Research Article

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Application of 4, 4′-Diaminobenzanilide Schiff Base Metal Complexes as Anti-Tumor and Anti-Dengue Agents

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ABSTRACT

A unique Schiff base ligand 4-(4-nitrobenzylideneamino)-N-(4-(4-nitroobenzy lideneamino) phenyl) benzamide L has been prepared by condensing 4,4′-diaminobenzanilide and p-nitrobenzaldehyde. The cobalt (II), nickel (II) and copper (II) complexes of L were also prepared. ^{1}H-NMR, FT-IR, UV-Vis., EPR and EI-mass techniques were used to confirm the formation and structure of the ligand and its complexes. Using Auto Dock vina and Discovery studio software, the synthesized complexes were docked with Human DNA topoisomerase I (PDB: 1SC7) and Dengue NS3 protease-helicase (PDB ID: 2VBC). The biological applications of the synthesized complexes were carried out by Cytotoxic screening analysis and DNA binding ability by using electronic spectra and Anti-Tumor activity by MTT assay. The results established that the synthesized transition metal complexes can act as good anti-tumor and anti-dengue agents.

Keywords: Anti-dengue drug; Docking; Schiff base ligand; 4,4′-diaminobenzanilide; p-nitrobenzaldehyde; NS3 protease-helicase.

1. INTRODUCTION

Schiff bases are condensation products of carbonyl compound, especially aldehydes or ketones, with primary amines and they were first reported by Hugo Schiff in 1864. Several investigations (Geary and Coord 1971) on Schiff base showed that the presence of lone pair of electrons in nitrogen atom of imine group is of considerable chemical and biological applications. Nowadays, the research field dealing with Schiff base coordination chemistry has expanded enormously. The importance of Schiff base complexes for bioinorganic chemistry, catalysis, biomedical applications, supramolecular chemistry and formation of compounds with unusual properties and structures has been well studied and reviewed (Abbaspour *et al.* 2002; Seleem and Chim 2003; Inas 2017). Interactions between transition metal complexes (typically Co (II), Ni (II), and Cu (II)) and DNA are studied in order to gain insight into the development of new chemotherapeutics and medicines. In cancer cells, interactions between small molecules and DNA frequently cause DNA damage (Alagesan *et al.* 2014). Dengue virus basically belongs to Flavivirus genus. This virus belongs to the Flaviviridae family. Humans and monkeys are the primary sources of dengue virus development. Humans infected with dengue fever, body pain, and a body temperature nearing 40 ºC are common symptoms, making them critically ill. Extreme symptoms of Dengue fever include severe headaches, facial flushing and skin rashes, and this severe condition is known as Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). It is impossible to carry out all of the experiments at a low cost. Docking is the only way to give newly synthesized drugs their exact results in relation to the target molecule (Bas *et al.* 2008; Irwin *et al.* 2005; Greenwood *et al.* 2010; Greco *et al.* 2007). Auto Dock vina and Discovery studio are useful tools for studying the ligand-target molecule interaction. This software aids in determining the ligand's binding mode, affinity, and exact stable configuration when it binds to the receptor (Iijima *et al.* 1999; Aoyama *et al.* 2009). We can understand the coordination mode and binding nature using this docking study. It will aid in the development of a suitable treatment for the disease (Hayashi *et al.* 2000; Aoyama *et al.* 2001; Iijima 1999). In this present work we plan to synthesize novel Schiff base ligand (L) and its cobalt (II), nickel (II) and copper (II) metal complexes, which were treated with human DNA topoisomerase I (PDB: 1SC7) and Dengue NS3 protease-helicase bi-functional enzyme (PDB ID: 2VBC) by docking. The biological applications of the synthesized complexes were carried out by Cytotoxic screening analysis and DNA binding ability by using

electronic spectra and Anti-Tumor activity by MTT assay.

2. MATERIALS AND METHODS

All the chemicals used in this study were purchased from commercial sources, and they were not purified before use. The following materials were purchased from Sigma Aldrich in the United States: 4,4'-diaminobenzanilide, *p*-nitrobenzaldehyde, cobalt chloride hexahydrate, nickel chloride hexahydrate and copper chloride dihydrate. Merck provided the solvents used in this study, which were used without further purification. ^{1}H-NMR spectra of the synthesized Schiff base ligands have been recorded in DMSO (d_6) by using TMS as an internal standard on a Bruker Advance DRX 300 FT-NMR instrument. The EI-Mass spectra were recorded using JEOL DX-303 EI mass spectrometer at Indian Institute of Technology, Chennai, India. Solid sample infrared spectra were recorded at 16 scans/min in a JASCO/FT-IR410 spectrometer in the range of 4000 – 400 cm^{-1}. For sample preparation, the potassium bromide disc method was used. Electronic spectra of the complexes were recorded using Perkin Elmer Lambda-25 UV-Vis. spectrophotometer using DMSO as solvent in the range of 200-800 nm. The room temperature X-band EPR spectra of the copper complex in DMSO were recorded on Varian E-4 X-band spectrometer using DPPH as the g-marker at Indian Institute of Technology, Chennai, India.

