Synthesis, Characterization, DNA binding, Anticancer and Antimicrobial activity , DFT, Molecular docking Studies of Copper (II) Complex with Neocuproine and L-Ornithine

Venkatesan Vinayagam[1,2], Shanmugam Rajalakshmi[1], M.N. Arumugham[1]
[1]*Department of Chemistry, Bio-inorganic Lab, Thiruvalluvar University, Serkadu, Vellore -632 115, Tamil Nadu, India*
[2]*Department of Science and Humanities, Thiruvalluvar College of Engineering and Technology, Vandavasi-604505, Tamil Nadu, India*

Abstract- The new complex, have been synthesized and characterized. The metal complex has been characterized by elemental analysis, UV, IR, conductivity measurements, EPR and magnetic studies. The interaction of complex with CT-DNA was investigated by carrying out UV- vis absorption spectrum and fluorescence ethidium bromide displacement experiment. The DNA-binding constants *K*b of complex were calculated to be 2.1059×10^{4} M^{-1}. The result indicated that these complexes interact with CT-DNA through intercalative mode. Competitive studies with ethidium bromide (EB) further showed that the complexes bind to DNA probably via intercalation. The anti-microbial activities and MTT-Assy studies have also been studied. In anti-microbial activity the complex showed good anti-microbial activity higher in gram-positive, gram-negative bacteria and fungi. The structure of the present complex has been optimized using the DFT/ B3LYP/6-31G(d,p)/LANL2DZ level of theory and cyclic voltametric studies, Molecular docking analysis was carried out.

Keywords: Neocuproine; DFT; DNA-binding; MTT Assay; L-Ornithine, docking.

1.INTRODUCTION

In past decades, coordination compounds have attracted much interest because of their diverse application in the field of bio-inorganic, biomedical and pharmaceuticals. Despite advances in the development of antimicrobial agents, infectious diseases remain amongst the top five causes of mortality in the world [1]. One of the most crucial problems is growing drug resistance, especially the antibiotic resistance of pathogenic bacteria. Because such resistance can develop very quickly, there is a dire necessity to develop new antimicrobial compounds [2,3]. Although medicinal chemistry was almost exclusively based on organic compounds and natural products, during the past three decades metal complexes have gained a growing interest as pharmaceuticals due to their potential therapeutic applications [4]. Copper is one of essential elements required for normal human metabolism [5]. Copper(II) is known to play a significant role in biological systems and also as a pharmaceutical agent [6]. Its antibacterial properties have been known for thousands of years. Synthetic copper(II) complexes have been reported to act as a potential anticancer and cancer inhibiting agents and a number of copper complexes have been found to be active both in vitro and in vivo [7,8]. Nitrogen-containing ligands has found wide applications in chemotherapy and asymmetric catalysis. Among them, l-ornithine and neocuproine have been the most attractive due to their various functions [9]. The neocuproine (neocup) and its derivatives exhibit antiviral, antifungal and antimycoplasmal activities [10].Numerous biological studies have demonstrated that deoxyribonucleic acid (DNA) is the primary target molecule for most anticancer and antiviral therapies [11]. The question of the interaction and reaction of metal complexes containing multidentate aromatic ligands, especially N-containing ligands, with DNA, therefore, has long concentrated much attention in the development of new reagents for medicine and biotechnology [12]. This is due to their possible application as therapeutic agents and photochemical properties which make them potential probes of DNA structure and conformation. There are three distinct modes of non-covalent interaction of these metal

complexes with DNA—intercalative association, DNA groove binding and electrostatic attraction—the nature of which is determined by the characteristics of the metal complexes. Metal complexes with a variety of organic chelating ligand are also of current interest owing to their biological activities. These include anti-inflammatory and anticonvulsant properties [13], cytotoxicity and antiviral activity [14]. Transition metal complexes of amino acids have a significant role in cancer chemotherapy. Mixed ligand complexes involving asparagine in combination with various metal ions have been suggested as possible antimetabolites in tumor cells [15]. Complexes of transition metals with amino acids in proteins and peptides are utilized in numerous biological processes like electron transfer, oxidation and as oxygen conveyers. In these processes, the enzymatic active site, which is very specific, forms complexes with divalent metal ions [16] L-ornithine is one of the four important amino acids having basic side chains, although it is not a constituent of proteins. L-ornithine is effective for stimulating, production and release of growth hormones and reducing hypertension [17]. Its function in the living systems is very important because of its role in Krebs cycle in the metabolism of mammals [18]. In the rapidly growing tumors, there is decarboxylase activity, and it can be reduced by chelating the carboxylate group of ornithine [19]. Potential medical uses of ornithine are, its antifatigue effect, cirrhosis and as weightlifting supplement [20]. In the literature, there are few reports concerning ternary complexes of Cu(II) with l-ornithine and substituted phenanthrolines [21–22]. Among them, bis(diisopropylsalicylato)-(2,9-dimethyl 1,10-phenanthroline)copper(II) complex seems to be the most potent. It exhibits cytotoxicity comparable with the anticancer drug, cisplatin [PtCl2(NH3)2] [23].Several types of biological drugs such as antiviral, anticancer, and antitumor can easily target nucleic acids and can bind to DNA by intercalative and non-intercalative mode of interaction [24]. A great number of DNA-targeting drugs have been reported as approved anticancer drugs. The efficacy of these drugs could be credited to their DNA-interacting capabilities. Hence, investigation of DNA-drug interaction paid more consideration in cancer chemotherapy [25-26]. Reactive oxygen species such as free radical that can attack on biological polymers like DNA, RNA, proteins, lipids and cell membranes, leading to the development of oxidative stress which in turn causes some degenerative and chronic diseases [27].Molecular docking study is a computational technique fascinated significant interest to researchers in the field of medicinal chemistry [28]. This method of approach is most frequently used in drug design and discovery to predict binding pattern and strength of small molecules with their targets. Biofilms are assemblies of microorganisms on biotic or abiotic surface surrounded by a polymer matrix. Since they offer protection for bacteria from antibiotics, disinfectants, extensive research have been devoted recently for the discovery of new biofilm inhibitors.

