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Synthesis, spectroscopic, thermal and biological aspect of mixed ligand copper(II) complexes

G. J. Kharadi · K. D. Patel

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Abstract Derivative of 8-hydroxyquinoline i.e. Clioquinol is well known for its antibiotic properties, drug design and coordinating ability towards metal ion such as Copper(II). The structure of mixed ligand complexes has been investigated using spectral, elemental and thermal analysis. In vitro anti microbial activity against four bacterial species were performed i.e. *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Bacillus substilis* and found that synthesized complexes (15–37 mm) were found to be significant potent compared to standard drugs (clioquinol i.e. 10–26 mm), parental ligands and metal salts employed for complexation. The kinetic parameters such as order of reaction ($n = 0.96$–1.49), and the energy of activation ($E_a = 3.065$–142.9 kJ mol^{-1}), have been calculated using Freeman–Carroll method. The range found for the pre-exponential factor (A), the activation entropy ($S^* = -91.03$ to -102.6 JK^{-1} mol^{-1}), the activation enthalpy ($H^* = 0.380$–135.15 kJ mol^{-1}), and the free energy ($G^* = 33.52$–222.4 kJ mol^{-1}) of activation reveals that the complexes are more stable. Order of stability of complexes were found to be [Cu(A^4)(CQ)OH] · 4H$_2$O > [Cu(A^3)(CQ)OH] · 5H$_2$O > [Cu(A^1)(CQ)OH] · H$_2$O > [Cu(A^2)(CQ)OH] · 3H$_2$O

Keywords Copper(II)—Clioquinol complexes · Spectroscopic · Freeman–Carroll method · TG/DTG analysis · Antibacterial activity

G. J. Kharadi · K. D. Patel (✉)
V. P. & R. P. T. P. Science College, Sardar Patel University, Vallabh Vidyanagar 388 120, Gujarat, India
e-mail: drkdpatel64@yahoo.co.in

G. J. Kharadi
e-mail: gaurangkharadi@yahoo.com

Introduction

Coumarin (2*H*-1-benzopyran-2-one), a naturally occurring plant constituent, has been used in the treatment of cancer [1], and oedemas [2], many of its derivatives shows biological activity. Biological effects observed include antibacterial [3], anti-thrombotic, vasodilatory [4], anti-mutagenic [5] and anti-tumourigenic [6–9] effects along with this they also as acting as lipoxygenase and cyclo-oxygenase inhibitors [10, 11]. Recently a number of studies have highlighted an antimicrobial activity of naturally occurring and synthetic coumarins [12–14]. Lately, a number of metal complexes of coumarins have been synthesized and their biological activity determined. Kostova et al. have shown the cytotoxic potential of coumarins complexed with cerium, lanthanum, zirconium and neodymium [15–19]. Previously we have been concerned with two main areas of coumarin chemistry, namely the chemotherapeutic [20–26] and antimicrobial [27] activity of functionalized coumarins.

5-chloro-7-iodo-8-hydroxyquinoline (clioquinol, CQ) attenuated AD symptoms in human clinical trials [28] incited renewed interest in its pharmacodynamics, linking its possible beneficial therapeutical action to the chelation of copper(II) in the brain. Recent reports indicate that that CQ also mitigates Huntington's disease neuropathological symptoms in a mouse model of this disorder [29]; CQ treatment decreased the accumulation of huntingtin aggregates, suggesting commonalities in the etiology of both neurological pathologies. These results suggest a broader clinical potential of CQ in the treatment of neurodegenerative deseases.

In spite of successful human trials with CQ for the treatment of (AD), the coordination chemistry of this ligand with copper(II) or zinc(II) and its interaction with

β-amyloid in the brain remains unknown. For a better understanding of its mechanism of action, we were incited to study the formation of CQ-metal complexes in a media with an ionic composition similar to the brain extracellular environment [30].

The thermal analysis techniques were extensively used in studying of the thermal behavior of metal complexes [31–34]. Kinetic studies of thermal decomposition reactions may become useful in calculating the parameters like order of reaction (n), activation energy (E_a), entropy change (S^*), enthalpy change (H^*), free energy change (G^*) and pre-exponential factor (A). Thermogravimetry is a process in which a substance is decomposed in the presence of heat, which causes bonds of the molecules to be broken [35, 36]. Present work describe synthetic, thermal, spectroscopic aspects and anti microbial activity of heterochelates. The suggested structure of the ligand is shown in Scheme 1.

Experimental

Materials

Reagent and solvents

All the chemicals used were of analytical grade salicylaldehyde, ethyl acetoacetate, piperidine, chloroform, hexane, benzaldehyde, anisaldehyde, vanillin, P-nitrobenzaldehyde, ethanol and copper salt were purchased form the E. Merck (India) Limited, Mumbai. Clioquinol was kindly provided by purchased form Atul Limited, Agro chemical Division, Atul, Valsald (India). Luria broth and agar-agar were purchased from SRL, India. Acetic acid and EDTA were purchased from Sigma Chemical Co., India. The organic solvents were purified by recommended method [37].

Synthesis of ligands

The neutral bidentate ligands were synthesized according to reported methods [38]. Structures of ligands A^1–A^4 are shown in Scheme 1.

Synthesis of 3-actyl coumarin

The 3-actyl coumarin was synthesized by the reported procedure [39].

