ejpmr, 2016,3(2), 238-244

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

SYNTHESIS, SPECTROSCOPIC CHARACTERIZATION AND BIOLOGICAL ACTIVITIES OF CO(II), NI(II), CU(II) AND ZN(II) COMPLEXES OF SCHIFF BASE DERIVED FROM ISATIN MONOHYDRAZONE AND FURFURALDEHYDE

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Article Received on 02/12/2015 **Article Revised on 23/12/2015** **Article Accepted on 12/1/2016**

ABSTRACT

A Schiff base, obtained by the condensation of isatin monohydrazone with furfuraldehyde (L_1) and its Co(II), Ni(II), Cu(II) and Zn(II) complexes as well as its mixed ligand complexes with 1,10-phenonthroline were synthesized. The ligands and their complexes were characterized by elemental analysis, molar conductance, FAB-mass, ^{1}HNMR, ^{13}CNMR, FT-IR, electronic spectra, magnetic moment, TGA and powder XRD studies. The analytical data show that the metal to ligand ratio is 1:1. The result showed that the Schiff base ligands act as a biidentate donor. The thermogravimetric analysis indicates the presence of lattice water molecules in the complexes. All the compounds have been tested in vitro against various pathogenic bacteria and fungi. DNA cleavage activity of the ligand and its complexes were assayed on Escherichia coli DNA using gel electrophoresis in the presence of H2O2. The anticancer activities of some selected complexes have also been studied towards human cervical cancer cell line (HeLa). Copper complexs was found to be most potent biologically active compound.

KEYWORDS: Schiff base, Metal complexes, Isatin, Furfuraldehyde, Antimicrobial, DNA cleavage.

1. INTRODUCTION

The rapid development of materials science has considerably promoted the uses of coordination complexes as functional materials such as catalysts, magnetic materials, non-linear optical materials and porous materials.[1-3] Transition metals, in view of their stereochemical versatility and rich redox reactivity, have an active part in small molecule binding and transport, electron transfer triggering, fine and selective catalysis. Due to these features, transition metals play an essential role in many biological processes and constitute the active sites in most enzymes.[4] There has been substantial interest in rational design of transition metal complexes which bind and cleave duplex DNA with sequence or structure selectivity.[5] DNA recognition by small transition-metal complexes has been aided by DNA cleavage chemistry that is associated with redox-active or photo-activated metal complexes.[6] Isatin, an endogenous indole and its derivatives exhibit a wide range of biological activities.[7] Isatin-based Schiff base copper(II) complex is related to the antiviral drug, methisazone. Schiff bases derived from isatin exhibit many neurophysiological and neuropharmacological effects like antimicrobial, antiviral, anticonvulsant, anticancer, antimycobacterial, antimalarial, cysticidal, herbicidal and antiinflammatory activity.[8–14] They also have anti-HIV, antiprotozoal and antihelminthic activities.[15–18] Recently they have found application as enzyme inhibitors in the inhibition of cysteine and serine proteases.[19] Varied applications of this type of Schiff base ligand and its complexes encouraged us for the synthesis, characterization and biological activity studies of some transition metal complexes of Schiff bases derived from Isatin with furfuraldehyde.

2. EXPERIMENTAL

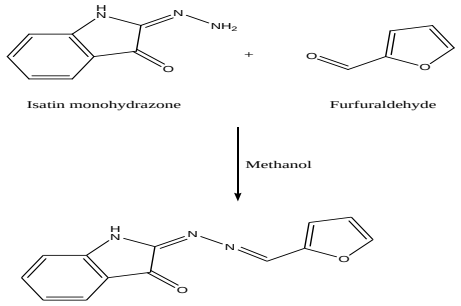
2.1. Materials

Isatin, hydrozene hydrate furfuraldehyde, 1,10-phenanthroline and were purchased from Himedia. Co(II)/Ni(II)/Cu(II)/Zn(II) acetate were purchased from Merck. All other reagents and solvents were purchased from commercial sources and are analytical grade.

