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Original article

Molecular drug design, synthesis and crystal structure determination of Cu^{II}–Sn^{IV} heterobimetallic core: DNA binding and cleavage studies

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ABSTRACT

A novel heterobimetallic Cu^{II}–Sn^{IV} complex **1** bearing bioactive 1,10-phenanthroline pharmacophore ligand scaffold was synthesized and characterized by elemental analysis, IR, UV–vis spectroscopy, Mass (ESI and FAB) and X–ray crystallography. The *in vitro* DNA binding studies of complex **1** with CT DNA was carried out by various biophysical and molecular docking techniques which revealed that complex **1** binds to DNA through intercalation in the minor groove having AT-rich sequences. Complex **1** exhibits high chemical nuclease activity cleaving supercoiled pBR322 DNA via hydrolytic pathway which was further evidenced by T4 DNA ligase assay. The complex **1** shows high inhibitory activity against Topo I at a very low concentration (15 μM), suggesting that complex **1** is an efficient catalytic inhibitor of human Topo I and further validated by molecular docking studies.

1. Introduction

Metal complexes that can bind to DNA are gaining considerable attention owing to their diverse applications in the field of bio-inorganic chemistry viz. diagnostic agents for medical applications, development of cleavage agents for probing nucleic acid structure [1,2] and identifiers of transcription start sites [3]. DNA is an important target of antitumor drugs and interactions of DNA with transition metal complexes are important for the design of efficacious drug entities which exhibit different properties than the mainstream protocol drugs viz. cisplatin, etc. which are currently in use [4].

Copper have been used since antiquity in metal-based therapies. Copper is a bioessential element which plays a key role in biological processes, and its complexes are preferred molecules for cancer inhibition by chemotherapy [5]. Among the copper complexes explored so far, considerable attention has been focused on 1,10-phenanthroline Cu^{II} complexes due to their high nucleolytic efficiency and numerous biological activities such as antitumor, antimicrobial, etc. Several copper complexes have been described as DNA cleaving agents and the best studied example is Sigman's $[Cu(phen)_2]^{2+}$ complex [6]. This complex is reduced in situ to $[Cu(phen)_2]^{+}$ and subsequently binds to minor groove of DNA, combines with molecular oxygen, to generate a non-diffusible oxidant and finally induces strand scission by oxidation of the ribose backbone.

Literature supports that Cu^{II} ions specifically bind to the N-7 guanine residue of DNA and cause strand breakage [7]. The kinetic analysis of copper DNA interaction and its site-specific binding with DNA have been well–documented [8]. On the contrary, Sn^{IV} complexes prefer to bind to the phosphate backbone of the DNA helix (Sn^{IV} ions have a hard Lewis acid nature, neutralize the negative charge of phosphate sugar, and bring conformational changes in DNA) [9]. Previous studies of Cu^{II} and Cu^{II}–Sn^{IV} complexes have shown interesting results against various cancerous cell lines (HeLa cells, T47D, HT29) [10]. It has been demonstrated that these complexes induce apoptosis via mitochondrial pathway. Thus, heterobimetallic complexes containing Cu^{II} and Sn^{IV} ions enhance the chemotherapeutic action many-fold as they provide a dual mode of binding at the molecular target site and also exhibit novelty due to preferential selectivity inside the

Abbreviations: UV–vis, UV–visible; CT DNA, Calf thymus DNA; Tris, tris(hydroxymethyl)aminomethane; EB, ethidium bromide; Phen, 1,10-phenanthroline; Topo I, topoisomerase I.

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cells [11]. Furthermore, some additional favorable non-covalent interactions such as hydrogen bonding, van der Waals forces within the groove of DNA can enhance DNA binding multi-fold and enforce specificity [12]. In the present work, we have designed a new ionic crystalline molecular entity **1** possessing Cu^{II}–Sn^{IV} heterodinuclear unit; such Cu^{II}–Sn^{IV} ionic motifs are scarce in literature, however, some ionic triphenyltin(IV)chloride carboxylate complexes exhibiting exceptional cytotoxicity have been reported earlier [13]. The molecule exhibits novelty, fulfilling all the pre-requirements for efficient chemotherapeutic drug design which include (i) good DNA binding propensity, (ii) specific tagging to drug target at molecular level, (iii) preferential selectivity to DNA base sites/minor groove binding, efficient DNA cleavage and potent Topo I activity, (iv) multifaceted binding modes, (v) unsaturated coordination geometry at the cation and anion metal centers. The computer-aided molecular docking study carried out in this work affords valuable information of drug binding mode (intercalative mode of binding, in addition to favorable hydrogen bonding interactions) in the active site of DNA–Topo I which lead to the rational design of new classes of anticancer drugs targeting Topo I. Literature reveals that minor groove analogs such as camptothecin derivatives, approved as anticancer drugs and selective DNA–Topo I inhibitors exhibited certain drawbacks such as chemically unstable structure and rapid efflux from the cell by the membrane pumps [14]. Thus, adapting a strategy based on metal and an intercalating scaffold with ionic recognition domain can lay design paradigm for new DNA–Topo I inhibitors.

