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Novel Heterocyclic Transition Metal Complexes: Synthesis, Characterization, Antimicrobial and Anticancer Activity

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A heterocyclic ligand, 5-(2-(4-chlorophenyl)-1*H*-benzo[*d*]imidazol-1-yl)quinolin-8-ol and its Co(II), Ni(II), Cu(II) and Zn(II) complexes were synthesized and characterized by elemental analysis and spectroscopic techniques. According to the spectral analysis, the ligand acts as a bidentate ligand and coordinating through the nitrogen and deprotonated oxygen atoms. For Cu(II) and Ni(II) complexes, spectral analysis reveals square planer geometry, whereas Co(II) and Zn(II) complexes have tetrahedral geometry. The antibacterial results show that Zn(II) complex is more effective than the other metal(II) complexes examined. The ligand and its metal complexes were tested for anticancer activity using the MTT assay with cisplatin as the reference drug against A549, MCF7, and HCT116 cancer cell lines. Results showed that the metal(II) complexes were shown to be more active than the ligand, especially Zn(II) complex being the most potent among this series.

Keywords: Metal(II) complex, Benzimidazole, Antimicrobial activity, Anticancer activity.

INTRODUCTION

Benzimidazole derivatives are a family of physiologically active compounds that have sparked the interest of medicinal chemists due to their diverse pharmacological characteristics [1-5] and prospective use as anticancer, antineoplastic, antiviral, and anti-inflammatory drugs [6-12]. Furthermore, due to their biological action, 5-chloro-8-hydroxyquinoline compounds are gaining popularity [13,14]. In addition, literature evidence shows that the heterocyclic-based ligands including N and O as donor atoms produce transition metal complexes [15]. The anticancer effects of the metal complexes are enhanced by their specific characteristics [15].

Metal capacity to generate positively charged ions in an aqueous solution, which can attach to negatively charged biological molecules, is an essential feature [16]. Complexes of the 3*d*-transition metal ions have lower toxicity and may easily permeate the cell membrane of microorganisms as compared to 4*d*- or 5*d*-metal complexes [17]. They also act as therapeutic agents in the treatment of a variety of diseases. Present work reports the synthesis of novel 5-(2-(4-chlorophenyl)-1*H*-benzo[*d*]imidazol-1-yl)quinolin-8-ol ligand and their Co(II), Ni(II), Cu(II) and Zn(II) complexes. Various analytical and spectroscopic techniques were used to characterize the novel ligand and its metal(II) complexes. Antibacterial and antifungal investigations were conducted on the synthesized ligand and its metal complexes. Furthermore, the cytotoxicity of these complexes was investigated with several human cancer cell lines.

EXPERIMENTAL

The chemicals *viz*. 1,2-phenylene diamine, 4-chlorobenzaldehyde, 5-chloro-8-hydroxyquinoline and tetrahydrofuran were purchased from Sigma-Aldrich and used without purification. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance-400 and Bruker Avance-100 MHz NMR instrument, respectively using TMS as an internal solvent and DMSO (d_6) as a solvent. Mass spectra were recorded on Perkin-Elmer LC-MS PE Sciex API/65 Spectrophotometer. FT-IR spectra were

measured with KBr discs on an Avatar 360 (Nicolet, Madison, USA) spectrophotometer in the wavenumber range of 4000-400 cm^{-1}. Melting points were determined in one end open capillary tubes on a Buchi 530 melting point apparatus and are uncorrected. The Merck made pre-coated TLC plates were used. Elemental analysis (C, H and N) was undertaken with the Perkin-Elmer model 240C analyzer**.** A UV-Vis double beam spectrophotometer (Systronics 118, India) was used to record the UV-Vis spectra. The molar conductance measurements were carried out using a digital electronic conductivity meter. Magnetic measurements were carried out by the Gouy method at room temperature (25 ± 1 ºC).

