ORIGINAL ARTICLE

Organotin Poly(ester ethers) from Salicylic Acid and Their Ability to Inhibit Selected Human Cancer Cell Lines

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ABSTRACT

Purpose/Method: We are searching for compounds that exhibit good inhibition of solid tumor cancers investigating structure/property relationships. To do this, we synthesize different organotin-containing polymers and then test their ability to inhibit the human cancer cell lines. **Results:** Organotin poly(ester ethers) were formed from the reaction of salicylic acid (SA) and organotin dihalides employing interfacial polycondensation. Product yield and chain length decrease as the alkyl group on the organotin increased consistent with the importance of the size of the alkyl group influencing both chain growth and product yield. Infrared spectral analysis shows the formation of Sn-0 and Sn-0-C=0 groups. Matrix-assisted laser desorption ionization mass spectroscopy analysis showed ion fragments to five units with good isotopic abundance values for tin. The products showed good inhibition of all of the tested human cancer solid tumor lines. **Conclusions:** The organotin polymers from SA exhibit good inhibition of all of the human cancer cell lines including two pancreatic and two breast cancer cell lines.

Key words: Breast cancer, matrix-assisted laser desorption ionization mass spectroscopy, organotin polymers, pancreatic cancer interfacial polycondensation, salicylic acid, tin-containing polymers

INTRODUCTION

This is a continuation of our efforts to discover materials that inhibit unwanted infectious agents and pathogens. The work also focuses on discovering the structure/property relations related to their biological behavior to accomplish this. One rational to our synthesis is to couple metal-containing moieties that are known to exhibit biological activity with Lewis bases that also exhibit biological activity hoping the combination will have a synergic effect.

Salicylic acid (SA) [Figure 1] is often the key biological agent in anti-acne medications and employed to treat aches and to reduce fevers. It plays critical roles in plants including their growth, transpiration, transport, ion uptake, and photosynthesis. Historically, it has played a role in a number of treatments and was originally obtained from the bark of the willow tree. It is a major part of aspirin.

Organotin compounds are well known for their biological activity. Some of our activity involving this has been recently reviewed.[1-3] More organotin compounds are available commercially than any other metal-containing organometallics.[4,5] Further, more organotin compounds have undergone testing as potential anticancer agents than any other single group of compounds.[1-5]

A number of polymers have incorporated the SA moiety into them. Essentially, all of them react either the hydroxyl group or from esters formed through the acid moiety but does not involve reaction with both reactive groups as we do in the present study. Following are examples of this.

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Chandorkar et al. initially reacted SA with diacid chlorides forming esters that then are reacted with alcohol containing reactants such as mannitol which is subsequently cross-linked.[6] Mitchell et al. formed an anti-inflammation bone treatment material from a SA -based poly(anhydride-ester) and a three-dimensional osteoconductive ceramic scaffold.[7]

A cross-linked material was made from a similar copolymer composed of SA and semicarbazide that was subsequently reacted with formaldehyde.[8] Zhao and Deng synthesized water-soluble polymers derived from aminosulfonate-phenol and SA then reacted with formaldehyde. These were added to concrete to enhance concrete properties such as flexural strength.[9]

Here, we describe the synthesis of organotin poly(ester ethers) from reaction of the salt of SA with various organotin dihalides-forming polymers with the repeat unit shown in Figure 2.

We have produced other similar products from reaction of compounds containing a single hydroxyl and acid group. These include cyano-4-hydrocinnamic acid [Figure 3][10] and glycyrrhetinic acid [Figure 4].[11]

The advantages of polymeric drugs have been recently reviewed emphasizing their use in combating cancer.[1] Following briefly presents some of these. First, polymers are filtered out by the kidneys more slowly than small compounds decreasing kidney damage and increasing body retention time allowing a greater time for cancer to be exposed to the polymeric drug. Second, polymers may be effective against tumors that have developed resistance to other chemotherapeutic agents because the polymer is not recognized by cellular resistance mechanisms because of their large size. The chemo agents that the cell has become resistant to are almost always relatively small molecules. This developed resistance is unfortunately not unusual in chemo patients. Our polymers have shown good inhibition of such resistant cancer cell lines. Third, the size and structure of polymers provide more binding sites to cellular targets, increasing effectiveness. Fourth, polymers typically accumulate in solid tumors more than in normal tissues because of the enhanced permeability and retention effect, resulting in high amounts of polymers in the interstitial space due to a leaky vasculature and limited lymphatic drainage typical of tumors. This effect is referred to as the enhanced permeability and retention effect, EPR effect. Fifth, the polymeric structure can permit easy coupling to other molecules, such as those that specifically target cancer cells, allowing delivery of a polymeric drug to a particular

