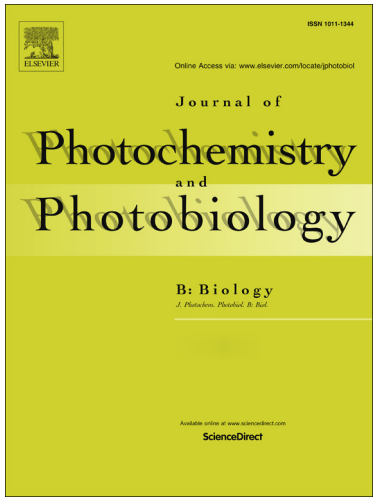

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Synthesis and characterization of Schiff base octaazamacrocyclic complexes and their biological studies

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ABSTRACT

A condensation reaction between 1, 2-diphenylethane-1,2-dione dihydrazone (DPEDDH) and dimethyl or diethyloxalate in methanol resulted in a novel Schiff base octaazamacrocyclic ligand, (L): (6,7,14,15-tetraoxa-2,3,10,11-tetraphenyl-1,4,5,8,9,12,13,16-octaazacyclohexadecane-1,3,9,11-tetraene). Subsequently metal complexes of the type $[MLX_2]$ and $[CuL]X_2$; (M = Mn(II), Co(II), Ni(II) and Zn(II) ; X = Cl or NO_3) were synthesized by the reaction of the free macrocyclic ligand (L) with the corresponding metal salts in 1:1 molar ratio. These complexes were characterized on the basis of analytical, conductivity and magnetic susceptibility measurements, ESI-mass, IR, NMR (^{1}H and ^{13}C), EPR and electronic spectral studies. The thermal stability of the complexes was also studied by TGA and DTA analyses. These studies show that all the complexes have octahedral arrangement around the metal ions except copper complexes which are square planar. The ligand and its complexes were screened for their antibacterial activity in vitro against Gram-positive *(Staphylococcus aureus)* and Gram-negative *(Escherichia coli)* bacteria and were also studied for their anticancer activity against the human cancer cells lines: HeLa (Human cervical carcinoma), MCF7 (Human breast adenocarcinoma) and Hep3B (Human Hepatocellular carcinoma). The recorded IC_{50} values for the tested compounds show moderate to good cytotoxicity against these cancer cell lines. The copper complex, $[CuL]Cl_2$, showed excellent antimicrobial activity against tested microorganisms which is almost equivalent to the standard drug ciprofloxacin.

KEYWORDS: Octaazamacrocyclic complexes, Spectral studies, Thermal analyses, Antibacterial activity and Anticancer activity.

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1. Introduction

The biomedical inorganic chemistry has been a fascinating research area due to broad applications of inorganic pharmaceuticals in various clinical therapy and diagnostics [1, 2]. Although platinum-based chemotherapeutic compounds have remarkable success with high efficiency against human testicular, ovarian, bladder, head and neck carcinomas, several side effects such as limited water solubility and the dose-dependent toxicities, mainly nephrotoxicity, cytotoxicity and emetogenesis are the major drawbacks associated with platinum based drugs [3, 4]. Thus in view of research for non platinum based drugs a large number of studies have recently been carried out [3, 5-7]. Specially Cu(II) ion is known to involve in many biological process having its role in increasing antitumor efficacy of organic molecules[8-10]. Thus the copper complexes are the theme of Scientists due to their potential use as antimicrobial, antiviral, anti-inflammatory and antitumor agents, enzyme inhibitors or chemical nucleases [8, 9]. Now a large number of copper(II) chelate complexes that exhibit cytotoxic activity through cell apoptosis or enzyme inhibition have been reviewed [8, 10]. The nitrogen and sulfur containing organic compounds and their metal complexes display a wide range of biological activity as antitumor, antibacterial, antifungal and antiviral agents [11, 12]. Macrocyclic Schiff base ligands have received special attention because of their mixed hard-soft donor character and versatile coordination behaviour [13] for their enantioselective catalytic reactions [14], biological activities, i.e., toxicity against bacterial growth, anticancerous and other biochemical properties [15]. The synthesis of azamacrocyclic compounds received considerable attention during the last decade because of their relationship to biomimetic and catalytic systems and their applications as chelating agents in biology and medicine [16, 17]. The presence of N=C-C=N structural unit in Schiff bases is responsible for the formation of a strong chelate ring resulting in possible electron delocalization associated with extended conjugation [13]. The polyamide macrocycles deserve special attention as they have been used for the construction of the corresponding polyazamacrocyclic complexes [18-20]. We have a long term interest in synthesis and characterization of macrocyclic complexes of a wide variety [19-23] and recently also studies

their biological properties [21-24]. Recently the antimicrobial and anticancer activities of other materials have also been reported from our laboratories [25-27]. Herein, we report a new series of novel Schiff base octaazamacrocyclic ligand (L):(6,7,14,15-tetraoxa-2,3,10,11-tetraphenyl-1,4,5,8,9,12,13,16-octaazacyclohexadecane-1,3,9,11-tetraene) and its transition metal complexes of the type, $[MLX_2]$ and $[CuL]X_2$, (M = Mn(II), Co(II), Ni(II) and Zn(II); X = Cl or NO_3), which were also studied their biological properties: antimicrobial and anticancer activities.

2. Experimental Section

2.1. Materials and Methods

The chemicals benzil, hydrazinehydrate (Merck), dimethyl and diethyloxalate (both Fluka) were used as received. Metal salts (all Merck) were commercially available pure samples. Benzildihydrazone (DPEDDH) was prepared by the reaction of benzil and hydrazinehydrate in methanol in 1:2 molar ratio. Ficoll-Histopaque, Fetal calf serum, RPMI1640 and antibiotic-antimycotic solutions were procured from Sigma Aldrich, USA. Hep3B (Human hepatocellular carcinoma), MCF7 (Human breast adenocarcinoma) and HeLa (Human cervical carcinoma) cell lines were procured from Cell Repository–National Centre for Cell Science Pune, (India).

2.2. Synthesis of Macrocyclic Ligand,

(L): 6,7,14,15-tetraoxa-2,3,10,11-tetraphenyl-1,4,5,8,9,12,13,16-octaazacyclohexadecane-1,3,9,11-tetraene)

A methanolic solution (25 cm^3) of 1,2-diphenylethane-1,2-dione dihydrazone (DPEDDH) (0.02mol, 4.76 gm) was placed in a round bottom flask, there a methanolic solution (25 cm^3) of dimethyl (2.36 gm, 0.02 mol) or diethyloxalate (2.92 gm , 0.02 mol) was slowly added with constant stirring for 8 h. The solution was then refluxed and stirred for 6 h. The reaction mixture was then kept for evaporation resulting in a light yellow solid product, which was filtered, washed several times with methanol and dried in vacuum.

2.3. Synthesis of Macrocyclic Complexes,

Synthesis of dichloro/nitrato (6,7,14,15-tetraoxa-2,3,10,11-tetraphenyl-1,4,5,8,9,12,13,16-octaazacyclohexadecane-1,3,9,11-tetraene) metal(II), $[MLX_2]$; (M = Mn(II), Co(II), Ni(II)

and Zn(II) ; X = Cl or NO_3 and (6,7,14,15-tetraoxa-2,3,10,11- tetraphenyl-1,4,5,8,9,12,13,16-octaazacyclohexadecane – 1,3,9,11-tetraene) copper(II) chloride and nitrate [CuL]X_2 (X = Cl or NO_3)

An equimolar amount of the methanolic solution of the macrocyclic ligand, (L) and corresponding metal salt (0.01mol) in 1:1 ratio were reacted in the round bottom flask. The reaction mixture was stirred with heating for 12h leading to the isolation of solid product which was filtered, washed several times with methanol and dried in vacuo.

3. Measurements

The elemental analyses for carbon, hydrogen and nitrogen were obtained from the Central Drug Research Institute Lucknow, India. The ^{1}H and ^{13}C-NMR spectra were recorded on a JEOL GSX 300 MHz FX-1000 spectrometer using DMSO-d_6 as a solvent and tetramethylsilane (Me_4Si) as an internal standard. The estimation of halogen was done gravimetrically [28] and the metals were estimated by titrating with standard EDTA solution [29]. The electronic spectra of the compounds in DMSO were recorded on a Pye-Unicam 8800 spectrophotometer at room temperature. The EPR spectra were recorded on a JEOL JES RE2X EPR spectrometer. Magnetic susceptibility were carried out at 25^0C using a Faraday balance. Electro spray ionization mass spectra (ESI-MS) of the complexes were recorded on a Q-Tof Micromass spectrometer from Guru Nanak Dev University (Amritsar, India). The IR spectra were recorded in the region 4000–400 cm^{-1} by using FT-IR Perkin Elmer (2400) spectrometer and in the region 700-30 cm^{-1} by using FT-IR/FIR Perkin Elmer (Frontier) spectrometer. The TGA and DTA were performed on a Schimadzu Thermal Analyze under nitrogen atmosphere using alumina powder as reference. The electronic spectra of the compounds were recorded on a Pye-Unicam 8800 spectrophotometer at room temperature. The antibacterial activity of the synthesized compounds was completed by the disc diffusion method and broth dilution methods [25, 30].

3.1. Cell viability assay (MTT)

The fresh Blood (10–15 mL) provided by Blood bank of Jawaharlal Nehru Medical College, A.M.U, Aligarh (India), was diluted with the same volume of phosphate buffered saline (PBS). The diluted blood sample was carefully layered on Ficoll-Histopaque. The mixture was centrifuged under at 900×*g* for 10 min at 20–22^0C. The undisturbed lymphocyte layer was carefully transferred out. The lymphocyte was washed and pelleted down with three

volumes of PBS for twice and resuspended RPMI-1640 media with 10% antibiotic and antimycotic solution v/v fetal calf serum (FCS) [26,31]. The anticancer activity in vitro was measured using the 3-(4, 5-dimethylthiazol-2-y1)-2, 5-diphenyltetrazolium bromide (MTT) assay. The HeLa (Human cervical carcinoma), MCF7 (Human breast adenocarcinoma) and Hep3B (Human hepatocellular carcinoma) cells were maintained in RPMI 1640 culture medium supplemented with 10% heat-inactivated fetal calf serum. The cells were plated at a density of 5×10^3 cells per well in a 96-well plate, and cultured for 24 h at 37°C. The cells were subsequently exposed to drugs. The plates were incubated for 48 h, and cell proliferation was measured by adding 20 µL of MTT dye (5 mg/mL in phosphate buffered saline) per well. The plates were incubated for a further 4 h at 37°C in a humidified chamber containing 5% CO_2. Formazan crystals formed due to reduction of dye by viable cells in each well were dissolved in 150 µL dimethyl sulfoxide, and absorbance was read at 570 nm. The absorption values were expressed as the cell present viability, according to the control group as 100%.

4. Results and Discussion

A [2+2] condensation reaction between 1, 2- diphenylethane - 1, 2-dione (DPEDDH) and dimethyl or diethyloxalate (1:1 molar ratio) in methanol resulted a novel Schiff base octaazamacrocyclic ligand, (L): (6,7,14,15-tetraoxa-2,3,10,11-tetraphenyl-1,4,5,8,9,12,13,16-octaazacyclohexadecane-1,3,9,11-tetraene) this ligand (L) upon subsequent treatment with appropriate metal salts in a 1:1 molar ratio in methanol yielded macrocyclic complexes of the type, $[MLX_2]$ and $[CuL]X_2$ (M = Mn(II), Co(II), Ni(II), and Zn(II) : X = Cl or NO_3) **(Scheme 1).** The ligand and its complexes are stable in at room temperature in atmosphere and are soluble in dimethyleformaldehyde and dimethyl sulfoxide. The formation of ligand framework and its complexes was further ascertained on the basis of results of elemental analyses, molecular ion peak in ESI-mass spectra **(Table 1),** characteristic bands in the FT-IR **(Table 2)** and resonance signals in the ^1H and ^{13}C NMR spectra. The overall geometry of the complexes was inferred from the observed values of magnetic moments and electronic spectra and the position of bands in the EPR for Cu(II) complexes **(Table 3).** The molar conductivities in DMSO indicate [32] that copper(II) complexes are electrolytic while others are non-electrolytic in nature.

