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Synthesis, spectral, thermal, DNA interaction and antimicrobial properties of novel Cu(II) heterochelates

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Novel dimeric Cu(II) heterochelates were synthesized using 1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-yl-1,4-dihydro-quinoline-3-carboxylic acid (Cpf) and eight different neutral bidentate ligands. All ligands were characterized by elemental analyses, melting point and IR, ^{1}H NMR and ^{13}C NMR spectra, while heterochelates were characterized by elemental analyses, IR spectra, reflectance spectra, magnetic measurements and thermogravimetric analyses. The DNA interaction and cleavage behaviors of the ligands and corresponding heterochelates were studied by UV–vis absorption titration and gel electrophoresis technique, respectively. The results indicate that heterochelates show larger DNA interaction and cleavage affinity than the ligands. The antimicrobial activities of heterochelates, ligands, cupric nitrate and standard drugs against six bacteria and three fungi were tested. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: absorption titration; DNA; heterochelates; antimicrobial activity; gel electrophoresis

INTRODUCTION

1-Cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-yl-1,4-dihydro-quinoline-3-carboxylic acid (Cpf) is a quinolone derivative and well known for its antibacterial activity, curing urinary tract and skin infections via inhibition of the synthesis of deoxyribonucleic acid.[1,2] Copper(II) and its complexes with drugs administrated for pharmacokinetic and therapeutic purposes are the subject of much research interest.[3–5] It is known that some clinical drugs work by chelation or by inhibiting the metalloenzymes. Therefore, metal ions like copper might play vital biological role in drug utilization and various biochemical processes in living systems.[6] Copper complexes are known to be site-specific radiopharmaceuticals, with antiamoebic, antibacterial, antifungal, antileukemic, antitumor, antiradical, anti-malarial and anti tuberculosis properties.[7–17] Copper and its complexes catalyze catecholase as well as phosphatase-like-activity and also have DNA cleavage and interaction properties.[18–20] Thus Cu(II) and its synergetic activity with quinolone drugs have been reported because if their improved pharmacological and molecular biochemical activity.[18–21,51] In the present work new dimeric Cu(II) heterochelates were synthesized using Cpf and different eight neutral bidentate ligands. They were characterized by spectroscopic technique and screened for antimicrobial activity. DNA interaction and cleavage studies were done using absorption titration and gel electrophoresis technique, respectively.

EXPERIMENTAL

Materials and methods

All the chemicals used were of analytical grade. Aniline, anthranilic acid, acetophenone, acetic anhydride, 2,3-butanedione, *p*-anisaldehyde, benzil, benzaldehyde, benzoyl chloride, hydrazine hydrate and cupric nitrate were purchased from the E. Merck (India) Limited, Mumbai. Ciprofloxacin hydrochloride was purchased form Bayer AG (Wyppertal, Germany). 1,8-Diaminonaphthalene was purchased from Lancaster, UK. Luria broth and agar-agar were purchased from Himedia, India. Sperm herring DNA was purchased from SRL, India. The organic solvents were purified by recommended methods.[22] Infrared spectra were recorded on an FT-IR Shimadzu spectrophotometer as KBr pellets in the range 4000–400 cm^{-1}. Carbon, hydrogen and nitrogen elemental analyses were performed with a model 240 Perkin Elmer elemental analyzer. The metal contents of the

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complexes were analyzed by EDTA titration[23] 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 differential scanning calorimetric study were obtained with a model 5000/2960 SDTA, TA instrument (USA). The ^1H and ^{13}C NMR were recorded on Bruker Avance (400 MHz). The diffuse reflectance spectra of the complexes were recorded in the range 1700–350 nm (as MgO disks) on a Beckman DK-2A spectrophotometer. Absorption titration was carried out using 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), with a Citizen balance. The diamagnetic correction was made using Pascal's constant.[24] All the complexes were insoluble in water, methanol and dimethyl formamide, but were soluble in dimethyl sulfoxide.

Synthesis of Schiff bases

N,N′-Dicyclohexylidene-naphthalene-1,8-diamine (A^1–dcnd)

The ethanolic solution (100 ml) of cyclohaxanone (1.96 g, 20 mmol) was added to ethanolic solution (100 ml) of 1,8-diamino naphthalene (1.58 g, 10 mmol). The mixture was stirred continuously for 4 h to obtain a fine yellow crystalline product, which was washed with *n*-hexane. The product was recrystallized in ethanol and dried in air. Yield 2.41 g, 68%; m.p. 135 °C; found (%): C 83.00, H 8.09, N 8.74. $C_{22}H_{26}N_2$ (318.45) requires (%): C 82.97, H 8.23, N 8.80.

N,N′-bis-(4-methoxy-phenyl)-1,2-diphenyl-ethane-1,2-diimine (A^2–bmpded)

An ethanolic solution (100 ml) of benzil (2.10 g, 10 mmol) and *p*-anisididine (2.46 g, 20 mmol) was refluxed in a water bath for 24 h, concentrated to one-third of its volume and kept overnight over a sulfuric acid desiccator. The obtained product was filtered, recrystallized in ethanol and washed with 1 : 1 absolute ether : hexane. Yield: 2.92 g, 64%; m.p. 120 °C; found (%): C 79.86, H 5.78, N 6.70. $C_{28}H_{24}N_2O_2$ (420.50) requires (%): C 79.98, H 5.75, N 6.66%.

N,N′-Bis-(phenyl)-1,2-dimethyl-ethane-1,2-diimine (A^3–bpdmed)

An ethanolic solution (100 ml) of aniline (1.86 g, 20 mmol) was added drop-wise to ethanolic solution (100 ml) of 2,3-butanedione (0.86 g, 10 mmol) and refluxed in a water bath for 8 h. The resulting mixture was filtered and the crystalline yellow product was further recrystallized in ethanol, washed with *n*-hexane and dried in air. Yield: 1.58 g, 58%; m.p. 114 °C; found (%): C 81.49, H 6.71, N 11.69. $C_{16}H_{16}N_2$ (236.31) requires (%): C 81.32, H 6.82, N 11.85%.

