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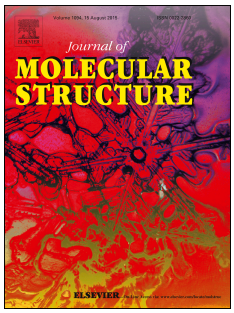

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Synthesis, characterization and biological studies of copper(II) complexes with 2-aminobenzimidazole derivatives

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Abstract

Novel series of four copper(II) complexes with 2-aminobenzimidazole derivatives (obtained from the Knoevenagel condensate of acetylacetone (obtained from acetylacetone and halogen substituted benzaldehydes) and 2-aminobenzimidazole) were synthesized. They were structurally characterized using elemental analysis, molar conductance, FAB mass, FT- IR, ^{1}H &^{13}C-NMR, UV–Vis., and EPR techniques. On the basis of analytical and spectral studies, the distorted square planar geometry was assigned for all the complexes. The antibacterial screening of the ligands and their copper complexes indicated that all the complexes showed higher anti microbial activities than the free ligands. Superoxide dismutase and antioxidant activities of the copper complexes have also been performed. In the electrochemical technique, the shift in ΔE_p, $E_{1/2}$ and Ipc values were explored for the interaction of the complexes with CT-DNA. During the electrolysis process, the present ligand system stabilizes unusual oxidation state of copper in the complexes. It is believed that the copper complexes with curcumin analogs may enhance chemotherapeutic behavior.

Keywords: DNA binding; Antioxidant; Complexes; Schiff base; Antimicrobial.

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1. Introduction

β-diketones have gained a lot of interest due to their potential importance as versatile ligands for chelation with different metals and also as intermediate in the core of heterocyclic systems such as flavones, benzodiazepine, pyrazole, isoxazole and pyrimidine. They are well known molecule exhibited keto-enol tautomerism [1-9]. Transition metal complexes containing β-diketone scaffolds are commonly found in biological systems and also play an important role in biochemical processes such as catalysis of drug interaction with bio molecules. Metal complexes with β-diketone derivatives were synthesized and showed broad spectrum of biological activities. Recently, the Cu(II), Ni(II), Co(II) and Zn(II) complexes were exhibited broad spectrum of biological and physic chemical properties and motivate researchers in the field of coordination chemistry of bio essential metal ions [10-14]. Many biologically active compounds were used as drugs possess modified pharmacological and toxicological potentials when administered in the form of metal-based compounds [15-16]. The structural modulation and coordination of ligands with different metal ions may significantly vary the pharmacological activities. In the present study, we have also decided to synthesize a series of novel *β*-diketone derivatives and copper complexes.

In view of the importance of metal complexes, the preparation, characterization and *in vitro* antibacterial and antifungal properties of copper complexes were performed. The newly synthesized β-diketone and their metal complexes were characterized by IR, ^{1}H NMR, ^{13}C NMR, molar conductance, magnetic moments, electronic and elemental analyses data. The synthesized ligands and their complexes have been screened for in vitro antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and antifungal activity .*In vitro* free radical scavenging activities of the ligands (L^1–L^4) and copper chelates were evaluated by DNA binding and anti oxidant assay methods [17-22].

2. Experimental

All chemicals and solvents were reagent grade and were purchased from Merck. All supporting electrolyte solutions were prepared using analytical grade reagents and doubly distilled water. Calf thymus DNA purchased from Genei Biolab, Bangalore, India.

Instrumentation

Elemental analysis of ligands and their copper complexes were carried out using Perkin-Elmer elemental analyzer. Molar conductance of the complexes was measured using a coronation digital conductivity meter. The ^{1}H & ^{13}C-NMR spectra of the ligands were recorded using TMS as internal standard. Chemical shifts are expressed in units of parts per million relative to TMS. The IR spectra of the ligands and their copper complexes were recorded on a Perkin-Elmer 783 spectrophotometer in 4000-200 cm^{-1} range using KBr disc. Electronic spectra were recorded in a Systronics 2201 Double beam UV-Vis., spectrophotometer within the range of 200-1100 nm region. Magnetic moments were measured by Guoy method and corrected for diamagnetism of the component using Pascal's constants. Cyclic voltammetry was performed on a CHI 604D electrochemical analyzer with three electrode system of glassy carbon as the working electrode, a platinum wire as auxiliary electrode and Ag/AgCl as the reference electrode. Tetrabutylammoniumperchlorate (TBAP) was used as the supporting electrolyte. Solutions were deoxygenated by eradication with N_2 previous to measurements. The interactions between metal complexes and DNA were studied using electrochemical and electronic absorption techniques. The FAB-mass spectra of ligands and their copper complexes were recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using Argon/Xenon (6 kV, 10 A) as the FAB gas. The Thermogravimetric analyses data were measured from 0ºC to 1000ºC temperature at a heating rate of 10ºC/min. The data were obtained by using a Perkin Elmer Diamond TG/DTA instrument.

Synthesis of ligands and their copper complexes

Synthesis of Knoevenagel condensate β-diketones

A series of Knoevenagel condensate of β-diketone with different substituted aromatic aldehydes [L^1/ 3-bromobenzaldehyde, L^2/3-fluorobenzaldehyde, L^3/3-chlorobenzaldehyde, L^4/3-cyanobenzaldehyde] was refluxed in the presence of potassium carbonate as catalyst in the ethanolic medium. The progress of reaction was monitored by TLC technique. After completion of reaction, the reaction mixture was poured into crushed ice. The yellow coloured knoevenagel condensate of β–diketone(s) was obtained. It was filtered, washed with ice cold water and dried *in vacuum*.

Synthesis of Schiff base ligands

Hot ethanolic solutions of 2-aminobenzimidozole (2 M) was added dropwise to Knoevenagel condensate of β-diketone (s) (1 M) was refluxed for 12 hrs in the presence of potassium carbonate as catalyst. The progress of reaction was monitored by TLC. After completion of reaction the solid material was removed by filtration and recrystallized.

Synthesis of copper complexes

Equimolar hot Ethanolic solutions of 2-aminobenzimidozole derivatives and copper acetate were refluxed for 6 hrs. The progress of reaction was monitored by TLC. Then, it was poured into crushed ice. The solid material was removed by filtration and recrystallized.

DNA Binding Studies

The binding interactions between metal complexes and DNA were studied using electrochemical and electronic absorption methods by using different concentrations of CT-DNA. Solutions of CT DNA in 50 mM NaCl/50 mM Tris-HCl (pH 7.2) gave the ratio of the UV absorbance at 260 and 280 nm, A260/A280, of ca. 1.8–1.9, indicating that the DNA was sufficiently free of protein contamination. The DNA concentration was determined by the UV absorbance at 260 nm after 1:100 dilution. The molar absorption coefficient was taken as 660 m 2 mol-1. Stock solutions were kept at 4 °C and used after not more than 4 days. Concentrated

stock solutions of the complexes were prepared by dissolving the complexes in DMSO and diluting suitably with the corresponding buffer to the required concentration for all of the experiments.

