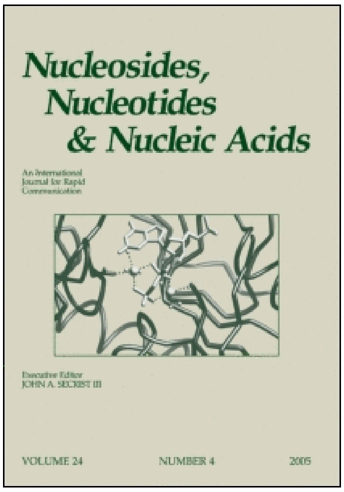

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Antimicrobial and DNA-Cleavage Studies of 22-Membered N_4 Tetraaza Macrocyclic Triazoles: Template Synthesis and Physicochemical Characterization

Sangamesh A. Patil[a]; Udaykumar V. Kamble[a]; Prema S. Badami[b]
[a] P. G. Department of Chemistry, Karnatak University, Dharwad, Karnataka, India [b] Department of Chemistry, Shri Sharanabasaveswar College of Science, Gulbarga, Karnataka, India

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ANTIMICROBIAL AND DNA-CLEAVAGE STUDIES OF 22-MEMBERED N_4 TETRAAZA MACROCYCLIC TRIAZOLES: TEMPLATE SYNTHESIS AND PHYSICOCHEMICAL CHARACTERIZATION

Sangamesh A. Patil,[1] Udaykumar V. Kamble,[1] and Prema S. Badami[2]
[1]*P. G. Department of Chemistry, Karnatak University, Dharwad, Karnataka, India*
[2]*Department of Chemistry, Shri Sharanabasaveswar College of Science, Gulbarga, Karnataka, India*

□ *A novel series of 22-membered macrocyclic complexes of the type [$MLCl_2$] ($M=Co^{2+}$, Ni^{2+} and Cu^{2+}) have been synthesized with newly derived biologically active ligands (L^I–L^{IV}). These ligands were synthesized by the condensation of* ortho*-phthalaldehyde and* bis*-(4-amino-5-mercapto-1, 2, 4-triazole-3-yl)alkanes. The mode of bonding and overall geometry of the complexes have been inferred through IR, EPR, electronic spectral studies, conductivity, magnetic, thermal, and electrochemical studies. All these complexes have been screened for their antibacterial (*Escherichia coli, Staphylococus aureus, Salmonella typhi, Pseudomonas aeruginosa) *and antifungal activities (*Aspergillus niger, Aspergillus flavus, and Cladosporium) *by the minimal inhibitory concentration (MIC) method. The DNA cleavage study was done by agarose gel electrophoresis technique.*

Keywords Synthesis; complex; macrocyclic; bistriazole; ortho-phthalaldehyde

1. INTRODUCTION

The interest in the study of macrocyclic complexes continues to expand because of their catalytic properties, which have led to industrial applications in addition to their involvement in many important biological processes such as photosynthesis and dioxygen transport.[1,2] The macrocyclic Schiff bases have been widely studied due to their selective chelation to certain metal ions depending on the number, type and position of their donor atoms, the ionic radius of metal ion and coordinating properties of counterions.[3–5] The peculiar chemical, structural, spectroscopic and magnetic

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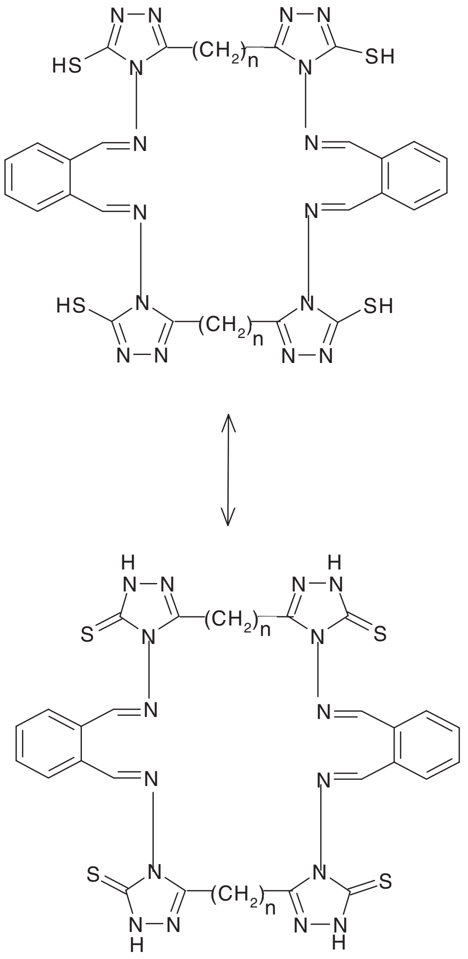
Address correspondence to Sangamesh A. Patil, P. G. Department of Chemistry, Karnatak University, Dharwad-580003, Karnataka, India. E-mail: patil1956@rediffmail.com

properties of Schiff base macrocycles and supramolecular structures involving trivalent lanthanide ions are useful for development of photonic light-converting devices and sensors,[6] contrast agents in magnetic resonance imaging,[7,8] potential radiopharmaceuticals,[9] sensitizers for photodynamic therapy and biomedical diagnostics.[10,11] The incorporation of metal centers into supramolecular system gives rise to novel electronic and/or magnetic properties as well as fascinating structural features. The importance of macrocyclic ligands and their metal complexes is obvious when seen in relationship to natural products such as metalloprotein, vitamin B_{12}, and chlorophyll.[12] A number of nitrogen donor macrocyclic derivatives have long been used in analytical, industrial, and medical applications.[13] Macrocyclic metal complexes are of great importance due to their resemblances to many natural systems such as porphyrins and cobalamines. Macrocyclic nickel complexes find use in DNA recognition and oxidation while the macrocyclic copper complexes find use in DNA binding and cleavage.[14,15] Several macrocyclic complexes with tetraaza macrocyclic ligand, such as cyclen, cyclam, or bicyclam were reported to exhibit antitumor activity.[16] The chemistry of macrocyclic complexes is also important due to their use as dyes and pigments as well as NMR shift reagents.[17] Macrocyclic complexes are also well known for their antibacterial and antifungal activities.[18,19] Herein, we report the synthesis, characterization, in vitro antimicrobial, and DNA-cleavage studies of metal complexes of macrocyclic ligands derived from *ortho*-phthalaldehyde and *bis*-(4-amino-5-mercapto-1, 2, 4-triazole-3-yl) alkanes (Figure 1).