4.1. ESI-Mass Spectra

Mass spectra of tetraiminetetraamide ligand and their complexes showed *m/z* peaks at 583.70 765.20, 717.10, 759.80, 712.20, 770.1, 720.1, 775.90, 715.20, 769.50 and 725.70 that correspond to $C_{32}H_{24}N_8O_4$, $C_{32}H_{24}N_{10}MnO_{10}$, $C_{32}H_{24}N_8O_4MnCl_2$, $C_{32}H_{24}N_{10}CoO_{10}$, $C_{32}H_{24}N_8O_4CoCl_2$, $C_{32}H_{24}N_{10}NiO_{10}$, $C_{32}H_{24}N_8O_4NiCl_2$, $C_{32}H_{24}N_{10}CuO_{10}$, $C_{32}H_{24}N_8O_4CuCl_2$, $C_{32}H_{24}N_8O_4ZnCl_2$ and $C_{32}H_{24}N_8ZnO_{10}$ moieties, respectively. The proposed molecular formulae of synthesized complexes were confirmed by comparing their molecular formula weights with respective m/z values **(Table 1)** which are in good agreement for aforementioned complexes shown in **(Figure 1).**

4.2. IR Spectra

The most significant feature of IR spectra **(Table 2)** of macrocyclic ligand and its complexes is the appearance of four characteristic bands in 1670-1715, 1510-1550, 1225-1255 and 635-670 cm^{-1} regions which may be assigned to amide I [υ(C=O), amide II υ(C-N) + δ(N-H)], amide III δ(N-H), and amide IV wagging [ϕ(C=O)], vibration, respectively [19]. The amide I υ(C=O) is observed in the region expected for the metal free amide group [33] ruling out the possibility of coordination through amide oxygen. Although the IR spectrum of (DPEDDH) there is appearance of two strong υ(N-H) bands at 3270 and 3220 but the IR spectra of macrocyclic ligand and its complexes show only single sharp υ(N-H) band in the 3230-3275 cm^{-1} region, which is attributable to the uncoordinated amide nitrogen [33]. Reported macrocyclic complexes exhibit a strong intensity band in the range of 1580–1595 cm^{-1} assignable [34] to the coordinated υ(C=N). There is observed increase in the υ(C=N) band intensity which may be due to the possible transformation of trans structure of the (DPEDDH) into cis configuration upon complexation. The cis structure is stabilized by the chelate ring formation, lowering the symmetry which consequently enhances the υ(C=N) band intensity. The bands at 425–470 cm^{-1}, originate from (M–N) vibration. The coordination of nitrato and chloro groups have been confirmed due to the appearance of bands in the 220-240 and 270-290 cm^{-1} regions which may be assigned to υ(M-O) and υ(M-Cl) respectively. In addition, the IR spectra of nitrato complexes exhibit bands at 1280, 1040 and 850 cm^{-1} which are consistent with the monodentate nature [19] of this group. However, free nitrate absorption in Cu(II) complex appeared at 1385 cm^{-1} which corresponds to an uncoordinated nitrato group.

4.3. ^1H - NMR Spectra

The ^{1}H NMR spectra of benzildihydrazone (DPEDDH) and the corresponding zinc complexes have been recorded and compared **(Figure 2).** Benzildihydrazone shows a multiplet at 5.12-6.03 ppm and a slightly broad signal at 3.93-4.71 ppm assignable [35] to the phenyl ring (C_6H_5, 10 H) and amino ($N\text{-}NH_2$, 4 H) protons of the hydrazine moiety. Both the zinc complexes showed a band at 7.074-7.625 ppm, reasonably ascribed [36] to the amide (N-NH-C, 4 H) protons. A multiplet at 5.39- 6.54 ppm in both complexes is characteristic of phenyl ring (C_6H_5, 20 H) protons. Absence of characteristic peaks corresponding to the protons of hydrazone and oxalate moieties, further confirm that the cyclization has indeed taken place.

4.4. ^{13}C- NMR Spectra

The ^{13}C-NMR spectrum of the mononuclear complexes gave signals characteristics of different carbon atoms of macrocyclic framework that appeared at their appropriate positions expected for proposed structure. However, the positions of resonance signals were found to be slightly downfield shifted [37] in complexes as compared with the free Schiff base macrocyclic ligand **(Figure 3)** confirming the coordination of the ligand to Zn(II) metal ion .

4.5. Magnetic Susceptibility Measurements

The magnetic moment values (Table 1) for Mn(II), Co(II) and Ni(II) complexes are in support of octahedral geometry around metal ions. Nickel(II) with d^8 configuration may have two unpaired electrons in both high spin and low spin (weak or strong ligand field) octahedral arrangements. However, it generally shows a tendency to form octahedral complexes with week field ligands and square planar complexes with strong field ligands. The observed magnetic moment values for Mn(II) and Co(II) complexes are consistent with the high spin octahedral geometry which indicates that the ligand field is weak. Thus it is probable that Ni(II) may assume an octahedral geometry rather than square planar. The magnetic moment for the mononuclear Cu(II) complexes having no major interaction between two copper moieties are reported in the range of 1.75 to 2.20 B.M. [38]. However, due to spin exchange phenomena between two Cu(II) ions lower magnetic moments (1.2-1.6 BM) were observed in the dimeric Cu(II) square planar complexes [39]. The observed magnetic moment values (1.87-1.92 B.M.) indicate that Cu(II) complexes are mononuclear.

4.6. Electronic Spectra

The electronic spectra **(Table 3)** of manganese complexes gave two bands in the 22400-22550 cm^{-1} and 18650-18850 cm^{-1} regions which may be assigned to $^6A_{1g} \rightarrow {}^4T_{2g}(F)$ and $^6A_{1g} \rightarrow {}^4T_{1g}(P)$ transitions, respectively, corresponding to an octahedral environment around the Mn(II) ion [20]. The cobalt complexes exhibit two bands appearing in the 21400-21700 and 15950-16200 cm^{-1} regions which may reasonably correspond to $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ and $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ transitions, respectively, consistent with octahedral geometry [20] around Co(II) ion. All the nickel complexes exhibit two electronic bands in 21200-24350 and 18200-18750 cm^{-1} regions which may be ascribed to $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ transitions, respectively, suggesting [18] an octahedral geometry around the metal ion. The energy level diagram for ligand field of distorted O_h or D_{4h} symmetry predicts three transitions: (I) $^2B_{1g} \rightarrow {}^2A_{1g}$ (υ_1), (II) $^2B_{1g} \rightarrow {}^2B_{2g}$ (υ_2) and (III) $^2B_{1g} \rightarrow {}^2E_g$ (υ_3). The electronic spectra of copper complexes show a broad band maximum at 16050 cm^{-1} which may be assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ transition. However, two weak shoulders appearing in the regions 21500-21750 and 11750-12450 cm^{-1} may be attributed to $^2B_{1g} \rightarrow {}^2E_g$ and $^2B_{1g} \rightarrow {}^2B_{2g}$ transitions, respectively, suggesting [40,41] a square-planar geometry around the Cu(II) ion. The conductivity data **(Table 1)** for the copper complexes show that Cl^- and NO_3^- groups are ionised and remain out of the coordination sphere. Further results of thermal analysis (TGA/DTA, Section 4.8) of the copper complex, $[CuL]Cl_2$, shows the absence of water/solvent molecules in/out of the coordination sphere. These studies rule out the possibility of distorted octahedral geometry and are in strong support of square planar structure for copper complexes. The EPR spectra further confirm square planar geometry for the copper complexes.

4.7. EPR Spectra

The EPR spectra of the powdered solid copper(II) macrocyclic complexes **(Figure 4)** were recorded at room temperature and their $g_{\|}$ and $g_{\perp}$ values have been calculated. Both the complexes exhibit a similar single broad absorption band. The absence of hyperfine lines in these complexes may be due to the strong dipolar and exchange interactions between the copper(II) ions in the unit cell [42]. The g values are related by the expression $G = (g_{\|} - 2) / (g_{\perp} - 2)$ and these complexes, $[CuL]X_2$ (X = Cl or NO_3), gave axial symmetry parameter, G in the 1.758 - 1.940 region (Table 3) indicating [42] very small exchange interaction among copper(II) ions in these complexes, as the G values are less than 4. It is known that with $g_{\|} > g_{\perp}$ the unpaired electron in tetragonal and square planar complexes lies in the dx^2-y^2

orbital giving $^2B_{1g}$ as the ground state [43]. The observed $g_{\parallel}$ and $g_{\perp}$ values **(Table 3)** appeared in the region 2.1782-2.0925 and 2.087-2.0526, respectively. Thus, for these copper complexes $g_{\parallel} > g_{\perp}$ which support the fact that the ground state of Cu(II) is predominantly $^2B_{1g}$ having unpaired electron in the $dx^2–y^2$ orbital. The unpaired electron will lie predominantly in the $dx^2–y^2$ orbital and two electrons in the dz^2 orbital. Thus ligands approaching along the Z – axis have to face very strong repulsive forces from the filled dz^2 orbital, consequently will not be successful to coordinate along the +Z and –Z directions which is also supported by the conductometric study and thermal analysis (Cl^-, NO_3^-, water or solvent molecules do not coordinate with the metal ions). The observed g values for both the copper complexes lie in the range reported for square planar complexes [43-45], thus the EPR spectral studies strongly support square planar structure for Cu(II) complexes. Their square planar geometry has also been corroborated from electronic spectra.

4.8. Thermal Analysis (TGA/DTA)

The thermal stability of the mononuclear octaazamacrocyclic complexes was investigated using TGA (Figure 5). The thermogravimetric analyses (TGA) were carried out at a heating rate of 20^0C/min in a nitrogen atmosphere over a temperature range of 10–800^0C. The complexes have a different decomposition process. The relative high thermal stability observed in all thermally investigated complexes may correspond to the absence of any solvent / water molecules in/out of the coordination sphere. This is proposed referring to the higher thermal stability observed for all investigated complexes in which the first decomposition step is started at a relatively higher temperature. The thermograms of TGA of mononuclear complexes exhibit decomposition between 100–550^0C, which may be due to the removal of the coordinated chloride or nitrate ions. The second decomposition stage followed the previous one ended at 800^0C, may be attributed to the removal of the organic part of the compounds into their corresponding oxides. Further horizontal constant curve may be due to the presence of metal oxides residue in the remaining part. The differential thermal analysis (DTA) curves **(Figure 5)** of the mononuclear complexes show endothermic peak in the temperature range 100–250^0C assigned to loss of coordinated chloride or nitrate ions. The sharp decomposition corresponding to the loss of organic moiety in complexes can be seen in the DTA curves that contained one sharp exothermic peak falling in the range of 250–450^0C and indicate the formation of metal oxides [46, 47].

4.9. Biological Activities

4.9.1. Antibacterial Studies

The newly prepared complexes were screened for their antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* bacterial strains by disc diffusion method [25, 30]. Standard inoculums (1-2 $X10^7$ CFU/ml 0.5 McFarland standards) were spread onto the surface of sterile agar plates. The discs measuring 6 mm in diameter were prepared using Whatman No.1 filter paper and were sterilized by dry heat at 140°C for 1 h. The sterile discs previously soaked in a known concentration of the test compounds were placed in the nutrient agar medium. Ciprofloxacin (30 µg) was used as positive control, while the disk poured in DMSO was used as negative control. The plates were inverted and incubated for 24 h at 37°C. The susceptibility was assessed on the basis of the diameter of the zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls (**Table 4** and **Figure 6).** Minimum inhibitory concentrations (MICs) were determined by the broth micro dilution method. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls were inoculated with approximately $5x10^5$ CFU/ml of actively dividing bacteria cells. The cultures of the bacterial strains were incubated for 24 h at 37°C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC). To obtain the minimum bacterial concentration (MBC), 0.1 ml volume was taken from each tube and spread on agar plates displayed in **Figure 7**. The number of CFU was counted after 18-24 h of incubation at 35°C. MBC was defined as the lowest drug concentration at which 99.9% of the inoculums were killed. The MIC and MBC are given in **Table 4**. The investigation of antibacterial screening data revealed that all tested compounds showed moderate to good antibacterial activity. Among the metal complexes studied the copper complex $[CuL]Cl_2$, showed highest antibacterial activity against *S. aureus* and *E. coli* nearly equivalent to standard drug ciprofloxacin. Further as reported earlier [5, 8] antibacterial activity of free ligand is found to be less than its metal complexes.

