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Synthesis, Characterization And Biological Studies of Metal Complexes With 3-Amino-5-Bromobenzofuran-2-Carboxamide Schiff Base

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ABSTRACT

In this study the new Schiff base 5-bromo-3-(((2-chloroquinolin-3-yl)methylene)amino)benzofuran-2-carboxamide and its metal complexes of Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II) have been synthesized. The structural features were determined on the basis of their elemental analysis, UV-Visible, magnetic susceptibility, molar conductance and characterized by IR, UV– Vis, ^1H NMR, mass data. All the complexes are of ligand is ML_2 which is 1:2 ratio of octahedral geometry. The antimicrobial activity is carried out and some of the complexes have exhibited good biological activity. In addition, the influence of DNA cleavage on all the complexes was analyzed by agarose gel electrophoresis method.

Keywords: Benzofuran, quinoline, Schiff base, DNA cleavage, antimicrobial activity.

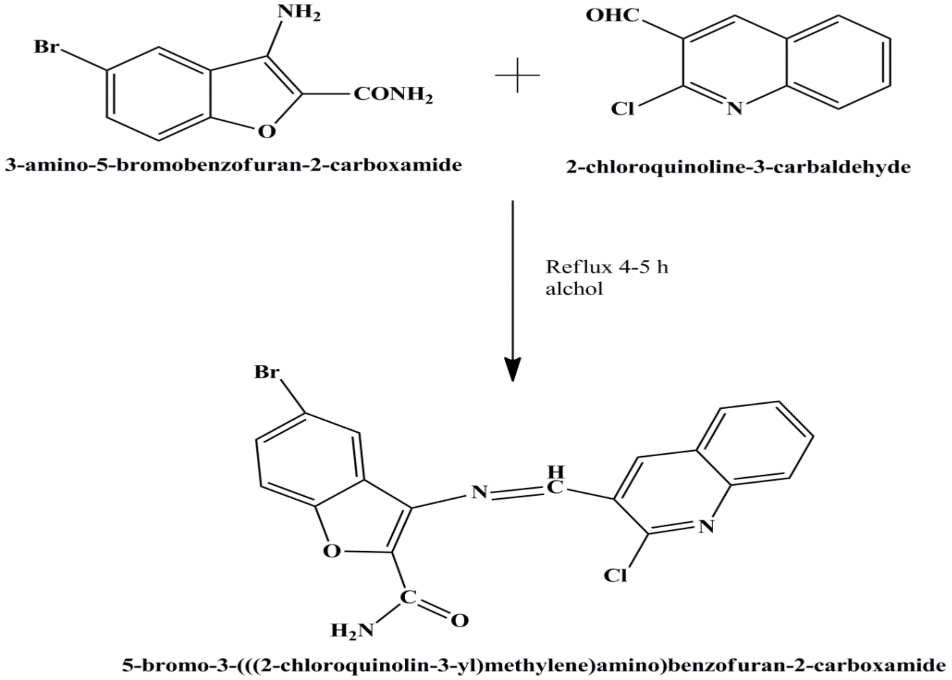
INTRODUCTION

The Schiff bases have wide applications in food industry, dye industry, analytical chemistry, catalysis, fungicidal, agrochemical and biological activities. Benzofuran compounds are abundant in nature, particularly among plants; often natural products possessing benzofuran have useful pharmacological properties. Benzofuran compounds occur in nature in a variety of structural forms, the seed oil of plant ‘‘Egonoki’’ which contains a benzofuran derivative called ‘‘Egonal,’’ an effective synergist for rotenone pyrethrum against house flies, mosquitoes, aphids and many other insects [1]. Potent pharmacological properties have been associated with 2-chloroquinoline-3-carbaldehydes and their derivatives [2]. These compounds have shown antimicrobial, antimalarial, anti-inflammatory, antitumor, and anti-parasitic activity [3-7]. Prompted by these observations we have synthesized some metal complexes of Schiff base formed by the condensation of 3-amino-5-bromobenzofuran-2-carboxamide and 2-chloroquinoline-3-carbaldehyde.