N,N′-Bis-(4-methoxy-phenyl)-1,2-dimethyl-ethane-1,2-diimine (A^4–bmpdme)

An ethanolic solution (100 ml) of *p*-anisididine (2.46 g, 20 mmol) was added drop-wise to ethanolic solution (100 ml) of 2,3-butanedione (0.86 g, 10 mmol) and refluxed in a water bath for 8 h. The resulting mixture was filtered and the crystalline yellow product was further recrystallized in ethanol, washed with *n*-hexane and dried in air. Yield: 1.86 g, 56%; m.p. 170 °C; found (%): C 72.83, H 6.64, N 9.48. $C_{18}H_{20}N_2O_2$ (296.36) requires (%): C 72.95, H 6.80, N 9.45%.

3-Amino-2-phenyl-3H-quinazolin-4-one (A^5–apq)

3-Amino-2-phenyl-3H-quinazolin-4-one was synthesized according to Deshmukh *et al.*[25]

N,N′-Bis-(1-phenyl-ethylidene)-naphthalene-1,8-diamine (A^6–bpend)

An ethanolic solution of (100 ml) 1,8-diaminonaphthalene (10 mmol, 1.58 g) was added to an ethanolic solution (100 ml) of acetophenone (20 mmol, 2.34 g), refluxed in a water bath for 8 h and kept for overnight in a refrigerator. The resulting mixture was filtered and the crystalline yellow product was further recrystallized in ethanol, washed with *n*-hexane and dried in air. Yield: 2.20 g, 56%; m.p. 242 °C; found (%): C 86.20, H 6.31, N 7.69. $C_{26}H_{22}N_2$ (362.46) requires (%): C 86.15, H 6.12, N 7.73%.

3-Acetyl-7-ethoxy-2-methyl-chromen-4-one (A^7–aemc)

The 3-acetyl-7-ethoxy-2-methyl-chromen-4-one was synthesized according to Kostanecki and Rozycki.[26]

3-(Benzylidene-amino)-2-phenyl-3H-quinazolin-4-one (A^8–bapq)

The 3-(benzylidene-amino)-2-phenyl-3H-quinazolin-4-one was synthesized by Deshmukh *et al.*[25]

Synthesis of heterochelates

$[Cu_2(Cip)_2(dcnd)_2(pip)]\cdot 5H_2O$

A methanolic solution (100 ml) of $Cu(NO_3)_2 \cdot 3H_2O$ (2.41 g, 10 mmol) was added to methanolic solution (100 ml) of dcnd(A^1) (3.18 g, 10 mmol), followed by addition of previously prepared solution (100 ml) of Cpf·HCl (3.67 g, 10 mmol) in water; the pH was adjusted to 6–7.5 pH with dilute NaOH solution. The resulting blue solution was refluxed for 0.5 h, evaporated to half its volume, and kept overnight at room temperature. The crystalline product was washed with ether and dried over a vacuum desiccator. The reaction was monitored using TLC. The experimental and physical parameters of the compounds are summarized in Table 1.

Antimicrobial activity

Preparation of stock solution

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

Determination of MIC value

The antimicrobial screening was carried out by minimal inhibitory concentration (MIC). MIC was determined using the method of progressive double dilution in liquid media

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Table 1. Experimental and physical parameter of the compounds

Compounds empirical formula	Elemental analyses % found (required)				m.p. (°C)	Yield (g/%)	Molecular weight
	C	H	N	Cu			
(I) $[Cu_2(Cip)_2(dcnd)_2(pip)] \cdot 5H_2O$	59.30	5.84	7.49	8.50	>360	6.29/68	1499.51
$C_{74}H_{86}Cl_2Cu_2F_2N_8O_{11}$	(59.27)	(5.78)	(7.47)	(8.48)			
(II) $[Cu_2(Cip)_2(bmpded)_2(pip)]5H_2O$	60.65	4.72	6.63	7.48	275	6.68/65	1703.61
$C_{86}H_{82}Cl_2Cu_2F_2N_8O_{15}$	(60.63)	(4.85)	(6.58)	(7.46)			
(III) $[Cu_2(Cip)_2(bpdmed)_2(pip)] \cdot 5H_2O$	55.78	5.04	8.40	9.61	265	5.06/60	1335.23
$C_{62}H_{66}Cl_2Cu_2F_2N_8O_{11}$	(55.77)	(4.98)	(8.39)	(9.52)			
(IV) $[Cu_2(Cip)_2(bmpdme)_2(pip)] \cdot 5H_2O$	54.58	5.12	7.68	8.75	260	5.42/60	1455.33
$C_{66}H_{74}Cl_2Cu_2F_2N_8O_{15}$	(54.47)	(5.13)	(7.70)	(8.73)			
(V) $[Cu_2(Cip)_2(apq)_2(pip)] \cdot 5H_2O$	52.16	4.25	10.50	9.41	190	5.66/67	1337.12
$C_{58}H_{56}Cl_2Cu_2F_2N_{10}O_{13}$	(52.10)	(4.22)	(10.48)	(9.50)			
(VI) $[Cu_2(Cip)_2(bpend)_2(pip)] \cdot 5H_2O$	62.18	4.79	7.05	8.03	255	5.72/59	1587.54
$C_{82}H_{78}Cl_2Cu_2F_2N_8O_{11}$	(62.04)	(4.95)	(7.06)	(8.01)			
(VII) $[Cu_2(Cip)_2(aemc)_2(pip)] \cdot 5H_2O$	53.36	4.95	4.26	9.57	240	5.32/63	1351.18
$C_{60}H_{66}Cl_2Cu_2F_2N_4O_{17}$	(53.33)	(4.92)	(4.15)	(9.41)			
(VIII) $[Cu_2(Cip)_2(bapq)_2(pip)] \cdot 5H_2O$	57.20	4.32	9.17	8.49	240	5.41/58	1513.33
$C_{72}H_{64}Cl_2Cu_2F_2N_{10}O_{13}$	(57.14)	(4.26)	(9.26)	(8.40)			

containing 1–50 ppm of the compound being tested.[27] The biological screening of all the experiments was carried out using MIC (2.5 μg/ml) and the results are expressed as zones of inhibition in millimeters. The antimicrobial activities of ofloxacin, levofloxacin, flucanozole, ligands, $Cu(NO_3)_2 \cdot 3H_2O$ 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 fungi cultures, namely *Aspergillus niger, Aspergillus flavus* and *Lasiodiplodia theobromae* using the Agar-plate technique.[28,29]

The zone of inhibition was measured (in mm) around the disk and the results are given in Table 7. Control experiments were performed in which only equivalent volume of solvents without added test compounds were applied to the petri dishes and the the zone of inhibition measured. All experiments were performed in triplicate and ofloxacin, levofloxacin and flucanozole were used as standard drugs. The growth was compared with solvent as control and is expressed as zone of inhibition vs control.