Synthesis of 3-(3-phenyl-acryloyl)-chromen-2-one (A^1)

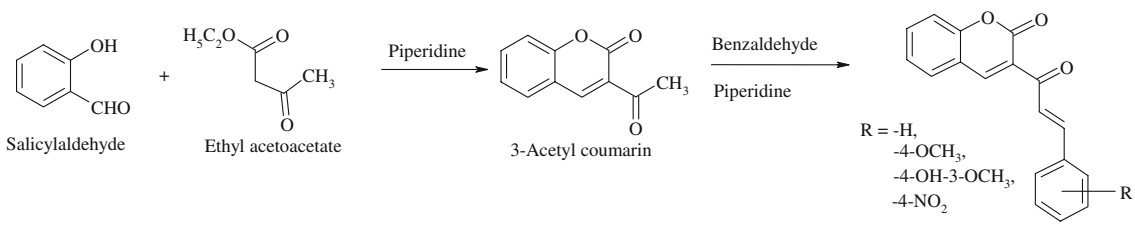
In a 100 ml round bottom flask 3-acetyl coumarin (0.01 mol, 1.88 g) and benzaldehyde (0.015 mol) were taken in 50 mL of ethanol. Catalytic amount of piperidine (1.0 mL) was added and the reaction mixture was stirred for 10 min at room temperature. The reaction mixture was then refluxed on water bath for 4 h. It was allowed to come at room temperature. A solid product separated out was filtered off, washed with cold ethanol and dried. It was recrystallized from ethanol. Yield 77.8%, m. p: 161 °C. Elemental analysis found (%) C 78.29; H 4.35%; Calculated for $C_{18}H_{12}O_3$: C 78.25; H 4.38%. FTIR (KBr, cm^{-1}): 1610 (α, β-unsaturated keton), 1743 (lactone carbonyl of coumarin). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.28–7.88 (11H, m, nine aromatic protons and two –CH = CH- protons merged), 8.56 (1H, s, C_4 proton).

Synthesis of 3-[3-(4-methoxy-phenyl)-acryloyl]-chromen-2-one (A^2)

A^2 was synthesized by same method used for A^1 by using anisaldehyde instead of benzaldehyde. Yield 70.1%, m. p: 167 °C. Elemental analysis found (%) C 74.57; H 4.66%; Calculated for $C_{19}H_{14}O_4$: C 74.50; H 4.61%. FTIR (KBr, cm^{-1}): 1609 (α,β-unsaturated keton), 1750 (lactone carbonyl of coumarin), 1247 (C–O–C, asymmetric), 1040 (C–O–C, symmetric). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 6.95–7.84 (10H, m, eight aromatic protons and two –CH = CH- protons merged), 8.52 (1H, s, C_4 proton), 3.90 (3H, s, $-OCH_3$).

Synthesis of 3-[3-(4-hyroxy-3-methoxy-phenyl)-acryloyl]-chromen-2-one (A^3)

A^3 was synthesized by same method used for A^1 by using vanillin instead of benzaldehyde. Yield 79.6%, m.



Scheme 1 General reaction scheme for synthesis of ligand

p: 191 °C. Elemental analysis found (%) C 70.77; H 4.40%; Calculated for $C_{19}H_{14}O_5$: C 70.80; H 4.38%. FTIR (KBr, cm^{-1}): 1613 (α,β-unsaturated keton), 1746 (lactone carbonyl of coumarin), 1241 (C–O–C, asymmetric), 1039 (C–O–C, symmetric), 3373 (O–H). ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) = 6.81–7.8 (9H, m, seven aromatic protons and two –CH = CH- protons merged), 8.51 (1H, s, C_4 proton), 3.86 (3H, s, $–OCH_3$), 11.62 (1H, S, –OH).

Synthesis of 3-[3-(4-nito-phenyl)-acryloyl]-chromen-2-one (A^4)

A^4 was synthesized by same method used for A^1 by using p-nitrobenzaldehyde instead of benzaldehyde. Yield 68.2%, m. p: 182 °C. Elemental analysis found (%) C 67.34; H 3.41; N 4.39%; Calculated for $C_{18}H_{11}NO_5$: C 67.29; H 3.45% N 4.36%. FTIR (KBr, cm^{-1}): 1620 (α,β-unsaturated keton), 1730 (lactone carbonyl of coumarin), 1523 ($ArNO_2$, asymmetric), 1347 ($ArNO_2$, symmetric). ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.35–8.01 (8H, m, six aromatic protons and two –CH = CH- protons merged), 8.2 (2H aromatic two protons ortho to nitro group), 8.57 (1H, s, C_4 proton).

Synthesis of metal complexes

$$\left[Cu\left(A^1\right)(CQ)OH\right] \cdot H_2O \quad (1)$$

An aqueous solution (100 mL) of copper(II) (10 mmol) was added to an ethanolic solution (100 mL) of ligand (A^1) (10 mmol), followed by addition of clioquinol (10 mmol) in ethanol; the pH was adjusted to 4.5–6.0 with diluted NaOH 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 colored crystalline product was obtained. The obtained product was washed with ether and dried over vacuum desiccators. The reaction scheme is shown in Scheme 2.

Compounds (2)–(4) were prepared according to same method and their physicochemical parameters are summarized in Table 1.

Methods

The metal content of the complexes were determined by the EDTA titration technique [40] after decomposing the organic matter with a mixture of $HClO_4$, H_2SO_4 and HNO_3 (1:1.5:2.5). Carbon, hydrogen and nitrogen were analyzed with the Perkin Elmer, USA 2400-II CHN analyzer. The magnetic moments were obtained by the Gouy's method using mercury tetrathiocyanatocobaltate(II) as a calibrant ($\chi_g = 16.44 \cdot 10^{-6}$ c.g.s. units at 20 °C). Diamagnetic corrections were made using Pascal's constant [41].

A simultaneous TG/DTG had been obtained by a model 5000/2960 SDT, TA Instruments, USA. The experiments were performed in N_2 atmosphere at a heating rate of 10 °C min^{-1} in the temperature range 50–800 °C, using Al_2O_3 crucible. The sample sizes are ranged in mass from 4.5–10 mg. The IR spectra were recorded on a FTIR Nicolet 400D Spectrophotometer using KBr pellets. NMR spectra were recorded on a model Avance 400 Bruker FT-NMR instrument using $CDCl_3$ as solvent. The reflectance spectra of the complexes were recorded in the range of 1,700–350 nm (as MgO discs) on a Beckeman DK-2A spectrophotometer.

