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P. Kavitha, M. Saritha, K. Laxma Reddy

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Synthesis, structural characterization, fluorescence, antimicrobial, antioxidant and DNA cleavage studies of Cu(II) complexes of formyl chromone schiff bases

P. Kavitha [a], M. Saritha[b], K. Laxma Reddy [a,]*

[a] Department of Chemistry, National Institute of Technology, Warangal 506004,Andhra Pradesh, India

[b] Department of Biochemistry, Sri Venkateswara University, Tirupati 517502, Andhra Pradesh, India

Email: laxmareddychem12@gmail.com

Tel: +91870-2462650 Fax: +91-8702459547

ABSTRACT

Cu(II) complexes have been synthesized from different schiff bases, such as 3-((2-hydroxy phenylimino)methyl)-4H-chromen-4-one (HL_1), 2-((4-oxo-4H-chromen-3-yl)methylneamino) benzoicacid (HL_2), 3-((3-hydroxypyridin-2-ylimino)methyl)-4H-chromen-4-one (HL_3) and 3-((2-mercaptophenylimino)methyl)-4H-chromen-4-one (HL_4). The complexes were characterized by analytical, molar conductance, IR, electronic, magnetic, ESR, thermal, powder XRD and SEM studies. The analytical data revealed that metal to ligand molar ratio is 1:2 in all the complexes. Molar conductivity data indicates that all the Cu(II) complexes are neutral. On the basis of magnetic and electronic spectral data, distorted octahedral geometry is proposed for all the Cu(II) complexes. Thermal behaviour of the synthesised complexes illustrates the presence of lattice water molecules in the complexes. X-ray diffraction studies reveal that all the ligands and their Cu(II) complexes have triclinic system with different unit cell parameters. Antimicrobial, antioxidant and DNA cleavage activities indicate that metal complexes exhibited greater activity as compared with ligands.

Key words: Antimicrobial activity, Antioxidant, DNA cleavage, Fluorescence, Formylchromone

1. Introduction

Synthesis of chromone derivatives have been of special interest due to their biological activities including antimycobacterial, anticonvulsant, antimicrobial, anticancer, antioxidant and mushroom tyrosinase inhibition activities [1□4]. These derivatives also serve as intermediates to many products of pharmaceuticals, agrochemicals and dyestuffs [5]. In addition, 3-formylchromone and its derivatives inhibit a human protein phosphatase PTP1B [6]. Schiff bases are an important class of ligands in catalysis and organic synthesis [7□10]. Furthermore, these ligands and their metal complexes had a variety of biological, clinical, analytical and industrial applications. Schiff bases of 3-formyl chromone and its complexes also have a variety of applications in biological, clinical and pharmacological areas [11□13]. A huge number of literature reports were available on DNA binding

studies of formyl chromone schiff bases and their metal complexes [14□16]. Knowledge of DNA binding parameters of these molecules explores their potential applications, since their binding mode could be associated with their ability to cause DNA damage [17□19]. This DNA damage is leading to the inhibition of uncontrolled growth of cancerous cells. Complexes of essential metals like copper and others are generally less toxic than that of non-essential ones and some of them even have important cellular cytotoxic effects. In addition, high nucleobase affinity and comparatively strong Lewis acidity of Cu(II) ion induce efficient DNA cleavage activity [20]. Prompted by the above literature reports, we report here the synthesis, characterisation and biological activity studies of Cu(II) complexes with 3-formyl chromone schiff bases such as 3-((2-hydroxy phenylimino)methyl)-4H-chromen-4-one (HL_1), 3-((3-hydroxypyridin-2-ylimino)methyl)-4H-chromen-4-one (HL_3) and 3-((2-mercaptophenylimino)methyl)-4H-chromen-4-one (HL_4). The synthesis, characterisation and antibacterial activity of 2-((4-oxo-4H-chromen-3-yl)methylneamino)benzoicacid (HL_2) and its Cu(II) complex was reported by C. Gyna Kumari et.al.[21]. However, we also report in this paper the synthesis of Cu(II) complex of HL_2 and its detailed characterisation, fluorescence and biological activity studies (antifungal, antioxidant and DNA cleavage).

2. Experimental

2.1. Materials

AR grade 2-hydroxy acetophenone, phosphorous oxychloride ($POCl_3$), 2-aminophenol, 2-amino-3-hydroxy pyridine, 2-amino benzoic acid, 2-amino thiophenol, copper acetate, 1,1-diphenyl-2-picryl hydrazyl (DPPH), butylated hydroxyl toluene (BHT) and organic solvents were used.

2.2. Synthesis of schiff bases

The ligands HL_1, HL_2 and HL_3 were prepared as per the reported methods [11,22]. HL_4 was prepared as per the procedure given below.

The preparation of schiff base (HL_4) was carried out by stirring the hot methanolic solutions of 3-formyl chromone [23] (1 mM) and 2-amino thiophenol (1 mM) at 40□50 ^{o}C up to 30 min. The progress of reaction was monitored by TLC. The product thus obtained was recrystallized in methanol. All these experimental details are given in Scheme 1.

2.3. Synthesis of Cu(II) complexes

A hot methanolic solution of schiff bases (2 mM) were added to a methanolic solution of $Cu(CH_3COO)_2{\cdot}H_2O$ (1 mM) and stirred for 1□2 h. After cooling, the green coloured complexes were separated and washed several times with hot methanol and dried in vacuum.

2.4. Physical measurements

Elemental analysis for C, H, N, and S was performed using Perkin Elmer CHNS analyser. Molar conductance of the complexes was measured in DMF using a Digisun conductivity meter. IR spectra of the ligands and their Cu(II) complexes were recorded on Tensor 2 FTIR spectrophotometer within the range 4000□400 cm^{-1} using KBr disc. The electronic spectra of the ligands and their Cu(II) complexes were recorded on JASCO V-670 spectrophotometer in the range of 250–1400 nm. Magnetic susceptibilities of the complexes were measured on a Guoy balance at room temperature, by making diamagnetic corrections using Pascal's constant. Thermo gravimetric analysis of the complexes were carried out on a Perkin Elmer diamond TGA instrument at a heating rate of 20 ^{o}C and nitrogen flow rate of 20 mL/min. The fluorescence spectra of ligands and their complexes were recorded on FLUOROLOG-FL3-11 spectroflourimeter. The solid state ESR spectra of the complexes were recorded using Bruker EMX EPR spectrometer at room temperature. The X-ray powder diffraction analysis was carried out by using Xpert-Pro X-Ray diffractometer with Cu Kα radiation. Scanning electron microscopy (SEM) of the complexes was obtained from Hitachi S-520 electron microscope at an accelerated voltage of 10 kV. ^{1}HNMR spectra of the ligands were recorded on Bruker-300 MHz spectrometer using $CDCl_3$ as a solvent and TMS as internal standard. Mass spectra of the ligands were recorded on a Jeol JMSD-300 spectrometer.

