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Research paper

Mononuclear copper(II) and binuclear cobalt(II) complexes with halides and tetradentate nitrogen coordinate ligand: Synthesis, structures and bioactivities

Mehul H. Sadhu [a], Sujit Baran Kumar [a,*], Jaswinder Kaur Saini [b], Sejal S. Purani [c], Tanvi R. Khanna [c]

[a] Department of Chemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara 390002, India
[b] Department of Microbiology and Biotechnology Centre, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara 390002, India
[c] Department of Biochemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara 390002, India

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ABSTRACT

In this work, we present the synthesis of four mononuclear copper(II) complexes [Cu(bdpab)X]Y and two binuclear cobalt(II) complexes $[Co(bdpab)Cl]_2Y_2$ (X = Cl^-, Br^-; Y = PF_6^-, BF_4^-) with tripodal N_4-coordinate *N*-benzyl-*N′*,*N′*-bis(3,5-dimethyl-1*H*- pyrazol-1-yl)-2-methy]-1,2-ethylenediamine (bdpab) and the complexes were characterized by elemental analyses, IR spectral data, molar conductivity measurement, EPR and crystal structure determination. Single crystal X-ray diffraction studies indicate that the copper centers in the complexes $[Cu(bdpab)Cl]PF_6$ and $[Cu(bdpab)Br]PF_6$ have distorted square pyramidal geometry and complex $[Co(bdpab)Cl]_2(PF_6)_2$ has octahedral geometry and two $[Co(bdpab)Cl]^+$ units are linked by two (μ-Cl) bridges. Copper(II) complexes form 1D supramolecular chain along c-axis through C-H-π interaction whereas cobalt(II) complex form 1D supramolecular chain along a-axis through π-π interaction. The antimicrobial activity of complexes $[Cu(bdpab)Cl]PF_6$, $[Cu(bdpab)Br]PF_6$ and $[Co(bdpab)Cl]_2(PF_6)_2$ were investigated against gram positive (*Bacillus subtilus*, *Streptococcus aureus*) and gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacterial strains by agar well dilution method and have demonstrated significant antimicrobial activity of the compounds. $[Co(bdpab)Cl]_2(PF_6)_2$ exhibited the best antibacterial activity among all the synthesized complexes. The studies on the interaction of complexes and DNA by agarose gel electrophoresis method revealed that the complexes can effectively cleave the circular plasmid DNA at very low concentrations. The cytotoxic activity of the complexes against A549 lung cancer cells showed that the complexes have better cytotoxic activity than corresponding metal salts and $[Cu(bdpab)Br]PF_6$ complex has best cytotoxic activity among the synthesized complexes.

1. Introduction

Since the discovery of cis-platin as an anti-cancer agent, design and synthesis of new transition metal compounds and their interaction with DNA have been an important area of research in bioinorganic chemistry for the development of new material as the therapeutic agent [1–4]. It is well known that gene has important role in the biological process and the study of the interaction of DNA with transition metal complexes is important for the development of less toxic, target specific and less side effect of the transition metal containing metallodrug [5–7]. Transition metal complexes are used as bioactive complex because they have different binding ability, coordination numbers and oxidation states etc. Among the transition metal complexes investigated as therapeutic agent, majority are from copper as it is an essential element for the biological system and has low toxicity [8–16]. Since cobalt is also important elements in the bio system, the interaction of cobalt with DNA has been attracted recently [17,18]. Since the DNA and transition metal interaction depends on the donor atom of the ligand, nitrogen coordinating ligands such as poly-pyridine are used mostly for the synthesis of bioactive transition metal complexes [19,20]. Many copper(II) and cobalt(II) complexes with tripodal ligands have been investigated for their anticancer properties [21–23]. There are few reports on the bioactivities on the model complexes of bleomycin with imidazole, pyrimidinyl amino and amino donor groups of ligands [24–26], Since there are some similarities between the metal binding of model complexes and metal complexes with tripodal pyrazole-based N_4-tetradentate ligands, we are interested to study the bioactivities of the copper(II) and cobalt(II) complexes with this pyrazolyl containing ligand.