2. EXPERIMENTAL

2.1. Physical Measurements

The infrared (IR) spectra of the complexes were recorded on HITACHI-270 IR spectrometer in the 4000–250 cm^{-1} region in KBr discs. The electronic spectra of the complexes were recorded in DMF on VARIAN CARY 50-BIO UV-spectrophotometer in the region of 200–1100 nm. The proton nuclear magnetic resonance (NMR) spectra were recorded in DMSO-d_6 on a BRUKER 300 MHz spectrometer at room temperature using tetra methyl silane (TMS) as an internal reference. Fast atom bombardment (FAB) mass spectra were recorded on a JEOL SX 102/DA-600 mass spectrometer/data system using argon/xenon (6KV, 10 Am) as the FAB gas. The accelerating voltage was 10 KV and the spectra were recorded at room temperature by using *m*-nitro benzyl alcohol as a matrix. The mass spectrometer was operated in the positive ion mode. The electron spin resonance (ESR) spectrum of the copper(II) (10) complex in polycrystalline state was recorded at 25°C using VARIAN E-4 X-band ESR spectrometer with cylindrical quartz sample tube



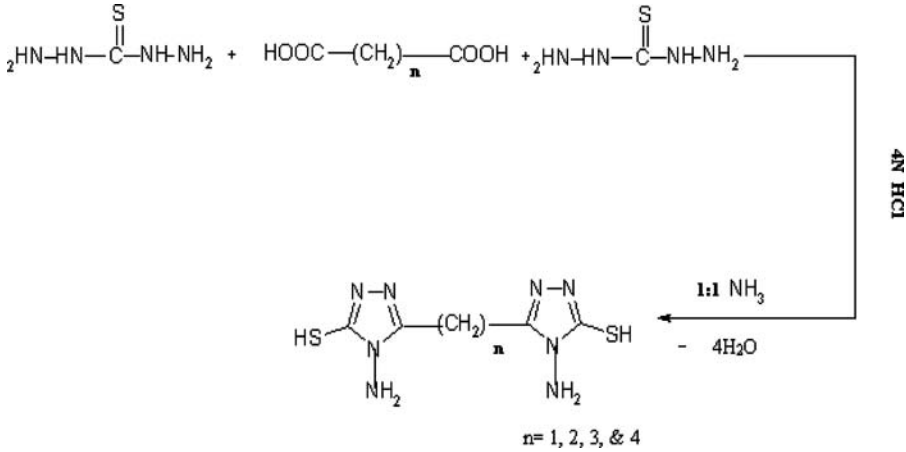
L^I : n=1, L^{II} : n=2, L^{III} : n=3, L^{IV} : n=4.

FIGURE 1 Structure of Schiff bases (L^I–L^{IV}) in thiol-thione tautomeric forms.

operating at microwave frequency ~ 9.1 GHz. Field calibration was checked using tetracynoethylene (TCNE) free radical for which g = 2.00277 at room temperature. Thermogravimetric analyses were measured from room temperature to 1000°C at heating rate of 10°C/min. The data were obtained by using a PERKIN-ELMER DIAMOND TG/DTG instrument. The electrochemistry of Cu(II) complexes were studied on CHI1110A-electrochemical analyzer (HCH Instruments, USA). Molar conductivity measurements were recorded on an ELICO-CM-82 T conductivity bridge with cell having cell constant 0.51 and magnetic moment was carried out on Faraday balance.

2.2. Synthesis

All the chemicals used for preparing *bis*-triazoles and their Schiff bases were of reagent grade (Aldrich Chemical Co., USA). The *bis*-(4-amino-5-mercapto-1,2,4-triazole-3-yl)alkanes prepared as reported[20] (Scheme 1). Purity of the samples were verified by elemental analyses(For compound $C_5H_8N_8S_2$, Found. C, 24.16%; H, 3.21%; N, 45.82%; S, 26.18%. Calcd. C, 24.59%; H, 3.27%; N, 45.90%; S, 26.22%. $C_6H_{10}N_8S_2$, Found. C, 27.73%; H, 3.79%; N, 43.36%; S, 24.78%. Calcd. C, 27.90%; H, 3.87%; N, 43.41%; S, 24.80%. $C_7H_{12}N_8S_2$, Found. C, 30.68%; H, 4.38%; N, 41.06%; S, 23.47%. Calcd. C, 30.88%; H, 4.41%; N, 41.17%; S, 23.52%. $C_8H_{14}N_8S_2$, Found. C, 33.48%; H, 4.74%; N, 39.12%; S, 22.34%. Calcd. C, 33.56%; H, 4.89%; N, 39.16%; S, 22.17%.). *Ortho*-phthalaldehyde was obtained from Aldrich.



SCHEME 1 Synthesis of *bis*-(4-amino-5-mercapto-1,2,4-triazol-3-yl)alkanes.

2.3. Synthesis of Macrocyclic Ligands (L^I–L^{IV})

Ortho-phthalaldehyde (2 mmol) in ethanol (25 mL) was added to an ethanolic solution of *bis*-(4-amino-5-mercapto-1,2,4-triazole-3-yl)alkanes (2 mmol, 25 mL) containing few drops of concentrated HCl. The reaction mixture was refluxed for 3 hours. The mixture was cooled to room temperature and the solvent removed under reduced pressure until solid formed that was washed with cold ethanol and dried under vacuum. M.P. (For compound L^I, 260°C; L^{II}, 262°C; L^{III}, 261°C; L^{IV}, 264°C), yield (60–65%), analytical data listed in Table 1.

2.4. Synthesis of Complexes (1–12)

A methanolic (25 mL) solution of metal salts (0.01 mol) was added to a magnetically stirred solution of *ortho*-phthalaldehyde (0.02 mol) and

TABLE 1 Analytical data of Schiff bases L^{I}–L^{IV}

Ligands	Empirical formula	C% Found.	C% calcd.	H% Found.	H% calcd.	N% Found.	N% calcd.	S% Found.	S% calcd.	%yield	m.p.°C
L^{I}	($C_{26}H_{20}N_{16}S_4$)	45.17	45.61	2.14	2.92	32.14	32.74	18.09	18.71	62	260
L^{II}	($C_{28}H_{24}N_{16}S_4$)	46.98	47.19	3.13	3.37	31.11	31.46	17.31	17.91	63	262
L^{III}	($C_{30}H_{28}N_{16}S_4$)	47.91	48.44	3.41	3.78	29.97	30.27	17.21	17.29	65	261
L^{IV}	($C_{32}H_{32}N_{16}S_4$)	49.54	50.00	3.98	4.16	28.94	29.16	16.43	16.66	60	264

bis-(4-amino-5-mercapto-1,2,4-triazole-3-yl)alkanes (0.02 mol) in methanol (50 mL) at room temperature. The reaction mixture was stirred for several hours, leading to precipitation of a solid product. The product was filtered off, washed with methanol, and dried in vacuum.

2.5. Analysis

The metal contents and sulfur were estimated gravimetrically by the standard method.[21] Carbon, hydrogen, and nitrogen were estimated using a C, H, N analyzer. Chlorides were determined by Volhard's method. The elemental analyses and molar conductance values are listed in Table 2.

3. RESULTS AND DISCUSSION

All the Co(II), Ni(II), and Cu(II) complexes are colored, stable in air, and nonhygroscopic solids. They are soluble in DMF and DMSO. The elemental analyses show that the Co(II), Ni(II), and Cu(II) complexes have 1:1 stoichiometry of the type [$MLCl_2$] ($M{=}Co^{2+}$, Ni^{2+}andCu^{2+}). The molar conductance values at the 10^{-3}M concentrations are too low to account for any dissociation of the complexes in DMF. Hence, the Co(II), Ni(II), and Cu(II) complexes may be regarded as nonelectrolytes.