4.9.2. Cytotoxic potential of macrocyclic complexes

The cytotoxic potential of the in house synthesized macrocyclic complexes against HeLa Hep3B, and MCF7 cell lines was assessed by determining the number of viable cells surviving after incubation with the macrocyclic complexes for the stipulated time period

using the MTT method as given in the experimental section. The cytotoxicity assay suggests a variable cytotoxicity of inhouse synthesized complexes for HeLa, MCF7 as well as Hep3B cell lines which can be attributed to the intrinsic anticancer property of these macrocyclic complexes. Curves of dose-dependent effects of all the complexes on cell viability of different human cancer cell lines (HeLa, MCF7, and Hep3B) are displayed in **Figure 8.** Experiments revealed that there was substantial increase in cytotoxicity in the cell lines with increasing exposure to drug concentration. The absorption values were expressed as the cell viability in percent, according to the control group as 100%. Assays were performed in triplicate on three independent experiments. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated using the software ''Prism 3.0''. For each of the tested complexes IC_{50} was calculated and the results are summarized in **Table 5**. It is apparent from the IC_{50} values that all the tested complexes show moderate to good cytotoxicity against different human cancer cell lines. Further as aspected cytotoxicity of metal complexes is comparatively more than the free ligand [7, 9]. It is remarkable that among these complexes the copper complex, $[CuL]Cl_2$, exhibits comparatively high cytotoxicity against all cancerous cell lines with IC_{50} values of 12.7±2.5 μM against HeLa, 11.5±2.5 μM against MCF7 and 12.53±1.9 μM against Hep3B cell lies, respectively.

Further, it is observed that both antibacterial and anticancer activities of metal complexes are comparatively higher than the metal free ligand under identical experimental conditions which is also in accordance with the chelation theory [21, 48]. This enhancement in their actvities may be due to the presence of azomethine bonds in the macrocyclic chelate ring. As a result of coordination of the metal ion through nitrogen of the azomethine bonds of the chelate ring the polarity of the metal atom is reduced due to partial sharing of its positive charge with the ligand, as there arises the possiblility of π electron delocalization within the chelate ring. Thus, in this process the lipophilic nature of the metal atom increases favouring its permeation more efficient through the lipid layer of cell membranes.

It is also remarkable that copper complex shows higher antibacterial and anticancer activities relative to other transition metals studied here [Mn(II), Co(II) and Ni(II)]. This behaviour of copper is in agreement with the earlier studies [8, 10, 49]. Except the role of copper as an essential trace element it exhibits considerable biochemical action forming complexes that interact with bio molecules, mainly protein and nucleic acids. The role of copper is significant as it can promote nucleic acid cleavage and therefore is used as metallodrug to cause DNA damage [10, 50, 51]. The earlier reports reveal that copper(II) complexes exhibit stronger DNA binding propensity [51-53] as compared to other transition metal complexes.

The later 3d transition metal ions, specially Cu(II), are classified as "borderline" between 'hard'(a-class) and 'soft' (b-class) metals. Regarding their metal-DNA interactions they show more affinity for both, the heterocyclic bases as well as phosphate group in contrast to 'soft' (b-class) metals like Pt(II), therefore there appears enhancement in their binding strength with DNA.

5. Conclusion

The newly synthesised free macrocyclic ligand (L): (6,7,14,15-tetraoxa-2,3,10,11-tetraphenyl-1,4,5,8,9,12,13,16-octaazacyclohexadecane-1,3,9,11-tetraene) after reaction with respective metal salts yielded transition metal macrocyclic complexes of the type $[MLX_2]$ and $[CuL]X_2$ (M = Mn(II), Co(II), Ni(II) and Zn(II) ; X = Cl or NO_3) having octahedral geometry around metal ions except the copper complexes which are square planar. After characterization further studied were carried out for their biological properties: antibacterial and anticancer activities. The ligand and its complexes were screened in vitro against *Staphylococcus aureus* and *Escherichia coli* bacteria and their cytotoxic potential was studied against different human cancer cell lines: HeLa, MCF7 and Hep3B. These studies reveal that metal complexes are comparatively more effective in their antibacterial and anticancer activities than the metal free ligand. Although copper complex shows moderate cytotoxicity against cancer cell lines but it shows excellent antimicrobial activity against tested microorganisms which is almost equivalent to the standard drug ciprofloxacin. These studies may explore further possibilities to provide new structural type macrocyclic complexes which may be applied in the development of novel antimicrobial and anticancer agents.

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Scheme 1 Formation and suggested structure of tetraiminetetraamide ligand and their complexes of the type $[MLX_2]$ and $[CuL]X_2$ [M = Mn(II), Co(II), Ni(II) and Zn(II) ; X = Cl or NO_3].

Table Caption

Table 1. Elemental analyses, m/z values, colors, yields, molar conductance, melting points and magnetic moments ($\mu_{eff.}$) of the complexes.

Table 2. IR spectral data of the complexes (cm^{-1}).

Table 3. Electronic spectral bands (cm^{-1}) with their assignments and EPR data of the complexes.

Table 4. MIC and MBC results of ligand and its complexes with positive control ciprofloxacin.

Table 5. The IC_{50} values ($\mu mol\ L^{-1}$) of the macrocyclic ligand and its complexes.

Figure caption

Figure 1. Mass spectra of (a) ligand and their complexes: (b) $[CoL(NO_3)_2]$ and (c) $[CuL](NO_3)_2$

Figure 2. ^{1}H- NMR spectra of (a) DPEDDH, (b) $[ZnL(NO_3)_2]$ and (c) $[ZnLCl_2]$.

Figure 3. ^{13}C- NMR spectra of mononuclear complexes: (a) Ligand (L), (b) $[ZnL(NO_3)_2]$ and (c) $[ZnLCl_2]$.

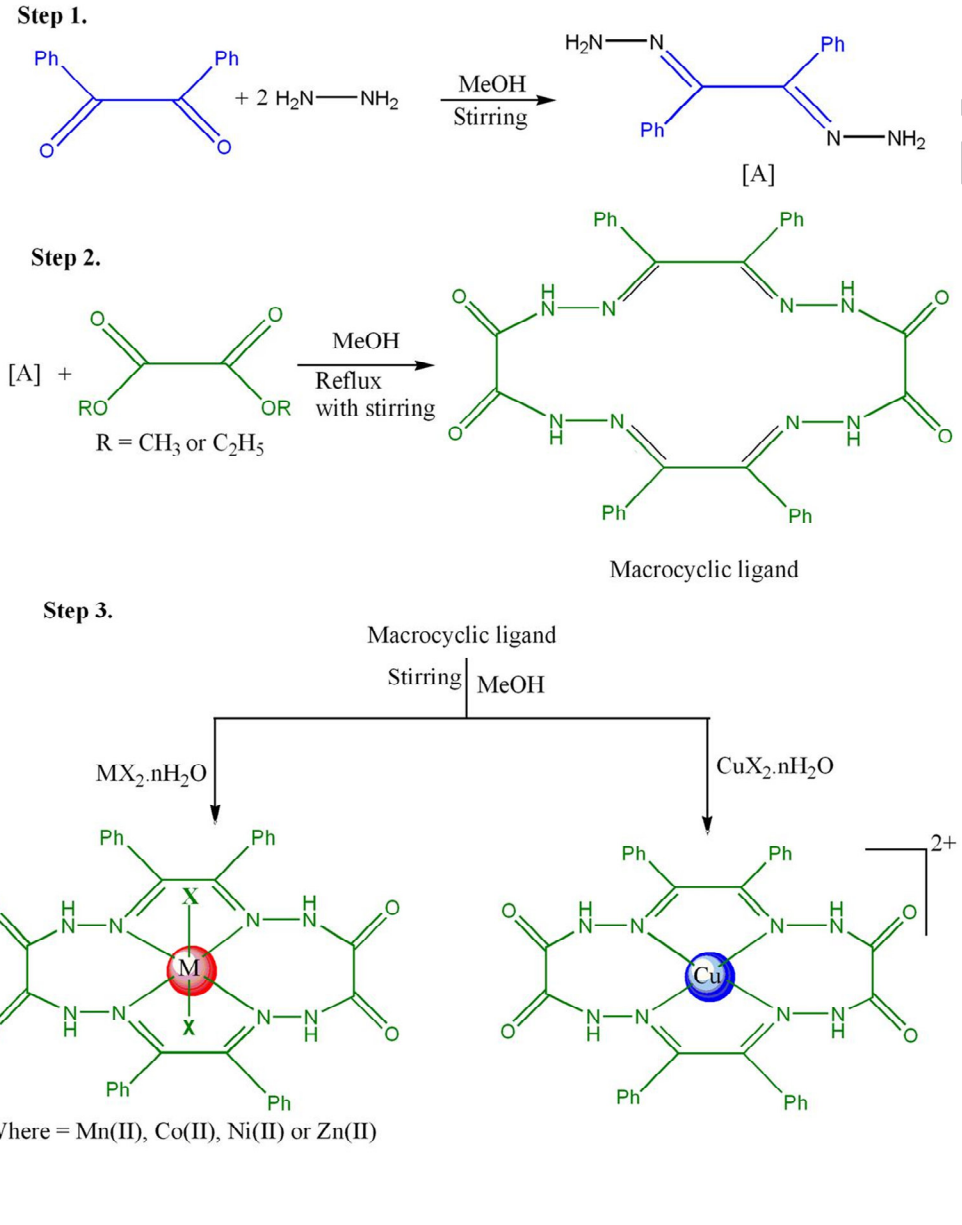
Figure 4. (a) EPR spectra of the $[CuL](NO_3)_2$ complex at room temperature; (b) EPR spectra of the $[CuL]Cl_2$ complex at room temperature.

Figure 5. TGA/DTA of the mononuclear complexes: (a) $[MnL(NO_3)_2]$, (b) $[CoLCl_2]$ and (c) $[CuL]Cl_2$.

