Synthesis and Characterization of Diorganotin(IV) Complexes of *N*-(2-Pyridylmethylene)-arylamines and Mutagenicity Testing *in vivo* of $Et_2SnCl_2 \cdot [L^4 = N\text{-(2-Pyridylmethylene)-4-toluidine}]$

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Diorganotin(IV) dichloride complexes of the type $R_2SnCl_2 \cdot L$ (R = methyl, ethyl, vinyl, t-butyl, n-butyl or phenyl; L = *N*-(2-pyridylmethylene)arylamine) have been synthesized and characterized on the basis of IR, NMR and ^{119}Sn Mössbauer studies. Investigation of the complexes indicated that *N*-(2-pyridylmethylene)arylamines form distorted *trans*-octahedral complexes with R_2SnCl_2 similar to the well-known $R_2SnCl_2 \cdot L$. Cytogenetic toxicology testing has been performed for $Et_2SnCl_2 \cdot L^4$ [L^4 = *N*-(2-pyridylmethylene)-4-toluidine] in mouse bone-marrow cells *in vivo* since such testing is a regulatory requirement before new drugs are released. This tin compound induced delay in cell-cycle kinetics and sister chromatid exchanges (SCEs) significantly. The effect of $Et_2SnCl_2 \cdot L^4$ was greater when endogenous glutathione (GSH) was depleted by buthionine sulphoximine (BSO). It seems that $Et_2SnCl_2 \cdot L^4$ induces SCEs due to formation of adduct by binding on DNA which could interfere in DNA synthesis and cause delay in cell proliferation. Depletion of GSH could reduce the shielding effect of GSH on chromatin and allows more $Et_2SnCl_2 \cdot L^4$ to bind on DNA. © **1998 John Wiley & Sons, Ltd.**

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INTRODUCTION

Diorganotin(IV) compounds, R_2SnCl_2 (X = anion) are often tetrahedral, and when appropriate nitrogen-chelating ligands are co-ordinated to the central metal, octahedral complexes $R_2SnCl_2 \cdot L$ (L = bidentate ligand) are obtained.[1,2] These complexes structurally resemble the active platinum compounds, i.e. *cis*-diamminedichloroplatinum(II) (cisplatin) and *cis*-diammine(cyclobutane-1,1-dicarboxylato)platinum(II) (carboplatin), and consequently a large number of such complexes have been tested for antitumour and anticancer activity. A structural correlation with biological activity for diorganotin complexes has shown that active

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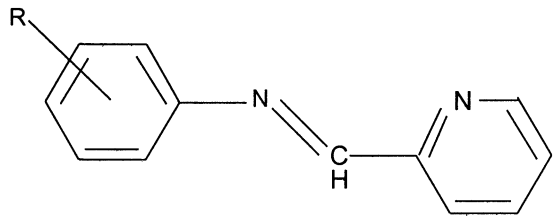

Figure 1 The ligands used in coordination with R_2SnCl_2 compounds. R = H (L^1), 2-CH_3 (L^2), 3-CH_3 (L^3), 4-CH_3 (L^4), 2-OCH_3 (L^5), 3-OCH_3 (L^6), 4-OCH_3 (L^7), 4-$C_6H_4NH_2$ (L^8) or 4-N=N-C_6H_5 (L^9).

species are associated with complexes having Sn–N bonds longer than 2.39 Å which in turn determine the formation of a tin–DNA complex.[3] Recently, the action of $R_2SnCl_2{\cdot}L$ (R = ethyl and L = 1,10-phenanthroline) towards DNA has been investigated.[4]

In view of that, and following our studies on the organotin(IV) complexes of nitrogen chelating ligand,[5,6] we report here on a series of diorganotin dichloride complexes of *N*-(2-pyridylmethylene)-arylamines (Fig. 1) and their characterization by spectroscopic techniques. In addition, the complex $Et_2SnCl_2{\cdot}L^4$ has been tested for mutagenicity since there is a regulatory requirement for such testing before new drugs are released.[7] These regulations generally require that a package of assays be done, using a number of different end-points, before it can be concluded that a chemical is non-mutagenic. Cytogenetic end-points in bone marrow and unscheduled DNA synthesis in rodent liver are required for mutagenecity testing; these assays may be supplemented by others, such as sister chromatid exchanges (SCEs).[7]

Therefore, in the present study we have made an attempt to determine the mutagenetic effect of $Et_2SnCl_2{\cdot}L^4$ in mouse bone-marrow cells *in vivo.* Organotin compounds have varying degrees of toxicological properties, depending on the nature and number of alkyl groups bonded to the tin atom. Et_2SnCl_2 is known to interact with cellular membranes such as rat liver mitochondria, erythrocytes of human and ox.[8,9] It also interacts with proteins like ATPase and hexokinase of trout, feline and human erythrocytes.[10] The possible interaction of this organotin compound with native DNA has still to be investigated. In this present investigation, established end-points such as sister chromatid exchanges (SCEs) and cell-cycle kinetics are used. Moreover, we have tried to determine the effect on cytotoxic potentialities of this new organotin compound in relation to the cellular glutathione (GSH) level since it plays an important role in cellular defence mechanisms.[11] It is known that GSH protects cells against radiation[12] and various toxic effects of xenobiotics[13] but not against radiomimetic drugs like bleomycin.[14,15] Recently it has been shown that the effect of GSH on the cytotoxicity of cisplatin and iproplatin in a human melanoma cell line was not a consequence of differences in GSH–Pt conjugation, but rather that it could be attributable to something else, such as effects on DNA repair, apoptosis, free-radical scavenging or other unknown mechanisms.[16] Therefore, an assessment of the influence of GSH on the activities of $Et_2SnCl_2{\cdot}L^4$ and other organotin compounds is warranted, in order to define better the compounds whose activity may be potentiated through appropriate modulations of GSH.

