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Synthesis, Characterization and Biological Evaluation: Copper(II) Complexes of Hydroxy coumarins with Ciprofloxacin

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ABSTRACT

Series of new Cu(II) complexes were synthesized by classical thermal technique. The biologically potent ligands (L) were prepared by refluxing 3-acetyl 4-hydroxy coumarin with aldehydes in the presence of piperidine in ethanol. The Cu(II) complexes have been synthesized by mixing an aqueous solution of $Cu(NO_3)_2$ in 1 : 1 molar ratios with ethanolic bidentate ligands and Ciprofloxacin. The structures of the ligands and their copper complexes were investigated and confirmed by the elemental analysis, FT-IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectral data. Thermal behavior of newly synthesized mixed ligand Cu(II) complexes were investigated by means of thermogravimetry, electronic spectra and magnetic measurements. The kinetic parameters such as order of reaction (n), energy of activation (Ea), entropy (S*), pre-exponential factor (A), enthalpy (H*) and Gibbs free energy (G*) have been calculated using Freeman-Carroll method. Ferric-reducing antioxidant power (FRAP) of all complexes were measured. All the compounds ware screened for their antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Streptococcus pyogenes, Bacillus subtilis* and antifungal activity against *Candida albicans* and *Aspergillus niger* have been carried out.

Keyword: Antioxidant, Ciprofloxacin, Hydroxycoumarin, Metal complexes, Cu(II)

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INTRODUCTION

4-Hydroxycoumarins are a class of vitamin K antagonist (VKA) anticoagulant drug molecules derived from coumarin .The synthetic drugs in the 4-hydroxycoumarin class are all noted primarily for their use as anticoagulants, though they can have several additional effects. Many coumarin derivatives are biologically active [1-3]. Much research has been focused on the inhibition of bacterial growth by naturally occurring coumarins (xanthoxin, herniarin, umbelliferone, and scopoletin) and on the antifungal activity of umbelliferone, scopoletin, and coumarin itself [4-7]. Some coumarin derivatives (novobiocin and analogues) have proven very active as antibiotics [8,9]. Among synthetic derivatives, several antibacterial 3-acyl [10-14] and 3-carbamoyl- 4-hydroxycoumarins [13,15] have been described. Coumarins are an important group of organic compounds that are used as additives to food, cosmetic [16] and optical brightening agent [17]. Along with these, coumarin derivatives have recently revealed new biological activities with interesting potential in therapeutic application besides their traditional employment as anticoagulant (antivitamin K activity) [18] and sustaining agents (photosensitizing action of furocoumarin) [19], they have yielded important results as an antibiotics (novobiocin and analogs) [20] and antitumor drug (geiparvarin) [21].

It is a second-generation fluoroquinolone antibacterial. It kills bacteria by interfering with the enzymes that cause DNA to rewind after being copied, which stops synthesis of DNA and of protein. Ciprofloxacin and other fluoroquinolones are valued for this broad spectrum of activity, excellent tissue penetration, and for their availability in both oral and intravenous formulations. Ciprofloxacin is used alone or in combination with other antibacterial drugs in the empiric treatment of infections for which the bacterial pathogen has not been identified, including urinary tract infections and abdominal infections [22] among others. It is also used for the treatment of infections caused by specific pathogens known to be sensitive. Ciprofloxacin is used to treat a number of infections including: infections of bones and joints, endocarditis, gastroenteritis, malignant otitis externa, respiratory tract infections, cellulitis, urinary tract infections, prostatitis, anthrax, chancroid, among others. Quinolones are a group of synthetic antibacterial agents now in clinical use already for over thirty years and ciprofloxacin is one of the widely used representatives [23-25]. The interactions of quinolones and metal ions have been thoroughly studied especially due to the interesting biological and chemical properties Ciprofloxacin can usually act as a bidentate ligand through the pyridone oxygen and one carboxylate oxygen. In the literature, diverse transition metal complexes of ciprofloxacin have been structurally characterized.

Synthesis, characteristic, spectroscopic properties and thermal aspects of newly coumarin based mixed ligand Cu(II) complexes as well as antioxidant and antimicrobial screening of newly synthesized compounds. Kinetic methods were employed in the present work to evaluate the kinetic parameters i.e. activation energies and the pre-exponential factor.

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MATERIAL AND METHODS

All reagents were of analytical reagent (AR) grade purchased commercially from Spectro chem. Ltd., Mumbai-India and used without further purification. Solvents employed were distilled, purified and dried by standard procedures prior to used [26]. Clioquinol was purchased from Agro Chemical Division, Atul Ltd., Valsad-India. The metal nitrates used were in hydrated form.

