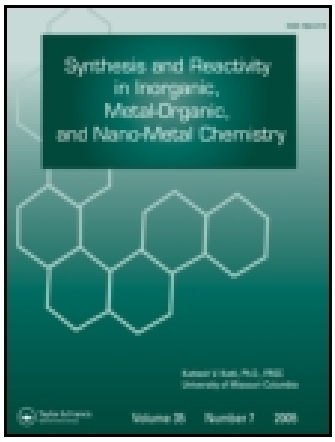

Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry

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Microwave-Assisted Synthesis of Mn(II), Co(II), Ni(II), Cu(II), and Zn(II) Complexes of Tridentate Schiff Base N-(2-hydroxyphenyl) 2-hydroxy-5-bromobenzaldimine: Characterization, DNA Interaction, Antioxidant, and In Vitro Antimicrobial Studies

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Microwave-Assisted Synthesis of Mn(II), Co(II), Ni(II), Cu(II), and Zn(II) Complexes of Tridentate Schiff Base N-(2-hydroxyphenyl) 2-hydroxy-5-bromobenzaldimine: Characterization, DNA Interaction, Antioxidant, and In Vitro Antimicrobial Studies

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Five mononuclear complexes of Mn(II), Co(II), Ni(II), Cu(II), and Zn(II) with the Schiff base N-(2-hydroxyphenyl) 2-hydroxy-5-bromobenzaldimine were synthesized under microwave irradiation. The microwave irradiation method was found remarkably successful and gave higher yield at a less reaction time. The complexes were fully characterized by elemental analyses, FT-IR, ^{1}H NMR, EPR, FAB mass spectra, electronic spectra, molar conductivity, magnetic susceptibility measurements, and thermogravimetric analyses (TGA). Structural compositions were assigned by mass spectral studies. Four-coordinate geometry has been assigned to these complexes tentatively. The interaction of the complexes with calf thymus DNA (CT-DNA) was investigated by absorption spectroscopy, emission spectroscopy, and viscosity measurements. The experimental evidences indicated that the binding strength follow the order Cu(II) > Mn(II) > Co(II) > Ni(II) >Zn (II). Moreover, the complexes were examined for their antioxidant activities against reactive oxygen species ($O_2^{\cdot-}$ and $HO^{\cdot}$ radicals). Additionally, the ligand and its complexes were screened in vitro for their antibacterial and antifungal activities. Preliminary information obtained from the present work would help in the development of nucleic acid molecular probes and new therapeutic reagents for diseases.

Keywords antimicrobial activity, antioxidant activity, characterization, complexes, DNA binding, microwave synthesis, O∩N∩O donor Schiff base

INTRODUCTION

Transition metal complexes have been widely applied in the field of medicine for centuries, although their molecular mechanism has not yet been entirely understood[1,2]. The medicinal properties of metal complexes depend on the nature of the metal ions and the ligands[3]. It is well known that metal ions present in the complexes are not only accelerating the drug action but also increase the effectiveness of the organic ligands[4].

A great number of chemotherapeutic anticancer drugs are those compounds interacting with DNA directly or preventing the proper relaxation of DNA. Also, over production of activated oxygen species in the forms of superoxide anion ($O_2^{\cdot-}$) and hydroxyl radical ($HO^{\cdot}$), generated by normal metabolic process, is considered to be the main contributor to oxidative damages to biomolecules such as DNA, lipids, and proteins, thus accelerating cancer, aging, inflammation, cardiovascular, and neurodegenerative diseases[5].

In this connection,studies of interaction between DNA and Schiff base metal complexes of multidentate aromatic ligands have been of current interest in the field of medicinal research and in the development of new therapeutic agents, mainly due to the fact that they exhibit novel electronic and/or magnetic properties with fascinating structural features, as well as the ability of these ligands to be modulated to DNA binding and cleaving abilities[6].

In the present work, the tridentate Schiff base ligand *N*-(2-hydroxyphenyl) 2-hydroxy-5-bromobenzaldimine (LH_2) and its Mn(II), Co(II), Ni(II), Cu(II), and Zn(II) complexes were synthesized by microwave-assisted synthesis as a powerful synthetic tool. In order to investigate the coordination mode of ligand, the complexes have been fully characterized by FT-IR, ^{1}H NMR, FAB-mass UV–VIS, magnetic measurements, and EPR studies. The DNA binding properties of the complexes have been investigated by absorption, fluorescence, and viscosity measurements. Moreover, the ligand and its complexes are also tested for their antioxidant in vitro antimicrobial activities.

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Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/lsrt.

EXPERIMENTAL

Materials

2-hydroxy-5-bromobenzaldehyde (5-Br-salicylaldehyde) was supplied from Merck, 2-aminophenol, $Mn(NO_3)_2.4H_2O$, $Co(NO_3)_2.6H_2O$, $Ni(NO_3)_2.6H_2O$, and $Cu(NO_3)_2.3H_2O$ and $Zn(NO_3)_2.6H_2O$ were supplied from Sigma–Aldrich. All solvents were of analytical grade.

Instruments

FT-IR spectra in KBr pellets (4000–400 cm^{-1}) were carried out on a Unicam-Mattson 1000 FT-IR. Elemental analyses were carried out on a JEOL JMS-AX500 Mass Spectrometer. ^1H NMR measurements were performed on a Spectrospin-Bruker AC 300 MHz spectrometer using DMSO-d_6 as a solvent and TMS as an internal reference. Fast atomic bombardment mass spectra (FAB-MS) were recorded with a VGZAB-HS spectrometer in a 3-nitrobenzylalcohol matrix. Molar conductivities of 1×10^{-3} M DMSO complex solutions were measured by using Jenway 4010 conductivity meter. Magnetic moments were determined on a Sherwood Scientific magnetic moment balance (Model N0: MK1) at room temperature (25°C) using $Hg[Co(SCN)_4]$ as a calibrant. Corrections for diamagnetism of the constituents were made using Pascal constants[7]. Thermogravimetric analyses (TGA) were carried out using a Shimadzu DT-50 thermal analyzer under nitrogen atmosphere with a heating rate of 10°C/min. Electron spin resonance (ESR) measurements of solid complexes were recorded at room temperature on Bruker EPR spectrometer at 9.706 GHz (X-band), the microwave power was (1.0 mW) with 4.0 G modulation amplitude, using 2,2-diphenylpyridylhydrazone (DPPH) as standard ($g = 2.0037$). Electronic spectra were recorded on a Shimadzu UV 1800 spectrophotometer, equipped with a PC, using UV Probe software, version 2. Fluorescence spectra were recorded on a Jenway6270Flourimeter at room temperature. Microwave-assisted synthesis was performed in an open glass vessel on a modified microwave oven model 2001 ETB with rotating tray and a power source 230 V, microwave energy output 800 W, and microwave frequency 2450 MHz.

Microwave-Assisted Syntheses

The use of microwave heating for the synthesis of the Schiff base ligand and its complexes allows a drastic reduction of the reaction times required by conventional heating and affords comparable or better yields.

Synthesis of N-(2-hydroxyphenyl) 2-hydroxy-5-brormobenzaldimine (LH_2)

0.1 mol of 2-amiophenol and 0.1 mol of 5-bromo-2-hydroxybenzaldehyde(5-Br-salicylaldehyde) were mixed well in a 50 mL Pyrex beaker and the mixture was irradiated in a microwave oven for 2 min. The reaction was monitored by thin layer chromatography (TLC). After completion of the reaction, the reaction mixture was allowed to attain room temperature and the yellow product obtained was recrystallized from ethanol.

Synthesis of Complexes

All the new Schiff base complexes were prepared by the same general procedure with stoichiometric amount of ligand: hydrated metal nitrate in a 1:1 mole ratio were mixed in a grinder. The reaction mixture was then irradiated in the microwave oven using 3–4 mL ethanol. The reaction was completed in ~ 6 min. The mixture was cooled to room temperature and poured into ice chilled ethanol, filtered, washed several times with hot petroleum ether (60–80°C) and dried in vacuum over P_2O_5.

Synthetic route of *N*-(2-hydroxyphenyl) 2-hydroxy-5-brormobenzaldimine and its M(II) complexes is shown in Scheme 1.