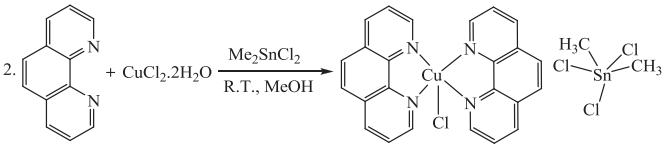
2. Chemistry

In order to elucidate the molecular recognition toward the minor groove of DNA and inhibit DNA processing enzymes, new drug candidate was designed and synthesized. The synthesis of novel heterobimetallic complex **1** was achieved by mixing stoichiometric amounts of Cu^{II} chloride dihydrate with 1,10-phenanthroline monohydrate followed by drop wise addition of dimethyltin dichloride (Scheme 1). The formulation of the complex **1** was confirmed by determination of the X-ray crystal structure. The resulting complex **1** is stable toward air and moisture and readily soluble in DMSO. Molar conductance value of complex in DMSO (1×10^{-3} M) at 25 °C suggests its 1:1 electrolyte nature ($74\ \Omega^{-1}\ cm^2\ mol^{-1}$).

3. Results and discussion

3.1. Electronic spectra

The complex **1** exhibited a broad d–d band at 726 nm and a low-energy shoulder band at 891 nm suggesting the Cu^{II} ion in a five-coordinate environment and strong charge transfer (CT) band near 271 nm followed by a shoulder band at 295 nm attributed to the π–π^* transition of the coordinated phenanthroline ligand (Supplementary material Fig. S1) [8,15]. The Sn^{IV} anion moiety did not exhibit any d–d band transitions.



Scheme 1. Synthetic route for the heterobimetallic complex **1**.

3.2. Mass spectral analysis

The nature of heterobimetallic complex **1** in solid and solution state were studied with FAB and ESI–MS, respectively (Fig. S2a and b). The FAB mass spectrum of complex **1** in solid state showed the molecular ion peak m/z at 712 which was assigned to $[C_{26}H_{22}Cl_4CuN_4Sn - 2H]^+$. The appearance of other peaks at m/z 677, 606 and 570 (50%) were ascribed to the species $[C_{26}H_{22}Cl_4CuN_4Sn - Cl + 2H]^+$ $[C_{26}H_{22}Cl_4CuN_4Sn - Cl_3 + 2H]^+$ and $[C_{26}H_{22}Cl_4CuN_4Sn - Cl_4 + 2H]^+$, respectively. However, ESI–MS spectra of the complex **1** in DMSO solution displayed molecular ion fragment peaks at m/z 458.8 and 254.3 corresponding to the complex cation and anion, respectively, which confirms the ionization of complex **1** in solution state and are well separated from each other leads to further fragmentation separately. The fragmentation peaks for complex cation observed at m/z 421.9 and 63.2 (50%) were ascribed to the species $[Cu(phen)_2C1 - Cl + H^+]^+$ and $[Cu(phen)_2C1\text{-}(phen)_2 + Cl + H^+]^+$, respectively. While in case of complex anion, fragmentation peaks after the successive expulsion of three chlorine atoms were obtained at m/z 218.4, 184.1 and 148.6. Another peak at m/z 118.7 was obtained by the removal of two $-CH_3$ groups and finally the Sn^{IV} metal ion was ionized. These mass spectral analysis (FAB and ESI) results indicate that complex **1** existed as a whole entity in solid state but undergoes self ionization in solution state.

3.3. Description of crystal structure

The single crystal X-ray analysis reveals that the title complex **1** crystallizes as monoclinic crystal system with space group $P2_1/c$. An ORTEP view of the complex together with atom-numbering scheme is illustrated in Fig. 1, while relevant bond lengths and bond angles are listed in Table 1. The crystal structure is quite interesting and displays the formation of a heterodinuclear unit having two different geometries with no evidence for even semi-coordination existing between them. However, both are held together by means of electrostatic forces and van der Waals interactions and separated by a distance of 6.04 Å. The molecular structure of the complex is composed of discrete $[Cu(phen)_2C1]^+$ cation and $[Me_2SnCl_3]^-$ anion in order to balance the charge and behaves as neutral molecule. The complex cation exhibits a five-coordinated $Cu^{II}N_4C1$ chromophore as slightly distorted square pyramid $(4+1)$ with the structural index parameter $\tau = 0.63$ [$\tau = (\beta - \alpha)/60$], where α and β are the two largest metal ligand bond angles in the complex, $\tau = 0$ and 1 for ideal square pyramidal and trigonal bipyramidal geometries, respectively. The plane of the phenanthroline ligands is inclined to each other with a dihedral angle of 46.9° showing these two rings are not in a good planarity. The $Cu^{II}N_4$ core, from two bidentate phen ligands, forming two five-membered chelated rings with intraligand N–Cu–N angles of 80.77(15) and 81.40(16)°, respectively, while the interligand N–Cu–N angles ranges between 112.39(15)–173.39(16)°, whereas apical position is occupied by the Cl atom, Cl(1). The Cu1–Nphen bond lengths fall within a narrow range of 2.001 (4)–2.143 (4) Å with an average value of 2.072 Å, which are significantly shorter than the Cu1–C1(1) bond length 2.3106 (13) Å (Table 1), consistent with the larger size of the chlorine atom, indicating that phenanthroline is more strongly coordinated to metal center than chlorine atom [16].

In the case of complex anion, the Sn^{IV} metal ion is five-coordinated in a trigonal bipyramidal geometry with the two methyl groups and one chlorine atom are placed in equatorial position while axial position is occupied by two chlorine atom. The bond length between Sn(1)–Cl(2), Sn(1)–Cl(3) and Sn(1)–Cl(4) are 2.368(12), 2.545(3) and 2.597(4), respectively, which indicates that