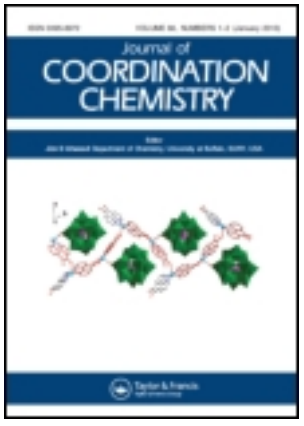

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Synthesis, crystal structure, spectroscopic, and biological studies on Cu(II) complexes of N,O donor dimethyl isoxazole Schiff bases

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Synthesis, crystal structure, spectroscopic, and biological studies on Cu(II) complexes of N,O donor dimethyl isoxazole Schiff bases

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Cu(II) complexes with Schiff bases DMIIMP, DMIIMBD, DMIIMBP, DMIIMCP, DMIIMMP, and DMIIMNP (see Introduction for definitions) are derived from condensation of 3,4-dimethyl 5-amino-isoxazole with salicylaldehyde and substituted salicylaldehydes. The newly synthesized ligands were characterized by IR, UV-Vis, ^{1}H NMR, ^{13}C NMR, mass spectra, and elemental analysis. The Cu(II) complexes were characterized by IR, UV-Vis, ESR, elemental analysis, magnetic moments, thermogram, DTA, and single crystal analysis. The complexes have general formula [M $(L)_2$]. The Schiff bases are bidentate coordinating through the azomethine nitrogen and phenolic oxygen of salicylaldehydes. Based on the analytical and spectral data, four-coordinate geometry is assigned for all the complexes. ESR and single crystal analysis suggests square planar geometry for all complexes. [Cu(DMIIMP)$_2$] crystallizes in the orthorhombic system. Antimicrobial studies of Schiff bases and their metal complexes show significant activity with the metal complexes showing more activity than corresponding Schiff bases. Cytotoxicity of the copper complexes on human cervical carcinoma cells (HeLa) was measured using the Methyl Thiazole Tetrazolium assay.

Keywords: Isoxazole Schiff base; Cu(II) complexes; Single crystal; Square planar; Antimicrobial activity

1. Introduction

Heterocyclic rings containing Schiff bases and their analogs exhibit an important role in several biological processes [1–4]. Schiff-based complexes were used as metal indicators in complexometric titrations and as colorimetric reagents [5–9]. Generally, Schiff bases exhibit good pharmacological properties such as antibacterial, antifungal, anticancer, anti-HIV activity, and also have applications as pesticides and insecticides [9, 10]. Studies on metal complexes of Schiff bases derived from 3-amino-5-methyl isoxazole and substituted salicylaldehydes were reported earlier and their antimicrobial activity enhances upon metal chelation [11–14]. Synthesis and characterization of 2-((3,4-dimethylisoxazol-5-ylimino) methyl)phenol (DMIIMP) was reported earlier [15]. In continuation of this field of

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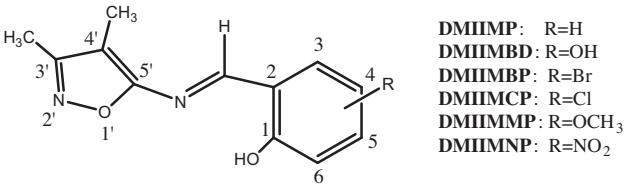

Figure 1. Structure of Schiff bases.

study, we report herewith synthesis and characterization of Schiff bases (see figure 1) of 2-((3,4-dimethylisoxazol-5-ylimino)methyl)benzene 1,4-diol) (DMIIMBD), 2-((3,4-dimethylisoxazol-5-ylimino)methyl)-4-bromophenol (DMIIMBP), 2-((3,4-dimethylisoxazol-5-ylimino)methyl)-4-chlorophenol (DMIIMCP), 2-((3,4-dimethylisoxazol-5-ylimino)methyl)-6-methoxyphenol (DMIIMMP), 2-((3,4-dimethylisoxazol-5-ylimino)methyl)-4-nitrophenol (DMIIMNP), and their Cu(II) complexes. $[Cu(DMIIMP)_2]$ was structurally characterized by single crystal X-ray crystallography. The biological activity of the Schiff bases and their metal complexes were carried out against bacteria and fungi. Cytotoxicity activity on human cervical carcinoma cells (HeLa) was measured using the methyl thiazole tetrazolium (MTT) assay.

