



Research Article

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Synthesis, characterization, DNA cleavage and antimicrobial studies of ternary Co(II) complexes of 3-formylchromone Schiff bases

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ABSTRACT

Four novel ternary cobalt(II) complexes of the composition $[CoLX]$ where $X = 1, 10$ phenanthroline, $L = 2$ -{[(4-oxo-4H-chromen-3-yl)methylene]amino}benzoic acid (CMABA), 4-chloro-2-{[(4-oxo-4H-chromen-3-yl)methylene]amino}benzoic acid (CCMABA), 5-nitro-2-{[(4-oxo-4H-chromen-3-yl)methylene]amino}benzoic acid (NCMABA) and N' -[(4-oxo-4H-chromen-3-yl)methylene] benzohydrazide (CMBH) were synthesized and characterized by using elemental analysis, FT-IR, UV-Vis, ESI-MS, molar conductance, thermal analysis (TG, DTA) and magnetic susceptibility measurements. The metal to ligand stoichiometry in all the complexes is 1:1:1. The complexes of CMABA, CCMABA and NCMABA are non-electrolytes; the complex of CMBH is 1:1 electrolyte in DMSO. CMABA, CCMABA and NCMABA act as mononegative, tridentate ligands coordinating through nitrogen of azomethine, oxygen of chromone-carbonyl and oxygen of carboxylic group. CMBH act as neutral, tridentate ligand, coordinating through nitrogen of azomethine, oxygen of chromone-carbonyl and oxygen of benzoyl group. 1, 10-phenanthroline acts as neutral bidentate ligand and coordinating through two nitrogen atoms. All these complexes are paramagnetic to the extent of one unpaired electron. Thermal analysis indicates the presence of coordinated water molecule in all the complexes. Based on analytical, conductance, magnetic, thermal and electronic spectral data, all complexes are assigned octahedral geometry. DNA cleavage studies shows that Co(II) complexes cleave DNA. The biological activity of the ligand and its complexes have been studied on four bacteria *E.coli*, *B.subtilis*, *Pseudomonas* and *Edwardella* and two fungi *penicillium* and *trichoderma* by well disc and fusion method and found that the ternary metal complexes are more active than the free Schiff base ligand.

Key words: 3-formylchromone, ternary complexes, DNA cleavage, antimicrobial studies

INTRODUCTION

Chromones have a wide range of biological activities including tyrosine and protein kinase C inhibitors, antiallergic, antiviral, antitubulin, antihypertensive as well being active at benzoazepine receptors, lipoxigenase, cyclooxygenase and modulating P-glycoprotein-mediated multidrug resistance [1,2]. Chromone derivatives are essential for the synthesis of many important oxygen heterocycles, pyrazoles and xanthenes [3]. Photosensitive chromone derivatives show prospects as light-sensitive components of recording media for archival three-dimensional optical memory [4]. Kawase [5] et al. examined several formyl chromones for their tumor cell-cytotoxic, anti-*Helicobacter pylori*, urease inhibitory and anti-HIV activity and observed that, 6,8-dichloro-3-formylchromone showed comparable anti-*Helicobacter pylori* activity with metronidazole and potent urease inhibition against jack bean urease. On the other hand, 6,8-dibromo-3-formylchromone exhibited potent inhibitory activity against the urease. It was found that these drugs showed increased activity, when administered as metal complexes rather than as organic compounds.

In recent years much attention has been paid to the studies of mixed ligand complexes of metals because of their wide applications in various fields of chemical importance and more particularly because of their presence in

biological systems. Mixed ligand metal complexes of transition metal ions involving Schiff bases found application towards understanding of many reactions in living processes [6]. The study of ternary complexes of metal ions with two different types of ligands is a topic of interest due to their presence in biological systems [7]. On the other hand, 1, 10-phenanthroline (1, 10-phen) the ligand moiety of the ternary complexes presented in this work is of considerable interest according to the biological and pharmacological properties [8]. Moreover, considerable attention was focused on the use of small metal complexes of the large planar aromatic chelating ligands (1, 10-phen or 2, 2-bipyridine) as DNA intercalating agents [9]. In recent years such complexes were studied as model compounds of biological importance [10]. The importance of ternary complexes in nature is evident since a great deal of biochemical reactions occurs within the coordination sphere of metal ions. Ternary complexes formation has important biological implications because of enhanced probability of bringing together two different kinds of ligands.