2.2. Synthesis of Schiff base ligand L_1

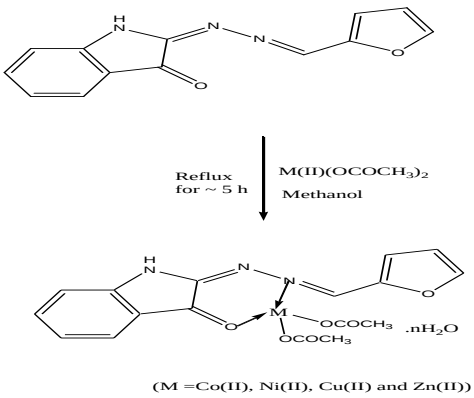
The synthesis of L is schematically presented in scheme 1. The Schiff base has been synthesized by refluxing hot methanolic solution (50 mL) of furfuraldehyde (10 mmol) and hot methanolic solution (40 mL) of isatin

monohydrazone (10 mmol) for 5 h with a few drops of hydrochloric acid. The product obtained after evaporation of the solvent was filtered and washed with cold methanol.



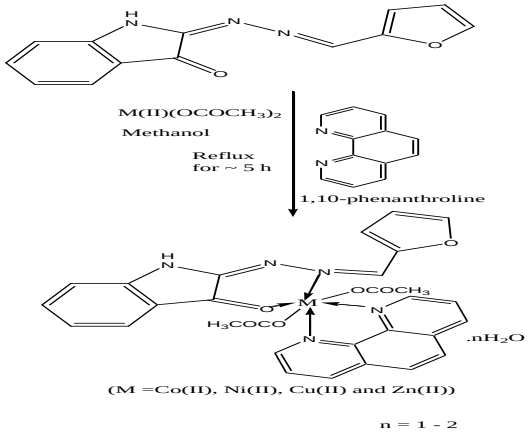
2.3. Synthesis of the Schiff Base Metal Complexes

Hot methanolic solution (25 mL) of Schiff base (1 mmol) was mixed with hot methanolic solution (15 mL) of Co(II)/Ni(II)/Cu(II)/Zn(II) acetate (1 mmol) and refluxed on a water bath for 5 h. The separated complex was filtered, washed thoroughly with water and methanol and dried in vacuum over fused $CaCl_2$.



2.4. Synthesis of mixed ligand Metal Complexes

Hot methanolic solution (25 mL) of Schiff base (1 mmol) was mixed with hot methanolic solution (15 mL) of Co(II)/Ni(II)/Cu(II)/Zn(II) acetate (1 mmol) and then mixed with methanolic solution of 1,10-phnanthroline and refluxed on a water bath for 5 h. The separated complex was filtered, washed thoroughly with water and methanol and dried in vacuum over fused $CaCl_2$.



2.5. Physical Measurements

Elemental analysis was carried out using a Perkin-Elmer elemental analyzer. The metal contents present in the complexes were determined by standard EDTA titration.[20] Molar conductance of the complexes was measured in (Dimethyl sulfoxide) DMSO (10^{-3} M) solutions using a Coronation Digital Conductivity Meter. The mass spectra were recorded on a JEOL JMS600H mass spectrometer. The ^{1}H and ^{13}C-NMR spectra were obtained on a JEOL GSX 400 FT–NMR spectrometer. IR(KBr) spectra were recorded on a JASCO FT/IR-410 spectrometer in the range 4000-400 cm^{-1}. The electronic spectra were recorded with Thermo Scientific UV-VIS spectrometer. Magnetic measurements were performed on a Guoy balance by making diamagnetic corrections using Pascal's constant at room temperature of about 30^{0}C. Thermal analysis was carried out on SDT Q 600/V8.3 build 101 thermal analyzer with a heating rate of 20^{0}C/min. Powder XRD of the prepared complexes were recorded on a Rigaku Dmax X-ray diffractometer with Cu-Kα radiation.