Absorption titration experiment

Absorption titration experiment was performed by maintaining a constant concentration of the complex while varying the nucleic acid concentration. This was achieved by dissolving an appropriate amount of the copper complex stock solution and by mixing various amounts of DNA stock solutions while maintaining the total volume constant. This resulted in a series of solutions with varying concentrations of DNA but with a constant concentration of the complex. The absorbance (A) of the most red-shifted band of complex was recorded after each successive additions of CT DNA. The intrinsic binding constant, K_b, was determined from the plot of [DNA]/(e_a- e_f) vs [DNA], where [DNA] is the concentration of DNA in base pairs, ea, the apparent extinction coefficient which is obtained by calculating A_{obs}/[complex] and e_f corresponds to the extinction coefficient of the complex in its free form. The data were fitted to the following equation where e_b refers to the extinction coefficient of the complex in the fully bound form.

$$[DNA]/(e_a - e_f) = [DNA]/(e_b - e_f) + 1/K_b(e_b - e_f) \quad \text{----------} \quad (1)$$

Each set of data, when fitted to the above equation, gave a straight line with a slope of $1/(e_b - e_f)$ and a y-intercept of $1/K_b(e_b - e_f)$. K_b was determined from the ratio of the slope to intercept.

Antimicrobial activities

The *in vitro* antimicrobial activities of the investigated compounds were tested against the bacterial species and fungal species. One day prior to the experiment, the bacterial and fungal cultures were inoculated in broth (inoculation medium) and incubated overnight at 37°C. Inoculation medium containing 24 hr grown culture was added aseptically to the nutrient medium and mixed thoroughly to get the uniform distribution. This solution was poured (25 mL in each dish) into petri dishes and then allowed to attain room temperature. Wells (6 mm in diameter) were cut in the agar plates using proper sterile tubes. Then, wells were filled up to the

surface of agar with 0.1 mL of the test compounds dissolved in DMSO (200 mM/mL). The plates were allowed to stand for an hour in order to facilitate the diffusion of the drug solution. Then the plates were incubated at 37°C for 24 hr for bacteria and 48 hr for fungi and the diameter of the inhibition zones were read. Minimum inhibitory concentrations (MICs) were determined by using serial dilution method. The lowest concentration (mg/mL) of compound, which inhibits the growth of bacteria after 24 hr incubation at 37°C, and of fungi after 48 hr incubation at 37°C was taken as the MIC. The concentration of DMSO in the medium did not affect the growth of any of the microorganisms tested.

Superoxide dismutase activity (SOD)

The superoxide dismutase activity (SOD) of the copper complexes were evaluated using alkaline DMSO as source of superoxide radicals ($O_2^{\bullet-}$) generating system in association with nitro blue tetrazolium chloride (NBT) as a scavenger of superoxide. Add 2.1 mL of 0.2 M potassium phosphate buffer (pH 8.6) and 1 ml of 56 µL of NBT solutions to the different concentration of copper complex solution. The mixtures were kept in ice for 15 min and then 1.5 mL of alkaline DMSO solution was added while stirring. The absorbance was monitored at 540 nm against a sample prepared under similar condition except NaOH was absent in DMSO.

Antioxidant Assay

The free radical scavenging activity of the Schiff base ligand (L) and its Cu(II), Co(II), Ni(II) and Zn(II) complexes was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Different concentrations of test compounds (12.5, 25, 50 and 100 mg) and standard Vitamin C was taken in different test tubes and the volume of each test tube was adjusted to 100 µL by adding distilled DMF. To the tubes containing sample solutions in DMF, 5 mL methanolic solution of DPPH (0.1 mM) was added to these tubes. The tubes were allowed to stand for 30 min. The control experiment was carried out as above without the test samples. The absorbance of test solutions was measured at 517 nm. The reduction of DPPH was calculated relative to the measured absorbance of the control. The radical scavenging activity was calculated using the following formula:

% Scavenging of DPPH = [(Control OD – Sample OD) /Control OD] X 100 --------- (2)

3. Results and discussion

The analytical, physical properties and molar conductance data of the complexes are given in Table1. The Cu (II) complexes were dissolved in DMSO and the molar conductivities of 10^{-3} M of their solution at room temperature were measured. The lower conductance values (6–11 $\Omega^{-1}cm^2mol^{-1}$) of the complexes support their non-electrolytic nature. Thus, the present complexes have non electrolytic nature as evidenced by the involvement of acetate ions in coordination. This result was further confirmed from the chemical analysis of CH_3COO^- ion, not precipitated by addition of $FeCl_3$.The elemental analysis data of the complexes are in good agreement with theoretical values presented in Table 1. The results obtained from micro analytical measurements, metal estimation, conductivity and mass spectral data confirm the stoichiometry of the copper complex as $[CuL(OAc)_2]$. The magnetic moments of copper(II) in any of its geometry lies around 1.85 B.M. which is very close to spin-only value i.e. 1.73 B.M. The values which we found in our case lie in the range, 1.80–1.84 B.M. These values are typical for mononuclear copper (II) compounds having d^9-electronic configuration. The observed magnetic moments of all the complexes correspond to distorted square planar Cu(II) complexes. However, the values are slightly higher than the expected spin-only values due to spin orbit coupling contribution.

IR Spectra

The IR spectrum of the ligand (L^1, fig. 1) showed a band at 1680 cm^{-1} for the imine υ(C=N) group which results from the Schiff base condensation of 2-aminobenzimidazole and Knoevenagel condensate. It has shifted to a lower frequency of 1663 cm^{-1} after complexation [23]. Moreover, the appearance of new bands at 466 cm^{-1} and 533 cm^{-1} corresponds to υ(M-N) and (M-O) [24]. Also, the new bands at 1357cm^{-1} at 1255 cm^{-1} corresponds to symmetric and asymmetric stretching for the υ(M-O) which evidence the participation of the COO^- ion in the

complexes. These facts are further supported by the appearance of bands between1380 cm^{-1}-1465 cm^{-1} and 1255 cm^{-1}-1357cm^{-1} attributed to $\upsilon_{asy}(COO^-)$ and $\upsilon_{sy}(COO^-)$, respectively in all copper complexes. The difference in $\Delta\upsilon$ between υasy(coo$^-$)and υsy(coo$^-$) in metal complexes are ~100 cm^{-1} (105-125 cm^{-1}) suggested the mode of coordination of carboxylate group in copper complexes as monodentate manner [25]. Finally, it was concluded that the ligand(s) was coordinated to copper atom through bidentate manner (imine nitrogen atoms). The coordination modes are imine nitrogen atoms and acetate ions, respectively. The IR spectrum of ligand (L^1) and its copper complex was shown in figs.1 &2.

^{1}H-NMR

The ^{1}H NMR spectrum of the ligands were recorded and presented. The ligand (L^1, fig. 3) exhibits different signals as follows: the peak was observed at 5.6 ppm as singlet which corresponds to –NH group (benzimidazole moiety) and D_2O exchangeable peak. The aromatic protons are observed in the range 7.32—7.80 ppm as multiplet peak. The signal was observed at 8.02 and 2.38-2.42 ppm which are assignable to the proton of the CH=C (Knoevenagel condensate) and methyl group as singlet peaks. The other ligands showed similar NMR spectral features (supplementary materials). There is a slight shift in aromatic proton peak was observed due to the presence of substituents in the benzaldehyde moiety.

