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ORIGINAL ARTICLE

DNA cleavage and antimicrobial studies of 17-membered schiff base macrocyclic triazoles: synthesis and spectroscopic approach

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Abstract A novel series of 17-membered 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 3-subtituted-4-amino-5-hydrazino-1,2,4-triazole with *bis*(phthalaldehyde)-ethylenediamine precursor. The structure of the complexes has been proposed by elemental analyses, 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 Schiff bases and their 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 Agar and Potato dextrose agar diffusion method. The DNA cleavage study was done by Agarose gel electrophoresis technique.

Keywords Phthalaldehyde · Triazole · Ethylenediamine · Macrocycle · Biologically active

Abbreviations used

BM	Bohr Magneton
EPR	Electron paramagnetic resonance
FAB	Fast atom bombardment
TG/DTG	Thermogravimetric/differential thermogravimetric analysis
DMF	*N*,*N* dimethyl formamide
DMSO	Dimethylsulphoxide
IR	Infra red
MIC	Minimum inhibitory concentration
NMR	Nuclear magnetic resonance
MRI	Magnetic resonance imaging
TMS	Tetramethylsilane

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Introduction

The field of macrocyclic chemistry of metals is developing very fast because of its variety of applications and importance in the area of coordination chemistry [1, 2]. The rational design and construction of inorganic and organometallic metallomacrocycles by transition metal directed multicomponent self assembly has major impact on supramolecular chemistry [3, 4]. The incorporation of metal centres 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 complexes is obvious when seen in relationship to natural products such as metalloprotein, vitamin B_{12} and chlorophyll [5]. A number of nitrogen donor macrocyclic derivatives have long been used in analytical, industrial and medical applications [6]. 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 [7, 8]. Macrocyclic metal complexes of the lanthanides e.g. Gd^{+3}etc. are used as MRI contrast agents [9–11]. Several macrocyclic complexes with tetraaza macrocyclic ligand, such as cyclen, cyclam or bicyclam were reported to exhibit antitumour activity [12]. The chemistry of macrocyclic complexes is also important due to their use as dyes and pigments as well as NMR shift reagents [13]. Macrocyclic complexes are also well known for their antibacterial and antifungal activities [14, 15]. 1,2,4-Triazole display biological activity such as inhibition of cholinesterase, interference with mitosis and reversible denaturation of serum proteins [16] and its derivatives have become very useful compounds in medicine, agriculture and in many fields of technology [17]. Chemistry of 1,2,4-triazole derivatives have attracted widespread attention due to their diverse biological activities and they are a new class of antimicrobial agents viz. Fluconazole and Itraconazole are used as antimicrobial drugs. The mercapto derivatives of triazoles have been proved to be effective pesticides and insecticides. 3-Amino-1,2,4-triazole under the trade name Amizol as a commercial herbicide and 4*n*-butyl-4H-1,2,4-triazole as a systemic protective fungicide against leaf rust for both spring and winter wheat. Recently, several complexes of various transition and inner transition metals with substituted 1, 2, 4-triazole ligands have been reported from our laboratory [18, 19]. There is growing interest in the studies on the metal complexes of macrocyclic Schiff bases derived from substituted triazoles which are well known bactericides, pesticides, insecticides and potential fungicides [20]. However, paucity of literature on the metal complexes of macrocyclic ligands derived from *bis*(phthalaldehyde)ethylenediamine and 3-subtituted-4-amino-5-hydrazino-1, 2, 4-triazole encourage us to synthesize the divalent cobalt, nickel and copper macrocyclic complexes.

Experimental

Physical measurements

Carbon, hydrogen and nitrogen were estimated by using Carlo Erba EA1108 Elemental analyzer. Chloride was determined by Volhard's method. The IR spectra of the Schiff bases and their 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 NMR spectra of the ligands 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 (6 kV, 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 matrix. The mass spectrometer was operated in the positive ion mode. The EPR spectrum of the copper(II) complex in polycrystalline state was recorded 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 heating rate of 10 °C/min. The data were obtained by using a PERKIN-ELMER DIAMOND TG/DTG instrument. Molar conductivity measurements were recorded on a ELICO-CM-82T conductivity bridge with cell having cell constant 0.51 and magnetic moment was carried out on Faraday balance.

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 [21, 22].

Synthesis of Bis*(phthalaldehyde)ethylenediamine*

A mixture of phthalaldehyde and ethylenediamine in 2:1 M proportions in an ethanolic medium (40 mL each) containing few drops of concentrated HCl was refluxed for 3–4 h. The product formed was filtered, washed with ethanol and recrystalized form EtOH.

Brown; Yield, 71%, M.P.192 °C, Selected IR Data (KBr ν cm^{-1}): 3206 ν (NH), 1712 ν (C = O), 1634 ν (C = N), 1443, 1091, 748 ν(Ring vibrations). ^{1}H-NMR-(300 MHz DMSO-d_6): δ (ppm) = 3.26 (s, 4H, CH_2–CH_2), 7.24 (m, 9H, Ar–H), 8.20 (s, 2H, –CH = N), 13.1 (s, 1H, –NH), 9.98 (s, 2H –CHO). MS: *m/z* (M^+) = 292. Elemental analyses: Found. C, 73.97%; H, 5.47%; N, 9.58%; Calcd. C, 74.91%; H, 5.41%; N, 9.51%.

