SYNTHESIS, SPECTROSCOPIC STUDIES AND BIOLOGICAL PERSPECTIVES OF TRANSITION METAL COMPLEXES OF N/S DONOR SCHIFF BASE

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Bidentate Schiff base, 4-[(p-dimethylaminobenzylidene)amino]-3-mercapto-6-methyl-1,2,4-triazin-5-one, and its Co(II), Ni(II), Cu(II) and Zn(II) complexes have been prepared and characterized with the aid of various physicochemical techniques like IR, NMR, ESR, elemental, electronic and thermal analysis. The conductance data suggested the non-electrolytic behaviour of the metal complexes. Fluorescence emission study demonstrated that the metal complexes possess more fluorescent intensity as compared to the Schiff base. Stability of the metal complexes has been checked by using Horowitz-Metzger method. The redox property of Cu(II) complexes has been investigated by using cyclic voltammetry. All the newly synthesized compounds have been screened for their *in vitro* antimicrobial activity against six microbial strains and it has been found that some of the tested compounds show good antimicrobial activity as compared to standard drug.

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Introduction

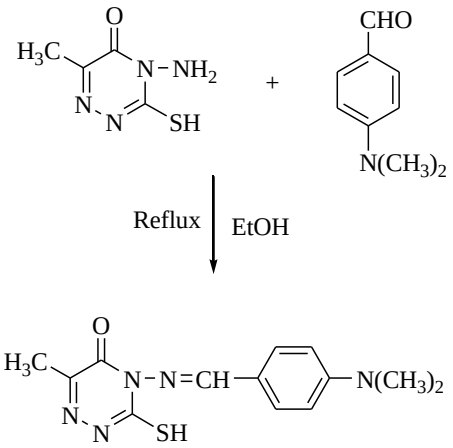
As Schiff bases have easy preparation, structural variety and varied denticities, they act as most potential group of chelators and have engrossed much attention for many years. In the field of inorganic chemistry, research is further spurred by the formation of chelate complexes of 3d transition metals with Schiff bases containing hard N atom and soft S donor atom.[1] Especially the 2p electrons of N atom of azomethine group and 3p electrons of S atom of thiol group take part in chelation with metal ions. Schiff base bind with metal ion through different types of binding modes.[2-4] Coordination of Schiff base with metal ions remarkably modulates the biological activity of metal complexes such as anticonvulsant,[5] antibacterial,[6] antifungal,[7] anti-HIV[8] and anticancer.[9,10] However, the biological properties of Schiff base depend on the ways in which it combines with metal ion. In many biological processes, metal ions accelerate the activity and efficacy of organic compound. Most of the N containing Schiff base ligand when combine with metal ion show carcinostatic and anti-HIV activities.[11-13] This broad pharmacological usage of Schiff base metal complexes encouraged us to put an effort towards the development of new Schiff base and its complexes with Co(II), Ni(II), Cu(II) and Zn(II) metal ion and further study their biological applications. In the preceding communications[14,15] structural elucidation and biological studies of metal complexes (3d metal ions with substituted 1,2,4-triazine Schiff bases) have been reported. Motivated by above findings, present article concentrate on the synthesis, characterization and antimicrobial studies of Co(II), Ni(II), Cu(II) and Zn(II) complexes derived from Schiff base 4-[(p-dimethylaminobenzylidene)-amino]-3-mercapto-6-methyl-1,2,4-triazin-5-one. Parent ligand and its metal complexes were examined for antimicrobial activity and the comparative account of antimicrobial activity has been discussed.

Experimental

All the chemicals and solvents used in present work were of analytical grade and purchased from Spectro Chem Ltd. IR spectral data of Schiff base and its metal complexes have been recorded in KBr pellets/Nujol mulls on a MB-3000 ABB spectrometer. ^{1}H-NMR of Schiff base and its Zn(II) complexes were examined on a Bruker ACF 300 spectrometer at 300 MHz in $CDCl_3$/DMSO-d_6 using TMS as an internal reference. Thermogravimetric (TG) analysis of the metal complexes were carried out on a Perkin Elmer (Pyris Diamond) instrument at a heating rate of 10 $^{\circ}$C min^{-1} by using alumina powder as reference compound. ESR spectra of the Cu(II) complexes were recorded under the magnetic field of 3000 Gauss at frequency 9.1 GHz by using Varian E-112 ESR spectrometer at SAIF, IIT, Bombay. Magnetic moment measurements were carried out on Vibrating Sample Magnetometer at Institute Instrumentation Centre, IIT, Roorkee. Electronic spectra of the metal complexes were recorded on T 90 (PG Instruments Ltd.) UV/VIS spectrometer in the region of 1100-200 nm by using DMF as solvent. Photoluminescence spectra of the Schiff base and its metal complexes were recorded on SHIMADZU RF-5301 PC spectrophotometer instrument. Electrochemical measurements of Cu(II) complexes were recorded on Ivium Stat Electrochemical Analyzer.

4-Amino-3-mercapto-6-methyl-1,2,4-triazin-5-one (AMMOT) was synthesized according to literature procedure.[16]

To prepare 4-[(p-dimethylaminobenzylidene)-amino]-3-mercapto-6-methyl-1,2,4-triazin-5-one (HL), an ethanolic solution of p-dimethylaminobenzaldehyde (1.034 g, 6.94 mmol) was mixed in ethanolic solution of AMMOT (1 g, 6.94 mmol) and the reaction mixture was refluxed for 8 h. The precipitated product was filtered, washed with ethanol, recrystallized from ethanol and dried under vacuum (Scheme 1), m.p. 232-234 °C. Anal. Calcd. for $C_{13}H_{15}N_5OS$: C, 53.96; H, 5.23; N, 24.20. Found C, 53.85; H, 5.16; N, 24.08.



Scheme 1. Synthesis of the Schiff base.

Synthesis of 1:1 metal:ligand complexes

An hot ethanolic solution of Schiff base (0.20 g, 0.69 mmol) was mixed with hot ethanolic solution of acetates of Co(II) (0.172 g, 0.69 mmol), Ni(II) (0.172 g, 0.69 mmol), Cu(II) (0.138 g, 0.69 mmol) or Zn(II) (0.152 g, 0.69 mmol). The solid product formed was filtered, washed successively with warm water, aqueous ethanol, and acetone and then dried.

Co(L)OAc.3H_2O: Anal. Calcd. for $C_{15}H_{23}CoN_5O_5S$: C, 40.54; H, 5.22; N, 15.76; Co, 13.26. Found: C, 40.44; H, 5.18; N, 15.70; Co, 13.19.