DNA interaction

Absorption titration study

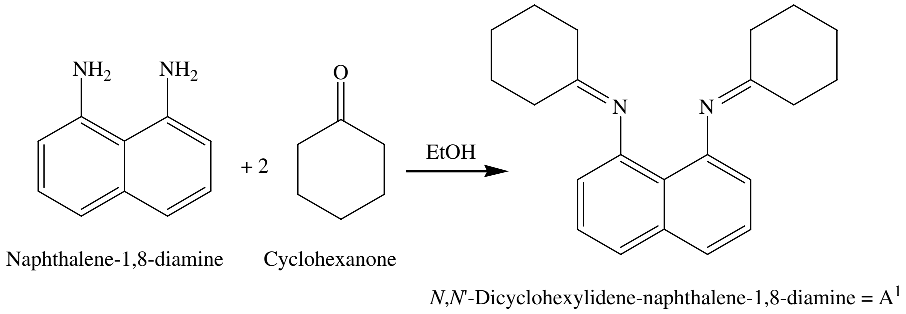
The DNA interaction study was performed on a UV–vis spectrophotometer. The absorption titration of compounds in DMSO and of the whole system in buffer (phosphate, pH 7.2) was carried out using a fixed amount of copper heterochelates (where compound: I = 14.99, II = 17.03, III = 13.35, IV = 14.55, V = 13.37, VI = 15.87, VII = 13.51, VIII = 15.13 μg) and a variable amount of DNA, i.e. 0–7 μg. Compound–DNA solutions were used to record the absorption spectra.

Gel electrophoresis study

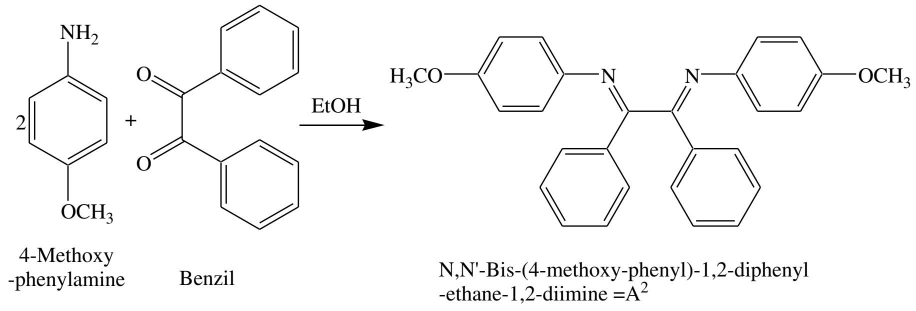
Inspection of supercoiled pBR322 was carried out in TAE [tris(hydroxymethyl)methylamine, acetic acid and EDTA] buffer, pH 8.0. DNA interaction experiments were carried out by mixing pBR322 (50 μM) in TE [40 mM Tris acetate and 1 mM EDTA] buffer (pH 8.0), and ligand or heterochelates (50 μM). The reaction mixture was incubated at room temperature for 1 h then adjusted with 6× 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 (Genei, 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; images were captured with an attached camera and analyzed using AlphaDigiDoc™ RT version V.4.1.0 PC-Image software.

RESULTS AND DISCUSSION

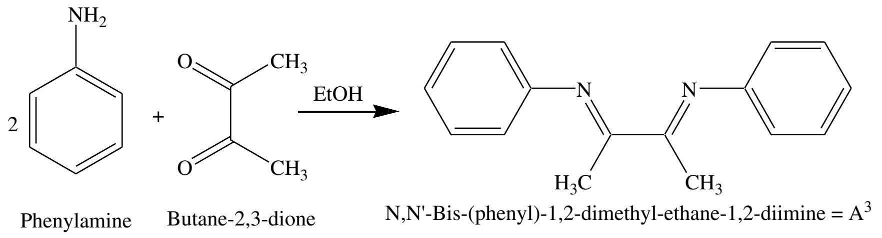
The neutral bidentate ligands A^1–A^4 were synthesized by reaction of ketone with amine in ethanolic solution, while ligands A^5–A^8 were prepared according to reported methods. Structural elucidations of all the synthesized ligands were done using elemental analyses, IR, ^1H and ^{13}C-NMR spectroscopy. The Cu(II) complexes were isolated by reaction of cupric nitrate and CpfHCl with variable ligands A^1–A^8 in 1 : 1:1 ratio. The Cu(II) coordinated to deprotonated carboxylate oxygen, pyridone oxygen and N–N/N–O/O–O of neutral bidentate ligands and nitrogen of piparazine ring to form a square pyramidal geometry. The thermal analysis suggests decomposition of crystalline water molecules and



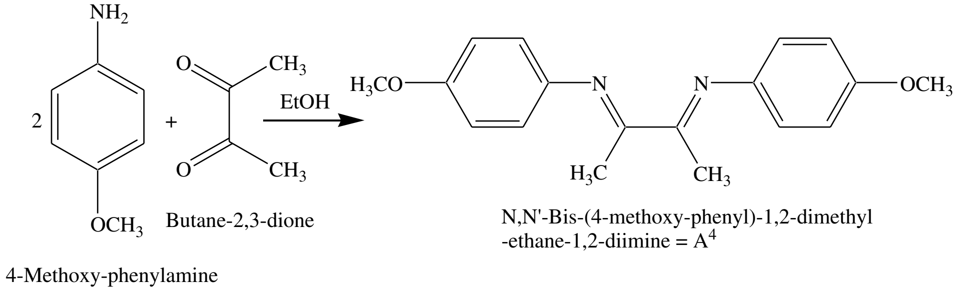
Scheme 1. Synthesis of N,N'-dicyclohexylidene-napthalene-1,8-diamine (A^1).



Scheme 2. Synthesis of N,N'-bis-(4-methoxy-phenyl)-1,2-diphenyl-ethane-1,2-diimine (A^2).



Scheme 3. Synthesis of N,N'-bis-(phenyl)-1,2-dimethyl-ethane-1,2-diimine (A^3).



