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Synthesis, characterization and *in vitro* antitumour activity of a series of substituted 2,2-di-n-butyl-4-oxo-benzo-1,3,2-dioxastannins

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The characterization by 1D and/or 2D ^{1}H NMR, ^{13}C NMR, ^{119}Sn NMR, Mössbauer and mass spectrometry of a series of di-n-butyltin derivatives of 3-, 4-, and 5-methyl-, 3-, 4-, and 5-methoxy-, 4- and 5-amino-substituted, and 3,5-diiodo-salicyclic acids is described. Their NMR spectra suggest that they are present as dimers in $CDCl_3$ solution, like the di-n-butyltin derivative of unsubstituted salicyclic acid. This has been confirmed by the observation of mixed dimers. The insoluble derivatives have been characterizerd by solid state cross polarization/magic angle spinning (CP/MAS) ^{13}C and/or ^{119}Sn NMR, Mössbauer and mass spectroscopy. These compounds are characterized *in vitro* by a lower inhibition dose than *cis*-platin or etoposide against a series of five human cell lines.

INTRODUCTION

We recently reported the synthesis and characterization of 2,2-di-n-butyl-4-oxobenzo-1,3,2-dioxastannin, which is dimeric in $CDCl_3$, as deduced from its ^{1}H and ^{13}C NMR spectra.[1] This compound exhibits a promising antitumour activity *in vitro*, characterized by inhibition concentrations IC_{50} values of 14×10^{-8} M and 40×10^{-8} M against P388 and L1210 respectively.[1] This activity is two to three times higher than for *cis*-platin, *cis*-$Pt(NH_3)_2Cl_2$. Therefore, we prepared a series of analogous compounds substituted in the aromatic ring in order to determine the influence of this type of substitution on the activity of the compound type. A recent paper[2] described the synthesis, the infrared and mass spectra, and the antimicrobial activity of 2,2-di-n-butyl-4-oxobenzo-1,3,2-di-oxastannin and its 3-methyl-substituted analogue. We present here the preparation (see Experimental section below) and characterization of an extended series of di-n-butyl derivatives of substituted salicylic acids, including ^{1}H, ^{13}C and ^{119}Sn NMR. Mössbauer and mass spectral data, together with the results of *in vitro* antitumour tests.

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RESULTS AND DISCUSSION

Synthesis and Mössbauer spectroscopic data

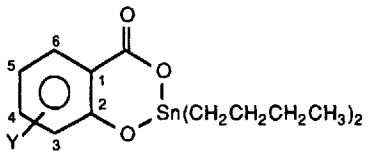
The melting points and Mössbauer data of some substituted 2,2-di-n-butyl-4-oxobenzo-1,3,2-dioxastannins, prepared[1] by the reaction of di-n-butyltin oxide with the corresponding substituted salicylic acid, are given in Table 1.

NMR data

The ^{1}H NMR data[a] of compounds **1** to **9** are summarized in Tables 2a and 2b.

The two-dimensional homonuclear correlation spectroscopy (COSY) spectrum of compound **6** allowed an unambiguous assignment of all signals

Table 1 Melting points, recrystallization solvents and Mössbauer data (isomer shift, IS: quadrupole splitting, QS; and line widths Γ) of compounds **1** to **9**



Compound	Y	M.p. (°C)	Recrystallization solvent	IS ($mm^{-1}s$)	QS ($mm^{-1}s$)	Γ_1 ($mm^{-1}s$)	Γ_2 ($mm^{-1}s$)
1	3-Me	94–96[a]	MeOH + DMSO	1.32	3.38	0.90	0.93
2	4-Me	173–175	MeOH + $CHCl_3$	1.40	3.78	0.94	0.88
3	5-Me	95–97	Hexane + $CHCl_3$	1.29	3.32	0.95	0.94
4	3-MeO	78–80	Hexane + $CHCl_3$	1.29	3.44	1.31	1.05
5	4-MeO	168–170	MeOH + $CHCl_3$	1.40	3.79	0.90	0.88
6	5-MeO	118–120	MeOH + hexane	1.32	3.40	1.07	0.92
7	3,5-I_2	234–237[b]	—	1.21	3.21	0.87	0.88
8	4-NH_2	>350	—	1.22	3.09	0.94	0.93
9	5-NH_2	105–107	—	1.27	3.19	1.11	0.96
		>350	Toluene				

[a] Literature: Ref. 2, 256–259°C.
[b] Literature: Ref. 3, 234–237°C.

except those of protons 8 and 9, which overlapped too strongly. The aliphatic region of the other compounds was assigned by analogy with the unambiguously assigned compound **6**, owing to chemical shift similarities.

