SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF TRIAZINE BASED SCHIFF BASE METAL COMPLEXES

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A new series of Co(II), Ni(II), Cu(II) and Zn(II) complexes with a Schiff base derived from 4-amino-3-mercapto-5-oxo-1,2,4-triazine and 2,4-dichlorobenzaldehyde have been synthesized and characterized by spectroscopic studies. The coordination possibility of the Schiff base towards the metal ions have been determined by analytical, spectral (IR, ^{1}H NMR, electronic spectroscopy, fluorescence, ESR) and thermal techniques. IR and thermal data support the presence of coordinated water in the metal complexes. The low molar conductance values in DMF indicate the non-electrolytic nature of the metal complexes. All the synthesized metal complexes show enhancement in fluorescence intensity in comparison to the ligand. The cyclic voltammetric studies of the Co(II), Ni(II) and Cu(II) complexes suggested the single electron transfer quasi-reversible nature of the complexes. Antimicrobial studies of the ligand and its metal complexes have been carried out *in vitro* against gram positive (*S. aureus, B. subtilis*), gram negative bacteria (*E. coli, P. aeruginosa*) and yeasts (*S. cerevisiae, C. albicans*).

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Introduction

The increasing number of infectious diseases and development of resistance by pathogens to most of the known antibiotics is becoming a serious health problem. To conquer this rapid development of drug-resistance, the design and synthesis of new compounds with effective and potent antimicrobial activities is of substantial need for medicinal chemists. Numerous 1,2,4-triazines are biologically active[1-4] and could be used as preventive and therapeutic agents against various diseases. The development of new drugs based on 1,2,4-triazines such as anticancer drug; tirapazamine and potent anticonvulsant; lamotrigine further reinforces the interest in synthesis of these compounds. Also, it has been reported that the triazine ring skelton possess significant antibacterial, antioxidant, antifungal, anti-tuberculosis and anti-inflammatory activities.[5-9] In addition, they have been used in dyes, agriculture[10] and present an important core in many natural[11-12] and synthetic[13-14] biologically active compounds. Triazine derivatives with additional N or S donor atoms exhibit strong chelating ability and provide potential binding sites for complexation with various metal ions.[15-16] Apart from the wide range of applications, the varied structural aspects of the coordination compounds derived from triazine derivatives cause considerable interest in their synthesis. The complexation of Schiff bases with transition metal ions further enhances their antimicrobial properties.[17-18] Recently, a novel antifungal agent named CTBT (7-chlorotetrazolo[5,1-c]benzo[1,2,4]triazine had been identified which induced oxidative stress in filamentous fungi[19] resulting in cell death. Encouraged by the above findings and as a part of our continuous effort, here we report the synthesis of some Schiff base metal complexes bearing the 1,2,4-triazine moiety with a view to explore their potency as better antimicrobial agents.

Experimental

Standard gravimetric methods were used to estimate the metal contents; cobalt was estimated as cobalt pyridine thiocyanate, nickel as nickel dimethyl glyoximate, copper was estimated as cuprous thiocyanate and zinc as zinc ammonium phosphate.[20] The IR spectra of the Schiff base and its Co(II), Ni(II), Cu(II) and Zn(II) complexes were recorded on a MB-3000 ABB Spectrophotometer in the 4000-250 cm^{-1} region. The electronic spectra of the complexes were recorded in DMF on a T 90 (PG Instruments ltd) UV/Vis spectrophotometer in the region of 1100-200 nm. The ^{1}H NMR spectra were recorded in DMSO-d_6 on a Bruker 300 MH_Z ACF 300 spectrometer at room temperature using TMS as an internal reference. The fluorescence spectra of the ligand and its metal complexes were recorded in DMF on SHIMADZU RF-5301 PC spectrophotometer. Thermal analysis of metal complexes in the range 50-800 °C was carried out on a Perkin Elmer (Pyris Diamond) instrument at a heating rate of 10°C Min^{-1}. The ESR spectra were performed on X-Band at a frequency of 9.1 GHz on a Varian E-112 ESR spectrometer under the magnetic field 3000 Gauss at SAIF, IIT Bombay. Cyclic voltammeter measurements were carried out in DMF on an Ivium Stat Electrochemical Analyzer using tetrabutylammonium perchlorate as supporting electrolyte. Magnetic moment measurements were carried out on a Vibrating Sample Magnetometer (Model 155) at Institute Instrumentation Centre, IIT Roorkee. The molar conductivity measurements of all the complexes were measured in DMF using 10^{-3} M solutions at room temperature on a Systronics-306 Conductivity Bridge.

