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Synthesis, characterization and biological studies of 1-D polymeric triphenyltin(IV) carboxylates

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ABSTRACT

The triphenyltin(IV) carboxylate compounds [{$SnPh_3(O_2CCH_2SXyl)$}$_\infty$] (**1**) (Xyl = 3,5-$Me_2C_6H_3$) and [{$SnPh_3(O_2CCH_2SMes)$}$_\infty$] (**2**) (Mes = 2,4,6-$Me_3C_6H_2$) have been synthesized by the reaction of $SnPh_3Cl$ with one equivalent of xylylthioacetic acid or mesitylthioacetic acid, respectively. **1** and **2** have been characterized by spectroscopic methods. The cytotoxic activity of **1** and **2** was tested against human tumour cell lines from four different histogenic origin: 8505C (anaplastic thyroid cancer), DLD-1 (colon cancer) and the cisplatin sensitive A253 (head and neck cancer) and A549 (lung carcinoma) and compared with those of the reference complex cisplatin. Interestingly, the cytotoxic activities of the carboxylate derivatives were higher than those of cisplatin against all the studied cells. DNA-interaction tests have been also carried out. Solutions of all the studied complexes have been treated with different concentrations of fish sperm DNA (FS-DNA), observing modifications of the UV spectra with intrinsic binding constants of 1.68×10^5 and 1.02×10^5, M^{-1} for **1** and **2**, respectively. In addition, the molecular structure of **2** has been determined by single crystal X-ray diffraction studies, observing that **2** consists of a 1-D coordination polymer in which the tin atoms present a five-coordinated geometry by coordination of two different oxygen atoms of two crystallographically dependent carboxylato ligands.

1. Introduction

Research on medicinal applications of metal complexes is an area of current interest and one of the most studied facets in biomedical and inorganic chemistry [1–3]. In this context, the potential therapeutic properties of tin compounds were observed as early as 1929 [4], however, the antiproliferative properties of these complexes were not studied in detail until 1980 [5]. More recently, different studies with very interesting results on the *in vitro* antitumour properties of organotin compounds against a wide panel of tumour cell lines of human origin have been reported [6–12]. Thus, tin(IV) derivatives such as cyclopentadienyltin(IV) derivatives have only recently been studied very briefly [13,14], while other tin(IV) complexes such as those with carboxylato [15–22], thiolato [23–29] and dithiocarbamato [30] ligands have been extensively studied, observing that tin(IV) carboxylate complexes present usually the highest cytotoxic activity [11]. Modification of the carboxylato ligands or the alkyl or aryl substituents at tin(IV) has a notable effect on the antiproliferative effect of di- or tri-alkyl or aryltin(IV) carboxylate complexes in anticancer tests [11,21]. Usually, triorganotin(IV) compounds display a higher biological activity than their di- and mono-organotin(IV) counterparts, which has been related to their ability to bind to proteins [31–33]. Thus, as a continuation of our work on the cytotoxic properties of metal carboxylate complexes [21,34–37], we decided to synthesize novel triphenyltin(IV) carboxylate complexes with the xylylthioacetato or mesitylthioacetato ligands, which seem to have a positive influence on the cytotoxicity of gallium [36] and titanium [34] complexes.

In this report we present the synthesis, characterization and study of the cytotoxicity of the triphenyltin(IV) carboxylate compounds [{$SnPh_3(O_2CCH_2SXyl)$}$_\infty$] (**1**) (Xyl = 3,5-$Me_2C_6H_3$) and [{$SnPh_3(O_2CCH_2SMes)$}$_\infty$] (**2**) (Mes = 2,4,6-$Me_3C_6H_2$). In addition, the molecular structure of **2** which consists of a 1-D coordination polymer is described.

2. Experimental

2.1. General manipulations

All reactions were performed using standard Schlenk tube techniques in an atmosphere of dry nitrogen. Solvents and NEt_3

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were distilled from the appropriate drying agents and degassed before use. $SnPh_3Cl$ and NEt_3 were purchased from Aldrich. Mesitylthioacetic acid and xylylthioacetic acid were prepared with slight modification of the literature procedure [38]. IR spectra (KBr pellets prepared in a nitrogen-filled glove box) were recorded on a Perkin–Elmer System 2000 or on a Nicolet Avatar FT-IR spectrometer in the range 350–4000 cm^{-1}. ^1H, $^{13}C\{^1H\}$ and ^{119}Sn NMR spectra were recorded on a Varian Mercury FT-400 spectrometer or on a Bruker AVANCE-400 and referenced to the residual deuterated solvent. UV–vis measurements were performed at room temperature with an Analytik Jena Specord 200 spectrophotometer between 190 and 900 nm. Microanalyses were carried out with a Perkin–Elmer 2400 or LECO CHNS-932 microanalyzer.

