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NOTE

Di- and Tri-organotin Derivatives of 3*S*,4*S*-3-[(*R*)-1-(tert-butyl-dimethylsilyloxy)ethyl]-4-[(*R*)-1-carboxyethyl]-2-azetidinone: Synthesis, Characterization and *in vitro* Antitumour Activity

Marcel Gielen[1]*, Hassan Dalil[1], Monique Biesemans[1,2], Bernard Mahieu[4], Dick de Vos[4] and Rudolph Willem[1,2]
[1]Department of General and Organic Chemistry, Faculty of Applied Sciences, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium
[2]High Resolution NMR Centre, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium
[3]Catholic University of Louvain, CPMC, B-1348 Louvain-la-Neuve, Belgium
[4]Medical Department, Pharmachemie BV, NL-2003 RN Haarlem, The Netherlands

Di-n-butyl-, triphenyl and tri-n-butyltin derivatives of 3*S*,4*S*-3-[(*R*)-1-(tert-butyldimethylsilyloxy)ethyl-4-[(*R*)-1-carboxyethyl]-2-azetidinone were synthesized and characterized. Their antitumour activity was screened against seven tumoural cell lines of human origin.

Keywords: azetidinones; organotin compounds; antitumour activity

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INTRODUCTION

Multifunctional di- and triorganotin carboxylates derived from bio-organic acids such as steroid carboxylic acids,[1] terebic acid,[2] 2,3:4,6-di-isopropylidene-2-keto-L-gulonic acid[3] and gibberellic acid[4] were found to exhibit interesting antitumour properties *in vitro*. On the other hand, numerous other organotin carboxylates have likewise been shown to exhibit high antitumour activity.[5–8]

In a similar way, the present report describes di- and tri-organotin carboxylates derived from 3*S*,4*S*-3-[(*R*)-1-(tert-butyldimethylsilyloxy)ethyl-4-[(*R*)-1-carboxyethyl]-2-azetidinone, hereafter R*COOH, and the results of their antitumour screening against six human tumoural cell lines. This carboxylic acid combines both the presence of multifunctionality and various heteroelements displayed also by the above-mentioned bio-organic ligands, and a pharmaceutical background, azetidinone and its derivatives being members of the carbapenem class of antibiotics.[9]

RESULTS AND DISCUSSION

Synthesis

The di-n-butyltin derivatives of the chiral carboxylic acid R*COOH (for structures and labelling scheme, see Fig. 1) were synthesized by a one-pot method described by Davies *et al*.[10] In a first step, tetra-n-butyldi-n-propoxydistannoxane is prepared from di-n-butyltin oxide and n-propanol in reflux-

* Correspondence to: Marcel Gielen, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium.
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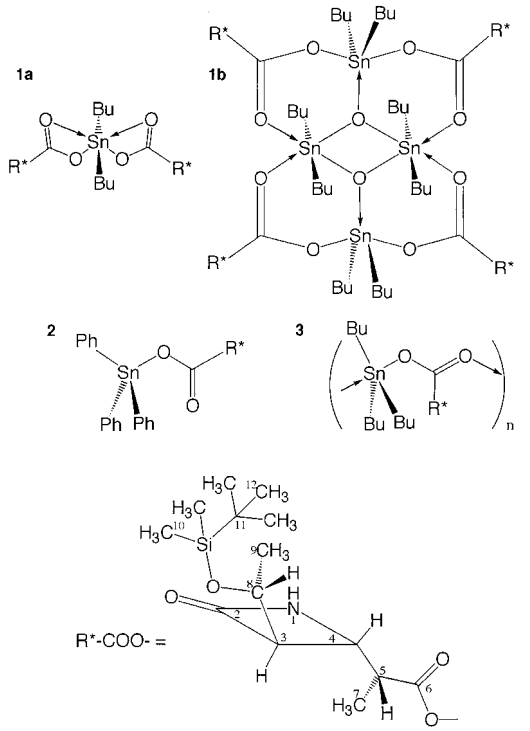

Figure 1 Structures proposed for **1a, 1b, 2** and **3** from Mössbauer data. R*COO represents the carboxylate residue shown.

ing benzene. This distannoxane subsequently reacts at room temperature with R*COOH.

With a tin/acid molar ratio of 1:2, the monomeric di-n-butyltin dicarboxylate (type **a**),[11,12] $Bu_2Sn(OOCR^*)_2$, **1a**, is obtained. A 1:1 molar ratio yields the dimeric tetra-n-butyldicarboxylatodistannoxane (type **b**),[11,12] $\{[Bu_2Sn(OOCR^*)]_2O\}_2$, **1b**.

