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Antimicrobial and DNA Cleavage Studies of New N_2O_2 Diazadioxa Macrocyclic Schiff Base Co(II), Ni(II) and Cu(II) Complexes Containing Triazole Head Unit: Synthesis and Spectroscopic Approach

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A novel series of N_2O_2 diazadioxa macrocyclic complexes [$MLCl_2$] (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 1, 6-*bis*(2-formylphenyl)hexane and 3-subtituted-4-amino-5-hydrazino-1, 2, 4-triazole. 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 the complexes are soluble in DMF and DMSO and are non-electrolytes. 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 MIC method. The DNA cleavage study was done by Agarose gel electrophoresis.

Keywords: Synthesis, complex, macrocyclic, triazole

1 Introduction

The coordination chemistry of macrocyclic ligands is a fascinating area of study for inorganic chemists (1). Schiff base macrocycles have been of great importance in macrocyclic chemistry (2). There is a continued interest in synthesizing macrocyclic complexes (3–5) because of their potential applications in fundamental and applied sciences (6) and importance in the area of coordination chemistry (7). The development of the field of bioinorganic chemistry has been another important factor in spurring the growth in interest in macrocyclic compounds (8). 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. 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 (9–11). The peculiar chemical, structural, spectroscopic and magnetic properties of Schiff base macrocyles and supramolecular structures involving trivalent lanthanide ions are useful for development of photonic light-converting devices and sensors (12, 13), contrast agents in magnetic resonance imaging (14), potential radiopharmaceuticals (15), sensitizers for photodynamic therapy and biomedical diagnostics (16, 17). The incorporation of metal centers into a supramolecular system gives rise to novel electronic and/or magnetic properties, as well as fascinating structural features. The importance of macrocyclic ligands and their complexes is obvious when seen in relationship to natural products such as metalloprotien, vitamin B_{12} and chlorophyll (18). A number of nitrogen donor macrocyclic derivatives have long been used in analytical, industrial and medical applications (19). Macrocyclic metal complexes are of great importance due to their resemblances to many natural systems such as porphyrins and cobalamines (20). Macrocyclic nickel

Abbreviations: BM-Bohr Magneton, EPR-Electron paramagnetic resonance, FAB-Fast atom bombardment, TG/DTG-Thermogravimetric/Differential thermogravimetric analysis, DMF-N, N dimethylformamide, DMSO-Dimethylsulphoxide, IR-Infra red, MIC-Minimum Inhibitory Concentration, NMR-Nuclear Magnetic Resonance.

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complexes find use in DNA recognition and oxidation while the macrocyclic copper complexes find use in DNA binding and cleavage (21, 22). Several macrocyclic complexes with tetraaza macrocyclic ligand, such as cyclen, cyclam or bicyclam were reported to exhibit antitumour activity (23). The chemistry of macrocyclic complexes is also important due to their use as dyes and pigments, as well as NMR shift reagents (24, 25). Herein, we report the synthesis, characterization, *in vitro* antimicrobial, DNA cleavage studies of metal complexes of macrocyclic ligands derived from 1,6-*bis*(2-formylphenyl)hexane and 3-subtituted-4-amino-5-hydrazino-1,2,4-triazole.

2 Experimental

2.1 Physical Measurements

The IR spectra of the complexes were recorded on HITACHI-270 IR spectrometer in the 4000-350 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 NMR spectra were recorded in DMSO-d_6 on a BRUKER 300 MHz spectrometer at room temperature using TMS as an internal reference. 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 EPR spectrum of the copper(II) complex in polycrystalline state were recorded at 25°C using VARIAN E-4 X-band EPR spectrometer with cylindrical quartz sample tube 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 a 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 (HCH Instruments) analyzer (USA). Molar conductivity measurements were recorded on a ELICO-CM-82 T conductivity bridge with a cell having cell constant 0.51 and a magnetic moment was carried out on Faraday balance.

2.2 Synthesis

All the chemicals used for preparing triazoles and their Schiff bases were of reagent grade. The 3-substituted-4-amino-5-mercapto-1,2,4-triazoles were prepared as reported (26, 27). The 1,6-*bis*(2-formylphenyl)hexane was prepared in the same way as in the literature (1,2).

2.3 Synthesis of 3-Subtituted-4-amino-5-hydrazino-1,2, 4-triazole

A mixture of 3-substituted-4-amino-5-mercapto-1,2,4-triazole and N_2H_4. H_2O in 1:1 molar proportions in EtOH was boiled under reflux for 4–5 h on a water bath. The reaction mixture was cooled at room temperature; within an hour the compound separated from the clear solution. It was filtered, washed and recrystallized from EtOH.

