

Synthesis and Initial Cell Line Results of Organotin Polyethers Containing Diethylstilbestrol

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Abstract Organotin polyethers containing the synthetic estrogen diethylstilbestrol have been made in moderate yield with chain lengths ranging from 20 to 200 repeat units employing the interfacial polycondensation procedure. Infrared spectroscopy shows the absence of the O–H group and formation of the Sn–O linkage consistent with the formation of the polyether. F-MALDI MS results are consistent with the presence of organotin-containing fragmentation clusters containing one and two tin atoms and ion fragments up to about 30 units long. The polymers all show moderate to good inhibition of a group of cancer cell lines including colon, breast and prostate cell lines. Many of them show significant CI_{50} values showing a preference to inhibit the cancer cell lines in preference to the normal cell lines.

Keywords Diethylstilbestrol · DES · Organotin polymers · Organotin polyethers · Cancer · Colon cancer · Breast cancer · Prostate cancer ·

This article is dedicated to Professor Astruc.

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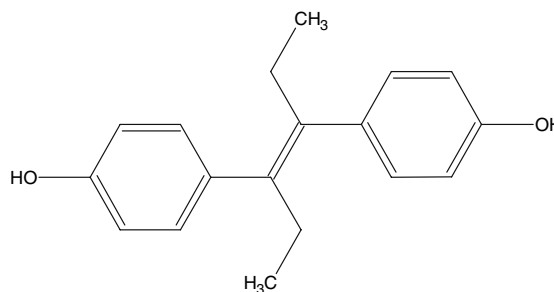
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1 Introduction

Diethylstilbestrol (4,4'[(1E)-1,2-ethenediyl]bisphenol; (E)-3,4-bis(4-hydroxyphenyl)-3-hexene), DES (Scheme 1), is a synthetic estrogen that mimics estrogen, one of the primary ovarian hormones. It is known by a number of common names including stilbesterol, stilboestrol and sold under a number of names including Apstil, cyren A, distilbene, and stilbetin.

DES was first used in 1938 for women in an effort to prevent miscarriage or premature deliveries. In 1953, a double-blind study showed that DES did little to improve premature deliveries or miscarriage. Even so, it was still widely marketed until the early 1970s for this use. By 1971 it was estimated that 5 to 10 million people were exposed to DES. In 1971 the Food and Drug Administration issued a Drug Bulletin advising physicians to halt prescribing DES. DES was linked to a rare vaginal cancer in female offspring. Further research has shown that DES is a teratogen that can cause malformation of an embryo or fetus.



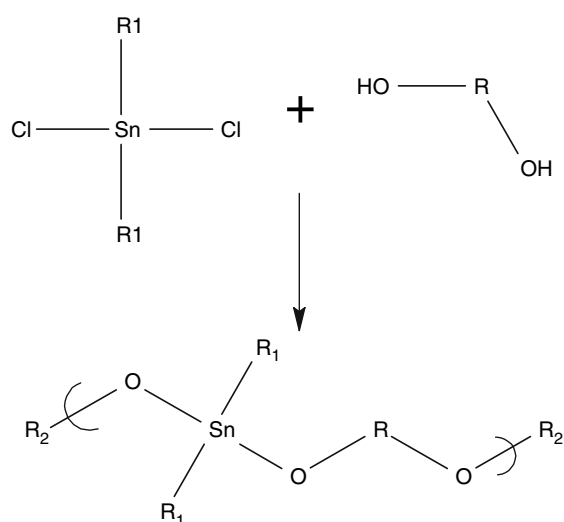
Scheme 1 DES

DES is currently used with animals. Its primary use is to treat urinary incontinence in spayed female cats and dogs. It has also been used to prevent unwanted pregnancy in dogs and cats. DES has been used to treat breast and prostate cancer but its use is limited because of relatively poor water solubility and a wide range of dose-related toxicities that includes nausea and vomiting, venous and arterial thrombosis, and fluid retention [1]. DES is effective against estrogen receptor positive (ER+) tumors [2, 3]. The use of estrogens as potent antiandrogens in hormonal therapy of metastatic prostate cancer has also been described [4]. Thus, there exist several studies that indicate the potential usefulness of DES as a positive drug in the treatment of specific cancers.

