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Water soluble molybdenocene complexes: Synthesis, cytotoxic activity and binding studies to ubiquitin by fluorescence spectroscopy, circular dichroism and molecular modeling

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ABSTRACT

Four new molybdenocene complexes, Cp_2Mo(L-ascorbato), Cp_2Mo(6-O-palmitoyl-L-ascorbato), [Cp_2Mo(ethyl maltolato)]Cl and Cp_2Mo((2*S*)-2-amino-3-methyl-3-thiolato-butanoato), were synthesized and structurally characterized by standard analytical methods. The cytotoxicity of these complexes was assessed on colon HT-29 and breast MCF-7 cancer cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. A higher cytotoxic activity was shown by all the new complexes on the MCF-7 cells over the Cp_2MoCl_2 complex. The complexes Cp_2Mo(L-ascorbato), Cp_2Mo(6-O-palmitoyl-L-ascorbato) and [Cp_2Mo(ethyl maltolato)]Cl displayed a stronger cytotoxic activity on colon cancer HT-29 cell line, over the molybdenocene dichloride (Cp_2MoCl_2). In contrast, Cp_2Mo((2*S*)-2-amino-3-methyl-3-thiolato-butanoato) exhibited proliferative properties on this cell line. Ubiquitin (Ub)–molybdenocene interactions were investigated using cyclic voltammetry, fluorescence quenching spectroscopy, circular dichroism (CD) and molecular modeling. The thermodynamic parameters (ΔH and ΔS) obtained using fluorescence quenching spectra and van't Hoff plot indicate the Ub–molybdenocene interactions are mainly hydrophobic. The CD data also support hydrophobic interactions with conformational changes in the Ub protein. Docking studies using molecular modeling revealed the amino acids involved in the Ub–molybdenocene interactions and corroborated the hydrophobic nature of the binding combined with hydrogen bonding.

1. Introduction

There is an increasing interest in the bioorganometallic chemistry of molybdenocene dichloride (Cp_2MoCl_2, Cp = η^5-cyclopentadienyl) due to the significant antitumor activity demonstrated on Ehrlich ascites tumor, Lewis lung carcinomas, and B16 melanoma and colon 38 cell lines [1–6]. In marked contrast to titanocene dichloride, the hydrolytic stability of Cp_2MoCl_2 at physiological pH offers the opportunity to explore structure modifications and develop the corresponding structure-activity relationship (SAR) [7].

The bioorganometallic chemistry of Cp_2MoCl_2 and closely related derivatives as metal-based anti-cancer drugs has been reviewed recently and highlights the basic features concerning its anti-proliferative activity [8]. For instance, inert Mo(IV)–L(ancillary) bonds inactivate the molybdenocene complexes leading to a non-cytotoxic response in cancer cell lines. Conversely, labile bonds combined with lipophilicity enhance the anti-proliferative activity of the resulting molybdenocenes. Other strategies such as the functionalization on the Cp rings with methoxyphenyl pendant groups have been pursued by Tacke and coworkers [9]. Adding 3,4-OMe- or 3,4,5-OMe-phenyl substituent on the Cp ring yields a molybdenocene dichloride showing low cytotoxicity, while the addition of 4-OMe-phenyl yields a highly cytotoxic compound toward cancer cells [9]. With this in mind, a new series of molybdenocenes containing four ligands with biological importance were synthesized, i.e., O,O′ and O,S bidentate ligands, L-ascorbic acid, a, 6-O-palmitoyl-L-ascorbic acid, b, ethyl maltol, c, and D-penicillamine ((2*S*)-2-amino-3-methyl-3-sulfanyl-butanoic acid), d, where the Mo(IV)–L(ancillary) bond stability, hydrophobic character and anti-proliferative activity could be modulated. Scheme 1 presents the ligands used in this study.

