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# Ni(II) and Zn(II) complexes of 2-((thiophen-2ylmethylene)amino)benzamide: Synthesis, spectroscopic characterization, thermal, DFT and anticancer activities



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#### HIGHLIGHTS

- Synthesis and characterization of ligand and there metal complexes.
- TGA results shows complexes are more stable as compared to ligand.
- Ligand bind neutral tridentate around Ni(II) and neural bidentate around Zn(II).
- *In vitro* anticancer activity against cell line HepG2 and MCF-7.

# G R A P H I C A L A B S T R A C T

TGA curves of (red) ligand (L), (voilet)  $[Ni(L^1)(OAc)_2(H_2O)](H_2O)$ , (green)  $[Zn(L^1)(OAc)_2]$  complexes in nitrogen atmosphere.



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## ABSTRACT

The paper presents the synthesis of Ni(II) and Zn(II) complexes of general composition  $M(L)X_2$  and  $M(L)_2X_2$  (M = Ni(II), Zn(II),  $X = Cl^{-1}$ ,  $OAc^{-1}$ ) with Schiff base obtained through the condensation of 2-aminobenzamide with thiophene-2-carbaldehyde. The characterization of newly formed complexes was done by <sup>1</sup>H NMR, UV–VIS, TGA, IR, mass spectrophotometry and molar conductivity studies. The thermal studies suggested that the complexes are more stable as compared to ligand. In DFT studies the geometries of Schiff's base 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 a distorted octahedral geometry has been assigned for Ni(II) complexes and tetrahedral geometry for Zn(II) complexes. The effect of these complexes on proliferation of human breast cancer cell line (MCF-7) and human hepatocellular liver carcinoma cell line (HepG2) were studied and compared with those of free ligand.

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# Introduction

Benzamides derivatives show significant antibacterial, antifungal, analgesic, antihelmintic, anti-inflammatory, antimalar-

ial, antitumor and antiallergic activities [1–7] and are still one of the most common functional groups employed in the preparation of an aromatic ligand. The ambidentate nature of amide group leads to the formation of regioisomers when forming a metal complex, as it is possible to coordinate either through the nitrogen or the oxygen atoms. The N and O chelating sites present in the amide group is the indispensable building block of biologically vital poly-

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mers, for instance peptides and proteins play a noteworthy function in biochemistry and biology. Schiff bases are potential anticancer drugs and when administered as their metal complexes, the anticancer activity of these complexes is enhanced in comparison to the free ligand [8]. Similarly, the role of thiophene derivates in medicinal chemistry is very well known for their therapeutic applications. Metal complexes containing amide functional groups confirm a wide spectrum of biological activities [9–14] and are also used as drugs in medicinal fields. Very limited research has been carried out on the synthesis of transition metal complexes with oxygen and nitrogen donor Schiff base derivatives of 2-aminobenzamide. Recently, Seyed et al. have reported a novel one pot synthesis method for the synthesis of anthranilamide Schiff bases [16]. Mixed metal complexes of 2-aminobenzamide with Cu(II) in the presence of some amino acids have been prepared, characterized and tested for their antibacterial activities [17]. The ambidentate nature of amide group and their resistance to hydrolysis (which means ligand cleavage are less likely to occur) makes it a preferred subject of study. Therefore, it is worthwhile to carry out the synthesis and study the spectral properties of the Schiff base derived from 2-aminobenzamide and thiophene-2-carbaldehyde. The goal of the study presented here is to synthesize the Ni(II) and Zn(II) metal complexes of Schiff base produced from condensation of 2-aminobenzamide & thiophene-2-carbaldehyde and to provide a baseline of structural data using various spectroscopic techniques, their thermal behavior, DFT studies and anticancer

#### Experimental

#### 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).

#### Synthesis of ligand

The Schiff base L (Fig. 1) was prepared by refluxing in ethanol (25–30 mL) an equimolar mixture of 2-aminobenzamide and thiophene-2-carbaldehyde for 3–4 h. On cooling the reaction mixture overnight, white product was precipitated out. It was filtered off, washed with cold ethanol and dried under vacuum over  $P_4O_{10}$ .

## Synthesis of metal complexes

Hot ethanolic solution (15 mL) of metal salt (acetate or chloride) (1 mmol) were mixed with a hot ethanolic solution (20 mL) of the ligand L (1 mmol or 2 mmol). The reaction mixture was refluxed for 8-24 h at 80-85 °C. On keeping the resulting mixture overnight at 0 °C, the product was separated out, which was fil-





#### Table 1

activity.

Physical and analytical data of the metal complexes  $(C_1-C_8)$  and ligand (L).

