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Organotin(IV) complexes of ethylsarcosine hydrochloride: synthesis, characterization and *in vitro* cytotoxic activity

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The synthesis and characterization of new coordination compounds of some organotin(IV) chlorides with ethylsarcosine hydrochloride (*N*-methylglycine ethyl ester hydrochloride) is reported; the ligand molecules appear to be bound to the tin atoms through the carbonyl oxygen atoms by means of dative bonds. As single crystals were not obtained, a number of experimental techniques were used to accomplish a definitive characterization and determination of their structure. The results obtained by ^{1}H, ^{13}C, ^{119}Sn NMR, FT-IR and ^{119m}Sn Mössbauer spectroscopy and thermogravimetric analysis suggested pentacoordination for the 1:1 (Sn:ligand (ESH)) derivatives, $[R_2SnCl_2(ESH)]^{+}Cl^{-}$, in a trigonal bipyramidal (TBP) structure, and hexacoordination for the 1:2 complexes, $[R_2SnCl_2(ESH)_2]^{2+}2Cl^{-}$, in an octahedral structure (R = Me, *n*-Bu, Ph; ESH = protonated ethylsarcosine, $CH_3CH_2O(O)CCH_2N(CH_3)H_2^{+}$). The triphenyltin(IV) chloride ethylsarcosine hydrochloride derivative exists only as a 1:1 pentacoordinated adduct in a strongly distorted TBP structure. Finally, the compounds have been tested for *in vitro* cytotoxic activity against human adenocarcinoma HeLa cells, showing, in some cases, a high potency, even at low concentration.

KEYWORDS: organotin(IV); ethylsarcosine hydrochloride; cytotoxicity; HeLa cells

INTRODUCTION

The widespread success of platinum compounds in the clinical treatment of certain types of neoplasia has placed their coordination chemistry in the front line in the fight against cancer. One of the major developments in the field of bioorganotin chemistry in the last two decades is the finding that certain organotin compounds play an important role in anticarcinogenesis.[1–7]

Although organotin(IV) compounds have been extensively studied as wood preservatives, fungicides, agrochemical miticides and fungicides,[8,9] biocides and environmental disinfectants[10] and as additives in the production of varnishes for ship hulls (antifouling paints),[11] due to their high biological activity, there is relatively little information available on organotin compounds as anticancer agents.

The diorganotins $[R_2SnX_2]$ (R = hydrocarbon group; X = halogen, —OR′, —NR′$_2$, —SR′, —OOCR′, etc.) are the largest group of tin compounds to have been studied for antitumour activity, but they are, in general, much less active than the platinum complexes.[12]

Brown,[13] in her doctoral work, concluded that a hydrolysable organotin compound, triphenyltin(IV) acetate, significantly retarded tumour growth, whereas the non-hydrolysable $[Ph_3SnCl]$ was inactive. In 1980, Crowe *et al.*[14] reported on the antitumour activity of a series of diorganotin(IV) dihalide and pseudohalide complexes $[R_2SnCl_2{\cdot}2L]$ (R = Me, Et, Pr, Bu, Ph; X = Cl, Br, I, NCS; L = bipyridyl, phenanthroline, 2-aminomethylpyridine, dimethyl sulfoxide (DMSO), pyridine, etc.), which were modelled on the active platinum complexes. They proposed that the mode of action might involve the initial transportation of the complex

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[$R_2SnCl_2{\cdot}2L$] into the tumorigenic cells followed by the reaction of [R_2SnCl_2] (or one of its hydrolysable products) and that a moderately stable complex is required for activity. Barbieri *et al.*[15] reported on the antitumour activity of [R_2Sn(adenine)$_2$] and [R_2Sn(glycylglycine)$_2$] complexes and suggested transportation of the complex species into the tumour cells, followed by attack of the hydrolysed R_2Sn moieties.