* Corresponding author.
E-mail address: sujit_baran@yahoo.com (S.B. Kumar).

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In this paper we report on the synthesis, characterization, structures and bioactivities of mononuclear copper(II) complexes [Cu(bdpad)X]Y and binuclear cobalt(II) complexes $[Co(bdpab)Cl]_2(Y)_2$ (X = Cl or Br and Y = BF_4 or PF_6). Crystal structures of three complexes $[Cu(bdpab)Cl]PF_6$, $[Cu(bdpab)Br]PF_6$ and $[Co(bdpab)Cl]_2(PF_6)_2$ have been solved by single crystal X-ray diffraction method. Antimicrobial activity, cytotoxicity and DNA cleavage study of the complexes have been investigated in detail.

2. Experimental

2.1. Materials

All chemicals and solvents were analytical grade reagents and purchased from commercial sources. Acetylacetone, paraformaldehyde, hydrazine hydrate, $CuCl_2{\cdot}2H_2O$, $CuBr_2{\cdot}4H_2O$ (Loba, India), $CoCl_2{\cdot}6H_2O$ (Siscochem, India), NH_4BF_4 and NH_4PF_6 (Aldrich) were reagent grade and used as received. *N*-(3,5-dimethyl-1H-pyrazole-1-yl)methanol [27] *N*-benzyl-*N′*,*N′*-bis(3, 5-dimethyl-1*H*- pyrazol-1-yl)-2-methy]-1,2-ethylenediamine (bdpab) were synthesized as per the published procedures [28].

2.2. Cell line and culture

Human lung carcinoma (A549) cells were procured from National Centre for Cell Science, Pune, India. The cell cultures were grown in Dulbecco's modified Eagle's medium (DMEM, Himedia) supplemented with 10% fetal bovine serum (Gibco-Invitrogen) and 1% antibiotic (Gibco-Invitrogen). Cell lines were maintained at 37 °C in a 5% (v/v) CO_2 atmosphere with 95% (v/v) humidity in a humidified incubator (Thermo Scientific). Cultures were passaged weekly using trypsin–EDTA (Himedia) to detach the cells from their culture flasks. The copper(II) and cobalt(II) salts and their complexes were dissolved in DMSO and diluted to the required concentration with culture medium. The DMSO content in the final concentrations did not exceed 0.1%.

2.3. Antimicrobial activity assay

The antimicrobial activity of the complexes were determine using various concentrations of Cu(II) and the complexes were prepared in distilled water with not more than 0.1% DMSO to assist dissolution. The screening was carried out using Gram positive (*Bacillus subtilis, Streptococcus aureus*) and Gram negative (*Escherichia Coli*, *Pseudomonas aeruginosa*) bacterial strains by agar well diffusion method. All the compounds were tested in duplicates. The Luria Bertani (LB) agar plates with 4 mm thickness were spread with 100 μl of overnight cultures. The test compounds at different concentrations were added to the wells (5 mm diameter) made in the agar plates. DMSO at the concentration of 0.1% was used as a negative control and 1 mg/mL of chloramphenicol was used as a positive control for the assay. The plates were incubated at 37 °C for 24 h. The plates were checked for zones of inhibition after incubation.

2.4. Cell proliferation assay (MTT)

Cell proliferation assay was performed [29] to check the in vitro anticancer activities of the two copper(II) and cobalt(II) salts and their complexes against A549 lung cancer cell line. The cells were seeded in 96-well microplates at a concentration of 5×10^4 cells/well. In the 24th hour, the fresh media modified with different concentrations of the test compounds was added. Each concentration was applied in triplicates. Untreated A549 cells grown in non-modified medium served as control. After 24 h incubation, the solutions were removed from the plates and (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) (5 mg/mL DMEM) solution was added followed by 3 h incubation. Medium with MTT was flicked off and the formazan crystals were solubilized in 100 μl DMSO. The absorbance of each well was measured at 540/620 nm by ELISA microplate reader (Thermo Scientific). Relative cell viability, expressed as a percentage of the untreated control (100% viability), was calculated for each concentration. Concentration-response curves were constructed for each experiment. The data are presented as means ± standard error of the mean. The IC_{50} values were calculated from the curves constructed by plotting cell survival (%) vs the complexes concentration (μg/mL). Statistical differences between control and treated cells at different concentrations were assessed by 2-way anova test in Graph-Pad Prism 5.0 software package.

