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Synthesis, spectroscopic characterization (IR, ^{1}H, ^{13}C and ^{119}Sn NMR, electrospray mass spectrometry) and toxicity of new organotin(IV) complexes with *N,N′,O*- and *N,N′,S*-scorpionate ligands

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New organotin(IV) derivatives containing the anionic ligands bis(3,5-dimethylpyrazol-1-yl)dithioacetate $[LCS_2]^-$ and bis(3,5-dimethylpyrazol-1-yl)acetate $[LCO_2]^-$ have been synthesized from reaction between $(CH_3)_2SnCl_2$ and lithium salts of the ligands. Mononuclear complexes of the type $\{[LCX_2](CH_3)_2SnCl\}$ (X = S or O) have been obtained and fully characterized by elemental analyses and FT-IR in the solid state and by NMR (^{1}H, ^{13}C and ^{119}Sn) spectroscopy, conductivity measurements and electrospray ionization mass spectrometry in solution. The acute toxicity of new organotin(IV) derivatives on rat was studied, comparing their effect with those of dimethyltin chloride $(CH_3)_2SnCl_2$. The comparison of LD_{50} of organotin(IV) complexes and $(CH_3)_2SnCl_2$ administered intraperitoneally, as a single dose, evaluated *in vivo* on rats, showed that toxicity decreases as follows: $(CH_3)_2SnCl_2 > LCO_2 > LCS_2$. The effect of these organotin(IV) complexes on DNA was evaluated *in vitro* and *in vivo* on rats treated with different doses of these compounds (1/20 LD_{50} and 1/100 LD_{50}). The lymphocyte DNA status was assessed by the comet assay, a rapid and sensitive single-cell electrophoresis technique, used to detect primary DNA damage in individual cells. After 36 h from the start of treatment the two new organotin(IV) derivatives induced a significant rise in comet assay parameters, indicating an increasing presence of damaged DNA. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: organotin(IV) compounds; *N,O,S*-chelating ligand; pyrazole; ^{119}Sn NMR; electrospray ionization mass spectroscopy; acute toxicity, rat; DNA damage; comet assay

INTRODUCTION

Poly(pyrazolyl)borates[1] and related scorpionate ligands, represent one of the most versatile types of tridentate σ-donor ligand that can coordinate to a wide variety of elements, e.g. from early to late transition metals, and the coordination chemistry of these systems has developed greatly in recent years.[2,3] Modifications of poly(pyrazolyl)borates can be made by replacement of the boron bridging atom by other elements such as carbon, silicon, or phosphorus; other important variations can be effected by replacing the pyrazolyl with triazolyl, imidazolyl, and methimazolyl moieties or by changing the substituents on the heterocyclic ring.[4] Such a change can either preserve or alter the charge of the ligand. In this research field, several recent contributions are related to the heteroscorpionate ligand derived from bis(pyrazol-1-yl)methanes of general structure $[RR'C(pz)_2]$, bearing a

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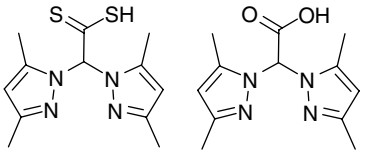


Scheme 1.

coordinating moiety (R′) such as acetate, dithioacetate, sulfonate, ethoxide, phenolate, thiolate and other classes of moieties.[5] Otero *et al*.[6] introduced for the first time the bis(3,5-dimethylpyrazol-1-yl)acetate, a scorpionate ligand with a carboxylate and two pyrazole donor groups. This ligand is analogous to poly(pyrazolyl)alkanes, but with a carboxylate fragment on the central carbon; it can be easily deprotonated and can act in the anionic form as a tripodal donor toward several metals, such as ruthenium, iron, zinc, niobium and titanium, where a κ^3-*N*,*N*,*O* is proposed.[6–11] The bis(3,5-dimethylpyrazol-1-yl)dithioacetates[12] (Scheme 1a) are analogues to bis(pyrazolyl)acetates (Scheme 1b), but with a soft dithiocarboxylate fragment on the central carbon. They can be easily deprotonated and can act in the anionic form like poly(pyrazolyl)borates.

