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Synthesis and spectral characterization of Schiff base complexes of Cu(II), Co(II), Zn(II) and VO(IV) containing 4-(4-aminophenyl) morpholine derivatives: Antimicrobial evaluation and anticancer studies

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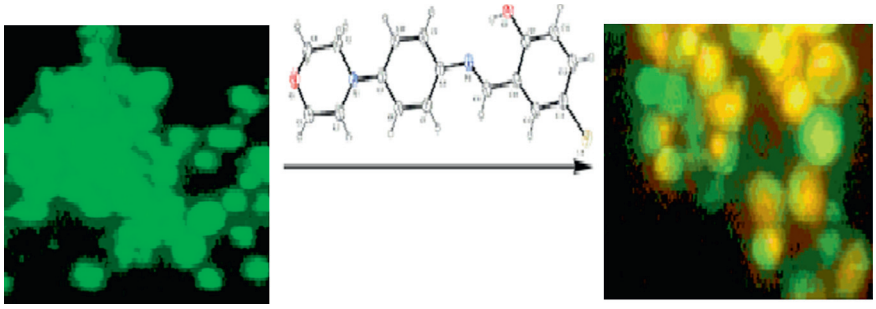
HIGHLIGHTS

- Synthesis of novel morpholinoaniline based Schiff bases.
- Reporting structurally solved morpholinoaniline for the first time.
- Complexes having potential antibacterial and antifungal activity.
- Good to moderate anticancer activity against HepG2.
- Zinc complex exhibits a good inhibitory activity against the human gastric cancer cells.

GRAPHICAL ABSTRACT



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ABSTRACT

Metal(II) chelates of Schiff bases derived from the condensation of 4-morpholinoaniline with substituted salicylaldehyde have been prepared and characterized by ^{1}H NMR, IR, electronic, EPR, and magnetic measurement studies. The complexes are of the type M(X-MPMP)2 [where M = Cu(II), Co(II)), Zn(II), or VO(IV); MPMP = 2-[(4 morpholinophenyl imino) methyl] 4-X-phenol, X = Cl, (L_1H), X = Br (L_2H)]. Single crystal X-ray crystallography studies confirm the structure of newly synthesized Schiff bases. The Schiff bases act as bidentate monobasic ligands, coordinating through deprotonated phenolic oxygen and azomethine nitrogen atoms. The free ligands and metal complexes are screened for their biopotency. Metal complexes exhibit better activity than ligands. Anticancer activity of ligands and their metal complexes are evaluated in human heptocarcinoma(**HepG2**) cells. The preliminary bioassay indicates that the Schiff base and its zinc complex exhibit inhibitory activity against the human gastric cancer cell lines.

Introduction

Schiff bases, a class of chelators are capable of forming coordinate bonds with many metal ions through both azomethine group and phenolic group or via its azomethine or phenolic groups [1–4]. The chemistry of Schiff base ligands and their metal complexes have attracted a lot of interest due to their facile synthesis and wide range of applications including antifungal, antibacterial, anticancer and catalytic fields [5–8]. The design, synthesis and structural characterization of salicylaldimine complexes are a subject of current interest [9]. Due to their interesting and versatile

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structural, magnetic, spectral, and catalytic and redox properties, they are used as models for metalloenzymes and various theoretical problems of chemistry [10].

4-Phenyl-morpholine derivatives are reported to possess antimicrobial [11,12], anti-inflammatory [13–15] and central nervous system activities [16–20]. Linezolide (commercially available antimicrobial) also possess a 4-phenyl-morpholine substituent. It is observed that 4-phenyl-morpholine is a potential substituent to impart significant antimicrobial property to quinazoline moiety [21]. These observations led to the conception that Schiff bases of 4-(4-aminophenyl)-morpholine would possess potential antimicrobial properties.

Cancer has overtaken heart disease as the world's top killer by 2010, part of a trend that should be more than double global cancer cases and deaths by 2030 [22]. Hepatocellular carcinoma is the most common type of liver cancer and causes more than 600,000 deaths in china each year [23]. Metal-based pharmaceuticals emerging from the interface of inorganic chemistry, pharmacology, toxicology and biochemistry have witnessed spectacular successes [24,25]. The discovery of the cytotoxic properties of cisplatin has provided enormous impetus for research into the use of metal complexes in the fight against cancer [26]. A broad array of medicinal applications of these compounds has been investigated, and some of them are found to be useful both as clinical diagnostic agents and in chemotherapeutic applications [27–36]. Though several Schiff base complexes are reported in the literature, morpholinoaniline Schiff base complexes have not been published. In continuation of our work in the area of Schiff base complexes [37], the synthesis, characterization, crystal structure determination and biological screening of metal (II) complexes of morpholinoaniline Schiff bases are described here.