On the basis of these last results, this work reports on the interaction of organometallic derivatives of tin(IV) with ethylsarcosine hydrochloride (ESHCl; *N*-methylglycine ethyl ester hydrochloride). The choice of such a ligand as the coordinating agent was made in order to increase the hydrolysability of the organotin(IV) precursors due to formation of Sn—O bonds,[16] thus permitting the attack of the hydrolysed R_2Sn/R_3Sn moieties on the tumour cells, leading to an antitumour activity perhaps comparable to platinum derivatives.

The characterization of the compounds synthesized has been carried out by means of elemental analysis, conductivity measurements, ^{1}H, ^{13}C, ^{119}Sn NMR, FT-IR and ^{119m}Sn Mössbauer spectroscopy and thermogravimetric (TG) analysis, and the complexes have been tested for *in vitro* cytotoxicity against human adenocarcinoma HeLa cells.

EXPERIMENTAL

Materials and measurements

ESHCl (Aldrich), triphenyltin(IV) chloride and dimethyl-, diphenyl- and di(*n*-butyl)tin(IV) dichloride (Fluka AG) were used as supplied. All other reagents and solvents were of high purity and were used as purchased without any further purification.

Human adenocarcinoma HeLa cells (kindly provided by Professor F. Majone, *Dipartimento di Biologia dell'Università, Padua, Italy*) were grown as monolayers in Hams-F12 medium (Wittaker Bioproduct) supplemented with 10% foetal calf serum (Biochrom-Seromed), and the antibiotics penicillin (50 units ml^{-1}) and streptomycin (50 mg ml^{-1}). Trypsin (0.25%, Boehringer) was routinely used for subcultures.

Melting points were determined on an Electrothermal IA9300 instrument.

Conductivity measurements were carried out with an Amel 134 type conductivity bridge using freshly prepared 10^{-3} M solutions in nitromethane at 25.0 ± 0.1 °C.

FT-IR spectra were recorded in Nujol between two polyethylene tablets on a Nicolet Vacuum Far FT-IR 20F spectrophotometer in the range 600–50 cm^{-1}, and in solid KBr on a Nicolet FT-IR 55XC spectrophotometer in the range 4000–400 cm^{-1}.

^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker Avance DRX400 spectrometer, equipped with a Silicon Graphics O2 workstation operating in Fourier transform, using tetramethylsilane (TMS) as internal standard. ^{13}C and ^{119}Sn NMR spectra were recorded on the same instrument using TMS and tetramethyltin(IV) (TMT) respectively as internal standards; proton noise decoupling was normally used to obtain an increase in the observed resonance peak intensities.

Elemental analyses were performed by Laboratorio di Microanalisi, *Dipartimento di Chimica Inorganica, Metallorganica ed Analitica dell'Università, Padua, Italy*, with a Carlo Erba 1108 CHNS-O microanalyser; Cl was determined by the Schöninger method.

The TG and thermodifferential analyses (DTA) curves were obtained using a Netzsch STA429 thermoanalyser. The measurements were carried out in the range 35–1200 °C in alumina crucibles under air (flux rate 30 cm^3 min^{-1}) and at a heating rate of 5 °C min^{-1}, using alumina as reference.

^{119m}Sn Mössbauer spectra were recorded at 80 K using an Air Liquide cryostat; the $Ca^{119m}SnO_3$ source (nominal strength 15 mCi, New England Nuclear Corporation) was moved at room temperature with constant acceleration, giving a triangular velocity waveform, and Lorentzian line shapes were fitted to the experimental spectra. The calibration was performed using an α-Fe foil at room temperature. The isomer shift values are relative to room temperature SnO_2.

Syntheses

General procedure

All of the diorganotin(IV) complexes were obtained by slow addition of the solid ethylsarcosine hydrochloride to a warm dichloromethane solution (*ca* 10 mL, 40 °C) of the appropriate diorganotin(IV) dihalide precursor in a 1:1 and 1:2 (Sn:ligand) ratio. The organotin solution, originally colourless, immediately turned to a pale yellow and became opalescent due to the formation of the stoichiometric adducts. The mixture was refluxed with stirring at 50 °C for 2 h and the solvent was then removed by slow evaporation under reduced pressure. The yellow oily substance obtained was washed with diethyl ether and dried *in vacuo*.

