SYNTHESIS, CHARACTERIZATION AND *IN VITRO* ANTICANCER ACTIVITY OF Co(II), Ni(II), Cu(II), AND Zn(II) COMPLEXES WITH 4-[{3-(4-BROMOPHENYL)-1-PHENYL-1H-PYRAZOL-4-YLMETHYLENE}-AMINO]-3-MERCAPTO-1,2,4-TRIAZIN-5-ONE

Kiran Singh[a]*, Ritu Thakur[a] and Gaurav Kumar[b]

Keywords: Schiff base; Metal complexes; Cell lines; ^{1}H-NMR; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide Assay; antimicrobial activity

Schiff base derived from the condensation of 3-(p-bromophenyl)-1-phenyl-1H-pyrazolecarboxaldehyde with 4-amino-3-mercapto-1,2,4-triazin-5-one and its Co(II), Ni(II), Cu(II) and Zn(II) metal complexes have been synthesized in 1:1 and 1:2 molar ratios. Ligand and its metal complexes are characterized by various physicochemical techniques. On the basis of these techniques, octahedral geometry deduced for Co(II), Ni(II) and Zn(II) complexes and square planar for Cu(II) complexes. Low molar conductance values of all the metal complexes reveal their non-electrolytic nature. All the synthesized complexes have been screened *in vitro* for antibacterial activity against *S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli* and antifungal activity against *C. albicans* and *S. cerevisiae*. It has been found that metal complexes show promising biological activity as compared to ligand. Schiff base and its metal complexes have been screened against human breast cancer cell lines by using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. *In vitro* anticancer cell lines results indicate that metal complexes exhibit significant activity on MCF-7.

* Corresponding Authors
E-Mail: kiransinghkuk@yahoo.co.in
[a] Department of Chemistry, Kurukshetra University, Kurukshetra 136119, Haryana, India
[b]Department of Biochemistry, Delhi University, Delhi 110021, India

Introduction

Today, cancer is a major health problem around the globe. It increases at an alarming rate and cause about 13% of all the death.[1] To overcome this problem, it is necessary to develop the new potent anticancer agents. Now a days, Pt(II) based anticancer drugs have been used for clinical chemotherapy but they have severe side effects. Therefore, more interest has been drawn in the synthesis of less toxic non-platinum metal complexes.[2] Coordination complexes of heterocyclic moiety especially pyrazole based derivatives paid much attention in the recent years. Pyrazoles emerged as powerful scaffold in the field of organic synthesis as they have been extensively used to design pharmaceuticals and agrochemicals.[3,4] Further, existing literature indicates that pyrazole moiety possess unique position in medicinal chemistry as they exhibit wide range of bioactivities like anticancer,[5] anticonvulsant,[6] antidepressant,[7] antipyretic,[8] anti-inflammatory,[9] antiviral,[10] antihistaminic,[11] etc. Due to their diverse biological applications, this structural motif has been used as starting material for the formation of various Schiff bases and their corresponding metal complexes.[12] Pyrazole based biologically active ligands and their complexes with transition metals such as Co, Ni, Cu, Zn, Pd and V show broad range of biological activities as well as medicinal properties.[13-15] Therefore, all aforementioned applications paved the way towards the development of Schiff bases and their metal complexes as new chemotherapeutic agents. Hence, all these findings prompted us to synthesize pyrazole based ligand and variety of metal complexes. Based on the above observations, present article focused on the synthesis of Co(II), Ni(II), Cu(II) and Zn(II) complexes with new Schiff base 4-[{3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-ylmethylene}-amino]-3-mercapto-1,2,4-triazin-5-one and screened them against human breast cancer cell lines.

Experimental

Materials and Methods All the used chemicals and solvents were of analytical grade. ^{1}H-NMR spectra of the newly synthesized compounds were recorded on Bruker ACF 300 spectrometer at 300 MHz in $CDCl_3$/d_6-DMSO using 'TMS' as reference compound. IR spectra of the Schiff base and its metal complexes have been examined in KBr pellets/Nujol mulls on a MB-3000 ABB spectrometer. Electronic spectra of metal complexes were recorded on T 90 (PG Instruments ltd) UV/VIS spectrometer in DMF in the region 1100-200 nm. Magnetic moment measurements were carried out on Vibrating Sample Magnetometer at Institute Instrumentation Centre, IIT Roorkee. Fluorescence spectra of the ligand and metal complexes were recorded on SHIMADZU RF-5301 PC spectrophotometer. ESR spectra of Cu complexes were recorded under the magnetic field 3000 Gauss at frequency 9.1 GHz by using Varian E-112 ESR spectrometer at SAIF, IIT Bombay. Cyclic voltammetry measurements of Cu(II) complexes was recorded on Ivium Stat Electrochemical Analyzer with three electrode system of glassy carbon as the working electrode, a platinum wire as auxiliary electrode and Ag/AgCl as the reference electrode. Thermogravimetric analysis was obtained on a Perkin Elmer (Pyris Diamond) instrument at heating rate of 10°C Min^{-1} by using alumina powder as reference.