2.1 Experimental Procedures

2.1.1 Preparation of Schiff base ligand L

A hot solution of 1.136 g (5 mmol) 4, 4'-diaminobenzanilide in 20 mL methanol was added slowly to a hot stirring solution of 1.5112 g (10 mmol) *p*-nitrobenzaldehyde in 20 mL methanol. The above mixture was stirred under reflux for 5 hours. On cooling to room temperature, the Schiff bases obtained are filtered, washed with diethyl ether and dried *in vacuo*.

2.1.2 Preparation of Cobalt (II), Nickel (II) and Copper (II) Schiff base (L1) Metal Complexes

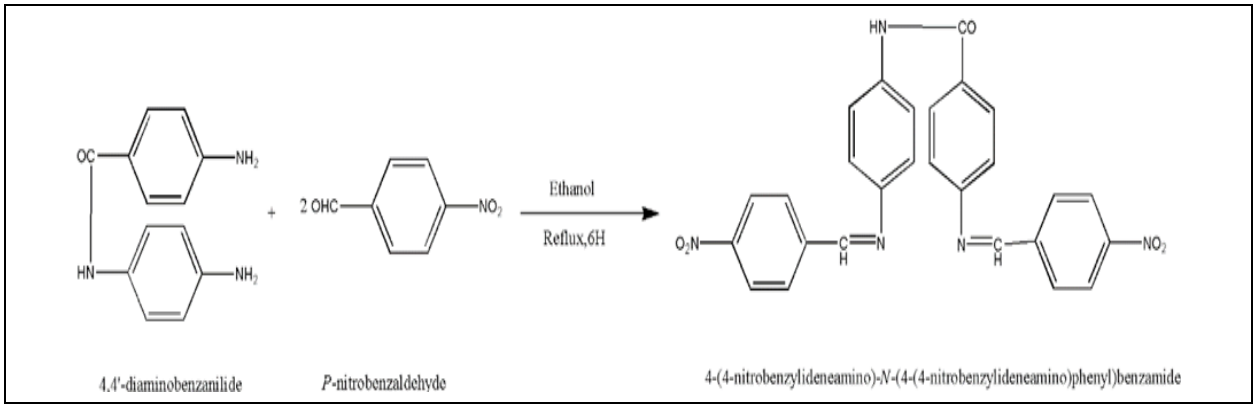
To the hot stirring solution of the 0.9869 g (2 mmol) Schiff base ligand L2 in 20 mL of methanol, the corresponding metal (II) chlorides [$CoCl_2$].$6H_2O$ (0.237 g, 1 mmol), $NiCl_2.6H_2O$ (0.2379 g, 1 mmol) and $CuCl_2.2H_2O$ (0.170 g, 1 mmol)] in 20 mL of methanol were added, stirred under reflux for 6 hours. Then the product obtained was filtered, washed and dried *in vacuo*.

2.1.3 DNA Binding Studies using Electronic Absorption Spectra

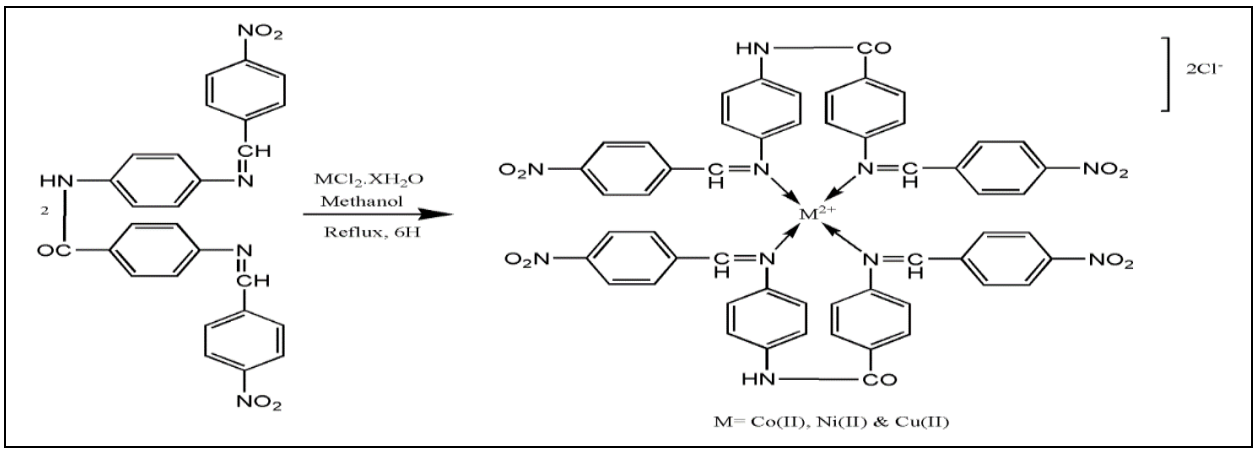
Electronic absorption spectrum of the complex was recorded before and after addition of CT-DNA in the presence of 50 mM Tris-HCl buffer (pH 7.5), Tris-hydrochloride (197 mg, 5 mM) and sodium chloride (730 mg, 50 mM) were accurately weighed and made up to 250 mL in a standard measuring flask using double distilled water. The pH of the solution was adjusted to 7.5 using 1 mM sodium hydroxide solution with the help of pH meter before making up to the mark. A fixed concentration of metal complexes (10 μM) was titrated with incremental amounts of CT-DNA over the range (0 – 200 μM).