The interaction of molybdenocene with transport proteins such as human serum albumin (HSA) has been investigated to understand and predict the overall drug distribution, efficacy, activity and toxicity [10,11]. Radio-labeled $Cp_2^{99}MoCl_2$-HAS binding studies provide evidence that Cp_2MoCl_2 binds the protein in a 9.4 to 1 ratio [10]. In other

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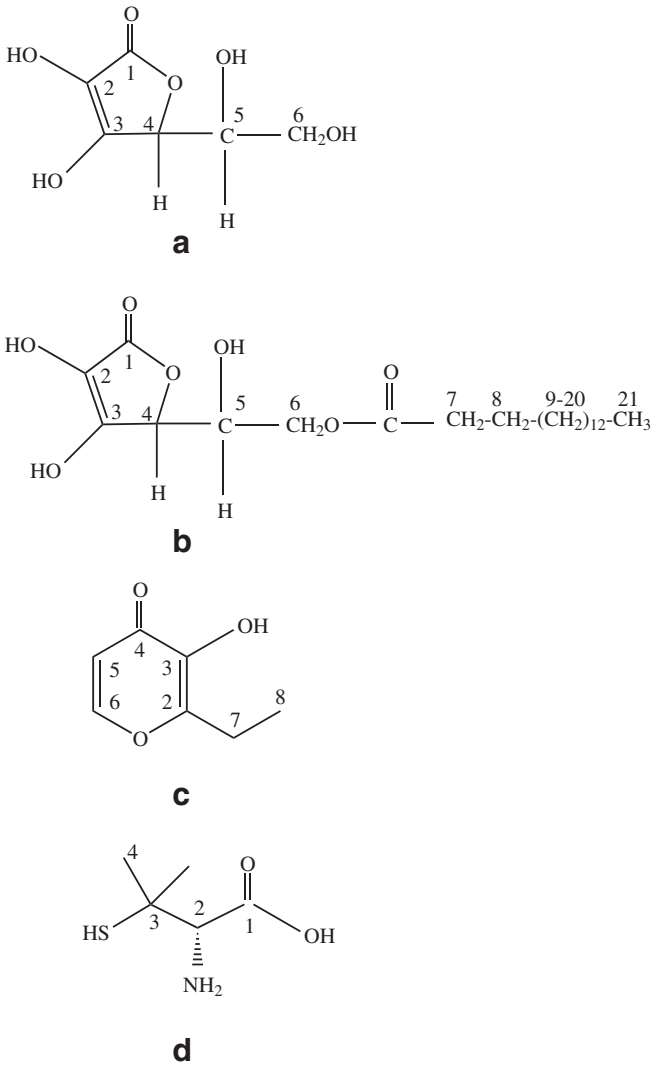

Scheme 1. Structure of ligands.

studies performed by our group we showed that, for Cp_2MoCl_2 and molybdenocene containing O,O′ bidentate ligands, there is no direct correlation between anti-proliferative activity on HT-29 and MCF-7 cells and binding affinity to HSA [11].

In our continuous efforts to understand the anti-cancer properties and mechanisms of action of molybdenocene complexes, we explored other potential biomolecular targets such as ubiquitin (Ub), a small highly conserved cytoplasmic protein of 76 residues which is highly compact and rigid [12]. This protein has a well-established role in regulating cellular proliferation and it has been implicated in the pathogenesis of cancer, tumor progression and neurodegenerative disorders (see reviews [13–16]). Therefore, it is a potential drug target in cancer cells. Ub is comprised of a β-sheet containing five strands, an α-helix and a short 3_{10}-helix. Two of the inner strands are parallel and the other three strands are antiparallel. The α-helix is located in the concavity formed by the β-sheet generating a hydrophobic core [12].

Several binding studies between Ub and metal ions such as Cu(II), Zn(II), Cd(II) and Hg(II) have revealed that Met-1, His-68, Glu-16/Met-1 and Glu-18 are possible coordination sites within the Ub structure [17,18]. Binding studies between platinum complexes and hUb revealed that the platination sites of hUb for cis-platin are the N-terminal methionine (Met-1) and His-68 [19–26]. Anti-cancer platinum drugs have been associated with the inhibition of ubiquitin-dependent proteolysis [27]. It is evident that Ub–metal interactions are important because they could serve as drug reservoirs, thus determining drug distribution, excretion, inactivation and toxicity. In addition, it could impair ubiquitin-dependent mechanisms that play an important role in tumor progression [13–16,27]. Given that Ub possesses several binding sites by either coordination to a metal center [17–26] or by hydrophobic interactions [28–30] and due to its role in cancer pathogenesis and tumor progression, we pursued an initial binding study with molybdenocene complexes. Herein we report our findings.

