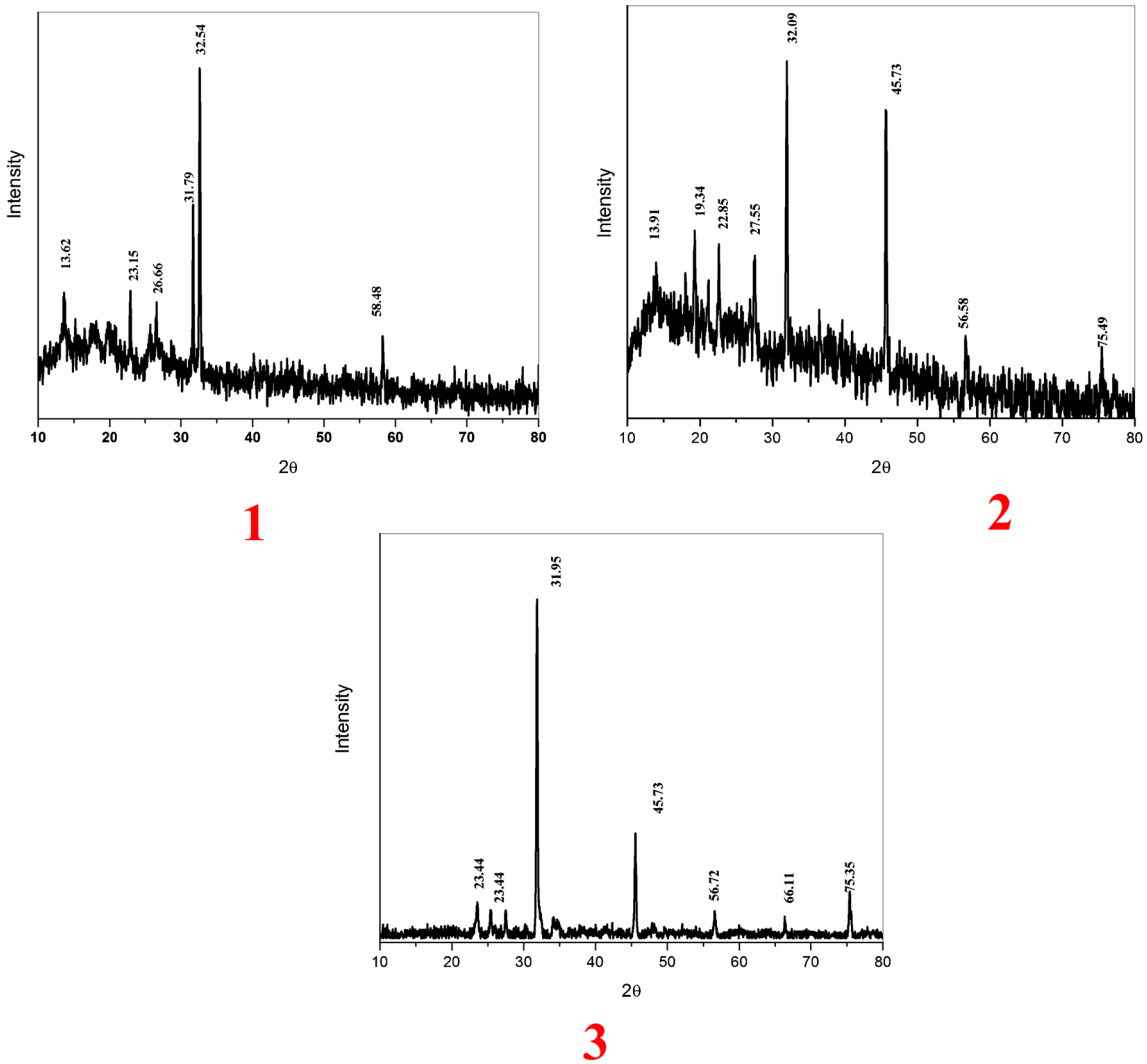


Fig. 2 Powder XRD of *1* [Co(L1)(A.A)(H2O)] *2* [Co(L2)(A.A)(H2O)] *3* [Cu(L3)(ABPH)(H2O)]

DNA containing solutions corrected from the flow time of buffer alone (t_0), $\eta=(t-t_0)$ [21]

Anti Oxidant Activity

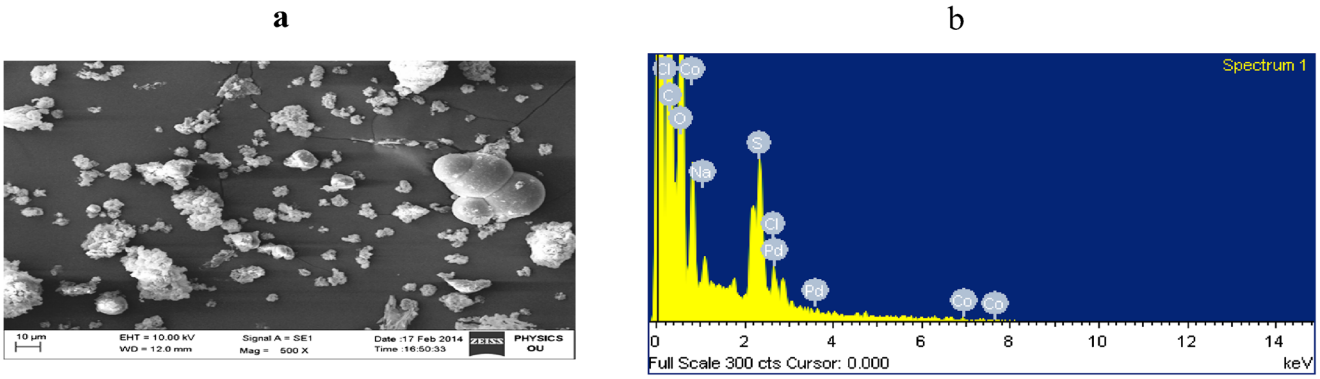
The Experiment [22] performed by the radical cation derived from Diphenyl picryl hydrazyl(DPPH) as stable free radical to assess anti oxidant activity and extracts .Different concentrations of the compounds were dissolved in methanol (25 to 150 mg/ml)0.0.5 ml of each compound was added to 1.5 ml of methanolic DPPH (0.004 %) solution. After 40 min of incubation at dark, the absorbance of the compounds was read at 517 nm by the UV–VIS Spectrophotometer. The percentage of inhibition of free radical DPPH was calculated according to the equation