Table 1. Analytical data of the ligand and its metal complexes

S. No.	Compound	Yield%	Elemental analysis calcd. (found) %			
			C	H	N	M
1	Schiff base ($C_{10}H_6N_4OSCl_2$)	84 %	39.88 (39.91)	2.01 (2.02)	18.60 (18.54)	-
2	Co(L)(OAc).3H_2O [$C_{12}H_{14}N_4O_6SCl_2Co$]	81 %	30.52 (30.47)	2.99 (3.02))	11.87 (11.83)	12.48 (12.42)
3	$Co(L)_2$.2H_2O [$C_{20}H_{14}N_8O_4S_2Cl_4Co$]	83 %	34.55 (34.57)	2.03 (2.03)	16.12 (16.18)	8.48 (8.46)
4	Ni(L)(OAc).3H_2O [$C_{12}H_{14}N_4O_6SCl_2Ni$]	82 %	30.54 (30.59)	2.99 (2.94)	11.87 (11.82)	12.44 (12.49)
5	$Ni(L)_2$.2H_2O [$C_{20}H_{14}N_8O_4S_2$ Cl_4Ni]	84 %	34.56 (34.49)	2.03 (2.08))	16.12 (16.11)	8.44 (8.41)
6	Cu(L)(OAc).H_2O [$C_{12}H_{10}N_4O_4SCl_2Cu$]	85 %	32.70 (32.65)	2.29 (2.32)	12.71 (12.68)	14.42(14.41))
7	$Cu(L)_2$ [$C_{20}H_{10}N_8O_2S_2Cl_4Cu$]	84 %	36.19 (36.23)	1.52 (1.54)	16.88 (16.84)	9.57 (9.58)
8	Zn(L)(OAc).3H_2O [$C_{12}H_{14}N_4O_6SCl_2Zn$]	77 %	30.11 (30.17)	2.95 (2.91)	11.71 (11.74)	13.66 (13.61)
9	$Zn(L)_2$.2H_2O [$C_{20}H_{14}N_8O_4S_2$ Cl_4Zn]	79 %	34.23 (34.21)	2.01 (2.06)	15.97 (15.94)	9.32 (9.34)

4-Amino-3-mercapto-5-oxo-1,2,4-triazine[21] was prepared by reported literature method.

4-(2,4-dichlorobenzylideneamino)-3-mercapto-5-oxo-1,2,4-triazine

To a solution of 4-amino-3-mercapto-5-oxo-1,2,4-triazine (0.5 g, 3.47 mmol) in absolute alcohol was added a solution of 2,4-dichlorobenzaldehyde (0.607 g, 3.47 mmol) in absolute alcohol and refluxed for four hours. The volume of the solution was reduced on a rotary evaporator. The product formed was filtered off, washed with ice cold methanol and recrystallized from the same solvent. Yield 84%; m.p.: 232-235°C

Synthesis of metal complexes (1:1)

The solutions of 0.20 g (0.66 mmol) of the ligand dissolved in ethanol were added with stirring to hot ethanolic solutions of Co(II) acetate (0.17 g, 0.66 mmol), Ni(II) acetate(0.17 g, 0.66 mmol), Cu(II) acetate (0.13 g, 0.66 mmol) and Zn(II) acetate (0.15 g, 0.66 mmol) respectively. The corresponding solid complexes were filtered, washed several times with warm water, followed by ethanol and acetone and finally dried.

Synthesis of metal complexes (1:2)

The hot ethanolic solutions of Co(II) acetate (0.17 g, 0.66 mmol), Ni(II) acetate (0.17 g, 0.66 mmol), Cu(II) acetate (0.13 g, 0.66 mmol) and Zn(II) acetate (0.15 g, 0.66 mmol) were added to the hot ethanolic solutions of the ligand (0.40 g, 1.32 mmol) with continuous stirring. The precipitates formed immediately were filtered off, washed with warm water, ethanol followed by acetone and dried.

Biological assay

The antimicrobial activity of the synthesized compounds was evaluated *invitro* by the agar well diffusion method against the bacteria (*Staphylococcus aureus* MTCC 96; *Bacillus subtilis* MTCC 121, *Escherichia coli* MTCC 1652; *Pseudomonas aeruginosa* MTCC 741) and yeasts (*Candida albicans* MTCC 227; *Saccharomyces cerevisiae* MTCC 170). The bacteria were subcultured on Nutrient agar whereas yeast on Malt yeast agar. Ciprofloxacin and amphotericin B was used as the standard antibacterial and antifungal drug respectively. All the microbial cultures were procured from Microbial Type Culture Collection (MTCC); IMTECH, Chandigarh. The activity was measured in terms of zone of inhibition appearing around the well against bacteria and fungus. The procedure was performed in three replicate plates for each organism and the mean values of the diameter of inhibition zones ± standard deviations were calculated.