Scheme 4. Synthesis of N,N'-bis-(4-methoxy-phenyl)-1,2-dimethyl-ethane-1,2-diimine (A^4).

stepwise decomposition of heterochelates. The synthesis of $[Cu_2(Cip)_2(bpy)_2(pip)]^{\bullet}6H_2O$ and its crystal structure was reported by Wu *et al.*;[30] they proposed a possible reaction scheme for dimeric heterochelates formation and liberation of piperazine ring from ciprofloxacin. It requires study of the polymorphisms of each intermediate; pH plays a vital role for the separation of piperazine from CpfHCl. In addition to this, a number of Fe(II) and Cu(II)

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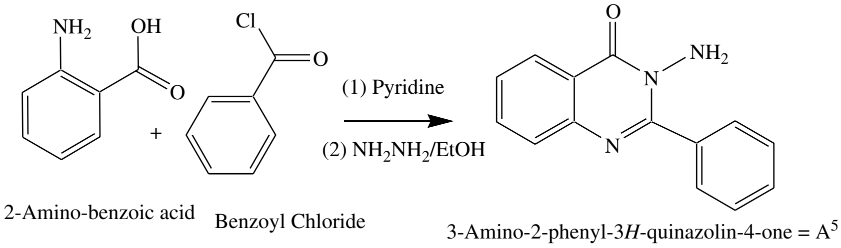
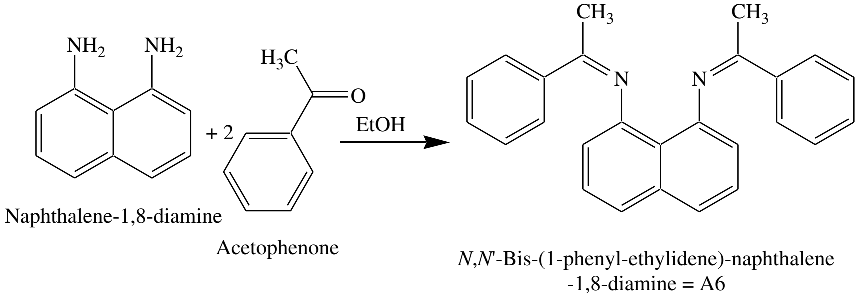
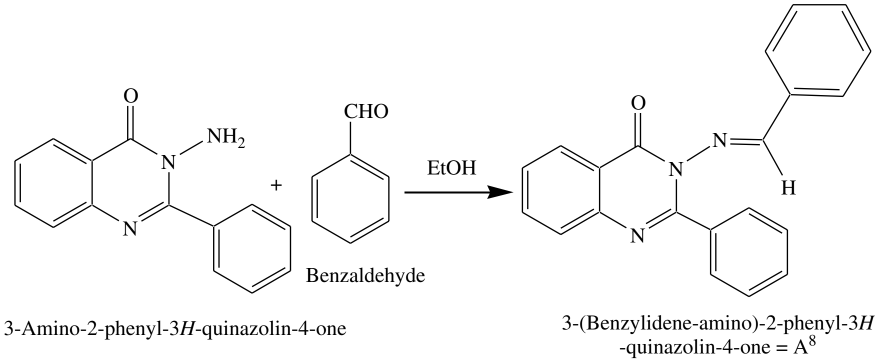


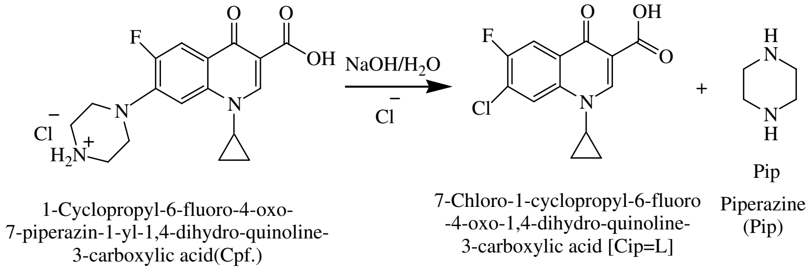
Scheme 5. Synthesis of 3-amino-2-phenyl-3H-quinazolin-4-one (A^5).



Scheme 6. Synthesis of *N*,*N*'-bis-(1-phenyl-ethylidene)-naphthalene-1,8-diamine (A^6).



Scheme 7. Synthesis of 3-(benzylidene-amino)-2-phenyl-3H-quinazolin-4-one (A^8).



Scheme 8. Possible reaction scheme for liberation of piperazine ring from ciprofloxacin.

compounds have been synthesized by Pansuriya *et al.*[31,32] The proposed reaction schemes are shown as Schemes 8 and 9.

All the heterochelates are insoluble in water and common organic solvents except DMSO, so it is difficult to grow single crystal for X-ray diffraction analyses. The elemental analyses

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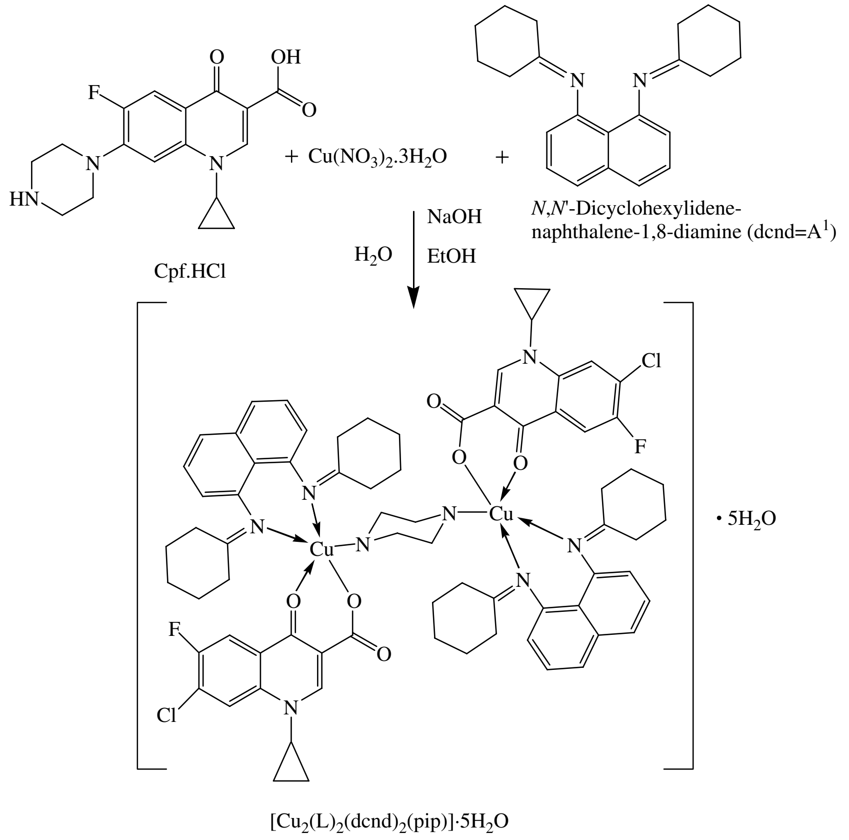

Scheme 9. Synthesis of dimeric heterochelate.

were in good agreement with proposed 1 : 1 : 1 Cu(II) : LH : A^n formulation of dimeric heterochelates.

