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The *in vitro* Antitumour Activity of Substituted Dibutyl-1,3,2-dioxastannolanes

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Six dibutyl-1,3,2-dioxastannolanes, including two enantiomeric pairs, exhibited greater *in vitro* antitumour activity towards a variety of human tumour cell lines than cisplatin but with little discrimination, suggesting hydrolysis to a common cytotoxic intermediate. A cell line hypersensitive to mitochondrial inhibitors (CI80–13S) was not sensitive to any of the test compounds, suggesting that cell mechanisms other than, or in addition to, mitochondrial function are targeted by tin antitumour agents. A pigmented melanoma cell line (MM418c5) was resistant to the test compounds, which were found to be sequestered by melanin. This resistance was not observed with triphenyltin hydroxide. © 1997 by John Wiley & Sons, Ltd.

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INTRODUCTION

Organotin compounds are one of the most widely studied class of metal-based antitumour agents.[1–3] This intensive investigation has led to the discovery of compounds with excellent *in vitro* antitumour activity but, in many cases, disappointingly low *in vivo* potency or high *in vivo* toxicity.[1–4] Design of improved organotin antitumour agents is unfortunately hampered by the paucity of information concerning the cellular targets of these compounds and their mechanism of action, although inhibition of mitochondrial oxidative phosphorylation appears to be an important mode of toxicity.[2,3]

Reviewing the extensive literature in this field reveals two classes of organotin compound with exceptionally high antitumour potency. Triphenyltin benzoates exhibit *in vitro* antitumour activity greater than that of cisplatin and comparable with that of mitomycin C.[5] The more active of these compounds have been patented.[6] We have recently reported, however, that while triphenyltin esters have a greater *in vitro* activity than cisplatin against four human tumour cell lines, this activity is independent of the structure of the ester moiety and comparable with that of triphenyltin hydroxide, suggesting hydrolysis to a common, cytotoxic intermediate.[7]

A large number of structurally diverse dibutylstannylene carboxylates and other Sn–O-bonded dibutyltin derivatives exhibit consistently high *in vitro* antitumour activity and some possess low mammalian toxicity and greater *in vivo* activity than cisplatin (for recent and selected examples, see Refs 8–13). This antitumour potency is, in general, structure-dependent, although for some compounds there is evidence of prior hydrolysis to a common $[Bu_2Sn]^{2+}$ equivalent species which is responsible for comparable activity.[13] Dibutylstannylene alkoxides and esters would appear to be more useful, therefore, in developing a structure–activity relationship. We also note that dibutylstannylene cyclic acetals are somewhat under-represented in previous studies of Sn–O-bonded dibutyltin derivatives and so we now report the *in vitro* antitumour activity of some structurally diverse dibutyl-1,3,2-dioxastannolanes (**1**–**4**; Fig. 1), including enantiomeric pairs prepared from diethyl D- or L-tartrate and (*R*,*R*) or (*S*,*S*)-2,3-di(benzyloxymethyl)ethanediol,[14]

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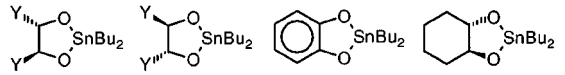

Figure 1 Dibutyl-1,3,2-dioxastannolanes synthesised for *in vitro* antitumour testing.

towards melanoma and other tumour cells, including a cell line highly sensitive to mitochondrial inhibitors.

EXPERIMENTAL

Melting points were obtained on a Thomas Hoover capillary melting-point apparatus and are uncorrected. NMR spectra were obtained on a Bruker ACF300 or Varian Gemini 200 spectrometer using $CDCl_3$ as solvent and $SiMe_4$ as internal reference unless otherwise stated. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Elemental analyses were performed on a Perkin-Elmer Model 240C elemental analyser. Reagents and solvents, obtained from commercial sources, were purified and dried in accordance with standard procedures, prior to use.

The synthesis of the dioxastannolanes **1**–**4** was readily accomplished by reaction of the corresponding diol with dibutyltin oxide in benzene or toluene/ethanol and azeotropic removal of the water formed using a Dean and Stark trap. The reactions were complete when the suspension of the insoluble oxide cleared.