Results and discussion

Physicochemical parameter of the ligand and complexes are presented in Table 1. The complexes were colored and stable in air. They are insoluble in water, alcohol and DMF but soluble in DMSO. All the heterochelates are non electrolyte in nature. The formation of the heterochelates are assumed according to the following balanced chemical Eq. 1

$$Cu(NO_3)_2 \cdot 3H_2O + A^n + CQ \rightarrow [Cu(A^n)(CQ)OH] \cdot xH_2O + 2HNO_3 + (n-x)H_2O \quad (1)$$

Where x = 1,3,4,5 and n = 1,2,3,4.

Scheme 2 Synthesis of $[Cu(A^1)(CQ)OH]$

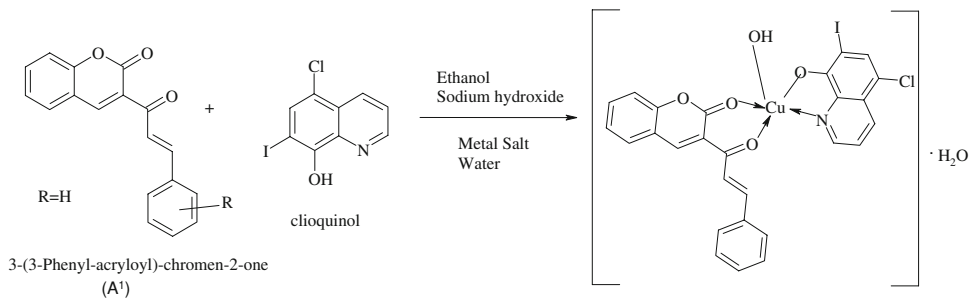


Table 1 Physicochemical parameter of the ligand and complexes

Ligand/Metal complexes	Molecular formula	Mol.Wt.	Yield (%)	Elemental analysis (%)						Metal (%)	
				C		H		N			
				Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found
A^1	$C_{18}H_{12}O_3$	276.29	77.0	78.25	78.29	4.38	4.35	_	_	_	_
A^2	$C_{19}H_{14}O_4$	306.32	70.1	74.50	74.57	4.61	4.66	_	_	_	_
A^3	$C_{19}H_{14}O_5$	322.32	79.6	70.80	70.77	4.38	4.40	_	_	_	_
A^4	$C_{18}H_{11}NO_5$	321.29	68.2	67.29	67.34	3.45	3.41	4.36	4.39	_	_
$[Cu(A^1)(CQ)OH] \cdot H_2O$ (1)	$C_{27}H_{19}O_6ClNCuI$	679.36	68	47.74	47.72	2.82	2.89	2.06	2.10	9.35	9.39
$[Cu(A^2)(CQ)OH] \cdot 3H_2O$ (2)	$C_{28}H_{25}O_9ClNCuI$	745.39	66	45.12	45.19	3.38	3.33	1.88	1.86	8.52	8.56
$[Cu(A^3)(CQ)OH] \cdot 5H_2O$ (3)	$C_{28}H_{29}O_{12}ClNCuI$	797.46	70	42.17	42.21	3.67	3.61	1.76	1.72	7.97	7.91
$[Cu(A^4)(CQ)OH] \cdot 4H_2O$ (4)	$C_{27}H_{24}O_{11}ClN_2CuI$	778.42	73	41.66	41.61	3.11	3.15	3.60	3.63	8.16	8.13

Infrared spectra

The important infrared spectral bands and their tentative assignments for the synthesized heterochelates were recorded as KBr disks and are summarized in Table 2.

In the 8-hydroxyquinoline complexes of divalent metals, the υ (C–O), appeared at 1,120 cm^{-1} region and the position of the band slightly varies with the metal [42]. The υ (C–O), observed in the free oxine molecule at 1,090 cm^{-1}, shifted to higher frequencies in all the mixed ligand complexes giving a strong absorption band at 1,110 cm^{-1}. This clearly indicates the coordination of 8-hydroxyquinoline in these complexes. In the investigated heterochelates, the band observed in the region 3400–3500, 1295–1300, 860–870 and 715–717 cm^{-1} are attributed to –OH stretching, bending, rocking and wagging vibrations, respectively due to the presence of water molecules [43]. The IR spectra of the coumarin derivatives show 1,610 and 1,743 cm^{-1} corresponding to (α, β-unsaturated ketone) and (lactone carbonyl ketone) respectively; on complexation these peaks shifted to a lower frequency 1,600 and 1,711 cm^{-1} due to complex formation. The weak band around 519 cm^{-1} and 776 cm^{-1} are attributed to the M–O and M–N stretching frequency.

Reflectance spectra and magnetic measurements

Copper(II) complexes (d^9 system) are known for their varieties of structures due to their various coordination numbers. Six coordinated copper(II) complexes possesses distorted octahedral geometry. The spectra of copper complexes are very difficult to assign even with relatively simple ligands because of the breadth of absorption band even at low temperature. The different coordination number having wide range of geometry. Generally for square pyramidal copper(II) complexes, bands observed in the region 9000–10000, 11500–16000 and 15000–19000 cm^{-1} have been ascribed to the transition $^2B_1 \rightarrow {}^2A_1$, $^2B_1 \rightarrow {}^2B_2$ and $^2B_1 \rightarrow {}^2E$ respectively. The diffuse reflectance spectra of square pyramidal copper(II) complexes $[Cu(A^n)(CQ)OH] \cdot xH_2O$ have been taken in solid state. The spectra of copper(II) complexes exhibit a broad band in the 16,980–12,770 cm^{-1} region [44–46]. These bands are characteristic of copper(II) *d-d* transition in tetragonal field in which copper(II) atom is in distorted square pyramidal coordination environment. The copper(II)-d^9 system and its compounds are expected to have magnetic moment close to be spin only value 1.73 B.M. Generally the magnetic moments of copper(II) in any of its geometry lies around

