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Synthesis, spectral, antimicrobial and antitumor assessment of Schiff base derived from 2-aminobenzothiazole and its transition metal complexes

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ABSTRACT

N-(thiophen-2-ylmethylene)benzo[*d*]thiazol-2-amine Schiff base (L) derived from 2aminobenzothiazole and 2-thiophenecarboxaldehyde was synthesized and characterized using elemental analysis, IR, mass spectra, ¹H NMR and UV-vis spectra. Its complexes with Cu(II), Fe(III), Ni(II) and Zn(II) were prepared and isolated as solid products and characterized by elemental and thermal analyses, spectral techniques as well as magnetic susceptibility. The IR spectra showed that the Schiff base under investigation behaves as bidentate ligand. The UV-vis spectra and magnetic moment data suggested octahedral geometry around Cu(II) and Fe(III) and tetrahedral geometry around Ni(II) and Zn(II). In view of the biological activity of the Schiff base and its complexes, it has been observed that the antimicrobial activity of the Schiff base increased on complexation with the metal ion. In vitro antitumor activity assayed against five human tumor cell lines furnished the significant toxicities of the Schiff base and its complexes.

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1. Introduction

Schiff bases form an interesting class of ligands that has enjoyed popular use in the coordination chemistry of transition, innertransition and main group elements [1,2]. Schiff bases have been reported to show a variety of biological actions by virtue of the azomethine linkage, which is responsible for various antibacterial, antifungal, herbicidal and clinical activities [3-5]. Polyfunctional ligands based on benzazole derivatives as 2-aminobenzothiazole, 2-aminothiazole and 2-aminobenzoxazole are relevant due to their biological activity as fungicides, antibiotics, pesticides and neuroprotectors [6-8]. Thiazole derivatives are widely used in the synthesis of the medicinal products such as sulphathiazole [9]. Thiazole and its derivatives play significant part in the animal kingdom. Vitamin B1, Penicillin and coenzyme cocarboxylase contain the thiazole ring. Benzothiazole ring is present in various marine or terrestrial natural compounds which have useful biological activities [10]. Transition metal ions play a vital role in a vast number of widely different biological processes [11]. Moreover, the activity can be enhanced when the biologically active ligand is coordinated to a transition metal ion. Metal complexes of Schiff bases derived from heterocyclic compounds containing nitrogen, sulfur and/or oxygen as ligand atoms are of interest as simple structural models of more complicated biological systems [12]. In the present work, we report the synthesis, characterization and biological activities of Cu(II), Fe(III), Ni(II) and Zn(II) complexes containing N-(thiophen-2-ylmethylene)benzo[d]thiazol-2-amine Schiff base derived from 2-aminobenzothiazole and 2-thiophenecarboxaldehyde. The antimicrobial assessment against the fungal and the bacterial microorganisms were studied. The cytotoxicities against five human cancer cell lines were examined.

2. Experimental

2.1. Materials and instrumentation

All chemicals used in this study were of the purest grade and purchased from Aldrich and used without further purification. Elemental analyses were performed on a Perkin-Elmer 2400 automatic elemental analyzer. Mass spectra were recorded using Finnigan MAT 8200 mass spectrophotometer. Infrared spectra were recorded on a Bruker vector 22 spectrophotometer applying KBr discs in the range of 4000–400 cm⁻¹. Electronic absorption spectra in the visible and ultraviolet regions were measured on a Shimadzu 3101 PC spectrophotometer as a Nujol mull matrix. The magnetic susceptibility was determined with a Magnetic susceptibility balance 436 Devon Park Drive (USA) using Hg[Co(SCN)₄] as calibrant. Nuclear magnetic resonance spectra were carried out on a Bruker DPX 200 spectrometer, using deuterated dimethylsulfoxide

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(DMSO-*d*₆) as a solvent. The thermogravimetric analysis (TGA) was performed on Shimadzu AT-50 thermal analyzer in the range 25 °C up to 800 °C with heating rate 10 °C/min in a nitrogen atmosphere.

