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Tetraethylammonium (diorgano)halogeno-(2,6-pyridinedicarboxylato)stannates: synthesis, characterization and *in vitro* antitumour activity

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The synthesis and characterization of tetraethylammonium (diorgano)halogeno(2,6-pyridinedicarboxylato)stannates are described. The solution structures of these complexes in $CDCl_3$ and DMSO are discussed on the basis of ^{119}Sn and ^{19}F NMR data. Their *in vitro* antitumour activities against two human tumour cell lines, MCF-7 and WiDr, are presented.

Keywords: Diorganotin, carboxylate, NMR, Mössbauer, antitumour

INTRODUCTION

Diorganotin 2,6-pyridinedicarboxylates exhibit interesting *in vitro* antitumour activities.[1] Atassi[2] assumed that water-soluble organotin compounds are likely to be more active than compounds soluble only in organic solvents. Therefore we prepared some tetraethylammonium (diorgano)-halogeno(2,6 - pyridinedicarboxylato)stannates, whose water solubility under physiological conditions is expected to be improved with respect to their parent compounds.

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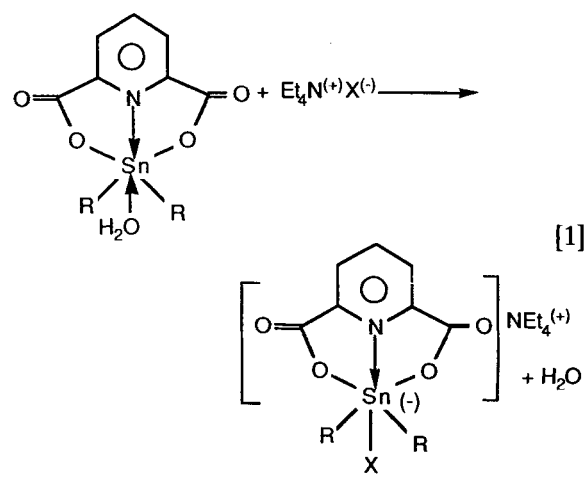
RESULTS AND DISCUSSION

Synthesis

The desired salts (Table 1) were obtained by reacting the parent diorganotin 2,6-pyridine-dicarboxylate with tetraethylammonium fluoride or chloride in acetonitrile (Eqn [1]) using the procedure for the analogous tetraethylammonium diorgano(halogeno)thiosalicylatostannates.[3,4] The new $Et_2Sn[(O_2C)_2C_5H_3N].H_2O$, compound, **5**, was also prepared by the procedure used to synthesize the corresponding di-n-butyltin compound.



[1]

The compounds **1–5** were characterized by Mössbauer spectrometry, and by ^1H, ^{13}C and ^{119}Sn NMR spectroscopy.

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Table 1 Melting points, recrystallization solvents and yields for the $[R_2Sn(O_2C)_2C_5H_3N)X]^-[NEt_4]^+$ salts **1–4**, and for the parent compound $Et_2Sn[(O_2C)_2C_5H_3N].H_2O$, **5**

Compound	R	X	M.p. (°C)	Recrystallization solvent	Yield (%)
1	Et	F	250–251	Acetonitrile	77
2	n-Bu	F	230–231	Acetonitrile	79
3	n-Bu	Cl	81–83	Acetonitrile	86
4	Ph	Cl	215–216	Acetonitrile	75
5	Et	—	279–280	Ethanol	90

Mössbauer parameters

The Mössbauer parameters of compounds **1–5** are given in Table 2.

These parameters have values similar to those of the corresponding parent compounds.[1] This observation suggests that the seven-coordination around the tin atom in the parent compounds[1,5] is maintained in the salts. This can be explained if it is assumed that the halide substitutes for the water molecule in one of the seven coordination sites. In order to obtain some evidence for this, TGA experiments were undertaken on one parent compound (**5**) and on one corresponding halide adduct (**1**). These data are described below.

TGA data

The TGA curves obtained for compounds **1** and **5** after thorough drying over P_2O_5 are given in Fig. 1. They clearly show that the crystals of **5** contain bonded water since, in a dynamically purged atmosphere, this is only released from 70 °C upwards. No weight loss due to the release of water is noticed for compound **1**, the fluoride adduct of compound **5**, giving evidence for our hypothesis.