The region 10.8–11.7 ppm is particularly interesting and informative. The compounds **1**, **2**, **3** and **6** in $CDCl_3$, and **9** in DMSO, exhibit broad resonances at 11.67, 11.34, 11.24 and 10.85, and 10.88 ppm respectively. The spectrum of a 1:1 mixture of compounds **3** and **6** exhibits a broad complex pattern between 10.89 and 11.25 ppm that could be described as two very broad triplets.

The high value of the chemical shifts are indicative of hydroxylic protons in a positively charged environment. Therefore, tentatively we propose that these data reflect the presence of one water molecule coordinated to tin. Such a water coordination was already observed previously.[4] Pattern superposition is attributed to the presence of several isomeric species, especially in mixed dimers (see below).

Solution ^{13}C NMR data[a] of compounds **1** to **9** are given in Tables 3a and 3b.

The two-dimensional heteronuclear ^{13}C–^{1}H COSY spectrum of compound **6** was used to assign all the ^{13}C resonances from the assigned ^{1}H resonances.

The assignment in the other compounds has been performed by comparison of the data with those of compound **6**, owing to chemical shift similarities. When this procedure was inoperative, we used DEPT (distortionless enhancement by polarization transfer) editing (proton pulse angle 135°) and/or chemical shift incremental rules.[5]

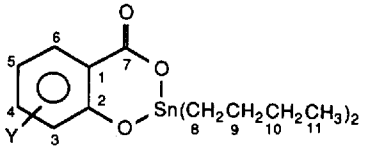
No ^{13}C NMR spectrum of compound **5** in solution could be recorded because its solubility was too low. Therefore, its solid-state ^{13}C cross-polarization–magic angle spinning (CP–MAS) NMR spectrum was recorded: it exhibits signals at 14.29 ppm (carbon 11), 26.39, 27.93 and 30.12 ppm (carbons 8 to 10), 55.81 ppm (methoxy), several overlapping lines between 105.1 and 110.5 ppm (of which one is clearly visible at 107.49 ppm) (carbons 1, 3, 5 and 6), at 134.0 ppm (carbon 4), 167.1 and 167.97 ppm (carbon 2) and 173.55 ppm (carbonyl).

The ^{119}Sn NMR data of compounds **1**, **3**, **4**, **6**, **8** and **9** in solution are given in Table 4.

The ^{1}H NMR spectra of $CDCl_3$ solutions of compounds **1**, **2**, **3**, **4**, **6** and **9** exhibit two diastereotopic methyl groups belonging to the Bu_2Sn moiety, as observed for the dibutylin derivative of the unsubstituted salicylic acid.[1] This observation was interpreted[1] by dimeric structures; see Fig. 1.

In the present study the existence of such dimers is strongly evidenced by the spectra of some 1:1 mixtures of pairs of substituted salicylic acid diorganotin derivatives. Thus, in the meth-

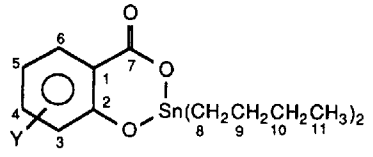
Table 2a ^{1}H NMR chemical shift δ (ppm) and coupling constant J(Hz) data of di-n-butyltin(IV) derivatives of some methyl- and methoxy-substituted salicylic acids (compounds **1**, **2**, **3**, **4** and **6**)