2.2. Synthesis of

N-(thiophen-2-ylmethylene)benzo[d]thiazol-2-amine Schiff base and their complexes

The Schiff base (L) was prepared by the condensation reaction of 2-aminobenzothiazole (0.15 g; 1 mmol) and 2-thiophenecarboxaldhyde (0.12 g; 1.1 mmol) dissolved in 30 mL absolute EtOH, in the presence of few drops of piperidine. The resulting mixture was stirred well, refluxed for 7 h. The mixture was concentrated to its half volume then cooled. Petroleum ether was added to the reaction mixture dropwise until a product began to precipitate. The formed product was filtered off, washed several times with EtOH, recrystalized from EtOH.

2.3. Synthesis of metal complexes

Cu(II) and Fe(III) complexes were prepared by mixing an ethanolic (20 mL) solution of L (2 mmol) with metal salts (1 mmol), namely, CuCl₂·2H₂O and FeCl₃·6H₂O in EtOH (10 mL) with continuous stirring. The mixture was then refluxed for 6–8 h. The solution was concentrated. The solid products obtained were filtered, washed with cold ethanol and dried in vacuum. Ni(II) and Zn(II) complexes were prepared by mixing the Schiff base (2 mmol) with metal salts, namely, Ni(AcO)₂·4H₂O and Zn(AcO)₂, in the solid state and melted in oil bath for 6–8 h. After cooling, the solid was finely ground and washed with diethyle ether.

2.4. Antimicrobial assessment

The fungal microorganisms chosen to test the antimicrobial activity of the synthesized compounds were Candida albicans, Aspergillus flavus, Asperigllus niger and Fusarium oxysporum. On the other hand, Escherchia coli, Klebsielia pneumoniae and Staphylococcus aureus were used as the bacterial microorganism. Nutrient agar was used for growing bacterial cultures. On the other hand, Sabouraud dextrose agar was used for the growth of C. albicans and Cazpek's dox agar was used for the growth of A. flavus and A. *niger*. The tested compounds were water insoluble; the cut plug method was employed to determine the antimicrobial activity of the synthesized compounds. A 0.5 mL spores or cell suspension were prepared and counted, then mixed with 9.5 mL of the corresponding sterilized melted media, and left to solidify at room temperature. Wells are made in seeded agar plate with different organisms under investigation by cork borer with diameter 8 mm and each one was filled with 5 mg of the tested compounds. All the plates were incubated at proper temperature (37 °C for S. aureus, E. coli, K. pneumonia, C. albicans and 27 °C for A. niger and A. flavus) for 24 h in case of bacteria and 72 h in case of fungi plates, and then the inhibition zone diameters were measured [13]. Nystatin used as antifungal control and ceftriaxone used as antibacterial control for comparing the results with nystatin and ceftriaxone inhibition zone.

2.4.1. Measuring the organisms surviving ratio

A suspension of each standard organism (0.5 mL) was mixed with 9.5 mL of the 10-fold diluted corresponding media broth in a sterile test tube containing the tested compound. The amount of compound present was such to give 5, 10, 15, and 20 mg/mL in the final solution. The seeded tubes were then shaken at 250 rpm (round/minute) at shaker incubator and incubated at proper temperature overnight. The solutions were then diluted 2-fold and 0.1 mL of each solution was spread onto agar plates of corresponding media. Controls without the compounds were prepared and the plates were incubated at proper temperature (as mentioned above) for 24 h, and then the numbers of colony forming units (CFU) were recorded. The surviving ratio (M/C) was calculated for each chosen organism at different compound concentrations against that of the control, where M is the number of organisms in presence of different compound concentrations and C is the number of organisms in absence of compound (control).

2.5. Antitumor activity

In vitro potential cytotoxicity of the complexes was tested using the method of Skehan et al. [14]. Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the complexes to allow attachment of cell to the wall of the plate. Different concentrations of the compounds under test (0, 1, 2.5, 5, 10, 15, 20 and 50 µg/mL) were added to the cell monolayer, triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 °C and in atmosphere of 5% CO₂. After 48 h, cells were fixed, washed and stained with Sulfo-Rhodamine-B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line after the specified compound.

