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Article

Ternary Copper(II) Complexes Containing Thiosemicarbazide: DNA Binding, Antimicrobial Activities, and DFT Studies

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ABSTRACT

Two copper complexes of type [Cu(phen)(L-Ser)(Tsc)]NO_3 (1), [Cu(bpy)(L-Ser)(Tsc)]NO_3 (2) (L-ser = L-serine, Tsc = Thiosemicarbazide, phen = 1,10-phenanthroline, and bpy = 2,2'-bipyridyl) have been synthesized and characterized by elemental analyses, ultraviolet-visible, infrared, and electron paramagnetic resonance (EPR) spectral studies. Based on elemental analyses and EPR studies confirm that two complexes have five coordinated geometry. DNA-binding properties of these metal complexes were investigated using absorption spectroscopy, fluorescence spectroscopy, viscosity measurements, and cyclic voltammetry. The binding ability of complexes differs in terms of binding constant; this may be attributed to difference in ligand coordinated the metal center, complex 1 shows higher binding affinity than complex 2. Complexes 1 and 2 have good antibacterial and antifungal activity on comparing with standard drugs.

Key words: DNA binding, Copper(II) complex, Thiosemicarbazide, L-serine.

1. INTRODUCTION

Transition metal complexes have potential DNA binding and cleavage properties in physiological condition and play a key role in medicinal biochemistry [1-4]. The use of such metal complexes as DNA-binding agents in sequence, as diagnostic agents in biochemistry applications and for drug development has generated much interest to develop this chemotherapy area further. The binding/cleavage of DNA targeted toward different constituents of DNA, namely, the phosphodiester linkage, deoxyribose, and heterocyclic bases. The DNA cleavage involves nucleobase degradation of the sugar moiety by abstraction of hydrogen atoms. Different modes of DNA cleavage, oxidative cleavage of DNA with irradiation of ultraviolet (UV)-visible light is much interest for medical applications of such complexes in treatment for cancer cells [5-9].

The photochemotherapeutic approach from the chemotherapeutic success of cis-platin as well as macrocyclic metal based complexes like phthalocyanine and related compounds [10-12]. Such complexes are used as potential anticancer drugs in chemotherapy. Such complexes are used as potential anticancer drugs in chemotherapy. Metal complexes showing DNA cleavage activity are limited to the complexes of polypyridyl bases for photophysical properties [13-15]. Recently, platinum(IV) complex as a potential anticancer drug cisplatin alternative is reported to show photocytotoxic activity [16]. DNA-binding activity of biologically important amino acid containing copper(II) complexes is really interested, recently, copper(II) complexes of the type amino acid-Cu-heterocyclic base show efficient DNA binding, cleavage, and cytotoxicity [17-22]. The present work deals to develop this chemistry further by synthesizing new ternary copper(II) complexes of L-serine and heterocyclic bases. This amino acid group has the potential to form hydrogen bonding interaction with double-stranded DNA and could show good DNA-binding propensity.

2. EXPERIMENTAL

2.1. Materials and Methods

Calf thymus DNA was obtained from Sigma–Aldrich, Germany, and was used as such copper(II) chloride dihydrate, 1,10-phenanthroline, L-serine, and semicarbazide were purchased from Merck, Plasmid pBR322 DNA was purchased from Genei, India. The DNA-binding titration was carried out in the buffer (50 mM NaCl–5 mM Tris–HCl, pH 7.1) at room temperature. Absorption spectra were recorded on a UV/visible Shimadzu 2450 spectrophotometer using cuvettes of 1-cm path length. Fourier-transform infrared (FT-IR) spectra were recorded on a FT-IR Perkin Elmer spectrophotometer with samples prepared as KBr pellets. Electron paramagnetic resonance (EPR) spectra were recorded on Varian E-112 EPR spectrometer at room temperature, the field being calibrated with DPPH = 1, 10-diphenyl-2-picrylhydrazyl (g =2.0037). Emission spectra were recorded on a JY Fluorolog-3-11 spectrofluorometer. Cyclic voltammetry (CV) experiments were recorded on CHI 602D (CH Instruments Co., USA) electrochemical analyzer under oxygen-free conditions using a three-electrode cell in aqueous solution with KCl (0.1 M) as the supporting electrolyte. A pt wire, glassy carbon, and the Ag/AgCl (in saturated KCl solution) electrodes were used as counter, working, and reference.

