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SYNTHESIS, SPECTROSCOPIC CHARACTERIZATION, DFT-STUDIES, MOLECULAR DOCKING, DNA BINDING ABILITY, MTTASSY AND ANTIMICROBIAL ACTIVITY OF NEW COPPER(II) COMPLEX WITH IMINODIACETIC ACID AND NEOCUPROINE

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Abstract

[Cu(neocuproine)(Imino)]$(NO_3)_2$ is a new copper (II) complex that was synthesised and characterised using elemental analysis, IR, EPR and UV-visible. A square-pyramidal (4+1) $Cu(NO_3)_2$ coordination sphere is visible in the complex. UV-Vis, DFT investigations, molecular docking, fluorescence, TGA, cyclic voltammetry, viscometric methods, and MTT assy were used to evaluate the current complex's binding relationship with calf thymus DNA (CT DNA) and propose a partial intercalation binding mode. The compound was discovered to have substantial cytotoxic effects against a human cell type (HepG2) as well as antibacterial and antifungal properties.

Keywords: Copper (II) complex; Iminodiacetic acid; neocuproine; Cytotoxicity; DNA binding , MTT assy, DFT, and docking.

1. Introduction

A precise understanding of the DNA-binding properties of metal complexes are driven by numerous motivations, which include therapeutic approaches, study of nucleic acid conformations and new tools for nanotechnology [1–4]. The characterization of DNA recognition by small redox- or photoactive transition metal complexes has been substantially aided by studying the DNA cleavage activity [5–8]. Double-strand breaks in duplex DNA are thought to be more significant sources of cell lethality than are single strand breaks, as they appear to be less readily repaired by DNA repair mechanisms [9–11]. A number of copper(II) complexes have been used as a candidates for mediation of strand scission of duplex DNA [12,13] and as probes of DNA structure. Over the last few years, more attention is given on the interaction between transition metal complexes and different ligand moieties [14-16]. The transition metal complexes provide immense opportunities to design and develop new drug compounds, which possess excellent biological activities with the available metals. The metal complexes are tuned into highly reactive as well as greater biologically active by modifying their geometry with ligands [17,18]. The nature of the metal ions, their oxidation states and the ligands attached to the metal ions are effectively influence the biological properties of the metal complexes [19]. DNA is the significant intracellular target molecule of several anticancer agents. In organisms the metal ions can play a vital role and their complexes can interact with nucleic acid by non-covalent interactions such as intercalation, minor and major groove binding and external electrostatic binding mode [20-22]. Cis-platin is a well-known and effective chemotherapeutic agent to treat the several cancers of human beings. However the drugs such as cis platin and its second generation drugs (carbo platin and Oxali platin) inherently possess few side effects and serious

toxicity [25]. To overcome these side effects, the drugs designed in such a way that they have less toxicity and minimal side effects, higher curative properties and cheaper drugs by using the different types of Schiff base complexes. Several transition metal complexes can be used as effective DNA cleavage agents and induced the cleavage mechanism in three types such as hydrolytic, oxidative and photolytic cleavages [26]. From the literature survey, it is found that the concept of DNA probing structures with isoxazole Schiff bases is authentic and warrants further development. The reason for the stupendous biological properties such as antibacterial, antifungal, antitumor, antioxidant and herbicidal properties [27, 28]. Recent investigations clearly suggests that the Cu(II) complexes are best suitable alternatives to platinum based chemotherapeutic drugs, [29] Cu(II) complexes interact with nucleic acid of tumour cells leading to cell death by blocking the uncontrolled replication of DNA, which stops the aggressive growth of cell division. Recently, we have reported, DNA interaction studies, antimicrobial investigations of different metal complexes from our laboratory [30–31].In view of the above facts, we have exclusively focused on synthesis, characterization, DNA binding, DNA cleavage, and antimicrobial activities Copper (II) complexe.

The copper (II) complex [Cu(neocuproine)(Imino)]$(NO_3)_2$ was prepared, characterised, and biological characteristics were described in this study. The findings demonstrated that the Cu(II) complex can bind to DNA effectively. The goal of the study is to create a ternary copper (II) complex containing an iminodiacetic acid and neocupproine that has DNA binding, antibacterial, and anti-cancer properties.

We synthesised and studied the complex [Cu(neocuproine)(Imino)]$(NO_3)_2$. EPR, TGA, UV, fluorescence spectroscopy, cyclicvoltammetry, and viscosity tests were used to investigate the Copper (II) complex's DNA binding mechanism. The antibacterial properties of optimal copper (II) complex against some human pathogenic microorganisms and the cytotoxic activity of Cu(II) complex against human liver cancer cell Hep-G2. The energies of the highest occupied and lowest unoccupied molecular orbitals (HOMO–LUMO) were calculated using the structural and vibrational parameters B3LYP/6-31G/LANL2DZ. Therefore, the obtained above results are well supported and give the new qualitative features of the copper complexes.

2.Experimental

2.1. Chemicals and Reagents

All chemicals, reagents and solvents used in the experiments were of analytical reagent grade and the solvents were purified by standard methods. Hydrated metal nitrate, Ethidium bromide, Neocuproine, Iminodiacetic acid and other chemicals used in this investigation were purchased from Sigma-Aldrich Chemicals, Hi Media Ltd. and Merck Company. Open glass capillary tubes were used to determine the melting points of synthesized compounds. CT–DNA were purchased from Sigma-Aldrich Chemicals, Hi Media Ltd. and Merck Company and stored at 4 ºC. The National Centre for Cell Science (NCCS) in Pune provided the human liver cancer cell line (HepG2), which was cultured in Eagles Minimum Essential Medium with 10% foetal bovine serum (FBS). The cells were kept at 37 ° C., 5% CO2, 95% air, and 100% relative humidity. The culture medium was replaced twice a week and the maintenance cultures were passaged once a week.

2.2. Instrumentation

Perkin Elmer 240C (USA) CHN analyser was used to estimate the percentage composition of carbon, hydrogen and nitrogen of synthesized compounds. Electronic spectra of compounds were recorded on a Shimadzu UV–2450 spectrophotometer in the range from 200 to 800 nm. The FT–IR studie of their metal complexe were recorded on Shimadzu IR Prestge-21 spectrophotometer using KBr pellets from 4000–400 cm^{-1}. The X–band ESR spectra of copper complexes were recorded by JES-FA200
ESR Spectrometer (JEOLJapan) at liquid nitrogen temperature. The thermal analyses (TGA) of complexes were determined in a dynamic nitrogen atmosphere (20 ml min−1) by using a Shimadzu DTG–60H simultaneous DTA–TG apparatus in the temperature range of 27–1000ºC. Fluorescence emission properties were performed on a perkin Elmer LS-45 spectrophotometer.Viscosity measurements were studied by using Ostwald's viscometer (Vensil).