Synthesis 4-amino-3-mercapto-1,2,4-triazin-5-one (AMOT) was synthesized according to reported procedure.[16]

Synthesis of 4-[{3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-ylmethylene}-amino]-3-mercapto-1,2,4-triazin-5-one (HL).

An ethanolic solution of AMOT (1.00 g, 6.94 mmol) was refluxed with an ethanolic solution of 3-(p-bromophenyl)-1-phenyl-1H-pyrazolecarboxaldehyde (2.27 g, 6.94 mmol) for 10 hours. The product formed was cooled to room temperature, filtered, washed with ethanol and recrystallized with same solvent and then dried (Fig. 1).

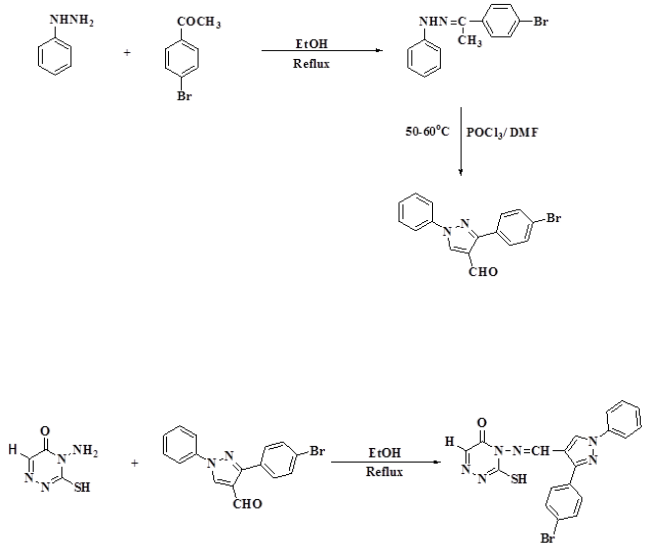

Figure 1. Synthesis of Schiff base

Synthesis of 1:1 metal:ligand complexes

Mixing the hot ethanolic solutions of Schiff base (0.20 g, 0.44 mmol) with hot ethanolic solutions of acetates of Co(II) (0.109 g, 0.44 mmol), Ni(II) (0.109 g, 0.44 mmol), Cu(II) (0.088 g, 0.44 mmol) and Zn(II) (0.096 g, 0.44 mmol). The colored product formed were immediately filtered, washed with warm water, aqueous ethanol, finally with acetone and then dried.

Synthesis of 1:2 metal:ligand complexes

Hot ethanolic solutions of metal acetates of Co(II) (0.109 g, 0.44 mmol), Ni(II) (0.109 g, 0.44 mmol), Cu(II) (0.088g, 0.44 mmol) and Zn(II) (0.096 g, 0.44 mmol) were treated with hot ethanolic solution of ligand (0.40 g, 0.88 mmol). The colored precipitates were formed immediately filtered, washed with warm water, aqueous ethanol and finally with acetone and then dried.

Antimicrobial Assay

The synthesized Schiff base and corresponding metal complexes were screened for antimicrobial activity against four bacterial strains (*Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 121, *Pseudomonas aeruginosa* MTCC 741 and *Escherichia coli* MTCC 1652) and two fungal strains (*Candida albicans* MTCC 227 and *Saccharomyces cerevisiae* MTCC 170). All the bacterial cultures were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh.

In vitro antimicrobial activity. Agar well-diffusion method was used to evaluate the newly synthesized Schiff base and its metal complexes. All the microbial culture were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of approximately $1.5x10^8$ cfu mL^{-1}. 20 ml of Muller Hinton agar medium was poured into each Petri plate and plates were swabbed with 100 µl inocula of the test microorganisms and kept for 15 minutes for adsorption using sterile cork borer of 8 mm diameter, wells were bored into the seeded agar plates and these were loaded with 100 µl volume (with concentration 4.0 $mgml^{-1}$) of each compound reconstituted in dimethyl sulphoxide (DMSO). All the plates were incubated at 37 °C for 24 hrs. Antimicrobial activity of each compound was evaluated by measuring the Zone of growth inhibition against the test organisms with zone reader (HiAntibiotic zone scale). DMSO was used as a negative control whereas Ciprofloxacin was used as positive control. This procedure was performed in three replicate plates for each organism.[17,18]

Minimum Inhibitory Concentration (MIC) MIC of the Schiff base and its metal complexes tested against bacterial stains through a modified agar well-diffusion method.[19] It is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation.

Anticancer Activity

Anticancer activity was carried out in human breast cancer cell lines (MCF-7), obtained from National Center for Cell Science, Pune, India. The cells were cultured in Dulbecco's modified Eagles medium (DMEM) containing 10 % fetal bovine serum (FBS) at 37 °C in an atmosphere containing 5 % CO_2.