2.6. Antimicrobial Activities

Antibacterial and antifungal properties of the ligand and its complexes were tested *in vitro* against the bacterial species *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Staphylococcus aureus*; fungal species are, *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans* by disc diffusion method. *Amikacin* was used as standard for antibacterial activity and n*ystatin* was used as a standard for antifungal activity. The test organisms were grown on nutrient agar medium in petri plates. The compounds were prepared in DMSO and soaked in filter paper disc of 5 mm diameter and 1 mm thickness. The discs were placed on the previously seeded plates and incubated at 37^{o}C and the diameter of inhibition zone around each disc was measured after 24 h for antibacterial and 72 h for antifungal activities. The minimum inhibitory concentration (MIC) was determined by serial dilution technique.[21]

2.7. DNA cleavage analysis

Cleavage reactions were run between the metal complexes and E. coli DNA and the solutions were diluted with loading dye using 1% agarose gel. Then 3 mL of ethidium bromide (0.5 mg mL 1) was added to the above solution and mixed well. The warmed agarose was poured and clamped immediately with a comb to form sample wells. The gel was mounted onto an electrophoretic tank and enough electrophoretic buffers were added to cover the gel to a depth of about 1 mm. The DNA sample (30 mmol L 1), 50 mmol L 1 metal complex and 500 mmol L 1 H2O2 in 50 mmol L 1 tris–HCl buffer (pH 7.1) were mixed with loading dye and loaded into the well of the submerged gel using a micropipette. Electric current (50 mA) was passed into running buffer. After 1–2 h, the gel was taken from the buffer. After electrophoresis, the gel was photographed under a UV transilluminator (280 nm) and documented.

2.8. In vitro anti cancer activity

The human cervical cancer cell line (HeLa) was grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). The cells were maintained at 37^0C, 5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week. The monolayer cells were detached with trypsin–ethylenediaminetetraacetic acid (EDTA) to make single cell suspension and viable cells were counted using a hemocytometer and diluted with a medium containing 5% FBS to give a final density of 1 x 10^5 cells/mL. One hundred microliters per well of cell suspension were seeded into 96-well plates at a plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h, the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethylsulfoxide (DMSO) to prepare the stock (200 mM) and stored frozen prior to use. At the time of sample addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with serum free medium. Additional three, 2-fold serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 lL of these different sample dilutions were added to the appropriate wells already containing 100 lL of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The medium without samples were served as control and a triplicate was maintained for all concentrations.[22]

MTT assay

MTT is a yellow water soluble tetrazolium salt [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide)]. Succinatedehydrogenase, a mitochondrial enzyme in living cells cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Thus, the amount of formazan produced is directly proportional to the number of viable cells. After 48 h of incubation, 15 lL of MTT (5 mg/mL) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4 h. The medium with MTT was then flicked off and formazan crystals obtained were solubilized in 100 lL of DMSO. The absorbance at 570 nm was measured using a micro plate reader.[23] The % cell inhibition was determined using the following formula.

% Cell inhibition = 100 – Abs(sample)/Abs(control) x 100.

Nonlinear regression graph was plotted between % cell inhibition and log10 concentration and IC50 was determined using Graph- Pad Prism software.

3. RESULTS AND DISCUSSION

3.1. Characterization of Schiff base ligands (L_1)

The Schiff base ligand (L_1) is dark red in colour and soluble in all common organic solvents. The elemental analysis data (Table 1) of ligands are in good agreement with those calculated for the suggested formula. The mass spectrum of the ligand L_1 shows a well-defined molecular ion peak at m/z = 241 [M^{+1} (92%)] which coincides with the formula weight of the Schiff bases. In the ^{1}H-NMR spectrum, the azomethine proton exhibits a singlet at 8.83 ppm. The methylene protons in the ring appear at 6.81-8.22 ppm. The FT-IR spectrum (Table 2) of the Schiff base ligand L_1 displayed a band at 1610 is the stretching vibrational frequency of azomethane group. The band corresponding to ketamine is shows at 1647. The Schiff base ligand L_1 exhibits a band at 1711 assigned to a stretching frequency of the carbonyl group of the isatin moiety. Sharp band present in the spectrum of the Schiff bases in the region 3321 is due to N-H stretching frequencies.[24] The electronic spectrum of the ligand L_1 exhibit an absorption band at 245 nm and 355 nm respectively are attributed to the π–π* transition of the azomethine chromophore.

