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Studies on DNA binding behaviour of biologically active transition metal complexes of new tetradentate N_2O_2 donor Schiff bases: Inhibitory activity against bacteria

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ABSTRACT

A series of Cu(II), Ni(II) and Zn(II) complexes of the type ML have been synthesized with Schiff bases derived from *o*-acetoacetotoluidide, 2-hydroxybenzaldehyde and *o*-phenylenediamine/1,4-diaminobutane. The complexes are insoluble in common organic solvents but soluble in DMF and DMSO. The measured molar conductance values in DMSO indicate that the complexes are non-electrolytic in nature. All the six metal complexes have been fully characterized with the help of elemental analyses, molecular weights, molar conductance values, magnetic moments and spectroscopic data. The analytical data helped to elucidate the structure of the metal complexes. The Schiff bases are found to act as tetradentate ligands using N_2O_2 donor set of atoms leading to a square-planar geometry for the complexes around all the metal ions. The binding properties of metal complexes with DNA were investigated by absorption spectra, viscosity measurements and cyclic voltammetry. Detailed analysis reveals that the metal complexes intercalate into the DNA base stack as intercalators. All the metal complexes cleave the pUC19 DNA in presence of H_2O_2. The Schiff bases and their complexes have been screened for their antibacterial activity against five bacterial strains (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*) by disk diffusion method. All the metal complexes have potent biocidal activity than the free ligands.

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1. Introduction

Transition metal complexes of N-donor ligands such as Schiff bases have inspired researchers due to their potent biological activities including antifungal, antibacterial, anticancer and herbicidal applications. Errors in gene expression can often cause disease and play a secondary role in the outcome and severity of human disease. Medicinal agents can affect gene expression by facilitating, mimicking or inhibiting any one of the properties that exists in typical transcriptional systems [1]. A large number of evidences indicate the mechanism of action of anticancer agents binds through distinctive binding modes to the DNA of cancer infected cell in such a way, that the cell cannot replicate further. This inhibition of replication finally leads to the death of the infected cell.

The binding modes are responsible for activity and the mechanism by which DNA replication is totally inhibited in cancer cells [2–5]. It is well known that DNA is the pharmacological target of cis-platin, the platinum atom forming covalent bonds to N7 positions of the adjacent purine bases, which conduces its cytotoxic activity [6,7]. Clinical problems of cis-platin include, acquired cisplatin resistance and limited spectrum of cancers that it can cure [8]. To overcome these limitations of cis-platin and to develop another class of metallodrugs which are less toxic and more effective for chemotherapeutic applications, researchers have been continuously investigating the interaction of other metal complexes with DNA.

The outstanding criteria for the development of metallodrugs as chemotherapeutic agents are the ability of the metallodrug to provoke DNA cleavage. A large number of transition metal complexes because of their redox properties, have been found to enhance DNA cleavage. A huge number of transition metal complexes have been shown to promote oxidative DNA cleavage in the presence of co-reagents [9]. The pharmacological efficacy of metal complexes depends on the nature of the metal ions and the ligands. It is declared in the literature that synthesized from same ligands with different metal ions possess different biological properties [10]. In the present research article we wish to report the synthesis, structure, anti-biogram and DNA binding and cleavage studies of transition metal complexes having *o*-acetoacetotoluidide derived Schiff base and its transition metal(II) complexes. The results should be beneficial in designing novel agents for targeting DNA as well as setting the stage for the synthesis of chemical antibacterial drugs.

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2. Experimental protocols

2.1. Reagents and instruments

All reagents, *o*-acetoacetotoluidide, 2-hydroxybenzaldehyde, *o*-phenylenediamine, 1,4-diaminobutane and metal(II) chlorides were of Merck products and used as supplied. Commercial solvents were distilled and then used for the preparation of ligands and their complexes. DNA was purchased from Bangalore Genei (India). Microanalyses (C, H and N) were performed in Carlo Erba 1108 analyzer at Sophisticated Analytical Instrument Facility (SAIF), Central Drug Research Institute (CDRI), Lucknow, India. Molar conductivities in DMSO (10^{-3} M) at room temperature were measured using Systronic model-304 digital conductivity meter. Magnetic susceptibility measurements of the complexes were carried out by Gouy balance using copper sulphate pentahydrate as the calibrant. The Infra-red spectra of the ligands and their complexes were obtained as KBr discs in the range 350–4500 cm^{-1} on a FT IR-8400S instrument recorded at University Science Instrumentation Centre (USIC), Madurai Kamaraj University, Madurai. NMR spectra were recorded on a Bruker Avance Dry 300 FT-NMR spectrometer in DMSO-d_6, using TMS as the internal reference. FAB-MS spectra were recorded with a VGZAB-HS spectrometer at room temperature in a 3-nitrobenzylalcohol matrix. EPR spectra were recorded on a Varian E 112 EPR spectrometer in DMSO solution both at room temperature (300 K) and liquid nitrogen temperature (77 K) using TCNE (tetracyanoethylene) as the g-marker. The absorption spectra were recorded using Shimadzu model UV-1601 spectrophotometer at room temperature.