2.4 Synthesis of 1, 6-*bis*(2-formylphenyl)hexane

To a stirred solution of salicylaldehyde (24.4 g, 0.2 mol) and K_2CO_3 (13.8 g, 0.1 mol) in DMF (100 mL) was added dropwise 1,6-dibromohexane (23.6 g, 0.1 mol) in DMF (100 mL). The reaction was continued for 4 h at 160–165°C and then 4 h at room temperature. After the addition was completed, 200 mL distilled water was added and it was put in a refrigerator; 1 h later, the precipitate was filtered and washed with 500 mL water, dried in air, recrystallized from ethanol and filtered in vacuum. Yield: 21.7 g (77%), Melting Point: 110–109°C, Color: Bright brown. (Scheme 1).

2.5 Synthesis of Schiff Bases (L^I-L^{IV})

A mixture of 1,6 *bis*(2-formylphenyl)hexane and 3-subtituted-4-amino-5-hydrazino-1,2,4-triazole in 1:1 molar proportion in methanol containing few drops of concentrated HCl was refluxed for 3–4 h. 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. The synthesized Schiff bases are shown in Figure 1. M.P. 256–268°C, yield (55–60%), analytical data listed in Table 1.

2.6 Synthesis of Complexes (1–14)

Co(II), Ni(II) and Cu(II) complexes(1-14)of the ligands (L^I-L^{IV}) were prepared by adapting template method

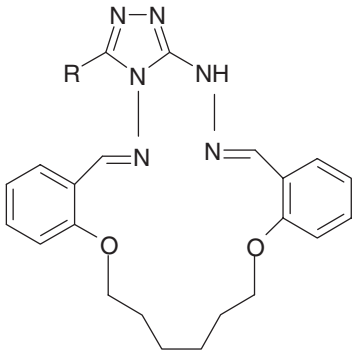
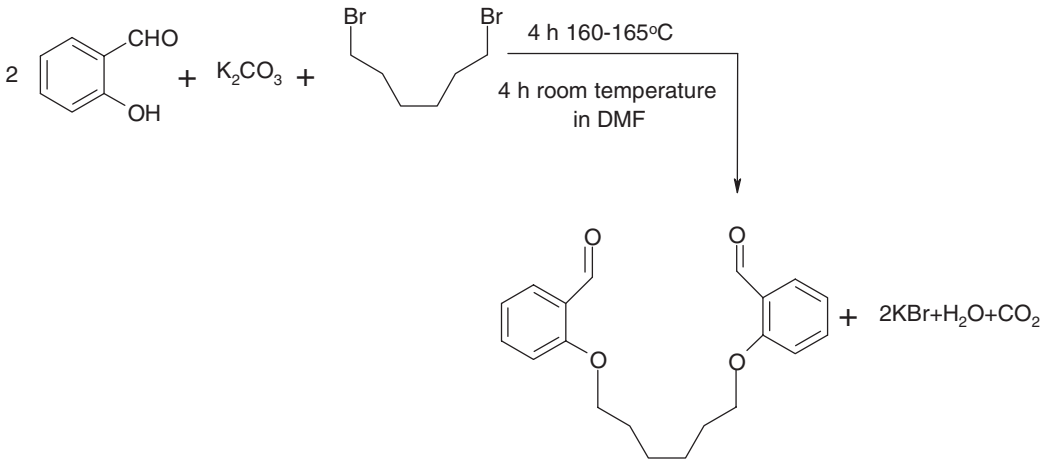


Fig. 1. Synthesized macrocyclic Schiff bases.



Sch. 1. Synthesis of 1, 6-*bis*(2-formylphenyl)hexane.

owing to the insolubility of the ligands in common organic solvents. To a stirred solution of 1,6 *bis*(2-formylphenyl)hexane (0.01 mol) and respective metal chloride (0.01 mol) in methanol (60 mL) was added dropwise 3-subtituted-4-amino-5-hydrazino-1,2,4-triazole (0.01 mol) in methanol (40 mL). After the addition was completed, the mixture was refluxed for 5–6 h. The separated complexes were collected by filtration, washed with hot methanol and dried under vacuum over $CaCl_2$. M.P. above 300°C, yield (50–58%), analytical data listed in Table 2.

2.7 Analysis

The metal contents were estimated gravimetrically by the standard method. Carbon, hydrogen, nitrogen were estimated using a C, H, N analyzer. Chlorides were determined by Volhard's method.

3 Results and Discussion

All the complexes are polycrystalline solids and the analytical data (Table 2) agrees well with the proposed mononuclear macrocyclic framework. The low conductivity values confirm the non-electrolytic nature of all the complexes. An interesting feature of the structures of the complexes under study is that the geometry about the oxygen atoms in the N_2O_2-donor complexes is almost identical with those about the corresponding nitrogen atoms in the N_4-donor complexes.

