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Synthesis and cytotoxic activity of metallic complexes of lawsone

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ABSTRACT

In the present study, a series of metallic complexes of the 1,4-naphthoquinone lawsone (**2**–**6**) were synthesized and evaluated for potential cytotoxicity in a mouse leukemic macrophagic RAW 264.7 cell line. Cell viability was determined by the MTT assay. Significant growth inhibition was observed for the copper complex **(4)** with an IC_{50} value of 2.5 μM. This compound was selected for further evaluation of cytotoxic activity on several human cancer cells including HT-29 (human colorectal adenocarcinoma), HepG2 (human hepatocellular carcinoma) and HeLa, (human cervical adenocarcinoma cells). Significant cell viability decrease was also observed in HepG2 cells. The apoptotic potential of this complex was evaluated in these cells. Compound **4** induced apoptosis by a mechanism that involves the activation of caspases 3, 8 and 9 and modulation of apoptotic-related proteins such as Bax, Bad, and p53. These results indicate that metal complexes of lawsone derivatives, in particular compound **4**, might be used for the design of new antitumoral agents.

1. Introduction

The quinonic moiety is considered by the National Cancer Institute (NCI) as an important biologically validated scaffold for the development of new bioactive compounds with good levels of cytotoxicity.[1,2] Indeed, many clinically important antitumoral drugs containing a quinone moiety such as anthracyclines, mitoxantrones and saintopin, show excellent anticancer properties.[3] A representative group of quinonoid compounds are 1,4-naphthoquinones which are widely distributed in nature and have been recognized to possess a wide range of biological activities such as antibacterial, anti-inflammatory, antifungal, antiviral.[4–7] Some illustrative examples of antitumoral naphthoquinones are plumbagin,[8] juglone,[9] β-lapachol[10] and rhinacanthone.[11]

Furthermore, transition metal-based drugs are increasing their importance in the therapy of cancer and other diseases. The best example of these metal-based drugs is cisplatin, one of the most used anticancer drugs.[12] Interestingly, metals can play an important role in modifying the pharmacological properties of known drugs.[13]

Quinones can bind potentially to metal ions in three different oxidations states: (i) quinone, (ii) its one electron reduced form semiquinone, (iii) catechol the two electrons reduced form. The binding ability of quinones in different oxidations states allows them to play an important role in biological systems.

There are only a few examples of metal complexes of 1,4-naphthoquinones. In this sense, Chen et al.[14] have recently published the synthesis, characterization and preliminary cytotoxicity evaluation of five lanthanide(III)–plumbagin complexes. Hernández-Molina et al.[15] reported the preparation of complexes of Co(II), Ni(II) and Cu(II) with the naturally occurring hydroxynaphthoquinone lapachol, and the Co(II) complex showed activity against the trophozoite stage of *Acanthamoeba castellanii* Neff.[16] Bustamante et al.[17] reported the isomerism and nuclearity control in bis(lawsonato)zinc(II) complexes. Some iron(II) complexes of *ortho*-functionalized *p*-naphthoquinones and the characterization by X-ray of a copper(II) complex of 2-hydroxy-1,4-naphthoquinone have also been described.[18]

In this paper we describe the preparation of five metal complexes of lawsone (**2–6**) and their cytotoxicity in human cancer

Abbreviations: MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; DMSO, dimethylsulphoxide; TLCK, N_{α}-Tosyl-L-lysine chloromethyl ketone hydrochloride.

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cells. The copper complex (**4**) was the most active compound of this series and the results presented here show that **4** significantly induced apoptosis in HepG2 human cancer cells by a mechanism that involves activation of caspases and modulation of apoptotic-related proteins.

2. Chemistry

2.1. Synthesis and structures of the metallic complexes of lawsone

Complexes **2**–**6** were synthesized by mixing ethanolic solutions of lawsone (**1**) and the corresponding metallic salts in the molar ratio ligand/metal 2:1 to afford the complexes as red solids (Fig. 1). Since the metallic salts used were acetates, it was not necessary the addition of a base to deprotonate lawsone. The reaction is driven to completion by the chelate effect and low solubility of the final products in ethanol. The lawsone–Metal(II) complexes $[Zn(Lw)_2(H_2O)_2]\cdot 2H_2O$ (**2**), $[Co(Lw)_2(H_2O)_2]$ (**3**), $[Cu(Lw)_2(H_2O)_2]$ (**4**), $[Ni(Lw)_2(H_2O)_2]$ (**5**), $[Mn(Lw)_2(H_2O)_2]$ (**6**) were characterized by elemental analyses and FT-IR spectroscopy. $[Zn(Lw)_2(H_2O)_2]\cdot 2H_2O$ (**2**) and $[Co(Lw)_2(H_2O)_2]$ (**3**) were furthermore characterized by diffraction of X-ray of suitable crystals grown from slow evaporation of the ethanolic solution (Fig. 2, Table S1 in Supplementary data contents), and in the case of the complex (**2**) by ^{1}H NMR spectrum.