MTT assay MTT assay was performed on MCF-7 breast cancer cells to determine cell viability.[20] Briefly, $4x10^3$ cells were seeded in 96 well plates. After incubation for 24 h at 37 °C under 5 % CO_2 in a humidified atmosphere, cells were exposed to different concentrations of cordycepin ranging from 0 to 100 µg mL^{-1} for 48 h. MTT solution (10 µL, 5 mg mL^{-1}) was added to each well and further incubated for 4 h at 37 °C. The medium was removed and formazan crystals were dissolved by adding 100 µL of DMSO into each well and then shaking for another 20 min. Optical density (OD) was measured at 570 nm with a micro plate reader (Bio-Rad) and percentage of viability (φ) was calculated as follows:

$$\varphi = 100 \frac{OD_{\text{test}}}{OD_{\text{control}}}$$

Percent (%) cytotoxicity = 100 – (Percent viability)

Table 1. Physical characterization and analytical data of the ligand and metal complexes

Compound	Color	MP (°C)	Yield (%)	Found (Calcd) %			
				C	H	N	M
HL $[C_{19}H_{13}N_6OSBr]$	Creamish yellow	232-234	85	50.24 (50.34)	2.60 (2.89)	18.28 (18.54)	-
$Co(L)(OAc).3H_2O$ $[C_{21}H_{21}BrCoN_6O_6S]$	Light green	244-248	81	40.31 (40.40)	3.28 (3.39)	13.10 (13.46)	9.25 (9.44)
$Co(L)_2.2H_2O$ $[C_{38}H_{28}Br_2CoN_{12}O_4S_2]$	Light green	250-254	79	45.58 (45.66)	2.75 (2.82)	16.70 (16.82)	5.77 (5.90)
$Ni(L)(OAc).3H_2O$ $[C_{21}H_{21}BrN_6NiO_6S]$	Light brown	270-274	83	40.28 (40.41)	3.28 (3.39)	13.20 (13.47)	9.26 (9.40)
$Ni(L)_2.2H_2O$ $[C_{38}H_{28}Br_2N_{12}NiO_4S_2]$	Light brown	264-268	77	45.46 (45.67)	2.77 (2.82)	16.73 (16.82)	5.75 (5.87)
$Cu(L)(OAc).H_2O$ $[C_{21}H_{17}BrCuN_6O_4S]$	Dark green	256-260	82	42.48 (42.54)	2.81 (2.89)	14.02 (14.17)	10.69 (10.72)
$Cu(L)_2$ $[C_{38}H_{24}Br_2CuN_{12}O_2S_2]$	Dark green	276-280	80	47.01 (47.14)	2.41 (2.50)	17.22 (17.36)	6.27 (6.36)
$Zn(L)(OAc).3H_2O$ $[C_{21}H_{21}BrN_6O_6SZn]$	Light yellow	280-282	78	39.90 (39.99)	3.30 (3.36)	13.25 (13.32)	10.24 (10.37)
$Zn(L)_2.2H_2O$ $[C_{38}H_{28}Br_2N_{12}O_4S_2Zn]$	Light yellow	278-282	75	45.28 (45.37)	2.70 (2.81)	16.65 (16.71)	6.43 (6.50)

Result and Discussion

Schiff base and its metal complexes are solid, colored, stable, non-hygroscopic in nature. All the metal complexes decomposed at high temperature on heating. They are insoluble in common organic solvent but soluble in DMF and DMSO. Their molar conductance values are low which consistent their non-electrolytic nature. The ligand and its metal complexes have been characterized with the help of IR, NMR, ESR, thermal, fluorescence, electron spectroscopic data, Magnetic moment measurements and cyclic voltammetry. Analytical data are presented in Table 1.

Vibrational Spectra

IR analysis of Schiff base indicates the bidentate nature of the ligand (Table 2). Schiff base shows characteristic band at 1597 cm^{-1} due to ν(-CH=N-) group. On complexation this band was shifted to lower frequency value it might be due to the formation of coordinate bond between azomethine N atom and metal ion.[21] A band appeared at 2793 cm^{-1} ascribed to ν(-SH) which was not observed in the spectra of metal complexes shows the deprotonation of thiol group and bonding through S atom[22] which again confirmed by a new band appeared ~ 756 cm^{-1} ascribed to ν (C-S). All the metal complexes show broad band located in the region 3271-3742 cm^{-1} which is attributed to ν(-OH) stretching frequency of coordinated water molecules. In metal complexes, ν(M-N) band appears in the region 460-535 cm^{-1} further confirms the chelation through N atom of azomethine group. Presence of ν(-$OCOCH_3$) group in 1:1 metal complexes highlighted by the band appeared in the region 1740-1744 cm^{-1}. The position of ν(-C=O) (at 1705 cm^{-1}) did not change on going from ligand to metal complexes implying the non-involvement of oxygen of keto group in coordination with metal ion.