From the literature survey we observed that 3-formyl chromone and ternary metal complexes are having important biological applications, so as ongoing research we thought that it is worthwhile to synthesize and study the ternary metal complexes with the title ligands. We present here, the synthesis, characterization and evolution of biological activities (DNA cleavage and antimicrobial) of ternary Co(II) complexes with CMABA / CCMABA / NCMABA / CMBH as primary ligand and 1,10-phenanthroline as secondary ligand.

EXPERIMENTAL SECTION

2.1 Chemicals: chromone-3-carbaldehyde, 2-amino benzoic acid, 2-amino-4-chloro benzoic acid, 2-amino-5-nitro benzoic acid and benzoylhydrazine were purchased from Sigma-Aldrich chemicals, remaining all chemicals and solvents were purchased from commercial sources and used as such without purification.

2.2 Synthesis of the Schiff base ligand:

A mixture of chromone-3-carbaldehyde (0.01 mol) and 2-amino benzoic acid / 2-amino-4-chloro benzoic acid / 2-amino-5-nitro benzoic acid / benzoylhydrazine (0.01 mol) was taken in ethanol and refluxed for 3 hours at 40°C on magnetic stirrer. The yellow colored Schiff bases obtained were recrystallised from petroleum ether and methanol. Purity of the compounds was checked by TLC. Yield: 80-90%.

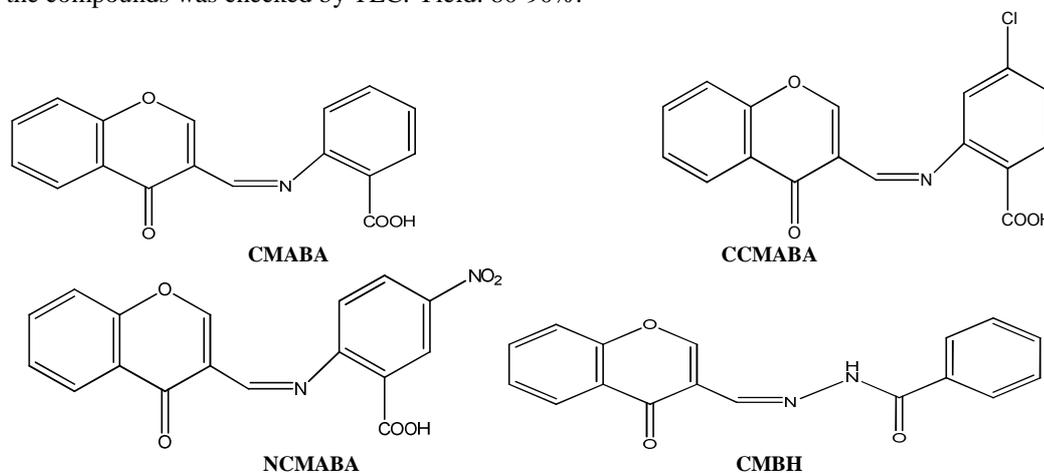


Fig. 1 Proposed structures of Schiff base ligands

2.3 Synthesis of ternary metal complexes: The appropriate quantity of CMABA / CCMABA / NCMABA / CMBH was dissolved in hot methanol and a hot solution of CoCl₂.6H₂O in methanol was added. The mixture solution was stirred for 40 minutes at 80°C, and then a solution of 1,10-phenanthroline in methanol was added dropwise. The reaction mixture was refluxed with stirring further for 2-3 hours at 80°C. The precipitated solid was then filtered, washed with cold methanol and dried in a vacuum desiccator over anhydrous CaCl₂.

2.4 Instruments used: The percentage composition of C, H and N of complexes and ligands were determined using micro analytical methods on Perkin Elmer 240C (USA) elemental analyzer. FT-IR spectra of the ligand and its complexes were recorded by using KBr pellets in the range 4000-400 cm⁻¹ on Perkin Elmer Infra red model 337. The UV-Visible spectra of the Schiff base ligand and its metal complexes were carried out in DMSO using a Shimadzu UV-1601 spectrophotometer. ¹H NMR spectrum of the ligand was recorded at 200MHz and 300MHz on Varian Gemini Unity Spectrometer using TMS as internal standard. The mass spectra of the compounds were recorded by ESI technique on VG AUTOSPEC mass spectrometer. TGA and DTA analysis of complex were carried on Mettler Toledo Star system in the temperature range 0-1000°C. The heating rates were controlled by 10°C min⁻¹.

