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Synthesis, characterization and biological aspects of novel five-coordinated dimeric-Cu(II) systems

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Abstract

The structural and spectroscopic properties of novel five-coordinated dimeric-Cu(II) system have been investigated. The biocidal activities of all eight compounds, ligands, cupric nitrate and standard drugs against six bacteria and three fungi were determined. The DNA interaction activity of complexes was studied using spectrophotometry and electrophoresis. The superoxide dismutase (SOD)-like activity of the complexes was compared with previously reported monomeric- and dimeric copper complexes. The results support the five-coordinated dimeric square pyramidal geometry for the quinolone-Cu(II) system.

Keywords: *Dimeric-Cu(II) system, fluoroquinolone, DNA binding, SOD activity, biocidal activity*

Introduction

The fluoroquinolone drugs are well known for their indistinguishable property of coordination with several transition metals [1]. Several spectrophotometry methods have been reported for the determination of fluoroquinolones in human blood and urine via metal chelation [2–4]. There is a drastic change in the biochemical properties of copper on chelation with drugs and it possess modified toxicological and pharmacological properties. Copper(II) is biologically essential and its compounds have proved beneficial in diseases like tuberculosis, rheumatoid arthritis, gastric ulcer, and cancer [5–7]. Some advanced copper-containing compounds have been reported as anti-inflammatory, antiamoebic, antimalerial, cytotoxic under hypoxic condition, and antiradical, antifungal [8–16]. Some dimeric Cu-proteins have an advanced application in oxygen activation and reduction [17]. Several monomeric and dimeric Cu(II) compounds are known to be biologically stable, membrane permeable and nontoxic as SOD mimics [18,19]. In the present study, eight dimeric Cu(II) compounds were synthesized and studied for different biochemical and thermal aspects.

Experimental

Materials and methods

All the chemicals used were of analytical grade. Cupric nitrate, 3-chloro aniline, cyclohaxanone, *o*-phenylenediamine, benzaldehyde, ethylenediamine, *p*-anisaldehyde and benzil, were purchased from the E. Merck (India) Ltd, Mumbai. Ciprofloxacin hydrochloride from Bayer AG (Wyppertal, Germany). Thiophene-2-carboxaldehyde, 2,2′-bipyridylamine, 2-aminopyridine, 1,8-diaminonaphthalene were from, Lancaster, England. Luria broth and agar-agar were from Himedia Laboratories Pvt. Ltd., India. Agarose and ethidium bromide were from Sigma Chemical Co., India. Bromophenol blue, xylene cyanol FF, tris(hydroxymethyl)methylamine, sucrose, acetic acid and EDTA were from Qualigens Fine Chemicals, India. NADH, nitroblue tetrazolium, phenazine methosulphate, and sperm herring DNA were from Sigma Chemical Co., USA. The organic solvents were purified by recommended methods [20]. Infrared spectra were recorded on a FT-IR Shimadzu spectrophotometer as KBr pellets in the range $4000-400\,cm^{-1}$. Carbon, hydrogen, nitrogen,

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and/or sulfur elemental analyses were performed with a model 240 Perkin Elmer elemental analyzer. The metal contents of the complexes were analyzed by EDTA titration [21] after decomposing the organic matter with a mixture of $HClO_4$, H_2SO_4, and HNO_3 (1:1.5:2.5). Thermogravimetric analyses and the differential scanning calorimetric study were done with a model 5000/2960 SDTA, TA instrument (USA). The ^1H NMR and ^{13}C NMR was recorded on a Bruker Avance (400 MHz). The diffuse reflectance spectra of the complexes were recorded in the range 1700–350 nm (as MgO discs) on a Beckman DK-2A spectrophotometer while electronic spectra were recorded on a Shimadzu UV-VIS spectrophotometer. The magnetic moments were measured by Gouy's method using mercury tetrathiocyanatocobaltate(II) as the calibrant ($\chi_g = 16.44 \times 10^{-6}$ cgs units at 20°C) on a Citizen Balance. The diamagnetic correction was made using Pascal's constant [22]. All the complexes were insoluble in water, methanol and dimethyl formamide, but were soluble in dimethyl sulphoxide.

Synthesis of schiff bases

The Schiff bases were synthesized according to reported methods and are summarized in Scheme I [23–26].

Synthesis of complexes:

$[Cu_2(Cip)_2(bipym)_2(pip)]\cdot5H_2O$ (I):. A methanolic solution of $Cu(NO_3)_2\cdot3H_2O$ (2.41 g, 10 mmol) was added to (2,2′bipyridylamine)bipym (1.71 g, 10 mmol) in methanol, followed by addition of a previously prepared solution of (ciprofloxacin)Cpf·HCl (3.67 g, 10 mmol) in water. The pH was adjusted to 6 ~ 7.5 pH with dilute HNO_3 or NaOH solution. The resulting blue solution was refluxed for 0.5 h., evaporated down to half volume, and kept overnight. The crystalline product obtained was washed with ether and dried over a vacuum desiccator. Yield: 80%, m.p.: 260°C, Found: C, 49.72, H, 3.42, N, 11.50; Cu, 10.49. $C_{50}H_{42}Cl_2Cu_2F_2N_{10}O_{11}$ (1204.92) requires: C, 49.84, H, 3.51, N, 11.62; Cu, 10.54%.

$[Cu_2(Cip)_2(bap)_2(pip)]5H_2O$ (II):. Prepared from bap (1.82 g, 10 mmol). Yield: 75%, m.p.: 293°C, Found: C, 52.81, H, 4.39, N, 9.05; Cu, 10.34. $C_{54}H_{54}Cl_2Cu_2F_2N_8O_{11}$ (1226.97) requires: C, 52.86, H, 4.43, N, 9.13; Cu, 10.35%.

