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Multiple heating rate kinetic parameters, thermal, X-ray diffraction studies of newly synthesized octahedral copper complexes based on bromo-coumarins along with their antioxidant, anti-tubercular and antimicrobial activity evaluation

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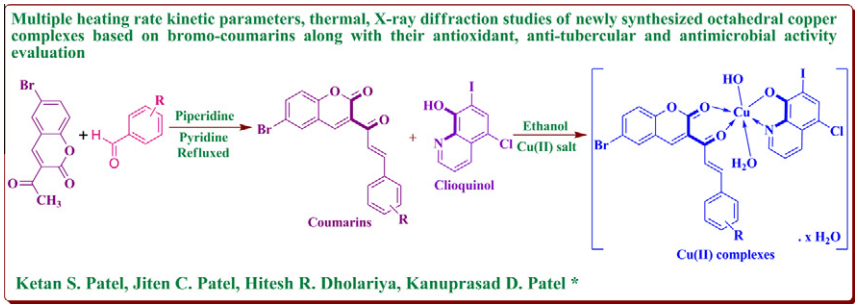
HIGHLIGHTS

- Mixed-ligand Cu(II) complexes based on bromo-coumarins with Clioquinol.
- Octahedral geometry was confirmed using electronic spectra and magnetic measurement.
- X-ray diffraction studies and multi-heating-rate kinetic parameters measurements of Cu(II) complexes.
- Antioxidant, anti-tubercular and antimicrobial studies of complexes.

GRAPHICAL ABSTRACT



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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 6-brom 3-acetyl 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 Clioquinol. The structures of the ligands and their copper complexes were investigated and confirmed by the elemental analysis, FT-IR, ^1H NMR, ^{13}C NMR, mass spectral and powder X-ray diffraction studies respectively. Thermal behaviour of newly synthesized mixed ligand Cu(II) complexes were investigated by means of thermogravimetry, differential thermogravimetry, differential scanning calorimetry, electronic spectra and magnetic measurements. Dynamic scan of DSC experiments for Cu(II) complexes were taken at different heating rates (2.5–20 °C min^{-1}). Kinetic parameters for second step degradation of all complexes obtained by Kissinger's and Ozawa's methods were in good agreement. On the basis of these studies it is clear that ligands coordinated to metal atom in a monobasic bidentate mode, by O—O and O—N donor system. Thus, suitable octahedral geometry for hexa-coordinated state has been suggested for the metal complexes. Both the ligands as well as its complexes have been screened for their *in vitro* antioxidant, anti-tubercular and antimicrobial activities. All were found to be significant potent compared to parent ligands employed for complexation.

1. Introduction

Interest in coumarin chemistry has flourished for many years; basically from a result of the wide spread use of coumarin derivatives. Coumarin was basic molecule found in family of many derivatives, like a simplest naturally occurring phenolic substance possessing fused benzene and α-pyrone rings. Naturally occurring as well as synthetic derivatives of coumarin compound have importance in wide range of fields such as medicinal chemistry, biochemistry, plant science and pharmacology [1]. A large number of structurally novel coumarin derivatives have been ultimately reported to show substantial cytotoxic and anti-HIV activity in vitro and in vivo [2]. The coumarin exists in a variety of forms, due to the various substitutions possible in their basic structure, which

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modulate their biological activity [3,4]. The coumarin derivatives are known to have diverse applications as anti-HIV [5–8], antibacterial [9,10], anthelminthic [11], anti-inflammatory [12–14] and antioxidant activities [15–14], anticoagulants, spasmolytics, anticancer drugs or as plant growth regulating agents [18–20]. Their complexation ability in respect to different metal ions has been studied and discussed widely in a considerable number of investigations [21–24]. It has been found that the binding of a metal to coumarin moiety retains or even enhances its biological activity [25–27]. In recent years the metal ions such as copper(II), iron(II), iron(III) or platinum(II) exert wide biological activity, for example against tumor cells. Also chromones, flavonoids and coumarins have been known for similar properties. From above facts those complexes of metals and ligands would be more active than the basic compounds. The biological activity of these complexes recently described in the literature is similar to widely used carboplatin [28–33]. Apart from the medicinal, biological and pharmacological applications coumarins are also used as sweeteners, fixatives of perfumes, additives in food, odour stabilizers in tobacco and an odour masker in paints and rubber [33].

Clioquinol (5-chloro-7-iodo-8-hydoxyquinoline) belongs to the quinoline class of compounds was first prepared in Germany by Ciba–Geigy during the last century. Clioquinol was used as antibiotics for the period of 1950s to 1970s [34,35]. Moreover, Clioquinol (CQ) is an antibiotic with metal-binding properties which has been shown to have anticancer activity in a number of experimental model systems [36,37]. Although Clioquinol has a long history of use in humans, it was observed that the cause of an epidemic of a rare neurological disease (subacute myeloptic neuropathy (SMON)) in Japan and was banned in many countries. Since that time, others have pointed out that SMON was not seen in other countries where Clioquinol was extensively used and have criticized the epidemiological data that led to its banning [38,39]. Because of optimistic data in animal studies, Clioquinol has been administered in clinical trials for Alzheimer's disease without reappearance of SMON [40,41], prompting careful re-evaluation of its use as a therapeutic agent. Clioquinol was widely used as an antibiotic for the treatment of amoebic dysentery and skin infection [42]. Regardless of it being a controversial compound, CQ can still serve as a model compound from which analogues could be developed that exploit its copper binding potential but avoid its negative associations. CQ is a lipophilic compound that is capable of forming stable complexes with Cu(II) ions [43]. Furthermore, the complexes of CQ with copper and zinc metal ions recognized for their biological effects which are significantly allied with protein aggregation and degeneration process in the brain [43], also these complexes have been used as an antimicrobial agent since many years [44]. Coumarin and CQ used as capable applicant for biological aspects [45]. Many reports were available on its credited [46].

Here the continuation of our earlier work [47,48], in present communication we describe synthesis, characteristic, spectroscopic properties, powder X-ray diffraction study and thermal aspects of newly coumarin based mixed ligand Cu(II) complexes as well as antioxidant, anti-tubercular and antimicrobial screening of newly synthesized compounds. Kissinger [49,50] and Flynn–Wall–Ozawa (FWO) [51,52] kinetic methods were employed to evaluate the kinetic parameters i.e. activation energies and the pre-exponential factor.

2. Experimental

2.1. Materials

All reagents were of analytical reagent (AR) grade purchased from Spectro Chem. Ltd., Mumbai-India and used without further purification. Solvents employed were distilled, purified and dried by standard procedures prior to use [53]. Clioquinol was purchased from Agro Chemical Division, Atul Ltd., Valsad-India. The metal nitrates were used in hydrated form.

2.2. Physical measurements

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 Perkin–Elmer, 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 ($g = 16.44 \times 10^{-6}$ c.g.s. units). Molar susceptibility was corrected using Pascal's constant [54]. Thermal decomposition (TG/DTG) analysis was obtained by a model Diamond TG/DTA, Perkin–Elmer, USA. The experiments were performed in N_2 atmosphere at a heating rate of 20 °C min^{-1} in the temperature range 30–840 °C. DSC analyses were carried out using Perkin–Elmer USA, Differncial Scanning Calorimetry (DSC-PYRIS-1). DSC analyses of complexes were also evaluated from dynamic scanning experiments at multiple heating rates of 2.5, 5, 10, 15 and 20 °C min^{-1}, respectively, with the best resolution and comparative results achieved at a scanning rate of 10 °C min^{-1}. The samples sizes were ranged in mass from 3 to 8 mg were heated in Al_2O_3 crucible. The electronic spectra were collected using LAMBDA 19 UV/Vis/NIR spectrophotometer in the region 200–1200 nm.

2.3. Crystallographic analysis

X-ray diffraction intensities were carried out with ±0.0025 accuracy using XRD Diffractometer (powder), Xpert MPD, Philips, Holland equipped with 2 kW power and Cu target X-ray tube used as a source of wavelength 1.542 Å, while the data were accumulate using JCPDF database. The detector used in the system was Xe-filled counterate and 2° θ measurement range of the instrument is 3° to 136°. The system contains goniometer was operated on vertical and horizontal mode with θ–θ and θ–2θ position respectively with radius 130–230 mm.