This method appears to have two advantages over that[13,14] by which dibutyltin oxide reacts with the carboxylic acid in refluxing ethanol/toluene; first, as the carboxylic acid is added at room temperature, organotin carboxylates unstable at higher temperatures can be prepared; second, the tetra-n-butyldi-n-propoxydistannoxane is synthesized in a water-free medium because the water is eliminated through a water/propanol/benzene azeotrope; hence water-sensitive organotin carboxylates can conveniently be prepared.

The triphenyl (**2**) and tri-n-butyltin (**3**) derivatives of R*COOH have been synthesized by reaction of the latter with triphenyltin hydroxide and tri-n-butyltin acetate,[15–17] respectively.

Table 1 ^{119}Sn Mössbauer parameters: quadrupole splitting (QS) and isomer shift (IS) (relative to $CaSnO_3$) of compounds **1a, 1b, 2** and **3**

Compound	QS (mm s^{-1})	IS (mm s^{-1})
1a	3.36	1.38
1b	2.96, 3.93	1.29, 1.36
2	2.92	1.12
3	3.57	1.48

Mössbauer data

The Mössbauer parameters of solids **1a, 1b, 2** and **3** are given in Table 1. The value of the quadrupole splitting of **1a** is compatible with the typical distorted bipyramidal trapezoidal geometry of diorganotin dicarboxylates.[18,19] Compound **1b** is characterized by two values of the quadrupole splitting, QS = 2.96 mm s^{-1}, compatible with the expected five-coordinate trigonal-bipyramidal tin, and QS = 3.93 mm s^{-1}, indicating a six-coordinate geometry which is unusual but nevertheless known for the endocyclic tin atoms of some compounds of this type.[18–22] The quadrupole splitting of the triphenyltin compound **2** is compatible with a monomeric structure, either with or without an intramolecular C=O→Sn coordination bond, characterized by QS values lying between 2.36 and 2.97 mm s^{-1}. By contrast, that of the tri-n-butyltin compound **3** is typical for a polymeric structure with intermolecular C=O→Sn coordination bonds and two oxygen atoms in a *trans* configuration, with values ranging from 3.0 to 4.1 mm s^{-1}.[14–16,23–25] The structure proposals resulting from these Mössbauer data are given in Fig. 1.

NMR data

The ^{1}H and ^{13}C NMR spectra of compounds **1a, 1b, 2** and **3** in $CDCl_3$ are described in Table 2. The aliphatic ^{1}H resonance assignment was based on homonuclear *J*-multiplet patterns and intensities of the resonances. The other ^{1}H and ^{13}C resonances have been assigned by ^{1}H–^{13}C correlations from 2D HMQC and HMBC NMR spectra.

The ^{117}Sn NMR data of compounds **1a, 1b, 2** and **3** are given in Table 3. For **1a**, the expected single ^{117}Sn resonance is observed with a chemical shift characteristic for a diorganotin dicarboxylate of type **a**.[11,12] Compound **1b** exhibits two ^{117}Sn resonances with a $^2J(^{117}Sn–O–^{119/117}Sn)$ coupling constant of 116 Hz,[26] with chemical shifts in

Table 2 ^{1}H and ^{13}C NMR data in $CDCl_3$ of compounds **1a, 1b, 2** and **3**: chemical shifts δ in ppm, $^{n}J(^{13}C–^{119/117}Sn)$ and $^{n}J(^{1}H–^{119/117}Sn)$ coupling constants in Hz (bold), $^{n}J(^{1}H–^{1}H)$ coupling constants in parentheses, with multiplet patterns