2.2 Synthesis of [Cu(neocuproine)(Imino)]$(NO_3)_2$

To a ethanolic solution (10 mL) of neocuproine (208.26 mg,1 mmol), $Cu(NO_3)_2 \cdot 3H_2O$ (241.61mg, 1 mmol) dissolved in ethanol (10 mL) was added dropwise with vigorous stirring. After 30 minutes, the ethanolic solution (10 mL) of Iminodiacetic acid (133.103 mg, 1 mmol) was added slowly, and then the solution was stirred for about 6 h at ambient temperature [32]. The resulting faint green solution was filtered and kept for slow evaporation (Schem.1).
After a few week, a suitable green precipetate separated for powder XRD experiments. Yield: 67%. Anal. Calcd (%) for $C_{18}H_{22}CuN_5O_{10}$: C 40.64, H 4.17, N 13.17, O 30.08; Found: C 40.72, H 4.10, N 13.24, O 30.15, FT-IR (KBr, cm−1): 3394 amine (N-H), 2984 methyl (C-H), 1712 carboxyli acid (C=O), 1270(m), 1007(m), 851(m), 541(m). UV–Vis spectrum (DMSO,λmaxin nm): 280 (π-π*in L_N).

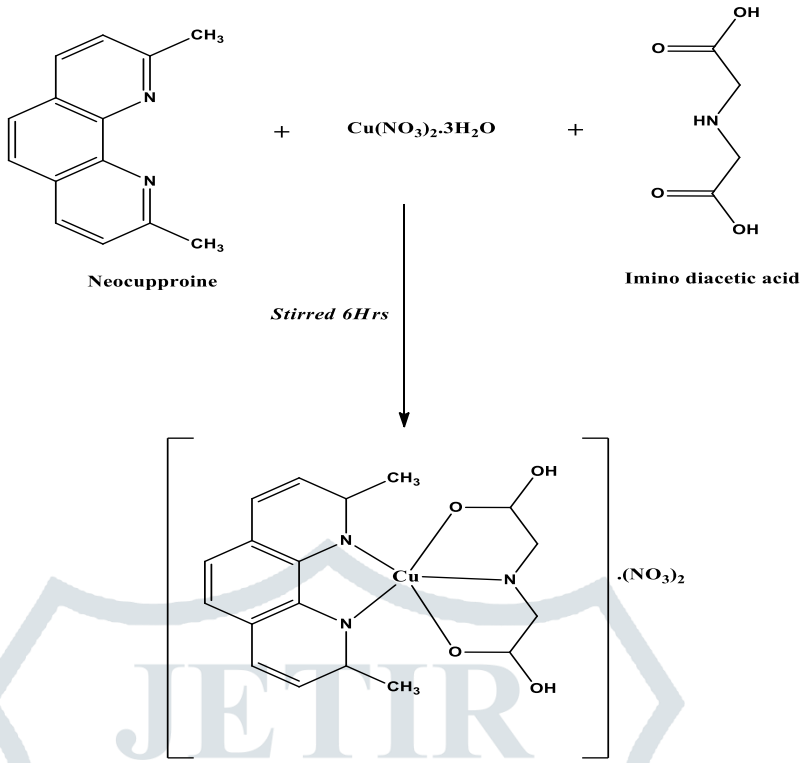


Figure 1 . Synthesis of [Cu(neocuproine)(Imino)](NO$_3$)$_2$

2.4. Spectroscopic studies on DNA interaction

2.4.1. Electronic absorption spectra.

The DNA binding study were accomplish at room temperature. The DNA concentration per nucleotide was determined by electronic absorption spectroscopy using 1 cm path length cuvettes [33]. DNA solutions in 5 mM Tris–HCl/50 mM NaCl buffer, pH 7.1 gave the ratio of UV absorbance at 260 and 280 nm, *A*260/*A*280, of 1.9, evidance that the DNA was adequately free of protein [34]. The DNA concentration was determined by measuring the UV absorption at 260 nm, taking the molar absorption coefficient (260) of CT-DNA as 6600 M–1 cm–1 [35]. The copper(II) complex absorption titration investigation in Tris–HCl buffer was completed utilising a fixed complex concentration and a supplement of DNA stock solutions. The absorption spectra of copper(II) complex-DNA solutions were recorded after a 10-minute incubation period. The fragmented change in absorption intensity as a function of DNA concentration was used to create titration curves. The intrinsic binding constant (Kb) can be calculated using the formula.

$$[DNA]/(\varepsilon a - \varepsilon f) = [DNA]/(\varepsilon b - \varepsilon f) + 1/Kb(\varepsilon b - \varepsilon f) \quad (1)$$

where an is the absorption coefficient measured at a specific DNA concentration, f is the absorption coefficient of a complex in the absence of DNA, b is the absorption coefficient of a complex when fully attached to DNA, and Kb is the intrinsic binding constant in M–1. Each set of data was fitted to the aforementioned equation, and plotting $[DNA]/(\varepsilon a - \varepsilon f)$ *vs* *[DNA]* yielded a slope and y-intercept of $1/(\varepsilon b - \varepsilon f)$ *and* $1/Kb(\varepsilon b - \varepsilon f)$, respectively. The intrinsic binding constant Kb was calculated using the slope to intercept ratio.

2.4.2. Fluorescence spectra.

Excitation at 480 nm and emission at 614 nm were used to record fluorescence spectra. Titrating complex (5 mM Tris–HCl/50 mM NaCl buffer) into a solution of DNA (110–4 M) and EtBr (810–5 M) was used to conduct the tests. The following expression was used to compute the Stern-Volmer quenching constant.[36].

$$I_0/I = 1 + Ksvr \quad (2)$$

Ksv is the linear Stern-Volmer quenching constant dependent on the ratio of the bound concentration of EtBr to the concentration of DNA, and r is the total concentration of complex to that of DNA. *Ksv* is defined as the ratio of slop to intercept in the plot of I_0/I versus *[Complex]/[DNA]*.

2.5 EPR study

In a Bruker EMX X band spectrometer operating at a field modulation of 100 kHz, modulation amplitude of 7G, and microwave radiation power of 10 mW at 90K, the X-band electron paramagnetic resonance (EPR) spectra was obtained.