2.4. Synthesis of 3-acetyl coumarin

3-Acetyl coumarin was prepared according to the reported method [55]. A mixture of 6-Bromo salicylaldehyde (0.1 mol, 12.2 g), ethyl acetoacetate (0.1 mol, 13.0 g) and 3–4 drop piperidine were stirred for 10 min at room temperature in a 100 mL round bottom flask. After 10 min it was heated for 30 min in water bath. A yellow solid obtained was taken out and washed with cold

ether. It was recrystallized from chloroform-hexane. Yield: 92%; m.p. 119.5 °C.

2.5. Synthesis of ligands (L^1–L^6)

The neutral bidentate ligands were synthesized using Claisen–Schmidt condensation [56]. General procedure for synthesis of the ligands (L) is shown in Scheme 1. The ligands were characterized using elemental analysis, FT-IR, Mass and NMR (^{1}H and ^{13}C) spectroscopy, while the Mass (L^2), NMR (L^6) and FT-IR (L^3) spectra is given in the supplementary data (S_1–S_3).

2.5.1. Synthesis of 6-bromo-3-(3-(3-chlorophenyl)acryloyl)-2H-chromen-2-one (L^1)

In a 100 ml round bottom flask 6-bromo 3-acetyl coumarin (0.01 mol, 1.88 g) and 3-chloro benzaldehyde (0.015 mol) were taken in 15 mL of pyridine. Catalytic amount of piperidine (1.0 mL) was added and the reaction mixture was stirred for 10 min at room temperature. After clear solution obtained, the reaction mixture was refluxed on oil bath. Completion of reaction was checked by TLC using mobile phase Ethyl acetate:Hexane (7:3). After the completion of reaction, subsequently it was allowed to room temperature. Afterwards it was pour into ice-cold water and adjusts the pH 4–5 using diluted HCl. A solid product separated out was filtered off, later on washed with cold ethanol and dried in air. It was recrystallized from ethanol. Yield: 86%, m.p. 164–165 °C. FT-IR (KBr, cm^{-1}): ν(C=O, α,β-unsaturated ketone) 1627, ν(C=O, lactone carbonyl of coumarin) 1745. ^{1}H NMR (DMSO-d_6 400 MHz): δ 6.88 (1H, d, J = 15.6, CH=CH– protons), δ 7.74–8.03 (7H, m, aromatic protons), δ 8.16 (1H, d, J = 15.6 CH=CH– protons), δ 8.59 (1H, s, C_4-H). ^{13}C NMR (DMSO-d_6 100 MHz): δ 118.1, 119.8, 124.8, 125.5, 126.2, 126.7, 128.4, 130.1, 130.8, 133.6, 134.0, 134.7, 136.2, 142.6, (14 different types of aromatic carbons), 147.3(C-4), 152.6(C-9), 158.7 (C=O, lactone carbonyl of coumarin), 182.9 (C=O, α,β-unsaturated ketone). MS (ESI) m/z 390.0 [M+H]$^+$, 392.1 [M+H]$^{2+}$, 394.0 [M+H]$^{4+}$; Elemental analysis found (%):C, 55.27; H, 2.34; calculated for $C_{18}H_{10}BrClO_3$ (389.63): C, 55.49; H, 2.59.

2.5.2. Synthesis of 6-bromo-3-(3-(4-chlorophenyl)acryloyl)-2H-chromen-2-one (L^2)

L^2 was synthesized by same method used for L^1 by using 4-chloro benzaldehyde instead of 3-chloro benzaldehyde. Yield: 76%, m.p. 164–165 °C FT-IR (KBr. cm^{-1}): ν(C=O, α,β-unsaturated ketone) 1621, ν(C=O, lactone carbonyl of coumarin) 1738. ^{1}H NMR (DMSO-d_6 400 MHz): δ 6.81 (1H, d, J = 16, CH=CH– protons), δ 7.34 (2H, d, J = 7.8, CH=CH– protons), δ 7.58 (2H, d, J = 7.8, CH=CH– protons), δ 7.78–8.08 (3H, m, three aromatic protons), δ 8.12 (1H, d, J = 16, CH=CH– protons), δ 8.59 (1H, s, C_4-H). ^{13}C NMR (DMSO-d_6 100 MHz): δ 118.7, 119.4, 124.6, 125.1, 128.2, 129.7, 129.3, 133.1, 133.8, 130.2, 134.9, 142.6, (12 different types of aromatic carbons), 147.6(C-4), 152.3(C-9), 159.7 (C=O, lactone carbonyl of coumarin), 183.8 (C=O, α,β-unsaturated ketone). MS (ESI) m/z 390.0 [M+H]$^+$, 392.0 [M+H]$^{+2}$, 394.0 [M+H]$^{4+}$; elemental analysis found (%): C, 55.27; H, 2.41; calculated for $C_{18}H_{10}BrClO_3$ (389.63): C, 55.49; H, 2.59.

2.5.3. Synthesis of 6-bromo-3-(3-(3-hydroxyphenyl)acryloyl)-2H-chromen-2-one (L^3)

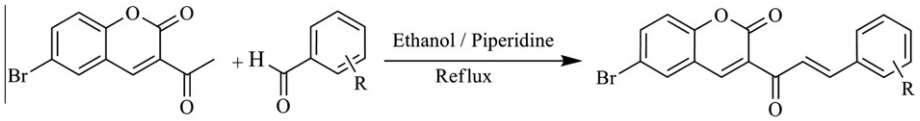
L^3 was synthesized by same method used for L^1 by using 3-hydroxy benzaldehyde instead of 3-chloro benzaldehyde. Yield: 72%, m.p. 167–169 °C FT-IR (KBr. cm^{-1}): ν(C=O, α,β-unsaturated ketone), ν(O—H) 3426, ν(C=O, lactone carbonyl of coumarin) 1740. ^{1}H NMR (DMSO-d_6 400 MHz): δ 6.77 (1H, d, J = 16, CH=CH– protons), δ 6.92–8.14 (7H, m, aromatic protons), δ 8.22 (1H, d, J = 16, CH=CH– protons), δ 8.56 (1H, s, C_4-H). δ 9.72 (1H, s, –OH). ^{13}C NMR (DMSO-d_6 100 MHz): δ 115.5, 117.9, 118.6, 120.2, 121.4, 124.8, 125.6, 130.2, 130.8, 134.5, 134.9, 135.4, 142.9 (13 different types of aromatic carbons), 147.2(C-4), 152.2(C-9), 158.4(C-16, carbon attach to phenolic OH), 160.5 (C=O, lactone carbonyl of coumarin), 183.2 (C=O, α,β-unsaturated ketone). MS (ESI) m/z 370.0 [M+H]$^+$, 372 [M+H]$^{2+}$; elemental analysis found (%): C, 58.08; H, 2.82; calculated for $C_{18}H_{11}BrO_4$ (371.18): C, 58.24; H, 2.99.

2.5.4. Synthesis of 6-bromo-3-(3-(4-hydroxyphenyl)acryloyl)-2H-chromen-2-one (L^4)

L^4 was synthesized by same method used for L^1 by using 4-hydroxy benzaldehyde instead of 3-chloro benzaldehyde. Yield: 74%, m.p. 158–160 °C FT-IR (KBr. cm^{-1}): ν(C=O, α,β-unsaturated ketone) 1617, ν(C=O, lactone carbonyl of coumarin) 1734, ν(O–H) 3426. ^{1}H NMR (DMSO-d_6 400 MHz): δ 6.88 (1H, d, J = 16, CH=CH– protons), δ 7.22 (2H, d, J = 7.8, CH=CH– protons), δ 7.56 (2H, d, J = 7.8, CH=CH– protons), δ 7.48–8.11 (3H, m, three aromatic protons), δ 8.20 (1H, d, J = 16, CH=CH– protons), δ 8.58 (1H, s, C_4-H), δ 9.76 (1H, s, –OH). ^{13}C NMR (DMSO-d_6 100 MHz): δ 114.9, 115.8, 118.1, 119.5, 124.2, 125.5, 127.3, 130.9, 134.7, 134.4, 142.6 (11 different types of aromatic carbons), 147.7(C-4), 152.8(C-9), 157.9(C-17, carbon attach to phenolic OH), 159.2 (C=O, lactone carbonyl of coumarin), 183.4 (C=O, α,β-unsaturated ketone). MS (ESI) m/z 370.0 [M+H]$^+$, 372.0 [M+H]$^{2+}$; elemental analysis found (%): C, 58.09; H, 2.78; calculated for $C_{18}H_{11}BrO_4$ (371.18): C, 58.24; H, 2.99.

