Synthesis, Characterization, DNA Binding, Molecular Docking, Cytotoxicity and Antimicrobial Studies of Transition Metal Complex with Iminodiacetic Acid and 1-Pentadecane Carboxylic Acid

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Abstract : **The Cu(II) complex have been synthesized from the iminodiacetic acid and 1-pentadecane carboxylic acid (palmitic acid). The nature of bonding and geometry of the transition metal complex have been deduced from elemental analysis, FT-IR, UV–vis, emission, EPR spectral studies. UV–vis, cyclic voltammetry titrations, and viscosity measurements were used to investigate the Cu(II) complex's interaction with CT- DNA. The complex binds to DNA through intercalative binding mode. The experimental data were confirmed by molecular docking studies, employing DNA sequences bind with DNA via a minor groove mode of binding. The biological activity of the metal complex has been studied on bacteria pathogenic strains *Enterobacter cloacae, Staphylococcus haemolyticus* and *Bacillus cereus* and fungi *Candida albicans* and *Aspergillus fumicatus* by well diffusion method.**

Keywords: **Iminodiacetic acid, DNA binding, Viscosity, 1-pentadecane carboxylic acid (palmitic acid), Biological activity and cytotoxicity.**

INTRODUCTION

The coordination chemistry of iminodiacetic acid (H_2IDA) has been a subject of incessant investigations due to its tridentate chelating performance towards metal ions resulting in complexes which show structural diversities [1],[2]. Moreover, some of these complexes play a conclusive role not only deactivation of enzymes but also in the stowage and transport of active substances through biological crusts[3]. The study of the structure of typical ternary complexes provides material's about how biological systems attain their specificity and stability, as well as styles to advance these features for biotechnological applications. Ternary complexes of oxygen donor ligands and heteroaromatic N-bases have been of distinct curiosity as they can display identical high stability. Copper is an indispensable bio element that plays a key role in biological progressions and its complexes are ideal molecules for cancer embarrassment[4]. Copper complexes containing phen ligands are useful probes for DNA duplexes. Copper(II) complexes are essential for the active positions of a vast number of metalloproteins in biotic organizations and have the potential to be used in a variety of catalytic processes in living organisms, such as electron transfer reactions or the induction of anticancer components[5] and, oxygen transport and endogenic oxidative DNA damage associated with aging and cancer[6].

Bioinorganic[7] and medicinal chemistry[8] both have elaborate versions of these procedures. Copper(II) chelates have been developed in detail to work with biological organizations and to exhibit antineoplastic drug activity[9]-[11] as well as antiseptic, antifungal[12],[13], and anticancer activity[14]. Because of their excellent binding ability with DNA base pairs, copper(II) N, S, O/N, N-donor chelators are respected anticancer delegates[15]. Pyridines are important heterocyclic composites in organic synthesis, serving primarily as agrochemicals and synthetic mediators. Pyridine byproducts, such as (aromatic) alkoxyl pyridine composites, amido pyridine, and its sprouts, which take the place of merging pyridine compounds, have previously been common in agrochemical harvest pitches[16].

Composites with the potential to confuse and cut double aground DNA farther down the functional conditions are being considered for use in medical

applications for genomic research as problem-solving go-betweens. In intercalative or indentation binding relationships, DNA base sets and amino sugar mediety are commonly implicated. Edmond Frémy discovered palmitic acid (1-pentadecane carboxylic acid) in saponified palm oil in 1840[17]. This miscellany covers the main industrial path for its development, which involves the hydrolysis of triglycerides (fats) in palm oil by high-temperature water and the subsequent distillation of the resulting mixture[18].

Palmitic acid is formed by the conversion of additional carbohydrates in the body. The predecessor of longer fatty acids is 1-pentadecane carboxylic acid, which was built over fatty acid combinations. Palmitic acid is a vital component of animal bodies as an importation. Single inquiry a newbie makes up 21-30% (molar) of human yard fat in humans[19], and it is a crucial, but wildly variable, lipid unit in human opposing milk. Palmitate wreaks havoc on acetyl-CoA carboxylase (ACC), the enzyme in charge of converting acetyl-CoA to malonyl-CoA, which is then utilized to add to the increasing acyl chain, hence preventing the production of further palmitate. The buildup of a palmitoyl assembly in a process known as palmitoylation is researched in almost all proteins. Many membrane proteins rely on palmitoylation for their localization. Palmitic acid and sodium salt innovation include application in meals because it is sensible and adds value and "mouthfeel" to achieved foods (suitability food). In organic goods, sodium palmitate is allowed as a common preservative[20]. Prompted by the biological activities of the transition metal ternary complexes, it was anticipated to strategy synthesis of mixed-ligand metal iminodiacetate complexes, M/IDA/Phen (1/1/1 mole ratio) and to chase in vitro antimicrobial and SOD activities.

Recently, our group interested to focus binding activity of DNA with amino acid containing copper (II) complexes, and heterocyclic base. The research work reveals that efficient DNA binding, cleavage, cytotoxicity and DFT properties for the complex have also been calculated[21]-[25]. In this study the transition metal complexes of iminodiacetic acid and 1-pentadecane carboxylic acid were assumed by using spectroscopic dealings of UV, FT-IR, EPR, viscosity, cyclic voltammetry and their biological activities such as antibacterial, antifungal and cytotoxicity were also premeditated. The DFT intentions were carried out with the Gaussian03 package and molecular docking were also accomplished.