Synthesis of 2-(4-chlorophenyl)-1*H*-benzo[*d*]imidazole (1): A solution of 1,2-phenylene diamine (5 mmol), 4-chlorobenzaldehyde (5 mmol) and NH_4Cl (10 mmol) were mixed in the minimum amount of THF and allowed to stir for 6 h at 60 ºC. The progress of the reaction was monitored by TLC. After completion of the reaction, the solvent was removed under reduced pressure and the residue was extracted with ethyl acetate to obtain desired compound **1** (**Scheme-I**). Yield: 93%. Anal. calcd. (found) % for $C_{13}H_9N_2Cl$: C, 68.28 (68.25); H, 3.97 (3.93); Cl, 15.50 (15.47); N, 12.25 (12.29). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 5.08 (s, 1H), 7.23-8.01 (m, 8H, Ar-H), mass: *m/z* 229 (M+1).

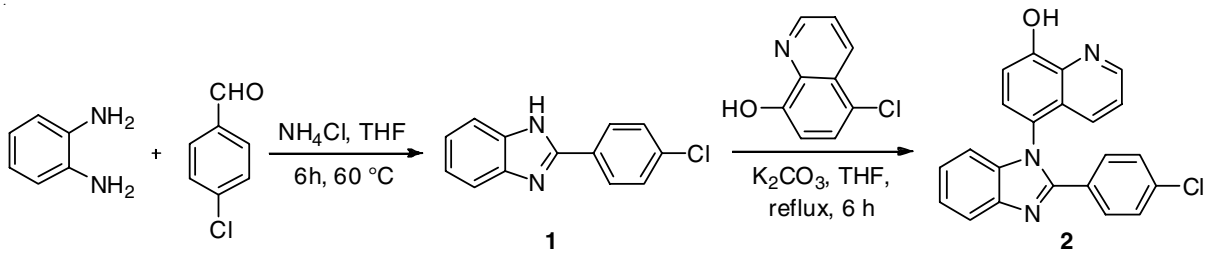
Synthesis of 5-(2-(4-chlorophenyl)-1*H*-benzo[*d*]imidazol-1-yl)quinolin-8-ol (2): Ligand (**2**) was synthesized by the reaction of compound **1** (2 mmol) with 5-chloro-8-hydroxyquinoline (2 mmol) in THF using anhydrous K_2CO_3. The above reaction mixture was allowed to stir for 6 h and the progress of the reaction mixture was monitored by TLC. After completion of the reaction (TLC; hexane:ethylacetate, 8:2 v/v), the reaction mixture was quenched in ice-cold water and extracted with dichloromethane. The organic layer was washed with 5% $NaHCO_3$ and dried over Na_2SO_4 and concentrated *in vacuo* to give the desired product (**2**) (**Scheme-I**). Yield: 91%. m.p.: 205 ºC. Anal. calcd. (found) % for $C_{22}H_{14}N_3OCl$: C, 71.07 (71.01); H, 3.80 (3.76); Cl, 9.54 (9.52); N, 11.30 (11.36). IR (KBr, ν_{max}, cm^{-1}): 3307.36 (-OH), 1670.00 (C=N), 1407.71 (CH), 1370.45 (C_6H_5), 825 (C-N), 783.36 (C=C), 739.23 (C-C). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.18-7.95 (m, 13H, Ar-H), 9.18 (s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 112.1, 113.1, 118.4, 118.7, 120.1, 122.1, 123.1, 128.9, 129.3, 131.9, 134.3, 135.1, 138.3, 141.3, 144.1, 149.4, 150.2, 152.7. Mass: *m/z* 372 (M+1). UV-visible: 292 nm (n-π*).

Synthesis of metal(II) complexes: Metal(II) complexes of 5-(2-(4-Chlorophenyl)-1*H*-benzo[*d*]imidazol-1-yl)quinolin-8-ol (**2**) were synthesized by adding 1 mmol of corresponding metal(II) acetate (M = Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}) to a stirred solution of ligand (2 mmol) in ethanol at 60 ºC. Following the completion of the reaction (TLC), the obtained precipitate was filtered off under vacuum and recrystallized using ethanol to get desired metal complex (**Scheme-II**).