Proton	Compound				
	1 (Y = 3-Me)	**2** (Y = 4-Me)	**3** (Y = 5-Me)	**4** (Y = 3-MeO)	**6** (Y = 5-MeO)
11	t, 0.806 [7.3] [^1J(C–H) = 113] t, 0.873 [7.3]	t, 0.794 [7.3] [^1J(C–H) = 112] t, 0.873 [7.3]	t, 0.894 [7.3] [^1J(C–H) = 118] t, 0.963 [7.3]	t, 0.796 [7.3] [^1J(C–H) = 116] t, 0.861 [7.3]	t, 0.822 [7] t, 0.874 [7]
10	m, 1.264–1.413	m, 1.262–1.401	m, 1.371–1.471	m, 1.363 [7.3]	m, 1.176–1.385
9, 8	m, 1.413–1.716	n, 1.401–1.704	m, 1.471–1.957	m, 1.542–1.970	m, 1.385–1.560
6	d, 7.620 [7.0]	d, 7.640 [8.0]	s, 7.577	d, 7.453 [6.6]	nrd, 7.283
5	nrt, 6.823	d, 6.790 [8.0]	Me, 2.422 (s) [^1J(C–H) = 128]	m, 6.784–6.875	MeO, 3.810 [^1J(C–H) = 129]
4	nrd, 7.310	Me, 2.373 (s) [^1J(C–H) = 136]	d, 7.260 [*ca* 9]	d, 7.052 [7.4]	dd, 7.079 [9.0; 2.7]
3	Me, 2.298 (s) [^1J(C–H) = 130]	s, 6.823	d, 6.905 [*ca* 9]	MeO, 3.923 (s) [^1J(C–H) = 132]	d, 6.920 [9.0]

[a] Abbreviations: b, broad; d, doublet; dd, doublet of doublets; m, complex pattern; nr, non-resolved; s, singlet; t, triplet. J values are given in square brackets. The solvent was $CDCl_3$ in each case.

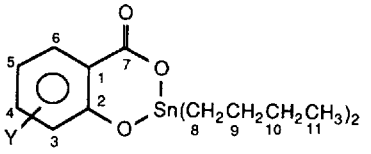
Table 2b ^{1}H NMR chemical shift δ (ppm) and coupling constant J (Hz) data[a] of di-n-butyltin(IV) derivatives of some di-iodo- and amino-substituted salicylic acids (compounds **7** to **9**)



Proton	Compound			
	7 (Y = 3,5-I_2) in $(CD_3)_2SO$	**8** (Y = 4-NH_2) in $(CD_3)_2SO/D_2O$	**9** (Y = 5-NH_2) in $(CD_3)_2SO$	**9** (Y = 5-NH_2) in $CDCl_3$
11	t, 0.792 [7.3] [^1J(C–H) = 127]	2nrt, 0.800 [^1J(C–H) = 125]	t, 0.802 [7.2]	t, 0.810 [7.2] t, 0.887 [7.2]
10	se, 1.297 [7.2]	m, 1.263–1.340	m, 1.192–1.352	m, 1.263–1.454
9	m, 1.350–1.385	m, 1.340–1.480		m, 1.668–1.758
8	m, 1.385–1.530 [^2J(Sn–C–H) = 145]	m, 1.480–1.740	m, 1.482–1.601	
6	d, 8.060 [2.4]	d, 7.446 [8.0]	s, 7.114	d (nr), 7.047
5		d, 6.075 [8.0]	NH_2, 3.957–3.975 (b)	NH_2, s, 3.5 (b)
4	d, 7.950 [2.4]	NH_2, s, 3.545 (b)	d, 6.632 [nv]	dd, 6.660 [8.6; 2.5]
3		s, 5.977	d, 6.555 [8.1]	d, 6.618 [8.6]

[a] Abbreviations: b, broad; d, doublet; dd, doublet of doublets; m, complex pattern; nr, non-resolved; s, singlet; t, triplet; se, sextet; nv, non-visible. J values are given in square brackets.

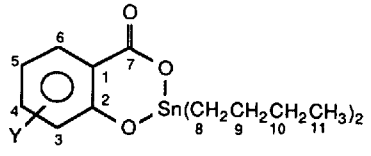
Table 3a ^{13}C NMR chemical shift δ (ppm) data of di-n-butyltin(IV) derivatives of some methyl- and methoxy-substituted salicylic acids (compounds **1**, **2**, **4**, **5** and **6**)



	Compound				
Carbon	**1** (Y = 3-Me)	**2** (Y = 4-Me)	**3** (Y = 5-Me)	**4** (Y = 3-MeO)	**6** (Y = 5-MeO)
11	13.52	13.51	13.47 13.49	13.46	13.52
10	26.75	26.76	26.75	26.70	26.75
8,9	27.37 27.79	27.37 27.79	27.37 27.79	27.32 27.73	27.40 27.78
7	175.50	172.20	175.64	175.66	175.49
6	130.69	136.03	130.60	116.47	113.80
5	120.18	128.37	127.90	118.18	152.08
4	117.75	126.58	136.19	122.11	122.89
3	146.59	118.18	117.33	148.83	118.33
2	161.86	160.31	159.77	152.34	156.18
1	112.25	114.18	114.44	115.09	114.66
Me	21.80	15.73	20.37	56.31	55.96

[a] The solvent was $CDCl_3$ in each case.