The ^{13}C NMR spectra of ligands were recorded and presented. In the ligand L^1(fig.4), the aromatic ring carbons are appeared in the range 124.8-127.6 ppm. The methyl carbons are also observed around 24.6 ppm. The peak was observed at 140.2 and 145.8 ppm which corresponds to –**C**H=C< and –**C**H=**C**<, respectively. The peak was also observed at 192.4 ppm corresponds to H_3C-**C**. The other ligands showed similar carbon NMR spectral features. There is a slight shift in aromatic ring carbon peaks were observed due to the presence of substituent's in the benzaldehyde moiety.

Mass Spectra

The FAB mass spectra of the Schiff bases and their corresponding copper complexes were recorded and compared their stoichiometry compositions. The $[CuL^1(OAc)_2]$ complex shows the molecular ion peak at m/z 757 and 759 (isotopic peaks of copper). This molecular ion undergoes dissociation which yielded a fragment ion peak at m/z 638 and further decomposition leads to the formation of demetallation to form the species $[L]^+$ gave fragment ion peak at m/z 575. The Copper complexes of $[CuL^2(OAc)_2]$ shows molecular ion peak (M+) at m/z = 745, respectively. Further, the fragmentation of two acetate ions was observed at m/z = 626. After losing their metal, the corresponding ligands ($[L]^+$) shows molecular ions peaks at m/z = 564.The mass spectrum of Cu(II) complex of $[CuL^3(OAc)_2]$ shows a molecular ion peak (M+) at m/z = 756. The second fragmentation of two acetate ions leads to the formation of ligand moiety of 2-aminobenzimidazole with molecular ion peak at m/z = 638, resulting from the elimination of metal results ion peak ($[L]^+$) at m/z = 574. The mass fragmentation pattern of copper complex . In case of $[CuL^4(OAc)_2]$ complex, the molecular ion peak observed at m/z = 737. This molecular ion further by losing two acetate atoms gave a fragment ion peak at m/z = 618 and these demetallation to form the species $[L]^+$ gave fragment ion peak at m/z = 555. Elemental analysis values are in close agreement with the values calculated from molecular formula of these complexes which is further supported by the FAB-mass studies of representative complexes.

EPR Spectra

The X- band EPR Spectrum of copper complexes was recorded in the solution state. The $[CuL^1(OAc)_2]$ complex at 300 K shows an intense absorption at high field. In the complex at frozen state shows four well-resolved peaks with different intensities in the low field region (Fig. 5) and one intense peak in the high field region. The magnetic susceptibility ~ 1.8 B.M indicates that the copper complex is mononuclear. This is also evident from the absence of a half field signal observed in the spectrum at 1600 G due to the ms=±2 transition, ruling out Cu-Cu interactions [26].

In the square planer complexes, the unpaired electron is in the d_{x2-y2} orbital given $^2B_{1g}$ as the ground state $g_{11} > g_\perp > 2$. The observed values ($A_{11=}$ 169>A⊥=52.g_{ll}=2.34>$g_\perp$=2.05>2) are well agreed with related square planer system [27]. In the axial spectrum, the "g" values are related with the exchange interaction is negligible. If the value of G is less than 4, the exchange interaction is considerable, For the present copper complexes, the G value of 6.8 suggest that the tetragonal axes are aligned parallel or slightly misaligned, consistent with d_{x2-y2} ground state. The in plane σ-bonding covalence parameters α^2, is related to A_{ll}, g_{ll} and $g_\perp$ according to the following equation,

$$\alpha^2 = A_{ll}/P + g_{ll} - 2.0023 + 3/7\ g_\perp - 2.0023 + 0.04$$

If the value of α^2=0.5 indicates covalent binding, while the values of α^2=1.0 suggested complete ionic bonding in complexes. The observed value of α^2=0.56 indicates that the complexes have some covalent character, the out of plane π bonding.γ^2 and in-plane π bonding (β^2) parameters are calculated from the following expression.

$$\beta^2 = (g_{ll} - 2.0023E/-8\lambda d^2)$$

$$\gamma^2 = g_\perp - 2.0023\ E/(-8\lambda d^2)$$

In these equations, λ= - 828cm^{-1} for the free metal ion. The observed β^2 (1.72) and α^2 (0.56) values indicate that there is interaction in the out of planeπ bonding where as in the in-plane π bonding is complex ionic. This is also confirmed by the orbital reducing factors which are estimated using the following reactions.

$$K_{ll} = g_{ll} - 2.0023\Delta E/8\lambda_0$$

$$K_\perp = g_\perp - 2.0027\Delta\ E/2\lambda_0$$

Where

λ_0 is the spin orbital coupling constant for the copper(II) ion is -828cm^{-1}

K_{ll} and $K_\perp$ are the parallel and perpendicular components of the orbital reduction factor(K), respectively.

The significant information about the nature of bonding in the copper(II) complexes can be derived from the relative magnitudes of $K_{II}<K_{\perp}$. In case of pure of σ bonding $K_{II}\approx K_{\perp}=0.77$.where as $K_{II}<K_{\perp}$ implies considerable in-planeπ bonding while her out of plane π bonding $K_{II} > K_{\perp}$, For the present complexes.the observed order is K_{II} (0.96)$>K_{\perp}$ (0.54) implying a greater than contribuction from out of planeπ bonding than from in-plane π bonding.

The degree of geometrical distortion plays a significant role in the biological activities. It was described as $f = g_{//}/A_{//}$ (index of tetragonal distortion). The "f" values are less than 135 cm associated with the square-planar geometry, whereas higher values indicate distortions towards tetrahedron. The f values of complexes were found to be in the range 126–141, indicating significant distortion from planarity in the synthesized copper complexes. The nature of ligand moieties and functional groups (in the ligands) were influenced on distortion from planarity. For the present complexes, the g_{II}/A_{II} is 142-148 which is in agreement with significant deviation from planarity and which is further confirmed by the bonding parameter α^2 whose value is less than unity.

TGA analysis

The thermal decomposition behavior of the complexes $[CuL^1(OAc)_2]$ (fig.6), $[CuL^2(OAc)_2]$ was recorded upto 800°c in nitrogen atmosphere. The correlations between the different decomposition steps of the complexes with the corresponding weight losses are discussed in terms of the proposed formula of the complexes.

The thermal decomposition pattern of the $[CuL^1(OAc)_2]$ complex displays three steps of weight loss: (i) the coordinate acetate ions are dissociated from the complex relatively low temperature range at 190-360°C, (ii) elimination of 2-aminobenzimidazole moiety was observed with partial decomposition of the parent organic ligand in the temperature range 360-580°C and (iii) the final decomposition step comprise the complete removal of the organic residual at the temperature range 580-800°C and the formation of the metallic oxide CuO as a final thermal decomposition product. The final decomposition products were identified by IR spectroscopy

with corresponding spectra obtained under the same condition as the acetate and imine groups. All other complexes showed similar thermogravimetric profiles.

DNA studies

DNA as a potential therapeutic target molecule has attracted by researchers towards therapeutic agents. The binding studies between molecules and DNA are significant and fruitful approach to develop efficient drug molecules. In the binding studies, calf thymus DNA was selected as DNA molecule because of its medical importance, low cost and ready availability properties to study the probable antimicrobial action mechanism of the effective compound at molecular level by UV–Vis spectroscopic and electrochemical methods.

