ORIGINAL RESEARCH



Thermal, kinetic, spectroscopic studies and anti-microbial, antituberculosis, anti-oxidant properties of clioquinol and benzocoumarin derivatives mixed complexes with copper ion

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Received: 4 January 2013 / Accepted: 5 March 2013 © Springer Science+Business Media New York 2013

Abstract A series of six Cu(II) complexes of cliquinol with benzo-coumarin derivatives have been synthesized. Physico-chemical, spectroscopic, and thermal properties of the complexes have been studied on the basis of infrared spectra, mass spectra, NMR spectra, electronic spectra, elemental analyses, and thermogravimetric analyses. The kinetic parameters such as order of reaction (n), energy of activation (E_a) , entropy (S^*) , pre-exponential factor (A), enthalpy (H^*) , and Gibbs free energy (G^*) have been calculated using Freeman-Carroll method. All the compounds were screened for their anti-bacterial activity against Escherichia coli, Pseudomonas aeruginosa, Streptococcus pyogenes, Bacillus subtilis, and anti-fungal activity against Candida albicans and Aspergillus niger. Ferric-reducing anti-oxidant power of all complexes were measured. Also the compounds against Mycobacterium tuberculosis shows clear enhancement in the anti-tubercular activity upon copper complexation.

 $\label{eq:comparison} \begin{array}{l} \mbox{Keywords} \quad \mbox{Benzo-coumarin derivative} \cdot \mbox{Cu(II) complex} \cdot \\ \mbox{Anti-microbial} \cdot \mbox{Anti-oxidant} \cdot \mbox{Anti-tuberculosis} \end{array}$

Electronic supplementary material The online version of this article (doi:10.1007/s00044-013-0576-6) contains supplementary material, which is available to authorized users.

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Abbreviations

- CQ Clioquinol
- L $L_1, L_2, L_3, L_4, L_5, L_6$
- L₁ 2-Cinnamoyl-*3H*-benzo[*f*]chromen-3-one
- L₂ (E)-2-(3-(3-Hydroxyphenyl)acryloyl)-3*H*-benzo[*f*] chromen-3-one
- L₃ (E)-2-(3-(4-Hydroxyphenyl)acryloyl)-*3H*-benzo[*f*] chromen-3-one
- L₄ (E)-2-(3-(4-Chlorophenyl)acryloyl)-*3H*-benzo[*f*] chromen-3-one
- L₅ (E)-2-(3-(4-Nitrophenyl)acryloyl)-*3H*-benzo[*f*] chromen-3-one
- L₆ (E)-2-(3-(2-Nitrophenyl)acryloyl)-*3H*-benzo[*f*] chromen-3-one
- $\mathbf{C} \qquad \mathbf{C}_1, \, \mathbf{C}_2, \, \mathbf{C}_3, \, \mathbf{C}_4, \, \mathbf{C}_5, \, \mathbf{C}_6$
- $C_1 \qquad [Cu(L_1)(CQ)(H_2O)(OH)]$
- $\mathbf{C_2} \qquad [\mathrm{Cu}(\mathrm{L}_2)(\mathrm{CQ})(\mathrm{H}_2\mathrm{O})(\mathrm{OH})]$
- $\mathbf{C_3} \qquad [\mathrm{Cu}(\mathrm{L}_3)(\mathrm{CQ})(\mathrm{H}_2\mathrm{O})(\mathrm{OH})]$
- $C_4 \qquad [Cu(L_4)(CQ)(H_2O)(OH)]$
- $\mathbf{C_5} \qquad [\mathrm{Cu}(\mathrm{L_5})(\mathrm{CQ})(\mathrm{H_2O})(\mathrm{OH})]$
- $C_6 \qquad [Cu(L_6)(CQ)(H_2O)(OH)]$
- B.M. Bohr magneton
- TG Thermo gravimetry
- DTG Differential thermogravimetric
- $E_{\rm a}$ Activation energy
- A Pre-expontial factor
- *N* Order of reaction

Introduction

Interest in coumarin chemistry has flourished for numerous years, largely as a result of extensive increase in the use of coumarin derivatives. Naturally occurring as well as synthetic coumarin derivatives find applications in various therapeutic

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areas (Olomola et al., 2010; Riveiro et al., 2010). Coumarin is a widely occurring secondary metabolite that occurs naturally in several plant families and essential oils, and has been used as sweeteners, fixatives of perfumes, additives in food, and cosmetics, odor stabilizers in tobacco and odor maskers in paints and rubber (Tyagi et al., 2005). In addition, heterocyclic compounds containing coumarin synthon have shown various activities such as anti-HIV, (Bedoya et al., 2005; Kirkiacharian et al., 2002; Lee, 2003; Thaisrivongs et al., 1994) anti-coagulant, (Kidane et al., 2004) anti-bacterial, (Khan et al., 2004) anti-cancer, (Radanyi et al., 2008) anthelminthic, (Lee et al., 1998) anti-inflammatory, (Ghate et al., 2003; Kontogiorgis and Hadjipavlou-Litina, 2004), and anti-oxidant (Kontogiorgis and Hadjipavlou-Litina, 2004; Nicolaides et al., 1998; Raj et al., 1998). The nature of substituent classifies that coumarin substitution at positions-3 is dominant for anti-microbial activity (Debeljak et al., 2007). Due to the known anti-microbial activity (Dawara and Singh, 2011; Hishmat et al., 1989; Kharadi and Patel, 2010), we were interested to prepare 3-acetyl coumarin derivatives and its metal complexes.

Derivative of 8-hydroxyquinoline i.e., clioquinol (CQ) is well-known for their anti-biotic properties in therapy due to its coordinating ability towards metal ions. CQ derivatives, especially 5-chloro-7-iodo-8-hydroxyquinoline (CQ) attenuated Alzheimer's disease (AD) symptoms in human clinical trials, belonging to the family of drugs called anti-infectives. CQ is an anti-bacterial and anti-fungal agent, which used widely as a topical cream for the treatment of skin infections such as eczema, athlete's foot, and other fungal infections (Ritchie et al., 2003; Yassin et al., 2000). Inciting renewed interest in its pharmacodynamics and linking its possible advantageous therapeutical action to the chelation of copper(II) in the brain. Metal ions and complexes have key roles in a broad range of processes necessary for brain function (Burdette and Lippard, 2003). Cu(II) plays a significant role in brain metabolism, as it is essential for the known enzymes CuZn, superoxide dismutase (SOD), ceruloplasmin, cytochrome C-oxidase, tyrosinase and dopamine b-hydroxylase. Copper is also associated with various biomolecules related to essential physiological activities in human organism. Until now numerous researches have been actively investigated copper compounds based on the hypothesis that endogenous metals may prove less toxic and more potent (Marzano et al., 2009). It has been well-established that the properties of copper complexes are largely determined by the nature of ligands and the donor atoms bound to the metal ion. Redox activity of Cu(II) ion plays a decisive role in addressing the main effects of this metal. Cu(II) may contribute to the production of free radicals and in regulation and in induction of apoptosis (Kozlowski et al., 2009).