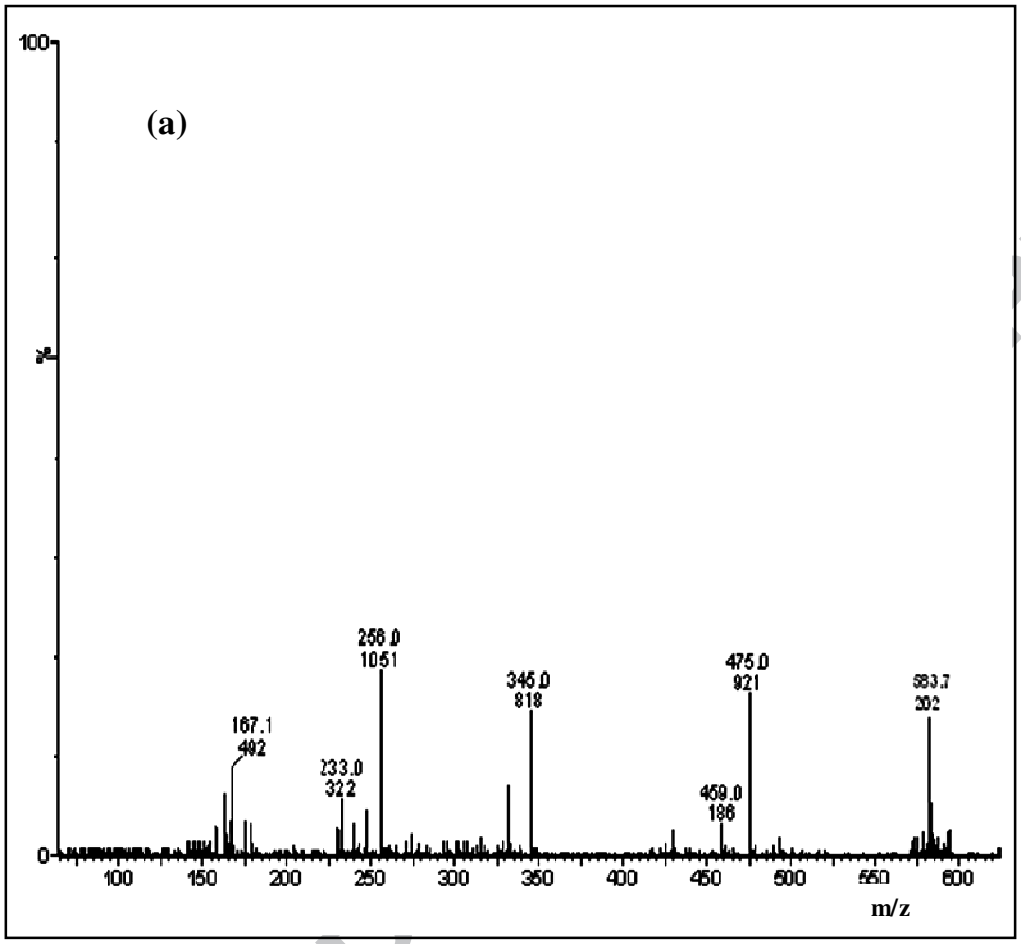
Figure 6. Zone of inhibition (in mm) of ligand and its complexes tested against *S. aureus and E. coli.*

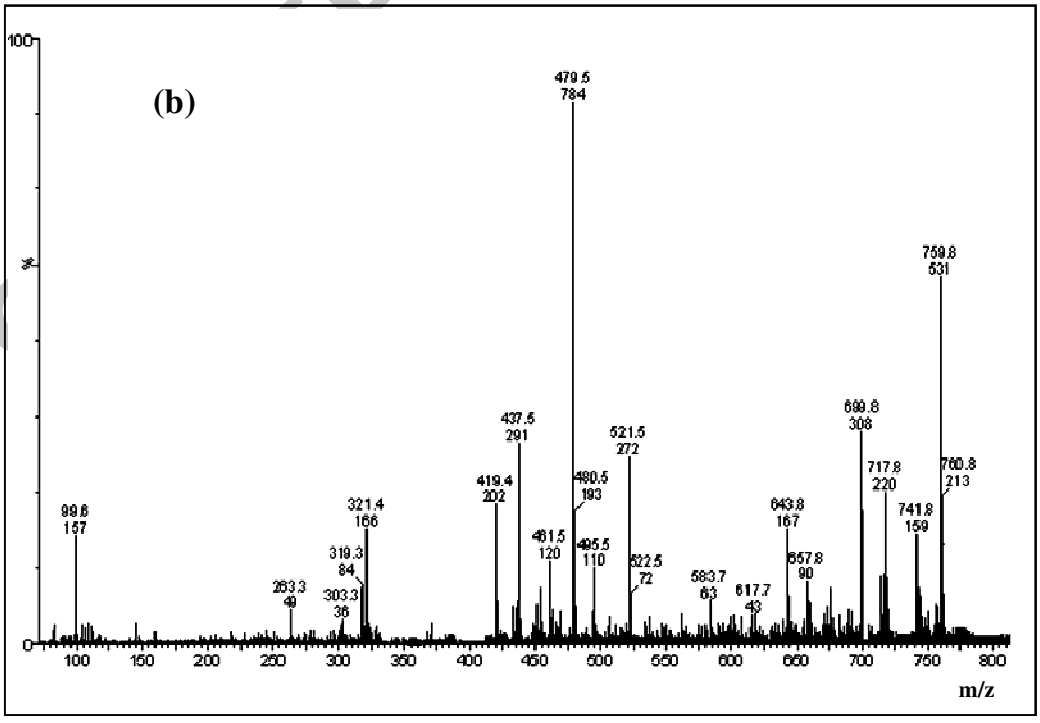
Figure 7 Antibacterial activity of ligand (a) (L), and its complexes (b) $[MnLCl_2]$, (c) $[CoLCl_2]$, (d) $[NiL(NO_3)_2]$ and (e) $[CuL]Cl_2$ shown by well-diffusion method.

Figure 8 Dose-dependent effects of (a) Ligand (L), (b) $[MnLCl_2]$, (c) $[CoLCl_2]$, (d) $[NiLCl_2]$, and (e) $[CuL]Cl_2$ complexes on different human cancer cell lines i.e., HeLa, MCF7 and Hep3B.



Scheme 1.





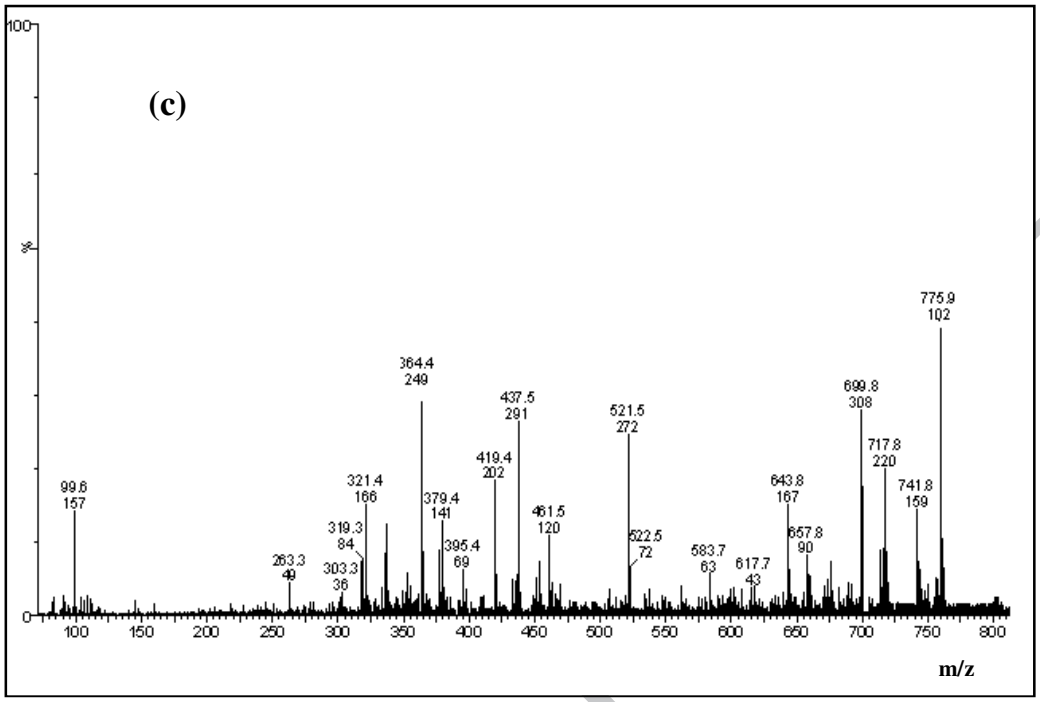
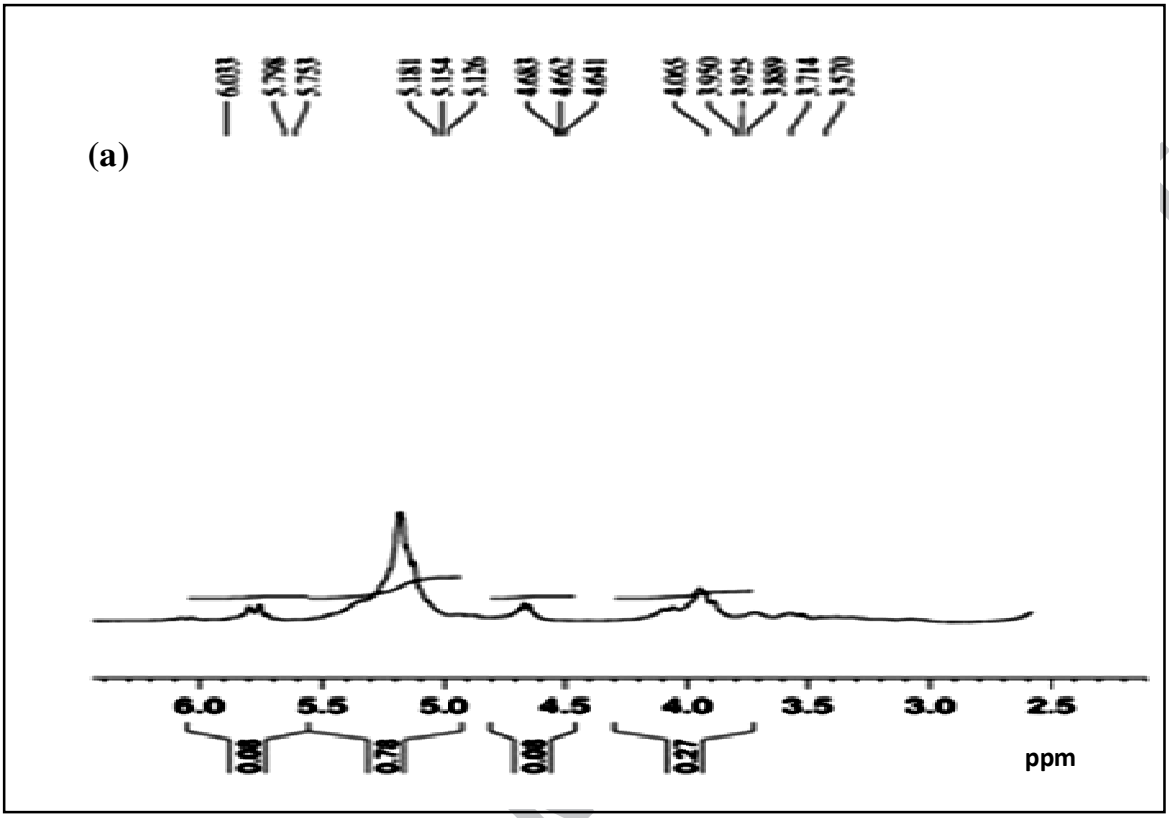


Figure 1. Mass spectra of (a) ligand and their complexes: (b) $[CoL(NO_3)_2]$ and (c) $[CuL](NO_3)_2$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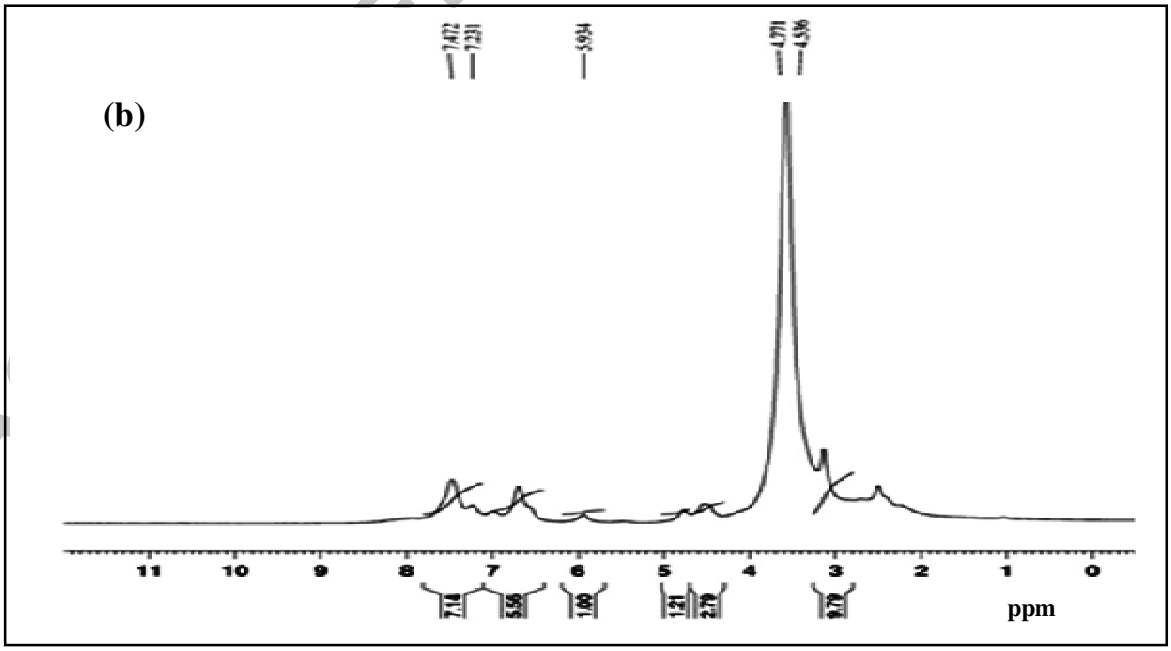
5.5
5.0
4.5
4.0
3.5
3.0
2.5
ppm