DES is reported to be susceptible to isomerization rendering it inactive [5]. We have found that incorporation of chemically sensitive molecules into polymer structures allows the moieties greater stability [6, 7]. Recently, several studies have looked at the incorporation of DES into polymers. Vicent and Duncan and co-workers reported the incorporation of DES into polymer structures for pH-triggered activation [8]. These ter-polymers were based on the use of PEG as the solubilizing moiety, an approach we also employed [9–12]. The products exhibited better cytotoxicity than DES alone against human and murine tumor cell lines. We have also synthesized a number of polyphosphate and polyphosphonate polymers containing DES [13].

We have been focusing attention on the synthesis and biological testing of metal-containing polymers as part of a battle against cancer and viruses [14–32]. Many of the compounds synthesized and tested contained known antibacterial and antiviral drugs such as norfloxacin, ampicillin, ciprofloxacin, and acyclovir. In general, the ability to inhibit cell growth, with respect to the alkyltins, is $\text{Bu} > \text{Pr} > \text{Et} > \text{Me} > \text{Oc} > \text{La}$, with the methyl, octyl, and lauryl essentially inactive. Our overall rationale for employing drugs coupled with organotin moieties with known biological effects is to create a compound that can interact with the target (cancer, bacteria, virus) at several venues, increasing the potential effectiveness of the combination drug against the target. Recently, we found that several simple non-drug containing polymers showed good inhibition of Balb 3T3 and human ovarian adenocarcinoma cells (Caov-3). One of these compounds was the polymer made from dibutyltin dichloride and 2-chloro-1,4-benzenediamine that showed a GI_{50} value of 0.04 $\mu\text{g}/\text{mL}$ against Balb 3T3 cells [33].

Some time ago we synthesized a number of organotin polyethers (Scheme 2) derived from reaction with various aliphatic and aromatic diols [34–37]. We found that a series of organotin polyethers derived from simple aliphatic diols showed good inhibition of Balb 3T3 cells [38, 39]. For instance, the product from dibutyltin dichloride and 1,6-hexanediol showed a GI_{50} of 5 $\mu\text{g}/\text{mL}$. The product from 1,4-butanediol and dibutyltin dichloride showed a

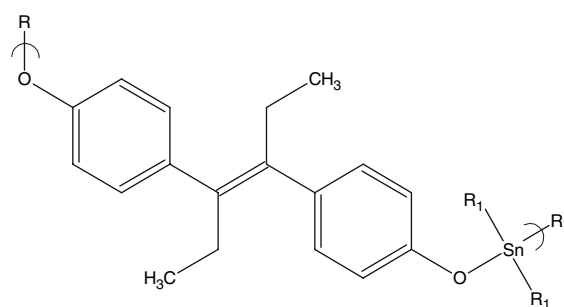


Scheme 2 General synthesis of organotin polyethers

GI_{50} of 0.25 $\mu\text{g}/\text{mL}$. And finally, the product of dibutyltin dichloride and 1,4-butanediol showed a GI_{50} value of 0.025 $\mu\text{g}/\text{mL}$, the lowest GI_{50} thus far found for organotin products. For comparison, the GI_{50} for cisplatin, the most widely used anticancer chemo drug, is 0.4 $\mu\text{g}/\text{mL}$.

This work suggested two structural windows that merited further investigation. These structural windows were: first, the activity increases as the distance between the oxygen atoms in the diols decreases. Second, the presence of a pi bond on the diol may contribute to the ability of the organotin polyether to inhibit cell growth. The present paper describes a glimpse into this second structural window.

We recently synthesized a series of dibutyltin polyethers that can be considered a series of ethylene glycols [9]. These polyethers inhibited a wide range of cancer tumors including breast, prostate, lung and colon tumors [40, 41]. As part of evaluating the structural window that the presence of unsaturation assists in the anticancer activity of organotin polyethers, we describe the synthesis and preliminary anticancer evaluation of organotin polyethers derived from DES. This is also part of our effort to include moieties within the organotin polymers that are known to exhibit biological activity. The products are believed to have a repeat unit structure shown in Scheme 3.