$[Cu_2(Cip)_2(tca)_2(pip)]\cdot5H_2O$ (III). Prepared from tca (1.87 g, 10 mmol. Yield: 70%, m.p.: 349°C, Found: C, 50.51, H, 4.25, N, 6.81; Cu, 10.25. $C_{52}H_{52}Cl_2Cu_2F_2N_6O_{11}S_2$ (1237.05) requires: C, 50.48, H, 4.39, N, 6.79; Cu, 10.27%.

$[Cu_2(Cip)_2(bendan)_2(pip)]\cdot5H_2O$ (IV). Prepared from bendan (3.34 g, 10 mmol). Yield: 63%, m.p.: 273°C, Found: C, 61.19, H, 4.45, N, 7.19; Cu, 8.18. $C_{78}H_{70}Cl_2Cu_2F_2N_8O_{11}$ (1531.35) requires: C, 61.17, H, 4.60, N, 7.31; Cu, 8.29%.

$[Cu_2(Cip)_2(benen)_2(pip)]\cdot5H_2O$ (V). Prepared from benen (2.36 g, 10 mmol). Yield: 75%, m.p.: 273°C, Found: C, 55.73, H, 5.01, N, 8.41; Cu, 9.47. $C_{62}H_{66}Cl_2Cu_2F_2N_8O_{11}$ (1335.15) requires: C, 55.77, H, 4.97, N, 8.39; Cu, 9.51%.

$[Cu_2(Cip)_2(bmbbd)_2(pip)]\cdot5H_2O$ (VI). Prepared from bmbbd (3.44 g, 10 mmol). Yield: 58%, m.p.: 284°C, Found: C, 57.11, H, 4.72, N, 7.16; Cu, 8.05. $C_{74}H_{74}Cl_2Cu_2F_2N_8O_{15}$ (1551.34) requires: C, 57.29, H, 4.80, N, 7.22; Cu, 8.19%.

$[Cu_2(Cip)_2(bcpded)_2(pip)]\cdot5H_2O$ (VII). Prepared from bcpded (4.29 g, 10 mmol). Yield: 50%, m.p.: 288°C, Found%: C, 57.18, H, 4.07, N, 6.41; Cu, 7.23. $C_{82}H_{70}Cl_6Cu_2F_2N_8O_{11}$ (1721.20) requires: C, 57.22, H, 4.09, N, 6.50; Cu, 7.38%.

$[Cu_2(Cip)_2(dcbd)_2(pip)]\cdot5H_2O$ (VIII). Prepared from dcbd (2.68 g, 10 mmol). Yield: 60%, m.p.: 251°C, Found: C, 56.49, H, 5.88, N, 7.97; Cu, 8.97. $C_{66}H_{82}Cl_2Cu_2F_2N_8O_{11}$ (1399.32) requires: C, 56.65, H, 5.90, N, 8.00; Cu, 9.08%.
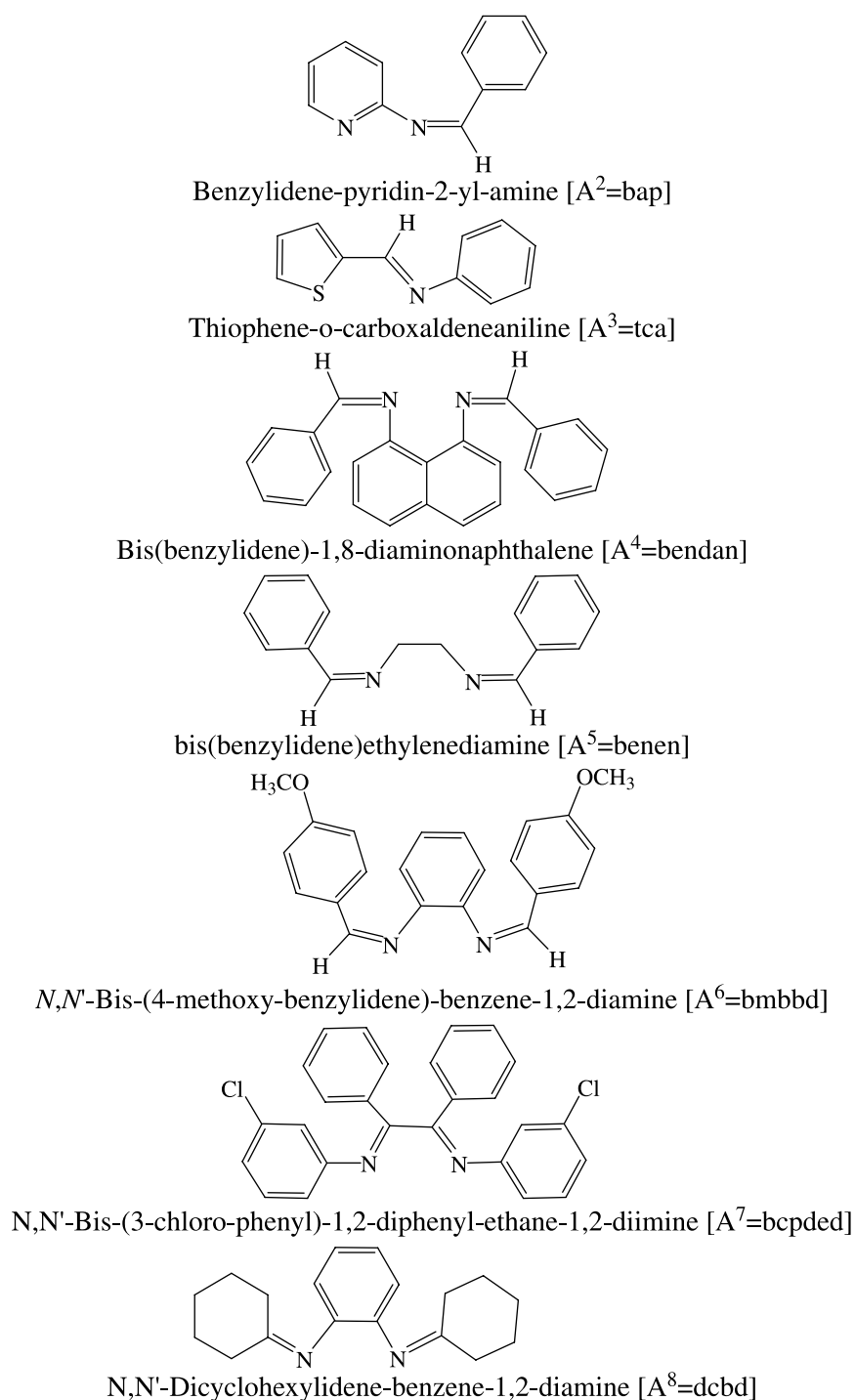
Preparation of stock solution

A stock solution of 2.5 ppm was made by dissolving 0.25 mg of each compound in 5% dimethyl sulphoxide solution.

