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Mixed-ligand copper(II) phenolate complexes: Synthesis, spectral characterization, phosphate-hydrolysis, antioxidant, DNA interaction and cytotoxic studies

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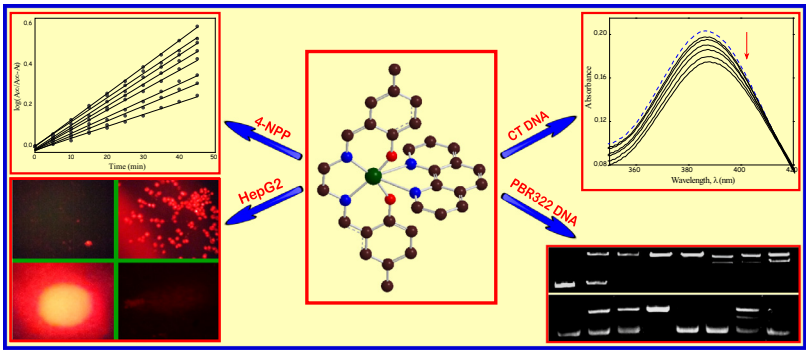
HIGHLIGHTS

- Eight mixed-ligand copper(II) complexes have been synthesized and characterized.
- Binding studies revealed the intercalative/groove binding of complexes with CT-DNA.
- The mechanism of DNA cleavage indicate the involvement of ROS.
- Cytotoxicity studies show the death of cancer cells *via* apoptosis.
- The complexes with hydrophobic —CH_3 substituent show higher biological activity.

GRAPHICAL ABSTRACT



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ABSTRACT

A series of phenol-based mixed-ligand copper(II) complexes of the type [CuL^{1-4}(diimine)] (**1**–**8**), where L^{1-4} = N^1,N^2-bis(5-substituted-2-hydroxybenzylidene)-1,2-ethylene/phenylenediimine and diimine = 2, 2′-bipyridyl (bpy) or 1,10-phenanthroline (phen), have been isolated and fully characterized by analytical and spectral techniques. Electronic spectra of complexes suggest Cu(II) cation has a d^9 electronic configuration, adopting distorted octahedral geometry with axial elongation, due to Jahn–Teller effect. Electrochemical studies of complexes evidenced one-electron irreversible reduction wave in the cathodic region. The observed rate constant (k) values for the hydrolysis of 4-nitrophenylphosphate (4-NPP) are in the range of 0.25–3.82 × 10^{-2} min^{-1}. The obtained room temperature magnetic moment values (1.79–1.90 BM) lies within the range observed for octahedral copper(II) complexes. Antioxidant studies revealed that these complexes possess considerable radical scavenging potency against DPPH. The binding studies of complexes with calf thymus DNA (CT-DNA) revealed intercalation with minor-groove binding, and the complex **4** exhibits highest binding activity than the other complexes. The cleavage activity on supercoiled pBR322 DNA revealed the involvement of hydroxyl radical and singlet-oxygen as reactive oxygen species, and complexes encourage binding to minor-groove. Further, the cytotoxicity of complex **4** on human hepatocellular liver carcinoma HepG2 cell line implies the cell death through apoptosis.

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Introduction

Hereditary material DNA is an important cellular receptor, many chemicals exert their antitumor effects by binding to DNA thereby changing the replication of DNA and inhibiting the growth of tumor cells, which is the basis of designing new and more effective antitumor agents, and their effectiveness depends on the binding mode and affinity [1,2]. Until the introduction of platinum-based chemotherapeutic drugs such as cisplatin and carboplatin, human heavily affected by different types of tumors. Initially, these drugs are widely and effectively applied in the treatment of various solid tumors, improving greatly the survival rates of patients. However, the dose-limiting, nephrotoxicity and the development of drug resistance prevents its potential efficacy [3,4]. The molecular mechanism of action of such DNA-targeting drugs involves covalent binding to nucleobase moieties, and low degree of selectivity [5]. The design and synthesis of new metal-based antitumor drugs that mimic the interaction mode of bio-molecules with enhanced selectivity and novel modes of DNA interaction like non-covalent interactions are the focus points of current research groups.

Next to ruthenium(II), copper(II) complexes have gained special attention as promising alternatives for antitumor agent [6]. Copper(II) ion is known to play a significant role in bio-systems, and its complexes follow different mode of action towards DNA (non-covalent) as compared to cisplatin (covalent). Generally, antitumor agents approved for clinical use are molecules which damage DNA and block DNA synthesis indirectly through inhibition of nucleic acid precursor biosynthesis/disrupt hormonal stimulation of cell growth [7]. Therefore, considerable attention has been focused on the development of new antitumor drugs based on the biocompatible transition metal complexes, which bind to and cleave DNA under physiological conditions. Also, these complexes must recognize nucleic acids, particularly, in a sequence-specific fashion and then bind to them in a way that alters their function. Copper(II) complexes, which possess biologically accessible redox potentials and demonstrate high nucleobase affinity, are potential reagents for cleavage of DNA both oxidatively and hydrolytically [8,9]. The appropriate redox property of Cu(II) ion is essential for various metabolic pathways like mitochondrial respiration, free radical scavenging, and iron absorption where it acts as a catalytic co-factor [10]. Therefore, Cu(II) based generic complexes exhibit higher antineoplastic potency towards human carcinomas as compared to cisplatin. In general, redox-active agents that damage DNA *in vitro* are thought to exhibit apoptotic activities in live cells by inducing oxidative stress and/or DNA damage [11,12]. Thus, complexes appear to form intracellular redox-active species in tumor cells, which generate cytotoxic reactive oxygen species *in vivo* [13].