Magnetic measurements were carried out on a Gouy balance model 7550 using $\text{Hg}[\text{Co}(\text{NCS})_4]$ as standard. The conductivity measurements were carried out in DMSO (10^{-3}M) using Digisum Electronic Digital conductivity meter, 0.01M KCl solution is used for calibration. Melting points of the ligand and decomposition temperature of complexes were determined on Polmen instrument (model no.MP-96). Molecular modelings of the compounds were carried out using Chem Office software.

2.5. DNA cleavage studies:

A DMSO solution containing the metal complexes ($15\ \mu\text{M}$) in a clean Eppendorf tube was treated with pUC19 plasmid DNA ($3.3\ \mu\text{l}$ of $150\ \mu\text{g}/\text{ml}$) in Tris-HCl buffer (0.10 M, pH 8.0) containing NaCl (50 mM) in presence and absence of additives. The contents were incubated for 1 h at $37\ ^\circ\text{C}$ and loaded onto a 1 % agarose gel after mixing $5\ \mu\text{l}$ of loading buffer (0.25 % bromophenol blue). The electrophoresis was performed at a constant voltage (80 V) until the bromophenol blue had travelled through 75 % of the gel. Subsequently, the gel was stained for 10 min by immersion in ethidiumbromide solution. The gel was then destained for 10 min by keeping it in sterile distilled water. The plasmid bands were visualized by viewing the gel under a transilluminator and photographed.

2.6. Biological activity:

2.6.1. Antibacterial activity: The antibacterial activity of Schiff base ligand and its transition metal complexes were studied against four bacteria, *E.coli*, *B.subtilis*, *Edwadrella* and *Trichoderma*. Each of the compounds is dissolved in DMSO at a concentration $1\text{mg}/\text{ml}$. Paper discs of Whatmann filter paper No.1 are used after sterilization. The paper discs were saturated with $10\ \mu\text{l}$ of the compound dissolved in DMSO solution and was placed in Petri dishes containing nutrient agar media inoculated with the above-mentioned bacteria separately. The inhibition zone was measured in millimeters after 24 hrs incubation at $37\ ^\circ\text{C}$.

2.6.2. Antifungal activity: The complexes were screened for their antifungal activity against fungi viz. *Penicillium* and *Trichoderma*. These fungal species were isolated from the infected parts of the host plants i.e. potato dextrose agar. The cultures of the fungi were purified by single spore isolation technique. A concentration of $1\ \text{mg}/\text{ml}$ of each metal complex compound in DMSO solution was prepared for testing against spore germination of each fungus. Filter paper discs of 5 mm in size were prepared by using Whatmann filter paper no. 1 (sterilized in an autoclave) and are saturated with $10\ \mu\text{l}$ of the metal complex compounds dissolved in DMSO solution. The fungal culture plates were inoculated at $25\pm 2\ ^\circ\text{C}$ for 48 h. The plates were then observed and the diameters of the inhibition zones (in mm) were measured and tabulated.

2.7. Molecular modeling's: Molecular modeling's for the proposed structures of metal complexes were carried out by using MM2CS Chem Office version 11.0 molecular modeling program.

RESULTS AND DISCUSSION

3.1. Physical characteristics of the complexes:

All the complexes are colored, stable at room temperature and are non-hygroscopic. On heating, they melt at high temperatures. The complexes are insoluble in water and are soluble in DMSO.

3.2. Elemental analysis:

The analytical data of the complexes are represented in **Table-1**. It is clear from the data that the experimental values shown for each of the complexes, are in good agreement with the theoretical values calculated for the complexes. The values reveal that the metal to ligand ratio is 1:1:1.

Table-1: Analytical data of ternary Cu(II) complexes

Complex	Formula	M.Wt.	C	H	N	M
$[\text{Co}(\text{II})\text{-CMABA-1,10-phen}]^+$	$[\text{CoC}_{29}\text{H}_{20}\text{N}_3\text{O}_5]$	549.4	63.18 (63.39)	3.91 (4.04)	9.83 (10.20)	10.56 (10.73)
$[\text{Co}(\text{II})\text{-CCMABA-1,10-phen}]^+$	$[\text{CoC}_{29}\text{H}_{19}\text{N}_3\text{O}_5\text{Cl}]$	583.8	59.14 (59.66)	3.16 (3.28)	7.01 (7.20)	9.83 (10.09)
$[\text{Co}(\text{II})\text{-NCMABA-1,10-phen}]^+$	$[\text{CoC}_{29}\text{H}_{19}\text{N}_4\text{O}_7]$	594.4	58.16 (58.60)	3.04 (3.22)	9.11 (9.43)	9.75 (9.91)
$[\text{Co}(\text{II})\text{-CMBH-1,10-phen}]^{2+}$	$[\text{CoC}_{29}\text{H}_{22}\text{N}_4\text{O}_4]$	549.4	63.12 (63.39)	3.94 (4.04)	9.23 (10.20)	10.66 (10.73)