With triphenyltin(IV) chloride, only the 1:1 adduct was synthesized. Using the above procedure, a yellow oily substance was obtained after slow solvent evaporation; and this was then stirred vigorously with diethyl ether, giving rise to a white powder. This was the only solid ESHCl derivative obtained.

All of the compounds synthesized are soluble in methanol, ethanol, chloroform, dichloromethane, acetone, DMSO, acetonitrile, dimethylformamide (DMF) and nitromethane, and insoluble in water, pentane, benzene and diethyl ether.

*[$Ph_3SnCl(ESH)]^+Cl^-$ (**1**)*

From 1.042 g of [Ph_3SnCl] (2.702 mmol) and 0.42 g of ESHCl (2.704 mmol). White powder. Anal. Found: C, 51.47; H, 5.20; N, 2.50; Cl, 12.96. Calc. for $C_{23}H_{27}Cl_2NO_2Sn$: C, 51.24; H, 5.05;

Table 1. Selected IR frequencies (cm^{-1})

Compound	$\nu_{a/s}(NH_2^+)$	$\nu(C{=}O)$	$\nu(C{-}O)$	$\nu_{a/s}(Sn{-}C)$	$\nu_{a/s}(Sn{-}Cl)$	$\nu(Sn{-}O)$
$(ESH)^+Cl^-$	2787/2707	1745	1239	–	–	–
[Me$_2$SnCl$_2$][a]	–	–	–	560/517	331/308	–
[(*n*-Bu)$_2$SnCl$_2$][b]	–	–	–	589/524	352/304	–
[Ph$_3$SnCl][c]	–	–	–	270/232	332	–
[Ph$_2$SnCl$_2$][c]	–	–	–	278/233	364/356	–
1	2774/2711	1735	1247	282/232	323	250
2	2787/2735	1735	1250	571/509	329	247
3	2781/2711	1734	1249	571	326	249
4	2795/2724	1734	1251	282/225	334	256
5	2781/2714	1734	1249	278	328	255
6	2781/2709	1735	1248	598/521	322	255
7	2781/2709	1735	1249	596	324	259

[a] Ref. 17.
[b] Ref. 18.
[c] Ref. 19.

N, 2.60; Cl, 13.15%. M.p. 59.0–60.4 °C. Yield 75.9%. Λ_M 83.8 Ω^{-1} cm^2 mol^{-1}.

*[Me$_2$SnCl$_2$(ESH)]$^+$Cl$^-$ (**2**)*

From 1.867 g of [Me$_2$SnCl$_2$] (8.500 mmol) and 1.306 g of ESHCl (8.501 mmol). Yellow oily substance. Anal. Found: C, 22.41; H, 5.09; N, 3.57; Cl, 28.22. Calc. for $C_7H_{18}Cl_3NO_2Sn$: C, 22.52; H, 4.86; N, 3.75; Cl, 28.49%. Yield 78.7%. Λ_M 85.3 Ω^{-1} cm^2 mol^{-1}.

*[Me$_2$SnCl$_2$(ESH)$_2$]$^{2+}$2Cl$^-$ (**3**)*

From 1.330 g of [Me$_2$SnCl$_2$] (6.052 mmol) and 1.860 g of ESHCl (12.110 mmol). Yellow oily substance. Anal. Found: C, 27.53; H, 5.92; N, 5.04; Cl, 26.72. Calc. for $C_{12}H_{30}Cl_4N_2O_4Sn$: C, 26.91; H, 5.74; N, 5.22; Cl, 26.91%. Yield 75.5%. Λ_M 157.9 Ω^{-1} cm^2 mol^{-1}.

