APPLIED ORGANOMETALLIC CHEMISTRY, VOL. 9, 251–257 (1995)

# Di(n-butyl)tin Bis(dihydroxybenzoate)s: Synthesis, Spectroscopic Characterization and *in vitro* Antitumour Activity

Marcel Gielen,* Abdeslam Bouhdid,* François Kayser,*† Monique Biesemans,*† Dick de Vos,‡ Bernard Mahieu§ and Rudolph Willem*†

* Department of General and Organic Chemistry, Faculty of Applied Sciences and † High Resolution NMR Centre, Free University of Brussels V.U.B., Room 8G512, Pleinlaan 2, B-1050 Brussels, Belgium, ‡ Pharmachemie BV, Medical Department, NL-2003 RN Haarlem, The Netherlands, and § Catholic University of Louvain, INAN, B-1348 Louvain-la-Neuve, Belgium

**Six di(n-butyl)tin(IV) and two dimethyltin(IV) bis-(dihydroxybenzoate)s were synthesized and characterized by $^{1}H$, $^{13}C$ and $^{119}Sn$ NMR and Mössbauer spectroscopy. Five of the di(n-butyl)tin compounds were screened *in vitro* against six human tumour cell lines, MCF-7, EVSA-T, WiDr, IGROV, M19 and A498. They are more active than carboplatin, cisplatin and 5-fluorouracil against all cell lines and of comparable activity or better than methotrexate. All dihydroxybenzoates with an *ortho*-hydroxyl group are more active against MCF-7 cells than substituted salicylates screened previously.**

**Keywords: butyltin; hydroxybenzoate ligand; synthesis; anticancer**

## INTRODUCTION

Bis{3-methoxysalicylato[di(n-butyl)]tin} oxide, $[2\text{-OH-3-CH}_3\text{O-C}_6\text{H}_3\text{COOSnBu}_2)_2\text{O}]_2$, obtained by the condensation of di(n-butyl)tin oxide and 3-methoxysalicyclic acid, exhibits a higher antitumour activity *in vitro* against MCF-7 (a mammary tumour) and WiDr (a colonic carcinoma) than the 5-methyl- and 4-methoxy-substituted analogues. Thus, the former compound is characterized by $ID_{50}$ values of 29 and 122 ng ml$^{-1}$, respectively,[1] instead of 90 and 377 ng ml$^{-1}$ for the 5-methyl analogue, and 190 and 1800 ng ml$^{-1}$ for the 4-methoxy analogue.[1]

The position of the methoxy substituent also influences the antitumour activity of the di(n-butyl)tin bis(methoxysalicylate)s, $(\text{CH}_3\text{O-2-OH-C}_6\text{H}_3\text{COO})_2\text{SnBu}_2$: for the 4-methoxy derivative, the $ID_{50}$ values are 131 and 1182 ng ml$^{-1}$, whereas for the 5-methoxy one, they are 54 and 611 ng ml$^{-1}$, respectively.

On the other hand, di(n-butyl)tin bis(4-hydroxy-3-methoxy-benzoate), $(4\text{-OH-3-CH}_3\text{O-C}_6\text{H}_3\text{COO})_2\text{SnBu}_2$, is characterized by excellent $ID_{50}$ values (44 ng ml$^{-1}$ and 82 ng ml$^{-1}$, respectively),[1] as compared with the former compounds.

We prepared and characterized a series of di(n-butyl)tin bis(dihydroxybenzoate)s in order to find out whether substituting an alkoxy or alkyl group by a further hydroxy group in the anion results in a noticeable change of the antitumour properties of its di(n-butyl)tin derivative. More precisely, we examined the influence of the relative positions of both hydroxy groups in the substituted benzoate moiety of the *in vitro* antitumour properties of five such compounds against a panel of six human tumour cell lines, MCF-7 (breast cancer), EVSA-T (breast cancer), WiDr (colonic carcinoma), IGROV (ovarian cancer), M19 (melanoma) and A498 (renal cancer).

