DI-n-BUTYL-, TRI-n-BUTYL- AND TRIPHENYLTIN dl-TEREBATES: SYNTHESIS, CHARACTERIZATION AND *IN VITRO* ANTITUMOUR ACTIVITY

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Abstract

Di-n-butyltin, tri-n-butyltin and triphenyltin terebates were screened against several human tumour cell lines and found comparably or more active than carboplatin, cis-platin, 5-fluorouracil, methotrexate and doxorubicin, some reference compounds used clinically.

Introduction

A large number of di- and triorganotin carboxylates exhibit interesting *in vitro* antitumour activities against human tumour cell lines[1].

Several 1:1 and 1:2 condensation compounds of di-n-butyltin oxide with mono-[2a], di-[2b], tri-, tetra-[2c] and pentafluorobenzoic acids[2d] are among such substances. Di-n-butyltin compounds are generally much more active than other diorganotin compounds.

Many substituted triphenyltin benzoates[3] are even more active. In contrast, tri-n-butyltin difluorobenzoates[4] are less active than the corresponding triphenyltin and di-n-butyltin compounds.

A series of tri- and diorganotin steroidcarboxylates were recently screened against seven human tumour cell lines, MCF-7 and EVSA-T, two breast cancers, WiDr, a colon cancer, IGROV, an ovarian cancer, M19 MEL, a melanoma, A498, a renal cancer, and H226, a non small cell lung cancer. The *in vitro* antitumour activities of the di-n-butyltin compound lie between those of 5-fluorouracil and doxorubicin. The activities of the triorganotin compounds are comparable to those of methotrexate or doxorubicin[5].

In the frame of our interest in biologically relevant organotin carboxylates, we report the synthesis and antitumour properties of di- and triorganotin derivatives of dl-terebic acid.

Results and Discussion

Di-n-butyltin diterebate, $(H_9O_2C_6\text{-COO})_2SnBu_2$, compound **1**, tri-n-butyltin terebate, $(H_9O_2C_6\text{-COO})SnBu_3$, compound **2**, and triphenyltin terebate, $(H_9O_2C_6\text{-COO})SnPh_3$, compound **3**, were synthesized by the condensation of dl-terebic acid, $(H_9O_2C_6\text{-COOH})$ with respectively di-n-butyltin oxide, tri-n-butyltin acetate and triphenyltin hydroxide.
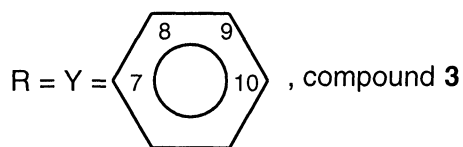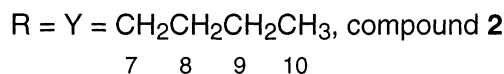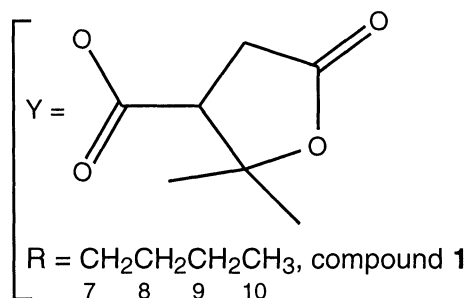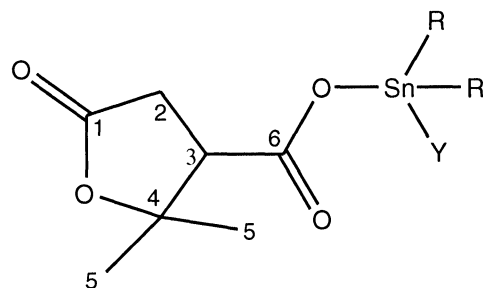
They were characterized by ^{1}H NMR, through multiplet patterns and resonance integrals, by ^{13}C NMR, supported by DEPT experiments, by [117]Sn NMR and by [119]Sn Mössbauer spectroscopy (see Experimental Section).

The $^1J(^{13}C\text{-}^{119/117}Sn)$ coupling constants (567/542 Hz) and the [117]Sn chemical shift (-131.6 ppm) of compound **1** lie in the ranges characteristic for di-n-butyltin dibenzoates[6] and -disalicylates[7][8] displaying a pseudo-octahedral or alternatively trapezoidal bipyramidal structure with two n-butyl groups in axial positions. The carboxylates coordinate the tin atom in the bidentate mode in equatorial positions with two adjacent short and two longer Sn-O bonds. All other NMR resonances are, accordingly, as expected for this type of structure.

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The $^1J(^{13}C-^{119/117}Sn)$ coupling constants (354/338 Hz) and the ^{117}Sn chemical shift (+125.4 ppm) of compound **2** are compatible with a monomeric tetrahedral structure found recently for a series of tri-n-butyl fluorobenzoates in solution[4], in agreement with earlier coupling data described by Holecek and Lycka[9].