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

A mixture of 3-subtituted-4-amino-5-mercapto-1, 2, 4 triazole and $N_2H_4.H_2O$ in 1:1 M proportion in an ethanolic medium (40 mL each) was boiled under reflux for 4–5 h. on water bath. The reaction mixture was cooled at room temperature; within 1 h the compound separated from the clear solution. It was filtered, washed and recrystalized from EtOH.

For Compound $C_2H_6N_6$, Colorless Solid; Yield, 78%, M.P. 98 °C, Selected IR Data (KBr ν cm^{-1}): 3209 ν (NH), 3288 ν asym. (NH_2), 3107 ν sym. (NH_2), 890 ν (N–N) of the hydrazone residue. ^{1}H-NMR-(300 MHz DMSO-d_6): δ (ppm) = 6.40 (s, 1H,), 4.95 (br, 2H, NH_2 of $NHNH_2$), 5.55 (s, 2H, NH_2), 8.81 (br, 1H, –NH). MS: *m/z* (M^+) = 114. Elemental analyses: Found. C, 21.05%; H, 5.26%; N, 73.68%. Calcd. C, 20.96%; H, 5.19%; N, 73.59%.

For Compound $C_3H_8N_6$, Colorless Solid; Yield, 77%, M.P.132 °C, Selected IR Data (KBr ν cm^{-1}): 3207 ν (NH), 3291 ν asym. (NH_2), 3108 ν sym. (NH_2), 892 ν (N–N) of the hydrazone residue. ^{1}H-NMR-(300 MHz DMSO-d_6): δ (ppm) = 2.51 (s, 3H, CH_3), 4.96 (br, 2H, NH_2 of $NHNH_2$), 5.57 (s, 2H, NH_2), 8.83 (br, 1H, –NH). MS: *m/z* (M^+) = 128. Elemental analyses: Found. C, 28.12%; H, 6.25%; N, 65.62%. Calcd. C, 28.06%; H, 6.19%; N, 65.61%.

For Compound $C_4H_{10}N_6$, Colorless Solid; Yield, 80%, M.P.162 °C, Selected IR Data (KBr ν cm^{-1}): 3209 ν (NH), 3368 ν asym. (NH_2), 3155 ν sym. (NH_2), 891 ν (N–N) of the hydrazone residue. ^{1}H-NMR-(300 MHz DMSO-d_6): δ (ppm) = 1.42 (t, 3H, CH_3), 2.96 (q, 2H, CH_2), 4.98 (br, 2H, NH_2 of $NHNH_2$), 5.81 (s, 2H, NH_2), 8.92 (br, 1H, –NH). MS: *m/z* (M^+) = 142. Elemental analyses: Found. C, 33.80%; H, 7.04%; N, 59.15%. Calcd. C, 33.62%; H, 7.01%; N, 59.11%.

For Compound $C_5H_{12}N_6$, Colorless Solid; Yield, 79%, M.P.172 °C, Selected IR Data (KBr ν cm^{-1}): 3210 ν (NH), 3376 ν assym. (NH_2), 3168 ν sym. (NH_2), 894 ν (N–N) of the hydrazone residue. MS: *m/z* (M^+) = 156. ^{1}H-NMR-(300 MHz DMSO-d_6): δ (ppm) = 1.01(t, 3H, CH_3), 1.65–1.90 (m, 2H, ß-CH_2), 2.97 (t, 2H, a-CH_2), 4.95 (br, 2H, NH_2 of $NHNH_2$), 5.62 (s, 2H, NH_2), 8.89 (br, 1H, –NH). Elemental analyses: Found. C, 38.46%; H, 7.69%; N, 53.84%. Calcd. C, 38.41%; H, 7.61%; N, 53.81%.

Synthesis of Schiff bases (L^I–L^{IV})

A mixture of *bis*(phthalaldehyde)ethylenediamine and 3-subtituted-4-amino-5-hydrazino-1, 2, 4-triazole in 1:1 M proportion in an alcoholic medium 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 have shown in Fig. 1.

For Compound L^I, ($C_{20}H_{18}N_8$), Pale yellow; Yield, 71%, M.P.198 °C, Selected IR Data (KBr ν cm^{-1}): 3210 ν (NH), 2940 ν (C–H), 1630 ν (C = N), 1420, 1080, 760 ν(Ring vibrations). ^{1}H-NMR-(300 MHz DMSO-d_6): δ (ppm) = 3.29 (s, 4H, CH_2–CH_2), 7.26 (m, 9H, Ar–H), 8.10 (s, 2H, –CH = N), 13.4 (s, 1H, –NH), D_2O exchangeable. MS: *m/z* (M^+) = 370. Elemental analyses: Found. C, 64.78%; H, 4.78%; N, 30.22%. Calcd. C, 64.86%; H, 4.86%; N, 30.27%;