Determination of the Metal Content of the Complexes

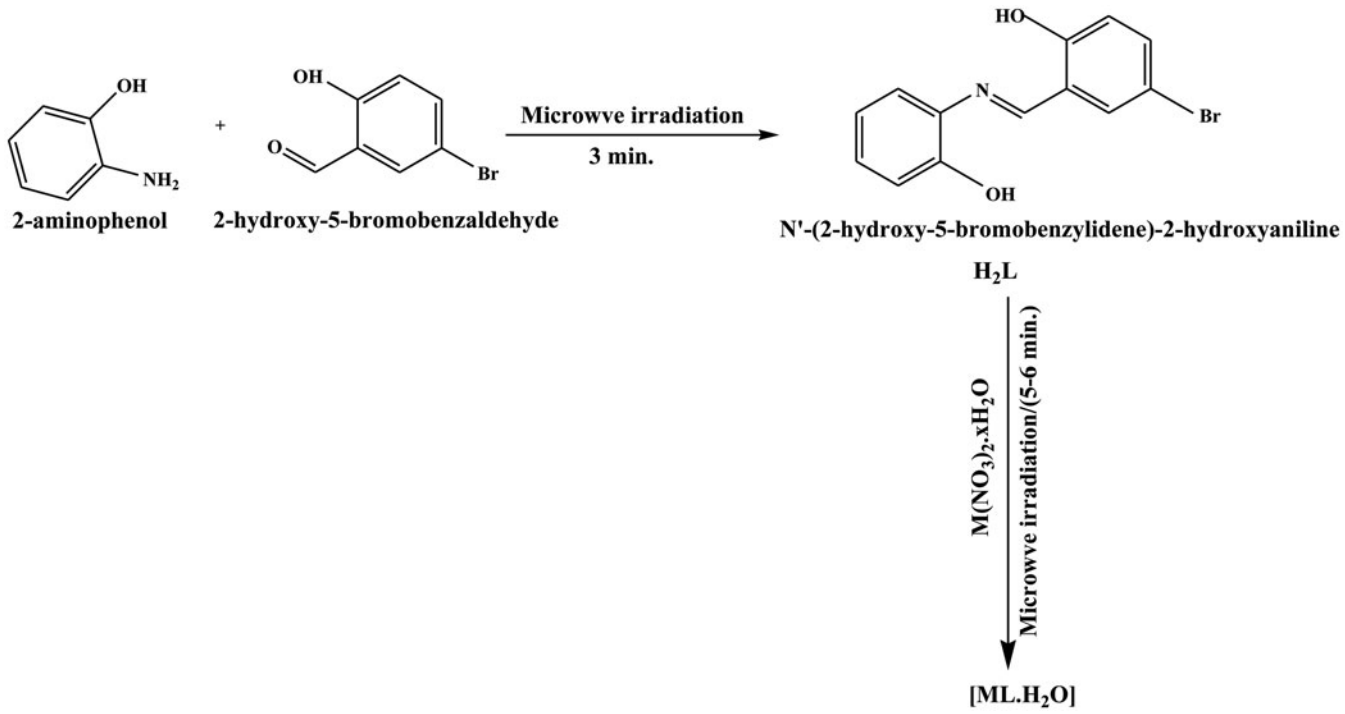
Thirty milligram of the different chelates was transferred to Kjeldahl flask. 5.0 mL concentrated nitric acid (1.0 M) was added initially to the powdered chelates, to start the fast wet oxidation digestion. This mixture had been digested by a gradual heating with dropping of H_2O_2 solution. This treatment was conducted until most of the powdered complexes were diminished and the remaining solution had the color of the corresponding metal salt. This solution was diluted up to 50 mL by deionized water and the metal content was determined by analytical methods described in the literature[8–10].

CT-DNA Interaction Studies

All experimental involving CT-DNA were performed in HCl/NaCl (5:50 mM) buffer solution (pH = 7.24). *Tris*-HCl was prepared using deionized and sonicated triple distilled water and kept at 4°C for 3 days. The absorption ratio of CT-DNA solutions A_{260}/A_{280} was 1.9:1, indicating that the DNA was sufficiently free from protein[11]. The DNA concentration was determined via absorption spectroscopy using the molar absorption coefficient of 6600 M^{-1} cm^{-1} (260 nm) for CT-DNA[12].

Stock solutions of metal complexes were prepared by dissolving them in dimethyl sulfoxide (DMSO) and suitably diluting them with the corresponding buffer to the required concentrations for all experiments. The extent of DMSO in the final concentration did not exceed 0.1% in the tested solutions. At this concentration, DMSO was not found to have any effect on DNA conformation.

Absorption titration experiments were carried out by varying the DNA concentration in the range of (0–10 μM) and maintaining the complex concentration constant at (50 μM). Upon measuring the absorption spectra, equal amount of DNA was added to both the complex solution and the reference solution to eliminate the absorbance of DNA itself. The reference solution was the corresponding buffer solution. Absorbance values were recorded after each successive addition of DNA solution and equilibration for ~ 10 min. Each sample was measured three times and an average value was calculated. The absorption data were analyzed for an evaluation of the intrinsic binding constant K_b of the complexes with CT-DNA.



SCH. 1.

DNA competitive binding studies with ethidium bromide solution (EB) were carried out by keeping the concentration of EB (30 μM) and DNA (200 μM) as constant and varying the concentration (0–50 μM) of the complexes. Before measurements, the resulting solutions were shaken up and incubated for 30 min. The emission spectra were recorded in the wavelength range of 500–700 nm at 595 nm (478 nm excitation wavelength).

Viscosity measurements were carried out using an Oswald microviscometer, maintained at constant temperature (30 $\pm$ 0.1°C) in a thermostat water bath. The DNA concentration was kept constant in all samples, but the ligand and its complexes concentration were increased from 10 to 50 μM. Mixing of the solution was achieved by bubbling the nitrogen gas through viscometer. The mixture was left for 10 min at 30 $\pm$ 0.1°C after addition of each aliquot of complex. The flow time was measured with a digital stopwatch. The experiment was repeated in triplicate to get the concurrent values. The data are presented as $(\eta/\eta_o)^{1/3}$ versus the ratio [complex]/[DNA], where η and η_o are the specific viscosity of DNA in the presence and the absence of the complex, respectively. The values of η and η_0 were calculated by the following equation:

$$\eta = (t - t_o)/t_o,$$

where t_o is the observed flow time of DNA containing solution and t is the flow time of buffer alone. Relative viscosities for DNA were calculated from the relation, η/η_o.

Antioxidant Activity

Scavenging Measurements of Superoxide Radical

The superoxide radicals were generated in the test system using NBT/VitB_2/MET and determined spectrophotometrically by the nitroblue tetrazolium photo reduction method[13–15]. The reported complexes (the final concentration: C_i (i = 1–6) = 5.0, 10.0, 20.0, 30.0, 40.0, 50.0 μM) were added to a solution containing [NBT (65 μM), L-MET (13 mM), VitB_2 (1.5 μM), EDTA (0.1 mM)] and the resulting solution was made up to 2 mL with phosphate buffer (10 mM, pH 7.0) in dark. The above mixture was illuminated with a white fluorescence lamp (15 W) for 15 min and the absorbance (A_i) was measured at 560 nm. The above mixture without no metal complex was used as a control and its absorbance was taken as A_o. All the experiments were conducted in triplicate and the data were expressed as the mean value. The suppression ratio was calculated by using the following equation:

$$O_2 \cdot^- \; scavenging \; activity\,(\%) = \frac{A_o - A_i}{A_o} \times 100.$$

Scavenging Measurements of Hydroxyl Radical

The hydroxyl radical (HO$^\cdot$) in aqueous media was generated through the Fenton reaction[16]. The solutions of the tested compounds were prepared with DMF. The reaction mixture contained 2.5 mL 0.15 M phosphate buffer (pH = 7.4), 0.5 mL 114 μM safranin, 1 mL 945 μM EDTA-Fe(II), 1 mL 3% H_2O_2,

TABLE 1
Elemental analyses, molar conductance and magnetic susceptibility data of the Schiff base ligand (LH_2) and its complexes

Compounds	Yield (%)	M.P. (°C)	M.Wt	Color	Calculated (Found) (%) C	H	N	Br	M	Λ_m (Ω^{-1} cm^2 mol^{-1})
(LH_2) $C_{13}H_{10}NBrO_2$	99	176	292.13	Orange	53.44 (53.32)	3.45 (3.38)	4.79 (4.76)	27.35 (27.16)	—	—
[MnL(H_2O)] (1) ($C_{13}H_{10}NBrO_3$)Mn	92	>300	363.06	Pale brown	43.00 (42.89)	2.77 (2.63)	3.85 (3.66)	22.00 (21.68)	15.13 (15.02)	7.3
[CoL(H_2O)] (2) ($C_{13}H_{10}NBrO_3$)Co	94	>300	367.06	Deep brown	42.53 (42.84)	2.74 (2.81)	3.81 (3.73)	21.77 (21.52)	16.05 (15.97)	16.5
[NiL(H_2O)] (3) ($C_{13}H_{10}NBrO_3$)Ni	95	>300	366.82	Brown	42.56 (42.48)	2.74 (2.62)	3.82 (3.77)	21.78 (21.66)	16.00 (15.96)	9.5
[CuL(H_2O)] (4) ($C_{13}H_{10}NBrO_3$)Cu	96	>300	371.67	Dark green	42.01 (42.09)	2.71 (2.75)	3.76 (3.66)	21.49 (21.38)	17.07 (17.00)	6.2
[ZnL(H_2O)] (5) ($C_{13}H_{10}NO_3$)Zn	97	>300	373.52	Yellow	41.80 (41.71)	2.69 (2.66)	3.74 (3.69)	21.39 (21.26)	17.50 (17.41)	4.1

and 30 μL the tested compound solution (the final concentration: C_i ($I = 1$–6) = 5.0, 10.0, 20.0, 30.0, 40.0, 50.0 μM). The sample without the tested compound was used as the control. The reaction mixtures were incubated at 37°C for 1 h in a water bath. Absorbances (A_i, A_o, and A_c) at 520 nm were measured. The suppression ratio for HO· was calculated from the following expression:

$$HO \cdot \text{scavengingactivity}\,(\%) = \frac{A_o - A_o}{A_c - A_o} \times 100,$$

where A_i is the absorbance in the presence of the tested compound; A_o is the absorbance in the absence of the tested compound; and A_c is the absorbance in the absence of the tested compound, EDTA-Fe(II) and H_2O_2.

