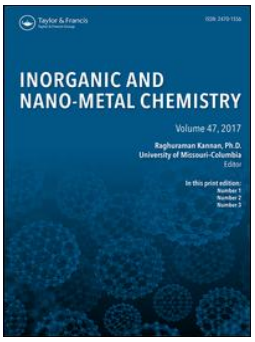

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Synthesis, spectral-characterization, biological and DFT studies of mixed ligand metal(II) complexes of 1,10-phenanthroline bearing 2-aminothiazole moiety

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ABSTRACT
The Schiff base ligand **L** is developed from furan-2-carboxaldehyde, 4-aminoantipyrine together with 2-aminothiazole. The mixed ligand complexes are developed from the condensation of above Schiff base ligand **L**, 1,10-phenanthroline as well as metal(II) acetates of Co(II), Ni(II), Cu(II), and Zn(II). The newly produced compounds were characterized using molar conductance, elemental analysis, XRD, mass, UV-Vis, ^{1}H NMR as well as IR spectral techniques. The X band ESR of [CuLphen] complex was recorded at 300 and 77 K. Geometry and Mulliken charge distribution of the **L** were studied using Density functional theory (DFT). *In vitro* biological analysis of **L** and its [CoLphen], [NiLphen], [CuLphen] as well as [ZnLphen] complexes were studied against bacterial variety such as *Pseudomonas aeruginosa*, *Escherichia coli* as well as *Staphylococcus aureus*. The fungal varieties include *Aspergillus niger* and *Candida albicans*. The potent DNA cleavage activities of **L** as well as its complexes were evaluated.

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1,10-Phenanthroline; pUC18 DNA; DFT; ESR; GC-MS

Introduction

The interesting invention of Schiff base ligand provides an array of valuable advantages in the study of coordination metal(II) chemistry through transition metal complexes. The **L** together with [CoLphen], [NiLphen], [CuLphen] and also [ZnLphen] having S, O, N donor atoms, show excellent stereochemical, electronic and electrochemical properties.[1] Schiff bases of mixed ligand complexes are produced by the condensation of aldehydes and amines/ketones, as a result, they form azomethine (>C=N) bond. Mainly Schiff bases with azomethine linkage are fundamental for various biological applications in the field of antitumor, antioxidant, catalysts, antifungal and antibacterial activity.[2–5] Compounds containing aminothiazole derivatives have potent biological applications in the path of the anti-inflammatory, anticancer, antitumor, antihypertensive, antidiabetic, antileishmanial, neuroprotective, and antiviral agent, DNA binding and cleavage, antituberculosis and in therapeutic fields.[6–8] Researchers discovered that the mixed ligand metal(II) complexes medicinally act as an exponent DNA-target substance for antiviral and anticancer treatment.

Investigators already reported the invention of Schiff base from 4-aminoantipyrine as well as furan-2-carboxaldehyde.[9,10] The Schiff base derivatives of 4-aminoantipyrine related compounds are reported to demonstrate biological, clinical, pharmacological and analytical applications.[11–14] Heterocyclic compounds like 2,2′-bipyridine and 1,10-phenanthroline act as a perfect ligand because of the availability of N atoms in a ring and a localized pair of electrons.[15] Compounds having heterocyclic moieties display biological activity.[16] Chemist in the recent years interested in Schiff base metal(II) complexes, due to their applications in catalysis, bio-inorganic systems and its thermal stability.[15,17] 4-Aminoantipyrine related ligands exhibit a tendency for coordination through metal centers.[18] Most of the copper complexes produce remarkable pharmacological activities. The relationship between DNA with a very tiny molecule is essential to design a new pharmaceutical material. Due to the significance of 4-aminoantipyrine, we have explored the production of mixed ligand complexes from 4-aminoantipyrine, 2-aminothiazole, and furan-2-carboxaldehyde along with Co(II), Ni(II), Cu(II), and Zn(II) metal acetates. The **L** as well as its metal(II) complexes, were technically characterized using different spectral studies including IR, UV, ^{1}H NMR, etc.

Experimental

Materials and methods

AnalaR grade reagents were used in the synthesis. 4-aminoantipyrine, furan-2-carboxaldehyde, 2-aminothiazole, and 1,10-phenanthroline were obtained from Sigma Aldrich. Metal(II) acetates along with solvents were purchased from Merck, which are not subjected to undergo any purification process. The purity of above starting materials is 99%.

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Instruments

Elemental analysis of **L** and its metal complexes was performed using Perkin–Elmer elemental analyzer. Molar conductance values were recorded using coronation digital conductivity meter with DMSO solution. IR spectra of **L** as well as metal complexes were taken from Perkin-Elmer FT-IR 783 spectrophotometer in 4000-400 cm^{-1}. ^1H NMR spectra were performed through Brucker 300 MHz spectrometer using $CDCl_3$ for ligand and DMSO-d_6 for Zn(II) complex with TMS as the internal standard. Electronic spectra were carried out on Perkin Elmer Lambda-25 UV–Vis. a spectrometer in the range 200–1100 nm. GC-MS spectrum was recorded on a JEOL GCMATE II GC-MS mass spectrometer. XRD studies were performed using XPERT-PRO X-ray diffractometer. ESR spectrum of the Cu(II) complex was performed at 300 as well as 77 K in DMSO solution with TCNE as a g-marker by Varian, USA E-112 ESR spectrometer.