2.2. Preparation of [{$SnPh_3(O_2CCH_2SXyl)$}$_\infty$] (**1**)

A solution of xylylthioacetic acid (0.26 g, 1.30 mmol) in toluene (50 mL) was added dropwise to a solution of $SnPh_3Cl$ (0.50 g 1.30 mmol) in toluene (50 mL) at room temperature. The reaction mixture was stirred for 20 min and NEt_3 (0.19 mL, 1.30 mmol) was then added dropwise. The reaction was then stirred at room temperature overnight. The mixture was filtered and the filtrate concentrated (10 mL) and cooled to −30 °C. Microcrystals of the title complex were isolated by filtration. Yield (calculated for the monomeric unit): 0.44 g, 62%. FT-IR (KBr): 1552 (s) (ν_a COO^-), 1426 (s) (ν_s COO^-), 694 (s) (ν Sn–C), 452 (m) (ν Sn–O); ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ 2.19 (s, 6H, *m*-Me of xylyl), 3.72 (s, 4H, CH_2), 6.75 (s, 2H, *o*-protons of xylyl), 6.95 (s, 1H, *p*-proton of xylyl), 7.46 (br m, 9H, *m*- and *p*- protons of $SnPh_3$), 7.70 (br m, 6H, *o*-protons of $SnPh_3$, ^3J (^1H–Sn) = ca. 50.2 Hz); $^{13}C\{^1H\}$ RMN (100.6 MHz, $CDCl_3$, 25 °C): δ 21.1 (*m*-Me of xylyl), 37.1 (CH_2), 128.9 (C-3 and C-5 of $SnPh_3$, ^3J (^{13}C–^{117}Sn) = 62.1 Hz and ^3J(^{13}C–^{119}Sn) = 64.7 Hz), 130.2 (C-4 of $SnPh_3$, ^4J(^{13}C–$^{117,119}Sn$) = 13.1 Hz), 136.7 (C-2 and C-6 of $SnPh_3$, ^2J ($^{13}C\{^1H\}$–$^{117,119}Sn$) = 48.7 Hz), 137.4 (C-1 of $SnPh_3$, ^1J (^{13}C–^{117}Sn) = 634.4 Hz and ^1J(^{13}C–^{119}Sn) = 662.8 Hz), 126.7 (C-2 and C-6 of xylyl), 128.0 (C-4 of xylyl), 135.6 (C-3 and C-5 of xylyl), 138.0 (C-1 of xylyl), 175.5 (COO) ppm. $^{119}Sn\{^1H\}$ NMR (149.2 MHz, $CDCl_3$, 25 °C): δ −101.9 ppm. FAB-MS (*m*/*e* (relative intensity)): 546 (1) [M^+], 367 (5) [M^+–$OCCH_2SMes$]. Elemental analysis, monomeric unit: $C_{28}H_{26}O_2SSn$ (545.28) calculated: C 61.67, H 4.81, found: C 61.29, H 4.70%.

2.3. Preparation of [{$SnPh_3(O_2CCH_2SMes)_\infty$] (**2**)

The synthesis of **2** was carried out in an identical manner to **1** starting from mesitylthioacetic acid (0.27 g, 1.30 mmol), $SnPh_3Cl$ (0.50 g, 1.30 mmol) and NEt_3 (0.19 mL, 1.30 mmol). Yield: 0.51 g, 70%. FT-IR (KBr): 1547 (s) (ν_a COO^-), 1392 (s) (ν_s COO^-), 695 (s) (ν Sn–C), 451 (m) (ν Sn–O); ^1H RMN (400 MHz, $CDCl_3$, 25 °C): δ 2.24 (s, 3H, *p*-Me of mesityl), 2.39 (s, 6H, *o*-Me of mesityl), 3.47 (s, 2H, CH_2), 6.82 (s, 2H, *m*-H of mesityl) 7.46 (br s, 9H, *m*- and *p*- protons of $SnPh_3$), 7.77 (br m, 6H, *o*-protons of $SnPh_3$, ^3J(^1H–Sn) = ca. 51.0 Hz). $^{13}C\{^1H\}$ RMN (100.6 MHz, $CDCl_3$, 25 °C): δ 21.0 (*p*-Me of mesityl), 21.7 (*o*-Me of mesityl), 37.1 (CH_2), 128.8 (C-3 and C-5 of $SnPh_3$, ^3J (^{13}C–^{117}Sn) = 61.7 Hz and ^3J(^{13}C–^{119}Sn) = 64.3 Hz), 130.2 (C-4 of $SnPh_3$, ^4J(^{13}C–$^{117,119}Sn$) = 13.4 Hz), 136.8 (C-2 and C-6 of $SnPh_3$, ^2J (^{13}C–$^{117,119}Sn$) = 48.3 Hz), 137.8 (C-1 of $SnPh_3$, ^1J(^{13}C–^{117}Sn) = 614.7 Hz and ^1J(^{13}C–^{119}Sn) = 640.4 Hz), 125.3 (C-4 of mesityl), 129.0 (C3-C5 of mesityl), 138.2 (C2-C6 of mesityl), 143.0 (C-1 of mesityl), 176.1 (COO) ppm. $^{119}Sn\{^1H\}$ NMR (149.2 MHz, $CDCl_3$, 25 °C): δ −102.7 ppm. FAB-MS (*m*/*e* (relative intensity)): 483 (5) [M^+–Ph], 351 (10) [M^+–$OOCCH_2SMes$], 195 (21) [M^+–$SnPh_3O$]. Elemental analysis, monomeric unit: $C_{29}H_{28}O_2SSn$ (559.31) calculated: C 62.28, H 5.05, found: C 62.11, H 5.09%.

2.4. Data collection and structural refinement of **2**

The data of **2** were collected with a CCD Oxford Xcalibur S ($\lambda(Mo_{K\alpha})$ = 0.71073 Å) using ω and φ scans mode. Semi-empirical from equivalents absorption corrections were carried out with SCALE3 ABSPACK [39]. All the structures were solved by direct methods [40]. Structure refinement was carried out with SHELXL-97 [41]. All non-hydrogen atoms were refined anisotropically, and hydrogen atoms were calculated with the riding model and refined isotropically. Crystallographic details are listed in Table 1.