Herein, we describe the synthesis and characterization of copper(II) complex of neocuproine and L-Ornithine ligand. The DNA binding capabilities of synthesized compounds were investigated by absorption, fluorescence, viscosity, ESR spectrum, and cyclic voltametric studies. The antimicrobial activity of the Cu(II) complex was investigated against a panel of fungai and bacterial strains. The toxicity of Cu(II) complex was determined on human HepG2 cells by MTT assay[29].

2.EXPERIMENTAL SECTION

2.1 Chemicals and regent

Copper (II) nitrate trihydrate ($Cu(NO_3)_2.3H_2O$) was obtained from Merck, Merk life science Pvt. Ltd, India. Neocuproine ($C_{14}H_{12}N_2$), L-ornithine ($C_5H_{12}N_2O_2$), and CT-DNA were collected through Sigma-Aldrich CoLtd, India. Ethidium bromide and DMSO were purchased from Avra Synthesis Pvt Ltd, India. Ethanol was brought from SDFCL, India. The entire chemicals and reagents used in the over experiments with analytical reagent grade were involved without any further purification.

2.2 Instrumental technique The DNA binding of Cu (II) complex was recorded using UV-Visible (the range from 200 to 800 nm) and fluorescence studies on the Shimadzu UV-2450 and Perkin Elmer LS-45 spectrophotometer. The X–band ESR spectrum of the copper complex was analyzed on a JES-FA200 ESR spectrometer at room temperature. The electrochemical properties of the obtained copper complex were carried out by using the cyclic voltammetry technique on the CHI608E instrument and using a three-electrode cell with a DMSO solution of $LiClO_4$ (0.1M) as the supporting electrode.

2.3 Synthesis of the [Cu(Neocup)(L-orn)NO_3] complex

To prepare Cu (II)complex. 1 mmol of $Cu(NO_3)_2$. $3H_2O$ and 1 mmol of $C_{14}H_{12}N_2$ were taken in two separate 50 MmL beakers containing dissolved with 10 MmL of ethanolic solution and the mixture was vigorously stirred for 30 min. Afterward, 1 mmol of $C_5H_{12}N_2O_2$ was added slowly and then the mixture was again stirred for 6h appropriate temperature[30]. The obtained green product was filtered and kept in evaporation at room temperature. The green precipitate was collected after a few days which is denoted as [Cu (Neocup)(L-ornithine)(NO_3)] complex as given in Fig.1. Then the yield was calculated as 70 %, the Elemental composition of $C_{19}HcuN_5O_5$ (%): C, 48.97; H,5.19; N,15.03; O, 17.17 Found (%): C,48.95; H,5.23; N, 15.06; O,17.20 IR (KBr, cm− 1): 3402 br, 3118w, 3241w, 2977w, 1596 s, 1522 vs, 1350s, 1284s, 1000 s, 769 m, 692 s, 538 m cm^{-1} (br, broad; vs, very strong; s, strong; m, medium; w, weak). UV-Vis (H_2O), λ/nm (ε/M^{-1} cm^{-1}): 275 (12,720 π–π*), 615 (226; d-d), mp:152.

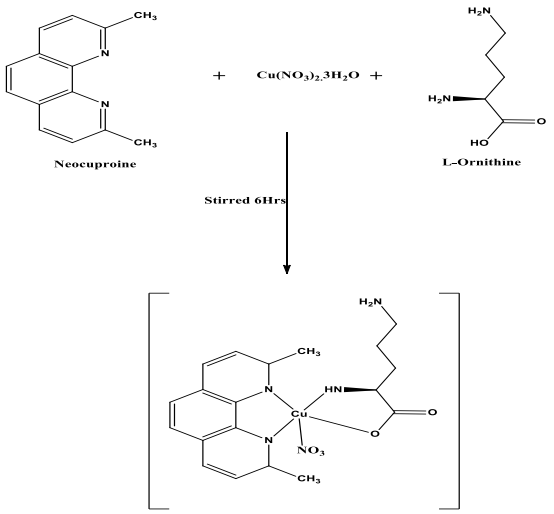

Figure 1 Synthesis Scheme of the Cu(II) Complex

2.4 DNA binding studies

2.4.1 Electronic Absorption Spectra

DNA and metal complex stock solutions were freshly prepared in Tris–HCl/NaCl buffer. Absorbance of DNA buffer solution was measured at 260 and 280 nm gave a ratio of ~1.9, indicating that the DNA was sufficiently free from protein [31] . The DNA concentration per nucleotide was determined the molar absorption coefficient 6600 M^{-1} cm^{-1} at 260 nm [32] . The titration of complexes were performed using a constant complex concentration (10 μM) to which increments of the DNA concentration (0–25 μM) was added. After the 30 min incubation of complex-DNA solutions at room temperature, the measurements were taken. While taking the analysis, equal amounts of DNA were added to both complex and reference solutions to eliminate the absorbance of DNA itself. Titration curves were constructed from the fractional change in the absorption intensity as a function of DNA concentration . The intrinsic binding con- stant K b , can be calculated by using the expression:

$$[DNA]/(\varepsilon a-\varepsilon f) = [DNA]/(\varepsilon b-\varepsilon f) + 1/K b\,(\varepsilon b-\varepsilon f) \quad \text{---------- (1)}$$

where [DNA] is the concentration of CT-DNA (per-nucleotide), εa is the absorption coefficient observed at a given DNA concentration, εf is the absorption coefficient of complex in the absence of DNA, εb is the absorption coefficient of complex when fully bound to DNA, and K b is the intrinsic binding constant in M^{-1} . The plot of *[DNA]/(ε a –ε f) versus* [DNA] gave a slope and the *y* -intercept which are equal to *1/(ε b –ε f) and 1/ K b (ε b –ε f)*, respectively, and the intrinsic binding constant K b was obtained from the ratio of the slope to the intercept.

2.4.2 Fluorescence Spectra

The fluorescence quenching experiments were carried out using ethidium bromide (EB, 75 μM) at room temperature in Tris–HCl/NaCl buffer (pH 7.2). The changes in emission intensities at 615 nm (480 nm excitation) of EB bound to DNA were measured with different complex concentrations. EB was nonemissive in buffer due to the fluorescence quenching of free EB by the solvent molecules. In the presence of DNA, EB showed enhanced emission intensity due to its intercalative bind- ing to DNA. Stern–Volmer quenching constants [33] were calculated using the equation

$$I_0 / I = 1 + K_{SV} r \quad \text{---------- (2)}$$

where I_0 and I are the fluorescence intensities in the absence and presence of complex, respectively, K_{SV} is the linear Stern–Volmer quenching constant dependent on the ratio of the bound concentration of ethidium bromide to the concentration of DNA, and r is the total concentration of complex to that of DNA. In the plot of I_0 / I *versus* [complex] / [DNA], K_{SV} is given by the ratio of slope to intercept.

2.5 Theoretical calculations

The rigid molecular docking studies were performed using Auto Dock Tools (ADT) version 1.5.6 and Auto Dock version 4.2.5.1 docking programs. Structure of the complexes was sketched by ChemSketch and save in mol format, then it was converted into pdb format by Open Babel GUI. The crystal structure of the B-DNA dodecamer d(CGCGAATTCGCG)2 was downloaded from the Protein Data Bank (PDB ID: 1BNA) (http://www. rcsb.org./pdb). For the DNA, hydrogen was added before computing Gasteiger charges, and then non-polar hydrogen atoms were merged. The distance between donor and acceptor atoms forming a hydrogen bond was defined as 1.9 A˚ with a tolerance of 0.5 A, ˚ and an acceptor hydrogen donor angle was not less than 120° The copper parameters, van der Waals (vdW) radii of 0.96 A˚ and vdW well depth of 0.01 kcal/mol were used in the docking calculation [34]. A grid box with dimensions of 60 × 60 × 110 A˚ 3 with 0.375 A˚ spacing and centered on (x, y, z) 14.779, 20.976, 8.804 was created that included the whole DNA. After the box center was set at the DNA center, grid energy calculations were carried out. Docking poses were visualized by Pymol (http://www.pymol. Sourceforget.net/) molecular graphics program.

2.6 Cyclic Voltammetry Studies:

Electrochemical techniques are best complementary to other related biophysical techniques that are applied to study the interaction of redox active species with nucleic acid [35] . The DNA and complex solutions were prepared by using Tris–HCl/NaCl buffer and DMF, respectively. The concentration of complexes and DNA can be taken as 0.1 mM. Solutions were deaerated by purging with N_2 gas for 10 min and N_2 was kept over the solution during the experiments.

2.7 Antimicrobial activity

Antimicrobial activity of the complex was evaluated by paper disc technique using nutrient agar as the medium. The in vitro antibacterial activity was tested against two Gram-positive bacteria, staphylococcus aureus (S.aureus) and two Gram-negative bacteria, Escherichia coli (E. coli), pseudomonas aeruginosa (P.aeruginosa). The antifungal activity was tested against candida albicans (C.albicans) Streptomycin and Mancozeb were the standard drugs for antibacterial and anti-fungal activities, respectively. The stock solution of each compound was prepared by dissolving 5mg of sample in 10 mL of DMSO to make the concentration of 500 lg/mL. The plates were allowed to stand for one hour in order to facilitate the diffusion of the drug solution. Then the plates were incubated at 37ºC for 24 h for bacteria and 48 h for fungi, subsequently, the zone of inhibition was observed, measured in mm and recorded. The activity of compounds were determined by using 25, 50, 100 lg/mL dilution method. The concentration (mg/mL) of compound, which inhibits the growth of bacteria after 24 h incubation and fungi after 48 h incubation at 37ºC, was taken. The concentration of DMSO in the medium did not affect the growth of any of the microorganisms tested. Minimum inhibitory concentrations (MIC) of the ligands and their metal complexes which exhibited antimicrobial activity were determined using literature methods [36]. All compounds diluted within the range of 100–10 lg/mL were mixed in the nutrient broth and 0.1mL of active inoculums was added to each tube. The MIC values, which were taken as being the lowest concentration of the compound that inhibited the growth of the microorganisms at 37ºC for 24 h for bacteria and at 28ºC for 48 h for fungi.