^{1}H-Nuclear Magnetic Resonance Spectra

^{1}H-NMR spectra of Schiff base and its Zn(II) metal complexes have been recorded in DMSO-d_6 by using 'TMS' as internal standard and data are given in Table 3. ^{1}H-NMR spectrum of Schiff base shows the following signals; δ_H (400 MHz, DMSO-d_6): 9.300 (1H, s, -CH=N-), 8.702 (1H, s, triazine-H), 7.907 (1H, s, pyrazole-H), 8.050 (2H, d, Ar-H), 7.580 (2H, t, Ar-H), 7.438 (1H, t, Ar-H), 7.787 (2H, d, Ar-H), 7.680 (2H, d, Ar-H), 13.90 (1H, br-s, -SH). The signal for azomethine proton deshielded in the spectra of Zn(II) complexes and appeared at δ 9.61 ppm indicates the complexation through azomethine nitrogen atom.[22] Signal of thiol group disappear in the spectra of Zn(II) complexes indicates the deprotonation of thiol group and complexation through S atom of thiol group.[23] Signals due to aromatic protons remain unaltered upon complexation. A new singlet observed at δ 4.009 ppm indicates the presence of coordinated water molecules in Zn(II) complexes. In 1:1 Zn(II) spectrum signal observed at δ 2.28 ppm due to methyl protons of –$OCOCH_3$ group.

Electronic spectroscopy and magnetic moment measurements

To obtain the information regarding the stereochemistry of metal complexes, the electronic spectral analysis of 1:1 and 1:2 metal complexes have been carried out in 10^{-3} mol L^{-1} solution of DMF and summarized in table 4.

The absorption spectra of Co(II) complexes display two absorption bands in the region 10500-20161 cm^{-1} and 10940-23108 cm^{-1} which was reasonably assigned to $^4T_{1g}$ (F)→ $^4T_{2g}$ (F) (ν_1) and $^4T_{1g}$ (F)→ $^4T_{1g}$ (P) (ν_3) transitions respectively. The coordination field parameters (D_q, B, β, β %) have been calculated by using Band-Fitting equation. The values of Racah parameter (B) were found to be in the range 636-743 cm^{-1} which is less than free ion value indicates overlapping of ligand metal orbitals.

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Table 2. Characteristics IR frequencies (cm^{-1}) of Schiff base and its metal complexes

Compound	ν(N=CH)	ν(C-S)	ν(S-H)	ν(OCOCH3)	ν(OH)	ν(M-S)	ν(M-N)
HL	1597	-	2777	-	-	-	-
$Co(L)(OAc).3H_2O$	1535	750	-	1744	3742	330	460
$Co(L)_2.2H_2O$	1535	756	-	-	3742	340	477
$Ni(L)(OAc).3H_2O$	1535	753	-	1740	3600	309	505
$Ni(L)_2.2H_2O$	1535	752	-	-	3742	333	496
$Cu(L)(OAc).H_2O$	1535	756	-	1744	3618	312	535
$Cu(L)_2$	1535	754	-	-	-	350	489
$Zn(L)(OAc).3H_2O$	1535	757	-	1740	3271	365	481
$Zn(L)_2.2H_2O$	1535	758	-	-	3742	331	517

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

Compound	^{1}H-NMR (ppm)
HL [$C_{19}H_{13}BrN_6OS$]	9.30 (s,1H, -C**H**=N-), 8.70 (s,1H, triazine-**H**), 7.90 (s, 1H, pyrazole-**H**), 8.05 (d, 2H, Ar-**H**), 7.58 (t, 2H, Ar-**H**), 7.43 (t, 1H, Ar-**H**), 7.79 (d, 2H, Ar-**H**), 7.68 (d, 2H, Ar-**H**), 13.90 (br-s, 1H, -SH)
$Zn(L)(OAc).3H_2O$ [$C_{21}H_{21}BrN_6O_6SZn$]	9.61 (s, 1H, -C**H**=N-), 8.67 (s, 1H, triazine-**H**), 7.89 (s, 1H, pyrazole-**H**), 8.02 (d, 2H, Ar-**H**), 7.56 (t, 2H, Ar-**H**), 7.42 (t, 1H, Ar-**H**), 7.78 (d, 2H, Ar-**H**), 7.66 (d, 2H, Ar-**H**), 2.28 (s, 3H, -OCOC**H**$_3$), 4.00 (s, 6H, -O**H**$_2$)
$Zn(L)_2.2H_2O$ [$C_{38}H_{28}Br_2N_{12}O_4S_2Zn$]	9.61 (s, 2H, -C**H**=N-), 8.67 (s, 2H, triazine-**H**), 7.89 (s, 2H, triazine-**H**), 8.02 (d, 4H, Ar-**H**), 7.56 (t, 4H, Ar-**H**), 7.41 (t, 2H, Ar-**H**), 7.78 (d, 4H, Ar-**H**), 7.67 (d, 4H, Ar-**H**), 4.00 (s, 4H, -O**H**$_2$)

The nephelauxetic ratios (β) are found to be less than one suggests the partial covalent character in the metal-ligand bond. Magnetic moment value of Co(II) complexes are found in the range of 4.4-4.9 BM which is in the expected range (4.3-5.0) of octahedral complexes.[24]