3. Results and discussion

The level of the purity of L has been checked by running TLC on a silica gel coated plate using EtOAc–EtOH (6:4, v/v) as the eluent. The elemental analysis data of the Schiff base and its metal complexes agree well with the proposed composition of the Schiff base and its metal complexes, Table 1.

3.1. Mass spectra

The mass spectrum of L, supplementary material, exhibits the molecular ion peak $[M^+]$ at m/z = 244 (80%). This molecular ion undergoes two major fragmentation pathways (Scheme 1). The first one includes the elimination of H[•] to give ion-peak at m/z = 243(base peak). This fragment undergoes cleavage to give ion peak at m/z = 135 (66%). This can be attributed to fragment ion of benzothiazole moiety. This fragment ion undergoes further fragmentation to give ion peak at m/z=77 (6%) which can be attributed to the phenyl radical ion. The second fragmentation pathway includes ion peak at m/z = 110 (6%) resulting from the elimination of benzothiazole moiety. Also, thiophene radical ion was observed at m/z = 82(26%). This fragment ion undergoes fragmentation by elimination of HS[•] radical to give fragment ion radical $[HC=C-C=C]^{+\bullet}$ at m/z = 50(9%). Analysis by mass spectroscopy for the metal complexes occurs mainly via initial loss of chlorine atoms and solvent molecules, followed by losing the metal ion. After losing the metal, the ligand molecular ion peak appeared at m/z = 244.

3.2. ¹H NMR

The formation of L was supported by the ¹H NMR spectrum, supplementary material, by the appearance of a sharp singlet at 9.40 ppm, corresponding to the azomethine proton. The multisignals within the range 7.32–7.58 ppm are assigned to the aromatic protons of benzothiazole ring. Moreover, the multisignals within the range 7.93–8.10 ppm are attributed to the protons of the thiophene ring. The ¹H NMR spectrum of the Zn(II) complex was compared with that of the parent Schiff base. The –CH=N– proton resonance appeared at 9.40 ppm as a sharp singlet is shifted to

Table 1
Analytical and physical data for the Schiff base and its complexes.

Compounds	Empirical formula	Color	Elemental analysis Found(Calc.)%			$\mu_{ m eff}$ (BM)	$\Lambda_{\rm m}~(\Omega^{-1}~{ m mol}^{-1}~{ m cm}^2)$
			%C	%Н	%N		
L	$C_{12}H_8N_2S_2$	Yellow	58.57(58.99)	3.44(3.30)	11.28(11.47)	-	-
[CuLCl (H2O)3]Cl	$CuC_{12}H_{14}N_2S_2O_3Cl_2$	Green	33.70(33.30)	3.20(3.30)	6.90(6.50)	1.82	70
[FeLCl ₂ (EtOH) ₂]Cl	FeC16H24N2S2O4Cl3	Orange	38.19(38.54)	4.40(4.04)	5.99(5.62)	3.48	98.5
[NiL(OAc) ₂]	NiC ₁₆ H ₁₄ N ₂ S ₂ O ₄	Green	45.05(45.63)	3.60(3.35)	6.70(6.65)	4.15	9.3
[ZnL(OAc) ₂]	$ZnC_{16}H_{14}N_2S_2O_4$	Orange	44.08(44.92)	3.57(3.30)	6.60(6.55)	diamagnetic	8.5

9.3 ppm in the Zn(II) complex with dramatic decrease in intensity suggest that the azomethine nitrogen is involved in coordination with Zn(II) in the complex. A new signal at 3.7 ppm may be assigned to the proton of the water molecules. The multisignals within the range of 2.4–2.5 ppm are assigned to the protons of the acetate groups in the complex.

3.3. Molar conductivity measurements

The molar conductivities of 10^{-3} M solution of the metal complexes in DMF were measured at $25 \,^{\circ}$ C. Table 1 shows the molar conductance (Λ_m) values of the metal complexes. It is concluded from the results that Cu(II) and Fe(III) complexes were found to have molar conductance values of 70 and 98.5 Ω^{-1} mol⁻¹ cm², respectively, indicating the ionic nature of these complexes [15]. Ni(II) and Zn (II) complexes were found to have molar conductance values of 9.3 and $8.5 \,\Omega^{-1}$ mol⁻¹ cm², respectively, supporting the non-electrolytic nature.