3.2. Characterization of metal Schiff base complexes

The analytical and physical characterization of metal complexes of the ligand L_1 is given in Table 1. The analytical data show that the metal to ligand ratio is 1:1 in all the complex systems. The composition of the complexes with ligand is $[ML_1(CH_3COO)_2].nH_2O$ and with mixed ligand is $[ML_1P(CH_3COO)_2].nH_2O$ where L_1 is the Schiff base ligands and P is 1,10-phenanthroline. The mass spectrum of the Co(II), Ni(II), Cu(II) and Zn(II) complexes of Schiff base ligand L_1 and its mixed ligand complexes show molecular ion peaks at m/z 453 (M^+ +1, 46%) for $[CoL_1(CH_3COO)_2].2H_2O$, 435 (M^+, 92%) for $[NiL_1(CH_3COO)_2].H2O$, 458 (M^++1, 47%) for $[CuL_1(CH_3COO)_2].2H_2O$, 459 (M^++1, 58%)] for $[ZnL_1(CH_3COO)_2].2H_2O$, 634 (M^+ +1, 34%) for $[CoL_1(Phen)(CH_3COO)_2].2H_2O$, 633 ($M^+$, 93%) for $[NiL_1(Phen)(CH_3COO)_2].2H_2O$, 620 ($M^+$+1, 37%) for $[CuL_1(Phen)(CH_3COO)_2].H_2O$, 621 ($M^+$+1, 30%)] for $[ZnL1(Phen)(CH_3COO)_2].H_2O$ respectively, which coincide with the formula weight of the Schiff base complexes. The low molar conductance values (Table 1) of the metal complexes reveal their non-electrolytic nature.[25]

Table 1. Analytical and physical data of the Schiff ligands and their complexes

Compound	Empirical formula	M.W	Elemental analysis Found (calcd) %				Λc (Ohm^{-1} cm^2mol^{-1})	λ_{max} (nm)	μ_{eff} (B.M)
			C	H	N	M			
L_1	$C_{13}H_9N_3O_2$	239.23	64.37 (65.27)	3.48 (3.79)	17.03 (17.56)	-		245, 355	-
$[CoL_1(CH_3COO)_2].2H2O$	$C_{17}H_{19}CoN_3O_8$	452.05	45.14 (45.05)	4.23 (4.63)	9.29 (10.10)	13.03 (13.16)	12	670	4.3
$[NiL_1(CH_3COO)_2].H2O$	$C_{17}H_{17}N_3NiO_7$	434.03	47.04 (47.78)	3.95 (3.63)	9.68 (10.11)	13.52 (13.11)	13	590	3.7
$[CuL_1(CH_3COO)_2].2H2O$	$C_{17}H_{19}CuN_3O_8$	456.07	44.69 (44.52)	4.19 (4.59)	9.20 (9.98)	13.91 (14.10)	12	600	2.0
$[ZnL_1(CH_3COO)_2].2H2O$	$C_{17}H_{19}N_3O_8Zn$	458.73	44.51 (44.31)	4.17 (4.58)	9.16 (9.94)	14.25 (15.07)	07	246, 357	dia
$[CoL_1(Phen)(CH_3COO)_2].2H_2O$	$C_{29}H_{27}CoN_5O_8$	632.50	55.07 (55.40)	4.30 (4.89)	11.07 (11.74)	9.32 (9.88)	12	450	4.9
$[NiL_1(Phen)(CH_3COO)_2].2H_2O$	$C_{29}H_{27}N_5NiO_8$	632.26	55.09 (55.42)	4.30 (4.89)	11.08 (11.75)	9.28 (9.84)	13	585, 425	3.1
$[CuL_1(Phen)(CH_3COO)_2].H_2O$	$C_{29}H_{25}CuN_5O_7$	619.09	56.26 (56.95)	4.07 (4.86)	11.31 (11.65)	10.26 (10.57)	12	430, 610, 745	1.8
$[ZnL_1(Phen)(CH_3COO)_2].H_2O$	$C_{29}H_{25}N_5O_7Zn$	620.93	56.10 (56.77)	4.10 (3.85)	11.40 (11.62)	10.53 (10.84)	07	246, 356	dia