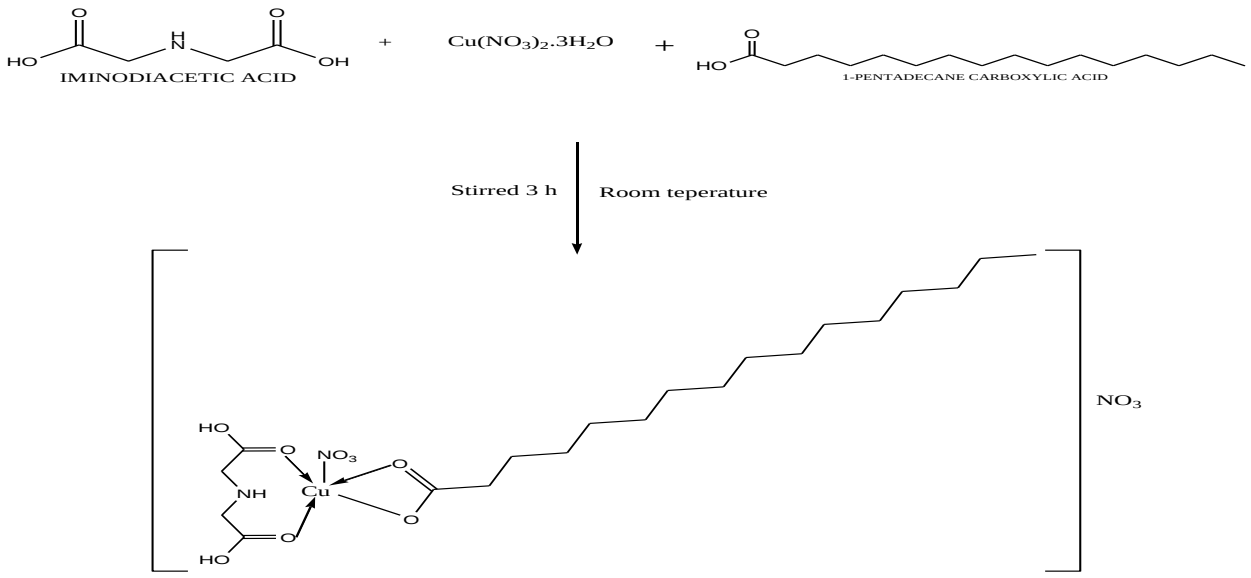
EXPERIMENTAL

Materials and Methods

All the chemicals used for the preparation of the ligands were of BDH quality, AR evaluation. The electronic spectra stayed recorded on PerkinElmer UV-visible Spectrophotometer Lambda 25 in DMSO. FT-IR spectra of the synthesized compounds were tested on PerkinElmer 100S spectrometer using KBr pellets. Elemental analysis was performed using a PerkinElmer CHN analyzer. The EPR spectrum of Cu(II) complex was noted using JOEL X-Band Electron Spin Resonance spectrometer at room temperature. The electro chemical tests were carried out using a Trace-Iab50 from radiometer. Potentials are expressed versus the Ag/AgCl electrode detached from the test solution by a salt bridge comprising the solvent/supporting electrolyte. A preerudite glassy carbon (GC) disc of 3 mm diameter (radiometer) was used as the working electrode and a platinum wire was the auxiliary electrode.

Synthesis of the Complex

The metal complex was formed by iminodiacetic acid (0.133 mg, 1mmol) was dissolved in ethanol (20 ml) and $Cu(NO_3)_2.3H_2O$ (0.241 mg, 1mmol) was then added. After 5 min, 1-pentadecane carboxylic acid (0.256 mg, 1mmol) was added into the solution and the solution was stirred for three hours at room temperature. Then a blue precipitate, Cu (II) complex (scheme.1) was formed in few weeks. It was purified by washing numerous times with ethanol, and then desiccated for 48 h in vacuum. Yield 67%. Analytical calculation for $C_{20}H_{38}CuN_2O_9$ (%): C, 46.73; H, 7.45; N, 5.45; Found (%): C, 46.69; H, 7.44; N, 5.48; FT-IR: 3270s, 3010w, 1723s, 1383b, 1114m, 734w, 594m (br, broad; s, strong; m, medium; w, weak) mp. 260 ^oC.



Scheme. 1 Synthesis of Cu(II) complex

DNA Binding Studies

Absorption titration experimentation was accomplished through fixed kindnesses of the complexes though progressively growing concentration of CT-DNA. Although degree the assignation spectra, a suitable amount of CT-DNA was further to composed composite solution and the position of solution to indifference the absorbance of CT-DNA the situation. Since the absorption titration data, the required constant (K_b) was firm by[26]

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

Where [DNA] denotes the concentration of DNA in base twosomes, a, f, and b correspond to A_{obsd}/[M], the extinction coefficient of the permissible amalgams and the extinction coefficient of the composite when wholly destined to DNA, respectively. K_b is agreed by the angle to capture ratio in plots of [DNA]/(a f) against [DNA].

Fluorescence Titration

The fluorescence titration approach was employed to better understand the complexes interaction behaviour with DNA. Fluorescence measurements were performed with a constant concentration of complex (15 M) and a range of CT-DNA concentrations from 10 to 100 M.

Cyclic Voltametric Studies

Cyclic voltammetry measurements of abilities are expressed versus the Ag/AgCl electrode disconnected from the test solution by a salt bridge comprising the solvent/ supporting electrolyte. A pre polished glassy carbon (GC) disc of 3 mm diameter (radiometer) was used as the working electrode and a platinum wire was the secondary electrode.