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2.2. Preparation of [Cu(phen)(L-Ser)(Tsc)]NO_3

Complex [Cu(phen)(L-Ser)(H_2O)]NO_3 was prepared in 72% yield from a reaction of copper nitrate (1 mmol) in water with a solution of phenanthroline (1 mmol) in methanol and L-serine (1 mmol) in NaOH solution. The reaction mixture was stirred for 5 h at room temperature. After the slow evaporation, blue colored [Cu(phen)(L-Ser)(H_2O)] NO_3 was obtained. The aqueous solution of complex (1.0 mmol) is treated with thiosemicarbazide and stirred for 4 h, the bluish-green color solution was filtered after the solvent evaporation, and the bluish-green complex 1 was washed with methanol and ether. Yield 56%; analytical (%) calculated for $C_{16}H_{19}CuN_7O_6S$: C, 38.36; H, 3.82; N, 19.57. Found: C, 37.01; H, 3.75; N, 18.66. Infrared (IR) (KBr pellet): 3366, 3213, 3134, 2949, 1751, 1633, 1586, 1519, 1427, 1381, 1311, 1265, 1124, 1041, 862, 719, 628, 572 cm^{-1}. UV-visible (λ, nm): 270 and 542 nm.

2.3. Preparation of [Cu(bpy)(L-Ser)(Tsc)]NO_3

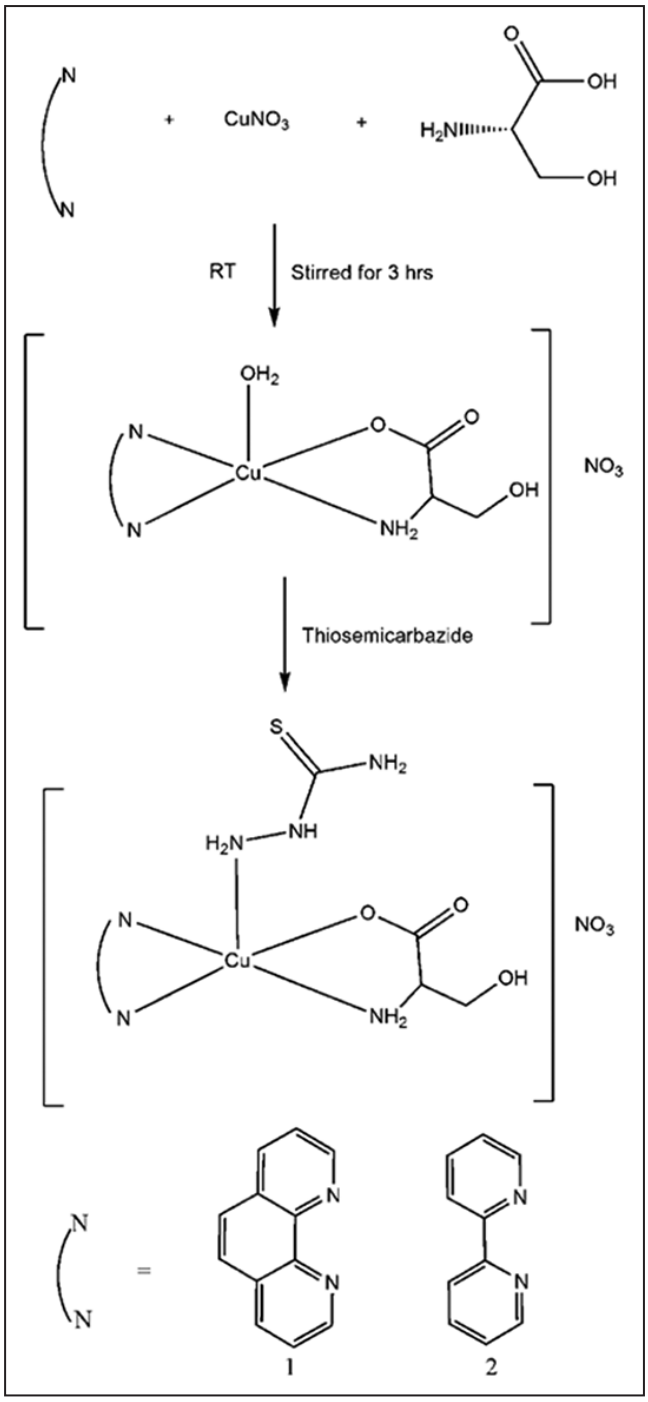
The preparation of complex **2** was described as above. Yield 62%; analytical (%) calculated for $C_{14}H_{19}CuN_7O_6S$: C, 35.25; H, 4.02; N, 20.56. Found: C, 34.54; H, 3.91; N, 19.90. IR (KBr pellet): 3145, 3089, 2933, 2875, 2771, 2627, 1585, 1566, 1506, 1386, 1373, 1360, 1319, 1269, 1232, 1136, 717, 540 cm^{-1}. UV-visible (λ, nm): 300 and 610 nm.