Comp.	Color	Molecular formula	Molecular	m/z	Yield	Element	Elemental analysis (%) found (calc.)			Molar conductance	Melting Pt.
no.			mass		(%)	С	Н	Ν	M <sup>a</sup>	$(\Omega^{-1} \operatorname{cm}^2 \operatorname{mol}^{-1})$	(°C)
L	White	$C_{12}H_{10}N_2OS$	230	231	82	62.32	4.04	12.08	-	-	188
						(62.59)	(4.38)	(12.16)			
C <sub>1</sub>	Light green	$[Ni(L)(OAc)_2(H_2O)](H_2O)$	442	443	74	43.25	4.45	6.23	13.10	98	>280
		C <sub>16</sub> H <sub>20</sub> N <sub>2</sub> NiO <sub>7</sub> S				(43.37)	(4.55)	(6.32)	(13.25)		
C <sub>2</sub>	Green	$[Ni(L)_2](OAc)_2$	636	637	62	52.54	4.01	8.76	9.15	194	>280
		$C_{28}H_{26}N_4NiO_6S_2$				(52.77)	(4.11)	(8.79)	(9.21)		
C <sub>3</sub>	Shiny	$[Ni(L)(Cl)(H_2O)_2]Cl \cdot H_2O$	412	413	68	34.77	3.77	6.71	14.01	96	>280
	green										
		$C_{12}H_{16}Cl_2N_2NiO_4S$				(34.82)	(3.90)	(6.77)	(14.18)		
$C_4$	Light	$[Ni(L)_2](Cl)_2 \cdot 2H_2O$	624	625	56	46.12	3.74	8.90	9.26	218	>280
	green										
		$C_{24}H_{24}Cl_2N_4NiO_4S_2$				(46.03)	(3.86)	(8.95)	(9.37)		
C <sub>5</sub>	White	$[Zn(L)(OAc)_2]$	412	413	76	46.33	3.78	6.62	15.71	11	>280
		$C_{16}H_{16}N_2O_5SZn$				(46.45)	(3.90)	(6.77)	(15.80)		
C <sub>6</sub>	White	$[Zn(L)_2](OAc)_2$	642	643	72	52.01	4.03	8.56	10.13	197	>280
_		$C_{28}H_{26}N_4O_6S_2Zn$				(52.22)	(4.07)	(8.70)	(10.15)		
C <sub>7</sub>	White	$[Zn(L)(Cl)_2]$	364	365	68	39.13	2.69	7.23	17.72	14	>280
_		$C_{12}H_{10}Cl_2N_2OSZn$				(39.32)	(2.75)	(7.64)	(17.84)		
C <sub>8</sub>	White	$[Zn(L)_2](Cl)_2 \cdot H_2O$	612	613	64	46.54	3.25	9.02	10.56	208	>280
		$C_{24}H_{22}CI_2N_4O_3S_2Zn$				(46.88)	(3.61)	(9.11)	(10.63)		

<sup>a</sup> M = Ni(II), Zn(II).

tered off, washed with cold ethanol & ether and dried under vacuum over  $P_4O_{10}$ .

#### 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 Quattrro mass spectrometer equipped with an elctrospray ionization source in the mass range of m/z 100 to m/z 1000. IR spectra (CsBr) were recorded on FTIR spectrum 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.

#### DFT calculations

The DFT calculations were performed using the B3LYP three parameter density functional, which includes Becke's gradient exchange correction [18], the Lee, Yang, Parr correlation functional [19] and the Vosko, Wilk, Nusair correlation functional [20]. The gas phase geometries of Ligand L and metal complexes ( $C_1$ — $C_8$ ) were fully optimized with respect to the energy using the 6-31+g(d,p) basis set using the Gaussian 09W suite [21]. Net atomic charges had been obtained using natural bond orbital (NBO) analysis of Weinhold and Carpenter [22].

#### In vitro studies (cell culturing)

The cell lines HepG2 (liver hepatocellular carcinoma) and MCF-7 (human breast adenocarcinoma) were cultured as monolayers and maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-gluta-mine, 100 U/ml penicillin and 100 mg/ml streptomycin in a humid-



Fig. 3. <sup>1</sup>H NMR spectra of ligand L.

ified atmosphere with 5% CO<sub>2</sub> at 37 °C in T-75 flasks and were sub cultured twice a week. For the assays, cells ( $2 \times 10^3$  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 L, (C<sub>1</sub>–C<sub>8</sub>) for 48 h. The cell proliferation of the control (untreated cells) was fixed to 100%. After completion of incubation period, 20 µl MTT (5 mg/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.

### **Results and discussion**

The Schiff base L (Fig. 1) was prepared by refluxing in ethanol (25–30 mL) an equimolar mixture of 2-aminobenzamide and thiophene-2-carbaldehyde. The structure of Schiff base thus formed was established by IR, <sup>1</sup>H NMR and Mass spectrophotometry.

