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Synthesis, Characterization and DNA Binding of Copper (II) Complexes with Mixed Ligands of 1, 10 - Phenanthroline / 2, 2'- Bipyridyl, L-Valine & Semicarbazide Studies on Anti-Microbial and Anti-Cancer Activities

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ABSTRACT

Ternary copper(II) complexes [Cu(phen)(L-val)SC)] 1 & [Cu(Bpy)(L-val)SC] 2 (phen = 1,10-phenanthroline Bpy = bipyridyl, L-Valine and SC= Semicarbazide), have been synthesized and characterized by CHN analysis, molar conductance, electronic absorption, IR and EPR spectral studies. They have been tested for their in vitro DNA binding activities by spectroscopic methods such as UV-Visible, Cyclic volumetric and viscosity measurement. Further, complexes 1 and 2 displayed significant cytotoxicity when examined *in-vitro* on a panel of cancerous cell line - human liver cancer cell line - HepG-2 cells (IC50= 52.41 and 48.67 μg/ml). Further complexes 1 & 2 were tested for their antimicrobial activities and it was found to have good antimicrobial activities.

INTRODUCTION

A number of metal chelates have been used as probes of DNA structure in solution[1], as agents for mediation of strand scission[2] of duplex DNA and as chemotherapeutic agents[3]. Many transition metal Schiff base complexes have been found to considerable interesting biological properties such as antibacterial, antitumour activity[4]. Catalysts for various organic reactions[5] metal complexes with polypyridyl ligands can bind to DNA both by metal coordination and through the polypyridyl ligands[6]. The coordination geometry of a Cu(II)complex bound to DNA affects the Cu(II)/Cu(I) redox behaviour and a change in the coordination geometry has been found to determine the properties of Cu(II) rather than Cu(I) complex species[7]. To improve the functions of metal complexes for application in the field of biotechnology, medicine and organic synthesis, one has to tune their properties such as redox potential to activate or deactivate oxidants, quantum yield in photochemical reactions, p*K*a of ligands in hydrolytic cleavage of nucleic acids, and hydrogen-bonding network in recognizing specific nucleotide base sequences, and their availability in drug delivery system. Among the various spectroscopic methods used to assess the DNA bound structures of paramagnetic metal complexes, DNA-fiber electron paramagnetic resonance (EPR) spectroscopy affords unique information on the binding structures, stereo-specificity, and dynamic properties of the complexes bound to DNA[8–11]. Copper (II) has been shown to bind to the DNA bases adenine, guanine and cytosine at the N(7) of purines and N(1)of pyrimidines[12]. Copper is a part of many redox-active metallo-enzymes[13]. An understanding of the modes of binding of metal complexes to DNA is required to illustrate the principles governing the DNA recognition by such functional molecules, that is, the factors that decide the affinity and specificity of the complexes for DNA base sequence. Cationic complexes have been found to both intercalate into DNA and bind non-covalently in a surface-bound groove-bound fashion[14]. Cisplatin is a widely used metal-based anticancer drug[15-17], but it is curative only in some selected tumors and due to side-effects as well as acquired cellular resistance its use is limited. So the development of more efficacious, less toxic and target specific non-covalent DNA binding anticancer drugs have received attention. Considerable efforts have been focused on the development of new anticancer drugs based on transition metal complexes. Copper is a bio-essential element, plays a key role in biological processes and its complexes are preferred molecules for anticancer inhibition[18]. Extended planar aromatic ring in the ligands allows better intercalation with DNA[19]. The role of ternary copper(II) complexes in biological systems is well known[20]. Among the transition metal

based DNA cleaving agents, particularly copper phenanthroline complexes, is primarily sugar directed. They are responsible for direct strand scission by hydrogen atom abstraction from the deoxyribose moiety. The dissimilar encodes present in the DNA are implicated in various regulatory processes such as gene expression, gene transcription, mutagenesis, carcinogenesis etc. The above mentioned processes can be modified by the interaction of drugs with specific regions of DNA[21, 22]. Recently, our group focused on the DNA binding activity of important amino acid containing copper(II) complexes are really interested, copper(II) complexes of the type amino acid-Cu-heterocyclic base show efficient DNA binding, cleavage and cytotoxicity[23-25]. In this paper, we discussed the effect of Semicarbazide with heterocyclic bases containing copper (II) complexes on DNA binding, antimicrobial and cytotoxic studies.

