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Exceptionally high *in vitro* antitumor activity of substituted triphenyltin benzoates including salicylates against a human mammary tumor, MCF-7, and a colon carcinoma, WiDr

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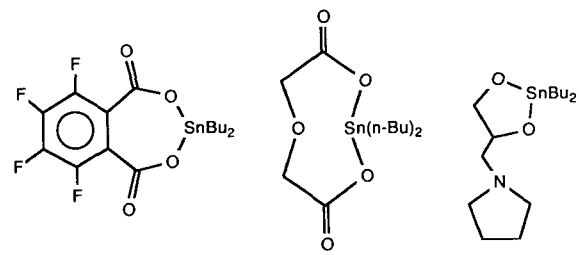
Nine new substituted triphenyltin benzoates of the type Ar_3Sn—OOC—C_6H_2XYZ [X=Y=H, Z= 2-OCH_3 and 4-F; X=H, Y=3-F, Z=5-F; X=H, Y=2-OH, Z=5-Cl, 5-NH_2, 5-OCH_3 and 5-SO_3H; X=2-OH, Y=3-$CH(CH_3)_2$ and Z=5-$CH(CH_3)_2$] were prepared and possess considerable *in vitro* antitumor activity against two human tumor cell lines (MCF-7, a mammary tumor, and WiDr, a colon carcinoma) comparable with that of mitomycin C.

Keywords: Triphenyltin, benzoates, antitumor, Mössbauer, NMR

INTRODUCTION

Narayanan reported that 48% out of the 129 *di*organotin compounds tested in 1982 by the National Cancer Institute (NCI) were found active against P388 leukemia *in vivo* in mice. In contrast, only 9% out of the 132 *tri*organotin compounds screened were found active as antitumor compounds.[1] As a consequence, many *di*organotin compounds were prepared of which a high percentage was found to be active *in vitro* against human tumor cell lines (see, for example, Refs 2–7).

The most active ones *in vitro* against two of these, MCF-7, a mammary tumor, and WiDr, a colon carcinoma, are bis[(substituted salicylato)-di-n-butyltin] oxides, $\{[XC_6H_3(2\text{-}OH)COO]Bu_2Sn\}_2O$, with X=5-Cl or 5-MeO, which provided ID_{50} values against MCF-7 and WiDr of respectively 31 and 256, and 29 and 122 ng cm^{-3}. The di-t-butyl analog of the 5-methoxysalicylato compound was characterized by ID_{50} values of 38 and 163 ng cm^{-3}.[2–7] These activities are significantly higher than those of cisplatin, an antitumor drug used clinically, characterized by ID_{50} values of 850 and 624 ng cm^{-3}. Di-n-butyltin dipicolinate $[(C_5NH_4COO)_2SnBu_2]$ and di-n-butyltin bis(4-hydroxy-3-methoxybenzoate) are comparably active *in vitro* against the same tumor cell lines, giving ID_{50} values of 43 and 92, and 44 and 82 ng cm^{-3}, respectively.[2–7] The compounds of Fig. 1 provided even more promising *in vitro* activities.[2–7]



ID_{50} values (ng cm^{-3})

MCF-7: 51	MCF-7: 28	MCF-7: 79
WiDr: 68	WiDr: 72	WiDr: 84

Figure 1 *In vitro* antitumor activities of three di-n-butyltin compounds against MCF-7 and WiDr cell lines.[2–7]

Triphenyltin hydroxide (Ph_3SnOH; Duter®)[8] and triphenyltin acetate ($Ph_3SnOCOMe$; Brestan®)[9] are pesticides used commercially in agriculture (see Ref. 10, for example), but are not carcinogenic.[8,9] Triphenyltin acetate even exhibits

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antitumor properties *in vivo* at low doses since it reduces the growth rate of tumors in mice when administered orally or intraperitoneally.[11] Since murine leukemias *in vivo* are no longer considered by the NCI as optimal models for antitumor pre-screening,[12] we prepared some triphenyltin derivatives in order to determine their antitumor activity *in vitro* against the human tumor cell lines MCF-7 and WiDr used recently as pre-screening tests.[2-7,12]

MATERIALS AND METHODS

Syntheses

Eight new substituted triphenyltin benzoates of the type $Ar_3Sn—OOC—C_6H_2XYZ$ [X = Y = H, Z = 2-OCH_3 and 4-F, compounds **1** and **2**; X = H, Y = 3-F, Z = 5-F, compound **3**; X = H, Y = 2-OH, Z = 5-Cl, 5-NH_2, 5-OCH_3 and 5-SO_3H, compounds **4–7**; X = 2-OH, Y = 3-$CH(CH_3)_2$ and Z = 5-$CH(CH_3)_2$, compound **8**] were typically prepared as follows. To a solution of 5.4 mmol $XYZC_6H_2COOH$ in a mixture of 150 cm^3 toluene and 50 cm^3 ethanol, 2 g (5.4 mmol) triphenyltin hydroxide was added. This mixture was refluxed for 6 h. The ternary azeotrope water/toluene/ethanol, and then half of the remaining solvent, were distilled off with a Dean–Stark funnel. The solution obtained was evaporated under vacuum. The oily compound obtained was crystallized in a suitable solvent (see below).

