



SYNTHESIS, STRUCTURAL AND BIOLOGICAL STUDIES OF Co(II), Ni(II), Cu(II) AND Zn(II) COMPLEXES OF 4-[(3-ETHOXY- 4-HYDROXYBENZYLIDENE)AMINO]-3-MERCAPTO-6- METHYL-5-OXO-1,2,4-TRIAZINE

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Keywords: Metal complexes; Schiff base; 4-[(3-ethoxy-4-hydroxybenzylidene)amino]-3-mercapto-6-methyl-5-oxo-1,2,4-triazine.

Co(II), Ni(II), Cu(II) and Zn(II) complexes with the bidentate ligand 4-[(3-ethoxy-4-hydroxybenzylidene)amino]-3-mercapto-6-methyl-5-oxo-1,2,4-triazine have been synthesized. The Schiff base and its metal complexes have been characterized by various physicochemical techniques like IR, ¹H-NMR, ESR, electronic and fluorescence spectroscopy and cyclic voltammetry. Elemental analysis, conductivity measurements and thermal analysis of synthesized compounds were also carried out. All the complexes were colored and non-electrolytic in nature. In vitro biological activities of the ligand and complexes have been checked against some pathogenic gram positive, gram negative bacteria and different fungi and then compared with some standard drugs as control.

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Here we report the synthesis and characterization of Co(II), Ni(II), Cu(II) and Zn(II) metal complexes of a newly synthesized ligand obtained from the condensation of 3-ethoxy-4-hydroxybenzaldehyde with 4-amino-3-mercapto-6-methyl-5-oxo-1,2,4-triazine.

Introduction

Schiff bases are generally formed by the condensation of an amine and an active carbonyl group containing compound.¹⁻³ During the recent years, there have been many investigations of Schiff bases possessing nitrogen-containing heterocyclic compounds.⁴ Amino-group containing heterocyclic compounds consisting of two or more potential donor centers play an important role in the study of competitive reactivity of a bidentate ligand system.⁵ The development of new drugs based on 1,2,4-triazines further enhances the interest in synthesis of these compounds. It has been well established that the triazine derivatives are potent antibacterial, antioxidant, antifungal, anti-tuberculosis and anti-inflammatory agents.⁶⁻¹³ In addition, their applications as herbicides, fungicides and insecticides are also well known.¹⁴⁻¹⁵ Metal ion complexes with Schiff bases are synthesized and their biological activities have been checked.¹⁶ These complexes can be used in various catalytic reactions and as models for biological systems. It has been well reported that some drugs possess higher activity when administered as metal complexes than as the free ligand. Increase of biological activity has been reported by the incorporation of transition metals in Schiff bases. Importance of Schiff base metal complexes has spurred the search for new metal-based drugs using different biologically activemetals.¹⁷⁻¹⁸

Triazine derivatives with additional N or S donor atoms exhibit strong chelating ability and provide potential binding sites for complexation with various metal ions. Certain cobalt, copper metal complexes are significant antiviral agents.¹⁹⁻²²

Experimental

All the chemicals and solvents used were of analytical grade. IR spectra of Schiff base and its metal complexes were recorded in KBr pellets/Nujol mulls on a MB-3000 ABB spectrometer. ¹H-NMR spectra of Schiff base and its Zn(II) complexes were recorded on bruker ACF 300 spectrometer at 300 MHz in DMSO-*d*₆ using TMS as a reference compound. Electronic spectra of metal complexes were recorded on T90 (PG instruments Ltd.) UV/VIS spectrophotometer in DMF solvent, in the region of 200-900 nm. Fluorescence spectra of ligand and metal complexes were recorded on SHIMADZU RF-5301 PC spectrofluorometer. Ivium Stat Electrochemical Analyzer was used for cyclic voltammetry measurement of Cu(II) complexes. Magnetic moment measurements were carried out on Vibrating Sample Magnetometer at the Institute Instrumentation Centre, IIT Roorkee.

Thermogravimetric analysis was carried out on the Perkin Elmer (Pyris Diamond) instrument at a heating rate of 10 °C min⁻¹ by using alumina powder as reference. ESR spectra of copper complexes were recorded under the magnetic field 0.3 T at frequency 9.1 GHz by using Varian E-112 ESR spectrometer at SAIF, IIT Bombay. Elemental analyses (C, H, N) of compounds were carried out by using a Perkin-Elmer 2400 Elemental analyzer. Gravimetric methods were used to determine the metal contents in synthesized metal complexes i.e. cobalt was estimated as cobalt pyridine thiocyanate, nickel as nickel dimethylglyoximate, copper as cuprous thiocyanate and zinc as zinc ammonium phosphate.²³

Synthesis of ligand

First, 4-amino-3-mercapto-6-methyl-5-oxo-1,2,4-triazine (AMMOT) was synthesized by a reported procedure.²⁴ Its ethanolic solution (1.50 g, 9.49 mmol) was mixed with an ethanolic solution of 3-ethoxy-4-hydroxybenzaldehyde (1.578 g, 9.49 mmol) and the reaction mixture was refluxed for 7 h. The progress of reaction was checked by TLC. The precipitated ligand, 4-[(3-ethoxy-4-hydroxybenzylidene)-amino]-3-mercapto-6-methyl-5-oxo-1,2,4-triazine (HL), was filtered, washed with ethanol, recrystallized from ethanol and then dried at room temperature, m.p. 182°-184 °C (Figure 1).