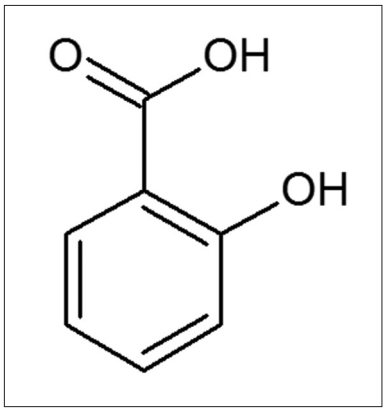


Figure 1: Chemical structure of salicylic acid

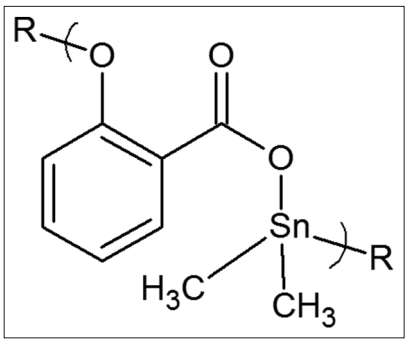


Figure 2: Repeat unit for reaction between salicylic acid and dimethyltin dichloride where R represents chain extension

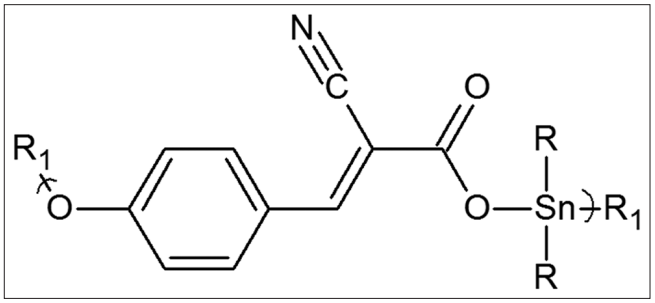


Figure 3: Repeat unit for the product of organotin dihalides and cyano-4-hydrocinnamic acid where R_1 represents chain extension

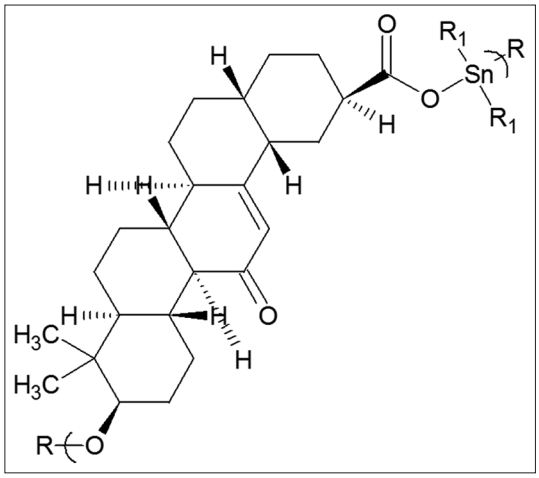


Figure 4: Repeat unit for the product of organotin dihalides and glycyrrhetinic acid where R_1 represents chain extension

site. Sixth, polymers can be designed to incorporate multiple anticancer agents within the same molecule that act against cancer cells by different mechanisms. Seventh, polymers can be designed as either a large stable compound that enters the cell by pinocytosis and is active in a polymeric form or as an unstable compound that slowly degrades into active monomeric units in a timed-release fashion. Our polymers inhibit cell growth as polymers rather than undergoing chain breakup. Thus, polymers have great flexibility in their design and many possible benefits compared to monomeric drugs.

EXPERIMENTAL

Synthesis

Reactions were carried out using the interfacial polycondensation technique, Briefly, an aqueous solution (30 ml) containing the SA (0.00300 mol) and sodium hydroxide (0.0060 mol) 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 hexane (30 ml) solution containing the organotin dihalide (0.00300 mol) was rapidly added through a hole in the jar lid using a powder funnel and the resulting solution blended for 15 s. The 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 using acetone and dried at room temperature.

Diphenyltin dichloride (1135-99-5), dimethyltin dichloride (753-73-1), SA (69-72-7), and dibutyltin dichloride (683-18-1) were purchased from Aldrich Chemical Co., Milwaukee, WS; diethyltin dichloride (866-55-7) was obtained from Peninsular Chemical Res., Gainesville, FL; dioctyltin dichloride (3542-36-7) was obtained from Ventron Alfa Inorganics, Beverly, Mass.

Characterization

Light scattering photometry was carried out employing a Brice-Phoenix Universal Light-Scattering Photometer Model 4000 with the polymers dissolved in dimethyl sulfoxide (DMSO). Infrared spectra were obtained employing attenuated total reflectance infrared spectroscopy utilizing a Thermo Scientific Nicolet iS5 Fourier-transform infrared equipped with an id5 attenuated total reflection attachment. 1H NMR spectra were obtained employing Varian Inova 400 MHz and Varian 500 MHz spectrometers.

High-resolution electron impact positive ion matrix-assisted laser desorption ionization mass spectroscopy (MALDI MS) time of flight (HR MALDI-time of flight), MS was carried out employing a Voyager-DE STR BioSpectrometer, Applied Biosystems, Foster City, CA. Standard settings were used which include a linear mode of operation and an accelerating voltage of 25,000 volts; grid voltage of 90%, and an acquisition mass range of 500–2500. Fifty to two hundred shots were typically taken for each spectrum. Results using graphene are included in the present paper. The solid product and graphene were mixed together employing copper spheres giving a fine powder that was employed to obtain the spectra.

Cell Testing

The toxicity of each test compound was evaluated using a variety of cancer cell lines and with human normal embryonic lung fibroblast (WI-38) as the standard. Following a 24-h incubation period, the test compounds were added at concentrations ranging from 0.0032 to 32 µg mL and allowed to incubate at 37°C with 5% CO2 for 72 h. Following incubation, CellTiter-Blue reagent (Promega Corporation) was added (20 uL/well) and incubated for 2 h. Fluorescence was determined at 530/590 nm and converted to % cell viability versus control cells.