	1a		**1b**		**2**		**3**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	s 6.30	—	s 5.90	—	s 5.77	—	s 5.90	—
2		168.5	—	168.4	—	168.4	—	168.4
3	dd (4, 4) 3.00	61.2	dd (5, 4) 2.92	60.9	dd (4, 4) 2.92	61.5	dd (4, 4) 2.92	61.3
4	dd (5, 5) 3.93	51.7	dd (5, 4) 3.89	51.5	dd (5, 5) 3.94	52.0	dd (5, 5) 3.87	52.0
5	dq (5, 7) 2.81	42.0	dq (5, 7) 2.80	43.0	dq (5, 7) 2.78	42.5	dq (5, 7) 2.66	42.7
6	—	184.3	—	181.8	—	180.4	—	179.4
7	d (7) 1.27	12.7	d (7) 1.20	11.9	d (7) 1.21	12.7	d (7) 1.17	12.6
8	dq (4, 7) 4.21	65.5	dq (5, 7) 4.17	65.5	dq (4, 6) 4.11	65.2	dq (5, 6) 4.15	65.3
9	d (7) 1.23	22.7	d (7) 0.91	22.6	d (6) 1.00	22.4	d (6) 1.16	22.4
10	s 0.08	−4.3, −4.9	s 0.06	−4.3, −5.0	s 0.02	−4.3, −5.1	s 0.03	−4.4, −5.1
11	—	17.9	—	17.9	—	17.9	—	17.9
12	s 0.89	25.7	s 0.86	25.7	s 0.83	25.7	s 0.83	25.6
α /i	1.63[a]	25.5 {**586/561**}	1.61[a]	25.5 {**576/553**}	—	137.9 {**652/623**}	t (8) 1.21 [**19**]	16.5 {**357/341**}
β/o	1.63[a]	26.7 {**37**}	1.61[a]	27.7 {**37**} 27.4 {**38**}	7.69 [**60**]	136.7 {**48**}	tt (7, 8) 1.56	27.8 {**21**}
γ/m	1.36[a]	26.2 {**98**}	1.30[a]	26.9 {**100**}	7.43 [**17**]	128.9 {**63**}	tq (7, 7)	26.9
δ/p	t (7) 0.92	13.5	t (7) 0.91	13.6	7.43	130.2	t (7) 0.87	13.6 {**17**}

[a] The chemical shift values given for the ^{1}H resonances of the n-butyl moieties correspond to the centre along the ^{1}H axis of the complex multiplet ^{1}H–^{13}C HMQC cross-peaks. t, Abbreviations: triplet; q, quartet; d, doublet.

agreement with two pairs of unequivalent endocyclic and exocyclic five-coordinate tin sites, as is usual for type **b** distannoxanes[11,12] in solution. Different geometries for the exocyclic tin atoms in solution and solid state are known.[20,27] The structure proposed for **1b** in solution is given in Fig. 2. Note the difference in coordination for the endocyclic tin atom, as compared with the solid state (Fig. 1)

The ^{117}Sn chemical shift as well as the $^{1}J(^{13}C–^{119/117}Sn)$ coupling constants of the triphenyltin compound **2** and of the tributyltin compound **3** solution (−107.8 and 113.9 ppm respectively) are typical for tetrahedral tin atoms.[17]

Table 3 ^{117}Sn NMR data of **1a, 1b, 2** and **3** in $CDCl_3$

Compound	δ (^{117}Sn) (ppm)
1a	−142.3
1b	−209.4, −214.7
2	−107.8
3	113.9

Antitumour activity

Compounds **1a**, **1b**, **2** and **3** in water/ethanol (99:1 v/v) solutions were screened *in vitro* against seven tumoural cell lines of human origin: MCF-7 and EVSA-T, two mammary tumours; WiDr, a colon carcinoma; IGROV, an ovarian cancer; M19 MEL, a melanoma; A498, a renal cancer; and H226, a non-small-cell lung cancer.[28,29] The screening results, expressed as inhibition doses ID_{50} (in ng ml^{-1}), are given in Table 4 together with those obtained under identical conditions for some clinically used reference antitumour drugs:[28,29] carboplatin, cisplatin, 5-fluorouracil, methotrexate and doxorubicin; and for organotin carboxylates of

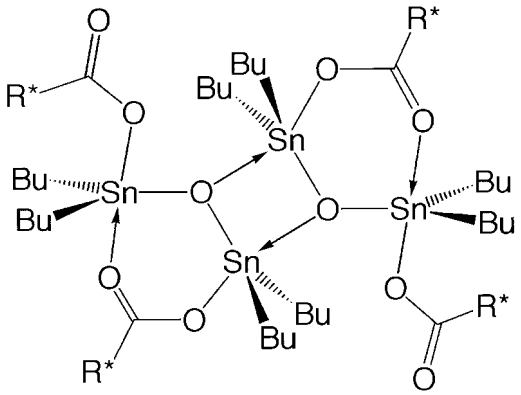

Figure 2 Solution structure of compound **1b**.

some bio-organic acids. Table 4 shows that the triorganotin compounds are more active than the di-n-butyltin ones. The triphenyltin compound **2** is comparable in activity with methotrexate and doxorubicin against all of the tumoural cell lines except H226. The tri-n-butyltin compound **3** is somewhat less active. Both **2** and **3** are more active than any reference compound against the H226 tumour. The activities of the di-n-butyltin compounds are comparable with or better than those of cisplatin and 5-fluorouracil. Compounds **1, 2** and **3** are comparable with, or less active than, the organotin derivatives (**4–13**) of some bio-organic acids.