Determination of MIC value

The biocidal screening was estimated by the minimal inhibitory concentration (MIC). MIC was determined using the method of progressive double dilution in liquid media containing 1ppm to 50ppm of the compound being tested. The biological screening of all the compounds was carried out at MIC (2.5 μg/mL) and results are expressed as zone of inhibition in mm. The biocidal activity of the ofloxacin, levofloxacin, flucanozole, ligands, $Cu(NO_3)_2\cdot3H_2O$, and its complexes were analyzed against various gram-negative and gram-positive bacterial cultures of *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Salmonella typhi, Escherichia coli and Serratia marcescens* and three fungal cultures namely *Aspergillus niger, Aspergillus flavus, Lasiodiplodia theobromae* using the Agar-Plate technique [27].

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

Benzylidene-pyridin-2-yl-amine [A^2=bap]

Thiophene-o-carboxaldeneaniline [A^3=tca]

Bis(benzylidene)-1,8-diaminonaphthalene [A^4=bendan]

bis(benzylidene)ethylenediamine [A^5=benen]

N,*N*'-Bis-(4-methoxy-benzylidene)-benzene-1,2-diamine [A^6=bmbbd]

N,N'-Bis-(3-chloro-phenyl)-1,2-diphenyl-ethane-1,2-diimine [A^7=bcpded]

N,N'-Dicyclohexylidene-benzene-1,2-diamine [A^8=dcbd]

Scheme 1. Synthesized Schiff bases.

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 a micropipette with sterilized tips; 100 μL of activated strain was placed onto the surface of an agar plate, and spread evenly over the surface by means of a sterile, bent glass rod. Then two wells in each plate having diameter 10 mm were made with the help of a sterilized borer.

Application of discs. Sterilized stock solutions (2.5 μg/mL) were used for application in the well of previously inoculated agar plates. When the discs were applied, they were incubated at 37°C for 24 h. The zone of inhibition was then measured (in mm) around the disc and the results are represented in Table I. Control experiments were performed where only the equivalent volume of solvent without added test compound was applied to the petri dishes.

All experiments were performed in triplicate and ofloxacin, levofloxacin, and fluconozole were used as standard drugs. The growth was compared with the control and is expressed as zone of inhibition *vs* control.

Super oxide dismutase activity

Measurement of SOD-mimic activity. The SOD-mimic activity of the copper(II) complexes was evaluated according to Viswanathan et al., [28], which was first proposed by McCord and Fridovich [18]. This *in vitro* method is based on the competing reactions of the tested compounds and nitroblue tetrazolium (NBT), phenazine methosulphate with NADH solution in distilled water. The reaction product is a blue formazan compound. The decrease in concentration of NBT is proportional to the SOD mimic activity of the tested compounds. The reaction solution consisted of a solution of tested compound in DMSO and NBT, phenazine methosulphate and NADH in distilled water. The formazan product was subsequently estimated at 560 nm using a Shimadzu UV-VIS spectrophotometer. The actual concentration of formazan dye produced was measured continuously at time intervals of 30 s.

DNA interaction absorption titration

A DNA interaction study was performed on a UV-VIS spectrophotometer. Absorption titration of compounds in DMSO, and whole system in buffer (phosphate, pH 7.2) were done by keeping fixed the amount of the copper compounds (where compound: I = 12.04, II = 12.26, III = 12.37, IV = 15.31, V = 13.35, VI = 15.51, VII = 17.21, VIII = 13.99 μgm) and the amount of DNA variable i.e. 2 to 7 μgm. Compound-DNA solutions were employed to record absorption spectra.

Gel analyses and quantification

The inspection of super coiled pBR322 was done in TAE [tris(hydroxymethyl)methylamine, acetic acid and EDTA] buffer pH 8.0. The pattern of inspection was designated as DNA alone (control), DNA in presence of ligands and DNA in presence of complex. Nuclease activity experiments conducted by mixing pBR322 (50μM) in TE [40 mM Tris acetate and 1 mM EDTA] buffer (pH 8.0), and ligand or complex (50 μM). The reaction mixture was incubated at room temperature for 1 h and then amended with 6 $\times$ loading buffer (40% sucrose, 0.02% bromophenol blue and 0.02% xylene cyanol FF) and loaded onto 0.8% agarose gel. Electrophoresis was carried out at constant voltage (100 V) in a Submarine Electrophoresis Unit (Genie, Banglore, India). The gel was stained with ethidium bromide. The same experimental conditions were maintained in control assays. The gels were viewed on a UV transilluminator, and images were captured with an attached camera and estimated using AlphaDigiDoc[TM] RT. Version V.4.1.0 PC-Image software.

Table I.   Biocidal activity data of compounds.