The $^1J(^{13}C-^{119/117}Sn)$ coupling constants (648/618 Hz) and the ^{117}Sn chemical shift (-97.6 ppm) of compound **3** are again in agreement with a tetrahedral monomeric structure[2][10][11].

R = CH₂CH₂CH₂CH₃, compound **1**

$R = CH_2CH_2CH_2CH_3$, compound **1**
   7   8   9   10

$R = Y = CH_2CH_2CH_2CH_3$, compound **2**
      7   8   9   10

$R = Y =$, compound **3**

Antitumour activities

The *in vitro* antitumour activities of compounds **1, 2** and **3** against several human cell lines, MCF-7 and EVSA-T, two breast cancers, WiDr, a colon cancer, IGROV, an ovarian cancer, M19 MEL, a melanoma, A498, a renal cancer, and H226, a non small cell lung cancer, are displayed in table 1, together with those of some reference compounds used clinically: carboplatin, cis-platin, 5-fluorouracil, methotrexate and doxorubicin.

Table 1 clearly shows that the compounds are comparably to more active than methotrexate and doxorubicin *in vitro* against almost all cell lines. The very high activity of compounds **2** and **3** against EVSA-T as well as of compound **2** in general should be outlined.

Experimental part

Syntheses

Compounds **1** to **3** were prepared by adding 5 mmole of di-n-butyltin oxide, tri-n-butyltin acetate or triphenyltin hydroxide, respectively, to a solution of 10 mmole, 5 mmole or 5 mmole of terebic acid in 150 cm³ toluene and 50 cm³ ethanol. After refluxing for 6 h, distilling off the ternary azeotrope

water/toluene/ethanol with a Dean Stark funnel and half of the remaining solvent, the resulting mixture was cooled down to room temperature, filtered and evaporated under vacuum. The residue was recrystallized from the appropriate solvent.

Compounds	MCF-7	EVSA-T	WiDr	IGROV	M19 MEL	A498	M226
1	27	25	134	18	27	61	104
2	3	<3	11	4	11	15	8
3	17	<3	17	19	42	58	39
Carboplatin[14]	10500	4500	3500	2400	5500	18000	25000
Cisplatin[14]	1400	920	1550	230	780	1200	3158
5-Fluorouracil[14]	350	720	440	850	310	340	5300
Methotrexate[14]	15	26	7	20	18	16	70
Doxorubicin[14]	25	13	18	150	21	55	180

Table 1. *In vitro* antitumour activities (ng/mL) of compounds **1** to **3**, together with those of some reference compounds used clinically.

Characterization

Mössbauer data: QS: quadrupole splitting; IS: isomer shift; Γ_1 and Γ_2: line widths, all in mm/s.
NMR data: all spectra were acquired from $CDCl_3$ solutions and referenced to the residual C^1HCl_3 resonance at 7.24 ppm for the ^1H spectrum, to the central $^{13}CDCl_3$ resonance at 77.0 ppm for the ^{13}C spectrum and to $\Xi(^{119}Sn) = 37.290665$ for the ^{119}Sn spectra[12].
Abbreviations for coupling patterns: s = singlet; d = doublet; t = triplet; td = triplet of doublets; tq = triplet of quartets; b = broad resonance; m = complex pattern; nv = non visible; coupling constants are given in Hz between parentheses for $^nJ(^1H-^1H)$ for ^1H spectra. Other coupling constants are indicated explicitly.
Compound **1**: recrystallization solvent: chlorofom/n-hexane; m.p.: 98-100°C; yield: 85%; Mössbauer data: QS: 3.44; IS: 1.34; Γ_1: 0.99; Γ_2: 0.91; ^1H NMR data: 2.988, dd (18, 9): H-2a; 2.700, dd (18, 9): H-2b; 3.196, dd (9, 9): H-3; 1.581, s: H-5a; 1.348, s: H-5b; 1.62 - 1.64, m: H-7 & H-8; 1.25 - 1.40, m: H-9; 0.873, t (7): H-10; ^{13}C NMR data: C-1: 174.1; C-2: 32.5; C-3: 50.5; C-4: 84.5; C-5a: 28.5; C-5b: 23.5; C-6: 179.5; C-7: 25.9 [$^1J(^{119/117}Sn-^{13}C)$ = 573/550]; C-8: 26.7 [$^2J(^{119/117}Sn-^{13}C)$ ≈ 31]; C-9: 26.4 [$^3J(^{119/117}Sn-^{13}C)$ = 100/96]; C-10: 13.5; ^{119}Sn NMR data: -134.0.
Compound **2**: recrystallization solvent: petroleum ether; m.p.: 50-51°C; yield: 95%; Mössbauer data: QS: 3.77; IS: 1.47; Γ_1: 0.85; Γ_2: 0.86; ^1H NMR data: 2.985, dd (17, 10): H-2a; 2.622, dd (17, 8): H-2b; 3.121, dd (10, 8): H-3; 1.562, s: H-5a; 1.297, s: H-5b; 1.50 - 1.65, m: H-7 & H-8; 1.20 - 1.40, m: H-9; 0.878, t (7): H-10; ^{13}C NMR data: C-1: 174.4; C-2: 32.7; C-3: 51.4; C-4: 84.8; C-5a: 28.5; C-5b: 23.3; C-6: 174.8; C-7: 16.7 [$^1J(^{119/117}Sn-^{13}C)$ = 356/338]; C-8: 27.8 [$^2J(^{119/117}Sn-^{13}C)$ ≈ 21]; C-9: 27.0 [$^3J(^{119/117}Sn-^{13}C)$ ≈ 64]; C-10: 13.6; ^{119}Sn NMR data: 123.4.
Compound **3**: recrystallization solvent: ethanol/petroleum ether; m.p.: 125-126°C; yield: 95%; Mössbauer data: QS: 3.52; IS: 1.31; Γ_1: 0.82; Γ_2: 0.79; ^1H NMR data: 3.065, dd (18, 10): H-2a; 2.669, dd (18, 9): H-2b; 3.231, dd (10, 9): H-3; 1.585, s: H-5a; 1.114, s: H-5b; 7.70-7.80, m: H-8; 7.30-7.50, m: H-9 & H-10; ^{13}C NMR data: C-1: 174.5; C-2: 32.7; C-3: 50.8; C-4: 84.8; C-5a: 28.5; C-5b: 23.2; C-6: 175.6; C-7: 137.5 [$^1J(^{119/117}Sn-^{13}C)$ = 648/618]; C-8: 136.9 [$^2J(^{119/117}Sn-^{13}C)$ = 51/49]; C-9: 129.1 [$^3J(^{119/117}Sn-^{13}C)$ = 65/62]; C-10: 130.5 [$^4J(^{119/117}Sn-^{13}C)$ ≈ 13]; ^{119}Sn NMR data: -98.3.
Instruments