Screening for Antimicrobial Activity

The antibacterial and antifungal activities of the newly synthesized compounds were evaluated by the agar well diffusion method[17] against the bacteria: gram +ve *(S. aureus and B. subtilis) and* gram −ve *(E. coli and P. aeruginosa)* and the fungi: *A. nig*er, *A. flavus, C. lunata*, and *C. albicans*. All the microbial cultures were adjusted to 0.5 McFarland Standard, which is visually comparable to a microbial suspension containing approximately 1.5×10^8 cfu/mL[18]. 20 mL of agar media was poured into each Petri plate and the plates were swabbed with 100 μL inocula of the test microorganisms and kept 15 min for adsorption. Using an 8 mm-diameter sterile cork borer, wells were bored into the seeded agar plates and these were loaded with 100 mL of a solution of each compound dissolved in the DMSO at a concentration of 1.0 mg/mL. All the plates were incubated for 24 h at 37°C in the case of bacteria strains and 48 h at 30°C in the case of fungi strains. The antimicrobial activity of all the synthesized compounds was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (Hiantibiotic zone scale). The medium with DMSO as solvent was used as the negative control, whereas media with *Streptomycin* (standard antibiotic) and *Nystatin* (standard antifungal drug) were used as the positive controls. The experiments were performed in triplicate.

RESULTS AND DISCUSSION

N-(2-hydroxyphenyl) 2-hydroxy-5-bromobenzaldimine Schiff base ligand and its metal complexes were synthesized under microwave irradiation and fully characterized by elemental analyses, FT-IR, ^{1}H NMR, EPR, FAB mass spectra, electronic spectra, molar conductivity, magnetic susceptibility measurements, and TGA. Elemental analysis data of the complexes indicated the formation of 1:1 [M:L] ratio. All the metal complexes were colored, stable toward air, insoluble in water, and common solvents but soluble in DMF and DMSO, except Zn(II) complex was soluble only in DMSO. The molar conductivities (Λ_m) of 1×10^{-3} M solutions of the complexes in DMSO fall in the range 4.1–16.5 Ω^{-1} mol^{-1} cm^2. These low values indicated that all of the complexes have non-electrolytic nature[19]. Elemental analyses and some physical properties of *N*-(2-hydroxyphenyl) 2-hydroxy-5-bromobenzaldimine (LH_2) and its reported complexes are listed in Table 1.

Characterization of the Complexes

FT-IR Spectra

The FT-IR spectra of the complexes are compared with that of the free ligand in order to determine the coordination sites which may involve in chelation. The characteristic FT-IR data of *N*-(2-hydroxyphenyl) 2-hydroxy-5-bromobenzaldimine (LH_2) and their metal complexes listed in Table 2. The FT-IR spectra of the free ligand exhibited a characteristic broad band due to υ(OH) at 3431 cm^{-1}. The broadness of the band was attributed to the presence of internal hydrogen bond OH·····N=C[20]. Furthermore, high intensity bands were observed at 1624 cm^{-1} and 1278 cm^{-1}, which are assigned to υ(C=N) and υ(C−O) for the phenolic C−O in the case of salicylideneanilines[21].

In comparison with the free Schiff base ligand, all the complexes exhibited a broadband in the range 3384–3432 cm^{-1}, which was assignable to υ(OH) of the coordinated water molecule associated with the complexes. The

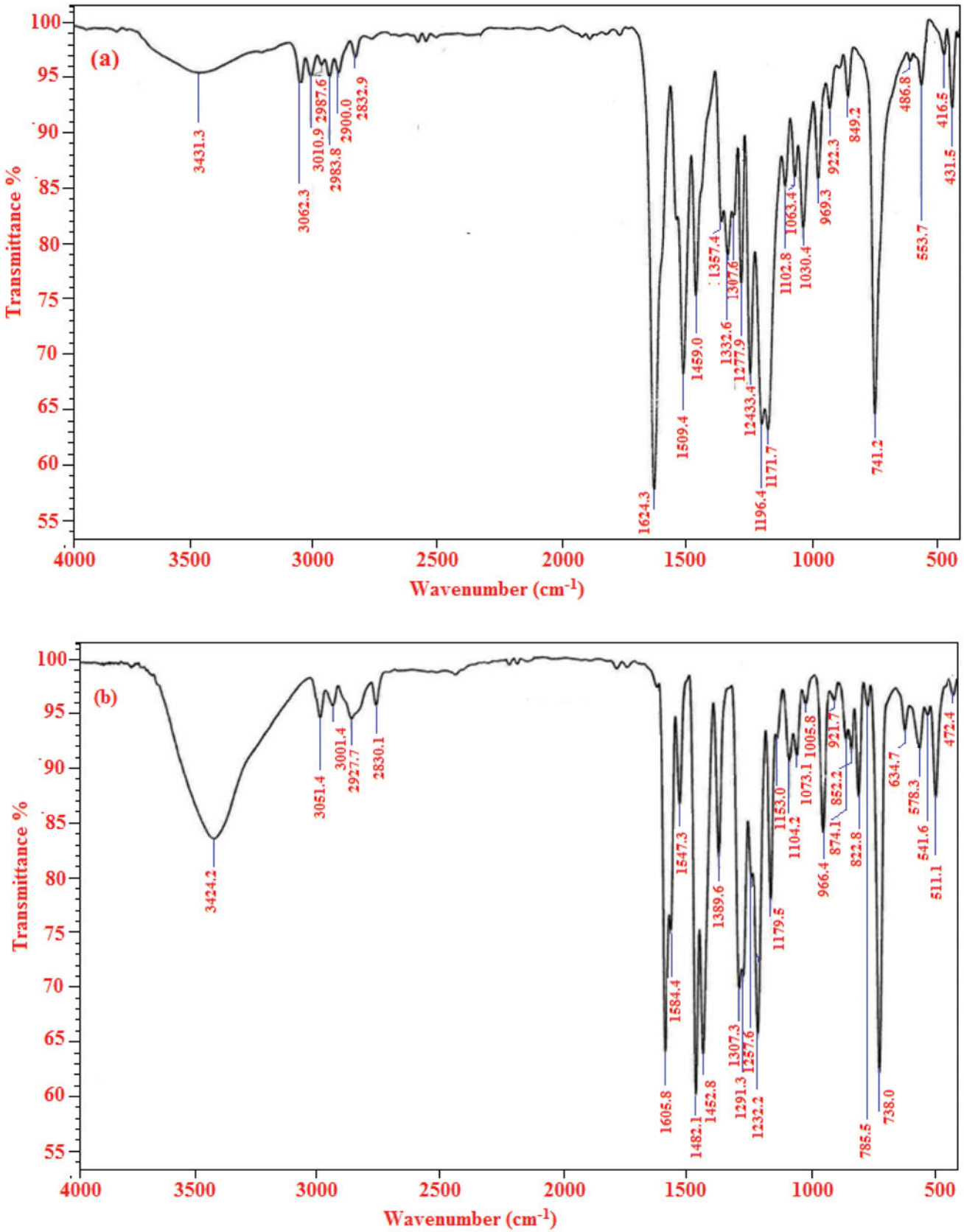


FIG. 1. FT-IR of LH_2 (a) and $[CuL(H_2O)]$ complex (b).

TABLE 2
Infrared (cm^{-1}, KBr) spectral data of LH_2 and its complexes

Compound	O—H	C=N	C—O	M—O	M—N
LH_2	3431(w, br.)	1624(s)	1278(m)	—	—
[MnL(H_2O)] (1)	3431(s, br.)	1605(s)	1289(m)	520(w)	427(w)
[NiL(H_2O)] (2)	3384(s, br.)	1607(s)	1297(m)	532(w)	434 (w)
[CoL(H_2O)] (3)	3618(w, br.)	1607(s)	1284(m)	512(w)	435(w)
[CuL(H_2O)] (4)	3424(s, br.)	1606(s)	1291(m)	511(w)	472(w)
[ZnL(H_2O)] (5)	3432(m, br.)	1610(s)	1290(m)	518(w)	442(w)

Notes.
*s: strong, m: medium, w: weak, br: broad.

presence of one water molecule was indicated from the thermogravimetric studies (vide infra). Moreover, in the FT-IR spectra of the complexes, the band of υ(C=N) is exhibited a shift to lower wave numbers in the region 1605–1610 cm^{-1} indicating that the nitrogen of azomethine group is coordinated to the metal ion[22]. Moreover, a medium to high intensity band appeared in the region 1284–1297 cm^{-1} due to phenolic υ(C–O), indicating the coordination of phenolic oxygen via deprotonation[24]. Finally, the most obvious feature after the complexation was found in the region 400–600 cm^{-1}. The non-ligand bands at 511–532 cm^{-1} and 427–442 cm^{-1} were observed due to M–N and M–O, which gave conclusive evidence regarding the bonding of azomethine nitrogen and phenolic oxygen of the Schiff base to the metal ion[20]. The FT-IR spectra of LH_2 and its Cu(II) complex as a representative example are shown in Figure 1.

^{1}H NMR Spectra of the Schiff Base and its Zinc(II) Complex

The ^{1}H NMR spectra of *N*-(2-hydroxyphenyl) 2-hydroxy-5-bromobenzaldimine (LH_2) and its diamagnetic Zn(II) complex recorded in d_6-DMSO at room temperature showed a sharp singlet signal at 8.67 and 8.85 ppm, respectively, which may be assigned to the azomethine hydrogen atom. The shift of the imine carbon proton signal to the downfield region in Zn(II) complex in comparison with that of the free ligand inferred coordination through the azomethine nitrogen atom of the ligand[23]. The multiplet signal due to the aromatic protons, exists in *N*-(2-hydroxyphenyl) 2-hydroxy-5-bromobenzaldimine at 6.99–7.78 ppm, exhibited a small shift in Zn(II) complex at 6.95–7.70 ppm. The small shift may be attributed to the changes in the electronic environment around the protons attached to the group that contain the site of donation and involvement in complexation[24]. Moreover, the ^{1}H NMR spectrum of the parent ligand showed two singlet signals at very downfield at δ 13.21 and 9.85 ppm, which were attributed to two phenolic –OH protons. A comparison of ^{1}H NMR spectrum of diamagnetic Zn(II) complex (Figure 2) showed that the chemical shift observed for the OH protons in the ligand was missed in the Zn(II) spectrum. The absence of –OH signals indicated deprotonation of the hydroxyl group of the Schiff base ligand prior to coordination with Zn(II) and confirmed the bonding of oxygen to the metal ions (C–O–M)[25]. The characteristic ^{1}H NMR data of *N*-(2-hydroxyphenyl) 2-hydroxy-5-bromobenzaldimine (LH_2) and its Zn(II) complex are listed in Table 3.