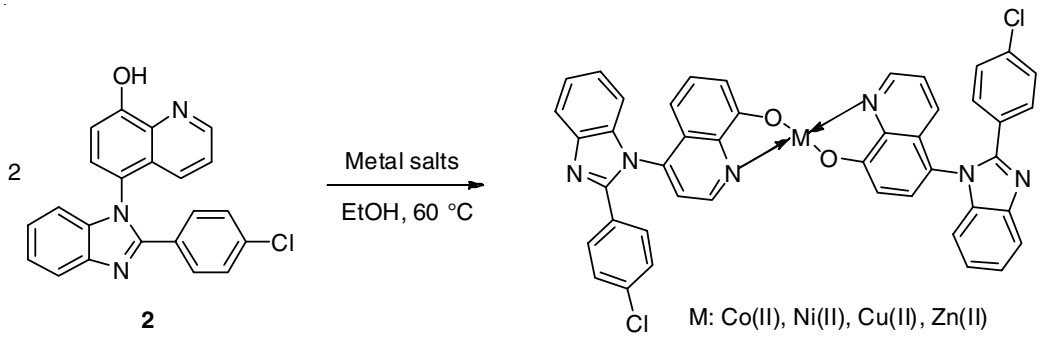
Co(II)complex: Yield: 73%, m.p.: 395 ºC. Anal. calcd. (found) % for $C_{44}H_{26}N_6O_2Cl_2Co$: C, 66.01 (65.97); H, 3.27 (3.23); Cl, 8.86 (8.83); Co, 7.36 (7.32); N, 10.50 (10.55). IR (KBr, cm^{-1}): 1571.35 (CH), 1499.44 (C=N), 530.88 (Co-N), 497.72 (Co-O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.07-7.11 (m, 6H, Ar-H), 7.25-7.33 (m, 4H, Ar-H), 7.51-7.54 (m, 6H, Ar-H), 8.01 (d, 6H, Ar-H), 8.12 (t, 4H, Ar-H). Mass: *m/z* 801 (M+1). Conductance (Λ_m, ohm^{-1} cm^2 mol^{-1}): 16.5.

Ni(II)complex: Yield: 78%, m.p.: 316 ºC. Anal. calcd. (found) % for $C_{44}H_{26}N_6O_2Cl_2Ni$: C, 66.03 (66.01); H, 3.27 (3.21); Cl, 8.86 (8.85); N, 10.50 (10.55); Ni, 7.33 (7.31). IR (KBr, ν_{max}, cm^{-1}): 1575.15 (CH), 1498.24 (C=N), 526.42 (Ni-N), 489.17 (Ni-O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.06-7.10 (m, 6H, Ar-H), 7.28-7.34 (m, 4H, Ar-H), 7.49-7.52 (m, 6H, Ar-H), 7.99 (d, 6H, Ar-H), 8.10 (t, 4H, Ar-H). Mass: *m/z* 802 (M+1). Conductance (Λ_m, ohm^{-1} cm^2 mol^{-1}): 13.1.

Cu(II)complex: Yield: 82 %, m.p.: 281 ºC. Anal. calcd. (found) % for $C_{44}H_{26}N_6O_2Cl_2Cu$: C, 65.63 (65.62); H, 3.25



Scheme-I: Synthesis of ligand



Scheme-II: Synthesis of transition metal complexes

(3.22); Cl, 8.81 (8.80); N, 10.44 (10.47); Cu, 7.89 (7.87). IR (KBr, ν_{max}, cm^{-1}): 1576.13 (CH), 1495.04 (C=N), 540.14 (Cu-N), 497.35 (Cu-O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.09-7.14 (m, 6H, Ar-H), 7.29-7.36 (m, 4H, Ar-H), 7.48-7.55 (m, 6H, Ar-H), 8.02 (d, 6H, Ar-H), 8.14 (t, 4H, Ar-H). Mass: *m/z* 806 (M+1). Conductance (Λ_m, ohm^{-1} cm^2 mol^{-1}): 20.8.

