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# Antibacterial, DNA interaction and cytotoxic activities of pendant-armed polyamine macrocyclic dinuclear nickel(II) and copper(II) complexes

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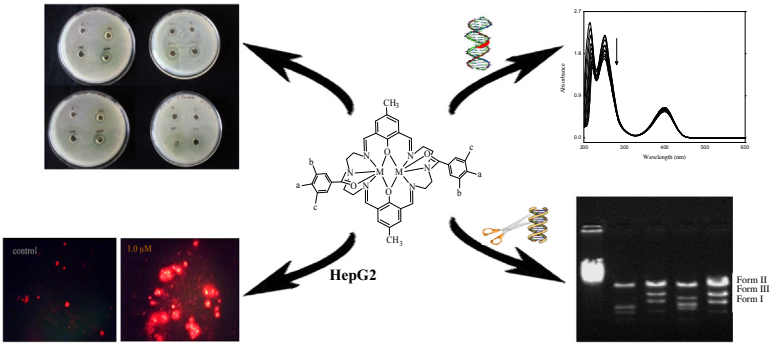
## HIGHLIGHTS

- Six pendant-armed complexes with benzoyl substituents have been synthesized.
- Antibacterial activity against Gram +ve and Gram −ve bacteria has been studied.
- DNA binding studies suggest the intercalative mode of binding.
- The synthesized complexes act as potent metallonucleases.
- Complexes can enter the nuclei of HepG2 cells and induce cell apoptosis.

## GRAPHICAL ABSTRACT

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## ABSTRACT

A series of dinuclear nickel(II) and copper(II) complexes (**1**–**6**) of hexaaza macrocycles of 2,6-diformyl-4-methylphenol with three different benzoyl pendant-arms, 2,2′-benzoyliminodi(ethylamine) trihydrochloride (L), 2,2′-4-nitrobenzoyliminodi(ethylamine) trihydrochloride (L′) and 2,2′-3,5-dinitrobenzoyliminodi(ethylamine) trihydrochloride (L″) have been synthesized and characterized by spectral methods. The electrochemical studies of these complexes depict two irreversible one electron reduction processes around $E^1_{pc} = -0.62$ to $-0.76$ V and $E^2_{pc} = -1.21$ to $-1.31$, and nickel(II) complexes (**1**–**3**) exhibit two irreversible one electron oxidation processes around $E^1_{pa} = 1.08$ to 1.14 V and $E^2_{pa} = 1.71$ to 1.74 V. The room temperature magnetic moment values ($\mu_{eff}$, 1.52–1.54 BM) indicate the presence of an antiferromagnetic interaction in the binuclear copper(II) complexes (**4**–**6**) which is also observed from the broad ESR spectra with a g value of 2.14–2.15. The synthesized complexes (**1**–**6**) were screened for their antibacterial activity. The results of DNA interaction studies indicate that the dinuclear complexes can bind to calf thymus DNA by intercalative mode and display efficient cleavage of plasmid DNA. Further, the cytotoxic activity of complexes **2**, **5** and **6** on human liver adenocarcinoma (HepG2) cell line has been examined. Nuclear-chromatin cleavage has also been observed with PI staining and comet assays.



## Introduction

The chemistry of macrocyclic compounds has become a fruitful research area due to the ability of these systems to interact

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with different substrates, such as metal ions or anionic species [1]. The term "macrocycle" is defined as a cyclic macromolecule or a cyclic compound with nine or more members [2]. Coordination chemists generally define a macrocycle more narrowly as a cyclic molecule with three or more potential donor atoms that can coordinate to a metal center. Cabbiness and Margerum introduced the term "macrocyclic effect" to explain the stability of macrocyclic polyamine when compared to their acyclic analogs [3]. Much effort has now been focused on pendant-armed macrocyclic complexes due to the fact that the ligating groups attached to the macrocyclic backbone can offer additional donor groups to produce important changes in the control of the stability, selectivity, stereochemistry and certain thermodynamic parameters [4,5] or promote the formation of dinuclear or polynuclear metal complexes with interaction between the metal centers as well as act as host for organic cations with different properties and applications [6]. The following two ways are involved in the process of making pendant-armed macrocyclic complexes: (i) The electrophilic substitution or addition reactions have been used in connecting the functional group with the ring framework (ii) "opened cryptands" can be generated by using analogous functional precursors with variable aromatic dialdehydes in the presence of certain metal ions [7].

