



Synthesis, crystal structures and biological activities of transition metal complexes of a salen-type ligand

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Abstract

Manganese(III), cobalt(II), nickel(II) and copper(II) complexes of a salen-type ligand, namely 6,6'-((1E,1'E)-(ethane-1,2-diylbis(azanylylidene))bis(methanylylidene))bis(5-isopropyl-2-methylphenol) (H₂L), have been synthesized and characterized by physicochemical and spectroscopic methods. In addition, single-crystal X-ray analysis confirmed the formulae of the manganese and nickel complexes as [Mn(OAc)(L)] and [Ni(L)], respectively. The free Schiff base and its complexes have been screened for in vitro antibacterial activity by colony count methods, and the antioxidant activity was assayed by DPPH radical scavenging. The ability of free H₂L and its complexes to mediate DNA cleavage was studied by agarose gel electrophoresis.

Introduction

DNA remains a long-term target for the diagnosis and treatment of human diseases [1]. DNA plays an essential role in the life process since it encodes all the genetic information required for the cellular functions [2]. Metal complexes of Schiff bases derived from salicylaldehyde have been found to be effective in DNA cleavage and can also possess anticancer and antibacterial activities [3–6]. DNA cleavage may lead to various pathological changes in living organisms and is also relevant to the synthetic restriction enzymes, chemotherapeutic drugs and DNA footprinting agents [7]. Recently, the ability of transition metal complexes to mediate DNA cleavage in the presence of oxidants such as O₂, H₂O₂ or peracids has been extensively studied. Efforts have been made to design transition metal complexes as chemical nucleases suitable for nicking or direct DNA strand scission [8, 9]. Cobalt complexes with different oxidation states have been found to possess DNA cleavage activity [10]. In this context, we report here the

synthesis of an N₂O₂ donor tetradentate Schiff base ligand H₂L-6,6'-((1E,1'E)-(ethane-1,2-diylbis(azanylylidene))bis(methanylylidene))bis(5-isopropyl-2-methylphenol), prepared by the condensation of ethane-1,2-diamine with 2-hydroxy-6-isopropyl-3-methyl benzaldehyde, together with its manganese(III), cobalt(II), nickel(II) and copper(II) complexes. All the compounds were characterized by physicochemical and spectroscopic methods and further screened for antibacterial, antioxidant and DNA cleavage activities.

Experimental

Materials and methods

Carvacrol, ethane-1,2-diamine, ethidium bromide, tris-boric acid-EDTA buffer (10X TBE buffer), loading dye mix, agarose gel, L-histidine, DABCO, D-mannitol and H₂O₂ 30% were purchased from Sigma-Aldrich. Sodium hydroxide, triethylamine, chloroform, ethanol, methanol, hexane, ethyl acetate, acetonitrile, DMF, DMSO, EDTA, manganese acetate tetrahydrate, cobalt acetate tetrahydrate, nickel chloride hexahydrate and copper(II) acetate monohydrate were Loba Chemie products. All the chemicals and solvents were used without further purification. The DNA pBR322 and DNA loading dye were purchased from Bangalore Genei. The electronic spectra were recorded as DMSO solutions on a Shimadzu UV 2450 series spectrophotometer in the range 200–800 nm. FTIR

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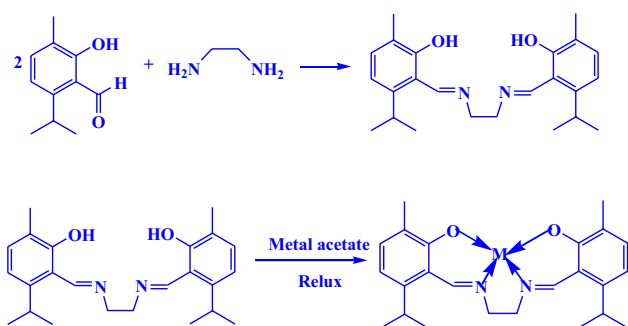
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spectra were recorded as KBr pellets on a Shimadzu FTIR-8400 spectrometer in the range 4000–400 cm^{-1} . NMR spectra were measured on a Bruker Avance III (400 MHz) spectrometer, and proton chemical shifts were recorded in ppm relative to tetramethyl silane as an internal standard using CDCl_3 as solvent. LCMS spectra were recorded on a Waters Micromass Q-ToF Micro instrument. The elemental analyses were obtained with a ThermoFinnigan elemental analyzer. The X-band ESR spectrum of the copper complex was recorded on a JES-FA200 ESR spectrometer at liquid nitrogen temperature (77 K) in DMF solution, using DPPH as a standard. Magnetic susceptibilities were measured at room temperature on a Gouy balance using $\text{Hg}[\text{Co}(\text{NCS})_4]$ as reference. Molar conductivities were measured on a Systronic conductivity bridge with a dip-tite cell using 10^{-3} M solutions in DMF.

