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RESEARCH ARTICLE

SYNTHESIS, STRUCTURAL CHARARCTERIZATION, ANTIMICROBIAL ACTIVITY AND DNA CLEAVAGE OF TRANSITION METAL (II) COMPLEXES DERIVED FROM 5-BROMO-3-HYDRAZONOINDOLIN-2-ONE, 3-((2-AMINOETHYL) AMINO) QUINOXALIN-2(1*H*)-ONE AND 2,2-DIMETHYL-1,3-DIOXANE-4,6-DIONE

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ABSTRACT

A new series of transition metal complexes of Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II) have been synthesized from the Schiff base (L) derived from 5-bromo-3-hydrazonoindolin-2-one 3-((2-aminoethyl)amino)quinoxalin-2(1*H*)-one and 2,2-dimethyl-1,3-dioxane-4,6-dione. All the complexes were characterized in the light of elemental analysis, molar conductance, FT IR, UV-VIS, magnetic, thermal and powder XRD studies. Analytical data reveal that the Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II) complexes possess 1:1 metal–ligand ratios. The ligand and its complexes have been screened for the antibacterial activities towards bacteria *E. coli, P. vulgaris* and *S. pyogones*, and antifungal activities towards fungi *A. flavus*, C. *albicans and F. solani*. The results of these studies revealed that the free ligand and its metal complexes showed significant antimicrobial potency. The DNA cleavage studies have also been carried out on Calf-thymus DNA and it is observed that these Co(II), Ni(II), Cu(II), Zn(II), Cd(II) shows complete DNA cleavage activity whereas Hg(II) show partial DNA cleavage in the presence of H_2O_2 and UV light.

INTRODUCTION

Schiff base ligand has been widely studied in the field of coordination chemistry mainly because of their facile synthesis, easy availability, electronic properties, and good solubility in common solvents (Smita Revankar *et al*., 2014). Schiff bases and their coordination compounds are well known to be biologically important. The presence of nitrogen and other donor atoms in its structure makes it biologically active. Recent literature has explored the biological importance of various structural derivatives of heterocyclic compounds. Schiff's bases, the condensed products of aromatic amines with aromatic aldehydes, have been known to possess a wide variety of biological applications like antibacterial, antifungal, antitumor, analgesic and anti-inflammatory activities (Yildirim *et al*., 2014). Thus, in recent years Schiff bases and their metal complexes have attained much attraction because of their extensive biological activities (Priya *et al*., 2013). Keeping the above fact in our mind and in continuation of our earlier work on transition metal complexes with Schiff bases, the ligand has been synthesized (Noorjahan begum *et al*., 2014). In this article, we report the synthesis, structural characterization and biological studies of 2,2-dimethyl-1,3-dioxane-4,6-dione based tetra dentate heterocyclic Schiff base derived from 5-bromo-3-hydrazonoindolin-2-one and 3-((2-aminoethyl) amino)quinoxalin-2(1*H*)-one and its complexes with Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II) metal ions.

MATERIALS AND METHODS

All the used chemicals and solvents were of analytical grade. All the reagents used for the preparation of the Schiff bases was obtained from Sigma Aldrich. Metal salts were purchased from Fluka.

Synthesis of Schiff base

The Schiff base has been synthesized by refluxing the equimolar mixture of hot methanolic solution of 2,2-dimethyl-1,3-dioxane-4,6-dione (0.001 mol), hot methanolic solution of 5-bromo-3-hydrazonoindolin-2-one (0.001 mol) and 3-((2-aminoethyl)amino)quinoxalin-2(1*H*)-one (0.001 mol) for 6-7 h in presence of catalytical amount of conc. HCl. After completion of the reaction, the resultant mixture was cooled to room temperature, filtered and reduced nearly half of its volume. It was then allowed to stay at room temperature for 3 h which resulted in the solid product. It was filtered, washed with

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methanol and finally recrystallized from methanol to afford Schiff base as shown in Fig. 1. The purity of the compound has been checked by TLC.

