ISSN 1070-3632, Russian Journal of General Chemistry, 2017, Vol. 87, No. 11, pp. 2654–2663. © Pleiades Publishing, Ltd., 2017.

Synthesis, Spectral Characterization, DNA Interaction, Antioxidant, and Antimicrobial Studies of New Water Soluble Metal(II) Complexes of Morpholine Based Ligand[1]

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Received June 13, 2017

Abstract—A new series of metal(II) complexes **1–5** of the type $[M(L)(AcO)_2H_2O]$, where L is a bidentate ligand, 2-(3-morpholinopropylimino)methylphenol and M is Mn(II) (**1**), Co(II) (**2**), Ni(II) (**3**), Cu(II) (**4**), and Zn(II) (**5**), have been isolated and characterized by physico-chemical and spectral methods. Spectroscopic data supported the square pyramidal geometry around the central metal ion in the complexes **1–5**. Absorption spectra and viscometric data indicated that the complexes **1–5** interacted with calf thymus (CT) DNA via intercalative strategy. Cleavage activities of complexes **1–5** with CT DNA were analyzed by gel-electrophoretic method. The *in vitro* antioxidant activity of complexes **1–5** was tested using the DPPH assay. The complexes **1–5** have been tested for antimicrobial activity against some pathogenic bacterial and fungal species by the agar well diffusion method.

Keywords: Schiff base, morpholine, DNA interaction, DNA cleavage, antioxidant, antimicrobial

DOI: 10.1134/S1070363217110214

Biologically active compounds and drugs containing morpholine [1, 2] as one of the substituents have been studied recently. Schiff bases ligands and their complexes derived from morpholine demonstrated high biological potential [3–7]. Schiff bases and metal complexes can easily bind to DNA [8, 9].

The present study is evaluating the DNA binding with M(II), formation of the corresponding complexes, structure activity relationship for the nuclease, the effect of various metals nature, influence of pH and hydrophobicity of DNA upon binding and cleavage of the complexes.

RESULTS AND DISCUSSION

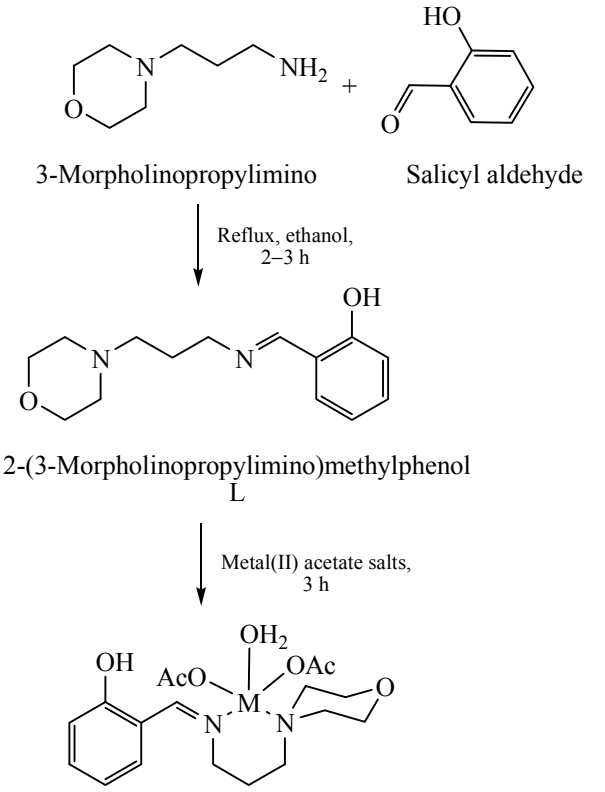
Synthetic pathway to complexes **1–5** is presented in Scheme 1, and their analytical and physicochemical characteristics [10] are listed in Table 1.

Electronic absorption spectra of the ligand L and the complexes **2–4** were recorded in ethanol at room temperature. The intense absorption bands observed at 254 and 315 nm were attributed to π→π* and *n*→π* transitions respectively. In spectra of complexes **2–4** these bands were shifted to a longer wavelength (257–396 nm) which was attributed to the intraligand charge transfer due to donation of the lone electrons pair of nitrogen atom of the ligand L to the central metal atom (N⟶M). The data for ligand L and its complexes **2–4** are presented in Table 2. The complexes **2–4** exhibited one *d–d* band at 633 (**2**), 594 (**3**) and 653 (**4**) nm, assigned to $^2E_g \longrightarrow {}^2T_{2g}$ transition that supported their square pyramidal geometry [11–13]. Diamagnetic ions Zn(II) and Mn(II) did not demonstrate any *d–d* transition in the visible region.