Experimental

Materials

5-Chlorosalicylaldehyde, 5-Bromosalicyladehyde and 4-Morpholinoaniline are procured from Sigma–Aldrich. Copper acetate, cobalt acetate, zinc acetate, vanadyl (IV) sulfate, N,N′-dimethylformaide, N,N-dimethylsulfoxide, acetonitrile, chloroform, ethanol and methanol are purchased form Merck.

Physical measurements

Crystal data collection APEX2 (Bruker, 2004); cell refinement: SAINT (Bruker, 2004), ^{1}H NMR spectra are recorded on a Bruker 300 MHz spectrometer. IR spectra are recorded in KBr disks with a Perkin Elmer FT-IR spectrophotometer. UV–Vis spectra of solution are recorded on a Shimadzu 1700 series spectrometer. Bio-Analytical Systems (BAS) model CV 50 electrochemical analyzer is used for cyclic voltammetric experiments. Magnetic parameters are measured with Lakeshore VSM 7304 with a maximum field of 20 KOe. ESR spectra are recorded on a Varian E-112 spectrometer.

Anti-bacterial screening

Antibacterial activities are investigated using agar well diffusion method. The activity of the free ligand, its metal complexes and standard drug Amikacin are studied against the *Chromobacterium vialacium*, *Staphylococcus aureus* (as grampositive bacteria), *Staphylococciaureus*, *Pseudomonasaeruginosa*, *and Shigella byogenes* (as gram negative bacteria). The solution of 2 mg/mL of each compound (free ligand, its metal complexes and standard drug Amikacin) in DMSO is prepared for testing against bacteria. Centrifuged pelletes of bacteria from a 24 h old culture containing approximately 10^4 to 10^6 CFU (colony forming unit) per mL are spread on the surface of Muller Hinton Agar plates. Wells are created in medium with the help of a sterile metallic bores and nutrients agar media (agar 20 g + beef extract 3 g + peptones 5 g) in 1000 mL of distilled water (PH 7.0), autoclaved and cooled down to 45 °C. Then, it is seeded with 10 mL of prepared inocula to have 10^6 CFU/mL. Petri plates are prepared by pouring 75 mL of seeded nutrient agar. The activity is determined by measuring the diameter of the inhibition zone (in mm). The growth inhibition is calculated according to Ref. [38].

Antifungal screening

The anti-fungal activity of the ligands and their metal complexes are studied by paper disc method [38]. *Aspergillusniger*, *Pencilliumchrysogenum* and *Candida albicans* are used as test organisms. Solution of desired concentration (1 mg/mL) was obtained by dissolving 2, 4, 6 mg of each compound in DMSO and added to Potato Dextrose Agar (PDA) medium in sterile Petri dishes. The sterilized medium with the added sample solution is poured into sterile Petri plates and allowed to solidify. Filter paper discs of 5 mm diameter are prepared prior to the experiment. The filter paper discs are placed on nutrient medium mixed with fungal strains. These Petri dishes are incubated at 35 °C for 48 h. The percent reduction in the radial growth diameter over the control is calculated. The growth is compared with dimethylsulfoxide as the control and Ketoconazole as a standard drug.

Cell viability test

The viability of cells is assessed by MTT assay using mononuclear cells. The assay is based on the reduction of soluble yellow tetrazolium salt to insoluble purple formazan crystals by metabolically active cells. Only live cells are able to take up the tetrazolium salt. The enzyme (mitochondrial succinate dehydrogenase) present in the mitochondria of the live cells is able to convert internalized tetrazolium salt to formazan crystals, which are purple in color. Then, the cells are lysed and dissolved in DMSO solution. The color developed is then determined in an ELISA reader at 570 nm.

The Hepatocellular carcinoma cells (HepG2 cells) are plated separately in 96 well plates at a concentration of 1×10^5 cells/well. After 24 h, cells are washed twice with 100 μl of serum-free medium and starved for an hour at 37 °C. After starvation, cells are treated with different concentrations of test compound (50–300 μg/ml) for 24 h. At the end of the treatment period, the medium is aspirated and serum free medium containing MTT (0.5 mg/ml) is added, Then it is incubated for 4 h at 37 °C in a CO_2 incubator.

The MTT containing medium is then discarded and the cells are washed with PBS (200 μl). The crystals are then dissolved by adding 100 μl of DMSO and this is mixed properly by pipetting up and down. Spectrophotometrical absorbance of the purple blue formazan dye is measured in a micro-plate reader at 570 nm [39].

Synthesis of Schiff base ligands

An ethanolic solution of 4-morpholinoaniline (5 mmol) is magnetically stirred in a round bottom flask followed by dropwise addition of appropriate substituted salicylaldehyde (5 mmol) containing 2–3 drops of glacial aceticacid. The reaction mixture is then refluxed for 3 h and upon cooling to 0 °C, crystalline solid precipitates from the mixture are separated out. Crystalline products are washed with ice cold ethanol and dried in vacuo over anhydrous $CaCl_2$. Single crystals suitable for the X-ray diffraction are obtained by slow evaporation of a solution of the title compound in DMF at room temperature (Supplementary files, Scheme 1).