DNA binding, cleavage, antimicrobial, and anticancer experiment details are given in literature [23].

3. RESULTS AND DISCUSSION

3.1. Synthesis and general aspects

Two copper(II) complexes (**1** and **2**) were synthesized (Scheme 1) by ligand substitution method from the complex [Cu(phen)(L-Ser)(H_2O)] NO_3. The complexes are soluble in water and other common organic solvents. From the analytical data, we confirmed that the metal-ligand ratio is 1:1:1 in complexes, which are consistent with the obtained elemental analysis. Molar conductance value indicated that complexes have 1:1 electrolytic behavior in solution. The complexes display an intense charge transfer band in 270 and 300 nm for complexes **1** and **2**, respectively, the observed charge transfer due to π–π* transition of N,N-donor coordinated phenanthroline and bipyridyl heterocyclic bases. The d-d band is observed in near 600 nm in aqueous medium and is in agreement with the square-pyramidal geometry. In infrared spectra, asymmetric and symmetric COO- stretching vibrations for complex **1** were observed at 1639 and 1311 cm^{-1}, the differences indicate that carboxylate ion coordinate with copper(II) by monodentate fashion. The complexes have one electron paramagnetic behavior at room temperature due to d^9 electronic configuration for the copper(II) center. The solid state EPR spectra of the copper(II) complexes were recorded in X-band frequencies. At room temperature, the complexes exhibit well defined isotropic feature g = 2.15 for complexes **1** and **2**, respectively. Such isotropic lines are usually the results of intermolecular spin exchange, which broaden the lines.



Scheme 1: Synthesis of complexes 1 and 2.

3.2. DNA-Binding Properties

3.2.1. Electronic spectral studies

The binding behavior of complexes 1 and 2 to calf thymus DNA has been studied by UV-visible titration. The absorption spectral traces of complexes **1** and **2** with increasing concentration of CT-DNA shown in Figure 1. We have observed a minor hypsochromic shift of 2 nm for complexes **1** and **2** and hyperchromicity, hypochromicity for phenanthroline, and bipyridyl complexes, respectively. The binding constants (K_b) of the complexes to CT-DNA are obtained by monitoring the change in absorption intensity of the charge transfer band with increasing concentration of CT-DNA, keeping the complex concentration constant [24]. The complex **2** shows weak binding to the DNA due to less extended planarity when compared to complex **1**, which is consistent with hypochromism behavior of complex 2. The Kb values of complexes **1** and **2** are 7.65×10^4 and 4.18×10^4, respectively. The higher binding constant of phen complex in comparison to bipyridyl complex could be due to the presence of planar rings facilitating non-covalent interactions with CT-DNA molecule.

3.3. Fluorescent Spectral Studies

The emission spectral studies are used to study the relative binding of complexes to CT-DNA. Ethidium bromide (EB) emits fluorescence light in the presence of DNA due to strong intercalation, and its fluorescence behavior quenched by successive addition of molecules.