Antimicrobial activity

In vitro antimicrobial screening of **L** along with its mixed ligand 1,10-phenanthroline complexes were treated against bacterial species such as *Staphylococcus aureus*, *Escherichia coli* as well as *Pseudomonas aeruginosa* by agar well diffusion method.[19] At first, the bacteria were re-energized by broth media at 37 °C and allowed to grow for 18 h. The wells were prepared in agar media plates. Each plate was inserted through 18 h old cultures (100 µl) as well as spread equally on every plate. Here chloramphenicol was used as a standard as well as dimethyl sulphoxide was used as a negative control. After 25 min, the prepared wells were filled with synthesized compounds around the concentrations of 50, 100, and 200 µg/mL, and then to record the minimum inhibitory concentration (MIC) value. At last, all the agar plates were kept for 24 h to incubated at 37 °C and then inhibition values were noted.

The antifungal ability of *Aspergillus niger* as well as *Candida albicans* were tested against the **L** and its mixed ligand and metal(II) complexes, first and foremost the collected cultures were re-energized by inoculating in wells of approximately 10 mm was bored using a good cutter in the plate. All plates were inoculated for 48 h old cultures (100 µl) in addition to spread the fungi uniformly on the plate. After 25 min, the compound at 25, 50, and 100 µg/ml concentrations have filled the wells, to record out the minimum inhibitory concentration (MIC) value. The clotrimazole was used as a standard also dimethyl sulphoxide (DMSO) was used as a negative control. Every plate was incubated for 72 h at 27 °C furthermore the inhibition values were noted.

Cleavage of pUC18 DNA

Agarose gel electrophoresis is a technique for separating as well as visualizing DNA fragments. Plasmid DNA nicking assay was performed using pUC18 plasmid DNA. A mixture of 10 µl of extract (10 µg) and plasmid DNA (10 µl) was incubated at room temperature for 30 min at 37 °C. A tube with plasmid was kept as negative control and one with 10 µl of Fenton's reagent as a positive control. The DNA samples were undergone electrophoresis on 1% agarose gel. The fragments are broken up by charge as well as the size and then move toward the agarose gel matrix when settled into an electric field. The electric field is produced by giving potential across the electrolyte (buffer). 6 µl of 10 mg/ml of ethidium bromide with gel comb was added into the gel casting apparatus. After setting, the comb and the gel were separated. The buffer has flowed through the gel tank and the platforms were placed with the gel. The gel was filled with the samples and run at 50 V for half an hour. The marked gel was visualized using a gel documentation system.

Synthesis of ligand of mixed ligand complexes

The **L** (furfuryl-4-aminoantipyrino-2-aminothiazolidine) was produced through the condensation reaction between furan-2-carboxaldehyde together with 4-aminoantipyrine[20] and the addition of ethanolic solution of 2-aminothiazole. The above solution was refluxed for 6 h until its volume will be reduced to one by third of its original volume. The yellow-color product was allowed to cool and then filtered. The product was purified through recrystallization using ethanol.[20,21] The purity of synthesized **L** is 90%. At room temperature, an ethanolic solution of Schiff base, metal acetates of Co(II)/Ni(II)/Cu(II)/Zn(II) as well as 1,10-phenanthroline were taken in an RB flask of ratio is about 1:1:1 was stirred magnetically for 3 h. Finally, the solid product was cooled, filtered, recrystallized with ethanol and then dried over desiccator.

Results and discussion

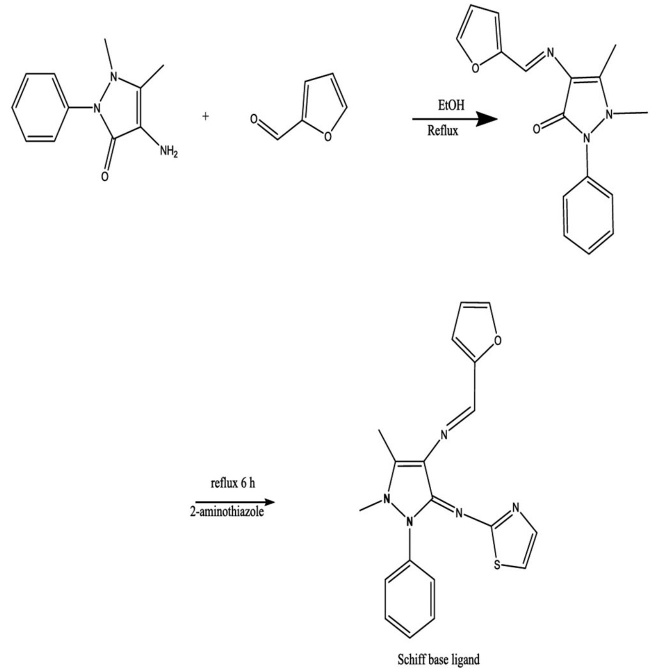
At room temperature, **L**, [CoLphen], [NiLphen], [CuLphen], and [ZnLphen] complexes are stable in nature. They are partially soluble in ethanol, methanol but finely soluble in THF, DMSO and DMF. The elemental analysis data is shown in (Table S1 in Supplementary Information).The schematic diagram for the preparation of Schiff base **L** is shown in Scheme 1.