2.5. Biological studies

2.5.1. Antimicrobial screening

In vitro antibacterial and antifungal activities of the ligands and their Cu(II) complexes were evaluated against gram-negative *(Proteus vulgaris, Klebsiella pnuemoniae)* and gram-positive bacteria *(Staphylococcus aureus, Bacillus subtilis)* and fungi strain (*Candida albicans*) using disc diffusion method. Kanamycin (30 µg/disc) and Clotrimazole (10 µg/disc) are the standards for antibacterial and antifungal activities. Sterile antibiotic discs (6 mm in diameter, prepared using Whatmann No. 1 paper) were placed over the nutrient agar medium. 100 µg of the compounds (initially dissolved in DMSO) were transferred to each disc with the help of a micropipette. After overnight incubation at 37 °C for bacteria and 25 °C for fungi, the zone of inhibition was measured in mm and compared with standard antibiotics. All the experiments were performed in triplicates and the average zones of inhibition was recorded. Control measurements were carried out with DMSO. The compounds which show significant activities were selected to determine the minimum inhibitory concentration (MIC) using disc diffusion technique.

2.5.2. DPPH radical scavenging activity

The free radical scavenging activity of the ligands and their Cu(II) complexes were determined by using DPPH according to the literature [24,25]. The principle for the reduction of DPPH free radicals is that the antioxidant reacts with stable free radical DPPH and it converts into 1,1-diphenyl-2-picrylhydrazine. The ability to scavenge the stable free radical DPPH is measured by decrease in the absorbance at 517 nm.

2.5.2.1. Qualitative analysis

All the ligands and their Cu(II) complexes were applied on a TLC plate as a spot (100 µg/mL) using mobile phase methanol:acetonitrile (7:1). It was allowed to develop the chromatogram for 30 min. After the completion of the chromatogram, the plate was sprayed with DPPH (0.2% w/v). The colour change (yellow spot on purple background on TLC plate) is an indication for the presence of antioxidant activity.

2.5.2.2. Quantitative analysis

Each compound was dissolved in methanol 10 mg/10 mL and it was used as stock solution. From the stock solutions 1 mL of each compound solution with different concentrations (0.25 µg□1.00 µg) were added to the 3 mL of methanolic DPPH (0.004%) solution. After 30 min, the absorbance of the test compounds was taken at 517 nm using UV-VIS spectrophotometer, which was compared with the corresponding absorbance of standard BHT concentrations (0.25 µg□1.00 µg). DPPH solution was used as control without the test compounds, whereas methanol was used as blank. The percentage of scavenging activity of DPPH free radical was measured by using the following formula

$$Scavenging\ activity\ (\%) = \left[\frac{(A_o - A_i)}{A_o}\right] \times 100$$

Where, A_o is the absorbance of the control and A_i is the absorbance of the sample. IC_{50} values were calculated for compounds, which exhibited the significant activity. IC_{50} is defined as concentration sufficient to obtain 50% of maximum scavenging activity.

2.5.3. DNA cleavage studies

Agarose gel electrophoresis was used to study the DNA cleavage activity of ligands and their Cu(II) complexes. pUC19 plasmid was cultured, isolated and used as DNA for the experiment. Test samples (1 mg/mL) were prepared in DMF. 25 µg of the test samples and 5 µL H_2O_2 (500 µM) were added to the isolated plasmid and incubated for 2 h at 37 ^oC. After incubation, 30 µL of plasmid DNA sample mixed with bromophenol blue dye (1:1) was loaded into the electrophoresis chamber wells along with the control DNA, 5M $FeSO_4$ (treated with DNA) and standard DNA marker containing TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/1 L). Finally it was loaded on to an agarose gel and

electrophoresed at a constant voltage of 50 V for 30 min. After the run, gel was removed and stained with 10.01 µg/mL ethidium bromide and image was taken in Versadoc (Biorad) imaging system. The extent of DNA cleavage and the results were compared with standard DNA marker.

3. Results and discussion

3.1. Characterisation of schiff base ligands

The schiff base ligands HL_1, HL_2 and HL_3 were characterised using ^{1}HNMR, ^{13}CNMR and Mass spectral data and were compared with the spectral data reported in the literature [11, 22]. However, the ligand HL_4 was characterized using analytical and ^{1}HNMR, ^{13}CNMR and Mass spectral data (supplementary material). ^{1}H NMR spectrum of HL_4 ligand showed a signal at 4.75 ppm (S, 1H, SH) is due to thiol proton. The methine proton of the azomethine group was observed as a singlet at 7.46 ppm (s, 1H, □CH=N□). The aromatic protons are appeared as a set of multiplets in the range 8.25–6.40 ppm (m, 9H, Ar CH). The ^{13}CNMR spectrum of HL_4 ligand showed a signal at 176.13 ppm is due to the carbonyl carbon of the pyrone ring. The imine carbon and carbon adjacent to the oxygen of the pyrone ring appear at 153.68 and 156.22 ppm, respectively. The aromatic carbons are observed in the range of 147.27□110.51 ppm. The mass spectra of HL_4 (Formula weight = 281) shows a molecular ion peaks at m/z 282.04 and 304.02 corresponding to [M+1] and [M+23], respectively. From all the spectral data, the proposed structures of the ligands are given in Fig. 1.

3.2. Characterisation of Cu(II) complexes

All the Cu(II) complexes were partially soluble in MeOH, $CHCl_3$ but freely soluble in DMF and DMSO. The analytical and physical data for the metal complexes are listed in Table 1. The analytical data listed in Table 1 indicate that the metal to ligand molar ratio is 1:2 for all the complexes. The conductivity data of all the metal complexes indicate that all are neutral in nature [26].

3.2.1. Determination of Cu(II) content in the complexes

Known amount (0.150 g) of the metal complexes were decomposed with concentrated nitric acid, the excess nitric acid is being expelled by evaporation with concentrated sulphuric acid. This process is repeated till the organic part of the complex got completely lost. The residue was cooled and dissolved in dilute nitric acid and the determination of Cu(II) was carried out using spectrophotometric method [27].