3.3. IR Spectra

The FTIR spectral data of the synthesized metal complexes are given in Table 2. The band at 1610 cm^{-1} for the azomethine group of free ligand L_1 was shifted to lower frequency in the range ~1602-1594 cm^{-1} in the complexes is indicative of the coordination of the azomethine nitrogen atom with the metal ion. The band at 1711 cm-1 for the carbonyl group of free ligand L1 was shifted to lower frequency in the range ~1703-1690 cm-1 in the complexes is indicated the coordination of the carbonyl oxygen with the metal ion. The new broad band appeared at ~3400 cm^{-1} in all the metal complexes can be attributed to the stretching vibration of the coordinated water molecules. A band at ~870 cm^{-1} in all the complexes is assigned to coordinated water molecule.[26] On complexation, the asymmetric and symmetric stretching bands of carboxylato groups of acetic acid are shifted to lower frequency for all the complexes, which reveals the formation of a linkage between the metal ion and carboxylato oxygen atom. Moreover, the difference (~200 cm^{-1}) between the asymmetric and symmetric stretching modes indicates the monodentate binding of the carboxylato group in the complexes.[27] The stretching frequency of N-H is overlap by the band of water molecules. The spectrum of all the metal complexes show new bands in the 548–585 cm^{-1} and 434–457 cm^{-1} regions, which may probably be due to the formation of M-O and M-N bonds respectively.[25]

Table 2. IR spectral data of the Schiff base ligands and their complexes (cm^{-1}).

Compound	ν_{azo}(C=N)	ν_{azo}(C=N)	ν(C=O)	ν(N-H)	$\nu(H_2O)$	ν(M-O)	ν(M-N)
L_1	1610	1647	1711	3321(s)	-	-	-
$[CoL_1(CH_3COO)_2].2H_2O$	1598	1648	1701	-	3325(b)	578	446
$[NiL_1(CH_3COO)_2].H_2O$	1595	1647	1697	-	3330(b)	585	453
$[CuL_1(CH_3COO)_2].2H_2O$	1602	1647	1694	-	3335(b)	563	434
$[ZnL_1(CH_3COO)_2].2H_2O$	1598	1646	1700	-	3332(b)	572	448
$[CoL_1(Phen)(CH_3COO)_2].2H_2O$	1601	1646	1698	-	3410(b)	565	455
$[NiL_1(Phen)(CH_3COO)_2].2H_2O$	1603	1649	1701	-	3335(b)	570	448
$[CuL_1(Phen)(CH_3COO)_2].H_2O$	1597	1648	1697	-	3340(b)	580	445
$[ZnL_1(Phen)(CH_3COO)_2].H_2O$	1598	1647	1699	-	3320(b)	585	438

3.4. Electronic Spectra and Magnetic Measurements

The electronic spectrum of Co(II) complex with L_1 shows two absorption bands (Table 1) at 670 nm region, which is due to the transition of $^4A_2(F) \rightarrow {}^4T_1(P)$. This indicates the tetrahedral geometry of the complex. The electronic spectrum of the Ni(II) complex of Schiff base ligand with L_1 show a bands in the region, 590 nm (Table 1) attributable to $^3T_1(F) \rightarrow {}^3T_1(P)$, suggesting an tetrahedral geometry around Ni(II) atom. The Cu(II) complex of L_1 display broad band in the ~600 nm region, which can be assigned to $d_x \rightarrow d_z^2$ transition, indicating the complex to have distorted square planar geometry. The magnetic susceptibility value is found to be 4.3 BM for $[CoL_1(CH_3COO)_2].2H_2O$ (normal range for tetrahedral Co(II) complexes is 4.2–4.8 BM), which indicated the tetrahedral geometry of the complex. The $[NiL_1(CH_3COO)_2].H2O$ complex reported herein is found to have a room temperature (30^0C) magnetic moment value of 3.7 BM, which is in the normal range observed for tetrahedral Ni(II) complexes (μ_{eff} 3.2 – 4.0 BM). The magnetic moment value of $[CuL_1(CH_3COO)_2].2H_2O$ is 2.0 BM (normal range for square planar Cu(II) complexes is 1.8–2.1 BM), which indicated the square planar geometry of the complex. $[ZnL_1(CH_3COO)_2].2H_2O$ complex is diamagnetic as expected and would have tetrahedral geometry. In the case of mixed ligand complexes, the electronic spectrum of Co(II) complex with L_1 and 1,10-phenanthroline show a absorption band (Table 1) at 450 nm region, which is assignable to the $^4T_1g(F) \rightarrow {}^4A_2g(F)$ transitions in an octahedral environment [28,29]. The electronic spectrum of the mixed ligand Ni(II) complex shows two bands in the region 585 and 425 nm (Table 1) attributable to $^3A_2g(F) \rightarrow {}^3T_1g(F)$ and $^3A_2g(F) \rightarrow {}^3T_1g(P)$ transitions, respectively, suggesting an octahedral geometry around Ni(II) atom. The mixed ligand Cu(II) complex display broad band in the 430, 610 and 745 nm region respectively, which can be assigned to $^2B_{1g} \rightarrow {}^2E_{1g}$, $^2B_{1g} \rightarrow {}^2B_{2g}$, and $^2B_{1g} \rightarrow {}^2A_{1g}$ transition respectively, indicating the complex to have distorted octahedral geometry.[28] In octahedral complexes, the Jahn–Teller effect is the most pronounced when an odd number of electrons occupy the Eg orbitals. The magnetic susceptibility value is found to be 4.9 BM for Co(II) (normal range for octahedral Co(II) complexes is 4.3–5.2 BM), which indicated the octahedral geometry of the complex.[30] The Ni(II) complex reported herein is 3.1 BM, which is in the normal range observed for octahedral Ni(II) complexes (μ_{eff} 2.9 – 3.3 BM).[30] The magnetic moment value of Cu(II) is 1.8 BM, which is normally observed for octahedral Cu(II) complexes.[29] Zn(II) complex is diamagnetic as expected and would have octahedral geometry.