2.1.4 Cytotoxicity screening analysis

The stock culture of bacteria was revived by inoculating in broth medium and grown at 37 ºC for 18 hours. The Lysogeny broth (LB) Agar plates were prepared and wells were made in the solidified LB agar plate. Each plate was inoculated with 18-hour old cultures (100 μL, 10^{-4} CFU) and spread evenly on the plate. After 20 min, the wells were filled with compound at different concentrations. Standard compound plate was also prepared in the same manner. All the plates were incubated at 37 ºC for 24 hours and the diameter of inhibition zone was noted.



Scheme 1. Preparation of Schiff base Ligand L



Scheme 2. Preparation of Schiff base metal complexes

2.1.5 MTT assay

To determine the cytotoxic effect of Schiff base copper complexes, cell viability study was done with the MTT reduction assay. Hep-2 cells were seeded in a 96-well plate at the density of 5 x 10^3 cells/well. The cells were allowed to attach and were grown in 96-well plate for 24 hours in 200 µL of Eagle's minimal essential medium (EMEM) with 10% Fetal Bovine Serum (FBS). After that the media was removed and replaced with suspension of various concentrations of Schiff base copper complexes (10 to 100 mg/mL) (minimum 4 wells seeded with each concentration), the cells were incubated for 48 hours. After the addition of (MTT), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (10 mL, 5 mg/mL), the cells were incubated at 37 ºC for another 4 hours. The medium was then removed, and 200 µL of DMSO was added to each well. Optical density of the formazan product was read at 620 nm using multi-well spectrophotometer. The results were given as the mean of four independent experiments. OD value was used to sort out percentage of viability by using the formula,

$$\text{Percentage of cell viability} = \frac{\text{OD value of the treated sample}}{\text{OD value of the control sample}} \times 100$$

2.1.6 Molecular Docking Study

Human-DNA-Topo-I complex (PDBID: 1SC7)] and Dengue NS3 protease-helicase bi-functional enzyme (PDB ID: 2VBC) were obtained from Protein Data Bank. Metal complexes were converted into PDB format using Mercury software (Greenwood *et al.* 2010). The 'receptor' (DNA/Dengue) and 'ligand' (metal complexes) files were docked using Auto Dock tools. The heteroatoms including water molecules and excess ligand were removed and also polar hydrogen atoms and Kollman charges were added to the receptor molecule to investigate the binding nature. The binding was enclosed in a box having number of grid points in (x) x (y) x (z) directions of 40 x 40 x 40 and a grid spacing of 0.35 Å. Docking studies were conducted using Auto Dock tools (ADT) version 1.5.4 and finalized by Auto Dock vina programme. The docked structures were exposed using Discovery studio software.

3. RESULTS AND DISCUSSION

3.1 ^{1}H-NMR Spectral Studies and Electron Ionization Mass Spectral analysis

The ^{1}H NMR spectra of the ligand L is provided in the Fig. 1. The appearance of signal at 8.477 ppm is attributed to azomethine group and the signal for amido proton appeared at 8.513 ppm (Abdulghani and Mohuee, 2016). The atomic protons of the ligand appeared in the range 7.2 – 7.9 ppm, which confirms the formation of the ligand. The EI mass spectra of ligand L is given in Fig. 2 which has the molecular ion M^+ peak at 492.9514 corresponding to the molecular weight of the ligand L. The peaks at 474.9596, 457.0669, 373.0051, 353.0565, 248.9471, 141.9279, 114.0282, 103.04 and 76.1198 corresponds to various fragments of ligand L.

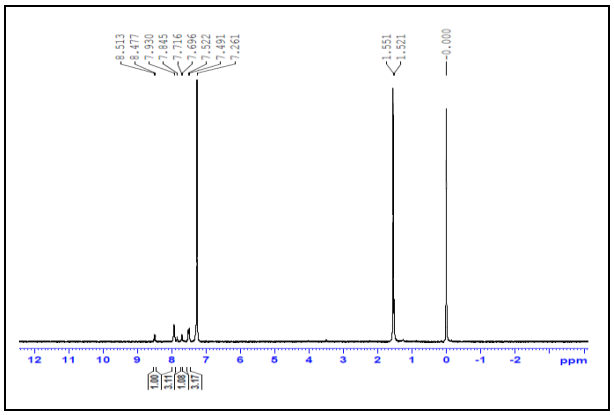


Fig. 1: ^{1}H-NMR spectrum of L

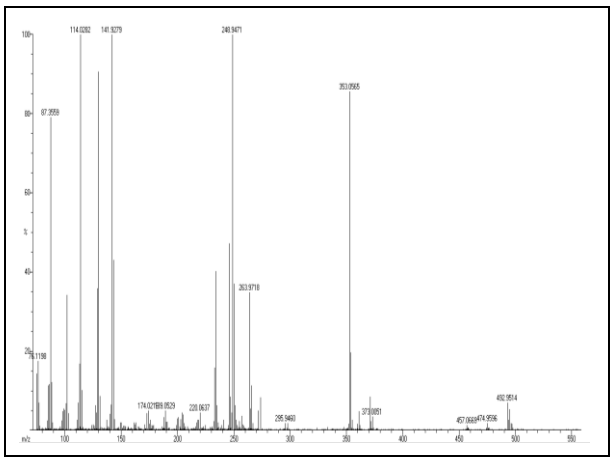


Fig. 2: EI-Mass spectrum of L

3.2 FT-IR Spectral Studies

The FT-IR spectrum of the Schiff base ligand L is given in Fig. 3 which has a strong band at 1598 cm^{-1}, which is attributed to the imino stretching frequency of the Schiff base ligand L (Singh *et al.* 2010).