Table 2 Mössbauer parameters of the $[R_2Sn(O_2CC_6H_4\text{-}2\text{-}S)X]^-[NEt_4]^+$ salts **1–4**, and of the parent compound $Et_2Sn[(O_2C)_2C_5H_3N].H_2O$, **5**

Compound	R	X	IS[a] (mm^{-1})	QS[b] (mm^{-1})	Γ_1 (mm^{-1})	Γ_2 (mm^{-1})
1	Et	F	1.30	4.23	0.90	0.93
2	n-Bu	F	1.27	3.50	0.91	0.95
3	n-Bu	Cl	1.50	4.28	0.87	0.88
4	Ph	Cl	1.23	3.99	0.96	0.90
5	Et	—	1.25	4.07	0.98	0.91

[a] IS, Isomer shift. [b] QS, quadrupole splitting.

^{1}H NMR data

The ^{1}H NMR parameters of compounds **1–5** are shown in Table 3.

In the ^{1}H NMR spectra of $CDCl_3$ solutions of $\{R_2Sn[2,6\text{-}(O_2C)_2C_5H_3N]F\}^-NEt_4^+$, compounds **1** and **2**. H-4 is slightly more shielded than H-3, these nuclei generating an AB_2 spectrum; on the contrary, H-4 is less shielded than H-3 for DMSO-d_6 solutions of compounds **3** and **4**, $\{R_2Sn[2,6\text{-}(O_2C)_2C_5H_3N]Cl\}^-NE_4^+$. This is also the case in $Et_2Sn[2,6\text{-}(O_2C)_2C_5H_3N].H_2O$, compound **5**.

^{13}C NMR data

The ^{13}C NMR data are given in Table 4.

The assignment of the ^{13}C is straightforward from the multiplicities observed in the selectively decoupled spectra and/or from the DEPT spectra, as well as from the intensities of the C(3) and C(4) carbon signals.

The rather high values of $^nJ(^1H, ^{117/119}Sn)$ and $^1J(^{13}C, ^{117/119}Sn)$ are in agreement with a hexa- or hepta-coordination[5,6] in solution.

^{119}Sn and ^{19}F NMR data

The ^{119}Sn NMR data are given in Table 5.

Whereas the analogous diorgano(halogeno)-thiosalicylatostannates $[R_2Sn(O_2C—C_5H_4\text{-}2\text{-}S)F]^-NEt_4^+$, exhibit characteristic $^1J(^{117/119}Sn,^{19}F)$ couplings in their ^{119}Sn NMR spectrum,[4] the $\{R_2Sn[2,6\text{-}(O_2C)_2C_5H_3N]F\}^-NEt_4^+$ salts all exhibit a single broad resonance in both $CDCl_3$ and DMSO-d_6. This line broadening can be explained as a coalescence due to an intermolecular fluorine exchange becoming rapid on the ^{119}Sn NMR timescale. The frequency of this exchange should be of the order of 2000 Hz, the value of the $^1J(^{117/119}Sn,^{19}F)$ coupling constant observed for the former compounds. This exchange is likely to be intermolecular because the $^1J(^{119}Sn,^{19}F)$ is lost. The resonance being likewise broad, the chlorides also probably undergo such exchange phenomena. However, in the case of the chlorides, this exchange implies at least two different species because the broad signal observed cannot be due to a single coalescence from a coupling doublet to a singlet since such couplings are not observable in the chlorides, as a consequence of the fast quadrupolar relaxation of chlorine nuclei. Actually it cannot be excluded that this broadening is induced by the latter quadrupolar relaxation itself. That the ^{1}H and ^{13}C NMR spectra of com-