3.1. Infrared Spectra

The Schiff bases (L^{I}–L^{IV}) have thiol and thione forms. A medium band at 3155 cm^{-1} due to ν(NH) indicates the thione form, while weak band centered at 2400 cm^{-1} due to ν(SH) is suggestive of thiole form. These observations confirm the thiole-thione tautomerism. A medium to high intensity band around 1630 cm^{-1} was assigned to ν(C=N) confirming the presence of *ortho*-phthalaldehyde. The NCSH and NHCS groups have thiamide-II vibrations, thiamide-III vibrations at 1045–1032 cm^{-1} and thiamide-IV vibrations (mainly from ν(C=S) located at 750 cm^{-1}. In addition to the above IR absorptions, medium intensity bands in the 1600–1575 cm^{-1} region are regarded

TABLE 2 Elemental analysis of Co(II), Ni(II), and Cu(II) complexes and their molar conductance data

Complex. No.	Empirical formula	Color/ Yield%	M.P. (°C)	M% Found. Calcd.	C% Found. Calcd.	H% Found. Calcd.	N% Found. Calcd.	S% Found. Calcd.	Molar conductance Ohm^{-1} cm^{2} $mole^{-1}$
1	$Co(C_{26}H_{20}N_{16}S_4)Cl_2$	Cream/68%	310	7.147.24	38.1838.332.37	2.4527.36	27.5215.64	15.72	19
2	$Co(C_{28}H_{24}N_{16}S_4)Cl_2$	Light pink/65%	305	6.917.00	39.9839.912.73	2.8526.28	26.6015.05	15.20	14
3	$Co(C_{30}H_{28}N_{16}S_4)Cl_2$	Pink/69%	315	6.586.7741.26	41.383.28	3.2125.68	25.7514.64	14.71	23
4	$Co(C_{32}H_{32}N_{16}S_4)Cl_2$	Violet/64%	325	6.626.5642.64	42.763.41	3.5624.82	24.9414.14	14.25	21
5	$Ni(C_{26}H_{20}N_{16}S_4)Cl_2$	green/69%	312	7.157.2138.42	38.342.36	2.4527.62	27.5315.64	15.73	17
6	$Ni(C_{28}H_{24}N_{16}S_4)Cl_2$	blue/60%	328	6.786.9739.86	39.922.66	2.8326.36	26.6115.14	15.20	11
7	$Ni(C_{30}H_{28}N_{16}S_4)Cl_2$	Darkgreen/66%	326	6.646.7541.24	41.393.14	3.2125.64	25.7514.58	14.71	13
8	$Ni(C_{32}H_{32}N_{16}S_4)Cl_2$	Light green/63%	330	6.486.5342.64	42.783.39	3.5624.83	24.9514.16	14.26	18
9	$Cu(C_{26}H_{20}N_{16}S_4)Cl_2$	Light green/62%	315	7.687.7638.01	38.122.32	2.4427.26	27.3615.47	15.63	16
10	$Cu(C_{28}H_{24}N_{16}S_4)Cl_2$	Blue/59%	328	7.397.5039.58	39.692.64	2.8326.35	26.4615.16	15.12	21
11	$Cu(C_{30}H_{28}N_{16}S_4)Cl_2$	brown/68%	342	7.147.2641.04	41.163.14	3.2025.41	25.6114.54	14.63	23
12	$Cu(C_{32}H_{32}N_{16}S_4)Cl_2$	Pale brown/64%	348	7.017.0442.46	42.553.41	3.5424.65	24.8214.12	14.18	26

as a combination of C=N of triazole ring and aromatic C=C stretching vibrations.

In the complexes the characteristic band due to ν(C=N) appeared around 1612–1605 cm^{-1} suggesting that the C=N group is coordinated to the metal through the nitrogen. The bands, due to C=N of the heterocyclic ring for the complexes, appeared almost in the same region as observed in the ligands.[22] All the complexes exhibited a broad medium intensity band in the 3150–3155 cm^{-1} region, and weak bands in the 2390–2400 cm^{-1} region are due to ν(NH) and ν(SH) vibrations. The band due to ν(C=S) at 750 cm^{-1} for ligands is unperturbed in the complexes indicating that sulfur does not coordinate. The presence of a medium-intensity band at 412–440 cm^{-1} region corresponding to the ν(M—N) vibration further confirms the formation of macrocyclic complex.[23] All the complexes show bands in the 1400–1460, 1080–1130, and 740–760 cm^{-1} regions assigned to phenyl ring vibrations.[24] The coordination of the chloro group has been ascertained by the band in the 280–310 cm^{-1} region, which may reasonably be assigned to ν(M—Cl).[25]

3.2. ^{1}H NMR Spectra

The ^{1}H-NMR spectra of all the Schiff bases exhibited signals at 13.6 and 13.4 ppm due to —NH protons. The resonance due to SH of all the compounds appeared at 3.5ppm indicating thiol-thione tautomerism. The azomethine protons in all the compounds appeared at 8.91ppm. Multiplets at 7.4–7.7 ppm are due to aromatic protons.

3.3. Electronic Absorption Spectra and Magnetic Studies

The electronic spectra of the cobalt(II) complexes showed two bands in the 13,350–14,000 and 20,500–22,500 cm^{-1} regions corresponding to $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$, and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ transitions, respectively.[26] However, the third band expected around 8500 cm^{-1} could not be properly resolved. The magnetic moment values of 4.52–4.64 BM further support the electronic spectra. The ligand field parameters Dq, B$'$, ν_2/ν_1, β, β%, and ligand field stabilization energy (LFSE) have been calculated and presented in Table 3.

The macrocyclic nickel(II) complexes showed two main bands in the regions 11,300–12,000 and 17,200–17,500 cm^{-1} assignable to $^3A_{2g} \rightarrow {}^3T_{1g}(F)$ and $^3A_{2g} \rightarrow {}^3T_{1g}(P)$ transitions, respectively, suggesting an octahedral geometry around the nickel(II) ion.[27] Further confirmation regarding the octahedral environment around the nickel(II) ion has been obtained from magnetic moment values of 3.14–3.19 BM.

The electronic spectra of the macrocyclic copper(II) complexes showed a broad band in the 16,200–16,350, and 20,100–20,450 cm^{-1} regions, which

TABLE 3 Ligand field parameters of Co(II) complexes (1–4) of the Schiff bases L^{I}–L^{IV}

Complex.	Transitions								
No.	ν_1	ν_2(Calcd.)	ν_3	Dq (cm^{-1})	B$^{|}$ (cm^{-1})	ν_2/ν_1	LFSE	β	β%
1	13350	27804	20500	1445.45	558.30	2.080	33.03	0.56	43.09
2	13520	28152	20700	1463.25	552.83	2.080	33.44	0.57	42.82
3	13720	28607	21400	1488.72	589.81	2.085	34.02	0.60	39.00
4	14000	29252	22500	1525.25	650.16	2.089	34.86	0.67	37.76

may be ascribed to $^2B_{1g} \rightarrow {}^2E_g$ and $^2B_{1g} \rightarrow {}^2B_{2g}$ transitions, respectively, corresponding to a distorted octahedral geometry around the copper(II) ion.[27] The magnetic moment values of 1.76 and 1.80 further support the observation of the electronic spectra.