The *in vitro* minimal inhibitory concentrations **(**MICs) of the various compounds were determined by modified agar well diffusion method[15].

Results and discussion

The elemental analysis data of the metal complexes is given in Table 1. The Schiff base is soluble in common organic solvents whereas the metal complexes are only soluble in DMF and DMSO. All complexes are colored non-hygroscopic solids and stable at room temperature. The molar conductance values found (12-16 ohm^{-1} cm^2 mol^{-1}) indicate non-electrolytic behavior of all the complexes.

IR spectra

The IR spectra of the complexes are consistent with the structural data given in this paper (Table 2). The band at 1605 cm^{-1} in the spectrum of the free Schiff base is assigned to the azomethine (–CH=N) group. This band is shifted to lower frequencies (1582-1589 cm^{-1}) in the spectra of metal complexes indicating the coordination of the –CH=N nitrogen to the metal ion.[22] A characteristic strong band at 2650 cm^{-1} due to -SH in the spectrum of ligand disappeared on complex formation[23] indicating deprotonation and coordination through sulphur. The spectra of the metal complexes exhibit a broad band around 3171-3410 cm^{-1} due to ν (OH) which is assigned to water molecules associated with the complexes.[24] The coordination of ligand to the metal centers through the azomethine nitrogen atom and sulfur atom of the triazine ring is further substantiated by ν (M-N)[25,26] and ν (M-S)[26] stretching bands in the range 460-470 cm^{-1} and 315-345 cm^{-1} respectively, in the spectra of metal complexes confirming binding of the ligand to center metal ions.

Table 2. Important IR spectral bands (cm^{-1}) of Schiff base and its metal complexes

Compound	ν(N=CH)	ν(C-S)	ν(S-H)	ν($OOCCH_3$)	ν(H_2O/OH)	ν(M-S)	ν(M-N)
Schiff base	1605	-	2650	-	-	-	-
Co(L)(OAc).3H_2O	1582	764	-	1736	3255	345	470
Co(L)$_2$.2H_2O	1589	756	-	-	3325	318	468
Ni(L)(OAc).3H_2O	1582	756	-	1744	3255	341	470
Ni(L)$_2$.2H_2O	1582	756	-	-	3374	342	470
Cu(L)(OAc).H_2O	1586	756	-	1774	3410	318	468
Cu(L)$_2$	1589	756	-	-	-	315	470
Zn(L)(OAc).3H_2O	1582	756	-	1744	3171	342	470
Zn(L)$_2$.2H_2O	1587	756	-	-	3286	342	468

^{1}H NMR Spectra

The ^{1}H NMR data of the Schiff base and its Zn(II) complexes have been summarized in Table 3. A singlet at δ 8.77 in the spectrum of ligand is assigned to the proton attached to imino (-CH=N-) group. The signal of azomethine proton shows a downfield shift in the spectra of 1:1 and 1:2 Zn(II) complexes indicating the coordination of nitrogen with the metal ion. The spectrum of the free ligand exhibits a signal at 13.7 ppm for the thiol proton. The absence of the signal for –SH proton in the spectra of Zn(II) complexes indicates the coordination of Schiff base through sulfur atom to the metal ion by deprotonation of the thiol group[15]. The aromatic protons of the Schiff base appeared in the region 7.38 - 8.19 ppm. These signals remained almost unaltered in the spectra of metal complexes.

Table 3. ^{1}H NMR spectral data of Schiff base and its Zn(II) complexes

Compounds	^{1}H NMR (ppm)
Schiff base	8.19 (d, 1H, Ar-H), 7.38 (s, 1H, Ar-H), 7.57(s, 1H, triazine -H), 7.26 (d, 1H, Ar-H), 8.77 (s,1H, -N=CH-), 13.7 (s, 1H, -SH)
Zn(L)(OAc).3H_2O	8.16 (d, 1H, Ar-H), 7.59 (s, 1H, triazine-H), 7.39 (s, 1H, Ar-H), 7.25 (d, 1H, Ar-H), 8.82 (s, 1H, -N=CH-)
Zn(L)$_2$ .2H_2O	8.18 (d, 2H, Ar-H), 7.57 (s, 2H, triazine -H), 7.37 (s, 2H, Ar-H), 7.16 (d, 2H, Ar-H), 8.86 (s, 2H, -N=CH-)