Preparation of the Schiff base (H_2L)

The Schiff base (H_2L) was prepared according to our previously reported method [11], in which a solution 2-hydroxy-6-isopropyl-3-methyl benzaldehyde (0.356 g, 2 mmol) plus ethane-1,2-diamine (0.060 g, 1 mmol) in methanol (30 ml) was boiled under reflux for 2 h (Scheme 1).

Yellow solid; Yield: 73%; Anal. calcd for $\text{C}_{24}\text{H}_{32}\text{N}_2\text{O}_2$ (%): C 75.7, H 8.4, N 7.3, Found: C 75.5, H 9.1, N 7.5; UV-Vis (DMF) λ_{max} (nm): 273, 334; FTIR (KBr pellet, cm^{-1}) ν_{max} : 3498 (OH), 1609 (C=N), 1462 (C=C), 1259 (C–O); $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) (δ , ppm): 14.60 (*brs*, 2H, OH), 8.72 (d, 2H, $-\text{HC}=\text{N}$), 7.09 (d, 2H, Ar-CH, $J = 8$ Hz), 6.61 (d, 2H, Ar-CH, $J = 8$ Hz), 3.95 (s, 4H, $-\text{CH}_2$), 3.28–3.21 (m, 2H, $-\text{CH}$ of isopropyl group), 2.18 (s, 6H, 2- CH_3), 1.11 (d, 12H, $J = 4$ Hz, 4- CH_3); $^{13}\text{C-NMR}$ (CDCl_3 , 400 MHz) (δ , ppm): 164.59, 160.74, 147.26, 133.74, 123.52, 114.30, 114.10, 59.63, 27.78, 23.90, 15.52; LCMS (*m/z*): calcd 381.52, obs 381.30.



Scheme 1 Synthesis of the salen-type Schiff base H_2L and its metal complexes. M = Mn, Co, Ni or Cu

Preparation of the manganese(III) complex

A solution of $\text{Mn}(\text{OAc})_2 \cdot 4\text{H}_2\text{O}$ (0.322 g, 1.31 mmol) in methanol (25 ml) was added to a stirred solution of H_2L (0.5 g, 1.31 mmol) in methanol (25 ml). The mixture was refluxed for 6–8 h; then the precipitate was filtered off, washed with methanol and dried under vacuum. Yield 0.5 g (78%).

Reddish brown Solid; Anal. calcd for $\text{C}_{26}\text{H}_{33}\text{MnN}_2\text{O}_4$ (%): C 63.4, H 6.7, N 5.6, Found: C 63.6, H 6.7, N 5.7; UV-Vis (DMF) λ_{max} (nm): 275, 333, 417; FTIR (KBr, pellet cm^{-1}) ν_{max} : 1585 (C=N), 1276 (C–O), 1379 (C=C), 518 (M–O), 452 (M–N); LCMS (*m/z*): calcd 492.49, obs 433.1 [M-OAc]; μ_{eff} : 4.82 B.M.; Conductance (Λ_{M} , $\Omega^{-1} \text{cm}^2 \text{mol}^{-1}$) in DMF: 19.60.

Preparation of the cobalt(II) complex

A solution of $\text{Co}(\text{OAc})_2 \cdot 4\text{H}_2\text{O}$ (0.327 g, 1.31 mmol) in methanol (25 ml) was added to a stirred solution of H_2L (0.5 g, 1.31 mmol) in methanol (25 ml). The mixture was refluxed for 6–8 h; then the precipitate was filtered off, washed with methanol and dried under vacuum. Yield 0.41 g (71%).