Synthesis of Cu(II), Co(II), Zn(II) and VO(IV) complexes

All the complexes are prepared using the following general procedure. Methanolic solutions (10 mL) of the Schiff base (1 mmol) and the metal acetate (0.5 mmol) (10 mL) are mixed thoroughly and boiled under reflux for 4–6 h, and then cooled to room temperature. The resulting precipitate is filtered, washed in the ice cold methanol and dried *'in vacuo'* (Supplementary files Scheme 2).

Results and discussion

Analytical, color and magnetic susceptibility data of all metal complexes are given in Table 1 and are in good agreement with proposed composition.

Crystal structure of Cl-MPMP and Br-MPMP

The crystals of present compounds belong to P21/C space group. The structures have been solved by direct method. The hydrogen positions have been fixed by a three dimensional difference Fourier synthesis. The Position coordinates and their temperature factors have been refined by least square method.

The geometric parameters like bond length and bond angle are listed in Table 2. Inter and intra molecular hydrogen bonding geometric parameters are listed in the Table 3. These parameters are similar in both the molecular structures. Most of the hydrogen bonding features are similar in both the molecular structures. The intra molecular and intermolecular interactions can be considered in structure analysis. The morphology of hydrogen bonded arrays present in these structures can be defined by the graph set assignments. The morpholine ring has found to assume a chair conformation in both the structures. Rings (C5···C10) and (C12···C17) are planar in both the structures and inclined about an angle 15.58° in Cl-MPMP. But both the rings are nearly in the same plane in the structure Br-MPMP. An intra molecular hydrogen bond O2—H2···N2 forms S(6) motif in graph set notation (Fig. 1. and Supplementary files Fig. S1). Due to the lack of more hydrogen bonds C—H···O hydrogen bonds determine the crystal packing. The H bond C2—H2A···O2 forms a linear chain with graph set motif C(3) along *X*-axis. The H bond C13—H13···O1 forms a srew related zig–zag chain in C(3) graph set motif along *Y*-axis.

An inversion related hetero dimer is found with H bonds C15—H15···Cl1 and C15—H15···Br1 and these hydrogen bonds form a motif $R_2^2(8)$. The molecules are packed as ridges in the structure along *Y*-axis. The packing of the molecules looks like a net across XY plane. The angle of inclination between the ridges is 64.54° in Br-MPMP and 66.48° in Cl-MPMP.

NMR spectra

The ^{1}H NMR spectra of the ligand and the zinc complex are recorded in $CDCl_3$ and DMSO-d6-solvent respectively (Supplementary files Figs. S2 and S3). Singlet peaks observed in the ^{1}H NMR spectra of the ligands at ~13.55 and 8.54 ppm are due to phenolic (—H) and azomethine(—CH=N) protons of ligands respectively (Table 4). The peak due to the phenolic proton is absent, while the peak due to the azomethine proton is shifted to 8.26 ppm in the spectrum of the Zn(II) complex. This observation clearly supports the involvement of the —OH chromophore and azomethine nitrogen in coordination. The aromatic ring protons are observed as multiplets in the region 6.74–7.48 ppm in the spectrum of ligand. These peaks are shifted slightly in the spectrum of the Zn(II) complex (Fig. 2). Multiplets observed in 3.19–3.22 ppm and 3.79–3.89 ppm ranges are due to aliphatic-CH_2 protons.

Infrared spectra

The IR spectral data for the ligands and their metal complexes are given in Table 5. The spectrum of free ligands has showed a band in 1655 cm^{-1} region characteristics of the $\upsilon_{C=N}$ stretching mode indicating the formation of the Schiff base product. This band is shifted towards lower frequencies in the spectrum of its metal complexes (1615–1613 cm^{-1}) and is compared with the above Schiff bases indicating the involvement of the azomethine nitrogen in coordination with metal ion. The C—O (phenolic) is observed in the range 1352–1301 cm^{-1}. Both the ligands in the present investigation exhibit a broadband centered at 3410–3450 cm^{-1}. This suggests the presence of a strongly hydrogen bonded OH group. It also suggests that the ligands exist in enol form in the solid state [40]. The coordination mode of ligand is supported by the disappearance of these bands in the spectra of complexes. [41,42]. Bands due to M–N appear in 647–669 cm^{-1} region.

Table 1
Analytical and physical data of the ligand and its metal complexes.

