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Synthesis, Characterization and Cytotoxic Activity of Complexes of Diorganotin(IV) Halides with *N*-Methyl-2,2′-bisimidazole

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The compounds $[SnR_2X_2(MBIm)]$ (MBIm = *N*-methyl-2,2′-bisimidazole; R = Me, Et, Bu, Ph; X = Cl or Br) have been synthesized and characterized by IR, Raman, Mössbauer and NMR spectroscopy, and their capacity to inhibit tumour cell division has been assayed. Measurements of conductivity in acetonitrile show the adducts to behave as non-ionogens in this solvent. The IR, Raman and Mössbauer data suggest that all the complexes have analogous pseudo-octahedral coordination geometries, with the R groups *trans* and MBIm bidentate. The ^{1}H NMR spectra show the MBIm ligand to be partially dissociated in $CDCl_3$. The most active compounds against the established cell line KB were the butyl derivatives.

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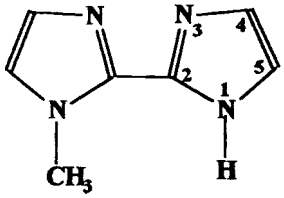
INTRODUCTION

Diorganotin(IV) complexes of the type $SnR_2X_2(L—L)$, where L—L is a bidentate N-donor ligand, are becoming increasingly important, not only from structural and preparative points of view[1] but also because a number of such compounds show antitumour activity towards P388 lymphocytic leukaemia cells.[2] As this activity has been related to the stability of the complexes, and bearing in mind the relatively low stability of complexes of 2,2′-bisimidazole with certain transition metals, we have previously used this ligand[3] and its *N*,*N*′-dimethyl derivative[4,5] to obtain complexes with SnR_2X_2. In continuation of this work we have now prepared a new series of compounds with the ligand *N*-methyl-2,2′-bisimidazole **1** (MBIm); this paper describes their preparation, structural characterization and *in vitro* cytostatic activity against the human carcinoma cell line KB.



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EXPERIMENTAL

Reagents

Dimethyltin dichloride (Aldrich); dimethyltin dibromide, diethyltin dichloride, diethyltin dibromide, dibutyltin dichloride and dibutyltin dibromide (Ventron); and diphenyltin dichloride (Alfa) were all used as supplied. Diphenyltin dibromide was prepared from the dichloride by an exchange reaction with NaBr. Solvents were purified by the usual methods. MBIm was prepared as described in the literature.[6]

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Preparation of complexes

All the complexes were prepared by addition of a solution of MBIm in dry CH_2Cl_2 (*ca* 10 ml) to a solution of the appropriate diorganotin(IV) derivative in *ca* 10 ml of the same solvent.

[$SnMe_2Cl_2$(MBIm)]
As above, from 0.219 g (1 mmol) of $SnMe_2Cl_2$ and 0.148 g (1 mmol) of MBIm. After stirring for two days, the reaction mixture was concentrated *in vacuo* to afford a solid product. Analysis: Calcd for $C_9H_{14}Cl_2N_4Sn$: C, 29.8; H, 4.1; N, 15.3. Found: C, 29.5; H, 3.8; N, 15.5%. Colour: salmon. Yield: 80%. M.p.: 172 °C. Molar conductivity Λ_M (MeCN, 10^{-3} M): 20.5 S cm^2 mol^{-1}.

[$SnMe_2Br_2$(MBIm)]
From 0.308 g (1 mmol) of $SnMe_2Br_2$ and 0.148 g (1 mmol) of MBIm. Analysis: Calcd for $C_9H_{14}Br_2N_4Sn$: C, 23.6; H, 3.3; N, 12.2. Found: C, 23.7; H, 3.2; N, 12.5%. Colour: Salmon. Yield: 80%. M.p.: 175 °C. Λ_M (MeCN, 10^{-3} M): 30.6 S cm^2 mol^{-1}.

[$SnEt_2Cl_2$(MBIm)]
From 0.247 g (1 mmol) of $SnEt_2Cl_2$ and 0.148 g (1 mmol) of MBIm. Analysis: Calcd for $C_{11}H_{18}Cl_2N_4Sn$: C, 33.2; H, 4.5; N, 14.1. Found: C, 33.5; H, 4.7; N, 14.3%. Colour: salmon. Yield: 75%. M.p.: 163 °C. Λ_M (MeCN, 10^{-3} M): 15.6 S cm^2 mol^{-1}.