The synthesized Schiff base was further used for the complexation with Ni(II) and Zn(II) metal ions, using the following metal salt: Ni(OAc)<sub>2</sub>.4H<sub>2</sub>O for complexes (C<sub>1</sub>) & (C<sub>2</sub>), NiCl<sub>2</sub>.6H<sub>2</sub>O for complexes  $(C_3)$  &  $(C_4)$ , Zn $(OAc)_2$ .2H<sub>2</sub>O for complexes  $(C_5)$  &  $(C_6)$  and  $ZnCl_2$  for complexes ( $C_7$ ) & ( $C_8$ ). 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 & DMSO. The molar conductance of the soluble complexes in DMF showed values indicating that complexes  $C_5 \& C_7 (11-14 \text{ ohm}^{-1} \text{ cm}^2 \text{ mol}^{-1})$  [16] are nonelectrolytes and complexes  $(C_1 - C_4)$ ,  $(C_6) \& (C_8) (96 - 218 \text{ ohm}^{-1})$ cm<sup>2</sup> mol<sup>-1</sup>) are electrolytic in nature. The elemental analysis data of the ligand are in agreement with the structure of the ligand. The elemental analysis data of the ligand (L) and metal complexes  $(C_1 - C_8)$  are given in Table 1.

#### Mass spectra

Table 3

The ESI mass spectrum of ligand showed a molecular ion peak at m/z = 231.2 amu corresponding to  $[M + H]^+$ , which confirms the proposed formula (Fig. 2). It also shows a series of peaks at 233.2, 214, 186, and 130 corresponding to various fragments. The

 Table 2

 Important infrared spectral bands (cm<sup>-1</sup>) and their assignments.

intensities of these peaks give the idea of the stability of the fragments. ESI mass spectrum of the Ni(II) and Zn(II) complexes showing the fragmentation model beside the m/z peak value are given in Table 1.

#### <sup>1</sup>H NMR spectra

The <sup>1</sup>H NMR spectra have been recorded for ligand L and Zn(II) complexes. The NMR spectra of the Zn(II) complexes indicated a shift of electron density from ligand to metal. The ligand L showed characteristic azomethine proton singlet at  $\delta$  8.45 ppm (Fig. 3). The characteristic signal, due to azomethine proton deshielded in the spectra of metal complexes, suggest a coordination of azomethine nitrogen atom. The azomethine proton of all zinc complexes appears as a singlet between 8.36 and 8.28 ppm. In addition, CH<sub>3</sub> hydrogen atoms of the acetate group appear as a singlet between 2.39 and 2.18 ppm for zinc complexes. The aromatic region is a set of doublets, triplets and multiplets in the range 7.75–6.67 ppm for the complexes while it is 7.61–6.68 ppm for the ligand. All the proton peaks were found to be in the expected regions.

### IR spectra

The IR spectrum of the ligand was compared with those of the metal complexes in order to confirm the binding mode of the Schiff base ligand to the corresponding metal ion. IR spectral data of the ligand L and its corresponding metal complexes  $(C_1 - C_8)$  are shown in Table 2. Peak corresponding to v(C=0) stretching vibrations was absent in IR spectra of L and, instead, a new band assigned to azomethine v(HC=N) linkage appeared at 1612 cm<sup>-1</sup> confirming the formation of Schiff base (Fig. 1). Band due to v(C=N) stretching frequencies shift towards lower side  $(1590-1578 \text{ cm}^{-1})$  in all complexes indicating that the azomethine nitrogen atom was coordinated to metal ion [23]. This is also confirmed by the appearance of new band in spectra of metal complexes in the range of 488-498 cm<sup>-1</sup>, which has been assigned to the v(M-N) bond [23]. The v(C=O) stretching vibration of amide group at 1670 cm<sup>-1</sup> free ligand shifts to a lower side (1668–1675 cm<sup>-1</sup>) in all metal complexes, indicating the coordination of the ligand through the oxygen of amide group [15]. The same is also supported by the appearance of band in region of 534–540 cm<sup>-1</sup>, which corresponds to v(M–O) bond. A noteworthy point in the IR spectra of all metal

Compound	$\nu(HC=N)$	v(C—S—C)	v(C=0)	v(M—N)	v(M—O)	v(M—S)	v(M—Cl)
L	1612	830	1685	-	-	-	-
C <sub>1</sub>	1590	842	1672	498	540	334	-
C <sub>2</sub>	1584	838	1668	490	536	339	-
C <sub>3</sub>	1583	845	1671	494	535	346	290
$C_4$	1578	840	1665	492	532	342	297
C <sub>5</sub>	1581	829	1665	488	539	-	-
C <sub>6</sub>	1585	830	1673	491	538	-	-
C <sub>7</sub>	1587	828	1672	490	537	-	315
C <sub>8</sub>	1581	830	1675	492	534	-	307

Magnetic moment, electronic spectral bands (cm<sup>-1</sup>) and ligand field parameters of Ni(II) complexes.