## EXPERIMENTAL

### Instruments

#### Mössbauer spectroscopy

The Mössbauer spectra were recorded in constant-acceleration mode and with a Ca $^{119m}SnO_3$ source from Amersham, UK, using a home-built (INAN, Université Catholique de Louvain) instrument, designed by the Instituut voor Kern- en Stralingsfysika (IKS, Katholieke Universiteit Leuven). The probe was maintained at a temperature between 90 and 100 K, and the source at room temperature. The digital data

CCC 0268–2605/95/030251–07


*Received 21 June 1994*
*Accepted 29 September 1994*

**Table 1** Melting points, recrystallization solvents and Mössbauer parameters for compounds of general formula $[(HO)_2C_6H_3COO]_2SnR_2$ with R = n-$C_4H_9$ (**1–6**) or $CH_3$ (**7** and **8**)

| | Compound | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| Melting point (°C) | >350 | 142–144 | 116–118 | 121–122 | >350 | 222–224 | 211–213 | >350 |
| Recryst. solvent | | $CHCl_3$ | $CHCl_3$ | $CHCl_3$ | EtOH/cyclohexane | EtOH | EtOH | EtOH |
| IS[a] ($mm^{-1}$) | 1.43 | 1.51 | 1.53 | 1.59 | 1.42 | 1.44 | 1.42 | 1.43 |
| QS[b] ($mm\ s^{-1}$) | 3.84 | 3.69 | 3.72 | 4.66 | 3.66 | 3.38 | 3.82 | 3.88 |
| $\Gamma_1$[c] ($mm\ s^{-1}$) | 1.00 | 0.92 | 1.09 | 0.92 | 0.90 | 0.96 | 0.81 | 0.96 |
| $\Gamma_2$[c] ($mm\ s^{-1}$) | 0.97 | 0.99 | 0.84 | 0.91 | 0.93 | 1.02 | 0.84 | 0.87 |

[a] Isomer shift. [b] Quadrupole splitting. [c] Line width.

were treated with a least-squares iterative program deconvoluting the spectrum as a combination of Lorentzians.

### NMR spectroscopy

All the NMR spectra were recorded on a Bruker AC250 instrument operating at 250.13 MHz proton frequency, equipped with a QNP ($^1H$, $^{19}F$, $^{13}C$, $^{119}Sn$) probe. Standard pulse programs and parameter sets available in the Bruker microprogram library were used.

## Syntheses

The compounds were prepared following the procedure used to make diorganotin salicylates.[2] The appropriate dihydroxybenzoic acid, $(HO)_2C_6H_3COOH$, was dissolved in a 4:1 mixture of toluene and ethanol. The diorganotin oxide was added to this solution in a molar ratio of acid/diorganotin oxide of 1:1 or 2:1. The reacting mixture is refluxed for 4–6 h. The ternary azeotrope water/ethanol/toluene, followed by the binary azeotrope ethanol/toluene, were distilled off with a Dean–Stark trap to 50% reduction of the initial volume. The remaining solution was evaporated under reduced pressure. The solid or oil obtained was purified by recrystallization in appropriate solvents (see Table 1).

## *In vitro* antitumour screening

The antitumour screening was performed by Mr H. J. Kolker, Dr J. Verweij, Professor Dr. G. Stoter and Dr J. H. M. Schellens, Laboratory of Experimental Chemotherapy and Pharmacology, Department of Medical Oncology, Rotterdam Cancer Institute, NL-3008 AE, Rotterdam, The Netherlands.

The cytotoxicity of compounds **1–4** and **6**, together with some reference compounds, was screened *in vitro* against six well-characterized human tumour cell lines by applying the microculture sulphorhodamine B test (SRB). The compounds were tested four times at ten concentrations varying by a factor of 3, ranging from 3 to 59050 ng $ml^{-1}$. Concentration response curves were determined and the $ID_{50}$ (drug concentration in ng $ml^{-1}$ at 50% growth inhibition) values were calculated.