2.6 Viscosity.

The viscosity of the complexes was measured using an Ostwald capillary viscometer. To maintain a consistent temperature of 30±0.1°C, a thermostatic water bath was used. The concentration of the complexes was increased from 0-100 M while DNA was employed at a concentration of 100 M. The flow timings were measured with a digital timer, and each sample was measured three times to get an exact figure, and an average flow time was determined. $(\eta/\eta o)^{1/3}$ vs [complex]/[DNA] was used to examine the

data[37]. Where, in the absence of the complex, o and o are the viscosities of CT DNA solutions, respectively. Where to, t1 and t are the flow times of TAE buffer alone and CT-DNA solutions in the absence and presence of the complexes in TAE buffer, respectively, and ηo= (t_1-to)/ to and η= (t-to)/ to are the flow times of TAE buffer alone and CT-DNA solutions in the absence and presence of the complexes in TAE buffer, respectively.

2.7 Cyclic Voltammetric Studies

Electrochemical tests were carried out in a single compartment three electrode cell design with a CH Instruments potentiostat. The reference electrode was a silver/silver chloride (3M KCl) electrode, while the counter electrode was a platinum wire. Tris–HCl/NaCl buffer and DMSO were used to make the DNA and complex solutions, respectively [38]. A 0.1 mM concentration of complexes and DNA can be used.

2.8 Antimicrobial Screening

The combination was tested in vitro against a variety of human-pathogenic Gram-positive *(Staphylococcus aureus)*, Gram-negative *(Pseudomonas and Escherichia coli)*, and fungal *(Candida albicans)* bacteria and fungi using the broth micro dilution method [39-40]. Mueller-Hinton agar (MHA) plates were used to disseminate the test pathogen. A sterile cork borer was used to create a well with a diameter of 6 mm that was then loaded with the desired concentration of medication over the agar. At 37°C, the test plates were incubated for 24 hours. The activity against the test pathogen was determined by reading the zone of inhibition (mm in diameter).
TESTED STRAIN: S.aureus, E.coli, P.aeruginosa, C.albicans. Zone of inhibition is expressed in mm . Positive Control :Ciprofloxacin-20 µg, Clotrimazole-20 µg*

2.8.1 MTT Assay

The monolayer cells were detached using trypsin-EDTA to generate single cell suspensions, and viable cells were counted using a hemocytometer and diluted with media containing 5% FBS to give a final density of $1x10^5$ cells/ml. At 37°C, 5% CO_2, 95% air, and 100% relative humidity, 100 microlitres of cell suspension per well were seeded into 96-well plates at a plating density of 10,000 cells per well and incubated to allow for cell attachment. The cells were treated with repeated concentrations of the test substances after 24 hours. They were first dissolved in neat dimethylsulfoxide (DMSO), and then an aliquot of the sample solution was diluted in serum free medium to twice the desired final maximum test concentration. A total of five sample concentrations were obtained by doing four further serial dilutions. The needed final sample concentrations were achieved by adding aliquots of 100 µl of each of these different sample dilutions to the appropriate wells already containing 100 µl of medium. Following the addition of the sample, the plates were incubated for an additional 48 hours at 37°C, 5% CO_2, 95% air, and 100% relative humidity. The medium containing no samples was used as a control, and all concentrations were done in triplicate.

3-[4,5-dimethylthiazol-2-yl] MTT (2,5-diphenyltetrazolium bromide) is a tetrazolium salt that is yellow and water soluble. The tetrazolium ring is cleaved by succinate-dehydrogenase, a mitochondrial enzyme that converts MTT to an insoluble purple formazan in living cells. As a result, the amount of formazan produced is equal to the number of viable cells. After 48 hours of incubation, 15l of MTT (5 mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated for 4 hours at 37°C. After flicking off the MTT media, the produced formazan crystals were solubilized in 100 µl of DMSO, and the absorbance at 570 nm was recorded using a microplate reader [41].

2.9 Theoretical calculations

The geometry optimization of the title complex was performed utilising the UB3LYP/6– 31G(d,p)/LANL2DZ level of theory in this study. The Gaussian 03 (Revision B.04) programme was used to perform quantum chemical calculations (DFT calculations) to get molecular geometries of minimal energies and molecular orbitals (HOMO–LUMO) [42]. Becke's three-parameter hybrid-exchange functional, the Lee, Yang, and Parr expression's nonlocal correlation, and the Vosko, Wilk, and Nuair 1980 local correlation functional (III) (B3LYP) [43] were employed. For C, N, and O, the 6–31G(d,p) basis set was employed. The LANL2DZ basis set [44] and pseudopotentials of Hay and Wadt were used for Cu atom [45]. The information about charge transfer within the molecule was elucidated using HOMO and LUMO analyses. The "Gauss view" is used to visualise molecular orbitals. These computations are useful for determining molecular properties such as bond lengths and bond angles. [46].

2.9.1 Molecular docking study

The interaction between DNA and copper(II) complexes was investigated in this study. The structure of the 355d B-DNA dodecamer was obtained from the RCSB website. PATCHDOCK, an online molecular docking server, performs nucleotide-metal complex docking. PATCH DOCK generates over 2,000 structures based on the shape complementarity concept, each with its own score and ACE value. Furthermore, the PATCH DOCK results are refined by another server, FIREDOCK, which returns the top ten structures. The resulting structures are sorted by their global energy. From the lowest global energy values, the best docked structure is chosen, which is -29.46 kcal/mol [47].

3.0 Result and discussion

3.1 Spectral studies

3.1.1 *UV-Visible spectra and Magnetic moments*

At room temperature, the copper(II) complex's UV-visible spectra are recorded in DMSO solution. The copper(II) complex exhibited two distinct absorption bands at 230 and 274-299 nm, which correspond to the aromatic ring's π– π* transition and the acetic group's

n– π* transition, respectively, and are -29.46 kcal/mol. This is where you'll find the best docking photos, Fig.1 [48]. However, in metal complexes, the positions of these respective bands are shifted to lower/higher wavelength indicating the coordination of the ligand to the metal ions. Further, all complexes exhibit an additional and characteristic broad band in the visible region, which was due to d – d transition. The copper(II) complex exhibit two bands at 502 and 550 nm, due to 1A1g → 1B1g and 1A1g → 1B1g transitions which is further ascertained by the magnetic moment values of copper(II) complex found to 1.74 BM.

3.1.2 IR spectral study

The early IR band assignments are beneficial in identifying the coordination behavior of ligands with copper(II) ions. Due to O–H stretching of water molecules, a broad centred band was formed in the region 3394–3250 cm-1, while an uncoordinated nitrate anion showed the stretching vibration in the region 1344–1340 cm-1.