2.2. Synthesis of 2-(2-aminophenylimino)methylphenol (L^1)/2-(4-aminobutylimino)methylphenol (L^2)

This monocondensation product was synthesized according to the literature procedure [11]. To the vigorously stirred solution of *o*-phenylenediamine/1,4-diaminobutane (10 mmol) was added dropwise a solution of appropriate 2-hydroxybenzaldehyde (10 mmol) in ethanol. After the addition was complete, the reaction mixture was heated for 1 h with constant stirring. The resulting solid product formed was filtered, washed, dried and recrystallized from ethanol.

2.2.1. Synthesis of Schiff base ligands H_2L^1/H_2L^2

o-Acetoacetotoluidide (10 mmol) was dissolved in ethanol (40 mL) and added to an ethanolic solution of 2-(2-aminophenylimino)methylphenol (L^1)/2-(4-aminobutylimino) methylphenol (L^2) (10 mmol) dissolved in hot ethanol (10 mL). The resulting mixture was refluxed for 1 h on a water-bath. On cooling, the yellowish orange/orange colored compound formed was filtered, washed, dried and recrystallized from ethanol. Schematic route for the synthesis of Schiff base ligands and their metal complexes is given in Scheme 1.

2.2.2. Synthesis of metal complexes

A solution of metal(II) chloride in ethanol (2 mmol) was refluxed with a hot ethanolic solution of the Schiff base (2 mmol), for *ca*. 3 h. Then the solution was reduced to one-third on a water bath. The solid complex precipitated was filtered off and washed thoroughly with ethanol and dried in *vacuo*.

2.3. DNA binding experiments

The interaction between metal complexes and DNA was studied using electronic absorption, viscosity and electrochemical methods. Disodium salt of calf thymus DNA was stored at 4 °C. Solution of DNA in the buffer 50 mM NaCl, 5 mM Tris–HCl (pH 7.2) in water gave a ratio 1.9:1 of UV absorbance at 260 and 280 nm, A_{260}/A_{280}, indicating that the DNA was sufficiently free from protein [12]. The concentration of DNA was measured using its extinction coefficient at 260 nm (6600 M^{-1} cm^{-1}) after 1:100 dilutions. Stock solutions were stored at 4 °C and used not more than 4 days. Doubly distilled water was used to prepare solutions. Concentrated stock solutions of the complexes were prepared by dissolving the complexes in DMSO and diluting suitably with the corresponding buffer to the required concentration for all the experiments. Absorption titration experiments were carried out by varying the DNA concentration and maintaining the complex concentration constant. Absorbance values were recorded after each successive addition of DNA solution and equilibration (*ca*. 10 min). The absorption data were analyzed for an evaluation of the intrinsic binding constant K_b using reported procedure [13].

Electrochemical studies were carried out using CHI Electrochemical analyzer, controlled by CHI620C software. CV measurements were performed using a glassy carbon working electrode and an Ag/AgCl reference electrode and supporting electrolyte was 50 mM NaCl, 5 mM Tris buffer (pH 7.2). All solutions were deoxygenated by purging with N_2 for 30 min prior to measurements.

Viscosity measurements at room temperature were carried out using a semi-micro dilution capillary viscometer. Each experiment was performed three times and an average flow time was calculated. Data were presented as (η/η_0) versus binding ratio, where η is the viscosity of DNA in presence of complex and η_0 is the viscosity of DNA alone.

2.4. Methodology for pUC19 DNA cleavage study

The cleavage of pUC19 DNA was determined by agarose gel electrophoresis. The gel-electrophoresis experiments were performed by incubation of the samples containing 30 μM pUC19 DNA, 50 μM metal complex and 50 μM hydrogen peroxide (H_2O_2) in Tris–HCl/NaCl buffer (pH 7.2) at 37 °C for 2 h. After incubation, the samples were electrophoresed for 2 h at 50 V on 1% agarose gel using Tris–acetic acid–EDTA buffer (pH 7.2). The gel was then stained using 1 μg cm^{-3} ethidium bromide (EB) and photographed under ultraviolet light at 360 nm. All the experiments were performed at room temperature unless otherwise stated.

2.5. Antimicrobial studies

Antibacterial activity of the Schiff base ligands and their metal complexes were tested *in vitro* against the bacterial species *viz. Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae* by the paper disk method using nutrient agar as the medium. Ampicillin was used as the standard antibacterial agent. The test organisms were grown on nutrient agar medium in petri plates. Disks were prepared and applied over the long culture. The compounds were prepared in DMSO and soaked in filter paper disk of 5 mm diameter and 1 mm thickness. The concentrations of ligand and the complexes used in this study were 0.01 μg/mL. The disks were placed on the previously seeded plates and incubated at 37 °C and the diameter of inhibition zone around each disk was measured after 24 h for antibacterial activity. Growth inhibition was calculated according to Ref. [14].

3. Results and discussion

The Schiff base ligands H_2L^1/H_2L^2 and their Cu(II), Ni(II) and Zn(II) complexes were synthesized and characterized by spectral and elemental analysis data. The complexes are found to be air

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