2.5. In vitro studies

2.5.1. Preparation of drug solutions

Stock solutions of studied tin(IV) compounds (**1**–**2**) were prepared, for solubility reasons, in dimethyl sulfoxide (DMSO, Sigma Aldrich) at a concentration of 20 mM, filtered through Millipore filter (0.22 μm) before use, and diluted by nutrient medium to various working concentrations. Nutrient medium was RPMI-1640 (PAA Laboratories) supplemented with 10% fetal bovine serum (Biochrom AG) and 1% penicillin/streptomycin (PAA Laboratories).

2.5.2. Cell lines and culture conditions

The cell lines 8505C, A253, A549 and DLD-1, included in this study, were kindly provided by Dr. Thomas Mueller, Department of Hematology/Oncology, Martin Luther University of Halle-Wittenberg, Halle (Saale), Germany. Cultures were maintained as monolayer in RPMI-1640 (PAA Laboratories, Pasching, Germany) supplemented with 10% heat inactivated fetal bovine serum (Biochrom AG, Berlin, Germany) and penicillin/streptomycin (PAA Laboratories) at 37 °C in a humidified atmosphere of 5% (*v*/*v*) CO_2.

2.5.3. Cytotoxicity assay

The cytotoxic activities of the compounds were evaluated using the sulforhodamine-B (SRB, Sigma Aldrich) microculture

Table 1
Crystal data and structure refinement for **2**.

Formula	$C_{29}H_{28}O_2SSn$
Fw	559.26
T (K)	130(2)
Cryst syst	Monoclinic
Space group	*C*2/c
a (pm)	2649.25(6)
b (pm)	992.66(2)
c (pm)	1945.19(4)
α (deg)	90
β (deg)	104.670(2)
γ (deg)	90
V (nm^3)	4.94871(18)
Z	8
Dc (Mg m^{-3})	1.501
μ (mm^{-1})	1.141
F(000)	2272
Cryst dimens (mm)	0.4 × 0.3 × 0.1
θ range (deg)	2.94–28.28
hkl ranges	$-35 \le h \le 35$, $-13 \le k \le 13$, $-25 \le l \le 25$
Data/parameters	6140/301
Goodness-of-fit on F^2	0.984
Final *R* indices [$I > 2\sigma(I)$]	$R_1 = 0.0210$, $wR_2 = 0.0477$
R indices (all data)	$R_1 = 0.0311$, $wR_2 = 0.0490$
Largest diff. peak and hole (e.$Å^{-3}$)	0.719 and −0.318

$R_1 = \sum ||Fo| - |FC|| / \sum |Fo|$; $wR_2 = [\sum[w(Fo^2 - Fc^2)^2] / \sum[w(Fo^2)^2]]^{0.5}$

colorimetric assay [42]. In short, exponentially growing cells were seeded into 96-well plates on day 0 at the appropriate cell densities to prevent confluence of the cells during the period of experiment. After 24 h, the cells were treated with serial dilutions of the studied compounds for 96 h. Final concentrations achieved in treated wells were 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0, 100.0 μM for **1** and **2**. Each concentration was tested in triplicates on each cell line. The final concentration of DMSO solvent never exceeded 0.5%, which was non-toxic to the cells. The percentages of surviving cells relative to untreated controls were determined 96 h after the beginning of drug exposure. After 96 h treatment, the supernatant medium from the 96 well plates was eliminated and the cells were fixed with 10% TCA. For a thorough fixation, plates were then allowed to stand at 4 °C. After fixation, the cells were washed in a strip washer. The washing was carried out four times with water using alternate dispensing and aspiration procedures. The plates were then dyed with 100 μL of 0.4% SRB for about 45 min. After dying, the plates were again washed to remove the dye with 1% acetic acid and allowed to air dry overnight. 100 μL of 10 mM Tris base solutions were added to each well of the plate and absorbance was measured at 570 nm using a 96 well plate reader (Tecan Spectra, Crailsheim, Germany). The IC_{50} value, defined as the concentrations of the compound at which 50% cell inhibition was observed, was estimated from the dose-response curves.