All cytotoxicity values are calculated against a baseline value for each line that was generated from "mock-treatment" of the normal and tumor cell lines with media supplemented with all diluents used to prepare the chemotherapeutic compounds. For example, if the compounds were dissolved in DMSO and serial dilutions prepared in MEM to treat the cells, then the mock-treated cells were "treated" with the same serial dilutions of DMSO without added chemotherapeutic compound. This was done to ensure that any cytotoxicity observed was due to the activity of the compound and not the diluents. For the studies reported here, the mock-treatment never resulted in a loss of cell viability of more than 1%, demonstrating that the activity observed was not due to cytotoxicity of any of the diluents used, but was due to the activity of the tested compounds.

Standard dilutions are employed beginning with the most concentrated with essentially total inhibition occurring to the most dilute where little or no inhibition occurs. The inhibition curve is sigmoid and the EC50 determined at the midpoint of the curve. Once inhibition begins, the concentration difference between the initial inhibition and final total inhibition is steep with the region between initial to final total inhibition essentially linear.

RESULTS AND DISCUSSION

Yield and Chain Length

Table 1 contains the yield and chain length for the synthesized poly(ether esters) produced from reaction with the salt of SA. The salt form is employed since the acid form is not sufficiently nucleophilic to react with organotin while the salt form is able to accomplish this.[1]

Both percentage yield and chain length, degree of polymerization decrease as the alkyl length on the tin

increases consistent with the possible importance of size considerations in the reaction with increased alkyl size inhibiting ready approach between the reactants.

Infrared Vibration Results

Infrared spectral analysis was carried out for all of the samples over the range of 4000–650 cm−1. All band locations are given cm^{-1}. Infrared spectral analysis is consistent with the proposed structure and with other reported analyses.[1,10-12] Bands derived from the monomers and polymers from dibutyltin and diphenyltin dichloride reacting with SA are given in Table 2. The spectra from all the products show bands characteristic of both reactants and new bands for the product assigned to the Sn-O and Sn-O-C(O) linkages [Table 2]. A new band for the Sn-O-C tin-ether linkage is found about 1090 and the Sn-O-C(O) linkage for the tin ester linkage is found about 1020. For C-H stretching about 3000, SA has bands about 3069 and 3037. Dibutyltin dichloride has bands at 2960, 2927, 2872, and 2858. The polymer shows bands at 3065, 3040, 2956, 2870, and 2856 showing bands from both the dibutyltin and SA moieties. Diphenyltin dichloride shows C-H stretching bands at 3068 and 3051 and the polymer shows bands at 3067 and 3056 from the diphenyltin moiety and bands at 3078 and 3056 consistent with the presence of units derived from SA. Bands characteristic of the carboxylic acid and hydroxyl proton about 3500 are missing as expected. Thus, infrared spectroscopy is consistent with the presence of units from both reactants and the formation of new bands consistent with the formation of the expected Sn-O and Sn-O-C(O) linkages.

SA, is not a symmetrical molecule, so there are more than one structural units formed about the organotin moiety. These are described in Figure 5 as being two symmetrical arrangements, Figure 5a and b, and the mixed arrangement 5c.

Table 1: Percentage yield and molecular weight as a function of the organotin dihalide

Organotin Moiety	% Yield	Molecular Weight	DP
Me_2Sn	66	4.1×10^5	1400
Et_2Sn	23	7.7×10^4	320
Bu_2Sn	9	9.0×10^4	240
Oc_2Sn	6	1.4×10^4	29
Ph_2Sn	99	1.9×10^5	460

DP: Degree of polymerization

Table 2: Selected infrared bands for the monomers and polymers associated with the dibutyltin and diphenyltin polymer

Band Assignment	SA	Bu_2SnCl_2	Bu_2Sn Polymer	Ph_2SnCl_2	Ph_2Sn Polymer
OH St	3505				
CH St Aromatic	3069,3037		3065,3040	3068, 3051	3078,3067,3056
CH Sym St Aliph		2960, 2927	2956, 2925		
CH Asym St Aliph	2803	2872, 2858	2870, 2856, 2817		2800
C=O St	1830		1691		1692
Ring CC ip St	1583,1526, 1447		1591, 1555, 1511, 1445		1591,1555, 1530, 1466
Sn-Ph St				1480, 1071	1482, 1066
CH_3 Sym St		1463	1464		
C=C St				1432, 1332	1437, 1333
CH_3 Asy Bend		1380	1390		
CH Wag C=C	1370		1378		1372
C-OH St Acid	1320				
Ring CC St	1030		1031		1031
Sn-O-C			1092		1092
Sn-O-C (O) St			1017		1031
Ring Breathing				996	997
CH_3 Rock		878	869		
Syn op Bend Ring Hydrogens				729	729
Asy op Bend Ring Hydrogens				691	695

Since the carboxyl moiety can exist as being bridged and non-bridged structures, there exist additional arrangements about the organotin unit. These are given in Figure 6 and consist of various bridged (as 6a) and non-bridged combinations along with ether linkages as shown in Figure 6b and e. We have found that asymmetric bridged structures as shown in Figure 6d and e are generally rare.[1,10,11]

Infrared spectroscopy is the easiest way to determine the presence of bridged and non-bridging structures.[1,10,11] Bridging asymmetric carbonyl absorptions are found around 1570. The bridging symmetric carbonyl band is found around 1410–1435. Non-bridging asymmetric carbonyl bands are found about 1600–1690; and the corresponding symmetric carbonyl bands are found about 1350–1370. Results for the products are given in Table 3.