Hypochromism and hyperchromism are significant spectral terms to distinguish the change of DNA double-helical structure in absorption spectroscopy. Due to the interaction between the electronic states of intercalating chromophore and that of the DNA base, the observed large hypochromism suggested a close proximity of the aromatic chromophore to the DNA bases with a fixed concentration of DNA, UV–vis absorption spectra were recorded with increasing amount of compound.

Electronic spectroscopy

The DNA binding efficiency of copper complexes were measured and presented. In the absorption spectrum of copper complex with L^1 showed an absorption band around 312.2 and 465 nm (due to π–π* and d-d transitions). In order to increase the concentration of DNA, there is change in absorption and shift in wavelength was observed (i.e., hypochromicity with a red-shifted). The observed hypochromicity was attributed to the π-stacking or hydrophobic inter-actions of the aromatic phenyl rings with amino acids in the DNA [28]. As compared the results with the standard intercalators, the copper complexes with electron withdrawing substituted ligand has shown higher binding affinity than other complexes. Therefore, we concluded that the planar nature of molecule, aromatic rings, conjugation effect and electron-withdrawing substituted groups may greatly promote the DNA-binding ability of their Knoevenagel condensate complexes.

Cyclic voltammetric studies

The cyclic voltammogram of the glassy carbon electrode in solutions containing $[CuL^1(OAc)_2]$ in the absence and in the presence of varying amount of DNA were shown in Fig.7. In the presence of DNA causes a considerable decrease in the voltammetric current of the redox wave with a slight shift in $E_{1/2}$ potential to positive region. The drop of the voltammetric currents in the presence of DNA may be attributed to slow diffusion of the copper complex bound to CT DNA. The net shift in $E_{1/2}$ and decrease in current intensity can be explained in terms of an equilibrium mixture of free and DNA-bound complexes to the electrode surface [29]. Finally, the conclusion arrived from the electrochemical behavior of copper complexes could bound with DNA by intercalative binding mode.

Anti-microbial studies

The *in vitro* antimicrobial activities of the investigated compounds were tested against the bacterial species, *Acietobacter baumannil, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* by disc diffusion method [30, 31] and summarized in table 3. The inhibitions around the antibiotic discs were measured after incubation and Streptomycin was used as standard drug.

The Schiff base and its complexes were screened for their anti microbial activity against invitro anti bacterial of the Complexes have been carried out against the both Gram positive and Gram negative bacteria using disc diffusion method. In the present study the results indicates that all complexes have a higher anti microbial activity than the free ligand. This is due to the greater lipophilic nature of the complexes. When concentration of the complexes increases, the activity also increases. The concentration plays a vital role in increasing the degree of inhibition. The higher anti microbial activity of the metal complexes compared to ligand may be due to the change in structure or coordination and chelating tends to make metal complexes act more powerful and potent bactereostatic agents, thus inhibiting the growth of the bacteria. This increase in the activity of the complexes can be explained on the basis of chelation theory.

Chelation considerably reduces the polarity of the metal ion because of partial sharing of its positive charge with donor groups and possible electron delocalization over the whole chelate ring. Such a chelation could enhance the lipophilic character of the central metal atoms.

Antibacterial activity of copper complexes was screened towards gram positive and gram negative bacteria. Further, the ligand showed low activity and the complexes moderate to higher activities as compared to standard drug towards the all organism tested due to the presence of -NH group present in benziimidazole moiety plays an important role in the biological activity. This group is believed to impart the transformation reaction in biological system. The antimicrobial activity of four complexes can be referred to the increase of their lipophilic character which in turn deactivates enzymes responsible for respiration processes and other cellular enzymes, which is playing a vital role in various metabolic pathways of the tested microorganisms.

Generally, metal complexes have shown higher activity against Gram-positive bacteria than against Gram negative pathogens. In the present study indicated that the Gram-positive bacteria were inhibited more strongly than Gram negative bacteria and explained on the basis of complex structure of cell wall. The gram-negative bacteria possessing an extra outer layer on top of the peptidoglycan and found to be highly impermeable. In the case of gram-positive bacteria, the polysaccharides in their cell wall called teichoic acid (negatively charged) and facilitated the passage of the positive metal ions.

The polysaccharides and lipids are prime important constituents of cell wall and membranes and favorable for metal ion interaction. This cell wall also constitutes carbonyl, phosphate and cystenyl ligands which help in maintaining the integrity of the membrane by performing as a diffusion barrier and also provide suitable sites for binding interaction. Moreover, the reduction in polarity enhances the lipophilic character of the copper chelates and interaction between the lipid and copper ion is favoured. This may lead to the breakdown of the permeability barrier of the cell resulting in intervention with the normal cell processes and

blocking of the metal binding sites in the enzymes of the microorganisms. These complexes may also disturb the respiration process of the cell and therefore block the protein synthesis, which confines further growth of the organism.

In addition to this, mode of action of these complexes may involve formation of a hydrogen bond through imine nitrogen atom with the active specific centers of cell constituents, resulting in interference with the normal cell process. The relative difference in the activity of the complexes against different kinds of microorganisms depend either on the difference in the ribosomes of the microbial cells or on the impermeability of the cells of the microbes. The low activity of some complexes may be ascribed due to low lipid solubility, consequently the metal ion may not be able to reach the favorable site of action of the cell wall to get interfere with the normal cell activity. Though chelation dominate in assessing the biological behavior of the complexes but simultaneously other factors such as dipole moment, solubility, size, redox potential of metal ions, coordinating sites, solubility, bond length between metal and the ligand, geometry of complexes, steric, pharmacokinetic, concentration and hydrophobicity have considerable influence on the antimicrobial potency. Hence, it may be concluded that the antimicrobial activity of the complexes may not be due to chelation alone but it is convoluted blend of numerous contributions.

SOD activity

Superoxide anions have a very short half life and produced continuously. In this colorimetric based assay, Inhibition of the reduction of nitroblue tetrazolium (NBT) to formazan (F) by the reported metal(II) complexes was used for detection of the SOD mimetic catalytic activity of these chelates in the phosphate buffer under similar biological conditions. As the reaction proceeding, the farmazan color is developed and the color changes from colorless to blue, which was associated with an increase in the absorbance at 560 nm. SOD reduces the superoxide ion concentration and thereby lowers the rate of formazan formation. In the SOD-like activity test, the metal complexes compete with NBT for oxidation of the generated superoxide

ions. The more efficient the complex, the lower the concentration that corresponds to 50% inhibition of NBT reduction; this concentration is termed IC_{50} for comparative purposes.

The data in Table 4 also reports the scavenging efficiency of each complex, giving its final concentration that produced efficient quenching of the superoxide anion radical. It also reveals that there are difference between the values of scavenging effectiveness and the catalytic constant of the complexes. This difference in the reactivates of these complexes can rationalized by means of correlation between the redox potential of the couples Cu^{II}/Cu^{I} during the catalytic cycle and the SOD-mimetic activity.