Compounds	Zone of Inhibition in mm					
	E. Coli	*B. Subtilis*	*S. aureus*	*S. typhi*	*B. cereus*	*S. marcesens*
Cantrol	11	11	10	11	11	11
$Cu(NO_3)_2 \cdot 3H_2O$	14	17	19	18	19	18
LH(Cpf. HCl)	28	34	40	32	31	37
Std. 1 (Ofl. HCl)	30	34	39	33	30	32
Std. 2 (Lef. HCl)	33	36	38	29	28	34
Std. 3 (flucanozole)	15	19	11	12	11	12
A^1	12	15	25	18	19	22
A^2	17	11	12	12	11	16
A^3	13	14	16	14	15	19
A^4	19	12	11	16	12	14
A^5	11	11	11	11	12	19
A^6	13	14	13	14	15	14
A^7	17	11	12	16	15	17
A^8	14	14	15	11	11	19
I	36	25	37	33	26	35
II	42	41	37	27	28	38
III	39	39	41	28	27	36
IV	32	30	31	26	25	30
V	41	40	36	31	31	38
VI	41	39	39	28	29	43
VII	40	40	39	28	33	35
VIII	42	38	37	30	25	52

Results and discussion

The ligands A^2–A^8 were prepared by the condensation reaction of aldehyde/ketone with amine in ethanol. The isolated ligands were characterized by elemental analyses, IR, ^{1}H and ^{13}C NMR spectroscopy. The copper(II) complexes of quinolone-3-carboxylic acid (cpf.HCl) were isolated by metathesis reaction of cupric nitrate and variable ligands A^1–A^8 in a 1:1:1 ratio. The copper(II) is coordinated to the carboxylate and pyridone oxygen of the quinolone-3-carboxylic acid to form a six membered ring. Copper complexes assumed five coordinated square pyramidal geometry coordinating with N-N/N-S of neutral bidentate ligands, two oxygens of cip.(L = 7-Chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid) and the nitrogen of the piperazine ring. The copper complexes are dimeric in nature. The $[Cu_2(Cip.)_2(bpy)_2(pip)]\cdot \cdot 6H_2O$ synthesis and its crystal structure has been reported by Wang et.al., who also proposed a possible reaction scheme for formation of the dimeric complex with liberation of piperazine ring from ciprofloxacin[29]. In continuation of earlier work [26] we have synthesized eight dimeric complexes which also follow the proposed reaction scheme (Scheme 2) for dimeric complexes. The synthesized dimeric compounds were characterized using IR spectra, electronic spectra, magnetic measurements and thermogravimetric analyses. The thermal decomposition suggests five water
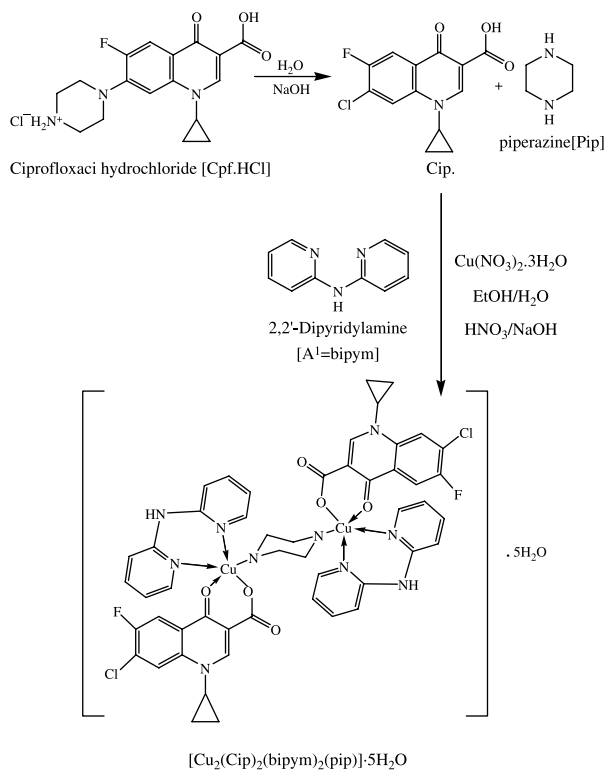
molecule of crystallization and stepwise decomposition of the complexes. The elemental analyses were in good agreement with the proposed 1:1:1; Cu(II): Cip:A^n formulation for all complexes.

^{1}H NMR and ^{13}C NMR spectra of Schiff bases

The ^{1}H NMR spectra and ^{13}C NMR spectra of the ligands in DMSO-d_6 are reported along with the possible assignment. The ^{1}H NMR spectra of ligands exhibited peaks at about 7.0–8.0 ppm assigned to the aromatic protons. The singlet peak which appeared at 7.8–9.1 ppm was assigned to the azomethine proton (−CH=N−). In the ^{13}C NMR spectra, the peaks observed at about 114.5–136.4, 113.5–144.5, and 128.5–143.0 ppm were assigned to aromatic, pyridine, and thiophene carbons respectively. Peaks observed at about 123.5–155.3, 155.3–161.2, 157.9–180.5 ppm were assigned to C−N, CH=N, and C=N carbons respectively.

IR spectra

The IR spectra of the complexes are shown in Table II. The ν(C=O) stretching vibration band appears at 1708 cm^{-1} in the spectra of ciprofloxacin, and in the complexes at 1626–1633 cm^{-1}; this shift towards lower energy suggests that coordination occurs through the carbonyl oxygen atom [30]. The sharp band in ciprofloxacin at 3520 cm^{-1} [31] is due to hydrogen bonding; which is contributed to ionic resonance structure and peak observed because of free hydroxyl stretching vibration. This band completely vanished in the spectra of the metal complexes indicating deprotonation of the carboxylic proton. The data is further supported by ν(M−O) [32] band which appeared at $502 \sim 525$ cm^{-1}. The strong absorption band obtained at 1624 cm^{-1} and 1340 cm^{-1} in ciprofloxacin is assigned to ν(COO)$_{asy}$ and ν(COO)$_{sym}$ respectively, while in the metal complexes these bands were observed at $\sim$ 1580 and $\sim$ 1370 cm^{-1}, respectively. The frequency separation ($\Delta\nu$) in the investigated complexes is greater than 200 cm^{-1}, suggesting a unidentate bonding nature for the carboxyl group [30,33–37]. In the investigated compound the ν (C=N) band of 2,2$'$ bipyridylamine appeared at 1580 cm^{-1}. which shifted to higher frequency at 1612 cm^{-1} [38,39] in the complexes indicating the bidentate N−N coordination of the ligand. Similarly for benzylidene-2-aminopyridine two strong bands were assigned at 1615 and 1593 cm^{-1} for the ν (C=N) stretching vibration of the azomethine and pyridine ring respectively which on complexation are shifted to 1666 cm^{-1} and 1620 cm^{-1}, respectively, suggesting the bidentate N−N participation in coordination [40]. The ν (C=N) peak for the Schiff bases A^3, A^4, A^5, A^6, A^7, and A^8 was observed at 1601 cm^{-1}–1629 cm^{-1} which on complexation were

Scheme 2.