Green solid; Anal. calcd for $\text{C}_{24}\text{H}_{30}\text{CoN}_2\text{O}_2$ (%): C 65.9, H 6.9, N 6.4, Found: C 65.0, H 7.3, N 6.9; UV-Vis (DMF) λ_{max} (nm): 269, 410, 632; FTIR (KBr pellet, cm^{-1}) ν_{max} : 1564 (C=N), 1234 (C–O), 1456 (C=C), 520 (M–O), 412 (M–N); LCMS (*m/z*): calcd 437.44, obs 437.2 μ_{eff} : 4.23 B.M. Conductance (Λ_{M} , $\Omega^{-1} \text{cm}^2 \text{mol}^{-1}$) in DMF: 14.35.

Preparation of the nickel(II) complex

A solution of $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (0.312 g, 1.31 mmol) in methanol (25 ml) was added to a stirred solution of H_2L (0.5 g, 1.31 mmol) in methanol (25 ml). The mixture was refluxed for 3 h; then the precipitate was filtered off, washed with methanol and dried under vacuum. Yield 0.45 g (79%).

Orange solid; Anal. calcd for $\text{C}_{24}\text{H}_{30}\text{N}_2\text{NiO}_2$ (%): C 65.9, H 6.9, N 6.4 Found: C 65.6, H 6.7, N 6.5; UV-Vis (DMF) λ_{max} (nm): 268, 349, 352, 442, 552; FTIR (KBr pellet, cm^{-1}) ν_{max} : 1560 (C=N), 1234 (C–O), 1452 (C=C), 474 (M–O), 432 (M–N); LCMS (*m/z*): calcd 437.20, obs 437; Conductance (Λ_{M} , $\Omega^{-1} \text{cm}^2 \text{mol}^{-1}$) in DMF: 15.64.

Preparation of the copper(II) complex

A solution of $\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$ (0.312 g, 1.31 mmol) in methanol (25 ml) was added to a stirred solution of H_2L (0.5 g, 1.31 mmol) in methanol (25 ml). The mixture was stirred

Fig. 1 ORTEP diagrams of [Mn(OAc)L] and [Ni(L)] complexes, respectively

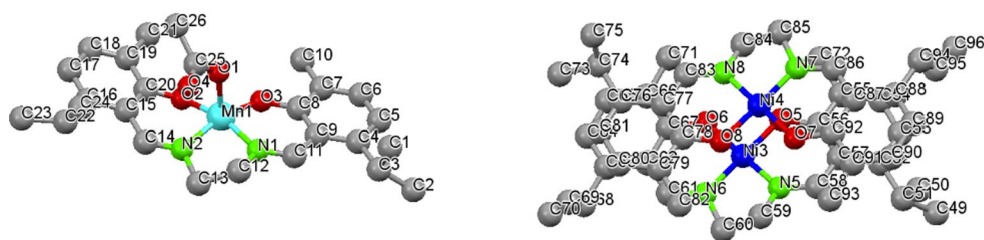


Table 1 Crystal data and structure refinement for [Mn(OAc)(L)] and [Ni(L)] complexes