2. Experimental

2.1. *Physical measurements*

^1H and ^{13}C NMR spectra of the ligands were recorded on a Bruker 400 MHz NMR instrument using tetramethyl silane as internal standard. EI mass spectra were recorded on a Vergleichdare Gerate (VG) micro mass 7070–H instrument; ESIMS spectra were recorded on a VG AUTOSPEC mass spectrometer. Digital conductivity meter of model DI-909 having a dip-type cell was calibrated with KCl solution. Electronic spectra of metal complexes in DMSO were recorded on a Shimadzu UV-Vis 1601 spectrophotometer. Magnetic susceptibilities of the complexes were determined on a Gouy balance model 7550 using $Hg[Co(NCS)_4]$ as standard. Diamagnetic corrections of the complexes were computed using Pascal's constants. Thermogram of complexes were carried out on a Mettler Toledo Star system in the temperature range of 0–1000 °C. Melting points of the ligands and decomposition temperature of complexes were determined on a Polmon instrument (model No. MP-96). IR spectra of the compounds were recorded using KBr pellets (4000–400 cm^{-1}) on a Perkin-Elmer Infrared model 337. The C, H, N compositions of the compounds were determined by using microanalytical techniques on a Perkin Elmer 240C (USA) elemental analyzer. EPR spectra of the copper complexes were recorded on an EPR Varian-E-112 at RT. The percentage composition of metal ions in solid metal complexes was determined by EDTA titration. All chemicals used were analytical reagent grade. Water, methanol, acetone, petroleum ether, and chloroform were purified by standard procedures [16].

2.2. *General procedure for the synthesis of isoxazole Schiff bases*

3,4-Dimethyl-5-amino-isoxazole (1.0 mM) was dissolved in hot methanol to which salicylaldehyde/5-hydroxy salicylaldehyde/5-bromo salicylaldehyde/5-chloro salicylaldehyde/3-methoxy salicylaldehyde and 5-nitro salicylaldehyde (1.0 mM) was added and the mixture was refluxed for 3 h under nitrogen. The dark yellow product formed was filtered and

washed with petroleum ether and recrystallized from methanol. Purity of the compounds was checked by TLC showing a single spot in petroleum ether and ethyl acetate (6:4) mixture. Yield: 80–85%.

2.3. *Microwave-assisted synthesis of isoxazole Schiff bases*

Microwave assisted condensation of 3,4-dimethyl-5-amino-isoxazole (1.0 mM) and salicylaldehyde/5-hydroxy salicylaldehyde/5-bromo salicylaldehyde/5-chloro salicylaldehyde/3-methoxy salicylaldehyde, and 5-nitro salicylaldehyde (1.0 mM) was carried out in 1 mL of methanol in a domestic oven (LG, 1300 W, 28 L capacity). The reaction mixture was subjected to microwave irradiation at 320 W for the period of time specified in table 1. Microwave-assisted synthesis brought down the reaction time from 3 h to 3 min and improved the yield from 80–85 to 90–95% in comparison with the conventional method. The purity of the compounds was checked by TLC showing a single spot in petroleum ether and ethyl acetate (6 : 4) mixture.

2.4. *Synthesis of Cu(II) metal complexes*

In preparation of metal complexes, the metal-to-ligand ratio was maintained at 1 : 2. Hot methanol solution of ligand (1.0 mM) and hot methanol solution of copper acetate monohydrate [$Cu(CH_3COOH)_2 \cdot H_2O$ (0.5 mM)] were mixed with constant stirring. The mixture was refluxed for 2–3 h at 70–80 °C on a water bath. On cooling, metal complexes precipitated. The products were filtered, washed with cold methanol, and dried under vacuum over P_4O_{10}.

2.5. *X-ray crystallographic procedures*

Dark green single crystals of [$Cu(DMIIMP)_2$] were grown by slow evaporation of methanol and chloroform mixture for three days at room temperature. A crystal of the complex was mounted on a glass fiber and used for data collection. Crystal data were collected at 298 K using a Bruker SMART APEX CCD single crystal diffractometer equipped with a graphite monochromator and Mo $K\alpha$ fine-focus sealed tube ($\lambda = 0.71073$ Å) operated at 2.0 kW, with increasing ω (width of 0.3°/frame) at a scan speed of 5 s/frame. Data integration and reduction were processed with SAINTPLUS software [17] and an empirical absorption correction was applied to the collected reflections with SADABS [18]. The crystal structure of the compound was solved by direct methods and refined on F^2 by full-matrix least-squares

Table 1. Comparison of conventional and microwave assisted syntheses of the compounds.