(b)
11
10
9
8
7
6
5
4
3
2
1
0
ppm

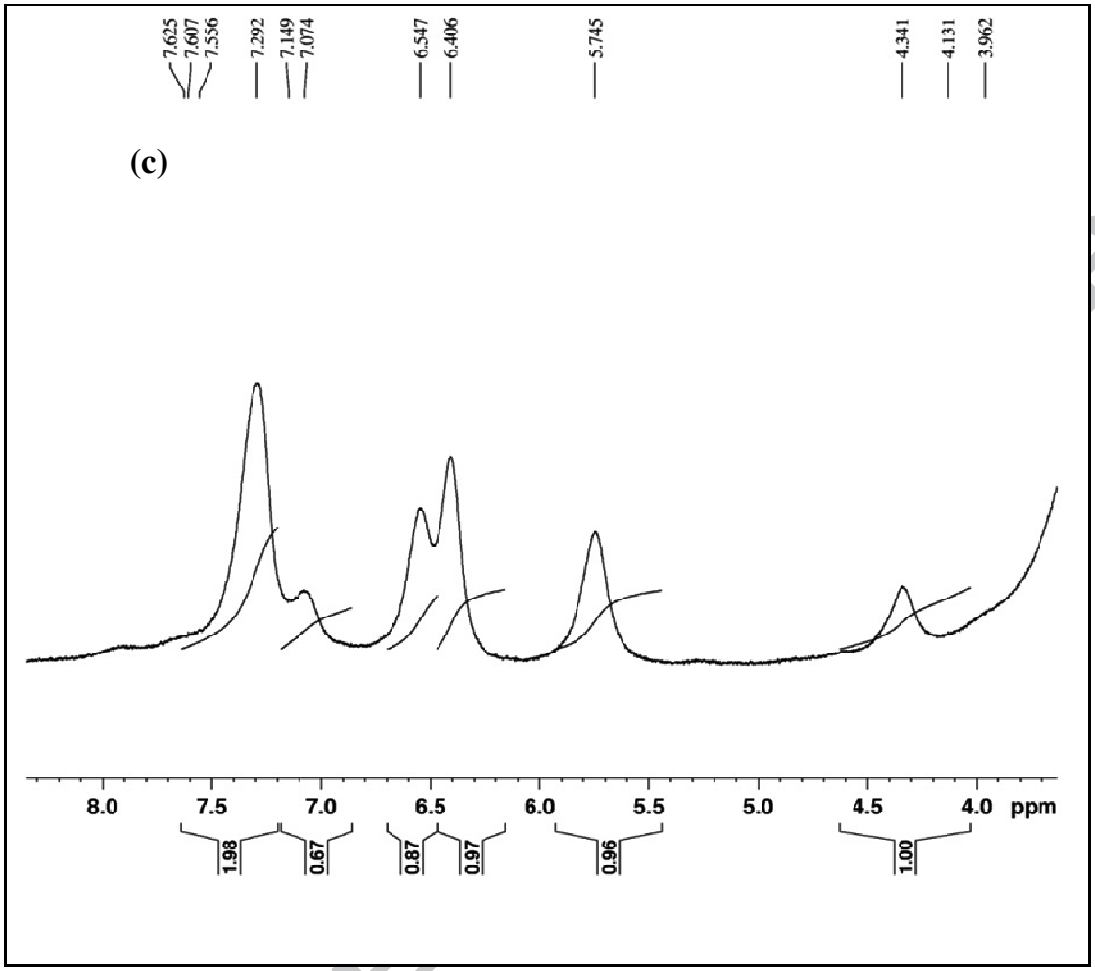
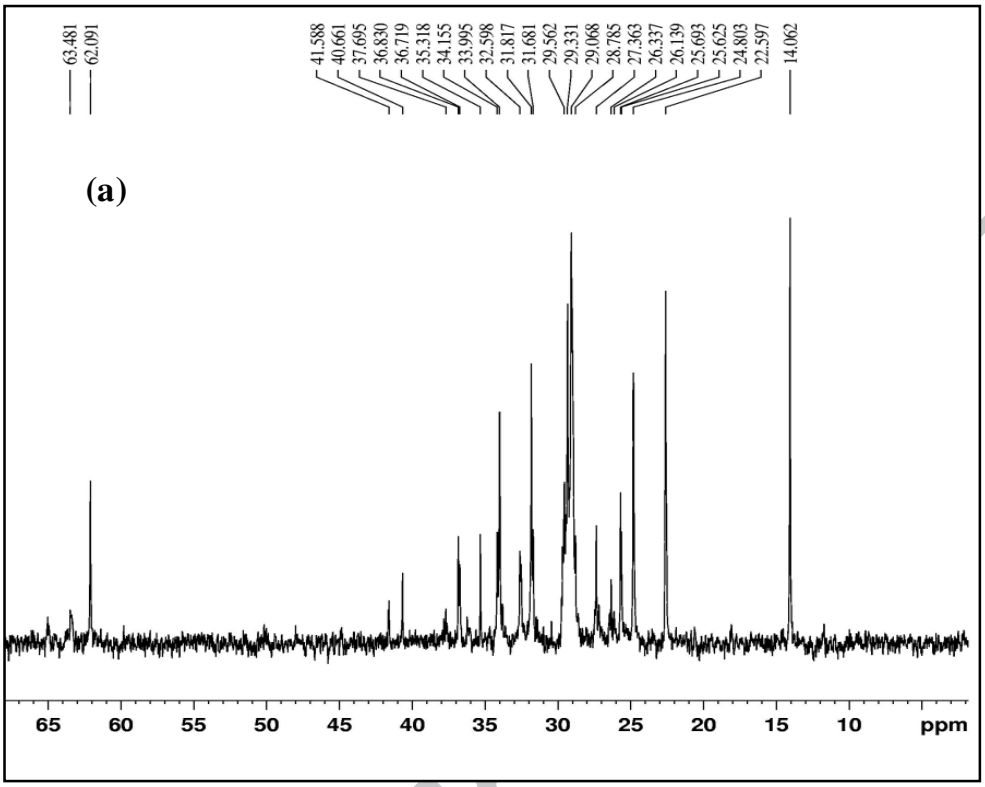


Figure 2. ^{1}H- NMR spectra of (a) DPEDDH mononuclear complexes, (b)[$ZnL(NO_3)_2$] and (c) [$ZnLCl_2$].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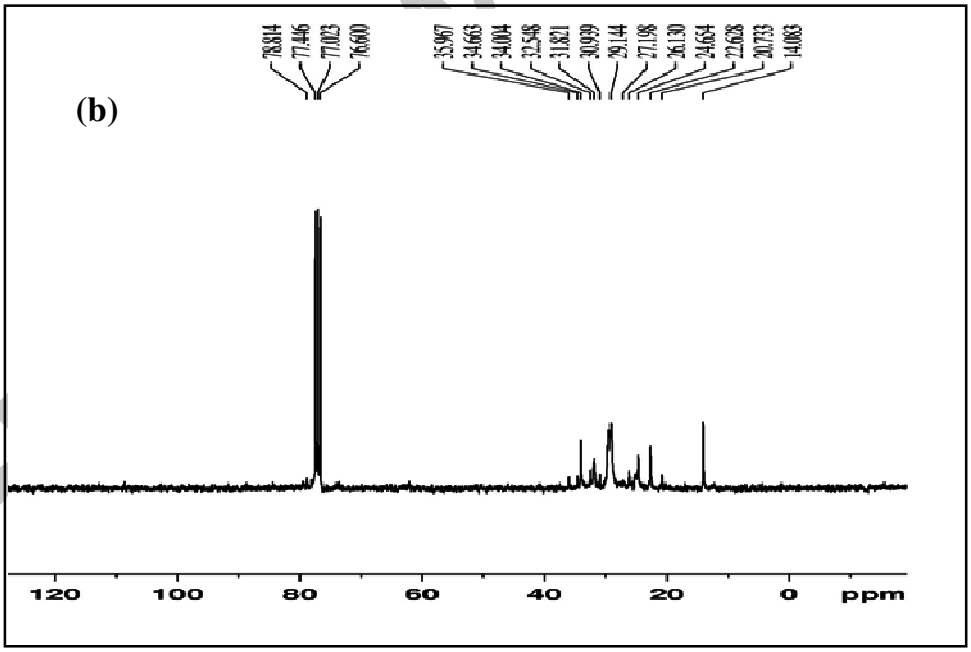
41.588
40.661
37.695
36.830
36.719
35.318
34.155
33.995
32.598
31.817
31.681
29.562
29.331
29.068
28.785
27.363
26.337
26.139
25.693
25.625
24.803
22.597
14.062
(a)
65
60
55
50
45
40
35
30
25
20
15
10
ppm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76.600
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34.004
32.548
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30.959
29.144
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22.628
20.733
14.083
(b)
120
100
80
60
40
20
0
ppm

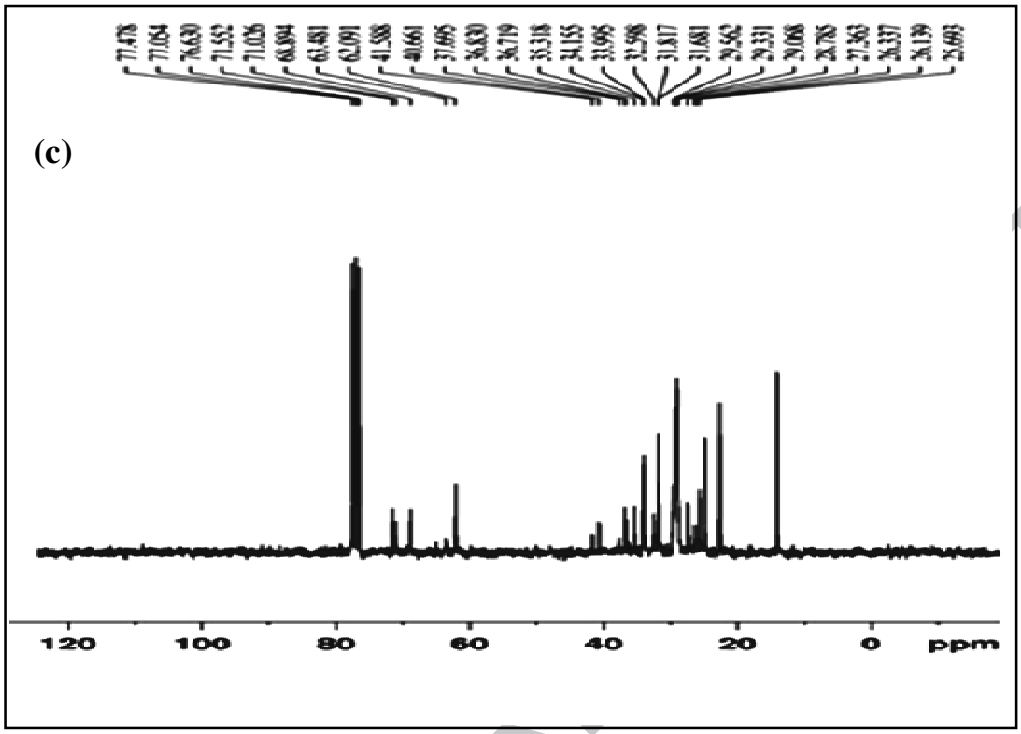


Figure 3. ^{13}C-NMR spectra of mononuclear complexes: (a) Ligand (L), (b) [ZnL(NO$_3$)$_2$]and (c) [ZnLCl$_2$].