2.6. DNA binding experiments monitored by UV–visible spectroscopy

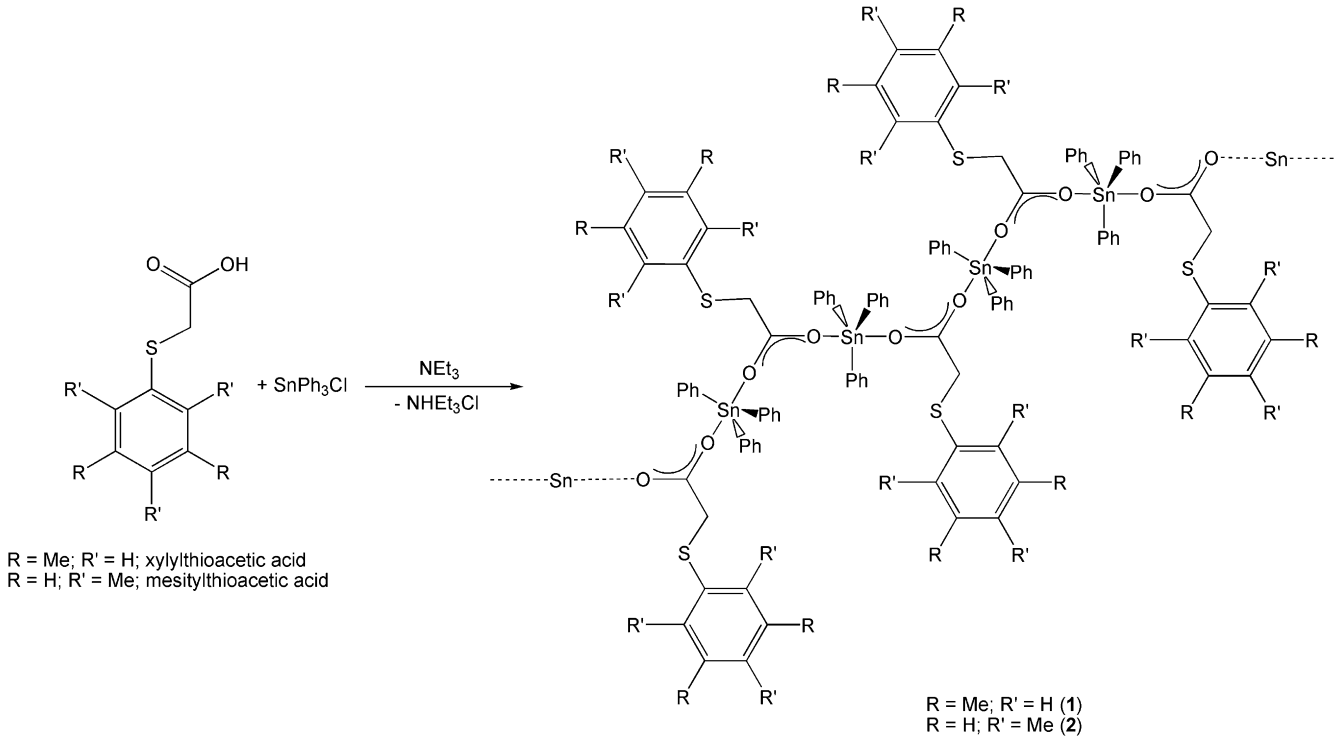
Fish sperm DNA (FS-DNA) was kindly provided by the Departamento de Ciencias de la Salud of the Universidad Rey Juan Carlos (Spain). The spectroscopic titration of FS-DNA was carried out in the buffer (50 mM NaCl–5 mM Tris–HCl, pH 7.1) at room temperature. A solution of FS-DNA in the buffer gave a ratio of UV absorbance 1.8–1.9:1 at 260 and 280 nm, indicating that the DNA was sufficiently free of protein [43]. Milli-Q water was used to prepare the solutions. The DNA concentration per nucleotide was determined adopting absorption spectroscopy using the known molar extinction coefficient value of 6600 M^{-1} cm^{-1} at 260 nm [44]. Absorption titrations were performed by using a fixed tin(IV) complex concentration to which increments of the DNA stock solution were added. Complex–DNA adducts solutions were incubated at 37 °C for 30 min before the absorption spectra were recorded.

3. Results and discussion

3.1. Synthesis and characterization of the triphenyltin(IV) complexes 1 and 2

Triphenyltin(IV) carboxylate complexes $[\{SnPh_3(O_2CCH_2SXyl)\}_\infty]$ (**1**) (Xyl = 3,5-$Me_2C_6H_3$) and $[\{SnPh_3(O_2CCH_2SMes)\}_\infty]$ (**2**) (Mes = 2,4,6-$Me_3C_6H_2$) were synthesized by the reaction of $SnPh_3Cl$ with one equivalent of xylylthioacetic acid or mesitylthioacetic acid in toluene at room temperature in the presence of stoichiometric amounts of NEt_3 (Scheme 1). Complexes **1** and **2** were isolated as colourless microcrystalline solids of high purity.

In the ^{1}H NMR spectrum of **1** the xylylthioacetato ligand gave a singlet at 2.19 ppm corresponding to the protons of the methyl groups of the xylyl moiety, a singlet at 3.72 ppm assigned to the methylene protons and a singlet at 6.75 ppm due to the aromatic protons of the phenyl ring. On the other hand, in the ^{1}H NMR spectrum of **2**, the mesitylthioacetato ligand gave two singlets at 2.24 and 2.39 ppm corresponding to the protons of the two different methyl groups of the mesityl moiety (*o*-methyl and *p*-methyl), one singlet at 3.47 corresponding to the methylene protons and one singlet at 6.82 ppm for the *m*-aromatic protons. In addition to these signals, two different multiplets, at ca. 7.5 ppm corresponding to the *m*- and *p*- protons and at 7.7 ppm assigned to the *o*-protons of $SnPh_3$ moiety, respectively, were observed. Satellite signals of the *o*-protons, due to coupling with the ^{117}Sn and



Scheme 1. Synthesis of triphenyltin(IV) complexes **1** and **2**.