Viscosity Measurements

Viscosity measurements were performed on an Ubbelohde viscometer in a 25°C thermostatic water-bath. By sonicating in the direction of minor problems climbing from DNA flexibility, DNA models with an average length of 200 bp were organized[27]. Titrations for the compounds (3 mM) were accomplished, and each compound was dominated by the CT-DNA solution (50 M) in the viscometer. The data was given as $(\eta/\eta_0)^{(1/3)}$, rather than the proportion of the complex's concentration to CT-DNA, where is the viscosity of CT-DNA in the complex's incidence, and η_0 is the viscosity of CT-DNA unsupported. The experimental flow time of CT-DNA realizing solutions was repaired commencing the flow time of buffer unaccompanied (t_0), resulting in a viscosity value of (t/t_0).

Molecular Docking Study

Docking studies were carried out using MGL tools 1.5.4 with AutoGrid4 and AutoDock4 to perform blind docking calculations Co(II) complexes and DNA sequence (pdb code: 3CO3). A full description is provided in SI together with the output files. The docked confirmations were then analyzed for hydrogen bonding and binding energy.

Antimicrobial Activity

The antibacterial activity of the complex was studied against pathogenic strains of bacteria *Enterobacter cloacae*, *Staphylococcus haemolyticus* and *Bacillus cereus*. These cultures were grown on appropriate medium at 37°C for overnight incubation and maintained at 4°C in a refrigerator. Well diffusion method for Muller Hinton agar medium. The fungal activity of human fungal pathogens like *Candida albicans* and *Aspergillus fumicatus* on the metal complex. The fungal cultures were maintained on nutrient agar medium and potato dextrose agar (PDA) medium respectively. Composition of PDA medium potato 200.0 g, dextrose 20.0 g, agar 15.0 g, distilled water 1000 ml and the pH maintained at 6.2 (Taylor *et al.*, 1995). The experiment was conducted in triplicates (n=3). The fungal cultures were maintained on nutrient broth (NB) at37°C and fungus was maintained on Potato dextrose agar (PDA) at 28°C.

Anticancer Study

The synthesized of the complex was tested on the human lung cancer cell line (A549). These cells were sowed in a 96 well plate and incubated. The cells were maintained at 37°C, 5% CO2, 95 % air and 100 % relative humidity and the cells were perused with concentrations. (6.5 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml) of the reported compound and permitted to incubate for the next 24h. Cell number/proliferation was measured after 48h of incubation using standard MTT assay. The cells exhibited dose-dependent growth repressive effect against the tested cell lines and IC 50 values were calculated.

RESULTS AND DISCUSSION

Absorption Titration

The electronic absorption spectroscopy is the most mutual way to examine the interactions of complexes with DNA. In universal, complex bound to DNA (CT-DNA) through intercalation frequently outcomes in hypochromism and red shift (bathochromism), due to the durable staking interaction among aromatic chromophore of the compound and the base pairs of DNA. The Cu(II) complex obtainable absorption bands at 227 nm. With growing concentration of DNA, the complex displayed hypochromicity and a red-shifted charge transference peak highest in the absorption spectra. Fig.1 shows the locations of the Cu(II) complex absorption bands in the absence and presence of CT-DNA. When the interleaved ligand container's π* detour interacts with the orbital of the DNA base pairs, the π-π* transition liveliness is reduced, resulting in bathochromism. By tracking the differences in absorbance, with increasing interest of DNA, the critical binding factors K_b of the complex to CT-DNA were established. A plot of [DNA]/(εa-εf) versus [DNA] will give a slope 1/(εb-εf) and an intercept 1/Kb (εb-εf). The K_b is the ratio of the slope and the intercept[28]. The K_b values used are intrinsic Cu(II) complex 2.8 x10^{-7} M^{-1} binding constant.

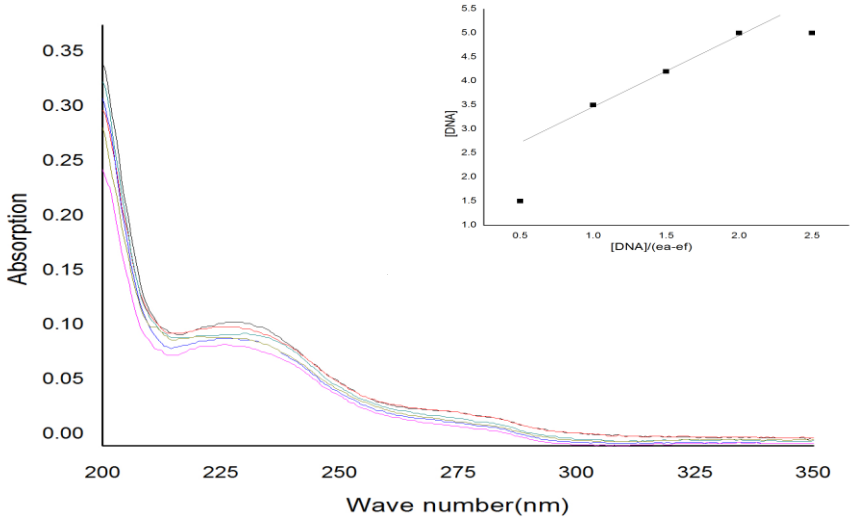


Fig. 1 Absorption spectral traces on addition of CT-DNA to complex inset plot of [DNA]/(ε_a-ε_f) vs [DNA] for absorption titration of CT-DNA with complex

Quenching Studies

Ethidium bromide (EB) emits intense fluorescence light in the presence of DNA, due to strong intercalation between the adjacent DNA base pairs. The extent of fluorescence of EB bound to DNA is used to determine the extent of binding between the second molecule and DNA. The emission spectra of EB bound to DNA in the absence and in the presence of Cu(II) complex is given in Fig. 2. The addition of the complex to DNA pretreated with EB causes increase in emission intensity, indicating that the complex competes with EB in binding to DNA. The classical Stern – Volmer equation is,

$$I_o/I = 1 + Ksv\ r \qquad (2)$$

Where I_o and I are the fluorescence intensities in the absence and presence of complex, respectively, Ksv is a linear Stern-Volmer quenching constant dependent on the ratio of r_{EB} (the bound concentration of EB to the concentration of DNA), and r is the ratio of total complex to DNA concentration. The quenching graphs show that the copper complex's quenching of EB attached to DNA agrees well with the linear Stern–Volmer equation, proving that the complex binds to DNA. The ratio of the slope to the intercept is Ksv in the linear fit plot of Io/I vs r. The complex has a Ksv value of 3.198 $x10^{-3}$ respectively.