2.8 Anticancer activity

The Liver cancer cell line (HEPG2) was plated separately using 96 well plates with the concentration of 1×10^4cells/ well in DMEM media with 1X Antibiotic Antimycotic Solution and 10% fetal bovine serum (Himedia, India) in a CO_2 incubator at 37°C with 5% CO_2. The cells were washed with 200 µL of 1X PBS, and then the cells were treated with various test concentrations of compounds in serum-free media and incubated for 24 h. The medium was aspirated from cells at the end of the treatment period. 0.5mg/mL MTT prepared in 1X PBS was added and incubated at 37°C for 4 h using a CO_2 incubator. After the incubation period, the medium containing MTT was discarded from the cells and washed using 200 µL of PBS. The formed crystals were dissolved with 100 µL of DMSO and thoroughly mixed. The colour intensity was evaluated at 570 nm. The formazan dye turns to purple-blue color. Spectrophotometrical observance of the purple, blue formazan dye was measured in a microplate reader at 570 nm (Biorad 680). Cytotoxicity was determined using Graphpad prism5 software. The growth inhibitory rate of treated cells was calculated by (OD control – OD test) / OD control × 100%. Detailed experimental procedures are similar to those reported previously[37].

3. RESULTS AND DISCUSSION

3.1 Spectral studies

3.1.1 Electronic spectral study

The electronic spectra in DMSO solution showing absorption of high energy 275-280 nm and 340-*490 nm are assigned to the intraligand π→π* and n→π* transitions respectively [38]. For* Cu(II) complex, d-d transition appeared below 600 nm, which is the characteristic feature of square planar structure .

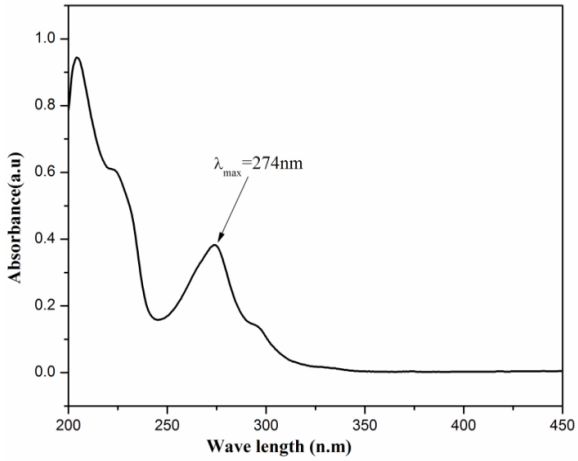

Figure 2UV-Visible studies of Cu(II) Complex

3.1.2 IR spectral study

In the IR spectra of the ligands the band at 3402 cm^{-1} is assigned to the phenolic OH group and

its frequency was found to disappear in the complexes fig 3. A band at 1596 cm-1 region is assigned to C=N absorption, this band of ligand undergoes small shifts to lower frequencies in the spectra of the complexes 1598-1566 cm-1 indicating coordination of the imine nitrogen [39]. Additional evidence for coordination of oxygen (M-O) and nitrogen (M-N) is the presence of the bands in the range 523- 673 and 433- 487 cm-1 respectively which is in agreement with the literature .

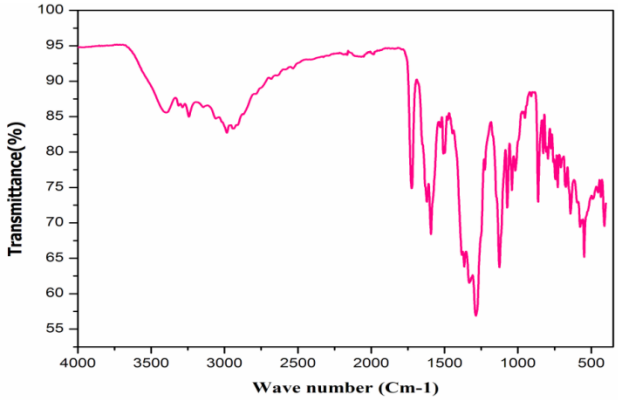

Figure 3 FT IR Spectrum of Cu(II) Complex

3.1.3 EPR spectral studies

The EPR spectrum of the copper complex was recorded at room temperature on X-band frequency of 9.86 GHz under the magnetic field strength varying from 2400 to 4400 G. Intense absorption signal appeared in the high field at 3400 G (Fig 4). As is apparent in the spectrum of copper complex, g_{II} (2.386) > $g_{\perp}$(2.203) indicating that the unpaired electron is in dx^2-y^2 orbital of square pyramidal complexes [40]. The anisotropy in g indicates the presence of a fairly large component of low symmetry in the ligand field.