EXPERIMENTAL

Materials

Dimethyltin dichloride (Fluka), diphenyltin dichloride, di(t-butyl)tin dichloride (Aldrich), divinyltin dichloride and di(n-butyl)tin dichloride (Stream chemicals) were used as such; diethyltin dichloride was prepared using standard procedure.

Measurements

Carbon, hydrogen and nitrogen analyses were performed with a Perkin-Elmer 2400 Series II instrument. IR spectra were obtained on a Perkin-Elmer 1720X FT spectrophotometer in KBr discs in the range 4000–400 cm^{-1}. ^1H and ^{13}C NMR spectra in $CDCl_3$ solution were recorded on a Bruker ACF 300 spectrometer measured at 300.13 and 75.47 MHz, respectively. The ^1H And ^{13}C chemical shifts were referred to Me_4Si set at 0.00 ppm and $CDCl_3$ set at 77.0 ppm, respectively. ^{119}Sn NMR spectra were recorded using a JEOL GX 270 MHz, FT NMR spectrometer at 100.75 MHz, with an inverse gated pulse delay of 2 s. ^{119}Sn Mössbauer spectra of diorganotin complexes were recorded on an Elscient–Laben spectrometer equipped with an AERE cryostat at liquid-nitrogen temperature. The $Ca^{119m}SnO_3$ Mössbauer source (10 mCi; Radiochemical Centre, Amersham, UK) moved with constant acceleration and triangular waveform. The velocity calibration was made using a ^{57}Co

Mössbauer source (10 mCi). An iron foil enriched to 95% in ^{57}Fe (DuPont Pharma Italia, Firenze, Italy), was used as the absorber. Intensity data for the crystals were measured at room temperature on a Rigaku AFC6R four-circle diffractometer. The other conditions and parameters are described elsewhere.[17]

Syntheses

A typical procedure is described below.

$Me_2SnCl_2 \cdot L^1$

A stirred solution of aniline (aminobenzene, $C_6H_5NH_2$; 0.50 g, 5.37 mmol) in dichloromethane (50 ml) was treated with a solution of Me_2SnCl_2 (1.18 g, 5.37 mmol) in dichloromethane (50 ml). The stirring was continued and the resulting white suspension was treated with a solution of pyridine-2-aldehyde (0.575 g, 5.37 mmol) in dichloromethane (40 ml). After addition was complete, the reaction mixture was stirred for another 30 min under ambient conditions. A brown microcrystalline solid was obtained which was filtered, washed with petroleum ether (60–80 °C), recrystallized from the same solvent and dried *in vacuo*.

The other diorganotin dichloride complexes of *N*-(2-pyridylmethylene)arylamine were prepared analogously using appropriate R_2SnCl_2 and ligands as described in Fig. 1).

Biological tests

Male Swiss albino mice, aged 2–3 months and weighing about 25–30 g [maintained in the laboratory in communal cages in room under controlled temperature (20 ± 2 °C) and lighting (12 h light/12 h dark) conditions on standard mouse diet (NMC Oil Mills Ltd, Pune, India) and water *ad libitum*] were used in all experiments. DL-Buthionine-*S*,*R*-sulphoximine (BSO) and Hoechst 33258 were purchased from Sigma Chemical Co. (USA). 5-bromodeoxyuridine (BrdU) tablets (50 mg) were obtained from Boehringer–Mannheim, Germany.

$Et_2SnCl_2 \cdot L^4$ (2 mg) was dissolved in 0.1 ml of ethanol and further diluted to 2 ml with double-distilled water to make a working solution. From the working solution 15 and 30 mg kg^{-1} was injected intraperitoneally (i.p.) 30 min after subcutaneous implantation of a BrdU tablet (50 mg).

In order to investigate the effect of $Et_2SnCl_2 \cdot L^4$ in glutathione depleted condition, BSO (200 mg kg^{-1}) was dissolved in phosphate buffered solution (pH 7.4) and the organotin compound was injected 10 h after BSO treatment (i.p.). Before cells were fixed at 20 h after $Et_2SnCl_2 \cdot L^4$ treatment, they were pretreated with colchicine (15 mg kg^{-1}) for 2 h. Animals were killed by cervical dislocation. The femurs were dissected out and the bone-marrow cells (BMCs) were obtained by injecting 2 ml of 0.075 M KCl (prewarmed at 37 °C, hypotonic solution). Cells were treated in hypotonic solution for 15 min and fixed in acetic acid/methanol (1:3). Slides were prepared by the flame-drying method and fluorescence plus Giemsa staining was performed according to Goto *et al.*[18] Slides were coded at random. Metaphase cells with differentially stained sister chromatids from each mouse were studied for evidence of SCEs. For scoring cell-cycle kinetics, metaphases were classified as being in the first (M1%), second (M2%) subsequent division (M3%) cycle, based on their differential staining pattern. The cell-cycle data were presented as average generation time (AGT), which is a ratio of BrdU duration (h) and replicative index (RI), where $RI = (1 \times M1 + 2 \times M2 + 3 \times M3)$/number of cells. For analysis of SCEs, metaphase cells with differentially stained sister chromatids from each mouse were studied. Data were subjected to parametric statistical analysis.

RESULTS AND DISCUSSION

The physical properties of the diorganotin(IV) complexes are listed in Table 1. The elemental analyses clearly indicate the formulation of product as $R_2SnCl_2 \cdot L$.