All reactions were monitored by thin-layer chromatography (TLC on alluminium plates coated with silica gel 60 F_{254}, 0.25 mm thickness, E. Merck, Mumbai-India) and detection of the components were measured under UV light or explore in Iodine chamber. Carbon, hydrogen and nitrogen were estimated by elemental analyzer PerkinElmer, USA 2400-II CHN analyzer. Metal ion analyses was carry out by the dissolution of solid complex in hot concentrated nitric acid, further diluting with distilled water and filtered to remove the precipitated organic ligands. Remaining solution was neutralized with ammonia solution and the metal ions were titrated against EDTA. ^{1}H and ^{13}C NMR measurements were carried out on Advance-II 400 Bruker NMR spectrometer, SAIF, Chandigarh. The chemical shifts were measured with respect to TMS which used as internal standard and DMSO-d_6 used as solvent. Infrared spectra of solids were recorded in the region 4000-400 cm^{-1} on a Nicolet Impact 400D Fourier-Transform Infrared Spectrophotometer using KBr pellets. The FAB mass spectrum of the complex was recorded at SAIF, CDRI, Lucknow with JEOL SX-102/DA-6000 mass spectrometer. Melting point of the ligands and metal complexes were measured by open capillary tube method. Solid state magnetic susceptibility measurements were carried out at room temperature using a Gouy's magnetic susceptibility balance with mercury tetrathiocyanato cobaltate(II) being used as a reference standard ($g = 16.44\times10^{-6}$ c.g.s. units). Molar susceptibility was corrected using Pascal's constant[12]. Thermal decomposition (TG/DTG) analysis was obtained by a model Diamond TG/DTA, PerkinElmer, U.S.A. The experiments were performed in N_2 atmosphere at a heating rate of 20 °C min^{-1} in the temperature range 30-840 °C. The electronic spectra were collected using LAMBDA 19 UV/Vis/NIR spectrophotometer in the region 200-1200 nm.

EXPERIMENTAL

3-Acetyl-4-hydroxycoumarin

To a solution of 4-hydroxy-2H-chromen-2-one (1.86 mmol) in acetic acid (16 ml) was added phosphorus oxychloride (4.3 ml). The mixture was heated at reflux for 30 min. After cooling, the precipitate was collected and recrystallized from ethanol to give 3-acetyl-4-hydroxy-2H-chromen-2-one as white needles. Yield 86%; mp 135°C.

General procedure for the preparation of Coumarine chalcone (L)

3-Acetyl-4-hydroxyxcoumarin (0.031 mol) and the substituted aromatic aldehyde (0.03 mol) were dissolved in 30 ml of chloroform. A catalytic amount of piperidine (0.02 mol) was added and the reaction mixture was refluxed for 1.5 h. The chloroform was distilled out and the

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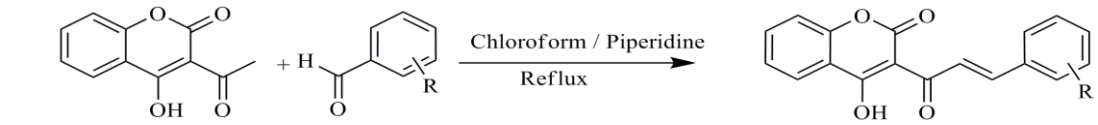
residue was washed with methanol. The synthetic route of targeted coumarin derivatives is shown in Scheme 1.



4-Hydroxy-3-acetyl coumarin Aldehyde Coumarin derivative (L)

Where R = H (L^1); *m*-Cl (L^2); *p*-Cl (L^3); *m*-NO_2 (L^4); *p*-CH_3 (L^5);

3-Cinnamoyl-4-hydroxy-2Hchromen-2-one (L^1)

L_1 was synthesized by the same method used for **L** by using benzaldehyde. $C_{18}H_{12}O_4$: Yield, 75%; m.p. 156-158°C. ESI-MS (m/z): 293.03[M]+. Found (%): C, 73.13, H, 4.03. calculated: C, 73.97, H, 4.14; ^{1}H NMR (DMSO-d_6 400 MHz): δ: 6.53-7.91 (11H, m, Ar-H), 12.10 (1H, -phenolic proton); ^{13}C NMR (DMSO-d_6 100 MHz): δ:98.54, 113.79,115.32, 122.70, 123.65, 124.05, 128.65, 129.27, 130.05, 133.42, 136.09, 142.44, 152.29 (15C, Ar-C), 158.47 (C=O, lactone carbon of coumarin), 183.05(C-4), 184.32 (C=O, *α, β*-unsaturated ketone); FTIR (KBr. cm^{-1}): 3145 (-OH), 1612 (C=O, *α, β*-unsaturated ketone), 1748 (C=O, lactone carbonyl of coumarin).

(E)-3-(3-(3-chlorophenyl)acryloyl)-4-hydroxy-2H-chromen-2-one(L^2)

L_2 was synthesized by the same method used for **L** by using 3-chlorobenzaldehyde. $C_{18}H_{11}ClO_4$: Yield, 70%; m.p. 162-163°C. ESI-MS (m/z): 326.12[M]+, 328.21[M+2]+. Found (%): C, 66.52, H, 3.74. calculated: C, 66.17, H, 3.39; ^{1}H NMR (DMSO-d_6 400 MHz): δ: 6.64-8.06 (10H, m, Ar-H), 12.24 (1H, -phenolic proton); ^{13}C NMR (DMSO-d_6 100 MHz): δ:97.59, 112.82, 115.61, 122.36, 124.09, 124.87, 125.53, 126.46, 128.65, 130.12, 132.49, 135.32, 136.47, 145.78, 153.07 (15C, Ar-C), 158.12 (C=O, lactone carbon of coumarin), 183.36 (C-4), 184.72 (C=O, *α, β*-unsaturated ketone); FTIR (KBr. cm^{-1}): 3140 (-OH), 1608 (C=O, *α, β*-unsaturated ketone), 1738 (C=O, lactone carbonyl of coumarin).