	[Mn(OAc)(L)] complex	[Ni(L)] complex
Empirical formula	C ₂₆ H ₃₃ MnN ₂ O ₄	C ₂₄ H ₃₀ N ₂ NiO ₂
Formula weight	492.48	437.21
Temperature	293(2) K	296(2) K
Crystal system	Monoclinic	Monoclinic
Space group	P2 ₁ /c	P2 ₁ /c
Unit cell dimensions Å	<i>a</i> = 10.4771(12) Å; <i>a</i> = 90° <i>b</i> = 17.693(2) Å; <i>b</i> = 90.009(2)° <i>c</i> = 13.2598(15) Å; <i>γ</i> = 90°	<i>a</i> = 12.0742(7) Å; <i>a</i> = 90° <i>b</i> = 31.331(2) Å; <i>b</i> = 90.305(2)° <i>c</i> = 23.0947(12) Å; <i>γ</i> = 90°
Volume	2457.9(5) Å ³	8736.5(9) Å ³
<i>Z</i>	4	16
Density (calculated)	1.331 Mg/m ³	1.330 Mg/m ³
Absorption coefficient	0.571 mm ⁻¹	0.910 mm ⁻¹
<i>F</i> (000)	1040	3712
Crystal size	0.250 × 0.200 × 0.200 mm ³	0.300 × 0.250 × 0.200 mm ³
Theta range for data collection	1.151°–24.999°	0.650°–25.919°
Index ranges	–12 ≤ <i>h</i> ≤ 12, –21 ≤ <i>k</i> ≤ 21, –15 ≤ <i>l</i> ≤ 15	–14 ≤ <i>h</i> ≤ 14, –38 ≤ <i>k</i> ≤ 38, –27 ≤ <i>l</i> ≤ 28
Reflections collected	30,427	154,854
Independent reflections	4263 [<i>R</i> (int) = 0.1011]	16,936 [<i>R</i> (int) = 0.1000]
Data/restraints/parameters	4263/0/299	16,936/3/1052
Goodness-of-fit on <i>F</i> ²	1.064	1.049
Final <i>R</i> indices [<i>I</i> > 2σ(<i>I</i>)]	<i>R</i> 1 = 0.0444, <i>wR</i> 2 = 0.0925	<i>R</i> 1 = 0.0503, <i>wR</i> 2 = 0.1191
Final <i>R</i> indexes [all data]	<i>R</i> 1 = 0.0808, <i>wR</i> 2 = 0.1109	<i>R</i> 1 = 0.1063, <i>wR</i> 2 = 0.1513
Largest diff. peak and hole	0.390 and –0.440 e Å ⁻³	0.694 and –0.456 e Å ⁻³

for 3–4 h; then the precipitate was filtered off, washed with methanol and dried under vacuum. Yield 0.42 g (72%).

Dark brown solid; Anal. calcd for C₂₅H₃₂CuN₂O₂(%): C 65.2, H 6.8, N 6.3, Found: C 65.6, H 6.9, N 6.6; UV–Vis: (DMF) λ_{max} (nm): 284, 379, 560, 572; FTIR (KBr, cm⁻¹) ν_{max}: 1560 (C=N), 1240 (C–O), 1408 (C=C), 520 (M–O), 410 (M–N); LCMS (*m/z*): calcd 442.05, obs 442.05. μ_{eff}: 1.69; Conductance (Λ_M Ω⁻¹ cm² mol⁻¹): 12.67.

Single-crystal X-ray diffraction

Crystals of the manganese and nickel complexes were each mounted on an APEX 2 (Bruker 2004) diffractometer, respectively. The cell refinement and data reduction were performed using APEX2/SAINT (Bruker 2004) and SAINT/XPREP (Bruker 2004), respectively [12]. The structures

Table 2 Selected bond lengths and angles for [Mn(OAc)(L)]

C(25)–O(4)	1.226(5) Å	O(3)–Mn(1)	1.854(2) Å
C(25)–O(1)	1.271(5) Å	O(3)–Mn(1)–O(2)	93.53(2)°
N(1)–Mn(1)	1.955(3) Å	O(3)–Mn(1)–N(1)	90.23(11)°
N(2)–Mn(1)	1.969(3) Å	O(2)–Mn(1)–N(1)	162.74(14)°
O(1)–Mn(1)	2.042(3) Å	O(3)–Mn(1)–N(2)	161.29(12)°
O(2)–Mn(1)	1.869(3) Å	O(2)–Mn(1)–N(2)	88.36(12)°

were solved by using SIR92 [13], and the structures were refined by SHELXL-2014/7 [14]. The computing molecular graphics were prepared with ORTEP 3.0 [15] and Mercury [16] software. Figure 1 shows ORTEP representations of [Mn(OAc)(L)] and [Ni(L)], with the common atomic numbering scheme. The crystallographic data and structure

refinement for both complexes are summarized in Table 1. Selected bond lengths and angles for [Mn(OAc)(L)] and [Ni(L)] are given in Tables 2 and 3, respectively.