MATERIALS AND METHODS

All the chemicals were of analytical grade. 3-amino-5-bromobenzofuran-2-carboxamide and 2-chloroquinoline-3-carbaldehyde were prepared by the literature procedure [8, 9].The metal and chloride contents are determined as per Vogel’s procedure [10].

Synthesis of Schiff base: A mixture of 3-amino-5-bromobenzofuran-2-carboxamide (0.01mol) and 2-chloroquinoline-3-carbaldehyde (0.01mol) in 40mL hot ethanolic medium was boiled under reflux for 4–5 h on a water bath. The light yellowish solid separated on partial evaporation of the solvent and subsequent cooling was filtered, washed with ethanol and finally recrystallized from ethanol. The purity of the Schiff base was checked by TLC.



Scheme 1. Synthesis of Schiff base ligand

Synthesis of metal complexes: The metal complexes were prepared using metal chlorides and the Schiff base by the general method. An ethanolic solution (40 mL) of Schiff base (2 mmol) and Co(II), Ni(II), Cu(II), Cd(II), Zn(II) and Hg(II) (1 mmol) chlorides was refluxed on water bath for about 4 h. An aqueous solution of sodium acetate was added to the reaction mixture to adjust the pH to 6.0-7.0 and reflux was further continued for about an hour. The separated solid complexes were filtered off, washed with little warm ethanol. The complexes obtained were finally dried under vacuum desiccator over fused $CaCl_{2.}$

Pharmacology: The Schiff base and Co(II), Ni(II), Cu(II), Zn(II), Cd(II), and Hg(II) complexes have been studied for their antibacterial and antifungal activities against *Bascillus subtilis* and *Escherichia coli* bacteria and *Aspergillus niger* and *Aspergillus flavus* fungi by the agar diffusion method in DMF as per the literature procedure [11, 12].

DNA Cleavage study

Agarose gel electrophoresis: Cleavage products were analyzed by agarose gel electrophoresis method [13]. Two hundred and fifty milligrams of agarose was weighed and dissolved in 25 ml of TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA L^{-1}) by boiling. When the gel attained 55 °C, it was poured into the gel cassette fitted with comb and left to get it solidified. The comb was carefully removed and gel was placed in the electrophoresis chamber flooded with TAE buffer. Twenty micro liters of DNA sample was loaded (mixed with bromophenol blue dye @ 1:1 ratio), carefully into the wells, along with standard DNA marker and a constant 50 V of electricity was passed for around 45 min. The gel was removed and carefully stained with ETBR solution (10 µg mL^{-1}) for 10-15 min and the bands were observed under UV transilluminator. The results were then compared with standard DNA marker.

RESULTS AND DISCUSSION

All the synthesized complexes were light in color, stable, non-hygroscopic in nature. These complexes are insoluble in common organic solvents but are remarkably soluble in DMSO and DMF. The analytical data (Table 1) showed that the complexes had stoichiometry.

Table 1. Elemental analysis, magnetic susceptibility and molar conductance data of Schiff base and its metal complexes.

Ligand / Complex	Mol. Wt. (g/mol)	M. p. (˚C)	Elemental Analysis Found(Calculated) %					μ_{eff} (BM)	Λ_M*
			C	H	N	Cl	M		
$C_{19}H_{11}BrClN_3O_2$	429.67	254	53.15 (53.24)	2.19 (2.59)	9.41 (9.80)	8.04 (8.27)	-	-	-
$[Co(C_{19}H_{11}BrClN_3O_2)_2Cl_2]$	988.27	>300	46.04 (46.23)	2.11 (2.25)	8.23 (8.51)	14.25 (14.37)	5.58 (5.97)	4.85	10.21
$[Ni(C_{19}H_{11}BrClN_3O_2)_2Cl_2]$	986.03	285	46.15 (46.42)	2.14 (2.25)	8.30 (8.52)	14.18 (14.37)	5.78 (5.95)	3.25	15.44
$[Cu(C_{19}H_{11}BrClN_3O_2)_2Cl_2]$	992.88	>300	45.85 (46.02)	2.14 (2.24)	8.30 (8.47)	14.25 (14.30)	6.25 (6.41)	1.92	11.25
$[Cd(C_{19}H_{11}BrClN_3O_2)_2Cl_2]$	1040.74	275	43.20 (43.86)	2.01 (2.13)	7.86 (8.08)	12.36 (13.63)	10.66 (10.80)	-	14.60
$[Zn(C_{19}H_{11}BrClN_3O_2)_2Cl_2]$	994.72	290	49.51 (45.93)	2.16 (2.23)	9.48 (8.46)	7.01 (14.27)	7.01 (6.58)	-	16.11
$[Hg(C_{19}H_{11}BrClN_3O_2)_2Cl_2]$	1028.92	>300	40.21 (40.43)	1.67 (1.96)	7.18 (7.44)	12.21 (12.56)	17.61 (17.77)	-	12.03

