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Diorganotin 2-fluorocinnamates and 4-fluorophenylacetates: synthesis, characterization and *in vitro* antitumour activity

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The synthesis and characterization by spectroscopy of several new di-n-butyltin and diethyltin 2-fluorocinnamates and 4-fluorophenylacetates are described. *In vitro* tests on two human tumour cell lines, MCF-7, a mammary tumour, and WiDr, a colon carcinoma, showed that two of these compounds are more active than cisplatin. Other *in vitro* tests performed by the NCI, USA on a panel of human tumour cell lines show that one of them, bis[di-n-butyl(2-fluorophenylacetato)tin] oxide, is characterized by statistically significant $D_{GI_{50}}$, D_{TGI} and $D_{LC_{50}}$ sensitivities, but non-significant D_H and MGD_H selectivities, whereas the analogous 2-fluorocinnamate shows no such significant values.

Keywords: Organotin, antitumour, fluorocinnamate, fluorophenylacetate

INTRODUCTION

Diorganotin mono- or di-fluorobenzoates exhibit interesting *in vitro* antitumour properties against MCF-7, a mammary tumour, and WiDr, a colon carcinoma.[1,2] As an extension of these studies based on fluorine-containing compounds, we prepared, characterized and tested some diorganotin fluorocinnamates and fluorophenylacetates in order to examine the influence on their activities of introducing a non-aromatic moiety between the fluorophenyl group and the carboxylate function.

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RESULTS AND DISCUSSION

The present fluorocinnamates and fluorophenyl acetates were synthesized in the same way as the fluorobenzoates described earlier,[1,2] viz. by the condensation of the appropriate carboxylic acid R′COOH and diorganotin oxide R_2SnO. Such condensations in molar ratio 2:1 provide monomeric diorganotin dicarboxylates, $(R'COO)_2SnR_2$, compounds of type **A** (Eqn [1]):

$$2R'COOH + R_2SnO \rightarrow H_2O + \underset{\text{Type A}}{R_2Sn(OOCR')_2} \quad [1]$$

In compound **1A**, R′COO = 2-fluorocinnamate, *o*-$FC_6H_4CH{=}CHCOO$, and R = n-Bu.

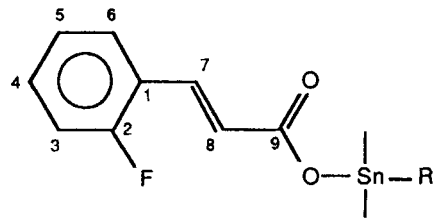
In molar ratio 1:1, dimeric distannoxanes, $\{[R_2SnOOCR']_2O\}_2$, compounds of type **B**, are obtained (Eqn [2]):

$$4R'COOH + 4R_2SnO \rightarrow 4H_2O + \underset{\text{Type B}}{\{[R_2SnOOCR']_2O\}_2} \quad [2]$$

In these compounds, the carboxylate and tin substituents were:

1B: R′COO = 2-fluorocinnamate, *o*-FC_6H_4CH = CHCOO, R = n-Bu
2B: R′COO = 2-fluorocinnamate, *o*-FC_6H_4CH = CHCOO, R = Et
3B: R′COO = 4-fluorophenylacetate, *p*-$FC_6H_4CH_2COO$, R = n-Bu
4B: R′COO = 4-fluorophenylacetate, *p*-$FC_6H_4CH_2COO$, R = Et

Table 1a ^{1}H NMR spectra[a] of organotin derivatives of 2-fluorocinnamic acid

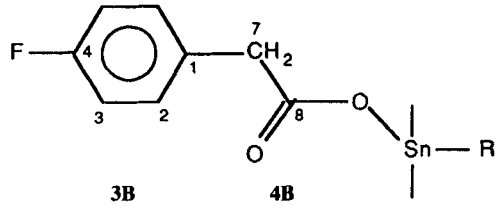


R = CH_2—CH_2—CH_2—CH_3 (10, 11, 12, 13)

R = CH_2—CH_3 (10, 11)