Scheme 3 Organotin Polyethers from DES

2 Experimental

2.1 Physical and Synthesis

Organotin dihalides were used as received. Dibutyltin dichloride (CAS # 683-18-1) was obtained from Alfin; dimethyltin dichloride (CAS # 753-73-1) was obtained from Aldrich; diethyltin dichloride (CAS # 866-55-7) from Peninsular; diphenyltin dichloride (CAS # 1135-99-5) from Aldrich, and dicyclohexyltin dibromide (CAS # 2954-94-1) from Ventron. Diethylstilbestrol (CAS # 56-53-1), DES, was obtained from Chemalog, South Plainfield, NJ.

Polymerization was accomplished employing the classical or aqueous interfacial polycondensation system. Briefly, an aqueous solution (30 mL) containing the diethylstilbestrol (0.00300 mol) and sodium hydroxide (0.0060 mole) was transferred to a one quart Kimax emulsifying jar fitted on top of a Waring Blender (model 1120; no load speed of about 18,000 rpm; reactions were carried out at about 25 °C). Stirring was begun and a heptane solution (30 ml) containing the organotin dihalide (0.00300 mol) was rapidly added (about 3–4 s) through a hole in the jar lid using a powder funnel. The resulting solution was blended for 15 s. The white precipitate was recovered using vacuum filtration and washed several times with deionized water and heptane to remove unreacted materials and unwanted by-products. The solid was washed onto a glass petri dish and allowed to dry at room temperature.

Solubilities were determined by placing 1–10 mg of polymer in 3 ml of liquid. The solid–liquid combinations were observed over a period of 2 to 4 weeks.

Light scattering was carried out employing a Brice-Phoenix BP 3000 Universal Light Scattering Photometer. Refractive indices were obtained using a Bauch & Lomb Abbe Model 3-L refractometer. Infrared spectra were obtained employing KBr pellets using a Mattson Instruments Galaxy Series 4020 FTIR using 32 scans and an instrumental resolution of 4 1/cm.

High resolution electron impact positive ion matrix assisted laser desorption ionization time of flight, HR MALDI-TOF; mass spectrometry was carried out employing a Voyager-DE STR BioSpectrometer, Applied Biosystems, Foster City, CA. The standard settings were used with a linear mode of operation and an accelerating voltage of 25,000 V; grid voltage 90% and an acquisition mass range of 2,000–100,000. Two hundred shots were taken for each spectrum. The matrix liquid was 2,5-dihydroxybenzoic acid.

2.2 Biological Characterization

Each of the cell lines were obtained from NCI and maintained in MEM-Eagles supplemented with 10% fetal bovine serum at 37 °C in a 5 % carbon dioxide atmosphere.

For testing of the compounds, cells were harvested, counted, and plated into 96-well plates at 1×10^4 cells per well in MEM-Eagles supplemented with 10% fetal bovine serum, and incubated for 24 h at 37 °C in a 5 % carbon dioxide atmosphere. A stock solution of the compound was prepared in DMSO at a known concentration. On day two 100 μ L MEM-Eagles supplemented with 10% fetal bovine serum and the indicated drug concentrations were added. Seventy-two h later the cells were assayed for proliferation using the CellTiter 96[®] Aqueous One Solution Cell Proliferation Assay by Promega Corporation. The assay is a colorimetric method for determining the number of viable cells in proliferation, cytotoxicity or chemosensitivity assays. The assay solution contained a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS^(a)] and an electron coupling reagent (phenazine ethosulfate; PES). Assays were performed by adding a small amount of the CellTiter 96 Aqueous One Solution Reagent directly to culture wells, incubating for 1–4 h and then recording absorbance at 490 nm with a 96-well plate reader. The quantity of formazan product as measured by the amount of 490 nm absorbance is directly proportional to the number of living cells in culture.

3 Results and Discussion

3.1 Synthesis and Structural Characterization

3.1.1 Product Yield and Chain Length

The reaction is general, occurring for all of the organotin dihalides. Percentage yields are generally in the moderate range (Table 1).