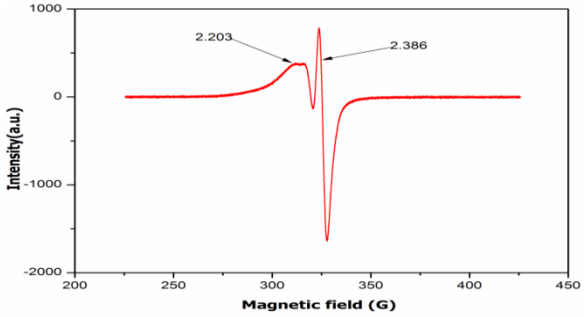

Figure 4 EPR Spectrum of Cu(II) Complex

3.2 DNA binding experiments

3.2.1 UV–Vis- absorption analysis

An efficient way to analyze the binding mode of the metal complex with DNA is electronic absorption spectroscopy [41]. The intrinsic constant of binding to CT-DNA was already calculated by tracking the absorption rate of the spectral band of charge transfer at 274 nm of the complex fig 5. The addition of growing quantities of CT-DNA, a significant “hyperchromic” effects have been observed with a mild red change of 2–3 nm, Indicative of DNA helix stabilization. The spectral features mean that either the external touch (electrostatic binding) or the major and minor DNA grooves are binding to the ligands and complexes. Moreover, this “hyperchromic effect” is depend on the two phenomena basis. First, the ligand has large surface area and there are planar aromatic chromophore promote a close binding relationship between the ligand and CT-DNA even more by, providing ample opportunity for the complex to bind with the CT-DNA, in between the stacking base pair, partial insertion of the aromatic moiety. The outcome of this Groove Binding in CT-DNA structural reorganisation that includes partial unwinding or damage to the external phosphate backbone of the double helix, leading to the creation of a cavity to accommodate the complex [42].. The Kb

values of the metal complex were calculated as Kb = 2.1059 X 10^4 M^{-1}

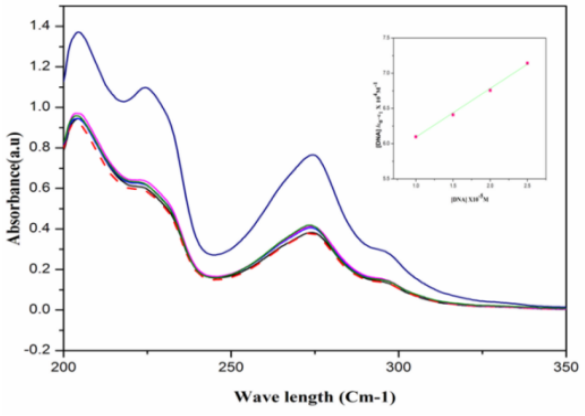

Figure 5 Absorption spectral identification on addition of CT DNA for Cu (II) complex (Inset plot of [DNA]/(εa-εf) Vs [DNA] for absorption titration of CT DNA with complex).

3.2.2 Fluorescence quenching study

Fluorescence spectral technique is an effective way to study metal interaction with CT DNA. Ethidium bromide (EB) is one of the most sensitive fluorescence probes that bind to DNA due to the intense increasing of the EB fluorescence after intercalating into CT DNA [43]. If a metal complex intercalates into DNA it leads to a decrease in the binding sites of DNA occupied by EB, resulting in the decrease of the fluorescence intensity of the EB-CT DNA system. Therefore, Cu(II) complex was added to CT DNA, pre-treated with EB. As can be seen in Fig.6, the intensity of fluorescence signal of the EB-CT DNA system decreased upon addition of complex. This is a clear indication that the complex competes with EB to the binding sites of DNA. Such behaviour is found for intercalative DNA interaction processes. Additionally the fluorescence quenching curve of the EB-CT DNA system for complex is in good agreement with the linear Stern-Volmer equation (see Eq. (2)) , $I_0 / I = 1 + K\,svr$ where I_0 and I are the fluorescence intensities of the CT DNA solutions in the absence and in the presence of the complex, respectively. Ksv is the Stern-Volmer dynamic quenching constant and [Q] is the total molar concentration of the quencher (in this case, the copper complex concentration). In the linear fit plot of I0/I versus [complex]/[DNA], K is given by the ratio of the slope to the intercept. The concentrations of the complex are taken for observing a 50% reduction of the emission intensity of EB , meaning that 50% of EB molecules have been replaced in EB-CT DNA system, at a concentration ratio of [complex 1]/[EB]=K1. Therefore, by taking Kapp = Kb (EB)/K1 and the DNA binding constant of 1.0 × 10^7 M−1 for EB the apparent DNA binding constant (Kapp) of 3.2705x10^{-5}M for complex 1 was obtained. This result suggests that the Cu(II) complex interacts with CT DNA via intercalation mode, which isfully consistent with the absorption spectral results undertaken in this study.

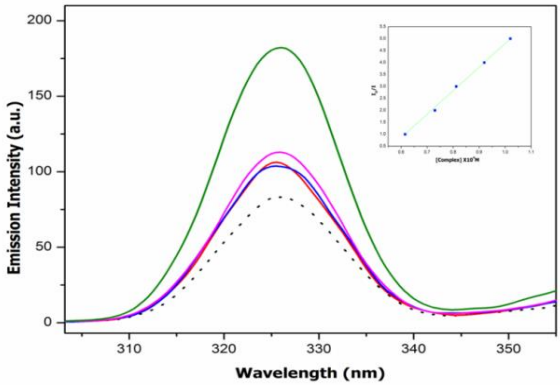

Figure 6 Fluorescence emission spectra (excited at 300 nm) of the CT DNA-EB (4 × 10^{-5} M ethidium bromide, 4 × 10^{-5} M CT DNA).

3.2.3 Viscosity measurements

We evaluated the changes DNA solution viscosity upon the addition of the complex and the EB to clarify the interactions between CT-DNA and the complex. The viscosity was measured to assess the complex–DNA binding mode because DNA viscosity increases with DNA length [44]. Intercalation elongates the DNA helices and increases DNA viscosity. The base pairs are disassociated to accommodate the binding ligands. On the contrary, nonclassical or partial intercalation bends the DNA helices and lowers DNA viscosity. Neither grooving nor electrostatic binding affects DNA viscosity . Figure 7shows that DNA viscosity increases with the [complex]/[DNA] ratio. Therefore, EB and both complex intercalatively to DNA, and this finding was corroborated by the absorption and emission spectra.