The unsymmetrical *N*-(2-pyridylmethylene)arylamine ligands (Fig. 1, L^1–L^9) are not planar and their conformation depends on the position of the nitrogen atoms as revealed by electronic excitation spectra,[21] preliminary CNDO/2 calculation with standard geometries[22] and X-ray structural analysis.[23] This class of ligands exist in the stable *trans*-isomeric form and possess pyridine and imine nitrogen atoms, both able to coordinate to a metal centre (electron acceptor); the situation is analogous to that of 2-(arylazo)pyridine.[6] Further, the π^*-acceptor orbital of the —HC=N— group in an *N*-(2-pyridylmethylene)arylamine has a higher energy than that of the —N=N— group in the corresponding 2-(arylazo)pyridine due to the less electronegative character of —HC=N— as compared with that of —N=N—. Hence, the —HC=N— group will less readily accept the electron density when bonded to the metal. However, the effectiveness of a ligand is a function

Table 1 The physical and analytical data of the complexes

Complex[a]	Colour	M.p. (°C)	Yield (%)	Elemental analysis Found (calcd) (%)			
				C	H	N	Sn
1 $Me_2SnCl_2{\cdot}L^1$	Yellow	178–179	28	41.50 (41.82)	4.02 (3.98)	7.18 (6.97)	29.50 (29.54)
2 $^nBu_2SnCl_2{\cdot}L^1$	Pale yellow	111–112	46	49.39 (49.41)	5.68 (5.76)	5.78 (5.76)	24.30 (24.43)
3 $Ph_2SnCl_2{\cdot}L^1$	Yellow	163–164	76	54.69 (54.78)	3.85 (3.80)	5.25 (5.32)	22.61 (22.57)
4 $Me_2SnCl_2{\cdot}L^2$	Yellow	173–174	81	43.30 (43.29)	3.98 (4.33)	6.80 (6.73)	28.40 (28.55)
5 $^nBu_2SnCl_2{\cdot}L^2$	Yellow	100–101	90	—[b]	—[b]	—[b]	23.60 (23.78)
6 $Ph_2SnCl_2{\cdot}L^2$	Pale yellow	99–100	75	55.70 (55.58)	4.20 (4.07)	5.00 (5.18)	21.50 (21.99)
7 $Me_2SnCl_2{\cdot}L^3$	Yellow	173–174	82	—[b]	—[b]	—[b]	28.30 (28.59)
8 $Ph_2SnCl_2{\cdot}L^3$	Peach	110–111	79	—[b]	—[b]	—[b]	22.10 (22.02)
9 $Me_2SnCl_2{\cdot}L^4$	Yellow	198–199	81	43.30 (43.29)	4.50 (4.32)	6.50 (6.73)	28.20 (28.59)
10 $Et_2SnCl_2{\cdot}L^4$	Pale yellow	196–197	46	45.91 (45.97)	4.85 (4.95)	6.11 (6.31)	26.65 (26.75)
11 $Vi_2SnCl_2{\cdot}L^4$	Yellow	193–194	40	46.30 (46.39)	4.09 (4.09)	6.32 (6.36)	26.50 (26.99)
12 $^nBu_2SnCl_2{\cdot}L^4$	Straw	119–120	68	50.36 (50.41)	5.85 (6.00)	6.01 (5.60)	23.50 (23.78)
13 $^tBu_2SnCl_2{\cdot}L^4$	Yellow	70–71	42	50.20 (50.41)	6.19 (6.00)	5.70 (5.60)	23.60 (23.78)
14 $Ph_2SnCl_2{\cdot}L^4$	Pale yellow	175–176	92	—[b]	—[b]	5.21 (5.18)	21.97 (22.02)
15 $Me_2SnCl_2{\cdot}L^5$	Yellow	165–166	79	41.20[c] (41.69)	4.26[c] (4.16)	6.22[c] (6.48)	27.60[c] (27.53)
16 $Vi_2SnCl_2{\cdot}L^5$	Yellow	183–184	45	46.30 (46.39)	4.00 (4.09)	6.13 (6.36)	26.80 (26.99)
17 $^nBu_2SnCl_2{\cdot}L^5$	Yellow	139–140	36	49.00 (48.86)	5.86 (5.81)	5.00 (5.42)	22.90 (23.01)
18 $Ph_2SnCl_2{\cdot}L^5$	Dark peach	75–76	76	—[b]	—[b]	4.87 (5.03)	21.10 (21.39)
19 $Me_2SnCl_2{\cdot}L^6$	Brown	209–210	78	41.76 (41.68)	4.30 (4.16)	6.58 (6.48)	27.10 (27.53)
20 $Ph_2SnCl_2{\cdot}L^6$	Yellowish green	186–187	78	—[b]	—[b]	—[b]	21.10 (21.39)
21 $Me_2SnCl_2{\cdot}L^7$	Pale yellow	186–187	76	41.70[d] (41.68)	4.30[d] (4.16)	6.40[d] (6.48)	27.40[d] (27.53)
22 $Vi_2SnCl_2{\cdot}L^7$	Yellow	176–177	48	—[b]	—[b]	6.10 (6.14)	25.90 (26.04)
23 $^nBu_2SnCl_2{\cdot}L^7$	Greenish yellow	105–106	48	48.80 (48.86)	5.79 (5.81)	4.38 (4.42)	22.89 (23.01)
24 $Ph_2SnCl_2{\cdot}L^7$	Yellow	194–195	71	53.67 (53.98)	4.02 (3.95)	5.00 (5.03)	21.10 (21.39)
25 $Me_2SnCl_2{\cdot}L^8$	Peach	136–137	72	48.50 (48.69)	4.36 (4.26)	8.52 (8.52)	23.97 (24.12)
26 $Me_2SnCl_2{\cdot}L^9$	Dark brown	126–127	56	47.10 (47.44)	3.95 (3.95)	11.10 (11.06)	23.50 (23.50)