We have decided to extend the coordination chemistry of bis(3,5-dimethylpyrazol-1-yl)acetate, $[LCO_2]^-$, and bis(3,5-dimethylpyrazolyl)dithioacetate, $[LCS_2]^-$, toward diorganotin(IV) acceptors. Our interest in the coordination chemistry of tin and organotin acceptors with poly(azolyl)alkanes,[13] β-diketones[14] and poly(azolyl)borates,[15–17] has a long history, and additional reasons for this research are based on the biological activity,[18] antifouling paints[19] and antitumour activity displayed by many organotin(IV) derivatives containing mixed *N*,*X*-ligands (*X* = O, S, P).[20] Organotin compounds are also of interest in view of the considerable structural diversity that they possess. This aspect has been attracting the attention of a number of researchers, and a multitude of structural types have been discovered.[21,22]

The acute toxicity (LD_{50}) of organotin(IV) complexes and dimethyltin chloride ($(CH_3)_2SnCl_2$) administered intraperitoneally as a single dose was evaluated on rat, according to the method of Thompson and Weil.[23] In addition, the effect of these new organotin(IV) complexes and $(CH_3)_2SnCl_2$ on lymphocyte DNA of rats was determined in *vivo* and *in vitro*. The level of DNA damage was investigated with the 'comet assay', which is a useful test with widespread potential applications in genotoxicity testing and biomonitoring.[24–26]

RESULTS AND DISCUSSION

Complex **1** has been synthesized by methathetic reaction of $Li(LCS_2)$ with $(CH_3)_2SnCl_2$ in CH_2Cl_2 solution at room temperature:

$$Li(LCS_2) + (CH_3)_2SnCl_2 \xrightarrow{CH_2Cl_2/r.t.} \underset{\mathbf{1}}{\left[(LCS_2)Sn(CH_3)_2Cl\right]} + LiCl \quad (1)$$

The derivative $[(LCS_2)Sn(CH_3)_2Cl]$ is reasonably stable in air; however, prolonged warming and/or storage under reduced pressure seems to induce some decomposition process via dethiocarbonylation with release of CS_2 and formation of a mixture of pyrazole-containing metal complexes and other unidentified products. Compound **1** shows good solubility in methanol, acetone and chlorinated solvents, and it is a non-electrolyte in CH_2Cl_2 solution.

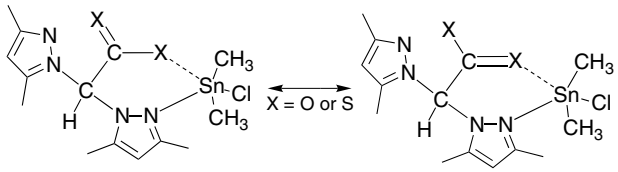
Complex **2** has been synthesized by methathetic reaction of $Li(LCO_2)$ with $(CH_3)_2SnCl_2$ in CH_2Cl_2 solution at room temperature:

$$Li(LCO_2) + (CH_3)_2SnCl_2 \xrightarrow{CH_2Cl_2/r.t.} \underset{\mathbf{2}}{\left[(LCO_2)Sn(CH_3)_2Cl\right]} + LiCl \quad (2)$$

The complex $[(LCO_2)Sn(CH_3)_2Cl]$ is reasonably stable in air and shows good solubility in methanol, acetone and chlorinated solvents; it is a non-electrolyte in CH_2Cl_2 solution.

Derivatives **1** and **2** have been characterized by analytical and spectral data. The IR spectra were obtained on solid samples (Nujol mull) and showed all the expected bands for the ligands and the tin moieties; weak absorptions in the range 3096–3130 cm^{-1} are due to the pz ring C–H stretchings, and the medium to strong absorptions near 1550 cm^{-1} are related to ring 'breathing' vibrations. The presence of the CS_2 group in derivative **1** is detected by intense absorptions at 1038 cm^{-1} and 810 cm^{-1}, due to the asymmetric and symmetric CS_2^- stretching modes respectively; a shift to red with respect to the free neutral ligands ($\nu_{asym}(CS_2^-) = 1078\ cm^{-1}$ and $\nu_{sym}(CS_2^-) = 831\ cm^{-1}$) is observed upon complex formation. These values fit those reported for analogous tutanium complexes.[12] The presence of the COO moiety in derivative **2** is detected by strong bands at 1652 cm^{-1} due to the asymmetric absorption mode, and a shift to red with respect to the free neutral ligands is observed upon complex formation. This is in accordance with electronic flow from the ligand toward the tin moiety, with consequent decreasing C═S or C═O bond order.

