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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.

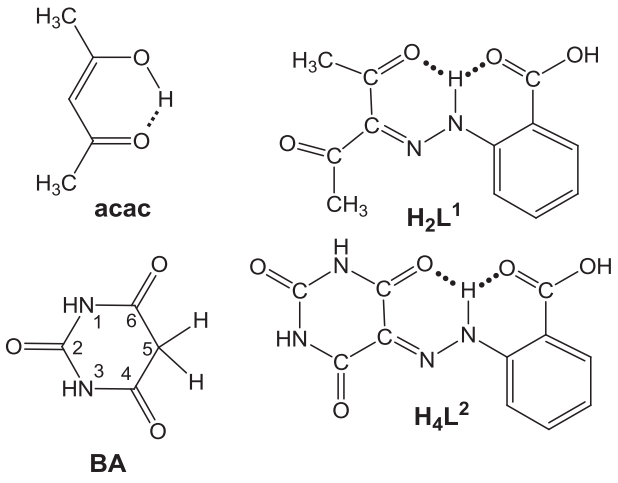
2. Experimental section

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

used to correct the data and potential volume of 229 Å^3 was found with 98 electrons per unit cell worth of scattering. These were removed from the models and not included in the empirical formula. The crystallographic details are listed in Table 1.

2.4. In vitro cytotoxicity assessment

Cell culture. Two tumor cell lines from human colorectal carcinoma (HCT116) and human hepatocellular carcinoma (HepG2) were grown as described [71]. For the dose–response curves, cells were seeded at a concentration of 7500 cells per well in 96-well plates. After a 24 h incubation period, old media was removed and replaced with fresh media containing **1** or **2** in concentrations 0.025–0.075 μM (for **1**) and 1–100 μM (for **2**) or 0.1% (v/v) DMSO (vehicle control). All the previous solutions were prepared from the 1000-times concentrated stock solutions of **1** or **2** to assure a maximum volume of DMSO in culture medium of 0.1% (v/v).

Viability assays. After 48 h of cell incubation with **1** or **2** and vehicle control, cell viability was evaluated using CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI, USA), as described [71]. All data were expressed as mean ± SEM from at least three independent experiments.

3. Results and discussion

3.1. Synthesis and characterization of *1* and *2*

H_2L^1 and H_4L^2 were synthesized by diazotation of 2-aminobenzoic acid and reaction of the corresponding diazonium salt with acetylacetone (acac) and BA, respectively, following the Japp–Klingemann procedure [60,65,66]. The analytic and spectroscopic characteristics of H_2L^1 and H_4L^2 support the proposed formulation (See Electronic Supplementary information for details) and agree with the reported data [60,65,66].

The reaction of Ph_3SnCl and H_2L^1 in the presence of triethylamine in hot anhydrous toluene for 1 h afforded **1** (Scheme 2) in 62% yield. The treatment of H_4L^2 with Et_2SnO in hot anhydrous toluene for 8 h gave a mixture of products; thus, compound **2** (Scheme 2) was isolated by following more laborious procedures which involved selective dissolutions and filtrations (see experimental for details). The IR spectra of **1** and **2** show the ν(NH), ν(C=O) and ν(C=O···H) vibrations at 3496, 1647 1612, and 2854, 1726, 1676 cm^{-1} respectively, while for H_2L^1 and H_4L^2 ν(NH), ν(C=O), and ν(C=O···H) are observed at 3482, 1638, 1601, and 2836, 1723, 1669 cm^{-1}, correspondingly.

Table 1
Crystallographic data and structure refinement details for **1** and **2**.

	1	**2**
Empirical formula	$C_{30}H_{26}N_2O_4Sn$	$C_{60}H_{68}N_{16}O_{22}Sn_4$
Fw	597.22	1840.06
λ (Å)	0.71069	0.71069
Cryst syst	Triclinic	Triclinic
Space group	$P-1$	$P-1$
a (Å)	11.5306 (14)	10.5505 (3)
b (Å)	14.418 (2)	11.1018 (4)
c (Å)	16.727 (4)	18.0829 (6)
α (°)	88.736 (5)	83.926 (2)
β (°)	79.344 (5)°	76.211 (3)
Γ (°)	72.819 (4)	66.051 (2)
V (Å^3)	2609.3 (8)	1879.81 (11)
Z	4	1
ρ_{calc} (Mg/m^3)	1.520	1.625
μ(Mo Kα) (mm^{-1})	1.018	1.394
No. reflns. read	23,947	20,704
No. reflns. unique	9244	7710
No. reflns. Obs.	6997	4833
GOOF	1.092	0.868
R_{int}	0.0515	0.0598
R1[a] ($I \geq 2\sigma$)	0.0675	0.0451
wR2[b] ($I \geq 2\sigma$)	0.1692	0.0997

[a] $R1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$.
[b] $wR2 = [\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]]^{1/2}$.