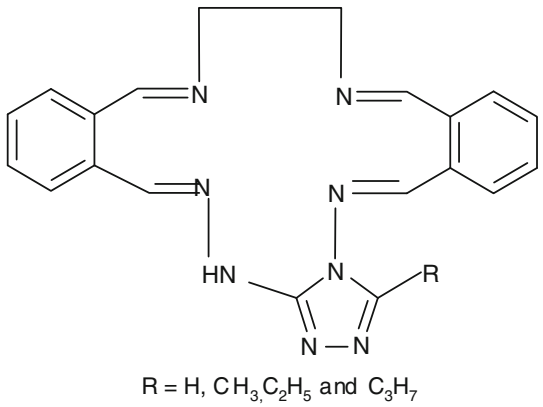

Fig. 1 Synthesized macrocyclic Schiff bases

For Compound L^{II}, ($C_{21}H_{20}N_8$), Cream; Yield, 69%, M.P. 201 °C, Selected IR Data (KBr ν cm^{-1}): 3212 ν (NH), 2924 ν (C–H), 1630 ν (C = N), 1440, 1095, 745 ν(Ring vibrations). ^{1}H-NMR-(300 MHz DMSO-d_6): δ (ppm) = 3.38 (s, 4H, CH_2–CH_3), 7.58 (m, 8H, Ar–H), 8.30 (s, 1H, –CH = N), 13.4 (s, 1H, –NH), D_2O exchangeable. MS: *m/z* (M^+) = 384. Elemental analyses: Found. C, 65.48%; H, 5.18%; N, 29.11%. Calcd. C, 65.62%; H, 5.20%; N, 29.16%.

For Compound L^{III}, ($C_{22}H_{22}N_8$), Cream; Yield, 73%, M.P. 206 °C, Selected IR Data (KBr ν cm^{-1}): 3211 ν (NH), 2926 ν (C–H), 1635 ν (C = N), 1400, 1130, 755 ν(Ring vibrations). ^{1}H-NMR-(300 MHz DMSO-d_6): δ (ppm) = 1.31 (t, 3H, CH_2–CH_3), 2.42 (q, 2H, CH_2–CH_3), 3.34 (s, 4H, –CH_2–CH_2), 7.61 (m, 8H, Ar–H), 8.31 (s, 4H, –CH = N), 13.60 (s, 1H, –NH, D_2O exchangeable). MS: *m/z* (M^+) = 398. Elemental analyses: Found. C, 66.36%; H, 5.48%; N, 28.01%. Calcd. C, 66.33%; H, 5.52%; N, 28.14%;

For Compound L^{IV}, ($C_{23}H_{24}N_8$), Yellow, 75%, M.P. 212 °C, Selected IR Data (KBr ν cm^{-1}): 3210 ν (NH), 2928 ν (C–H), 1633 ν (C = N), 1460, 1100, 770 ν (Ring vibrations). ^{1}H-NMR-(300 MHz DMSO-d_6): δ (ppm) = 1.01 (t, 3H, –CH_2–CH_2–CH_3), 1.72 (m, 2H, –CH_2–CH_2–CH_3), 2.62 (t, 2H, –CH_2–CH_2–CH_3), 3.37 (s, 4H, –CH_2–CH_2), 7.80 (m, 8H, Ar–H), 8.40 (s, 1H, –CH = N), 13.5(s, 1H, –NH, D_2O exchangeable). MS: *m/z* (M^+) = 412. Elemental analyses: Found. C, 66.48%; H, 5.96%; N, 27.02%. Calcd. C, 66.99%; H, 5.82%; N, 27.02%.

Synthesis of the complexes (1–16)

Co(II), Ni(II) and Cu(II) complexes of the ligands (L^I–L^{IV}) were prepared by adapting template method owing to the insolubility of the ligands in common organic solvents. To a stirred solution of *bis*(phthalaldehyde)ethylenediamine

(0.01 mol), and respective metal chloride (0.01 mol) in methanol(40 mL each) was added drop wise methanolic solution of 3-subtituted-4-amino-5-hydrazino-1, 2, 4-triazole (0.01 mol 40 mL). After the addition was completed the mixture was refluxed for 4–5 h. The separated complexes were collected by filtration, washed with hot methanol and dried under vacuum over $CaCl_2$.

Analyses

The metal contents were estimated gravimetrically by the standard method [23]. Carbon, hydrogen, nitrogen were estimated using a C, H, N analyzer. The results of elemental analyses and molar conductance values are listed in Table S1 (Supplementary information).

Result and discussion

All the Co(II), Ni(II) and Cu(II) complexes are colored, stable in air and non-hygroscopic 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+} and Cu^{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 non-electrolytes.