Table II. Infrared spectral data of complexes.

Compounds	ν (C=O) Pyridone cm^{-1}	ν (COO)$_{asy}$ cm^{-1}	ν (COO)$_{sym}$ cm^{-1}	$\Delta\nu$ cm^{-1}	ν (C–Cl) cm^{-1}	ν (C=N) cm^{-1} Azo methine	ν (C=N) cm^{-1} Ring	ν (M–N) cm^{-1}	ν (M–O) cm^{-1}	ν (M–S) cm^{-1}
I	1632	1583	1381	202	1111	–	1612	539	504	–
II	1628	1581	1376	205	1147	1666	1620	545	511	–
III	1626	1589	1381	208	1128	1575	–	520	515	420
IV	1632	1578	1371	207	1179	1602	–	540	505	–
V	1627	1580	1370	210	1146	1605	–	542	511	–
VI	1629	1588	1382	206	1145	1570	–	541	514	–
VII	1631	1578	1377	201	1102	1575	–	540	510	–
VIII	1633	1584	1383	201	1111	1574	–	537	502	–

shifted to 1570 cm^{-1}–1605 cm^{-1}, indicating the N—S and N—N bidentate coordination of the ligand [41–44]. This data was further supported by ν(M–N) [45] which appeared at 520 ~ 545 cm^{-1}. In the case of [Cu$_2$(Cip)$_2$(tca)$_2$(pip)]·5H$_2$O, the ν(C—S) band of tca observed at 765 cm^{-1} is shifted lower to 750 cm^{-1} in the spectra of the complex indicating the participation of the sulfur atom of the thiophene ring. This data was further supported by a new band observed at 420 cm^{-1} which can be assigned to ν (M–S) [46–48] mode.

Electronic spectra and magnetic properties

The electronic spectral data and magnetic moments are summarized in Table III. The diffuse reflectance spectra of dimeric copper(II) complexes [Cu$_2$(L)$_2$ (A^n)$_2$(pip)] ·5H$_2$O were taken in solid as well as liquid (DMSO) states; both states showed quite similar results. The spectra of Cu(II) complexes exhibited a broad band in the 589–783 nm region [49–51,17]. These bands are characteristic of a Cu(II) d–d transition in a tetragonal field in which the Cu(II) atom is in a distorted square pyramidal coordination environment. The magnetic moments of all compounds was obtained between 1.76–2.10 B.M and is good agreement for a five-coordinated dimeric copper(II) mixed-ligand system and consistent with the presence of a single unpaired electron [52,53].

TGA and DSC

The thermogravimetric (TGA) and differential scanning calorimetric (DSC) data for the synthesized complexes is given in Table IV. The dehydration processes are interpreted by endothermic peaks in the DSC curves. The DSC curves showed the melting process for I to VIII complexes at 260, 293, 349, 273, 273, 284, 288, and 251, respectively, decomposition is followed by an exothermic continuous plot. The initial weight loss which occurred in the 24–110°C temperature range for all complexes is attributed to a

Table III.   Electronic spectral data of complexes.

Compounds	d–d transition in solid state cm^{-1}	λ_{max} in DMSO d–d transition nm(cm^{-1})	μ_{eff} B.M.
I	12770	783 (12771)	1.87
II	13765	726 (13774)	2.10
III	16980	589 (16977)	1.90
IV	13150	760 (13157)	1.76
V	14180	705 (14184)	1.84
VI	13100	762 (13123)	1.99
VII	14550	688 (14534)	1.94
VIII	14280	700 (14285)	1.80

Table IV. Thermal data of complexes.