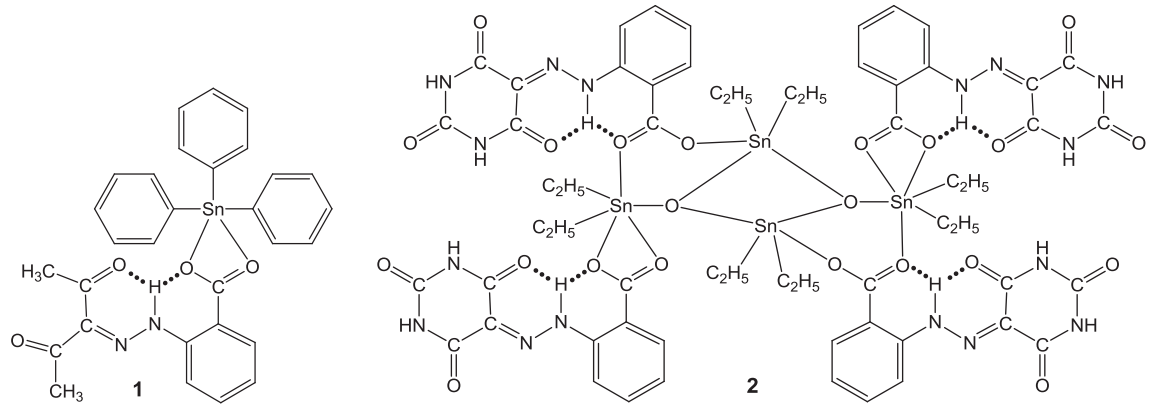
The observed bathochromic shift of the IR wavenumbers in **1** and **2** can be related to a weakening of the intramolecular resonance assisted hydrogen bond (RAHB) [72–76] in the complexes in comparison to the free organic compounds. This conclusion is also supported by the ^{1}H NMR spectra where the signals for the hydrazone proton were detected at slightly lower fields (δ 15.86 and 15.78 for **1** and **2**, respectively) relatively to those for H_2L^1 (δ 15.71) and H_4L^2 (δ 15.27). Hence, upon complexation to a Sn^{IV} centre these protons become less shielded and their mobility in the three-centred RAHB increases. In this respect, if compared with the BA moiety, the acac fragment increases the mobility of the NH proton in the free ligands and in complexes.

Although, the AHMACs ligands in **1** and **2** preserve the three-centred RAHB system linking the NH moiety to the O_{ketone} and to the $O_{carboxyl}$ atoms, the N···O_{ketone}, N···$O_{carboxyl}$ and O_{ketone}···$O_{carboxyl}$ distances in the three-centred H_2L^1 RAHB system change upon deprotonation of the carboxylic moiety varying from 2.668, 2.582 and 3.031 Å in the neutral organic molecule to 2.596, 2.627 and 3.000 Å in the anionic group [66], values that are very close to the average found in complex **1** (see below and Fig. 1).

3.2. Description of the X-ray crystal structures

The asymmetric unit of **1** comprises two independent molecules of the mononuclear tin compound (Fig. 1). Each molecule contains a five coordinate Sn(IV) metal centre in distorted trigonal bipyramidal environments (τ_5 = 0.45 and 0.51), with the anionic $(HL^1)^-$ group acting as a bidentate μ_1-O,O chelator; the remaining coordination sites are occupied by the carbon atoms of three phenyl groups. The geometry of the two complex molecules differ slightly as a result of the relative orientations of the phenyl ligands, with the angles between the least–square planes of the rings in the Sn1 molecule being considerably shorter than those in the Sn2 one (Fig. S1). In each molecule of **1**, and as expected, one of the metal-oxygen bond distances is longer than the other (*viz*. Fig 1 legend). However, the Sn1–O1 length [2.528(6) Å] is considerably shorter than the related Sn2–O22 [2.604(6) Å], though in the range of those found in other tin-carboxylate compounds [21,77–79]. Despite the fact that a base (triethylamine) was used for the syntheses of **1**, the RAHB system was not destroyed and the hydrazone hydrogen atoms participate in intramolecular three-centred RAHB systems (Fig. 1). Resulting from such contacts, the carbon–oxygen bonds of the ketone moieties in the ligands [1.228(10) and 1.224(11) Å for the Sn1 and the Sn2 molecules, respectively] are slightly longer than the non-interacting ketone groups [1.216(10) and 1.209(11) Å for the Sn1 and the Sn2 molecules, respectively].