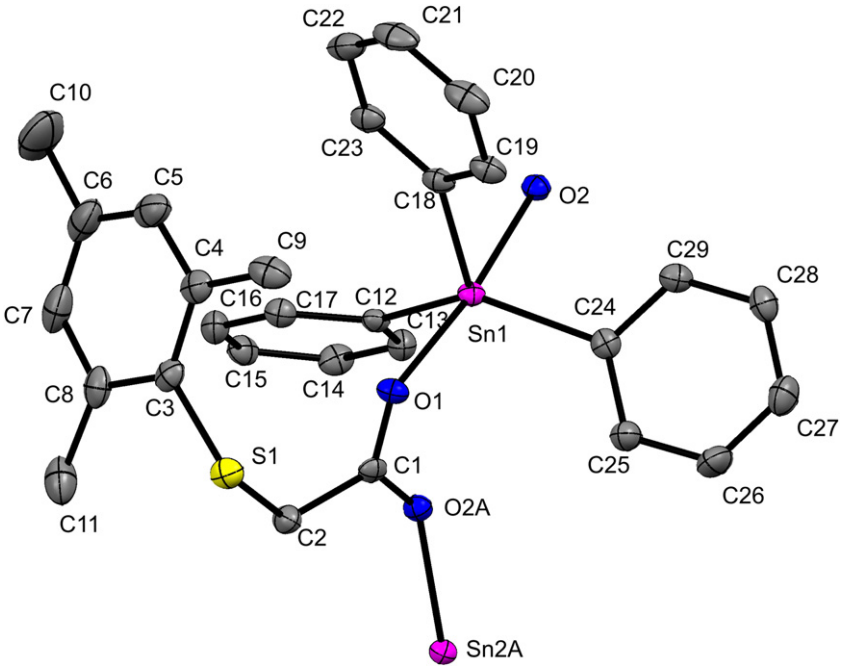


Fig. 1. Molecular structure and atom-labelling scheme for the monomeric unit of 2 with thermal ellipsoids at 50% probability (hydrogen atoms have been omitted for clarity).

^{119}Sn isotopes at a three bond distance were also observed, however, we were unable to resolve the independent satellite signals corresponding to the two tin nuclei. Therefore, the observed coupling constant for these protons of approximately 50 Hz is an approximate value that can be applied to either nucleus.

In the $^{13}C\{^1H\}$ NMR spectra of **1**–**2**, four signals at ca. 129, 130, 137 and 138 ppm were observed for the C-3/C-5, C-4, C-2/C-6 and C-1 carbon atoms of the phenyl groups. Tin-carbon coupling constants for these signals gave values of approximately $^1J(^{13}C–Sn)$ 610–660 Hz, $^2J(^{13}C–Sn)$ 48 Hz, $^3J(^{13}C–Sn)$ 65 Hz, and $^4J(^{13}C–Sn)$ 13 Hz. The $^1J(^{13}C–Sn)$ 610–660 Hz coupling constants show that the C–Sn–C angles in solution may be similar to those found in the solid state (see Section 3.2).

In addition to the signals assigned to the phenyl groups the carboxylato ligands showed the expected signals.

The IR spectra of the complexes **1** and **2** show strong bands in two different regions at ca. 1550 and 1400 cm^{-1}, which correspond to the asymmetric and symmetric vibrations, respectively, of the COO moiety. The differences between the asymmetric and symmetric vibrations of about 150 cm^{-1} indicate bridging bidentate coordination of the carboxylato ligand [45]. This phenomenon was also confirmed by X-ray diffraction studies (see Section 3.2).

3.2. Structural studies

2 crystallizes in the monoclinic space group *C*2/c with eight molecules of the monomeric unit located in the unit cell. The asymmetric unit of **2** (Fig. 1) consists of a carboxylato ligand coordinated to a $SnPh_3$ unit by a single oxygen, which aggregates *via* further Sn–O interactions to form a zig-zag 1-D coordination polymer (Fig. 2). Generally, triorganotin(IV) carboxylates with bulky R groups attached to the Sn atom favour tetrahedral monomeric structures, while sterically less demanding R groups favour bridged polymeric structures [46]. However, the molecular structure of **2** reveals that the central tin atom is penta-coordinated in slightly distorted trigonal bypyramidal geometry with the phenyl groups in equatorial positions and two oxygen atoms of two different carboxylato ligands in axial positions. This geometry is

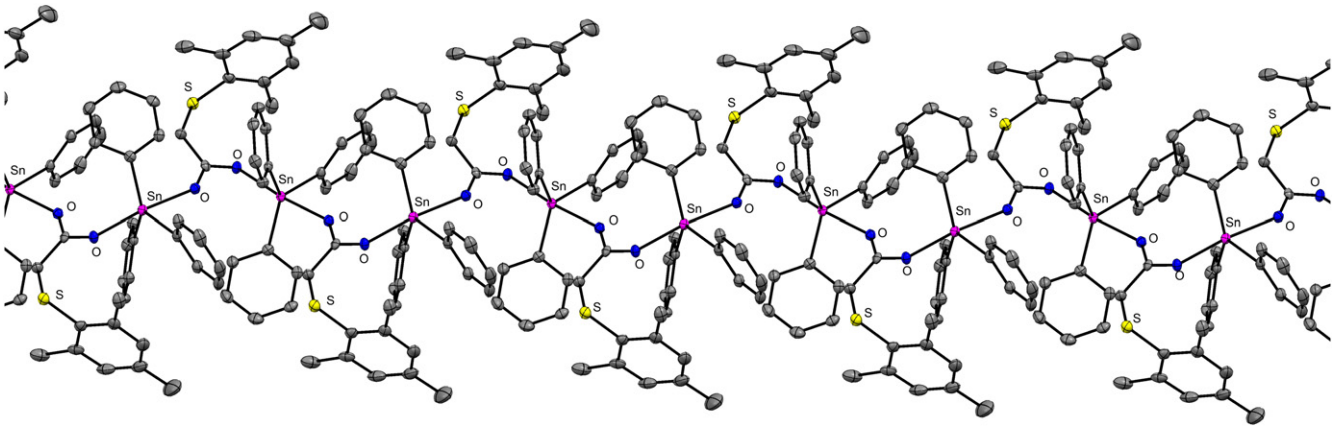


Fig. 2. 1-D zig-zag chain structure of **2**, propagation *via* O → Sn coordination.