The absorption spectra of Ni(II) complexes show three absorption bands in the range 9665-10600 cm^{-1}(ν_1), 16720-17621 cm^{-1} (ν_2) and 23818-24607 cm^{-1} (ν_3) assigned to $^3A_{2g}$ (F)→ $^3T_{2g}$ (F) (ν_1), $^3A_{2g}$ (F)→ $^3T_{1g}$ (F) (ν_2) and $^3A_{2g}$ (F)→ $^3T_{1g}$ (P) (ν_3) transitions respectively. The coordination field parameters (D_q, *B*, β, β %) have also been calculated for Ni(II) complexes which are indicative of octahedral geometry.[25] The values of Racah parameter (B) were found to be in the range 759-769 cm^{-1} which is less than free ion value (1041 cm^{-1}) indicates overlapping of ligand metal orbitals. The nephelauxetic ratios (β) are found to be less than one suggests the partial covalent character in the metal-ligand bond. In addition to this, the ratio of ν_2/ν_1 (1.71-1.73) indicates the octahedral geometry and observed magnetic moment values are found in the range of 3.2-3.4 BM which lies in the expected range of reported octahedral complexes.

A band observed in case of Cu(II) complexes in the region of 18510-19565 cm^{-1} assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ indicates the square planar geometry of the copper complexes which is further confirmed by magnetic moment values 1.8-2.0 BM which is in the expected range of square planar complexes.

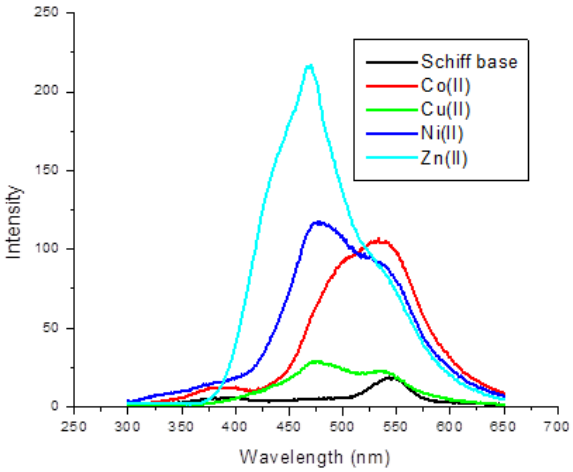

Figure 2. Fluorescence spectra of Schiff base and its metal complexes

ESR Spectra

ESR spectra of solid Cu(II) complexes were analyzed on X band at frequency 9.1 GHz under the magnetic field strength of 3000 G by using DPPH free radical as standard. The observed g values for $Cu(L)OAc.H_2O$ ($g_\parallel$ = 2.12, $g_\perp$ = 2.06, g_{av} = 2.08, *G* = 2.03) and for $Cu(L)_2$ ($g_\parallel$ = 2.13, $g_\perp$= 2.08, g_{av} = 2.09, *G* = 1.64). From experimental the $g_\parallel$ and $g_\perp$ values are more than 2.04 suggest the axial geometry pattern for Cu(II) ion. The expression $g_\parallel > g_\perp > 2.0023$ indicates that electron lies in d_{x2-y2} orbital giving $^2B_{1g}$ as the ground state and suggest the square planar geometry for Cu(II) complexes.[26]

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

Compound	Λ_{max} (cm^{-1})	Band Assignment	D_q (cm^{-1})	B (cm^{-1})	ν_2/ν_1	β	β %
$Co(L)(OAc).3H_2O$	10500	$^4T_{1g}$ (F) →$^4T_{2g}$ (F) (ν_1)	1039	636	1.99	0.655	34.5
	20890*						
	20161	$^4T_{1g}$ (F) →$^4T_{1g}$ (P) (ν_3)					
$Co(L)_2.2H_2O$	10940	$^4T_{1g}$ (F) →$^4T_{2g}$ (F) (ν_1)	1216.8	743	2.11	0.765	23.5
	23108*						
	20865	$^4T_{1g}$ (F) →$^4T_{1g}$ (P) (ν_3)					
$Ni(L)(OAc).3H_2O$	9665	$^3A_{2g}$ (F) →$^3T_{2g}$ (F) (ν_1)	966.5	769	1.73	0.739	26.1
	16720	$^3A_{2g}$ (F) →$^3T_{1g}$ (F) (ν_2)					
	23818	$^3A_{2g}$ (F) →$^3T_{1g}$ (P) (ν_3)					
$Ni(L)_2.2H_2O$	10281	$^3A_{2g}$ (F) →$^3T_{2g}$ (F) (ν_1)	1028	759	1.71	0.729	27.1
	17621	$^3A_{2g}$ (F) →$^3T_{1g}$ (F) (ν_2)					
	24607	$^3A_{2g}$ (F) →$^3T_{1g}$ (P) (ν_3)					

The *G* (Axial symmetry parameter) value of the complex found to be less than 4.0 indicating the considerable exchange interaction in the Cu(II) centers.[27] The value of g_{av} > 2.0023 (free electron) estimated from the expression:

$$g_{av} = 1/3\ (g_{\parallel} + 2g_{\perp})$$

This is consistent with partial covalent property of Cu(II) complexes.