*[Ph$_2$SnCl$_2$(ESH)]$^+$Cl$^-$ (**4**)*

From 1.131 g of [Ph$_2$SnCl$_2$] (3.289 mmol) and 0.505 g of ESHCl (3.290 mmol). Yellow oily substance. Anal. Found: C, 40.91; H, 4.27; N, 2.80; Cl, 21.42. Calc. for $C_{17}H_{22}Cl_3NO_2Sn$: C, 41.05; H, 4.46; N, 2.82; Cl, 21.38%. Yield 77.2%. Λ_M 79.1 Ω^{-1} cm^2 mol^{-1}.

*[Ph$_2$SnCl$_2$(ESH)$_2$]$^{2+}$2Cl$^-$ (**5**)*

From 0.933 g of [Ph$_2$SnCl$_2$] (2.713 mmol) and 0.834 g of ESHCl (5.426 mmol). Yellow oily substance. Anal. Found: C, 40.38; H, 5.71; N, 4.20; Cl, 21.93. Calc. for $C_{22}H_{34}Cl_4N_2O_4Sn$: C, 40.59; H, 5.26; N, 4.30; Cl, 21.78%. Yield 78.2%. Λ_M 162.3 Ω^{-1} cm^2 mol^{-1}.

*[(n-Bu)$_2$SnCl$_2$(ESH)]$^+$Cl$^-$ (**6**)*

From 1.119 g of [(*n*-Bu)$_2$SnCl$_2$] (3.684 mmol) and 0.566 g of ESHCl (3.685 mmol). Yellow oily substance. Anal. Found: C, 33.96; H, 6.53; N, 2.91; Cl, 26.72. Calc. for $C_{13}H_{30}Cl_3NO_2Sn$: C, 34.13; H, 6.61; N, 3.06; Cl, 23.25%. Yield 69.2%. Λ_M 86.1 Ω^{-1} cm^2 mol^{-1}.

*[(n-Bu)$_2$SnCl$_2$(ESH)$_2$]$^{2+}$2Cl$^-$ (**7**)*

From 0.945 g of [(*n*-Bu)$_2$SnCl$_2$] (3.110 mmol) and 0.955 g of ESHCl (6.220 mmol). Yellow oily substance. Anal. Found: C, 35.29; H, 6.92; N, 4.56; Cl, 22.96. Calc. for $C_{18}H_{42}Cl_4N_2O_4Sn$: C, 35.38; H, 6.93; N, 4.58; Cl, 23.21%. Yield 69.7%. Λ_M 167.0 Ω^{-1} cm^2 mol^{-1}.

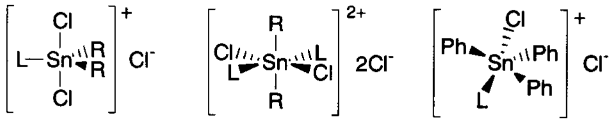
RESULTS AND DISCUSSION

FT-IR spectra

The most significant bands recorded in the FT-IR spectra of the reagents and the complexes have been collected in Table 1.

For the ESHCl adducts, we have been able to deduce pentacoordination for the [R$_2$SnCl$_2$(ESH)]$^+$Cl$^-$ complexes, with a trigonal bipyramidal (TBP) tin atom geometry having the R groups in equatorial positions (Fig. 1a), and hexacoordination for the [R$_2$SnCl$_2$(ESH)$_2$]$^{2+}$2Cl$^-$ compounds, in an octahedral structure with the R groups in the axial positions (Fig. 1b). The triphenyltin(IV) chloride ESHCl derivative exists only as a 1:1 pentacoordinated adduct in a strongly distorted TBP structure with the three phenyl groups occupying all equatorial positions (Fig. 1c).