Table 1 Product yield, molecular weight and chain length

Organotin dichloride	Product yield (%)	dn/dc	Molecular weight	DP
Me ₂ SnCl ₂	75	– 1.1	7.0×10^4	160
Et ₂ SnCl ₂	70	– 0.9	2.1×10^4	48
Pr ₂ SnCl ₂	75	– 1.5	1.2×10^4	26
Bu ₂ SnCl ₂	67	– 1.2	1.6×10^4	32
Cy ₂ SnBr ₂	62*	– 1.5	1.2×10^4	22
Ph ₂ SnCl ₂	39*	1.1	1.1×10^5	200

Reaction conditions: Organic phase containing 30 mL heptane and 0.00300 mole of organotin dihalide added to rapidly stirred (18,000 rpm, no load) aqueous solutions containing 0.00300 moles of diethylstilbestrol and 0.0060 moles of sodium hydroxide with stirring for 15 s

* 30 mL chloroform

The products were generally soluble in HMPA and 1-methyl-2-pyrrolidinone but less soluble or insoluble in DMSO, acetone, and DMF. Since the polymers exhibited the best solubility in HMPA, it was employed in determining the weight average molecular weight via light scattering photometry (Table 1). The products were low to intermediate length polymers with average degrees of polymerization in the range of about 20 to 200. Chain length decreased as the size of the alkyl group increased consistent with steric requirements being important. Chain length was studied for a period of over two months (Table 2). The products were generally stable for about 45 days after which there is some decrease in chain length. This is consistent with other organotin polymers where chain length remained essentially the same for about a month.

The lack of solubility in a commercially available deuterated solvent prevents solution proton NMR from being run.

3.1.2 Vibrational Spectroscopy

Infrared spectral results are consistent with the proposed repeat unit and are based on literature values for the

Table 4 Selected infrared vibration assignments for dibutyltin dichloride and diethyltin dichloride and the polymers derived from their reaction with diethylstilbestrol

Assignment	Bu ₂ SnCl ₂	Bu ₂ Sn polymer	Et ₂ SnCl ₂	Et ₂ Sn polymer
CH ₃ Asym. St.	2,959	2,961	2,978	2,967
CH ₂ Asym. St.	2,927	2,930	2,962	2,930
CH ₃ & CH ₂ Asy. St.	2,871/2,858	2,870/2860	2,929/2,869	2,871
CH ₃ Asy. Bending	1,463	1,465	1,448	1,438
CH ₃ Sym. Bending	1,380	1,387	1,380	1,372
C–C St.	1,178	1,172	1,228	1,248
CH ₃ rocking	878	853	870	860

organotin [14, 16, 30, 32, 41, 42] and for DES [43, 44]. Only selected bands will be described here. Table 3 focuses on bands derived from DES and bands associated with the formation of the Sn–O linkage and presence of the organotin moiety. Table 4 focuses on bands directly derived from the organotin moiety, that are not cited in Table 3. Following, results from the dibutyltin-DES polymer are described. DES itself shows an intense band with a

Table 2 Chain length, degree of polymerization, as a function of time in solution

Time (days) organotin	0	7	17	45	67
Me ₂ Sn	7×10^4	7×10^4		7×10^4	4.5×10^4
Et ₂ Sn	2.1×10^4	2.0×10^4		1.1×10^4	1.0×10^4
Pr ₂ Sn	1.2×10^4	1.2×10^4	1.1×10^4	7.1×10^3	6.7×10^3
Bu ₂ Sn	1.6×10^4	1.6×10^4	1.4×10^4	1.6×10^4	1.6×10^4
Cy ₂ Sn	1.2×10^4	1.2×10^4		1.0×10^4	7.4×10^3
Ph ₂ Sn	1.1×10^5	1.1×10^5	4.8×10^4	2.4×10^4	1.0×10^4

Table 3 Selected infrared vibration assignments for diethylstilbestrol and organotin polymers derived from reaction with diethylstilbestrol