Mass Spectral Studies

The FAB-mass spectrum of Schiff base ligand (LH_2) depicted in Figure 3a showed a molecular ion peak [M^+] at *m/z* 292 which is equivalent to its molecular weight corresponding with formula, $C_{13}H_{10}NBrO_2$. In addition, the fragment peaks observed at *m/z* 185 and 107 were assigned to the fragment ions $[C_6H_5NO]^+$ and $[C_7H_5BrO]^+$, respectively. The FAB-mass spectra of Mn(II), Co(II), Ni(II), Cu(II), and Zn(II) complexes showed a molecular ion peak M^+ corresponding to *m/z* 363, 367, 366, 371, and 373, respectively, consistent with monomeric nature of complexes. The FAB-mass spectrum of Cu(II) complex (Figure 3b) showed a fragmentation peak at *m/z* 353, corresponding to the loss of water molecule and a fragmentation peak at *m/z* 308, corresponding to demetallation of [CuL(H_2O)] to form the species $[L + H]^+$. The other reported complexes showed similar patterns. Thus, the mass spectral data results along with elemental analyses agree with the formation of [ML(H_2O)] complexes with 1:1 stoichiometry.

Magnetic Properties of Complexes

Magnetic measurements were recorded in order to obtain information about the geometry of the complexes. The magnetic

TABLE 3
^{1}H NMR spectral data (δ, ppm) of LH_2 and Zn(II) complex

Compound	δ (ppm)	Assignments
LH_2	13.21, 9.85	(s, 2H, –OH)
	8.67	(s, 1H, –CH=N)
	6.99–7.78	(m, 7H, ArH)
[ZnL(H_2O)] (5)	—	—
	8.85	(s, 1H, —CH=N)
	6.95–7.70	(m, 7 H), ArH)

*s: singlet, m: multiplet.

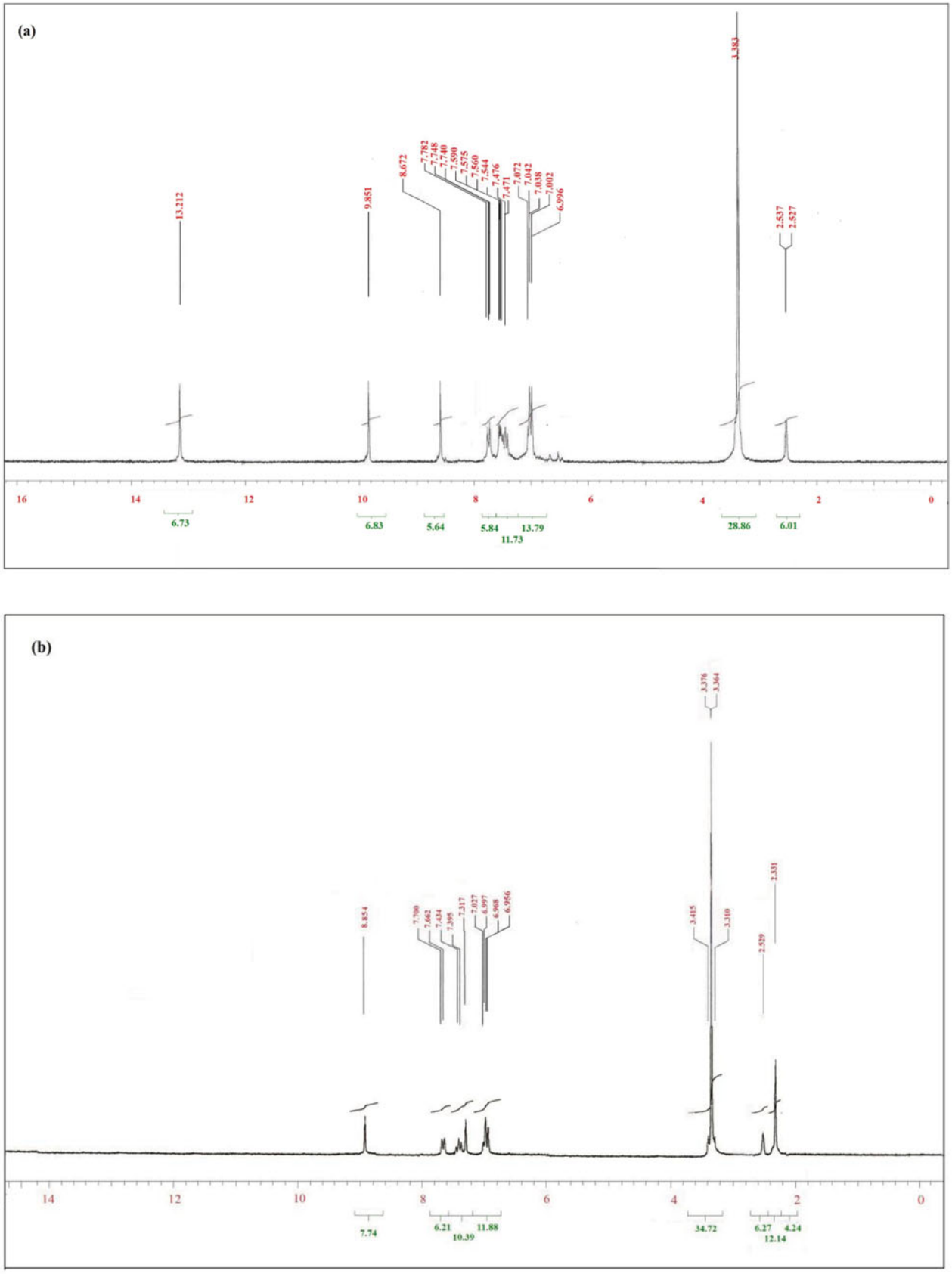


FIG. 2. ^{1}H NMR of LH_2 (a) and [ZnL (H_2O)] complex (b).

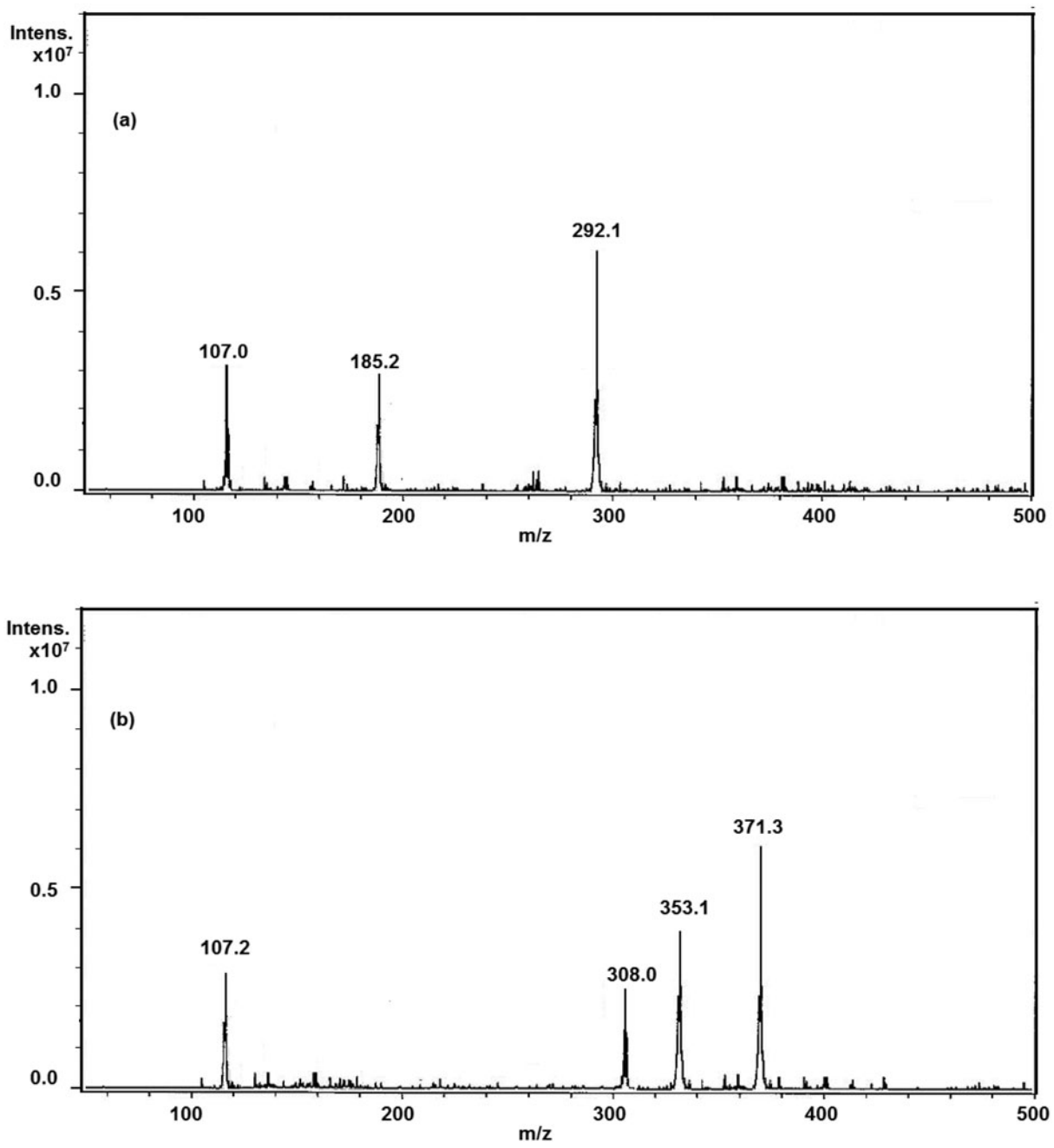


FIG. 3. FAB-mass of LH_2 (a) and [$CuL(H_2O)$] complex (b).