Compound	Empirical formula (formula weight)	Color	Elemental analysis(found)			Magnetic moment (μ_B)
			C	H	N	
L1-Cl-MPMP (1)	$C_{17}H_{17}N_2O_2Cl$ (316.81)	Pale yellow	64.46 (64.31)	5.41 (5.51)	8.84 (8.67)	–
L2-Br-MPMP (2)	$C_{17}H_{17}N_2O_2Br$ (361.23)	Pale yellow	56.52 (56.64)	4.74 (4.54)	7.75 (7.98)	–
$Cu(L1)_2$ (3)	$C_{34}H_{32}N_4O_4Cl_2Cu$ (695.09)	Brown	58.75 (59.89)	4.64 (4.49)	8.06 (7.68)	1.86
$Cu(L2)_2$ (4)	$C_{34}H_{32}N_4O_4Br_2Cu$ (784)	Brown	52.09 (52.33)	4.11 (4.21)	7.15 (6.89)	1.81
$Co(L1)_2$ (5)	$C_{34}H_{32}N_4O_4Cl_2Co$ (690.48)	Red	59.14 (58.89)	4.67 (4.54)	8.11 (7.89)	4.72
$Co(L2)_2$ (6)	$C_{34}H_{32}N_4O_4Br_2Co$ (779.38)	Red	52.4 (52.25)	4.14 (4.23)	7.19 (6.79)	4.56
$VO(L1)_2$ (7)	$C_{34}H_{32}N_4O_5Cl_2V$ (698.49)	Brown	58.46 (58.66)	4.62 (4.39)	8.02 (7.89)	1.87
$VO(L2)_2$ (8)	$C_{34}H_{32}N_4O_5Br_2V$ (789.49)	Brown	51.86 (52.34)	4.1 (4.28)	7.12 (7.27)	1.92
$Zn(L1)_2$ (9)	$C_{34}H_{32}N_4O_4Cl_2Zn$ (696.94)	Dark yellow	58.59 (58.67)	4.63 (4.42)	8.04 (7.82)	–
$Zn(L2)_2$ (10)	$C_{34}H_{32}N_4O_4Br_2Zn$ (785.84)	Dark yellow	51.97 (51.75)	4.1 (4.37)	7.13 (7.42)	–

Table 2
Crystal data and structure refinement for Cl-MPMP and Br-MPMP.

Empirical formula	$C_{17}H_{17}ClN_2O_2$	$C_{17}H_{17}BrN_2O_2$
Formula weight	316.78	361.24
Temperature	T = 293(2) K	T = 293(2) K
Wavelength	0.71073 Å	0.71073 Å
Crystal system, space group	Monoclinic, $P2_1/c$	Monoclinic, $P2_1/c$
Unit cell dimensions	a = 10.2620(6) Å σ = 90°	a = 10.2981(7) Å σ = 90°
	b = 8.0531(5) Å β = 99.465 (2)°	b = 8.1334(6) Å β = 99.673(2)°
	c = 18.6368 (11) Å γ = 90°	c = 18.7663(13) Å γ = 90°
Volume	1519.20 (16) Å3	1549.49(19) $Å^3$
Z, Calculated density	4,1.385 Mg/m^3	8, 1.549 Mg/m^3
Absorption coefficient	0.260 mm^{-1}	2.662 mm^{-1}
F(000)	664	736
Crystal size	0.30 × 0.20 × 0.20 mm	0.30 × 0.20 × 0.20 mm
Theta range for data collection	θ = 2.2–26.00°	q = 2.20–26.00°
Limiting indices	$h = -12 \rightarrow 12$	$-17 \leqslant h \leqslant 17$,
	$k = -9 \rightarrow 9$	$-11 \leqslant k \leqslant 11$,
	$l = -21 \rightarrow 22$	$-20 \leqslant l \leqslant 20$
Reflections collec/unique	15,357/2976 [R_{int} = 0.0285]	15,323/3028 [R(int) = 0.0291]
Completeness to Δ = 26.29	99.90%	99.50%
Max. and min. transmission	0.9498 and 0.9260	0.6181 and 0.5023
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/parameters	2976/0/200	3028/0/200
Goodness-of-fit on F^2	1.05	1.076
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.031, wR_2 = 0.0837	R_1 = 0.0257, wR_2 = 0.0744
R indices (all data)	R_1 = 0.038, wR_2 = 0.0895	R_1 = 0.0361, wR_2 = 0.0805
Largest diff. peak and hole	−0.16 (e $Å^{-3}$)	−0.250 (e $Å^{-3}$)

Table 3
Hydrogen bonds for Cl-MPMP and Br-MPMP (A and °).

D—H···A	d(D—H)	d(H···A)	d(D···A)	<(DHA)
O(2)—H(2)···N(2)	0.82	1.83	2.5604(16)	147.5
O(2)—H(2)···N(2)	0.82	1.84	2.569(2)	147.7

Symmetry transformations used to generate equivalent atoms.

