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Mixed ligand complexes of Cu^{II} with pyridoxal thiosemicarbazone and dipeptides: Synthesis, characterization, DNA binding, cleavage, antibacterial and docking studies†

Ch. Venkata Ramana Reddy[a]*, Saritha Aduri[b] and B. Sireesha[c]

[a]Department of Chemistry, Jawaharlal Nehru Technological University Hyderabad, Hyderabad-500 085, India

E-mail: vrr9@yahoo.com

[b]Department of Chemistry, St. Francis College for Women, Hyderabad-500 016, India

[c]Department of Chemistry, Nizam College, Osmania University, Hyderabad-500 001, India

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Mixed ligand complexes of Cu^{II} of the type, [CuAL] where, A = pyridoxal thiosemicarbazone (PLTSC) and L = dipeptide viz. glycyl-glycine (gly-gly), glycyl-L-leucine (gly-leu), glycyl-L-tyrosine (gly-tyr) and glycyl-L-valine (gly-val), have been synthesized. They were characterized by elemental analyses, LC-MS, FTIR, UV-Vis and ESR studies, thermal analysis, molar conductance and magnetic susceptibility measurements. The ligand, A is coordinated through O,N,S donor atoms while, L is coordinated through the O,N,N donor atoms. However, in the complexes [Cu(PLTSC)(gly-leu)(H_2O)] and [Cu(PLTSC)(gly-tyr)(H_2O)], the PLTSC is coordinated only through O,N donor atoms. The interaction of the complexes with calf thymus DNA (CT-DNA) was investigated by UV-Vis absorption and fluorescence emission spectroscopy and also by viscosity measurements, which suggested intercalative mode of binding between the complexes and CT-DNA. The mode of DNA binding was further analyzed by docking studies, which supports intercalative binding. The complexes demonstrate excellent nuclease activity on super coiled plasmid pBR322 DNA in the studies by agarose gel electrophoresis. The metal complexes also exhibit moderate inhibition activity against Gram-positive bacteria.

Keywords: Mixed ligand complexes, pyridoxal thiosemicarbazone, dipeptides, antibacterial, DNA binding, cleavage, docking.

Introduction

Mixed ligand complexes play significant role in biological processes and act as models for mimicking the role of metal ions in biological systems[1]. Copper is an essential cofactor for many enzymes, which catalyze various biochemical reactions[2]. Cu^{II} is known to form stable binary and ternary complexes with a vast variety of ligands[3]. Pyridoxal thiosemicarbazone (PLTSC), a Schiff base derived from pyridoxal and thiosemicarbazide is known to act as a potential ligand with three donor atoms O,N,S. Its binary complexes are known to exhibit anticancer, antileukemic and antiamoebic activities[4–6].

Dieptides are good chelating agents with three or four donor atoms[7]. Transition metal complexes of dipeptides were reported to possess alkaline phosphatase activity, antiinflammatory, antibacterial, antitumor and effective cardiovascular activities[8–10]. The biological and chelating features of PLTSC and dipeptides have generated interest to investigate their mixed ligand complexes with Cu^{II}. Accordingly, we report the synthesis, characterization and biological activity studies of ternary complexes of Cu^{II} involving PLTSC (A) and dipeptides (L) viz. glycyl-glycine (gly-gly), glycyl-L-leucine (gly-leu), glycyl-L-tyrosine (gly-tyr) and glycyl-L-valine (gly-val).

Experimental

Materials:

All the chemicals and dipeptides used were of analytical grade, procured from Sigma Aldrich. PLTSC was prepared by a reported procedure[11].

Physical measurements:

CHN analysis was obtained from Thermo Finnigan 1112 elemental analyzer. Mass spectra of the complexes were re-

†**Invited Lecture.**

corded on LCMS 2010A, Schimadzu spectrometer. The other spectroscopic measurements were made using Schimadzu IR Prestige-21 Spectrometer (KBr, 4000–250 cm^{-1}), Systronics UV-Vis Double beam spectrophotometer 2201, JEOL JES-FA 200 ESR spectrometer at room temperature and Schimadzu Spectrofluorometer, RF-5301 (520–770 nm). The molar conductivity of freshly prepared (10^{-3} *M*) solutions of complexes in DMSO was measured using a Digisun digital conductivity bridge. Thermo gravimetric analyses (TGA) were performed using Schimadzu TGA-50H in nitrogen atmosphere in the temperature range of 0°C to 1000°C with a heating rate of 20°C per min. Magnetic susceptibilities were measured at room temperature on Faraday balance, model 7550 using $Hg[Co(NCS)_4]$ as an internal standard. Diamagnetic corrections were made using Pascal's constants[12]. DNA cleavage experiments were performed with the help of an electrophoresis system (Biotech) supported by a Genei power supply over a potential range of 50–500 V. It was subsequently visualized and photographed by a Transilluminator system (Biotech).