(*R,R*)-Diethyl 1,3,2-dioxastannolane-4,5-dicarboxylate (1a)

Diethyl D-tartrate (0.83 g, 4.03 mmol) and dibutyltin oxide (1 g, 4.02 mmol) in benzene (30 ml) were heated under reflux in a Dean and Stark apparatus to remove the water formed by the azeotropic distillation. After 2 h, the suspension of the tin oxide cleared, whereupon reflux was continued for an additional 3 h. The reaction mixture was allowed to cool and the solvent removed *in vacuo*. The crude product was recrystallized from hexane–chloroform (7:1) to afford the title compound (1.59 g). Yield = 91%, m.p. 138–139 °C; ^{1}H NMR δ 0.87 (t, 7 Hz, 6H), 1.28 (t, 7 Hz, 6H), 1.4 (m, 12H), 4.17 (m, 4H), 4.54 (s, 2H); ^{13}C{^{1}H} NMR δ 13.5 (CH_3), 14.3 (CH_3), 27.1 (CH_2), 27.4 (CH_2), 62.2 (CH_2), 72.4 (CH_2), 173.2 (C); $[\alpha]_D^{25}$ −26.3 (c = 1 in chloroform). Analysis: Calcd for $C_{16}H_{30}O_6Sn$: C, 43.96; H, 6.92; Sn, 27.16. Found: C, 43.66; H, 6.56; Sn, 26.80%.

(*S,S*)-Diethyl 1,3,2-dioxastannolane-4,5-dicarboxylate (1b)

The compound (**1b**) which was generated using the method above has m.p. 137.5–139 °C; its ^{1}H NMR was identical to the isomer **1a**; $[\alpha]_D^{25}$ +25.5 (c = 1 in chloroform). Analysis: Calcd for $C_{16}H_{30}O_6Sn$: C, 43.96; H, 6.92; Sn, 27.16. Found: C, 44.01; H, 6.76; Sn, 27.59%.

(*S,S*)-4,5-Benzyloxymethyl-1,3,2-dioxadibutylstannolane (2a)

(*S, S*) - 2, 3 - Di(benzyloxymethyl)ethanediol[14] (1.30 g, 4.3 mmol) and dibutyltin oxide (1.1 g, 4.4 mmol) in benzene (30 ml) were heated under reflux in a Dean and Stark apparatus for 5 h. Upon removal of solvent and trituration with ether, an off-white crude crystalline product was obtained. Recrystallization from hexane afforded the title compound (2.0 g). Yield = 90%; m.p. 86.5–88 °C; ^{1}H NMR δ 0.90 (t, 6H), 1.90–1.0 (m, 12H), 3.20–3.70(m, 6H), 4.38–4.60 (m, 4H), 7.31–7.21 (m, 10H); $[\alpha]^{25}_D$ +14.3 (c = 1 in chloroform). Analysis: Calcd for $C_{26}H_{38}O_4Sn$: C, 58.56; H, 7.18; Sn, 22.26. Found: C, 59.01; H, 6.95; Sn, 22.08%.

(*R,R*)-4,5-Benzyloxymethyl-1,3,2-dioxadibutylstannolane (2b)

Repeating the procedure for **2a** above, but using (*R,R*)-2,3-di(benzyloxymethyl)ethanediol[14] instead, afforded the title compound; its m.p. and ^{1}H NMR data were identical with compound **2a**; $[\alpha]_D^{25}$ −14.1 (c = 1 in chloroform). Analysis: Calcd for $C_{26}H_{38}O_4Sn$: C,58.56; H, 7.18; Sn, 22.26. Found: C, 58.38, H, 6.73; Sn, 22.34%.

2,2-Dibutyl-1,3,2-benzodioxastannolane (3)

Catechol (4.97 g; 0.02 mol), di-n-butyltin oxide (2.20 g; 0.02 mol) and benzene (700 ml) were heated under reflux in a Dean and Stark apparatus for three days. Removal of the solvent and recrystallization from hot dimethylformamide followed by washing with ether provided the title compound as colourless needles (6.75 g).

Yield = 99%; m.p. > 200 °C (slow dec.); ^{1}H NMR (DMF-d_7) δ 0.82 (t, 6H), 1.25–1.43 (m, 8H), 1.57–1.61 (m, 4H), 6.35–6.55 (AA′BB′, 4H); ^{13}C {^{1}H} NMR δ 14 (CH_3), 24 (CH_2), 27 (CH_2), 28 (CH_2Sn), 114.5 (CH), 117 (CH), 155 (C). Analysis: Calcd for $C_{14}H_{22}O_2Sn$: C 49.30; H, 6.50. Found: C, 49.28; H, 6.61%.