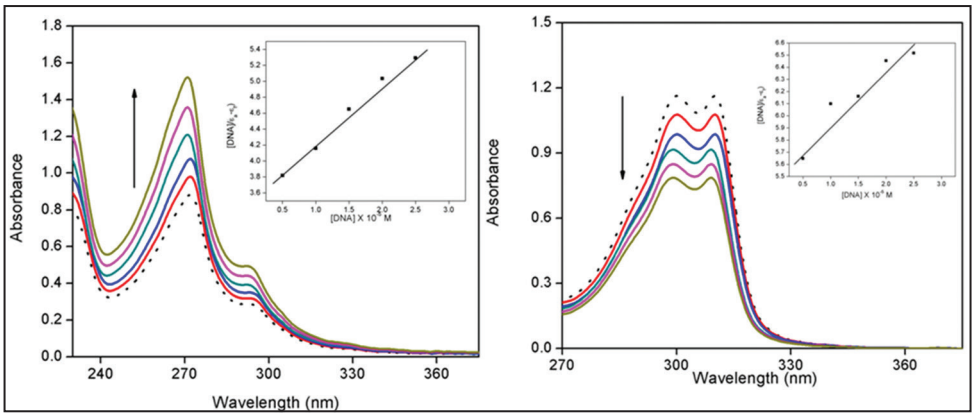


Figure 1: Absorption spectral traces on addition of CT-DNA to complexes 1 and 2 (shown by arrow). Inset plot of [DNA]/(ε_a-ε_f) versus [DNA] for absorption titration of CT-DNA with complex at 270 and 300 nm.

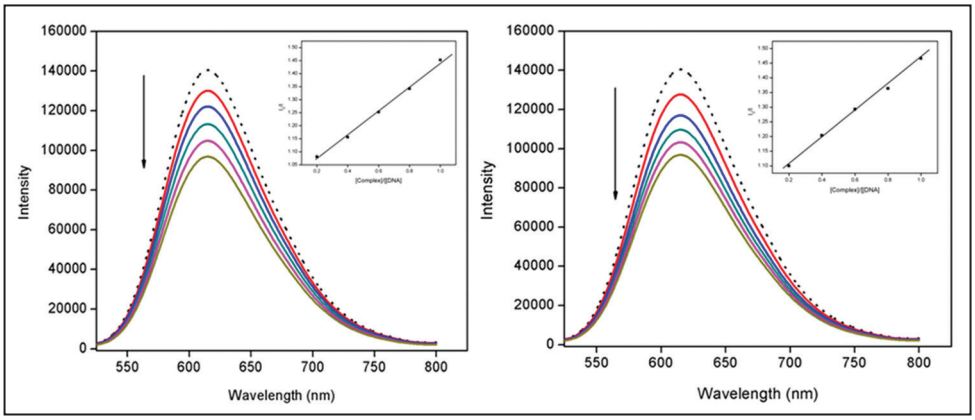


Figure 2: Emission spectra of EB bound to DNA in the absence (dotted line) and the presence (dashed line) of complexes 1 and 2. Arrow (↓) shows the intensity changes on increasing the concentration of the complex. Inset: Stern–Volmer quenching curves.

In Figure 2, the emission spectra of EB bound to DNA in the absence and presence of complexes **1** and **2** are shown, the addition of the complexes to DNA with EB produced significant decrease in emission intensity, indicating that the complexes bound with EB in DNA binding [25]. The quenching plots (Figure 3) demonstrate that the quenching of the EB by complexes **1** and **2** is in good agreement with the linear Stern–Volmer relationship, which confirms that the two complexes (**1** and **2**) bind to DNA. In the plot of I_0/I versus [complex]/[DNA], K_{sv} is given by the ratio of the slope to the intercept. From these data, K_{sv} values of 0.464 and 0.446 were determined for **1** and **2**, respectively.