Zn(II)complex: Yield: 81 %, m.p.: 273. ºC. Anal. calcd. (found) % for $C_{44}H_{26}N_6O_2Cl_2Zn$: C, 65.49 (65.46); H, 3.25 (3.22); Cl, 8.79 (8.76); N, 10.41 (10.45); Zn, 8.10 (8.07). IR (KBr, ν_{max}, cm^{-1}): 1576.91 (CH), 1493.27 (C=N), 528.41 (Zn-N), 489.16 (Zn-O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.09-7.13 (m, 6H, Ar-H), 7.29-7.34 (m, 4H, Ar-H), 7.46-7.51 (m, 6H, Ar-H), 7.99 (d, 6H, Ar-H), 8.11 (t, 4H, Ar-H). Mass: *m/z* 808 (M+1). Conductance (Λ_m, ohm^{-1} cm^2 mol^{-1}): 15.4.

Antimicrobial activity: The newly synthesized ligand and its metal(II) complexes were tested for antibacterial activity against a range of bacteria, including *Staphylococcus aureus* (NCIM 5021), *Bacillus subtilis* (NCIM 2999) and *Escherichia coli* (NCIM 2574), and fungi, Candida albicans (NCIM 3471). Microbial strains were cultivated overnight at 37 ºC in nutrients and potato dextrose agar medium. The antibacterial activity and minimum inhibitory concentration (MIC) of the ligand and its metal complexes were assessed using agar disc diffusion [18,19] and broth dilution techniques [20], respectively. The inhibitory zone was measured in millimeter for all samples evaluated in triplicate. The MIC was determined to be the lowest chemical concentration that inhibited the development of the macroscopic microorganism.

Cytotoxicity assay: MTT assay [21] was used to assess the cytotoxic activity of the synthesized ligand and its metal(II) complexes. Exponentially cell proliferating were planted three times in six-well plates at a density of 1×10^5 cells per well. After 24 h, the cells were treated with all of the test compounds at various doses for another 24 h. After 48 h of treatment, 20 µL of MTT (5 mg/mL) was added to all wells and incubated for 4 h at 37 ºC. After carefully removing the medium, 40 µL of DMSO was added to each well. At a wavelength of 595 nm, the plates were analyzed using an ELISA plate reader. The IC_{50} of each compound was defined as the concentration of the compound that inhibited 50% of growth in distinct cell lines when compared to the control culture.

RESULTS AND DISCUSSION

Chemistry: By cyclocondensing 1,2-phenylenediamine with 4-chlorobenzaldehyde in THF at 60 ºC for 6 h in the presence of NH_4Cl, benzimidazole core (**1**) was synthesized. The inclusion of 5-chloro-8-hydroxyquinoline backbone in the benzimidazole core appears to be of importance due to the biological and therapeutic relevance of this molecule. As a result, 5-chloro-8-hydroxy quinoline was allowed to condensed with compound **1** in THF to obtain the desired ligand **2** with a 91% yield. The chemical pathway for ligand synthesis is shown in **Scheme-I**. The ^{1}H NMR spectrum results agreed with the predicted ligand structure in terms of the number of protons and their chemical shifts. For ligand **2**, ^{1}H NMR spectrum data revealed resonance at δ 8.86 ppm (s, 1H, OH). Furthermore, the proton spectrum data of compound **1** revealed a singlet peak for the -NH group at δ 5.08 ppm, but it was not observed in compound **2**. In FTIR, the presence of hydroxyl groups at 3307.6 cm^{-1} and the absence of an NH stretching peak at 3460 cm^{-1} were observed in the IR spectra. For metal(II) complexation, ligand **2** was allowed to react with metal(II) acetate in a 2:1 molar ratio in ethanol. The mononuclear complexes were formed when ligand **2** interacts with metal acetate in a 2:1 molar ratio.