$$I(\%) = \frac{(Ao-Ai)}{Ai} \times 100$$

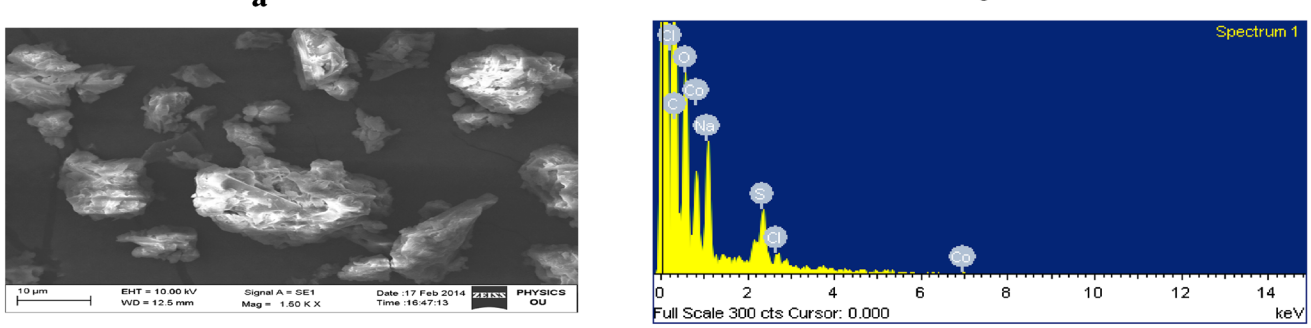
where, A_0 is the absorbance of the control and A_i is the absorbance of the sample.

Antibacterial Studies

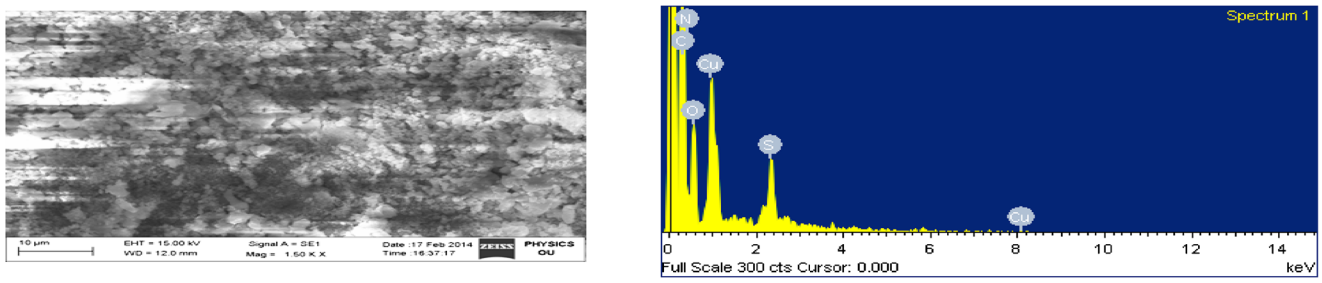
The agar disc diffusion method was employed for the determination of antibacterial activities of the Metal Complexes. 0.1 ml of the 5 pathogenic bacteria Bacillus subtilis, Klebsiella



SEM EDAX OF [Co L_1(A.A)(H_2O)]



SEM EDAX OF [Co L_2(A.A)(H_2O)]



SEM EDAX OF [Cu L_3(ABPH)(H_2O)]

Fig. 3 Images of the metal complexes deposited on a 52,100 steel substrate after thermolysis of compounds . **a** Scanning electron microscopy(SEM). **b** Energy-dispersive X-ray spectroscopy (EDAX) spectrum of complexes

pneumonia, Staphylococcus aureus, Pseudomonas putida and Escherichia coli suspensions were spread on different plates nourished with LB (Luria Bertani) media. Filter paper discs (5 mm in diameter) were placed on the plates and then on to the discs Metal Complexes (10 μl) were impregnated in different concentrations. Ampicillin 5 μl (10 μg/10 μl concentration) served as the standard for measuring the antibacterial activity. The plates were then incubated at 37 °C for 24 h. The zones of inhibition were measured in (mm).[23]

Docking Studies

Materials and Methods

Freely accessible Softwares are used in this study. The Protein Data Bank (PDB) is a worldwide source for handling and distribution of three dimensional biological macromolecular structure data. The protein structure of (3HUF) [24] was downloaded from Protein Data Bank. The drug molecule 1) [Co $(L_1)(A.A)(H_2O)$], 2) $[Co(L_2)(A.A)(H_2O)]$, and 3) $[Cu(L_3)(ABPH)(H_2O)]$ were synthesized the molecules in the laboratory as shown in synthesis part. The PDB file of 3HUF obtained from the Protein Data Bank was visualized using SSViewer. Docking results were also analyzed and visualized using Pymol. The docking was achieved using the docking software AutoDock 4.2 (The Scripps Research Institute, "www.scripps.edu"), with the support of AutoDock Tools (ADT)—an accessory program the user to interact with Auto Dock from a Graphic User Interface (GUI). Auto Dock is a suite of automated docking tools designed to predict how small molecules/ligands such as substrates or drug molecules bind to a receptor/protein of known 3D structure.

Receptor Ligand Docking

For getting the drug-receptor binding energy and inhibition constant, molecular docking was performed. The detailed procedure followed is:

Preparation of Ligands and Receptor Files

The PDB files deposited in Protein Data Bank are often far perfect for docking analysis and are present with potential problems like missing hydrogen atoms, multiple molecules, added waters etc. The CT-DNA (CTP-1 Endonuclease) was downloaded from PDB database (PDB ID 3HUF). It was first read in ADT, added water removed and polar hydrogens were added. Kollman charges were added. Finally file was saved with .pdbq extension (q = charge). In a similar procedure, the ligand files were read in ADT, all hydrogens added, charges added and non-polar hydrogens merged and saved with pdbqt extension. ADT then automatically predicted the best root. The ligand files were then saved with pdbqt extension (q = charge).