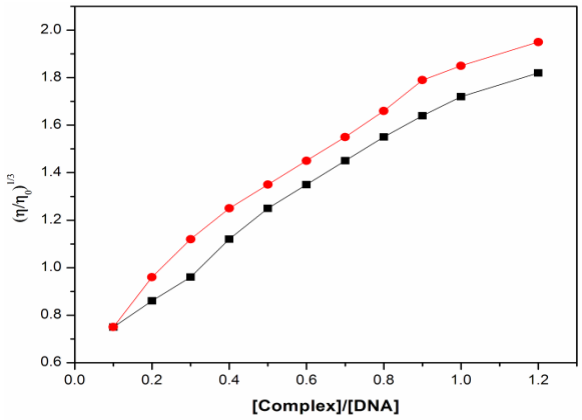

Figure 7 Effect of relative viscosity with an increasing amount of CT-DNA at room temperature

3.2.4 Cyclic Voltammetry Studies

The cyclic voltammetry to the metal complex was further carried out to find the interaction between the metal complex and DNA. It is confirmed that the useful result well matches to the before utilized technique of UV–V and viscosity methods [45]. The a typical three-electrode setup was employed in the cyclic voltammogram route which was constructed with a 0.01mM solution of copper (II) complex without and with DNA is measured by the platinum as the working electrode in DMSO solution. The corresponding result is shown in Fig.8, resulting in the forwarding scan a single oxidation peak is observed that corresponds to the oxidation peak of the metal complex. While the reverse scan can exhibit a single cathodic peak that is also implying the cathodic peak of the metal complex. When the presence of CT-DNA the anodic peak intensity considerably increased (Fig.8) at the same time the cathodic peak intensity decreased with small shitting and indicate that well defusing of CT-DNA with metal complex on the electrode surface. Thus, the CT-DNA and metal complex is bounded together implying from the intensity variation in metal complex with DNA moieties.

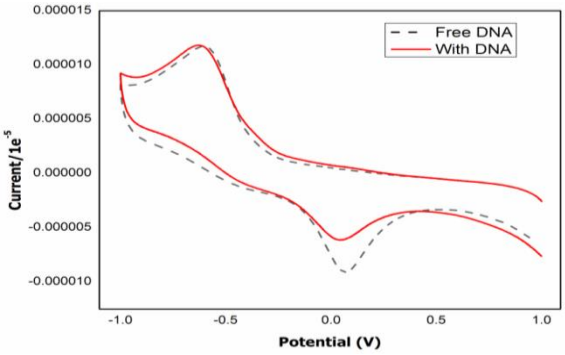

Figure 8 Cyclic voltammetry of Cu(II) complex

3.2.5 Anticancer assay

The cytotoxicity of the complex to be used as chemotherapeutic agents was studied using MTT assay. The ability of the complex on HepG2 cells was tested with or without various concentrations (6.5–100µg/ml) of the complex for 24 h. Cells incubated with different concentrations using normal as control. After the incubation period, MTT assay was carried out to calculate the cell death percentage. For each concentration of the complex cells were incubated in triplicate. Fig. 9clearly illustrates that there is a clear decrease in the viable cell number in the cells incubated with complex in a concentration-dependent manner. The viability of cells incubated without any compound was considered as 100%, and the percentage[46] of viable cells incubated with compounds are given as relative to the control. The IC50 and R2 values of an optimized complex of Cu(II)are noted as 60.19 and 0.9983 (fig 10), respectively. The results revealed that copper complex showed potent cytotixic effects against human cell line(HepG2) which concludes its potential part in medicine an anticancer and antiseptic agent.

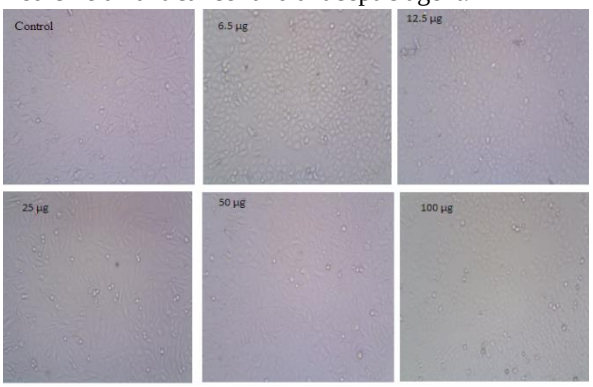

Figure 9 Anticancer activity of Cu (II) complex

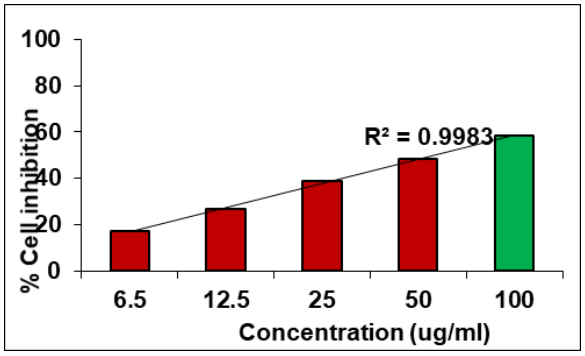

Figure 10 Cell viability of HepG2 cells after treatment in Cu(II) complex at various concentration

3.3 Antimicrobial Assay

The antimicrobial activity of copper(II) nitrate and synthesized complex [Cu(Neocup)(L-orn)NO_3] was screened against a panel of pathogenic bacterial strains using the microbroth dilution method. Compounds with a amino acids like L-ornithine containing terminal amine moiety easily penetrate through cell walls due to their ionic character[46]. The antimicrobial screening data indicate that the metal salt, free acid as well as the complex aredisplaying fairly good antimicrobial activity against all the four mentioned bacteria. The complex is most effective antimicrobial agent against *S.aureus*, *E.coli*,*P.aeruginosa*,*C.albicans* bacterial, fungal strains (Fig. 11). MIC values (lg/ml) for the metal salt and its complex with L-ornithine were summarized in Table 1. The amino acid complex was active against bacteria under very low concentration and minimizing the possible toxic effects.

