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Synthesis of new 4-methylesculetin complexes as anti-neoplastic agents and X-ray structure of dimeric bis-bipyridyl-bis-4-methylesculetinato zinc(II)

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ABSTRACT

The new complexes of 4-methylesculetin (H_2mesc), $[Zn_2(bpy)_2(mesc)_2]$, $[Zn(PPh_3)_2(mesc)]$, $Na_2[MoO_2(mesc)_2]$, $(PPh_4)_2[Mo_2O_5(mesc)_2]$, [Pd(bpy)(mesc)], [Pd(phen)(mesc)], $[Pd(PPh_3)_2(mesc)]$, $[Pt(PPh_3)_2(mesc)]$ and $[Ag(bpy)_2]$. (bpy)H_2mesc(NO_3), are reported. The complexes were characterized on the bases of elemental analysis, spectroscopic (IR, UV–Vis, mass, ^{1}H, ^{13}C and ^{31}P NMR) and physical (conductivity and thermal) techniques. The ligand ($mesc^{2-}$) behaves in a binegative bidentate fashion, chelating the metal ions through the deprotonated hydroxy oxygen atoms, except in case of Ag(I), where it was found outside the coordination sphere of the complex. The X-ray crystal structure of $[Zn_2(bpy)_2(mesc)_2]$ has been determined. The bond length of Zn–O(7) is shorter than Zn–O(6). A theoretical study on H_2mesc and its dianion $mesc^{-2}$ was undertaken through computational conformational analysis, indicating the higher basicity of O(7) with respect to O(6). H_2mesc and the complexes have been tested as anti-neoplastic agents against human prostate cancer (DU 145) and human breast cancer (MDA-MB231) cell lines.

1. Introduction

Cancer is one of the most leading causes of death, and many efforts have been made to discover agents endowed with cytotoxic action [1,2]. For several chemotheraputic agents, a direct correlation between anticancer efficacy and the ability to induce apoptosis has been established. Thus, the development of new anticancer agents aimed to promote apoptosis in cancer cells [3,4].

Coumarins owe their class name to "COUMAROU", the vernacular name of the tonka bean, from which coumarin itself was isolated in 1820. Coumarins belong to benzo-α-pyrone families. 6,7-Dihydroxycoumarin (esculetin; Fig. 1) and 4-methyl-6,7-dihydroxycoumarin (methylesculetin; Fig. 2) are of interest since they contain the non-innocent catecholato group and function as dianionic chelating agents (Fig. 3) [5]. Coumarins exist in a variety of forms, due to the various substitutions possible in their basic structure, which modulate their biological activity [6]. They are found in a number of natural products including the antibiotic novobicin, which inhibits DNA-gyrase. Esculetin is known to be a lipogenase inhibitor, reducing the selective cytotoxicity of the dilinoleoyl glycerol (DLG) mediate, which is transformed by the E1A gene in 3Y1 cells [7]. Multidrug resistance (MDR) of human tumors is one of the major reasons for the failure of chemotherapy in refractory cancer patients [8]. Unfortunately, the concentration of many of these agents necessary to reverse drug resistance is difficult to achieve *in vivo* [9]. Thus, there is considerable interest in the search for new P-glycoprotein (P-gp) inhibitors that do not show significant toxicity at doses required for P-gp inhibition [10]. 6,7-Dihydroxycoumarin derivatives have been screened for their cytotoxic activity against human tumor cells and some of them exhibit potent cytotoxic activity [11]. They have been investigated as potential candidates for cancer therapy and exhibited antiproliferative effect in leukemia cells by inducing apoptosis [10,11]. It is also suggested that proper substitution at the 3 and/or 4 positions of the molecule makes it possible to design more cytotoxic agents. Complexes bearing coumarin functionality have been employed to design site-specific antitumour agents, they are selective for solid malignant cells with reduced toxicity [12,13]. A number of coumarin complexes were evaluated for their *in vitro* antiproliferative activity [14,15]. Moreover, the *in vitro* cytotoxicity of coumarin complexes of cerium(I) against Burkitt

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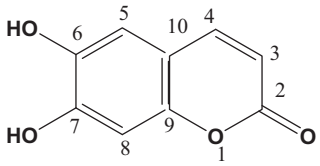

Fig. 1. Structure of esculetin (H_2mesc).

