Asian Journal of Chemistry; Vol. 28, No. 11 (2016), 2487-2494

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2016.20039

Synthesis, Characterization and Biological Activity of Zn(II) Complexes with Dibasic Tridentate ONS-Donor Ligand

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Received: 13 April 2016; *Accepted*: 30 July 2016; *Published online*: 10 August 2016; AJC-18024

A new kind of zinc(II) complexes **1** and **2** with new Schiff base ligand **(L)** have been synthesized and characterized by ^{1}H NMR, X-ray crystallography, IR and UV-visible spectroscopic studies. The trigonality index τ of 0.63 for complex **2** indicates that the coordination geometry around zinc is intermediate between trigonal bipyramidal and square pyramidal geometries and is better described as trigonal bipyramidal distorted square based pyramid (TBDSBP) with zinc displaced above the N(2), N(4), O(1) and S(1) coordination plane and towards the elongated apical N(1) atom. The newly synthesized Schiff base ligand **(L)** and its Zn(II) complexes **1**-**2** were assayed for *in vitro* antibacterial activity against two Gram-positive bacteria strains *(Stapholococcus aureus*, *Pseudomonas aeruginosa)* and Gram-negative bacteria *E. coli.* The cancer cell line studies of the effect of the ligand **(L)** and its Zn(II) complexes **1**-**2** on a MCF-7 cancer cell line by an MTT assay indicates that the ligand **(L)** exhibits higher activity towards the metal complexes **1** and **2** when compared with cyclophosphamide as reference drug.

Keywords: Thiosemicarbazone ligand, Zn(II) complexes, Crystal structure, Cell line studies.

INTRODUCTION

Thiosemicarbazones and their metal complexes exhibit a wide range of applications that extend from their use in analytical chemistry through pharmacology to nuclear medicine [1-4]. Schiff bases are regarded as "privileged ligands" due to their capability to form complexes with a wide range of transition metal ions yielding stable and intensely coloured metal complexes. Some of them have been shown to exhibit interesting physical and chemical properties and potential biological activities [5-10]. Attempts are being made to replace these platinum-based drugs with suitable alternatives and numerous metal complexes are synthesized and screened for their anticancer activities [11,12]. A wide repertoire of Zn(II) complexes have been utilized as radio protective agents [13] tumor photosensitizers [14] antidiabetic insulin-mimetic [15] and antibacterial or antimicrobial agents [16]. It is also well-known that Zn(II) is useful to reduce the cardio and epatotoxicity induced by some anticancer drugs [17]. However, very little data on the cytotoxicity of zinc-based compounds against human cancer cell lines are as yet available [18].

In continuation of our work in the area of Schiff base complexes [19,20] a new type of Zn(II) complexes have been synthesized and characterized by NMR, IR, UV-visible spectroscopic methods and single crystal X-ray studies for the newly synthesized Zn(II) complexes **1** and **2**. Based on these studies a trigonal bipyramidal distorted square based pyramid (TBDSBP) has been proposed for the Zn(II) complexes. Apart from these studies in order to get a clear cut idea about the pharmacological properties of the Zn(II) complexes, antibacterial, antifungal and cancer cell line studies have been carried out.

EXPERIMENTAL

3-Ethoxysalicylaldehyde (Sigma-Aldrich) and N(4)-phenylthiosemicarbazide (Sigma-Aldrich), $Zn(OAc)_2{\cdot}2H_2O$, 2,2'-bipyridine (bpy) (Sigma-Aldrich), 1,10-phenanthroline (Phen) (E-Merck) were obtained commercially and used without further purification. Double distilled water is used for all the experiments. All the reagents and solvents were analytical, spectroscopic grade and they were used without further purification for the preparation of thiosemicarbazone ligand **(L)** and Zn(II) complexes **1** and **2**.

Physical measurements: Crystal data collection APEX2 (Bruker, 2004); cell refinement: SAINT (Bruker, 2004), ^{1}H NMR spectra are recorded on a Bruker 300 MHz spectrometer. IR spectra are recorded in KBr disks with a Perkin Elmer FT-IR spectrophotometer. UV-visible spectra of solution are recorded on a Shimadzu 1700 series spectrometer.

Antifungal screening: The antifungal activity of the ligand (**L**) and their Zn(II) complexes (**1-2**) are studied by paper disc method [21]. *Candida albicans sp*, *Aspergillus niger sp* and *Macrophonia sp* are used as test organisms. Solution of desired concentration (1 mg/mL) was obtained by dissolving 2, 4, 6 mg of each compound in DMSO and added to potato dextrose agar (PDA) medium in sterile Petri dishes. The sterilized medium with the added sample solution is poured into sterile Petri plates and allowed to solidify. Filter paper discs of 5 mm diameter are prepared prior to the experiment. The filter paper discs are placed on nutrient medium mixed with fungal strains. These Petri dishes are incubated at 35 °C for 48 h. The per cent reduction in the radial growth diameter over the control is calculated. The growth is compared with dimethyl sulfoxide as the control and *Ketokonazole* as a standard drug.