(±)trans-2,2-Dibutylhexahydro-1,3,2-benzodioxastannolane (4)

trans-Cyclohexanediol (697 mg, 6 mmol), dibutyltin oxide (1.49 g, 6 mmol), toluene (150 ml) and ethanol (60 ml) were heated under reflux in a Dean and Stark apparatus for 16 h. Removal of the solvent and recrystallization from ethanol, followed by recrystallization from methylene chloride, yielded the titled compound (1.46 g). Yield = 70%; m.p. 223 °C; ^{1}H NMR δ 0.92 (t, 7 Hz, 6H), 1.32 (m, 12H), 1.67 (m, 6H), 1.91 (m, 2H), 2.99 (br s, 2H); ^{13}C{^{1}H} NMR δ 13.64 (CH_3), 24.44 (CH_2), 25.17 (CH_2), 27.02 (CH_2), 27.52 (CH_2), 34.12 (CH_2), 78.77 (CH). Analysis: Calcd for $C_{14}H_{28}O_2Sn$: C, 48.45; H, 8.13. Found: C, 48.64; H, 8.38%.

In vitro cytotoxicity assays

Compounds were dissolved in either dimethyl sulphoxide (DMSO) or ethanol diluted with culture medium (5% adult donor bovine serum in RPMI 1640) and treatment was carried out in microtitre trays with cultures of 2×10^4–5×10^4 cells. Cells were either expoxed to the drug for 2 h and incubated for three days at 37 °C (Table 1) or exposed for the full period of incubation (Table 2) labelled with [^{3}H]thymidine (10 μCi ml^{-1}) for 3 h, and then harvested (trypsin) on to glass fibre mats before scintillation counting. Survivial curves were obtained by plotting the log of the percentage survival compared with the control against the drug concentration, using five concentrations (in quadruplicate) which spanned the 1–100% survival range. Standard errors for D_{37} values were less than 20%.

The *in vitro* cytotoxicity of compound **3** and triphenyltin hydroxide (**5**) were also determined after premixing with melanin (Table 3, below). To 0.2 ml of a 2 mg ml^{-1} suspension of melanin in culture medium was added 0.2 ml of a 10 μg ml^{-1} solution of compound **3** or **5**. These mixtures, together with appropriate control samples (Table 3) were then incubated for 1 h at 37 °C with occasional mixing, microfuged and the supernatant assayed against HeLa cells for three days as described above.

Table 1. *In vitro* cytotoxicity of enantiomers of 2,2-dibutyl-1,3,2-dioxadibutylstannolanes **1** and **2** against HeLa[a] cells (2 h drug exposure)

Compound	D_{37} (μM)[b]
1a	1.5
1b	1.5
2a	1.2
2b	0.8
Bu_2SnCl_2	1.0

[a] Mer$^-$ human cervical carcinoma.
[b] Concentration required to reduce cell survival by a factor of 0.37 relative to untreated controls.

Table 2. *In vitro* cytoxicity of **1**–**4** and reference compounds towards four human tumour cell lines (three-day drug exposure)

	D_{37} (μM)[a]			
Compound	HeLa[b]	MM96L[c]	MM418c5[d]	CI80–13S[3]
1a	0.2	nt[f]	3.7	0.3
2a	0.2	nt[f]	3.5	0.3
3	0.4	0.4	1.1	0.3
4	0.5	0.3	0.8	0.3
Bu_2SnCl_2	0.5	nt[f]	2.0	0.4
Cisplatin	0.7	nt[f]	2.0	5.0

[a] Concentration required to reduce cell survival by a factor of 0.37 relative to untreated controls.
[b] Mer$^-$ human cervical carcinoma.
[c] Human melanoma.
[d] Human melanoma with high melanin content.
[e] DNA crosslinking-resistant human ovarian carcinoma.
[f] nt, not tested.