Proton	**1A** R = n-butyl	**1B** R = n-butyl	**2B** R = ethyl
3	7.10 (ddd, **11**,8,1)	7.10 (dd, **8**,8)	7.10 (dd, **8**,8)
4	7.37 (dddd, 8,8,**5**,2)	7.35 (bddd, 7,7,**6**)	7.35 (dddd, 8,8,**6**,1)
5	7.17 (ddd, 8,8,1)	7.17 (dd, 8,8)	7.17 (dd, 8,8)
6	7.55 (ddd, **8**,8,2)	7.60 (bdd, **7**,7)	7.59 (ddd, **8**,8, 1)
7	7.91 (d, 16)	7.74 (d, 16)	7.75 (d, 16)
8	6.63 (d, 16)	6.54 (d, 16)	6.55 (d, 16)
10			1.56 (q, 8, ^2J(H–Sn) = 82)
	1.70–1.93 (m)	1.54–1.80 (m)	and 1.63 (q, 8, ^2J(H–Sn) = 82)
11			1.40 (t, 8, $^3J(^1H-^{119/117}Sn)$ = 153/146) and
			1.41 (t, 8, $^3J(^1H-^{119/117}Sn)$ = 145/139)
12	1.42 (tq, 7,7)	1.41 (tq, 7,7) and 1.38 (tq, 7,7)	—
13	0.92 (t, 7)	0.92 (t, 7) and 0.88 (t, 7)	—

Table 1b ^{1}H NMR spectra of organotin derivatives of 4-fluorophenylacetic acid



R = CH_2—CH_2—CH_2—CH_3 (9, 10, 11, 12)

R = CH_2—CH_3 (9, 10)

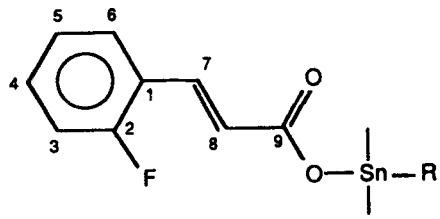
Proton	**3B** R = n-butyl	**4B** R = ethyl
2	7.19 (dd, 9,**6**)	7.20 (dd, 9,**6**)
3	6.97 (dd, **9**,9)	6.97 (dd, **9**,9)
7	3.44 (s)	3.47 (s)
9		1.250 (q, 8) and 1.253 (q, 8)
	1.45–1.48 (m)	
10		1.13–1.20 (m)
11	1.11–1.29 (m)	—
12	0.84 (t, 7)	

[a] Abbreviations: d = doublet; t = triplet; q = quartet; m = complex pattern; b = broad.

Spectroscopic characterization of compound 1A

The ^{1}H NMR data of compound **1A** are given in Table 1a. The expected resonances were assigned through their multiplicity and intensity patterns, as well as by the coupling constants characterizing their multiplets and/or the tin satellites. The ^{13}C NMR spectral data, displayed in Table 2a, combined with DEPT experiments, confirm the proposed structure (see Fig. 1). The aromatic carbon resonances were assigned by comparison of experimental chemical shifts with those calculated with increments from literature data.[3] The unde-

Table 2a ^{13}C NMR data of organotin derivatives of 2-fluorocinnamic acid[a]



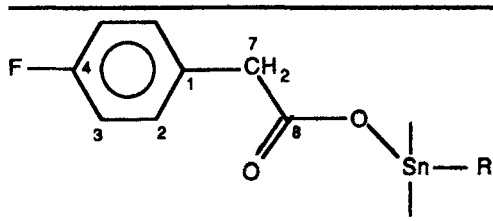
R = CH_2—CH_2—CH_2—CH_3 (10 11 12 13)

R = CH_2—CH_3 (10 11)

Carbon[b]	**1A** R = n-butyl	**1B** R = n-butyl	**2B** R = ethyl
1 (calcd 124.4)	122.4 (d, ^2J (^{13}C–^{19}F) = 12)	123.1 (d, $^2J(^{13}C–^{19}F) = 11$)	123.0 (d, $^2J(^{13}C–^{19}F) = 12$)
2 (calcd 161.0)	161.3 (d, $^1J(^{13}C–^{19}F) = 254$)	161.3 (d, $^1J(^{13}C–^{19}F) = 253$)	161.3 (d, $^1J(^{13}C–^{19}F) = 253$)
3 (calcd 115.4)	116.1 (d, $^3J(^{13}C–^{19}F) = 22$)	116.1 (d, $^3J(^{13}C–^{19}F) = 22$)	116.1 (d, $^3J(^{13}C–^{19}F) = 22$)
4 (calcd 129.3)	131.6 (d, $^2J(^{13}C–^{19}F) = 8$)	131.1 (d, $^2J(^{13}C–^{19}F) = 7$)	131.2 (d, $^2J(^{13}C–^{19}F) = 8$)
5 (calcd 124.0)	124.3	124.4	124.4
6 (calcd 127.8)	129.1	129.0	129.0
7	120.4 (d, $^3J(^{13}C–^{19}F) = 6$)	124.4 (d, $^3J(^{13}C–^{19}F) = 6$)	124.4 (d, $^3J(^{13}C–^{19}F) = 6$)
8	138.8	136.0	136.1
9	175.8	172.4	172.7
10	25.2, $^1J(^{13}C–^{119/117}Sn) = 584/558$	29.5, $^1J(^{13}C–^{119/117}Sn) = 731/695$ 27.2, $^1J(^{13}C–^{119/117}Sn) = 693/663$	19.9, $^1J(^{13}C–^{119/117}Sn) = 722/695$ 22.1 $^1J(^{13}C–^{119/117}Sn) = 752/721$
11	26.5, $^2J(^{13}C–Sn) = 34$	27.8, $^2J(^{13}C–Sn) = 36$ 27.5, $^2J(^{13}C–Sn) = 33$	9.8, $^2J(^{13}C–Sn) = 36$ 10.0, $^2J(^{13}C–Sn) = 42$
12	26.2, $^3J(^{13}C–Sn) = 97$	27.0, $^3J(^{13}C–Sn) = 130$ 26.9, $^3J(^{13}C–Sn) = 117$	—
13	13.4	13.7	—