Infrared spectra

Infrared spectra frequencies of Schiff bases and their Co(II), Ni(II) and Cu(II) complexes are presented in Table S2 and S3 respectively (Supplementary information). The free ligands showed a medium intensity band in the 3212–3210 cm^{-1} region assigned to ν NH vibrations [24], which has been observed in the 3210–3207 cm^{-1} region in the case of complexes. It can be observed that, there is no considerable shift in the ν NH vibrations in case of complexes compared to the ligands, indicates non-involvement of NH group in the coordination. The ligands coordinate to the metal ions through the nitrogen of C = N group is supported by the presence of the νC = N band in the 1605–1595 cm^{-1} range in the case of macrocyclic Co(II), Ni(II) and Cu(II) complexes. However, these bands appeared in the 1635–1630 cm^{-1} range in the IR spectra of the macrocyclic ligands [25]. The presence of a medium-intensity band at 440–410 cm^{-1} region corresponding to the ν (M–N) vibration further confirms the formation of macrocyclic complex. All the complexes show bands in the 1460–1400, 1130–1080 and 760–740 cm^{-1} regions assigned to phenyl ring vibrations. The coordination of the chloro group has been ascertained by band in the 310–280 cm^{-1} region, which may reasonably be assigned to ν(M–Cl) [26]. The two moderately strong bonds appearing at 890 and 720 cm^{-1} can be attributed respectively to ν(N–N) of the hydrazone residue and inplane deformation of the triazole ring [27, 28].

^{1}H and ^{13}C NMR spectra

The ^{1}H NMR spectral data of the macrocyclic Schiff bases give some important information to conclude to the formation of macrocyclic ligands. The disappearance of the primary amino proton signal and the appearance of a singlet observed in the region 3.29–3.37 ppm in the ligands may be assigned to methylene protons adjacent to nitrogen indicated that the proposed macrocyclic skeleton has been formed [29]. This fact was also supported by the disappearance of peaks around 9.98 ppm corresponding to aldehydic protons [30]. The ^{1}H NMR spectra of all Schiff bases exhibited signals in the range 13.6 and 13.4 ppm due to NH protons these protons are D_2O exchangeable and confirming the assignment [31]. Multiplets observed in the range of 7.2–7.8 ppm have been assigned to the aromatic protons (Table 1).

The ^{13}C NMR spectra of the ligands exhibited signals in the range 152.22–154.65 ppm indicating the presence of carbon which is doubly bonded to nitrogen [32]. The ligand which is having methylenic carbon adjacent to nitrogen atom contains signal in the range of 45.3–59.4 ppm indicate the presence of N-CH_2 linkage [33]. The aryl carbons are resonated in the range of 131.8–140.1 ppm.

Electronic absorption spectra and magnetic studies

The electronic spectra of the macrocyclic Co(II) complexes showed two bands in the region 14260–14400 and 21150–21500 cm^{-1}, which may be reasonably assigned to the $^4T_{1g}$ (F) → $^4A_{2g}$ (F) and $^4T_{1g}$ (F) → $^4T_{1g}$ (P) transitions,

Table 1 ^{1}H NMR spectroscopic data of the of Schiff bases (L^I–L^{IV})

Schiff base	NMR data
$C_{20}H_{18}N_8$	3.29(s, 4H, –CH_2–CH_2), 7.26(m, 9H, Ar–H), 8.10(s, 4H, –CH = N), 13.4(s, 1H, –NH, D_2O exchangeable).
$C_{21}H_{20}N_8$	2.12 (s, 3H, CH_3), 3.33(s, 4H, –CH_2–CH_2), 7.58(m, 8H, Ar–H), 8.30(s, 1H, –CH = N) 13.4(s, 1H, –NH, D_2O exchangeable).
$C_{22}H_{22}N_8$	1.31(t, 3H, CH_2–CH_3), 2.42(q, 2H, CH_2–CH_3), 3.34(s, 4H, –CH_2–CH_2), 7.61(m, 8H, Ar–H), 8.31(s, 4H, –CH = N), 13.60(s, 1H, –NH, D_2O exchangeable).
$C_{23}H_{24}N_8$	1.01(t, 3H, –CH_2–CH_2–CH_3), 1.72(m, 2H, –CH_2–CH_2–CH_3), 2.62(t, 2H, –CH_2–CH_2–CH_3), 3.37(s, 4H, –CH_2–CH_2), 7.80(m, 8H, Ar–H), 8.40(s, 1H, –CH = N), 13.5(s, 1H, –NH, D_2O exchangeable).

respectively, suggesting an octahedral geometry around the cobalt (II) ion [34, 35]. However, the third band expected around 8000 cm^{-1} could not be properly resolved. The magnetic moment values of 4.57 and 4.56 BM further support the electronic spectral observations. The ligand field parameters Dq, B′, v_2/v_1, β, $\beta\%$ have been calculated and presented in Table 2. The electronic spectra of the macrocyclic nickel(II) complexes exhibited two bands in their electronic spectra in the regions 11,400–11,600 and 18,100–18,300 cm^{-1}, which may be ascribed to $^3A_{2g}$ (F) → $^3T_{1g}$ (F) and $^3A_{2g}$ (F) → $^3T_{1g}$ (P) transitions, respectively, are consistent with an octahedral geometry around nickel(II)ion [35]. Further confirmation regarding the octahedral environment around the nickel(II) ion has been obtained from magnetic moment values of 3.15 and 3.18 BM.

The electronic spectra of the macrocyclic Copper (II) complexes showed band in the 16,250–16,400 and 20,300–20,500 cm^{-1}, regions which may be ascribed to $^2B_{1g}$ → ^2E_g and $^2B_{1g}$ → $^2B_{2g}$ transitions, respectively, corresponding to distorted octahedral geometry around Cu(II)ions. The magnetic moment values in the range 1.78–1.82 further support the electronic spectral data.