Table 2 The characteristic IR bands of heterochelates and magnetic moments

Complexes	ν(O-H) cm^{-1} (br)	ν(C = N) cm^{-1} (w)	ν(M-O) cm^{-1} [CQ] (m)	ν(M-N) cm^{-1}(w)	ν(C = O)cm^{-1} α, β-unsaturated ketone (s)	ν(C = O)cm^{-1} lactone carbonyl ketone (s)	ν(M-O) cm^{-1} [A^n]	μ_{eff} / B.M
$[Cu(A^1)(CQ)OH] \cdot H_2O$ (1)	3,510–3,300	1,588	500	776	1,600	1,711	519	1.79
$[Cu(A^2)(CQ)OH] \cdot 3H_2O$ (2)	3,540–3,200	1,602	500	769	1,599	1,733	515	1.93
$[Cu(A^3)(CQ)OH] \cdot 5H_2O$ (3)	3,500–3,320	1,588	500	769	1,603	1,725	513	1.77
$[Cu(A^4)(CQ)OH] \cdot 4H_2O$ (4)	3,620–3,200	1,593	500	771	1,610	1,703	518	1.98

s strong, *m* medium, *w* weak, *br* broad *CQ* clioquinol

1.8 B.M. at room temperature. The magnetic moment value of copper(II) complexes obtained in the range of 1.77–1.98 B.M., which are close to the spin-only values expected in S = ½ system (1.73 B.M.) and may be indicative of five coordinating copper(II) complexes and consistent with the presence of single unpaired electron [47, 48].

Calculation of activation thermodynamic parameters of the decomposed complexes

The thermodynamic activation parameters of the decomposition process of the complexes such as energy of activation (E_a) and order of reaction (n) were evaluated graphically by employing the Freeman–Carroll [49] method using the following relation:

$$[(-E_a/2.303R)\Delta(1/T)]\Delta\log w_r = -n + \Delta\log(dw/dt)/\Delta\log w_r \quad (2)$$

Where T is the temperature in K, R is gas constant, $w_r = w_c - w$; w_c is the mass loss at the completion of the reaction and w is the total mass loss up to time t. E_a and n are the energy of activation and order of reaction, respectively. A typical curve of [$\Delta\log(dw/dt)/\Delta\log w_r$] *vs.* [$\Delta(1/T)\Delta\log w_r$] for the copper(II) complex is shown in Fig. 1. The slope of the order of reaction (n) was determined from the intercept.

The thermodynamic activation parameters of the decomposition process of dehydrated complexes such as entropy (S^*), preexponential factor (A), enthalpy (H^*) and free energy of the decomposition (G^*), were calculated using the following relations [50, 51].

$$E_a/RT_s^2 = A/\Phi\exp(-Ea/RT_s) \quad (3)$$

$$S^* = 2.303(\log Ah/KT)R \quad (4)$$

$$H* = E_a - RT* \quad (5)$$

$$G* = H* - T_s S* \quad (6)$$

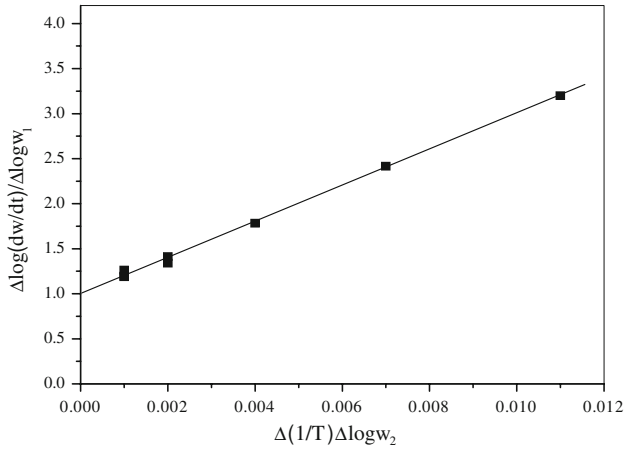


Fig. 1 Freeman–Carroll plot for thermal dehydration of [Cu(A^1)(CQ)OH] · H_2O

Where Φ is the heating rate, K is the Boltzman constant, h is the Plank constant and T_s is the peak temperature from DTG curve. According to the kinetic data obtained from DTG curves, all the complexes have negative entropy, which indicates that the studied complexes have more ordered systems than reactants [52].

The thermal behavior of the prepared complexes

In the following paragraphs the thermal behavior of the synthesized complexes, characterized on the basis of TG and DTG methods are described. Thermal data and kinetic parameters of the complexes are given in Tables 3 and 4, respectively.

$$\left[\mathrm{Cu}\left(\mathrm{A}^1\right)(\mathrm{CQ})\mathrm{OH}\right] \cdot \mathrm{H_2O} \quad (1)$$

TG/DTG curves of the complex [Cu(A^1)(CQ)OH].H_2O are represented in Tables 3 and 4, respectively. The [Cu(A^1)(CQ)OH] · H_2O complex undergoes decomposition in four stages. The first DTG peak at 85 °C, in the temperature range 80–140 °C, with the mass loss (obs. 2.91%; calcd. 2.89%) corresponds to the loss of one moles of lattice water molecules. The loss of one mole of lattice water molecules is a first order and the value of the energy of activation for the dehydration process is found to be 3.45 kJ mol^{-1}. The second stage between 150–180 °C corresponds to the decomposition of one hydroxyl molecules [53].The observed mass loss is 2.69% which is consistent with the theoretical value of 2.79%. Two well-separated peaks are displayed on the DTG curve i.e. Figure 2 indicates that the decomposition of the ligand divides into two steps. The third stage, which occurs in the temperature range 280–320 °C, with a DTG peak at 306 °C, corresponds to the decomposition of one part of the ligand. The observed mass loss for this stage is 48.32%. The fourth stage occurs between 400–720 °C, corresponding to the decomposition of remaining part of the ligand, with the mass loss of 42.71% (Table 3).The final residue, estimated as copper oxide.