MATERIALS AND METHODS

Synthesis of [Cu(phen)(L-valine)SC]NO_3 (1)

The complex [Cu(phen)(L-val)(H_2O)](NO_3) was synthesized according to a published method[26]. To the aqueous solution of [Cu(phen)(L-val)(H_2O)](NO_3) (1 mmol) was added semicarbazide (1 mmol) the colour of the solution change from blue to light yellow. The resulting solution was stirred for 6 hrs and then solution of complex **1** was filtered. The filtrate was kept for slow evaporation, after two weeks, the light yellow colored complex was separated out. Yield: 68%; Anal. (%) Calc. for $C_{18}H_{23}CuN_7O_6$: C, 43.50; H, 4.66; N, 19.73. Found: C, 41.22; H, 4.38; N, 18.95. IR (KBr pellet): 3417, 3225, 2914, 2182, 1636, 1385, 1082, 860, 732 cm^{-1}. UV-Vis (λ, nm): 272, & 600.

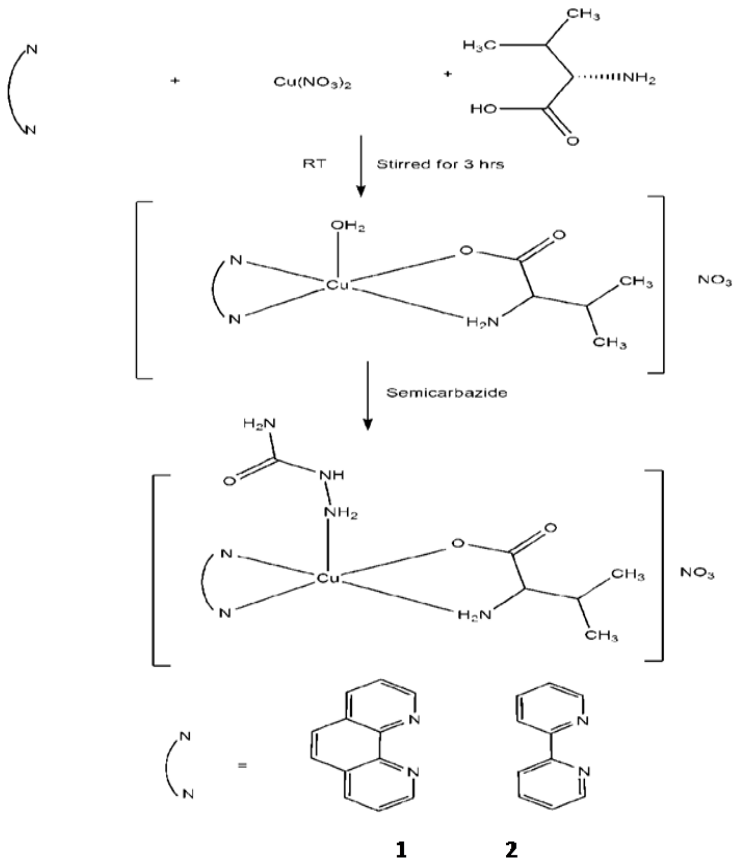
Synthesis of [Cu(bpy)(L-val)SC]NO_3 (2)

Synthesis of complex **2** is same as described above. Yield: 66%; Anal. (%) Calc. for $C_{16}H_{23}CuN_7O_6$: C, 40.63; H, 4.90; N, 20.73. Found: C, 39.41; H, 4.67; N, 20.06. IR (KBr pellet): 3417, 3187, 2426, 1628, 1605, 1378, 1178, 838, 728 cm^{-1}. UV-Vis (λ, nm): 300 & 622 nm.

