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Synthesis and mechanistic insight of glycosylated Cu^{II}/Ni^{II}–Sn_2^{IV} heterobimetallic DNA binding agents: Validation of a specific Cu^{II}–Sn_2^{IV} chemotherapeutic agent for human leukemic cell line K-562

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ABSTRACT

Carbohydrate-conjugated heterobimetallic Cu^{II}/Ni^{II}–Sn_2^{IV} chemical entities **1**–**4** were designed, synthesized and characterized by elemental analysis and spectroscopic methods *viz.*, UV–vis, IR, ESI–mass and NMR (^{1}H, ^{13}C, and ^{119}Sn; in case of Ni^{II}–Sn_2^{IV} analogs **2** and **4**) and EPR (in case of Cu^{II}–Sn_2^{IV} analogs **1** and **3**) to act as specific antitumor chemotherapeutics. Interaction of **1** and **3** with CT DNA was carried out by optical methods to ascertain the mode of binding, mechanism of action and their therapeutic potential. **1** and **3** bind to DNA *via* electrostatic mode involving phosphate oxygen of DNA-sugar backbone. The intrinsic binding constant, K_b values of **1** and **3**, were calculated and found to be 2.76×10^4 and 3.84×10^4 M^{-1}, respectively which implicates greater binding propensity of diphenyltin analog **3**. Furthermore, a mechanistic insight with 5′-GMP was envisaged for **1** and **3**. The cleavage activity of **3** was investigated with supercoiled pBR322 DNA by gel electrophoresis which demonstrated efficient cleavage activity *via* an oxidative pathway. The molecular docking technique was also utilized to ascertain the mechanism and mode of action of diphenyltin analog **3**. The *in vitro* antitumor activity of **3** was evaluated by SRB assay against a panel of human cancer cell lines which clearly revealed good cytotoxic activity against K-562 human leukemic cells with GI_{50} values <10 μg/ml.

1. Introduction

Cancer is a deadly menace which is the main cause of man's mortality and morbidity. A recent survey by WHO reports 12 million new cases of cancer every year and ~27 million new cancer cases and 11.5 million deaths are expected in the next two decades [1]. It is generally assumed that cancers are derived from numerous tissues with multiple etiologies and endless combination of genetic and/or epigenetic alterations, therapies for cancers must be as diverse as the disease itself. Studying cancer treatments at the molecular basis holds promise for developing more effective cancer therapy strategies. In past few years, there has been an exponential growth in the area of medicinal inorganic chemistry due to significant achievements both in cancer diagnostics and therapeutics. The most considerable challenge facing effective cancer therapy is systemic toxicity of chemotherapeutic drugs, resistance, their lack of tumor localization and an even distribution throughout the body including tumor tissues.

A rational drug design is required to achieve specific targeting features and to control toxicity, by controlling thermodynamic and kinetic processes of metal complexes *viz.*, choice of metal ion and its oxidation state [2] or tailoring of ligand scaffold. The cytotoxicity of drug candidate is also determined to some extent by lipophilicity *i.e.*, the most lipophilic compounds are most cytotoxic [3]. Ligands possessing biologically active pharmacophore and biocompatible properties are tethered to other ligands having multifunctional groups with N and O donor metal binding sites to obtain modulated ligand scaffold which can mute the potential toxicity of metallodrugs [4]. In view of the above criteria for effective drug design, metal complexes of carbohydrates have been investigated due to their –OH rich periphery, good coordinating ability, homochirality, stereospecificity, weak immunogenicity and low toxicity [5,6]. Apart from biological area, metal carbohydrate interactions are also important in metal-catalyzed enantioselective synthesis, as carbohydrates represent enantiomerically pure compounds isolated from the chiral pool [7,8].

Previous literature reveals the condensation of aldehydes with D-glucosamine yields Schiff base sugar-imines [9]. In the recent

Abbreviations: CT DNA, calf thymus DNA; EB, ethidium bromide; En, ethylenediamine; GlcN, glucosamine; UV–vis, UV–visible.

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past, substituted benzaldehyde of vanillin series was condensed with D-glucosamine hydrochloride; however its biological implication was scarcely unexplored [10]. 2-Hydroxy-3-methoxybenzaldehyde (o-vanillin) tethered to a bioactive pharmacophore such as D-glucosamine; which is potentially capable of targeting cancer cells by exploiting the increased glucose uptake resulting from over expression of glucose transporters and glycolytic enzymes [11,12] and therefore could prove as a cancer chemotherapeutic drug entity. The complexes of carbohydrate framework show improved efficacy, good cytotoxicity with specific selectivity for tumor cells as compared to healthy cells and better cellular uptake than cisplatin [13].

Copper being a physiologically essential transition metal ion, plays an important role in the endogenous oxidative DNA damage associated with aging and cancer. However, tin a non-transition element possesses a hard Lewis acid nature bring conformational changes in DNA. Organotin(IV) complexes have displayed remarkable *in vitro* and *in vivo* antiproliferative properties, owing to which they play a crucial role in cancer oncology [14,15]. Further, organotin(IV) complexes with Schiff base ligands have received considerable attention owing to their fascinating chemical behavior and potential applications in biotechnology [16]. There has been many-fold enhancement in DNA binding profile and chemotherapeutic action of copper complexes by the incorporation of another metallic center, particularly organotin(IV) scaffold which bears testimony as antitumor and apoptosis director. Currently, serious toxicity and poor antitumor efficacy resulting from the induction of drug resistance in hematological malignancies and other tumors are common hindrances to successful systemic chemotherapy [17]. Chronic myelogenous leukemia accounts for 15% of adult leukemias. Although antitumor agent adriamycin has high efficacy against chronic myelogenous leukemia, resistance to adriamycin is a major clinical problem and an important cause for treatment failure [18].