Table 3b ^{13}C NMR chemical shift δ (ppm) data[a] of di-n-butyltin(IV) derivatives of some di-iodo- and amino-substituted salicylic acids (compounds **7** to **9**).

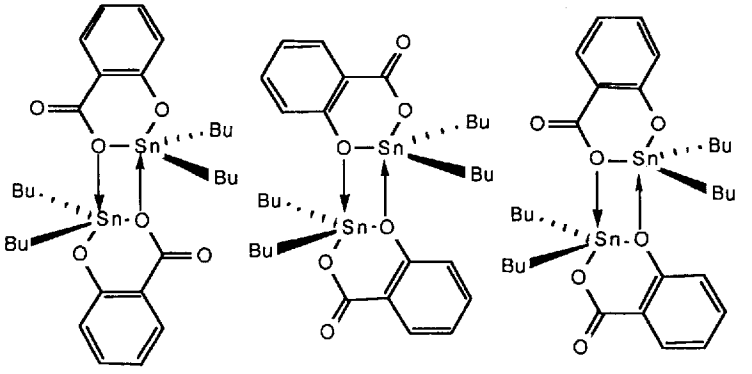


	Compound			
Carbon	**7** (Y = 3,5-I_2) in $(CD_3)_2SO$	**8** (Y = 4-NH_2) in $(CD_3)_2SO/D_2O$	**9** (Y = 5-NH_2) in $(CD_3)_2SO$	**9** (Y = 5-NH_2) in $CDCl_3$
11	13.43	13.27; 13.38	13.43	13.43; 13.52
8–10	25.52 26.55 27.99	25.72; 26.29 26.49; 26.63 26.82; 26.98	10: 25.73 9: 26.52 (^2J(Sn–C) = 56) 8: 24.88 [^1J($^{119/117}$Sn–C) = 717/689]	10: 26.71 27.36 27.82
4,6	140.78 147.85	6: 131.87 4: 154.70	121.26 3, 4, 6 121.26 117.30	6: 115.67 4: 123.88
3, 5	76.63 96.09	5: 105.70 3: 98.91	5: 137.15	5: 138.44 3: 118.12
1	122.62	102.89	120.08	114.83
2,7	162.49 166.41	2: 162.67 7: 174.55	2: 155.93 7: 168.88	2: 154.86 7: 175.33

[a] J values are given in square brackets.

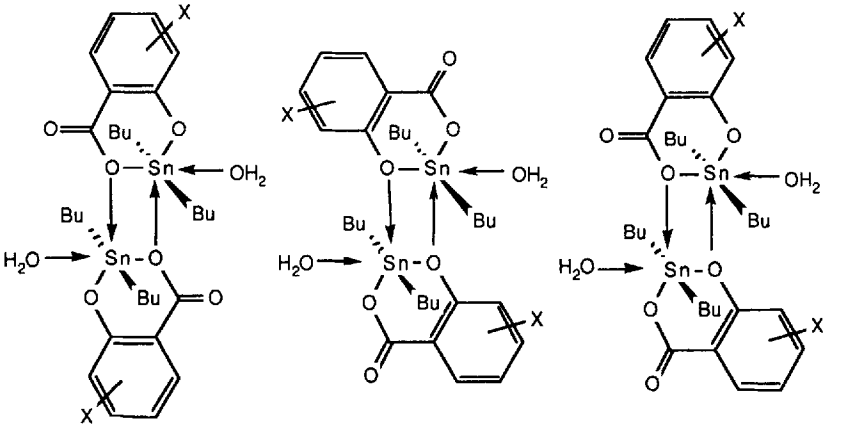
Table 4 ^{119}Sn NMR data of compounds **1**, **3**, **4**, **6**, **8** and **9** in solution