FTIR studies: Fig. 1 shows the IR spectra of Co(II) and Zn(II) complexes. The absence of the band attributed to ν(O-H) in the IR spectra of metal(II) complexes conclusively supports the deprotonation of ligand molecule before complexation. After deprotonation, the ligand might form a bond with the metal ion at the oxygen of the hydroxyl group. When complexation occurred, the band of O-H in the ligand disappeared as a result of the metal ion complexing with the ligand *via* an oxygen atom. For the free ligand, the stretching frequency for the hydroxyl group was found at 3307.6 cm^{-1} and this peak was disappeared in the metal(II) complexes spectra. In addition,

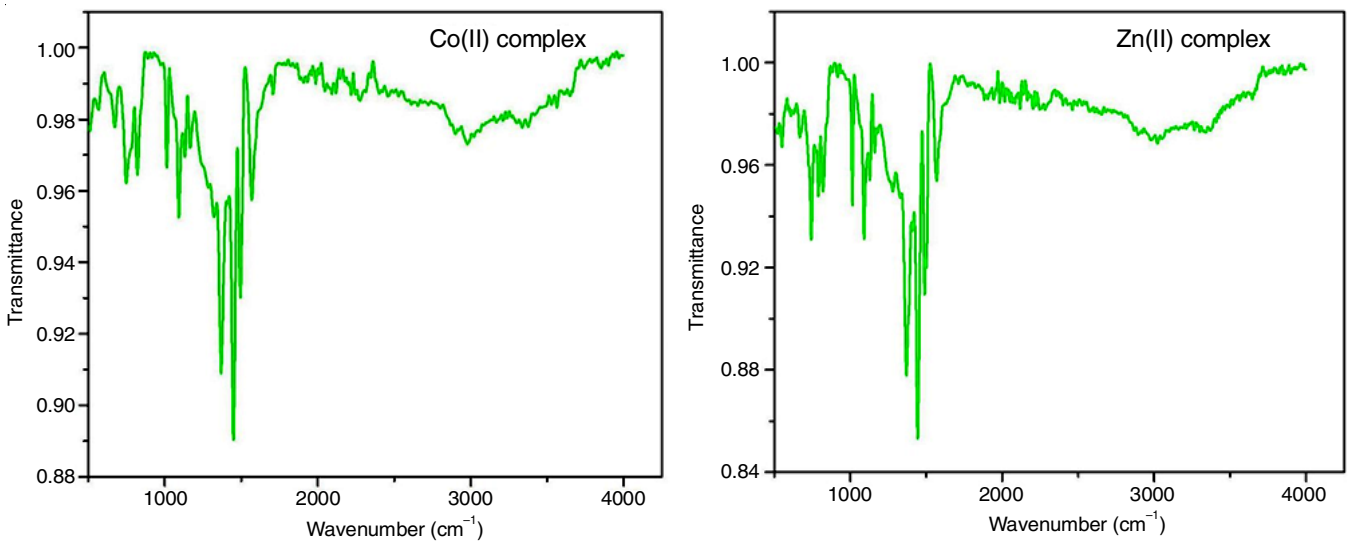


Fig. 1. IR spectra of Co(II) and Zn(II) complexes

the bands at 540-526 and 497-489 cm^{-1} are attributed to the M-N and M-O bonds, respectively. This showed that heterocyclic ligand coordinated to the metal *via* nitrogen and deprotonated oxygen atoms. Furthermore, the absence of the proton signal of the OH moiety in the ^1H NMR spectrum of the metal(II) complex indicated deprotonation during coordination. It is also noticed that the intensity of the benzene ring signals decreases and shifts to a lower field, which implied that the complexation impacts the electron density distribution through the nitrogen and deprotonated oxygen atoms to some extent.

In DMF, the UV-Vis spectra of metal complexes were obtained in the range of 200-720 nm (Fig. 2). A band at 633 nm is observed in the electronic spectra of the Co(II) complex. The $^4A_{2f} \rightarrow {}^4T_{1p}$ transition might be assigned to this band (Table-1). This transition determines the likelihood of Co(II) complex tetrahedral geometry [22]. The absorption spectra of Ni(II) complex revealed bands at 336 and 508 nm, respectively, corresponding to the $^1A_{1g} \rightarrow {}^1A_{2g}$ and $^1A_{1g} \rightarrow {}^1B_{1g}$ transitions, confirming the square planar shape [23]. The Cu(II) complex has an absorption band at 642 nm, indicating a square planar geometry with the $^2B_{1g} \rightarrow {}^2A_{1g}$ transition. No bands were found in the visible region for the d^{10} electronic configuration of Zn(II) complex, which indicates the tetrahedral geometry of Zn(II) complex [24].