Compounds	Process	TGA		DSC°C	
		Temperature range°C	Mass loss % Found/Calc.	Endo peaks	Melting point
I	$[Cu_2(L)_2(A^1)_2(pip)]\cdot5H_2O \rightarrow Cu_2(L)_2(A^1)_2(pip)] + 5H_2O$	30–110	7.32/7.46	128.59	260
	$[Cu_2(L)_2(A^1)_2(pip)] \rightarrow [Cu_2(L)_2(A^1)_2] + pip$	170–215	7.10/6.99		
	$[Cu_2(L)_2(A^1)_2] \rightarrow [Cu_2(A^1)_2] + 2L$	245–480	46.78/46.67		
	$[Cu_2(A^1)_2] \rightarrow 2Cu + 2A^1$	520–660	28.21/28.40		
II	$[Cu_2(L)_2(A^2)_2(pip)]\cdot5H_2O \rightarrow [Cu_2(L)_2(A^2)_2(pip)] + 5H_2O$	30–105	7.31/7.33	114.61	293
	$[Cu_2(L)_2(A^2)_2(pip)] \rightarrow [Cu_2(L)_2(A^2)_2] + pip$	175–210	6.79/6.87		
	$[Cu_2(L)_2(A^2)_2] \rightarrow [Cu_2(A^2)_2] + 2L$	250–490	45.73/45.83		
	$[Cu_2(A^2)_2] \rightarrow 2Cu + 2A^2$	510–670	29.76/29.70		
III	$[Cu_2(L)_2(A^3)_2(pip)]\cdot5H_2O \rightarrow [Cu_2(L)_2(A^3)_2(pip)] + 5H_2O$	26–105	7.30/7.27	125.63	349
	$[Cu_2(L)_2(A^3)_2(pip)] \rightarrow [Cu_2(L)_2(A^3)_2] + pip$	150–205	6.94/6.81		
	$[Cu_2(L)_2(A^3)_2] \rightarrow [Cu_2(A^3)_2] + 2L$	240–475	45.42/45.45		
	$[Cu_2(A^3)_2] \rightarrow 2Cu + 2A^3$	510–650	30.29/30.27		
IV	$[Cu_2(L)_2(A^4)_2(pip)]\cdot5H_2O \rightarrow [Cu_2(L)_2(A^4)_2(pip)] + 5H_2O$	24–110	5.90/5.87	110.02	273
	$[Cu_2(L)_2(A^4)_2(pip)] \rightarrow [Cu_2(L)_2(A^4)_2] + pip$	180–220	5.69/5.50		
	$[Cu_2(L)_2(A^4)_2] \rightarrow [Cu_2(A^4)_2] + 2L$	240–480	36.85/36.72		
	$[Cu_2(A^4)_2] \rightarrow 2Cu + 2A^4$	510–670	43.54/43.67		
V	$[Cu_2(L)_2(A^5)_2(pip)]\cdot5H_2O \rightarrow [Cu_2(L)_2(A^5)_2(pip)] + 5H_2O$	32–105	6.72/6.74	124.24	273
	$[Cu_2(L)_2(A^5)_2(pip)] \rightarrow [Cu_2(L)_2(A^5)_2] + pip$	165–200	6.37/6.31		
	$[Cu_2(L)_2(A^5)_2] \rightarrow [Cu_2(A^5)_2] + 2L$	220–460	42.18/42.11		
	$[Cu_2(A^5)_2] \rightarrow 2Cu + 2A^5$	490–640	35.45/35.39		
VI	$[Cu_2(L)_2(A^6)_2(pip)]\cdot5H_2O \rightarrow [Cu_2(L)_2(A^6)_2(pip)] + 5H_2O$	45–110	5.68/5.80	92.72	284
	$[Cu_2(L)_2(A^6)_2(pip)] \rightarrow [Cu_2(L)_2(A^6)_2] + pip$	170–220	5.38/5.43		
	$[Cu_2(L)_2(A^6)_2] \rightarrow [Cu_2(A^6)_2] + 2L$	250–490	36.23/36.24		
	$[Cu_2(A^6)_2] \rightarrow 2Cu + 2A^6$	520–700	44.31/44.40		
VII	$[Cu_2(L)_2(A^7)_2(pip)]\cdot5H_2O \rightarrow [Cu_2(L)_2(A^7)_2(pip)] + 5H_2O$	36–105	5.20/5.22	115.61	288
	$[Cu_2(L)_2(A^7)_2(pip)] \rightarrow [Cu_2(L)_2(A^7)_2] + pip$	140–200	4.91/4.89		
	$[Cu_2(L)_2(A^7)_2] \rightarrow [Cu_2(A^7)_2] + 2L$	240–480	32.87/32.67		
	$[Cu_2(A^7)_2] \rightarrow 2Cu + 2A^7$	515–710	49.93/49.88		
VIII	$[Cu_2(L)_2(A^8)_2(pip)]\cdot5H_2O \rightarrow [Cu_2(L)_2(A^8)_2(pip)] + 5H_2O$	45–110	6.52/6.43	118.50	251
	$[Cu_2(L)_2(A^8)_2(pip)] \rightarrow [Cu_2(L)_2(A^8)_2] + pip$	175–205	6.07/6.02		
	$[Cu_2(L)_2(A^8)_2] \rightarrow [Cu_2(A^1)_2] + 2L$	245–480	39.96/40.18		
	$[Cu_2(A^8)_2] \rightarrow 2Cu + 2A^8$	510–670	38.20/38.36		
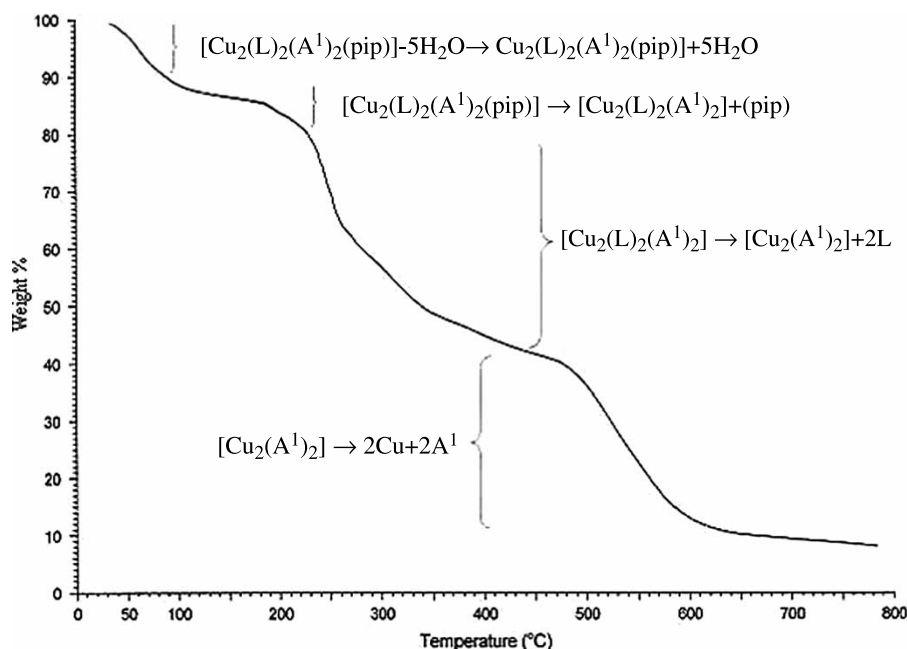
Figure 1. TGA of $[Cu_2(Cip)_2(bipym)_2(pip)]\cdot 5H_2O$.

loss of five water molecules of crystallization. In the second step weight loss during 140–220°C corresponding to the (pip) molecule, followed by liberation of (L) in between 220–490°C. Finally, decomposition of A^n occurs in the temperature range 510–710°C, and the remaining weight is in good agreement with two copper atoms. The TGA of $[Cu_2(Cip)_2(bipym)_2(pip)]\cdot 5H_2O$ is shown in Figure 1.