[$SnEt_2Br_2$)MBIm)]
From 0.336 g (1 mmol) of $SnEt_2Br_2$ and 0.148 g (1 mmol) of MBIm. Analysis: Calcd for $C_{11}H_{18}Br_2N_4Sn$: C, 27.2; H, 3.7; N, 11.5. Found: C, 27.6; H, 3.8; N, 11.7%. Colour: pink. Yield: 77%. M.p.: 178 °C. Λ_M (MeCN, 10^{-3}M): 25.7 S cm^{-2} mol^{-1}.

[$SnBu_2Cl_2$(MBIm)]
From 3.03 g (1 mmol) of $SnBu_2Cl_2$ and 0.148 g (1 mmol) of MBIm. Analysis: Cald for $C_{15}H_{26}Cl_2N_4Sn$: C, 39.7; H, 5.7; N, 12.3. Found: C, 40.0; H, 5.8; N, 12.8. Colour: fuschia. Yield: 80%. M.p.: 146 °C. Λ_M (MeCN, 10^{-3} M): 30.9 S cm^2 mol^{-1}.

[$SnBu_2Br_2$(MBIm)]
From 0.392 g (1 mmol) of $SnBu_2Br_2$ and 0.148 g (1 mmol) of MBIm. Analysis: Calcd for $C_{15}H_{26}Br_2N_4Sn$: C, 33.2; H, 4.8; N, 10.3. Found: C, 33.4; H, 4.5; N, 9.9%. Colour: fuschia. Yield: 80%. M.p.: 162°C. Λ_M (MeCN, 10^{-3} M): 26.5 S cm^2 mol^{-1}.

[$SnPh_2Cl_2$(MBIm)]
From 0.343 g (1 mmol) of $SnPh_2Cl_2$ and 0.148 g (1 mmol) of MBIm. Analysis: Calcd for $C_{19}H_{18}Cl_2N_4Sn$: C, 46.3; H, 3.8; N, 11.4. Found: C, 46.4; H, 3.7; N, 11.6%. Colour: salmon. Yield: 71%. M.p. 232 °C. Λ_M (MeCN, 10^{-3} M): 20.2 S cm^2 mol^{-1}.

[$SnPh_2Br_2$(MBIm)]
From 1 mmol of $SnPh_2Br_2$ and 0.148 g (1 mmol) of MBIm. Analysis: Calcd for $C_{19}H_{18}Br_2N_4Sn$: C, 39.2; H, 3.1; N, 9.6. Found: C, 38.9; H, 3.1; N, 9.6%. Colour: pink. Yield: 75%. M.p.: 227 °C. Λ_M (MeCN, 10^{-3} M): 83.2 S cm^2 mol^{-1}

Physical measurements

C, H and N were determined using a Carlo Erba 1108 microanalyser. Melting points were determined in a Büchi apparatus. IR spectra were recorded in Nujol mulls or KBr pellets with a Bruker IFS66v FT-IR spectrometer. Raman spectra were recorded on the Bruker FT-IR spectrometer using an FRA-106 accessory and polycrystalline samples. Molar conductivities of 10^{-3} M solutions in acetonitrile were measured with a WTW LF-3 conductivity meter. ^{1}H NMR spectra were recorded in $CDCl_3$ at room temperature on a Bruker WM-250 operated at 250.13 MHz and are referred to TMS. Mössbauer spectra were recorded at 80.0 K in a Harwell cryostat; the $Ca^{119m}SnO_3$ source (15 mCi, NEN) was kept at room temperature and moved with a triangular velocity waveform; suitable computer programs were employed to fit Lorentzian lineshapes to the experimental data.

In vitro cytostatic activity evaluation

Cytostatic activity was assayed with the established cell line KB, which derives from an oral epidermoid human carcinoma.[7] Stock cultures were grown in 25 cm^2 flasks containing 10 ml of buffered Eagle's minimum essential medium (MEM) supplemented with glutamine, non-essential amino-acids (1%) and newborn calf serum (10%), as prevously described.[8,9] The cell population doubling time was *ca* 24 h. The cells were dissociated with 0.05% trypsin solution, plated at a density of 5×10^5 cells per well in 24-well cell culture clusters (Costar) containing

1.0 ml of MEM per well, and preincubated for 24 h to allow adhesion to the substrate.