2. Results and discussion

2.1. Synthesis and characterization

Four new molybdenocene complexes were synthesized in aqueous solution, according to Scheme 2, varying the donor atoms, charge and lipophilicity of the ligands and pH. Ascorbato and 6-O-palmitoyl-L-ascorbato are O,O′ di-anionic bidentate ligands while penicillamine ((2*S*)-2-amino-3-methyl-3-thiolato-butanoato) is a O,S di-anionic bidentate ligand. These three molybdenocene derivatives are neutral species but differ in their lipophilic character. Ethyl maltolato is a O,O′ anionic bidentate ligand and produces a cationic molybdenocene species. These complexes were purified by column chromatography. After chromatographic separation and crystallization, these molybdenocenes are isolated as pure powders in about 60% yield. These species are highly hygroscopic and very soluble in water, with exception of Cp_2Mo(6-O-palmitoyl-L-ascorbato) which is partially soluble but, soluble in a 5% DMSO aqueous solution. At physiological pH they are soluble and stable for a period of over 12 h as evidenced by ^1H NMR spectroscopy.

The selection of the ligands is based on the following criteria. Ascorbic acid is well known for its antioxidant properties and protective effects in cancer patients, showing an inhibitory control of cell growth and division. But in presence of metal ions such as Fe(II) and Cu(I), ascorbic acid has pro-oxidative properties producing reactive oxygen species (ROS), H_2O_2 and HO, which can result in cell damage and death [31–35]. On the other hand, at high doses L-ascorbic acid and 6-O-palmitoyl-L-ascorbic acid (an ester formed from vitamin C and the saturated palmitic acid) have shown to have selective cytotoxicity toward tumor cells through hydrogen peroxide generation [33–38]. Of particular importance, 6-O-palmitoyl-L ascorbic acid showed a stronger anti-proliferative effect than L-ascorbic acid, apparently due to the balance between lipophilicity and hydrophilicity [38]. The selection of D-penicillamine ((2*S*)-2-amino-3-methyl-3-sulfanyl-butanoic acid) was based on the coordinating properties shown by its chelating capacity in Wilson's disease and antirheumatic drugs [39–41] while maltol was chosen based on its extensive use as O,O′ chelating ligand with many biologically important metals, providing aqueous solubility to the metal ions as well as some degree of hydrophobicity [42–47].

The new species were characterized by spectroscopic and analytical methods. The IR spectra of Cp_2Mo(L-ascorbato) (1) and Cp_2Mo(6-O-palmitoyl-L-ascorbato) (2) have prominent peaks at 3114 (1) and 3116 cm^{-1} (2) corresponding to the Cp(C–H) stretching, at 1750 (1) and 1734 (2) cm^{-1} corresponding to the ν(C═O) of the ester groups and at 1620 and 1614 cm^{-1} for the C═C groups respectively. Cp_2Mo(L-ascorbato) has one peak in the aliphatic region at 2915 cm^{-1} whereas Cp_2Mo(6-O-palmitoyl-L-ascorbato) has three peaks in this region at 2960, 2915 and 2850 cm^{-1}. Cp_2Mo((2*S*)-2-amino-3-methyl-3-thiolato-butanoato) has two peaks at 3486 and 3470 cm^{-1} corresponding to the stretching of the NH_2 group, the Cp(C–H) stretching at 3094 cm^{-1}, the ν(C≡O) (carbonyl) stretching at 1614 cm^{-1} and N–H bending at 1640 cm^{-1}. Of particular importance, the ν(S–H) stretching at 2650 cm^{-1} is not present indicating the thiol group has been deprotonated and is engaged in bonding to Mo(IV). In the IR spectrum of [Cp_2Mo(ethyl maltolato)]Cl besides the Cp peak at 3094 cm^{-1}, the ketonic carbonyl at 1640 cm^{-1} and the ν(C═C) stretching at 1600 cm^{-1} are clearly present. The complete IR spectral data is included in the Supplementary Information Section.