Accepted Manuscript

Design, spectral characterization, thermal, DFT studies and anticancer cell line activities of Co(II), Ni(II) and Cu(II) complexes of Schiff bases derived from 4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol

Prateek Tyagi, Sulekh Chandra, B.S. Saraswat, Deepak Yadav

PII:	S1386-1425(15)00330-3
DOI:	http://dx.doi.org/10.1016/j.saa.2015.03.034
Reference:	SAA 13448
To appear in:	Spectrochimica Acta Part A: Molecular and Biomo- lecular Spectroscopy
Received Date:	18 November 2014
Revised Date:	24 February 2015
Accepted Date:	1 March 2015



Please cite this article as: P. Tyagi, S. Chandra, B.S. Saraswat, D. Yadav, Design, spectral characterization, thermal, DFT studies and anticancer cell line activities of Co(II), Ni(II) and Cu(II) complexes of Schiff bases derived from 4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* (2015), doi: http://dx.doi.org/10.1016/j.saa.2015.03.034

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Design, spectral characterization, thermal, DFT studies and anticancer cell line activities of Co(II), Ni(II) and Cu(II) complexes of Schiff bases derived from 4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol

Prateek Tyagi^{a,b}, Sulekh Chandra^{a,*}, B S Saraswat^b, Deepak Yadav^c

^a Department of Chemistry, Zakir Husain Delhi College, University of Delhi, JLN-Marg, New Delhi 110002, India

^b Department of Chemistry, School of Sciences, IGNOU, Maidan Garhi, New Delhi 110068, India

^c Faculty of Medicine, Jamia Hamdard, New Delhi 110062, India

*Corresponding author Tel.: +91-11-22911267; Fax: +91-11-23215906 Email: schandra_00@yahoo.com

Abstract

A series of two biologically active Schiff base ligands L^1 , L^2 have been synthesized in equimolar reaction of 4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol with thiophene-2carbaldehyde and furan-2-carbaldehyde. The synthesized Schiff bases were used for complexation with different metal ions like Co(II), Ni(II) and Cu(II) by using a molar ratio of ligand : metal as 1:1 and 2:1. The characterization of Schiff bases and metal complexes was done by ¹H NMR, UV–Vis, TGA, IR, Mass spectrometry and molar conductivity studies. The In DFT studies the geometries of Schiff bases and metal complexes were fully optimized with respect to the energy using the 6-31+g(d,p) basis set. On the basis of the spectral studies an octahedral geometry has been assigned for Co(II), Ni(II) and Cu(II) complexes. The effect of these complexes on proliferation of human breast cancer cell line (MCF-7) and human hepatocellular liver carcinoma cell line (Hep-G2) were studied and compared with those of free ligand. The anticancer cell line results reveal that all metal complexes show moderate to significant % cytotoxicity on cell line HepG2 and MCF-7.

Key Words: Schiff Base, Co(II), Ni(II) and Cu(II) complexes, Spectroscopic Studies, DFT, Anticancer cell line activity.

1. Introduction

Cancer, a diverse group of diseases characterized by the proliferation and spread of abnormal cells, is a major worldwide problem [1–2]. Therefore, the search and development of new potent anticancer drugs is highly desirable today in cancer research. The biological significance of 1,2,4-triazole derivatives is very well known because of their diverse pharmacological properties [3–8]. A wide variety of therapeutically interesting molecule that after inclusion of 1,2,4-triazole moiety, transforms into better drugs has also been reported in literature. Fluconazole, a triazole derived compound is broad spectrum antifungal agent [9]. Similarly, trazodone is a very well known antidepressant [10]. 1,2,4-triazole ring substituted chemotherapeutics, such as Vorozole, Letrozole and Anastrozole (Fig. 1), are currently being used for the treatment of breast cancer [11]. The chemistry of 1,2,4-triazole and its fused heterocyclic derivatives has received considerable attention owing to their synthetic and effective biological importance. Schiff base of 1,2,4-triazole derivatives have also been found to possess extensive biological activity.

The role of N and S atoms in the coordination of metals at the active sites of numerous metallo-biomolecules is very well known. It has been reported in literature that binding of drug to a metallo element enhances its activity and in some cases the complex possesses even more healing properties than the parent drug [12]. Metal elements such has cobalt, nickel and copper have small size, higher nuclear charge and thus have a great affinity for coordination. A detail survey of the literature revealed that no work has been carried out on the synthesis of metal complexes derived from 4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol. The synthesized Schiff bases have coordination sites with SNOS sequence and varied coordination abilities. In view of the significant structural and biological applications of triazole compounds, it is worthwhile to report the synthesis of cobalt(II), nickel(II) and copper(II) complexes (1–12) of triazole Schiff base derivatives L^1 , L^2 , derived from the condensation reaction of 4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol with thiophene-2-carbaldehyde and furan-2-carbaldehyde, respectively (Scheme 1). These compounds have

been investigated for their *in vitro* anticancer cell line activity against human breast cancer cell line (MCF-7) and human hepatocellular liver carcinoma cell line (HepG2).

Insert Fig. 1.

2. Experimental

2.1 Materials and Methods

All the chemicals were used of Anala R grade and received from Sigma-Aldrich and Fluka. Metal salts were purchased from E. Merck and used as received. MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide) and 0.25% trypsin and 0.02% EDTA mixture was purchased from Himedia (India). Fetal bovine serum (FBS) was purchased from Biowest (USA).