Molar conductance

Molar conductance information of metal complexes in DMSO solution (10^{-3}M) at 30 °C was shown in (Table S1 in Supplementary Information). The non-electrolytic behavior of the metal(II) complexes is evidenced by the lowest molar conductance values in the range 5–20 Ω^{-1} cm^2 mol^{-1}.[22]

IR spectra

IR spectrum was useful to predict the skeleton of the complexes, coordination modes upon the chelation process and the detection of the donor sites. The similarity study of



Scheme 1. Synthetic route for preparation of Schiff base **L**.

Table 1. IR spectral data of **L** and its complexes (cm^{-1}).

Compound	$\nu_{(CH=N)}$	$\nu_{(C=N)}$	$\nu_{(OH)}$	$\nu_{(C=N)}$ (1,10-Phen moiety)	$\nu_{(M-N)}$	$\nu_{(M-O)}$
L	1592	1647	–	–	–	–
[CoLphen]	1525	1624	3428	711	457	574
[NiLphen]	1523	1623	3516	720	480	505
[CuLphen]	1527	1637	3525	714	480	671
[ZnLphen]	1525	1625	3425	723	495	669

1,10-phenanthroline metal(II) complexes with **L** suggests that most of the peaks are disappeared, newly formed or shifted. In the IR spectra of the complexes, azomethine bands are shifted to lower wavenumber among the complexes. The spectral results are given in Table 1. The **L** shows –NH stretching frequency at the range of $3688\,cm^{-1}$. IR spectrum shows two strong bands of Schiff base ligand at 1592 and $1647\,cm^{-1}$. The above two bands indicate stretching vibrations of (–CH=N) and (>C=N) units respectively.[7] In the case of mixed ligand complexes of 1,10-phenanthroline the (–CH=N) and (>C=N) stretching remains changed. The (–CH) and (>C=N) stretching of 1,10-phenanthroline accompanied at the range of $1055–1018\,cm^{-1}$ and $723–701\,cm^{-1}$ in the metal complexes. The metal complexes show the IR spectrum at a region $521–597\,cm^{-1}$ for M–O bond in addition to $421–442\,cm^{-1}$ owing to the development of M–N bonds. The bands formed at ($3425–3525\,cm^{-1}$) characteristic of H_2O molecule.[23] From this, it is finalized that the presence of (–CH=N) together with (>C=N) groups of **L** acts as a bidentate ligand.

^{1}H NMR spectra

^{1}H NMR range of **L** and [ZnLphen] complex is specified in (Figures S1 and S2 in Supplementary Information).The singlet signal for (–CH=N–) in the **L** is formed at 9.4 ppm and for [ZnLphen] complex is 9.6 ppm. This is because of the deshielding effect of azomethine group in coordination with Zn(II) ion in [ZnLphen] complex. In ligand, the multiplet signals are seen in 7.3–7.8 ppm range, this is for aromatic protons.[24] The peaks at 2.5 ppm as well as 3.3 ppm are assigned for ($–CH_3$) and ($>N-CH_3$) groups respectively.[25] The signals for furfural moieties are at 6.6 and 6.9 ppm.

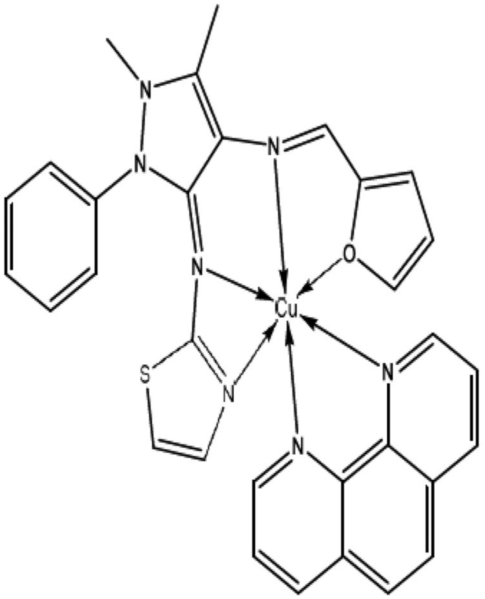


Figure 1. Proposed structure of [CuLphen].