			Conventional		Microwave at 320 W	
S.No.	Ligand	M.P (°C)	Time (h)	Yield (%)	Time (s)	Yield (%)
1	DMIIMP	128	3	75	60	94
2	DMIIMBD	162	4	75	90	92
3	DMIIMBP	147	2	85	40	95
4	DMIIMCP	281	2	76	45	94
5	DMIIMMP	110	3	80	95	93
6	DMIIMNP	232	2	78	30	96

using SHELXTL-97 [19]. All nonhydrogen atoms were refined anisotropically and hydrogens on carbons were added from calculation.

2.6. *Cell culture*

The human cervical carcinoma cell lines (HeLa) were cultured as a monolayer with Roswell Park Memorial Institute medium (RPMI-1640), supplemented with 10% (v/v) fetal bovine serum (FBS), 2 mM L-glutamine, 4.5 g L^{-1} glucose, 1 × nonessential amino acids, and 1 × antibiotics consisting of penicillin/streptomycin, gentamycin, amphotericin B, and nystatin at 37 °C, in a humidified atmosphere of 5% CO_2, in a CO_2 incubator.

2.6.1. Cytotoxicity assay (MTT assay). The MTT assay was used to assess cytotoxicity [20]. HeLa were obtained from the National Center for Cell Science (NCCS), Pune, India. Copper complexes (1–6) were dissolved in DMSO, diluted in culture medium, and used to treat the cancer cell, with the complex at 2–10 μg mL^{-1}, for 72 h. DMSO diluted in the culture medium was used as the solvent control. A miniaturized viability assay using 3-[4,5-dimethyl thiazole-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) was carried out according to the method described by Mosmann [21]. HeLa cells growing exponentially were added to 96-well plates (Orange Scientific) at a density of 3×10^3 per well after counting on a Bright Line Haemocytometer (Sigma Ltd.). Compounds (1–100 μM) were then added to the wells, ensuring an equal volume of 200 μL across the plates. Cell number/proliferation was measured at 72 h using a standard-based assay (MTT assay) without modification. MTT (Hi Media Ltd.) was added to each well to yield a working concentration of 0.4 mg mL^{-1} and the plates were returned to the incubator for an additional 2 h. Then the medium was aspirated, 200 μL of DMSO (Sigma Ltd.) was added to each well and the plates were agitated gently for 5 min before measuring the optical density at 600 nm using a Thermo Scientific Multi Skan EX Elisa reader. The IC_{50} value was determined as concentration of the complex that is required to reduce the absorbance to half that of the control.

3. Results and discussion

3.1. *Characterization of metal complexes*

All the complexes are stable at room temperature and nonhygroscopic. On heating, they decompose at high temperatures. The complexes are insoluble in water but are soluble in DMSO.

3.1.1. Elemental analysis. The analytical data of the ligands, complexes, and the composition assigned to the complexes are presented in table 2. From the data, it is clear that the experimental values shown for each of the complexes are in agreement with the theoretical values calculated for 1 : 2 ratios.

3.1.2. IR spectra. In order to study binding of the Schiff bases to copper, IR spectra of the free ligands are compared with spectra of corresponding complexes. The important absorption frequencies of all complexes with their ligands and their assignments are given in table 3.

Table 2. Analytical data of Schiff bases and their metal complexes.

Complex	Formula	M.Wt.	C	H	N	O	M
DMIIMP	$C_{12}H_{12}N_2O_2$	216	66.56 (66.65)	5.56 (5.59)	12.83 (12.96)	14.24 (14.80)	
DMIIMBD	$C_{12}H_{12}N_2O_3$	232	61.95 (62.06)	5.19 (5.21)	11.97 (12.06)	20.54 (20.67)	
DMIIMBP	$C_{12}H_{11}BrN_2O_2$	296	48.76 (48.84)	3.58 (3.76)	9.34 (9.49)	10.54 (10.84)	
DMIIMCP	$C_{12}H_{11}ClN_2O_2$	250	57.05 (57.49)	4.34 (4.42)	11.07 (11.17)	12.24 (12.76)	–
DMIIMMP	$C_{13}H_{14}N_2O_3$	246	63.25 (63.40)	5.60 (5.73)	11.27 (11.38)	19.38 (19.49)	–
DMIMNP	$C_{12}H_{11}N_3O_4$	261	55.06 (55.17)	4.10 (4.24)	16.01 (16.09)	24.34 (24.50)	–
[Cu(DMIIMP)$_2$]	$[CuC_{24}H_{22}N_4O_4]$	493	57.85 (58.35)	4.01 (4.49)	12.07 (12.86)	12.24 (12.95)	12.54 (12.86)
[Cu(DMIIMBD)$_2$]	$[CuC_{24}H_{22}N_4O_6]$	525	54.42 (54.80)	4.13 (4.22)	9.94 (10.05)	18.01 (18.25)	11.91 (12.08)
[Cu(DMIIMBP)$_2$]	$[CuC_{24}H_{20}N_4O_4Br_2]$	653	44.05 (44.23)	2.98 (3.09)	8.37 (8.60)	9.26 (9.82)	9.24 (9.75)
[Cu(DMIIMCP)$_2$]	$[CuC_{24}H_{20}N_4O_4Cl_2]$	563	51.09 (51.21)	3.19 (3.58)	9.74 (9.95)	11.11 (11.37)	11.07 (11.29)
[Cu(DMIIMMP)$_2$]	$[CuC_{26}H_{26}N_4O_6]$	553	56.28 (56.36)	4.61 (4.73)	9.47 (10.10)	6.91 (7.33)	11.14 (11.47)
[Cu(DMIIMNP)$_2$]	$[CuC_{24}H_{20}N_6O_8]$	583	48.55 (49.36)	3.05 (3.45)	12.79 (14.39)	21.36 (21.92)	10.36 (10.88)