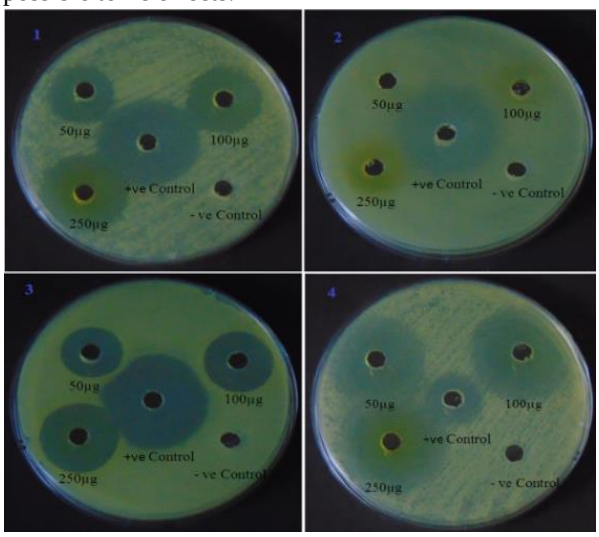

Figure 11 (1-3) Antimicrobial and(4) antifungal activity of Cu (II) complex at 6 mm zone inhibition diameter.

Table 1 Antibacterial activity of Cu(II) complex

S. No	Microorganism	Control		Zone inhibition of Complex		
		-Ve	+ve	50µg /ml	100µg/ ml	250µ g/ml
1.	*S.aureus*	-	31	20	23	25
2.	*E.coli*	-	31	-	-	-
3.	*P.aeruginosa*	-	33	19	21	25
4.	*C.albicans*	-	16	21	24	26

3.4 Theoretical calculations

DFT was further applied to find the ground-state structure of the optimized Cu(II) complex using the B3LYP/6-31G(d,p) level of theory as illustrated in Fig. 12. It is observed that the copper atom is five coordinated under distorted square-pyramidal geometry [47]. As resulted in Table 2, the bond lengths and bond angles measured by the DFT studies and the complex maximum varying of bond length 2.043 (Å) for C3-N1-Cu49 bond and bond angle is noted as 112.4 and 103.09 (Å) for O-Cu-N1 and O-Cu-N3. The stability of molecules is influenced by the energy gap and dipole moment. The frontier molecule orbital's HOMO and LUMO are very important tools for chemical reaction and take a portion in chemical stability. The electron transitions can be revealed from HOMO to LUMO which determines the way in which one molecule interacts with other molecules. Because electron conductivity is measured, it may be confirmed that the molecular electron transport activity exists. In this Cu (II) complex, the HOMO is localized entirely in the terminal N-group and heterocyclic bases whereas LUMO is highest circulated in a fully metal center. The energy gap of HOMO–LUMO was confirmed (2.2300 eV) in the charge transfer process within the Cu(II) complex.

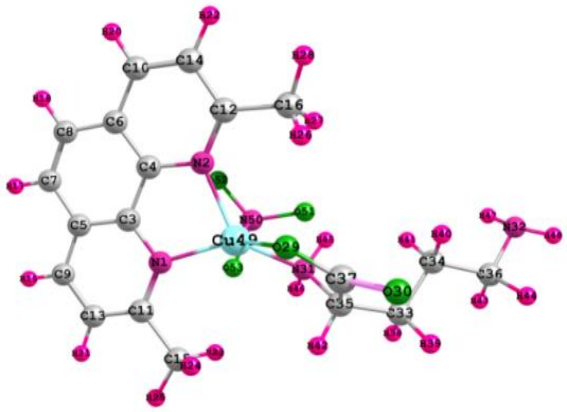

Figure 12 optimised structure of Cu(II) complex

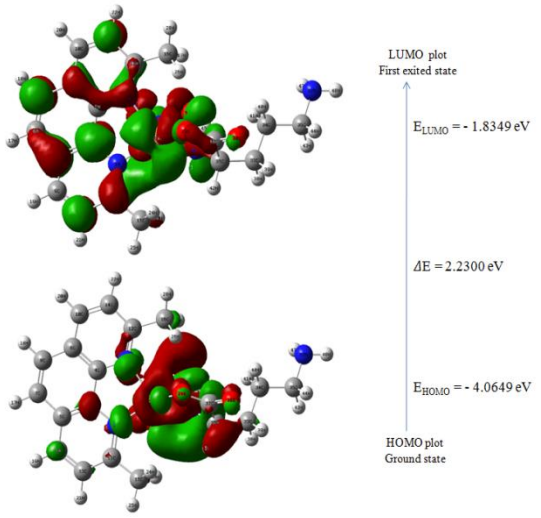

Figure 13 Geometries of Cu(II) complex, and Energy gap of HOMO and LUMO on Frontier molecular orbitals