Compound	Solvent	δ (ppm) relative to Me_4Sn	$\nu_{1/2}$ (Hz)
1	$CDCl_3$	−202.3 and −190.3	71 and 190
3	$CDCl_3$	−198.8 and −192.6	83 and 75
4	$CDCl_3$	−187.4 and −200.1	51 and 63
		−233.9 and −253.3	50 and 40
6	$CDCl_3$	−195.0 and −198.0	76 and 87
8	DMSO-d_6	−245.6	
9	DMSO-d_6	−235.5	



A **B** **C**

Figure 1 The three possible dimers for the dibutyltin derivative of unsubstituted salicylic acid.[1]



A' **B'** **C'**

Figure 2 The three possible dimers for the dibutyltin derivatives of substituted salicylic acids **1** to **6**.

oxy or methyl region of their ^{1}H NMR spectra, the 1:1 mixtures of **3** and **6**, and **1** and **4**, exhibit additional new resonances showing chemical shifts slightly different from those of the resonances of the originating components and also higher intensities than these. This observation is in agreement with the formation of mixed dimers present in higher proportions than the

dimers from the pure compounds (typically >3:1, not precisely measurable because of resonance overlapping).

Taking into account the presence of bound water signals not observed in the unsubstituted salicylic acid tin derivative, we propose dimeric six-coordinate structures for compounds **1** to **6** involving a water molecule coordinated at tin (see Fig. 2).

For these dimers A′, B′ and C′, one, one and two methyl triplets, respectively, are expected in the butyl region of the ^{1}H NMR spectrum. This is overall more than what is experimentally observed. Either structure C′ alone or both the two dimers A′ and B′ are in agreement with the two triplets observed experimentally. Because the ^{119}Sn NMR spectrum of the dibutyltin derivative of the unsubstituted salicylic acid[1] exhibits two minor very noisy lines of comparable intensities and two major lines of comparable but more significantly different intensities, the most straightforward interpretation was that the major dimers are A and B, and that the minor dimer is C, not visible in the ^{1}H and ^{13}C NMR probably because of accidental isochrony of minor C resonances with those of the major A and B dimers (see Fig. 1).

The data obtained from the aliphatic methyl ^{1}H resonances and the ^{119}Sn solution signals of compounds **1**, **3** and **6** do not support this proposal, because their pairwise intensity ratios are not significantly different from 1:1. Hence, for this series of compounds, we propose the dimeric structure C′ involving coordinated water (see Fig. 2). We have no explanation for the difference in dimer structure between the unsubstituted and substituted salicylic acid tin derivatives. Nevertheless the high sensitivity of the dimeric isomer distribution to the nature of the substituent is confirmed by the fact that compound **4** exhibits four ^{119}Sn resonances, in agreement with the presence of all three dimers A′, B′ and C′. Thus the major signals of identical intensity observed in compound **4** at −187.4 and −200.1 ppm are atrributed to the dimer C′, and the two less intense ones at −233.3 and −253.3 ppm to the symmetrical dimers A′ and B′.

On the contrary, for DMSO solutions, only one methyl triplet is seen for compounds **7** and **9**, which can be explained by the destruction (by the very strongly nucleophilic DMSO) of the dimeric structure present in $CDCl_3$. In a mixture of DMSO and D_2O, two methyl triplets are again visible in the aliphatic region of the ^{1}H NMR spectrum of compound **8** and, in the ^{13}C NMR, this compound exhibits two sets of butyl signals, which suggests the partial restoration of the structure of Fig. 2, competing with the more specific DMSO complexation observed in pure DMSO.

The solid-state ^{119}Sn CP–MAS NMR spectroscopic data of compounds **1**, **2**, **5**, **7** and **9** are summarized in Table 5.

The isotropic ^{119}Sn chemical shifts δ for compounds **1**, **2**, **5**, **7** and **9** characterize these as species with strong intra- and/or inter-molecular association in the solid state. The solution-state ^{119}Sn chemical shifts for compound **1** in the non-coordinating solvent $CDCl_3$ are very similar to the isotropic chemical shifts for compound **1** in the solid state. Therefore, it is legitimate to assume an identical structure for compound **1** in solution and in the solid state. Since the isotropic chemical shifts for the solid compounds **1**, **2**, **5**, **7** and **9** all occur in the same region and all show a very similar shielding pattern (see Fig. 3), it is certainly justified to postulate identical solution and solid-state structures for all these compounds. It is not straightforward to assign a specific coordination number from the isotropic chemical shifts alone, but coordination in the solid state must be at least five-fold. All spectra show very large chemical shift anisotropies of the order of 1000 ppm, together with a spinning sideband pattern typical for an asymmetric shielding tensor (see Fig. 3). Such asymmetric, but less extensive, shielding