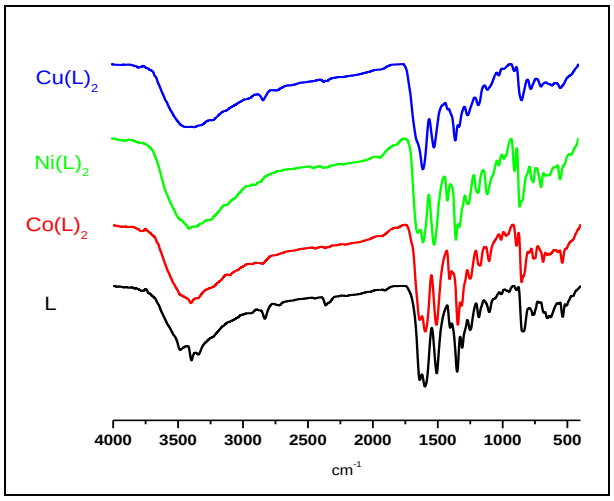


Fig. 3: FT-IR Spectra of Schiff base ligand L1 and its complexes

There is a strong peak at 3396 cm^{-1}, which is the characteristic N-H stretching frequency of the secondary amide group of 4, 4'-diaminobenzanilide moiety. The band around 3000 cm^{-1} is assigned to C-H stretching of aromatic group. The peak at 1638 cm^{-1} is due to the stretching of C=O group of 4, 4'diaminobenzanilide moiety. The band around 1500 cm^{-1} and 1349 cm^{-1} are attributed to the symmetric and asymmetric stretching frequencies of N=O group (Kemp and William 1991). The band at 838 cm^{-1} is due to the para substituted aromatic deformation frequency. This confirms the effective condensation of 4, 4-diaminobenzanilide and *p*-nitrobenzaldehyde. In order to study the binding mode of the Schiff base ligand L2 to the metal in the complexes, the IR spectrum of the free ligand is compared with those of the complexes. The band near 1598 cm^{-1} is shifted to lower frequencies in complexes. This clearly indicates the coordination of the amino-nitrogen to the metal center (Raman and Sobha, 2012). Further, the IR spectra of the complexes show some new sharp signals in the region 462, 464 and 463 cm^{-1} for Co $(L)_2$, Ni $(L)_2$ and Cu $(L)_2$ complexes respectively, which corresponds to metal-nitrogen stretching formed by the coordination of imino nitrogen and metal centers (Thomas *et al.* 1995). Thus, the FT-IR spectra confirm the formation of Schiff base metal complexes.

3.3 Electronic Spectral Analysis

The electronic spectra of complexes Co $(L)_2$, Ni $(L)_2$ and Cu $(L)_2$ are recorded in the range of 200-800 nm in DMSO and are depicted in Fig. 4.

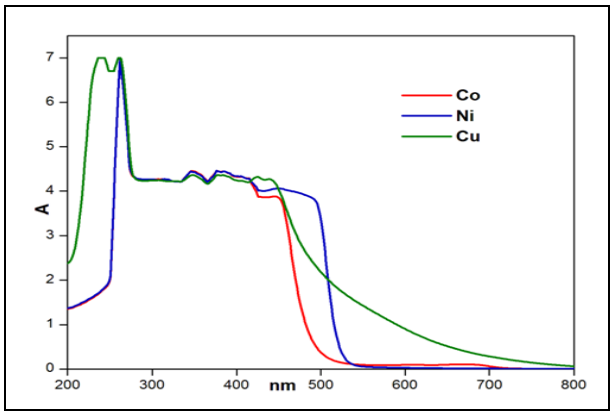


Fig. 4: UV-Vis. Spectra of Schiff base metal complexes

The spectral profiles below 350 nm are similar for all the complexes and are ligand-centered transitions (π-π^* and n- π^*) of benzene and non-bonding electrons present on the nitrogen of azomethine group in the Schiff base complexes. In the electronic spectrum of the cobalt complex, the band around 380 nm is assigned to LMCT transition while the d-d band observed in the lower energy region around 420 nm is assigned to the combination of $^2B_{1g} \rightarrow {}^1A_{1g}$ and $^1B_{1g} \rightarrow {}^2E_g$ transitions. This transition represents a square planar geometry (Chen *et al.* 1978). In the electronic spectrum of the nickel complex, the band around 360 nm is assigned to LMCT transition. Additionally, a broad band observed in the lower energy region around 370-410 nm is assigned to d-d $^1A_{1g} \rightarrow {}^1B_{1g}$ transition. This transition represents the square planar geometry for the nickel complex (Del and David 1983). The electronic spectrum of copper complex shows three major peaks at around 271, 325 and 450 nm. The first two peaks can be attributed to the transitions from ligand moiety. The band at ~325 mm is due to the LMCT transition. In addition, the charge transfer transitions from metal to ligand or ligand to metal may also be the reason for the emergence of this band in the electronic spectrum of copper complex. Besides, the expected d-d transition band is noted at ~450

nm which is attributed to the combination of $^2B_{1g} \rightarrow {}^2E_g$ and $^2B_{1g} \rightarrow {}^2B_{2g}$ transitions. This further stands as the evidence for the square planar geometry of d^9 Cu (II) system.