In the far-IR region, medium to strong absorptions appear upon coordination, due to stretching modes of Sn–S or Sn–O, Sn–N, Sn–C and Sn–Cl.[27] In the IR spectra of **1**, one absorption assigned to Sn–S has been detected at 402 cm^{-1}.[28] In the IR spectra of **2**, one absorption assigned to Sn–O has been detected at 334 cm^{-1}. The Sn–Cl stretching frequencies appear as strong broad bands at 250 and 243 cm^{-1} in derivative **1** and at 291 and 281 cm^{-1} in derivative **2**. The Sn–C stretching frequencies appear as medium or strong absorptions in the range 574–520 cm^{-1}; these absorptions



Scheme 2.

agree well with the trends previously observed in similar *N*-donor complexes.[29]

In the ^{1}H NMR spectra of complexes **1** and **2** in $CDCl_3$ solution (see Experimental section), the signals due to the pyrazolyl rings are always deshielded with respect to those in the spectra of the free donor, confirming the existence of the complexes in solution. The room-temperature ^{1}H NMR spectra of derivatives **1** and **2** exhibit only one set of signals for the protons of the pyrazolyl rings of the ligands, resulting from dynamic exchange processes. This is common in complexes of corresponding poly(pyrazolyl)borates,[30] suggesting highly fluxional species or complete dissociation and reassociation of the pyrazolyl nitrogen atoms, which occur rapidly even at lower temperatures. For derivative **1** it was possible to slow down the rate of the dynamic process responsible for the spectra obtained at room temperature, and static spectra were obtained at 218 K: the resonances due to the pyrazole protons split into two sets of signals, one of them being assignable to the free pyrazole ring. This pattern indicates that the two pyrazolyl rings are magnetically different according to the hypothesized five-coordinate tin core (Scheme 2).

The ^{119}Sn chemical shifts of diorganotin(IV) derivatives **1** and **2**, at −188.6 ppm and −280.7 ppm respectively, are in accordance with those of five-coordinate diorganotin(IV)halides complexes involving *S*-, *O*- or *N*-donors.[31–33] The tin–proton coupling constants $^2J(^{119}\text{Sn}, ^1\text{H})$ and $^2J(^{117}\text{Sn}, ^1\text{H})$ are 75 Hz and 72 Hz respectively in compound **1** and 81 Hz and 78 Hz respectively in compound **2**, falling in the range for penta-coordinate dimethyltin(IV) species (Scheme 2).[34,35] The tin–carbon coupling constants $^1J(^{119}\text{Sn}, ^{13}\text{C})$ are 613 Hz (compound **1**) and 692 Hz (compound **2**); on the basis of Lockarts's equation,[35] the Me–Sn–Me angles for **1** and **2** are estimated to be 130° and 137° respectively, suggesting a distorted equatorial disposition of the Me groups (Scheme 2).

Electrospray ionization (ESI) is considered a 'soft' ionization technique. Consequently, few ions are produced, usually the molecular ion plus some adduct ion from the mobile-phase solutions.[36,37] ESI mass spectrometry is particularly suitable for the study of labile organotin systems in solution. In the discussion of the mass spectra of the diorganotin(IV) derivatives, only the most abundant ion of the isotope cluster will be mentioned.

A very simple fragmentation pattern was detected in the positive- and negative-ion spectra of derivatives **1** and **2**, dissolved in acetone solution and detected at a fragmentation voltage of 30 V. For derivative **1**, significant fragments at m/z 428 (100%) and m/z 463 (100%) have been attributable to loss of one chloride anion or one proton, in the positive- and negative-ion spectra respectively, from the monomeric species $[(LCS_2)Sn(CH_3)_2Cl]$. Peaks at m/z 247, 249, 271 and 520, due to the free *O*-donor ligand, are present in the spectra of derivative **2**, due to the inferior stability of this diorganotin derivative with respect to complex **1** in acetone solution.