moments obtained at room temperature showed paramagnetism for Mn(II), Co(II), Ni(II), and Cu(II) complexes and dimagnetism for Zn(II) complex (Table 4). The magnetic susceptibility value of Cu(II) complex (1.80 BM) was an indicative of one unpaired electron per Cu(II) ion and suggested a square-planar geometry[26]. The magnetic moment of Ni(II) complex 3.84 BM was close to that of 3.0–3.5 B.M for the more distorted tetrahedral complexes[27]. The Co(II) complex has a magnetic susceptibility value of 4.74 BM which lies in the range (4.2–4.8 BM) known for most of the mononuclear Co(II) complexes in the tetrahedral environment[28]. In the case of Mn(II) complex, the magnetic moment value was found to be 5.23 BM, indicating the presence of Mn(II) complex in the tetrahedral structure. Finally, Zn(II) complex showed diamagnetism as expected for d^{10} configuration, in analogy with those described for Zn(II) complexes containing N–O donor Schiff bases[29] and according to the empirical formulae, a tetrahedral geometry was proposed for the Zn(II) complex.

TABLE 4
Electronic spectra and magnetic properties of LH_2 and its complexes

Compound	UV–VIS (DMSO, 10^{-3} M)	$\mu_{eff.}$ (BM)
LH_2	226[a], 346[b]	—
[MnL(H_2O)] (1)	238[a], 270[b], 440[c]	5.23
[NiL(H_2O)] (2)	232[a], 264[b], 446[c]	3.84
[CoL(H_2O)] (3)	242[a], 268[b], 402[c]	4.74
[CuL(H_2O)] (4)	268[a], 298[b], 442[c]	1.80
[ZnL(H_2O)] (5)	260[a], 304[b], 434[c]	Dia

Notes.
a: $\pi \rightarrow \pi^*$; b: n$\rightarrow\pi^*$; c: LMCT.

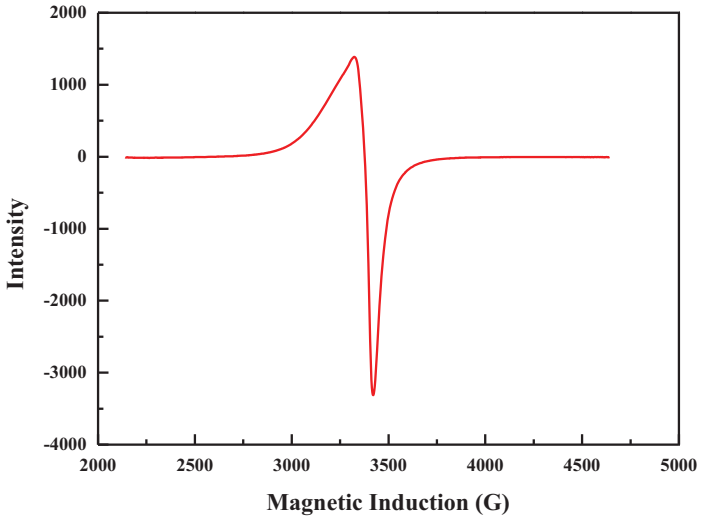


FIG. 4. EPR spectrum of [CuL(H_2O)] complex at 298 K.

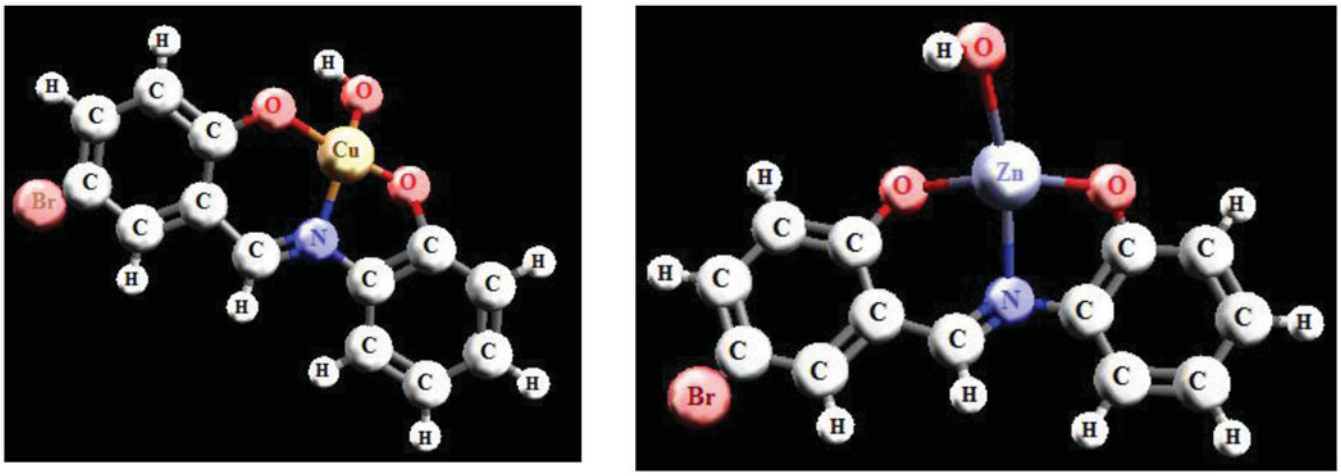
EPR Spectra of Cu(II) Complex

The EPR spectrum of solid Cu(II) complex (Figure 4) recorded at room temperature (298 K) was consistent with the square-planar geometry.[30] The "*g*" tensor values of the Cu(II) complexes could be used to obtain the ground state[31]. The Cu(II) complex (5) exhibited $g_{\|}$ value of 2.09 and g value of 2.02. The trend $g_{\|} > g > 2.0023$ indicate that the unpaired electron is localized in the d_{x2-y2} orbital of the Cu(II) ion[32,33] and characteristic of axial symmetry. The deviation of the calculated g_{av} value (2.07) from that of the free electron (2.0023) is due to the covalence property[34], which was supported by Kivelson and Neiman[35]. According to Hathaway[32], if the anisotropic value $G > 4$, the exchange interaction is negligible, while $G < 4$ indicates a considerable exchange interaction in the complex. The calculated G value for Cu(II) complex was 4.5, which suggested that the local tetragonal axis is aligned parallel or slightly misaligned and consistent with $d_{x2\text{-}y2}$ ground state. Moreover, this result indicates that the exchange interaction between Cu(II) centers is negligible[32,35]. The EPR spectra of the solid Mn(II), Co(II), and Ni(II) complexes at room temperature do not show EPR signal because the rapid spin lattice relaxation of the Mn(II), Co(II), and Ni(II) broaden the lines at higher temperatures[36].

From the above arguments, the proposed structure of Cu(II) complex and Zn(II) complex as an example of tetrahedral complexes is shown in Scheme 2.

Thermal Analysis Studies

Thermogravimetric analysis (TGA) is a very useful method for studying the thermal decompositions of solid substances, including simple metal salts and complex compounds[37]. Thermal studies of all the complexes were performed under nitrogen atmosphere in the temperature range of 20–1000°C. The thermal curves of the [MnL(H_2O)] (1) and [CuL(H_2O)] (4) as representative examples are given in Figure 5. Thermal analysis of the synthesized complexes confirmed the presence of a coordinated water molecule. The decomposition mass losses were found in agreement with the



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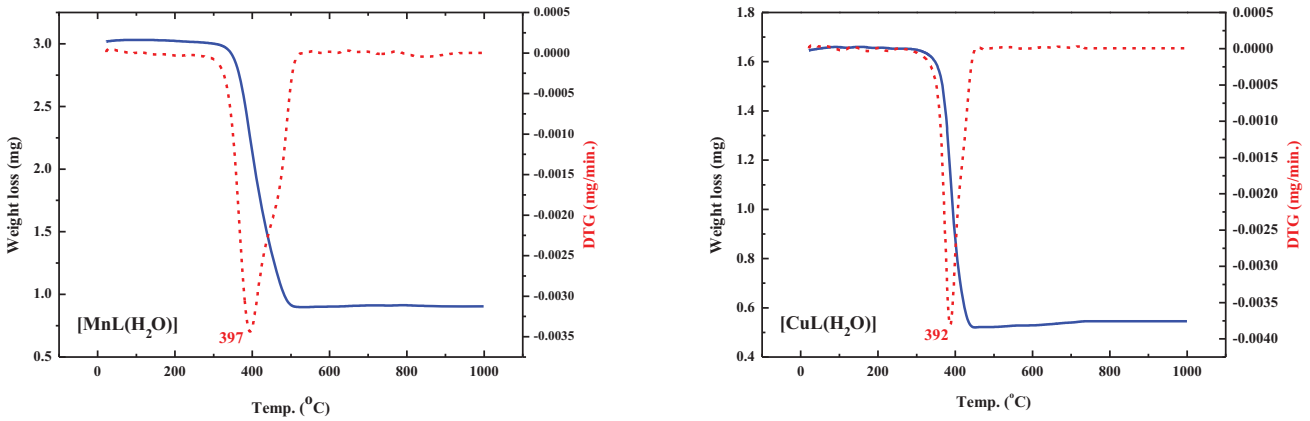

FIG. 5. The thermal curves of [MnL(H_2O)] and [CuL(H_2O)] complexes.

formula weightsss of each complex proposed from the elemental analysis. The thermal behavior of all the complexes is almost same. The TG data revealed that the complexes decomposes in one step with an endothermic peak in the DTA curves at around 262–669°C, corresponding to the elimination of coordinated water and organic ligand moieties, leaving metallic oxide as the final decomposition product. The residual metal oxide percentages were coincided fairly with the theoretical values. The nature of proposed chemical change with temperature and the percent of metal oxide obtained are given in Table 5.