*Values mentioned within the bracket are calculated.

Table 3. IR absorption frequencies (cm^{-1}) of Schiff bases and complexes.

Compound	ν (OH)	ν (CH=N)	ν (C–O)	ν (M–O)	ν (M–N)	Other bands
DMIIMP	3427w, br	1598s	1164m	–	–	–
$[Cu(DMIIMP)_2]$	–	1611s	1179w	580m	453m	–
DMIIMBD	3416w 3325m	1572s	1171s	–	–	–
$[Cu(DMIIMBD)_2]$	3365 br	1586m	1194m	521w	421w	–
DMIIMBP	3446w, br	1641s	1182s	–	–	779s ν(C–Br)
$[Cu(DMIIMBP)_2]$	–	1647s	1174s	542m	455m	
DMIIMCP	3453w	1605s	1183s	–		778(s) ν(C–Cl)
$[Cu(DMIIMCP)_2]$	–	1607s	1176s	545w	456w	
DMIMNP	3447w, br	1602m	1293	–		–
$[Cu(DMIIMNP)_2]$	–	1604s	1311m	523m	417m	–
DMIIMMP	3444w, br	1597s	1255s	–	–	–
$[Cu(DMIIMMP)_2]$	–	1610s	1249s	525w	454w	–

In Schiff bases, azomethine stretches appear as split bands with two maxima at 1646–1602 and 1598–1561 cm^{-1}. These bands shift to higher frequency by 10–35 cm^{-1} in complexes, indicating nitrogen of azomethine is coordinated [22–25]. A broad band at 3447 to 3416 cm^{-1} due to phenolic OH disappears in their complexes, indicating coordination through phenolic hydroxyl [26]. For DMIIMBD, two medium intensity bands at 3416 and 3325 cm^{-1} are due to stretch of the phenolic OH and the second OH para with respect to the phenolic OH. A medium intensity band at 1293 to 1164 cm^{-1} due to phenolic υC–O shifted to higher or lower frequency by 8–23 cm^{-1} in the complexes, suggesting participation of the oxygen of the hydroxyl in bonding [27, 28]. Shifts are due to coordination of ligand to copper by the azomethine nitrogen and phenolic oxygen. This is also supported by υM–O and υM–N vibrations in the far infrared region (523–580 and 417–456 cm^{-1}), respectively [29–32].

3.1.3. Thermal analysis. The thermogram of Cu-DMIIMP is given in (Supplementary material with heating rate of 10 °C min^{-1} under nitrogen, from ambient temperature to 1000 °C. Thermal decomposition of $[Cu(DMIIMP)_2]$ can be divided into four stages 113–283, 284–368, 369–453, and 454–708 °C, respectively. The mass loss of 34.51, 10.34, 11.25, and 7.70% against calculated values of 39.64, 11.87, 12.92, and 8.84% show partial decomposition of the ligand. There is no clear plateau observed in the DT curve of the complex, *i.e.* the intermediate is unstable, continuing to lose mass. The final residue estimated as copper with 0.26 ligand is obtained at 708 °C and has mass loss of 23.45% against the calculated value of 26.73%.

3.1.4. Magnetic susceptibility and electronic spectra. Electronic spectra and magnetic moment of the metal complexes are listed in table 4. Magnetic moment values of Cu(II) complexes are 1.84–1.98 B.M. The Cu(II) complexes show a single broad band at 15,455 to 16,920 cm^{-1} due to $^2B_{1g} \rightarrow {}^2E_g$, suggesting square planar geometry. Square planar Cu(II) complexes are expected to give three bands. However, these bands usually overlap, giving only one broad absorption. The electronic spectra and magnetic moment data coupled with the analytical and conductance data suggest square planar geometry for the Cu(II) complexes.