IR spectra (Table 3) of the ligand L demonstrated the characteristic band at 1630 cm^{-1} assigned to ν (CH=N), which was shifted to lower frequencies in the spectra of the complexes **1–5** (1592–1598 cm^{-1}) indicating the involvement of CH=N nitrogen in coordination to the metal ion [14, 15]. Morpholine ν(C–N) band (1375 cm^{-1}) was shifted to lower frequencies in the spectra of all complexes (1362–1329 cm^{-1}) indicat-

[1] The text was submitted by the authors in English.

Scheme 1. Synthesis and proposed geometry of complexes **1–5**.



M = Mn(II) (**1**), Co(II) (**2**), Ni(II) (**3**), Cu(II) (**4**), and Zn(II) (**5**).

ing the involvement of morpholine ring nitrogen in coordination to the central metal ion. The IR spectral data indicated that the ligand L acted as the bidentate chelating agent and the phenolic OH group was not involved in the complexes formation. The difference between asymmetric and symmetric frequencies recorded for carboxylate of the acetate group ($\Delta\nu_{as,s} \geq 200$ cm^{-1}) suggested the unidentate coordination [16]. The new bands in the regions 667–601 and 535–482 cm^{-1} indicated formation of the M–O and M–N bonds.

^{1}H NMR spectra of the ligand L and complex **5** confirmed that both the azomethine group and nitrogen of morpholine moiety were involved in bonding with central metal ion. The acetate **5** and water protons signals were recorded at 1.25 and 4.9 ppm due to linkage to the metal ion. The signal of morpholine protons **5** was shifted downfield with respect to ligand L due to participation of nitrogen atom in complexation.

ESR spectrum of the complex **4** recorded at 300 K demonstrated an intense absorption band (Table 4) in the high field region which was isotropic due to tumbling motion of the molecules. For computing the values from the spectrum, tetracyanoethylene (TCNE) free radical as a *g*-marker was used. The observed $A_{||}$, $A\perp$, $g_{||}$, and $g\perp$ values were 175, 71, 2.23, and 2.03 respectively. The g tensor values of complex **4** could be used to derive the ground state. The spectra also supported the fact that the unpaired electron lied predominantly in the $d_{x2} - d_{y2}$ orbital [17–20] which was evident from the value of the exchange interaction term *G*, estimated from the Eq. (1).

$$G = \frac{g_{||} - 2.0023}{g_{\perp} - 2.0023}. \quad (1)$$

The observed value of the exchange interaction parameter for the copper complex (G = 6.77) suggested that the local tetragonal axes were aligned parallel or slightly misaligned and the unpaired electron was present in the $d_{x2} - d_{y2}$ orbital. The result indicated that the exchange coupling effects were not operative [21]. The ESR parameters and the *d–d* transition energies were used for evaluating the

Table 1. Analytical, molar conductance and magnetic susceptibility data for ligand L and complexes **1–5**

Comp. no.	Formula	Color	Yield, %	Elemental analysis data, %								Molar conductance Λ_M, Ω^{-1} cm^2 mol^{-1}	μ_{eff}, BM
				found				calculated					
				M	C	H	N	M	C	H	N		
L	$C_{14}H_{20}N_2O_2$	Orange	79	–	67.1	8.9	11.7	–	67.7	8.1	11.3	–	–
1	$MnC_{18}H_{28}N_2O_7$	Dark brown	58	12.4	49.1	6.1	6.6	12.5	49.2	6.4	6.4	25.1	5.40
2	$CoC_{18}H_{28}N_2O_7$	Dark green	62	13.4	48.6	6.4	6.5	13.3	48.8	6.3	6.3	24.4	2.6
3	$NiC_{18}H_{28}N_2O_7$	Pale green	65	13.5	49.1	6.2	6.4	13.3	49.0	6.4	6.4	18.7	–
4	$CuC_{18}H_{28}N_2O_7$	Green	69	14.1	48.4	6.4	6.2	14.2	48.2	6.3	6.3	24.4	1.73
5	$ZnC_{18}H_{28}N_2O_7$	Yellow	60	14.3	48.2	6.2	6.4	14.5	48.0	6.2	6.2	17.4	–

Table 2. Electronic absorption spectral data for ligand L and complexes **1–5** in ethanol

Comp. no.	Absorption band, λ_{max}, cm^{-1}	Band assignment	Suggested geometry
L	25062 (399) 31746 (315) 39370 (254)	INCT[a] INCT INCT	–
2	15798 (633)	$^2E_g \rightarrow {}^2T_{2g}$	Square Pyramidal
3	16835 (594)	$^2E_g \rightarrow {}^2T_{2g}$	Square Pyramidal
4	15314 (653)	$^2E_g \rightarrow {}^2T_{2g}$	Square pyramidal