RESULTS AND DISCUSSION

General Aspects

These complexes are synthesized by ligand substitution method; the synthetic strategy of the complexes is outlined in Scheme 1 given below. The synthesized complexes are more stable and they are soluble in water and in other organic solvents. The elemental analysis data of the copper(II) complexes agree with the theoretical values.



Scheme 1: Synthesis of complexes 1 and 2.

In the UV region, the complex presented in Figure 1, bands around 273 and 300 nm which can be attributed to $\pi \rightarrow \pi^*$ transition of the coordinated phenanthroline ligand, and the complexes **1** and **2** exhibits d-d band at UV-Visible spectra. The complexes are in good agreement with the previously reported square pyramidal geometry of the complexes.

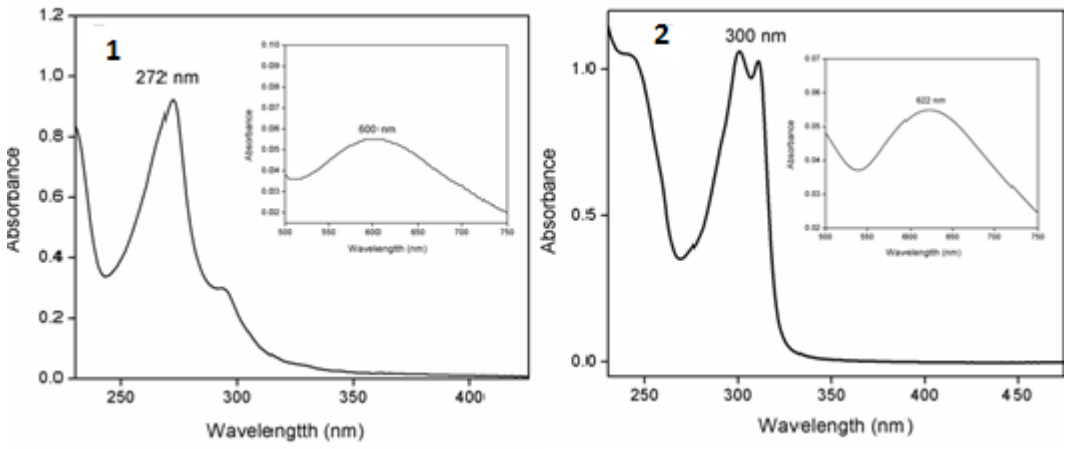


Figure 1: UV-Visible spectra of complexes 1 and 2.

In the IR region, for complexes, the band around 3417 cm^{-1} for both complexes (**1**) and (**2**) can be assigned to γ (N-H) stretching frequency of amino acid. The coordination of nitrogen atoms of heterocyclic base with copper metal ion can be examined by δ(C-H) for phenanthroline 853 cm^{-1} is shifted to 860 cm^{-1} and the band around 1385 cm^{-1} (**1**) and 1378 cm^{-1} (**2**) has been assigned for γ(N-O) of nitrate ion (Figure 1).

The solid state EPR spectra of the copper (II) complexes were recorded in X-band frequencies (Figure 3). At room temperature, complexes **1** and **2** exhibit well defined single isotropic lines. Such isotropic lines are usually the results of intermolecular spin exchange, which broaden the lines. This intermolecular type of spin exchange is caused by the strong spin coupling which occurs during a coupling of two paramagnetic species. EPR spectra of mononuclear complexes copper(II) species with S=1/2, those with two signals ($g_{\perp}$and $g_{\parallel}$), on comparing these two signals $g_{\perp}(x,y) > g_{\parallel}(z)$ ($B_{\perp}(x,y) < B_{\parallel}(z)$) representing the elongated axial symmetry of the spin tensor.