The intraperitoneal injection of the organotin(IV) complexes $[(LCS_2)SnMe_2Cl]$ (**1**) and $[(LCO_2)SnMe_2Cl]$ (**2**) and of the acceptor $(CH_3)_2SnCl_2$ was immediately followed by decreased motor activity, prostration but not tremors. Deaths occurred in the first 4–6 h. When the animals survived the treatment, all the above symptoms disappeared completely. LD_{50} and 95% confidence limits of the organotin(IV) compounds, as well as those of $(CH_3)_2SnCl_2$, are reported in Table 1. Based on the LD_{50} values, $(CH_3)_2SnCl_2$ ($LD_{50} = 28.51 \pm 0.21$ mg kg^{-1}) is 1.6-fold and 2.4-fold significantly more toxic than $[(LCO_2)SnMe_2Cl]$ (**2**; $LD_{50} = 45.91 \pm 0.08$ mg kg^{-1}) and $[(LCS_2)SnMe_2Cl]$ (**1**; $LD_{50} = 69.08 \pm 0.14$ mg kg^{-1}) respectively. Derivative **2** is 1.5-fold significantly more toxic than derivative **1**.

The effect of the different organotin compounds under study on the lymphocyte DNA was quantified using the comet assay.[25] Cells with increased DNA damage display an increased migration of genetic material in the direction of electrophoresis. DNA damage is quantified by measuring the displacement of the genetic material between the cell nucleus (comet 'head') and the resulting 'tail'. The parameters used as an index of DNA damage are tail length (TL), tail intensity (TI) and tail moment (TM); the latter is one of the best indices of induced DNA damage among the various parameters calculated by computerized image analysis. It considers both the length of DNA migration in the comet tail (TL) and the percentage of nuclear material migrated out from the comet head in the comet tail (TI); therefore, this was the parameter evaluated in this work.

Figure 1 shows the mean TM values of rat lymphocytes treated for 30 min at 37 °C with different organotin compounds. The presence of 10 μM of each compound gave similar values for TM as the control. The values of TM increased significantly after 30 min of incubation only in the sample incubated with compound **2**.

Table 1. The acute toxicity (LD_{50} plus/minus confidence interval limits σ) to rats of $(CH_3)_2SnCl_2$ acceptor and diorganotin(IV) complexes $[(LCS_2)SnMe_2Cl]$ (**1**) and $[(LCO_2)SnMe_2Cl]$ (**2**)

Compound	$LD_{50} \pm \sigma$ (mg kg^{-1})
$(CH_3)_2SnCl_2$	28.51 ± 0.21
Complex **1**	69.09 ± 0.14[a]
Complex **2**	45.91 ± 0.08[a,b]

[a] $P < 0.05$ compared with $(CH_3)_2SnCl_2$.
[b] $P < 0.05$ compared with complex **1**.