Scheme 1. Proposed fragmentation route of Schiff base L.

3.4. IR spectra

Preliminary identification regarding the formation of L was obtained from the absence of the IR bands characteristic for the amino group of 2-aminobenzothiazole and the carbonyl group of 2-thiophenecarboxaldehyde. This is further confirmed by the appearance of new bands at 1642 and 1014 cm⁻¹ characteristic of $v_{C=N}$ and $\gamma_{CH=N}$ of the azomethine group, Table 2. The bands observed at 1572 and 710 cm⁻¹ are attributed to $v_{C=N}$ and v_{C-S-C} of thiazole ring [16]. The bands located at 1530 and 1445 cm⁻¹ can be assigned to the $\nu_{C=C}$ of the aromatic ring. The two bands at 3056 and 2980 cm⁻¹ are due to the asymmetric and symmetric stretching vibrations of the CH group of the aromatic ring. The complexation of L with Cu(II), Fe(III), Ni(II) and Zn(II) leads to small shift in the bands assigned to $v_{C=N}$ of the azomethine group and v_{C-S-C} of thiazole ring. This is probably due to the coordination properties of the ligand and suggesting the involvement of sulfur atom in the bonding with the metal ions [17]. Also, the IR bands near 430, 500 and 555 cm⁻¹ are ascribed to ν_{M-S} , ν_{M-O} and ν_{M-N} [18], respectively. This presents a good evidence for the participation of thiophene sulfur atom in bond formation. Moreover the IR spectra of Cu(II) and Fe(III) complexes exhibit a medium band around 280 cm^{-1} , which are assigned to v_{M-CI} [19]. All complexes display bands near $3400 \,\mathrm{cm}^{-1}$, which are assigned to v_{OH} of the water or ethanol molecules [20].

3.5. Thermal analysis

The weight loss of thermal gravimetric analysis (TGA) for L and its complexes was calculated, Table 3, for the different steps and compared with those of theoretically calculated for the suggested formula based on the results of elemental analysis and mass spectra. Differential thermal analysis (DTA) curve of L shows an endothermic peak at 125 °C. TG curve exhibits no mass loss at that temperature range. This DTA peak can be assigned to the lattice or chemical rearrangement of the compound. The endothermic peak observed at 280 °C, with mass loss (100%) is assigned to the complete decomposition of L. The thermograms of the complexes exhibit thermal stability from room temperature up to 155 °C (Cu), 70 °C (Fe), 175 °C (Ni) and 158 °C (Zn), indicating that no water of crystallization are present in the complexes. TGA and DTA curves suggest that the complexes loss the coordinated water or ethanol molecules followed by chlorine atoms and acetate anions. The exothermic decomposition for all complexes began at ca. 260 °C by the elimination of thiophene moiety followed by benzothiazole moiety and finally the formation of the residue.

3.6. Electronic spectra and magnetic moments

The electronic spectrum of the L, as Nujol mull matrix, Fig. 1, exhibits absorption bands at ca. 210 nm (47 619 cm⁻¹) and 265 nm (37,735 cm⁻¹), which are corresponding to π - π * transitions of the benzenoid system of benzothiazole moiety which are due to (${}^{1}L_{a} \leftarrow {}^{1}A$) and (${}^{1}L_{b} \leftarrow {}^{1}A$) states, respectively [21]. The absorption band at 230 nm (43,478 cm⁻¹) is due to the π - π * transitions

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Table 2 IP spectral bands (cm^{-1}) of the Schiff base

IR spectral bands (cm^{-1}) of the Schiff base and its metal complexes.

Compounds	ν_{CH} Aromatic	$\nu_{\rm C=N}$ thiazole	$\nu_{C=C}$	$\nu_{\rm C=N}$ azomethine	γнс=Ν	ν_{C-S-C}	ν_{M-N}	ν_{M-O}	ν_{M-S}	ν_{M-Cl}
L	3056, 2980	1572	1530, 1445	1642	1015	710	-	-	-	-
[CuLCl(H ₂ O) ₃]Cl	3103, 2984	1576	1540, 1463	1647	1013	706	555	502	426	288
[FeLCl ₂ (EtOH) ₂]Cl	3057, 2970	1581	1467	1653	1014	707	586	482	417	263
[NiL(OAc) ₂]	3000, 2983	1615	1547, 1454	1638	1019	721	547	502	430	-
$[ZnL(OAc)_2]$	3100, 2998	1588	1532, 1458	1649	1014	721	559	502	435	-

Table 3

Thermal gravimetric analysis data for the investigated complexes.