In general, all of the products except for the octyl, exhibit larger carbonyl bands associated with non-bridging. We also find that bridging occurs when asymmetric structures are present (6b), that is, structures that contain one ether and one ester linkage.[10,11,13-15]

Nuclear Magnetic Resonance (NMR) Spectral Analysis

NMR was conducted employing d-6 DMSO for the various products. Here, we describe results for SA, dibutyltin and diphenyl dichlorides, and the products with SA. Figure 7 contains the structure indicating proton locations described for the SA and the butyl chain from dibutyltin dichloride. The protons at the hydroxyl and acid groups are missing as expected for the polymers. For the butyltin chain in the polymer, the bands are found at α 1.51; β 1.61; γ 1.31; δ 0.83. The associated SA bands in the polymer are found at 7.12, 7.39, 7.58, and 8.60. From the diphenyltin dichloride, bands are found at (ortho) 7.31, (meta) 7.41, and (para) 7.81 assigned to the phenyl moiety. For the diphenyl polymer bands appear at (ortho) 7.91 and 7.82; and for the meta protons at 6.90 and 6.88; and for the para proton at 7.58. For the SA bands appear at about 6.9, 7.0, 7.4, and 7.9 for the polymer. For the phenyl polymer, the bands directly adjacent to the acid and alcohol are shifted mildly downfield and for the dibutyltin and other alkyl organotin polymers, the bands are largely unchanged consistent with polymer formation having minimal effect on the NMR. Thus, NMR is consistent with the presence of both units within the product and formation of the linkages from the absence of the associated protons. Further analysis is not made because of the low solubility of the polymer in the d-6 DMSO.

R-O-Sn-O-R	R-C(O)-O-Sn-O-C(O)-R	R-C(O)-O-Sn-O-R
(a)	(b)	(c)

Figure 5: Arrangements of the salicylic acid-derived moiety about the tin atom

MALDI MS

Usual MALDI MS suffers from the major limitation that the samples much be soluble in volatile liquids. The most widely used solvent is water that allows intimate contact between the matrix and sample. This requirement is typically not fulfilled for most polymers. For over a decade, we and others have been employing MALDI MS for the identification of non-volatile metal and non-metal containing polymers looking at the fragments rather than the actual intact polymer chains. This approach is applicable to soluble and insoluble products so has wide potential for application. This technique has been recently reviewed.[16-19]

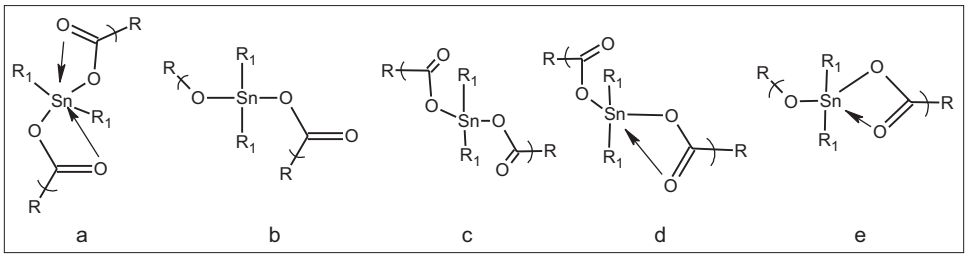


Figure 6: Geometrical arrangements about the metal atom

Table 3: Presence of bridging and non-bridging associated bands and location

Organotin Moiety	Asym Non-bridging	Sym Non-bridging	Asym Bridging	Sym Bridging
Me_2Sn	1686(m)	1351(m)	1557(s)	1448(s)
Et_2Sn	1687(m)	1360(s)	1595(s)	-
Bu_2Sn	1691(m)	1357(m)	1591(s)	-
Oc_2Sn	1687(m)	1332(m)	1593(m)	1430(m)
Ph_2Sn	1692(m)	1356(m)	1591(m)	1437(s)

Where l=large, m=moderate, s=small

In the current study, graphite is used as the matrix agent. This use has been recently reviewed.[19] The advantage of graphite is that no major ion fragment clusters are produced below 500 Da and for our experiments, this is below the typical mass range studied.

Two mass spectra modes were employed in studying the MALDI MS for the SA polymers. The linear mode is employed when high-mass ions are the major focus, while the reflective mode is employed when greater precision is the major focus. Several abbreviations are employed in describing the tentative assignment for a particular ion fragment cluster. These abbreviations are U = one unit, 2U = two units, and SA minus two protons. Na is sodium which is present as a contaminant.

Figure 8 and Table 4 presents MALDI MS data for the product of dioctyltin dichloride and SA.

Ion fragment clusters to five units are found.