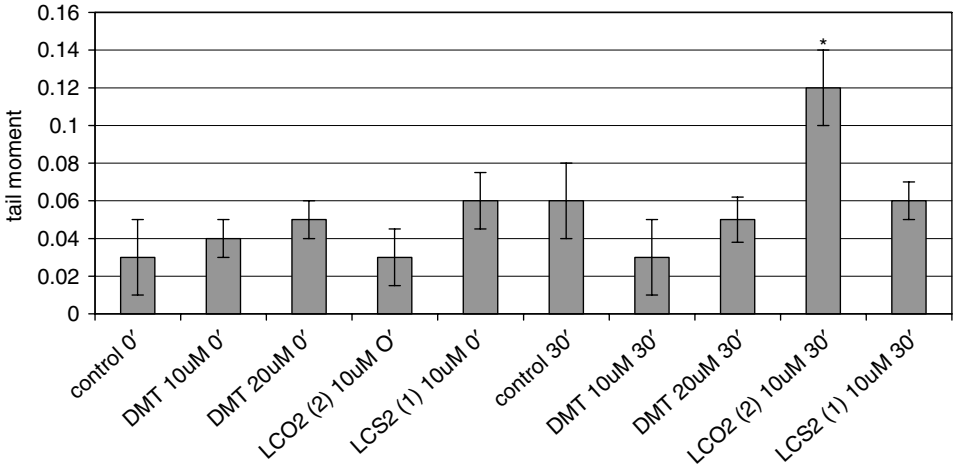


Figure 1. Observed distribution of comet parameter TM (mean plus/minus standard error of the mean) in lymphocytes from rats incubated in the presence of different organotin(IV) complexes in phosphate-buffered saline (PBS), at 37 °C for 30 min. Data (at least 150 scores/sample) are mean values of three replicated experiments. $^*P < 0.05$ compared with other groups..

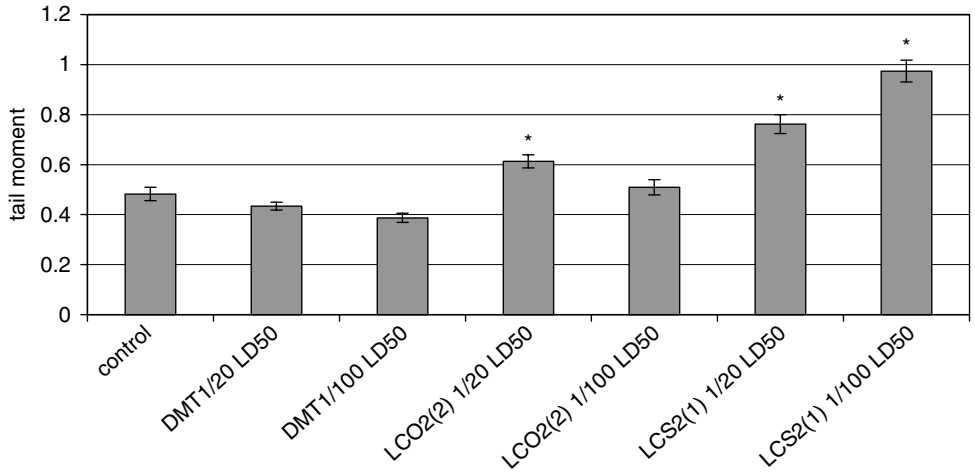


Figure 2. Observed distribution of comet parameter tail moment (mean plus/minus standard error of the mean) in lymphocytes from rats after 36 h from the start of treatment with different doses (1/20 LD_{50} and 1/100 LD_{50}) of various organotin(IV) complexes. Data (at least 150 scores/sample) are mean values of three replicated experiments. $^*P < 0.05$ compared with control group, $(CH_3)_2SnCl_2$ 1/20 LD_{50} and $(CH_3)_2SnCl_2$ 1/100 LD_{50} groups..

In order to evaluate the '*in vivo*' effect of these organotin compounds on rat lymphocyte DNA we treated rats with different amounts of organotin compounds. The treatment of rats with 1/10 LD_{50} and 1/2 LD_{50} for 36 h induced excessive lymphocyte DNA damage such that it was not possible to measure the comet parameters (all cells were damaged). For this reason, we reduced the dose of $(CH_3)_2SnCl_2$ and diorganotin(IV) compounds to 1/20 LD_{50} and 1/100 LD_{50}.

A rise in DNA damage was observed after 36 h from the start of treatment with derivative **2** at the highest dose (1/20 LD_{50}); the TM increased significantly compared with the control and $(CH_3)_2SnCl_2$ (1/20 LD_{50} − 1/100LD_{50}). Derivative **1** also induces a significant increase in TM at lower dose (there is no significant difference between the two doses; Fig. 2).