Ni(L)OAc.3H_2O: Anal. Calcd. for $C_{15}H_{23}N_5NiO_5S$: C, 40.56; H, 5.22; N, 15.77; Ni, 13.22. Found: C, 40.50; H, 5.15; N, 15.70; Ni, 13.12.

Cu(L)OAc.H_2O: Anal. Calcd. for $C_{15}H_{19}CuN_5O_4S$. C, 42.00; H, 4.46; N, 16.33; Cu, 14.81. Found: C, 41.91; H, 4.37; N, 16.24; Cu, 14.79.

Zn(L)OAc.3H_2O: Anal. Calcd. for $C_{15}H_{23}N_5O_5SZn$: C, 39.96; H, 5.14; N, 15.53; Zn, 14.50. Found: C, 39.90; H, 5.10; N, 15.39; Zn, 14.44.

Synthesis of 1:2 metal:ligand complexes

Hot ethanolic solution of ligand (0.40 g, 1.38 mmol) was mixed with a hot ethanolic solution of acetates of Co(II) (0.172 g, 0.69 mmol), Ni(II) (0.172 g, 0.69 mmol), Cu(II) (0.138 g, 0.69 mmol) and Zn(II) (0.152 g, 0.69 mmol). The product formed immediately was filtered off, washed successively with warm water, aqueous ethanol, and acetone and then dried in desiccator.

$Co(L)_2.2H_2O$: Anal. Calcd. for $C_{26}H_{32}CoN_{10}O_4S_2$: C, 46.49; H, 4.80; N, 20.85; Co, 8.77. Found: C, 46.40; H, 4.75; N, 20.70; Co, 8.71.

$Ni(L)_2.2H_2O$: Anal. Calcd. for $C_{26}H_{32}N_{10}NiO_4S_2$: C, 46.51; H, 4.80; N, 20.86; Ni, 8.74. Found: C, 46.46; H, 4.77; N, 20.73; Ni, 8.69.

$Cu(L)_2$: Anal. Calcd. for $C_{26}H_{28}CuN_{10}O_2S_2$: C, 48.78; H, 4.41; N, 21.88; Cu, 9.93. Found: C, 48.70; H, 4.34; N, 21.78; Cu, 9.87.

$Zn(L)_2.2H_2O$: Anal. Calcd. for $C_{26}H_{32}N_{10}O_4S_2Zn$. C, 46.05; H, 4.76; N, 20.66; Zn, 9.64. Found: C, 45.92; H, 4.70; N, 20.61; Zn, 9.57.

Antimicrobial assay

The Schiff base and its metal complexes were individually tested against a penal of microorganisms viz., *Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 121, *Pseudomonas aeruginosa* MTCC 741 and *Escherichia coli* MTCC 1652 (bacterial strains) and *Candida albicans* MTCC 227 and *Saccharomyces cerevisiae* MTCC 170 (fungal strain). All the bacterial cultures were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh.

For *In vitro* antimicrobial activity, the Schiff base and its metal complexes were assayed through Agar well diffusion method.[17] 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 the plates were swabbed with 100 µL inocula of the test microorganisms and kept for 15 min 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 mg mL-1 of each compound reconstituted in DMSO. All the plates were incubated at 37 °C for 24 h. 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 each treatment was repeated thrice for each organism.

Minimum inhibitory concentration (MIC)

MIC is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. MIC of the Schiff base and its metal complexes was tested against bacterial strains through a modified agar well-diffusion method.[17]

Results and discussion

All the newly synthesized compounds are coloured, stable in air, non-hygroscopic in nature, soluble in DMF and DMSO. All the metal complexes were non-electrolytic in nature which indicated their stability toward dissociation in DMF solution.

Table 1. IR Frequencies of Schiff base and the metal complexes.

Compound	υ (N=CH)	υ (C-S)	υ (S-H)	υ ($OCOCH_3$)	υ (H_2O/OH)	υ (M-S)	υ (M-N)
HL	1589	-	2854	-	-	-	-
Co(L)(OAc).3H_2O	1574	818	-	1740	3425	328	450
Co$(L)_2$.2H_2O	1582	818	-	-	3550	305	480
Ni(L)(OAc).3H_2O	1574	818	-	1744	3433	333	492
Ni$(L)_2$.2H_2O	1582	756	-	-	3433	360	460
Cu(L)(OAc).H_2O	1582	810	-	1744	3487	352	530
Cu$(L)_2$	1582	810	-	-	-	347	508
Zn(L)(OAc).3H_2O	1574	810	-	1744	3580	343	522
Zn$(L)_2$.2H_2O	1582	810	-	-	3495	370	474

IR spectra

The comparative IR frequencies of HL and all the metal complexes have been recorded in the region 4000-700 cm^{-1} and the important bands are listed in table 1. IR frequencies reveal the binding mode of HL with metal ions. In HL, a characteristic band observed at 1589 cm^{-1} due to υ (-CH=N-) stretching vibration which underwent to lower frequency (1574-1582 cm^{-1}) in the spectra of metal complexes, corroborating the coordination through azomethine nitrogen atom.[18,19] It is further supported by a new band (in far IR) which appeared in the region 450- 530 cm^{-1} ascribed to υ (M-N) stretch. Another characteristic band appeared at 2854 cm^{-1} due to υ (-SH) stretching vibration in the ligand, which is not observed in the spectra of the metal complexes shows the deprotonation of thiol group and complexation through S atom of thiol group. The deprotonation was further supported by a new stretching vibration (in far IR) observed in the region of 305-370 cm^{-1} due to υ (M-S).

In metal complexes, stretching vibration appeared in the region 3425-3580 cm^{-1} which can be assigned to υ (-OH) stretch. A characteristic band was observed at 1697 cm^{-1} in Schiff base as well as in the metal complexes due to υ (C=O) stretch which concluded the non-participation of keto group in chelation. IR spectra of 1:1 metal complexes exhibited a band in the region 1740-1744 cm^{-1} due to υ (-$OCOCH_3$) stretch. Furthermore, the absorption band ~1612 cm^{-1} and ~1435 cm^{-1} corresponding to the asymmetric and symmetric stretch of acetate group being an indication of monodentate coordination of acetate ion.[20,21]

^{1}H-NMR spectra

The chemical shift value of different types of protons of Schiff base and its Zn(II) complexes have been recorded in DMSO-d_6 solvent using TMS as internal standard. Schiff base displays two characteristics peaks at δ = 8.20 and 10.21 ppm due to imine proton (-CH=N-) and proton of thiol group (-SH) respectively. In Zn(II) complexes, the signal due to imine proton is deshielded and observed at δ 8.87 ppm which indicates the linkage through imine N atom. Signal due to thiol proton of Schiff base is absent in the complexes indicating the deprotonation and subsequent participation in complexation.[22,23] In addition, the spectrum of 1:1 Zn(II) complex shows singlet at δ + 2.40 ppm due to 3H of –$OCOCH_3$ group.