Complex	$\mu_{ m eff}$ BM	$\lambda_{max}$ (cm <sup>-1</sup> )	$\lambda_{\max}$ (cm <sup>-1</sup> )		$Dq (cm^{-1})$	<i>B</i> (cm <sup>-1</sup> )	β	LFSE (kJ mol <sup>-1</sup> )
		<i>v</i> <sub>1</sub>	<i>v</i> <sub>2</sub>	<i>v</i> <sub>3</sub>				
C <sub>1</sub>	2.85	11,764	16,260	25,641	1176	440	0.42	168
$C_2$	2.94	11,520	16,000	24,570	1152	400	0.38	165
C <sub>3</sub>	2.97	11,428	15,748	24,937	1142	427	0.41	164
C <sub>4</sub>	2.91	10,869	16,051	23,529	1086	465	0.45	156

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Thermal analysis data for the Schiff's base and its complexes.

Comp. no.	Molecular formula	Stages	Temp. (°C)	Decomposition species	Residual species	Mass loss	(%)
						Found	Calc.
L	$C_{12}H_{10}N_2OS$	1st	150-270	C <sub>4</sub> H <sub>3</sub> S	-	100	100
		2nd	270-460	$C_8H_7N_2O$			
C <sub>1</sub>	$[Ni(L)(OAc)_2(H_2O)](H_2O)$	1st	80-130	Loss of H <sub>2</sub> O	NiO	16.8	16.74
	C <sub>16</sub> H <sub>20</sub> N <sub>2</sub> NiO <sub>7</sub> S	2nd	130-320	C <sub>8</sub> H <sub>11</sub> O <sub>5</sub> S			
		3rd	320-540	$C_8H_7N_2$			
C <sub>2</sub>	$[Ni(L)_2](OAc)_2$	1st	135-230	$C_4H_6O_4$	NiO	11.5	11.63
	$C_{28}H_{26}N_4NiO_6S_2$	2nd	230-410	$C_8H_6S_2$			
		3rd	410-555	$C_{16}H_{14}N_4O$			
C <sub>3</sub>	$[Ni(L)(Cl)(H_2O)_2]Cl \cdot H_2O$	1st	70-145	HCl, 1/20 <sub>2</sub>	NiO	18.65	17.96
	C <sub>12</sub> H <sub>16</sub> Cl <sub>2</sub> N <sub>2</sub> NiO <sub>4</sub> S	2nd	145-360	C <sub>5</sub> H <sub>9</sub> O <sub>2</sub> SNCl			
		3rd	360-560	C <sub>7</sub> H <sub>6</sub> N			
$C_4$	$[Ni(L)_2](Cl)_2 \cdot 2H_2O$	1st	70-195	Loss of 2HCl, $H_2O$ , $1/2O_2$	NiO	12.74	11.85
	C24H24Cl2N4NiO4S2	2nd	195-390	C <sub>8</sub> H <sub>6</sub> S <sub>2</sub> (2 thiophene ring)			
		3rd	390-550	C <sub>16</sub> H <sub>14</sub> N <sub>4</sub> O (organic moiety)			
C <sub>5</sub>	$[Zn(L)(OAc)_2]$	1st	125-360	C <sub>8</sub> H <sub>9</sub> O <sub>4</sub> S	ZnO	19.7	19.41
	$C_{16}H_{16}N_2O_5SZn$	2nd	360-600	$C_8H_7N_2$			
C <sub>6</sub>	$[Zn(L)_2](OAc)_2$	1st	110-235	$C_4H_6O_4$	ZnO	12.47	12.46
	$C_{28}H_{26}N_4O_6S_2Zn$	2nd	235-390	$C_8H_6S_2$			
		3rd	390-550	$C_{16}H_{14}N_4O$			
C <sub>7</sub>	$[Zn(L)(Cl)_2]$	1st	120-375	$C_4H_3Cl_2S$	ZnO	22.04	21.97
	C <sub>12</sub> H <sub>10</sub> Cl <sub>2</sub> N <sub>2</sub> OSZn	2nd	357-550	$C_8H_7N_2$			
C <sub>8</sub>	$[Zn(L)_2](Cl)_2 \cdot H_2O$	1st	60-110	Loss of H <sub>2</sub> O	ZnO	13.21	13.07
	$C_{24}H_{22}Cl_2N_4O_3S_2Zn$	2nd	110-380	Loss of C <sub>8</sub> H <sub>6</sub> OCl <sub>2</sub> S <sub>2</sub>			
		3rd	380-545	C <sub>16</sub> H <sub>14</sub> N <sub>4</sub> (organic moiety)			