3.1 IR Spectra

The IR spectra of the ligands showed a medium intensity band at 3250 cm^{-1} assigned to ν NH vibrations (28), which has been observed in the 3250–3245 cm^{-1}region in the case of complexes. It can be observed that, there is no considerable shift in the ν NH vibrations in the case of complexes compared to the ligands, indicates non-involvement of NH group in the coordination. A medium of high intensity band at 1680 cm^{-1}in the case of ligands is assigned to ν C=N. The band due to ν C=N observed at 1680 cm^{-1} in the case of ligands has shown negative shift of 45-40 cm^{-1} in the complexes which suggests the involvement of azomethine group (C=N) in the coordination to metal ions and bonded through the nitrogen atoms (29). Spectra of all the complexes are dominated by bands between 2956–2854 cm^{-1} due to ν (Alph.–CH) groups. Conclusive evidence of the bonding is also shown by the observation that new bands in the IR spectra of the metal complexes appeared at 542–504 cm^{-1} and 486–470 cm^{-1} assigned to ν (M–O) and ν (M–N) stretching vibrations. All the complexes showed bands in the 1454–1398, 1125–1070 and 750–745 cm^{-1} regions assigned to phenyl ring vibrations.

The two moderately strong bands appearing at 890 and 720 cm^{-1} can be attributed respectively to ν(N-N) of the

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

		C%		*H%*		*N%*			
Ligands	*Empirical Formula*	*Found*	*Calcd.*	*Found*	*Calcd.*	*Found*	*Calcd.*	*yield (%)*	*M.P. °C*
L^{I}	$(C_{20}H_{20}N_6O_2)$	63.74	63.84	5.14	5.31	22.28	22.34	60	256
L^{II}	$(C_{21}H_{22}N_6O_2)$	63.22	64.61	5.66	5.64	21.42	21.53	58	262
L^{III}	$(C_{22}H_{24}N_6O_2)$	65.09	65.34	5.67	5.94	20.67	20.79	55	264
L^{IV}	$(C_{23}H_{26}N_6O_2)$	65.91	66.02	6.12	6.22	19.98	20.09	59	268

Table 2. Elemental analysis of C(II), Ni(II) and Cu(II) complexes and their molar conductance data

Complex No.	Empirical formula	Color/ Yield (%)	M.P. (°C)	M%		C%		H%		N%		μ_{eff} (BM)	Molar conductance $Ohm^{-1}cm^2mole^{-1}$
				Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.		
1	$Co(C_{20}H_{20}N_6O_2)Cl_2$	Pink/58%	320	11.52	11.63	47.51	47.44	3.99	3.95	16.46	16.60	4.70	10
2	$Co(C_{21}H_{22}N_6O_2)Cl_2$	Light pink/55%	325	11.22	11.33	47.99	48.47	4.01	4.23	16.11	16.15	4.84	16
3	$Co(C_{22}H_{24}N_6O_2)Cl_2$	Pink/57%	325	10.94	11.03	48.68	49.45	4.37	4.49	15.64	15.73	4.90	21
4	$Co(C_{23}H_{26}N_6O_2)Cl_2$	Violet/54%	315	10.68	10.75	50.12	50.38	4.67	4.74	15.13	15.33	4.94	19
5	$Ni(C_{20}H_{20}N_6O_2)Cl_2$	Light blue/58%	322	11.52	11.60	47.21	47.46	3.88	3.95	16.48	16.66	2.70	15
6	$Ni(C_{21}H_{22}N_6O_2)Cl_2$	blue/50%	328	11.18	11.29	48.17	48.49	4.09	4.23	16.11	16.16	3.23	14
7	$Ni(C_{22}H_{24}N_6O_2)Cl_2$	Darkgreen/56%	326	10.91	10.99	49.39	49.47	4.23	4.49	15.59	15.74	3.34	11
8	$Ni(C_{23}H_{26}N_6O_2)Cl_2$	Light green/53%	330	10.64	10.71	50.23	50.40	4.63	4.74	15.21	15.33	3.40	18
9	$Cu(C_{20}H_{20}N_6O_2)Cl_2$	Light green/52%	335	12.30	12.44	46.94	47.01	3.83	3.91	16.31	16.45	1.78	17
10	$Cu(C_{21}H_{22}N_6O_2)Cl_2$	Blue/58%	338	12.09	12.11	48.01	48.05	4.13	4.19	15.91	16.01	1.83	22
11	$Cu(C_{22}H_{24}N_6O_2)Cl_2$	brown/58%	322	11.76	11.80	48.91	49.03	4.39	4.45	15.28	15.60	1.86	21
12	$Cu(C_{23}H_{26}N_6O_2)Cl_2$	Pale brown/54%	328	11.42	11.50	49.81	49.96	4.59	4.70	15.19	15.20	1.89	25

hydrazone residue and inplane deformation of the triazole ring (30).