Prior to the experiments, a mycoplasma test was carried out on all cell lines and found to be negative. All cell lines, except EVSA-T, were maintained in a continuous logarithmic culture in RPMI medium with Hepes and Phenol Red supplemented with 10% bovine calf serum (BCS), penicillin (111 IU $ml^{-1}$) streptomycin (111 μg $ml^{-1}$), gentamycin (46 μg $ml^{-1}$) and insulin (10.6 μg $ml^{-1}$). EVSA-T was maintained in DMEM with 5% BCS and antibiotics as described. The cells were mildly trypsinized for passage and for use in experiments.

RPMI, DMEM and SRB (sulphorhodamine B) were obtained from Brunschwig (Amsterdam, The Netherlands). BCS was obtained from Hyclone (Logan, UT, USA), DMSO from Baker (Deventer, The Netherlands), phosphate-buffered saline (PBS) from Boom (Meppel, The Netherlands) and insulin Neerlandicum from Organon (Oss, The Netherlands). Streptomycin, penicillin, gentamycin and trypsin were obtained from Gibco (Breda, The Netherlands).

The test and reference compounds were dissolved to a concentration of 177,147 ng $ml^{-1}$ as follows:

Organotin compounds: 1.0–2.2% DMSO in full growth RPMI medium.
Carboplatin: 10% water in full growth RPMI medium
Cisplatin: 0.17% DMSO in full growth RPMI

medium

No additional pretreatment (ultrasonication) was needed for complete dissolution of all compounds.

On day 1, 200 µl of trypsinized tumour cells (2000 cells/well) were plated in 96-well flat-bottomed microtitre plates (Costar, no. 3799, Badhoevedorp, The Netherlands). The plates were preincubated for 24 h at 37 °C, 5% $CO_2$, to allow the cells to adhere.

On day 2, 100 µl of a solution with the highest drug concentration was added to the wells of column 12 and from there diluted three-fold to column 3 by serial transfer of 100 µl using an eight-channel micropipette. The final volume of column 3 was adjusted to 200 µl with PBS. Column 2 was used for the blank. PBS was added to column 1 to diminish interfering evaporation.

On day 7, the incubation was terminated by washing the plates twice with PBS. Subsequently, the cells were fixed with 10% trichloroacetic acid in Milli Q water (Millipore, Etten Leur, The Netherlands) and kept at 4 °C for 1 h.

After five washings with tap-water, the cells were stained for 15 min with 0.4% SRB, dissolved in 1% acetic acid, and then washed with 1% acetic acid to remove the unbound stain. The plates were air-dried and the bound protein stain was dissolved by 150 µl of 10 mM Tris base. The absorbance was read at 540 nm using an automated microplate reader (Titertec, Flow Laboratories Ltd, Irvine, Scotland).

## RESULTS AND DISCUSSION

The compounds **1** to **6** of general formula $[(HO)_2C_6H_3COO]_2Sn(n\text{-}C_4H_9)_2$ were synthesized from the corresponding dihydroxybenzoic acid and di(n-butyl)tin oxide in a 2:1 molar ratio, using the procedure described earlier for the synthesis of substituted diorganotin salicylates[3–5] or benzoates.[7–11] Their melting points and recrystallization solvents are listed in Table 1.

The characteristics of two dimethyltin compounds of the same type, $[(HO)_2C_6H_3COO]_2Sn(CH_3)_2$, compounds **7** and **8**, are also given in Table 1.

### Mössbauer spectra

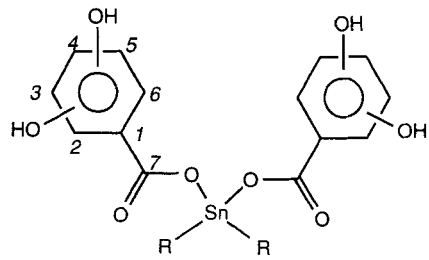
The Mössbauer data for compounds **1–8** are likewise presented in Table 1.