DNA Binding Studies

Absorption Spectral Studies

The application of electronic absorption spectroscopy is one of the most useful techniques to investigate the binding mode of DNA with metal complexes[38]. The electronic absorption spectra of the investigated ligand and its complexes (1–5) consist of two or three well-resolved bands in the range of 226–446 nm. The high-energy absorption band appeared in the spectra of the ligand and its complexes below 400 nm are assigned π–π^* and n–π^* transitions[39] and the band observed around 400 nm in the complexes spectra may be assigned to LMCT transition, usually observed in metal Schiff base complexes containing phenolato ligands due to "phenolato-to-M" charge transfer[40]. The absorption spectra of the ligand and its complexes in the absence and the presence of CT-DNA are given in Figure 6. In the case of the ligand, with increasing DNA concentrations, a small change was observed in the absorption intensity which is consistent with its weak external contact with the duplex. For the complexes, the well-defined LMCT band observed was used to monitor absorption titration of the complexes with DNA solution. With increasing concentration of DNA, all the complexes showed hypochromicity and a redshifted charge transfer peak maxima, indicating the complexes are actively in associating with CT-DNA. These spectral characteristics obviously suggested that the titled complexes most likely interact with DNA through a mode of stacking interaction between aromatic chromophore of the complexes and the base pairs of DNA. For the quantitative investigation of the binding strength of the ligand and its complexes to CT-DNA, the intrinsic binding constants K_b of the complexes with CTDNA were calculated using the following function equation[41]:

$$\frac{[\mathrm{DNA}]}{(\varepsilon_a - \varepsilon_f)} = \frac{[\mathrm{DNA}]}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{[K_b\,(\varepsilon_b - \varepsilon_f)]}$$

TABLE 5
Thermogravimetric characteristics of the reported complexes

Complex	Temp. Range (°C)	Mass loss (%) Calculated (Found)	Assignment	Residue
[Mn(L)(H_2O)] (1) [Mn($C_{13}H_8NBrO_2$)(H_2O)]	290–530	76.05 (75.95)	$C_6H_4N + C_7H_4 + {}^1/_2\,Br_2 + H_2O$	MnO_2
[Co(L)(H_2O)] (2) [Co($C_{13}H_8NBrO_2$)(H_2O)]	396–669	79.58 (79.18)	$C_6H_4N + C_7H_4O + {}^1/_2\,Br_2 + H_2O$	CoO
[Ni(L)(H_2O)] (3) [Ni($C_{13}H_8NBrO_2$)(H_2O)]	389–558	79.63 (78.96)	$C_6H_4N + C_7H_4O + {}^1/_2\,Br_2 + H_2O$	NiO
[Cu(L)(H_2O)] (4) [Cu($C_{13}H_8NBrO_2$)(H_2O)]	262–455	78.59 (78.54)	$C_6H_4N + C_7H_4O + {}^1/_2\,Br_2 + H_2O$	CuO
[Zn(L)(H_2O)] (5) [Zn($C_{13}H_8NBrO_2$)(H_2O)]	438–668	78.21 (78.00)	$C_6H_4N + C_7H_4O + {}^1/_2\,Br_2 + H_2O$	ZnO

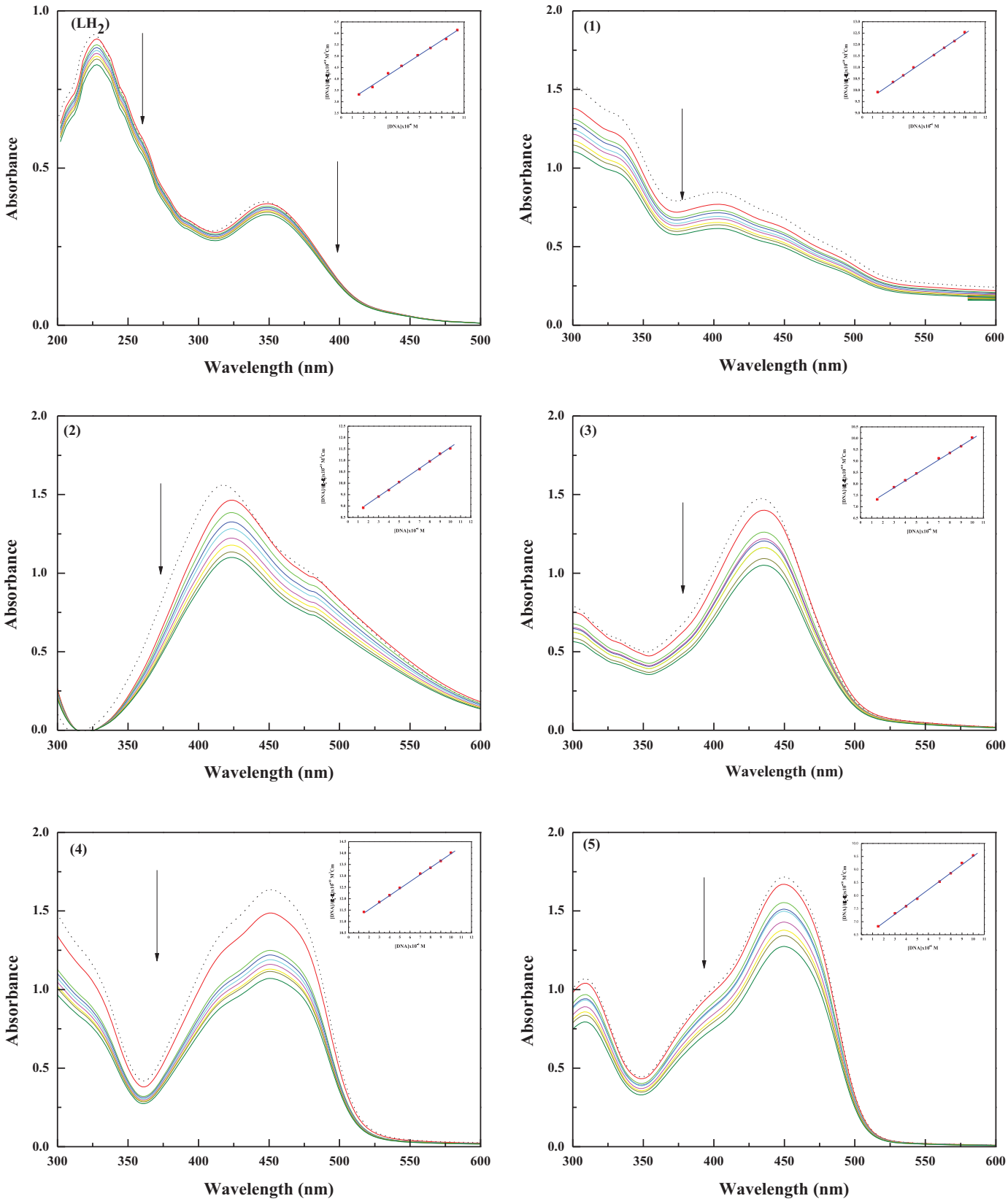

FIG. 6. Electronic absorption spectra of LH_2 and complexes (50 μM, DMSO) in the absence (·····) and presence (—) of increasing amounts of CT-DNA (0–10 μM). Arrows show the changes in absorbance with respect to an increase in the DNA concentration (Inset: Plot of [DNA] vs. [DNA]/($e_a - e_f$)).