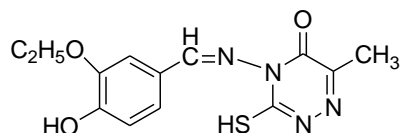


Figure 1. Schiff Base: 4-[(3-ethoxy-4-hydroxybenzylidene)-amino]-3-mercapto-6-methyl-5-oxo-1,2,4-triazine.

Synthesis of metal complexes

Synthesis of 1:1 metal complexes

The metal complexes were prepared by the reaction of the ligand (0.4 g, 1.30 mmol) with the respective acetates of Co (0.326 g, 1.30 mmol), Ni (0.325 g, 1.30 mmol), Cu (0.261 g, 1.30 mmol) and Zn (0.286 g, 1.30 mmol) in ethanolic solution. The solid products formed were filtered, washed with warm water, alcohol and acetone and then dried in desiccator.

Synthesis of 1:2 metal complexes

Hot ethanolic solutions of ligand (0.8 g, 2.61 mmol) were mixed with hot ethanolic solutions of acetates of Co (0.326 g, 1.30 mmol), Ni (0.325 g, 1.30 mmol), Cu (0.261 g, 1.30 mmol), Zn (0.286 g, 1.30 mmol). The products formed were filtered off, washed with warm water, alcohol and finally with acetone and then dried in desiccator.

Pharmacology

The newly synthesized ligand and its metal complexes have been screened in vitro for their antimicrobial activity against two Gram-positive (*Staphylococcus aureus* MTCC 96 and *Bacillus subtilis* MTCC 121), two Gram-negative (*Escherichia coli* MTCC 1652 and *Pseudomonas aeruginosa* MTCC 741) and two fungi (*Candida albicans* MTCC 227 and *Saccharomyces cerevisiae* MTCC 170) by agar well diffusion method as reported in previous papers from our laboratory.²⁵ All the bacterial cultures were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh. Ciprofloxacin and amphotericin B were used as standard antibacterial and antifungal drugs respectively.

The agar well diffusion method was used to check the antibacterial activity of synthesized compounds.²⁶ All the

microbial cultures were adjusted to 0.5 McFarland Standard, which is visually comparable to a microbial suspension of approximately 1.5×10^8 cfu mL⁻¹. 20 mL of Mueller Hinton agar medium was poured into each Petri dish and dishes were swabbed with 100 μ L inoculum of the test microorganisms and kept for 20 minutes for adsorption. Using sterile cork borer of 8 mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100 μ L volume with concentration of 4.0 mg mL⁻¹ of each compound reconstituted in DMSO. All the plates were incubated at 36 °C for 24 h. Antibacterial activity of each compound was analyzed by measuring the zone of growth inhibition against the test organisms with zone reader (Hi Antibiotic zone scale). This procedure was performed in three replicate plates for each organism.

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. MIC of the various compounds against bacterial strains was tested through a modified agar well-diffusion method.

Results and discussion

Schiff base and its metal complexes are colored and non-hygroscopic in nature. Though Schiff base is soluble in common organic solvents such as ethanol and methanol, its metal complexes are insoluble in common organic solvents but soluble in DMF and DMSO. The molar conductance of all the complexes was measured in DMF using 10^{-3} M solutions at room temperature. The molar conductance values (36.89 – 43.29 S cm² mol⁻¹) indicate that all the complexes are non-electrolytic in nature. The purity of the ligand and its metal complexes was checked by TLC. Analytical data of all compounds are given in Table 1.

IR spectra

The comparative IR frequencies of ligand and its metal complexes were recorded in the region 400-4000 cm⁻¹. The important IR frequencies of Schiff base and metal complexes are summarized in Table 2. In the free ligand, a characteristic band observed at 1697 cm⁻¹ due to azomethine $\nu(-CH=N)$ group,^{27,28} which is shifted to lower frequency (20-30 cm⁻¹) in spectra of the metal complexes, indicating a coordination through the nitrogen of azomethine group. A weak band observed at 2893 cm⁻¹ due to ν (SH) group in the ligand, has disappeared in the metal complexes, which indicates the deprotonation of thiol group. In metal complexes, a broad band appeared in the region 3232-3742 cm⁻¹ due to ν (OH/H₂O), which is assigned to phenolic OH and water molecules associated with the complexes. A characteristic band was observed at 1582 cm⁻¹ in Schiff base and its metal complexes due to $\nu(C=O)$ stretch, its unaltered position indicates the non-participation of keto group in chelation. In 1:1 metal complexes, band was observed at 1744 cm⁻¹ due to acetate group.²⁵ The coordination of ligand to the metal through the azomethine nitrogen atom and sulfur atom is further supported by (M-N) and (M-S) bands in the range 482-488 cm⁻¹ and 323-342 cm⁻¹ respectively.^{29,30}

Table 1. Analytical data of the synthesized compounds .