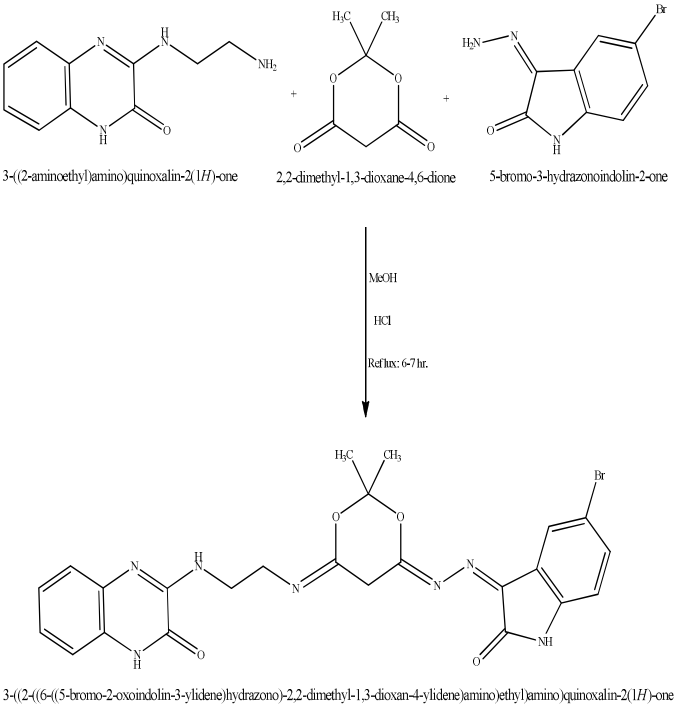

Fig. 1. Schematic representation of Schiff base

Synthesis of metal complexes

Hot ethanolic solution of Co(II), Ni(II), Cu(II), Cd(II), Zn(II) and Hg(II) chlorides and solution of respective ligand were mixed in 1:1 molar ratio. The resulting solution was refluxed with stirring for 4-5 h. Then, to the reaction mixture sodium acetate was added to adjust the pH to 6.0–7.0, after which immediately solid, intense colored complexes precipitated out. The formed complexes were filtered, collected and then washed with hot ethanol until the filtrate becomes colourless. The complexes were dried in a desiccators. Elemental analysis data has been shown in Table 1. The complexes were analyzed for their metal and chloride contents by standard methods (Vogel, 1968).

Table 1. Physical and analytical data of the ligand and its metal (II) complexes

Compound	Molecular formula	Yield (%)	Found (Calculated) % C	H	N	M	Cl	Ωm (Ω^{-1} cm^2 mol^{-1})	μ_{eff} (BM)
L	$C_{24}H_{22}N_7O_4Br$	82	52.18 (52.01)	4.01 (3.92)	17.75 (17.64)	-	-	-	-
[$CoLCl_2$]	[$CoC_{24}H_{22}N_7O_4BrCl_2$]	72	42.25 (42.10)	3.25 (3.11)	14.37 (14.22)	8.63 (8.59)	5.19 (5.11)	15.26	4.75
[$NiLCl_2$]	[$NiC_{24}H_{22}N_7O_4BrCl_2$]	78	42.27 (42.13)	3.25 (3.18)	14.38 (14.22)	8.60 (8.53)	5.19 (5.11)	17.77	2.80
[$CuLCl_2$]	[Cu $C_{24}H_{22}N_7O_4BrCl_2$]	69	41.97 (41.82)	3.23 (3.17)	14.28 (14.10)	9.25 (9.08)	5.16 (4.94)	19.22	1.99
[$ZnLCl_2$]	[Zn $C_{24}H_{22}N_7O_4BrCl_2$]	65	41.86 (41.74)	3.22 (3.15)	14.24 (14.19)	9.49 (9.39)	5.14 (4.94)	22.27	-
[$CdLCl_2$]	[Cd $C_{24}H_{22}N_7O_4BrCl_2$]	68	39.18 (39.00)	3.01 (3.00)	13.33 (13.28)	15.27 (15.14)	4.81 (4.78)	23.99	-
[$HgLCl_2$]	[Hg $C_{24}H_{22}N_7O_4BrCl_2$]	70	34.99 (34.87)	2.69 (2.55)	11.90 (11.85)	24.34 (23.56)	4.30 (4.28)	28.88	-