The higher biological activities of copper complexes may be attributed to the flexible ligands, which are able to accommodate the geometrical change from Cu^{II} to Cu^{I}, specially the labile water molecules, which are proposed to be easily substituted by the substrate $O_2^{\bullet-}$, in the catalytic process, just like the $O_2^{\bullet-}$, in place of H_2O bound to copper site in the mechanism of dismutation of $O_2^{\bullet-}$ by native SOD. The mechanism proposed for the dismutation of superoxide anions by both superoxide dismutase and complexes 2 and 3 is thought to involve redox cycling of metal(II) ions (eqs. 1 and 2):

$$M^{2+} + O_2^{-} \rightarrow M^{+} + O_2$$

$$M^{+} + O_2^{-} + 2H^{+} \rightarrow M^{+} + H_2O_2$$

It has been proposed that electron transfer between copper(II) and superoxide anion radicals occurs through direct binding. Therefore, copper complexes exhibited higher SOD activity than other metal complexes. This observation was confirmed by distortion of geometry ("f" factor value). The synthesized copper complexes have higher distortion of geometry.

Comparative study

Researchers have synthesized different copper complexes and performed different biological studies for chemotherapeutic agents. Mahdi Behzad et al [32] have performed antibacterial activity for the synthesized copper and nickel complexes and compared. The observations indicated that copper complexes with square planar geometry showed higher activity. The nature of ligands, central metal and geometry will decide the biological activities.

Mohamed M. Ibrahim [33] synthesized a series of albendazole-based copper(II) complexes and structurally characterized. Authors have observed two different geometries for copper complexes. Among the copper complexes, the complex with square planar environment exhibited higher biological activities. Nasibeh Imani [34] have arrived conclusion that the prepared copper complex with distorted square planar geometry have shown higher antibacterial activity. Generally, Copper(II) complexes are appeared as potential drug candidates for chemotherapeutic agents [35–37]. The DNA-interaction and cytotoxicity of curcumin analogs [38–41] along with methylacetoacetate with copper(II) complexes as similar to cisplatin analogs. Ali and coworkers have reported that the Knoevenagel's condensate of a curcumin derivative showed better pharmaceutical activity [42]. These Cu(II) complexes have two labile chlorides similar to cisplatin, they may interact covalently with DNA bases and form stable complexes. We inspired from the above facts, in the present study, the copper complex with electron withdrawing substituted ligands (-Cl group) possessed higher biological activities. Here, the complex geometry, ligand structural core and copper atom may enhance biological activities.

Conclusion

The design and synthetic approach was focused on the development of copper complexes with curcumin analogs with enhanced biological activities. The lower molar conductance values of the complexes which corresponds to non-electrolytic nature. The antibacterial screening of the ligands and their copper complexes indicated that all the complexes showed higher anti microbial activities than the free ligands. Superoxide dismutase and antioxidant activities of the copper complexes have also been performed. In the electrochemical technique, the shift in ΔE_p, $E_{1/2}$ and Ipc values were explored for the interaction of the complexes with CT-DNA.

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Figure captions

Figure 1 IR spectrum of ligand L^1

Figure 2 IR spectrum of copper complex of L^1

Figure 3 ^1H NMR spectrum of L^1

Figure 4 ^{13}C NMR spectrum of L^1

Figure 5 ESR spectra of copper complex of L^1 at 77 K

Figure 6 TGA profile of copper complex of L^1

Figure 7 The absorption spectra of complexes in the absence and presence of CT-DNA of $[CuL^1(OAc)_2]$ complex. Arrow shows the absorbance changing upon the increase of DNA concentration.

Figure 8 The cyclic voltammogram of copper complex of L^1 in the absence and presence of DNA at various concentrations. Arrow indicates the changes in voltammetric currents upon increasing the DNA concentration.