Subsequently the agents to be tested were added. The compounds were dissolved immediately before use in sterile acetone, and these solutions were diluted with the growth medium to the desired concentrations. The final acetone concentration in the culture medium, 0.5%, showed no cytotoxic effect in preliminary tests. At least five concentrations of each compound were used, with eight cell culture wells for each concentration. Each agent was assayed on at least three separate occasions. Each test included a blank containing complete medium without cells.

The cells were incubated with the test compounds at 37 °C in an atmosphere that was 5% CO_2 and had a relative humidity of 100%. The incubation time was 72 h, during which period the control wells showed exponential cell growth.

The end-point of cell growth was determined by *in situ* fixation of cells, followed by staining with the protein-binding dye sulforhodamine B (SRB).[10] Briefly, adherent cell cultures were fixed *in situ* by addition of 250 μl of cold 50% (wt/vol) trichloroacetic acid (TCA) and were kept for 60 min at 4 °C. The supernatant was then discarded and the plates were washed three times with deionized water and dried. SRB solution (500 μl, 0.4% wt/vol in 1% acetic acid) was added to each well, and the cells were allowed to stain for 20–30 min at room temperature. Unbound SRB was removed by washing three times with 1% acetic acid. Then the plates were air-dried. Bound stain was solubilized with unbuffered Tris base [Tris(hydroxymethyl)aminomethane] and the optical densities at 565 nm were read on a Perkin–Elmer 550 SE spectrophotometer.

The SRB assay was also used to measure the cell population density at time 0 (the time at which the test compounds were added).

Cytostatic activity was evaluated from the inhibition of cell growth in the treated cultures with respect to the controls. The statistical significance of these results was estimated by means of Student's *t* test ($P < 0.01$). IC_{50}, the concentration of test compound (mg/ml medium and micromolar) at which cell proliferation was 50% of that observed in control cultures, was determined by linear regression analysis.

RESULTS AND DISCUSSION

The reactions of SnR_2X_2 with MBIm yielded solid complexes with low melting points that are stable to light and in dry air but not in moist air, and are more soluble in polar than in non-polar solvents.

Mössbauer spectra

The Mössbauer spectra (Table 1) shows slightly distorted quadrupole split doublets, the parameters of which indicate octahedral coordination geometry with the organic moieties occupying the apical positions. Steric hindrance among the various groups bonded to the tin centre seems to have a strong influence on the isomer shifts of the six alkyltin derivatives, which increase slightly but steadily in the order Me < Et < Bu,

Table 1. Mössbauer data of the prepared compounds

Compound	δ^a	$\Delta E_Q{}^b$	Γ^c	$A_{2/1}$	$\Gamma_{2/1}$
$[SnMe_2Cl_2(MBIm)]$	1.51	3.90	0.88	0.89	0.90
$[SnMe_2Br_2(MBIm)]$	1.56	3.86	0.82	0.95	1.06
$[SnEt_2Cl_2(MBIm)]$	1.67	4.02	0.81	1.00	1.05
$[SnEt_2Br_2(MBIm)]$	1.76	4.02	0.83	1.02	1.01
$[SnBu_2Cl_2(MBIm)]$	1.72	4.03	0.90	0.98	0.97
$[SnBu_2Br_2(MBIm)]$	1.79	3.97	1.03	0.97	0.99
$[SnPh_2Cl_2(MBIm)]$	1.39	3.42	0.92	0.95	0.99
$[SnPh_2Br_2(MBIm)]$	1.44	3.40	0.85	0.98	0.98

[a] Isomer shift (mm s^{-1}).
[b] Quadrupole splitting (mm s^{-1}).
[c] Full width at half peak height (mm s^{-1}).

bisimidazole[3] < methylbisimidazole and Cl < Br (this last case also reflects the low electronegativity of the bromide ion compared with that of the chlorine ion) so that the smallest isomer shift is that of $[SnMe_2Cl_2(MBIm)]$, 1.51 mm s^{-1}, and the largest that of $[SnBu_2Br_2(MBIm)]$, 1.79 mm s^{-1}. The quadrupole splitting values, on the contrary, are practically constant and very close to that calculated using the point-charge model. Hence a structure with *trans* alkyl groups and a C—Sn—C bond angle close to 180° may be proposed for all these compounds. Similar behaviour is shown by the two diphenyltin derivatives, except that both the isomer shift and the quadrupole splitting have lower values as a consequence of the different electronic properties of the phenyl group.