All NMR spectra were recorded on a Bruker AC250 instrument, using a QNP probe tuned at 250.13, 62.93 and 93.28 MHz for ^1H, ^{13}C and ^{119}Sn nuclei, respectively.
Mössbauer spectra were obtained as described previously[8].
In vitro tests

The following human tumor cell lines were used: - MCF7 Breast cancer - EVSA-T Breast cancer - WiDr Colon cancer - IGROV Ovarian cancer - M19 MEL Melanoma - A498 Renal cancer - H226 Non small cell lung cancer.

MCF7 is estrogen receptor ER+ /progesterone receptor PgR+ and EVSA-T is ER-/PgR-.

Cell lines WIDR, M19 MEL, A498, IGROV and H226 belong to the currently used anti-cancer screening panel of the National Cancer Institute, USA[13]. The *in vitro* cytotoxicity of the compounds was determined using SRB[14] as a cell viability test.

Prior to the experiments a mycoplasma test was carried out on all cell lines and found to be negative. All cell lines were maintained in a continuous logarithmic culture in RPMI 1640 medium with Hepes and phenol red. The medium was supplemented with 10% FCS, penicillin 100 IU/ml:md streptomycin 100 µg/ml. The cells were mildly trypsinized for passage and for use in experiments. RPMI and FCS were obtained from Life technologies (Paisley, Scotland). SRB, DMSO, penicllin and streptomycin were obtained from Sigma (St. Louis, MO, USA), TCA and acetic acid from Baker B.V. (Deventer, NL) and PBS from NPBI B.V. (Emmer-Compascuum, NL).

The test and reference compounds were dissolved to a concentration of 238095 ng/ml in full medium, by 21 fold dilution of a DMSO solution which contained 1 mg compound / 200 µl . The experiment was started on day 0.

On day 0 150 µl of trypsinized tumor cells (1500 - 2000 cells/well) were plated in 96-wells flatbottom microtiter plates (falcon 3072, BD). The plates were preincubated 48 hr at 37°C, 8,5 % CO_2 to allow the cells to adhere.

On day 2, a three fold dilution sequence of ten steps was made in full medium, starting with the 238095 ng/ml stock solution. Every dilution was used in quadruplicate by adding 50 µl to a column of four wells.

This results in a highest concentration of 59523 ng/ml present in column 12. Column 2 was used for the blank. To column 1 PBS was added to diminish interfering evaporation.

On day 7 the incubation was terrninated by washing the plate twice with PBS. Subsequently the cells were fixed with 10% trichloroacetic acid in PBS and placed at 4°C for one hour. After five washings with tap water, the cells were stained for at least 15 minutes with 0,4% SRB dissolved in 1% acetic acid. After staining the cells were washed with 1% acetic acid to remove the unbound stain. The plates were air dried and the bound stain was dissolved in 150 µl tris base. The absorbance was read at 540 nm using an automated microplate reader (Labsystems Multiskan MS). Data were used for construction of concentration-response curves and determination of the ID_{50} value by use of Deltasoft 3 software.

All raw data and the mastercopy of this report have been filed in the archives of the laboratory of medical oncology of the Rotterdam Academical hospital.

Acknowledgements

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