[a] Intra ligand charge transfer band.

bonding parameters α^2, β^2 and γ^2 that measured the covalence of σ-bonds (in-plane) and π bonds (in-plane and out-of-plane), respectively. The in-plane σ bonding parameter α^2 was calculated using the Kivelson and Neiman expression [Eqs. (2)–(4)] [22]:

$$\alpha^2 = \frac{A_{||}}{0.036} + (g_{||} - 2.0023) + \frac{3}{7}(g_{\perp} - 2.0023) + 0.04, \quad (2)$$

$$\beta^2 = \frac{(g_{||} - 2.0023)E}{-8\lambda\alpha^2}, \quad (3)$$

$$\gamma^2 = \frac{(g_{||} - 2.0023)E}{-2\lambda\alpha^2}, \quad (4)$$

where, $\lambda = 828$ cm^{-1} for the free ion and E is an electronic transition energy. The values presented in Table 4 indicated that there was a substantial interaction in the in-plane σ-bonding, whereas π bonding coefficients were almost ionic. The co-factors of degree of geometrical distortion $f_{||} = 127$ cm^{-1} indicated slightly tetrahedral distortion and square pyramidal geometry around Cu(II) ion [23–26].

Mass spectra of Schiff base ligand L and the complexes confirmed the stoichiometry of metal chelates as ML type.

Cyclic voltammogram of the complexes **1–5** were recorded in ethanol solution (potential range from 1.0 to –1.0 V) and demonstrated a well-defined redox process (Table 5) which correspond to the formation of M(II)/M(I) couple. Each of the **1–5** complexes demonstrated a reversible voltametric cathodic peak at –0.26, –0.27, –0.29, –0.20, and –0.31 V, and also reversible anodic peak at 0.13, 0.1, 0.18, 0.08, and 0.17 V, respectively. The quasi-reversible peaks were obtained

Table 3. FT–IR spectral data for the ligand L and complexes **1–5**

Comp. no.	FT–IR, cm^{-1}					
	ν(CH=N)	ν(C–N)	ν(COO)	ν(H_2O)	ν(M–O)	ν(M–N)
L	1630	1375	–	–	–	–
1	1593	1340	1662 (as) 1398 (s)	3250 860 (s)	616	517
2	1598	1352	1667 (as) 1400 (s)	3270 845 (s)	636	515
3	1594	1329	1664 (as) 1404 (s)	3240 868 (s)	667	482
4	1592	1362	1665 (as) 1390 (s)	3244 831 (s)	631	512
5	1596	1341	1664 (as) 1402 (s)	3267 853 (s)	600	535

Table 4. ESR spectral data for the complex **4** in methanol at 77 K[a]

Comp. no.	Tensor, G, cm^{-1}						Bonding parameters							$f_{||}$
	$A_{||}$	$A\perp$	A_{iso}	$g_{||}$	$g\perp$	g_{iso}	α^2	β^2	γ^2	$K_{||}$	$K\perp$	K^2	G	
4	175	71	106	2.23	2.03	2.13	0.89	0.85	0.50	0.77	0.11	0.31	6.77	127

[a] $g_e = 2.00277$; Microwave frequency (γ') = 9.114×10^9 cycle/s; 1 $G = 10^{-4}$ cm^{-1}; $f_{||} = g_{||}/A_{||}$.

Table 5. Cyclic voltammetric data for the complexes **1–5** in ethanol (0.001 M) containing 0.1 M (TBAP) and scan rate 100 mV s^{-1}

Comp. no.	Couple	E_{pa}, V	E_{pc}, V	ΔE_p, V	I_{pa}, μA	I_{pc}, μA	I_{pa}/I_{pc}, μA
1	Mn(II)/Mn(I)	0.13	–0.26	0.13	0.95	0.98	0.97
2	Co(II)/Co(I)	0.10	–0.27	0.17	–2.58	2.92	0.88
3	Ni(II)/Ni(I)	0.18	–0.29	0.11	–2.76	3.07	0.90
4	Cu(II)/Cu(I)	0.08	–0.20	0.12	–2.83	3.02	0.93
5	Zn(II)/Zn(I)	0.17	–0.31	0.14	0.86	0.93	0.92

Table 6. Intrinsic binding constant (K_b) and chromism (H%) of the complexes **1–5** at pH = 4.0