Electronic absorption spectra and magnetic moment measurements

The electronic absorption spectra of 10^{-3} M solutions of 1:1 and 1:2 metal complexes have been recorded in DMF at room temperature. The electronic absorption spectra of 1:1 and 1:2 Co(II) complexes exhibit two bands in the region 10500-11476 cm^{-1} and 20254-20841 cm^{-1} attributable to $^4T_{1g}$ (F) → $^4T_{2g}$ (F) (υ_1) and $^4T_{1g}$ (F) → $^4T_{1g}$ (P) (υ_3) transitions respectively. These two transitions support the octahedral geometry of Co(II) complexes. Band-Fitting equation has been used to calculate the ligand field parameters (Table 2) like D_q, B, β, β % .The values of D_q were found in the range 1160-1270 cm^{-1} which again confirms the octahedral geometry for Co(II) complexes. Racah interelectronic repulsion parameter (B) was found to be less than free ion value, which clearly indicates the orbital overlap and delocalization of d-electrons. The values of β were less than unity, supporting the partial covalent character of M-L bond. The magnetic moment values for Co(II) complexes were found in the range 4.4-4.8 BM, which in the expected range (4.3-5.0 BM) of octahedral complexes[24] and clearly indicates the presence of three unpaired electrons.

The electronic absorption spectra of 1:1 and 1:2 Ni(II) complexes exhibit three absorption bands in the region 9687-10698 cm^{-1}, 16918-17655 cm^{-1} and 24487-24721 cm^{-1} assigned to $^3A_{2g}$ (F) → $^3T_{2g}$ (F) (υ_1), $^3A_{2g}$ (F) → $^3T_{1g}$ (F) (υ_2) and $^3A_{2g}$ (F) → $^3T_{1g}$ (P) (υ_3) transitions respectively. The ligand field parameters like D_q, B, β, β % have also been calculated for Ni(II) complexes. The values of D_q were found to be 960-1070 cm^{-1} which supports an octahedral geometry. The values of B were less than free ion value, which shows the orbital overlap and delocalization of d-electrons. The values of β were less than unity signifying the partial covalent nature of metal-ligand bond. The observed magnetic moment values for Ni(II) are in the range of 3.3-3.5 BM, which is in good agreement with the reported range of octahedral complexes[24,25] and indicates the presence of two unpaired electron.

The electronic absorption spectra of 1:1 and 1:2 Cu(II) complexes exhibit one absorption band in the region 18610-19781 cm^{-1} attributed to $^2B_{1g}$ → $^2A_{1g}$ transition, which is indicative of square planar geometry of copper complexes. Square planar geometry is further confirmed by the observed magnetic moment value (1.9-2.0 BM), which is in the expected range of square planar complexes[24] and indicates the paramagnetic nature of Cu(II) complexes.

Table 2. Some electronic spectral data of metal complexes.

Compounds	D_q cm^{-1}	B cm^{-1}	υ_2 / υ_1	B	β %
Co(L)(OAc).3H$_2$O	1169.3	729.8	1.09	0.751	24.9
Co(L)$_2$.2H$_2$O	1269.4	705.5	2.10	0.726	27.4
Ni(L)(OAc).3H$_2$O	968.7	822.9	1.74	0.790	21.0
Ni(L)$_2$.2H$_2$O	1069.8	685.5	1.65	0.658	34.2

Fluorescence spectra

Fluorescent spectra of Schiff base and its 1:2 metal complexes have been recorded in 10^{-3} M solution in DMF at EX wavelength of 265 nm. Fluorescent data helps us to investigate the changes in fluorescent property of Schiff base when it binds with metal ion.[26] The emission spectra are presented in Figure 1.

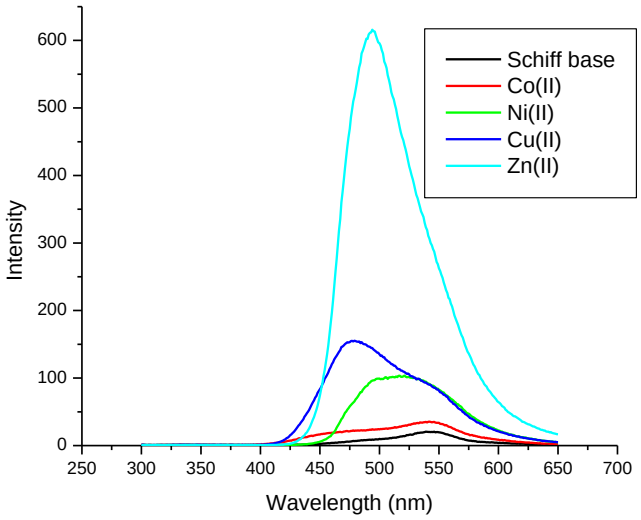


Figure 1. Photoluminescence spectra of Schiff base and its 1:2 metal complexes

The emission wavelengths were observed at 542 nm, 521 nm, 479 nm, and 494 nm for Co(II), Ni(II), Cu(II) and Zn(II) complexes respectively. A weak emission band was observed at 539 nm for Schiff base. Metal complexes show highest fluorescent intensity as PET process is blocked due to engagement of lone pair of electrons of ligand with metal ion. Among all the complexes, Zn(II) complex shows highest fluorescent intensity. On the other hand, coordination between ligand and metal ion reduces the loss of energy via radiation less thermal vibrations.[27] Thus, enhancement in fluorescent intensity was observed and it is evident that fluorescence emission intensity of ligand increase upon coordination and order of their fluorescent intensity are Zn(II) > Cu(II) > Ni(II) > Co(II) > Schiff base.

