

Biological activity, Spectroscopic and DNA Cleavage of Binuclear Schiff Base Complexes

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ABSTRACT

A new series of transition metal complexes of Cu(II), Ni(II) and VO(II) have been synthesized from the Schiff base (L) derived from 5-iodosalicylaldehyde and 3,3',4,4'-tetraminobiphenyl. Structural features were obtained from their spectral and analytical data. The data show that these complexes have composition of $[M_2(L)]X$ type. (where $M = (Cu(II), Ni(II), \text{ and } VO(II))$ $X = ClO_4^-, SO_4^{2-}$ $L =$ binucleating tetradentate ligand). The spectral data of the complexes suggest a square-planar geometry around the central metal ion except VO(II) complex which has square-pyramidal geometry. The redox behavior of copper and vanadyl complexes was studied by cyclic voltammetry. The pUC18 DNA cleavage study was monitored by gel electrophoresis method. The results suggest that binuclear Cu(II), Ni(II) and VO(II) complexes cleaves pUC18 DNA in presence of the oxidant H_2O_2 . The invitro antimicrobial activities of the synthesized compounds have been tested against the gram negative and gram positive bacteria's. The binuclear Schiff base complexes were found to be higher antibacterial activity than the free ligand.

Keywords : 3,3',4,4'-tetraminobiphenyl, Schiff base, binuclear, Antimicrobial, CT-DNA.

I. INTRODUCTION

Schiff base complexes have been extensively investigated for more than a century and have been employed in areas that include magnetochemistry [1], non-linear optics [2] photophysical studies [3], catalysis [4] and materials chemistry [5]. Schiff bases derived from an amine and any aldehyde are a class of compounds which co-ordinate to metal ions via the azomethine nitrogen [6]. Metal complexes of Schiff bases derived from substituted salicylaldehydes and various amines have been widely investigated because of their wide applicability [7-10]. The oxovanadium(IV) ion, VO^{2+} is considered to be the most stable oxocation of the first-row transition metal ion [11]. It forms stable anionic, cationic and neutral complexes with various types of ligands. Monomeric and five coordinate oxovanadium(IV) complexes are formed with several bidentate Schiff bases. Chelating ligands containing O and N donor atoms show broad biological activity and are of special interest because of the variety of ways in which they are bonded to metal ions [12]. It is well known that several Schiff base complexes have anti-inflammatory, antipyretic, analgesic, anti-diabetic, anti-

bacterial, anti-cancer and anti-HIV activity [13, 14]. The interaction of transition metal complexes with DNA has been extensively studied in the development of new tools for nanotechnology [15, 16]. In the present investigation we report here the synthesis, spectroscopic, redox, DNA cleavage studies and antimicrobial studies of new tetradentate N_2O_2 donor type Schiff base and their metal complexes. In this paper the synthesis of new tetradentate N_2O_2 donor type Schiff base and its metal complexes (Cu(II) Ni(II) and VO(II)) derived by the condensation of 3,3',4,4'-tetraminobiphenyl and 5-iodosalicylaldehyde is described.

II. EXPERIMENTAL

2.1 Materials and methods

All the chemicals used were chemically pure and AR grade. Solvents were purified and dried according to standard procedures [17]. Metals were purchased from Merck. 3,3',4,4'-tetraminobiphenyl & 5-iodosalicylaldehyde were obtained from Aldrich. Other chemicals were also purchased from Merck and Aldrich.

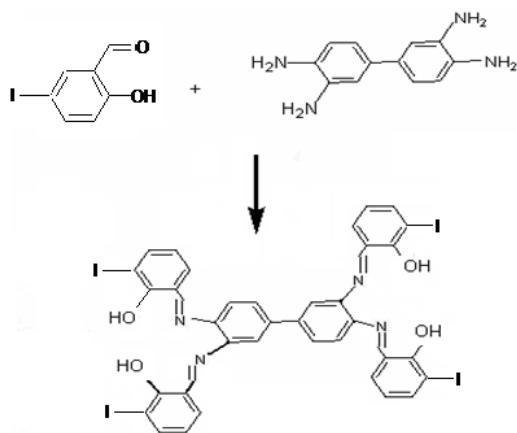
Synthesis: Caution! Perchlorate salts are potentially explosive and were handled only in small quantities with care.