(E)-3-(3-(4-chlorophenyl)acryloyl)-4-hydroxy-2H-chromen-2-one(L^3)

L_3 was synthesized by the same method used for **L** by using 4-chlorobenzaldehyde. $C_{18}H_{11}ClO_4$: Yield, 68%; m.p. 168-169 °C. ESI-MS (m/z): 326.34[M]+, 328.04[M+2]+. Found (%): C, 66.59, H, 3.57. calculated: C, 66.17, H, 3.39; ^{1}H NMR (DMSO-d_6 400 MHz): 6.58-7.96 (10H, m, Ar-H), 12.05 (1H, -phenolic proton); ^{13}C NMR (DMSO-d_6 100 MHz): δ:98.23, 114.75,115.81, 123.08, 124.12, 125.32, 128.46, 130.17, 131.24, 137.17, 138.42, 144.25, 152.64 (15C, Ar-C), 158.18 (C=O, lactone carbon of coumarin), 183.47(C-4), 184.36 (C=O, *α, β*-unsaturated ketone); FTIR (KBr. cm^{-1}): 3165 (-OH), 1610 (C=O, *α, β*-unsaturated ketone), 1728 (C=O, lactone carbonyl of coumarin), 1092 (p-substituted (C-Cl)).

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(E)-4-hydroxy-3-(3-(4-nitrophenyl)acryloyl)-2H-chromen-2-one(L^4)

L_4 was synthesized by the same method used for **L** by using 4-nitrobenzaldehyde. $C_{18}H_{11}NO_6$: Yield, 66%; m.p. 174-175 °C. ESI-MS (m/z): 338.17[M]+. Found (%): C, 64.71, H, 3.64, N, 3.98. calculated: C, 64.10, H, 3.29, N, 4.15; ^{1}H NMR (DMSO-d_6 400 MHz): δ: 6.47-8.16 (10H, m, Ar-H), 12.37 (1H, -phenolic proton); ^{13}C NMR (DMSO-d_6 100 MHz): δ:98.29, 113.53,116.23, 122.67, 123.94, 124.74, 125.56, 130.87, 135.27, 136.14, 143.92, 146.26, 153.24 (15C, Ar-C), 158.29 (C=O, lactone carbon of coumarin), 182.94(C-4), 184.03 (C=O, *α, β*-unsaturated ketone); FTIR (KBr. cm^{-1}): 3160 (-OH), 1615 (C=O, *α, β*-unsaturated ketone), 1725 (C=O, lactone carbonyl of coumarin), 1518 (Ar-NO_2, asymmetric), 1356 (Ar-NO_2, symmetric).

(E)-4-hydroxy-3-(-3-P-tolylacryloyl)-2H-chromen-2-one(L^5)

L_5 was synthesized by the same method used for **L** by using 4-methyalbenzaldehyde. $C_{19}H_{14}O_4$: Yield, 78%; m.p. 182-183 °C. ESI-MS (m/z): 306.76[M]+. Found (%): C, 75.84, H, 4.73. calculated: C, 75.50, H, 4.61; ^{1}H NMR (DMSO-d_6 400 MHz): ^{1}H NMR (DMSO-d_6 400 MHz): 2.16 (3H, s, Ar-H_{15}), 6.43-8.02 (10H, m, Ar-H), 12.35 (1H, -phenolic proton); ^{13}C NMR (DMSO-d_6 100 MHz): δ: 21.05(C-18), 97.86, 113.27,115.34, 123.63, 124.09, 124.97, 128.62, 130.82, 132.08, 136.12, 137.23, 144.78, 153.06 (15C, Ar-C), 158.22 (C=O, lactone carbon of coumarin), 183.15(C-4), 184.29 (C=O, *α, β*-unsaturated ketone); FTIR (KBr. cm^{-1}): 3173 (-OH), 1618 (C=O, *α, β*-unsaturated ketone), 1745 (C=O, lactone carbonyl of coumarin), 2930(asym), 2732(sym) ν(CH_3) cm^{-1}, 1443(asym), 1346(sym) δ(CH_3) cm^{-1}.

Synthesis of metal complexes

[Cu(L^1)(CF) (H_2O)OH] (C^1)

An aqueous solution of $Cu(NO_3)_2 \bullet 3H_2O$ salt (10 mmol) was added into ethanolic solution of ligand (L^1) (10 mmol) and subsequently an ethanolic solution of ciprofloxacin (10 mmol) was added with continuous stirring. Then the pH was adjusted in between 4.5-6.0 by addition of diluted NH_4OH solution. The resulting solution was refluxed for 5 h and then heated over a steam bath to evaporate up to half of the volume. The reaction mixture was kept overnight at room temperature. A fine coloured crystalline product was obtained. The obtained product was washed with ether and dried over vacuum desiccators. Complexes C^2-C^5 was prepared according to same method and their physicochemical parameters are summarized in Table 1. The synthetic protocol of complexes is shown in Scheme 2.