EPR spectrum of copper (II) complex (12)

The EPR spectrum of polycrystalline macrocyclic Cu(II) (12) complex studied here recorded at 25 °C did not show any hyperfine splitting, it exhibited only single signal. The analyses of the spectrum gives $g_{\|} = 2.178$, $g\perp = 2.040$, which support that $d_x^2 - _y^2$ may be the ground state. The observed $g_{\|}$ value for the complex is less than 2.3 which is in agreement with the covalent character of the metal–ligand bond [36]. The $g_{\|}$ (2.178) > g⊥ (2.040) observed was found to be in accordance with the criterion of Kivelson and Neiman, implying the presence of unpaired electron is localized in $dx^2 - y^2$ orbital for the Cu(II) ion characteristic of the axial symmetry. Tetragonally elongated structure is thus confirmed for Cu(II) complex. The parameter G was calculated by using the expression i.e. $G = (g_{\|} - 2)/(g\perp - 2)$. According to Hathaway, the G value measures the exchange interaction between metal centers in polycrystalline solids. In case G > 4, the exchange interaction is negligible, but if G < 4 considerable interaction occurs in the complexes [36]. The calculated G value is larger than four suggesting that there is no interaction between the copper centers.

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 500 °C. All the metal complexes showed only a single decomposition curve between 309 and 345 °C corresponding to the loss of organic moiety. Above 500 °C, organic moieties in these macrocyclic compounds were decomposed leading to the formation of metal oxide.

FAB mass spectra of Schiff base $L^{II;}$ and its Ni(II) complex (2)

The FAB mass spectrum of Schiff base (L^{II}) showed a molecular ion peak at *m/z* 384 which is equivalent to its molecular weight. The fragments in the spectrum leading to the formation of the species $[C_{21}H_{20}N_8]^+$. The FAB mass spectrum of Ni(II) complex (2) exhibited molecular ion peaks $[M]^+$ at *m/z* 513 $[M]^+$, 515$[M + 2]^+$ and 517$[M + 4]^+$ which are equivalent to its molecular weight of the Ni(II) complex (2). Some other peaks appeared at *m/z* 238, 340 and 436 corresponds to the $[Ni(C_9H_{10}N_2)Cl]^+$, $[Ni(C_{16}H_{14}N_3)Cl]^+$ and $[Ni(C_{19}H_{16}N_7)Cl]^+$ species which are resulted from the parent compound (Fig. 2). The two peaks appeared at *m/z* 443 and 478 due to loss of two chlorine atoms and one chlorine atom respectively. All these fragmentation patterns are well observed in the FAB mass spectra.

Fab mass spectral results of complexes: $Co(C_{20}H_{18}N_8)Cl_2$: *m/z* $(M^+) = 498$, *m/z* $[M + 2]^+ = 500$, *m/z* $[M + 4]^+ = 502$. $Co(C_{21}H_{20}N_8)Cl_2$: *m/z* $(M^+) = 513$, *m/z* $[M + 2]^+ = 515$, *m/z* $[M + 4]^+ = 517$. $Co(C_{22}H_{22}N_8)Cl_2$: *m/z* $(M^+) = 527$, *m/z* $[M + 2]^+ = 529$, *m/z* $[M + 4]^+ = 531$. $Co(C_{23}H_{24}N_8)Cl_2$: *m/z* $(M^+) = 541$, *m/z* $[M + 2]^+ = 543$, *m/z* $[M + 4]^+ = 545$. $Ni(C_{20}H_{18}N_8)Cl_2$: *m/z* $(M^+) = 498$, *m/z* $[M + 2]^+ = 500$, *m/z* $[M + 4]^+ = 502$. $Ni(C_{21}H_{20}N_8)Cl_2$: *m/z* $(M^+) = 513$, *m/z* [M +

Table 2 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})	v_2/v_1	LFSE	β	$\beta\%$
	v_1	v_2 (Cacd.)	v_3						
1	14260	29623	21150	1536.37	532.91	2.077	35.11	0.5511	44.889
2	14310	29728	21230	1541.82	535.21	2.077	35.24	0.5534	44.651
3	14352	29817	21350	1546.77	541.18	2.077	35.35	0.5596	44.035
4	14400	29929	21500	1552.94	548.63	2.078	35.49	0.5673	44.264