Grid Parameter File Preparation

For the prediction of docking interaction energy, a three-dimensional box (grid) was created in which the protein molecule is enclosed. The grid volume was kept large in order to permit the ligand to rotate freely, even with its most fully extended conformation. The parameters essential to create such a grid were stored in the Grid Parameter File with gpf extension.

Running Auto Grid

Autogrid 4 generates one map for each kind of atom in the ligand. For instance, a molecule containing carbon, nitrogen, oxygen, hydrogen, maps will be generated as molecule.C.map, molecule.N.map, molecule.O.map, molecule.H.map. These are grid maps in ASCII format and are readable by Auto Dock [25]. Auto Grid also creates corresponding output of the macromolecular file with the extension glg.

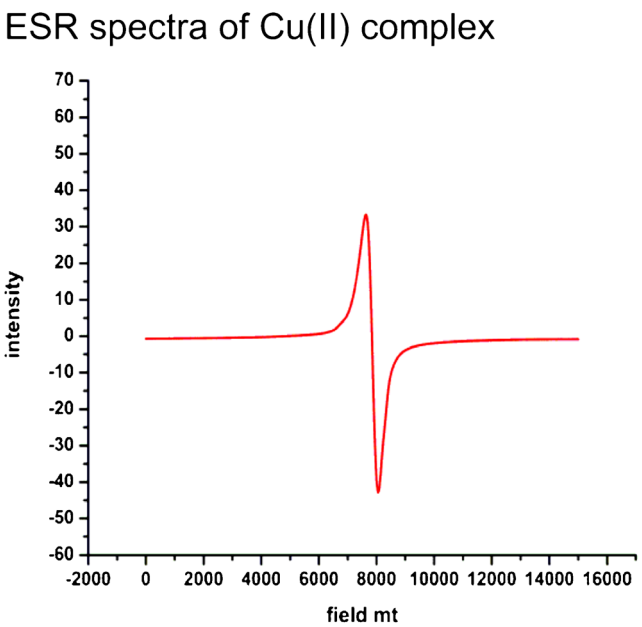
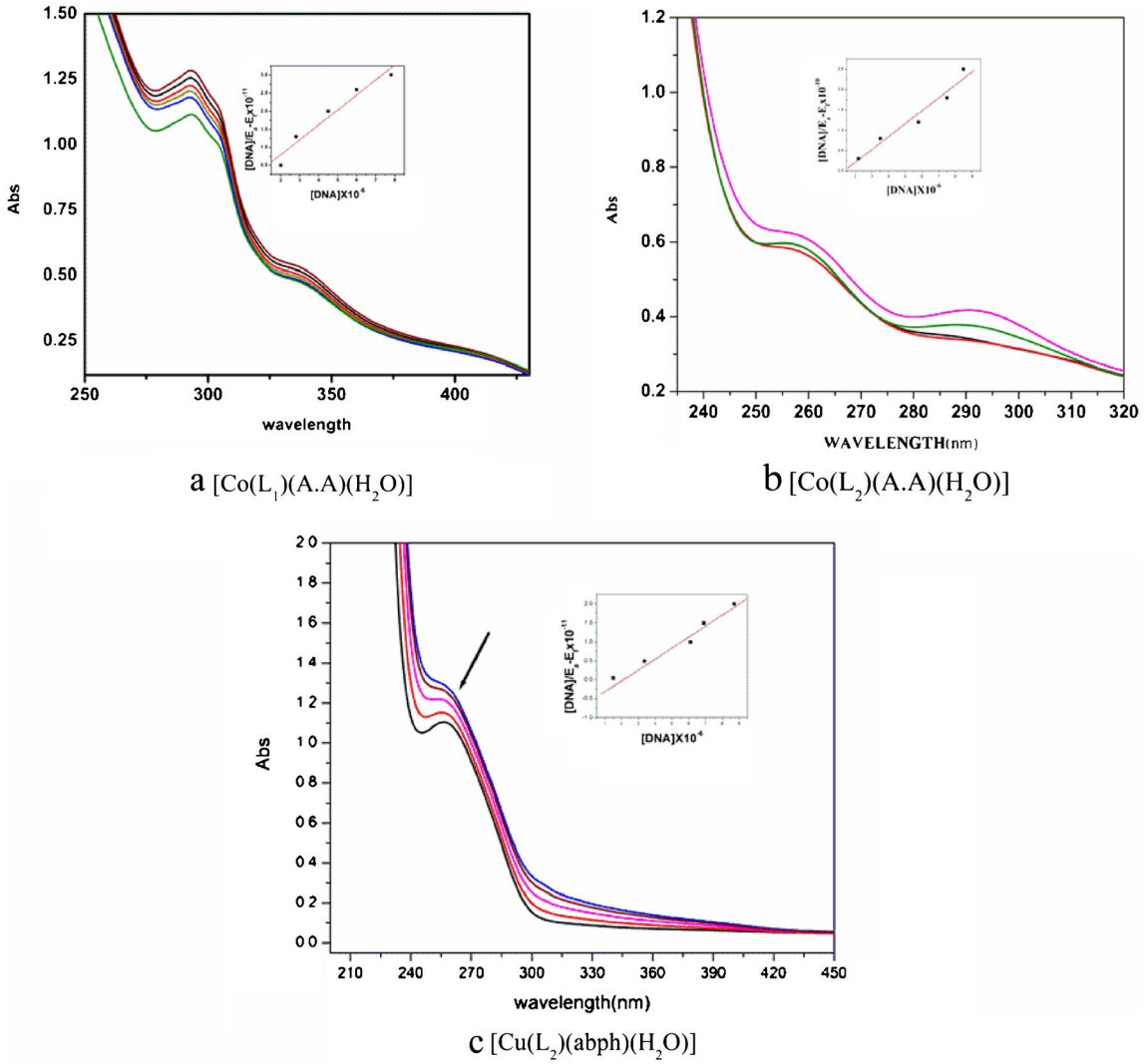


Fig. 4 ESR spectra of Cu(II) complex



a [Co(L$_1$)(A.A)(H$_2$O)] b [Co(L$_2$)(A.A)(H$_2$O)]

c [Cu(L$_2$)(abph)(H$_2$O)]

Fig. 5 Electronic spectra of three metal complexes (100 μM), complex **a** [Co(L$_1$)(A.A)(H$_2$O)] **b** [Co(L$_2$)(A.A)(H$_2$O)] **c** [Cu(L$_3$)(ABPH)(H2O)] in the absence and presence of increasing amounts of CT-DNA, [DNA]= 10–100 μM. ([DNA] =2.3×10−4 M). *Arrow* shows the absorbance changes upon increasing DNA concentration. Inset: Linear plots for the calculation of intrinsic binding constant Kb

Docking Parameter File Preparation

The docking parameter file, trains Auto Dock about the map files to use, the ligand to move, and other properties used for the ligand. Auto Dock's search approaches comprise the Monte Carlosimulated annealing (SA), local search (LS), the Genetic Algorithm (GA) and the hybrid genetic algorithm with local search (GALS). The latter one also referred to as the Lamarckian genetic algorithm (LGA) [26] was the chosen algorithm for this analysis.