Methods:

DNA binding activity by electronic absorption spectra:

The binding of mixed ligand metal complexes with CT-DNA was studied in potassium phosphate buffer solution of pH 7.2. A solution of DNA in the buffer gave a ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280} of 1.85–1.9, indicating that the DNA was sufficiently free of protein. The concentration of DNA was determined from the UV absorbance at 260 nm using the extinction coefficient, ε_{260} = 6600 M^{-1} cm^{-1}. The absorbance titrations were carried out at a fixed concentration of complexes, to which increments of the CT-DNA stock solutions (2–20 μL) were added. CT-DNA was added to both the complex solution and the reference solution to eliminate the absorbance by CT-DNA itself. The stock solutions of the complexes were prepared in DMSO and diluted suitably with the phosphate buffer to the required concentration of 20 μM. After the addition of DNA to the metal complex, the resulting solution was allowed to equilibrate for 10 min. Later, the absorption was noted. Change in absorption was then fitted to the following equation to obtain intrinsic binding constant (K_b).

$$[DNA]/[\varepsilon_a - \varepsilon_f] = [DNA]/[\varepsilon_b - \varepsilon_f] + 1/K_b[\varepsilon_b - \varepsilon_f] \quad (1)$$

where, $\varepsilon_a = A_{obs}/[complex]$, ε_f and ε_b are molar extinction coefficients of unbound and fully bound complexes respectively. The plots $[DNA]/(\varepsilon_a - \varepsilon_f)$ versus [DNA] have a slope equal to $1/(\varepsilon_a - \varepsilon_f)$ and the intercept equal to $1/K_b$ $(\varepsilon_a - \varepsilon_f)$. The intrinsic binding constant, K_b is the ratio of slope to intercept[13].

Competitive DNA binding fluorescence studies:

Binding of the complexes to CT-DNA was further studied by fluorescence spectroscopy, using ethidium bromide (EB) bound CT-DNA solution in phosphate buffer of pH 7.2. In a typical experiment, 480 μL of CT-DNA solution was added to 2020 μL of EB in buffer solution. The fluorescence intensity was measured upon excitation at λ_{max} 520 nm. The changes in fluorescence emission intensities of EB bound to DNA in the range of 530 to 750 nm were recorded with an increasing amount of the complex concentration from its 50 μM stock solution. Stern-Volmer quenching constants were calculated using the equation,

$$I_0/I = 1 + K_{sv}.r \quad (2)$$

where, I_0 and I are the fluorescence intensities in the absence and presence of the complex respectively, K_{sv} is a Stern-Volmer quenching constant and r is the ratio of total concentration of complex to that of DNA. The value of K_{sv} is given by the ratio of slope to intercept in a plot of I_0/I vs [complex]/[DNA][14,15].

Viscosity measurements:

The viscosity measurements were carried out using the Ostwald viscometer, maintained at 25°C in a thermostatic water bath. Each complex (50 μM) was introduced into CT-DNA solution (300 μM) in phosphate buffer (pH 7.2) present in the viscometer. Flow time of the solutions was recorded in triplicate for each sample and an average flow time was calculated. Data were presented as $(\eta'_{sp}/\eta_{sp})^{1/3}$ versus the ratio of the concentration of the complex to CT-DNA, where, η'_{sp} is the viscosity of CT-DNA in the presence of the complex and η_{sp} is the viscosity of CT-DNA alone[16].

DNA cleavage studies:

Agarose gel electrophoresis technique was used to monitor the DNA cleavage ability of the metal complexes on super coiled pBR 322 plasmid DNA. In the experiment, plasmid DNA (300 ng/3 μL) was treated with the complexes in DMSO (20–60 μM) in 5 mM Tris-HCl/50 mM NaCl buffer (pH 7.2). The mixture was incubated for 1 h at 37°C. A loading

buffer containing 1% bromophenol blue and 40% sucrose (1 μL) was added and loaded onto a 0.8% agarose gel containing EB (1 μg/ml). The gel was run in TAE buffer (40 mM Tris base, 20 mM acetic acid, 1 mM EDTA) of pH 8.3, at a constant voltage, 60 V for 2 h until the bromophenol blue had traveled through 75% of the gel. The bands were visualized by viewing the gel on a transilluminator and photographed[17].

Antibacterial activity:

The complexes were screened for their antibacterial activity using agar well diffusion method[18] against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*E. coli* and *Pseudomonas aeruginosa*) at a concentration of 100 μg per well.

Docking studies:

Docking of the metal complexes with DNA was carried out in Autodock 4.2 as per reported works[19,20]. Crystal structure of DNA was downloaded from protein data bank (www.rcsb.org)pdb id: 1N37[21], which was prepared by protein preparation wizard applying OPLS 2005 force field in Schrodinger suite. A grid was prepared around the intercalation site by selecting the co-crystallized ligand. Metal complexes were constructed and optimized in ChemDraw. These were docked into DNA intercalation site using Autodock 4.2. Molecular interaction diagrams are obtained from PMV[22].

Synthesis of mixed ligand Cu^{II} complexes:

A common method is followed for the synthesis of all the ternary complexes, [CuAL] except for the variation of in dipeptide, L as follows:

Synthesis of [Cu(PLTSC-H)(gly-gly-H)] (1):

0.4838 g (1.7 mmol) of PLTSC and 0.3293 g (1.7 mmol) of gly-gly were added simultaneously to an aqueous solution containing 0.2983 g (1.7 mmol) of $CuCl_2.2H_2O$. An immediate color change was observed and the mixture was refluxed for 3 h. A green colored precipitate was obtained by adjusting the pH to 7–8 with a solution of ammonium hydroxide in methanol. The reaction mixture was continued to reflux for an hour and later filtered, washed several times with hot distilled water, followed by petroleum ether and finally air dried. (Yield: 71%). Anal. Calcd. for $C_{13}H_{18}N_6O_5SCu$: C, 35.89; H, 4.13; N, 19.34. Found: C, 35.86; H, 4.14; N, 19.31; APCI-MS in MeOH: *m/z* 435 [M^+]; IR (KBr) cm^{-1}: 1581 (C=N), 1132 (Ar.C-O), 800 (C=S), 1558 (peptide -NH), 1616 (-COO^- asym), 1392 (-COO^- sym); UV-Vis nm: λ_{max} = 257, 336, 414, 587; μ_{eff} = 1.85 B.M. ΛM [Ω^{-1} cm^2 M^{-1}, 10^{-3}, DMSO]: 009.