These hypotheses are confirmed by the FT-IR spectra. In fact, [R$_2$SnCl$_2$(ESH)]$^+$Cl$^-$ complexes exhibit both ν_a(Sn—C) and ν_s(Sn—C) vibrational bands, indicating a non-linear C—Sn—C moiety consistent with their proposed structure,[20–22] whereas, [R$_2$SnCl$_2$(ESH)$_2$]$^{2+}$2Cl$^-$ compounds show only one band, due to the SnC$_2$ asymmetric stretching vibration and



(1.a) **(1.b)** **(1.c)**

R = Me, *n*-Bu, Ph; L = ESH

Figure 1. Structure of the 1:1 (**a**) and 1:2 (**b**) adducts. Possible structure of $[Ph_3SnCl(ESH)]^+Cl^-$ (**c**).

suggesting that the C—Sn—C skeleton of these compounds is linear.[22,23]

The same considerations may be applied to the vibrational modes assignable to ν(Sn—Cl): the presence in the spectra of all of the synthesized compounds of one band due to the $SnCl_2$ asymmetric stretching indicates a linear Cl—Sn—Cl fragment.[23]

The existence of an $—[N(CH_3)H_2]^+Cl^-$ saline group is underlined by the presence of the $\nu_{a/s}(NH_2^+)$ in the 2795–2711 cm^{-1} region and the consistent absence of a weak thin band in the 3350–3310 cm^{-1} range, commonly attributed to ν(N—H) for a neutral amino group.[24,25] Each ionic positively charged amino group is neutralized by the presence of a Cl^- counter ion (as confirmed by the conductivity measurements); if the Cl^- counter ion was not present to make the $—N(CH_3)H_2^+$ structure rigid, a band would have been expected at ~500 cm^{-1} due to the torsional oscillation of the pyramidal system (shown in Fig. 2) around the C—N bond axis.[24]

The carbonyl stretching frequency appears as a strong band at 1745 cm^{-1} in the ligand and shifts by ~10 cm^{-1} in all the tin complexes, indicating an interaction between the tin and the carbonyl oxygen atoms.[25] In analogous compounds,[27–29] the presence of tin–carbonyl oxygen bonds is evidenced by a negative shift by 25–80 cm^{-1} of the ν(C═O) band. In the present situation, however, the generalization that the larger the magnitude of $\Delta\nu$(C═O) is for a given carbonyl ligand then the stronger is the donor interaction[30] does not hold, as ν(C═O) is probably strongly coupled with other modes.[31]

For all compounds, only one band assignable to ν(Sn—O)[32] is found in the range 259–247 cm^{-1}, indicating, particularly for the compounds **3**, **5** and **7**, linear O—Sn—O moieties according to the structure shown in Fig. 1b.

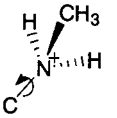


Figure 2. Torsional oscillation of the $—N(CH_3)H_2$ group around the C—N bond axis.

Table 2. ^{119m}Sn Mössbauer spectral data (mm s^{-1})

Compound	Isomer shift	Quadrupole splitting
$[Me_2SnCl_2]$[a]	1.66	3.56
$[(n\text{-}Bu)_2SnCl_2]$[a]	1.75	3.50
$[Ph_3SnCl]$[a]	1.39	2.50
$[Ph_2SnCl_2]$[a]	1.45	2.76
1	1.34	2.57
2	1.64	3.49
3	1.63	4.01
4	1.44	2.99
5	1.41	3.24
6	1.73	3.69
7	1.70	3.79

[a] Ref. 33.

^{119m}Sn Mössbauer spectra

The Mössbauer spectral parameters of all of the compounds synthesized are summarized in Table 2. The isomer shifts and linewidths of the complexes are typical of a single tin(IV) site.[34] A slight lowering of the isomer shift values is observed (compared with the organotin precursors) owing to a reduction in the tin atom 5s orbital electronic density; this is caused by coordination sphere expansion changing the atomic hybridization by additionally using the 5d orbital with a reduction in the s-electronic density.[35]

A comparison between the quadrupole splitting data for the ESHCl organotin(IV) derivatives and those reported in literature for analogous compounds[36] leads to the conclusion that, in the solid state, the $[R_2SnCl_2(ESH)]^+Cl^-$ complexes are pentacoordinated in a TBP structure with the hydrocarbon groups occupying the equatorial positions,[37] while the $[R_2SnCl_2(ESH)_2]^{2+}2Cl^-$ complexes are hexacoordinated with an octahedral structure with the organic groups occupying the axial positions.[38] These results are in full agreement with the structural hypotheses based upon the FT-IR study.