[a] $L^1 – L^9$ as described in Fig. 1; Me = methyl, Et = ethyl, Vi = vinyl, Bu = butyl and Ph = phenyl.
[b] Not analysed.
[c,d] Data taken from Refs 19 and 20, respectively.

of both its π-acceptor and its σ-donor abilities. In such a case, both ν(C=N) (of pyridine and imine) vibrations are expected to be sensitive to coordination. On this basis, we discuss the interaction between R_2SnCl_2 and *N*-(2-pyridylmethylene)arylamine derivatives.

IR spectra

A strong IR band in the region 1620–1600 cm^{-1} for the uncomplexed ligand is characteristic of the azomethine group —HC=N—. The band due to the pyridine ν(C=N) is also reported to occur in this region. Owing to this, normally a strong broad band is observed for the ligand, possibly overlapped, at 1600–1580 cm^{-1}, instead of two separate bands. However, two distinct bands have been detected in the case of L^2, due to ν(C=N). The band appearing at the higher wavenumber, at $\sim$1630 cm^{-1}, is assigned to ν(C=N) (imine) whereas the one appearing at $\sim$1600 cm^{-1} is assigned to ν(C=N) (pyridine).[24] With this background, the mode of coordination is assessed for ν(C=N) from IR spectroscopic data of the complexes. In the complexes, both the bands due to ν(C=N) (imine) and ν(C=N) (pyridine) have been detected and these frequencies are lowered by $\sim$10 cm^{-1}. However, in the case of complexes **2, 5, 19** and **20,** only one broad band is observed at $\sim$1613 cm^{-1}. Such shifts of ν(C=N) frequencies are due to the coordination of both imino and pyridine nitrogen atoms to tin. In the low-frequency infrared region, the bands due to ν(Sn−R) and ν(Sn−Cl) have been detected (where possible), they are presented in Table 2.

NMR spectra

In general, the ^{1}H and ^{13}C NMR spectra of the complexes displayed the expected resonances due to both the *N*-(2-pyridylmethylene)arylamine skeleton and the Sn−R skeleton. The integration values correspond to the formulations of the products. Efforts to assign the individual ^{1}H and ^{13}C NMR signals were not made since the signals do not provide any useful information about the co-ordination owing to the direct coordination of the DMSO solvent, as expected. However, in the

Table 2 Characteristic IR bands (cm^{-1}) of the complexes

	ν(C=N)		ν(Sn−R)		ν(Sn−Cl)	
Complex	Imine	Pyridine	ν_{as}	ν_s	ν_{as}	ν_s
1 $Me_2SnCl_2{\cdot}L^1$	1622	1590	574	522	275	237
2 $^nBu_2SnCl_2{\cdot}L^1$	1613[a]	1613[a]	—[b]	—[b]	—[b]	—[b]
3 $Ph_2SnCl_2{\cdot}L^1$	1621	1592	—[b]	—[b]	—[b]	—[b]
4 $Me_2SnCl_2{\cdot}L^2$	1625	1588	568	505	308	258
5 $^nBu_2SnCl_2{\cdot}L^2$	1616[a]	1616[a]	—[b]	—[b]	—[b]	—[b]
6 $Ph_2SnCl_2{\cdot}L^2$	1651	1599	262	233	—[b]	—[b]
7 $Me_2SnCl_2{\cdot}L^3$	1627	1591	567	492	312	254
8 $Ph_2SnCl_2{\cdot}L^3$	1638	1599	266	229	—[b]	—[b]
9 $Me_2SnCl_2..L^4$	1622	1589	569	524	300	246
10 $Et_2SnCl_2{\cdot}L^4$	1623	1589	—[b]	—[b]	—[b]	—[b]
11 $Vi_2SnCl_2{\cdot}L^4$	1624	1590	—[b]	—[b]	—[b]	—[b]
12 $^nBu_2SnCl_2{\cdot}L^4$	1624	1590	—[b]	—[b]	—[b]	—[b]
13 $^tBu_2SnCl_2{\cdot}L^4$	1622	1586	—[b]	—[b]	—[b]	—[b]
14 $Ph_2SnCl_2{\cdot}L^4$	1622	1598	270	233	—[b]	—[b]
15 $Me_2SnCl_2..L^5$	1625	1589	571	502	308	246
16 $Vi_2SnCl_2{\cdot}L^5$	1640	1590	—[b]	—[b]	—[b]	—[b]
17 $^nBu_2SnCl_2{\cdot}L^5$	1631	1592	—[b]	—[b]	—[b]	—[b]
18 $Ph_2SnCl_2{\cdot}L^5$	1622	1592	—[b]	—[b]	—[b]	—[b]
19 $Me_2SnCl_2{\cdot}L^6$	1613[a]	1613[a]	558	518	270	—[b]
20 $Ph_2SnCl_2{\cdot}L^6$	1613[a]	1613[a]	—[b]	—[b]	—[b]	—[b]
21 $Me_2SnCl_2{\cdot}L^6$	1623	1597	574	516	279	242
22 $Vi_2SnCl_2{\cdot}L^7$	1623	1597	—[b]	—[b]	—[b]	—[b]
23 $^nBu_2SnCl_2{\cdot}L^7$	1621	1595	—[b]	—[b]	—[b]	—[b]
24 $Ph_2SnCl_2{\cdot}L^7$	1622	1596	283	231	—[b]	—[b]
25 $Me_2SnCl_2{\cdot}L^8$	1590	1590	570	501	283	242
26 $Me_2SnCl_2{\cdot}L^9$	1646	1586	—[b]	—[b]	—[b]	—[b]