In recent years, metal complexes of pendant-armed macrocyclic polyamines are of growing interest for applications in biological and medicinal chemistry, in addition to catalysis. Macrocyclic polyamines bearing pendant-arms can effectively bind specific metals with suitable complexation kinetics and stability while attached to a tumor-targeting vector or peptide. These compounds are used in the radiopharmaceuticals for positron-emission tomography (PET), different phases of biological testing against HIV or antitumor activity and DNA interaction [8–10]. Studies on the interaction of pendant-armed macrocyclic complexes with DNA not only gives information about the reactive modes for protein–DNA interactions and probes of DNA structure but also explains the techniques of molecular biology and drug design [11]. In some cases, the toxicity of macrocyclic polyamines was found to decrease with the increased anti-HIV activity when they are coordinated with bivalent metals like nickel and copper [12]. Based upon these criteria researchers are further involved to exploit their derivatives. Nickel(II) and copper(II) complexes have shown notable different biological activities. Nickel was long thought not to be a metal of biological importance. However, Zerner discovered that urease is a nickel enzyme [13]. Since then, other important enzymes that depend on nickel for activity have been identified. For this reason, the synthesis of complexes oriented to mimic metal sites of different types of metalloproteins constitutes an important branch in both inorganic and organic chemistry [14]. The role of copper compounds as pharmaceutical drugs in the treatment of numerous chronic diseases is well established. The macrocyclic ligands in the complexes are smaller than enzymes which allow the attack at the macromolecular region which are beyond the reach of enzymes. Due to its unique properties it is used as adjuvant in PCR diagnostics, cleaving agents and attacking agents [15,16].

It is significant to note that pendant-armed macrocyclic nickel(II) and copper(II) complexes have been rarely used for bimetallic biosite [17,18]. This has prompted us to design and synthesis a series of dinuclear nickel(II) and copper(II) complexes with pendant-armed polyamine macrocycles of diethylenetriamine derivatives with N-substituted groups (at the secondary amine nitrogen atom) and 2,6-diformyl-4-methylphenol by template method. The biological significance of the synthesized pendant-armed complexes has been investigated in term of antibacterial, DNA interaction and cytotoxic activities.

## Experimental

### Materials

2,6-Diformyl-4-methylphenol has been synthesized according to the literature method [19]. Benzoyl chloride, nitrobenzoyl chlorides, diethylenetriamine, salicylaldehyde and metal(II) perchlorate salts were commercial products (from Merck and Aldrich). Solvents were dried and purified before being used according to standard procedure. Tetra(*n*-butyl)ammonium perchlorate (TBAP) used as supporting electrolyte in the electrochemical measurements was purchased from Fluka and recrystallized from hot methanol. (***Caution!*** TBAP is potentially explosive; hence care should be taken in handling the compound). Tris–HCl buffer (5 mM Tris–HCl/50 mM NaCl, pH 7.2), Tris = Tris(hydroxymethyl)aminomethane, solution was prepared using deionized double distilled water. Calf thymus DNA (CT-DNA) and pBR322 DNA were purchased from Bangalore Genei (India).

### Physical measurements

The elemental analysis (C, H and N) was measured with a Carlo Erba elemental analyzer Model 1106. $^1$H NMR spectral data were collected on Varian-VNMRS-400 in $CDCl_3$ and DMSO solution with tetramethylsilane (TMS) as an internal standard at ambient temperature. IR spectra were recorded on ABB Instruments, MB-3000 spectrophotometer using KBr pellets in the range 4000–400 $cm^{-1}$. The mass data of ligands were obtained on a JEOL GC mate GC–MS spectrometer and the mass spectra for complexes were taken on Q-TOF 6000 ESI mass spectrometer. Electronic spectra of complexes were recorded on Perkin Elmer Lambda-45 spectrophotometer, in the range of 200–1100 nm using 1 cm quartz micro cuvettes. X-band EPR spectra were recorded on a Varian EPR-E 112 spectrophotometer using diphenylpicrylhydrazine (DPPH) as the reference. Room temperature magnetic moments were recorded on a PAR Vibrating sample magnetometer model 155. Cyclic voltammograms were obtained on a CHI-602D electrochemical analyzer using a three-electrode cell in which a glassy carbon electrode was the working electrode, a saturated Ag/AgCl electrode was the reference electrode and platinum wire was used as the auxiliary electrode. A ferrocene/ferrocenium (1+) couple was used as an internal standard and $E_{1/2}$ of the ferrocene/ferrocenium ($Fc/Fc^+$) couple under the experimental condition was 470 mV. Tetra(*n*-butyl)ammonium perchlorate (TBAP) was used as the supporting electrolyte (0.1 M) and all complex solutions were around $10^{-3}$ M concentration. All electrochemical measurements were carried out in solutions purged with pure nitrogen for 30 min in advance.

### Synthesis of ligands

#### 2,2′-Benzoyliminodi(ethylamine) trihydrochloride (L)

A solution of diethylenetriamine (1.08 mL, 10 mmol) in ethanol (20 mL) was added dropwise to a solution of salicylaldehyde (2.1 mL, 20 mmol) in ethanol (20 mL). The mixture was stirred for 2 h and then refluxed for 6 h. Then the reaction mixture was cooled to room temperature, and a solution of benzoyl chloride (1.16 mL, 10 mmol) in ethanol (20 mL) followed by $Na_2CO_3$ (1.06 g, 10 mmol) were added. The resulting solution was refluxed for 36 h, cooled to room temperature, an excess solid $Na_2CO_3$ was filtered off and the filtrate was concentrated. A yellowish hygroscopic product 2,2′-(benzoyliminodiethylene)bissalicylidene was obtained which was used without further purification.

Yield: 3.25 g (78%). Analytical data for $C_{25}H_{25}N_3O_3$: Selected IR (KBr) ($\nu$/$cm^{-1}$): 3442 $\nu$(OH), 1732 $\nu$(C=O), 1631 $\nu$(C=N). $^1$H NMR