Table 4. Electronic spectral data and magnetic susceptibilities.

Complex	Frequency in cm^{-1} (nm)	$\varepsilon = 10^2\ M^{-1}\ cm^{-1}$	μ_{eff} (BM)
$[Cu(DMIIMP)_2]$	16,920 (591)	0.045	1.87
$[Cu(DMIIMBD)_2]$	15,455 (647)	0.051	1.84
$[Cu(DMIIMBP)_2]$	16,233 (616)	0.059	1.98
$[Cu(DMIIMCP)_2]$	16,891 (592)	0.061	1.92
$[Cu(DMIIMMP)_2]$	15,954 (627)	0.072	1.89
$[Cu(DMIIMNP)_2]$	16,273 (614)	0.049	1.96

3.1.5. ESR Spectra. ESR spectra of the Cu(II) complexes were recorded as polycrystalline samples on X band at 9.3 GHz under magnetic field strength 3400G. The complexes show one intense isotopic absorption in the high field region due to tumbling of the molecules in solution. The EPR parameters of *viz*. $g_\parallel$, $g_\perp$, Δg, and G are presented in table 5. Values of $g_\parallel$ and $g_\perp$ are 2.149–2.159 and 2.039–2.043, respectively; $g_\parallel > g_\perp > 2.0023$ (g_e) suggest the unpaired electron is in the $d_{x^2-y^2}$ orbital giving $^2B_{1g}$ as the ground state, consistent with square planar geometry. The $g_\parallel$ values for all Cu(II) complexes are less than 2.3, in agreement with covalent metal ligand bond. The g values are related to axial symmetry parameter G by the Hathway expression, *i.e*. $G = (g_\parallel - 2.0023)/(g - 2.0023)$. According to the data, the G values for the Cu(II) complexes are less than four, indicating the ligands are strong field and the metal ligand bonding in these complexes is covalent [33, 34].

3.1.6. Structural elucidation of $[Cu(DMIIMP)_2]$ by single crystal analysis. Single crystal X-ray diffraction reveals that $[Cu(DMIIMP)_2]$ consists of $[C_{24}H_{22}CuN_4O_4]$ and crystallizes in centrosymmetric orthorhombic *Pbca* space group with Cu(II) on a special position. The X-ray crystal structure of $[Cu(DMIIMP)_2]$ shows half of the complex in its asymmetric unit and eight molecules in the unit cell; relevant parameters are tabulated in table 6. An ORTEP representation is shown in figure 2 with the atom numbering scheme; atoms shown with (A) are generated through symmetry relation and the relevant symmetry codes are (A) #1 $-x+1,-y,-z+1$. Selected bond lengths and angles are given in table 7. Copper lies on a crystallographic twofold rotational axis and is coordinated to two Schiff bases. The crystal structure consists of discrete molecular species in which Cu(II) is square planar, ligated through deprotonated phenolate oxygen O1 and the azomethine nitrogen N1. The observed bond distances from metal center to donor sites are Cu(1)–O(1) = 1.8743(19) Å and Cu(1)–N(1) = 1.984(2) Å, in agreement with reported values [35]. The O–Cu–O, N–Cu–N angles are 180.00 and 179.99, O–Cu–N angles are 90.92–89.08 [O(1)–Cu(1)–N(1) = 90.92° and O(1) #1–Cu(1)–N(1) = 89.08°] which are ideal values for square planar geometry. The unit cell parameters of the complex are $a = 10.0321(19)$ Å, $b = 9.8785(18)$ Å, $c = 22.786(4)$ Å, and $\alpha = \beta = \gamma = 90°$.

Table 5. ESR data of copper complexes.

Complex	Temperature (K)	$g_\parallel$	$g_\perp$	Δg	G
$[Cu(DMIIMP)_2]$	300	2.1594	2.0431	0.1162	3.6928
$[Cu(DMIIMBD)_2]$	300	2.1539	2.0402	0.1137	3.8279
$[Cu(DMIIMBP)_2]$	300	2.1498	2.0423	0.1075	3.5416
$[Cu(DMIICP)_2]$	300	2.1510	2.0391	0.1119	3.8624
$[Cu(DMIIMMP)_2]$	300	2.1497	2.0411	0.1086	3.6437
$[Cu(DMIIMNP)_2]$	300	2.1526	2.0426	0.1099	3.5807

Table 6. Crystal data and structure refinement details for $[Cu(DMIIMP)_2]$.