[a] $^nJ(^{13}C–Sn)$ = unresolved $^{119}Sn/^{117}Sn$ satellites. [b] Calculated chemical shifts with increments from literature data[3]

Table 2b ^{13}C NMR data of diorganotin derivatives of 4-fluorophenylacetic acid[a]



R = CH_2—CH_2—CH_2—CH_3 (9 10 11 12)

R = CH_2—CH_3 (9 10)

Proton	**3B**	**4B**
1 (calcd 128.3)	131.5	131.4
2 (calcd 130.5)	130.6 (d, $^3J(^{13}C–^{19}F) = 8$)	130.8 (d, $^3J(^{13}C–^{19}F) = 8$)
3 (calcd 116.7)	115.1 (d, $^2J(^{13}C–^{19}F) = 21$)	115.2 (d, $^2J(^{13}C–^{19}F) = 21$)
4 (calcd 162.4)	161.7 (d, $^1J(^{13}C–^{19}F) = 245$)	161.8 (d, $^1J(^{13}C–^{19}F) = 245$)
7	42.8	42.8
8	177.2	177.6
9	28.6, $^1J(^{13}C–^{119/117}Sn) = 741/717$ 27.4, $^1J(^{13}C–^{119/117}Sn) = 703/669$	19.7, $^1J(^{13}C–^{119/117}Sn) = 723/692$ 21.4, $^1J(^{13}C–^{119/117}Sn) = 752/723$
10	27.4, $^2J(^{13}C–Sn) = 41$ 27.2, $^2J(^{13}C–Sn) = nv$[b]	9.5, $^2J(^{13}C–Sn) = 37$ 9.7, $^2J(^{13}C–Sn) = 45$
11	26.7, $^3J(^{13}C–Sn) = 130$ 26.6, $^3J(^{13}C–Sn) \triangleq 126$	—
12	13.4	—

[a] $^nJ(^{13}C–Sn)$ = unresolved $^{119}Sn/^{117}Sn$ satellites. [b] Abbreviation: nv, non-visible.

Table 3 ^{119}Sn and ^{19}F NMR, and Mössbauer parameters, of compounds **1A**, **1B**, **2B**, **3B** and **4B**

Compound	^{119}Sn NMR (ppm)	^{19}F NMR (ppm)	Mössbauer parameters			
			QS (mm s^{-1})	IS (mm s^{-1})	Γ_1 (mm s^{-1})	Γ_2 (mm s^{-1})
1A	−148.6	−114.3	3.51	1.44	0.96	0.99
1B	−216.2, −206.8	−115.2	3.39	1.33	0.94	0.96
$^2J(^{119}SnO^{117/119}Sn)$	[118]					
2B	−209.1, −216.3	−115.1	3.39	1.30	0.87	0.88
$^2J(^{119}SnO^{117/119}Sn)$	[113]					
3B	−207.9, −215.8	−116.4	3.19	1.30	1.09	1.05
$^2J(^{119}SnO^{117/119}Sn)$	[121]					
4B	−213.9, −209.5	−116.8	3.18	1.31	1.00	1.03
$^2J(^{119}SnO^{117/119}Sn)$	[126]					

Abbreviations: QS, quadrupole splitting; IS, Isomer shift; Γ_1, Γ_2, linewidths.