2.5.5. Synthesis of 6-bromo-3-(3-(3-hydroxy,4-methoxyphenyl)acryloyl)-2H-chromen-2-one (L^5)

L^5 was synthesized by same method used for L^1 by using 3-hydroxy,4-methoxybenzaldehyde instead of 3-chloro benzaldehyde. Yield: 80%, m.p. 169–170 °C FT-IR (KBr. cm^{-1}): ν(C=O, α,β-unsaturated ketone) 1618, ν(C=O, lactone carbonyl of coumarin) 1735, (C–O–C, asymmetric) 1240, (C–O–C, symmetric) 1042. ^{1}H NMR (DMSO-d_6 400 MHz): δ 3.79 (3H, s, –OCH_3), δ 6.82 (1H, d, J = 15.6, CH=CH– protons), δ 6.98–7.59 (6H, m, six aromatic protons), δ 8.18 (1H, d, J = 15.6, CH=CH– protons), δ 8.59 (1H, s, C_4-H), δ 11.10 (1H, s, –OH). ^{13}C NMR (DMSO-d_6 100 MHz): δ 56.8 (C-18, OCH_3), 112.4, 117.5, 118.7, 120.4, 122.6, 127.7, 124.6, 125.7, 130.9, 134.8, 134.7, 142.5, 149.7 (13 different types of aromatic carbons), 146.8(C-4), 147.5 (C-17, carbon attach to phenolic OH), 152.7(C-9), 159.6 (C=O, lactone carbonyl of coumarin), 183.7 (C=O, α,β-unsaturated ketone). MS (ESI) m/z 400.0 [M+H]$^+$, 402.0 [M+H]$^{2+}$; elemental analysis found (%):C, 56.55; H, 3.03; calculated for $C_{19}H_{13}BrO_5$ (399.99): C, 56.88; H, 3.27.



6-Bromo-3-acetyl coumarin Aromatic Aldehyde Coumarin derivative (L)

Where R = *m*-Cl (L^1); *p*-Cl (L^2); *m*-OH (L^3); *p*-OH (L^4); *p*-OH-*m*-OCH_3 (L^5); H (L^6)

Scheme 1. General procedure for synthesis of ligands (L).

2.5.6. Synthesis of 6-bromo-3-cinnamoyl-2H-chromen-2-one (L^6)

L^6 was synthesized by same method used for L^1 by using benzaldehyde instead of 3-chloro benzaldehyde. Yield: 79%, m.p. 166–169 °C FT-IR (KBr. cm^{-1}): ν(C=O, α,β-unsaturated ketone) 1602, ν(C=O, lactone carbonyl of coumarin) 1743, (C–O–C, asymmetric) 1242, (C–O–C, symmetric) 1040. ^{1}H NMR (DMSO-d_6 400 MHz): δ 6.86 (1H, d, J = 16, CH=CH– protons), δ 7.21–8.05 (8H, m, eight aromatic protons), δ 8.25 (1H, d, J = 16, CH=CH– protons), δ 8.59 (1H, s, C_4-H). ^{13}C NMR (DMSO-d_6 100 MHz): 118.1, 119.5, 124.1, 124.9, 126.8, 128.4, 129.2, 130.3, 134.5, 134.9, 135.7, 142.6 (12 different types of aromatic carbons), 147.7(C-4), 152.4(C-9), 159.6 (C=O, lactone carbonyl of coumarin), 183.7 (C=O, α,β-unsaturated ketone). MS (ESI) m/z 355.0 $[M+H]^+$, 357.0 $[M+H]^{2+}$; elemental analysis found (%): C, 60.52; H, 3.03; calculated for $C_{18}H_{11}BrO_3$ (355.18): C, 60.67; H, 3.12.

2.6. Synthesis of metal complexes

2.6.1. [Cu(L^1)(CQ) (H_2O)OH]·2H_2O (C^1)

An aqueous solution of $Cu(NO_3)_2{\cdot}3H_2O$ salt (10 mmol) was added into ethanolic solution of ligand (L^1) (10 mmol) and subsequently an ethanolic solution of Clioqunol (10 mmol) was added with continuous stirring. Then the pH was adjusted in between 4.5 and 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 product was obtained in powder form. The obtained product was washed with ether and dried over vacuum desiccators.

Complexes C^2–C^6 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, while FT-IR spectrum of C^3 is given in the supplementary data (S_4).

2.7. Antioxidant study

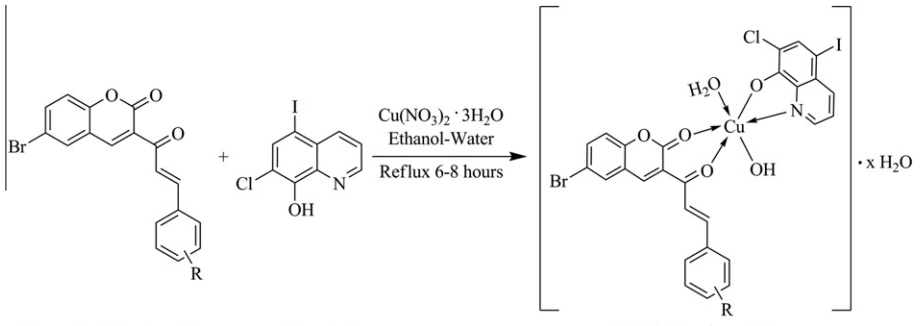
Ferric reducing antioxidant power (FRAP) was determine using an adapted method [57,58]. The antioxidant potentials of the compounds were examine by their reducing power of the TPTZ–Fe(III) complex to TPTZ–Fe(II) complex for the total antioxidant capacity of tested samples. This method was employed because of its simple, fast and also reproducible results can be obtained. Initially following solutions were prepared, (A) acetate buffer, 300 mM pH 3.6 (3.1 g sodium acetate trihydrate and 16 ml conc. acetic acid per L of buffer solution), (B) 10 mM 2,4,6-tripyridyl-*s*-triazine in 40 mM HCl, (C) 20 mM $FeCl_3{\cdot}6H_2O$ in distilled water, (D) 1 mM of ascorbic acid dissolved in 100 mL distilled water. FRAP working solution was prepared by mixing the above (A), (B) and (C) solutions in the ratio of 10:1:1, respectively. A mixture of 40.0 μL, 0.5 mM sample solution and 1.2 mL FRAP reagent was incubated at 37 °C for 15 min. The working solution was necessary to use as freshly prepared. The ascorbic acid was used as a standard antioxidant compound and results were expressed with compared to ascorbic acid.

2.8. Anti-tubercular activity

Test compounds were evaluated for *in vitro* anti-tubercular activity. The MICs were determined and interpreted for *M. tuberculosis* H37Rv according to the procedure of the approved micro dilution reference method of antimicrobial susceptibility testing [59,60]. Compounds were taken at concentrations of 100, 50, 25 and 12 μg/mL in DMSO, 1.0 ml of each concentration was used for the study. To this, 9.0 ml of Lowenstein–Jensen medium was added. A sweep from *M. tuberculosis* H37RV strain culture was discharged with the help of nichrome wire loop with a 3 mm external diameter into a vial containing 4 ml of sterile distilled water. The vial was shaken for 5 min. Then using nichrome wire loop suspension was inoculated on the surface of each of Lowenstein–Jensen

Table 1
Analytical and physiochemical parameters of ligands and complexes.