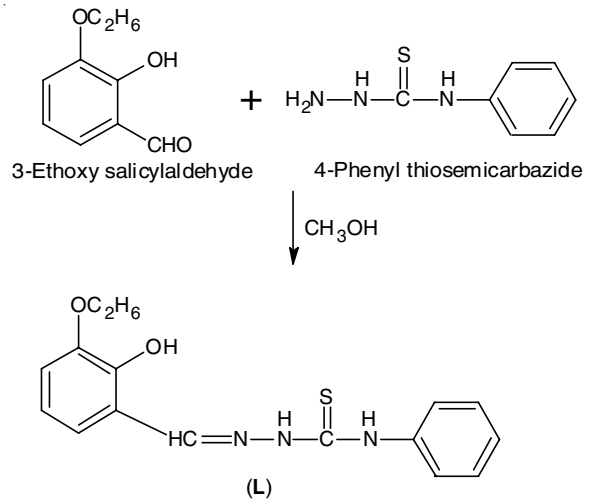
Antibacterial screening: Antibacterial activities are investigated using agar well diffusion method. The activity of the free ligand (**L**) and its Zn(II) complexes **1-2** and standard drug amikacin are studied against the Gram-positive bacteria strains (*Stapholococcus aureus*, *Pseudomonas aeruginosa*) and Gram-negative bacteria *E. coli*. The solution of 2 mg/mL of each compound [free ligand (**L**)] and its Zn(II) complexes (**1-2**) and standard drug (amikacin) in DMSO is prepared for testing against bacteria. Centrifuged pellets of bacteria from a 24 h old culture containing approximately 10^4 to 10^6 CFU (colony forming unit) per mL are spread on the surface of Muller Hinton agar plates. Wells are created in medium with the help of a sterile metallic bores and nutrients agar media (agar 20 g + beef extract 3 g + peptones 5 g) in 1000 mL of distilled water (pH 7.0), autoclaved and cooled down to 45 °C. Then, it is seeded with 10 mL of prepared inocula to have 10^6 CFU/mL. Petri plates are prepared by pouring 75 mL of seeded nutrient agar. The activity is determined by measuring the diameter of the inhibition zone (mm). The growth inhibition is calculated according to Kumar *et al.* [21].

Cell viability test: The viability of cells is assessed by MTT assay using mononuclear cells. The assay is based on the reduction of soluble yellow tetrazolium salt to insoluble purple formazan crystals by metabolically active cells. Only live cells are able to take up the tetrazolium salt. The enzyme (mitochondrial succinate dehydrogenase) present in the mitochondria of the live cells is able to convert internalized tetrazolium salt to formazan crystals, which are purple in colour. Then, the cells are lysed and dissolved in DMSO solution. The colour developed is then determined in an ELISA reader at 570 nm.

The Hepatocellular carcinoma cells (HepG2 cells) are plated separately in 96 well plates at a concentration of 1 × 10^5 cells/well. After 24 h, cells are washed twice with 100 µL of serum-free medium and starved for 0.5 h at 37 °C. After starvation, cells are treated with different concentrations of test compound (50-300 µg/mL) for 24 h. At the end of the treatment period, the medium is aspirated and serum free medium containing MTT (0.5 mg/mL) is added, then it is incubated for 4 h at 37 °C in a CO_2 incubator.

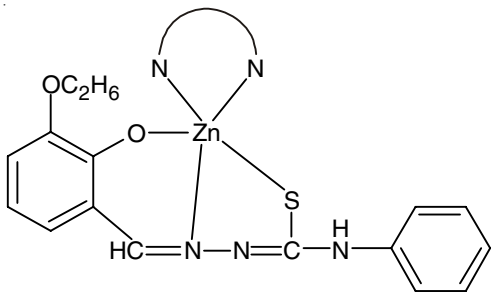
The MTT containing medium is then discarded and the cells are washed with PBS (200 µL). The crystals are then dissolved by adding 100 µL of DMSO and this is mixed properly by pipetting up and down. Spectrophotometrical absorbance of the purple blue formazan dye is measured in a micro-plate reader at 570 nm [22].

Synthesis of Schiff base ligand (L): 3-Ethoxy salicylaldehyde (0.5 mmol) in methanol (0.83 g) was taken in a round bottomed flask and stirred by a magnetic stirrer followed by dropwise addition of methanolic solution of 4-phenylthiosemicarbazide (3.0 mmol) for 2 to 4 h (**Scheme-I**). The resulting white solid was removed by filtration and washed with cold ethanol and dried *in vacuo* over anhydrous $CaCl_2$ to remove any moisture. m.p.: 210 °C, Yield: 82 %



Scheme-I: Synthesis of the new thiosemicarbazone ligand (**L**)

Synthesis of the Zn(II) complexes 1-2: A solution of $Zn(OAc)_2{\cdot}2H_2O$ (0.55 g, 0.25 mmol) in ethanol, was added to a solution of thiosemicarbazone ligand (**L**) (0.79 g, 0.25 mmol) in a round bottomed flask with constant stirring. After 0.5 h, the base (0.25 mmol 2,2'-bipyridine)/(0.25 mmol 1,10-phenanthroline) dissolved in ethanol was added in the round bottomed flask. The stirring was continued for about 1 h and the yellow colour compound formed was filtered, washed with cold ethanol and ether and dried *in vacuo* over anhydrous $CaCl_2$. A single orange colour crystal suitable for the X-ray diffraction was obtained by slow evaporation of a solution of chloroform (**Scheme-II**).