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

The ^{1}H NMR spectra and ^{13}C NMR spectra of the ligands have been taken in DMSO-d_6. The IR, ^{1}H NMR and ^{13}C NMR spectral data are reported along with the possible assignment in Tables 2–4, respectively. The ^{1}H NMR spectra of ligands exhibit peaks at 6.5–8.2 ppm assigned to the aromatic protons. The singlet peak appeared at 8.06 ppm was assigned to azomethine proton (–CH=N–). In the case of ^{13}C NMR spectra, peaks observed at 105.9–136.4 and 15.4–36.9 ppm were assigned to aromatic and aliphatic carbons, respectively, while the peak at ~55.3 ppm was assigned to methoxy carbons. Peaks observed at ~145.0, ~165.5, ~164.1 and ~166.1 ppm were assigned to C–N, C=N, CH=N, and (C=O) carbons, respectively.

Table 2. IR spectral data of ligands

Compounds	ν(C=O) cm^{-1}	ν(C=N) cm^{-1} azomethine	ν(C=N) cm^{-1} ring	ν(C=C) cm^{-1} aromatic
A^1	—	1600	—	1570
A^2	—	1601	—	1574
A^3	—	1613	—	1572
A^4	—	1610	—	1570
A^5	1680	—	1590	1545
A^6	—	1630	—	1575
A^7	1680, 1660	—	—	1550
A^8	1680	1618	1584	1564

IR spectra

The IR spectra of heterochelates are shown in Table 5. The absorption band observed at 1624 and 1340 cm^{-1} in ciprofloxacin is assigned to $\nu(COO)_{asy}$ and $\nu(COO)_{sym}$, respectively, while in heterochelates these bands were observed at ~1600 and ~1380 cm^{-1}. The frequency separation ($\Delta\nu = \nu COO_{asy} - \nu COO_{sym}$) in investigated heterochelates was greater than 200 cm^{-1}, suggesting a unidentate bonding nature for the carboxylato group.[33–35] The ν(C=O) stretching vibration band appeared at 1708 cm^{-1} in the spectra of ciprofloxacin; the heterochelates showed this band at 1609–1627 cm^{-1}. The band shifted towards lower energy, suggesting that coordination occurs through the carbonyl oxygen atom.[36] The sharp band in ciprofloxacin at 3520 cm^{-1}[37] is due to hydrogen bonding, which contributes to the ionic resonance structure and the peak observed because of free hydroxyl stretching vibration. This band completely vanished in the spectra of heterochelates, indicating deprotonation of

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Table 3. ^{1}H NMR of ligands

Compounds	δ ppm (aromatic)	δ ppm (aliphatic)	δ ppm (CH=N, azomethine)
A^1	6.50–7.28(6H, m, Ar–H)	1.51–2.37 (20H, CH_2)	—
A^2	6.65–7.91(18H, m, Ar–H)	3.72(6H, s, OCH_3)	—
A^3	6.81–7.42(10H, m, Ar–H)	2.19 (6H, s, CH_3)	—
A^4	6.67–6.86(8H, m, Ar–H)	2.07 (6H, s, CH_3), 3.73(6H, s, OCH_3)	—
A^5	7.28–8.19(9H, m, Ar–H)	—	—
A^6	7.24–7.67(16H, m, Ar–H)	2.50(6H, s, CH_3)	—
A^7	7.17–8.24(3H, m, Ar–H)	1.64(3H. t, CH_3), 2.38(3H. s, CH_3), 2.35 (2H. m, CH_3) 2.65 (2H. m, OCH_2)	
A^8	7.24–8.20(14H, m, Ar–H)	—	8.06(1H, s, CH=N)

Table 4. ^{13}C NMR of ligands

Compounds	δ ppm (aromatic)	δ ppm (C=N)	δ ppm (C–N)	δ ppm (CH=N)	δ ppm (aliphatic)
A^1	105.9, 113.4, 116.9, 127.1, 134.7	173.0	140.0	—	22.3, 25.3, 36.9
A^2	113.9–134.4(Ar), 157.3(C–O)	165.4	135.4	—	55.3 (OCH_3)
A^3	118.8–129.0(Ar)	168.3	150.9	—	15.4(CH_3)
A^4	114.2–120.6(Ar), 158.6(C–O, Ar)	168.9	144.1	—	15.4(CH_3), 55.4(OCH_3)
A^5	127.02–133.02(Ar), 164.7.6(C=O)	165.3(C=N, ring)	145.5	—	—
A^6	119.39–136.45(Ar)	172.4	148.9	—	16.8(CH_3)
A^7	110.7–127.4(Ar), 154.7, 155.8, 168.5 (C–O), 175.2(C=O)	—	—	—	19.79(CH_3), 21.18(CH_3), 32.25(CH_3), 63.25(OCH_2), 200.4(C=O)
A^8	120.8–134.5(Ar), 166.1(C=O)	163.6(C=N, ring)	138.8	164.1(azomethine)	—

Table 5. Infrared spectral data of heterochelates

Compounds	ν(C=O) cm^{-1} pyridone	$\nu(COO)_{asy}$ (cm^{-1})	$\nu(COO)_{sym}$ (cm^{-1})	Δν (cm^{-1})	ν(C–Cl) (cm^{-1})	ν(C=N) (cm^{-1}) azomethine	ν(C=N) (cm^{-1}) ring	ν(M–N) (cm^{-1})	ν(M–O) (cm^{-1})
I	1618	1606	1390	216	1148	1570	—	535	510
II	1627	1608	1384	224	1145	1560	—	540	509
III	1609	1600	1380	205	1144	1575	—	540	511
IV	1617	1607	1383	224	1145	1569	—	535	510
V	1619	1590	1384	206	1160	—	1600	540	515
VI	1623	1608	1388	220	1146	1602	—	538	510
VII	1619	1600	1374	226	1150	—	—	540	508
VIII	1620	1598	1375	223	1130	1570	1600	537	512

carboxylic proton. The ν(C=O) peak for A^5, A^7 and A^8 observed at ~1680 cm^{-1} (cyclic) ~1660 cm^{-1} (acetyl) which is shifted to 1575 cm^{-1} on formation of heterochelates.[38,39] These data are further supported by the ν(M–O)[31] band appearing at 508–515 cm^{-1}. The ν(C=N) band for A^1–A^4, A^6 and A^8 were observed at ~1612 cm^{-1}. This ν(C=N) band shifted at ~1575 cm^{-1} in heterochelates due to chelation, indicating the bidentate N–N coordination of the ligand.[40,41] These data are further supported by ν(M–N)[32] appear at ~540 cm^{-1}.