Compounds empirical formula	Elemental analyses, % found (calc.)				Colour	M.p. (°C)	Yield (%)	Molecular weight	μ_{eff} B.M.
	C	H	N	Cu(II)					
C^1 $C_{28}H_{24}BrCl_2CuINO_8$	38.86(38.57)	2.87(2.65)	1.66(1.45)	7.53(7.31)	Greenish Yellow	>350	65	843.75	1.80
C^2 $C_{28}H_{22}BrCl_2CuINO_7$	40.73(40.42)	2.69(2.43)	1.70(1.47)	7.70(7.42)	Greenish Yellow	>300	60	825.74	1.79
C^3 $C_{28}H_{23}BrClCuINO_8$	41.66(40.46)	2.27(2.08)	1.74(1.44)	7.87(7.53)	Greenish Yellow	>350	77	807.29	1.87
C^4 $C_{28}H_{25}BrClCuINO_9$	40.75(40.52)	3.05(2.76)	1.70(1.41)	7.70(7.44)	Greenish Yellow	>300	75	825.31	1.85
C^5 $C_{29}H_{29}BrClCuINO_{11}$	39.88(39.72)	3.35(3.08)	1.60(1.47)	7.28(7.08)	Greenish Yellow	>350	70	873.35	1.82
C^6 $C_{28}H_{23}BrClCuINO_7$	41.56(41.78)	2.42(2.60)	1.58(1.80)	8.02(8.19)	Greenish Yellow	>300	72	776.26	1.83



Coumarin derivatives (L) Clioquinol Cu (II) Complexes (C)

Where R = *m*-Cl (C^1); *p*-Cl (C^2); *m*-OH (C^3); *p*-OH (C^4); *p*-OH-*m*-OCH_3 (C^5); H (C^6)

Scheme 2. General synthetic route of complexes (C).

medium containing the test compounds. Further test media was incubated for four weeks at 37 °C. Readings were taken at the end of fourth week. The appearance of turbidity was considered as bacterial growth and indicates resistance to the compound. Test compounds were compared to reference drugs Isoniazid (MIC = 0.025 μg/mL), Streptomycin (MIC = 6.25 μg/mL) and Ethambutol (MIC = 20 μg/mL). Lowenstein–Jensen medium containing standard drugs as well as DMSO was inoculated with *M. tuberculosis* H37RV strain. The anti-tubercular activity tests were run in triplicate.

2.9. Antimicrobial activity

The *in vitro* antimicrobial activity of all the synthesized ligand and corresponding Cu(II) complexes were screened for their antibacterial against two Gram(⁺ve) *Staphylococcus aureus*, *Bacillus subtilis* and two Gram(⁻ve) *Escherichia coli*, *Pseudomonas aeruginosa*, where antifungal against *Candida albicans*, *Aspergillus niger* and *Aspergillus clavatus* using the broth dilution method [61]. All the ATCC culture was collected from institute of microbial technology, Bangalore. 2% Luria broth solution was prepared in distilled water while, pH of the solution was adjusted to 7.4 ± 0.2 at room temperature and sterilized by autoclaving at 15 lb pressure for 25 min. The tested bacterial and fungal strains were prepared in the Luria broth and incubated at 37 °C and 200 rpm in an orbital incubator for overnight. Sample solutions were prepared in DMSO for concentration 200, 150, 100, 50, 25, 12 and 6 μg/mL. The standard drug solution of streptomycin (antibacterial drug) and nystatin (antifungal drug) were prepared in DMSO. Serial broth micro dilution was adopted as a reference method. Ten microliter solution of test compound was inoculated in 5 mL Luria broth for each concentration respectively and additionally one test tubes was kept as control. Each of the test tubes was inoculated with a suspension of standard microorganism to be tested and incubated at 35 °C for 24 h. At the end of the incubation period, the tubes were examined for the turbidity. Turbidity in the test tubes indicated that microorganism growth has not inhibited by the antibiotic contained in the medium at the test concentration. The antimicrobial activity tests were run in triplicate.

3. Result and discussion

The synthesized Cu(II) complexes were characterized by elemental analysis, FTIR and mass (ESI–MS and FAB) spectra, while geometry of the complexes were confirmed using electronic spectra, magnetic moment, thermal properties and kinetic measurements. However, ligands and its complexes have been screened for their *in vitro* antioxidant, anti-tubercular and antimicrobial activities.

3.1. Elemental analysis

The analytical and physiochemical data of the complexes are summarized in Table 1. 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:

$$Cu(NO_3)_2\cdot 3H_2O + L + CQ \rightarrow [Cu(L)(CQ)(H_2O)OH].\times H_2O + 2\,HNO_3 + nH_2O$$

(where $L = L^1, L^2, L^3, L^4, L^5, L^6$; $x = 2,1,1,2,3,1$ and $n = 1,2,2,1,0,2$).

3.2. FT-IR spectra

The significant infrared spectral bands and their assignments for the synthesized ligands and their complexes were recorded as KBr disks and are presented in Table 2. The IR data of the free ligands and its metal complexes were carried out within the IR range 4000–400 cm^{-1}. The vibrational frequencies of complexes were comparing with their free ligand. The IR spectrum of the compounds L^3, L^4 and L^5 shows a strong OH stretching band in between 3460 and 3520 cm^{-1}, while spectra of the mixed-ligand Cu(II) complexes reveals that a broad band in the region 3410–3450 cm^{-1} due to stretching vibration of OH group which indicates formation of complexes and other bands at ~850 cm^{-1} and ~715 cm^{-1} due to the rocking and wagging vibration of the OH group respectively, caused by the water molecule present in synthesized complexes [62]. The IR spectrum of the free ligand shows a very stronger bands at ~1633 and ~1598 cm^{-1} due to stretching frequency of C=N present in Clioquinol moiety. These bands were shifted to lower frequencies in the complexes ~30–38 cm^{-1}, which clearly indicate that it has been affected upon complexation via metal ions. The complexes of 8-hydroxyquinoline with divalent metals, the observed band of C–O stretching frequency appeared at ~1120 cm^{-1} region and this band faintly varies with the metal [63]. In the free oxine molecule the C–O stretching frequency observed at ~1090 cm^{-1}, which is shifted to higher frequencies in all the mixed ligand complexes with a strong absorption band at ~1110 cm^{-1}. This result indicates the coordination of 8-hydroxyquinoline in these complexes. In IR spectrum all the complexes shows the bands at ~549 and ~456 cm^{-1} due to the Cu–O and Cu–N respectively. Mehmet Tumer et al. mentioned the weak band around ~550 cm^{-1} and ~468 cm^{-1} were attributed to the Cu–O and Cu–N stretching frequency [64,65]. The IR spectra of the coumarin derivatives shows ~1610 and ~1743 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 ~1730 cm^{-1} due to complex formation.

Table 2
FT-IR data of synthesized compounds.

Compounds	ν(O–H) cm^{-1}(br)	ν(C=N) cm^{-1}(w)	α,β-Unsaturated ν(C=O) cm^{-1}(s)	Lactone carbonyl ν(C=O) cm^{-1}(s)	ν(Cu–N) cm^{-1}(w)	ν(Cu–O) cm^{-1}(w)
L^1	–	–	1614	1739	–	–
L^2	–	–	1616	1736	–	–
L^3	3464	–	1620	1741	–	–
L^4	3486	–	1625	1743	–	–
L^5	3482	–	1623	1741	–	–
L^6	3480	–	1624	1742	–	–
C^1	3420	1555	1602	1742	547	455
C^2	3432	1554	1608	1738	545	457
C^3	3423	1558	1612	1740	551	452
C^4	3435	1550	1610	1745	552	458
C^5	3440	1556	1605	1741	549	453
C^6	3442	1553	1602	1743	550	451

s = strong, w = weak, br = broad.

3.3. 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 [66,67], however the known preference for Jahn–Teller distortions in cases of trigonal and octahedral geometry. A very weak low intense absorption band associated with *d–d* transition for Cu(II) complexes at 465, 532 nm was regular octahedral transition, its indicate the octahedral geometry of complexes [68]. On the other hand, many copper compounds show temperature dependent geometric distortions and single copper ions in a host lattice of regular symmetry may reveal interesting spectroscopic properties [69].

In the electronic spectra of the complexes, the wide range bands were observed due to either the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ of C=N chromophore or charge transfer transition arising from π electron interactions between the metal and ligand, which involves either a metal to ligand or ligand to metal electron transfer [70]. The electronic spectra of hexa coordinate Cu(II) complexes were shows D_{4h} or C_{4v} symmetry in which 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 [71,72]. The electronic spectra of Cu(II) complexes (C^1–C^6) display three prominent bands. Low intensity broad band in the region 16,900–17,900 cm^{-1} was assigned as 10 Dq band corresponding to $^2E_g \rightarrow {}^2T_{2g}$ transition [73]. In addition, there was a high intensity band in the region 22,900–27,100 cm^{-1}. This band is due to symmetry forbidden ligand → metal charge transfer transition [74]. The band above 27,100 cm^{-1} was assigned as ligand band. Therefore distorted octahedral geometry around Cu(II) ion was suggested on the basis of electronic spectra [75], which was further discovered by its magnetic moment value of 1.79–1.87 BM falls within the range generally observed for octahedral Cu(II) complexes [76,77]. In general, the electronic spectral data and magnetic moment data support the octahedral geometry of the all complexes. The electronic spectra of C^3 is given in Supplementary material (S_5).