Running Autodock

Lastly, Auto Dock was run from the GUI of ADT and docked ligand files were used for the analysis. The dlg files were read in ADT and SSViewer [27] for the binding energies prediction in the docked ligand protein complexes.

Results and Discussion

Characterization by TGA

The thermo gravimetric analysis technique is the most important in studying the solvent molecules in attachment with the complex molecule. Thermo gravimetric studies for the complexes were performed within the temperature range from 30 to 800 °C . All metal complexes decomposed at 140–800 °C range. The first decomposition step within the temperature range 150–250 °C may be attributed to the liberation of

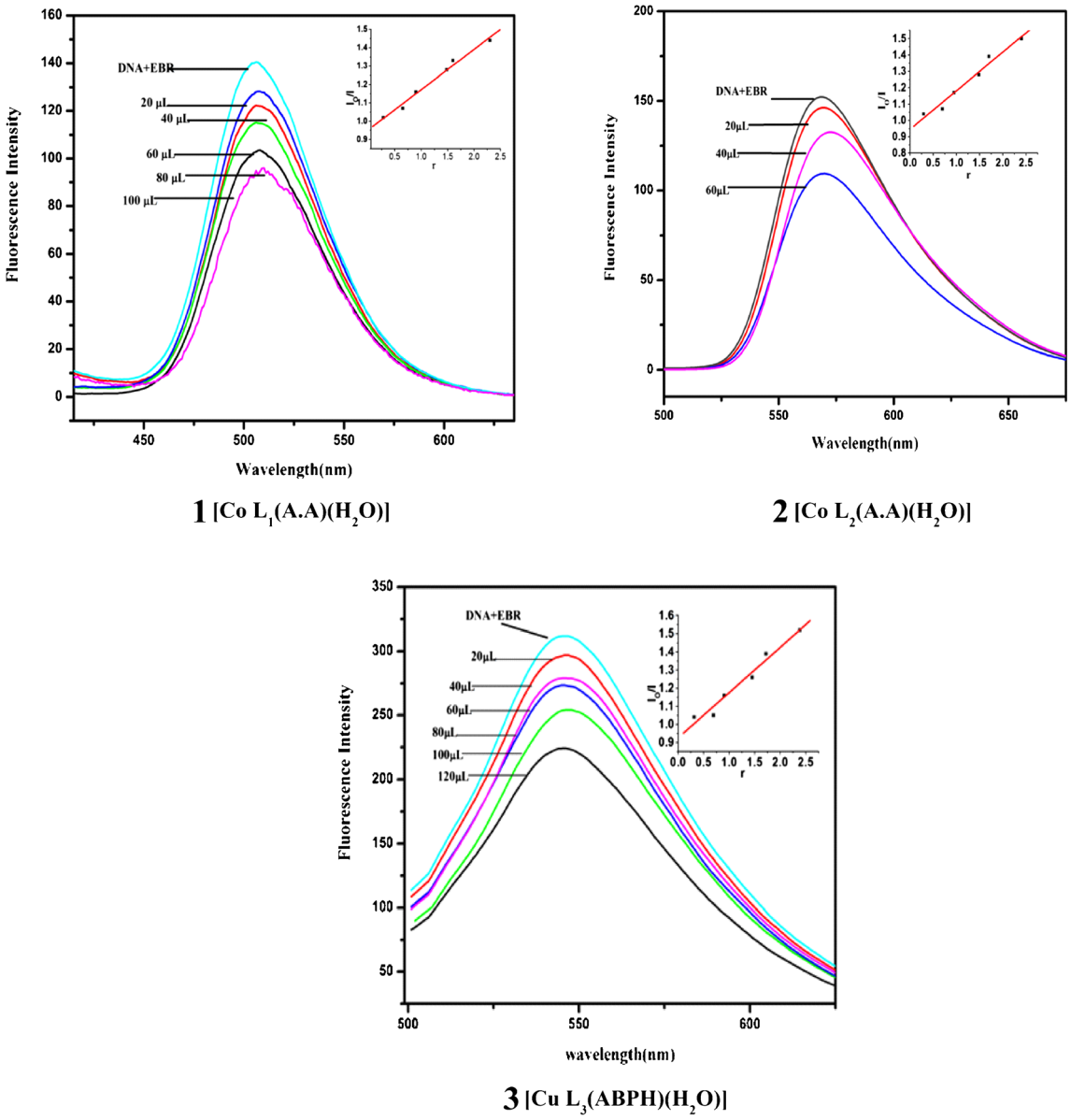


Fig. 6 Emission spectra of EB bound to DNA ([DNA] =2.4×10−4). in the absence and presence of complexes (100 µM) complex *1* [Co(L_1)(A.A)(H_2O)] *2* [Co(L_2)(A.A)(H_2O)] *3* [Cu(L_3)(ABPH)(H_2O)] Inset: Stern-Volmer quenching curves

coordinated H_2O molecule. The weight loss in the range of 380 to 800 °C are attributed to decomposition of complexes (Fig. 1).

X-ray Diffraction and SEM Analysis

To obtain extra support about the structure of the metal complexes X-ray diffraction was performed. The diffractograms obtained for the metal complexes are given in (Fig. 2). The XRD patterns indicate crystalline nature for the complexes. Which may be attributed to the formation of a well defined distorted crystalline structure. Probably, this behavior is due to the incorporation of water molecules into the coordination sphere. The morphology of the metal complexes has been illustrated by the scanning electron microscopy (SEM) (Fig. 3). Illustrate the SEM photographs of the metal complexes.