Electron Spin Resonance spectra

The powder ESR spectra of Cu(L)(OAc).H$_2$O and Cu(L)$_2$ have been recorded which resolved the parallel and perpendicular features of *g*-factor. The ESR parameters, calculated for Cu(L)(OAc).H$_2$O, are $g_{\parallel}$= 2.12, $g_{\perp}$= 2.06, g_{av} = 2.08, *G* = 2.04) and for Cu(L)$_2$ are ($g_{\parallel}$ = 2.14, $g_{\perp}$= 2.07, g_{av} = 2.09, *G* = 2.03). This g value is the characteristics of axial symmetry and trends $g_{\parallel} > g_{\perp} > g_e$ (2.0023) indicates that electron is most likely to be in the $d_{x^2-y^2}$ orbital.[28] In addition, the exchange interaction parameter is calculated by Hathaway expression (eqn. 1).

$$G = \frac{g_{\parallel} - 2.0023}{g_{\perp} - 2.0023} \qquad (1)$$

The *G* value is found to be less than 4.0 indicating considerable exchange interaction in Cu(II) complexes.[29,30] Furthermore, the observed g value was less than 2.3 which suggests the partial covalent nature of metal-ligand bond.[28] Thus, these ESR parameters support the square planar geometry of the Cu(II) complexes.[31]

Thermogravimetric Analysis

The thermal analysis of 1:2 complexes of Co(II), Ni(II), Cu(II) and Zn(II) metal ion have been studied in temperature range of 50-700 °C under air atmosphere by using α-Al_2O_3 as reference. TG curves are presented Figure 2. The mass loss were obtained from this TG curve is compared with the calculated values. This TG data is further supported by DTA curves.

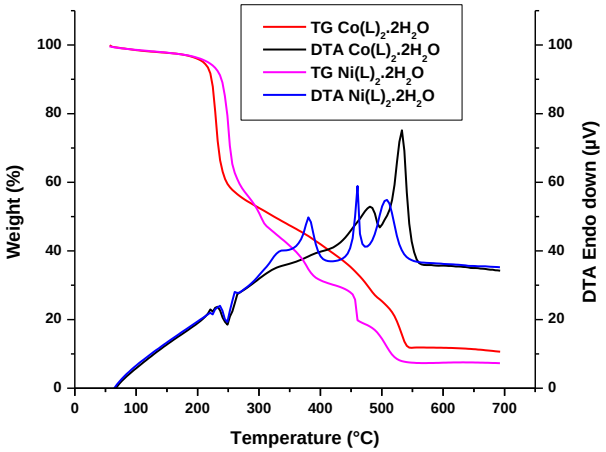


Figure 2a. TG and DTA plot of CoL$_2$.2H$_2$O and NiL$_2$.2H$_2$O

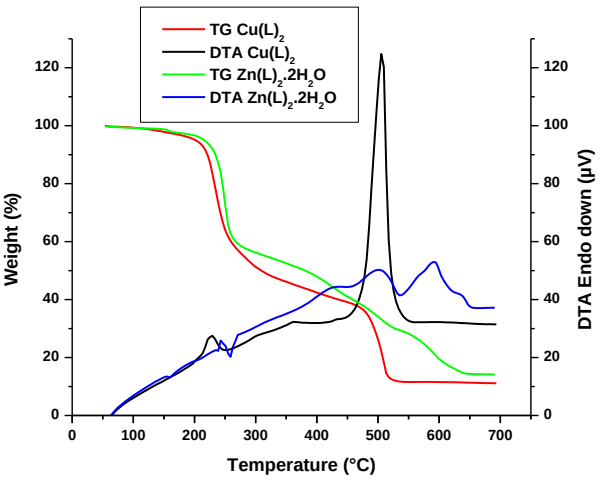


Figure 2b. TG and DTA plot of CuL$_2$ and ZnL$_2$.2H$_2$O

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The TG curve of $Co(L)_2.2H_2O$ shows decomposition in three successive steps. First decomposition step from 95-220°C, results in mass loss 5.00 % (Calcd. 5.36 %) associated with the removal of coordinated water molecules. Second step shows decomposition within temperature range 221-310 °C due to loss of organic moiety with estimated mass loss of 43.10 % (Calcd. 43.82 %). Third decomposition step (311-520°C) involved mass loss 41.45 % (Calcd. 42.03 %) confined to the removal of triazine ring. The final weight of the residue corresponds to CoO.

TG degradation of $Ni(L)_2.2H_2O$ also occurred in three steps. First decomposition step attributed to the loss of coordinated water molecules in the temperature range 110-220 °C with mass loss 5.05 % (Calcd. 5.36 %). Second decomposition step (221-285 °C) reasonably accounted for the mass loss 43.15% (Calcd. 43.83 %) confined to the loss of organic moiety. Third decomposition step has been observed from 286-500 °C assigned to the removal of triazine ring with mass loss 42.00 % (Calcd. 42.04 %) leaving behind the NiO as residue.

In case of $Cu(L)_2$, degradation took place in two major steps. First degradation step has been observed from 120-290 °C results in mass loss 45.09 % (Calcd. 45.97 %) associated with removal of organic moiety. Second degradation step (291-495 °C) indicates the removal of triazine ring with mass loss 44.01 % (Calcd. 44.09 %) leaving CuO as residue.

The TG curve of $Zn(L)_2.2H_2O$ shows three step degradation. First degradation step was observed from temperature range 100-220 °C with mass loss 5.21 % (Calcd. 5.31 %) attributed to the removal of two water molecules. Second step (221-280 °C) exhibited mass loss 43.12 % (Calcd. 43.38 %) indicates the loss of organic moiety. Third decomposition involved mass loss 41.29 % (Calcd. 41.61 %) corresponds to the release of triazine ring in the temperature range from 281-550 °C. The remaining residue was estimated as ZnO.

Table 3. Calculated Activation Energies (kJ mol^{-1}) for Individual Stages in degradation of metal complexes.

Compounds	E_1^* (r^2)	E_2^* (r^2)	E_3^* (r^2)
$Co(L)_2.2H_2O$	5.87 (0.94)	1.94 (0.95)	1.85 (0.98)
$Ni(L)_2.2H_2O$	6.89 (0.96)	23.9 (0.91)	2.66 (0.97)
$Cu(L)_2$	16.2 (0.92)	1.15 (0.94)	-
$Zn(L)_2.2H_2O$	9.94 (0.97)	22.7 (0.91)	1.75 (0.98)

*1,2 and 3 stands for first, second and third stage, r^2 stands for regression coefficient; Heating rate = 10 °C min^{-1}.

Kinetic Calculations

Horowitz-Metzger method[32] is used for non-isothermal degradation of $Co(L)_2.2H_2O$, $Ni(L)_2.2H_2O$, $Cu(L)_2.2H_2O$ and $Zn(L)_2.2H_2O$.

Table 4. Biological activity of Schiff base and its Metal Complexes.