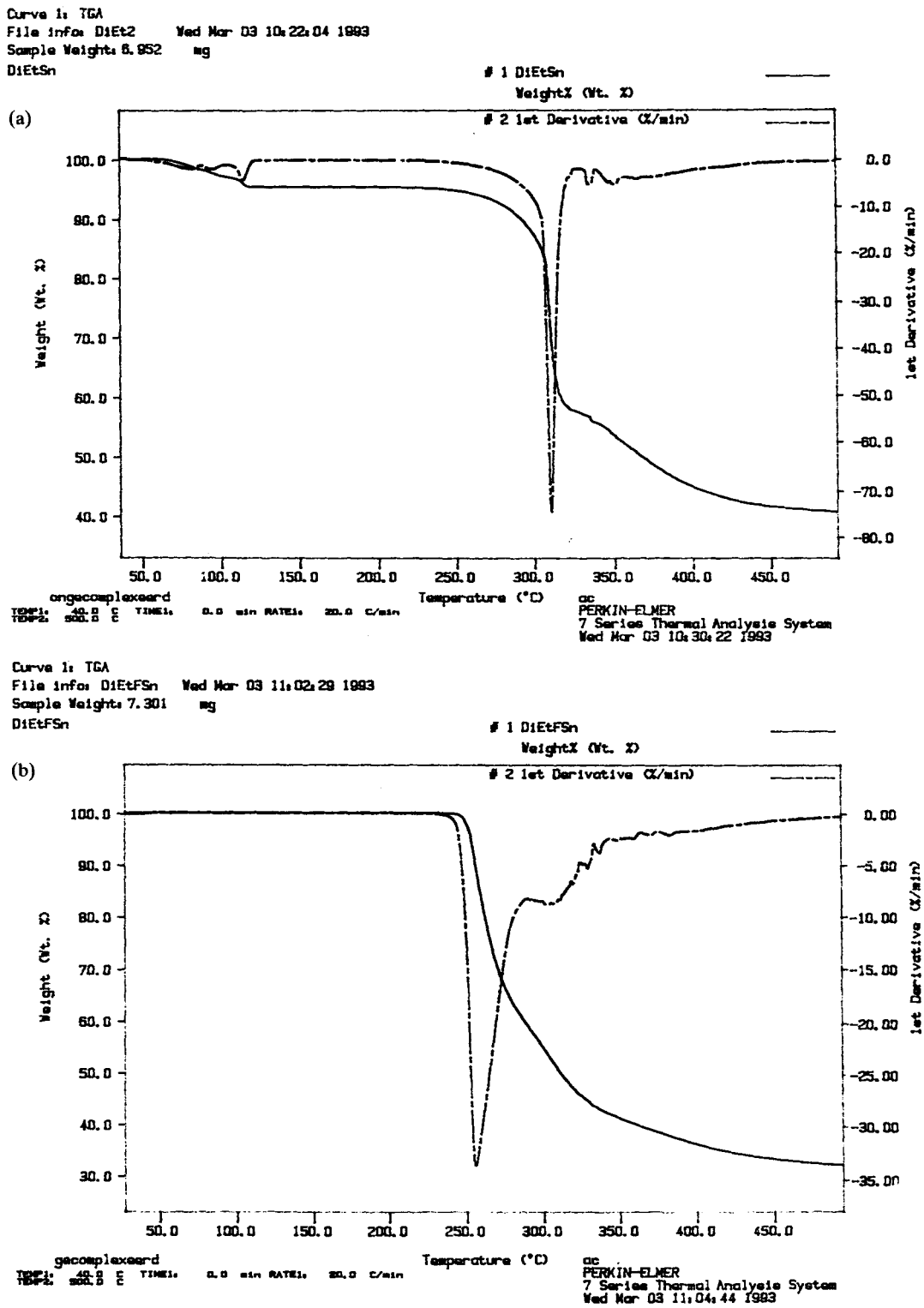
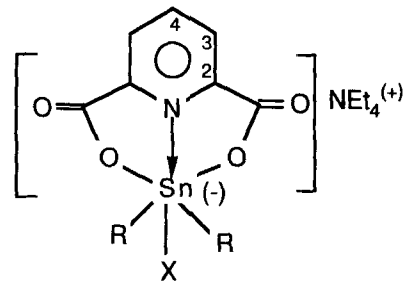


Figure 1 TGA curves obtained for (a) $Et_2Sn[(O_2C)_2C_5H_3N].H_2O$, compound **5**, and (b) $Et_2Sn[(O_2C)_2C_5H_3N]F^-[Et_4N^+]$, compound **1**.