Electronic spectra and magnetic moment studies

The electronic spectral and magnetic moment data are given in Table 4. Two absorption bands in the region, 10893 cm^{-1} – 11,086 cm^{-1} (ν_1) and 20492 - 21008 cm^{-1} (ν_3) have been observed in the electronic spectra of the 1:1 and 1:2 Co(II) complexes indicative of $^4T_{1g}$ (F) → $^4T_{2g}$ (F) and $^4T_{1g}$ (F) → $^4T_{1g}$ (P) transitions[27] respectively. These transition are suggestive of octahedral geometry around the Co(II) ion. The magnetic moment values of 4.4 and 4.6 BM for the 1:1 and 1:2 Co(II) complexes further support octahedral geometry[23]. The electronic spectra of the Ni(II) complexes exhibits three absorption bands in the region 10,148 - 10,173 cm^{-1} (ν_1), 17,212 – 17,241 cm^{-1} (ν_2) and 23,981 – 24,272 cm^{-1} (ν_3) due to $^3A_{2g}$ (F) → $^3T_{2g}$ (F), $^3A_{2g}$ (F) → $^3T_{1g}$ (F) and $^3A_{2g}$(F) → $^3T_{1g}$ (P) transitions, respectively. These transitions support octahedral geometry for the Ni(II) ion[26]. The magnetic moment values (3.18 and 3.23 BM) further confirm the above geometry[28]. The ligand field parameter[29] Dq, B, β, β % have been calculated for the Co(II) and Ni(II) complexes (Table 4). The values of these parameters indicate partial covalent character of the metal-ligand bond.

In the electronic spectra of 1:1 and 1:2 Cu(II) complexes, a single absorption band at 18904 and 18248 cm^{-1} respectively, has been assigned to $^2B_{1g}$ → $^2A_{1g}$ transition, characteristic of square planar geometry around the Cu(II) ion[30]. The magnetic moment values for the Cu(II) complexes (1.81 and 1.83) are in agreement with those reported for square planar complexes.

Fluorescence Spectral Studies

The fluorescence properties of the ligand and its metal complexes have been studied at room temperature in 10^{-3} DMF solutions with excitation wavelength 290 nm. The Schiff base shows a weak band with fluorescence intensity of 80 (345 nm). The complexes exhibit strong bands with fluorescence intensity of 263 (334 nm) for Co(II), 143 (344 nm) for Ni(II), 264 (344) nm for Cu(II) and 677 (348) nm for Zn(II) complexes (Fig. 1).

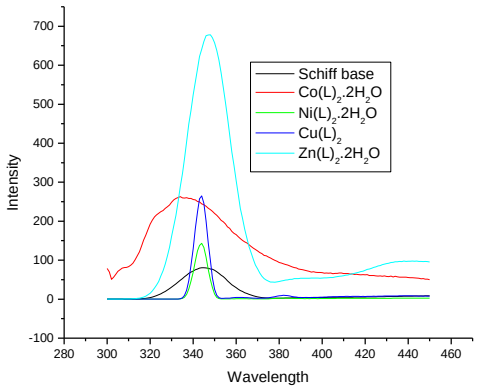

Figure 1. Fluorescence spectra of Schiff base and its metal complexes.

The increase in emission maxima is in the order of Schiff base < Ni(II) < Co(II) < Cu(II) < Zn(II). The higher fluorescence intensity of the complexes as compared to the ligands may be attributed to increased rigidity of the ligand on coordination, inhibition of the PET process, etc., thereby, reducing the loss of energy by thermal vibrational decay.[31,32]

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Table 4. Electronic spectral data and ligand field parameters of metal complexes

Compound	Transitions (cm^{-1})			*Dq* cm^{-1}	*B* cm^{-1}	ν_2/ν_1	β	β%	μ_{eff} (BM)
	ν_1	ν_2	ν_3						
$Co(L)(OAc).3H_2O$	11086	23374†	20492	1228.7	707	2.10	0.73	27.17	4.40
$Co(L)_2.2H_2O$	10893	23024†	21008	1223.1	757	2.11	0.78	22.05	4.60
$Ni(L)(OAc).3H_2O$	10173	17212	24272	1017.3	731	1.69	0.70	29.78	3.18
$Ni(L)_2.2H_2O$	10148	17187	24226	1014.8	731	1.69	0.70	29.75	3.23

†Calculated value

Table 5. Thermogravimetric data of metal complexes

Compound	Temperature, °C	Decomposed moiety	Mass %		Residue %	
			Calcd.	Found	Calcd.	Found
$Co(L)_2.2H_2O$	110-215	H_2O	5.18	5.27	10.77	10.86
	215-420	Organic moiety	45.73	45.56		
	421-515	Triazine	38.25	38.14		
$Ni(L)(OAc).3H_2O$	115-250	H_2O	11.44	11.62	15.8	15.74
	250-400	Organic moiety	46.19	46.11		
	400-510	Triazine	26.48	26.55		
$Cu(L)(OAc).H_2O$	110-170	H_2O	4.08	4.10	18.03	18.09
	170-410	Organic moiety	49.46	49.35		
	410-650	Triazine	28.36	28.29		
$Zn(L)_2.2H_2O$	105-220	H_2O	5.13	5.15	11.6	11.44
	220-410	Organic moiety	45.31	45.87		
	410-495	Triazine	37.91	37.23		