[a] Vibrations due to both imine and pyridine and appeared as broad peak.
[b] Not identified with reasonable certainty.

case of Me_2SnCl_2·L complexes, a sharp singlet at ~1.2 ppm along with two doublet satellite resonances appear at a relative intensity of ~4% of the main $-CH_3$ resonance, due to coupling of methyl protons with ^{117}Sn and ^{119}Sn nuclei with a coupling constant of $^2J(^{117}Sn-^1H) \cong 93$ Hz and $^2J(^{119}Sn-^1H) \cong 98$ Hz. The observed increase in the above coupling constants compared with those in Me_2SnCl_2[$^2J(^{117}Sn-^1H) = 66$Hz; $^2J(^{119}Sn-^1H) = 70$ Hz] is suggested to be due to an increase in the coordination number of tin as a result of complex formation.[25] The magnitude of ^2J is comparable with those observed for the complexes with octahedral geometry in solution.[26] Furthermore, the observation of only a single sharp resonance, and only set of spin–spin coupling constants between tin nuclei and methyl protons indicates that only one isomer, probably the *trans-form*, exists in solution[27] (see the discussion under X-ray results, below, for additional support). Similarly, in ^{13}C NMR spectra, a signal at ~18.0 ppm is observed in $CDCl_3$ and DMSO-d_6 (1:4) mixture (Me_2SnCl_2: ~6.45 ppm ($CDCl_3$) and 10.60 ppm (DMSO-d_6)). Thus, a substantial shift of around 7.0 ppm is could be due to the coordination effect. ^{119}Sn NMR spectra of Me_2SnCl_2 complexes show a broad signal at approximately -240 ± 10 ppm. The observed values are close to that of the Me_2SnCl_2·2DMSO complex (−246 ppm in DMSO-d_6) and hence coordination effects could not be determined.

^{119}Sn Mössbauer spectra

In order to obtain a deeper insight into the structure, ^{119}Sn Mössbauer spectra of the complexes were recorded in the solid state. Since this technique precludes a solvent effect on the complexes, a precise interpretation concerning the structure is possible; the results are presented in Table 3.

The quadrupole splitting parameters (QS), with the application of the results of point-charge calculations,[28] have proved useful in distinguishing between *cis* and *trans* configurations in the R_2SnX_4–type complexes with octahedral geometries. The QS values[29] for diorganotin complexes are 1.7–2.2 and 3.5–4.2 mm s^{-1} for *cis* and *trans* octahedral complexes, respectively. The observed QS values for the complexes of the present investigation lie inside the range delimited for *trans*-R_2Sn octahedral geometry. The slight difference in QS values of the complexes can arise from differences in the electron-donation properties of the ligands and the charge distribution around the metal atom, which can originate in the steric demands of the ligands. This causes the N—Sn—N bite angle to deviate from 90° and thus distorts the geometry from the ideal six-coordinated structure. Further, the non-linearity of the C—Sn—C group has been calculated using the Parish equation and the values are given in Table 3. It is also noteworthy that the decreases in *s*-electron density at the tin nucleus and in the asymmetry of

Table 3 ^{119}Sn Mössbauer parameters (mm s^{-1})[a] of some complexes

	^{119}Sn Mössbauer spectra				
Complex[b]	IS	QS	Γ_1	Γ_2	C—Sn—C (°)[b]
1 $Me_2SnCl_2·L^1$	1.48	3.93	0.96	0.98	164
2 $^nBu_2SnCl_2·L^1$	1.51	3.45	0.94	0.93	143
3 $Me_2SnCl_2·L^2$	1.29	3.55	0.94	0.95	156
4 $Me_2SnCl_2·L^2$	1.38	4.00	1.95	0.94	164
6 $Ph_2SnCl_2·L^2$	1.50	3.47	0.93	0.96	152
7 $Me_2SnCl_2·L^3$	1.41	3.84	0.94	0.97	159
9 $Me_2SnCl_2·L^4$	1.43	3.99	1.02	1.03	170
10 $Et_2SnCl_2·L^4$	1.53	3.87	1.01	1.17	161
12 $^nBu_2SnCl_2·L^4$	1.57	3.91	0.84	0.83	163
14 $Ph_2SnCl_2·L^4$	1.28	3.47	0.84	0.84	152
15 $Me_2SnCl_2·L^5$	(1.46)	(4.32)	(—)	(—)	(—)
17 $^nBu_2SnCl_2·L^5$	1.55	4.04	0.92	0.93	180
21 $Me_2SnCl_2·L^7$	1.49 (1.49)	3.95 (3.99)	0.94 (—)	0.85(—)	166 (—)
24 $Ph_2SnCl_2·L^7$	1.30	3.30	0.93	0.95	145
25 $Me_2SnCl_2·L^8$	1.37	3.74	0.94	1.09	154
26 $Me_2SnCl_2·L^9$	1.38	3.76	0.93	1.10	155

[a] Values in parentheses are taken from Refs 19 and 20 for complexes **15** and **21**, respectively.
[b] C—Sn—C angles are calculated using the Parish equation: $QS = 4[R]\ (1 - \frac{3}{4})\ \sin^2\theta]^{\frac{1}{2}}$

Table 4 Selected bond distances (Å) and bond angles (°) in the complexes of Me_2SnCl_2 with NN chelating ligands[a]