3.2.2. Infrared spectral studies

Infrared spectral data of ligands and their Cu(II) complexes are listed in Table 2. All the schiff base ligands have the most characteristic band of azomethine (C=N) group appeared in the range of 1600□1550 cm^{-1} [28]. In all their Cu(II) complexes this band was shifted about 10□25 cm^{-1} to lower wave number. It indicates the involvement of nitrogen atom of the azomethine group in coordination

to the metal ion. A broad band appeared at 3250 cm^{-1} in HL_1 and HL_3 ligands is due to phenolic υ(O□H) group, which was disappeared in their complexes, confirms the participation of oxygen of O□H group in coordination to the metal ion. The stretching vibrations of S□H have no significant role, since its band is very weak in HL_4 ligand. However, participation of the SH group in chelation is ascertained from the shift of υ(C□S) at 790 cm^{-1} in the ligand to lower frequencies by 40 cm^{-1} in the complex [29]. In HL_2 ligand, a strong band appeared at 1365 cm^{-1} is due the υ(C□O) of carboxylic group. In its Cu(II) complex it was shifted to 1351 cm^{-1}, indicating the coordination of carboxylic group through the oxygen atom to the metal ion [21]. In all the schiff base ligands, appearance of a band in the range of 1650□1620 cm^{-1} assignable to the υ(C=O) of chromone moiety [30]. In their Cu(II) complexes this band was shifted to lower wave number by about 10□50 cm^{-1}, which confirms the participation of oxygen atom of carbonyl group in coordination to the metal ion. Participation of oxygen and nitrogen atoms in the coordination is further confirmed by far IR spectral bands at around 600□400 cm^{-1} range corresponding to υ(M□N) and υ(M□O), respectively. All complexes showed a broad band around 3400 cm^{-1} due to υ(OH) from water molecules. The IR spectral data confirms, all the schiff base ligands coordinated to a Cu(II) ion in a tridentate manner through ONO/ONS.

3.2.3. Electronic spectral and magnetic studies

The geometry of the Cu(II) complexes was deducted from the electronic spectra. The electronic spectra of HL_1 and its Cu(II) complex are shown in Fig. 2. Electronic spectral data of all ligands and their Cu(II) complexes and magnetic moment values are depicted in Table 3. The electronic spectra of all ligands show two bands. One band within the range of 25,000□26,000 cm^{-1} is attributed to the $n \rightarrow \pi^*$ transitions. Another band within the range of 31,000□34,000 cm^{-1} is due to the $\pi \rightarrow \pi^*$ transitions. All the Cu(II) complexes show a band 18,000□19,000 cm^{-1} range and a shoulder on the lower energy side at 13,000□15,000 cm^{-1} range indicates that these complexes are in distorted octahedral structure [31]. The two bands observed in their electronic spectra due to $^2B_{1g} \rightarrow {}^2B_{2g}$ and $^2B_{1g} \rightarrow {}^2E_g$ transitions, respectively. These transitions are characteristic of distorted octahedral geometry [32].

The magnetic moment values (Table 3) of all Cu(II) complexes are observed in the range of 1.74□2.01 BM, which infer the complexes are in distorted octahedral structure [33]. The magnetic moment values are higher than normal range of Cu(II) complexes which may be referred to spin orbit coupling.

3.2.4. ESR spectral studies

ESR spectra of all the Cu(II) complexes were recorded at room temperature in the solid state. The spectral data of all the complexes are given in Table 4. The complexes, $Cu(L_3)_2$ and $Cu(L_4)_2$ showed a

broad band centred at g=2.09 and 2.06, respectively without resolved hyperfine structure. $[Cu(L_2)_2].H_2O$ complex exhibits four well resolved hyperfine peaks and $[Cu(L_1)_2].2H_2O$ complex with unresolved peaks (Fig. 3). The values of the complexes are $g_{\|} > g_{\perp} > 2.0023$, indicating that the unpaired electron in the ground state of Cu(II) is predominately lies in $d_{x^2-y^2}$ orbital [34] and also suggesting a tetragonal distortion around Cu(II) ions [35]. Kivelson and Neiman showed that for an ionic environment $g_{\|}$ is normally 2.3 or larger, but for covalent environment $g_{\|}$ is less than 2.3. In the present study, $g_{\|}$ value for the Cu(II) complexes are in the range of 2.19□2.20, consequently the environment is covalent. The $(g_{\|}/A_{\|})$ values also indicates the stereochemistry of the Cu(II) complexes. Reported range for square planar complexes is 105□135 cm^{-1} and for tetragonal distorted complexes is >135 cm^{-1} [36]. For the present Cu(II) complexes $g_{\|}/A_{\|}$ value is >135 cm^{-1}, indicates the tetragonal distortion. The metal-ligand σ bonding parameter α^2 is calculated using the literature [37]. The value of exchange interaction term G was calculated from the following expression

$$G = \frac{g_{\|} - 2.0023}{g_{\perp} - 2.0023}$$

According to Hathaway [38], if the value of G is greater than 4, the exchange interaction between Cu(II) centres in the solid state is negligible. Whereas its values are less than 4, a considerable exchange interaction exists in the solid complex. The G values obtained in the present Cu(II) complexes are greater than 4, indicating the absence of exchange interaction between Cu(II) centres in the solid state [39]. Further, orbital reduction parameters like $K_{\|}^2 = \alpha^2\beta^2$, $K_{\perp}^2 = \alpha^2\gamma^2$ are calculated using following expressions [40, 41].

$$g_{\|} = 2.0023 - \left[\frac{8 \times \lambda_0 \times \alpha^2\beta^2}{\Delta E_{xy}}\right],$$

$$g_{\perp} = 2.0023 - \left[\frac{2 \times \lambda_0 \times \alpha^2\gamma^2}{\Delta E_{xz}}\right]$$

Here α^2 measures the in-plane σ bonding, β^2 measures the in-plane π-bonding and γ^2 is the out of plane π-bonding. A number of conclusions are deduced from these parameters. α^2 values for purely σ bonding ligands such as ammonia and ethylenediamine lies in the range of 0.73□0.77. For completely covalent bond α^2 is 0.5 and for purely ionic bond α^2 is 1. In the present complexes, α^2 values fall in the range 0.6□0.7 indicating appreciable covalency in the σ bond. $K_{\perp}^2 > K_{\parallel}^2$ is for in-plane π-bonding and $K_{\perp}^2 < K_{\parallel}^2$ is for out of plane π-bonding. In the present complexes $K_{\perp}^2 > K_{\parallel}^2$, this indicates that the complexes are in plane π-bonded. The spin orbit coupling constant for the complexes was found to be less than that of the free metal ion (-828 cm^{-1}), suggesting considerable mixing of ground and excited terms. The values obtained for hyperfine splitting and covalency parameters are well agreement with literature reports [42].

3.2.5. Thermal studies

TG graphs of all Cu(II) complexes are shown in Fig. 4. The decomposition stages, temperature range, as well as the observed and calculated mass loss percentages of all the complexes are illustrated in Table 5. Thermo gravimetric analysis (TGA) was used as a probe to establish the presence of water molecules either in the coordination sphere or in the ionic sphere [43]. Generally in TG Analysis, lattice water molecules were lost in the temperature range of 50□120 ^oC and coordinated water molecules at 120□250 ^oC range.