3.4. Thermal studies of Cu(II) complexes

Thermal behaviour of the complexes was studied using TG, DTG and DSC analysis. The thermal decomposition TG curves corresponding to the complex (C^3) is presented in Fig. 1, whereas data for all complexes are given in Table 3. The thermal decomposition occurs in four steps in air are observed. According to the mass losses, the following degradation pattern might be proposed for complex $[Cu(L^3)(CQ)(H_2O)OH]\cdot H_2O$ (C^3) is represented in Scheme 3. All the compounds decompose with time respectively. Thermal decomposition started by dehydration process and was accompanied by endothermic effect between 70 and 100 °C due to loss of one lattice water molecules in first step. The observed mass loss was 2.58% which was nearly equal to theoretical value 2.23%. In the second step, exothermic decomposition between 200 and 240 °C corresponds to loss of one coordinated water and one hydroxyl molecule. The observed mass loss was 4.53% which was nearly equal to theoretical value 4.33%. Next two steps were also exothermic and endothermic related with removal of coordinated Clioquinol as well as ligands respectively. As temperature raise, the intermediate complexes $[Cu(L^3)(CQ)]$ (340–400 °C) and [Cu(CQ)] (510–610 °C) convert to CuO residue of fragments. The observed mass loss for third and fourth stage was 38.07% (calc. 37.84%) and 42.59% (calc. 45.97%), respectively. The final solid product of decomposition was CuO (obs. 12.03%; calc.9.63%) accompanied by broad exothermic effect on above 600 °C.

DTG curves corresponding to the complex (C^3) is represented in Fig. 2. First step of decomposition was dehydration process with endothermic effect on DTG curve at 86 °C, while increasing in temperature of $[Cu(L^3)(CQ)(H_2O)OH]$ and $[Cu(L^3)(CQ)]$ shows exothermic effect at 229 °C and 344 °C, respectively. Endothermic DTG peak at 552 °C associated with elimination of coumarins. The final residue predicted as copper oxide.

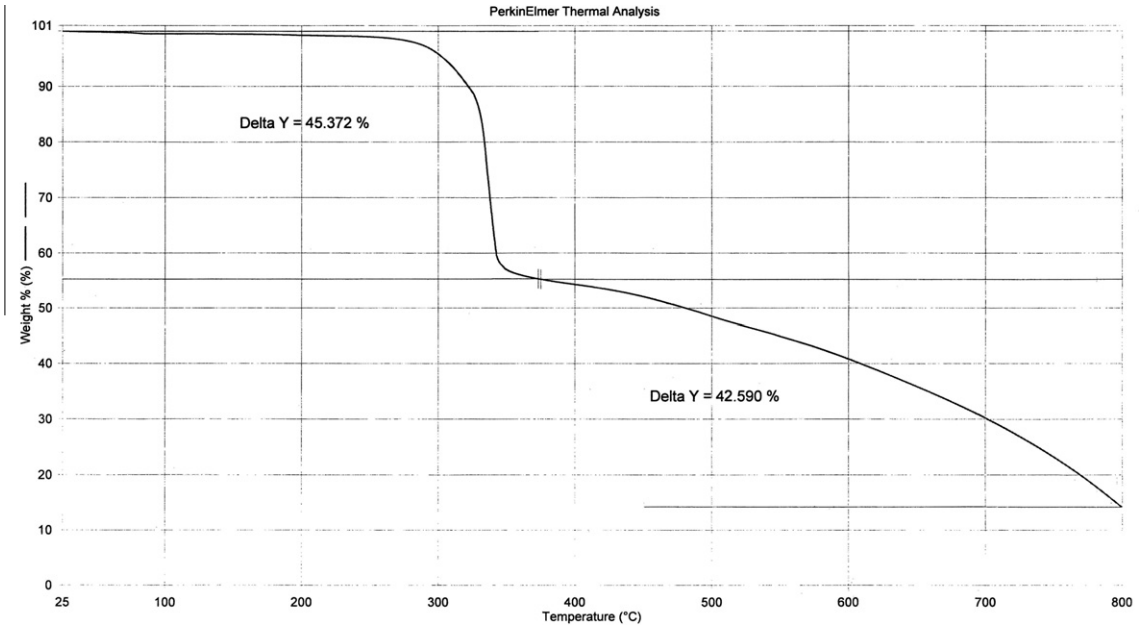
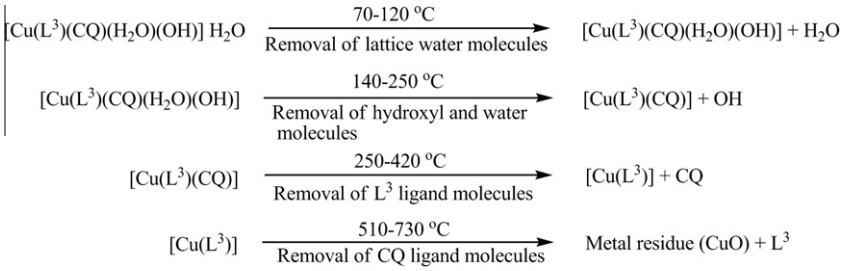


Fig. 1. TG curve of the complex $[Cu(L^3)(CQ)(H_2O)OH]\cdot H_2O$.

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

Complexes	TG range °C	DTG_{max} °C	Mass loss% obs. (calc.)	Assignment
C^1	70–390	96	46.77(46.55)	Loss of two lattice water molecules
		225		Loss of one –OH and H_2O molecules
		335		Removal of Clioquinol
	390–800	570	48.23	Removal of L^1 ligand
		>650	4.72	Leaving CuO residue
C^2	90–370	95	44.52(44.36)	Loss of one lattice water molecules
		240		Loss of one –OH and H_2O molecules
		346		Removal of Clioquinol
	370–800	560	48.20	Removal of L^2 ligand
		>650	8.65	Leaving CuO residue
C^3	70–380	86	45.37(45.31)	Loss of one lattice water molecules
		229		Loss of one –OH and H_2O molecules
		344		Removal of Clioquinol
	380–800	552	42.590	Removal of L^3 ligand
		>650	12.26	Leaving CuO residue
C^4	70–360	92	47.74(47.65)	Loss of two lattice water molecules
		242		Loss of one –OH and H_2O molecules
		352		Removal of Clioquinol
	360–800	545	42.590	Removal of L^4 ligand
		>650	9.86	Leaving CuO residue
C^5	80–385	98	45.98(45.90)	Loss of three lattice water molecules
		230		Loss of one –OH and H_2O molecules
		355		Removal of Clioquinol
	385–800	565	48.96	Removal of L^5 ligand
		>650	5.21	Leaving CuO residue
C^6	85–380	97	46.11(46.02)	Loss of one lattice water molecules
		235		Loss of one –OH and H_2O molecules
		350		Removal of Clioquinol
	380–800	564	45.75	Removal of L^6 ligand
		>650	8.47	Leaving CuO residue



Scheme 3. Thermal fragmentation of complex $[Cu(L^3)(CQ)(H_2O)OH]\cdot H_2O$.