The molecular structure of **2** consists of a distannoxane dimer, which is built up around a centrosymmetric cyclic $Et_4Sn_2O_2$ core (Fig. 2) where the anionic $(H_3L^2)^-$ ligands act as chelating bidentate (to Sn2) and as bridging bidentate (between Sn1 and Sn2) chelators. The bridging carboxylate group belongs to a half-chair 6-membered SnOCOSn*O* unit, with puckering parameters Q_T = 0.641(4), φ = 331.5(4)° and θ = 88.4(4)° [80], and contains a three-coordinate *O* atom; through a Sn*O* edge, that ring connects to the central 4-membered core. The coordination geometry of Sn1 is distorted trigonal bipyramidal formed by three oxygen atoms in the equatorial positions (two μ_3-O and one O atom of the bridging carboxylate) and two C atoms of the ethyl groups in the apical sites. However, via an intramolecular contact with one of the oxygen



Scheme 2. Schematic representations of complexes **1** and **2**.

atoms of the neighbouring chelating bidentate carboxylate group (Sn1···O21 3.087(4) Å], this metal atom features a skewed trapezoidal bipyramidal geometry. This type of coordination environment is recognized for Sn2 where the basal plane includes both oxygen atoms of the chelating bidentate and one of the bridging bidentate carboxylate units, as well as the μ_3-oxygen; two C atoms of the ethyl groups occupy the axial positions. Similarly to what occurred with the other tin atom, the coordination number of Sn2 can be foresaw as pentagonal bipyramidal by means of the (comparably much weaker) intramolecular interaction with the O13 carbonyl atom [3.211(4) Å]. The Sn–O distances in the central core differ significantly [2.028(4) and 2.157(3) Å] and agree with those found in the literature [81,82]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Similarly to what has previously been reported [40,46,66,83], the molecular structure of **2** contains two intramolecular six-membered hydrogen-bonded rings. As was mentioned above, this behaviour is well described by the so-called RAHB model which relates to the synergistic mutual reinforcement of intramolecular hydrogen bonding due to π-electron delocalization [72–76]. The geometry, the typical double bond length of C8–N12 [1.295(7) Å] and the formation of intramolecular N–H···O hydrogen bonds indicate that the hydrazone is the preferred tautomeric form of the molecule. However, the N11–N12 distance [1.312(6) Å] suggests a partial double bond character for this bond and thus some degree of conjugation with the aromatic ring.

Besides the RAHB, which is present in both Sn complexes, **2** also displays intramolecular and intermolecular hydrogen contacts (Fig. S2) the latter leading to a 1D chain with base vector [1–21].