3.4 EPR Spectral Analysis

The EPR spectra of copper complex provide information of importance in studying the metal ion environment. The copper complex $Cu(L)_2$ exhibits an isotropic signal, without any hyperfine splitting, with g_{iso} = 2.104, as shown in Fig. 5. The g value obtained in the present study when compared to the g value of a free electron (2.0023), indicate an increase of the covalent nature of the bonding between the metal ion and the ligand molecule. Isotopic lines usually result due to occupancy of the unpaired electron in a degenerate orbital in square planar geometry.

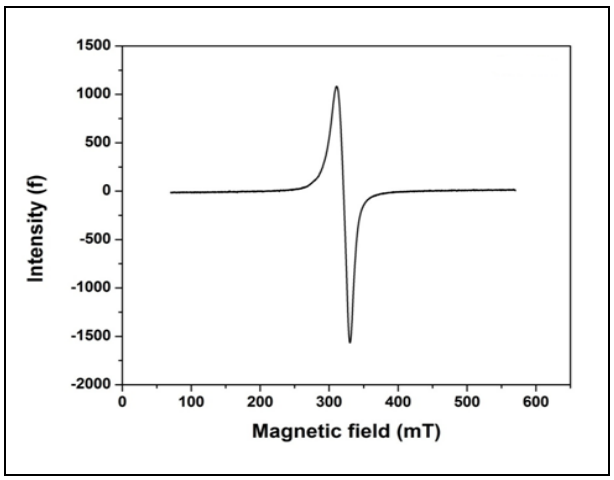


Fig. 5: EPR Spectrum of $Cu(L)_2$

3.5 Cytoxicity Screening

There are several methods for assessing the carcinogenic or mutagenic properties of the given chemical structure. The method followed here is a bacteria strain-based assay, which includes *E. coli* AB1157 which is a wild-type strain proficient in DNA damage repair. The bacterial strain is incubated with the compounds of interest for the analysis of any associated lethl effects. On incubation with the compounds, any free radical generation by the compounds lead to the lethality of the cells. This cytotoxic potentiality of the compound will be displayed in terms of zone of inhibition. The cytotoxic screening analysis of the complexes showed that copper complex alone exhibit exellent cytotoxicity screening effects at MIC value of 0.25 mg.

Table 1. Zone of Inhibition (mm) for Cytotoxicity Screening Analysis

Compounds	0.125 mg	0.25 mg	0.5 mg	1.0 mg	2.0 mg	MIC (mg)
Co $(L)_2$	0	0	3	7	10	0.5
Ni $(L)_2$	0	0	0	0	5	2.0
Cu $(L)_2$	0	2	4	5	10	0.25
Stannous chloride	0	0	3	8	15	0.5

3.6 DNA Binding Studies Using Electronic Spectra

The interactions of metal complexes with DNA are of interest in order for the development of chemotherapeutic agents. Electronic aborption spectroscopy is one of the most useful techniques for DNA binding studies of metal complexes. The interactions of copper complex Cu $(L)_2$ with CT-DNA were investigated by UV-Vis. absorption titrations and shown in Fig. 6.