Biological studies

Antibacterial activity

The antibacterial activities of the compounds were assayed by the colony count method, using the selected test organisms *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In this procedure, cells of the test organisms were grown in nutrient broth until mid-log phase and used as inoculums for performing antibacterial tests. Approximately 1×10^6 cells/ml of the test organisms were each inoculated with 0–500 $\mu\text{g/ml}$ concentrations of the test compounds and incubated for 16–18 h at 37 °C. After incubation, the number of viable cells was assayed by spreading an aliquot from the broth on Muller–Hilton agar and counting the number of colony forming units per milliliter (CFU/ml). DMSO was used as negative control. Minimum inhibitory concentration (MIC) was determined. Ciprofloxacin, ampicillin and streptomycin were used as positive controls for the comparison of antibacterial activity [17].

DPPH radical scavenging activity

Radical scavenging activity was assayed by the bleaching of a purple-colored methanol solution of the stable radical 1,1-diphenyl-1-picrylhydrazyl (DPPH) by the test compound [18]. Aliquots (1 ml) of the required test compound concentrations (5, 10, 25, 50 and 100 $\mu\text{g/ml}$) in methanol were added to 4 ml of a 0.004% (w/v) methanol solution of DPPH. After 30 min of incubation at room temperature, the absorbance was measured against blank at 517 nm. The percent inhibition ($I\%$) of free radical production from DPPH was calculated by the following equation:

$$\% \text{ scavenging} = [(A \text{ control} - A \text{ sample})/A \text{ blank}] \times 100$$

where 'A control' is the absorbance of the control reaction (containing all reagents except the test compound) and 'A sample' is the absorbance of the test compound. Tests were carried out in triplicate.

Table 3 Selected bond lengths and angles for [Ni(L)]

N(1)–Ni(1)	1.829(4) Å	N(2)–Ni(1)–O(1)	178.99(18)°
N(2)–Ni(1)	1.825(4) Å	N(1)–Ni(1)–O(1)	93.64(16)°
Ni(1)–O(1)	1.831(3) Å	N(2)–Ni(1)–O(2)	93.28(16)°
Ni(1)–O(2)	1.832(3) Å	N(1)–Ni(1)–O(2)	178.78(18)°
N(2)–Ni(1)–N(1)	87.08(18)°	O(1)–Ni(1)–O(2)	86.02(15)°

DNA cleavage experiments

The DNA cleavage potential of the free Schiff base and its complexes was studied by gel electrophoresis on a total sample volume of 10 μl in 0.5 ml transparent Eppendorf microcentrifuge tubes containing pBR322 DNA (200 ng). For the gel electrophoresis experiments, supercoiled pBR322 DNA was treated with the test compounds concentrations (10, 20, 50, 100, 150 and 200 μM) and the mixtures were incubated in the dark for 30 min at 37 °C. The reaction was quenched by adding 2 μl DNA loading dye (two dye mixture containing bromophenol blue and xylene cyanol), and the samples were loaded in wells prepared for 1% agarose gel electrophoresis (tris-boric acid-EDTA (TBE) buffer, pH 8.2) for 3 h at 40 V. The pBR322 DNA bands were stained using ethidium bromide, and the level of cleavage of pBR 322 DNA was determined by measuring the intensities of the bands using a UVITECH gel documentation system. For mechanistic investigations, initial experiments were carried out in the presence of H_2O_2 at different sample concentrations [19].

Results and discussion

The manganese(III), cobalt(II), nickel(II) and copper(II) complexes were synthesized by reactions of the corresponding metal salts with 6,6'-((1E,1'E)-(ethane-1,2-diylbis(azanylylidene))bis(methanylylidene))bis(5-iso-propyl-2-methylphenol) Schiff base (H_2L) (Scheme 1). Coordination of the ligand to each metal through its oxygen and nitrogen donor sites was confirmed by elemental analysis, electronic spectra, FTIR spectra, ESR and LCMS spectroscopy, magnetic susceptibility and molar conductance measurements. Furthermore, the structures of the manganese(III) and nickel(II) complexes were confirmed by single-crystal X-ray crystallography.