Scheme-II: Proposed structure of the complexes **1** and **2**

TABLE-1
CRYSTAL DATA AND STRUCTURE REFINEMENT FOR COMPLEX [ZnL(phen) (**1**) AND [ZnL(bpy)] (**2**)

Crystal data	Compound **1**	Compound **2**
Empirical formula	$C_{29}H_{24}N_5O_2SZnCl_3$	$C_{55}H_{52}N_{10}O_5S_2Zn_2$
Formula weight	678.31	1127.93
Wavelength	0.71073 Å	0.71073 Å
Crystal system	Monoclinic	Monoclinic
Space group	C2/c	P21/n
a, b, c (Å)	25.6400(6), 14.7350(4), 15.7130(3)	13.6702(4), 14.6101(5), 26.4561(8)
α, β, γ (°)	90, 90.7280(10), 90	90, 93.9540(10), 90
Volume	5936.0 (2) $Å^3$	5271.3 (3) $Å^3$
Z, Calculated density	8, 1.518 Mg/m^3	4, 1.421 Mg/m^3
F(000)	2768	2336
Crystal size	0.35 × 0.35 × 0.30 mm^3	0.30 × 0.20 × 0.20 mm^3
Temperature	293 (2) K	293 (2) K
θ Min-Max	2.05 to 25.00°	1.59 to 25.00°
Completeness to θ	25.00 99.9 %	25.00 99.8 %
Max. and Min. transmission	0.7536 and 0.6536	0.8563 and 0.7236
Final R indices [I >2σ(I)]	R1 = 0.0329, wR2 = 0.0828	R1 = 0.0360, wR2 = 0.0780
R indices (all data)	R1 = 0.0443, wR2 = 0.0916	R1 = 0.0648, wR2 = 0.0935
Largest diff. peak and hole	0.543 and -0.362 e.$Å^{-3}$	0.331 and -0.246 e.$Å^{-3}$

RESULTS AND DISCUSSION

Crystal structure of complex 1: The molecular structure of the complex along with atomic numbering is given in Fig. 1, Crystallographic parameters and selected bond lengths and angles are given in Tables 1 and 2. The compound crystallizes in a monoclinic lattice with space group C2/c. The zinc in the mononuclear complex is five-coordinate and is having approximately trigonal bipyramidal geometry. The basal coordination positions are occupied by the phenolato oxygen, O(1), azomethine nitrogen, N(5) and thiolate sulfur, S(1), of the thiosemicarbazone and the phen nitrogen, N(2). In a five-coordinate system, the angular structural parameter (τ) is used to propose an index of trigonality. In a five-coordinate system, the angular structural parameter (τ) is used to propose an index of trigonality. The trigonality index τ of 0.55 [According to Addison *et al.* [23], τ = (β-α)/60, where β = N(5)-Zn(1)-N(1) = 174.81(8)° and α = O(1)-Zn(1)-S(1) = 141.29(6)°] for perfect square pyramidal and trigonal bipyramidal geometries the values of τ are zero and unity, respectively [23]. This indicates that the coordination geometry around zinc is intermediate between trigonal bipyramidal and square pyramidal geometries and is better described as trigonal bipyramidal distorted square based pyramid (TBDSBP) with zinc displaced above the N(1), N(5), O(1) and S(1) coordination plane and towards the elongated apical N(2) atom [24]. The four base atoms are coplanar showing a significant distortion form a trigonal bipyramidal distorted square based pyramid geometry indicated by O(1)-Cu(1)-S(1) bond angle (141.29°). The central zinc atom is displayed from the basal plane in the direction of the axial nitrogen, which is evident from the bond angles of N(5)-Zn(1)-N(1), (174.81°), O(1)-Zn(1)-N(5), (89.95°). The bond angles O(1)-Zn(1)-N(2), (106.06°), N(2)-Zn(1)-N(1), (76.91°), N(5)-Zn(1)-N(2), (99.20°) indicate the distortion from a trigonal bipyramidal distorted square based pyramid geometry (τ = 0.55). One of the reasons for the deviation from an ideal stereochemistry is the restricted bite angle imposed by both the ligand and phen ligands. The O(1)-Zn(1)-N(2) bond angle 106.06° and

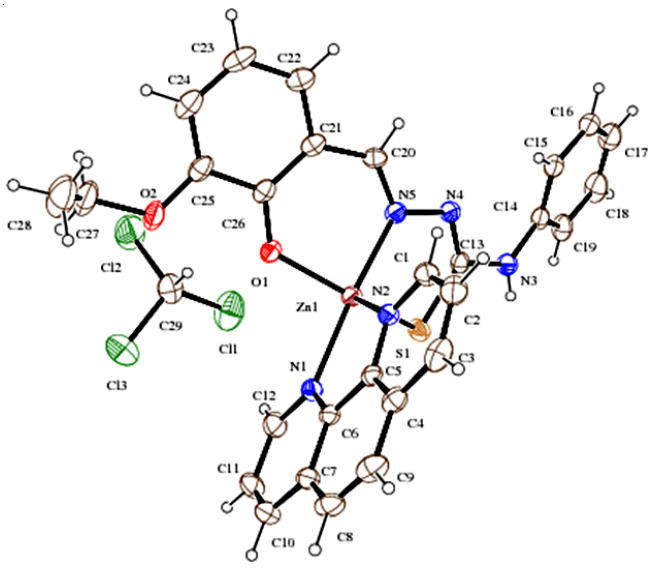


Fig. 1. Structure and labeling diagram of the complex **1**

TABLE-2
SELECTED BOND LENGTHS AND ANGLES FOR COMPLEXES **1-2**

Atoms	Compound **1**	Compound **2**
Bond lengths		
N(1)-Zn(1)	2.189(2)	2.117(2)
N(2)-Zn(1)	2.132(2)	2.171(2)
O(1)-Zn(1)	1.9625(16)	1.944(2)
S(1)-Zn(1)	2.3737(7)	2.3362(8)
Bond angles		
O(1)-Zn(1)-N(2)	106.06(8)	76.27(10)
O(1)-Zn(1)-N(1)	94.42(7)	101.59(9)
O(1)-Zn(1)-S(1)	141.29(6)	134.92(7)
N(2)-Zn(1)-S(1)	112.57(5)	96.13(7)
N(1)-Zn(1)-S(1)	96.49(6)	123.46(7)