3.4. Viscosity Studies

To understand the interaction mode between the complex and DNA, viscosity measurements were carried out. The intercalation mode of complexes with CT-DNA results in increase length of the DNA helix while the base pairs are separated to accommodate the metal complexes, which leads to the increase of DNA viscosity [26]. The effect of complexes **1** and **2** on the viscosity of CT-DNA solution is given in Figure 3. The plot shows that the relative viscosity of CT-DNA increased with the addition of the complex **1** in comparison of complex **2**, but the increase is less than that observed for the typical intercalator EB, indicating that intercalative interaction between the complex **1** and CT-DNA is weaker than that of the typical intercalator EB, which is consistent with the above absorption spectroscopic result.

3.5. CV Studies

The CV behavior for complexes **1** and **2** in Tris–HCl buffer (pH 7.28) in the presence and absence of CT-DNA is shown in Figure 4. In the

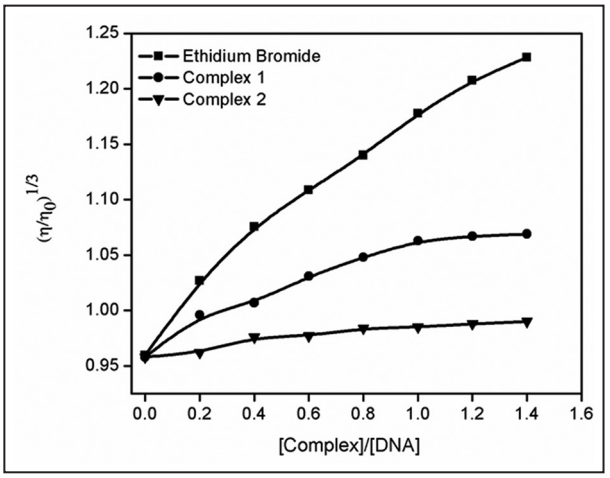


Figure 3: Effect of increasing amounts of complexes on the relative viscosities of CT-DNA at 25°C.

forward scan, a single cathodic and anodic peak was observed, which indicates that the process is reversible. When CT-DNA is added to a solution of complexes, marked decrease in the peak current and potential values were observed. The CV behavior was not affected by the addition of CT-DNA indicating that the decrease of peak current of complexes after the addition of DNA due to the binding of complex to the DNA [27]. When concentration of DNA increased, the changes in peak current and potential become slow. This reveals that the complexes were interact with calf thymus DNA.

Table 1: Antimicrobial activity of copper (II) complexes

Zone of inhibition (mm)				
Micro organisms	**Complex 1**	**Complex 2**	**Copper nitrate**	**Ciprofloxacin/amphotericin-B**
Bacteria				
Escherichia coli	26	22	12	18
Enterococcus faecalis	26	20	18	30
Staphylococcus aureus	29	35	33	34
Fungi				
Aspergillus fumigatus	22	16	15	12
Mucor sps	18	11	11	15

Table 2: Bond length and bond angle of complexes 1 and 2.

Parameters	Complex 1	Complex 2
Cu-N1	2.06	2.04
Cu-N2	2.04	2.03
Cu-N3	2.06	2.06
Cu-N4	2.39	2.39
Cu-O1	1.95	1.94
N1-Cu-N2	81.8	80.8
N3-Cu-N2	156.03	156.27
N1-Cu-O1	173.01	172.79

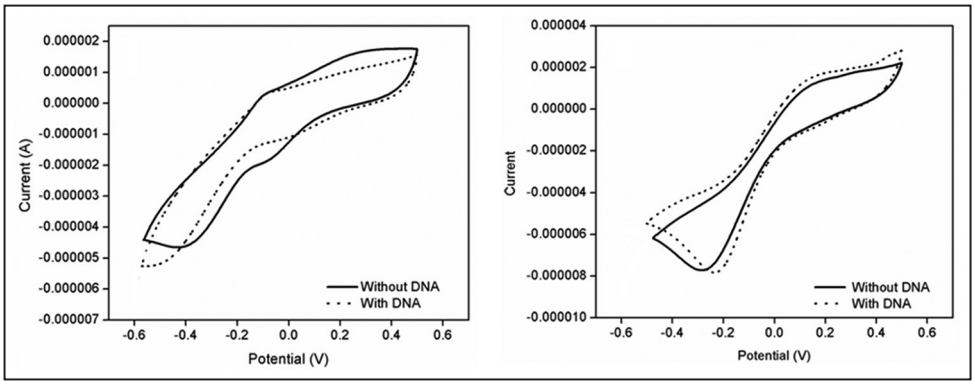


Figure 4: Cyclic voltammogram of complexes (1 and 2) in the absence (dashed line) and presence (dotted line) CT-DNA.