Synthesis of [Cu(PLTSC)(gly-leu-2H)H_2O] (2):

The same procedure was followed as explained for complex **1**, using 0.4838 g (1.7 mmol) of PLTSC, 0.3293 g (1.7 mmol) of gly-leu and 0.2983 g (1.7 mmol) of $CuCl_2.2H_2O$. (Yield: 68%). Anal. Calcd. for $C_{17}H_{28}N_6O_6SCu$: C, 40.15; H, 5.51; N, 16.53. Found: C, 40.18; H, 5.46; N, 16.51; APCI-MS in MeOH: *m/z* 508 [M^+], *m/z* 490 [M^+-H_2O]; IR (KBr) cm^{-1}: 1606 (C=N), 1151 (Ar.C-O), 835 (C=S), 1620 (-COO^- asym), 1381 (-COO^- sym); UV-Vis nm: λ_{max} = 258, 341, 420, 593; μ_{eff} = 1.94 B.M. ΛM [Ω^{-1} cm^2 M^{-1}, 10^{-3}, DMSO]: 008.

Synthesis of [Cu(PLTSC)(gly-tyr-2H)H_2O] (3):

The same procedure was followed as explained for complex **1**, using 0.4838 g (1.7 mmol) of PLTSC, 0.4168 g (1.7 mmol) of gly-tyr and 0.2983 g (1.7 mmol) of $CuCl_2.2H_2O$. (Yield: 64%). Anal. Calcd. for $C_{20}H_{26}N_6O_7SCu$: C, 43.01; H, 4.66; N, 15.05. Found: C, 43.05; H, 4.69; N, 15.01; APCI-MS in MeOH: *m/z* 558 [M^+], *m/z* 540 [M^+-H_2O]; IR (KBr) cm^{-1}: 1556 (C=N), 1141 (Ar.C-O), 837 (C=S), 1598 (-COO^- asym), 1381 (-COO^- sym); UV-Vis nm: λ_{max} = 257, 337, 418, 595; μ_{eff} = 2.03 B.M. ΛM [Ω^{-1} cm^2 M^{-1}, 10^{-3}, DMSO]: 009.

Synthesis of [Cu(PLTSC-H)(gly-val-H)] (4):

The same procedure was followed as explained for complex **1**, using 0.4838 g (1.7 mmol) of PLTSC, 0.3048 g (1.7 mmol) of gly-val and 0.2983 g (1.7 mmol) of $CuCl_2.2H_2O$. (Yield: 71%). Anal. Calcd. for $C_{16}H_{24}N_6O_5SCu$: C, 40.34; H, 5.50; N, 19.27. Found: C, 40.37; H, 5.51; N, 19.23; APCI-MS in MeOH: *m/z* 476 [M^+]; IR (KBr) cm^{-1}: 1610 (C=N), 1172 (Ar.C-O), 800 (C=S), 1544 (peptide -NH), 1598 (-COO^- asym), 1394 (-COO sym); UV-Vis nm: λ_{max} = 290, 337, 413, 598; μ_{eff} = 1.92 B.M. ΛM [Ω^{-1} cm^2 M^{-1}, 10^{-3}, DMSO]: 005.

Results and discussion

Elemental analysis and physical properties:

The mixed ligand complexes, [Cu^{II}-PLTSC-dipeptide] obtained were colored solids, amorphous and stable at room temperature. They are soluble in DMSO and DMF. The observed CHN analysis results are in good agreement with the calculated values. The low molar conductivity (05–07 Ω^{-1} cm^2 mol^{-1}) of the complexes indicate their non-electrolytic nature. The magnetic moment, μ_{eff} values of the complexes

found between 1.85–2.03 B.M., suggest the presence of one unpaired electron in Cu^{II} and the paramagnetic nature of the complexes.

Mass spectra:

The APCI(+) mass spectrum of [Cu^{II}-C(PLTSC)(gly-gly)] (**1**) (Fig. S1) showed peak at *m/z* 435 for [Cu(PLTSC-H)(gly-gly-H]$^+$. The APCI (–) mass spectrum of [Cu^{II}-(PLTSC)(gly-leu)] (**2**) (Fig. S2) showed peaks at *m/z* 508 due to [Cu(PLTSC)(gly-leu-2H)(H_2O)]$^+$ and *m/z* 490 corresponding to [Cu(PLTSC)(gly-leu-2H)]$^+$. The APCI (–) mass spectrum of [Cu^{II}-(PLTSC)(gly-tyr)] (**3**) (Fig. S3) showed peaks at *m/z* 558 due to [Cu(PLTSC)(gly-leu-2H)(H_2O)]$^+$ and *m/z* 540 with respect to [Cu(PLTSC)(gly-leu-2H)]$^+$. The APCI (+) mass spectrum of [Cu^{II}-C(PLTSC)(gly-val)] (**4**) (Fig. S4) showed peak at *m/z* 477 for [Cu(PLTSCH)(gly-val-H]$^+$. The *m/z* values of the four mixed ligand complexes suggest the stoichiometry of the complexes in 1:1:1 (M:A:L) ratio.