	Complex[a]			
Parameter	**4**	**7**	**9**	**15**
Reference	This work	This work	This work	Ref. 19
Bond distances				
Sn–Cl(1)	2.486(3)	2.508(2)	2.503(2)	2.496(2)
Sn–Cl(2)	2.507(3)	2.486(3)	2.495(2)	2.519(2)
Sn–N(1)	2.429(9)	2.427(8)	2.444(5)	2.456(5)
Sn–N(2)	2.444(8)	2.500(7)	2.484(5)	2.459(5)
C(5)–C(6)	1.470(1)	1.470(1)	1.475(8)	1.454(9)
C(6)–N(2)	1.280(1)	1.300(1)	1.261(7)	1.274(9)
C(7)–N(2)	1.440(1)	1.430(1)	1.440(7)	1.425(8)
Bond angle				
C(21)-Sn-C(31)	171.5(5)	171.7(4)	171.6(3)	172.8(4)
Cl(1)-Sn-Cl(2)	101.2(1)	101.6(1)	99.04(6)	101.2(6)
N(1)-Sn-N(2)	69.3(3)	67.8(3)	68.0(2)	68.1(2)

[a] Complexes are numbered as in Table 1.

the *p*-orbital population observable in the pairs of complexes (Me and Ph) are consistent with the decreasing inductive effect of phenyl in comparison with methyl. The isomer shifts of the complexes vary from 1.3 to 1.5 mm s^{-1}, compare well with the precedent literature and are typical of quadrivalent tin in organometallic compounds.[6]

Thus, the spectroscopic data of the complexes suggest a distorted octahedral geometry with a *cis* arrangement of the chloro ligands and a *trans* arrangement of the R_2Sn groups; the equatorial positions are completed by the two nitrogen atoms of the ligand.

X-ray results

The molecular structures of the complexes **2, 4, 7, 9, 10** and **17** were determined by X-ray crystallography and the results are reported elsewhere.[17] Selected bond distances and bond angles of $Me_2SnCl_2{\cdot}L^2$ (**4**), $Me_2SnCl_2{\cdot}L^3$ (**7**) and $Me_2SnCl_2{\cdot}L^4$ (**9**) are given in Table 4 along with the data of closely related known system(s).

The structure of $Me_2SnCl_2{\cdot}L$ ($L = L^2 - L^4$) is essentially molecular and features an octahedral Sn atom which is co-ordinated by two methyl substituents, two chloride atoms and two nitrogen-donor atoms derived from the ligand, L. For convenience, the discussion is based on the parameters of complex **4**. The arrangement of the donor atoms is such that the two organic substituents occupy positions approximately *trans* to each other [C(21)—Sn—C(31) is ~171.5(5)°; see Table 4 for specific values]. The major distortion from the regular octahedral geometry is manifested in the N(1)—Sn—N(2), i.e., chelate, angle of 69.3(3)° arising from the restricted bite distance of L. The five-membered chelate ring is essentially planar, as seen in the two Sn/N(1)/C(5)/C(6) and Sn/N(2)/C(6)/C(5) torsion angles of −2(1)° and −9(1)°, respectively. Chemically non-equivalent N(1) and N(2) atoms have Sn–N bond distances of 2.429(9) Å [Sn–N(1)] and 2.444(8) Å [Sn–N(2)], which are equal within experimental error, and Sn–Cl separations of 2.486(3) Å [Sn–Cl(1)] and 2.507(3) Å [Sn–Cl(2)]. The sequence of C(5)–C(6), C(6)–N(2) and C(7)–N(2) bond distances of 1.47(1) Å, 1.28(1) Å and 1.44(1) Å indicates that there is relatively little conjugation over this chromophore despite the planarity of the constituent atoms; the N(1)/C(5)/C(6)/N(2) and C(5)/C(6)/N(2)/C(7) torsion angles are 7(2)° and −172(1)°, respectively. It is noteworthy that the *o*-tolyl residue is not coplanar with the remaining portion of L, as seen in the C(6)/N(2)/C(7)/C(8) torsion angle of −124(1)°. The parameters obtained for complex **4** are very close to those of three other derivatives, i.e. **7, 9** and **15**, and thus the complexes all are isostructural in the solid state. A change in the ring substituents on the aryl moiety of the ligand does not normally change the fundamental structure of the complex.

Selected bond distances and bond angles of the rest of the complexes, i.e. $Et_2SnCl_2{\cdot}L^4$ (**10**), $^nBu_2SnCl_2{\cdot}L^1$ (**2**) and $^nBu_2SnCl_2{\cdot}L^5$ (**17**) are given in Table 5 along with the data of closely related known system(s).

The structure of $R_2SnCl_2{\cdot}L$ (R = ethyl and n-

Table 5 Selected bond distances (Å) and bond angles (°) in the complexes of R_2SnCl_2 (R = ethyl or n-butyl) with NN chelating ligands

	Complex[a]			
Parameter Reference	**10** This work	Et_2SnCl_2·Bipy Ref. 30	**2** This work	**17** This work
Bond distances				
Sn–Cl(1)	2.516(2)	2.529(1)	2.467(3)	2.518(4)
Sn–Cl(2)	2.480(2)	2.545(1)	2.499(2)	2.524(3)
Sn–N(1)	2.452(6)	2.368(4)	2.506(6)	2.450(1)
Sn–N(2)	2.559(6)	2.382(4)	2.575(6)	2.490(1)
C(5)–C(6)	1.470(1)	1.507(10)	1.460(1)	1.470(2)
C(6)–N(2)	1.276(8)	1.340(12)	1.280(1)	1.260(1)
C(7)–N(2)	1.443(9)	—	1.432(9)	1.45(2)
Bond angles				
C(21)—Sn—C(31)	164.6(4)	175.8(2)	164.8(4)	174.8(6)
Cl(1)—Sn—Cl(2)	96.45(8)	104.2(3)	101.24(8)	102.0(1)
N(1)—Sn—N(2)	66.8(2)	69.0(1)	66.2(2)	67.2(3)