Biocidal activity

Comparative analysis showed higher antibacterial activity for the complexes than the free ligands, metal salt, fluconazole (fungicide) and control (DMSO) against *S. aureus etc.*, while good activities were noted as compared to the standard drugs ofloxacin and levofloxacin. In the case of fungi no significant antifungal activity was observed against *Aspergillus niger etc.* The increase in biocidal activity of the complexes over that of the ligand may be due to the effect of the metal ion on the normal cell process. A possible mode for increase in biocidal activity may be considered in the light of Overton's concept [54] and the chelation theory [55]. According to Overton's concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid-soluble materials so that liposolubility is an important factor which controls biocidal activity. On chelation, the polarity of the metal ion will be reduced to a great extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the

delocalization of π-electrons over the whole chelate ring and enhances the lipophilicity of the complexes.

SOD mimic activity

The superoxide dismutase-like activity of the dimeric complexes was determined (Table V). The IC_{50} values of the complexes are lower than that of free Cu(II) ($IC_{50} = 0.450\,\mu M$)[17], suggesting that the improvement in activity is not due to dissociation of the complexes. The superoxide scavenging data suggest that all the complexes exhibit higher activity than the previously reported activity of monomeric and dimeric copper complexes [56–60]. It is noted that the activity

Table V. IC_{50} values of copper complexes.

Compounds	IC_{50} (μM)	References
I	0.058	This work
II	0.055	This work
III	0.052	This work
IV	0.048	This work
V	0.056	This work
VI	0.055	This work
VII	0.052	This work
VIII	0.090	This work
$[Cu_2(L^4)_2(OCH_3)_2(NH_3)_2]$	0.103	17
$[Cu_2(L^4)_2(OCH_3)_2(DMSO)_2]$	0.059	17
$[Cu(L^1)_2(NH_3)_2].MeOH$	0.244	17
$[Cu(L^2)_2(DMSO)_2]$	0.188	57
$[Cu(L^4)_2(py)_2]$	0.146	56
$[Cu(stz)_2(py)_3Cl]$	1.310	58
$[Cu(stz)_2(Him)_2].MeOH$	0.664	59
$[Cu(stz)_2(4-mHim)_2]$	0.586	60
Cu_2Zn_2SOD	0.006	56

exhibited by complexes I–VIII is only 8.0–15.0 fold respectively, less than that of the native enzyme ($IC_{50} = 0.006 \mu M$). It has been reported that a more distorted geometry leads to higher SOD activity [17,61]. An important point observed here is that all eight complexes have almost identical IC_{50} values. The similar activity of complexes can be explained on the basis of almost identical geometry, limited steric hindrance, and vacant coordination sites that permit the access and the successful coordination of the $O_2^{\cdot-}$ radical. In addition to these, a favorable response of electrons of the C=N bond and aromatic ligand could be to stabilize the $Cu-O_2^-$ interaction.

DNA interaction

Absorption spectroscopy is widely well known to determine the binding of complexes with DNA. Complexes bound to DNA through interaction result in hypochromism (blue shift) and bathochromism (red shift) due to interaction between chromophores and the base pairs of DNA. The extent of hypochromism is commonly consistent with the strength of the intercalative interaction [62–64]. The DNA interaction data with the complexes are shown in Table VI. The absorption spectra of the complex $[Cu_2(Cip)_2(bipym)_2(pip)].5H_2O$ is shown in Figure 2. A maxima at about 275 nm is observed in the spectrum of complex without DNA, which was decreased as the amount of DNA increased and observed at about 258 nm in presence of 7 μgm of DNA. Thus, the red shift (bathochromism) is due to interaction between chromophores and the base pair of DNA, suggesting that the complexes were bound to DNA through intercalation [65–67].

Electrophoretic behavior of complex-DNA systems

The effect of the binding of complexes on supercoiled (SC) pBR322 was determined by its ability to make it bulky by binding with reactive sites of pBR322 DNA and produce changes in its conformation from

Table VI.   DNA interaction data with the complexes.