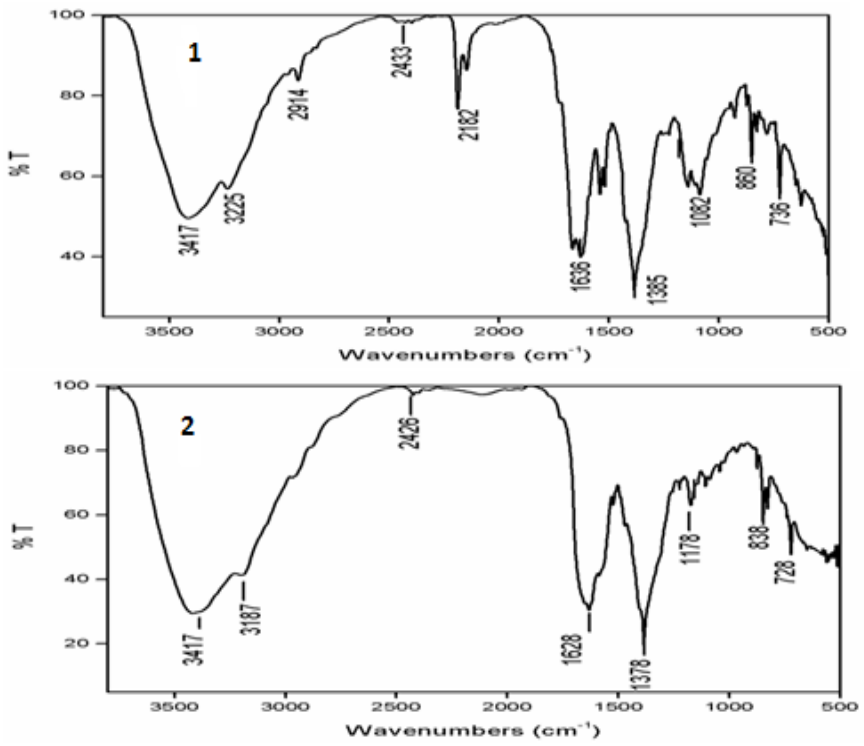


Figure 2: Infrared spectra of complexes 1 and 2.

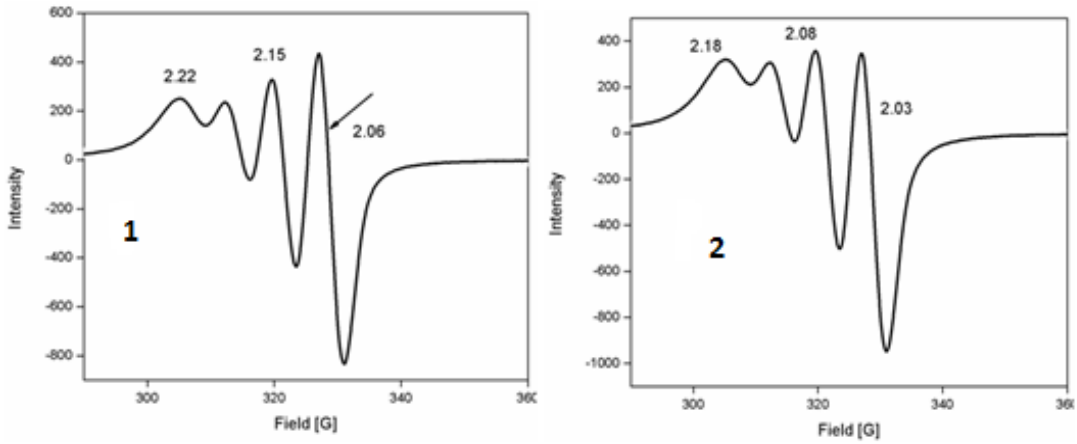


Figure 3: EPR spectra of complexes 1 and 2.

DNA binding studies

Electronic spectral studies

Electronic absorption spectroscopy was an effective method to examine the binding mode of DNA with metal complexes. In general, hypochromism and redshift are associated with the binding of the complex to the helix by an intercalative mode, involving strong stacking

interaction of the aromatic chromophore of the complex between the DNA base pairs. Figure 4 shows the UV absorption spectral study of copper(II) complex in the absence and presence of DNA. The absorption intensity of the complexes **1** and **2** increased (hyperchromism and blue shift) evidently after the addition of DNA, which indicated the interactions between DNA and the complex through intercalative mode.