Compound	Temperature (°C)	Weight loss % Found(Calc.)	Weight of residue % Found(Calc.)	Assignment
[CuLCl (H ₂ O) ₃]Cl	27-155	_		Thermally stable
	155–470	50.0(48.34)		3 coordinated water, 2 chlorine and thiophene moiety
	470-655	35.6(34.70)		Complete decomposition and elimination of benzothiazole moiety
	655-800		14.4(14.7)	Residue: Cu metal
[FeLCl ₂ (EtOH) ₂]Cl	27-70	-		Thermally stable
	70-160	9.98(9.24)		1 coordinated ethanol
	160-255	31.42(30.60)		1 coordinated ethanol and 3 chlorine
	255-370	17.00(16.87)		Elimination of thiophene moiety
	370–500	30.35(29.92)		Complete decomposition and elimination of benzothiazole moiety
	500-800		11.25(10.44)	Residue: Fe metal
[NiL(OAc) ₂]	27-175	-		Thermally stable
	175-250	28.10(28.02)		2 coordinated acetate groups
	250-370	19.20(19.80)		Elimination of thiophene moiety
	370-700	32.00(32.10)		Elimination of benzothiazole +
		6.08(6.41)		HCN
	700-800		14.62(13.77)	Residue: Ni metal
[ZnL(OAc) ₂]	27-158	-		Thermally stable
	158-245	27.45(27.58)		2 coordinated acetate groups
	245-350	19.65(19.67)		Elimination of thiophene moiety
	350-580	24.74(24.11)		Elimination of benzothiazole +
		5.94(6.32)		HCN
	580-800		22.22(22.78)	Residue: ZnS



Fig. 1. Nujol mull electronic spectra of L and its metal complexes (a) L, (b) [CuLCl(H₂O)₃]Cl, (c) [FeLCl₂(EtOH)₂]Cl, (d) [NiL(OAc)₂], and (e) [ZnL(OAc)₂].