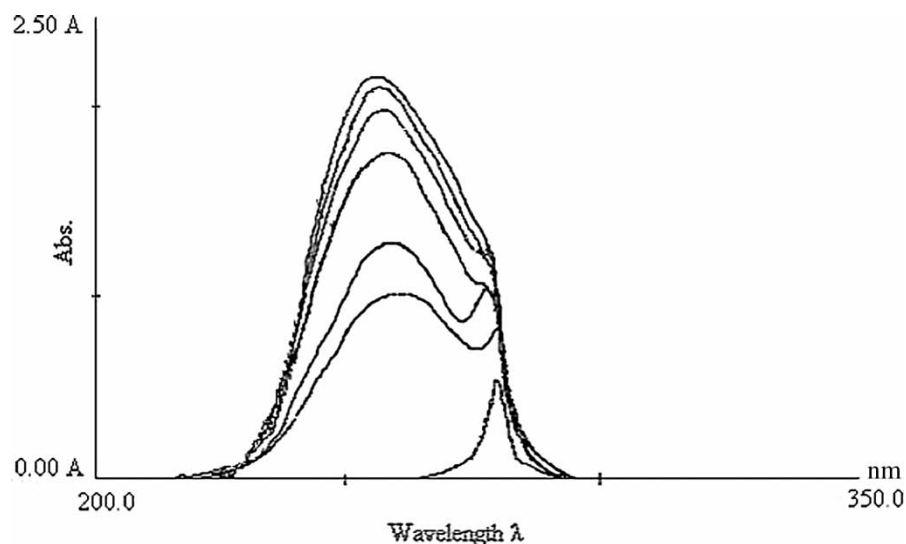| Compound	DNA μgm	Compound λ_{max} nm	A^n λ_{max} nm	L λ_{max} nm	Cu(II) λ_{max} nm
I	0	277	260.4	260.0	260.0
$[Cu_2(L)_2(A^1)_2(pip)]\cdot 5H_2O$	3	261	259.6	259.2	258.0
	5	259	258.6	258.8	257.2
	7	258	245.0	247.4	256.4
II	0	273	260.6	260.0	260.0
$[Cu_2(L)_2(A^2)_2(pip)]\cdot 5H_2O$	3	262	258.4	259.2	258.0
	5	260	258.0	258.8	257.2
	7	259	249.4	247.4	256.4
III	0	273	260.0	260.0	260.0
$[Cu_2(L)_2(A^3)_2(pip)]\cdot 5H_2O$	3	261	258.2	259.2	258.0
	5	257	257.8	258.8	257.2
	7	256	246.6	247.4	256.4
IV	0	270	263.0	260.0	260.0
$[Cu_2(L)_2(A^4)_2(pip)]\cdot 5H_2O$	3	259	259.2	259.2	258.0
	5	258	258.2	258.8	257.2
	7	257	244.2	247.4	256.4
V	0	273	265.2	260.0	260.0
$[Cu_2(L)_2(A^5)_2(pip)]\cdot 5H_2O$	3	263	259.2	259.2	258.0
	5	260	258.2	258.8	257.2
	7	258	246.6	247.4	256.4
VI	0	273	261.7	260.0	260.0
$[Cu_2(L)_2(A^6)_2(pip)]\cdot 5H_2O$	3	264	258.8	259.2	258.0
	5	262	258.0	258.8	257.2
	7	259	244.6	247.4	256.4
VII	0	275	259.0	260.0	260.0
$[Cu_2(L)_2(A^7)_2(pip)]\cdot 5H_2O$	3	263	258.4	259.2	258.0
	5	261	258.0	258.8	257.2
	7	258	245.4	247.4	256.4
VIII	0	270	260.3	260.0	260.0
$[Cu_2(L)_2(A^8)_2(pip)]\cdot 5H_2O$	3	262	259.4	259.2	258.0
	5	259	258.4	258.8	257.2
	7	258	247.4	247.4	256.4

Figure 2.   Absorption spectra of complex $[Cu_2(L)_2(A^1)_2(pip)]\cdot5H_2O$ in absence and presence of [DNA].

supercoiled(SC) to nicked open circular(OC) form. When pBR322 is subjected to electrophoresis, the fastest migration is observed for SC. If one strand is cleaved due to binding with a reactive species, the SC form is converted into the OC form. Figure 3 shows the electrophoresis of all eight complexes after incubation for one hour by comparison with the same experiments carried out with Cu(II) and ligands. Complexes exhibit higher activity nuclease than that of Cu(II).
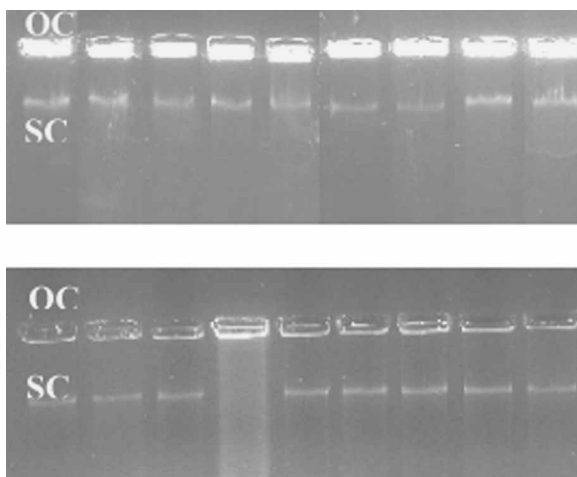
The greater cleavage activity of the complexes with respect to the ligands is clearly seen from Figure 3 and data (Table VII). It can be seen that SC migrated faster than OC. SC is a smear on the gel while OC remains in the well. There may be two reasons for this, (I) OC become bulky having a higher molecular weight due to intercalation of compounds. (II) OC requires more time to run on the gel than SC. From the experiment we can conclude that the conversion of SC to OC is higher in the presence of complexes than in the presence of free ligands or Cu(II).

Conclusion

All the copper complexes are dimeric in nature having a distorted square pyramidal geometry. The complexes exhibit good biocidal activities as when compared to the standard drugs ofloxacin and levofloxacin. In the case of fungi no significant antifungal activity was observed. All eight compounds possess good SOD activity and results lead to the indication that the dimeric nature increases the ability

Figure 3. Electropheresis of pBR 322 after incubation with different agents. (A) Lane 1: pBR322 (control), lane 2: pBR322 + I, lane 3: pBR322 + II, lane 4: pBR322 + III, lane 5: pBR322 + IV, lane 6: pBR322 + V, lane 7: pBR322 + VI, lane 8: pBR322 + VII, lane 9: pBR322 + VIII (B) Lane 1: pBR322 + Cu(II), lane 2: pBR322 + A^1, lane 3: pBR322 + A^2, lane 4: pBR322 + A^3, lane 5: pBR322 + A^4, lane 6: pBR322 + A^5, lane 7: pBR322 + A^6, lane 8: pBR322 + A^7, lane 9: pBR322 + A^8.

Table VII.   Cleavage of pBR322 DNA.

Compounds	DNA %		Compounds	DNA %	
	SC	OC		SC	OC
Control	100.0	00.00	Cu(II)	72.15	27.85
A^1	54.57	45.43	I	36.63	63.37
A^2	75.00	25.00	II	71.19	28.81
A^3	48.17	51.83	III	45.05	54.95
A^4	84.95	15.05	IV	36.37	63.63
A^5	47.35	52.65	V	34.31	65.69
A^6	83.94	16.06	VI	58.29	41.71
A^7	81.12	18.88	VII	69.13	30.87
A^8	86.99	13.01	VIII	44.62	55.38

of the compounds to dismutate the superoxide radical. Absorption titration and gel electrophoresis analyses suggest that the complexes have good DNA binding properties.

Acknowledgements

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