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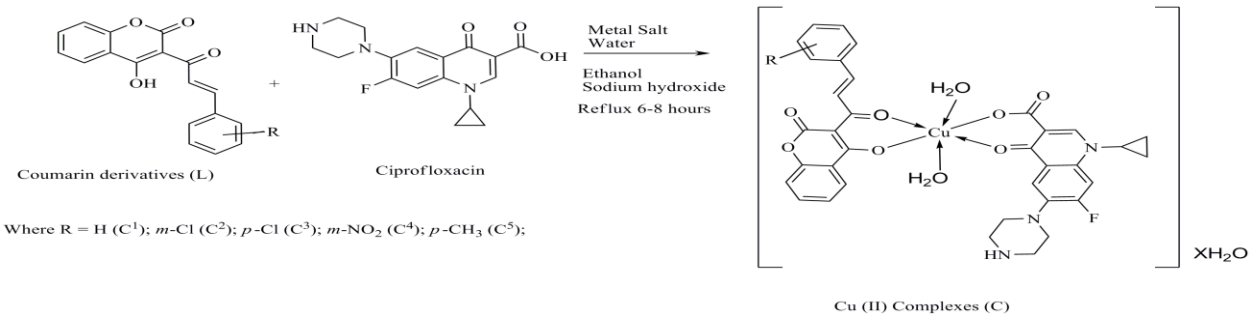

Table 1 Analytical and physical parameters of complex

Compounds/ empirical formula	Elemental analyses, % found (required)				M.p. (°C)	Yield (%)	Mol.wt. (gm)	μ_{eff} (B.M.)
	C	H	N	Cu(II)				
$C^1/C_{35}H_{32}CuFN_3O_9$	58.12 (58.29)	4.34 (4.47)	5.69 (5.83)	8.70 (8.81)	>350	69	721.19	1.81
$C^2/C_{35}H_{35}ClCuFN_3O_{11}$	52.98 (53.10)	4.31 (4.46)	5.19 (5.31)	7.88 (8.03)	>350	71	791.66	1.84
$C^3/C_{35}H_{33}ClCuFN_3O_{10}$	54.21 (54.34)	4.17 (4.30)	5.30 (5.43)	8.06 (8.21)	>300	68	773.65	1.79
$C_4/C_{35}H_{33}CuFN_4O_{12}$	53.48 (53.61)	4.09 (4.24)	7.02 (7.14)	7.98 (8.10)	>350	77	784.20	1.80
$C^5/C_{36}H_{38}CuFN_3O_{11}$	55.87 (56.06)	4.80 (4.97)	5.32 (5.45)	8.11 (8.24)	>300	72	771.24	1.85

Antimicrobial activity

Peptone (5 g), sodium chloride (5 g) and beef extract (1.5 g) were suspended in 1000 mL distilled water. The solution was boiled to dissolve all the ingredients completely. The pH of the solution at 25 °C was adjusted to 7.4±0.2 and sterilized by autoclaving at 15 lb pressure (121 °C) for 15 min. One day prior to the test, bacterial and fungal strains were made in the sterile nutrient broth and incubated at 37 °C overnight. Sample solutions were prepared by dissolving 1mg of sample in 10 mL of 2% DMSO to give the concentration 100 µg/mL. The standard solutions of Streptomycin (antibacterial drug) and Flucinozole (antifungal drug) were prepared in 2% DMSO to give concentration of 100µg/mL. Serial broth microdilution was adopted as a reference method. Serial dilutions of test compounds were made in broth, after which a standardized microorganism suspension was added. Quantities of test compounds were serially diluted to attain the final concentrations of 100, 50, 25, 12.5, 6.25 and 3.125µg/mL. One of the test tubes was kept as control. Each of the 10 test tubes was inoculated with a suspension of microorganism to be tested and incubated at 35 °C for 18 h. At the end of the incubation period, the tubes were visually examined for the turbidity. Cloudiness in the test tubes indicated that microorganism growth has not inhibited by the antibiotic contained in the medium at the test concentration.

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Antioxidant activity

Ferric reducing antioxidant power (FRAP) was measured by a modified method of Benzie and Strain [27,28]. The antioxidant potentials of the compounds were estimated as their power to reduce the TPTZ–Fe(III) complex to TPTZ–Fe(II) complex (FRAP assay), which is simple, fast, and reproducible. FRAP working solution was prepared by mixing a 25.0 mL, 10 mM TPTZ solution in 40 mM HCl, 20 mM $FeCl_3.6H_2O$ and 25 mL, 0.3 M acetate buffer at pH 3.6. A mixture of 40.0 mL, 0.5 mM sample solution and 1.2 mL FRAP reagent was incubated at 37 °C for 15 min. Absorbance of intensive blue colour [Fe(II)–TPTZ] complex was measured at 593 nm. The ascorbic acid was used as a standard antioxidant compound. The results are expressed as ascorbic equivalent (mmol/100 g of dried compound). All the tests were run in triplicate and are expressed as the mean and standard deviation (SD).