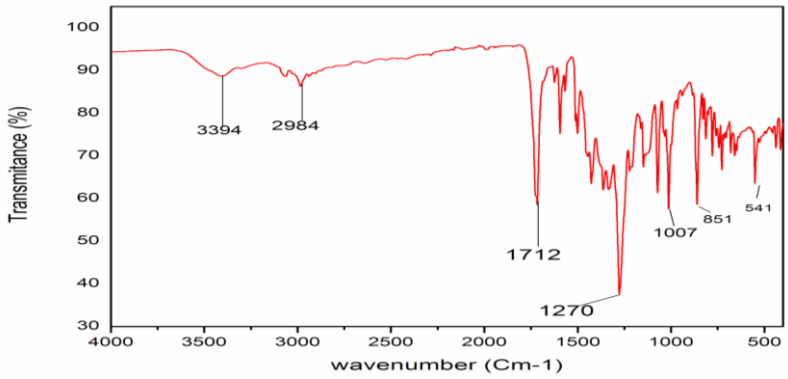


Fig.2 FTIR spectra of copper(II) complex

3.1.3 EPR and magnetic studies

At ambient temperature, one-electron paramagnetic complexes had magnetic moment (eff) values in the range of 1.62–1.68 BM, which is somewhat lower than the spin-only value of 1.73 BM for a high spin Cu(II) centre with S = 1/2, indicating discrete mononuclear complexes [49]. At ambient temperature, the X-band EPR spectra of dry and powdered mixed-ligand copper(II) complex samples were recorded at a magnetic field strength of 3210 G (frequency 9.423 GHz) (Fig. 3). The trend g || (2.176) > g (2.133) > ge (2.0023) denotes the presence of an unpaired electron in the dx2 –y2 orbital (2B1g state) and the complex's square-based geometrie.

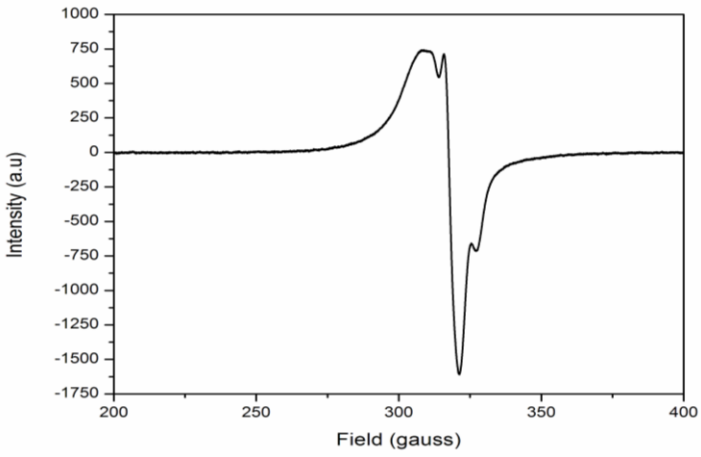


Fig. 3 EPR spectrum of Cu complex

3.1.4 Thermal analyses of the complexes

Figure. 3 – shows TGA curves for [Cu(neocuproine)(Imino)]$(NO_3)_2$ complex. The complex is stable up to 124 ºC and then gradually loses its weight to decompose. In the weight loss occurs at 124 to 147 ºC corresponding to the loss of lattice water (Calculated: 6.7%; observed 6.4%). The second weight loss the range of 147 to 258.3 ºC is due to the elimination of nitrate ions (Calculated: 12.2%; observed 11.9%). The third weight loss the range of 258.3 to 348.9 ºC is due to the elimination of immino diacetic acid (Calculated: 24.41%; observed 24.3%) above the 348.9ºC the complex was decomposed.[50]

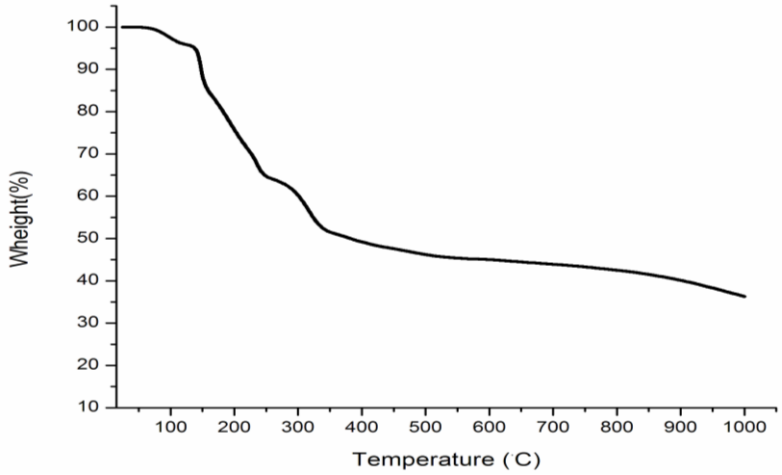


Fig. 4 TGA analysis of Cu(II) complex

3.2 DNA binding experiments

3.2.1 UV-Vis absorption analysis

The interactions of metal complexes with DNA have been the subject of interests for the development of effective chemotherapeutic agents. The binding modes to DNA would give in sights into the understanding of the biochemical mechanism of action of the complexes. Recently, studies on some ternary complexes [Cu(neocuproine)(Imino)]$(NO_3)_2$ indicated that the size, shape, and polarity of the side chains of different ligands in these ternary complexes may influence their binding mode to DNA [51]. The absorption spectra of [Cu(neocuproine)(Imino)]$(NO_3)_2$, in the absence and presence of CT-DNA were shown in Fig. 4. In the UV region, the complex presented two bands 231 (Kb=3.22X 10^4 M^{-1} cm^{-1}) and 275 nm
(Kb= 1.19 X 10^4 M^{-1} cm^{-1}), which can be attributed to the π-π* transition of the coordinated neocuproine ligand.

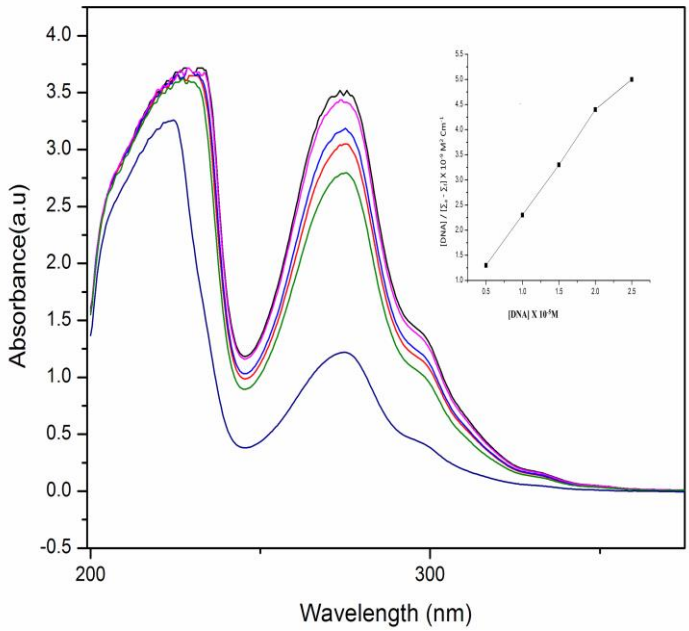


Fig. 5 Absorption spectral identification on addition of CT DNA for Cu (II) complex (Inset plot of [DNA]/(εa-εf) Vs [DNA] for absorption titration of CT DNA with complex).