Table 3 ^{1}H NMR data for $\{R_2Sn[2,6\text{-}(O_2C)_2C_5H_3N]X\}^-$ NEt_4^+, compounds **1–4**, and for compound **5**, $Et_2Sn[2,6\text{-}(O_2C)_2C_5H_3N].H_2O$[a]



Group	**1** (R = Et, X = F) in $CDCl_3$	**2** (R = Bu, X = F) in $CDCl_3$	**3** (R = Bu, X = Cl) in DMSO-d_6	**4** (R = Ph, X = Cl) in DMSO-d_6	**5** (R = Et) in DMSO-d_6
H_3C	1.16(t, 7)	1.47(t, 7)	1.57(tt, 7, 2*)	1.20(tt, 7, 2*)	—
CH_2N	3.71(q, 7)	3.65(q, 7)	3.72(q, 7)	3.27(q, 7)	—
H_3C	0.74(t, 8) [^3J = 169/177]	0.60(t, 7)	0.86(t, 7)	*m* and p-C_6H_5: 7.25–7.28(m)	H_3C: 0.69(t, 8) [J = 165/172]
CH_2	—	0.95–1.23(m)	1.30(tq, 7, 7)	*o*-C_6H_5:	—
CH_2	—	0.95–1.23(m)	1.38–1.50(m)	7.47(dd, 8, 2)	—
CH_2Sn	1.36(q, 8) [$^2J \approx 119$]	1.25–1.32(m)	1.38–1.50(m)	[^3J = 114]	CH_2Sn: 1.36(q, 8) [^2J = 108/114]
3-H**	8.28	8.24	8.54	8.27	8.28
4-H**	8.11	8.07	8.69	8.41	8.46

[a] Chemical shifts in ppm (multiplicities, $^nJ(^1H,^1H)$ coupling constants in Hz); the values of the $^nJ(^1H,^{117/119}Sn)$ coupling constants are given between brackets and represented as ^nJ. Abbreviations: m, complex pattern; d, doublet; t, triplet; q, quartet. * Coupling with ^{14}N (I = 1), 1:1:1 triplet; ** ν_A and ν_B of an A_2B system with $^3J_{AB}$ = 7 Hz.

pounds **1–4** show no broadening is explained by the intermolecular exchange being rapid on the ^1H and ^{13}C NMR timescale.

The chemical shift of the fluoride **1** in $CDCl_3$ is high-field shifted by *ca* 100 ppm with respect to its value in DMSO-d_6. This suggests a stronger coor-

Table 4 ^{13}C NMR data for $\{R_2Sn[2,6\text{-}(O_2C)_2C_5H_3N]X\}^-$ NEt_4^+, compounds **1–4**, and for compound **5**, $Et_2Sn[2,6\text{-}(O_2C)_2C_5H_3N].H_2O$[a]

	1 (R = Et, X = F) in $CDCl_3$	**2** (R = Bu, X = F) in $CDCl_3$	**3** (R = Bu, X = Cl) in DMSO-d_6	**4** (R = Ph, X = Cl) in DMSO-d_6	**5** (R = Ft) in DMSO-d_6
H_3C	7.4	7.4	7.2	7.2	—
CH_2N	52.7	52.7	51.6	51.6	—
H_3C	9.8 [^2J = 84]	13.2	13.4	*p*-C_6H_5: 128.1	H_3C: 9.8 [^2J = 84]
CH_2	—	26.1 [^3J = 163]	26.9 [^3J = 153]	*m*-C_6H_5: 127.8 [^3J = 122]	—
CH_2	—	27.5 [^2J = 68]	26.9 [^2J = 54]	*o*-C_6H_5: 133.6	—
CH_2Sn	23.5 [^1J = 1124/1175]	30.9 [^1J = 1091/1141]	32.6 [^1J = 1348]*	[^2J = 67] i-C_6H_5: 151.2	CH_2Sn: 23.1 [^1J = 952/1005]
C(2)	147.7	147.5	146.6	146.1	146.8
C(3)	124.5	124.5	125.7	125.5	125.5
C(4)	140.4	140.4	144.5	144.1	143.0
COO	166.2	166.1	163.7	163.9	163.9

[a] Chemical shifts in ppm; $^nJ(^{13}C,^{117/119}Sn)$ coupling constants in Hz are given between brackets. [b] Badly resolved, poor signal-to-noise ratio.