Thermal studies

From decomposition process, the thermodynamic activation parameters of the complexes such as energy of activation (*Ea*) and order of reaction (*n*) were evaluated graphically by employing the Freeman–Carroll [29] method using the following relation:

$$[(-Ea/2.303R)\ \Delta(1/T)]\ \Delta\log wr = - n + [\Delta\log(dw/dt)/\Delta\log wr]$$

Where R is gas constant, T is the temperature in K, wr = wc–w; wc is the mass loss at the completion of the reaction and w is the total mass loss up to time t. Ea and n are the energy of activation and order of reaction, respectively. A typical plots of $[\Delta\log(dw/dt)/\Delta\log wr]$ vs $[\Delta(1/T)/\Delta\log wr]$ were linear for all of the decomposition steps. The energy of activation *Ea* was calculated from the slopes of these plots for all stages and the order of reactions (*n*) determined from the intercept, which shows first order reaction over the whole range of decomposition for all complexes. A typical plot for the thermal degradation of $[Cu(A^2)(CF)(H2O)(H_2O)OH]\bullet H_2O$ is shown in Figure 2. The thermodynamic activation parameters of the decomposition process of dehydrated complexes such as entropy (*S**), pre-xponential factor (*A*), enthalpy (*H**) and free energy of the decomposition (*G**), were calculated using the following relations [30,31].

$$Ea/RTs^2 = A/\Phi \exp(-Ea/RTs).$$

$$S^* = 2.303(\log Ah/KTs)R,$$

$$H^* = Ea - RTs^*$$

$$G^* = H^* - Ts\ S^*$$

Where h is the plank constant, Φ is the heating rate, K is the Boltzmann constant, and Ts is the temperature of peak from DTG curve.

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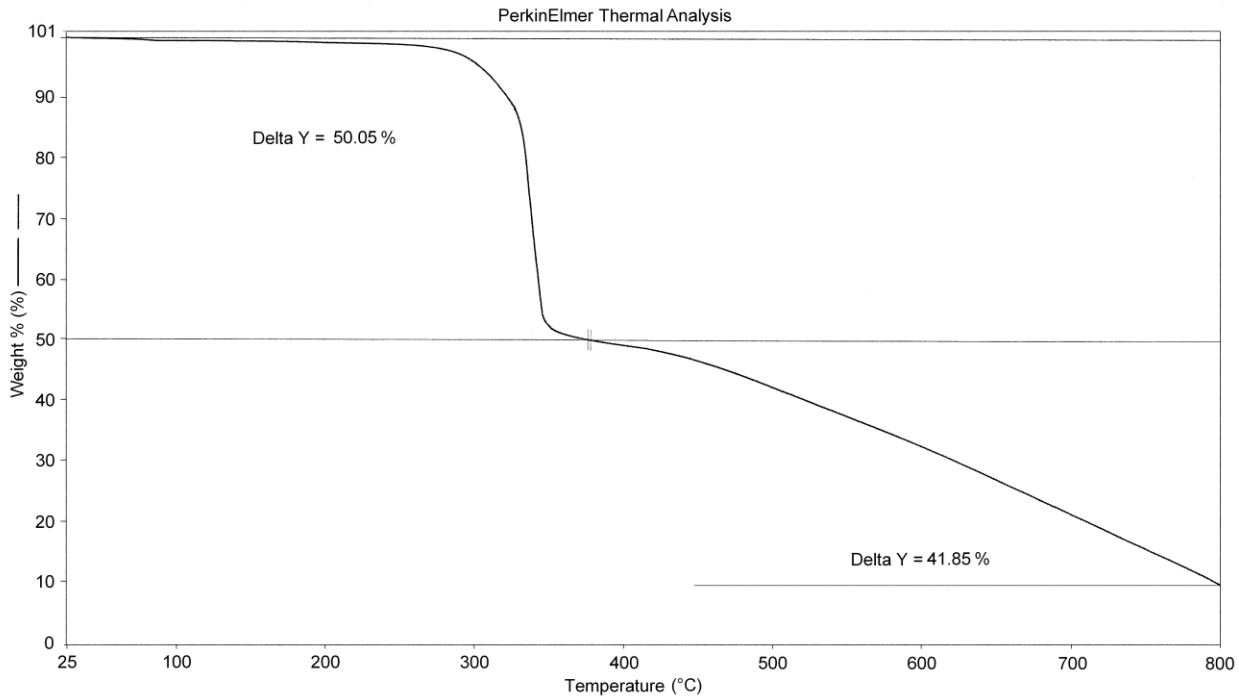

RESULT AND DISCUSSION

The synthesized Cu(II) complexes were characterized by elemental analysis, FTIR and mass (ESI-MS & FAB) spectra, The metal ion in their complexes were determined after mineralization. The metal content in chemical analysis was estimated by complexometrically [32], while geometry of the complexes was confirmed from electronic spectra, magnetic moment, thermal properties and kinetic measurements. However, ligands and its complexes have been screened for their *in vitro* antioxidant and antimicrobial activities.

Elemental analysis

The analytical and physiochemical data of the complexes are summarized in Table 1. The experimental data were in very good agreement with the calculated ones. The complexes were colored, insoluble in water and commonly organic solvents while soluble in DMSO as well as stable in air. The structure of the complexes is assumed according to the chemical reaction as shown below;

$$Cu(NO_3)_2 \cdot 3H_2O + L + CF \longrightarrow [Cu(L)(CF)(H_2O)OH] \bullet x H_2O + 2HNO_3 + n H_2O$$

(Where L = L^1, L^2, L^3, L^4, L^5; x = 0, 2, 1, 1, 2 and n = 3, 1, 2, 2, 1)