Table 5 ^{119}Sn solid-state CP–MAS NMR data of compounds **1**, **2**, **5**, **7** and **9**

	Compound				
	1	**2**	**5**	**7**	**9**
δ (relative intensity)	−208.3 (4.5) −214.2 (2.0) −223.6 (2.5)	−275.0	−278.5	−221.8	−234.0
$\nu_{1/2}$ (Hz)	200	900	500	350	1800

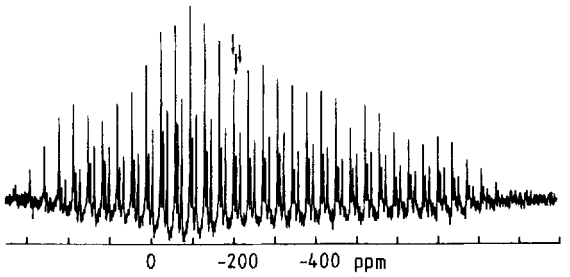


Figure 3 ^{119}Sn CP–MAS NMR spectrum of compound **1**. The centre bands are marked with arrows. The depicted spectrum was obtained at a spinning rate of 4 kHz. The recycle delay was 5 s, the contact time 1 ms; 10 000 transients were accumulated.

tensors, along with similar half-height linewidths, have been observed for polymeric dialkyltin oxide $[R_2SnO]_n$.[6] This is strong evidence for a highly distorted coordination sphere of the various tin sites in compounds **1**, **2**, **5**, **7** and **9**, in agreement with the structures proposed in Fig. 2, especially for compound **1**, with its three centre bands in a 4.5:2.0:2.5 ratio. It should be mentioned, however, that this finding can also be explained by polymorphism in the solid state, a common property in organotin chemistry. The observed half-height linewidths for compounds **1**, **2**, **5**, **7** and **9** vary between 200 and 1800 Hz. As high-resolution solid-state NMR spectroscopy, especially of heavy-metal spin-1/2 nuclei, responds very strongly to short-range order effects, compound **9** (and possibly compound **2**) must be considered as highly disordered, i.e. amorphous. The chemical shift dispersion for compounds **1**, **5** and **7** is much smaller. At our field strength of 7 T, ^{119}Sn NMR linewidths of 200 or 350 Hz are a good indication for fairly high short-range order. It should be stated, however, that highly crystalline molecular organotin compounds usually yield even narrower resonances with linewidths as small as 10 Hz—at least in the absence of further complicating effects.

Mass spectroscopic data

The 70-eV mass spectra of compounds **1–9** are described in Table 6. The fragments containing no substituted salicylic acid ligand, obeyed the usual[7] fragmentation rules. The presence of $[Ar(O)COO]_2Sn_2Bu_3^+$, observed for compounds **1**, **2**, **5** and **8**, might be due to the dimeric character of these compounds even in the gas phase. This reinforces the existence of dimeric structures presumed from liquid- and solid-state

Table 6 Peak intensities as percentages of the base peak in the 70 eV mono-isotopic mass spectra of compounds **1**, **2**, **5**, **7** and **8** of the type $ArO(COO)SnBu_2$

	Compound								
	1[a]	**2**[b]	**3**	**4**[c]	**5**[d]	**6**[e]	**7**[f]	**8**	**9**[g]
$Sn^{\cdot+}$ ($m/z = 120$)	24	15	9	17	7	7	10	7	7
HSn^+ ($m/z = 121$)	14	16	9	20	12	18	9	12	6
$HOSn^+$ ($m/z = 137$)	32	—	17	32	15	27	—	15	7
$BuSn^+$ ($m/z = 177$)	13	22	7	—	16	18	7	16	—
$BuSnH_2^+$ ($m/z = 179$)	—	—	—	—	—	—	9	13	5
Ar—O—Sn^+	100	97	42	81	100	72	100	100	54
Ar—O—$SnH^{+\cdot}$	27	30	—	—	31	—	26	31	14
$ArO(COO)Sn^{+\cdot}$	—	—	—	—	—	—	10	—	61
$ArO(COO)SnH^+$	91	80	41	72	31	58	9	31	13
$M^{\cdot+}$	66	—	100	100	—	—	15	—	100
M + 1	36	100	16	11	67	100	4	67	5
$[Ar(O)COO]_2Sn_2Bu_3^+$	7	33	Trace	—	4	—	—	4	—