In addition, the JCPDS was used to match all of the peaks on the XRD of Zn(II) complex. The obtained XRD (Fig. 3) results of Zn(II) complex are consistent with a tetragonal crystal

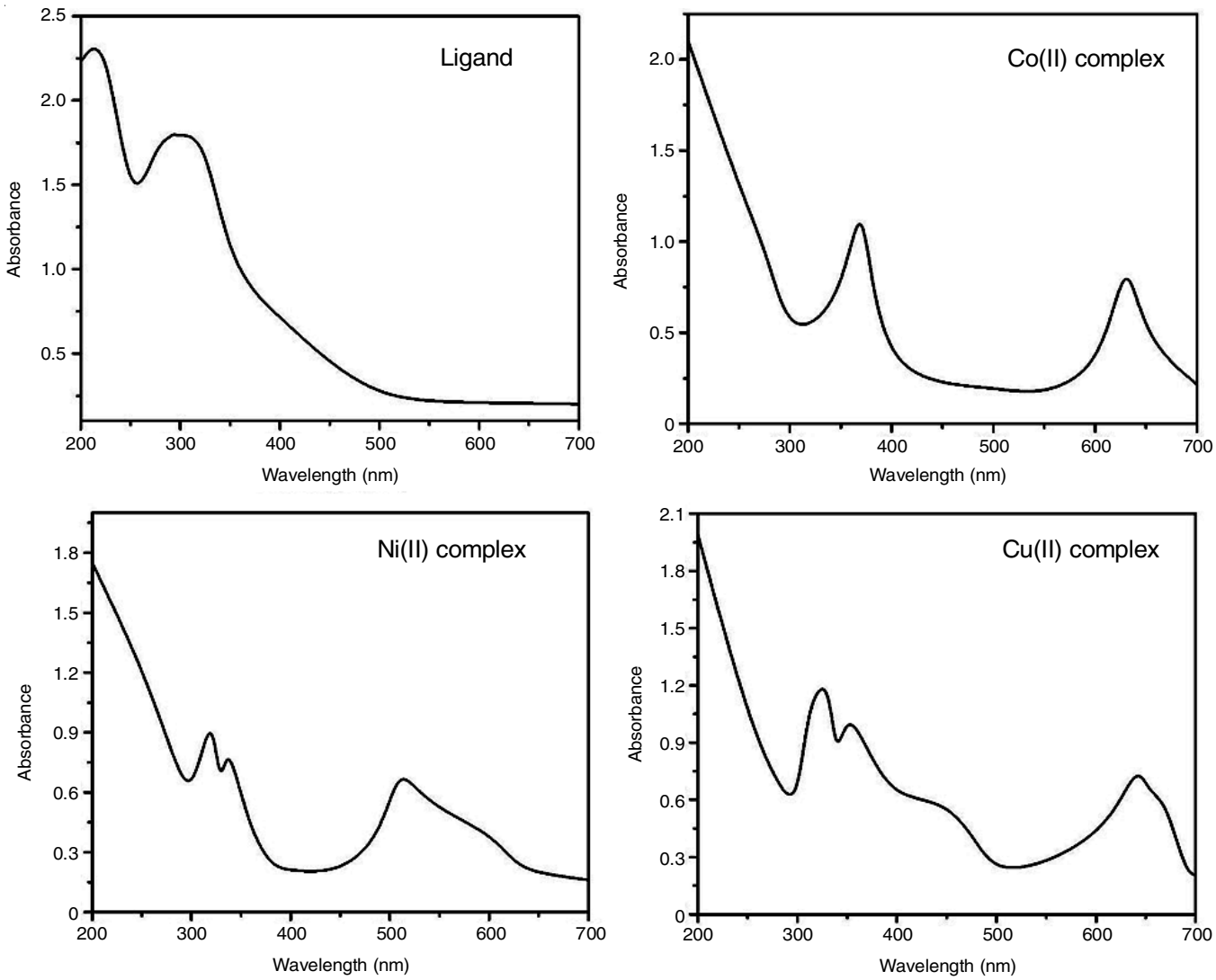


Fig. 2. Absorption spectra of ligand and its metal complexes

TABLE-1
ELECTRONIC SPECTRAL ASSIGNMENTS, MAGNETIC MOMENT AND GEOMETRY OF METAL COMPLEXES

Complexes	λ_{max} (nm)	Band assignments	μ_{eff} (B.M.)	Geometry
Co(II) complex	369, 633	$^4A_{2f} \rightarrow {}^4T_{1p}$	4.23	Tetrahedral
Ni(II) complex	318, 336, 508	$^1A_{1g} \rightarrow {}^1A_{2g}$, $^1A_{1g} \rightarrow {}^1B_{1g}$	Diamagnetic	Square Planar
Cu(II) complex	328, 349, 642	$^2B_{1g} \rightarrow {}^2A_{1g}$	1.81	Square Planar
Zn(II) complex	–	–	Diamagnetic	Tetrahedral

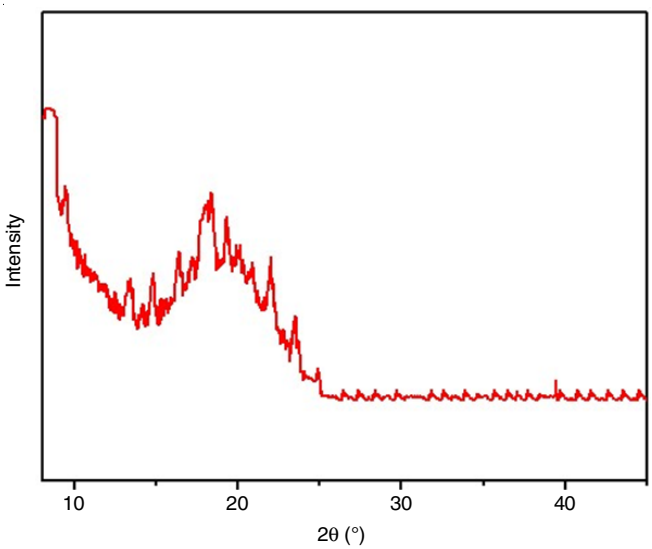


Fig. 3. Powder XRD diffractogram of Zn(II) complex

structure, thus confirms the tetrahedral geometry for Zn(II) complex. Table-1 shows the magnetic susceptibility and electronic spectral assignments of the synthesized metal(II) complexes. According to the results, Co(II) and Cu(II) complexes are paramagnetic, whereas Ni(II) and Zn(II) complexes are diamagnetic. The molar conductivities of prepared complexes indicate that all the metal(II) complexes are non-electrolytes.