ESR Spectra

To acquire further information about the stereochemistry and the site of the metal ligand bonding, ESR spectra of copper(II) complex was recorded in the solid state.(Fig 4). Represents the ESR spectrum of Cu(II) complex. The g values of the complex are $g_{\parallel}$ (2.240)>$g_{\perp}$ (2.072) >2.0023, indicating that the unpaired electron in the ground state of Cu(II) is predominantly

in d_{x2-y2}. The value of exchange interaction term G, estimated from the following expression is 3.8.

$$G = g\|-2.0023/g\perp-2.0023$$

These values are most closely associated with copper(II) ions with a distorted octahedral geometry [28].The spin-orbit coupling constant, value (−715 cm^{-1}) calculated by the formula, $g_{av}=1/3[g_{\|}+2g\perp]$ and $g_{av}=2(1\text{-}2\lambda/10Dq)$, is less than the free Cu(II) ion (−832 cm^{-1}) which supports covalent nature.

Electronic Absorption Titrations

Electronic absorption spectroscopy was used to determine the binding characteristics of metal complexes with DNA. The binding of the Cu(II),Co(II) complexes to the DNA helix was characterized by resulting hypochromism in intensity and red shift in wavelength due to the strong stacking interaction between an aromatic chromophore and the base pairs of DNA. It indicates intercalative mode of binding. The absorption spectra of the three complexes in the absence and presence of DNA are shown in (Fig. 5). The $[Cu(L_2)(ABPH)H_2O]$ complex exhibits two absorption bands one at 310 nm and another at 250 nm, the intrinsic binding constant value K_b is 2.5×10^4 M^{-1}.The $[Co(L_1)(A.A)(H_2O)]$ complex exhibits two absorption bands one at 330 nm and another at 280 nm, the intrinsic binding constant value K_b is 1.2×10^4 M^{-1}. The$[Co(L_3)(A.A)(H_2O)]$ complex exhibits two absorption bands one at 300 nm and another at 260 nm, the intrinsic binding constant value K_b is 1.1×10^4 M^{-1}, With increasing DNA concentration, resulting in a tendency to hypo chromism and slight red shifts, which indicates that the complexes can bind with DNA through intercalative mode.

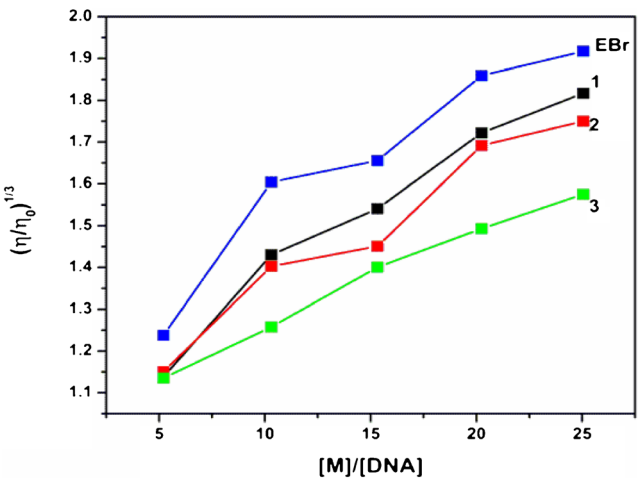


Fig. 7 Effects of increasing amount of Ethidium bromide (EBr), complexes *1* $[Co(L_1)(A.A)(H_2O)]$ *2* $[Co(L_2)(A.A)(H_2O)]$ *3* $[Cu(L_3)(ABPH)(H_2O)]$ on the relative viscosity of CT-DNA at 29 °C± 0.1, [DNA]=20 μM

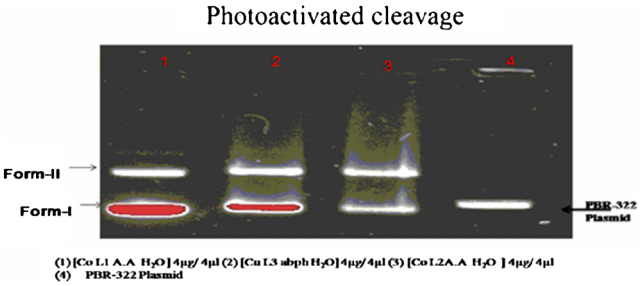


Fig. 8 Photo activated cleavage of PBR322 DNA in the presence of *1* $[Co(L_1)(A.A)(H_2O)]$ *2* $Co(L_2)(A.A)(H_2O)]$ *3* $[Cu(L_3)(ABPH)(H_2O)]$ after irradiation at 365 nm.Lane 4 control plasmid DNA(untreated pBR322), lanes 1–3 addition of complexes at 20, 40, and 60

For metallo intercalators, DNA binding is associated with hypochromism and a redshift in the metal to-ligand charge transfer (MLCT) and ligand bands [36]. This is primarily due to the intercalation mode involving strong stacking interactions between an aromatic chromophore and the base pairs of DNA. The extent of the hypochromism in a UV–visible band is consistent with the strength of the interaction [37, 38]

Fluorescence Studies

Metal complexes emits intense fluorescence in the presence of CT-DNA, due to their strong intercalation between the adjacent CT-DNA base pairs. Ethidium bromide (EB) is one of the most useful fluorescent probes that bind to DNA through intercalation [20] .The binding of complexes to CT-DNA was also studied by fluorescence attempts by evaluating the fluorescence emission intensity of the DNA with EB system