3.4. Electron Spin Resonance Spectral Studies

The ESR spectrum of polycrystalline macrocyclic Cu(II) (10) complex studied here recorded at 25°C did not show any hyperfine splitting exhibiting only a single signal. The analyses of the spectrum gives $g\| = 2.171$, $g\perp = 2.031$, which supports the presence of an unpaired electron in the $d_{x2\text{-}y2}$ orbital. The macrocyclic Cu(II) (10) complex showed that $g\| < 2.3$ indicating that the present complex exhibited appreciable covalent nature.[28] The G values [$G = (g\|\text{-}2)/(g\perp\text{-}2)$] which measure-+ the exchange interaction between the copper centers in polycrystalline compounds have been calculated. According to Hathway,[28] if $G > 4$, it indicates a exchange interaction is negligible and if $G < 4$, it indicates a considerable exchange interaction in solid compounds.[28] The calculated G value is larger than four suggesting that there is no interaction between the copper centers.[29]

3.5. Thermogravimetric Analyses

Thermogravimetry (Tg) and derivative thermogravimetry (DTG) studies were carried out for some of the complexes. These complexes decompose gradually with the formation of respective metal oxide above 500°C shown in Figure 2. All the metal complexes showed only a single decomposition curve between 280°C and 310°C corresponding to the loss of organic moiety. Above 500°C, metal(II) complexes were decomposed leading to the formation of their respective metal oxides.

3.6. Fast Atom Bombardment Mass Spectral Studies of Schiff Base L^{I} and Its Co(II) (1) Complex

The FAB mass spectrum of L^{I} shows a molecular ion peak at *m/z* 684 equivalent to its molecular weight. The fragments in the spectrum lead to the

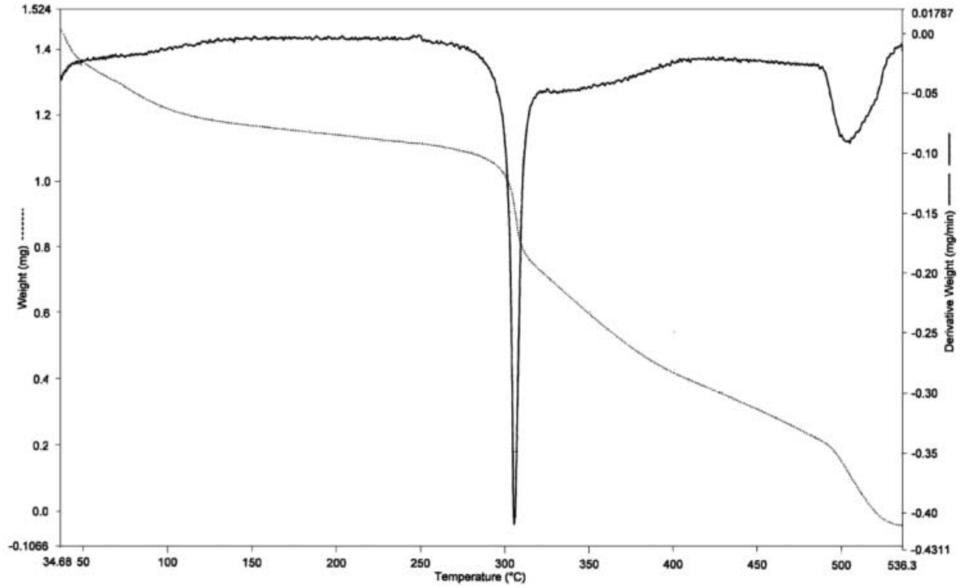


FIGURE 2 Thermogravimetric (TG/DTG) curves of Ni(II) (4) complex.

formation of the species $[C_{26}H_{20}N_{16}S_4]^+$. The FAB mass spectrum of Co(II) complex (1) showed molecular ion peaks $[M]^+$ at *m/z* 813, $815[M+2]^+$ and $817[M+4]^+$, which are equivalent to the molecular weight of the Co(II) complex (1) shown in Figure 3. Some other peaks appeared at m/z 552, 446, and 285 correspond to the $[Co(C_{21}H_{16}N_9S_2)Cl]^+$, $[Co(C_{13}H_{10}N_7S_2)Cl]^+$ and $[Co(C_7H_5N_2)Cl]^+$ species which resulted from the loss of $C_5H_4N_7S_2Cl$, C_8H_6N and C_6H_5N fragments from the parent compound. The two peaks appeared at m/z 743 and 778 are due to loss of two chlorine atoms and

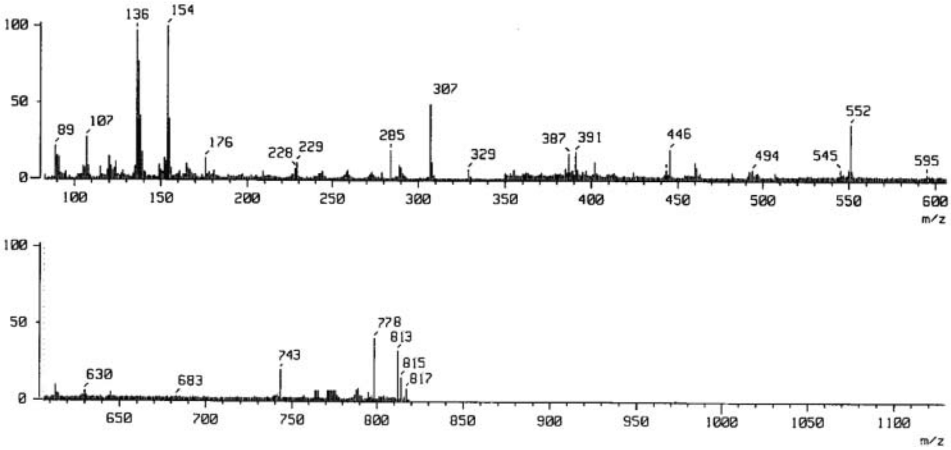


FIGURE 3 Fast atom bombardment mass spectrum of Co(II) complex (1).

one chlorine atom, respectively. All these fragmentation patterns are well observed in the FAB mass spectrum.