Based on the elemental analysis and spectral data, the following structure (Fig. 4) is proposed for these metal(II) complexes. Based on molar conductance, magnetic movement and UV-visible spectral analysis, the geometry of the synthesized Co(II), Ni(II), Cu(II) and Zn(II) complexes to be concluded that tetrahedral, square planar, square planar and tetrahedral, respectively.

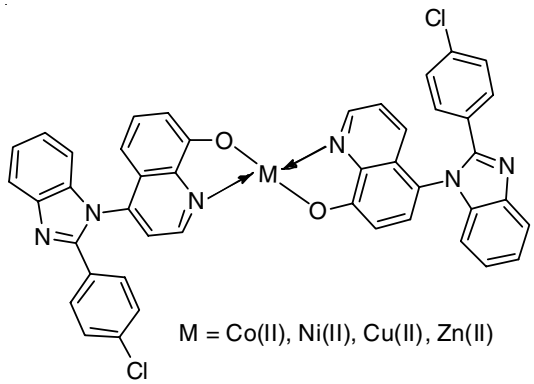


Fig. 4. Proposed structure of the synthesized metal(II) complexes

Antimicrobial activity: The disc diffusion method was used to screen the synthesized ligand and its transition metal complexes *in vitro* antibacterial and antifungal activity against *S. aureus*, *B. subtilis*, *E. coli*, and antifungal activity against *C. albicans* strains. The broth dilution technique was used to determine the minimum inhibitory concentration (MIC). The MICs of the synthesized ligands and their metal complexes against bacterial and fungal species are shown in Table-2. All the metal(II) complexes had lower MIC values than the ligand alone. According to the literature, metal ions, geometry surrounding metal ions and counter ions all have a significant impact on the suppression of antimicrobial activity [25]. In comparison to other examined Co(II), Ni(II) and Cu(II) complexes, these results demonstrate that Zn(II) complex are most potent against tested bacterial and fungal strains.