Table 2
Selected Bond Lengths (pm) and Angles (°) for **2**.

	2
Sn(1)–C(24)	211.9(2)
Sn(1)–C(18)	212.3(2)
Sn(1)–C(12)	212.3(2)
Sn(1)–O(1)	219.9(2)
Sn(1)–O(2)	233.7(1)
O(1)–C(1)	126.7(2)
O(2A)–C(1)	125.2(2)
C(24)–Sn(1)–C(18)	108.80(7)
C(24)–Sn(1)–C(12)	134.67(6)
C(18)–Sn(1)–C(12)	115.95(7)
C(24)–Sn(1)–O(1)	96.96(6)
C(18)–Sn(1)–O(1)	91.71(5)
C(12)–Sn(1)–O(1)	88.77(5)
C(24)–Sn(1)–O(2)	90.32(5)
C(18)–Sn(1)–O(2)	88.05(5)
C(12)–Sn(1)–O(2)	84.51(5)
O(1)–Sn(1)–O(2)	172.39(4)

Symmetry transformations used to generate equivalent atoms: A = −x+1/2, y+1/2, −z+3/2.

indicated by the sum of the angles C(24)–Sn(1)–C(18) 108.80(7)°, C(24)–Sn(1)–C(12) 134.67(6)°, C(18)–Sn(1)–C(12) 115.95(7)° which is 359.42° and shows that Sn(1), C(12), C(18) and C(24) are coplanar. In addition, the angles C(24)–Sn(1)–O(1) 96.96(6)°, C(18)–Sn(1)–O(1) 91.71(5)°, C(12)–Sn(1)–O(1) 88.77(5)°, C(24)–Sn(1)–O(2A) 90.32(5)°, C(18)–Sn(1)–O(2) 88.05(5)°, C(12)–Sn(1)–O(2) 84.51(5)° (close to 90°) and O(1)–Sn(1)–O(2) 172.39(4)° (close to 180°) show that O(1) and O(2) are located in axial positions.

The Sn–O bond lengths Sn(1)–O(1) 219.9(2) Sn(1)–O(2) 233.7(1) pm, and the Sn–C bond lengths, Sn(1)–C(24) 211.9(2), Sn(1)–C(18) 212.3(2), Sn(1)–C(12) 212.3(2) pm are similar to those found in other similar triorganotin(IV) carboxylate complexes [47–51]. With these distances one can envisage that the carboxylato ligand chelates to two symmetry related Sn atoms giving rise to different Sn–O bond distances. The inequality in the Sn–O bonds is reflected in the associated C–O bond distances (C(1)–O(2A) 125.2(2) and C(1)–O(1) 126.7(2) pm), the longer C–O bond (C(1)–O(1) 126.7(2) pm) is involved with the shorter Sn–O interaction (Sn(1)–O(1) 219.9(2) pm).

These structural parameters are in agreement with the difference of ca. 150 cm^{-1} between the asymmetric and symmetric vibration of the COO moiety in the IR spectra (see Section 3.1) which indicates bridging behaviour [45,52].

Selected bond lengths and angles for **2** are given in Table 2.

According to the different IR and NMR data, the solid-state structure of **1** should present a similar structure; however, we were unable to obtain crystals suitable for their analysis by X-ray diffraction studies.

Additionally, it seems that the polymeric chain is not retained in solution of donor solvents such as DMSO, which coordinate to the metal centre leading to a mononuclear species, as observed in NMR spectroscopy.

3.3. Cytotoxic studies

The *in vitro* cytotoxicities of organotin(IV) compounds **1** and **2** against human tumour cell lines 8505C anaplastic thyroid cancer, A253 head and neck tumour, A549 lung carcinoma and DLD-1 colon carcinoma were determined by using the SRB microculture colorimetric assay [42]. In addition, cytotoxicities of cisplatin and related titanocene(IV) [37] and organogallium(III) [36] compounds with the same carboxylato ligands have been included for comparison (Table 3).

The studied organotin antitumour agents showed a dose-dependent antiproliferative effect toward all the studied cancer cell lines (Fig. 3). Estimates based on the IC_{50} values show that the studied tin complexes are more active than cisplatin and all the titanocene(IV) and organogallium(III) derivatives against all the studied human cancer cell lines.

Taking into account the standard deviation, there is not a substantial higher activity of any of the tin complex over the other, with the exception of the DLD-1 cells where the IC_{50} value for **1** is 0.060 ± 0.001 μM while for **2** is much higher, 0.178 ± 0.002 μM, indicating a preference of complex **1** on DLD-1 cells.

On direct comparison with cisplatin, the cytotoxic activity of complexes **1** and **2** is from 8 to 85 times higher. Again, the highest activity ratio cytotoxicity of organotin complex/cisplatin activity was observed for **1** in the DLD-1 cell. Also the fact that greater tolerances of high tin concentrations in biological systems may be possible (in direct contrast to the large number of side-effects associated to very low concentrations of platinum), makes these tin compounds ideal candidates for further studies.

In addition, **1** and **2** present activities up to 285 and 2520 times higher than their gallium(III) and titanocene(IV) analogues, respectively.

3.4. DNA-interaction studies

The R_3Sn^+ moieties have been observed to directly affect DNA [53] as well as binding to membrane proteins or glycoproteins, or to cellular proteins; e.g. to ATPase, hexokinase, acetylcholinesterase of human erythrocyte membrane or to skeletal muscle membranes [54]. In addition, a wide number of reports have been published concerning the possible mechanisms for the interaction of alkyl or aryltin moieties with the membrane or constituents within the cell [55–58], although the exact mechanism is still unclear. However, it is generally agreed that the R_3Sn^+ fragments may bind to the phosphate groups in DNA [59–61], changing the intracellular metabolism of the phospholipids of the endoplasmic reticulum [62,63].