Sample	DES polymer	Me ₂ Sn polymer	Et ₂ Sn polymer	Pr ₂ Sn polymer	Bu ₂ Sn polymer	Cy ₂ Sn polymer	Ph ₂ Sn
OH St	3,427						
CH St. Ar.	3,020	3,021	3,022	3,021	3,020	3,036	3,064, 3,030
CH St. Ali.	2,973, 2,945	2,969, 2,930	2,967, 2,930	2,958, 2,930	2,961, 2,930	2,960, 2,923	2,974
	2,874	2,870	2,871	2,869	2,870, 2,860	2,848	2,920, 2,872
C=C St. Alkene	1,651	1,650					
C=C St. Ar.	1,607	1,601	1,601	1,601	1,611	1,608	1,608
Phenylene Skeletal	1,523	1,509	1,500	1,499	1,514	1,511	1,511
CH ₂ Scissoring	1,462	1,459	1,458	1,458	1,448	1,450	1,450
C–O St.	1,418, 1113	1,428, 1110	1,430, 1098	1,428, 1,095	1,430, 1,100	1,427, 1,100	1,429, 1,096
C–C St.	1,045	1,045	1,045	1,066	1,050	1,040	1,022
CH op Def.	831	832	832	832	831	830	831
CH ₂ Rocking	722	720	720	720	720	722	723
Sn–C Assym. St.		585	585	590	590	590	571
Sn–C Sym. St.		518	533	517	522	555	515
Sn–O St.		417	420	422	420	418	422

maximum at 3,427 (all bands given in cm^{-1}). It is the most intense band in the spectrum of DES. This band is missing in the spectra of the polymers though the polymers typically show a small band at about 3,377 probably due to the presence of water. DES shows bands due to the aromatic C–H at 3,020 and aliphatic C–H bands at 2,973, 2,945, and 2,874. Dibutyltin dichloride shows C–H aliphatic stretching bands at 2,959, 2,927, 2,871, and 2,858. The product shows bands at about 2,961 (Sn–Bu), 2930 (Sn–Bu), 2870 (Sn–Bu & DES), and 2,860 (Sn–Bu). It is normal for the Sn–Bu C–H stretch associated bands to be relatively intense. The Sn–O stretching band is generally found over a wide range of 400 to 700 but for acyclovir-associated polymers and the analogous organotin polyethers derived from PEG, the band is found at about 420. The new band at 420 is then assigned to the Sn–O moiety.

Similar results were found for the other polymers (Tables 3 and 4). The CH aliphatic bands were broader for the polymers in comparison to either monomer and only the most intense bands are given in Table 4.

In summary, infrared spectral results are consistent with the proposed structure.

3.1.3 Mass Spectrometry

High resolution fragmentation matrix assisted laser desorption/ionization mass spectrometry, HR F MALDI MS, was run on the samples. Only high abundance (>five times the background and greater) ion fragments were given in these tables. A number of abbreviations were employed to describe proposed assignments: S = DES – 2H; Bu = C_4H_9 , U = one unit or one repeat unit, 2U = two units and OPh is oxygen–phenylene. For high mass ion fragments, precise mass were employed such as C = 12.01, Sn = 118.71, etc.

Results for only the dibutyltin product are given here. The dibutyltin product was chosen since it offers the best overall inhibition of cell lines. While 2,5-dihydroxybenzoic acid, BA, has been recommended as a preferred matrix material for polymeric materials, we recently found that at low masses some of the major ion fragments may be derived from reaction of the matrix, BA, with the organotin moiety [45]. This was found for the current products.

Table 5 contains the most abundant ion fragments for the 500–5,000 Da range for the dibutyltin polymer. The ion fragments at 614, 671, and 793 Da were derived from the reaction of the organotin moiety with the matrix. Tin containing ion fragments formed clusters that contained ion fragments present in the predicted isotopic abundances for tin. While tin has ten isotopes, only isotopes that are present in 10% and greater will be considered. While ion