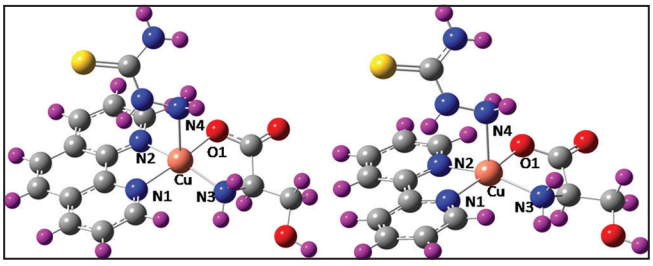


Figure 5: Optimized geometry of complexes 1 and 2.

3.6. Antibacterial and Antifungal Activity

The complexes **1** and **2** were screened *in vitro* for its microbial activity against certain bacterial and fungal species using disc diffusion method. The complexes were found to exhibit considerable activity against bacteria and the fungus. Our group recently reported that amino acid containing complexes have good antimicrobial activity [28]. Zoroddu *et al*. [29] reported that copper complexes show considerable activity against the bacteria. Recently, Patel *et al*. [30] have indicated that the copper(II) complexes with L-phenylalanine have exhibited considerable activity against some human pathogens. In our biological experiments, using copper(II) complexes [31], we have observed antibacterial activity and antifungal activity. The complexes **1** and **2** have shown high antibacterial activity (Table 1) against *Staphylococcus aureus* when comparing with *Escherichia coli* and *Enterococcus faecalis*, similarly, complex **1** has shown high antifungal activity against *Aspergillus fumigatus* than *Mucor* sps. It may be concluded that our complexes **1** and **2** inhibit the growth of bacteria and fungi to a greater extent.

3.7. DFT Study

The two copper complexes optimized using the B3LYP/LANL2DZ basis set [32], optimized geometry of complexes **1** and **2** was shown in Figure 5. The interligand and intraligand angles are equal to 156°

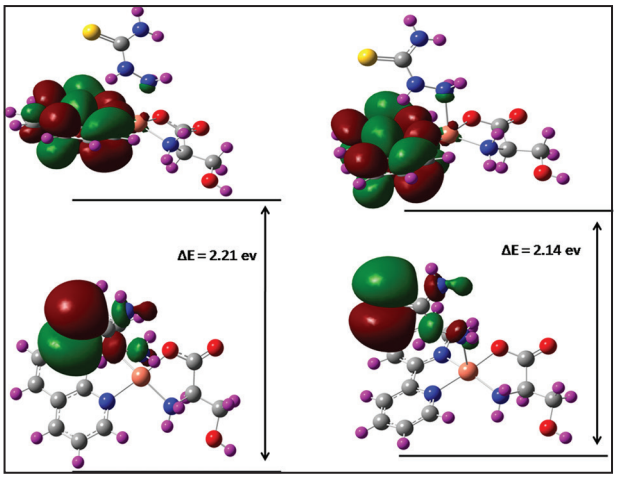


Figure 6: Energy gap of complexes 1 and 2.

and 81° for complex **1,** respectively, almost same value obtained for complex **2**. The interligand bond angle was slightly higher in complex 2 than complex 1. Moreover, bond length was equal in both complexes **1** and **2** (Table 2). Phen complex has shown higher band gap than bipy complex (Figure 6).

4. CONCLUSION

The synthesis, characterization, DNA binding, and biological activities of complexes were reported. G-value suggested that copper(II) complexes are five coordination geometry. The DNA-binding results revealed that the complex 1 can bind though intercalation mode and complex 2 interact through partial intercalation. As the mixed ligand complexes containing amino acid and heterocyclic bases show a unique DNA-binding property, good antibacterial and antifungal agents.

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***Bibliographical Sketch**

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