2.2 General procedure for the synthesis of Ligands $L^{1} - L^{2}$

Intermediate triazoles **3a** was synthesized from corresponding acid hydrazide (**1a**) through multi-step reactions (Scheme 1) as per the reported literature [13–14]. The hydrazide was converted to potassium salt (**2a**) by reacting with carbon disulphide and potassium hydroxide in absolute ethanol. On reacting with hydrazine hydrate, the salt undergoes ring closure to yield the triazole, **3a**. The desired Schiff base L^1 was obtained in good yield by refluxing equimolar amount of 4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (**3a**) with thiophene-2-carbaldehyde using acetic acid as a solvent. The mixture was stirred at room temperature for 10 h (Scheme 1). The reaction mixture was kept overnight, pale yellow product was precipitated out. It was filtered, washed with ice cold water, methanol and recrystallized with a mixture of DMF & methanol. The same procedure was used for the synthesis of ligand L^2 .

(E)-5-(pyridin-4-yl)-4-((thiophen-2-ylmethylene)amino)-4H-1,2,4-triazole-3-thiol L^{1}

Yield: 74(%). Color (pale-yellow). M.p. 254–256 °C. IR (KBr, cm⁻¹): 1618 (HC=N), 2710 (SH), 1103 (C=S). ¹H NMR (DMSO- d_6 , δ , ppm) 7.29–7.31 (m, 1H, Ar–H), 7.80–7.87 (m, 3H, Ar–H), 8.0 (d, 1H, J = 7.2 Hz, Ar–H), 8.75–8.81 (m, 2H, Ar–H), 9.92 (s, 1H, N=CH), 14.45 (s, 1H, triazole –SH). Anal. Calcd. for C₁₂H₉N₅S₂ (287.36): C: 50.16; H: 3.16; N: 24.37; Found: C: 50.19; H: 3.17; N: 24.39%. Mass spectrum (ESI) [M]⁺ = 287.97

(E)-4-((furan-2-ylmethylene)amino)-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol L^2

Yield: 76(%). Color (pale-yellow). M.p. >280 °C. IR (KBr, cm⁻¹): 1616 (HC=N), 2714 (SH), 1107 (C=S). ¹H NMR (DMSO- d_6 , δ , ppm) 6.81–6.83 (m, 1H, Ar–H), 7.42–7.43 (m, 1H, Ar– H), 7.81–7.87 (m, 2H, Ar–H), 8.11–8.12 (m, 1H, Ar–H), 8.75–8.81 (m, 2H, Ar–H), 9.61 (s, 1H, N=CH), 14.45 (s, 1H, triazole –SH). Anal. Calcd. for C₁₂H₉N₅OS (271.30): C: 53.13; H: 3.34; N: 25.81; Found: C: 53.17; H: 3.35; N: 25.83%. Mass spectrum (ESI) [M]⁺ = 271.88

2.3 General procedure for the synthesis of metal complexes 1-12

Preparation of Co(II) complexes with (E)-5-(pyridin-4-yl)-4-((thiophen-2-ylmethylene)amino)-4H-1,2,4-triazole-3-thiol L^1 (1)

Hot methanolic solution (15 mL) of CoCl₂.6H₂O (2 mmol) was added drop wise to a magnetically stirred solution of (E)-5-(pyridin-4-yl)-4-((thiophen-2-ylmethylene)amino)-4H-1,2,4-triazole-3-thiol L^1 (2 mmol) in methanol (20 mL) (scheme 1). The resultant mixture was refluxed for 4 h and the clear solution was allowed to cool at room temperature. The solid product precipitated out which was filtered, washed with methanol, diethyl ether and dried under vacuum over P₄O₁₀. The same method was used for the preparation of metal complexes **2–5** in metal : ligand as 1:1. The metal complexes **6–12** was synthesized by using the above mentioned same procedure by taking metal chloride : ligand in a 1:2 molar ratio. Physical, analytical and spectral data of ligands and metal complexes are given in Table 1.

2.4 Analysis

The carbon and hydrogen were analyzed on Carlo-Erba 1106 elemental analyzer. The nitrogen content of the complexes was determined using Kjeldahl's method. Molar conductance was measured on the ELICO (CM82T) conductivity bridge. ESI-MS spectra were obtained using a VG Biotech Quattro mass spectrometer equipped with an elctrospray ionisation source in the mass range of m/z 100 to m/z 1000. IR spectra (CsBr) were recorded on FTIR BX-II spectrophotometer. NMR spectra were recorded with a model Bruker Advance DPX-300 spectrometer operating at 400 MHz using DMSO-d6 as a solvent and TMS as internal standard. The electronic spectra were recorded in DMSO on Shimadzu UV mini-1240 spectrophotometer. Thermogravimetric analysis (TGA) was carried out in dynamic nitrogen atmosphere (30 ml/min) with a heating rate of 10 °C/min using a Schimadzu TGA-50H thermal analyzer. EPR spectra of the Cu(II) complexes were recorded

as polycrystalline sample at room temperature on E4-EPR spectrometer using the DPPH as the g-marker.

2.5 DFT Calculations

The DFT calculations were performed using the B3LYP three parameter density functional, which includes Becke's gradient exchange correction [15], the Lee, Yang, Parr correlation functional [16] and the Vosko, Wilk, Nusair correlation functional [17]. The gas phase geometries of Ligand L^1 , and metal complexes (1–3) were fully optimized with respect to the energy using the 6-31+g(d,p) basis set using the Gaussian 09W suite [18].