The absorption intensity of the complex increased (hyperchromism) evidently after the addition of DNA, which indicated the interactions between DNA and the complex. A similar hyperchromism was also observed for a copper(II)complex with a ligand bearing OH group.[52] As DNA double helix possesses many hydrogen bonding sites which are accessible both in the minor and in the major grooves, it is likely that the OH group of the ternary complex forms hydrogen bonds with DNA, which may contribute to the hyperchromism observed in absorption spectra. The complex bound to DNA through intercalation, which involves a strong stacking interaction of the planar aromatic rings of the coordinated ligand with the base pairs of DNA, usually result in hyperchromism and blueshift.[53] The observed hyperchromism and blue shift indicates that complex involves in partial intercalation to the base pairs of DNA. The binding constant Kb of 1.76 X 10^4 M^{-1} was determined from the plot of [DNA]/($[\sum_a - \sum_f]$) versus [DNA] (inset of Fig. 4), and the K_b value is higher than those for related other Cu(II)complexes such as [Cu(phen)(L-Thr)H_2O]ClO_4 ($6.35\times10^3M^{-1}$),[43] [Cu(Sal-L-val)phen] ($6.48\times10^3M^{-1}$)[44] and π[Cu(naph-Leu)(phen)]$CH_3OH.5H_2O$ ($4.87\times10^3M^{-1}$),[45] [Cu(phen)(pro)(H_2O)]ClO4 ($3.86\times10^3M^{-1}$),[46] [Cu(bipy)(pro)(H_2O)]ClO_4 (4.6×10^3 M^{-1}).[47]

3.2.2 Fluorescence quenching study

Another method for probing the complex's interaction to CT–DNA is fluorescence spectroscopy. As a spectral probe, the intensity of ethidium bromide emission was employed. In Tris–HCl buffer, the inherent fluorescence intensities of DNA and ethidium bromide are low, but the fluorescence intensity of ethidium bromide is increased when DNA is added due to intercalation. If the complex also substantially intercalates with DNA, ethidiumbromide binding to DNA will be reduced, resulting in a reduction in fluorescence. [54]. The fluorescence intensity at 540 nm due to ethidium bromide binding to DNA reduced dramatically with increasing complex concentration in our experiment (figure 6). This could be due to the metal complex competing with ethidium bromide for DNA binding sites, displacing the ethidium bromide (whose fluorescence is heightened when DNA is bound), or a direct quenching contact with DNA. As with other copper(II) complexes, the former is most likely to occur. [55, 56]. The binding constant, *K*sv, is$(2.5\pm0.2) \times 10^4 M^{-1}$.

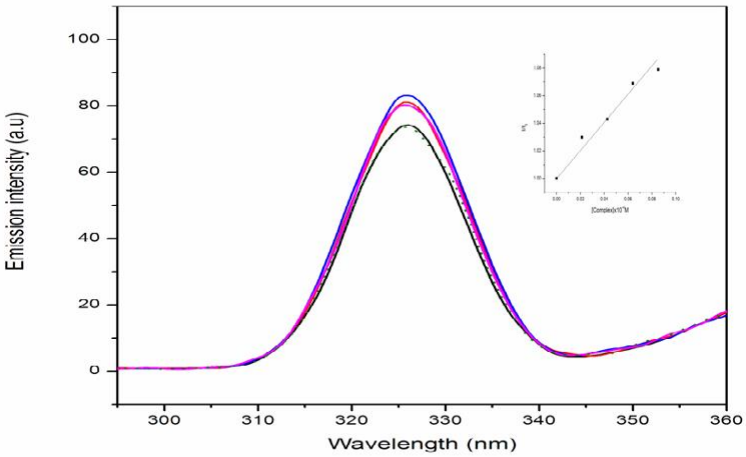


Fig. 6 Fluorescence emission spectra (excited at 300 nm) of the CT DNA-EB (4×10^{-5} M ethidium bromide, 4×10^{-5} M CT DNA).

3.2.3 Viscosity measurements

Viscosity measurements were taken to learn more about the copper(II) complex's DNA binding mechanism. Because the relative specific viscosity of (η/η_0) (η and η_0 are the specific viscosities of DNA in the presence and absence of the complex, respectively) DNA reflects the increase in contour length generated by intercalation, a traditional intercalator such as ethidium bromide could cause a large rise in the viscosity of DNA solutions. A partial and/or non-classical intercalation of the ligand, on the other hand, can bend or kink DNA, resulting in a reduction in effective length and an increase in viscosity [56, 57], whereas electrostatic and groove binding have little or no influence on the relative viscosity of DNA solutions[52]. As a result, viscosity measures, which are sensitive to changes in DNA contour length, are a good way to look for complexes that intercalate DNA. Figure 7 depicts the plots of relative specific viscosities against 1/R = ([Complex]/[DNA]). As the concentration of the complex rises, so does the relative specific viscosity. However, as compared to traditional intercalators such as ethidium bromide in the same DNA concentration range, the rise in viscosity was significantly lower [58]. This finding backs with the spectrum investigations that show the complex only weakly intercalates with DNA base pairs. Because base pairs are split to accommodate the binding ligand, partial intercalation causes the DNA helix to stretch, causing the solution's viscosity to increase.