Table 5 ^{119}Sn NMR data for $\{R_2Sn[2,6\text{-}(O_2C)_2C_5H_3N]X\}^-\ NEt_4\}^+$, compounds **1–4**, and for data compound **5**, $Et_2Sn[2,6\text{-}(O_2C)_2C_5H_3N].H_2O$

	(R = Et, X = F)		2 (R = Bu, X = F)	3 (R = Bu, X = Cl)	4 (R = Ph, X = Cl)	5 (R = Et)
	$CDCl_3$	DMSO-d_6	$CDCl_3$	DMSO-d_6	DMSO-d_6	DMSO-d_6
$\delta(^{119}Sn)$, ppm	−476.5	−380.9	−477.2	−352.8	−579.9	−408.5

dination of the fluoride in $CDCl_3$ than of DMSO in DMSO solutions. The fact that the apparently more weakly coordinating DMSO is able to substitute the more strongly coordinating fluoride can be explained by the law of Mass Action, in an equilibrium of the type

$$[Et_2SnLF]^- + n\ DMSO \rightleftarrows Et_2SnL(DMSO)_n + F^-$$

in which DMSO is used in a large excess (L = 2,6-pyridinedicarboxylate).

Table 6a gives ^{119}Sn and ^{19}F NMR data of compound **1** in various mixtures of $CDCl_3$ and DMSO-d_6. The ^{119}Sn chemical shift variation as a function of solvent composition confirms the above interpretation. The weak maximum exhibited in this variation at a composition of 50/50 (v/v) $CDCl_3$/DMSO-d_6 probably reflects a poorer coordination ability of DMSO-d_6 in the presence of large amounts of $CDCl_3$ than in pure DMSO-d_6, possibly as a consequence of attracting dipole–dipole interactions between $CDCl_3$ and DMSO-d_6 molecules. The high-field shift of the ^{19}F chemical shift of the fluorine atom of **1** in pure DMSO-d_6 (−109.9 ppm) with respect to that in $CDCl_3$ (−89.9 ppm) reflects the higher ionic character of the fluoride ligand in the former than in the latter solvent, confirming the above interpretation. We attribute the existence of a maximum in this ^{19}F chemical shift in 50/50 (v/v) $CDCl_3$/DMSO-d_6 to a higher shielding by the lone pairs of the fluoride anions than in pure DMSO-d_6. In the latter, which is more polar, the lone pairs are expected to be stabilized by the solvent cage around the fluoride anion. This causes a slight deshielding of the ^{19}F nucleus, which should be most shielded by the lone pairs of the fluoride anion when the latter is least solvated.

Table 6a ^{119}Sn and ^{19}F NMR data of compound **1**, $[Et_2SnLF]^-\ Et_4N^+$, in various mixtures of $CDCl_3$ and DMSO-d_6[a]

Solvent composition (%)			
$CDCl_3$	DMSO-d_6	$\delta(^{119}Sn)$[b]	$\delta(^{19}F)$[b]
100	0	−476.5	−89.9
ca 100	1 drop	−462.9	−92.8
75	25	−376.9	−110.9
50	50	−364.0	−115.0
25	75	−369.2	−113.6
0	100	−380.9	−109.9

[a] Concentrations of **1** in $CDCl_3$ and DMSO-d_6 were 0.15 M. [b] ^{119}Sn and ^{19}F chemical shifts are given in ppm with respect to tetramethyltin and fluorotrichloromethane in DMSO-d_6, taken respectively as external references.

Table 6b gives ^{119}Sn and ^{19}F chemical shift data of various mixtures of compound **1**, $[Et_2SnLF]^-\ NEt_4^+$ and compound **5**, $Et_2SnL.H_2O$, in DMSO-d_6. Compound **5** is insoluble in $CDCl_3$. The observation of a single, rather broad, ^{119}Sn resonance confirms the existence of a rapid intermolecular transfer of the fluoride anion from compound **1** to **5**. The single resonance observed in the ^{19}F spectrum and the total absence of any $^1J(^{19}F\text{–}^{119/117}Sn)$ coupling satellites confirms this view. The ^{119}Sn chemical shift data reflect a slightly lower coordination ability of DMSO-d_6 towards tin in the ionic compound, **1**, than in the neutral one, **5**. This proposal is in good agreement with the stabilization of the fluoride (F^-) anion by DMSO proposed above, and suggests the existence of at least a weak residual fluoride coordination to tin through solvated ion–metal pairing. The ^{19}F NMR data are in agreement with this proposal. Thus, the ^{19}F chemical shift is slightly more low-field shifted in the weakly bound state of the solvated $[Et_2SnLF]^-\ NEt_4^+$ complex than in the more unbound ionic state of the fluoride anion in mixtures containing higher amounts of the neutral complex $Et_2SnL.H_2O$, where the free anions have a longer lifetime.