RESULTS AND DISCUSSION

Enantiomers commonly exhibit quite different biological properties. We started our investigation of the *in vitro* cytotoxicity of dibutyl-1,3,2-dioxastannolanes, therefore, with a comparison of the antitumour activity of the enantiomers of **1** and **2** against the human cervical carcinoma cell line, HeLa. Cell death did not appear to be turnover-dependent but rather was relatively rapid, as evidenced by only

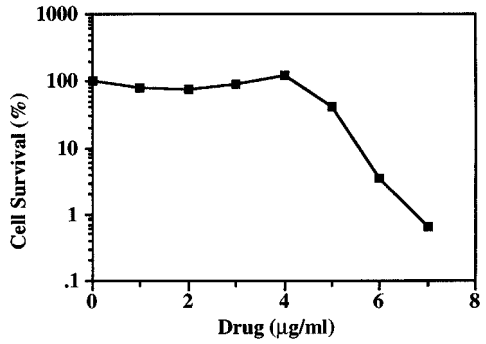

Figure 2 Dose–response curve for treatment of MM418c5 with **1a**.

a small difference in D_{37} on exposure for 2 h (Table 1) or for three days (Table 2). Table 1 revealed no significant difference in cytotoxicity towards HeLa cells between **1a**/**1b** and **2a**/**2b** or between these compounds and dibutyltin dichloride. This lack of discrimination is consistent with hydrolysis to a common cytotoxic Bu_2Sn^{2+} species. A similar conclusion can be drawn from a comparison of the cytotoxicity of these substrates with that of catecholate **3** and of the *trans*-cyclohexanediol equivalent **4** (Table 2). While these tin compounds generally exhibited a greater potency than cisplatin, there was again no significant discrimination between agents.

Diorganotin compounds are known to inhibit mitochondrial oxidative phosporylation.[2,3] Interestingly, however, the ovarian cancer line CI80–13S, which is highly sensitive to killing by sodium azide, cadmium chloride[15] and other mitochondrial inhibitors (P. G. Parsons, unpublished results), showed similar levels of survival to HeLa and MM96L (Table 2). It may be that these agents do inhibit mitochondrial oxidative phosphorylation, but the comparable cytotoxicity results obtained with CI80–13S suggest that some other mechanism of action may be more significant. The other noteworthy result from Table 2 is the decreased cytotoxicity of these tin compounds towards the heavily pigmented human melanoma MM418c5. The dose–response curve for treatment of MM418c5 with **1a** (Fig. 2) showed a plateau until a saturation dose at approximately 4 $\mu g\ ml^{-1}$. This resistance was not evident for cisplatin or for the triphenyltin carboxylates previously studied.[7]

Melanins are largely poly(5,6-dihydroxyindole)s and so it seemed reasonable to us that the resistance of MM418c5 could result from association of Bu_2Sn^{2+} with the catechol moieties of these polymers after uptake of the cytotoxic tin species into the cell. To test this hypothesis, HeLa cells were treated with **3**, and also with **3** which had been premixed with melanin. The results in Table 3 clearly indicate a decrease in cytoxicity for the sample which had been premixed with melanin. This decrease was not observed with a sample of triphenyltin hydroxide (**5**) premixed with melanin relative to **5** alone which exhibited identical *in vivo* cytotoxicities. This increased affinity of melanin for Bu_2Sn^{2+} relative to Ph_3Sn^+ is presumably due to bidentate coordination by the catechol moieties to the former.

While dibutyl-1,3,2-dioxastannolanes exhibit high *in vitro* potency against mammalian tumour cells, their apparent hydrolytic instability under biological conditions precludes them as suitable candidates for structure–activity relationship studies. The relative insensitivity of the CI80–13S cell line does suggest, however, that inhibition of mitochondrial oxidative phosphorylation may not be the primary mechanism of cell death and that other targets such as DNA[2,3] or sulphydryl proteins in the cytoplasm or plasma membrane should be considered as more likely. We have also observed an interesting resistance of pigmented cells towards dibutyltin species which is not observed for triphenyltin species. It may be that the former could be developed as possible imaging agents for melanoma.

Table 3. *In vitro* cytotoxicity of **3** and **5** towards HeLa cells with and without melanin premixing

Compound	D_{37} (μM)
3	0.4
3 + melanin	1.6
Melanin	Non-toxic
5	0.3
5 + melanin	0.3
Melanin	Non-toxic

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