N(1)-Zn(1)-S(1) bond angle 141.29°, indicate a slight tilting of the axial Zn(1)-N(1) bond in the direction of the O(1)-Zn(1) bond away from the S(1)-Zn(1) bond. The variation in Zn-N bond distances, Zn(1)-N(1) (2.189 Å), Zn(1)-N(2) (2.132 Å)

and Zn(1)-N(5) (2.059 Å) indicate differences in the strength of the bond formed by each of the coordinating nitrogen atoms. The difference in bond lengths can be attributed to the difference in the extent of π back-bonding between the 1,10-phenanthroline and thiosemicarbazone moieties. However, the large bond distance at the axial Zn (1)-N(1) positions supports the lack of significant out of plane π-bonding. Coordination to Zn(II) lengthens the C-S bond substantially to 1.752 Å from 1.680 Å in unsubstitued salicylaldehyde N(4) phenylthiosemicarbazone [25] as would be expected on coordination of thiolate sulfur. Hydrogen bonding interactions for complex **1** is shown in Fig. 2 and unit cell packing diagram of the compound is shown in Fig. 3.

Crystal structure of complex 2: The molecular structure of the compound **2** along with the atom numbering scheme is represented in Fig. 4. Crystallographic parameters and selected bond lengths and angles are given in Table-2. Suitable pale yellow crystals were obtained from a solution of **2** in a solvent of acetone. The compound **2** is monoclinic with a space group P21/n. This complex is mononuclear and five coordinated. In the complex [ZnLbpy], Zn(II) is located in an approximately trigonal bipyramidal geometry in which the equatorial positions are occupied by the S(1), O(1), N(1) and the axial positions by N(2) and N(4) [Zn(1)-N(2), 2.171(2), Zn(1)-N(4), 2.072(2) Å] with the N(4)-Zn(1)-N(2) angle of 173.06(9)° being close to the 'ideal' value of 180° which is usual for such systems [26]. In a five-coordinate system, the angular structural parameter (τ) is used to propose an index of trigonality. The trigonality index τ of 0.63 {According to Addison *et al.* [23], τ = (β-α)/60, where β = N(4)-Zn(1)-N(2) = 173.06(9)° and α