On comparing the Kb values (1.07 X 10^6 M^{-1}) (**1**) and 7.12 X 10^5 M^{-1}) (**2**) of complexes, complex **1** have higher value than complex **2**. So the binding propensity of the phen complex **1** is high due to the presence of an extended planar aromatic ring in phen. Earlier studies on bis-phen copper complex have shown that this complex binds to DNA either by partial intercalation or binding of one phenanthroline ligand to the minor groove while the other phen making favourable contacts within the groove[27, 28].

Fluorescent spectral studies

As the copper (II) complexes are non-emissive, competitive binding studies with EtBr were carried out to gain support for the mode of binding of the complexes with DNA. The study involves addition of the complexes to DNA pretreated with EtBr ([DNA]/ [EtBr] = 1) and then measurement of intensity of emission.

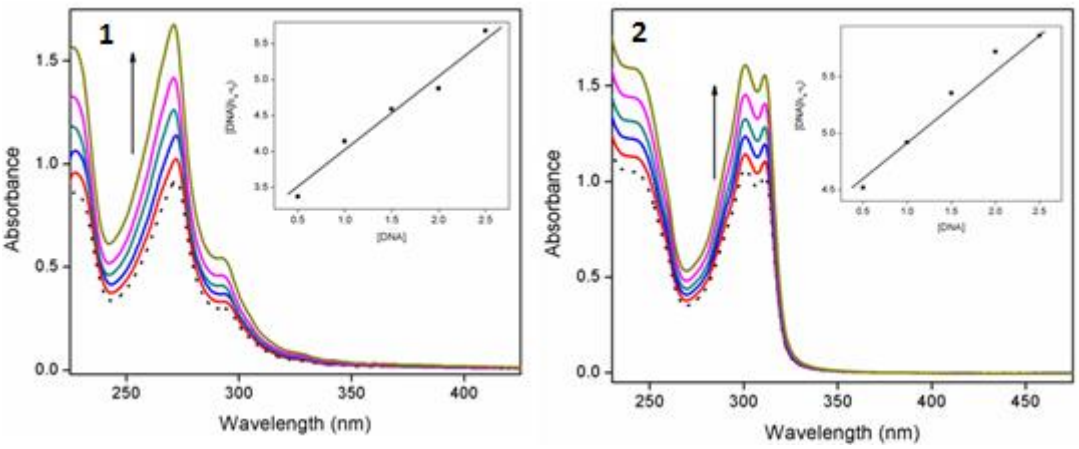


Figure 4: Absorption spectral traces on addition of CT DNA to complexes 1 and 2 (shown by arrow). Inset plot of [DNA]/(εa-εf) vs [DNA] for absorption titration of CT DNA with complexes.

The observed enhancement in the emission intensity of EtBr bound to DNA is due to intercalation of the fluorophore in between the base pairs of DNA and stabilization of its

excited state (Figure 5). Addition of all the complexes to CT-DNA incubated with EtBr decreases the DNA induced enhancement in emission to the same extent. This suggests that the complexes displace DNA-bound EtBr and bind to DNA at the intercalation sites with almost the same affinity, which is consistent with the above spectral results suggesting partial intercalation of the phenanthroline ring[29].

Viscosity measurements

To further explore the binding mode of the copper(II) complex with DNA, viscosity measurements were carried out. Since the relative specific viscosity ($\eta/\ \eta_0$) (η and η_0 are the specific viscosities of DNA in the presence and absence of the complex, respectively) of DNA reflects the increase in contour length associated with separation of DNA base pairs caused by intercalation, a classical intercalator such as ethidium bromide could cause a significant increase in viscosity of DNA solutions.