* Molar conductance values in ohm^{-1}cm^{2} mol^{-1}

IR studies: The IR spectral data of the Schiff base and its complexes are presented in table 2. The IR spectrum of the free Schiff base ligand showed a band at 3280 cm^{-1} are assigned to NH_2 stretching vibrations of primary amide group in Schiff base, which was practically remain unchanged in the spectra of its complexes suggesting nonparticipation of NH_2 group on chelation [8]. The strong band observed at 1680 cm^{-1} in the free ligand was attributed to ν (C=O) stretch of carbonyl group. This band shifted to a lower wave number side in all the complexes indicates the participation of the carbonyl oxygen in bonding with metal ions. A band observed at 1552 cm^{-1} was attributed to ν(C=N) stretch of the quinoline ring which remains unaltered in all the complexes confirming non coordination of ring nitrogen in bonding. A medium-to strong intensity band at 1589 cm^{-1} in the free ligand was attributed to ν(C=N) stretch of the azomethine group. Coordination of the Schiff's base to the metal ions through the nitrogen atom is expected to reduce electron density in the azomethine link and lower the ν(C=N) stretching absorption frequency. This band shifted to a lower wave number side in all the complexes indicates the participation of the azomethine nitrogen in coordination with metal ions. ν(C–O–C) stretching vibrations of furan moiety in the region 1031–1,247 cm^{-1} have been reported in literature [1]. In this study, the ν(C–O–C) stretching vibration is observed at 1,206 cm-1, remains unaltered in the metal complexes, indicating non-participation of the furan ring oxygen atom in bonding with the metal ions. The weak intensity non-ligand bands in the region 554–566 and 448-468 cm^{-1} in the spectra of the complexes are assigned to frequencies of ν(M–O) and ν(M–N) stretching vibrations, respectively [14].

Table 2. The infrared frequencies (in cm^{-1}) of the Schiff base and its complexes

Ligand / Complex	ν_{NH}	$\nu_{C=O}$	$\nu_{C=N}$	ν_{M-O}	ν_{M-N}
$C_{19}H_{11}BrClN_3O_2$	3280	1680	1589	-	-
$[Co(C_{19}H_{11}BrClN_3O_2)_2Cl_2]$	3286	1659	1577	566	460
$[Ni(C_{19}H_{11}BrClN_3O_2)_2Cl_2]$	3274	1651	1563	561	467
$[Cu(C_{19}H_{11}BrClN_3O_2)_2Cl_2]$	3285	1646	1574	566	463
$[Cd(C_{19}H_{11}BrClN_3O_2)_2Cl_2]$	3284	1669	1578	562	448
$[Zn(C_{19}H_{11}BrClN_3O_2)_2Cl_2]$	3288	1634	1556	561	468
$[Hg(C_{19}H_{11}BrClN_3O_2)_2Cl_2]$	3281	1670	1582	554	464

^{1}H NMR studies: The ^{1}H NMR spectrum of the Schiff base ligand (Fig. 1)was recorded in DMSO-d_6. The signal at δ (9.23) (s, 1H, >CH=N) due to azomethine proton, δ (5.62) (s, 2H, NH_2) due to amine respectively. Upon complex formation, the downfield shift of azomethine proton from δ 9.23 (s, 1H, >CH=N) of the Schiff base to δ 9.65 (s, 1H, >CH=N) in complexes, indicate the involvement of >CH=N nitrogen. The aromatic protons of the Schiff base ligand at δ (6.86-8.51) (m, 8H) shifted downfield in the complexes.