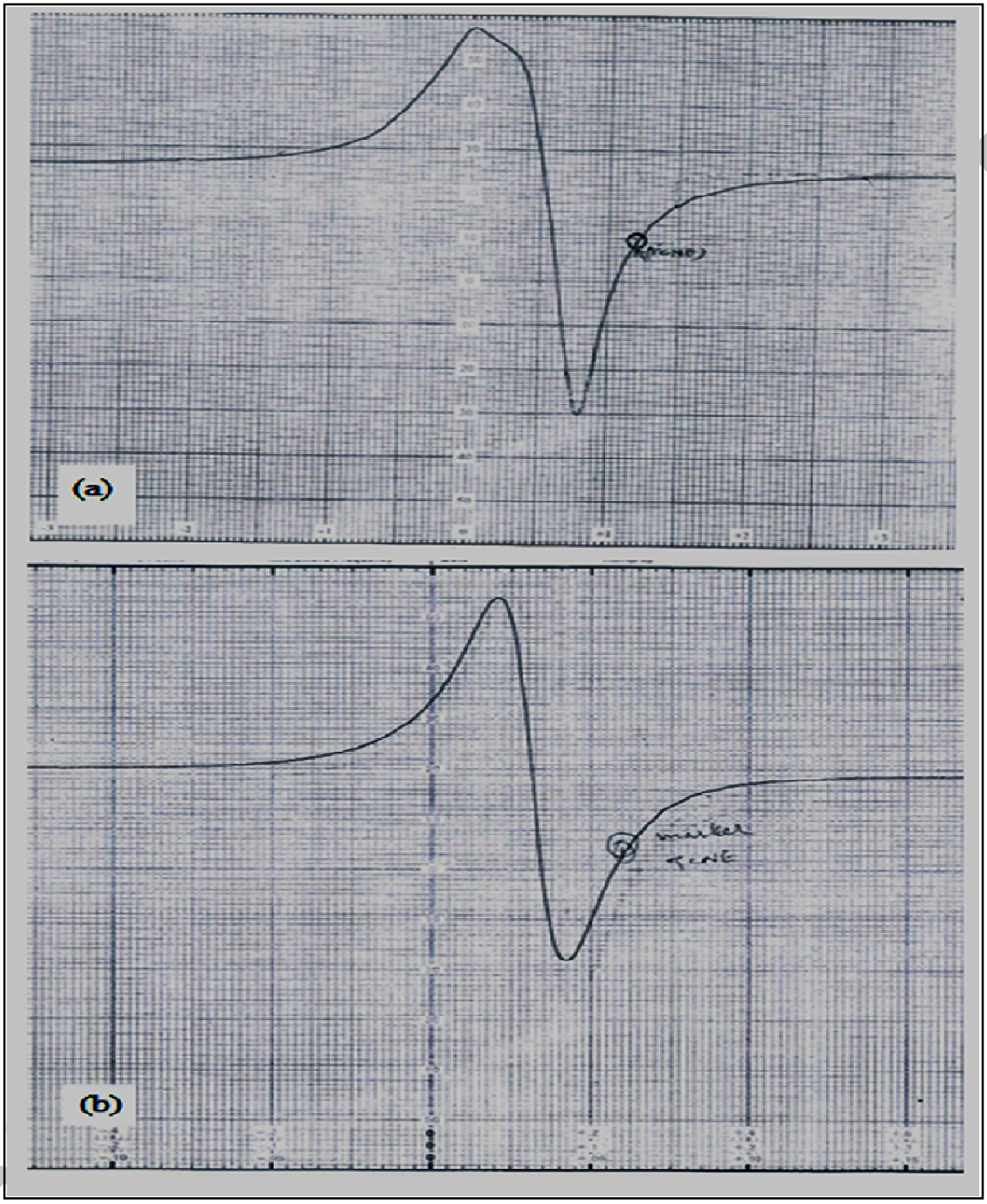


Figure 4. (a) EPR spectra of the $[CuL](NO_3)_2$ complex at room temperature; (b) EPR spectra of the $[CuL]Cl_2$ complex at room temperature.

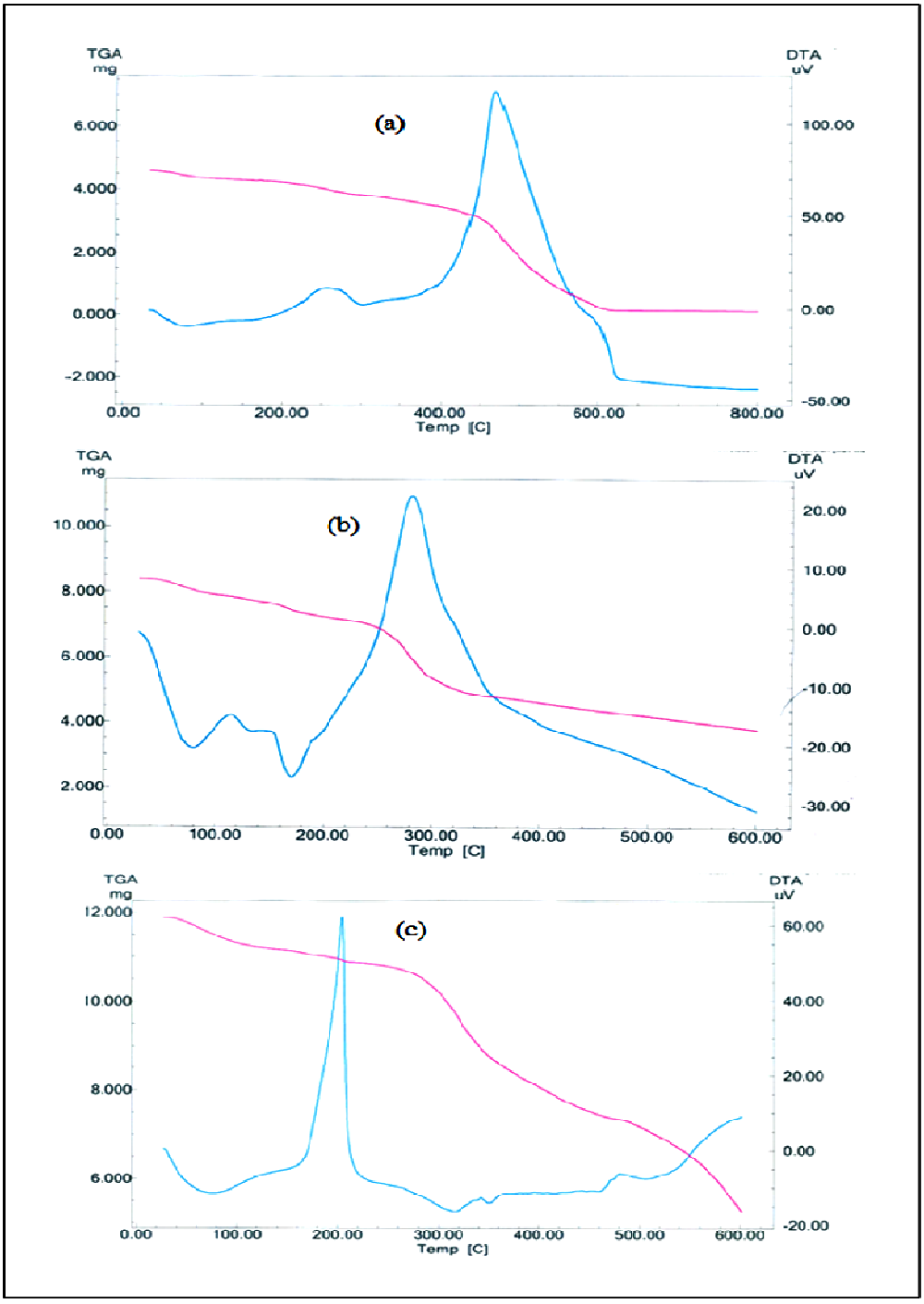


Figure 5. TGA/DTA of the mononuclear complexes; (a) $[MnL(NO_3)_2]$, (b) $[CoLCl_2]$ and $[CuL]Cl_2$

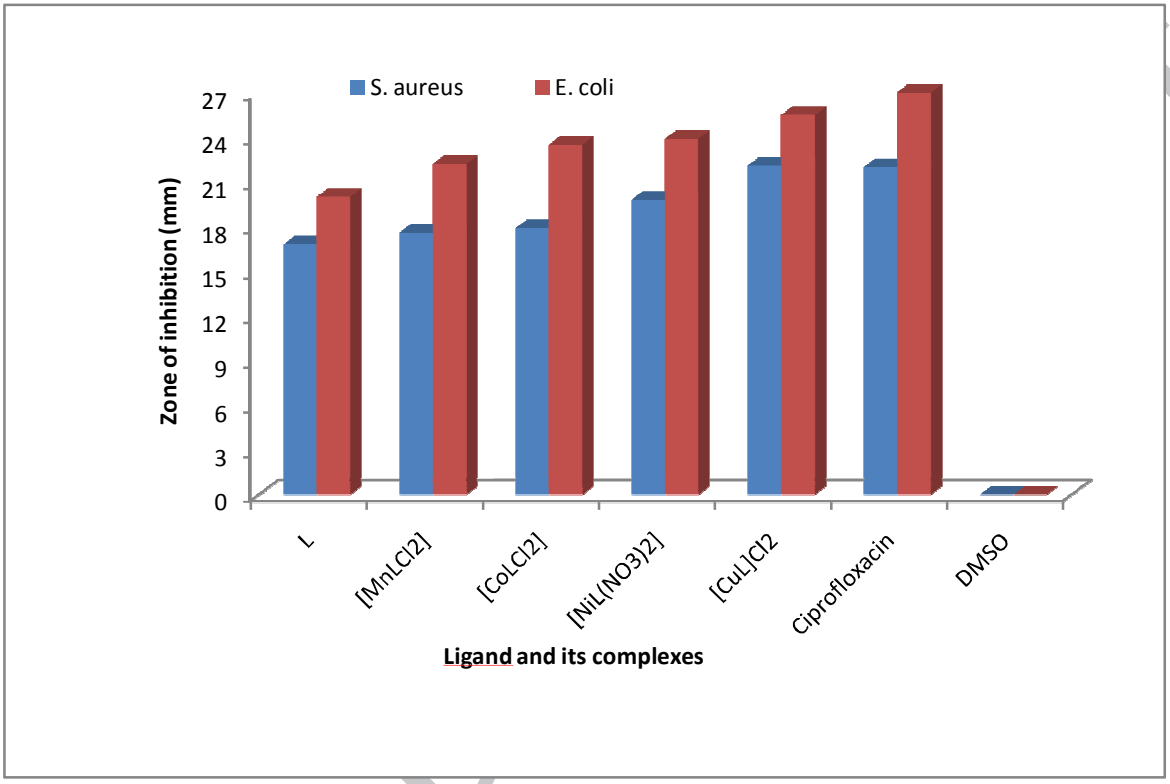


Figure 6. Zone of inhibition (in mm) of ligand and its complexes tested against *S. aureus* and *E. coli.*