The electronic spectrum of the free Schiff base shows two intense bands at 273 and 332 nm which can be assigned to the $\pi \rightarrow \pi^*$ transitions of the aromatic rings and the imines group, respectively [20]. These bands were also observed at similar positions in the UV–Vis spectra of the complexes. A new band at 417 nm and weak absorbance at higher wavelengths in the spectrum of the manganese complex are assigned to metal-to-ligand charge transfer and $d-d$ transitions, respectively, in accordance with previously reported observations closely for related manganese(III) complexes [21]. A band at 632 nm in the spectrum of the cobalt(II) complex is indicative of tetrahedral geometry. The electronic spectrum of the nickel(II) complex shows an absorption band below 600 nm, and the lack of any transition at longer wavelength indicates a large crystal-field splitting, consistent with a square-planar geometry for nickel(II). The spectrum of the copper(II) complex shows a shoulder at 560 nm which

is assigned to $d-d$ transitions in a square-planar geometry [22].

The FTIR spectrum of the free Schiff base shows a $\nu(\text{OH})$ vibration at 3438 cm^{-1} which is attributed to the phenolic OH groups. This band is absent from the spectra of the metal complexes. An absorption in the region of $1609\text{--}1560\text{ cm}^{-1}$ in the spectrum of H_2L is assigned to the azomethine group ($-\text{C}=\text{N}$), and the position of this band is found to be shifted upon complexation. The characteristic absorption bands for the $\text{C}=\text{C}$ and $\text{C}-\text{O}$ groups of H_2L were observed at 1462 and 1259 cm^{-1} , respectively. There was a significant change in the positions of both of these bands upon complexation. An absorption in the ranges of $410\text{--}452$ and $510\text{--}525\text{ cm}^{-1}$ in the spectra of the metal complexes can be assigned to the (M–N) and (M–O) stretching vibrations [23, 24].

In the ^1H NMR spectrum of H_2L , peaks in the region $6.61\text{--}7.09\text{ }\delta$ are attributable to the aromatic ring protons. A sharp singlet at 8.72 ppm assigned to the azomethine proton ($-\text{CH}=\text{N}-$) confirms formation of the Schiff base linkage. Another singlet at 2.18 ppm is observed for the methyl group protons, while a broad peak at 14.60 ppm is assigned to the phenolic OH group. A doublet and multiplet at 1.13 and 3.2 ppm are assigned to isopropyl group CH_3 and CH protons, respectively. A singlet at $3.95\text{ }\delta$ is assigned to the ethylene diamine protons.

The LCMS spectrum of the free Schiff base showed the molecular ion at $m/z = 381.3$, i.e., M^+ [Calcd. 380.52] confirming the empirical formula. The LCMS spectra of the manganese(III), cobalt(II), nickel(II) and copper(II) complexes each showed the molecular ion peaks exactly equivalent with the empirical formulas (see “[Experimental](#)” section).

The molar conductivities of 10^{-3} M solutions of the complexes in DMF were measured at room temperature. The observed values were in the range of $12.67\text{--}19.60\text{ mol}^{-1}\text{ cm}^{-2}$, showing that all of these complexes are nonelectrolytic [25].

The magnetic moments of the manganese(III), cobalt(II) and copper(II) complexes were 4.82 , 4.23 and 1.69 B.M. , respectively. These values are consistent with square pyramidal, tetrahedral and square-planar geometries, respectively [26, 27]. The nickel(II) complex was found to be diamagnetic, as expected for the square-planar geometry confirmed through the single-crystal structure.

The EPR spectrum of the copper complex at 77 K was recorded in DMF solvent. The g -tensor values of copper complexes can be used to derive the ground state [28]; hence, from the ESR spectrum of this complex the values of $g_{\parallel} = 2.43$, $g_{\perp} = 2.077$, $G = 5.76$, $g_{\text{avg}} = 2.195$, $f = 136.01$ were derived. These values show that $g_{\parallel} > g_{\perp} > g_e$ with f in the range of $105\text{--}135\text{ cm}^{-1}$; this pattern has been reported for the square-planar complexes. The data indicate that the unpaired electron lies in the $d_{x^2-y^2}$ orbital, giving $^2\text{B}_1\text{g}$ as the

ground state [29]. Furthermore, g_{\parallel} at 2.34 is within the range of $2.3\text{--}2.4$ indicating the presence of mixed copper–nitrogen and copper–oxygen bonds in this complex [30]. The geometric parameter, $G = (g_{\parallel} - 2/g_{\perp} - 2)$, for axial spectra measures the exchange interaction between copper(II) centers in the polycrystalline state; if $G > 4.0$, the exchange interaction is negligible, whereas if it is less than 4.0 considerable exchange interaction is indicated. In the present complex, G is 5.76 indicating negligible exchange [31].