Tin contains ten isotopes with seven having a relative isotopic abundance of five percent and greater. The presence of tin within the ion clusters is indicated by the "tell-tale" fingerprints caused by the isotopic abundance of these tin isotopes as seen in Figure 8 about 722, 845, 936, 961, 1101, etc. Table 5 contains isotopic abundance matches for two ion fragment clusters containing two tin atoms each [Table 5]. The matches are reasonable and consistent with the ion fragment clusters containing two tin atoms. Presence of isotopes is both an advantage and disadvantage. It is a disadvantage because the intensity of a "single" tin-containing ion is divided into the various isotopic containing fragments diminishing the specific overall intensity caused by that structure. It is an advantage because agreement of the relative intensities of tin-containing fragments with its known isotopic natural abundance, given in the tables as the "Standard," gives greater confidence of the ion fragment cluster containing tin.

Figure 9 contains a portion of the refractive MALDI MS for the product of dimethyltin dichloride and SA and Table 6 contains the major ion fragment clusters found for the same polymer.

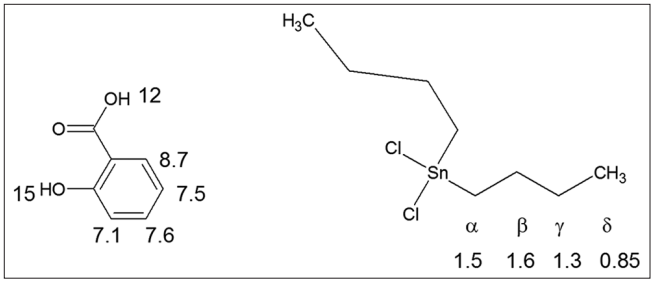


Figure 7: Location of bands for SA and dibutyltin dichloride

Ion fragment clusters to 8 units are found for the linear mode and three units for the reflective mode.

Table 4: Major ion fragments derived from the MALDI MS of the product of dioctyltin dichloride and SA

Mass, Da/Linear	Mass, Da/Reflective	(Tentative) Assignment
527		U+SA-2CO_2
572	573	U+SA-CO_2
	596	U+SA-O
	713	U+OcSn
	749	U+OcSn, 2O
845	843	U+Oc_2Sn, O
936		U+Oc_2Sn, 2CO_2, Na
	944	2U-O
961		2U
	971	2U-O+Na
987		2U+Na
	1000	2U+CO_2
1101	1102	2U+SA
	1177	2U+OcSn, O
1203		2U+OcSn
	1227	2U+OcSn, Na
1316	1318	2U+Oc_2Sn
1426		3U-O
	1461	3U+O
1480	1480	3U+CO_2
	1539	3U+SA-CO_2
1568		3U+SA-O
	1596	3U+SA-O+Na
1716		3U+OcSn, CO_2
	1786	3U+Oc_2Sn
	1824	3U+Oc_2Sn, O, Na
1878	1874	3U+Oc_2Sn, 2CO_2
1914		4U-O
2008		4U+SA-CO_2, O
2161		4U+OcSn
	2185	4U+OcSn, O
	2241	4U+SA-CO_2
2288		4U+Oc_2Sn, O
	2313	5U-CO_2
2348		5U
2425		5U+SA-CO_2, O

MALDI MS: Matrix-assisted laser desorption ionization mass spectroscopy, SA: Salicylic acid

Table 5: Isotopic abundances for two ion fragments derived from reaction of dioctyltin dichloride and SA containing two tin atoms (for isotopes whose relative abundances are>10%; reflective)

Standard for 2 Sn		U+Oc$_2$Sn, O		2U+Na	
Isotopic Mass	**Rel. Intensity**	**Mass**	**Rel. Intensity**	**Rel. Intensity**	**Rel. Intensity**
232	12	839	13	983	12
233	13	840	15	984	14
234	43	841	45	985	46
235	35	842	35	986	36
236	94	843	94	987	93
237	51	844	52	988	49
238	100	845	100	989	100
239	35	846	33	990	34
240	81	847	78	991	78
242	32	849	32	993	32
244	22	851	26	995	21

Left two columns give the natural abundance mass and relative intensity for that particular mass. The next two sets contain the found mass for the ion fragment pictured above and experimentally found relative abundance found

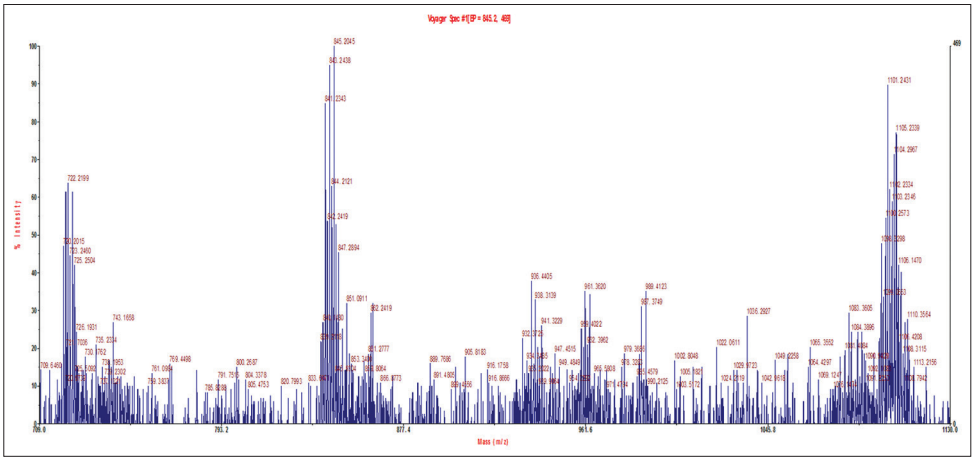


Figure 8: Linear matrix-assisted laser desorption ionization mass spectroscopy over the approximate mass range of 700–1150 Da for the product of dioctyltin dichloride and salicylic acid