Although from the toxicity study the LD_{50} for the $(CH_3)_2SnCl_2$ acceptor showed higher toxicity with respect to the other organotin(IV) complexes, the lymphocyte DNA damage was significantly increased in the presence of complexes **1** and **2**. This behaviour could be linked with different reactivities of these compounds; in fact,

$(CH_3)_2SnCl_2$ is less reactive compared than the two organotin(IV) complexes, which split to give the more reactive dimethyltin compared with $(CH_3)_2SnCl_2$. Anyway, the different effects of complexes **1** and **2** on DNA could be linked with the different fates of two complexes, the degradation of which could follow different pathways and so modulate their bioavailability. Besides, another factor that likely could influence DNA damage is the structural effect of these three compounds; in particular, in complex $[(LCS_2)SnMe_2Cl]$ (**1**) the presence of sulfur in the coordination environment makes the system more dynamic, permitting an easier interaction with DNA compared with complex $[(LCO_2)SnMe_2Cl]$ (**2**). Another factor that should be considered is the influence of the hydrophobic character of the two complexes, which influences the permeability and consequently the ability to cross the membrane. The structural influence and the different reactivities may also explain the differences measured between *in vivo* and *in vitro* studies, not forgetting the diverse biological transformation.

Further studies should be performed to identify the metabolites formed in the animals treated with these complexes in order to understand better the mechanisms of their biological interaction. Hence, the information obtained with this study is important for planning the synthesis of new biologically relevant organototin(IV) complexes in the antitumour activity field.[20]

EXPERIMENTAL

Chemistry

All syntheses were carried out under a nitrogen atmosphere. All reagents were purchased from Alfa (Karlsruhe) and Aldrich (Milwaukee) and used as received. The ligands $Li[LCS_2]$[12] and $Li[LCO_2]$[6] were prepared according to literature methods. All solvents were distilled and degassed with dry nitrogen prior to use. The samples for microanalysis were dried *in vacuo* to constant weight (20 °C, *ca* 0.1 Torr). Elemental analyses (carbon, hydrogen, nitrogen, sulfur) were performed with a Fisons Instruments 1108 CHNS-O elemental analyser. IR spectra were recorded from 4000 to 100 cm^{-1} with a Perkin–Elmer System 2000 FT-IR instrument. ^1H, ^{13}C and ^{119}Sn NMR spectra were recorded on a VXR-300 Varian instrument operating at room temperature (at 300 MHz for ^1H, 75 MHz for ^{13}C and 111.9 MHz for ^{119}Sn). Melting points were taken on an SMP3 Stuart Scientific Instrument. ESIMS spectra were obtained in positive- or negative-ion mode on a Series 1100 MSD detector HP spectrometer, using an acetone mobile phase. The compounds were added to the reagent-grade methanol to give solutions of approximate concentration 0.1 mM. These solutions were injected (1 μl) into the spectrometer via an HP 1090 Series II high-performance liquid chromatograph fitted with an autosampler. The pump delivered the solutions to the mass spectrometer source at a flow rate of 300 μl min^{-1}, and nitrogen was employed both as a drying and nebulizing gas. Capillary voltages were typically 4000 V and 3500 V for the positive- and negative-ion modes respectively. Confirmation of all major species in this ESIMS study was aided by comparison of the observed and predicted isotope distribution patterns, the latter calculated using the IsoPro computer program.[38]