The Zn(II) complexes exhibit highest fluorescence emission which may be due to stable d^{10} configuration. Enhanced fluorescence properties exhibited by the metal complexes may offer wide applicability as fluorescent probes, sensor devices and in various fields of medicine.

Thermal analysis

Thermogravimetric analysis of $Co(L)_2.2H_2O$, $Ni(L)OAc.3H_2O$, $Cu(L)(OAc).H_2O$ and $Zn(L)_2.2H_2O$ was carried out in atmospheric air at a heating rate of 10 °C min^{-1} up to 800 °C. The data are provided in Table 5. The TG curve of $Co(L)_2.2H_2O$ indicates first weight loss of 5.27 % (calcd. 5.18 %) in the temperature range 110-215 °C attributed to loss of three coordinated water molecules. The second step represents a mass loss of 45.56 % (calcd. 45.73 %) from 215 – 420 °C corresponding to the loss of organic moiety. The third step shows decomposition of triazine ring with mass loss of 38.14 % (calcd 38.25 %) from 421 °C to 515 °C leaving CoO as residue. For complex, $Ni(L)OAc.3H_2O$, first weight loss of 11.62 % (calcd. 11.44 %) occurs between 115 ˚C and 250 ˚C, attributed to the loss of three coordinated water molecules. The subsequent steps with weight loss of 46.11% (calcd. 46.19 %) within the temperature range 250 - 400˚C and weight loss of 26.55 % (calcd. 26.48 %) from 400 – 510 ˚C correspond to the decomposition of organic moiety and triazine ring respectively. The TG curve of $Cu(L)(OAc).H_2O$ shows three decomposition steps within the temperature range 110-170 ˚C, 170-410 ˚C and 410-650 ˚C corresponding to loss of organic moiety and triazine ring respectively whereas the complex $Zn(L)OAc.2H_2O$ shows three decomposition steps in the temperature range 105-220˚C, 220-410 ˚C and 410-495 ˚C respectively (Table 5, Fig. 2).

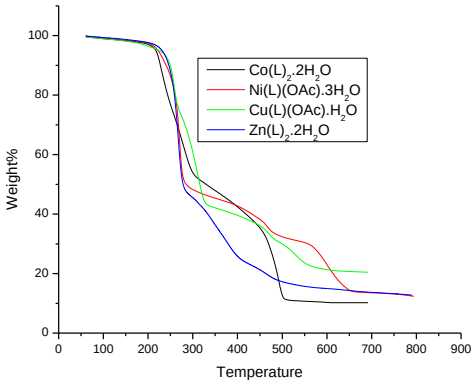

Figure 2. TG curves of metal complexes.

ESR spectra

The ESR spectra of the 1:1 and 1:2 Cu(II) complexes have been recorded. The observed g values for the complex $Cu(L)OAc.H_2O$ are $g_{\parallel} = 2.18$, $g_{\perp} = 2.07$, $g_{av} = 2.11$ and for $Cu(L)_2$, $g_{\parallel} = 2.19$, $g_{\perp} = 2.05$, $g_{av} = 2.10$. The g values can be used to derive the ground state. The results obtained from the ESR spectra of 1:1 and 1:2 Cu(II) complexes follow the trend, $g_{\parallel} > g_{\perp} > 2.0023$, indicating the unpaired electron to be in the $d_{x^2-y^2}$ orbital and corresponding to square planar geometry around the Cu(II) ion.[33] The small difference in the $g_{\parallel}$ and $g_{\perp}$ values indicates that the complexes exhibit typical axial behavior. The values of exchange interaction coupling constant (G) are 2.62 and 3.3, respectively for the 1:1 and 1:2 complexes, suggesting that there is considerable exchange coupling in the solid complexes.[34] The value of $g_{\parallel} < 2.3$ is in agreement with the covalent character of the metal ligand bond.

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Table 6. *In vitro* antimicrobial activity of synthesized compounds through agar well diffusion method.