$[Cu(L_1)_2].2H_2O$ complex was decomposed in four steps. The first step is corresponding to the loss of two lattice water molecules in the temperature range between 40□144 ^oC with a mass loss of 5.39%. The second (145□289 ^oC) and third steps (290□460 ^oC) are the decomposition of schiff base ligand with the mass loss 23.09% and 16.93%, respectively. The final step found in the range of (461□779 ^oC) with the mass loss of 41.82% can be regarded as the total loss of schiff base ligand. The remaining mass 12.77% is due to the formation of CuO as residue.

$[Cu(L_2)_2].H_2O$ complex is decomposed in three steps. The first step is due to the loss of lattice water molecule below the 120 ^oC with the mass loss of 2.65%. The second step (121□532 ^oC) corresponds to the loss of 61.76% which may be due to the removal of schiff base ligand. The third step (533□827

^{o}C) is corresponding to the removal of a fragment of the schiff base ligand with the mass loss 23.54%. The remaining mass 12.05% is corresponds to the formation of CuO as residue.

$Cu(L_3)_2$ complex is stable up to 137 ^{o}C after that it is decomposed in two steps. In the first step complex shows the mass loss of 18.04% in the temperature range (137□368 ^{o}C), corresponding to the partial decomposition of schiff base ligand. And the second step found in the range (369□885 ^{o}C) with a mass loss of 68.45% was assigned to the complete removal of schiff base moiety. The residue with a mass of 13.51% was regarded as CuO.

$Cu(L_4)_2$ complex is decomposed in two steps. The first (40□374 ^{o}C) and second (375□852 ^{o}C) steps are due to the loss of ligand molecules with the mass loss of 61.78% and 25.55% respectively. The remaining mass 12.57% is regarded CuO as residue.

The overall weight loss and weights of residues are well in agreement with the experimental error.

On the basis of the above studies the proposed structures of the Cu(II) complexes are shown in Fig. 5.

3.2.6. Fluorescence studies

The emission spectra of all the ligands and their Cu(II) complexes were recorded in the solid state. The emission spectra of HL_3 and its Cu(II) complex are shown in Fig. 6. The HL_1 ligand was characterised by an emission band around 540 nm upon photo excitation at 398 nm. Cu(II) complex of HL_1 shows three emission bands at 541, 604 and 727 nm. The HL_2 ligand exhibits a band at 492 nm and their Cu(II) complex was characterised by the emission bands at 494, 540 and 652 nm. HL_3 and its Cu(II) complex exhibit emission bands at 524 and 552, 621, 748 nm respectively. HL_4 and its Cu(II) complex was characterised by an emission bands at 492 and (440, 490, 599) nm respectively upon photo excitation at different wavelengths. There was a decrease in the intensities of the emission bands of complexes compared to the respective ligands. Earlier reports showed that transition metals decrease the fluorescence intensity very effectively [44□46].

3.2.7. Powder XRD and SEM studies

The Powder XRD patterns of HL_3 and its Cu(II) complex are shown in Fig. 7. The observed unit cell parameters are given in Table 6. The Powder XRD patterns of all ligands and their Cu(II) complexes were recorded. Peak positions of all ligands were different from its Cu(II) complexes indicating the formation of metal complexes. Unit cell parameters were found by using trial and error methods. All compounds are triclinic with different unit cell parameters. The XRD patterns of all complexes are similar and suggest that all the complexes possess similar structure. The average crystallite sizes for all compounds is calculated using Debye Scherrer's formula [47]

$$d_{xrd} = \frac{0.9 * \lambda}{\beta cos\theta}$$

Where λ is the wavelength of Cu-Kα radiation (1.5406 Å), β is the full width at half maximum and θ is the Braggs reflection angle. The crystallite sizes are found to be in the range of 10□100 nm for all the compounds. The morphology and grain size of the metal complexes have been illustrated using the scanning electron macrograph (SEM). The SEM micrographs of $[Cu(L_1)]_2.2H_2O$ and $[Cu(L_3)]_2$ are shown in Fig. 8. SEM images of the metal complexes with uniform matrix imply their homogeneous nature. $[Cu(L_1)]_2.2H_2O$ and $[Cu(L_3)]_2$ complexes show plate and semi needle like morphologies, respectively.

3.2.8. Biological activity studies

3.2.8.1. Antimicrobial activity

The MIC values of the antimicrobial activity of all compounds are presented in Table 7. All the complexes exhibited moderate to good activities when compared with the standard antibiotics. The ligands hold less activity except HL_2, against tested strains. All Cu(II) complexes show marked anti microbial activity against all strains when compared to their corresponding ligands. The MIC values were calculated, which have significant activity (Table 7). Higher activity of the complexes was explained based on Overtones concept and chelation theory. According to Overtones concept of cell permeability, the lipid membrane that surrounds the cell favours the passage of only lipid soluble materials. Liposolubility is an important factor which controls the antimicrobial activity. On chelation, the polarity of the metal ion is reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of positive charge of the metal ion with donor groups. Further, it increases the delocalisation of π-electrons over the whole chelating ring and enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms. There are also other factors which increase the activity are solubility, conductivity and bond length between the metal and ligand [48□54].

3.2.8.2. DPPH radical scavenging activity

The scavenging activity was increased by increasing the concentration of the compounds. DPPH antioxidant assay is based on ability of decolourization of DPPH (a stable free radical) in the presence of antioxidants. This radical contains an odd electron responsible for absorbance at 517 nm. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured by changes in absorbance. In the present study, based on the IC_{50} values, the scavenging activities of ligands and their Cu(II) complexes are calculated and those values are given in Table 8. From the results, it was found that the IC_{50} values of $[Cu(L_1)_2].2H_2O$ is 0.16 μg/mL. $[Cu(L_1)_2].2H_2O$ show good activity compared to remaining complexes. HL_1 and HL_2 ligands do not show any scavenging activity. The radical scavenging activity of the compound depends on the

structural factors such as the phenolic hydroxyl, carboxylic groups and other structural features [55]. The order of the scavenging activity of all the complexes according to their IC_{50} values is given below.