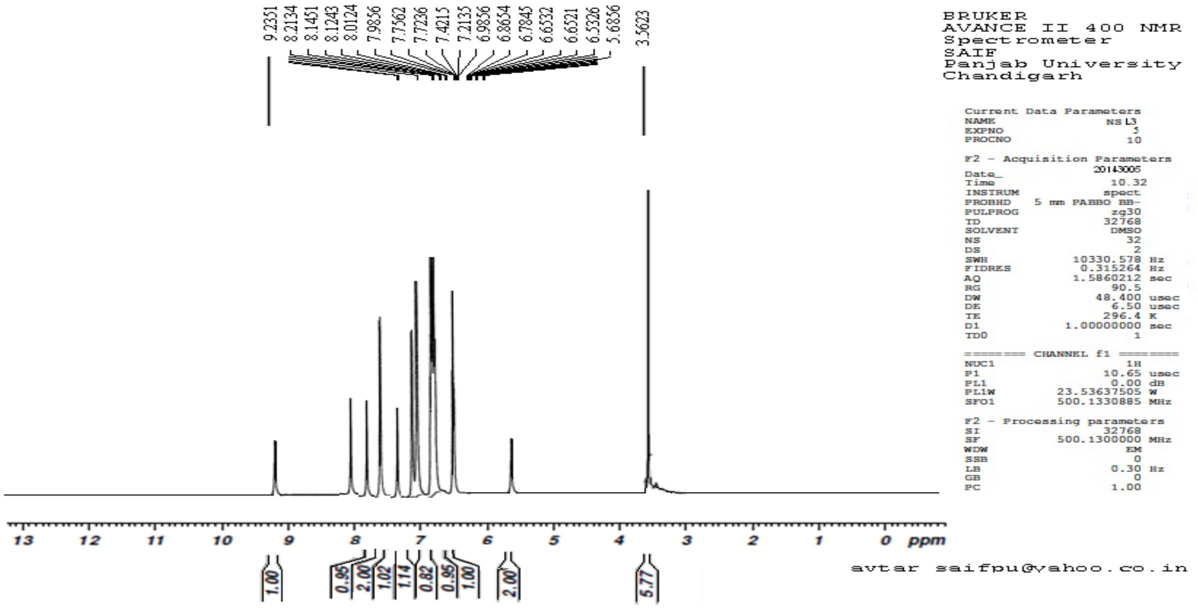


Fig. 1: ^{1}HNMR spectra of ligand

Mass studies: Mass spectroscopy has been increasingly used as a powerful structural characterization technique in coordination chemistry. The LC–MS of the Schiff's base showed a molecular ion peak at m/z 429, which is equivalent to its total molecular weight of the ligand. The mass spectrum of Zn(II) complex showed a molecular ion peak at m/z 994.72 which is equivalent to the molecular weight of the complex.

Electronic spectral studies: The electronic absorption spectra of the Co(II), Ni(II) and Cu(II) complexes were recorded in freshly prepared DMF solution (10^{-3} M) at room temperature and the spectral data are presented in table 3. The electronic spectrum of Co(II) complex displayed bands at 16865 and 19678 cm^{-1}, assignable to $^4T_{1g}$ (F) → $^4A_{2g}$ (F) (ν_2) and $^4T_{1g}$ (F) → $^4T_{2g}$ (P) (ν_3) transitions respectively, in an octahedral environment [15]. The ν_1 band was calculated using the band fitting procedure suggested by Underhill and Billing [16]. The electronic spectrum of Ni(II) complex exhibited two absorption bands, at 18942 and 20890 cm^{-1}. These two bands are assignable to $^3A_{2g}$ (F) → $^3T_{1g}$ (F) (ν_2) and $^3A_{2g}$(F) → $^3T_{1g}$ (ν_3) transitions, respectively, in an octahedral environment.