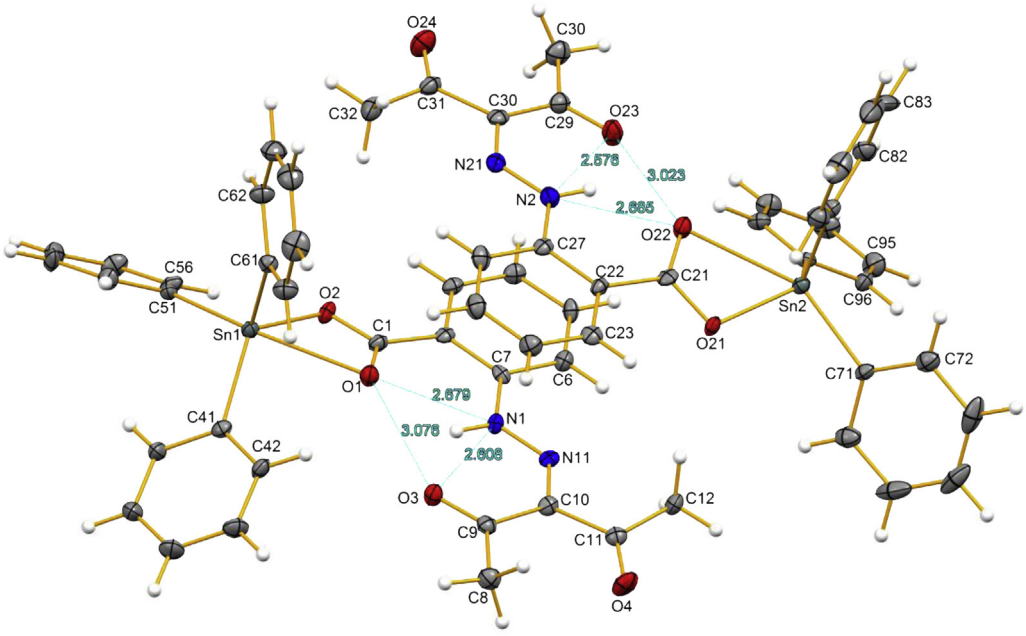


Fig. 1. ORTEP diagram of the molecules of complex **1** with atomic numbering scheme and indicating the distances (lines and values in light blue) in the three-centered H_2L^1 RAHB system. Ellipsoids are drawn at 30% probability. Selected bond distances (Å) and angles (°): C1–O1 1.229(10), C7–N1 1.396(10), N1–N11 1.309(9), O1–Sn1 2.528(6), O2–Sn1 2.096(6), C41–Sn1 2.123(8), C51–Sn1 2.148(8), C61–Sn1 2.114(8); C27–N2 1.414(11), C21–O21 1.318(10), C21–O22 1.232(10), N2–N21 1.316(10), O21–Sn2 2.092(6), O22–Sn2 2.604(6), C71–Sn2 2.137(8), C81–Sn2 2.120(8), C91–Sn2 2.125(8). O2–Sn1–O1 55.1(2), C41–Sn1–O1 84.1(3), C51–Sn1–O1 148.9(3), C61–Sn1–O1 90.6(3), O2–Sn1–C41 122.1(3), O2–Sn1–C51 96.0(3), O2–Sn1–C61 107.3(3), C61–Sn1–C41 112.8(3), C61–Sn1–C51 110.8(3), C41–Sn1–C51 106.4(3); O21–Sn2–C71 97.5(3), O21–Sn2–C81 104.2(3), O21–Sn2–C91 120.9(3), C81–Sn2–C71 108.1(3), C81–Sn2–C91 116.5(3), C91–Sn2–C71 107.5(3).