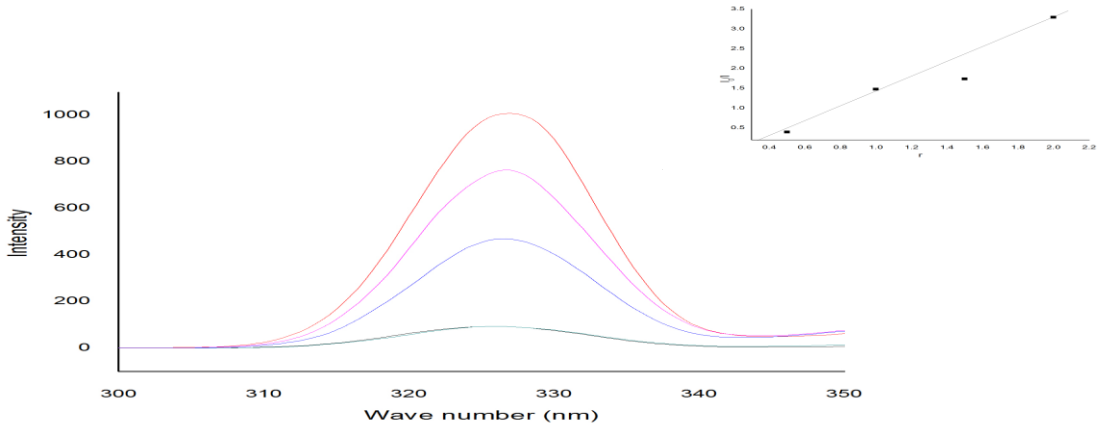


Fig. 2 Effect of increasing amount of Cu complex on the relative viscosity of CT-DNA at 28 °C±0.1, [DNA]=15 μM

Cyclic Voltammetry

To assist the nature of binding of the Cu(II) complex with nonappearance and occurrence of DNA are accessible in Fig. 3 With the count of CT-DNA to the complex, the voltammetric current coupled with positive shift in $E_{1/2}$ is observed to reduction significantly. When the complex binds with DNA, a non-electro dynamic product is molded which decreases the concentration of the electro active kind existing in the solution thus causing a drop in the peak current[29]. The present complex decrease in Voltametric current in the incidence of DNA in this case can be linked to the purposeful circulation of the metal complex that is guaranteed to CT DNA. The complex intercalates into the DNA dual helix when E_{pc} or E_{pa} shifts to the positive side[30].

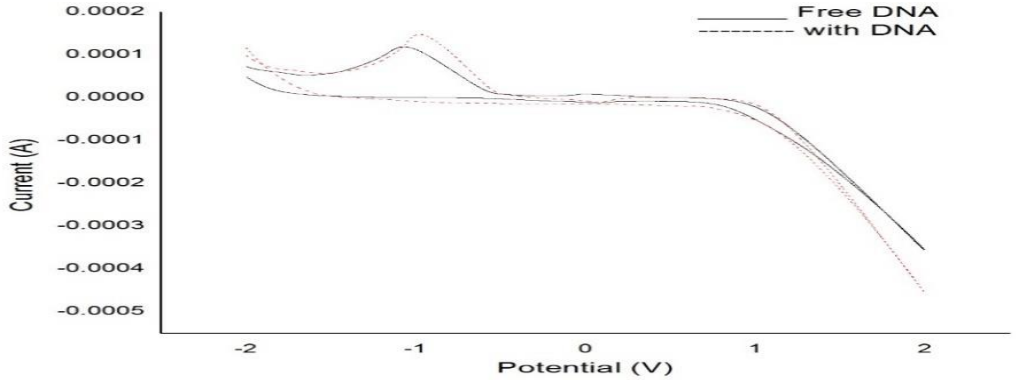


Fig. 3 Structure of Cyclic voltammetry copper complex (presence and absence of CT-DNA)

Viscosity Studies

Optical photo physical concerts are frequently employed to investigate the binding characteristics of ligands, metal compounds and DNA, but they do not provide enough information to construct a binding model. As a result, viscosity scopes were chosen to expose the interaction between metal complex and DNA. Complexes that bind fully in the DNA trenches by defective and/or non-classical intercalation, under similar conditions, typically cause fewer discernible (positive or negative) or no changes in DNA solution viscosity[31]. In contrast to [compound]/ [DNA], the morality of (0)1/3 were concocted (Fig. 4). As the amount of chemical in the Cu(II) complex increases, the viscosity of DNA decreases progressively. The lower simulated viscosity of DNA may be explained by a binding mechanism that caused bends or twists in the DNA, compressing its effective span and thus its viscosity. The findings suggest that the Cu(II) complex binds to DNA by incomplete embolism