| Compounds | Color | Mp. °C, dec* | Yield, % | Elemental analysis, calculated (found), % | | | |
|--|-----------------|--------------|----------|---|-------------|---------------|---------------|
| | | | | C | H | N | M |
| Schiff Base, HL C ₁₃ H ₁₄ N ₄ O ₃ S | Light yellow | 182 | 84 % | 50.98 (50.32) | 4.57 (4.18) | 18.30 (18.09) | |
| Co(L)(OAc).3H ₂ O | Dark Green | 200 | 81 % | 37.74 (37.64) | 4.61 (4.56) | 11.74 (11.44) | 12.35 (11.93) |
| Co(L) ₂ .2H ₂ O | Dark Green | 202 | 80 % | 44.26 (43.91) | 4.25 (4.13) | 15.88 (15.80) | 8.36 (7.81) |
| Ni(L)(OAc).3H ₂ O | Yellowish Green | 224 | 77 % | 37.76 (36.39) | 4.61 (4.18) | 11.75 (11.69) | 12.31 (11.87) |
| Ni(L) ₂ .2H ₂ O | Yellowish Green | 210 | 78 % | 44.27 (44.13) | 4.26 (4.11) | 15.89 (15.43) | 8.32 (8.12) |
| Cu(L)(OAc).H ₂ O | Brown | 214 | 84 % | 37.38 (37.10) | 3.74 (3.15) | 11.63 (11.56) | 13.18 (12.35) |
| Cu(L) ₂ | Brown | 196 | 82 % | 46.32 (45.74) | 3.86 (3.08) | 16.63 (15.97) | 9.42 (9.06) |
| Zn(L)(OAc).3H ₂ O | Light Yellow | 240 | 80 % | 37.24 (37.02) | 4.55 (4.26) | 11.59 (11.08) | 13.52 (12.89) |
| Zn(L) ₂ .2H ₂ O | Light Yellow | 260 | 82 % | 43.86 (43.23) | 4.22 (3.19) | 15.74 (14.82) | 9.19 (8.23) |

d* = decomposed

Table 2. Important IR frequencies (cm⁻¹) of the Schiff base and its metal complexes.

| Compound | $\nu(\text{N}=\text{CH})$ | $\nu(\text{C}-\text{S})$ | $\nu(\text{S}-\text{H})$ | $\nu(\text{OCOCH}_3)$ | $\nu(\text{H}_2\text{O}/\text{OH})$ | $\nu(\text{M}-\text{S})$ | $\nu(\text{M}-\text{N})$ |
|---------------------------------------|---------------------------|--------------------------|--------------------------|-----------------------|-------------------------------------|--------------------------|--------------------------|
| HL | 1697 | - | 2893 | - | - | - | - |
| Co(L)(OAc).3H ₂ O | 1674 | 741 | - | 1744 | 3232 | 340 | 485 |
| Co(L) ₂ .2H ₂ O | 1674 | 779 | - | - | 3240 | 341 | 485 |
| Ni(L)(OAc).3H ₂ O | 1671 | 779 | - | 1745 | 3232 | 342 | 486 |
| Ni(L) ₂ .2H ₂ O | 1674 | 779 | - | - | 3240 | 341 | 485 |
| Cu(L)(OAc).H ₂ O | 1674 | 779 | - | 1744 | 3395 | 325 | 488 |
| Cu(L) ₂ | 1682 | 779 | - | - | 3742 | 323 | 488 |
| Zn(L)(OAc).3H ₂ O | 1674 | 771 | - | 1744 | 3456 | 342 | 482 |
| Zn(L) ₂ .2H ₂ O | 1651 | 771 | - | - | 3742 | 342 | 485 |

¹H-NMR spectra

The ¹H-NMR spectral data of Schiff base and Zn(II) complexes are displayed in Table 3.

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

| Compounds | δ (ppm) |
|---------------------------------------|---|
| Schiff Base | 7.46 (s, 1H, Ar-H), 7.31 (d, 1H, Ar-H), 6.96 (d, 1H, Ar-H), 4.11 (q, 2H, -OCH ₂ -CH ₃), 1.39 (t, 3H, -OCH ₂ CH ₃), 2.27 (s, 3H, triazine-CH ₃), 8.39 (s, 1H, -N=CH-), 13.64 (s, 1H, -SH), 4.08 (m, 1H, -OH) |
| Zn(L)(OAc).3H ₂ O | 7.44 (s, 1H, Ar-H), 7.27 (d, 1H, Ar-H), 6.93 (d, 1H, Ar-H), 2.27 (s, 3H, triazine-CH ₃), 4.07 (q, 2H, -OCH ₂ -CH ₃), 8.36 (s, 1H, -N=CH-), 1.34 (t, 3H, -OCH ₂ CH ₃), 4.08 (m, 1H, -OH) |
| Zn(L) ₂ .2H ₂ O | 7.43 (s, 2H, Ar-H), 7.25 (d, 2H, Ar-H), 6.93 (d, 2H, Ar-H), 2.27 (s, 6H, triazine-CH ₃), 4.09 (q, 4H, -OCH ₂ -CH ₃), 8.36 (s, 2H, -N=CH-), 1.34 (t, 6H, -OCH ₂ CH ₃), 4.08 (m, 1H, -OH) |

¹H-NMR spectra of Schiff base and its metal complexes were recorded in DMSO-*d*₆. In Schiff base, a peak appears at $\delta = 8.39$ ppm due to azomethine proton, which is deshielded in the spectra of Zn(II) complexes, indicating the complexation through azomethine nitrogen atom.

The signal for the SH proton appearing at $\delta = 13.64$ ppm in the spectrum of the free ligand has disappeared in the spectra of Zn(II) complexes, further supporting the complexation through S atom of the thiol group.^{29,30} The peak appearing at $\delta = 2.27$ ppm due to CH₃ group present in triazine ring did not change in the spectra of metal complexes.

Electronic spectra and magnetic moment measurements

The electronic spectral and magnetic moment data are given in Table 4. In order to understand the nature of the M-L bond, the electronic spectral data of the complexes were calculated in DMF. Co(L)(OAc).3H₂O and Co(L)₂.2H₂O complexes of HL exhibit two absorption bands, which fall in the region 10230-10900 cm⁻¹ and 19615-20271 cm⁻¹, attributed to ⁴T_{1g}(F) → ⁴T_{2g}(F) (ν_1) and ⁴T_{1g}(F) → ⁴T_{1g}(P) (ν_3) transitions,³¹ respectively. ν_2 was not observed but it could be calculated by using the relation $\nu_2 = \nu_1 + 10 D_q$. By using band-fitting equation,³² the ligand field parameters (D_q , B , β , $\beta\%$) were also calculated.