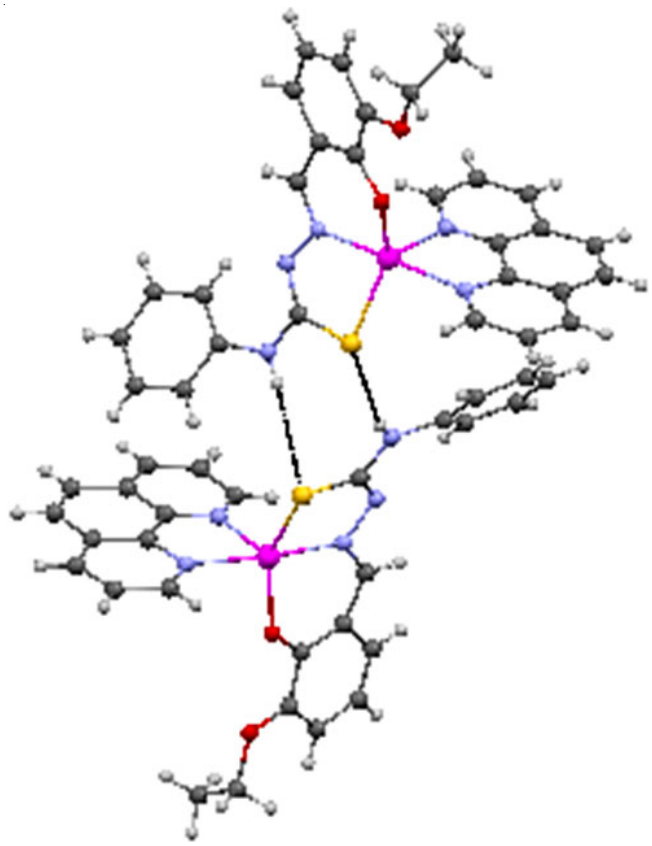


Fig. 2. Hydrogen bonding interaction for complex **1**

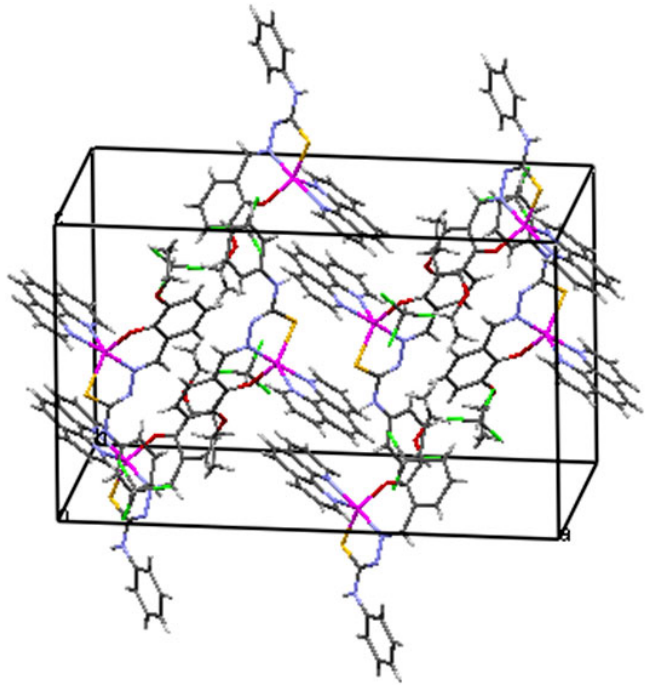


Fig. 3. Unit cell packing diagram of the complex **1**

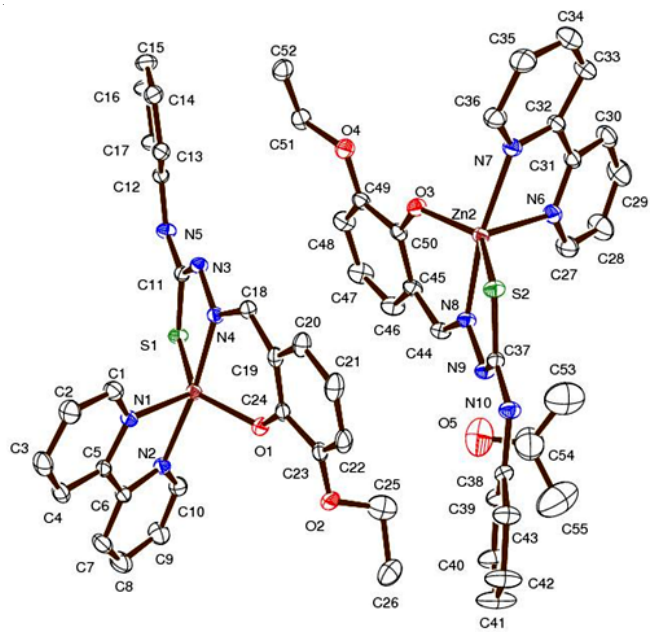


Fig. 4. Structure and labeling diagram of the complex **2**

= O(1)-Zn(1)-S(1) = 134.92(7)°; for perfect square pyramidal and trigonal bipyramidal geometries the values of τ are zero and unity, respectively [27]} indicates that the coordination geometry around zinc is intermediate between trigonal bipyramidal and square pyramidal geometries and is better described as trigonal bipyramidal distorted square based pyramid (TBDSBP) with zinc displaced above the N(2), N(4), O(1) and S(1) coordination plane and towards the elongated apical N(1) atom [28]. One of the reasons for the deviation from an ideal stereochemistry is the restricted bite angle imposed by both the $(L)^{2-}$ and 2,2'-bipyridine ligand. The bite angle around the metal *viz.* N(1)-Zn(1)-N(2) of 76.27(10)° may be considered normal, when compared with an average value of 77°

cited in the literature [35-37]. The variation in Zn-N bond distances, Zn(1)-N(1), 2.117(2), Zn(1)-N(4), 2.072(2) and Zn(1)-N(2), 2.171(2) indicate differences in the strengths of the bonds formed by each of the coordinating nitrogen atoms. The Zn-N bond lengths are shorter than those reported for mononuclear Zn(II) complexes, while there is no significant variation in the Zn-S bond lengths reported [29]. The dihedral angle formed by the least square plane for the compound **2**. The imine bond formation is evidenced from N(4)-C(18) and N(3)-C(11) distances of 1.283(4) Å and 1.296(4) Å. The C-N bond length of 1.396(4) Å and C-S bond length of 1.750(3) is similar to those reported for coordination of thiosemicarbazone in the thiolate form [30,31]. For complex **2**, hydrogen bonding inter-action (Fig. 5) and unit cell packing diagram of the compound is shown in Fig. 6. The molecules in the crystal lattice are stabilized by combination of hydrogen bonding and π-π interactions between aromatic rings.