Fluorescence Spectral Studies

Fluorescent emission spectra of Schiff base and its 1:2 metal complexes have been recorded in DMF with 10^{-3} molar concentration (Fig. 2). The fluorescent property of the ligand shows significant changes (enhancement in fluorescent intensity, shift of emission wavelength) when it is coordinated in metal and form complexes.[28] Fluorescence of Schiff base was quenched by the PET process due to presence of lone pair of electrons on N atom of Schiff base.[29] Metal ions are engaged with lone pair of electrons by formation of coordinate bonding, PET process is blocked and fluorescent intensity increases in metal complexes. Zn(II) metal complexes show more enhancement with emission wavelength of 470 nm whereas Co(II), Ni(II) and Cu(II) complexes show emission wavelength at 533 nm, 478 nm, and 535 nm respectively. A weak emission band observed at 543 nm for Schiff base. Enhancement order of fluorescence: Schiff base < Cu(II) < Co(II) < Ni(II) < Zn(II).

Thermogravimetric Analysis

Thermogravimetric analyses of $Co(L)(OAc).3H_2O$, $Ni(L)_2.2H_2O$, $Cu(L)(OAc).H_2O$ and $Zn(L)_2.2H_2O$ complexes have been carried out in temperature range of 50-700 °C in air atmosphere at heating rate of 10 °Cmin^{-1} by using α-Al_2O_3 as reference. Different decompositions are represented in table 5 and Fig. 3. The Thermogravimetric curves are further supported by the DTA curves.

Decomposition of $Co(L)(OAc).3H_2O$ took place in three steps. First decomposition step (100-220 °C) involved mass loss of 8.10 % (Calcd. 8.65 %) consistent with removal of three water molecules. Second decomposition step confined to removal of organic and acetate moieties with mass loss 59.80 % (Calcd. 61.54 %) in the temperature range 221-510 °C. Third decomposition step has been observed in the range 511-570 °C with mass loss 19.50 % (Calcd. 20.35 %) corresponds to removal of triazine ring leaving CoO as residue.

Thermal study of $Ni(L)_2.2H_2O$ has been carried out in three stages. First stage occurred between 95-210 °C with mass loss 3.00 % (Calcd. 3.60 %) assigned to removal of two water molecules. Second stage (211-450 °C) results in mass loss 63.75 % (Calcd. 65.07 %) corresponds to loss of organic moiety. Third stage (451-620 °C) involved mass loss of 23.00 % (Calcd. 25.43 %) assigned to removal of triazine ring leaving NiO as residue.

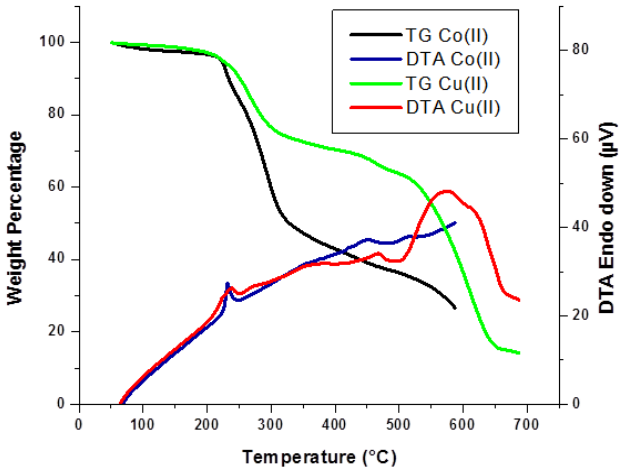

Figure 3. Thermogravimetric and DTA plot of a) $Co(L)(OAc).3H_2O$ b) $Cu(L)(OAc).H_2O$

Thermal data of $Cu(L)(OAc).H_2O$ suggest the copper complex is stable up to 90 °C. First degradation step exhibited mass loss 3.00 % (Calcd. 3.03 %) in temperature range 90-150 °C, associated with loss of one water molecule. In second step organic and acetate moieties were removed in temperature range 151-590 °C with mass loss 63.45 % (Calcd. 64.80 %). Third step (591-660 °C) associated with loss of triazine ring with mass loss 20.35 % (Calcd. 21.43 %) leaving CuO as residue.