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

| Compounds | Transitions (cm ⁻¹) | | | D_q (cm ⁻¹) | B (cm ⁻¹) | ν_2/ν_1 | β | $\beta\%$ |
|---------------------------------------|---------------------------------|--------------------|---------|---------------------------|-------------------------|---------------|---------|-----------|
| | ν_1 | ν_2 | ν_3 | | | | | |
| Co(L)(OAc).3H ₂ O | 10230 | 21615 ^a | 19615 | 1138.56 | 702.70 | 2.11 | 0.724 | 27.6 |
| Co(L) ₂ .2H ₂ O | 10900 | 22989 ^a | 20271 | 1208.98 | 704.05 | 2.109 | 0.725 | 27.5 |
| Ni(L)(OAc).3H ₂ O | 10523 | 16530 | 23813 | 1052.3 | 584.93 | 1.57 | 0.562 | 43.8 |
| Ni(L) ₂ .2H ₂ O | 10618 | 17681 | 24940 | 1061.8 | 717.80 | 1.665 | 0.689 | 31.1 |
| Cu(L)(OAc).H ₂ O | 18681 | | | - | - | - | - | - |
| Cu(L) ₂ | 19952 | | | - | - | - | - | - |

The calculated values of crystal field splitting energy (D_q) are 1138.56 and 1208.98 cm⁻¹ for 1:1 and 1:2 Co(II) complexes, respectively. These values lie within the range reported for octahedral complexes. The value of Racah parameter (B) was less than free ion value (971 cm⁻¹), indicating the orbital overlap and delocalization of d -electron on the ligand. The nephelauxetic ratio (β) was found to be less than 1, which indicated the partial covalent nature of metal ligand bonds.

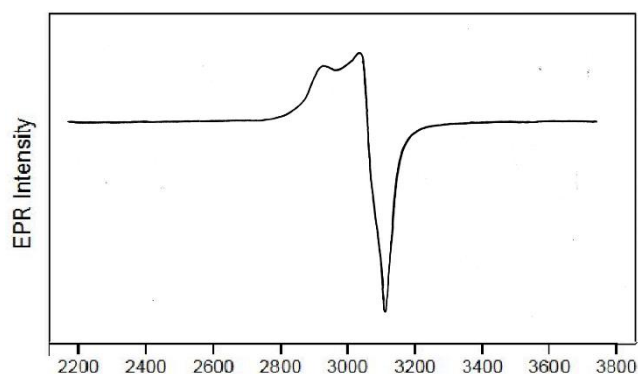
The magnetic moment data of Co(II) complexes indicate the presence of three unpaired electrons. The magnetic moment values were found in the range of 4.5-5.0 BM, which is in the expected range (4.3-5.2 BM) of octahedral complexes.³³

Ni(L)(OAc).3H₂O and Ni(L)₂.2H₂O complexes exhibit three absorption bands in the region 10523-10618 cm⁻¹ (ν_1), 16530-17681 cm⁻¹ (ν_2) and 23813-24940 cm⁻¹ (ν_3), attributed to ${}^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ (ν_1), ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ (ν_2) and ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ (ν_3) transitions, respectively. The ligand field parameters (D_q , B , β , $\beta\%$) were also calculated for Ni(II) complexes. These parameters indicate octahedral arrangement around the Ni(II) complexes³⁴ and suggest the partial covalent nature of the metal ligand bond. The calculated values of crystal field splitting energy (D_q) are 1052.3 cm⁻¹ and 1061.8 cm⁻¹ for 1:1 and 1:2 Ni(II) complexes respectively. The observed magnetic moment values were found in the range of 3.3-3.4 BM, which is in the range of reported octahedral compounds.³⁵

For 1:1 and 1:2 Cu (II) complexes, a band observed in the region of 18681-19952 cm⁻¹, assigned to ${}^2B_{1g} \rightarrow {}^2A_{1g}$, indicating the square planar geometry of the copper complexes.³⁶ Further confirmation was achieved by magnetic moment measurements 1.9-2.0 BM, which is well within expected range of square planar geometry of Cu(II) complexes.

ESR spectral studies

ESR spectra for 1:1 and 1:2 Cu(II) complexes i.e., Cu(L)(OAc).H₂O and Cu(L)₂ were recorded at room temperature on the X-Band at 9.1 GHz under the magnetic field set 0.3 T. The observed g values for Cu(L)(OAc).H₂O ($g_{\parallel}=2.197$, $g_{\perp}=2.129$, $g_{av}=2.152$) and Cu(L)₂ ($g_{\parallel}=2.197$, $g_{\perp}=2.126$, $g_{av}=2.150$) corresponds to square planar geometry of both the complexes. The trend $g_{\parallel} > g_{\perp} > 2.0023$ was observed for Cu(II) complexes, indicates that the unpaired electron is localized in the $d_{x^2-y^2}$ orbital of the Cu(II) ion and corresponds to square planar geometry around Cu(II) ions.³⁷

**Figure 2.** ESR spectrum of Cu(L)(OAc).H₂O.

The $g_{\parallel} < 2.3$ value confirms the covalent character of the metal ligand bond. According to Hathaway and Billing³⁸ if axial symmetry parameter i.e. $G > 4$, the exchange interaction is negligible, but if $G < 4$, a considerable exchange interaction in the solid complexes would be found. The 1:1 and 1:2 Cu(II) complexes gave the G value in the range of 1.537-1.574 indicating exchange interaction in solid complexes. ESR spectrum of Cu(L)(OAc).H₂O is shown in Figure 2.