2.6 In vitro studies (Cell culturing)

The cell lines HepG2 (liver hepatocellular carcinoma) and MCF-7 (human breast adeno carcinoma) were cultured as monolayers and maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin in a humidified atmosphere with 5% CO₂ at 37 °C in T-75 flasks and were sub-cultured twice a week. For the assays, cells (2 x 10^3 cells/well in 200 µl of complete DMEM) were placed in each well of a 96 well flat bottom plate. Cells were allowed to adhere for overnight, and then treated with two concentrations (1 and 10 µM) samples of each L¹, L² and metal complexes (1 – 12) for 48 h. The cell proliferation of the control (untreated cells) was fixed to 100%. After completion of incubation period, 20 µl MTT (5mg/ml) was added to each well for 2 h. Following which media was removed and 100 µl of DMSO was added to each well in order to solubilize the formazan. The plate was read using the ELISA reader at a wavelength of 540 nm.

3. Results and discussion

The Schiff base L^1 (scheme 1) was prepared by refluxing in acetic acid an equimolar amount of 4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol and thiophene-2-carboxaldehyde. The same method was used for the preparation of the ligand L^2 . The structure of Schiff bases thus formed was established by IR, ¹H NMR, Mass spectrometry and CHN analysis.

Insert Scheme 1

The synthesized Schiff bases were soluble in DMF, DMSO and methanol on heating only. The composition of the ligands were consistent with their NMR, IR, mass spectral and CHN data. The synthesized Schiff bases were further used for the complexation with Co(II), Ni(II) and Cu(II) metal ions, using the following metal salt: CoCl₂.6H₂O for complexes **1,4,7** and **9**, NiCl₂.6H₂O for complexes **2, 5, 8** and **11**, CuCl₂for complexes **3, 6, 9** and **12**. The obtained complexes are microcrystalline solids which are stable in air and decompose above 280 °C (Table 1). They are insoluble in common organic solvents such as acetone & chloroform, sparingly soluble in ethanol, methanol and completely soluble in DMF and DMSO. The molar conductance of the soluble complexes in DMF showed values indicating that complexes 1-12 (11–17) ohm⁻¹ cm² mol⁻¹) are non-electrolytes [19]. Physical measurements and analytical data of the complexes 1-12 are given in Table 1.

Insert Table 1

3.1 Mass Spectra

The ESI mass spectrum of ligand showed a molecular ion peak at m/z = 287.97 amu corresponding to $[M]^+$, which confirms the proposed formula $[C_{12}H_9N_5S_2]^+$ and its base peak was observed at m/z 178.95 (Fig. 2). Similarly the molecular ion peak of ligand L^2 shows a molecular ion peak at 271.88 and its base peak was observed at m/z 178.88. The mass spectrum of L^1 shows a series of peaks at 180.81, 179.68, 177.95, 101.98, and 73.99 corresponding to various fragments. The intensities of these peaks give the idea of the stability of the fragments. The m/z peak values of the ligand L^1 , L^2 and the metal complexes 1–12 are given in Table 1.

Insert Fig. 2

3.2 ¹H NMR Spectra

The ¹H NMR spectra have been recorded for ligand L^1 and L^2 . The spectra of ligand L^1 (Fig. 3) displayed azomethine (-HC=N) proton as a singlet at 9.92 ppm. The aromatic protons appeared as a set of doublet and multiplet in the region 8.81–7.29 ppm. The triazole –SH proton appear as a weak singlet at 14.45 ppm for the ligand L^1 . Similarly, in the spectra of the ligand L^2 the azomethine (-HC=N) proton appeared as a singlet at 9.61 ppm. The signals of all the protons of the ligand L^1 , L^2 were found as to be in their expected region.

Insert Fig. 3

3.3 IR Spectra

The characteristic bands of IR spectra of ligands L^1 , L^2 and their metal(II) complexes (1–12) are reported in order to confirm the binding mode of the Schiff base ligands to the corresponding metal ion. Both ligands possessed potential donor sites like azomethine linkage (-C=N), triazole (-SH) and S/O of thiophene/furan, which have tendency to coordinate with metal ions (Table 2). Peak corresponding to v(C=O) stretching vibrations was absent in IR spectra of L^1 and, instead, a new band assigned to azomethine v(HC=N) linkage appeared at 1618 cm⁻¹ confirming the formation of Schiff base. Similarly, the peak at 1616 cm⁻¹ in L^2 corresponds to v(HC=N) linkage [20]. The IR spectra of the ligands L^1 , L^2 show characteristic bands due to v(SH) at 2710 and 2714 cm⁻¹, respectively [21].

The comparison of the IR spectra of Schiff base ligands with corresponding metal complexes gave clue of binding modes of the Schiff base ligand to the corresponding metal ion. The IR band due to azomethine C=N group shifts to lower frequency (12-18 cm⁻¹) 1598-1606 cm⁻¹, representing the coordination of azomethine N in complex formation [22]. This is also confirmed by the appearance of new band in spectra of metal complexes in the range of 462-498 cm⁻¹, which has been assigned to the v(M-N) bond. The deprotonation of the thiol group is indicated by the absence of a band in the metal complexes in range 2710-2714 cm⁻¹, which is due to v(S-H) of Schiff bases, indicating that the metal is coordinated through thiol sulphur [22]. This is supported by the lower frequency shift which appears around 680-670 cm^{-1} in the metal complexes due to v(C-S). The specific band of v(C-S-C) stretching vibration of thiophene ring at 834 cm⁻¹ in free ligand L^1 shifts to (849–864 cm⁻¹) in complexes 1–3 and 7–9. This is also supported by the appearance of new band in range of 338–356 cm⁻¹ of far IR spectra, which has been assigned to v(M-S) bond. Similarly, the band at 1018 cm⁻¹ in ligand L^2 corresponding to v(C–O–C) stretching vibration of the furan ring shifts to a lower value of 998–1004 cm⁻¹ in metal complexes **4–6** and **10–12** [23]. The band in complexes 4-6 and 10-12 in the region 510-534 cm⁻¹ is due to metal-oxygen bond formation [24].