The IS values observed are typical for diorganotin(IV) compounds. The QS values of all compounds except **4** are compatible with a skew-trapezoidal bipyramid or bicapped tetrahedron structure in the solid state, comparable with that of dimethyltin diacetate[12] and of di(n-butyl)bis(*o*-aminobenzoato)- or di(n-butyl)bis(5-chloro-2-hydroxybenzoato)-tin.[13, 14]

Assuming that tin acquires such a pseudo-octahedral configuration in these compounds, partial quadrupole splitting (pqs) calculations suggest a C–Sn–C angle of 140–160° based on pqs values of zero for the anions and −1.03 for each butyl group.[15] The much larger quadrupole splitting found for compound **4** is consistent with a heptacoordinate structure like that of dimethyltin dipicolinate in the solid state.[16]

We cannot exclude the compounds **1**, **5** and **8** being polymers, as their melting points exceed 350 °C.

The corresponding condensation products obtained from a 1:1 molar ratio of di(n-butyl)tin oxide and dihydroxybenzoic acid, and expected from previous studies[17–21] to be of the type $(\{[(HO)_2C_6H_3COO](n\text{-}C_4H_9)_2Sn\}_2O)_2$, were found to be insoluble in the usual organic sol-




*8* *9* *10* *11*

R = $CH_2$ - $CH_2$ - $CH_2$ - $CH_3$
or
R = $CH_3$

| Compound | $(OH)_2$ | R |
|---|---|---|
| **1** | 2,3- | n-Bu |
| **2** | 2,4- | n-Bu |
| **3** | 2,5- | n-Bu |
| **4** | 2,6- | n-Bu |
| **5** | 3,4- | n-Bu |
| **6** | 3,5- | n-Bu |
| **7** | 2,4- | Me |
| **8** | 2,6- | Me |

**Table 2** $^1$H NMR data[a] of di-n-butyltin compounds of general formula $[(HO)_2C_6H_3COO]_2Sn(n\text{-}C_4H_9)_2$, **1–6**

| | Compound | | | | | |
|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** |
| Proton | Solvent DMSO-$d_6$ | $CDCl_3$ | $CDCl_3$ | $CDCl_3$ | DMSO-$d_6$ | DMSO-$d_6$ |
| *2* | — | — | — | — | d: 7.28 [2] | d: 6.76 [2] |
| *3* | — | d: 6.42 [2] | d: 6.90 [9] | d: 6.50 [8] | — | — |
| *4* | dd: 6.70 [8; 1] | — | dd: 7.06 [9; 3] | t: 7.36 [8] | — | t: 6.33 [2] |
| *5* | dd: 6.42 [8; 8] | dd: 6.41 [8; 2] | — | ≡3 | d: 6.72 [8] | — |
| *6* | dd: 6.88 [8; 1] | d: 7.86 [8] | d: 7.41 [3] | — | dd: 7.23 [8; 2] | ≡2 |
| *8* | m: 1.43–1.52 | m: 1.65–1.86 | t: 1.87 [7] | t: 1.96 [7] | t: 1.48 [8] | m: 1.35–1.51 |
| *9* | m: 1.30–1.42 | m: 1.65–1.86 | tt: 1.71 [7; 7] | tt: 1.75 [7; 7] | tt: 1.23 [8; 8] | m: 1.35–1.51 |
| *10* | tq: 1.19 [7; 7] | tq: 1.39 [7; 7] | tq: 1.39 [7; 7] | tq: 1.41 [7; 7] | m: 1.01–1.13 | tq: 1.22 [7; 7] |
| *11* | t: 0.75 [7] | t: 0.89 [7] | t: 0.89 [7] | t: 0.93 [7] | t: 0.75 [8] | t: 0.73 [7] |
| OH | s: 13.7 | bs: 10.8 | bs: 10.2 | bs: 10.1 | bs: 11.8 | bs: 9.4 |

[a] Multiplicity: chemical shift in ppm with respect to TMS [$^nJ_{H-H}$ in Hz]; bs, broad singlet; d, doublet; dd, doublet of doublets; m, complex pattern; t, triplet; tq, triplet of quartets.

vents, including dimethyl sulphoxide (DMSO). Only their melting points and Mössbauer parameters were determined, viz:

2,3-Dihydroxybenzoate, m.p. >350 °C; QS 3.83; IS 1.42; $\Gamma_1$, $\Gamma_2$ 1.00, 0.97 mm s$^{-1}$
2,4-Dihydroxybenzoate, m.p. 235–238 °C; QS 3.10; IS 1.27; $\Gamma_1$, $\Gamma_2$: 1.11, 1.04 mm s$^{-1}$
2,5-Dihydroxybenzoate, m.p. 238–241 °C; QS 3.63; IS 1.42; $\Gamma_1$, $\Gamma_2$: 1.00, 0.97 mm s$^{-1}$
2,6-Dihydroxybenzoate, m.p. 155–157 °C; QS 3.98; IS 1.47; $\Gamma_1$, $\Gamma_2$: 0.88, 0.86 mm s$^{-1}$
3,4-Dihydroxybenzoate, m.p. >350 °C; QS 3.54; IS 1.40; $\Gamma_1$, $\Gamma_2$: 1.05, 1.09 mm s$^{-1}$
3,5-Dihydroxybenzoate, m.p. >350 °C; QS 3.11; IS 1.28; $\Gamma_1$, $\Gamma_2$: 1.06, 0.99 mm s$^{-1}$

Again, polymeric structures cannot be excluded, if the total insolubility of these compounds is taken into account.

## NMR spectra

The $^1$H NMR data of the di(n-butyl)tin compounds **1–6** are described in Table 2.

The assignment of the resonances is based on the proton–proton coupling patterns and/or chemical shifts. All compounds exhibit a broad resonance in the range 9–13 ppm, which is assigned to the hydroxyl protons.

The $^{13}$C NMR data of the di(n-butyl)tin compounds **1–6** are reported in Table 3. The assignment of the aromatic $^{13}$C resonances was performed from calculations using aromatic chemical shift increments,[22] and from distortionless enhancement by polarization transfer (DEPT) spectra. The $^nJ(^{119/117}Sn–^{13}C)$ coupling constants facilitated the assignment of the aliphatic $^{13}$C resonances of the n-butyl groups. The $^1J(^{119/117}Sn–^{13}C)$ values in $CDCl_3$ for compounds **2**, **3** and **4** are typical for the usual trapezoidal bipyramidal structure of diorganotin dicarboxylates.[18–21] Those in DMSO for the 3,5-dihydroxy compound **5** suggest at least a six-coordinate structure likely to involve DMSO molecules as ligands.

The $^1$H and $^{13}$C NMR data of the dimethyltin compounds **7** and **8** in DMSO-$d_6$ are presented in Table 4. The same conclusion holds as for the di(n-butyl)tin compounds.

The $^{119}$Sn NMR data of compounds **1–8** are summarized in Table 5. The $^{119}$Sn NMR data in DMSO solutions reveal three types of spectral behaviour. Compounds **1** and **5** exhibit a single $^{119}$Sn resonance at an unusually high frequency for such compounds in DMSO solutions. These data suggest that the trapezoidal bipyramidal structure[18–21] characteristic for $CDCl_3$ solutions is preserved in DMSO for **1** and **5**. These compounds **1** and **5** have in common the presence of two hydroxy groups in *ortho* positions to one another, but the role of this property remains unclear. The $^1J(^{119/117}Sn–^{13}C)$ couplings, only slightly larger than the values observed in $CDCl_3$, are in agreement with this proposal.

In contrast, compounds **4**, **6**, **7** and **8** exhibit a

**Table 3** $^{13}C$ NMR data[a] of di-n-butyltin compounds of general formula $[(HO)_2C_6H_3COO]_2Sn(n\text{-}C_4H_9)_2$, **1–6**