Reactive oxygen species (ROS) are formed by aerobic organisms as an unavoidable consequence of cell metabolism.

Although effective natural defense mechanisms against these highly reactive species exist (e.g., natural anti-oxidants such as vitamins E and C, β -carotene, and polyphenolic flavonoids), excessive ROS production which escorts many pathophysiological conditions (oxidative stress) poses the need of further anti-oxidant protection. As a result, research for the development of novel small molecules with anti-oxidant activity is constantly attracting attention. Hence, our effort is focused on these metal-ion complexes because of their promising uses as anti-oxidant agents (Colak *et al.*, 2010).

The aim of this study was to prepare the mixed-ligand complexes of Cu(II) using CQ with coumarin derivatives and to determine their properties. In our previous reports, we have mentioned a series of fused coumarin derivatives and its transition metal complexes (Kharadi and Patel, 2009a; b). In continuation of our preceding work, we describe here synthesis, characterization, and spectroscopic features of new mixed-ligand Cu(II) complexes of CQ with coumarin derivatives along with anti-microbial, antioxidant, and anti-tubercular activities. Thermal behavior of the complexes has been investigated using thermogravimetric (TG) analysis. Thermogravimetry is a process in which a substance is decomposed in the presence of heat, which causes bonds of the molecules to be broken (Albano et al., 2000; Carrasco, 1993). Kinetic parameters like order of reaction (*n*), activation energy (E_a), entropy change (S^*), enthalpy change (H^*) , free energy change (G^*) , and preexponential factor (A) are carried out using kinetic studies of thermal decomposition reactions.

Experimental

Material

All chemicals were purchased from the following commercial sources: Spectrochem Pvt. Ltd., Mumbai, E. Merck Pvt. Ltd., Mumbai, S D Fine Chem Limited, Mumbai. All chemicals were of reagent grade and solvents of analytical grade were distilled, purified and dried using appropriate methods (Vogel and Furniss, 1989). All reactions were monitored by thin-layer chromatography (TLC on aluminium plates coated with silica gel 60 F_{254} , 0.25 mm thickness, Merck) and detection of the components were done under UV light or explore in iodine chamber. The metal nitrates were used in hydrated form.

Physical measurement

Elemental analysis was performed by elemental analyzer PerkinElmer, Cambridge, MA, USA 2400-II CHN. Analyses of metal ions was carried out by the dissolution of the solid complexes in hot concentrated nitric acid, further diluted

with distilled water (DW) and filtered to remove the precipitated organic ligands. Remaining solution was neutralized with ammonia solution and the metal ions were titrated against EDTA. Melting points were measured using open capillary. Infrared spectra were recorded in the region of 4,000-400 cm⁻¹ on Shimadzu FTIR 8401 spectrophotometer using KBr pellets. ¹H and ¹³C NMR measurements were carried out on Avance-II 400 Bruker NMR spectrometer. Chemical shifts were measured with respect to TMS as internal standard and DMSO- d_6 used as solvent. FAB mass spectrums of complexes were recorded at CDRI, Lucknow with JEOL SX-102/DA-6000 mass spectrometer at room temperature using Argon/Xenon as the FAB gas. Electronic spectra were recorded on a LAMBDA 19 UV/VIS/NIR in the region of 200-1,200 nm. TG scans were run on a model Diamond TG/DTA, PerkinElmer, Cambridge, MA, USA. The analysis carried out under a nitrogen atmosphere at a heating rate of 20 °C min⁻¹ from 30 to 840 °C. Effective magnetic susceptibility measurement were calculated by the Gouy's method using mercury tetrathiocyanatocobaltate (II) as a calibrant ($\chi = 16.44 \times 10^{-6}$ c.g.s. units at 20 °C). Molar susceptibility was corrected using Pascal's constant (Mabbs and Machin, 2008).

Preparation of ligands

2-Acetyl-3*H*-benzo[f]chromen-3-one was prepared according to reported method (Bischler, 1892). General procedure for the synthesis of ligands (**L**) is shown in Scheme 1.

In a 250 mL three-neck round bottom flask, a mixture of 2-hydroxynaphthaldehyde (0.1 mol) and ethylacetoacetate (0.1 mol) was taken in 50 mL ethanol. Catalytic amount of piperidine (3–4 drops) was added and the reaction mixture was stirred for 10 min at room temperature. It was further heated for 2 h; in water bath. A yellow solid obtained was separated by filtration, washed with cold ether and dried over the vacuum pump. It was recrystallized from chloroform–hexane. Yield, 85 %; m.p. 189 °C. ESI–MS (m/z): 238.8 (M)+.

2-Cinnamoyl-3H-benzo[f]chromen-3-one (L_1)

A mixture of 2-acetyl-3H-benzo[f]chromen-3-one (0.01 mol) and benzaldehyde (0.015 mol) in chloroform (50 mL) was taken in 100 mL round bottomed flask fitted with a reflux condenser. 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 mixture was refluxed for 6 h and progress of the reaction was monitored by TLC. After completion of reaction (as evidenced by TLC), the reaction mixture was cooled to room temperature. A solid product separated out was filtered out, washed with cold ethanol and dried in air. It was recrystallized from ethanol-DMF (9:1) mixture. C₂₂H₁₄O₃: yield, 80 %; m.p. 207 °C. ESI-MS (m/z): 326.3[M]+. Found (%): C, 80.71, H, 4.03. Calculated: C, 80.97, H, 4.32; ¹H NMR (DMSO-d₆ 400 MHz): δ: 7.46–7.82 (10H, m, Ar-H and H_{12}), 8.06 (1H, d, J = 7.8 Hz, Ar-H₉), 8.30 (1H, d, J = 9.0 Hz, H₁₃), 8.62 (1H, d, J = 8.2 Hz, Ar-H₅), 9.35 (1H, s, Ar-H₄); ¹³C NMR (DMSO-*d*₆ 100 MHz): δ: 112.61 (C-4a), 116.33 (C-10), 122.27, 123.84, 124.49, 126.35, 128.57, 128.90, 128.97, 129.30, 129.86, 130.66, 134.44, 135.92 (14C, Ar-C), 142.79 (C-14), 143.81 (C-13), 151.22 (C-4), 155.17 (C-10a), 158.42 (C=O, lactone carbon of coumarin), 186.64 (C=O, α , β -unsaturated ketone); FTIR (KBr cm⁻¹): 1610 (C=O, α , β -unsaturated ketone), 1722 (C=O, lactone carbonyl of coumarin).