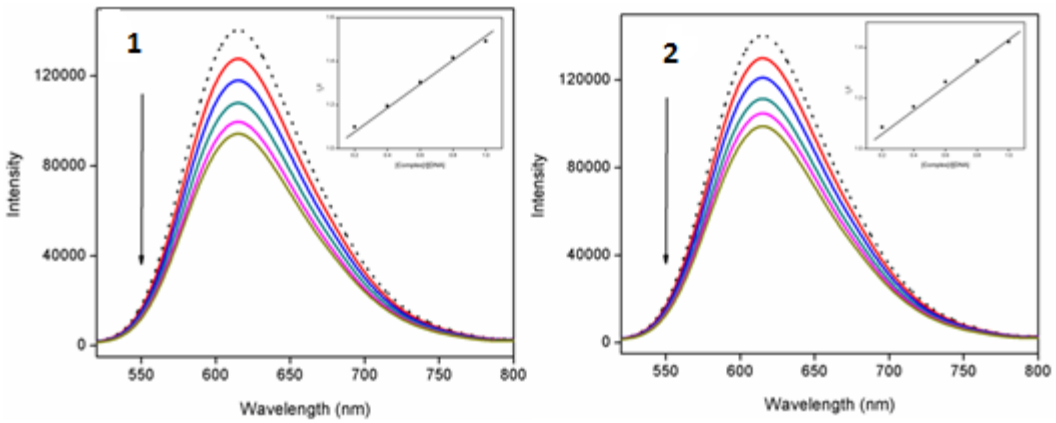


Figure 5: Emission spectra of EB bound to DNA in the absence (dotted line) and the presence (dashed line) of complexes 1 and 2. Arrow (↓) shows the intensity changes upon increasing the concentration of the complex. Inset: Stern–Volmer quenching curves.

In contrast, a partial and/or non-classical intercalation of the ligand could bend or kink DNA, resulting in a decrease in its effective length with a concomitant increase in its viscosity[30, 31], while the electrostatic and groove binding cause little or no effect on the relative viscosity of DNA solutions. Therefore viscosity measurements, which are sensitive to the changes in the contour length of DNA, are useful to probe for DNA intercalation by complexes.

The plots of relative specific viscosities versus 1/R = ([Complex]/[DNA]) are shown in Figure 6. The relative specific viscosity increases with increasing concentration of the complex. However, the increase in the viscosity was much less compared to that of classical intercalators like ethidium bromide in the same DNA concentration range. This observation supports the above spectral studies which suggest that the complex **1** intercalates with the DNA base pairs and complex **2** involve through groove binding. Intercalation results in lengthening of the DNA helix due to base pairs being separated to accommodate the binding ligand, leading to an increase in viscosity of the solution.

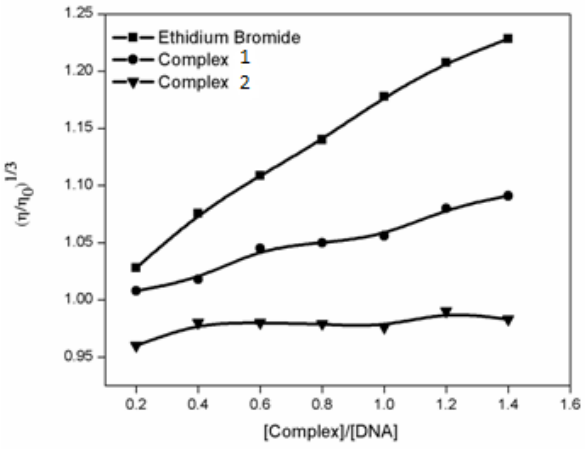


Figure 6: Viscosity of complexes 1 and 2 with CT-DNA.

Cyclic voltammetry studies

The cyclic voltammetric (CV) response for complexes **1** and **2** in Tris–HCl buffer (pH 7.28) in the presence and absence of CT DNA is shown in Figure 7. In the forward scan, a single cathodic and anodic peak were observed, which corresponds to the reduction and oxidation of complexes, 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 was observed. The cyclic voltammetric behavior was not affected by the addition of very large excess of DNA, indicating that the decrease of peak current of complexes after the addition of DNA due to the binding of the complex to the DNA[32]. When the concentration of DNA increased, the changes in peak current and potential become slow. This reveals that the complexes were interacting with Calf thymus - DNA.