Synthesis

*$[(LCS_2)SnMe_2Cl]$ (**1**).* To a CH_2Cl_2 solution (50 ml) of $(CH_3)_2SnCl_2$ (0.220 g, 1.0 mmol), $Li(LCS_2)$ (0.286 g, 1.0 mmol) was added. The mixture reaction was stirred 4 h at room temperature, then solvent was removed on a rotary evaporator and chloroform was added (20 ml). The LiCl was removed by filtration and the filtrate reduced to half volume. Then diethyl ether was added (40 ml) and a yellow precipitate afforded, which was filtered off, washed with *n*-hexane (10 ml) and dried to constant weight under reduced pressure. Recrystallization from petroleum ether gives complex **1** as a microcrystalline solid (85% yield). M.p.: 155–158 °C dec. IR (Nujol mull, cm^{-1}): 3130w, 3096w (CH), 1558s (C═N, C═C), 1038m ($\nu_{asym}(CS_2^-)$), 810m ($\nu_{sym}(CS_2^-)$), 550s, 520s (Sn–C), 402w (Sn–S), 306w (Sn–N), 250s, 243s (Sn–Cl). ^1H NMR ($CDCl_3$, 293 K): δ1.04 (s, 6H, Sn–CH_3, $^2J(^{119}Sn-^1H) = 75$ Hz, $^2J(^{117}Sn-^1H) = 72$ Hz), 2.19 (s, 6H, 3- or 5-CH_3), 2.45 (s, 6H, 3- or 5-CH_3), 6.05 (s, 2H, 4-C*H*), 7.05 (s, 1H, C*H*). ^1H NMR ($CDCl_3$, 218 K): δ1.16 (sbr, 6H, Sn–CH_3), 2.15 (s, 3H, 3- or 5-CH_3), 2.20 (s, 3H, 3- or 5-CH_3), 2.25 (s, 3H, 3- or 5-CH_3), 2.45 (s, 3H, 3- or 5-CH_3), 5.95 (s, 1H, 4-C*H*), 6.09 (s, 1H, 4-C*H*), 7.05 (s, 1H, C*H*). ^{13}C NMR ($CDCl_3$, 293 K): δ 10.89 (s, Sn–CH_3, $^1J(^{119}Sn,^{13}C) = 613$ Hz), 12.31 (sbr, 3- or 5-CH_3), 13.69 (sbr, 3- or 5-CH_3), 76.0 (s, *C*H), 107.00 (sbr, 4-CH), 145.96 (sbr, 3- or 5-*C*CH_3), 148.04 (sbr, 3- or 5-*C*CH_3). ^{119}Sn NMR ($CDCl_3$, 293 K): δ − 188.6 (s). ESIMS (acetone) m/z (%): (−) 463 (100) $[(LCS_2)SnMe_2Cl - H^+]^-$. ESIMS (acetone) m/z (%): (+) 428 (100) $[(LCS_2)(SnMe_2)]^+$, 873 (20) $[(LCS_2)_2(SnMe_2)_2(OH)]^+$. Anal. Found: C, 36.45; H, 4.48; N, 12.23; S, 13.51. Calc. for $C_{14}H_{21}ClN_4S_2Sn$: C, 36.27; H, 4.57; N, 12.08; S, 13.83%.

*$[(LCO_2)SnMe_2Cl]$ (**2**).* Compound **2** was prepared similarly to compound **1**, by using Me_2SnCl_2 (0.220 g, 1.0 mmol) and $Li(LCO_2)$ (0.254 g, 1.0 mmol). Recrystallization from *n*-hexane and diethyl ether gave complex **2** as a microcrystalline solid in 83% yield. M.p.: 68–70 °C. IR (Nujol, cm^{-1}): 3120w (C–H), 1652sbr, 1411m (CO), 1558s (C═N, C═C), 574mbr, 523mbr (Sn–C), 334s (Sn–O), 291s, 281sh (Sn–Cl). ^1H NMR ($CDCl_3$): δ0.98 (s, 6H, Sn–CH_3, $^2J(^{119}Sn-^1H) = 81$ Hz, $^2J(^{117}Sn-^1H) = 78$ Hz), 2.32 (s, 6H, 3- or 5-CH_3), 2.36 (s, 6H, 3- or 5-CH_3), 5.92 (s, 2H, 4-C*H*), 6.56 (s, 1H, C*H*). ^{13}C NMR ($CDCl_3$, 293 K): δ 9.69 (s, Sn–CH_3, $^1J(^{119}Sn,^{13}C) = 692$ Hz), 10.90 (sbr, 3- or 5-CH_3), 12.79 (sbr, 3- or 5-CH_3), 67.5 (s, *C*H), 106.90 (sbr, 4-CH), 140.94 (sbr, 3- or 5-*C*CH_3), 148.94 (sbr, 3- or 5-*C*CH_3), 166.23 (s, *C*O_2). ^{119}Sn NMR ($CDCl_3$):

δ −280.7. ESIMS (acetone) m/z (%): (−) 247 (100) $[(LCO_2)]^-$, 467 (20) $[(LCO_2)(SnMe_2Cl)Cl]^-$. ESIMS (acetone) m/z (%): (+) 249 (70) $[(LCO_2H) + H^+]^+$, 271 (100) $[(LCO_2H) + Na^+]^+$, 520 (40) $[(LCO_2H)_2 + Na^+]^+$, 616 (30) $[(LCO_2)(SnMe_2Cl)_2]^+$, 835 (20) $[(LCO_2)(SnMe_2Cl)_3Cl]^+$. Anal. Found C, 39.04; H, 5.16; N, 12.67. Calc. for $C_{14}H_{21}ClN_4O_2Sn$: C, 38.97; H, 4.91; N, 12.98%.