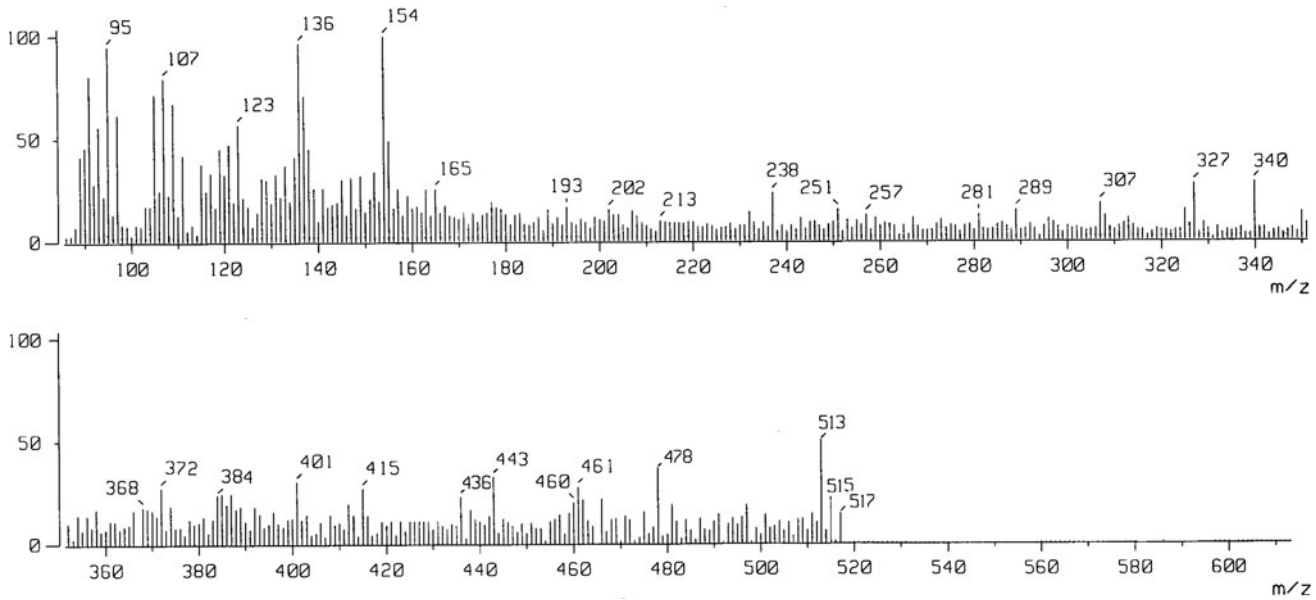

Fig. 2 FAB-mass spectrum of Ni (II) (6) complex

$2]^+ = 515$, *m/z* $[M + 4]^+ = 517$. $Ni(C_{22}H_{22}N_8)Cl_2$: *m/z* $(M^+) = 527$, *m/z* $[M + 2]^+ = 529$, *m/z* $[M + 4]^+ = 531$. $Ni(C_{23}H_{24}N_8)Cl_2$: *m/z* $(M^+) = 541$, *m/z* $[M + 2]^+ = 543$, *m/z* $[M + 4]^+ = 545$. $Cu(C_{20}H_{18}N_8)Cl_2$: *m/z* $(M^+) = 503$, *m/z* $[M + 2]^+ = 505$, *m/z* $[M + 4]^+ = 507$. $Cu(C_{21}H_{20}N_8)Cl_2$: *m/z* $(M^+) = 531$, *m/z* $[M + 2]^+ = 533$, *m/z* $[M + 4]^+ = 535$. $Cu(C_{22}H_{22}N_8)Cl_2$:: *m/z* $(M^+) = 545$, *m/z* $[M + 2]^+ = 547$, *m/z* $[M + 4]^+ = 549$.

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 Co(II) (3) displays a reduction peak at $E_{pc} = -1.0400$ V with corresponding oxidation peak at $E_{pa} = -0.5922$ V (Fig. 3). The peak separation (ΔE_p) of this couple is 0.44 V at scan rate 0.1 V and increases with scan rate. The most significant feature of the Co(II) complex is the Co(II)/Co(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. The separation in peak potentials increases at higher scan rates consistent with quasi-reversibility of the Co(II)/Co(I) couple. The Cu(II) (9) complex exhibits a reduction peak at $E_{pc} = 0.5824$ with direct re-oxidation peak at $E_{pa} = 0.3120$ V corresponding to the Cu(II)/Cu(I) couple (Fig. 4). The peak separation (ΔE_p) is 0.270 V. This Cu(II) complex is also quasi-reversible as the separation in peak potential is higher than 59 mV and the peak current rise with