[a] Complexes are numbered as in Table 1.

butyl; L = L^1, L^4 or L^5) is molecular and features an octahedral Sn atom which is co-ordinated by two R substituents, two chloride atoms and two chemically non-equivalent nitrogen donor atoms in the ligand. The structure is similar to that of Me_2SnCl_2·L. In the complexes **10, 2** and **17**, the organic substituents assume positions approximately *trans* to each other and C(21)–Sn–C(31) bond angles are 164.6(4)°, 164.8(4)° and 174.8(6)°, respectively. In complexes **10** and **2**, the bond angles C(21)–Sn–C(31) are of the same order of magnitude within experimental error, thus reflecting the fact that the ring substituents on the aryl moiety of L and the ethyl or butyl groups attached to the tin do not affect the structural features. In the case of complex **17**, the observed C(21)–Sn–C(31) bond angle is 174.8(6)° and is higher with respect to the other two complexes. In general, the complexation does not change the bite angles of N(1)–Sn–N(2) in any of these cases.

Mutagenicity tests

It was possible to distinguish unequivocally the number of divisions in the presence of BrdU, as well as the number of SCEs in second-replication cycle cells. Table 6 shows that both concentrations of Et_2SnCl_2·L^4 induced a significant delay in the cell cycle in bone-marrow cells. The percentage of first-cycle metaphases (M1) was higher, indicating a delay in cell-cycle progression. Compared with the control, the AGT was significantly increased after organotin-compound treatment. The extent of delay was increased further after organotin treatment of BSO-treated mice. This enhancement in delay in cell-cycle kinetics was prominent for 30 mg kg^{-1} of Et_2SnCl_2·L^4 where 97% or more cells were in M1. However, it is worth mentioning that 67% of mice failed to survive treatment with plus BSO 30 mg kg^{-1} Et_2SnCl_2-L^4. From the data it is clear that the mortality rate of mice was increased when organotin compound was treatment was given to BSO-treated mice.

Table 6 also shows that Et_2SnCl_2·L^4 induced SCEs in a dose-dependent manner in mouse bone-marrow cells harvested at 20 h, since at 17 h fixation time not many cells were available for SCE study due to induction of delay in the cell-cycle kinetics. The frequency of SCEs was further increased when organotin (15 mg kg^{-1}) was injected in to BSO-treated mice.

The effect of BSO alone has been studied in two mice only. Significant induction of SCEs was observed without showing any significant rise in AGT values.

SCEs have proved to be a sensitive indicator of DNA damage.[31] Although most of the anticancer drugs currently in the clinic are effective in causing chromosomal damage, there is considerable variability in their ability to induce SCE.[32] For many of the alkylating agents, including cisplatin, SCE does indeed occur at lower concentrations and appears to be a more sensitive assay than direct measurement of chromosome aberrations.[33] The present study *in vivo* shows that exogenous addition of Et_2SnCl_2·L^4 induces SCEs and delay in cell-cycle kinetics in mouse bone-marrow cells. However, the present bone-marrow cell system might become an inap-

Table 6 Effect of $Et_2SnCl_2{\cdot}L^4$ and BSO alone or in combination on cell-cycle kinetics and the frequency of SCEs in mouse bone-marrow cells *in vivo*

Experimental conditions	Fixation (h)	No. of treatments (No. of dead)[c]	Total metaphases	M1 (%)	AGT[d] (h)	Mean AGT	Cell score	SCE/M[e]	SCE/M per mouse (± SEM)
Untreated	17	6 (0)	302	49	10.62		44	2.52	
			160	34	0.83	09.37	41	2.80	2.66 ± 0.10
	20		178	08	08.56		37	2.37	
			080	28	09.92		35	2.77	
			156	24	09.62		65	2.38	
			130	29	09.62	09.43	41	2.90	2.60 ± 0.12
BSO	20	2 (0)	352	45	11.39		59	4.47	
			287	52	11.88	11.64	51	4.60	4.54 ± 0.10[a]
$Et_2SnCl_2{\cdot}L^4$	17	10 (1)	403	94	16.66				
(15 mg kg^{-1})			299	90	16.05				
			246	89	15.90				
			102	90	15.13	15.93[b]			
	20		253	79	16.80		32	3.57	
			306	66	15.07		45	3.45	
			276	88	18.30		20	3.80	
			166	71	15.89		26	4.25	
			134	63	14.96	16.20[b]	24	4.75	3.96 ± 0.24[a]
BSO + $Et_2SnCl_2{\cdot}L^4$	17	10 (5)	211	86	17.90				
(15 mg kg^{-1})			458	66	15.20	16.55			
	20		326	38	12.57		114	6.48	
			406	45	13.22		164	7.20	
			135	78	16.80	14.20	19	5.70	6.96 ± 0.43[b]
$Et_2SnCl_2{\cdot}L^4$	17	09 (4)	310	82	14.80	14.80[b]			
(30 mg kg^{-1})	20		354	63	14.66		85	4.50	
			274	40	12.20		68	5.30	
			275	44	12.89		71	4.48	
			213	68	12.00	12.94[b]	94	5.78	5.02 ± 0.03[b]
BSO + $Et_2SnCl_2{\cdot}L^4$	20	09 (6)	125	100	20.50				
(30 mg kg^{-1})			180	100	20.50				
			102	97	20.10	20.30[b]			