(E)-2-(3-(3-Hydroxyphenyl)acryloyl)-3Hbenzo[f]chromen-3-one (L₂)

L₂ was synthesized by the same method used for L₁ using 3-hydroxybenzaldehyde. C₂₂H₁₄O₄: yield, 76 %; m.p. 252 °C. ESI–MS (*m*/*z*): 342.6[M]+. Found (%): C, 77.48, H, 4.42 %. Calculated: C, 77.18, H, 4.12; ¹H NMR (DMSO-*d*₆ 400 MHz): δ : 6.87–7.79 (9H, m, Ar–H and H₁₂), 8.08 (1H, d, *J* = 8.0 Hz, Ar–H₉), 8.31 (1H, d, *J* = 9.2 Hz, Ar–H₁₃), 8.63 (1H, d, *J* = 8.4 Hz, Ar–H₅), 9.30 (1H, s, Ar–H₄), 9.71 (1H, br s, Ar–OH); ¹³C NMR (DMSO-*d*₆ 100 MHz): δ : 112.63 (C-4a), 114.48 (C-10),



R =(-H, -3-OH, -4-OH, -4-Cl, -4-NO₂, -2-NO₂)

Scheme 1 General procedure for synthesis of ligands (L)

116.48, 118.16, 120.11, 122.45, 124.10, 124.54, 126.46, 128.98, 129.02, 129.25, 129.85, 130.06, 135.73 (13C, Ar–C), 135.87 (C-14), 142.56 (C-13), 144.12 (C-4), 155.06 (C-10a), 157.75 (C–OH), 158.48 (C=O, lactone carbon of coumarin), 186.99 (C=O, α , β -unsaturated ketone); FTIR (KBr cm⁻¹): 3380 (O–H, -stretching), 1610 (C=O, α , β -unsaturated ketone), 1743 (C=O, lactone carbonyl of coumarin).

(E)-2-(3-(4-Hydroxyphenyl)acryloyl)-3Hbenzo[f]chromen-3-one (L₃)

 L_3 was synthesized by the same method used for L_1 using 4-hydroxybenzaldehyde. C₂₂H₁₄O₄: yield, 78 %; m.p. 259 °C. ESI-MS (m/z): 342.8[M]+. Found (%): C, 77.42, H, 4.38. Calculated: C, 77.18, H, 4.12; ¹H NMR (DMSO- d_6 400 MHz): δ: 6.81-7.92 (9H, m, Ar-H and H₁₂), 8.21 (1H, d, J = 8.8 Hz, Ar-H₉), 8.42 (1H, d, J = 9.6 Hz, Ar-H₁₃), 8.75 (1H, d, J = 8.0 Hz, Ar–H₅), 9.42 (1H, s, C₄–H), 9.89 (1H, s, -OH); ¹³C NMR (DMSO-*d*₆ 100 MHz): δ: 111.64 (C-4a), 114.87 (C-10), 117.54, 120.76, 122.64, 124.69, 124.87, 126.12, 128.62, 129.56, 129.94, 130.23, 135.46 (13C, Ar-C), 136.32 (C-14), 143.54 (C-13), 146.12 (C-4), 155.06 (C-10a), 157.56 (C-OH), 159.24 (C=O, lactone carbon of coumarin), 187.09 (C=O, α , β -unsaturated ketone); FTIR (KBr cm⁻¹): 3385 (O-H, -stretching), 1615 (C=O, α , β -unsaturated ketone), 1740 (C=O, lactone carbonyl of coumarin).

(E)-2-(3-(4-Chlorophenyl)acryloyl)-3Hbenzo[f]chromen-3-one (L₄)

L₄ was synthesized by the same method used for L₁ using 4-chlorobenzaldehyde. C₂₂H₁₃ClO₃: yield, 68 %; m.p. 230 °C. ESI–MS (*m*/*z*): 360.5[M]+, 362.2[M+2]+. Found (%): C, 73.59, H, 3.93. Calculated: C, 73.24, H, 3.63; ¹H NMR (DMSO-*d*₆ 400 MHz): δ: 7.43–7.85 (9H, m, Ar–H and H₁₂), 8.18 (1H, d, *J* = 8.0 Hz, Ar–H₉), 8.45 (1H, d, *J* = 10.0 Hz, Ar–H₁₃), 8.63 (1H, d, *J* = 8.8 Hz, Ar–H₅), 9.38 (1H, s, C₄– H); ¹³C NMR (DMSO-*d*₆ 100 MHz): δ: 113.23 (C-4a), 114.44 (C-10), 115.48, 118.54, 122.21, 124.65, 125.19, 125.84, 126.46, 127.28, 128.35, 130.46, 133.5, 134.23 (14C, Ar–C), 135.67 (C-14), 142.46 (C-13) 144.42 (C-4), 155.36 (C-10a), 158.68 (C=O, lactone carbon of coumarin), 186.49 (C=O, α, *β*-unsaturated ketone); FTIR (KBr cm⁻¹): 1605 (C=O, α, *β*-unsaturated ketone), 1725 (C=O, lactone carbonyl of coumarin), 1090 (*p*-substituted (C–Cl)).

(E)-2-(3-(4-Nitrophenyl)acryloyl)-3Hbenzo[f]chromen-3-one (L₅)

 L_5 was synthesized by the same method used for L_1 using 4-nitrobenzaldehyde. $C_{22}H_{13}NO_5$: yield, 64 %; m.p. 244 °C.