Empirical formula	$C_{24}H_{22}CuN_4O_4$
Formula weight	494.00
Temperature	298(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	*Pbca*
Unit cell dimensions	$a = 10.0321(19)$ Å $b = 9.8785(18)$ Å $c = 22.786(4)$ Å $\alpha = 90.00$ $\beta = 90.00$ $\gamma = 90.00$
Volume	2258.2(7) Å^3
Z	4
Density (calculated)	1.453 Mg m^{-3}
Absorption coefficient	1.006 mm^{-1}
F(0 0 0)	1020
Crystal size	0.42 × 0.32 × 0.16 mm^3
Theta range for data collection	1.79–26.02°
Index ranges	−12<=h<=11, −12<=k<=12, −27<=l<=27
Reflections collected	19,924
Independent reflections	2213 [R(int) = 0.0545]
Completeness to theta = 26.02°	99.1%
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.8556 and 0.6774
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	2213/0/153
Goodness-of-fit on F^2	1.077
Final *R* indices [$I > 2\sigma(I)$]	R1 = 0.0481, wR2 = 0.1141
R indices (all data)	R1 = 0.0643, wR2 = 0.1224
Largest diff. peak and hole (eÅ^{-3})	0.332 and −0.386

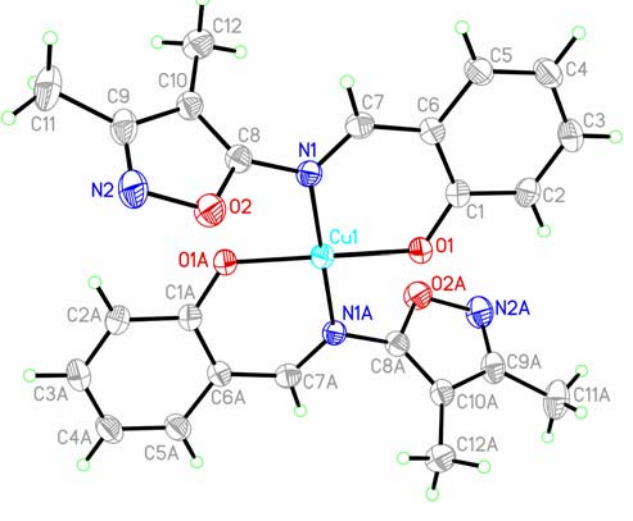

Figure 2. Thermal ellipsoidal plot of $[Cu(DMIIMP)_2]$ with atom labeling scheme. Displacement ellipsoids are drawn at 30% probability level except for the H atoms, which are shown as circles of arbitrary radius. Atoms shown with (A) are generated through symmetry relation and the relevant symmetry codes are (A) #1 −x + 1,−y,−z + 1.

Table 7. Selected bond lengths [Å] and angles [°] for [Cu(DMIIMP)$_2$].

C(7)–N(1)	1.299(4)
C(8)–O(2)	1.340(3)
C(8)–C(10)	1.341(4)
C(8)–N(1)	1.397(3)
C(9)–N(2)	1.289(5)
C(9)–C(11)	1.491(5)
C(10)–C(12)	1.482(4)
Cu(1)–O(1)	1.8743(19)
Cu(1)–O(1)#1	1.8743(19)
Cu(1)–N(1)#1	1.984(2)
Cu(1)–N(1)	1.984(2)
N(2)–O(2)	1.409(3)
O(1)–Cu(1)–O(1)#1	180.0
O(1)–Cu(1)–N(1)#1	89.08(9)
O(1)#1–Cu(1)–N(1)#1	90.92(9)
O(1)–Cu(1)–N(1)	90.92(9)
O(1)#1–Cu(1)–N(1)	89.08(9)
N(1)#1–Cu(1)–N(1)	179.998(1)

Symmetry transformations used to generate equivalent atoms: #1 $-x+1,-y,-z+1$.

3.1.7. Antimicrobial activity. DMIIMP, DMIIMBD, DMIIMBP, DMIIMCP, DMIIMMP, and DMIIMNP and their binary complexes with Cu(II) have been screened for antimicrobial activity against bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and fungi (*Aspergillus niger* and *Rhizopus oryzae*) by paper disc method. The susceptibility of the ligands and their metal complexes were determined by measuring the size of inhibition diameter. The concentration for these samples was maintained as 1 mg ml^{-1} in DMSO. The results thus obtained (table 8) are explained on the basis of Overtone's concept and Chelation theory [36, 37]. The mode of action of the compounds may involve formation of a hydrogen bond through the azomethine group with the active centers of cell constituents, resulting in interference with normal cell process [38].