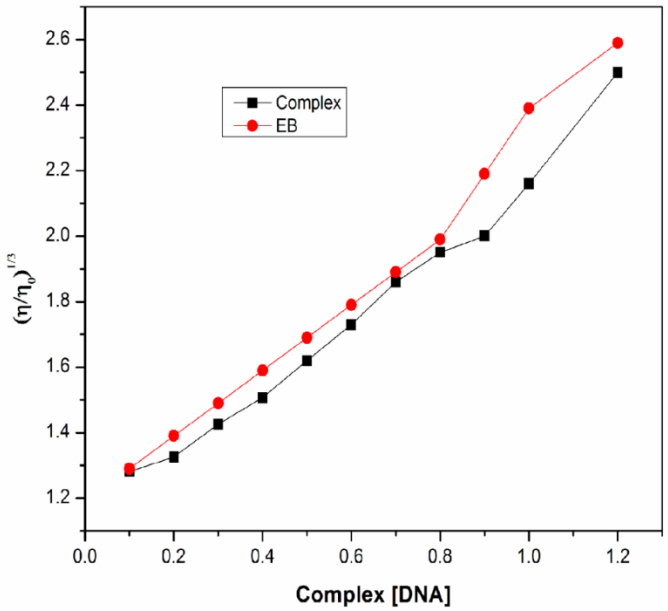


Fig. 7 Effect of relative viscosity with an increasing amount of CT-DNA at room temperature

3.2.4 Cyclic voltammetry

The application of cyclic voltammetry to study of the interaction between complex and DNA provides a useful compliment to the previously utilized methods of investigation such as UV-Vis and viscosity experiments. The typical cyclic voltammogram of a 0.01 mM solution of

[Cu(neocuproine)(Imino)]$(NO_3)_2$ complex with out and with DNA at glassy carbon (GC) electrode in DMSO were carried out (Fig. 7). In the forward scan, a single cathodic peak was observed, which corresponds to the reduction of complex. In the reverse scan, no anodic peak was observed, which indicates that the process is irreversible. When CT-DNA is added to a solution of complex, marked decrease in the peak current height and shifts of peak potential to more -ve values were observed.

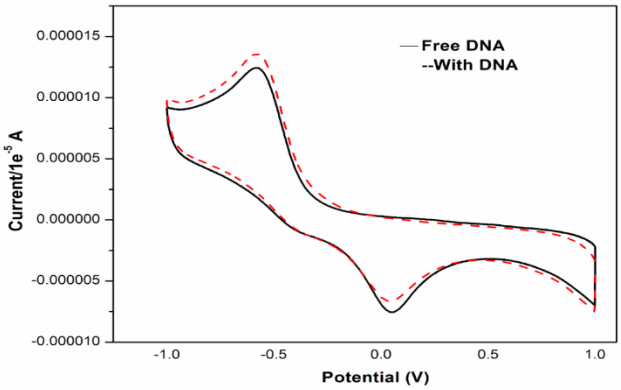


Fig. 8 Cyclic voltammetry of Cu(II) complex

The cyclic voltammetric behavior was not affected by the addition of very large excess of DNA, indicating that the decrease of peak current of complex after the addition of DNA due to the binding of [Cu(neocuproine)(Imino)]$(NO_3)_2$ complex to the DNA. When the concentration of the DNA increased with changes the in peak current and potential become slowly. This reveals that the complex were interacting with CT-DNA.[59]

3.2.5 Cytotoxic activity

[Cu(neocuproine)(Imino)]$(NO3)_2$ was used to test the cytotoxic effects in vitro on tumour cell lines (HepG2). According to the data reported in (Fig. 9), a lower dose of copper(II) complex has no cytotoxic effect on HepG2 cells after a 48-hour treatment. The complex solution was made at a concentration of 10^{-4}M. Copper(II) complex treatment, on the other hand, reduced cell viability by 70%, 50%, 40%, and 30%, respectively, at doses of (6.5, 12.5, 25, 50, 1005g/ml). After 48 hours, the copper(II) complex-induced cell death IC50 (inhibitory concentration 50%) was around 15L. The cell morphology transformed to a spheroid shape, and chromatin condensation occurred (Fig. 9). These findings show that apoptosis is the primary cause of copper(II) complex-induced cell death [60].

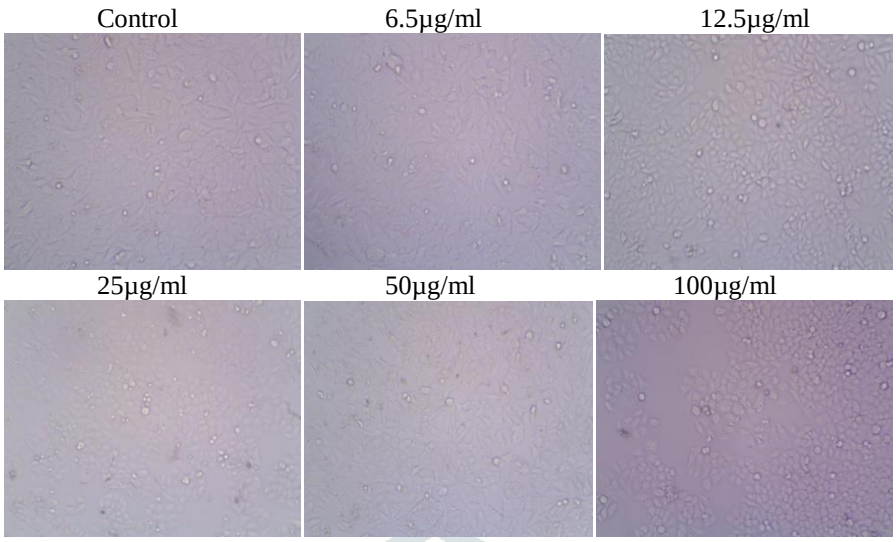


Fig. 9 Anticancer activity of Cu (II) complex

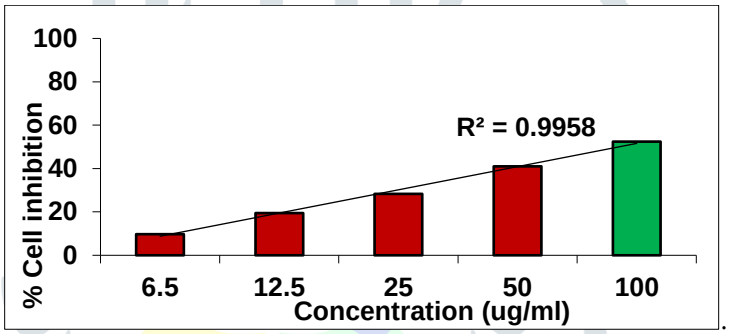


Fig.10 Cell viability of HepG2 cells after treatment in Cu(II) complex at variousconcentration

3.3 Antifungal activity

The copper(II) complex are screened for their antifungal activity against A. S.aureus, E.coli , P. Aeruginosa and C. albicans. The minimum inhibitory concentration (MIC) values of the investigated compound are summarized in Table 1. A comparative study of MIC values of complexes (3.2–7.1µg/mL) against all the fungi indicates that the metal complex have higher antifungal activity. Such increased activity on metal chelation can be explained on the basis of Tweedy's chelation theory [61]. Chelation reduces the polarity of the metal ion considerably because of the partial sharing of its positive charge with the donor groups and also due to electron delocalization on the whole chelating ring. The results above clearly show that the complex's negative bacteria will be inactive against bacteria and fungal species. At the same time, the Cu(II) combination has antibacterial and antifungal properties.