3.5. Kinetic measurement by isoconversion methods

The isoconversion method was advanced tool for investigation of the kinetics parameters at the start and end of the chemical reaction. The kinetic parameters can easily interpreted by a comparison of measurements at different heating rates [78]. Multiple dynamic DSC experiments of all complexes were carried out at heating rates of 2.5, 5, 10, 15 and 20 °C min^{-1}, respectively, while Fig. 3 shows DSC scans of the complexes C^3 for second step decomposition at different heating rates. An isconversional method, which presumes the activation energy and pre-exponential factor were both functions of the degree of heat flow, can be used to investigate the multi-heating-rate scan data [79]. Similarly, this approach was useful to estimate the order of reaction (n). Moreover, results obtained from multi heating rate dynamic scans experiments were investigated using Kissinger's [49,50] and Ozawa's [51,52] approaches for determination of kinetic parameters. The Kissinger's approach work on the principle, the peak temperature is a function of the heating rate. In case of complex C^3 the exothermic peak temperature was shifted to higher temperatures with increase in heating rate and according to Kissinger's approach, the maximum reaction rate $d\alpha/dt$ occurs at T_p, where $d^2\alpha/dt^2 = 0$, and the kinetic Eq. (1) can be expressed through the following first order reaction

$$\ln\left(\frac{\beta}{T_p^2}\right) = \ln\left(\frac{AR}{E_a}\right) - \frac{E_a}{RT_p} \quad (1)$$

where T_p is the peak temperature of the DSC curve, β represents the heating rate and R is the gas constant. This equation was employ with a sensible estimation even to an nth order but it is not considerable order. The reaction progress under appropriate conditions, when thermal equilibrium was constantly maintained, Fig. 4 shows a plot $\ln(\beta/T_p^2)$ vs. $1/T_p$ gives a straight line with a slope equal to $-E_a/R$ which gives the values of activation energy while the values of the pre-exponential factor A was found from the intercept.

The isoconversional Flynn Wall Ozawa method was the integral method which provided a basic correlation between the activation

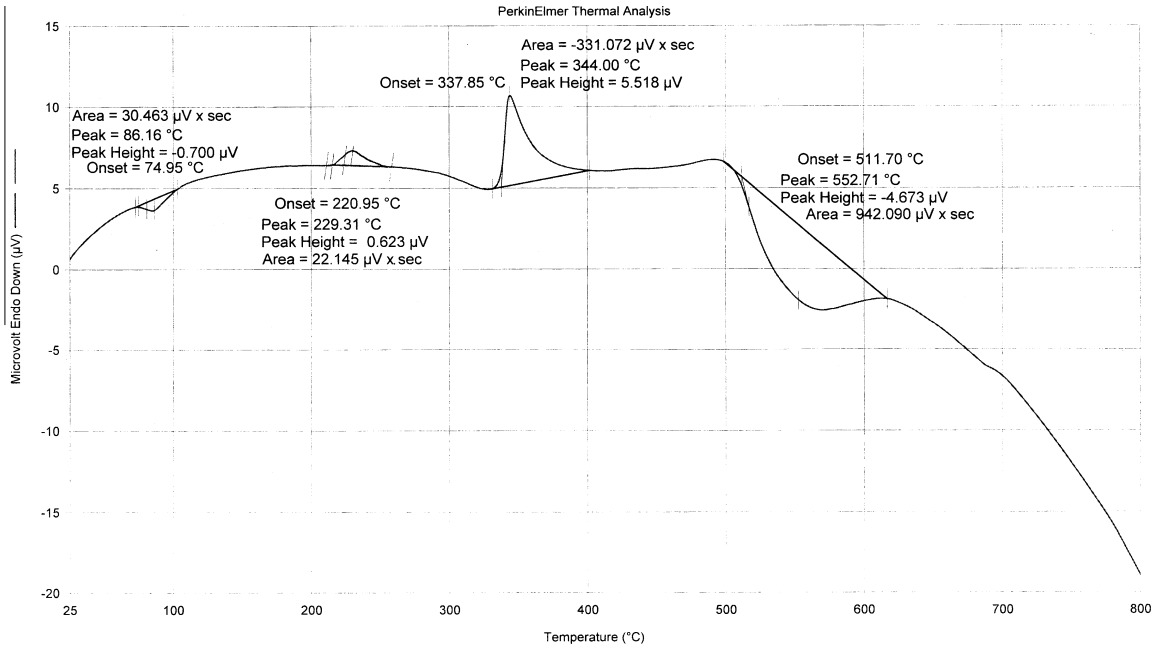


Fig. 2. DTG curve of the complex $[Cu(L^3)(CQ)(H_2O)OH]\cdot H_2O$.

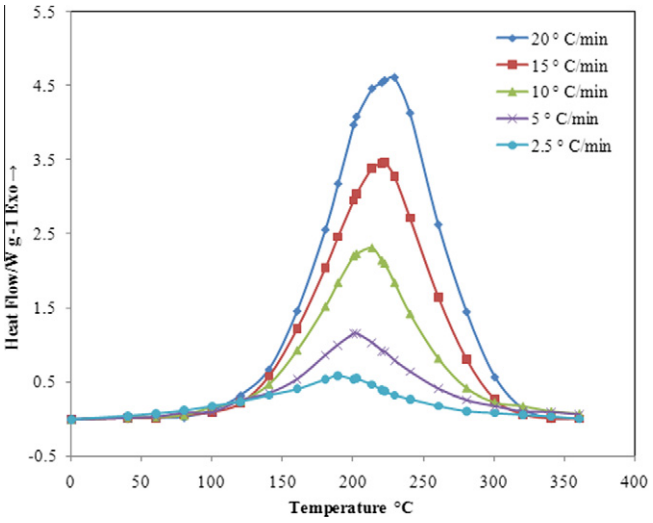


Fig. 3. Dynamic scan DSC curve of complex C^3.

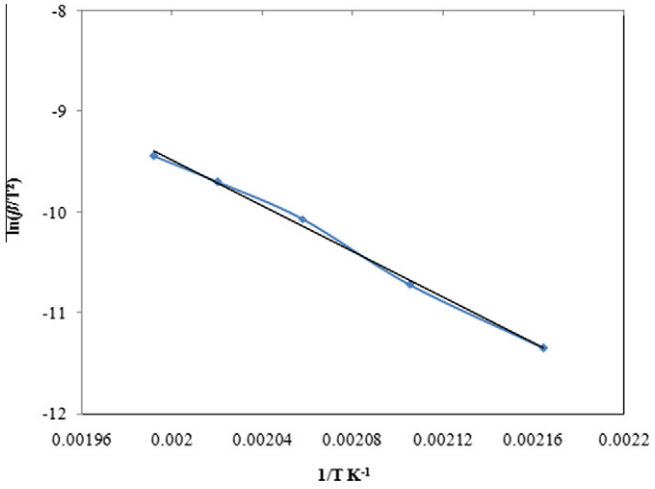


Fig. 4. Kissinger's approach to determine kinetic parameters for complex C^3.

energy, the heating rate and isoconversion temperature shown in Eq. (2),

$$\log \beta = -\frac{0.4567E_a}{RT_i} + A' \quad (2)$$

where for each degree of conversion, A′ is a constant that can be expressed as Eq. (3),

$$A' = \log\left(\frac{AE_a}{g(\alpha)R}\right) - 2.315 \quad (3)$$

where E_a is the activation energy, A the pre-exponential factor, R the gas constant, β the heating rate, α the degree of conversion, $g(\alpha)$ is the integral function of conversion and $f(\alpha)$ the differential function of conversion, For each degree of conversion α, the plot of log β vs. $1/T$ obtained at different heating rates should be a straight line which gives a slope proportional to the corresponding activation energy E_a and an intercept of the pre-exponential factor A shown in Fig. 5. The relationship of activation energy and conversion can be measured through the whole reaction. The activation energy in the starting decreases with rising value of conversion, getting a least value at around 20% conversion. Then activation energy was increase up to 60% conversion, prior to decreasing again at high conversions. From plots of each method, the experimental data sensibly fine during most of the temperature range but deviate considerably in the higher temperature area. Activation energy (E_a) shows a similar fashion as A with conversion in Fig. 6, which recommended linear correlation between activation energy and pre-exponential factor. The calculated results from the Kissinger's method are shown in Table 4. Ozawa's method gives an average value of complexes C^3 91.824 kJ mol^{-1}, which is a little bit lesser that obtained from Kissinger's method. Kissinger's and Ozawa's methods equally show an outstanding linear correlation between kinetics parameters based on the experimental figures.