3.2 ^{1}H-NMR Spectra

The ^{1}H-NMR spectra of the Schiff bases were recorded in DMSO-d_6 solution. The spectra of the Schiff bases showed two singlets in the range of 8.16-8.24 ppm, assignable to the two imine protons (–CH=N, 2H) (31). The spectra exhibited a multiplet in the range approximately 6.90-7.80 ppm corresponding to aromatic protons(8H), 1.55 ppm due to $CH_2\underline{CH_2}CH_2$ protons, 1.85 ppm due to $\underline{CH_2}CH_2O$ protons, 4.20 ppm due to $CH_2\underline{CH_2}O$ protons (29). The ^{1}H-NMR spectra of the all the ligands exhibited signals in the range 13.2-13.4 ppm, because of NH protons these protons are D_2O exchangeable and confirm the assignment. All the above results, along with the absence of any signal corresponding to free amine or alcoholic protons, strongly suggest that the proposed macrocyclic frameworks have been formed.

3.3 Electronic Spectra

3.3.1. *Cobalt complexes*

The electronic spectra of the cobalt complexes showed two bands in the 13,320–14,020 and 20,550–22,700 cm^{-1} regions corresponding to $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ transitions, respectively (32). However, the third band expected around 8500 cm^{-1} could not be properly resolved. The magnetic moment values of 4.70–4.94 BM further support the electronic spectra. The ligand field parameters Dq, B/, $\nu_2/\ \nu_1$, β, β% and LFSE (Ligand field stabilization energy) have been calculated and presented in Table 3.

3.3.2. *Nickel complexes*

The electronic spectra of Ni(II) complexes exhibited a well discernable band with a shoulder on the low energy side. The other two bands generally observed in the region at ca. 16,650–17,050 cm^{-1}(ν_2), and 27,740–28210 cm^{-1} (ν_3), are assigned to $^3A_{2g} \rightarrow {}^3T_{1g}(F)$ (ν_2) and $^3A_{2g} \rightarrow {}^3T_{1g}(P)$ (ν_3) transitions, respectively. The first two bands result from the splitting of one band, ν_1 and are in the range ca. 9720–10,400 and 11,815–12,330 cm^{-1}, which can be assigned to $^3B_{1g} \rightarrow {}^3E_g$ and $^3B_{1g} \rightarrow {}^3B_2g$, transitions, assuming the effective symmetry to be D_{4h} (component of $^3T_{2g}$ in Oh symmetry) (33). The intense higher energy band at ca. 34,520 cm^{-1} may be due to a $\pi - \pi^*$ transition of the (C=N) group. Various bands do not follow any regular pattern and seem to be anion independent. The spectra are consistent with distorted octahedral nature of these complexes.

3.3.3. *Copper complexes*

The electronic spectra of the copper(II) complexes exhibited bands in the region ca. 17,810–19,630 cm^{-1} with a shoulder on the low energy side at ca. 14,620–16,040 cm^{-1} and show that these complexes are distorted octahedral (32). Assuming tetragonal distortion in the molecule, the d-orbital energy level sequence for these complexes may

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

Complex No.	Transitions			Dq (cm^{-1})	B$^{|}$ (cm^{-1})	ν_2/ν_1	LFSE	β	β%
	ν_1	ν_2(Calcd.)	ν_3						
1	13320	27751	20550	1443.14	556.09	2.083	32.98	0.575	42.49
2	13350	27920	21750	1457.04	641.36	2.091	33.30	0.663	33.67
3	13395	28076	22550	1468.11	696.07	2.085	33.55	0.719	28.01
4	14020	29309	22700	1528.92	663.28	2.090	34.94	0.685	31.40

be: x^2–$y^2 > z^2 > xy > xz > yz$ and the shoulder can be assigned to: $z^2 \rightarrow x^2 - y^2$ ($^2B_{1g} \rightarrow {}^2B_{2g}$) and the broad band contains both the $xy \rightarrow x^2 - y^2$($^2B_{1g} \rightarrow {}^2E_g$) and xz, yz $\rightarrow x^2 - y^2$ ($^2B_{1g} \rightarrow {}^2A_{2g}$) transitions (33). The band separation of the spectra of the complexes is of the order of 2500 cm^{-1}, which is consistent with proposed geometry of the complexes (33).Therefore, it may be concluded that, all the complexes formed by macrocycles with Cu (II) metals are distorted octahedral.

3.4 Magnetic Studies

The magnetic moments obtained at room temperature are listed in Table 2. The magnetic measurements for Co(II) and Ni(II) complexes showed magnetic moment values of 4.70–4.94 BM and 2.7–3.4 BM respectively, suggesting the consistency with their octahedral environment (34). The Cu(II) complexes show magnetic moments of 1.78–1.89 BM is slightly higher than the spin-only value 1.73 BM which is expected for one unpaired electron and it offers possibility of an octahedral geometry (35).