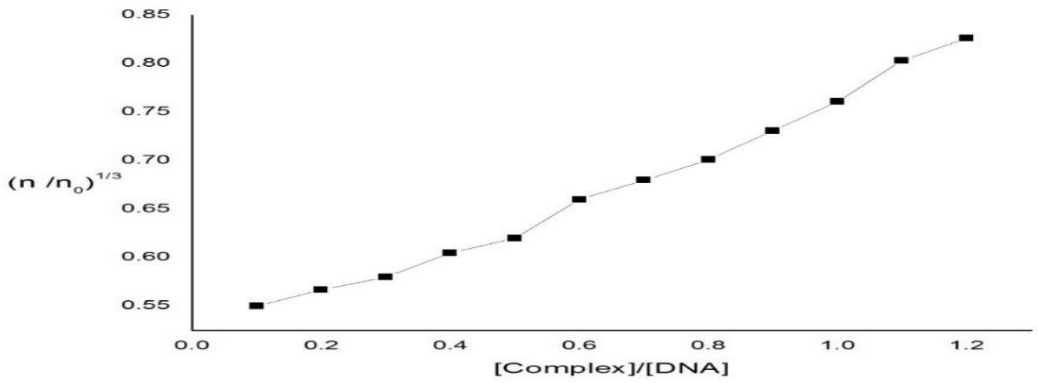


Fig. 4 Effect of increasing amounts of complex on the relative viscosity of calf thymus DNA at 27ºC

EPR Spectroscopy

The EPR spectrum of copper(II) complex provides information, important in studying the metal ion environment. The EPR spectra were recorded in DMSO at Room Temperature (Fig. 5). At room temperature it shows a forceful absorption crew in the high pitch district and is isotropous due to the dipping motion of the molecules[32]. Nevertheless, this complex at fluid nitrogen temperature display well committed points in low turf area due to the link of the electron spin of the 63Cu nucleus (I = 3/2). The peaks are broad and have the appearance of ill resolved. The peaks can be attributed to hyperfine splitting of the nitrogen atom (I = 1) of the ligand. At room temperature, the complex exhibit well defined single isotropic lines at a g value of the axially elongated g‖ value of complex is 2.199 respectively. g‖ values are better than $g^{\perp}$ value 2.045. The trend, g ‖> $g^{\perp}$>Ge (2.002) showed that the unpaired electrons was localized in the dx^2-y^2 orbital of copper(II)[33].

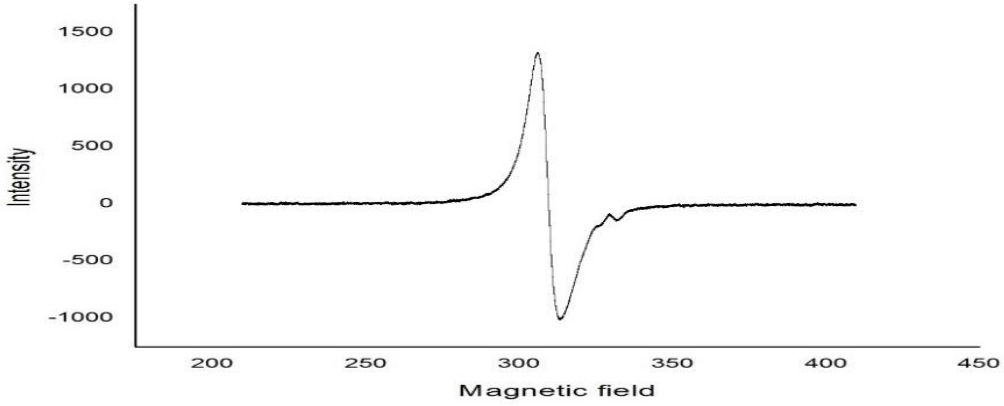
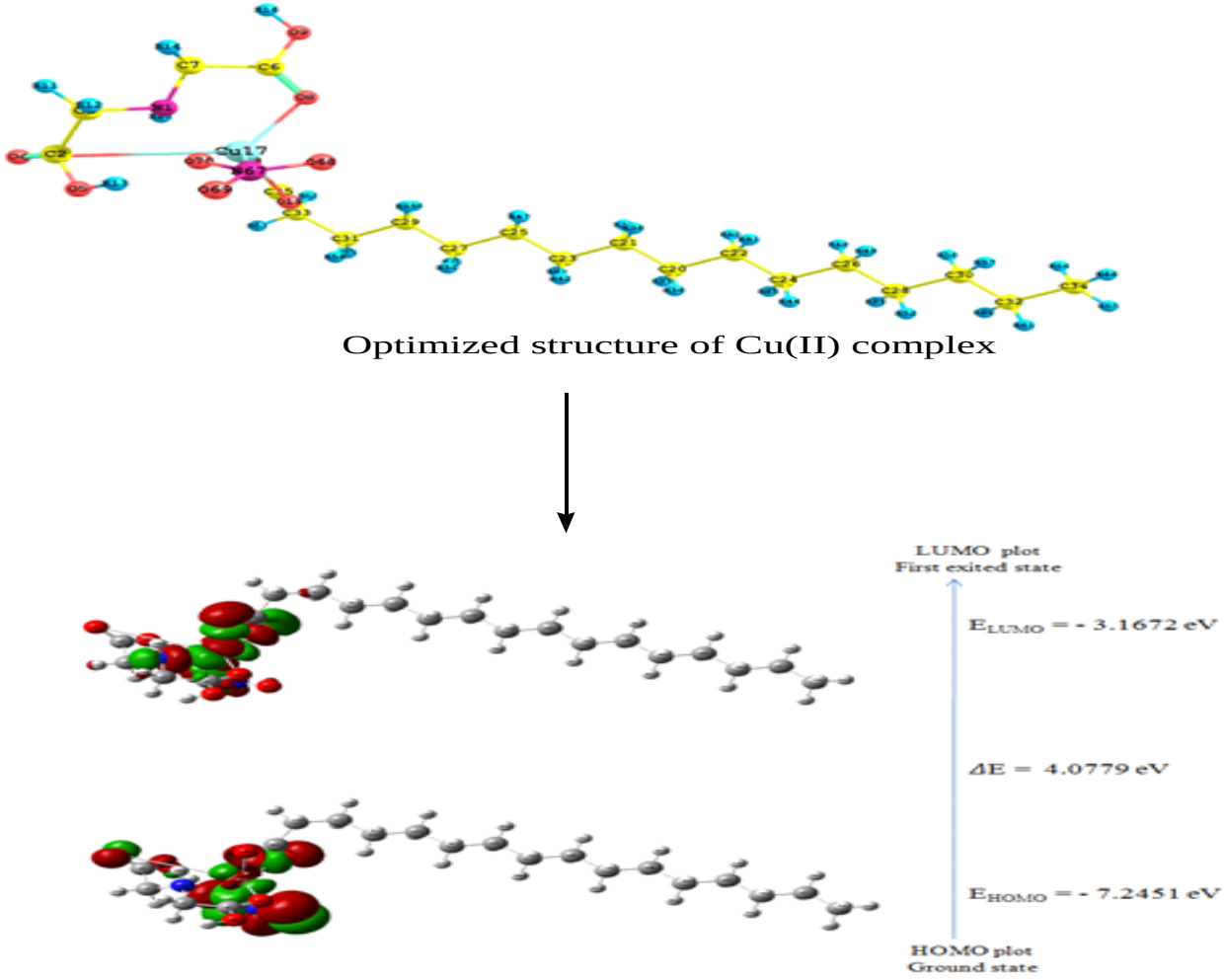