Table. 2a Bond length and bond angle of Cu (II) complex

S.No	Parameter	B3LYP/6-31G(d,p)
1.	Bond length	
2.	N1-Cu49	1.969
3.	N2-Cu49	2.038
4.	O29-Cu49	1.951
5.	N31-Cu49	2.043
6.	Cu49-N50	1.264

Table. 2b Bond length and bond angle of Cu (II) complex

S.No	Parameter	B3LYP/6-31G(d,p)
	Bond Angle	
1.	C3-N1-Cu49	112.4
2.	C11-N1-Cu49	129.4
3.	N1-Cu49-N2	83.4
4.	N1-Cu49-O29	134.5
5.	N1-Cu49-N31	122.1
6.	C4-N2-Cu49	110.5
7.	C12-N2-Cu49	130.4
8.	N2-Cu49-O29	108.8
9.	N2-Cu49-N31	128.6
10.	C37-O29-Cu49	115.8
11.	O29-Cu49-N31	84.9
12.	C35-N31-Cu49	104.6
13.	H45-N31-Cu49	109.5
14.	H46-N31-Cu49	119.2
15.	N1-Cu49-N50	98.9
16.	N2-Cu49-N50	70.7
17.	O29-Cu49-N50	126.6
18.	N31-Cu49-N50	62.3
19.	Cu49-N50-O51	102.9
20.	Cu49-N50-O52	99.3
21.	Cu49-N50-O53	67.4

3.5 Docking Studies

Molecular docking was done on complex to make available acumens to the interactions between complex and A-DNA. Docking results within the Root Mean Square Deviation RMSD value 25.254 Å is considered acceptable [47]. The RMSD values of docked complex are below 25.254A^{o} The docking experiment done on complexes with A-DNA tetradecanucleotide sequence d(CCCCGGTACCGGGG)2 with C2(Table.3). G13 base pair identified the major-groove as the binding site. As seen , the most favorable energetically docked pose. (Figure 14). These resultsagree with the experimental findings and will provide more information on groove binding.

Figure 14 Molecular docking of copper (II) complexes with CT-DNA.

Table. 3 Bond length and bond angle of Cu (II) complex

S.No	Parameter	Values
1.	Property	AntiCancer
2.	PDB ID	303D
3.	Binding Energy (kcal/mol)	-9.35
4.	Inhibition constant, ki (μm)	139.97
5.	RMSD (Å)	25.254
6.	Intermolecular Energy (kcal/mol)	10.54
7.	Vdw + Hbond + desolv Energy (kcal/mol)	-7.04
8.	Binding Nature	Groove Binding

3.6. Molecular electrostatic potential

MEP (Molecular electrostatic potential) Fig.15 is as a significant tool to predict the electrophilic and nucleophilic attacks for the biological interactions. The MEP of the title compound was optimized geometry using b3lyp method 6-311+g (d, p) was calculated. As can be observed in Fig.5. The different colors in this plot are indicated different values of the electrostatic potential. Red<orange< yellow<green<blue. The blue illustrates the strongest attraction. The positive area is located around vinyl and phenyl groups. These areas are

having positive potential. The negative area is related to C=O group. In these areas having negative potential are over the electronegative atoms such as oxygen. The red indicates the strongest repulsion. These regions of negative potential are associated with the lone pair of electronegative atoms. The residuals species are surrounded by zero potential. The nitrogen of NH and oxygen of OH are as anions coordination to palladium and the formation of complex.

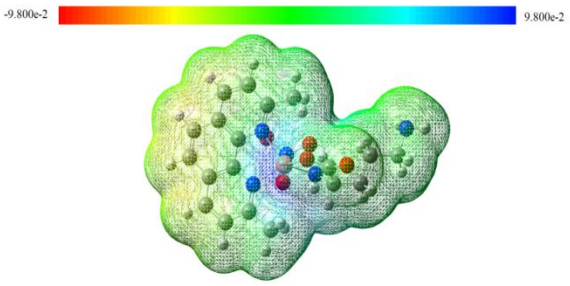

Figure 15 Molecular electrostatic potential of Cu(II) Complex

4.CONCLUSION

In this conclusion, the copper (II) complex[Cu (Neocup)(L-orni)(NO_3)] have been fabricated and analysed byUV–visible, emission, EPS, and DFT studies. The complex exhibits a distorted square-pyramidal geometry. In the present investigation, The binding mode of the complex with CT-DNA has been performed by UV-Vis absorption spectroscopy method, ethidium bromide displacement evaluation, viscometrical technique and CV studies The equilibrium binding constant(K_b)displays that the DNA-binding affinity increases with increasing DNA addition. Furthermore, the molecular docking studies were confirmed the Cu (II) complex injected into the base pairs of CT-DNA. Therefore, the partial intercalative binding intercalation of Cu(II)complex with DNA was confirmed from the titration experiments. DFT studies of the structural properties such bond lengths, geometries, bond angles, and torsion angles and spectroscopic properties of HOMO–LUMO of Cu (II) complex have been reported and the theoretical result also compared with experimental data's. The spectroscopic finding is in great qualitative agreement with the optimized experimental and theoretical structure. It is predicted that the DFT studies performed at DFT/B3LYP/6-31G*(d,p)/LANL2DZ level are the suitable quantum chemical technique for repeating the experimental results for the obtained complex. The energy gap diagram of HOMO–LUMO gaps revealed that the charge transfer interactions take place within the copper complex.

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