ESI–MS (*m*/*z*): 371.3[M]+. Found (%): C, 71.37, H, 3.74, N, 3.98. Calculated: C, 71.16, H, 3.53, N, 3.77; ¹H NMR (DMSO-*d*₆ 400 MHz): δ: 6.90–7.65 (9H, m, Ar–H and H₁₂), 8.15 (1H, d, *J* = 8.4 Hz, Ar–H₉), 8.25 (1H, d, *J* = 9.6 Hz, Ar–H₁₃), 8.47 (1H, d, *J* = 8.0 Hz, Ar–H₅), 9.38 (1H, s, C₄–H); ¹³C NMR (DMSO-*d*₆ 100 MHz): δ: 111.73 (C-4a), 114.20 (C-10), 116.69, 118.34, 120.91, 122.65, 124.65, 126.76, 128.08, 129.22, 129.95, 130.35, 135.13 (13C, Ar–C), 135.89 (C-14), 143.16 (C-13), 144.52 (C-4), 147.42 (C-17, carbon attach to NO₂), 155.46 (C-10a), 159.28 (C=O, lactone carbon of coumarin), 187.29 (C=O, α, β-unsaturated ketone); FTIR (KBr cm⁻¹): 1608 (C=O, α, β-unsaturated ketone), 1735 (C=O, lactone carbonyl of coumarin), 1510 (Ar–NO₂, asymmetric), 1345 (Ar–NO₂, symmetric).

(E)-2-(3-(2-Nitrophenyl)acryloyl)-3Hbenzo[f]chromen-3-one (L₆)

 L_6 was synthesized by the same method used for L_1 using 2-nitrobenzaldehyde. C₂₂H₁₃NO₅: yield, 65 %; m.p. 218 °C. ESI-MS (m/z): 371.6[M]+. Found (%): C, 71.42, H, 3.76, N, 3.96. Calculated: C, 71.16, H, 3.53, N, 3.77; ¹H NMR (DMSO-d₆ 400 MHz): δ : 6.80–7.20 (9H, m, Ar-H and H_{12}), 7.80 (1H, d, J = 8.8 Hz, Ar-H₉), 8.05 (1H, d, J = 10.4 Hz, Ar-H₁₃), 8.18 (1H, d, J = 8.4 Hz, Ar-H₅), 9.23 (1H, s, C₄-H); ¹³C NMR (DMSO- d_6 100 MHz): δ : 111.73 (C-4a), 113.56 (C-10), 116.67, 118.86, 120.31, 122.68, 124.21, 124.25, 126.87, 128.78, 129.42, 129.35, 132.55, 133.06, 134.28 (13C, Ar-C), 135.65 (C-14), 142.32 (C-13), 144.56 (C-4), 152.22 (C-15, carbon attach to phenolic NO₂), 155.26 (C-10a), 158.44 (C=O, lactone carbon of coumarin), 186.39 (C=O, α , β -unsaturated ketone); FTIR (KBr cm⁻¹): 1605 (C=O, α , β -unsaturated ketone), 1738 (C=O, lactone carbonyl of coumarin), 1495 (Ar-NO₂, asymmetric), 1330 (Ar-NO₂, symmetric).

Synthesis of complexes (C₁–C₆)

All these complexes (C_1-C_6) were prepared by the general experimental procedure as described below and synthetic route of complexes (C) shown in Scheme 2.

A hot ethanolic solution of hydrated metal nitrate i.e., $Cu(NO_3)_2 \cdot 3H_2O(1.0 \text{ mmol})$ and ligand (L) (1.0 mmol) was slowly added to a ethanolic solution of CQ (1.0 mmol). The resultant mixture was neutralized (pH = 7) using dilute solution of NaOH in water. Furthermore, the mixture was heated under reflux for 6–8 h; after that solution was evaporated to half of its original volume. The formed precipitates were filtered off, washed with ethanol followed by diethyl ether and dried under vacuum. The physico-chemical data of the synthesized complexes are listed in Table 1.

Scheme 2 General synthetic

route of complexes (C)



R=-H,-3-OH,-4-OH,-4-Cl,-4-NO₂,-2-NO₂

Table 1	Analytical	and p	hysiochemical	parameters	of the	complexes
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Entry	Empirical formula	Elemental ana	Elemental analyses, % found (required)				Yield Mo	Molecular	$\mu_{\rm eff}$
		С	Н	Ν	Cu(II)	(°C)	(%)	mass	B.M.
C ₁	$\begin{matrix} [Cu(L_1)(CQ)(H_2O)(OH)] \\ C_{31}H_{21}ClCuINO_6 \end{matrix}$	51.37 (51.05)	2.73 (2.90)	1.59 (1.92)	8.93 (8.71)	>300	75	727.47	1.91
C ₂	$ \begin{bmatrix} Cu(L_2)(CQ)(H_2O)(OH) \end{bmatrix} \\ C_{31}H_{21}ClCuINO_7 $	49.73 (49.95)	2.66 (2.84)	1.69 (1.88)	8.81 (8.53)	>300	72	743.65	1.88
C ₃	$ \begin{bmatrix} Cu(L_3)(CQ)(H_2O)(OH) \end{bmatrix} \\ C_{31}H_{21}ClCuINO_7 $	49.67 (49.95)	2.69 (2.84)	1.63 (1.88)	8.37 (8.53)	>300	77	743.72	1.90
C ₄	$ \begin{bmatrix} Cu(L_4)(CQ)(H_2O)(OH) \end{bmatrix} \\ C_{31}H_{20}Cl_2CuINO_6 $	48.52 (48.74)	2.88 (2.64)	1.57 (1.83)	8.61 (8.32)	>300	72	761.44	1.93
C ₅	$ \begin{bmatrix} Cu(L_5)(CQ)(H_2O)(OH) \end{bmatrix} \\ C_{31}H_{20}ClCuIN_2O_8 $	48.35 (48.08)	2.82 (2.60)	3.50 (3.62)	8.52 (8.21)	>300	65	772.75	1.92
C ₆	$\begin{array}{l} [Cu(L_6)(CQ)(H_2O)(OH)] \\ C_{31}H_{20}ClCuIN_2O_8 \end{array}$	48.42 (48.08)	2.84 (2.60)	3.48 (3.62)	8.47 (8.21)	>300	62	772.51	1.87

The complexes comprise high melting points (>300 °C) and are insoluble in common organic solvents but partially soluble in DMF as well as DMSO.

