Inorganica Chimica Acta 479 (2018) 229–239

Contents lists available at ScienceDirect

Inorganica Chimica Acta

journal homepage: www.elsevier.com/locate/ica

Research paper

Cu^{II}-Na^{I} heteronuclear complex as anticancer entity against human breast cancer cell lines: DNA binding, cleavage, and Computational studies

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ARTICLE INFO

Article history:
Received 15 January 2018
Received in revised form 8 April 2018
Accepted 18 April 2018
Available online 19 April 2018

Keywords:
Cu^{II}-Na^{I} heterobimetallic complex
Crystal structure
DFT
Hirshfeld surface analyses
DNA cleavage activity
Cytotoxicity

ABSTRACT

Herein, we report the synthesis and structural investigation of Cu^{II}-Na^{I} heterobimetallic complex **1**, which comprises of the compartmental Schiff-base ligand (**H_2L**) derived from DACH (1,2-Diaminocyclohexane) and o-vanillin. B3LYP/TZVP DFT calculation was performed to get a deeper insight of the ground state electronic structure, and quantitative analysis of non-covalent interactions was carried out using Hirshfeld surface analysis to explore H-bonding, C-H···π, Cu···C-H and Cu···H-C interactions. Furthermore, *in vitro* DNA binding studies with Complex **1** demonstrated the electrostatic mode of interaction at the major groove of the DNA. Complex **1** showed the oxidative damage of pBR322 DNA via ROS generation. Additionally, *in vitro* cytotoxicity and genotoxicity of complex **1** were investigated on human breast cancer cells (MCF-7), revealed concentration-dependent cell viability at micromolar concentration level. Flow cytometric analysis confirmed the cytotoxic potential of complex **1** as the percentage of apoptotic cells were increased in the treatment group. Genotoxicity was evident in the induction of micronucleus and DNA fragmentation.

1. Introduction

Cancer is one of the leading causes of morbidity and mortality, with approximately 14 million new cases in 2012 and 8.8 million cancer deaths globally in 2015 [1]. Altogether, nearly 1 in 6 deaths is due to cancer. According to agencies, 21.7 million new cancer cases can increase the global burden, and about 13 million cancer deaths are expected by 2030 [2]. Therefore, a major challenge for chemists is to develop new anticancer drugs with reduced toxicity and superior chemical and pharmacological properties (*viz.,* solubility, cellular uptake, kinetically stable and metabolic clearance) to increase the survival rates of patients [3]. Since Rosenberg's serendipitous discovery opened the pathway for the introduction of metal complexes in antineoplastic chemotherapy, several Pt(II) complexes (i.e., cisplatin, carboplatin, and oxaliplatin) have become backbones in cancer treatment [4]. However, the cross-resistance and severe side effects of platinum drugs have limited their clinical application, to a great extent [5]. To search for the answer of the above mentioned problems, many drug design strategies have been used, and over the last 25 years, one attractive procedure the so-called "metal-drug synergism" can be achieved by combining a pharmacologically active organic scaffold and a metal-based complex, searching for synergistic action against several pathogens, including tumor cells [6]. These chemotherapeutic agents act by inhibition of the synthesis of DNA, the primary intracellular target for several anticancer drugs. The interaction between small molecules and DNA can lead to DNA damage in cancer cells by blocking the division of cancer cells and causing cell death. Studies on the non-covalent interactions of molecules with the major groove of DNA are promising as potential new therapeutic agents [7] (See Scheme 1).

In continuation of our quest for robust design of alternative target specific metal-based anticancer agents, thus we considered new approach towards the designing of potential metallo-drugs by combining two different endogenous metal ions, copper, and

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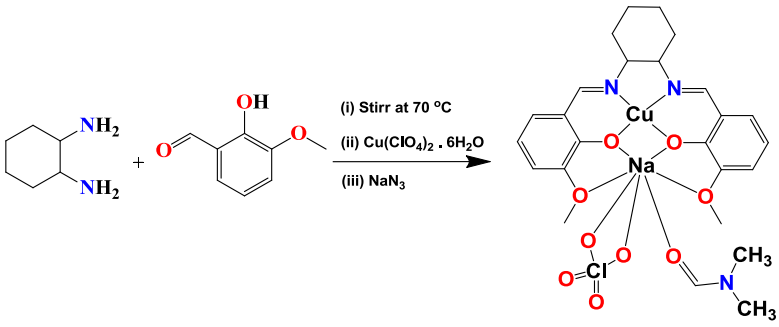
https://doi.org/10.1016/j.ica.2018.04.036
0020-1693/



Scheme 1. Schematic representation of the synthesis of complex **1**.

sodium into the pharmacologically active organic scaffold that are biocompatible. Copper accumulates in tumors due to selective permeability of cancer cell membrane to copper compounds; thereby they can act as "artificial nucleases" for the sequence-specific disruption of gene function [8]. While the role of sodium during apoptosis is essential, evidence in literature has supported that sodium channels could be promising targets for cancer therapy in regards to sensitizing tumor cells to die [9]. Sodium ions induce early apoptosis and play a significant role in essential cellular functions like solute migration and differentiation, gene expression, excitation-contraction coupling and intercellular communication, etc. [10]. We have demonstrated earlier in our previous studies that heterobimetallic complexes of the copper exhibit a remarkable antiproliferative profile and show preferential selectivity inside the cells, inducing apoptosis. The strategic design and synthesis of heteronuclear complexes are quite challenging. The metal centers in heteronuclear systems promote cooperative synergic interactions, for example in biological systems, cooperative interactions are commonly observed which accomplish an extraordinary range of catalytic transformations [11].