Fluorescence spectral studies

The fluorescence spectra of the ligand and its Co(II), Ni(II), Cu(II) and Zn(II) complexes (10^{-3} M) were recorded in DMF solution at room temperature with excitation wavelength at 265 nm.

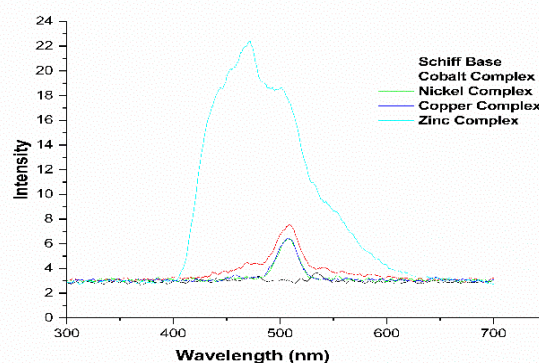
**Figure 3.** Fluorescence spectra of the Schiff base and its metal complexes.

Table 5. In vitro antimicrobial activity of synthesized chemical compounds and standard drugs.

| S. No. | Compounds | Diameter of growth of inhibition zone (mm) ^a | | | | | |
|--------|---------------------------------------|---|------------------|------------------------|----------------------|--------------------|----------------------|
| | | Gram positive bacteria | | Gram negative bacteria | | Fungi | |
| | | <i>B. subtilis</i> | <i>S. aureus</i> | <i>E. coli</i> | <i>P. aeruginosa</i> | <i>C. albicans</i> | <i>S. cerevisiae</i> |
| 1 | Schiff Base (HL) | 18 | - | - | - | - | - |
| 2 | Co(L)(OAc).3H ₂ O | 16 | 21 | 16 | 16 | 15 | 16 |
| 3 | Co(L) ₂ .2H ₂ O | 15 | - | - | 23 | 7 | 16 |
| 4 | Ni(L)(OAc).3H ₂ O | 11 | 13 | 9 | - | - | 18 |
| 5 | Ni(L) ₂ .2H ₂ O | 12 | 18 | 7 | - | 6 | 18 |
| 6 | Cu(L)(OAc).H ₂ O | 8 | - | 8 | 18 | - | 16 |
| 7 | Cu(L) ₂ | 7 | - | 8 | 15 | 6 | 15 |
| 8 | Zn(L)(OAc).3H ₂ O | 22 | 25 | 20 | 20 | 21 | 20 |
| 9 | Zn(L) ₂ .2H ₂ O | 21 | 32 | 19 | 13 | 21 | 20 |
| 10 | Ciprofloxacin | 24.0 | 26.6 | 25.0 | 22 | - | - |
| 11 | Amphotericin-B | - | - | - | - | 16.6 | 19.3 |

(-) No activity; ^a Values, not including diameter of the well (8mm), are means of three replicates.

Table 6. MIC ($\mu\text{g ml}^{-1}$) of synthesized chemical compounds and standard drugs.

| S. No. | Compounds | Gram positive bacteria | | Gram negative bacteria | | Fungi | |
|--------|---------------------------------------|------------------------|------------------|------------------------|----------------------|--------------------|----------------------|
| | | <i>B. subtilis</i> | <i>S. aureus</i> | <i>E. coli</i> | <i>P. aeruginosa</i> | <i>C. albicans</i> | <i>S. cerevisiae</i> |
| 1 | Schiff Base | 25 | - | - | - | - | - |
| 2 | Co(L)(OAc).3H ₂ O | 25 | 25 | 25 | 50 | 50 | - |
| 3 | Co(L) ₂ .2H ₂ O | 50 | - | - | 12.5 | - | 50 |
| 4 | Ni(L)(OAc).3H ₂ O | 50 | 50 | - | - | - | - |
| 5 | Ni(L) ₂ .2H ₂ O | 50 | 25 | - | - | - | 50 |
| 6 | Cu(L)(OAc).H ₂ O | - | - | - | 50 | - | - |
| 7 | Cu(L) ₂ | - | - | - | 50 | - | - |
| 8 | Zn(L)(OAc).3H ₂ O | 12.5 | 12.5 | 25 | 12.5 | 12.5 | 50 |
| 9 | Zn(L) ₂ .2H ₂ O | 25 | 6.25 | 50 | 50 | 12.5 | 50 |
| 10 | Ciprofloxacin | 6.25 | 6.25 | 6.25 | 12.5 | - | - |
| 11 | Amphotericin-B | - | - | - | - | 12.5 | 12.5 |

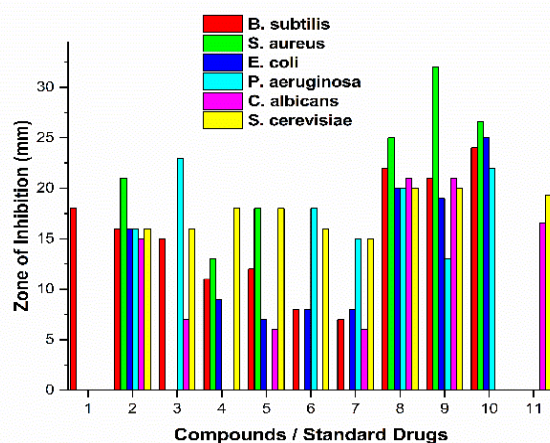
The data obtained indicate about the changes in fluorescence property of Schiff base, when it binds with metal ions. All the metal complexes show increased fluorescence intensity with strong emission at 510 nm for Co(II), 508 nm for Ni(II), 507 nm for Cu(II) and 472 nm for Zn(II) complexes. The enhanced fluorescence intensity of metal complexes in comparison to ligand may be due to the formation of coordination bonds between metal ions and ligand which increases the rigidity of the complex thereby reducing the loss of energy through vibrational motion.^{39,40} The decrease in emission maxima was in the order of Zn(II) > Co(II) > Ni(II) > Cu(II) > Schiff base (Figure 3).