XRD studies

The crystalline character of the complexes was predicted by using powder X-ray diffraction studies. The crystalline dimension of **L** and its complexes were derived using Scherrer's formula. $d_{XRD} = 0.9\lambda/\beta\cos\theta$. In the present study **L**, [CoLphen], [NiLphen], [CuLphen], and [ZnLphen] complexes are nanocrystalline with grain size 70, 73, 70, 59, and 80 nm, respectively.[26] The XRD pattern of **L**, [CoLphen], [NiLphen], [CuLphen] and [ZnLphen] complexes are displayed in Figure S3a–e in Supplementary Information.

Mass spectra

GC-MS spectrum of **L** and mixed ligand of [CoLphen], [NiLphen], [CuLphen], and also [ZnLphen] complexes were recorded. The **L** ($C_{19}H_{17}N_5OS$) shows the molecular ion peak at 363 *m/z*. Molecular ion peaks of [CoLphen], [NiLphen], [CuLphen] and [ZnLphen] complexes were found at 606, 605, 610, and 611 *m/z*, which exactly predict the stoichiometry of the metal complexes. Thus the GC-MS spectral data suggest that the ion peaks values of the molecules have good conformity with the structures recommended by spectral studies and also elemental analysis. Proposed structure of mixed ligand metal complex of Cu(II) is shown in Figure 1.

Figure 2. ESR spectrum of [CuLphen] complex.

Electronic absorption spectra and magnetic moment studies

The electronic absorption spectra of **L** and mixed ligand complexes of 1,10-phenanthroline were carried out in DMSO solution. These spectra demonstrate the geometry of transition metal(II) complexes. The site of the absorption region, band assignments together with the geometry of the prepared complexes and magnetic moment values are given in (Table S2 in Supplementary Information). The **L** displays broadband at 320 nm together with 369 nm which developed π–π^* transition of the azomethine (–CH=N) unit. The spectrum for the complex of [CoLphen] exhibits the absorption region at 1033 nm which recognized to $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ transition suggesting octahedral geometry. The μ_{eff} (effective magnetic moment) value of [CoLphen] is 5.14 BM. The [NiLphen] complex provides the absorption band at 1080 nm range assignable to $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ transition and μ_{eff} value of [NiLphen] is 3.23 BM characteristic of six coordinated octahedral geometry of [NiLphen] complexes. The [CuLphen] complex displays band in the electronic spectrum at 735 nm indicates $^2B1_g \rightarrow {}^2A1_g$ transition characteristic of distorted octahedral geometry with μ_{eff} value 1.89 BM. The [ZnLphen] complex is diamagnetic, because of d_{10} configuration. So the expected geometry of [ZnLphen] complex is octahedral and also exhibits INCT bands.[27]

ESR studies

ESR spectrum of [CuLphen] complex has been carried out at 300 K as well as 77 K in DMSO is shown in Figure 2. At 300 K one intense absorption band of [CuLphen] complex possess isotropic signal in high field region, the reason is the tumbling movement of the molecules. At 77 K [CuLphen] complex produces four well-fined peaks. Among the four peaks, one peak is more intense which is found in the high field region. The remaining three peaks are found in the low field regions which have low intensity.[28] The order of *g* is $g_\parallel > g_\perp > 2.0027$ denotes the occurrence of an unpaired electron present in the d_{x^2}-d_{y^2} orbital. The experimental values are $A_\parallel = 156 > A_\perp = 52$; $g_\parallel = 2.37$; $g_\perp = 2.03 > 2.0027$ moreover the ESR parameters of the [CuLphen] complex recommend that the complex has distorted octahedral geometry. This provides a clear idea about the unpaired electrons usually situated in the $d_x{}^2$-$d_y{}^2$ orbital.

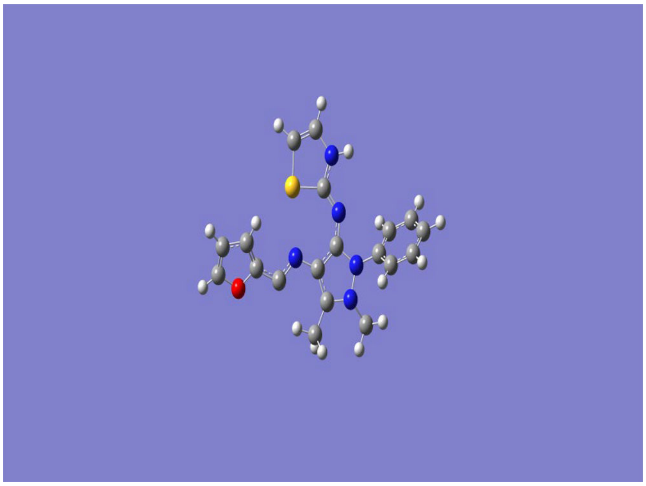


Figure 3. Optimized structural geometry of **L**.

