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Synthesis, spectroscopic and electrochemical studies of *N*,*N*-bis[(*E*)-2-thienylmethylidene]-1,8-naphthalenediamine and its Cu(II) complex: DNA cleavage and generation of superoxide anion

Mohammad Shakir [a,*], Mohammad Azam [a], M.F. Ullah [b,1], S.M. Hadi [b]

[a] Department of Chemistry, Aligarh Muslim University, Aligarh 202 002, India
[b] Department of Biochemistry, Aligarh Muslim University, Aligarh 202 002, India

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ABSTRACT

A novel tetradentate Cu(II) complex of the type, $[CuL](NO_3)_2$ was synthesized by the interaction of Schiff base ligand, *N*,*N*-bis[(*E*)-2-thienylmethylidene]-1,8-naphthalenediamine, L obtained by the condensation of thiophene-2-carboxaldehyde and 1,8-diaminonaphthalene. The formation of Schiff base ligand, L and its Cu(II) complex was confirmed on the basis of results of elemental analyses, mass, FT-IR, ^1H and $^{13}C\{^1H\}$ NMR spectral studies. UV–Vis, EPR and magnetic susceptibility data support a square planar environment around Cu(II) ion. However, molar conductance values confirmed 1:2 electrolytic nature for the Cu(II) complex. The electrochemical studies of Cu(II) complex was carried out by using cyclic voltammetry which revealed the complex to exhibit quasi reversible process. The biological activity of Cu(II) complex such as ability to bind DNA and DNA cleavage were studied where the Cu(II) complex was shown to cause considerable DNA cleavage and also generated reactive oxygen species such as superoxide anion. Since it is known that various anticancer drugs act through induction of oxidative stress that is mediated by reactive oxygen species, our results suggest a putative role of Cu(II) complex similar to various anticancer drugs.

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1. Introduction

Schiff bases (imines) constitute one of the most widely used families of organic compounds not only as synthetic intermediates but also in coordination chemistry [1]. They are studied extensively due to their synthetic flexibility, selectivity and sensitivity towards the central metal atom, structural similarities with natural biological substances and also due to presence of imine group (—CH=N—) which imports in elucidating the mechanism of transformation and resemination reaction in biological system [2]. Several studies have shown that the presence of a lone pair of electrons in sp2 hybridized orbital of nitrogen atom of the azomethine group is of considerable chemical and biological importance [2]. Different studies have also indicated a strong relationship between the metal ions and/or their complexes with the potential ligands as promising antitumor [3,4] and antibacterial agents [5]. The sulfur containing ligands are wide spread among coordination compounds and are important components of biological transition metal complexes [6]. It has been reported that activities, viz, antifungal, insecticidal, acaricidal, antibacterial, anticarcinogenic and tuberculostatic, etc. of sulfur containing ligand increases on complexation [7]. The study of the reactivity of Schiff bases complexes involving heteroaromatic moiety has received a great deal of attention during the past decades because of their interaction, specifically, with square planar complexes that efficiently bind and cleave DNA under physiological conditions and have wide applications in nucleic acid chemistry as foot printing and sequence specific binding agents, for modeling the restriction enzymes in genomic research, and as new structural probes in diagnostic medicinal applications for the treatment of cancer [6,8–11]. Numerous studies have demonstrated that DNA is the primary intracellular target of anticancer drugs due to the interaction between small molecules and DNA, which can cause DNA damage resulting in cell death [12,13]. In addition, some of these drugs are also known to enhance oxidative stress in cancer cells through generation of reactive oxygen species [14] and may lead to apoptosis [15]. Schiff bases have been shown as important anticancer drug candidates as they exhibit remarkable biological activities such as DNA damage, plasmid cleavage, protein cleavage and apoptosis [16–19]. Recently, redox active metal complexes have been shown to possess strong anticancer properties and have been used for DNA cleavage reactions in the presence of oxidants viz, O_2, H_2O_2

* Corresponding author. Tel.: +91 9837430035.
E-mail addresses: shakir078@yahoo.com (M. Shakir), mazamchem@gmail.com (M. Azam).
[1] Present address: Department of Pathobiology, University of Tennessee, 2407 River Drive, Knoxvilli, TN 37996, USA.

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or a peracid [20]. Literature supports that many chemically or electrochemically generated metal in high oxidation state can act as an oxidant in the presence of a reductant [21]. Copper(II) is a biologically active essential metal ion; its chelating ability and positive redox potential allows participation in number of biological processes [22,23] such as the role played by its complexes for cancer inhibition [24]. Copper complexes usually do not mediate nucleobase oxidation, but are responsible for direct strand scission by hydrogen atom abstraction from the deoxyribose moiety [21]. Sigman and coworkers have shown that *bis*-(1,10-phenanthroline)copper(I) complex in presence of H_2O_2 efficiently cleaves DNA [25]. The importance in DNA binding and cleavage by redox and photoactive metal complexes is primarily for examining the sequence specificities of DNA binding using a variety of intercalating ligands by 'footprinting' methods [26]. It has been demonstrated that the copper accumulates in tumors due to the selective permeability of cancer cell membranes to copper compounds [27]. Thus, a number of copper complexes have been screened for anticancer activity, and some of them were found to be active both *in vitro* and *in vivo* [28]. Literature supports that cupric ions have been shown to bind the DNA bases, adenine, guanine and cytosine at the N(7) of purines and N(1) of pyrimidines [29]. These ions can be reduced and then oxidized by dioxygen leading to hydroxyl radical production, close to the metal binding site, which can damage DNA in site specific reaction [29]. In continuation of our efforts in the preparation and characterization of Schiff base ligands, derived from heterocyclic aldehydes, and their metal complexes [30,31], Herein, We report the synthesis and spectroscopic characterization of Schiff base ligand, *N*,*N*-bis[(*E*)-2-thienylmethylidene]-1,8-naphthalenediamine, L and its Cu(II) complex of the type, $[CuL](NO_3)_2]$ obtained by the condensation reaction of 1,8-diaminonaphthalene and thiophene-2-carboxaldehyde and the biological activity of the synthesized ligand, L and its Cu(II) complex with a view to evaluate their pharmacological significance.