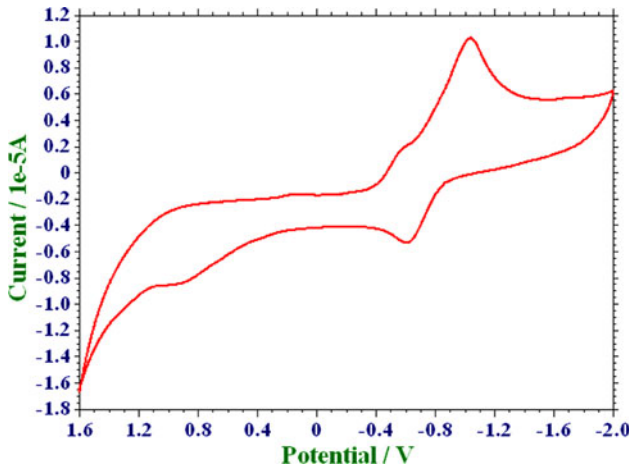

Fig. 3 Cyclic voltamogram of Co (II) (3) complex

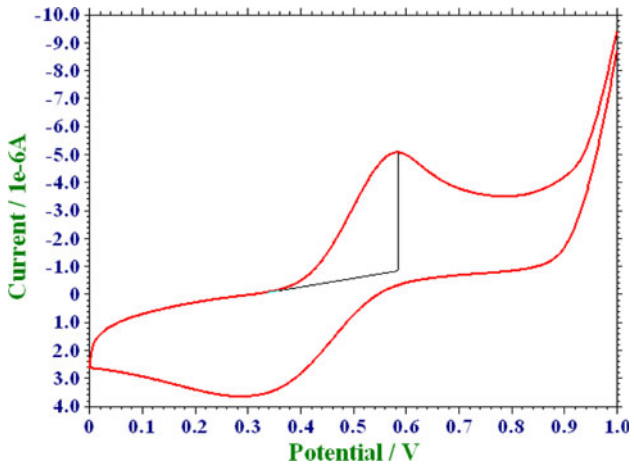

Fig. 4 Cyclic voltamogram of Cu (II) (9) complex

increasing square root of the scan rate [37]. 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 cyclic voltammetric responses with the scan rate varying 50–250 mV/s gives the evidence for quasi-reversible one electron oxidation.

Biological activities

In vitro antibacterial and antifungal assay

The synthesized Schiff bases and their corresponding Co(II), Ni(II) and Cu(II) 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* and *Cladosporium* by the reported method [38, 39]. 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 solvent DMF on the biological screening, separate studies were carried out with solvent DMF only and it showed no activity against any microbial strains.

Minimum inhibitory concentration (MIC)

Some compounds showing promising antibacterial/antifungal activities were selected for minimum inhibitory concentration studies [40, 41].

Antimicrobial results

The microbial results were systematized in Tables 3 and 4. 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 [42, 43] 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. 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 case of bacteriological studies, the results were compared with the standard drug (Gentamycine). It was observed that, some of the Schiff bases were found

Table 3 Antibacterial and antifungal results of Schiff bases (L^{I}–L^{IV})

Schiff bases	Conc. (μg mL^{-1})	Anti bacterial activity (zone of inhibition in %)				Antifungal activity (zone of inhibition in %)		
		E. coli	*S. aureus*	*S.typhi*	*P.aeruginosa*	*A. flavus*	*Cladosporium*	*A. niger*
L^{I}	100	50	45	48	67	85	74	75
	50	49	44	49	66	84	73	74
	30	50	44	50	66	83	72	73
L^{II}	100	58	59	43	60	88	71	78
	50	58	59	42	59	87	69	76
	30	57	58	43	59	86	70	77
L^{III}	100	61	57	57	69	87	51	84
	50	58	57	56	70	86	50	71
	30	59	56	57	71	88	51	74
L^{IV}	100	81	60	46	63	74	72	86
	50	80	59	46	62	75	72	88
	30	81	60	45	61	74	71	88
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 4 Anti bacterial and anti fungal results of Co(II), Ni(II) and Cu(II) complexes (1–12) and standard

Complex no.	Conc. ($\mu g\ mL^{-1}$)	Anti bacterial 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	51	54	62	70	86	79	79
	50	54	55	63	71	84	80	78
	30	54	54	62	70	85	80	78
2	100	68	60	50	64	86	71	79
	50	67	61	51	63	88	73	80
	30	66	61	50	62	87	74	81
3	100	70	62	68	73	89	59	79
	50	69	60	69	74	88	61	78
	30	69	61	70	74	88	61	80
4	100	84	68	50	69	74	85	98
	50	85	68	50	67	75	84	99
	30	85	69	53	68	78	85	97
5	100	81	56	69	80	86	78	80
	50	80	60	67	80	87	79	80
	30	80	61	68	81	85	80	81
6	100	69	62	58	65	89	80	84
	50	69	64	58	65	88	81	82
	30	68	59	58	66	88	81	81
7	100	71	64	68	74	89	58	84
	50	71	64	69	74	90	57	81
	30	70	64	70	73	91	57	82
8	100	81	73	61	82	89	82	89
	50	81	71	64	84	89	80	88
	30	81	72	61	82	89	81	88
9	100	54	60	65	72	88	81	82
	50	54	59	65	69	88	82	82
	30	54	62	65	70	88	83	79
10	100	69	64	54	66	90	83	80
	50	69	64	54	68	90	82	81
	30	68	64	56	68	91	81	80
11	100	68	65	70	74	89	58	81
	50	68	65	70	75	90	62	83
	30	68	65	71	75	90	59	84
12	100	80	71	64	81	90	83	91
	50	80	72	63	83	89	81	89
	30	80	70	65	81	88	82	90
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

potentially active against all bacterial strains. Compound (L^{III}) shows high activity against all bacterial strains especially with *P.aeruginosa* and *E. coli*, 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 show high activity against fungal species. Compound (L^{I}) and (L^{IV}) show very high activity, an interesting feature is that, the compound (L^{IV}) shows high activity against *A. niger*, 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 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, 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 [46]. It has also been observed that some moieties such as the azomethine linkage or heteroaromatic nucleus introduced into such compounds exhibited 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 [47].

The minimum inhibitory concentration 10 μg/mL was shown by compound L^{IV} against *E. coli* and *A. niger* and compound 8 against *A. flavus* and *P.aeruginosa* compound 5 shown MIC 10 μg/mL against *S. typhi* and *A. flavus*. In all other cases, the compounds exhibited MICs ranging from 10–100 μg/mL against all the microbial strains and some of are given in Table 5.