$[Cu(L_1)_2].2H_2O > [Cu(L_2)_2].H_2O > [Cu(L_3)_2] > [Cu(L_4)_2]$

3.2.8.3. DNA cleavage activity

DNA cleavage activity was studied for all the ligands and their Cu(II) complexes using gel electrophoresis method against pUC19 DNA in the presence and absence of H_2O_2 as an oxidant. The results of the DNA cleavage studies are depicted in Fig. 9. When circular plasmid was subjected to gel electrophoresis, the fastest migration will be observed for the supercoil form (Form I). If scission occurs on one strand it is called as nicked circular (Form II). The supercoil will relax to generate a slower moving nicked circular form (Form II). If both strands are cleaved, a linear form (Form III) that migrates between Form I and Form II will be generated [56]. To investigate the mechanism of nucleolytic activity, experiments were also performed in the presence of H_2O_2. All the ligands exhibited significant activity in the presence of H_2O_2. In the absence of H_2O_2, all ligands do not show cleavage activity but their Cu(II) complexes are able to convert supercoiled (Form I) to nicked circular (Form II) and open circular form (Form III). Thus revealed that all Cu(II) complexes except $[Cu(L_3)_2]$ show good nuclease activity in the absence of any additives. Control (DNA alone) does not show any cleavage. $FeSO_4$ is used as standard, it shows complete DNA cleavage activity. In the presence of H_2O_2, the intensity of the treated DNA samples has diminished because of the DNA cleavage [57]. Ligands HL_1, HL_2 and HL_4 show cleavage activity except HL_3. $[Cu(L_3)_2]$ complex shows moderate activity compared to the remaining complexes. In all the ligands (except HL_3) and their Cu(II) complexes, more pronounced activity was observed in the presence of oxidant compared to the absence of oxidant, may be due to the increased production of hydroxyl radicals. These results indicate that the Cu(II) ion also plays an important role in the cleavage activity. As the compound has DNA cleavage activity, it can be concluded that the compound inhibits the growth of the pathogenic organism by cleaving the genome [58].

4. Conclusions

In the present study, we synthesised 3-formyl chromone schiff bases and their Cu(II) complexes. All synthesized complexes were characterized by using various spectroscopic techniques. The results revealed that all the ligands were coordinated to the Cu(II) ions in tridentate manner and geometrical structures of these complexes were found to be tetragonally distorted octahedral. From the thermograms the number of lattice water molecules present in the complexes was calculated. The well defined crystalline and homogeneous nature of the Cu(II) complexes was observed from PXRD and

SEM studies. The schiff bases and their Cu(II) complexes are fluorescent in nature. All the Cu(II) complexes exhibited prominent antimicrobial activity than ligands. All the Cu(II) complexes show good DPPH scavenging activity. DNA cleavage activity was observed in the presence of H_2O_2 for all ligands (except HL_3) and their Cu(II) complexes.

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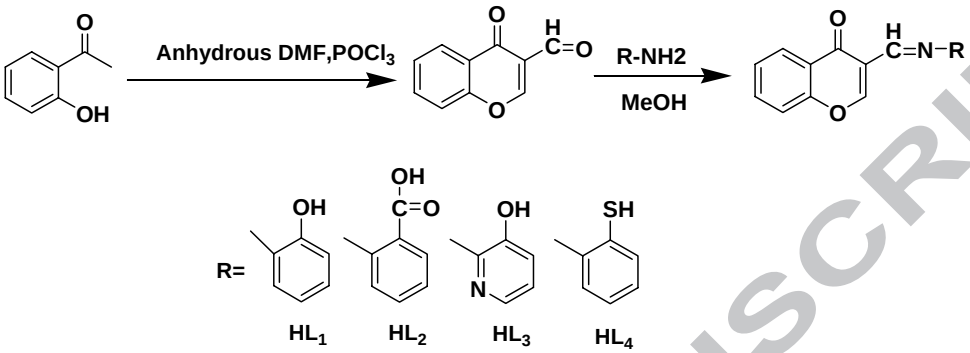
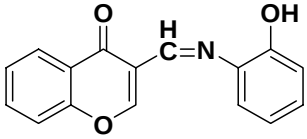
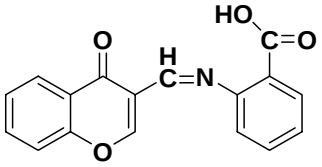
FIGURES



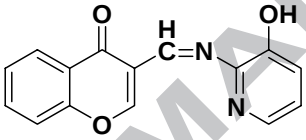
Scheme 1. Synthesis of schiff base ligands



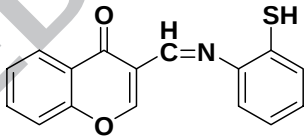
3-(-(2-hydroxyphenylimino)methyl)-4*H*-chromen-4-one
(HL_1)



2-((4-oxo-4*H*-chromen-3-yl)methyleneamino)benzoic acid
(HL_2)



3-((3-hydroxypyridin-2-ylimino)methyl)-4*H*-chromen-4-one
(HL_3)



3-(-(2-mercaptophenylimino)methyl)-4*H*-chromen-4-one
(HL_4)

Figure 1. Structures of schiff base ligands

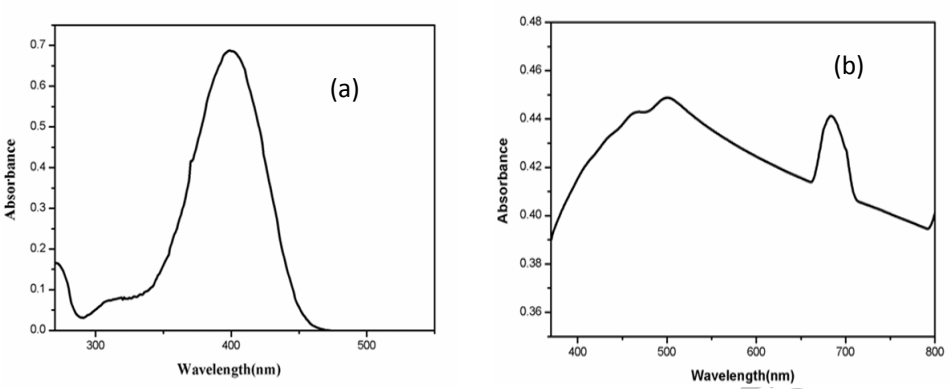


Figure 2. Electronic spectra of (a) HL_1 (b) $[Cu(L_1)_2].2H_2O$

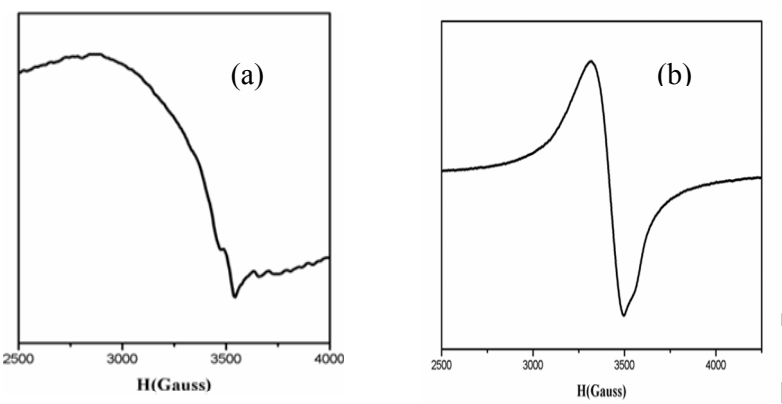


Figure 3. ESR spectra of (a) $[Cu(L_1)_2].2H_2O$, (b) $[Cu(L_4)_2]$.