Biological activity of metal chelates

Bacteria are both valuable and detrimental to environment. Many bacteria are pathogenic to human leading to number of diseases such as tetanus, typhoid fever, pneumonia, food-borne illness, leprosy, tuberculosis, etc. Bacteria are not only harmful to humankind but are harmful to plants and animals too. Bacteria have been classified in two groups on the basis of the constituents of the cell wall and they are Gram-positive and Gram-negative; Cell wall of Gram-positive bacteria are made up of thick peptidoglycan or murein layer and teichoic acid; while cell wall of Gram-negative are thin peptidoglycan and lipopolysaccharide-

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

Complexes	TG range/ °C	DTG_{max}/ °C	Mass loss/% obs. (calcd.)	Assignment
$[Cu(A^1)(CQ)OH] \cdot H_2O$ (1)	80–140	85	2.91(2.89)	Loss of 1 lattice water molecules
	150–180	170	2.69(2.79)	Loss of hydroxyl molecules
	280–320	306	48.32	Removal of A^1 ligand
	400–720	520	42.71	Removal of clioquinol ligand
			91.1(91.21)	Leaving CuO residue
$[Cu(A^2)(CQ)OH] \cdot 3H_2O$ (2)	50–120	79	7.01(7.08)	Loss of 3 lattice water molecules
	150–180	140	2.51(2.53)	Loss of hydroxyl molecules
	200–320	280	46	Removal of A^2 ligand
	600–750	670	45.2	Removal of clioquinol ligand
			71.35(71.37)	Leaving CuO residue
$[Cu(A^3)(CQ)OH] \cdot 5H_2O$ (3)	80–140	89	12.9(12.92)	Loss of 5 lattice water molecules
	150–180	167	2.53(2.57)	Loss of hydroxyl molecules
	220–320	286	47.03	Removal of A^3 ligand
	650–750	700	44.2	Removal of clioquinol ligand
			90.9(90.82)	Leaving CuO residure
$[Cu(A^4)(CQ)OH] \cdot 4H_2O$ (4)	80–140	97	10.5(10.4)	Loss of 4 lattice water molecules
	150–180	171	2.58(2.54)	Loss of hydroxyl molecules
	250–320	291	47.6	Removal of A^4 ligand
	600–720	641	44.1	Removal of clioquinol ligand
			73.94(73.96)	Leaving CuO residure

Table 4 Thermodynamic data of the thermal decomposition of metal complexes

Complex	TG range/°C	E_a/kJ mol^{-1}	n	A/s^{-1}	S^*/J K^{-1} mol^{-1}	H^*/kJ mol^{-1}	G^*/kJ mol^{-1}
$[Cu(A^1)(CQ)OH] \cdot H_2O$ (1)	50–80	3.45	0.96	0.143	−102.1	0.767	33.77
	180–250	15.22	1.48	2.473	−98.48	10.96	61.48
	280–320	25.13	1.00	7.258	−97.55	19.70	83.40
	400–720	71.70	1.00	920.6	−95.08	63.87	153.4
$[Cu(A^2)(CQ)OH] \cdot 3H_2O$ (2)	50–80	3.065	0.98	0.110	−102.6	0.380	33.52
	200–320	21.49	1.20	9.064	−97.514	16.89	70.82
	600–750	142.9	1.00	16126677.4	−92.599	135.15	222.4
$[Cu(A^3)(CQ)OH] \cdot 5H_2O$ (3)	50–160	6.141	0.96	0.697	−100.0	3.456	35.78
	220–320	17.24	1.49	21.98	−97.166	13.89	53.056
	650–750	129.39	1.00	8.494	−91.03	124.79	175.13
$[Cu(A^4)(CQ)OH] \cdot 4H_2O$ (4)	80–180	8.34	0.98	0.669	−99.87	4.9161	46.164
	250–320	30.51	1.10	91.473	−96.21	25.913	79.139
	600–720	88.56	1.00	8390.0	−94.36	80.641	170.57

containing. Herein we have chosen four bacterial specie two of each kind namely *Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens* and *Bacillus substilis.* First two are Gram-positive and remaining two are Gram-negative.

Kolokolov et al., have suggested that the transition metal complexes with biologically active ligands frequently exhibit higher biological activity and lower toxicity than the initial ligands, which makes possible their use in medicine and biochemistry [54]. Raman et al., have reported that complex exhibit higher antimicrobial activity than the free ligands [55]. Legler et al., have reported the synthesis and antimicrobial activity of silver complex of arginine and glutamic acid and observed that the complexes exhibit higher antibacterial activity [56]. Sharma et al., have synthesize copper(II) complexes with thiocarbazones of thiophene-2-carboxaldehyde and studied their antiamoebic activity against *E. histolytica* [57]. Complexes of

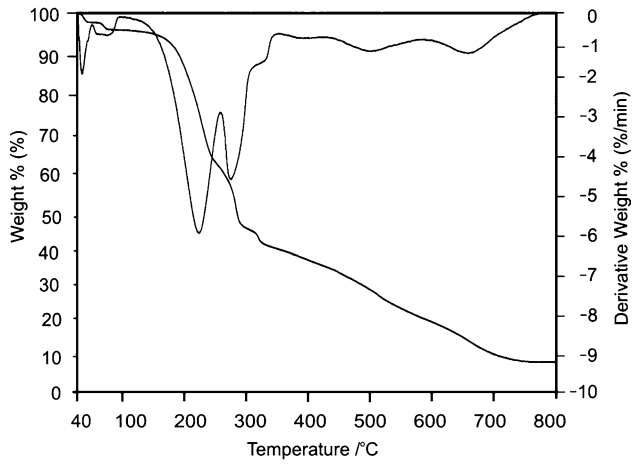


Fig. 2 TG and DTG Curve of $[Cu(A^1)(CQ)OH] \cdot H_2O$

manganese(II), iron(II), cobalt(II), nickel(II), copper(II), and zinc(II) with a bishydrazone have been screened for antimicrobial activity by Mohanan and Murukan [58].

Preparation of stock solution

A stock solution of 10 mg/mL was made by dissolving compound in minimum amount of DMSO and making it up with sterile D.D.Water.

Preparation of agar plates

The media was made up by dissolving bacteriological agar (20 gm) and Luria broth (20 gm) (SRL, India) in 1 L sterile distilled water. The mixture was autoclave for 15 min at 120 °C and then dispended into sterilized Petri dishes, allowed to solidify, and then used for inoculation.