S. No.	Compound	Diameter of growth of Inhibition zone (mm)[a]					
		B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***	***C. albicans***	***S. cerevisiae***
1a	Schiff base	26±2.16^{ab}	24±2.03^{b}	17±1.59^{c}	30±2.32^{a}	24±1.72 ^{b}	26±1.99^{ab}
2a	Co(L)(OAc).3H_2O	17±1.64^{b}	16±1.6^{b}	15±1.35^{c}	21±1.75^{a}	15±1.90^{c}	20±1.67^{a}
3a	Co(L)$_2$.2H_2O	15±1.28^{d}	14±1.3^{de}	13±1.08^{e}	17±1.44^{c}	25±1.26^{a}	22±1.19^{b}
4a	Ni(L)(OAc).3H_2O	18±1.65^{c}	16±1.42^{cd}	15±1.38^{d}	19±1.92^{bc}	20±1.86^{b}	22±2.06 ^{a}
5a	Ni(L)$_2$.2H_2O	19±1.88^{a}	15±1.72^{b}	10±0.42^{d}	16±1.03^{b}	12±0.81^{c}	19±0.98^{a}
6a	Cu(L)(OAc).H_2O	20±1.36^{a}	18±1.54^{b}	14±1.12^{d}	16±0.94^{c}	10±0.57^{e}	20±1.64^{a}
7a	Cu(L)$_2$	24±1.99^{ab}	23±1.74^{b}	25±1.66^{a}	23±2.49^{b}	-	24±2.04^{ab}
8a	Zn(L)(OAc).3H_2O	26±2.18^{b}	25±1.73^{c}	25±1.61^{c}	26±1.87 ^{b}	-	30±2.56^{a}
9a	Zn(L)$_2$.2H_2O	21±2.12^{b}	18±0.94^{c}	17±0.88^{c}	21±2.23^{b}	12±0.68^{d}	32±2.34^{a}
10a	Ciprofloxacin	24±1.68 ^{b}	26.6±3.24^{a}	25±2.02^{ab}	22±1.72^{c}	-	-
11a	Amphotericin B	-	-	-	-	16.6±1.17^{b}	19.3±1.14^{a}

-: No activity. All values are Mean ± S.E of mean. Means with different letters in the same row are significantly (P<0.05) different. (Data were analyzed by Duncan's Multiple Range test). a Values, including diameter of the well (8mm), are means of three replicates

Electrochemistry

The cyclic voltammograms of the all the metal complexes were recorded in DMF at a scan rate of 0.1 V s^{-1} in the potential range -1.5 to 0.5 V. Tetrabutylammonium perchlorate was used as a supporting electrolyte. The cyclic voltammogram of the Co(II) complex exhibits a cathodic reduction peak at -1.3 V and associated anodic oxidation peak at -0.9 V corresponding to formation of Co(II)/Co(I) couple.[33] The peak separation (ΔE_p = 0.4 V) indicates a quasi reversible one electron process. The Ni (II) complex shows a cathodic peak at -1.14 V characteristic of the Ni(II) → Ni(I) couple and associated anodic peak at -0.6 V indicating Ni(I) → Ni(II) couple (Fig. 3a). The peak separation value (ΔE_p = 0.54 V) between the cathodic and anodic potential is high indicating one electron, quasi-reversible process[23].

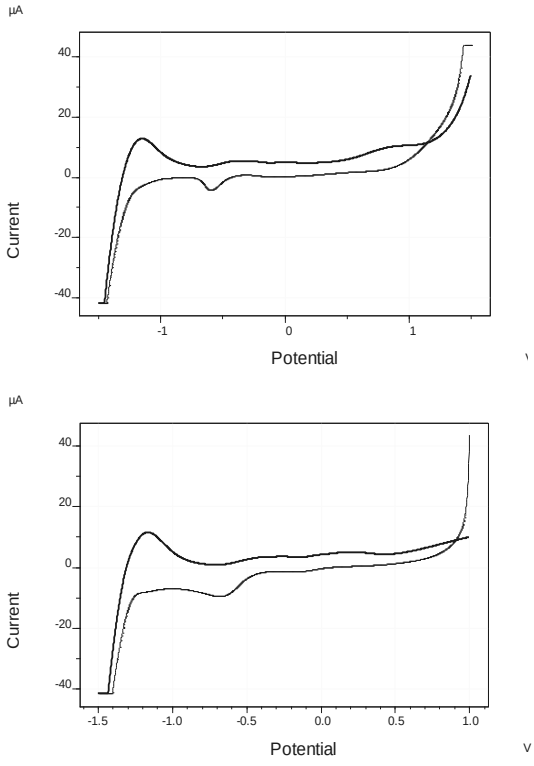

Figure 3. Cyclic voltammogram of (a) Ni(L)$_2$.2H_2O (b) Cu(L)OAc.H_2O.