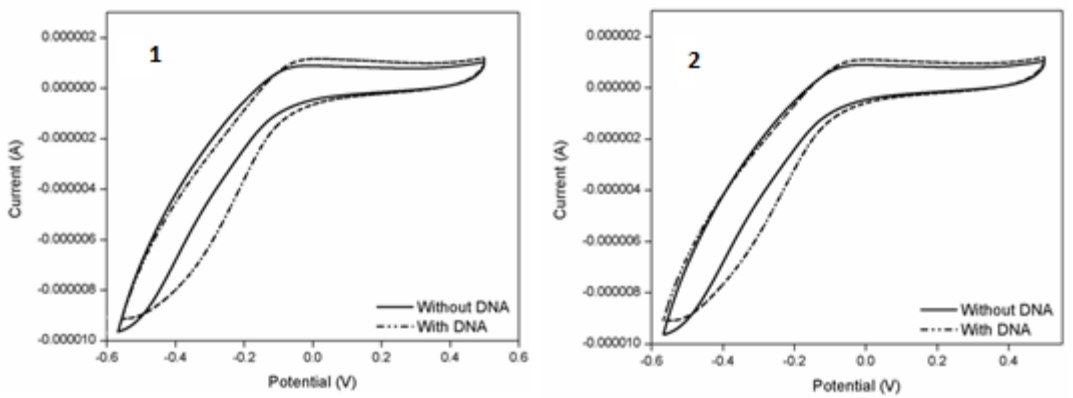


Figure 7: Cyclic voltammogram of complexes (1 and 2) in the absence and presence of CT DNA.

Antibacterial and antifungal activity

The copper (II) complexes were tested *in vitro* for its antibacterial and antifungal activity against certain pathogenic 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 has good antimicrobial activity[33]. Copper complexes show remarkable activity against the bacteria, the copper(II) complexes with L-phenylalanine has exhibited considerable activity against some human pathogens (Figure 8). In our biological experiments, using copper(II) complexes, we have observed antibacterial activity antifungal activity. The complex **1** and **2** exhibit higher antibacterial activity (Table 1) against *staphylococcus aureus*. On comparing complexes **1** and **2,** complex **1** has shown high antifungal activity against *Aspergillus fumicatus*. It may be concluded that our complexes **1** and **2** inhibit the growth of bacteria and fungi to a greater extent.

Table 1: Antibacterial and antifungal activity of complexes 1 and 2.

Sr. No.	Micro Organisms	Complex 1	Complex 2	Copper Nitrate	Ciproflaxacin/ Amphotericin-B
		Zone of Inhibition (mm)			
Bacteria					
1	*Escherichia coli*	22	21	11	20
2	*Enterococcus faecalis*	24	20	16	26
3	*Staphylococcus aureus*	27	22	19	32
Fungi					
4	*Aspergillus fumicatus*	18	23	12	17
5	*Mucor sps*	14	15	11	16

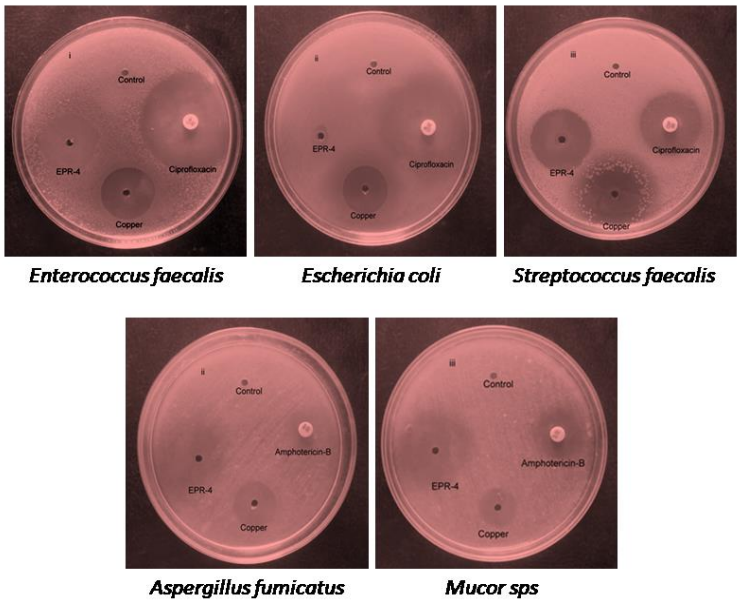


Figure 8: Antibacterial and antifungal activity of complex 1.