Experimental outcome of α^2 (0.88) reveals the covalent character of [CuLphen] complex in addition to β^2 (1.23) and also γ^2 (0.32), respectively. The bonding capacity of [CuLphen] has been derived using magnitudes of $K_{||}$ along with $K\perp$ values. The relationship between pure σ-bonding, $K_{||} \approx K\perp = 0.77$, moreover $K_{||} < K\perp$ stands for in-plane π-bonding, and $K_{||} > K\perp$ for out-of-plane π-bonding.[29,30] In the present [CuLphen] the predicted values are showing $K_{||}(1.0) > K\perp$ (0.28) which implies a larger involvement from out-of-plane π-bonding and the absence of in-plane-π-bonding to the complex.

DFT computational studies

The optimized structure of **L** was obtained from computational studies of the Gaussian 6.0 program DFT-B3LYP/6-312G. The optimized **L** structure was shown in Figure 3 and the bond length and bond angle parameters were given in Table 2. The bond length and bond angle values of calculated and experimental results are almost similar in range.

Mulliken charge distribution

Mulliken charge distribution of the optimized geometry of **L** structure was analyzed and the results are given in Table 3 as well as atomic charges of the optimized molecular structure of **L** were shown in Figure 4. This result shows that the electronegativity of the atoms plays a key role in Mulliken charge distribution. The connection between two atoms shows higher and lower electronegativity. Studies show that the higher electronegativity of the atom possesses negative charge and lower electronegativity possesses a positive charge.[31] Heteroatoms like N(4), N(5), N(24), N(25), and O(37) showing negative charges with electron-donating property and S(28) is having a positive charge. The negative atom bearing heteroatoms tends to coordinate with metals. Mulliken charge distribution says that except C(10) and

Table 2. Bond length and bond angle parameters.

Bond lengths (Å)		Bond angles (°)	
C(1)–C(2)	1.391	C(2)–C(1)–N(5)	108.0043
C(1)–N(5)	1.345	C(2)–C(1)–N(25)	134.8416
C(1)–N(25)	1.4077	N(5)–C(1)–N(25)	117.1097
C(2)–C(3)	1.3898	C(1)–C(2)–C(3)	106.6529
C(2)–N(26)	1.3888	C(1)–C(2)–N(26)	121.7294
C(3)–N(4)	1.4864	C(3)–C(2)–N(26)	131.4653
C(3)–C(10)	1.405	C(2)–C(3)–N(4)	108.8807
N(4)–N(5)	1.4656	C(2)–C(3)–C(10)	128.9191
N(4)–C(6)	1.4284	N(4)–C(3)–C(10)	122.1717
N(5)–C(14)	1.0974	C(3)–N(4)–N(5)	107.8497
C(6)–H(7)	1.09	C(3)–N(4)–C(6)	124.5318
C(6)–H(8)	1.0895	N(5)–N(4)–C(6)	119.1217
C(6)–H(9)	1.099	C(1)–N(5)–N(4)	108.336
C(10)–H(11)	1.0987	C(1)–N(5)–C14)	127.7195
C(10)–H(12)	1.1043	N(4)–N(5)–C(14)	121.4629
C(10)–H(13)	1.4011	N(4)–C(6)–H(7)	111.2423
C(14)–C(15)	1.4029	N(4)–C(6)–H(8)	110.3744
C(14)–C(16)	1.3977	N(4)–C(6)–H(9)	107.5357
C(15)–C(17)	1.0826	H(7)–C(6)–H(8)	109.4356
C(15)–H(18)	1.3982	H(7)–C(6)–H(9)	109.2988
C(16)–C(19)	1.0846	H(8)–C(6)–H(9)	108.9009
C(16)–H(20)	1.4006	C(3)–C(10)–H(11)	111.9822
C(17)–C(21)	1.085	C(3)–C(10)–H(12)	112.3386
C(17)–H(22)	1.4001	C(3)–C(10)–H(13)	105.5111
C(19)–C(21)	1.0849	H(11)–C(10)–H(12)	108.3592
C(19)–H(23)	1.0849	H(11)–C(10)–H(13)	105.2778
C(21)–H(24)	1.3012	H(12)–C(10)–H(13)	113.1918
N(25)–C(27)	1.34	N(5)–C(14)–C(15)	119.4453
N(26)–C(34)	1.8482	N(5)–C(14)–C(16)	120.0289
C(27)–S(28)	1.3777	C(15)–C(14)–C(16)	120.5251
C(27)–N(43)	1.8457	C(14)–C(15)–C(17)	119.4633
S(28)–C(31)	1.3446	C(14)–C(15)–H(18)	119.3923
C(29)–H(32)	1.0807	C(17)–C(15)–H(18)	121.1437
C(29)–N(43)	1.397	C(14)–C(16)–C(19)	119.5545
H(30)–N(43)	1.0097	C(14)–C(16)–H(20)	119.5444
C(31)–H(33)	1.0778	C(19)–C(16)–H(20)	120.8953
C(34)–C(35)	1.4467	C(15)–C(17)–C(21)	120.3654
C(35)–C(36)	1.385	C(15)–C(17)H(22)	119.5678
C(35)–O(37)	1.4247	C(21)–C(17)–H(22)	120.0625
C(36)–C(38)	1.4313	C(16)–C(19)–C(21)	120.2203
C(36)–H(39)	1.0781	C(16)–C(19)–H(23)	119.6007
O(37)–C(40)	1.3807	C(21)–C(19)–H(23)	120.1732
C(38)–C(40)	1.3709	C(17)–C(21)–C(19)	119.8594
C(38)–H(41)	1.0802	C(17)–C(21)–H(24)	120.0891
C(40)–H(42)	1.0767	C(19)–C(21)–H(24)	120.0514
		C(1)–N(25)–C(27)	132.3628
		C(2)–N(26)–C(34)	117.0618
		N(25)–C(27)–S(28)	133.5578
		N(25)–C(27)–N(43)	118.3981
		S(28)–C(27)–N(43)	108.0408
		S(27)–C(28)–C(31)	88.5633
		C(31)–C(29)–N(32)	127.9211
		C(31)–C(29)–H(43)	113.4248
		H(32)–C(29)–N(43)	118.6541
		S(28)–C(31)–C(29)	111.9386
		S(28)–C(31)–H(33)	120.044
		C(29)–C(31)–H(33)	128.0135
		N(26)–C(34)–C(35)	114.1938
		C(34)–C(35)–C(36)	137.5837
		C(34)–C(35)–O(37)	115.1268
		C(36)–C(35)–O(37)	106.9776
		C(35)–C(36)–C(38)	108.567
		C(35)–C(36)–H(39)	124.464
		C(38)–C(36)–H(39)	126.963
		C(35)–O(37)–C(40)	107.6267
		C(36)–C(38)–C(40)	106.6195
		C(36)–C(38)–H(41)	127.1336
		C (40)–C(38)–H(41)	126.2465
		O(37)–C(40)–C(38)	110.1979
		O(37)–C(40)–H(42)	116.2116
		C(38)–C(40)–H(42)	133.5899
		C(27)–N(43)–C(29)	118.0286
		C(27)–N(43)–H(30)	117.9283
		C(29)–N(43)–H–(30)	124.0426