3.5 EPR Spectrum of Copper (II) Complex (9)

The EPR spectrum of polycrystalline macrocyclic Cu(II) (9) complex studied here recorded at 25°C did not show any hyperfine splitting, it exhibited only a single signal. The analysis of the spectrum gives $g_{||} = 2.150$, $g_{\perp} = 2.030$, which supports the presence of unpaired electron in the $d_{x^2-y^2}$ orbital. The macrocyclic Cu(II)(9) complex showed that $g_{||} < 2.3$ indicating that the present complex exhibit appreciable covalent nature (36, 37). The G value [$G = (g_{||}\text{-}2)/(g_{perp}\text{-}2)$] which measures the exchange interaction between the copper centers in polycrystalline complexes has been calculated. According to Hathway, if $G > 4$, then exchange interaction is negligible and if $G<4$, it indicates a considerable exchange interaction in solid compounds (37). The calculated G value is larger than four suggesting that there is no interaction between the copper centers.

3.6 Thermogravimetric Analyses

TG and DTG studies were carried out for some of the complexes. These complexes decompose gradually with the formation of respective metal oxide above 700°C shown in Figure 2. All the metal complexes showed only a single decomposition curve between 341 and 360°C corresponding to the loss of organic moiety. Above 700°C, metal (II) complexes were decomposed leading to the formation of their respective metal oxides.

3.7 FAB Mass Spectral Studies of Schiff Base L^I and its Co (II) (1) Complex

The FAB mass spectrum of L^I showed a molecular ion peak at *m/z* 376 equivalent to its molecular weight. The fragments in the spectrum leading to the formation of the species $[C_{26}H_{20}N_6O_2]^+$ The FAB mass spectrum of Co(II)complex(2) showed molecular ion peaks $[M]^+$ at *m/z*519, 521$[M+2]^+$ and 523$[M+4]^+$which are equivalent to its molecular weight of the Co(II) complex (2) shown in Figure 3. Some other peaks appeared at *m/z* 412, 316 and 213 corresponds to the $[Co(C_{17}H_{22}N_6O)Cl]^+$, $[Co(C_{14}H_{10}N_2O)Cl]^+$ and

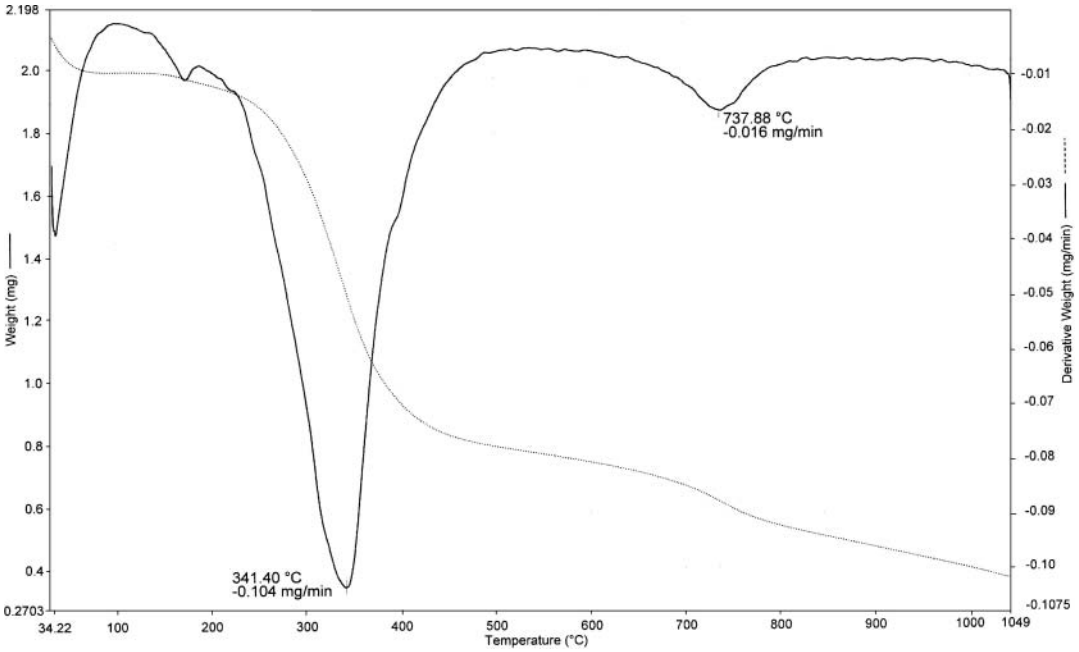


Fig. 2. Thermogravimetric (TG/DTG) Curves of Ni (II) (2) complex.