IR spectra:

The characteristic IR bands of the ligands and ternary complexes observed from the IR spectra (Figs. S5-S8) are listed in Table 1. IR spectral data of mixed ligand complexes indicate the coordination of PLTSC (A) through O,N,S donor atoms in the complexes **1** and **4**. In the complexes **2** and **3** the PLTSC is coordinated only through N,O donor atoms. The band attributed for Py-NH^+ due to the migration of Py-OH proton to Py-N in PLTSC[23] around 2850 cm^{-1} has disappeared in **1** and **4**, while it is retained in the complexes **2** and **3**. In all the ternary complexes, PLTSC is coordinated to Cu^{II} ion via azomethine nitrogen and oxygen atom of Py-OH. A difference was observed in the coordination of S atom of PLTSC, wherein the stretching frequency of C=S has been shifted to lower values from 841 cm^{-1} to 800 cm^{-1} in the complexes **1** and **4**, while in case of complexes **2** and **3**, no such change was observed. This indicates the non-coordination of 'S' of thioamide as also reported by others[24].

Coordination of the free amino group in all the dipeptides has been indicated by an increase in the stretching frequency of -NH_2 by about 50–70 cm^{-1} around 3300–3340 cm^{-1} in the complexes. The coordination of peptide -NH in **1** and **4** was indicated by the shift in the stretching frequency from 3055–3070 cm^{-1} to around 3200 cm^{-1} and bending frequency from 1520–1530 cm^{-1} to about 1545 cm^{-1}. However, the absence of bending vibration of peptide NH in the complexes **2** and **3** indicate its coordination after the loss of proton. The difference [$\Delta = \nu_{as}(COO^-) - \nu_s(COO^-)$] above 200 cm^{-1}, for all the complexes were larger than the Δ values of the free dipeptides (150–185 cm^{-1}), indicate the coordination of carboxylate oxygen[25].

Table 1. Characteristic IR bands (cm^{-1}) of the ligands and ternary complexes

Ligand/complex	$\nu(NH_2)$	ν(NH)	ν(Py-NH^+)	ν(C=N)	ν(Py ring)	$\nu(COO^-)$	ν(Ar.C-O)	ν(NH) bend	ν(C=S)
PLTSC	3296	3184	2823	1625	1529		1126		841
	3184				1504		1184		
gly-gly	3286	3055				1575		1533	
						1408			
1	3358	3176		1604	1494	1616	1151	1558	800
	3290					1392			
gly-leu	3257	3068				1556		1514	
						1406			
2	3329	3192	2846	1606	1500	1620	1151		835
	3242				1452	1381			
gly-tyr	3361	3030				1604	1145	1517	
						1419	1111		
3	3329	3192	2864	1610	1498	1598	1151		837
	3226					1381			
gly-val	3251	3070				1558		1519	
						1406			
4	3334	3143		1610	1492	1598	1172	1544	800
	3290					1394			

Thermal analysis:

The thermograms of the complexes **1** and **4** (Figs. S9 and S12) show a weight loss from 230–250ºC, accompanied by an exothermic peak in the DTA curve, indicate the decomposition of the complex. Whereas, a weight loss in the thermograms of **2** and **3** (Figs. S10 and S11) between 150–180ºC along with an endothermic peak in the DTA curve at 170ºC, suggest the presence of one mole of coordinated water in these complexes. From 250ºC onwards all the complexes exhibited a similar gradual decomposition pattern forming metal oxide as the final residue.

Electronic spectra:

The electronic spectra of the mixed ligand complexes (Figs. S13-S16) showed bands of low intensity at 588 nm (17,000 cm^{-1}) assigned to d-d transition, $^2B_{1g} \rightarrow {}^2B_{2g}$ with Cu^{II} ion in the tetragonally distorted octahedral geometry[26]. Additional bands at 290–257 nm (34,482–38,910 cm^{-1}), 340–336 nm (29,360–29,800 cm^{-1}) and at 417 nm (24,000 cm^{-1}) corresponding to π-π^* and n-π^* transitions of PLTSC and charge transfer transitions were observed.

ESR spectra:

A single resonance line was observed in the powder ESR spectra of the mixed-ligand complexes of **1** and **4** (Figs. S17 and S20). However, the ESR spectra of **2** and **3** (Figs. S18 and S19) demonstrated a hyperfine structure, with $g_{||} > g_{\perp} > g_e$, which is typical for an axially elongated symmetry, with the unpaired electron localized in the $d_{x^2-y^2}$ orbital of the Cu^{II} ion[27]. The $g_{||}$ values of the complexes were also less than 2.3, indicating a significant covalent nature of M-L bonds.

The spectral and analytical data supports the tetragonally distorted octahedral geometry of the complexes. The proposed structures of the complexes are presented in Fig. 1.