Compound	**Diameter of growth of inhibition zone in mm[a]**					
	B. subtilis	***S. aureus***	***E.coli***	***P. aeruginosa***	***C. albicans***	***S. cerevisiae***
HL	17	14	13	14	10	16
$Co(L)(OAc).3H_2O$	12	14	12	16	21	14
$Co(L)_2.2H_2O$	14	15	10	17	19	19
$Ni(L)(OAc).3H_2O$	15	17	15	18	18	15
$Ni(L)_2.2H_2O$	14	15	16	18	16	16
$Cu(L)(OAc).H_2O$	14	14	14	12	12	12
$Cu(L)_2$	18	16	14	18	18	15
$Zn(L)(OAc).3H_2O$	18	15	22	18	18	19
$Zn(L)_2.2H_2O$	17	20	20	20	19	19
Ciprofloxacin	24.0	26.6	25.0	22	-	-
Amphotericin-B	-	-	-	-	16.6	19.3

[a]Values, including diameter of the well (8 mm), are means of three replicates.

Table 5. MIC (μg mL^{-1}) of the synthesized compounds.

Compounds	***B. subtilis***	***S. aureus***	***E. coli***	***P. aeruginosa***	***C. albicans***	***S. cerevisiae***
HL	50	-	-	-	-	50
$Co(L)(OAc).3H_2O$	-	-	-	50	25	-
$Co(L)_2.2H_2O$	-	-	-	50	25	25
$Ni(L)(OAc).3H_2O$	-	50	-	50	50	-
$Ni(L)_2.2H_2O$	-	-	50	50	50	50
$Cu(L)(OAc).H_2O$	-	-	-	-	-	-
$Cu(L)_2$	50	50	-	50	50	-
$Zn(L)(OAc).3H_2O$	50	-	25	50	50	25
$Zn(L)_2.2H_2O$	50	25	25	25	50	25
Ciprofloxacin	6.25	6.25	6.25	12.5	-	-
Amphotericin-B	-	-	-	-	12.5	12.5

The kinetic parameters like α (degree of conversion), T_s (temperature at which 1/(1- α) = 1/exp = 0.368), θ, E (calculated from the slope of the graph between ln ln [1/1-α] and θ) and ln ln[1/1- α] can be calculated by using the eqn. 2, where *R* is Universal Gas Constant.

$$\ln \ln [1/1-\alpha] = E\theta/RT_s^2 \quad (2)$$

The following stability order of the complexes $Cu(L)_2$ > $Zn(L)_2.2H_2O$ > $Ni(L)_2.2H_2O$ > $Co(L)_2.2H_2O$ has been suggested by Horowitz-Metzger method and their activation energy values are presented in Table 3 and Figure 3.

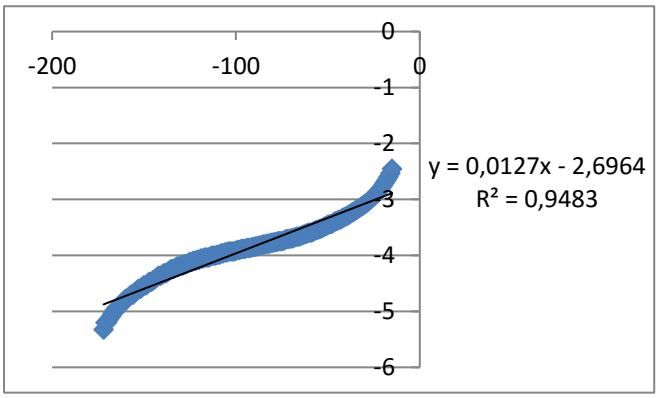

Figure 3a. Horowitz-Metzger plot of ln ln[1/1-α] against θ for first stage decomposition of $Co(L)_2.2H_2O$

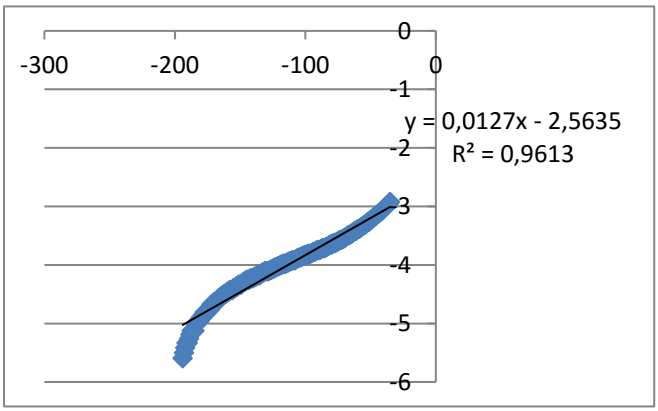

Figure 3b. Horowitz-Metzger plot of ln ln[1/1-α] against θ for first stage decomposition of $Ni(L)_2.2H_2O$

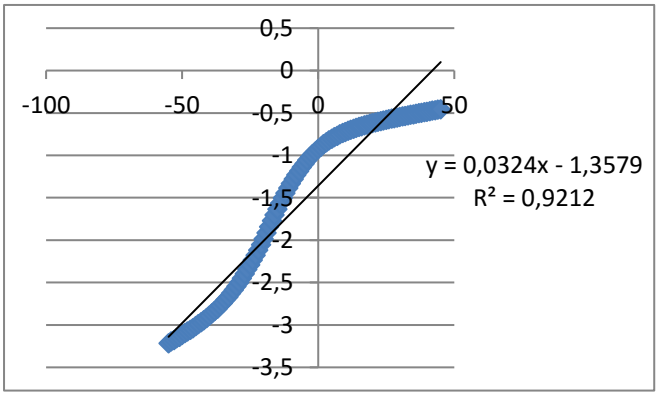

Figure 3c. Horowitz-Metzger plot of ln ln[1/1-α] against θ for first stage decomposition of $Cu(L)_2$

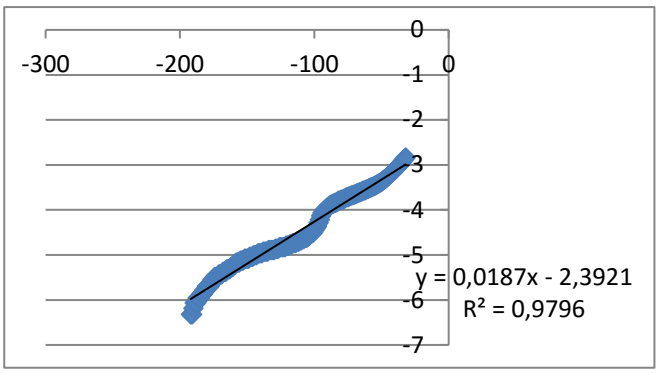

Figure 3d. Horowitz-Metzger plot of ln ln[1/1-α] against θ for first stage decomposition of $Zn(L)_2.2H_2O$

Electrochemical Studies

Cyclic Voltammogram of copper complexes have been recorded in DMF with a scan rate of 0.5 to -1.3 V by using $n Bu_4N^+ClO_4^-$ (TBAP) as supporting electrolyte (Figure 4). $Cu(L)(OAc).H_2O$ and $Cu(L)_2$ show reduction peaks in forward sweep at E_{Pc} = - 0.1 V and E_{Pc} = - 0.99 V assigned to $Cu^{2+/+}$ couple and oxidation peaks at E_{Pa} = - 0.65 V and E_{Pa} = - 0.60 V assigned to $Cu^{+/2+}$ couple.