The variation in the activity of different complexes against different organisms depend either on the impermeability of the cells of the microbes or difference in ribosome of microbial cells. A comparison of the biological activity of the synthesized compounds with some known antibiotics (Gentamycin) is presented in table 8. [Cu(DMIICP)$_2$], [Cu

Table 8. Antimicrobial activities of the Schiff bases and metal complexes.

Complex	*E. coli*	*P. aeruginosa*	*R. oryzae*	*A. niger*
DMIIMP	++	+	+	+
[Cu(DMIIMP)$_2$]	++	++	++	++
DMIIMBD	++	++	+	+
[Cu(DMIIMBD)$_2$]	+++	+++	++	++
DMIIMBP	++	++	++	++
[Cu(DMIIMBP)$_2$]	+++	+++	++	++
DMIIMCP	+	++	+	+
[Cu(DMIIMCP)$_2$]	+++	+++	++	++
DMIIMMP	+	+	+	+
[Cu(DMIIMMP)$_2$]	++	++	++	++
DMIMNP	+	+	+	+
[Cu(DMIIMNP)$_2$]	+	+	+	+
Gentamycin (Standard)	++	++	–	–

Highly active = +++ (inhibition zone > 15 mm); Moderatively active = ++ (inhibition zone > 10 mm); Slightly active = + (inhibition zone > 5 mm); Inactive = – (inhibition zone < 5 mm).

Table 9. *In vitro* cytotoxicities (HeLa cell line) of Cu(II) complexes.

Conc. (μg ml^{-1})	Absorbance	Cell viability (%)	Toxicities (%)	Conc. (μg ml^{-1})	Absorbance	Cell viability (%)	Toxicities (%)
Control	0.1165	100	0	Control	0.1165	100	0
[Cu(DMIIMP)$_2$]				[Cu(DMIIMCP)$_2$]			
2	0.0760	65.23	34.77	2	0.0915	78.54	21.46
4	0.0615	52.78	47.22	4	0.0820	70.38	29.62
6	0.0615	52.78	47.22	6	0.0670	57.51	42.49
8	0.0595	51.07	48.93	8	0.0515	44.20	55.80
10	0.0580	49.78	50.22	10	0.0425	36.48	63.52
[Cu(DMIIMBD)$_2$]				[Cu(DMIIMMP)$_2$]			
2	0.1055	90.55	9.45	2	0.0855	73.39	
4	0.1030	88.41	11.59	4	0.0810	69.52	0.0915
6	0.0885	75.96	24.04	6	0.0765	65.66	34.34
8	0.0680	58.36	41.64	8	0.0735	63.09	36.91
10	0.0540	46.35	53.65	10	0.0630	54.07	45.93
[Cu(DMIIMBP)$_2$]				[Cu(DMIIMNP)$_2$]			
2	0.1040	89.27	10.73	2	0.1085	93.13	6.87
4	0.0615	52.78	47.22	4	0.1025	87.98	12.02
6	0.0590	50.64	49.36	6	0.1010	86.69	13.31
8	0.0540	46.35	53.65	8	0.0750	64.37	35.63
10	0.0530	45.49	54.51	10	0.0735	63.09	36.91
				12	0.0612	52.53	47.47
				14	0.0531	45.57	54.43

$(DMIIMBD)_2]$, and $[Cu(DMIIMBP)_2]$ show good activity against bacteria and fungi. The Schiff bases show moderate activity and all Cu(II) complexes show appreciable activity, suggesting increase in activity upon complexation. On comparison with earlier reported similar N,O donor Schiff base Cu(II) complexes, activity enhances upon complexation [39, 40].

3.1.8. Cytotoxic activity. The cytotoxic activity of copper complexes was examined on cultured HeLa for 72 h to medium containing the respective complexes at 2–10 μg ml^{-1} using MTT assay and results are given in table 9. The cytotoxic activity determined according to dose values of the exposure of the complex required to reduce survival of the cell is given in figure 3. Toxicities of 10 μg ml^{-1} of $[Cu(DMIIMP)_2]$, $[Cu(DMIIMBD)_2]$, $[Cu(DMIIMBP)_2]$, $[Cu(DMIIMCP)_2]$, $[Cu(DMIIMMP)_2]$, and $[Cu(DMIIMNP)_2]$ are 50.22, 53.65, 54.51, 63.52, 45.93, and 47.47%, respectively. $[Cu(DMIIMCP)_2]$ is a good antitumor agent on HeLa cell lines. The IC_{50} values are given in table 10. The IC_{50} values