Method of anti-microbial assay

The minimum inhibition concentration (MIC) of synthesized compounds was carried out using Kirby-Bauer disk diffusion method according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) (Clinical and Laboratory Standards Institute (Clsi) (formerly Nccls) Performance Standards for Anti-microbial Disk Susceptibility Tests Approved Standard, 2006). Anti-bacterial activity was screened against two Gram-positive (B. subtilis ATCC 11774, S. pyogenes ATCC12384) and two Gram-negative (E. coli ATCC 25922, P. aeruginosa ATCC 25619) bacteria by taking ciprofloxacin and norfloxacin as standard antibacterial drugs. Anti-fungal activity was screened against two fungal species (C. albicans ATCC 66027 and A. niger ATCC64958), where flucanazole and nystatin were used as standard anti-fungal drugs. All ATCC cultures were collected from Bangalore and tested against above-mentioned known drugs. The standard solutions of anti-bacterial drugs and anti-fungal drugs were prepared in DMSO. The results including standard drugs are summarized in Table 2.

In this method, anti-biotics are impregnated onto paper disks and then placed on a seeded Mueller–Hinton agar plate using a mechanical dispenser or sterile forceps. Plate is then incubated for 16–18 h at 37 °C and diameter of zone of inhibition around disk is measured to the nearest millimeter. Solution of all newly synthesized compounds and standard drugs were prepared at 600, 400, 200, 100, 70, 40, 20 μ g mL⁻¹ and at 100, 80, 60, 40, 35, 30, 20, 15, 10, 5, 2, 1 μ g mL⁻¹ concentrations, respectively using micro dilution method, in the wells of microplates by plating in MHB. The lowest concentration of compound that completely inhibits macroscopic growth was determined and minimum inhibitory concentrations (MIC) are reported in Table 2.

Method of anti-oxidant assay

Ferric-reducing anti-oxidant power (FRAP) was measured by a modified method (Parmar *et al.*, 2012). The antioxidant potentials of compounds were estimated as their power to reduce the TPTZ–Fe(III) complex to TPTZ–Fe(II) complex. This method is simple, fast, and reproducible results can be obtained. Total anti-oxidant capacity of

 Table 2
 Anti-microbial and anti-oxidant activities of synthesized compounds

Anti-microbial a	Anti-oxidant activity							
Entry	Gram-negative bacteria		Gram-positive bacteria		Fungus		FRAP value (mmol 100 g^{-1})	
	E. coli	P. aeruginosa	S. pyogenes	B. subtilis	C. albicans	A. niger		
L ₁	400	400	400	>600	400	200	NT	
L_2	400	200	200	600	200	200	NT	
L ₃	200	200	400	400	200	400	NT	
L_4	100	100	100	200	200	200	NT	
L ₅	100	200	100	200	200	200	NT	
L ₆	200	400	200	400	400	200	NT	
C ₁	100	100	100	100	200	100	332	
C ₂	100	100	100	200	100	100	402	
C ₃	70	100	100	100	100	100	367	
C ₄	40	70	40	40	100	100	306	
C ₅	70	100	70	100	100	100	335	
C ₆	100	200	100	200	100	100	353	
Ampicillin	100	100	100	100	NT	NT	NT	
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

biological samples is expressed as ascorbic equivalent (mmol 100 g^{-1} of dried compound).

Preparation of solution

- Acetate buffer, 300 mM pH 3.6 (3.1 g sodium acetate trihydrate and 16 ml concentrated acetic acid per L of buffer solution).
- b) 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) (MW 312.34) in 40 mM HCl.
- c) 20 mM FeCl₃·6H₂O (MW 270.30) in DW.
- d) 1 mM of ascorbic acid (MW 176.13 g mol⁻¹) dissolved in 100 mL DW.

FRAP working solution: mix the above prepared (a), (b), and (c) solutions in the ratio of 10:1:1, respectively. A mixture of 40.0 μ L, 0.5 mM sample solution, and 1.2 mL FRAP reagent was incubated at 37 °C for 15 min. Working solution must be always freshly prepared. The ascorbic acid was used as a standard anti-oxidant compound.

In vitro evaluation of anti-tuberculosis activity

An entitle compounds were evaluated for in vitro antitubercular activity. The MICs were determined and interpreted for M. tuberculosis H37Rv according to the procedure of the approved micro dilution reference method of anti-microbial susceptibility testing (Akhaja and Raval, 2011; Andrew, 2001; Rattan and Churchill, 2000). Compounds were taken at concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 μ g mL⁻¹ 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 DW. The vial was shaken for 5 min. Then using nichrome wire loop suspension was inoculated on the surface of each of Lowenstein-Jensen medium containing the test compounds. Further test media was incubated for 28 days at 37 °C. Readings were seen after 28 days of incubation. The appearance of turbidity was considered as bacterial growth and indicates resistance to the compound. Test compounds were compared to reference drugs isoniazid, ethambutol, and streptomycin. Lowenstein-Jensen medium containing standard drugs was inoculated with M. tuberculosis H37RV strain. Anti-tubercular activity test was run in triplicate and the deviation for any triplicate results was not more than $\pm 1-5$ % and results are summarized in Table 3.

Table 3 Anti-tuberculosis activity of synthesized compounds

Entry	MIC μ g mL ⁻¹ <i>M. tuberculosis</i> (MTCC 200)	% Inhibition	
L ₁	100	18	
L_2	100	23	
L_3	50	46	
L_4	12.5	88	
L ₅	50	54	
L ₆	50	37	
C ₁	100	21	
C ₂	>50	29	
C ₃	25	52	
C ₄	3.125	96	
C ₅	12.5	92	
C ₆	25	43	
Streptomycin	6.25	98	
Isoniazid	0.25	99	
Ethambutol	3.125	99	

Results and discussion

The properties of Cu(II) complexes with coumarin derivatives were studied. The synthesized compounds were characterized and discussed on the basis of elemental analysis, infrared spectra, electronic spectra, FAB mass spectroscopy, thermal methods, magnetic and kinetic measurements. Further anti-microbial, anti-tuberculosis, and anti-oxidant activities for the same compounds were discussed.

Elemental analysis

Analytical and physicochemical data of the complexes are summarized in Table 1. The complexes are colored and stable in air. They are insoluble in water and in most organic solvents but partially soluble in DMF as well as DMSO. The structure of the complexes is assumed according to the chemical reaction as shown below:

 $\begin{array}{l} Cu(NO_3)_2 \cdot 3H_2O + L \\ + CQ \longrightarrow dil. NaOH[Cu(L)(CQ)(H_2O)(OH)] \\ + 2NaNO_3 + xH_2O \end{array}$

where $L = L_1$, L_2 , L_3 , L_4 , L_5 , L_6 .