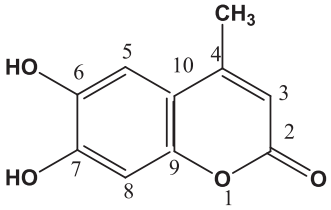

Fig. 2. Structure of 4-methylesculetin (H_2mesc).

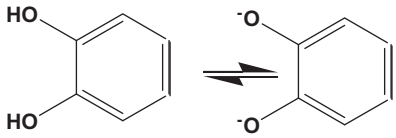

Fig. 3. Dianionic form of 1,2-dihydroxybenzene derivatives.

lymphoma (P3HR1) and leukemic (THP-1) cell lines were investigated [16]. In our laboratory over the past few years, we have developed some Zn(II), Ag(I), Pd(II) and Pt(II) complexes as potential anticancer agents [17–23]. Esculetin is a weak acid (pK_{a1} = 7.3 and pK_{a2} = 8.0). The OH(7) is very reactive and its dissociation decreases when a nucleophilic (Me or Ph) group is substituted at position 4, i.e., it becomes less acidic [24]. We have reported earlier a number of esculetin (H_2esc) second- and third-row transition metal complexes [25], and herein we have synthesized a number of mixed ligand complexes of Zn(II), Mo(VI), Pd(II), Ag(I) and Pt(II) with H_2mesc as the primary chelating agent and 2,2′-dipyridyl (bpy), 1,10-phenanthroline (phen) and triphenylphosphine (PPh_3) as the secondary chelating agents. A computational conformational analysis on H_2mesc and its dianion $mesc^{-2}$ was undertaken through. Since coumarin and substituted coumarins are associated with low toxicity, the anti-neoplastic activities of H_2mesc and the complexes against human prostate cancer (DU 145) and human breast cancer (MDA-MB231) cell lines were examined.

2. Experimental

2.1. Materials

All reagents were purchased from Alfa-Aesar and Aldrich Chemical Co. and all manipulations were performed under aerobic conditions using materials and solvents as received. $[Pd(L)Cl_2]$ (L = bpy, phen) [25], $[M(PPh_3)_2Cl_2]$ (M(II) = Pd, Pt) [25] and $[Zn(PPh_3)_2Cl_2]$ [26] were synthesized by the literature methods. DMSO-d_6 was used for the NMR measurements, which were referenced against TMS.

The human prostate cancer (DU 145) and human breast cancer (MDA-MB231) cell lines were obtained from the American Type Culture Collection (ATCC catalog number). Cells were maintained in Dulbecco's Modified Eagle Medium (Wisent Inc., St-Bruno, Canada) supplemented with 10% FBS, 10 mM HEPES, 2 mM L-gutamine and 100 μg/ml penicillin/streptomycin (GibcoBRL, Gaithersburg, MD). In all assays cells were plated 24 h before drug treatment.

2.2. Instrumentation

Elemental analyses and X-ray crystallography were performed in the Department of Chemistry, Université de Montréal. The crystal structure was measured at the X-ray Crystal Structure Unit, using a Bruker Platform diffractometer, equipped with a Bruker MART 4 K Charger-Coupled Device (CCD) Area Detector using the program APEX II and a Nonius Fr591 rotating anode (Copper radiation) equipped with Montel 200 optics. The crystal-to-detector distance was 5 cm and the data collection was carried out in 512 × 512 pixel mode. The initial unit cell parameters were determined by the least-squares fit of the angular setting of strong reflections, collected by a 10.0° scan in 33 frames over three different parts of the reciprocal space (99 frames total) and one complete sphere of data was collected.. IR spectra were measured as KBr discs on a Nicolet 6700 Diamond ATR spectrometer in the 4000–200 cm^{-1} region. NMR spectra were recorded on a VNMRS 500-MHz spectrometer in DMSO-d_6 using TMS as a reference. Mass spectra, ESI-MS and EI-MS were recorded using LCQ Duo and double-focusing MS25RFA instruments, respectively. Electronic spectra were measured in DMF using a Hewlett-Packard 8453 spectrophotometer. Thermal analysis studies were made in the 20–800 °C range at a heating rate of 20 °C min^{-1} using Ni and NiCo as references, on a TA instrument TGA model Q500Analyzer TGA-50. Molar conductivity measurements were carried out at room temperature on a YSI Model 32 conductivity bridge. The GAUSSIAN 03 rev B-02 suite of programs was used in the computational calculations [27].