Comp. no.	λ_{max}, nm		Δλ, nm	Chromism, H%[a]	K_b[b]×10^4, M^{-1}	Type of chromism and shift
	free	bound				
1	311	314	3	31.32	1.42	Hypo and red
2	388	390	2	43.67	2.95	Hypo and red
3	327	331	4	39.63	2.04	Hypo and red
4	329	332	3	48.69	6.72	Hypo and red
5	332	336	4	37.43	1.97	Hypo and red

[a] $H\% = [(A_{free} - A_{bound})/A_{free}]\times 100\%$.
[b] K_b is an intrinsic DNA binding constant.

for the complexes **1–5** at 0.13, 0.17, 0.11, 0.12, and 0.14 V, respectively, with scan rate of 100 mV/s. The ratio of anodic and cathodic peak current ($I_{pc}/I_{pa} \approx 1$) corresponded to one electron transfer process [27].

Binding of the complexes **1–5** to DNA helixes (Fig. 1) were characterized by monitoring the changes in the absorbance of π–π* bands and shift in wavelength upon each addition of DNA solution to the complex. As the concentration of DNA increased, hypochromism (pH 10.0), 37.42 (**1**), 46.57 (**2**), 42.84 (**3**), 53.21 (**4**), and 38.43% (**5**), was observed in the charge transfer (CT) band of each complex along with the red shift of about 2–5 nm, which suggested the intercalative binding of complexes **1–5** with DNA [28, 29]. The intrinsic binding constant (K_b) values were calculated from the perturbation observed in CT band of the complexes. The absorption data was analyzed to evaluate K_b, which could be determined from a plot of [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA] using Eq. (5) [30, 31].

$$\frac{[DNA]}{\varepsilon_a - \varepsilon_f} = \frac{[DNA]}{\varepsilon_b - \varepsilon_f} = \frac{1}{K_b(\varepsilon_b - \varepsilon_f)}, \quad (5)$$

where [DNA] is the concentration of DNA in base pairs. The apparent extinction coefficient (ε_a) observed for the MLCT absorption band at the given DNA concentration was obtained by calculating Abs/[complex], ε_f and ε_b correspond to the extinction coefficient of the complex free (unbound) and fully bound to DNA. The K_b values could be obtained from the ratio of the slope to the intercept of the plots of [DNA]/($\varepsilon_a - \varepsilon_f$) vs [DNA]. The order of binding constants (K_b) of the complexes **1–5** at various pH media was the following: pH 10.0 > 4.0 > 7.0. The values of K_b are given in Table 6. The complexes **1–5** exhibited higher binding activity in alkaline media than in acidic or neutral.

Viscosity measurements were carried out on CT–DNA by varying concentration of the added complexes **1–5**. Degree of viscosity probably depended on the binding affinity to DNA (Fig. 2). Viscosity of DNA increased with rising ratio of complexes **1–5** to DNA, indicating binding of the complexes to DNA [32–34]. Relative specific viscosity for DNA either in the presence or absence of complexes **1–5** was calculated from Eq. (6).

$$\left(\frac{\eta}{\eta_0}\right)^{1/3} = \frac{\left[\frac{(t_{complex} - t_0)}{t_0}\right]}{\left[\frac{(t_{DNA} - t_0)}{t_0}\right]}, \quad (6)$$