Fig. 4. Electronic spectra of Ni(II) complexes.

complexes is the position of v(N—H) stretching vibration of NH<sub>2</sub> of amide group. Sharp bands at 3120–3123 cm<sup>-1</sup> for all complexes have been found to be unaltered and are in the same position as in ligand L, which indicates the non-involvement of amido-N atom in complex formation [24]. Two new bands appeared in complexes  $C_1 \& C_5$  at 1570–1598 cm<sup>-1</sup> and 1366–1386 cm<sup>-1</sup> are ascribed to v(COO<sup>-</sup>)<sub>as</sub> and v(COO<sup>-</sup>)<sub>s</sub> of carboxylato group and the magnitude of  $\Delta v$  values (204–212 cm<sup>-1</sup>) suggests the coordination of carboxylato group of the acetate molecule is in monodentate fashion [24,25].

A broad band appeared in complexes  $C_1 \& C_3$  at 3300– 3400 cm<sup>-1</sup> and 825–838 cm<sup>-1</sup> region are attributed to the stretching and rocking mode of v(OH) vibration of coordinated water molecule [24]. Also, the specific band of v(C–S–C) ring vibration at 830 cm<sup>-1</sup> in free ligand shifts to (838–845 cm<sup>-1</sup>) in all Ni complexes [26]. This is also supported by the appearance of new band



**Fig. 5.** TGA curves of (red) ligand (L), (voilet)  $[Ni(L^1)(OAc)_2(H_2O)](H_2O)$ , (green)  $[Zn(L^1)(OAc)_2]$  complexes in nitrogen atmosphere. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



**Fig. 6.** Geometry optimized structures of (a) ligand L, (b) complex  $C_1$ , (c) complex  $C_3$ , (d) complex  $C_5$ , (e) complex  $C_7$  and (f) complex  $C_6$  (Color Code: H = White, C = Gray, N = Blue, O = red Ni = Silver Gray, Zn = Gray). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

in range of 334–346 cm<sup>-1</sup>, which has been assigned to v(M-S) bond. However, there is no change in the band for v(C-S-C) ring vibration in zinc complexes, which indicates that S of thiophene coordinates to complexes of Ni only. Based on these observations it may be appreciated that the ligand L coordinate neutral tridentately around the Ni(II) ion and neutral bidendately around the Zn(II) ion.

(e)

## Magnetic moments

The magnetic moment observed for Ni(II) complexes lies in the range 2.85–2.97 B.M suggesting a distorted octahedral geometry [27]. Similarly, the Zn(II) complexes are found to be diamagnetic

in nature. The observed magnetic moments of Ni(II) complexes are given in Table 3.

(f)

## Electronic Spectra

The electronic absorption spectra of the Ni(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. Spectra of all Ni(II) complexes are very similar (Fig. 4) and the distorted octahedral geometry around the metal ion has been confirmed from the nature of electronic transitions [28,29]. The electronic spectra of Ni(II) complexes display bands at 10,869–11,764 cm<sup>-1</sup>, 15,384–16,260 cm<sup>-1</sup> and 23,529–25,641 cm<sup>-1</sup>, which may be assigned due to <sup>3</sup>A<sub>2g</sub>

 $(F) \rightarrow {}^{3}T_{2g}(F), {}^{3}A_{2g}(F) \rightarrow {}^{3}T_{1g}(F)$  and  ${}^{3}A_{2g}(F) \rightarrow {}^{3}T_{1g}(P)$ , transitions respectively, in approximately octahedral environment. The ligand field parameters (Dq, *B*,  $\beta$ , LFSE) have also been calculated for Ni(II) complexes by using band-fitting equation. These parameters indicate significant covalent character of the metal ligand bonds.

## Thermal analysis

Thermal behavior of the complexes has been studied using thermogravimetric analysis from ambient temperature to 800 °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. Typical TG curves for Ni(II) and Zn(II) complexes ( $C_1 \& C_5$ ) in comparison to ligand L are presented in Fig. 5. The temperature ranges and percentage mass losses of the decomposition reaction are given in Table 4 together with evolved moiety and the theoretical percentage mass losses. The TG curve of ligand L, refers to two stages of mass losses at temperature ranges from 150 to 460 °C. These stages involved mass losses of 100.0% and

mass losses may be due to the successive losses of  $C_{12}H_{10}N_2OS$  at the given temperature ranges. All metal complexes undergo decomposition in two to three stages. In the first stage, a peak corresponding to the loss of the uncoordinated water molecule or due to the loss of coordinated or uncoordinated anions (chloride or acetate) was observed. The coordinated anion (acetate/chloride) with subsequent removal of the thiophene ring was observed in the second stage. In the third stage, there was a loss in mass corresponding to the decomposition of primary 2-aminobenzamide ligand and as a final stage; it left air stable metal oxide as a residue.