The $[Ph_3SnCl(ESH)]^+Cl^-$ adduct shows a quadrupole splitting value intermediate between that assignable to a pentacoordinated complex in a TBP structure with all the phenyl groups in the equatorial positions (3.00–4.00 mm s^{-1}) and that with a phenyl group in the axial position and the other two aryl moieties in the equatorial sites (1.70–2.40 mm s^{-1}).[39] This result is in accord with the hypothesis of a weak Sn—O interaction, leading to a pentacoordinated complex in a strongly distorted TBP geometry.[40]

Solution studies

The conductivity measurements confirm the conducting behaviour of the compounds in solution. For **1, 2, 4** and **6** the Λ_M values are in accord with these complexes being formulated as 1:1 electrolytes (75–95 Ω^{-1} cm^2 mol^{-1} in

Table 3. ^{1}H, ^{13}C, ^{119}Sn NMR data ($CDCl_3$, 25.0 °C, ppm)[a]

Compound	δ(Sn—R)	δ(ligand)	δ(COO)	δ(Sn)
$(ESH)^+Cl^-$	–	1.32 (CH_3—C, *t*) 2.85 (CH_3—N, *s*) 3.88 (CH_2—N, *s*) 4.30 (CH_3—O, *q*) 9.80 (NH_2^+, *t*, *br*)	165.97	–
$[Me_2SnCl_2]$[b]	1.21 (CH_3, *s*)	–	–	142.25[e]
$[(n\text{-}Bu)_2SnCl_2]$[c]	0.96 (CH_3, *t*) 1.40 (—CH_2—, *m*) 1.78 (—CH_2—, *m*) 1.84 (CH_2—Sn, *m*)	–	–	128.28[f]
$[Ph_3SnCl]$[d]	7.44–7.75 (C—H, *m*)	–	–	−45.24[g]
$[Ph_2SnCl_2]$[e]	7.49–7.81 (C—H, *m*)	–	–	−28.99[g]
1	7.44–7.69 (C—H, *m*)	1.31 (CH_3—C, *t*) 2.82 (CH_3—N, *s*) 3.81 (CH_2—N, *s*) 4.29 (CH_3—O, *q*) 9.78 (NH_2^+, *t*, *br*)	168.32	−57.53
2	1.37 (CH_3, *s*)	1.35 (CH_3—C, *t*) 2.92 (CH_3—N, *s*) 3.97 (CH_2—N, *s*) 4.33 (CH_3—O, *q*) 8.88 (NH_2^+, *t*, *br*)	171.13	−63.58
3	1.34 (CH_3, *s*)	1.33 (CH_3—C, *t*) 2.88 (CH_3—N, *s*) 3.91 (CH_2—N, *s*) 4.31 (CH_3—O, *q*) 9.32 (NH_2^+, *t*, *br*)	169.52	−103.13
4	7.26–8.08 (C—H, *m*)	1.26 (CH_3—C, *t*) 2.63 (CH_3—N, *s*) 3.67 (CH_2—N, *s*) 4.20 (CH_3—O, *q*) 9.64 (NH_2^+, *t*, *br*)	170.52	−185.89
5	7.28–8.13 (C—H, *m*)	1.27 (CH_3—C, *t*) 2.65 (CH_3—N, *s*) 3.69 (CH_2—N, *s*) 4.22 (CH_3—O, *q*) 8.84 (NH_2^+, *t*, *br*)	169.58	−239.03
6	0.90 (CH_3, *t*) 1.33 (—CH_2—, *m*) 1.56 (—CH_2—, *m*) 1.77 (CH_2—Sn, *m*)	1.27 (CH_3—C, *t*) 2.71 (CH_3—N, *s*) 3.91 (CH_2—N, *s*) 4.25 (CH_3—O, *q*) 9.17 (NH_2^+, *t*, *br*)	172.08	−60.84
7	0.94 (CH_3, *t*) 1.31 (—CH_2—, *m*) 1.44 (—CH_2—, *m*) 1.90 (CH_2—Sn, *m*)	1.32 (CH_3—C, *t*) 2.87 (CH_3—N, *s*) 3.93 (CH_2—N, *s*) 4.29 (CH_3—O, *q*) 9.25 (NH_2^+, *t*, *br*)	170.41	−105.55