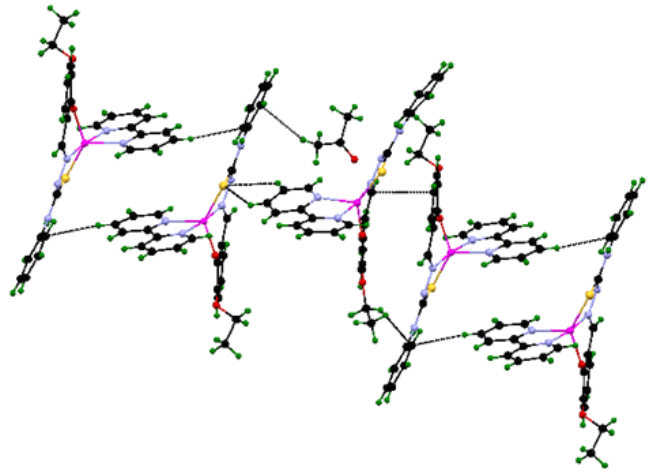


Fig. 5. Hydrogen bonding interaction for complex **2**

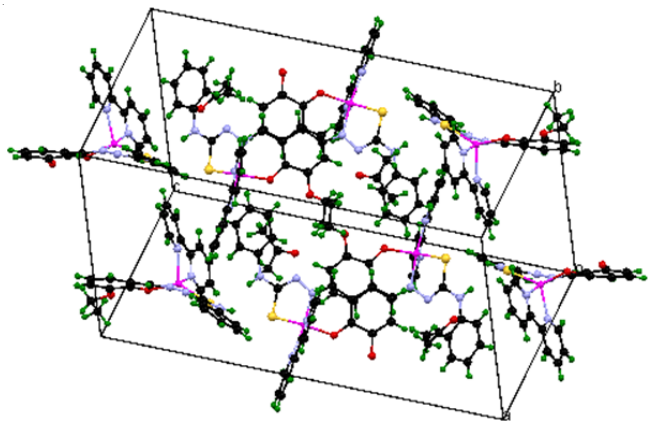


Fig. 6. Unit cell packing diagram of the complex **2**

^{1}H NMR spectra: ^{1}H NMR spectra of ligand **(L)** and its Zn(II) complexes (**1** and **2)** are recorded in DMSO-d_6 and the corresponding spectrum is given in Fig. 7. A singlet observed at δ 11.78 ppm in ligand **(L)** due to phenolic OH proton has disappeared in the spectra of complexes **1** and **2** [29]. The singlet appeared at δ 8.51 ppm attributed to azomethine proton of the free ligand **(L)** was shifted to δ 8.76 ppm in the complex **2**. The thiosemicarbazone ligand (**L**) shows a singlet at

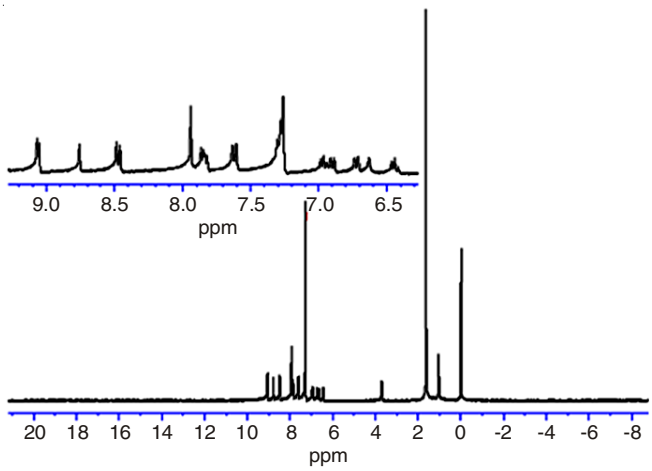


Fig. 7. ^{1}H NMR spectra of complex **2**

δ 10.03 ppm for NH proton and it is absent in the spectra of complex **2**, which clearly indicates the enolization of –NH-C=S group of the ligand (**L**) followed by deprotonation prior to coordination of the thiolate sulfur [32]. The two NH signals of the ligand **(L)** at δ 9.08 ppm and δ 10.04 ppm, respectively, were absent in the complex **2**. (One NH signal of the complex is disappeared, probably exchanged with the solvent) Signals observed in the ^{1}H NMR spectra of ligand at δ 10.03 and δ 8.51 ppm is due to the presence of NH (NH^1) and phenyl NH (NH^2) protons, respectively. The peak due to NH^1 has absent and the peak due to the phenyl NH proton shifted to downfield in the spectrum of complex **2**. The peaks displayed in the region of δ 7.2-7.5 ppm in the complex **2** are due to the aromatic protons of thiosemicarbazone and phenanthroline ligands. The ethoxy protons appeared as a quartet and a triplet at δ 3.00 and δ 1.22 ppm, respectively. Similar observations are observed for complex **1**. From the spectral data, it is clear that the coordination occurs through ONS donor ligand **(L)** for both the complexes **1** and **2**.

IR spectra: IR spectral data of the ligand (**L**) and complexes **1**-**2** are given in Table-3. The intense band in the region 1593 cm^{-1} in the IR spectrum of Schiff base ligand (**L**) is associated with C=N stretching vibration and is shifted to lower frequencies 1539 cm^{-1} in the spectrum of corresponding complex **1** and this change in value indicate the coordination of azomethine nitrogen to the metal ion [20a]. The band due to phenolic OH group disappeared in the IR spectrum of complex **1** in the region 3394-3299 cm^{-1} (Fig. 8) suggesting deprotonation of metal ion. In addition, a band appeared at 1275 cm^{-1} due to phenolic C-O stretching in the Schiff base ligand (**L**) has been shifted to 1308 cm^{-1} in the IR spectrum of the complex **1** indicating the coordination through phenolic oxygen atom. IR spectrum of free ligand (**L**) showed one band at 3468 cm^{-1} due to terminal NH^2 and this bands position altered in the spectrum of the corresponding complex **1**, revealing non participation of NH^2 in coordination [20b]. A band which appeared in the region 3174-2980 cm^{-1} due to N-H in the ligand (**L**) disappeared on complexation. Further, a band due to C=S (781 cm^{-1}) which appeared in the ligand has completely disappeared in the spectrum of the complexes and a new band appeared at 756 (**1**) and 737 (**2**) cm^{-1} (for C-S) due to enolization of -NH-C=S group of the ligand, followed by