Antimicrobial activity

Schiff base and its metal complexes were checked for their in vitro antimicrobial activity by agar well diffusion method against two gram positive bacteria (*B. subtilis* and *S. aureus*), two gram negative bacteria (*E. coli* and *P. aeruginosa*) and two fungi (*C. albicans* and *S. cerevisiae*).

Schiff base (HL) and its metal complexes possessed variable biological activity against *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans* and *S. cerevisiae* (Table 5). Zones of inhibition were observed in the range 7-32 mm, 7-23 mm and 6-21 mm against gram positive bacteria, gram negative bacteria and fungi, respectively (Figure 4). On the basis of growth of inhibition zone, compound

Zn(L)(OAc).3H₂O and Zn(L)₂.2H₂O were found to be most effective against *B. subtilis* and *S. aureus* with inhibition zone 22 mm, 25 mm and 21 mm, 32 mm, respectively, Compounds Zn(L)(OAc).3H₂O, Zn(L)₂.2H₂O show good activity against *E. coli* with inhibition zone 20 mm and 19 mm respectively. Zn(L)(OAc).3H₂O and Co(L)₂.2H₂O exhibit good activity against *P. aeruginosa* with inhibition zone 20 mm and 23 mm and Zn(L)₂.2H₂O was found to be most effective against *C. albicans* with inhibition zone found 21 mm.

**Figure 4.** Comparison of Zone of inhibition of compounds with standard drugs.

Zn(L)₂.2H₂O, Ni(L)(OAc).3H₂O and Ni(L)₂.2H₂O were found to be effective against *S. cerevisiae* with inhibition zone 20 mm, 18 mm & 18 mm respectively.

The MIC of the tested compounds have been observed from 6.25-50 µg mL⁻¹ against gram positive and gram negative bacteria and (12.5-50) µg mL⁻¹ in case of fungi (Table 6). Among these compounds, Zn(L)₂.2H₂O shows best antibacterial activity against *S. aureus* and shows lowest MIC value 6.25 µg mL⁻¹. Compounds Zn(L)(OAc).3H₂O and Zn(L)₂.2H₂O show good activity against *E. coli*, *C. albicans* and *P. aeruginosa*, *S. cerevisiae* respectively with lowest MIC values 12.5 µg mL⁻¹. MIC values of the metal complexes indicate moderate activity against the microbial strains as compared to standard drugs, Ciprofloxacin and amphotericin B.

Thermal analysis

To determine the thermal stability and chemical composition of 1:1 Co(II), Ni(II), Cu(II) and Zn(II) complexes, thermogravimetric analysis has been carried out over a temperature range of 50-600 °C by using alumina as a reference compound. The percentage mass loss of the complexes with temperature is shown in the Figure 5.

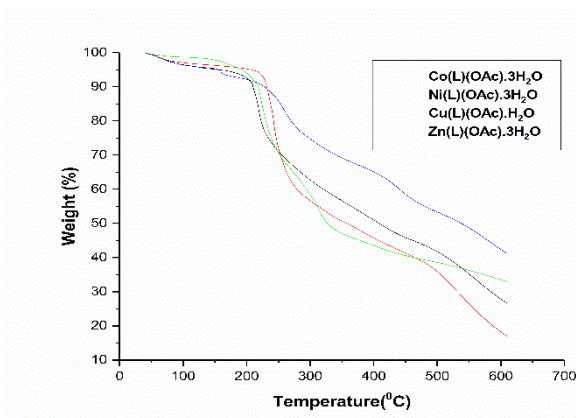


Figure 5. TG curves of synthesized metal complexes.

Table 7. Calculated activation energies (kJ mol⁻¹) for different stages in degradation of metal complexes.^a

| Compounds | E ₂ * (R ²) | E ₃ * (R ²) |
|------------------------------|------------------------------------|------------------------------------|
| Co(L)(OAc).3H ₂ O | 50.26 (0.9892) | 100.38 (0.9384) |
| Ni(L)(OAc).3H ₂ O | 73.65 (0.9972) | 126.0 (0.9132) |
| Cu(L)(OAc).H ₂ O | 60.54 (0.9794) | 43.00 (0.9895) |
| Zn(L)(OAc).3H ₂ O | 28.754 (0.9901) | 26.168 (0.9941) |

^aHeating rate was 10 °C min⁻¹. *R² stands for regression coefficient and 2,3 represents second and third stages of degradation.