| | Compound | | | | | |
|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** |
| | Solvent | | | | | |
| Carbon | DMSO-$d_6$ | $CDCl_3$ | $CDCl_3$ | $CDCl_3$ | DMSO-$d_6$ | DMSO-$d_6$ |
| 1 | 113.6 | 105.8 | 112.3 | 99.6 | 121.7 | 132.6 |
| 2 | 155.5* | 163.4* | 155.7* | 161.8 | 117.6* | 106.7* |
| 3 | 153.1* | 103.0 | 116.0 | 107.9 | 144.6 | 157.3 |
| 4 | 116.4** | 162.6* | 124.5** | 137.4 | 149.7 | 105.5* |
| 5 | 117.0** | 108.0 | 147.9* | ≡3 | 116.4* | ≡3 |
| 6 | 117.1** | 133.5 | 118.4** | ≡2 | 119.9* | ≡2 |
| 7 | 167.6 | 177.1 | 177.1 | 178.3 | 168.6 | 171.8 |
| 8 | 26.2 | 26.4 | 26.4 | 28.0 | 24.6 | 28.6 |
| $^1J$(Hz) | ~686† | 564/538 | 572/546 | 554/528 | 656/625 | 852/812 |
| 9 | 26.4 | 26.5 | 26.3 | 26.6 | 25.6 | 25.8 |
| $^2J$ (Hz) | 39 | 34 | 32 | 36 | 36 | 40 |
| 10 | 25.5 | 26.3 | 26.2 | 26.5 | 26.3 | 24.6 |
| $^3J$(Hz) | 110 | 97 | 98 | 100 | nv | 135 |
| 11 | 13.4 | 13.3 | 13.3 | 13.4 | 13.3 | 12.6 |

[a] Chemical shift in ppm with respect to TMS; nv, non-visible; * and ** permutable assignments; $^1J$ represents the pair of $^1J(^{119/117}Sn-^{13}C)$ coupling constants; $^nJ$ ($n = 2, 3$) represents the unresolved $^nJ(^{119/117}Sn-^{13}C)$ coupling constant; † non-resolved $^1J(^{119/117}Sn-^{13}C)$ satellites (broad; poor signal-to-noise ratio).

broader $^{119}Sn$ resonance below −286 ppm. These data suggest six-coordinate complexes involving DMSO as ligands,[3–6] as well as the higher $^1J(^{119/117}Sn-^{13}C)$ couplings in DMSO than in $CDCl_3$.

Finally, two compounds, **2** and **3**, exhibit two

**Table 4** $^1H$ and $^{13}C$ NMR spectra[a] in DMSO-$d_6$ of dimethyltin compounds of general formula $[(HO)_2C_6H_3COO]_2Sn(CH_3)_2$, **7** and **8**

| | Compound | | | |
|---|---|---|---|---|
| | **7** | | **8** | |
| Atom | $^1H$ | $^{13}C$ | $^1H$ | $^{13}C$ |
| 1 | | 105.6 | | 102.8 |
| 2 | | 163.0* | | 161.6 |
| 3 | d: 6.23 [2] | 102.1 | d: 6.19 [8] | 106.1 |
| 4 | | 163.4* | t: 7.09 | 133.6 |
| 5 | dd: 6.30 [9; 2] | 107.4 | ≡3 | ≡3 |
| 6 | d: 7.6 [9] | 131.9 | | ≡2 |
| 7 | | 172.7 | | 174.5 |
| $CH_3$ | s: 0.90 | 11.4 | s: 0.92 | 13.3 |
| | $[^2J_{Sn-H} \approx 103]$ | $[^1J_{Sn-C} = 754/718]$ | $[^2J_{Sn-H} \approx 108]$ | $[^1J_{Sn-C} = 628/601]$ |

[a] Chemical shifts in ppm with respect to TMS; $^nJ_{H-H}$ coupling constants, expressed in Hz between brackets; * assignment permutable; $^2J_{Sn-H}$ represents the unresolved $^2J(^{119/117}Sn-^1H)$ coupling constant; $^1J_{Sn-C}$ represents the pair of $^1J(^{119/117}Sn-^{13}C)$ coupling constants.