Procedure of inoculation

The target microorganism cultures were prepared separately in 15 mL of liquid LB medium for activation. Inoculation was done with the help of micropipette with sterilized tips; 100 µL of activated strain is placed onto the surface of an agar plate, and spread evenly over the surface by means of a sterile, bent glass rod. Then two well having diameter of 10 mm is done with the help of sterilized borer in each plate.

Application of discs

Sterilized stock solutions (10 mg/mL) were used for the application in the well of earlier inoculated agar plates. When the discs were applied, they were incubated at 30 °C (Gram positive) and 37 °C (Gram negative) for 24 h. The zone of inhibition was then measured (in mm) around the disc. The control experiments were performed where only equivalent volume of solvents without added test compounds and measured the zone of inhibitions (in mm). All experiments were performed in triplicate and clioquinol was used as standard drugs. The growth was compared with solvent as the control and is expressed as zone of inhibition (in mm).

Metal complexes exhibit higher biocidal activity as compared to the free ligands, metal salts, control (DMSO) where as are in competition with clioquinol. From comparative analysis as shown in Fig. 3, it is observed that all the metal complexes are more potent biocidal than the ligands A^1–A^4. The zone of inhibition was measured (in mm) around the disc and the results are represented in Table 5. From the graph it is clear that copper(II)-A^3 is highly active among the complexes of the respective metal, this may be due to presence of methoxy and hydroxyl group of ligand where as copper(II)-A^3 is most active among all which may be due to combine effect of copper(II) and functional groups on the ligand.

This improvement in activity of complexes is also be rationalized on the basis of their structure activity relationship.

A feasible manner for raise in biocidal activity may be explained on the basis of chelation theory [59] or/and may be due to light of Overtone's concept [60]. Chelation reduces the polarity of the metal ion considerably, mainly because of the partial sharing of its positive charge with donor groups and possible π-electron delocalization on the whole chelate ring. Polysaccharides and lipids are some important constituent of cell wall and membranes, Chelation can considerably reduce the polarity of the metal ion,

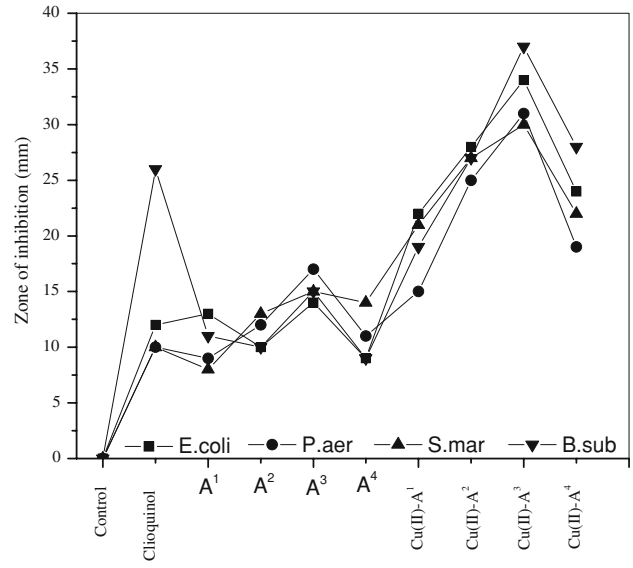


Fig. 3 Comparative analysis for biological activity

Table 5 Agar plate technique

Compound	Zone of inhibition (mm)			
	Gram +ve		Gram -ve	
	Escherichia coli	*Pseudomonas aeruginosa*	*Serratia marcescens*	*Bacillus substilis*
Control (DMSO)	0	0	0	0
Clioquinol	12	10	10	26
A^1	13	9	8	11
A^2	10	12	13	10
A^3	14	17	15	15
A^4	9	11	14	9
Cu(II)–A^1	22	15	21	19
Cu(II)–A^2	28	25	27	27
Cu(II)–A^3	34	31	30	37
Cu(II)–A^4	24	19	22	28

which in turn increases the lipophilic character of the chelates. Thus, the interaction between metal ion and the lipid is favored. This may lead to the breakdown of the permeability barrier of the cell, resulting in interference with the normal cell processes. Presence of lypophilic and polar substituents are expected to enhance biocidal activity. Heterocyclic ligand with multifunctionality have greater chance of interaction either with nucleoside bases (even after complexation with metal ion) or with biologically essential metal ions present in the biosystem can be promising candidates as bactericides since they always look to enact especially with some enzymatic functional groups, to achieve higher coordination number. Thus, the antibacterial property of metal complexes can not be ascribed to chelation alone but it is an intricate blend of all the above contributions.