Insert Table 2

The presence of coordinated water molecules in the complexes **1–6** is indicated by a broad band in the region 3220–3412 cm⁻¹ [21] and two weaker bands in the region 755–795 and 702–718 cm⁻¹ due to v(–OH) rocking and wagging mode of vibrations [25], respectively. Thus the IR spectral data results provide strong evidences for the complexation of the potentially tridentate Schiff bases and also suggests that the metal(II) coordinated through azomethine nitrogen, thiol sulphur and through sulphur/oxygen of the thiophene/furan ring. Based on these observations it may be appreciated that the ligands L¹, L² coordinate uninegative tridentately around the Co(II), Ni(II) and Cu(II) ion.

3.4 Conductance and magnetic susceptibility measurements

The molar conductance values of metal complexes **1–12** were obtained in DMF as a solvent at room temperature and their results in (Ω^{-1} cm²mol⁻¹) are recorded as in Table 3. Generally, higher molar conductance values are indicative of the electrolytic nature of the metal complexes and lower values show their non-electrolytic nature. The molar conductance of the metal complexes in DMF showed values indicating that complexes **1–12** (11–17 ohm⁻¹ cm² mol⁻¹) are non-electrolytes. The magnetic moment (B.M.) values of all the metal complexes, **1–12** at room temperature were recorded in Table 3. The magnetic moment values of Co(II) complexes were found in the range of 4.81–4.97 B.M. suggesting the Co(II) complexes as high-spin with three unpaired electrons in an octahedral environment. The Ni(II) complexes exhibited magnetic moment values in the range of 2.92–2.99 B.M. representative of two unpaired electrons per Ni(II) ion suggesting these complexes to have octahedral geometry [26]. The experimental magnetic moment values of 1.93–1.96 B.M. for Cu(II) complexes are indicative of one unpaired electron per Cu(II) ion for d⁹ system suggesting spin-free distorted octahedral geometry.

Insert Table 3

3.5 Electronic spectra

The electronic absorption spectra of the Co(II), Ni(II) and Cu(II) complexes in DMSO were recorded at room temperature and the band positions of the absorption maxima, band assignments and the proposed geometry are listed in Table 3. The electronic spectra of Co(II)

complexes generally showed three absorption bands in the region at 9,778–9,874, 17,330– 17,774 and 29,718–30,256 cm⁻¹ assigned to transitions $4T_{1g}\rightarrow {}^{4}T_{2g}(F)$, ${}^{4}T_{1g}\rightarrow {}^{4}A_{2g}(F)$ and ${}^{4}T_{1g}\rightarrow {}^{4}T_{g}(P)$, showing an octahedral geometry around the Co(II) ion [20]. The electronic spectral data of Ni(II) complexes showed d-d bands in the region 10,815–11,643, 15,383– 16,476 and 23,165–25,715 cm⁻¹, respectively, assigned to the transitions ${}^{3}A_{2g}(F) \rightarrow {}^{3}T_{2g}(F)$, ${}^{3}A_{2g}(F) \rightarrow {}^{3}T_{1g}(F)$ and ${}^{3}A_{2g}(F) \rightarrow {}^{3}T_{2g}(F)$, which are characteristic of Ni(II) in octahedral geometry [22]. Electronic spectra of Cu(II) complexes show the d–d transition bands in the range 12,195–15,524, 18,598–19,112 and 24,435–27,465 cm⁻¹. These bands correspond to ${}^{2}B_{1g}\rightarrow {}^{2}A_{1g}(d_{x2-y2}\rightarrow d_{z2}), {}^{2}B_{1g}\rightarrow {}^{2}B_{2g}(d_{x2-y2}\rightarrow d_{xy})$ and ${}^{2}B_{1g}\rightarrow {}^{2}E_{g}(d_{x2-y2}\rightarrow d_{xz}, d_{yz})$ transitions, respectively. On the basis of electronic transitions, a distorted octahedral geometry is suggested for Cu(II) complexes.

3.6 Thermal Analysis

Thermal behaviour of the metal complexes has been studied using thermogravimetric analysis from ambient temperature to 1000 °C in nitrogen atmosphere. Based on the thermograms; decomposition stages, temperature ranges, decomposition product as well as weight loss percentages of the complexes were calculated. The TG curves were redrawn as % mass loss vs. temperature (TG) curves. The thermal behaviour of complexes 4-6 is almost same. Typical TGA/DTG curve for Co(II) (4) is presented in Fig. 4. The temperature ranges and percentage mass losses of the decomposition reactions are given in Table 4 together with evolved moiety and the theoretical percentage mass losses. The TGA curves of the complexes 4-6 undergo decomposition in two to three stages. In the first stage, a peak corresponding to the loss of coordinated water molecule and chloride, followed with subsequent removal of the five membered heterocyclic ring in the second stage. In the third stage, there was a loss in mass corresponding to the decomposition of primary 1,2,4-triazole moiety and as a final stage, it left air stable metal oxide as a residue. The three step decomposition of complexes 4-6 as indicated by DTG peaks around 130–190, 310–385 and 570–680 °C corresponding to the mass loss of coordinated water molecule and chloride, furan moiety and 1,2,4-triazole moiety, respectively.