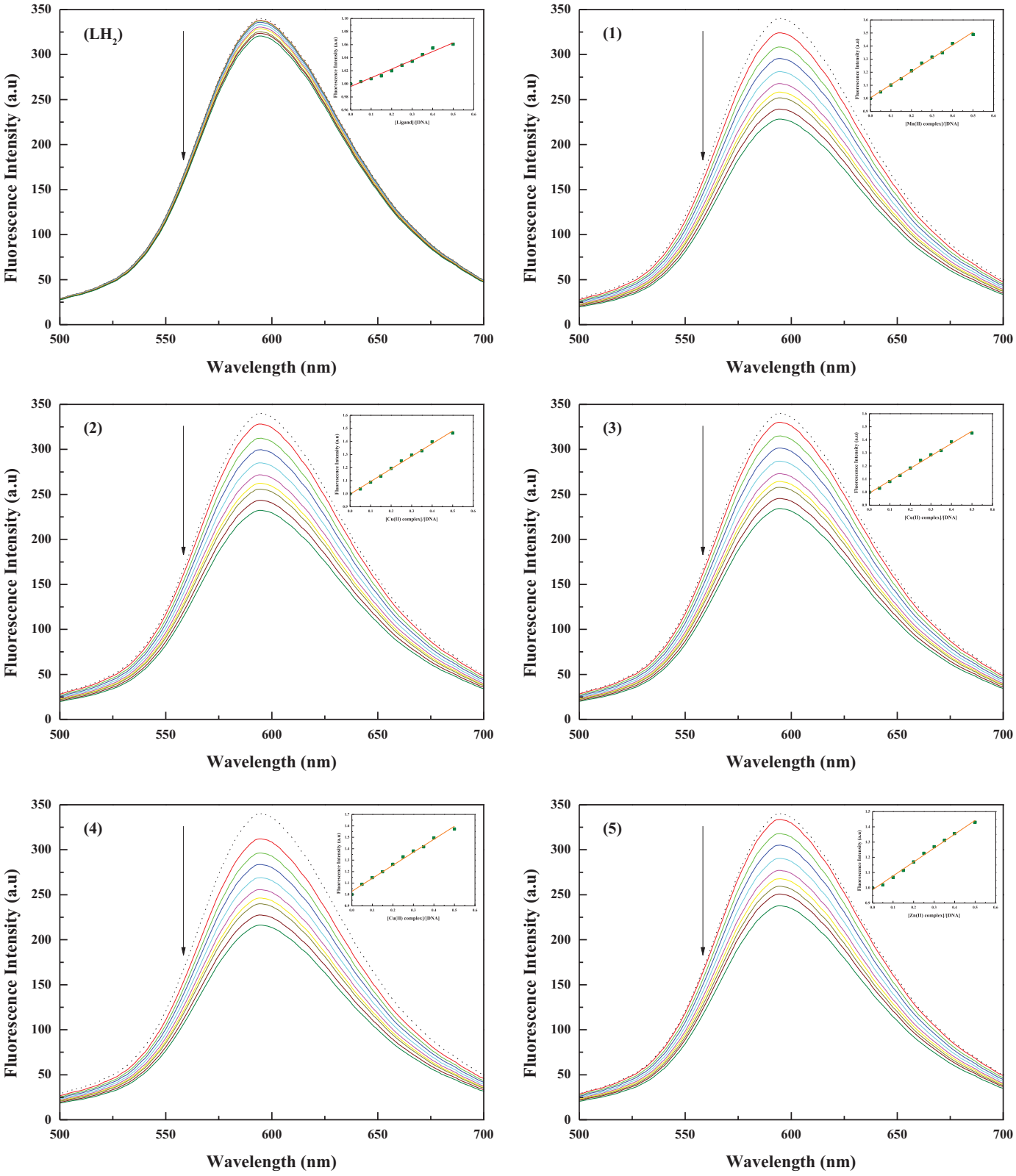

FIG. 7. Emission spectra of DNA-bound EB in Tris buffer [EB] = 30 μM, [DNA] = 200 μM in the absence (·····) and presence (—) of increasing amounts of LH_2 and complexes (0–50 μM). Arrows show the changes in emission intensity with respect to an increase complex concentration (Inset: Plot of Stern–Volmer plot of I_o/I vs. [LH_2]/[DNA].and [$M^{(II)}$ complex]/[DNA].

TABLE 6
UV–VIS absorption properties of LH_2 and its complexes in the presence of CT-DNA

Complex	$K_b \times 10^4$ (M^{-1})	% Hyporchromism[a]	Redshift (nm)
LH_2	0.933	1.8	0.5
[MnL(H_2O)] (1)	3.078	21	12
[CoL(H_2O)] (2)	2.276	15	7
[NiL(H_2O)] (3)	2.261	13	5
[CuL(H_2O)] (4)	3.569	26	3
[ZnL(H_2O)] (5)	1.999	9	2

where [DNA] is the concentration of DNA in base pairs, the apparent extinctions coefficient ε_a, ε_b, and ε_f correspond to $A_{obs.}$/[compound], respectively, the extinction coefficient of the compound in the free solution and the extinction coefficient of the compound in the fully bound form, respectively. In plots [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA] (Figure 6, inset), the intrinsic binding constants (K_b) value, given by the ratio of the slope to the intercept of the linear fit of data were found to be 0.933×10^4 M^{-1}, 3.078×10^4 M^{-1}, 2.276×10^4 M^{-1}, 2.261×10^4 M^{-1}, 3.569×10^4 M^{-1}, and 1.999×10^4 M^{-1} for LH_2, Mn(II), Co(II), Ni(II), Cu(II), and Zn(II) complexes, respectively, indicating a moderate intercalation between the complexes and CT-DNA. These (K_b) values are much smaller than the typical classical intercalators (e.g., EB-DNA, $\sim 10^6$ M^{-1})[42] and have the same level as those of some well-established intercalation agents ($\sim 10^4$)[43,44]. The K_b value (Table 6) revealed that the comparative binding strength of the complexes with CT-DNA were in the order 4 > 1 > 2 > 3 > 5 > LH_2, suggesting that Cu(II) intercalates more strongly than other complexes, which may be explained as in the square-planar complex the more extended π-area of planar aromatic ring of the ligand could intercalate better between the adjacent base pairs of DNA than that of other complexes having tetrahedral geometries.

Fluorescence Spectral Studies

Ethidium bromide (EB) was used to investigate the potential DNA binding mode of the ligand and its complexes (1–5). EB emits intense fluorescence at 595 nm in the presence of CT-DNA due to its strong intercalation between the adjacent DNA base pairs. The addition of a second molecule which binds to DNA more strongly than EB would quench the DNA-induced EB emission[45]. The extent of quenching of the fluorescence of EB bound to DNA would reflect the extent of the DNA binding of the second molecule. The ligand, complexes (1–5) and DNA independently or in combination do not give luminescence spectra in buffer and DMSO solutions. The emission spectra of DNA-bound EB in the absence and the presence of the ligand and its complexes (1–5) are shown in Figure 7. It was clearly seen that the addition of the ligand and its complexes (1–5) to DNA pretreated with EB caused appreciable reduction in emission intensity, indicating that the complexes binds to DNA at the sites occupied by EB. The quenching of the EB bound to DNA by the complex is in agreement with the linear Stern–Volmer equation[46]:

$$I_o/I = 1 + K_{SV}\frac{[M^{(II)}Complex]}{[DNA]}$$

where I_o and I represent the fluorescent intensities in the absence and the presence of the complex, respectively, and Ksv is the linear Stern–Volmer quenching constant. The K_{sv} value is obtained from the slope of the I_o/I versus [$M^{(II)}$complex]/[DNA] linear plot (Figure 7, inset) and is found to be 0.864, 1.54, 1.17, 1.05, 1.93, and 1.01 for LH_2, Mn(II), Co(II), Ni(II), Cu(II), and Zn(II) complexes, respectively, indicating that DNA binding affinity of Cu(II complex is higher than those of other complexes. Also, the data revealed that DNA-bound EB can be more readily replaced by the complexes in the order 4 > 1 > 2 > 3 > 5 > LH_2, which is consistent with the above UV–VIS observations.

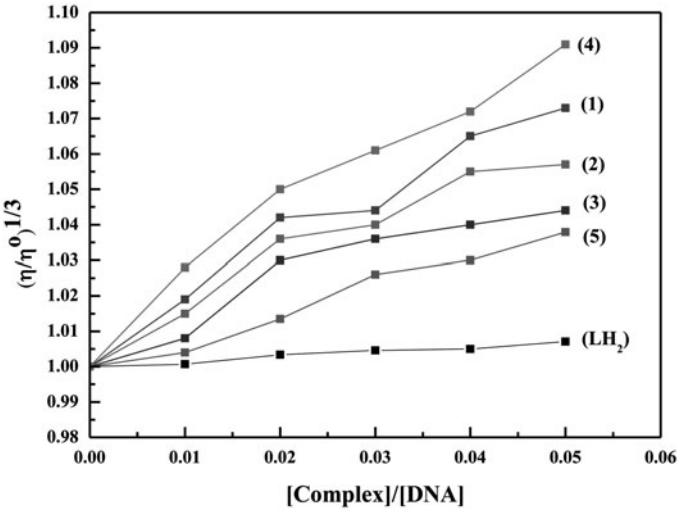

FIG. 8. The effect of increasing concentrations of LH_2 and complexes on the viscosity of CT-DNA at 30.0 ± 0.1°C.

Viscosity Measurements

Due to its sensitivity to the change of length of DNA, viscosity measurements may be the most effective means to study the binding mode of complexes to DNA. In classical intercalation, the DNA helix lengthens as base pairs are separated to accommodate the bound ligand leading to an increase in DNA viscosity, whereas a partial, non-classical ligand intercalation causes a bend (or kink) in DNA helix reducing its effective length and thereby its viscosity[47]. The effect of the ligand and its complexes on the viscosity of CT-DNA is shown in Figure 8. The viscosity measurements clearly showed that as the amount of the ligand (LH_2) and complexes (1–5), the relative

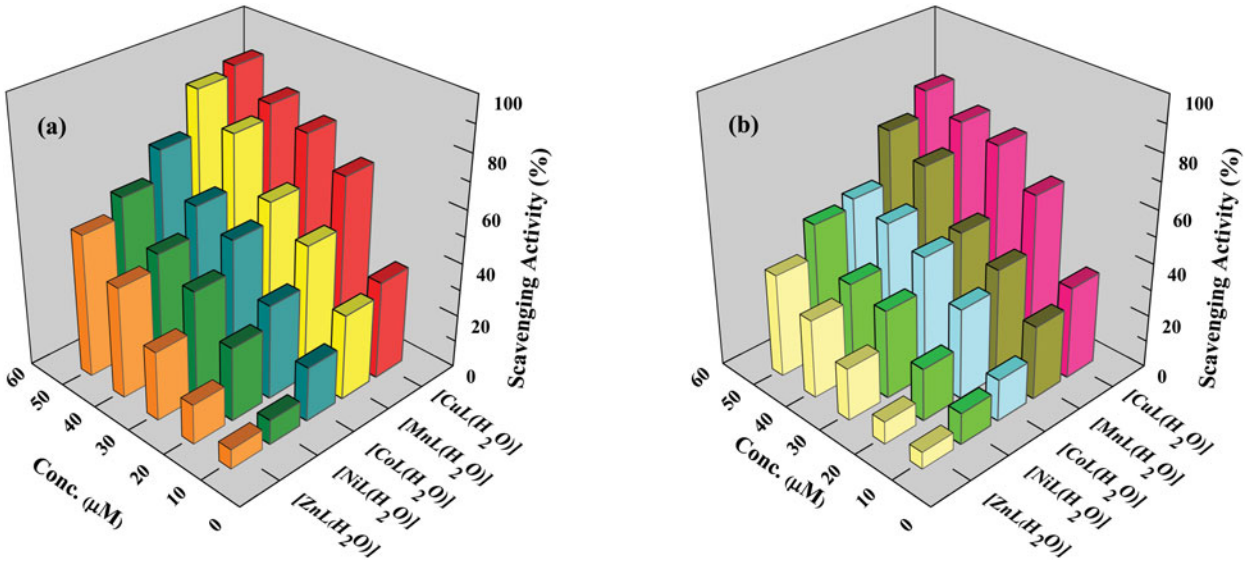

FIG. 9. Scavenging activity of complexes on superoxide radical (a) and hydroxyl radical (b).

viscosity of CT-DNA increases steadily, which proved that all the compounds can intercalate between adjacent DNA base pairs, causing an extension in the DNA helix[48]. The increased degree of viscosity, which may depend on its affinity to DNA, follows the order of 4 > 1 > 2 > 3 > 5 > LH_2.