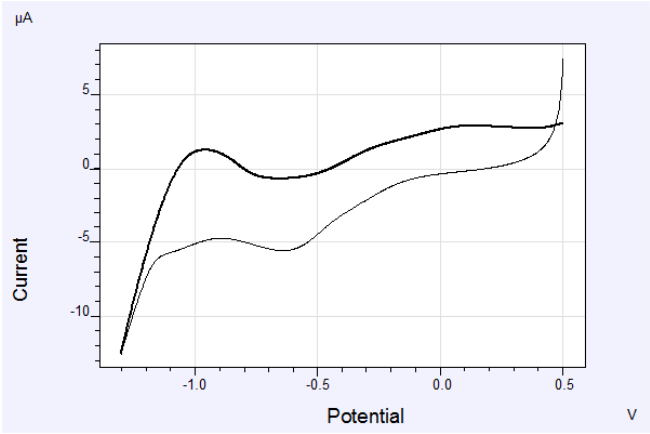

Figure 4. Cyclic Voltammogram of $Cu(L)_2$

For $Cu(L)(OAc).H_2O$ and $Cu(L)_2$ half wave potentials ($E_{1/2}$) were found to be 0.375 V and 0.795 V respectively, indicating a one-electron transfer. The peak to peak separation value (ΔE_P = 0.55 V and 0.39) between cathodic and anodic potential is high. Above data deduced that redox couples are related to quasi reversible one electron transfer process.[33,34]

Antimicrobial activity

Schiff base and its respective metal chelates were screened for antimicrobial activity against four bacterial strains and two fungal strains by using agar well diffusion method.[35] All the data of antimicrobial evaluation are summarized in Table 4 and Figure 5.

For gram positive bacteria inhibition zone were observed in the range 12-20 mm, 10-22 mm for gram negative bacteria and 10-21 mm for yeast. $Cu(L)_2$ and $Zn(L)(OAc).3H_2O$ exhibit maximum inhibition zone of 18 mm against *B. subtilis* while $Zn(L)_2.2H_2O$ exhibit maximum inhibition zone of 20 mm towards *S. Aureus* and *P. aeruginosa*. $Zn(L)(OAc).3H_2O$ showed significant activity (18-22 mm) against *E. coli*, *C. albicans* and *S. cerevisiae*. $Co(L)(OAc).3H_2O$ shows the highest activity (21 mm) against *C. albicans*. Beside this, $Co(L)_2.2H_2O$ and $Zn(L)_2.2H_2O$ exhibit good activity (19 mm) towards *C. albicans* and *S. cerevisiae* respectively.

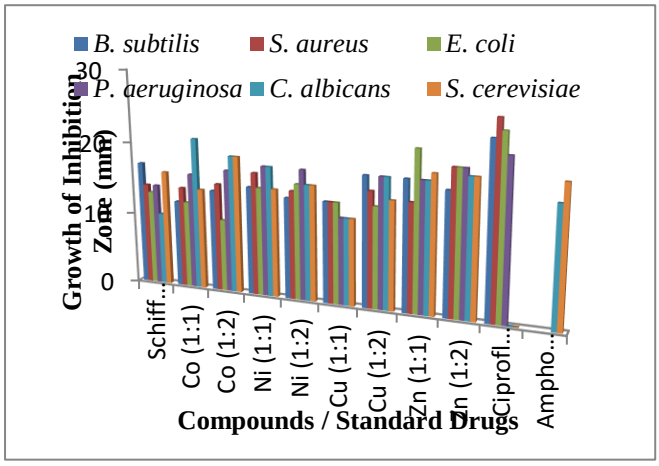

Figure 5. Antibacterial activities of synthesized compounds

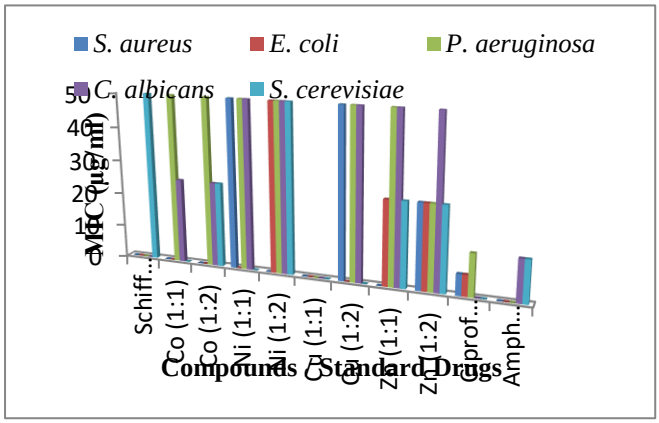

Figure 6. Comparison of MIC of Schiff base and its metal complexes with standard drugs

Against most of the strains, Zn(II) complexes showed the highest activity and follow the order *C. albicans* ≈ *S. cerevisiae* > *P. Aeruginosa* > *E. coli.* The data showed that metal complexes show better biological activity as compared to Schiff base.[36,37] It is explained by

Overtone's concept[38] of cell permeability and Tweedy's Chelation theory.[39] According to the Overtone's concept of cell permeability, the lipid membrane surrounding the cell favors the passage of only lipid-soluble material; therefore, liposolubility is an important factor which controls the antimicrobial activity. On chelation, polarity of the metal ion is reduced to a greater extent due to the overlapping of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Moreover, delocalization of the π-electrons over the whole chelate ring is increased and the lipophilicity of the complex is enhanced.[39,40] The increased lipophilicity enhances the penetration of the complexes into the lipid membranes and blocks the metal binding sites in the enzymes of microorganisms.