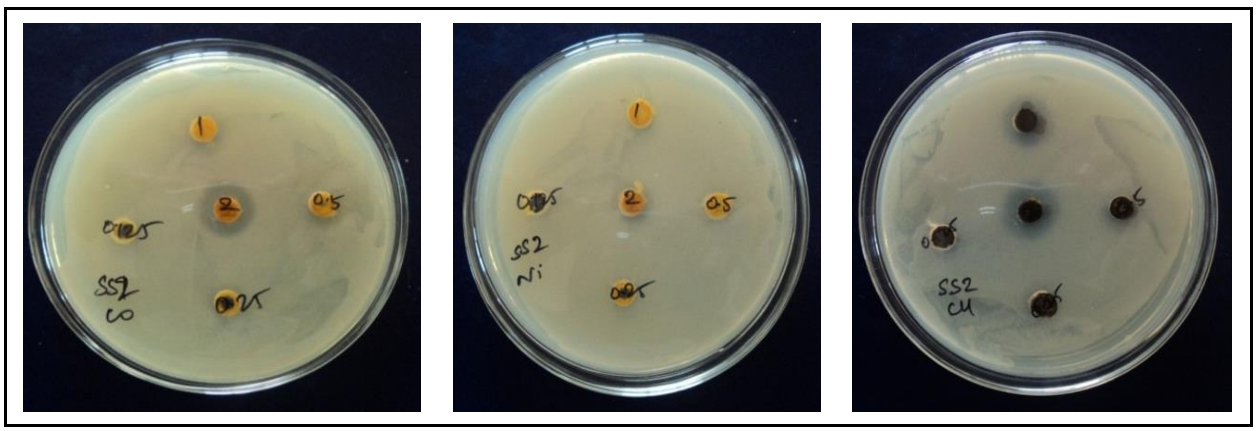


Fig. 6: Cytotoxicity Screening Analysis of Complexes

Upon addition of increasing amount of CT-DNA from 0-200 µL, a significant "hyperchromic" effect of the intra-ligand bands at 257.8-300 nm was accompanied by a red shift of 2-7 nm, indicative of the breakage of the DNA helix (Herebian *et al.* 2002; Asadi *et al.* 2004). There is no appreciable change in the charge transfer band. As the concentration of the DNA was increased, the absorption bands of the copper complex initially showed hyperchromism, but on further increasing concentration, hyperchromism with blue shift is obtained (Fig. 7).

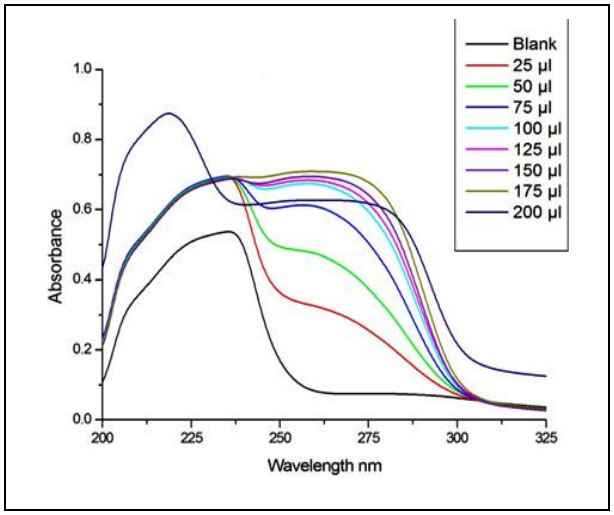


Fig. 7: DNA Binding interactions of $Cu(L)_2$

The *in vitro* cytotoxicity of the copper complex $Cu(L)_2$ was evaluated against Larynx Cancer Cells Hep_2 and given in Fig. 8. The IC_{50} (the concentrations inhibited in 50% of the cellular proliferation) of the studied complex is 40 µg.

Molecular docking with human DNA topoisomerase I

Human DNA topoisomerase I and II were the selective targeting area for synthesizing the anticancer drug (Pommier, 2006). The molecular docking of Cobalt (II), Nickel (II) and Copper (II) complexes were performed to determine the value of binding affinity and the selected binding residue, along with the sterically suitable conformations. The low value of the binding energy shows the more effective binding affinity between the 'receptor' and the 'ligand' molecules. The various conformations of docked molecular complexes were analyzed in terms of binding energy, hydrogen bonding and hydrophobic interaction between receptors and the acceptor. More negative value of the relative binding values suggests that the interaction between the DNA and ligand is so strong, due to the extended aromatic ring. Phenyl ring has higher free binding energy which gives a better binding affinity value compared with a compound containing bipyridyl ring. From these works, mono-nuclear complexes gives better results towards the HDNA. The binding energy values of the Co (II), Ni (II) and Cu (II) complexes are -11.6, -6.9 and -10.4 kcal mol^{-1} respectively, towards human DNA topoisomerase I. This shows that Cobalt (II) and Copper (II) molecules easily bind with the DNA helix and the Nickel (II) complex prefer to bind with the outermost protein's amino acid residue. The docked images were shown in Fig. 9.

NS3 protease-helicase (dengue virus) is a very important target area which should be docked. Cobalt (II), Nickel (II) and Copper (II) complexes exhibit very low binding energy value; it means that complexes are having very high binding affinity towards NS3 protease-helicase. The distance between the selected receptor to targeted molecule is also low. The binding energy values of Co (II), Ni (II) and Cu (II) complexes are: -12.7, -12.9 and -12.6 kcal mol^{-1}, respectively. The binding interactions were shown in Fig. 10. From the theoretical point of view, these complexes are considered to be good anti-dengue drugs.