Complex **1** structure was elucidated by single crystal X-ray diffraction and other spectroscopic techniques. Although there are a few literature reports of heteronuclear complexes incorporating sodium and copper, the aqua-soluble Cu/Na heteranuclear complex examples remain very scant [12]. The binding affinity towards DNA has been studied by using absorption, emission spectroscopy, and DNA electrophoresis. The anti-cancer potential of complex **1** was evaluated in human breast adenocarcinoma MCF-7 cells through cytotoxicity, apoptosis and DNA damage assays.

2. Experimental section

2.1. Materials

$Cu(ClO_4)_2{\cdot}6H_2O$ (Sigma-Aldrich), o-vanillin (Sigma-Aldrich), 1,2-Diaminocyclohexane (Alfa Aesar) and calf thymus DNA (CT DNA) (Sigma–Aldrich), pBR322 DNA (Genei) used as received. From Invitrogen (Carlsbad, CA, USA), FBS (Fetal bovine serum), penicillin-streptomycin and trypsin/ EDTA were obtained. PBS (phosphate buffered saline), dimethyl sulfoxide (DMSO), ethidium bromide, acridine orange**,** Trypan blue, Cytochalasin-B, agarose for electrophoresis and Dulbecco's Modified Eagle's medium (DMEM), were obtained from Sigma-Aldrich (St Louis, MO, USA). The Cell Titer 96® (Non-radioactive Cell Proliferation Assay kit), purchased from Promega (Madison, WI, USA). All the culture wares and consumables used in these experiments were from Nunc, Denmark.

2.2. Methods and instrumentation

Instruments used for Microanalysis was CE–440 elemental analyzers (Exeter Analytical Inc.), FT-IR was carried out on Perkin–Elmer Model 1320 spectrometer (KBr disk, 400–4000 cm^{-1}), Perkin-Elmer UV–vis spectrophotometer, Shimadzu RF–5301 PC spectrofluorophotometer. Axygen horizontal electrophoretic assembly with power supply and Vilber-Infinity gel documentation system for imaging.

2.3. Synthesis of $[Cu^{II}Na^{I}L(DMF)(ClO_4)]$ (**1**)

A solution of o-vanillin (2 mmol, 10 mL MeOH) was mixed with DACH (2 mmol, 0.24 mL). The mixture was allowed to stirring at 70 °C for a period of 3 h to give deep yellow clear solution. A methanolic solution of $Cu(ClO_4)_2{\cdot}6H_2O$ (0.37 g, 1 mmol) was added to the above reaction mixture which was refluxed with constant stirring for 2 h. After this was added, sodium azide (1 mmol, 65 mg) in a methanol-DMF (1:2) mixture (15 mL) to above reaction mixture and stirred for 2 h. After 2–3 weeks crystals suitable for X-ray were obtained on slow evaporation in a refrigerator of brown color.

Yield 88%, M.P. 210 °C. Anal. Calc. for $[C_{25}H_{31}ClCuN_3NaO_9]$ (%): C, 46.95; H, 4.89; N, 6.57; Found: C, 46.84; H, 4.78; N, 6.51.

2.4. Single crystal X–ray crystallography

To obtain crystallographic data of complex **1**, Bruker SMART APEX CCD diffractometer at 100 K on a using graphite monochromatic MoK_α radiation ($\lambda = 0.71073$ Å) [13–17] (for details see SI). The refinement and crystal data are presented in Table 1. Selective bond distances and angles are given in Tables S1 and S2.

2.5. Theoretical calculations

The ORCA 3.0.1 programmed was used for theoretical calculations [18]. The quantum chemical calculations were performed by applying the DFT method with Becke-3-Lee-Yang-Parr (B3LYP) supplemented with the balanced polarized triple-zeta def2-TZVP basis set for all atoms [19]. The initial coordinates was taken from the single crystal X-ray structural data. The resolution of identity approximation with decontracted auxiliary def2-TZV/J coulomb fitting basis set and the chain-of-spheres approximation were subjected, to fasten the calculations [20]. The Crystal Explorer software was used for mapping the Hireshfeld surface by taking crystal structure data from .cif file [21].

AutoDock Vina software package was utilized for the molecular docking studies [22]. The Pre-docking preparation of the ligand (complex **1**) and receptor (DNA: PDB; 1BNA) was done by MGL Tool-1.5.6. Discovery Studio 4.1 and Pymol molecular graphic visualization programs were used for the imagining of most favorable docked poses [23].