IR and Raman spectra

In the 4000–600 cm^{-1} range the free bisimidazole ligand bands undergo small shifts upon coordination, those of the ring stretching vibrations (1600–1300 cm^{-1}) being similar to those found for bidentate 2,2′-bisimidazole[3] and *N*,*N*′-dimethyl-2,2′-bisimidazole[4,5] in diorganotin (IV) dihalide complexes and in keeping with bonding through the pyridinic nitrogen.

Table 2 shows selected infrared and Raman data in the range 700–100 cm^{-1}. Structurally the most important bands are the Sn–C, Sn–X and Sn–N stretching bands but the Sn–N bands are very difficult to identify due to their overlapping ligand bands. The ν(Sn–C) vibrations are found at positions close to those associated with the known *trans* arrangement of the R groups in $SnR_2X_2L_2$ complexes in which L is a nitrogen-bearing monodentate ligand.[11–14] Both these bands are also close to the positions reported for the corresponding unsubstituted and dimethyl-substituted 2,2′-bisimidazole complexes[3–5] except in the case of the dibutyl compounds; identification of the ν(Sn–C) bands in the IR spectra of the unsubstituted and dimethyl-substituted butyl derivatives was not, as in the present work, supported by Raman data.

Although the bidentate character of the ligand and the *trans* C–Sn–C arrangement postulated on the basis of the Mössbauer and vibrational data imply a *cis* X–Sn–X arrangement, potentially generating two IR and two Raman bands, only a single strong or medium broad IR band is clearly attributable to ν(Sn–X). The missing bands are no doubt included among the numerous additional shoulders or weak bands present in both the IR and Raman spectra.

Solution studies

The solubility of the complexes in acetonitrile was sufficient for measurement of their conductivities. The molar conductivity values are in all cases lower than those for 1:1 electrolytes in acetonitrile (120–160 S cm^2 mol^{-1}).[15]

Since previously studied complexes between

Table 2. Significant IR and Raman (R) bands[a] for $[SnR_2X_2(MBIm)]$ complexes in the 700–100 cm^{-1} region

Compound		ν_{as}(Sn–C)	ν_s(Sn–C)	ν(Sn–X)
$[SnMe_2Cl_2(MBIm)]$	IR	577vs	515s	245s,b
	R		514s	—
$[SnMe_2Br_2(MBIm)]$	IR	577s	515m	188sh
	R		505s	—
$[SnEt_2Cl_2(MBIm)]$	IR	537m	487m	214s,b
	R		489m	—
$[SnEt_2Br_2(MBIm)]$	IR	532s	483m	164s,b
	R		483m	—
$[SnBu_2Cl_2(MBIm)]$	IR	625w	597w	237s,b
	R		599m	—
$[SnBu_2Br_2(MBIm)]$	IR	624w	595w	156s,b
	R		592w	—
$[SnPh_2Cl_2(MBIm)]$	IR	288s	235m	225m,b
$[SnPh_2Br_2(MBIm)]$	IR	286m	234m	169m,b

[a] Abbreviations: vs, very strong; w, weak; m, medium; s, strong; b, broad.

Table 3. ^{1}H NMR parameters[a] (δ in ppm and J in Hz)