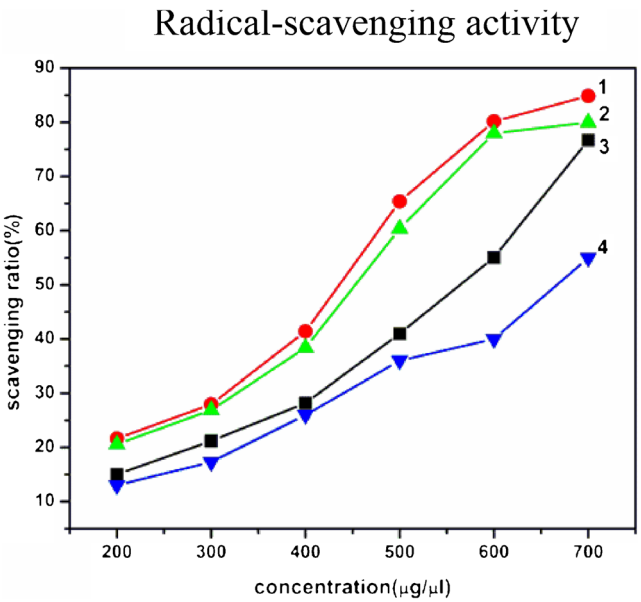


Fig. 9 Radical-scavenging activity on DPPH radicals (%) of the compounds

Table 1 The values of the anti microbial activity of the metal complexes

S. No	Name of bacteria's	[Co L_2AAX]	[CuL_3ABX]	[CoL_1AAX]	Ampicilline(10 μg/10 μl)
1	Klebsiellapneumonia	2.74	6.95	2.63	10.62
2	Pseudomonas putida	4.00	5.99	2.70	9.79
3	Bacillus subtilis	3.46	3.15	7.13	15.76
4	Escherichia coli	2.58	7.77	2.20	9.64
5	Staphylococcusaurous	2.42	9.15	3.78	11.26

by the addition of three compounds. In this assay, if a compound is replacing the EB, then the emission intensity at 550 nm of EB will be decreased (31–33). Certainly, when metal complexes were introduced into a solution of the DNA

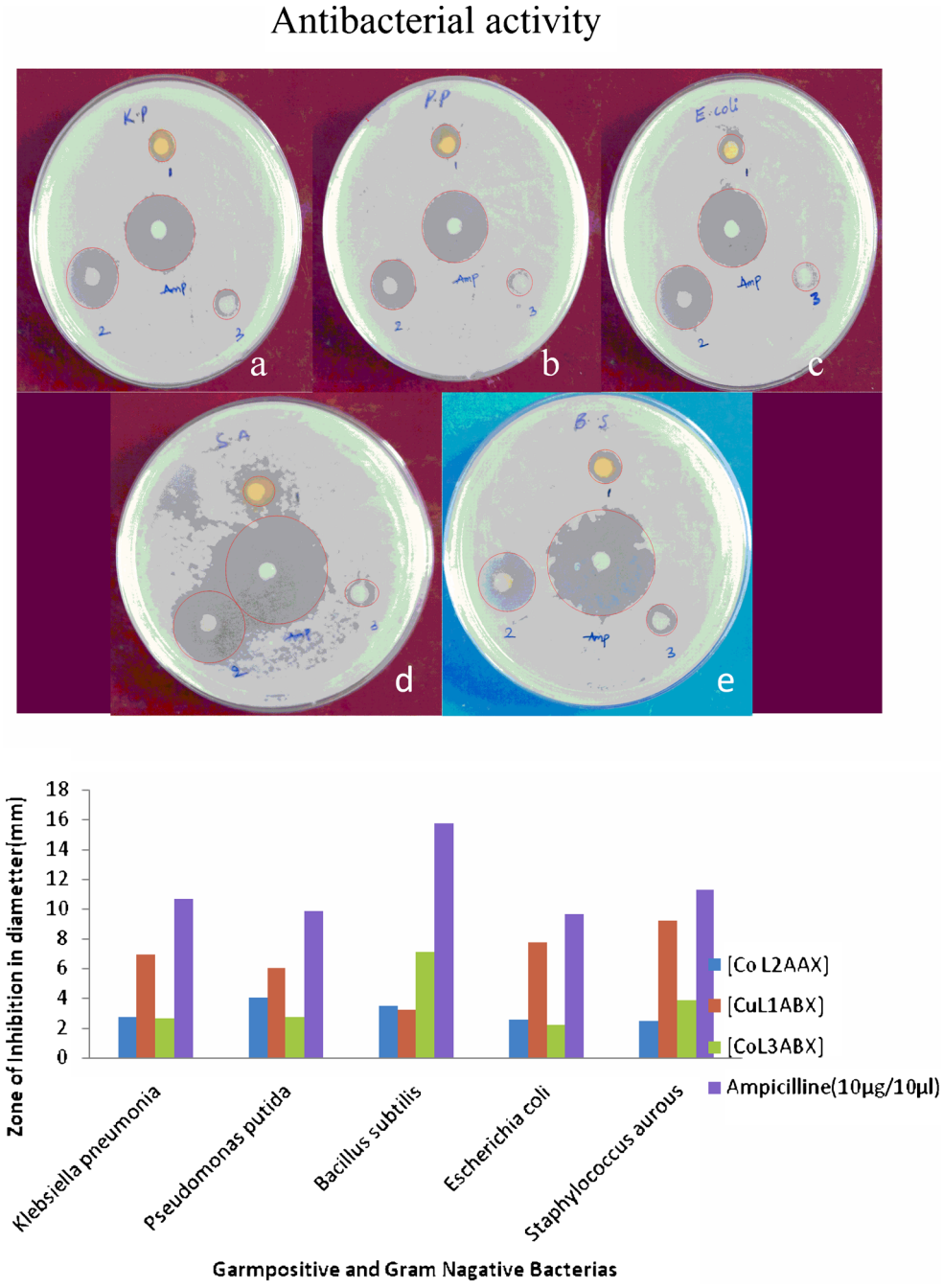


Fig. 10 Antibacterial activity of metal complexes

containing EB system (Fig. 6), the three compounds produced a partial replace of EB, giving linear Stern−Volmer plots fitting the classical equation $I_0/I = 1 + K_{SV}$ r. In this equation, I_0 and I are the fluorescence intensities in the absence and presence of the quencher respectively, and r is the concentration of the quencher, evaluating the linear Stern−Volmer quenching constant, K_{SV}, from the slope. The quenching constants, K_{SV}, obtained were 2.9×10^4 M^{-1}, 1.8×10^4 M^{-1} and 1.9×10^4 M^{-1} for three complexes, which confirms that copper complex has a higher DNA affinity than other two.