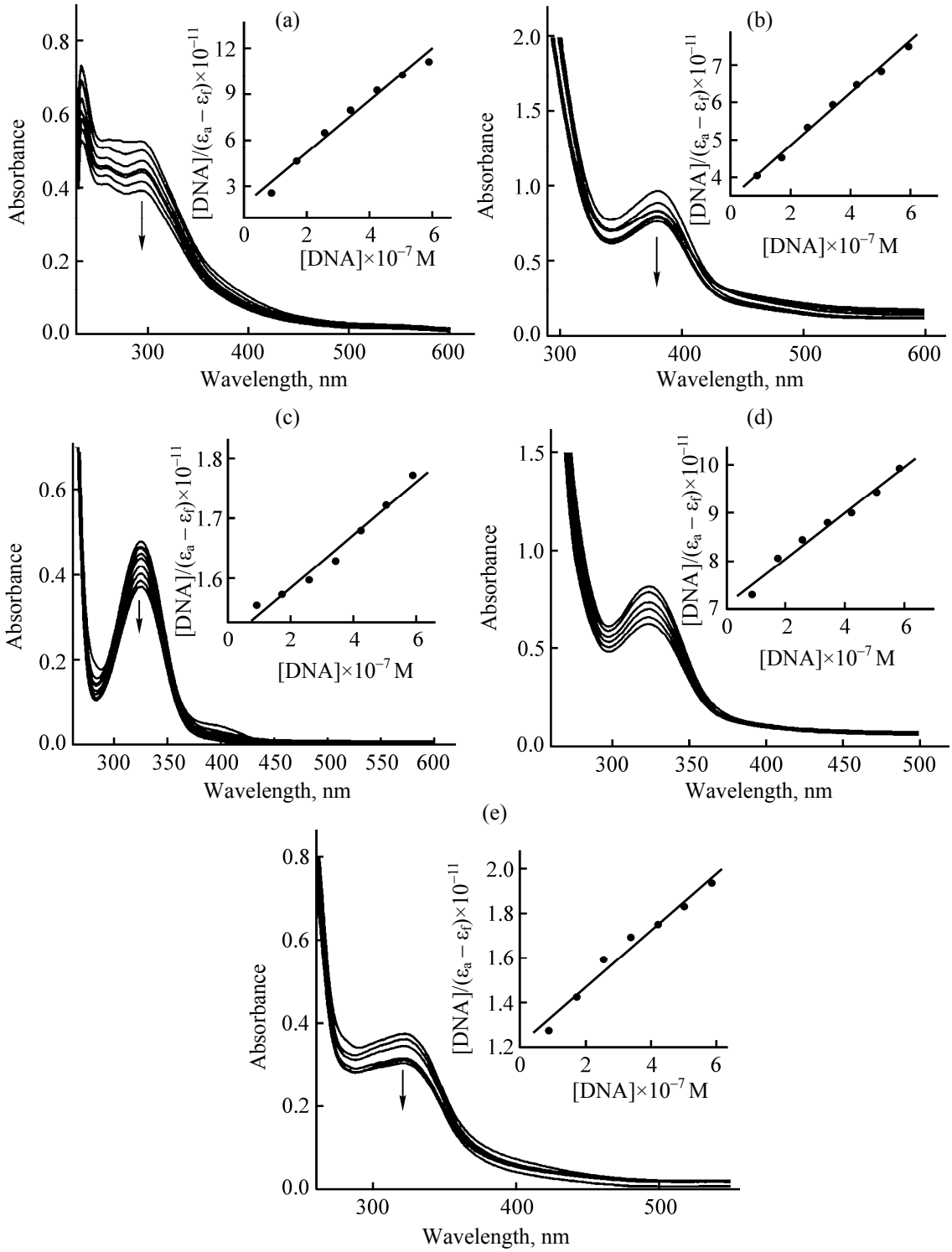


Fig. 1. Specrophotometric absorption titration of complexes (a–c) **1–5** in the presence and absence of CT-DNA at pH 4.0. The arrows indicate decrease in absorption intensity upon successive addition of DNA. Inside: a typical plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$ versus [DNA].

where η is the specific viscosity of DNA in the presence of the complex and η_0 is the specific viscosity of DNA alone; $t_{complex}$, t_{DNA}, and t_0 are the average flow time for the DNA in the presence of the complex, DNA alone and Tris-HCl buffer respectively. The values of $(\eta/\eta_0)^{1/3}$ were plotted against [complex]/[DNA].

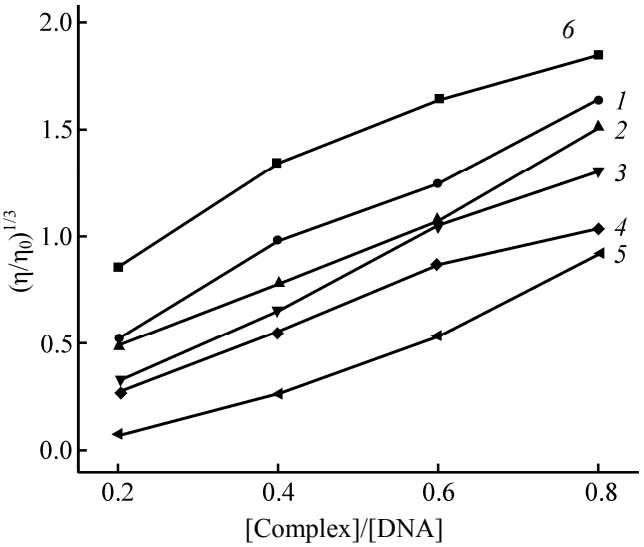


Fig. 2. Effect of increasing amounts of the complexes **1–5** on the relative viscosities of DNA: (*1*) **1**, (*2*) **2**, (*3*) **3**, (*4*) **4**, (*5*) **5**, and (*6*) EtBr.