**Table 5** $^{119}$Sn chemical shifts[a] of compounds of the type $[(HO)_2C_6H_3COO]_2SnR_2$ with R = n-$C_4H_9$ (**1–6**) or $CH_3$ (**7** and **8**)

| | Compound | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Solvent | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| $CDCl_3$ | — | −125.6 | −121.8 | −122.3 | — | — | — | — |
| DMSO | −171.6 | −256.1<br>−293.1(b) | −240.1<br>−291.4(b) | −377.2 | −134.4 | −303.5 | −286.9 | −344.3 |

[a] Chemical shifts $\delta$ ($^{119}$Sn) in ppm with respect to tetramethyltin; b, broad resonance (>3000 Hz).

$^{119}$Sn resonances, one *ca* 1000 Hz broad, at −256 ppm and −240 ppm, respectively, and a second, more than 3000 Hz broad, around −290 ppm. We tentatively propose that these compounds may exist as an interconverting mixture of the two types of structures observed for the other compounds in DMSO. This interconversion appears to coalesce on the $^{119}$Sn NMR timescale ($\Delta\nu \approx 8000$ Hz) and is, accordingly, fast on the $^1$H and $^{13}$C NMR timescales.

## *In vitro* antitumour screening

Compounds **1–4** and **6** were screened *in vitro* against several human tumour cell lines: MCF-7 and EVSA-T (two breast cancers), WiDr (a colonic carcinoma), IGROV (an ovarian cancer), M19 MEL (a melanoma) and A498 (a renal cancer). MCF-7 is an oestrogen receptor (ER+)/progesterone receptor (PgR+), and EVSA-T is ER/PgR−. The other four cell lines belong to the currently used anticancer screening panel of the National Cancer Institute, Bethesda, MD, USA.

The $ID_{50}$ values (ng ml$^{-1}$) obtained for these compounds (shown in Table 6) are compared with the cytotoxicities of two commonly used metal-based drugs (carboplatin and cisplatin). Table 6 indicates that all the organotin compounds tested were more active *in vitro* against all cell lines than carboplatin and cisplatin.

All dihydroxybenzoates with an *ortho*-hydroxyl group turn out to be more active against MCF-7 than the di(n-butyl)tin methoxysalicylates screened previously,[1] showing an activity improvement upon introduction of an additional OH group. Except for one methoxysalicylate, which is of comparable activity, the same conclusion holds for the WiDr cell line. However, the 3,5-dihydroxybenzoate, having no *ortho*-hydroxyl group, is significantly less active than the methoxysalicylates and dihydroxybenzoates with at least one *ortho*-hydroxyl group. Within the latter subclass, no significant differences of activities are observable.

*Acknowledgements* This research was supported by the Belgian 'Nationaal Fonds voor Wetenschappelijk Onderzoek', NFWO (grant no S2/5 CD F198, MG), by the Belgian 'Nationale Loterij' (grant nos 9.0050.90 and 9.0006.93, RW, MB) and by the Human Capital and Mobility Programme of the European Community (Contract no ERBCHRXCT920016). The 'Ministère des Affaires Culturelles du Luxembourg' (grant no BFR90/036; FK), the 'Comité National des Bourses

**Table 6** Inhibition doses $ID_{50}$ (ng ml$^{-1}$) of compounds **1–4** and **6**, and of two commonly used metal-based antitumour drugs, against six human tumour cell lines

| Compound | MCF-7 (breast cancer) | EVSA-T (breast cancer) | WiDr (colonic carcinoma) | IGROV (ovarian cancer) | M19 (melanoma) | A498 (renal cancer) |
|---|---|---|---|---|---|---|
| **1** $[2,3\text{-}(OH)_2C_6H_3COO]_2SnBu_2$ | 7 | 43 | 90 | 51 | 50 | 50 |
| **2** $[2,4\text{-}(OH)_2C_6H_3COO]_2SnBu_2$ | 16 | 54 | 120 | 85 | 58 | 130 |
| **3** $[2,5\text{-}(OH)_2C_6H_3COO]_2SnBu_2$ | 4 | 48 | 115 | 60 | 65 | 100 |
| **4** $[2,6\text{-}(OH)_2C_6H_3COO]_2SnBu_2$ | 15 | 58 | 130 | 110 | 65 | 130 |
| **6** $[3,5\text{-}(OH)_2C_6H_3COO]_2SnBu_2$ | 130 | 130 | 500 | 120 | 190 | 280 |
| Carboplatin | 5500 | 1100 | 1500 | 780 | 5300 | 3500 |
| Cisplatin | 800 | 1200 | 650 | 79 | 530 | 1200 |

OTAN' (FK), and the 'Ministère de l'Education Nationale du Luxembourg' (FK) are gratefully acknowledged.