Viscosity Measurements

To understand the nature of the DNA binding of the complexes, viscosity measurements were carried out on CT DNA by increasing the concentration of the added complexes. In which solution viscosity of DNA is changes to alters in effective length of DNA. The viscosity of the CT- DNA sample increases with the addition of the complex. The viscosity studies give a strong evidence for intercalation. EB causes a most increment in viscosity of DNA solutions. Values of $(\eta/\eta_0)^{1/3}$, where η and η_0 are the specific viscosity of DNA in the presence and absence of complex, were plotted against [compound]/[DNA], as shown in (Fig. 7). The results enlighten that all complexes bound to CT-DNA explain increase in relative viscosities with an increase in the [compound]/[DNA] ratio proposing the intercalative binding mode of the complex with DNA [21].

DNA Cleavage Studies

Metal complexes can provoke numerous changes in DNA conformation after the complex has been bound. Upon the gel electrophoresis of the PBR322 buffer, the complexes showed efficient cleavage activities and converted the double-stranded SC DNA (form I) into the nicked circular(NC) form (form II) (Fig. 8). The photograph shows bands of metal complexes with DNA and the control. DNA is negatively charged and when kept in an electric field, it moves toward the anode. The two forms of PBR322 DNA can be identified by their dissimilar mobilities on agarose gel electrophoresis. In this experiments clearly indicated that the metal complexes has a nuclease activity on DNA. Earlier reported that [20] upon single-strand cleavage, the super coiled structure slow down, producing the nicked circular form with deliberate movement.

Anti Oxidant Activity

In this method the stable DPPH radical scavenging is used to assess antioxidant activities .In DPPH free radical scavenging activity, antioxidants are reacting with the stable free radical (DPPH) producing a colorless 1,1-diphenyl-2-picryl hydrazine. When DPPH accept an electron or hydrogen radical to get stability, its absorption decreases .

The antioxidant activities of the metal complexes, as found by scavenging DPPH radical, are shown in (Fig. 9). When the concentration of compounds was increased from 200 μg μL^{-1} to 700 μg μL^{-1}, DPPH radical scavenging activities were not

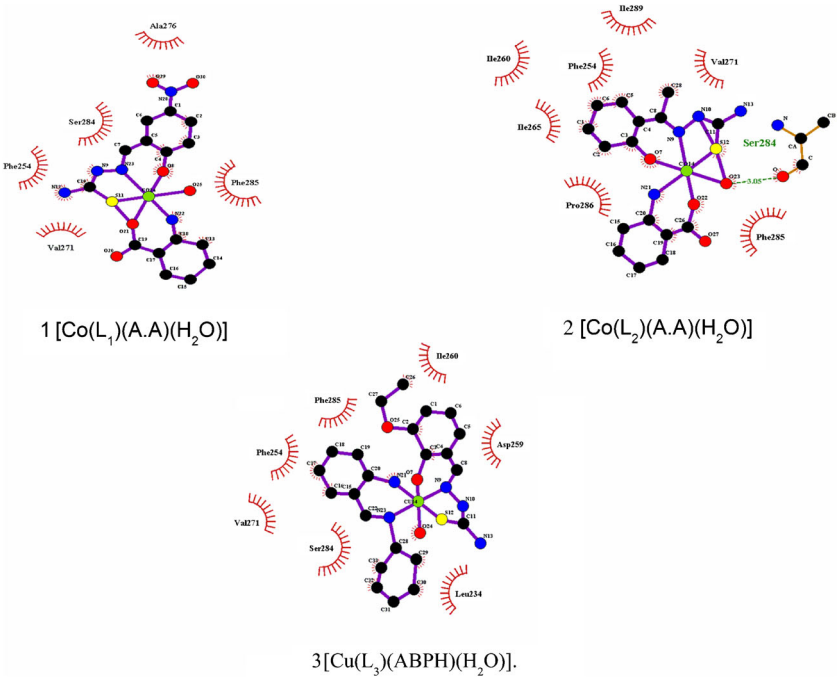


Fig. 11 Docking Images of *1* $[Co(L_1)(A.A)(H_2O)]$ *2* $[Co(L_2)(A.A)(H_2O)]$ *3* $[Cu(L_3)(ABPH)(H_2O)]$

increased significantly. The DPPH radical scavenging activities were found to be 24.9 % for 1, 23.7 % for 2, and 17.3 % for 3 and 17.1 % for 4 at concentration of the 200 μg μL^{-1}. Among the DMSO solution of compounds the DPPH radical scavenging activity, 2 was showed the maximum activity (23.7 %). Ascorbic acid exhibited higher DPPH scavenging activity, than the compounds at all the concentrations and their DPPH radical scavenging activity were found to be 76.8 %,75.3 % and 58 %, at concentration of 700 μg μL^{-1}, respectively[29].

Anti Bacterial Activity

All the complexes were tested for anti-bacterial activity against the 5 pathogenic bacteria Bacillus subtilis, Klebsiella pneumonia, Staphylococcus aureus, Pseudomonas putida and Escherichia coli suspensions were spread on different plates nourished with LB (Luria Bertani) media. The values of the anti microbial activity of the metal complexes are displayed in Table 1. Ampicillin was taken as the positive control (Standard) Drug for the measurement of Zone of Inhibition (in mm). The Metal Complexes have shown referable antibacterial activity (Fig. 10). Cu(II) complex show the highest zone of inhibition.

Docking Studies

The Auto Dock program computes the binding and docking energies. The energy terms include intermolecular energy (comprised of van der Waals energy, hydrogen bonding energy, desolation energy and electrostatic energy), internal energy and torsional energy. The first two energies make up docking energy, the first and the third items constitute the binding energy. 1) $[Co(L_1)(A.A)(H_2O)]$, 2) $[Co(L_2)(A.A)(H_2O)]$ and 3) $[Cu(L_3)(abph)(H_2O)]$ is docking energies are 38.447 kcal/mol, 37.938 kcal/mol and, 47.66kal/mol respectively. The docking energies show the same propensity as the binding energy. Inhibition constant was also calculated with the order of $[Co(L_2)(A.A)(H_2O)]>[Co(L_1)(A.A)(H_2O)]>[Cu(L_3)(abph)(-H_2O)]$. The surface view of receptor with different ligands (Fig. 11).The binding site of the receptor is colored in red and the ligands are in yellow color.