[a] A fragment-ion at $m/z = 253$ has also been observed ($I = 17\%$). It might be due to $C_7H_5(O)COOSn^+$. [b] A fragment-ion at $m/z = 253$ has also been observed ($I = 29\%$); cf, footnote a. [c] A fragment-ion at $m/z = 199$ has also been observed ($I = 12\%$), probably due to $PhSnH_2^+$. Fragment-ions at $m/z = 269$ (37%), 242 (79%) and 228 (55%) have also been observed. [d] A fragment-ion at $m/z = 227$ has also been observed (23%), probably due to $MeOPhSn^+$. [e] A fragment-ion at $m/z = 227$ has also been observed ($I = 23\%$); cf, footnote d. [f] A fragment-ion at $m/z = 199$ has also been observed ($I = 12\%$); cf, footnote c. [g] Fragment-ions at $m/z = 285$ (Ar—O—$SnBu^+$, $I = 7\%$) and at $m/z = 342$ (Ar—O—$SnBU_2^+$, $I = 7\%$) have also been observed.

Table 7 Inhibition doses (ID_{50} values) *in vitro* (in ng cm^{-3}) of compounds **1**, **2**, **6**, **7** and **8**, and of some reference compounds,[8] against five human tumour cell lines

Organotin compound	Human tumour cells				
	A204	MCF-7	724	WiDr	IgR-37
1	97	44	86	330	675
2	105	51	84	316	667
6	69	29	46	122	547
7	161	72	117	454	970
8	105	42	70	330	642
Doxorubicin	10	63	25	31	63
Cis-platin	817	850	268	624	878
Etoposide	91	187	457	624	427
Mitomycin C	18	3	15	17	4

data as well as from previous work.[1] For all compounds, the fragments observed are in agreement with the structures proposed.

In vitro anticancer screening

Compounds **1**, **2**, **6**, **7** and **8** were submitted to *in vitro* tests against five human tumour cells lines, A204 (rhabdomyosarcoma), MCF-7 (mammary tumour), T24 (bladder carcinoma), WiDr (colon carcinoma) and IgR-37 (melanoma). The results are given in Table 7.

The five compounds exhibited similar activity against each of the cell lines tested: the ID_{50} values are not very different from each other. Depending on the cell line, the ID_{50} values are lower (up to 20 times) than those obtained earlier for *cis*-platin or etoposide.[8] Our compounds are however less active than doxorubicin and mitomycin C. The MCF-7 was the most sensitive of the five cell lines used.

EXPERIMENTAL

Synthesis

The syntheses are similar to that used for the preparation of the unsubstituted salicylic acid derivative:[1] for compounds **1**–**6**, 4.97 g (0.02 mol) of di-n-butyltin oxide and 0.02 mol of the substituted salicylic acid were refluxed in 200 cm^3 ethanol and 500 cm^3 toluene. The ternary azeotrope, water/ethanol/toluene, was distilled off with a Dean–Stark funnel. After 6 h the solvent was evaporated. For compounds **2** and **5**, the solid obtained was recrystallized from chloroform/methanol. For compound **1**, the oil obtained was dissolved in the smallest possible volume of methanol/DMSO. After one week in the refrigerator, the crystals obtained were filtered and washed several times with petroleum ether. For compounds **3**, **4** and **6**, the same procedure was used except that hexane/chloroform was used instead of methanol/DMSO. For compound **4**, the oil obtained solidified in the refrigerator and was characterized as such. For compounds **8** and **9**, the reaction was carried out in benzene. After 24 h of reflux, the mixture became homogeneous for compound **9**. The solvent was evaporated and a yellow compound was obtained that was left under vacuum for 4 h. On the contrary, the mixture was still heterogeneous for compound **8** after 24 h of reflux. The solids obtained were filtered and washed several times with petroleum ether.

Instruments

The ^1H and ^{13}C NMR spectra were recorded on a Brüker WM 250 instrument (chemical shifts versus TMS as internal standard). The ^{119}Sn NMR and ^{13}C CP–MAS NMR spectra were recorded on a Varian 200 instrument [chemical shifts versus TMS (^{13}C NMR) or tetramethyltin (^{119}Sn NMR) as external standard].