Physical measurements

Elemental analysis carbon, hydrogen and nitrogen analysis was carried out using a Heracus Carlo Erba 1108 CHN analyzer at STIC, Cochin. The IR spectra of the Schiff base and its Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II) complexes were recorded in KBr pellets in the region of 4000-450 cm^{-1} on a Perkin Elmer 783 FT-IR spectrophotometer. The electronic spectra of the Co(II), Ni(II) and Cu(II) complexes were recorded on an ELICO SL-164 double beam UV-visible spectrophotometer in the range of 200-1200 nm in DMF (10^{-3} M) solution. Magnetic susceptibility measurements were made at room temperature on a Gouy balance using $Hg[Co(NCS)_4]$ as the calibrant. Molar conductivity measurements were recorded on an ELICO CM-180 conductivity bridge in DMF solution (10^{-3} M) using a dip-type conductivity cell fitted with a platinum electrode. The ^{1}H-NMR spectra were recorded in DMSO-d_6 on a Bruker 500 MHz spectrophotometer using TMS as an internal standard. The mass spectra were recorded on a JEOL GC mate mass spectrophotometer. The ESR spectrum of the Cu(II) complex in the polycrystalline state was recorded on a Varian-E-4X band EPR spectrophotometer using TCNE as the 'g'marker (g = 2.00277) at room temperature. Thermal analyses were measured from room temperature to 1000°C in N_2 on a perkin Elmer, Diamond TG/DTA model thermal analyzer at STIC, Cochin, with a heating rate of 10°C min^{-1}. The XRD patterns of the Cu(II) complex were recorded on a Bruker AXS D8 Advance X-ray diffractometer using Cu Kα = 1.5404 radiation (λ Å).

Pharmacology

In vitro antimicrobial activity

Antimicrobial activity of the ligand and its complexes were tested in vitro against the bacterial species *E. coli, P. vulgaris* and *S. pyogones*, fungal species, *A. flavus*, C. *albicans and F. solani* by the disc diffusion method (Bauer *et al.*, 1966). Ciprofloxacin was used as the standard antibacterial agent whereas nystatin was used as the standard antifungal agent. The stock solution of each compound was prepared by dissolving 10 mg of each test compound in 10 mL of freshly distilled DMSO. The various concentrations of the test compounds (100, 75, 50, 25 and 12.5 µg/mL^{-1}) were prepared by diluting the stock solution with the required volume of freshly distilled DMSO. The MIC was measured after 24 h in case of antibacterial activity, 48 h for antifungal activity (Siddappa and Nabiya sultana mayana, 2014).

DNA cleavage activity

The compounds were added separately to the Calf-thymus DNA sample (Sambrook *et al.*, 1989). The samples mixtures were performed under aerobic conditions with H_2O_2 as an oxidant by incubation at 37 °C for 2h. The electrophoresis of the samples was done according to the following procedure. CT DNA 50 µM of Schiff base and its complexes, 50 µM of H_2O_2 in 0.05 M Tris–HCl buffer (pH = 7.2). After incubation, 1 µL of loading buffer (bromophenol blue in H_2O) was added to each tube and the mixed samples were loaded on 1 % agarose gel. The samples were electrophoresed at a constant voltage (50 V) for 2 h in Tris–acetic acid–EDTA buffer (pH 8.3). After electrophoresis, the gel was stained for 30 min by immersing it in 1 µg / cm^3 ethidiumbromide (EB) solution. The cleavage was visualized by viewing the gel under UV light and photographed (Nagajothi *et al*., 2012).

RESULTS AND DISCUSSION

The analytical data of the complexes are given in Table 1. All these complexes are analyzed for 1:1 stoichiometry of the type $MLCl_2$. On the basis of these characterizations it has been found that all the complexes are non-hygroscopic, stable at room temperature, insoluble in water but fairly soluble in DMSO and DMF. The molar conductance values in DMF (10^{-3}M) solution at room temperature shows all the complexes are non electrolytes (Geary, 1971).