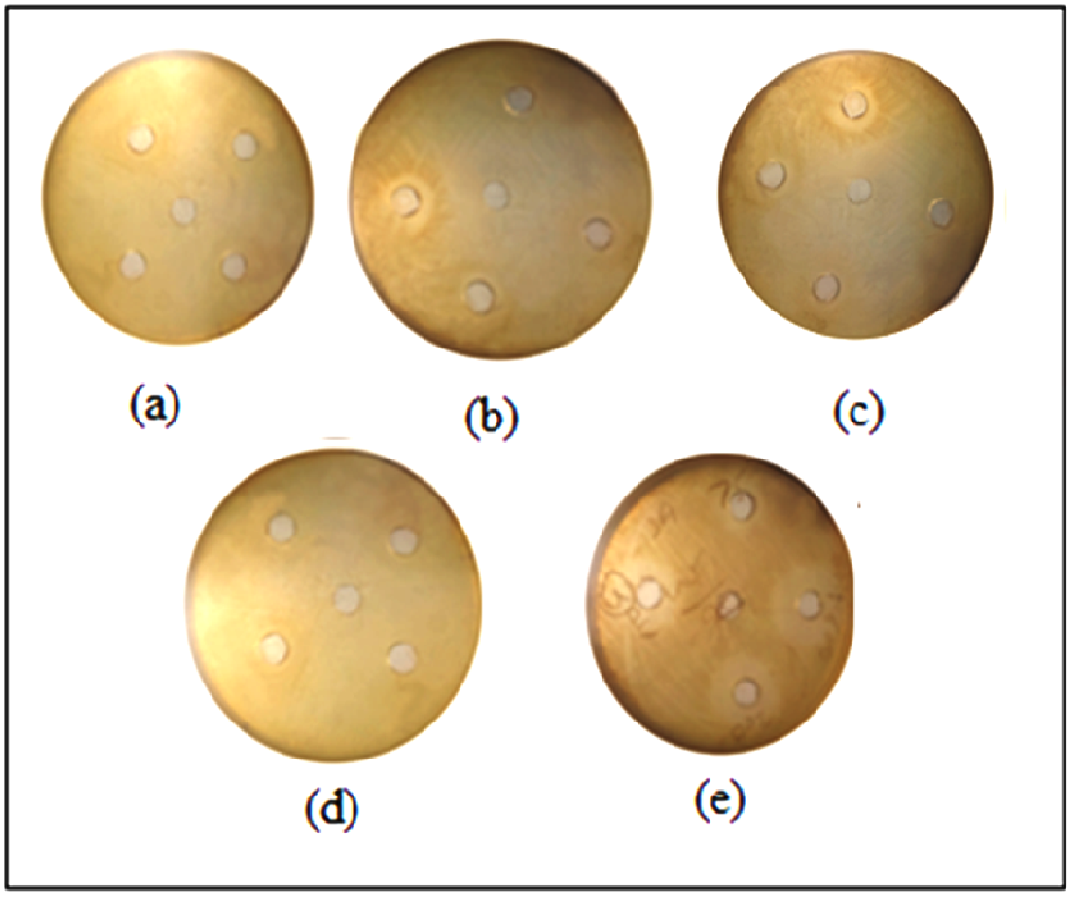


Figure7. Antibacterial activity of ligand (a) (L), and its complexes (b) $[MnLCl_2]$, (c) $[CoLCl_2]$, (d) $[NiL(NO_3)_2]$ and (e) $[CuL]Cl_2$ shown by well-diffusion method.

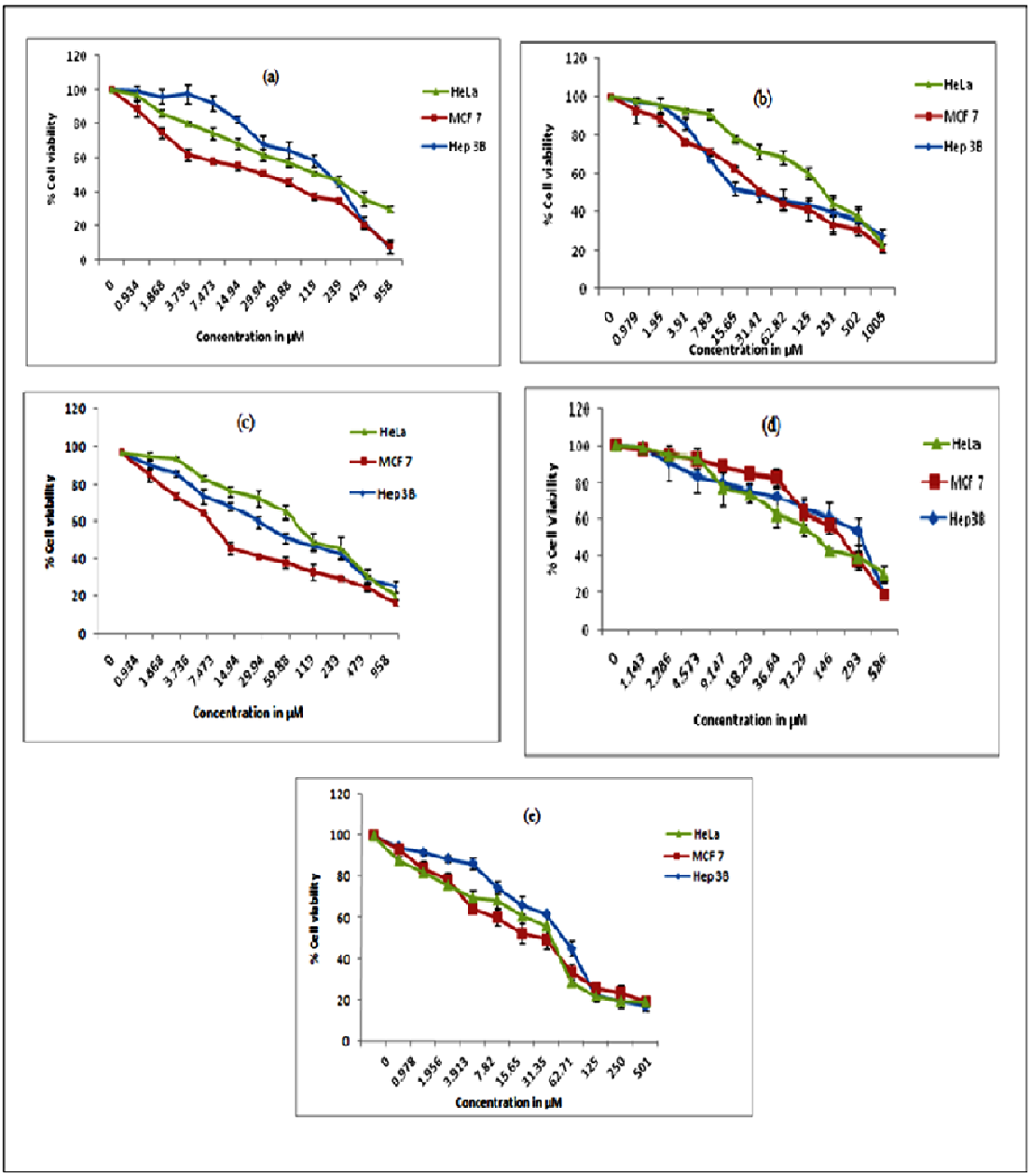


Figure 8. Dose-dependent effects of (a) Ligand (L), (b) [$MnLCl_2$], (c) [$CoLCl_2$], (d) [$NiLCl_2$] and (e) [$CuL]Cl_2$ complexes on different human cancer cell lines i.e., HeLa, MCF7 and Hep3B cell line.

Table 1. Elemental analyses, m/z values, Colors, yields, molar conductance, melting points and magnetic moments ($\mu_{eff.}$) of the complexes.

Complexes	m/z Found (calc.)	Color	Found (calc.) % M	C	H	N	Molar Conductivity ($ohm^{-1}cm^2\ mol^{-1}$) /m.p.(°C)	$\mu_{eff.}$ (BM)
DPEDDH $C_{14}H_{14}N_4$	240.00 (238.29)	White	-	69.00 (70.50)	6.70 (5.20)	23.60 (23.51)	- /170	-
Ligand $C_{32}H_{24}N_8O_4$	583.70 (584.59)	Light Yellow	-	53.20 (50.33)	4.50 (3.16)	19.90 (18.34)	- /180	-
$[MnL(NO_3)_2]$ $C_{32}H_{24}N_{10}MnO_{10}$	765.20 (763.54)	Pink	7.20 (7.35)	47.20 (50.0)	3.20 (3.60)	17.20 (18.25)	22.3/190	5.76
$[MnLCl_2]$ $C_{32}H_{24}N_8O_4MnCl_2$	717.10 (711.08)	Light pink	7.92 (7.72)	50.00 (54.04)	3.10 (3.40)	14.00 (15.84)	19.6/193	5.71
$[CoL(NO_3)_2]$ $C_{32}H_{24}N_{10}CoO_{10}$	759.80 (767.54)	Dark pink	6.90 (7.67)	49.2 (50.0)	3.20 (3.15)	17.90 (18.25)	20.1/200	4.66
$[CoLCl_2]$ $C_{32}H_{24}N_8O_4CoCl_2$	712.20 (714.93)	Light pink	8.30 (8.24)	(53.70 (53.75)	3.20 (3.38)	15.20 (15.75)	18.4/220	4.58
$[NiL(NO_3)_2]$ $C_{32}H_{24}N_{10}NiO_{10}$	770.10 (767.29)	Green	7.60 (7.64)	50.09 (50.08)	3.12 (3.15)	18.20 (18.25)	20.6/210	3.18
$[NiLCl_2]$ $C_{32}H_{24}N_8O_4NiCl_2$	720.10 (714.84)	Light green	7.71 (8.21)	52.76 (53.76)	3.67 (3.38)	14.90 (15.70)	21.2/212	3.13
$[CuL](NO_3)_2$ $C_{32}H_{24}N_{10}CuO_{10}$	775.90 (772.15)	Brown	8.29 (8.22)	48.77 (49.77)	2.90 (3.13)	17.70 (18.14)	110/220	1.92
$[CuL]Cl_2$ $C_{32}H_{24}N_8O_4CuCl_2$	715.20 (719.65)	Dark Brown	7.99 (8.83)	49.20 (53.40)	3.29 (3.36)	18.00 (15.65)	120/225	1.87
$[ZnL(NO_3)_2]$ $C_{32}H_{24}N_8ZnO_{10}$	769.50 (774.02)	Off White	7.20 (7.61)	45.25 (49.65)	2.90 (3.12)	17.22 (18.10)	20.0/195	-
$[ZnLCl_2]$ $C_{32}H_{24}N_8O_4ZnCl_2$	725.70 (721.41)	Off white	8.90 (9.06)	49.0 (52.99)	3.24 (3.35)	16.00 (15.61)	19.9/199	-

*DPEDDH = 1,2-diphenylethane-1,2-dione dihydrazon.

Table 2. IR spectral data of the complexes (cm^{-1}).

Complexes	υ(NH_2 / NH)	Amide bands				υ(C = N)	υ(M-N)
		I	II	III	IV		
DPEDDH	3270 3220	-	-	-	-	1615	-
Ligand	3230	1670	1520	1230	640	1600	-
$[MnL(NO_3)_2]$	3250	1690	1530	1240	650	1595	440
$[MnCl_2]$	3270	1680	1510	1255	670	1580	425
$[CoL(NO_3)_2]$	3275	1710	1520	1245	640	1585	460
$[CoLCl_2]$	3275	1695	1525	1230	635	1590	470
$[NiL(NO_3)_2]$	3265	1700	1540	1235	665	1595	450
$[NiLCl_2]$	3240	1715	1530	1250	650	1585	435
$[CuL](NO_3)_2$	3250	1695	1545	1250	670	1580	455
$[CuL]Cl_2$	3245	1680	1550	1245	655	1590	450
$[ZnL(NO_3)_2]$	3250	1685	1535	1230	655	1595	450
$[ZnLCl_2]$	3265	1690	1520	1225	635	1585	445

Table 3. Electronic spectral bands (cm^{-1}) with their assignments and EPR data of the complexes.