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Table 5. Thermogravimetric results of 1:1and 1:2 metal(II) complexes

Compound	TG range (°C)	% Mass loss Found (Calcd.)	Decomposed moiety	Residue, % Found (calcd).	Residue
$Co(L)(OAc).3H_2O$	100-220	8.65 (8.10)	H_2O	9.5 (12)	CoO
$[C_{21}H_{21}CoN_6O_6SBr]$	221-510	61.54 (59.80)	Organic & OAc moiety		
	511-570	20.35 (19.50)	Triazine ring		
$Ni(L)_2.2H_2O$	95-210	3.00 (3.60)	H_2O	6.50 (7.48)	NiO
$[C_{38}H_{28}N_{12}NiO_4S_2Br_2]$	211-450	63.75 (65.07)	Organic moiety		
	451-620	23.00 (25.43)	Triazine ring		
$Cu(L)(OAc).H_2O$	90-150	3.03 (3.00)	H_2O	10.74 (13.41)	CuO
$[C_{21}H_{17}CuN_6O_4SBr]$	151-590	64.80 (63.45)	Organic & OAc moiety		
	591-660	21.43 (20.35)	Triazine ring		
$Zn(L)_2.2H_2O$	110-230	2.85 (3.58)	H_2O	7.00 (8.12)	ZnO
$[C_{38}H_{28}N_{12}O_4S_2Br_2Zn]$	231-350	62.75 (64.62)	Organic moiety		
	351-600	23.50 (25.26)	Triazine ring		

The TG curve of $Zn(L)_2.2H_2O$ shows three degradation steps. First degradation step has been observed with mass loss 2.85 % (Calcd. 3.58 %) from temperature range 110-230 °C corresponds to removal of two water molecules. Second degradation step (231-350 °C) assigned to removal of organic moiety with mass loss 62.75 % (Calcd. 64.62 %). Third mass loss 23.50 % (Calcd. 25.26 %) observed in temperature range 351-600 °C indicates the removal of triazine ring leaving ZnO as residue.

Cyclic voltammogram

The electrochemical feature of Cu(II) complexes was investigated in DMF solution by taking tetrabutylammoniumperchlorate as supporting electrolyte at room temperature. The repetitive scan were carried out at 100 mV s^{-1} within potential range of 1.0 to -1.0 V. Forward scan of Voltammogram of $Cu(L)(OAc).H_2O$ and $Cu(L)_2$ show reduction peaks at E_{Pc} = 0.10 V and E_{Pc} = 0.09 V respectively associated with $Cu^{2+/+}$ couple and reverse scan show oxidation peaks at E_{Pa} = -0.90 V and E_{Pa} = -0.65 V respectively associated with $Cu^{+/2+}$ couple. The peak separation data found to be ΔE_P = 0.8 V and 0.56 V. The analysis of cyclic voltammogram indicates the quasireversible one electron transfer process.[30,31]

Biological Screening

Biological screening of Schiff base and its metal complexes have been studied against standard microbial strains of *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans* and *S. cerevisiae* (Fig. 4). To find out the minimum concentration of ligand and its complexes which inhibit the visible growth of microbes, the compounds were tested for *invitro* biological evaluation. It was observed that ligand was biologically active but their metal complexes show more pronounced activity which can be rationalized by Overtone's concept[32] and Tweedy's chelation theory.[33] According to Overtone's concept of cell permeability, the lipid membrane surrounding the cell favors the passage of only lipid-soluble material; therefore, liposolubility is a crucial factor which controls the antimicrobial activity. Tweedy suggest that chelation could facilitate the passage of complexes across the cell membrane. On chelation polarity of metal ion reduces because of partial sharing of its positive charge with donor groups and increase in the delocalization of π-electrons over the whole chelate ring. Among the complexes, $Co(L)(OAc).3H_2O$, $Co(L)_2.2H_2O$ and $Cu(L)(OAc).H_2O$ were highly active against gram positive bacteria with diameter of inhibition zone ranging between 20-22 mm, 19-23 mm and 17-23 mm respectively. $Cu(L)(OAc).H_2O$ and $Cu(L)_2$ show high activity against gram negative bacteria with inhibition zone ranging between 19-23 mm and 20-22 mm respectively. Compounds $Co(L)_2.2H_2O$, $Zn(L)(OAc).3H_2O$ and $Zn(L)_2.2H_2O$ were very effective against yeast with inhibition zone between 13-17 mm, 14-20 mm and 13-23 mm respectively.

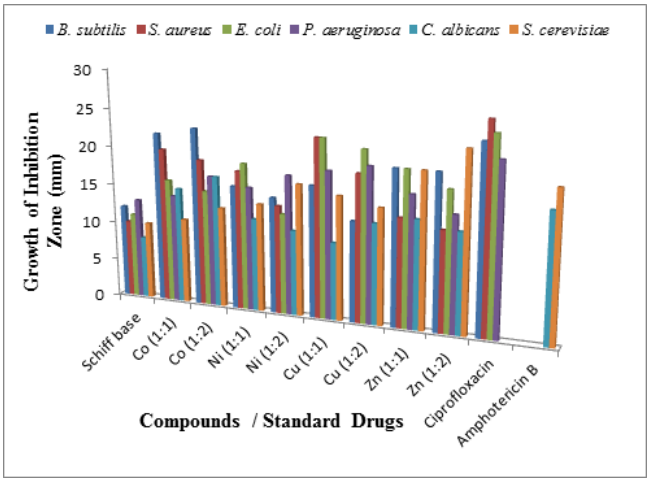

Figure 4. Biological activity of Schiff base and metal complexes with standard drugs

The compounds which shows highest diameter of growth of inhibition zone were selected to test their MIC values. Compounds $Co(L)(OAc).3H_2O$, $Co(L)_2.2H_2O$ and $Cu(L)(OAc).H_2O$ shows lowest MIC value 12.5 µg mL^{-1} against gram positive bacteria and compounds $Co(L)(OAc).3H_2O$ and $Co(L)_2.2H_2O$ shows lowest MIC value 12.5 µg mL^{-1} against *C. albicans*.