Thus, the binding behaviour of the studied organotin(IV) compounds to DNA helix has been followed through absorption spectral titrations, because absorption spectroscopy is one of the most useful techniques to study the binding of any drug to DNA [64–67]. The absorption spectra of the complexes in the absence

Table 3
IC_{50} (μM) for the 96 h of action of **1**, **2**, [Ti(η5–C5H5)(η5–C5H4{CMe2(CH2CH2CH=CH2)})(O2CCH2SXyl)2], [Ti(η5–C5H5)(η5–C5H4{CMe2(CH2CH2CH=CH2)})(O2CCH2SMes)2] [37], [{Me2Ga(μ–O2CCH2SMes)}2] [36] and cisplatin on 8505C anaplastic thyroid cancer, A253 head and neck tumor, A549 lung carcinoma and DLD-1 colon carcinoma determined by sulforhodamine-B microculture colorimetric assay.

Complex	IC_{50} ± SD [μM]			
	8505C	A253	A549	DLD-1
1	0.132 ± 0.010	0.081 ± 0.003	0.094 ± 0.013	0.060 ± 0.001
2	0.172 ± 0.003	0.100 ± 0.014	0.129 ± 0.014	0.178 ± 0.002
[Ti(η5–C5H5)(η5–C5H4{CMe2(CH2CH2CH=CH2)})(O2CCH2SXyl)2]	182.3 ± 2.5	182.6 ± 2.0	192.5 ± 1.1	151.2 ± 4.2
[Ti(η5–C5H5)(η5–C5H4{CMe2(CH2CH2CH=CH2)})(O2CCH2SMes)2]	190.8 ± 2.2	131.2 ± 0.5	144.6 ± 2.9	115.7 ± 2.9
[{Me2Ga(μ–O2CCH2SMes)}2]	20.5 ± 2.3	7.7 ± 0.3	26.9 ± 7.0	12.4 ± 0.1
cisplatin	5.0 ± 0.2	0.81 ± 0.02	1.51 ± 0.02	5.1 ± 0.1

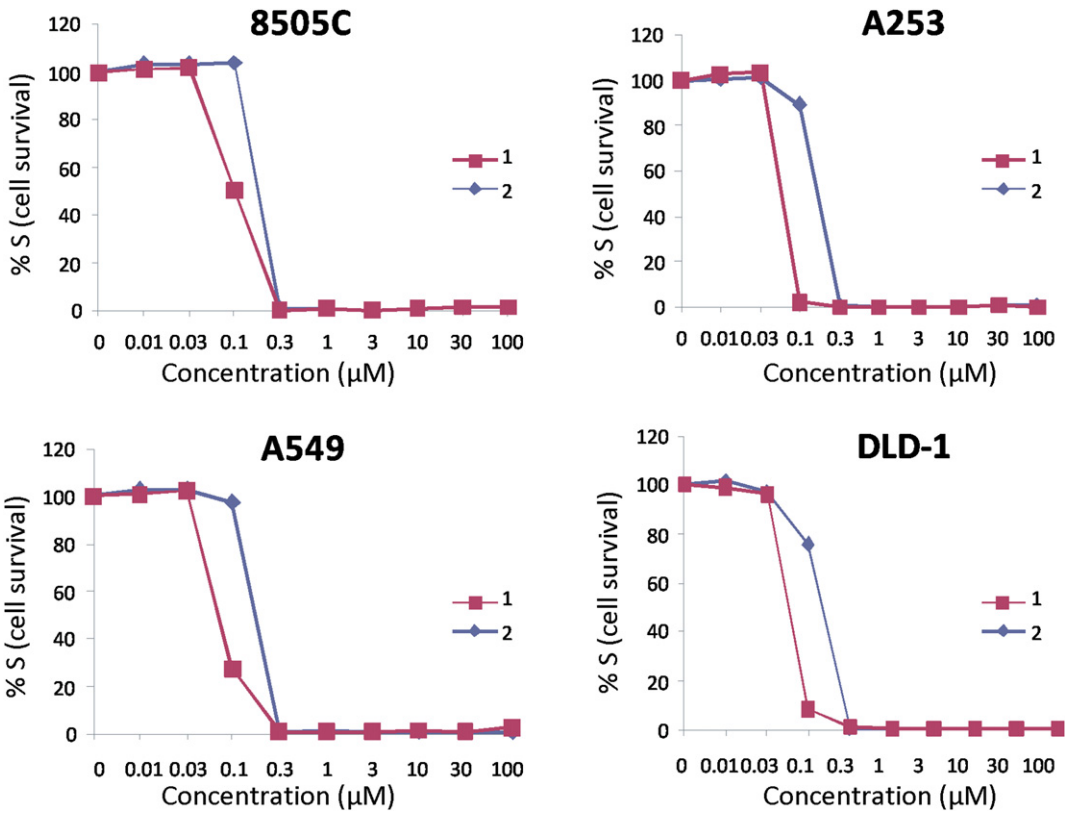


Fig. 3. Representative graphs show survival of 8505C, A253, A549 and DLD-1 cells grown for 96 h in the presence of increasing concentrations of **1** and **2**. Standard deviations (all less than 10%) are omitted for clarity.

and in the presence of FS-DNA (fish sperm DNA) have been recorded. With increasing concentrations of FS-DNA, the absorption bands of the complexes were affected, resulting in the tendency of hyperchromism and a very slight blue shift. The organotin(IV) compounds **1** and **2** may bind to the DNA in different modes on the basis of their structure and charge. The organotin(IV) compounds (**1** and **2**) may be charged (R_3Sn^+) and there could be classical electrostatic interactions which may be responsible for the spectral changes observed in the study. However, other electrostatic effects such as hydrogen bonding between the complexes and the base pairs in DNA may also be present [68–71].