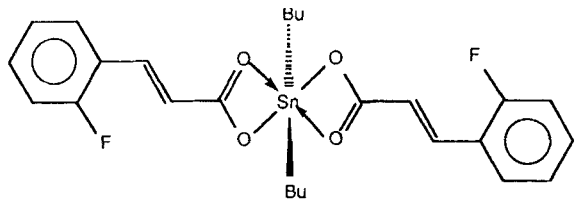


Figure 1 Structure proposed for compound **1A**.

coupled (ddd, 11, 7 and 5 Hz) ^{19}F NMR spectrum confirmed the multiplicities observed in the proton spectrum. The ^{119}Sn NMR spectrum displays a singlet at −148.6 ppm in agreement with the chemical shifts of analogous compounds.[4–8] The hexacoordination of such compounds is also supported by the Mössbauer parameters (see Table 3).

Spectroscopic characterization of compounds 1B, 2B, 3B and 4B

The ^{1}H NMR data of compounds **1B–4B** are described in Tables 1a and 1b. They exhibit the expected resonance multiplicities (coupling with ^{1}H, ^{19}F and/or $^{119/117}$Sn satellites) and intensities. Two triplets are visible for the methyl groups, which confirms the di-n-butyltin and diethyltin moieties to be non-equivalent, in agreement with the dimeric structure of type **B** distannoxane compounds[4–8] (see Fig. 2). The two quartets likewise observed for the methylene moieties confirm the ethyltin groups to be heterotopic.

The ^{13}C NMR data of compounds **1B–4B**, displayed in Tables 2a and 2b, are likewise compatible with the proposed structure. Each of the three methylene groups of the n-butyl substituent, the signals of which are easily assigned by the value of the tin–carbon coupling constant,

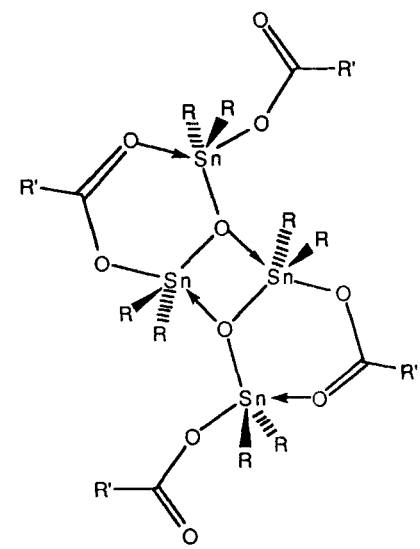


Figure 2 Structure proposed for compounds **1B–4B**.

Table 4 ID_{50} values (ng cm^{-3}) of compounds **1B** and **3B**, and of reference compounds, tested[9] against two human tumour cell lines, MCF-7 and WiDr

Compound	MCF-7		WiDr	
	In DMSO	In EtOH	In DMSO	In EtOH
1B	42	28	337	368
3B	42	13	323	268
Cisplatin[9]	850	—	624	—
Etoposide[9]	187	—	624	—
Doxorubicin[9]	63	—	31	—
Mitomycin C[9]	3	—	17	—

Table 5 NCI *in vitro* screening data review checklist for compounds **1B** and **3B**

Nr.	NSC no.	Response parameters			Selectivity analysis					$D_{GI_{50}}$	
		log GI_{50}	log TGI	log LC_{50}	ΔGI_{50} range	ΔTGI range	ΔLC_{50} range	Response parameter	Subpanel sensitivity	D_{TGI} $D_{LC_{50}}$	D_H MGD_H
1B	643 860	−6.11	−5.67	−5.15	1.30	1.36	1.25	GI_{50}		45 (−6)	70 (−4)
					1.91	2.18	2.40			25 (−6)	62
										30 (−5)	
3B	643 859	−6.93	−6.15	−5.68	1.07	1.59	1.67			51 (−7)	63 (−7)
					2.30	3.74	3.35	TGI	Kidney	57 (−6)	56
										51 (−6)	

also appear pairwise, as expected for dimeric distannoxanes. The ^{19}F NMR spectral data are summarized in Table 3. All ^{19}F resonances are broad so that the $J(^{19}F–^{1}H)$ coupling constants determined from the ^{1}H spectra could not be confirmed from the ^{19}F NMR data. The ^{119}Sn NMR spectra (Table 3) show two signals and tin satellites due to the tin–tin coupling between the two heterotopic tin atoms. The presence of the two heterotopic tin atoms is not evidenced by the Mössbauer spectra showing only one doublet with normal width. It is not unusual that Mössbauer spectra do not reveal such heterotopism.[4–8]

In vitro antitumour activity of compounds 1B and 3B against MCF-7, a human mammary tumour, and WiDr, a human colon carcinoma

The results of the *in vitro* tests against two human tumour cell lines, MCF-7, a mammary tumour, and WiDr, a colon carcinoma, performed[9] on compounds **1B** and **3B**, are given as ID_{50} values in Table 4. Data on some compounds currently used clinically as antitumour agents are given for comparison.