### Geometry optimization and NBO analysis

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, Ni(II) complexes and Zn(II) complexes are shown in Fig. 6. The numbering scheme for ligand L, Ni(II) and Zn(II) complexes are given in Scheme 1. The molecular structure of Ni(II) complexes show a distorted octahedral geometry around the Ni(II) center as revealed from the calculated bond lengths and bond angles (Table



Scheme 1. Numbering scheme of the optimized structures (a) ligand L, (b) complex C1 & C3 and (c) complex C5 & C7.

Table 5											
Optimized	geometry of	L and its metal	complexes	(bond	length in	Angstroms	and	bond	angle i	n degr	ree)

Parameters <sup>a</sup>	Ligand (L)	Complex C <sub>1</sub> <sup>b</sup>	Complex C <sub>3</sub> <sup>c</sup>	Complex C <sub>5</sub> <sup>d</sup>	Complex C7 <sup>e</sup>
M-N <sub>1</sub>	-	1.86	1.87	2.14	2.15
М—О	-	2.69	2.42	2.19	2.13
M—S	-	2.71	2.54	-	-
M—X	-	1.91	2.29	2.1	2.36
M—X'	-	-	-	2.13	2.25
M—Y	-	2.04	1.92	-	-
M—Y′	-	2.12	1.87	-	-
C7-0	1.22	1.27	1.25	1.27	1.26
C <sub>6</sub> —N <sub>1</sub>	1.40	1.48	1.50	1.49	1.42
$N_1 - C_8$	1.27	1.35	1.34	1.32	1.30
C <sub>8</sub> -C <sub>9</sub>	1.54	1.45	1.42	1.42	1.44
C <sub>9</sub> —S	1.67	1.77	1.79	1.76	1.82
OMY	-	63.73	72.4	-	-
OMN <sub>1</sub>	-	96.20	95.2	-	-
YMX	-	85.25	87.4	-	-
XMS	-	96.57	94.2	-	-
SMY'	-	86.21	83.5	-	-
N <sub>1</sub> MY′	-	91.67	92.2	-	_
XMX'	-	-	-	112.1	114.2
XMO	-	-	-	109.8	111.2
XMN <sub>1</sub>	-	-	-	107.3	103.3
N1MX′	-	-	-	110.1	109.2

L = 2-((thiophen-2-ylmethylene)amino)benzamide.

<sup>a</sup> See Scheme 1 for numbering.

<sup>b</sup> [M(L)(YY')X], M = Ni, X =  $H_2O$ , Y = Y' = OAc.

<sup>c</sup> [M(L)(YY')X], M = Ni, X = Cl, Y = Y' = H<sub>2</sub>O.

<sup>d</sup> [M(L)(XX')], M = Zn, X = X' = OAc.

 $^{e}$  [M(L)(XX')], M = Zn, X = X' = Cl.

5). The ligand L interacts with metal ion through azomethine nitrogen, oxygen of amide group and through sulfur of thiophene moiety in a tridendate fashion. The thiophene moiety is bent out of the coordination plane and move far from Ni(II) ion, which results in the elongation of Ni-S bond length. Similarly, the C=O bond of the amide group moves out of the plane, which results in increase of Ni-O bond length. The Ni-S and Ni-O bond lengths in complexes C<sub>1</sub> and C<sub>3</sub> lies in the range of 2.54–2.71 Å and 2.42–2.69 Å respectively. This elongation in Ni-S bond length caused a distortion from the regular octahedral geometries. It is due to distortion that bond angle O–Ni–Y reduces from 90° to 63.73° in complex C<sub>1</sub> and  $72.4^{\circ}$  in complex C<sub>3</sub> respectively. The two axial positions in complex  $C_1$  are occupied by acetate ion, while at the sixth position a water molecule is coordinated. Similarly, in complex C<sub>3</sub> the two axial positions are occupied by water molecule and chloride ion coordinates at the sixth position. Attempt to optimize the complexes C<sub>2</sub> & C<sub>4</sub> was not successful.