Insert fig. 4

Insert Table 4

3.7 EPR spectra

EPR spectra of Cu(II) complexes were recorded at room temperature as polycrystalline sample, on X band at frequency of 9.1 GHz under the magnetic-field strength of 3000G. The analysis of Cu complex (**3**) gives $g_{\parallel} = 2.191$, $g_{\perp} = 2.056$ and G = 3.41 (Table 3). The observed g_{\parallel} values for the complex are less than 2.3 in agreement with the covalent character of the metal ligand bond. The trend $g_{\parallel} > g_{\perp} > 2.0023$ observed for the complex indicates that unpaired electron is localized in $d^{x_2 \cdot y_2}$ orbital of the Cu(II) ion and the spectral features are a characteristic of axial symmetry. Thus, a tetragonal geometry is confirmed for the aforesaid complex [27]. $G = (g_{\parallel} - 2)/(g_{\perp} - 2)$, which measures the exchange interaction between the metal centers in a polycrystalline solid, has been calculated. According to Hathaway, if G > 4, the exchange interaction is negligible, but G < 4 indicates considerable exchange interaction in the solid complexes [28]. The "G" value for Cu complexes reported in this paper, is <4 indicating the exchange interaction in solid complexes. The g_{\parallel} , g_{\perp} and G values for complexes **3**, **6**, **9** and **12** are given in Table 3.

3.8 Geometry optimization

Geometry Optimization was done using B3LYP functional with 6-31+g(d,p) basis sets as incorporated in the Gaussian 09W programme in gas phase. The fully optimized geometries of the ligand L¹ and complexes **1–3** are shown in Fig. 5. The numbering scheme for ligand L¹, and complexes **1–3** are given in Scheme 2. The molecular structure of Complex **1** shows octahedral geometry around the Co(II) centre as revealed from the calculated bond lengths and bond angles (Table 5). The C₁–S₁, C₄–S₁, C₅–N₁, N₁–N₂ and C₆–S₂ bond lengths become slightly longer in complexes as ligand L¹ coordinates via azomethine nitrogen, deprotonated sulphur of triazole moiety and through sulphur of thiophene. The thiophene moiety is bent out of the coordination plane and move far from Co(II) ion, which results in the slight elongation of Co–S bond length. Similarly, the bond length between Co and sulphur of triazole elongates to 2.228 A^o. The Co–S₁, Co–N₁, Co–O₁, Co–O₂ and Co–Cl bond lengths are found to be 2.224, 2.102, 2.014, 2.017 and 2.313 A^o, respectively. This elongation in Co– S and Co–N bond lengths caused a slight distortion from the regular octahedral geometries. The bond angles in the coordination sphere of Co(II) complexes are found approximately near to the perpendicular value.

Insert Fig. 5

Insert Scheme 2

Insert Table 5

Similar bonding behaviour is observed in case of complex 2 having Ni(II) as metal centre. All the bond lengths and bond angles have very close values as compared to complex 1. However, complex 3 having Cu(II) metal centre shows a large degree of distortion from the regular octahedral geometry. It is because of this distortion that the axial Cu–S₁ and Cu–S₁['] elongates to 2.556 and 2.634 A^o, respectively. This elongation in bond lengths causes a change in the bond angle values of coordination sphere. The bond angles N₁–Cu–N₂ and N₁–Cu–N₂['] reduces 69.23^o and 72.12^o, respectively. The bond angle N₁–Cu–Cl is found to be 161.42^o. The bond lengths and the bond angles within the thiophene moiety and 1,2,4-triazole moiety do not change significantly on coordination with metal ion. On the basis of the above discussion following (Fig. 6) structures can be proposed for the synthesized complexes.

Insert Fig. 6

3.8 Cell viability determination

The *in vitro* cytotoxicity of the ligand L and metal complexes (1-12) on human cell lines HepG2 and MCF-7 was determined by a MTT based assay. The results are expressed as the percentage of cell toxicity with respect to the control and are represented in Table 6. The in vitro screening revealed that ligand L¹, L² and metal complexes 1-12 have very less effect on % inhibition on cell proliferation on cell lines HepG2 and MCF-7 at concentration of 1 μ M. But at concentration of 10 μ M complexes 6, 9, 11 and 12 show a significant increase in % inhibition on cell proliferation on cell line HepG2 (Fig. 7). Similarly, the complexes 6, 10 and 11 shows a significant increase in % inhibition on cell proliferation on cell line MCF-7.

Insert Table 6

Insert Fig. 7

Conclusion

The newly synthesized Schiff base ligands L^1 , L^2 act as tridentate ligands, and all these are coordinated through the azomethine-N, thiol sulphur and through sulphur of thiophene/oxygen of furan to the metal ion. All the synthesized metal(II) complexes possessed an octahedral geometry except the Cu(II) complexes which showed a distorted octahedral geometry. The reasonable agreement between the theoretical and experimental

data reflects to the great extent the suitability of the applied basis set, 6-31+g(d,p) for this type of work and confirms the suggested structure. The findings of anticancer cell line activity reveal that all metal complexes show moderate to significant % inhibition on the cell proliferation on cell line HepG2 and MCF-7. Interestingly, the complexes **6** and **9** shows 38 and 49 % inhibition on cell proliferation against cell line HepG2, whereas complexes **9**, **10** and **11** greatly reduce the malignant MCF-7 cell growth by 34, 38 & 33%, respectively. This inhibition on cell proliferation might be due to azomethine (-HC=N-) linkage and/or hetero atoms present in these compounds. Further structural optimization studies might thus represent a rationale for further investigation.

5. Acknowledgements

We thank the Principal, Zakir Husain Delhi College for providing lab facilities, Jamia Hamdard, New Delhi for Anticancer cell line activities. Authors are thankful to DRDO, for financial assistance.