The tested compounds exhibit slightly higher activities against WiDr, than cisplatin or etoposide. Their activity is comparable with that (200–300 ng cm^{-3}) of the monofluorobenzoate analogue.[10] Against MCF-7, they are even more active than doxorubicin; when dissolved initially in ethanol instead of DMSO, they score even better.

In vitro antitumour activity of compounds 1B and 3B against 60 human tumour cell lines

The screenings were performed at the National Cancer Institute (NCI) using the standard protocols developed there according to its new investigational *in vitro*, disease-oriented, primary antitumour screen.

The data were treated by the NCI protocols described elsewhere.[11] In summary, $D_{GI_{50}}$, D_{TGI} and $D_{LC_{50}}$ are subpanel sensitivities calculated from the dose-response parameters GI_{50}, TGI and LC_{50}, which represent the interpolated concentrations at which the percentage growth (PG) is +50, 0 and −50, respectively. Computer simulations by the NCI suggest a value of $D_{GI_{50}}$, D_{TGI} and $D_{LC_{50}} \geq 50$ to represent a statistically significant antitumour differential sensitivity.[11] D_H and MGD_H are selectivity parameters evaluating cell-line subpanel selectivities of the antitumour activity of the compounds. NCI simulations suggest that values of D_H and $MGD_H \geq 75$ represent statistically significant subpanel selectivities.[11]

According to these criteria, Table 5 shows that compound **1B** is inactive. For compound **3B**, only the sensitivity parameters $D_{GI_{50}}$, D_{TGI} and $D_{LO_{50}} \sim 50$ are satisfactory, but D_H and MGD_H below 75 reveal no significant subpanel selectivity.

EXPERIMENTAL

Instruments

The Mössbauer spectra were recorded as described previously.[12]

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM 270 instrument at 270.13 and 67.93 MHz respectively. The ^{119}Sn NMR spectra were obtained on a Bruker WM 500 instrument at 186.5 MHz. The ^{19}F NMR spectra were recorded on a Bruker AC250 instrument at 235.36 MHz.

Syntheses

Compounds of type **A** were typically prepared as follows: 1.00 g (4.0 mmol) di-n-butyltin oxide or 0.86 g (4.0 mmol) diethyltin oxide were added to

8.0 mmol of the appropriate organic acid dissolved in 150 cm^3 of toluene and 50 cm^3 of ethanol. The mixture was refluxed for 6 h and the ternary azeotrope water/ethanol/toluene was distilled off with a Dean–Stark funnel. Half of the remaining solution was evaporated under vacuum. The oily compound obtained was crystallized from ethanol.

The synthesis of compounds of type **B** occurs similarly but only half the amount of the organic acid is used, i.e. 4.0 mmol.

1A, R'COO = *o*-FC_6H_4CH=CHCOO, R = n-Bu: yield 92%, recrystallized from ethanol; m.p. 95–96 °C.
1B, R'COO = *o*-FC_6H_4CH=CHCOO, R = n-Bu: yield 94%, recrystallized from chloroform + ethanol; m.p. 88–90 °C.
2B, R'COO = *o*-FC_6H_4CH=CHCOO, R = Et: yield 97%, recrystallized from ethanol; m.p. 203–205 °C.
3B, R'COO = *o*-$FC_6H_4CH_2COO$, R = n-Bu: yield 83%, recrystallized from chloroform + ethanol; m.p. 74–75 °C.
4B, R'COO = *o*-$FC_6H_4CH_2COO$, R = Et: yield 78%, recrystallized from chloroform + ethanol; m.p. 103–104 °C.

In vitro tests against MCF-7 and WiDr

Drug activity was determined using an automated *in vitro* technique as described previously.[9]

In vitro tests against the NCI panel

The cell panel consists of 60 lines against which the compounds are tested at five concentrations differing by 10-fold dilutions from 10^{-4} to 10^{-8} mol dm^{-3}. A 48-h continuous drug exposure protocol was used. A sulforhodamine B (SRB) protein assay allowed the estimation of cell viability or growth.[13] Protocols and activity parameters have been described elsewhere.[11,13]

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