Similarly, the Zn(II) complexes were fully optimized in gas phase and the molecular structure revealed a tetrahedral environment around the Zn(II) ion. The ligand L interacts with metal ion in a bidendate fashion through azomethine nitrogen and oxygen of amide group. The remaining two positions are occupied by anions (acetate/chloride) in complexes  $C_5 \otimes C_7$ . The complex  $C_6$ , which is  $M(L)_2$  type has all the four position occupied by nitrogen and sulfur atoms of the ligand L. The bond angles in the Zn(II) complexes are found near to the approximate value. The X–Zn–X', X–Zn–N<sub>1</sub>, X–Zn–O, N<sub>1</sub>–Zn–X' bond angles in complex  $C_5$  are 112.1°, 101.1°, 109.8° and 110.1° respectively. Similarly the calculated bond angles X–Zn–X', X–Zn–N<sub>1</sub>, X–Zn–O, N<sub>1</sub>–Zn–X' in complex  $C_7$  are 114.4°, 103.3°, 111.2° and 109.2° respectively. Natural Bond Orbital analysis was done on complexes  $C_1 \& C_7$ and the results revealed that the electronic configuration of Ni in complex  $C_1$  is: [core]4s<sup>0.34</sup>3d<sup>8.45</sup>4p<sup>0.32</sup>4d<sup>0.03</sup>5p<sup>0.06</sup>, 18 core electrons, 9.20 valence electrons, and 0.016 Rydberg electrons with 27.216 electrons as a total electrons that is in agreement with the calculated natural charge (+0.780 *e*) on nickel atom. Similarly, the electronic configuration of Zn in  $C_7$  is [core]4s<sup>0.61</sup>3d<sup>9.90</sup>4p<sup>0.98</sup> 5p<sup>0.01</sup>: 18 core electrons, 11.5 valence electrons (on 4s, 3d, 4p and 5p atomic orbitals), and 0.015 Rydberg electrons with 29.511 as a total electrons and +0.4882 *e* as a natural charge. The calculated occupancies (electron densities) on NBO orbitals, hybrids on atoms and polarities of bonds between atoms are given in Table 6.

We also calculated the energies for Highest occupied molecular orbital (HOMO) and Lowest occupied molecular orbital (LUMO). The calculated energies of the HOMO and LUMO for ligand are -0.226 hartree and 0.017 hartree, respectively. The HOMO of the ligand is concentrated on carbonyl oxygen of amide group, while LUMO is concentrated on  $C_{11}=C_{12}$  bond of thiophene moiety. The 0.020 hartree isovalue contours for the HOMO and LUMO are displayed in Fig. 7. On the basis of the above discussion following (Fig. 8) structures can be proposed for the synthesized complexes.

#### Cell viability determination

The *in vitro* cytotoxicity of the ligand L and metal complexes  $(C_1-C_8)$  on human cell lines HepG2 and MCF-7 was determined by a MTT based assay. The results are expressed as the percentage of viable cells with respect to the control and are represented in Table 7. The *in vitro* screening revealed that ligand L and complexes

Table 6

Occupancy (e) and polarity (%) of natural bond orbitals (NBOs) and hybrids calculated for complex	K C <sub>1</sub>	and complex $C_7$ .
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Molecule <sup>a</sup>	NBO orbital <sup>a,b</sup>	Occupancy (e)	Polarity (%) <sup>c</sup>	NBO hybrid	AO (%) <sup>d</sup>
$[Ni(L)(OAc)_2(H_2O)]H_2O$	σ(Ni—Y)	1.812	11.64 (Ni)	pd <sup>1.61</sup>	s(0.92) p(41.26) d(61.12)
			88.36 (Y)	sp <sup>8.6</sup>	s(10.4) p(89.6)
(Complex C <sub>1</sub> )	$\sigma(Ni-Y')$	1.756	4.69 (Ni)	sp <sup>2.74</sup> d <sup>1.44</sup>	s(19.32) p(52.91) d(27.76)
			95.31 (Y')	sp <sup>5.5</sup>	s(15.3) p(84.2) d(0.5)
	$LP(N_1)$	1.797		sp <sup>3.73</sup>	s(21.13) p(78.87)
	LP(O)	1.936		sp <sup>0.69</sup>	s(59.34) p(40.66)
	LP(S)	1.797		sp <sup>3.73</sup>	s(21.13) p(78.87)
	LP(X)	1.986		Sp <sup>5.47</sup>	s(15.46) p(84.54)
$[Zn(L)Cl_2]$	$\sigma(Zn-X')$	1.987	15.57 (Zn)	sp <sup>1.47</sup> (Zn)	s(40.22) p(59.09) d(0.69)
			84.43 (X')	sp <sup>1.85</sup> (X')	s(35.06) p(64.94)
(Complex C <sub>7</sub> )	σ(Zn—X)	1.984	15.67 (Zn)	sp <sup>1.36</sup> (Zn)	s(42.06 p(57.15) d(0.02)
			84.33 (X)	sp <sup>2.01</sup> (X)	s(33.26) p(66.74)
	$LP(N_1)$	1.893		sp <sup>4.40</sup>	s(18.53) p(81.47)
	LP(O)	1.808		sp <sup>3.46</sup>	s(22.44) p(77.56)

Calculations performed at the B3LYP/6-31+g(d,p) level of theory.