DNA cleavage experiment

Preparation of Culture media

DNA cleavage experiments were done according to the literature [48]. Nutrient broth (peptone, 10; Yeast extract, 5; NaCl, 10 in (g/L)) was used for culturing of *E. coli* and Potato dextrose broth (potato, 250; dextrose, 20, (g/L)) was used for the culture of *A. niger*. The 50 mL media was prepared and autoclaved for 15 min at 121 °C under 15 lb pressure. The autoclaved media was inoculated with the seed culture *E. coli* was incubated for 24 h and *A. niger* for 48 h at 37 °C.

Isolation of DNA

The fresh bacterial culture (1.5 mL) is 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 min. Then, it was centrifuged at 10,000 rpm for 10 min and equal volume of chloroform : isoamyl alcohol (24:1) and 1/20th volume of 3 M sodium acetate (pH 4.8) was added to this supernatant and centrifuged at 10,000 rpm for 10 min. To this supernatant three volumes of chilled absolute alcohol was added. The precipitated DNA was separated by centrifugation. Dried the pellet and dissolved in TE buffer (10 mM tris pH 8.0, 1 mM EDTA) and stored in cold condition.

Agarose gel electrophoresis

Cleavage products were analyzed by agarose gel electrophoresis method. Test samples (1 mg/mL) were prepared in DMF. The samples (25 μg) were added to the isolated DNA of *E. coli* and *A. niger*. 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) was 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 L) and finally loaded on agarose gel and passed the constant 50 V of electricity for around 30 min. Removed the gel and stained with 10.0 μg/mL ethidium bromide for 10–15 min and the bands observed under UV transilluminator and photographed to determine the extent of DNA cleavage and the results were compared with standard DNA marker.

Table 5 Minimum inhibitory concentration (μg mL^{-1}) results for some compounds

Compds.	Anti bacterial activity (zone of inhibition in %)				Antifungal activity (zone of inhibition in %)		
	E. coli	*S. Aureus*	*S.typhi*	*P.aeruginosa*	*A. flavus*	*Cladosporium*	*A. niger*
IV	10	20	15	10	20	15	10
5	15	25	10	15	10	15	25
6	15	25	15	15	20	15	25
8	10	25	25	15	15	20	10
10	20	25	20	25	20	10	20
12	25	20	20	20	15	15	15

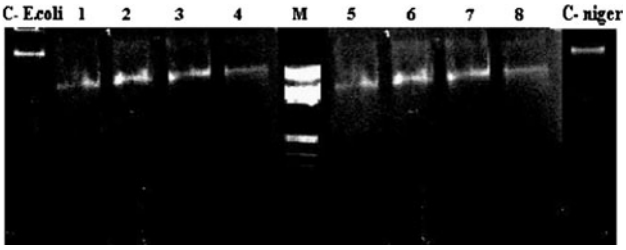

Fig. 5 M: Standard Molecular weight Marker; C-*E. coli*- Control DNA of *E. coli*; Lane 1–4: *E. coli* DNA treated with Schiff base (L^{IV}) and it's Cu(II) Co(II) and Ni(II) complexes respectively. Lane 5–8: *A. niger* DNA treated with Schiff base (L^{IV}) and it's Cu(II) Co(II) and Ni(II) complexes respectively. C-niger is Control DNA of *A. niger*

Electrophoretic analysis result

The Schiff base (L^{II}) and its Co(II), Ni(II) and Cu(II) complexes were studied for their DNA cleavage activity by agarose gel electrophoresis method and presented in the 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 Zinc (II) are suitable for hydrolytic cleavage of DNA. Sigman and co-worker 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 [49]. Similarly, the anticancer antibiotic bleomycins containing iron cleave DNA in an oxidative manner [50, 51].

The gel after electrophoresis clearly revealed that, both the Schiff base (L^{IV}) and it's complexes 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 1–8) compared to the control DNA of *E. coli* and *A. niger*. This shows that, the control DNA alone does not show any apparent cleavage where as 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 compound inhibits the growth of the pathogenic organism by cleaving the genome.

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

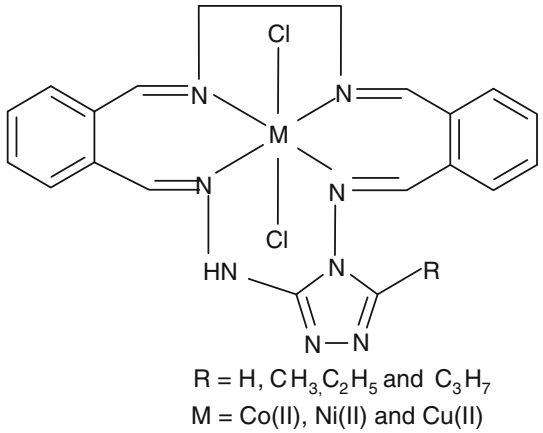

Fig. 6 Proposed structure for metal (II) complexes

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 these complexes with DNA was investigated by gel electrophoresis technique. From the observation, it was found that Schiff base (L^{IV}) and its metal (II) complexes cleave DNA more efficiently. All these observations put together lead us to propose the following structure shown in Fig. 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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