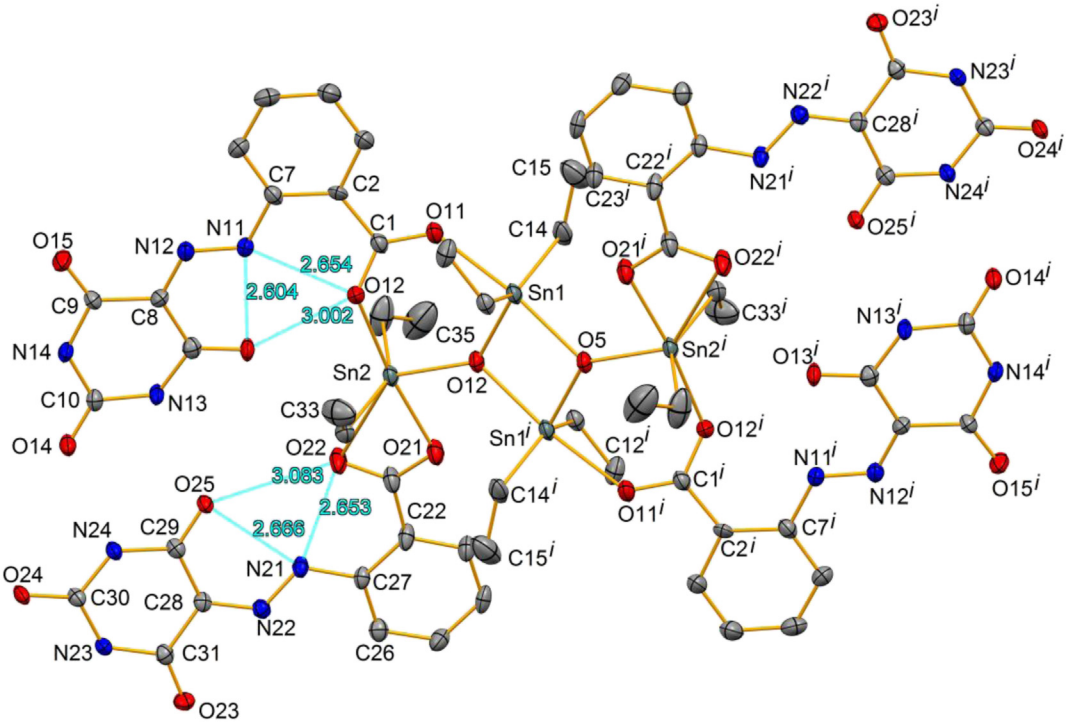
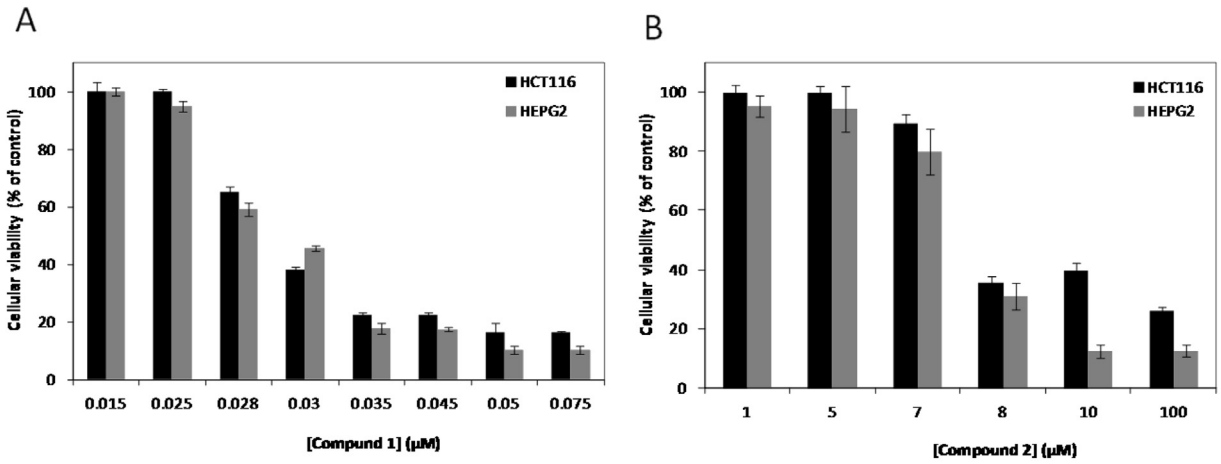


Fig. 2. ORTEP diagram of the molecules of complex **2** with atomic numbering scheme and indicating the distances (lines and values in light blue) in the three-centered H_2L^1 RAHB system. Ellipsoids are drawn at 30% probability and hydrogen atoms were omitted for clarity. Selected bond distances (Å) and angles (°): C1–O11 1.242(6), C1–O12 1.261(6), C7–N11 1.412(7), C8–N12 1.295(7), C21–O21 1.283(6), C21–O22 1.245(6), N11–N12 1.312(6), N21–N22 1.295(6), C7–N11 1.412(7), C8–N12 1.295(7), C27–N21 1.401(6), C28–N22 1.326(6), C12–Sn1 2.130(5), C14–Sn1 2.123(6), C32–Sn2 2.110(5), C34–Sn2 2.113(7), O5–Sn1 2.028(4), O11–Sn1 2.252(4), O5–Sn2 2.055(3), O5–Sn1^i 2.157(3), O12–Sn2 2.307(4), O12–Sn2 2.307(4), O21–Sn2 2.192(4), Sn1–Sn1^i 3.2467(8). Sn1–O5–Sn2 132.92(18), Sn1–O5–Sn1^i 101.69(14), Sn2–O5–Sn1^i 124.50(18), C14–Sn1–C12 141.0(3), C34–Sn2–C32 145.8(3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



C

Tumor cell line	IC_{50} values (μM)	
	Compound 1	Compound 2
HCT116	0.0284 ± 0.0001	7.35 ± 0.01
HEPG2	0.287 ± 0.0001	7.52 ± 0.07

Fig. 3. Dose-dependent cytotoxicity of the organotin compounds **1** (A) and **2** (B) in HCT116 (black) and HEPG2 cells (dark grey). Cells were incubated in the presence of the compounds for 48 h and their viability was evaluated by MTS assay. The corresponding IC_{50} values (C) were calculated by nonlinear regression analysis using GraphPad Prism (Graph Pad Software Inc, San Diego). The results are expressed as the mean ± SEM percentage compared to controls (0.1% (v/v) DMSO) from three independent experiments.