Scheme 1 Schematic presentation of synthesis of copper complexes

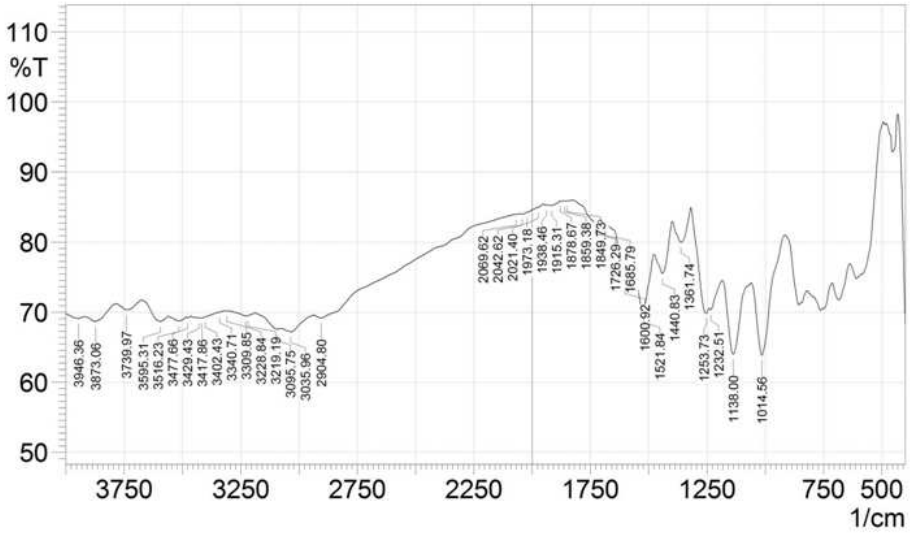


Figure 1

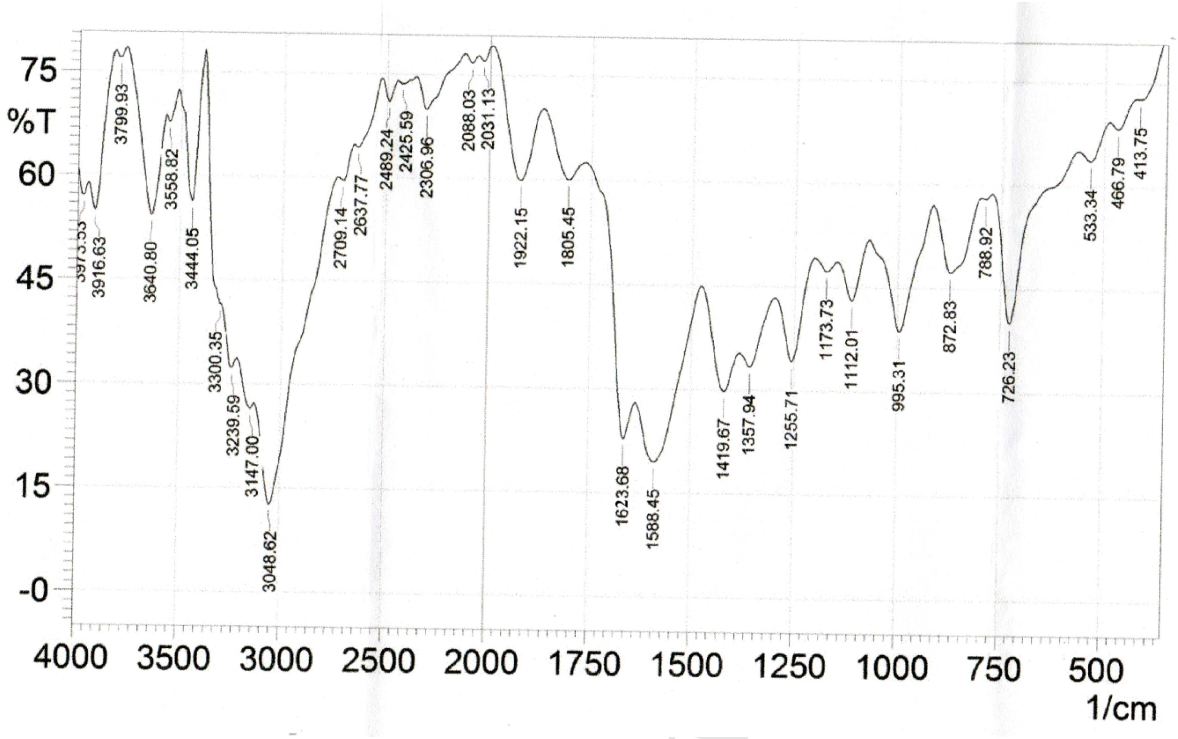


Figure 2

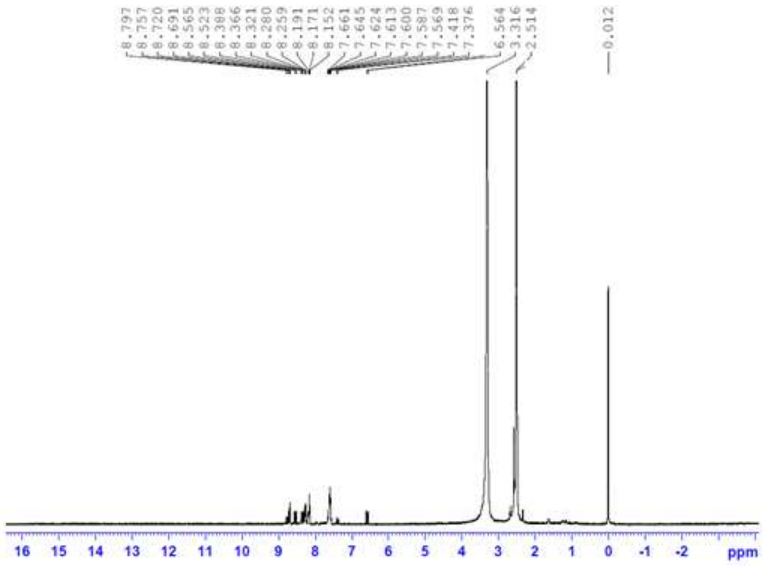


Figure 3

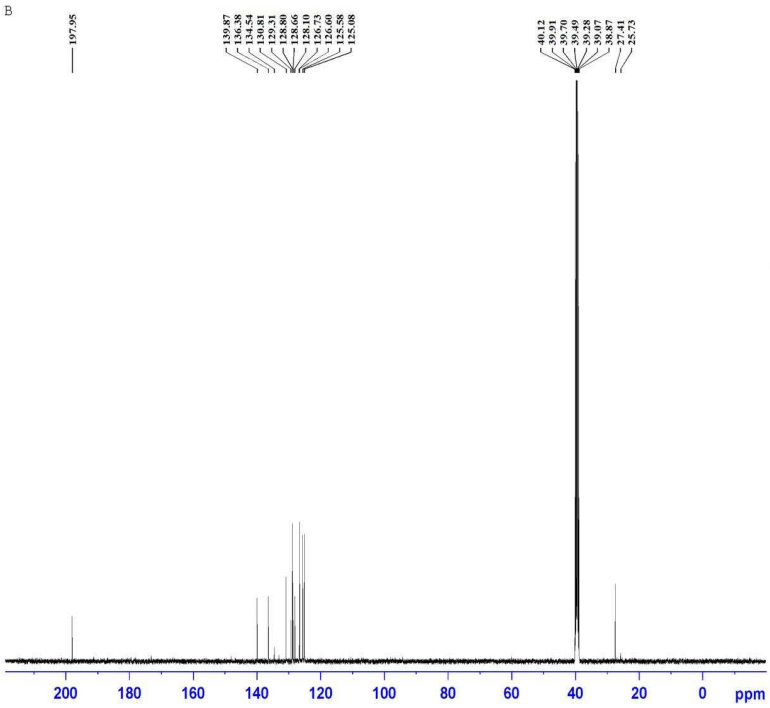


Figure 4

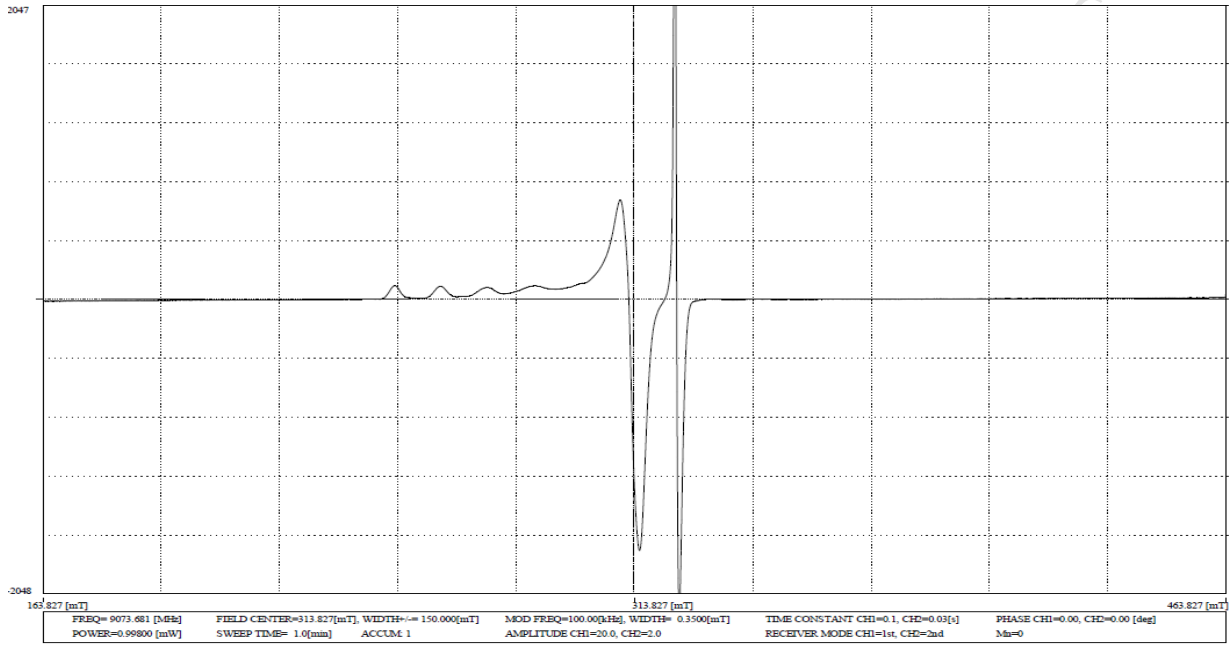


Figure 5

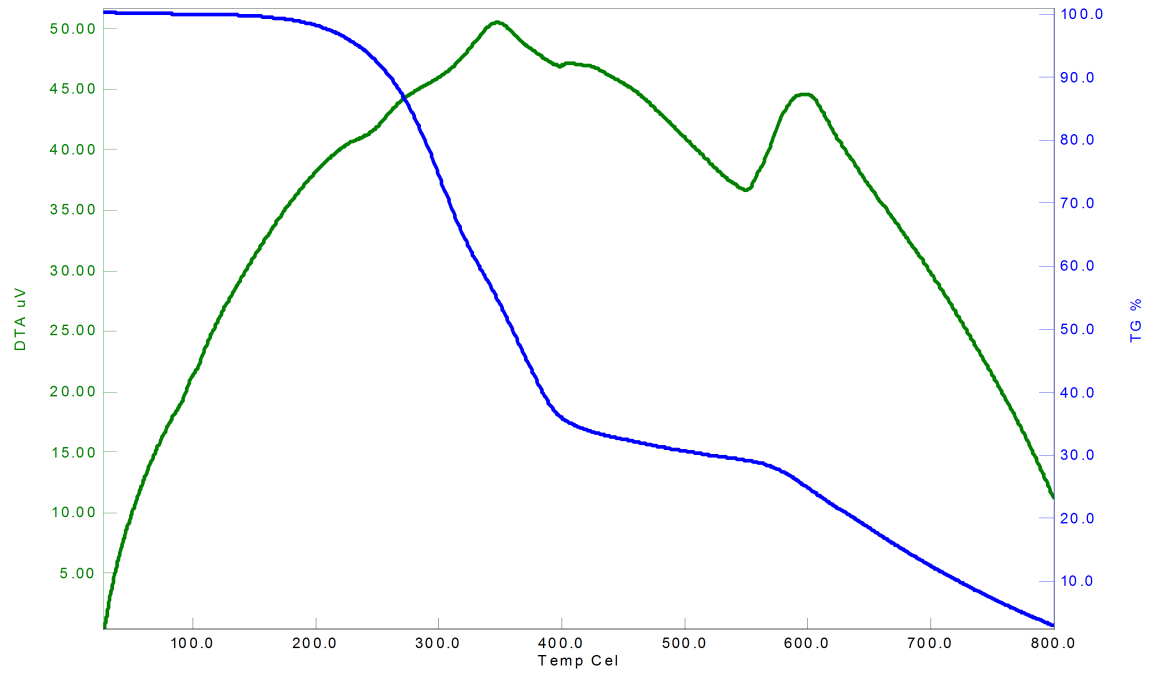


Figure 6

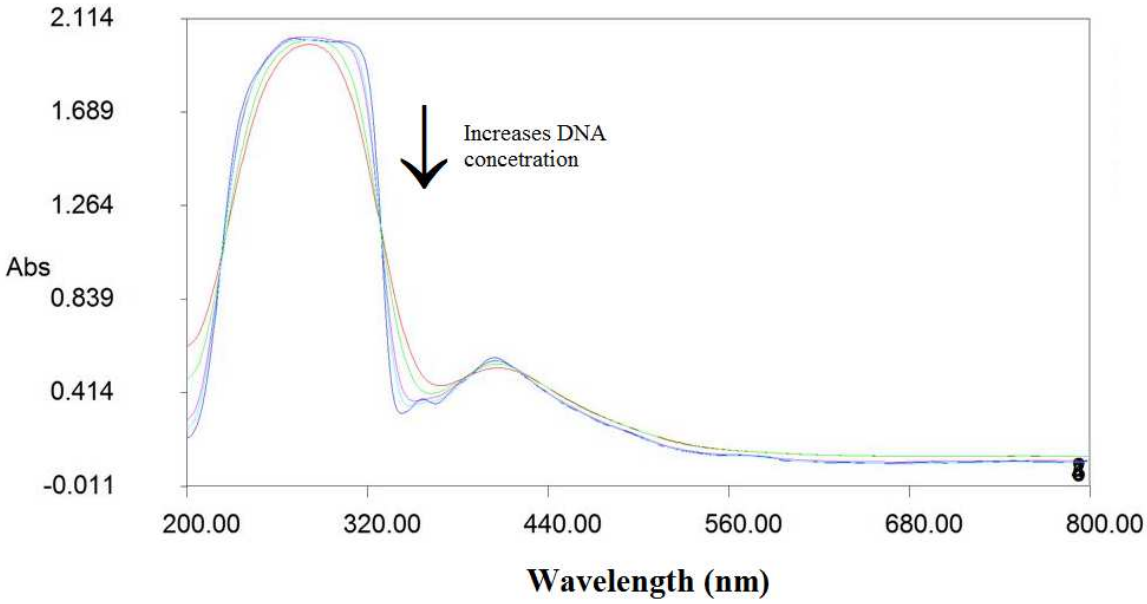


Figure 7

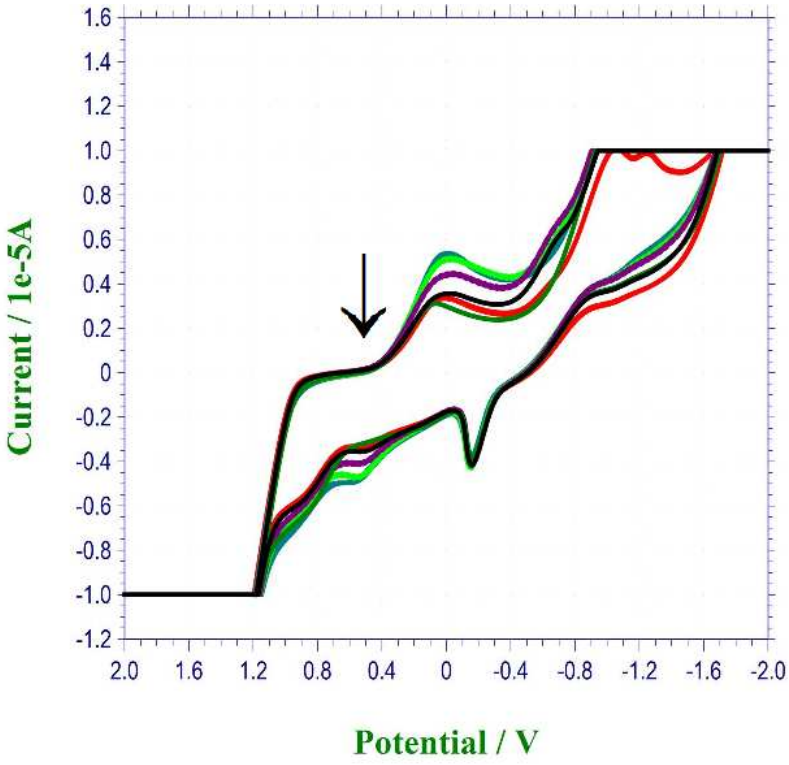
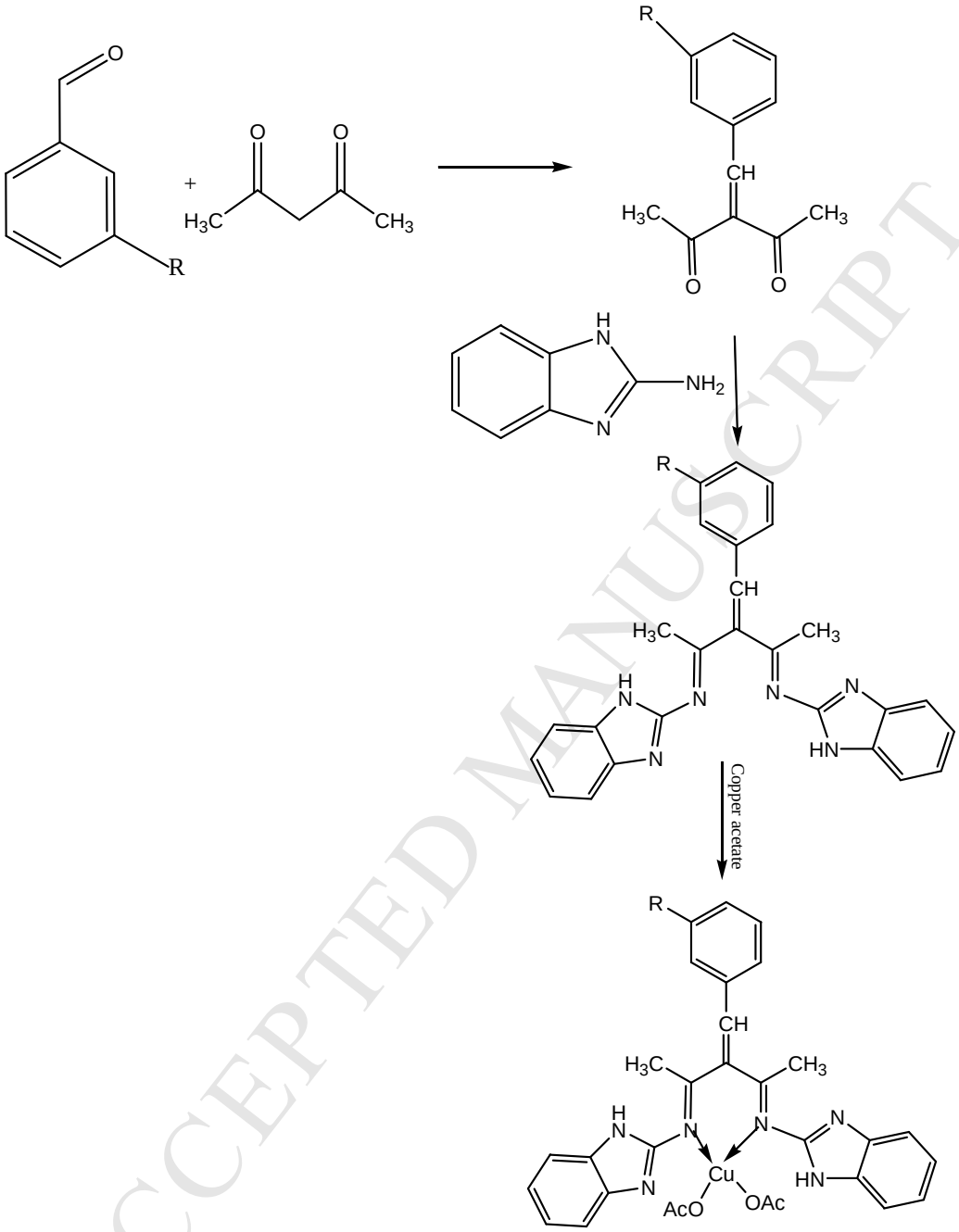


Figure 8



Where

R = -Br (L^1), -F (L^2), -Cl (L^3), -CN (L^4)

Scheme 1 Schematic presentation of synthesis of copper complexes

Table captions

Table 1 Elemental analysis, yield, colour, magnetic moments and molar conductance values of ligands and their copper complexes

Table 2 IR spectral data (cm^{-1}) for the free ligands and their copper complexes

Table 3 Minimum inhibitory concentration values of the synthesized compounds against the growth of bacteria (in μM).