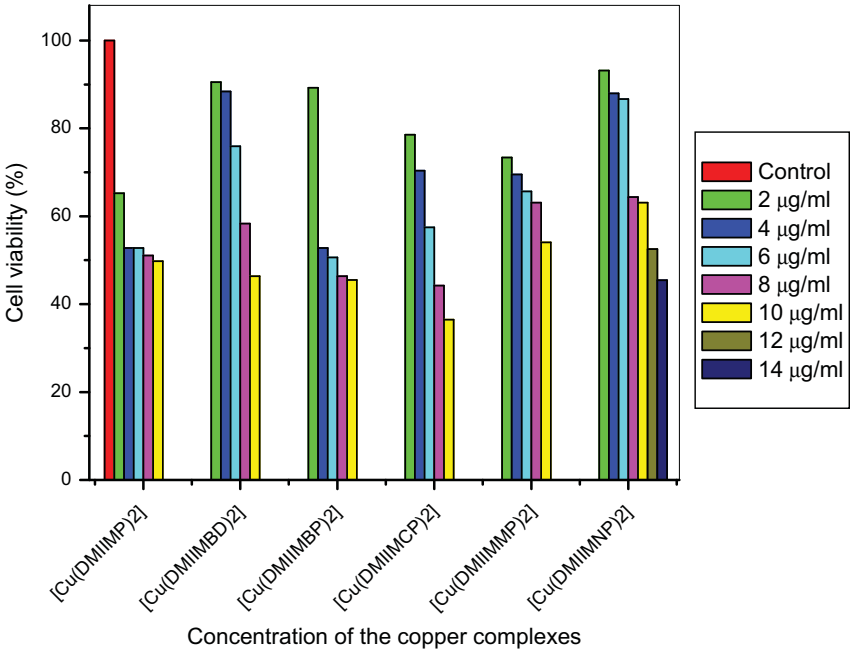

Figure 3. Percentage of cell viability *vs*. different concentrations for HeLa cells exposed to the copper complexes after 72 h incubation.

Table 10. IC_{50} range of Cu(II) complexes for HeLa cells.

Complex	IC_{50} (μg ml^{-1})
$[Cu(DMIIMP)_2]$	10 ± 0.04
$[Cu(DMIIMBD)_2]$	10 ± 0.7
$[Cu(DMIIMBP)_2]$	6 ± 0.08
$[Cu(DMIIMCP)_2]$	6 ± 0.9
$[Cu(DMIIMMP)_2]$	10 ± 0.8
$[Cu(DMIIMNP)_2]$	12 ± 0.6

of $[Cu(DMIIMP)_2]$, $[Cu(DMIIMBD)_2]$, $[Cu(DMIIMMP)_2]$, and $[Cu(DMIIMMNP)_2]$ are higher for the 72 h treatment groups, in the range of 10 ± 0.04–12 ± 0.61 μg ml^{-1}, whereas $[Cu(DMIIMBP)_2]$ and $[Cu(DMIIMCP)_2]$ are 6 ± 0.90 μg ml^{-1}.

4. Conclusions

Complexes of Cu(II) with N,O donor Schiff bases DMIIMP, DMIIMBD, DMIIMBP, DMIIMCP, DMIIMMP, and DMIIMNP have been synthesized and characterized by analytical, conductance, IR, electronic, ESR spectral data, magnetic moments, and single crystal X-ray crystallographic data. The metal ligand stoichiometries in these complexes are 1 : 2. The Schiff-based ligands are mononegative, bidentate, coordinating through nitrogen of azomethine and phenolic oxygen. Based on analytical data, all these complexes are square planar. The ligands and metal complexes were screened against bacteria and fungi as size of inhibition diameter. The Schiff bases show moderate activity and all Cu(II) complexes show appreciable activity. $[Cu(DMIICP)_2]$, $[Cu(DMIIMBD)_2]$, and $[Cu(DMIIMBP)_2]$ exhibit more potent antibacterial activity than the antibiotic. The cytotoxicity of all the Cu(II) complexes was studied and $[Cu(DMIIMCP)_2]$ at 10 μg ml^{-1} concentration shows 63.52% toxicity, suggesting a good antitumor agent on HeLA.

Supplementary material

Crystallographic details of $[Cu(DMIIMP)_2]$ in the form of CIF file is available with the CCDC, number 851,651. This data can be obtained free of charge from the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB21EZ, UK; Fax: (+44) 1223-336-033 or E-mail: deposit@ccdc.cam.ac.uk.

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