Table 3. Mulliken charges (*e*) for the **L**.

Atom	Charge/e
C(1)	0.448849
C(2)	0.133349
C(3)	0.399648
N(4)	−0.40523
N(5)	−0.56971
C(6)	−0.25083
H(7)	0.173467
H(8)	0.175688
H(9)	0.185176
C(10)	−0.46061
H(11)	0.154958
H(12)	0.12404
H(13)	0.207032
C(14)	0.253749
C(15)	−0.08249
C(16)	−0.08988
C(17)	−0.15537
H(18)	0.154491
C(19)	−0.14977
H(20)	0.157771
C(21)	−0.10395
H(22)	0.136737
H(23)	0.140011
H(24)	0.13635
N(25)	−0.53465
N(26)	−0.51938
C(27)	0.202046
S(28)	0.561898
C(29)	0.205973
H(30)	0.343331
C(31)	−0.53189
H(32)	0.172404
H(33)	0.169692
C(34)	0.070074
C(35)	0.059686
C(36)	−0.11505
O(37)	−0.49342
C(38)	−0.19388
H(39)	0.142635
C(40)	0.111612
H(41)	0.122916
H(42)	0.145335
N(43)	−0.63273

C(31) atoms, all the other atoms participated in metal coordination. The two atoms C(10) and C(31) having a comparatively large negative charge and they do not involve in coordination bonding with metals because of steric hindrance among the C(10) and C(31) methyl groups.

Antimicrobial analysis

Agar well diffusion experimental technique was adopted for the *in vitro* antimicrobial analysis of selected bacterial as well as fungal strains. The synthesized **L**, [CoLphen], [NiLphen], [CuLphen] and also [ZnLphen] complexes were treated against bacterial species such as *P. aeruginosa*, *S. aureus* as well as *E. coli* together with fungal species *A. niger* and *C. albicans*. The results of antimicrobial studies are provided in Table 4. The standard chloramphenicol used for antibacterial in addition to clotrimazole as standard for antifungal studies.