References

- 1. D.M. Parkin, F. Bray, J. Ferlay, P. Pisani, CA Can. J. Clin. 55 (2005) 74.
- 2. S. Whittaker, R. Marais, A.X. Zhu, Oncogene 29 (2010) 4989.
- 3. A.M. Isloor, B. Kalluraya, P. Shetty, Eur. J. Med. Chem. 44 (9) (2009) 3784.
- 4. J. Patole, D. Shingnapurkar, S. Padhye, C. Ratledge, Bioorg. Med. Chem. Lett. 16 (2006) 1514.
- P. Valentina, K. Ilango, M. Deepthi, P. Harusha, G. Pavani, K.L. Sindhura, C.H. Guru Keerthanan, J. Pharm. Sci. Res. 1 (2) (2009) 74.
- 6. B.S. Holla, B. Veerendra, M.K. Shivananda, B. Poojary, Eur. J. Med. Chem. 38 (2003) 759.
- 7. S.V. Bhandari, K.G. Bothara, M.K. Raut, A.A. Patil, A.P. Sarkate, V.J. Mokale, Bioorg. Med. Chem. 16 (2008) 1822.
- 8. K.V. Sujith, J.N. Rao, P. Shtty, B. Kalluraya, Eur. J.Med.Chem. 44 (2009) 3697.
- 9. T. Propst, W. Vogel, A. Propst, O. Dietze, H. Braunsteiner, J. Mol. Med. 70 (1992) 55.
- 10. S.M. Stahl, CNS Spectr. 13 (2008) 1027.
- G. Capranico, G. Zagotto, M. Palumbo, Curr. Med. Chem. Anticancer Agents 4 (2004) 335.

- S.J. Lippard, J.M. Berg, Principles of Bioinorganic Chemistry, University Science Books, Mill Valley, CA, 1999.
- R.M. Abdel-Rahman, K.O. AL-Footy, F.M. Aqlan, Int.J. ChemTech Res. 3 (1) (2011)
 423.
- 14. H.K. Fun, C.K. Quah, A.M. Vijesh, S. Malladi, A.M. Isloor, Acta Cryst. E66 (2010) 29.
- 15. M A.D. Becke, Phys. Rev. A 38 (1988) 3098.
- 16. C. Lee, W. Yang, R.G. Parr, Phys. Rev. B 37 (1988) 785.
- 17. S.H. Vosko, L. Wilk, M. Nusair, Can. J. Chem. 58 (1980) 1200.
- M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery, Jr., J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, O. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski, D.J. Fox, Gaussian, Inc., Wallingford CT, 2009.
- J. Dharmaraja, T. Esakkidurai, P. Subbaraj, S. Shobana, Spectrochimica Acta A 114 (2013) 607.
- 20. M. Tyagi, S. Chandra, P. Tyagi, Spectrochimica Acta A 117 (2014) 1.
- 21. G. Singh, P.A. Singh, K. Singh, D.P. Singh, R.N. Handa, S.N. Dubey, Proc. Natl. Acad. Sci. Ind. 72A (2002) 87.
- 22. P. Tyagi, S. Chandra, B. S Saraswat, Spectrochimica Acta A 134 (2015) 200.
- 23. B. K. Patel, S. C. Chaudhury, J. Indian Chem. Soc., 1988, LXV, 661.
- 24. M. Tyagi, S. Chandra, A. Akhtar, D. Chand, Spectrochimica Acta A 118 (2014) 1056.
- 25. W.J. Geary, Coord. Chem. Rev. 7 (1971) 81.
- 26. S. Chandra, Ruchi, K. Qanungo, S. K. Sharma, Spectrochimica Acta A 79 (2011) 1326.
- 27. S. Chandra, S. Bargujar, R. Nirwal, N. yadav, Spectrochimica Acta A 106 (2013) 91.
- B.J. Hathaway, J.N. Bardley, R.D. Gillard (Eds.), Essays in Chemistry, Academic Press, New York, NY, USA, 1971.



Fig. 1. Some triazole based market available drugs



Scheme 1. Preparation of the ligands L^1 , L^2 and their metal complexes 1–12

CCFA



Fig. 2. Mass Spectrum of ligand L¹





Fig. 4. Thermogravimetric (TGA/DTG) curves of Co(II) complex (4)



Fig. 5. Geometry Optimized structures of (a) Ligand L^1 , (b) Complex 1, (c) Complex 2, (d) Complex 3 (Colour Code: H=White, C=Grey, N= Blue, O=red, Co= Grey, Ni= Silver Grey, Cu=Pink)



Scheme 2. : Numbering Scheme of the optimized structures (a) Ligand L^1 , (b) Complexes 1–3



Fig. 6. Proposed Structure of the newly obtained metal complexes



Fig. 7. Ligand L¹, L² and metal complexes (1–12) inhibited the cell viability of HepG2 & MCF-7 cells (Data were assayed by ANOVA and Student's *t*-test. Differences between means were considered significant when yielding a P < 0.05. Results are presented as means \pm S.D.)

Table 1

Physical measurements and analytical data of the ligand (L^1, L^2) and metal complexes(1-12).