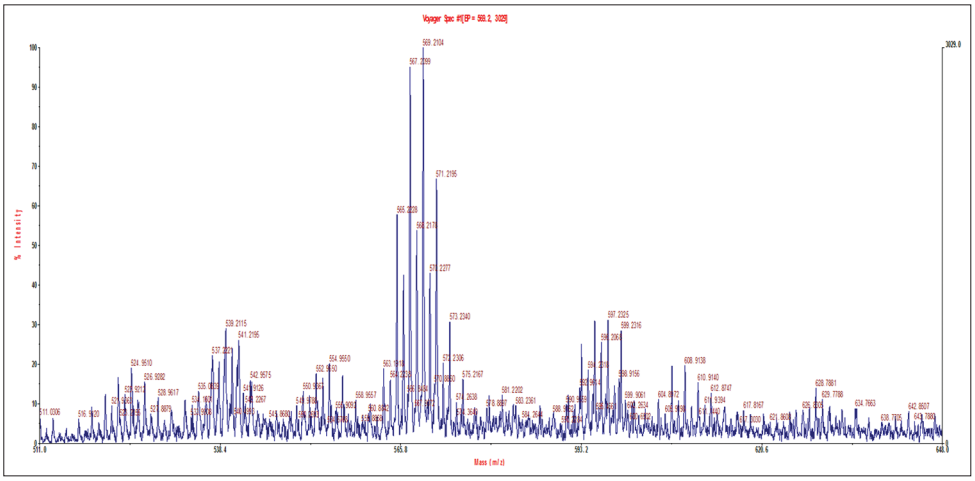


Figure 9: Reflective matrix-assisted laser desorption ionization mass spectroscopy for the product of dimethyltin dichloride and salicylic acid over the mass range of 500–650 Da

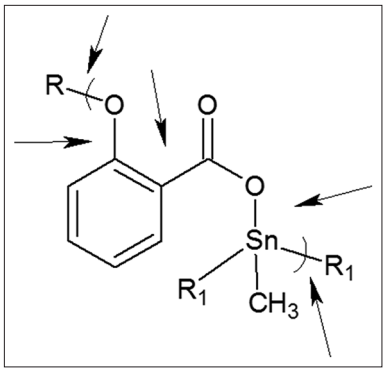


Figure 10: Preferred scission sites for the polymer from salicylic acid and organotin dichlorides

Table 6: Major ion fragments found in the MALDI MS for the product of dimethyltin dichloride and SA

Mass, Da/Linear	Mass, Da/Reflective	(Tentative) Assignment
534		2U-CO_2
552	554	2U+Na-CO_2
	569	2U
597		2U+Na
609	609	2U+O, Na
628	629	2U+CO_2, Na-Me
705		2U+SA
714		2U+SA, Na-O
	728	2U+Me_2Sn
736	736	2U+Me_2Sn, O
763		2U+Me_2Sn, CO_2
774		2U+Me_2Sn, 2O, Na
783		2U+Me_2Sn, CO_2, O
807		2U+Me_2Sn, 2CO_2
	868	3U+O
900		3U+SA-2CO_2
	919	3U+SA-2CO_2
925		3U+SA, Na-2CO_2
993		3U+SA
	1001	3U+Me_2Sn
	1019	3U+Me_2Sn, O
	1075	3U+Me_2Sn, CO_2, Na
	1141	4U
	1262	4U+SA-O
	1584	5U+SA
	2002	7U
	2288	8U

MALDI MS: Matrix-assisted laser desorption ionization mass spectroscopy, SA: Salicylic acid

Table 7 contains isotopic abundance matches for two ion fragment clusters each containing two tin metals.

Similar results are found for the MALDI MS for the other products. As in other cases, the major sites for chain scission are the hetero backbone sites as shown in Figure 10.

Cell Analysis Results

Human cell lines employed in the current study are given in Table 8.

The cells represent a broad range of solid tumor cancers along with the WI-38 standard cell line.

One recent emphasis is to produce compounds that inhibit pancreatic cancer because pancreatic cancer does not have a generally accepted "cure." Thus, the set includes two widely employed human pancreatic cell lines. These are AsPC-1, which represents about 80% of the human pancreatic cancers. It is an adenocarcinoma pancreatic cell line. The second pancreatic cancer cell the PANC-1, which is the cancer cell line in about 10% of human cancer. It is an epithelioid carcinoma pancreatic cell line.