*Found (Calculated)

3.3. Conductivity:

The conductance values for the present ternary Co(II) complexes of CMABA, CCMABA and NCMABA are around $60\ \text{Ohm}^{-1}\cdot\text{cm}^2\cdot\text{mol}^{-1}$ in DMSO ($10^{-3}\ \text{M}$) indicating electrolytic (1:1) nature, where as the conductance value for ternary Co(II) complex of CMBH is $140.3\ \text{Ohm}^{-1}\cdot\text{cm}^2\cdot\text{mol}^{-1}$ indicating that the complex is 1:2 electrolyte [11].

3.4. Thermal analysis (TGA/DTA): From the thermograms of these complexes, it is concluded that the coordinated water molecules are eliminated in the temperature range of 150-200°C [12]. Further the ligands gradually decompose to their corresponding metal oxides at high temperatures. The thermogram of CMABA and CMBH are given in Fig-2. Presence of water molecule is further confirmed by the endothermic bands observed in the respective DTA curve in the temperature region where the TGA curves should loss in weight. In addition to the endothermic bands, the DTA curves of complexes also show exothermic bands. These bands appeared at higher temperatures, which represent phase transition, oxidation and/or decomposition of the compound.

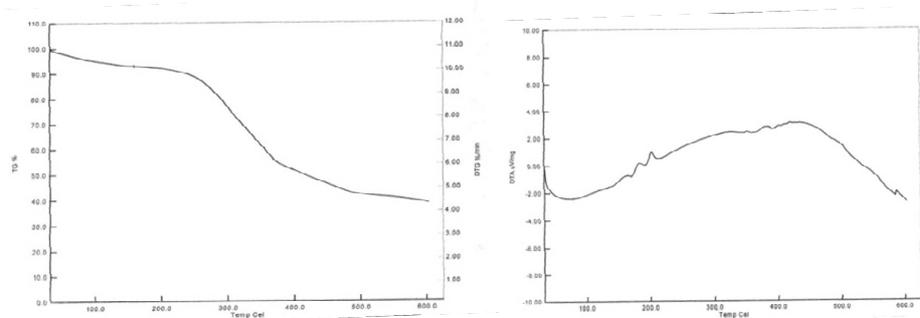


Fig-2. TGA/DTA spectrum of Co(II)-CMBH-1,10-phen

3.5. Infrared spectra:

The important absorption frequencies of Co(II) complexes are given in Table-2. IR spectra of the ligands (CMABA / CCMABA / NCMABA / CMBH) displayed medium intensity band around 1670 cm^{-1} due to chromone carbonyl group is shifted to lower frequency region in the ternary complexes suggesting the participation of oxygen of chromone-carbonyl group in coordination [13]. The IR spectrum of free Schiff base ligands shows an absorption band around 1620 cm^{-1} assigned for the $\nu(\text{C}=\text{N})$ of azomethine group. In the IR spectra of corresponding cobalt complexes, this absorption band have been shifted to lower region by about 10 - 20 cm^{-1} , which confirms the coordination of azomethine group through nitrogen atom [14].

The $\nu(\text{OH})$ (carboxylic acid) and $\nu(\text{C}=\text{O})$ (carboxylic acid) stretching vibrations are observed around 3457 and 1641 cm^{-1} , respectively, for CMABA, CCMABA and NCMABA. The participation of the carboxylate oxygen atom in the complexes formation was evidenced from the disappearance of $\nu(\text{OH})$ and $\nu(\text{C}=\text{O})$ stretching vibrations in the spectra of the complexes. In the same way was the coordination of carboxylic group as carboxylate ion through oxygen atom is evidenced by the appearance of $\nu_{\text{sym}}(\text{COO}^-)$ and $\nu_{\text{asym}}(\text{COO}^-)$ vibrations in the spectra of the complexes in the regions around 1383 and 1538 cm^{-1} , respectively [15]. A band at 1638 cm^{-1} due to $\nu(\text{C}=\text{O})$ of benzoyl hydrazine of CMBH is shifted to lower frequency region in the complex suggesting the participation of oxygen of benzoyl-carbonyl group in coordination [16].