				Elemen	ntal Ana	lysis (%)) found	
No	Molecular mass/ molecular formula	m/z M. P	Yield		(ca	alc.)	0	
•		(°C)	(%)	С	Н	N	M ^a	
L^1	C ₁₂ H ₉ N ₅ S ₂ [287.36]	287.48 254	74	50.19	3.15	24.40	_	
L^2	C ₁₂ H ₉ N ₅ OS [271.30]	271.31>280	76	53.15	3.33	(24.37) 25.82	-	
	$[C_{0}(1^{-1} - H)C](H_{2}O_{2})][416.76]$. 0		(53.13)	(3.34)	(25.81)	14 12	
1	$C_{12}H_{12}ClCoN_5O_2S_2$	417.08>280	64	(34.58)	(2.90)	(16.80)	(14.14)	
2	[Ni(L ¹ –H)Cl(H ₂ O) ₂] [416.52]	416 67 >280	68	34.72	2.93	16.78	14.04	
4	$C_{12}H_{12}CIN_5NiO_2S_2$	416.67 >280	00	(34.60)	(2.90)	(16.81)	(14.09)	
3	[Cu(L ¹ –H)Cl(H ₂ O) ₂] [421.38]	422.32>280	58	34.14	3.93	16.55	15.02	
	$C_{12}H_{12}ClCuN_5O_2S_2$			(34.20)	(2.87)	(16.62)	(15.08)	
4	$[Co(L^2-H)Cl(H_2O)_2]$ [400.70] $C_{12}H_{12}ClCoN_5O_3S$	400.81 >280	66	35.98	3.02	(17.48)	14.69 (14.71)	
E	[Ni(L ² –H)Cl(H ₂ O) ₂] [400.46]	400 40 > 280	56	35.98	3.02	17.44	14.67	
5	C ₁₂ H ₁₂ ClN ₅ NiO ₃ S	400.40 >280	50	(35.99)	(3.02)	(17.49)	(14.66)	
6	[Cu(L ² –H)Cl(H ₂ O) ₂] 405.32]	405.29 > 280	60	35.58	2.99	17.27	15.68	
0	$C_{12}H_{12}ClCuN_5O_3S$		-	(35.56)	(2.98)	(17.28)	(15.68)	

7	$[Co(L^1-H)_2]$ [631.64]	631 78 \280		45.67	2.54	22.20	9.35
Ι	$C_{24}H_{16}CoN_{10}S_4$	031.78 >280	J 4	(45.64)	(2.55)	(22.18)	(9.33)
0	[Ni(L ¹ –H) ₂] [631.40]	621 21 - 280	56	45.65	2.58	22.22	9.33
8	$C_{24}H_{16}N_{10}NiO_2S_2\\$	031.31 >280		(45.66)	(2.55)	(22.18)	(9.30)
0	$[Cu(L^1-H)_2]$ [636.25]	626 42 - 280	62	45.33	2.53	22.05	10.01
У	$C_{24}H_{16}CuN_{10}O_2S_2$	030.42 >280		(45.31)	(2.53)	(22.02)	(9.99)
10	$[Co(L^2-H)_2]$ [599.51]	500 87 \ 280	52	48.10	2.70	23.35	9.84
10	$C_{24}H_{16}CoN_{10}O_2S_2$	J99.07 ~200	52	(48.08)	(2.69)	(23.36)	(9.83)
11	[Ni(L ² –H) ₂] [599.27]	598 89 >280	66	48.09	2.71	23.35	9.80
11	$C_{24}H_{16}N_{10}NiO_2S_2\\$	550.057200		(48.10)	(2.69)	(23.37)	(9.79)
12	$[Cu(L^2-H)_2]$ [604.13]	604 12 > 280	65	47.74	2.67	23.22	10.51
12	C ₂₄ H ₁₆ CuN ₁₀ O ₂ S ₂	0011127200		(47.72)	(2.67)	(23.19)	(10.52)
^a M =	Co(II), Ni(II), Cu(II)						
	G						
C)						
P							

Table 2

Important infrared spectral bands (cm⁻¹) and their assignments

Compound	v(HC=N)	v(C-S-C)	v(C-O-	v(M-O)	$\nu(M-N)$	ν (M-N)
			C)			
\mathbf{L}^{1}	1618	834	-	-	-	
L^2	1616	-	1018	-		
1	1599	854	-	-	468	483
2	1601	852	-	-	476	498
3	1598	849	-	-	462	478
4	1606	-	999	522	480	498
5	1599	-	1002	516	473	491
6	1604	-	1004	510	470	492
7	1604	851	-	-	470	487
8	1602	864	-	-	465	485
9	1598	856	-	-	475	495
10	1599	-	998	528	462	484
C ₁₁	1600	-	1001	534	478	495
12	1605	-	1004	524	465	488

Table 3

Conductivity, magnetic, electronic spectra and EPR spectra of metal complexes 1-12

Ma	$\Omega_{ m M}$	B.M	(am^{-1})	~	~	C
INO.	$(\Omega^{-1} cm^2 mol^{-1})$	(μ_{eff})	$\lambda_{\rm max}$ (cm)	g⊫	g⊥	G
1	12.6	4.87	9778, 17665, 29974	-	-0	-
2	14.2	2.93	10855, 15383, 25165	-	6	-
3	11.0	1.95	13468, 18598, 25378	2.191	2.056	3.41
4	13.4	4.89	9812, 17774, 30256		-	-
5	12.8	2.98	11643, 16476, 23165	-	-	-
6	16.8	1.93	15524, 19112, 27465	2.241	2.074	3.25
7	15.2	4.81	9874, 17330, 29718	-	-	-
8	16.1	2.92	10815, 15922, 25715	-	-	-
9	17.2	1.96	12195, 18712, 24435	2.290	2.095	3.05
10	15.6	4.97	9860, 17568, 30140	-	-	-
11	12.9	2.99	11434, 15960, 24546	-	-	-
12	13.7	1.95	14265, 19004, 26810	2.154	2.045	3.42