Among the [CoLphen], [NiLphen], [CuLphen], and [ZnLphen] metal(II) complexes [CuLphen] complex exhibits higher antibacterial activity. Other complexes like

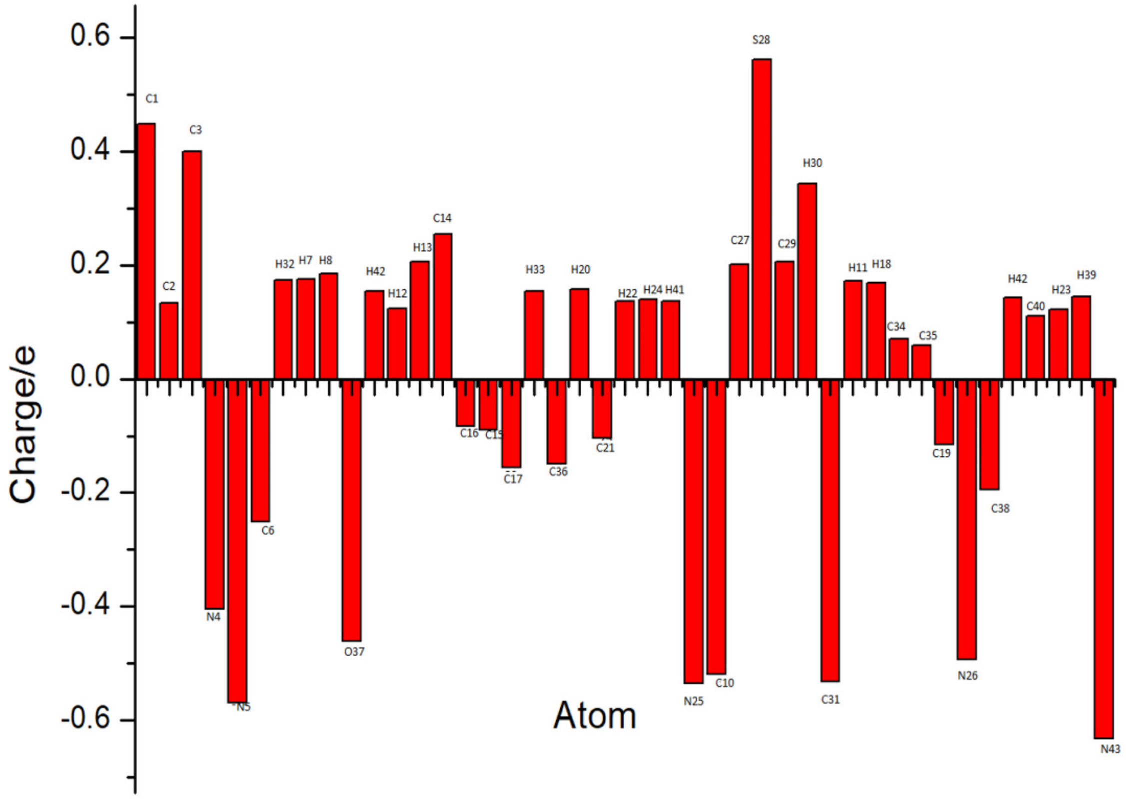


Figure 4. Atomic charges of the optimized molecular structure of **L**.

Table 4. Antimicrobial results of **L** and its complexes.

Compound	Antibacterial activity						Antifungal activity			
	Inhibition zone (mm) ± SD						Inhibition zone (mm) ± SD			
	P. aeruginosa		*E. coli*		*S. aureus*		*C. albicans*		*A. niger*	
Conc(μg/ml)	15	30	15	30	15	30	15	30	15	30
L	10 ± 0.13	12 ± 0.22	13 ± 0.14	13 ± 0.1	11 ± 0.01	12 ± 0.15	13 ± 0.14	14 ± 0.13	13 ± 0.22	13 ± 0.14
[CoLphen]	7 ± 0.07	9 ± 0.21	9 ± 0.21	11 ± 0.03	7 ± 0.07	10 ± 0.13	14 ± 0.16	14 ± 0.24	12 ± 0.11	12 ± 0.01
[NiLphen]	10 ± 0.13	12 ± 0.2	12 ± 0.22	16 ± 0.07	10 ± 0.03	11 ± 0.03	15 ± 0.18	16 ± 0.01	12 ± 0.14	12 ± 0.01
[CuLphen]	12 ± 0.22	14 ± 0.21	13 ± 0.14	23 ± 0.21	15 ± 0.42	18 ± 0.5	14 ± 0.16	18 ± 0.61	17 ± 0.41	17 ± 0.48
[ZnLphen]	13 ± 0.14	26 ± 0.19	12 ± 0.22	17 ± 0.09	10 ± 0.13	12 ± 0.22	13 ± 0.14	15 ± 0.04	13 ± 0.35	13 ± 0.21
Chloramphenicol	25 ± 0.28	28 ± 0.65	30 ± 0.8	34 ± 0.78	26 ± 0.46	28 ± 0.6	–		–	
Clotrimazole	–		–		–		24 ± 0.35	26 ± 0.15	23 ± 0.24	25 ± 0.31

SD: Standard deviation.