The bis(lawsonato)copper(II) complex was prepared using $Cu(OAc)_2$ as metal source instead of $CuCl_2$ in the presence of Et_3N to ensure the full deprotonation of the hydroxyl group of the ligand as previously reported by Salunke-Gawali et al.[19] Recently, Bustamante et al.[17] have reported the structures of different isomers of bis(lawsonato)zinc complexes. These complexes have been prepared using zinc(II) acetate and triethylamine to increase the pH. The formation of the *cis* and the *trans* isomers depends on the order of the addition of the reagents being the *cis* isomer obtained when a methanolic solution of lawsone was added into a solution of zinc(II) acetate in water followed by the addition of NEt_3. The sequence of addition of the reagents for the formation of the *trans* isomer is just the opposite and zinc(II) acetate dissolved in water was slowly added to a solution of lawsone and triethylamine. In our case the *cis* isomer was obtained by addition of an ethanolic solution of zinc(II) acetate to an ethanolic solution of lawsone (1:2 ratio).

The lawsone–Co(II) complex is hexacoordinated and centrosymmetric with two bidentate ONQ ligands and two water molecules. The Co(II) environment is approximately octahedral. The basal plane of the complex is occupied by the quinone carbonyl oxygen atoms O(1) and O(1′) at 2.136(2) Å and the phenoxy oxygens O(3) and O(3′) of ONQ at 2.032(7) Å. The apical position are occupied by the two oxygen atoms of water O(1w) and O(1w) at a distance of 2.092(12) Å in *trans* position. The structure with the water in *trans* position is also found for the bis(lawsonato)copper(II)[19] complexes and for the *trans* isomer of the Zn(II) bis(lawsonato) complex[17] and the bond distances for the Zn–O apical in the *trans* isomer complex is 2.141(3) Å and the corresponding to the copper(II) complex is 2.007(3) Å. The binding angle of the ligand for the two groups O(1)-Co-O(3) are and 78.91(8)° and 101.09(8)°, respectively, while the bond angle between O1w–Co–O3 is 89.40(8)° and 90.60(8)° which are quite close to the right angle for an octahedral geometry. Selected bond distances and angles are given in Table S2 in Supplementary data contents.

Supramolecular assemblies via H-bonding formed between one of the hydrogen of the water ligand and the phenoxy oxygen of a nearby molecule and between the second hydrogen of the water ligand and oxygen of the uncoordinated carbonyl group of a quinone ring of the next molecule is an interesting part of the molecular structure (See Fig. S1, Supplementary data contents). A table showing the distances of the hydrogen bonding for the cobalt(II)–lawsonato is provided (Table S4, Supplementary data Contents). Lawsone with its *ortho*-hydroxy group is known to exhibit keto/enol tautomerism. In the present compound the shorter bond distance of C(7)–O(1) (1.230(5) Å) compared to the bond distances C(10)–O(2) and C(8)–O(3) of 1.239(5) Å and 1.287(3) Å, respectively, alongside with the increase in the bond distance of C(7)–C(8) (1.515(7) Å) is indicative of a carbonyl character for the former bond. For this complex C(1)–O(1) and the C(8)–O(3) bond distances Å are shorter than the typical carbonyl bonds of semiquinonate (1.27–1.30 Å) and catecholate (1.30–1.36 Å)[20] forms of the ligand implying that the two lawsonate anions are coordinated to the Co(II) cation in their fully oxidized form.

One striking difference of the Zn–Lw complex prepared in this work with respect to that of cobalt complex is that the water molecules are situated in *cis* position contrarily to the Co–Lw complex, where the water molecules are *trans* to each other. However, in a recent paper on isomerism and nuclearity control in bis(lawsonato)zin(II) complexes, the isomers *cis* and *trans* were isolated and their structures determined by X-ray. The addition sequence of the reagents during the reaction seems to play a crucial role in the isolation of the *cis* or the *trans* isomers. When the reaction mixture was allowed to stand for a long period both isomers *cis* and *trans* were obtained, which can be identified and separated by the different form of the crystals.[17] The zinc(II) structure consists of a distorted octahedron with one water molecule occupying an equatorial position alongside three phenoxy oxygens and the other water molecule occupying the apical position. The Zn–Ow distance is of 2.044(3) Å for the water axial and 2.106(3) Å for the water equatorial. The Zn–O distances of the phenoxy groups are Zn–O(1) 2.180(3) Å, Zn–O(3) 2.050(3) Å, Zn–O(4) 2.311(3) Å and Zn–O(6) 1.992(3) Å. The O(1)–C(7) and the O(3)–C(8) distances are 1.229(5) and 1.281(4) Å. The hydroxyl groups and the water ligands are involved in an extensive hydrogen bonding network. These structural features are in good agreement with those reported for the Zn(II) *cis* isomer.

The complexes show a broad hydroxyl absorption band centered around 3400 cm^{-1}, indicative of the water molecules present in the complexes. The bands occurring at 1680–1700 cm^{-1} are typical of 1,2-quinone carbonyl group.

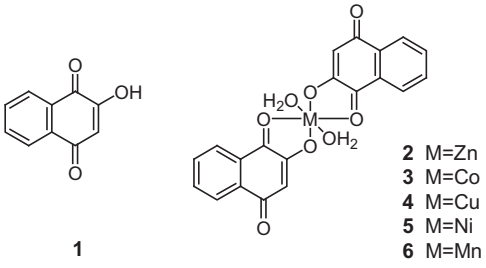

Figure 1. Structure of lawsone (**1**) and general structure of the metallic complexes of lawsone (**2**–**6**).

3. Results and discussion

3.1. Compound 4 decreases cell viability in a dose-dependent manner

To establish the pharmacological potential of the lawsone metal complexes synthesized (Fig. 1), the compounds were initially tested for cytotoxicity in a mouse leukemic macrophagic RAW