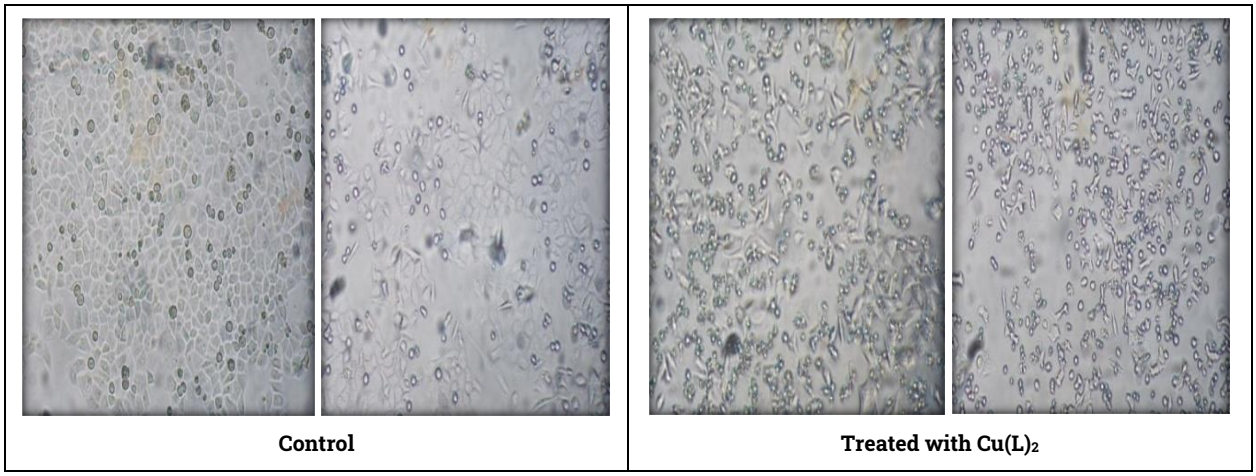


Fig. 8: Anti-Tumor Activity of $Cu(L)_2$

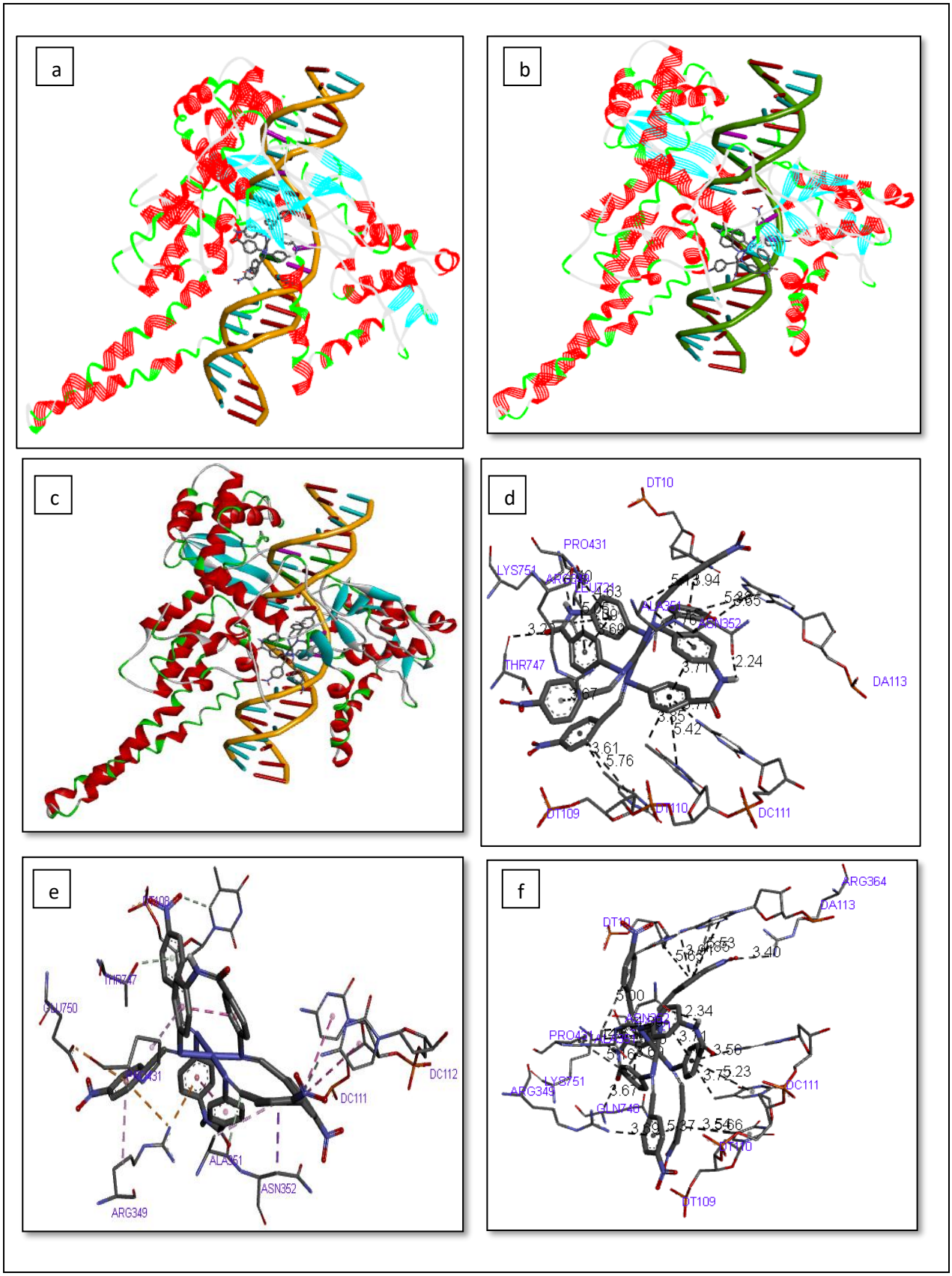


Fig. 9: The binding of Co (II), Ni (II) and Cu (II) complexes with (a), (b) and (c): active site of human DNA topoisomerase I and (d), (e) and (f): selective nucleotide of human DNA topoisomerase I