The ^{119}Sn CP–MAS NMR spectra were obtained on a Brüker MSL 300 spectrometer, equipped with a double-bearing probe and 7 mm o.d. ZrO_2 rotors. All ^{119}Sn chemical shifts are given with respect to tetramethyltin, using the shift of solid tetracyclohexyltin (−97.35 ppm) as a secondary reference. The procedure to set the Hartmann–Hahn matching condition for $^1H/^{119}Sn$ cross polarization has been described elsewhere.[6] The proton 90° pulse length was set to 5 μs; a contact time of 1 ms was used throughout. Recycle delays were 5 s, spinning speeds were 3.0–4.5 kHz. To obtain a sufficient signal-to-noise ratio, 1000 to 10 000 transients were accumulated. All spectra were re-run at a second, sufficiently different, spinning speed in order to assign centre bands.

The Mössbauer spectra were recorded in the constant acceleration mode on an Elscint Promeda instrument with a MVT4 transducer ($Ca^{119m}SnO_3$ source from Amersham; sample temperature 90–100 K).

The mass spectra were recorded on a VG M Micromass 7070F instrument (source temperature 180–200°C, pressure $\sim 10^{-5}$–10^{-7} mbar).

In vitro tests

Drug activity was determined using an automated *in vitro* technique.[8] In summary, human tumour cells were plated in the wells of 96-well flat bottom microtitre plates (Falcon, type 3070). The plates were incubated for two days at 37°C (5% CO_2) to allow the cells to adhere and resume exponential growth prior to the addition of the drugs. After two days, 50 μl of the highest drug concentrations were added to the wells of column 12 and from there serially diluted three-fold to row 1 by serial transfer of 50 μl using an eight-channel micropipette. The final volume of row 1 was adjusted to 100 μl. No additions were made to the wells of rows A and B, which served as controls. All drugs were tested in duplicate.

The plates were further incubated for five days at 37°C (5% CO_2). On day 7, the cultures were terminated by the addition of 100 μl saline containing 0.002% (w/v) propidium iodide, 0.3% drawing ink and 0.5% Triton X-100. The plates were kept overnight at 4°C before reading. Fluorescence intensity was measured by a photomultiplier. Dose–response curves were obtained and ID_{50} values were calculated.

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REFERENCES

1. Gielen, M, Vanbellinghen, C, Gelan, J and Willem, R *Bull. Soc. Chim. Belg.* 1988, 97: 873
2. Obafemi, C A, Ejenavi, A B, Kolawole, D O and Oloke J K *Inorg. Chim. Acta*, 1988, 151: 21
3. Gielen, M, Mancilla, T, Ramharter, J and Willem, R, *J. Organomet. Chem.*, 1987, 328: 61
4. Gielen, M, Joosen, E, Mancilla, T, Jurkschat, K, Willem, R, Roobol, C, Bernheim, J, Atassi, G, Huber, F, Hoffmann, E, Preut, H and Mahieu, B *Main Group Met. Chem.*, 1987, 10: 147; Huber, F, Preut, H, Hoffmann, E and Gielen, M *Acta Cryst.*, 1989, C45:51
5. Silverstein, R M, Bassler, G C and Morrill, T C, *Spectrometric Identification of Organic Compounds*, 4th edn, Wiley, New York, 1981
6. Harris, R K and Sebald, A *Magn. Reson. Chem.*, 1987, 25: 1058
7. Gielen, M, Barthels, M R, De Clercq, M and Nasielski, J, *Bull. Soc. Chim. Belg.*, 1971, 80: 89; Gielen, M and Topart, J, *Bull. Soc. Chim. Belg.*, 1974, 83: 249; Fish, R H, Holmstead, R L, Gielen, M and De Poorter, B, *J. Org. Chem.*, 1978, 43: 4969; Gielen, M, Simon, S and Van de Steen, M *Organic Mass Spectrometry*, 1983, 18: 451; Gielen, M *Organic Mass Spectrometry*, 1983, 18: 453; Gielen, M, Tzschach, A and Jurkschat, K *Bull. Soc. Chim. Belg.*, 1985, 94; 359; Gielen, M *Bull. Soc. Chim. Belg.*, 1985, 94: 1075
8. van Lambalgen, R and Lelieveld, P *Invest. New Drugs*, 1987, 5: 161