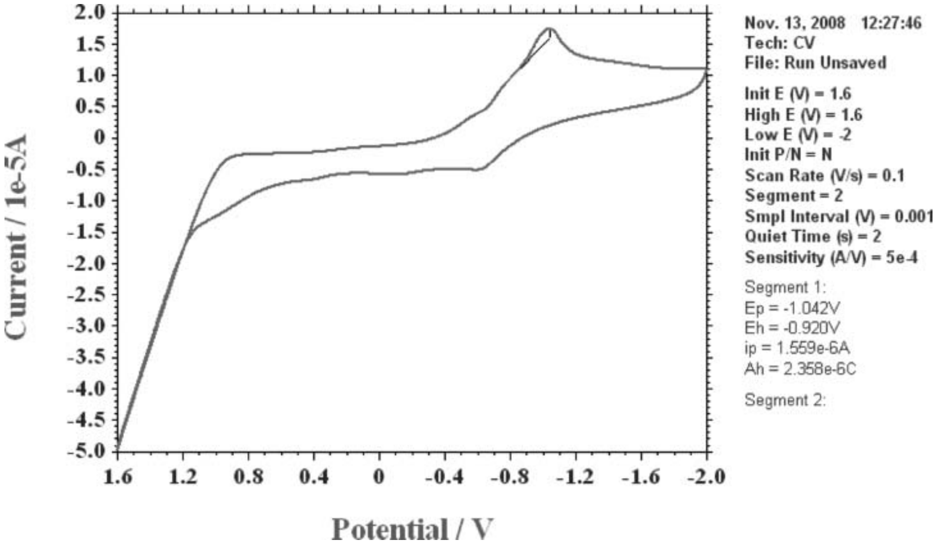
3.7. Electrochemical Studies

Electrochemical properties of the copper(II) complex was studied on a CHI1110A-Electrochemical analyzer in *N,N*-dimethyl formamide (DMF) containing 0.05 M n-Bu_4NClO_4 as the supporting electrolyte. A cyclic voltammogram of representative Cu(II) (**7**) complex shown in Figure 4 displays a reduction peak at $E_{pc} = 0.1078V$ with corresponding oxidation peak at $E_{pa} = 0.6291V$. The peak separation (ΔE_p) of this couple is 0.5213V at scan rate 0.1 V and increases with scan rate. The most significant feature of the Cu(II) complex is the Cu(II)/Cu(I) couple, which is a quasi-reversible one electron oxidation. The ratio of cathodic to anodic peak height was less than one; however, the peak current increases with increase of the square root of the scan rate, establishing a diffusion controlled electrode process.[30] The separation in peak potentials increases at higher scan rates consistent with quasi-reversibility of the Cu(II)/Cu(I) couple.

4. BIOLOGICAL ACTIVITIES

4.1. In Vitro Antibacterial and Antifungal Assay

The synthesized macrocyclic Schiff bases and their complexes were screened for their biological activities by using four bacteria, namely *E. coli, S. aureus, S. typhi*, and *P. aeruginosa*, and three fungi namely *A.niger, A. flavus,*



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Ah = 2.358e-6C

Segment 2:
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FIGURE 4 Cyclic voltammogram of Cu(II) complex (**7**).

and *Cladosporium* by the reported method.[31,32]All the uncoordinated metal salts exhibited negligible activities towards all bacteria and fungi species.[33] The DMF was used as solvent control.

The bacteria were subcultured in agar medium. The Petri dishes were incubated for 24 hours at 37°C. The standard antibacterial drug (Gentamycine) was also screened under similar conditions for comparison. The fungi were subcultured in potato dextrose agar medium. A standard antifungal drug (Fluconazole) was used for comparison. The Petri dishes were incubated for 48 hours at 37°C. The wells were dug in the agar media using sterile metallic borer. Activity was determined by measuring the diameter of the zone showing complete inhibition (mm). Growth inhibition was compared with standard drugs.

In order to clarify any effect of DMF on the biological screening, separate studies were carried out with solvent DMF only.

4.2. Minimum Inhibitory Concentration

Some compounds showing promising antibacterial/antifungal activities were selected for minimum inhibitory concentration (MIC) studies.

4.3. Antimicrobial Results

The microbial results are systematized in Tables 4 and 5. The antibacterial and antifungal studies suggested that all the Schiff bases were found to be biologically active and their metal(II) complexes showed significantly enhanced antibacterial and antifungal activities. It is, however, known that chelation tends to make the Schiff bases act as more powerful and potent bactereostatic agents, thus, inhibiting the growth of bacteria and fungi more than the parent Schiff bases.[34,35] It is suspected that factors such as solubility, conductivity, dipole moment, and cell permeability mechanism (influenced by the presence of metal ions) may be the possible reasons for the increase in activity. In the case of bacteriological studies, the results were compared with the standard drug (Gentamycin). It was observed that, some of the Schiff bases were found potentially active against all bacterial strains. Compound (I) shows high activity against all bacterial strains especially with *S. aureus* and *S. typhi* whereas metal(II) complexes (1–12) of these Schiff bases (I-IV) were also screened against the same bacterial strains. It was evident that overall potency of the uncoordinated compounds was enhanced on coordination with metal ions. In the case of antifungal activity, the results were compared with the standard drug Fluconazole. All Schiff bases showed high activity against fungal species. Compounds L^{I} and L^{IV} showed very high activity, an interesting feature is that the compound L^{IV} shows high activity against *Cladosporium*, However, the Co(II), Ni(II), and Cu(II) complexes (1–12) of these Schiff bases showed much enhanced activity as compared to the

TABLE 4 Antibacterial and antifungal results of Schiff bases (L^{I}–L^{IV})

		Antibacterial activity(Zone of inhibition in%)				Antifungal activity(Zone of inhibition in%)		
Ligands.	Conc. (μgmL^{-1})	*E. coli*	*S. aureus*	*S. typhi*	*P. aeruginosa*	*A. flavus*	*Cladosporium*	*A. niger*
L^{I}	100	50	78	88	67	64	70	68
	50	51	80	90	66	65	68	71
	30	53	81	91	65	64	69	69
L^{II}	100	51	57	58	61	65	71	74
	50	50	56	58	58	65	69	71
	30	50	56	59	59	68	68	73
L^{III}	100	64	59	50	68	69	80	68
	50	62	58	51	67	70	79	69
	30	60	59	52	67	71	79	70
L^{IV}	100	61	67	46	87	61	88	68
	50	58	68	48	88	63	89	69
	30	57	66	—	90	62	90	70
DMF	100	6	6	6	6	6	6	6
	50	6	6	6	6	6	6	6
	30	6	6	6	6	6	6	6
Standard	100	99	99	99	99	98	98	100
	50	100	99	100	100	100	98	99
	30	98	99	100	99	99	99	100

uncoordinated compounds. This enhancement in the activity may be rationalized on the basis of the presence of the C=N bond. It has been suggested that chelation/coordination reduces the polarity of the metal ion mainly because of partial sharing of its positive charge with a donor group within the whole chelate ring system.[36,37] This process of chelation thus increases the lipophilic nature of the central metal atom, which in turn, favors its permeation through the lipoid layer of the membrane thus causing the metal complex to cross the bacterial membrane more effectively so increasing the activity of the complexes. Besides this, many other factors such as solubility, dipole moment, conductivity influenced by metal ion may be the possible reasons for the remarkable antibacterial activities of these complexes.[38] It has also been observed that some moieties such as the azomethine linkage or heteroaromatic nucleus introduced into such compounds exhibit extensive biological activities that may be a result of the increase in hydrophobic character and liposolubility of the molecules in crossing the cell membrane of the microorganism and enhance the biological utilization ratio and activity of complexes.[39]

The minimum inhibitory concentration 10 μg/mL was shown by compound L^{I} against *S. aureus* and *S. typhi and* compound L^{IV} against *Cladosporium* and *P.aeruginosa* compound 9 shown MICs 10 μg/mL against *S. typhi and A. Flavus*. In all other cases, the compounds exhibited MICs ranging

TABLE 5 Antibacterial and antifungal results of Co(II), Ni(II), and Cu(II) complexes (1–12) and standard