Compound	$\delta(CH_3–Sn)$	$\delta(CH_2)$	$\delta(CH_2)$	$\delta(CH_2)$	δ(Ligand)
MBIm					4.13(s) (CH_3); 6.90(d); 7.02(d) (H5′,H4′) 7.05(s); 7.15(s) (H5, H4)
$[SnMe_2Cl_2(MBIm)]$[b]	1.11(s)				4.28(s) (CH_3); 7.01(d); 7.13(d) (H5′, H4′); 7.39(s) (H5, H4)
$[SnMe_2Br_2(MBIm)]$[c]	125(s)				4.29(s) (CH_3); 7.04(d); 7.15 (d); (H5′, H4′); 7.42(s) (H5, H4)
$[SnEt_2Cl_2(MBIm)]$	1.29(t)	1.71(q)			4.30(s) (CH_3); 7.07(b); 7.21(b) (H5′, H4′); 7.41(b) (H5, H4)
$[SnEt_2Br_2(MBIm)]$	1.30(t)	1.78(q)			4.31(s) (CH_3); 7.05(d); 7.19(d) (H5′, H4′); 7.42(b) (H5, H4)
$[SnBu_2Cl_2(MBIm)]$	0.88(t)	1.35(m)	1.70(m)	1.76(m)	4.29(s) (CH_3); 7.05(d); 7.16(d) (H5′, H4′); 7.38(s) (H5, H4)
$[SnBu_2Br_2(MBIm)]$	0.91(t)	1.38(m)	1.68(m)	1.80(m)	4.31(s) (CH_3); 7.06(d); 7.18(d); (H5′, H4′); 7.41(s) (H5, H4)

[a] Abbreviations: s, singlet; b, broad; d, doublet; t, triplet; q, quadruplet; m, multiplet.
$^2J(^{117/119}Sn–^1H)$; [b] 78.5/81.8; [c] 73.3/76.5.

diorganotin(IV) halides and bidentate ligands coordinating via nitrogen are totally dissociated in dimethyl sulphoxide (DMSO) but only partially dissociated in $CDCl_3$, in this work we obtained ^{1}H NMR spectra in $CDCl_3$ so as to be able to assess the stability of the new compounds by comparison of their degrees of dissociation. The chemical shifts of the alkyl compounds (Table 3) confirm that the ligand remained at least partially coordinated, but because of poor solubility it was impossible to obtain the specta of the phenyl compounds and only in the case of the methyl compounds was it possible to calculate $^2J(^{117/119}Sn–^1H)$, which is strongly dependent on the coordination number of the tin and therefore useful for estimation of the extent of dissociation. The values of $^2J(^{117/119}Sn–^1H)$ for $[SnMe_2Cl_2(MBIm)]$ and $[SnMe_2Br_2(MBIm)]$, 78.5/81.8 and 73.3/76.5 respectively, were slightly sensitive to concentration (as reported for other compounds),[12] and larger than the values of 65.7/68.7 and 63.2/66.1 reported for the respective free acceptors,[4] which confirms the persistence of some degree of coordination in $CDCl_3$. Comparison of the values obtained for the chloride with those obtained for the analogous unsubstituted bisimidazole, 75.7/79.2,[3] shows that monomethylation of the ligand increased only very slightly the stability of its complexes with diorganotin(IV) halides.

Table 4. Results of *in vitro* cytostatic assay against cell line KB

Compound	IC_{50} (μg/ml medium)	(μM)
$[SnEt_2Cl_2(MBIm)]$	0.600	1.52
$[SnEt_2Br_2(MBIm)]$	0.540	1.11
$[SnBu_2Cl_2(MBIm)]$	0.023	0.05
$[SnBu_2Br_2(MBIm)]$	0.170	0.31
$[SnPh_2Cl_2(MBIm)]$	0.299	0.61
$[SnPh_2Br_2(MBIm)]$	0.296	0.51
cis-$[PtCl_2(NH_3)_2]$	0.110	0.37

In vitro cytostatic activity

Table 4 shows the IC_{50} values, expressed as μg/ml^{-1} and as micromolar concentrations of the ethyl, butyl and phenyl derivatives (the methyl derivative may be expected to be less active),[3] and, for comparison, *cis*-$[PtCl_2(NH_3)_2]$. The butyl derivatives were the most active, even more so than *cis*-$[PtCl_2(NH_3)_2]$, and the ethyl derivatives the least. This behaviour parallels that found previously in similar complexes of 2,2′-bisimidazole.[3] The nature of the halogen bound to the metal atom does not seem to have any influence on activity, except for the butyl complexes, for which the chloride compound is much the more active.

In all cases, the activity of these compounds was dose-dependent only up to a concentration of about IC_{80}; further concentration increments caused neither significant further reduction in cell division nor cell lysis as observed with, for example, *cis*-$[PtCl_2(NH_3)_2]$.

Experiments to explain this phenomenon are currently in progress.

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