The bands observed in the region 1500-1300 cm^{-1} for the free phenanthroline ligand are modified in the spectra of ternary Co(II) complexes. In particular, the peaks corresponding to the ring stretching frequencies $\nu\text{C}=\text{C}$ and $\nu\text{C}=\text{N}$ at 1505 and 1421 cm^{-1} undergo shifts to higher frequencies at around 1515 and 1428 cm^{-1} , indicating the coordination of the 1,10-phenanthroline nitrogen atoms to the metal ion [17]. The characteristic out-of-plane hydrogen bending modes of free phenanthroline at 855 and 738 cm^{-1} , shift to lower frequency region, indicating the participation of the 1,10-phenanthroline nitrogen atoms in coordination with the metal ion [18]. The far-infrared region of the spectra shows new peaks around 430 and 520-568 cm^{-1} are assigned to the $\nu\text{M-N}$ and $\nu\text{M-O}$ vibrations respectively [19].

Table-2 IR Absorption frequencies of ternary Co(II) complexes

Complex	$\nu\text{C}=\text{O}$ (γ -pyrone)	Coordinated water	$\nu\text{C}=\text{N}$	$\nu\text{C}=\text{O}$ (benzoyl)	$\nu_{\text{as}}(\text{COO}^-)$ (carboxylate)	$\nu_{\text{s}}(\text{COO}^-)$ (carboxylate)	$\nu\text{M-N}$	$\nu\text{M-O}$
[Co(II)-CMABA-1,10-phen] ⁺	1642	727	1607	-	1517	1380	424	558
[Co(II)-CCMABA-1,10-phen] ⁺	1657	728	1605	-	1516	1353	423	547
[Co(II)-NCMABA-1,10-phen] ⁺	1681	727	1605	-	1516	1328	424	568
[Co(II)-CMBH-1,10-phen] ²⁺	1644	764	1614	1614	-	-	424	550

3.6. Magnetic moments:

The magnetic moment values of the ternary Co(II) complexes are listed in Table-3. Magnetically Co(II) complexes are of two types, one having three unpaired electrons as in the case of high-spin octahedral and tetrahedral geometries and the other having one unpaired electrons as in the case of low-spin octahedral geometries. The

magnetic moments of the present Co(II) complexes lie in the range 4.87-5.01 B.M. suggesting high-spin octahedral geometry [20].

Table-3 Electronic spectral data for ternary Co(II) complexes

Complex	Frequency (cm ⁻¹)	μ_{eff} B.M.
[Co(II)-CMABA-1,10-phen] ⁺	13106, 19724, 23697	4.92
[Co(II)-CCMABA-1,10-phen] ⁺	10905, 15823, 23256	4.87
[Co(II)-NCMABA-1,10-phen] ⁺	12626, 15337, 23148	4.89
[Co(II)-CMBH-1,10-phen] ²⁺	10560, 12547, 22371	5.01

3.7. Electronic spectra:

The electronic spectral data of the ternary Co(II) complexes of all the ligands are presented in **Table-3**. Electronic spectra of some representative complexes of CMABA and NCMABA are shown in **Fig-3**. All the present Co(II) complexes show each three peaks around 10560 - 13106, 12547 - 19724 and 22371 - 23697 cm⁻¹ which are attributed to ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ transitions. These transitions suggest an octahedral geometry [21].

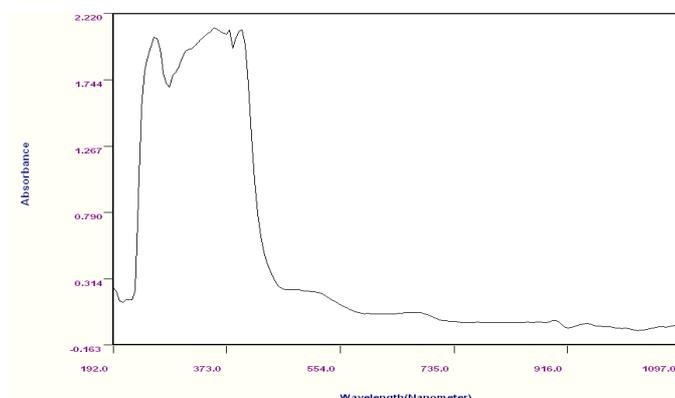


Fig-3. UV-Vis spectrum of Co(II)-CMBH-1,10-phen

3.8. ¹H NMR / ¹³CNMR spectra:

The ¹H NMR spectrum of the Schiff base ligand is recorded in DMSO-d₆. In the ¹H NMR spectra of Schiff base ligand a peak at 10.26 δ is assigned for the proton of carboxylic acid of Schiff base and a singlet at 8.93 δ assignable for proton of azomethine group.

The ¹³C NMR spectrum of the Schiff base ligand is recorded in DMSO-d₆. In the ¹³C NMR spectra of Schiff base ligand a peak at 188.4 δ is assigned for the carbon of chromone carbonyl, a peak at 168.8 is assigned for the carbon of carboxylic group and a peak at 163.4 is assigned for azomethine carbon.

3.9. Mass spectra of the compounds:

The mass spectral data of Schiff base ligand and its metal chelates are in good agreement with the theoretical values.

3.10. DNA cleavage studies:

The characterization of DNA recognition by transition metal complexes has been aided by the DNA cleavage chemistry that is associated with redox-active or photo-activated metal complexes [22,23]. DNA cleavage is controlled by relaxation of supercoiled circular form of pUC19 DNA into nicked circular form and linear form. When circular plasmid DNA is conducted by electrophoresis, the fastest migration will be observed for the supercoiled form. If one strand is cleaved, the supercoils will relax to produce a slower-moving open circular form. If both strands are cleaved, a linear form will be generated that migrates in between.

The cleavage efficiency of the complexes compared to that of the control is due to their efficient DNA binding ability. The proposed general oxidative mechanisms and account of DNA cleavage by hydroxyl radicals via abstraction of a hydrogen atom from sugar units that predict the release of specific residues arising from transformed sugars, depending on the position from which the hydrogen atom is removed. The cleavage is inhibited by free radical scavengers implying that hydroxyl radical or peroxy derivatives mediate the cleavage reaction. The reaction is modulated by a metal complex bound hydroxyl radical or a peroxy species generated from the co-reactant H₂O₂.

In the present study, the pUC19 plasmid DNA gel electrophoresis experiment was conducted at 37°C using our synthesized complexes in the presence of H₂O₂ as an oxidant.

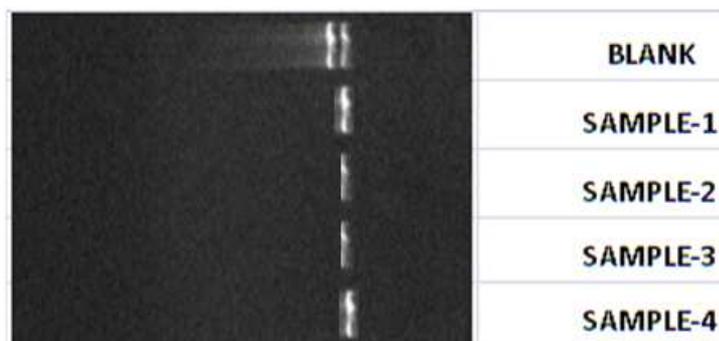


Fig-4. Changes in the Agarose gel electrophoretic pattern of pUC19 plasmid DNA, induced by H₂O₂ and metal complexes: DNA alone (Blank); DNA + Co(II)-CMABA + H₂O₂ (sample-1); DNA + Co(II)-CCMABA + H₂O₂ (sample-2); DNA + Co(II)-NCMABA + H₂O₂ (sample-3) and DNA + Co(II)-CMBH + H₂O₂ (sample-4)

From the Fig - 4, it is observed that, at high concentration (50 µM), complexes exhibit nuclease activity in the presence of H₂O₂. Control experiment using DNA alone (blank) does not show any significant cleavage of plasmid DNA even on longer exposure time. From the observed results, it is concluded that the supercoiled DNA was converted to nicked DNA in case of complexes, Co(II)-CMABA-1,10-phen (sample-1), Co(II)-CCMABA-1,10-phen (sample-2), Co(II)-NCMABA-1,10-phen (sample-3) and Co(II)-CMBH-1,10-phen (sample-4). All these complexes cleave DNA as compared to control DNA.