Fig. 5 EPR spectra of the Cu(II) complex

Density Functional Theory

The DFT calculations were carried out B3LYP/6-31G with the Gaussian03 package. The geometrical parameters viz. calculated bond distances, bond angles and detected bond lengths and angles in complex are given in Table. 1. In general, there is good arrangement between the calculated and experimental Cu-N and Cu-O bond lengths of the iminodiacetic acid and 1-pentadecane carboxylic acid ligands. The energies of HOMO to LUMO orbitals are exposed given in Fig. 6. It has been clearly pointed out that the HOMO–LUMO energy gap decreases with the increase in aromatic replacement. Thus, the HOMO–LUMO energy gap of ΔE=4.0779eV the copper complex. The electrostatic potential (ESP) mapping (Fig. 7) displayed electrophilic region for copper complex. Additionally, some parameters are calculated like ionization potential, electron affinity, electronegativity and chemical potential, these values are 7.245, 3.167, 5.206, and -5.206. The chemical hardness, softness and electrophilicity index also calculated, these values are 2.039,0.024 and 6.646 respectively.



Fige. 6 The optimized and HOMO-LUMO structure of Cu(II) complex

Table. 1 Bond length and bond angle of Cu(II) complex

Parameters	Complex
O8 - Cu	2.242
O4 - Cu	4.328
Cu- N	2.886
Cu-O18	2.024
Cu-O19	2.035

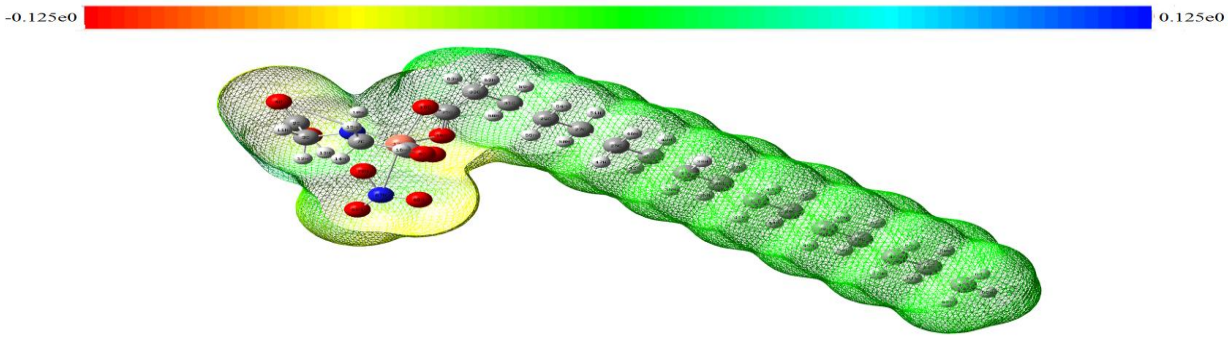


Fig. 7 Electrostatic surface mapping of copper(II) complex

Molecular Docking Studies

Molecular docking is an appealing method to shed light into drug-biomolecule infrastructures. Taking into account the experimental data of DNA binding for molecular docking studies were proficient in order to identify the most required binding site on the biomolecule, condescending that DNA and copper complex are rigid. Complex was studied concluded 1D and 2D NMR, indicating that both isomers Λ-and Δ-interact with oligonucleotides by the same mode and can afford us a good pointer for our docking calculations[34]. A series of DNA duplex arrangements known for groove binding were tested, and the ones with the lower (more stable) energy are agreed thereafter. The results of docking are manageable in Table. 2 and confirm the tentative data. According to our docking calculations, complex interact successfully with DNA duplex sequences (TCATAAATGTATCTAAGTAG)2 by groove binding. Actually, complex docks to the former sequence modes, through the π-aromatic system of Cu(II) complex (Fig. 8) Visualization of the interaction of to the specific region of the sequence of DNA (5D2Q) respectively. The binding energy is at -4.44 calmol^{-1} respectively.