Table 3. Electronic spectral data and ligand field parameters of Cu(II), Ni(II) and Co(II) Complexes

Complexes	Absorption bands(cm^{-1})			Dq (cm^{-1})	B′ (cm^{-1})	β	β%	$\nu_{2/}\ \nu_1$	LFSC (Kcal)
	ν_1	ν_2	ν_3						
[$Cu(C_{19}H_{11}BrClN_3O_2)_2Cl_2$]	12678-18412			1524	-	-	-	-	-
[$Co(C_{19}H_{11}BrClN_3O_2)_2Cl_2$]	8240	16865	19678	862	845	0.87	11.2	1.93	14.7
[$Ni(C_{19}H_{11}BrClN_3O_2)_2Cl_2$]	9521	18942	20890	942	771	0.79	20.5	1.98	16.1

The light green colored Cu(II) complex exhibited low intensity single broad asymmetric band at 12678-18412 cm^{-1}. The broadness of the band indicates the three transitions $^2B_{1g}$ → $^2A_{1g}$ (ν_1), $^2B_{1g}$ → $^2B_{2g}$ (ν_2) and $^2B_{1g}$ → ^2E_g (ν_3), which are of similar in energy and give rise to only one broad absorption band. The broadness of the band may be due to dynamic Jahn–Teller distortion. All of these data suggested a distorted octahedral geometry around the Cu(II) ion [17]. The octahedral geometry was further supported by the values of ligand field parameters, such as the Racah inter electronic repulsion parameter (B′), ligand field splitting energy (10 Dq), nephelauxetic parameter (β) and ligand field stabilization energy (LFSE) [18]. The (B′) values for the complexes were lower than the free ion values, which indicate the orbital overlap and delocalization of d-orbitals. The (β) values obtained were less than unity, suggesting a considerable amount of covalency for the metal–ligand bonds. Further, the (β) value for the Ni(II) complex was less than that of the Co(II) complex, indicating the greater covalency of the M–L bond .

Table 4. Antimicrobial activity results of the Schiff base and its metal complexes.

Compound	Concentration (μgmL^{-1})	Zone of inhibition against bacteria (mm)		Zone of inhibition against fungi (mm)	
		B. subtilis	*E. coli*	*A. flavus*	*A. niger*
$C_{19}H_{11}BrClN_3O_2$	50	8	9	8	8
	100	11	14	12	12
	200	16	17	14	15
[$Cu(C_{19}H_{11}BrClN_3O_2)_2Cl_2$]	50	15	15	13	12
	100	18	22	18	16
	200	25	27	23	20
[$Ni(C_{19}H_{11}BrClN_3O_2)_2Cl_2$]	50	14	14	11	10
	100	19	18	15	13
	200	23	26	20	19

$[Co(C_{19}H_{11}BrClN_3O_2)_2Cl_2]$	50	12	13	12	11
	100	16	16	15	14
	200	22	24	21	18
$[Zn(C_{19}H_{11}BrClN_3O_2)_2Cl_2]$	50	12	14	10	10
	100	16	18	15	13
	200	25	26	21	19
$[Cd(C_{19}H_{11}BrClN_3O_2)_2Cl_2]$	50	10	11	10	10
	100	15	16	14	14
	200	18	20	18	16
$[Hg(C_{19}H_{11}BrClN_3O_2)_2Cl_2]$	50	12	13	11	12
	100	14	16	14	15
	200	20	22	17	15
Gentamicine	200	27	30	-	-
Fluconazole	200	-	-	25	23