3.3. Biological activity studies

The *in vitro* cytotoxicity of **1** and **2** was evaluated in HCT116 and HEPG2 tumor cell lines using MTS metabolic assay and expressed as concentration of complex required to inhibit 50% of cell proliferation when compared to untreated cells (IC50; μM) [71] (Fig. 3). After 48 h of incubation, a decrease of cell viability was observed, in a dose-dependent manner, for both complex **1** and **2** in HCT116 and HEPG2 tumor cell lines (Fig. 3). The complex **1** possesses the highest activity against both tumor cell lines, with IC_{50} values of 0.0284 ± 0.0001 μM in HCT116 cells and 0.287 ± 0.0001 μM in HEPG2 cells (Fig. 3), when compared with IC_{50} values for complex **2** of 7.35 ± 0.01 μM (HCT116 cells) and 7.52 ± 0.07 μM (HEPG2 cells) (Fig. 3). Complex **2** presents IC_{50} values slightly higher than those of common chemotherapeutic agents such as cisplatin and doxorubicin (IC_{50} of 4.14 μM [84] and 1.4 μM [85], respectively) in colorectal carcinoma cell line, nevertheless, the most outstanding results was obtained from the activity of complex **1**, which is 145 times and 5 times better than that of cisplatin and doxorubicin, respectively, in colorectal carcinoma cell line evaluated.

In order to demonstrate that this antiproliferative potential observed was indeed due to an effect of the complexes, the dose-dependent cytotoxicity of the free ligands was assessed (Fig. S3). As we can observe for all the tested concentrations of the free ligands (1–100 μM) no loss of cell viability was observed (Fig. S3). These results clearly indicate that the high loss of cell viability observed in Fig. 3 is due to the presence of complexes **1** and **2**.

Previously, our group have also demonstrate the antiproliferative potential of a di-organotin complex in several tumor cell lines (IC_{50} values for malignant cell lines of 0.238 ± 0.011 μM (HCT116) and 0.199 ± 0.003 μM (HepG2)) [86]. The comparison of our previous results [86] with the results presented here indicates that the complex **1** has an IC_{50} value 10 times lower than the corresponding value for the di-organotin Ref. [86].

Several other organotin complexes with promising *in vitro* [15,20,77,87–89] antiproliferative activities in human tumor cell lines have been reported, most of them presenting IC_{50} in the same order of magnitude of the corresponding value for cisplatin but higher than the values for doxorubicin Refs. [15,20,87,89]. Nevertheless, a triphenyltin(IV) complex [SnPh3(DMFU)] and a tri-*n*-butyltin(IV) complex with non-enolic bidentate O,O-coordination of Schiff base ligand presented IC_{50} that were 100× smaller than the corresponding value for cisplatin [77,88] and very similar to the complex **1** presented here. Taking together, these results indicate that despite these are preliminary results, further studies on the antitumor effects of complex **1** is highly recommended.

4. Conclusions

Our results reveal the potential interest of the AHMAC ligands as building blocks in the construction of assemblies which involve organotin–oxygen clusters. In fact, we have synthesized and fully characterized two new organotin(IV) compounds, $[Sn(C_6H_5)_3HL^1]$ (**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**) containing, as ligands, arylhydrazones of methylene active acetylacetone or barbituric acid. While in **1** the ligated $(HL^1)^-$ behaves as a chelating bidentate ligand, in **2** the $(H_3L^2)^-$ moiety presents both this mode of linkage and the bridging bidentate one. Compound **2** appears to be the first example of a tetranuclear oxoalkylyltin(IV) AHMAC compound to be fully described. The antiproliferative activity of **1** and **2** were also evaluated, the activity of the former being higher than that of the latter. Complexes **1** and **2** seem to be promising candidates for further *in vitro* investigations on other tumor cell lines and compound **1** for possible *in vivo* tests.

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Appendix A. Supplementary material

Analytical data for compounds H_2L^1 and H_4L^2. CCDC 964527, and 964528 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Appendix B. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jorganchem.2013.12.019.

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