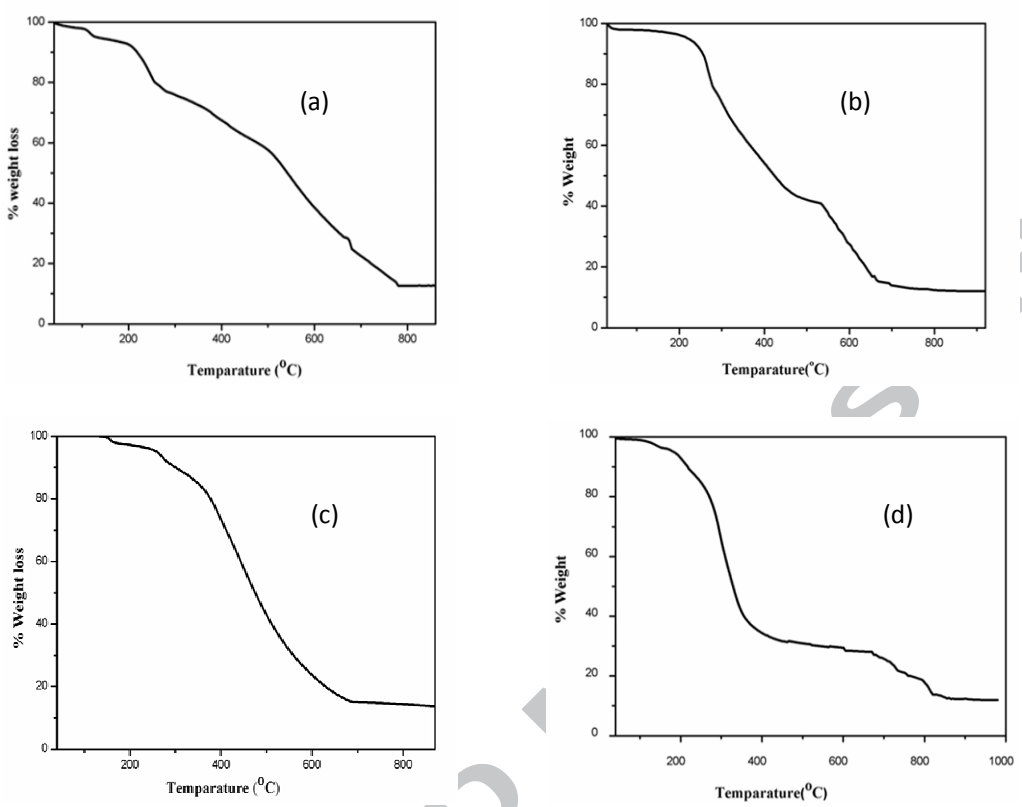


Figure 4. TG graphs of (a) $[Cu(L_1)_2].2H_2O$, (b) $[Cu(L_2)_2].H_2O$, (c) $[Cu(L_3)_2]$, (d) $[Cu(L_4)_2]$