Table 4

			Temp		Residual	Mass L	oss (%)
Comp. No	Molecular Formula	Stages	(°C)	Possible Evolved Species	Species	Found	Calc.
		1^{st}	155-265	C ₄ H ₄ N			
\mathbf{L}^{1}	$C_{12}H_9N_5S_2$	2^{nd}	265-480	C ₈ H ₇ N ₂ O	5	100	100
		1^{st}	130-190	H ₄ O ₂ Cl			
4	$[\operatorname{Co}(\mathrm{L}^2-\mathrm{H})\operatorname{Cl}(\mathrm{H}_2\mathrm{O})_2]$	2^{nd}	310–385	Ċ ₅ H ₄ N	CoO	18.81	18.69
•	$C_{12}H_{12}CICoN_5O_3S$	3 rd	570–680	C7H4N4S			
		1^{st}	140-220	H ₄ O ₂ Cl			
5	$[Ni(L2-H)Cl(H2O)2]$ $C_{12}H_{12}ClN_5NiO_3S$	2 nd	220-360	C ₅ H ₄	NiO	18.98	18.65
		3 rd	450-715	$C_7H_4N_5S$			
	$[Cu(L^2-H)Cl(H_2O)_2]$	1 st	160-465	C ₅ H ₈ O ₂ NCl	CuO	19 75	19.62
6	$C_{12}H_{12}ClCuN_5O_3S$	2^{nd}	465-675	$C_7H_4N_4S$	euo	17.13	17.02
P							

Thermal Analysis data the ligand (L^1) and metal complexes (4-6).

Table 5

Optimized geometry of the ligand L^1 and metal complexes 1-3 (bond lengths in Angstroms; bond angles in degrees).

Par	rameters ^a	Ligand L ¹	Complex 1 ^b	Complex 2 ^c	Complex 3 ^d
($C_1 - S_1$	1.799	1.806	1.805	1.812
($C_4 - S_1$	1.816	1.831	1.833	1.828
($C_4 - C_5$	1.427	1.454	1.456	1.452
($C_5 - N_1$	1.300	1.309	1.311	1.313
Ν	$N_1 - N_2$	1.337	1.342	1.345	1.355
Ν	$N_2 - C_6$	1.485	1.498	1.497	1.496
($C_6 - S_2$	1.906	1.922	1.927	1.925
($C_{6} - N_{3}$	1.463	1.502	1.506	1.498
1	$M - S_1$		2.224	2.237	2.556
Ν	$M - N_1$		2.102	2.117	2.124
1	$M - S_2$	-	2.228	2.231	2.634
Ν	$M - O_1$	-	2.014	2.234	2.384
Ν	$M - O_2$	-	2.017	2.232	1.989
	M – Cl	-	2.313	2.324	2.397
Z	S_1MN_1	-	81.49	83.71	87.45
Z	M_1MS_2	-	87.54	81.24	69.23
Z	S_2MO_2	-	88.12	86.43	84.32

$\angle S_1 W O_1$	-	85.32	81.19	72.12
∠O ₁ MCl	-	88.39	87.24	78.74
∠O ₂ MCl	-	85.11	83.27	81.34
∠N ₁ MCl	-	172.16	174.22	161.42
^a Scheme 1 for num	bering			
^b M=Co, ^c M=Ni, ^d	M=Cu		.59	
		UN.		
	2			

Table 6

Effect of a ligand L^1 , L^2 and series of metal complexes (1–12) at two concentrations of 1 and 10 μ M on the HepG2 and MCF-7 cell proliferation.^a

	Survival cell		Cell growth		Survival cell		Cell growth	
Compound	fraction (%)) at 1 µM	inhibition	(%) at 1 µM	fraction (%) at 10 µM	inhibition (%) at 10 µM
	HepG2	MCF-7	HepG2	MCF-7	HepG2	MCF-7	HepG2	MCF-7
Control	100 ± 2	100 ± 1	-	-	100 ± 1	100 ± 2	-	-
L^1	96 ± 3	97 ± 3	4	1	94 ± 3	95 ± 2	6	5
L^2	97 ± 2	98 ± 2	3	2	96 ± 2	97 ± 1	4	3
1	93 ± 4	95 ± 4	7	5	82 ± 3	89 ± 4	18	11
2	95 ± 3	96 ± 3	5	4	78 ± 5	83 ± 3	22	17
3	92 ± 5	95 ± 3	8	5	76 ± 3	78± 5	24	22 ^b
4	94 ± 3	96 ± 3	6	4	83 ± 4	81 ± 2	17	19
5	92 ± 3	93 ± 4	8	7	72 ± 2	77±3	28	23 ^b
6	91 ± 5	90 ± 1	9	10 ^b	62± 1	73±2	38 ^b	27 _b
7	94 ± 3	97 ± 2	6	3	71 ± 2	80 ± 2	29	20
8	90 ± 2	96 ± 3	10	4	69 ± 1	73± 1	31 ^b	27
9	88 ± 4	93 ± 2	12 ^b	7	51 ± 3	66 ± 2	49 ^b	34 ^b
10	92 ± 5	94 ± 5	8	6	65± 5	62±4	35 ^b	38 ^b
11	89 ± 4	92 ± 3	11 _b	8	76± 3	67±2	24	33 ^b
12	87 ± 3	91 ± 3	13 ^b	9	68±2	76± 1	32 ^b	24 ^b

^a The results are expressed as the percentage of viable cells with respect to the control and are presented as mean \pm SD.

^b Significantly different from the control



Thermogravimetric (TGA/DTG) curves of Co(II) complex (4)



Highlights

- Synthesis of 1,2,4-triazole derived Schiff bases and their metal complexes. •
- Characterization by physical, spectral and analytical data. ٠
- Acctinition