The DNA binding studies have been described in this study show that changing the metal environment can modulate the binding property of the complex with DNA[49].

Antioxidant Activity

Schiff base metal complexes are known to show various biological activities including biocidal and antioxidant activities[50,51]. Reactive oxygen species (ROS), e.g., superoxide ($O_2^{\cdot -}$) and hydroxyl ($HO^{\cdot}$) radical are responsible for cell membrane disintegration, membrane protein damage, DNA mutation, and further initiate or propagate the development of many diseases. Since the reported complexes exhibited good DNA-binding affinity, it was considered worthwhile to study other potential aspects of these compounds such as antioxidant activity. Figure 9 depicts the inhibitory effect of the complexes on $O_2^{\cdot -}$ and hydroxyl $HO^{\cdot}$. As shown in Figure 9, the inhibitory effect of the complexes (1–5) on $O_2^{\cdot -}$ and hydroxyl $HO^{\cdot}$ was concentration dependent and the suppression ratio increased with increasing of sample concentration (5–50 μM). Among the studied complexes, Cu(II) and Mn(II) complexes showed highest superoxide scavenging ability of 91% and 88% respectively at a concentration of 50 μM. Also, Co(II), Ni(II), and Zn(II) complexes also showed considerably high scavenging activity of 72%, 61%, and 54%, respectively. The complexes at the same concentration (10–50 μM) were also screened for the $HO^{\cdot}$ radical scavenging activity and it was evident from the results that Cu(II) and Mn(II) complexes are better scavenging

TABLE 7
Antimicrobial activity of LH_2 and its complexes

	Antibacterial activity of zone of inhibition (mm)				Antifungal activity of zone of inhibition (mm)			
Compound	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*A. niger*	*A. flavus*	*C. lunata*	*C. albicans*
LH_2	8	9	10	10	14	13	11	14
[$MnL(H_2O)$] (1)	21	21	17	17	18	19	19	17
[$NiL(H_2O)$] (2)	17	15	16	16	16	17	13	18
[$CoL(H_2O)$] (3)	19	17	16	18	17	20	23	21
[$CuL(H_2O)$] (4)	23	24	20	20	21	22	27	25
[$ZnL(H_2O)$] (5)	11	18	13	12	13	16	16	18
Streptomycin	28	27	24	24	—	—	—	—
Nystatin	—	—	—	—	21	21	22	22
DMSO	0	0	0	0	0	0	0	0

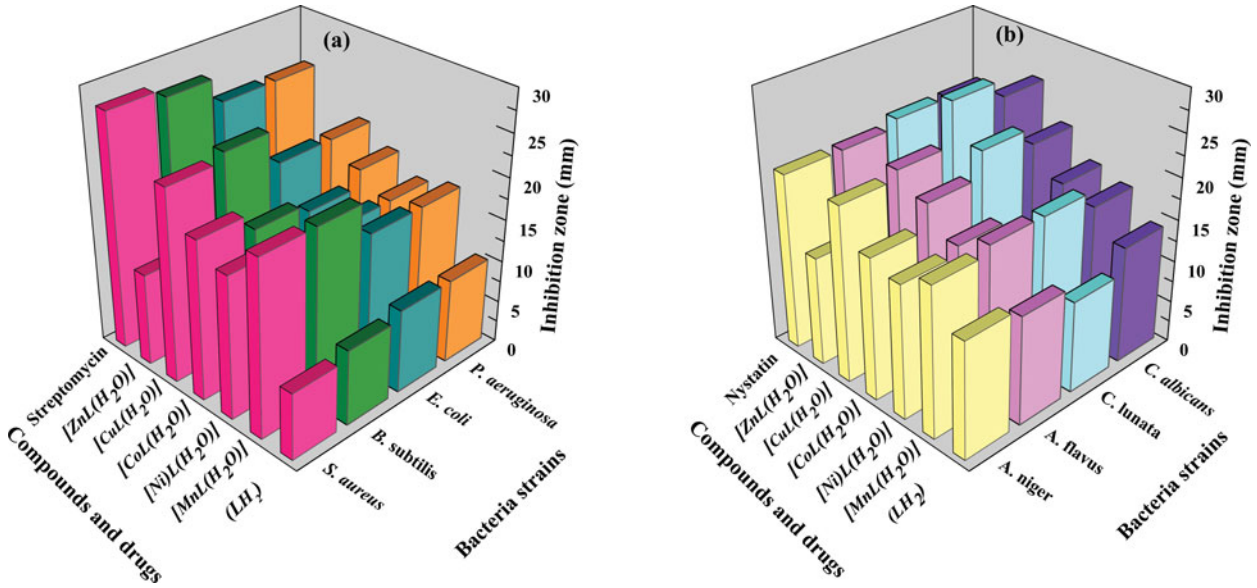

FIG. 10. Biological diagram of LH_2 and its complexes against bacteria strains (a) and fungi strains (b).

agents (84% and 73%) than Co(II), Ni(II), and Zn(II) which showed 54%, 50%, and 39% scavenging activities respectively at a concentration of 50 μM.

In Vitro Antimicrobial Activity

Antibacterial and antifungal activities of the *N*-(2-hydroxyphenyl) 2-hydroxy-5-bromobenzaldimine (LH_2) and its metal complexes (1–5) were tested for their inhibitory effects on the growth of bacteria: *S. aureus, B. subtilis*, *E. coli*, and *P. aeruginosa* and the fungi: *A. niger, A. flavus, C. lunata,* and *C. albicans* because such organisms can achieve resistance to antibiotics through biochemical and morphological modification[52]. The inspection of microbial results is listed in Table 7 and represented graphically in Figure 10. The results have indicated that, in comparison with the Schiff base *N*-(2-hydroxyphenyl) 2-hydroxy-5-bromobenzaldimine (LH_2), Mn(II) and Cu(II) complexes showed good antibacterial and antifungal activity, whereas the Co(II), Ni(II), and Zn(II) showed moderate activity. The variation in the activity of different metal complexes against different microorganisms depends on the impermeability of the cell or differences in the ribosomes in the microbial cells[53]. Such increased activity of the metal chelates can be explained on the basis of chelation theory. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalization of π-electrons over the whole chelate ring and enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms. In general, metal complexes are more active than the ligands because metal complexes may serve as a vehicle for activation of ligands as the principle cytotoxic species[54]. The studies showed that the newly synthesized compounds possess higher antifungal activity than the antibacterial property.

CONCLUSION

A series of mononuclear four coordinated transition metal (II) complexes (M = Mn, Co, Ni, Cu, and Zn) were synthesized under microwave conditions by the reaction hydrated metal nitrate salt with the tridentate ONO Schiff base N-(2-hydroxyphenyl) 2-hydroxy-5-bromobenzaldimine (LH_2). The analytical, FT-IR, UV–VIS, EPR, FAB-mass, magnetic and thermal studies have been confirmed the bonding of Schiff bases to metal ions through the azomethine-nitrogen and phenolic-oxygen atoms forming stable four coordinated complex, where the fourth site was occupied by a water molecule. Based on the characterization data and keeping in mind the preferred geometries, the square-planar geometry was proposed for Cu(II) complex, whereas the tetrahedral geometry was proposed for Mn(II), Co(II), Ni(II), and Zn(II) complexes, respectively. The interaction of the ligand and its complexes with CT-DNA was performed with UV spectroscopy, fluorescence study, and viscometric measurements and it revealed that the reported compounds can bind to CT-DNA via intercalation mode. The binding studies have been shown that Cu(II) complex has stronger binding strength as compared to the other complexes. Additionally, the complexes also exhibited good antioxidant ($O_2^{\cdot -}$ and $HO^{\cdot}$ radical scavengers) and the suppression ratio follow the order of Cu(II) > Mn(II) > Co(II) > Ni(II) > Zn(II) > LH_2. Moreover, in vitro antimicrobial activities of the new compounds were studied against the bacteria strains (*S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*) and the fungi strains (*A. niger*, *A. flavus*, *C. lunata*, and *C. albicans*). The data have been shown that the metal complexes show enhanced inhibitory activity compared to the parent ligand under

identical experimental conditions. These findings clearly indicated that the complexes have many potential practical applications, like the development of nucleic acid molecular probes and new therapeutic reagents for diseases.

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