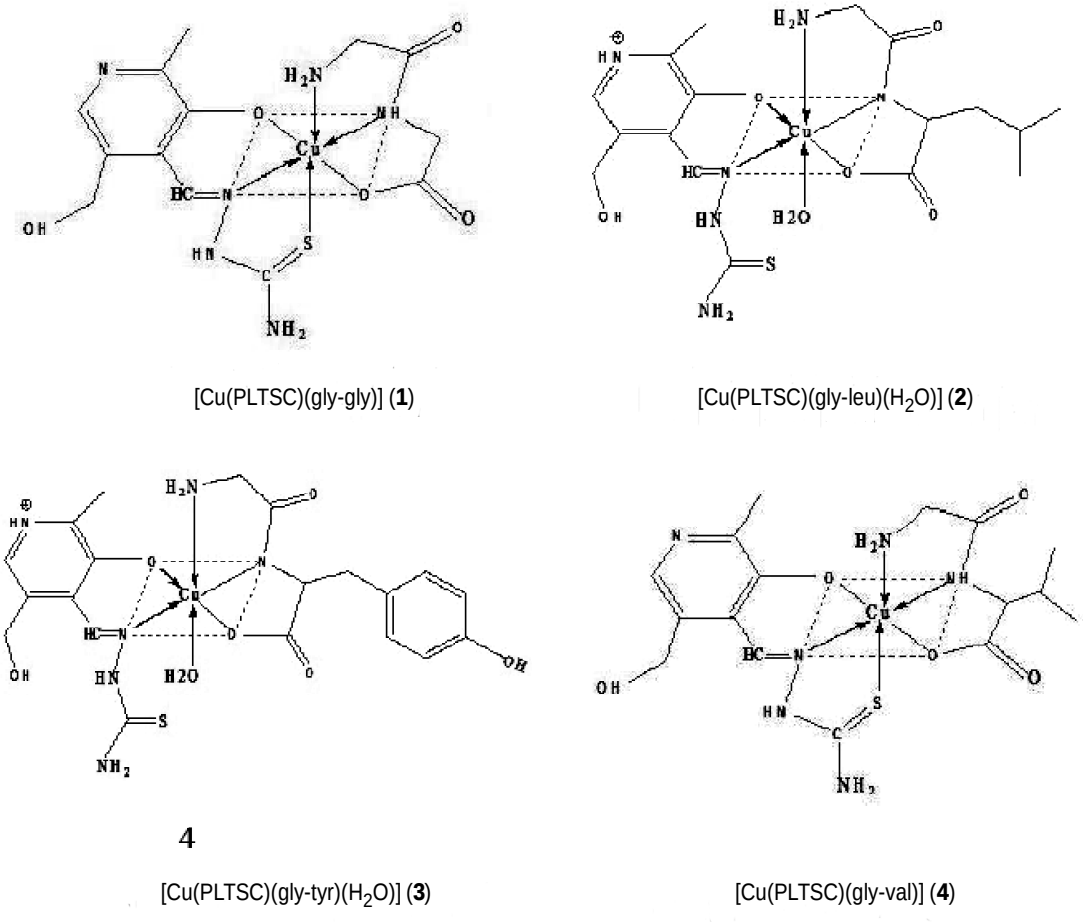


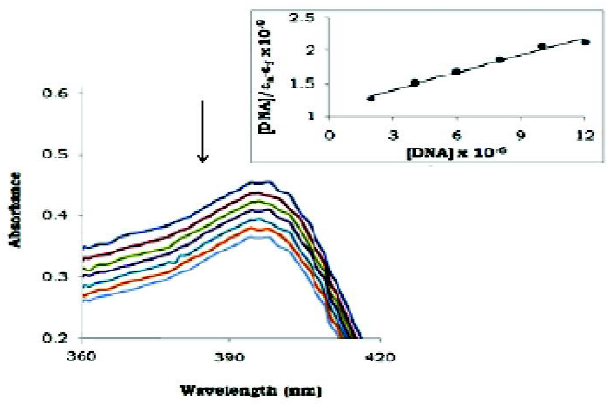
Fig. 1. Proposed structures of the [Cu^{II}AL].

DNA binding studies:

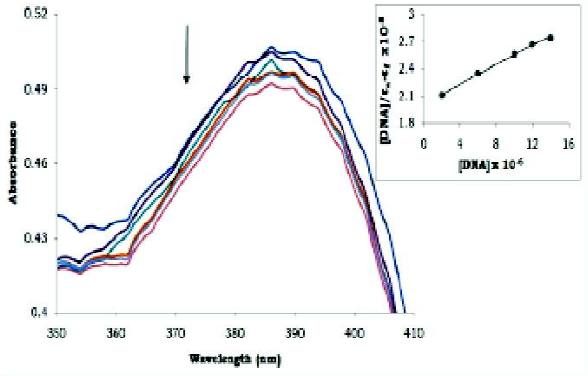
Electronic absorption spectral titration:

The binding of transition metal complexes to the DNA can be conveniently studied by UV-Vis absorption titrations, by recording the changes in the absorption of the metal complex with the increase in concentration of DNA. The interaction between the metal complex and DNA can be either covalent or non-covalent. Covalent binding involves the replacement of the labile ligand by a nitrogen base of DNA, while the non-covalent interaction may include electrostatic, groove binding and intercalation. Usually intercalation of a complex to DNA leads to hypochromism associated with red or blue shift, due a strong π-π interaction between the DNA base pairs and the hetero atomic aromatic ring of the ligand. The strength of intercalation is indicated by the extent of hypochromism[28].