Electronic spectra and magnetic properties

Electronic spectral data and magnetic moments are summarized in Table 6. Electronic spectra of $[Cu_2(L)_2(A^1)_2(pip)] \cdot 5H_2O$ are shown in Fig. 1. The diffuse reflectance spectra of heterochelates $[Cu_2(L)_2(A^n)_2(pip)] \cdot 5H_2O$ have been taken in solid states. The spectra of Cu(II) heterochelates show a broad band in the ~13, 750 cm^{-1} region.[42,43] These bands are characteristic of Cu(II) d–d transition in the tetragonal field in which the Cu(II) atom is in a distorted square pyramidal coordination environment. The magnetic moments of all compounds

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Table 6. Electronic spectral data of ligands and heterochelates

Compounds	d–d transition in solid state (cm^{-1})	μ_{eff} (B.M.)
I	14 180	1.89
II	14 080	1.77
III	13 825	1.84
IV	13 590	1.91
V	13 500	1.80
VI	13 970	1.87
VII	14 285	1.93
VIII	12 840	1.96

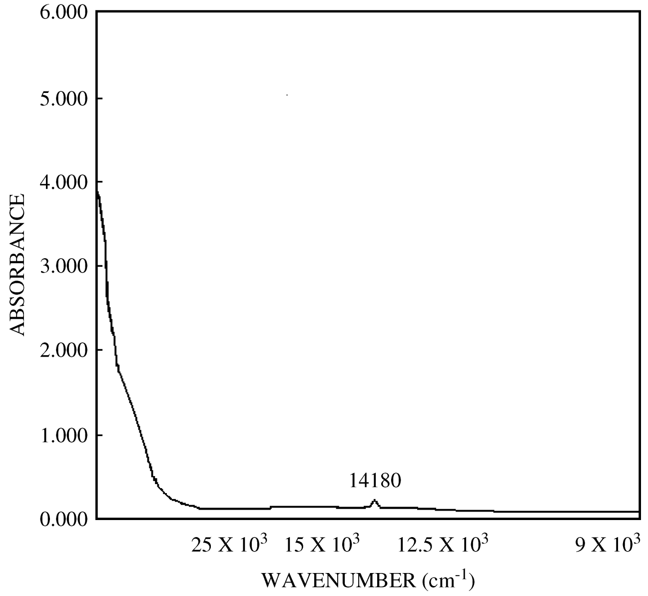

Figure 1. Electronic spectral curve of $[Cu_2(L)_2(A1)_2(pip)]\cdot 5H_2O$.

obtained between 1.77 and 1.96 B.M. is in good agreement for five coordinated dinuclear copper(II) heterochelates and consistent with the presence of a single unpaired electron.[44,45]

TGA

The thermogravimetric (TGA) analysis for the synthesized heterochelates obtained at a heating rate of 10 °C/min in N_2 atmosphere over the range 0–800 °C. The TGA curve of compound $[Cu_2(L)_2(A^1)_2(pip)]\cdot 5H_2O$ is shown in Fig. 2. The initial weight loss occurring in the 30–110 °C temperature range for all complexes is attributed to a loss of five water molecules of crystallization. In the second step weight loss during 130–235 °C corresponded to (pip) molecule, followed by liberation of (L) between 240 and 500 °C. Finally, decomposition of A^n occurs at 510–730 °C, and the remaining weight is in good agreement with that of two copper atoms.[32]

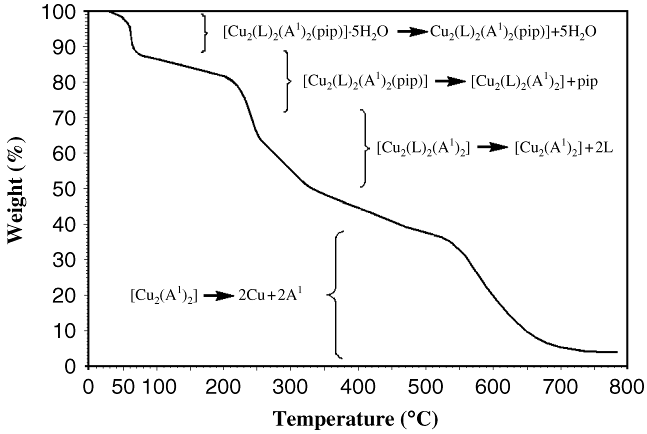

Figure 2. TGA curve of $[Cu_2(L)_2(A1)_2(pip)]\cdot 5H_2O$.

Antimicrobial activity

The increase in antimicrobial activity may be considered in light of Overtone's concept[46] and Tweedy's chelation theory.[47] According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favors the passage only of lipid-soluble materials due to which liposolubility is an important factor controlling the antimicrobial activity. On chelation, the polarity of the metal ion will be reduced to a greater 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. This increased lipophilicity enhances the penetration of the heterochelates into lipid membranes and blocks the metal binding sites in the enzymes of microorganisms. These heterochelates also disturb the respiration process of the cell and thus block the synthesis of proteins which restrict further growth of the organisms. Furthermore, the mode of action of the compounds may involve the formation of a hydrogen bond through the azomethine/carbonyl/amine group with the active centre of cell constituents, resulting in interference with the normal cell process.[48] Comparative analysis shows that the heterochelates exhibit higher antimicrobial activity than that of free ligands, metal salt and the control (DMSO). The heterochelates exhibit good activities (Table 7) as compared with the standard drugs ofloxacin, levofloxacin and flucanozole. In case of all three fungi, no significance antifungal activity was observed.