IR spectra

The important infrared spectral bands and their tentative assignments for the synthesized compounds were recorded as KBr disks and are summarized in Table 4. The IR data of free ligands and its metal complexes were carried out within the IR range $4.000-400 \text{ cm}^{-1}$. In 8-hydroxyquinoline complexes of divalent metals, (C-O) band appeared at $\sim 1,120 \text{ cm}^{-1}$ region and position of the band slightly varies with the metal (Charles et al., 1956), furthermore (C-O) peak observed in free oxine molecule at $\sim 1.090 \text{ cm}^{-1}$. which shifted to higher frequencies in all mixed-ligand complexes giving a strong absorption band at $\sim 1,110 \text{ cm}^{-1}$. This clearly indicates that 8-hydroxyquinoline has been affected to leading complexation through metal ion. The IR spectrum of the ligands L₂ and L₃ shows a strong -OH stretching band between 3,350 and 3,400 cm⁻¹, while spectra of mixed-ligand Cu(II) complexes indicates a broad band in the region $3,300-3,500 \text{ cm}^{-1}$ due to stretching vibration of OH group which revels formation of complexes and other bands at ~850 and ~715 cm⁻¹ due to rocking and wagging vibration of the water molecule and OH group. respectively (Nakamoto, 2009). In addition, IR spectra of the coumarin derivatives $[L_1-L_6]$ shows 1,610 and 1,743 cm⁻¹ bands corresponding to α , β -unsaturated ketone and lactone carbonyl ketone, respectively, upon complexation these peaks shifted to a lower frequency 1,600 and 1,700 cm⁻¹ due to complex formation. Moreover, weak bands around 519 cm⁻¹ (Patel *et al.*, 2011) and 776 cm⁻¹ (Tümer *et al.*, 1999) are attributed to Cu-O and Cu-N stretching frequency, respectively. Tumer et al. mentioned that week band around 500 and 770 cm^{-1} were attributed to the Cu–O and Cu-N stretching frequency (Tümer et al., 1999).

Table 4 FT-IR data of the complexes

Complexes (C ₁ –C ₆)	$v(O-H)^{br} (cm^{-1})$	$v(C=N)^w (cm^{-1})$	α, β-unsaturated $v(C=O)^{s} (cm^{-1})$	Lactone carbonyl $v(C=O)^{s} (cm^{-1})$	$v(Cu-N)^{w} (cm^{-1})$	$v(Cu-O)^w (cm^{-1})$
C ₁	3,375	1,565	1,604	1,710	750	505
C ₂	3,350	1,575	1,600	1,700	770	500
C ₃	3,355	1,570	1,605	1,710	760	505
C ₄	3,400	1,560	1,600	1,705	765	495
C ₅	3,385	1,570	1,602	1,725	770	490
C ₆	3,410	1,565	1,600	1,715	750	500

s strong, w weak, br broad

FAB spectra

The fast atom bombardment (FAB) mass spectra of all complexes were obtained using *m*-nitrobenzyl alcohol as matrix. FAB-mass spectra of complexes and its fragmentation scheme of the complex C_1 ($C_{31}H_{21}ClCuINO_6$) given in supplementary material. This spectrum reveals that molecular ion peak at m/z 727 of complex (without water of crystallization) along with several other peaks observed at 710, 583, 531, 440, 389, 349, 324, 280, 147, and 146 m/z value. Thus, the m/z of all fragments of complex with relative intensity confirms the stoichiometry of complex. Apart from it several peaks found at 136,137,154, and 307 m/z value are due to the usage of matrix.

Reflectance spectra and magnetic properties

The magnetic moments of mixed-ligand Cu(II) complexes $(d^9 \text{ system})$ are known for their varieties of structures due to their various coordination numbers. Six coordinated Cu(II) complexes possess distorted octahedral geometry. Magnetic moments of the Cu(II) complexes lies in between 1.87 and 1.97 BM range. These values are typical of mononuclear Cu(II) compounds with d^9 electronic

Table 5 Thermo analytical data of Cu(II) complexes

configuration. The observed magnetic moments of all complexes correspond to characteristic high-spin octahedral complexes. However, these values are slightly higher than the expected spin-only values due to spin-orbit coupling contribution (Cotton, 1999). Cu(II) complexes with octahedral geometry reveals that the absorption spectra are in visible region and only one broad band about 615 nm (Lewis *et al.*, 1960; Patel *et al.*, 2010). Here in the case of our spectra the only λ_{max} was found at about 615 nm which indicated an octahedral geometry for metal complexes.

Thermal studies of Cu(II) complexes

Thermal decomposition data of all complexes are summarized in Tables 5 and 6. Thermogravimetric analysis (TG and DTG) representative curves corresponding to complex (C_2) are presented in Figs. 1 and 2. Thermal decomposition occurs in three steps. In first step, endothermic decomposition between 180 and 240 °C attributed to dehydration process, which is due to loss of coordinated water molecule and hydroxyl ion among which the observed mass loss is 4.62 %, which is nearly equal to theoretical value 4.70 %. Loss of coordinated water molecule and hydroxyl ion is a first-order and value of energy of activation for dehydration

Complexes [formula]/(code)	TG range (°C)	DTG _{max} (°C)	Mass loss/% obs. (calculated)	Assignment
$[Cu(L_1)(CQ)(H_2O)(OH)]$	190–250	232	4.49 (4.81)	Removal of 1 mol –OH and H ₂ O
(C ₁)	280-380	354	41.62	Removal of L ₁ ligand
	380-800	529	39.32	Removal of CQ ligand
			14.57	Leaving CuO residue
$[Cu(L_2)(CQ)(H_2O)(OH)]$	180-240	229	4.62 (4.70)	Removal of 1 mol –OH and H ₂ O
(C ₂)	290-380	352	39.69	Removal of L ₂ ligand
	380-800	535	43.32	Removal of CQ ligand
			12.37	Leaving CuO residue
$[Cu(L_3)(CQ)(H_2O)(OH)]$	170-220	227	4.94 (4.70)	Removal of 1 mol –OH and H ₂ O
(C ₃)	280-360	355	42.29	Removal of L ₃ ligand
	360-800	537	41.23	Removal of CQ ligand
			11.54	Leaving CuO residue
$[Cu(L_4)(CQ)(H_2O)(OH)]$	180-250	235	4.39 (4.59)	Removal of 1 mol –OH and H ₂ O
(C ₄)	280-390	353	44.23	Removal of L ₄ ligand
	390-800	532	39.36	Removal of CQ ligand
			12.02	Leaving CuO residue
$[Cu(L_5)(CQ)(H_2O)(OH)]$	200-260	225	4.92 (4.53)	Removal of 1 mol –OH and H ₂ O
(C ₅)	260-370	339	43.33	Removal of L ₅ ligand
	370-800	533	39.23	Removal of CQ ligand
			12.52	Leaving CuO residue
$[Cu(L_6)(CQ)(H_2O)(OH)]$	160-230	225	4.39 (4.53)	Removal of 1 mol –OH and H ₂ O
(C ₆)	260-390	359	43.63	Removal of L_6 ligand
	390-800	552	39.22	Removal of CQ ligand
			12.76	Leaving CuO residue