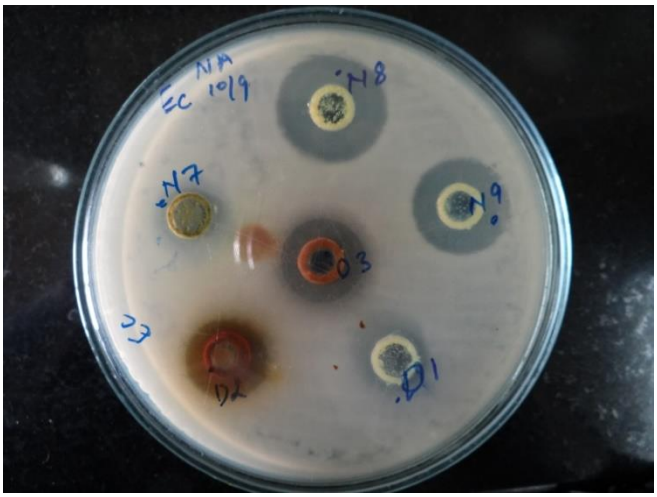

Figure 7a. Inhibition zone of Zn^{II} complexes (N8 and N9) against bacteria *E. coli*

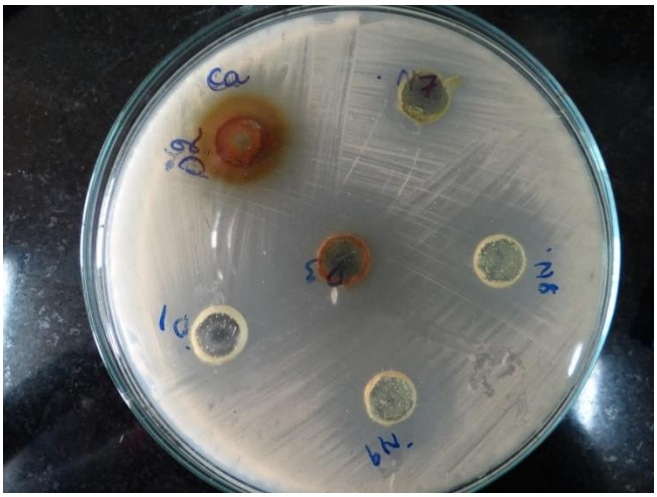

Figure 8b Inhibition zone of Zn^{II} complexes (N8 and N9) against fungi *C. albicans*

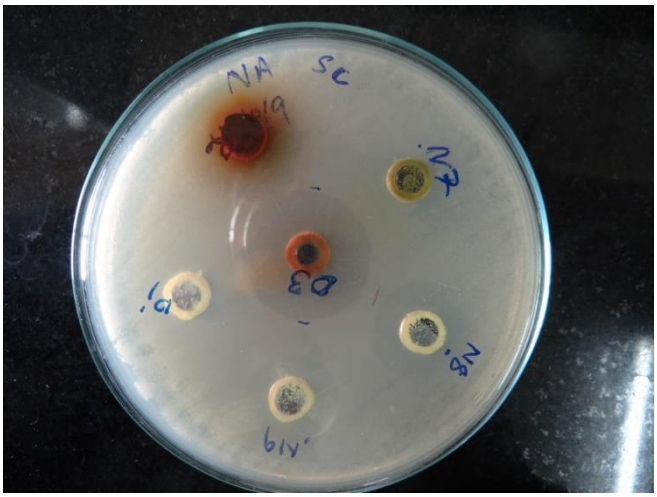

Figure 8c Inhibition zone of Zn^{II} complexes (N8 and N9) against fungi *S. cerevisiae*

MIC value of the tested compound was found to be in the range of 25-50 μg mL^{-1} and presented in Table 5 and Figure 6. The Zn(II) complexes exhibit better antibacterial and antifungal activities against *C. albicans*, *S. cerevisiae*, *P. Aeruginosa* and *E. coli*, (Figure 7) than the others..

The obtained data reveals that Co(II), Ni(II) and Zn(II) complexes exhibited octahedral geometry while Cu(II) complexes exhibit square planar geometry. The proposed structures are presented on Figure 8.

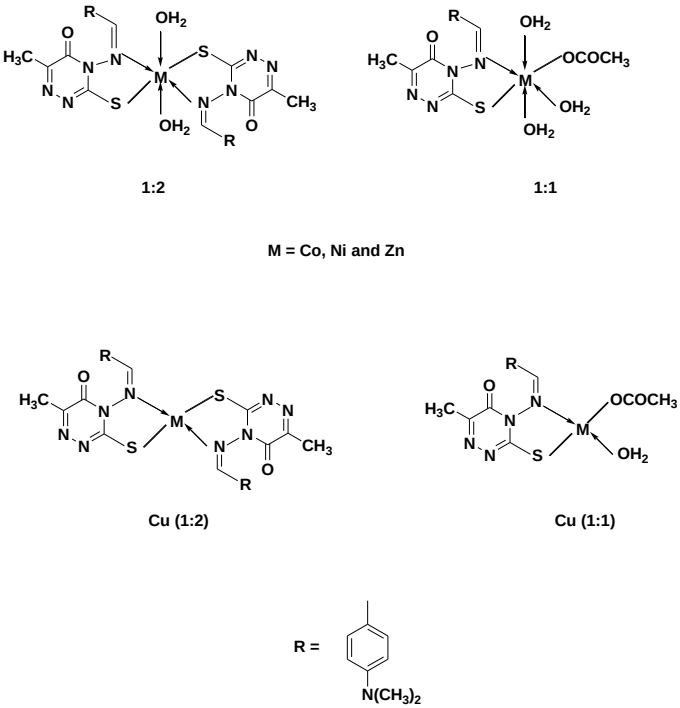

Figure 7. Proposed structures of Schiff base metal complexes.

Conclusions

Various physicochemical techniques have been used to characterize the Schiff base as well as metal complexes. IR spectra reveal that Schiff base act as bidentate ligand as they are coordinated with metal centre via N and S atoms. TG and kinetic studies have been used to determine the thermal stability of the complexes. Presence of lattice water molecules are calculated by TG. The Co(II), Ni(II) and Zn(II) complexes exhibit octahedral geometry while Cu(II) complexes exhibit square planar geometry. Biological experiments indicated that the metal complexes exhibit higher biological activity as compared to noncoordinated Schiff base. The Zn(II) complexes exhibit the best antibacterial and antifungal activities against *C. albicans*, *S. cerevisiae*, *P. Aeruginosa* and *E. coli* among the studied complexes.