The Cu(II) complex displays a reduction peak at E_{pc} = -1.16 V with a corresponding oxidation peak at E_{pa} = -0.65 V (ΔE_p = 0.51 V) indicating quasi-reversible one electron Cu(II)/Cu(I) couple (Fig. 3b).

The Zn(II) complexes do not show any oxidation or reduction peak. The value of half wave potentials, $E_{1/2}$ was found to be -1.1 V, -0.87 V and -0.91 V for the Co(II), Ni(II) and Cu(II) complexes respectively attributed to one electron transform.

Antimicrobial discussion

The enhanced lipophilic character favours the permeation of the central metal ion through the lipid layer of the microorganisms thus inhibiting the growth of the microorganisms and destroying them more aggressively.

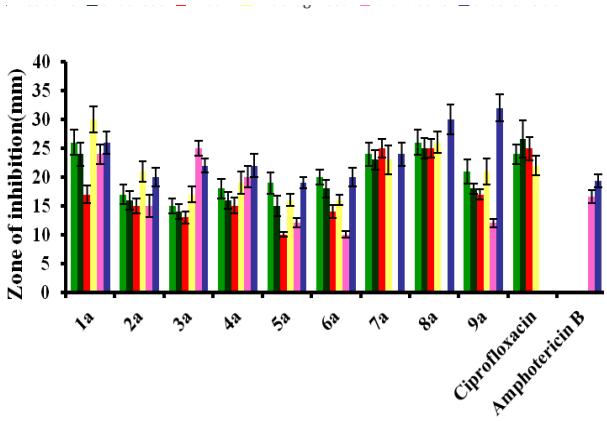

Figure 4a. Antimicrobial activity of compounds/standard drugs showing zone of inhibition.

The antimicrobial studies inferred that the complexes act as more powerful and potent antimicrobial agents as compared to the Schiff base. This may be due to change in structure due to coordination thereby reducing the polarity of metal ion due to partial sharing of its positive charge with the donor groups within the chelate ring system which, in turn, increases the lipophilic nature of the central metal atom.[17]

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Table 7. Minimum inhibitory concentration (MIC) (in µg mL^{-1}) of compounds

S. No.	Compound	*B. subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*	*S. cerevisiae*
1a	Schiff base	12.5	25	50	6.25	25	12.5
2a	$Co(L)(OAc).3H_2O$	50	50	nt	25	nt	25
3a	$Co(L)_2.2H_2O$	nt	nt	nt	50	12.5	25
4a	$Ni(L)(OAc).3H_2O$	50	50	nt	25	25	25
5a	$Ni(L)_2.2H_2O$	25	nt	nt	50	nt	25
6a	$Cu(L)(OAc).H_2O$	25	50	nt	50	nt	25
7a	$Cu(L)_2$	25	25	12.5	25	-	12.5
8a	$Zn(L)(OAc).3H_2O$	12.5	12.5	12.5	12.5	-	6.25
9a	$Zn(L)_2.2H_2O$	25	50	50	25	nt	6.25
10a	Ciprofloxacin	6.25	6.25	6.25	12.5	-	-
11a	Amphotericin B	-	-	-	-	12.5	12.5

- : No activity

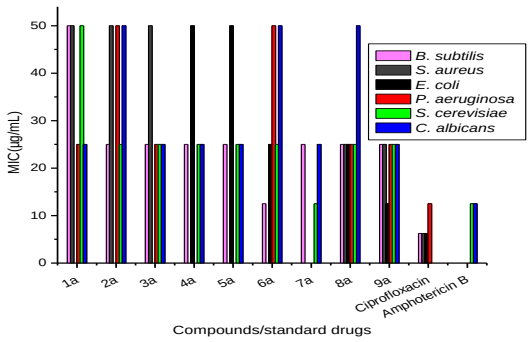


Figure 4b. Comparison of MIC of compounds with standard drugs.

The results of the antibacterial studies reveal that all the Co(II), Ni(II), Cu(II) and Zn(II) complexes (**2a-9a**) possess strong antibacterial activities against *Bacillus subtilis* with low MIC values in the range of 6.25 - 25 µg mL^{-1}. The Zn(II) complexes exhibit good antibacterial activities against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* whereas other compounds shown moderate activities against these bacteria. All the complexes also show potential antifungal activities against the tested fungi that are better than the reference drug amphotericin B. The The antimicrobial activities of the investigated compounds are shown in Table 6, Fig. 4a. and the minimum inhibitory concentration (MIC) values are summarized in Table 7 (Fig. 4b).

Statistical analysis

Significant differences among treatment groups were tested by Analysis of variance (ANOVA) Duncan's multiple range tests for the experiment (Table 6). Statistical significance was settled at a probability value of $P<0.05$. All statistics was performed using SPSS Version 11.5 for Windows.