All these results are in agreement with the existence of an equilibrium, rapid on both ^{119}Sn and ^{19}F NMR timescales, of the type:

$$F^- + Et_2SnL.H_2O \rightleftarrows Et_2SnLF^- + H_2O$$

Table 6b ^{119}Sn and ^{19}F NMR data of various mixtures of compound **1**, $[Et_2SnLF]^-\ Et_4N^+$, and compound **5**, $Et_2SnL.H_2O$, in DMSO-d_6[c]

Compound composition (%)				
$[Et_2SnLF]^-\ Et_4N^+$	$Et_2SnL.H_2O$	Solvent	$\delta(^{119}Sn)$[b]	$\delta(^{19}F)$[b]
100	0	DMSO-d_6	−380.9	−109.9
75	25	DMSO-d_6	−387.9	−110.7
50	50	DMSO-d_6	−393.5	−111.6
25	75	DMSO-d_6	−397.1	−112.7
0	100	DMSO-d_6	−408.5	—

[b] ^{119}Sn and ^{19}F chemical shifts are given in ppm with respect to tetramethyltin and fluorotrichloromethane in DMSO-d_6, taken respectively as external references. [c] Concentrations of **1** and **5** were 0.15 M.

In vitro antitumour activity

The ID_{50} values obtained as described previously[10–15] for compounds **1**, **3** and **5**, and for the parent di-n-butyl compound[1] against two human tumour cell lines, MCF-7, a mammary tumour, and WiDr, a colon carcinoma, are summarized in Table 7.

From Table 7, it is clear that the ionic compounds have no improved *in vitro* antitumour activity with respect to the parent diorganotin 2,6-pyridinedicarboxylate, but on the contrary are even less active. Since our ionic compounds have a higher solubility than the corresponding parent compounds, this observation does not support the hypothesis of Atassi[2] that water-soluble tin compounds might exhibit a higher antitumour activity, at least for the present cell lines. It should be outlined that, once more,[8,9] the di-n-butyltin compound exhibits a much higher activity than the corresponding diethyltin compound in contrast to most tin compounds tested *in vivo* against P388 leukemia in mice.[2] Furthermore, the di-n-butyltin compound is quite active against WiDr, which is usually not the case for most di-n-butyltin derivatives as they exhibited promising activity mainly against MCF-7.[8]

Table 7 Inhibition doses, ID_{50} (ng cm^{-3}) for compounds **1**, **3** and **5**, for the parent di-n-butyl derivative and for some reference compounds[7] against MCF-7 and WiDr human tumour cell lines

Compound	MCF-7	WiDr
5, $Et_2Sn[2,6\text{-}(O_2C)_2C_5H_3N].H_2O$	822	1290
1, $\{Et_2Sn[2,6\text{-}(O_2C)_2C_5H_3N]F\}^-NEt_4^+$	1002	2495
n-$Bu_2Sn[2,6\text{-}(O_2C)_2C_5H_3N].H_2O$	54	76
3, $\{n\text{-}Bu_2Sn[2,6\text{-}(O_2C)_2C_5H_3N]Cl\}^-\ NEt_4^+$	118	220
Doxorubicin[7]	63	31
Cis-platin[7]	850	624
Etoposide[7]	187	624
Mitomycin C[7]	3	17

Equipment

The Mössbauer spectra were recorded as described previously.[10–15] The TGA spectra were recorded on a Perkin–Elmer TGA7 instrument. The mass spectra were recorded on an AEI MS 902S instrument coupled to a NOVA computer. Samples were introduced via the direct insertion probe. The ^1H and ^{13}C NMR spectra were recorded at 270.13 and 67.93 MHz respectively on a Bruker AM 270 instrument. The ^{19}F and ^{119}Sn NMR spectra were recorded at 235.34 and 93.28 MHz respectively on a Bruker AC 250 instrument.

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