Electronic spectral and magnetic studies

The magnetic moments and electronic spectral bands of the complexes are summarized in Table 2. The electronic spectrum of the Co(II) complex exhibit absorption bands at 16129 and 21276 cm^{-1} corresponding to ν_2 and ν_3 transitions, respectively $^4T_{1g}$ (F) → $^4A_{2g}$ (F) (ν_2) and $^4T_{1g}$ (F) → $^4T_{2g}$ (P) (ν_3) The ν_1 transition is not observed in these Co(II) complex because of its proximity to the strong ν_3. Cobalt (II) complex display a magnetic moment value of 4.75 BM, which is within the range of 4.46-5.53 BM which correspond to high spin octahedral environment around the metal ion (Methaq, 2014).

The Cu(II) complex a band observed at 14705–17271 cm^{-1} was assigned to the transitions $^2B_{1g} \rightarrow {}^2A_{2g,}$ (ν_1), $^2B_{1g} \rightarrow {}^2E_g$ (ν_2) and $^2B_{1g}$ → $^2B_{2g}$ (ν_3), which is characteristic of octahedral geometry. The Cu(II) complex showed magnetic moment value of 1.99 BM, which is within the range of 1.75–2.20 BM is consistent with octahedral geometry (Prakash *et al*., 2008). The ligand field parameters Dq, *B'*, *β*, *β%*, $\nu_{2/}\nu_1$, LFSE have been calculated using the procedure given by Drago (Drago, 1968).

IR spectral studies

The important IR spectral data are given in Table 3. On complexation the bands at 1590 and 1594 cm^{-1} for the N-N=C and CH_2-N=C groups in the free ligand was shifted to lower frequency in the range ~1580-1566 and ~1590-1561 cm^{-1} indicating the coordination of the C=N nitrogen atom to the metal ion (Usharani *et al*., 2013). The sharp bands at 1689 cm^{-1} for indole C=O group and 1680 cm^{-1} for C=O of the free ligand has been shifted to ~1671-1665 and 1675-1660 cm^{-1} in the complex indicating the linkage between the metal ion and carbonyl oxygen atom (Disha *et al*., 2013). Bands of indole NH, C-NH and O=C-NH and ring C=N remains almost unafected indicating the non participation of these groups in coordination (Ajaykumar *et al*., 2009). Further, the spectrum of all the metal complexes show new bands in the 445–477 and 350-377 cm^{-1} region, which may probably be due to the formation of M-N and M-Cl bonds, respectively (Aurora *et al*., 2014).

Mass spectral studies

The mass spectrum of the Schiff base showed a molecular ion peak at *m/Z* 552, which is equivalent to its molecular weight. The mass spectrum of Zn(II) complex showed a molecular ion peak at *m/Z* 688 which is equivalent to its molecular weight of the complex. Hence supporting the suggested structre for the complex.

Table 2. Electronic spectral bands and ligand field parameters of the Co(II), Ni(II) and Cu(II) complexes in DMF (10^{-3} M) solution

Complexes	Transitions in cm^{-1} ν_1	ν_2	ν_3	Dq (cm^{-1})	*B'* (cm^{-1})	*β*	*β%*	$\nu_{2/}\nu_1$	LFSE (k cal)
[$CoLCl_2$]	7389	16129	21276	873	1015	0.97	2.32	2.18	14.98
[$NiLCl_2$]	9705	15625	25641	970	810	0.77	22.11	1.60	32.27
[$CuLCl_2$]	-	14705-17271	-	1598	-	-	-	-	27.40

Table 3. IR spectral bands of the ligand and its metal complexes (cm^{-1})

Tentative assignments	L	[$CoLCl_2$]	[$NiLCl_2$]	[$CuLCl_2$]	[$ZnLCl_2$]	[$CdLCl_2$]	[$HgLCl_2$]
N-N=C	1590	1580	1575	1568	1575	1566	1578
CH_2-N=C	1594	1590	1561	1577	1583	1588	1583
Indole *ν*(C=O)	1689	1671	1666	1678	1665	1678	1680
ν(C=O)	1680	1670	1668	1660	1671	1675	1673
ν(M-N)	-	445	451	450	452	461	477
ν(M-Cl)	-	350	355	351	358	370	377

The Ni(II) complex show two bands at 15625 and 25641 cm^{-1} assigned to $^3A_{2g}$ (F) → $^3T_{1g}$ (F) (ν_2), $^3A_{2g}$ (F) → $^3T_{1g}$ (P) (ν_3) transitions, respectively, in an octahedral environment. The lowest band ν_1 was not observed due to limted range of the instrument used. Ni(II) complex showed the magnetic moment value of 2.80 BM, which is within the range of 2.7-3.3 BM suggesting consistency with their octahedral environment (Priya and Lakshmi, 2014).