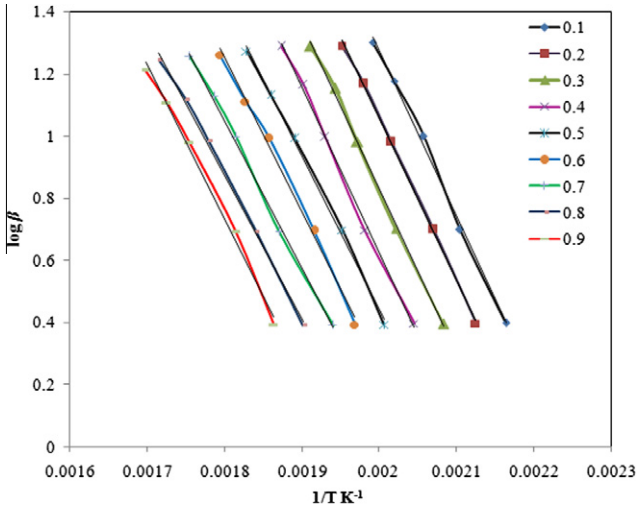


Fig. 5. Kinetic parameters of complex C^3 using Ozawa's approach on.

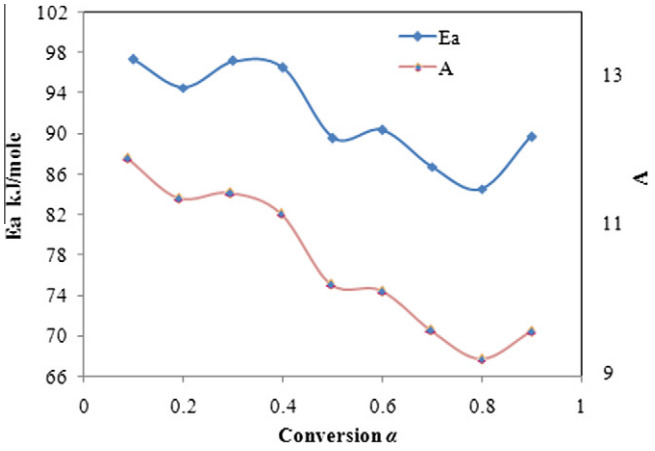


Fig. 6. Linear relationship between α, E_a and A for complex C^3: Ozawa's approach.

Table 4
Kinetic parameters of Cu(II) complexes by Kissinger's approach.

Heating rate °C min^{-1}	T_p °C for C^3	Kissinger values for C^3	
		E_a kJ mol^{-1}	lnA
20	229	94.098 ± 0.5	13.14 ± 0.1
15	222		
10	213		
5.0	202		
2.5	189		

3.6. FAB mass spectra

The fast atom bombardment (FAB) mass spectra of the complex C^6 and its fragmentation scheme are given in** Supplementary material (S_6–S_7). FAB mass spectrum reveals that isotropic peak at *m/z* 757 of complex without water of crystallization, where as several peaks observed at 740.82, 613.92, 561.16, 471.11, 391.20, 352.18, 326.16, 282.17, 147.97 and 146.07 *m/z* value. Thus the *m/z* of all the fragments of complex with the relative intensity confirms the stoichiometry of the complex.

3.7. X-ray diffraction studies

The feasible lattice dynamics of the final powdered compound $[Cu(L^6)(CQ)(H_2O)OH]\cdot H_2O$, structure was presumed on the basis of X-ray powder diffraction studies. The observed inter planar spacing values i.e. *d*-spacing were measured from the diffractogram of the compound. Moreover, the Miller indices *h*, *k* and *l* were assigned to each *d* value along with 2-Theta angles are given in Table 5. The results show that the compound belongs to hexagonal crystal system having unit cell parameters such as $a = 4.9168$, $b = 4.9168$, $c = 5.4089$ with maximum deviation of 2-Theta = 0.025 and Alpha = 90, Beta = 90, Gama = 120 at the wavelength = 1.540598. We have tried to isolate single crystal of Cu(II) complex for accurate X-ray crystal study but could not succeed to develop single crystal, it might be due to polycrystalline nature of complex. However, the structural information on the majority of inorganic metal complexes is not available because of the powder or polycrystalline nature of these materials. Generally it is often difficult to grow good quality single crystals of these inorganic complexes. In such cases, the powder X-ray diffraction studies might be useful. Even with some inherent limitations, this method yield valuable information about the characteristics of the crystal w3x. Singh et al. [80] were recently reported the X-ray powder diffraction studies for structure of hexa-coordinated Tin(IV) complex in which the ligand adopts the most stereo-chemically favorable orientation. On the basis of the above discussion, following structures shown in Figs. 7 and 8 were proposed for the complexes.

3.8. Antioxidant studies

Antioxidant power was specifically the ability of transfer a single electron for compound. The antioxidant capacity of complexes C^1–C^6 was determined by a FRAP method. The FRAP results 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^3 and C^5 showed relatively high antioxidant activity while compound C^1 shows poor antioxidant power (Table 6). Among the tested compounds hydroxyl group substituted derivatives possess more ferric reducing power compared to that of chloro and methoxy group substituted derivatives. Thus form the data obtained, the potency order on the basis of various substitutions on *p*-position of the phenyl ring is given as *p*-OH > *p*-OH-*m*-OCH_3 > *m*-OH > *p*-Cl > *m*-Cl. However, none of the compounds have been found more potent than standard ascorbic acid.

3.9. Anti-tubercular activity

The anti-tubercular activities of all the synthesized compounds were assessed against *M. tuberculosis* H37RV at 25, 50 and 100 μg/mL. The minimum inhibitory concentrations of compounds compared with the standard drugs isoniazid, streptomycin and ethambutol, and are summarized in Table 6. Ligands show inhibition at concentration 100 μg/mL. Complexes C^5 also exhibits activity at 50 μg/mL concentration while C^3 and C^4 complexes have shown enhancement in activity with MIC of 25 μg/mL. None of the tested compounds have the inhibition more than standards.

3.10. Antimicrobial bioassay

All the synthesized ligands and complexes were evaluated for their antibacterial and antifungal studies. The antibacterial and antifungal tests were carried out using the serial broth dilution method. The *in vitro* antimicrobial activities of the investigated compounds were screened against the bacterial species *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa* and fungal species *C. albicans*,

Table 5
Observed and calculated X-ray diffraction data of $[Cu(L^6)(CQ)(H_2O)OH]\cdot H_2O$ complex.

h	*k*	*l*	2 Theta (Obs.)	2 Theta (Calc.)	2 Theta (Diff.)	*d* (Obs.)	*d* (Calc.)	*d* (Diff.)	Intensity (Obs.)
0	0	1	16.4458	16.375	0.708	5.39023	5.40890	−0.01860	9.12
1	0	0	21.0783	20.845	0.233	4.2149	4.25807	−0.04317	18.82
1	0	1	26.4939	26.622	−0.128	3.3643	3.34573	0.01860	100.00
1	0	2	38.9913	39.440	−0.4478	2.31002	2.28291	−0.02710	18.44
2	0	1	44.7847	45.763	−0.9783	2.02374	1.98109	0.04265	18.10
1	1	2	50.8907	50.104	0.7867	1.79285	1.81913	−0.02628	18.48

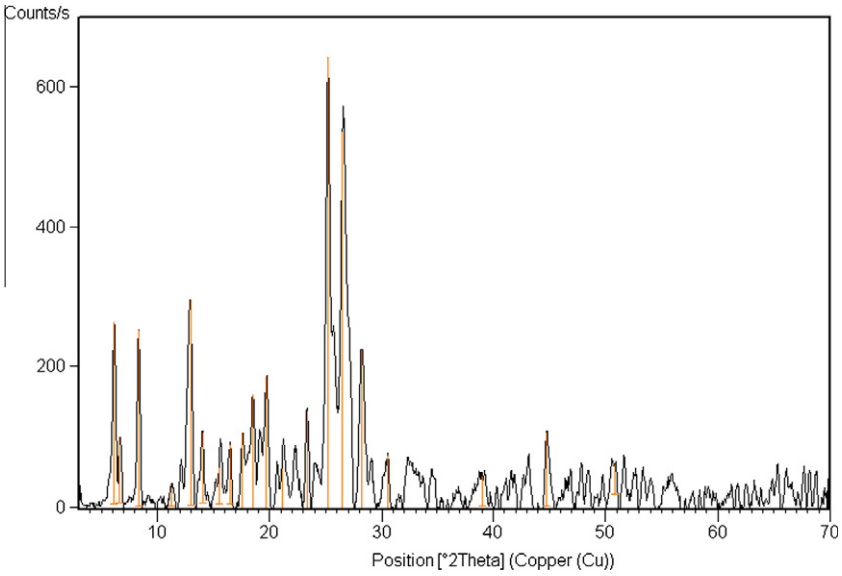


Fig. 7. X-ray patterns of complex C^6 powder samples.