TABLE-2
in vitro ANTIMICROBIAL ACTIVITY OF LIGAND AND ITS METAL COMPLEXES

Compound	Minimum inhibitory concentration (MIC)[a] (μM)			
	Gram-positive		Gram-negative	Fungi
	S. aureus	*B. subtilis*	*E. coli*	*C. albicans*
Ligand	24.1±1.39	28.3±1.15	42.9±1.93	28.5±0.95
Co(II) complex	21.9±1.07	24.1±1.33	29.6±1.16	23.6±0.55
Ni(II) complex	16.3±1.52	22.8±0.95	33.4±1.74	26.2±1.29
Cu(II) complex	8.9±0.91	10.6±0.78	17.5±0.15	10.5±1.72
Zn(II) complex	9.3±1.02	10.1±1.26	16.7±0.18	10.3±1.25
Rifampicin	0.5±0.61	0.5±0.17	4.1±1.02	Nt
Flucanazole	–	–	–	4.2±1.17

[a]The values given are means of three experiments; Nt: not tested

Anticancer activity: The cytotoxicity of the synthesized ligand (**2**) and its metal(II) complexes was assessed using the MTT assay with cisplatin as the reference on various cancer cell lines, including human lung carcinoma A549, human breast MCF7 and human colon cancer HCT116. The findings of the cytotoxic activities were calculated based on the doses of possible drug exposure necessary to reduce cell line survival to 50% (IC_{50}). Ligand **2** exhibited the least level of anticancer action, but the metal(II) complexes had a moderate anticancer activity (Table-3). The IC_{50} of the synthesized metal complexes against tested human cancer cell lines revealed that Zn(II) complex had the lowest IC_{50} values. The structure-activity relationship assessment of the synthesized metal(II) complexes revealed that the ligand had modest activity against the tested microbial strains. Zinc(II) complex has substantially higher activity than cobalt(II), nickel(II) and copper(II) complexes.

TABLE-3
ANTICANCER ACTIVITY OF LIGAND AND THEIR METAL COMPLEXES ON CANCER CELLS

Compound	IC_{50} (μM)[a]		
	A549	MCF-7	HCT116
Ligand	18.36 ± 1.29	13.81 ± 1.77	23.67 ± 1.09
Co(II) complex	16.18 ± 1.36	15.32 ± 0.81	21.97 ± 1.27
Ni(II) complex	13.16 ± 1.28	11.28 ± 1.21	16.13 ± 1.39
Cu(II) complex	14.19 ± 0.85	11.48 ± 1.15	15.36 ± 1.96
Zn(II) complex	10.91 ± 1.72	10.16 ± 0.68	11.95 ± 0.52
Cisplatin	3.62 ± 0.68	3.75 ± 1.26	8.15 ± 0.17

[a]The values given are means of three experiments.

Conclusion

A new heterocyclic ligand, 5-(2-(4-chlorophenyl)-1*H*-benzo[*d*]imidazol-1-yl)quinolin-8-ol and its Co(II), Ni(II), Cu(II) and Zn(II) complexes were synthesized and characterized. Several analytical and spectroscopic approaches were used to characterize the metal(II) complexes. Using UV-Vis

and other spectrum analyses, it was possible to establish that the complex geometry is tetrahedral for Co(II) and Zn(II) complexes and square planar for Ni(II) and Cu(II) complexes. In contrast to ligand, the antibacterial activity of all the metal(II) complexes was much higher. Among the synthesized metal(II) complexes, Zn(II) complex has been found to be effective. The anticancer study results indicated these complexes, notably Zn(II) complex, have better anticancer activity against tested human cancer cell lines, and this insight may be useful in designing novel anticancer metallodrugs.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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