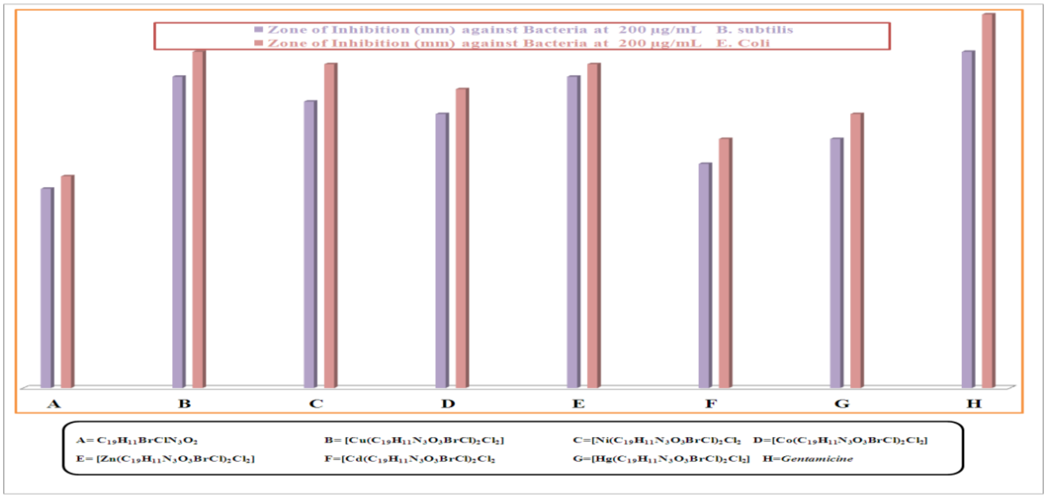


Fig. 2 Antibacterial results of Schiff base and its metal complexes.

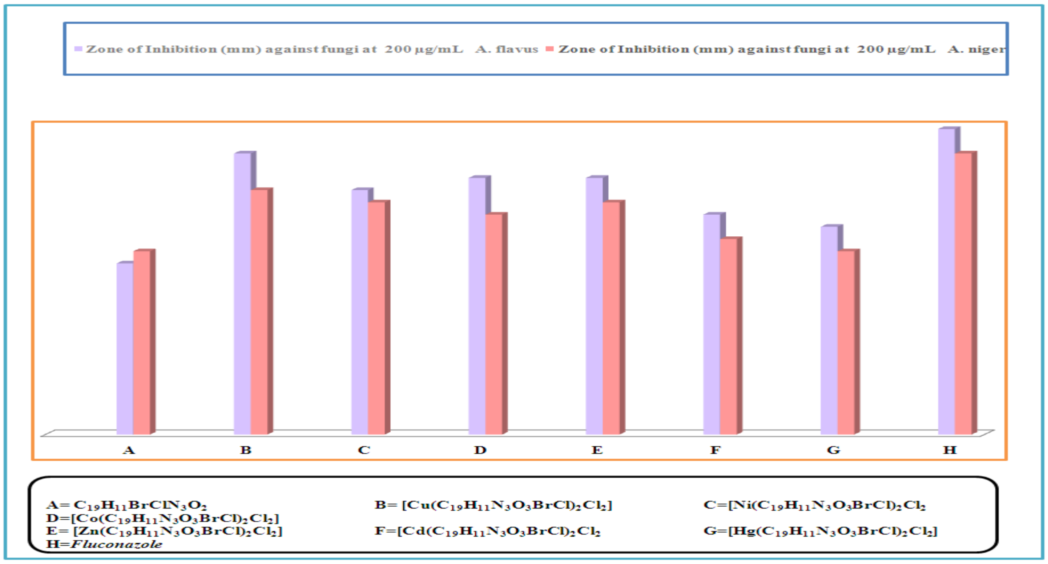


Fig. 3 Antifungal results of Schiff base and its metal complexes.

APPLICATIONS

Pharmacology Results

Antibacterial and Antifungal Analyses: The in vitro evaluation of antimicrobial activity of the Schiff base and its complexes used in the present study were screened against *E. coli, B. subtilis* bacteria and *A. niger, A. flavus* fungi. The microbial screening results were presented in table 4 and fig 2-3. The results reveal that the Schiff base was active and its metal complexes showed increased antibacterial and antifungal activity. Comparative study of the ligands and their complexes indicates complexes have higher antimicrobial activity than the free ligands. Antimicrobial activity of all the compounds was increased on coordination. The enhanced activity of the metal complexes over the Schiff base may be explained by chelation theory. On chelation the positive charge of metal is partially shared with the donor atoms present in the ligands, there may be π- electron delocalization over the whole chelating. This increases the lipophilic character of the metal chelate and favors its penetration through the lipid layer of the bacterial membranes [19].