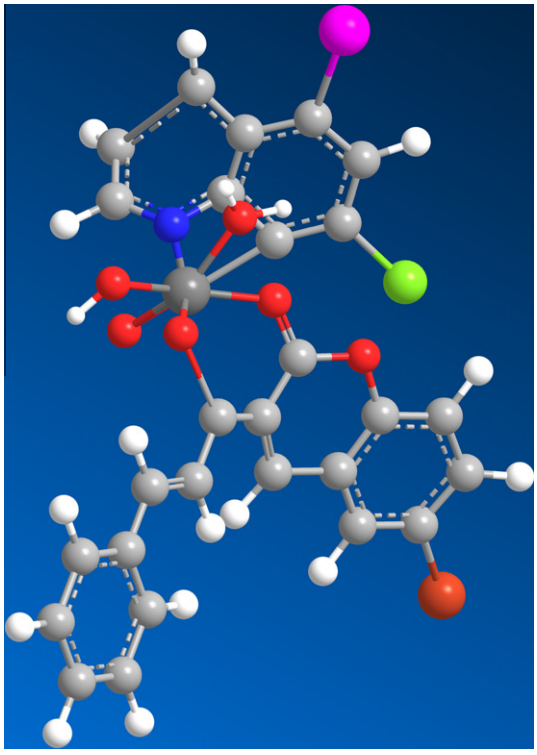


Fig. 8. 3D Molecular structures of complex C^6.

A. Niger and A. Clavatus. The minimum inhibitory concentration (MIC) values of the compounds are summarized in Table 6.

A relative study for MIC values of the ligands and their complexes signify that complexes display higher antimicrobial activity than the free ligands. In present investigation, the antimicrobial activity of the ligands may be due to the heteroaromatic residues. Compounds containing C=N group have improved antimicrobial activity than C=C group. The growth of certain microorganisms takes place even in the absence of oxygen. Hence, compounds containing C=C group still capable of absorbing oxygen which are not related with the growth of microorganisms. The greater activity of the complexes can be clarified on the basis of Overtone's concept [81] and Tweedy's chelation theory [82]. According to Overtone's concept of cell permeability, the lipid membrane surroundings in the cell was permit only the lipid–soluble resources, which makes liposolubility a key part that control the antimicrobial activity. During complexation, the polarity of the metal ion will be decrease up to a certain level due to the overlapping of the ligand orbital and partial sharing of the positive charge of the Cu(II) ion with hetero atoms. Furthermore, it enhances the delocalization of π-electrons around the entire complex ring and enhances the lipophilicity of the complexes. This increased lipophilicity enhances the permeation of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms. These complexes also troubled the respiration process of the cell and hence block the synthesis of proteins, which restricts additional growth of the organism and as a result microorganisms died. The experimental deviation in the activity of the Cu(II) complexes across the different classes of organisms studied may be characteristic

Table 6
Antimicrobial, anti-tubercular and antioxidant results of compound.

Compounds	Minimum inhibition concentration[a] of microorganisms (μg/mL)							Anti-tubercular activity[a]	Antioxidant activity[b]
	Bacteria				Fungi				
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. Niger*	*A. Clavatus*		
L^1	200	>150	200	200	>200	200	200	>100	NT
L^2	150	150	200	150	200	150	>150	100	NT
L^3	100	50	100	100	150	200	200	100	NT
L^4	100	50	150	150	100	150	150	>50	NT
L^5	150	100	150	150	150	>150	150	100	NT
L^6	>200	200	200	200	200	200	200	>100	NT
C^1	>100	150	100	100	100	150	200	100	311.70
C^2	100	100	150	100	100	100	150	>50	337.23
C^3	25	>25	25	25	25	50	25	25	407.02
C^4	25	25	>25	25	25	25	25	25	345.74
C^5	50	50	>50	>50	50	>50	>50	50	387.44
C^6	200	150	150	200	150	200	200	100	302.56
CQ	12	12	>6	12	>12	12	12	NT	NT
Isoniazide	NT	NT	NT	NT	NT	NT	NT	0.025	NT
Ethambutol	NT	NT	NT	NT	NT	NT	NT	20	NT
Streptomycin	12	6	6	12	NT	NT	NT	6.25	NT
Nystatin	NT	NT	NT	NT	6	12	12	NT	NT
Ascorbic acid	NT	NT	NT	NT	NT	NT	NT	NT	500

NT = not tested.
[a] Average value of triplicate results.
[b] FRAP results expressed in mM of ascorbic acid per 100 g of sample i.e. mmol/100 g.

to differences in cell wall and/or membrane construction (Gram-positive bacteria, Gram-negative bacteria and fungi). It is expected that the more extensive hetero-aromatic ring system of Clioquinol and the presence of the lipophilic group C═N would give better lipophilicity on the Cu(II) complexes and permit it to penetrate the cell wall and inhibit intracellular interactions. At the same time, hydroxylated derivatives had excellent antibacterial activity, it was unexpected that the other Cu(II) complexes were really moderate against all of the microbial species tested. Although the range of functionalities on the aromatic ring of the coumarin core is significant only in the presence of a hydroxyl group on the aromatic ring shown antimicrobial activity for the subsequent Cu(II) complexes. The role of the hydroxyl group in this activity is complicated to find out but the metal complexes of other hydroxylated derivatives of coumarin have been earlier shown to have excellent antimicrobial activity. Examples contain Cu(II) and Ni(II) complexes of 4-hydroxycoumarins [83,84]. In a previous study on the antimicrobial activity of catechols, the position and number of hydroxyl groups on the aromatic ring were responsible for their relative toxicity towards microorganisms, with facts that increasing hydroxylation results in an enhance in antimicrobial activity [85]. The mechanism recommended being liable for catechol toxicity to microorganisms contain enzyme inhibition by the oxidized compounds. The results would be indicate that substitution of the hydroxyl groups on the aromatic ring of the coumarin ligand was also vital for giving antimicrobial activity onto the Cu(II) complexes.

In fact, coordination, locking the polar electronegative atoms in the inner core around the metal and confining the apolar residues in an external lipophilic envelope, favor diffusion through biomembranes [86]. It is also suspected that factors such as solubility, conductivity, dipole moment may be the possible reasons for the increase in activity. Since all complexes have not shown enhanced activity as compared to parent ligand were rationalized the fact that, steric and pharmacokinetic factors also play a decisive role in deciding the potency of an antimicrobial agent [87]. In review, the antimicrobial testing results reveal that complexes possess higher activity at lower concentration compared to parent ligand. All ligands show inhibition concentration between 200 and 100 μg/mL. Complexes C^5 also exhibits activity at 50 μg/mL concentration while C^3 and C^4 complexes have shown enhancement in activity with MIC of 25 μg/mL.

4. Conclusions

Present studies describe the synthesis of biological active coumarin derivatives (L^1–L^6) and their Cu(II) complexes (C^1–C^6). The structures of the ligands were investigated and confirmed by the elemental analysis, FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectral studies. The ligand coordinates to metal ion through phenolic OH via deprotonation, eneamino nitrogen, lactone carbonyl oxygen and α,β-unsaturated carbonyl oxygen. The octahedral geometry were assigned for Cu(II) complexes on the basis of electronic, magnetic moment and thermogravimetric analysis. On the basis of these studies it is clear that ligands coordinated to metal atom in a monobasic bidentate mode, by O—N and O—O donor system. X-ray diffraction results show that the compound belongs to hexagonal crystal system with polycrystalline nature of complex which was further conformed octahedral geometry. Activation energy and pre-exponential factor calculated by isoconversional methods. Kissinger's and Ozawa's methods equally show an outstanding linear correlation between kinetics parameters based on the experimental figures and the reaction is first order. All the complexes found antioxidant significant activity compared to parent ligands employed for complexation. *In vitro* anti-tubercular and antimicrobial 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. Anti-tubercular activity results show that the complexation of ligand with Cu(II) metals has doubled its activity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2012.05.057.

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