Instruments

The Mössbauer spectra were recorded as described previously for other compounds.[8]

The ^1H 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.

In vitro tests

Compounds **1–8** were tested *in vitro* against two human tumor cell lines: MCF-7 mammary carcinoma (ITRI-TNO) and WiDr colon carcinoma. The test experiments were performed as described previously.[13]

RESULTS

The results of the *in vitro* tests performed against MCF-7 and WiDr on a series of triphenyltin benzoates, $(C_6H_5)Sn—OOC—C_6H_2XYZ$, compounds **1–8**, including salicylates (Y = 2-OH), compounds **4–8**, are given in Table 1 and compared with those of some reference compounds, **9–12**.[13]

DISCUSSION

From Table 1, it is clear that all triphenyltin benzoates, except compound **7**, score comparably with mitomycin C against WiDr, and much better

Table 1 Inhibition doses ID_{50} (ng cm^{-3}) obtained for a series of triphenyltin benzoates, $(C_6H_5)_3Sn—OOC—C_6H_2XYZ$, **1–8**, and for four reference compounds, **9–12**, against two human tumor cell lines, MCF-7 and WiDr

Compound	X	Y	Z	MCF-7	WiDr
1	H	H	2-OCH_3	16	15
2	H	H	4-F	15	14
3	H	3-F	5-F	18	17
4	H	2-OH	5-Cl	11	18
5	H	2-OH	5-NH_2	14	17
6	H	2-OH	5-OCH_3	6	15
7	H	2-OH	5-SO_3H	100	131
8	2-OH	3-$CH(CH_3)_2$	5-$CH(CH_3)_2$	8	13
9 Cisplatin[13]	—	—	—	850	624
10 Etoposide[13]	—	—	—	187	624
11 Doxorubicin[13]	—	—	—	63	31
12 Mitomycin C[13]	—	—	—	3	17

than the other three reference compounds against MCF-7. Compound **6** even scores comparably with mitomycin C againt MCF-7. The improved activities of these triphenyltin compounds with respect to di-n-butyltin analogs is striking since the latter provided ID_{50} values against the same tumor cell lines never lying below 50 and 100 ng cm^{-3}, as mentioned before.

These triphenyltin compounds are now being tested *in vivo* in order to determine whether the very high activities found *in vitro* remain comparable on *in vivo* models. These studies could provide some information about correlations between *in vitro* and *in vivo* activities.

EXPERIMENTAL

Characterizations of compounds 1–8

Triphenyltin *o*-methoxybenzoate,[14–16] compound 1
Recrystallized from ethanol; m.p. 102–103 °C; yield 68%.

R_f on Polygram® SIL G/UV_{254} TLC plates from Macherey–Nagel & Co.: 0.80 (elution with cyclohexane/dioxane, 1:1.

Mössbauer parameters: quadrupole splitting (QS) 2.33; isomer shift (IS) 1.22; band widths Γ_1 and Γ_2 0.92 and 0.90 mm s^{-1}.

^{1}H NMR ($CDCl_3$) [proton number, multiplicity, chemical shift in ppm (coupling constants in Hz)]: H-3, d, 6.94 (8); H-4, dd, 6.95 (7, 7); H-5, hidden under the signals of *m*- and *p*-protons; H-6, dd, 7.98 (8, 2); *o*-H, m, 7.78–7.82; *m*- and *p*-H, m, 7.39–7.46; CH_3O, s, 3.88.

^{13}C NMR ($CDCl_3$): ipso-C, 138.7 [$^1J(^{119/117}Sn-^{13}C) = 648/620$]; *o*-C, 137.0, [$^2J(Sn-C) = 48$]; *m*-C, 128.8 [$^3J(Sn-C) = 63$]; *p*-C, 130.0; C-1, 120.3 (*calcd 115.8*); C-2, 159.6 (*161.8*); C-3, 112.2 (*113.8*); C-4, 133.4 (*134.0*); C-5, 120.0 (*120.5*); C-6, 133.0 (*131.4*); CO, 172.4; CH_3O, 13.9.