Conclusion

In this paper we report the synthesis of mixed ligand Cu(II),Co(II) complexes and characterized by spectral and analytical data. Thermal studies provided the information of coordinated water molecules in the complexes SEM analysis of three metal complexes revealed their homogeneous nature. The metal complexes were screened for antioxidant activity. DNA binding of 1, 2 and 3 complexes were investigated by electronic absorbance titrations, viscosity, and fluorescence measurements. The results strongly favor the binding of the complexes with CT DNA via intercalation mode. complex 3 displaying a higher binding affinity as compared to other complexes. The photolytic cleavage experiment is performed using super coiled PBR322 plasmid DNA. The result of agarose gel electrophoresis indicates that the Cu(II), Co(II) complexes are able to carry out an efficient cleavage of PBR322 DNA. Additionally, all the compounds were screened for anti-bacterial activity. From the docking studies we found that $[Cu(L_3)(ABPH)(H_2O)]$ shown the good binding affinity with the receptor when compare with other two molecules. Further it is also confirmed by the antibacterial studies.

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References

1. Guo Z, Sadler PJ (1999) Metals in medicine. Angew Chem Int Ed 38: 1512–1531
2. Dyson P J, and Sava G (2006) Metal-based anti tumor drugs in the post genomic era. Dalton Trans 1929–1933. DOI: 10.1039/B601840H
3. Williams DR (1972) Metals, ligands, and cancer. Chem Rev 72:203–213
4. Romero-Canelón I, Sadler PJ (2013) Next-generation metal anticancer complexes: multitargeting via redox modulation. Inorg Chem 52: 12276–12291
5. Bruijnincx PCA, Sadler PJ (2008) New trends for metal complexes with anticancer activity. Curr Opin Chem Biol 12:197–206
6. Campbell MJM (1975) Coord Chem Rev 15:279–312, 241–249
7. John RP, Sreekanth A, Kurup MRP, Usman A, Razak IA, Fun HK (2003) Spectral studies and structure of a 2-hydroxyacetophenone 3-hexamethyleneiminyl thiosemicarbazonate(−2) copper(II)complex containing 1,10- phenanthroline Spectrochem. Acta A 59:1349–1358
8. Liberta AB, West DX (1992) Antifungal and antitumor activity of heterocyclic thiosemicarbazones and their metal complexes: current status. BioMetals 5:121–126
9. Ali MA, Livingiston SE (1974) Coord Chem Rev 13:273
10. Yamada S (1999) Coord Chem Rev 192:537
11. Erkkila KE, Odom DT, Barton JK (1999) Chem Rev 99:2777
12. Armitage B (1998) Chem Rev 98:1171
13. Pogozelski WK, Tullius TD (1998) Chem Rev 98:1089
14. Mrksich M, Dervan PB (1993) Antiparallel side-by-side hetero dimer for sequence-specific recognition in the minor groove of DNA by a distamycin/1-methylimidazole-2-carboxamide-netropsin pair. J Am Chem Soc 115(7):2572–2576
15. Jiang GB, Xie YY, Lin GJ, Huang HL, Liang ZH, Liu YJ (2013) Synthesis, characterization, DNA interaction, antioxidant and anticancer activity studies of ruthenium(II) polypyridyl complexes. J Photochem Photobiol B 5(129):48–56
16. Marmur J (1961) A procedure for the isolation of deoxyribonucleic acid from micro-o rganisms. J Mol Biol 3:208–218

17. Saeed A, Al-Masoudi NA, Latif M (2013) Synthesis and antiviral activity of New substituted methyl [2-(arylmethylene-hydrazino)-4-oxo-thiazolidin-5-ylidene]acetates. Arch Pharm Chem Life Sci 346: 618–625
18. Sukriye G, Ozdemir N, Bal-Demirci T, Ulkuseven B, Dincer M, Andac O (2010) Quantum-chemical, spectroscopic and X-ray diffraction studies on nickel complex of 2-hydroxyacetophenone thiosemicarbazone with triphenylphospine. Polyhedron 29:2393–2403
19. Xue L-W, Wang X, Zhao G-Q (2012) Synthesis, crystal structure, and atimicrobial activity of a novel trinuclear Cadmium(II) complex derived from 2-ethoxysalicylaldehydethiosemicarbazone synthesis and reactivity in inorganic. Metal-Org Nano-Metal Chem 42(9): 1334–1338
20. Shobha Devi C, Anil Kumar D, Singh SS, Gabra N, Deepika N, Praveen Kumar Y, Satyanarayana S (2013) Synthesis, interaction with DNA, cytotoxicity, cell cycle arrest and apoptotic inducing properties of ruthenium(II) molecular "light switch" complexes. Eur J Med Chem 64(2013):410–421
21. Tan CP, Liu J, Chen LM, Shi S, Ji LN (2008) Synthesis, structural characteristics, DNA binding properties and cytotoxicity studies of a series of Ru(III) complexes. J Inorg Biochem 102(8):1644–1653
22. Cheung LM, Cheung PCK, OoiV EC (2003) Antioxidant activity and total phenolics of edible mushroom extracts. Food Chem 81:249–255
23. Anupama B, Sunita M, Shiva Leela D, Ushaiah B, Gyana Kumari C (2014) Synthesis, spectral characterization, DNA binding studies and antimicrobial activity of Co(II), Ni(II), Zn(II), Fe(III) and VO(IV) complexes with 4-aminoantipyrine schiff base of ortho-vanillin. J Fluoresc 24:1067–1076
24. Williams RS, Dodson GE, Limbo O, Yamada Y, Williams JS, Guenther G, Classen S, Glover JN, Iwasaki H, Russell P, Tainer JA (2009) Nbs1 flexibly tethers Ctp1 and Mre11-Rad50 to coordinate DNA double-strand break processing and repair. Cell 139(1):87–99. doi:10.1016/j.cell.2009.07.033
25. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ (2009) AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. J Comput Chem 30:2785–2791
26. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ (1998) Automateddocking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem 19:1639–1662
27. Perugu S, Jalli H, Bhanoori M (2013) SSViewer: sequence structure viewer. Int J Innov Technol Exploring Eng (IJITEE) 3(4):2278–3075
28. Kavitha P, Saritha M, Laxma Reddy K (2013) Synthesis, structural characterization, fluorescence, antimicrobial, antioxidant and DNA cleavage studies of Cu(II) complexes of formyl chromone Schiff bases. Spectrochim Acta A Mol Biomol Spectrosc 102:159–168