Crystal structure studies

X-ray studies of the manganese and nickel complexes reveal that each central metal is coordinated by a single ligand through one five-membered and two six-membered chelate rings. The manganese(III) complex has an irregular square pyramidal five-coordinate environment, with the basal plane formed by N_2O_2 atoms of the Schiff base ligand from its two deprotonated phenol oxygens and two imino nitrogens. An acetate ligand occupies the apical position. The observed Mn–N, Mn–O and Mn–OAc bond lengths are in the ranges of previously reported manganese complexes [32]. Similarly, in the nickel complex the Ni–O and Ni–N distances are within the ranges of $1.83\text{--}1.87$ and $1.83\text{--}1.89\text{ \AA}$, respectively, as described for N_2O_2 square-planar $[\text{Ni}(\text{L})]$ complexes [33]. In $[\text{Ni}(\text{L})]$, the Ni–O and Ni–N bond lengths are in the ranges of $1.831\text{--}1.832$ and $1.829\text{--}1.825\text{ \AA}$, respectively. The bond angles around the metal center $\text{N}2\text{--Ni--O}2$, $\text{O}1\text{--Ni--O}2$, $\text{N}2\text{--Ni--N}1$ and $\text{O}1\text{--Ni--N}1$ take values of $93.95(15)$, $87.47(12)$, $85.7(3)$ and $92.8(3)^\circ$, respectively. The $\text{N}(2)\text{--Ni}(1)\text{--O}(1)$ and $\text{N}(1)\text{--Ni}(1)\text{--O}(2)$ bond angles are $178.99(18)$ and $178.78(18)^\circ$, respectively. These values indicate that the complex has a distorted square-planar geometry. Both structures are monoclinic with a beta angle close to 90° . They have been refined as pseudomerohedral twins.

Antibacterial activity

Both the free Schiff base ligand and its complexes were tested for antibacterial activity against *P. aeruginosa* as a Gram-negative bacteria and *B. subtilis* and *S. aureus* as Gram-positive bacteria, with the results presented as MIC values (Table 4).

The results indicated that the Schiff base possesses activity against *B. subtilis* and *S. aureus* which is comparable to the standard ampicillin, while the manganese(III) complex shows excellent activity with MIC values of 85 , 87 and $70\text{ }\mu\text{g/ml}$ against *B. subtilis*, *S. aureus* and *P. aeruginosa*, respectively. However, all of these complexes displayed poor activities as compared to ciprofloxacin and streptomycin [34].

Table 4 Representation of antibacterial activity of H₂L and its metal complexes

Compounds name	MIC (μg/ml)		
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>
H ₂ L	240	143	225
Manganese(III)	85	87.5	70
Cobalt(II)	250	140.5	159.5
Nickel(II)	276	175	250
Copper(II)	152	114	172
Ciprofloxacin	50	50	25
Ampicillin	250	250	100
Streptomycin	25	25	12.5

Table 5 Representation of antibacterial activity of H₂L and its metal complexes

Compounds	IC ₅₀ values
H ₂ L	49.55
Manganese(III)	55.36
Cobalt(II)	18.25
Nickel(II)	14.21
Copper(II)	15.29
BHT	16.50
Ascorbic acid	12.80
α-Tocopherol	12.20

Antioxidant activities

The antioxidant activities of the complexes were assayed by quenching of the DPPH radical, which absorbs at 517 nm. Results are reported as the IC₅₀ values (Table 5).

The antioxidant activities of free H₂L and its complexes were compared with those of butylated hydroxyl toluene (BHT), ascorbic acid and α-tocopherol as standards. Free H₂L was found to have poor antioxidant activity; however, several of its complexes showed much better activities. In particular, the nickel(II) and copper(II) complexes showed the activities better than BHT, and comparable to ascorbic acid and α-tocopherol. In contrast, the manganese(III) complex exhibited poor activity.