2. Experimental

2.1. Materials

All chemicals used were of analytical reagent grade (AR) and of highest purity available. They included thiophene-2-carboxaldehyde, 1,8-diaminonaphthalene, all metal salts $M(NO_3)_2{\cdot}6H_2O$ [M = Co(II), Ni(II) and Zn(II)], $Cu(NO_3)_2{\cdot}3H_2O$ and methanol as a solvent (All E. Merck). Plasmid pBR 322 (GE NEI, Bangalore) CT-DNA (Sigma, St. Louis, USA).

2.2. Physical measurements

Microanalyses (C, H, N) recorded on Perkin–Elmer 2400 C H N elemental analyzer, ^1H and ^{13}C NMR spectra recorded in $CDCl_3$ using Avance II 400 NMR spectrometer, ESI-mass spectra recorded on Q-ToF micro (Waters Company) were obtained from SAIF, Punjab University, Chandigarh (India). FT-IR spectra (4000–200 cm^{-1}) were recorded as a KBr/CsI disk on a Perkin–Elmer 621 spectrophotometer. Electronic spectrum in DMSO was recorded on a Pye-Unicam 8800 spectrophotometer. EPR spectrum of Cu(II) complex was recorded on a Varian-4 spectrometer (X-band) using diphenylpicrylhydrazide (DPPH) (g = 2.0036) as a calibrant, at IIT, Madras. The electrical conductivities of 10^{-3} M solution in DMSO were obtained on a Systronic type 302 conductivity bridge equilibrated at 25 ± 0.01 °C. Magnetic susceptibility measurements were carried out using Faraday balance at room temperature. Fluorescence measurements were made on Shimadzu spectrofluorimeter Model RF-1501. Electrochemical study (CV) was carried out on Metrohm 797 VA Coputrance.

2.3. Synthesis of ligand, N,N-bis[(E)-2-thienylmethylidene]-1,8-naphthalenediamine, L

A methanolic solution of thiophene-2-carboxaldehyde (2 mM, 0.185 ml) was added to the methanolic solution of 1,8-diaminonaphthalene (1 mM, 0.158 g) with constant stirring in a molar ratio of 2:1. The resultant reaction mixture was refluxed for 5 h leading to the formation of a golden yellow colored solution. The resulting solution was then kept for evaporation at room temperature for few days to give fine micro crystalline yellow product. The product was filtered, washed and dried in vacuo.

Yield: 53%, mp. 118 °C, Anal. found (%): C, 69.30; H, 4.02; N, 8.05; S, 18.43, Calc., C, 69.33; H, 4.07, N; 8.08; S, 18.50%, ^1H NMR ($CDCl_3$) δ (ppm), 9.91 ppm (—CH=N), ^{13}C NMR ($CDCl_3$): δ (ppm) 144.1 ppm (—CH=N).

2.4. Synthesis of $[CuL](NO_3)_2$: dinitrato(N,N-bis[(E)-2-thienylmethylidene]-1,8-naphthalenediamine) Cu(II)

One mM solution of hydrated metal(II) nitrate dissolved in 25 cm^3 methanol was added dropwise into a methanolic solution (20 cm^3) of ligand, L (1 mM) taken in a round bottom flask. The reaction mixture was stirred for an hr followed by refluxing for 4–5 h which resulted the isolation of colored solid product. The product thus formed was filtered, washed with methanol and vacuo dried.

Yield: 65%, mp. >300, Anal. found (%): C, 44.96, H, 2.60; N, 10.45; S, 11.95; Cu, 11.85 Cal., C, 44.98; H, 2.64; N, 10.49; S, 12.00, Cu, 11.90%.

2.5. The fluorescence studies of ethidium bromide bound to DNA in the presence of ligand and Cu(II) complex

Experiment was carried out at pH 7.0 in the buffer containing 50 mM NaCl and 5 mM Tris–HCl. DNA and ethidium bromide (EB) were dissolved in buffer at the concentrations of 3 and 1 μg/ml, respectively. The concentration of ligand and complex was 50 μM. EB displays very weak fluorescence in aqueous solution. However, in the presence of DNA, it exhibits intense fluorescence because of the intercalation to base pairs in DNA. Ligand and complex were added to EB bound with ct DNA and the intensity of fluorescence of EB was measured. Fluorescence spectra were recorded using excitation wavelength of 478 nm and the emission range set between 485 and 685 nm. Before examining the fluorescent properties of EB, it was checked that the tested compounds did not quench the EB fluorescence (Fig. 1).

2.6. Reaction with plasmid pBR322 DNA

Reaction mixtures (30 μl) contained 10 mM Tris–HCl, pH 7.5, 0.5 μg plasmid DNA and other components (Fig. 2). Incubation was carried out at 37 °C for 2 h. After the incubation, 10 μl of a solution containing 40 mM EDTA, 0.05% bromo phenol blue tracking dye and 50% (v/v) glycerol was added and the mixture was subjected to electrophoresis on 1% agarose gel. The gel was stained with ethidium bromide (0.5 mg/ml), viewed and photographed on a UV transilluminator.

2.7. Reaction of ligand and complex with calf thymus DNA and digestion with S_1 nuclease

Reaction mixtures (0.5 ml) containing 10 mM Tris–HCl (pH 7.5), 500 μg DNA and various concentrations of ligands/Cu(II) complex

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