[CoLphen], [NiLphen] and also [ZnLphen] complexes exhibit better activity in the path of microorganisms than the **L**. The potent antibacterial function of Cu(II) complex has been studied in the consequences of Cu(II) ion in the normal cell method. Compounds having –CH=N unit shows better antimicrobial effect than > C=C < unit. Most of the microorganisms inclusive of > C=C < unit are responsible for taken up O_2, so they are not involved in the growth of microorganisms. The better activities of metal(II) complexes have been interpreted in terms of Overtone's concept together with Tweedy's Chelation Theory.[32] A literature report of Overtone's concept explained that cell permeability together with lipophilicity is a major condition for antimicrobial activity, which controls the antibacterial activity. The lipophilic nature of the central metal ion is lifted by chelation. The MIC value for investigated bacterial species of *P. aeruginosa* 17 μg/mL, *E. coli* 32 μg/mL as well as *S. aureus* 30 μg/mL. In the case of fungal species, [CoLphen], [NiLphen] and also [ZnLphen] complexes display moderate activity toward *A. niger* as well as *C. albicans.* The [CuLphen] complex possesses higher activity towards the two fungi compared to **L**.

DNA cleavage studies

The cleavage capacity of prepared complexes together with DNA is examined by using agarose gel electrophoresis in the path of nicking assay (Figure 5). It is an efficient technique used for the interpretation of DNA that affects the double helix and strand breaks. Their relative charge and size are essential for the separation of analytes. This experiment was conducted in metal complexes with the presence

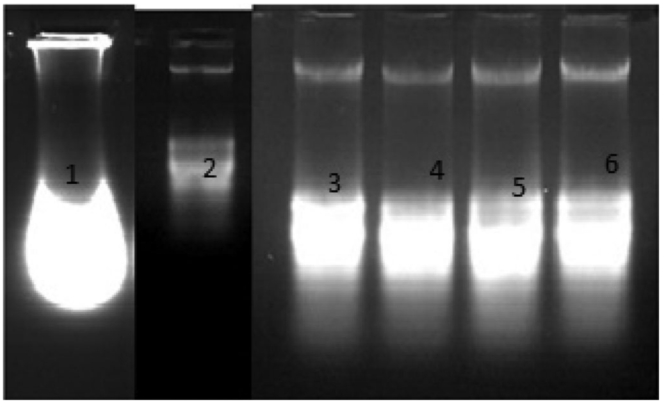


Figure 5. Agarose gel electrophoresis possess cleavage of pUC18 DNA by lane 1: DNA control, lane 2: **L** + pUC18 DNA, lane 3: [CoLphen] + pUC18 DNA + H_2O_2; lane 4: [NiLphen] + pUC18 DNA + H_2O_2; lane 5: [CuLphen] + pUC18 DNA + H_2O_2; lane 6: [ZnLphen] + pUC18 DNA + H_2O_2.

as well as the absence of H_2O_2 as an oxidant and pUC18 DNA. Cleavage of DNA is controlled by slow down the supercoiled spherical shape of pUC18 DNA into a nicked spherical shape as well as linear shape. The electrophoresis technique were adopted for the plasmid DNA, we observed the very quick movement of the supercoiled shape (Form I). Now the single strand of the supercoiled shape (Form I) was cleaved and generated a slow movable open circular shape (Form II). Finally, the above two strands were cleaved and a linear shape (Form III) will be generated. The above linear shape should be moved in between the supercoil as well as the open circular shape.[33] The [CoLphen], [NiLphen], [CuLphen] in addition to [ZnLphen] complexes have appreciable growth in the concentration of bands in open circular shape. Thus the above complexes have significant nicking activity. The DNA is cleaved through complexes effectively in the existence of oxidant that is due to the development of OH˙ Radical.[34] The biochemical reaction with mixed ligand metal complexes as well as an oxidant can produce OH˙ radical, this was explained by the following equation.

$$\mathbf{H_2O_2 + M^{n+} \rightarrow M^{n+1} + {}^{\cdot}OH + OH^-}$$

Conclusion

The biologically active **L**, [CoLphen], [NiLphen], [CuLphen], and [ZnLphen] complexes were developed and also characterized based on spectro-analytical data. Molar conductance measurements confirm the non-electrolytic environment of **L** as well as its metal complexes. ^{1}H NMR spectra confirm the coordination of azomethine proton. IR spectra specify, on comparison with **L** the (–CH=N–) band is shifted to lower wavenumber among the complexes. Electronic absorption spectra and magnetic moment studies indicate that the [CoLphen], [NiLphen] and [ZnLphen] have octahedral geometry and [CuLphen] possess distorted octahedral geometry. Powder XRD records suggest nanocrystalline character of the complexes. ESR calculations pointed out the distorted octahedral geometry of [CuLphen] complex. DFT studies provide information about the optimized geometry of **L**, reactivity parameters and Mulliken charge distribution of the atoms. Among the complexes [CuLphen] shows potent biological activity toward antimicrobial analysis. pUC18 DNA cleavage studies exhibit effective cleavage of DNA via the hydrolytic pathway. Thus the prepared 1,10-phenanthroline mixed ligand metal(II) complexes possess remarkable biological application suggests that have been used for significant drug design.

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