Table 4 SOD activity of ligands and their copper complexes (μM)

Table 1

Compound	Yield	Colour	Found (calc)					μ_{eff}(BM)
			Cu	C	H	N	(Ω cm^2 mol^{-1})	
L1	62	Dark brown	-	68.25 (68.20)	4.67 (4.64)	9.50 (9.48)	-	-
L2	68	Light yellow	-	67.54 (67.50)	4.24 (4.30)	10.02 (10.01)	-	-
L3	58	Dark brown	-	67.05 (67.15)	4.04 (3.98)	12.56 (12.52)	-	-
L4	73	Dark brown	-	67.68 (67.73)	4.34 (4.25)	10.15 (10.12)	-	-
$CuL^1(OAc)_2$	65	Black	8.45 (8.42)	58.03 (58.00)	4.18 (4.05)	7.53 (7.42)	11	1.83
$CuL^2(OAc)_2$	74	Black	8.59 (8.50)	57.42 (57.40)	3.82 (3.91)	7.62 (7.50)	9	1.80
$CuL^3(OAc)_2$	54	Pale brown	8.68 (8.56)	55.54 (42.65)	3.65 (3.75)	6.98 (6.86)	7	1.85
$CuL^4(OAc)_2$	62	Light black	7.60 (7.48)	57.38 (57.31)	4.28 (4.14)	7.52 (7.42)	6	1.84

Table 2

Compound	υ(C=N) (cm^{-1})	υ(C=N) (cm^{-1})	υ(M-O) (cm^{-1})	υ(M-N) (cm^{-1})	υasy (COO-) (cm^{-1})	υsy (COO^-) (cm^{-1})
L^1	1642	1630	–	–	–	–
L^2	1638	1632	–	–	–	–
L^3	1645	1636	–	–	–	–
L^4	1640	1638	–	–	–	–
$CuL^1(OAc)_2$	1621	1620	540	470	1340	1298
$CuL^2(OAc)_2$	1635	1623	533	466	1357	1255