Complex [formula]/(code)	TG range (°C)	$E_{\rm a} ({\rm kJ} {\rm mol}^{-1})$	п	$A (s^{-1})$	S* (J K ⁻¹ mol ⁻¹)	$H^* (kJ mol^{-1})^{-1}$	$G^* (kJ mol^{-1})$
$[Cu(L_1)(CQ)(H_2O)(OH)]$	190–250	3.45	1.00	0.012	-104.3	0.163	31.53
(C ₁)	280-380	15.49	1.30	0.164	-99.27	19.84	42.73
	380-800	74.84	0.97	7.12×10^2	-97.63	67.55	176.89
$[Cu(L_2)(CQ)(H_2O)(OH)]$	180-240	3.81	1.03	0.067	-102.93	0.154	37.78
(C ₂)	290-380	18.11	1.12	0.135	-97.46	15.45	45.44
	380-800	72.57	1.17	7.25×10^2	-95.16	64.48	157.07
$[Cu(L_3)(CQ)(H_2O)(OH)]$	170-220	3.57	1.05	0.024	-101.3	0.147	35.75
(C ₃)	280-360	14.21	1.27	0.113	-97.46	12.76	43.18
	360-800	67.27	1.12	7.45×10^2	-96.57	61.72	152.43
$[Cu(L_4)(CQ)(H_2O)(OH)]$	180-250	3.78	0.99	0.065	-109.51	0.176	36.27
(C ₄)	280-390	19.65	1.19	0.174	-96.52	19.69	42.76
	390-800	73.29	1.12	7.32×10^{2}	-90.6	63.59	163.97
$[Cu(L_5)(CQ)(H_2O)(OH)]$	200-260	3.34	1.15	0.041	-105.5	0.191	36.634
(C ₅)	260-370	16.67	1.20	0.125	-98.78	21.763	48.374
	370-800	64.53	1.00	7.74×10^{2}	-93.26	54.641	167.476
$[Cu(L_6)(CQ)(H_2O)(OH)]$	160-230	3.51	1.10	0.023	-102.3	0.175	33.72
(C ₆)	260-390	20.82	1.32	0.165	-98.14	13.25	42.76
	390-800	76.17	1.02	7.63×10^{2}	-97.17	60.96	152.25

Table 6 Kinetic parameters of Cu(II) complexes

process is found to be 3.81 kJ mol⁻¹. In second step, exothermic decomposition between 290 and 380 °C corresponds to loss of coordinated ligand with observed mass loss 39.69 %. Next step was also exothermic and associated with elimination of coordinated CQ, respectively. As temperature increases (380–800), intermediate complexes [Cu(CQ)] converted to CuO residue of fragments. Observed mass loss in this step is 43.32 %. Final product estimated as CuO, has observed mass of 12.37 % compared to the calculated value of 10.61 %. First is dehydration process with endothermic effect on DTG curve at 229 °C, while increase in the temperature of [Cu(L₂)(CQ)], shows exothermic effect at 352 °C in second step. In third step, exothermic DTG peak at 535 °C is associated with elimination of CQ. Final residue

predicted as CuO, while thermal fragmentation scheme for $[Cu(L_2)(CQ)(H_2O)(OH)]$ is shown in Scheme 3.

Kinetic measurement and thermal stability

In past decade there has been major interest in determining rate-dependent parameters of solid-state non-isothermal decomposition reactions by analysis of TG curves. A number of equations (Šesták *et al.*, 1973; Wendlandt, 1974) have been reported to analyze a TG curve and to obtain values for kinetic parameters. Wendlandt et al. (Šesták *et al.*, 1973; Wendlandt, 1974) has discussed advantages of this method over the conventional isothermal method. Rate



Fig. 1 TG curve of the complex C_2



of a decomposition process can be described as product of two separate functions of temperature and conversion (Šesták *et al.*, 1973). Some of the kinetic parameters such as activation energy and order of reaction for initial decomposition reaction were calculated and relationship between thermal stability and chemical structure of complexes is discussed. Commonly used methods for this purpose are differential method of Freeman and Carroll (1958) integral method of Coats and Redfern (Refat *et al.*, 2008) and the approximation method of Horowitz and Metzger (Refat *et al.*, 2008).

In our investigation, thermal behaviors of all complexes in terms of stability, peak temperatures, and values of kinetic parameters were investigated. Kinetic parameters of decomposition method such as entropy (S^*), enthalpy (H^*), pre-exponential factor (A), and Gibbs free energy (G^*) were studied using the Horowitz-Metzger equation method and discussed in brief as below. Results obtained by this method are well-correlated with each other and are summarized in Table 6. The pre-exponential factor (A) was calculated from the equation: $E_a/RT_s^2 = A/\Phi \exp(-E_a/RT_s)$. The entropy (S^*), enthalpy (H^*), and Gibbs free energy (G^*), were calculated from: $S^* = 2.303(\log Ah/KT_s)$ R, $H^* = E_a - RT_s^*$ and $G^* = H^* - T_s$ S^* respectively, where *h* is the Plank constant, \mathcal{O} is the heating rate, *K* is the Boltzmann constant, and *T*_s is temperature of peak for DTG curve. Each compound (C₁–C₆) has followed decomposition process as shown below

 $Solid - 1 \rightarrow Solid - 2 \, + \, Gas$

All complexes were evaluated for their thermally active parameters (E_a and n) of decomposition process using following equation:

$$[(-E_a/2.303R)\Delta(1/T)]\Delta \log w_r$$

= -n + [\Delta \log(dw/dt)/\Delta \log w_r]

where *R* is gas constant, *T* is temperature in *K*, $w_r = w_c - w$; w_c is the mass loss at completion of reaction, and *w* is total mass loss up to time *t*. E_a and *n* are energy of activation and order of reaction, respectively. The method reported by Freeman and Carroll (1958) has been assumed. Plots of $[\delta \log(dw/dt)/\delta \log w_r]$ versus $[\delta(1/T)/\delta \log w_r]$ were linear for all decomposition steps. Energy of activation E_a was calculated from slopes of these plots for all stages and order of reactions (*n*) determined from intercept, which shows first-order reaction over whole range of decomposition for all complexes. A typical plot for thermal degradation of $[Cu(L_2)(CQ)(H_2O)(OH)]$ is shown in (Fig. 3). All