2.2 Physical measurements

The elemental analysis were carried out with a Carlo-Erba 1106-model 240 Perkin Elmer analyzer. The solution conductivity measurements were performed to establish the charge type of the complexes. The complexes were dissolved in MeCN/DMF/DMSO and molar conductivities of 10^{-3} M of their solutions at 28 °C were measured. Infrared spectra were recorded on the Perkin Elmer FT-IR-8300 model spectrometer using KBr disc and Nujol mull techniques in the range of 4000-400 cm^{-1} . Electronic absorption spectra in the UV-Visible range were recorded on Perkin Elmer Lambda - 25 between 200-800 nm by using DMF as the solvent. Cyclic voltammetry studies were performed on a CHI760C electrochemical analyzer in single compartmental cells at 29 °C with $\text{H}_2\text{O}/\text{DMSO}$ (95:5) solution using tetrabutylammonium perchlorate (TBAP) as a supporting electrolyte.

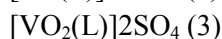
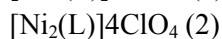
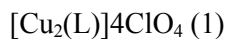
2.3 Preparation of binucleating tetradentate Schiff base ligand

The binucleating tetradentate Schiff base was prepared by condensation of tetramine with appropriate aldehydes (Scheme 1). 3,3',4,4'-tetraminobiphenyl (1 mmol) in 20 ml of methanol was stirred with 5-iodosalicylaldehyde (4 mmol) in 20 ml of methanol for 4 h. The resulting yellow orange solid was separated and dried in vacuum. Yield: 85%.

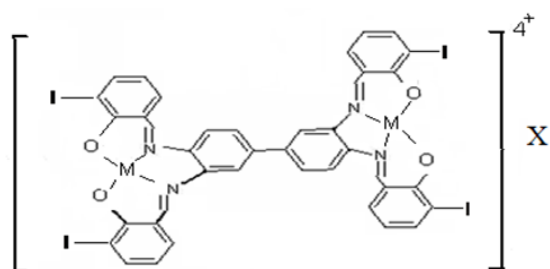


Scheme 1 Structure of binucleating tetradentate Schiff base ligand

2.4 Synthesis of binuclear Schiff base complexes



Metal(II) perchlorates of $[\text{Cu}(\text{II})]$, $[\text{Ni}(\text{II})]$ and $[\text{VO}(\text{II})]$ sulphate (0.2 mmol) and the potential binucleating Schiff base ligand (0.1 mmol) were dissolved in DMF (20 ml) and the mixture was heated to reflux for 3 h and the reactions were monitored by TLC. After partial evaporation of the solvent, solid (60–70%) metal(II) Schiff base complexes (Scheme 2) were separated and dried in vacuum.



Scheme 2 Structure of binuclear $\text{Cu}(\text{II})$, $\text{Ni}(\text{II})$ and $\text{VO}(\text{II})$ Schiff base complexes

Where, $\text{M} = \text{Cu}(\text{II})$, $\text{Ni}(\text{II})$ and $\text{VO}(\text{II})$ $\text{X} = 4\text{ClO}_4^-$, 2SO_4^{2-}

2.5 Cyclic voltammetry

All voltammetric experiments were performed with a CHI760C electrochemical analyzer, in single compartmental cells using Tetrabutylammonium perchlorate as a supporting electrolyte. The redox behavior of the complexes have been examined in absence and in presence of CT-DNA at a scan rate 0.2 Vs^{-1} in the potential range +1.2 to -2.0 V. A three-electrode configuration was used, comprised of a glassy carbon electrode as the working electrode, a Pt-wire as the auxiliary electrode, and an Ag/AgCl electrode as the reference electrode. The electrochemical data such as cathodic peak potential (E_{pc}) and anodic peak potential (E_{pa}) were measured.

2.6 Gel electrophoresis

The cleavage of pUC18 DNA was determined by agarose gel electrophoresis [18]. The gel electrophoresis

experiments were performed by incubation of the samples containing 40 μM pUC18 DNA, 50 μM metal complexes and 50 μM H_2O_2 in Tris-HCl buffer (pH 7.3) at 39°C for 2 h. After incubation, the samples were electrophoresed for 3 h at 55 V on 1% agarose gel using Tris-acetic acid-EDTA buffer (pH 7.3). The gel was then stained using 1 $\mu\text{g cm}^{-3}$ ethidium bromide (EB) and photographed under ultraviolet light at 355nm. All the experiments were performed at room temperature unless otherwise mentioned.