2.5. Cleavage experiment

The bacterial plasmid pBS KS (+) was isolated from *Escherichia coli* strain by alkaline lysis method [30]. The ratio 260 to 280 nm (A260/A280) absorbance was checked to be ~1.86, which indicated that the DNA is sufficiently free from protein [31]. The concentration of DNA was determined spectrophotometrically (UV 1601 UV–Visible spectrophotometer, SHIMADZU) at 260 nm using the known molar extinction coefficient value of 6700 M^{-1} cm^{-1} [32]. The DNA was stored at −20 °C until used. Visualization of DNA under UV was done using Alpha imager HP System, Alpha innotech.

The Cu(II) complexes **1**, **3** and **5** were examined for their ability to cleave DNA. For this 3 μM PBS KS(+) plasmid DNA was treated with Cu(II) complexes at various concentration ranging from 10 μM-50 μM along with the addition of 5 μM hydrogen peroxide. A plasmid with volume made up with sterile distilled water was kept as an untreated control. A reaction with 5 μM H_2O_2 alone and with 50 μM of the complexes alone were also kept to check their individual effect on the plasmid. After overnight incubation at 37 °C, 5 μl from each reaction was loaded using bromophenol blue (0.25%) and glycerol (30%) loading dye onto 0.8% agarose gel containing ethidium bromide (final 0.5 μg/ml). The gel was observed under UV trans illuminator at 360 nm.

2.6. Syntheses

2.6.1. Synthesis of complex $[Cu(bdpab)Cl]PF_6$ (**1**)

A solution of ligand bdpab (0.176 g, 0.5 mmol) in methanol (10 ml) was added drop by drop to a stirred light green solution of $CuCl_2{\cdot}2H_2O$ (0.085 g, 0.5 mmol) in the same solvent (10 ml) and the colour changed to dark green immediately. After 10 min, NH_4PF_6 (0.082 g, 0.5 mmol) in methanol (10 ml) was added drop by drop and resulting green color solution was stirred for another 3 h at room temperature, filtered it and kept the filtrate for slow evaporation at room temperature. Light green color single crystals were obtained after 5 days.

Yield. 0.110 g (74%). Found C = 40.78, H = 4.88, N = 14.21%. Anal calc for $C_{20}H_{28}ClCuF_6N_6P$: C = 40.27, H = 4.73, N = 14.09%. IR (KBr pellet) cm^{-1}; *ν*(-NH), 3329 vs; *ν*(C═C)/ph ring, 1600 s; *ν*(C═C) + *ν*(C═N)/pz ring 1551 s, 1474 s; *ν*(PF_6^-), 848 s. UV–Vis spectra: λ_{max}/nm (ε_{max}/$mol^{-1}cm^{-1}$). 682(171), 279(4568), 226(18067). Λ_M ($\Omega^{-1}cm^2$ mol^{-1}) = 120. μ_{eff} = 1.76 BM.

2.6.2. Synthesis of complex $[Cu(bdpab)Cl]BF_4$ (**2**)

This complex was prepared by following the same procedure as that of complex **1** except NH_4BF_4 was used in place of NH_4PF_6.

Yield. 0.105 g (77%). Found C = 44.77, H = 5.32, N = 15.78%, Anal calc for $C_{20}H_{28}ClCuF_4N_6B$: C = 44.63, H = 5.24, N = 15.61%. IR (KBr pellet) cm^{-1}; *ν*(-NH), 3132 vs; *ν*(C═C)/ph ring, 1600 s; *ν*(C═C) +