DNA cleavage activity

We have utilized pBR322 DNA in order to study agarose gel electrophoresis. DNA cleavage is controlled by relaxation of supercoiled circular form DNA into nicked circular and linear forms. When circular DNA is analyzed by electrophoresis, the fastest migration will be observed for the supercoiled form (Form I). If one strand is cleaved, the supercoils will convert to a slower-moving open-circular

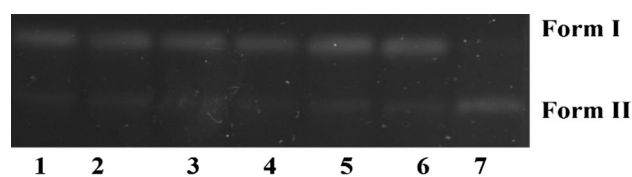


Fig. 2 Changes in the agarose gel electrophoretic pattern of pBR 322 DNA induced by H₂O₂ and the cobalt(II) complex. Lane 1, DNA + H₂O₂; lane 2, DNA + 10 μM sample + H₂O₂; lane 3, DNA + 20 μM sample + H₂O₂; lane 4, DNA + 50 μM sample + H₂O₂; lane 5, DNA + 100 μM sample + H₂O₂; lane 6, DNA + 150 μM sample + H₂O₂; lane 7, DNA + 200 μM sample + H₂O₂

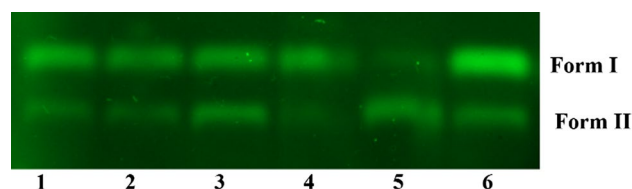


Fig. 3 Changes in the agarose gel electrophoretic pattern of pBR 322 DNA. Lane 1, DNA + 200 μM sample; lane 2, DNA + 200 μM sample + D-mannitol; lane 3, DNA + 200 μM sample + DMSO; lane 4, DNA + 200 μM sample + DABCO; lane 5, DNA + 200 μM sample + H₂O₂; lane 6, DNA + 200 μM sample + L-histidine

form (Form II), and if both the strands are cleaved, a linear form (Form III) will be generated; this migrates in between Forms I and II. We found that the free Schiff base ligand and its manganese(III), nickel(II) and copper(II) complexes do not have any effect on DNA in the presence or absence of hydrogen peroxide, suggesting that these compounds do not mediate the oxidative cleavage of DNA. However, the cobalt(II) complex possesses DNA cleavage activity at higher concentration, 200 μM in the presence of oxidant H₂O₂, as evident (Lane 7) from Fig. 2, where the conversion of supercoiled DNA to nicked DNA occurs (Fig. 3).

Thereafter, we carried out experiments with the cobalt(II) complex at a constant concentration (200 μM) in the presence of different radical scavengers, namely D-mannitol, DMSO, DABCO, H₂O₂ and L-histidine. In the presence of H₂O₂, the Form I DNA was to Form II, while partial conversion occurred in the presence of DABCO and there was no effect of D-mannitol, DMSO and L-histidine on Form I DNA. This suggests that oxidative DNA cleavage is mediated by hydroxyl radicals in the presence of the complex. The mechanism of DNA cleavage in similar systems has been described in the literature, and mechanistic studies are in progress [35].

Conclusion

Manganese(III), cobalt(II), nickel(II) and copper(II) complexes of a salen-type Schiff base ligand were synthesized and characterized. Square pyramidal and distorted square-planar geometries were confirmed through single-crystal X-ray crystallographic techniques. The manganese(III) and copper(II) complexes were found to possess antibacterial activities against the *B. subtilis*, *S. aureus* and *P. aeruginosa* as compared to standard ampicillin. The nickel(II), copper(II) and cobalt(II) complexes exhibited good antioxidant activities. The cobalt(II) complex was found to cleave pBR322 DNA in the presence of H₂O₂.

Supplementary materials

‘CCDC numbers 1554257 and 1544860 contain the supplementary crystallographic data for this paper. The data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures.’

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