^{1}H-NMR spectral studies

^{1}H-NMR spectra of the ligand and its Zn(II) complex was recorded in DMSO-d^6. The proton NMR spectra of Schiff base displayed singlet each at 2.2, 2.4, 10.1, 10.2, 10.4 ppm and 1.37 ppm respectively, due to the 2 proton of $–CCH_2C-$, 2 protons of H_2C-N=C, one protons of C-NH, one proton of indole NH, one proton of O=C-NH and six protons of 2 methyl groups. The aromatic protons of Schiff base resonated as multiplets in the region 7.3-7.9 ppm (m, 7H). The aromatic protons singles in the spectrum of the Zn(II) complex is shifted downfield compared to the ligand (Siddappa and Nabiya sultana, 2014).

Thermo gravimetric analysis

Thermal analyses (TGA and DTA) of the Cu(II) complex is used to get information about the thermal stability of this copper complex and suggest a general scheme for thermal decomposition of this copper complex. In the present investigation, heating rates were suitably controlled at 10°C /min under nitrogen atmosphere, and the weight loss was measured from the ambient temperature up to 40–750°C (Gehad geindy *et al.*, 2006). The complex is stable up to 200°C and exhibits a two step decomposition pattern, as is evident from the TG-DTA profile. The mass loss in the first step occurred at 289.14°C which can be attributed to the loss of chloride molecule. The complex exhibited second decomposition step at 370.91°C which can be attributed to the loss of ligand moiety further leaving behind the metal oxide residue. The complex shows gradual degradation upto 708.9°C (Prasad *et al.*, 2011).

ESR spectrum of Copper complex

ESR spectrum of copper complex has been recorded. The Cu(II) complex exhibit typical axial behavior with slightly different $g_{||}$ and $g_{\perp}$ values. The geometric parameter G, which is a measure of exchange interaction between the copper centres in the polycrystalline compounds, is calculated by using the expression $G = (g_{||} - 2.00277) / (g_{\perp} - 2.00277)$ (Lekha *et al.*, 2013). For copper complex, $g_{||} = 2.177$ $g_{\perp} = 2.058$, $g_{av} = 2.058$ and G = 3.122 have been observed at room temperature. The axial symmetry parameter G is less than 4 indicating considerable exchange interaction in the solid complex (Kiran *et al.*, 2014).

Powder X-ray diffraction

The x-ray powder diffraction patterns for the new Cu(II) complex is depicted in Fig. 2. There are 8 reflections (2θ) between 26.915 to 79.550 with maxima at 2θ = 40.608 corresponding to the value of d = 2.219. The XRD data of Cu(II) complex is given in the Table 4.