DNA interactions

Absorption spectroscopy is used to determine the interaction of the chelates with DNA. Heterochelates bound to DNA through interaction result in bathochromism (red shift) and hypochromism (blue shift) due to interaction between chromophores and the base pair of DNA. The extent of hypochromism is commonly consistent with the strength of intercalative interaction.[49–51] Figure 3 shows absorption

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Table 7. Antimicrobial data of ligands and complexes

Compounds	Zone of inhibition (mm)					
	E. coli	*B. substilis*	*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
Standard 1 (Ofl. HCl)	30	34	39	33	30	32
Standard 2 (Lef. HCl)	33	36	38	29	28	34
Standard 3 (flucanozole)	15	19	11	12	11	12
A^1	13	15	12	11	13	14
A^2	11	11	11	14	11	12
A^3	11	11	14	12	13	11
A^4	12	12	15	11	14	12
A^5	17	18	19	16	16	17
A^6	18	12	12	17	13	14
A^7	16	16	18	15	16	17
A^8	18	19	19	18	18	19
I	40	37	36	27	28	42
II	46	39	43	30	32	43
III	37	35	37	34	29	41
IV	38	35	38	35	33	42
V	36	37	33	37	35	34
VI	33	31	31	27	27	32
VII	38	34	32	33	36	35
VIII	37	37	36	37	36	39

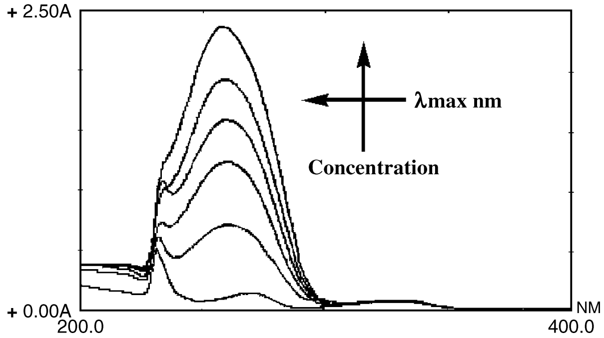


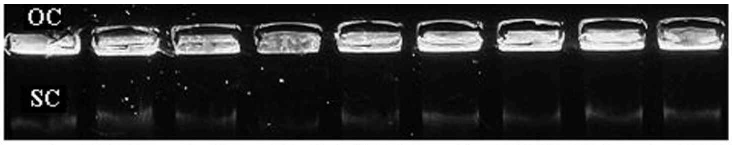
Figure 3. DNA absorption titration curve of $[Cu_2(L)_2(A1)_2 (pip)]^{\bullet}5H_20$.

titration curve of compound $[Cu_2(L)_2(A^1)_2(pip)] \cdot 5H_2O$. A maximum at about ~275 nm was observed in the spectrum of the complex without DNA, which decreased as the amount of DNA increased and was observed at about ~258 nm in the presence of 6 μg of DNA. For variable ligands (A^1–A^8), $Cu(NO_3)_2.3H_2O$ and ciprofloxacin maxima were observed at about ~261 nm in the absence of DNA and ~257 nm in the presence 6 μg of DNA. All data lead to the suggestion that, in the presence of 6 μg of DNA, the whole complex dissociated, and free Cu(II), constant ligand (Cip) and variable ligands (A^1–A^8) interacted with DNA or the complex interacted with DNA (i.e. N of purines or pyrimidines ring) through the coordinating atoms of the complex [i.e. Cu(II), N and O].

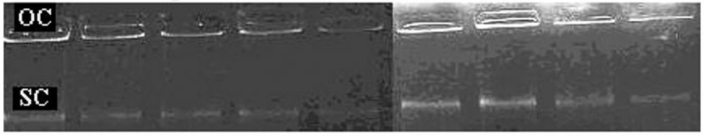
Gel quantification of heterochelate–DNA systems

The interaction of heterochelates on supercoiled (SC) pBR322 was determined by its ability to make it bulky by changing the conformation of pBR322 DNA due to binding with reactive sites of DNA. When pBR322 is subjected to electrophoresis, the fastest migration is observed for supercoiled DNA. If one strand is cleaved due to binding with reactive species, the SC form is converted in open nicked circular DNA (OC) form. Figure 4 shows the electrophoretic process of all eight heterochelates and comparisons of these experiments were carried out with Cu(II) and ligands. Heterochelates exhibit higher nuclease activity than those of Cu(II).

The greater cleavage activity of heterochelates compared with the ligands is clearly shown in Fig. 4 and Table 8. The SC smear on the gel while OC remain in the well. This may be due to OC becoming bulky, having high molecular weight due to intercalation of compounds, and/or OC requiring more time to run on the gel than SC. From the experiment we can conclude that the conversion of SC to OC is greater in the presence of



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



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

Figure 4. Gel of pBR322 with compounds.

Table 8. Gel electrophoresis data of ligands and complexes with DNA

Compounds	DNA %		Compounds	DNA %	
	SC	OC		SC	OC
Control	100	00	Cu(II)	72	28
A^1	84	16	I	59	41
A^2	67	33	II	55	45
A^3	80	20	III	76	24
A^4	74	26	IV	46	54
A^5	75	25	V	44	56
A^6	70	30	VI	56	44
A^7	67	33	VII	33	67
A^8	85	15	VIII	26	74

heterochelates than in the presence of free ligands and Cu(II).