Complexes	Electronic Spectra (cm^{-1})	Assignments	EPR Parameters		
			$g_{\parallel}$	$g_{\perp}$	G
$[MnL(NO_3)_2]$	18,650	$^6A_{1g} \rightarrow {}^4T_{1g}$ (P)			
	22,550	$^6A_{1g} \rightarrow {}^4T_{2g}$ (F)	-	-	-
$[MnCl_2]$	18,850	$^6A_{1g} \rightarrow {}^4T_{1g}$ (P)			
	22,400	$^6A_{1g} \rightarrow {}^4T_{2g}$ (F)	-	-	-
$[CoL(NO_3)_2]$	16,200	$^4T_{1g}$ (F) $\rightarrow {}^4T_{1g}$ (P)			
	21,400	$^4T_{1g}$ (F) $\rightarrow {}^4T_{1g}$ (F)	-	-	-
$[CoLCl_2]$	15,950	$^4T_{1g}$ (F) $\rightarrow {}^4T_{1g}$ (P)			
	21,700	$^4T_{1g}$ (F) $\rightarrow {}^4A_{2g}$ (F)	-	-	-
$[NiL(NO_3)_2]$	18,200	$^3A_{2g}$(F) $\rightarrow {}^3T_{1g}$(F)			
	24,350	$^3A_{2g}$(F) $\rightarrow {}^3T_{1g}$(P)	-	-	-
$[NiLCl_2]$	18,750	$^3A_{2g}$(F) $\rightarrow {}^3T_{1g}$(F)			
	21,200	$^3A_{2g}$(F) $\rightarrow {}^3T_{1g}$(P)	-	-	-
$[CuL](NO_3)_2$	11,750	$^2B_{1g} \rightarrow {}^2B_{2g}$			
	16,050	$^2B_{1g} \rightarrow {}^2A_{1g}$	2.1782	2.0872	1.940
	21,500	$^2B_{1g} \rightarrow {}^2E_g$			
$[CuL]Cl_2$	12,450	$^2B_{1g} \rightarrow {}^2B_{2g}$	2.0925	2.0526	1.758
	16,000	$^2B_{1g} \rightarrow {}^2A_{1g}$			
	21,750	$^2B_{1g} \rightarrow {}^2E_g$			

Table 4. MIC and MBC results of ligand and its complexes with positive control ciprofloxacin.

Complexes	Gram positive bacteria		Gram negative bacteria	
	S. aureus		*E. coli*	
	MIC	MBC	MIC	MBC
L	>100	100	>100	100
$[MnLCl_2]$	>100	>100	100	>100
$[CoLCl_2]$	25	50	25	100
$[NiL(NO_3)_2]$	50	>100	100	>100
$[CuL]Cl_2$	>25	50	50	>50
Standard ciprofloxacin	6.25	12.5	6.25	12.5

MIC (µg/ml) = minimum inhibitory concentration, i.e. the lowest concentration of the compound to inhibit the growth of bacteria completely; MBC (µg/ml) = minimum bacterial concentration, i.e., the lowest concentration of the compound for killing the bacteria complete

Table 5. The IC_{50} values (μmol L^{-1}) of the macrocyclic ligand and its complexes.

Complexes	HeLa	MCF7	Hep3B
Ligand	20.23±2.6	19.40±1.9	21.21±1.4
$[MnLCl_2]$	18.23±2.6	18.56±1.5	17.16±1.8
$[CoLCl_2]$	13.78±2.3	15.70±1.3	13.20±2.7
$[NiLCl_2]$	13.60±1.7	16.74±2.8	13.60±1.7
$[CuL]Cl_2$	12.70±2.5	11.50±2.5	12.53±1.9
(Doxo)*	4.10±0.7	2.90±2.5	2.30±0.6
5-FU*	6.20±0.8	4.80±1.7	4.6±1.2

* Doxorubicin (Doxo) and 5 – Fluorouracil (5-FU) are drugs of reference

Fig. 2

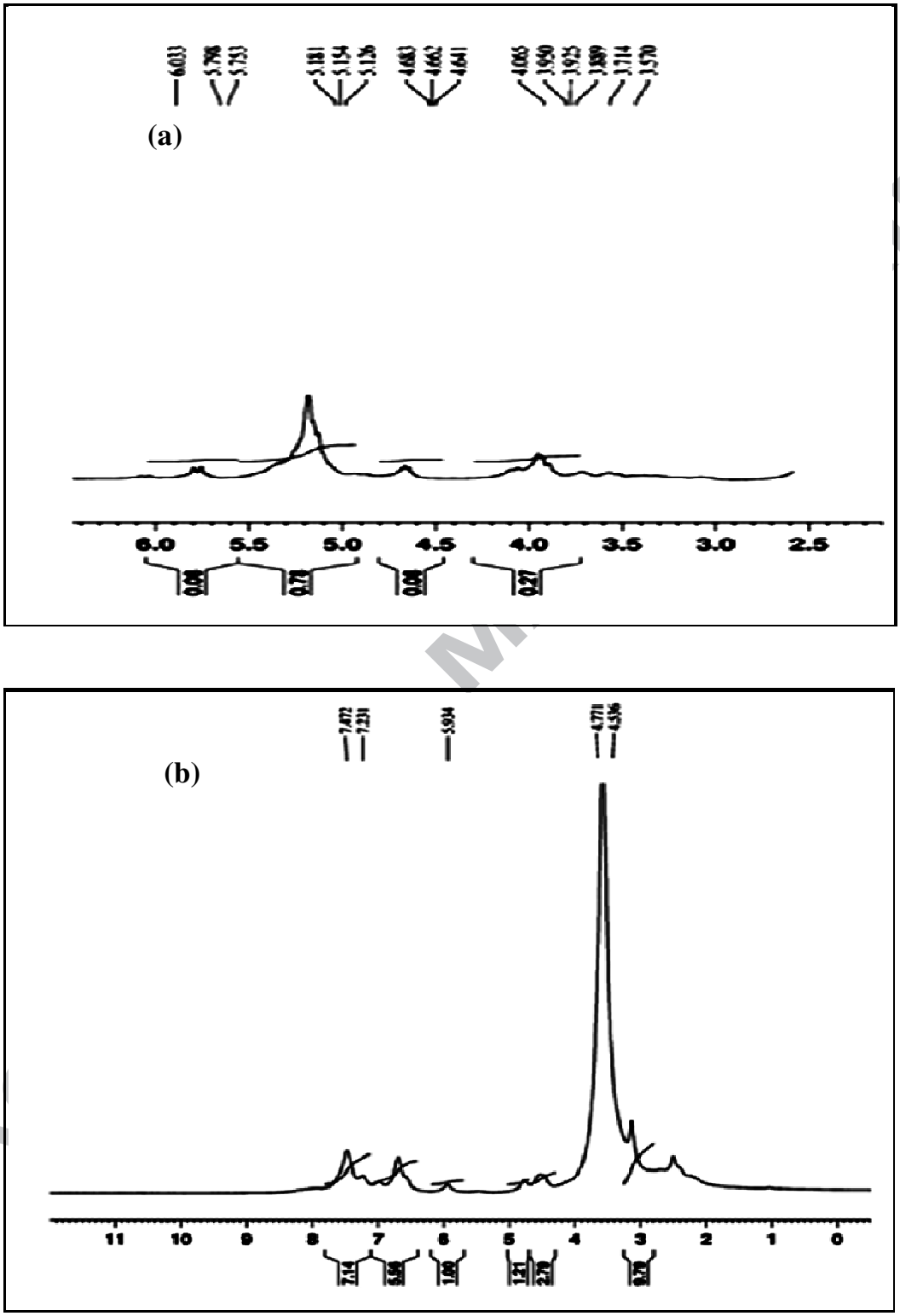


Figure 2. (a) and (b)

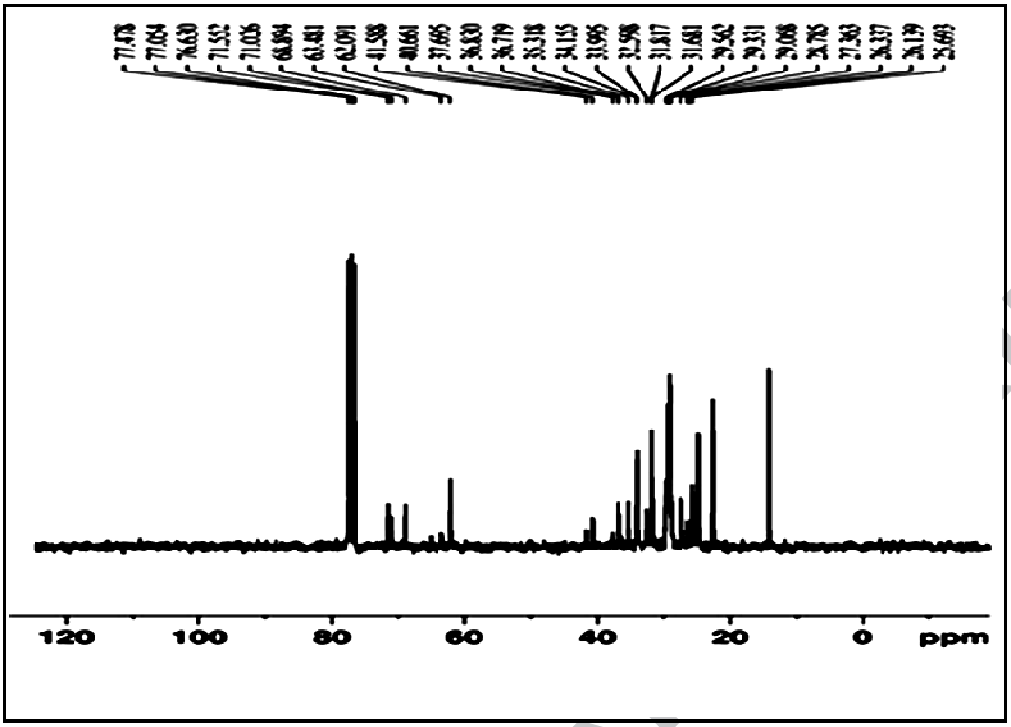
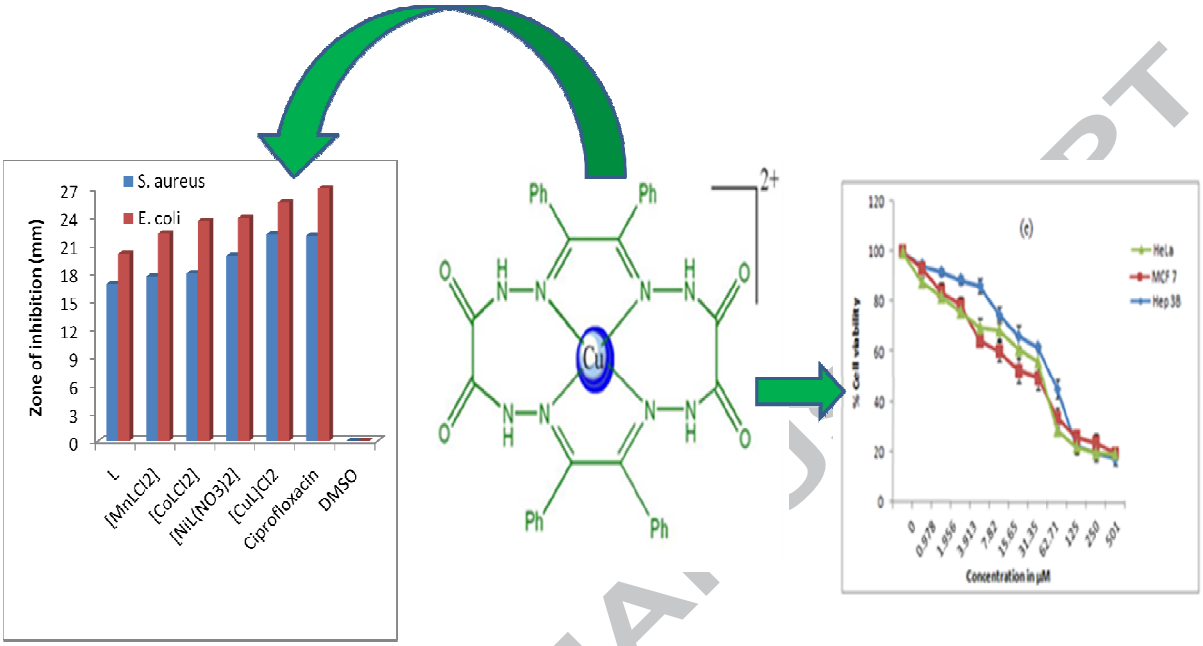


Figure 3. (c)

HIGHLIGHTS

- 16-membered octaazamacrocyclic complexes
- Octahedral and square planar geometry around the metal ions
- Antibacterial study by disc diffusion method
- Anticancer activity by using MTT assay

Graphical Abstract



- ***Synthesize macrocyclic copper complex (in middle), dose response curve for cancer cell lines (right) and antibacterial activity graph (left)***