2.7 Antimicrobial activity

The *in vitro* antibacterial activity of the ligand and the complexes were tested against the bacteria *Bacillus subtilis*, *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus aureus* by well diffusion method using nutrient agar as the medium. Streptomycin was used as standard for bacteria. The stock solution (10^{-2} mol L^{-1}) was prepared by dissolving the compound in DMF and the solution was serially diluted in order to find minimum inhibitory concentration (MIC) values. In a typical procedure, a well was made on the agar medium inoculated with microorganisms in a Petri plate. The well was filled with the test solution and the plate was incubated for 24 h for bacteria at 40 °C. During the period, the test solution diffused and the growth of the inoculated microorganisms was affected. The inhibition zone was developed, at which the concentration was noted.

III. RESULTS AND DISCUSSION

The binuclear Schiff base ligand prepared and reacts with transition metals Cu(II), Ni(II), and VO(II). The Schiff base ligand has been synthesized from 5-iodosalicylaldehyde and 3,3',4,4'-tetraminobiphenyl (scheme 1) in 4:1 mole ratio. The results of elemental analyses were in good agreement with those required by the proposed formulae. The data in consistent with the earlier reports support the proposed formulation of the binuclear complexes (scheme 2). The higher conductance values (Table 3) of chelates support the electrolytic (1:2) nature of metal complexes.

3.1 IR spectra

The important IR absorption frequencies of the synthesized complexes are shown in Table 1. The

azomethine nitrogen $\nu_{\text{C=N}}$ stretching frequency of the free ligand appears around 1625 cm^{-1} , which is shifted to lower frequencies in the spectra of all the complexes ($1600\text{-}1610 \text{ cm}^{-1}$). These bands are shifted to lower wave numbers indicating the involvement of azomethine nitrogen in coordination to the metal ion [19]. This is further supported by the disappearance of the ν_{OH} in the range of $3400\text{-}3440 \text{ cm}^{-1}$ in all the complexes. Accordingly, the ligand acts as a tetradentate chelating agent bonded to the metal ion via two -C-O groups & two -C=N azomethine nitrogen atoms of the Schiff base (scheme 2). Assignment of the proposed coordination sites is further supported by the appearance of medium bands at $480\text{-}510 \text{ cm}^{-1}$ and $430\text{-}470 \text{ cm}^{-1}$ which could be attributed to M-O, M-N respectively [20]. In addition, the Oxovanadium complexes shows a band at 970 cm^{-1} - 980 cm^{-1} attributed to V=O stretching frequency [21]. A further examination of Infrared spectra of complexes shows the presence of a band in the $1070\text{-}1115 \text{ cm}^{-1}$ region. The strong band is ascribable to ClO_4^- & SO_4^{2-} ions [22].

Table 1. Infrared spectral data for the ligand and binuclear Schiff base complexes

Complexes	$\nu(\text{-C=N})$ (cm^{-1})	$\nu(\text{-OH})$ (cm^{-1})	$\nu(\text{V=O})$ (cm^{-1})	$\nu(\text{M-O})$ (cm^{-1})	$\nu(\text{M-N})$ (cm^{-1})	$\text{ClO}_4^-/\text{SO}_4^{2-}$ (cm^{-1})
(Ligand)	1625	3400	-	-	-	-
$[\text{Cu}_2(\text{L})]4\text{ClO}_4^-$	1600	3410	-	10	460	1080
$[\text{Ni}_2(\text{L})]4\text{ClO}_4^-$	1610	3440	-	490	470	1115
$[\text{VO}_2(\text{L})]2\text{SO}_4^{2-}$	1602	3405	980	480	430	1070