TABLE-3
IR SPECTRAL DATA (cm^{-1}) AND UV SPECTRAL DATA (nm) OF FREE LIGAND (**L**) AND ITS Zn(II) COMPLEXES **1-2**

Compound	ν(CH=N)	ν(C-O)	ν(N-H)	ν(C=S)	ν(O-H)	ν(Zn-N)	ν(Zn-O)	λ_{max} (nm)
L	1593	1275	3468	781	3286	–	–	299, 336
1	1581	1308	3476	756	–	435	525	376
2	1583	1309	3482	737	–	483	575	375

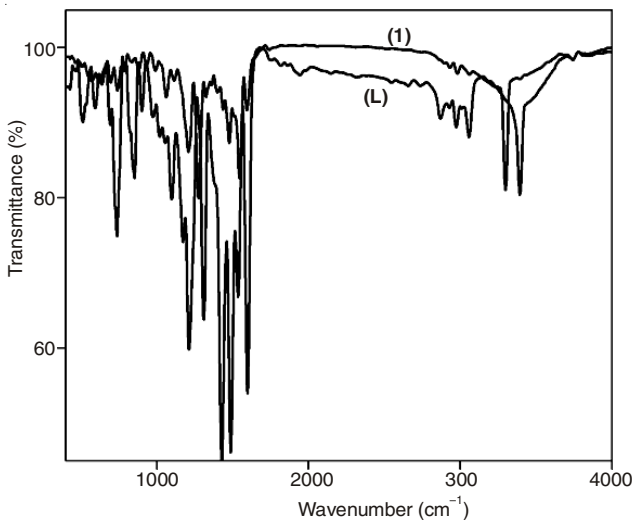


Fig. 8. IR spectra of ligand (**L**) and complex **1**

deprotonation prior to coordination of the thiolate sulfur [33]. The non-ligand bands observed in far IR region for complex **1** are assigned to ν(M-N) (483-435, cm^{-1}) and ν(M-O) (575-525, cm^{-1}) stretching vibrations. Two strong bands at 1580 and 1560 cm^{-1} assigned to ν(-C=C-) and ν(-C=N-) (of 2,2'-bipyridine), are shifted to higher frequencies by 12-29 cm^{-1}. Similarly, the strong bands observed at 1430, 1580 and 1560 cm^{-1} assigned to ν(ring), ν(C=C) and ν(C=N), respectively for 1,10-phenanthroline, are shifted to higher frequencies by 29-58 cm^{-1}. This indicates that the nitrogen atoms in phen and bpy coordinate to the complexes **1-2** [33,34].

Electronic spectra: The electronic spectra of the ligand (**L**) and its Zn(II) complexes **1-2** recorded in DMSO solvent and it shows four bands in the region 247, 297, 334 and 375 nm. The peaks below 300 nm are assigned to intraligand transitions, $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$.The bands appeared in the region 377 nm (Table-3) are assigned to MLCT, respectively.

Antifungal activity: Table-4 indicates that the ligand as well as the complexes **1** and **2** has a significant degree of antifungal activity against, *Candida albicans*, *Aspergillus niger* and *Macrophonia* at 2 mg/mL concentration. The effect is susceptible to the concentration of the compound used for inhibition. The complex **2** shows greater activity against *Candida albicans* species. The antifungal activity of the ligand **(L)** and its Zn(II) complexes **1** and **2** varies in the following order of fungal species *Macrophonia* > *Candida albicans* > *Aspergillus niger*. The antifungal experimental results of the compounds were compared with the standard antifungal drugs ketokonazole at the same concentration. The complex **2** exhibit greater antifungal activities against *Candida albicans sp* and *Aspergillus niger sp* and the complex **1** is resistant to the above fungus. They also show low activity against *Macrophonia sp* than complex **1**. From the observed data it shows that the antifungal activity depends upon the type of metal complexes and varies in the following order of the metal complexes **2 > 1**.

Antibacterial activity: The antibacterial activity of the newly synthesized ligand **(L)** and its Zn(II) complexes **1** and **2** were determined by the standard 'disc diffusion' method. The ligand **(L)** it Zn(II) complexes **1** and **2** with the standard drug amikacin were screened separately for their antibacterial activity against the Gram-positive bacteria *Stapholococcus aureus*, *Pseudomonas aeruginosa* and Gram-negative bacteria *E. coli*. The results of the bacterial study of the synthesized compounds are shown in Table-5. The antibacterial activity of the ligand **(L)** and its Zn(II) complexes **1** and **2** varies in the following order of fungal species *Pseudomonas aeruginosa* > *Stapholococcus aureus* > *E. coli*. The antibacterial experimental results of the compounds were compared with the standard antibacterial drug amikacin at the same concentration. The complex **2** exhibit greater antibacterial activities against *Pseudomonas aeruginosa, Stapholococcus aureus* and *E. coli* and the complex **1** is resistant to the bacteria *E. coli*. Complex **1** also shows low activity against *Pseudomonas aeruginosa, Stapholococcus aureus* than complex **2**. From the observed data it shows that the antibacterial activity depends upon the type of Zn(II) complexes and varies in the following order of the metal complexes **2 > 1**. The increased activity of the Zn(II) chelates can be explained on the basis of chelation theory. It is known that chelation tends to make the metal complexes act as more powerful and potent bactericidal agents, thus killing more of the bacteria than the ligand **(L)**. It is observed that, in a complex, the positive charge of the metal is partially shared with the donor atoms present in the ligands and there may be π-electron delocalization over the whole chelating [35]. This increases the lipophilic character of the metal chelate and favours its permeation through the lipoid layer of the bacterial