2.3. Synthesis

2.3.1. Cis-$Na_2[MoO_2(mesc)_2].2.5H_2O$

An aqueous solution of $Na_2[MoO_4].2H_2O$ (0.242 g, 1 mmol; 5 mL) was added to H_2mesc (0.192 g, 1 mmol) in methanol (15 mL). The mixture was stirred for 2 h and the resulting red-brown precipitate was filtered off, washed with ice-cold water, methanol and air-dried. *Anal.* Calc. For $C_{20}H_{17}MoNa_2O_{12.5}$: C, 40.1; H, 2.8; Na, 7.8; Mo, 16.0%, Found: C, 40.1, H, 2.7; Na, 7.7; Mo, 16.2%. Conductivity data (10^{-3} M in DMF): Λ_M = 160 Ohm^{-1}. IR (cm^{-1}) ν(C=O) 1660; ν(C—C) 1492; ν(C—O) 1257; ν_s(Mo—O) 929; ν_{as}(Mo—O) 909; ν(MoO_2) 743. ^{1}H NMR (d_6-DMSO/TMS, ppm) δ: CH_3, 3.33; H(3), 5.63; H(8), 5.98; H(5), 6.42. ESI-MS: *m*/*z*: 509.7 (Calcd 508.94) $[MoO_2(mesc)_2]^+$, 350.9 (Calcd 349.9) $[MoO_2(mesc)O_2]^+$. 318.2 (Calcd 317.9) $[MoO_2(mesc)]^+$.

2.3.2. Cis-$(PPh_4)_2[Mo_2O_5(mesc)_2].2H_2O$

An aqueous solution of $Na_2[MoO_4].2H_2O$ (0.242 g, 1 mmol; 5 mL) was added to H_2mesc (0.192 g, 1 mmol) in water (20 mL). To the resulting red-brown solution, tetraphenylphosphonium chloride (0.76 g, 2 mmol) in water (5 mL) was added. A red-brown precipitate was produced, filtered off, washed with water and air-dried. *Anal.* Calc. For $C_{68}H_{56}Mo_2O_{15}P_2$: C, 59.7; H, 4.1; Mo, 14.0%, Found: C, 59.6, H, 4.0; Mo, 14.1%. Conductivity data (10^{-3} M in DMF): Λ_M = 42 Ohm^{-1}. IR (cm^{-1}) ν(C=O) 1662; ν(C—C) 1491; ν(C—O) 1255; ν_s(Mo—O) 930; ν_{as}(Mo—O) 910; ν(MoO_2) 745. ^{1}H NMR (d_6-DMSO/TMS, ppm) δ: CH_3, 3.31; H(3), 5.65; H(8), 6.01; H(5), 6.44. ESI-MS: *m*/*z*: 493.2 (Calcd 493.88) $[Mo_2O_5(mesc)O_2]^+$.

2.3.3. $[M(PPh_3)_2(mesc)]$ (M(II) = Pd, Pt)

H_2mesc (0.096 g, 0.5 mmol) was added to a suspension of $[M(PPh_3)_2Cl_2]$ (M(II) = Pd, Pt) (0.5 mmol) in dichloromethane (10 mL). The mixture was heated under reflux for 36 h. The yellow