FT-IR spectra

The analysis of the FT-IR spectra of both ligands and complex provided information on the coordination mode between the ligands and the metal ion IR Spectra. The IR spectral data are summarized in Table 2. The infrared spectra of fluoroquinolones are quite complex due to the presence of the numerous functional groups in the molecules, therefore their

interpretation is based on the most typical vibrations [33] being the most important region in the IR spectra of fluoroquinolones between ~1810 and ~1320 cm^{-1} [34]. Spectra of the mixed-ligand Cu(II) complexes reveals that a broad band in the region ~3430-3470 cm^{-1} is due to stretching vibration of OH group. The *ν*(C=O) stretching vibration band appears at ~1706 cm^{-1} in the spectra of ciprofloxacin, and the complexes show this band at ~1630 cm^{-1}; this band shifted towards lower energy, suggesting that coordination occurs through the pyridone oxygen atom [35]. The strong absorption bands obtained at ~1627 and ~1385 cm^{-1} in ciprofloxacin are observed at ~1560-1580 and ~1350-1380 cm^{-1} for $\nu(COO)_a$ and $\nu(COO)_s$ in the complexes, respectively; in the present case the separation frequency $\Delta\nu > 200$ cm^{-1} ($\Delta\nu = \nu COO_a - \nu COOs$), suggesting unidentate binding of the carboxylato group [36-38]. The IR spectra of the coumarin derivatives shows ~1615 and ~1745 cm^{-1} bands corresponding to *α, β*-unsaturated ketone and lactone carbonyl ketone respectively, on complexation these peaks shifted to a lower frequency ~1600 and ~1740 cm^{-1} due to complex formation. In all the complexes, a new band is seen in the ~ 540-550 cm^{-1} region, which can be attributed to υ(Cu-O) [39,40].

Table 2 FT-IR data of synthesized compounds

Comp.	υ(O–H)^{br} cm^{-1}	υ(COO) sym	υ(COO) asym	α, β-unsat. υ(C=O)^{s} cm^{-1}	lactone carbonyl υ(C=O)^{s} cm^{-1}	υ(C=O) of pyridone	υ(M–O)^{w} cm^{-1}
C^1	3438	1379	1592	1602	1723	1615	525
C^2	3436	1371	1585	1601	1735	1622	512
C^3	3421	1373	1589	1607	1739	1636	516
C^4	3439	1377	1580	1603	1726	1622	527
C^5	3441	1370	1593	1609	1731	1626	521

^{s} = strong, ^{w} = weak, ^{br} = broad

Electronic spectra and magnetic measurement

Electronic spectral data along with magnetic susceptibility measurements gave sufficient support to determine the geometry of metal complexes. The electronic spectra of complexes were recorded in DMF solution with scan range 200-1200 nm. Usually octahedral geometry was found for Cu(II) complexes [41,42], then again, several copper compounds show temperature dependent geometric distortions and single copper ions in a host lattice of regular symmetry may reveal interesting spectroscopic properties [43].

The electronic spectra of hexa coordinate Cu(II) complexes were either D_{4h} or C_{4v} symmetry, the E_g and T_{2g} level of ^2D free ion term will split into B_{1g}, A_{1g}, B_{2g} and E_g levels, respectively under the influence of the distortion, Which cause the two transitions such as $^2B_{1g} \rightarrow {}^2B_{2g}$ and $^2B_{1g} \rightarrow {}^2A_{1g}$. This promotes the distorted octahedral Cu(II) complex which was usual in the d^9 system [44,45]. The electronic spectra of Cu(II) complexes (C^1-C^5) display three prominent bands. Low intensity broad band in the region 16,900-17,600 cm^{-1} was assigned as 10 Dq band corresponding to $^2E_g \rightarrow {}^2T_{2g}$ transition [46]. In addition, there was a high intensity band in the region 22,600-27,000 cm^{-1}. This band was due to symmetry forbidden ligand →

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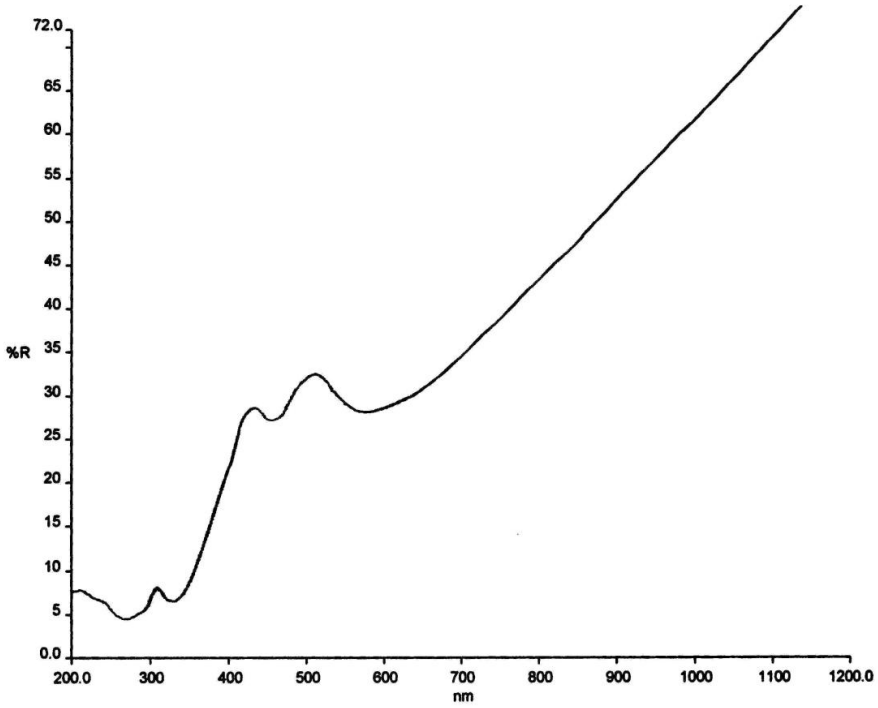
metal charge transfer transition [47]. The band above 27,000 cm^{-1} was assigned as ligand band. Therefore distorted octahedral geometry around Cu(II) ion was suggested on the basis of electronic spectra [48], which was further revealed by its magnetic moment of 1.79-1.86 BM falls within the range generally observed for octahedral Cu(II) complexes [49,50]. Therefore the electronic spectral data and magnetic moment data supports the octahedral geometry of the all complexes. The electronic spectra of complex C^2 is given in Figure 1.