<sup>a</sup> See numbering Scheme 1 [Complex  $C_1$ : X = O(H<sub>2</sub>O), Y = Y' = O(OAc), Complex  $C_7$ : X = X' = Cl].

<sup>2</sup> LP(N), LP(O), LP(S) are the valence lone pair orbital present on nitrogen, oxygen and sulfur atom.

<sup>c</sup> Values for the A-B sigma molecular orbital.

<sup>d</sup> Percentage contribution of atomic orbitals in NBO hybrid.



Fig. 7. Plot of HOMO and LUMO of ligand L



Fig. 8. Proposed structure of the newly obtained metal complexes.

## Table 7

Effect of a ligand L, and series of metal complexes ( $C_1-C_8$ ) at two concentrations of 1 and 10  $\mu$ M on the HepG2 and MCF-7 cell proliferation.<sup>a</sup>

Compound	Survival cell	fraction (%) at 1 $\mu$ M	Cell growth inhibition (%) at 1 $\mu M$		Survival cell fraction (%) at 10 $\mu M$		Cell growth inhibition (%) at 10 $\mu M$	
	HepG2	MCF-7	HepG2	MCF-7	HepG2	MCF-7	HepG2	MCF-7
Control	100 ± 2	100 ± 2	-	-	100 ± 2	100 ± 2	-	-
L	98 ± 2	99 ± 2	2	1	97 ± 3	95 ± 5	3	5
C <sub>1</sub>	97 ± 5	98 ± 2	3	2	90 ± 10	92 ± 8	10	8
$C_2$	96 ± 4	93 ± 7	4	7 <sup>b</sup>	87 ± 13	85 ± 15	13	15
C <sub>3</sub>	97 ± 3	96 ± 4	3	4	84 ± 16	72 ± 21	16 <sup>b</sup>	28 <sup>b</sup>
$C_4$	92 ± 8	94 ± 6	8 <sup>b</sup>	6	52 ± 48	58 ± 42	48 <sup>b</sup>	42 <sup>b</sup>
C <sub>5</sub>	98 ± 2	99 ± 1	2	1	91 ± 9	86 ± 14	9	14
C <sub>6</sub>	95 ± 5	97 ± 3	5	3	$80 \pm 10$	83 ± 13	20 <sup>b</sup>	17 <sup>b</sup>
C <sub>7</sub>	98 ± 2	97 ± 3	2	3	86 ± 14	81 ± 19	14	19 <sup>b</sup>
C <sub>8</sub>	$94 \pm 6$	95 ± 5	6	5	72 ± 28	61 ± 39	28 <sup>b</sup>	39 <sup>b</sup>

<sup>a</sup> The results are expressed as the percentage of viable cells with respect to the control and are presented as mean ± SD.

<sup>b</sup> Significantly different from the control.



**Fig. 9.** Ligand L and complexes  $(C_1-C_8)$  inhibited the cell viability of HEP-G2 & 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 ± S.D.).

 $(C_1-C_8)$  has very less effect on % inhibition on cell proliferation on cell lines HepG2 & MCF-7 at concentration of 1  $\mu$ M. But at concentration of 10  $\mu$ M complexes C<sub>3</sub>, C<sub>4</sub>, C<sub>6</sub> & C<sub>8</sub> shows a significant increase in % inhibition on cell proliferation on cell line HepG2 (Fig. 9). Similarly, the complexes C<sub>3</sub>, C<sub>4</sub>, C<sub>6</sub>, C<sub>7</sub> & C<sub>8</sub> shows a significant increase in % inhibition on cell proliferation on cell line MCF-7.

## Conclusion

The analytical and physico-chemical analyses confirmed the composition and the structure of the newly obtained complex combinations. On the basis of the spectral studies Ni(II) complexes were found to have a distorted octahedral geometry whereas Zn(II) complexes has a tetrahedral geometry. In all Ni(II) complexes the ligand L acts as neutral tridentate around the metallic ion, while in Zn(II) complexes the ligand act as neutral bidentate. 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. Results of the antitumor activity screening indicated that the ligand L has very less effect on the % inhibition on cell proliferation at the two tested concentrations on HepG2 & MCF-7. Interestingly, the complex C<sub>4</sub> shows a 48% inhibition on cell proliferation against HepG2, whereas complexes C<sub>4</sub> and C<sub>8</sub> greatly reduce the malignant MCF-7 cell growth by 42% & 29% respectively. Further structural optimization studies might thus represent a rationale for further investigation.

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