TABLE-4
MINIMUM INHIBITION CONCENTRATION (MIC) DATA OF THE SYNTHESIZED LIGAND (**L**) AND Zn(II) COMPLEXES **1-2** AGAINST GROWTH OF BACTERIA AND FUNGI

Compound	Microorganism (MIC) values					
	E. coli	*S. aureus*	*P. aeruginosa*	*C. albicans*	*A. niger*	*Macrophonia*
L	3	R	6	R	R	R
1	R	7	12	R	R	12
2	7	13	14	12	5	10

TABLE-5
PERCENTAGE OF CELL VIABILITY AND DEATH ANALYSIS IN DUPLICATE STUDY MODEL FOR LIGAND (L) AND Zn(II) COMPLEXES **1-2**

Compound	12.5 µg	25 µg	50 µg	100 µg
L	52.49	51.77	36.56	19.63
1	88.75	79.93	61.60	52.48
2	89.05	70.21	51.87	40.62
Cyclophosphamide	100	100	100	100

membranes. There are other factors which also increase the activity, which are solubility, conductivity and bond length between the metal and the ligand.

Cytotoxicity: The *in vitro* cytotoxic activities of the synthesized Schiff base ligand (**L**) and its Zn(II) complexes **1-2** are studied on human breast cancer cell lines (MCF-7) by applying the MTT colorimetric assay (Table-6). The calculated values, that is, the concentration (µg/mL) of a compound able to cause 50 % of cell death with respect to the control culture, are presented in Fig. 9. Cyclophosphamide is used as a reference compound. The MCF-7 cells are sensitive to the O-N-S Schiff base with the cell viability value of ligand **(L)** and their complexes **1-2** in µg. Taking into the account that thiosemicarbazone molecules exhibit cytotoxicity activity [36]. We have tested the ability of the ligands (**L**) and its Zn(II) complexes **1-2** inhibit the tumor cell growth. On comparison with the ligand and its Zn(II) complexes **1-2**, Ligand (**L**) show a higher value than Zn(II) complexes **1-2** which indicate the presence of bulky groups at position N(4) of the thiosemicarbazone moiety and heterocyclic bases 1,10-phenanthroline, 2,2′-bipyridine enhanced the anti-tumour activity [37-40]. The complexes **1** and **2** are cytotoxic to the breast cancer cell lines. Although the schiff base ligand (**L**) and its Zn(II) complexes

TABLE-6
IC_{50} VALUES FOR THE LIGAND (**L**) AND COMPLEXES **1-2**

Sample	IC_{50} values
Ligand (L)	25.5
[ZnL(phen)]	198.0
[ZnL(bpy)]	103.0

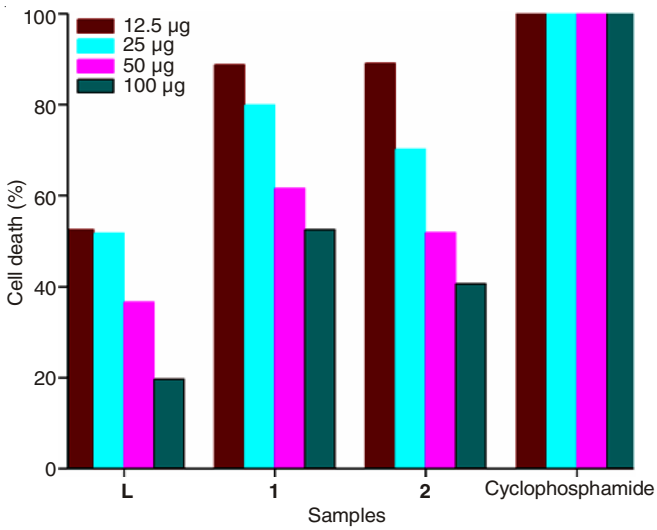


Fig. 9. Percentage of cell death against cyclophosphamide, ligand (L) and Zn(II) complexes **1-2**

are less effective than cyclophosphamide, the schiff base ligand (**L**) is more effective than the complexes **1-2**. The different activities are currently being investigated in terms of the mechanism of action of these compounds at the cellular level [41].

IC_{50} values (compound concentration that produces 50 % of cell death) were calculated for the free ligand (**L**) and the title complexes **1-2** against human breast cancer cell lines (MCF-7). The ligand (**L**) and its Zn(II) complexes **1-2** exhibited significant anticancer activity. It is worth nothing that the free ligand (**L**) showed a lower IC_{50} value than the Zn(II) complex **1-2**, indicating that the antitumor activity of the ligand (**L**) is greater than that of the complexes **1-2**. Further, as revealed by the observed IC_{50} values, the potency of the ligand and its Zn(II) complexes to kill the cancer cells follows the order **L > 2 >1**, revealing that it varies with the mode and extent of interaction of the complexes with cyclophosphamide.

Supplementary data

CCDC 964627 and CCDC 964628 contain the supplementary crystallographic data for **1** and **2**. These data can be obtained free of charge viahttp://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk.

ACKNOWLEDGEMENTS

Financial assistance received from the Department of Science and Technology, New Delhi, India [Grant No. SR/FTP/CS-40/2007] and University Grants Commission, New Delhi, India [F.No: 38-70/2009 (SR)] are gratefully acknowledged.

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