3.11. Biological activity studies:

The antimicrobial activity of the Schiff base ligand and its metal complexes were tested against the four bacteria *E.coli*, *Edwardella*, *Pseudomonas* and *B.subtilis* and two fungi *penicillium* and *trichoderma* by the well disc and fusion method. The test solutions were prepared in DMSO at a concentration of 1mg/ml. The zone of inhibition values were measured in millimeter after 24 hrs incubation at 37°C. The antimicrobial results are given in table-4.

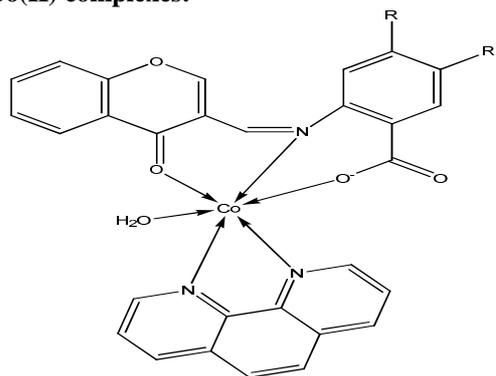
Table-4. Antimicrobial activity in centimeter (cm)

Complex	<i>E.coli</i>	<i>B.subtilis</i>	<i>Edwardella</i>	<i>Pseudomonas</i>	<i>Penicillium</i>	<i>Trichoderma</i>
Co(II)-CMABA-1,10-phen	+	+	++	++	++	++
Co(II)-CCMABA-1,10-phen	+++	++	++	++	++	+
Co(II)-NCMABA-1,10-phen	+++	+	+	+++	+	++
Co(II)-CMBH-1,10-phen	++	++	+++	+	+++	+

The value in the above table indicates that the activity of the Schiff base ligand became more pronounced when coordinated with the metal ions. The presence of azomethine moiety and chelation effect with central metal enhances the antibacterial activities. This enhancement in antibacterial activity of these metal complexes can be explained based on the chelation theory [24].

When a metal ion is chelated with a ligand, its polarity will be reduced to a greater extent due to the overlap of ligand orbital and the partial sharing of the positive charge of the metal ion with donor groups. Furthermore, the chelation process increases the delocalization of the π-electrons over the whole chelate ring, which results in an increase in the lipophilicity of the metal complexes. Consequently, the metal complexes can easily penetrate into the lipid membranes and block the metal binding sites of enzymes of the microorganisms. These metal complexes also affect the respiration process of the cell and thus block the synthesis of proteins, which restrict further growth of the organism.

Proposed structures of ternary Co(II) complexes:



Ternary Co(II) complex of CMABA / CCMABA / NCMABA

Where R=R₁=H, CMABAR=Cl, R₁=H, CCMABAR=H, R₁=NO₂, NCMABA

Molecular modeling's of proposed structures of ternary Co(II) complexes:



Co-CMABA-1,10-Phen

Co-CMBH-1,10-Phen

CONCLUSION

The ternary Co(II) complexes of CMABA, CCMABA, NCMABA and CMBH have been structurally characterized. The metal to ligand stoichiometry in all the complexes is 1:1:1. The complex contains one coordinated water molecule. The complexes of CMABA, CCMABA and NCMABA are 1:1 electrolytes and the complex of CMBH is 1:2 electrolyte in DMSO. CMABA, CCMABA and NCMABA act as mononegative, tridentate ligands coordinating through nitrogen of azomethine, oxygen of chromone-carbonyl and oxygen of carboxylic group. CMBH act as neutral, tridentate ligand, coordinating through nitrogen of azomethine, oxygen of chromone-carbonyl and oxygen of benzoyl group. 1,10-phenanthroline acts as neutral bidentate ligand and coordinating through two nitrogen atoms. All these complexes are paramagnetic to the extent of three unpaired electrons. Based on analytical, conductance, magnetic and electronic spectral data, all complexes are assigned six-coordinated geometry. The nuclease activity of the above ternary complexes shows that all the complexes cleave DNA through redox chemistry. The biological activity of the ligand and its ternary complexes have been studied on four bacteria *E.coli*, *B.subtilis*, *Pseudomonas*, *Edwardella* and two fungi *penicillium* and *trichoderma* by well disc and fusion method and found that the metal complexes are more active on microorganisms than the free Schiff base ligand.

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