In this connection, the mixed-ligand complexes which show varied molecular structures, DNA structural selectivity and great DNA binding affinity have attracted special attention [14,15]. Much attention has been paid on DNA intercalation, while the other DNA binding modes such as groove binding, partial intercalation (also known as semi-intercalation) and insertion need more exploration for structure–function relationship [16]. From the literature evidence, the groove binders as with intercalators can also be used for clinical treatment of cancer, bacterial and fungal infections [17]. In general, compounds with antioxidant activity have been found to posses anticancer, anti-cardiovascular, anti-inflammatory and many other activities [18].

In view of all these observations, herein we report the synthesis, spectral characterization, phosphate-hydrolysis, antioxidant, DNA binding and cleavage activities of salen-diimine based mixed-ligand copper(II) complexes of N^1,N^2-bis(5-substituted-2-hydroxybenzylidene)-1,2-ethylene/phenylenediimine and diimine (2, 2′-bipyridyl (bpy) or 1,10-phenanthroline (phen)). The complex **4** has been tested for its anticancer activity on HepG2 cell line. The mode of cell death was studied using PI staining and comet assay.

Experimental

Materials

The N,N′-bis(salicylaldehyde)ethylenediimine (salen) type ligands, H_2L^{1-4}, (H_2L^1 – N^1,N^2-bis(5-methyl-2-hydroxybenzylidene) -1,2-ethylenediimine, H_2L^2 – N^1,N^2-bis(5-methyl-2-hydroxybenzylidene)-1,2-phenylenediimine, H_2L^3 – N^1,N^2-bis(5-bromo-2-hydroxy benzylidene)-1,2-ethylenediimine and H_2L^4 – N^1,N^2-bis(5-bromo-2-hydroxybenzylidene)-1,2-phenylenediimine) were prepared by [2+1] Schiff base condensation reaction [19]. Solvents used for spectroscopic studies were HPLC grade. Chemicals were commercial product and purified by standard procedure before use. The CT-DNA and supercoiled pBR322 DNA were purchased from Bangalore Genei (India). 5 mM Tris–HCl [Tris(hydroxymethyl)aminomethane hydrochloride]/50 mM NaCl buffer was prepared in deionized water in which the pH was adjusted (till pH 7.2) with HCl/NaOH solution.

***Caution*!** During handling of perchlorate salts of metal ion with organic ligands, care should be taken because of explosion.

Physical methods

CHN micro-analysis was performed using Carlo Erba model 1106 elemental analyzer. FT IR spectra were recorded on a JASCO FT/IR-4100 spectrophotometer using KBr pellets in the range 4000–400 cm^{-1}. Electronic spectra of complexes were recorded in HPLC grade DMF on Agilent-8453 spectrophotometer in the range of 200–900 nm at ambient temperature. Electrospray ionization (ESI) mass spectra were recorded on Q-Tof mass spectrometer. The powder X-ray diffraction data of complexes were collected on Rich Siefert 3000 diffractometer with graphite-monochromated Cu $K\alpha_1$ radiation (λ = 1.5406 Å). Redox properties of complexes were performed on CHI 602D (CH Instruments Co., USA) electrochemical analyzer. The measurements were carried out under N_2 atmosphere using a three-electrode cell in which a glassy carbon, saturated Ag/AgCl and platinum wire are used as the working, reference and auxiliary electrode, respectively. A ferrocene/ferrocenium (Fc/Fc^+) couple was used as an internal standard. Concentration of complexes was taken around 0.1 mM and supporting electrolyte (TBAP) was at 0.1 M. X-Band EPR spectra of complexes were recorded on Varian EPR-E 112 spectrometer at room temperature and polycrystalline DPPH (2,2′-diphenyl-1-picrylhydrazyl) with a '*g*' value of 2.0023 was used as a standard field marker. The room temperature magnetic moment was measured on a PAR (model-155) vibrating sample magnetometer.

General procedure for synthesis of mixed-ligand copper(II) complexes

To a methanolic solution of $Cu(ClO_4)_2{\cdot}6H_2O$ (1 mmol, 0.37 g), appropriate ligands, H_2L^{1-4} (1 mmol) in methanol:acetonitrile (4:1 v/v) solution and equimolar triethylamine was added with stirring, followed by bpy/phen (1 mmol) in methanol at room temperature. Then, the reaction was continued for 6 h at 80 °C. After cooling the reaction mixture to room temperature, the resulting solid product was collected by filtration, washed with cold methanol and fully dried in *vacuo*. Efforts taken to get single crystals in different conditions and solvents went unsuccessful.

[CuL^1(bpy)], ***1***

Yield: 0.41 g (79.7%). Color: Green. Anal. Calc. for $C_{28}H_{26}N_4O_2$-Cu: (FW: 514.1): C, 65.42; H, 5.10; N, 10.90%. Found: C, 65.39; H,