$[Cu(L^2)(CF)(H_2O)_2]\ H_2O \xrightarrow[\text{Removal of lattice water molecules}]{80\text{-}130\ ^oC} [Cu(L^2)(CF)(H_2O)_2] + H_2O$

$[Cu(L^2)(CF)(H_2O)_2] \xrightarrow[\text{Removal of two co-ordinated water}]{150\text{-}280\ ^oC} [Cu(L^2)(CF)] + (H_2O)_2$

$[Cu(L^2)(CF)] \xrightarrow[\text{Removal of } L^2 \text{ ligand molecules}]{290\text{-}400\ ^oC} [Cu(CF)] + L^2$

$[Cu(CF)] \xrightarrow[\text{Removal of CF ligand molecules}]{410\text{-}800\ ^oC}$ Metal residue (CuO) + CF

Thermal studies of synthesized complexes

Thermal data and kinetic parameters of the complexes are given in Tables 3 and 4, respectively. The typical TG curves of the complexes $[Cu(L^2)(CF)(H_2O)_2]\bullet 2H_2O$ are characterized in Figure 2. Thermal fragmentation for complexes $[Cu(L^2)(CF)(H_2O)_2]\bullet 2H_2O$ is shown in Scheme 3. The anhydrous complexes show enormous thermal stability up to 250 °C. Next two steps were occurring exothermic and endothermic related with removal of coordinated ligand (coumarins) as well as Ciprofloxacin respectively. In the third subsequent stage, the decomposition and combustion of ligand(loss 45.23%) occurs at 260-430 °C. Where in fourth subsequent stage for complex show the decomposition and combustion of Ciprofloxacin (loss

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41.85%) occurs at 410-800°C. The removal of Ciprofloxacin undergoes decomposition forming CuO as the final residue.

Table 3 Thermoanalytical results (TG and DTG) of metal complexes

Complexes	TG range/ °C	Mass loss% obs. (calcd.)	Assignment
C_1	70-370	45.12(45.38)	Loss of two H_2O molecules
			Removal of L^1 ligand
	380-810	45.94	Removal of Ciprofloxacin ligand
		9.94	Leaving CuO residue
C_2	80-400	50.05(50.24)	Loss of two lattice water molecules
			Loss of two H_2O molecules
			Removal of L^2 ligand
	410-800	41.85	Removal of Ciprofloxacin ligand
		8.10	Leaving CuO residure
C_3	80-390	50.25(50.37)	Loss of one lattice water molecules
			Loss of two H_2O molecules
			Removal of L^3 ligand
	390-800	42.82	Removal of Ciprofloxacin ligand
		6.93	Leaving CuO residure
C_4	70-380	49.58(49.76)	Loss of one lattice water molecules
			Loss of two H_2O molecules
			Removal of L^4 ligand
	380-800	42.25	Removal of Ciprofloxacin ligand
		9.22	Leaving CuO residure
C_5	70-370	49.02(49.18)	Loss of two lattice water molecules
			Loss of two H_2O molecules
			Removal of L^5 ligand
	370-800	42.96	Removal of Ciprofloxacin ligand
		9.02	Leaving CuO residure

Table 4 Kinetic parameters of Cu(II) complexes

Complex	TG range/ °C	E_a/kJ mol^{-1}	n	A/s^{-1}	S*/J K^{-1} mol^{-1}	H*/kJ mol^{-1}	G*/kJ mol^{-1}
C^1	70-370	3.58	1.02	0.212	-101.42	1.754	38.47
	380-810	5.65	1.40	0.164	-99.26	1.996	46.66
C^2	80-400	3.87	1.23	0.075	-101.34	0.154	36.71
	410-800	12.14	1.07	0.235	-97.24	2.453	45.48
C^3	80-390	3.89	1.02	0.036	-102.31	0.136	35.72
	390-800	9.21	1.15	0.213	-98.46	8.764	42.18
C^4	70-380	3.73	0.98	0.058	-103.51	1.175	39.23
	380-800	9.75	1.03	0.274	-99.54	2.695	41.47
C^5	70-370	3.25	1.18	0.079	-106.54	0.190	33.63
	370-800	6.37	1.23	0.225	-98.98	1.766	49.31