Electronic spectra

The electronic spectra are recorded in $CHCl_3$. The ligand field spectra of the copper complex in $CHCl_3$ show a band around 700 nm due to a combination of $^2B_{1g} \rightarrow {}^2A_{1g}$ and $^2B_{1g} \rightarrow {}^2B_{2g}$ transitions [43]. For a vanadyl complex, in a square pyramidal environment, three transitions are expected [44]. In the present case, an intense charge transfer band masks the higher energy bands and hence only one band is observed. In chloroform solution, a band at 655 nm is observed which is assigned to the transition $^2B_2 \rightarrow {}^2E$ (υ_1) [45–49].

Magnetic moment

The magnetic susceptibility measurements in the solid state show that the present complexes are paramagnetic at room temperature. The $\mu_{eff.}$ value of the copper(II) and oxovanadium(IV) complexes fall in the range of 1.80–1.92 B.M. corresponding to one unpaired electron [50,51]. For Co(II) complexes, the observed magnetic moment values suggest a high spin octahedral structure.

Electrochemistry

Cyclic voltammograms of the present complexes are recorded in CH_3CN and scanned in the cathodic direction at a rate of 100 mV/s using tetrabutylammonium perchlorate as the supporting electrolyte in the potential +1.6 V to −1.6 V range. The representative cyclic voltammogram of the complex is given in Fig. 3.

In general, redox data have been used to identify the model system among the synthetic complexes. In the copper complexes, the redox couple Cu(II)/Cu(I) is important and is known to be influenced by the ligand factors such as nature of donor atoms and their structural arrangement around the copper ion [52]. Copper complexes show an irreversible redox couple with a cathodic and anodic peak potential at E_p^c = −650 mV and E_p^a = −950 mV respectively. Complexes 3 and 4 show the negative reduction potential, which is typically seen in many other simple square planar complexes [53,54].

EPR spectra

The X-band EPR spectra of copper complexes are recorded in acetonitrile solution. The EPR spectral parameters derived from their spectra are presented in Table 6 (Supplementary files Fig. S4a). The EPR spectra of copper complexes in solution exhibit a set of four well resolved peaks in the high field region. The "g" tensor values of the copper(II) complexes can be used to derive the ground state [50,55–57]. In axially elongated octahedral and square planar complexes, the unpaired electron occupies the $d_{x^2-y^2}$ orbital with $^2B_{1g}$ ground state resulting in $g_{\|} > g_{\perp}$. However, in a compressed octahedron the unpaired electron occupies the d_z^2 orbital with $^2A_{1g}$ ground state having $g_{\perp} > g_{\|}$. The observed "g" values suggest that the unpaired electron lies predominantly in the $d_{x^2-y^2}$ orbital.

For all vanadyl complexes, the ESR. spectra are recorded in toluene/acetonitrile solutions at RT and LNT. The solution spectra consist of eight hyperfine lines (Supplementary files Fig. S4b). The observed spin Hamiltonian parameters $A_{\|}$ and $g_{\|}$ (Table 6) are in good agreement with the values generally expected for a vanadyl complex with square pyramidal geometry having a VON_2O_2 chromophore [58,59]. From the 'g' values, it is evident that the unpaired electron is in d*xy* orbital.

Antimicrobial activity

The synthesized ligand and the complexes are tested for their in vitro antimicrobial activity. They are tested against the bacteria *C.vialacium*, *S. Flexinari*, *S.aureus*, *P.aeruginosa*, *S.byogenes*, *P. chrysogenum*, *A. niger*, and *C. albicans*. The minimum inhibitory concentration (MIC) values of the investigated compounds against bacteria and fungi are summarized in Tables 7 and 8 respectively.