Table 1 Antimicrobial activity of Cu(II) complex

S.No	Microorganism	Control		Zone inhibition of complex		
1.		-Ve	+Ve	50µg/ml	100µg/ml	250µg/ml
2.	*S.aureus*	-	30	25	27	29
3.	*E.coli*	-	32	-	-	-
4.	*P.aeruginosa*	-	31	16	17	20
5.	*C.albicans*	-	16	22	25	27

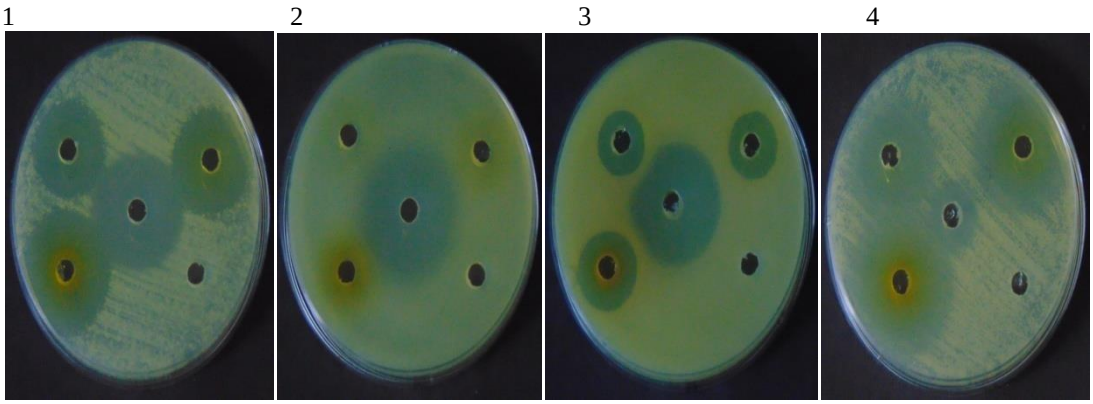


Fig. 11 (1-3) antimicrobial and (4) antifungal activity of Cu(II) complex at 6mm zone inhibition diameter

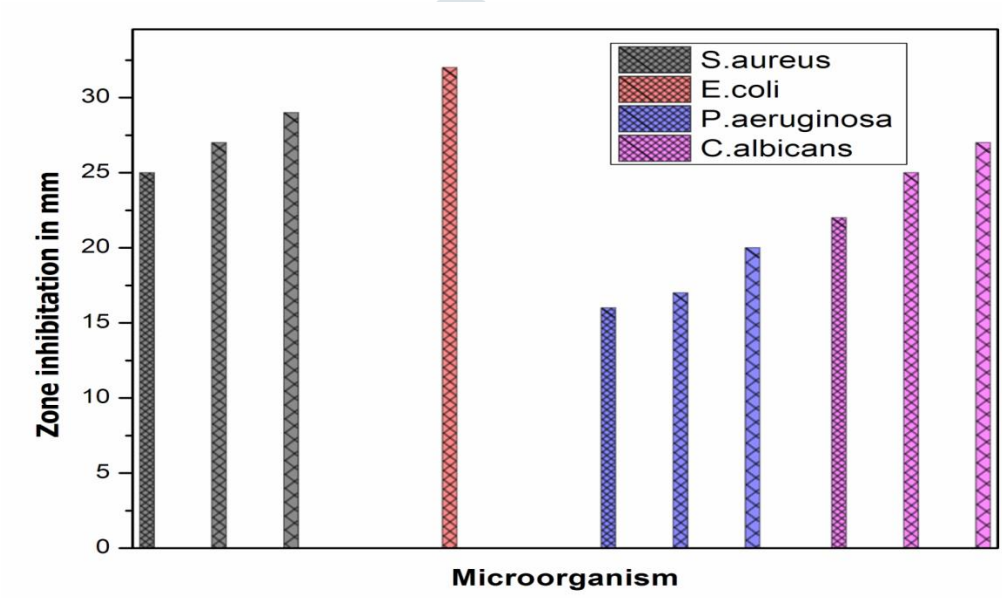


Fig. 12 Bar graph indicates antifuncal activities of Cu(II) complex.

3.4 DFT Study

Fig.13 shows the optimal shape of a copper complex optimised using the B3LYP/LANL2DZ basis set [62]. For the Cu(II) complex, the intra ligand angles are 135.75° for N1-Cu-N3 and 137.78° for N2-Cu-N3. N2-Cu-N3 complexes had a slightly larger inter-ligand bond angle than N1-Cu-N3 complexes. Furthermore, both complexes N1 and N2 had the same bond length (Table 2). The band gap of the neocuperion complex is higher than that of other ligand complex (Figure 13). The energy gap and dipole moment have an impact on molecule stability. The HOMO and LUMO orbitals of frontier molecules are key tools for chemical reactions and contribute to chemical stability. The electron transitions from HOMO to LUMO can be seen, which govern how one molecule interacts with other molecules. Because electron conductivity is measured, the presence of molecular electron transport activity may be confirmed. The HOMO is completely isolated in the terminal N-group and heterocyclic bases in this Cu (II) complex, whereas the LUMO is most widely diffused in the totally metal core. In the charge transfer mechanism within the Cu(II) complex, the HOMO–LUMO energy gap (1.02 eV) was confirmed.

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

S.NO	Bonding Parameter	Bond distance (ºA) / Bond angle (ºA)
1.	Cu-N1	2.01
2.	Cu-N2	2.00
3.	Cu-N3	2.06
4.	Cu-O1	3.68
5.	Cu-O2	3.68
6.	N1-Cu-N3	135.75
7.	N2-Cu-N3	137.78