3.2 Electronic spectra

The UV-visible spectra are often very useful in the evaluation of results furnished by other methods of structural investigation. The electronic absorption spectra of the Schiff base ligand and its complexes were recorded in DMF solution in the range of 200 to 800 nm regions and the data are presented in Table 2. The absorption spectrum of free ligand consist of an intense bands centered at 340 nm attributed to $\text{n-}\pi^*$ transitions of the azomethine group. Another intense band in higher energy region of the spectra of the free ligand was related to $\pi\rightarrow\pi^*$ transitions of benzene rings. These transitions are also found in the spectra of the complexes, but they shifted towards lower frequencies, confirming the coordination of the ligand to the metal ions. Further, the d-d transition of the complex showed a broad band

centered at 440 nm for Cu(II) complex. This is due to $^2B_{1g} \rightarrow ^2A_{1g}$ transition [23]. The spectra of Ni(II) complex in the visible region at about 370 and 380 nm is assigned to $^1A_{1g} \rightarrow ^1A_{2g}$, $^1A_{1g} \rightarrow ^1B_{1g}$, transitions, suggesting an approximate square planar geometry of the ligand around the metal ions [24]. The intense charge transfer band at 410-425 nm in Oxovanadium(IV) complex assigned to $^2B_2 \rightarrow ^2A_1$, $^2B_2 \rightarrow ^2E$ transitions. This is due to electron delocalization over whole molecule on complexation. Based on these data, a square planar geometry has been assigned to the complexes except VO(II) complex which has square pyramidal geometry. These values are comparable with other reported complexes [25].

Table 2. Absorption spectral data of the ligand and binuclear Schiff base complexes

Complexes	Absorption (λ_{max})(nm)			
	d-d	$\pi \rightarrow \pi^*$ Benzene/ imino	$n \rightarrow \pi^*$ Azomethine	L $\rightarrow$ MCT
Ligand	-	273,254	340	-
[Cu ₂ (L)]4ClO ₄	440	279,245	334	440
[Ni ₂ (L)]4ClO ₄	490	270,250	332	370
[VO ₂ (L)]2SO ₄	470	275,242	333	425

3.3 Molar Conductance studies

The complexes are insoluble in most common polar and non-polar organic solvent. They are soluble in DMSO, DMF and CH₂Cl₂. The molar conductivities of the complexes were measured in DMSO for freshly prepared solutions and after standing for two weeks. The conductivity increased very slightly with time in DMSO for all the complexes. The present coordination moieties cannot be replaced by the solvent molecules. The value of these conductance's are in the range 25-30 ohm⁻¹ cm² mol⁻¹ in DMSO and are in accordance with those reported for electrolyte in this solvent. This is indicative that these complexes dissociate very slightly in this solvent. As observed in the IR spectra, where the compounds were formulated as electrolytes the molar conductivities show that all the anions are present

outside the coordination sphere in solution [26-31]. The data are presented in Table 3.

Table 3. Molar conductance data of the binuclear Schiff base the complexes

Complexes	Solvent	Molar conductance Λ_m (ohm ⁻¹ cm ² mol ⁻¹)	Types of electrolyte
[Cu ₂ (L)]4ClO ₄	MeCN	240	1:2
	DMF	200	1:2
[Ni ₂ (L)]4ClO ₄	MeCN	110	1:2
	DMSO	125	1:2
[VO ₂ (L)]2SO ₄	MeCN	163	1:2
	DMSO	132	1:2

3.4 Cyclic voltammetry studies

Cyclic voltammetry is the most versatile electroanalytical technique for the study of electroactive species. The important parameters of a cyclic voltammogram are the magnitudes of the anodic peak potential (*E_{pa}*) and cathodic peak potential (*E_{pc}*). Cyclic voltammetry has been employed to study the interaction of complex with CT-DNA. The cyclic voltammogram of complex 1 in the absence of CT-DNA shows in Fig. 1(a) reveals non-Nernstain but fairly reversible/quasi-reversible one electron redox process involving Cu(II)/Cu(I) couple. The cyclic voltammograms of complexes were obtained in H₂O/DMSO (95:5) solution, at scan rate 0.2 Vs⁻¹ over a potential range from +1.2 to -2.0 V. In the absence of CT-DNA, complex 1 and other complexes data are listed in Table 4. the anodic peak potential (*E_{pa}*) of complex 1 appeared at - 0.412 V and the cathodic (*E_{pc}*) at -1.30 V. The cyclic voltammograms of complex 1 reveals a one electron quasireversible wave attributed to the redox couple Cu(II)/Cu(I) with the formal electrode potential, $E^0 = -0.863$ V, and $\Delta E_p = -1.712$ V which is larger than the Nernstian value observed for the one electron transfer couple. On addition of CT-DNA, the complex 1 Fig. 1(b) shows a shift in *E_{pc}*= (-1.110 V), *E_{pa}*= (+0.210 V) and $\Delta E_p = (0.900V)$ values indicating strong binding of binuclear complex with CT-DNA. The decrease in ratio of anodic to cathodic peak signify that adsorption of Cu(I) is enhanced in the presence of CT-DNA. Further,

the shift in E^0 value and increase in peak heights potentials suggest that both Cu(II) and Cu(I) form of complex 1 bind to CT-DNA [32].