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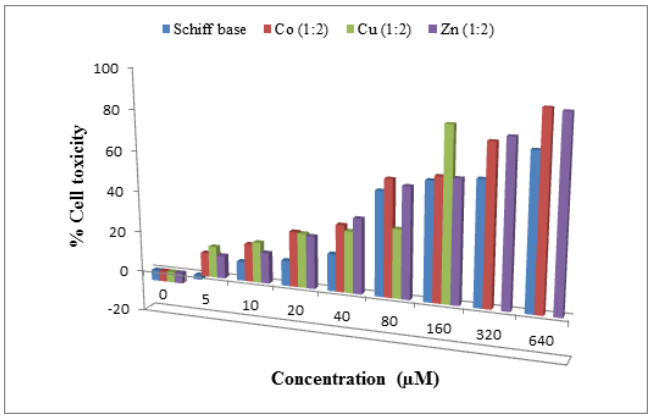

Figure 5. Effect of concentration of Schiff base and its metal complexes on % cytotoxicity of human breast cancer cell line

Anticancer activity

In order to check the cytotoxicity of complexes, Schiff base and its metal complexes has been evaluated against human breast cancer cell lines (MCF-7) within 5-640 µM concentration range (Fig. 5). Doxorubicin was taken as reference compound. The IC_{50} value obtained from *in vitro* evaluation demonstrate the significant cytotoxicity of the tested metal complexes against MCF-7 cell lines (Fig. 6). Metal complexes are found to be more cytotoxic (IC_{50} = 98.66-106.90 µM) against MCF-7 cell lines as compared to Schiff base. Compound 4 i.e. Zn (1:2) complex shows highest cytotoxicity with IC_{50} value 98.66 µM followed by Co (1:2) complex with IC_{50} value 100.04 µM and then Cu (1:2) complex with IC_{50} value 106.90 µM. In this series, metal complexes were found to be most active as compared to Schiff base and their descending order are Zn(II) > Co(II) > Cu(II) > Schiff base.

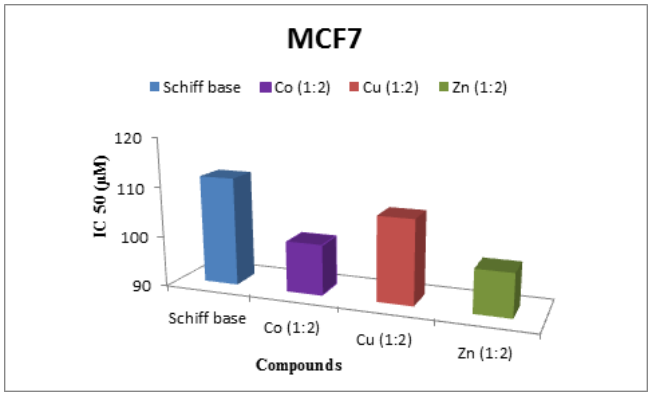

Figure 6. Comparative IC_{50} value of Schiff base and its metal complexes against MCF-7 cell lines

This enhancement of anticancer activity in metal complexes may be due to coordination.[34] Pyrazoles is a prevalence scaffold in the synthesis of anticancer active compounds and their efficiency increases when they coordinated with metal ion. Structure activity relationship (SAR) study of complexes reveals that coordination via azomethine N atom and S atom enhances the anticancer activity.

Stastical Analysis

By using SPSS Stastical software (version 16), level of significance was analyzed through one way ANOVA followed by Duncan test. It was observed that $^{*}P < 0.05$ and $^{**}P < 0.01$, ns = non-significant. All the values were expressed as mean ± standard error of mean (SEM) with % cytotoxicity.

Conclusions

By combining the information coming from different conventional techniques, octahedral geometry has been deduced for Co(II), Ni(II), Zn(II) complexes and square planar for Cu(II) complexes (Fig. 7). Antimicrobial screening indicates that metal complexes are more active as compared to Schiff base. Specially, Co(II) and Zn(II) complexes have shown more activity. *In vitro* anticancer cell lines activity reveals that metal complexes show moderate anticancer activity over Schiff base. Among them Zn(II) complex found to be more efficient against MCF-7 cell lines. It is concluded that metal complexes can be used as lead molecule for new anticancer agents.

Supplementary Information

All the information pertaining to characterization of Schiff base and its metal complexes using ^{1}H-NMR (Fig. S1, S2, S3), ESR spectra (Fig. S4) and thermogravimetric plot (Fig. S5), Biological data (tables S1, S2) and Stastical data of tested compounds (S3) are given in the supporting information.

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