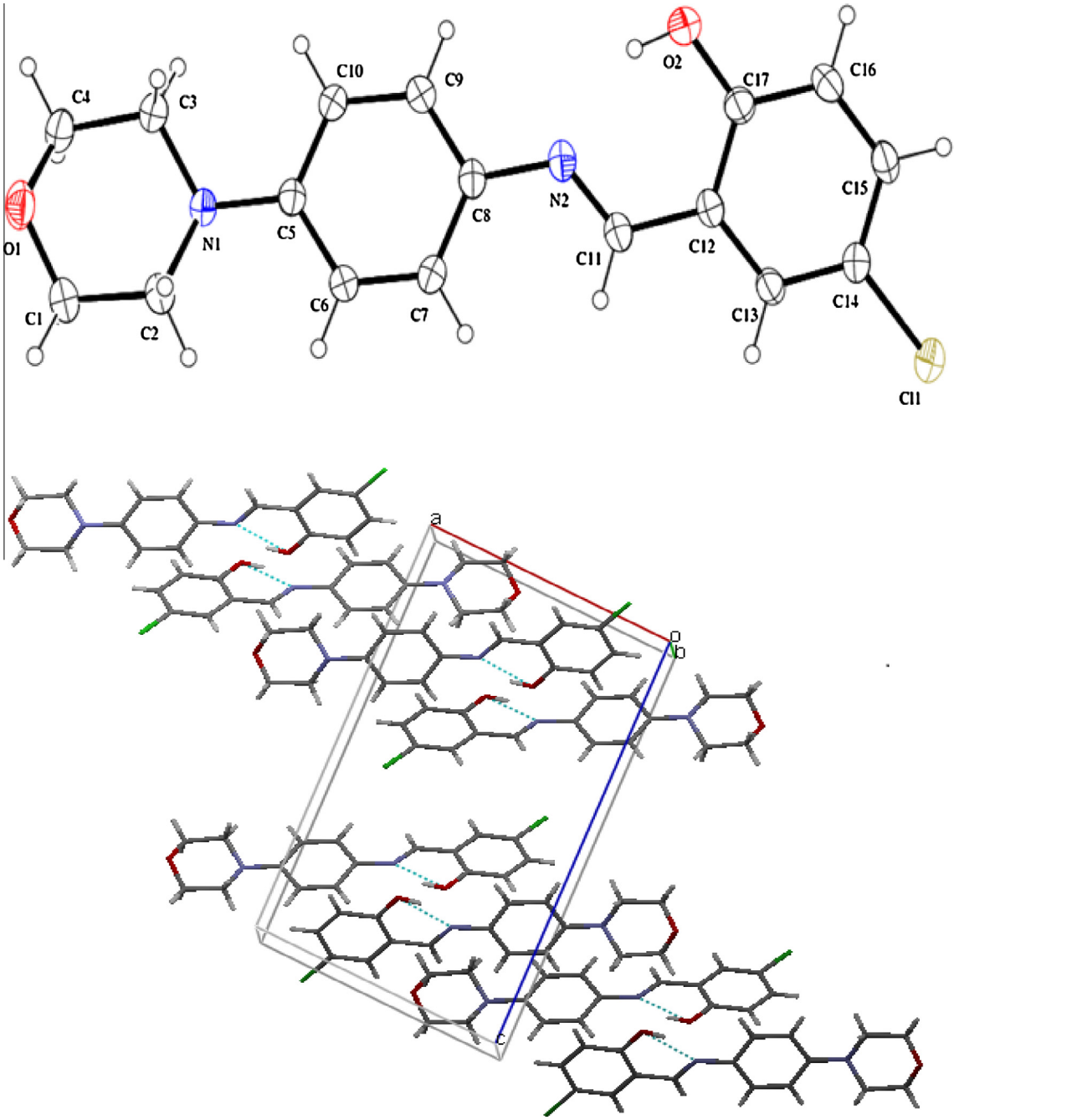

Fig. 1. Crystal structure and unit cell packing diagram for L1-(Cl-MPMP).

Table 4
^{1}NMR spectral data of the ligand and its metal complexes (in ppm).

Compound	□(O—H)	□(CH═N)	Aromatic	Aliphatic
Br-MPMP	13.55	8.545	6.89–6.95(m,3H) 7.21–7.48(m,4H)	3.86–3.89(m,4H) 3.19–3.22(m,4H)
Cl-MPMP	13.52	8.551	6.92–6.96(m,3H) 7.26–7.34(m,4H)	3.86–3.89(m,4H) 3.19–3.22(m,4H)
Zn(Cl-MPMP)	–	8.26	6.74–7.32(m,14H)	3.79–3.81(m,4H) 3.08–3.11(m,4H)

Representative inhibitory images are presented in Supplementary files Figs. S5 and S6 respectively. A comparative study of MIC values of ligand and the complexes indicate that the metal complexes exhibit higher antimicrobial activity than the free ligand. Such increased activity of the complexes can be explained on the basis of the Tweedy's chelation theory [60]. Chelation reduces the polarity of the metal ion considerably because of the partial sharing of its positive charge with the donor group and also due to π-electron delocalization on the whole chelate ring. The lipids and polysaccharides are some important constituents of the cell wall and membranes which are preferred for metal ion interaction. Apart from this, the cell walls also contain many phosphates, carbonyl and cystenyl ligands which maintain the integrity of the membrane by acting as a diffusion barrier and also provide suitable sites for binding. Furthermore, the reduction in polarity increases the lipophilic character of the chelates and an interaction between the metal ion and the lipid is favored. This may lead to the breakdown of the permeability barrier of the cell resulting in