[a] *s* = singlet; *t* = triplet; *q* = quadruplet; *m* = multiplet; *br* = broad.

[b] Ref. 42.

[c] Ref. 43.

[d] Ref. 44.

[e] Ref. 45.

[f] Ref. 46.

[g] Ref. 47.

Table 4. Thermal data for the reaction:

$$3\ \text{Haloorganotin(IV)} \xrightarrow{1} (Cl_2SnO)_3 \xrightarrow{2} \tfrac{3}{2}SnCl_4(g) + \tfrac{3}{2}SnO_2(s)$$

	TG analysis			Weight loss (%)	
Compound	Process	T (°C)	DTA	Found	Calculated
1	Melting	59–60	endo	–	–
	Step 1	132–261	exo	−62.4	−63.1
	Step 2	387–273	exo	−85.9	−86.5
2	Step 1–2	37–225	exo	−79.8	−80.7
3	Step 1–2	38–224	exo	−86.6	−86.1
4	Step 1	123–266	exo	−58.0	−58.7
	Step 2	365–765	exo	−84.1	−84.9
5	Step 1	120–306	exo	−69.1	−68.4
	Step 2	306–496	exo	−88.0	−88.4
6	Step 1–2	68–228	exo	−82.8	−83.5
7	Step 1–2	96–225	exo	−88.2	−87.7

nitromethane),[41] whereas the molar conductivity values of **3, 5** and **7** indicate 1:2 electrolytes (150–180 $\Omega^{-1}\,cm^2\,mol^{-1}$ in nitromethane).[41]

^1H NMR measurements for all of the complexes performed in $CDCl_3$ solution are reported in Table 3. It may be noted that the chemical shifts are very similar to those of the free organotin(IV) chlorides and the ligand. These findings may be explained by a soft interaction in solution between the organotin precursors and the ESHCl ligand.

The most interesting aspect, however, is the increase in the $^2J(^{117/119}Sn{-}C{-}^1H)$ coupling constants passing from the uncomplexed $[Me_2SnCl_2]$ (65.49/68.45 Hz) to $[Me_2SnCl_2(ESH)]^+Cl^-$ (73.63/78.05 Hz) and $[Me_2SnCl_2(ESH)_2]^{2+}2Cl^-$ (86.90/90.13 Hz). Moving from the organotin precursors (tetrahedral) to the 1:1 compounds (TBP), the tin orbitals change their hybridization from sp^3 to sp^3d, and, similarly, the 1:2 complexes show an octahedral structure with sp^3d^2 metal orbital hybridization. The tin orbital s-character increase is therefore expressed by a further increase in $^2J(^{117/119}Sn{-}C{-}^1H)$.[42,48] It is of interest to note that the values of the angles $CH_3{-}Sn{-}CH_3$ calculated by means of the Lockhart and Manders equation[49] support pentacoordination around the tin atom for **2** and hexacoordination around the tin centre for **3**.