The Co(II) complex decomposes in the three stages. The TG curve of Co(L)OAc.3H₂O indicates first weight loss of 8.26 % (calcd. 11.32 %) in the temperature range 40-205 °C attributed to loss of three coordinated water molecules. The next step represents a mass loss of 40.87 % (calcd. 43.82 %) from 205-400 °C corresponding to the loss of organic moiety. The final step resulted in the decomposition of triazine ring with mass loss of 30.42 % (calcd. 32.50 %) from 400°C to 610 °C.⁴¹

The TG curve of Ni(L)(OAc).3H₂O exhibits first weight loss of 9.64 % (calcd. 11.32 %) at 40-230°C, attributed to the loss of three coordinated water molecules. The subsequent steps correspond to the decomposition of organic moiety with weight loss of 42.66 % (calcd. 43.84 %) within the temperature range 230-380 °C and triazine ring with weight loss of 30.73 % (calcd. 32.51 %) from 380-610 °C. The TG curve of Cu(L)(OAc).H₂O shows three decomposition steps within the temperature range 40-160°C, 160-310°C and 310-600°C corresponding to loss of one water molecule, organic moiety and triazine ring respectively⁴² whereas the complex Zn(L)OAc.3H₂O shows three decomposition steps in the temperature range 40-210°C, 210-400°C and 400-595°C respectively. Activation energies for different stages in degradation of metal complexes are shown in Table 7. The TG curves are further supported by the DTA curves Figure 6.

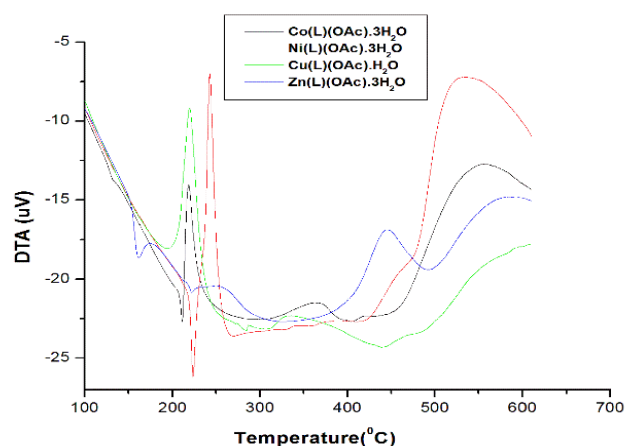


Figure 6. DTA curves of synthesized metal complexes.

Determination of degradation activation energy

For a second order reaction, Coats and Redfern⁴³ provided an approximation. This is an integral form of the rate equation. The simplified form of the equation is

$$\ln \left[\frac{g(\alpha)}{T^2} \right] = \ln \left[\frac{AR}{E\beta} \right] - \frac{E}{RT} \quad (1)$$

where,

T = temperature,

A = pre-exponential term (min⁻¹),

R = gas constant,

E = energy of activation,

B = heating rate (°C min⁻¹),

α = degree of conversion and

g(α) = degradation mechanism but $g(\alpha) = (1/1 - \alpha) - 1$

For second order mechanism *E* can be calculated from the slope of the graph between $\ln[g(x)/T^2]$ and 1/*T*. Table (7) presents the activation energy values for second and third decomposition stages obtained using Coats – Redfern method and corresponding regression coefficients (R²).

Table 8. Selected calculated bond angles (°) and bond lengths (Å) for ligand and its 1:1 and 1:2 copper metal complexes.

| Compounds | Atoms | Bond Angles (°) | Atoms | Bond Lengths (Å) |
|-----------------------------|--------------------|-----------------|--------------|------------------|
| Schiff Base | N(5)-N(14)-C(15) | 120 | C(4)-S(12) | 1.815 |
| | C(4)-S(12)-H(13) | 109.5 | N(14)-C(15) | 1.26 |
| | N(5)-C(6)-O(7) | 123.43 | C(15)-C(17) | 1.503 |
| | C(15)-C(17)-C(22) | 119.9 | C(6)-O(7) | 1.208 |
| Cu(L)(OAc).H ₂ O | C(4)-S(13)-Cu(21) | 109.5 | N(12)-Cu(21) | 2.491 |
| | H(19)-O(18)-Cu(21) | 109.5 | O(14)-Cu(21) | 1.81 |
| | N(5)-N(12)-C(22) | 120 | O(18)-Cu(21) | 1.81 |
| | N(12)-Cu(21)-O(14) | 109.5 | S(13)-Cu(21) | 1.247 |
| Cu(L) ₂ | N(5)-N(12)-Cu(30) | 97.882 | C(4)-S(16) | 1.856 |
| | N(5)-C(4)-S(16) | 109.5 | S(16)-Cu(30) | 1.644 |
| | C(4)-S(16)-Cu(30) | 109.5 | N(18)-N(29) | 1.352 |
| | S(16)-Cu(30)-S(28) | 128.169 | N(12)-Cu(30) | 2.502 |

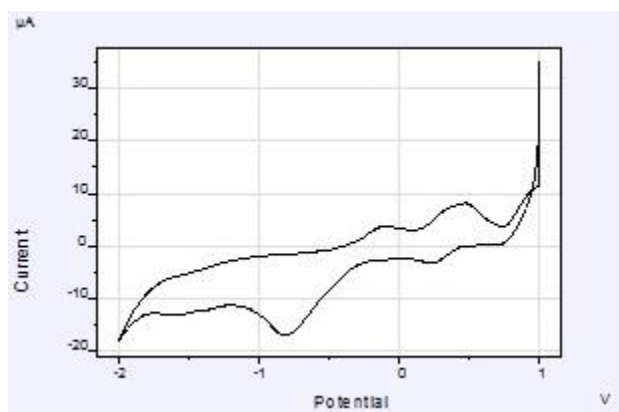
Based on activation energy values (E_2) and (E_3) as deduced using second order model, following stability order is suggested:



Electrochemical behavior

Electrochemical properties of 1:1 Cu(II) complex (10^{-3}M) in DMF was investigated with the help of cyclic voltammetry in potential ranging from -2.0 to +1.0 V in DMF solution with a scan rate of 0.1 V/s, containing tetrabutylammonium perchlorate as the supporting electrolyte. Cyclic voltammogram (CV) is shown in Figure 8.