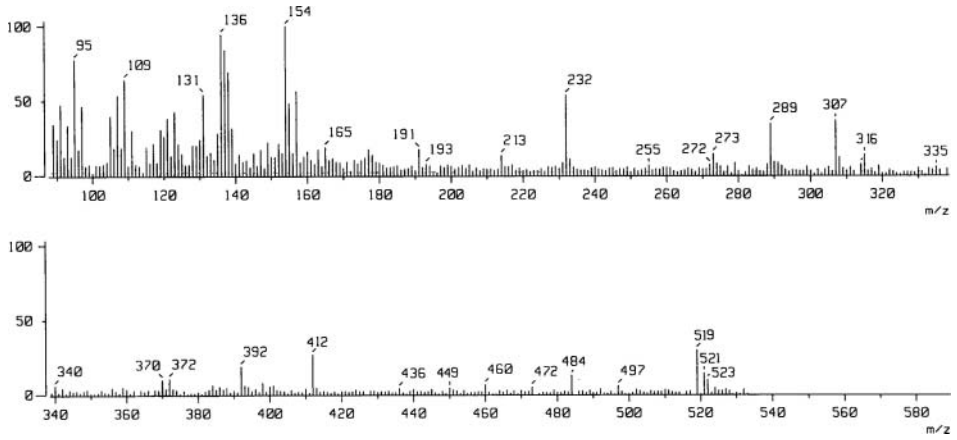


Fig. 3. FAB-mass spectrum of Co(II) (2) complex.

$[Co(C_7H_5NO)Cl]^+$ species which resulted from the loss of C_4H_8OCl, $C_3H_4N_4$ and C_7H_5N fragments from the parent compound. The two peaks appeared at m/z 449 and 484 are due to loss of two chlorine atoms and one chlorine atom, respectively. All these fragmentation patterns are well observed in the FAB mass spectra.

3.8 Electrochemical Studies

Electrochemical properties of the complexes were studied on a CHI1110A-Electrochemical analyzer in *N*, *N*-dimethylformamide (DMF) containing 0.05 M n-Bu_4NClO_4 as the supporting electrolyte. A cyclic voltammogram of Cu(II) (9) displays a reduction peak at $E_{pc} = -0.7$ V with a corresponding oxidation peak at $E_{pa} = -1.24$ V (Fig. 4.). The peak separation of this couple (ΔE_p) is 0.54 V at a scan rate 0.1 V and increases with scan rate. This Cu(II) complex is quasi-reversible as the separation in peak potential is higher than 59 mV and the peak current rise with increasing square root of the scan rate. The difference between forward and backward peak potentials can provide a rough evaluation of the degree of the reversibility of one electron transfer reaction. The analyses of cyclovoltammetric responses with the scan rate varying 50 to 250 mV/s provides evidence for quasi-reversible one electron oxidation.

The ratio of cathodic to anodic peak height was less than one. This establishes the electrode process as diffusion controlled (38). These characteristic features are consistent with the quasi-reversibility of the Cu(II)/Cu(I) couple.

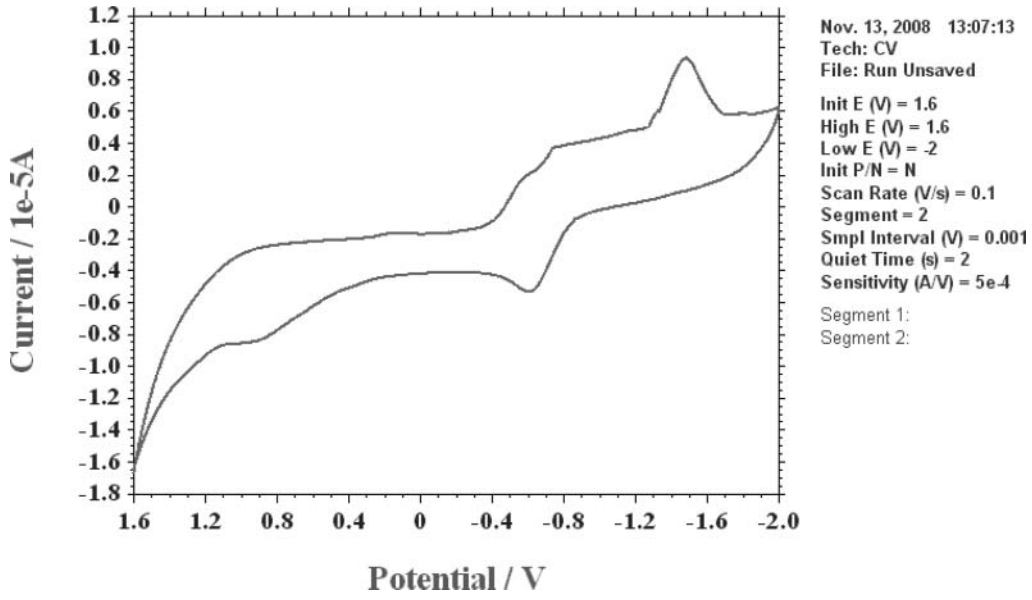


Fig. 4. Cyclic voltammogram of Cu (II) (9) complex.