^{119}Sn NMR ($CDCl_3$): −115.1.

Triphenyltin *p*-fluorobenzoate,[17–19] compound 2
Recrystallized from ethanol; m.p. 86–87 °C; yield 24%.

R_f on Polygram® SIL G/UV_{254} TLC plates: 0.51 (elution with petroleum ether/acetic acid, 6:1.

Mössbauer parameters QS 2.54; IS 1.27; Γ_1 and Γ_2 0.85 and 0.86 mm s^{-1}.

^{1}H NMR ($CDCl_3$): H-2 and H-6, dd, 8.14 (9, 6); H-3 and H-5, dd, 7.05 (9, 9); *o*-H, m, 7.76–7.81; *m*- and *p*-H, m, 7.45–7.51.

^{13}C NMR ($CDCl_3$): ipso-C, 138.3 [$^1J(^{119/117}Sn-^{13}C) = 649/620$]; *o*-C, 136.8 [$^2J(Sn-C) = 48$]; *m*-C: 128.8 [$^3J(Sn-C) = 63$]; *p*-C, 130.1; C-1, 127.0; C-2 and C-6, d, 133.1 [$^3J(^{19}F-^{13}C) = 9$]; C-3 and C-5, d, 115.1 [$^2J(^{19}F-^{13}C) = 22$]; C-4, d, 165.6 [$^1J(^{19}F-^{13}C) = 253$]; CO, 171.6.

^{119}Sn NMR ($CDCl_3$): −110.2.

Triphenyltin 3,5-difluorobenzoate, compound 3
Recrystallized from ethanol; m.p. 121–122 °C; yield 30%.

R_f on Polygram® SIL G/UV_{254} TLC plates: 0.53 (elution with cyclohexane/dioxane, 1:1).

Mössbauer parameter (mm s^{-1}) QS 2.61: IS 1.26; Γ_1 and Γ_2 0.90 and 0.89.

^{1}H NMR ($CDCl_3$): H-2 and H-6, dd, 7.62 (8, 2); H-4, tt, 6.94 (9, 2); *o*-H, m, 7.76–7.82; *m*- and *p*-H, m, 7.45–7.51.

^{13}C NMR ($CDCl_3$): ipso-C, 137.8 [$^1J(^{119/117}Sn-^{13}C) = 647/617$]; *o*-C, 136.8 [$^2J(Sn-C) = 48$]; *m*-C, 129.0 [$^3J(Sn-C) = 64$]; *p*-C, 130.3; C-1, 134.5 [$^3J(^{19}F-^{13}C) = 9$]; C-2 and C-6, d, 113.4 [$^2J(^{19}F-^{13}C) = 26$]; C-3 and C-5, dd, 115.1 [$^1J(^{19}F-^{13}C) = 249$; $^3J(^{19}F-^{13}C) = 12$]; C-4, d, 107.7 [$^2J(^{19}F-^{13}C) = 25$]; CO, 170.0.

^{119}Sn NMR ($CDCl_3$): −100.3.

Triphenyltin 5-chlorosalicylate, compound 4
Recrystallized from CH_2Cl_2/petroleum ether; m.p. 122–123 °C; yield 75%.

R_f on Polygram® SIL G/UV_{254} TLC plates: 0.76 (elution with cyclohexane/dioxane, 1:1).

Mössbauer parameters (mm s^{-1}) QS 2.79; IS 1.32; Γ_1 and Γ_2 0.90 and 0.89.

^{1}H NMR ($CDCl_3$): H-3, d, 6.88 (9); H-6, d, 7.94 (2); *o*-H, m, 7.65–7.90 [$^3J(Sn-H) = 64$]; H-4, *m*- and *p*-H, m, 7.45–7.53; OH, bs, 11.06.

Triphenyltin 5-aminosalicylate, compound 5
Recrystallized from CH_2Cl_2/petroleum ether; m.p. 145–146 °C; yield 78%.

R_f on Polygram® SIL G/UV_{254} TLC plates: 0.73 (elution with cyclohexane/dioxane, 1:1).

Mössbauer parameters (mm s^{-1}): QS 3.10; IS 1.30; Γ_1 and Γ_2 0.90 and 0.89.

^{1}H NMR ($CDCl_3$): H-3 and H-4, AB part of an ABX system with $\nu_A = 6.75$, $\nu_B = 6.79$, $J_{AB} = 9$, $J_{AX} = 0$ and $J_{BX} = 2$; H-6, d, 7.292 (2); *o*-H, m, 7.64–7.90 [$^3J(Sn-H) = 54$]; *m*- and *p*-H, m, 7.40–7.50; NH_2, m, 2.71–3.42.