Table 5 Most abundant ion fragment clusters for the product from di-*n*-butyltin dichloride and DES; 500–5,000 daltons

m/e	(Proposed) assignment
557	U – Et + OPh
614	2Bu ₂ Sn + BA
699	U + S – OPh
925	2U – Bu, O
1,161	2U + S – OPh
1,586	3U + OPh
2,506	5U + O
2,693	5U + S – Bu, O
3,075	6U + OPh
3,499	7U
3,962	8U – Et
4,632	9U + BuSn
579	U + Ph
671	3Bu ₂ Sn + BA – 3Bu
793	3Bu ₂ Sn + BA – Bu
961	2U – O, Et
1,473	3U – O
1,999	4U
2,620	5U + S – OPh, Bu
3,029	6U + O
3,256	6U + S
3,610	7U – Bu + OPh
4,118	8U + BuSn

where U = one repeat unit; 2U = two repeat units; etc

Table 6 Isotopic abundance matches for selected ion fragments containing one tin atom derived from the product of DES and di-*n*-butyltin dichloride

m/e	% Nat. Abun	U – Et + OPh		U + Ph		U + S – OPh	
		m/e	% Abun	m/e	% Abun	m/e	% Abun
116	45	553	35	575	44	695	25
117	24	554	20	576	30	696	25
118	74	555	85	577	74	697	60
119	26	556	30	578	30	698	26
120	100	557	100	579	100	699	100
122	14	559	20	581	17	701	20
124	18	561	18	583	18	703	–

fragment clusters containing two and three tin atoms were present, for brevity, only three ion fragment clusters containing one tin are reported here (Table 6).

Table 7 contains high mass ion fragments derived from the dibutyltin polymer. High intensity ion masses greater than its molecular weight of 16,000 Da are not found.

Table 7 Most abundant ion fragment clusters for the product from di-*n*-butyltin dichloride and DES; >10,000 daltons

m/e	(Proposed) Assignment
10,014	20U
10,328	21U – O, Ph, Bu
11,027	22U + OPh – Bu
11,983	24U
13,306	27U – S
10,061	20U + OPh
10,801	22U – S
11,253	23U – Bu ₂ Sn
12,761	26U – Bu ₂ Sn
15,114	30U + S – O

3.2 Biological Results

The tested cell lines represented a wide range of cancers including breast (NCI Designation MDA MB-231 and MCF-7), colon (NCI Designation HT-29), and prostate (NCI Designation PC-3) cancer cell lines. They also included two “healthy” cell lines, the WI-38 human cells and 3T3 cells that are mouse cells that were partially transformed. Results from them were compared to results from the various cancer cell lines giving measures of the ability of the tested drug to differentiate between healthy and cancer cell lines. Of the two, the WI-38 is the most widely used and accepted healthy cell line for such comparisons. Also included in the test samples are cisplatin, the most widely employed anticancer drug, as a comparison to a known anticancer agent, and dibutyltin dichloride to evaluate the effect of presence of the dibutyltin moiety with respect to cancer cell line inhibition.

Different measures are employed in the evaluation of cell line results. Here we used the two most widely employed- GI₅₀ values which are: the lowest concentration

where growth is inhibited by 50% and the Chemotherapeutic Index, CI₅₀ which is a measure of the amount needed to inhibit 50% cell growth, GI₅₀, for the normal cell lines, here WI-38 and 3T3 cell lines, divided by the amount needed to inhibit 50% cell growth for one of the cancer cell lines. It is to be noted that different researchers generally emphasize one of these measures over the other with neither measure universally accepted. Thus, results from both of these measures were presented. Table 8 contains the GI₅₀ values for the polymers, dibutyltin dichloride, and cisplatin.

The GI₅₀ values for the polymers were generally in the same range and lower than cisplatin, Bu₂SnCl₂, and DES. Unlike other studies, there was no clear trend with respect to cell inhibition and nature of the alkyl unit on tin. With the exception of the WI-38 cells, there was a relationship between general inhibition for the polymers and DES itself but not between the polymers and dibutyltin dichloride itself. Further, the breast cancer cell line without estrogen (MDA MB-231) showed better test results than the breast cancer cell line that was positive for estrogen (MCF-7), perhaps because some of the drug was bound to the estrogen receptors and not available to act within the cell. This was consistent with the finding that DES is effective against estrogen receptor positive (ER+) tumors [2, 3].