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	53	68	58	68	63	86	78
	50	52	66	55	67	64	88	78
	30	53	67	57	66	64	86	79
L^{II}	100	81	67	57	87	66	73	71
	50	80	66	57	88	66	72	71
	30	80	66	57	89	68	71	72
L^{III}	100	63	57	53	61	60	60	61
	50	61	57	52	62	61	59	62
	30	60	58	53	62	61	59	61
L^{IV}	100	60	57	46	67	64	68	67
	50	59	58	48	68	64	69	68
	30	59	57	47	67	63	70	69
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

4 Biological Activities

4.1 *In Vitro* Antibacterial and Antifungal Assay

The synthesized macrocyclic Schiff bases and their complexes were screened for their biological activity by using four bacteria, namely *E. coli, S. aureus, S. typhi* and *P. aeruginosa* and three fungi namely *A.niger, A. flavus* and *Cladosporium* by the reported method (39, 40). All the uncoordinated metal salts exhibited negligible activities towards all bacterial and fungi species (41, 42). The DMF was used as solvent control.

The bacteria were subcultured in agar medium. The Petri dishes were incubated for 24 h 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. Standard antifungal drug (Fluconazole) was used for comparison. The Petri dishes were incubated for 48 h 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 and it showed no activity against any microbial strains.

4.2 Minimum Inhibitory Concentration (MIC)

Some compounds showing promising antibacterial/antifungal activities were selected for minimum inhibitory concentration 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 (42, 43). 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 activities. In case of bacteriological studies, the results were compared with the standard drug (Gentamycine). It was observed that, some of the Schiff bases were found potentially active against all bacterial strains. Schiff base (L^{II}) shows high activity against all bacterial strains especially with *E. coli* and *P. aeruginosa* where as metal (II) complexes (1-12) of these Schiff bases (L^I- L^{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 case of antifungal activity, the results were compared with the standard drug (Fluconazole). All Schiff bases were showed high activity against fungal species. Schiff bases (L^I) and (L^{II}) showed very high activity, an interesting feature is that, the Schiff base (L^I) 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 uncoordinated compounds. It was evident from the

Table 5. Antibacterial and antifungal results of Co (II), Ni (II) and Cu (II) complexes (1-12) and standard

		Antibacterial activity (Zone of inhibition in %)				*Antifungal activity (Zone of inhibition in %)*		
Compds.	*Conc.* (μgmL^{-1})	*E. coli*	*S. aureus*	*S.typhi*	*P.aeruginosa*	*A. flavus*	*Cladosporium*	*A. niger*
1	100	63	69	60	69	68	88	80
	50	64	68	61	69	69	86	79
	30	63	68	62	70	70	86	80
2	100	81	69	63	88	73	76	78
	50	82	70	63	88	71	76	77
	30	83	70	63	88	70	77	76
3	100	66	64	66	67	64	64	69
	50	65	66	67	69	65	62	68
	30	67	66	68	69	66	62	69
4	100	65	69	54	71	65	71	73
	50	66	71	56	74	66	73	73
	30	68	71	56	73	68	73	74
5	100	63	70	64	70	73	91	88
	50	62	71	63	71	72	93	87
	30	63	71	62	70	73	91	87
6	100	86	69	66	94	70	77	79
	50	88	69	66	95	71	77	80
	30	88	68	66	93	71	78	81
7	100	67	66	58	64	64	68	70
	50	69	65	59	66	65	69	71
	30	69	65	59	65	64	69	71
8	100	64	66	58	70	66	74	78
	50	64	65	58	71	67	73	76
	30	66	66	59	77	69	73	77
9	100	61	69	65	74	68	92	89
	50	63	69	66	74	69	92	89
	30	63	69	66	75	68	92	89
10	100	86	75	69	93	70	74	78
	50	88	74	68	93	71	72	77
	30	88	74	69	93	72	71	76
11	100	65	71	61	66	74	91	87
	50	66	71	62	66	73	92	86
	30	64	741	61	67	72	93	88
12	100	65	69	64	71	65	76	78
	50	65	68	65	70	66	75	79
	30	65	68	64	70	65	75	80
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

data that, this activity significantly increased on coordination. This enhancement in the activity may be rationalized on the basis of the presence of 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 (44, 45). This process of chelation thus increases the lipophilic nature of the central metal atom, which in turn, favours 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 (46). 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

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*
I	25	20	25	20	20	10	10
II	10	15	20	10	25	20	20
5	20	25	20	20	20	15	10
6	10	20	20	10	20	20	25
9	20	25	20	25	25	15	10
10	15	20	25	10	20	20	20

membrane of the microorganism and enhance the biological utilization ratio and activity of complexes (47).

The minimum inhibitory concentration 10 μg/mL was shown by compound L^{II} against *E. coli* and *P. aeruginosa*, compound L^{I} against *A.niger* and *Cladosporium* compound 6 shown MICs 10 μg/mL^{-1} against *E. coli* and *P. aeruginosa*. In all other cases, the compounds exhibited MICs ranging from 10 μg/mL–100 μ g/mL against all the microbial strains, some of 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 15 min. at 121°C under 15 lb pressure. The autoclaved media were inoculated with the seed culture and incubated at 37°C for 24 h.