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REFERENCES

1. Sundquist WT, Lippard SJ. *Coord. Chem. Rev.* 1990; **100**: 293.
2. Pedreño E, López-Contreras AJ, Cremades A, Peñafiel R. *J. Inorg. Biochem.* 2005; **99**: 2074.
3. Weder JE, Dillon CT, Hambley TW, Kennedy BJ, Lay A, Biffin JR, Regtop HL, Davies NM. *Coord. Chem. Rev.* 2002; **232**: 95.
4. Kato M, Muto Y. *Coord. Chem. Rev.* 1988; **92**: 45.
5. Efthimiadou EK, Sanakis Y, Katsarou M, Raptopoulou CP, Karaliota A, Katsaros N, Psomas G. *J. Inorg. Biochem.* 2006; **100**: 1378.
6. Peisach J, Aisen P, Blumberg W. *The Biochemistry of Copper.* Academic Press: New York, 1966.
7. Holland JP, Aigbirhio FI, Betts HM, Bonnitcha PD, Burke P, Christlieb M, Churchill GC, Cowley AR, Dilworth JR, Donnelly PS, Green JC, Peach JM, Vasudevan SR, Warren JE. *Inorg. Chem.* 2007; **46**: 465.
8. Sharma S, Ather A, Maurya MR, Azam A. *Euro. J. Med. Chem.* 2005; **40**: 1414.
9. Iqbal MS, Bukhari IH, Arif M. *Appl. Organomet. Chem.* 2005; **19**: 864.
10. Briganti F, Scozzafava A, Supuran CT. *Eur. J. Med. Chem.* 1997; **32**: 901.
11. Ruiz M, Perelló L, Ortiz R, Castiñeiras A, Maichle-Mössmer C, Cantón E. *J. Inorg. Biochem.* 1995; **59**: 801.
12. Panchal PK, Pansuriya PB, Patel MN. *J. Enz. Inhib. Med. Chem.* 2006; **21**(2): 203.
13. Belicchi-Ferrari M, Bisceglie F, Casoli C, Durot S, Morgenstern-Badarau I, Pelosi G, Pilotti E, Pinelli S, Tarasconi P. *J. Med. Chem.* 2005; **48**(5): 1671.
14. Adsule S, Barve V, Chen D, Ahmed F, Dou QP, Padhye S, Sarkar FH. *J. Med. Chem.* 2006; **49**(24): 7242.
15. Vančo J, Švajlenová O, Račanská E, Muselík J, Valentová J. *J. Trace Elem. Med. Biol.* 2004; **18**: 155.
16. Thompson KH, Orvig C. *Dalton Trans.* 2006; 761.
17. Williuns DR. *The Metal of Life.* Reinhold: London, 1971.
18. Rey NA, Neves A, Bortoluzzi AJ, Pich CT, Terenzi H. *Inorg. Chem.* 2007; **46**: 348.
19. Pedreño E, López-Contreras AJ, Cremades A, Peñafiel R. *J. Inorg. Biochem.* 2005; **99**: 2074.
20. Macías B, Villa MV, Gómez B, Borrás J, Alzuet G, González-Álvarez M, Castiñeiras A. *J. Inorg. Biochem.* 2007; **101**: 444.
21. Turel I. *Coord. Chem. Rev.* 2002; **27**: 232.
22. Furniss BS, Hannaford AJ, Smith PWG, Tatchell AR. *Vogel's Textbook of Practical Organic Chemistry*, 5th edn. ELBS and Longman: London, 2004.
23. Vogel AI. *Textbook of Quantitative Inorganic Analysis*, 4th edn. ELBS and Longman: London, 1978.
24. Weiss A, Witte H. *Magnetochemie.* VCH: Weinheim, 1973.
25. Deshmukh MB, Deshmukh DS. *J. Ind. Chem. Soc.* 1995; **72**: 847.
26. Kostanecki SV, Rozycki A. *J. Chem. Soc.* 1901; **34**: 102.
27. Dimitra KD, Demertzis MA, Filiou E, Pantazaki AA, Yadav PN, Miller JR, Zheng Y, Kyriakidis DA. *Biometals* 2003; **16**: 411.
28. Pelczar MJ, Chan ECS, Krieg NR. *Microbiology*, 5th edn, Vol. 23. Tata McGraw-Hill: New Delhi, 1993; 488.
29. Pansuriya PB, Patel MN. *Appl. Organomet. Chem.* DOI: 10.1002/aoc.1246.
30. Wu G, Wang G, Fu X, Zhu L. *Molecules* 2003; **8**: 287.

31. Pansuriya PB, Dhnadhukia P, Thakkar V, Patel MN. *J. Enz. Inhib. Med. Chem.* DOI: 10.1080/14756360701228988.
32. Pansuriya PB, Patel MN. *J. Enz. Inhib. Med. Chem.* DOI: 10.1080/14756360701384039.
33. Deacon GB, Philips RJ. *Coord. Chem. Rev.* 1980; **23**: 227.
34. Chohan ZH, Suparan CT, Scozzafava A. *J. Enz. Inhib. Med. Chem.* 2005; **20**(3): 303.
35. Nakamoto K. *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, 4th edn. Wiley Interscience: Chichester, 1986.
36. Turel I, Leban I, Bukovec N. *J. Inorg. Biochem.* 1999; 241.
37. Silverstein RM, Webster FX. *Spectrometric Identification od Organic Compounds*, 6th edn. Wiley: New York, 2004.
38. Chohan ZH, Suparan CT. *J. Enz. Inhib. Med. Chem.* 2005; **20**(5): 463.
39. Chohan ZH, Rauf A, Naseer MM, Somra MA, Suparan CT. *J. Enz. Inhib. Med. Chem.* 2006; **21**(2): 173.
40. Raman N, Kulandaisamy A, Jayasubramananian K. *Polish J. Chem.* 2002; **76**: 1085.
41. Chohan ZH, Pervez H, Rauf A, Khan KM, Supuran CT. *J. Enz. Inhib. Med. Chem.* 2004; **19**(5): 417.
42. González-Alvarez M, Borrás J, Alzuet G, Agudo LC, Garciá-Grunda S, Montejo-Benardo JM. *Inorg. Chem.* 2005; **44**: 9424.
43. Melnik M. *Coord. Chem. Rev.* 1981; **36**: 287.
44. Figgis BN, Lewis J. In: *Modern Coordination Chemistry: Principles and Methods*, Lewis J, Wilkins RG (eds). Interscience: New York, 1960; 400.
45. Carbello R, Castiñeiras A, Covelo B, Garcí-Martínez E, Niclós J, Váquez-López EM. *Polyhedron* 2004; **23**: 1505.
46. Parekh HM, Pansuriya PB, Patel MN. *Polish J. Chem.* 2005; **79**: 1843.
47. Tweedy BG. *Phytopathology* 1964; **55**: 910.
48. Chohan ZH, Khan KM, Supuran CT. *Appl. Organomet. Chem.* 2004; **18**: 305.
49. Tysoe SA, Margan RJ, Baker AD, Strekas TC. *J. Phys. Chem.* 1993; **97**: 1707.
50. Kelly TM, Tossi AB, McConnell DJ, Strekas TC. *Nucl. Acid. Res.* 1985; **13**: 6017.
51. Efthimiadou EK, Sanakis Y, Raptopoulou CP, Karaliota A, Katsaros N, Psomas G. *Bioorg. Med. Chem. Lett.* 2006; **16**(14): 3864–3867.