The second measure was the 50% chemotherapeutic index, CI₅₀. As noted before, the chemotherapeutic index is the concentration of the compound that inhibits the growth of the normal cells (here the WI-38 and 3T3 cells) by 50% divided by the concentration of the compound that inhibits the growth of the tumor cells by 50%. Larger values were desired since they indicate that a larger concentration was required to inhibit the healthy cells in comparison to the cancer cells or stated in another way, larger values indicated some preference for inhibiting the cancer cells in preference to the normal cells. In general, CI₅₀ values larger than 2 are considered significant. CI₅₀ values were

Table 8 GI₅₀ concentrations (micrograms/mL) for organotin polyethers for tested cell lines. Values given in () are standard deviations for each set of measurements

Sample	WI-38	3T3	PC-3	MDA*	HT29	MCF-7
DES	0.25(0.2)	0.11(0.05)	0.67(0.05)	0.05(0.01)	0.22(0.02)	0.64(0.05)
Bu ₂ SnCl ₂	0.20(0.05)	0.20(0.05)	1.40(0.11)	1.40(0.12)	1.20(0.11)	0.70(0.06)
Me ₂ Sn polym.	1.60(0.5)	0.90(0.01)	5.30(0.05)	0.47(0.04)	0.42(0.03)	0.65(0.05)
Et ₂ Sn polym.	0.05(0.01)	0.33(0.09)	0.09(0.01)	0.16(0.01)	0.26(0.01)	0.55(0.05)
Pr ₂ Sn polym.	2.30(0.5)	0.10(0.03)	0.81(0.05)	0.09(0.01)	0.49(0.04)	0.66(0.05)
Bu ₂ Sn polym.	2.50(0.5)	0.08(0.01)	0.74(0.05)	0.05(0.01)	0.45(0.04)	0.62(0.05)
Cy ₂ Sn polym.	0.22(0.02)	0.17(0.02)	0.10(0.01)	0.21(0.02)	0.22(0.01)	0.50(0.05)
Ph ₂ Sn polym.	2.30(0.5)	0.08(0.01)	0.85(0.05)	0.11(0.02)	0.12(0.01)	0.65(0.05)
Cisplatin	0.05(0.04)	3.00(0.29)	1.00(0.10)	1.00(0.10)	2.00(0.21)	3.00(0.28)

* MDA MB-231

Table 9 Chemotherapeutic index-50% for the samples for each cell line

Sample ID	WI-38 PC-3	WI-38 MDA*	WI-38 HT-29	WI-38 MCF-7	3T3 PC-3	3T3 MDA*	3T3 HT-29	3T3 MCF-7
DES	0.37	5.0	1.1	0.39	0.16	2.2	0.50	0.17
Bu ₂ SnCl ₂	0.14	0.14	0.17	0.29	0.14	0.14	0.17	0.29
Me ₂ Sn polym.	3.0	3.4	3.8	2.5	0.17	0.19	0.21	0.14
Et ₂ Sn polym.	0.56	0.31	0.19	0.09	3.7	2.1	1.3	0.60
Pr ₂ Sn polym.	2.8	2.6	4.7	3.5	1.2	1.1	0.20	0.15
Bu ₂ Sn polym.	3.4	5.0	5.6	4.0	0.11	1.6	0.18	0.13
Cy ₂ Sn polym.	2.2	1.0	1.0	4.4	1.7	0.81	0.77	0.34
Ph ₂ Sn polym.	2.7	2.1	1.9	3.5	0.09	0.73	0.67	0.12
Cisplatin	0.05	0.05	0.03	0.02	3	3	1.5	1.0

* MDA MB-231

given in Table 9. Those CI₅₀ values equal to and greater than two were highlighted by bold type for ready identification.

Several features were apparent. First, there was a difference in CI₅₀ values between the WI-38 and 3T3 cells. CI₅₀ values for the WI-38 were almost all greater than one with the majority of them being greater than two (75%), the generally accepted threshold for there being a significant difference in cell inhibition between the cancer cell lines and the healthy WI-38 cell line. For the 3T3 cell line comparisons, values were generally less than one (92%), consistent with a tendency of the compounds to favor inhibition of the 3T3 cells in comparison to the cancer cells. One difference between the WI-38 and 3T3 cells was that the WI-38 are human in origin whereas the 3T3 cells were mouse. Additionally the WI-38 cells were truly normal, were mortal and were very sensitive to contact inhibition. The 3T3 cells were somewhere down the path to transformed as they were no longer mortal but immortal,

but were still sensitive to contact inhibition. Second, as in the case with the GI₅₀ values, there was a correlation between the CI₅₀ values for DES and the polymer results but not the CI₅₀ values for Bu₂SnCl₂. Thus, the primary “driving force” for distinguishing cell inhibition appeared to be the DES in the present study. Third, the nature of the organotin moiety appeared to be a secondary factor in determining the ability to inhibit the cells. The most effective polymers contained the dibutyltin moiety followed closely by the diphenyltin and dipropyltin moiety but this trend was not dominant.