5.2 Isolation of DNA

The fresh bacterial culture (1.5 mL) was centrifuged to obtain the pellet, which was 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 min, then it was centrifuged at 10,000 rpm for 10 min. 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 min. To this supernatant 3 volumes of chilled absolute alcohol was added. The precipitated DNA was separated by centrifugation. Dried the pellet and dissolve 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 agarose gel electrophoresis method. Test samples (1mg/mL) were prepared in DMF. The samples (100 μg) were added to the isolated DNA of *E. coli.* The samples were incubated for 2 h 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 min. Then removed the gel and stained with 10.0 μg/mL ethidium bromide for 10–15 min. The bands observed under UV transilluminator and photographed to determine the extent of DNA cleavage and the results were compared with standard DNA marker.

5.4 Electrophoretic Analysis

Four copper (II) complexes (9–12) were studied for their DNA cleavage activity by Agarose gel electrophoresis method (Fig. 5). DNA cleavage reactions generally proceed via two major pathways (I) Oxidative cleavage of the sugar and/or nucleobase moiety and (II) 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

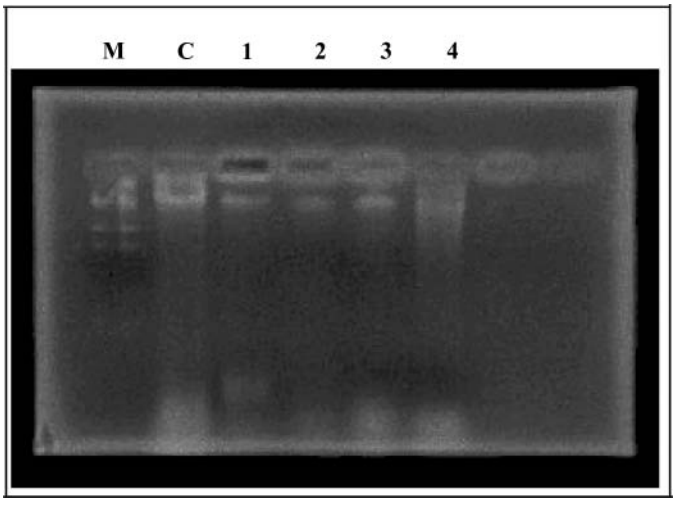


Fig. 5. M: Standard Molecular weight Marker; C-*E. coli*- Control DNA of *E. coli*; Lane 1- 4: *E. coli* DNA treated with Cu (II) (9–12) complexes, respectively.

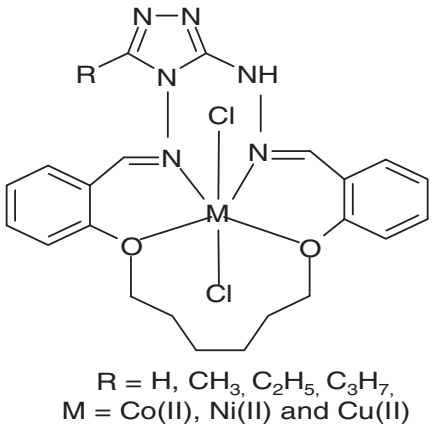


Fig. 6. Proposed structure for metal (II) complexes.

Zinc (II) are suitable for hydrolytic cleavage of DNA. Sigman et al. have reported *bis*(phen)copper (I) complex as first "copper based chemical nuclease" that cleaves the DNA in presence of H_2O_2 and thiol (48, 49). Similarly, the anticancer antibiotic bleomycins containing iron cleave DNA in an oxidative manner (50).

The gel after the electrophoresis clearly revealed that, the gel shows that all compounds have the cleavage activity. Compounds 1, 2 and 3 have acted on DNA as there was molecular weight difference between the control and the treated DNA samples. The difference was observed in the bands (Lane 1, 2 and 3) compared to the control DNA of *E.coli.* Whereas compound 4 showed such difference along with a streak, indicating unspecific cleavage too. This shows that, the control DNA alone does not show any apparent cleavage where as Cu (II) complexes shown. However, the nature of reactive intermediates involved in the DNA cleavage by the complexes has not been 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 inhibits the growth of the pathogenic organism by cleaving the genome.

6 Conclusions

The synthesized Schiff bases act as tetradentate ligand through the coordination of two azomethine nitrogen and two oxygen atoms to the metal ion. The bonding of ligand to metal ion was confirmed by the analytical, IR, electronic, magnetic, EPR, FAB-mass, thermal and electrochemical studies. In biological results it confirms 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 gel electrophoresis technique. From the observation, it was found that all copper (II) complexes cleave DNA more efficiently. All these observations put together lead us to propose the following structure shown in Figure 6, in which, the complex having the stoichiometry of the type $[MLCl_2]$ (M=Co (II), Ni (II) and Cu (II)).

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