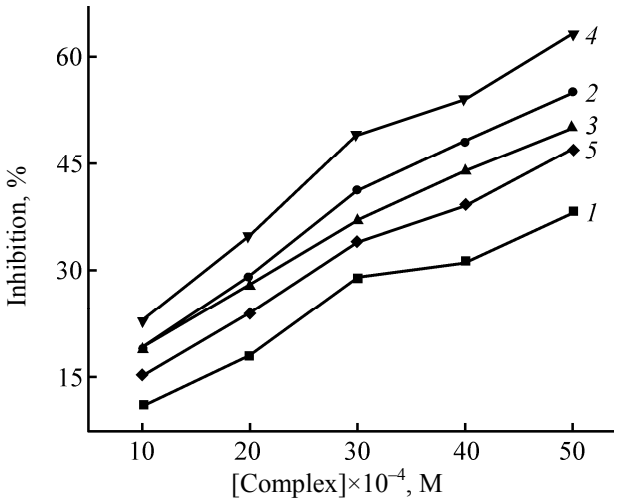


Fig. 3. Percent of inhibition DPPH vs concentratiion of complexes (*1*) **1**, (*2*) **2**, (*3*) **3**, (*4*) **4**, and (*5*) **5**.

The CT–DNA cleavage study by gel electrophoresis method performed with the ligand L and complexes **1–5** at 37°C indicated that the complexes **1**, **4** and **5** cleaved the DNA appreciably as compared to the control DNA. The complexes **2**, **3** and the ligand L did not exhibit the cleavage even upon long exposure time. It was also observed that free radical scavengers inhibited the DNA cleavage which confirmed involvment of the free radical [36, 37].

Antioxidant activity of complexes **1–5** were studied by using the DPPH assay method. The method depends on the ability of an antioxidant to donate its electron to DPPH which in turn depends on the ability of DPPH to change color from purple to yellow.

According to the accumulated data (Fig. 3) the complex **4** had higher scanging ability than complexes **1–3** and **5** probably due to the presence of the morpholine and salicylaldehyde substituents.

Antimicrobial activity of the ligand L and its complexes **1–5** were tested against seven pathogenic bacteria and three fungal microorganism species by the well diffusion method (Table 7). The study indicated that the complexes **1–5** exhibited higher antimicrobial activity than the free ligand L and their activity depended upon the size and charge distribution of metal ions, shape and redox potential of the metal chelates [38]. The complexes **4** and **5** were more potent than the other ones. The increased inhibition activity of complexes can be explained on the basis of the Overtone's concept [39] and Tweedy's Chelation hypothesis [40, 41]. The observed zones of inhibition for the complexes **1–5** followed the order: control > **4** > **3** > **5** > **1** > **2** > L that matched the Irving-William's order of stability. This can be elucidated on the basis of Structure-Activity Relationship [40] (SAR). The low activity of complexes **1** and **2** could be due to the low lipophilicity of M(II) ion and low penetration of these complexes through the lipid membrane. Such activity of the complexes could serve as a good projection for treating some common diseases like urinary tract and hospital-acquired infections [42].

EXPERIMENTAL

All reagents used were extra pure AR grade (Sigma, Aldrich) and used without further purification. Solvents used for physical measurements were of AR grade and purified by the standard methods [43]. Melting points were determined on a Gallen Kamp apparatus in open glass capillaries. CHN analysis was carried out on a Gouy balance at room temperature. Calf thymus (CT) DNA was purchased from GENEI (India). Magnetic susceptibility measurements were performed according to the Gouy method using Hg[Co $(SCN)_4$] as the calibrant. UV-Vis spectra were recorded in ethanol on a Shimadzu UV-1800 spectrophotometer. IR spectra were recorded using KBr pellets on a Shimadzu FT-IR spectrophotometer. Cyclic voltammetric measurements were performed using a glassy carbon working electrode (3 mm), Pt

Table 7. Antibacterial and antifungal activities of the ligand L and its complexes **1–5** at a concentration of 10^{-2} M determined by the well diffusion method