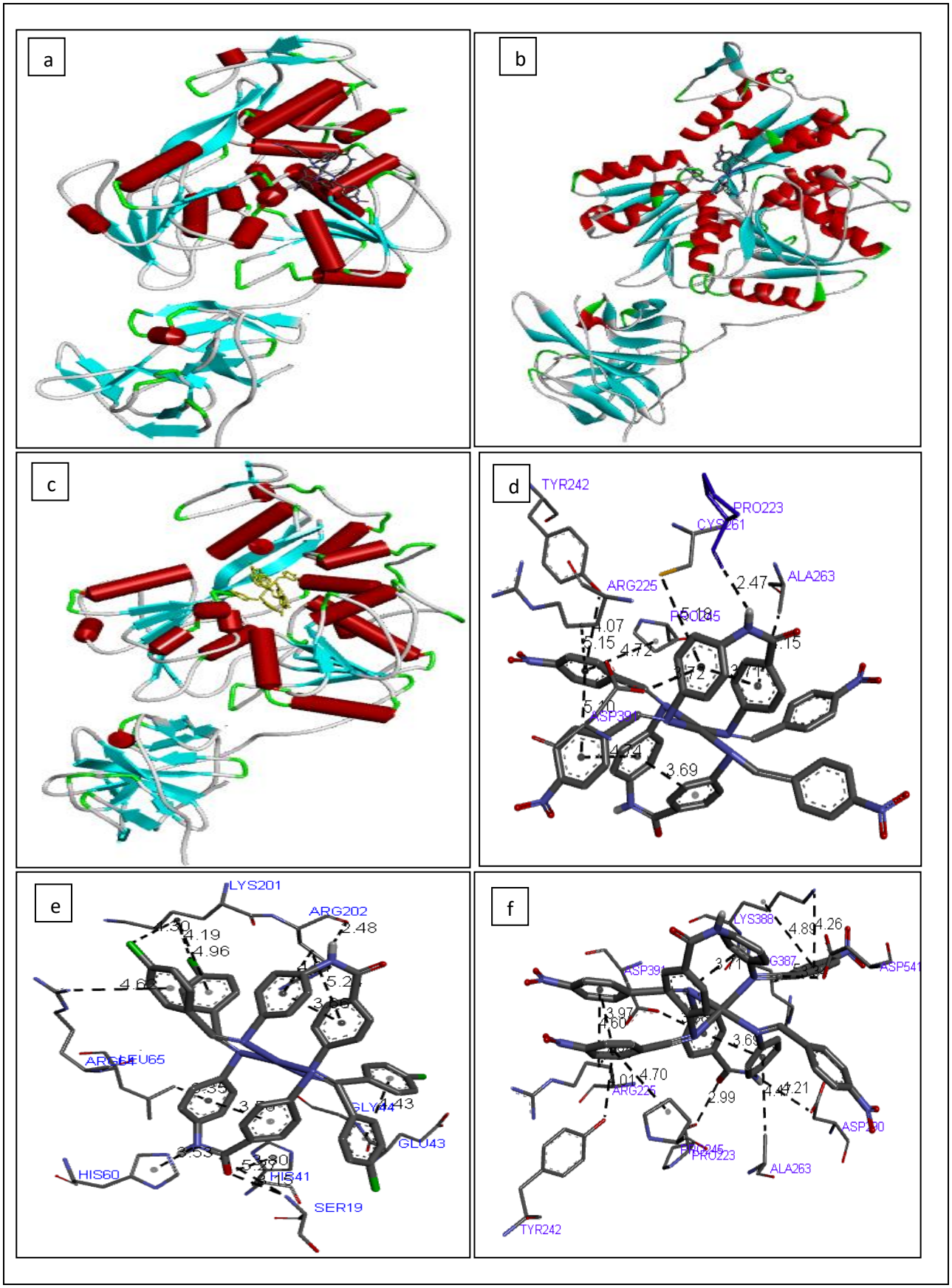


Fig. 10: The binding of Co (II), Ni (II) and Cu (II) complexes with (a), (b) and (c): active site of NS3 protease-helicase and (d), (e) and (f): selective amino acid residue of NS3 protease-helicase

4. CONCLUSION

The cobalt (II), nickel (II) and copper (II) complexes of a new Schiff base ligand L with several phenyl rings were synthesized. Various spectroscopic techniques confirmed the formation of the ligand and its nanometal complexes. The biological applications of the synthesized complexes were carried out by Cytotoxic screening analysis and DNA binding ability using Electronic spectra and Anti-Tumor activity by MTT assay. The results obtained have shown that among the synthesized complexes copper complex has potential biological activity. The docking studies were carried out using synthesized nanometal complexes with human DNA topoisomerase I (PDB: 1SC7) and Dengue NS3 protease-helicase bi-functional enzyme (PDB ID: 2VBC) using Auto Dock vina and Discovery studio software. The binding energy values of Co (II), Ni (II) and Cu (II) complexes have shown that these types of compounds can act as potential Anti-Dengue and Anticancer agents.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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