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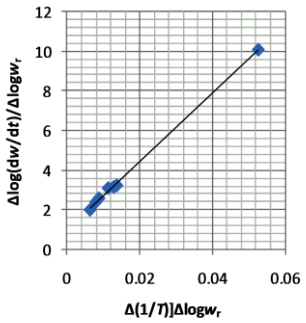
The thermodynamic activation parameters of the decomposition process of dehydrated complexes, such as activation entropy (*ΔS#*), pre-exponential factor (*A*), activation enthalpy (*ΔH#*) and free energy of activation (*ΔG#*), were calculated using the reported equations [51] Freeman-Carroll plot of complex C^2 is given in figure 3 and kinetic data for all complexes are tabulated in Table 4. All the complexes have negative entropy, which indicates that the studied complexes have more ordered systems than reactants [52]. The kinetic parameters, mainly energy of activation (*E*a) was useful in conveying the strength of the bonding of ligand moieties with the metal ion. The calculated *E*a values of the investigated complexes for the dehydration stage of ligand (A^n) were in the range 3.20-3.82 kJ mol^{-1}. The relatively high *E*a value indicates that the ligand (A^n) is strongly bonded the metal ion. The final solid product of decomposition was CuO (8.10%) accompanied by broad exothermic effect on above 800 °C.



Antimicrobial bioassay

All synthesized compounds were evaluated for their antimicrobial activity against various microorganisms such as *E. coli, P. aeruginosa, S. pyogenes, B. subtilis, A. niger and C. albicans* using Kirby- Bauer disk diffusion method and results were compared with standard drugs as summarized in (Table 5). Kolokolov *et al.* have suggested that the transition metal complexes with biologically active ligands frequently exhibit higher biological activity and lower toxicity than initial ligands, which makes possible their use in medicine and biochemistry [53]. Raman *et al.* have reported that complex exhibit higher antimicrobial activity than free ligands [54]. An increased in activity of metal chelates can be explained on the basis of chelation theory [55], according to which polarities [56,57] of ligands and central metal atoms are reduced through charge equilibration over whole chelate ring. This increases lipophilic character of metal chelate and favors its permeation through lipid layer of bacterial membranes [58]. The results were compared with those of the standard drug. All the metal complexes were more potent bactericides than the ligand. C^1 complex was much less microbially active than the other complexes. From Table 5, it can be seen that the highest inhibition of growth occurred on C^2 complex against the microorganism, while C^3, C^4 and C^5 shows enhance activity than C^1 but less potent than C^2.

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Table 5 Antimicrobial and antioxidant activities of synthesized compounds

Antimicrobial Activity (Minimal Inhibition Concentration, in µg/mL)							Antioxidant Activity
Entry	Gram negative bacteria		Gram positive bacteria		Fungus		FRAP value (mmol/100 g)
	E. coli	*P. aeruginosa*	*S. pyogenes*	*B. subtilis*	*C. albicans*	*A. niger*	
L_1	400	400	400	>600	400	200	NT
L_2	100	100	100	200	200	200	NT
L_3	100	200	100	200	200	200	NT
L_4	400	200	200	600	200	200	NT
L_5	200	200	400	400	200	400	NT
C_1	100	100	100	100	200	100	76.86
C_2	40	70	40	40	100	100	83.94
C_3	70	100	70	100	100	100	83.33
C_4	100	100	100	200	100	100	55.02
C_5	70	100	100	100	100	100	62.90
Ciprofloxacin	20	10	20	05	NT	NT	NT
Norfloxacin	10	10	10	10	NT	NT	NT
Flucanazole	NT	NT	NT	NT	10	10	NT
Nystatin	NT	NT	NT	NT	100	100	NT
E. Coli= ATCC25922; *P. aeruginosa*= ATCC25619; *S. pyogenes*= ATCC12384 ; *B. subtilis*= ATCC11774 ; *C.albicans*= ATCC 66027; *A.niger*= ATCC 64958 NT= Not tested							

Antioxidant studies

A capacity to transfer a single electron i.e. the antioxidant power of all compounds was determined by a FRAP assay. The FRAP value was expressed as an equivalent of standard antioxidant ascorbic acid (mmol/100 g of dried compound). FRAP values indicate that all the compounds have a ferric reducing antioxidant power. The compounds C^2 and C^3 showed relatively high antioxidant activity while compound C^1, C^4 and C^5 shows poor antioxidant power (Table 5).

In conclusion, the antimicrobial testing results reveal that complexes possess higher activity at lower concentration compared to parent ligand. It is known that chelation tends to make the Schiff bases more powerful and potent bacteriostatic agents [59].

CONCLUSION

Here elucidate the synthesis of biological active coumarin derivatives (L^1-L^5) and their Cu(II) complexes (C^1-C^5). The structures of the ligands were investigated and confirmed by the elemental analysis, FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral studies. Octahedral geometry were allocate for Cu(II) complexes on the basis of electronic, magnetic moment and TG analysis. Complexes shows momentous effective antioxidant activities compared to their ligands employed for complexation. *In vitro* antimicrobial activity and antioxidant activity of all synthesized compounds show good results with an enhancement of activity on complexation

with metal ions. This enhancement in the activity may be attributed to increased lipophilicity of the complexes. In review, the antimicrobial testing results reveal that complexes possess higher activity compared to parent ligand. The kinetic parameters, especially energy of activation (*E*a), are helpful in assigning the strength of the complexes. The calculated *E*a values of the investigated complexes for the first dehydration step were in the range 3.20–3.92 kJ mol^{-1} (Table 4). Based on the activation energy values the thermal stabilities of complexes in the decreasing order are: $C^3 > C^2 > C^4 > C^1 > C^5$.

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