[a] $P < 0.01$, [b] $P < 0.001$ compared with the respective control, Student's *t*-test.
[c] Number of mice treated (Number of mice that failed to survive).
[d] Average generation time.
[e] Metaphase.

propriate tissue to sample for cytogenetic analysis of new organotin compounds which could be a potential antitumour drugs in future. This could lead to a problem in selecting the most appropriate end-point(s) for testing *in vivo*. Virtually all the drugs used clinically do cause chromosomal events, and therefore, this is a logical end-point for mutagenecity tests *in vivo* which could easily be studied in bone-marrow system.[7]

The present findings of cell cycle delaying effects of $Et_2SnCl_2{\cdot}L^4$ does support the previous observation regarding the antiproliferative effect of di- and tri-alkyltin compounds.[34] Such antiproliferation properties may induce SCEs.[35] Two forms of SCE induction are known: (1) by damaging DNA[36] and (2) by inhibiting DNA synthesis.[35] It seems that in the present case $Et_2SnCl_2{\cdot}L^4$ induces SCEs through formation of an adduct by binding on DNA which could interfere in DNA synthesis, causing delay in cell proliferation. It is worth mentioning that the bond lengths Sn–N(1) and Sn–N(2) of $Et_2SnCl_2{\cdot}L^4$ are much higher than in other reported organotin compounds and therefore this complex could easily bind on DNA.

A novel aspect of the present study is the analysis of the influence of BSO on the effect of $Et_2SnCl_2{\cdot}L^4$. The rationale for BSO treatment is based on the premise that GSH serves as a major endogenous cellular defence against various toxic effects of xenobiotics,[37] and GSH depletion itself may lead to significant sensitization. The present observed BSO-mediated increase in $Et_2SnCl_2{\cdot}L^4$ sensitivity could be attributed to depletion of endogenous GSH. The total GSH in the mouse bone marrow (eight mice in each category) was estimated by following the method of Theodorous

et al.[37] The data indicate that 10 h of incubation with BSO could deplete the GSH level significantly (52% of the control value).[38] Treatment with BSO produces a rapid decrease in the GSH levels of the various tissues.[39] Intermediate rates of depletion[40] were seen in the bone marrow, with a nadir at 8–12 h. Therefore, the incubation period of BSO treatment was kept at 10 h in the present study.

BSO-mediated GSH depletion exacerbated the mutagenic effect of $Et_2SnCl_2{\cdot}L^4$; this could be due to a reduced shielding effect of GSH on chromatin. It seems that both the structural arrangement of the chromatin and the presence of DNA-bound protein offer a far more efficient protection against mutagen-induced DNA damages than intracellular scavengers of radicals.[41,42] Although the production of reactive oxygen species and free radicals by $Et_2SnCl_2{\cdot}L^4$ is not known, being structurally similar to complexes of platinum, $Et_2SnCl_2{\cdot}L^4$ could disrupt the DNA helix by cross-linking with DNA and interfering with replication. Such an adduct-forming ability by $R_2SnX_2{\cdot}L_2$ compounds has already been reported.[43] Therefore, depletion of GSH by BSO reduces the shielding effect of GSH on chromatin and allows more $Et_2SnCl_2{\cdot}L^4$ to bind on DNA. In fact, it is known that protein-bound GSH amounts to 60% of the cellular free GSH content and 35% of the total cellular GSH.[44] Evidence was obtained that at least two peptides, one being GSH, was bound to the nucleoproteins, as mixed disulphides.[45] Moreover, aminothiol radioprotectors, in general, have been reported to bind and slow down DNA strand separation for replication.[46] Thus BSO-mediated GSH depletion could lead more binding of $Et_2SnCl_2{\cdot}L^4$ on DNA. However, in addition to this mechanism one cannot rule out the possibility of $Et_2SnCl_2{\cdot}L^4$ conjugation with GSH since this is an important route for detoxification of several compounds of exogenous origin.[47] This could be the reason why the presence of BSO potentiates $Et_2SnCl_2{\cdot}L^4$-mediated death of mice. Although the exact reason for the death of mice by $Et_2SnCl_2{\cdot}L^4$ treatment is not known, it could be due to its neurotoxic potentiality, since Et_2SnCl_2 exhibits neurotoxic properties.[48]

BSO alone induced SCEs significantly with respect to the untreated control. It was reported previously that BSO could induce SCEs in mouse bone-marrow cells.[49] This observation is an indication of the important protective role of endogenous GSH in cells against peroxides and free radicals which are formed by normal metabolic pathways.[50]

It has been shown that organotin compounds seem to have a vast potential for use as antitumour agent.[2,51] Unfortunately, even for the most widely used anticancer drugs, there are gaps in the literature that make it difficult to assess the relative mutagenic potential of different types of compound.[32] The present compound, $Et_2SnCl_2{\cdot}L^4$, shows ability in blocking cell proliferation, which is a positive effect of several anticancer drugs.[52] Moreover, being structurally similar to cisplatin, it is expected that the mode of action of the tin complexes could be similar to that of cisplatin[2]. Therefore, the present block in cell proliferation could be due to cross-linking of $Et_2SnCl_2{\cdot}L^4$ to the N^7 position of two consequtive guanine molecules in DNA, thus disrupting the DNA helix. It has been proposed that the N^7 position of guanine is important for chemotherapy,[53] and therefore much information is needed regarding the mutagenic potential of this and other organotin compounds before it is considered as an antitumour agent.

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