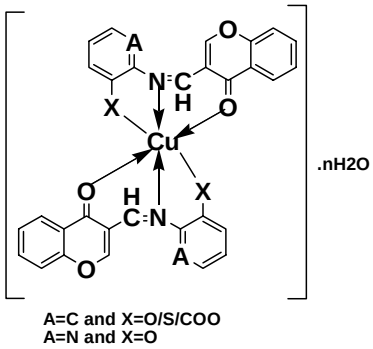


Figure 5. Structure of Cu(II) complexes

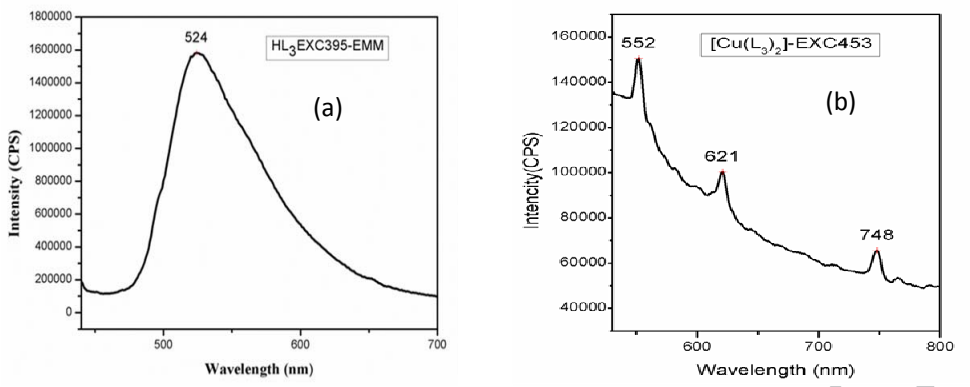


Figure 6. Emission spectra of (a) HL_3 (b) $[Cu(L_3)_2]$

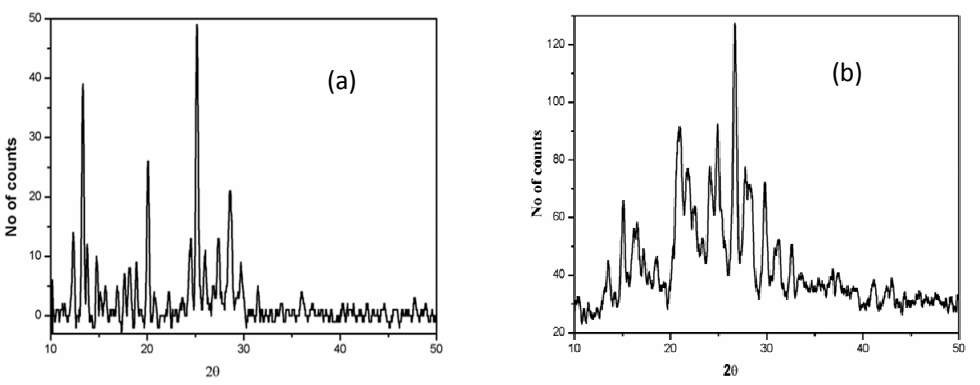


Figure 7. PXRD patterns of (a) HL_3 (b) $[Cu(L_3)_2]$

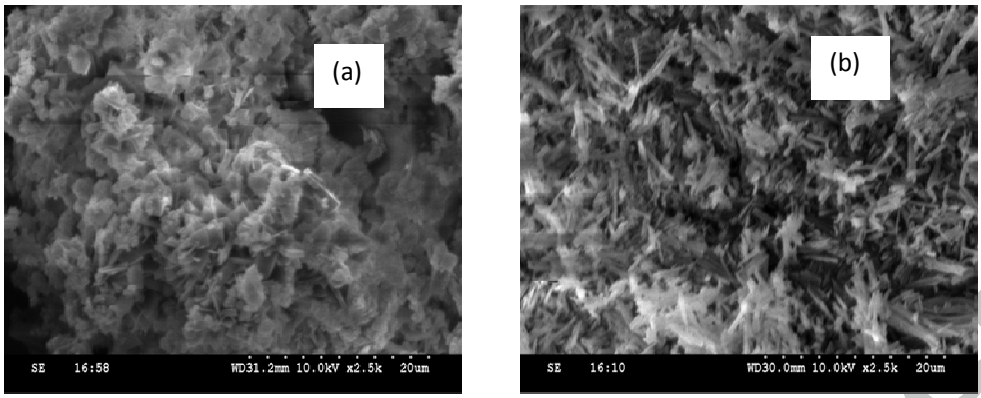


Figure 8. SEM micrographs of (a) $[Cu(L_1)_2].2H_2O$ (b) $[Cu(L_3)_2]$

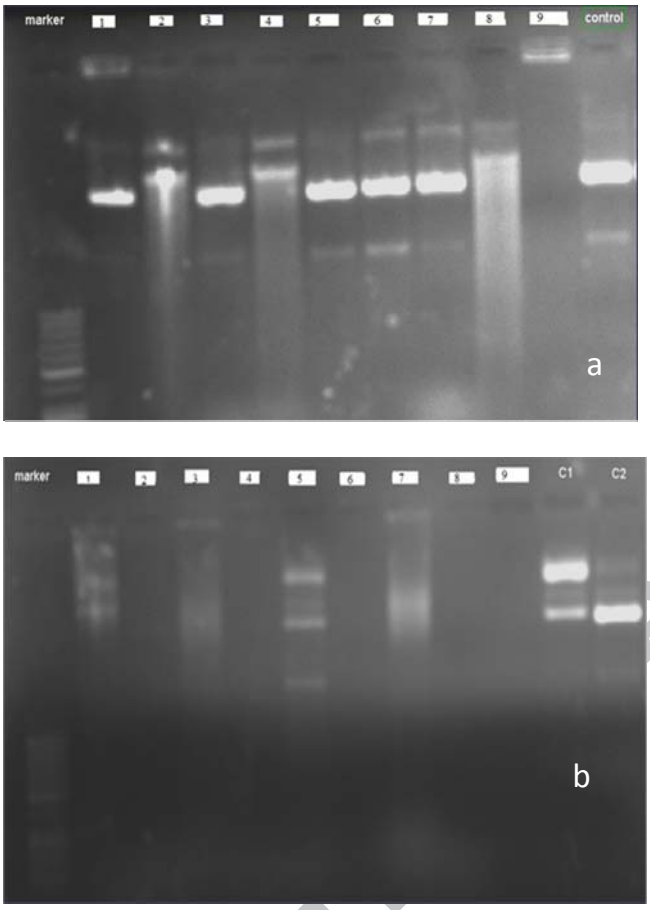


Figure 9. DNA cleavage studies of ligands and their Cu(II) complexes.

a. DNA cleavage activity in the absence of H_2O_2. Lane 1: DNA + HL_1, Lane 2: DNA + $[Cu(L_1)_2].2H_2O$, Lane 3: DNA + HL_2, Lane 4: DNA + $[Cu(L_2)_2].H_2O$, Lane 5: DNA + HL_3, Lane 6: DNA + $[Cu(L_3)2]$, Lane 7: DNA + HL_4, Lane 8: DNA + $[Cu(L_4)_2]$, Lane 9: DNA + $FeSO_4$, Lane control : DNA alone.
b. DNA cleavage in the presence of H_2O_2. Lane 1: DNA + HL_1 + H_2O_2, Lane 2: DNA + $[Cu(L_1)_2].2H_2O$ + H_2O_2, Lane 3: DNA + HL_2+ H_2O_2, Lane 4: DNA + $[Cu(L_2)_2].H_2O$ + H_2O_2, Lane 5: DNA + HL_3 + H_2O_2, Lane 6: DNA + $[Cu(L_3)_2]$ + H_2O_2, Lane 7: DNA + HL_4 + H_2O_2, Lane 8: DNA + $[Cu(L_4)_2]$ + H_2O_2, Lane 9: DNA + $FeSO_4$ + H_2O_2, Lane C1: DNA + H_2O_2, Lane C2: DNA alone.

Table1. Physical, analytical and molar conductivity data of Cu(II) complexes

Molecular Formula	Mol. Wt	Colour (%yield)	% Found (cald)					Molar Conductivity ($mho^{-1}mol^{-1}cm^2$)
			C	H	N	S	Cu	
$[Cu(L_1)_2].2H_2O$ $[Cu(C_{16}O_3NH_{10})_2].2H_2O$	627.5	Green (75)	61.19 (61.19)	3.75 (3.82)	4.39 (4.46)		10.09 (10.11)	20
$[Cu(L_2)_2].H_2O$ $[Cu(C_{17}O_4NH_{10})_2].H_2O$	665.5	Dark green (80)	61.59 (61.30)	3.49 (3.30)	4.15 (4.20)		9.30 (9.54)	18
$[Cu(L_3)_2]$ $Cu[(C_{15}O_3N_2H_9)_2]$	593.5	Leaf green (80)	60.55 (60.65)	3.04 (3.03)	9.51 (9.43)		10.82 (10.70)	14
$[Cu(L_4)_2]$ $Cu(C_{16}O_2NSH_{10})_2$	623.5	Green (76)	61.54 (61.58)	3.19 (3.20)	4.38 (4.49)	10.25 (10.26)	10.24 (10.19)	15

Table 2. FTIR spectral data of ligands and their Cu(II) complexes

Compound	υ(OH)	υ(C═O)	υ(C═N)	υ(CO)	υ(C-S)	υ(M-N)	υ(M-O)
HL_1	3241	1643	1605				
HL_2		1651	1605	1365			
HL_3	3246	1647	1591				
HL_4		1622	1597		790		
$[Cu(C_{16}O_3NH_{10})_2].2H_2O$		1603	1587			439	532
$[Cu(C_{17}O_4NH_{10})_2].H_2O$		1621	1589	1351		449	528
$[Cu(C_{15}O_3N_2H_9)_2]$		1599	1580			446	511
$[Cu(C_{16}O_2NSH_{10})_2]$		1644	1572		749	468	523