Compound	Gram positive bacteria			Gram negative bacteria				Fungi		
	Bacillus subtilis	*Staphylococcus aureus*	*Streptococcus faecalis*	*Escherichia coli*	*Salmonella typhimurim*	*Klebsiella Pneumonia*	*Shigella boydii*	*Candida albicans*	*Aspergillus niger*	*Enterobacter species*
L	7.7	8.4	10.2	11.5	7.2	15.7	10.6	11.3	10.2	9.5
1	18.4	18.4	19.4	16.3	15.6	18.8	18.7	19.5	21.4	19.5
2	18.5	15.3	15.2	20.2	13.2	18.4	16.6	15.4	16.2	15.3
3	18.5	16.8	22.8	19.7	15.2	21.4	23.3	23.2	22.4	20.4
4	20.3	23.5	22.6	25.1	22.2	22.5	23.3	21.7	20.6	19.8
5	22.7	21.2	22.7	23.1	20.4	23.5	23.3	21.7	22.5	21.4
Streptomycin	28.7	27.2	28.7	26.1	27.4	25.5	27.3	28.7	28.6	29.4

wire was an auxiliary electrode and an Ag/AgCl reference electrode. All solutions were purged with N_2 for 30 min prior to each set of experiments. Tetrabutylammonium perchlorate (TBAP) was used as a supporting electrolyte. The X-band ESR spectra of the samples in ethanol were obtained at 300 K and 77 K on a Varian $E_{1/2}$ spectrometer. Viscosity experiments were carried out on an Ubbelodhe viscometer at 30±0.1°C. The standard nutrient and dextrose agar was used as the medium for testing activity of microorganisms as antibacterial and antifungal agents. DNA binding, CT–DNA cleavage and antioxidant studies were recorded in EtOH solution using UV-1800 spectrophotometer and UV-Transilluminator at Chemistry Research Centre, MSEC, Kilakarai.

Synthesis of 2-(3-morpholinopropylimino)methylphenol (L). Ethanolic solution (20 mL) of 3-morpholinopropylamine (1.44 g, 10 mmol) and salicylaldehyde (1.22 g, 10 mmol) was refluxed intensivly for 2–3 h, then cooled down to room temperature. The solvent was evaporated slowly from the reaction mixture to yield yellow orange liquid of L which was purified by column chromatography using petroleum ether and methanol (1 : 2) mixture as an eluent. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.8 p (2H), 2.2 t (4H), 2.4 t (2H), 3.6 t (2H), 3.7 t (4H), 6.9–7.3 m (4H), 8.35 s (1H), 13.5 s (1H). UV-Vis spectrum (EtOH), λ_{max}, nm: 315, 254.

Synthesis of complexes 1–5. A solution of L (0.248 g, 1 mmol) in ethanol (20 mL) was added to a solution of a metal(II) acetate salt (1 mmol) in 20 mL ethanol and the mixture was refluxed for 3 h. The volume of solvent was then reduced to one third and the resulting solution was cooled down to room temperature. The precipitated complex was filtered off, washed thoroughly with ethanol and dried in vacuum. All complexes were recrystallized from ethanol.

Spectral, physico-chemical and analytical data for complexes **1–5** are presented in Tables 1–3. Melting points of complexes **1–5,** °C**:** 154 (**1**), 163 (**2**), 159 (**3**), 116 (**4**), 104 (**5**).

Complex 5. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.25 s (3H), 1.8–2.0 p (2H), 2.82–2.85 m (6H), 3.4–3.6 t (2H), 3.7 t (4H), 4.9 s (2H), 6.9–7.4 m (4H), 8.45 s (1H), 13.5 s (1H).

DNA interaction. Experiments of interaction of the complexes **1–5** with CT–DNA were carried out at various pH (4.0, 7.0, 10.0) using different buffers (acidic-sodium acetate, neutral-Tris-HCl and alkaline-sodiumbicarbonate) at room temperature. A solution of DNA in a buffer gave a ratio of UV absorbance at 260 and 280 nm of about 1.8–1.9, indicating that DNA was sufficiently free from protein [44]. DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient of 6600 M^{-1}/cm at 260 nm [45]. The complexes **1–5** were dissolved in a mixture of 5 % methanol and 95% buffer solution. Stock solutions were stored at 4°C and used within 4 days. Absorption titration experiments were performed with fixed concentration of the

complexes **1–5** (30 μM) with varying concentration of DNA (0–50 μM). An equal amount of DNA was added to all the reference and the test solutions to eliminate the absorbance of DNA itself [46].

Viscosity measurements. Viscosity measurements were carried out using a semi micro viscometer at room temperature using Tris-HCl buffer (pH 7.0) at a constant temperature of 27.0±0.1°C. Titrations were performed for the complexes **1–5** and control ethidium bromide (EtBr) (0.2×10^{-5} M) and each complex was introduced into the CT–DNA solution (10^{-4}M) present in the viscometer [47]. Flow time of each sample was measured three times. Data were represented graphically as $(\eta/\eta_0)^{1/3}$ vs concentration ratio ([Complex]/[DNA]), where h is viscosity of DNA in the presence of a complex and h_0 is viscosity of DNA [48]. Viscosity values were calculated from the observed flow time of DNA-containing solutions (t > 100 s) and corrected for the flow time of a buffer (t_0).