## REFERENCES


1. M. Gielen, P. Lelieveld, D. de Vos and R. Willem, *In vitro* antitumour activity of organotin compounds. Chapter 2 in: *Metal-Based Antitumour Drugs*, Gielen, M. (ed.), Freund, London, 1992, pp. 29–54.
2. M. Gielen, C. Vanbellinghen, J. Gelan and R. Willem, *Bull. Soc. Chim. Belg.* **97**, 873 (1988).
3. M. Bouâlam, R. Willem, M. Biesemans, B. Mahieu, J. Meunier-Piret and M. Gielen, *Main Group Met. Chem.* **14**, 41 (1991).
4. M. Bouâlam, R. Willem, M. Biesemans and M. Gielen, *Appl. Organomet. Chem.* **5**, 497 (1991).
5. M. Gielen and R. Willem, *Anticancer Res.* **12**, 257 (1992).
6. R. Willem, M. Biesemans, F. Kayser, M. Bouâlam and M. Gielen, *Inorg. Chim. Acta* **197**, 25 (1992).
7. A. Meriem, M. Biesemans, R. Willem, B. Mahieu, D. de Vos, P. Lelieveld and M. Gielen, *Bull. Soc. Chim. Belg.* **100**, 367 (1991).
8. M. Gielen, A. El Khloufi, M. Biesemans, B. Mahieu and R. Willem, *Bull. Soc. Chim. Belg.* **101**, 243 (1992).
9. M. Gielen and R. Willem, *Anticancer Res.* **12**, 269 (1992).
10. M. Gielen, J. Meunier-Piret, M. Biesemans, R. Willem and A. El Khloufi, *Appl. Organomet. Chem.* **6**, 59 (1992).
11. M. Gielen and R. Willem, *Anticancer Res.* **12**, 1323 (1992).
12. T. P. Lockhart, J. C. Calabrese and F. Davidson, *Organometallics* **6**, 2479 (1987).
13. A. Meriem, R. Willem, J. Meunier-Piret, B. Mahieu and M. Gielen, *Main Group Met. Chem.* **13**, 167 (1990).
14. M. Gielen, M. Bouâlam, B. Mahieu and E. R. T. Tiekink, *Appl. Organomet. Chem.* **8**, 19 (1994).
15. R. V. Parish, in *Mössbauer Spectroscopy Applied to Inorganic Chemistry*, Long, G. J. (ed.), Plenum, New York, 1984, p. 527.
16. T. P. Lockhart and F. Davidson, *Organometallics* **6**, 2471 (1987).
17. E. R. T. Tiekink, *Appl. Organomet. Chem.* **5**, 1 (1991).
18. M. Bouâlam, R. Willem, M. Biesemans, B. Mahieu, J. Meunier-Piret and M. Gielen, *Main Group Met. Chem.* **14**, 41 (1991).
19. M. Gielen, J. Meunier-Piret, M. Biesemans, R. Willem and A. El Khloufi, *Appl. Organomet. Chem.* **6**, 59 (1992).
20. M. Gielen, J. Meunier-Piret, M. Biesemans, A. El Khloufi and R. Willem, *Polyhedron* **11**, 1961 (1992).
21. M. Gielen, M. Biesemans, A. El Khloufi, J. Meunier-Piret and R. Willem, *J. Fluorine Chem.* **64**, 279 (1993).
22. H. O. Kalinowski, S. Berger and S. Braun, *Carbon-13 NMR Spectroscopy*, John Wiley, New York, 1988.
23. S. Roelens and M. Taddei, *J. Chem. Soc., Dalton Trans. II*, 299 (1985).