Complex. No.	Conc. (μgmL^{-1})	Antibacterial activity (Zone of inhibition in%)				Antifungal activity(Zone of inhibition in%)		
		E. coli	*S. aureus*	*S. typhi*	*P. aeruginosa*	*A. flavus*	*Cladosporium*	*A. niger*
1	100	60	80	90	68	70	75	75
	50	61	81	91	69	69	78	79
	30	64	83	93	71	70	79	80
2	100	58	60	64	65	75	77	80
	50	59	63	63	66	76	80	81
	30	60	63	63	68	78	81	81
3	100	70	62	55	67	74	84	74
	50	68	63	54	69	75	82	73
	30	69	63	53	69	76	82	74
4	100	65	70	53	93	65	90	70
	50	66	71	52	95	66	92	73
	30	68	73	50	95	68	94	74
5	100	59	80	84	66	71	74	71
	50	58	78	83	65	72	74	70
	30	58	76	82	64	73	71	70
6	100	60	66	66	67	74	76	80
	50	63	69	66	68	72	80	80
	30	62	64	66	69	74	80	80
7	100	71	61	58	66	77	78	80
	50	69	63	59	65	76	79	81
	30	69	63	60	65	74	79	81
8	100	60	74	61	90	64	88	68
	50	61	75	64	88	66	90	68
	30	62	76	66	87	62	90	69
9	100	71	80	65	94	68	70	69
	50	71	81	66	94	69	69	69
	30	70	86	67	95	68	68	67
10	100	66	76	86	70	70	74	78
	50	68	77	85	71	71	72	77
	30	64	74	84	71	72	71	76
11	100	64	73	70	70	64	71	77
	50	66	74	71	72	63	72	76
	30	64	74	71	72	62	73	74
12	100	65	74	66	91	65	90	70
	50	64	75	65	90	66	88	71
	30	64	76	64	94	64	89	74
DMF	100	6	6	6	6	6	6	6
	50	6	6	6	6	6	6	6
	30	6	6	6	6	6	6	6
Standard	100	99	99	99	99	98	98	100
	50	100	99	100	100	100	98	99
	30	98	99	100	99	99	99	100

TABLE 6 Minimum inhibitory concentration (μgmL^{-1}) results for some compounds

	Antibacterial activity (Zone of inhibition in%)				Antifungal activity (Zone of inhibition in%)		
Compds.	*E. coli*	*S. aureus*	*S. typhi*	*P. aeruginosa*	*A. flavus*	*Cladosporium*	*A. niger*
L^{I}	20	10	15	25	20	15	15
L^{IV}	15	15	15	20	15	10	20
9	20	15	10	10	20	25	15
10	25	20	20	15	10	20	20
11	10	25	10	25	25	15	25
12	15	20	25	20	20	20	10

from 10 μg/mL to 100 μg/mL against all the microbial strains and some of them are given in Table 6.

5. DNA CLEAVAGE EXPERIMENT

5.1. Preparation of Culture Media

Nutrient broth (peptone, 10; yeast extract, 5; NaCl, 10 in (g/l)) was used for culturing of *E. coli*. The 50 mL media was prepared and autoclaved for 15minutes at 121°C under 15 pounds of pressure. The autoclaved media were inoculated with the seed culture and incubated at 37°C for 24 hours.

5.2. Isolation of DNA

The fresh bacterial culture (1.5 mL) was centrifuged to obtain the pellet, which was then dissolved in 0.5 mL of lysis buffer (100 mM tris pH 8.0, 50 mM EDTA, 10% SDS). To this 0.5 mL of saturated phenol was added and incubated at 55°C for 10 minutes. Then it was centrifuged at 10,000 rpm for 10 minutes. Then equal volume of chloroform : isoamyl alcohol (24:1) and 1/20th volume of 3M sodium acetate (pH 4.8) was added to this supernatant and centrifuged at 10,000 rpm for 10 minutes. To this supernatant 3 volumes of chilled absolute alcohol was added. The precipitated DNA was separated by centrifugation. Dry the pellet and dissolved in TE buffer (10 mM tris pH 8.0, 1 mM EDTA) and stored in cold condition.

5.3. Agarose Gel Electrophoresis

Cleavage products were analyzed by the agarose gel electrophoresis method. Test samples (1 mg/mL) were prepared in DMF. The samples (100 μg) were added to the isolated DNA of *E. coli*. The samples were incubated for 2 hours at 37°C and then 20 μL of DNA sample (mixed with bromophenol blue dye at 1:1 ratio) were loaded carefully into the electrophoresis chamber wells along with standard DNA marker containing TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/1 ltr) and finally loaded on agarose gel and pass the constant 50 V of electricity for around 30 minutes. Then the gel was removed and stained with 10.0 μg/mL ethidium bromide for 10–15 minutes. The bands were observed under UV transilluminator and photographed to determine the extent of DNA cleavage and the results were compared with a standard DNA marker.

5.4. Electrophoretic Analysis

Four Cu(II) (9–12) complexes were studied for their DNA cleavage activity by the agarose gel electrophoresis method (Figure 5). DNA cleavage reactions generally proceed via two major pathways: (1) oxidative cleavage of the sugar and/or nucleobase moiety and (2) hydrolytic pathway involving the phosphate group. Iron and copper complexes are known to be useful for oxidative cleavage of DNA involving nucleobase oxidation and/or degradation of sugar by abstraction of deoxyribose hydrogen atoms while complexes containing strong Lewis acids like copper(II) and zinc (II) are suitable for hydrolytic cleavage of DNA. Sigman et al. have reported a *bis*(phen) copper(I) complex as the first "copper based chemical nuclease" that cleaves the DNA in presence of H_2O_2 and thiol.[40] Similarly, the anticancer antibiotic bleomycins containing iron cleave DNA in an oxidative manner.[41,42]

The gel after the electrophoresis clearly revealed that all compounds have the cleavage activity. Complexes 1, 2, and 3 have acted on DNA as there was a molecular weight difference between the control and the treated DNA samples. The difference was observed in the bands (lanes 1, 2, and 3) compared to the control DNA of *E. coli*. Compound 4, however, showed such a difference along with a streak, indicating unspecific cleavage too.

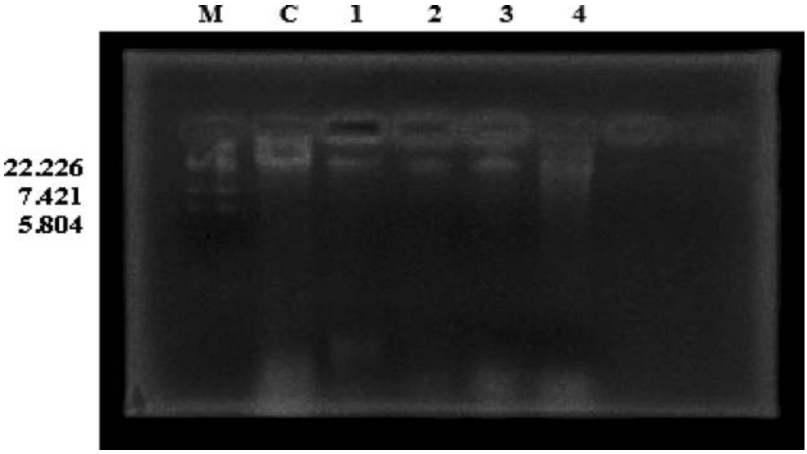

FIGURE 5 M: standard molecular weight marker; C-*E. coli*—control DNA of *E. coli*; lanes 1–4: *E. coli* DNA treated with Cu(II) complexes (9–12), respectively.