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References

1. Egan DA, O'Kennedy R, Moran E, Cox D, Prosser E, Thornes RD. The pharmacology, metabolism, analysis, and applications of coumarin and coumarin related compounds. Drug Metab Rev. 1990;22:503–29.
2. Casley Smith J, Coll JR. Inhibition of monoamine oxidases by coumarin-3-acyl derivatives: oedemas and computational study. Phys Surg. 1993;22:67–71.
3. Laurin P, Klich M, Dupuis-Hamelin C, Mauvais P, Lassaigne P, Bonnefoy A, et al. Synthesis and *in vitro* evaluation of novel highly potent coumarin inhibitors of gyrase B. Bioorg Med Chem Lett. 1999;9:2079–84.
4. Hoult JRS, Paya M. Pharmacological and biochemical actions of simple coumarins: natural products with therapeutic potential. Gen Pharmacol. 1996;27:713–22.
5. Pillai SP, Menon SR, Mitscher LA, Pillai CA, Shankel DA. Umbelliferone analogues and their potential to inhibit benzo(a)pyrene- and hydrogen peroxide-induced mutations. J Nat Prod. 1999;63:1358–62.
6. Maucher A, Kager M, von Anger E. Evaluation of the antitumour activity of coumarin in prostate cancer models. J Cancer Res Clin Oncol. 1993;119:150–4.
7. Sharma S, Stutzman D, Kellof JG, Steele VE. Screening of potential chemopreventive agents using biochemical markers of carcinogenesis. Cancer Res. 1994;54:5848–55.
8. Egan D, James P, Cooke D, O'Kennedy R. Studies on the cytostatic and cytotoxic effects and mode of action of 8-nitro-7-hydroxycoumarin. Cancer Lett. 1997;118:201–11.
9. Maucher A, Kager M, von Anger E. Antitumour activity of coumarin in prostate and mammary cancer models. J Cancer Res Clin Oncol. 1994;120:S14–6.
10. Kimura Y, Okuda H, Arichi S, Baba K, Kozawa M. Inhibition of the formation of 5-hydroxy-6,8,11,14-eicosatetraenoic acid from arachidonic acid in polymorphonuclear leukocytes by various coumarins. Biochim Biophys Acta. 1985;834:224–9.
11. Hoffmanova J, Kozubik A, Dusek L, Pachernik J. Inhibitors of lipoxygenase metabolism exert synergistic effects with retinoic acid on differentiation of human leukemia HL-60 cells. Eur J Pharmacol. 1998;350:273–84.
12. Borges F, Roleira F, Milhazes N, Santana L, Uriarte E. Simple coumarins and analogues in medicinal chemistry: occurrence, synthesis and biological activity. Curr Med Chem. 2005;12:887–916.
13. Khan IA, Kulkarni MV, Gopal M, Shahabuddin MD, Sun COM. Synthesis and biological evaluation of novel angularly fused ploycyclic coumarins. Bioorg Med Chem. 2005;15:3584–7.
14. Devienne KF, Goncalves Raddi MS, Gomes Coelho R, Vilegas W. Structure–antimicrobial activity of some natural isocoumarins and their analogues. Phytomedicine. 2005;12:378–81.
15. Kostova I, Momekov G, Zaharieva M, Karaivanova M. Cytotoxic activity of new lanthanum (III) complexes of bis-coumarins. Eur J Med Chem. 2005;40:542–51.
16. Kostova I, Manolov I, Momekov G. Cytotoxic activity of new neodymium (III) complexes of bis-coumarins. Eur J Med Chem. 2004;39:765–75.
17. Kostova I, Manolov I, Karaivanova M. Synthesis, physicochemical characterization, and cytotoxic screening of new zirconium complexes with coumarin derivatives. Arch Pharm. 2001;334:157–62.
18. Kostova I, Manolov I, Karaivanova M. New lanthanide complexes of 4-methyl-7-hydroxycoumarin and their pharmacological activity. Eur J Med Chem. 2001;36:339–47.

