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Di- and tri-organotin(IV) complexes of arylhydrazones of methylene active compounds and their antiproliferative activity

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ABSTRACT

Two organotin(IV) complexes, $[Sn(C_6H_5)_3HL^1]$ (**1**) and $[Sn(C_2H_5)_2(1\kappa O,2\kappa O\text{-}H_3L^2)(1\kappa O^2\text{-}H_3L^2)(\mu_3\text{-}O)]_2$ (**2**), were isolated upon interaction of Ph_3SnCl and Et_2SnO with 2-(2-(2,4-dioxopentan-3-ylidene)hydrazinyl) benzoic acid (H_2L^1) and 2-(2-(2,4,6-trioxotetrahydropyrimidin-5(2H)-ylidene) hydrazinyl)benzoic acid (H_4L^2), respectively, in toluene solution. Complexes **1** and **2** were characterized by IR and NMR spectroscopies, elemental and single crystal X-ray diffraction analyses. While in **1** the $(HL^1)^-$ ligand binds the metal in a chelating bidentate mode, in **2** the $(H_3L^2)^-$ anion acts not only as a chelating bidentate but also as a bridging bidentate donor. The *in vitro* antiproliferative activity against human colorectal carcinoma (HCT116) and human hepatocellular carcinoma (HEPG2) cells lines demonstrated that compound **1** possesses high *in vitro* antiproliferative activity with IC_{50} values of 0.0284 ± 0.0001 μM and 0.287 ± 0.0001 μM for HCT116 and HEPG2 cells, respectively.

1. Introduction

Organotin(IV) compounds have emerged as potential candidates for metallopharmaceuticals, in particular for cancer chemotherapy, due to their apoptotic inducing character [1–3] and antiproliferative properties [4–6]. The antiproliferative activity, in some cases, is higher than the corresponding activity of cisplatin or other drugs used for the clinical cancer treatment. Some organotin complexes have been tested *in vivo* with encouraging results [7–11].

Although the mechanism of their activity is not well established, it has been suggested [12] that organotin(IV) compounds yield antiproliferative effects through binding to the thiol groups of proteins, hence, differing from the behaviour of other cytotoxic complexes which usually interact with DNA [13,14]. In particular, organotin(IV) carboxylates have attracted much interest because of their bioactivities as antiviral, antibacterial and antifungal agents, wood preservatives, pesticides, etc. [15–20]. It was demonstrated that the activities are essentially related to the number and nature of the organic groups attached to the central Sn atom, but the role of carboxylate ligands, of the molecule structure and of the metal coordination number are also very important [16,20,22–30]. Thus, triorganotin(IV) compounds display a larger array of biological activity than their di- and mono-organotin(IV) analogues. The tin(IV) atom in organotin carboxylates can either be four-, five- or six-coordinated, while mono- and bidentate modes of carboxylate group in such compounds were reported [22,23]. Evidencing the role of carboxylate groups in the geometry of tin compounds, a bridging mode usually leads to a polynuclear compound with pentacoordinated Sn(IV) while a monomeric compound results when the carboxylate ligand acts as a nonbridging bidentate ligand [21,22].

Additionally, arylhydrazones of methylene active compounds (AHMACs) are of a great potential in medicinal chemistry [31–38]. They have been tested as potential analgesic [31,32], antipyretic [32], antibacterial [33–37] and antifungal [38] drugs. It was demonstrated that AHMACs can form complexes with various metals which possess interesting structural, analytical, magnetic and/or catalytic properties [39–52]. The preparative procedures for these complexes are usually rather straightforward giving high yields of final products. As far as we know, only one Sn^{IV} complex with an AHMAC ligand has been reported, together with its *in vitro* antifungal activity [38].