^1H NMR measurements for all of the complexes were also performed in DMSO-d_6 solution, showing that no dissociation occurs in this solvent over 48 h, contrary to the findings for analogous compounds.[50,51]

In addition, ^{13}C NMR spectra of the free ligand and the complexes were registered in the same solvent; the most informative data regard the chemical shift of the —COO ligand carbon atom (Table 3). The slight deshielding, compared with the same signal of the free ESHCl, is indicative of a coordination through the carbonyl oxygen atom in all of the complexes, which leads to a decrease of electron density on the carboxylic carbon atom.[31]

In Table 3, ^{119}Sn NMR chemical shifts are also reported. The experimental data show a significant shift of the resonance peaks to higher fields in moving from the starting organotin(IV) reagents to the 1:1 and 1:2 adducts, as reported for analogous organotin(IV) derivatives.[52–55] These results are in agreement with the hypothesis of an increase in the coordination number of the tin atom in the complexes and, hence, of the tin nuclear shielding.[52–55]

Thermal behaviour

The thermal behaviour of the compounds synthesized has been studied in order to establish the different decomposition steps, to confirm the proposed stoichiometry and to support the conclusions of the various spectroscopic techniques.

The results of these analyses have been summarized in Table 4 and a good correlation exists between the calculated and found values.

The first TG step leads to a trimeric intermediate, $(Cl_2SnO)_3$,[56,57] and, subsequently, a very intense exothermic effect is recorded at higher temperatures that corresponds to the removal of the chlorine atoms, leading to SnO_2 formation as the residue.[58]

In vitro cytotoxicity studies

The compounds were dissolved in DMSO (4.5 mM) and, immediately prior to the experiments, a calculated amount of the drug solution was added to the growth medium until a final solvent concentration of 0.5% had been reached, which had no discernible effect on cell killing. The cytotoxicity was evaluated by means of the tetrazolium salt reduction (MTT) test.[59,60]

Table 5. Detection of *in vitro* cytotoxic activity with MTT test

	Cell vitality ± SD (%)			
Compound	0.0 µM	12.5 µM	25.0 µM	50.0 µM
1	100.0	11.5 ± 4.5	0.4 ± 3.8	0.2 ± 3.4
2	100.0	97.2 ± 3.4	90.9 ± 4.6	89.9 ± 4.0
3	100.0	99.4 ± 2.4	99.3 ± 4.7	94.1 ± 2.1
4	100.0	23.3 ± 2.7	8.9 ± 3.4	7.7 ± 3.6
5	100.0	38.0 ± 2.2	9.15 ± 3.3	7.8 ± 3.6
6	100.0	15.3 ± 4.5	6.0 ± 3.2	4.2 ± 2.5
7	100.0	10.37 ± 3.4	9.58 ± 2.9	4.6 ± 2.3
cis-DDP	100.0	39.0 ± 4.8	24.5 ± 3.9	14.2 ± 2.6

All of the complexes are stable in DMSO over 48 h, as proved by ^{1}H NMR spectra performed at different times.

HeLa cells (5×10^4 cells ml^{-1}) were seeded in 96-well microplates in the growth medium (100 µl) and then incubated at 37 °C in a 10% carbon dioxide atmosphere. After 24 h, the medium was removed and replaced with a fresh substrate containing the compound to be studied at the appropriate concentrations (12.5, 25 and 50 µM). Quadruplicate cultures were established for each treatment. After another 24 h, each well was treated with 10 µl of a 5 mg ml^{-1} MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) saline solution and, after 5 h incubation, 100 µl of a sodium dodecylsulfate solution in 0.1 M HCl were added. After an overnight incubation, the inhibition of cell growth by the various complexes was detected by measuring the absorbance of each well at 570 nm using a Camberra–Packard microplate reader. For comparison purposes, the cytotoxicity of *cis*-diamminodichloroplatinum(II) (*cis*-DDP, cisplatin) was evaluated under the same experimental conditions.

The *in vitro* cytotoxic activities reported in Table 5 show that the dimethyltin(IV) derivatives are essentially inactive, whereas the other complexes are much more effective than cisplatin, even at low concentrations. It is additionally possible to observe that virtually no cytotoxicity difference occurs between the 1:1 and 1:2 derivatives of the same organotin(IV) substrate, indicating that the activity level is mainly due to the organotin(IV) fragment.

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