 $({}^{1}L_{b} \leftarrow {}^{1}A)$ state of the thiazole moiety [22]. The two bands at 215 nm (46,512 cm⁻¹) and 255 nm (39,215 cm⁻¹) are attributed to the $\pi - \pi^*$ transition of the thiophene moieties due to $({}^{1}L_{a} \leftarrow {}^{1}A)$ and $({}^{1}L_{h} \leftarrow {}^{1}A)$ states, respectively [23]. The shoulder band at 335 nm $(29,851 \text{ cm}^{-1})$ may be assigned to the $\pi - \pi^*$ transition [18,24]. This band was found in the spectra of all complexes with small shift. This may be attributed to the donation of the lone pairs of electron of nitrogen atom of Schiff base to the metal ion [8]. While the broad band at $375 \text{ nm} (26,667 \text{ cm}^{-1})$ can be attributed to the transition within the molecule. essentially an intramolecular charge transfers interaction. The electronic absorption spectrum of Cu(II) complex exhibits three broad, low intensity band centered at 655 nm $(15,267 \text{ cm}^{-1})$, 625 nm $(16,000 \text{ cm}^{-1})$ and 581 nm $(17,211 \text{ cm}^{-1})$. These bands may reasonably be assigned to Cu(II) d-d transitions $^{2}B_{1g} \rightarrow ^{2}A_{1g} (d_{x2-y2} - d_{z2}), ^{2}B_{1g} \rightarrow ^{2}B_{2g} (d_{x2-y2} - d_{xy}) \text{ and } ^{2}B_{1g} \rightarrow ^{2}E_{g}$ $(d_{x2-v2}-d_{xz}, d_{yz})$ transitions, respectively. These d-d transitions are normally close in energy [18]. These transitions are due to the ${}^{2}E_{g}$ and ${}^{2}T_{2g}$ states of the octahedral Cu(II) ion (d⁹) split under the influence of the asymmetric filling causing the tetragonal distortion. The energy level sequence will depend on the amount of tetragonal distortion due to the ligand field and the Jahn-Teller effect [25]. The magnetic moment of 1.82 BM, is slightly higher than the spin-only value 1.73 BM expected for one unpaired electron, which offers possibility of an octahedral geometry [26]. A shoulder band observed at $460 \text{ nm} (21,739 \text{ cm}^{-1})$ is due to ligand-metal change transfer (LMCT) [18]. The electronic spectrum of Fe (III) complex displays absorption bands of low intensity at 655 nm (15,267 cm⁻¹), 625 nm $(16,000 \text{ cm}^{-1})$ and 581 nm $(17,211 \text{ cm}^{-1})$. These bands are assigned to octahedral geometry around Fe(III) [20]. The magnetic moment value of 3.50 BM is lower than that of high spin d⁵ configuration, indicating the probability of equilibrium between high and low spin states [20]. The electronic spectrum of the green Ni(II) complex is consistent with tetrahedral geometry showing d-d transition at 581 nm (17,211 cm⁻¹) assignable to ${}^{3}T_{1(F)} \rightarrow {}^{3}T_{1(P)} \nu_{3}$ [27]. The weaker bands on either side of this transition being assigned to spin forbidden bands [28]. These transitions suggest the tetrahedral geometry around the Ni(II) ion. The shoulder band observed at 440 nm $(22,727 \text{ cm}^{-1})$ corresponds to the excited state derived from the ¹G term [29]. The magnetic moment value (4.15 BM) is near to the value expected for tetrahedral Ni(II) [30]. The electronic spectrum of the orange Zn(II) complex shows broad band at 550 nm (18,182 cm⁻¹) corresponds to LMCT. On the other hand, the Zn(II) complex is found to be diamagnetic as expected for d¹⁰ configuration. It has been reported that for tetracoordinated Zn(II) complexes, tetrahedral is the most preferred one [31].

3.7. EPR

The EPR spectrum of Cu(II) complex at room temperature (300 K), Fig. 2, exhibits an axial pattern with no obvious hyperfine structure. The absence of hyperfine structure at room temperature may be attributed the strong dipolar and exchange interactions between the Cu(II) ions [32]. The $g_{||}$ (2.09) and g_{\perp} (2.05) values suggest that d_{x2-y2} may be the ground state as $g_{||} > g_{\perp} > 2.04$, which is consistent with elongated tetragonally distorted octahedral Cu(II) complexes [33]. Also, the trend $g_{\parallel} > g_{\perp} > g_e$ (2.0023) observed for this complex indicated that the unpaired electron is most likely in the $d_{x^2-y^2}$ orbital and the spectral features are of axial symmetry [30]. It has been reported that g_{II} value of Cu(II) complex can be used as a measure of the covalent character of the metal-ligand bond. The g_{\parallel} < 2.3 in agreement with the covalent character of the metal ligand bond [34]. The axial symmetry parameter G, determined as $G = (g_{\parallel} - 2)/(g_{\perp} - 2)$. The parameter G is found to be much less than 4 suggesting considerable exchange interaction in the solid state Cu(II) complex [33,35]. The EPR spectrum of Fe(III) complex, Fig. 2,



Fig. 2. X-band ESR spectra of (a) [CuLCl(H₂O)₃]Cl and (b) [FeLCl₂(EtOH)₂]Cl.

shows broad signal with of axial symmetry with g value at 2.1 corresponds to low spin iron (III) [36,37]. In addition to a weak signal at 3.9, this is known to be characteristic of distorted high spin iron (III) [38]. These two signals confirm the probability of the equilibrium between high and low spin states.