Fig. 3 Thermal degradation plot of complex C2

complexes have negative entropy according to the kinetic data obtained from DTG curves specify that synthesized complexes have higher controlled systems than reactants and are stable (Refat *et al.*, 2008) also higher activation energy reveal the high stability of such complexes due to their covalent bond character (Hatakeyama and Quinn, 1999; Olomola *et al.*, 2010). The improved stability of the benzo-coumarin metal complexes showed a correlation with increased anti-microbial activity.

Anti-microbial

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 2. Fursina 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 (Fursina et al., 2002). Raman et al. have reported that complex exhibit higher anti-microbial activity than free ligands (Raman et al., 2003). An increased activity of metal chelates can be explained on the basis of chelation theory (Raman et al., 2009) according to which polarities (Keshavan and Gowda, 2002; Srivastava, 1981) 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 (Varnes et al., 1972). As shown in Table 2, all complexes possessed good to better activity against bacterial and fungal species. Compounds C_3 , C_4 , and C_5 have found excellent activity compared with standard drug ampicillin against E. coli as well as against *P. aeruginosa*, compound C_4 showed better activity compared to standard drug. In Gram-positive bacteria, compounds C4 and C5 towards S. pyogenes as well as compound C₄ towards B. subtilis displayed superior inhibitory action than that of ampicillin. Majority of the complexes showed parallel inhibitory action compared to standard ampicillin. Reviewing of the anti-fungal activity indicated that all the compounds have exhibited good activity against *A. niger* and *C. albicans* compared to standard fungicidal nystatin. As per results, the comparative study between ligands and their complexes showed increase in the activity of all the complexes than ligands due to their coordination to metal.

Furthermore, complex C_2 having 3-hydroxy substitution on phenyl ring have been found activity (100 µg mL⁻¹) against *E. coli* and (200 µg mL⁻¹) against *B. subtilis* but changing the position of hydroxyl group to 4-position of phenyl ring (complex C_3), resulted increase the activity towards *E. coli* (70 µg mL⁻¹) and *B. subtilis* (100 µg mL⁻¹). Similar situation observed in case of complex C_6 carrying nitro substitution on phenyl ring at 2-position have been found moderate activity against all bacterial species. While, replacing the 2-position of nitro group to 4-position of phenyl ring (complex C_5), resulted increase the anti-bacterial potency towards all bacterial species. It has been observed that the complexes having substitution at 4-position on phenyl ring showed better anti-microbial potency than substitution at other position.

Interestingly, the complexes C_4 and C_5 with electron negative groups at 4-position on the phenyl ring displayed excellent inhibitory action against most of the employed strains than (un)substitutions (C_1) and electron donating group at 3 and 4-position (C_2 and C_3) on the phenyl ring. The complexes C_4 and C_5 have been found as leading antimicrobial members against most of the employed stains. Thus from the data obtained, the potency order on the basis of various substitutions on the phenyl ring is given as $4-Cl > 4-NO_2 > 4-OH > 3-OH = -H > 2-NO_2$.

Anti-oxidant

In vitro anti-oxidant activity of complexes C_1-C_6 was measured using FRAP assay and results expressed in mM of ascorbic acid per 100 g of sample (Table 2), exposed moderate to good activity. Among the all test compounds, hydroxyl group substituted derivatives posses more ferricreducing power compared to that of chloro, nitro or unsubstituted one. Thus from the data obtained, potency order is given on basis of various substitutions attached to phenyl ring as $-OH > -NO_2 > -H > -Cl$. However, all the complexes have been found to show significant activity compared to the standard ascorbic acid.

Anti-tubercular activity

The anti-tubercular activities of all the synthesized compounds were assessed against *M. tuberculosis* H37RV at different concentration 3.125, 6.25, 12.5, 25, 50, and 100 µg mL⁻¹. The MIC of test compounds compared with standard drugs Isoniazid, Ethambutol, and Streptomycin as well as % inhibition are summarized in Table 3. Ligands show inhibition concentrations 100 and 50 µg mL⁻¹ except L₄ 12.5 µg mL⁻¹. A complex C₁ also exhibits activity at 100 µg mL⁻¹ concentration, while C₄ and C₅ complexes have shown enhancement in activity with MIC of 3.125 and 12.5 µg mL⁻¹ along 96 and 92 %, respectively, where other complexes exhibits at 25 µg mL⁻¹ as MIC. Compound C₄ has been emerged as the promising anti-tubercular member due to better activity along with compared to streptomycin.

Conclusion

The synthesized Cu(II) complexes are confirmed based on the data obtained from physico-chemical, spectroscopic, magnetic and thermal properties. TG curves of complexes shows weight loss of 4.6 % in temperature range 180-250 °C may be assigned to coordinated hydroxyl ion and water molecule. Structures for all metal complexes suggest octahedral geometry in which 1,3-diketone and eneamino linkage of ligands are OOON donors to metal. Furthermore, the geometry was confirmed using electronic spectra and magnetic moment value. Anti-microbial studies revealed that the metal complexes are more effective than that of relevant free ligands. Apart from this, compounds C₄ and C₅ showed excellent activity compared with standard drugs while other compounds exhibit good to moderate activity against all bacteria. In review, the complexes C₄ and C₅ have been found as leading anti-microbial members against most of the employed stains due to the complexes having electron negative groups at 4-position on the phenyl ring. In vitro anti-tubercular activity of all synthesized compounds shows good results with an enhancement of activity on complexation among metal ions. Especially, compound C_4 has been emerged as the hopeful anti-tubercular member due to excellent activity along with compared to streptomycin, while good antioxidant power was shown by complexes as compared to ascorbic acid.

Acknowledgments Authors are grateful to the Principal, V. P. & R. P. T. P. Science College, Sardar Patel University, Vallabh Vidyanagar for providing research facilities. Authors are also thankful to Head/Director of SICART, V. V. Nagar and SAIF, Chandigarh for providing instrumental facilities.

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