19. Kostova I, Manolov I, Nicolova I, Danchev ND. New metal complexes of 4-methyl-7-hydroxycoumarin sodium salt and their pharmacological activity. Farmaco. 2001;56:707–13.
20. Finn G, Craven B, Egan D. Study of the in vitro cytotoxic potential of natural and synthetic coumarin derivatives using human normal and neoplastic skin cell lines. Melanoma Res. 2001;11:461–7.
21. Finn G, Craven B, Egan D. In vitro cytotoxic potential and mechanism of action of selected coumarins, using human renal cell lines. Cancer Lett. 2002;183:61–8.
22. Finn G, Craven B, Egan D. Investigation of intracellular signalling events mediating the mechanism of action of 7-hydroxycoumarin and 6-nitro-7-hdroxycoumarin in human renal cells. Cancer Lett. 2003;205:69–79.
23. Finn G, Craven B, Egan D. Activation of mitogen activated protein kinase pathways and melanogenesis by novel nitroderivatives of 7-hydroxycomarin in human malignant melanoma cells. Eur J Pharm Sci. 2005;26:16–25.
24. Finn G, Craven B, Egan D. A study of the role of cell cycle events mediating the action of coumarin derivatives in human malignant melanoma cells. Cancer Lett. 2004;214:43–54.
25. Finn G, Craven B, Egan D. Daphnetin induced differentiation of human renal carcinoma cells and its mediation by p38 mitogen-activated protein kinase. Biochem Pharmacol. 2004;67:1779–88.
26. Finn G, Craven B, Egan D. Modulation of mitogen-activated protein kinases by 6-nitro-7-hydroxycoumarin mediates apoptosis in renal carcinoma cells. Eur J Pharmacol. 2003;481: 159–67.
27. Creaven BS, Egan DA, Kavanagh K, McCann M, Mahon M, Noble A, et al. Synthesis and antimicrobial activity of copper(II) and silver(I) complexes of hydroxynitrocoumarins: X-ray crystal structures of $[Cu(hnc)2(H_2O)_2] \cdot 2H_2O$ and [Ag(hnc)] (hncH = 4-hydroxy-3-nitro-2H-chromen-2-one). Polyhedron. 2005;24:949–57.
28. Ritchie CW, Bush AI, Mackinnon A, Macfarlane S, Mastwyk M, MacGregor L. Metal-protein attenuation with iodochlorhydroxyquin (clioquinol) targeting a amyloid deposition and toxicity in alzheimer disease: a pilot phase 2 clinical trial. Arch Neurol. 2003; 60:1685–1691.
29. Nguyen T, Hamby A, Massa S. Clioquinol down-regulates mutant huntingtin expression *in vitro* and mitigates pathology in a Huntington's disease mouse. Proc Natl Acad Sci. 2005;102: 11840–5.
30. Di Vaira M, Bazzicalupi C, Orioli P, Messori L, Bruni B, Zatta P. Clioquinol, a drug for alzheimer's disease specifically interfering with brain metal metabolism: structural characterization of its zinc(ii) and copper(ii) complexes. Inorg Chem. 2004;43: 3795–7.
31. Soliman AA. Thermogravimetric and spectroscopic studies on cadmium complexes with two salicylidene thiophenol Schiff bases. J Therm Anal Calorim. 2001;63:221–31.
32. Mohamed GG, Abdel-Wahab ZH. Salisaldehyde-2-aminobenzimidazole Schiff base complexes of Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II). J Therm Anal Calorim. 2003;73:347–59.
33. El-Borai HA. Structural and thermal studies of some aroylhydrazone Schiff's bases-transition metal complexes. J Therm Anal Calorim. 2005;81:339–46.
34. Patel SH, Pansuriya PB, Chhasatia MR, Parekh HM, Patel MN. Coordination chain polymeric assemblies of trivalent lanthanides with multidentate Schiff base synthetic, spectral investigation and thermal aspects. J Therm Anal Calorim. 2008;91:413–8.
35. Albano CL, Sciamanna R, Aquino T, Martinez JJ. European congress on computational methods in applied sciences and engineering, ECOMAS 2000, Barcelona, 11–14 Sept 2000.
36. Carrasco F. The evaluation of kinetic parameters from thermogravimetric data: comparison between established methods and the general analytical equation. Thermochim Acta. 1993;213:115–34.
37. Vogel AI. Textbook of practical organic chemistry. 5th ed. London: Longman; 1989.
38. Micky JAA, Farrug AA. Synthesis and reactions of 3-cinnamoyl coumarin derivatives with activated nitriles. Indian J Chem. 1997; 36B:357–60.
39. Bischler A. Ueber die Entstehung einiger substituirter Indole. Chem Ber. 1892;25:2860–79.
40. Jeffrey GH, Bassett J, Mendham J, Denney RC. Vogel's textbook of quantitative chemical analysis. 5th ed. Harlow: Longman; 1989.
41. Weiss A, Witte H. Magneto chemie. Weinheim: Verlag Chemie; 1973.
42. Charles RG, Freiser H, Friedel R, Hilliard LE, Johnston WD. Infra-red absorption spectra of metal chelates derived from 8-hydroxyquinoline, 2-methyl-8-hydroxyquinoline, and 4-methyl-8-hydroxyquinoline. Spectrochim Acta. 1956;8:1–8.
43. Panchal PK, Patel MN. Synthesis, structural characterization, and antibacterial studies of some mixed-ligand first row d-transition metal complexes. Synth React Inorg Met-Org Chem. 2004;34: 1277–89.
44. Carballo R, Castineiras A, Covelo B, Garcia-Martinez E, Nicolas J, Vazquez-Lopez EM. Solid state coordination chemistry of mononuclear mixed-ligand complexes of Ni(II), Cu(II) and Zn(II) with α-hydroxycarboxylic acids and imidazole. Polyhedron. 2004; 23:1505–18.
45. Iskander MF, EL-Sayed L, Salem NMH, Werner R, Haase W. Synthesis, characterization and magnetochemical studies of dicopper(II) complexes derived from bis(N-salicylidene)dicarboxylic acid dihydrazides. J Coord Chem. 2005;58:125–39.
46. Mendoza-Diaz G, Martineza-Auguilera LMR, Perez-Alonso R, Solans X, Moreno-Esparza R. Synthesis and characterization of mixed ligand complexes of copper with nalidixic acid and (N—N) donors. Crystal structure of $[Cu(Phen)(Nal)\text{-}(H_2O)]NO_3 \cdot 3H_2O$. Inorg Chim Acta. 1987;138:41–7.
47. Melnik M. Mono-, bi-, tetra- and polynuclear copper(II) halogenocarboxylates. Coord Chem Rev. 1981;36(1):1–44.
48. Figgis BN, Lewis J. In: Lewis J, Wilkins RG, editors. Modern coordination chemistry: principles and methods. New York: Interscience; 1960. p. 400.
49. Freeman ES, Carroll B. The application of thermoanalytical techniques to reaction kinetics: the thermogravimetric evaluation of the kinetics of the decomposition of calcium oxalate monohydrate. J Phys Chem. 1958;62:394–7.
50. Nath M, Arora P. Spectral and thermal studies of cobalt(II), nickel(II) and copper(II) complexes of Schiff bases obtained from o-hydroxyacetophenone and amino acids. Synth React Met-Org Chem. 1993;23:1523–46.
51. Sekerci, Yakupoglu F. Thermal analysis study of some transition metal complexes by TG and DSC methods. J Therm Anal Calorim. 2004;75:189–95.
52. Mohamed GG, Nour El-Dien FA, El-Gamel NEA. Thermal behaviour of metal complexes of 6-(2-pyridylazo)-3-acetamidophenol . J Therm Anal Calorim. 2002;67:135–46.
53. Masoud MS, Ali AE, Mohamed RH, Abd El Zahar Mostafa M. Dielectric relaxation spectroscopy of heteronuclear cobalt(II)–copper(II) complex of 1-phenyl-3-methyl-5-pyrazolone. Spectrochim Acta Part. 2005;62:1114–9.
54. Kolokolov FA, Fursina AB, Pavlov PA, Terekhov VI. Synthesis, antimicrobial and antimycotic activity of the complexes of d-elements with furan-containing carboxamidrazones. Pharma Chem Jou. 2002; 36:307–8.
55. Raman N, Kulandaisamy A, Thangaraja C, Jeyasubramanian K. Redox and antimicrobial studies of transition metal(II) tetradentate Schiff base complexes. Trans Met Chem. 2003;28:29–36.
56. Legler AV, Kazachenko AS, Kazbanov VI, Per'yanova OV, Veselova OF. Synthesis and antimicrobial activity of silver

complexes with arginine and glutamic acid. Pharma Chem J. 2001;35:501–3.
57. Sharma S, Athar F, Maurya M, Azam A. Copper(II) complexes with substituted thiosemicarbazones of thiophene-2-carboxaldehyde: synthesis, characterization and antiamoebic activity against *E. histolytica*. Eur J Med Chem. 2005;40:1414–9.
58. Mohanan K, Murukan B. Complexes of manganese(II), iron(II), cobalt(II), nickel(II), copper(II), and zinc(II) with a bishydrazone. Synth React Inorg Met-Org Nano-Met Chem. 2005;35:837–44.
59. Taylor DM, William DR. Trace element medicine and chelation theory. London: Royal Society of Chemistry; 1998.
60. Anjaneyula Y, Rao RP. Preparation, characterization and antimicrobial activity studies on some ternary complexes of Cu(II) with acetylacetone and various salicylic acids. Synth React Inorg Met-Org Chem. 1986;16:257–72.