Structural information by computational methods

Chem 3D Ultra was used to perform the geometrical optimization calculations. The optimized geometries and the plot of highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO) of the ligand and some of the metal complexes are shown in Figs. 5-7.

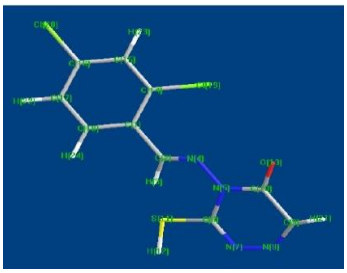


Figure 5a. Optimized geometry of Schiff base

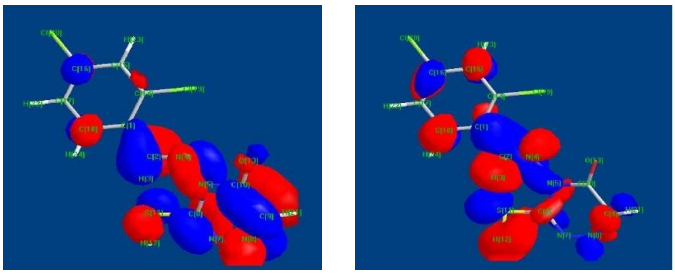
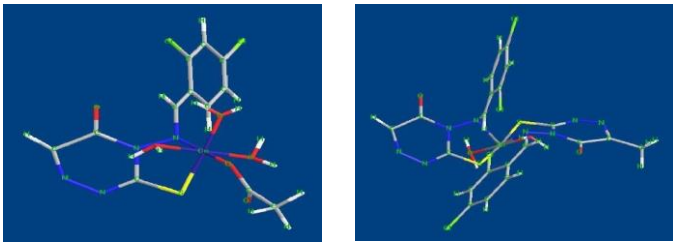
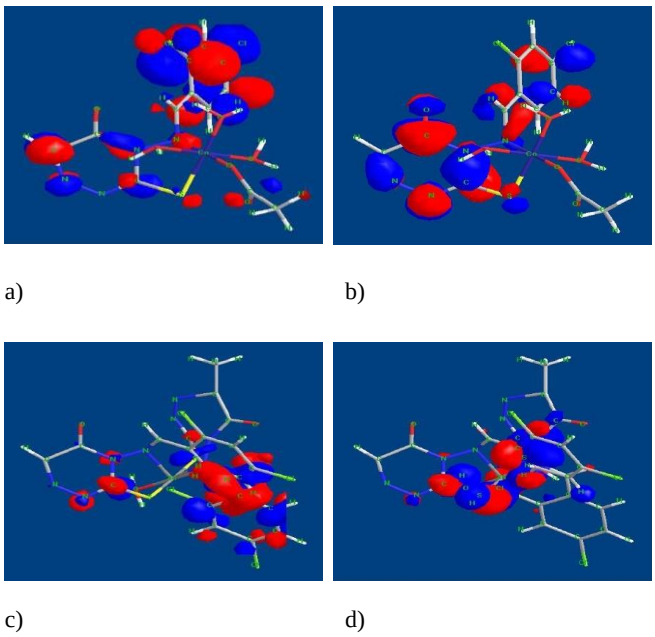


Figure 5b. Plot of HOMO, LUMO, HOMO-1 and LUMO+1 calculated molecular orbital levels for Schiff base.



a) **b)**

Figure 6. (a) The optimized geometry of $Co(L)(OAc).3H_2O$, **(b)** The optimized geometry of $Zn(L)_2.2H_2O$.



a) b)

c) d)

Figure 7. (**a**) HOMO level for Co(L)(OAc).3H_2O complex , **(b)** LUMO level for Co(L)(OAc).3H_2O complex, (c) HOMO level for $Zn(L)_2$.2H_2O complex **(d)** LUMO level for $Zn(L)_2$.2H_2O complex.

Conclusions

The Co(II), Ni(II), Cu(II) and Zn(II) complexes derived from the Schiff base, 4-(2,4-dichlorobenzylideneamino)-3-mercapto-5-oxo-1,2,4-triazine were synthesized and characterized on the basis of analytical and spectral data. The results of the investigation suggest octahedral geometry for the Co(II), Ni(II) and Zn(II) complexes and square planar geometry around the Cu(II) ion. The Schiff base and their metal complexes were found highly active against the bacterial and fungal species and therefore can be used as new antimicrobial drugs after testing their toxicity.

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Conflicts of interest

The author(s) declare that there is no conflict of interest regarding the publication of this manuscript.

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