Herein, we report the synthesis and characterization of Cu^{II}/Ni^{II}–Sn_2^{IV} (**1**–**4**) heterobimetallic chemical entities derived from D-glucosamine ligand. Further, preliminary *in vitro* DNA binding studies of Cu^{II}–Sn_2^{IV} (**1** and **3**) analogs and the cleavage activity of diphenyltin analog **3** supports its potential use as cancer chemotherapeutic agent particularly selective for leukemia.

2. Experimental section

2.1. Materials and measurements

Reagent grade chemicals were used without further purification for all syntheses and experiments. Ethylenediamine (Loba Chem.), 2-hydroxy-3-methoxybenzaldehyde (o-vanillin), D-glucosamine hydrochloride, dimethyltin(IV) dichloride, diphenyltin(IV) dichloride, tris(hydroxymethyl)aminomethane or tris buffer, NaN_3, DMSO, SOD, methyl green, DAPI (Sigma–Aldrich), copper(II) chloride dihydrate, nickel(II) chloride hexahydrate (E. Merck), 6× loading dye (Fermentas Life Science), 5′-GMP (Fluka) and supercoiled plasmid pBR322 DNA (Genei) were utilized as received. Disodium salt of CT DNA purchased from Sigma Chem. Co. and was stored at 4 °C.

The ^{1}H, ^{13}C and ^{119}Sn NMR spectra were obtained on a Bruker DRX-400 spectrometer operating at 400, 100 and 150 MHz, respectively. Infrared spectra were recorded on Interspec 2020 FTIR spectrometer in KBr pellets from 400 to 4000 cm^{-1}. Electrospray mass spectra were recorded on Micromass Quattro II triple quadrupol mass spectrometer. Microanalyses (C, H and N) were performed on an Elementar Vario EL III. EPR spectra of copper complexes were recorded on Varian E 112 spectrometer at the X-band frequency (9.1 GHz). Electronic spectra were recorded on a UV-1700 PharmaSpec UV–visible Spectrophotometer (Shimadzu). Fluorescence measurements were made on Shimadzu RF-5301PC Spectrofluorophotometer. Viscosity measurements were carried out from observed flow time of CT DNA containing solution ($t > 100$ s) corrected for the flow time of buffer alone (t_0), using Ostwald's viscometer at 25 ± 0.01 °C. Flow time was measured with a digital stopwatch. Molar conductance was measured at room temperature on Eutech con 510 electronic conductivity bridge.

2.2. DNA binding and cleavage experiments

DNA binding experiments which include absorption spectral titrations, fluorescence titrations and viscosity measurements conformed to the standard methods and practices previously adopted by our laboratory [19–22]. DNA cleavage experiment has been performed by the standard protocol as described in [23].

2.3. Molecular docking

The rigid molecular docking studies were performed by using HEX 6.3 software [24], which is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein, enzymes and DNA molecule. The structure of the complex was sketched by CHEMSKETCH (http://www.acdlabs.com) and converted to pdb format from mol format by OPENBABEL (http://www.vcclab.org/lab/babel/). The crystal structure of the B-DNA dodecamer $d(CGCGAATTCGCG)_2$ (PDB ID: 1BNA) was downloaded from the protein data bank (http://www.rcsb.org./pdb). Visualization of the docked pose has been done by using CHIMERA (www.cgl.ucsf.edu/chimera) and PyMol (http://pymol.sourceforget.net/) molecular graphics program.

2.4. In vitro antitumor studies

The cell lines used for *in vitro* antitumor activity were A2780 (ovary), Hop62 (lung), U373MG (CNS), PC3 (prostrate), MCF7 (human breast), K-562 (leukemia) and HCT15 (colon). These human malignant cell lines were procured and grown in RPMI-1640 medium supplemented with 10% fetal bovine serum and antibiotics to study growth pattern of these cells. The proliferation of the cells upon treatment with chemotherapy was determined using the sulforhodamine B (SRB) assay. Cells were seeded in 96 well plates at an appropriate cell density to give optical density in the linear range (from 0.5 to 1.8) and were incubated at 37 °C in CO_2 incubator for 24 h. Stock solutions of the complexes were prepared as 100 mg/ml in DMSO, and four dilutions, that is 10, 20, 40, and 80 ml, in triplicates were tested; each well receiving 90 ml of cell suspension and 10 ml of the drug solution. Appropriate positive control (Adriamycin) and vehicle controls were also run. The plates with cells were incubated in CO_2 incubator with 5% CO_2 for 24 h followed by drug addition. The plates were incubated further for 48 h. Termination of experiment was carried out by gently layering the cells with 50 ml of chilled 30% TCA (in case of adherent Q18 cells) and 50% TCA (in case of suspension cell lines) for cell fixation and kept at 4 °C for 1 h. Plates were stained with 50 ml of 0.4% SRB for 20 min. All experiments were made in triplicate.

2.5. Syntheses

2.5.1. Synthesis of ligand, *L*

The N-glycoside ligand, **L** was synthesized according to the procedure reported earlier [25] by the condensation of D-glucosamine hydrochloride (2.15 g, 10 mmol) with o-vanillin (1.52 g, 10 mmol) in 1:1 stoichiometric ratio in MeOH under reflux for 4–5 h.