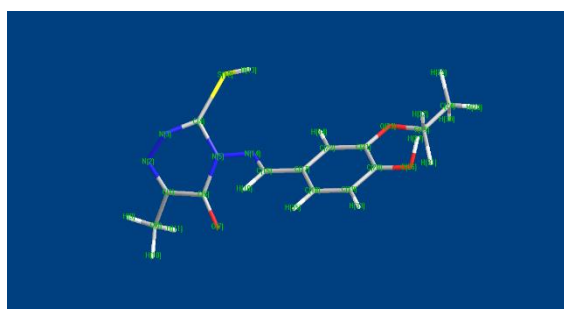
The complex Cu(L)(OAc).H₂O exhibits two reduction peaks at $E_{pc} = 0.5$ V and $E_{pc} = -0.12$ V for Cu(II) to Cu(I) and Cu(I) to Cu(0) and corresponding two oxidation peaks at $E_{pa} = -0.82$ V and $E_{pa} = +0.26$ V for Cu(0) to Cu(I) and Cu(I) to Cu(II) complexes. ΔE for these processes is very high, which is consistent with the quasi reversible process. The ratio of cathodic to anodic peak current ($I_c/I_a = 0.214$) was found to be less than one.⁴⁴

**Figure 7.** Cyclic Voltammogram of Cu(L)(OAc).H₂O.

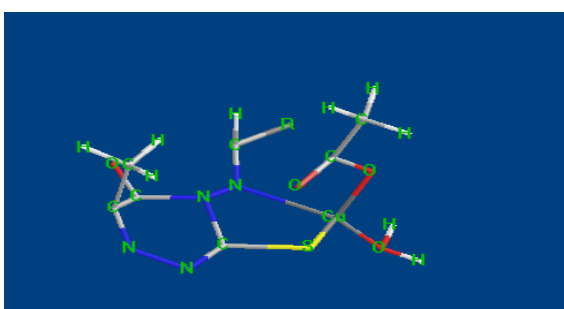
Structural determination by computational method.

The optimized geometries of Schiff base and its some 1:1 and 1:2 metal complexes are shown in Figure 8. Bond length and bond angles of some complexes are depicted in Table 8 respectively.

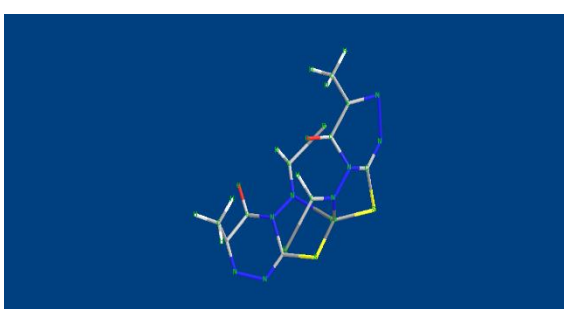
(A)



(B)



(C)



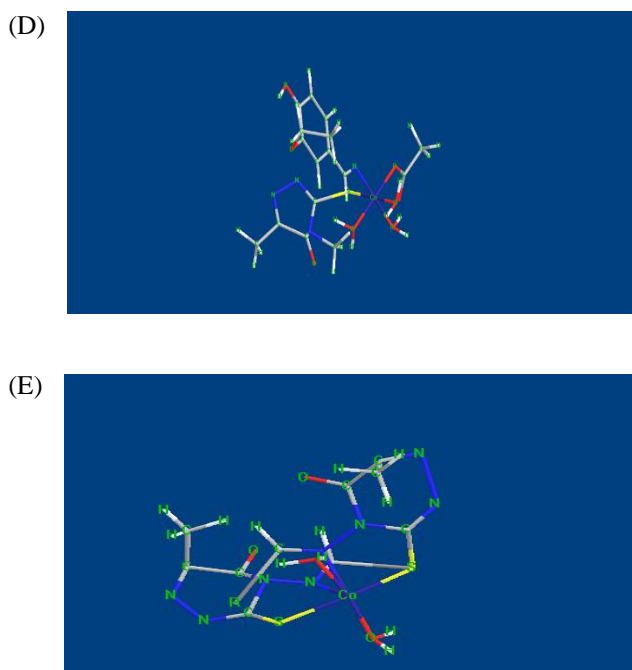


Figure 8. 3D structures of Schiff base (A), Cu(L)(OAc).H₂O (B), Cu(L)₂ (C), Co(L)(OAc).3H₂O (D) and Co(L)₂.2H₂O (E)

Conclusion

The synthesized 4-[(3-ethoxy-4-hydroxybenzylidene)-amino]-3-mercapto-6-methyl-5-oxo-1,2,4-triazine (HL) Figure 1. Schiff base is a bidentate ligand, coordinates through azomethine nitrogen and sulphur to the different metal ions. Synthesized compounds were characterized with the help of IR, NMR, ESR, electronic, Fluorescence spectroscopy, cyclic voltammetry, magnetic and thermal studies.

The results of the investigations suggest octahedral geometry for the Co(II), Ni(II) and Zn(II) complexes and square planar geometry around the Cu(II) metal ion. On the basis of above studies, Schiff base is versatile ligand which can coordinate with different metal ions and also biologically active. Metal complexes exhibit enhanced antimicrobial and antifungal activities against various microbial strains as compared to parent ligand and also possess comparable activities with standard drugs.

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