The pair of breast cancer cell lines deserves special comment. They represent a matched pair of cell lines. The MDA-MB-231 (strain number 7233) cells are estrogen-independent, estrogen-receptor (ER) negative, while the MCF-7 (strain line 7259) cells are ER positive.[1,20-22] Recently, the American Medical Society recommended that certain breast cancers need not be treated with chemo drugs but rather treated with removal and hormone treatments. In some studies involving organotin polymers, we found there was a marked difference

Table 7: Isotopic abundances for two ion fragments derived from reaction of dimethyltin dichloride and SA containing two tin atoms (for isotopes whose relative abundances are >10%; reflective mode)

Standard for 2 Sn		2U		2U+Na	
232	12	563	14	591	13
233	13	564	14	592	13
234	43	565	45	593	44
235	35	566	37	594	35
236	94	567	94	595	95
237	51	568	51	596	52
238	100	569	100	597	100
239	35	570	35	598	35
240	81	571	78	599	80
242	32	573	32	601	30
244	22	575	25	603	25

SA: Salicylic acid

Table 8: Cell lines employed in the current study

Strain #	NCI Desig.	Tumor origin	Histological Type
3465	PC-3	Prostate	Carcinoma
7233	MDA MB-231	Pleural effusion breast	Adenocarcinoma
1507	HT-29	Rectosigmoid colon	Adenocarcinoma
7259	MCF-7	Pleural effusion-breast	Adenocarcinoma
ATCC CCL-75	WI-38	Normal embryonic lung	Fibroblast
CRL-1658	NIH/3T3	Embryo-continuous cell line of highly contact-inhibited cells	Fibroblast
	AsPC-1	Pancreatic cells	Adenocarcinoma
	PANC-1	Epithelioid pancreatic cells	Carcinoma

Table 9: EC_{50} concentrations (μg/mL) for the tested compounds. Values given in are standard deviations for each set of measurements

Sample	WI-38	PANC-1	AsPC-1	
Me_2SnCl_2	0.22(.1)	0.80(.1)	0.71(.1)	
Me_2Sn/SA	0.62(.6)	0.61(.6)	0.66(.7)	
Et_2SnCl_2	0.20(.1)	0.48(.1)	0.90(.1)	
Et_2Sn/SA	0.59(.5)	0.55(.5)	0.59(.6)	
Bu_2SnCl_2	0.20(.05)	0.0032(.001)	0.012(.01)	
Bu_2Sn/SA	0.60(.5)	0.62(.6)	0.67(.6)	
Oc_2SnCl_2	0.30(.1)	0.85(.1)	0.85(.1)	
Oc_2Sn/SA	0.61(.5)	0.63(.5)	0.64(.5)	
Ph_2SnCl_2	0.25(.1)	0.71(.1)	0.83(.1)	
Ph_2Sn/SA	0.62(.6)	0.61(.6)	0.62(.6)	
SA	0.49(.1)	0.51(.1)	0.51(.1)	
Cisplatin	0.019(.01)	0.0023(.005)	0.0035(.005)	
Sample	***PC-3***	***MDA-MB-231***	***HT-29***	***MCF-7***
Me_2SnCl_2	0.51(.1)	0.44(.1)	0.56(.1)	0.66(.1)
Me_2Sn/SA	0.60(.6)	0.64(.7)	0.62(.6)	0.62(.6)
Et_2SnCl_2	0.61(.1)	0.64(.1)	0.71(.1)	0.77(.1)
Et_2Sn/SA	0.58(.6)	0.57(.5)	0.54(.5)	0.61(.5)
Bu_2SnCl_2	1.4 (1.1)	1.4 (1.3)	1.2(.1)	0.7(.06)
Bu_2Sn/SA	0.54(.6)	0.57(.7)	0.56(.7)	0.58(.7)
Oc_2SnCl_2	0.55(.1)	0.65(.1)	0.65(.1)	0.70(.1)
Oc_2Sn/SA	0.61(.5)	0.62(.5)	0.64(.6)	0.67(.6)
Ph_2SnCl_2	0.82(.1)	0.76(.1)	0.56(.1)	0.68(.1)
Ph_2Sn/SG	0.61(.6)	0.62(.6)	0.59(.6)	0.60(.5)
SA	0.50(.1)	0.51(.1)	0.50(.1)	0.50(.1)
Cisplatin	0.0044(.004)	0.0029(.002)	0.0041(.003)	0.0057(.003)

between the ability to inhibit the two cell lines dependent on polymer structure,[1,20-22] with those polymers containing the phenylene-O moiety showing much-lowered ability to inhibit certain breast cancer cell lines, namely MCF-7 cells, compared to the MDA cell line. The phenylene-O structure presents in many hormone treatments. Thus, in treating breast cancer patients, the structure of the hormone treatment and cell line should be reviewed with this in mind.

The PC-3 (3465) cells are of interest because this particular prostate cell line is viewed as one of the most resistant of the prostate cancer cell lines.

While different measures have been employed in the evaluation of cell line results the two most widely employed are used here. The most widely employed involves the concentration, dose, needed to reduce the growth of the particular cell line. Here, we will use effective concentration, EC, values. The concentration of a drug, antibody, or toxicant that induces a response halfway between the baseline and maximum after a specified exposure time is referred to as the 50% response concentration and is given the symbol EC_{50}.