3.5. Thermal Analysis

The thermal stability data of the complexes are listed in Table 3. All the complexes undergo similar decomposition mainly in three stages. The first stage taking place in the 80–100^0C range corresponds to dehydration of lattice water molecules. The second decomposition step indicates the removal of organic

ligand moiety in the 300–700^{0}C range with the formation of metal oxide as the final product.

3.6. Powder XRD

The powder XRD pattern of the Schiff base complexes were recorded over the 2h = 0–80 range and $[ZnL_1(CH_3COO)_2].2H_2O$ complex display sharp crystalline peaks indicating their crystalline nature, whereas other complexes with L1 ligand do not exhibit well-defined crystalline peak indicating their amorphous behavior. The mixed ligand complexes with L1 and 1,10-phenanthroline $[CoL_1(Phen)(CH_3COO)_2].2H_2O$, $[CuL1(Phen)(CH_3COO)_2].H_2O$, and $[ZnL_1(Phen)(CH_3COO)_2].H_2O$ complexes are showes sharp crystaline peaks indicating their crystalline nature. Mixed ligand Ni(II) complex is amorphus in nature.

4. Biological Studies

4.1. Antimicrobial activity

The results of the antimicrobial activities are summarized in Table **3**. The standard error for the experiment is ± 0.001 cm and the experiment was repeated thrice under similar conditions. DMSO was used as a negative control and amikacin, was used as positive standards for antibacterial studies. Nystatin was used as a reference for antifungal studies. These compounds exhibit moderate to strong antimicrobial activity. The copper(II) and Zinc(II) complexes show a remarkable activity, especially against the bacteria such as *E. coli*., *S. Auveus* and *K. Pneumonia.* The copper complex shows better activity against *E.Coli* compared to the standared amikacin. The zinc and copper complexes displays good activity against the fungal species *C. albicans*. The antimicrobial activity of the complexes is greater than that of the free ligand, this indicates that the complexation to metal enhances the activity of the ligand. This is explained on the basis of Overtone's concept and chelation theory.[31] Chelation tends to make the ligand a more powerful and potent bacterial agent. A possible explanation for this increase in the activity upon chelation is that, in a chelated complex, the positive charge of the metal is partially shared with donor atoms present in the ligands and there is an electron delocalization over the whole chelated ring. This, in turn, increases the lipoid layers of the bacterial membranes. Generally, it is suggested that the chelated complexes deactivate various cellular enzymes, which play a vital role in various metabolic pathways of these microorganisms.

Table 3. In vitro antimicrobial activity (MIC, µg/mL) of the ligands, complexes and standard reagents.