In order to compare the binding strengths of the complexes, the intrinsic binding constant, K_b, was determined using the following equation [72]:

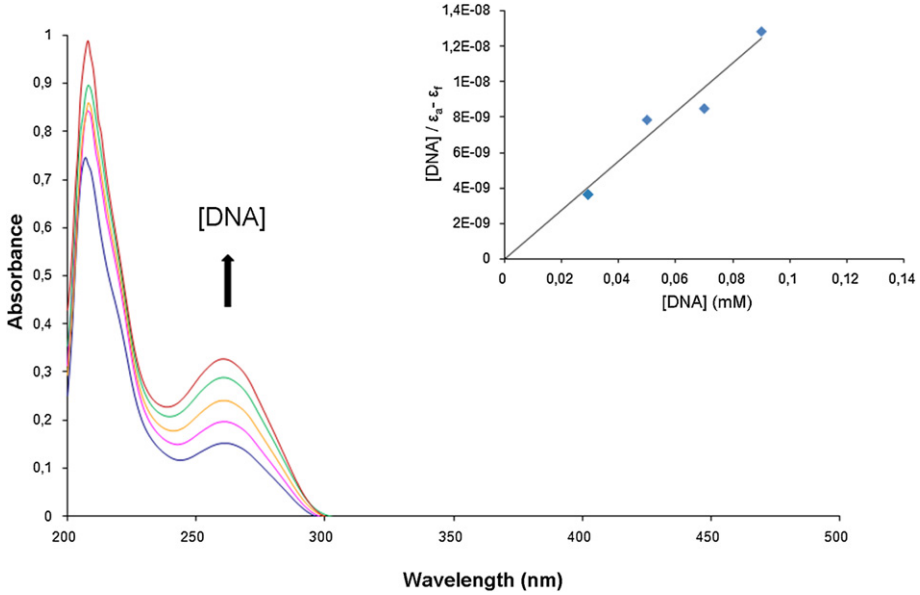


Fig. 4. Absorption spectra of **1** in the presence of increasing amounts of DNA. Arrow indicates that absorbance changes upon increasing DNA concentrations. Inset: plot of $[DNA]/\varepsilon_a - \varepsilon_f = [DNA]/\varepsilon_0 - \varepsilon_f + 1/K_b(\varepsilon_0 - \varepsilon_f)$, experimental data points; solid line, linear fitting of the data.

$$\frac{[DNA]}{\varepsilon_a - \varepsilon_f} = \frac{[DNA]}{\varepsilon_0 - \varepsilon_f} + \frac{1}{K_b\left(\varepsilon_0 - \varepsilon_f\right)}$$

where [DNA] is the concentration of DNA in base pairs, ϵ_a, ϵ_f and ϵ_0 correspond to A_{obs}/[Complex], the extinction coefficient of the free tin complexes and the extinction coefficient of the complexes in the fully bound form, respectively, and K_b is the intrinsic binding constant. The ratio of slope to intercept in the plot of [DNA]/($\epsilon_a - \epsilon_f$) versus [DNA] gives the value of K_b (inset Fig. 4).

As an example, Fig. 4 shows the absorption spectra of complex **1** in the presence of increasing amounts of DNA.

Thus, the intrinsic binding constants of 1.68×10^5 and 1.02×10^5, M^{-1} for **1** and **2**, respectively, have been successfully calculated, observing that complex **1**, which is the most cytotoxic compound, gives a K_b slightly higher than that of **2**, indicating a slightly higher affinity from DNA, which may be implicated in the higher cytotoxic activity shown by **1**.

4. Conclusions

Two new triphenyltin(IV) carboxylate complexes have been synthesized and characterized. The molecular structure of **2** consists of a carboxylato ligand coordinated to a $SnPh_3$ unit by a single oxygen, which aggregates *via* further Sn–O interactions to form a zig-zag 1-D coordination polymer, which is surprising because generally, triorganotin(IV) carboxylates with bulky R groups attached to the Sn atom favour tetrahedral monomeric structures. The cytotoxic activity of these compounds has been tested against human tumour cell lines observing that both tin(IV) compounds present higher cytotoxic activity than cisplatin and titanocene(IV) and organogallium(III) compounds with the same ligands. From the studied cell lines compound **1** presents the highest cytotoxicity (IC_{50} of 0.060 ± 0.001 μM) against DLD-1 cell line. In addition, DNA-interaction tests have been carried out, observing classical electrostatic interactions of all the complexes with DNA with intrinsic binding constants of 1.68×10^5 and 1.02×10^5, M^{-1} for **1** and **2**, respectively.

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Appendix. Supplementary material

Crystallographic data for the structural analysis of **2** have been deposited with the Cambridge Crystallographic Data Centre, CCDC-772114 (**2**). Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44 1223 336033; E-mail: deposit@ccdc.cam.ac.uk or http//www.ccdc.cam.ac.uk).

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jorganchem.2010.04.029.

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