3.8. Antimicrobial assessment

The results of antimicrobial assessment, Table 4, exhibit that L has a high antifungal activity against *A. niger* with 17 mm inhibition zone. Cu(II), Ni(II) and Zn(II) complexes have high antimicrobial activity against *E. coli*, the inhibitions zones are 18, 14 and 11 mm, respectively. Zn(II) complex has antibacterial activity against *Klebsielia* sp. with 16 mm inhibition zone. L and its Cu(II) and Zn(II) complexes exhibit large effect on bacterial and fungal species because they show large clear inhibition zones with the tested bacteria and fungi on solid agar media: the diameters of inhibition zones ranged between (18–25 mm) by the end of incubation. L had no antiyeast activity against *C. albicans*. It was observed that antimicrobial activity increased when tested compound is coordinated with Cu, Zn and Ni. Moreover, Zn(II) complex had antifungal activity ity against *A. niger* more than nystatin which was used as control. On other hand, Cu(II) complex had high antibacterial activity against



Fig. 3. Growth inhibition of different concentration of Zn(II) complex.



Fig. 4. Survival curves of some tumor cell lines.

Table 4

Diameters of inhibition zone (mm) of tested compounds against different species of microorganisms.

Compounds	Inhibition zone/mm									
	E. coli	Staphylococcus aureus	Klebsiella sp.	Candida albicans	A. niger	A. flavus var. coulmnaris	Fusarium oxysporum			
L	0	0	0	0	17	0	0			
[CuLCl (H ₂ O) ₃]Cl	18	0	0	0	20	0	0			
[FeLCl ₂ (EtOH) ₂]Cl	0	0	9	0	0	0	0			
[NiL(OAc) ₂]	14	0	14	0	0	0	0			
$[ZnL(OAc)_2]$	11	0	16	0	25	0	0			
Nystatin	0	0	0	0	20	20	13			
Ceftriaxone	21	13	17	0	0	0	0			

Table 5

 $IC_{50}\ (\mu M)$ dose of the compound which survival to 50%.

Compounds	Cell lines							
	HELA MCF7		HEPG2	HCT116	HEP2			
	IC ₅₀ (μΝ	1)						
L	0.186	-	-	-	-			
[CuLCl(H ₂ O) ₃]Cl	0.027	0.049	-	-	-			
[NiL(OAc) ₂]	0.049	-ve	-	-	-			
$[ZnL(OAc)_2]$	0.025	0.027	0.048	0.0042	-ve			

E. coli compared with ceftriaxone. The growth inhibiting effect was quantitatively determined by the (M/C) ratio of the surviving cell number. The results also show that the inhibitory effect is increased with an increase in concentration of the compounds, Fig. 3. Biological and pharmaceutical activates of Schiff base derived from 2-thiophene carboxaldehyde and aminobenzoic acid have been studied in comparison to Cu, Fe, Zn, Ni and Co complexes [18]. These compounds were screened for the antimicrobial activity against bacterial species and fungal species. *E. coli* was inhibited by Fe, Cu and Zn complexes at concentration 5 mg which formed inhibition zones ranged between 11 and 15 mm. In addition to complexes had anticandidal activity against *Candida* sp. with low inhibition zone sized 5 mm.

3.9. Antitumor activity

In vitro potential cytotoxicity of Cu(II), Ni(II) and Zn(II) complexes was tested against cervical carcinoma (HELA) and breast carcinoma (MCF 7). The relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line, Fig. 4, IC50 values are cited in Table 5. The results of the cytotoxic assays showed that the complexes have significant bioactivity towards human HELA cell lines than the free Schiff base L. The Zn(II)-complex showed the best cytotoxic activity towards this cell line. Cu(II)-complex showed slight cytotoxic activity towards breast carcinoma (MCF 7) cell line. On the other hand, Ni(II)-complex does not show any cytotoxic activity towards human MCF7 cell line. According to the significant cytotoxic activity of Zn-complex towards (HELA) and (MCF 7) cell lines, in vitro potential cytotoxicity of Zn (II) complex was tested against liver carcinoma (HEPG2), colon carcinoma (HCT116) and larynx carcinoma (HEP2) cell lines. The results show that the Zn-complex has significant cytotoxic activity towards all cell lines except HEP2 cell line. In conclusion, the Zn-complex exhibits significant decrease in surviving fraction of HCT116, MCF7 and HELA human cancer cells and induced apoptosis of these cell lines.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.saa.2011.04.064.

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