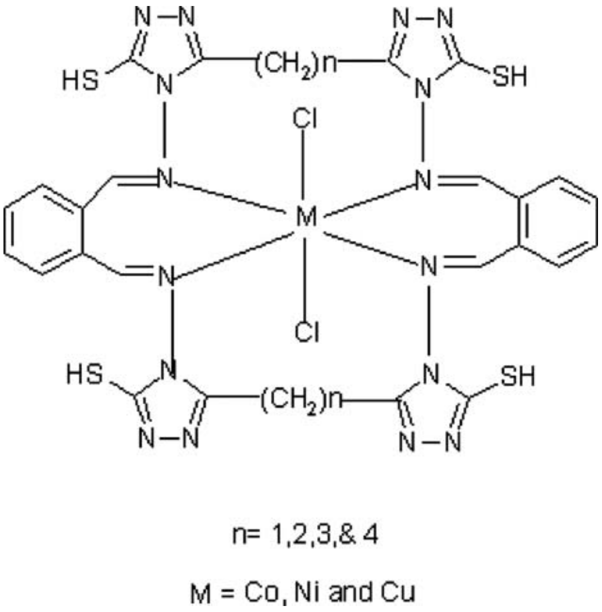

FIGURE 6 Proposed structure for metal(II) complexes.

This shows that, the control DNA alone does not show any apparent cleavage whereas Cu(II) complexes do show cleavage. However, the nature of reactive intermediates involved in the DNA cleavage by the complexes is not clear. The results indicated the important role of metal in these isolated DNA cleavage reactions. As the compound was observed to cleave the DNA, it can be concluded that, the compounds inhibit the growth of the pathogenic organism by cleaving the genome.

6. CONCLUSION

The synthesized Schiff bases act as tetradentate ligands through the coordination of four azomethine nitrogen atoms to the metal ion. The bonding of ligand to metal ion was confirmed by the analytical, IR, electronic, magnetic, ESR, FAB mass, thermal, and electrochemical studies. The biological results demonstrated that all the Schiff bases are biologically active and their metal(II) complexes have shown more promising activities than the Schiff bases. The interaction of copper(II) complexes with DNA was investigated by utilizing gel electrophoresis. It was found that all copper(II) complexes cleave DNA efficiently. All these observations put together lead us to propose the structure shown in Figure 6, in which the complex has the stoichiometry of the type [$MLCl_2$] (M=Co(II), Ni(II), and Cu(II)).

REFERENCES

1. Champness, N.R.; Frampton, C.S.; Reid, G.; Tocher, D.A. Mixed phospha-thia macrocyclic chemistry: synthesis and characterisation of [M(Ph_2[14]aneP_2S_2)]2+,M = Pd, Pt and [RhCl_2(Ph_2[14]aneP_2S_2)]+. single crystal structures of [Pt(Ph_2[14]aneP_2S_2)](PF_6)2.MeNO_2 and trans-[RhCl_2(Ph_2[14]aneP_2S_2)]PF_6. (Ph_2[14]aneP_2S_2 = 1,5-diphenyl-1,5-diphospha-8,12-dithiacyclotetradecane). *J. Chem. Soc., Dalton. Trans.* **1994**, 20, 3031–3038.
2. Fraustoda Silva, J.J.R.; Williams, R.J.P.; *The Biological Chemistry of the Elements*, Clarendon Press, Oxford, 1991.
3. Vallarino, L.M.J.; In: *Handbook on the Physics and Chemistry of Rare Earths*, Gschneidner Jr., K.A.; Eyring, L. (Eds.). Elsevier, Amsterdam, 1991, pp. 191–285.
4. Hernandez-Molina, R.; Mederos, A. In: *Comprehensive Coordination Chemistry II*, McCleverty, J.A.; Meyer, T.J. (Eds.). Elsevier, Amsterdam, 2004, pp. 12–28.
5. Gerbeleu, N.V.; Arion, V.B.; Burges, J. *Template Synthesis of Macrocyclic Compounds*, Wiely-VCH, Weinheim, 1999.
6. Parker, D.; Dickins, R.S.; Purschmann, H.; Crossland, C.; Howard, J.A.K. Being excited by lanthanide coordination complexes: aqua species, chirality, excited-state chemistry, and exchange dynamics. *Chem. Rev.* **2002**, 102, 1977–2010.
7. Toth, E.L.; Helm, A.E.; Merbach. In: *Comprehensive Coordination Chemistry II*, McCleverty, J.A., Meyer, T.J. (Eds.). Elsevier, Amsterdam, 2004, pp. 35–49.
8. Woods, M.; Kovacs, Z.; Zhang, S.; Sherry, A.D. Towards the rational design of magnetic resonance imaging contrast agents: isolation of the two coordination isomers of lanthanide DOTA-type complexes. *Angew. Chem. Int. Ed.* **2003**, 42, 5889–5892.
9. Keire, D.A.; Jang, Y.H.; Li, L.; Dasgupta, S.; Goddard III, W.A.; Shively, J.E. Chelators for radioimmunotherapy: I. NMR and ab initio calculation studies on 1,4,7,10-tetra(carboxyethyl)-1,4,7,10-tetraazacyclododecane (DO4Pr) and 1,4,7-Tris(carboxymethyl)-10-(carboxyethyl)-1,4,7,10-tetraazacyclododecane (DO3A1Pr). *Inorg. Chem.* **2001**, 40, 4310–4318.
10. Williams, L.A.D.; Howell, R.C.; Young, R.; Kahwa, I.A. Neuropharmological actions of some binuclear lanthanide(III) complexes. *Comp. Biochem. Physiol. C* **2001**, 128, 119–125.
11. Woods, M.; Kovacs, Z.; Sherry, A.D. Targeted complexes of lanthanide(III) ions as Therapeutic and diagnostic pharmaceuticals. *J. Supramolecular Chem.* **2002**, 2, 1–15.
12. Keypour, H.; Khanmohammadi, H.; Wainwright, K.P.; Taylor, M.R. Syntheis, crystal structure, NMR and ab initio molecular-orbital studies of some magnesium(II) macrocyclic Schiff-base complexes, with two 2-aminoethyl pendant arms. *Inorg. Chim. Acta.* **2004**, 357, 1283–1291.
13. Ma, W.; Tian, Y.; Zhang, S.; Wu, J.; Fun, H.; Chantrapromma, S. Synthesis and characterization of 1,8-bis(ferrocenylmethyl)-5,5,7,12,12,14-hexamethyl-1,4,8,11-tetraazacyclote tradecane, a macrocyclic ligand and its complexes. *Transition Met. Chem.* **2006**, 31, 97–102.
14. Muller, J.G.; Chen, X.; Dadiz, A.C.; Rokita, S.E.; Burrows, C.J. Macrocyclic nickel complexes in DNA recognition and oxidation. *Pure. Appl. Chem.* **1993**, 65, 545–550.
15. Liu, J.; Lu, T.B.; Deng, H.; Ji, L.N.; Qu, L.H.; Zhou, H. Synthesis, DNA-binding and cleavage studies of macrocyclic copper(II) complexes. *Transition Met. Chem.* **2003**, 28, 116–121.
16. Kong, D.; Xie, Y. Synthesis, structural characterization of tetraazamacrocyclic ligand, five coordinated zinc(II)complex. *Inorg. Chim. Acta.* **2002**, 338, 142–147.
17. Seto, J.; Tamura, S.; Asai, N.; Kishii, N.; Kijima, Y.; Matsuzawa, N. Macrocyclic functional dyes: applications to optical disk media, photochemical hole burning and nonlinear optics. *Pure. Appl. Chem.* **1996**, 68, 1429–1434.
18. Chaudhary, A.; Bansal, N.; Gajraj, A.; Singh, R.V. Antifertility, antibacterial, antifungal and percent disease incidence aspects of macrocyclic complexes of manganese(II). *J. Inorg. Biochem.* **2003**, 96, 393–400.
19. Singh, R.V.; Chaudhary, A. Biologically relevant tetraaza macrocyclic complexes of manganese: Synthetic, spectral, antimicrobial, antifertility and anti-inflammatory approach. *J. Inorg. Biochem.* **2004**, 98, 1712–1721.
20. Avagi, P.G.; Patil, S.A. Synthesis, spectral, thermal, solid state d.c. electrical conductivity, fluorescence and biological studies of lanthanum(III) and thorium(IV) complexes of 24-membered macrocyclic triazoles. *J. Coord. Chem.*, **2008**, 61, 2570–2583.
21. Vogel, AIA. *A Text Book of Quantitative Inorganic Analyses*, 3rd ed., ELBS Longman's Green and Co. Ltd., London, 1962.