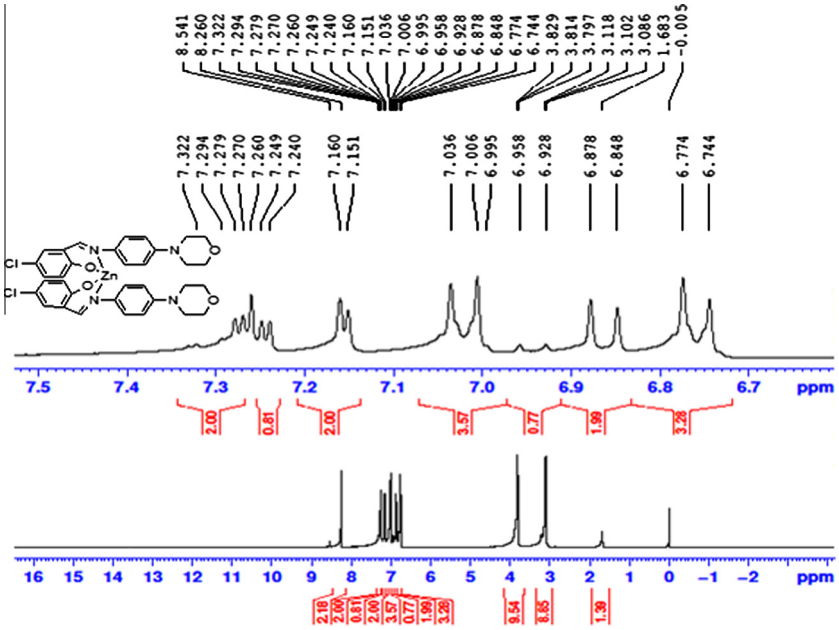

Fig. 2. ^{1}H NMR spectrum of Zn (Cl-MPMP)$_2$ in $CDCl_3$.

Table 5
IR spectral data of the ligand and its metal complexes (in cm^{-1}).

Compound	υ(C═N)	υ(C—O)
L1-Cl-MPMP (1)	1655	1352
L2-Br-MPMP (2)	1655	1342
Cu(L1)2 (3)	1615	1308
Cu(L2)2 (4)	1612	1306
Co(L1)2 (5)	1604	1320
Co(L2)2 (6)	1610	1296
VO(L1)2 (7)	1610	1304
VO(L2)2 (8)	1613	1307
Zn(L1)2 (9)	1606	1321
Zn(L2)2 (10)	1614	1301

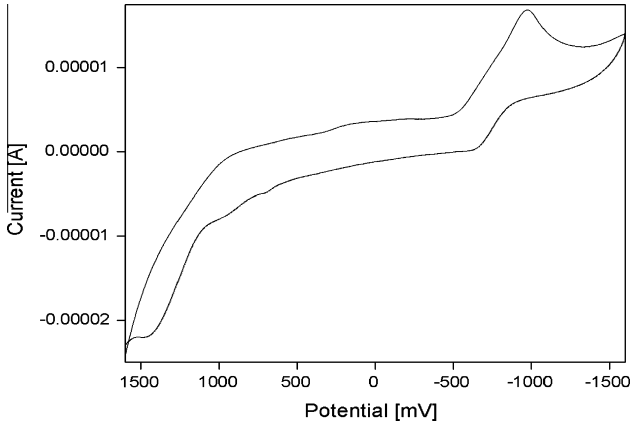

Fig. 3. Cyclic voltammogram of [Cu(L$_1$)$_2$].

interference with the normal cell processes. Besides this, the complexes may also indulge in the formation of hydrogen bonded interaction through the coordinated anions and azomethine group with the active centers of the cell constituents. Factors capable of increasing lipophilic nature are expected to enhance the antimicrobial property. The synthesized Schiff base ligand has moderate inhibitory effects on the growth of tested microorganism. This is due to the presence of azomethine groups which have chelating properties. These properties may be used in metal transport across the bacterial membranes or to attach to the bacterial cells at a specific site from which it can interfere their growth. Ligands exhibit MIC in the range of 3–15 and 3–16 2 mg/mL for all the bacteria and 9–17 and 11–18 2 mg/mL for all the fungi, respectively. The antimicrobial activity of zinc complex shows greater bactericidal and fungicidal activities against *P. aerugonisa* and *C. albicans* as compared to their corresponding Schiff base. The present investigated antimicrobial activity data indicate that all the newly synthesized complexes exhibit a slightly different antimicrobial activity as compared to that of the control drugs [60].

Table 6
EPR spectral data of copper and vanadium complexes.

Compounds	$g_{\|}$	$g_{\perp}$	$A_{\|}$ ($\times10^{-4}$ cm^{-1})	$A_{\perp}$ ($\times10^{-4}$ cm^{-1})
[Cu(L1)$_2$] (3)	2.231	2.059	154.7	15.5
[Cu(L2)$_2$] (4)	2.240	2.067	153.6	16.4
[VO(L2)$_2$] (8)	1.951	1.989	164.7	56.5

Cytotoxic activity

The in vitro cytotoxic activities of the synthesized Schiff base and its complexes are studied on hepatoma cell lines (HepG2) by applying the MTT colorimetric assay (Table 9). The calculated IC_{50} values, that is, the concentration (μg/mL) of a compound able to cause 50% of cell death with respect to the control culture, are presented in Figs. S7–S9 (Supplementary files). Cyclophosphamide is used as a reference compound. The HepG2 cells are sensitive to the O—N Schiff base with the IC_{50} value of 860 μg cm^{-3}. The Zn(II) complex is cytotoxic to the gastric cancer cell lines with IC_{50} value of 706 μg cm^{-3} (Table 10). Although the Schiff base and zinc complex are both less effective than cyclophosphamide, the Zn(II) complex is more effective than the Schiff base ligand towards cancer cell lines. The complexation of metal ions enhances the anticancer behavior as is evidenced by the lower IC_{50} values of the complex

Table 7
Minimum inhibition concentration (MIC) data of the synthesized compounds against growth of bacteria.