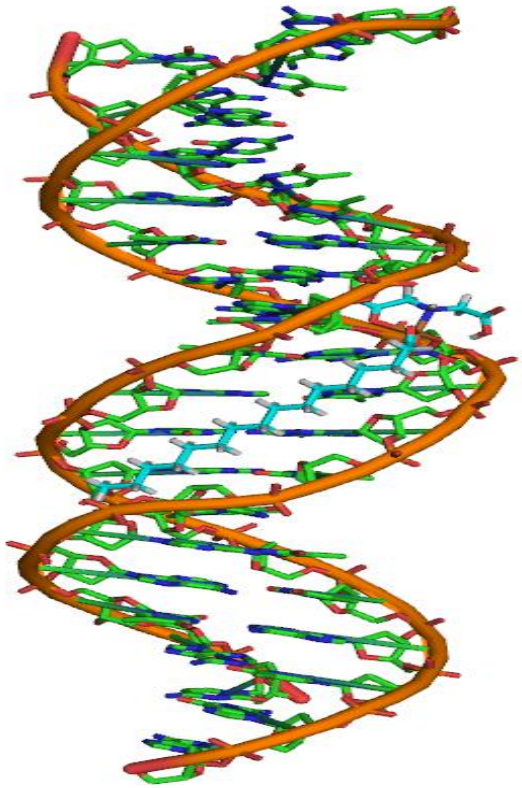


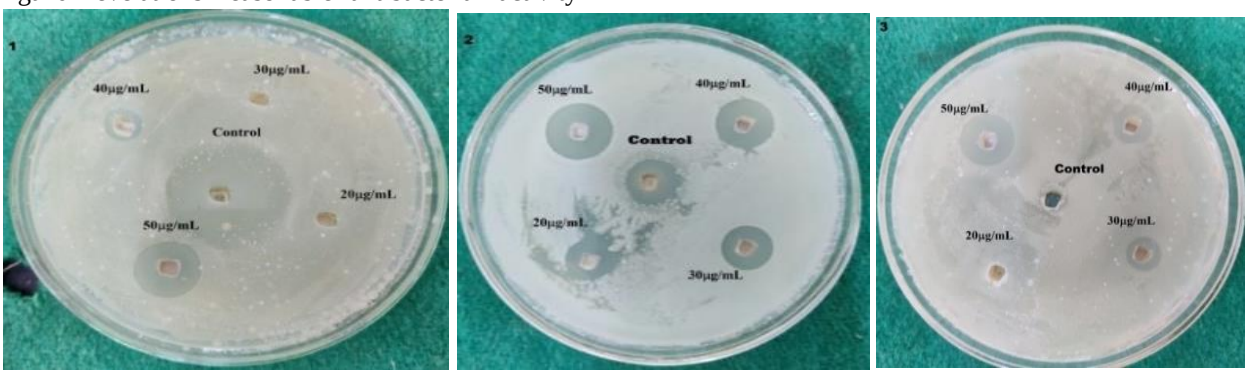
Fig. 8 Visualization of the interaction sequence of DNA (TCATAAATGTATCTAAGTAG)2 (5D2Q)

Table 2 Molecular docking studies for Cu(II) complex with the sequence (TCATAAATGTATCTAAGTAG)2 (pdb code: 5D2Q)

Pdb code	Complex	Binding energy (kcal·mol−1)	Intermolar energy (kcal·mol−1)	Inhibition constant (μM)	Electrostatic energy (kcal·mol−1)
5D2Q	[Cu(imino)(1-penta)] NO_3	-4.44	-4.44	552.13	-4.45

Antibacterial Activity

For in vitro antimicrobial activity, the studied compounds were tested in contradiction of the bacteria such as the pathogenic strains (Fig. 9) *Enterobacter cloacae*, *Staphylococcus haemolyticus* and *Bacillus cereus*. The zone inhibition concentration standards of the compound against the growth of microorganisms are abridged in Table. 3. Recently our group testified that amino acid comprising complex have good antimicrobial action[35]. The results designate that the ligand revelations reasonable antibacterial activity with respect to the complex against the same microorganisms under indistinguishable experimental conditions. Current studies disclose that the high atomic radius and electronegative metal ions in their metal complexes show high antimicrobial activity. Sophisticated electro negativity and great atomic radius decrease the effective positive controls on the metal complex molecules which facilitates their interaction with the highly delicate cellular membranes near the charged particle[36].



Enterobacter cloacae *Staphylococus Haemolytics* *Bacillus cereus*

Fig. 9 Antibacterial activity of the copper (II) complex

Table 3 Antibacterial activity zone inhibition of copper (II) complex

ZONE OF INHIBITION in mm						
S.NO	Test Organism	50μg/ml	40μg/ml	30μg/ml	Sample 20μg/ml	Control (Ciproflaxcin)
1	*Enterobacter cloacae*	11	10	8	-	11
2	*Staphylococus Haemolytics*	10	9	8	-	10
3	*Bacillus cereus*	11	10	8		10

Antifungal Activity Studies

The results of the antifungal broadcast of the copper metal complex with *Candida albicans* and *Aspergillus fumigates* at 10 μg/ml, 20 μg/ml and 30 μg/ml concentrations of the synthesized silver nanoparticles were to be found on test organism-seeded plates are given in the Fig. 10. Metal complex have stronger antifungal activity than free ligands, according to comparative investigations of the ligands and their complexes. *Fluconazole* used as positive control. The activity was resolute after 72hrs of gestation at 28°C. The inhibition zones were measured in mm (Table. 4). The antifungal activity grades exposed that the ligands and their Cu(II) complex have shown weak to good activity against *Candida albicans* and *Aspergillus fumigates*.