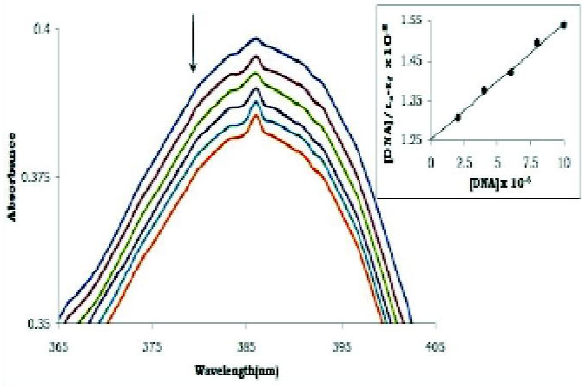
In the present study, electronic absorption titrations of the ternary Cu^{II} complexes with CT-DNA showed hypochromism with red or blue shift as shown in Fig. 2, indicating their binding through intercalation, owing to the extended π-electron system of the ligands. Quantitative comparison of the binding affinity of the complexes can be made by their



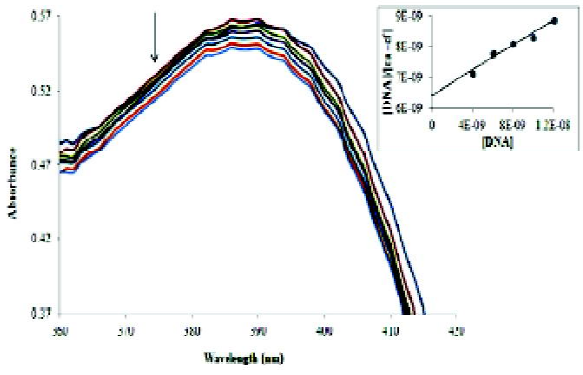
[Cu(PLTSC)(gly-gly)] (**1**)



[Cu(PLTSC)(gly-leu)(H_2O)] (**2**)



[Cu(PLTSC)(gly-tyr)(H_2O)] (**3**)



[Cu(PLTSC)(gly-val)] (**4**)

Fig. 2. Absorption spectra of mixed ligand Cu^{II} complexes (20 μM) in phosphate buffer (pH 7.2) upon addition of CT-DNA (0–20 μM). Arrow indicates hypochromism with increase in concentration of DNA. Inset: Plot of [DNA]/($\varepsilon_b - \varepsilon_f$) versus [DNA].

intrinsic binding constants, K_b.

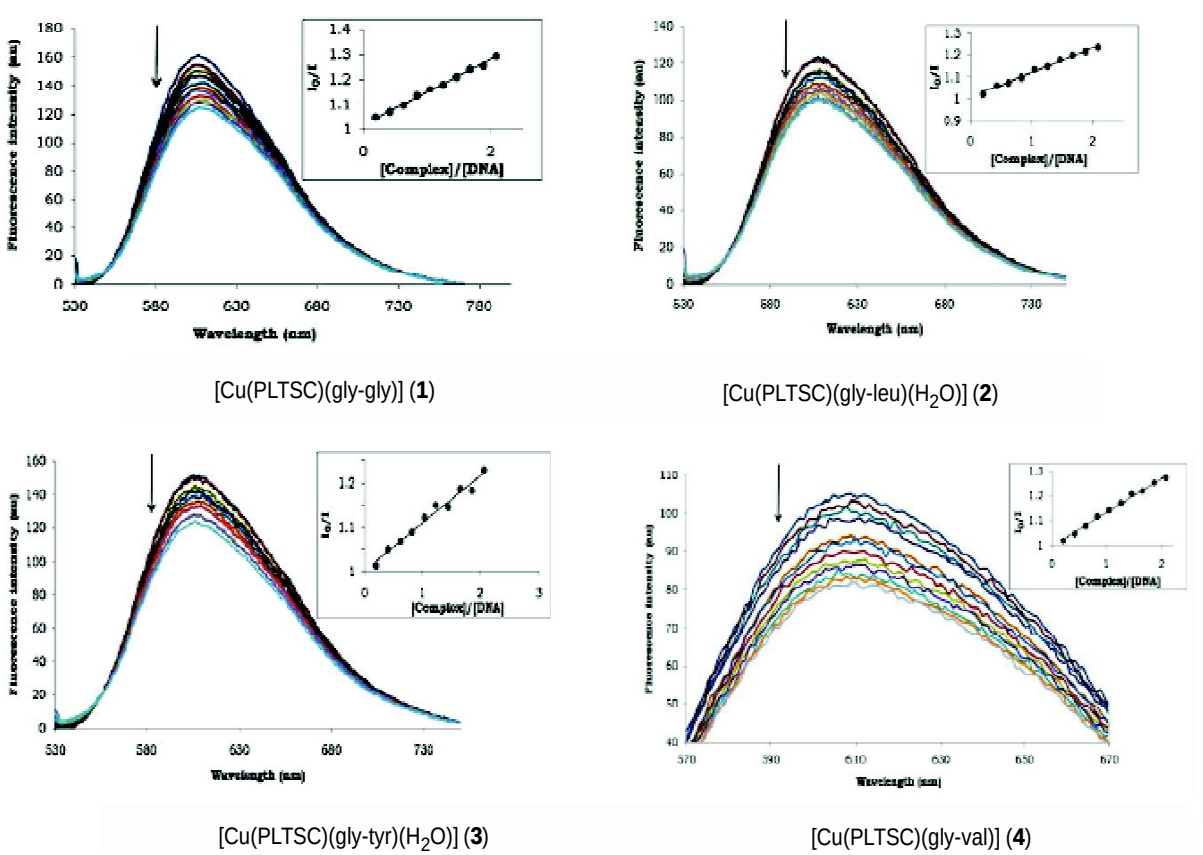
The K_b values were found to be 7.4588×10^4, 2.5755×10^4, 2.2328×10^4 and 3.3333×10^4 respectively for, [Cu^{II}-PLTSC-(gly-gly)], [Cu^{II}-PLTSC-(gly-leu)], [Cu^{II}-PLTSC-(gly-tyr)] and [Cu^{II}-PLTSC-(gly-val)], which indicate strong binding affinity of the complexes towards CT-DNA.