22. Avagi, P.G.; Patil, S.A. Synthesis, spectral characterization and microbiological studies of Co(II), Ni(II) and Cu(II) complexes with some novel 20-membered macrocyclic hydrazino-1,2,4-triazole Schiff bases. *J. Enz. Inhib. Med. Chem.* **2009**, 24, 140–150.
23. Patil, S.A.; Kamble, U.V.; Badami, P.S. Template synthesis, characterization, in vitro antimicrobial, and DNA cleavage studies of Co(II), Ni(II), Cu(II), and Zn(II) complexes with 15-membered N_2O_2 diazadioxa macrocycles. *Main Group Chem.* **2009**, 8, 189–206.
24. Shakir, M.; Kumar, O.; Varkey, S.P. 3-mercapto-1,2-propanediol and its 1-methoxy and 1-ethoxy derivatives, $HSCH_2CH(OH)CH_2OR'$ ($R' = H, CH_3, C_2H_5$), as uninegative bidentate bridging ligands: Synthesis and characterization of dimeric complexes with cobalt, copper and silver. *Polyhedron* **1992**, 11, 2831–2836.
25. Rana, V.B.; Singh, P.; Singh, D.P.; Teotia, M.P. Trivalent chromium, manganese, iron and cobalt chelates of a tetradentate N6 macrocyclic ligand. *Transition Met. Chem.* **1982**, 7, 174–177.
26. Shakir, M.; Varkey, S.P.; Firdaus, F.; Hameed, P.S. Template synthesis of iron(II), cobalt(II), copper(II) and zinc(II) complexes of diamidediimine macrocycles. *Polyhedron* **1994**, 15, 2319–2325.
27. Lever, A.B.P.; *Inorganic Electronic Spectroscopy*. Elsevier, Amsterdam, 1984.
28. Chandra, S.; Gupta, S. Copper(II) complexes with twelve to fourteenmembered ring macrocyclic ligands. *J. Ind. Chem. Soc.*, **2005**, 82, 1087–1090.
29. Shakir, M.; Chishti, H.T.N.; Chingasubam, P. Metal ion-directed synthesis of 16-membered tetraazamacrocyclic complexes and their physico-chemical studies. *Spectrochim Acta* **2006**, 64(A), 512–517.
30. Bard, A.-J.; Izatt, L.-R. *Electrochemical Methods Fundamentals and Applications*, 2nd ed., Wiley, New York, 2001.
31. Sadana, A.K.; Miraza, Y.; Aneja, K.R.; Prakash, O. Hypervalent iodine mediated synthesis of 1-aryl/hetryl-1,2,4-triazolo[4,3-*a*] pyridines and 1- aryl/hetryl 5-methyl-1,2,4-triazolo[4,3-*a*]quinolines as antibacterial agents. *Eur. J. Med. Chem.* **2003**, 38, 533–536.
32. Greenwood, D.; Snack, R.; Peurtherer, J. *Medical Microbiology: A Guide to Microbial Infections: Pathogensis, Immunity, Laboratory Diagnosis and Control*, 15th ed., Churchill Livingstone, Edinburgh, UK, 1997.
33. Efthimiadou, E.K.; Thomadaki, H.; Sanakis, Y.; Raptopoulou, C.P.; Katsaros, N.; Scorilas, A.; Karaliota, A.; Psomas, G. Structure and biological properties of the copper(II) complex with the quinolone antibacterial drug *N*-propyl-norfloxacin and 2,2′-bipyridine. *J. Inrg. Biochem.*, **2007**, 101, 64–73.
34. Chohan, Z.H.; Supuran, C.T.; Scozzafava, A. Metalloantibiotics: Synthesis and antibacterial activity of cobalt(II), copper(II), nickel(II) and zinc(II) complexes of kefzol. *J. Enz. Inhib. Med. Chem.* **2004**, 19, 79–84.
35. Chohan, Z.H.; Praveen, M. Synthesis, characterization, coordination and antibacterial properties of novel asymmetric 1,1′-disubstituted ferrocene-derived Schiff-base ligands and their Co(II), Cu(II) Ni(II) and Zn(II) complexes. *Appl. Organomet. Chem.* **2001**, 15, 617–625.
36. Chohan, Z.H.; Pervez, H.; Rauf, A.; Khan, K.M.; Supuran, C.T. Isatin-derived antibacterial and antifungal compounds and their transition metal complexes. *J. Enz. Inhib. Med. Chem.* **2004**, 19, 417–423.
37. Chohan, Z.H.; Supuran, C.T.; Scozzafava, A. Metal binding and antibacterial activity of ciprofloxacin complexes. *J. Enz. Inhib. Med. Chem.* **2005**, 20, 303–305.
38. Chohan, Z.H.; Scozzafava, A.; Supuran, C.T. Unsymmetrical 1,1′-disubstituted ferrocenes: synthesis of Co(II), Cu(II), Ni(II) and Zn(II) chelates of ferrocenyl-1-thiadiazolo-1′-tetrazole, -1-thiadiazolo-1′-triazole and -1-tetrazolo-1′-triazole with antimicrobial properties. *J. Enz. Inhib. Med. Chem.* **2002**, 17, 261–266.
39. Chohan, Z.H.; Hassan, M.U.; Khan, K.M.; Supuran, C.T. In-vitro antibacterial, antifungal and cytotoxic properties of sulfonamide-derived Schiff's bases and their metal complexes. *J. Enz. Inhib. Med. Chem.* **2005**, 20, 183–188.
40. Sigman, D.S.; Graham, D.R.; Aurora, V.; Stern, A.M. Oxygen-dependent cleavage of DNA by the 1,10-phenanthroline.cuprous complex. Inhibition of escherichia coli DNA polymerase. *J. Biol. Chem.*, **1979**, 254, 122–169.
41. Umezawa, H. Structure and action of bleomycin. *Prog. Biochem. Pharmacol.*, **1976**, 11, 18–27.
42. Burger, R.M. Cleavage of nucleic acids by bleomycin. *Chem. Rev.*, **1998**, 98, 1153–1169.