^{13}C NMR ($CDCl_3$): ipso-C, 137.7 [$^1J(Sn-C) = 630$]; *o*-C, 136.7 [$^2J(Sn-C) = 47$]; *m*-C, 128.9 [$^3J(Sn-C) = 64$]; *p*-C, 130.3; C-1,

113.1; C-2, 154.8; C-3, 116.5; C-4, 124.0; C-5, 137.9; C-6, 117.6; CO, 174.8.

^{119}Sn NMR ($CDCl_3$): −116.1.

Triphenyltin 5-methoxysalicylate, compound 6

Recrystallized from CH_2Cl_2/petroleum ether; m.p. 137–138 °C; yield 72%.

R_f on Polygram® SIL G/UV_{254} TLC plates: 0.37 (elution with cyclohexane/ethanol 4:1).

Mössbauer parameters (mm s^{-1}): QS 2.75; IS 1.28; Γ_1 and Γ_2: 0.91 and 0.90.

^{1}H NMR ($CDCl_3$): CH_3O, s: 3.74; H-3, d, 6.864 (9); H-4, dd, 7.024 (9, 3); *o*-H, m, 7.65–7.91 [^3J(Sn–H) = 63]; H-6, *m*- and *p*-H, m: 7.42–7.53; OH, bs, 10.6.

^{13}C NMR ($CDCl_3$): CH_3O, 55.8; ipso-C, 137.9 [^1J(Sn–C) = 632]; *o*-C, 136.7 [^2J(Sn–C) = 47]; *m*-C, 128.9 [^3J(Sn–C) = 62]; *p*-C, 130.3; C-1, 114.0; C-2, 156.0; C-3, 118.0; C-4, 123.5; C-5, 151.8; C-6, 113.1; CO, 174.7.

^{119}Sn NMR ($CDCl_3$): −97.6.

Triphenyltin 5-hydroxysulfonylsalicylate, compound 7

Recrystallized from ethanol; m.p. > 350 °C; yield 76%.

R_f on Polygram® SIL G/UV_{254} TLC plates: 0.20 (elution with cyclohexane/ethanol, 4:1); Mössbauer parameters (mm s^{-1}): QS 4.36; IS 1.32; Γ_1 and Γ_2 0.87 and 0.93.

^{1}H NMR (DMSO-d_6): H-3, d, 6.818 (8); H-6, d, 8.105 (2); *o*-H, d, 7.771(7) [^3J(Sn–H) = 106]; H-4, *m*- and *p*-H, m, 7.34–7.46; OH, bs, 11.6; SO_3H, 3.39–3.47.

^{13}C NMR (DMSO-d_6): ipso-C, 144.6; *o*-C, 139.5; *m*-C, 133.6; *p*-C, 134.3; C-1, 118.3; C-2, 165.8; C-3, 121.2; C-4, 137.5; C-5, 152.9; C-6, 132.7; CO, 179.3.

^{119}Sn NMR (DMSO-d_6): −276.7.

Triphenyltin 3,5-di-isopropylsalicylate, compound 8

Recrystallized from ethanol; m.p. 150–151 °C; yield 90%.

R_f on Polygram® SIL G/UV_{254} TLC plates: 0.62 (elution with cyclohexane/ethanol, 4:1.

Mössbauer parameters (mm s^{-1}): QS, 2.56; IS 1.28; Γ_1 and Γ_2 0.86 and 0.92.

^{1}H NMR ($CDCl_3$): 3-i-Pr: CH, sept, 3.344 (7); CH_3, d, 1.231 (7); 5-i-Pr: CH, sept., 2.843 (7); CH_3, d, 1.220 (7); H-4, d, 7.236 (2); H-6, d, 7.715 (2); *o*-H, m, 7.67–7.91 [^3J(Sn–H) = 59]; *m*- and *p*-H, m, 7.40–7.49; OH, bs, 11.3.

^{13}C NMR ($CDCl_3$): 3-i-Pr: CH_3, 24.1; CH, 33.4; 5-i-Pr: CH_3, 22.4; CH, 26.9; ipso-C, 137.9 [$^1J(^{119/117}Sn–^{13}C)$ = 648/618]; *o*-C, 138.0 [^2J(Sn–C) = 48]; *m*-C: 128.9 [^3J(Sn–C) = 64]; *p*-C: 130.3 [^4J(Sn–C) = 13]; C-1, 112.1; C-2, 157.4; C-3, 136.2; C-4, 130.6; C-5, 138.6; C-6, 125.9; CO, 175.7.

^{119}Sn NMR ($CDCl_3$): −101.4.

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