Competitive DNA binding studies of the complexes:

The competitive DNA binding of the ternary complexes was evaluated by monitoring the changes in the fluorescence emission intensity of ethidium bromide (EB) bound to CT-DNA with the increase in the concentration of the complex. When the metal complexes were added to the EB-DNA adduct, the DNA induced emission intensity of EB gets reduced due to the displacement of EB by the complex[29]. The quenching in the fluorescence intensity of DNA bound EB with the increase in the concentration of complexes is presented in Fig. 3. The competitive DNA binding ability of the complexes in terms of Stern-Volmer constant were found to be 0.1294, 0.1112, 0.1058 and 0.1388 for [Cu^{II}-PLTSC-(gly-gly)], [Cu^{II}-PLTSC-(gly-leu)], [Cu^{II}-PLTSC-(gly-tyr)] and [Cu^{II}-PLTSC-(gly-val)] respectively.

Viscometric measurements:

When a metal complex binds to DNA through intercalation, it causes a significant increase in the viscosity of DNA solution. This is due to the increase in overall DNA contour length by the insertion of the complex in between the base pairs leading to increase in separation of base pairs at intercalation sites. But no change or less change occurs in the viscosity of DNA solution when a complex binds in the DNA grooves[30]. The effect of EB and the complexes on the viscosity of DNA was studied to understand their mode of binding with the DNA and the results are shown in Fig. 4. The increase in the viscosity of DNA solution, upon the addition



[Cu(PLTSC)(gly-gly)] (**1**) [Cu(PLTSC)(gly-leu)(H_2O)] (**2**)

[Cu(PLTSC)(gly-tyr)(H_2O)] (**3**) [Cu(PLTSC)(gly-val)] (**4**)

Fig. 3. Emission spectra of EB bound CT-DNA in the absence and presence of Cu^{II} complexes. Arrow shows the decrease in emission intensity with the increase in complex concentration. Inset: Plot of I_0/I versus [Complex]/[DNA].

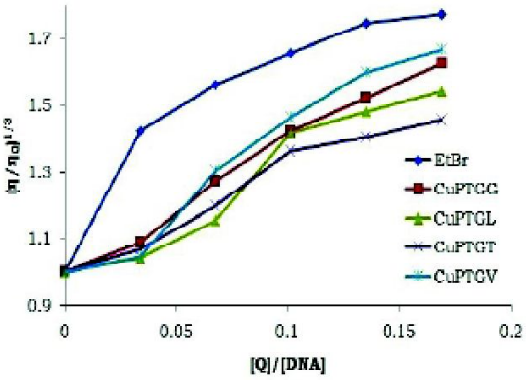


Fig. 4. Effect of increasing amounts of EB and mixed ligand complexes of Cu^{II} on the relative viscosities of CT-DNA at 25°C. (In the figure, EtBr = EB; PT = PLTSC; GG = gly-gly; GL = gly-leu; GT = gly-tyr and GV = gly-val).

of the complexes indicate their intercalative mode of binding to CT-DNA.

DNA cleavage study:

When plasmid DNA is subjected to electrophoresis, the fastest migration will be observed for the super coiled form (Form I). When cleavage or nicking of one strand takes place, the super coiled form relaxes to produce a slow moving open circular or nicked form (Form II). If both strands are cleaved, a linear form (Form III) is generated, which migrates in between[31].

The nuclease activity of the metal complexes on pBR 322 DNA studied by gel electrophoresis is presented in Fig. 5. The results show that the ternary Cu^{II} complexes are able to induce hydrolytic cleavage of super coiled DNA from super coiled form to nicked form. This may be due to the presence of nucleophiles like water or hydroxyl groups in the metal complexes, which facilitate the cleavage[32].

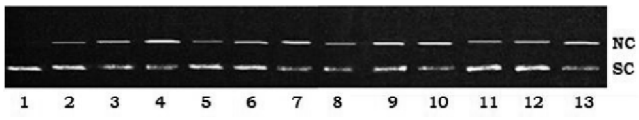


Fig. 5. Agarose gel electrophoresis pattern for the cleavage of supercoiled pBR 322 DNA by mixed ligand Cu^{II} complexes. Lane **1**: DNA control, Lanes **2-4:** DNA + [Cu^{II}-PLTSC-(gly-gly)] (20, 40, 60 μM respectively), Lanes **5-7:** DNA + [Cu^{II}-PLTSC-(gly-leu)] (20, 40, 60 μM respectively), Lanes **8-10:** DNA + [Cu^{II}-PLTSC-(gly-tyr)] (20, 40, 60 μM respectively) and Lanes **11-13:** DNA + [Cu^{II}-PLTSC-(gly-val)] (20, 40,60 μM respectively).