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References

[1]Krishnan,B., Vijayanthimala, R., *J. Chem. Biol. Phyd. Sci*, **2015**, *5*, 1139.

[2]Ejidike, I. P., Ajibade, P. A., *Molecules*, **2015**, *20*, 9788.

[3]Mahalakshmi, N., Rajavel, R., *Arabian J. Chem*., **2014**, *7*, 509.

[4]Nagesh, G. Y., Mahadev, U. D., Mruthyunjayaswamy, B. H. M., *Int., J. Pharm. Sci. Rev. Res*., **2015**, *31*, 190.

[5]Sridhar, S. K., Pandeya, S. N., Stsbles, J. P., Ramesh, A., *Eur. J. Pharm. Sci*., **2002**, *16*, 129.

[6]Prakash, A., Singh, B. K., Bhojak, N., Adhikari, D., *Spectrochim. Acta, Part A*,**2010**, *76*, 356.

[7]Bharti, S. K., Nath, G., Tilak, R., Singh, S. K., *Eur. J. Med. Chem*., **2010**, *45*, 651.

[8]Al-masoudi, N. A., Aziz, N. M., Mohammed, A. T., *Phosphorus, Sulphur Silicon Relat. Elem*., **2009**, *184*, 2891.

[9]Creaven, B. S., Devereux, M., Foltyn, A., McClean, S., Rosair, G., Thangella, V. R., Walsh, W., *Polyhedron*, **2010**, *29*, 813.

[10]Shakir, M., Azam, M., Ullah, M. F., Hadi, S. M., *J. Photochem. Photobiol*, **2011**, *104*, 449.

[11]Chohan, Z. H., Kausar, S., *Met. Based Drugs*, **2000**, *7*, 17.

[12]Chohan, Z. H., Jaffery, M. F., Supuran, C. T., *Met. Based Drugs*, **2001**, *8*, 95.

[13]Racane, L., Kulenovic, V. T., Jakic, L. F., Boykin, D. W., Zamola, G. K. ,*Heterocycles*, **2001**, *55*, 2085.

[14]Abou-Hussein, A. A., Linert, W. ,*Spectrochim. Acta, Part A*, **2015**, *141*, 223.

[15]Naik, K. H. K., Ashok, B., Naik, N., Mulla, J. A. S., Prakasha, A., *Spectrochim. Acta, Part A*, **2015**, *141*, 88.

[16]Singh, K., Kumar, Y., Puri, P., Sharma, C., Aneja, K. R., *Int. J. Inorg. Chem*., **2012** doi: 10.1155/2012/873272.

[17]Aneja, K. R., Sharma, C., *Judishapur J. Microbiol*., **2011**, *4*, 175.

[18]Shanker, K., Rohini, R., Kumar, K. S., Reddy, P. M., Ho, Y. P., Ravinder, V., *J. Indian Chem. Soc*., **2009**, *86*, 153.

[19]Keypour, H., Shayesteh, M., Golbedaghi, R., Blackman, A. G., Cameron, S. A., *Transition Met. Chem*., **2013**, *38*, 611.

[20]Atkins, A. J., Black, D., Finn, R. L., Marin-Becerra, A., Blake, A. J., Ruiz-Ramirej, L., Li, W-S., Schroder, M., *Dalton Trans*., **2003**, *9*, 1730.

[21]Singh, A. K., Pandey, O. P., Sengupta, S. K., *Spectrochim. Acta, Part A*, **2012**, *85*, 1.

[22]Singh, K., Raparia, S., Surain, P., *Med. Chem. Res*., **2014**, doi: 10.1007/s00044-014-1298-0.

[23]Singh, K., Kumar, Y., Puri, P., Sharma, C., Aneja, K. R., *Arabian J. Chem*., **2013** doi: 10.1016/j.arabjc.2012.12.038.

[24]Kumar, D., Syamal, A., Jaipal, Gupta, P. K., *J. Indian Chem. Soc*., **2007**, *84*, 217.

[25]Kumar, D., Chadda, S., Sharma, J., Surain, P., *Bioinorg. Chem. Appl*., **2013**, doi: 10.1155/2013/981764.

[26]Shanmugakala, R., Tharmaraj, P., Sheela, C. D., Chidambaranathan, N., *Med. Chem. Res*., **2014**, *23*, 329.

[27]Das, D., Chand, B. G., Sarker, K. K., Dinda, J., Sinha, C., *Polyhedron*, **2006**, *25*, 2333.

[28]Kivelson, D., Neiman, R., *J. Chem. Phys*., **1961**, *35*, 149.

[29]Bagihalli, G. B., Avaji, P. G., Patil, S. A., Badami, P. S., *Eur. J. Med. Chem*., **2008**, *43*, 2639.

[30]Hathaway, B. J., Billing, D. E., *Coord. Chem. Rev*., **1970**, *5*, 143.

[31]Boraey, H. A. E., Rahman, R. M. A., Atia, E. M., Hilmy, H., *Central Eur. J. Chem*., **2010**, *8*, 820.

[32]Singh, K., Ritu,Vikas K., *J. Chem. Biol. Phys. Sci*., **2015**, *5*, 2691.

[33]Hazra, M., Dolai, T., Pandey, A., Dey, S. K., Patra, A., *J. Saudi Chem. Soc*., **2014**, doi: 10.1016/j.jscs.2014.02.009.

[34]Wu, H., Kou, F., Jia, F., Liu, B., Yuan, J., Bai, Y., *Bioinorg. Chem. Appl*., **2011**, doi: 10.1155/2011/105431.

[35]Okeke, M. I., Iroegbu, C. U., Eze, E. N., Okoli, A. S., Esimone, C. O., *J. Ethnopharmacol*., **2001**, *78*, 119.

[36]Singh, K., Kumar, Y., Puri, P., Kumar, M., Sharma, C.,*Eur. J. Med. Chem*., **2012**, 52, 313.

[37]Patil, S. A., Unki, S. N., Kulkarni, A. D., Naik, V. H., Badami, P. S., *Spectrochim. Acta, Part A*, **2011**, *79*, 1128.

[38]Anjaneyula, Y., Rao, R. P., *Synth. React. Inorg. Met. Org. Chem*., **1986**, *16*, 257.

[39]Dharamraj, N., Viswanathamurthi, P., Natarajan, K., *Transition Met. Chem*., **2001**, *26*, 105.

[40]Raman, N., Kulandaisamy, A., Jeyasubramanian, K., *Polish J. Chem*., **2002**, *76*, 1085.

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