Compound	Microorganism (MIC) values				
	Chromobacterium Vialacium	*Shigella Flexinari*	*Staphylococci aureus*	*Pseudomonas aeruginosa*	*Streptococci byogenes*
L1-Cl-MPMP (1)	11	3	3	15	R
L2-Br-MPMP (2)	12	R	3	16	R
$Cu(L1)_2$ (3)	13	3	17	12	R
$Cu(L2)_2$ (4)	12	R	3	18	8
$Co(L1)_2$ (5)	15	5	6	14	R
$Co(L2)_2$ (6)	7	R	R	12	R
$VO(L1)_2$ (7)	12	R	8	12	R
$VO(L2)_2$ (8)	8	10	5	10	5
$Zn(L1)_2$ (9)	14	R	15	7	R
$Zn(L2)_2$ (10)	13	R	16	14	3
Amikacin	20	20	18	17	18

Table 8
Minimum inhibition Concentration (MIC) data of the synthesized compounds against growth of fungi.

Compound	% Inhibition of spore germination								
	Pencillium chrysogenum			*Candida albicans*			*Aspergillus niger*		
	2	4	6	2	4	6	2	4	6
L1-Cl-MPMP (1)	9	13	15	14	16	17	13	15	17
L2-Br-MPMP (2)	12	15	17	11	13	16	14	16	18
$Cu(L1)_2$ (3)	14	14	16	15	16	18	12	14	15
$Cu(L2)_2$ (4)	11	13	16	10	13	16	11	14	17
$Co(L1)_2$ (5)	15	17	19	15	16	18	13	15	16
$Co(L2)_2$ (6)	10	14	16	12	16	19	14	17	18
$VO(L1)_2$ (7)	15	17	19	16	19	21	13	14	15
$VO(L2)_2$ (8)	11	13	16	12	14	17	13	15	19
$Zn(L1)_2$ (9)	12	14	19	12	15	13	11	13	15
$Zn(L2)_2$ (10)	16	18	21	14	16	17	15	19	21
Ketoconazole	18	18	18	17	17	17	20	20	20

Table 9
Percentage of cell viability and death analytsis in duplicate study model.

Samples	50 μg	100 μg	200 μg	300 μg
L1(Cl-MPMP)	91.9	66.2	64.2	70.6
Cu(L1)	91	95.2	90.6	87.5
Co(L1)	94.1	94.5	86.8	93.7
VO(L1)	76.2	93.8	91.8	89.7
Zn(L1)	92.2	69.1	65.3	54.6
Cyclophosphamide	100	100	100	100

Table 10
IC_{50} values of the ligand and the metal complexes.

Compounds	Values in($\mu g\ cm^{-3}$)
P.C	1002
N.C	897
L1-(Cl-MPMP)	860
Cu(L1)	2920
Co(L1)	3321
Vo(L1)	1486
Zn(L1)	706

P.C = Positive control (cyclophosphamide).
N.C = Negative control (untreated cell).

compared with the uncoordinated ligand. This may be attributed to the increase in conjugation R═N─R′, in the ligand moiety on complexation. The preliminary bioassay indicates that the Schiff base and its zinc complex exhibit inhibitory activity against the human gastric cancer cell lines. The different activities are currently being investigated in terms of the mechanism of action of these compounds at the cellular level [61].

Conclusions

The present study describes the synthesis of new Schiff bases derived from morpholinoaniline and mutisubstituted salicylaldehydes. The X-ray crystallography study confirms the structure of newly synthesised Schiff bases. The spectral data show that the Schiff bases act as monobasic bidentate NO chelating agents coordinating the metal ion via the azomethine nitrogen atom and the phenolic oxygen atom. Further, the promising results have been observed for the antimicrobial screening especially for the metal complexes against both the fungi and bacteria and it is attributed to the fact that the metal complexes are potentially active against bacterial cells than fungi cells. The free ligand and its metal complexes show anticancer activity against HepG2 cells. In view of the biological activity, the ligands and the complexes have shown a moderate activity.

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Appendix A. Supplementary material

CCDC 848959 and 848960 contain the supplementary crystallographic data for Br-MPMP and Cl-MPMP respectively. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336 033; or e-mail: deposit@ccdc.cam.ac.uk.

Further, the schemes and figures related to this work are available in the Supplementary files. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2013.07.101.

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