Table 9 contains the EC50 values for the current polymers and monomers. Values for cisplatin are included. Cisplatin is among the most widely employed chemo drugs in the treatment of a wide variety of cancers. It is quite toxic and offers many unwanted side effects.

The polymers show good inhibition of all of the tested cell lines. There does not appear to be a difference in the ability to inhibit growth for the two breast-associate cell lines, though the repeat unit structure does contain the phenylene-O-Sn group. Furthermore, the polymers exhibit decent inhibition of the two pancreatic cancer cell lines consistent an ability to inhibit other pancreatic cell lines. The polymers also exhibit decent inhibition of the cell lines of the other cancer-associated cells.

For most of the organotin polymers studied by us, the organotin presence is considered to be the major consideration with the Lewis base essentially non-active. But for the present case, the EC_{50} values for SA are of the same order as the polymers so that the toxicity may be due to the polymer itself, presence of the organotin moiety, and/or presence of the SA or some combination. It is also important to note that in the previous studies for similar polymers that the polymers themselves act intact as the anticancer agent so that the inhibition data is due to the polymer and not to released "monomer"-derived species.[1]

In other studies, we observed a marked ability for polymers containing the dibutyltin moiety, followed by those containing the diphenyltin moiety, to exhibit lower EC50 values.[1] In the current study, the EC50 values are similar. This mean that treatments using the polymers derived from dibutyltin dichloride can be employed to inhibit the cancer cell lines. This offers some advantages briefly described following. Dibutyltin dichloride, in comparison to other organotin halides, offers the least toxicity toward human beings. Next, it is the least expensive of the organotin dihalides. Third, it is the most widely used of the organotin dihalides available in the gram to ton quantity. Fourth, its widespread use means that there is available lots of technical and biological data. Finally, it decomposes to tin oxide which is environmentally considered non-toxic.

The second widely employed analysis of cell data involves the comparison of the amount of drug needed to inhibit the standard cell line compared to amount of drug necessary to inhibit the particular cell line. It is then simply the ratio of EC50 found for the standard cell line, WI-38 listed in Table 10 as simply WI, divided by the EC50 for the particular cell line. This data is displayed in Table 10. Larger values are preferred. For the current study, the values are all about one signifying that the test compound, here polymer, has little preference for inhibiting the cancer cell line compared to inhibiting the standard cell line.

Thus, the organotin polymers inhibit all of the tested solid cancer cell lines giving good EC50 values.

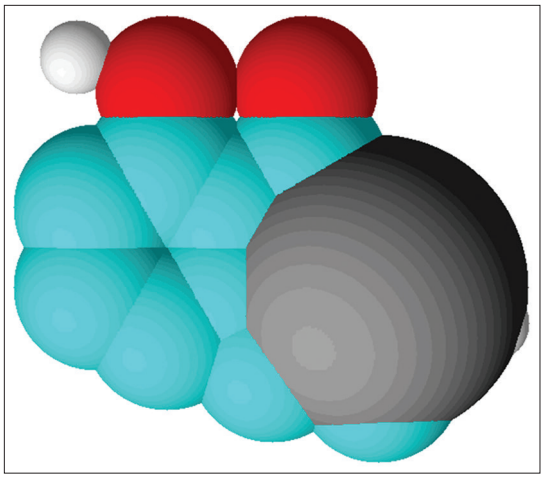
Summary

Polymers derived from the reaction of organotin dichlorides and SA have been synthesized employing the interfacial polycondensation system. The interfacial system has been used to commercially synthesize aramids and polycarbonates.[23,24] The synthesis employs commercially available reactants. Synthesis takes about 15 s using simple equipment. This means that the synthesis of these materials should be somewhat straightforward from gram to ton amounts. FT-IR shows that the products are largely composed of alternate R-O-C(O)-Sn-O-R units. Bands showing the ether and ester linkages are found. MALDI MS shows ion fragment to greater than five units. The polymers show good inhibition of all of the tested human cancer cell lines including two pancreatic cancer cell lines representing about 90% of the observed human pancreatic cancer and two breast cancers.

New Paper ML Salicylic acid Sn

Table 10: CI_{50} results for values calculated from data given in Table 9

Sample	EC_{50}WI/EC_{50} PANC-1	EC_{50}WI/EC_{50} AsPC-1	EC_{50} WI/EC_{50} PC-3	EC_{50}WI/EC_{50} MDA	EC_{50}WI/EC_{50} HT-29	EC_{50}WI/EC_{50} MCF-7
Me_2Sn/SA	1.0	0.94	1.0	0.94	1.0	1.0
Et_2Sn/SA	1.1	1.0	1.0	1.0	1.1	0.99
Bu_2Sn/SA	0.99	0.90	1.1	1.1	1.1	1.0
Oc_2Sn/SA	0.99	0.95	1.0	0.99	0.95	0.91
Ph_2Sn/SA	1.0	1.0	1.0	1.0	1.1	1.0



Space-filling model for one repeat unit of the polymer from salicylic acid and dimethyltin dichloride.

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How to cite this article: Carraher CE, Roner MR, Lynch M, Moric-Johnson A, Miller L, Slawek P, Mosca F, Frank J. Organotin Poly(ester ethers) from Salicylic Acid and Their Ability to Inhibit Selected Human Cancer Cell Lines. J Clin Res Oncology 2018;1(1):1-11.