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One of the methylene active compounds used as starting material in the synthesis of biologically active molecules is acetylacetone (acac, Scheme 1), and some of its derivatives have already been commercialized [53–56]. Another perspective moiety to be introduced is barbituric acid (BA, Scheme 1). The biological activity of barbiturates is well renown [49,50] and was shown to be related with the nature of the substituent in position C-5 [57,58]. An arylhydrazone moiety can be easily introduced to the C-5 position by treatment of BA with aromatic diazonium salts in ethanolic solution (Japp–Klingemann reaction) [59–64]. The arylhydrazones of acetylacetone and BA can be further used as intermediates in organic synthesis [64] or as ligands in coordination chemistry [59–63], however their antiproliferative properties have not yet been studied. On the other hand, introduction of the –COOH group to the aryl moiety improves the coordination ability of an AHMAC ligand and enhances its affinity *e.g.* to Sn(IV) [38].

We focused this work on the following aims: i) to prepare, by an easy and convenient way, new Sn^{IV}–AHMAC complexes with alkyl, aryl, acac, barbiturate moieties and the –COOH substituent; ii) to study the *in vitro* antitumor activity of the synthesized complexes. To reach these aims, 2-(2-(2,4-dioxopentan-3-ylidene)hydrazinyl) benzoic acid (H_2L^1) and 2-(2-(2,4,6-trioxotetrahydro-pyrimidin-5(2H)-ylidene)hydrazinyl)benzoic acid (H_4L^2) (Scheme 1) were chosen as ligand precursors and Ph_3SnCl and Et_2SnO as tin(IV) sources.

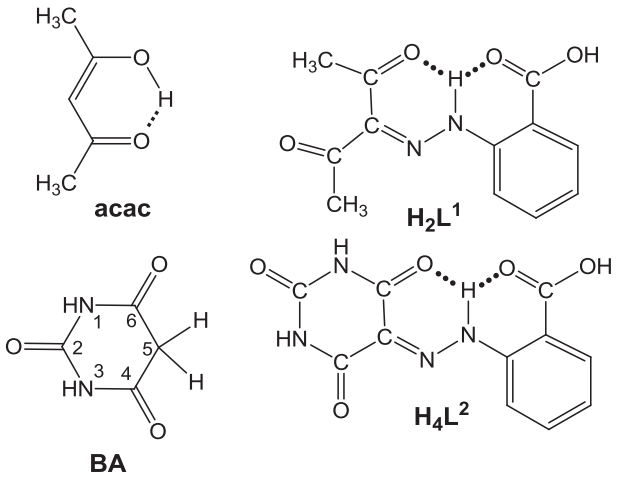
2. Experimental section

2.1. Materials and instrumentation

All the chemicals were obtained from commercial sources (Aldrich) and used as received. Infrared spectra (4000–400 cm^{-1}) were recorded on a BIO-RAD FTS 3000MX instrument in KBr pellets. ^1H and ^{13}C $\{^1H\}$ NMR spectra were recorded on a Bruker Advance II+ 300.13 (75.468 carbon-13) MHz (UltraShield™ Magnet) spectrometer at ambient temperature. C, H and N elemental analyses were carried out by the Microanalytical Service of the Instituto Superior Técnico.

2.2. Syntheses of organotin(IV) complexes

The syntheses and characterization of the AHMACs compounds H_2L^1 [65,66] and H_4L^2 [60] were reported earlier and will not be discussed here.



Scheme 1.

2.2.1. Synthesis of **1**

0.050 g (0.20 mmol) of H_2L^1 was dissolved in hot anhydrous toluene (30 mL) and this solution was added dropwise with continuous stirring to a hot anhydrous toluene solution (30 mL) of Ph_3SnCl (0.075 g, 0.20 mmol). The reaction mixture was refluxed for 2 h, then triethylamine (0.040 g, 0.40 mmol) was added, and the refluxing was continued for additional 1.5 h. The reaction mixture was cooled to r.t. and filtered to remove Et_3NHCl. The filtrate was collected and taken to dryness. The residue was dissolved in hexane with heating and filtered while hot. The crude product was obtained after evaporation of hexane and was then recrystallized from a mixture of toluene–hexane (1:1), yielding the yellow crystalline product **1**.