Biological studies

Materials

All reagents used in this study were of analytical grade.

Animals and drug administration. Male Wistar rats (n = 80; Charles River, Calco, LC, Italy) weighing 225–250 g were employed. The animals were housed in plastic (Makrolon) cages (five rats per cage), in a temperature-controlled room (21 ± 0.5 °C), and maintained on a laboratory with diet and water *ad libitum*. Light/dark cycle was 07 : 00h to 19 : 00h. Prior to administration of compounds, rats were fasted 16 h. After compound administration, food pellets and tap water were freely available. The substances were dissolved in water/alcohol 40/60 v/v and administered by intraperitoneal injection (2 ml kg^{-1}).

Acute toxicity study. The LD_{50} and its confidence interval were determined by using 10 animals per dose level, with four or more dose levels being tested per substance, and with the logarithms of successive dose levels different by a constant. At least two separate experiments were used to evaluate the lethality of the new organotin(IV) complexes. The percentage mortality was determined at 24 h and values of LD_{50} and 95% confidence limits were calculated according to the methods of Thompson and Weil.[23]

Lymphocyte DNA damage

Lymphocyte separation. Lymphocyte separation was performed by means of a Ficol density gradient (obtained from Nycomed Pharma AS, Oslo, Norway, and Dulbecco's Modified Eagle from Life Techologies, Paisley, Scotland). Whole blood was diluted (1 : 1) in PBS and stratified on a solution of lymphoprep and then centrifuged for 20 min at 3000 rpm. Peripheral blood lymphocytes (PBLs) were separated from erythrocytes and washed with PBS.

Comet assay. For *in vitro* study, the compounds used in this study dissolved in ethanol (100%) were added to the lymphocytes (400 000 cells/slide) to a final concentration of 10 μM. Samples incubated with 10 μM and 20 μM of $(CH_3)_2SnCl_2$ were used to compare the effect of diorganotin(IV) complexes **1** and **2**. The lymphocytes were tested immediately after addition of compounds (incubation time 0 min) and after incubation at 37 °C for 30 min (incubation time 30 min).

For *in vivo* study, we separated lymphocytes from blood after 36 h from the start of treatment with the different compounds studied. DNA single-strand breaks or alkali-labile sites are induced by a great variety of genotoxic substances. The comet assay[24,39] used to measure the DNA strand breaks in individual cells was essentially the same as that described previously.[40] Cells were suspended in 0.7% low-melting-point agarose in PBS and pipetted into microscope slides precoated with a layer of 1% normal-melting-point agarose. The agarose with the cell suspension was allowed to set on the precoated slides at 4 °C for 10 min. Subsequently, another top layer of 0.7% low-melting-point agarose was added and allowed to set at 4 °C for 10 min. The slides were then immersed in lysed solution (1% sodium *n*-lauroyl-sarcosinate, 2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris HCl pH 10, 1% Triton X-100 and 10% dimethylsulfoxide) overnight at 4 °C in the dark in order to lyse the embedded cells. Slides were next exposed to alkaline buffer (1 mM Na_2EDTA, 300 mM NaOH) for 20 min to allow for DNA unwinding, and then subjected to 20 min electrophoresis at 25 V in the same alkaline buffer. After that, they were washed with 0.4 M Tris HCl buffer (pH 7.5) to neutralize excess alkali and to remove detergents before staining with ethidium bromide (2 μg ml^{-1}). Cells were examined with an Axioskop 2 plus microscope (Carl Zeiss, Germany) equipped with an excitation filter of 515–560 nm and a magnification of ×20. Imaging was performed using a specialized analysis system ('Metasystem' Altlussheim, Germany) to determine TL, TI and TM; all parameters correlated with the degree of DNA damage in the single cells.

Statistical analysis. The experimental data are expressed as mean values plus/minus standard error of three different experiments performed in triplicate. Statistical analysis was carried out using the one-way analysis of variance followed by the Newman–Keuls test. A value of $P < 0.05$ was considered statistically significant.

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