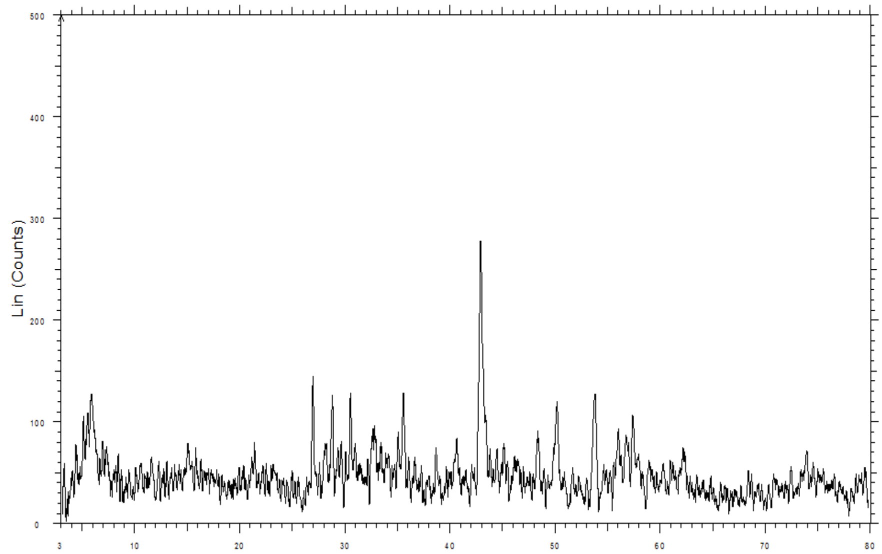

Fig. 2. Powder XRD pattern of Cu(II) Complex

Table 4. X-ray diffraction data of Cu(II) Complex

Peak no.	2θ	θ	Sin θ	$\sin^2\theta$	$h^2+k^2+l^2$	hkl	d Value		a in Å
							abs	Cal	
1	26.915	13.457	0.2327	0.0541	1	1 0 0	3.3099	3.3086	3.308
2	28.811	14.405	0.2487	0.0618	1.1427 (1)	1 0 0	3.1710	3.0950	3.308
3	28.743	14.371	0.2482	0.6160	1.1374 (1)	1 0 0	3.1034	3.1022	3.308
4	30.514	15.257	0.2631	0.0692	1.2785 (1)	1 0 0	2.9272	2.9260	3.308
5	32.777	16.388	0.2821	0.0796	1.4698 (1)	1 0 0	2.7301	2.7290	3.308
6	35.544	17.772	0.3052	0.0931	1.7201 (2)	1 1 0	2.5236	2.5226	3.308
7	38.687	19.343	0.3312	0.1097	2.0257 (2)	1 1 0	2.3255	2.3246	3.308
8	40.608	20.304	0.3470	0.1204	2.2232 (2)	1 1 0	2.2198	2.2190	3.308
9	42.917	21.458	0.3658	0.1338	2.4709 (2)	1 1 0	2.1056	2.1048	3.308
10	48.371	24.185	0.4096	0.1678	3.0990 (3)	1 1 1	1.8801	1.8794	3.308
11	50.182	25.091	0.4240	0.1798	3.3202 (3)	1 1 1	1.8164	1.8157	3.308
12	53.783	26.891	0.4523	0.2045	3.7772 (4)	2 2 0	1.7030	1.7024	3.308
13	56.803	28.405	0.4756	0.2262	4.1772 (4)	2 2 0	1.6194	1.6188	3.308
14	57.417	28.708	0.4803	0.2307	4.2602 (4)	2 2 0	1.6036	1.6029	3.308
15	79.550	39.775	0.6397	0.4093	7.5573 (8)	2 2 0	1.2038	1.2035	3.308

The interplanar spacing (d value, Angstrom) has been calculated. The values of d value (the volume average of the crystal dimension normal to diffracting plane) full width at half maximum of prominent intensity peak and particle size of Cu(II) complex was estimated. The $h^2+k^2+l^2$ values of the copper complex were found to be 1, 1, 1, 1, 2, 2, 2, 2, 3, 3, 4, 4, 4, 8. The absence of forbidden number indicates that the Cu(II) complex may belongs to cubic symmetry (Foziah, 2014).

Pharmacology Results

In vitro antimicrobial screening results

The *in vitro* antimicrobial screening results are given in Table 5. The synthesized Schiff base has an inhibitory effect (MIC values of 75–100 mg mL^{-1}) on growth of the tested bacterial strains. All complexes showed greater bactericidal activities against *E. coli* (MIC 12.50–50 µg mL^{-1}), *P. vulgaris* (MIC 12.50–50 µg mL^{-1}) and *S. pyogones* (MIC 12.50–50 µg mL^{-1}) than the ligand. In the fungal studies, the ligand had an inhibitory effect (MIC values in range 50–100 µg mL^{-1}) on the growth of the tested strains and complexes again showed greater fungicidal activities against *A. flavus* (MIC 12.50–25 µg mL^{-1}), *C. albicans* (MIC 12.50–50 µg mL^{-1}) and *F. solani* (MIC 12.50–50 µg mL^{-1}). Cd(II) and Hg(II) complexes had greater bacterial and fungal activities than the ligand. All of the tested compounds showed good biological activity against microorganism. The results of the investigations account for the antipathogenic behavior of the compounds and this efficacy is positively modified on complexation (Fakruddin *et al*., 2014).