DNA Cleavage Result: The Co(II), Ni(II), Cu(II), Zn(II), Cd(II), and Hg(II) complexes were studied for their DNA cleavage activity by the agarose gel electrophoresis method and are presented in figure 4. The gel after electrophoresis clearly revealed that the intensity of all of the treated DNA samples diminished, possibly because of the cleavage of the DNA and all the complexes have acted on DNA effectively. Since, as there was molecular weight difference between the control and the treated DNA samples. A difference was observed in the bands of the complexes (lanes 1-6) compared to that of the control DNA of *E. coli*. This indicates that the control DNA alone did not show any apparent cleavage, while the complexes did. However, the nature of the reactive intermediates involved in the DNA cleavage by the complexes is not clear. The results indicated the important role of metal ions 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 [19].

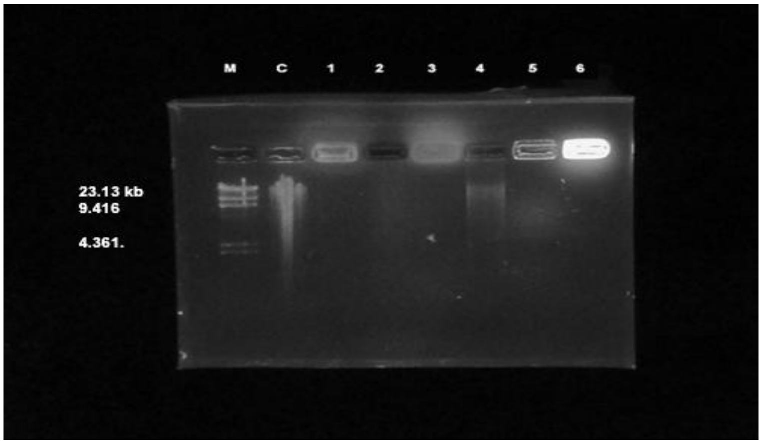


Figure 4. DNA cleavage activity **of** Cu(II), Ni(II), Co(II), Zn(II), Cd(II) and Hg(II) complexes

CONCLUSIONS

Based on the elemental analysis, spectral studies, conductance data and magnetic susceptibility data, these ligands are neutral bidentate, coordinating through the amide O and N of the azomethine group. The antimicrobial activity results indicate that all complexes have been found to be more effective than its ligand as the process of chelation dominantly affects the overall biological behavior of the compounds. All the complexes show good efficiency towards DNA cleavage. Based on the analytical and spectral studies, we propose octahedral geometry to all the complexes (Figure. 5).

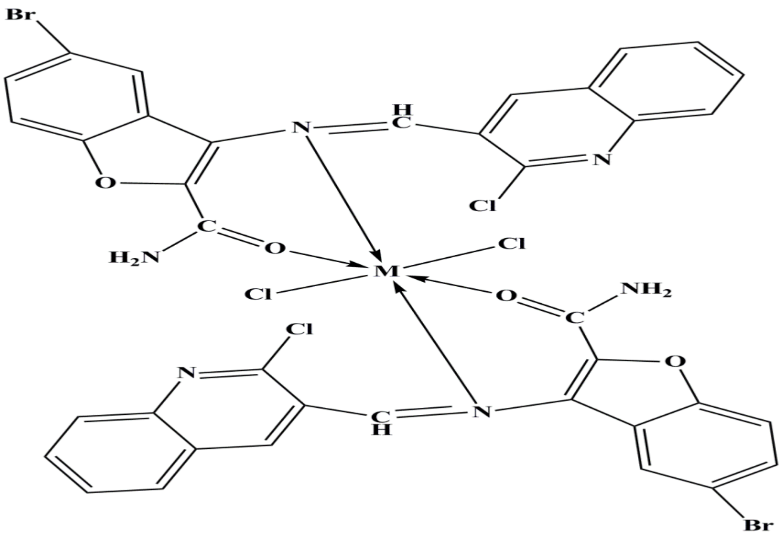


Figure 5. Proposed Structures of the complexes [M= Cu(II), Ni(II), Co(II), Zn(II), Cd(II) and Hg(II)].

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