EXPERIMENTAL

Syntheses

Compounds 1a and 1b

Dibutyltin oxide (0.83 g, 3.32 mmol for **1a** and 1.66 g, 6.64 mmol for **1b**) and an excess (6 ml) of propanol were refluxed in 150 ml of benzene for 3 h. The ternary azeotrope benzene/water/propanol was removed using a Dean–Stark apparatus. The reaction mixture was cooled to room temperature; a solution of *3S,4S*-3-[(*R*)-1-(tert-butyldimethylsilyl-oxy)ethyl]-4-[(*R*)-1-carboxyethyl]-2-azetidinone [2 g (6.64 mmol) in 30 ml of benzene/methanol (2:1 v/v)] was added dropwise and the mixture was stirred for one night at room temperature. The solvent was removed under reduced pressure. The compounds **1a** and **1b** were recrystallized from

Table 4 *In vitro* inhibition doses ID_{50} $(ng\ ml)^{-1}$ against seven tumoural cell lines of human origin obtained for compounds **1a** and **2**, some reference antitumour drugs (Refs 28, 29) and some representative organotin carboxylates

	Cell line						
Compound[a]	MCF-7	EVSA-T	WiDr	IGROV	M19 MEL	A498	H226
1a	146	125	657	234	127	292	363
1b	289	188	545	289	199	314	298
2	14	9	14	19	24	36	31
3	34	26	35	125	85	136	90
Cisplatin	699	422	967	169	558	2253	3269
5-Fluorouracil	750	475	225	297	442	143	340
Methotrexate	18	5	<3	7	23	37	2287
Doxorubicin	10	8	11	60	16	90	199
4	498	180	781	666	70	331	1302
5	27	25	134	18	61	61	104
6	262	244	401	245	247	327	306
7	18	<3	36	18	51	42	61
8	17	<3	17	19	42	42	39
9	<3	<3	<3	7	5	20	—
10	18	<3	15	17	32	53	53
11	3	<3	11	4	11	15	8
12	102	53	74	116	111	170	146
13	16	<3	15	<3	51	138	76

[a] Compounds **4–13**: **4**, tetra-n-butylbis(2,3:4,6-di-isopropylidene-2-keto-L-gulonato)distannoxane (Ref. 3); **5**, di-n-butyltin diterebate (Ref. 2); **6**, di-n-butyltin digibberellate (Ref. 4); **7**, di-n-butyltin bis(deoxycholate) (Ref. 1); **8**, triphenyltin terebate (Ref. 2); **9**, triphenyltin gibberellate (Ref. 4); **10**, triphenyltin deoxycholate (Ref. 1); **11**, tri-n-butyltin terebate (Ref. 2); **12**, tri-n-butyl gibberellate (Ref. 4); **13**, tri-n-butyl lithoxycholate (Ref. 1)

hexane (m.p. 119–121 °C; yield 78%) and benzene/hexane (m.p. 170–173 °C; yield 65%), respectively.

Compounds 2 and 3

Stoichiometric amounts of 3*S*,4*S*-3[(*R*)-1-(tert-butyldimethylsilyoxy)ethyl]-4-[(*R*)]-1-carboxyethyl]-2-azetidinone (1.00 g, 3.32 mmol) and tributyltin acetate (1.16 g, 3.32 mmol) or triphenyltin hydroxide (1.22 g, 3.32 mmol) were refluxed in 150 ml of toluene/ethanol (4:1 v/v) for 5–6 h. The ternary azeotrope toluene/water/ethanol was removed using a Dean–Stark apparatus. The solvent was removed under reduced pressure. The triphenyltin compound, **2**, was recrystallized from hexane, m.p. 57–59 °C; yield 75%. The tributyltin compound **3** was an oil which was purified by chromatography on Sephadex LH-20 (Pharmacia Biotech) with chloroform as eluent; yield 92%.

Characterization methods

The Mössbauer spectra were recorded as described elsewhere.[30]

The NMR spectra were recorded from $CDCl_3$ solutions on a Bruker AC250 spectrometer, using a QNP probe tuned at 250.13, 62.93 and 89.15 MHz for ^{1}H, ^{13}C and ^{117}Sn nuclei, respectively. ^{1}H and ^{13}C resonances were referenced to the solvent peak at 7.24 and 77.0 ppm respectively, while Ξ (^{117}Sn) = 35.632295 was used for the ^{117}Sn resonances as absolute external reference[31]. The 2D gradient-enhanced ^{1}H–^{13}C HMQC and HMBC spectra were recorded on a Bruker AMX500 instrument, as described elsewhere.[32]

Antitumour screening

The protocol followed for the antitumour screenings has already been reported.[28,29]

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