DNA cleavage studies. DNA cleavage activity of complexes **1–5** with CT–DNA were monitored by the agarose gel electrophoresis method [49, 50]. Gel electrophoresis experiments were performed under aerobic conditions with H_2O_2 upon incubation at 37°C for 2 h. The mixture consisting of CT–DNA (15 μL, 30 μM), a complex **1–5** (5 μL, 50 μM), Tris-HCl/NaCl buffer solution (pH = 7.0) (29 μL, 50 mM /18 mM), and H_2O_2 (1 μL, 500 μM) was shacked well and maintained at room temperature for 2 h. Bromophenol blue dye (1 μL) solution was added to the mixture, which was injected into 1% agarose gel chamber wells. The gel was stained by immersing it in a tank buffer solution containing ethidium bromide (0.5 μg/mL). Upon power supply (50 V) DNA migration was occurred towards the positive pole. After completion of DNA migration the gel layer was taken out from the solution tank and snapped under a UV Transilluminator and the bands indicated the extent of DNA cleavage and compared with the standard DNA marker.

Antioxidant activity. The complexes **1–5** were tested for *in vitro* antioxidant activities at 37°C by 2,2-diphenyl-1-(2,4,6-trinirophenyl)hydrazyl (DPPH) free radical scavenging assay method [51]. Complexes **1–5** were dissolved in ethanol at different concentrations (10–50 μM). Sample solution of different concentrations (1 mL) and 4 mL of 0.1 mM DPPH solution were loaded in different test tubes and shaken vigorously for 2–3 min. Then the test tubes were incubated in dark room for 20 min at 37°C. A blank DPPH solution without the sample was used for the baseline correction and it gave a strong absorption maximum at 517 nm (purple color with $\varepsilon = 8.32\times10^3$ M^{-1}/cm). In the course of DPPH reaction with an antioxidant complex the colour changed from purple to light yellow indicating formation of a stable macromolecular radical. After incubation, the absorbance value for each sample was measured at 517 nm [52].

Antimicrobial activity. Antimicrobial activity of complexes **1–5** was tested against three gram positive bacteriae (*Bacillus subtilis, Staphylococcus aureus,* and *Streptococcus faecalis*), four gram negative bacteriae (*Escherichia coli, Salmonella typhimurim, Klebsiella Pneumonia,* and *Shigella boydii*) using Muller Hinton nutrient agar media and three fungi (*Candida albicans, Aspergillus niger,* and *Enterobacter species*) using potato dextrose agar as the medium by the well diffusion technique [53–55]. The results were recorded as zones of inhibition in mm and compared with the standard drug streptomycin for antibacterial and antifungal studies.

CONCLUSIONS

A N_2 type ligand L was synthesized by condensation of 3-morpholinopropyl amine with salicyladehyde. Novel complexes **1–5** containing M(II) ion were synthesized from morpholine based Schiff base ligand L and characterized by physico-chemical, spectral and analytical techniques. UV-Vis, MS and EPR spectral data suggested that the structure of complexes **1–5** adopted the square pyramidal geometry. The bidentate ligand L bind to the M(II) ions through two nitrogen atoms. The chelates were non-electrolytes and magnetic susceptibility values indicated their monomeric nature. The intercalative binding of complexes **1–5** with DNA was confirmed by a red shift along with a hypochromism and the viscosity study. The DNA cleavage activity of complexes **1**, **4** and **5** with DNA demonstrated remarkable activity, while complexes **2**, **3** and the ligand L have shown no activity under aerobic condition and in the presence of H_2O_2. Antimicrobial and antioxidant activities of the complexes **1–5** were higher than those of the free ligand L which was probably due to the presence of both electron withdrawing and electron donating moieties in the chelate ring.

ACKNOWLEDGEMENTS

The authors express their sincere thanks to the

Department of Science and Technology (DST) – Science and Engineering Research Board (SERB-Ref. SR/FT/CS-117/2011 dated 29.06.2012), Government of India, New Delhi for their funding assistance, the Managing Board, Dean, Principal, Head and staff members in Chemistry Research Centre of Mohamed Sathak Engineering College, Kilakarai. They also express their gratitude to the Managing Board, Principal and the Department of Chemistry of Sri Vidya college of Engineering and Technology, Virudhunagar and V.O.C College of Arts & Science, Tuticorin for providing all the research and instrumental facilities.

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