Table 4. Cyclic voltammetric data of the binuclear Schiff base Complexes in DMSO solution.

Complexes	Couple	E _{pc} (V)	E _{pa} (V)	ΔE _p (m v)
[Cu ₂ (L)]4Cl O ₄	Cu(II)/ Cu(I)	- 0.41 2	- 1.300	-1.712
[Ni ₂ (L)]4Cl O ₄	Ni(II)/Ni(I)	1.65	1.75	0.10
[VO ₂ (L)]2SO ₄	VO(IV)/ VO(III) VO(IV)/VO(V)	-1.50	-0.40	-1.10

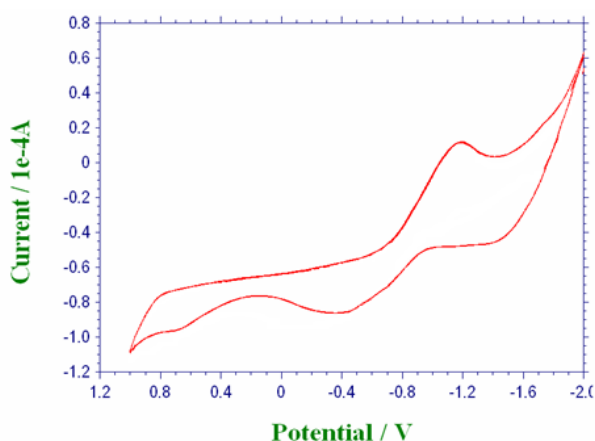


Figure 1a. Cyclic voltammogram of complex 1 alone

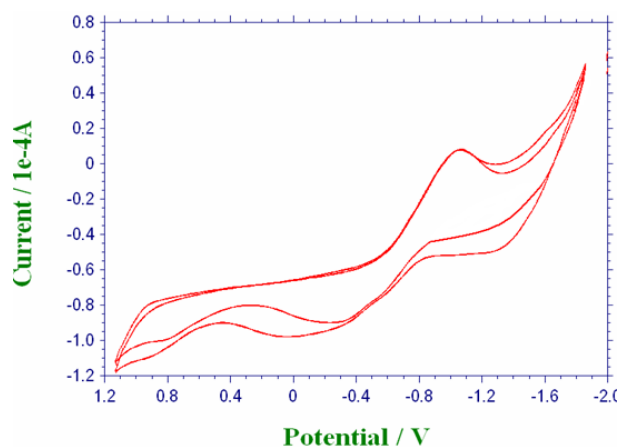


Figure 1b. Complex 1 in presence of CT-DNA
[Complex 1]

3.5 Cleavage of Plasmid pUC18 DNA

DNA cleavage is controlled by relaxation of supercoiled circular conformation of pUC18 DNA to nicked circular conformation and linear conformation. When circular plasmid DNA is conducted by electrophoresis, the fastest migration will be observed for the supercoiled form (Form I). If one strand is cleaved, the supercoils will relax to produce a slower-moving open circular form (Form II). If both strands are cleaved, a linear form (Form III) will be generated that migrates in between. Figure 2 illustrates the gel electrophoresis experiments showing the cleavage of plasmid pUC18 DNA induced by the three binuclear complexes. The control experiments did not show any apparent cleavage of DNA (lane 1 & 2). Copper binuclear complex in the presence of H₂O₂ (lane 1) at higher concentration (50μM) shows more cleavage activity compared to binuclear Nickel and Oxovanadium(IV) complexes. The supercoiled plasmid DNA was completely degraded. This shows that a slight increase in the concentration over the optimal value led to extensive degradations, resulting in the disappearance of bands on agarose gel [33]. Nickel binuclear complex in the presence of H₂O₂ resulting the conversion of supercoiled form (Form-I) into linear form (Form-III) (lane 4). Oxovanadium binuclear complex in the presence of H₂O₂ (lane 5) at higher concentration (50μM) shows cleavage activity in which supercoiled DNA (Form-I) cleaved and supercoiled form converted to open circular form (Form-II). The results revealed that the Cu(II), Ni(II) complexes have more cleavage than VO(II) complex. Probably this may be due to the formation of redox couple of the metal ions and its behaviour. Further the presence of a smear in the gel diagram indicates the presence of radical cleavage [34].