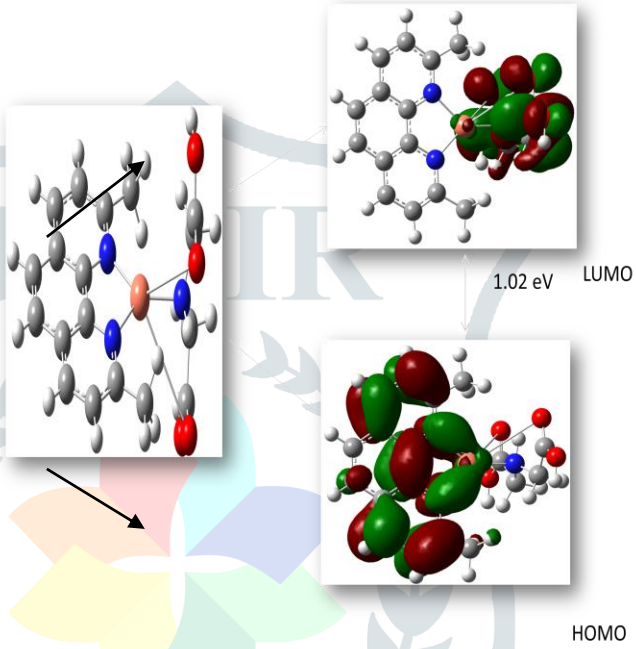


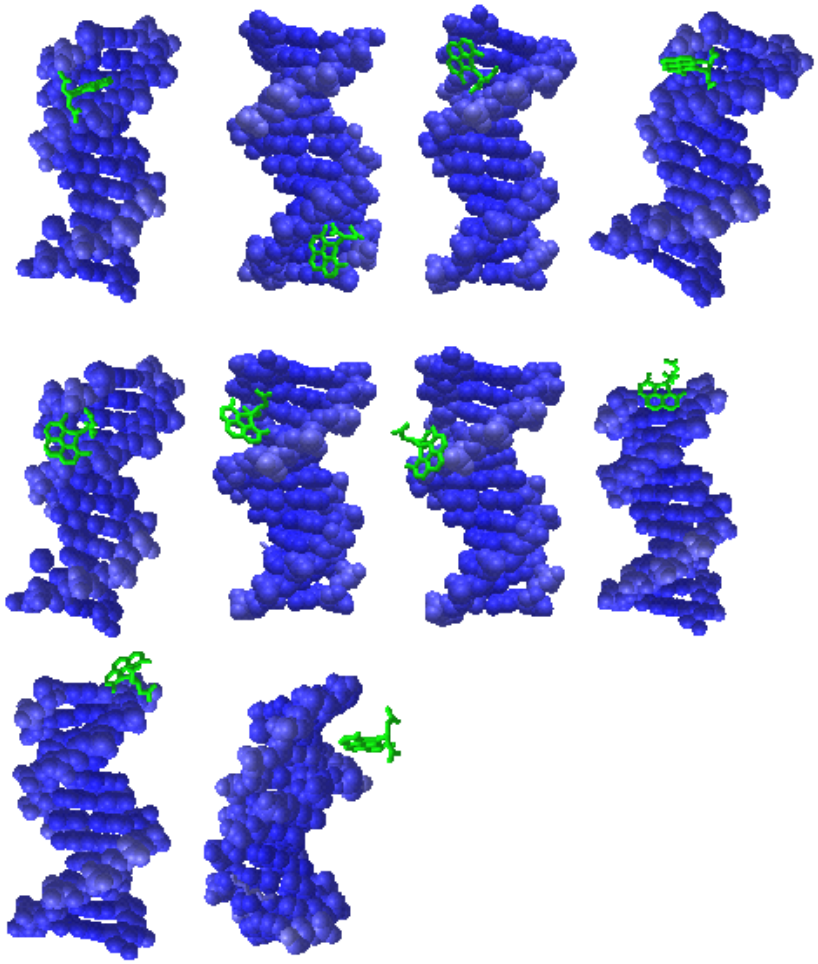
Fig. 13 (a)Geometries of Cu(II) complex, (b) Energy gap of HOMO and LUMO on Frontier molecular orbitals.

3.5 Molecular docking Studies

The interaction of DNA and copper(II) complexes was investigated in this study. The structure of 355d B-DNA dodecamer was derived from the RCSB website. PATCHDOCK, an online molecular docking server, is used to dock nucleotide-metal complexes. PATCH DOCK generates almost two thousand structures based on the shape complementarity concept, each with its own score and ACE value. PATCH DOCK's results are further improved by another server, FIREDOCK, which returns the top ten structures [63-64]. The structures that emerge are sorted by their global energy. The best docked structure is chosen from the global energy values with the lowest value of -29.46 kcal/mol. Figure 14 shows the best-docked images, which include copper complex and DNA. The compounds had a greater bonding connection than the free ligand, and the complex showed stronger interaction with CT-DNA than the ligand, according to docking experiments.

Table 3 The best docked pictures are enclosed here. Computed using PatchDock and FireDock servers scores of the top 10 docked.

S.No	Patchdock Server		FireDockServerS			
	Score	Area	Global Energy kcal/mol	Attractive VdW kcal/mol	Repulsive VdW kcal/mol	ACE Kcal/mol
1	3628	509.70	-35.21	-15.05	4.65	-13.90
2	3568	505.80	-32.75	-15.03	7.56	-14.24
3	3512	425.60	-27.38	-14.70	12.90	-13.25
4	3496	467.40	-20.81	-15.60	25.15	-13.23
5	3494	485.20	-16.52	-10.79	6.44	-7.30
6	3482	479.20	-8.32	-4.85	2.80	-4.15
7	3472	561.40	-6.71	-5.24	3.07	-3.82
8	3454	481.50	-5.57	-4.17	0.52	-3.26
9	3440	475.60	-4.28	-3.93	1.35	-3.70
10	3400	360.30	3.64	-0.67	0.00	-0.40



Models of complex 1:355d.

Fig.14 Molecular docking of copper (II) complex with CT-DNA

3.6 Conclusion.

The copper (II) complex [Cu (Neocup)(Immino)H_2O)$(NO_3)_2$ has been synthesised and studied using UV–visible, emission, EPS, TGA, and DFT. A deformed square-pyramidal geometry may be seen throughout the complex. UV-Vis absorption spectroscopy, ethidium bromide displacement evaluation, viscometrical approach, and CV studies were used to determine the binding mode of the complex with CT-DNA in this study. The equilibrium binding constant (Kb) shows that as the amount of DNA added, the DNA-binding affinity increases. The Cu (II) complex injected into the base pairs of CT-DNA was also validated by molecular docking investigations. As a result of the titration experiments, the partial intercalative binding intercalation of Cu(II)complex with DNA was confirmed. DFT analyses of structural features such as bond lengths, geometries, bond angles, and torsion angles, as well as spectroscopic properties of Cu (II) complex HOMO–LUMO have been described, with theoretical results compared to experimental data. The spectroscopic finding is in great qualitative agreement with the optimised experimental and theoretical structure. DFT studies at the DFT/B3LYP/6-31G*(d,p)/LANL2DZ level are anticipated to be the best quantum chemical approach for reproducing the experimental results for the produced complex. The charge transfer interactions take place within the copper complex, according to the energy gap diagram of HOMO–LUMO gaps.

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