Compound	Bacterial species				Fungal species		
	E. coli	***K. Pneumonia***	***S. Auveus***	***P. Valgaris***	***A. niger***	***A. flavus***	***C. albicans***
L_1	78	>100	90	>100	>100	86	>100
$[CoL_1(CH_3COO)_2].2H2O$	26	48	38	96	>100	>100	79
$[NiL_1(CH_3COO)_2].H2O$	88	75	69	>100	92	98	>100
$[CuL_1(CH_3COO)_2].2H2O$	5	6	5	73	98	96	5
$[ZnL_1(CH_3COO)_2].2H2O$	31	22	20	75	87	>100	19
$[CoL_1(Phen)(CH_3COO)_2].2H$	32	54	28	93	52	63	54
$[NiL_1(Phen)(CH_3COO)_2].2H_2$	83	64	62	74	46	57	53
$[CuL_1(Phen)(CH_3COO)_2].H_2$	4	5	4	63	81	75	5
$[ZnL_1(Phen)(CH_3COO)_2].H_2$	6	4	5	62	84	73	4
Amikacin[a]	05	05	04	05	-	-	-
Nystatin[a]	-	-	-	-	06	06	05

[a,] Standards

4.2. DNA cleavage analysis

Gel electrophoresis experiments using *E. Coli* DNA was performed with the ligand and its complexes in the presence and absence of H_2O_2 as an oxidant. The results indicate that all the complexes could interact with DNA in the presence of H_2O_2. All the complexes cleave DNA completely. The metal complexes seem to catalyze the generation of highly reactive hydroxyl radicals from H_2O_2. These hydroxyl radicals participate in the oxidation of the deoxyribose moiety, followed by the hydrolytic cleavage of the sugar-phosphate backbone. Cu(II) and Zn(II) complexes with L1 ligand completely cleaved the DNA by generating the hydroxyl radicals oxidant H_2O_2. In the case of mixed ligand complexes, the copper and nickel complexes are completely cleaved the DNA.

4.3. Anticancer activity

To find out the biological effects of the ligand and its complexes on cancer cells, we used the compounds to treat HeLa (Human Cervical Cancer Cells) at the concentrations of 6.25, 12.5, 25, 50 and 100µM for 48h. The untreated cells were used as a control. Cell growth inhibition was analyzed by MTT assay and the results showed that the complexes and the ligand exhibited an inhibitory effect on the proliferation of HCT116 and HeLa cells in a dose-dependent manner (Table. 4). The IC_{50} values for the complexes Cu(II) and Zn(II) are very low compared with the other complexes and the free ligand. This indicates that these compounds possess pronounced anti-proliferative effects on HeLa cancer cells.

Table 4. IC_{50} values of the ligand and the complexes.

Compound	HeLa
L_1	56.6
$[CoL_1(CH_3COO)_2].2H2O$	35.8
$[NiL_1(CH_3COO)_2].H2O$	46.3
$[CuL_1(CH_3COO)_2].2H2O$	22.1
$[ZnL_1(CH_3COO)_2].2H2O$	24.5
$[CoL_1(Phen)(CH_3COO)_2].2H_2O$	62.7
$[NiL_1(Phen)(CH_3COO)_2].2H_2O$	41.3
$[CuL_1(Phen)(CH_3COO)_2].H_2O$	21.8
$[ZnL_1(Phen)(CH_3COO)_2].H_2O$	23.1

5. CONCLUSION

Co(II), Ni(II), Cu(II) and Zn(II) complexes with the Schiff base ligand derived from the condensation of isatin monohydrazone with furfuraldehyde (L_1) and its mixed ligand complexes with 1,10-phenonthroline were synthesized. From the spectral data it was found that the coordination of the Schiff base to the metal atom was found to be through the azomethine nitrogen and carbonyl group of free ligand. Tetrahedral and octahedral geometry was assigned for different complexes. Presence of water molecule and the elemental composition were confirmed by the thermogravimetric analysis. Powder XRD results show that some complexes are crystalline and other complexes are amorphous in nature. The antimicrobial studies reveal that the complexes show higher activity than the ligand. The DNA cleavage studies shows, Cu(II) Zn(II) and Ni(II) cleaved the DNA completely. In the case of anticancer studies, Cu(II) and Zn(II) complexes are more active than the other complexes and the free ligand.

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