Table 3. Electronic spectral data of ligands and their Cu(II) complexes

Compound	Band position (cm^{-1})	Assignment	μ_{eff}(B.M)
HL_1	26000	$n \rightarrow \pi^*$	-
	33003	$\pi \rightarrow \pi^*$	
HL_2	26315	$n \rightarrow \pi^*$	-
	32258	$\pi \rightarrow \pi^*$	
HL_3	25316	$n \rightarrow \pi^*$	-
	37037	$\pi \rightarrow \pi^*$	
HL_4	27027	$n \rightarrow \pi^*$	-
	32467	$\pi \rightarrow \pi^*$	
$[Cu(L_1)_2].2H_2O$	14598	$^2B_{1g} \rightarrow {}^2B_{2g}$	1.86
	19762	$^2B_{1g} \rightarrow {}^2E_g$	
$[Cu(L_2)_2].H_2O$	14405	$^2B_{1g} \rightarrow {}^2B_{2g}$	1.92
	18726	$^2B_{1g} \rightarrow {}^2E_g$	
	23075	Charge transfer	
$[Cu(L3)_2]$	13700	$^2B_{1g} \rightarrow {}^2B_{2g}$	1.74
	19840	$^2B_{1g} \rightarrow {}^2E_g$	
$[Cu(L_4)_2]$	13513	$^2B_{1g} \rightarrow {}^2B_{2g}$	2.01
	19607	$^2B_{1g} \rightarrow {}^2E_g$	

Table 4. ESR spectral data of Cu(II) complexes

ESR spectral data	$[Cu(L_1)_2].2H_2O$	$[Cu(L_2)_2].H_2O$	$[Cu(L_3)_2]$	$[Cu(L_4)_2]$
$g_{\|}$	2.20	2.19	-	-
$g_{\perp}$	2.04	2.05	-	-
$\|g\|$	2.09	2.09	2.09	2.06
$A_{\|} \times 10^{-4}$ cm^{-1}	157	137	-	-
$A_{\perp} \times 10^{-4}$ cm^{-1}	65	49	-	-
$\|A\| \times 10^{-4}$ cm^{-1}	96	78	-	-
G	5.24	4.00	-	-
$g_{\|}/A_{\|}$ (cm^{-1})	140	160		
α^2	0.68	0.63	-	-
β^2	0.64	0.57	-	-
γ^2	0.66	0.85	-	-
$K_{\|}^2$	0.44	0.36	-	-
$K_{\perp}^2$	0.45	0.54	-	-
$-\lambda$(cm^{-1})	493	444	-	-

Table 5. TG data of Cu(II) complexes

Compound	Temperature range(°C)	Mass loss Found (cald%)	Assignment
$[Cu(L_1)_2].2H_2O$	28-144	5.39(5.74)	$2H_2O$
$[Cu(C_{16}O_3NH_{10})_2].2H_2O$	145-289	23.09(23.11)	$C_9O_2H_5$
	290-460	16.93(16.41)	C_7NH_5
	461-779	41.82(42.07)	$C_{16}O_3NH_{10}$
	>780	12.77(12.67)	CuO(Residue)
$[Cu(L_2)_2].H_2O$	40-120	2.65(2.70)	H_2O
$[Cu(C_{17}O_4NH_{10})_2].H_2O$	121-532	61.76(61.61)	$C_{24}O_5N_2H_{14}$
	533-827	23.54(23.74)	$C_{10}O_2H_6$
	>828	12.05(11.95)	CuO (Residue)
$[Cu(L_3)_2]$	137-368	18.04(18.02)	$C_5ON_2H_3$
$[Cu(C_{15}O_3N_2H_9)_2]$	369-885	68.45(68.57)	$C_{25}O_4N_2H_{15}$
	>886	13.51(13.41)	CuO(Residue)
$[Cu(L_4)_2]$	40-374	61.78(61.92)	$C_{22}ON_2S_2H_{14}$
$[Cu(C_{16}O_2NSH_{10})_2]$	375-852	25.55(25.33)	$C_{10}O_2H_6$
	>853	12.67(12.75)	CuO(Residue)

Table 6. Unit cell parameters of ligands and their Cu(II) complexes

Compound	a(A^o)	b(A^o)	c(A^o)	α(°)	β(°)	γ(°)	V(A^o)^3	d_{xrd}(nm)
HL_1	10.28	8.44	7.78	102.58	105.63	95.69	675	53
HL_2	11.85	8.14	9.54	108.64	115.19	93.89	920	105
HL_3	10.84	7.44	7.37	98.64	117.46	99.48	595	22
HL_4	8.68	6.42	6.18	98.70	103.05	102.70	345	45
$[Cu(L_1)_2].2H_2O$	10.74	7.67	6.81	99.73	120.01	93.58	561	16
$[Cu(L_2)_2].H_2O$	12.94	8.42	9.64	105.18	119.04	91.49	1052	22
$[Cu(L_3)_2]$	9.64	8.35	8.18	93.56	105.01	95.16	660	27
$[Cu(L_4)_2]$	8.24	6.61	6.28	96.86	102.54	111.21	343	12

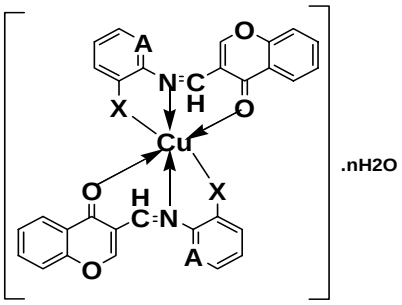
Table 7. MIC values of antimicrobial activity of ligands and their Cu(II) complexes (in μg/mL)

Compound	*Bacillus subtilis*	*Staphylococcus aureus*	*Proteus vulgaris*	*Klebsiella pneumoniae*	*Candida albicans*
HL_1	-	-	-	-	-
HL_2	80	80	80	80	80
HL_3	-	-	-	-	-
HL_4	-	-	-	-	-
$[Cu(L_1)_2].2H_2O$	30	30	30	30	35
$[Cu(L_2)_2].H_2O$	30	30	30	30	33
$[Cu(L_3)_2]$	30	20	30	20	30
$[Cu(L_4)_2]$	55	55	55	55	55
Kanamycin	04	10	08	11	--
Clotrimazole	--	--	--	--	10

Table 8. IC_{50} values of DPPH scavenging activity of ligands and their Cu(II) complexes

Compound	IC_{50}(µg/mL)
HL_3	1.27
HL_4	0.40
$[Cu(L_1)_2].2H_2O$	0.16
$[Cu(L_2)_2].H_2O$	0.51
$[Cu(L_3)_2]$	0.70
$[Cu(L_4)_2]$	0.91
BHT	0.67

Graphical absract



A=C and X=O/S/COO
A=N and X=O

Highlights

- Formyl chromone schiff bases and their Cu(II) complexes have been synthesised and characterised.
- The synthesised complexes acts as potential anti oxidant agents.
- All ligands and their Cu(II) complexes exhibit good fluorescence.
- Complexes displayed an efficient DNA cleavage activity when compared with the ligands.