Aspergillus fumicatus *Candida albicans*

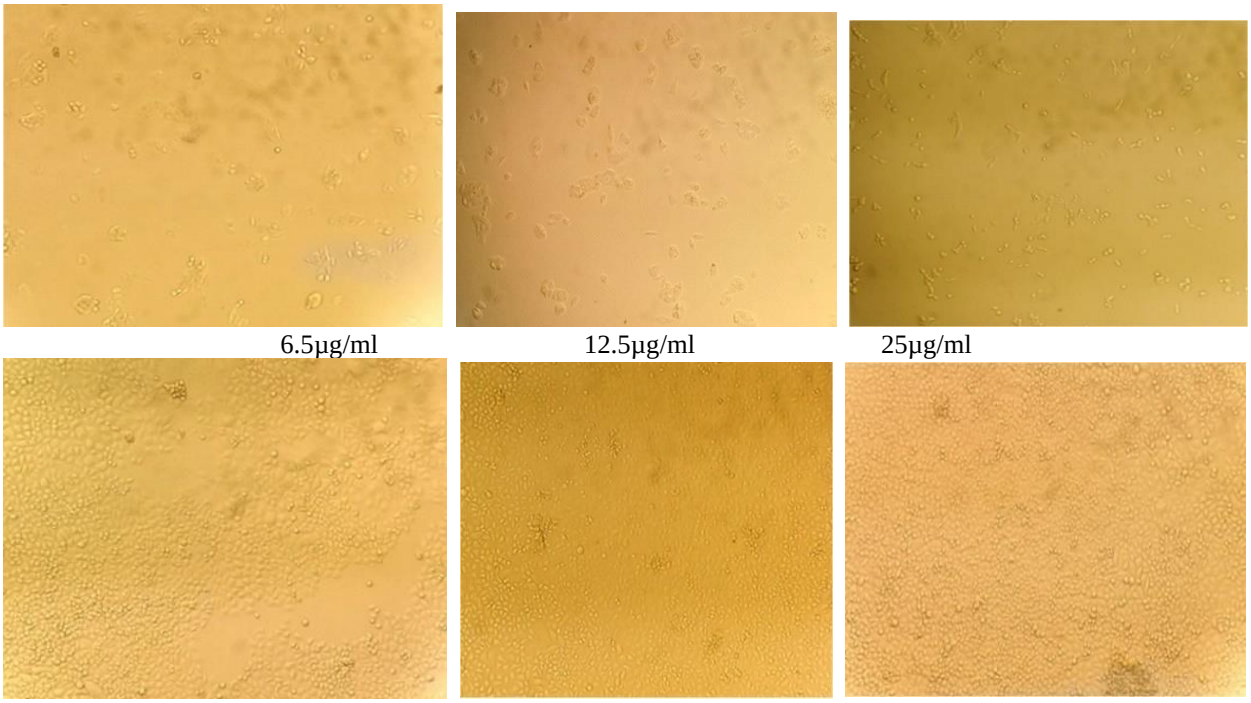
Fig. 10 Antifungal activity of the Cu(II) complex

Table. 4 Antifungal activity of the Cu(II) complex

S.No	Pathogenic fungus	Zone of inhibition (mm)			Standard (Fluconazole)
		10 ug	20 ug	30 ug	-
1.	*Candida albicans*	00	05	16	22
2.	*Aspergillus fumicatus*	00	04	14	24

Anticancer Study

In vitro percentage embarrassments of lung cancerous cells i.e. A549 were studied at (6.5 µg/ml-100 µg/ml) concentrations of the testified complex are given in Fig. 11 separately. The percentage of inhibition activity (Fig. 12) with A549 cell line at these concentrations were 13.24, 28.45, 40.31,53.95 and 62.84%. These values visibly specified that copper metal ion complex is more active against A549 cell line. Additionally, IC50 and R^2 values were calculated and were found to be 35.76 and 0.993 respectively.



6.5µg/ml 12.5µg/ml 25µg/ml

50µg/ml 100µg/ml A-549 Control

Fig. 11 Anticancer activity of the copper (II) complex against A549 cell line

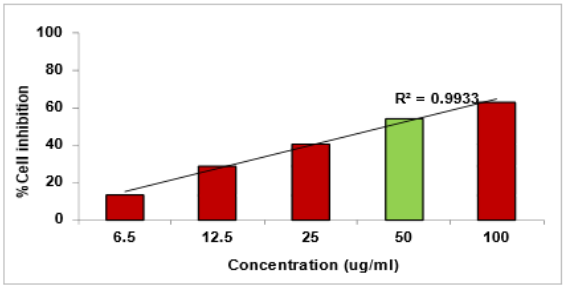


Figure. 12 Anticancer activity of the copper (II) complex against A549 cell lin

CONCLUSION

In this study based on analytical, infrared, EPR, viscosity, all these complexes are dispensed to be display coordination number was five. The DNA-binding assets of synthetic metal complexes have been widely studied by dissimilar methods with electronic absorption spectra and cyclic voltammetry. The docking experiments reveal that both complex interact as groove binders with the duplex sequences (TCATAAATGTATCTAAGTA)2 binding mode. The showing of biological activities of complex against the bacteria (*Enterobacter cloacae, Staphylococcus aemolyticus* and *Bacillus cereus*) and fungi (*Candida albicans* and *Aspergillus fumigates*) indicates that the complex show the enhanced activity in comparison to free ligand. The results show that complex 1 exhibited potent cytotoxic effects against human lung cancerous cells i.e. A549 concludes its potential role in medicine as antiseptic and anticancer agent.

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