MTT assay

The cytotoxicity of the complexes **1** and **2** to be used as anticancer agents were studied using MTT assay (Table 2). The ability of the complexes on HepG2 cells was tested with or without various concentrations (7.8–1000 µg/ml). Cytotoxicity of all the synthesized compounds against HepG2 was determined by MTT assay. Growth of this cancer cells was measured by the ability of living cells to reduce the yellow MTT to purple formazan products. Evaluation of in vitro anticancer properties towards HepG2 cancer cell lines revealed that thirteen compounds exhibited good to moderate cytotoxicity, out of which, two compounds 1 and 2 exhibited good anticancer potency with an IC50 value of 52.41 and 48.67 µg/ml, respectively. The level of anticancer potential was studied by automated docking of ligands to the binding sites of ALK. Further investigation concerning an enantioselective version of this reaction and the mechanism of apoptosis induced by these compounds in HepG2 cancer cell lines is currently ongoing and will be reported in due course. Cells incubated with different concentration of Doxorubicin served as positive control. After incubation period, MTT assay was carried out to calculate the cell death percentage. For each concentration, of the complexes, cells were incubated in triplicate[34-37]. The (Figure 9) clearly illustrates that there is a clearly damage in the live cells number in the cells incubated with complex in a concentration dependent manner. Viability of cells incubated without any

compound was considered as 100% and the percentage of live cells incubated with compound are given as relative to the control.

Table 2: MTT Assay of complexes 1 and 2.

Concentration (µg/ml)	Dilutions	Absorbance (O. D.)		Cell viability (%)	
		Complex 1	Complex 2	Complex 1	Complex 2
1000	Neat	0.182	0.190	21.43	20.22
500	1:1	0.240	0.255	28.26	26.58
250	1:2	0.312	0.320	36.74	33.26
125	1:4	0.375	0.348	44.16	41.73
62.5	1:8	**0.445**	**0.405**	**52.41**	**48.67**
31.2	1:16	0.511	0.463	60.18	58.28
15.6	1:32	0.580	0.531	58.77	64.85
7.8	1:64	0.651	0.632	66.31	74.63
Cell control	-	0.849	0.839	100	100

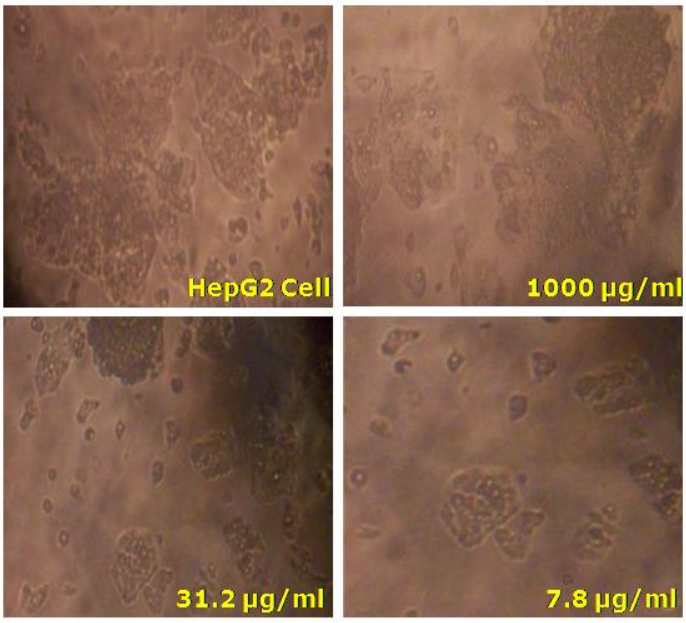


Figure 9: HepG2 Cell morphology of complex 1

CONCLUSION

The synthesis, characterization, DNA binding and biological activities of complexes was reported. g-value suggested that copper(II) complexes are five coordination geometry. The DNA binding results revealed that 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. Complexes **1** and **2** have significant role against HepG2 cells.

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