$CuL^3(OAc)_2$	1629	1628	520	440	1338	1270
$CuL^4(OAc)_2$	1625	1622	510	450	1328	1260

Table 3

Compound	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumaniae*	*Pseudomonas aeruginosa*	*Proteus vulgaris*
L^1	0.64	0.72	0.68	0.76	0.60
L^2	0.78	0.80	0.66	0.82	0.88
L^3	0.72	0.76	0.80	0.86	0.92
L^4	0.70	0.78	0.68	0.72	0.78
$[CuL^1(OAc)_2]$	0.26	0.20	0.24	0.28	0.18
$[CuL^2(OAc)_2]$	0.16	0.24	0.26	0.24	0.22
$[CuL^3(OAc)_2]$	0.32	0.30	0.22	0.22	0.26
$[CuL^4(OAc)_2]$	0.18	0.26	0.24	0.14	0.18
Streptomycin	0.02	0.04	0.03	0.06	0.05

Table 4

Sl.No	Compounds	IC_{50} values (μM)
1	L^1	0.86
2	L^2	0.92
3	L^3	0.74
4	L^4	0.68
5	$[CuL^1(OAc)_2]$	0.22
6	$[CuL^2(OAc)_2]$	0.18
7	$[CuL^3(OAc)_2]$	0.25
8	$[CuL^4(OAc)_2]$	0.20
9	Native enzyme	0.04

Highlights

- Copper complexes with 2-aminobenzimidazole derivatives were synthesised
- Copper complexes were characterized using spectral and analytical techniques
- Copper complexes are similar to cis-platinum analogs were screened against microbes
- SOD activity was also performed.