DNA cleavage activity results

Agarose gel electrophoresis experiment using Calf-thymus DNA were performed with Schiff base and its Co(II), Ni(II), Cu(II), Cd(II), Zn(II) and Hg(II) complexes in the presence of H_2O_2 as an oxidant. From Fig. 3, it is evident that the Co(II), Ni(II), Cu(II), Cd(II) and Hg(II) complexes cleave DNA completely whereas the Zn(II) show partial cleavage activity in the presence of H_2O_2 (Jeena and Abbs, 2014). Probably this may be due to the formation of redox couple of the metal ions and its behaviour. Further, the presence of a smear in the gel diagram indicates the presence of radical cleavage (Raman *et al*., 2007).

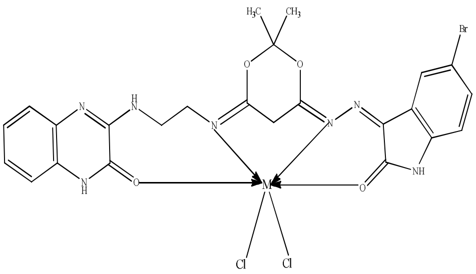

Fig. 4. **Proposed structure of M = Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II) metal complexes**

Conclusion

In this research article, we have synthesized a new Schiff base and its metal complexes. The bonding of ligand to the metal ions has been confirmed by the analytical data and various spectral studies.

Table 5. Antimicrobial activity of ligand and its metal (II) complexes evaluated by MIC (µg mL^{-1})

Compounds	*E. coli*	*P. vulgaris*	*S. pyogones*	*A. flavus*	*C. albicans*	*F. solani*
L	75	75	100	50	75	100
[$CuLCl_2$]	50	50	12.50	25	12.50	50
[$CoLCl_2$]	25	50	50	25	50	25
[$NiLCl_2$]	50	25	12.50	25	25	12.50
[$HgLCl_2$]	12.50	12.50	12.50	12.50	12.50	12.50
[$CdLCl_2$]	12.50	12.50	12.50	12.50	12.50	12.50
[$ZnLCl_2$]	25	50	25	25	25	25
Ciprofloxacin	12.50	12.50	12.50	-	-	-
Nystatin	-	-	-	12.50	12.50	12.50

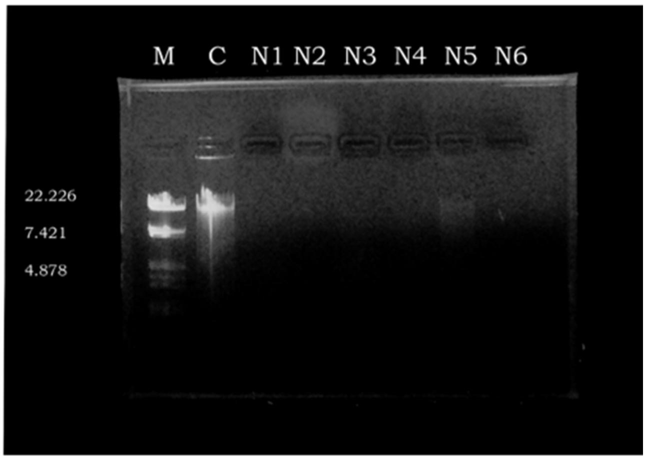

Fig. 3. Gel picture showing the cleavage analysis of samples

The Cd(II) and Hg(II) complexes have exhibited an enhanced antimicrobial activity whereas Co(II), Ni(II), Cu(II) and Zn(II) complexes have shown a moderate activity than its ligand. The DNA cleavage studies revealed that the metal complexes showed good efficiency towards DNA cleavage. On the basis of above mentioned studies we proposed the octahedral geometry for the metal complexes (Fig. 4).

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