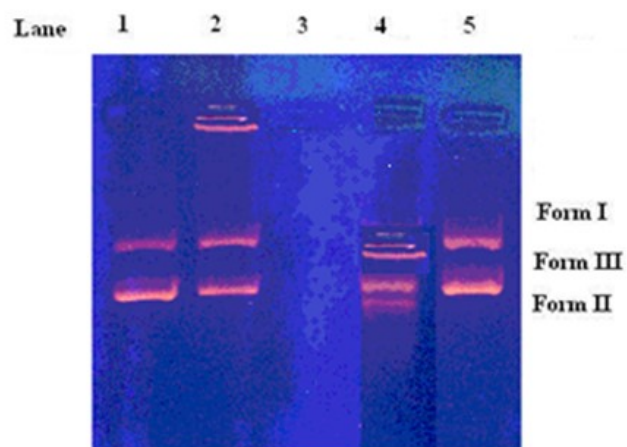


Figure 2. Changes in the agarose gel electrophoretic pattern of pUC18DNA induced by H₂O₂ and metal complexes: Lane 1, DNA alone; Lane 2, DNA alone + H₂O₂; Lane 3, DNA + Cu binuclear complex + H₂O₂; Lane 4, DNA + Ni binuclear complex + H₂O₂; Lane5, DNA + VO binuclear complex + H₂O₂.

3.6 Antimicrobial activity

The ligand and their complexes have been tested for *invitro* growth inhibitory activity against *gram-positive* microbes *Bacillus subtilis*, *staphylococcus aureus* and *gram-negative* microbes *Klebsiella pneumonia*, *Escherichia coli* by using well-diffusion method. As the

test solution concentration increases, the biological activity also increases. It is found that the activity increases upon co-ordination. The increased activity of the metal chelates can be explained on the basis of chelation theory [35]. The orbital of each metal ion is made so as to overlap with the ligand orbital. Increased activity enhances the lipophilicity of complexes due to delocalization of pi-electrons in the chelate ring [36]. In some cases increased lipophilicity leads to breakdown of the permeability barrier of the cell [37]. The results revealed that the metal complexes Cu(II), Ni(II) and VO(II) have higher antimicrobial activity than the ligand are shown in Table 5.

Table 5. Antibacterial activity of the ligand and binuclear Schiff base complexes

	Klebsiella pneumoniae (mm)				Escherichia coli (mm)				Staphylococcus aureus (mm)				Bacillus subtilis (mm)			
Complexes	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100
	(μl)				(μl)				(μl)				(μl)			
Ligand	11	13	12	14	12	13	14	15	11	13	14	16	12	13	15	18
[Cu ₂ (L)]4Cl O ₄	10	14	13	16	13	14	16	16	12	15	16	18	10	16	18	19
[Ni ₂ (L)]4Cl O ₄	11	15	18	19	14	15	17	18	14	16	17	19	13	15	19	20
[VO ₂ (L)]2S O ₄	14	16	17	18	15	16	18	19	15	16	18	19	12	14	18	19

IV. CONCLUSION

The N₂O₂ type Schiff base ligand is synthesized from 5-iodosalicylaldehyde and 3,3',4,4'-tetraminobiphenyl. It acts as a tetradentate ligand and forms stable complexes with transition metal ions such as Copper(II), Nickel(II), and Oxovanadium(IV). The ligand and its complexes are characterized using spectral and analytical data. The interaction of these complexes with CT-DNA was

investigated by gel electrophoresis. All the transition metal complexes have higher activity than the control CT-DNA. The Cu(II), Ni(II) complexes have more activity than VO(IV) complex and the control CT-DNA. . The metal complexes have higher antimicrobial activity than the free ligand.

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