Antibacterial activity:

The results of the antibacterial studies presented in Table 2, indicate moderate antibacterial activity of the complexes against Gram-positive bacteria, *S. aureus* and *B. subtilis*. However, the complexes were inactive against Gram-negative bacteria, *E. coli* and *P. aerugenosa*. This may be due to the outer membrane of Gram-negative bacteria, which provides an extra layer of protection and a formidable barrier for many antibiotics[33].

Table 2. Zone of inhibition values (mm) of Gram-positive bacteria by the metal complexes

Sr. No.	Complex	*Staphylococcus aureus*	*Bacillus subtilis*
1.	Cu^{II}-PLTSC-(gly-gly)	15	12
2.	Cu^{II}-PLTSC-(gly-leu)	16	12
3.	Cu^{II}-PLTSC-(gly-tyr)	11	8
4.	Cu^{II}-PLTSC-(gly-val)	18	16
	Amikacin (30 μg) (standard)	19	22

Docking studies:

Docking studies provide the information regarding the possible binding mode of compounds with the macromolecule like DNA and the approximate binding affinity. In the present study, all the metal complexes were docked into the intercalation site of DNA using Autodock 4.2. The dock score provided in Table 3 represents the binding affinity of the Cu^{II} complexes, **1** to **4**. All the complexes showed equivalent dock score ranging from –7.13 to –6.57 kcal/mol. The dock score is in good agreement with the experimental binding constant values. The dock score and estimated inhibition constant, K_i indicated highest binding of complexes **2** and **4**. Analysis of docking pose of complex **4** was carried out to understand the binding mode. Fig. 6 shows the dock pose of complex **4** in the intercalation site of DNA and Figs. 7 and 8 show the interaction of metal complex with DNA bases. Complex **4**

Table 3. Dock score and calculated inhibition constant from DNA docking studies in AutoDock 4.2

Complex	Dock score (kcal/mol)	Estimated inhibition constant K_i (μM)
1	–6.87	9.17
2	–7.13	5.92
3	–6.57	15.35
4	–7.09	6.32

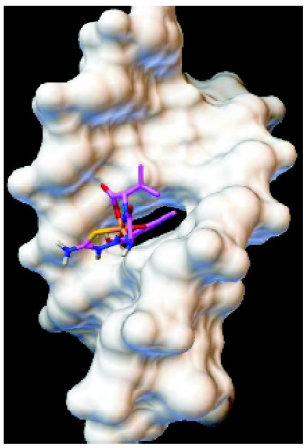


Fig. 6. Dockpose of complex **4** into intercalation site of DNA:DNA in molecular surface form.

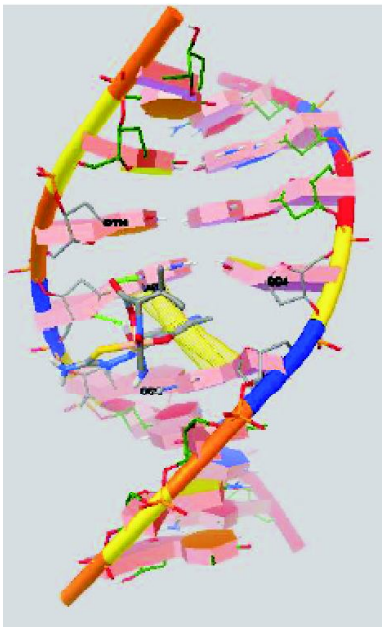


Fig. 7. Dockpose of complex **4** into intercalation site of DNA:DNA in ribbon form showing hydrogen bond interaction and π-π interaction with DNA bases.

forms two hydrogen bonds with G13 base. The pyridine moiety of the complex intercalates between the bases pairs G13:C4 and C12:G5, forming strong π-π interaction with DNA bases G13 and G5. All the complexes have pyridine moiety and hence show the similar kind of π-π interaction resulting in closer binding affinity values. Thus, the docking studies indicate intercalative mode of binding of the complexes with the DNA, which is confirmed from the experimental studies.

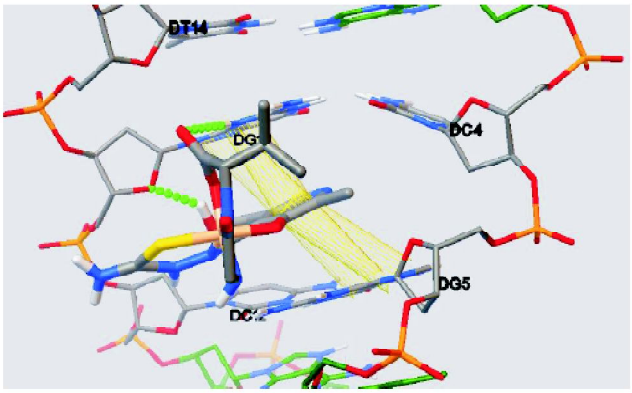


Fig. 8. Dockpose of complex **4** into intercalation site of DNA showing hydrogen bond interaction with DNA base G13 and π-π interaction with DNA bases G13 and G5.

Conclusion

The new mixed ligand Cu^{II} complexes with PLTSC and dipeptides were characterized by spectral and analytical techniques. The results indicated the distorted octahedral geometry of the complexes. All the complexes exhibited good binding activity towards CT-DNA by intercalative mode, which was also supported by the docking studies. The complexes were found to show hydrolytic cleavage of pBR 322 plasmid DNA. The complexes inhibited the growth of Gram-positive bacteria.

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