Yield: 62% (based on Sn). Calcd. for $C_{30}H_{26}N_2O_4Sn$ ($M = 597.25$): C, 60.33; H, 4.39; N, 4.69; Found: C, 60.23; H, 4.21; N, 4.52%. IR, cm^{-1}: 3496 ν(NH), 1685 ν(C=O), 1647 ν(C=O), 1612 ν(C=O···H), 1584 ν(C=N). ^1H NMR (300.13 MHz, DMSO-d_6) δ: 2.48 (s, CH_3), 2.57 (s, CH_3), 7.41–7.53 ($3C_6H_5$), 7.64–7.89 (C_6H_4), 15.86 (s, 1H, NH). ^{13}C $\{^1H\}$ NMR (75.468 MHz) in DMSO, internal TMS, δ (ppm): 26.90 (CH_3), 31.63 (CH_3), 114.92 (Ar–CC=O), 120.71 (Ar–NH–N), 123.76 (Ar–H), 128.65 (3Ar–H), 131.47 (Ar–H), 132.41 (6Ar–H), 132.69 (Ar–H), 134.33 (Ar–H), 136.21 (6Ar–H), 138.74 (3Ar–Sn), 143.45 (C=N), 168.22 (C=O), 195.06 (C=O), 197.12 (C=O). Due to the low solubility of **1**, the ^{119}Sn NMR was not performed.

2.2.2. Synthesis of **2**

0.050 g (0.20 mmol) of H_4L^2 and the equimolar amount of $(Et_2Sn)O$ were dissolved in hot anhydrous toluene (80 mL). The reaction mixture was refluxed for 8 h in a Dean and Stark apparatus. The water generated from the reaction mixture was separated azeotropically. The reaction mixture was cooled to r.t. and filtered to remove the formed admixtures; the filtrate was collected and taken to dryness. The residue was washed with hexane, dissolved in toluene and the solution was filtered. The crude product was obtained after evaporation of toluene; it was then recrystallized from a mixture of toluene–chloroform (1:1), affording the yellow crystalline product **2**.

Yield: 56% (based on Sn). Calcd. for $C_{60}H_{68}N_{16}O_{22}Sn_4 \cdot CHCl_3$ ($M = 1864.00$): C, 38.79; H, 3.69; N, 12.02; Found: C, 38.81; H, 3.60; N, 12.01%. IR (KBr): 3069 and 2854 ν(NH), 1726 ν(C=O), 1676 ν(C=O···H), 1590 ν(C=N) cm^{-1}. ^1H NMR (300.130 MHz) in DMSO, internal TMS, δ (ppm): 1.01 (s, $2CH_3$), 1.97 ($2CH_2$), 7.06–7.85 (4H, Ar–H), 11.38 and 11.53 (2H, N–H), 15.78 (1H, N–H). ^{13}C $\{^1H\}$ NMR (75.468 MHz) in DMSO, internal TMS, δ (ppm): 14.43 ($2CH_3$), 22.34 ($2CH_2$), 117.04 (Ar–H), 117.78 (Ar–H), 120.37 (Ar–H), 125.45 (Ar–H), 131.88 (C=N), 135.12 (Ar–NH–N), 144.69 (Ar–CC=O), 150.43 (C=O), 160.65 (C=O), 161.07 (C=O), 168.72 (ArCOOH). Due to the low solubility of **2,** the ^{119}Sn NMR was not performed.

2.3. X-ray structure determination

Crystals of **1** and **2** suitable for X-ray structural analysis were grown by slow evaporation, at r.t., of their toluene solutions. The data were collected using a Bruker AXS-KAPPA APEX II diffractometer with graphite-monochromated Mo-Kα radiation. Data were collected at 150 K using omega scans of 0.5° per frame, and a full sphere of data was obtained. Cell parameters were retrieved using Bruker SMART software and refined using Bruker SAINT [67] on all the observed reflections. Absorption corrections were applied using SADABS [67]. Structures were solved by direct methods using the SHELXS-97 package [68] and refined with SHELXL-97 [68]. Calculations were performed using the WinGX System–Version 1.80.03 [69]. There were disordered molecules present in the structure of **2** with no obvious major site occupations found and thus it was not possible to model them. PLATON/SQUEEZE [70] was