An additional question concerned the influence of simply having the dibutyltin moiety present in a polymer. Table 10 contains the CI₅₀ values normalized against the CI₅₀ value for dibutyltin dichloride or diethylstilbestrol. This ratio may offer some measure of the effectiveness of having the two monomer moieties within the polymer. Values greater than one would be consistent with the presence of that moiety present in the polymer having a

Table 10 Chemotherapeutic index-50% for the samples for each cell line normalized against values for dibutyltin dichloride and diethylstilbestrol

Sample	WI-38** PC-3	WI-38 MDA*	WI-38 HT-29	WI-38 MCF-7	3T3 PC-3	3T3 MDA*	3T3 HT-29	3T3 MCF-7
Bu₂SnCl₂	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Bu ₂ Sn Polymer	24	360	33	14	0.79	11	1.1	0.45
DES	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Me ₂ Sn Polymer	8.1	0.68	3.5	6.4	1.1	0.09	0.42	0.82
Et ₂ Sn Polymer	1.5	0.06	0.17	0.23	22	0.95	2.6	3.5
Pr ₂ Sn Polymer	7.6	0.52	4.3	9.0	0.75	0.50	0.40	0.88
Bu ₂ Sn Polymer	9.2	10	5.1	10	0.69	0.73	0.36	0.76
Cy ₂ Sn Polymer	5.9	0.20	0.91	11	11	0.37	1.5	2.0
Ph ₂ Sn Polymer	7.3	4.2	17	9.0	0.56	0.33	1.3	0.71

* MDA MB-231

** CI₅₀ for WI-38/PC3 for the dibutyltin polymer divided by the CI₅₀ for WI-38/PC3 for dibutyltin dichloride itself

favorable effect. These values were highlighted in bold for easy identification.

The division between values related to the WI-38 and 3T3 cells continued. For the WI-38 most of the values (71%) were greater than one, but for the 3T3 cells most values were less than one (67%). For both the WI-38 and 3T3 cell results, values often deviate greatly from one consistent with the units being part of a polymer affecting their ability to inhibit cell growth. If the influence was simply related to the ratio of each reactant within the polymer then values should be within the realm of 0.5 to 2 because the organotin and DES moieties have approximately the same mass and same frequency factor with each chain unit having one organotin and one DES-derived moiety.

No norm has been established for this kind of comparison.

As noted before, the inhibition results for the polymers appeared to closely resemble those of DES rather than the nature of the organotin moiety. In previous studies where the Lewis base moiety was kinetin, [24] ticarcillin, [46] ciprofloxacin, [27, 47] 1,1'-ferrocene dicarboxylic acid, [42] and cephalixin [47] the inhibition trends appeared to be largely dependent on the organotin moiety where there were decades difference between the GI_{50} values where the overall inhibition trend was (lowest GI_{50} concentration) $Bu_2Sn > Ph_2Sn > Pr_2Sn > Et_2Sn > Me_2Sn$. In all of these cases, the Lewis base drug showed little or no ability to inhibit the test cell lines. For acyclovir [41] the difference was less prominent, with the difference in GI_{50} concentration being about 10 fold but where the overall trend generally followed the same order as noted before. Acyclovir showed mild inhibition of the tested cell lines. For the present study, the overall trend appeared to be about the same but the difference was generally less than 10 fold. Unlike the previous studies, DES did show a pronounced ability to inhibit cell line growth and this appears to be the primary factor in the ability of the polymers ability to inhibit cell growth. As noted before, WI-38 cell results were consistent with the polymers showing better cell growth inhibition in comparison to DES alone, so that the coupling of the organotin moiety with the DES provided a positive factor in cell line inhibition.

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